

ICP Waters Report 153/2023

Responses of benthic invertebrates to chemical recovery from acidification



International Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes

Convention on Long-Range Transboundary Air Pollution



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<p>Summary</p> <p>The report provides an assessment of biological recovery from acidification in freshwater environments in Europe. The report consists of two parts, a regional data analysis based on an international dataset of biological and water chemical records, and a collection of national contributions on monitoring and assessment of biological recovery in different countries. The regional analysis showed that 47% of all included rivers (21 sites, for the period 1994-2018) and 35% percent of all lakes (34 sites, for the period 2000 to 2018) showed significant increases in species richness. Correlations between species diversity and water chemical components (ANC, pH, SO₄) were found, supporting that the biological responses were related to chemical recovery. Additionally, the composition of functional traits in rivers underwent significant changes over time. Both parts of the report demonstrate ongoing biological recovery from acidification in European acid-sensitive freshwater environments.</p>	
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CONVENTION ON LONG-RANGE
TRANSBOUNDARY AIR POLLUTION

INTERNATIONAL COOPERATIVE PROGRAMME ON
ASSESSMENT AND MONITORING THE EFFECTS OF
AIR POLLUTION ON RIVERS AND LAKES

Responses of benthic invertebrates to chemical recovery from acidification

Prepared at the ICP Waters Programme subcentre
NORCE Norwegian Research Center
Bergen, June 2023

Preface

The International Cooperative Programme on Assessment and Monitoring of the Effects of Air Pollution on Rivers and Lakes (ICP Waters) was established under the Executive Body of the UNECE Convention on Long-range Transboundary Air Pollution (CLRTAP) in July 1985. Since then, ICP Waters has been an important contributor to document the effects of implementing the Protocols under the Convention. ICP Waters has prepared numerous assessments, reports and publications that address the effects of long-range transported air pollution.

ICP Waters and its Programme Centre is chaired and hosted by the Norwegian Institute for Water Research (NIVA). A programme subcentre is established at NORCE, Bergen. ICP Waters is supported financially by the Norwegian Ministry of Climate and Environment and the Trust Fund of the UNECE LRTAP Convention.

The main aim of the ICP Waters programme is to assess, on a regional basis, the degree and geographical extent of the impact of atmospheric pollution, in particular acidification, on surface waters. More than 20 countries in Europe and North America participate in the programme on a regular basis.

An important basis of the work of the ICP Waters programme is the data from existing surface water monitoring programmes in the participating countries, collected through voluntary contributions. The ICP Waters network of monitoring sites is geographically extensive and includes long-term data series (more than 25 years). The programme conducts annual chemical intercomparison and biological intercalibration exercises.

This report presents a regional analysis of biological recovery by means of chemical and biological data from ICP Water sites in Europe, as well as national chapters on biological recovery produced by National Focal Centres (NFCs). Gaute Velle and Christian Lucien Brodin at the ICP Waters subcentre have been the main responsible for the data compilation, statistical analyses and writing of the report. Heleen de Wit (Chair of ICP Waters) and Kari Austnes (Head of the ICP Waters Programme Centre) have contributed at all stages of the report. The other co-authors have also provided valuable feedback on the joint part of the report. In particular we would like to thank Don Monteith and Angela Boggero for significant contributions during the writing of the report.

We wish to thank the NFCs for their efforts in compiling and submitting chemical and biological data and producing national chapters. The report could not have been produced without the active involvement by the NFCs.

Bergen, 08.06.2023

Gaute Velle
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Summary

The report provides an assessment of the biological recovery from acidification in freshwater environments in Europe. The report consists of two parts; first an analysis of an international dataset of biological and water chemical records, secondly a collection of national contributions on monitoring and assessment of biological recovery.

The regional analysis evaluates the biological recovery from acidification from 104 acid-sensitive rivers and lakes from the Czech Republic, Germany, Italy, Norway, Sweden, the UK, and Switzerland. The assessment includes data on aquatic macroinvertebrate EPT taxa (Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)) as indicators of acidification. EPT taxa are particularly useful for evaluating biological responses to acidification because they are sensitive to acid components of the water, and their identification has been consistently performed at the species level throughout the monitoring period. Changes in species diversity and their functional traits (attributed to each species based on information from literature) were assessed. Functional traits describe the main mechanisms through which organisms contribute to the stability of ecosystem functions, such as productivity, litter breakdown, and water filtration. Also, a statistical analysis linked the biological changes to chemical recovery. The contributed data records were filtered based on criteria for sufficient data length for trend analysis, resulting in a dataset of 21 rivers and 34 lakes.

Forty-seven percent of all included rivers (21 sites, for the period 1994-2018) and thirty-five percent of all lakes (34 sites, for the period 2000 to 2018) showed significant increases in species richness. The smaller changes for lakes compared with rivers could be related to the shorter time period, but biological communities in lakes can also be less responsive than in rivers because of lower rates of habitat recolonization. Correlations between species diversity and water chemical components (ANC, pH, SO₄) were found, supporting that the biological responses were related to chemical recovery.

Additionally, the composition of functional traits in rivers underwent significant changes over time. Both species richness and functional traits exhibited more abrupt changes after 2012, and the proportion of predators in the EPT community was unexpectedly low towards the end of the period. The alterations in functional traits within acid-sensitive surface waters in Europe can potentially have profound effects on ecosystem functioning. It should be noted that the functional trait analysis was limited to EPT taxa, which represent varying proportions of the total biological community in rivers. Further analyses are necessary to consider the impacts of other components of the biological community and other potential drivers, such as catchment properties and climate change.

The national sections were contributed by Italy, Switzerland, Norway, Sweden, and the UK. These contributions highlight the significance of national monitoring and assessment activities for evaluation of biological recovery in lakes and rivers. Responses to reduced surface water acidification were evaluated, suitable biological indices were identified, and the recovery of benthic invertebrates was assessed. The results varied across sites, with a decrease in acidophilic species in Italian lakes from 1991 to 2000 and limited recovery in Swiss acid-sensitive sites. Norway observed significant biological recovery, while Sweden saw positive trends in taxon richness but slower recovery in low-pH sites. The UK reported positive trends in acid-neutralizing capacity and changes in diatom and macroinvertebrate communities, although not all sites exhibited macroinvertebrate recovery. Additionally, methods and tools for assessing acidification effects on benthic fauna were shared.

Overall, the report highlights the ongoing biological recovery from acidification in European acid-sensitive freshwater environments. The biological recovery commenced from the 1990s and is still ongoing. The recovery is observed in terms of a gradual return of acid-sensitive taxa and increasing biodiversity and is seen across benthic macroinvertebrates and diatoms (diatoms only considered in the national contributions). The report also emphasizes the significance of integrated biological and water chemical monitoring programs, as well as the potential of such data to investigate the intricate relationship between environmental disturbances, species diversity, functional traits, and the recovery of freshwater ecosystems.

1 Introduction and aim of the report

1.1 Background

Atmospheric pollution peaked in Europe during the 1980s and has gradually decreased from the 1990s towards the present. The reduced pollution has resulted in improved chemical conditions in freshwaters, as indicated by increasing pH, and a reduction in the concentration of labile aluminum, sulphate and, to a lesser degree, nitrate. This chemical recovery is a precondition for biological recovery, i.e., the return of acid-sensitive organisms. The Convention on Long-range Transboundary Air Pollution (LRTAP Convention) has an effect-based approach, which implies that documentation of reduced pollution on ecosystem status is essential for assessing the effectiveness of the emission reduction policies. Therefore, data on biological recovery in rivers and lakes are extremely valuable under the Convention.

In acidified surface waters, long-term records of macroinvertebrates are especially suitable for assessing biological recovery. Aquatic macroinvertebrates spend most of their life cycle in water, feeding on plants, bacteria, and decaying organic material - and serving as a food source for other organisms. EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa are particularly useful for evaluating biological responses to acidification because they are sensitive to acid components in water. In ICP Waters, these taxa have been identified at the species level throughout the monitoring period.

1.2 Aims and call for contributions

In 2021, ICP Waters sent out a call for national contributions for a report on biological recovery from acidification. The aims of the report are to document temporal trends in biological recovery and compare national or regional analyses of biological responses, and thereby demonstrate the usefulness of biological monitoring data. An additional feature of the analysis is the focus on functional traits (feeding and movement mode) of benthic invertebrates. Here, we hypothesize that biological recovery is associated with a shift in functional traits of the invertebrate community leading to changes in ecosystem function.

To meet the aims for the analysis, the call for contributions included:

- i) A call for data (temporal records of benthic invertebrates, water chemistry and functional feeding groups, if available, from acid-sensitive rivers and lakes) for a joint, regional assessment, to be conducted by the programme subcenter.
- ii) National assessments of biological recovery. The national assessments were required to have sections on acid-sensitivity of monitored regions, description of monitoring methods and how biological responses to acidification are assessed (use of indices), i.e. not restricted to benthic invertebrates.

The regional and national assessments are thus complementary, where the strength of the regional analysis is to use a similar methodology across all countries for assessing temporal change. The strength of the national contributions was the freedom to use other organism groups than benthic invertebrates and regionally adapted acidification indices, thus providing a potentially more complete overview of ecological status. Acidification indicators rely on knowledge of presence and acid-

sensitivity of species that often have a regional or local occurrence and therefore cannot be applied universally.

The following contributions were made: Czech Republic, Germany, Italy, Norway, Sweden, Switzerland, and the UK submitted temporal records of benthic invertebrates and water chemistry while Italy, Switzerland, Norway, Sweden, and the UK submitted national assessments.

The report first presents the regional data analysis of benthic invertebrates (Chapter 2), followed by the national assessments (Chapters 3 to 8), each of which are shortly summarized in Chapter 9.

2 Analyses of benthic invertebrate recovery of ICP Waters sites from the Czech Republic, Germany, Italy, Norway, Sweden, Switzerland, and UK

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2.1 Introduction

Freshwaters are heavily impacted by human perturbations, and freshwater ecosystems may be the most endangered ecosystems on Earth (Sala et al. 2000, Dudgeon et al. 2006). Yet, there is a lack of data on population trends for a large proportion of freshwater species in Europe (Cuttelod et al. 2011, Gozlan et al. 2019). The major types of human-induced stressors that may affect freshwater ecosystems include over-exploitation, pollution, habitat degradation, species invasion and flow modification (Dudgeon et al. 2006). Air pollution and the associated impacts on water quality represent a major large-scale environmental impact on freshwater ecosystems (Schindler 1988, Stoddard et al. 1999). Toxic effects of elevated hydrogen and aluminum ion concentrations caused by acid deposition (colloquially referred to as acid rain), have represented major drivers of loss of acid sensitive organisms (Raddum and Skjelkvale 2001). For example, declining fish populations in lakes and streams and detrimental effects on macroinvertebrates (i.e., invertebrate animals >0.25 mm in length (Rosenberg and Resh 1993)) were reported from the beginning of the 20th century in Norway and Sweden, and from the mid-20th century in Canada and the United States (Schofield 1976, Raddum et al. 1984). The UNECE Convention on Long-range Transboundary Air Pollution (LRTAP Convention), established in 1979, aimed to reduce air pollution using an effect-based approach (Grennfelt et al. 2020). It has led to substantial reductions in emissions of sulfur (S) and nitrogen (N) to the atmosphere, resulting in reduced acid deposition and substantial chemical recovery of surface waters in Europe and North America (Stoddard et al. 1999, Garmo et al. 2014). Biological recovery from acidification, in terms of a reappearance of acid-sensitive taxa and an increase in biological diversity, is also evident in many sites (Gunn and Sandøy 2003), however, it has repeatedly been suggested that biological recovery is lagging behind the chemical recovery (Monteith et al. 2005, Hesthagen et al. 2011). Since the LRTAP Convention assesses the need for further reduction of S and N emissions to the atmosphere based on effects, i.e., damage to ecosystems, it is important to provide evidence of responses of aquatic biological communities to chemical recovery.

The term biological diversity or “biodiversity” refers to the variety and variability of life on Earth. Biodiversity commonly includes many aspects that describe the variability of life-forms, such as the variation of genes, the number and the evenness of species, or the diversity of ecosystems (Purvis and

Hector 2000). It may also cover measures of the value and range of functional traits in an ecosystem, i.e., characteristics that reflect a species' adaptation to its environment (Hooper et al. 2005). Examples of traits include feeding mechanisms, modes of movement or organism size and shape. Ecosystem processes rely heavily on traits (Chapin et al. 1998) since the traits influence how an ecosystem operates or functions, e.g., recycling of organic matter and nutrients or filtering of water (Leenhardt et al. 2015). An ecosystem's health is often defined by its ability to maintain structures and functions at a natural rate, as well as its resilience to abiotic changes, like pollution or human interference (Grootjans et al. 2012, van Andel and Aronson 2012). Species react differently to certain stresses, and thus the presence, absence, and abundance of species within a functional trait group will determine the resilience of the function and of the ecosystem itself (Walker et al. 1999). Healthy ecosystems tend to be sufficiently diverse in species to provide functional redundancy, when it comes to morphological, physiological, or phenological characteristics that influence growth, reproduction, and survival (Violle et al. 2007). This means that fluctuations in the abundance of some species can be offset by fluctuations of others (Valencia et al. 2020), thereby providing ecosystem stability. Analysis of functional traits therefore provides the link between ecosystem functioning and the more commonly used measures of biodiversity (Schmera et al. 2017).

Macroinvertebrates occur in large numbers and include many species in freshwaters. They are important in the ecosystem and deliver many ecosystem services, especially since (1) they facilitate breakdown of particulate organic matter and contribute to carbon and nitrogen cycling (Schmera et al. 2017), (2) they are a major source of food for higher trophic levels in their ecosystem, like fish, birds, amphibians, and invertebrate predators, (3) they filter the water, and (4) they transfer energy from freshwaters to terrestrial environments. Macroinvertebrates feed in a variety of ways, e.g., by scraping algae, shredding coarse detritus, consuming fine organic matter, or filtering water for particles and free-living phytoplankton (Gamito and Furtado 2009). As such, they can reveal knowledge that is central for monitoring, restoring, and preserving freshwater ecosystems (Heino 2005).

The widely reported improvement in water chemistry is expected to lead to the return of acid sensitive macroinvertebrate species and communities, and potentially also, to an increase in functional diversity. In this report, we will assess evidence for, and the extent of, biological recovery from acidification of benthic invertebrates in acid-sensitive surface waters in Europe. We have used extensive monitoring data on benthic macroinvertebrates and water chemistry from mid-1980 and up to the present from sites in the Czech Republic, Germany, Norway, Sweden, Switzerland, the UK, and Italy. These countries participate in hydrochemical and macroinvertebrate intercomparison schemes that ensure quality and international comparability. We focus on species of Ephemeroptera, Plecoptera, and Trichoptera (EPT) because these have been identified to species level consistently over the monitoring period, are common in freshwaters, have fairly well-known traits, and are highly sensitive to environmental perturbations (Wallace and Webster 1996, Sandin and Johnson 2000).

Our primary objectives have been to use EPT to (1) quantify trends in biological diversity as determined by the number of species, (2) quantify trends in functional traits in rivers, and (3) examine whether observations of biological change can be interpreted specifically as biological recovery from acidification, via comparison with water chemical records.

2.2 Methods

2.2.1 Study sites

Study sites include lakes and rivers from the Czech Republic, Germany, Italy, Norway, Sweden, Switzerland, and UK (Table 1, Figure 1, Figure 2, Figure 3, Appendix 1). The full data set includes 515,000 individual EPT specimens from 4771 samples in 62 lakes and 49 rivers collected between 1982 and 2021 (Figures 2 and 3). Most study sites represent nutrient poor, acidified waters in natural or semi-natural catchments that have been exposed to long-range transported air pollution, in particular S and N deposition. These sites form part of national biological and chemical monitoring programs in running and standing freshwaters (Halvorsen et al. 2002, Horecký et al. 2002, Horecký et al. 2006, Johnson and Goedkoop 2007, Schaumburg et al. 2008, Kernan et al. 2010, Horecký et al. 2013). The bedrock varies among sampling regions, but is mostly acid-sensitive, consisting of gneiss, granite, or quartzite. In terms of vegetation, the northernmost sites are situated in the boreal zone and the southernmost sites are situated in the cool temperate zone.

The temporal records differ in sampling frequency (regular sampling: annual, biannual, every four years; irregular) and period covered (the longest records cover 30 years, shorter records start after 2000). Striving for most consistency regarding period covered while still being able to include as many sites as possible, we used the following site selection criteria:

- i. Lakes with records covering 2000-2018, with at least one year of data before 2003 and at least one year of data after 2015.
- ii. Rivers covering the period 1994-2018, with at least one year of data before 1997 and at least one year of data after 2015.

Sites that did not meet the criteria were excluded from the analyses of temporal variation.

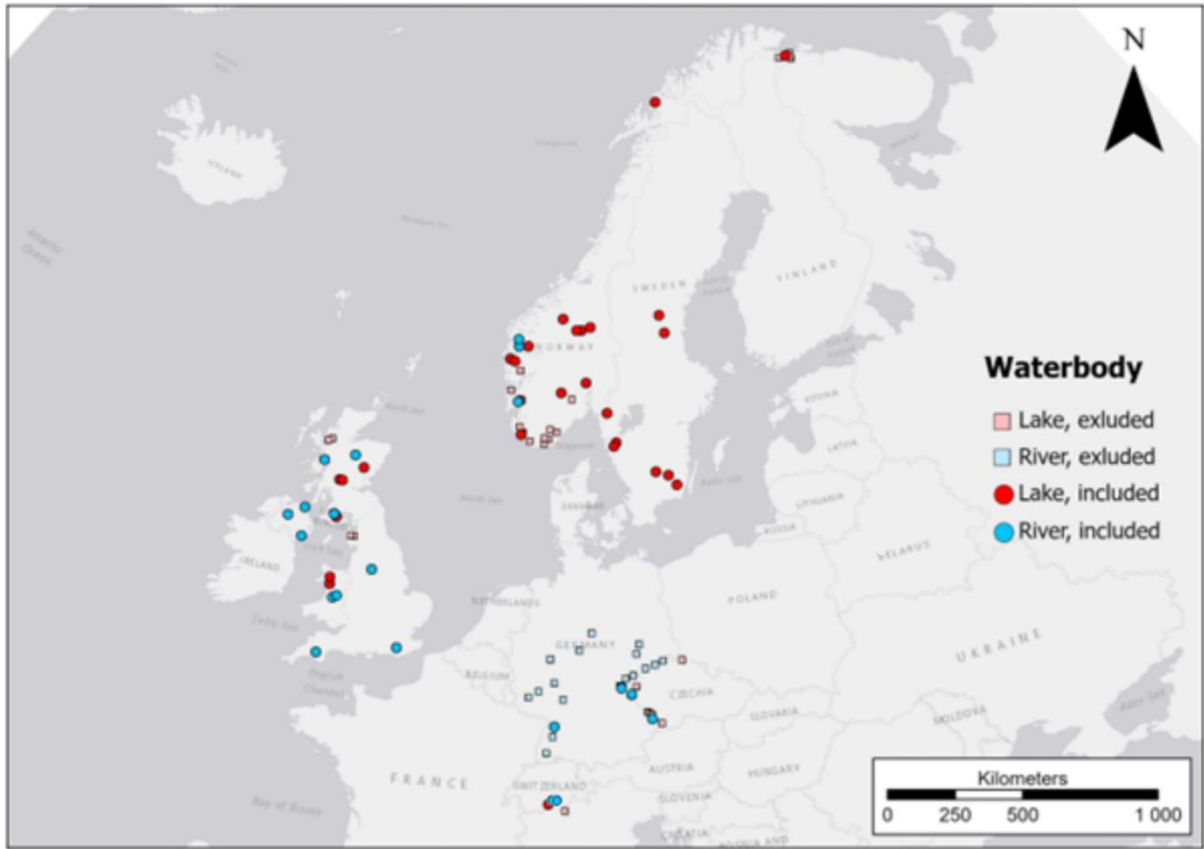
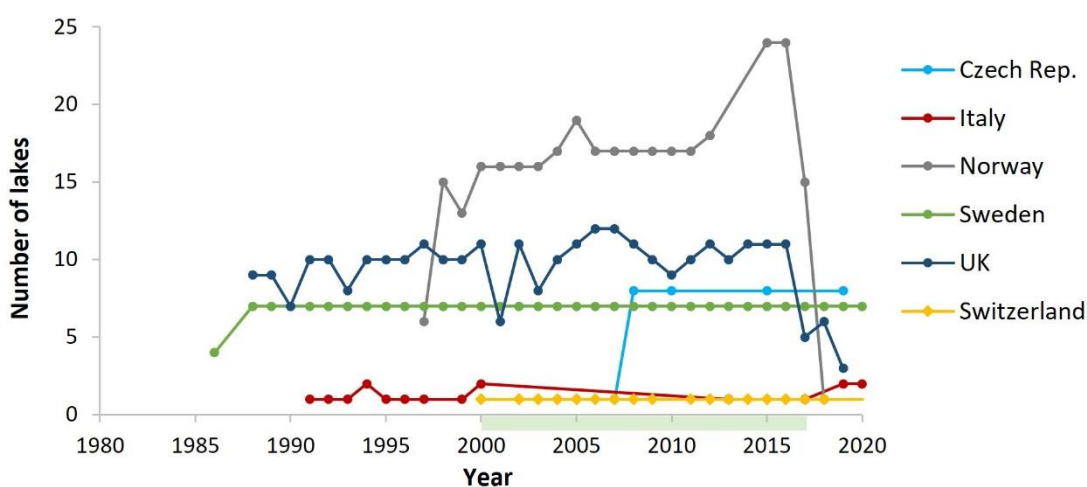


Figure 1. Study sites in the Czech Republic, Germany, Italy, Norway, Sweden, Switzerland, and UK.

Table 1. Study sites of biological samples. The number of samples per site is given in Appendix 1.

Country	Lakes			Rivers		
	Number	Time period	Long term sites* (n)	Number	Time period	Long term sites** (n)
Czech Rep.	8	2007-2019	0	1	2000-2017	0
Germany	0			27	1982-2020	6 (annual)
Italy	2	1991-2020	1 (11)	3	1991-2020	2 (annual)
Norway	24	1997-2018	14 (annual)	5	1982-2020	3 (annual/biannual)
Sweden	7	1983-2021	7 (annual)	0		
Switzerland	1	2000-2021	1 (annual)	3	1991-2018	2 (annual from 2000)
UK	12	1988-2019	7 (annual)	11	1988-2019	10 (annual)

* lakes with records covering 2000-2018, with at least one year of data before 2003 and at least one year of data after 2015. ** rivers covering the period 1994-2018, with at least one year of data before 1997 and at least one year of data after 2015. n = sampling frequency or nr of years with observations.

**Figure 2.** Number of lakes monitored per year per country. The green shading along the x-axis indicates the minimum period required for a site to be included in the trend analysis. See Appendix 1 for number of samples per lake.

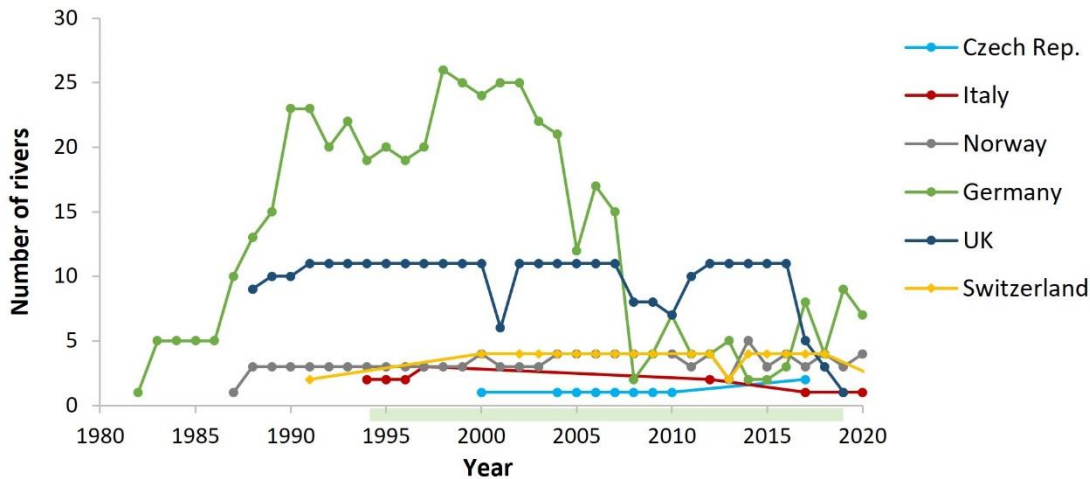


Figure 3. Number of rivers monitored per year per country. The green shading along the x-axis indicates the minimum period required for sites to be included in the trend analysis. See Appendix 1 for number of samples per river.

Some of the monitoring sites in Germany include sites that are limed, sites that are affected by mining, or sites in deeply weathered soils with a high sulfate-adsorption capacity (see Appendix). These were excluded from the analyses of overall trends since they are clearly impacted by chemical factors other than air pollution.

We predicted that the extent of biological recovery would vary according to the site-specific sensitivity to acidification and the initial and most recent levels of acid deposition. To reduce noise and ease interpretation of results, the sites were clustered into two groups based on sensitivity to acidification and strength of chemical recovery:

1. Highly acid-sensitive sites with a strong chemical recovery, implying a significant temporal change in both pH and ANC (“strong”)
2. Sites with a weak- to intermediate acid-sensitivity and chemical recovery, implying sites with either no change in pH and ANC, or with only significant temporal change in either pH or ANC (“weak”)

2.2.2 Biological data

The benthic invertebrate samples in our dataset were collected by kick sampling (Frost et al. 1971), following the ICP Waters manual (Wathne et al. 2010). Most sampling was done in April-June (39% of all samples), or October-November (48%). Each sample was sorted under a stereomicroscope in the laboratory, and macroinvertebrates subsequently identified. The consistency of the taxonomic resolution through the 30-year sampling period was checked and corrected by methods described in (Velle et al. 2013). In short, data from sites with inconsistent taxonomic resolution were reduced to a coarser taxonomic level prior to numerical analysis. The sampling interval varied among sites and years, with an average of about 1.5 samples per sampling station per year from the time most monitoring programs began in the 1980s and up to the present (Appendix 1).

All contributing countries identify mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) (EPT taxa) to species, and data for these groups only were therefore retained in the

analyses. The benthic invertebrates were identified with the use of extensive national and international keys (e.g., Askew 1988, Lillehammer 1988, Wallace et al. 1990, Nilsson 1996).

2.2.3 Functional groups and traits

In comparison to rivers, lakes exhibit a lower proportion of EPT taxa in their benthic macroinvertebrate communities. The EPT community in rivers displays longer functional response gradients compared to lakes. This difference is particularly noticeable in some of the study lakes, which are oligotrophic. The implication is that we have analyzed functional groups and traits exclusively for rivers.

We analyzed two functional groups: 1. feeding mode, since feeding is strongly related to natural environmental gradients, organic matter processing, energy flow, trophic relationship, and management activities (Statzner and Bêche 2010, Birara et al. 2020) and 2. movement mode, since movement is strongly related to habitat use and dispersal ability (Rabeni et al. 2005, Heino 2008). Feeding mode represents the most common traits included in analyses of function, perhaps due to the direct link between feeding and ecosystem functions (Schmera et al. 2017). Feeding impacts the way species interact by predation, break down detritus, feed on plants, and filter water, potentially altering the food sources and physical properties in an ecosystem.

Traits of the two functional groups were assigned to each EPT species based on Cummings and Merritt (1996), Schmidt-Kloiber and Hering (2015), Moog and Hartmann (2017), and Usseglio-Polatera et al. (2000). Several species are attributed to more than one trait within these functional groups. We selected the dominating trait (> 35% of the 10 pt system used by freshwaterecology.info/traits), and two traits if there were two dominating traits. No species were attributed to more than two traits for feeding mode and movement mode separately. We identified 10 traits within the two functional groups in the species lists (Table 2, Appendix 2). The final metric represents the proportion of taxa with a specific trait, i.e., the relative prevalence of a trait in the population.

Table 2. Functional groups and traits of aquatic macroinvertebrates included in the study. A complete species list with traits is given in Appendix 2.

Functional group	Trait group	Description
Feeding mode	Gatherers/Collectors	Feed on fine particulate detritus on stream bottom
	Filterers	Filter suspended particulate material from water column
	Predators	Consume other animals and engulf whole prey or suck body fluids
	Grazers/Scrapers	Feed on periphytic algae and associated material on mineral or organic substrate
	Shredders	Feed on living or decomposing vascular plant tissue, coarse particulate organic material by chewing large pieces
	Other	Other modes of feeding
Movement mode	Swimming	Swim through water
	Burrowing/Boring	Burrow in soft substrates or bore in hard substrates
	Sprawling/Walking	Move actively over surfaces with legs, pseudopods or on mucus
	Sessile / Semi-sessile	Fasten to hard substrates, plants, or other animals
	Others	Other modes of locomotion

2.2.4 Chemical data

We focused on three chemical variables that are strongly related to acidification; pH, sulphate (SO_4^{2-}), and acid-neutralizing capacity (ANC). ANC was calculated as the difference in the sums of equivalent concentrations of base cations ($\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+$) and acid anions ($\text{Cl}^- + \text{SO}_4^{2-} + \text{NO}_3^-$). For a detailed description of measured water chemistry, see Skjelkvåle and de Wit (2011). See Appendix 1 for a full list of sites. A comprehensive analysis of trends in water chemistry at ICP Water sites between 1990 and 2016 is published in Garmo et al. (2020). Most lakes in the study are small and homogeneous, and the chemistry was sampled in one sampling station in the lake or at the outflow as representative of the full lake. All chemical samples were averaged to annual means and were included in the analysis of temporal trends in water chemistry. Details on paring of biological and chemical data are described in 3.2.5.

2.2.5 Numerical analysis

All statistical analysis was done in the R statistical environment (version 4.1.1; R Core Team 2021). The analyses included three main steps:

First, we compiled a combined data set that included paired annual invertebrate samples and chemistry samples. All biological monitoring stations were included in the analyses of diversity and functional groups. Usually, water samples were taken at the biological sampling stations for chemical analysis, but there were exceptions. Also, the timing of chemical and biological sampling did not always coincide. For sites lacking fully harmonized biological and chemical monitoring in space and time, we imposed as criterion for the inclusion in our analyses that the water samples had to be taken within the year that the biological sample was taken. Where more than one macroinvertebrate sample or water chemistry sample was provided for a given year, we took the average number of unique EPT taxa or the averaged chemical concentrations per station for that year.

Secondly, we conducted tests to examine temporal trends. The non-parametric Sen's slope estimator (Sen 1968) was utilized with a significance level of 0.05, employing a Mann-Kendall test. This was done using annual samples for pH, SO_4^{2-} , ANC, and the number of EPT taxa. The number of EPT taxa was calculated for all sites that met the site selection criteria (section 3.2.1), also including sites with missing chemical data. To visualize the temporal variation in the number of EPT taxa in the grouped data, we employed normalization by subtracting the mean EPT taxa for a given year and dividing by the standard deviation based on all years. A Mann-Kendall test with a significance level of 0.05 was also employed to analyse the temporal changes in the proportion of functional traits. The magnitude of change and direction was calculated using a Sen's slope, which indicates the magnitude (in percentages) of change per year. Each trait was tested separately. The prevalence of functions over time was depicted using a LOESS smoother with a span of 0.75 (Cleveland 1993). Box plots were used to illustrate the variation in water chemistry based on yearly averages.

Thirdly, we determined whether water chemistry was a probable driver of the observed changes in diversity. This was done by analysing the correlations between species diversity and pH, ANC, and SO_4^{2-} using paired annual invertebrate samples and chemistry samples and testing for significance ($p < 0.05$) using a Pearson correlation in the base package. Stations not monitored for water chemistry (see Appendix 1) were excluded in the correlation analysis.

2.3 Results

2.3.1 Water chemistry

There were substantial changes in pH, ANC, and sulphate over time at the sites (Figure 4 and Figure 5) and in the remaining sites (Appendix 3 and 4). A significant decrease in sulphate occurred in 33 lakes and 20 rivers, pH increased significantly in 29 lakes and 18 rivers, and ANC increased significantly in 34 lakes and 19 rivers. There were five lakes and five rivers with a weak to intermediate chemical recovery, and 29 lakes and 16 rivers with a strong chemical recovery.

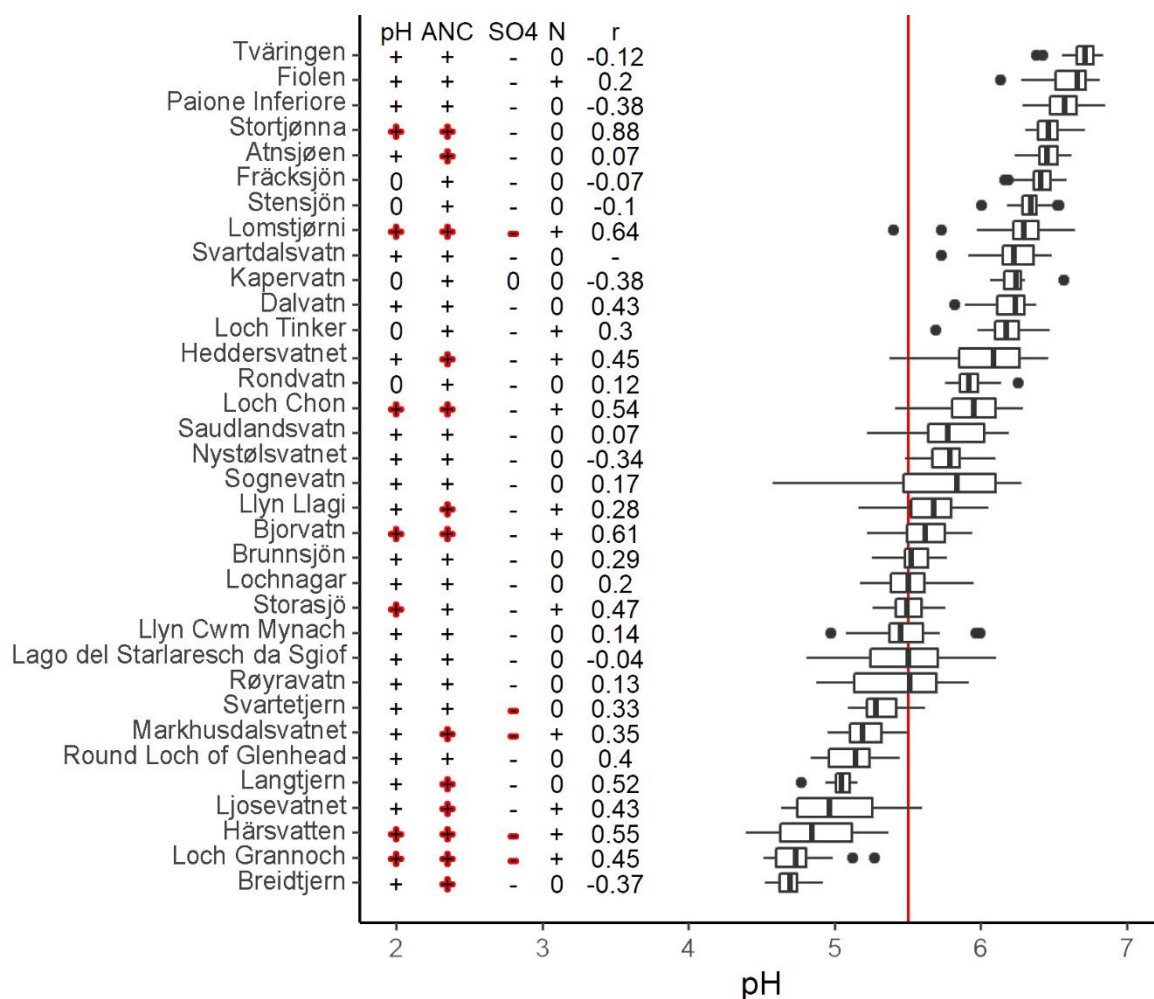


Figure 4. Boxplot of pH for lakes that are part of the analysis of trends, The red line denotes a pH of 5.5. The columns on the left-hand side indicate sites with a significant ($p < 0.05$) increase (+), decrease (-) or no change (0) in pH, ANC, SO_4^{2-} , and species diversity (N), as assessed by a Sen slope. r represents the relationship between species diversity and pH (Pearson correlation coefficient). Red symbols indicate a significant relationship between the variable (pH, ANC, or SO_4) and species diversity (N).

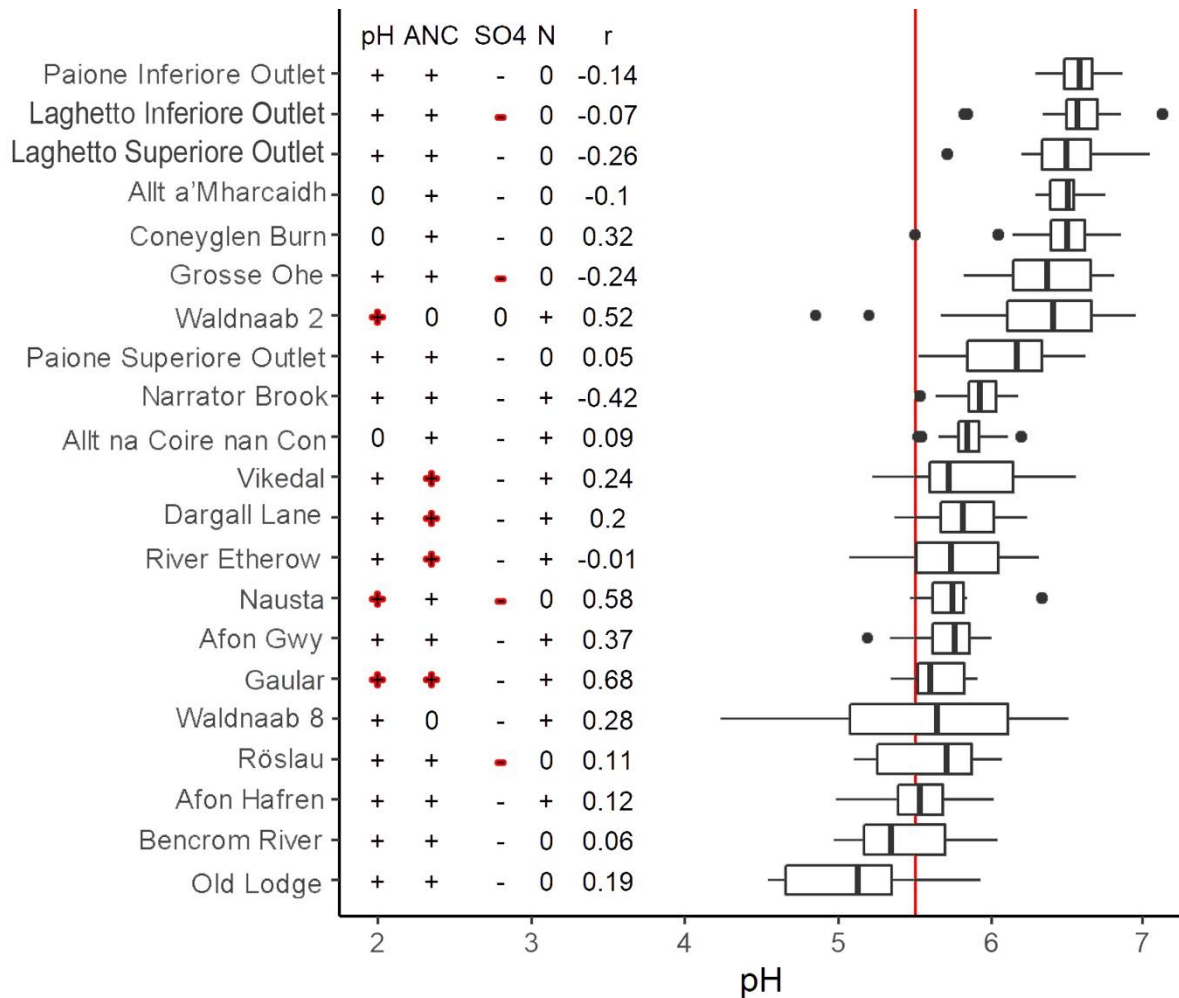


Figure 5. Boxplot of pH for rivers that are part of the analysis of trends. The red line denotes a pH of 5.5. The columns on the left-hand side indicate sites with a significant ($p < 0.05$) increase (+), decrease (-) or no change (0) in pH, ANC, SO_4^{2-} , and species diversity (N), as assessed by a Sen slope. r represents the relationship between species diversity and pH (Pearson correlation coefficient). Red symbols indicate a significant relationship between the variable (pH, ANC, or SO_4) and species diversity (N).

2.3.2 Species diversity

A total of 34 lakes from Italy, Norway, Sweden, Switzerland, and UK and 21 rivers from Germany, Italy, Norway, Switzerland, and UK met the criterion on minimum length of time series and were included in the analyses of biological trends (Table 3). Species diversity, as represented by the number of EPT taxa, increased significantly in 12 and 10 long-term monitoring lakes and rivers, respectively (Table 3, Figures 4 and 5). No sites showed a significant decrease in this metric. The standardised data reveals a tendency for diversity to increase across sites with a strong chemical recovery and to a lesser extent sites with a weak to intermediate chemical recovery (Figure 6). However, the increase was more pronounced for rivers than for lakes (Figure 6). There were large fluctuations in diversity after about 2012. When it comes to individual sites, the Pearson analysis indicates a stronger correlation between the temporal variation in species diversity and ANC than between species diversity and pH or sulphate (Figure 4 and Figure 5). Species diversity was significantly correlated ($p < 0.05$) to pH for 7 lakes and 3 rivers, to sulphate for 5 lakes and 4 rivers, and to ANC for 13 lakes and 4 rivers.

Table 3. Number of sites in the analyses of trends. The numbers in brackets indicate the percentage of sites with a significant increase in diversity, as assessed by Sen's slope and a Mann-Kendall test with significance level 0.05t. All= all sites. Strong = sites where pH and ANC have increased significantly. Weak = sites with either a significant temporal change in pH or ANC, or no change in pH and ANC.

	All	Weak	Strong
River	21 (48%)	5 (60%)	16 (44%)
Lake	34 (35%)	5 (20%)	29 (38%)

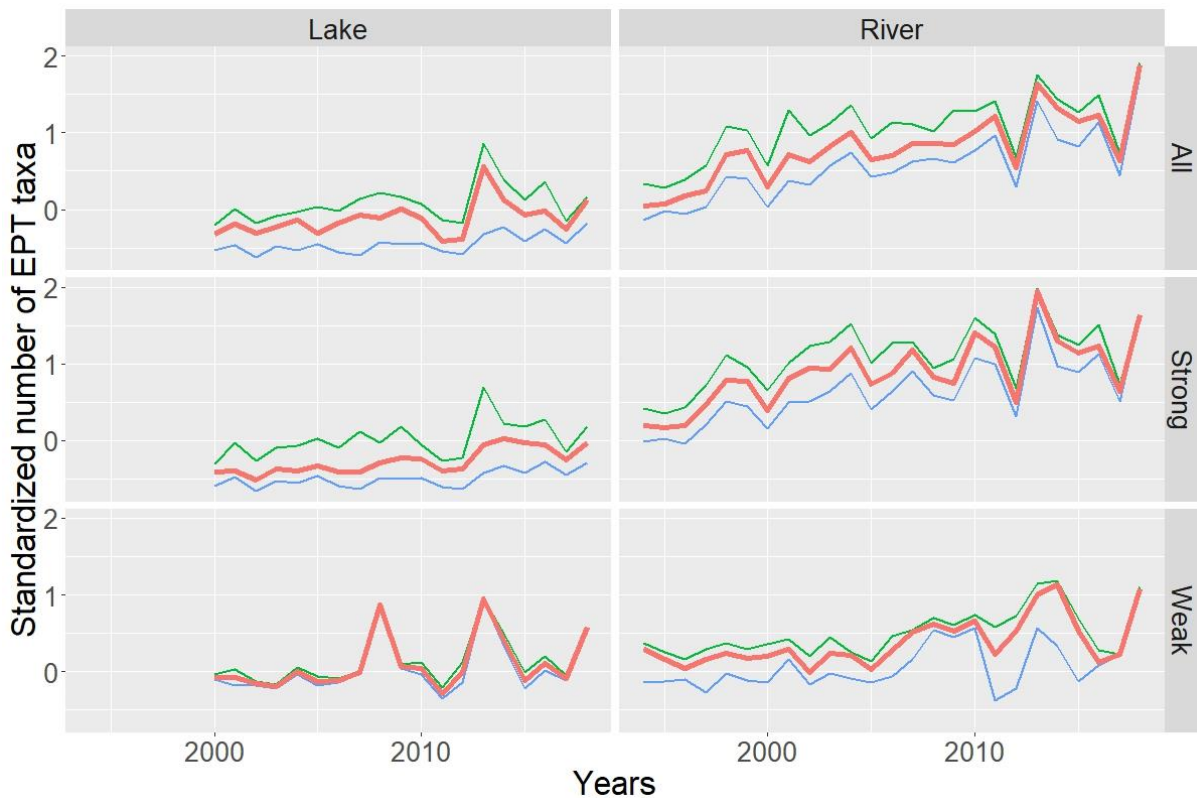


Figure 6. Temporal variation in the number of species of Ephemeroptera, Plecoptera, and Trichoptera (EPT) in lakes and rivers grouped according to chemical recovery. All= all sites. Strong = sites where pH and ANC have increased significantly. Weak = sites with either a significant temporal change in pH or ANC, or no change in pH and ANC. The middle red line represents the median, the upper green line is the 75th percentile and the lower blue line is the 25th percentile.

2.3.3 Variation in functional traits

Most EPT taxa in the river sites that were included in the analyses of temporal variation belonged to grazers/scrapers, shredders, and sprawlers (Figure 7). With respect to feeding mode, the proportion of gatherers/collectors had the higher abundance (Figure 8). This trait comprised about 30 to 50% of the species over time. Filterers had the lowest representation (around 5 to 10%), while the other groups differed roughly between 10 to 40%. Sprawlers dominated among the traits of movement

mode with a relative abundance of 50 to 70% (Figure 8). Sessile/Semi-Sessile had the lowest representation (around 5 to 8%) of movement modes. The proportion of several traits changed significantly over time (Table 4). However, the development in the proportion of traits was predominantly stable until around 2010 to 2012, from which the proportion of filterers, gatherers/collectors, predators, sprawlers, and swimmers indicate faster shifts or opposite changes (Figure 8, and Figure 9). The number of traits that changed significantly over time was higher in the sites with a weak to intermediate chemical recovery than in the sites with a strong chemical recovery. This is opposite from the EPT taxa, which exhibited more changes at sites where chemical recovery was strongest ('strong' group). There were also some notable differences in the two groups of sites. This especially concerns the gatherers/ collectors and the shredders, which show an opposite pattern over time in the two groups of sites.

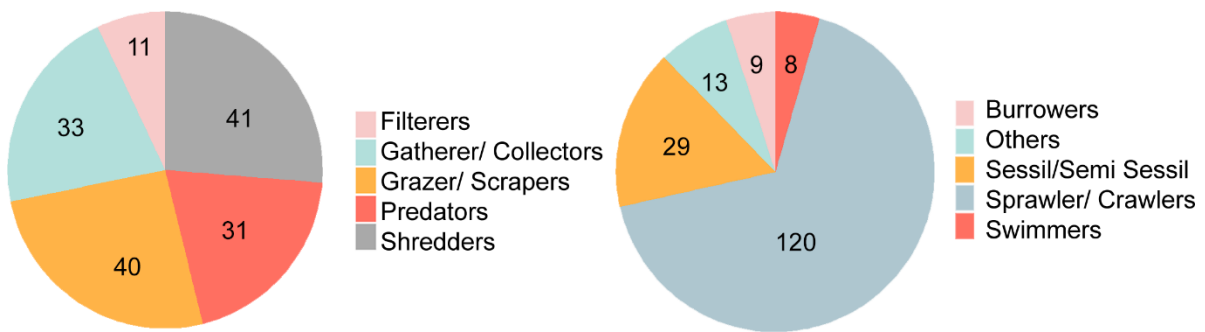


Figure 7. Total number of species with given feeding traits (left) and movement traits (right) within Ephemeroptera, Plecoptera, and Trichoptera in rivers.

Table 4. Changes in the proportion of functional traits of EPT taxa over time in rivers expressed as Sen slopes. Values of the slopes indicate percentage change in the trait per year. All = all sites. Strong = sites where pH and ANC have increased significantly. Weak = sites with either a significant temporal change in pH or ANC, or no change in pH and ANC. * and bold denotes significant change using a Mann-Kendall test with a significance level of 0.05.

	All	Strong	Weak
Moving Burrowing	-0.92*	-0.61	-1.50*
Other	0.49	0.63	-0.05*
Sessile/semi-sessile	0.06	0.07*	0.05
Sprawlers	0.11	-0.18	0.73*
Swimmers	0.25	0.09	0.77*
Feeding Filterers	0.02	0.10	-0.17
Gatherers	-0.68	-0.71	-0.51*
Grazer	0.26	0.04*	0.93*
Predators	-0.47	-0.49*	-0.30
Shredders	0.86	1.05	0.05*

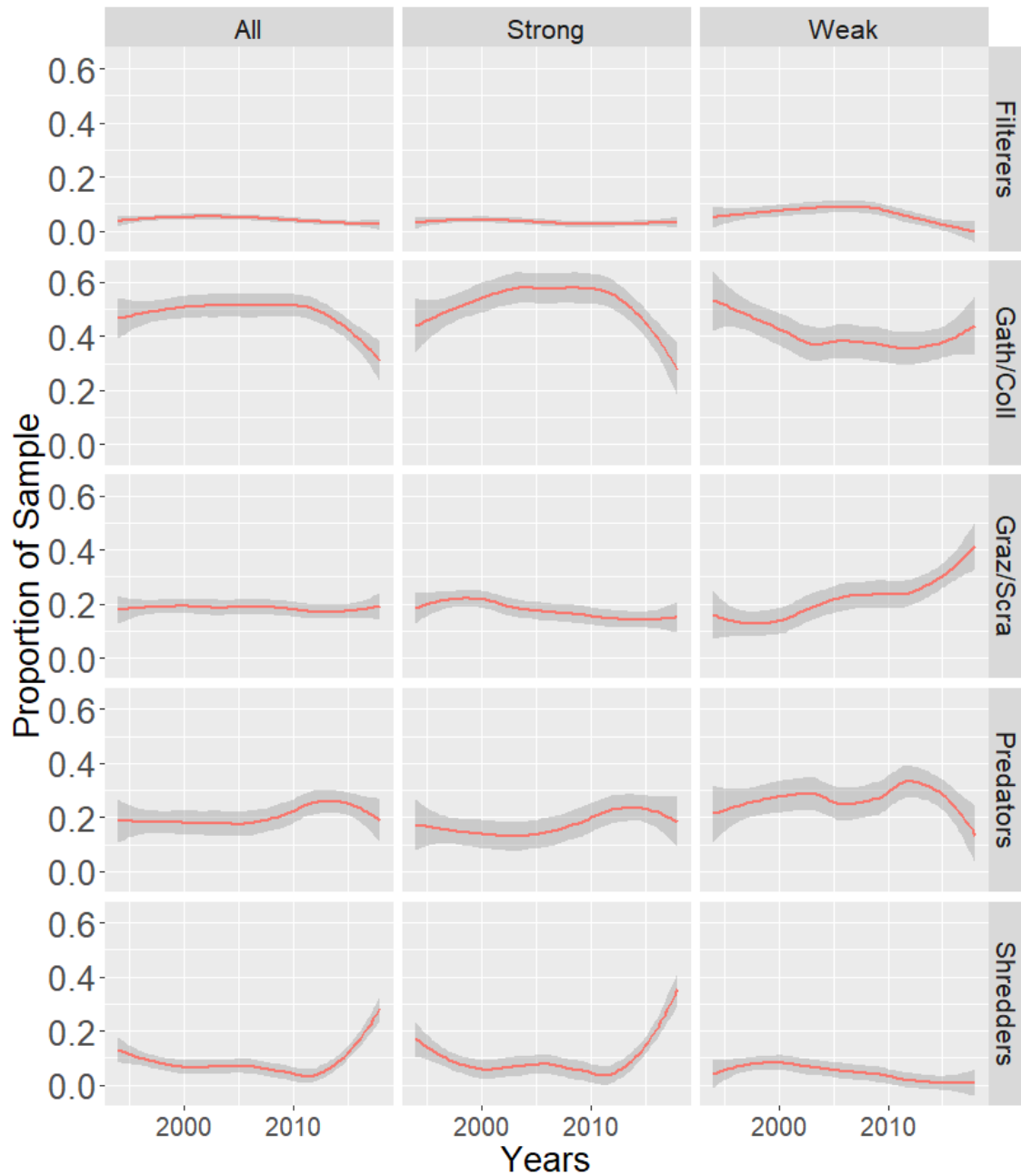


Figure 8. Temporal variation in the proportion of individuals with different traits of **feeding mode** added a LOESS smoother for species within Ephemeroptera, Plecoptera, and Trichoptera in rivers grouped according to the degree of chemical recovery. All= all sites. Strong = sites where pH and ANC have increased significantly. Weak = sites with either a significant temporal change in pH or ANC, or no change in pH and ANC. The grey shading indicates confidence intervals.

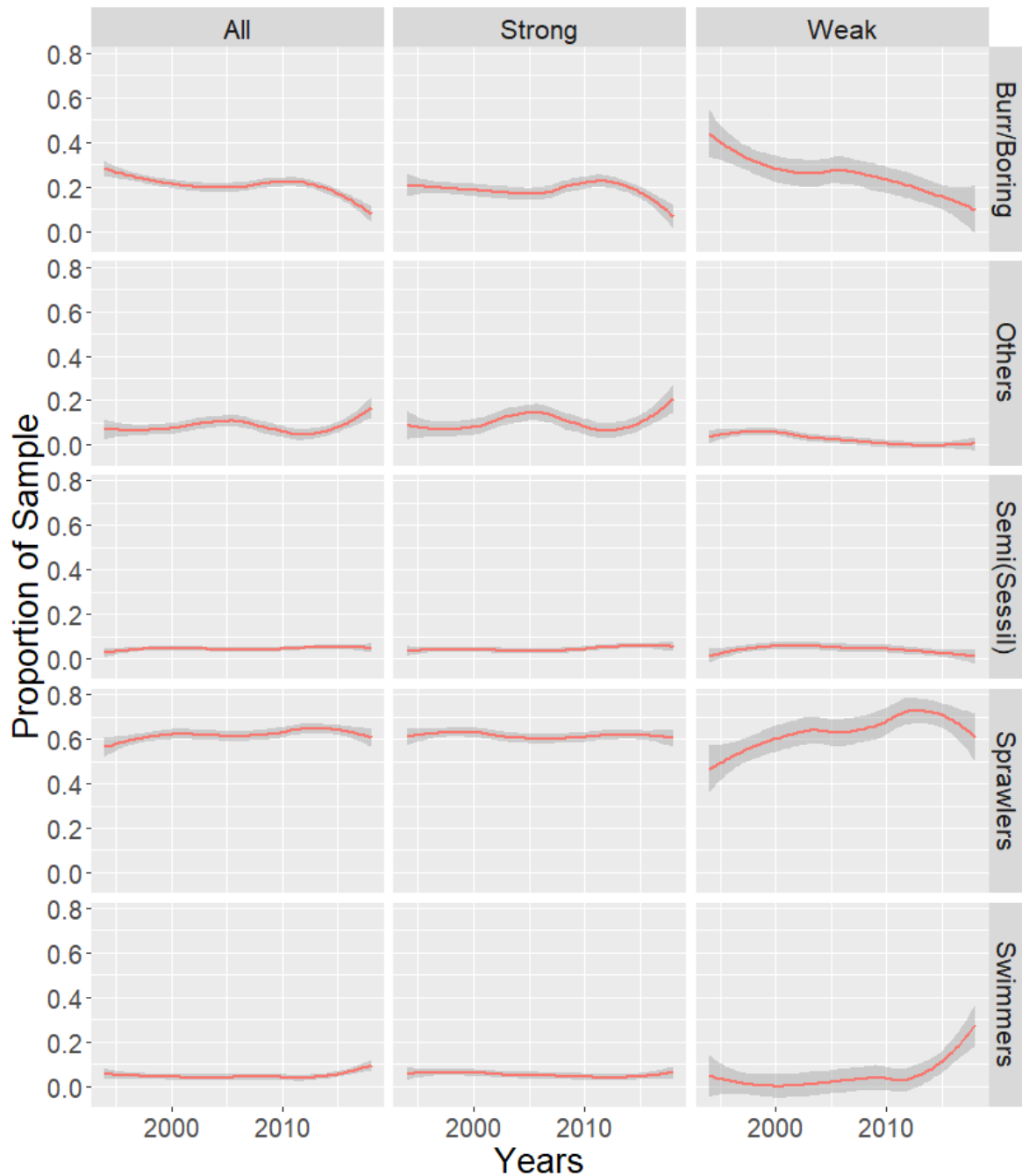


Figure 9. Temporal variation in the proportion of individuals with different traits of **movement mode** added a LOESS smoother for species within Ephemeroptera, Plecoptera, and Trichoptera in rivers grouped according to the degree of chemical recovery. All= all sites. Strong = sites where pH and ANC have increased significantly. Weak = sites with either a significant temporal change in pH or ANC, or no change in pH and ANC. The grey shading indicates confidence intervals.

2.4 Discussion

2.4.1 Chemical recovery

A decrease in sulphate and increase in pH and ANC were evident at most sites. The decrease in sulphate was significant at all sites except one German river and one Norwegian lake (Figure 4 and Figure 5). This suggests that most sites have undergone a chemical recovery of acidification, which agrees with Garmo et al. (2020). Data on DOC were not available for the clearwater lakes in Switzerland and Italy since DOC in these lakes is usually below detection limit. Therefore, trends in DOC were not evaluated. We did not analyze dissolved organic carbon (DOC), however, analysis of ICP Waters data has previously demonstrated that reductions in acid anions have been accompanied by significant increases in DOC (Monteith et al. 2007, De Wit et al. 2021). Thus, most sites have become less acidic and more light-limited.

2.4.2 Biological recovery

Expected response

There is a long tradition of using EPT taxa as indicators of degradation and recovery of freshwater ecosystems caused by human-induced perturbations (Loch et al. 1996, Ligeiro et al. 2013). The community of EPT taxa often consists of many species, especially in rivers, and EPT taxa often perform better than other indices when it comes to assessing the impact from acidification and chemical-induced disturbance (Wallace et al. 1996, Sandin and Johnson 2000). Acidification is known to have had a negative impact on the biodiversity of freshwater macroinvertebrates (Guerold et al. 2000, Traister et al. 2013). For example, the abundance of macroinvertebrates during acidification can be a tenth of the abundance before acidification (Rosemond et al. 1992, Olofsson et al. 1995). Chemical recovery from acidification would therefore be expected to promote the gradual reappearance of sensitive taxa, thus altering species composition, and increasing species richness and density of the community (Fjellheim and Raddum 1995, Sandøy and Romundstad 1995). When toxicity is too great for acid-sensitive taxa, local survival is only possible in refuges, perhaps downstream or in neighbouring catchments, which are less impacted by acidification.

As conditions improve, the sensitive taxa reappear from the source populations. The timescale for biological improvement can be difficult to predict. It is anticipated that there will be a continued rise in diversity as biological recovery typically lags improvements in water chemistry (Murphy et al. 2014). Several hypotheses have been put forward to elucidate this delay (e.g., Ledger and Hildrew 2005, Monteith et al. 2005): (1) acid-sensitive taxa struggle to reestablish their functional niches, which have been occupied by acid-tolerant generalist species, (2) re-colonization takes time since source populations of a species are lacking, (3) a chronic reduction in the buffering capacity in the catchment may have left the chemistry of runoff vulnerable to minor- or short-term episodes of acidification (snow melt, sea salt episodes) that may continue to exert detrimental effects on sensitive species, (4) the quality of algae as food for benthic macroinvertebrates in streams that are still somewhat acidified may be low (Herrmann et al. 1993), and (5) other environmental conditions may also have shifted, due for example to lower availability of base cations, warmer water temperatures etc. The strength of these five processes may be site dependent. Hence, the collapse of biological populations during acidification should not be expected to be mirrored during the chemical recovery phase. In line with this, the patterns of species recovery often vary among sites (Stendera and Johnson 2008, Angeler and Johnson 2012, Velle et al. 2013) and there is no evidence of macroinvertebrate communities approaching equilibrium for sites elsewhere, or reaching pre-acidification conditions (Wallace 1990, Guerold et al. 2000, Baker et al. 2021b). The large fluctuations in diversity in the present study also suggest that is not straightforward to predict the future trajectory of diversity. We lack information on

the reference state of diversity and biological composition at the sites with the implication that we cannot predict the end point of the biological recovery at the sites. When it comes to functional traits, biological communities frequently exhibit a stable composition of traits (de Bello et al. 2021). Consequently, we can anticipate that the composition of functional traits will stabilise once the sites have achieved pre-acidified chemical conditions, even if the species community itself does not reach a pre-acidified composition.

Temporal variation in biological diversity

We found a significant increase in the number of EPT taxa towards the present in 35% of the lakes and 48% of the rivers (Figure 4 and Figure 5, Table 2). A smaller change in lakes can be attributed to several potential reasons: 1. Lakes were monitored over a shorter time, with monitoring that perhaps started after most of the chemical recovery had already occurred; (2) EPT taxa are often more diverse and abundant in rivers than lakes and therefore have longer biological response gradients; (3) river environments tend to be more physically and chemically variable than lakes, so that physical and/or chemical alteration during hydrological episodes could promote more rapid species turnover; (4) biological recovery from acidification requires source populations for sensitive species that may have been lost during the acidification phase and therefore need to re-immigrate. Since the connectivity of lakes in the landscape is generally poor relative to river networks, biological communities often are more isolated (Loch et al. 1996, Ligeiro et al. 2013), which could delay the biological recovery.

The sites in the current study have undergone a significant chemical recovery that coincide with the increase in diversity. The correlation between diversity and acid components of the water was significant in some, but not all sites in the present study (Figure 4 and Figure 5). There was no apparent difference in the proportion of sites exhibiting a significant increase in diversity between the group with weak to moderate chemical recovery and the group with strong chemical recovery. This is somewhat surprising, particularly given that the chemical gradient is longer in the sites with a strong chemical recovery, which is expected to result in a stronger biological response. However, all the sites are considered acid sensitive, and ANC increased significantly in eight of the ten sites with a weak to moderate chemical recovery. This may suggest that the chemical gradient is of sufficient length for a biological response also at sites with a weak to moderate chemical recovery. It should also be noted that the magnitude of change in diversity was larger at sites with a strong chemical recovery. Previous analyses of the ICP Waters dataset that is part of the current study suggested very clear relationships between acid chemistry and diversity amongst both rivers and lakes up to 2010-2014 and that a reduction of acidifying components in the water had a strong influence on species diversity in remote surface waters across north-western Europe (Velle et al 2013, 2016). Other findings also support a correlation between diversity of benthic macroinvertebrates and chemical recovery (Wallace et al. 1996, Sandin and Johnson 2000). We conclude that the observed increases in EPT taxa are most likely related to chemical recovery. However, the large fluctuations in diversity from about 2012 are not reflected in pH, ANC, and SO_4 (Appendix 4, Garmo et al. 2020) and need further analyses.

Biological response to acid conditions

Mean annual diversity over time was significantly correlated to mean annual ANC at more sites than for SO_4 and pH. Diversity was significantly correlated to ANC in about 40% of the lakes and 20% of the rivers. A clearer response to ANC in lakes than rivers may potentially be caused by point samples for ANC in rivers being highly sensitive to short-term variation in hydrology, meaning that annual average lake ANC may be more representative of recent conditions (i.e., prior to biological sampling) than the annual average river ANC. Hence, fluctuating ANC values in rivers may distort a correlation to diversity.

ANC is a measure of the overall buffering capacity against acidification of water and represents the balance between base cations and strong acid anions. Acidification is associated with increased concentrations of labile aluminium. In fish, accumulation of aluminum on the gills affects the species' ability to regulate ions, like salts, and inhibit respiratory functions, like breathing (Rosseland et al. 1990). A stronger biological response to ANC than to other acid components of the water may potentially be caused by the exchange of ions in benthic macroinvertebrates. The exchange of ions is affected by the ion balance in the water and may be adversely affected during acidic conditions. Macroinvertebrates continuously exchange ions with water over permeable parts of their skin to maintain acid-base balance and ionic equilibrium. Gills are highly permeable and play a major role in the uptake of ions in macroinvertebrates, especially through excretion of ammonia and ammonium ions (Houlihan et al. 1982). Cations move inwards to maintain electroneutrality during excretion, and the level of active uptake depends on external concentrations (Houlihan et al. 1982). This suggests that the energy requirements are high for gill-breathing invertebrates when the concentration of base cations is low under acidic conditions (Sutcliffe and Hildrew 1989). Even if sensitive taxa can tolerate some acidity, they are eliminated due to competition for food and (or) predation pressure rather than direct toxicity during acidic conditions (Havas and Likens 1985). In addition, elevated concentration of aluminum can cause direct toxicity on sensitive macroinvertebrates by affecting osmoregulation and leaving less energy for their growth and reproduction (Herrmann and Andersson 1986, Olofsson et al. 1995). However, the effect of aluminum is complex since (1) aluminum to some extent also can ameliorate the toxic effects of low pH on survival and osmoregulation (Herrmann et al. 1993, Herrmann 2001) by blocking the membrane permeability of hydrogen ions (Herrmann et al. 1993) and (2) humic substances can ameliorate the effects by complex binding of metals (Herrmann 2001).

2.4.3 Functional traits

Even though analysis of functional traits to understand ecosystem processes have become common in freshwaters during the recent years (Schmera et al. 2022), assigning functional traits to macroinvertebrates is not a straightforward task (Petchev and Gaston 2002, 2006, Zhu et al. 2017). This is due to several factors: some species have poorly known traits, certain traits exhibit continuous variation, and some species are generalists that can switch between traits depending on factors such as body size, competition, and environmental conditions. For example, some shredders can switch to scraping and feed on algae in acidic conditions and in the absence of scrapers (Ledger and Hildrew 2005). The implication is that there inevitably are uncertainties involved in analyses and interpretation of traits. We have analyzed traits for EPT taxa to reduce the uncertainties since traits are more well known in EPT taxa than among many other benthic invertebrates. EPT taxa dominate the macroinvertebrate community in rivers. However, this also means that we lack an analysis of traits for non-EPT taxa. We have also included data over many years, many taxa, and many sites to increase the signal to noise ratio.

The diversity-based approach indicates an increase in the number of species towards the present (Figure 6), while the trait-based approach indicates a noisy pattern with fluctuations that deviated among the groups of sites (Figure 8 and Figure 9, Table 4). Biodiversity is considered to enhance ecosystem stability and ecological quality (de Bello et al. 2021), particularly in terms of the resistance of ecosystem productivity to climatic extremes (Isbell et al. 2015). Environmental perturbations often cause a decrease in taxonomic richness and altered species composition, but do not necessarily influence the composition of functional traits. This is due to functional redundancy, where many species have similar traits such that species replacements that occur have limited impact on the overall composition of traits or ecosystem processes (Schmera et al. 2017). Biological impacts of acid deposition, such as declining populations of fish and reduced species richness of invertebrates, had

significant effects on the occurrence and abundance of functional traits in the ecosystems (Ledger and Hildrew 2005). Hence, we can also expect a change in the composition of functional traits as a response to chemical recovery. Our results indicate that the proportion of some traits in the macroinvertebrate community changed significantly over time and that the speed of the shifts in traits accelerated after about 2012 (Figure 8 and Figure 9). This may suggest that the response to environmental variables is strong enough to overcome functional redundancy.

However, the magnitude of increase in EPT taxa was strongest where chemical recovery was strongest (Figure 6), whereas the changes in proportion of functional traits was often most distinct where chemical recovery was weak to moderate. So, the functional trait analysis results are not completely consistent with the changes found in EPT species richness. A difference between changes in traits and changes in diversity may suggest a non-directional and complex relationship between functional traits, biodiversity, and recovery from acidification, or a lag between taxonomic diversity and function. An effect where changes in functional traits of benthic macroinvertebrates lag taxonomic diversity has been found previously and was then linked to the recovery from acidification (Baker et al. 2021a). Possibly, the presence of species other than EPT taxa may have had a large imprint on the biological communities in some rivers, in addition to other driving factors, in particular climate change. The rivers in this analysis encompass wide ecological and biodiversity gradients, differences in climate and climate change, and substantial variation in catchment properties. Possibly, analysis of functional traits for a region should be preceded by studies of functional traits in single, data rich rivers.

Feeding mode

There was a significant change in grazers and predators in the group of sites with a strong chemical recovery. There was a total of 31 predatory species in the data (Figure 7). Most predatory species in the data belong to Trichoptera, and include species that are highly sensitive towards acidification, such as *Hydropsyche pellucidula* and *Lepidostoma hirtum*, and species that are non-sensitive, such as *Rhyacophila nubila* and *Polycentropus flavomaculatus* (Fjellheim and Raddum 1992).

Numerous studies have found correlations between the abundance of predators and prey (Jeffries and Lawton 1985, Arditi and Ginzburg 1989, Warren and Gaston 1992). In freshwater benthic macroinvertebrates, the average predator-prey ratio is approximately 0.48 in small, species-poor communities and 0.29 in large, species-rich communities (Jeffries and Lawton 1985). The average ratio is around 0.36 (Jeffries and Lawton 1985, Warren and Gaston 1992). In the current study, the overall predator-prey ratios increased slightly towards 2012 and then decreased more sharply to below initial levels in the present, as indicated by the proportion of predators (Figure 8). Predator-prey interactions typically provide stability for ecosystems and enable the coexistence of many species (Lancaster 1990, Allesina and Tang 2012). The number of predatory species depends on the availability and types of prey, meaning that an increase in species richness leads to higher diversity in prey items, allowing for a greater variety of predation niches (Jeffries and Lawton 1985). Given the commonly observed correlation between the number of prey and predators, as well as the correlation between biodiversity and ecosystem stability (Loreau and De Mazancourt 2013), it is surprising that the predator-prey ratio decreased towards the present while the number of EPT species was increasing. The commonly reported predator-prey ratios pertain to the invertebrate community (Jeffries and Lawton 1985), and there is little reason to believe that predatory fish were introduced into the rivers in about 2012 or that the abundance of fish increased after 2012. However, we cannot rule out the possibility that the absence of EPT predators was compensated by non-EPT predators in the macroinvertebrate community.

The proportion of gatherers/collectors changed significantly in sites with a weak to intermediate chemical recovery. Gatherers/collectors are generalist feeders on detritus, and a change in gatherers-collectors may be interpreted to reflect changes in the amount of available detritus, for example decaying microphyte algae. The proportion of grazers also increased significantly over time with a smaller relative increase at sites with strong chemical recovery compared to sites with a weak to intermediate chemical recovery. Grazers are usually scarce or absent at pH below 5.4, whereas shredders can prevail at low pH (Ledger and Hildrew 2005). Some of the lakes and one river still have pH below 5.4 (Figure 4 and Figure 5), and we can expect that the abundance of grazers will increase at these sites as the chemical recovery commences. The implication is that grazers and collectors can replace shredders as the dominant group when pH increases above 5.4 (Ledger and Hildrew 2005). Grazers feed on green algae and small detritus particles, and can affect algal growth in rivers where light is not a limiting factor (Wallace and Webster 1996). An increase in grazers could be caused by an increase in DOC, since DOC and microorganisms form a heterotrophic biofilm (Komínková et al. 2000) that grazers can feed on. The increase in grazers in the group of sites with a weak chemical recovery could be attributed to the rather fast ability of highly acid-sensitive species, like the mayfly *Baetis rhodani* (a grazer), to recolonize areas during the recovery from acidification, either by drifting from local refuges or aerial dispersal from nearby sites (Fjellheim and Raddum 1995, Sandøy and Romundstad 1995).

Shredders also increase in proportion in the group with weak to intermediate recovery. Shredders are generally tolerant of acidic conditions (Herrmann et al. 1993). Plants constitute a source of food and structure for macroinvertebrates (Velle et al. 2022), and especially shredders. The increase in shredders may be caused by an increased abundance of aquatic plants in rivers and lakes worldwide (Hussner et al. 2017, Verhofstad et al. 2017, Kagami et al. 2019). While the abundance of macrophytes is linked to increased fertilization and river regulation (Moe et al. 2013), an increasing occurrence of filamentous green algae in streams is linked to both a fertilization effect and reduced acidification (Kinross et al. 2000, Schneider et al. 2018). Algae and plants are often limited by phosphorus in clear water sites, even though a reduced atmospheric deposition of nitrogen may suggest a shift towards nitrogen limitation (Thrane et al. 2021).

Movement mode

Several traits (burrowers, other, sprawlers, and swimmers) changed significantly in the group of sites with weak to intermediate chemical recovery, while sessile/semi-sessile trait group changed significantly in the sites with strong chemical recovery. Movement traits may affect species' ability to withstand predatory pressure or abiotic stressors, like floods, by affecting their ability to inhabit an area under periodic stress. Movement by sprawling/walking was most common throughout the study period. Sprawlers are related to substrate conditions and prefer fine sediments (Murphy et al. 2017). Invertebrates that are visible on the lakebed, like sprawlers, are especially vulnerable to predation from visual predators (Kohler and McPeck 1989, Blake and Hart 1995), suggesting that reduced visibility may be favorable for sprawlers and negatively impact visual predators, like fish. Visual cues are important in aquatic environments and visibility can often be constrained (Aksnes and Giske 1993, Hartman and Abrahams 2000). Many freshwaters are browning due to an increase in DOC during the recovery from acidification (Monteith et al. 2007), suggesting that the increased abundance of sprawlers towards 2012 could be caused by browning. Swimmers also increased in abundance towards the present, which is likely not related to the biological recovery per se, but rather an increase in grazers/gatherers that also swim, like the mayfly *B. rhodani*.

2.5 Conclusions

Our primary objectives have been to use EPT to (1) quantify trends in biological diversity, (2) quantify trends in functional traits, and (3) examine whether the biological change can be interpreted specifically as biological recovery from acidification.

Our findings indicate that in a variety of streams and lakes undergoing chemical recovery, there is a simultaneous increase in the EPT richness. The results provide evidence of biological recovery from acidification in surface waters, demonstrating that the reduction of air pollution contributes to increased biological diversity. Temporal variation in biological diversity shows an increase in EPT taxa in a higher fraction of rivers than lakes. The smaller change in EPT taxa in lakes could be related to the shorter time studied in lakes, but biological communities in lakes can also be less responsive than in rivers because of more stable environmental conditions, less EPT species, and lower rates of habitat recolonization. The biological response to acid conditions shows a stronger correlation with ANC than other acid components.

Furthermore, the proportion of functional traits of EPT taxa in rivers exhibited significant changes over time, particularly in rivers that displayed minimal chemical recovery, which contrasts with the changes in EPT richness. Both species richness and functional traits showed more pronounced changes after 2012, and the proportion of predators in the EPT community was unexpectedly low towards the present. These findings may suggest that ecosystems previously impacted by acidification may be vulnerable to environmental disturbances. It should be noted that the functional trait analysis was limited to EPT taxa, which represent varying proportions of the total biological community in the rivers included in this dataset. While the functional analysis suggests changes in the composition of traits in acidified and recovering rivers, further analyses are necessary to assess the impacts of other components of the biological community and other potential drivers, such as catchment properties and climate change.

In conclusion, our study emphasizes the significance of integrated biological and water chemical monitoring programs, as well as the potential of such data to investigate the intricate relationship between environmental disturbances, species diversity, functional traits, and the recovery of freshwater ecosystems.

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3 Diatoms as a biological indicator to support ecosystem monitoring under the NEC Directive in Italy

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3.1 Introduction

In 2019, the National Emission Ceilings Directive (NECD - 2016/2284/EU) was launched by the European Union to set objectives for emission reduction for SO₂, NO_x, NMVOCs, NH₃ and PM_{2.5} for each Member State. Besides that, an innovative aspect of NECD is Article 9, that mentions the issue of “monitoring air pollution impacts” on ecosystems and sets parameters and indicators to be adopted for the monitoring of these impacts on both terrestrial and freshwater ecosystems.

Within the activities for the implementation of the NECD in Italy, eight high altitude alpine lakes in the N-W Italian Alps, for which long-term data are available (since the 1980s), were selected for a survey in 2019 and 2020. The selected lakes have the characteristics required by the ICP Waters Program for the purpose of their use for monitoring the effects and response of water to air pollution. They lie on a gradient of sensitivity to acidification (as the main pressure), especially in relation to the lithological composition of the basin, and are affected by medium-high nitrogen deposition, therefore potentially at risk of enrichment in nitrates (Rogora et al., 2001; 2013). Besides sampling for chemical analysis, samples have been collected for macroinvertebrates and epilithic diatoms, both included among the biological indicators suggested by Annex V of the NEC Directive, following a European wide standardized sampling protocol. Focus of the monitoring was the assessment of chemical and biological responses to reduced surface water acidification, by analysing temporal trends, describe the extent and degree of acid sensitivity and possibly identify suitable biological indices for Italian sites. An in-depth analysis was performed for Lakes Paione Inferiore and Superiore, belonging to both the ICP Waters and LTER networks (LTER sites EU-IT-088 and EU-IT-089): for these lakes long-term data exist, thanks to previous monitoring within EU-funded projects (Rogora et al., 2013).

3.2 Methods

The main characteristics of the study lakes can be found in Fornaroli & Boggero (chapter 4 in this issue). Diatoms sampling was performed in early autumn which appears to be the most representative period for monitoring purposes (Rott et al., 2006), following a European wide standardized sampling protocol (Manuals ICP Waters, 2010) for high altitude lakes (<http://www.icp-waters.no/publications/#icpwmanual>). Epilithic diatoms were sampled on cobbles of considerable size, preferably free of filamentous algae. At least 5 cobbles were collected and scraped with a toothbrush on a total area of about 1 m² of lake shoreline. In the laboratory, the samples were then treated with hot hydrogen peroxide and hydrochloric acid following standard procedures (Battarbee et al., 2001), and finally mounted using Naphrax on permanent slides for species identification (Zeiss Axiolab, magnification 1000x). The taxonomic recognition was based on Krammer and Lange-Bertalot

(1986–2004), Lange-Bertalot (2001), Krammer (2000; 2002; 2003), Lange-Bertalot et al. (2011) Cantonati et al. (2017) integrated with the paper on the *Achnanthydium minutissimum* species complex by Potapova and Hamilton (2007). For each sample, a minimum of 400 valves were identified and results expressed as relative abundances (%).

3.3 Results

A total of 112 taxa, belonging to 37 genera and 120 taxa, belonging to 40 genera have been detected, to the highest taxonomic resolution possible, respectively in 2019 and 2020. The eight lakes have communities characterized mainly by species acidophilic or neutrophilic, and only in low percentage acidobiontic or alkaliphilic (Fig 1), according to the van Dam et al. (1994) classification. Between the two years, all the lakes showed quite stable communities.

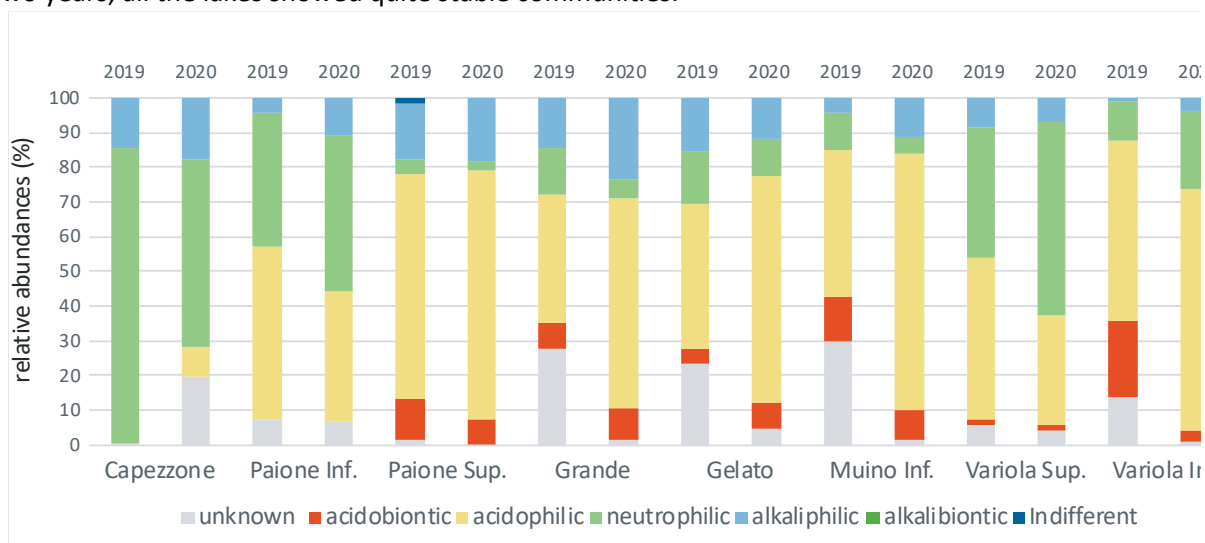


Fig. 1 - Diatom pH requirement according to van Dam et al. (1994).

The main indices of richness, diversity (H' - Shannon and Weaver, 1949) and evenness (Pielou, 1969) were applied to highlight between-lakes differences and to evaluate the possible anthropogenic pressure effects (in particular acidification) on the diatomic community (Table 1).

Tab. 1 - Richness, diversity (Shannon) and evenness (Pielou) index applied to diatom community in the lakes under study.

lakes	genera number		species number		H'		Evenness	
	2019	2020	2019	2020	2019	2020	2019	2020
Paione Inferiore	19	18	36	32	3.22	3.43	0.62	0.69
Paione Superiore	17	15	28	28	2.93	3.4	0.61	0.71
Grande	16	21	30	36	3.59	3.76	0.73	0.73
Capezzzone	10	20	18	36	2.26	3.98	0.54	0.77
Gelato	15	17	29	34	4.01	4.07	0.82	0.8
Muino Inferiore	20	16	40	41	4.05	3.88	0.76	0.72
Variola Superiore	18	21	37	43	3.77	4.19	0.72	0.77
Variola Inferiore	18	21	33	41	4.08	4.02	0.82	0.75

In 2019 Lake Capezzone, the only one without acidophilic diatoms, showed the lowest values of all indices but in 2020 their values were comparable to those of other diatom communities and all lakes that showed greater biodiversity. Unfortunately, the limited number of samples do not allow us to assess whether this change is temporary, linked to specific conditions in 2020, or whether it is permanent.

The data of the abundances of epilithic diatoms collected in the Lakes Paione were included in a dedicated dataset, as well as the morphological description of the species and the hydromorphological characteristics of the basins. These data have been compared with the previous ones available from 1991, to evaluate the main long-term trends, in relation to pH (Fig. 2). In the last decade, diatoms taxonomy has undergone a profound revision, with the definition of new genera and the reassignment or identification of new species. For this purpose, taxonomy of historical data sets has been updated and uniformed according to the software Omnidia 6.09 (Leiconte et Al. 1993). The flora of both lakes indicates a chemical recovery towards less acidic conditions. In lake Paione Superiore, the acidobiontic species decreased with an increase in the acidophilic species. In lake Paione Inferiore acidophilic species decreased with an increase in neutrophilic species. These lakes are still monitored with annual samplings starting in 2019 and the trend is more evident during the most recent years. In the recent years, the share of acidophilic species is higher than (Lake Paione Superiore) or similar to (Lake Paione Inferiore) 1991 levels, suggesting that the lakes remain acid-sensitive. Lake Paione Inferiore has a greater biodiversity than Lake Paione Superiore, probably due to both a lower altitude and a lower impact of acidification. For both lakes, the pH values were always > 5.5 (AWIC_{sp} index just over 5).

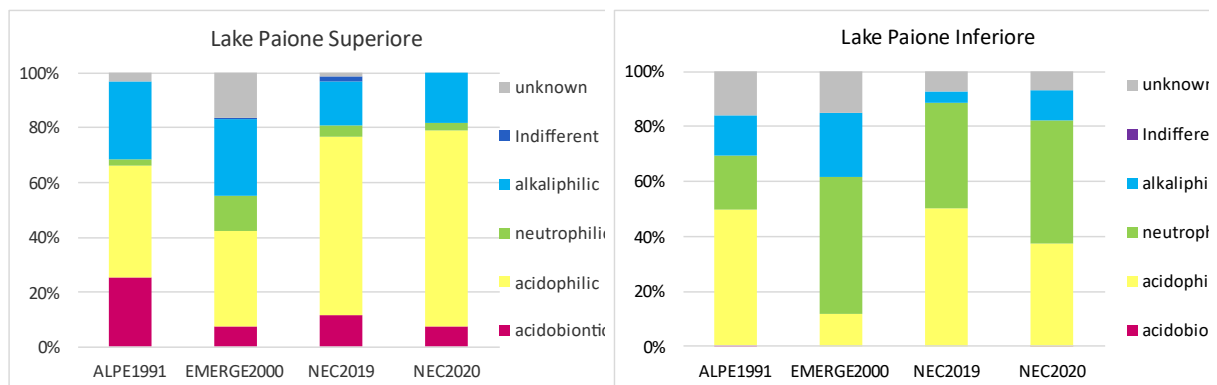


Fig. 2- Compositional variation of diatom assemblages in Lakes Paione during the last 30 years, according to their pH preferences.

3.4 Acknowledgements

This activity was partially funded by a 2-year cooperation agreement between the Italian Minister for Ecological Transition (MiTE) and Carabinieri-CUFA (Command Unit for forest, agri-food and environmental protection; formerly National Forest Service) for the development of the Italian monitoring network under the NEC Directive (NEC Italy).

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4 Italian biological monitoring of freshwaters, data sharing and acidification indices tool

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4.1 Introduction

In the last decades, within the EU there has been a strong commitment by the Commission to increase the availability of environmental data and promote data sharing. The most important impulse was introduced with the INSPIRE Directive (INfrastructure of SPatial InfoRmation in Europe - 2007/2/EC), a Community law which obliges Member States to adopt measures for the creation of services that allow the sharing of spatial data among all public authorities, with the aim of ensuring that future environmental policies can count on a greater quantity of high level information and therefore be more effective. Other Directives support the INSPIRE Directive, requiring public authorities to release their own data when required (Environmental Information Directive - 2003/4/EC) and to facilitate their reuse (Public Sector Information Directive - 2003/98/EC). European legislation therefore agrees that all territorial data (i.e., those relating to a specific location or geographical area) held by or on behalf of a public authority are archived, made available and stored at a more suitable level.

Among them, biodiversity data are of foremost importance within the ICP Waters and more generally within the monitoring programs aiming to provide data to assess the biological response to atmospheric pollution under the National Emission Ceilings (NEC) Directive, as well as within the Water Framework Directive 2000/60/EC to evaluate the improvement of the environmental quality status of freshwaters following the main goals defined by the European Community regarding water protection. Our proposal is thus, within the several freshwater monitoring programs envisaged by the above Directives and Plans, to share the tools developed in Italy (Boggero et al., 2021) to facilitate the sharing of the data obtained at a national and an international level by each Member State. In particular, we focused on high altitude freshwater ecosystems, which have been identified as sensitive and reliable monitoring sites to assess the response of freshwater biota to air pollution (Battarbee et al., 2009). The present goal is also in line with two main sustainability objectives for 2030 United Nations Agenda relative to:

- 1) 6.6 – Protect and restore water-related ecosystems including mountains, forests, swamps, rivers, aquifers and lakes;
- 2) 15.5 – Take effective and immediate actions to reduce the degradation of natural environments, halt and reversing biodiversity loss, and protect the endangered species.

This contribution is also in line with the European and Italian biodiversity strategies for 2030 and the National Recovery and Resilience Plan (PNRR), as well as with local strategies that aim to preserve nature and reverse the degradation process of ecosystems, to restore our natural capital ensuring in the meantime its resilience to future challenges posed by climate change. Indeed, the data were collected as part of research programs funded by the European Union and by national authorities, with the aim of improving and deepening the environmental knowledge of our country. The knowledge acquired make it possible to give an address to future national legislation.

4.2 Study area

All the study lakes are in the Ossola Valley (Fig. 1), a main valley consisting of 7 secondary Alpine valleys fan-like distributed in the northern edge of Piedmont Region at the border with Switzerland and with Lake Maggiore area to the south, surrounded by the Pennine Alps on the western side and by the Lepontine Alps on the northern and eastern sides. The lakes occupy only 4 of them (Tab. 1).

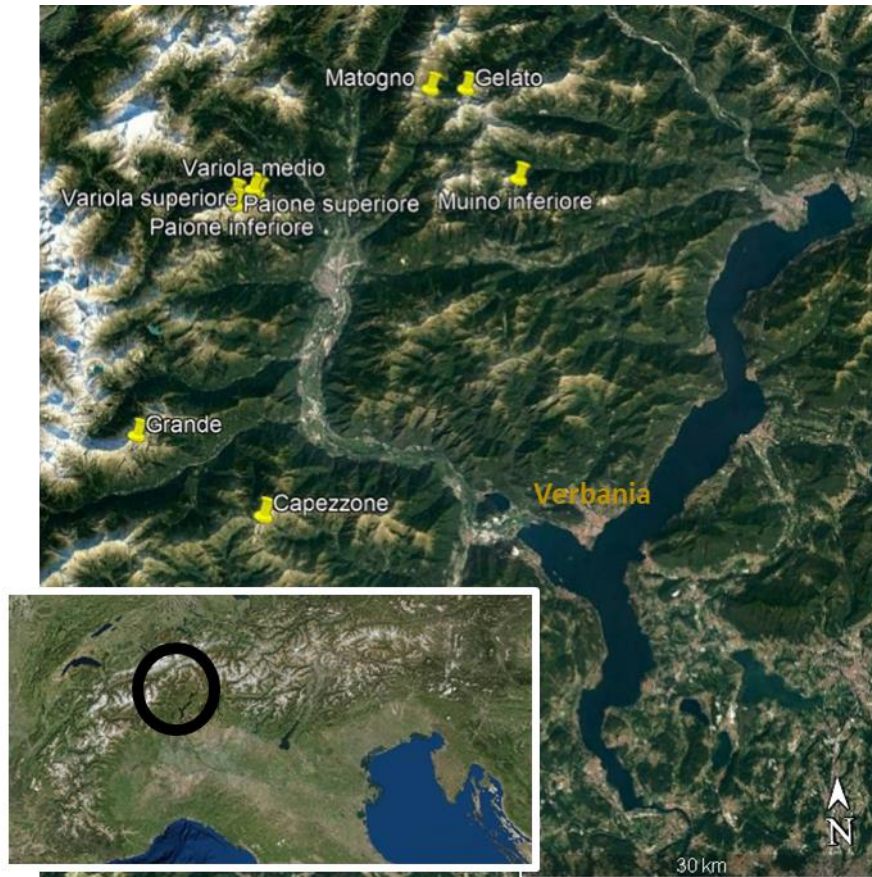


Fig. 1 - Distribution of the study lakes within the Ossola Valley, N-W Italy. Yellow pointers: lakes.

Tab. 1 - Main geographic characteristics of the study lakes with presence of river network.

Valley	Lake name	Altitude m a.s.l.	Latitude N WGS 84 decimal degrees	Longitude E	inlet	outlet
Agarina	Gelato	2418	46.24843	8.44028	no	no
Agarina	Matogno	2087	46.24947	8.40237	x	x
Anzasca	Grande	2214	46.00184	8.07801	no	x
Bognanco	Paione inferiore	2006	46.16924	8.19024	x	x
Bognanco	Paione di mezzo	2145	46.17252	8.19076	x	x
Bognanco	Paione superiore	2251	46.17591	8.18991	no	x
Bognanco	Variola medio	2137	46.17707	8.21402	x	x
Bognanco	Variola superiore	2198	46.17980	8.21010	no	x
Strona	Capezzone	2100	45.93810	8.20895	no	x
Vigizzo	Muino inferiore	1886	46.18085	8.49251	no	x

Habitats were defined according to the second-level EUNIS classification system (<http://eunis.eea.europa.eu/> - Davies et al., 2004), a pan-European system for habitat identification, including standing waters (C1.1: Permanent oligotrophic lakes, ponds and pools; 10 sites), and running waters (C2.5: Surface temporary running waters; 13 sites). Some of these high altitude lakes have been monitored since the '80s in the context of EU research projects on the effects of transboundary air pollution and climate change on mountain lakes (e.g., AL:PE1, AL:PE2, MOLAR, EMERGE) and are sensitive to acidification, particularly those lying in watersheds composed of acid rocks: the lakes are not only affected by atmospheric deposition pollution, but they also undergo indirect anthropogenic pressure such as climate change (Rogora et al., 2013). Their reduced watersheds consist mainly of bare rocks, scarce patches of alpine meadows and very thin soils. The watersheds are mainly dominated by acidic rocks, although small amounts of carbonate rocks are also present: since lithology varies from one basin to another, this implies that lakes are differently affected by acidification.

4.3 Results

Biomonitoring is crucial to assess and preserve the quality status of freshwaters, mainly at high altitude where climatic change have a strong influence. Here we share a dataset relative to long-term data on macroinvertebrates of lakes Paione developed since the '90s, and a spatial dataset on other high altitude lakes and their river network with data relative to the last years (2019-2020) (Boggero et al., 2023) useful to test acidification indices use. As regards acidification and its effects on the benthic fauna of lakes and their river network, several indices can be applied, some of them considering the whole macroinvertebrate community, some other using specific taxon. However, the application of those indices is generally confined within the country in which they were developed, making the sharing of methods among researchers difficult. Moreover, the indices used to evaluate the effects of the acidification through the presence/ absence, or the relative abundance of sensitive taxa were mainly developed for Northern European lotic environments, and it was not possible to apply them to most of the Italian data due to the absence of indicator taxa. Therefore, our work aims to cover this gap by developing an R tool for the consistent use of already available acidification indices and to provide the basis for the development of new ones. The tool was specifically developed to allow the calculation of specific acidification indices based on the occurrence or on the relative abundance of

taxa sensitive to acidification effects. The indices for which the calculation is already implemented are reported below:

1. Raddum 1988 index (Raddum et al., 1988), developed to evaluate the effects of acidification on rivers and lakes in Norway;
2. Raddum 1990 index (Fjellheim & Raddum, 1990), developed to evaluate the effects of acidification on rivers and lakes in Norway;
3. NIVA index (Bækken & Kjellberg, 2004), developed to evaluate the effects of acidification on humus-rich streams in eastern Norway;
4. AWIC_{fam} index (Davy-Bowker et al., 2003, 2005), developed to evaluate the effects of acidification on streams and rivers in England and Wales;
5. AWIC_{sp} index (Davy-Bowker et al., 2003), developed to evaluate the effects of acidification on streams and rivers in England and Wales;
6. Braukmann index (Braukmann & Biss, 2004), developed to evaluate the effects of acidification on streams and rivers in Germany;
7. LAMM index (McFarland et al., 2010), developed to evaluate the effects of acidification on clear and humic lakes in the UK;
8. TL index (Hämäläinen & Huttunen, 1990), developed to evaluate the effects of acidification on streams and rivers in Finland.

Moreover, the tool allows to calculate generic metrics related to diversity, richness and functional aspects of the whole macroinvertebrates assemblage or to specific groups:

- 1) total number of taxa (Ofenböck et al., 2004);
- 2) number of taxa, families and relative abundance of Ephemeroptera, of Plecoptera, of Trichoptera, and of the whole EPT (Böhmer et al., 2004; Ofenböck et al., 2004);
- 3) number of taxa and relative abundances of Diptera chironomids and of oligochaetes (Wiederholm, 1980);
- 4) Shannon diversity index (Shannon & Weaver, 1948).

The tool uses three data input:

1. a table with the list of taxa with related information such as taxonomic classification (phylum, class, order, family, genus, species) and indices scores (when available);
2. a table with the list of samples with the relevant information such as location, date of sampling, chemical and other environmental characteristics;
3. a table with the presence/abundance of each taxon in each sample.

A taxa list with 663 entry is already available and cover all the taxa within the taxa lists of the indices already implemented and the taxa sampled in the Italian sites that were not included in those taxa lists.

The standard output of the tool is a table with the information related to each sample, the values of the different indices (when available) and the list of taxa with assigned score for each index. R scripts, the taxonomic database and the Italian data to be used as test case were actually uploaded to GitHub (<https://github.com/RiccardoFornaroli/AcificationIndexes/>), and the list of uploaded files is:

1. "Indexes_formulas.r" is the R script with the function "ACI_R" that evaluate the indices for any sample;

2. "Acidification_index.r" is the R script that apply the function "ACI_R" to the Italian data as a test case;
3. "Taxonomy_18_01_2022.csv" is the taxonomic database used to classify taxa and store sensitivity level for each index, when available;
4. "Bio_lit_Samples.csv" is the list of Italian samples to be used as a test case;
5. "Bio_lit_UPDATED.csv" is the file with the abundance of taxa in each sample in long format.

This tool can eventually be included in some existing R packages such as biomonitoR (Laini et al. 2018 - <https://github.com/alexology/biomonitoR>), a package for managing taxonomic and functional information and for calculating indices for biomonitoring of running waters, with a focus on macroinvertebrates. On our opinion, it is advisable to adopt this type of tools within monitoring programs, to allow a wider sharing of the data and better comparability of the results gathered by different countries and research groups.

4.4 Acknowledgements

This activity was partially funded by a 2-year cooperation agreement between the Italian Ministry for Ecological Transition (MiTE) and Carabinieri - CUFA (Command of Forestry, environmental and Agri-food Units; formerly National Forest Service) for the development of the Italian monitoring network under the NEC Directive (NEC Italy).

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5 Biological responses to reduced acidification in surface waters in Switzerland

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5.1 Acid sensitivity

Most freshwaters in Switzerland are protected from acidification by calcareous rock strata. Potentially acid-sensitive water bodies have small catchments and are situated on slow-weathering crystalline bedrock at higher altitudes. Based on the geology (slow weathering acidic rocks), the altitude (>1500 m a.s.l.) and the lake area (> 0.5 ha), Rhim (1994) identified 79 potentially acid sensitive lakes. These are all situated in the Southern Swiss Alps (Fig. 1).

5.2 Monitoring and assessment approach

In order to monitor and assess acidification of freshwaters in Switzerland, up to 50 acid sensitive Alpine lakes were monitored at irregular intervals between 1980 and 2000. Since 2000, water chemistry of 20 acid sensitive high-altitude Alpine lakes and three mountain rivers (Maggia, Vedeggio, Verzasca) have been monitored regularly by the Canton of Ticino on behalf of the Federal Office for the Environment. Lakes were sampled two to three times a year, rivers monthly. Since 2000, six of these lakes (Starlarescio, Tomeo, Inferiore, Superiore, Nero, Alzasca) and the three rivers belong to the monitoring network of ICP Waters. Results of the monitoring, including trend analysis, are published in yearly and thematic reports (<http://www4.ti.ch/dt/da/spaas/uacer/temi/aria/per-saperne-di-piu/rapporti-e-studi>).

For the assessment of biological responses to recovery from acidification, benthic invertebrates were monitored in four high-altitude mountain lakes: the acidic ($\text{Alk} < 0 \text{ meq m}^{-3}$) lakes Starlarescio and Tomeo and the acid-sensitive ($0 < \text{Alk} < 50 \text{ meq m}^{-3}$) lakes Inferiore and Superiore. Additional monitoring was conducted in three mountain rivers: the neutral river Maggia and the low alkaline ($50 < \text{Alk} < 200 \text{ meq m}^{-3}$) rivers Vedeggio and Verzasca. Invertebrates were sampled by "kick sampling" 4 to 8 times a year in the rivers and 2 to 3 times a year in the lakes, according to the ICP Waters Manual (Wathne et al. 2010). The rivers Maggia and Vedeggio were sampled from 2000 to 2011, river Verzasca from 2000 to 2017. Between 2000 and 2013 the lakes were sampled both in the littoral and in the outlet. From 2013 to 2018 sampling was restricted to the outlets which are more likely inhabited by indicator species for acidity (Steingruber et al. 2013). In fact, many of these acid sensitive species were determined for rivers and are therefore current loving. After 2018 monitoring of benthic invertebrates has been reduced to the two still acidic lakes Tomeo and Starlarescio. The biological monitoring programme has been progressively reduced at the non-acidic sites ($\text{pH} > 6$) because of the absence of a temporal trend in the invertebrate population and because of economic constraints. Catchment characteristics of the presented invertebrate monitoring sites are presented in Table 1.

Figure 1. Potentially acid sensitive areas (rose shadow) and lakes (points) in Switzerland (Relief map ©2011 swisstopo).

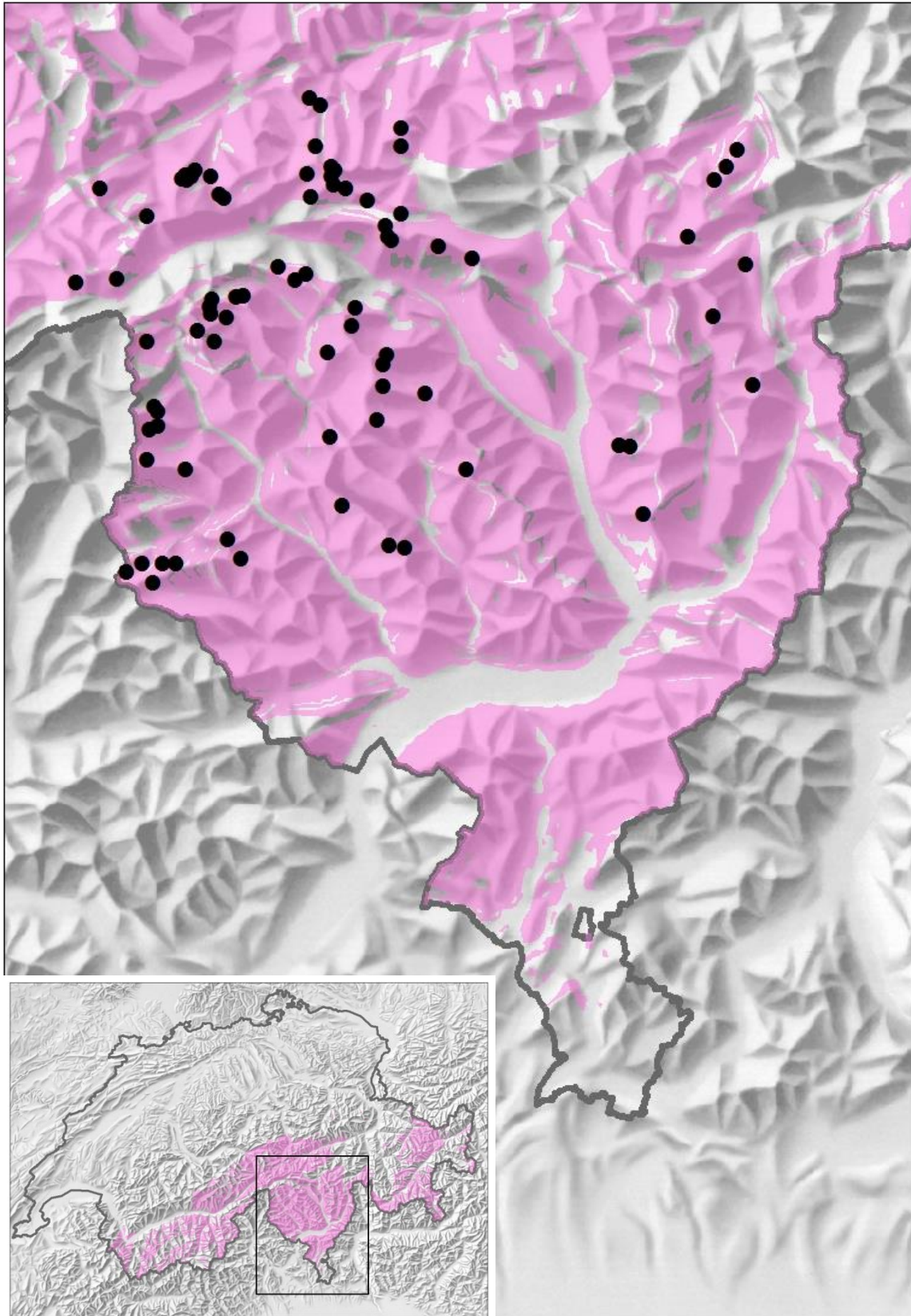


Table 1 Basic catchment characteristics of the monitored lake outlets and rivers

Site	Acronym	Latitude	Longitude	Altitude (m a.s.l.)	Catch. area (ha)
Inferiore	INF	46°28'34''	8°35'34''	2074	182
Superiore	SUP	46°28'34''	8°35'05''	2128	125
Tomeo	TOM	46°21'47''	8°41'23''	1692	294
Starlarescio	STA	46°16'26''	8°46'25''	1985	23
Maggia	MAG	46°21'16''	8°38'08''	610	~18900
Vedeggio	VED	46°07'45''	8°59'24''	740	2000
Verzasca	VER	46°21'24''	8°47'33''	918	~2700

In Switzerland, the assessment of invertebrate river samples is based on the standardized calculation of the IBCH quality index (BAFU, 2019), that is itself based on the taxonomic diversity (VT) and the indicator group (GI) and is characterized by values ranging between 0 and 1. For its calculation the semi-quantitative sampled invertebrates are determined mainly at the family taxonomic level or at coarser taxonomic levels (e.g., oligochaetes). The Swiss Macroinvertebrate Index IBCH_2019 permits to roughly determine the biological condition of the watercourse and to assign one of the following five condition classes: very good ($IBCH \geq 0.8$), good ($0.6 \leq IBCH < 0.8$), moderate ($0.4 \leq IBCH < 0.6$), insufficient ($0.2 \leq IBCH < 0.4$), bad (< 0.2). It is indicative of deficits in water quality and in the structural habitat diversity. A more specific indicator for acidification does not exist in Switzerland.

However, Steingruber et al. (2007) observed a good overlap between the pH of river Maggia, Vedeggio and Verzasca and the German acidification classes developed for mountain streams of average altitude (400-1500 m a.s.l.) by Braukmann and Biss (2004). Their scale consists of the following five acidification classes: 1 continuously neutral (pH 6.5 to >7.0, never below 6.0); 2 predominantly neutral to episodically acidic (pH 6.5 to 7.0, rarely below 5.5); 3 periodically critically acidic (pH 5.5 to 6.5, sometimes below); 4 periodically strongly acidic (pH around 5.5, periodically below); 5 continuously extremely acidic (pH 4.3 to 5.5, often below). Not surprisingly, these classes are not suitable for assessing the acidity of the high-altitude lake outlets. In fact, the lake outlets are characterized by low invertebrate taxa diversity and by very low relative abundances of EPT taxa (*Ephemeroptera*, *Plecoptera*, *Trichoptera*) and high relative abundances of chironomids independently of the water acidity (Boggero and Lencioni 2006, Füreder et al. 2006, Steingruber et al. 2013). A decrease of the EPT species richness with increasing elevation and decreasing catchment areas has also been reported by Altermatt et al. (2013). Nonetheless, the method proposed by Braukmann and Biss (2004) could be adapted to high-altitude outlets of mountain lakes. In the original version, to determine the acidification class, the indicator taxa are sorted first by the indicator value (from 1 (acid sensitive organisms) to 5 (very acid-resistant organisms)) and then by the relative abundance (from the highest to the lowest). Afterwards the relative abundances are cumulatively added up to a dominance of 10%. The indicator value of the last added taxa corresponds then to the acidification class. We saw that the resulting acidification classes fit quite well with the lake pH of high-altitude lake outlets if the abundances are added up to a dominance of 1% instead of 10% (Steingruber et al. 2013). Next to the indicator taxa, also more general invertebrate indicators like the total number of taxa and the number of EPT taxa (especially mayflies and stoneflies) have been shown to correlate positively with the pH of the lake outlets (Steingruber et al. 2013).

In order to avoid differences in the taxa number caused by different identification levels used through time, we defined an identification level for each taxonomic group and ignored information beyond this

level. The identification levels were the following: Annelida -> class, Arachnida -> subcohort, Coleoptera -> genus, Diptera -> family, Ephemeroptera -> genus, Heteroptera -> genus, Megaloptera -> genus, Odonata -> genus, Trichoptera -> genus, Mollusca -> class, Plathelminthes -> family. Moreover, since the sample sizes varied greatly from year to year and it is known that the number of taxa/species increases with the number of individuals, the number of families needed for the calculation of the IBCH_2019 index and the number of total taxa and EPT taxa were standardized. For each sampling site a logarithmic regression was calculated between the logarithm of the number of families and the number of individuals in a sample. Similarly, a linear regression was calculated between the logarithm of the yearly total number of taxa and EPT taxa and the number of individuals sampled in a year. With this function for each year the taxa numbers were standardized to a sample size of 1000 individuals.

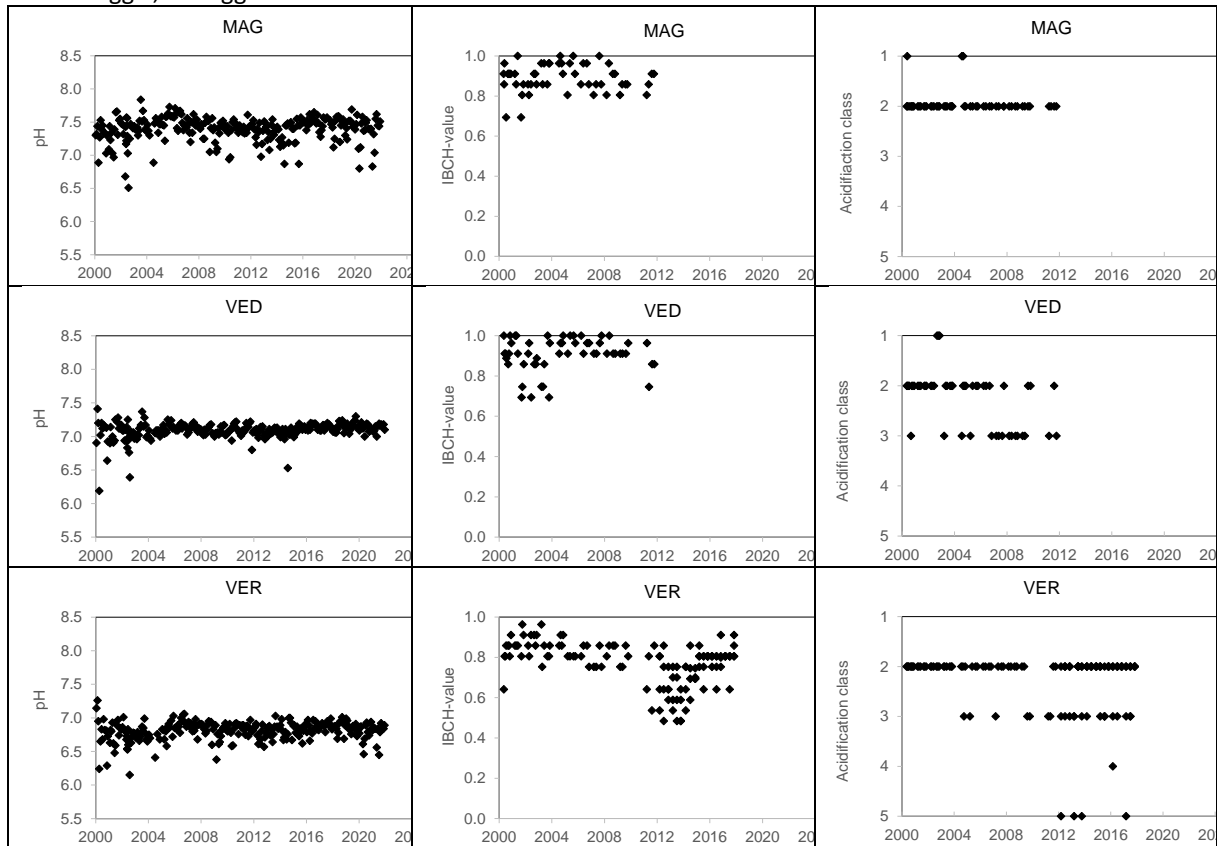
5.3 Acidification status

We here present the temporal trends of the invertebrate indicators. For the mountain rivers Maggia, Vedeggio and Verzasca we show the temporal evolution of the IBCH_2019 index and the acidification classes according to Braukmann and Biss (2004). For the high-altitude lakes Inferiore, Superiore, Tomeo and Starlarescio, in addition to the change over time of the IBCH_2019 index and the modified acidification index, we also present the total number of taxa and the number of EPT taxa.

The pH in river Maggia varied between 6.5 and 7.8 and was on average 7.4, in river Vedeggio it varied between 6.2 and 7.4 and was on average 7.1 and in river Verzasca it varied between 6.2 and 7.3 and was on average 6.8 (Fig. 2). Concentrations of H⁺ did not change significantly over time, while alkalinity increased significantly in all three rivers (Steingruber 2021). From 2000 to 2011, the IBCH_2019 index of the three rivers did not change over time, with values mainly >0.8, indicating very good water condition. In river Verzasca the values were mostly slightly lower compared to river Maggia and Vedeggio (Fig. 2). After 2012, in river Verzasca sampling at “pool” sites has been initiated separately from the “riffle” sites. Most samples after 2012 are still indicative for good water condition. However, occasionally “pool” samples had IBCH_2019 indices below 0.8, indicative of “only” moderate to good water condition. These samples were mainly characterized by the absence of *Perlidae*, *Perlododae* and *Philopotamidae*. We think that the occurrence of single samples with lower IBCH_2019 indices is rather related to the morphology of the sampling sites and not to the water chemistry. This is also confirmed by the fact that the acidification classes of the rivers did not change over time. River Maggia showed values of 2, typical for predominantly neutral to episodically acidic waters with pH values around 6.8-7.0. Values of rivers Vedeggio and Verzasca fluctuated between 2 and 3 (periodically critically acidic with pH values around 5.5-6.5) (Fig. 2). The acid status suggested by the invertebrate population thus slightly underestimates the measured chemical values. In river Verzasca few samples with acidification classes smaller than 3 occurred after 2012 in some “pool samples” that were characterized by the absence or the presence of very low relative abundances of mayfly taxa. Again, we think that this can rather be attributed to the “pool site” sampling than to a temporal trend.

All the high-altitude lakes were characterized by an increase of the pH. Since 2000, average autumn values increased from 6.6 to 6.9 in lake Inferiore, from 6.4 to 6.9 in lake Superiore, from 5.7 to 6.0 in lake Tomeo and from 5.2 to 5.8 in lake Starlarescio (Fig. 3 and Fig. 4). Reconstruction of the pre-acidification pH by Rogora (2013) suggests that today, the pristine pH has already been reached for lakes Inferiore and Superiore, but not for the other two lakes. Modelled pre-acidified pH values by means of the MAGIC model were ~6.8, ~6.9, ~6.7 and ~6.5 for lakes Inferiore, Superiore, Tomeo and Starlarescio, respectively.

Figure 2. pH, IBCH_2019 index and the acidification class calculated according to Braukmann and Biss (2004), in rivers Maggia, Vedeggio and Verzasca.



The acidification classes were generally higher at the lakes with lower pH and lower at the lakes with higher pH (lowest in lakes Inferiore and Superiore, followed by lake Tomeo and lake Starlarescio) (Fig. 3 and Fig. 4). They were on average 2.6 in lake Inferiore, 2.8 in lake Superiore, 4.2 in lake Tomeo and 4.6 in lake Starlarescio, suggesting that outlets of lakes Inferiore and Superiore are situated between class 2 (pH 6.5-7.0) and 3 (pH 5.5-6.5), the outlet of lake Tomeo close to class 4 (around 5.5) and the outlet of lake Starlarescio between class 4 and 5 (4.3-5.5). These pH classes obtained from the invertebrate population are all slightly below the measured pH (around 0.5 units), indicating that the adaptation of the acidification class from mountain streams to outlets of high-altitude lakes can be improved. However, it reflects well the actual pH differences among the lakes. Similarly, the yearly total number of taxa was on average 13 in lake Inferiore and Superiore, 10 in lake Tomeo and 11 in lake Starlarescio, while the yearly number of EPT taxa was on average 7 in lake Inferiore, 8 in lake Superiore, 4 in lake Tomeo and 2 in lake Starlarescio. *Ephemeroptera*, the most acid-sensitive EPT order, were absent in lake Tomeo and Starlarescio. Also, *Perlodidae* one of the most acid-sensitive families of the *Plecoptera* order were regularly found only in lakes Inferiore and Superiore.

The IBCH_2019 values did not relate to the acid status of the lakes and suggested moderate water conditions of lakes Inferiore, Superiore and Tomeo, and an insufficient condition of lake Starlarescio. The reason for this result is related to the fact that the IBCH method underestimates the water quality at altitudes above the epirhithral (trout zone), because in these cold waters the biodiversity is naturally poor (BAFU, 2019).

Figure 3 pH, for IBCH_2019 index and the acidification class calculated according to Braukmann and Biss (2004), the yearly number of total and EPT taxa and yearly mean relative abundance of chironomids in lakes Inferiore and Superiore.

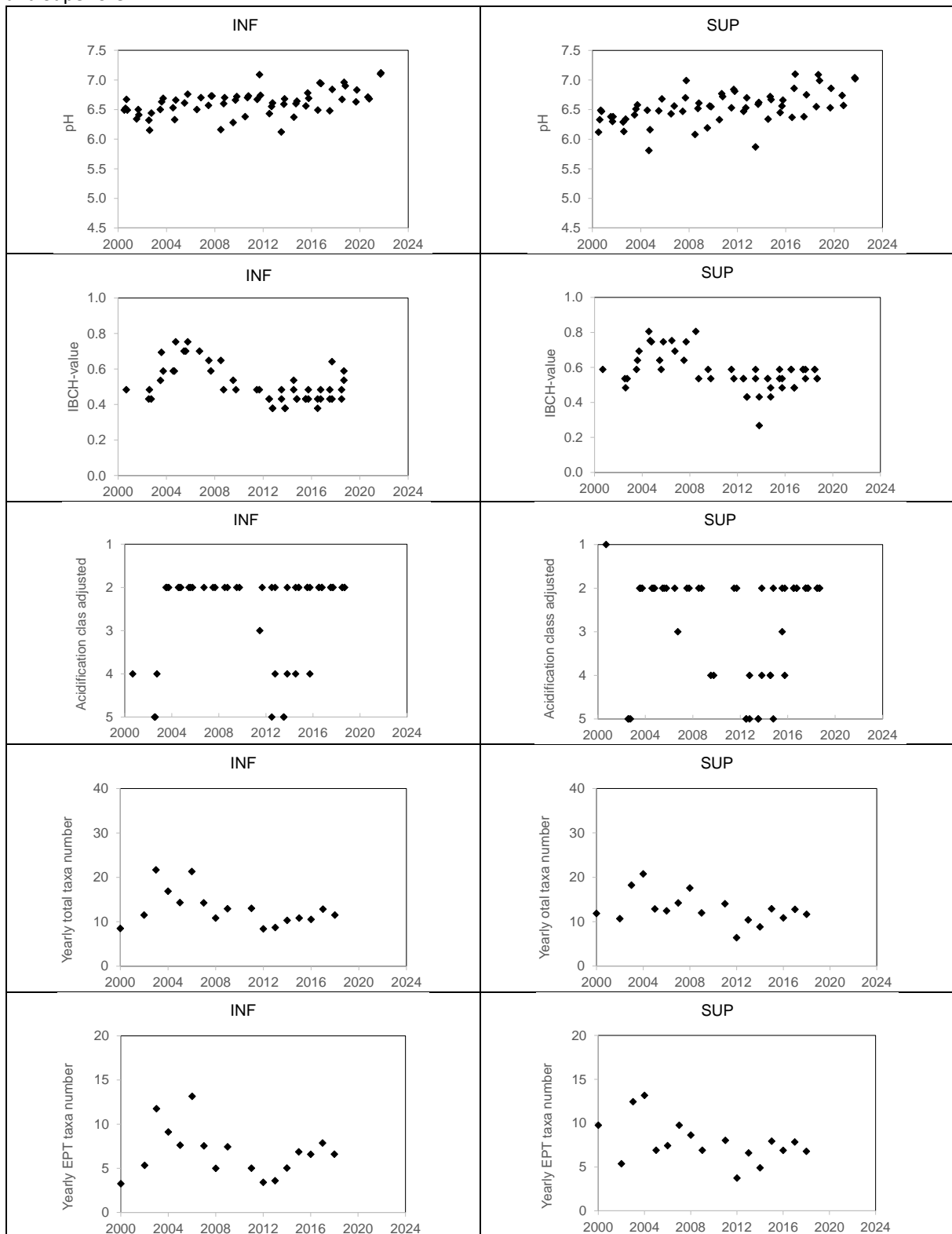
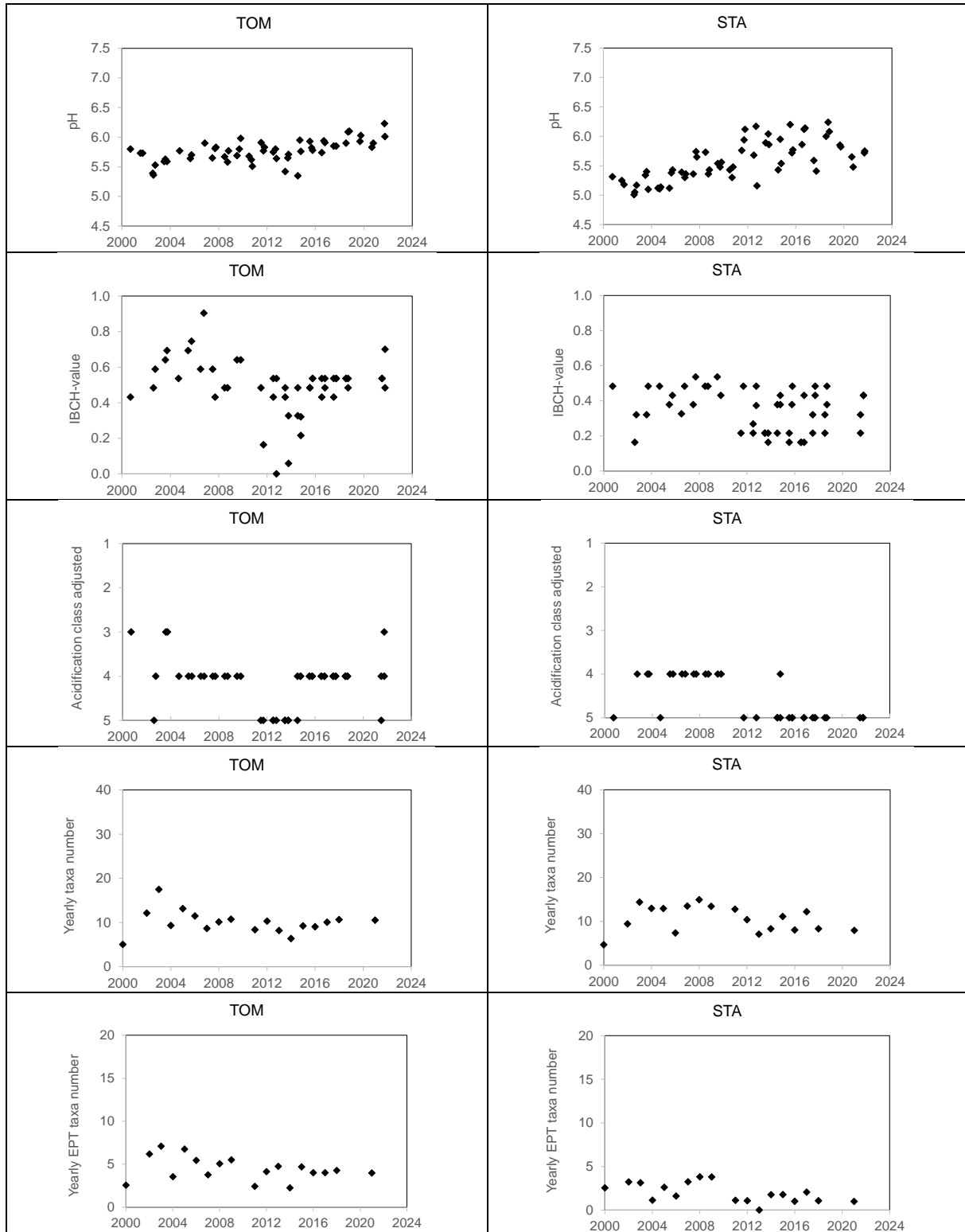


Figure 4 pH, for IBCH_2019 index and the acidification class calculated according to Braukmann and Biss (2004), the yearly number of total and EPT taxa and the yearly mean relative abundance of chironomids in lakes Tomeo and Starlarescio.



The average pH and the acidification class of the rivers Maggia, Vedeggio and Verzasca barely changed during the last 20 years. pH values were permanently well above the threshold of 6, below which negative impacts on the acid-sensitive benthic invertebrates is expected (Keller et al. 1999). It is therefore not surprising that no change of invertebrate indicators was observed. For the lakes instead, there is a certain discrepancy between the increasing pH values and the absent change of the invertebrate indicators. For the lakes Inferiore and Superiore it can be argued that the good state of their invertebrate community, considered their peculiar ecosystem (high-altitude oligotrophic lake outlet with a short growing season), can be attributed to consistent pH values above 6. In fact, they are inhabited by acid sensitive mayfly genera (*Ecdyonurus*, *Epeorus*, *Rhithrogena*), even though not at great abundances as observed at the lower situated rivers Maggia, Vedeggio and Verzasca. Only at the end of the 1980's lake Inferiore and Superiore had pH values slightly below 6. It is possible that a biological recovery occurred during the decade between 1990 and 2000, when monitoring did not occur. Differently, a recovery from acidification of the invertebrate population can still be expected in the lakes Tomeo and Starlarescio. In both lakes the threshold of pH 6 has been reached only recently and in lake Starlarescio the pH is occasionally still below this threshold. As deposition of sulphur is already very low, a significant further chemical recovery largely depends on future reduction of nitrogen deposition (Steingruber et al. 2021). In addition, it also depends on the colonization of acid-sensitive insects from other freshwater ecosystems.

Future trend analysis of benthic invertebrate fauna indices must also consider the influence of climate change. For example, Hutter et al. (2019) observed a recent increase of the number of EPT genera as a consequence of the temperatures increase in Swiss watercourses.

To summarize, recovery of benthic invertebrates from acidification could not be observed despite generally increasing pH values. This is probably because the investigated water bodies already had pH values above the critical threshold for sensitive species when monitoring was started, or, for lakes Tomeo and Starlarescio, because this threshold is still not fully met. However, considering that from 20 regularly monitored lakes, well representing the potentially acid sensitive lakes in Switzerland, only three lakes occasionally show critical conditions for acid-sensitive benthic invertebrates (pH <6), we conclude that freshwater acidification in Switzerland is now limited to few sites only. Moreover, it is important not to forget that freshwaters in crystalline bedrocks at high altitudes can also be naturally acidic, as they usually have small catchments and almost no soil, with very limited capacity to neutralize rainwater. Nevertheless, it has also to be noted that disturbances due to excessive nitrogen inputs have not been resolved in these lakes.

5.4 Acknowledgments

This study was supported by the Swiss Federal Office for the Environment through the ICP Waters Programme.

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6 Monitoring of acidification in Norway

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6.1 Introduction

There are three programs in Norway that monitor effects of long-transported air pollution on ecosystems: monitoring of freshwaters, monitoring of damage to forests and program for monitoring of terrestrial nature. Depending on program, these programs monitor air, water, forests, and aquatic- and terrestrial fauna. The aim of the monitoring is to gain knowledge on acidification and effects of acidification, to assess the need to implement abatement measures and to evaluate existing measures.

The program for monitoring of freshwater adheres to the EU Water Framework Directive (WFD). In Norway, the assessment of ecological status under the WFD is water type specific (Direktoratsgruppen vanndirektivet 2018) and is separated into surveillance monitoring and operational monitoring. Both types of monitoring are performed with standardized methods and a representative selection of sites from different ecoregions, water types and ecological status, which allows spatial and temporal comparison. Both lakes and rivers are monitored. The monitoring of acidified lakes is more extensive than the monitoring of rivers and includes zooplankton, benthic, invertebrates, fish, pH, ANC and labile aluminum, while the monitoring of rivers includes only benthic invertebrates. The data collected in the monitoring of benthic invertebrates are used to calculate several acidification indices (Table 1).

Table 1. Indices on benthic invertebrates that are used for the monitoring of acidification in freshwaters in Norway.

Index	River	Lake	Intercalibrated	Comments	Reference
Acidification index 1	Yes	Yes	No	Most used index, especially in rivers	Raddum and Fjellheim 1984
Acidification index 2	Yes	Yes	No	Modified index 1	Raddum 1999, Raddum and Fjellheim 1995
RAMI (River Acidification Macroinvertebrate Index)	Yes	No	Yes	Developed by Northern Intercalibration Group for Norway, Sweden and UK	B. McFarland et al. unpublished, Direktoratgruppen 2018
LAMI (Lake Acidification Macroinvertebrate Index)	No	Yes	No	Littoral inverts. Developed by Northern Intercalibration Group for Norway, Sweden and UK	B. McFarland et al. unpublished, Direktoratgruppen 2018
MultiClear (Multimetric index Clear lakes)	No	Yes	Yes	Littoral inverts in clear lakes. Developed by Northern Intercalibration Group for Norway, Sweden and UK	B. McFarland et al. unpublished, Sandin et al. 2014

6.2 Methods

There are 42 lakes and five rivers in the monitoring programs for acidification. Each lake has two sampling stations (littoral and outlet) that have been sampled annually (most sites) or every fourth

year since 1997, while each river has many sampling stations (7 to 16) that have been sampled annually or every second year since 1981 (Table 2). All sites have clear waters and a low natural content of calcium and other major ions and are considered acid sensitive. None of the sites are limed. Details on the sites and sampling interval can be found in Schartau et al. (2021). Benthic animals are sampled spring and autumn with the use of a kick-net with mesh size 250 μm . We have used Acidification index 1 (Raddum and Fjellheim 1984) to find the effect of acidification on benthic invertebrates in the sites. In lakes, the index is calculated for amalgamated samples (littoral + outlet). We have also used the number of EPT taxa, which are mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera), to depict biological diversity over time. Trends in index 1 and number of EPT are assumed to give a representative overview of the effects of acid deposition, especially since many sites have been sampled over many years with the use of consistent methods.

Table 2. Rivers that are monitored for the effects of long-transported aerosols on benthic invertebrates in Norway with details on the sampling. All sites are sampled each spring and fall during years of monitoring.

River	Start	Frequency of sampling	Sampling stations
Nausta	1981	Annual to 2001, every other since	14
Gaular	1984	Annual to 1997, every other since	16
Vikedal	1982	Annual	12
Ogna	1983	Annual to 2002, every other since	9
Farsund	1981	Annual	7

6.3 Results and discussion

The monitoring of macroinvertebrates in lakes indicates an increase in acidification index 1 for most regions since the late 1990s and towards the present (Figure 1), suggesting a reduction in acidifying components of the water. This is in line with the observed increase in ANC and pH, and a reduction in labile aluminum of the sites (Schartau et al. 2021). The improving water chemistry has been especially evident in southern Norway, which originally received the most acidification (Schartau et al. 2021). However, the reduction is not always evident and varies among regions. In region 2 (mid-Norway), 8 (Eastern Norway) and region 9 (Northern Norway), the results indicate an increased acidification. The increase is minor and data from more years are needed to confirm the results since these lakes are only sampled every fourth year. For some sites, the chemical conditions have also reached a plateau during the last 7-10 years (Schartau et al 2021).

The monitoring of benthic invertebrates in rivers indicates that acidification has decreased in all rivers from the 1980s and up to the present (Figure 2 and Figure 3). According to acidification index 1, the acidification was most severe in the river Farsund, and least severe in the river Nausta. However, the index indicates that the fauna is little influenced by acidification today and has reached the environmental target (better than the moderate/ good boundary).

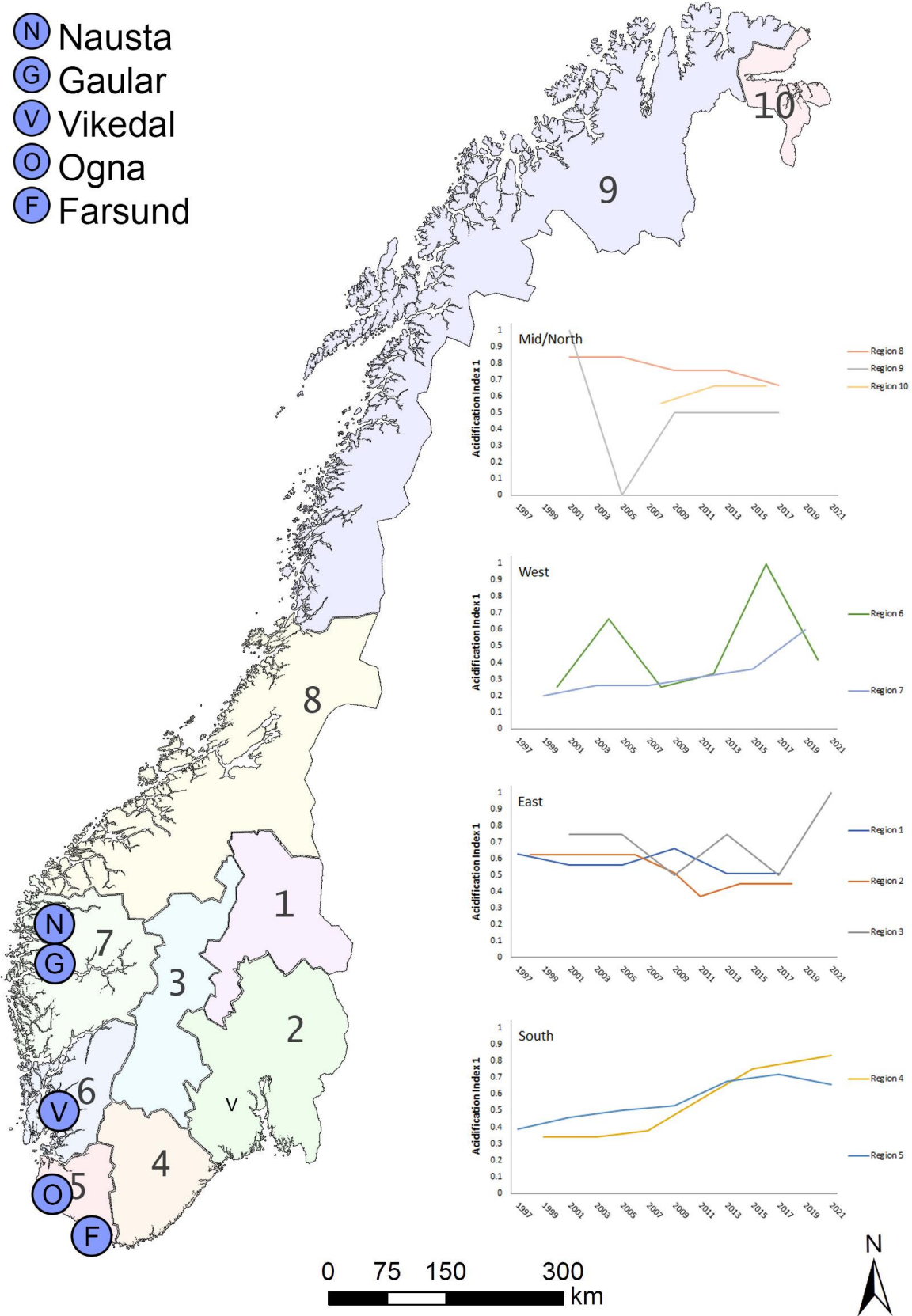


Figure 1. Map of Norway showing the sites of monitoring in rivers (denoted with letters) and the regions of monitoring of lakes. The graphs show trends in acidification index 1 over time for lakes per region.

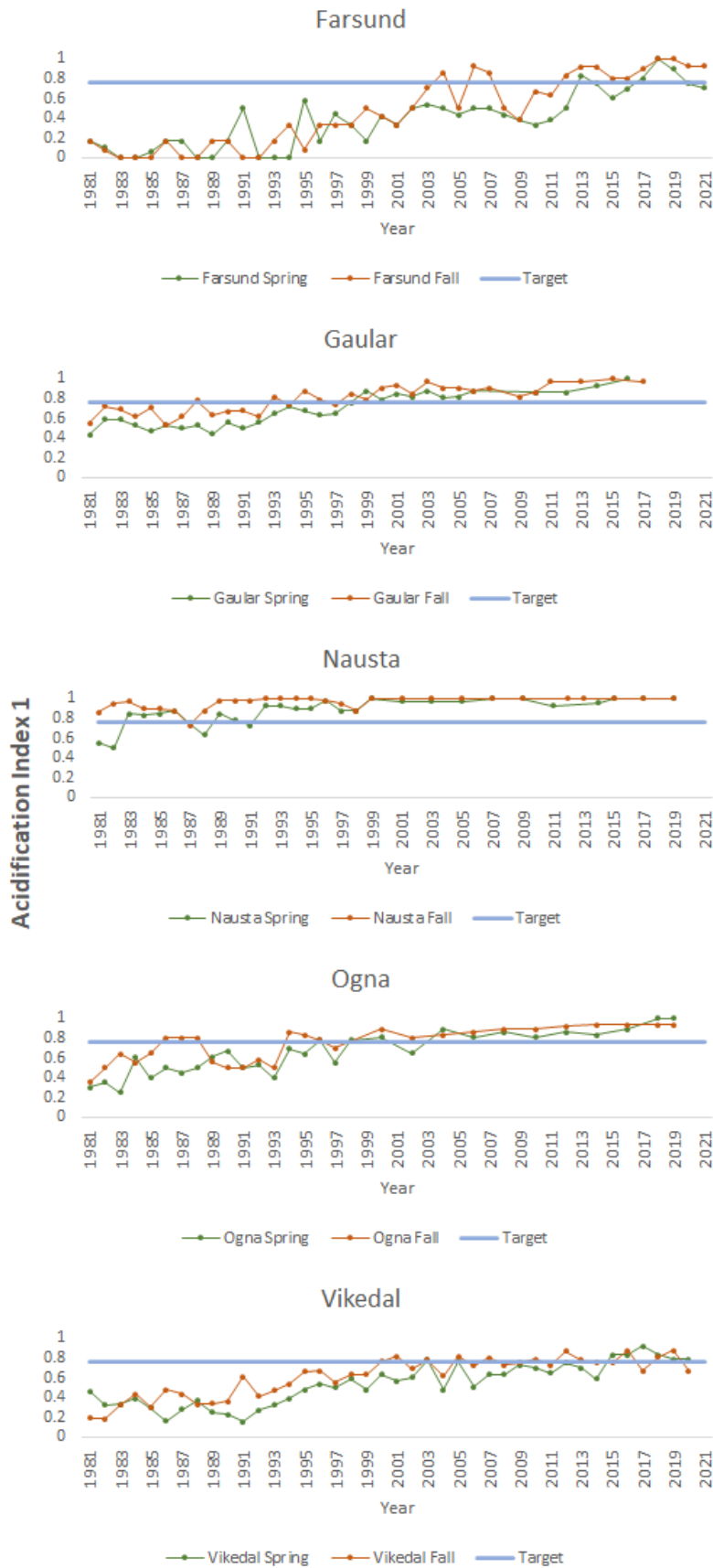


Figure 2. Trends in acidification index 1 based on benthic invertebrates in five rivers in Norway.

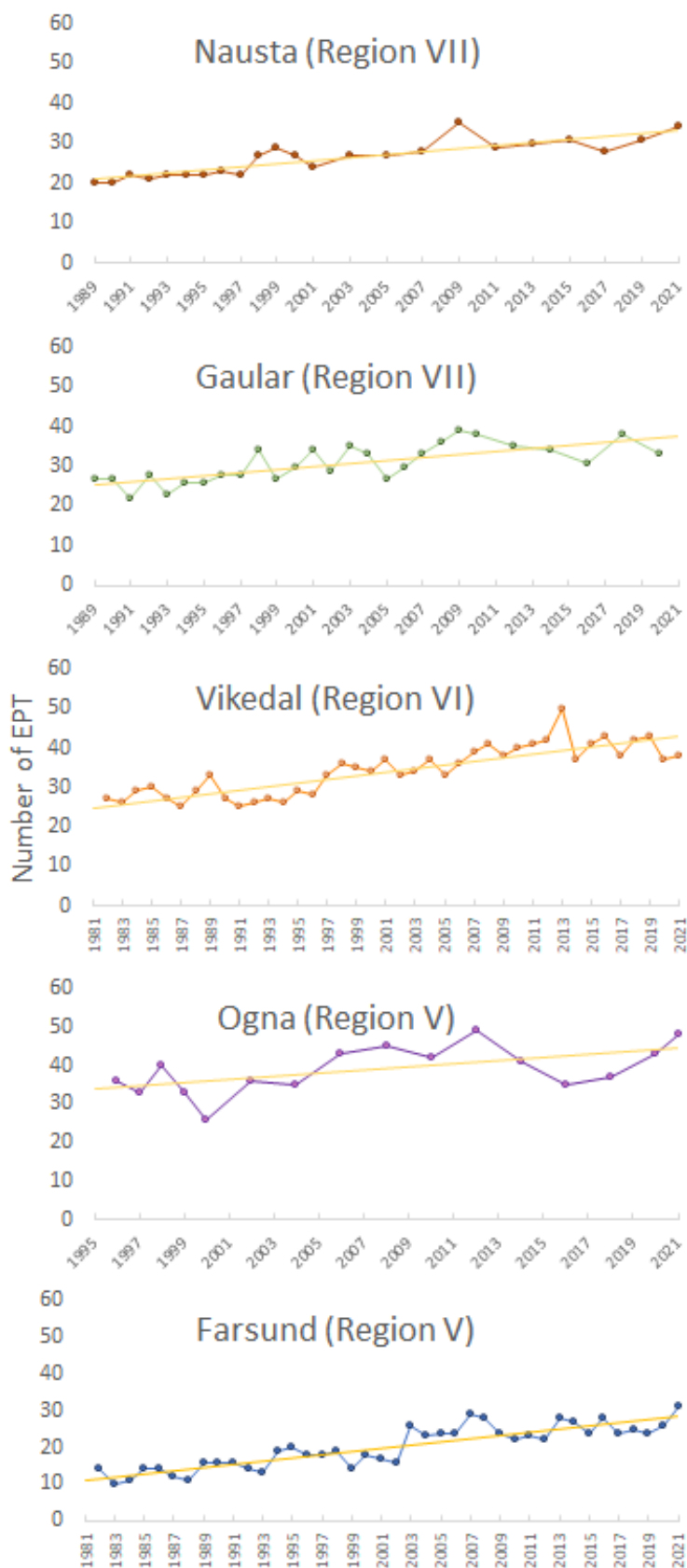


Figure 3. Trends in the number of species of Ephemeroptera, Plecoptera and Trichoptera (EPT-taxa) in rivers in Norway.

There are relatively large annual fluctuations in acidification index 1 in rivers (Figure 2). The acidification and recovery history can be divided into three phases from the 80s and up to the present: 1) A decade from the early 80s: The fauna was negatively affected by acidification and with large between-year variations. 2) A decade from the early 1990s: There was a marked increase in index values, suggesting decreased acidification. Three of the rivers (Nausta, Gaular and Oгна) reached the environmental target, while the environmental conditions in Vikedal and Farsund varied above and below the target. 3) From the early 2000s and up to the present: All sites indicate that acidification is slowly decreasing. The water during spring can be acidified in the most sensitive rivers Farsund and Vikedal during some years, but the ecological status has mostly been above the environmental target from 2016 onwards.

Monitoring of biological diversity (number of EPT taxa) in rivers indicates a slow recolonization of species and increasing diversity from the 1980s and up to the present (Figure 3). This is evident in all rivers. The increase in diversity is most pronounced in Farsund and Vikedal, mirroring the acidification index results and suggesting that these rivers were most influenced by acidification. It is too early to conclude whether the diversity of rivers has reached a maximum, or whether the increase will continue.

It can to some extent be expected that the chemical improvement is not always evident in the biota of the lakes. The biological monitoring of the lakes started in the late 1990s after the most pronounced chemical recovery. We lack information on the biological recovery prior to the onset of the biological monitoring. We should expect a delay between recovery of water chemistry and biota. The delay is caused by effects, such as 1) acidic episodes that are not evident in the monitoring of water chemistry, 2) access to the sites from source populations of sensitive taxa, including geographical distance and barriers for spread, 3) new stable equilibria that include fauna consisting of acid tolerant species that also have competitive advantages compared to re-establishing species, 4) negative effects caused by increased TOC or reduced content of calcium (Hessen et al. 2017) and 5) interactions with other environmental variables, such as climate.

A comparison between the acidification index in rivers and lakes indicates a higher amplitude of change and a clearer signal in rivers. This either suggests that the fauna of rivers is more sensitive to acidification than the fauna of lakes, that the fauna of rivers have a greater colonizing ability than the fauna of lakes, or that the environmental conditions of lakes are more stable than in rivers. It could also be caused by the later start of the monitoring of lakes than rivers. Other studies have also found a stronger signal in rivers compared to lakes (Velle et al. 2013, 2016), suggesting that the fauna in lakes will likely respond more slowly to acidifying components in the water and a chemical recovery than the fauna in rivers.

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7 Responses of benthic macroinvertebrate assemblages to reduced acidification in streams and lakes in Sweden

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7.1 Acid sensitivity

Sweden is mostly covered by granitoid moraines with a low weathering rate, hence most lakes and streams have soft water and are generally acid-sensitive. Exceptions are the agricultural plains in southern Sweden with more weatherable soils and regional occurrence of calcareous bedrock, both giving well-buffered soils. Precipitation shows a decreasing trend from west to east in Sweden, leading to an increasing trend in alkalinity from west to east. Acid deposition shows a strong decreasing gradient from south to north. According to the national survey of lake water chemistry based on a stratified random selection of all Swedish lakes larger than 1 ha, 6.7% of these lakes were regarded as acidified, with the majority in the south-western region.

7.2 Monitoring and assessment approach

National monitoring of acidification impacts on benthic macroinvertebrates in streams and lakes of Sweden started in the 1980s (Johnson 1999, Fölster et al. 2014). In the early years sampling was for some waters conducted in spring, but starting from 1995 (for lakes) or 1996 (for streams) it has been consistently conducted in autumn with international standard methods. For this national report, we analyzed data from 1996-2020 for streams and from 1995-2019 for lakes to get consistent data with autumn samples. Data were extracted from <https://miljodata.slu.se/mvm/>. Sites were selected based on four criteria: (i) no liming in the catchment; (ii) <1% urban land in the catchment; (iii) <10% agricultural land in the catchment; (iv) mean acid neutralizing capacity (ANC) <300 meq L⁻¹, i.e., being acid-sensitive.

In total 18 streams and 78 lakes were selected (Table 1). These sites covered wide ranges of catchment area (0.4–370.0 km²), ecosystem size especially for lakes (surface area <0.1–7.1 km²), and elevations (19–975 m asl). Thus, the data were judged representative for acid-sensitive water bodies in Sweden.

Table 1. Numbers of selected streams and lakes in the ecoregions of Sweden. See text for site selection criteria. Sites were categorized into different classes based on their interannual averages of pH: high-pH (pH≥6.5), mid-pH (5.5≤pH<6.5), and low-pH (pH<5.5).

Ecoregion	Stream pH class			Lake pH class		
	High	Mid	Low	High	Mid	Low
Borealic Uplands	2		1	7		
Fennoscandian Shield	6	3	1	16	5	
Central Plains		3	2	24	19	7

Taxon richness and composition were analyzed for trends in assemblage changes. To account for

differences in taxonomic resolution between sites and years, data were first aggregated to genus level. The number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa and the multimetric index for lake acidification (MILA; Johnson and Goedkoop 2007, HaV 2018) were used as indicators for acidification in streams and lakes, respectively. The ecological quality index (EQ) derived from MILA was used to indicate the status of lakes in the Central Plains (HaV 2018). Temporal changes in assemblages in lakes were analyzed using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities. To determine whether the assemblage changes were related to acidification, the water chemistry data (pH, acid neutralizing capacity (ANC), and concentrations of base cations (BC; \log_{10} -transformed) and sulphate (SO_4 ; \log_{10} -transformed)) were fitted on the NMDS ordination and tested for statistical significance with random permutations. For all analyses, the sites were categorized into three classes based on their interannual mean of pH in 1995-2019 (lakes) or 1996-2020 (streams): high-pH ($\text{pH} \geq 6.5$), mid-pH ($5.5 \leq \text{pH} < 6.5$), low-pH ($\text{pH} < 5.5$). Taxon richness and biological indices were compared among pH classes and/or ecoregions using Kruskal-Wallis test (for ≥ 3 groups) with the post hoc Dunn's test, or by Wilcoxon test (for 2 groups). Analyses for streams were not separated by ecoregions due to the limited number of sites (Table 1).

7.3 Acidification status

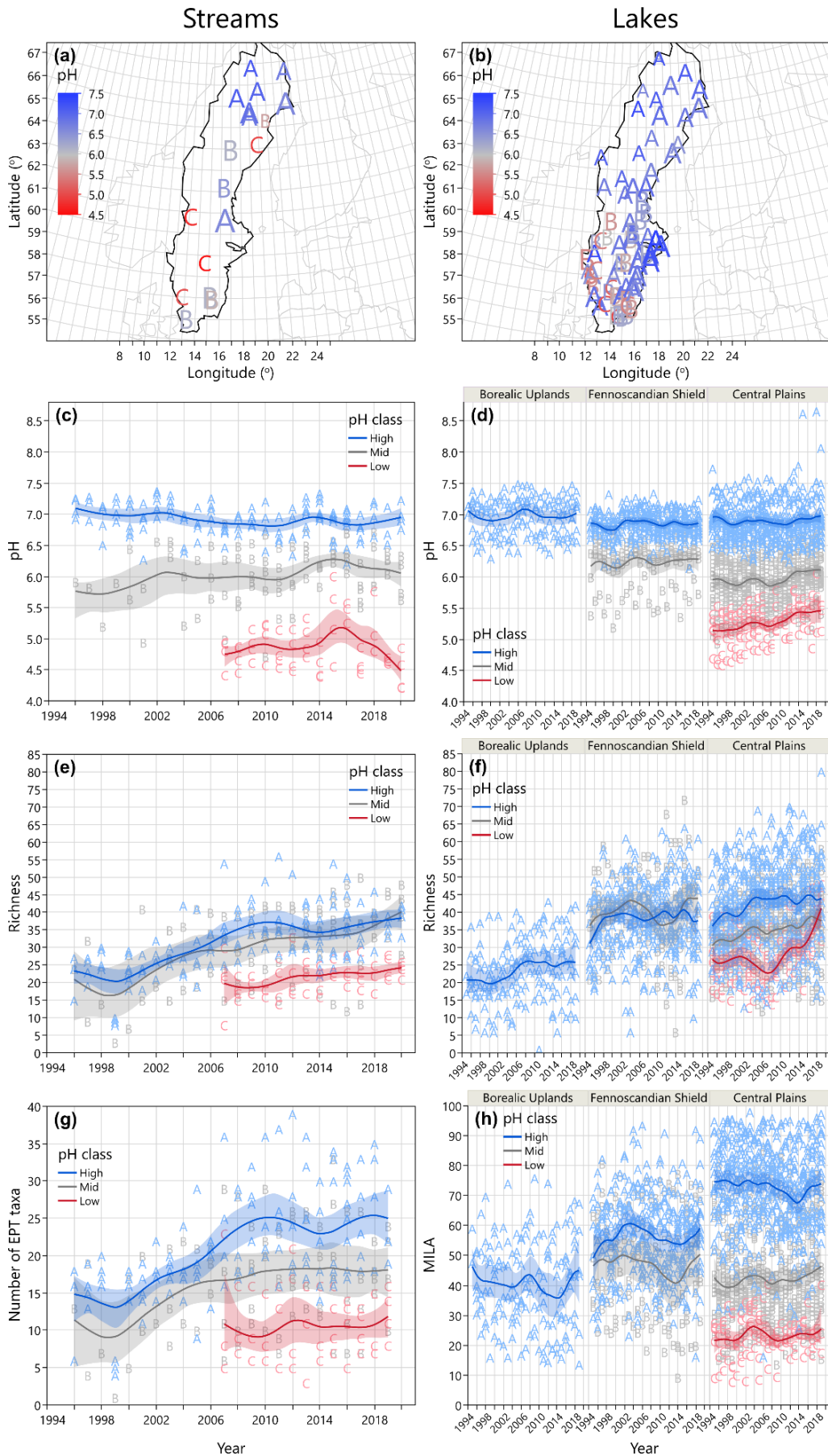
In Sweden, acidity is classified from macroinvertebrates in lakes by the MILA index. If MILA indicates acidic conditions, water chemistry should be used to distinguish between natural and anthropogenic acidification by a site-specific classification (HaV, 2019). Four of the 18 streams and 22 of the 78 lakes were acidified according to water chemistry.

7.4 Trend analysis

Trends in water chemistry – There were significant trends of decreasing SO_4 in 8 streams (44%), 46 lakes (92%) in the Central Plains, 19 lakes (90%) in the Fennoscandian Shield, and 4 lakes (57%) in the Boreal Uplands. Only 1 stream overall (6%) and 2 lakes (29%) in the Boreal Uplands showed significant increasing SO_4 trends. Acidic sites were more common in the Central Plains, i.e. southern Sweden, than in the Boreal Uplands and the Fennoscandian Shield (Table 1; Fig. 1a,b). Trends of slightly increasing pH were observed mainly in the mid-pH streams and the mid- and low-pH lakes, while no temporal changes were found in the high-pH streams and lakes and the low pH-streams (Fig. 1c,d). The pH of low-pH streams increased in 2007-2016, but decreased after 2016 (Fig. 1c).

Trends in benthic macroinvertebrates – Taxon richness was on average 9-11 lower in low-pH sites than in high- and mid-pH sites overall (all $p < 0.001$; Fig. 1e,f), indicating the negative impacts of acidity on the recovery of zoobenthos assemblages at the low-pH sites. Interestingly, taxon richness in high- and mid-pH streams increased over time (Fig. 1e). Also, the EPT taxa increased by 64% and 38% in high- and mid-pH streams, i.e. from 14 to 23 and from 13 to 18, respectively, between 1996 and 2019 (Fig. 1g). The low-pH streams, which had shorter time-series data, did not show any temporal trends neither in taxon richness nor the number of EPT taxa (Fig. 1e,g). These results further support the limited recovery of zoobenthos assemblages in the low-pH streams.

Figure 1 (next page). Locations and the interannual averages of pH of the 18 streams (a) and 78 lakes (b) in Sweden with an ANC of $< 300 \text{ meq L}^{-1}$. The sites were categorized into different classes based on their interannual averages of pH: high-pH ($\text{pH} \geq 6.5$; A), mid-pH ($5.5 \leq \text{pH} < 6.5$; B), and low-pH ($\text{pH} < 5.5$; C). Symbol size is proportional to the taxon richness of benthic macroinvertebrates in the streams and the littoral habitats of lakes. Temporal trends in pH (c,d), taxon richness (e,f), total number of Ephemeroptera, Plecoptera, and Trichoptera taxa (g), and multimetric index for lake acidification (h) of the sites were visualized using cubic splines (solid lines) \pm 95% confidence limits (shades) with $\lambda = 0.05$. Data in (c,e,g) and (d,f,h) are from streams in 1996-2020 and lakes in 1995-2019, respectively. Each data point is a site in the specific year. In (d,f,h), the lake data are presented based on their ecoregions, i.e. Boreal Uplands, Fennoscandian Shield, and Central Plains.



Trends in taxon richness and MILA in lakes differed between ecoregions. In the Fennoscandian Shield, taxon richness was similar between high- and mid-pH lakes ($p = 0.098$; Fig. 1f), but MILA was higher in high-pH lakes than in mid-pH lakes ($p < 0.001$; Fig. 1h). In the Central Plains, both taxon richness and MILA followed the same order as for pH: high-pH lakes > mid-pH lakes > low-pH lakes (all $p < 0.001$; Fig. 1d,f,h).

Mid-pH lakes in the Fennoscandian Shield had higher richness (mean \pm SD = 40 ± 11) and MILA (47 ± 18) than those in the Central Plains (richness 35 ± 9 ; MILA 42 ± 15) (all $p \leq 0.020$; Fig. 1f,h), likely reflecting the higher overall pH in mid-pH lakes in the Fennoscandian Shield (6.2 ± 0.3) than in the Central Plains (6.0 ± 0.4) ($p < 0.001$; Fig. 1d). Among the high-pH lakes, however, taxon richness and MILA were highest in the Central Plains (richness 42 ± 12 ; MILA 73 ± 14), intermediate in the Fennoscandian Shield (richness 38 ± 10 ; MILA 56 ± 14), and lowest in the Borealic Uplands (richness 23 ± 9 ; MILA 41 ± 15) (all $p < 0.001$; Fig. 1f,h). These differences did not conform the pH differences of high-pH lakes between ecoregions (Fig. 1d). Results from mid- and high-pH lakes thus suggest interacting effects of acidity and climate on lake zoobenthos assemblages (Johnson and Angeler 2010).

Taxon richness followed the temporal trends in pH for the low- and mid-pH lakes. For example, at the low-pH sites, both pH and richness decreased after 2002, and increased after 2007 and 2014 (Fig. 1d,f). The low-pH lakes in the Central Plains showed the largest increase in richness (Fig. 1f). Richness also increased in the high-pH lakes at all ecoregions in 1995-2019, although there were no overall changes in pH (Fig. 1d,f). These increases in richness were likely related to warming (Johnson and Angeler 2010, Lau et al. 2022, Lento et al. 2022).

MILA did not show any overall trends for all lake classes in the ecoregions (Fig. 1h). However, increases or peaks in MILA followed those in pH, but decreases or troughs in MILA of high- and mid-pH lakes were also found when there were no obvious changes in pH, e.g. in 2014-2015 (Fig. 1d,h).

Lake benthic macroinvertebrate assemblages differed between pH classes, with greatest differences between high- and low-pH lakes. Temporal trends in assemblages were mainly observed in the low-pH sites of the Central Plains (Fig. 2a,b). Trends of slightly increasing pH and decreasing SO_4 in these lakes were accompanied by increasing abundances of EPT genera that are acid-sensitive (*Oecetis*) or acid-tolerant (*Kageronia*, *Nemoura*, and *Molannodes*) (Schartau et al. 2008), with large fluctuations between years indicating small populations and slow and unstable assemblage recovery. The limited temporal changes in assemblages of mid- and high-pH lakes in individual ecoregions suggest that the macroinvertebrate assemblages were relatively stable or that the differences among sites were larger than those among years. An example was illustrated by the mid-pH lakes in the Central Plains (Fig. 2c,d). These results corresponded to the small or lack of temporal trends in pH and MILA (Fig. 1d,f).

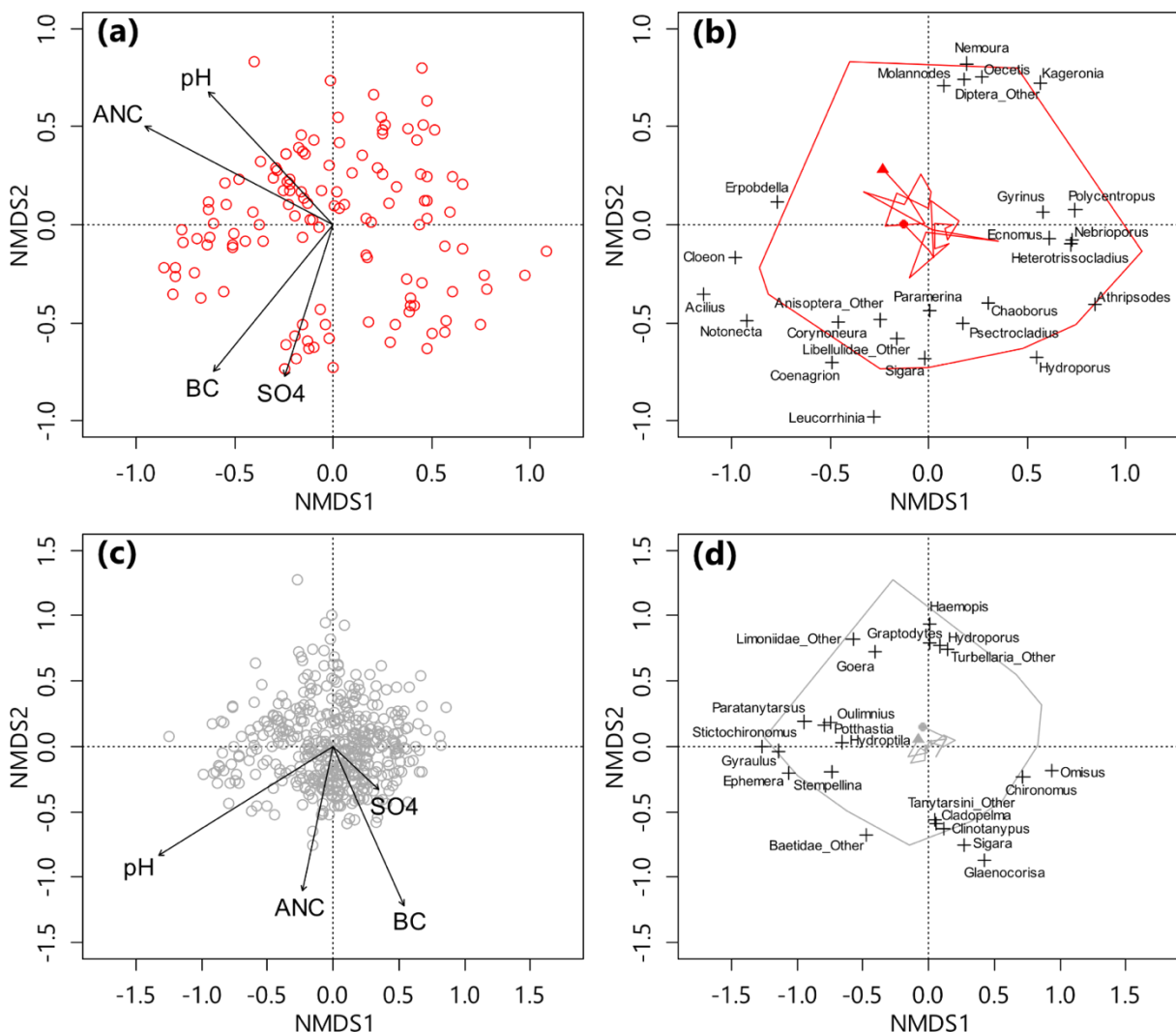
At the high- and mid-pH streams, the trends of increasing taxon richness and number of EPT taxa indicate their generally improved status. In contrast, at the low-pH streams, the lower values and the absence of positive trends in these indicators suggest their worse and unimproved status.

We found positive trends in taxon richness of lakes, but trends in MILA were lacking and so the overall averages of EQ of lakes in individual pH classes in the Central Plains were used to indicate their status. Based on these EQ, status was high for the high-pH lakes (EQ = 1.04 ± 0.20), moderate for the mid-pH lakes (EQ = 0.60 ± 0.21), and unsatisfactory for the low-pH lakes (0.33 ± 0.10) in the Central Plains, according to the national environmental quality standards (HaV 2018). The contrast in benthic macroinvertebrate assemblages between low- and high-pH lakes in the Central Plains also supports the poorer status of the low pH lakes.

Overall, the results from streams and lakes indicate that, despite significant trends in chemical recovery, the recovery of benthic macroinvertebrates from acidification was especially limited at acid-sensitive, low-pH sites in the Central Plains, where acidification has been most severe. Yet, richness

and/or acid-sensitive EPT taxa increased over time in many high- and mid-pH acid-sensitive sites, and were likely partly driven by warming (Johnson and Angeler 2010, Lau et al. 2022, Lento et al. 2022). The absence of similar increases in richness and EPT taxa at the low-pH sites suggests slower recovery due to a 'impoverished' species pool in the Central Plains that was strongly affected by acidification. These results stress the need for quantifying the impact of climate change on biological responses to acidification.

Figure 2. Non-metric multidimensional scaling (NMDS) of benthic macroinvertebrate assemblages at the seven low-pH (a,b; 3D stress = 0.19) and 19 mid-pH (c,d; 3D stress=0.20) lakes in central Plains of Sweden in 1995–2019. (a,c) Ordination of the lakes. Each data point is a site in a specific year. Arrows indicate fits with pH, acid neutralizing capacity (ANC), and concentrations of base cations (BC; \log_{10} -transformed) and sulphate (SO_4 ; \log_{10} -transformed) of lake water. All fits are significant based on permutation tests (all $p \leq 0.007$). (b,d) Convexhulls of data, within which the lines indicate trajectories of assemblage changes over time, i.e. across group centroids of individual years. Solid circles and triangles indicate start and end years, respectively. Only taxa of top 50% most common and top 30% (b) or 20% (d) best fit with the NMDS axes are shown.



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8 Assessing biological recovery from acidification in the UK

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8.1 Introduction

The UK UWMN (formerly known as the Acid Waters Monitoring Network or AWMN) was established in 1988 by the UK's Department of the Environment (DoE). It aimed to assess the efficacy of controls on the atmospheric emission of acidic pollutants in improving the ecological health of the UK's acidified surface waters. It initially comprised 22 lakes and streams, mostly draining catchments underlain by acid-sensitive geologies, and distributed across a large acid deposition gradient. The majority of its lakes were known to have acidified over the course of the previous two centuries as a direct consequence of anthropogenic acid deposition. More details on the history, structure, and outputs of the UWMN can be found on the UWMN website (<https://uwmn.uk/>).

Consistent with approaches in several other countries, a critical level of Acid Neutralising Capacity (ANC_{crit}) of $20 \mu\text{eq L}^{-1}$ was adopted as a water chemical recovery target for acid-sensitive UK waters. Levels of ANC for the majority of UWMN sites at the onset of monitoring were substantially below the ANC_{crit} (i.e. $20 \mu\text{eq L}^{-1}$). Over the 30 years of monitoring, the majority of UWMN sites have shown strong positive trends in ANC, in many cases from consistently negative values to levels substantially above $20 \mu\text{eq L}^{-1}$. The strength of the ANC increases correlate with rates of reduction in acid anion concentration – in turn a response to large reductions in acid deposition. A subset of sites (in this summary referred to as “chemical control sites”) are either situated in remote regions that have never received sufficient acid deposition to acidify, and/or are sufficiently well buffered by their underlying geology to have resisted effects of acid deposition.

Chemical and biological monitoring has been maintained according to consistently applied protocols at nearly all originally selected sites up to the present day. Here we provide a brief summary of more detailed assessments of trends in epilithic diatom and macroinvertebrate populations provided within a wider interpretation of the first three decades of UWMN data (Monteith et al., 2022). UWMN monitoring also includes the monitoring of diatom assemblages collected annually in sediment traps (to allow comparisons with historical trends recorded in sediment cores), multi-annual surveys of aquatic macrophyte assemblages. Annual surveys each autumn of salmonids in streams and lake outflows were discontinued in 2015.

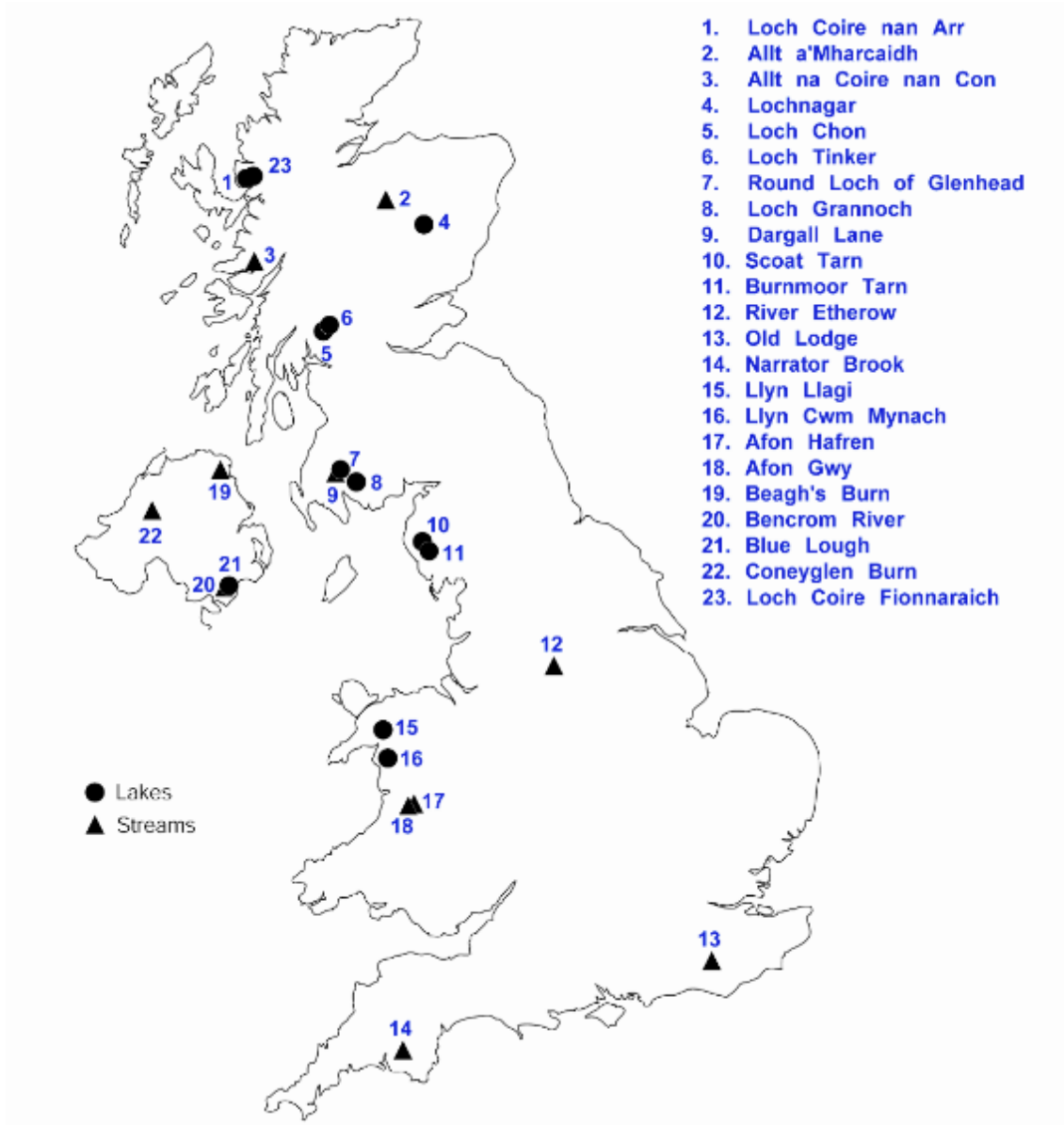


Figure 1. Location of UK Upland Waters Monitoring Network lakes and streams

8.2 Methods

8.2.1 Biological sampling and sample preparation

Samples of epilithic diatoms and macroinvertebrates are collected each summer and spring respectively from UWMN lakes and streams. Epilithic diatoms growing in the biofilm of 3-5 permanently submerged horizontal stone surfaces are removed using a toothbrush and preserved using lugol's iodine. Three to four replicate samples are taken from around lake shorelines and along a 50 metre section of UWMN streams. Samples are then "cleaned" using hydrogen peroxide, microscope slides prepared using Naphrax high refractive index mounting medium, and 500 individual diatom valves identified and counted using a light microscope at x 1000 magnification.

Five replicate macroinvertebrate samples are taken from riffle locations in the UWMN streams and from multiple shallow littoral habitat types in UWMN lakes. The biological samples are analysed by

taxonomic specialists to determine the abundance of each species, in the case of epilithic diatoms, or at mixed taxon level (i.e. species, genus or family) with respect to macroinvertebrates.

8.2.2 Statistical methods

Diatom species counts for each site and sampling year were aggregated, converted to percentages and the percentage data square-root transformed prior to all statistical analysis. Macroinvertebrate counts were also pooled for each site and year, but not transformed prior to analysis. Turnover in species composition, or β -diversity, was quantified using detrended canonical correspondence analysis (DCCA) with “sample year” as the sole constraining variable. The gradient length of the first DCCA axis provides a measure of turnover. A turnover of 1 or 2 standard deviation units represents an approximately 50% change or complete exchange in species composition respectively over the sampling period (Hill and Gauch, 1980; ter Braak and Verdonschot, 1995). Redundancy analysis (RDA) was used to test for a linear trend in assemblage composition, with sample year again the sole explanatory variable. The presence of a trend was determined using a restricted permutation test in which the ordering of samples was maintained with starting samples selected via random cyclic shifts of the time series. The single constrained eigenvalue from each RDA (RDA1) quantifies the fraction of total variance in the species data that can be explained by a linear trend. The RDA1 scores were also compared to an equivalent measure from an unconstrained Principle Components Analysis (PCA1) to calculate the fraction of the main pattern of variance in diatom data that can be explained by a linear trend. Finally, in the case of the epilithic diatom data, a principal curve (PrC) was fitted to the species data at each site to summarise the trajectory of change. Principal curve is a non-linear curve fitted through the data in multivariate space and can provide a more parsimonious fit than other ordination methods if there is a single dominate gradient (Simpson and Birks, 2012).

To explore the extent to which the epilithic diatom trends at each site can be accounted for by changes in acidity, we calculated the diatom acidification metric (DAM; Juggins et al., 2016) for each sample. This metric encapsulates the pH range of the constituent taxa, and ranges from zero for an assemblage dominated by acidobiontic taxa (i.e., those most common at pH less than 5) to 100 for an assemblage dominated by taxa most abundant at pH greater than 7. For streams, we used DAM as described in Juggins et al. (2016) and, because some taxa common in lakes are not recorded in the original DAM metric, we used a modified version for lakes based on species optima from the SWAP lake-sediment diatom calibration dataset (Birks et al., 1990). The significance of trends in DAM at each site was assessed using a Sen’s slope estimator (Wilcox, 2010), and visualized using a smoother based on a generalized additive model with spline smoother and a first order autoregressive CAR(1) process for the residuals (a similar approach to that applied to the water chemistry metrics). Periods along the trend where there is a significant increase or decrease in DAM were identified by computing the 95% point-wise confidence intervals of the first derivative of the fitted smooth function (see Monteith et al. (2014) for details). The same technique was used to highlight periods of significant change in the PrC scores.

Separate acidification metrics were used to represent lake and stream macroinvertebrates: the lake acidification macroinvertebrate metric (LAMM) for lakes (WFD-UKTAG, 2008); and the acid waters indicator community index (AWICsp) for streams (Murphy et al., 2013). LAMM assigns a sensitivity score to each taxon and then calculates an abundance-weighted average for all scored taxa in a sample. Similarly, AWICsp assigns a sensitivity score to each of 48 stream macroinvertebrate taxa with the final AWICsp index value representing the average for scored taxa. LAMM and AWICsp scores were calculated for each lake and stream site in each year from the pooled list of taxa from replicate kick samples. Non-parametric Mann-Kendall tests were applied to determine the likelihood of monotonic change in index values over time, with the strength of trends described by the Sen slope statistic. All

statistical analyses were performed using R software for statistical (R Core Team, 2020) with the additional packages: vegan (PCA and RDA: Oksanen et al., 2022), prncurve (PrC: Hastie and Stuetzle, 1989), darleq3 (DAM: Juggins et al. 2016), mvabund (mGLM: Wang et al. 2016).

8.3 Results

8.3.1 Epilithic diatoms

Most sites, other than the chemical control sites, have undergone significant trends in species composition as assessed by RDA. Fourteen sites have species turnover greater than 1 SD unit, indicating an approximately 50% change in species composition at these locations. Sixteen sites showed a RDA1/PCA1 ratio over 0.5, i.e. that linear change with time was the dominant pattern of variation in the species data. The principal curve analysis (Figure 2a) provides an indication of how linear compositional change has been, and helps to identify where and when change has been most rapid or has levelled off. Four sites (all chemical control sites) showed no significant periods of change, 11 showed a sustained rate of turnover throughout the monitoring period, or at least the last 20 years, 3 sites showed sustained change in the early part of the record but little or no in the last c. 8-10 years, and 5 showed more complex patterns of turnover.

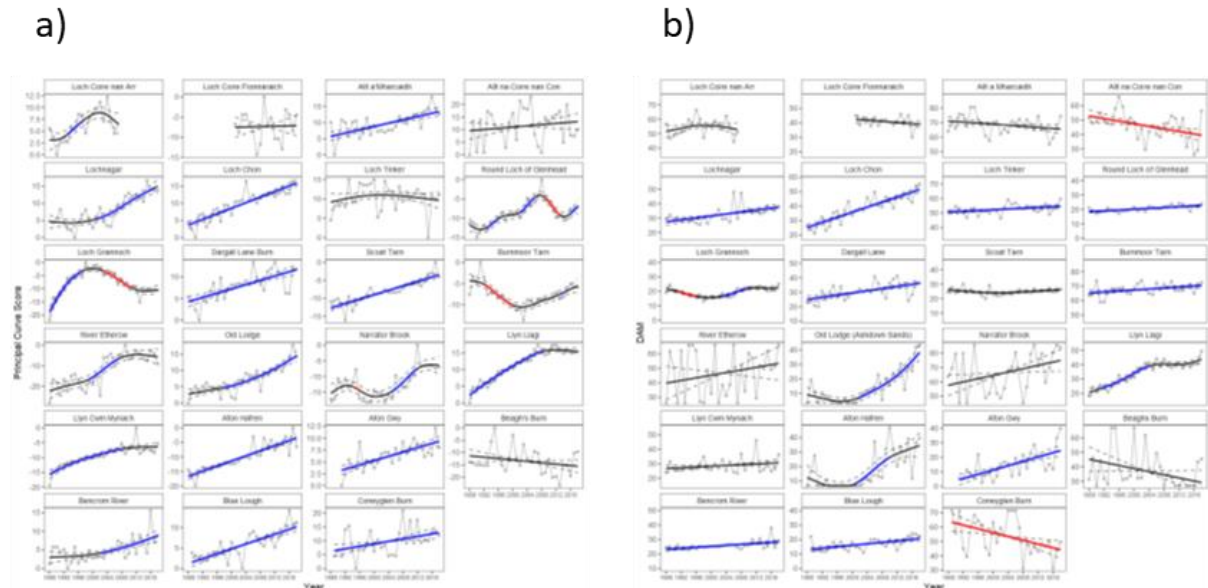


Figure 2. a) *Principal curve (PrC) trajectories for each site, with GAM smoother highlighting periods of significant species change (blue = significant increase; red = significant decrease).* b) *Trends in diatom acidification metric (DAM) for each site, with GAM smoother indicating periods of significant change (blue = significant increase; red = significant decrease).*

The more detailed analysis presented in Monteith et al. (2022), demonstrates that progressive changes in composition at the non-control sites is consistent with trends in increasing lake- or stream-water pH. Of the 14 sites that show a significant increase in pH, all also exhibit a significant trend in epilithic diatoms that reflect transitions to less acid tolerant taxa and this is also reflected in the DAM scores (Figure 2b). Across the UWMN lake sites there is a strong correlation between the magnitude of diatom species change over the period of monitoring (i.e., turnover) and the magnitude of pH change. However, the relationship between the absolute magnitude of diatom species change and pH change is not significant for streams. The stream diatom flora shows much greater inter-annual variation than the lake flora, reflecting not only the degree of recovery but also responsiveness to antecedent flow

conditions and related short-term variation in chemistry. Some sites in the network show a remarkable degree of biological recovery. Loch Chon and the River Etherow, for example, have evolved from a chronically acidified state with a flora dominated by acid tolerant taxa to one characterized by acidiphilous (acid loving) diatoms indicative of naturally acid soft waters. However, by comparing the epilithic diatom floras of the UWMN lake sites with pre-acidification communities occurring in the deeper sediments of these sites (representing conditions from more than 200 years ago), only three (Loch Chon, Loch Tinker and Llyn Llagi) have current diatom assemblages considered close to a pre-acidification flora. The remaining six non-control lakes, although showing varying degrees of recovery, are still characterized by acidobiontic taxa indicative of acute acidification and lack acid sensitive taxa that were present in the pre-acidification flora.

8.3.2 Aquatic macroinvertebrates

Lakes and streams exhibited similar ranges of macroinvertebrate turnover over the monitoring period. At five lakes and seven streams, taxa turnover was greater than the 1 standard deviation unit – indicative of an approximate 50% change in species composition. Significant directional change in macroinvertebrate community composition, as determined by RDA, was observed at 12 sites: six lakes and six streams (Figure 3a).

Variation in the biological community was significantly linked to at least one hydrochemical (acidity) predictor variable in four lakes (Loch Chon, Loch Tinker, Round Loch of Glenhead and Llyn Llagi) and six streams. The most frequently related variable at the lake sites was water pH, whereas ANC was the dominant explanatory variable in the UWMN streams (significant at six of the 11 sites). The acidification index scores showed significant increases (indicative of a reduction in acidification stress) at 14 UWMN sites; seven lakes and seven streams (Figure 3b). The greatest rates of increase in the LAMM metric were at Round Loch of Glenhead and Loch Chon where, by 2016, values were at, or exceeding, levels observed in other non-acidified “control” lakes in the network. More modest rates of increase were evident at Blue Lough and Llyn Cwm Mynach, but also at the non-acidified Burnmoor Tarn; all three having asymptotic response curves with increases most pronounced prior to 2005. LAMM values at the highly acidic Blue Lough were low throughout, but particularly so over the first 10 years of monitoring when they were often at the minimum possible score (2.0). In contrast, LAMM values at Burnmoor Tarn confirm that the site was never greatly affected by acidification with values initially varying around 5.0: over the past 10 years they have usually been greater than 5.6, possibly reflecting a response to a sustained increase in bicarbonate alkalinity at the site. Lochnagar and Scoat Tarn, both highly acidified but chemically recovering sites, were the only UWMN lakes not to show evidence of macroinvertebrate recovery.

Seven UWMN streams underwent significant increases in AWICsp score; the low deposition montane Allt a’Mharcaidh and two streams in Northern Ireland were the only sites where there was no evidence for a change in the score. A decreasing trend in AWICsp scores at the low deposition Coneyglen Burn in Northern Ireland reflect a few recent sampling occasions when only 2-3 AWICsp scoring taxa were recorded. The strongest rate of recovery was seen in the River Etherow where AWICsp values have gone from among the lowest in the network in the late 1980s to among the highest in 2016. Alkalinity and ANC have also recovered strongly at this site, while labile aluminium concentrations fell substantially over the first 20 years of monitoring.

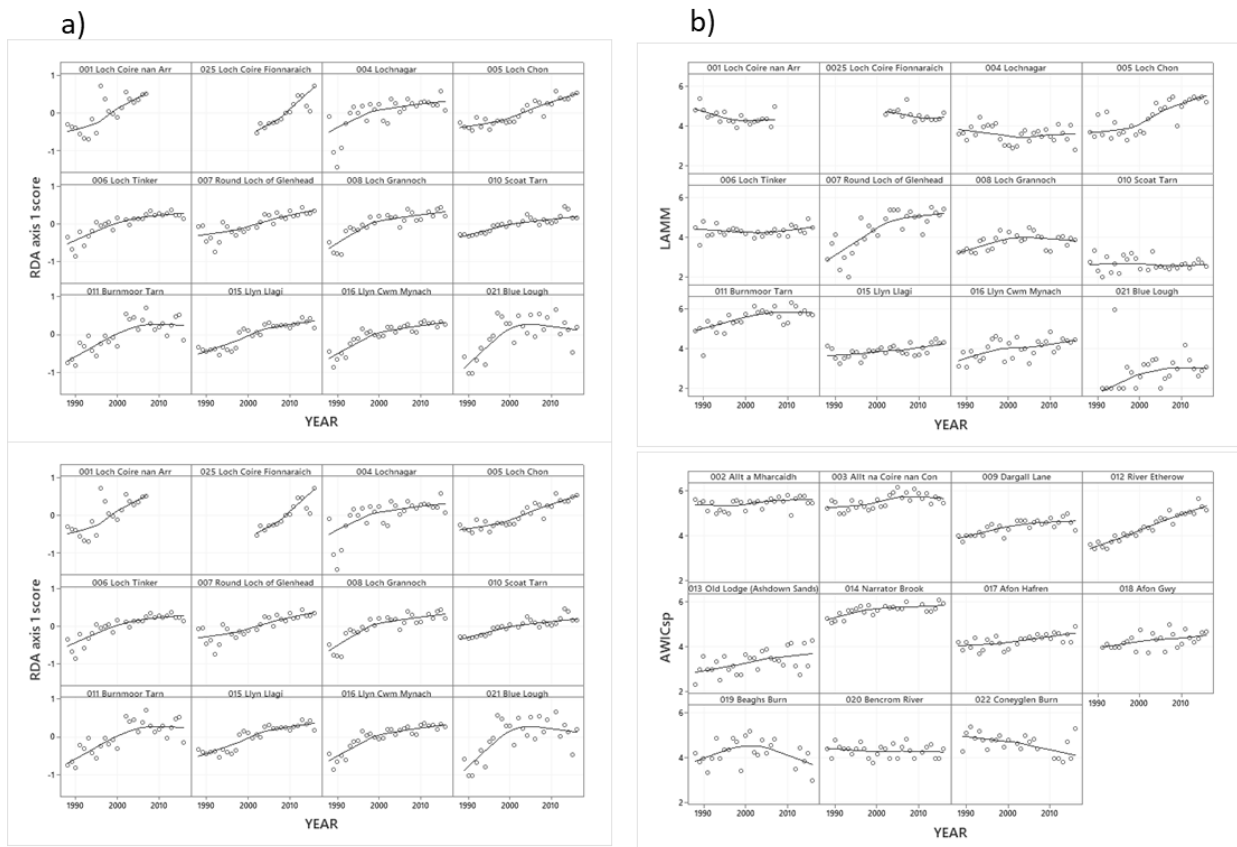


Figure 3. a) Variation in axis 1 scores from redundancy analysis fitted to macroinvertebrate data from each of the UWMN lake and stream sites, constrained by sampling year. The solid line represents a LOESS smoother (degree of smoothing = 0.75, number of steps = 2). b) Variation in LAMM and AWICsp values at each of the UWMN lake and stream sites, respectively. The solid line represents a LOESS smoother (degree of smoothing = 0.75, number of steps = 2).

8.4 Conclusions

Our analysis of the first three decades of UWMN biological data provides widespread evidence for improvements in epilithic diatom and macroinvertebrate communities that are consistent with partial recovery from the effects of chronic acidification. In general, there is much less indication of biological change in the lakes and streams situated in historically low acid deposition regions (e.g. Loch Coire Fionnaraich, Allt a'Mharcaidh) and those situated on more chemically buffered geology, although there are exceptions (such as the slight increase in the macroinvertebrate acidity metric in the relatively well buffered Burnmoor Tarn).

It is also clear that while recovery is extensive at some sites, it remains largely incomplete. This is most obvious with respect to the lake diatom communities for which pre-acidification references are available in the form of the lake sediment fossil record. There would seem, therefore, to be the potential for further biological improvement providing further chemical recovery is possible. There is no indication of a systematic difference in the extent to which either biological group is responding to reductions in acidification of fresh waters in lakes and streams. This suggests fears that the more episodic chemistry (and hence the continued occurrence of acid episodes) of some running waters might impose a barrier to recovery have not been realised.

The apparent reduction, and even cessation, in the rate of biological recovery in both biological groups at a number of sites over the last 10 or so years is perhaps surprising, but broadly consistent with a general flattening out of acid deposition and corresponding water acidity. There are a number of possible obstacles to biological recovery keeping pace with chemical recovery (Monteith et al., 2005; Murphy et al., 2014) including variability in the chemical recovery trend (Kowalik et al., 2007), limitations on the dispersal of acid-sensitive colonists from unimpacted source areas (Gray and Arnott, 2011), hysteresis in the recovery trajectory due to biological interactions within the acid-tolerant assemblage (Monteith et al., 2005), and climate change or other effects disrupting a straightforward recovery from acidification (Johnson and Angeler, 2010). Further monitoring is therefore likely to reveal continued gradual biological improvement as one or more of these impediments is gradually overcome. As direct acid deposition effects begin to dwindle, however, effects of other factors on water pH, such as changes in hydrology and episodicity, nutrient enrichment (from N deposition and catchment land-use), climate and dissolved organic matter inputs will become increasingly important in influencing the epilithic diatom community. These rare long-term studies should therefore continue to serve as hugely valuable ecological barometers of long-term environmental change in these sensitive upland ecosystems well after the direct effects of chemical recovery from acidification have stabilised.

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9 Short summary of national contributions of biological monitoring and assessment

9.1 Italy

The Italian assessment of acidification concerns the collection of water chemistry, diatoms, and benthic macroinvertebrates in 2019 and 2020 in lakes to meet the National Emission Ceilings Directive (NECD). The focus was to assess the chemical and biological responses to reduced surface water acidification and identify suitable biological indices for Italian sites. Two lakes, Paione Inferiore and Superiore, received in-depth analysis due to their inclusion in the ICP Waters and LTER networks, and the availability of long-term data from previous monitoring projects. Several indices were applied to evaluate the differences among lakes and assess the potential anthropogenic pressures, particularly acidification, on the diatom communities.

The results showed variations in biological indices among lakes, and some lakes exhibiting greater biodiversity than others. The lakes exhibited communities dominated by acidophilic or neutrophilic species, with a small percentage of acidobiontic or alkaliphilic species. The communities remained relatively stable between the two years. Lake Capezzone, which lacked acidophilic diatoms, showed lower index values in 2019 but comparable values to other lakes in 2020. The limited number of samples prevented a conclusive determination of whether this change was temporary or permanent. The abundances of epilithic diatoms in Lakes Paione were compared to previous data from 1991 to evaluate long-term trends in relation to pH. The analysis showed a decrease in acidophilic species from 1991 to 2000, particularly in Lake Paione Inferiore. In recent years, the abundance of acidophilic species has either been higher or like 1991 levels. Both lakes maintained pH values above 5.5. Overall, the survey provided insights into the chemical and biological responses of alpine lakes in the Italian Alps to reduced surface water acidification. It highlighted the stability of diatom communities and indicated potential long-term changes in the composition of acidophilic species.

9.2 Switzerland

The contribution from Switzerland describes the monitoring and assessment of acidification of freshwater bodies. The study focuses on Alpine water bodies situated on slow-weathering crystalline bedrock. The monitoring and assessment approach involved regular water chemistry measurements in acid-sensitive lakes and rivers from 2000 onwards. Benthic invertebrates were sampled to assess biological responses to recovery from acidification.

The results showed that the pH of the monitored rivers remained within the neutral to episodically acidic range, while alkalinity increased. The invertebrates indicated good water condition, with occasional lower values in river Verzasca. The acidification classes of the rivers did not change significantly over time. The pH of the high-altitude lakes increased, reaching pristine pH levels in lakes Inferiore and Superiore. In summary, the recovery of benthic invertebrates from acidification could not be observed despite an overall increase in pH values. This lack of recovery may be because the water bodies already had pH values above the critical threshold for sensitive species when monitoring began, or in the case of lakes Tomeo and Starlarescio, because this threshold has still not been fully met. However, it is worth noting that out of the 20 regularly monitored lakes in Switzerland, which are representative of potentially acid-sensitive lakes, only three lakes occasionally exhibit critical

conditions for acid-sensitive benthic invertebrates ($\text{pH} < 6$). This suggests that freshwater acidification in Switzerland is now limited to only a few sites. Additionally, it is important to consider that freshwater bodies in areas with crystalline bedrocks at high altitudes can be naturally acidic. These bodies usually have small catchments and minimal soil, resulting in limited capacity to neutralize rainwater.

9.3 Norway

The Norwegian contribution describes monitoring activities to gain knowledge about acidification and its impacts, evaluate the need for implementing abatement measures, and assess the effectiveness of existing measures. Both lakes and rivers are monitored, with more extensive monitoring for lakes compared to rivers. Here, acidification indices and the number of EPT taxa are presented to assess the effects of acid deposition and biological diversity over time. The biological recovery in lakes is particularly noticeable in southern Norway, which initially experienced the most significant acidification. However, there are also regions where increased acidification is observed, but more data is needed to confirm these results. In rivers, the monitoring of benthic invertebrates suggests a decrease in acidification since the 1980s, and that most years and sites now have reached the environmental target. The monitoring of biological diversity in rivers also shows a slow recolonization of species and increasing diversity over time. The results indicate a delay between the recovery of water chemistry and biota, which potentially may be caused by acidic episodes not detected in water chemistry monitoring, access of the fauna to sites from source populations, new equilibria with acid-tolerant species, effects of increased total organic carbon, and interactions with other environmental variables, such as climate.

A comparison between the acidification index in rivers and lakes suggests a stronger signal and greater changes in rivers, indicating that the fauna in rivers may be more sensitive to acidification or have better colonizing abilities compared to lakes. Also, the monitoring of lakes started later than the monitoring of rivers, which could contribute to the differences observed. Overall, the monitoring programs provide valuable information about the status of freshwater ecosystems in Norway, the trends in acidification, and the recovery of biological communities.

9.4 Sweden

The Swedish contribution presents the monitoring and assessment of acidification impacts on benthic macroinvertebrates in streams and lakes in Sweden. The section sums up data collected from 1996 to 2020 for streams and 1995 to 2019 for lakes, focusing on acid-sensitive water bodies.

Four of the 18 streams and 22 of the 78 lakes were acidified according to water chemistry. The trends in water chemistry showed a decreasing trend in sulphate concentrations, particularly in the Central Plains. The pH levels varied, with slight increases observed in mid-pH streams and lakes, while no significant changes were found in high-pH sites. Taxon richness and biological indices for acidification were compared among different pH classes and ecoregions. High- and mid-pH streams showed increasing trends in taxon richness and the number of EPT taxa, suggesting a biological recovery. The low-pH lakes in the Central Plains showed limited recovery and poorer status, while high-pH lakes in the Central Plains exhibited the highest taxon richness.

The study highlights the limited recovery of benthic macroinvertebrates from acidification, particularly in acid-sensitive, low-pH sites in the Central Plains. However, there were positive trends in taxon richness and EPT taxa in many high- and mid-pH acid-sensitive sites, potentially influenced by warming. The absence of similar increases in richness and EPT taxa at the low-pH sites suggests slower recovery

due to an impoverished species pool in the Central Plains, which was strongly affected by acidification. The study emphasizes the need to quantify the impact of climate change on biological responses to acidification.

9.5 The UK

The UK contribution presents three decades of data from the UWMN (Upland Waters Monitoring Network). UWMN was established in 1988 to assess the effectiveness of controls on acidic pollutants in improving the ecological health of acidified lakes and rivers in the UK. Biological monitoring included the sampling and analysis of epilithic diatoms and macroinvertebrates. At the beginning of the monitoring, most sites had ANC levels well below the target. However, over the 30-year monitoring period, most sites showed significant positive trends in ANC, with many reaching levels well above the target.

The analysis of epilithic diatoms revealed significant changes in species composition at most sites, except for the chemical control sites. The diatom communities and the acidification metric for diatoms indicates a reduction in acidity. The extent of diatom species change was strongly correlated with the magnitude of pH change in lake sites but showed greater inter-annual variation in stream sites. Some sites showed remarkable biological recovery, transitioning from chronically acidified states to diatom assemblages indicative of naturally acid soft waters. However, comparing the current diatom communities with pre-acidification communities preserved in sediment cores revealed that only a few sites closely resembled the pre-acidification flora. Acidification metrics for macroinvertebrates indicated strong rates of recovery for most sites, while others, despite chemical recovery, did not show evidence of macroinvertebrate recovery.

This suggests the potential for further biological improvement if chemical recovery continues. There is no systematic difference in the response of diatoms and macroinvertebrates to reductions in acidification, indicating that episodic chemistry and acid episodes have not hindered recovery. The apparent slowdown in biological recovery over the past decade aligns with the overall decline in acid deposition and water acidity.

9.6 Italian contribution on sharing of methods

This Italian contribution provides an important step towards to the sharing of biological data and tools. The authors are developing tools in the software R to calculate acidification indices to assess the effects of acidification on the benthic fauna. The goal is to provide a consistent methodology for applying existing indices and to lay the foundation for developing new ones. The R tool allows for the calculation of specific acidification and calculation of generic metrics related to diversity, richness, and functional aspects of the macroinvertebrate assemblage. The developed tool and associated files have been uploaded to GitHub, allowing researchers to access and utilize them. It is suggested that such tools be included in existing R packages for biomonitoring, promoting data sharing and comparability of results across different countries and research groups. Overall, this initiative aims to enhance the assessment and preservation of freshwater quality through biomonitoring efforts and the sharing of standardized tools and data.

Appendices for the regional assessment

Appendix 1: Study sites

Study sites with year of start and end of the biological- and chemical time series, and number of years with biological samples. * sites are impacted by liming, mining or weathering (leaching) processes. Sites included in the analyses of trends include: S highly acid-sensitive sites with a strong chemical recovery, implying a significant temporal change in both pH and ANC and W sites with a weak- to intermediate acid-sensitivity and chemical recovery, implying sites with either no change in pH and ANC, or with only significant temporal change in either pH or ANC. ¹ Includes 7 sampling stations ² Includes 12 sampling stations, ³ Include 14 sampling stations, ⁴ Include 10 sampling stations, ⁴ Include 10 sampling stations, ⁵ Include 10 sampling stations. ⁵ missing ANC data.

Country	Type	Name	Biol. data start	Biol. data end	Biol. sampling years	Chem. data start	Chem. Data end
Czech Rep	Lake	Grosser Arbersee	2007	2019	4		
Czech Rep	Lake	Kleiner Arbersee	2007	2019	4		
Czech Rep	Lake	Laka	2007	2019	4	1990	2019
Czech Rep	River	Lysina	2000	2017	9	1990	2019
Czech Rep	Lake	Plesne, jezero	2007	2019	4	1990	2019
Czech Rep	Lake	Prasilske, jezero	2007	2019	4	1990	2019
Czech Rep	Lake	Rachelsee	2007	2019	4	1990	2016
Czech Rep	Lake	Sern, jezero	2007	2019	4		
Czech Rep	Lake	Sertovo jezero	2007	2019	4		
Czech Rep	Lake	Uhlirska	2017	2017	1	1990	2019
UK	Lake	Blue Lough	1994	2016	18	1990	2019
UK	Lake	Burnmoor Tarn	1988	2016	27	1990	2019
UK	Lake S	Llyn Cwm Mynach	1988	2017	24	1990	2019
UK	Lake S	Llyn Llagi	1988	2017	29	1990	2019
UK	Lake S	Loch Chon	1988	2019	31	1990	2019
UK	Lake	Loch Coire Fionnaraich	2002	2018	17	2001	2019
UK	Lake	Loch Coire nan Arr	1988	2007	20	1990	2019
UK	Lake S	Loch Grannoch	1988	2019	29	1990	2019
UK	Lake W	Loch Tinker	1988	2018	28	1990	2019
UK	Lake S	Lochnagar	1988	2018	31	1990	2019
UK	Lake S	Round Loch of Glenhead	1991	2019	22	1990	2019
UK	Lake S	Scoat Tarn	1988	2016	27	1990	2019
UK	River S	Afon Gwy	1991	2017	27	1991	2019
UK	River S	Afon Hafren	1988	2017	29	1990	2019
UK	River W	Allt a Mharcaidh	1988	2018	31	1990	2019
UK	River W	Allt na Coire nan Con	1988	2018	30	1990	2019
UK	River	Beaghs Burn ⁵	1988	2016	25	1990	2019
UK	River S	Bencrom River	1988	2016	28	1990	2019
UK	River W	Coneyglen Burn	1989	2016	24	1990	2019
UK	River S	Dargall Lane	1988	2019	31	1990	2019
UK	River S	Narrator Brook	1988	2016	25	1991	2019
UK	River S	Old Lodge (Ashd. Sands)	1988	2017	30	1990	2019
UK	River S	River Etherow	1988	2016	29	1990	2019
Italy	River	Paione Inferiore	1991	2019	11	1990	2019
Italy	Lake	Paione Superiore	1999	2019	3	1990	2019
Norway	Lake S	Atnsjøen	1998	2017	17	1998	2021

Norway	Lake S	Bjorvatn	1997	2016	18	1998	2021
Norway	Lake S	Breidtjern	1997	2017	17	1990	2022
Norway	Lake S	Dalvatn	2000	2017	10	1996	2021
Norway	Lake S	Heddersvatnet	1997	2017	19	1990	2022
Norway	Lake W	Kapervatnet	1999	2017	13	1990	2022
Norway	Lake S	Langtjern	1998	2017	18	1972	2021
Norway	Lake	Lille Hovvatnet	2004	2016	11	1995	2021
Norway	Lake S	Ljosvatnet	1998	2018	19	1990	2021
Norway	Lake S	Lomstjørni	1998	2017	16	1995	2021
Norway	Lake S	Markhusdalsvatnet	1998	2017	18	1998	2021
Norway	Lake S	Nystølsvatnet	1998	2017	15	1990	2022
Norway	Lake W	Rondvatnet	1999	2017	14	1997	2021
Norway	Lake S	Røyrvatnet	1998	2017	17	1990	2021
Norway	Lake S	Saudlandsvatnet	1997	2016	18	1995	2021
Norway	Lake S	Sognevatn	1998	2016	16	1990	2021
Norway	Lake S	Stortjørna	1998	2017	12	1998	2021
Norway	Lake S	Svartdalsvatnet	2000	2017	7	1990	2022
Norway	Lake S	Svartetjern	1997	2017	19	1994	2021
Norway	Lake	Øvre Jerpetjern	1997	2010	14	1987	2011
Norway	River	Farsund ¹	2003	2020	18		
Norway	River S	Gaular ²	1989	2020	26		
Norway	River S	Nausta ³	1989	2019	22	1989	2009
Norway	River	Ogna ⁴	2000	2020	11		
Norway	River S	Vikedal ⁵	1987	2020	34	1972	1993
Sweden	Lake S	Brunnsjön	1986	2020	34	1990	2021
Sweden	Lake S	Fiolen	1988	2021	34	1990	2021
Sweden	Lake W	Fräcksjön	1986	2020	34	1990	2021
Sweden	Lake S	Härsvatten	1988	2021	34	1990	2021
Sweden	Lake W	Stensjön	1986	2021	35	1990	2021
Sweden	Lake S	Storasjö	1986	2021	35	1990	2021
Sweden	Lake S	Tvåringen	1988	2021	34	1990	2021
Switzerland	River S	Laghetto Superiore outlet	1991	2021	18	1991	2021
Switzerland	River S	Laghetto Inferiore outlet	1991	2018	18	1991	2021
Switzerland	River	Lago di Tomè outlet	2000	2018	18	2000	2021
Switzerland	Lake S	Lago del Starlaresc da Sgiof	2000	2021	17	2000	2021
Italy	River S	Paione Inferiore outlet	1991	2017	11	1990	2019
Italy	River	Paione Inferiore inlet	1993	2020	3	1990	2019
Italy	River S	Paione Superiore outlet	1994	2020	13	1990	2019
Germany	River	Dürreychbach	1987	2009	18	1990	2019
Germany	River	Elberndorfer Bach*	1988	2019	24	1990	2016
Germany	River	Ettelsbach *	1992	2005	14	1992	2005
Germany	River	Eger*	1989	2019	20	1990	2019
Germany	River	Goldersbach	1986	2009	19	1990	2019
Germany	River S	Grosse Ohe	1983	2020	27	1990	2019
Germany	River	Grosse Pyra *	1992	2020	26	1992	2019
Germany	River	Gräfenbach *	1982	2015	11	1990	2019
Germany	River	Heidelbach *	1993	2006	13	1992	2006
Germany	River	Hinterer Schachtenbach	1983	2011	23	1990	2012
Germany	River	Kleine Kinzig	1985	2009	20	1990	2019
Germany	River	Lange Bramke	1986	2009	15	1990	2019
Germany	River	Nieste	1987	2004	11	1990	2004

Germany	River W	Oberpfälzer Wald. Waldnaab 8#	1984	2019	26	1990	2019
Germany	River W	Oberpfälzer Wald. Waldnaab 2#	1985	2019	27	1990	2019
Germany	River	Rombach	1987	2004	11	1990	2004
Germany	River	Rote Pockau *	1992	2020	23	1992	2018
Germany	River S	Röslau	1989	2019	21	1990	2019
Germany	River	Schmerbach	2000	2004	5	1990	1998
Germany	River	Seebach*	1983	2011	20	1990	2012
Germany	River	Taubenbach *	1992	2006	15	1993	2006
Germany	River	Traunbach *	1983	2015	14	1990	2019
Germany	River	Vorderer Schachtenbach	1983	2011	22	1990	2012
Germany	River	Wilde Weisseritz *	1992	2020	23	1990	2018
Germany	River	Wolfsbach *	1992	2012	17	1992	2018
Germany	River	Zinnbach	1989	2012	21	1990	2019
Germany	River	Zinse*	1988	2019	24	1990	2019

Appendix 2: Functional traits

Functional traits of the species in the data sets. Functional feeding traits include grazers (GR), shredders (SH), Gatherers/Collectors (GA), filterers (FI), predators (PR), and other modes of feeding (OT). Functional movement traits include swimming (SW), burrowing/ boring (BU), sprawling/ walking (SP), sessile/semi-sessile (SM), and other modes of locomotion (OT).

Functions	Feeding mode						Movement mode				
Species/ taxon	GR	SH	GA	FI	PR	OT	SW	BU	SP	SM	OT
<i>Adicella filicornis</i>	2	8							7	3	
<i>Adicella reducta</i>	2	8							7	3	
<i>Agapetus fuscipes</i>	8		2						3	7	
<i>Agapetus nimbulus</i>	8		2						3	7	
<i>Agapetus ochripes</i>	8		2						3	7	
<i>Agrypneta crassicornis</i>	1	3			1				5		
<i>Agrypnia obsoleta</i>		3	1		6				10		
<i>Agrypnia pagetana</i>		3	1		6				10		
<i>Agrypnia picta</i>		3	1		6				10		
<i>Agrypnia varia</i>		3	1		6				10		
<i>Allogamus uncatus</i>	2	6			2				7	3	
<i>Alainites muticus</i>											
<i>Allogamus antennatus</i>	1	3			1				5		
<i>Allogamus auricollis</i>	3	2		3	2				7	3	
<i>Allogamus uncatus</i>	2	6			2				7	3	
<i>Ameletus inopinatus</i>	5		5				5		5		
<i>Amphinemura borealis</i>	5	2	3						7		3
<i>Amphinemura standfussi</i>	5	2	3						7		3
<i>Amphinemura sulcicollis</i>	3	3	4						7		3
<i>Anabolia nervosa</i>	2	5	1		2				7	3	
<i>Arcynopteryx compacta</i>	2				8			2	8		
<i>Arthroplea congener</i>				10					10		
<i>Athripsodes aterrimus</i>		5	3		2				7	3	
<i>Athripsodes bilineatus</i>		5	3		2				7	3	
<i>Athripsodes cinereus</i>		3	3		4				7	3	
<i>Baetis alpinus</i>	5		5				3		4		
<i>Baetis buceratus</i>	5		5				8		2		
<i>Baetis digitatus</i>	5		5				3		4		
<i>Baetis fuscatus</i>	5		5				6		4		
<i>Baetis lutheri</i>	5		5				3		4		
<i>Baetis melanonyx</i>	3		1				3		4		
<i>Baetis muticus</i>	5		5					5	5		
<i>Baetis niger</i>	5		5				6		4		
<i>Baetis pentaplebodes</i>	1		3				3		4		
<i>Baetis rhodani</i>	5		5				6		4		
<i>Baetis scambus</i>	5		5				6		4		
<i>Baetis subalpinus</i>	5		5				3		4		
<i>Baetis vernus</i>	5		5				8		2		

Beraea maurus	3	7					10	
Brachycentrus montanus	2			5	3		5	5
Brachycentrus subnubilus	2			5	3		5	5
Brachyptera risi	7		3				3	7
Brachyptera seticornis	7		3				3	7
Brachyptera starmachi	7		3				3	7
Caenis horaria				10				10
Caenis luctuosa				10				10
Capnia atra	2	6	2				1	6
Capnia nigra	1	4	5				1	6
Capnia pygmaea		5	5				1	6
Capnia vidua	2	6	2				1	6
Centroptilum luteolum	7		3			6		4
Ceraclea albimacula	1				9		7	3
Ceraclea annulicornis	1	3	1		1	1	7	3
Ceraclea nigronervosa						10	7	3
Chaetopteryx major	2	6			2		7	3
Chaetopteryx villosa	3	4	2		1		7	3
Chloroperla susemicheli	1	1	2		6		5	5
Chloroperla tripunctata	1	1	2		6		5	5
Cloeon dipterum	5		5			8		2
Cloeon inscriptum	5		5					
Cloeon praetextum	5		5					
Cloeon simile	5		5			6		4
Crunoecia irrorata	5	3			2		7	3
Crunoecia kempnyi		3			2	5	7	3
Cyrnus flavidus				1	9		4	6
Cyrnus insolutus				1	9		4	6
Cyrnus trimaculatus				1	9		4	6
Dictyogenus fontium	1	1	1		7		2	8
Dinocras cephalotes	1				9		2	8
Diplectrona felix				3	1		5	5
Diura bicaudata					10		2	8
Diura nanseni					10		5	
Drusus annulatus	8	1	1				7	3
Drusus biguttatus	8	1	1				7	3
Drusus discolor	1			4	5		7	3
Drusus improvisus	8	1	1				5	
Drusus monticola	8	1	1				7	3
Drusus muelleri	2			4	4		5	
Drusus trifidus	8	1	1				7	3
Ecclisopteryx dalecarlica	8	1	1				7	3
Ecclisopteryx guttulata	8	1	1				7	3
Ecclisopteryx madida	8	1	1				7	3
Ecdyonurus helveticus	5		5			2	8	
Ecdyonurus picteti	5		5			1	5	
Ecdyonurus submontanus	5		5			1	5	

Ecdyonurus torrentis	5	5			1	5	
Ecdyonurus venosus	5	5			2	8	
Ecnomus tenellus			1	9		5	5
Electrogena lateralis	5	5				10	
Electrogena ujhelyii	5	5				10	
Epeorus assimilis	10				1	8	1
Ephemera danica		3	7		1	9	
Ephemera vulgata		2	8		1	9	
Ephemerella aurivillii	4	1	5		1	5	
Ephemerella ignita	2	2	1	1	1	5	
Ephemerella major	5		5			10	
Ephemerella mucronata	5		5			10	
Erotesis baltica		10				7	3
Glossosoma boltoni	8		2			3	7
Glossosoma conformis	8		2			3	7
Glossosoma intermedium	8		2			3	7
Glyptotaelius pellucidus	1	6		3		7	3
Goera pilosa	9		1			3	7
Grammotaulius submaculatus		10				7	3
Habroleptoides confusa			10			5	5
Habrophlebia fusca	2		8		2	8	
Habrophlebia lauta	2		8		2	8	
Halesus digitatus		3				5	
Halesus radiatus	1	7		2		7	3
Halesus rubricollis	1	7		2		7	3
Halesus tessellatus		3				5	
Heptagenia dalecarlica	3	2	1		1	5	
Heptagenia sulphurea	5		5		4	6	
Holocentropus dubius			1	9		4	6
Holocentropus insignis			1	3		4	6
Holocentropus picicornis			1	9		4	6
Hydropsyche angustipennis	2		5	3		5	5
Hydropsyche dinarica	2		5	3		5	5
Hydropsyche exocellata	2		5	3		5	5
Hydropsyche fulvipes	2		5	3		5	5
Hydropsyche instabilis	2		5	3		5	5
Hydropsyche pellucidula	2		5	3		5	5
Hydropsyche saxonica	2		5	3		5	5
Hydropsyche silfvenii	2		5	3		5	5
Hydropsyche siltalai	2		5	3		5	5
Hydropsyche tenuis	2		5	3		5	5
Isogenus nubecula				10		2	8
Isoperla carbonaria				10		5	
Isoperla difformis	1	1	1	7		2	8
Isoperla grammatica	1	1	1	7		2	8
Isoperla obscura	1	1	1	7		2	8
Isoperla oxylepis	1	1	1	7		5	

Isoperla rivulorum	1	1	1	7	2	8	
Isoperla silesica	1	1	1	7	2	8	
Isoperla sudetica	1	1	1	7	2	8	
Ithytrichia lamellaris	10					7	3
Kageronia fuscogrisea	5		5		3	7	
Lepidostoma hirtum	8	2				7	3
Leptophlebia marginata			10		3	7	
Leptophlebia vespertina			10		3	7	
Leuctra albida	3	3	4			5	5
Leuctra alpina	3	3	4			5	5
Leuctra autumnalis	3	3	4			5	5
Leuctra braueri	3	3	4			5	5
Leuctra digitata	3	3	4			5	5
Leuctra fusca	3	3	4			5	5
Leuctra geniculata	2	2	5	1		10	
Leuctra hexacantha	3	3	4			5	5
Leuctra hippopus	3	3	4			5	5
Leuctra inermis	3	3	4			5	5
Leuctra leptogaster	3	3	4			5	5
Leuctra major	3	3	4			5	5
Leuctra moselyi	3	3	4			5	5
Leuctra nigra	3	3	4			7	3
Leuctra prima	3	3	4			5	5
Leuctra pseudocingulata	3	3	4			5	5
Leuctra pseudosignifera	3	3	4			5	5
Leuctra rauscheri	3	3	4			5	5
Leuctra rosinae	3	3	4			5	5
Leuctra teriolensis	3	3	4			5	5
Limnephilus bipunctatus	2	5		3		7	3
Limnephilus borealis	2	5		3		7	3
Limnephilus centralis	2	5		3		7	3
Limnephilus coenosus	2	5		3		7	3
Limnephilus decipiens	2	5		3		7	3
Limnephilus extricatus	2	5		3		7	3
Limnephilus fuscicornis	2	5		3		7	3
Limnephilus lunatus	2	5		3		7	3
Limnephilus marmoratus	2	5		3		7	3
Limnephilus rhombicus	2	5		3		7	3
Limnephilus sparsus	2	5		3		7	3
Limnephilus vittatus	2	5		3		7	3
Lype phaeopa	10					3	7
Lype reducta	10					3	7
Melampophylax melampus	2	6		2		7	3
Mesophylax impunctatus	2	6		2		7	3
Metalyte fragilis	8		1	1		3	7
Micrasema gelidum	5	5				3	1
Micrasema longulum	5	5				8	2

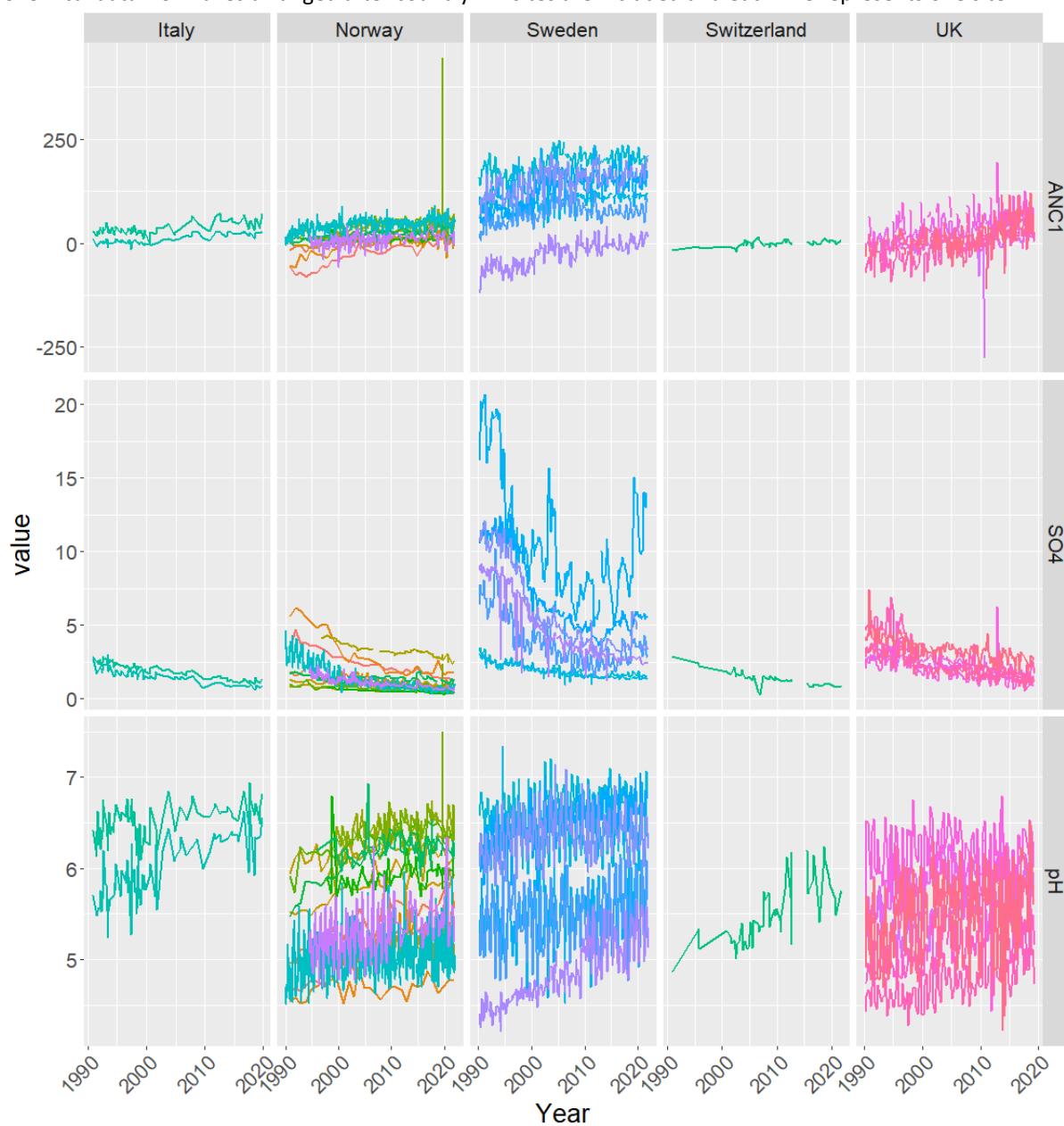
Micrasema minimum	5	5				8	2	
Micropterna lateralis	2	6		2		7	3	
Micropterna nycterobia	2	6		2		7	3	
Micropterna sequax	2	6		2		7	3	
Molanna albicans	1		1	0	3	7	3	
Molanna angustata			3		7	7	3	
Molanna nigra	1		1		3	7	3	
Molannodes tinctus			3		7	7	3	
Mystacides azurea	2	2	5		1	7	3	
Mystacides longicornis	2	2	5		1	7	3	
Mystacides nigra	2	2	5		1	7	3	
Nemotaulius punctatolineatus		3				7	3	
Nemoura avicularis		7	3			5		5
Nemoura cambrica		7	3			5		5
Nemoura cinerea		7	3			5		5
Nemoura dubitans		7	3			5		5
Nemoura erratica		7	3			5		5
Nemoura flexuosa		7	3			5		5
Nemoura marginata		7	3			5		5
Nemoura minima		7	3			5		5
Nemoura mortoni	1	2	7			5		5
Nemoura obtusa		7	3			5		5
Nemoura sciurus		7	3			5		5
Nemoura viki		7	3			5		5
Nemurella pictetii	2	4	4			5		5
Neureclipsis bimaculata				1	9	4	6	
Notidobia ciliaris		10				8	2	
Odontocerum albicorne	3	3			4	7	3	
Oecetis lacustris					10	7	3	
Oecetis ochracea					10	7	3	
Oecetis testacea					10	7	3	
Oligoplectrum maculatum	2		1	4	3	5	5	
Paraleptophlebia cincta			10			1	4	
Paraleptophlebia submarginata			10			3	7	
Paraleptophlebia submarginata			10			3	7	
Perla marginata	1				9	2	8	
Perlodes dispar	2				8	2	8	
Perlodes microcephalus	2				8	2	8	
Philopotamus montanus				10		4	6	
Philopotamus variegatus				10		4	6	
Phryganea bipunctata	1	2	1		6	5		
Phryganea grandis	1	2	1		6	10		
Plectrocnemia conspersa				1	9	4	6	
Plectrocnemia geniculata				1	9	4	6	
Polycentropus flavomaculatus	2			3		2	3	
Polycentropus irroratus				1	9	4	6	
Potamophylax cingulatus	2	6			2	7	3	

Potamophylax latipennis	2	6		2		7	3
Potamophylax luctuosus							
Potamophylax nigricornis	2	6		2		7	3
Potamophylax rotundipennis	2	6		2		7	3
Procloeon bifidum			10		6	4	
Procloeon pennulatum	5		5		6	4	
Protonemura auberti	3	5	2			5	5
Protonemura austriaca	3	5	2			5	5
Protonemura caprai	3	5	2			5	5
Protonemura hrabei	3	5	2			5	5
Protonemura intricata	3	5	2			5	5
Protonemura lateralis	3	5	2			5	5
Protonemura meyeri	3	5	2			5	5
Protonemura montana	3	5	2			5	5
Protonemura nimborum	3	5	2			5	5
Protonemura nitida	3	5	2			5	5
Protonemura praecox	3	5	2			5	5
Psychomyia fragilis	8		1	1		3	7
Psychomyia pusilla	6		2	1	1	3	7
Rhadicoleptus alpestris	1	6		3		7	3
Rhithrogena germanica	10					7	3
Rhithrogena hercynia	10				1	6	
Rhithrogena picteti	10				1	6	
Rhithrogena semicolorata	10					10	
Rhyacophila aurata				10		8	2
Rhyacophila dorsalis				10		8	2
Rhyacophila evoluta				10		8	2
Rhyacophila fasciata				10		8	2
Rhyacophila glareosa				10		8	2
Rhyacophila hirticornis				10		8	2
Rhyacophila italica				10		8	2
Rhyacophila munda				10	2	3	1
Rhyacophila nubila				10		8	2
Rhyacophila oblitterata				10		8	2
Rhyacophila occidentalis	1	1		8		8	2
Rhyacophila polonica				10		8	2
Sericostoma personatum		9		1		8	2
Sericostoma schneideri	1	3				1	5
Serratella ignita	2	2	1	1	1	5	
Setodes argentipunctellus	1	3				7	3
Setodes punctatus	1	3				7	3
Sialis fuliginosa				10	4	6	
Sialis lutaria				10	4	2	4
Sialis sordida				10	4	1	5
Silo nigricornis	9		1			3	7
Silo pallipes	9		1			3	7
Siphonurus alternatus			10		8	2	

Siphonurus lacustris			10		9	1	
Siphonoperla burmeisteri	1	1	2	6		1	5
Siphonoperla torrentium	1	1	2	6		1	5
Stenophylax mucronatus		3				7	3
Stenophylax permistus		5	5			7	3
Stenophylax vibex		3				7	3
Taeniopteryx hubaulti	3	2	5			5	5
Taeniopteryx nebulosa	3	2	5			5	5
Tinodes dives	8		1	1		3	7
Tinodes pallidulus	8		1	1		3	7
Tinodes rostocki	8		1	1		3	7
Tinodes waeneri	7		1	1	1	3	7
Trienodes bicolor	5	5				7	3
Wormaldia occipitalis				10		4	6
Wormaldia subnigra				10		4	6

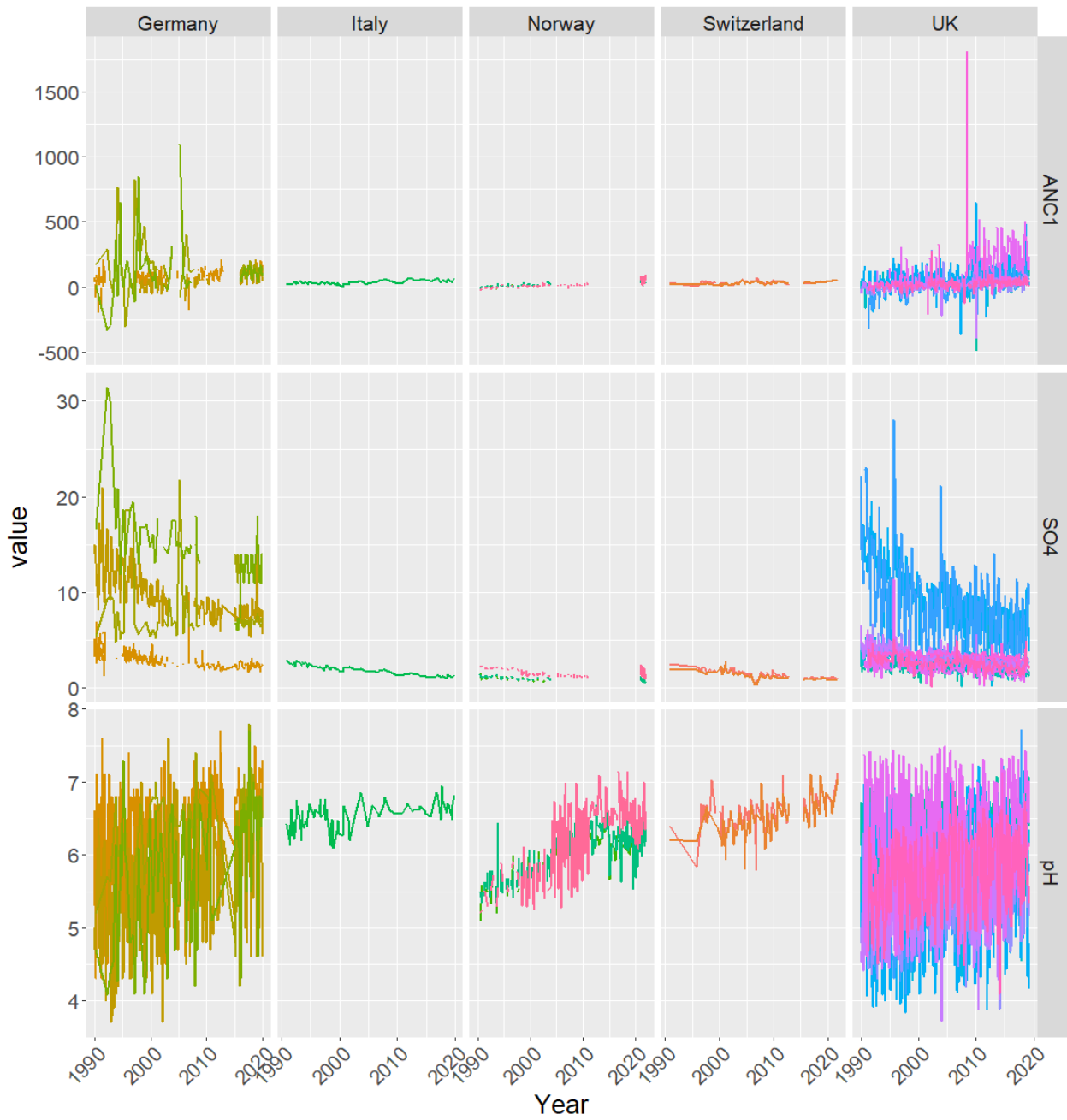
Appendix 3: Chemical data from lakes

Chemical data from lakes arranged after country. All sites are included and each line represents one site.



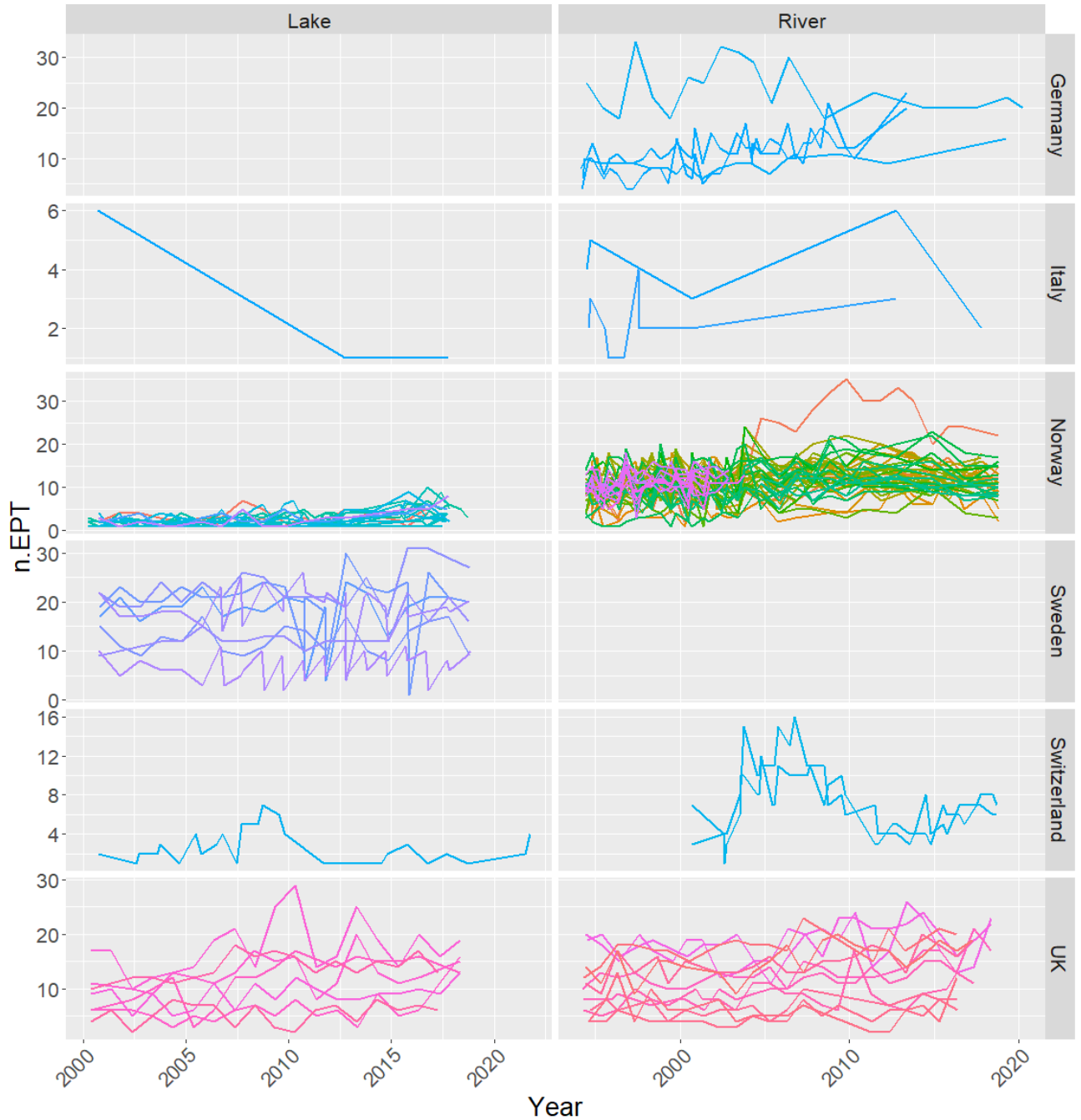
Appendix 4: Chemical data from rivers

Chemical data from long-term monitoring rivers divided into country. Each line represents one site.



Appendix 5: Species diversity of lakes and rivers

Temporal variation in the number of species (n.) of Ephemeroptera, Plecoptera, and Trichoptera (EPT) from long-term monitoring rivers and lakes divided into country. Each line represents one site.



Appendix: Thematic reports from the ICP Waters programme

Since its establishment in 1985, the ICP Waters programme has prepared numerous assessments, reports and publications that address the effects of long-range transported air pollution, including thematic reports, chemical intercalibrations, biological intercalibrations, proceedings of Task Force meetings, and peer-reviewed articles. Reports and publications are available at the ICP Waters website; <http://www.icp-waters.no/>

Thematic reports from the ICP Waters programme from 2000 up to present are listed below.

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- De Wit, H. A., Garmo Ø. A. and Fjellheim A. 2015. Chemical and biological recovery in acid-sensitive waters: trends and prognosis. **ICP Waters Report 119/2014.**

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