Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Norwegian Institute for Water Research

Dep. of aquatic Sciences and Assessment

Acidified or not? A comparison of Nordic systems for classification of physicochemical acidification status and suggestions towards a harmonised system

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SLU, Vatten och miljö: Rapport 2021:1

Cover photo: Jens Fölster (Grövelsjön on the border between Norway and Sweden)
Printed: Digital only
Printed year: 2021

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Org.nr 202100-2817	

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Preface

This report is a result of several projects financed by the Swedish Agency of Marine and Water Management and the Norwegian Environment Agency. The authors are currently active at SLU (Jens Fölster, Peter Carlson, Richard Johnson, Simon Hallstan, Kerstin Holmgren), NIVA (Øyvind A. Garmo, Kari Austnes), NORCE (Gaute Velle), NINA (Ann Kristin Schartau), IVL (Filip Moldan) and YMPARISTO (Jukka Aroviita). In addition to the authors, Randi Saksgård (NINA) Jukka Ruuhijärvi (LUKE) as well as several co-workers at these institutes were helpful in compiling data and taking part in workshops. . Øyvind A. Garmo was main author of Chapter 3, comparing national classification systems, Peter Carlson was main author of Chapter 4, statistical analysis of the data, and Jens Fölster was main author of Chapter 5, developing suggestions to a new harmonised classification system.

Summary

Acidification of lakes and streams from long-range transboundary air pollution is one of the most severe and spatially extensive environmental problems in northern Europe and North America. The Nordic countries, with acid sensitive soils and located downwind of the industrial areas of western and central Europe were particularly affected, with local extinctions of fish populations and other harmful effects on the aquatic ecosystems. Although the deposition of acidic pollutants today is tenfold lower than during peak years in the 1980s, acidification is still a major problem due to legacy acidification of the soils in the catchments of lakes and streams.

The Nordic countries have developed different criteria to classify acidification from chemical parameters and to distinguish anthropogenically acidified waters from naturally acidic waters. In brief, the different systems reflect dissimilarities in geology and climate and different forms of management. This has resulted in acidification assessments that are not directly comparable. In international reporting, for example to the UN-ECE Air convention and the EU Water Framework Directive, discrepancies among the Nordic countries reflect more the different classification systems used rather than environmental conditions. To address this issue, the Swedish Agency of Marine and Water Management and the Norwegian Environment Agency initiated a project to assess the possibility of harmonising classifications of acidification across the Nordic countries, as well as to lay a foundation for improved and harmonised systems and reporting. The project focused on analyses of a joint database, comprising data on water chemistry and biology, which was compiled by representatives from Norway, Sweden and Finland.

Comparisons of the national classification systems showed marked differences. The Finnish system focuses only on rivers, with primary attention given to acidification caused by the draining of sulphide soils. Both the Norwegian and the Swedish systems focus more on anthropogenic-induced acidification by deposition and both are based on reference values calculated using the MAGIC model. However, while the Norwegian system, like the Finnish, is based on water body types and type-specific class boundaries, the Swedish system is object specific. Furthermore, the Swedish system is based on changes in the whole macroinvertebrate community (i.e. including species with varying degrees of sensitivity/tolerance to acidification), while the Norwegian system is based on empirically derived critical levels of a single species (brown trout). A comparison of the different systems showed that classification using the Swedish system was much stricter: 74 of 373 water bodies (20 %) were considered acidified (moderate status or worse) according to the Swedish system, compared to 34 of 205 streams (17 %) using Finnish system and only 10 of 470 waters (2 %) using the Norwegian system.

The Nordic dataset with chemistry and biology included 165 lakes with data on littoral invertebrates, 114 lakes with data on fish, 99 streams with data on invertebrates and 80 streams with data on fish. The first objective of our study was to determine and quantify acidification indicator(s) that are robust predictors of biological change. Gradient forest and generalised additive modelling showed that the acid neutralising capacity (ANC), calculated as the difference between base cations (calcium, magnesium, sodium and potassium) and strong acid anions (sulphate, chloride and nitrate), was the strongest predictor. Our analyses also revealed that pH was a relatively poor predictor, a finding that contrasted with earlier studies on national datasets. This discrepancy might be explained by our use of a larger dataset, covering broader environmental gradients in ion concentrations and natural organic acids, compared to the earlier studies. The advantage of using ANC was further supported by analysis of interactions between environmental variables, e.g., responses between pH and biology were confounded by interactions with other environmental parameters, to a much higher degree than ANC.

For lake invertebrates and fish gradient forest revealed pronounced upper thresholds at around 150 µeq/l ANC with one or two peaks between 90 and 140 µeq/l ANC. The upper threshold in the most important community changes for both stream invertebrates and fish occurred at around 200 µeq/l. The higher threshold in rivers is likely due to the higher temporal variability of acidic conditions in streams, with the biotic responses reflecting the most acidic conditions. In our analysis we used mean values since the sampling frequency was highly variable and therefore it was unlikely that acidic episodes were captured in the chemical sampling of most streams. Mean values can then be interpreted as the risk of ANC levels below the critical levels during extreme events.

Here we propose an approach for Nordic classifications and exemplify this approach using the Swedish acidification index for macroinvertebrates in lakes (the MILA index). Similarly, this approach could also be applied to other indices, to streams and for fish. If decided that the approach should be developed further, we suggest that new indices are developed for ANC for both lakes and rivers using the Nordic dataset. A common Nordic classification for macroinvertebrates in lakes and rivers could then underpin classifications using ANC.

For sites with circumneutral and alkaline reference conditions, the class boundaries for ANC can be set in relation to the biological classification. For naturally acidic sites, we recommend an approach where the class boundaries are expressed as an EQR instead of a fixed ANC value. The EQR-derived class boundaries should be based on a biological classification system but should be adapted to reflect sensitivities across different ANC-ranges. For example, for lakes a smaller change in ANC is accepted for good status in the range of 90-150 µeq/l ANC where most of the change in species composition for both invertebrates and fish occur.

The MAGIC model, currently used for estimating reference values in both Norway and Sweden, cannot be applied to all water bodies requiring status classification. Results from our study showed that a simple regression model for reference ANC, as a function of BC, SO_4 and Cl, could be calibrated using data from MAGIC-modelled lakes and rivers distributed across all of Sweden. Hence, following validation, it is expected this simple regression model could be used for Norway and Finland as well.

Our approach can potentially be developed into a harmonised Nordic classification system for acidification. However, the benefits of a revised system have to be weighed against other aspects that are important for society and decision makers. For example, should thresholds be based on the environmental requirements of single, relatively sensitive, species deemed important by society, or as a gradual change in species composition from a reference condition (sensu EU Water Framework Directive) as suggested in this report? Should ANC be used as single indicator for acidification as suggested here, or is pH preferred since it is well-known and widely used, or should inorganic aluminium be used since it is more directly related to toxicity? Should an object-specific system be chosen since it results in lower classification errors, or is a type-specific system preferred due to its simplicity? Even if the different countries decide differently to these and other questions, we hope that this report provides a good foundation for continued dialog in order to ultimately achieve a more harmonised classification of acidification between countries and between chemical and biological quality elements.

1 Introduction

Acidification from long range transboundary pollution is one of the most severe and spatially extensive environmental problems in Northern Europe and Northern America (Grennfelt et al, 2020). Already in the early stages of industrialisation, sulphuric acid from combustion of fossil fuel caused harmful effects on human health and environment close to the factories. These local problems were solved by building high smokestacks ultimately turning what was originally a local environmental problem into transboundary and international issues. The Nordic countries were particularly affected due to low buffering soils and their location downwind to the most intensive industrial parts of Europe. Norway has experienced the strongest impacts, due to high levels of deposition and the prevalence of thin and poorly buffered soils. In Finland, humic acids and oxidation of sulphidic soils are now considered as more important determinants of aquatic acidity than acidic deposition. Sweden is somewhat in-between, i.e. impacted both by anthropogenic and natural acids. All three countries show large geographical gradients in acidic deposition related to average precipitation and proximity to pollution sources.

Although sensitive ecosystems were probably affected much earlier, the link between long range transboundary pollution and acidification of freshwater ecosystems was first suggested in 1959 (Dannevig, 1959), and it was not until 1967 that acidification was acknowledged as a large-scale problem by the general public (Odén, 1967). The issue was first brought up by the OECD and UNECE which subsequently resulted in the formation of the Air Convention in 1979 (UNECE Convention on Long-range Transboundary Air Pollution). International acceptance and collaboration have resulted in a reduction of acid deposition to levels comparable to the beginning of the last century. However, despite decreased emissions and acidic deposition, freshwater ecosystems are still showing the effects of acidification due to legacy acidification of soils in their catchments and due to deposition at some sites remaining at non-sustainable levels.

Within water management, different criteria for acidification have been developed. In Sweden, implementation of an extensive liming programme in the 1980s to mitigate acidification was based on classification by pH and titrated alkalinity (Naturvårdsverket 1990). Work by the Air Convention of finding credible targets for emission reductions resulted in the concept of Critical Load (Henriksen and Posch, 2001). Accordingly, the maximum level of deposition that an ecosystem can tolerate without experiencing long-term damage, reflects among-region differences in vulnerability. The chemical threshold used in the calculation of critical loads is referred to as the critical limit. Acid neutralising capacity (ANC), based on ion balances, is commonly used and well suited for estimating critical loads for surface waters. ANC has also been shown to be a good predictor of trout populations in lakes (Lien et al. 1996). In 2000, the Water Framework Directive (WFD) was adopted by the EU Member States and in 2006 also by Norway. The directive mandates the classification of ecological status of all waterbodies. As part of the implementation of the WFD, classification systems for biological quality elements were intercalibrated by the member states. However, since the WFD has a strong focus on the biological quality elements, physicochemical quality elements were not included in the intercalibration exercises. This has resulted in strongly diverging classification systems for acidification as well as for nutrients (Kelly et al., 2019). Norway and Finland developed a system for classification of acidification based on grouping water bodies into types and different class boundaries were established for chemical indicators for each type. Sweden, on the other hand, developed a system based on estimated change in pH for individual waterbodies. Sweden also chose this approach for critical limit in the calculation of critical load. This led to a much higher exceedance of critical load in Sweden compared to

Norway, which was attributed mainly to the different approaches used in estimating the chemical criterion for acidification (Moldan et al., 2015).

These method related differences in defining acidification threatens the credibility of environmental management and of reporting to international agencies. To address this issue, the Swedish agency of Marine and Water Management and the Norwegian Environment Agency initiated a project to investigate the possibility of harmonising classifications of acidification across the Nordic countries, as well as to lay a foundation for improved and harmonised systems and reporting. The project focused on analyses of a joint database, comprising data on water chemistry and biology, which was compiled by representatives from Norway, Sweden and Finland in 2017.

1.1 Aim

The aim of this project was to evaluate and compare the different classification systems for acidification in Norway, Sweden and Finland. Further we compiled a joint Nordic dataset with biology and enough chemistry parameters in lakes and rivers to evaluate the relation between biological parameters and relevant chemical acidity indicators. The questions to be answered were:

- What results will the different classification systems give when applied to the same dataset representing a wide range of ionic strength and organic content and what explains the differences?
- What chemical indicators for acidity gives the best correlation to biological quality elements?
- Are there any pronounced thresholds in the relation between biota and the chemical acidity indicators?

Finally, we aimed to give a suggestion on how a common classification system for acidification could be designed based on the findings from the project.

1.2 Background

1.2.1 Chemical indicators for acidity

Several parameters indicating the acidity status of the water have been used here and elsewhere (Box 1). The negative logarithm of the hydronium ion activity (pH) is frequently used in assessing acidity and acidification. It is well known by most citizens and commonly used in most classification systems. Low pH negatively affects the osmoregulation of many aquatic organisms (e.g. Fromm, 1980). However, when acidification was recognised as an important driver of biodiversity loss, it soon became clear that inorganic labile aluminium (Ali) deleteriously affects acid-sensitive organisms, especially fish (Gensemer & Playle, 1999). Methods for analysing the toxic inorganic fractions of aluminium in water were established, and concentration thresholds for toxic effects on biota were established (Driscoll et al. 2001). The buffering capacity for acidity, alkalinity, is measured by titration with an acid down to a defined pH value. Alkalinity mainly depends on the concentration of hydrogen carbonate and carbonate in the water, originating from the weathering of minerals in the catchment soils, but is affected by the humic acid concentration. Methods for measuring alkalinity differ due to the use of different pH endpoints and results can also differ due to other procedures. Since the Nordic countries use different methods, this parameter could not be used in this study. An alternative measure of the buffering capacity is the Acid Neutralising Capacity (ANC); calculated as the difference between base cations (BC) and strong acid anions (SAA). These ions are chemically well defined and the consistency between different analytical methods is higher compared to titrated alkalinity. Since BCs tend to originate from the same weathering processes as carbonates, ANC reflects the balance between alkalinity production from soil processes and acid deposition. One drawback of ANC is that for acidified waters, ANC often is calculated as a small difference between relatively large BC and SAA giving a high calculation error. ANC could then alternatively be calculated from titrated alkalinity and DOC (Hemond, 1990). ANC was introduced in the calculations of Critical Load, since it is well suited to the models based on ion balances (e.g. Henriksen et al., 1995) and because there was a well-defined threshold for fish (Lien et al., 1996).

Use of ANC has, however, been questioned because it neglects the importance of organic acids for both acidity and sensitivity of aquatic organisms to acidification. For example, a brown water system could have a relatively low pH and yet ANC could be moderately high. It has been argued that this is not an issue as organic matter complex binds aluminium ions, the most toxic ions associated with acidification. However, this might have been an oversimplification since the concentration of total aluminium is strongly correlated to organic matter (Köhler et al, 2014). In Norway, the influence of organic acids was addressed by assuming that one-third of the organic acids could be regarded as strong acids anions, and included in the calculation of ANC (Lydersen 2004). This modified ANC was denoted ANCoaa and was used in the Norwegian calculation of critical loads (e.g. Larssen et al., 2008a). In Sweden, debate on the choice of acidity parameters initiated a study to assess relationships between water chemistry and biology in lakes and streams (Fölster et al 2007). This study found that pH was the best predictor of biology and therefore pH was used in the Swedish classification system. Similar results were found in a Norwegian study on lake trout, and the authors argued that if ANC is used as an acidity parameter then different boundaries for different TOC concentrations need to be determined (Hesthagen et al. 2008).

Box 1. Acidity indicators

pH = -log10 $\{H^+\}$

Inorganic labile aluminium: Ali = $Al^{3+} + Al(OH)^{2+} + Al(OH)_2^+ + AlSO_4^+ + AlF^{2+} + AlF_2^+$

Alkalinity: Amount of acid needed to decrease pH down to a defined value.

BC (base cations) = $Ca^{2+} + Mg^{2+} + Na^+ + K^+$

SAA (strong acid anions) = $SO_4 2- + Cl^- + NO_3^-$

ANC = BC – SAA

ANCo1 = ANC – 10/3 * TOC (mg/l)

ANCo2 = ANC – 10*2/3 * TOC (mg/l)

(All units except TOC are in mekv/l)

1.2.2 Assessing acidification

The buffering capacity of many lakes and streams in Scandinavia is often low due to an overall low rate of weathering, sometimes coupled also with very thin soil cover. Further, the concentrations of natural organic acids can be relatively high resulting in natural acidic conditions. This means that the pH or buffering capacity of naturally acidic sites is often below thresholds for biological effect even when the sites are not acidified. Hence, for naturally acidic sites these thresholds are not recommended when assessing and classifying the effects of anthropogenic acidification. Instead, the present state should ideally be compared to the reference state. Acid deposition changes the composition of both anions and cations in the water due to interactions with the soil in the catchment. To compensate for these interactions a dynamic model is needed to calculate more accurate reference conditions. In Sweden and Norway, the Model of Acidification of Groundwater in Catchments (MAGIC) is used (Cosby et al. 2001, Moldan et al. 2013).

The classification of ecological status according to the WFD is done primarily using biological quality elements (BQE), and physicochemical quality elements are only regarded as supporting variables. Therefore, it is important that classifications using physicochemical variables are harmonised on average to result in the same classification achieved using BQEs. The class boundaries for acidity are often set by relating BQEs to a chemical acidity indicator using gradient analyses and identifying thresholds. Biological thresholds for acid-sensitive organisms typically occur around pH 5.5, resulting in the placement of the good/moderate (G/M) boundary (Lindegarth et al., 2016). When the reference value for acidity is far above the designated threshold, the class boundaries for chemical indicators of acidity can be set in direct relation to the biological classification. The methods for setting class boundaries for nutrients developed by the ECOSTAT group could then be applied even for acidification (Phillips et al. 2017). However, for naturally acidic waters, when the reference value of acidity is close to, or even below thresholds for acid-sensitive organisms, this approach is not applicable.

The Finnish and Norwegian classification system is based on water body types. In the Norwegian system, which includes 15 acid-sensitive water types, the reference values and the high/good boundary for each type are based on MAGIC model results for waters within each type. The remaining class boundaries were set by expert judgement, based on relationships between ANC and status (unaffected, damaged or extinct) for trout populations for 5 broad types representing very low to low calcium levels and very low to moderate humus levels. The class boundaries are based on the assumption of increasing ANC requirements with increasing calcium and humic content (Direktoratsgruppa Vanndirektivet, 2013)

The Swedish classification system does not use water body types and biological quality elements (BQE), calibrated against pH, provide only a measure of acidity, i.e. not acidification. When classification indicates acidic conditions, it is recommended that the final classification is done based on chemical criteria and classification to distinguish between natural acidity and anthropogenic acidification (HaV 2019). Classifications did not differ among water body types, individual waterbody classifications were not developed for Sweden (Drakare et al. 2017). The Swedish classification for acidification using chemical variables was developed prior to the last revisions and development of biologial classifications, and the two approaches have to date not been harmonised. Chemical classifications are based on deviation in pH (dpH) from a site-specific reference value calculated using the MAGIC model, and the class boundaries for dpH were set by expert judgement (Fölster et al. 2007).

Parallel to this development of management tools for acidification in Scandinavia, there was a large amount of scientific work on biological effects of acidification. These studies were usually based on intensive studies of single or only a few sites and included a high number and frequency of potential drivers of change; hence, their results may be difficult to apply to monitoring data where sampling frequency and number of analytic variables are usually limited. As an alternative approach, in this study we are analysing relationships between chemistry and biology using a relatively large, spatially and temporally extensive, dataset compiled from national monitoring programmes. These types of data are likely not be optimal to reveal the underlying mechanisms of biological change from acidification because the most critical harmful events are seldom captured by routine water chemistry monitoring. On the other hand, the use of monitoring data is anticipated to provide robust statistical models and predictions of biota using water chemistry in general and specifically the effects of acidification.

2 Materials and methods

2.1 Compilation of a Nordic dataset of chemistry and biology

This project was preceded by workshops, with participants from Norway, Sweden and Finland, on evaluation and development of classification of ecological status from physicochemical quality elements. It was then concluded that all countries suffered from limitations in their national data in terms of number of sites and coverage of physicochemical and regional gradients. A merging of the national datasets would improve the statistical analysis and also give more credible results when comparing the classification systems between countries and developing more harmonised classification systems for physicochemical quality elements. The participants then decided on a compilation of a Nordic data set with data on physicochemical and biological parameters from sites with both types of data. However, in contrast to these earlier compilations, in which only averages over time were collected, this compilation should include data from single measurement from each site in order to allow a deeper analysis on time effects and importance of variability. It also gave flexibility to aggregate the data as suitable for the purpose and to calculate biological indicators from species data. The focus was on recent data (last decade), but older data could be delivered when a country regarded it as relevant.

Data were collected from lakes and rivers with data for at least one biological quality element and a minimum of chemistry including either TotP and Chla for nutrients assessments or pH, Ca, Mg, Na, K, SO4, Cl, TOC for assessments of acidification (abbreviations explained in Box 2). When available, Colour, Secci depth, Cond, Turb , Temp, Al, Ali, F, Fe, Mn, Si, PO4-P, NO3, NH4 and TotN were also included in the database.

Box 2. Parameter abbreviations

TotP = Total phosphorus
Chla = Chlorophyll a
Ca = Calcium
Mg = Magnesium
Na = Sodium
K= Potassium
SO_4 = Sulphate
Cl = Chloride
F = Fluoride
Si = Silica
TOC = Total organic carbon
Turb = Turbidity

Cond. = Electric conductivity
Al = Aluminium
Ali = Inorganic aluminium (labile)
Fe = Iron
Mn = Manganese
PO_4-P = Phosphate
NO_2+NO_3-N = Nitrate + nitrite
NH_4 = Ammonium
TotN = Total nitrogen
Temp. = Water temperature

Data on phytoplankton in lakes was delivered as abundance (mm^3/l) of single taxa (mostly species or genus). Over 19000 samples were included.

Macrophyte data from lakes were delivered in slightly different forms from the three countries, as survey methods differs, and have not been compiled or harmonised at this stage. Macrophyte data from streams, on the other hand, consisted of harmonized data from the Intercalibration work from 2014-2016 in the Northern Geographical Intercalibration Group.

Phytobenthos data was delivered as relative abundance of taxa (Finland) or the number of counted valves (Sweden). As Norway does not monitor diatoms routinely, they only delivered index data from non-diatomaceous benthic algae, but this index was intercalibrated with other Nordic countries' indices.

Macroinvertebrate data was delivered for samples from rivers as well as from littoral and profundal zones in lakes. Data was delivered as abundances for samples from littoral zones and rivers, and as abundance per square meter for profundal samples. Subsamples were aggregated.

Lake fish were sampled with multimesh Nordic gillnets, according to European standard (CEN 2015), The lake fish data were delivered as abundance and biomass, expressed as numbers and biomass (g) per gillnet and night (Npue and Bpue), for each fish species in the catch. Stream fish were sampled by electrofishing by wading, also according to a European standard (CEN 2003). The stream fish data were delivered as numbers of fish caught in one or more electofishing runs and as estimated abundance per 100 m^2 for each fish species caught. Since Norway delivered salmon and trout in streams lumped together, these two species were lumped for the whole stream fish dataset.

Basic site data on identification, pressures, land use and other geographical data was delivered. The intention was that each country should identify sites, with data from different quality elements as well as chemistry, and assign them unique IDs, so that chemistry and biology could be matched. However, this was not always possible. For some waters, chemistry was sampled at multiple sites with different sampling programmes and not coinciding with the biological quality elements. In those cases the merging of sites had to be done not at site level, but on for example lake or stream segment level. Which level that is suitable differs depending on what biological quality element and chemical parameters that should be analysed. Instead of one big database, the different national data from each quality element was stored as separate files. The final merging of data therefore has to be made for each evaluation, using a suitable ID (for lake, water body, stream segment etc).

The dataset consisted of data from around 1 900 sites with data from chemistry and at least one biological quality element. In Table 1 the distribution of sites with different data in lakes and rivers in the three countries is presented. Sampling of the different quality elements does not always overlap.

Table 1. Number of monitoring sites in lakes and rivers in the three countries with data on chemistry and the biological quality elements Phytobentos (PB), Phytoplankton (PP), Aquatic macroinvertebrates (BF, for lakes both in the littoral and profundal zones) Fish and Macrophytes.

country	lake/river	PB	PP	BF	BF profundal	Fish	Macrophytes
NO	lake	4	591	1079	0	68	216
NO	river	171	1	116		43	67
SE	lake	18	918	1079	448	461	119
SE	river	491		1511		120	68
FI	lake	0	2096	196	935	220	351
FI	river	320		890		36	141

To save time and effort, no data on methods or known limitations of the data was included in the data table. Instead, this important information was kept as comments in the deliveries or as soft knowledge within the project group. To avoid misinterpretation of the data by persons not aware of these limitations, the data were not made publicly available although all data was extracted from open data sources. However, the data will be available for persons outside the project group after consultation with someone inside the group.

A subset of the data in the Nordic database was derived by including only chemistry samples where Ca, K, Mg, Na, pH, SO_4 Cl, and TOC was analysed, and where chemistry and biology were sampled the same year. When available, nutrients and other chemical data were included. During the project period, additional data was included from Norwegian reference rivers (surveillance monitoring). Hence different datasets are used in the different analysis presented in this report.

2.2 Calculations

When labile aluminium (Ali) was available, that was included in the data selection. For Swedish sites, the fraction of positively charged Ali was modelled with a geochemical equilibrium model (VisualMINTEQ) using pH, total concentration of aluminium, Ca, Mg, Na, K, SO_4, Cl, F, Fe and TOC as input (Sjöstedt et al., 2010).

ANC was calculated as BC – SAA (see box 1). If no NO_3 data was available, ANC was calculated with only SO_4 and Cl as anions. NO_3 is negligible for the ion balance in large parts of Scandinavia and then not analysed. Only in regions with high nitrogen deposition it can become significant and is then often analysed. It is therefore acceptable to calculate ANC without NO_3 when the concentration is known to be negligible.

Two alternative modified ANC values were calculated with either 1/3 (ANCo1) or 2/3 (ANCo2) of the organic acids regarded as strong. The concentration of organic acidity was set to 10.2 µeq/mg C (Lydersen et al. 2004).

2.3 Statistical methods

Basic statistical analysis such as linear regression was performed with JMP® Pro 15.2.1 by the SAS Institute Inc. For the statistical analysis of biological responses to selected predictors of acidity in chapter 4, the robust methods Gradient Forest and General Additive Models (GAMs) were used.

Gradient Forest

Gradient Forest (Ellis, Smith & Pitcher 2012) was used to explore the predicative importance of acidity indicators and environmental/spatial factors (*objective 1*) and identify any important thresholds to establish where along the range of these gradients the important changes of species composition occur (*objective 3*). Advantages of Gradient Forest are that it does not require the specification of a functional form, no single dominant data structure is required, pre-selection of variables is not needed (a robust stepwise selection method is used) and the variables can be a mixture of continuous and categorical variables, the same variable can be reused in different parts of a tree because context dependency is automatically recognized, and these methods are robust to the effects of outliers and missing data.

To account for low counts and many zeros in the datasets, mean abundances were relativized by Hellinger transformations (Legendre & Gallagher 2001), hereafter referred to as relative abundance. The Hellinger transformation comprises dividing each value in a data matrix by its row sum, and taking the square root of the quotient, defined as:

$$y\acute{i}j = \sqrt{\frac{yij}{yi.}}\,,$$

where j indexes the species, i the site/sample, and $i.$ is the row sum for the ith sample. To expand the range of environmental controls affecting metrics of population/community status we also examined responses of taxa presence/absence data which indicates a stronger threshold response to hydrochemistry rather than a continuous response as shown by taxon relative abundance (Johnson et al., 1993). As such, separating the effects of acidification metrics from other environmental influences would be challenging using relative abundance alone. Given this context, including analysis of the presence data is more conducive to the derivation of environmental standards. All analyses were performed using R 3.5.3, (R core team, 2020) with the R packages *extendedForest and gradientForest* (Ellis et al., 2012).

General Additive Models (GAMs)

Analyses using General Additive Models (GAMs) compared how relationships between predictors of water acidity and biological composition depend on interactions with spatial/environmental variables. GAMs relax the assumption of linearity between predictors and response variable, i.e. if relationships are best approximated by a smoother. To account for any threshold changes in biological composition we used adaptive splines, which would make it possible to model sudden changes in the response. Further, our model included shrinkage splines to eliminate predictor variables with very small or no effect in the model. All analyses were performed using R 3.5.3, R package *mgcv* (Wood, 2017).

Preceding analysis with GAMs, correspondence analysis (CA) was done to create response variables that describe gradients of compositional change in fish and macroinvertebrate assemblages. CA uses X^2 distance and is recommended over other ordination methods (e.g., Principal Components Analysis) when using rank abundances or when the data have numerous 0 values (Legendre and Legendre 1998). For each of four datasets (lake and stream invertebrates and lake and stream fish), CAs were performed on square-root transformed means of taxa abundances with rare taxa down-weighted (SQRT), and taxa presence/absence (P/A). From each analysis the first (CA1) and second (CA2) axis was retained resulting in four response variables ($CA1_{SQRT}$, $CA2_{SQRT}$, $CA1_{P/A}$, $CA2_{P/A}$) for each dataset (i.e. lake invertebrate, lake fish and stream fish) (see *objective 2*, Appendix 1, Tables A.1.1 and A.1.2). All calculations of CA-axis were done using Canoco 5 (ter Braak and Šmilauer 2012).

To reduce the number of spatial/environmental descriptors principal component analysis (PCA) on centered and standardized variables was used to create index score variables that are an optimally weighted combination of a group of correlated variables. Separate PCAs were conducted on lake and stream datasets and the first (PC1), second (PC2), and third axis (PC3) were retained from the PCA as variables to characterize most of the among-site spatial/environmental variation (see *objective 2*, Tables 4-5, Figs. 6-7). All analyses were done using Canoco 5 (ter Braak and Šmilauer 2012).

Subsequently, CAs for biological response data (n=4), PCs for spatial/environmental data (n=3) were included in separate GAMs and analysis performed separately for four of the five acidification indicators as data was insufficient to test interactions of Ali. For lake macroinvertebrates GAMs included main effects and two-way interactions between the acidification indicator and all three PCs in one model, resulting in 16 models (4 CAs × 4 acidification indicators). Because fewer sites were available in the stream invertebrate and lake and stream fish datasets model complexity was reduced to include main effects and two-way interactions between the acidification indicator for each PC in a separate model, resulting in 48 models each for stream invertebrate and lake fish datasets (4 CAs × 4 acidification indicators × 3 PCs). All analyses were done using R 3.5.3, R package mgcv (Wood, 2017).

3 Differences between Norwegian, Swedish and Finnish systems for assessment of chemical acidification of surface waters.

3.1 Introduction

Acidification has been and is recognised as a significant pressure on water bodies in Norway, Sweden and to some extent Finland. The countries have therefore developed methods to classify acidification status, which is listed among the chemical and physicochemical quality elements supporting the biological quality elements in Annex V of the Water Framework Directive (2000/60/EC). Low pH in water bodies is caused by acids of natural or anthropogenic origin. Organic acids arising from the decomposition of natural organic matter are in the former category. Anthropogenic acids are released through emissions of sulphur, nitrogen and chlorine to air and falls as acid rain. The biological quality elements are not well suited to distinguish between natural and artificial causes of acidity. So called "supporting elements" are therefore of some importance when deciding where mitigating measures such as liming is required to achieve or maintain good status. Interestingly, the systems for classification of chemical acidification developed in Norway, Sweden and Finland are rather different. In this work package we will describe how they differ and explore the consequences of the differences. We start with a description of the three systems before we look at how the outcomes differ when we apply them to the same set of data from across the Nordic countries

Norway. In Norway water bodies with calcium concentration lower than 4 mg/l are considered as being sensitive to acid deposition. These acid sensitive waters are further divided into 15 types according to concentrations of calcium and total organic carbon (TOC, alternatively DOC) (Direktoratsguppen-Vanndirektivet, 2018). Typification should be made on means of minimum 4 samples in lakes and monthly samples from rivers. The type should reflect the calcium and TOC levels the water body would have if it was undisturbed by human activity. If the calcium or TOC level is close to the threshold between types, so close that the typification is highly uncertain, the one with the strictest, classification should be selected. The most acid-sensitive water-bodies are divided in more types (12 types representing water-bodies with Ca lower than 1 mg/l) than the moderately acid-sensitive water-bodies (3 types with Ca 1-4 mg/l). For each of the 15 types, reference values and boundaries separating the classes high and good, have been defined for the parameters pH, ANC and Ali (or LAl) based on the extent of deviation from the reference value (Table 2) which is supposed to represent a perceived undisturbed state for the particular water type. The important boundary between good and moderate status for ANC and Ali were based on statistical analysis of the relation between ANC and brown trout population status in 790 lakes (see Hesthagen et al., 2008 for a general introduction to how this was done). All lakes were considered as acid-sensitive (< 4 mg Ca/L) and most lakes had < 1 mg Ca/L. The original analyses were conducted for three broad types defined by TOC content (< 2, 2-5, > 5 mg C/L). This was followed by new analyses in 2013. The dataset was the same as described by Hesthagen et al. (2008), and the analysis was repeated for the 2 broad types with calcium lower than 1 mg/L (0-0.5 mg Ca/L, 0.5-1 mg Ca/L) and TOC lower than 2 mg C/L. The 790 lakes were among the 1500 sampled for water chemistry in 1995 (1006 from the stratified random selection and about 500 from the 1986 selection). Of the 790 lakes, 83 % had TOC lower than 5 mg/l at the time, i.e. the data on humic lakes was somewhat limited. Data on population status were obtained through mail questionnaires about historic and current fish status in the individual lakes. The answers were compiled together with the results from the water chemistry survey, allowing analysis of dose-response. The G/M boundary for ANC and Ali was originally defined as the values corresponding to a 90 % probability of the lake brown trout population being "healthy" (the other nominal categories were "damaged" and "extinct"), but thresholds for ANC were adjusted somewhat to account for a delay in biological recovery (expert judgment). The reference value and the high good boundary for pH was derived from ANC (see Wright and Cosby, 2012 for a description of how). The criteria for rivers are the same as those for lakes except for anadromous stretches (see footnote 1). The good-moderate boundaries for ANC are fairly harmonised with the variable limits for ANCooa (ANC modified for organic acids) used in the calculation of critical loads and exceedances for Norway (Austnes and Lund, 2014). The ratios of pH, ANC and Ali to the respective reference values (the so-called ecological quality ratio (EQR)) are normalized according to individual scales and combined to a single normalized EQR (nEQR), which determines the state of chemical acidification. For ANC, 100 was added to the values to avoid negative numbers. The reference values come from hindcasts obtained with a dynamic model (MAGIC) for lakes in the Norwegian 1000 lake survey from 1995 (Wright and Cosby, 2012).

Table 2. Reference values for pH and pH boundaries between the different classes for the 15 different acid sensitive water body types defined in Norway. Similar tables exist for the parameters ANC and Ali (DirektoratsguppenVanndirektivet, 2018). Innsjøtype = Lake type, Elvetype = River type, Typebeskrivelse = Type description, Ref. Verdi = Reference value, Svært god = High, God = Good, Moderat = Moderate, Dårlig = Poor, Svært dårlig = Bad *.

Tabell 7.2 Grenseverdier for pH i Innsjøer og elvestrekninger uten anadrom fisk. **a)** Absolutt verdier for pH.

Innsjøtype (nr)	Elvetype (nr)	Type-beskrivelse	Kalsium (mg Ca/l)	TOC (mg C/l)	pH (absolutte verdier)					
					Ref. verdi	Svært god	God	Moderat	Dårlig	Svært dårlig
L101a, L201a, L301a	R101a,R201a, R301a	Svær kalkfattig, svært klar	<0,25	<2	5,9	6,1-5,7	5,7-5,4	5,4-4,9	4,9-4,7	< 4,7
L101b, L201b, L301b	R101b, R201b, R301b		0,25-0,5	<2	6,4	6,6-6,1	6,1-5,7	5,7-5,1	5,1-4,8	< 4,8
L101c, L201c, L301c	R101c, R201c, R301c		0,5-0,75	<2	6,6	6,7-6,3	6,3-5,9	5,9-5,3	5,3-4,9	< 4,9
L101d, L201d, L301d	R101d, R201d, R301d		0,75-1	<2	6,7	6,8-6,5	6,5-6,2	6,2-5,5	5,5-5,0	< 5,0
L102a, L202a, L302a	R102a, R202a, R302a	Svært kalkfattig, klar	<0,25	2-5	5,1	5,3-5,0	5,0-4,8	4,8-4,6	4,6-4,5	< 4,5
L102b, L202b, L302b	R102b, R202b, R302b		0,25-0,5	2-5	5,8	6,2-5,1	5,1-4,9	4,9-4,7	4,7-4,6	< 4,6
L102c, L202c, L302c	R102c, R202c, R302c		0,5-0,75	2-5	6,3	6,5-5,8	5,8-5,1	5,1-4,8	4,8-4,6	< 4,6
L102d, L202d, L302d	R102d, R202d, R302d		0,75-1	2-5	6,5	6,7-6,2	6,2-5,6	5,6-5,0	5,0-4,7	< 4,7
L103a, L203a, L303a	R103a, R203a, R303a	Svært kalkfattig, humøs	<0,25	5-15	4,8	5,0-4,7	4,7-4,6	4,6-4,5	4,5-4,4	< 4,4
L103b, L203b, L303b	R103b, R203b, R303b		0,25-0,5	5-15	5,0	5,6-4,7	4,7-4,6	4,6-4,5	4,5-4,4	< 4,4
L103c, L203c, L303c	R103c, R203c, R303c		0,5-0,75	5-15	5,4	6,1-4,8	4,8-4,7	4,7-4,5	4,5-4,4	< 4,4
L103d, L203d, L303d	R103d, R203d, R303d		0,75-1	5-15	6,1	6,4-5,3	5,3-5,0	5,0-4,7	4,7-4,5	< 4,5
L104, L204, L304	R104, R204, R304	Kalkfattig, svær klar	1-4	<2	7,0	7,3-6,7	6,7-6,1	6,1-5,7	5,7-5,1	< 5,1
L105a, L105b, L205, L305	R105, R205, R305	Kalkfattig, klar	1-4	2-5	7,0	7,3-6,6	6,6-5,9	5,9-5,2	5,2-4,9	< 4,9
L106, L206, L306	R106, R206, R306	Kalkfattig, humøs	1-4	5-15	6,8	7,2-6,2	6,2-4,9	4,9-4,6	4,6-4,5	< 4,5

***** After the analysis was done it was found out that for types R106,R206 and R306 (last row) the class boundaries for pH were incorrectly reported. Correct boundaries are (G/M: 5.6, M/P: 4.9, P/B: 4.6)

Sweden. In Sweden, the criteria are defined as a pH depression compared to the estimated pre-industrial pH for each specific water body (HVMFS, 2013; Naturvårdsverket, 2007). The degree of chemical acidification is classified according to the magnitude of the depression (Table 3). The pH change (dpH) is derived from the change in ANC assuming constant DOC (dissolved organic carbon) and CO_2 partial pressure. Variation in dpH is therefore fully explained by variation in dANC. The acidity of DOC is modelled as described by Hruška et al. (2003), and CO_2 is considered to be a linear function of DOC, as described by Sobek et al. (2003). A more detailed description is provided in Handbok 2007:4 (Naturvårdsverket, 2007).

pH was selected as parameter based on comparison studies of the relationship between water chemistry and littoral fauna and fish (Holmgren and Buffam 2005, Fölster et al. 2007, Johnson et al. 2007). pH then came out as better correlated to biota compared to ANC, titrated alkalinity and modelled inorganic aluminium for lakes in southern Sweden. The same results were obtained from unpublished studies in Swedish streams. Defined changes in pH as a criterion for the class boundaries for ecological status was chosen based on the linear relationship between pH and an acidification index for littoral fauna and further supported by a similar response for epiphytic diatoms (Kahlert and Gottschalk, 2014). In this way, the assessment reflects the response of the whole community and not just presence of a single species. Further many waters are naturally acidic which means that their reference value might be below a critical level e.g. for brown trout. A change in pH of 0.4 units was chosen as the good/moderate boundary. The choice of a dpH of 0.4 as the threshold was a pragmatic choice that corresponds approximately to a change of one unit in the biological acidification index used for littoral fauna, and is slightly larger than the difference between the 10 and 90 % levels in the logistic regression of acid sensitive fish in southern Sweden (Fölster et al., 2007). The effects of changing water chemistry on the aquatic communities was regarded to be gradual with no clear thresholds or safe levels, i.e. any artificial change in pH could have an effect.

Table 3. Swedish criteria for chemical acidification. The criteria only apply to waters with mean pH lower than 7.3 and/or mean calcium concentration lower than 8 mg/l.

Estimated pH depression since pre-industrial times (pH units)	State of chemical acidification
<0.2	High
0.2-0.4	Good
0.4-0.6	Moderate
0.6-0.8	Poor
>0.8	Bad

Finland. Acidification has in recent times not been considered a major problem for Finnish lakes, and chemical criteria have therefore not been defined. The acidification state of running waters is classified according to the annual pH minimum levels. These criteria are primarily aimed at effects of runoff from acidic sulphate soils rather than air pollution. Six types of rivers have pH criteria and the threshold between “moderate” and “good” state is mean annual minimum pH < 5.4-5.6, depending on type (Aroviita et al., 2012), i.e. the thresholds are quite similar for all 6 types (Table 4). The approach differs from the Norwegian and Swedish in the (almost) constant threshold condition. The “good-moderate” boundary is linked to fish response to pH (Sari Mitikka, personal communication).

Table 4. Finnish criteria for chemical acidification of rivers. *Tyyppi* = Type, *Muuttuja* = Variable, *Kausi* = Season, *Yksikkö* = Unit, *Vertailuolot* = Reference state, *Luokkarajat* = Class boundaries.

Tyyppi	Muuttuja	Kausi	Yksikkö	Vertailu-olot	Luokkarajat			
					E/Hy	Hy/T	T/V	V/Hu
St ja ESt Suuret ja erittäin suuret turvemaiden joet	kok. P	vuosi	µg/l	<20	20	40	60	90
	kok. N	vuosi	µg/l	<450	450	900	1500	2500
	pH-minimi	vuosi		>5,7	5,7	5,5	5,0	4,8
Sk ja ESk Suuret ja erittäin suuret kangasmaiden joet	kok. P	vuosi	µg/l	<15	15	35	55	85
	kok. N	vuosi	µg/l	<335	335	800	1400	2400
	pH-minimi	vuosi		>5,8	5,8	5,6	5,1	4,9
Ssa Suuret savimaiden joet	kok. P	vuosi	µg/l	<40	40	60	100	130
Kt Keskisuuret turvemaiden joet	kok. P	vuosi	µg/l	<20	20	40	60	90
	kok. N	vuosi	µg/l	<450	450	900	1500	2500
	pH-minimi	vuosi		>5,7	5,7	5,5	5,0	4,8
Kk Keskisuuret kangasmaiden joet	kok. P	vuosi	µg/l	<15	15	35	55	85
	kok. N	vuosi	µg/l	<335	335	800	1400	2400
	pH-minimi	vuosi		>5,8	5,8	5,6	5,1	4,9
Ksa Keskisuuret savimaiden joet	kok. P	vuosi	µg/l	<40	40	60	100	130
Pt Pienet turvemaiden joet	kok. P	vuosi	µg/l	<20	20	40	60	90
	kok. N	vuosi	µg/l	<450	450	900	1500	2500
	pH-minimi	vuosi		>5,6	5,6	5,4	5,0	4,8
Pk Pienet kangasmaiden joet	kok. P	vuosi	µg/l	<15	15	35	55	85
	kok. N	vuosi	µg/l	<335	335	800	1400	2400
	pH-minimi	vuosi		>5,8	5,8	5,6	5,1	4,9
Psa Pienet savimaiden joet	kok. P	vuosi	µg/l	<40	40	60	100	130

3.2 Selection of sites and data from the database

The database contained data from 6 986 sites at the time of extraction. The criteria for inclusion were as follows:

- Sites with data on benthic fauna and/or fish as well as water chemistry from the period 2014-2016
- Sites sampled for water chemistry in the period 2014-2016
- Limed sites were excluded
- Only water samples analysed for a minimum set of parameters including pH, calcium, magnesium, sodium, potassium, sulphate, chloride and total organic carbon were considered
- For lakes only samples from the surface (top three meters) were considered for water chemistry

For water bodies where several sites met the criteria, an average value for the sites was calculated. A total of 470 waterbodies had sites that passed these criteria (Table 5).

Table 5. Waters included in the present study.

	Lakes	Streams/rivers
Finland	65	143
Norway	47	5
Sweden	153	57
Total	265	205

3.3 Estimated or derived data

The Swedish classification system requires estimates of pre-industrial water chemistry (ANC and pH) for the water body in question. For the Swedish sites the change in ANC in the individual lake or stream/river since year 1860 was estimated using MAGIC, which is a dynamic model simulating changes in soil and water chemistry as a response to acid deposition (Cosby et al., 1985). Sweden has a library of frequently updated MAGIC calibrations for lakes and streams, and a matching routine which can be used in cases where a suitable calibration does not exist for the water body of interest (Moldan et al., 2020), which is normally the case. The MAGIC model and library is a convenient and scientifically sound way to simulate historic (and future) water chemistry. However, with only Swedish sites in the MAGIC library, the library could not be used directly for Norwegian and Finnish sites.

For Norwegian sites changes in ANC were simulated by the use of 990 statistically selected Norwegian lakes sampled during the regional survey in 1995. The water chemistry trajectories of these lakes have been modelled with MAGIC. The modelling was done as described by (Larssen et al., 2008); the only change being a recalibration with updated deposition scenarios (Austnes et al., 2016). The Norwegian sites selected from the Nordic database were subsequently matched to one of the 990 lakes, using the MAGIC library routine, which requires geographical coordinates, runoff, lake area as well as water chemistry as input. It was the simulated water chemistries of the 990 lakes for the year 1995 (preferably) or 2014-2016 that was used for matching the Norwegian sites in the Nordic database with one of the 990 lakes (using data from the same year/time period). The MAGIC model does not simulate DOC dynamics, so if the matching was made for 2014-2016 DOC change from 1995 to 2015 had to be estimated. This was done as described by Austnes et al. (2016). The matching was done by IVL, the institution responsible for the Swedish MAGIC library. The pre-industrial (1860) and current (2015) ANC for the sites were taken from the modelled time series of the matched lake (same as current practice in Sweden).

A somewhat different approach was chosen for estimating ANC changes at Finnish lake sites. Here, a metamodel based on MAGIC simulations for 2 439 Swedish lakes was developed in order to relate contemporary lake water chemistry observations to ANC in 1860. The procedure is as described in Chapter 5 in this report (Equation 4). The use of Swedish instead of Finnish lake data for construction of the metamodel introduces some extra uncertainty, but we consider it acceptable for our purpose here. No attempt was made to estimate ANC change at Finnish stream sites. The method used to derive dpH from dANC was the same for all three countries and is described in the previous chapter.

The Norwegian system considers labile aluminium (Ali) as one of three parameters in classification of acidification status. This parameter is therefore routinely measured in Norwegian water samples, but not in samples from the Swedish and Finnish sites. For Swedish sites, however, the modelled fraction of positively charged inorganic aluminium was included in the data.

3.4 Data treatment and classification

Remarks concerning the data treatment and classification are listed below.

Norwegian method. Arithmetic means for the period 2014-2016 were used for typification and classification of waters (for pH on back logged data). An exception is Ali for which the 90th percentile from the whole period was used. Present calcium and TOC concentrations were used for typification, instead of estimates of pre-industrial levels (see Austnes et al., 2016). Water bodies were typified according to the mean calcium and TOC calculated, i.e. waters were not recategorized to a more sensitive type when the mean calcium or TOC concentration was close to thresholds between water types. We chose this simplest of procedures in order to avoid adding another layer of complexity and subjectivity. To set the acidification status class, the median of the nEQR for pH, ANC and Ali (if data was available) was chosen. Using the arithmetic mean instead of the median was also tested. Of the 470 waters selected, 181 (70 lakes and 111 streams) had mean calcium concentrations above 4 mg/l. These are considered alkaline or non-sensitive according to the Norwegian system and were not assigned an nEQR or state of acidification. Waters with mean TOC levels higher than 15 mg/l were assessed using the thresholds defined for humic waters (5-15 mg TOC/L) (state of acidification has not been defined for very humic waters).

Swedish method. Arithmetic means for the years 2014-2016 or 1995 were used to match the water body to a MAGIC modelled trajectory as described above. An exception was Swedish running waters where flow weighted means were used. The dpH determined the state of acidification (Table 3). The 10 lakes and 62 streams with mean pH higher than 7.3 and/or mean calcium concentration higher than 8 mg/l were considered alkaline or non-sensitive. The dpH was not estimated for these waters.

Finnish method. The mean annual minimum pH for the period 2006 -2012 was used to classify running waters according to Table 4. This method was applied to all 205 rivers.

3.5 Results

3.5.1 Water chemistry in the selected lakes and rivers/streams

The water chemistry of the lakes and rivers/streams on which the classification systems were tested, varied across spatial gradients (Figure 1). Ion concentrations and TOC increased from west to east corresponding to gradients from high to low precipitation, and from mountain areas with thin and patchy soils to thick soils with forests and mires. More detailed descriptions and explanations of regional variations are found in Skjelkvåle et al. (2001). Waters in the Skåne, Stockholm's län and Osthrobothnia area have high levels of sulphate and calcium and are likely to be more affected by sulphate rich soils than air pollution.

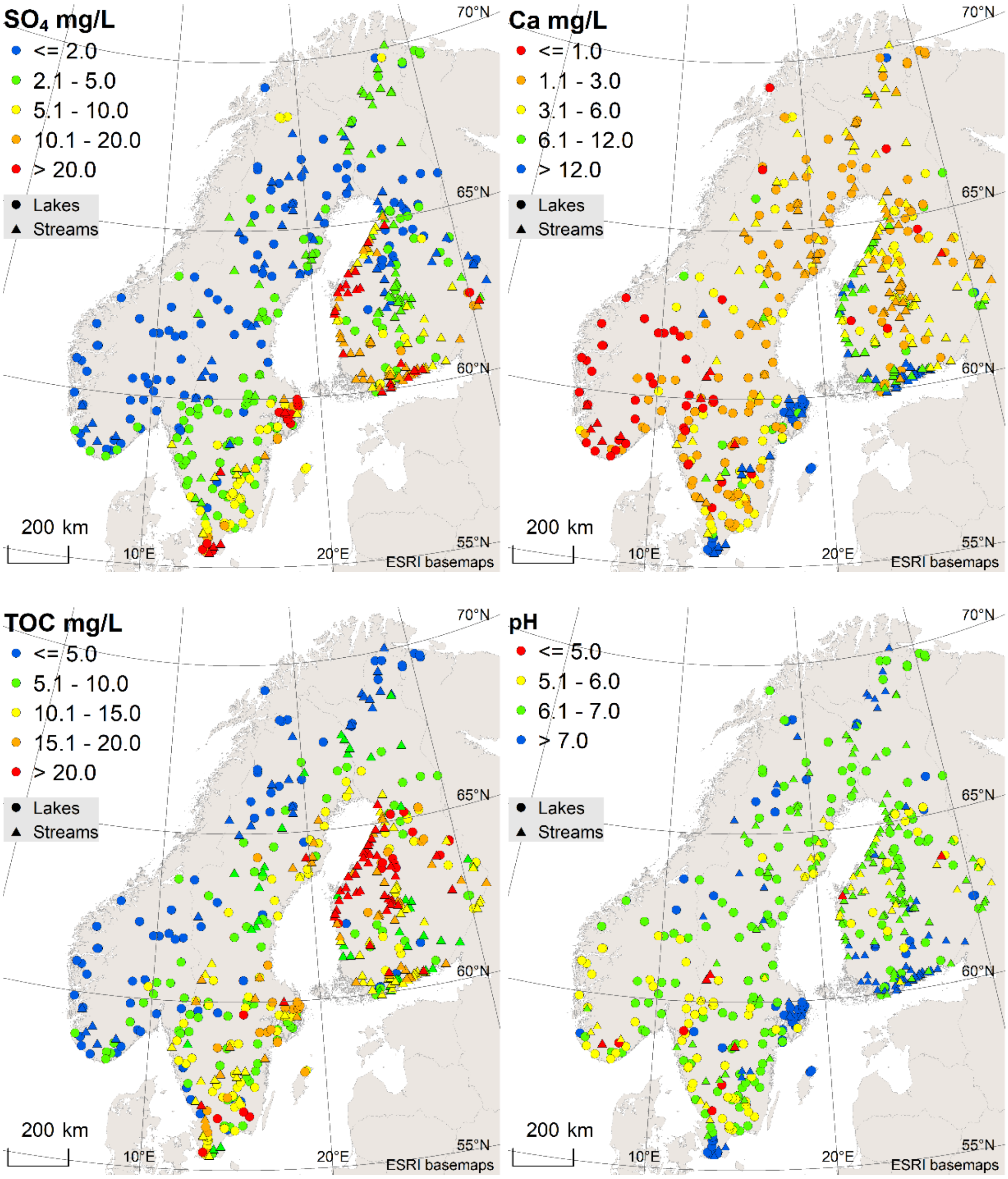


Figure 1. Mean concentration of sulphate, calcium, TOC and pH between 2014 and 2016 for the 265 lakes and 205 rivers/streams.

3.5.2 State of acidification – Norwegian and Swedish systems

Both the Norwegian and Swedish systems consider deviation from a reference state estimated for the year 1860. Let us first verify that the systems agree concerning the undisturbed pre-industrial ANC and pH. Agreement was expected since both countries base their estimates on the same model (MAGIC). A comparison showed that there were indeed no significant differences between the reference values for very calcium-poor waters (t-test, $p<0.05$). Figure 2 illustrates the difference between having types (categories) instead of lake or river specific reference values. For clear and humic waters with calcium concentration between 1-4 mg/l, comprising two Norwegian types assigned the same ANC reference of 125 µEq/L, the specific reference ANC varies between 80 and 380 µEq/L. The variation is much less for the very calcium poor waters where the type resolution is much higher with 12 different types defined for calcium levels below 1 mg/l. The correlation between the Norwegian and Swedish reference values was slightly higher for ANC than for pH. The reference pH is derived from ANC, DOC and pCO_2, and different assumptions concerning the effects of the two latter could affect the correlation for pH.

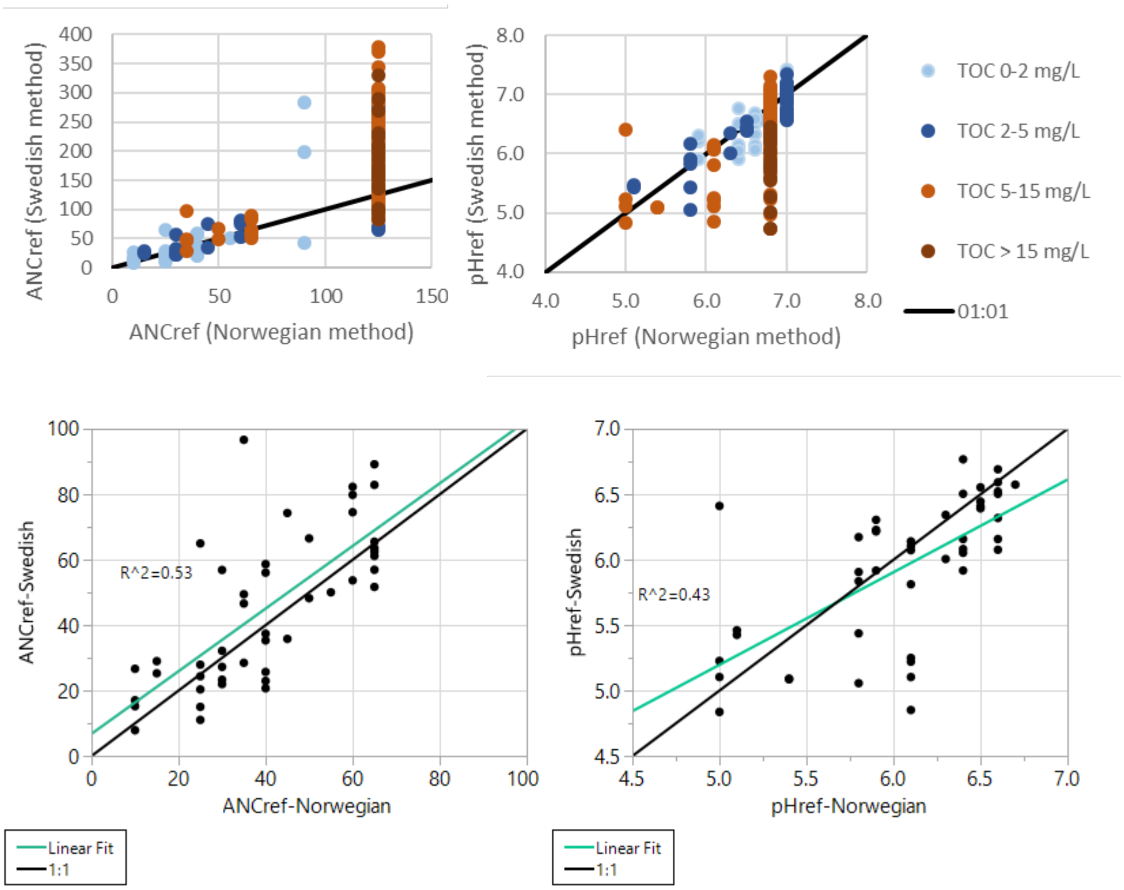


Figure 2. Estimated year 1860 ANC (left panels) and pH (right panels) for the individual waters plotted against the reference ANC and pH of the relevant water type according to the Norwegian system. The bottom panels show the results for the very calcium poor waters (Ca < 1 mg/l), i.e. with calcium poor (Ca 1-4 mg/l) waters excluded.

There is poor agreement between the systems regarding how large the deviation from the reference state has to be in order for the water body to fall below the important "good/moderate" threshold (Figure 3). For most of the waters considered here, the Norwegian system accepts a larger pH depression than the Swedish without relegating it to "moderate" or worse state. Only three of the 15 Norwegian types can be relegated to “moderate” or worse state for pH depressions of 0.4 or less (Figure 4). These are of the very calcium poor types.

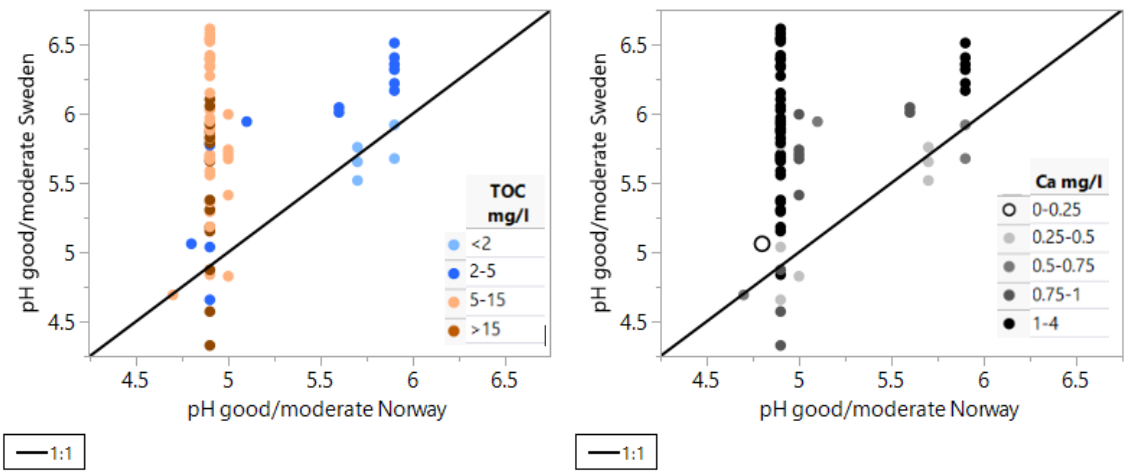


Figure 3. The pH separating “good” and “moderate” acidification state according to the Swedish system (i.e. reference pH – 0.4) versus the corresponding boundary for the Norwegian water types. The waters included in these plots were classified as moderate or worse according to the Norwegian and/or the Swedish system.

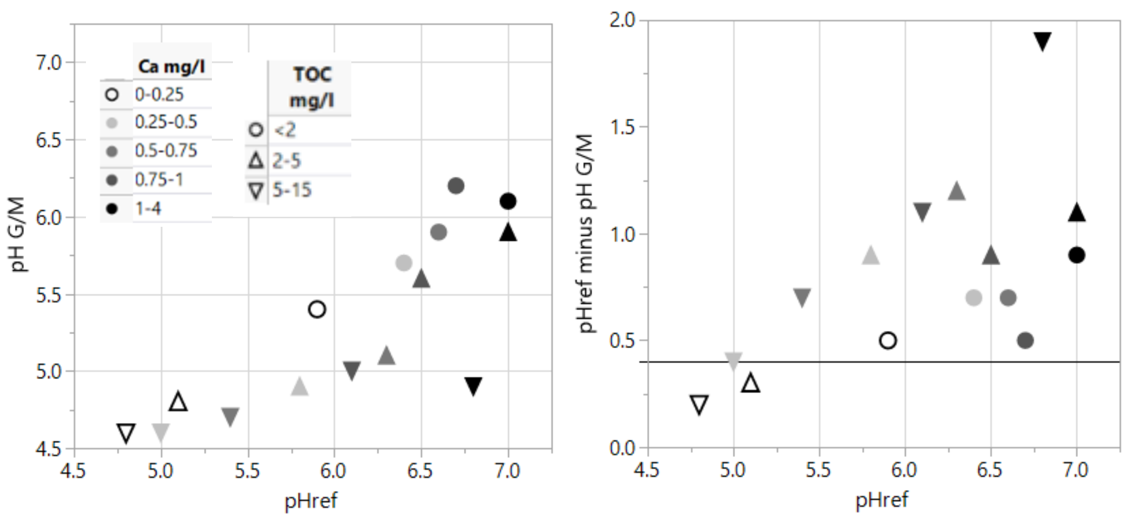


Figure 4. Left panel: The pH separating “good” and “moderate” state plotted against reference pH defined for the 15 Norwegian water types. Right panel: The difference between reference pH and the “good”/”moderate” pH boundary plotted against reference pH. The horizontal line represents the pH depression of 0.4 that corresponds to the Swedish “good”/”moderate” boundary.

As expected, this resulted in differences between the assessments made with the Norwegian and Swedish classification system (Figure 5). Only 10 of the 470 waters were classified as “moderate” or worse with the Norwegian system, whereas the extent of acidification was more widespread according to the Swedish system. The geographical pattern is similar for lakes and rivers/streams. However, the spatial coverage of rivers is poor in the west, and the dpH was not estimated for Finnish rivers/streams. Below we consider the differences between the Norwegian and Swedish systems in more detail.

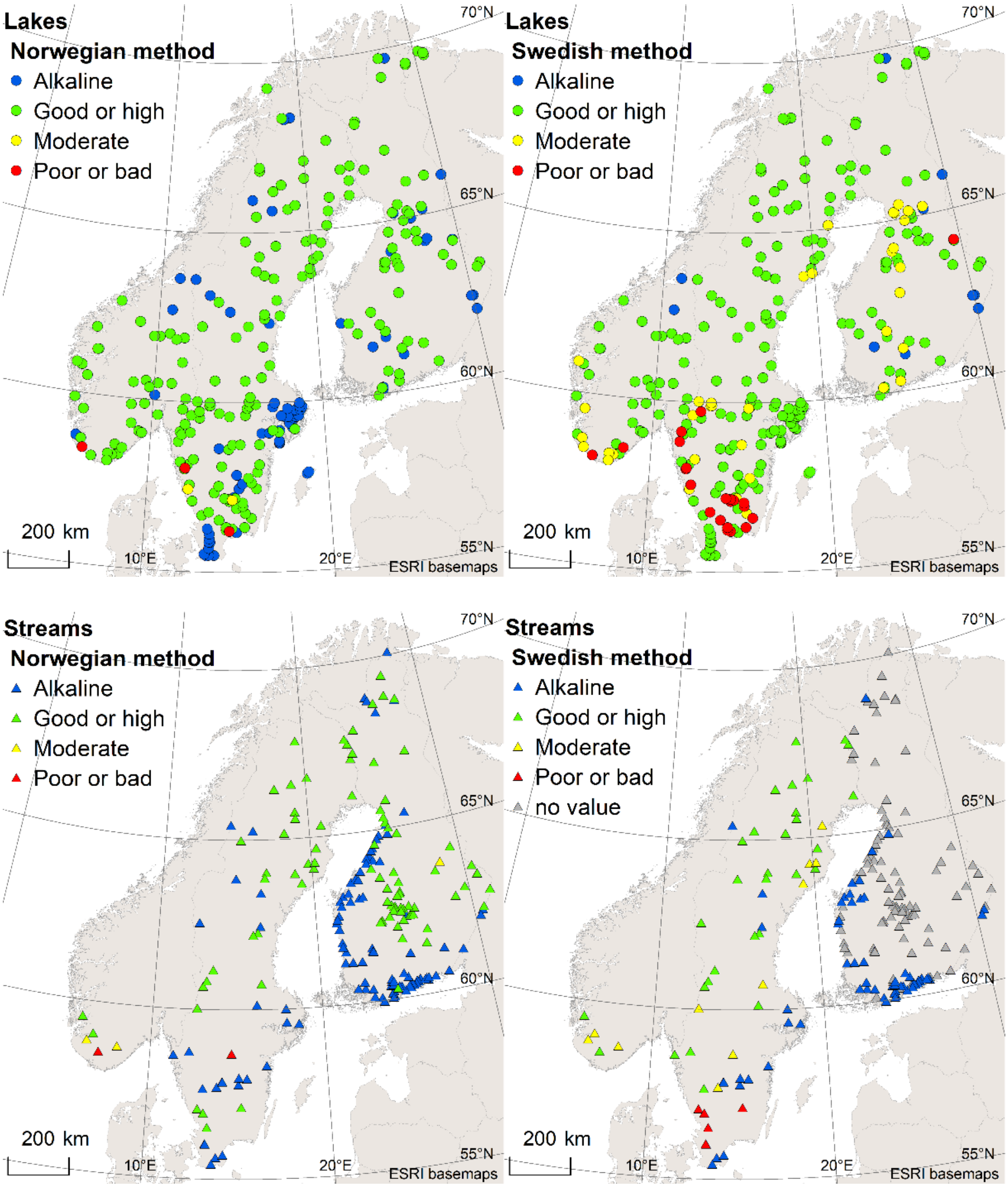


Figure 5. State of acidification in Nordic lakes (top panels) and rivers/streams (bottom panels) classified according to the Norwegian (left panels) and Swedish (right panels) systems.

The results show that water bodies whose acidification states were classified as "moderate" or worse with the Norwegian system, received the same assessment with the Swedish system (
Figure 6). There were two exceptions, and they were close to the threshold. About half of the water bodies that met the Norwegian criterium for good or high status, failed to meet the corresponding Swedish criterium. It was usually pH or ANC that determined the median nEQR and thereby

acidification status according to the Norwegian system. The nEQR of both pH and ANC was correlated to dpH (R^2= 0.39 and 0.29, respectively), which is not surprising since pH is derived from ANC and both nEQR and dpH indicate deviation from a reference state. The nEQR for Ali was usually lower than for pH and ANC. Furthermore, the correlation of nEQR for Ali to estimated dpH was weaker than for the nEQRs of ANC and pH (r^2=0.23). It follows that compared to nEQR of ANC and pH, less of the variation in the nEQR of Ali is explained by dpH. Note also from
Figure 6 that several lakes with dpH < 0.4 are not “good” according to nEQR of Ali. It is not clear why the pattern differs for aluminium. There are several possible explanations: 1. It is well known that concentrations of Ali can vary considerably at the same (acidic) pH value. 2. There are other factors besides annual mean ANC and pH that affects Ali values during episodes. 3. The use of the 90^{th} percentile gives weight to extreme values, which may be erroneous. Aluminium is the primary toxicant for algae and fish in acidic waters and is therefore very important. Unfortunately, lack of data on aluminium fractions and methodological differences hampers in-depth analysis of the current dataset.

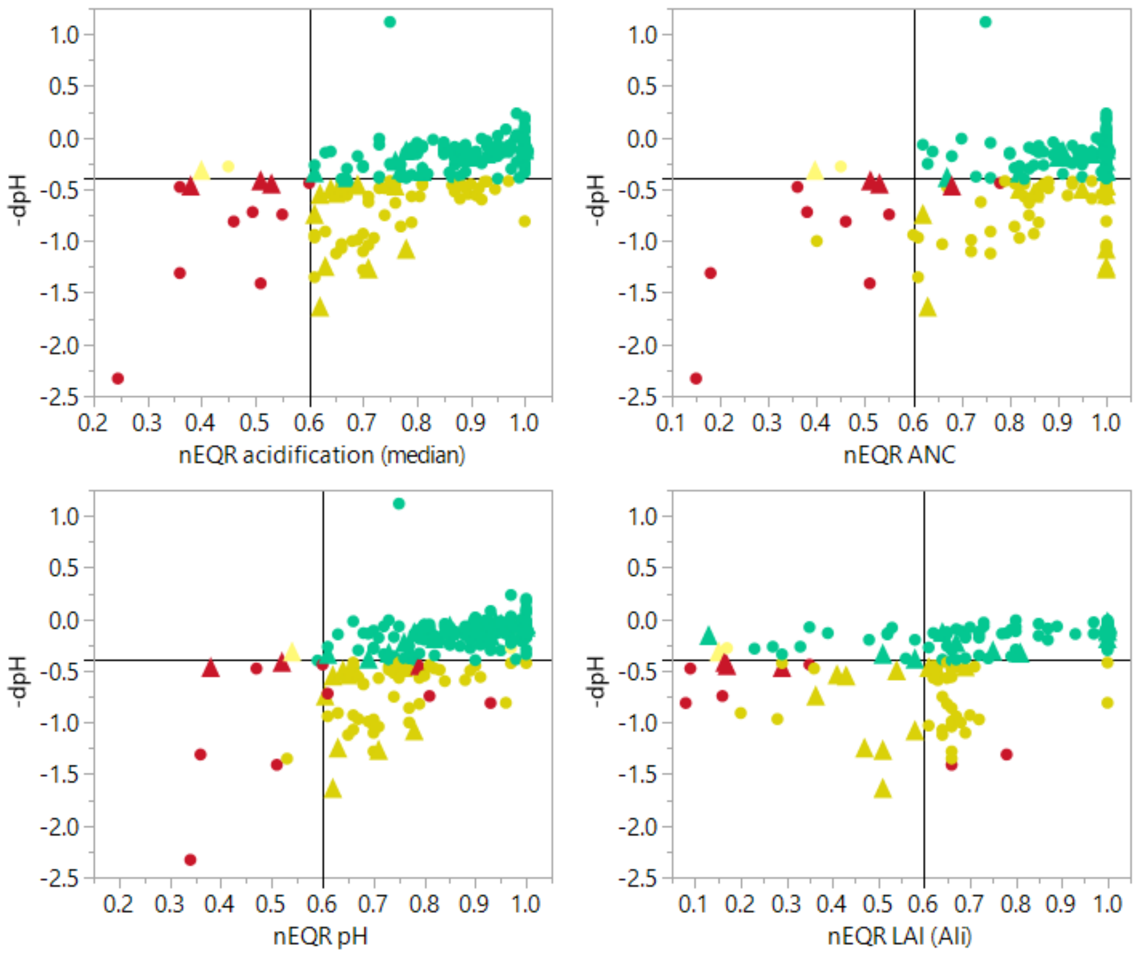


Figure 6. Estimated pH depression since 1860 (dpH) plotted against the median of the normalized ecological quality ratios (nEQR) for ANC, pH and Ali (top left panel). The vertical and horizontal straight lines represent the threshold between good and moderate status of the Norwegian and Swedish classification system, respectively. The circles represent lakes and the triangles rivers/streams. Green and red colour indicate that the assessment is similar according to both systems, i.e. good or high status as green and moderate, poor or bad as red, respectively. Yellow colour indicates that the assessments made with the Swedish and Norwegian system end up on the opposite side of the good-moderate boundary. The two points with a brighter hue of yellow represent the only waters where the Swedish system indicated good or high state and the Norwegian system moderate or worse state The top right and bottom panels show how the nEQRs of the three individual parameters comprising the Norwegian system are distributed compared to the median that determines the status.

The waters that were classified as moderate or worse according to the Norwegian system, had low or very low calcium, low TOC, low pH and high Ali compared to the rest of the waters (Figure 7). The systems were also more in agreement concerning the acidification state of these very calcium poor (< 1 mg/l Ca) and clear (< 5 mg/l TOC) waters compared to the state of the browner more calcium-rich waters. This reflects the high resolution of discrete Norwegian low calcium - low TOC water types compared to the cruder categories for browner waters (see Chapter 3.1). Calcium-poor waters tend to have a lower natural (i.e. pre-industrial) pH than more alkaline waters. A decline in pH by 0.4 from e.g. 6.0 will be more critical for an acid sensitive organism such as brown trout than a decline by 0.4 from higher pH. Both changes have consequences for some organisms, but the countries differ in their acceptance of these changes. The Swedish system might in this case classify sites as acidified although the biological effect is subtle. However, since the buffering capacity increases markedly as pH increases above 6, this potential over-sensitivity is a minor problem. Only 7 of 301 waters had an estimated pH depression higher than 0.4 despite having a measured pH above 6 (note that dpH is not derived directly from measured pH but from the trajectory simulated by MAGIC for the match).

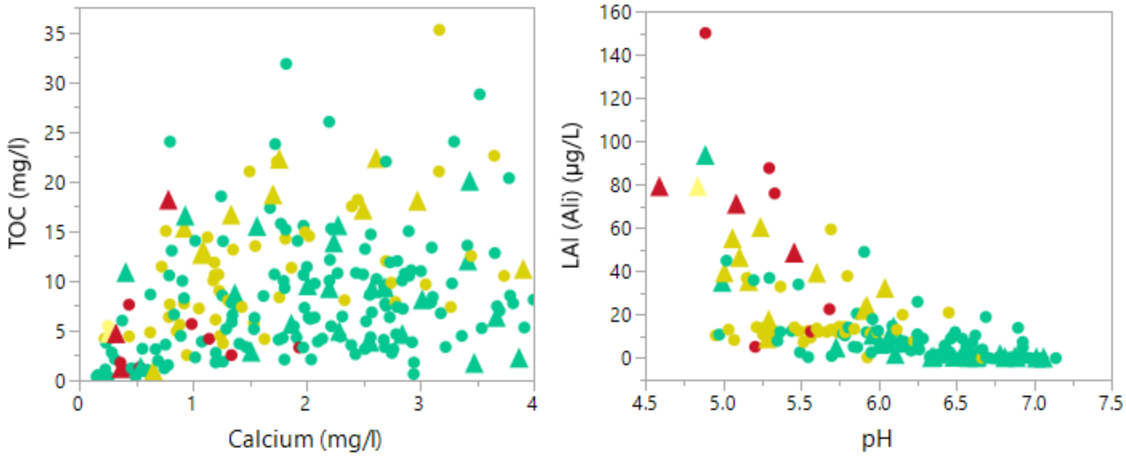


Figure 7. Concentration of TOC versus calcium (left panel) and concentration of Ali versus measured pH. The colour and symbols are explained in the caption of
Figure 6.

3.5.3 The Finnish system for rivers/streams

The result of the Finnish assessment of rivers/streams is displayed below (Figure 8). The Finnish and Swedish system showed surprisingly good agreement with respect to the important good/moderate boundary considering how different they are (Figures 5 and 8). Unfortunately, the Swedish system was not applied to the Finnish streams, precluding a more detailed comparison. A comparison with the Norwegian system showed that the systems largely agree about the acidification state of the most calcium-poor and clear waters (Figure 9). The disagreement, i.e.good or better state according to the Norwegian system and “not good” according to the Finnish, mostly arose for rivers/streams with calcium and TOC concentration higher than 1 and 10 mg/l, respectively.

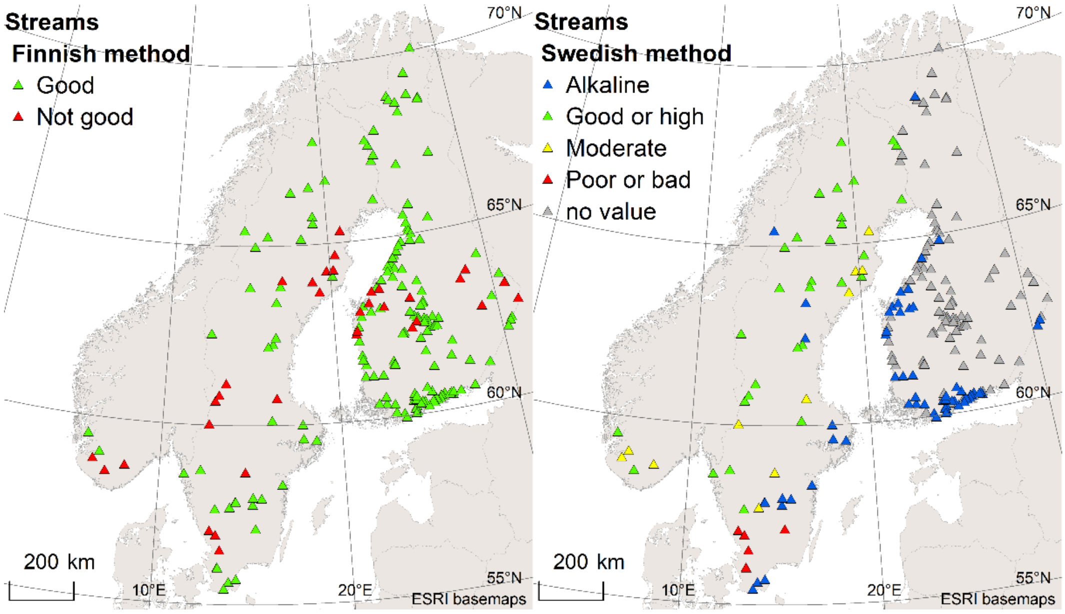


Figure 8. Acidification of rivers/streams according to the Finnish and Swedish system.

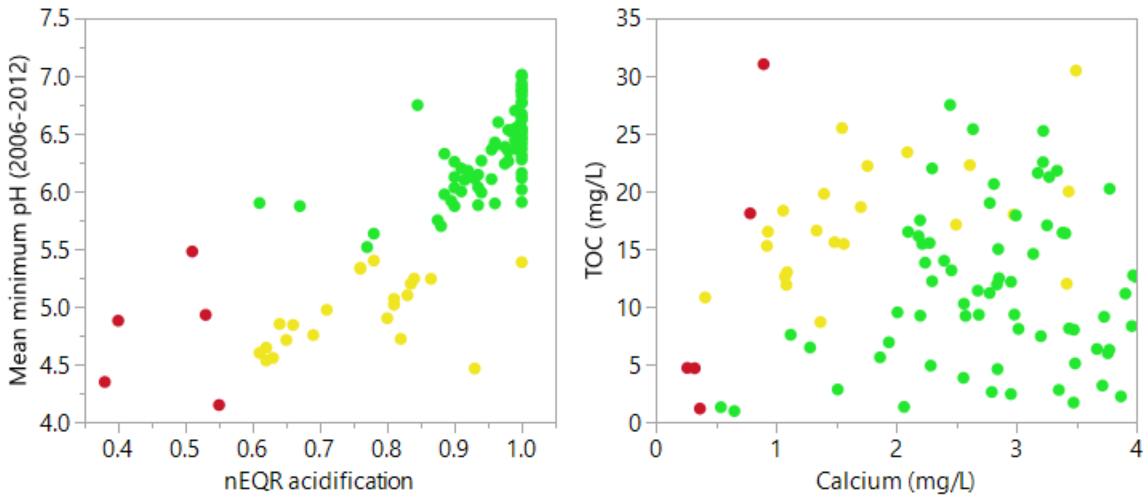


Figure 9. Mean minimum pH of rivers/streams for the period 2006-2012 plotted against nEQR acidification (left panel) and stream/river concentration of TOC versus calcium concentration (right panel). The green and red colour represent rivers/streams where both the Finnish and the Norwegian system indicate good or better or moderate or worse state of acidification, respectively. The yellow colour indicates moderate or worse state according to the Finnish system and good or better state according to the Norwegian system. There were no rivers/streams were the respective assessments were the other way around.

3.6 Discussion

Making a classification system for acidification is a large challenge where the effects on the aquatic communities by a century of acid deposition including soil interactions should be assessed based on a limited number of water chemistry samples. The Nordic countries have solved this in different ways reflecting the differences in deposition history, geology and climate, administrative demands and access to data. The different classification systems all consider changes from a perceived reference state, but the systems differ with respect to parameters, definition of unacceptable acidification, the environmental targets for waters of different composition, data requirements and complexity in use. It was therefore not surprising that they led to different assessments although the degree to which they differed was larger than expected. Only 10 of the 470 waters (2 %) were considered acidified according to the Norwegian system. The Swedish system found 74 of 373 waters (20 %) and the Finnish 34 of 205 streams (17 %) to be acidified. The difference was largest for brown waters with medium levels of calcium. The agreement was better for clear lakes (there were few clear streams or rivers included in the analysis).

The main cause of the discrepancy is the different biological responses that are used to define the threshold between “good” and “moderate” state. The importance in handling organic acids was also differing between the countries. The decrease in acid deposition and the increase in DOC that has been observed over the last decades, imply that natural acidity has become more important. A reassessment of the physicochemical criteria used to assess acidification is therefore called for, and it would be timely to use this opportunity to consider full or partial harmonisation of approaches across the borders. The joint dataset compiled for this project gives the potential for a far better scientific fundament for a classification system compared to earlier work since it includes a large number of sites with a width of chemical parameters and species abundancies of both fish and macroinvertebrates in both lakes and rivers and covering larger geographical and chemical gradients.

4 Analysis of biological responses to selected predictors of water acidity.

In this chapter we explore the performance of different chemical acidity indicators to predict response in biological communities. We do this by using state of the art statistical methods on the joint Nordic dataset on water chemistry, fish and invertebrates in lakes and streams from Norway, Sweden and Finland.

Objectives for this study included extraction of a relevant dataset from the Nordic database, statistical analysis of the relation between biological and physicochemical parameters and evaluation of different chemical acidity indicators (pH, ANC, ANCo1, ANCo2, Ali). The focus of the first part of the analyses was to quantify and select acidification indicator/s with the most importance to explain and predict biological composition at a Nordic scale. The first criterion in acidification indicator selection was consistency of high predictive importance for changes in biological communities across data sets/treatments (*objective 1*). The second criterion in choice of an acidification indicator was that an acidification indicator be robust against interactions with other parameters (*objective 2*). Interaction effects occur when the effect of one variable depends on the value of another variable. If biological response to an acidification indicator significantly depends on other parameters it is critical to incorporate the interacting parameters in the model because you can't interpret the main effects without considering the interactions. This type of effect makes the model more complex, requiring more data and complicating decisions on waterbody classification and response to acidification. The focus of the second part of the analyses was to explore the empirical shape and magnitude of changes in composition along acidification gradients, and identify any critical values of acidity indicators along these gradients that correspond to threshold changes in biological composition (*objective 3*).

4.1 Data treatment and description

Fish, invertebrate and chemistry data was retained from the Nordic database, which contains environmental monitoring data from lakes and streams. Stream chemistry was considered relevant if it was taken at the same site as the biological samples, or within the same stream segment. Lake chemistry could be from anywhere in the lake. In some cases, biology was matched to chemistry using the IDs in the databases (MVM-data). A subset of the data in the Nordic database was derived by including only chemistry samples where Ca, Cl, K, Mg, Na, pH, SO_4 and TOC were analysed, and where chemistry and biology were sampled the same year, and for sites which had four or more water chemistry sampling occasions. The time period was limited to 2005–2016 for lake and stream invertebrates and lake fish, while for stream fish the time period was limited to 1996–2019. Furthermore, for invertebrates, only littoral kick-samples taken in September, October or November were considered. In the subsequent datasets the arithmetic means of taxa abundance, environmental descriptors, and acidity indicators were calculated for each site and then filtered by removing sites with mean pH>7 and ANCo2>200 µeq/l. Lastly, all data sets were filtered by removing Swedish sites known to be limed to mitigate acidification. The dataset included invertebrates from 165 lakes and 99 streams and fish from 114 lakes and 80 streams (Table 6).

Table 6. Number of sites.

Dataset	Sweden	Norway	Finland	Total
Lake invertebrate communities	105	48	12	165
Stream invertebrate communities	46	53	0	99
Lake fish communities	83	22	9	114
Stream fish communities	37	43	0	80

4.1.1 Lake and stream invertebrates

Correspondence Analysis (CA) on square-root transformed means of taxa abundances with rare taxa down-weighted was done to examine the relationship of among-site invertebrate composition and assigned waterbody classification (lake/stream) using Canoco 5 (ter Braak & Šmilauer 2012). A clear separation was detected between lake and stream assemblage composition along axis 1 of the CA, with few deviances from their original waterbody classification (lakes=lakes=94.5%, streams=streams=97.7%) (Figure 10). Of the 12 originally classified as streams within the lake cluster (CA axis 1 negative values, Figure 10) two were impoundments, seven within 200 meters from a lake or between lakes, and one was 500 meters from a lake. The three originally classified as lakes within the stream cluster (CA axis 1 positive values, Figure 10) were long narrow Norwegian lakes within a stream system, with an inlet and outlet at the narrow ends of the lake. Accordingly, we divided the dataset for further analysis based on site association to "lake" and "stream" invertebrate composition cluster along the first axis of the CA (Figure 10).

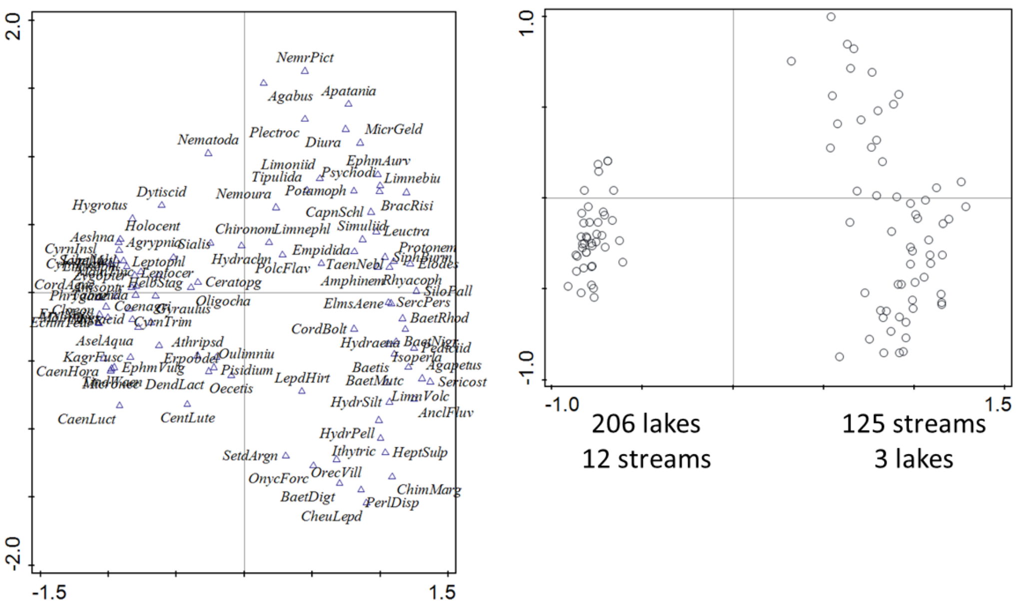


Figure 10. CA of lake and stream invertebrate taxa and sites. Taxa scores (left) and site scores (right) are shown for CAI and CA2.

4.1.2 Lake and stream fish

For lake and stream fish, environmental and chemical acidity indicator data were retained from the Nordic data based on annual correspondence of physical-chemical parameters (mean during September to August, i.e. approximately one year before fish sampling).

4.1.3 Predictors - lake and stream invertebrates

Along with the five acidification indicators, data by site included water chemistry (TOC, TotP, NO_2+NO_3-N, Ca, K, SO_4, BC), catchment size and land use characteristics (% agriculture, % forest, % water, % wetland), and spatial components (altitude, latitude, longitude) (Table 7)

Table 7. Mean and range of acidity indicators and environmental descriptors for lake and stream invertebrate sites.

	Lake			Stream		
Acidity indicator	**MEAN**	**MINIMUM**	**MAXIMUM**	**MEAN**	**MINIMUM**	**MAXIMUM**
pH	6.14	4.53	6.98	6.22	4.93	7.00
ANC (µeq/l)	120	-25	291	116	7	291
ANCo1 (µeq/l)	89	-37	231	92	-8	234
ANCo2 (µeq/l)	59	-97	189	67	-53	194
Ali (µg/l)	15.1	0.1	251.4	15.1	0.1	82.9
Environmental descriptor						
TOC (mg/l)	8.96	0.35	29.92	7.08	0.43	21.54
Tot-P (µg/l)	10	2	61	8	2	50
NO_2+NO_3-N (µg/l)	62	2	354	68	1	581
Ca (µeq/l)	110	8	330	91	15	246
K (µeq/l)	12	2	40	8	1	23
SO_4 (µeq/l)	65	9	29	38	8	214
BC (µeq/l)	303	29	756	232	45	675
% agriculture	1.2	0.0	37.6	0.6	0.0	8.8
% forest	62.6	0.0	95.9	52.3	0.0	99.5
% water	12.7	0.0	31.8	4.0	0.0	24.8
% wetland	4.7	0.0	41.8	3.1	0.0	22.8
catchment size (km^2)	34.9	0.25	1407	102	0.51	1165
altitude	245	27	1166	487	39	1438
longitude	14.7	5.3	30.8	13	5.6	30.4
latititude	60.3	56.2	69.7	61.8	56	70.5

4.1.4 Predictors – lake and stream fish

Along with the five acidification indicators, stream and lake data by site included parameters of water chemistry (TOC, TotP, NO_2+NO_3-N, Ca, K, SO_4, BC), catchment size and land use characteristics (% agriculture, % forest, % water, % wetland), and spatial components (altitude, latitude, longitude) (Table 8). Lake data by site also included lake area and maximum lake depth (m) (Table 8).

Table 8. Mean and range of acidity indicators and environmental descriptors for lake and stream fish sites.

	Lake			Stream		
Acidification indicator	**MEAN**	**MINIMUM**	**MAXIMUM**	**MEAN**	**MINIMUM**	**MAXIMUM**
pH	6.21	4.71	6.95	6.29	5.02	6.96
ANC (µeq/l)	129	6	288	123	22	312
ANCo1 (µeq/l)	97	-12	242	98	8	254
ANCo2 (µeq/l)	65	-85	200	74	-44	196
Ali (µg/l)	9.6	0.01	57.9	13.8	0.1	74.2
Environmental descriptor						
TOC (mg/l)	9.40	0.31	33.52	7.35	0.43	22.67
Tot-P (µg/l)	11	1	51	7	2	50
NO_2+NO_3-N (µg/l)	60	2	617	64	6	492
Ca (µeq/l)	118	11	387	101	15	249
K (µeq/l)	13	2	39	8	1	22
SO_4 (µeq/l)	74	10	295	40	8	167
BC (µeq/l)	320	29	871	242	58	580
maximum depth (m)	18	2	80	NA	NA	NA
lake area (km^2)	1.38	0.02	34.1	NA	NA	NA
% agriculture	0.9	0.0	12.2	0.9	0. 0	9.1
% forest	66.4	0.0	99.4	59.4	0.7	97.9
% water	13.2	1.2	37.0	3.6	0.0	24.8
% wetland	4.9	0.0	42.5	7.6	0.0	44.0
catchment size (km^2)	22.4	0.20	478.2	104.7	1.3	1164.9
altitude	242	39	1166	522	25	1438
longitude	15.2	5.3	30.4	12.3	5.6	23.2
latitude	60.3	56.1	69.7	61.4	56.3	69.6

4.2 Results and discussion:

4.2.1 Objective 1, Predictor Importance:

Lake macroinvertebrate communities

Results indicated the most consistent acidity indicator across models, i.e.high importance predictor of community change in lake macroinvertebrates, was ANCo1 followed closely by ANC and pH (Figure 11, a-f). In the Nordic dataset the predictive importance was highest in acidity indicators ANCo1 and ANC (Figure 11 a, d). When the data was divided into national (Swedish and Norwegian) datasets, some deviating results were found. In the Swedish sub-dataset, pH and ANCo2 had higher performance than ANCo1 and ANC compared to the Nordic dataset (Figure 11 b, e). For the Norwegian dataset, using relative abundances the predictive importance was highest in acidity indicators Ali, pH and ANCo1, respectively (Figure 11 c). However, for taxa presence absence, ANC and ANCo1 had higher performance than Ali, pH and ANCo2, respectively (Figure 11 f). The differences in performance for the different national sub-datasets might reflect the different chemical ranges of the two sub-datasets (see Appendix 1). Spatial predictors were consistently of high importance across models, particularly longitude at the Nordic scale (Figure 11 e), latitude and altitude in the Swedish sub-dataset (Figure 11 e), and altitude in the Norwegian dataset (Figure 11 c). Although spatial parameters were important, the consistent high importance of ANCo1 for driving community change of lake benthic macroinvertebrates in Swedish and Norwegian sub-datasets corroborates with results in the Nordic dataset. With the exception of percent forest for Norway (Figure 11, a, c) catchment land use, catchment size, TOC and nutrients were of intermediate or low importance as predictors (Figure 11, a-f).

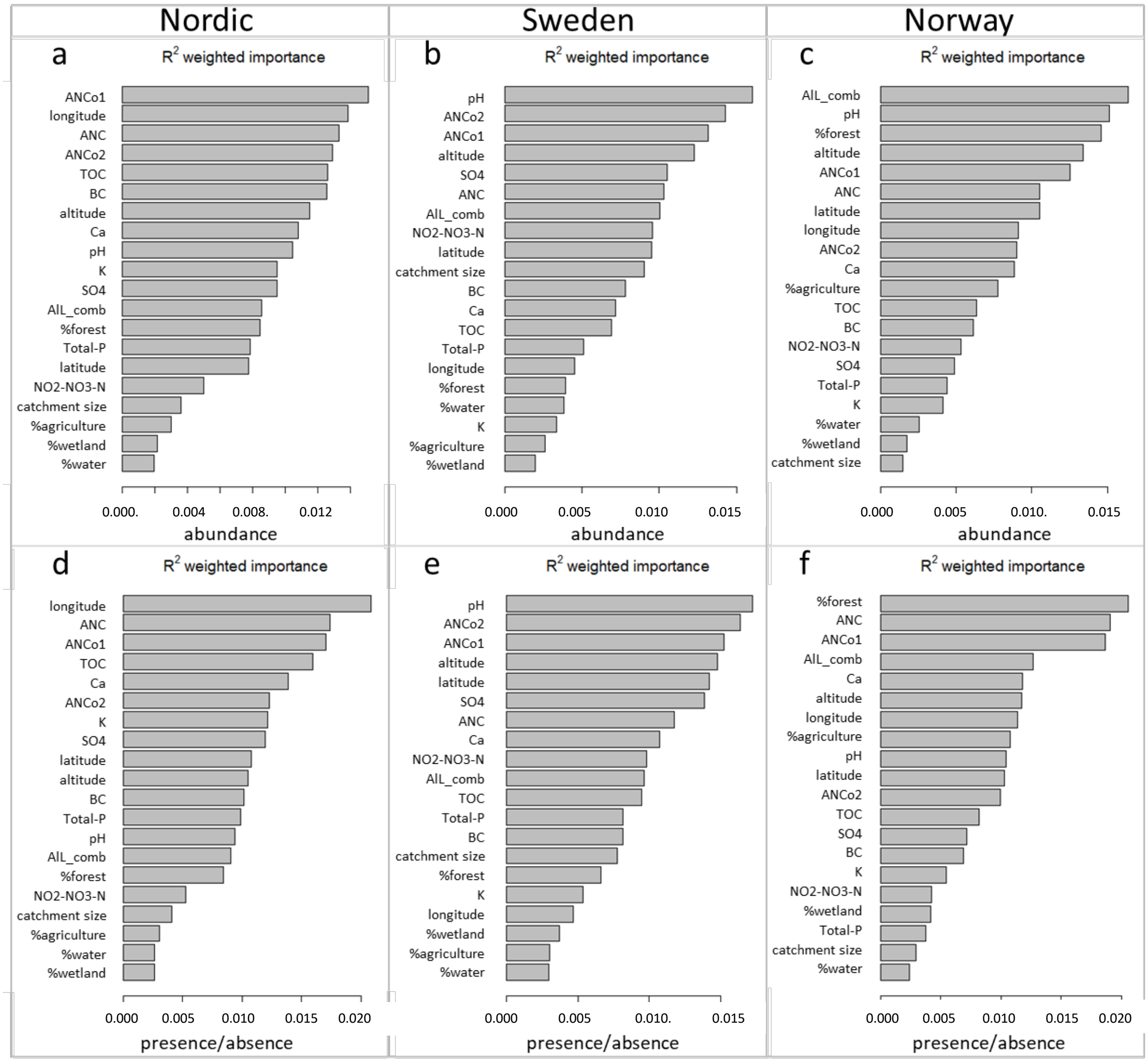


Figure 11. Lake invertebrate overall conditional importance of the acidity indicators and environmental spatial/environmental descriptors as predictors of relative taxa abundance (a, b, c) and presence/absence (d, e, f) for Nordic scale (a, d), Swedish sites (b, e), and Norwegian sites (c, f). Ali is denoted AlL_comb.

Stream macroinvertebrate communities

Results in river macroinvertebrate datasets indicated the Ali was the most consistent acidity indicator across models as a high importance predictor of community change (Figure 11 a-f, Figure 12 a-f). However, in the full (Nordic) dataset the predictive importance of ANC followed closely behind Ali (Figure 12 a, d). In the Swedish sub-datasets acidity indicator Ali had the highest predictive importance (Figure 12 b,e) followed by ANCo1 in relative abundance (Figure 12 b), and pH for taxa presence/absence (Figure 12 e). In the Norwegian dataset the predictive importance of pH exceeded Ali for relative taxa abundance (Figure 12 c), while for taxa presence/absence the predictive importance of ANCo2 was only slightly lower than Ali (Figure 12 f). Spatial predictor latitude was consistently of highest importance across sub-dataset models (Figure 12 a-f), while longitude had greater importance in the full Nordic dataset (Figure 12 a,d). Catchment size, a proxy for stream size, was consistently of high or intermediate importance across sub-dataset models (Figure 12 b,c,e,f), but of lower importance in the Nordic dataset (Figure 12 a,d). K had intermediate importance in the full Nordic dataset (Figure 12 a,d). Other predictors were of intermediate or low importance (Figure 12 a-f).

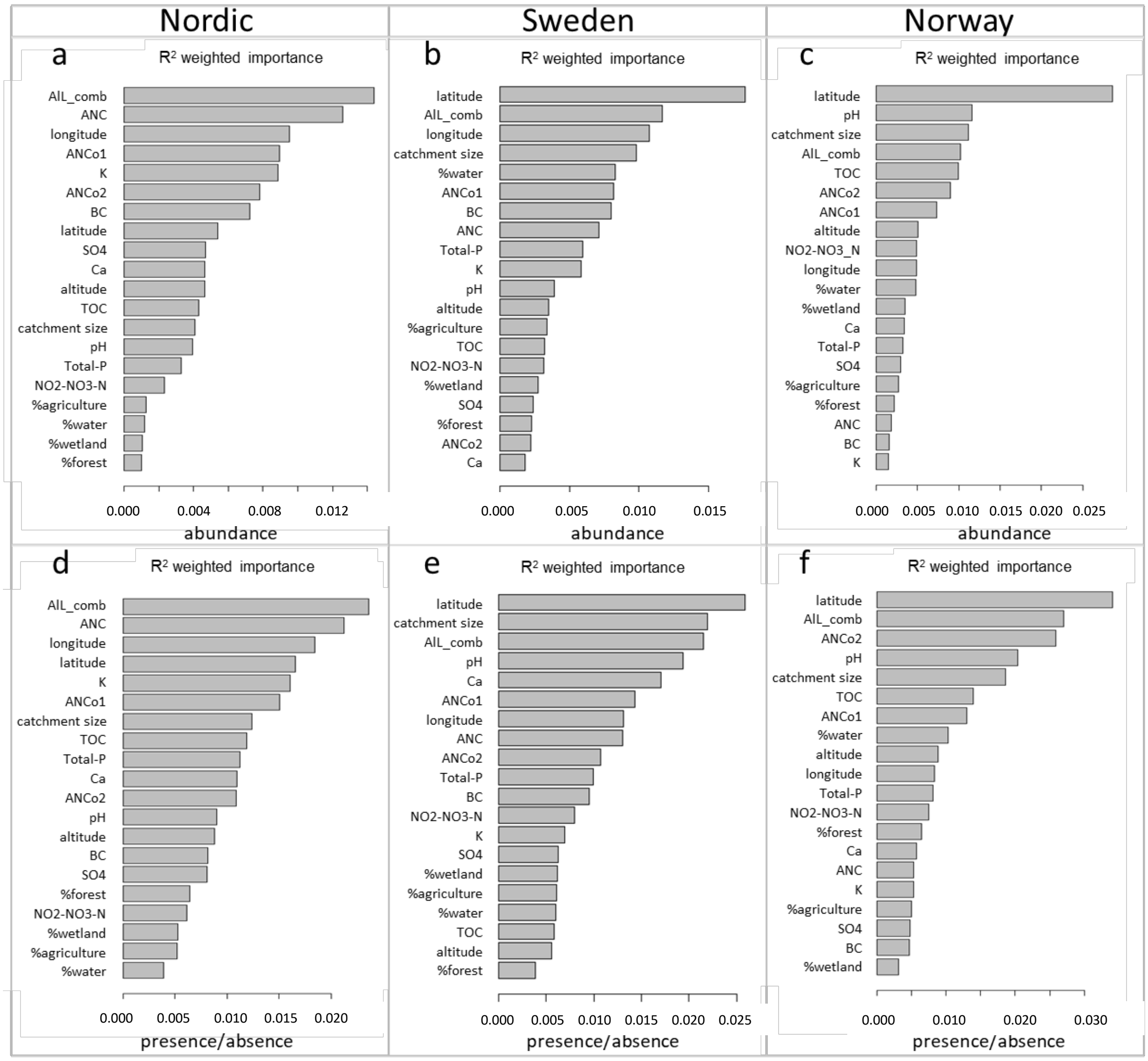


Figure 12. Stream invertebrate overall conditional importance of the acidity indicators and environmental spatial/environmental descriptors as predictors of relative taxa abundance (a, b, c) and presence/absence d, e, f) response for all sites (a, d), Swedish sites (b, e), and Norwegian sites (c, f). Ali is denoted AlL_comb,

Lake fish communities

Results indicated ANC was the most consistent acidity indicator across models as a high importance predictor of community change in lake fish (Figure 13, a-d). In the Nordic dataset, ANC had the greatest predictive importance followed closely by ANCo1 (Figure 13 a, c). In the Swedish relative abundances sub-dataset pH had the greatest predictive importance followed by ANCo2, ANCo1, and ANC, respectively (Figure 13 b). Using species presence/absence in the Swedish sub-dataset ANC had the greatest predictive importance followed very closely by ANCo1 and ANCo2 (Figure 13 d). For lake fish, compared to lake macroinvertebrates, acidity indicators were of moderate to low importance relative to several environmental/spatial predictors that were of high importance (Figure 13 a-d). TOC was of high importance in all models except in the Swedish sub-dataset using species presence/absence data (Figure 13 d). Altitude was of high importance across all models (Figure 13 a-d), and longitude in the Nordic dataset (Figure 13 a, c). Characteristics of lake size (catchment size, lake area, maximum depth) were of high to intermediate importance in the Swedish sub-dataset (Figure 13, b, d). Nutrients were of intermediate importance along with percent forest area in the catchment, while other categories of catchment land use were of lower importance (Figure 13 a-d). There were no separate analyses of Finnish or Norwegian data due to the low number of lakes in these datasets.

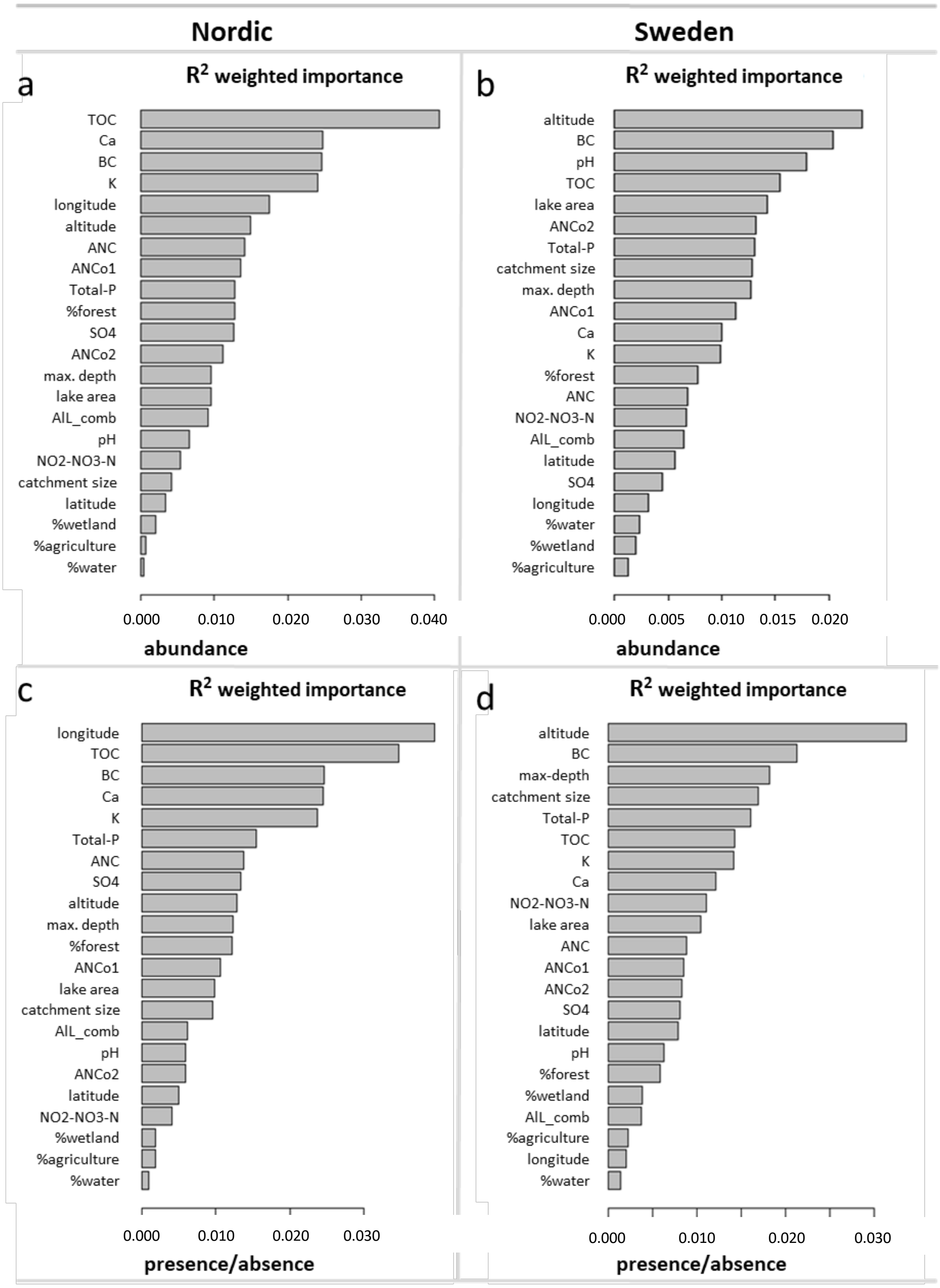


Figure 13. Lake fish overall conditional importance of the acidity indicators and environmental spatial/environmental descriptors as predictors of relative taxa abundance (a, b) and presence/absence (c, d) response for all sites (a, c), and Swedish sites (b, d). Ali is denoted AlL_comb.

Stream fish communities

Results of stream fish community relative abundance indicated that Ali was the acidity indicator of highest importance followed by ANCo2 and ANC with intermediate importance (Figure 14, a). For the presence/absence dataset, ANC had the highest importance followed very closely by Ali (Figure 14, b). Longitude, altitude, TOC, and potassium (K) were predictors of high importance (Figure 14, a-b). All other predictors were of intermediate or low importance (Figure 14, a-b). There were no separate analyses of Norwegian data due to the low number of river sites in this dataset.

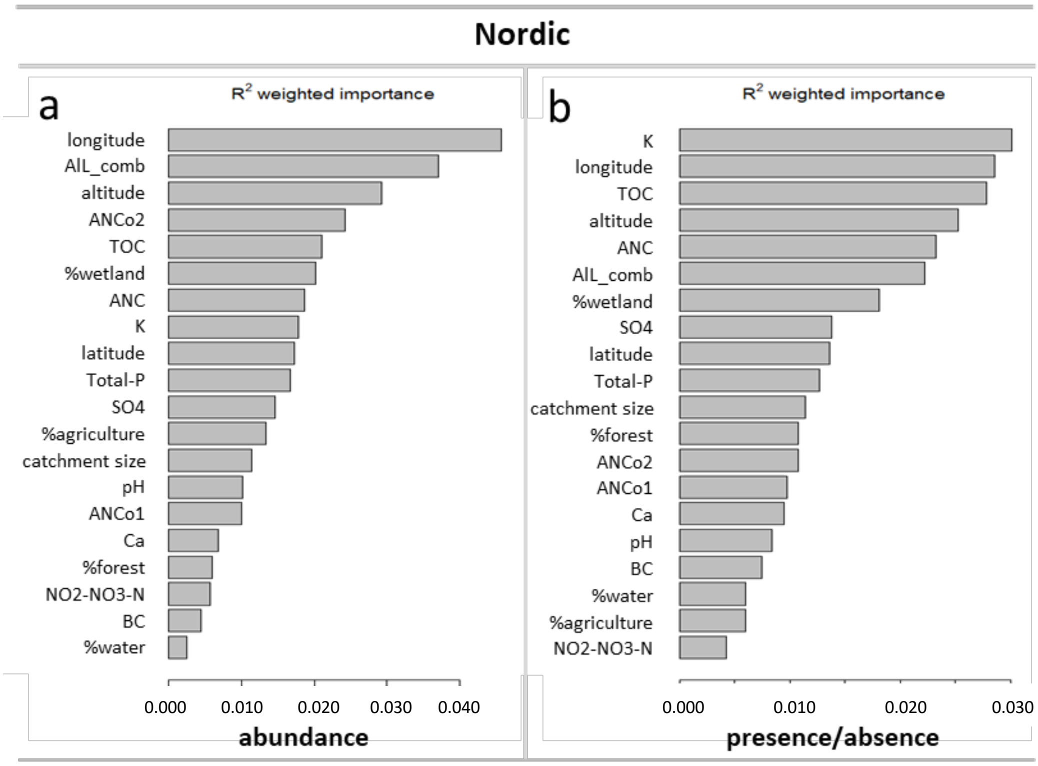


Figure 14. Stream fish overall conditional importance of the acidity indicators and environmental spatial/environmental descriptors as predictors of relative taxa abundance (a) and presence/absence (b) response for all sites. Ali is denoted AlL_comb,

4.2.2 Objective 2, Interactions with spatial/environmental variables.

Lake and stream invertebrates

Results of PCA for both lake and stream were similar; the first (PC1) and second (PC2) PC axis described the broad environmental gradients within a Nordic spatial context, while the third PC (PC3) axis was most strongly driven by catchment size (Table 9).

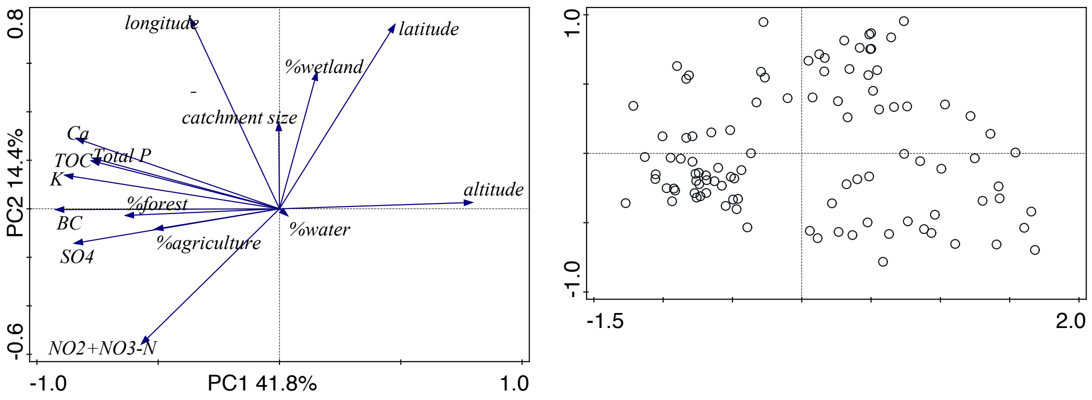
The first PC axis for lake invertebrate sites (PC1, eigenvalue 0.418) was related to increased nutrient enrichment (TotP, $NO_2 + NO_3$), humic acids (TOC), other water chemistry parameters (Ca, K, SO_4, BC) and agriculture and forest area in the catchment, and to decreased altitude (Table 9, Figure 15).

The first PC axis for stream invertebrate sites (PC1, eigenvalue 0.394) was similar to lakes except for NO_2+NO_3-N and the percent agricultural land use that had stronger influence in PC2 (Table 9, Figure 15). For lakes the second PC axis (PC2, eigenvalue = 0.144) was related to increase in wetland area, latitude and longitude (Table 9, Figure 15) and the third PC axis (PC3, eigenvalue = 0.109) was related to variables indicative of decrease in the percent water in the catchment with an increase in catchment size (Table 9). For streams the second PC axis (PC2, eigenvalue = 0.172) was related to an increase in nitrogen (NO_2+NO_3-N), agricultural land use, and percent wetland in the catchment with decreasing latitude and longitude (Table 9, Figure 15) The third PC axis for streams (PC3, eigenvalue = 0.111) was related to catchment size (Table 9).

Table 9. Loading matrix of PC1, PC2, & PC3 from principal component analysis for Nordic scale lake and stream spatial/environmental descriptors at invertebrate sites. Shaded bold cells indicate the strongest drivers of the PC gradient and shaded non-bolded cells indicate moderate drivers of the PC gradient.

	Lakes			Streams		
	PC1	PC2	PC3	PC1	PC2	PC3
TOC (mg/l)	**-0.7708**	0.2088	-0.1570	**0.8157**	-0.0486	0.4111
Tot-P (µg/l)	**-0.7761**	0.1982	-0.2388	**0.7920**	-0.1000	0.0806
NO2+NO3-N (µg/l)	-0.5694	-0.5580	-0.3218	0.2373	**0.7995**	-0.1360
Ca (µeq/l)	**-0.8387**	0.2904	0.1057	**0.7037**	0.0025	-0.4548
K (µeq/l)	**-0.8853**	0.1387	0.0698	**0.8154**	-0.0376	-0.3722
SO4 (µeq/l)	**-0.8456**	-0.1424	0.2800	**0.8029**	0.1777	-0.2044
BC (µeq/l)	**-0.9229**	-0.0035	0.1045	**0.812**	0.0965	-0.2544
% agriculture	-0.5141	-0.0842	-0.4066	0.4915	**0.6048**	-0.2143
% forest	-0.6353	-0.0275	-0.0332	**0.6843**	0.0342	0.5133
% water	0.0339	-0.0287	**0.6160**	-0.3865	0.2874	0.1939
% wetland	0.1548	0.5641	-0.4679	-0.1627	0.5208	-0.2815
catchment size (km^2)	-0.0032	0.3562	**-0.6427**	-0.4372	0.0451	**-0.5988**
altitude (masl)	**0.7955**	0.0262	-0.1367	**-0.7916**	-0.0083	-0.2792
latitude (WGS84)	0.4760	**0.7599**	0.1200	-0.0509	**-0.8424**	-0.3718
longitude (WGS84)	-0.3676	**0.7853**	0.3669	0.6193	**-0.6741**	-0.1473

a) Lake invertebrates



b) Stream invertebrates

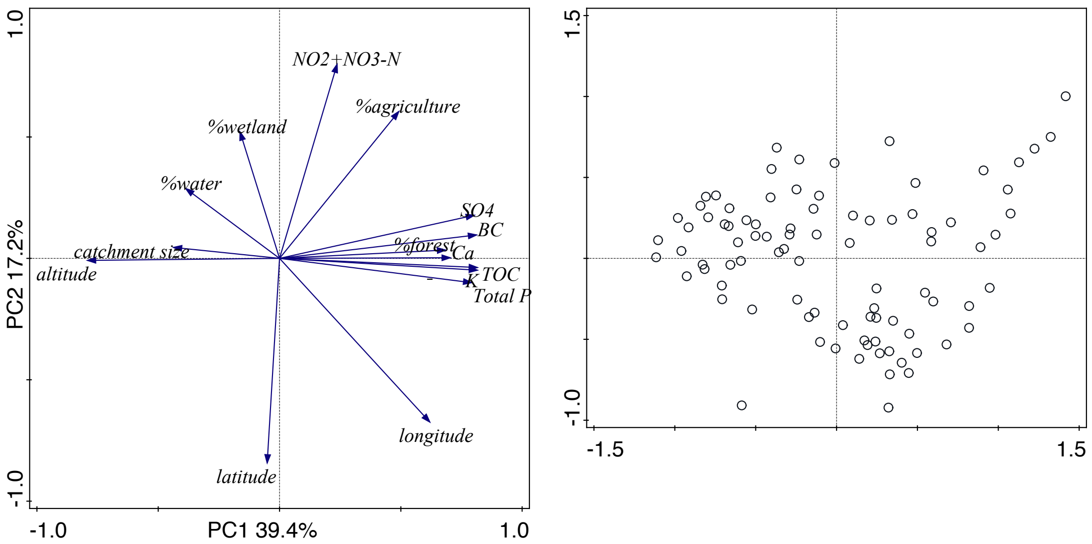


Figure 15. PCA of lake (a) and stream (b) spatial/environmental predictors at invertebrate sites. Variable scores (left) and site scores (right) are shown for PCI and PC2.

Lake and stream fish

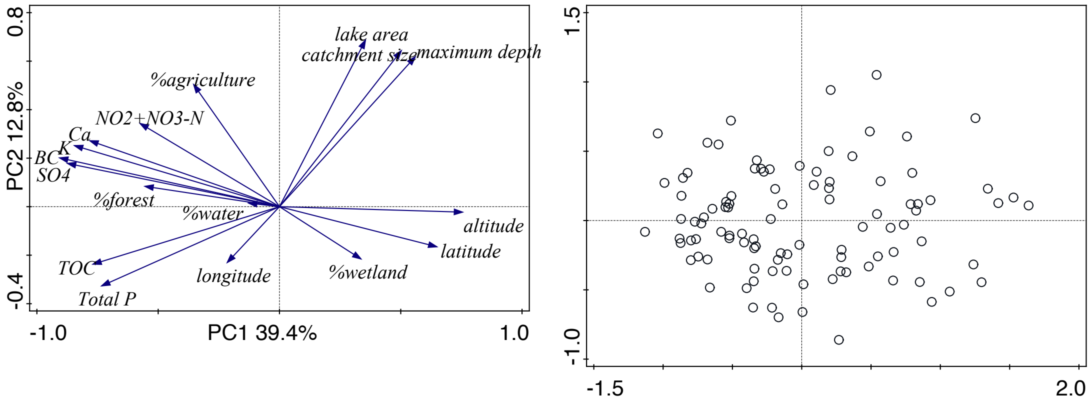
The first PC axis for lake fish sites (PC1, eigenvalues 0.394) was related to an increase in K, humic acids (TOC), SO4, nutrient enrichment (Tot-P, NO2 + NO3), and forest area in the catchment and to decreased latitude and altitude (Table 10, Figure 16). The second PC axis (PC2, eigenvalue = 0.128) was related to increase in lake size (lake area, lake depth), catchment size, and agricultural land use area in the catchment (Table 10, Figure 16). The third PC axis (PC3, eigenvalue = 0.121) was related to an increase in wetland area in the catchment and to increasing longitude and latitude (Table 10).

The first PC axis for stream fish sites (PC1, eigenvalues 0.422.) was related to an increase in BC, SO4, K, Ca, TOC, and total P at lower elevations (decreased altitude) and with increased longitude and increase in forest and agricultural area in the catchment (Table 10, Figure 16). The second PC axis (PC2, eigenvalue = 0.177) was related to increase in nitrogen (NO_2+NO_3-N) and to decrease in wetland area in the catchment a latitude (Table 10, Figure 16). The third PC axis (PC3, eigenvalue = 0.112) was related to catchment size (Table 10).

Table 10. Loading matrix of PC1, PC2, & PC3 from principal component analysis for lake and stream spatial/environmental descriptors for fish sites. Shaded bold cells indicate the strongest drivers of the PC gradient and shaded non-bolded cells indicate moderate drivers of the PC gradient.

	Lakes			Stream		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
TOC (mg/l)	**-0.7688**	-0.2216	0.2852	**0.8333**	-0.1251	0.3814
Tot-P (µg/l)	**-0.7269**	-0.3152	0.2471	**0.7995**	-0.0028	0.0487
NO_2+NO_3-N (µg/l)	**-0.5638**	0.3143	-0.4332	0.3107	**0.8551**	-0.1499
% agriculture	-0.3649	**0.5013**	-0.0153	**0.6267**	0.4822	-0.2649
% forest	**-0.6294**	0.1010	0.2089	**0.7332**	-0.0306	0.4884
% water	-0.1742	0.0095	-0.0712	-0.3994	0.2231	-0.2994
% wetland	0.3254	-0.1770	**0.5368**	0.2041	**-0.7337**	-0.0341
Ca (µeq/l)	**-0.7808**	0.2989	0.3654	**0.7074**	-0.1618	-0.4516
K (µeq/l)	**-0.8419**	0.2575	0.0895	**0.8305**	0.0275	-0.291
SO4 (µeq/l)	**-0.8739**	0.1680	-0.1338	**0.8175**	0.2086	-0.284
BC (µeq/l)	**-0.906**	0.2077	0.1164	**0.8025**	0.0045	-0.3056
catchment size (km^2)	0.4885	**0.6775**	0.3086	-0.3163	-0.1541	**-0.7082**
altitude (masl)	**0.7545**	-0.020	-0.0290	**-0.8188**	0.0781	-0.2313
latitude (WGS84)	**0.6406**	-0.1202	**0.6276**	-0.155	**-0.8118**	-0.2902
longitude (WGS84)	-0.2218	-0.1809	**0.7400**	**0.6819**	-0.5765	-0.051
maximum depth (m)	0.5614	**0.6140**	-0.1121	NA	NA	NA
lake area (km^2)	0.3401	**0.7203**	0.4082	NA	NA	NA

a) Lake fish



b) Stream fish

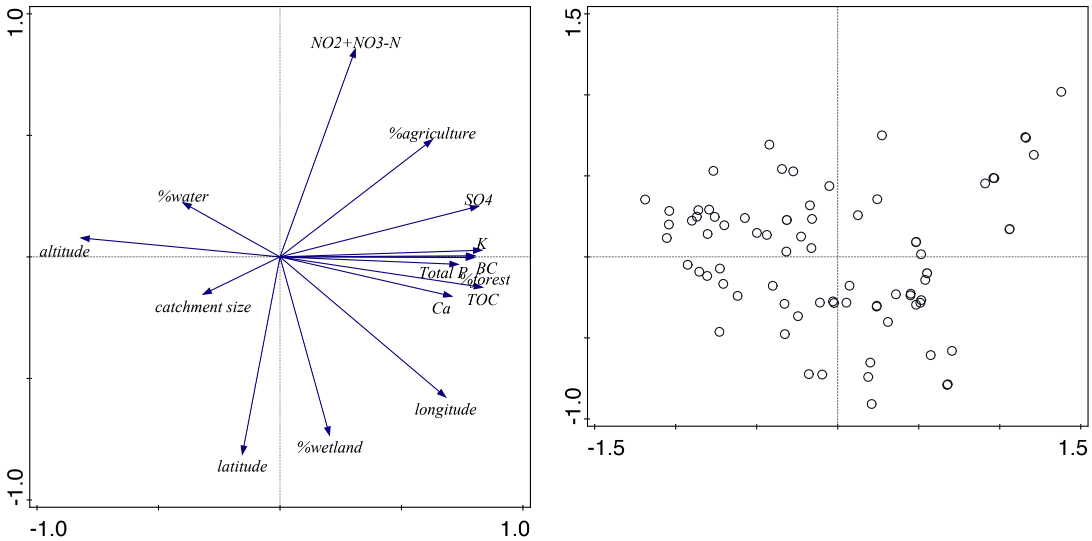


Figure 16. PCA of lake (a) and stream (b) spatial/environmental predictors at fish sites. Variable scores (left) and site scores (right) are shown for PCI and PC2.

Interactions

The second criterion in choice of an acidity indicator was that an acidity indicator is robust against interactions with other parameters. Data was insufficient to test interactions of Ali. In all models effects of pH were more often dependent on interacting parameters compared to ANCo2, ANCo1 and ANC (Table 11).

Table 11. Number of significant interactions between acidity indicators and spatial/environmental gradients (PC1, PC2, PC3) GAMs for lake invertebrates, stream invertebrates, lake fish, and stream fish response variables of abundance ($CA1_{SQRT}$, $CA2_{SQRT}$), and presence/absence ($CA1_{P/A}$, $CA2_{P/A}$).

		number of significant interactions			
		total	PC1	PC2	PC3
Lake invertebrates	pH	**7**	3	2	2
	ANCo1	**0**	0	0	0
	ANC	**0**	0	0	0
	ANCo2	**0**	0	0	0
River invertebrates	pH	**8**	4	2	2
	ANCo1	**1**	1	0	0
	ANC	**4**	2	2	0
	ANCo2	**2**	1	1	1
Lake fish	pH	**2**	2	0	0
	ANCo1	**1**	1	0	0
	ANC	**2**	2	0	0
	ANCo2	**0**	0	0	0
River fish	pH	**5**	1	1	3
	ANCo1	**3**	1	2	0
	ANC	**0**	0	0	0
	ANCo2	**1**	1	0	0
TOTAL	**pH**	**22**	**10**	**5**	**7**
	ANCo1	**5**	**3**	**2**	**0**
	ANC	**6**	**4**	**2**	**0**
	ANCo2	**3**	**2**	**1**	**0**

4.2.3 Objective 3, Gradient Responses and Thresholds

The focus of the second part of the analyses was to explore the empirical shape and magnitude of changes in composition along acidification gradients and identify any critical values of acidity indicators along these gradients that correspond to threshold changes in biological composition. Based on results considering the first and second criteria (objectives 1 and 2) we focused our analysis of gradient responses and thresholds based on values of ANC.

Macroinvertebrates

The frequency distributions of split importance showed that changes in lake and stream macroinvertebrate assemblage along acidification gradients were nonuniform. Locations on the gradient where the splits density (black line) was greater than data density (red line) (ratio > 1, e.g. Figure 17 a, c) indicate higher relative importance for compositional change. Data densities were biased because of unequal distribution along acidification indicator values (e.g., red 'density of data' line). To overcome this, the density of splits was standardized by the density of data to get the ratio of density (blue line) (e.g. Figure 17 a, c). Locations on the gradient where the splits density was greater than data density (ratio > 1, e.g. Figure 17 a, c) indicate higher relative importance for compositional change, and locations of high relative rates of assemblage change where ratio density (e.g. Figure 17 a, c, blue line) was greater than data density (e.g. Figure 17 a, c, red line). Critical threshold values along acidification indicator gradients are indicated by the initial (green solid arrow) and secondary (green dashed arrow) high relative rate (blue>red line) of assemblage change encountered starting from the greatest acidification indicator values (x axis far right) (e.g. Figure 17 a, c). For example, along the ANC gradient in the relative abundance lake macroinvertebrate dataset, four important splits occurred at *c*. 260 *c*. 160, *c*. 110, and *c*. 20 µeq/l, indicating large changes in taxa abundance and composition corresponding to thresholds of community change (Figure 17 a). The standardized and accumulative split importance values show the shapes of cumulative change in abundance of each taxon (e.g. Figure 17 b, d). Changes for individual taxa varied in magnitude and threshold values along these gradients, and those contributing to overall compositional change can be identified. In these nonlinear curves, shallow slopes indicate low rates of change, whereas steep slopes indicate high rates (e.g. Figure 17 b, d).

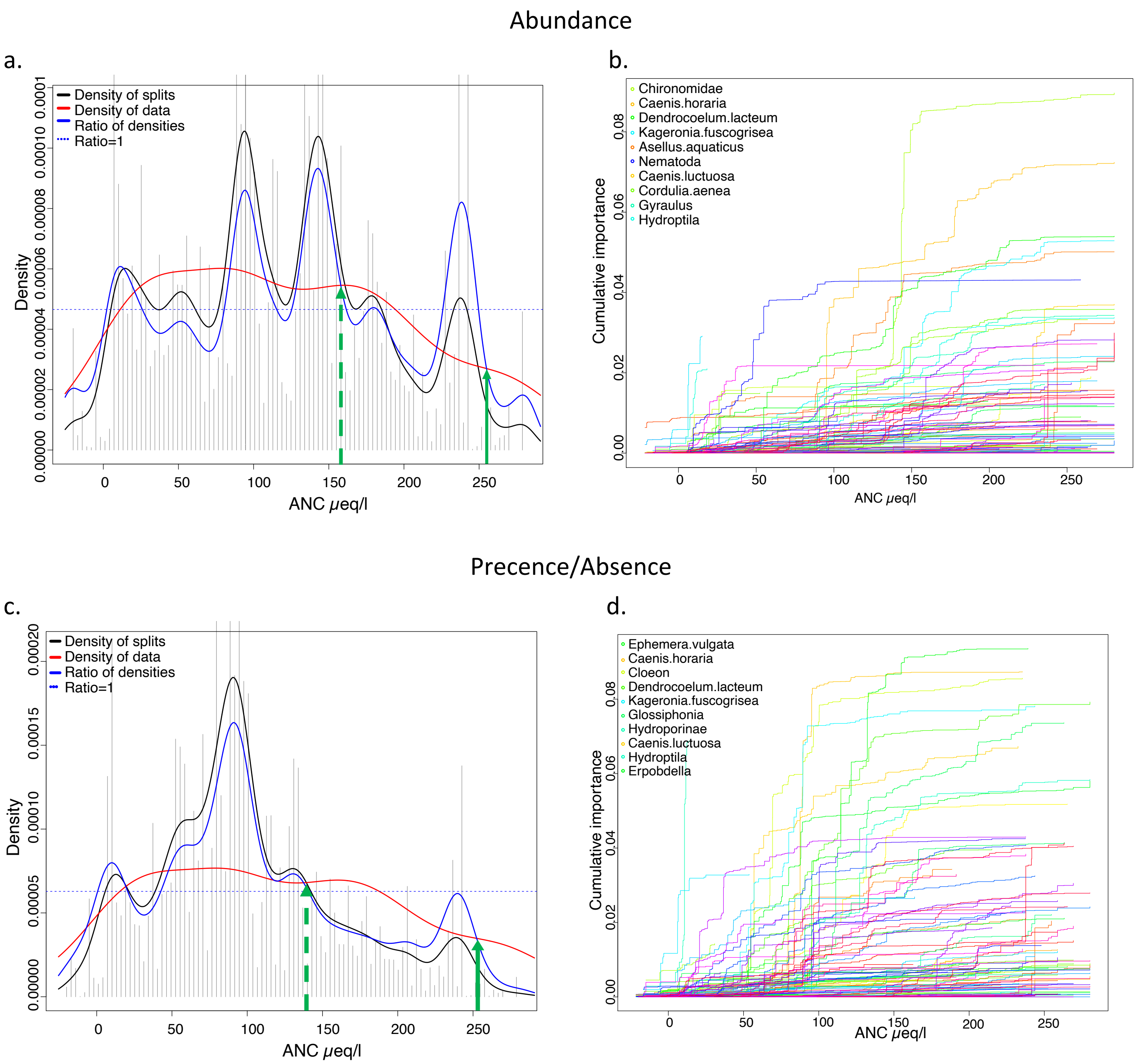


Figure 17. Lake macroinvertebrate responses of relative abundance (a, b) and presence/absence (c, d). Density plots (a, c) of splits location and importance on gradient (histogram), density of splits (black line _) and observations (red line _) and ratio of splits standardized by observation density (blue line _) (a, c). Ratios >1 indicate locations of relatively greater change in composition. Initial critical threshold (solid green arrow), secondary critical threshold (dashed green arrow). Cumulative distributions of standardized splits importance plots (b, d) for each species scaled by R^2. The most important taxa driving community change are listed in the upper left corner of b and d.

The greater splits densities indicated greater community change in lake macroinvertebrates after the tertiary threshold in relative abundance data and secondary in presence/absence data (Figure 17 a, c). The greatest community change of lake macroinvertebrates using relative abundance and presence/absence data coincided in the range of *c*. 160-80 μeq/l ANC in terms of splits densities (Figure 17 a, c), although cumulative distributions of standardized splits importance for each species differed between datasets (Figure 17 b, d). In the threshold range of *c*. 160-125 μeq/l ANC for relative abundance data the taxa most important for driving community changes were Chironomidae, *Cordulia aenea*, *Asellus aquaticus*, and the flatworm species *Dendrocoelum lacteum* (Figure 17 b). In the threshold, range of *c*. 110-50 μeq/l ANC for relative abundance data the taxa most important for driving community changes was *Caenis horaria* (Figure 17 b). In the threshold range of c. 120-45 μeq/l ANC for relative presence/absence data the taxa most important for driving community changes were *Caenis horaria*, *Kageronia fuscogrisea*, the mayfly genus *Cloeon*, the mayfly species *Ephemera vulgata*, and *Dendrocoelum lacteum*, respectively (Figure 17 d).

Inspection of site distributions in lake macroinvertebrates at the secondary threshold in relative abundance data corresponded to diminishing site occurrences of three mayfly taxa *Ephemera vulgata*, *Hydroptila* sp., *Caenis horaria*, *Gyraulus* sp., and *Glossiphonia* sp. (25% quartile site occurrence mean=132 μeq/l ANC, range=127-138 μeq/l ANC) (Figure 17 b, d, Table 12). The next most sensitive taxa corresponded to diminishing site occurrences of *Caenis luctuosa*, *Cloeon* sp., and the flatworm *Dendrocoelum lacteum* at higher ANC values (25% quartile site occurrence ANC mean=113 μeq/l, range=108-116 μeq/l), and at lower ANC values the *Kageronia fuscogrisea*, *Cordulia aenea*, *Turbellaria* sp., and *Asellus aquaticus* (25% quartile site occurrence ANC mean=96 μeq/l, range=91-99 μeq/l) (Figure 17 b, d, Table 12). The less sensitive taxa important for community change included decreasing occurrences of *Laccophilus* sp., and Chironomidae (25% quartile site occurrence ANC mean=12 μeq/l, range= -15-47 μeq/l) (Figure 17 b, d, Table 12).

Table 12. Median, 25% quartile, and minimum ANC values (μeq/l) at site occurrences of the most important macroinvertebrate taxa contributing to overall compositional change in lakes, for combined results of Gradient Forest using relative abundance and presence/absence of macroinvertebrate taxa.

			ANC (μeq/l)			
Phylum/Order/Class	Family	Genis species	minimum	25% quartile	median	N
Annelidia						
Arhynchobdellida	Erpobdellidae	Erpobdella sp.	13	96	153	73
Rhynchobdellida	Glossiphoniidae	Glossiphonia sp.	71	134	159	38
Malacostraca						
Isopoda	Asellidae	*Asellus aquaticus*	-25	97	158	105
Mollusca						
Gastropoda	Planorboidae	*Gyraulus sp.*	45	127	174	55
Platyhelminthes						
Rhabditophora	Planarian	*Dendrocoelum lacteum*	28	116	158	59
Turbellaria	Turbellaria sp.	Turbellaria sp.	8	98	154	60
Insecta						
Coleoptera	Dytiscidae	*Laccophilus sp.*	-15	-15	-5	4
	Dytiscidae (Hydroporinae)	Hydroporinae sp.	-15	4	8	7
Diptera	Chironomidae	Chironomidae sp.	-25	47	107	164
Ephemeroptera	Caenidae	*Caenis horaria*	64	127	169	78
	Caenidae	*Caenis luctuosa*	45	114	166	68
	Baetidae	*Cloeon sp.*	17	108	157	72
	Ephemeridae	*Ephemera vulgata*	25	138	177	57
	Heptageniidae	*Kageronia fuscogrisea*	21	99	159	100
Odonata	Corduliidae	*Cordulia aenea*	7	91	144	58
Trichoptera	Hydroptilidae	*Hydroptila sp.*	64	134	172	48

In streams, compared to lakes, the initial threshold occurred at higher values of ANC (e.g. relative abundance *c*. 290 μeq/l ANC) (Figure 18 a). This result corresponds with the higher temporal variability of water acidity in streams compared to lakes with a deviation of annual minimum mean from mean values of 75 μeq/l ANC (standard deviation = 48 μeq/l) for streams and 44 μeq/l ANC(standard deviation = 39 μeq/l) for lakes. For relative abundance data, the important changes after the initial threshold covered a broad range *c*. 290-135 μeq/l ANC, peaking at *c*. 260 μeq/l ANC followed by another increase at *c*. 175 μeq/l ANC (Figure 18 a). For presence/absence data, the greatest changes occurred after the secondary threshold peaking at *c*. 170 μeq/l ANC, thus correlating with the smaller peak observed in relative abundance data (Figure 18 a, d). For the presence/absence data after the initial threshold, community change indicated a smaller peak at *c*. 250 μeq/l ANC (Figure 18 c), roughly correlating with the greatest peak observed with relative abundance (Figure 18 a). For relative abundance data, important taxa for driving community changes at the initial peak (*c*. 260 μeq/l ANC) was *Anispotera* (Figure 18 b). The taxa most important at the increase in community change observed at *c*. 135-190 μeq/l ANC with relative abundance data were *Hydraena*, *Ithytrichia* and *Hyptagenia* respectively (Figure 18 b). For presence/absence data, taxa most important for driving community changes after the threshold c. 250 μeq/l ANC was *Silo pallipes* (Figure 18 d). Taxa most important for driving community changes for the peak at c. 120-190 μeq/l for presence/absence data were *Hydraena*, *Sericostoma personatum*, the mayfly *Baetis niger* and *Agapetus* sp., respectively (Figure 18 d).

Inspection of site distributions in stream macroinvertebrates indicated the most sensitive taxa corresponded to diminishing site occurrences of *Agapetus* sp. and *Hydropsyche angustipennis* (25% quartile site occurrence ANC mean=178 μeq/l, range=169-187 μeq/l) (Figure 18 b. d, Table 13). The next most sensitive taxa corresponded to diminishing site occurrences of *Silo pallipes*, *Hydraena* sp., *Centroptilum luteolum*, *Sericostoma personatum*, and *Baetis muticus* (25% quartile site occurrence ANC mean=147 µeq/l, range=135-153 µeq/l) (Figure 18 b. d, Table 13), followed by *Capnopsis schilleri*, *Baetis niger*, *Heptagenia dalecarlica*, *Baetis* sp., Muscidae sp., Psychodidae sp., *Ithytrichia* sp., and *Gyraulus* sp. (25% quartile site occurrence ANC mean=110 µeq/l, range=96-120 µeq/l) (Figure 18 b. d, Table 13). The less sensitive taxa important for community change included decreasing occurrences of *Heptagenia* sp., *Elmis aenea*, *Anisoptera* sp., and *Tipulidae* sp. (25% quartile site occurrence ANC mean=82 µeq/l, range=68-88 µeq/l) (Figure 18 b. d, Table 13).

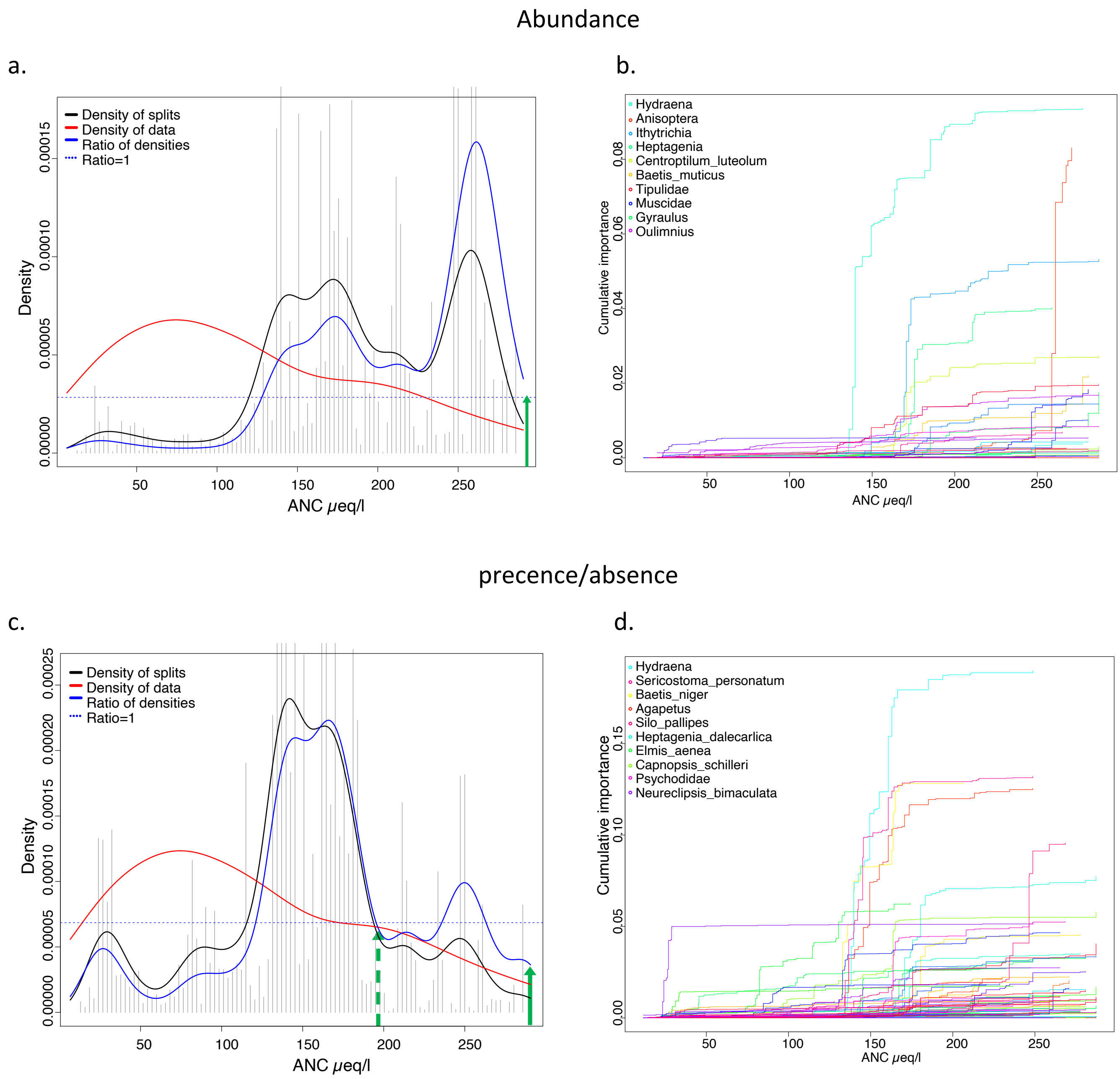


Figure 18. Stream macroinvertebrate responses of relative abundance (a, b) and presence/absence (c, d). Density plots (a, c) of splits location and importance on gradient (histogram), density of splits (black line _) and observations (red line _) and ratio of splits standardized by observation density (blue line) (a, c). Ratios >1 indicate locations of relatively greater change in composition. Initial critical threshold (solid green arrow), secondary critical threshold (dashed green arrow). Cumulative distributions of standardized splits importance plots (b, d) for each species scaled by R^2. The most important taxa driving community change are listed in the upper left corner of b and d.

Table 13. Median, 25% quartile, and minimum ANC values (µeq/l) at site occurrences of the most important macroinvertebrate taxa contributing to overall compositional change in streams, for combined results of Gradient Forest using relative abundance and presence/absence of macroinvertebrate taxa.

			ANC (µeq/l			
Phylum/Class/Order	Family	Genis species	minimum	25% quartile	median	N
Mollusca						
Gastropoda	Planorboidae	*Gyraulus sp.*	45	96	198	13
Insecta						
Coleoptera	Elmidae	*Elmis aenea*	22	86	140	54
	Hydraenidae	*Hydraena sp.*	24	150	188	31
Diptera	Muscidae	Muscidae *sp.*	22	109	182	13
	Psychodidae	Psychodidae *sp.*	24	108	181	27
	Tipulidae	Tipulidae *sp.*	7	68	124	53
Ephemeroptera	Baetidae	*Baetis sp.*	24	113	202	20
		Baetis muticus	24	135	202	24
		Baetis niger	33	118	182	42
		Centroptilum luteolum	84	149	182	15
	Heptagenidae	*Heptagenia sp.*	45	88	164	24
		Heptagenia dalecarlica	65	115	194	24
Odonata	Anisoptera sp.	Anisoptera *sp.*	29	84	166	11
Plecoptera	Capniidae	*Capnopsis schilleri*	74	120	180	30
Trichoptera	Hydropsychidae	*Hydropsyche angustipennis*	81	187	210	8
	Hydroptilidae	*Ithytrichia sp.*	22	104	182	25
	Glossosomatidae	*Agapetus sp.*	62	169	205	21
	Sericostomatidae	*Sericostoma personatum*	45	146	184	34
	Goeridae	*Silo pallipes*	105	153	185	16

Lake fish

The peaks range of important community change of lake fish was similar using relative abundance and presence/absence data in terms of splits densities (*c*. 150-70 µeq/l ANC) and threshold values of ANC (Figure 19, Table 14), although cumulative distributions of standardized splits importance for each species differed between datasets (Figure 19, Table 14). For relative abundance data from the major peak *c*. 150-70 µeq/l ANC roach (*Rutilus rutilus*) were the most important species driving community change followed by pike (*Esox lucius*), perch (*Perca fluviatilis*), and smelt (*Osmerus eperlanus*), respectively (Figure 19 b). For presence/absence data from the major peak threshold (*c*. 40-145 µeq/l ANC), roach were the most important species driving community change followed by ruffe (*Gymnocephalus cernuus*), perch, and pike, respectively (Figure 19 d).

Inspection of site distributions in lake fish indicated the most sensitive species corresponded to diminishing site occurrences of eight species within five families; smelt, bream (*Abramis brama*), roach, rudd (*Scardinius erythrophthalmus*), vendace (*Coregonus albula*), burbot (*Lota lota*), ruffe, and bleak (*Alburnus alburnus*) (25% quartile site occurrence ANC mean=130 µeq/l, range=120-157 µeq/l) (Figure 19 b, d, Table 14). The next most sensitive species corresponded to diminishing site occurrences of pike, perch, and Eurasian minnow (*Phoxinus phoxinus*) (25% quartile site occurrence ANC mean=91 µeq/l, range=76-103 µeq/l) (Figure 19 b, d, Table 14). Brown trout were the least sensitive species important for community change (25% quartile site occurrence ANC mean=29 µeq/l) (Figure 19 b, d, Table 14).

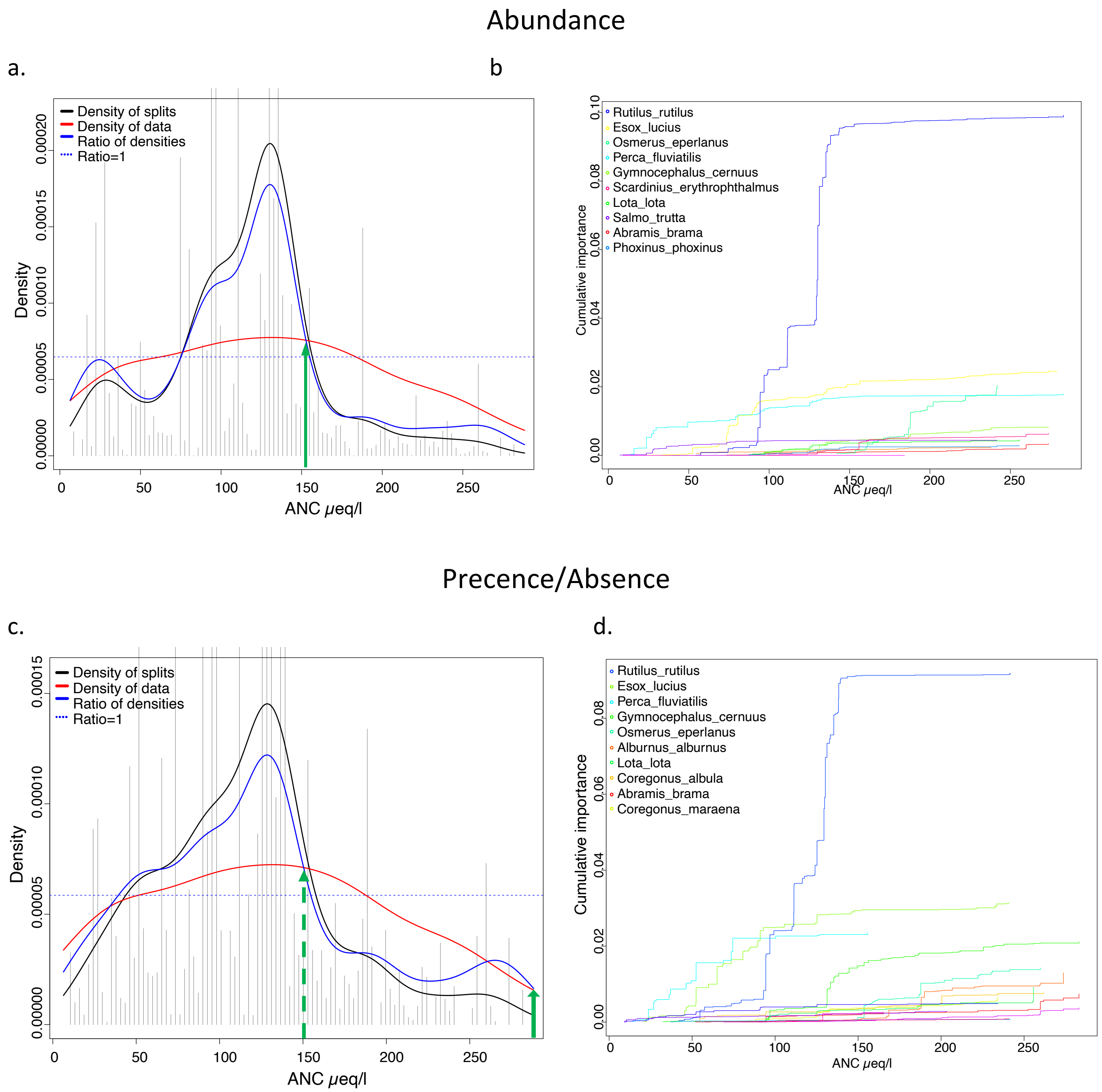


Figure 19. Lake fish responses of relative abundance (a, b) and presence/absence (c, d). Density plots (a, c) of splits location and importance on gradient (histogram), density of splits (black line _) and observations (red line _) and ratio of splits standardized by observation density (blue line _) (a, c). Ratios >1 indicate locations of relatively greater change in composition. Initial critical threshold (solid green arrow), secondary critical threshold (dashed green arrow). Cumulative distributions of standardized splits importance plots (b, d) for each species scaled by R^2. The most important taxa driving community change are listed in the upper left corner of b and d.

Table 14. Median, 25% quartile, and minimum ANC values (μeq/l) at site occurrences of the most important fish species contributing to overall compositional change in lakes, for combined results of Gradient Forest using relative abundance and presence/absence of fish species.

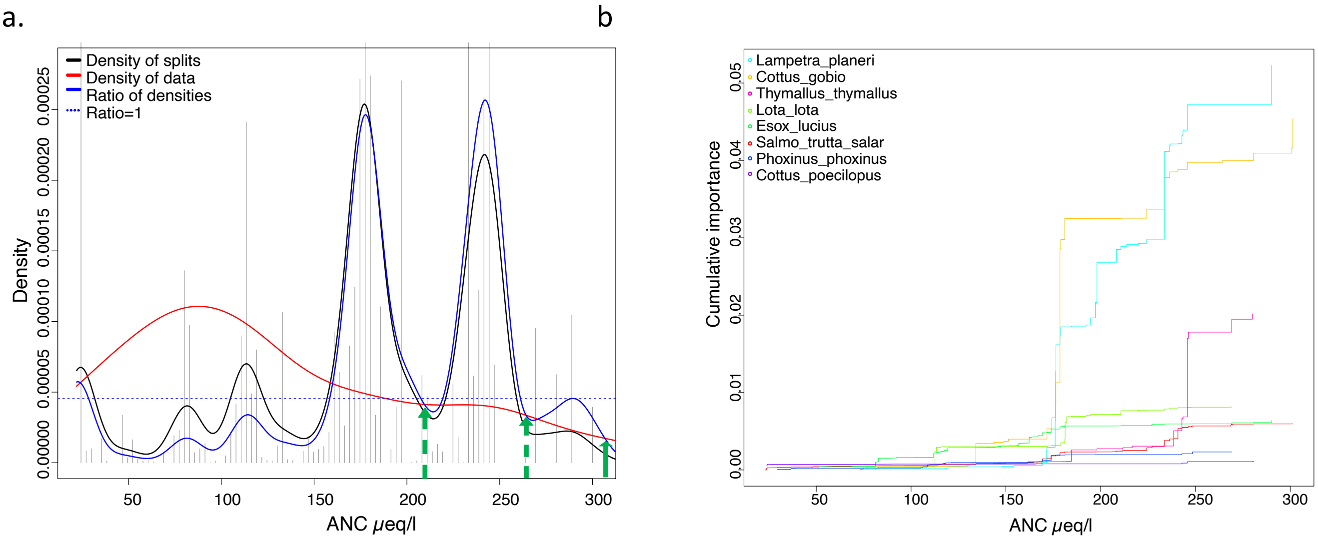
		ANC (μeq/l)			
Family	Genis species	minimum	25% quartile	median	N
Cyprinidae	*Abramis brama*	77	135	159	16
	Alburnus alburnus	98	120	188	11
	Phoxinus phoxinus	45	76	123	11
	Rutilus rutilus	57	130	168	63
	Scardinius erythrophthalmus	57	130	160	15
Esocidae	*Esox lucius*	24	103	149	80
Lotidae	*Lota lota*	98	122	171	14
Osmeridae	*Osmerus eperlanus*	120	157	188	10
Percidae	*Gymnocephalus cernuus*	31	121	162	36
	Perca fluviatilis	21	94	142	97
Salmonidae	*Coregonus albula*	60	125	172	13
	Salmo trutta	6	29	60	26

Stream fish

The most important community change of stream fish using relative abundance and presence/absence corresponded at values of *c*. 175 μeq/l ANC (Figure 20, Table 15). However, for relative abundance data another high peak of important community changes occurred at greater values of ANC after the secondary threshold (*c*. 240 μeq/l) (Figure 20 a). For the peak at *c*. 240 μeq/l ANC observed using relative abundance data brook lamprey (*Lampetra planeri*) were the most important species driving community change followed by European bullhead (*Cottus gobio*), grayling (*Thymallus thymallus*), respectively (Figure 20 a, b). At the secondary and greatest peak observed for relative abundance data (*c*. 175 μeq/l ANC) European bullhead and brook lamprey were the most important species driving community change followed by pike (*Esox lucius*), and burbot (*Lota lota*), respectively (Figure 20 a, b). For the corresponding presence/absence data splits densities peak at *c*. 215-140 μeq/l ANC the most important species driving community change were European bullhead and brook lamprey, respectively (Figure 20 c, d).

Inspection of site distributions in stream fish indicated the most sensitive species was grayling (25% quartile site occurrence 212 μeq/l ANC) (Table 15). However this result should be interpreted with caution as grayling only occurred at five sites and it is probable that these results are more related to regional distribution and habitat preference rather than a response to increasing acidity. Site distributions of the most sensitive species following grayling corresponded to diminishing site occurrences of brook lamprey (25% quartile site occurrence 175 μeq/l ANC) (Table 15). The next most sensitive taxa corresponded to diminishing site occurrences of European bullhead, pike, roach, burbot, Eurasian minnow, and perch (25% quartile site occurrence ANC mean=113 μeq/l, range=104-120 μeq/l) (Table 15). Brown trout/Atlantic salmon and alpine bullhead were the least sensitive taxa important for community change (25% quartile site occurrence ANC mean=76 μeq/l, range=69-82 μeq/l) (Table 15).

Abundance



Precence/Absence

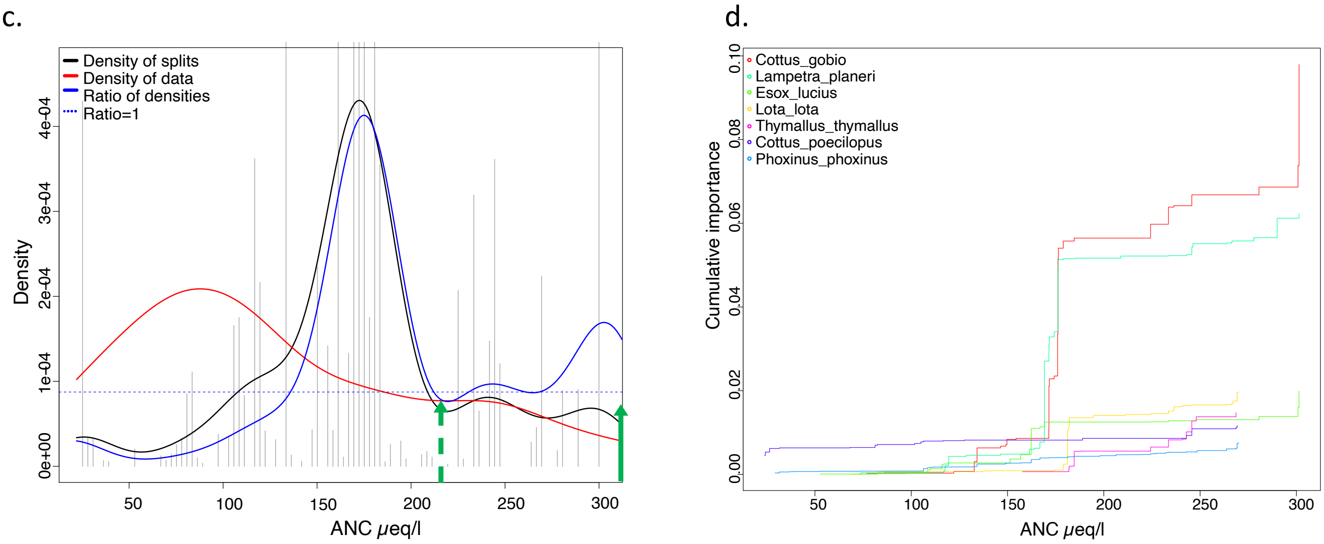


Figure 20. Stream fish responses of abundance (a, b) and presence/absence (c, d). Density plots (a, c) of splits location and importance on gradient (histogram), density of splits (black line _) and observations (red line _) and ratio of splits standardized by observation density (blue line _) (a, c). Ratios >1 indicate locations of relatively greater change in composition. Initial critical threshold (solid green arrow), secondary critical threshold (dashed green arrow) and tertiary critical threshold (dotted green arrow). Cumulative distributions of standardized splits importance plots (b, d) for each species scaled by R^2. The most important taxa driving community change are listed in the upper left corner of b and d.

Table 15. Median, 25% quartile, and minimum ANC values (µeq/l) at site occurrences of the most important fish species contributing to overall compositional change in streams, for combined results of Gradient Forest using relative abundance and presence/absence of fish species.

		ANC (µeq/l)			
Family	Genis species	minimum	25% quartile	median	N
Cottidae	*Cottus gobio*	64	120	179	11
	Cottus poecilopus	24	82	248	7
Cyprinidae	*Phoxinus phoxinus*	34	109	138	21
	Rutilus rutilus	66	114	174	9
Esocidae	*Esox lucius*	66	118	178	23
Lotidae	*Lota lota*	66	113	198	14
Percidae	*Perca fluviatilis*	24	104	174	14
Petromyzontidae	*Lampetra planeri*	66	175	197	12
Salmonidae	*Salmo trutta/salar*	22	69	109	80
	Thymallus thymallus	185	212	248	5

4.2.4 Time series analysis

The main approach of this project was to analyse the relation between water chemistry and biological quality elements for means over several years from each site. This between site relation will then be used to predict the sensitivity of the species communities to acidification over time for single sites. An attempt to test this space for time replacement was made by a time series analysis found in Appendix 2. The trends in both chemistry and biology, however, were too weak for the time period analysed to make any conclusions.

4.3 Discussion

The statistical evaluation of the joint dataset indicated ANC as the most relevant acidity chemistry predictor for biota. This contrasts to some degree with earlier studies showing pH as a superior predictor compared to ANC (Fölster 2007, Hesthagen 2008). Biological acidity indices are often based on the relation to pH, sometimes because pH was available for many more sites than other acidity predictors, as when developing the Norwegian-Swedish acidity index for lake fish (Holmgren et al. 2018). However, this study has revealed that pH is sub-optimal because; i) the predictive importance of pH was lower than ANC in all models using a larger scale Nordic dataset, and ii) relationships between pH and biota were highly dependent on other environmental factors (i.e. significant interactions). In contrast to pH, all ANCs displayed much fewer interactions with environmental/spatial variables. The modifications of ANC to account for that a part of the organic acids can be regarded as strong acids, ANCo1 and ANCo2, did not result in a higher prediction power. Rather the opposite. Further, the modified ANCs only gave slightly fewer interactions in the GAM analysis compared to ANC. The lack of importance of strong organic acids was somewhat surprising and contrasts with earlier studies (e.g. Hesthagen 2008).

Present Nordic ecological quality criteria based on pH or modified ANC are more or less sensitive to the concentration of DOC and how it has changed over time. This is especially true for the Swedish system based on change in pH. With a system based on ANC, the importance of changes in DOC on acidification classification will be less. If DOC is mainly controlled by the near stream zone, as found by Ledesma et al. (2016), the effect of DOC changes on ANC dynamics is negligible. However, if

DOC is originating from the whole catchment it will have an effect on the ion balance of soil water that is important to the dynamics of ANC (Hruska, 2014).

For lake invertebrate and fish, for both relative abundance and presence/absence, there were a pronounced upper thresholds at around 150 µeq/l ANC with one or two peaks between 90 and 140 µeq/l ANC. In rivers, an additional peak starting above 300 µeq/l for invertebrate and 260 µeq/l for fish was found for the relative abundance data. In the presence/absence data, these peak were only hinted. Likewise, the upper threshold in the greatest community changes in presence/absence data for both stream invertebrates and fish started around 200 µeq/l. The higher threshold in rivers is likely due to the higher temporal variability in acid condition in streams, with the biotic responses reflecting the most acid conditions. Although speculative, as our dataset of water chemistry did not cover extreme events, the mean values we used can be interpreted as the risk for getting ANC levels below the critical levels during extreme events.

The thresholds of greatest importance (initial and secondary) corresponded with invertebrate taxa shown to be sensitive or highly sensitive to acidity in previous studies (e.g. Moe et al. 2010) particularly species of mayflies, caddisflies, mollusks (e.g. snails), and leeches. Other taxa were important for overall community turnover, such as the riffle beetle *Elmis aenea*, the fly (dipteran) families Muscidae and Tipulidae, but were not necessarily correlated with or driving important thresholds associated with acidity.

For lake fish, roach were by far the most acid sensitive species, a finding that corroborates with earlier studies of lake acidification in the Nordic boreal region (e.g. Rask et al. 1995). For stream fish, brook lamprey and European bullhead were the most acid sensitive species, a finding supported by other studies that have shown negative effects of increasing acid waters on these species (Goodwin et al. 2008; Degerman and Appelberg 1992). However, in our study minimum values of ANC at site occurrences of brown trout (lakes) and brown trout/Atlantic salmon (streams) contrasts with the fact that trout is used as an acidification indicator species. In Norway, brown trout is the fish species with by far the most lost or affected stocks due to acidification (Tammi et al. 2003). Brown trout is also the only fish species in many Norwegian lakes, partly because of prehistorical introduction in lakes. It is also chosen as indicator species because of its economic importance and because much information spanning many decades exist on the density and population structure for many brown trout populations (Bulger et al., 1993; Enge et al., 2017). Speculatively, our contrasting results could be a result of migratory behaviour and/or resistance of population strains representing genetic adaptations to waterbodies that are naturally acidified (Swarts, Dunson, & Wright 1978). Nevertheless, our findings suggest that when brown trout and Atlantic salmon are used as acidification indicator species, inclusion of other sensitive species, particularly those which are non-migratory, would improve the determination of critical thresholds and development of biological indices.

The analysis of the time series gave some support to the conclusions from the static analysis with Gradient Forest. Due to weak trends in both chemistry and biology during the time span, the data did not allow for an in-depth analysis of the effect on biota from changes in acidity over time. The availability of intercomparable data from the preceding time periods with more dramatic changes from acidification and recovery is low. This means that we are limited to spatial comparisons, like the one in chapter 4, if we want to base the classification on the relationship between water chemistry and the whole ecosystem and not just single well-known species.

5 Quantification of thresholds, absolute change or relative change of physiochemical parameters as criteria for acidification.

In this chapter we propose that classification of acidification is based on the results from the Nordic dataset on biology and chemistry and less on expert judgement. The demand from the WFD is that the classification from water chemistry, when applicable, should reflect a classification from biological quality elements, at least on average. We here exemplify how this can be done by using the Swedish macroinvertebrate acidity index (MILA) used for classifying lake acidity. Since the MILA index was intercalibrated within the Northern GIG, these drafts for classification has relevance even outside of Sweden (Poikane et al. 2016). Given that the MILA index did not show any difference in response for different water body types in the WFD, typologies to partition natural variability were not used in our calculations (Lindegarth et al. 2016). Similar principles can be applied to other indices, and if it is revealed that responses differ by water body types, the calculations can be revised accordingly. The same approaches used here for lakes could later be used for rivers as well. We develop classifications for ANC since it was earlier shown to be preferred as chemical indicator for acidity.

5.1 For naturally circumneutral waters (ANC > upper level off effect):

Thresholds for ANC classes were set by regressing (linear) the Swedish MILA index calibrated using littoral lake macroinvertebrates against ANC (Figure 21,

Table 16). Regression showed that the relation between ANC and MILA was poor; a finding that is not too surprising as the index was originally calibrated against mean pH. Most likely an index developed and calibrated against ANC would result in lower uncertainties. Regression of MILA against ANC resulted in a G/M class boundary of 124 µeq/l, a value that is high compared to other class boundaries established for ANC. In the Norwegian WFD classification system, type-specific G/M boundaries range between 0 and 40 µeq/l, and for the calculation of critical load 20 µeq/l has been used as critical value for ANC (now a variable ANCo1 is used).

These class boundaries derived from the biological classification can be used for sites with a reference value of ANC above the max value of the MILA index, 254 µeq/l. Above this value acid-sensitive organisms are not affected by ANC. This circumneutral group could be used to form one water body type.

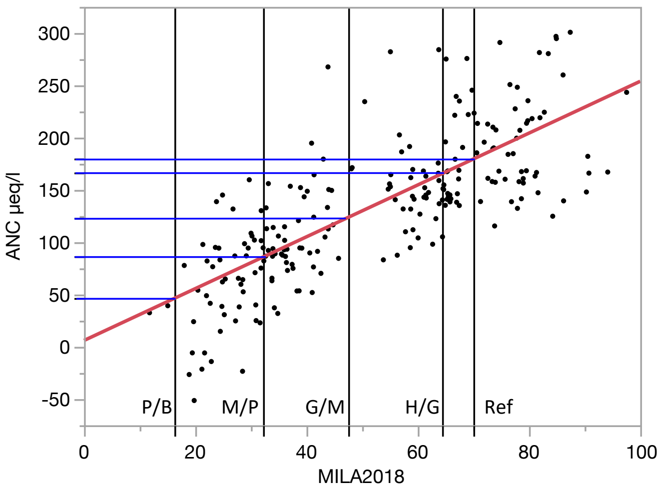


Figure 21 Linear regression of ANC against the macroinvertebrate acidity index MILA2018 for 44 lakes in southern Sweden. Three-year averages 2000-2017. r^2 = 0.57. The black lines denote class boundaries for MILA. The blue lines show proposed class boundaries for ANC.

Table 16. Class boundaries of ANC in natural circumneutral lakes based on the relationship with the MILA-index.

	EQR_{MILA}	MILA	ANC
Max		100	254
Ref	1	70	180
H/G	0.92	64.4	166
G/M	0.68	47.6	124
M/O	0.46	32.2	86
O/D	0.23	16.1	46
Min		0	6

5.2 Thresholds for natural acidic sites (ANC_{ref} < level of effect)

For naturally acidic sites, here defined as an ANC_{ref} < the level of effect for the acidity index, the ecosystem is partly controlled by acidity already under reference conditions. We then suggest that the class boundaries are set in relation to the site specific reference levels (Figure 22). Three approaches are presented for naturally acidic sites. One is based on the relation between biological indices and ANC and setting the EQR (ecologic quality ratio) over a larger ANC range below the level of effect. The second approach uses the response curves extracted from the gradient forest tree analysis of the Nordic dataset. The third consists of using the class boundaries from the biological classifications and the response curves from gradient forest analysis in combination.

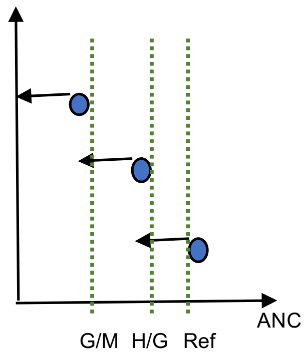


Figure 22. Conceptual figure of the problem with fixed class boundaries for acidification when the reference states (the blue dots) are near or below the reference state of the classification system.

5.2.1 Draft 1. Thresholds based on the relation with an index

Thresholds for ecological status (ES) are usually set by an EQR calculated as the measured value divided by the reference value. For ANC this approach is problematic since ANC can have negative values. One way to resolve this is to calculate the EQR based on the difference from the ANC corresponding to the theoretical high or low value of the response metric. As a high value, the maximum level of effect between ANC and a biological acidification index was chosen. ANC above this threshold is regarded as high status. Similarly, a low value could be chosen as the ANC corresponding to the lowest theoretical value of the response index. This approach results in a value of 60 µeq/l ANC for the MILA index. However, both of these values are higher than many values for ANC_{obs} and ANC_{ref}. An alternative approach to establish the minimum ANC value is to choose the lowest value of ANC for waters with better status class than bad (poor or better). In the Norwegian EQC, the lowest threshold for bad status is -20 µeq/l. A similar threshold was found in the Swedish classifications; the lowest ANC value for non-limed lakes (N = 4 357) in the national lake survey 2011-2016 with poor quality or better was -25 µeq/l. Although classification systems differ between Sweden and Norway there appears to be consensus that waters with ANC < -20 or -25 µeq/l can be classified as bad quality. Here we chose the lower of these two values (-25 µeq/l) as the minimum value to be used in our calculations and subtracted from the reference value and measured value when calculating EQR. A third alternative is to choose the lowest ANC value observed in naturally acidic waters. This approach, used in the Norwegian classification system, resulted in an ANC value of -100µeq/l. This value seems reasonable when compared to data from the non-limed lakes in the Swedish national lake survey 2011 – 2016. The lowest measured ANC was -98 µeq/l when a few lakes with extreme chemistry were excluded (both cations and anions were above 500 µeq/l).

The three alternative calculations of EQR were calculated as:

Equations 1 a-c

a $EQR = (ANC_{high} - ANC_{ref})/(ANC_{high}-ANC_{obs})$

where ANC_{high} here is chosen as the ANC related to MILA = 100, which is 254 µeq/l

b, c $EQR = (ANC_{obs} - ANC_{low})/(ANC_{ref}-ANC_{low})$

where ANC_{low} is -25 µeq/l (b) or -100 µeq/l (c).

Thresholds for the status classes can be calculated by inserting the ANC-values given in Table 17 and using them for all measures below ANC_{max}.

Table 17. Class boundaries for EQR for ANC with three approaches based on the MILA index.

	EQR_{MILA}	MILA	ANC µeq/l	EQR = (ANChigh - ANCref)/ (ANChigh-ANCobs)	EQR = (ANCobs - ANClow)/ (ANCref-ANClow)	
					ANClow = -25 µeq/l	ANClow = -100 µeq/l
Max		100	254			
Ref	1	70	180			
H/G	0.92	64.4	166	0.84	0.93	0.95
G/M	0.68	47.6	124	0.57	0.73	0.80
M/O	0.46	32.2	86	0.44	0.54	0.66
O/D	0.23	16.1	46	0.36	0.35	0.52
Min		0	6			

When a fixed EQR is used as a G/M boundary, the absolute change between reference value and the G/M boundary will depend on the reference value. When the EQR is calculated from two differences, the slope of this relation will depend on how the differences are calculated. To illustrate this, we calculated the difference between ANC_{ref} and $ANC_{(G/M)}$, here referred to as $dANC_{(G/M)}$, as a function of ANC_{ref} for the three alternatives. The equations 2 a-c were derived from equations 1 a-c:

Equations 2 a-c:

$$ANC_{(G/M)a} = ANC_{high} – (ANC_{high} – ANC_{ref})/EQR_{(G/M)a}$$

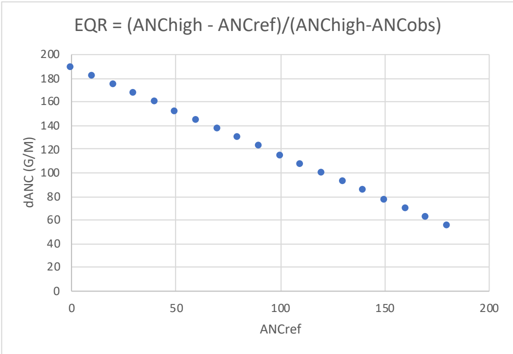
$$ANC_{(G/M)b} = EQR(G/M)b * (ANC_{ref} – (-25)) + (-25)$$

$$ANC_{(G/M)c} = EQR_{(G/M)c} * (ANC_{ref} – (-100)) + (-100)$$

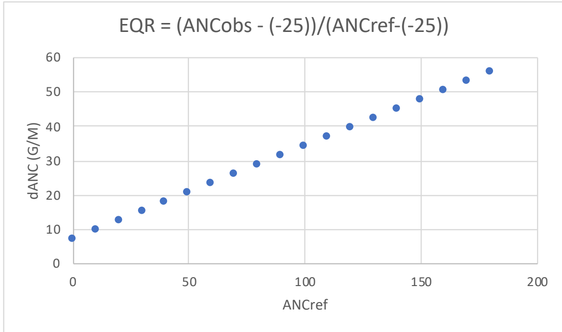
$$dANC_{(G/M)} = ANC_{ref} – ANC_{(G/M)}$$

When ANCref is the value corresponding to the reference value of the MILA index (180 µeq/l) there is no difference in dANC(G/M). However, when ANCref is lower, there is a pronounced difference between the alternatives (Figures 23 a-c).

a)



b)



c)

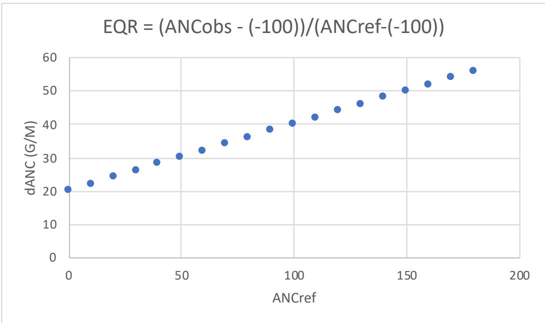


Figure 23 a-c. Relation between accepted absolute change in ANC for good status for three ways of calculating EQR. EQR is calculated either as the difference between a high ANC-value and measured vs reference values of ANC, here 254 µeq/l (a) or by a low value, here -25 µeq/l (b) or -100 µeq/l (c). In both cases the absolute change in ANC (dANC) for the G/M boundary depends on ANCref which is shown by the graphs.

For approach a) $dANC_{(G/M)}$ increases as ANC_{ref} decreases, which is not desirable. If ANC_{ref} is 50 µeq/l it allows a large decrease of 150 µeq/l to -100 µeq/l for the G/M boundary. The lower sensitivity in ion weak waters compared to well buffered waters is not acceptable (Figure 23 a).

The approach with subtracting a low ANC value (b and c) is more useful with an increased sensitivity as ANC_{ref} decreases (Figures 23 b and c). The accepted change in ANC (dANC) for the G/M boundary and for a certain ANC_{ref}, however, will depend on the ANC_{min} used. If -100 µeq/l is chosen instead of -25 µeq/l, the slope of dANC to ANC_{ref} will be flatter, resulting in a less sensitive classification for low ion weak waters. In the further work we chose to use -25 µeq/l since it is more sensitive for ion weak waters and results in larger contrasts to the alternative approach presented below. This value is also in agreement with the Norwegian classification system where the G/M boundary of ANC for the water type with a reference value for ANC of 10 µeq/l (*sv. kalkfattig, sv. klar*) has a G/M boundary 10 µeq/l lower than the reference value.

5.2.2 Draft 2. Class boundaries based on the density plots from the Random Forest analysis in Chapter 4

An alternative approach to set class boundaries is to use the density plots from the random forest analysis presented in Figures 17 to 20 in Chapter 4. The presence/absence plots were more uniform and showed on major peaks around 100 µeq/l for fish and macroinvertebrates in lakes and streams. The peaks are at higher ANC values for streams than lakes, and below these major peaks no or only minor peaks were observed. The method focuses on highlighting major shifts in community composition and should not be interpreted as there is no effect on biota as ANC, for example, decreases from 50 to -50 µeq/l. Due to few species the change is not as dramatic as around ANC of 100 µg/l, when a large number of sensitive species is no longer recorded. To overcome this issue, we decided to use the width of the major peaks as a measure of the change in ANC that is acceptable for the G/M boundary and simply apply this range on the whole ANC-range below the level of effect.

The width of the peaks is around 70 µeq/l for all relationships, with the exception of fish in streams where the peak was 90 µeq/l. The class boundaries can be set related to the peak widths according to Table 18. This approach differs from the former in that it has the same absolute change in ANC for each class boundary for all naturally acid waters.

Table 18. Class boundaries based on peak widths from random forest.

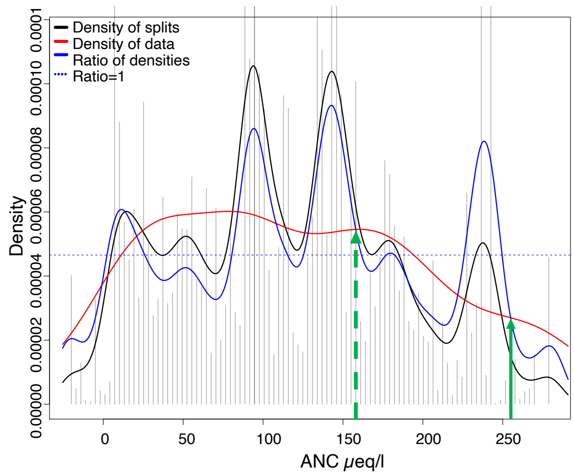
Border	Fraction of peak width	dANC eq/l
H/G	0.25	17
G/M	0.5	35
M/P	1	70
P/B	1,5	105

5.2.3 Draft 3. Combining class boundaries and response curves.

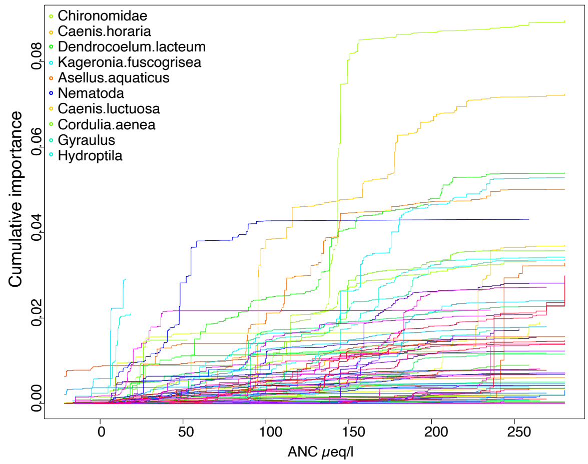
The graph from the gradient forest analysis that was used for approach "Draft 2" was the "density plot" (Figure 24a). This relationship was used to find thresholds, focussing on where the most dramatic biological changes occur. The "cumulative importance" plot for single species gives a more nuanced view of the changes along the ANC gradient (Figure 24b). Shifts in community composition are reflected in species "disappearing", "appearing" and "disappearing" along the gradient. Changes in community composition occurs all along the ANC gradient, although most changes in the cumulative importance plot occur at peaks in the density plot. A third plot, not shown in Chapter 4 is the overall cumulative importance plot (Figure 24c). This relationship reflects the gradual change in the macroinvertebrate community across the ANC gradient, with slightly steeper slopes at the ANC values of the major peaks in the density plots. A third approach for setting class boundaries for ANC was based on this plot, in combination with the relationship to the MILA-index.

Since the slope is steeper between ANC 85 and 145 µeq/l, the classification should be more sensitive in that region. To adjust for a non-linear relationship, thresholds could be based on relative changes in cumulative importance. To establish threshold values, we used the same relation between the Swedish MILA-index and ANC as above (Figure 21). This links chemical criteria with the biological classification which is required by the WFD. The EQR for ANC was calculated as the difference to a chosen low value to avoid negative ratios. In this way, it is similar to Draft 1 but has narrower class ranges in the region between ANC 80 and 145, according to Figure 24. The slopes of the different ANC regions were calculated by visually fitting lines to the cumulative importance plot. We chose -20 µeq/l to subtract from ANC in the EQR ratio since it corresponds to the cumulative importance of 0 (Figure 25).

a.



b.



c.

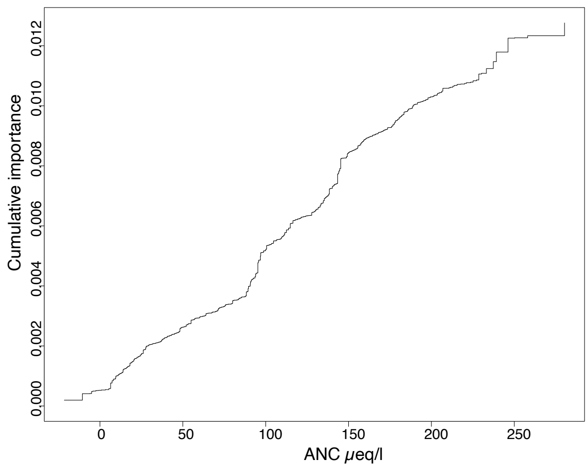


Figure 24. Density plot (a), cumulative importance of single species (b) and overall cumulative importance from gradient forest analysis of abundance of macroinvertebrates in lakes. Figures a and b are the same as figures 17a and b in Chapter 4.

The class boundaries for cumulative importance (CI) is set using the ANC values corresponding to the MILA-index and the broken linear curve in Figure 25 (Table 19). New H/G class boundaries for ANC related to the reference value were then calculated as:

$$ANC_{H/G} = ANC_{ref} - (CI_{ref} - CI_{H/G})/slope \qquad \text{(Equation 3)}$$

Boundaries for the other classes were calculated in a similar way. Different sets of class boundaries were calculated from the two slopes. Depending on the measured (ANC_t) and reference (ANC_{ref}) values of ANC, different class boundaries are used according to:

$ANC_{ref} > 115$ and $ANC_t < 115$ use $EQR_{high\ slope}$

else use $EQR_{low\ slope}$

Hence, if the ANC value has passed the centre value of the sensitive area (115 μeq/l), the EQR from the higher slope relationship should be used, otherwise the EQR from the lower slopes is used. The calculation could be refined by weighted averaging of the two slopes for each site.

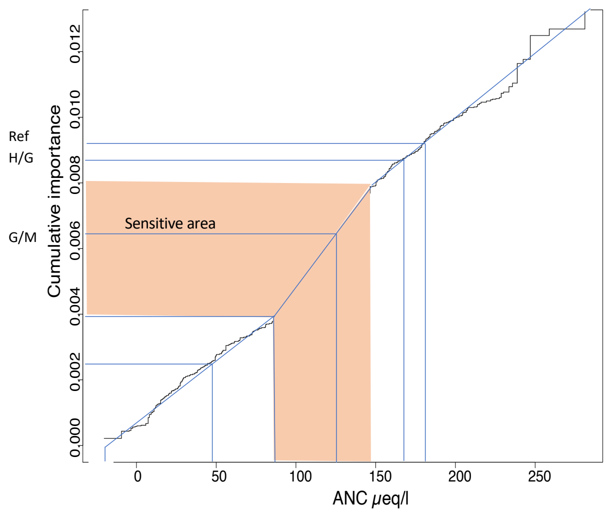


Figure 25. Overall cumulative importance of single species from gradient forest analysis of abundance of macroinvertebrates in lakes. Linear lines are fitted to the curve and a region with a steeper slope is marked as a sensitive area. Blue lines mark class boundaries for ecological status based on the relation of ANC to the Swedish macroinvertebrate MILA index for lakes.

Table 19. EQR class boundaries for ANC with two approaches based on the MILA index and the slope of cumulative importance to ANC from gradient forest analysis. MILA index, ANC and cumulative importance values are also given. ANC_{min} is set to -20 µeq/l.

	EQR MILA	MILA	ANC	Cumulative importance	EQR ANC		
					overall	high slope	low slope
Ref	1	70	180	0.00920			
H/G	0.92	64.4	166	0.00865	0.93	0.96	0.93
G/M	0.68	47.6	124	0.00645	0.72	0.79	0.63
M/O	0.46	32.2	86	0.00395	0.53	0.60	0.30
O/D	0.23	16.1	46	0.00250	0.33	0.49	0.10
Min			-20	0.00000			

5.3 Evaluation of the proposed approaches

The three new suggestions were compared with the Norwegian and the Swedish classification systems by comparing the acidification classes assigned using the different systems. The three approaches are hereafter referred to as Draft 1 to 3 according to the definitions above. Objects with diverging classification were then given some attention. The reference values were calculated by a regression model based on MAGIC simulations (Equation 4 below). Lakes diverging two classes or more between the different methods are given in Appendix 3. Some of the lakes occurred in more than one example.

5.3.1 Comparison with the Norwegian system.

The Norwegian classification could be applied to 195 lakes in the Nordic dataset.

Draft 1

Draft 1 was in general more sensitive compared to the Norwegian system (Table 20). Only three out of 195 lakes had a slightly higher classification by Draft 1, while 128 had a lower classification. Of those, 28 lakes were lowered two status class steps by Draft 1 (examples 1 to 28 in Appendix 3). Many of these lakes had relatively high Ca concentrations but also high SO_4 concentrations. Examples 20 to 28 had ANC-values > 100 µeq/l, which might reflect that the Norwegian system was based on trout, while the new approaches include many macroinvertebrate species where some are more acid sensitive than trout. However, 11 of the sites with large deviation in classification had an ANC < 50 µeq/l. The largest deviations in classification, three steps, were in example 1 and 11. Example 1 had a reference ANC and present ANC of 63 and 0 µeq/l, respectively and a pH of 5.1 and inorganic labile aluminium (Ali) of 63.7 µeg/l. Example 11 decreased from 224 to 53 µeq/l in ANC and with a pH of 5.5.

Table 20. Comparison of lake classifications of acidification according to the Norwegian system for ANC with a proposed system based on a constant EQR for ANC (Draft 1). Red text denotes deviations of two or more classes.

	Draft 1				
Norw.	H	G	M	P	B
H	***43***	*70*	*19*	*0*	*0*
G	*3*	***19***	*24*	*6*	*2*
M	*0*	*0*	***2***	*3*	*1*
P	*0*	*0*	*0*	***0***	*3*
B					

Draft 2

For Draft 2 large differences were noted in the classification of the lakes, with a strong bias towards more stricter classifications by Draft 2, but also in a few cases the mismatch was in the other direction (Table 21, examples 29 – 70 in Appendix 3Table). Twelve out of 42 lakes with more than two class steps difference by Draft 2 compared to the Norwegian system had ANC over 150 µeq/l (examples 59 – 70). For these lakes Draft 2 is probably overestimating acidification. Example 29 is an example where Draft 2 probably gives a more relevant classification compared to the Norwegian system. In this lake, ANC decreased from 109 to 32 µeq/l with a pH of 5.7. Example 71 shows an obviously acidified lake that is missed by Draft 2 with an ANC decline from 22 to 8 µeq/l and a pH of 5.

Table 21. Comparison of lake classifications of acidification according to the Norwegian system for ANC with a proposed system based on a constant change in ANC (Draft 2). Red text denotes deviations of two or more classes.

	Draft 2				
Norw.	H	G	M	P	B
H	**58**	41	26	7	0
G	11	**13**	21	5	4
M	1	1	**2**	2	0
P	0	0	1	**1**	1
B					

Draft 3

Differences between Draft 3 and the Norwegian classification were slightly smaller than for Draft 1, but Draft 3 was still stricter than the Norwegian system with 22 lakes classified as two classes lower by Draft 3 (Table 22). The lakes with large discrepancies had relatively high ANC values, more than 40 µeq/l, although with large changes in ANC from the reference values (examples 111-130 in Appendix 3). One lake classified as having good status by the Norwegian system and as having poor status by Draft 3, had an ANC decrease from 63 to 0 µeq/l. For this lake Draft 3 is certainly more appropriate.

Table 22. Comparison of lake classifications of acidification according to the Norwegian system for ANC with a proposed system based on the relation of ANC to the cumulative importance from the gradient forest analysis of macroinvertebrates (Draft 3). Red text denotes deviations of two or more classes.

	Draft 3				
Norw.	H	G	M	P	B
H	**43**	76	12	1	0
G	3	**25**	17	5	4
M	0	2	**3**	1	0
P	0	0	0	**1**	2
B	0	0	0	0	**0**

5.3.2 Comparison with the Swedish system

The Swedish system could be applied to all 265 lakes in the dataset.

Draft 1

Looking at classification mismatches of more than two classification steps, Draft 1 was slightly less sensitive than the Swedish system. Seven lakes out of 265 had status class two or more steps higher using Draft 1 compared to the Swedish system (Table 23, examples 72-78 in Appendix 3). In example 77, ANC had decreased from 293 to 172 µeq/l with a pH of 5.5 and TOC of 22.6 mg/l. The classification from Draft 1 as high status seems more realistic to this naturally acidic site compared to the Swedish system. The same can be expressed for example 78. In example 79, where Draft 1 gave a two steps lower classification than the Swedish system, ANC declined from 166 to 110 µeq/l with a pH of 6.9. The ANC of 110 µeq/l is just at the upper boundary of a major peak (Figure 17) and together with the high pH, acidification is probably overestimated by Draft 1.

Table 2. Comparison of lake classifications of acidification according to the Swedish system for dpH with a proposed system based on a constant EQR for ANC (Draft 1). Red text denotes deviations of two or more classes.

	Draft 1				
Swe.	H	G	M	P	B
H	**104**	58	1	0	0
G	10	**27**	15	0	0
M	1	4	**19**	0	0
P	1	0	5	**3**	0
B	0	0	5	6	**6**

Draft 2

Draft 2 resulted in a two steps lower classification for 14 lakes (Table 24, example 80-93 in Appendix 3). In all cases the lakes had both high SO_4 and ANC and most had pH values > 6.5 The opposite pattern was found in 15 lakes (examples 94 to 108). Examples 96 to 101 were classified as having high or good status by Draft 2, although ANC was < 20 µeq/l. These were all relatively clear water lakes with low ANC_{ref}, which reflects the shortcoming of using a constant dANC for G/M boundary. By contrast, examples 107 and 108 were brown water lakes (TOC 22.6 and 35.2 mg/l) with high ANC (172 and 200 µeq/l) and were classified as having high status by Draft 2. The pH value is just below 5.6 where the buffering capacity is extremely low. The Swedish system based on dpH classified these lakes as poor and moderate status, while the other classifications were high or good.

Table 24. Comparison of lake classifications of acidification according to the Swedish system for dpH with a proposed system based on a constant change in ANC (Draft 2). Red text denotes deviations of two or more classes.

	Draft 2				
Swe.	H	G	M	P	B
H	**121**	33	9	0	0
G	16	**7**	24	5	0
M	2	9	**9**	4	0
P	1	4	2	**0**	2
B	0	2	6	6	**3**

5.3.3 Draft 3

Draft 3 resulted in two or more steps higher classification compared to the Swedish classification for 14 lakes (Table 25, examples 133-146 in Appendix 3). Most of these lakes had a pH around 5.6 where even small changes in ANC can lead to large differences in pH. In example 135, a Finnish site, measured pH was 6, but the modelled pH was 5.4. Two lakes were classified two steps lower by Draft 3 (examples 131 and 132). These lakes had relatively high pH, 6.3 and 6.9, but relatively high changes in ANC, 68 and 55 µeq/l. Measured ANC values were 77 and 110 µeq/l, both below the peaks in the density plots from the gradient forest analysis for macroinvertebrate abundance (Figure 17) indicating that these lakes are affected by the ANC, resulting in a biological shift.

Table 3. Comparison of lakes classifications of acidification according to the Swedish system for dpH with a proposed system based on the relation of ANC to the cumulative importance from the gradient forest analysis of macroinvertebrates (Draft 3). Red text denotes deviations of two or more classes.

	Draft 3				
Swe.	H	G	M	P	B
H	**104**	58	1	0	0
G	10	**28**	13	1	0
M	1	13	**8**	2	0
P	1	4	2	**0**	2
B	0	0	8	5	**4**

5.3.4 Comments on the proposed new classification systems

From the three approaches, Draft 3 has the highest potential for further development into a classification system for naturally acid sites. It links the biological and chemical classifications, like Draft 1, but also takes into account that there are larger changes in biota when ANC passes through the critical area indicated by the peaks in the density plots and the steeper slopes from the gradient forest analysis.

In the present state it is inferred from the MILA index that was developed to predict pH for a Swedish dataset. If a new classification for macroinvertebrates is developed from the Nordic dataset and related to ANC, a similar approach as the present Draft 3 could potentially be used to develop a new Nordic classification system.

Comparisons between the different classification systems showed that the Norwegian system was less sensitive compared to the new approaches, which agrees with an earlier comparison with the Swedish system in Chapter 3.

Here we only evaluated the approaches based on lakes, since there is no official index for macroinvertebrates in rivers in Sweden allowing an analogous approach for rivers. If a common Nordic index is developed, this could easily be done.

5.4 Setting site specific reference values for acidity

Acid deposition from long-range transboundary air pollution shows a relatively even distribution over the landscape with large gradients across large spatial scales (Andersson et al. 2018). This means that all ecosystems within an impacted region are more or less chemically affected by acidification and no reference sites for acidification chemistry can be found within the same region. Further, there is almost no reliable pre-acidification water chemistry data that can be used for reference values, and consequently reference values need to be modelled. The most commonly used model to estimate reference conditions is the dynamic MAGIC model (Cosby et al. 2001). Extensive application of the MAGIC model has been made for a large number of lakes and rivers in Sweden by using data from national monitoring of freshwater and soils (Moldan et al. 2013). For lakes and streams with no MAGIC model information available, a reference value can be calculated using a meta model based on MAGIC models. The meta model currently used in the Swedish classification system, the MAGIC library (Moldan et al., 2020), uses a matching routine where the water body to be classified is matched to the most similar lake or stream according to the shortest weighted Euclidian distance for a set of relevant chemical and geographical parameters, with the weighing factors determined by a regression model . Besides a reference value, the MAGIC library gives the whole time series of water chemistry since preindustrial conditions. Since the method only matches to one modelled water body, it does not provide a quantitative measure of uncertainty. Further, the error depends on how many similar lakes that are present in the library. Moldan et al. (2020) tested the frequency at which MAGIC model based acidification assessment deviate from assessment done with the MAGIC library. That provides an indication of uncertainty in the assessment. An alternative meta model was suggested by (Erlandsson et al. 2008). A reference value for ANC was then calculated by a regression model with the reference ANC from MAGIC as dependent variable and BC, SO_4 and Cl from a certain year as independent variable. Different regression coefficients then need to be estimated for each year. This approach gave an estimation of the error by the meta model but had the drawback that the data for calibration of the regression model was restricted to a limited number of water bodies with time series. Further, the classification became unstable between years since the regression coefficients were dependent on between-year variation in the calibration data set. To further explore the issue of uncertainty we tried an alternative to the Erlandsson et al. (2008) approach by using modelled data from the MAGIC model for contemporary years as independent data. Since the MAGIC model was run with constant average hydrology, between-year variation in the regression coefficients only reflected the gradual change in the relation between present and preindustrial chemistry due to the recovery from acidification, but not any random, weather-driven variation. Further, this method can be calibrated with the 2439 lakes and 244 rivers in the Swedish MAGIC library.

ANC_{1860} was modelled by BC, SO_4, and Cl from 2010 with a multiple regression for all 2439 lakes in the MAGIC library (Equation 4, all units in µeq/l).

$$ANC_{ref} = -4.695 - 0.374 * SO4 + 1.006 * BC - 0.907 * Cl \qquad \text{(Equation 4)}$$

The r^2 for the model was 0.97 (Figure 26 a). This regression-based meta model could be compared with the matching routine by performing a jack-knife test. Each lake in the library was then removed from the database and matched by the matching routine to get the ANC_{ref} from the most similar lake in the database. Values of ANC_{ref} from this jack-knife test were then evaluated against the MAGIC-value itself by a linear regression, resulting an r^2 value of 0.91 (Figure 26b).

a. Regression model **b. Matching routine**

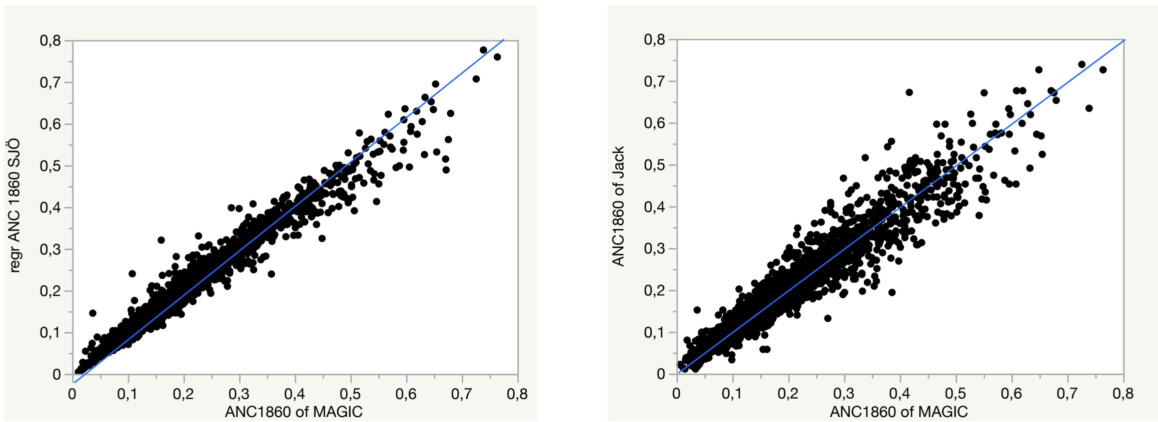


Figure 26. Predicted ANC_{ref} against MAGIC ANC_{ref}. (a). Prediction by regression model. (b). Prediction with the matched lake from the MAGIC library (jack knife).

The better performance of the regression model was even more pronounced when comparing the pH values calculated from ANC_{ref} and measured TOC (Figure 27 a and b). For the calculation of pH, the triprotic model by (Köhler 2014) was used and pCO_2 was estimated according to (Sobek et al. 2003).

a. Regression model **b. Matching routine**

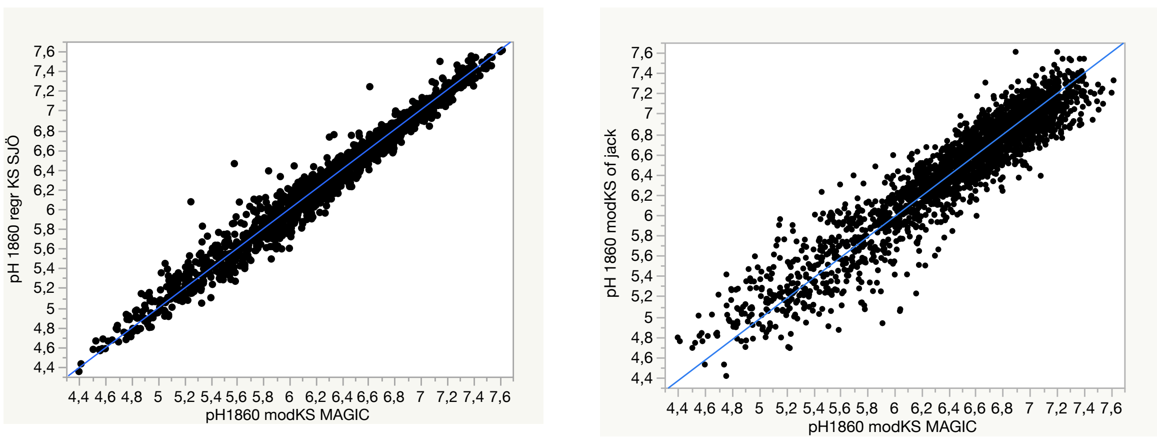


Figure 27. Predicted pH_{ref} by a) regression model (r^2=0.98) and b) matching against MAGIC(r^2=0.88). pH calculated by ANC_{ref}, TOC and pCO_2 estimated from TOC according to (Sobek et al. 2003) and a triprotic model for organic acids (Köhler 2014).

Further evaluation was done by comparing pH_{ref} calculated from ANC_{ref} with pH_{ref} from paleolimnological reconstructions (Erlandsson et al. 2008). The r^2-value was lower for the matching routine (0.41) than for the MAGIC model (0.45) (Figure 28). The regression model, however, gave a higher r^2-value compared to the MAGIC model (0.49). This somewhat surprising result might be explained in that the input data to the regression model comprised 5-year means of water chemistry, while the MAGIC models in most cases was calibrated with data from one year. This could be evaluated further.

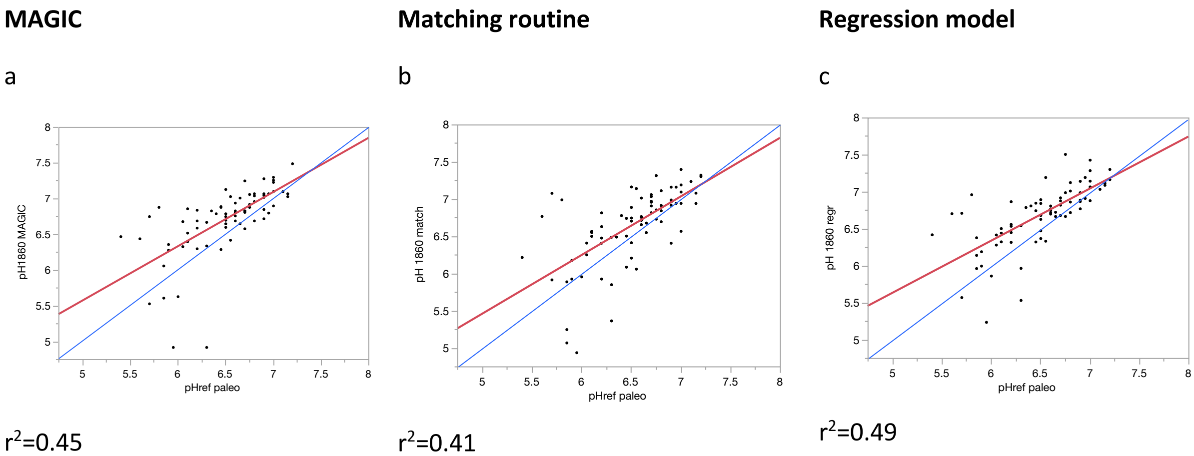


Figure 28. Comparison of pre-industrial pH from paleolimnological reconstructions with MAGIC models (a), matching with the MAGIC library (b) and a regression model (c). The blue lines denote a 1:1 line and the red line regression lines.

Data from MAGIC models of both lakes and rivers were combined to calibrate a regression model including both categories. A dummy variable for rivers was used to test if the same regression model could be used for both lakes and rivers. All cross factors for the dummy variable were included in the model. Although the dummy variable and the cross factors all were significant, their contribution to the model was negligible (Table 26). This suggests that the same regression model could be used for both lakes and rivers.

Table 26. Multiple linear regression of ANC_{ref} from MAGIC as a function of SO_4, Cl and BC for 2010 in 2683 lakes and rivers in the MAGIC library. A dummy variable for the rivers and the cross factor for the dummy variable and the other independent variables were included in the model. The table shows an effect test where the contribution (%) of each component to the model is calculated as ratio between the sum of squares for each component and the total sum of squares.

Variable	Sum of squares	Model contribution (%)	F-value	P
SO_4 2010	235851	0.68	527	<0.0001*
Cl 2010	6382430	18.31	14248	<0.0001*
BC 2010	22439726	64.37	50095	<0.0001*
VDR	2952	0.01	7	0.0103*
SO_4 2010*VDR	12558	0.04	28	<0.0001*
Cl 2010*VDR	3816	0.01	9	0.0035*
BC 2010*VDR	2361	0.01	5	0.0218*
Totalt	34860599			

The same approach was used to test if the same regression model could be used in both northern and southern Sweden. A dummy variable was then made for northern Sweden. Only the cross factor with SO_4 was statistically significant (Table 27). Although the contribution to the model only was 0.34 %, it is relatively large in relation to the contribution from SO_4, 0.98 %. It is likely that SO_4 is important for estimating the change in ANC from acidification, i.e. the difference between ANC_t and ANC_{ref}. This implies that the regional dependence of the regression parameter for SO_4 should be further investigated.

Table 27. Multiple linear regression of ANC_{ref} from MAGIC as a function of SO_4, Cl and BC for 2010 in 2683 lakes and rivers in the MAGIC library. A dummy variable for northern Sweden and the cross factor for the dummy variable and the other independent variables were included in the model. The table shows an effect test where the contribution of each component to the model (in percent) is calculated as the ratio between the sum of squares for each component and the total sum of squares.

Variable	Sum of squares	Model contribution (%)	F-value	P
SO4 2010	343264	0.98	916	<0.0001*
Cl 2010	2167475	6.22	5783	<0.0001*
BC 2010	20751106	59.53	55369	<0.0001*
North	315	0	1	0.359
SO4 2010*North	119433	0.34	319	<0.0001*
Cl 2010*North	90	0	0	0.6238
BC 2010*North	20	0	0	0.8186
Totalt	34860599			

6 Conclusions and final discussion

Comparisons of the three different Nordic systems currently used for classifying acidification of surface waters using water chemistry data revealed differences. The Finnish system focuses only on rivers with primary attention given to acidification caused by the draining of sulphide soils. Both the Norwegian and the Swedish systems focus more on anthropogenic-induced acidification by deposition and both are based on reference values calculated using the MAGIC model. However, while the Norwegian system, like the Finnish, is based on water body types and type-specific class boundaries, the Swedish system is object specific. Furthermore, the Swedish system is based on changes in the whole macroinvertebrate community (i.e. including species with varying degrees of sensitivity/tolerance to acidification), while the Norwegian system is based on empirically derived critical levels of a single species (brown trout). Classification using the Swedish system is, therefore, much stricter compared to the type-specific approaches, resulting in more sites classified as having moderate or worse status when compared to Norway. Here we proposed a new classification approach based on analyses and findings using a joint Nordic dataset including water chemistry, macroinvertebrates and fish from lakes and streams.

We propose a new classification approach based on ANC, since this variable was found to be the most consistent acidity indicator with highest predictive power across all biological datasets using the full (Nordic) dataset. Both ANC and modified ANCs were more robust predictors of biological change compared to pH; modelling using pH often revealed significant, confounding interaction terms with other environmental variables. Further, ANC is less affected by changes in DOC compared to the other indicators, in particular pH. This implies that classifications based on ANC will be more robust against browning.

The MAGIC model was used for estimating reference values in both Norway and Sweden, The model has been applied to a large number of sites, but cannot be used for all water bodies that should be classified. We show that a simple regression model for reference ANC, as a function of BC, SO_4 and Cl, could be calibrated using data from MAGIC-modelled lakes and rivers in all of Sweden. With some validation by modelled waters it could potentially be used for Norway and Finland as well. However, small but significant differences in regression parameters between different regions in Sweden indicates that precision would be increased by using regionally calibrated models. Moreover, the regression model approach resulted in less uncertainty compared to the matching procedure currently used in Swedish assessments, is simpler to use and requires fewer sites to achieve the same level of statistical power if used in a new region. It has, however, a drawback that it will use one average set of parameters which will consequently be more suitable for assessment of the lakes that are closer to an average values of all the regression parameters. It will be less reliable for lakes different from average. This will not improve by adding more “different” lakes to the regression.

Our suggested approach is exemplified here by using the Swedish acidification index for macroinvertebrates in lakes (the MILA index). The approach could also be applied to streams and for fish. Using a species assemblage as opposed to a single indicator species is also more consistent with the objectives stipulated by the Water Framework Directive. If it is decided that the suggested approach should be further developed, we suggest that new indices are developed for ANC for both lakes and rivers using the Nordic dataset. A common Nordic classification for macroinvertebrates in lakes and rivers could then underpin classifications using ANC.

For sites with circumneutral and alkaline reference conditions, the class boundaries for ANC can be set in relation to the biological classification. However, since many lakes and streams are naturally acidic with the species composition already controlled by acidity, class boundaries for ecological status cannot simply be set using biological and chemical thresholds alone. A reference value needs to be determined and compared to the present state for each site or for water body types.

For naturally acidic sites, we recommend an approach where the class boundaries are expressed as an EQR instead of a fixed value of ANC. Since ANC can be negative, the ratio has to be calculated after addition of a fixed value to both the reference and present values of ANC. The EQR-derived class boundaries should be based on biological classification but should be modified according to sensitivities across different ANC-ranges according to a gradient forest analysis expressed as cumulative importance.

The path to harmonisation has some crossroads where science alone cannot be the sole guidance, i.e. society/environmental management need to be involved in the decision making processes. The most important issues to discuss are:

1. How to define unacceptable acidification. Should thresholds be based on the requirements of single relatively sensitive species that is deemed important by society, or as a gradual change of a species community from a reference condition, following the Water Framework Directive as suggested in this report? The answer to this question will have implications for the methods that can be used to establish the biological and chemical thresholds. For a high-profile species such as brown trout, historical data is often available encompassing the whole period of widespread acidification. This is not possible for other species or communities, leaving the "space for time" approach as the only alternative (notable exception: paleo reconstruction using diatoms in lakes). Both approaches have strengths and weaknesses, a full discussion of which is outside of the scope here. Full harmonisation across the Nordic borders would require choosing the same biological response variable, but partial harmonisation is also possible, e.g. by agreeing on point 2 and/or 3 below.

2. Which parameters should be used to determine acidification? Alkalinity, ANC, ANCoaa, pH, Ali or a combination? In this report we propose the use of ANC since it was the best predictor of biota in the analysis and since it is the easiest to model. Additionally, ANC is what MAGIC hindcasts. Intensity parameters such as pH and Ali are more closely linked to toxicity but these variables add complexity and uncertainty because they require additional modelling of DOC and CO_2. Moreover, additional models will rely on predicted changes in ANC, as is the case for the current Swedish method. Estimating ANC requires accurate and precise determination of seven ions. In areas affected by sea salt, ANC is a small difference between relatively large concentrations of ions leading to large errors associated with calculations. In such situations, alkalinity from acidimetric titration could be an alternative, or alternatively ANC could be calculated from alkalinity, DOC and Ali as is currently done in the UK.

3. Should a type-specific (as the Norwegian) or an object-specific system (as the Swedish) be selected? Both consider deviation from a reference state which must be defined. The object-specific approach offers the most accurate classification and is perhaps required if the Swedish system with gradual change and a relatively strict good/moderate boundary is chosen as a model (see point 1). Having a type-specific system is simpler but, depending on parameters chosen under point 2 and thresholds chosen under point 1, will require a large number of water body types to achieve the same level of precision and accuracy.

We hope that this report provides a good foundation for continued dialog on these points and ultimately results in a more harmonised classification of acidification between countries and between chemical and biological quality elements.

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Appendix 1. Supplementary tables and figures to Chapter 4

Table A.1.1. Summary statistics of correspondence analysis (CA1 & CA2) of Nordic scale lake and stream macroinvertebrate square-root transformed means of taxa abundances (SQRT), and taxa presence/absence (P/A).

	Lake invertebrates		Stream invertebrates	
	Total variation 2.215		Total variation 2.077	
	$CA1_{SQRT}$	$CA2_{SQRT}$	$CA1_{SQRT}$	$CA2_{SQRT}$
Eigenvalues	0.21	0.16	0.25	0.16
Exp. variation (cum.)	9.52	16.85	12.06	19.97
	Total variation 6.726		Total variation 4.20	
	$CA1_{P/A}$	$CA2_{P/A}$	$CA1_{P/A}$	$CA2_{P/A}$
Eigenvalues	0.26	0.21	0.27	0.21
Exp. variation (cum.)	3.94	7.01	6.35	11.37

Table A.1.2. Summary statistics of correspondence analysis (CA1 & CA2) of Nordic scale lake and stream fish square-root transformed means of species abundances (SQRT), and taxa presence/absence (P/A).

	Lake fish		Stream fish	
	Total variation 2.519		Total variation 1.653	
	$CA1_{SQRT}$	$CA2_{SQRT}$	$CA1_{SQRT}$	$CA2_{SQRT}$
Eigenvalues	0.85	0.46	0.39	0.37
Exp. variation (cum.)	33.66	52.01	23.72	46.18
	Total variation 4.30		Total variation 2.699	
	$CA1_{P/A}$	$CA2_{P/A}$	$CA1_{P/A}$	$CA2_{P/A}$
Eigenvalues	0.74	0.31	0.58	0.42
Exp. variation (cum.)	28.13	40.08	21.51	37.24

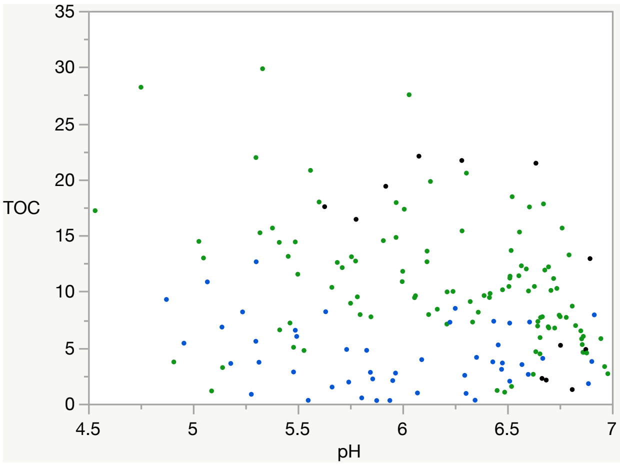

Figure A.1.1. TOC and pH in lake invertebrate sites. N=165. Blue = Norwegian sites. Green = Swedish sites. Black is Finnish sites.

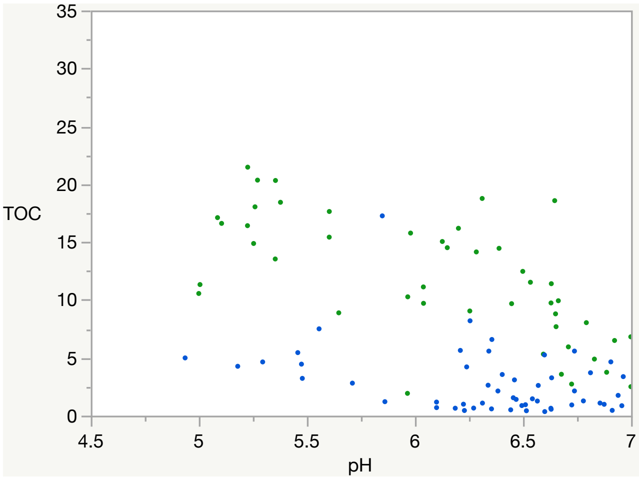

Figure A.1.2. TOC and pH in stream invertebrate sites. N=99. Blue = Norwegian sites. Green = Swedish sites.

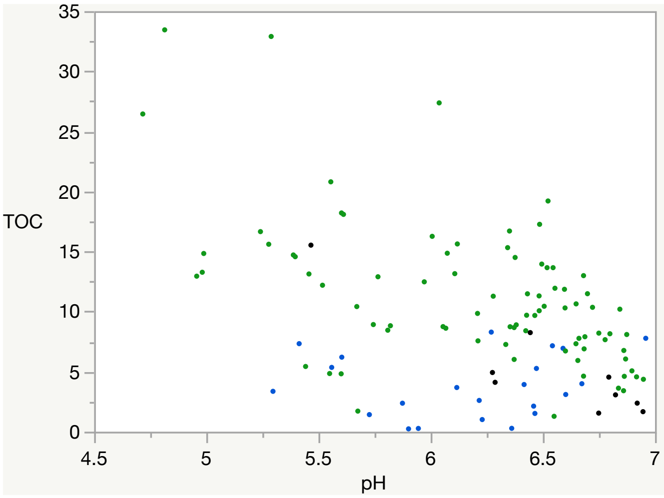

Figure A.1.3. TOC and pH in lake fish sites. N=114. Blue = Norwegian sites. Green = Swedish sites.

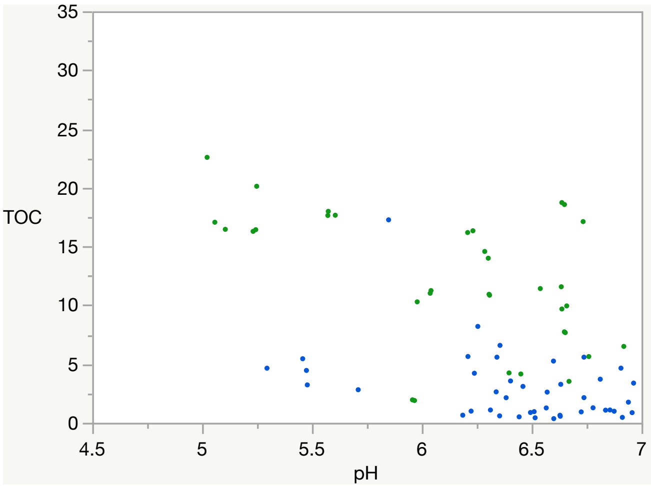

Figure A.1.4. TOC and pH in stream fish sites. N=80. Blue = Norwegian sites. Green is Swedish sites.

Appendix 2. Time series analysis

By Gaute Velle and Robert Lennox

Introduction

Acidification has seriously affected the biota of many European regions. In Norway, detrimental effects on Atlantic salmon (*Salmo salar* L) and brown trout (*Salmo trutta* L.) begun one century ago (Huitfeldt-Kaas 1922). Detrimental effects on benthic invertebrates likely began prior to the effects on fish since some of the invertebrate species are more acid sensitive than fish (Raddum et al. 1984). Now, monitoring programmes indicate a reduction in atmospheric pollution since the late 1980s, causing improved water quality and improved ecological state in a broad range of geographical areas (Stoddard et al. 1999, Evans et al. 2001, Halvorsen et al. 2003, Monteith et al. 2005, Stendera and Johnson 2008, Johnson and Angeler 2010, Hesthagen et al. 2011, Lento et al. 2012). The biological recovery typically includes reappearance, followed by a modest or pronounced increase in acid-sensitive taxa (Raddum and Fjellheim 1995, Hesthagen et al. 2011). However, the effects on benthic invertebrates over time is poorly investigated. A pertinent question is how the recovery has affected the biodiversity and the composition of the benthic invertebrate assemblages. In addition, it is important to know the environmental drivers of potential changes in the invertebrate assemblages.

In this project, we have investigated the time series data of benthic invertebrates from Norway, Sweden and Finland. The aims are to find whether the composition of assemblages have changed over time and whether the biodiversity has changed over time. The data are from 2005 and up to the present, suggesting that some of the most pronounced changes in the biological assemblages had already occurred, concurrent with the most pronounced reduction in acidification in the 1990s.

Methods

Data treatment and description

Invertebrate, environmental, and acidification indicators were retained from the Nordic data based on annual correspondence of parameters (2005-2016) and for sites which had four or more water chemistry sampling occasions. The data set was filtered by removing Swedish sites known to be limed. There were 165 lakes sites and 55 stream sites in the final data set (Table A.2.1).

Table A.2.1. Number of sites

	Number of sites				
Dataset	Sweden	Norway	Finland	Total	Total with limed sites removed
lake invertebrate communities	158	48	12	218	165
stream invertebrate communities	120	8	0	128	55

Predictors – lake and stream invertebrates

Along with the five acidification indicators (pH, ANC, ANCo1, ANCo2 and Ali), data by site included water chemistry (TOC, TotP, NO_2+NO_3-N), catchment size and land use characteristics (% agriculture, % forest, % water, % wetland), and spatial components (altitude, latitude, longitude) (Table A.2.2). Sulphate was not included because of high inflation (co-linearity with ANC).

Table A.2.2. Mean and range of acidification indicators and environmental indicators for lake and stream invertebrate sites (for calculation of ANC, ANCo1, ANCo2, see main report. All units for ions are in mekv/l).

	Lake			**Stream**		
Acidification indicator	**MEAN**	**MINIMUM**	**MAXIMUM**	**MEAN**	**MINIMUM**	**MAXIMUM**
pH	6.14	4.53	6.98	6.05	4.93	7
ANC	118	-25	291	145	7	291
ANCo1	88	-37	231	108	-8	234
ANCo2	58	-97	189	71	-53	194
Ali	16	0	251	17	0	83
Environmental descriptor						
TOC (mg/l)	8.9	0.4	29.9	10.5	0.7	21.5
Tot-P (µg/l)	10	2	61	10	2	27
NO_2+NO_3-N (µg/l)	60	1	353	77	1	581
% agriculture	1	0	10	1	0	9
% forest	73	0	100	77	1	100
% water	13	0	35	3	0	14
% wetland	5	0	47	9	0	50
catchment size (km2)	35	0.25	1407	50.1	0.51	494

Biological diversity and changes in species composition

Biodiversity can be measured in many ways, implying that there exist several biodiversity indices. An ideal biodiversity index is able to reduce complex information on structure and abundance to simple numerical metrics. However, it is important to be aware of two main limitations to the concept of biodiversity: (1) the term is artificial implying that biodiversity is not an intrinsic property in nature and (2) biodiversity is a simplification of nature and it is necessary to consider that information is lost when complex processes are reduced to a single number (Hurlbert 1971).

In his development of a conceptual family of species diversity indices, Whittaker (1960) determined the total diversity in the landscape (γ-diversity) by the diversity at one site (α -diversity) and the assemblage difference among sites or with time (β -diversity). For the basic unit of biological classification, the species, α -diversity is expressed as a function of the number of species and their frequency (Chapin Iii et al., 2000; Tuomisto, 2010). We have adopted the Shannon diversity index as α –diversity. To track changes in the composition of the assemblages over time, we use Bray-Curtis dissimilarity as β –diversity.

Biological sensitivity to acidification or climate varies according to site and region (Raddum and Skjelkvale 2001, Moe et al. 2010). We can also expect that the response to acidification may override the response to climate for sites with a dominating change along the acidification axis. Hence, in an attempt to reduce noise and ease interpretation of results, the sites were clustered into four groups based on likely sensitivity to acidification. To classify sites, we used results from Chapter 4 (time for space) on important chemical variables and threshold values. The clusters of sites are:

Group 1. All sites
Group 2. Sites where ANCo1 always has remained above 100 µekv/l (least acid sensitive sites)
Group 3. Sites where ANCo1 has remained between 40 and 100 µekv/l (transition sites)
Group 4. Sites where ANCo1 always has remained below 40 µekv/l (acid sensitive sites)

We calculated diversity as a function of taxon richness and abundance using the Shannon diversity index in the R package vegan (Oksanen 2016). The diversity was then modelled as a function of sampling date and habitat type (river and lakes) using mixed effects model (R package nlme: Pinheiro et al. 2013). To find the changes in β –diversity over time, we used the abundance matrix of benthic invertebrates and measured Bray-Curtis dissimilarity, then selected consecutive sampling time points, divided dissimilarity by the number of intervening years, and plotted by country and habitat.

To find the drivers of diversity and species composition over time, we used mixed effects models with random intercept of site and random slope of habitat type. Here, the chemical variables except pH were log-scaled. We also detected the biological composition patterns along environmental gradients and over time using ecological data using Gradient Forest (Ellis et al. 2012) in the r-package gradientForest. We also used Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMEANOVA; Anderson 2001) to find environmental predictors that can explain the species assemblages over time (R- package vegan).

As a first attempt to produce a model that classifies acidification status based on the biota (that is, a biological index), we used a unimodal-based technique of weighted averaging partial least squares regression (WA-PLS: ter Braak and Juggins 1993) using the rioja package in r (Juggins 2015). This is a three-step approach: 1. Find environmental variables that are important drivers for the species' distribution and abundance. 2. Model each species response to the important environmental variable (s). In this step, we collect biota and environmental variables at many sites. This collection of data is referred to as a training set. 3. In the final step, we use the species composition of a single site to infer the environmental variable of interest at the site. To do this, we need information from the second step. The performance of the model is evaluated by comparing the measured environmental variable at all sites with the inferred variable of the sites. Simulation studies, theoretical analyses, and palaeoenvironmental applications suggest that WA, and in particular WA-PLS is a robust method that performs well with noisy, species rich, compositional data with many zero values, and in no-analogue situations (ter Braak and Juggins 1993, Birks 1995, ter Braak 1995, Birks 1998).

Results

Not surprisingly, many of the chemical variables are highly correlated (Figure A.2.1). This implies that some of the co-varying variables should be disregarded in the numerical analyses. A selection of which variables to pertain in the numerical analyses was based on results from the co-variance matrix, results on important chemical variables found in Chapter 4 and from biological knowledge on the variables that we expect to influence invertebrates. Co-varying environmental variables also implies that a combination of variables best describes the biological assemblages (Figure A.2.2). The first and most important is related to acid-base related components of the water, where these co-vary.

Trends in diversity

The overall change in the taxon abundance and richness (Shannon diversity index) of the benthic invertebrates from 2005 to 2016 indicates a slight increase in diversity in the rivers and a reduction in the lakes (Figure A.2.3). Most of the changes in diversity were gradual and small at single sites, implying no significant change in diversity at most sites over the time covered by the data (Figure A.2.4). Overall, there was a significant increase in diversity for eight of the sites and a significant decrease at one site.

When the sites are sorted according to sensitivity towards acidification, there were no changes in diversity at the most acid sensitive sites, while a decrease in diversity occurred in both the least acid sensitive lake sites and in the transitionary acid sensitive lake sites (Figure A.2.5). This may indicate that other drivers of diversity also influence the invertebrates. In the rivers, there was a short period of rapid increase in diversity in the most acid sensitive sites, while the transitionary acid sensitive sites had a small and gradual increase in diversity.

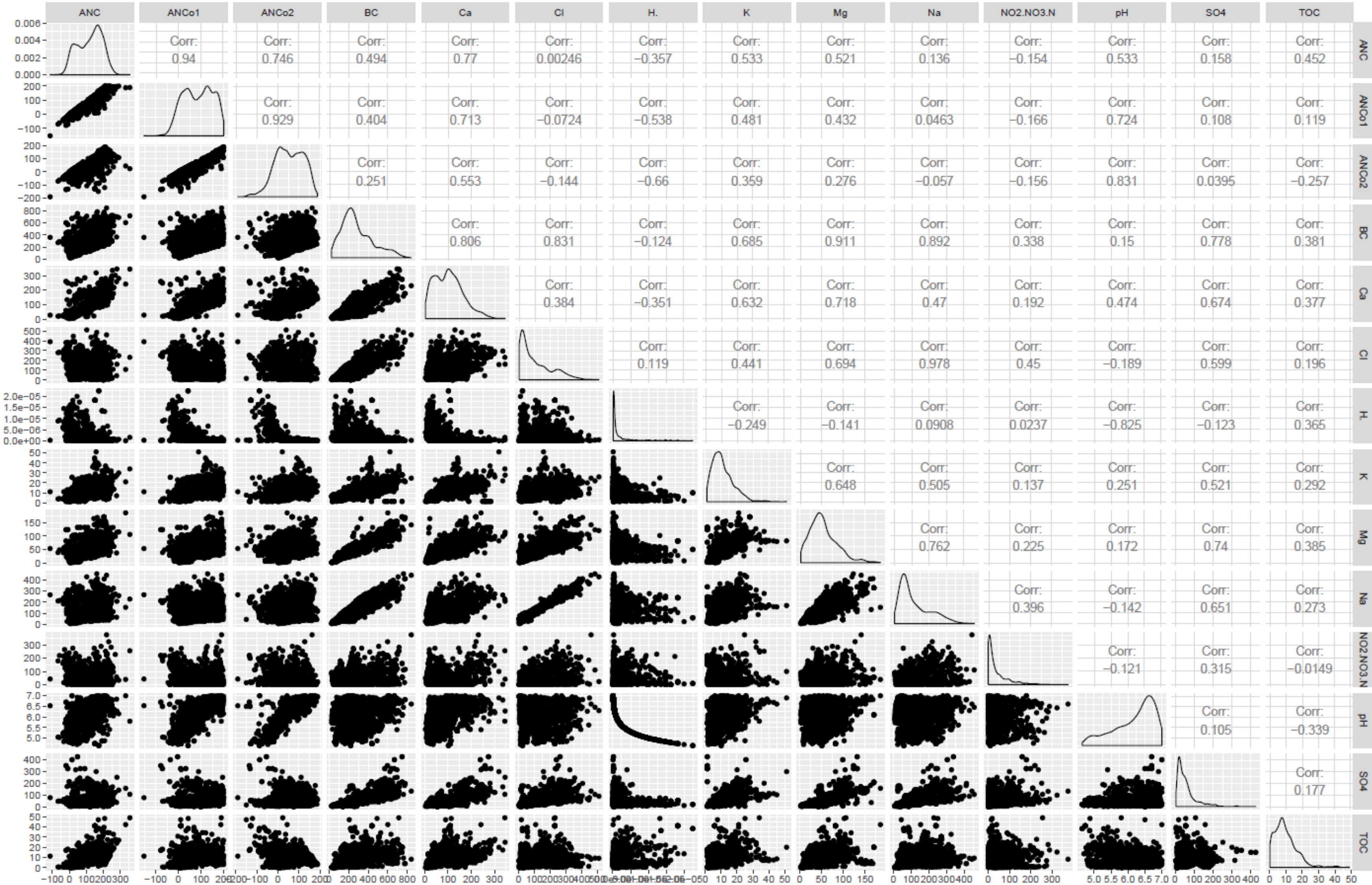

Figure A.2.1. Covariance matrix of environmental variables.

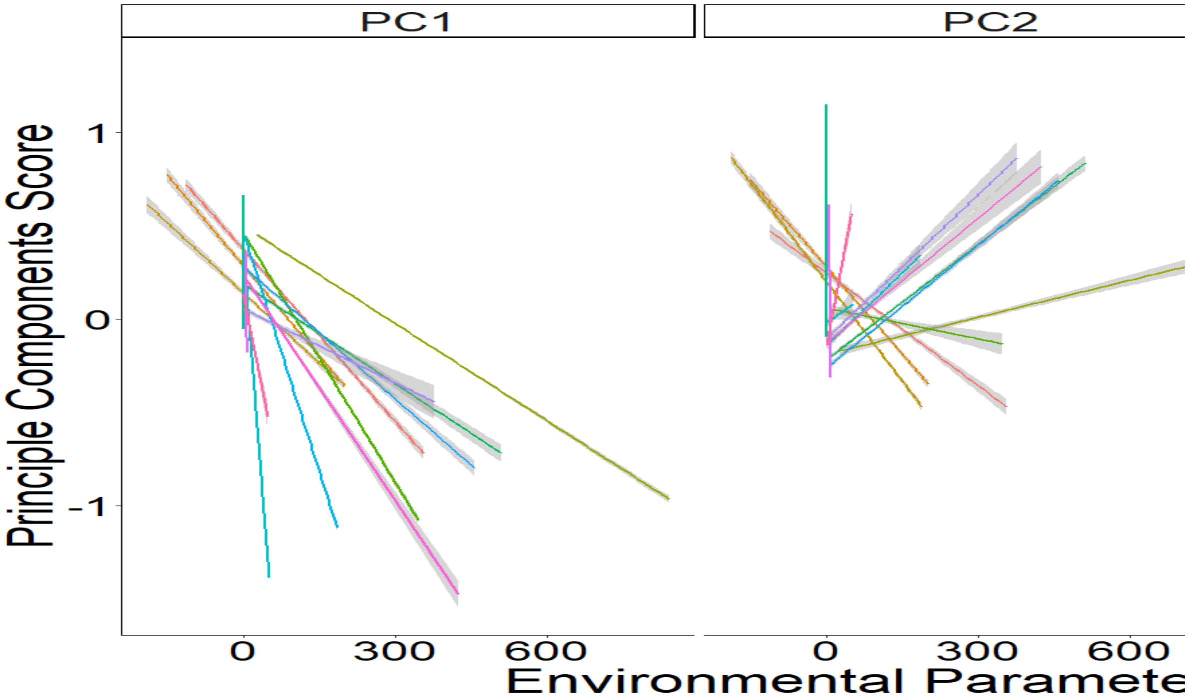

Figure A.2.2. Principle component scores that indicate the combination of environmental variables that best describe the biological community. All three axes are significant.

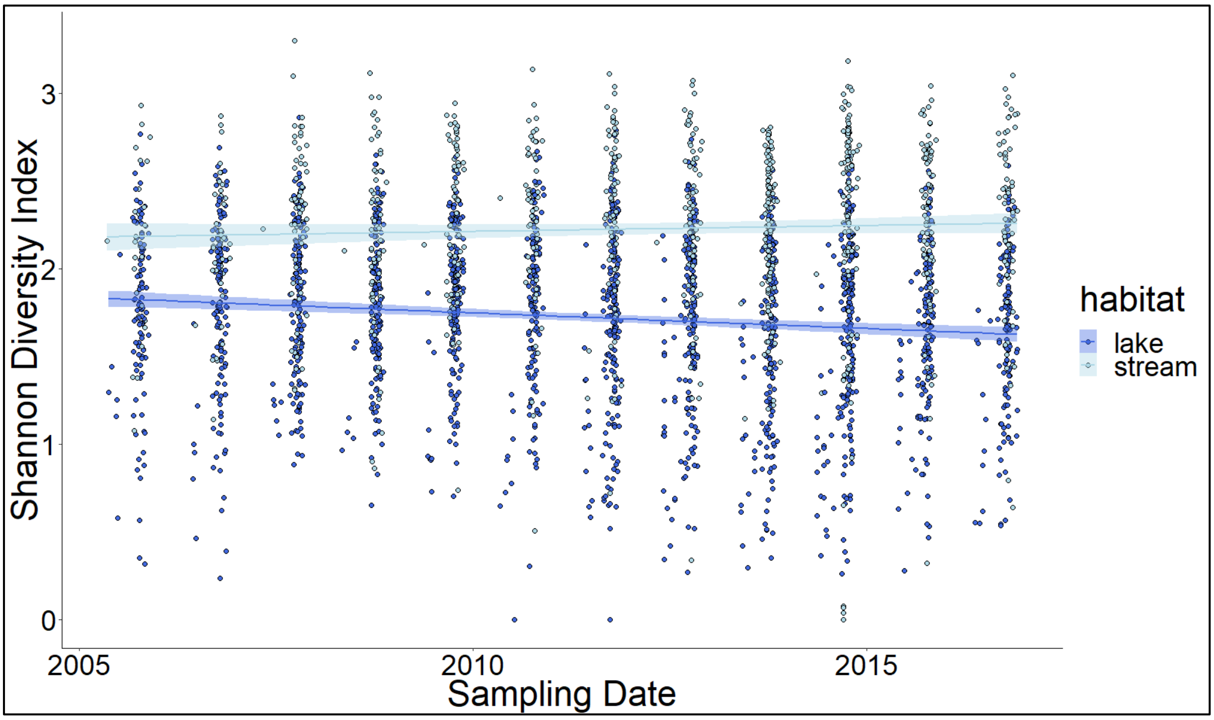

Figure A.2.3. Overall trends in diversity for all sites from Finland, Sweden and Norway, divided into rivers and lakes.

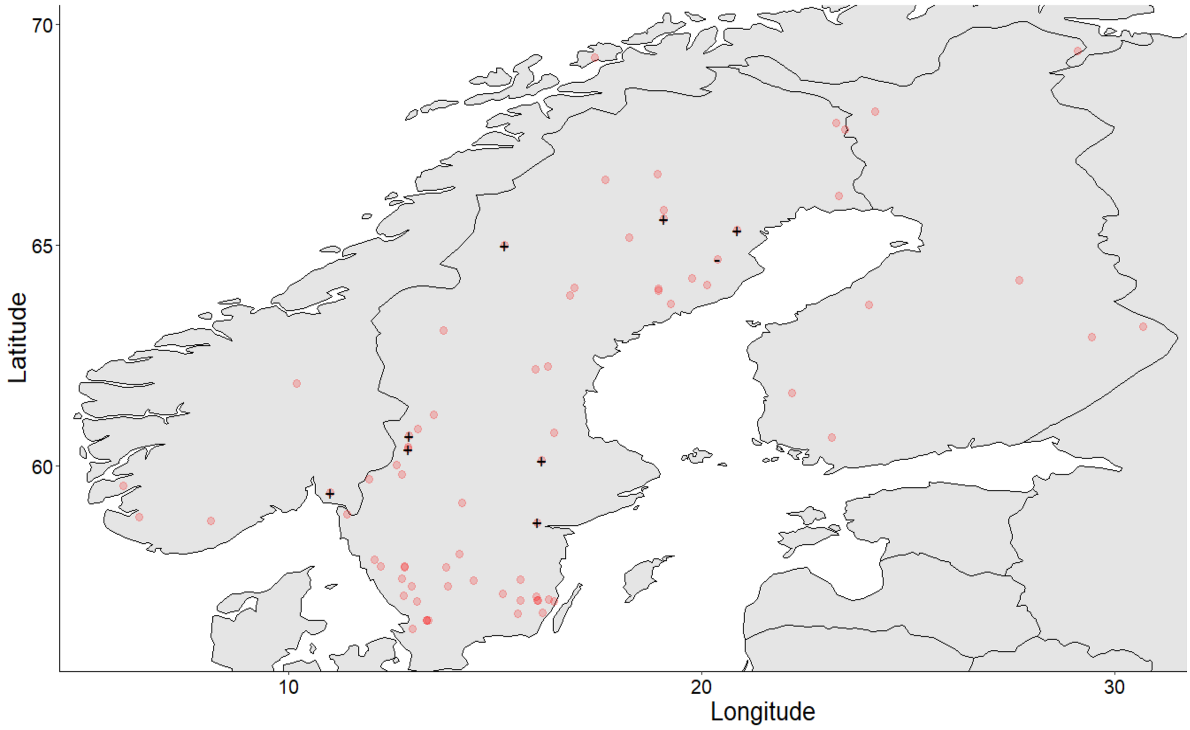

Figure A.2.4. Trends in α- diversity (Shannon) for zoobenthos in all rivers and lakes in the study. The linear trends are from a least squares model where + indicates a significant overall increase since the start of the site- specific sampling programme and – denotes a decrease. A red dot denotes a site with no significant change in diversity.

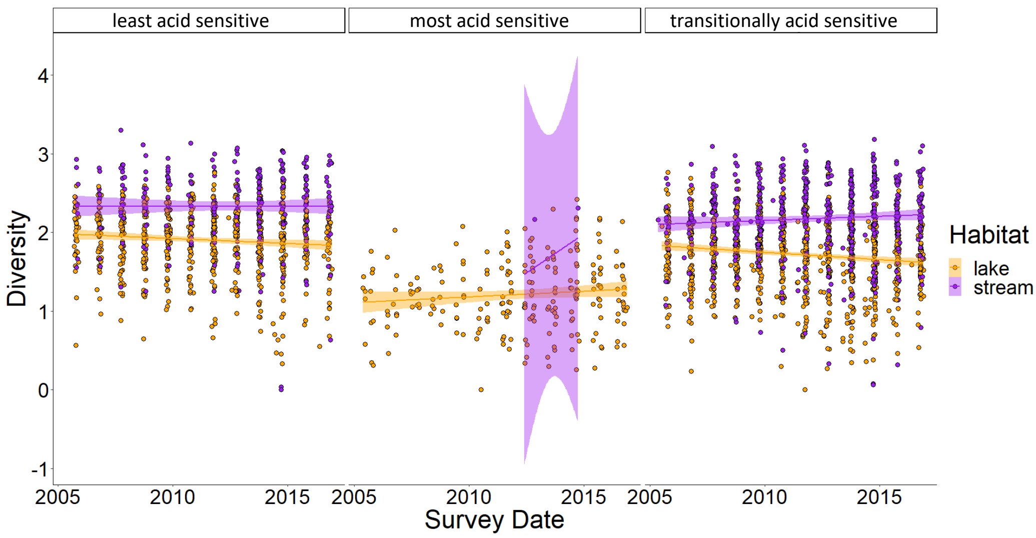

Figure A.2.5. Trends in diversity (Shannon) over time lakes and streams. The sites are clustered in to three categories based on ANCo1: least acid sensitive (ANCo1 > 100), most acid sensitive (ANCo1 < 40) and transitionally acid sensitive (ANCo1 40-100).

There was a positive linear relationship between diversity and acid-base related components of the water (Figure A.2.6 and Figure A.2.7, Table A.2.3 and Table A.2.4) where higher diversity was accompanied with less acidity. This relationship was evident in lakes and streams. Interestingly, there was also a positive linear relationship between diversity and TOC and especially in lakes (Figure A.2.8, Table A.2.3 and Table A.2.4). Time was not significant as explanatory variable for the species diversity in streams (Table A.2.3), agreeing with the finding that the diversity did not change significantly over time for most sites (Figure A.2.3). Time was significant in lakes (Table A.2.3).

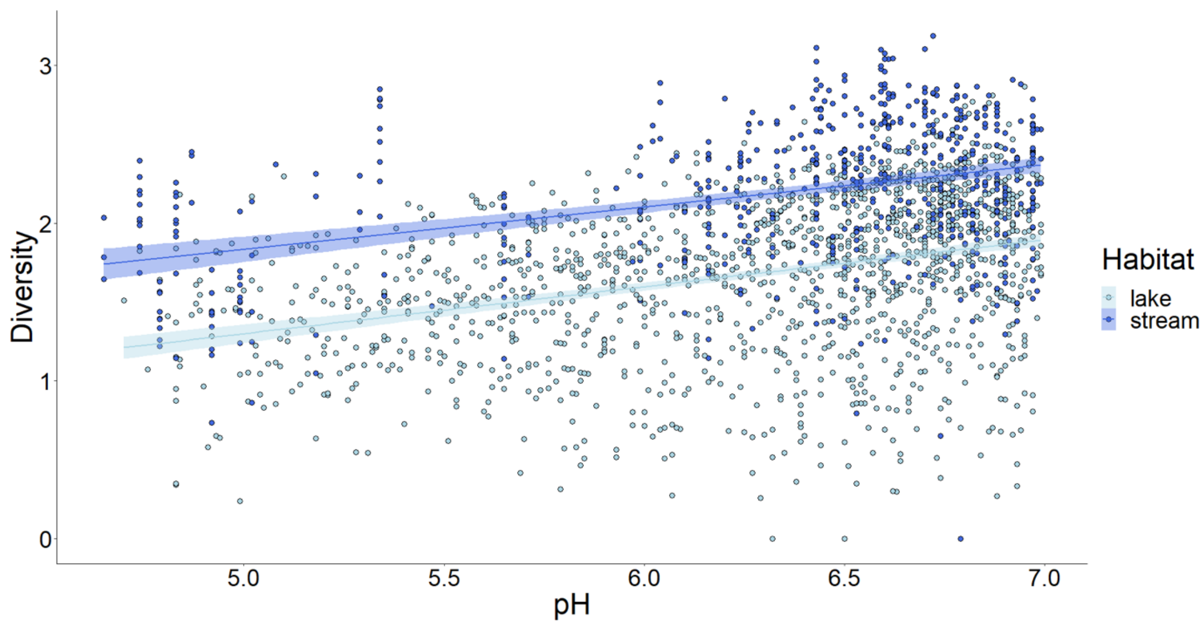

Figure A.2.6. Relationship between pH and diversity (Shannon). The plot is based on a mixed effects model with random intercept of site and random slope of habitat type. pH is truncated to maximum 7.0.

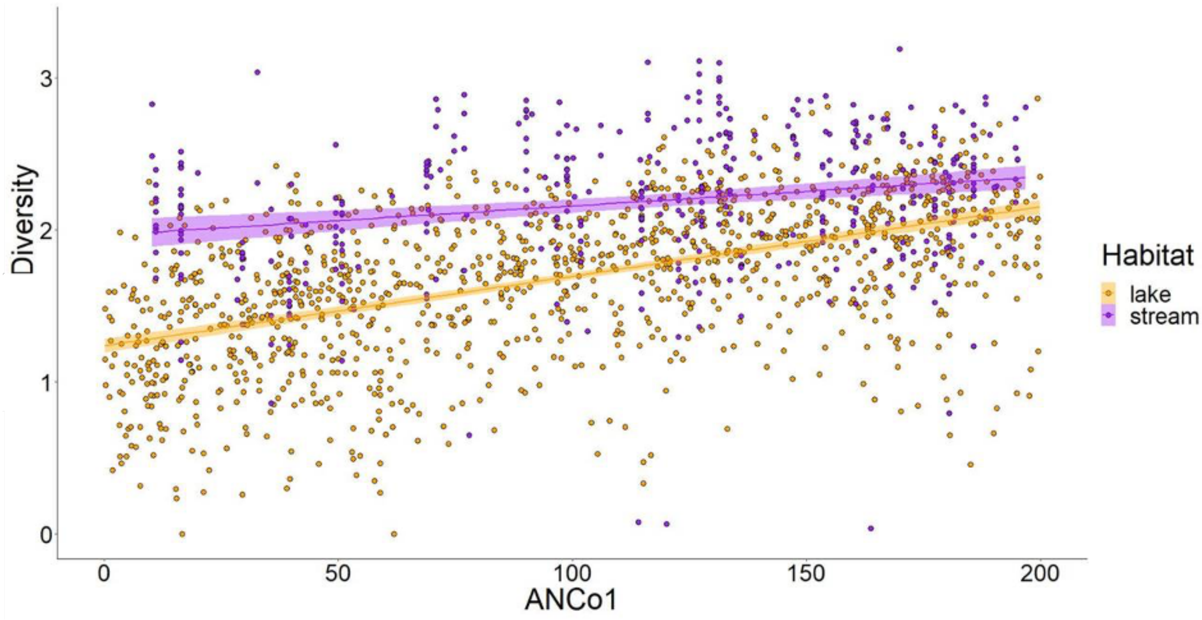

Figure A.2.7. Relationship between ANCo1 and diversity (Shannon). The plot is based on a mixed effects model with random intercept of site and random slope of habitat type. ANCo1 is truncated to maximum 200.

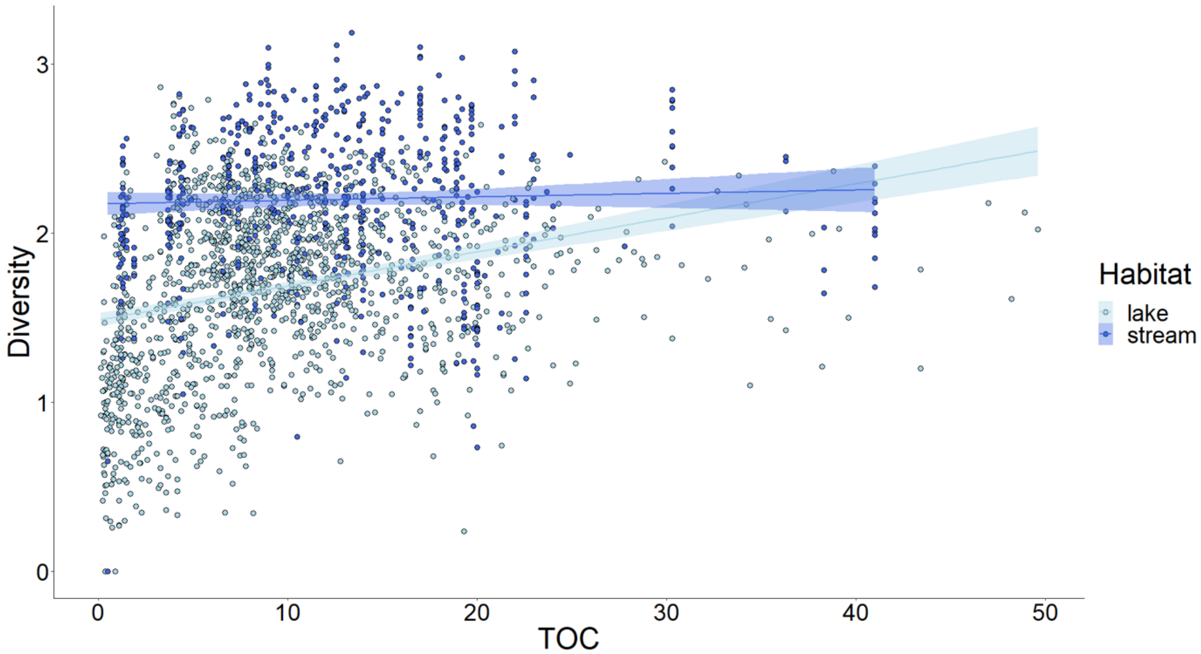

Figure A.2.8. Relationship between TOC and diversity (Shannon). The plot is based on a mixed effects model with random intercept of site and random slope of habitat type. Only sites with pH < 7.0 are included to have a comparable selection of sites as the other analyses of chemical drivers of diversity

Table A.2.3**.** Mixed effects model with random intercept of site and random slope of habitat type for lakes.

Variable	**Value**	**Std.Error**	**DF**	**t-value**	**p-value**
(Intercept)	1.09	0.27	1215.00	4.08	0.00
pH	0.05	0.04	1215.00	1.14	0.25
ANCo1	0.00	0.00	1215.00	3.55	0.00
TOC	0.01	0.00	1215.00	5.15	0.00
Date (scaled)	-0.03	0.01	1215.00	-3.54	0.00

Table A.2.4**.** Mixed effects model with random intercept of site and random slope of habitat type for streams.

Variable	**Value**	**Std.Error**	**DF**	**t-value**	**p-value**
(Intercept)	-0.60	0.43	423.00	-1.41	0.16
pH	0.42	0.06	187.00	6.54	0.00
ANCo2	0.00	0.00	187.00	-2.26	0.02
TOC	0.02	0.00	187.00	3.60	0.00
Date (scaled)	0.01	0.02	423.00	0.79	0.43

Change in species assemblages over time

The rate of change in the species assemblage occurred more rapidly over time in streams than in lakes (Figure A.2.9). It seems the rate of change in lakes has increased towards the present, while the rate of change was constant over time in streams. When it comes to country, the most pronounced changes in the species assemblages occurred in Sweden, while the least changes occurred in Norway (Figure A.2.10). Especially in the Norwegian streams, the turnover is constant and low, indicating little among-year variation in the assemblages. Most likely, the assemblage shift were more pronounced prior to 2005 concurring with the most pronounced changes in acidification.

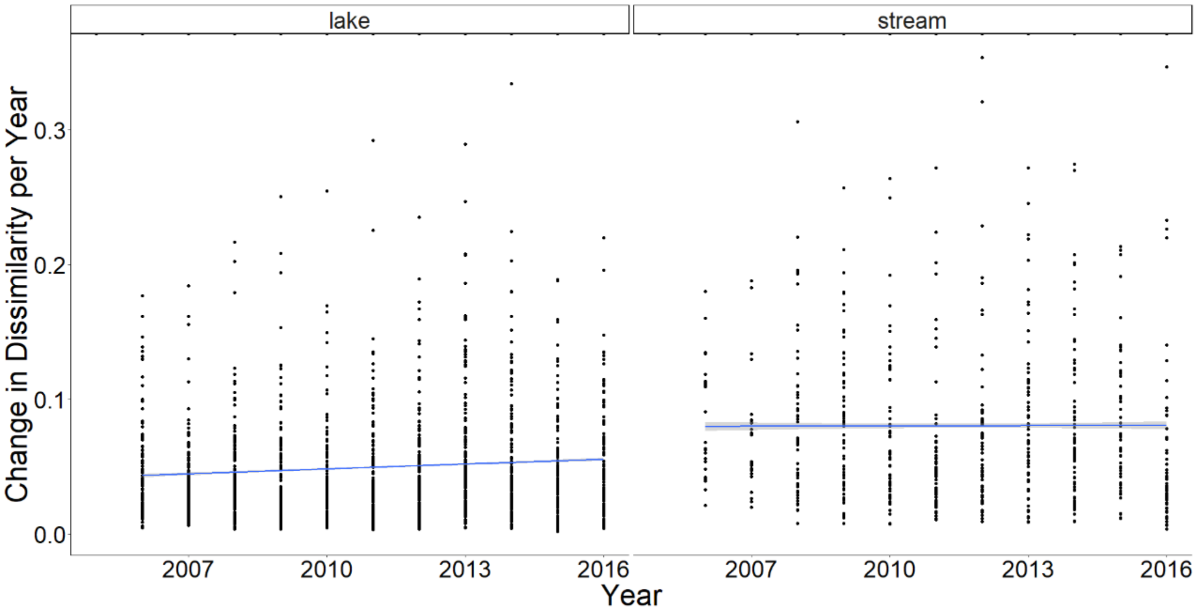

Figure A.2.9. Overall change in the species assemblages over time for streams and lakes.

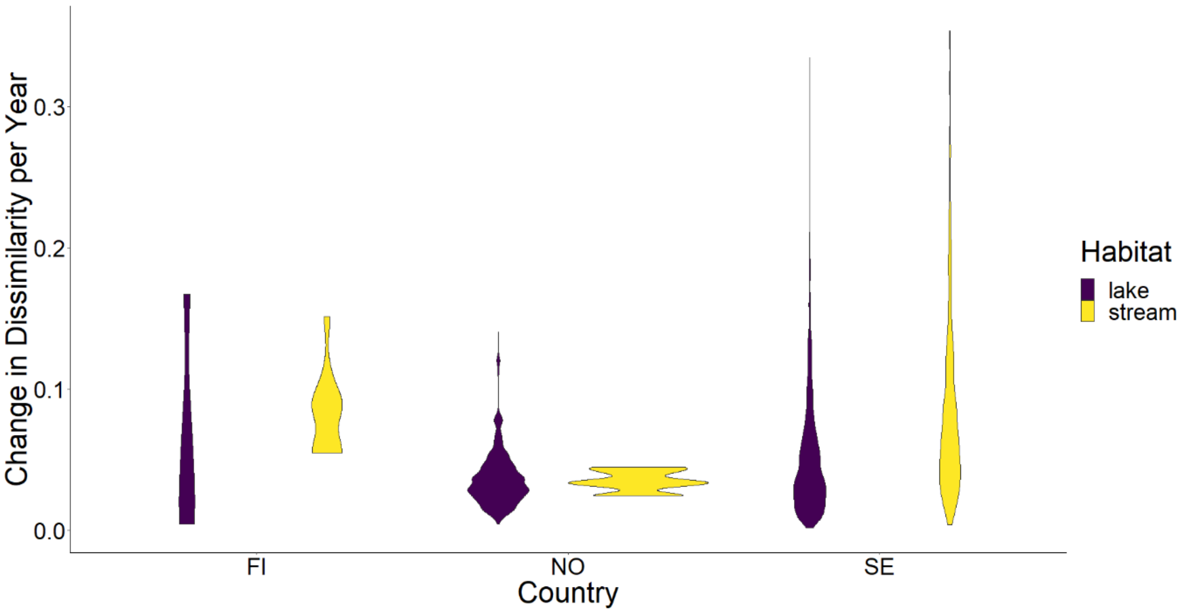

Figure A.2.10. Change in the species assemblages over time for lakes and streams in Finland, Norway and Sweden. Wider shape indicates where the changes in dissimilarity occur most often.

The rates of change were similar for the categories of sites, suggesting that the rates of change in the species assemblages did not vary according to sensitivity towards acidity (Figure A.2.11). Results from the Gradient forest and PERMEANOVA still indicate that acidifying components of the water were correlated to the assemblage changes. In streams, pH was the most important explanatory variable for the species assemblages (Figure A.2.12 and Table A.2.5). In lakes, ANCo1 was the most important explanatory variable (Figure A.2.13, Table A.2.6). Time was less important than the chemical variables as explanatory variable for the species assemblages. However, time was still a significant variable, and most changes were gradual.

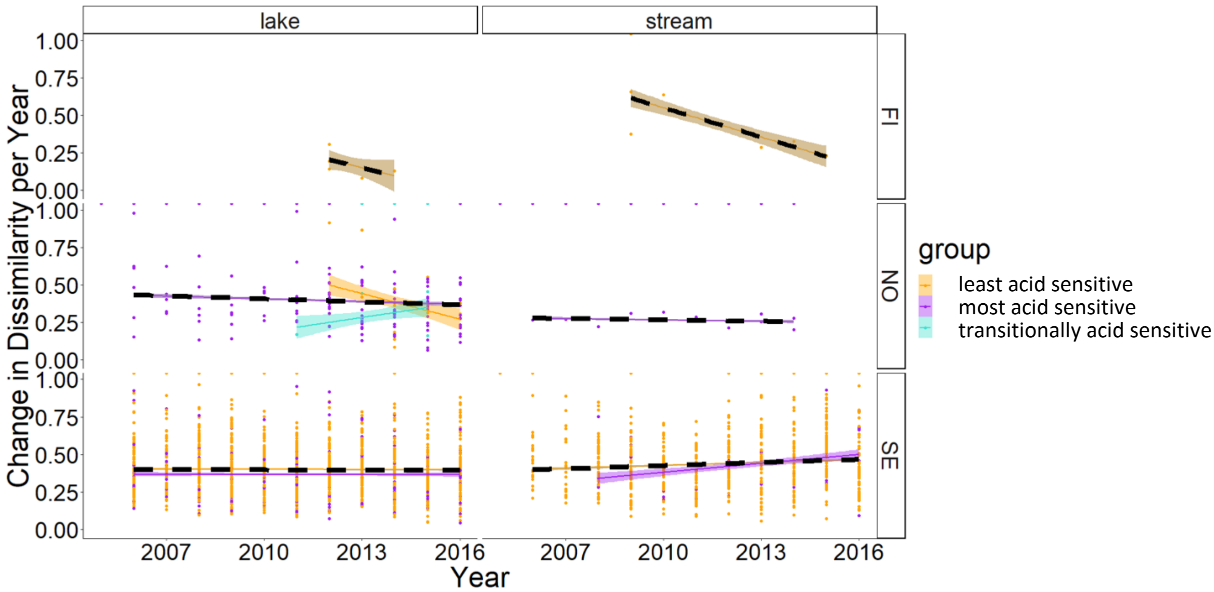

Figure A.2.11. Change in the species assemblages over time for lakes and streams in Finland, Norway and Sweden. The sites are clustered in to three categories based on ANC1: least acid sensitive (ANCo1 > 100), most acid sensitive (ANCo1 < 40) and transitionally acid sensitive (ANCo1 40-100).

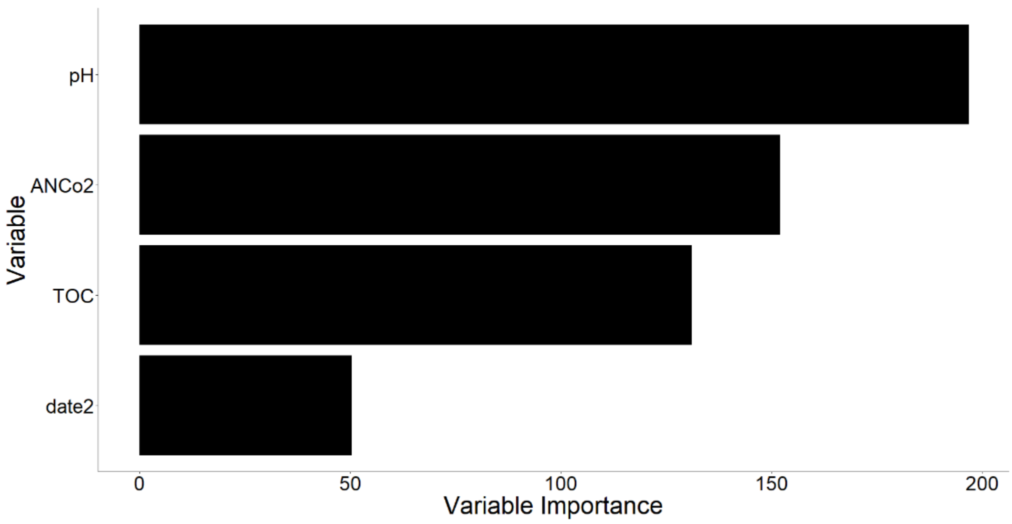

Figure A.2.12. Gradient forest on the importance of some selected variables on the variability of invertebrate assemblages in streams.

Table A.2.5**.** Permeanova indicating the significance for the chemical variables and time as explanatory variables for the biota over in streams.

Variable	**DF**	**Sum-sOfSqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**	**Signifi-cance**
ANCo2	1	3.913	3.9130	14.0039	0.02793	0.001	***
TOC	1	2.384	2.3838	8.5311	0.01701	0.001	***
pH	1	4.993	4.9927	17.8682	0.03563	0.001	***
Time	1	1.140	1.1401	4.0801	0.00814	0.001	***

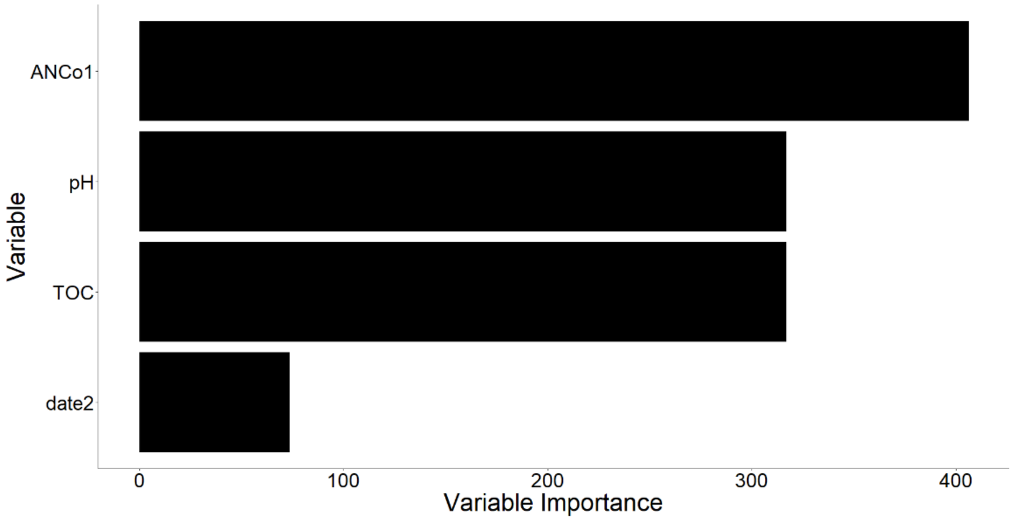

Figure A.2.13. Gradient forest on the importance of some selected variables on the variability of invertebrate assemblages in lakes.

Table A.2.6. Permeanova indicating the significance for the chemical variables and time as explanatory variables for the biota in lakes.

Variable	**Df**	**Sum-sOfSqs**	**MeanSqs**	**F.Model**	**R2**	**Pr(>F)**	**Signifi-cance**
ANCo1	1	21.4	21.402	96.344	0.06733	0.001	***
TOC	1	7.54	7.54	33.945	0.02372	0.001	***
pH	1	2.41	2.41	10.835	0.00757	0.001	***
Time	1	1.93	1.93	1.933	0.00608	0.001	***

Conclusions

It is evident that acid-base related components of the water are strong and significant predictors of both species diversity and assemblage shifts. It is not straightforward to select the best predictor since chemical variables linked to acidification are highly correlated. Still, it seems that ANCo1 and ANCo2 are important predictors in both streams and lakes. In lakes TOC is an additional important predictor, which may be indirectly linked to acidification through brownification of the water (Monteith et al. 2007). The results indicate that the assemblages respond to these variables both over space and over time.

The overall change in diversity index of the benthic invertebrates from 2005 to 2016 was only significant for eight of the sites in the analyses. Most likely, the diversity has already increased in many sites prior to 2005 as a response to reduced acidification (Velle et al. 2013).

Acidification may influence the invertebrates in diverse ways. Especially, measures of ANC are likely important since zoobenthos actively use ions for their acid-base balance and ionic equilibrium between blood and tissue. The animals lose some ions by diffusion over gills and permeable parts of the body. They also excrete ammonia or ammonium via the gills, and need to actively take up cations to maintain electroneutrality (Morris et al. 1989).

The strong response to acid-base related components suggests that we can build a robust model to predict the ecological status based on fauna at any one site - that is an acidity index. The advantage with a data set consisting of many sites, such as the Nordic data base used in the current study, is that we can examine whether the responses are universal, or whether the responses vary according to type of water body. If the responses are universal, we can build common ecological indices with common threshold values. For examples, the Norwegian classification system used under the EU Water framework directive includes several water body types where each has unique threshold values that indicate the ecological status of the site (Veileder 02:2018). Still, the low end of the gradient including calcium poor sites with (very) clear water are poorly represented in the data. It would be an advantage to add more sites in this end of the gradient, and especially sites with running water. There are currently few such sites in the data set. A common acidity index with common threshold values would ease the intercalibration work and ensure common practices among countries.

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Appendix 3. Examples of lakes with large differences in classifications for different systems

Classifications with Norwegian (Norw.) and Swedish (Swe) and three draft suggestions to new classification represent high (H), good (G), moderate (M), poor (P) and bad (B) ecological status. Lakes sites are described by country (F = Finland, N = Norway and S = Sweden), site-ID, current mearuments of Ca, TOC, ANC and Ali, as well as estimated reference value of ANC (ANC_{ref}) and current ANC deviation from ANC_{ref} (dANC).

Example	Norw	Swe	Draft 1	Draft 2	Draft 3	Country	site-ID	Ca µeq/l	TOC mg/l	pH	ANC µeq/l	SO4 µeq/l	Al/L µg/l	ANC_{ref} $MAGIC_{regr}$	dANC SWE
1	G	B	B	M		S	643914-127698-NW643960-127717	21.9	4.4	5.1	0	56.7	63.7	63	63
2	H	P	M	G		N	067-26000-L	11.2	4.2	5.2	13.4	21.8	27.7	35	22
3	M	B	B	P		S	637260-128728-SE637260-128728	66.9	2.5	5.9	13.9	92.8		110	96
4	H	P	M	G		N	067-26133-L	11.8	3.8	5.3	19.4	18	32.7	38	18
5	G	B	P	M		N	024-21894-L	47.4	2.5	5.8	25.9	50.5	18.2	91	65
6	H	M	M	G		N	044-22101-L	34	3.1	5.9	30	24	10.3	64	34
7	G	B	P	P		S	632515-146675-SE632515-146675	61.9	4.5	5.7	31.6	104.3		109	77
8	G	P	P	B		S	758677-161050-SE758677-161050	146.9	0.6	6.5	40.4	179.6		152	112
9	H	M	M	G		S	664603-136484-NW664597-136454	36.3	11.4	5.0	46.5	40.2		74	27
10	H	M	M	G		F	3540	44.2	5.6	6.0	49.8	52.5		83	33
11	G	B	B	B		S	623624-141149-NW623507-141145	186.6	10.5	5.5	53.4	215.8		224	170
12	G	B	P	P		S	638665-129243-NW638595-129158	65.4	8	5.6	54.1	66.3		129	75
13	H	M	M	M		S	640609-148673-NW640599-148678	46	7.7	5.7	55.2	52.8		93	38
14	H	M	M	M		F	1310	76.7	5.8	6.2	71.9	85		127	56
15	G	B	P	B		S	624486-141154-NW624492-141135	137.1	9.8	6.0	72	205.6		231	159
16	H	G	M	M		N	247-64713-L	73.2	3.4	6.6	80.2	84.6	6	137	56
17	G	P	P	B		S	624421-147234-SE624373-147299	163.2	7.4	6.1	86.5	206.5		238	151
18	H	G	M	M		F	1375	104.8	3.9	6.8	87.1	72.9		142	55
19	H	G	M	M		F	2782	125.5	3.2	6.8	95.4	74.4		144	49
20	H	M	M	P		S	624038-143063-NW623984-143051	127.6	8.6	6.3	101.1	104.6		188	87
21	H	G	M	M		F	1255	77	6.3	6.5	106.7	72.1		159	52
22	H	H	M	M		N	247-64482-L	90.2	2.1	6.9	110.2	76.3	3.2	166	55
23	H	M	M	P		S	646293-126302-NW646288-126346	109.9	10	6.3	116.2	81.9		208	92
24	H	G	M	P		S	634057-144257-NW634057-144257	116.5	8.1	6.2	136.9	118.5		224	87
25	H	G	M	P		S	642489-151724-SE642489-151724	142.2	7.2	6.7	142.9	118.2		225	82
26	H	G	M	P		S	633025-142267-SE633025-142267	138.8	7.9	6.7	150.2	102		231	81
27	H	G	M	P		S	645289-128665-NW645343-128665	150.1	11	6.4	153.7	75.3	6	233	79
28	H	G	M	P		S	644987-152393-SE644987-152393	158.8	6.8	6.8	165.9	103.1		238	72
29	G	B	P	P		S	632515-146675-SE632515-146675	61.9	4.5	5.7	31.6	104.3		109	77
30	G	P	P	B		S	758677-161050-SE758677-161050	146.9	0.6	6.5	40.4	179.6		152	112
31	G	B	B	B		S	623624-141149-NW623507-141145	186.6	10.5	5.5	53.4	215.8		224	170
32	G	B	P	P		S	638665-129243-NW638595-129158	65.4	8	5.6	54.1	66.3		129	75
33	H	M	M	M		S	640609-148673-NW640599-148678	46	7.7	5.7	55.2	52.8		93	38
34	G	M	M	P		F	1365	81.4	4.2	6.2	58.2	96.9		128	70

Exam-ple	Norw	Swe	Draft 1	Draft 2	Draft 3	Country	site-ID	Ca µeq/l	TOC mg/l	pH	ANC µeq/l	SO4 µeq/l	Al/L µg/l	ANCref MAGICregr	dANC SWE
35	H	M	M	M		F	1310	76.7	5.8	6.2	71.9	85		127	56
36	G	B	P	B		S	624486-141154-NW624492-141135	137.1	9.8	6	72	205.6		231	159
37	H	G	M	M		N	247-64713-L	73.2	3.4	6.6	80.2	84.6	6	137	56
38	G	B	M	P		S	633989-140731-NW634041-140729	101	14.5	5.3	83.1	87.4		156	73
39	G	P	P	B		S	624421-147234-SE624373-147299	163.2	7.4	6.1	86.5	206.5		238	151
40	H	G	M	M		F	1375	104.8	3.9	6.8	87.1	72.9		142	55
41	H	G	G	M		F	90	61.5	9	5.9	87.7	50		126	38
42	H	G	G	M		N	012-5771-L	89.4	4.3	6.7	88.1	41.3	12.4	125	37
43	H	G	G	M		S	655209-126937-SE655209-126937	18.9	6	6	90.7	39.3		131	40
44	H	G	M	M		F	2782	125.5	3.2	6.8	95.4	74.4		144	49
45	H	M	M	P		S	624038-143063-NW623984-143051	127.6	8.6	6.3	101.1	104.6		188	87
46	H	G	G	M		F	3634	80.9	8.5	6.4	104.2	61.3		147	43
47	H	G	M	M		F	1255	77	6.3	6.5	106.7	72.1		159	52
48	H	H	M	M		N	247-64482-L	90.2	2.1	6.9	110.2	76.3	3.2	166	55
49	H	M	M	P		S	646293-126302-NW646288-126346	109.9	10	6.3	116.2	81.9		208	92
50	H	G	G	M		S	656263-156963-NW656322-156971	98.5	15.5	5.5	129.8	54.7		168	38
51	H	H	G	M		N	017-6701-L	146.2	3.8	6.9	131.2	41.9	13	172	41
52	H	H	G	M		S	672467-148031-SE672467-148031	114.8	4.4	6.5	132.4	76.1		183	51
53	H	G	G	M		S	664715-151400-NW664774-151407	103.5	9.7	6.4	134.8	77.5		188	54
54	G	M	M	P		S	633738-142203-NW633823-142163	122.3	18.2	5.6	134.8	88.1		208	73
55	H	G	M	P		S	634057-144257-NW634057-144257	116.5	8.1	6.2	136.9	118.5		224	87
56	H	G	G	M		S	655587-158869-SE655605-158820	142.9	10.7	6.4	137.1	85.6		196	59
57	H	G	M	P		S	642489-151724-SE642489-151724	142.2	7.2	6.7	142.9	118.2		225	82
58	H	G	G	M		S	644463-139986-NW644467-139971	133.9	9.5	5.5	146	70.6		202	56
59	H	G	M	P		S	633025-142267-SE633025-142267	138.8	7.9	6.7	150.2	102		231	81
60	H	G	M	P		S	645289-128665-NW645343-128665	150.1	11	6.4	153.7	75.3	6	233	79
61	H	G	G	M		S	635878-137392-NW635849-137394	141.8	10	6.3	154.8	68.7		213	58
62	H	G	G	M		S	637121-151366-NW637090-151377	121.6	10.8	6.4	155.1	73.4		214	59
63	H	H	G	M		F	2182	136.4	6.3	6.7	157.5	95.8		221	63
64	H	G	G	M		F	1295	144.7	15	6.6	165.5	85.4		220	55
65	H	H	G	M		F	6464	124.8	10.7	6.8	165.5	74.3		214	48
66	H	G	M	P		S	644987-152393-SE644987-152393	158.8	6.8	6.8	165.9	103.1		238	72
67	H	G	G	M		F	3146	109.8	26	5.7	183	68.7		229	46
68	H	H	G	M		S	662322-139339-SE662322-139339	128	4.3	6.8	183.5	58		228	44
69	H	H	G	M		S	664197-149337-SE664197-149337	154.6	7.8	6.7	188	69.2		246	58
70	H	H	G	M		S	650061-142276-NW650033-142304	134.8	7.5	6.6	198.9	51.8		240	41
71	M	G	M	H		N	020-10069-L	12.7	5.5	5	7.6	16.7	55.8	22	14
72	G	B	M	G		F	12035	27.8	3.2	6	17	44.3		45	28
73	G	B	M	G		S	662682-132860-NW662756-132817	31.2	4.8	5.5	19.3	45.8	27.1	53	34
74	G	B	M	M		S	652902-125783-NW652888-125811	54.5	12.5	5.2	62.7	50.3		115	53
75	G	B	M	M		S	647050-130644-NW647037-130646	99.2	14.9	5.3	77.4	63.6		143	66
76	G	B	M	P		S	633989-140731-NW634041-140729	101	14.5	5.3	83.1	87.4		156	73
77	G	P	H	H		S	627443-149526-NW627437-149509	182.3	22.6	5.5	171.9	160.1		293	121
78	G	M	H	H		S	626898-138855-NW626873-138871	158.2	35.2	5.3	200.2	89.3		288	88
79	H	H	M	M		N	247-64482-L	90.2	2.1	6.9	110.2	76.3	3.2	166	55

Exam-ple	Norw	Swe	Draft 1	Draft 2	Draft 3	Country	site-ID	Ca µeq/l	TOC mg/l	pH	ANC µeq/l	SO4 µeq/l	Al/L µg/l	ANCref MAGICregr	dANC SWE
80	H	H	M	M		N	247-64482-L	90.2	2.1	6.9	110.2	76.3	3.2	166	55
81	H	H	G	M		N	017-6701-L	146.2	3.8	6.9	131.2	41.9	13	172	41
82	H	H	G	M		S	672467-148031-SE672467-148031	114.8	4.4	6.5	132.4	76.1		183	51
83	H	G	M	P		S	634057-144257-NW634057-144257	116.5	8.1	6.2	136.9	118.5		224	87
84	H	G	M	P		S	642489-151724-SE642489-151724	142.2	7.2	6.7	142.9	118.2		225	82
85	H	G	M	P		S	633025-142267-SE633025-142267	138.8	7.9	6.7	150.2	102		231	81
86	H	G	M	P		S	645289-128665-NW645343-128665	150.1	11	6.4	153.7	75.3	6	233	79
87	H	H	G	M		F	2182	136.4	6.3	6.7	157.5	95.8		221	63
88	G	H	G	M		S	673534-153381-SE673534-153381	125.6	13.2	6.2	163.8	55.8		200	36
89	H	H	G	M		F	6464	124.8	10.7	6.8	165.5	74.3		214	48
90	H	G	M	P		S	644987-152393-SE644987-152393	158.8	6.8	6.8	165.9	103.1		238	72
91	H	H	G	M		S	662322-139339-SE662322-139339	128	4.3	6.8	183.5	58		228	44
92	H	H	G	M		S	664197-149337-SE664197-149337	154.6	7.8	6.7	188	69.2		246	58
93	H	H	G	M		S	650061-142276-NW650033-142304	134.8	7.5	6.6	198.9	51.8		240	41
94	P	B	B	M		N	026-21438-L	21.6	1	5.3	-6.9	37.7	54.1	48	55
95	G	B	B	M		S	643914-127698-NW643960-127717	21.9	4.4	5.1	-0.1	56.7	63.7	63	63
96	G	M	G	H		N	061-26511-L	7.7	0.4	5.4	4.9	9.5	7.7	14	9
97	H	P	M	G		N	067-26000-L	11.2	4.2	5.2	13.4	21.8	27.7	35	22
98	M	P	M	G		N	038-22548-L	18.1	1.8	5.7	14.1	19	14.3	36	22
99	G	B	M	G		F	12035	27.8	3.2	6	17	44.3		45	28
100	G	B	M	G		S	662682-132860-NW662756-132817	31.2	4.8	5.5	19.3	45.8	27.1	53	34
101	H	P	M	G		N	067-26133-L	11.8	3.8	5.3	19.4	18	32.7	38	18
102	G	B	P	M		N	024-21894-L	47.4	2.5	5.8	25.9	50.5	18.2	91	65
103	M	B	P	M		N	022-11592-L	49.4	5.7	5.3	26.5	43.4	53	80	54
104	G	P	M	G		N	021-11147-L	39.4	6.4	5.4	33.5	34.7	27.7	67	33
105	G	B	M	M		S	652902-125783-NW652888-125811	54.5	12.5	5.2	62.7	50.3		115	53
106	G	B	M	M		S	647050-130644-NW647037-130646	99.2	14.9	5.3	77.4	63.6		143	66
107	G	P	H	H		S	627443-149526-NW627437-149509	182.3	22.6	5.5	171.9	160.1		293	121
108	G	M	H	H		S	626898-138855-NW626873-138871	158.2	35.2	5.3	200.2	89.3		288	88
109	G	B	B	M	P	S	643914-127698-NW643960-127717	21.9	4.4	5.1	-0.1	56.7	63.7	63	63
110	H	M	M	G	M	N	044-22101-L	33.6	3.1	5.9	30	23.9	10.3	64	34
111	G	P	P	B	B	S	758677-161050-SE758677-161050	146.9	0.6	6.5	40.4	179.6		152	112
112	G	B	B	B	B	S	623624-141149-NW623507-141145	186.6	10.5	5.5	53.4	215.8		224	170
113	G	B	P	P	P	S	638665-129243-NW638595-129158	65.4	8	5.6	54.1	66.3		129	75
114	G	M	M	P	P	F	1365	81.4	4.2	6.2	58.2	96.9		128	70
115	H	M	M	M	M	F	1310	76.7	5.8	6.2	71.9	85		127	56
116	G	B	P	B	B	S	624486-141154-NW624492-141135	137.1	9.8	6	72	205.6		231	159
117	G	G	M	M	P	N	020-11074-L	95.5	4.3	6.3	76.6	64.6	14	145	68
118	H	G	M	M	M	N	247-64713-L	73.2	3.4	6.6	80.2	84.6	6	137	56
119	G	B	M	P	P	S	633989-140731-NW634041-140729	101	14.5	5.3	83.1	87.4		156	73
120	G	P	P	B	B	S	624421-147234-SE624373-147299	163.2	7.4	6.1	86.5	206.5		238	151
121	H	G	M	M	M	F	1375	104.8	3.9	6.8	87.1	72.9		142	55
122	H	G	G	M	M	F	90	61.5	9	5.9	87.7	50		126	38
123	H	G	G	M	M	N	012-5771-L	89.4	4.3	6.7	88.1	41.3	12.4	125	37
124	H	G	G	M	M	S	655209-126937-SE655209-126937	18.9	6	6	90.7	39.3		131	40

Exam-ple	Norw	Swe	Draft 1	Draft 2	Draft 3	Country	site-ID	Ca µeq/l	TOC mg/l	pH	ANC µeq/l	SO4 µeq/l	Al/L µg/l	ANCref MAGICregr	dANC SWE
125	H	G	M	M	M	F	2782	125.5	3.2	6.8	95.4	74.4		144	49
126	H	M	M	P	P	S	624038-143063-NW623984-143051	127.6	8.6	6.3	101.1	104.6		188	87
127	H	G	G	M	M	F	3634	80.9	8.5	6.4	104.2	61.3		147	43
128	H	G	M	M	M	F	1255	77	6.3	6.5	106.7	72.1		159	52
129	H	H	M	M	M	N	247-64482-L	90.2	2.1	6.9	110.2	76.3	3.2	166	55
130	H	M	M	P	M	S	646293-126302-NW646288-126346	109.9	10	6.3	116.2	81.9		208	92
131	G	G		M	P	N	020-11074-L	95.5	4.3	6.3	76.6	64.6	14	145	68
132	H	H		M	M	N	247-64482-L	90.2	2.1	6.9	110.2	76.3	3.2	166	55
133	H	P		G	G	N	067-26000-L	11.2	4.2	5.2	13.4	21.8	27.7	35	22
134	M	P		G	G	N	038-22548-L	18.1	1.8	5.7	14.1	19	14.3	36	22
135	G	B		G	M	F	12035	27.8	3.2	6	17	44.3		45	28
136	G	B		G	M	S	662682-132860-NW662756-132817	31.2	4.8	5.5	19.3	45.8	27.1	53	34
137	H	P		G	G	N	067-26133-L	11.8	3.8	5.3	19.4	18	32.7	38	18
138	M	B		P	M	S	633437-143286-NW633400-143306	57.1	4.2	5.6	23.4	109.1		105	82
139	G	B		M	M	N	024-21894-L	47.4	2.5	5.8	25.9	50.5	18.2	91	65
140	M	B		M	M	N	022-11592-L	49.4	5.7	5.3	26.5	43.4	53	80	54
141	G	B		P	M	S	632515-146675-SE632515-146675	61.9	4.5	5.7	31.6	104.3		109	77
142	G	P		G	G	N	021-11147-L	39.4	6.4	5.4	33.5	34.7	27.7	67	33
143	G	B		M	M	S	652902-125783-NW652888-125811	54.5	12.5	5.2	62.7	50.3		115	53
144	G	B		M	M	S	647050-130644-NW647037-130646	99.2	14.9	5.3	77.4	63.6		143	66
145	G	P	H	H	H	S	627443-149526-NW627437-149509	182.3	22.6	5.5	171.9	160.1		293	121
146	G	M	H	H	H	S	626898-138855-NW626873-138871	158.2	35.2	5.3	200.2	89.3		288	88