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# Assessing acidity impacts in Nordic lakes and streams: Development of a macroinvertebrate-based multimetric index to quantify degradation and recovery

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ABSTRACT

1) Emissions of acidifying compounds have decreased over several decades, nevertheless acidification impacts on aquatic ecosystems remains as a regionally important environmental driver resulting in biodiversity loss and impaired function in many lakes and streams.
2) Many metrics based on macroinvertebrates are currently used to assess the biological impacts of acidification. However, few include measures of community composition, abundance, diversity, and the presence/absence of tolerant/sensitive taxa, and fewer still are calibrated simultaneously for both lentic and lotic waters and across large geographic regions.
3) Data on water chemistry and benthic macroinvertebrates was extracted from a database compiled by representatives from Norway, Sweden and Finland. Using lake- and stream data on water chemistry and macroinvertebrates from Norway and Sweden, we developed a Nordic macroinvertebrate-based multimetric index (MMI) for acidity (NAMI) to assess impacts of and recovery from acidity using information on measures of community structure and traits.
4) Lake and stream datasets were explored together and independently for correlation between measures of community structure and traits to acid neutralizing capacity (ANC), modified ANC regarding 1/3 of the organic acids as strong (ANCo1) and pH. Significantly correlated candidate metrics with highest correlations were chosen using forward stepwise linear regression models against ANC, ANCo1 and pH. Results showed that the combined lake and stream MMI had the highest correlation (r-squared) with ANCo1.
5) Seven metrics were included in NAMI: one measure of composition (sum of the combined relative abundance of Gastropoda, Bivalvia and Crustacea), two measures of diversity (the number of Ephemeroptera taxa excluding leptophlebiids and the number of Bivalvia taxa), one effect trait (taxa with life cycle duration $>$ 1 year), two response traits (taxa with resistance forms: eggs and plastron respiration) and one tolerance trait (preference $>$ 5 pH $<$ 5.5).
6) The NAMI is a promising metric to standardize lake and stream macroinvertebrate assessments of acidity impacts and recovery across the Nordic countries, and to harmonize chemical and biological classifications of water quality, including progress towards achieving international objectives.

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## 1. Introduction

The acidification of surface waters has been a severe and spatially extensive environmental issue in Europe and in the northern regions of North America (Muniz, 1990; Havas and Rosseland, 1995; Grennfelt et al., 2020). Atmospheric deposition of acid components had a significant impact on lakes and rivers, particularly until the 1990s (Schindler et al., 1989; Skjelkvåle et al., 2005). This led to marked decreases in pH and alkalinity and associated increases in sulphate and toxic aluminium. Moreover, intensive land use in regions with acid sulphate soils causes mobilisation of acidity from the soil that via runoff results in severe acidification in waterbodies (e.g. Corfield, 2000; Fältmarsch et al., 2008). Degraded water quality due to acidification results in biodiversity loss and a general shift in community structure from acid-sensitive to more acid-tolerant taxa (Larsen et al., 1996; Sandin et al., 2004; Fölster et al., 2021). Although decreased emissions and acidic deposition have resulted in improvements in surface water quality (Stoddard et al., 1999; Skjelkvåle et al., 2005), biological recovery of acidified ecosystems is often slow due to legacy acidification of soils and due to deposition at some sites remaining at non-sustainable levels (e.g. Stendera and Johnson, 2008; Johnson and Angeler, 2010a; Angeler and Johnson, 2012). Thus, acidification persists as one of the foremost problems affecting the biodiversity and functioning of inland surface waters (e.g. Driscoll and Wang, 2019; Laudon et al., 2021; Angeler et al., 2021).

In Europe, the Nordic countries were especially impacted by atmospheric acidification due to low buffering soils and location downwind to intensive industrial regions. Norway experienced the strongest impacts, due to high levels of deposition and the prevalence of thin, siliceous soils (Henriksen et al., 1998). In Finland, humic acids and oxidation of sulphide soils were considered as more important determinants of aquatic acidity than acidic deposition (Henriksen et al., 1998). Sweden was somewhat in-between as lakes and streams were impacted both by anthropogenic and natural acids (Henriksen et al., 1998). All three countries show relatively large spatial gradients in acidic deposition related to average precipitation and proximity to pollution sources (Henriksen et al., 1998).

For the classification of acidification of streams as part of implementing the EU Water Framework Directive the Nordic countries currently use different classification systems based on surface water chemistry (Fölster et al., 2021). Norway and Finland use water body type-specific criteria, whereas Sweden uses a site-specific geochemical model to estimate change in pH relative to reference conditions (Moldan et al., 2013). The Nordic countries also use different approaches for calculating critical loads for acid deposition. These calculations serve as a foundation for the negotiation of reductions of acid deposition within the UN-ECE Convention for Long Range Transboundary Pollutants (de Vries et al., 2015). For estimating critical limits in the calculation of critical loads, Norway and Sweden use a site-specific modelling approach, but with different critical values, while Finland uses typology-based approaches. Discrepancies in the approaches used to classify acidification and estimate critical load have led to a higher exceedance of critical load in Sweden when compared to Norway (Moldan et al., 2015). Some water bodies are even classified differently when they are located between two national borders. These method-related differences in defining acidification threaten the credibility of environmental management and reporting to international agencies. Ideally, a more harmonized classification would involve relating classifications to water quality and biological responses, similar to the European intercalibration exercise (Poikane et al., 2015). In this exercise, member states quantified biological responses and harmonized assessment approaches to different pressures (e.g. Heiskanen et al., 2004). In intercalibrating methods to assess of acidification, member states compared the responses of benthic macroinvertebrates (Sandin et al., 2014) and fish (Olin et al., 2014) across a common set of sites that spanned an acidic gradient of high to low impact.

Benthic macroinvertebrates comprise a diverse and generally abundant group of organisms that are commonly used in monitoring and assessment due to a wide range of environmental tolerances and preferences (Johnson et al., 1993). Low pH values associated with acidification frequently result in changes in benthic macroinvertebrate community structure and function (e.g. Raddum and Fjellheim, 1984; Larsen et al., 1996). Given the relatively strong and quantifiable effects of acidification on macroinvertebrate assemblages, with the loss of sensitive species and increased relative abundances of tolerant species, early efforts to develop response metrics focused solely on the presence/absence of indicator species (Raddum and Fjellheim, 1984; Fjellheim and Raddum, 1992). Many of these relatively simple indices continue to be used in national and European assessments of acidification (Schartau et al., 2008; Sandin et al., 2014). Although the use of simple indices is cost effective as only the presence/absence of a few indicator species need to be identified, it simultaneously ignores potentially important but subtle changes within the assemblage. Moreover, due biogeographical differences in species distributions and regional or local differences species' sensitivities to acidity, caution is advised when using these taxon-based indices outside of the range of the original calibration dataset. Finally, the use of species' traits together with assemblage composition is considered as a powerful approach for assessing impacts across large geographic regions as it alleviates many issues related to biogeographic distributions (e.g. Statzner et al., 2001).

A multimetric index (MMI) combines metrics that characterize biotic communities, such as abundance, composition, richness, diversity and trait attributes, into one composite metric to assess degradation (Karr, 1981). Globally, MMI approaches have been developed to monitor the ecological conditions of lakes and streams (e.g. Barbour, 1999; Smith et al., 1999; Hawkins et al., 2000; Baptista et al., 2007; Golfieri et al., 2018). In this study, we explore the potential of developing an MMI for benthic macroinvertebrate communities in streams and lakes to assess the impacts of acidification across the Nordic countries. Our main aims were to (i) calibrate an MMI to assess the impact of acidity and recovery across the Nordic region and (ii) ultimately develop a common tool for assessing water quality and harmonizing assessments in the Nordic countries. As a case study using three Swedish lakes, our secondary aim was to evaluate if the MMI we developed could indicate temporal recovery from acidification.

## 2. Methods

### 2.1. Data treatment and description

A joint Nordic database, comprising data on water chemistry and benthic macroinvertebrates and compiled by representatives from Norway, Sweden and Finland was used in this study. The Nordic database consists of sites with a minimum of five annual samples that comprised water chemistry, where pH, Ca, Cl, K, Mg, Na, $NO_3$ pH, SO4 and TOC were taken and analysed according to international (ISO) or European (EN) standards (ICP-Waters Programme Centre, 2010). Stream water chemistry was sampled at the same site or stream segment as macroinvertebrates. Samples for lake water chemistry were taken from a mid-lake site at depths 0.5 – 2 m. Acid neutralising capacity (ANC) was calculated according to Table 1, along with modified ANC where 1/3 (ANCo1) or 2/3 (ANCo2) of the organic acids were assessed

**Table 1**
Measures of acidity.

| Acidity related chemical indicators* |
|---|
| pH = −log10 {H+} |
| 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*1/3 * TOC (mg/l) |
| ANCo2 = ANC – 10*2/3 * TOC (mg/l) |

* All units except TOC are in μeq/l

as strong (Lydersen, 2004).

Only data with concurrent samples of macroinvertebrates and water chemistry from 2000 to 2019 were included from the database. Sites that had 18 or more water chemistry sampling occasions were selected to enhance the precision of mean values. This extraction resulted in a dataset of sites representing a gradient of increasing acidity, as characterized by decreasing pH and increasing ANC(s) (Table 2). To minimize noise or error related to the response to acidification in the dataset, we removed sites that are limed to mitigate acidification and sites with significant agricultural impact. Sites were excluded if they exhibited agricultural land use in the catchment exceeding 12 %, total phosphorus above 35 μg/l, and/or inorganic nitrogen exceeding 600 μg/l. Furthermore, all sites with an altitude exceeding 500 m a.s.l. were excluded because of naturally low temperature, reduced nutrient availability and channel instability at higher altitudes. These conditions often result in low diversity and density, and altered species composition (Brittain et al., 2001; Lods-Crozet et al., 2001). The final dataset consisted of 62 lake- and 58 stream sites (Table 2). No sites from Finland met the criteria for inclusion. Therefore, only sites from Sweden and Norway were considered further.

Macroinvertebrates were sampled using a kick-net in autumn (September – November) in stream riffle sections and in stony lake littoral zones following standardized national protocols of the Water Framework Directive (WFD) (Järvinen et al., 2021; Norwegian Environment agency, 2018; Johnson and Hallstan, 2016). The standardized national bioassessment protocols focus on stream riffle sections and stony lake littoral zones because these habitats are expected to have the greatest biodiversity resulting in higher statistical power to detect changes in water quality. All macroinvertebrate samples were sorted and taxonomically identified according to national quality control and assurance protocols (e.g. Velle et al., 2020). Taxonomic identification was done to the lowest taxonomic unit possible, usually to species or species groups, except for oligochaetes and chironomids, and taxonomy harmonized prior to merging the data from Norway and Sweden into one database.

In all analyses, we used the arithmetic means for taxon abundance, water chemistry and the subsequent acidity indicators pH and ANC. Different fractions of organic acids, including ANC, ANCo1 and ANCo2 (Table 1), were calculated for each site. Furthermore, other environmental variables (e.g. water chemistry [total organic carbon [TOC], total phosphorus [TotP], inorganic nitrogen [$NO^2 + NO^3$-N], calcium [Ca], potassium [K], sulphate [SO4], base cations [BC]), catchment characteristics [size and land use: % agriculture, % forest, % water, % wetland] and spatial components [altitude, latitude, longitude] were compiled to characterize the study sites (Table 2).

### 2.2. Defining the calibration and reference datasets

The development of a multimetric index requires the establishment of a pressure gradient represented by minimally disturbed and putatively impacted sites (Dahl and Johnson, 2004; Hering et al., 2006a). Ideally, reference sites should represent the full range of naturally occurring conditions within the region (e.g. Norris and Hawkins, 2000; Hawkins et al., 2010), while impacted sites used in the calibration dataset should preferably only represent the pressure of interest (e.g. Dahl and Johnson, 2004). Accordingly, the calibration dataset was selected from an acidity range for stream sites with ANC < 190 and/or pH < 6.5 and lake sites with ANC < 150 and/or pH < 6 (Table 3, Table 4). This truncation was performed to remove noise or error from sites expected not to be affected by acidity and resulted in a calibration dataset of 33 streams and 36 lakes. Finding representative non-acidified sites can be challenging since airborne acid deposition typically is distributed evenly across the landscape, although site-specific effects are

**Table 3**
Parameters and values used in filtering to obtain the calibration dataset.

| | STREAM | | LAKE | |
|---|---|---|---|---|
| | **Removed if** | **Range in dataset** | **Removed if** | **Range in dataset** |
| **CALIBRATION DATASET** | | | | |
| altitude m a.s.l. | >500 | 56–483 | >500 | 58–382 |
| % agricultural land use | >12 | 0–7 | >12 | 0–7 |
| pH | >6.5 | 4.5–6.48 | >6 | 4.68–5.96 |
| ANC (μeq/l) | >190 | 9.5–184.6 | >150 | −25.4–145.1 |
| **REFERENCE DATASET** | | | | |
| altitude m a.s.l. | >500 | 4–499 | >500 | 29–488 |
| % agricultural land use | >12 | 0–7 | >12 | 0–7 |
| pH | <6.5 | 6.55–7.3 | <6.5 | 6.51–7.15 |
| ANC (μeq/l) | <200 | 205.5–435.9 | <200 | 202.6–428.1 |

**Table 2**
Mean and range of acidity indicators and environmental descriptors for Nordic lake and stream study sites.

| | Lakes (N = 62) | | | Streams (N = 58) | | |
|---|---|---|---|---|---|---|
| | **MEAN** | **MINIMUM** | **MAXIMUM** | **MEAN** | **MINIMUM** | **MAXIMUM** |
| **Acidity indicator** | | | | | | |
| pH | 6.0 | 4.7 | 7.2 | 6.2 | 4.5 | 7.3 |
| ANC (μg/l) | 155 | −25.4 | 428 | 170 | −9.5 | 436 |
| ANCo1 (μg/l) | 119 | −36.6 | 389 | 136 | −22.6 | 383 |
| ANCo2 (μg/l) | 84.0 | −97.0 | 350 | 101 | −81.3 | 330 |
| Al/L_comb (μg/l) | 22.7 | 0.02 | 251 | 15.4 | 0.01 | 86.5 |
| **Environmental variable** | | | | | | |
| TOC (mg/l) | 10.4 | 0.9 | 28.3 | 10.2 | 0.8 | 21.5 |
| Tot-P (μg/l) | 10.3 | 2.5 | 29.5 | 9.9 | 1.5 | 24.9 |
| $NO^2 + NO^3$-N (μg/l) | 66.0 | 6.4 | 210 | 79.3 | 5.4 | 581 |
| Ca (μeq/l) | 145 | 11.2 | 391 | 154 | 16.8 | 418 |
| K (μeq/l) | 14.4 | 3.3 | 30.2 | 11.4 | 2.5 | 36.1 |
| $SO_4$ (μeq/l) | 80.4 | 20.7 | 229 | 71.5 | 20.4 | 235 |
| BC (μeq/l) | 363 | 120 | 681 | 352 | 79.2 | 717 |
| % agriculture | 1 | 0 | 7 | 1 | 0 | 7 |
| % forest | 70 | 5 | 92 | 67 | 9 | 99 |
| % water | 13 | 2 | 31 | 4 | 0 | 25 |
| % wetland | 5 | 0 | 42 | 9 | 0 | 42 |
| catchment size ($km^2$) | 41.6 | 0.3 | 845 | 130 | 0.5 | 1469 |
| altitude m a.s.l. | 173 | 29 | 488 | 233 | 4 | 499 |
| longitude | 14.49 | 5.25 | 23.05 | 14.95 | 6.00 | 23.47 |
| latitude | 59.47 | 56.21 | 68.31 | 61.64 | 56.04 | 69.49 |

**Table 4**
Mean and range of acidity indicators and environmental descriptors for the combined lake and stream sites used in the final MMI calibration and reference datasets.

| | Calibration | | | Reference | | |
|---|---|---|---|---|---|---|
| | MEAN | MINIMUM | MAXIMUM | MEAN | MINIMUM | MAXIMUM |
| **Acidity indicator** | | | | | | |
| pH | 5.5 | 4.5 | 6.5 | 6.9 | 6.5 | 7.3 |
| ANC | 79.6 | −25.4 | 185 | 274 | 203 | 436 |
| ANCo1 | 42.9 | −36.6 | 150.2 | 241 | 162 | 389 |
| ANCo2 | 6.17 | −97 | 132 | 209 | 117 | 350 |
| Al/L_comb | 29.4 | 0.37 | 251 | 1.41 | 0.01 | 8.7 |
| **Environmental variable** | | | | | | |
| TOC (mg/l) | 10.8 | 0.91 | 28.3 | 9.6 | 0.77 | 18.7 |
| Tot-P (μg/l) | 10 | 2.08 | 29.5 | 10.2 | 1.48 | 25.2 |
| $NO^2 + NO^3$-N (μg/l) | 73.9 | 5.39 | 581 | 70.4 | 6.41 | 272 |
| Ca (μeq/l) | 78 | 11.2 | 231 | 245 | 110 | 418 |
| K (μeq/l) | 9.48 | 2.53 | 24 | 17.6 | 4.68 | 36.1 |
| $SO_4$ (μeq/l) | 64.5 | 20.7 | 229 | 91.8 | 20.4 | 235 |
| BC (μeq/l) | 272 | 79.2 | 675 | 474 | 249 | 717 |
| % agriculture | 0.01 | 0.00 | 0.07 | 0.01 | 0.00 | 0.07 |
| % forest | 0.71 | 0.05 | 0.99 | 0.65 | 0.08 | 0.85 |
| % water | 0.09 | 0.00 | 0.31 | 0.08 | 0.00 | 0.26 |
| % wetland | 0.07 | 0.00 | 0.42 | 0.07 | 0.00 | 0.42 |
| catchment size (km2) | 16.5 | 0.25 | 143 | 176 | 0.74 | 1469 |
| altitude | 195 | 56 | 483 | 211 | 4 | 499 |
| longitude | 13.66 | 5.25 | 23.47 | 16.14 | 10.81 | 23.26 |
| latitude | 59.89 | 56.04 | 69.19 | 61.37 | 56.52 | 69.49 |

moderated by local geology. In this study, the dataset representing least-disturbed reference conditions was defined by sites expected not to be affected by acidity (ANC > 200 and/or pH > 6.5) based on thresholds for biological change in response to increasing acidity, as obtained by gradient forest analyses (Fölster et al. 2021) (Table 3, Table 4). This resulted in a reference data set for streams (N = 25) and lakes (N = 26) (Table 3, Table 4).

### 2.3. Analyses

In the subsequent analyses, the chemical acidity indicators pH, ANC and ANCo1 were compared to macroinvertebrate responses to identify the variable with the strongest response. Only the calibration dataset was used in steps 1–5, while the reference dataset was also included in step 6.

*Step 1: Calculation and selection of candidate metrics*

A total of 474 metrics were calculated from ASTERICS PERLODES (https://www.gewaesser-bewertung-berechnung.de) and the invertebrate trait database from Tachet et al. (2010) and explored for their relationship with measures of acidity. Three other metrics were further calculated as combinations of metrics, including the sum of the relative abundance (%) of Gastropoda + Bivalvia + Crustacea or by removing taxa known to be tolerant in an otherwise sensitive taxa group, e.g. removal of Leptophlebiidae from Ephemeroptera (Murphy et al., 2013; Johnson and Hallstan, 2016). Metrics were retained if they correlated significantly (Spearman $\rho < 0.05$) with pH and/or ANCs and if the direction of response was as anticipated by literature and similar across datasets for lakes, streams, and lakes and streams combined (Appendix C and D). For the metrics with significant correlation to acidity indicators, a sub-selection was made based on predicted responses to acidity and that the metrics should be applicable across the Nordic region. The resulting candidate metrics were then divided into five attribute groups: (1) Composition metrics include characteristics of taxonomic abundance or relative abundance, (2) diversity measures include characteristics of the number of taxa within taxonomic groups, (3) effect trait measures are characteristics of an organism's phenotype that affect both its fitness and its effects on ecosystem processes, such as resource acquisition and biomass production rates (Verberk et al., 2013; Violle et al., 2007), (4) response trait measures are characteristics of an organism's phenotype that regulate its environmental responses, reflecting especially its environmental tolerances and ecological flexibility (Violle et al., 2007) and (5) tolerance measures include metrics representing acidity preference or tolerance.

*Step 2: Selecting metric combinations*

Multiple stepwise regression, with forward selection, was run using individual acidity indicators (pH, ANC, or ANCo1) as the dependent and the candidate metrics as the independent variables for each of the five attribute groups. Based on Akaike information criterion (AIC), only candidate metrics that contributed significant additional information in the regression model were chosen for possible inclusion in the final multimetric index. Furthermore, the possible inclusion of a metric was based on theoretical rationale and that the metrics should not be redundant (e.g. either number of Gastropoda taxa or number of taxa within Gastropoda + Bivalvia + Crustacea were selected). Forward selection continued until all attribute groups were represented by at least one metric. This procedure was carried out separately for each dataset and acidity indicator.

Next, ecoregion delineations were used to partition large-scale natural (biogeographic) variability that included the three main ecoregions Central Plains, the Fenno-Scandian Shield and the Borealic Uplands described in the European Water Framework Directive (EC, 2014) and Illies (1978). The forward selection procedure was then carried out on the three datasets split geographically by ecoregion (Fig. 1).

*Step 3: Scaling of metrics prior to creating MMI's*

Before the candidate metrics were combined and used for an MMI each metric value was normalized (*LH Value*) between 0 and 1 based on the lowest and highest metric values in the dataset as recommended by Hering et al. (2006b) when reference data are not included. For metrics decreasing with increasing acidity we used:

$$LHValue = \frac{\text{Metric result} - \text{Lowest metric result in dataset}}{\text{Highest metric result in dataset} - \text{Lowest metric result in dataset}}$$

and for metrics increasing with increasing acidity we used:

$$LHValue = 1 - \frac{\text{Metric result} - \text{Lowest metric result in dataset}}{\text{Highest metric result in dataset} - \text{Lowest metric result in dataset}}$$

MMI's were calculated as the mean of the 0 to 1 scores of all Core Metrics of each MMI.

*Step 4: Performance evaluation of MMI's.*

Least squares regression of each MMI was carried out separately for each dataset (lake, stream, and combined lake and stream) and acidity

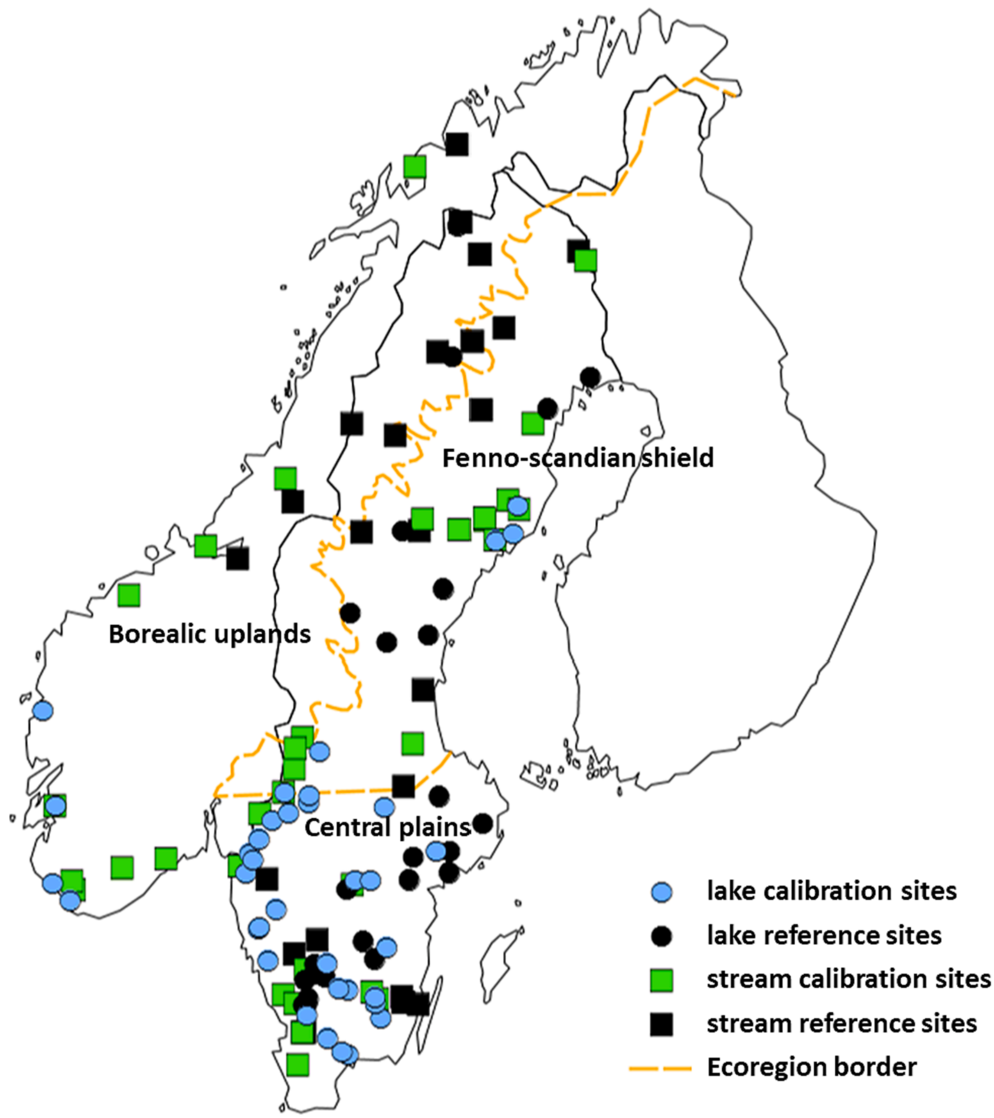


**Fig. 1.** Map of sites and three main ecoregions across Norway and Sweden.

indicator (pH, ANC and ANCo1). As was for step 3, the procedure was then carried out on the three datasets split geographically by the three main ecoregions (Fig. 1).

*Step 5: Recalibration of the MMI using 90th and 10th percentiles as upper and lower limits with final data set including both lake and stream, and both acidity gradient and reference data sets.*

The final MMI dataset of 120 sites included 69 combined lake and stream calibration sites, and 51 reference sites (Table 3, Table 4). The 90 and 10 percentiles for each of the metrics that composed the MMI were used as the upper and lower limits (*PER Value*). Since reference data are included here we followed the recommendation by Hering et al. (2006b) to use the 90 and 10 percentiles as the upper and lower limits (*PER Value*) for each of the metrics that composed the MMI.

For metrics decreasing with increasing acidity we used:

$$PERValue = \frac{\text{Metric result} - \text{10th percentile of metric}}{\text{90th percentile of metric} - \text{10th percentile of metric}}$$

and for metrics increasing with increasing acidity we used:

$$PERValue = 1 - \frac{\text{Metric result} - \text{10th percentile of metric}}{\text{90th percentile of metric} - \text{10th percentile of metric}}$$

Negative values (those that fall below the 10 percentile) were given a value of zero, and all values above 1 (those that fall above the 90 percentile) were given a value of one.

*Step 6: Validation of the MMI*

Validation was achieved by comparing the MMI values between the calibration (impacted by acidity) and reference data sets using *t*-tests. Based on previous work using gradient forest, we identified a threshold ANCo1 concentration where changes in benthic macroinvertebrate assemblages were minimal (Fölster et al. 2021). Combined with other variables characterising low pressures (e.g. land use) we expect our reference sites to be representative of a group of minimally disturbed sites. With a functional MMI, we expect a significant difference between the means of the calibration and reference data.

All statistical analyses were done in JMP 14.0.0 (SAS Institute Inc. JMP 1989–2021).

### 2.4. Evaluation of temporal recovery in three Swedish lakes

To evaluate if the index also could indicate a temporal recovery from acidification, we applied the MMI to macroinvertebrate data from three lakes representing recovery from different levels of acidity in the Central Plains ecoregion of Sweden (Supplementary data, Table 1). Data were selected from three lakes in the dataset utilized for development of the MMI. These lakes had a continuous time series of water chemistry and macroinvertebrate data of up to 33 years and showed a pronounced chemical recovery within ranges where a change in the macroinvertebrate community could be expected. We calculated the MMI value and ANCo1 for each annual sample over a thirty-year period for each of the three lakes (Supplementary data, Table 1).

## 3. Results

### 3.1. Multimetric index development

Correlation analyses (step 1) indicated that 375 metrics were significantly related to acidity variables (Appendix C and D). After accounting for redundancy, predicted response and geographic relevance, a subset of 29 candidate metrics was selected. These candidate metrics were divided into the five attribute groups (Table 5).

Multiple stepwise regression (step 2) resulted in 72 combinations of the 29 candidate metrics potentially contributing to the final MMI's. The calibration dataset least squares regression (step 4) indicated that the acidity indicator including ANCo1 and seven metrics resulted in the MMI with the highest performance (greatest $R^2$) (Fig. 2a, b, Table 5). This is hereafter referred to as the Nordic Acidity Macroinvertebrate Index (NAMI). The seven metrics included in the NAMI comprised one composition metric (the sum of the relative abundance of Gastropoda, Bivalvia and Crustacea), two measures of diversity (number of Ephemeroptera (- Leptophlebiidae) and number of Bivalvia taxa), one effect trait (life cycle duration > 1 year), two resistant traits (eggs and plastron respiration) and the pH tolerance trait (preference > 5 pH < 5.5).

**Table 5**
The 29 candidate metrics divided into five attribute groups. Values in bold text show the seven core metrics used in the NAMI. CODE refers to metric(s) in Appendix A and B. TRAIT STATE/METRIC refers to a subcomponent of TRAIT/METRIC.

| CODE | ATTRIBUTE GROUP | TRAIT/METRIC | TRAIT STATE/METRIC |
|---|---|---|---|
| A308 | composition | Taxonomic group (abundance) | Diptera |
| A248 | composition | Taxonomic group (abundance) | EPT [%] (abundance classes) |
| A225 | composition | Taxonomic group [%] | Bivalvia [%] |
| A229 | composition | Taxonomic group [%] | Crustacea [%] |
| A240 | composition | Taxonomic group [%] | Diptera [%] |
| A224 | composition | Taxonomic group [%] | Gastropoda [%] |
| A224 + 225 + 229 | composition | Taxonomic group [%] | **Gastropoda [%] + Bivalvia [%] + Crustacea [%]** |
| A236 | composition | Taxonomic group [%] | Megaloptera [%] |
| A2 | diversity | Number of Taxa | Number of Taxa |
| A264_noLepto | diversity | Taxonomic group (number of taxa) | **Ephemeroptera (- Leptophlebiidae)** |
| A258 | diversity | Taxonomic group (number of taxa) | **Bivalvia** |
| A262 | diversity | Taxonomic group (number of taxa) | Crustacea |
| A279 | diversity | Taxonomic group (number of taxa) | EPT/Diptera |
| A257 | diversity | Taxonomic group (number of taxa) | Gastropoda |
| A257 + 258 + 262 | diversity | Taxonomic group (number of taxa) | Gastropoda + Bivalvia + Crustacea |
| T16 | effect trait | Aquatic stages | adult |
| A188 | effect trait | Feeding types | [%] Predators |
| T9 | effect trait | Life cycle duration | **> 1 year** |
| T2 | effect trait | Maximal potential size | > 0.25-0.5 cm |
| T12 | effect trait | Potential number of cycles per year | > 1 |
| T29 | response trait | Resistance forms | **egg stages** |
| T35 | response trait | Respiration | gill |
| T36 | response trait | Respiration | **plastron** |
| T34 | response trait | Respiration | tegument |
| T100 | response trait | Temperature | psychrophilic |
| A62 | tolerance | Acid Index (Hendrikson & Medin) | Acid Index (Hendrikson & Medin) |
| A350 | tolerance | AWIC Index | AWIC Index |
| T111 | tolerance | pH (preferendum) | **> 5–5.5** |
| T113 | tolerance | pH (preferendum) | > 6 |

Slopes of NAMI regressed against ANCo1 differed between streams ($p = 0.002$) and lakes ($p = 0.003$) (Fig. 2). However, as the ANCo1 gradients differed in length between impacted stream sites and impacted lake sites (–22 – 150 ANCo1 μeq/l for streams and −37 – 94 for lakes) (Fig. 2), we compared slopes using the same gradient length for both streams and lakes (–22 to 100 ANCo1). Results showed no difference between streams and lakes ($p = 0.237$) (Appendix E).

Recalibration of the NAMI using 90th and 10th percentiles as upper and lower limits and using the combined stream and lake reference data sets (step 5) resulted in standard normalization values for the seven metrics included in the NAMI (Table 6).

The *t*-test results (step 6) showed that the mean of the NAMI from the putatively impacted sites differed from reference sites. NAMI for reference sites was significantly greater than the mean of the index for impacted stream sites ($t = -5.30$, $P < 0.001$) (Fig. 3a) and impacted lake sites ($t = -7.16$, $P < 0.001$) (Fig. 3b). A Wilcoxon Signed-Rank Test indicated that the median post-test ranks were statistically significantly higher than the median pre-test ranks $Z = 4.38$, $p = <0.0001$ for streams and $Z = 5.37$, $p < 0.0001$ for lakes.

### 3.2. Evaluation of temporal recovery in three Swedish lakes

In the three lakes, NAMI follows the chemical recovery of ANCo1 and pH, with some delay (Fig. 4). The biological recovery, as indicated by the NAMI, was similar across lakes regardless of the initial level of acidity (Fig. 4).

## 4. Discussion

The significant difference between the mean value of NAMI in calibration and reference datasets indicates a working MMI. The NAMI was significantly correlated to ANC's and pH, but less so in the latter. This contrasts with earlier studies on more geographically limited datasets showing that pH was more significantly correlated to changes in macroinvertebrate assemblages (Fölster et al., 2007). Earlier work using the Nordic dataset demonstrated that for larger gradients in acidity and TOC, the response of biota was more strongly correlated to ANC or modified ANC than pH (Fölster et al., 2021). Furthermore, Fölster et al. (2021) recommend the use of ANC instead of pH for assessing acidification as biological responses to pH are often confounded by covariables such as latitude, altitude, forest cover and total phosphorus. Use of titrated alkalinity, commonly used in assessing acidification (Centre, I.-W. P. 2010), was not possible due to the different pH titration endpoints used by the Nordic countries (NIVA, 2021). ANC has been criticised for not accounting for organic acids and reflecting biological responses in relation to acidification (Lydersen et al., 2004). For example, brown water lakes and streams can have relatively high buffering capacity (high ANC) but low acidity (low pH) (Lydersen et al., 2004). The modified ANC used here (ANCo1 and ANCo2) accounts for the stronger fractions of organic acids, contributing to a better understanding of macroinvertebrate responses to ANC.

The NAMI includes biodiversity metrics responding to general disturbance, while specifically targeting acidity by including the relative abundance of sensitive and tolerant species. The diversity metrics, the number of Ephemeroptera (excluding Leptophlebiidae) and the number of Bivalvia taxa, were significantly correlated with acidity. The finding

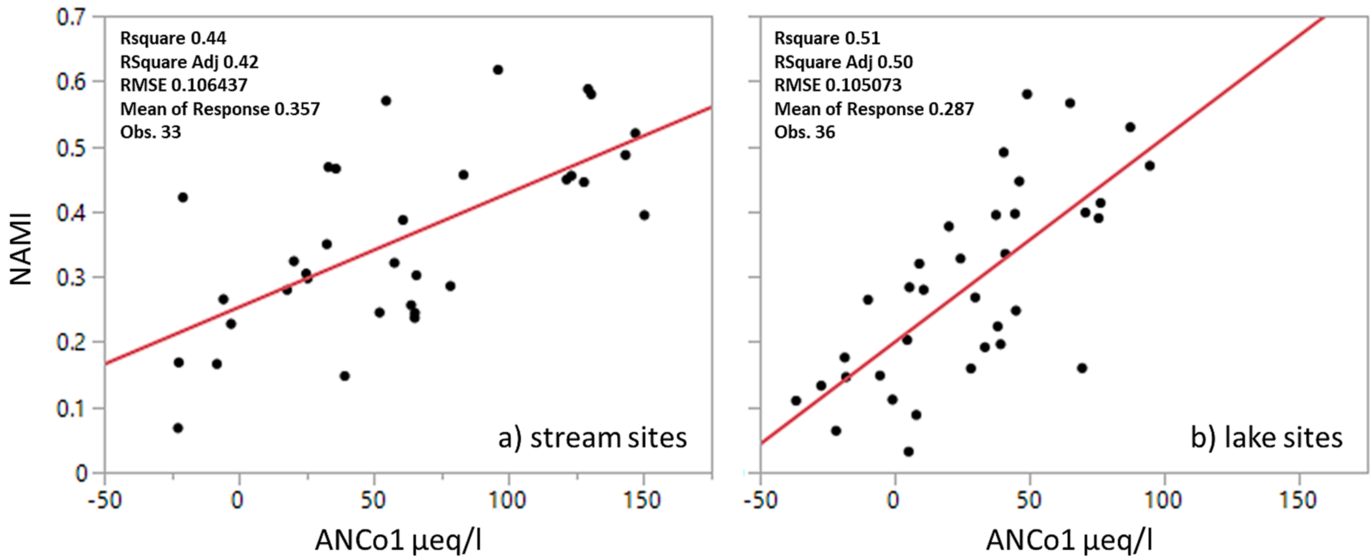


**Fig. 2.** Relationship of NAMI to ANCo1 in stream (a) and lake sites (b).

**Table 6**
Normalization of index values for the seven simple metrics included in the NAMI to values between 0 and 1.

| CODE | TRAIT STATE/METRIC | $Index_{90th\ percentile} = 1$ | $Index_{10th\ percentile} = 0$ | TRAIT/METRIC INDEX VALUE |
|---|---|---|---|---|
| A224 + 225 + 229 | Gastropoda [%] +Bivalvia [%] +Crustacea [%] | >22.2 | <0 | $Value = \frac{\text{Metric result} - 0}{18.225 - 0}$ |
| A258 | Bivalvia | >2 | <0 | $Value = \frac{\text{Metric result} - 0}{2 - 0}$ |
| A264_noLepto | Ephemeroptera (- Leptophlebiidae) | >8 | <0 | $Value = \frac{\text{Metric result} - 0}{8.9 - 0}$ |
| T9 | > 1 year | >0.279 | <0.07 | $Value = \frac{\text{Metric result} - 0.091}{0.321 - 0.091}$ |
| T29 | egg stages | >0.255 | <0.008 | $Value = \frac{\text{Metric result} - 0.012}{0.258 - 0.012}$ |
| T36 | plastron | >0.036 | <0 | $Value = \frac{\text{Metric result} - 0}{0.051 - 0}$ |
| T111 | > 5–5.5 | >0.22 | <0.186 | $Value = 1 - \frac{\text{Metric result} - 0.187}{0.22 - 0.187}$ |

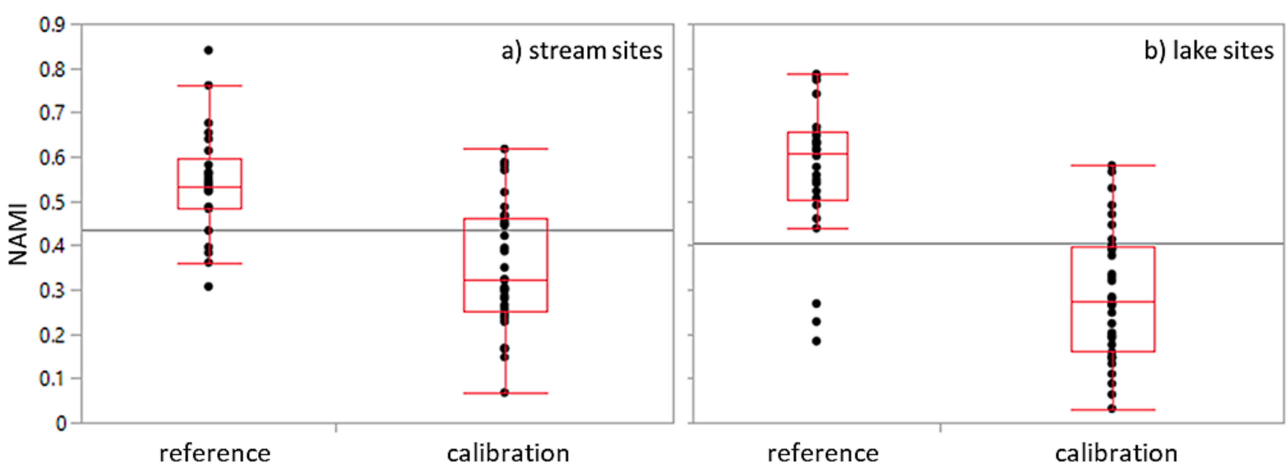


**Fig. 3.** Box and whisker plots showing median and interquartile ranges (10th, 25 th, 50 th, 75 th, 90 th percentiles) of the NAMI and in stream sites (a), and lake sites (b) characterized as calibration and reference. Also original values are shown as black dots.

that biodiversity loss was strongly correlated with acidity is not surprising, as many studies have shown low densities and loss of acid-sensitive taxa associated with acidification (e.g. Økland and Økland, 1986; Townsend et al., 1983; Raddum and Fjellheim, 1984; Smith et al., 1990; Økland, 1992; Johnson and Angeler, 2010b). Our finding that a significant tolerance trait including pH preference above 5 to 5.5, is consistent with many earlier studies that have shown marked changes in community composition at pH levels below 6 (e.g. Økland and Økland, 1986; Mason, 1996; Johnson et al., 2007; Johnson and Hallstan, 2016).

Significant declines in mayflies, caddisflies, beetles, molluscs and

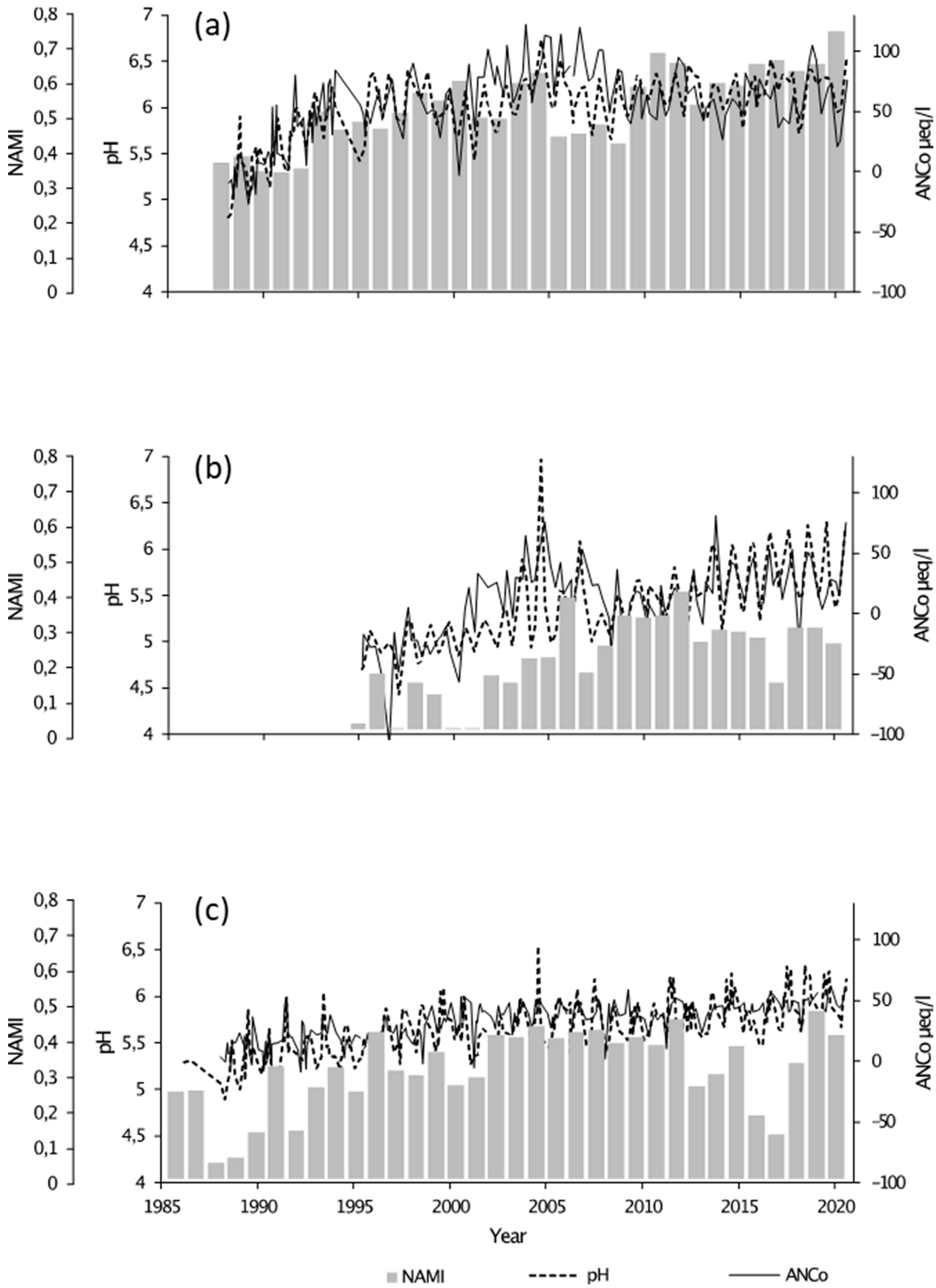


**Fig. 4.** The Nordic acidification multimetric index NAMI and the chemical recovery from acidification in three Swedish lakes: (a) Sännen, (b) Lilla Öresjö, and (c) Övre Skärsjön.

crustaceans have been noted in streams at pH < 5.7 (Reynolds and Ormerod, 1993) and community change between pH 5.5 and 6.0 (Johnson et al., 2007). By contrast, several leptophlebiid mayfly species are relatively tolerant to acidity (Johnson and Hallstan, 2016) and consequently were excluded in the metrics used in our study. ANC is a measure of the buffering capacity of water against acidification and represents the balance between cations and anions. A direct response to ANC in macroinvertebrates can potentially be explained by the exchange of ions, which is influenced by the ion balance in the water. Macroinvertebrates continuously exchange ions with the water through permeable parts of their bodies to maintain acid-base balance and ionic equilibrium. The gills, being highly permeable, play a significant role in ion uptake, particularly through the excretion of ammonia and ammonium ions (Houlihan et al., 1982). Cations move inward to maintain electroneutrality during excretion, and the level of active uptake depends on external concentrations (Houlihan et al., 1982). This suggests that gill-breathing invertebrates have high energy requirements when the concentration of ions, such as cations in acidic waters, is below saturation levels (Sutcliffe and Hildrew, 1989). While some sensitive taxa can tolerate certain levels of acidity, they can be eliminated due to competition for food and/or predation pressure rather than direct toxicity (Havas and Likens, 1985). Additionally, elevated concentrations of aluminum at low pH can directly affect osmoregulation and reduce energy available for growth and reproduction, causing direct toxicity for sensitive macroinvertebrates (Herrmann and Andersson, 1986; Olofsson et al., 1995).

Furthermore, earlier studies have shown bivalve taxon richness and the relative abundance of molluscs and crustaceans to be negatively correlated with acidity (e.g. Økland, 1992; Dolmen and Kleiven, 2008). Low calcium carbonate concentration, needed for shells and exoskeletons, is considered as the main factor controlling their distributions. In poorly buffered waters, exoskeleton, shell and mantle dissolution results in microbial infections (Kat, 1982) and impaired reproduction (Servos et al., 1985).

The ability to avoid or withstand impacts are two traits that allow organisms to survive and reproduce in disturbance prone systems. The response traits, organisms with egg stages and plastron respiration and the effect trait, life spans > 1 year, were significantly correlated with acidity. Macroinvertebrates with plastron respiration include many disturbance-tolerant taxa, such as coleopterans and hemipterans, that often increase in relative abundance in acidified aquatic systems (Johnson et al., 1993). Acidification often manifests as a pulse-type disturbance, with, for example, episodic acidic events during spring snow melt and autumn precipitation. Eggs are often more resistant to stress than other life-cycle stages (Alekseev et al., 2007; Arnott and Yan, 2002) increasing an organism's ability to withstand acidic episodes. Conversely, macroinvertebrates with a relatively long aquatic life span (>1 year) not only have a higher probability of being affected by an acidic event but can also experience delayed recolonization and recovery as acidification decreases. Acidic episodes impact the recovery of acid-sensitive invertebrates (Bradley and Ormerod, 2002), other organisms (Johnson and Angeler, 2010b) and food webs (Angeler and Goedkoop, 2010; Lau et al., 2017) in systems where rehabilitation (liming) resulted in increased pH. Liming can be a "command-and-control" management form that often fails to restore a waterbody to preliming conditions once management is ceased (Baho et al., 2014; Angeler et al., 2021).

Analyses (t-tests) showed that mean NAMI values at putatively impacted sites differed from reference sites. However, our results also showed a number of potential false negative (type 2) errors, i.e. impact is potentially occurring but not detected, particularly for impacted sites with NAMI values > 0.4. These sites all had pH above 5.3, ANCo1 values between −20.8 and 150 μeq/l and aluminium concentrations between 3.67 and 32.5 μg/l. The higher NAMI values at these sites could be correlated to higher values of calcium (mean = 114 μeq/l) compared to sites with NAMI values below 0.4 (mean = 62 μeq/l) and with pH higher than 5.3 ($p = 0.0004$). Calcium concentrations are mainly controlled by soil weathering, but also track closely to the trends of sulphate in Swedish waterbodies (Weyhenmeyer, 2008) and elsewhere (Jeziorski et al., 2008). Unlike sulphate, calcium has a more direct physiological effect, as it is required for shell and mantel formation in molluscs, exoskeleton synthesis in crustaceans (Thorp and Covich, 2001) and ion balance during excretion in gill breathing macroinvertebrates (Houlihan et al., 1982). Sites with NAMI values above 0.4 had greater relative abundance of the collective Gastropoda, Bivalvia and Crustacea (mean = 9.6) compared to sites with NAMI values values below 0.4 (mean = 4.0), and similarly for the number of Bivalvia taxa (mean = 1.1 and 0.56, respectively). Indeed, all the simple metrics that compose the NAMI were higher in sites with higher calcium values, except for a pH preference > 5 to 5.5 and the egg stage as a resistance form. However, more work on ion regulation in macroinvertebrates is required before trends can be identified with certainty, as physiological differences exist between insects, molluscs, and crustaceans in ion regulation responses to acid stress (e.g. the ability of crayfish to mobilize calcium from their exoskeleton and energy requirements during excretion in gill breathing insects). Nevertheless, these results suggest that a NAMI value of 0.4 is an important threshold for acidity impacts. Seven reference sites (13.7 %) had NAMI values below 0.4 and these sites had no indication of being acidic, thus their low NAMI values were likely a result of some unmeasured factor other than acidity, or NAMI indicator taxa were missed in sampling.

Our sites were not evenly distributed across the Nordic countries potentially resulting in geographic bias and representativity. More sites were in southern Norway and Sweden compared to the North. Given the low number of sites in northern regions caution is advised in using the NAMI until more data are available. Likewise, availability of sufficient quality data is required for validating the index for Finland. However, despite regional bias, the NAMI has potential to complement or replace national assessment methods and to harmonise Nordic assessments.

Our case study results support NAMI as a tool for assessing the relationship of biological and chemical recovery in terms of rising ANCo1 and pH. Biological responses in all three lakes showing chemical recovery also showed biological recovery, typically occurring within 4 to 5 years after increases in ANCo1 and pH. Increases in NAMI values over time were likely not only dependent on taxon-specific critical thresholds for acid sensitivity, but also related to the vicinity of source populations and taxon differences in mechanisms for dispersal. Biological responses could occur quite rapidly if nearby sources exist (Niemi et al., 1990). However, species with life cycles restricted to the aquatic environment and limited mechanisms of dispersal (e.g. Mollusca and Crustacea) are likely slower to recolonize compared to insects that have an adult flight stage, such as Ephemeroptera. Accordingly, it is possible that initial increases in NAMI were driven by metrics such as Ephemeroptera minus Leptophlebiidae, while later increases were driven by metrics such as percent Gastropoda + Bivalvia + Crustacea, and number of Bivalvia taxa. Beyond the number and rate of colonization of individuals, the interaction of other factors could affect the macroinvertebrate community, such as their generation time, fecundity of established colonizers, and interactions with established taxa (Niemi et al., 1990, Keller and Yan, 1998). A delay in biological recovery can also be attributed to acidic episodes that may not be readily apparent in water chemistry monitoring. Negative effects can arise from increased total organic carbon (TOC) or reduced calcium content (Hessen et al., 2017), as well as interactions with other environmental variables, such as climate. In two lakes, there were occasions with low NAMI values during the recovery phase. These low measures could indicate episodic acidic events that can occur in lake littoral regions during late winter and spring snowmelt, but are not observed in water chemistry sampling, and/or poor recolonization or low abundances of acid sensitive taxa.

## 5. Conclusion

In conclusion, the multimetric NAMI demonstrates its potential as a reliable tool for quantifying the effects of and recovery from acidity in lake and stream ecosystems in the Nordic countries, and potentially in other regions. It also serves as a valuable tool for harmonizing chemical and biological monitoring and assessments, including progress towards achieving international objectives. A common Nordic acidity index for macroinvertebrates is a first step towards a harmonized classification of acidification within the WFD and calculation of critical loads for the United Nations Economic Commission for Europe's Long-Range Transboundary Air Pollution (UN-ECE LRTAP). To understand the full potential of NAMI, future studies are needed that cover a broader geographic range in detecting degradation from acidification and quantifying biological recovery.

## CRediT authorship contribution statement

**Peter E. Carlson:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Richard K. Johnson:** Conceptualization, Methodology, Writing - original draft. **Jukka Aroviita:** Conceptualization, Methodology, Writing - original draft. **Gaute Velle:** Conceptualization, Methodology, Writing - original draft. **Jens Fölster:** Conceptualization, Methodology, Writing - original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.111028.

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