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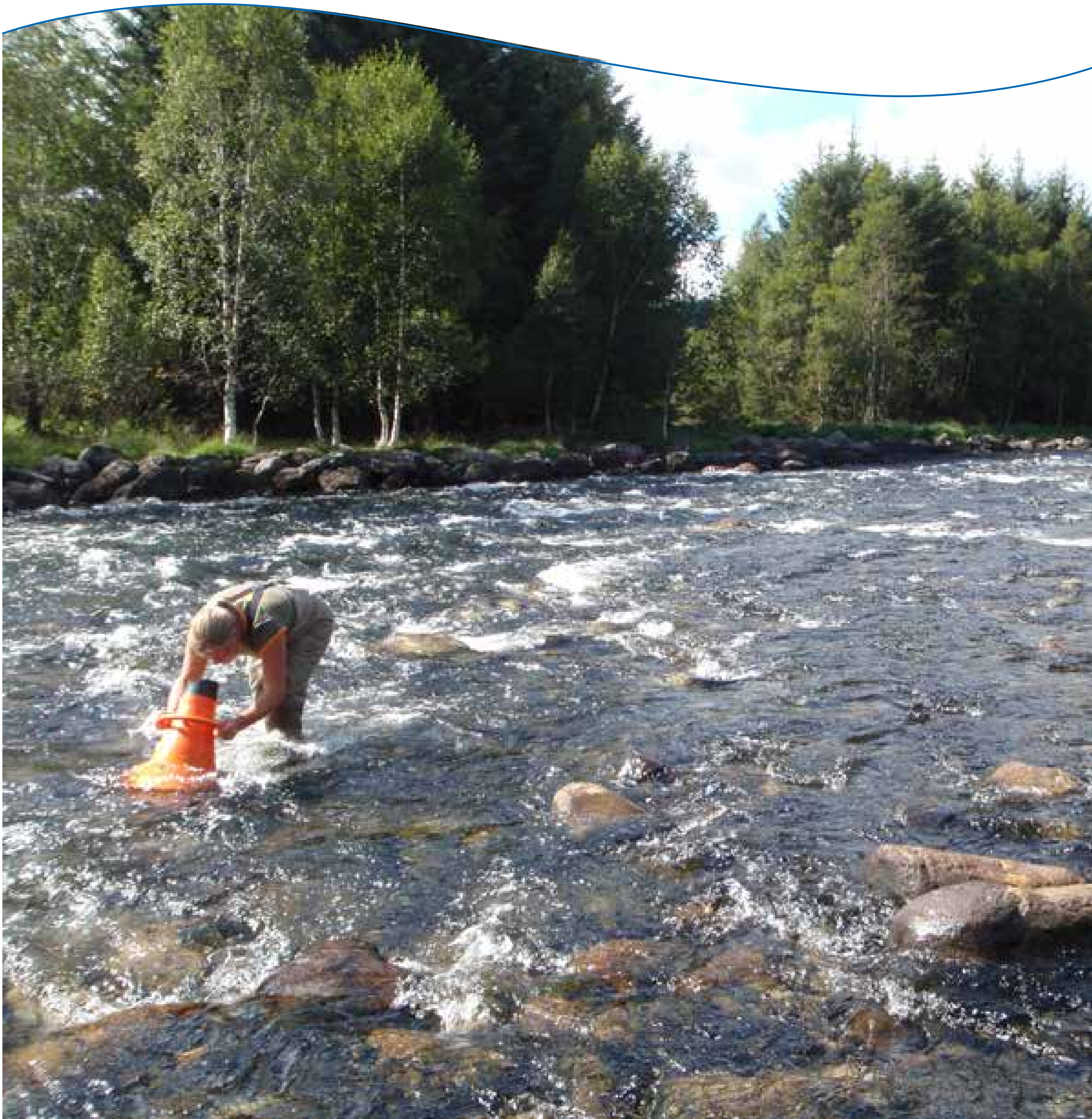
Nitrogen dose-response relationships: benthic algae and macroinvertebrates in running water



Norwegian Institute for Water Research
– an institute in the Environmental Research Alliance of Norway

REPORT

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Abstract

Nitrogen deposition affects freshwater biodiversity in two ways: by contributing to acidification via nitrate in runoff, and by acting as a nutrient. We used data for two organism groups, benthic algae (224 sites) and benthic invertebrates (62 sites), to test if N as a nutrient affects the species numbers of these groups in running water. Neither of these groups showed significant relationships with nitrogen, except for a positive relationship between nitrate concentration and the number of blue green algae with heterocysts. It is not possible to set a critical limit for N as a nutrient, at least for these organism groups in running water.

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1. Biodiversitet	1. Biodiversity
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Nitrogen dose-response relationships: benthic algae and macroinvertebrates in running water

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Preface

Norway participates in several of the activities of the Working Group on Effects, part of the Convention on Long Range Transboundary Air Pollution. One of these activities is to provide estimates of critical loads of sulphur, nitrogen and acidity. In 2012 the Coordinating Centre of Effects (CCE) of the International Cooperative Programme for Modelling and Mapping (ICP M&M) issued a "Call for Data" aimed at formulating nitrogen dose-response relationships, as a step in setting critical loads for nitrogen as a nutrient. The overall goal is to protect ecosystems from "no net loss of biodiversity". In February 2013 NIVA suggested that Norway respond to this call for data by developing such nitrogen dose-response relationships for two organism groups in surface waters. This work has been conducted under contract 7013519 from the Norwegian Climate and Pollution Agency (KLIF) (now part of the Norwegian Environment Agency --Miljødirektoratet). Contact person at KLIF was Tor Johannessen. The work was conducted as part of the National Focal Centre for ICP M&M. At NIVA Tor Erik Eriksen conducted the analyses of macroinvertebrates. Susanne Schneider conducted the analyses of the benthic algae. Richard F. Wright prepared the report. Markus Lindholm carried out the quality control of the report.

Oslo, November 2013

Thorjørn Larssen

Contents

Summary

Here we use data from running waters in Norway to assess whether N deposition affects biodiversity of two organism groups: benthic algae (periphyton) and macroinvertebrates. This information is then used to evaluate the empirical critical loads for N as a nutrient for surface waters. pH and total phosphorus (TP) are also included in this analysis as these factors are known to affect both organism groups. The goal is to isolate and quantify the role of N.

Benthic algae species and associated water chemistry data were extracted from NIVAs benthic algae database. A total of 224 sites have chemistry data for pH, and concentrations of nitrate (NO_3) and total phosphorus (TP), as well as numbers of species for the algal groups bluegreen (blue), bluegreen with heterocysts (blue.hcyst), green (green), other species (rest), and total number of non-diatom species (=sum of blue+green+rest) (nondia).

Benthic invertebrate species and associated water chemistry data were taken from sites in one ecoregion (eastern Norway: counties of Telemark, Oppland, Buskerud, Akershus, Oslo, Hedmark and Østfold). A total of 62 sites had data for pH, NO_3, TP and total nitrogen (TN).

For the benthic algae none of the species groups was significantly related to NO_3 concentration. As expected, however, the total number of non-diatom benthic algae taxa was significantly related to pH and total phosphorus. Similarly, for the benthic invertebrates there were no effects from total nitrogen (TN) or nitrate (NO_3) on any of the tested metrics. Again as expected, there were, however, significant relationships between pH and all the tested taxa count metrics.

There was a significant negative relationship between NO_3 concentration and the number of blue green algae with heterocysts. Heterocysts function as locations for fixing N_2. The most likely explanation for this relationship is that at lower concentrations of NO_3 the algae have a greater need to fix N_2 to obtain the N needed.

In the absence of significant dose-response relationships between concentrations of NO_3 and measures of species composition, it is not possible to set a critical limit for N as a nutrient, at least for these organism groups in running water.

The critical loads for acidity for lakes in Norway calculated using the FAB model for the 2300 12 x 12 km grids have values as low as 8.4 meq/m^2/yr, with 50 % of the values less than about 60 meq/m^2/yr (Larssen and Høgåsen 2003). If all this acidity is assumed to come from N deposition, then this corresponds to critical loads for N as low as 1.2 kgN/ha/yr, with 50 % of the grid squares below about 8.5 kgN/ha/yr. Thus the current objective of protecting fish populations in Norway by limiting the acidifying effects of S and N deposition will most likely also satisfy the proposed empirical critical loads for N as a nutrient to protect other organisms groups as well.

Sammendrag

Data fra rennende vann i Norge blir her brukt til å vurdere hvorvidt deposisjon av nitrogen (N) påvirker det biologiske mangfoldet av to organismegrupper: bentiske alger (periphyton) og bentiske makroinvertebrater (bunndyr). Denne informasjonen blir så brukt til å vurdere de empiriske tålegrensene for N som et næringsstoff for overflatevann. pH og total fosfor (TP) er også inkludert i denne analysen da disse faktorene er kjent for å påvirke begge organismegruppene. Målet er å isolere og kvantifisere nitrogens rolle.

Artsdata forbentiske alger med tilhørende vannkjemiske data ble hentet fra NIVAs databaser. Det var tilsammen 224 lokaliteter med tilgjengelige kjemidata med hensyn på pH, konsentrasjoner av nitrat (NO_3) og total fosfor (TP), samt antall arter for algegruppene blågrønn (blå), blågrønn med heterocyster (blue.hcyst), grønn (grønn), andre arter (resten), og totalt antall ikke-kiselalger (= summen av blå + grønn + resten) (nondia).

Bunndyr med tilhørende vannkjemiske data ble hentet fra NIVAs databaser. Det ble kun brukt data fra én økoregion (Øst-Norge: Telemark, Oppland, Buskerud, Akershus, Oslo, Hedmark og Østfold). Totalt 62 lokaliteter hadde tilgjengelige data for pH, NO_3, TP og total nitrogen (TN).

For bentiske alger var ingen av artsgruppene signifikant relatert til konsentrasjon av NO_3. Som forventet var imidlertid det totale antall ikke-diatomeer av bentiske alge taksa signifikant relatert til pH og total fosfor. Tilsvarende gav ingen av de utførte beregningene effekter av total nitrogen (TN) eller nitrat (NO_3) på bunndyr. Det var imidlertid en signifikant sammenheng mellom pH og alle undersøkte indekser basert på taksatellinger, akkurat som forventet.

Det var en signifikant negativ sammenheng mellom NO_3 konsentrasjonen og antall blå grønne alger med heterocystser. Nitrogenfiksering foregår i heterocystsene, og den mest sannsynlige forklaring på denne sammenhengen er at alger har et større behov for å fiksere N_2 ved lave konsentrasjoner av NO_3. Dette for å dekke algens behov for N.

I fravær av signifikante dose-respons-forhold mellom NO_3 konsentrasjoner og målinger av artssammensetningen er det ikke mulig å sette en kritisk grense for N som næringsstoff for disse organismegruppene i rennende vann.

Tålegrenser for surhetsgrad i innsjøer i Norge, beregnet etter FAB-modellen for 2300 12 x 12 km rutenett, viser verdier så lave som 8,4 meq/m2/yr, med 50 % av verdiene mindre enn ca 60 meq/m2/yr (Larssen og Høgåsen 2003). Antatt at all aciditet skyldes N deponering vil dette tilsvare tålegrenser så lave som 1,2 kgN/ha/år, med 50 % av rutenett under ca 8,5 kgN/ha/år. Dermed vil nåværende mål om å beskytte fiskebestander i Norge ved å begrense de forsurende effektene av deposisjon av S og N mest sannsynlig også tilfredsstille de foreslåtte empiriske tålegrensene for N som et næringsstoff for å beskytte andre organismegrupper også.

1. Introduction

The Convention on Long Range Transboundary Air Pollution (LRTAP) is an international body under the United Nations Economic Commission for Europe (UNECE). The Convention aims to reduce the adverse effects of air pollution. Recent protocols have been science-based and are intended to reduce emissions of sulphur (S) and nitrogen (N) compounds such that the exceedence of critical loads to ecosystems is minimised. Both terrestrial and aquatic ecosystems are included. This work is led by the Coordinating Centre for Effects (CCE) under the International Cooperative Programme for Modelling and Mapping (ICP M&M).

At its 25th session in 2007 the Executive Body of the LRTAP Convention agreed to encourage the Working Group on Effects " … to increase its work on quantifying effects indicators, in particular for biodiversity. These should also be linked to the integrated assessment modelling activities" (ECE/EB.AIR/91, para. 31). At the CCE workshop in Warsaw 16-19 April 2012 a way forward was proposed to enable the (trans-boundary) comparison of effect indicator-values in a harmonized way. The aim is to assess to which extent "no net loss of biodiversity" is achieved using suitable biodiversity endpoints (e.g. protection of rare species, provisioning, regulating or cultural services) of interest on a regional scale.

In Norway the focus in the critical loads work has been on surface water acidification and effects on fish (salmon in running water and brown trout in lakes)(Henriksen et al. 1992, Henriksen et al. 1999). The links between deposition of S and N, surface water chemistry and biological effects have been based on the acid neutralising capacity (ANC) in water; an ANC_{limit} has been set for salmon and trout such that at ANC values above the limit, there is less than 5% risk of adverse effects. In this critical load of acidity, the role of N (and S) is simply that of a strong-acid anion (nitrate and sulphate) that can accompany the cations aluminium and acid, both of which are toxic to fish.

N acts also as a nutrient in surface waters. Deposition of N might lead to unacceptable effects on aquatic ecosystems, independent of the acidifying effects of nitrate. Critical loads for N as a nutrient have generally been obtained by empirical methods. A review and revision of these empirical critical loads was made at an expert workshop in 2010 (Bobbink and Hettelingh 2011). Chapter 5 of the report from this workshop deals with the effects of N deposition on inland surface water habitats (EUNIS class C). EUNIS, the European Nature Information System, is part of the Biodiversity data centre (BDC) operated by the European Environmental Agency. The empirical critical loads for N as a nutrient take into account information summarised in a recent review by de Wit and Lindholm (2010). They concluded that N deposition can alter the species composition of the primary producers algae and macrophytes in oligotrophic lakes; information on other organism groups in lakes and all groups in running waters was less extensive.

In November 2012 the CCE issued a "Call for Data" with deadline March 2014 requesting information from the national focal centres (NFC) for ways to lay the ground for formulating N dose-response relationships on a regional (EUNIS) scale, upscaling from individual sites. The call focused on terrestrial biodiversity and soil-vegetation interactions: «The objective of this Call for Data is to compile output variables of soil-vegetation models for every EUNIS class (level 3) within the country (preferably in Natura2000 or other protected areas). This should enable the calculation of (country-specific) biodiversity indicators for (scenario) assessment of changes in biodiversity on a regional scale.»

The focus on the soil-vegetation system is probably due to the fact that in most countries in Europe adverse effects of air pollutants are much more prevalent in terrestrial ecosystems. In Norway, however, aquatic ecosystems have been widely affected. Here we use data from running waters in Norway to assess whether N as a nutrient affects biodiversity of two organism groups: benthic algae (periphyton) and macroinvertebrates. This information is then used to evaluate the empirical critical loads for N as a nutrient for surface waters (EUNIS class C) suggested by Bobbink and Hettelingh (2011) based on various biodiversity indicators. pH and total phosphorus (TP) are also included in this analysis as these factors are known to affect both organism groups. The goal is to isolate and quantify the role of N.

2. Materials and methods

2.1 Benthic algae

Benthic algae species and associated water chemistry data were extracted from NIVAs benthic algae database. A total of 224 sites have chemistry data for pH, and concentrations of nitrate (NO_3) and total phosphorus (TP), as well as numbers of species for the algal groups bluegreen (blue), bluegreen with heterocysts (blue.hcyst), green (green), other species (rest), and total number of non-diatom species (=sum of blue+green+rest) (nondia) (**Table 1**).

Non-diatom benthic algae, i.e. algae that live attached to the river bottom or in close contact on or within patches of attached aquatic plants, were surveyed once at each site during summer/autumn according to European standard procedures (EN 15708:2009) along an approximately 10-m length of river bottom using an aquascope (i.e. a bucket with a transparent bottom). At each site, percent cover was noted for each form of macroscopically visible benthic algae, and samples were collected and stored separately in vials for species determination. All benthic algae were examined under a light microscope (100 to 400 or 1000 times magnification) and identified as close to species level as possible (EN 15708: 2009). Identification literature comprised several floras and monographs (Komarek and Anagnostidis 1999, Eloranta and Soininen 2002, Gutowski and Forster 2009, John et al. 2011). Details are given by Lindstrøm et al. (2004).

We calculated non-diatom taxon richness (i.e. number of taxa), and richness of cyanobacteria (n.cyano), of cyanobacteria having heterocysts (n.cyano.hcyst), of "classical green algae" (=Viridiplantae; n.green), and of all other algae taxa except cyanobacteria and green algae (n.rest). We refer to "taxa" rather than "species" to recognize that entities identifiable with the light microscope and current literature may not always reflect true biological species. Assignment of non-diatoms into taxa was primarily based on the determination guides cited above. For some genera of filamentous green algae whose vegetative forms cannot be determined to species level (e.g. Spirogyra Link or Mougeotia C.Agardh) categories which are based mainly on filament width were used (see Schneider and Lindstrøm (2009) and Schneider and Lindstrom (2011) for further details). The same taxonomic levels were used consistently for analysis of all sites.

Water chemistry samples were taken at the sampling sites between one and 24 times within the same year the benthic algae samples were collected, and the results were stored in the database of the Norwegian Institute for Water Research (NIVA). All chemistry analyses were carried out at NIVA according to Norwegian standard (NS) procedures during all years (pH: NS 4720; total phosphorus (TP): NS 4725; NO_3: NS-EN ISO 10304-1).

Benthic algae and associated water chemistry data originated from numerous projects and were collected between 1976 and 2010. For the sites from where observations from several years exist, mean values for both explanatory and response variables were calculated. We thus ensured that one site occurred exactly once in the dataset. The complete dataset of non-diatom benthic algae richness as well as nitrate, TP and pH included 224 sites over all Norway.

Table 1. Summary statistics for chemical and algal parameters for the 224 sites in this study. Units: NO_3 µgN/l; TP µgP/l.

	logNO_3	**pH**	**logTP**	**blue**	**blue.hcyst**	**green**	**rest**	**nondia**
average	1.78	6.55	0.72	5.43	1.57	6.15	1.02	12.60
SD	0.53	0.78	0.30	3.41	1.44	3.21	1.04	5.38

These data were approximately normally distributed, with the exception of the number of other algae species (rest). All three explanatory variables are correlated with each other, but the Pearson r is not particularly large, and the strongest autocorrelation occurs between pH and NO_3 (**Table 2**).

Table 2. Correlations (Pearson r) between the three chemical variables. Marked correlations are significant at $p < .05$. N=224. Units: NO_3 µgN/l; TP µgP/l.

	logNO3	**pH**	**logTP**
logNO3	1.00	-.36 p<.001	.20 p<.003
pH		1.00	.26 p<.000
logTP			1.00

The data were then fitted to a multivariate second-order polynomial function similar to the one used by Schneider et al. (2013), but additionally including logNO_3 as a third explanatory variable.

richness =
$b0+b1*pH+b2*(pH)^2+b3*logTP+b4*(logTP)^2+b5*logNO_3+b6*(logNO_3)^2+b7*pH*logTP+b8*pH*logNO_3+b9*logNO_3*logTP+b10*pH*logTP*logNO_3$

All data were centred and scaled to one standard deviation for the modelling, because this allows use of the regression coefficients as measures of effect size (Schielzeth 2010). ANOVA was used to find the variables which significantly contribute to explained variation.

2.2 Benthic invertebrates

Species and associated water chemistry data were taken from sites in one ecoregion (eastern Norway: counties of Telemark, Oppland, Buskerud, Akershus, Oslo, Hedmark and Østfold) because taxa richness of macroinvertebrates is dependent on ecoregion. Sites which were polluted by mining, industrial or wastewater pollution were excluded. Sites affected by liming activities (mitigation of acidification) were also excluded. All samples are from autumn (September, October and November). We excluded samples that were influenced by slow flowing, turbid rivers. At sites that had more than one sample, average values (of metrics and chemistry) were used; this avoids pseudo replicates. A total of 62 sites had data for pH, NO_3, TP and total nitrogen (TN). The range of chemistry data was NO_3 (2-555 µgN/L), pH (4.8-7.8), TP (1.8-25 µgP/L), TN (85-860 µgN/L). Significant autocorrelations occurred only between log(TN) and log(NO_3) and log(TN) and log(TP).

Data were centred and scaled to one standard deviation for the modelling. ANOVA was used to find the variables which significantly contribute to explained variation.

Table 3. Correlations (Pearson r) between the four chemical variables tested. Marked correlations are significant at $p < .05$. Units: NO_3 µgN/l; TN µgN/l; TP µgP/l.

	logNO3	**pH**	**logTP**	**logTN**
logNO3	1.0000	-.14 p =.366	- 0.21 p = .120	0.58 p < 0.001
pH		1.0000	-.134 p=.299	-0.046 p = 0.710
logTP			1.0000	0.441 p < 0.001
logTN				1.0000

We analysed diversity with two types of indices. Firstly, we looked at taxa count indices, which are indices that summarize the number of taxa within chosen taxonomic levels. We looked at the total numbers of taxa (n taxa) and numbers of ephemeropterans, plecopterans and trichopterans (EPT taxa). We know from the literature that several taxa are acid sensitive (Raddum and Fjellheim 1984a, Larsen et al. 1996). We hypothesise that sensitive taxa are lost from the macroinvertebrate assemblages and without being replaced by other (acid tolerant) taxa, thus acidity should lead to fewer taxa present. We expect no response from NO_3.

Secondly, we analysed chosen groups with a Shannon –Wiener diversity index (Shannon 1948). This is defined as

$$SWd = exp\left(-\sum_{i=1}^{s} p_i \, lnp_i\right)$$

where p is the proportion of individuals belonging to the species i.

This index compares the numbers of taxa and their relative distribution. Its value increases both when the number of taxa and evenness increases. Thus, a high species count with even distributions gives a high diversity. SWd is a "blind" index in the sense that it compares numbers of species without taking pollution sensitivity into account. However, despite being indiscriminative to pollution sensitive taxa, this index could give interesting results when looking at sensitive taxa groups. We looked at SWd for all present taxa, EPT taxa and the composition of functional feeding groups of EPT. To avoid noise in the data, we analyzed the SWd of functional feeding groups of EPT and only from the four most dominant groups: Grazers and scrapers, gatherers/collectors, predators and shredders. Acid stress can lead to shifts in the composition of functional groups and that this ultimately can lead to retarded ecosystem functioning (Pye et al. 2012). Our hypotheses were that acid stress would: 1) lead to less diverse communities of taxa by removing acid sensitive species, and 2) lead to higher evenness of feeding groups by reducing populations of dominant groups, like grazers and scrapers and gatherers/collectors (*Baetis rhodani* makes up 50 % of each of these feeding groups). This could lead to assemblages of EPT with higher relative proportions of predators and shredders. We expect no response from NO_3 on these indices.

3. Results

3.1 Benthic algae

The total number of non-diatom benthic algae taxa was significantly related to a convex function of pH (Schneider et al. 2013 confirmed), and decreased linearly with increasing logTP (Schneider et al. 2013 confirmed), but there was no interaction between logTP and pH (Schneider et al. 2013 not confirmed). Nothing else was significant (**Table 4**).

Table 4. Estimated effect sizes from the multivariate model of numbers of algal species on the three explanatory chemical variables pH, TP and NO_3. Data were centred and scaled. Only p-values < 0.1 for the ANOVA are shown. Bold values denote p-values < 0.05. +/- indicates a positive or negative direction of the model coefficient (linear: indicates decrease; quadratic: indicates concave response) or positive (linear: increase, quadratic: convex). nondia = taxon richness non-diatom benthic algae; n.blue = taxon richness cyanobacteria; n.green = taxon richness green algae; n.rest = taxon richness all others except blue and green; n.blue.hcyst = taxon number cyanobacteria having heterocysts. Units: NO_3 µgN/l; TP µgP/l.

	nondia	blue	green	rest	blue.hcyst
pH	**-0.00**	**0.12**		**0.16**	**0.08**
$(pH)^2$	**-0.27**	**-0.24**	**-0.17**		-0.14
logTP	**-0.29**	**-0.30**	**-0.27**	**0.32**	**-0.32**
$(logTP)^2$					
$logNO_3$					**-0.07**
$(logNO_3)^2$		0.12			**0.15**
pH*logTP					
pH*$logNO_3$					
logTP*$logNO_3$					-0.14
pH*logTP*$logNO_3$					

The total number of benthic cyanobacteria (blue) reacted in the same way, i.e. it was significantly related to a convex function of pH and decreased linearly with increasing logTP. The fact that the linear term is kept in the model does not change this statement. Nothing else was significant, but the p-value for the quadratic term to NO_3 was quite low (0.08; see **Table 4**). The coefficient for the quadratic term is positive, that means the response has a minimum (concave shape) rather than a maximum (convex shape).

The total number of green algae reacted similarly, i.e. it was significantly related to a convex function of pH, and decreased linearly with increasing logTP. Nothing else was significant.

In contrast, the number of "other algae" (i.e. not green and not cyanobacteria) significantly increased linearly with logTP and increased linearly with pH. Nothing else was significant.

The number of cyanobacteria with heterocysts (blue.hcyst) decreased with increasing TP and also significantly decreased with increasing NO_3 (no linear decrease, but the minimum of the quadratic term is at a larger concentration than the range in the data) (**Figure 1**). Nothing else was significant.

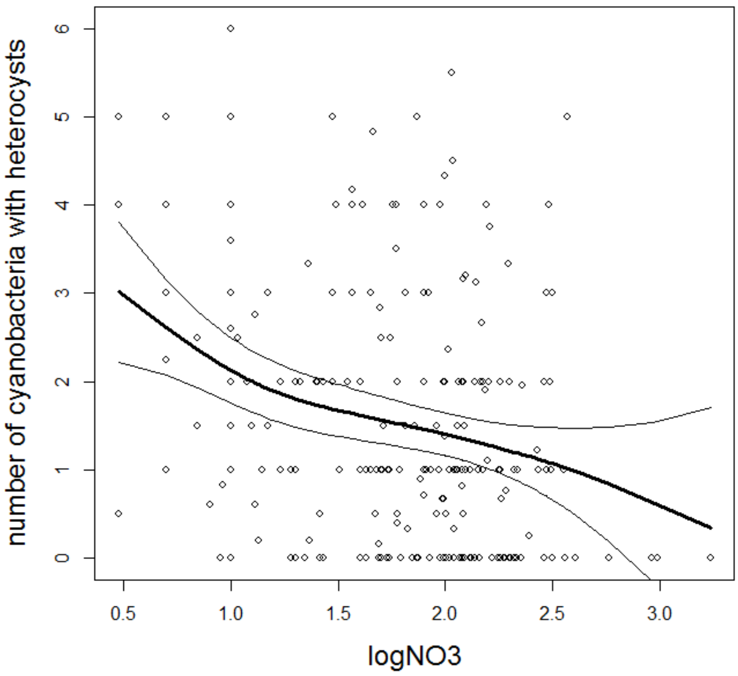

Figure 1. GAM-fit (generalized additive model) of the number of cyanobacteria-taxa having heterocysts to logNO_3 (µgN/l). Explained variation is 8.5% (from adjusted R-squared). Lines indicate model fit +- 2 standard errors of model fit.

For the number of green algae, the number of cyanobacteria, the number of "rest" algae, and the total number of benthic non-diatoms, TP and pH are much more important than NO_3. In contrast, for the number of cyanobacteria having heterocysts, TP is most important, followed by NO_3 (before pH).

3.2 Benthic invertebrates

There were no effects from total nitrogen (TN) or nitrate (NO_3) of the tested metrics (**Figure 2**). There were, however, significant relationships between pH and all the tested taxa count metrics (**Figure 3**) (**Table 5**). The total number of taxa (nTaxa), the total number of EPT-taxa (nEPT), the number of Ephemeroptera taxa (nE), number of Plecoptera taxa (nP) had also a significant response from total phosphorus (TP). There were no interactions between any of the chemical variables and any metric.

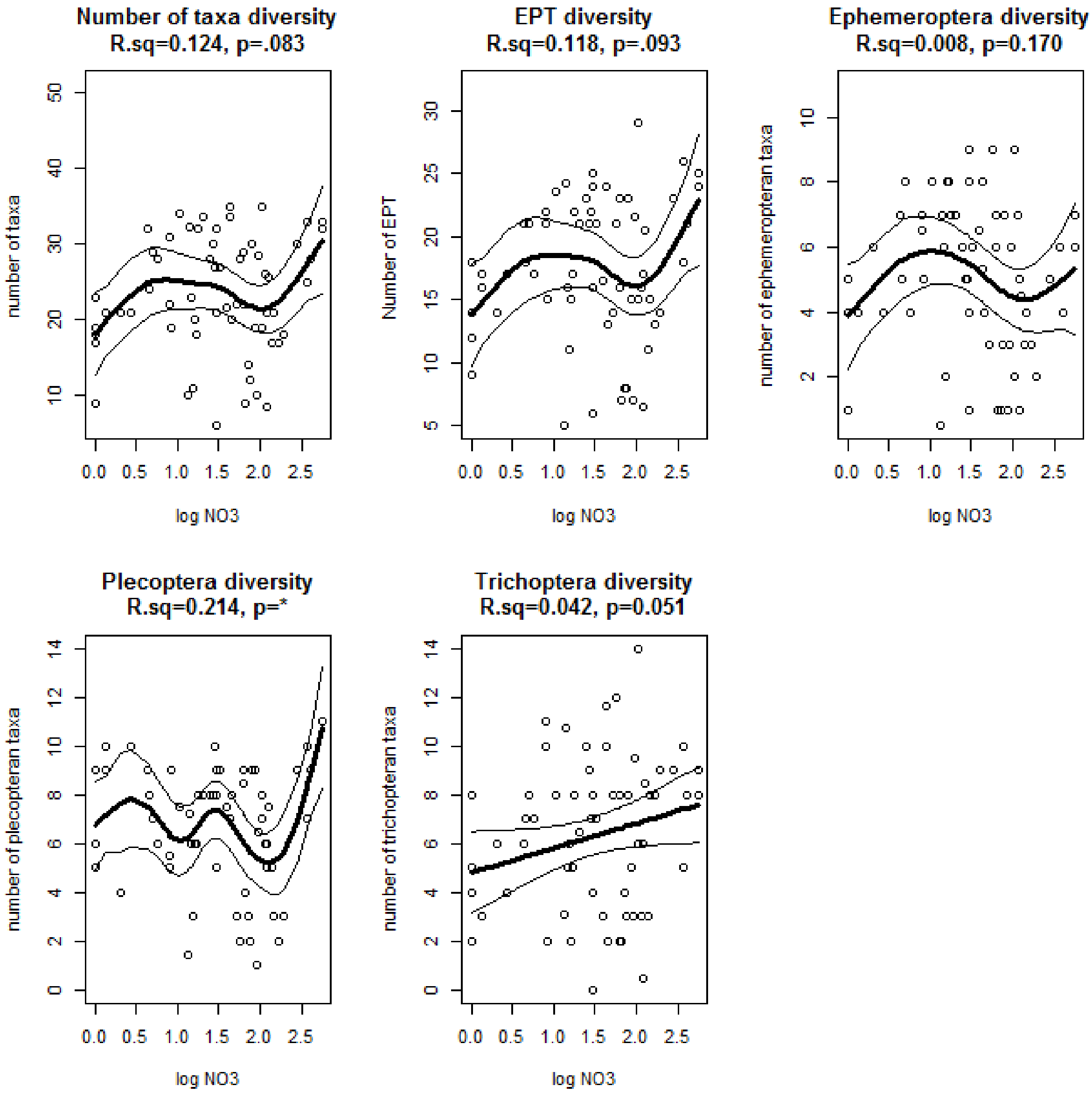

Figure 2. GAM-fit (generalized additive model) for chosen taxa count metrics and log(NO_3). Lines indicate model fit +- 2 standard errors of model fit. None of the relationships are strongly significant. Units: NO_3 µgN/l.

Table 5. Estimated effect sizes from the multivariate model from chosen metrics on the four explanatory chemical variables: pH, total phosphorus (TP), total nitrogen (TN) and nitrate (NO3). Data were centred and scaled. Only p-values < 0.05 for the ANOVA are shown. +/- indicates a positive or negative direction of the model coefficient (linear: indicates decrease; quadratic: indicates concave response) or positive (linear: increase, quadratic: convex): nTaxa= the total number of taxa, nEPT= the total number of EPT-taxa, nE = number of Ephemeroptera taxa, nP = number of Plecoptera taxa, nT = number of Trichoptera taxa, SWd taxa = Shannon-Wiener diversity for the total number of taxa, SWd EPT = Shannon-Wiener diversity for EPT taxa and SWd funk. Group = Shannon-Wiener diversity for functional groups of EPT taxa. Significance levels are indicated by: * (p<0.05), ** (p<0.01), and *** (p<0.001).

	Taxa count diversity metrics					**Shannon-Wiener diversity metrics**		
	nTaxa	**nEPT**	**nE**	**nP**	**nT**	**SWd Taxa**	**SWd EPT**	**SWd funk. group**
pH	9.21 (***)	5.84 (***)	3.61 (***)	1.01 (*)	1.35 (*)			
$(pH)^2$	0.100 (***)	1.00 (***)	1.45 (***)	0.63 (*)	0.35 (*)			
logTP	6.23 (**)	2.00 (*)	2.17 (**)	0.95 (*)				-0.21 (**)
$(logTP)^2$	0.24 (**)	0.17 (*)	0.28 (**)	0.11 (*)				-0.25 (**)
$logNO_3$								
$(logNO_3)^2$								
logTN								
$(logTN)^2$								-1.85 (*)
pH*logTP								
pH*$logNO_3$								
pH*logTN								
logTN*$logNO_3$								
logTP*log NO_3								
logTP*log TN								

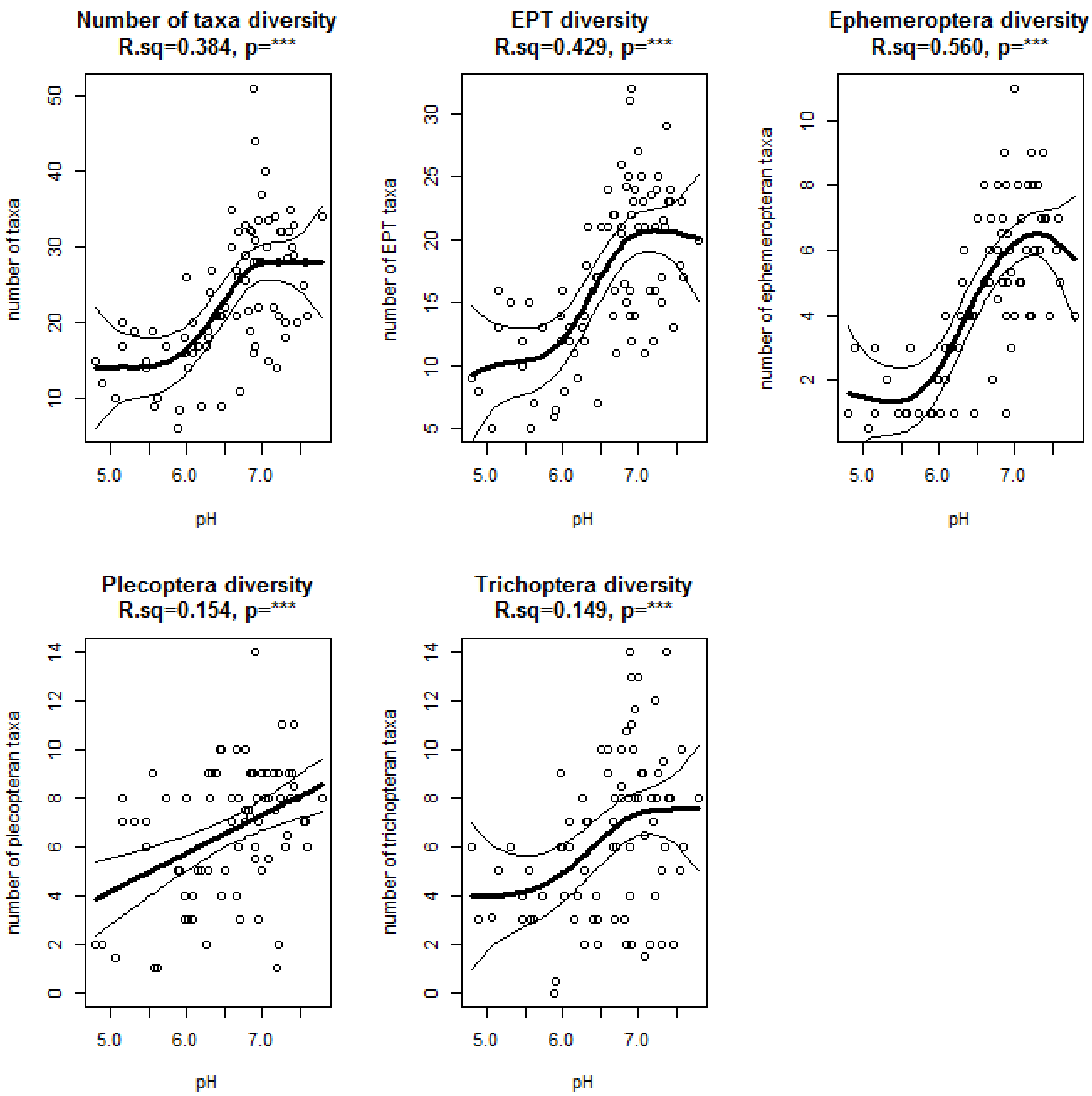

Figure 3. GAM-fit (generalized additive model) for chosen taxa count metrics and pH. Lines indicate model fit +- 2 standard errors of model fit. The data show the expected relationship with lower numbers of species at lower pH.

The Shannon-Wiener diversity (SWd) of the composition of functional feeding groups of EPT taxa had a negative trend with increasing TP and TN. No other metric based on SWd showed a signifcant relationship with the tested chemistry parameters.

4. Discussion

We have used data from running waters in Norway to identify possible indicators of changes in biodiversity in response to changes in concentrations of N as a nutrient. For two organism groups, benthic algae and benthic invertebrates, the data did not show significant dose-response relationships between concentrations of NO_3 and several measures of species composition (number of taxa of various groups, Shannon-Weiner index).

There was, however, a significant negative relationship between NO_3 concentration and the number of blue green algae with heterocysts. Heterocysts function as locations for fixing N_2. The most likely explanation for this relationship is that at lower concentrations of NO_3 the algae have a greater need to fix N_2 to obtain the N needed. This agrees with findings by Liess et al. (2009) in a study of benthic algae in 13 oligotrophic Swedish lakes; they found that the proportion of N-fixing algae was higher in lakes with lower tot N concentrations.

For the benthic invertebrates, the taxa count indices decreased with increasing acid stress but showed no significant responses to NO_3. In contrast to taxa count metrics, SWd metrics did not respond to increasing acidity. This could mean that, in terms of SWd, the removal or reduction of populations of some acid sensitive taxa leads to more even communities of EPT. This is not unlikely, because populations of the acid sensitive ephemeropterans *Baetis spp.* (members of the Empheroptrea order) can be dominant in many Norwegian rivers. It is not unlikely that the composition of functional feeding groups could increase from acid stress. This illustrates how SWd is not always a good measure of ecosystem health.

In the absence of significant dose-response relationships between concentrations of NO_3 and measures of species composition, it is not possible to set a critical limit for N as a nutrient, at least for these organism groups in running water. A critical load is defined as "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grennfelt 1988). Setting a critical load for N as a nutrient requires two steps. First a critical limit must be established between the concentration of one or more N compounds in water and an "unacceptable" harmful effect, i.e. loss of biodiversity. Second, a relationship between N deposition and the concentration of N in water must be established.

de Wit and Lindholm (2010) recently reviewed the literature on nutrient enrichment effects of N deposition on biology of oligotrophic surface waters. Their report includes information from the UK summarised by Curtis and Simpson (2007), as well as conclusions from the 2002 Expert Workshop on Critical Loads for Nitrogen held in Berne (Achermann and Bobbink 2002), and the 2010 Expert Workshop on Critical Loads held in Noordwijkerhout (Bobbink and Hettelingh 2011). The summary table from de Wit and Lindholm (2010) was appended to the Noordwijkerhout report and indicates proposed new empirical critical loads for nutrient N in freshwaters. The values range from as low as 1 kgN/ha/yr for arctic lakes, to 10-20 kgN/ha/yr for lakes in sand dunes in the Netherlands. The values in this table are for lakes only. Some information is given for running waters, but no empirical critical loads for running waters were proposed.

No summary of empirical critical *limits* for either lakes or running waters is available, however. This makes the use of these empirical critical loads contingent upon assumptions regarding the retention of N deposition in terrestrial ecosystems and the leaching of N to freshwaters.

Deposition of inorganic N compounds can affect aquatic ecosystems in two ways: (1) by contributing to acidity through leaching of NO_3 accompanied by acidifying cations H+ and Al, and (2) by enriching nutrient N for primary producers. Both acidification and nutrient N enrichment can result in cascading effects on various organism groups and ecosystem functions. The acidifying effect of N deposition is wellaccounted for in modelling and mapping of critical loads. For surface waters the National Focal Centre in Norway uses the steady-state water chemistry (SSWC) model and first-order mass balance model (FAB) to determine critical loads for acidity, which in turn can be expressed in terms of S and N deposition (Henriksen and Posch 2001). These two models differ primarily in the manner N leaching is handled; the SSWC model assumes that the fraction N retained or lost in the terrestrial catchment and lake remains constant at present-day rates, whereas the FAB model assumes that most N deposited is leached to surface waters. The SSWC model thus assumes no change in nitrogen saturation of terrestrial ecosystems, whereas the FAB model assumes that at steady-state all terrestrial ecosystems will be fully nitrogen saturated. For Norway the critical loads obtained by these models is based on the critical limits for ANC to protect brown trout populations in lakes and Atlantic salmon in rivers (Henriksen et al. 1999). It is also well known that acidification affects other organism groups, including benthic algae (Schneider and Lindstrøm 2009) and benthic invertebrates (Raddum and Fjellheim 1984b, Raddum and Skjelkvåle 1995).

The FAB model is more conservative, in that it adheres to the precautionary principle and sets lower values for tolerable N deposition in the future. Critical loads based on the FAB model were used in the Gothenburg protocol and its recent revision.

The critical loads for acidity for lakes in Norway calculated using the FAB model for 12 x 12 km grids have values as low as 8.4 meq/m²/yr, with 50% of the values less than about 60 meq/m²/yr (Larssen and Høgåsen 2003). If all this acidity is assumed to come from N deposition, then this corresponds to critical loads for N as low as 1.2 kgN/ha/yr, with 50% of the grid squares below about 8.5 kgN/ha/yr (**Figure 4**). Thus the current objective of protecting fish populations in Norway by limiting the acidifying effects of S and N deposition will most likely also satisfy the proposed empirical critical loads for N as a nutrient to protect other organisms groups as well.

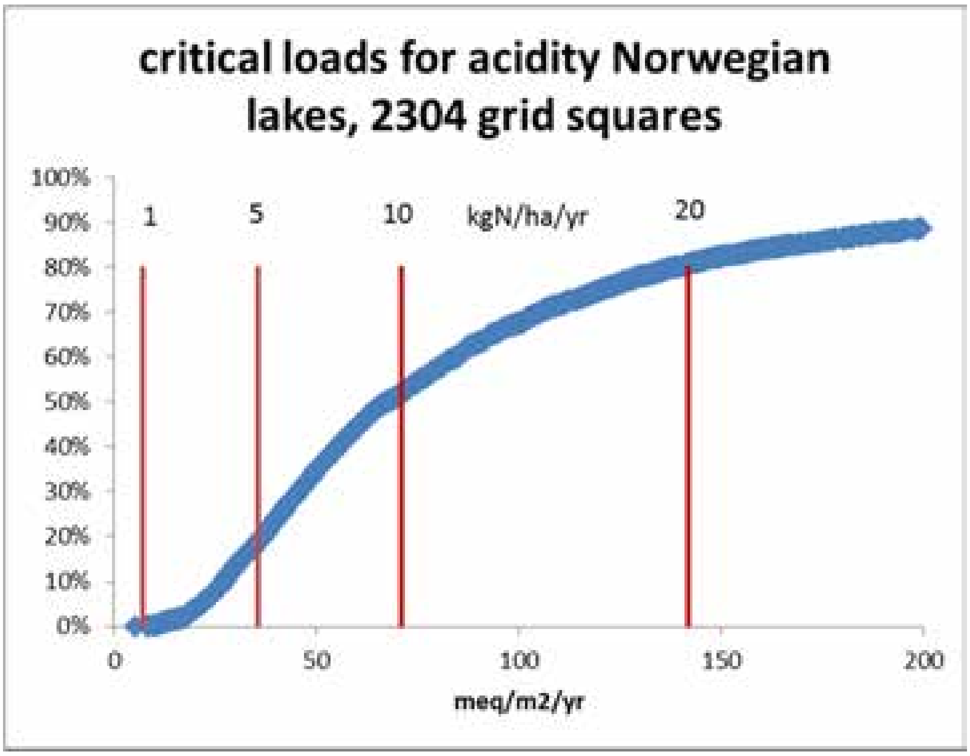

Figure 4. Critical loads for acidity, Norwegian surface waters, calculated for 2303 12x 12 km grid squares covering the whole country, using the FAB model (Larssen and Høgåsen 2003). Also shown are the corresponding values for N deposition in kgN/ha/yr, if it is assumed that all the acidity is due to N deposition and leaching of NO_3 to surface waters.

The effects-based protocols to the CLRTAP have aimed to minimise the exceedence of critical loads to terrestrial and aquatic ecosystems. The choice of which element of the ecosystem is to be protected from significant harmful effects has been left up to the individual countries to determine. In Norway the critical loads for freshwaters submitted to the CCE have been based on protecting brown trout populations in lakes and salmon populations in running waters. Only critical loads for acidity have been submitted. Heretofore, no critical loads have been submitted for other organism groups such as phytoplankton, macrophytes, benthic algae, zooplankton, or macroinvertebrates. The proposed goal of "no net loss of biodiversity" necessitates that critical loads for both acidity and N as a nutrient be developed for other organism groups.

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