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Liming effects on ecosystem structure, function and trophic relationships in lakes

FÖRFATTARE

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GENERAL INTRODUCTION

Freshwater liming programs have been established in several industrialized countries to alleviate the effects of anthropogenic acid deposition. Sulphur and nitrogen deposition can have profound impacts on surface aquatic ecosystems, altering biotic community structure and functional processes in ecosystems (McKie et al., 2006). Liming as a mitigation measure adds CaCO_3 to anthropogenically acidified waters to neutralize H^+ ions and facilitate precipitation of toxic ions, especially aluminum, that become soluble at lower pH (Weatherly, 1988). Liming has raised pH in many acidified systems, often allowing recovery of acid-sensitive organisms (Bradely & Ormerod, 2002). However, the systematic alkalinization of streams and lakes itself constitutes a substantial ecosystem-level perturbation (Weatherly, 1988; Schreiber, 1996). Liming can increase turbidity and the inorganic content of particulate matter consumed by many invertebrates (Kullberg, 1987), and may also precipitate dissolved organic carbon (DOC), removing it from microbial food webs and reducing its buffering potential in humic systems (Kullberg et al., 1993). Furthermore, any additional inflow of acid water from runoff or tributaries into limed systems can create an aluminum chemistry that is potentially more toxic than that of untreated water (Rossland et al., 1992; Teien et al., 2004).

The Swedish liming program was established during the 1970s to mitigate extensive acidification of poorly buffered freshwaters. In particular, the IKEU program was initiated in 1989 and comprises lakes that have been extensively monitored with regard to important biological and abiotic variables. Several recent reports (Holmgren, 2008; Persson, 2008a; Stendera, 2008; Sundbom, 2008a; Östlund, 2008) and also previous publications using IKEU data (e.g., Appelberg, 1998; Persson, 2001; Willén, 2006; Persson, 2008b), often only focus on single communities and/or single habitats which limits an overall assessment of overall biological responses to liming from a structural point of view. Only the paper of Persson and Appelberg (2001) provide a comparison of plankton, benthos and fish communities from a production perspective, and Holmgren (2001) studied biomass size spectra based on plankton and fish communities to provide a means of an integrated structural (community structure) and functional (trophic relationships between communities and energy flow) analysis. Finally, Sundbom (2008b) provided a comparative long-term analysis of quantitative structural and functional aspects of plankton, benthos and fish communities. He showed that the biomass levels and temporal trends of different functional groups

were significantly different between limed lakes and reference lakes. His results provide a basis for a more exhaustive exploration of community structure using comparative univariate and multivariate statistics.

Comparative analyses using different statistical methods could provide a means to assess whether different lake communities in different habitat types of limed lakes converge with those in circumneutral reference lakes. If this is the case, this could indicate that liming has potential to restore lake communities of anthropogenically acidified lakes to conditions of circumneutral reference lakes; this could support liming applications as a mitigation measure. Alternatively, if communities in limed lakes are structurally different from those in acid and circumneutral reference lakes, liming likely fails to achieve restoration goals, and magnitudes of differences between the structures of different communities may help evaluate to what extent liming can constitute a form of anthropogenic perturbation. In the first part of the present report, we test these assumptions by means of a standardized comparison of phytoplankton, zooplankton, and macroinvertebrates in three habitat types (littoral, sublittoral and profundal). Our analysis spans a period from 2000 to 2004, which allows assessing magnitudes of structural differences between lake categories over several years between communities in a standard way. Even though much larger time series are available in the programme, the high heterogeneity (missing data, different sampling resolutions) of these data sets precluded their use for a standardized comparison between multiple communities. In the second part of this report, we focus on a quantitative analysis of trophic relationships in food webs of limed acid and circumneutral lakes using stable isotopes. We determine whether benthic and pelagic food web structure differs in limed lakes compared to acid and circumneutral references.

PART 1. ANALYSIS OF BENTHIC AND PELAGIC COMMUNITY STRUCTURE

Material and Methods

Data assembly

We evaluated data of phytoplankton, zooplankton in the pelagic and macroinvertebrate communities in three benthic habitat types (littoral, sublittoral, and profundal) available in the IKEU and national lake monitoring databases. Data have been collected since 1986, but the databases were highly heterogeneous with regard to temporal sampling resolution of communities in acid reference lakes (hereafter referred to

as acid lakes), circumneutral reference lakes (circumneutral lakes) and limed lakes. This was primarily due to repeated adjustments of the sampling frequencies and differences in sampling methods. For example, phytoplankton communities were sampled between 2 and 7 times per year while macroinvertebrates were sampled only once a year during most of the program. In order to be able to make standardized comparisons between communities, all analyses for the present report are based on a single yearly sampling occasion (August for phyto- and zooplankton communities, October for macroinvertebrates). Extracting a single yearly value from the databases also helped to avoid potential problems, which would arise from the calculation of annual means based on irregular intra-annual sample sizes among lakes and communities. Our final analysis is restricted to the 5-year period between 2000 and 2004 ultimately constrained by methodological differences in the sampling of littoral macroinvertebrate communities in IKEU (limed lakes) and monitoring programs of acid and circumneutral lakes.

The lakes summarized in Table 1 and Figure 1 met our final selection criteria for standardized comparisons. Four of these lakes are acid lakes, seven are circumneutral lakes, while eleven lakes are limed lakes. Some of their water quality variables are also shown in Table 1. For the present study all analyses except littoral macroinvertebrates are based on biomass data (mm^3/L for phytoplankton, mm^3/m^3 for zooplankton, g/m^2 for sublittoral and profundal macroinvertebrates). Littoral macroinvertebrate samples were collected by standardized kick samples, thus resulting in semi-quantitative abundance data.

Sampling procedures

For water quality analysis we used August values of surface-water samples (0–2 m), which were collected, in the open-water mid-lake station in each lake. Water was collected with a Plexiglas sampler and kept cool during transport to the laboratory. Samples were analyzed for alkalinity, and concentrations of Ca, Mg, Na, K, SO_4 , Cl, F, $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}+\text{NO}_3\text{-N}$, total N, $\text{PO}_4\text{-P}$, total P, remaining P (total P – $\text{PO}_4\text{-P}$), Si, total organic carbon (TOC) and Chlorophyll *a*. Secchi depth, water temperature, dissolved oxygen concentration, conductivity, and pH were measured in the lakes. These water quality variables helped to delineate lake types, i.e. while limed lakes clearly comprised one treatment group, we discerned between acid and circumneutral reference lakes, chiefly on the basis of their pH, ANC and alkalinity values (Table 1).

FIGURE 1: Localization of study lakes. Lake categories: acid reference lakes (A), circumneutral reference lakes (N), limed lakes (L). 1 = Ejgdesjön (L), 2 = Rotehogstjärnen (A), 3 = Fräcksjön (N), 4 = Härsvatten (A), 5 = Stora Härsjön (L), 6 = Gylltigesjön (L), 7 = Stora Skärsjön (N), 8 = Stengårdshultasjön (L), 9 = Älgarydssjön (A), 10 = Gyslättasjön (L), 11 = Fiolen (N), 12 = Storasjö (A), 13 = Brunnsjön (A), 14 = Allgjuttern (N), 15 = Stora Envättern (N), 16 = Stensjön (L), 17 = Övre Skärsjön (A), 18 = Västra Skälsjön (L), 19 = Tryssjön (L), 20 = Bösjön (L), 21 = Stensjön (N), 22 = Källsjön (L), 23 = Remmarsjön (N), 24 = Lien (L). Encircled numbers indicate lakes that were sampled for analysis of stable isotopes (i.e. part 2 of this project).



All physicochemical analyses were done at the Department of Aquatic Sciences and Assessment following international (ISO) or European (EN) standards when available (Wilander et al. 2003). Littoral macroinvertebrate samples were collected once in autumn (between September and November) from stony habitats (wind exposed littoral regions) using standardized kick sampling and a handnet (European Committee for Standardisation, 1994) with a 0.5-mm

TABLE 1. Morphological and water chemistry characteristics of lakes included in the study. Values represent the interannual mean values based on summer values (August) \pm 1 standard error for the time period 2000–2004.

SMHI X SMHIY	Lake area (km ²)	Max. Depth (m)	pH	Alkalinity meq/l	Ca meq/l	Mg meq/l	Na meq/l	K meq/l	SO ₄ meq/l	Cl meq/l	F mg/l	TOC mg/l	PO ₄ -P µg/l	NO ₂ +NO ₃ - N µg/l
Acid ref. lakes														
627443	0.11	10.60	5.71	<0.01	0.20	0.12	0.21	0.02	0.20	0.17	0.13	20.42	4.20	53.40
149526			\pm 0.10	\pm <0.01	\pm 0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm 0.01	\pm <0.01	\pm 4.51	\pm 0.86	\pm 11.13
643914	0.19	26.20	4.84	-0.02	0.03	0.06	0.24	0.01	0.09	0.26	0.03	2.38	1.40	55.00
127698			\pm 0.07	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm <0.01	\pm 0.23	\pm 0.24	\pm 8.22
652902	0.17	9.40	5.88	0.02	0.07	0.07	0.21	0.01	0.07	0.18	0.05	13.26	2.60	4.60
125783			\pm 0.03	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm <0.01	\pm 1.65	\pm 0.68	\pm 1.33
663532	1.74	32.00	5.87	0.01	0.07	0.07	0.06	0.01	0.09	0.04	0.11	4.72	1.20	95.20
148571			\pm 0.18	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 1.43	\pm 0.20	\pm 17.94
Circumneutral ref. lakes														
642489	0.19	40.70	6.79	0.07	0.17	0.10	0.13	0.01	0.17	0.09	0.22	7.08	1.80	4.00
151724			\pm 0.15	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm <0.01	\pm 0.01	\pm 0.23	\pm 0.37	\pm 1.05
633025	1.65	10.50	6.72	0.07	0.15	0.09	0.17	0.04	0.13	0.17	0.07	7.64	1.40	5.40
142267			\pm 0.14	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm 0.01	\pm <0.01	\pm 0.96	\pm 0.24	\pm 1.29
645289	0.28	14.50	6.68	0.08	0.17	0.09	0.26	0.02	0.10	0.26	0.07	9.42	2.40	7.60
128665			\pm 0.07	\pm 0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm <0.01	\pm 0.42	\pm 0.40	\pm 2.73
708619	1.37	14.40	6.40	0.06	0.09	0.04	0.06	0.01	0.03	0.02	0.18	9.08	3.40	6.40
162132			\pm 0.11	\pm 0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm 1.11	\pm 0.93	\pm 1.72
683673	0.57	8.50	6.37	0.04	0.06	0.03	0.05	0.01	0.04	0.02	0.08	6.26	2.40	6.80
154083			\pm 0.05	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm 0.56	\pm 0.51	\pm 2.82
655587	0.38	11.20	6.70	0.05	0.17	0.07	0.10	0.01	0.12	0.08	0.11	9.40	1.60	3.40
158869			\pm 0.12	\pm 0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm 0.61	\pm 0.40	\pm 0.24
628606	0.31	11.50	6.99	0.13	0.18	0.16	0.32	0.01	0.17	0.31	0.07	5.10	1.80	4.20
133205			\pm 0.08	\pm 0.01	\pm <0.01	\pm <0.01	\pm 0.01	\pm <0.01	\pm 0.01	\pm 0.01	\pm <0.01	\pm 0.99	\pm 0.37	\pm 1.59

	SMHI X SMHIY	Lake area (km ²)	Max. Depth (m)	pH	Alkalinity meq/l	Ca meq/l	Mg meq/l	Na meq/l	K meq/l	SO4 meq/l	Cl meq/l	F mg/l	TOC mg/l)	PO ₄ -P µg/l	NO ₂ +NO ₃ - N µg/l	
Limed lakes																
	680235	1.15	17.00	6.75	0.09	0.12	0.02	0.04	0.01	0.03	0.01	0.35	6.72	1.80		5.40
Bösjön	141799			±0.17	±0.02	±0.02	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±0.02	±0.91	±0.37		±2.29
	653737	0.83	28.60	7.49	0.25	0.33	0.06	0.28	0.01	0.08	0.28	0.09	5.78	1.80		116.00
Eigdesjön	125017			±0.12	±0.03	±0.02	±<0.01	±<0.01	±<0.01	±<0.01	±0.01	±<0.01	±0.75	±0.58		±14.52
	629489	0.40	20.00	7.04	0.26	0.39	0.11	0.21	0.01	0.10	0.21	0.09	15.68	5.00		154.20
Gyltigesjön	133906			±0.10	±0.03	±0.03	±0.01	±0.01	±<0.01	±0.01	±0.01	±<0.01	±1.85	±1.26		±14.96
	633209	0.33	9.80	6.87	0.12	0.29	0.06	0.15	0.01	0.13	0.13	0.08	12.90	1.60		4.20
Gysiättasjön	141991			±0.06	±0.01	±0.01	±<0.01	±0.01	±<0.01	±0.01	±0.01	±<0.01	±1.01	±0.24		±0.92
	683582	0.22	17.40	6.78	0.15	0.24	0.04	0.06	0.01	0.04	0.02	0.09	15.24	3.00		4.60
Källsjön	154935			±0.09	±0.01	±0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±1.72	±0.55		±1.47
	663216	1.53	29.20	6.85	0.12	0.18	0.07	0.10	0.01	0.09	0.07	0.12	7.24	2.40		15.80
Lien	148449			±0.08	±0.02	±0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±0.66	±0.60		±7.00
	638317	4.98	26.80	7.07	0.18	0.29	0.08	0.15	0.02	0.09	0.16	0.07	10.14	2.40		53.80
Stengårdshultasjön	138010			±0.04	±0.01	±0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±0.43	±0.75		±14.37
	656419	0.39	20.60	6.89	0.10	0.22	0.08	0.10	0.02	0.17	0.09	0.07	8.50	1.80		14.20
Stensjön	164404			±0.06	±0.01	±0.02	±0.01	±<0.01	±<0.01	±0.02	±0.01	±<0.01	±0.48	±0.37		±9.04
	640364	2.57	42.00	7.36	0.28	0.39	0.08	0.30	0.02	0.12	0.33	0.07	4.80	1.20		133.40
Stora Härsjön	129240			±0.12	±0.01	±0.01	±<0.01	±0.01	±<0.01	±<0.01	±0.01	±<0.01	±0.19	±0.20		±15.53
	670275	0.30	19.60	6.52	0.06	0.16	0.03	0.05	0.01	0.03	0.03	0.07	Nd	Nd		4.00
Tryssjön	146052			±0.09	±0.01	±0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	Nd	Nd		±1.38
	664620	0.41	18.70	7.00	0.14	0.20	0.03	0.06	0.01	0.09	0.04	0.08	12.58	1.60		3.40
V. Skällsjön	148590			±0.15	±0.02	±0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±<0.01	±2.37	±0.40		±0.51

mesh size, and preserved in 70% ethanol. Samples of sublittoral and profundal invertebrates were sampled using an Ekman grab (surface area 0.025 m²), screened in a 0.5 mm sieve and preserved in 70% ethanol. Five replicate samples were collected and biomasses were determined by weighing (ethanol weight); the average of the five replicates is used for analyses. In the laboratory, samples were sorted under 10x magnification, identified using dissecting and light microscopy. Organisms were identified to the lowest taxonomic unit possible, generally to the species level, although exceptions occurred with some chironomid larvae and immature oligochaetes.

Zooplankton was sampled quantitatively in August using a 55-cm Plexiglas tube (i.d. 10 cm) equipped with a closing mechanism triggered by a messenger. Samples were generally collected at 2-m intervals from the surface down to 8-m depth. Samples were pooled, screened (40 µm), and preserved in acid Lugol's solution. Taxonomic analyses, enumeration, and length measurements were done using an inverted microscope. Biovolumes were calculated from length measurements and known relationships for different taxa, life stages and/or size classes.

Epilimnetic, integrated samples (0–4 m) of phytoplankton samples were collected in August with a tube sampler, usually from 5 sites per lake, pooled and preserved in Lugol's solution. Taxonomic analyses and species enumeration was done under an inverted microscope using the Utermöhl technique (Olrík et al. 1989). Biovolumes were calculated from geometric shapes following Blomqvist & Herlitz (1998).

Structural community metrics and functional groups

For all communities we used structural metrics that are routinely used in the analysis of ecological communities (i.e. total biomass/abundance, species/taxon richness and Shannon-Wiener index). Regarding the functional classification, we followed to a great extent the schemes used by Sundbom (2008). For example, phytoplankton was divided into autotrophic, mixotrophic and heterotrophic biomass groups following the classification scheme of Jansson et al. (1996). Bacillariophyceae, Conjugatophyceae, Cryptophyceae, Cyanophyceae, Loxophyceae, Prasinophyceae, Xanthophyceae and Chlorophyceae were considered to be functionally autotrophic, except the green algae *Polytoma* and *Polytomella* (mixotrophic taxa). Many groups that are known to have many mixotrophic species are Chrysophyceae, Craspedophyceae, Dinophyceae, Euglenophyceae, Haptophyceae and Raphidophyceae. Taxa were considered

heterotrophic when they contained no photosynthetic apparatus (e.g., euglenozoan flagellates).

Zooplankton was divided into the functional groups predators and filter-feeders (Gliwicz 1969ab). The latter group not necessarily ingests only bacterioplankton and phytoplankton, but also preys on small animals. However, as filter feeders “passively prey” on animals with a smaller body size, they are not dealt with as predators in the strict sense. We considered as predators all cyclopoid copepods, the calanoid copepods *Heteroscope* and *Eurytemora*, the cladocerans *Bythotrephes* and *Leptodora*, and the rotifer *Asplanchna*. The remaining taxa were assigned as filter-feeders.

Functional guilds of macroinvertebrates were according to the descriptions by Moog (1995). Although Moog's scheme differentiates between 10 groups we focused on the broader categories Detritivores, Predators, Filter Feeders, and Grazers. Many species could sometimes be assigned simultaneously to several guilds, i.e. due to ontogenetic feeding shifts. When this occurred we used the dominant functional category to characterize a species functional role. However, if the relative distribution among different guilds was more even, then we considered this species to have a very broad feeding plasticity during their life span, and will refer to this group as “Multiple group”, reflecting their functionally multiple roles in aquatic ecosystems.

Statistical analyses

Analysis of variance (ANOVA) was carried out in SPSS v.12 (SPSS Inc, Chicago, Illinois, USA) to test for differences in selected water quality variables (pH, Alkalinity, Ca, Mg, total N, total P and Si), community metrics (total biomass/abundance, species richness and Shannon-Wiener biodiversity) and functional groups for each of the studied communities between lake type (acid lakes, circumneutral lakes and limed lakes; fixed factor) and over the study years (random factor). In addition, we contrasted in the same way the Median Acidity Index calculated for littoral macroinvertebrates (Hendriksson and Medin 1986). The ANOVA models were calculated on the basis of Type III sums of squares to take the unbalanced design into account. Likewise, the Scheffe test, which is a valid, fairly conservative test, sufficiently generalised to be applicable to unequal designs, was used to determine pairwise differences in treatment means. All dependent variables were log (x+1)-transformed to fulfill the requirements of parametric tests.

Non-metric multidimensional scaling (NMDS) was done in Primer v.6 (Primer-E Ltd, Plymouth, UK) to explore the similarity of community trends over

the study period across lake types. As a nonlinear technique, NMDS ranks points in ordination space in a way that the distance between sampling points (in this study aquatic communities) reflects community similarity (ter Braak, 1995). The ordination is based on a Bray-Curtis dissimilarity matrix derived from average values of all replicate lakes and log (x+1)-transformation of the sample by species matrix. In addition, a NMDS analysis was carried out for water quality; in this case the ordination is based on a Euclidean distance matrix derived from standardized and log(x+1)-transformed water chemistry data, including Secchi depth, water temperature, dissolved oxygen concentration, conductivity, pH, Alkalinity, and concentrations of Ca, Mg, Na, K, SO₄, Cl, F, NH₄-N, NO₂-N+NO₃-N, total N, PO₄-P, total P, remaining P (total P – PO₄-P), Si, total organic carbon (TOC) and Chlorophyll *a*. The final solutions for each community and the water quality analysis are based on 999 permutations. Pearson correlation analyses were carried out in SPSS to explore the relationship of NMDS dimensions with dominant taxa, community metrics, functional groups and water quality variables (Table 1).

Analysis of Similarity (ANOSIM; 999 permutations) was also run in Primer to test if significant differences in biomass/abundance of communities occurred among lake types. This analysis is an approximate non-parametric analogue of the standard univariate analysis of variance ANOVA, and it uses the R statistic to test differences between groups (R=0, no differences; R=1, all dissimilarities between groups are larger dissimilarity within groups). In the present study, the ANOSIM analysis was used to complement the NMDS analyses. As such it was of prime interest to use the same samples as those used for the ordination. This means that we first calculated the yearly average for each lake type. This resulted in 5 replicates (5 study years) x 3 lake types (acid lakes, circumneutral lakes, limed lakes) = 15 samples for the analysis. Similarity Percentage routine (SIMPER; also included in Primer v.6) was used to reveal which taxa contributed to dissimilarity between lake types.

Results and Discussion

Lake characteristics and water quality

Most of the lakes had a surface area <1 km², but some lakes were up to 5-times larger. Brunnsjön was the smallest lake (0.11 km²) while Stengårdshultasjön was the largest (4.98 km²) (Table 1). The lakes also showed a depth gradient, with circumneutral Stensjön being the shallowest ($Z_{\max} = 8.5$ m) and the limed Stora Härsjön being the deepest ($Z_{\max} = 42$ m). With regard to trophic state characteristics acid lakes, circumneutral lakes and limed lakes showed average

total P concentrations of 9.45, 7.43 and 8.18 µg L⁻¹, respectively, and average total N concentrations of 399.98, 356.97 and 383.50 µg L⁻¹, respectively. The concentrations of these nutrients were not significantly different between lake types (Table 2; Figure 2). However, substantial differences in water quality were observed among lake types with regard to water quality variables that are most affected by acidification and liming treatments. For example, the mean pH of acid reference lakes was always below 6, while circumneutral reference lakes showed pH values between 6.4 and 7.0, and some limed lakes showed a pH > 7.0. These values were significantly different between lake types (Table 2; Figure 2). Also Ca and Mg concentrations, but not Si concentrations, were significantly different between lake types (Table 2). As a result of their ionic composition, lake types had also significantly different alkalinity values (Table 1). The differences in water chemistry among lake types were well captured in multivariate ordination space (Figure 3), and an analysis of similarity showed significant differences in water quality between lake categories (ANOSIM: global R = 0.996, P < 0.001).

Univariate analyses of community structure

Our analyses based on functional categories and traditional community metrics, i.e. total biomass or total abundance, species richness and biodiversity indices such as the Shannon-Wiener index, vary among the analyzed communities. With regard to community metrics, phytoplankton showed significant differences in species richness and Shannon-Wiener biodiversity, while littoral macroinvertebrates showed differences only in total abundance. Zooplankton, sublittoral and profundal macroinvertebrates showed no significant differences in community metrics between lake categories (Table 2, Figures 4-8).

From a diversity point of view, these results suggest that phytoplankton has lower species richness and Shannon-Wiener biodiversity in acid lakes than in circumneutral lakes and limed lakes (Figure 4). This could be partly due to negative effects of low pH in acid lakes, but also indicate a beneficial effect of liming on phytoplankton diversity to similar levels as those observed in natural reference lakes. We highlight however that we miss any intra-annual variability and that our findings are limited to summer conditions. Furthermore, the lack of data before implementing liming as an acidification mitigation measure in these lakes does not allow us to determine with certainty to what extent liming increases phytoplankton diversity.

Functional group characteristics also seemed to vary as a function of lake type. Higher heterotrophic phytoplankton biomass was found in

TABLE 2. ANOVA results showing degrees of freedom, mean squares, F-ratios and significance levels of structural and functional community attributes of phytoplankton, zooplankton, and macroinvertebrates in three habitat types (littoral, sublittoral, and profundal) of three lake types (acid lakes, circumneutral lakes, and limed lakes). Significance levels: * P < 0.05, ** P < 0.01, *** P < 0.001, NS not significant. Note for macroinvertebrates that the “Multiple group” represents those organisms that share various functional characteristics according to Moog (1995). See Material and Methods for details.

		pH			Alkalinity			Ca			Mg			Si			Total P			Total N		
		MS	F		MS	F		MS	F		MS	F		MS	F		MS	F		MS	F	
Lake Type	2, 8	0.05	364.4***	0.03	350.01***	0.03	797.0***	<0.01	307.8***	0.03	NS	0.09	NS	<0.01	NS	0.05	<0.01	NS	<0.01	NS	6.27**	NS
Years	4, 11.26	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	7.58**	NS	0.02	NS	<0.01	NS	0.05	<0.01	NS	<0.01	NS	NS	NS
Interaction	8, 95	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	0.01	NS	<0.01	NS	<0.01	NS	<0.01	<0.01	NS	<0.01	NS	NS	NS
Error		<0.01		<0.01		<0.01				0.03		0.06				0.02						

		Biomass			Species richness			Shannon-Wiener diversity			Autotrophic biomass			Heterotrophic biomass			Mixotrophic biomass		
		MS	F		MS	F		MS	F		MS	F		MS	F		MS	F	
Lake Type	2, 8	0.01	NS	0.58	70.52***	0.14	46.11***	0.01	11.85**	<0.01	38.60***	0.01	NS	<0.01	NS	0.01	NS	NS	NS
Years	4, 11.26	0.01	NS	0.02	NS	0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	<0.01	NS	NS
Interaction	8, 95	<0.01	NS	0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	NS	<0.01	<0.01	NS	NS
Error		0.02		0.01		0.01		<0.01		<0.01		<0.01		<0.01		<0.01		<0.01	

		Biomass			Species richness			Shannon-Wiener diversity			Filter feeders			Predators		
		MS	F		MS	F		MS	F		MS	F		MS	F	
Lake Type	2, 8	0.31	NS	0.01	NS	0.01	0.01	1.17	9.68**	1.44	1.44	NS	NS	1.44	NS	NS
Years	4, 11.26	0.76	6.25**	<0.01	NS	<0.01	NS	0.92	7.39**	0.38	0.38	NS	NS	0.38	NS	NS
Interaction	8, 95	0.11	NS	<0.01	NS	0.01	NS	0.12	0.74	0.12	0.12	NS	NS	0.12	NS	NS
Error		0.23		0.01		<0.01		0.16								

TABLE 2. Continued.

Littoral macroinvertebrates

df	Biomass		Species richness		Shannon-Wiener diversity		Acidity index		Detritivore biomass		Filter feeder biomass		Grazer biomass		Predator biomass		Multiple groups biomass	
	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Lake Type	0.13	8.86**	0.66	NS	<0.01	NS	0.01	11.85***	0.28	NS	2.80	63.05***	4.34	84.35***	0.43	NS	1.09	7.84*
Years	0.02	NS	0.08	NS	<0.01	NS	<0.01	NS	0.20	NS	0.16	NS	0.18	NS	0.14	NS	0.04	NS
Interaction	0.01	NS	0.22	NS	<0.01	NS	<0.01	NS	0.32	NS	0.04	NS	0.05	NS	0.10	NS	0.14	NS
Error	0.03		0.19		<0.01				0.21		0.36		0.31		0.12		0.24	

Sublittoral macroinvertebrates

df	Biomass		Species richness		Shannon-Wiener diversity		Detritivore biomass		Filter feeders biomass		Grazer biomass		Predator biomass		Multiple group biomass	
	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Lake Type	0.26	NS	0.26	NS	0.07	NS	0.62	88.10***	0.16	10.32**	0.07	9.36**	0.57	28.19**	0.03	NS
Years	0.08	NS	0.11	NS	0.02	NS	0.03	NS	0.01	NS	0.01	NS	0.04	NS	0.04	NS
Interaction	0.05	NS	0.05	NS	0.01	NS	0.01	NS	0.02	NS	0.01	NS	0.02	NS	0.01	NS
Error	0.10		0.11		0.01		0.05		0.03		0.02		0.06		0.02	

Profundal macroinvertebrates

df	Biomass		Species richness		Shannon-Wiener diversity		Detritivore biomass		Filter feeders biomass		Grazer biomass		Predator biomass		Multiple group biomass	
	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Lake Type	0.26	NS	0.29	NS	0.05	NS	0.13	NS	0.01	NS	0.01	NS	0.94	53.96**	<0.01	NS
Years	0.05	NS	0.06	NS	0.01	NS	0.01	NS	<0.01	NS	<0.01	NS	0.06	NS	<0.01	NS
Interaction	0.04	NS	0.04	NS	0.01	NS	0.01	NS	<0.01	NS	<0.01	NS	0.02	NS	<0.01	NS
Error	0.18		0.19		0.02		0.08		<0.01		<0.01		0.15		<0.01	

FIGURE 2. Comparison of selected *water quality variables* between acid lakes, circumneutral lakes and limed lakes. Shown are the means \pm standard errors of 4 (acidic ref. lakes), 7 (circumneutral ref. lakes) and 11 (limed lakes) replicates. The letters highlight significant differences between lakes from a pairwise comparison (Scheffe test).

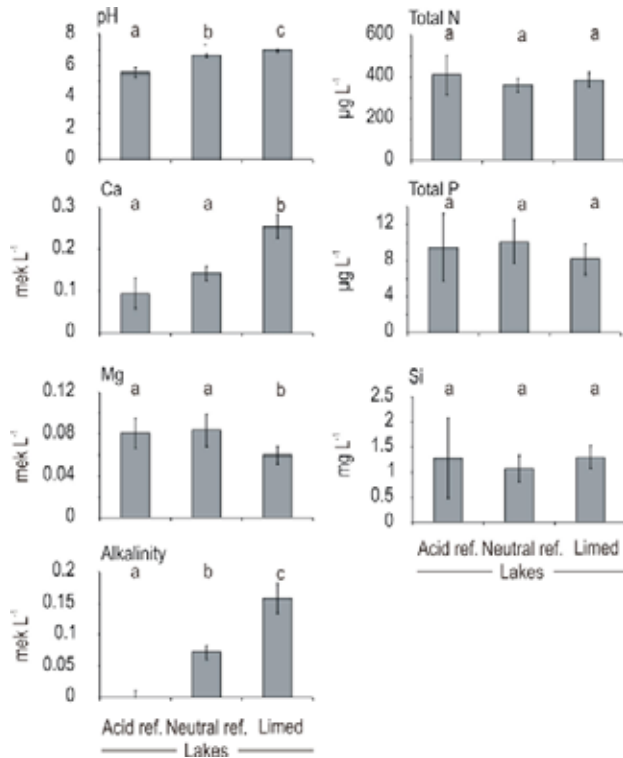
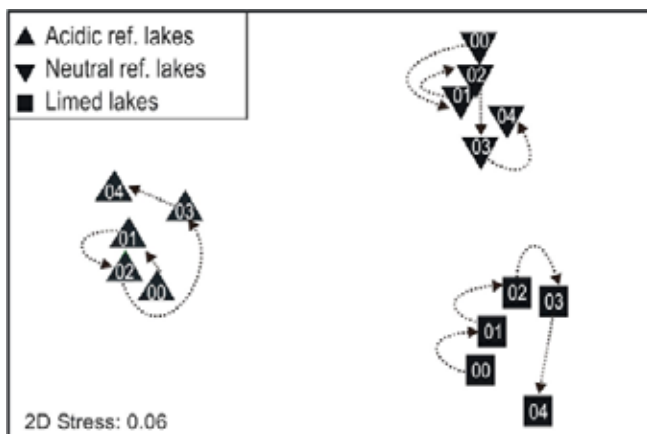


FIGURE 3. Nonmetric multidimensional scaling ordination showing temporal trends of water quality in acid lakes, circumneutral lakes and limed lakes between 2000 [00] and 2004 [04].



circumneutral lakes and acid lakes than in limed lakes (Table 2; Figure 4). Predator biomass among zooplankton was not significantly different across lake types but filter feeder biomass was higher in circumneutral lakes than in limed lakes. Filter feeder biomass had similar values in circumneutral lakes and acid lakes but was higher in limed lakes (Table 2; Figure 5). The biomass/abundance of detritivores in the macroinvertebrate communities differed according to habitat characteristics. While no significant differences of detritivores were found in littoral and profundal habitats across lake types, the sublittoral habitat showed a significantly lower biomass of this group in limed and circumneutral lakes (Table 2; Figures 6-8). The biomass of filter feeders and grazers seemed to be consistently lower in acid references compared to circumneutral references and limed lakes, but a significant difference could only be found for littoral and sublittoral habitats (Table 2; Figures 6-8). Predator biomass showed opposite patterns in the different lake types. The predator guild in littoral habitats was significantly lower in acid lakes. The opposite was observed in the sublittoral and profundal habitats, where predator biomass was significantly higher in circumneutral lakes and limed lakes (Table 2; Figures 6-8). Finally, also the biomass of species which showed a broad feeding plasticity (i.e., multiple

groups), varied among lake types, with the only significant difference occurring in the littoral community where circumneutral lakes had a higher biomass than limed lakes and acid lakes (Table 2; Figures 6-8).

While most differences were found in the structural and functional composition of planktonic and benthic communities, the effect of time was only significant for total biomass and filter feeder biomass for zooplankton, which could be due to chance events. However, the interaction term (lake type x time) was not significant in any ANOVA model, which suggests that the community characteristics observed on a single sampling data were temporally stable in all lake types during the study period. This finding suggests that liming does not substantially impact on community characteristics of limed lakes relative to circumneutral and acid lakes.

Structural and functional responses to liming varied between the different communities. This suggests that environmental monitoring and assessment of impacts of liming as a mitigation measure based on a single organism group and community (i.e. planktonic algae, littoral macroinvertebrates) is not straightforward. Even within a single organism group responses can vary as a function of habitat characteristics. This calls for studies that combine the information of multiple organism groups and habitats to unravel the whole

FIGURE 4. Comparison of *phytoplankton* community metrics and the biomass of functional guilds between acid lakes, circumneutral lakes and limed lakes. Shown are the means \pm standard errors 4 (acidic ref. lakes), 7 (circumneutral ref. lakes) and 11 (limed lakes) replicates. The letters highlight significant differences between lakes in a pairwise comparison (Scheffe test).

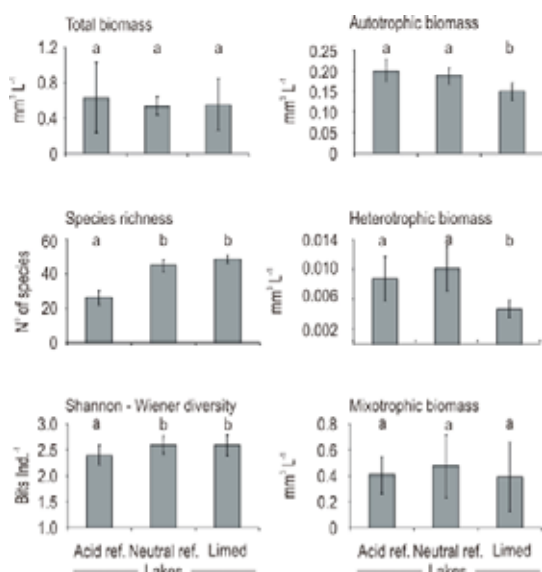


FIGURE 5. Comparison of *zooplankton* community metrics and the biomass of functional guilds between acid lakes, circumneutral lakes and limed lakes. Shown are the means \pm standard errors of 4 (acidic ref. lakes), 7 (circumneutral ref. lakes) and 11 (limed lakes) replicate lakes. The letters highlight significant differences between lakes in a pairwise comparison (Scheffe test).

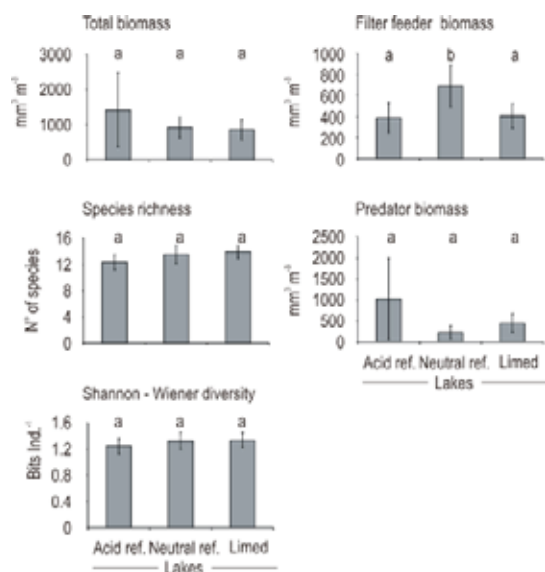


TABLE 3. Results of ANOSIM (Analysis of Similarity) tests showing the R statistic and P levels of global models and pairwise comparisons of different communities across lake types (acid lakes, circumneutral lakes and limed lakes).

	Phytoplankton		Zooplankton		Macroinvertebrates Littoral		Macroinvertebrates Sublittoral		Macroinvertebrates Profundal	
	R	P	R	P	R	P	R	P	R	P
Global model	0.83	0.001	0.67	0.001	0.68	0.001	0.80	0.001	0.82	0.001
Acid x Neutral	1	0.008	0.90	0.001	0.94	0.008	0.99	0.008	1	0.008
Acid x Limed	0.47	0.008	0.67	0.001	1	0.008	0.99	0.008	0.74	0.016
Circumneutral x Limed	0.93	0.008	0.57	0.001	0.35	0.06	0.41	0.016	0.74	0.008

FIGURE 6. Comparison of *littoral macroinvertebrate* community metrics and the biomass of functional guilds between acid lakes, circumneutral lakes and limed lakes. Shown are the means \pm standard errors of 4 (acidic ref. lakes), 7 (circumneutral ref. lakes) and 11 (limed lakes) replicate lakes. The letters highlight significant differences between lakes in a pairwise comparison (Scheffe test).

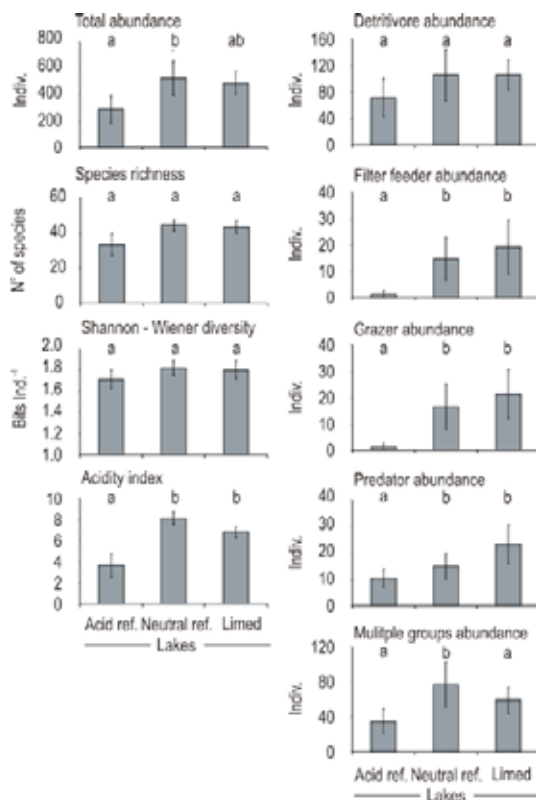
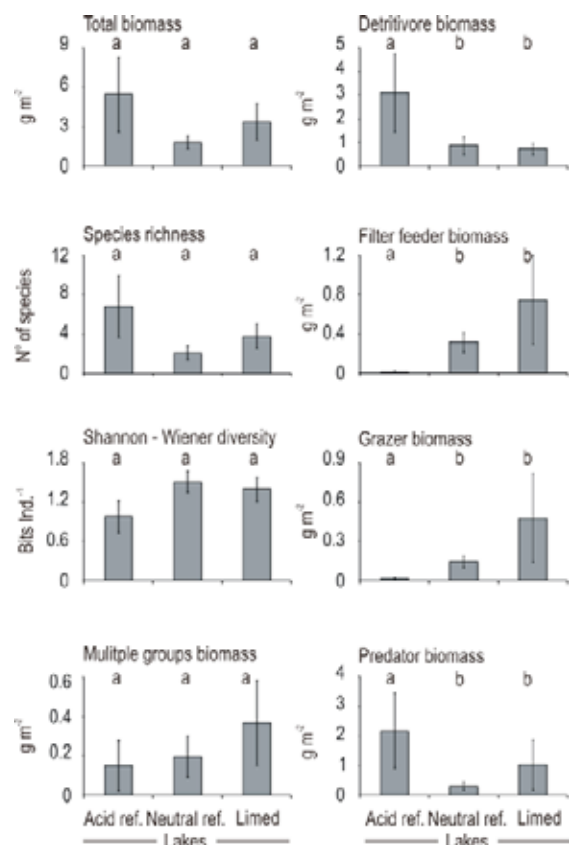


FIGURE 7. Comparison of *sublittoral macroinvertebrate* community metrics and the biomass of functional guilds between acid lakes, circumneutral lakes and limed lakes. Shown are the means \pm standard errors of 4 (acidic ref. lakes), 7 (circumneutral ref. lakes) and 11 (limed lakes) replicate lakes. The letters highlight significant differences between lakes in a pairwise comparison (Scheffe test).



magnitude of environmental impacts in general in boreal lake ecosystems and specifically that of liming. Stendera and Johnson (2008) reached similar conclusions in their study of community recovery from anthropogenic acidification of several Swedish lakes (some of them included in the present study), mediated in part by habitat characteristics.

Multivariate analyses of community structure

The analyses based on nonmetric multidimensional scaling show a clear separation of all communities among acid lakes, circumneutral lakes and limed lakes. For each analysis the stress value of the obtained

ordinations was < 0.1 which according to Clarke (1993) can be regarded as a good ordination with no real risk of drawing false inferences. Most communities in limed lakes seemed to occupy intermediate positions compared with acid and circumneutral lakes (Figure 9). Analysis of similarity indicated that these differences were significant for all groups except for littoral macroinvertebrates, which did not differ between circumneutral lakes and limed lakes (Table 3). This suggests that the majority of communities of limed lakes do not converge with those in circumneutral lakes as a result of liming.

Similarity percentages (SIMPER) analyses allowed

TABLE 4. Results of SIMPER (Similarity Percentages) analysis, showing the percentage of phytoplankton species contributing to community structure, based on their biomass.

Species	Acid	Neutral	Limed
Bacillariophyceae			
Aulacoseira alpigena		2.63	2.95
Aulacoseira distans			1.81
Aulacoseira distans var. tenella	1.7		0.53
Cyclotella spp. (10-15 µm)			1.13
Tabellaria flocculosa var. asterionelloides		0.81	1.01
Chlorophyta			
Botryococcus terribilis	3.28	6.63	
Botryococcus spp.			0.91
Monoraphidium dybowskii	2.21	2.48	1.61
Oocystis sp.		0.71	0.46
Uroglena sp.		1.76	1.57
Unidentified chlorococcales	2.4	2.54	1.02
Dinophyta			
Ceratium furcoides			0.6
Ceratium hirundinella		2.25	1.59
Gymnodinium fuscum			0.6
Gymnodinium spp. (10-14 µm)		1.5	
Gymnodinium uberrimum	4.13	3	
Peridinium inconspicuum	2.14	3.43	1.93
Peridinium willei	2.17		
Peridinium sp.			0.47
Rhodophyceae			
Cryptomonas marssonii (<20 µm)			1.62
Cryptomonas spp. (<20 µm)		6.34	3.05
Cryptomonas spp. (<20-40 µm)	9.83	15.76	3.73

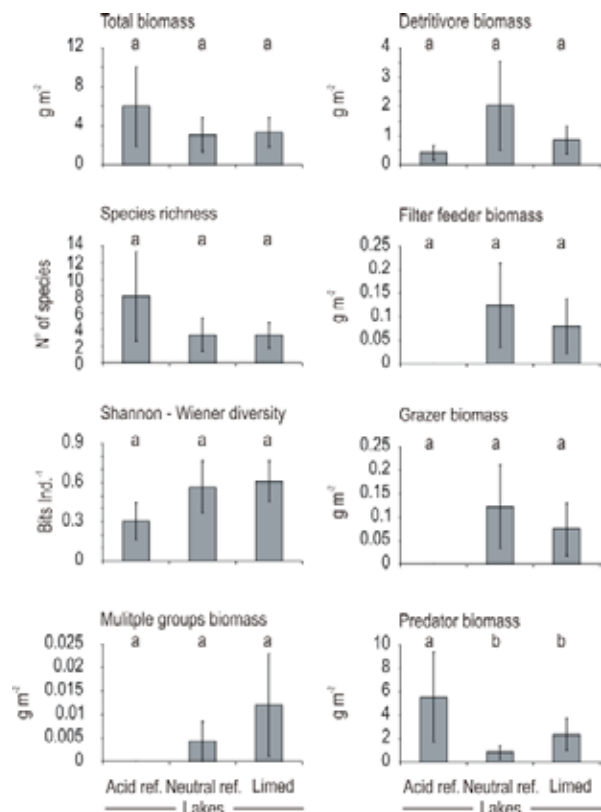
Species	Acid	Neutral	Limed
Rhodomonas lacustris		7.18	3.3
Chrysophyceae			
Chrysidiastrum catenatum			0.64
Chrysochromulina parva		1.5	1.22
Chrysooccus sp.			0.51
Monosigales spp.		0.55	
Pseudopedinella sp.		2.25	3.46
Stichogloea doederleinii		0.89	
Spiniferomonas sp.		0.93	
Synurophyceae			
Mallomonas allorgei		0.6	
Mallomonas caudata		1.06	0.45
Mallomonas sp.			1.2
Mallomonas crassisquama		0.8	
Raphidophyceae			
Gonyostomum semen	54.47		41.97
Cyanoprokaryota			
Merismopedia tenuissima	2.12	5.32	0.48
Woronichinia naegeliana		0.72	
Woronichinia naegeliana		0.72	
Colourless flagellates			
Katablepharis ovalis		3.15	1.44
Unidentified taxa			
Unidentified monads (<3 µm)		0.67	
Unidentified monads (3-5 µm)	4.2	7.53	2.33
Unidentified monads (5-7 µm)	2.1	4.56	3.09
Unidentified monads (7-10 µm)		2.46	2.28
Unidentified monads (>10 µm)			1.45

us to identify species that characterize the communities in the different lake types, i.e. in reference lakes and limed lakes. With regard to phytoplankton, acid reference lakes were characterized by fewer species relative to circumneutral lakes and limed lakes. This was due to the relatively high occurrence of the raphidophycean flagellate *Gonyostomum semen* that comprised more than 50% of phytoplankton biomass in acid lakes. Similarly, in acid lakes, Chrysophyceae and Synurophyceae were absent while Cryptophyceae biomass was markedly lower than in the other lake types. Circumneutral lakes and limed lakes shared many species, but their relative contribution varied between both lake types, underlying the significant difference in community structure found in the multi-variate analyses (Figure 9, Table 4)

The correlation analysis relating the NMDS dimensions to phytoplankton taxa was in good agreement with the SIMPER analysis (Table 5). The positive correlation of many phytoplankton taxa with NMDS dimension 1 (horizontal dimension) suggests that these species become more abundant towards the right side of the ordination coincident with the location of limed lakes and circumneutral lakes in the ordination. Among these species we find e.g., *Katablepharis ovalis*, *Gymnodinium* sp., *Rhodomonas lacustris* and *Chrysochromulina parva*. The negative correlation of *Gonyostomum semen* with NMDS 1 clearly supports the high dominance of this species in acid lakes, while the negative correlation of autotrophic biomass with NMDS further suggests that this functional group was less important in these lakes. With regard to environmental variables, the negative correlation of $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ and TOC with NMDS 1 suggests that limed lakes and circumneutral references had a lower water color and a lower amount of nitrogen compounds relative to acid lakes. NMDS 2 (vertical dimension) was positively correlated with Na, SO_4 , Mg and Cl and negatively correlated with pH, conductivity, alkalinity and Ca. The correlations also reflect nicely the separation of limed lakes from the other lake categories along this dimension in the ordination, with limed lakes showing positions in the ordination where e.g., pH, alkalinity and Ca concentrations were high (negative correlations with NMDS 2).

Zooplankton communities in acid, circumneutral and limed lakes were clearly separated along NMDS 1, with limed lakes occupying an intermediate position to acid and circumneutral lakes (Figure 9). The SIMPER analysis showed that acid lakes had a higher share of rotifer species than circumneutral and limed lakes. Other notable differences were found in the cladocerans, where *Ceriodaphnia quadrangula* was

FIGURE 8. Comparison of *profunda* macroinvertebrate community metrics and the biomass of functional guilds between acid lakes, circumneutral lakes and limed lakes. Shown are the means \pm standard errors of 4 (acidic ref. lakes), 7 (circumneutral ref. lakes) and 11 (limed lakes) lakes. The letters highlight significant differences between lakes in pairwise comparisons (Scheffe test), i.e. lakes having no letters in common are significantly different.



more abundant in acid lakes than in circumneutral and limed lakes, respectively, while *Holopedium gibberum*, absent from acid lakes, contributed on average at least 10% to zooplankton biomass in circumneutral and limed lakes. Also, *Daphnia cristata* was more abundant in limed and circumneutral lakes, respectively, than in acid lakes. There were also several indifferent species that had similar biomasses in all lake types, e.g., *Diaphanosoma brachyurum* and *Eubosmina coregoni* (Cladocera) and *Conochilus unicornis* (Rotifera). This could indicate their broad ecological plasticity and/or tolerance to acid conditions or liming treatments of lakes (Table 6).

Correlation of NMDS dimensions with water chemistry variables, zooplankton taxa, community metrics and functional guilds further helped to evaluate forcing functions for community structure in the three lake types (Table 7). Being in agreement with the SIMPER analysis, NMDS dimension 1 correlated

TABLE 5. Pearson correlations between NMDS dimensions and water quality variables, phytoplankton taxa, and phytoplankton functional groups. For phytoplankton taxa only correlation coefficients > 0.75 are shown where all correlations were significant at P< 0.001.

	MDS 2	
Water quality variables		
F	0,71**	
K	0,87***	
NO ₂ -N+NO ₃ -N	-0,88***	
TOC	-0,64**	
Na		0,84***
SO ₄		0,73**
Mg		0,71**
Cl		0,68**
pH		-0,63*
Conductivity		-0,71**
Alkalinity		-0,80***
Ca		-0,82***
Taxa		
<i>Katablepharis ovalis</i>	0,86	
<i>Gymnodinium sp. (10-14 µm)</i>	0,84	
Unidentified monads (<3 µm)	0,80	
<i>Spiniferomonas sp.</i>	0,78	
<i>Chrysochromulina parva</i>	0,77	
<i>Rhodomonas lacustris</i>	0,75	
<i>Gonyostomum semen</i>	-0,90	
<i>Cyclotella sp. (15-20 µm)</i>		-0,77
Functional groups		
Autotrophic biomass	-0,78***	

positively with *Daphnia cristata*, *D. cucullata*, *Daphnia sp.*, *Holopedium gibberum*, *Kellicottia longispina*, *Limnospida frontosa* and *Polyarthra vulgaris*. The analysis therefore shows that these species are more abundant in circumneutral and limed lakes than in acidic lakes. Conversely, the negative correlation of *Ceriodaphnia quadrangula*, *Kellicottia bostoniensis*, *Trichocerca capucina*, and *Polyarthra remata* with NMDS 1 indicates a more “acidophilic” characteristics of these species. Furthermore, the positive correlation of species richness with NMDS 1 further suggests that circumneutral and limed lakes had higher species richness than acid lakes. These trends are shown in Figure 5 even though there was no significant treatment effect. The biomass of filter feeding zooplankters was also higher in circumneutral and

TABLE 6. Results of SIMPER (Similarity Percentages) analysis, showing the percentage of zooplankton species contributing to community structure based on their biomass.

Species	Acid	Circum-neutral	Limed
Cladocera			
<i>Ceriodaphnia quadrangula</i>	14.92	2.4	11.31
<i>Daphnia cristata</i>	7.99	15.75	14.08
<i>Daphnia cucullata</i>		3.86	
<i>Daphnia galeata</i>	2.81	3.39	10.85
<i>Daphnia sp.</i>	13.97	14.89	15.79
<i>Diaphanosoma brachyurum</i>	7.11	6.27	5.61
<i>Eubosmina coregoni</i>	17.49	15.29	14.35
<i>Holopedium gibberum</i>		11.33	9.95
<i>Limnospida frontosa</i>		2.69	
Copepoda			
<i>Diaptomus graciloides</i>		1.99	
Rotifera			
<i>Conochilus unicornis</i>	3.56	3.52	3.15
<i>Kellicottia bostoniensis</i>	8.03		
<i>Keratella cochlearis f. typica</i>	2.6		2.09
<i>Kellicottia longispina</i>		2.15	
<i>Ploesoma hudsoni</i>	2.89		
<i>Polyarthra remata</i>	4.88		
<i>Polyarthra vulgaris</i>	3.29	6.98	4.83
<i>Trichocerca capucina</i>	2.77		

limed lakes than in acid lakes, while the opposite was true for predator biomass. The three lake types also differed with respect to several water quality variables. According to the zooplankton ordination, lake types were separated chiefly on the basis of nitrogen compounds (negative correlation with NMDS 1; reflecting acidic conditions) and F1, pH, and Cl (positive correlation with NMDS 1; reflecting conditions in circumneutral and limed lakes). Alkalinity, Ca, and phosphates correlated negatively with NMDS 2 reflecting conditions in limed lakes, while the positive correlation of sulphates and Mg with NMDS 2 reflect conditions of reference lakes.

Littoral macroinvertebrates were also clearly separated along NMDS dimension 1 in the multivariate ordination, even though communities of limed

lakes clustered closer to those of circumneutral lakes than those of acid lakes (Figure 9). The separation in ordination space of communities of limed lakes and circumneutral lakes was not significant according to the ANOSIM analysis. However, it must be highlighted that the analysis of littoral macroinvertebrate communities is based on abundance data while that for the other organism groups was done on biomass data. Possibly, biomass-based analyses more accurately detect differences among lake types as it includes a quantitative dimension rather than information on abundance.

The SIMPER analysis shows that circumneutral and limed lakes share many macroinvertebrate taxa that equally contributed to community composition (Table 8). Indeed, the ANOSIM analysis did not detect significant differences in macroinvertebrate community structure between these lake types. Several species were relatively important in these lake types while they were absent from acid lakes (e.g., the ephemeropterans *Caenis horaria*, *C. lucturosa*, *Centroptilium luteolum* and *Ephemera vulgaris*, the coleopteran *Oulimnius troglodytes-tuberculatus* or the plecopteran *Nemoura avicularis*, and several dipterans). Other species were exclusively found in acid lakes; however, each comprised a relatively low individual share in community structure, e.g. the chironomids *Phaenospectra* sp., *Stenochironomus* sp. and unidentified Tanypodinae, the trichopterans *Cyrnus insolutus* and *Holocentropus* sp., the odonate *Erythromma najas*, and the coleopteran *Hygrotus* sp. Yet other species were more or less indifferent and occurred in all three lake types, e.g. the bivalve *Pisidium* sp., the chironomid *Psectrocladius* sp., the ephemeropteran *Leptophlebia vespertina*, water mites (Hydracarina, Hydrachnida), and the isopod *Asellus aquaticus* (Crustacea).

Results of the Pearson correlation analysis of water chemistry variables and littoral macroinvertebrate taxa with the NMDS dimensions are shown in Table 9. The positive correlation of phosphate and ammonium-N concentrations with NMDS 1 suggests that limed and circumneutral lakes had higher nutrient concentrations than acid lakes. By contrast, the analysis suggests that acid lakes were characterized by higher Si contents, indicated by the negative correlation of Si with NMDS 1. All lakes were separated with regard to Cl concentrations along NMDS 2, which suggests that all lakes generally had higher Cl concentrations during earlier years of the study. Conspicuously, many water chemistry variables indicative of acidity/acidification (e.g., pH, alkalinity) were not significant in the correlation analysis with littoral macroinvertebrate communities.

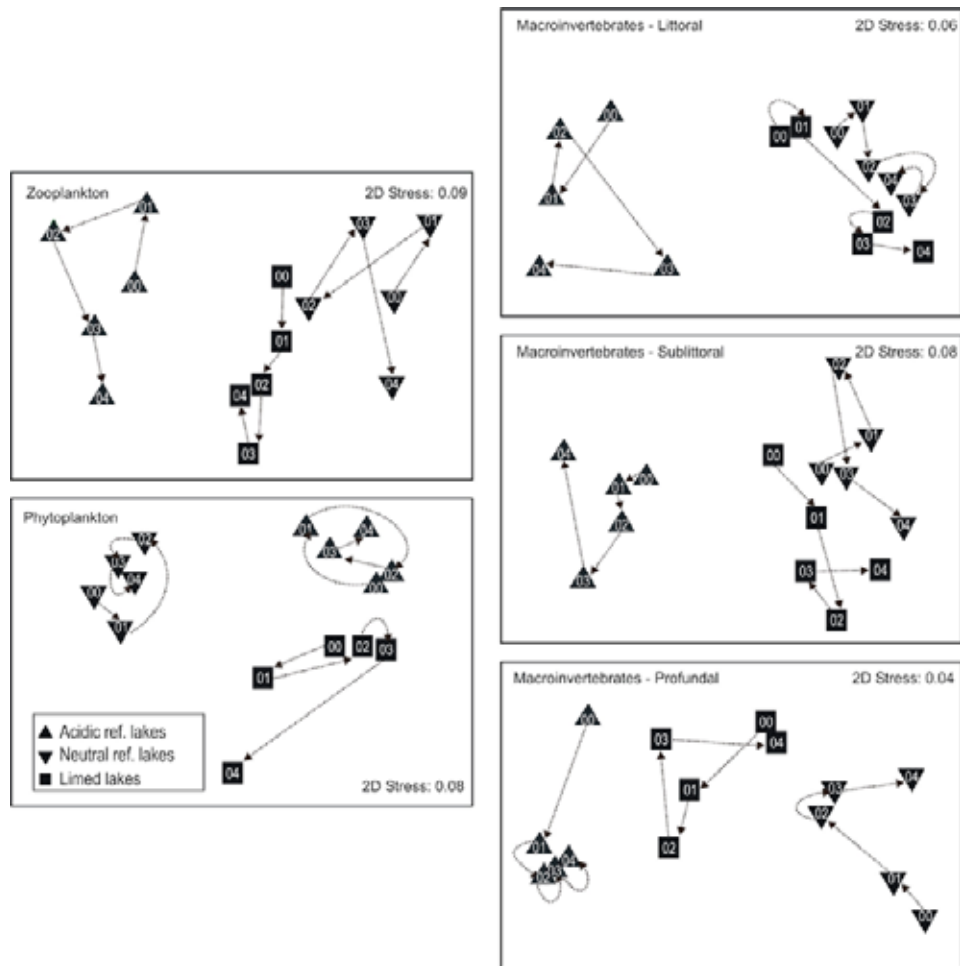
TABLE 7. Pearson correlations between NMDS dimensions and environmental characteristics, zooplankton taxa, community metrics and zooplankton functional groups. Correlation coefficients and corresponding P values are given.

* P < 0.05, ** P < 0.001, *** P < 0.001.

	MDS 1	MDS 2
Environmental variables		
Fluoride	0,90***	
pH	0,77***	
K	0,74**	
Cl	-0,57*	
NO2-N + NO3-N	-0,76***	
SO4		0,68**
Mg		0,63*
Alkalinity		-0,54*
Ca		-0,54*
PO4-P		-0,52*
Taxa		
<i>Daphnia cristata</i>	0,87***	
<i>Daphnia cucullata</i>	0,70**	
<i>Daphnia</i> sp.	0,83***	
<i>Holopedium gibberum</i>	0,92***	
<i>Kellicottia longispina</i>	0,55*	0,58*
<i>Limnoscia frontosa</i>	0,70**	
<i>Polyarthra vulgaris</i>	0,72**	
<i>Ceriodaphnia quadrangula</i>	-0,77***	
<i>Kellicottia bostoniensis</i>	-0,70**	
<i>Trichocerca capucina</i>	-0,82***	
<i>Polyarthra remata</i>	-0,57*	0,63*
<i>Eubosmina coregoni</i>		0,77*
<i>Conochilus unicornis</i>		0,61*
<i>Diaphanosoma brachyurum</i>		0,73**
<i>Diaptomus graciloides</i>		0,62*
<i>Keratella cochlearis f. typica</i>		0,59*
Community metrics		
Species richness	0,60*	
Functional groups		
Biomass filter feeders	0,52*	0,75*
Biomass predators	-0,59*	

This contrast with the analyses for phytoplankton, zooplankton and sublittoral and profundal macroinvertebrates, and highlights that other factors than acidity/acidification alone can be relevant for structuring the communities in boreal lakes. Stendera and Johnson (2008) made similar observations regarding the recovery of benthic communities from anthropogenic acidification. However, rather than in littoral

FIGURE 9. Nonmetric multidimensional scaling ordinations showing temporal trends of phytoplankton, zooplankton, and macroinvertebrate (in littoral, sublittoral and profundal habitats) communities in acid reference lakes, circumneutral lakes and limed lakes between 2000 [00] and 2004 [04]. Note that the ordinations are based on biomass values of individual taxa, except for littoral macroinvertebrates, which are based on abundance



habitats, they found community responses to be uncoupled from acidification in profundal habitats of their study lakes. Correlations of macroinvertebrate taxa with the NMDS dimensions is in good agreement with the SIMPER analysis, with *Endochironomus* sp. and *Sialis lutaria* seemed to be representative of acidic lakes, while *Oulimnius* sp. and *Mystacides longicornis/nigra* apparently being more abundant in limed and circumneutral lakes than in acid lakes. The positive correlation of several species with NMDS 2 is a consequence of the fact that these species were more abundant during earlier years of the study, as the distribution patterns of study years in the NMDS ordinations indicate (Figure 9).

The NMDS ordination of sublittoral macroinvertebrates is similar to that obtained for littoral macroinvertebrates (Figure 9) with the communities in limed and natural lakes clustering close together. However,

as indicated by the ANOSIM analysis, these sublittoral communities were significantly different. The SIMPER analysis showed that circumneutral and limed lakes had a relatively high biomass of *Pisidium* sp. (Mollusca, Bivalvia), *Asellus aquaticus* (Isopoda, Crustacea) and *Ephemera vulgata* (Ephemeroptera) (Table 10). On the other hand, the higher relative biomass of *Cyrmus flavidus* and *Sialis lutaria* in the sublittoral of acid lakes is in good agreement with the results found for the littoral macroinvertebrate communities. Conspicuous is the massive occurrence of the predatory dipteran *Chaoborus flavicans* in acid lakes and limed lakes (Table 10).

The correlation analysis of NMDS dimensions with water chemistry variables and community structural and functional metrics is in agreement with observations made for phytoplankton and zooplankton communities. Water quality variables related to

TABLE 8. Results of SIMPER (Similarity Percentages) analysis, showing the percentage of littoral macroinvertebrate species contributing to community structure (based on their abundance) in acid reference lakes, circumneutral reference lakes, and limed lakes.

Species	Acid	Circumneutral	Limed
Diptera			
<i>Ablabesmyia longistyla</i>		0.71	1.41
<i>Ablabesmyia monilis</i>			0.55
<i>Ceratopogonidae</i>	2.1	2.77	3.9
<i>Cladopelma sp.</i>		0.6	0.56
<i>Cladotanytarsus sp.</i>	2.37	3.92	2.12
<i>Cricotopus sp.</i>	0.99	0.69	1.24
<i>Demicryptochironomus vulneratus</i>		0.97	0.52
<i>Dicrotendipes sp.</i>	3.6	1.64	1.45
<i>Empididae</i>			0.65
<i>Endochironomus sp.</i>	2.23	1.57	0.96
<i>Epoicocladus ephemeræ</i>			0.6
<i>Glyptotendipes sp.</i>		0.57	
<i>Heterotanytarsus apicalis</i>			0.43
<i>Lauterborniella agrayloides</i>	1.46	1.73	
<i>Microtendipes sp.</i>			0.8
<i>Pagastiella orophila</i>	0.83	2.02	1.79
<i>Parakiefferiella sp.</i>		0.64	0.54
<i>Paramerina sp.</i>	2.83	0.58	1.16
<i>Paratanytarsus sp.</i>		2.09	1
<i>Phaenopsectra sp.</i>	0.76		
<i>Polypedilum breviantennatum</i>	0.98		0.56
<i>Polypedilum sp.</i>			0.41
<i>Procladius sp.</i>	3.51	2.1	2.4
<i>Psectrocladius sp.</i>	8.62	4.03	5.35
<i>Pseudochironomus prasinatus</i>	1.23	1.41	0.75
<i>Stenochironomus sp.</i>	0.67		
<i>Tanypodinae, unidentified</i>	0.56		
<i>Tanytarsus sp.</i>	3.9	4.69	3.55
<i>Thienemannimyia sp.</i>	1.32		1.44
Trichoptera			
<i>Cyrnus flavidus</i>	2.14	1.35	0.88
<i>Cyrnus insolutus</i>	1.21		
<i>Cyrnus trimaculatus</i>			1.23
<i>Ecnomus tenellus</i>		0.94	
<i>Holocentropus sp.</i>	0.72		
<i>Hydroptila sp.</i>			1.98
<i>Lepidostoma hirtum</i>	0.51	1	
<i>Limnephilus sp.</i>	0.91		0.41
<i>Limnephilidae unidentified</i>	1.31		0.4
<i>Molannodes tinctus</i>			0.5
<i>Mystacides azurea</i>		1.03	1.6
<i>Mystacides longicornis/nigra</i>	0.87	1.09	1.58
<i>Oecetis testacea</i>			0.85

TABLE 8. Continued.

Species	Acid	Circumneutral	Limed
<i>Oxyethira</i> sp.	0.67	2.02	0.6
<i>Tinodes waeneri</i>		0.5	
Ephemeroptera			
<i>Caenis horaria</i>		3.9	4.23
<i>Caenis luctuosa</i>		4.5	5.42
<i>Centroptilum luteolum</i>		1.79	1.18
<i>Cloeon dipterum</i>	1.48	2.28	2.01
<i>Ephemera vulgata</i>		0.65	2.31
<i>Kageronia fuscogrisea</i>	4.63	1.74	3.15
<i>Leptophlebia marginata</i>	0.59	2.93	2.8
<i>Leptophlebia vespertina</i>	14.4	6.78	6.88
Hydrachnida			
<i>Argyroneta aquatica</i>	0.53	0.78	
<i>Hydracarina</i>	4.11	1.83	1.65
Crustacea			
<i>Asellus aquaticus</i>	12.77	8.66	7.64
Annelida			
<i>Erpobdella octoculata</i>		1	
Odonata			
<i>Erythromma najas</i>	0.57		
<i>Platycnemis penn.-Pyrrhosoma nymph.</i>		0.57	
Zygoptera		0,99	
Mollusca			
<i>Gyraulus albus</i>			1.36
<i>Pisidium</i> sp.	2.5	4.62	5.39
Coleoptera			
<i>Hygrotus</i> sp.	0.76		
<i>Oulimnius</i> sp.		0.5	
<i>Oulimnius troglodytes-tuberculatus</i>		0.79	0.81
Heteroptera			
<i>Micronecta</i> sp.		2.69	
Plecoptera			
<i>Nemoura avicularis</i>		0.88	0.55
Megaloptera			
<i>Sialis lutaria</i>	1.62	0.85	0.57
Plathelminthes			
<i>Turbellaria</i>		0.74	

acidity reflecting the distribution of lakes types in the ordinations. For example, alkalinity, and concentrations of Ca, K, oxygen and F correlated positively with NMDS dimension 1, indicating that circumneutral lakes and limed lakes have higher values of these variables in comparison with acid lakes which had a higher amount of Na, Cl and nitrogen compounds

(negative correlation of these variables with NMDS 1; Table 11). Also with this community, the results from the correlation analysis are in good agreement with the SIMPER analysis regarding the distribution of taxa. Species with negative correlations with NMDS 1 (e.g. *Cyrnus flavidus*, *Sialis lutaria*) seemed to have relatively higher biomasses in acid lakes,

TABLE 9. Pearson correlations between NMDS dimensions and environmental characteristics, littoral macroinvertebrate taxa. Shown are correlation coefficients and corresponding P values. * P < 0.05, **, P < 0.001, *** P < 0.001. Community metrics and functional groups were not significant.

	MDS 1	MDS 2
Water quality variables		
PO4-P	0,83***	
NH4	0,54*	
Si	-0,58*	
Cl		0,52*
Taxa		
<i>Endochironomus sp.</i>	-0,61*	
<i>Sialis lutaria</i>	-0,56*	
<i>Oulimnius sp.</i>	0,58*	
<i>Mystacides longicornis/nigra</i>	0,67**	
<i>Erythromma najas</i>		0,70**
<i>Paramerina sp.</i>		0,63*
<i>Procladius sp.</i>		0,61*
<i>Dicrotendipes sp.</i>		0,58*
<i>Cyrrus flavidus</i>		0,56*

while species that positively correlated with NMDS1 (e.g. *Asellus aquaticus*, *Ephemera vulgata*) seemed more abundant in circumneutral and limed lakes. Interestingly, *Chaoborus flavicans*, which strongly contributed to the biomass of sublittoral macroinvertebrates in acid and limed lakes correlated negatively with NMDS 2. This suggests that this species was

particularly abundant during the study years 2003 in acid lakes and during 2002–2004 in limed lakes (Figure 9). The latter shows how large interannual variation in species biomass/abundance can affect the analysis.

NMDS dimensions of the sublittoral macroinvertebrate community ordination also showed significant correlations with structural community metrics and functional feeding groups (Table 11). These correlations are in good agreement with the results obtained from the univariate analysis (Figure 7). Shannon-Wiener biodiversity seemed to be higher in circumneutral and limed lakes (positive correlation with NMDS 1), while total biomass and species richness was higher in acid lakes (negative correlations with NMDS 1) (Table 11). Also with regard to functional groups, the biomass of filter feeder was higher in limed lakes (positive correlation with NMDS 1 and negative correlation with NMDS 2), whilst the biomass of predators and detritivores was higher in acid lakes (Figure 7, Table 11).

Finally, profundal macroinvertebrate community structure also showed a clear separation by lake type, with limed lakes occupying an intermediate position between acid and circumneutral lakes in the ordination (Figure 9). SIMPER analysis revealed a high contribution of *Chaoborus flavicans* to the profundal benthos communities in all lake types, while *Pisidium* also contributed to explain community structure in circumneutral lakes (Table 12). Despite *Chaoborus* also being dominant in limed lakes, other rare species (*Pisidium* sp, Hydracarina), that do not appear due to the 90%-cutoff level applied in statistical analysis (see

TABLE 10. Results of SIMPER (Similarity Percentages) analysis, showing the percentage of sublittoral macroinvertebrate species contributing to community structure (based on their abundance) in acid reference lakes, circumneutral reference lakes, and limed lakes.

Species	Acid	Neutral	Limed
Mollusca			
<i>Pisidium sp.</i>		27.36	24.51
Crustacea			
<i>Asellus aquaticus</i>		11.04	9.33
Trichoptera			
<i>Cyrrus flavidus</i>	12.88		2.18
<i>Molanna angustata</i>			1.99
<i>Ephemeroptera</i>			
<i>Ephemera vulgata</i>		21.2	11.06
Diptera			
<i>Chaoborus flavicans</i>	59.63	18.34	42.81
Megaloptera			
<i>Sialis lutaria</i>	21.92	12.66	

TABLE 11. Pearson correlations between NMDS dimensions and environmental characteristics, sublittoral macroinvertebrate taxa, community metrics and sublittoral macroinvertebrate functional groups. Shown are correlation coefficients and corresponding P values.

* P < 0.05, ** P < 0.001, *** P < 0.001.

	MDS 1	MDS 2
Water quality variables		
F	0,96***	
Alkalinity	0,75***	
Ca	0,61**	
K	0,59*	
O2	0,58*	
NO2-N + NO3-N	-0,58*	
Na	-0,76***	
Cl	-0,77***	
Mg		0,59*
Taxa		
<i>Asellus aquaticus</i>	0,69**	-0,52*
<i>Ephemera vulgata</i>	0,62*	-0,53*
<i>Cyrrus flavidus</i>	-0,62*	
<i>Sialis lutaria</i>	-0,65**	
<i>Chaoborus flavicans</i>		-0,58*
Community metrics		
Shannon-Wiener diversity	0,72**	
Species richness	-0,62*	
Total biomass	-0,72**	-0,65**
Functional groups		
Filter feeders	0,70**	-0,57*
Predators	-0,76***	
Detritivores	-0,90***	

methods section), have contributed to explain some 8% of community structure.

The correlation analysis of NMDS dimensions with environmental variables, profundal macroinvertebrate taxa, community structural attributes and functional guilds shows both similarities and differences with regard to the macroinvertebrate communities in shallower habitat types (littoral and sublittoral). The correlation of *Pisidium* and *Chaoborus* are in good agreement with the results from the SIMPER analysis. Also the correlation of structural community metrics agrees with the results observed in the sublittoral benthos community with Shannon-Wiener diversity being higher in circumneutral lakes and limed lakes (positive correlation with NMDS 1), while total species richness and total biomass was higher in acid lakes (negative correlation with NMDS 2). Also with regard to functional groups the higher biomass of filter feeders in circumneutral and limed lakes and

TABLE 12. Results of SIMPER (Similarity Percentages) analysis, showing the percentage of profundal macroinvertebrate species contributing to community, based on their biomass.

Taxa	Acid	Neutral	Limed
Mollusca			
<i>Pisidium sp.</i>		14.91	
Diptera			
<i>Chaoborus flavicans</i>	99.95	85.01	92.02

TABLE 13. Pearson correlations between NMDS dimensions and environmental characteristics, profundal macroinvertebrate taxa, community metrics and profundal macroinvertebrate functional groups. Shown are correlation coefficients and corresponding P values.

* P < 0.05, ** P < 0.001, *** P < 0.001.

	MDS 1	MDS 2
Water quality variables		
F	0.86***	
K	0.73**	
pH	0.69**	
NO2-N+NO3-N	-0.83***	
Conductivity		0.55*
Taxa		
<i>Pisidium sp.</i>	0.81***	
<i>Chaoborus flavicans</i>	-0.93***	
Community metrics		
Shannon-Wiener diversity	0.59*	0.53*
Species richness	-0.53*	
Total biomass	-0.64**	-0.72**
Functional groups		
Detritivores	0.86***	
Filter feeders	0.82***	
Grazers	0.81***	
Predators	-0.94***	

the higher biomass of predators in acid lakes agree with the results from the analysis of sublittoral communities. The only notable difference is the positive correlation of detritivores which seem to be higher in circumneutral lakes, thereby contrasting with the lower biomass found in the sublittoral of acidic lakes. Notwithstanding, the overall results of the multivariate analysis of profundal macroinvertebrates matches again well the results from the univariate analysis.

PART 2: ANALYSIS OF FOOD WEB STRUCTURE USING STABLE ISOTOPES

We used natural stable isotopes to estimate the sources of carbon and nitrogen assimilated by different trophic levels in benthic and pelagic food webs of limed, acid and circumneutral lakes. Analysis of carbon stable isotopes is frequently carried out to discriminate between carbon originating from different sources (Peterson and Fry, 1987; Post, 2002). The basis is that physical and biological turnover of elements affects the proportions of light and heavy isotopes. Both photosynthesis and diffusion in water reduce the carbon isotope ($^{13}\text{C}/^{12}\text{C}$) compared to atmospheric CO_2 . Consequently, the lowest ratio is found among pelagic primary producers and less reduced ratios in terrestrial primary production. Because both enzymatic metabolism and excretion discriminate against the heavy nitrogen isotope, the nitrogen ratio ($^{15}\text{N}/^{14}\text{N}$) will increase with trophic level in the food chain. The analyses of the stable isotope composition of C and N are time-integrated representations of the food sources assimilated by the consumers (Lajtha and Michener, 1995; Vander Zanden et al. 1999). Standard fractionation with trophic level are about 1 ‰ for C and about 3.4 ‰ for N (Peterson and Fry, 1987; Cabana and Rasmussen, 1994), but several studies have shown that these values show large variation (see review by Vander Zanden and Rasmussen 2001).

Material and methods

Study design

A subset of nine monitoring lakes, three each of limed lakes and acid and circumneutral reference lakes (Table 14), respectively, were visited three times during 2006–2007 (in summer, fall, and spring) to collect samples of seston, epilithic biofilms, zooplankton, benthic invertebrates, and fish for stable isotope (SI) analyses. Life samples were filtered/sorted and snap-frozen in the field.

Seston samples (<65 μm) were collected by filtering (GF/C, precombusted) a mixed epilimnetic sample, or a whole water-column sample during unstratified conditions. An appropriate volume of water was filtered in the field, but on a few occasions, due to extreme weather conditions, this was done in the lab within 8 hours after sampling. Filters containing seston were wrapped in aluminum-foil and snap frozen in liquid nitrogen. A subsample of the water was fixed in Lugol's solution for taxonomic analyses. Additionally, humic and fulvic acids (collectively referred to as dissolved organic matter; DOM) were extracted from a 25-L mixed epilimnetic sample, or

a whole water-column sample during unstratified conditions. DOM in this water sample was trapped by adding 1 g/L of DAEA-cellulose (diethylaminoethylcellulose, Aldrich) during repeated agitation for at least one hour. During this time DOM sorbes to the DAEA-cellulose, resulting in a clear overlying water phase. DAEA was then allowed to settle and regained on a paper filter, and stored cool (2–3 °C) until desorption in the lab. After a washing step (deionized water) desorption of DOM was accomplished by adding 0.3 M NaOH and subsequent filtration to remove the DAEA. Desorbed, concentrated DOM was collected, frozen, and lyophilized (i.e. the material is rapidly frozen and dehydrated).

Samples of epilithic biofilms were collected by brushing the surface of 3–5 stones collected from the littoral zone with minor additions of ambient lake water in a tray. The suspension of epilithic biofilms was transferred to 50-mL polyethene flasks and frozen in dry ice. A subsample was preserved in 70% ethanol for taxonomic analysis of algae.

Samples of littoral macroinvertebrates were collected with hand nets (0.5 mm-mesh) in stony habitat (0–1 m depth) and among the sparse vegetation. Individuals of dominant taxa were sorted, identified to the lowest possible taxonomic unit (i.e. species, genus or in some cases family-level), transferred to cryonic vials, and snap-frozen in liquid nitrogen. Profundal invertebrates were sampled from the deepest part of the lake using an Ekman grab and subsequent sieving (0.5 mm). Also here several individuals of abundant chironomid taxa and the phantom midge *Chaoborus flavicans* were transferred to cryonic vials and frozen as described above.

Fish samples were obtained either from ongoing fish-monitoring programs run by the Swedish Fisheries Board, or from own sampling trips. From fish collected from monitoring programs, a sample of dorsal muscle was dissected in the field and frozen within hours in cryonic vials. Coding of the vials guaranteed linking to fish data (species, age, length, weight, etc.). Fish samples collected during our own sampling events consisted of whole individuals that were frozen in the field. From selected individuals a sample of dorsal muscle was prepared from partly thawed fish preventing the loss of water and effects in stable isotope composition (e.g. Feuchtmayr and Grey 2003). Samples of Perch (*Perca fluviatilis*) were collected for three different size classes, i.e. 4–6 cm, 8–12 cm, and >15 cm, to reflect the species' ontogenetic feeding shifts from planktivorous to benthivorous, and further to piscivorous size classes. Unfortunately, no fish samples were collected in Lake Storås.

TABLE 14. Lake and predominant catchment characteristics and mean values of selected water chemistry variables for limed lakes (L), acid lakes (A), and circumneutral lakes (C) that were part of the stable isotope study. Abbreviations: Agri=Agriculture; Alk=Alkalinity; TOC,=Total Organic Carbon.

Lake name and coordinates	Lake type	Size (ha)	Catchment (ha)	% Forest	% Agri	% Water	Zmean (m)	pH	Alk (meq/l)	Tot-P (µg/l)	TOC (mg/l)
Stora Härsjön 640364, 129240	L	257	2270	65.4	3.0	23.0	14	6.8	0.195	8	5
Gyltigesjön 629489, 133906	L	40	17200	60.7	7.7	6.0	9.1	6.5	0.108	9	6
Gyslättsjön 633209, 141991	L	32	280	70.4	6.2	10.3	2.8	7.7	0.178	15	12
Älgarydssjön 633989, 140731	A	32	345	86.9	3.0	9.3	1.6	5.5	-0.002	18	15
Rotehogtjärnen 652902, 125783	A	16	380	93.2	0.5	4.4	3.6	5.5	0,0004	14	12
Storasjö 631360, 146750	A	35	212	83.2	0.0	16.7	3.5	5,4	-0.003	17	8
Fiolen 633025, 142267	C	155	548	40.2	16.0	28.2	3,9	6.5	0,05	11	6
Fräcksjön 645289, 128665	C	27	398	88.5	0.0	6.7	4.1	6,4	0.06	10	8
St. Skärsjön 628606, 133205	C	33	246	86.3	0.0	13.5	3,9	6.8	0.11	8	5

For stable isotope analyses, all samples were lyophilized and appropriate amounts of dry weight were transferred to tin capsules, packed, and sent off to the stable isotope facility at UC-Davies (California, USA). Note that composite samples were analyzed for invertebrates, while muscle tissue collected from single individuals of fish were analyzed. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, as well as contents of nitrogen and carbon were analyzed on a gas chromatograph coupled to a mass spectrometer. $\delta^{13}\text{C}$ values are reported relative to the V-PeeDee Belemnite (PDB, limestone) Standard, i.e. $\delta^{13}\text{C}$ in ‰, is the deviation of the isotopic ratio of the sample from that of the standard; $\delta^{13}\text{C}$ (‰) = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where $R = {}^{13}\text{C}/{}^{12}\text{C}$. A positive $\delta^{13}\text{C}$ value means that the sample has more of the heavier isotope (${}^{13}\text{C}$) than the standard. $\delta^{15}\text{N}$ in ‰, is the deviation of the isotopic ratio of the sample from that of the atmospheric nitrogen standard; $\delta^{15}\text{N}$ (‰) = $(R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where $R = {}^{15}\text{N}/{}^{14}\text{N}$.

Classification of functional groups and food webs

We discerned between the benthic and the pelagic trophic pathways, to assess whether liming impacts food web structure and trophic relationships in contrasting lake habitats. These pathways were treated separately, based on the evidence that these food webs are separated in terms of their carbon flows (France 1995). In order to circumvent problems

associated with the quantification of algal or detrital food resources and provide better predictions of the trophic position of organisms, molluscan primary consumers have been suggested as baseline indicator organisms in stable isotope studies (e.g. Post, 2002; Vadeboncoeur et al., 2003). However, as these taxa are largely absent from the lake types studied here we used epilithic biofilms, seston (<65 µm) to characterize the base of the food webs. These trophic pathways were based on the compartments outlined below. We wish to point out that temporal variability in species occurrences and the lack of acid sensitive taxa in acid and limed lakes (i.e. differences among communities, see also part 1) in some cases limit statistical comparisons of lake categories.

Benthic food web:

EPILITHON: Algae, protozoa, fungi, bacteria and other microbes growing on the surfaces of stones were considered to build the base of the benthic food web.

GRAZERS: This trophic level represents the “herbivores” in the benthic food web. We used the isotope signatures of the mayflies *Heptagenia fuscogrisea* and *Heptagenia* sp. (Ephemeroptera) which are expected to feed on epilithon. Although a few specimens of these taxa were found in acidic lakes because of their acid sensitivity, these were the only grazer species available for the comparative study.

SHREDDERS: This functional feeding group is expected to convert coarse organic matter into finer fractions of organic matter. The crustacean *Asellus aquaticus* is an acid-tolerant species, and it could be sampled in all study lakes. Leaf litter serves as a food source of this species and we used the stable isotope fractionation values provided by Bohman (2005) for assessing the importance of leaf litter as a food source in the benthic food web.

SHREDDERS/GRAZERS: This group is comprised of species that share functional characteristics of grazers and shredders, and which can be regarded to graze either on epilithon or to transform coarse organic matter. For this study, Limnephilidae were well represented in all lake categories and were used to represent this group of broader feeding plasticity.

INVERTEBRATE PREDATORS: Members of this group prey on other invertebrates. In this study we used larvae of *Sialis lutaria* (Megaloptera), *Cordulia aenea* (Odonata), *Aeshna* sp. (Odonata), *Erythromma najas* and other unidentified Zygoptera, and predatory leeches (Hirudinea).

VERTEBRATE (FISH) PREDATORS: In this study we considered benthivorous perch (*Perca fluviatilis*) as the top predator in the benthic food web. Size classes of 8-12 cm of perch are known to feed preferentially on benthic invertebrates (Quevedo and Olsson 2006).

Pelagic food web:

DISSOLVED ORGANIC MATTER (DOM) AND SESTON: In this study we measured the stable isotope signatures of dissolved organic matter. Humic matter can be “channeled” through the microbial loop to consumers at higher trophic levels (herbivorous zooplankton, invertebrate and fish predators). Seston (< 65 µm), by contrast, can be assimilated directly by grazers, which themselves serve as food source for invertebrate and fish predators. Seston and DOM can therefore be regarded as the base of the pelagic food web.

INVERTEBRATE PREDATORS: The phantom midge (*Chaoborus flavicans*; Diptera) was well represented in all lakes, and its intermediate position in the pelagic food web allows us to assess whether energy from the food web base (dissolved organic matter and seston) is transferred to top predators.

PLANKTIVOROUS FISH: In this study we considered perch fry (young life history stages; 4-6 cm size class [Magnus Dahlberg, The Swedish Board of Fisheries, personal communication]) and roach (*Rutilus rutilus*) to prey preferentially on zooplankton. Unfortunately, the occurrence of perch fry was highly heterogeneous between lakes and no data were available for

the acidic Storasjö, limiting the comparison between lake categories.

PISCIVOROUS FISH: Pike (*Esox lucius*) and adult perch (body lengths > 15 cm) comprised the top predators in the pelagic food webs. As was the case with perch fry, the occurrence of individuals of predatory fish varied substantially even within a single lake category and no data were available for Storasjö, thereby limiting comparisons between acid, circumneutral and limed lakes.

Statistical analyses

We used the Wilcoxon test to compare the delta N and delta C fractionation in the different trophic levels composing the benthic and the pelagic food webs between limed, circumneutral and acid lakes. We pooled data from the three sampling periods because the food web bases (epilithon in the benthic food web, and seston in the pelagic food web) were not significantly different between sampling dates. Furthermore, pooling of the data was necessary because species of selected higher trophic levels showed strong temporal variability, i.e. were missing from our samples due to ontogenetic reasons (e.g. small life stages that were not caught by our hand nets). Some size classes of macroinvertebrate species and fish were present in very low abundances during some of the samplings or absent from some lakes (particularly acid-sensitive taxa in acid lakes). Although, temporal within-taxa variability in stable isotope composition may occur in selected macroinvertebrates (e.g. Bohman 2005), we assume that environmental constraints inherent to each lake category ultimately dictate the feeding behavior of organisms in the longer term. Thus, if liming as a mitigation measure significantly affects lake ecosystem structure and function, variability among lake categories should override temporal variability within a set of specific lakes. Hence we assume that food web structure in limed, acid and circumneutral lakes can be depicted in a confident way.

Results and Discussion

A number of recent studies have claimed that corrections of the isotopic signal (i.e. $\delta^{13}\text{C}$) are needed for lipid-rich tissue or whole animals in order to make sound interpretations of stable isotope data, in particular concerning the origin of their food (Mateo et al. 2008, Logan et al. 2008). The reason behind this is that lipids have more negative $\delta^{13}\text{C}$ -values than proteins due to kinetic isotope effects that occur during lipid synthesis. Variability in lipid content of tissue or whole animals may affect $\delta^{13}\text{C}$ and can thus erroneously be interpreted as habitat shifts or food sources

originating from terrestrial and aquatic sources, respectively. This effect is most pronounced for lipid-rich tissues in fish (e.g. liver) and for zooplankters that are relatively rich in lipids. The atomic C/N ratio of samples, analyzed simultaneously with carbon and nitrogen isotopes, have been suggested as a proxy for the lipid contents of samples. However, Logan et al. (2008) conclude that the C/N ratio accounts for less of the variation in invertebrate samples, with $\delta^{13}\text{C}$ signatures increasing only slightly more than 1 ‰ upon lipid extraction for samples with bulk C/N between 4 and 8 (their Figure 4). In our study, samples of fish tissue had C/N ratios that were consistently lower than 4.1, while the 90-percentile for C/N ratios of invertebrate samples was 7.9. For these reasons we have chosen to present uncorrected data in this report.

Benthic food web

In general epilithon, grazers, grazers/shredders, and benthivorous perch showed similar $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in acid, limed and circumneutral lakes (Figure 10), which suggests that their carbon sources and trophic structure are not affected by liming. Significant differences were detected only for shredders and invertebrate predators that showed a significantly lower $\delta^{15}\text{N}$ in acid lakes than in limed and circumneutral lakes, respectively (Figure 10). This suggests that these functional groups occupy lower trophic positions in the food web of acid lakes compared with circumneutral and limed lakes. In other words, there is less trophic fractionation (fewer intermediate trophic levels) below shredders and invertebrate predators in acid lakes. This may be a result of the inhibition of microbial degradation of leaf litter and the disappearance of small, acid-sensitive invertebrates at intermediate trophic levels. Also predators showed $\delta^{13}\text{C}$ values that were unique to each lake category, with the lowest $\delta^{13}\text{C}$ values found in limed lakes and the highest in acid lakes. This suggests that invertebrate predators in limed lakes rely more on lake internal carbon production, while those in acid lakes depend more on terrestrial sources of carbon. The observation that there is substantial C-fractionation in limed and acid lakes whereas all trophic levels are in the range of -27 to -29 in circumneutral lakes is also interesting. These differences are likely a consequence of the fact that our acid and limed lakes were more humic-rich and thus presumably more heterotrophic, resulting in a relatively larger assimilation of respired carbon (e.g. del Giorgio and Peters 1994). Despite these differences between lake types, the results suggest that liming does not have systematic effects on the stable isotope ratios of taxa within functional guilds in benthic habitats.

Also other functional feeding groups in acid lakes tended to have lower $\delta^{13}\text{C}$ values in acid lakes than in the other lake categories, although the differences were not significant ($P > 0.05$). However, these patterns are not a consequence of the acid status of lakes in general, but rather strongly dependent on the catchment characteristics of the different study lakes. For example, organisms belonging to the same functional feeding group or similar taxa in Lake Älgarydssjön had substantially more negative $\delta^{13}\text{C}$ values than their counterparts in the other two acidic lakes, Rotehogstjärnen and Storasjö. Organisms belonging to the same functional feeding groups or taxonomic units in the latter lakes showed $\delta^{13}\text{C}$ values that were similar to those in limed and circumneutral lakes. These differences are likely a consequence of the fact that the highly humic Lake Älgarydssjön has a food web where assimilation of respired carbon (by microbes) is quantitatively more important than in the other lakes. The assimilation of respired carbon implies several fractionation steps, resulting in more depleted (more negative) $\delta^{13}\text{C}$ values (e.g. del Giorgio and Peters, 1994). Differences in the degree of assimilation of respired carbon are also apparent for $\delta^{13}\text{C}$ signatures in the epilithic samples, which tended to be higher in neutral lakes than that in the other lake types. This is probably due to a higher incorporation of inorganic carbon from catchment erosion (HCO_3^-) in these lakes (that have a higher alkalinity) and a lower degree of assimilation of carbon originating from lake internal processes (i.e. respiration). Repeated liming should theoretically result in higher $\delta^{13}\text{C}$ values, as the $\delta^{13}\text{C}$ of limestone is zero. However, the extent of this cannot be extracted from our results.

Several interesting patterns emerge from a schematic benthic food web using isotopic data (Figure 12). Most notably are the low $\delta^{15}\text{N}$ values for grazers, being in the same range or lower than $\delta^{15}\text{N}$ values for their presumed food source, epilithon. These data suggest a very low degree of isotopic fractionation, i.e. the difference between consumers and their food source, between epilithon and grazers. Isotopic fractionation between epilithon and invertebrate grazers was much less than the frequently assumed $\delta^{15}\text{N}$ of 3.4‰ across trophic levels (Minagawa and Wada, 1984). Indeed high food-quality resources may be transferred to grazer/detritivores with only low N-fractionation (e.g. Vanderklift and Ponsard 2003, Goedkoop et al. 2006). However, it must be emphasized that only a single sample for grazers was available for Lake Älgarydssjön, a sample that may not be representative of the other acid lakes. This calls for some caution in the interpretation of the results.

FIGURE 10. Stable isotope signatures (means \pm 1 SE) of compartments of the littoral benthic food web (i.e. epilithon, invertebrate grazers, grazers/shredders, shredders and predators, as well as benthivorous perch) in limed, circumneutral and acid lakes. Significant differences (Wilcoxon test) in a pairwise comparison are highlighted (p values are shown). Note the differences in scales.

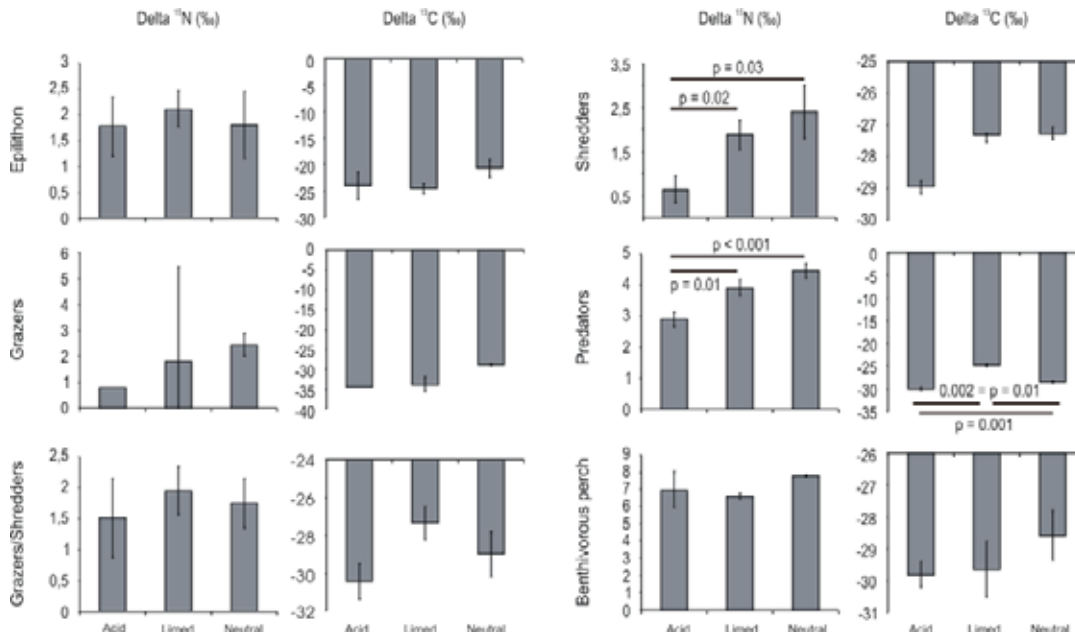
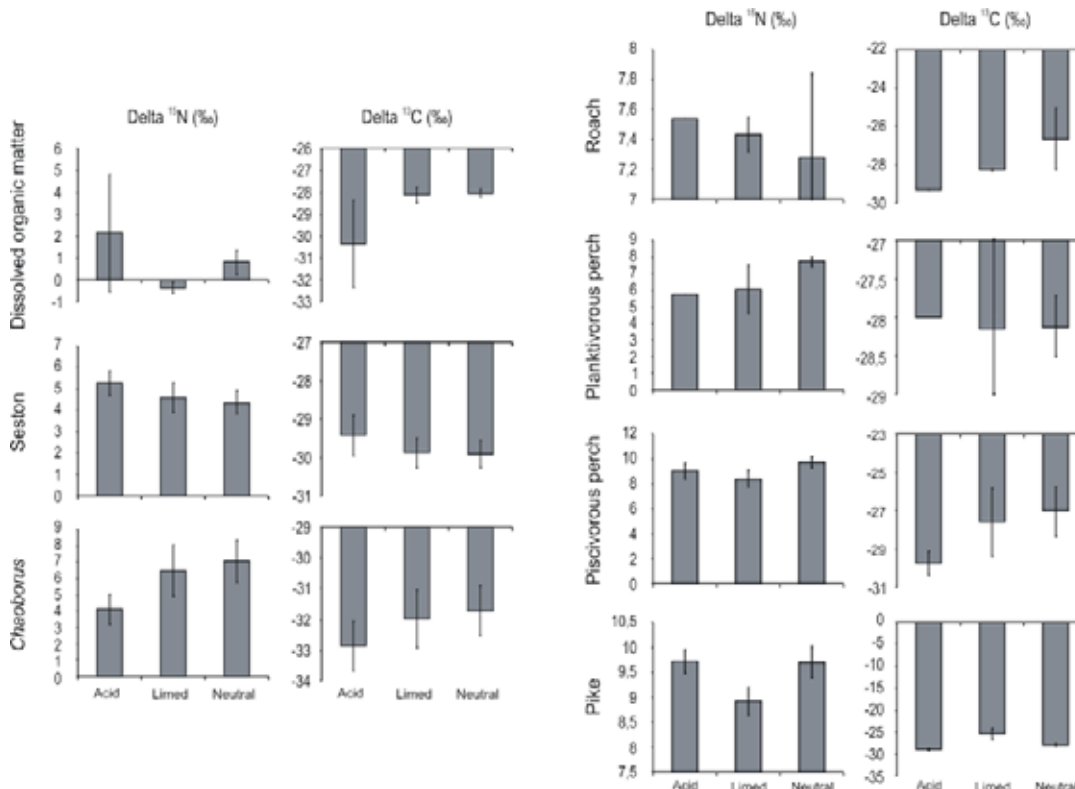


FIGURE 11. Stable isotope signatures (means \pm 1 SE) of compartments of the pelagic food web (i.e. dissolved humic matter, seston, roach, perch and pike) in limed, circumneutral and acid lakes. Note that no significant differences were found in pairwise comparisons (Wilcoxon tests). Note also the difference in scales.



Conspicuous are also the reoccurring patterns of increasing $\delta^{15}\text{N}$ values across gradients of acidification (i.e. acid to limed to circumneutral lakes) for grazers, shredders and invertebrate predators (Figure 10). These patterns show that similar taxonomic or functional groups have lower $\delta^{15}\text{N}$ values in acid lakes than in circumneutral lakes, while limed lakes have an intermediate position. This increase was most pronounced for shredders (i.e. *Asellus aquaticus*) that had a much higher $\delta^{15}\text{N}$ in circumneutral lakes than in acid lakes. The differences may be a consequence of the differences in leaf processing between lake types and/or the utilization of other food sources. For example, the reduced competition due to the disappearance of acid-sensitive taxa (in acid lakes, but partly also in limed lakes) may allow species with a high functional plasticity to feed on alternative food sources. This conjecture is supported by ^{13}C -data showing that shredders (i.e. *Asellus aquaticus*) are more depleted in ^{13}C in acid lakes than in limed and circumneutral lakes, suggesting a larger share of autochthonous benthic algal production in their food in limed and circumneutral lakes (and likely microfauna associated with these biofilms). Indeed, the acid lakes sampled in this study were much more humic than the other lake types, thus causing light-limitation and lower autochthonous production of benthic algae in acid lakes. Moreover, epilithic samples from circumneutral lakes were less depleted in ^{13}C , further supporting the conjecture that alternative food sources were utilized in these lakes. As expected, ^{15}N -enrichment was higher for invertebrate predators and fish, due to repeated fractionation at consecutive lower trophic levels.

Pelagic food web

The various compartments of the pelagic food webs of limed, acid and circumneutral lakes showed different degrees of variability in their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (Figure 11). Partly due to the relatively high variability in some taxonomic and/or functional feeding groups it was not possible to detect significant differences between lakes. For example, for the invertebrate predator *Chaoborus* and fish predators no significant differences were detected as a consequence of the low sample size and high variability within some of the groups (e.g., planktivorous perch and roach in acid lakes). Again, the low sample size was a consequence of a lack of acid sensitive taxa/groups in acid lakes.

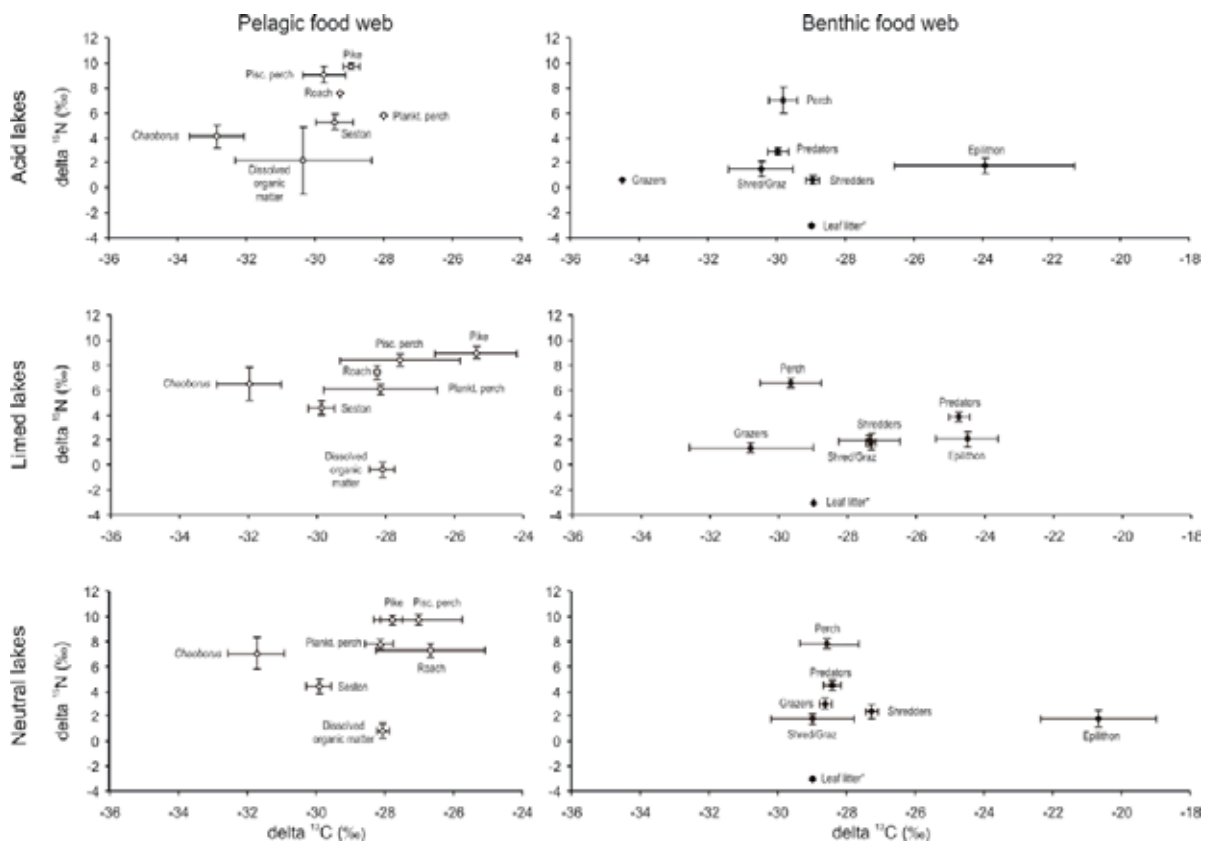
Despite the lack of significant differences among lake categories, the data reveal some interesting trends in stable isotope composition between lake types. For example, at the base of pelagic trophic pathways, isolated dissolved organic matter were more depleted in $\delta^{13}\text{C}$ in acid lakes than in limed lakes and

circumneutral lakes, -30.33 (average) ± 1.98 (SE), -28.11 ± 0.36 and -28.05 ± 0.17 , respectively for the three lake types. These values are in the same range as $\delta^{13}\text{C}$ signals reported for DOM in four nutrient-poor forest lakes in Northern Sweden, range -27.7 to -29.9 (Karlsson et al. 2003). Most depleted in $\delta^{13}\text{C}$ were dissolved organic matter from Älgarydsjön (-34.3), that also caused the high variation in $\delta^{13}\text{C}$ among acid lakes. The reason for the lower $\delta^{13}\text{C}$ values for dissolved organic matter in Lake Älgarydsjön is unclear. Possibly, a relatively large share of exudates from the Raphidophycean *Gonyostomum semen* may have contributed to this observation. Lysis of *Gonyostomum* cells upon handling and addition of the ion exchanger may have contributed to a large pool of algal exudates in the sample obtained from this lake. Algal carbon and thus excreted sugars and polymers will be more depleted in $\delta^{13}\text{C}$ than dissolved organic matter (e.g. Karlsson et al. 2003).

Seston samples in our lakes had $\delta^{13}\text{C}$ values between -29 and -30 and were close to the values for forest lakes reported by Karlsson et al (2003). Seston samples consisted of a mixture of bacteria and algal cells, largely reflecting the size fraction available for zooplankton. Zooplankton samples have been collected, divided into cladocerans, herbivorous copepods and carnivorous copepods, and will be analyzed during the winter of 2009. These samples of zooplankton, although not specifically part of this project, will provide additional information about the $\delta^{13}\text{C}$ signature of the edible fraction of the seston and the transfer of nutrients and energy from seston to the invertebrate predator *Chaoborus* and to planktivorous fish. *Chaoborus* larvae were some 2% more depleted than seston samples, which is in line with an assumed 1% fractionation per trophic level (Petersen and Fry, 1987). Interestingly, *Chaoborus* larvae were much more depleted than the samples of invertebrates, planktivores and other fish (Figures 11 and 12), suggesting a strong link to food sources obtained in the benthic habitat where consecutive fractionations by microbes and small invertebrates provide a ^{13}C -depleted food source (e.g. Grey et al. 2004). Many of the relatively small lakes investigated here, in particular the more dystrophic (humic) lakes, have long periods of hypoxia during summer and fall, likely resulting in a potentially important role of methanogenic bacteria (Jones and Grey 2004, Grey et al. 2004). Deposit-feeding chironomid larvae of the genus *Chironomus*, collected in the profundal sediments of some of our lakes had low $\delta^{13}\text{C}$ -values of -31.1 to -36.1 , lending support to this conjecture.

No significant differences in $\delta^{15}\text{N}$ between lake types were found for any of the investigated samples,

FIGURE 12. Schematic presentation of the benthic and pelagic trophic pathways in limed, acid and circumneutral lakes based on the stable isotope composition of their biota. Data from leaf litter, obtained from Bohman (2005), are marked with an asterisk. Plots show means \pm standard errors.



i.e. seston dissolved organic matter, invertebrates or fish. However, some interesting observations are worth commenting upon. The apparent decreasing trend in seston $\delta^{15}\text{N}$ across the gradient from acid to limed to circumneutral lakes may indicate that the base of the food web in the acid lakes (that are more humic than the other types of lakes) is dominated more than in the other lake types by mixotrophic species. Mixotrophy implies trophic fractionation and thus enrichment in $\delta^{15}\text{N}$. Conversely, as light conditions are better, seston in the clearer limed and circumneutral to a larger share may include autotrophic species, likely explaining the lower $\delta^{15}\text{N}$ -values. Also the lower mean $\delta^{15}\text{N}$ in the predator-/phantom midge *Chaoborus flavicans* in acid lakes (by more than 2 ‰) are conspicuous. These data (near-significant at $P = 0.07$) suggest a more efficient transfer of nutrients to *Chaoborus* larvae in acid lakes than in the other two lake types. Interestingly, a similar tendency of lower mean $\delta^{15}\text{N}$ in acid lakes is found for planktivorous perch (Figure 11). Possibly the occurrence of repeated summer blooms of the mixotrophic flagellate *Gonyostomum semen* in

these slightly acidic, humic lakes and/or differences in stratification patterns among lake types (longer stratification in acid brown-water lakes) may affect the differences in $\delta^{15}\text{N}$ for these zooplankton-feeding larvae. For example, although mixotrophy generally implies an increase in $\delta^{15}\text{N}$ (i.e. enrichment, see above), the mass occurrence of *Gonyostomum* during the most productive months of the year may be efficiently transferred to higher trophic levels by yet unknown trophic pathways. We can only speculate about possible causes for this observation, but possibly future analyses of stable isotopes in preserved samples of different zooplankton taxa/groups may help to reveal the mechanism behind these observations.

Pike in acid and limed lakes was more enriched in ^{15}N than was piscivorous (large) perch (Figure 12), whereas these two fish groups had similar $\delta^{15}\text{N}$ in circumneutral lakes. However, rather than resulting from liming, we attribute this difference to ontogenetic characteristics of the sampled pike populations. One of the sampled individuals in the limed lakes was very small (136 cm) leading to a slightly lower average size of pike in limed lakes (483 ± 126.6 cm)

relative to acid (528 ± 26.7 cm) and circumneutral references (546 ± 115 cm). In other words, we ascribe this result to the differences in size (age) of the few pikes that were caught, as larger pike is known to be more depleted in ^{15}N than smaller individuals (e.g. Meili et al. 1993).

SUMMARY AND FUTURE PERSPECTIVES

Our study of communities of different organism groups, almost consistently based on biomass data (part 1) points at systematic and significant differences, with communities in limed lakes repeatedly being the intermediate between acid and circumneutral lakes. These results likely reflect that community compositions and biomass in limed lakes are kept at some intermediate state due to liming. Alternatively, the intermediate state indicates how far these lakes have come in the transition from an acidified to a fully restored state since the liming began. In acid lakes, acid-sensitive taxa have disappeared and biomass production is frequently lower, while circumneutral lakes have more taxa and higher biomasses within these taxa. Univariate analysis of structural (total biomass, species richness and Shannon-Wiener diversity) and functional (feeding guilds) characteristics showed community and lake-type-specific responses.

Our study of the isotopic composition of organisms in acid, circumneutral and limed lakes (part 2) does not point at systematic changes in the isotopic signature of fish, pelagic and benthic invertebrates, and their food sources as a result of liming. However, some interesting differences between lake types were found. For example, our study showed that shredders (i.e. *Asellus aquaticus*) had a much higher $\delta^{15}\text{N}$ in circumneutral lakes than in acid lakes, suggesting differences in leaf processing between lake types and/or the utilization of other food sources due to ecological release as a consequence of the disappearance of acid sensitive taxa. Repeated liming should theoretically result in higher $\delta^{13}\text{C}$ values as the $\delta^{13}\text{C}$ of limestone is zero. Such lime-induced modifications of the $\delta^{13}\text{C}$ signature of organisms should primarily occur in phytoplankton and epilithic algae that assimilate HCO_3^- originating from lime stone additions. However, our results did not show significant differences in the $\delta^{13}\text{C}$ of seston and epilithon between lake types, partly due to relatively high variability of lakes within each type. The question whether liming has affected the isotopic signature of seston and epilithon (and higher trophic levels) calls for an alternative study design. We therefore will propose the hind casting of changes in stable isotope composition of faunal elements (from the

sediment archive) and fish (samples from banking). The temporal change of stable isotope patterns could be compared with genetic and autecological traits of organisms hatched from different sediment strata (Angeler 2007). We anticipate that such a paleolimnological approach can elucidate structural and functional shifts, particularly in isotopic signatures of key taxa (cladocerans, reflecting also seston isotopic changes, chironomids) during both acidification and liming.

Determining an appropriate isotopic signature of phytoplankton, bacterioplankton, or epilithic algae is tricky, as a pure sample of cells is difficult to obtain (but see Vuorio et al 2006). Our samples of seston and epilithic communities were also a mixture of algal and bacterial cells, likely also “contaminated” with protozoans and ciliates. In other words, these composite samples may not give good estimates of the isotopic composition of the food source of grazers. Future analyses of zooplankton samples will provide further information, as the $\delta^{13}\text{C}$ signal of herbivorous zooplankton should be close to that of their food source.

During 2009 we will continue to work on the dataset generated in this study. The inclusion of isotopic data for zooplankton samples will further improve the data set and allow for a more thorough analysis. We aim at producing at least two scientific publications. Reprints will be sent to the Swedish EPA as a means of additional reporting.

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