Phytoplankton responses to nitrogen and phosphorus enrichment in unproductive Swedish lakes along a gradient of atmospheric nitrogen deposition

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ABSTRACT: Lake sampling and *in situ* nutrient enrichment enclosure experiments with nitrogen (N) and phosphorus (P) were conducted in unproductive Swedish lakes along a gradient of increasing atmospheric N-deposition. The regional and seasonal patterns of nutrient limitation of phytoplankton were clearly related to the amounts of N-deposition and N-inputs the lakes received. In areas of low N-deposition in northern Sweden, N-limitation of phytoplankton was evident throughout the summer season due to high catchment N-retention and very low dissolved inorganic N (DIN) inputs during the early summer. High N-deposition in the south was accompanied by high lake DIN-concentrations during the early summer and subsequent P-limitation of phytoplankton. However, P-limitation did not persist over the summer and, as a consequence of a declining DIN-pool, the lakes switched to dual- and co-limitation by N and P, and then to N-limitation. Generally, the lakes were N-limited rather than P-limited during the summer. We conclude that N-limitation is probably a natural state of the unproductive lakes studied, but P-limitation of variable intensity and duration has been induced by elevated atmospheric N-deposition.

KEY WORDS: N-limitation · P-limitation · Phytoplankton · Lakes · N-deposition · Seasonal variation · Nutrients

INTRODUCTION

Escalating fossil fuel combustion, agricultural intensification, and forestry fertilizer applications (Galloway & Cowling 2002) have led to an almost 10-fold increase in anthropogenic nitrogen (N) emissions during the 20th century. Furthermore, current anthropogenic contributions to the global N-cycle are estimated to be of the same magnitude as natural rates of N-mobilization (Steffen et al. 2004), resulting in a large-scale alteration of the global N-cycle (Steffen et al. 2004). N-emissions can be transported long distances and affect aquatic environments far from their sources (Holland et al. 2005). Debates concerning large-scale aquatic N-eutrophication have mainly focused on coastal marine systems (Vitousek et al. 1997, Howarth & Marino 2006), while lakes have been largely neglected, probably because production in lakes has generally been considered to be phosphorus (P)-limited (Kalff 2002).

However, in the 1990s the P-limitation paradigm (Kalff 2002) was questioned after evidence indicated that lake phytoplankton can be primarily or co-limited by N (Elser et al. 1990).

The possibility that amelioration of N-limitation by N-deposition may have profound effects was subsequently raised by Bergström et al. (2005) and Bergström & Jansson (2006), who showed that N-deposition had caused large-scale eutrophication of unproductive lakes across substantial areas of Europe and North America. In addition, N-limitation of phytoplankton was found in regions with low rates of N-deposition (<250 kg N km^{-2} yr^{-1}), while higher rates (≥500 kg N km^{-2} yr^{-1}) had caused increases in lake inorganic N-concentrations and, consequently, eutrophication. The cited authors suggested that many unproductive lakes in the northern hemisphere are (and probably were for a long time) naturally N-limited, but that P-limitation has resulted in many cases from alterations in the bal-

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ance between N and P through increased anthro-
pogenic N-inputs (Bergström & Jansson 2006). These
suggestions are also supported by paleolimnological
evidence from the Colorado Front Range (Baron et
al. 2000, Wolfe et al. 2001, 2002) and the eastern
Canadian Arctic (Wolfe et al. 2006), indicating that
enhanced N-deposition since the mid-20th century has
increased productivity and caused changes in phyto-
plankton compositions favoring more mesotrophic
species.

However, the conclusions in the studies presented
by Bergström et al. (2005) and Bergström & Jansson
(2006) were based on the indirect evidence of a cor-
relation between the phytoplankton biomass to total
phosphorus ratio (chl a:P_{Tot}) and rates of N-deposition.
Furthermore, the background data were mostly aver-
ages obtained from a few samples collected during the
summer, and it was also not clear whether nutrient lim-
itation was imposed by a single nutrient during the
entire summer season, or whether shifts in nutrient
(N or P) limitation occurred. Hence, the hypothesis of
induced P-limitation lacked experimental support, and
the effect of this factor in lakes subjected to atmos-
pheric N-deposition during the summer was unknown.

Therefore, to test the hypothesis and examine the
effects of N-deposition in more detail, we first exam-
ined the mid-summer nutrient limitation of phyto-
plankton by conducting nutrient enrichment experi-
ments in unproductive lakes along an N-deposition
gradient in Sweden, and then investigated seasonal
variations in nutrient limitation using a subset of lakes

investigated in the nutrient enrichment study. We
hypothesized that lakes receiving low atmospheric N-
deposition are primarily N-limited during the summer,
while increases in N-deposition rates will shift lakes to
being primarily P-limited during the summer.

MATERIALS AND METHODS

Study area. We sampled lakes and performed
enrichment experiments along a roughly north–south
axis (with an associated gradient in rates of N-deposi-
tion, ranging from 100 to 1000 kg N km^{-2} yr^{-1}) in 4
regions of boreal Sweden (Fig. 1) during the summers
of 2004 to 2006. The influence of agricultural activities
and sewage-water inputs on the studied lakes is minor.
Thus, apart from liming (which occurs in Regions 1
to 3), the only significant anthropogenic influences are
from forestry (which is performed at similar intensities
throughout the country) and atmospheric deposition.

Experiments and lake sampling. Nutrient enrich-
ment experiments were initially performed during
2004 and 2005 in 11 or 12 lakes per region in late
July to early August (Region 1: July 29–August 4,
2005; Region 2: July 20–23, 2005; Region 3: August
4–7, 2004; Region 4: July 27–29, 2004). These experi-
ments were performed to characterize the mid-summer
nutrient limitation of phytoplankton in each region. A
second set of experiments was conducted in 2006 in 3
to 4 lakes from each region (included in the 2004 and
2005 experiments) to monitor the seasonal variations
in nutrient limitation. These experiments were carried
out on 2 or 3 different occasions during the summer
(Region 1: May 5–8, June 8–11, July 25–28; Region 2:
May 10–13, June 13–16, July 30–August 2; Region 3:
May 29–June 1, August 8–11; Region 4: June 12–15,
July 28–31). Lakes in Regions 1 and 2 were sampled 3
times because in Sweden summer is longer in the
south than in the north (Raab & Vedin 1995).

Initially, temperature and conductivity profiles were
measured to determine stratification conditions. All
the lakes from Regions 1 to 3 were stratified (depths
between 3 and 6 m), while in Region 4 most (8 out of
12) of the lakes were unstratified. Water was then
pumped from 1 to 2 m depth (i.e. from the trophogenic
layer of the epilimnion or, in unstratified lakes, the
surface layers) through a filter (50 µm mesh size) to
remove large zooplankton and, hence, minimize graz-
ing. The water was collected in 20 l transparent low-
density polyethylene (LDPE) containers (Cole-Parmer)
with 80% ambient solar irradiance (photosynthetically
active radiation, PAR) transmittance. Nutrients were
added to the containers to increase the concentrations
of N by 1 mg l^{-1} and/or the concentrations of P by
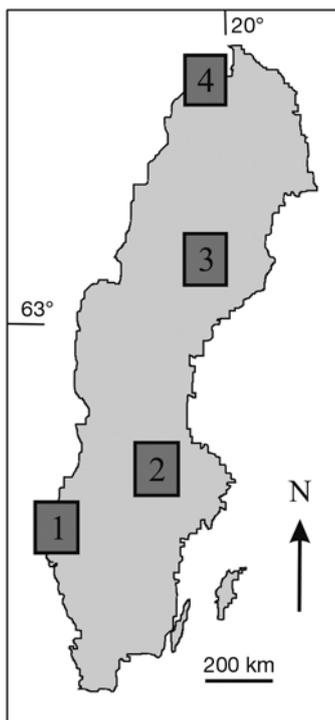100 µg l^{-1} (molar N:P ratio, 23:1) according to treat-

Fig. 1. The Swedish
regions investigated in
the present study

ment. N was added as NH_4NO_3 and P as KH_2PO_4 (Jansson et al. 1996), and overall there were 4 separate treatments: no addition (control, C), N-addition (N), P-addition (P), and N- plus P-addition (N+P). There were 3 replicates of each treatment per lake, and the containers were incubated in the lakes at 1.5 m depth for 4 d in each year, except in 2006 (this duration was selected after a test experiment, performed over 2 to 10 d in one of the lakes in Region 4 during 2003, showed that nutrient-mediated increases in phytoplankton biomass virtually ceased after 3 to 4 d; data not shown). After incubation, water samples were taken from each container and analyzed for chlorophyll a (chl a). The procedure was similar each year, although the containers were only incubated for 3 d in 2006.

In addition, during each experiment water samples were taken from the epilimnion at 1.5 m depth with a Ruttner sampler (2 l) for analyses of water chemistry variables and chl a. The light conditions in the containers and the mixed layer of the lakes were calculated following Ramberg (1979) using: mean daily incident PAR (μmol m^{-2} s^{-1}) data obtained from the Swedish Meteorological and Hydrological Institute (SMHI) (www.smhi.se), the depths of the mixed layers (m), and diffuse light attenuation coefficients (k_d) calculated from lake dissolved organic carbon (DOC) concentrations (Bukaveckas & Robbins-Forbes 2000).

Analyses of nutrient enrichment experiments. Spatial and seasonal variations in nutrient limitation were evaluated by measuring chl a responses in the nutrient enrichment enclosure experiments. For each specific lake experiment, 1-way ANOVAs, with Dunnett's post hoc test against a single control group ($p < 0.05$), were used to examine for significantly higher values in fertilized relative to untreated enclosures (i.e. controls). These analyses were performed on log-transformed data. One-way ANOVAs, with Dunnett's post hoc test against a single control group ($p < 0.05$), were also performed on pooled data from each specific region and time period. In these analyses, the data from each lake experiment were first normalized [(chl a/mean chl $a_{experiment}$) − (SD chl $a_{experiment}$)] to reduce variance between lake experiments, and then log-transformed to obtain a quasi-normal distribution, satisfying the requirements of the subsequent analyses. The different types of nutrient limitation were defined as: N-limitation, which shows a significantly greater response in chl a compared to the control after N-addition, and P-limitation, which shows a significantly greater response in chl a compared to the control after P-addition. Dual limitation by N and P was equated to a significantly higher response in chl a relative to controls when both N- and P-additions alone led to increased chl a (Jansson et al. 2006). Co-limitation by N and P was equated with an increase in the N+P-treatment relative to the control (Jansson et al. 2006) when there was no response to N added alone or to P added alone.

The mean degree of N- and P-limitation was also estimated for each of the regions during the summer of 2006 by dividing the mean summer chl a concentrations in N- and P-treated enclosures, respectively, by the mean concentrations in the corresponding controls. The mean degree of N- and P-limitation during the summer was then related to the annual wet dissolved inorganic N-deposition (DIN-deposition).

Analyses of lake water samples. pH was measured in the laboratory using an Orion portable meter (Model 230). Samples to be used for ammonia (NH_4) and nitrate (NO_3) analyses were filtered (using 0.45 μm Sartorius cellulose acetate filters) then frozen and stored until analysis, while samples to be used for total nitrogen (N_{Tot}) and phosphorus (P_{Tot}) were frozen and stored unfiltered. The contents of these nutrients in the samples were subsequently analyzed at the Evolutionary Biology Center (EBC) Limnology Section, Uppsala, Sweden, following procedures of Bergström & Jansson (2000). Samples to be used for DOC determinations were filtered using precombusted GF/F filters (0.7 μm, 2 cm diameter), and the resulting filtrates were then stored in a freezer until their DOC contents were determined, after Sobek et al. (2003), using a Shimadzu TOC 5000 at the EBC for samples taken during 2004 and 2005, and a Shimadzu TOC V$_{CPH}$ at the Abisko Scientific Research Station, Abisko, Sweden for samples taken in 2006. In addition, known volumes of samples to be used for chl a determinations (both lake and experiment samples) were GF/F filtered (0.7 μm, 4.25 cm diameter) in the dark, and the filters were then stored in a freezer until chlorophyll was extracted from them (within 1 mo of sampling) by placing each filter in 10 ml of ethanol for 24 h in the dark and at room temperature. A luminescence spectrofluorometer (Perkin Elmer, LS 55), was then used to estimate the chl a contents of the extracts by measuring emissions at 673 nm following excitation by 433 nm light. Extracted chlorophyll was not corrected for pheophytin interference using acidification.

Atmospheric deposition. Measurements of wet bulk atmospheric DIN-deposition were obtained from the Swedish Environmental Research Institute (IVL) (www.ivl.se) for each sampling year (Bergström et al. 2005). In addition, annual wet DIN-deposition rates for 2004 to 2006 were estimated using data from monitoring stations situated close to the studied regions. We also estimated deposition between January and July in 2004 and 2005 for each region, and the wet DIN-deposition in each region during the period before the sampling dates, i.e. January to May (only Regions 1 and 2), January to June, and January to July, in 2006.

DIN-concentrations in the Swedish Monitoring Program of streams and lakes. DIN-estimates for the lakes in each of the regions were obtained using data gathered by the Swedish Reference Monitoring Program for running waters and lakes, provided by the Department of Environmental Assessment Analyses, Swedish University of Agriculture Sciences, Uppsala, Sweden (www.ma.slu.se). Monthly DIN-concentration data were collected for small streams and lakes (lake surface area: 0.06 to 2.0 km^2) from forested catchments in each of the 4 regions. The stream and lake locations chosen had very small upstream lakes (or none) in their respective drainage areas, and all chosen lakes had an inlet stream. The collated data spanned different time periods, from just 1 yr to 23 consecutive years. Data for 3 streams (828 samples) and 5 lakes (519 samples) were collated for Region 1, 5 streams (921 samples) and 8 lakes (764 samples) for Region 2, 9 streams (1408 samples) and 7 lakes (682 samples) for Region 3, and 4 streams (799 samples) and 5 lakes (338 samples) for Region 4. Monthly DIN means and standard deviations were calculated for the streams and lakes from each region.

RESULTS

Lake characteristics

Physical, chemical and biological characteristics of the lakes sampled are given in Tables 1 & 2. The surface areas of the lakes used in the enrichment experiments ranged between 0.02 and 2.48 km^2. The annual wet DIN-deposition rates decreased across the gradient south to north; from 800 N km^{-2} yr^{-1} in Region 1 (southernmost) to <100 kg N km^{-2} yr^{-1} in Region 4 (northernmost). Lake conductivity also declined south to north, from 60 to 32 µS cm^{-1}. In addition, lake water temperatures in Region 4 were somewhat lower, while pH measurements were higher, on average, than in the other regions. Lakes were most humic in Region 3 (mean DOC: 12.8 mg l^{-1}), followed by Regions 2 (8.4 mg l^{-1}), 1 (7.6 mg l^{-1}), and 4 (6.1 mg l^{-1}). The highest mean P_{Tot} concentration was also found in Region 3, which was unsurprising since the mean DOC was highest in this region (Meili 1992). Mean N_{Tot} concentrations ranged between 217 and 280 µg l^{-1}, and DIN-concentrations ranged between 9 and 26 µg l^{-1}. The phytoplankton biomass (expressed as chl *a*) was

Table 1. Characteristics of the lakes sampled from late July to early August during 2004 (Regions 3 and 4) and 2005 (Regions 1 and 2). Values presented as averages ± SD; 12 lakes were studied in each region (11 in Region 2). Cond.: conductivity; DOC: dissolved organic carbon; N_{Tot}: total nitrogen; DIN: dissolved inorganic nitrogen; P_{Tot}: total phosphorus; chl *a*: chlorophyll *a*; DIN_{dep1}: wet DIN-deposition for the time period January to July; DIN_{dep2}: annual wet DIN-deposition for 2004 and 2005

Region	Lake area (km^2)	Temp. (C°)	Cond. (µS cm^{-1})	pH	DOC	N_{Tot} (mg l^{-1})	DIN	P_{Tot} (µg l^{-1})	DIN: P_{Tot}	Chl *a* (µg l^{-1})	Chl *a*: P_{Tot}	DIN_{dep1} (kg N km^{-2} yr^{-1})	DIN_{dep2}
1	0.41 ± 0.64	19.3 ± 0.9	60 ± 12	6.5 ± 0.5	7.6 ± 2.8	264 ± 48	11 ± 6	10 ± 7	1.7 ± 1.3	3.7 ± 4.3	0.38 ± 0.25	492	800
2	0.41 ± 0.19	20.3 ± 0.7	46 ± 13	6.6 ± 0.2	8.4 ± 2.5	280 ± 53	9 ± 4	9 ± 2	1.0 ± 0.5	2.7 ± 0.7	0.31 ± 0.08	252	434
3	0.49 ± 0.41	21.4 ± 1.0	34 ± 5	6.2 ± 0.4	12.8 ± 3.4	247 ± 45	20 ± 8	18 ± 5	1.2 ± 0.5	2.9 ± 2.0	0.16 ± 0.10	142	195
4	0.17 ± 0.16	15.7 ± 1.2	32 ± 17	7.0 ± 0.3	6.1 ± 2.4	217 ± 119	26 ± 22	10 ± 4	2.9 ± 2.7	1.6 ± 0.9	0.16 ± 0.09	52	88

Table 2. Characteristics of the lakes included in the 2006 seasonal study. Time periods: Regions 3 and 4, Jun = early summer, Jul/Aug = summer; Regions 1 and 2, May = early summer, Jun = summer, Jul/Aug = summer. DIN_{dep1}: wet DIN-deposition for the time period January to May (only Regions 1 and 2), January to June, and January to August; DIN_{dep2}: annual wet DIN-deposition during 2006. Values presented as averages ± SD; 3 lakes were sampled in Region 4, while 4 lakes were sampled in the other regions. Other abbreviations, see Table 1

Region	Time	Temp. (C°)	Cond. (µS cm^{-1})	pH	DOC (mg l^{-1})	N_{Tot}	DIN (µg l^{-1})	P_{Tot}	DIN: P_{Tot}	Chl *a* (µg l^{-1})	Chl *a*: P_{Tot}	DIN_{dep1} (kg N km^{-2} yr^{-1})	DIN_{dep2}
1	May	11.8 ± 1.3	51 ± 8	6.2 ± 0.7	7.9 ± 2.7	185 ± 7	30 ± 13	6.0 ± 0.7	5.2 ± 2.8	1.3 ± 0.6	0.20 ± 0.09	320	920
	Jun	19.0 ± 1.7	49 ± 8	6.3 ± 0.7	8.5 ± 2.7	166 ± 17	11 ± 6	7.3 ± 1.1	1.5 ± 0.8	2.6 ± 0.8	0.37 ± 0.12	403	
	Jul/Aug	24.5 ± 1.4	54 ± 11	6.6 ± 0.7	7.9 ± 2.2	159 ± 17	11 ± 7	6.3 ± 0.4	1.8 ± 1.2	1.4 ± 0.3	0.22 ± 0.03	478	
2	May	15.1 ± 0.8	42 ± 9	6.0 ± 0.3	10.2 ± 1.9	210 ± 56	25 ± 16	8.1 ± 1.5	3.0 ± 1.7	2.1 ± 0.9	0.25 ± 0.07	278	670
	Jun	23.5 ± 1.0	40 ± 9	6.3 ± 0.1	10.4 ± 1.6	200 ± 33	12 ± 7	8.3 ± 1.5	1.3 ± 0.7	2.3 ± 1.4	0.26 ± 0.14	308	
	Jul/Aug	22.6 ± 1.0	42 ± 9	6.3 ± 0.2	10.2 ± 2.0	199 ± 32	8 ± 4	8.3 ± 1.7	0.9 ± 0.5	2.5 ± 1.0	0.29 ± 0.07	335	
3	Jun	11.2 ± 0.7	31 ± 2	5.7 ± 0.4	10.6 ± 3.1	151 ± 28	7 ± 3	11.1 ± 1.6	0.7 ± 0.3	2.5 ± 1.2	0.24 ± 0.13	121	256
	Jul/Aug	21.3 ± 0.9	30 ± 1.0	6.6 ± 0.4	9.4 ± 2.4	147 ± 15	7 ± 3	9.5 ± 1.2	0.8 ± 0.3	2.4 ± 0.7	0.26 ± 0.08	151	
4	Jun	10.4 ± 1.4	14 ± 6	6.7 ± 0.3	4.2 ± 1.1	78 ± 12	3 ± 0	6.2 ± 2.3	0.6 ± 0.4	1.3 ± 0.2	0.25 ± 0.11	11	40
	Jul/Aug	15.4 ± 0.9	14 ± 6	6.9 ± 0.4	4.7 ± 1.7	93 ± 28	6 ± 0	6.1 ± 2.1	1.0 ± 0.2	0.7 ± 0.3	0.12 ± 0.02	23	

generally low, and the mean decreased across Region 1 to Region 4 (from 3.7 to 1.6 µg l^{-1}). The mean ratio of chl a:P$_{Tot}$ followed the same pattern, decreasing by a factor of about 2.4 across Region 1 to Region 4. Thus, phytoplankton biomass per unit phosphorus was higher in regions with high atmospheric N-deposition.

Both the accumulated N-deposition and the annual wet DIN-deposition decreased across Region 1 to Region 4 in 2006. P$_{Tot}$ concentrations were rather stable seasonally, whereas DIN-concentrations varied between regions and over the summer. DIN-concentrations were low over the summer in the northern regions (Regions 3 and 4), and the DIN:P$_{Tot}$ ratios varied between 0.3 and 1.3. The highest DIN-concentrations were measured in the southern regions (1 and 2) during the early summer; over the summer these declined, and the DIN:P$_{Tot}$ ratios consequently decreased from ca. 5 to 2 in Region 1 and from ca. 3 to 1 in Region 2.

DIN-concentrations in Monitoring Program streams and lakes

Large changes in the DIN-concentrations of both small streams and lakes were seen over the year. The temporal variations were largest in Regions 1 and 2 (south) (Fig. 2), and the lake DIN-concentrations were strongly correlated with the stream water concentrations (r^2 values varied from 0.73 to 0.87, p ≤ 0.002).

Experiments

Mean daily light intensities in the experimental containers ranged between 70 and 500 µmol m^{-2} s^{-1}, and in the mixed layers of experimental lakes they ranged between 45 and 400 µmol m^{-2} s^{-1}. These levels greatly exceeded reported irradiance levels of potential light limitation in phytoplankton (<7 µmol m^{-2} s^{-1}) (Koch et al. 2004), implying that the lakes were primarily nutrient limited. The results from the mid-summer nutrient enrichment enclosure experiments of 2004 and 2005 (Fig. 3A) show that chl a concentrations significantly increased (p < 0.05) after N+P-addition in every lake and region studied (the latter based on 1-way ANOVAs applied to pooled data for each region). In addition, the response of phytoplankton was significantly stronger (p < 0.05) after N-addition alone than after P-addition alone (Fig. 3A,B) Data for individual lakes showed that the phytoplankton biomass was significantly higher (p < 0.05) after N-addition, relative to the control treatment, in 9 lakes out of 12 in Region 4 (Fig. 3B). In the other lakes, phytoplankton were either co-limited (1 lake) or dual-limited (2 lakes). In Region 3, N-limitation was even more frequent (10 N-limited lakes out of 12 lakes). In Region 2, there was a response to P-addition in 1 lake, but most lakes responded to N-addition (Fig. 3B). In Region 1, two lakes responded to P-addition, although most of the lakes responded to N-addition (Fig. 3B). Therefore, N-limited lakes clearly
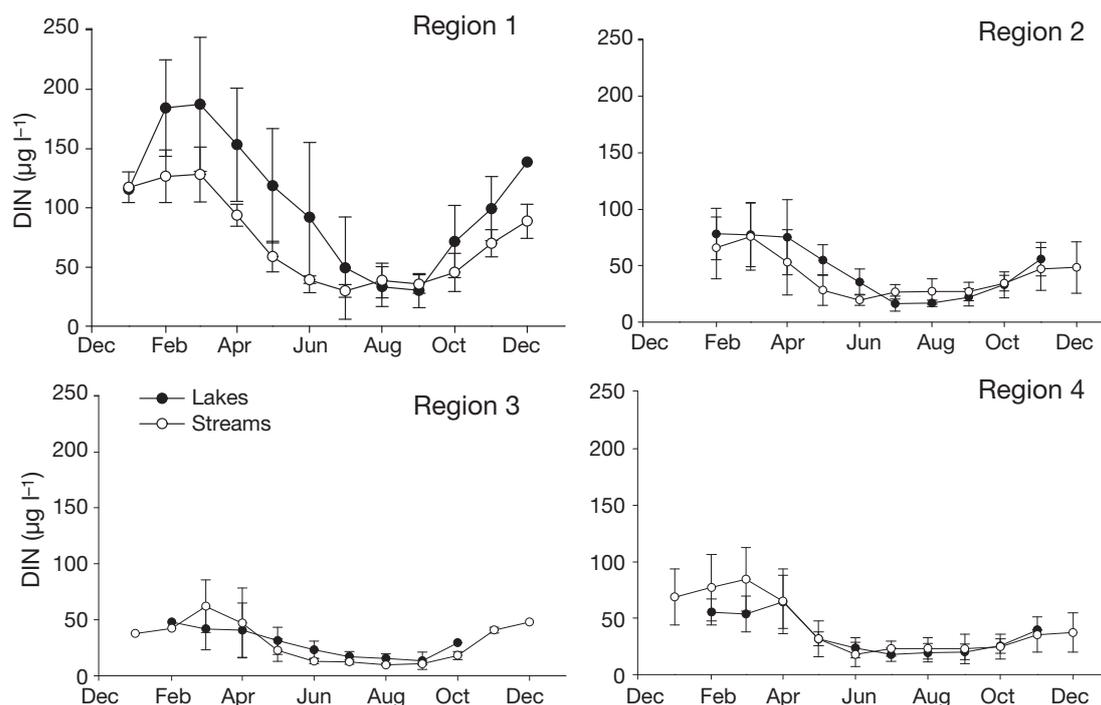

Fig. 2. Mean monthly concentrations of dissolved inorganic nitrogen (DIN) in small headwater streams and lakes in Regions 1 to 4
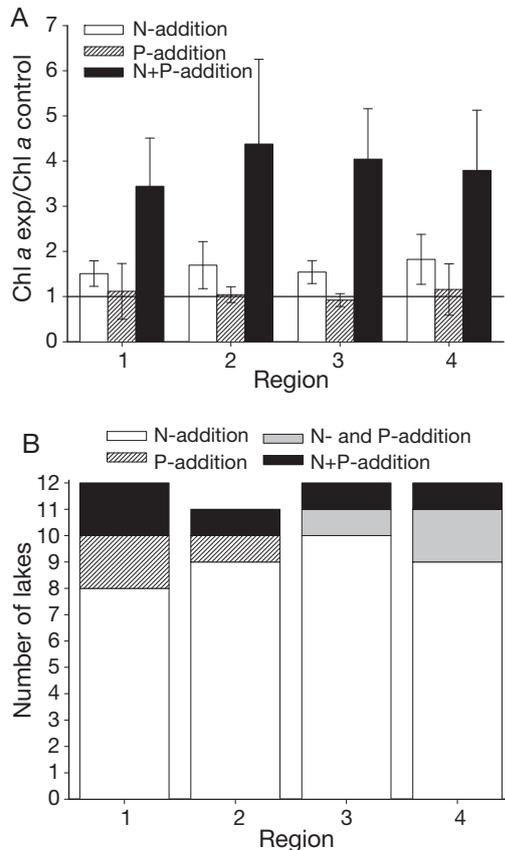
Fig. 3. (A) Average responses (±SD) in chl *a* concentrations relative to corresponding controls in enclosures with different nutrient additions (N, P, and N+P); 12 lakes were tested in each region (11 in Region 2). (B) Number of lakes showing significant (p < 0.05) positive responses (relative to controls) to the specific additions

Fig. 4. (A) Average responses (±SD) in chl *a* concentrations relative to corresponding controls in the enclosures with different nutrient additions (N, P, and N + P) at different times. Experiments were conducted in May, June, and July/August (Jul) in Regions 1 and 2, but only in June and July/August (Jul) in Regions 3 and 4. (B) Number of lakes showing significant (p < 0.05) positive responses (relative to controls) to the specific additions

represented the majority in all regions during mid-summer (Fig. 3A,B).

However, seasonal patterns in nutrient limitation differed between the southern Regions 1 and 2 and the northern Regions 3 and 4, as shown in Fig. 4A,B. When analyzed separately, lakes in Regions 3 and 4 were clearly N-limited throughout the summer, although 1 lake in Region 3 did not respond to any nutrient addition (Fig. 4A,B). In Region 2, the lakes generally switched from P- to N-limitation over the summer (May to July). The pattern was similar in Region 1, but the switch was not as clear, since P-limitation persisted in one lake in June (Fig. 4A). One lake in Region 1 was also co-limited during July. Thus, the switch in nutrient limitation of phytoplankton, from clear P-limitation to clear N-limitation, was more pronounced and occurred earlier in Region 2 than in Region 1 (Fig. 4A,B). One-way ANOVAs (p < 0.05) applied to pooled data for each region and time period revealed that lakes in Regions 3 and 4 were clearly N-limited over summer; lakes in Region 2 switched from P- to N-limitation by
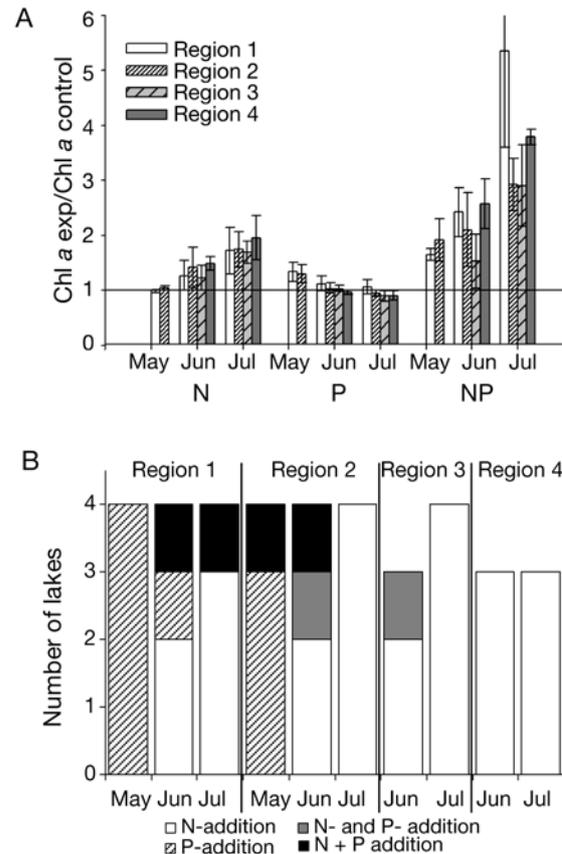
June, and N-limitation persisted until July; while lakes in Region 1 switched from P-limitation to co- and N-limitation over the summer. Thus, in all regions, the degree of N-limitation of phytoplankton was enhanced over the summer, whereas the opposite occurred for P-limitation (Fig. 4A). Phytoplankton also became increasingly nutrient limited, since the response to N+P-addition, relative to the controls, increased over the summer (Fig. 4A).

The degree of P- and N-limitation relative to the controls was related to the seasonal variation in $DIN:P_{Tot}$ ratios over the summer. N-limitation predominated at $DIN:P_{Tot}$ ratios <1.5, whereas P-limitation dominated at higher ratios (Fig. 5). The mean degree of N- and P-limitation observed in each region was related to the annual wet DIN-deposition (Fig. 6). In Regions 3 and 4 (low N-deposition), N-limitation was constant throughout the summer, and P did not stimulate phytoplankton development. P-limitation only occurred in Regions 1
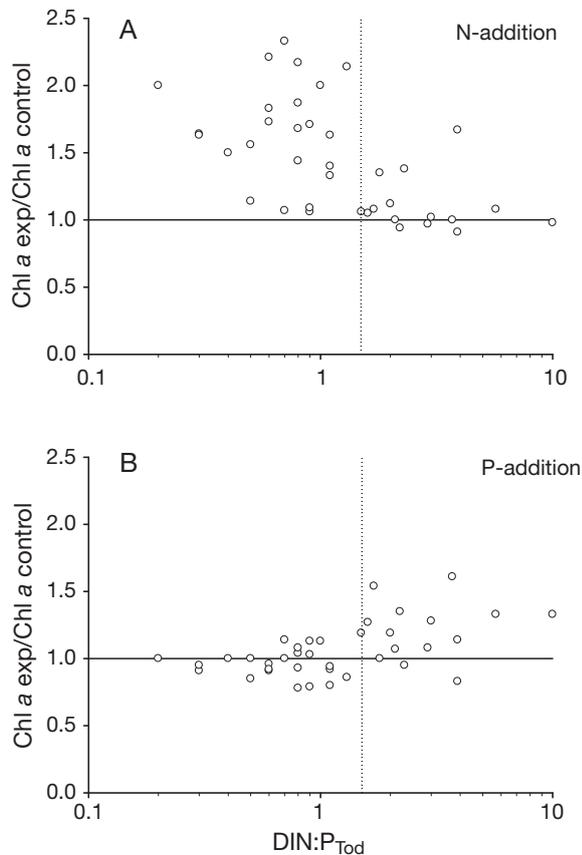
Fig. 5. Relationship between lake DIN:P$_{Tot}$ ratios and the chl *a* concentration responses, relative to controls, in the summer of 2006, to: (A) N-additions and (B) P-additions. Dotted line indicates a DIN:P$_{Tot}$ ratio of 1.5
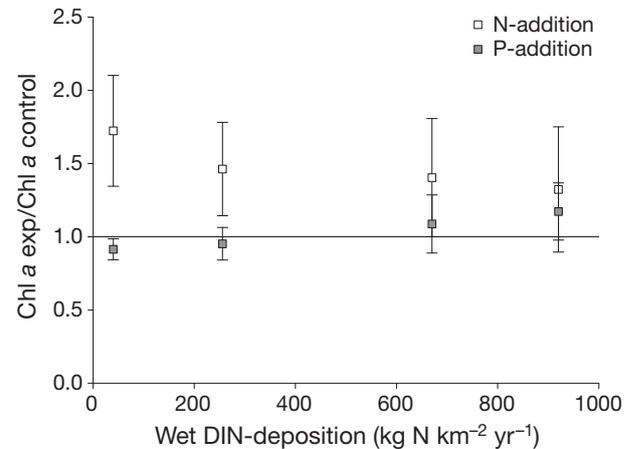

Fig. 6. Relationship between annual wet DIN-depositions and the mean responses in chl *a* concentrations, relative to controls in the summer of 2006, to N- and P-additions from Regions 1 to 4 (mean values ± SD for all experiments)

and 2 (high N-deposition), with the mean summer degree of P-limitation highest in Region 1. However, mean summer degrees of N-limitation were still higher than those of P-limitation in Regions 1 and 2 (Fig. 6).

DISCUSSION

Short-term mesocosm experiments were used to assess regional and seasonal variations in the nutrient limitation of phytoplankton in unproductive Swedish lakes. The experimental results represent snap-shot indices of spatial and temporal changes in the nutrient limitation of natural phytoplankton assemblages, as affected by variations in nutrient loadings and lake DIN:P$_{Tot}$ ratios. This experimental approach, therefore, lacks some ecological realism since, inter alia, it neglects predation (Schindler 1998). The exhaustion of dissolved inorganic carbon (DIC) and nitrogen gas (N_2) is a potential problem associated with enclosures, since carbon dioxide (CO_2) and N_2 diffusion from the atmosphere are prevented (Schindler 1977). Hence,

DIC-concentrations were measured in the enclosures at the beginning and end of the experiments in the lakes with the lowest DIC-concentrations (Region 4). The DIC-concentrations remained at 70 to 100 % of the initial concentrations after 3 to 4 d incubation. In most of the study lakes the CO_2 was a large part of the total DIC and varied between 20 and 100 % (median: 44 %), as calculated from pH (Table 2; see Wetzel 2001). Tests where net primary production was measured in N+P-enriched mesocosms during 4 d in some of the lakes showed that estimated CO_2 consumption was well below the CO_2 pool at the start of the experiment (A.-K. Bergström unpubl. data). Hence, we consider that biomass development and phytoplankton production were not limited by DIC-exhaustion in the enclosures. Furthermore, it is very unlikely that N_2-fixation would ever be limited by N_2 in the enclosures, since: surface water N_2 concentrations vastly exceed the half-saturation constant for N-uptake by phytoplankton (Bryhn & Blenckner 2007); the planktonic N-fixation rates reported for oligo- to mesotrophic lakes are low (Wetzel 2001); and the incubation times were short, 3 to 4 d. Consequently, the results from our enrichment enclosure experiments can be used to assess nutrient limitation of phytoplankton.

The regional differences in nutrient limitation of phytoplankton in unproductive Swedish lakes were related to atmospheric N-deposition and lake DIN-concentrations. Phytoplankton were N-limited in the northern lakes subject to low N-deposition (Regions 3 and 4), while in the southern lakes (Regions 1 and 2), higher N-deposition was accompanied by increased lake DIN-concentrations and a switch from N- to P-limitation of phytoplankton. These findings are consis-

tent with previously reported results based on monitoring data, which also showed regional differences in nutrient limitation related to N-deposition (Bergström et al. 2005).

There were clear regional differences in the observed changes in nutrient limitation over the summer season. Whereas N-limitation persisted over the entire summer in lakes in northern Sweden (Regions 3 and 4), lakes were generally P-limited for a short period during the early summer in southern Sweden (Regions 1 and 2). Furthermore, the degrees of N- and P-limitation were directly related to the DIN:P_{Tot} ratios. During the early summer, the DIN pool declined in Regions 1 and 2, which contributed to decreases in the DIN:P_{Tot} ratios and a switch from P-limitation to dual- (N- and P-), co- (N+P), and N-limitation of phytoplankton over the summer season. Phytoplankton were generally N-limited when DIN:P_{Tot} ratios were <1.5, in approximate accordance with observations in unproductive lakes in the USA (Morris & Lewis 1988, Axler et al. 1994).

Nutrient limitation was not only related to N-deposition, but also to differences in climatic and hydrological parameters. In southern Sweden (Regions 1 and 2), high winter discharge due to rain events (Raab & Vedin 1995), together with high N-deposition and N-saturation of catchments (Binkley & Högberg 1997), led to high DIN-concentrations in lakes during winter (November to April) and early summer. Conversely, the hydrology in northern Sweden (Regions 3 and 4) is characterized by low winter discharge and pronounced spring floods in May/early June following snow melt (Raab & Vedin 1995). These conditions, together with low N-deposition and N-deficiency in the catchments (Binkley & Högberg 1997), result in very low DIN-concentrations in lakes during the early summer (May to June). Therefore, the observed early summer DIN-concentrations in unproductive Swedish lakes (Table 2, Fig. 2) match the spatial pattern of wet DIN-deposition (Bergström et al. 2005), boosted by hydrological differences across the deposition gradient. For these reasons, lakes in southern Sweden have far higher DIN:P_{Tot} ratios during the early stratification period than lakes in the north.

Elevated lake DIN-concentrations in southern Sweden (Regions 1 and 2) caused eutrophication, as shown by the increase in chl a:P_{Tot} ratios (Table 1) (Bergström et al. 2005, Bergström & Jansson 2006) and DIN-assimilation by phytoplankton. This contributed, in turn, to a decrease in lake DIN-concentrations. However, the DIN-decline was probably related to other processes as well, such as denitrification and outlet losses. The importance of hydrology was evident from the high seasonal variations in DIN-concentrations in small streams and lakes. Furthermore, this seasonal variation was particularly pronounced in south-

ern Sweden (Regions 1 and 2), where lowered stream concentrations were followed by decreased lake concentrations (Fig. 2). It is not possible to derive quantitative estimates of the relative importance of processes responsible for DIN-losses (e.g. biological uptake, dissimilatory nitrate reduction, and various abiotic processes) from our data. However, Molot & Dillon (1993) reported that ~20% of the annual bulk atmospheric N-deposition (wet DIN-deposition: ~900 kg N km^{-2} yr^{-1}) on forested catchments in Ontario was exported downstream from lakes, whereas losses due to sedimentation and denitrification were lower (4 and 12%, respectively). The co-variation between stream and lake DIN indicates that stream water characteristics and lake water renewal times were also important determinants of lake DIN-concentrations in the present study. Consequently, hydrology probably had a greater influence on nutrient limitation than biological processes in small lakes with short water renewal times (1 to 3 mo).

Due to the geographical and seasonal variations in lake DIN concentrations and DIN:P_{Tot} ratios, the mean degrees of N- and P-limitation clearly differed between regions over the entire summer period. Nevertheless, in accordance with previously reported findings (Jansson et al. 1996, 2001, Bergström et al. 2005, Karlsson et al. 2005), single nutrient limitation by N was the natural state for lakes under low N-deposition (northern Sweden). In addition, the mean degree of P-limitation increased along the N-deposition gradient, directly reflecting the relative increase in DIN-availability in the southern, high-deposition areas (Regions 1 and 2). However, the mean degree of N-limitation was still higher than the mean degree of P-limitation in those areas over the summer season (Fig. 6).

There was also an increase in chl a responses, relative to control treatments, after N+P-additions, implying that phytoplankton became more nutrient limited over the summer. More severe nutrient limitations could be caused by decreasing pools of available limiting nutrients, or higher phytoplankton growth rates induced by increased lake water temperatures during late summer (Kalff 2002). Therefore, our results are in partial agreement with those from a global analysis of nutrient limitations of primary producers presented by Elser et al. (2007), showing that there are positive synergistic effects of combined N+P-enrichments in the pelagic zone of lakes, and that single nutrient enrichment by either N or P most likely induces limitation by the other element. On the other hand, our results differed in this respect from the study by Elser et al. (2007), since our studied lakes showed different growth responses to N- and P-enrichment alone (related to N-deposition rates and DIN:P_{Tot} ratios of the lakes). In addition, N-limitation was clearly more

pronounced, and both spatially and temporally more widespread, than P-limitation.

Previous studies have shown that increases in the abundance of N_2-fixing cyanobacteria can compensate for N-limitation of phytoplankton in productive lakes (Schindler 1977, Schindler et al. 2008). This kind of ecosystem response might not be detected in short-term enclosure experiments such as ours, since N_2-fixing cyanobacteria are slow-growing organisms (Chan et al. 2004). However, increases in the abundance of N-fixing cyanobacteria are not a universal response to N-limitation. Unproductive lakes of northern Sweden have a naturally low abundance of N-fixing cyanobacteria, and dense populations of cyanobacteria do not develop even though N-limited conditions prevail throughout the summer season (Holmgren 1983, Jansson et al. 1996, 2001). Moreover, dense populations of cyanobacteria do not develop in naturally N-limited unproductive lakes in northern Sweden, even under extremely N-limited conditions induced by P-enrichment in whole-lake continuous P-addition experiments over the summer (Jansson et al. 2001). Overall, this indicates that N-limitation does not necessarily induce N_2-fixation in planktonic communities of unproductive lakes.

In summary, we showed that N-limitation of phytoplankton dominated in low atmospheric N-deposition areas in boreal Swedish lakes. P-limitation was observed during a relatively short period in the early summer in lakes in southern Sweden, where it was induced by high atmospheric N-deposition, high DIN-inputs via catchment runoff, and consequently high DIN-concentrations in the lakes. As the summer progressed, P-limitation in these systems switched to dual- and co-limitation of N and P, and to N-limitation, due to exhaustion of the DIN-pool in the lakes.

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