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Nitrogen, phosphorus and *Daphnia* grazing in controlling phytoplankton biomass and composition – an experimental study

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Abstract

The role of nitrogen as a factor controlling phytoplankton biomass was studied in nutrient enrichment incubations in the laboratory using water from pelagic region of two mesotrophic lakes in eastern Finland, Lake Kallavesi (in year 1994) and Lake Juurusvesi (in year 1995). We used different combinations of phosphorus and nitrogen additions in a total of eight experiments. Furthermore, we included *Daphnia* grazing treatment to the experimental design in Lake Juurusvesi experiments. The nitrogen treatments did not increase chlorophyll *a* concentration in any of the experiments compared with the controls. Chlorophyll *a* content was highest in those nutrient treatments where phosphorus was added with or without nitrogen. *Daphnia* grazing decreased chlorophyll *a* concentration compared with non-grazed treatments. In some cases grazing also caused higher ammonium concentrations. These experiments, as well as the nutrient ratio of the lake water used, suggest that phosphorus is likely to control the amount of phytoplankton biomass.

Introduction

The importance of phosphorus in the eutrophication process of originally oligotrophic freshwaters is well documented, whereas the role of nitrogen is more controversial. There is relatively little experimental work on the responses of freshwater phytoplankton communities to nitrogen additions. Studies performed in the Baltic Sea (i.e. Tamminen, 1990) reveal the key role of nitrogen in controlling primary productivity in brackish water ecosystems.

Effects of nutrients on the biomass of phytoplankton, commonly used to indicate eutrophication, are usually predicted on the basis of the absolute and relative amounts of nutrients in the watershed (Forsberg et al., 1978). However, these predictions do not take into account, for instance, the range of organisms and their biological demands for nutrients, or the ecological relationships present in the studied area. Furthermore, predictions on the effects of incoming nutrients focus on the responses of primary producers measured as changes in biomass, but not on the species composition of phytoplankton. Since the demands of inorganic nutrients vary greatly according to species and along changing environmental conditions, it is necessary to try to incorporate these latter factors in studies focusing on nutrient ratios.

Both abiotic and biotic environmental factors may influence the responses of a lake system to nutrient additions. Of the biotic factors, the impact of zooplankton grazing on phytoplankton may also influence nutrient conditions. Grazers may, for instance, produce small scale local patches of nutrients affecting the nutrient ratios and, consequently, the limiting nutrient if nutrients are excreted at a different ratio than they exist in the surrounding water (Elser & George, 1993) or in the material grazed (Sterner, 1990). It has been shown that some algae can directly utilize phosphorus excreted by grazers (Lehman & Scavia, 1982). Natural ecosystems are full of small scale patches. This

Table 1. Incubation experiments I–VIII were performed in years 1994 and 1995. Sampling site and time, and some basic chemical characteristics of the studied lake waters.

Year	Site	Experiment number	Date	chl-*a* $\mu g\ l^{-1}$	tot–N $\mu g\ l^{-1}$	NO_3–N $\mu g\ l^{-1}$	NH_4–N $\mu g\ l^{-1}$	PO_4–P $\mu g\ l^{-1}$	tot–P $\mu g\ l^{-1}$
1994	Lake	I	9 Jun	12	770	180	39	6	35
	Kallavesi	II	4 Jul	6	680	220	11	5	27
		III	1 Aug	9	680	140	37	3	23
		IV	29 Aug	8	770	170	108	< 2	21
1995	Lake	V	6 Jun	8	690	150	22	< 2	24
	Juurusvesi	VI	5 Jul	7	550	170	11	< 2	19
		VII	2 Aug	9	520	79	15	< 2	19
		VIII	30 Aug	6	610	140	14	< 2	15

heterogeneity is, however, usually ignored in routine fieldwork.

Planktonic algae are capable of using both ammonium and nitrate as a nitrogen source. Ammonium is usually preferred because of its energetic advances (McCarthy et al., 1982; Wetzel, 1983; Miyazaki et al., 1989; Gu & Alexander, 1993), but the use of different nitrogen forms is dependent on their availability and abiotic conditions (Whalen & Alexander, 1986). We included the two nitrogen forms to investigate the possible differences in phytoplankton responses. We studied experimentally in the laboratory, (1) whether additions of inorganic nitrogen, ammonium and nitrate, have effects on phytoplankton biomass in waters from two mesotrophic lakes, and (2) whether the experimentally produced nitrogen enrichment causes changes in the phytoplankton composition.

We also took into account in our experiments the possible effects of zooplankton grazing and asked (3) whether the possibly strengthened recycling effects of *Daphnia* grazing change the responses of the phytoplankton in situation of relatively high nitrogen and low phosphorus content.

Materials and methods

Study sites

Lakes Kallavesi and Juurusvesi (Finland) are two different parts of the Vuoksi watersystem, which runs to the Gulf of Finland. Lake Kallavesi is 51 700 ha in area and has a maximum depth of 69 m. According to phytoplankton biomass, Lake Kallavesi is meso-eutrophic (measured as chlorophyll *a*) (Table 1). The sampling site was located in the pelagic region in the northern part of South-Kallavesi (area 31 500 ha) near a narrow strait, which separates the southern part of the lake from the northern part. Depth in the sampling site was 19 m. Lake Juurusvesi is mesotrophic (Table 1), it is 15 900 ha in area and the maximum depth is about 54 m. The study site was at Kuuslahti (maximum depth about 44 m, depth in the sampling site was 22 m), a narrow bay at the northern part of Lake Juurusvesi. Both Lake Kallavesi and Lake Juurusvesi are to some extent disturbed by industry.

Water samples for laboratory experiments were collected one day prior to the beginning of each incubation. Water was collected with a Limnos-sampler from 0–5 m so that the total water volume collected was 110–125 l in experiments I–IV and 250–287 l in experiments V-VIII. Water was sieved immediately through 150 μm filter (Elser & George, 1993) for the removal of the largest zooplankters (Suttle & Harrison, 1988; Dodds & Priscu, 1990), and stored in a dark and cool (+ 4 ˚C) place overnight.

Experimental design and treatments

In a series of experiments, the treatments used were nutrient additions and incubation time in experiments I–IV (year 1994, Lake Kallavesi), whereas in experiments V–VIII (year 1995, Lake Juurusvesi) we included an additional treatment, zooplankton grazing (with and without addition of *Daphnia*). The nutrient addition treatments consisted of six different levels: control, two different nitrogen additions (N), two (exp. V–VIII, year 1995) and three (exp. I–IV, year 1994) types of nitrogen + phosphorus additions (NP) (Table 2) and at Lake Juurusvesi (exp. V–VIII, year 1995) one manipulation where phosphorus was added alone (P) (Table 2). The levels of the additions, made once at the beginning

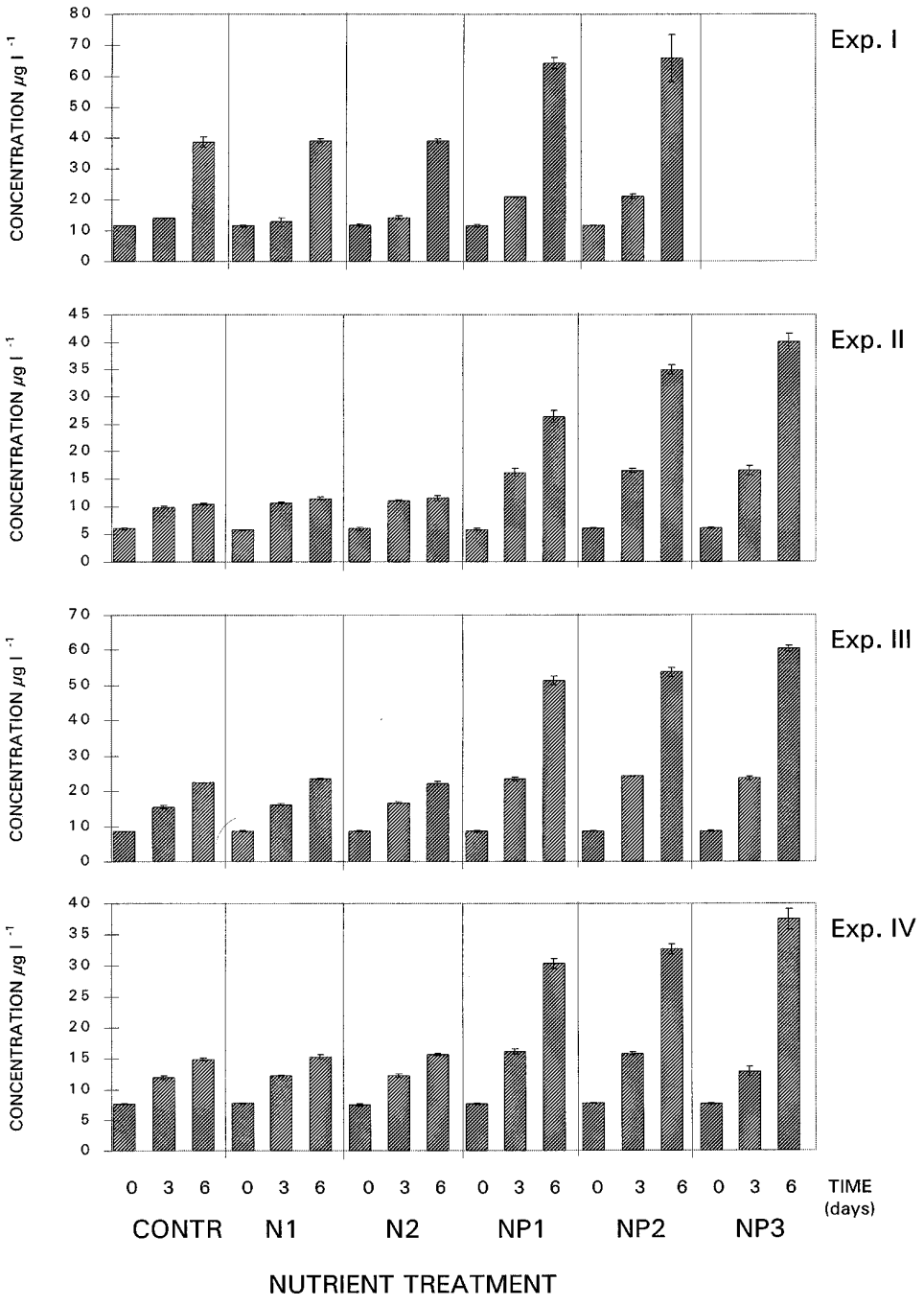

Figure 1. The mean chlorophyll *a* concentrations (± SD) in experiments I–IV in year 1994 in different treatments. Nutrient treatment abbreviations are explained in Table 2.

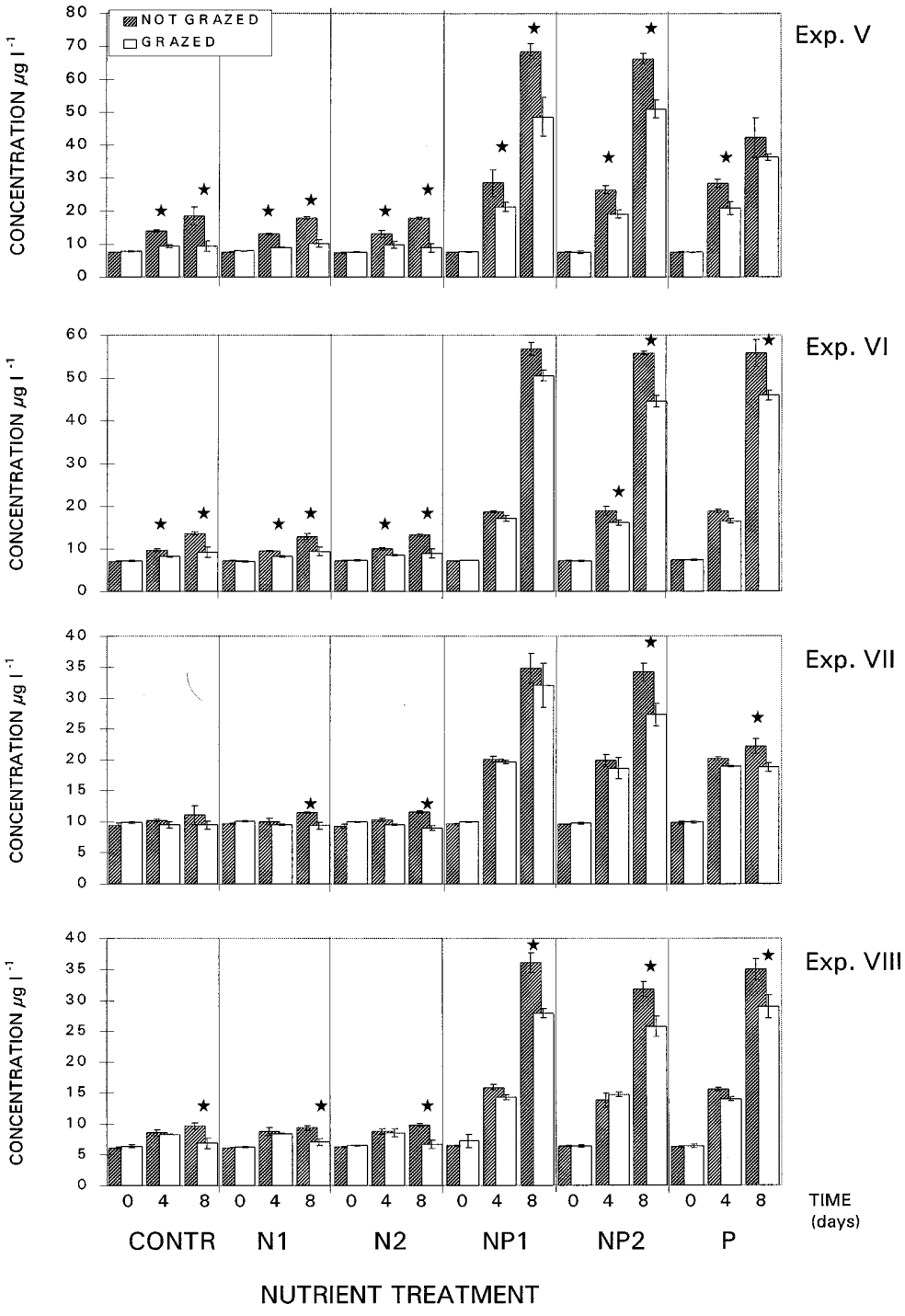

Figure 2. The mean chlorophyll *a* concentrations (± SD) in experiments V–VIII in year 1995 in different treatments. Nutrient treatment abbreviations are explained in Table 2. Statistically significant differences between *Daphnia* grazed and non-grazed treatments (Tukey, $p < 0{,}05$) are indicated with asterisks.

Table 2. Nutrient treatments of the experiments in years 1994 (Lake Kallavesi, experiments I–IV) and 1995 (Lake Juurusvesi, experiments V–VIII) and the abbreviations used in the figures and text.

Nutrient treatment		1994		1995	
		Nitrogen addition $\mu g\ l^{-1}$	Phosphorus addition $\mu g\ l^{-1}$	Nitrogen addition $\mu g\ l^{-1}$	Phosphorus addition $\mu g\ l^{-1}$
Control		–	–	–	–
Nitrogen (1)	N1	300	–	300	–
Nitrogen (2)	N2	1000	–	1000	–
Nitrogen + phosphorus (1)	NP1	300	10	300	15
Nitrogen + phosphorus (2)	NP2	1000	33	1000	15
Nitrogen + phosphorus (3)	NP3	300	125	(not performed in 1995)	
Phosphorus	P	(not performed in 1994)		–	15

of each experiment, were based on the existing nutrient loads to the areas studied. The incubation times were 0, 3 and 6 days in experiments I–IV and 0, 4 and 8 days in experiments V–VIII. In each treatment combination we had three replicate experimental units (incubation bottles). The total number of units was 45 in exp. I, 54 in exp. II–IV and 108 units in exp. V–VIII.

The experiments were conducted in the laboratory in 2 l Duran glass bottles under a light cycle of 16 h light/8 h dark ($8 \pm 0{,}8$ W m^{-2}, Osram daylight lamps 36 W/12,) and at constant temperature of 20 ± 1 °C. Four experiments were run during the summer of 1994 (experiments I–IV) with the water from Lake Kallavesi and in 1995 (experiments V–VIII) with the water from Lake Juurusvesi. Three replicate bottles for each treatment combination were filled with sieved lake water. For N and NP-treatments, the chemical used in experiments I–IV was NH_4Cl (Suttle & Harrison, 1988; Wehr, 1989) and in experiments V-VIII $NaNO_3$ (Mazumder & Lean, 1994). KH_2PO_4 (Suttle & Harrison, 1988; Aldridge et al., 1993) was used as a source of phosphorus in P and NP treatments. All chemicals were Merck's pro analyse quality. Incubation bottles were located randomly at the table and their places were altered daily to prevent any site effects. Water was mixed by stirring with a glass tube twice a day during the incubations.

Laboratory strain of *Daphnia pulex* De Geer, originating from small fishless pond Kupittaa from southern Finland was used as a grazer in the 1995 experiments. Group of *D. pulex* individuals from a laboratory strain were acclimated in sieved water from Lake Juurusvesi at the incubation temperature three weeks prior to the experiments. These individuals and their offspring were used as grazers in all experiments performed. Six to eight individuals, consisting of both juveniles and adults, were added to the respective units. With use of different age classes we wanted to insure that mortality in one age class would not diminish the grazing effect. At the end of each experiment, we collected the daphnids from the water with a 300 μm sieve for further analyses. During the incubations dead individuals were counted daily by visual observation. At the end of the incubations the numbers of egg-bearing adult *Daphnia* females were counted. In all experiments, the number of these females increased towards the end, indicating relatively good potential of population growth. The effect of sieving of *Daphnia*, done also for non-grazed units, on the phytoplankton content was at maximum 2.1% of the chlorophyll *a* concentration.

Phytoplankton and chemical analyses

All chemical analyses were performed at the laboratory of North Savo Regional Environment Centre according to Finnish Standard methods. NO_2–N + NO_3–N (further called as NO_3–N, reduction of NO_3–N to NO_2–N with Cu–Cd column, colorimetric determination of azo-colour, autoanalyzer), NO_2–N (only in 1994 experiments, colorimetric determination of azo-colour), NH_4–N (spectrophotometric determination with hypochlorite and phenol), total N (oxidation with $K_2S_2O_8$ to NO_3–N, measured as NO_2–N), PO_4–P (spectrophotometric determination as phosphomolybdate) and total P (preservated with H_2SO_4, oxidated with $K_2S_2O_8$, spectrophotometric determination as phosphomolybdate) were measured at the beginning, at the middle and at the end of the experiments. Chlorophyll *a* content, used as an indicator of phytoplankton biomass, was analysed spectrophotometrically using

ethanol extraction method. Subsamples for identifying phytoplankton species composition were taken from each bottle prior to chemical analyses and were preserved with acetic Lugol solution. Phytoplankton > 3 μm in size was identified from day zero situation and controls, N2 and NP2 treatments from the last incubation day (day six) from two replicate experimental units in the year 1994 experiments, totalling 32 phytoplankton samples. In year 1995 experiments, just one phytoplankton sample from day zero and control, N2, NP2 and P from day eight with and without *Daphnia* grazing was analysed, totalling 36 phytoplankton samples. Utermöhl (1958) method was used for phytoplankton composition analyses. Phytoplankton cells were identified until 1500 units were counted. Cell volumes used in biomass estimations were either the measured volumes from the samples or the cell volumes for algae of corresponding size from Biological database (Finnish Environment Institute).

Statistical analyses

Multivariate analysis of variance was used in statistical testing of the results. In the experiments I–IV, all chemical parameters and chlorophyll *a* content, and in the experiments V–VIII, chlorophyll *a* and NH_4–N content were included in the model. Tukey's test was used as an *a posteriori* test. Logarithmic transformation was used if the data was not normally distributed or due to the heteroscedasticity of variances. The results of chemical parameters NO_3–N, PO_4–P, total N and total P, in experiments V–VIII were transformed to yield change from the mean at the beginning of the experiments. These transformations made the variables dependent and therefore they were tested with nonparametric methods (Kruskall-Wallis).

We present here the results of chlorophyll *a* data of the experiments performed in years 1994 and 1995, and the key data of the chemical analyses from the year 1995. Concerning the phytoplankton results, we present the data from one experiment in 1995 (exp. VII), which is a representative example of changes in phytoplankton composition. Other results from the experiments I–IV are presented and discussed in Karjalainen et al. (1996).

Table 3. Multivariate analysis of variance using factors nutrient treatment (NU) and incubation time (*T*) in the experiments I–VI in year 1994, showing only results of the variable chlorophyll *a*.

		Chlorophyll *a*		
Exp.		df	*F*	*p*
I	NU	4	108.97	0.000
	T	2	3317.46	0.000
	T × NU	8	28.68	0.000
II	NU	5	834.33	0.000
	T	2	3855.65	0.000
	T × NU	10	466.37	0.000
III	NU	5	1294.55	0.000
	T	2	23293.26	0.000
	T × NU	10	468.04	0.000
IV	NU	5	881.40	0.000
	T	2	13094.65	0.000
	T × NU	10	377.92	0.000

Results

Chlorophyll a *data for years 1994 and 1995*

The single effects of incubation time, nutrient treatments (in years 1994 and 1995) and *Daphnia* grazing (in year 1995) on chlorophyll *a* concentration were statistically significant in every experiment I–VIII (Tables 3 and 4, Figures 1 and 2). Nutrient treatments and time in all experiments I–VIII and *Daphnia* grazing and time in experiments V–VIII had interactive effects on the chlorophyll *a*. Nutrient treatments and grazing had interactive effects in two (exp. V and VI) of the four experiments performed (Table 4 and Figure 2).

Phytoplankton biomass, measured as chlorophyll *a* content, increased during the incubation periods (Tables 3 and 4, Figures 1 and 2). At the end of the experiments the increase was the highest in those nutrient treatments where phosphorus was added with or without nitrogen. The phytoplankton biomass was higher in NP and P treatments than in the controls or N treatments at the end of the study in all experiments I–VIII (Tukey $p < 0{,}05$). Concerning phytoplankton biomass, N treatments did not differ from the controls in any of the experiments (Figures 1 and 2) and the effects of two N addition levels (N1 and N2) did not differ from each other. The NP treatments, NP1 and NP2, differed significantly (Tukey $p < 0{,}05$) just in experiments II, IV and VIII. NP3 treatment caused a slightly higher biomass than the other combined nutrient treatments in experiments II–IV (Figure 1). Phosphorus treatment

Table 4. Multivariate analysis of variance using factors nutrient treatment (NU), incubation time (*T*) and *Daphnia* grazing (*G*) in the experiments V–VIII in year 1995.

Exp.		Chlorophyll *a*			NH_4-N		
		df	*F*	*p*	df	*F*	*p*
V	NU	5;72	519.37	0.000	5;72	22.88	0.000
	T	2;72	2536.39	0.000	2;72	346.31	0.000
	G	1;72	329.21	0.000	1;72	7.47	0.008
	$T \times G$	2;72	99.69	0.000	2;72	9.04	0.000
	$T \times$ NU	10;72	173.24	0.000	10;72	13.98	0.000
	NU $\times G$	5;72	4.35	0.002	5;72	1.74	0.137
	T $\times$ NU $\times G$	10;72	5.07	0.000	10;72	2.06	0.039
VI	NU	5;72	1729.77	0.000	5;72	38.27	0.000
	T	2;72	7354.45	0.000	2;72	451.73	0.000
	G	1;72	287.13	0.000	1;72	23.25	0.000
	$T \times G$	2;72	100.15	0.000	2;72	10.87	0.000
	$T \times$ NU	10;72	629.61	0.000	10;72	15.22	0.000
	NU $\times G$	5;72	5.00	0.001	5;72	4.99	0.001
	$T \times$ NU $\times G$	10;72	2.69	0.007	10;72	4.69	0.000
VII	NU	5;71	756.07	0.000	5;71	117.11	0.000
	T	2;71	1169.14	0.000	2;71	338.80	0.000
	it G	1;71	52.70	0.000	1;71	64.67	0.000
	$T \times G$	2;71	44.91	0.000	2;71	10.40	0.000
	$T \times$ NU	10;71	219.97	0.000	10;71	37.78	0.000
	NU $\times G$	5;71	1.16	0.339	5;71	3.12	0.013
	$T \times$ NU $\times G$	10;71	0.76	0.666	10;71	4.58	0.000
VIII	NU	5;72	773.05	0.000	5;72	32.33	0.000
	T	2;72	2585.01	0.000	2;727	66.08	0.000
	G	1;72	81.70	0.000	1;72	81.70	0.000
	$T \times G$	2;72	86.12	0.000	2;72	10.78	0.000
	$T \times$ NU	10;72	279.00	0.000	10;72	17.50	0.000
	NU $\times G$	5;72	0.85	0.519	5;72	5.12	0.000
	$T \times$ NU $\times G$	10;72	2.18	0.029	10;72	5.26	0.000

(P, in year 1995) caused a lower chlorophyll *a* concentration compared with NP treatments in experiments V and VII (Tukey, $p < 0{,}05$) (Figure 2).

Chemical data for year 1995

The PO_4–P concentrations in the lakes studied, especially in Lake Juurusvesi, were low (Table 1). In the treatments where PO_4–P was added, amounts of it decreased significantly during the incubations (Table 5) and at the end of the experiments concentrations were ≤ 3 μg l^{-1} in every treatment. Also the amount of inorganic nitrogen lowered towards the end (Table 4 and 5, Figures 3 and 4). The decrease was the highest in treatments where phosphorus was added with nitrogen. In non-grazed P treatments, both ammonium (≤ 4 $\mu g\, l^{-1}$ NH_4–N) and nitrate (≤ 10 $\mu g\, l^{-1}$ NO_3–N) concentrations were low at the end of the experiments. In experiments V and VII nitrate concentrations were relatively low (< 60 $\mu g\, l^{-1} NO_3$–N) already at day four (Figure 3).

The effects of grazing

In experiments V–VIII, the chlorophyll *a* concentration was generally lower in the grazed treatments than in the treatments without added *Daphnia* (Table 4, Figure. 2). The difference in chlorophyll *a* was statistically significant (Tukey, $p < 0{,}05$) in almost all nutrient treatments at the end of each experiment (Figure 2). Grazing had also effects on the ammonium concentration of the water (Table 4, Figure 4). In some grazed controls and N treatments, ammonium concentrations were higher than in respective treatments without grazing (Tukey,

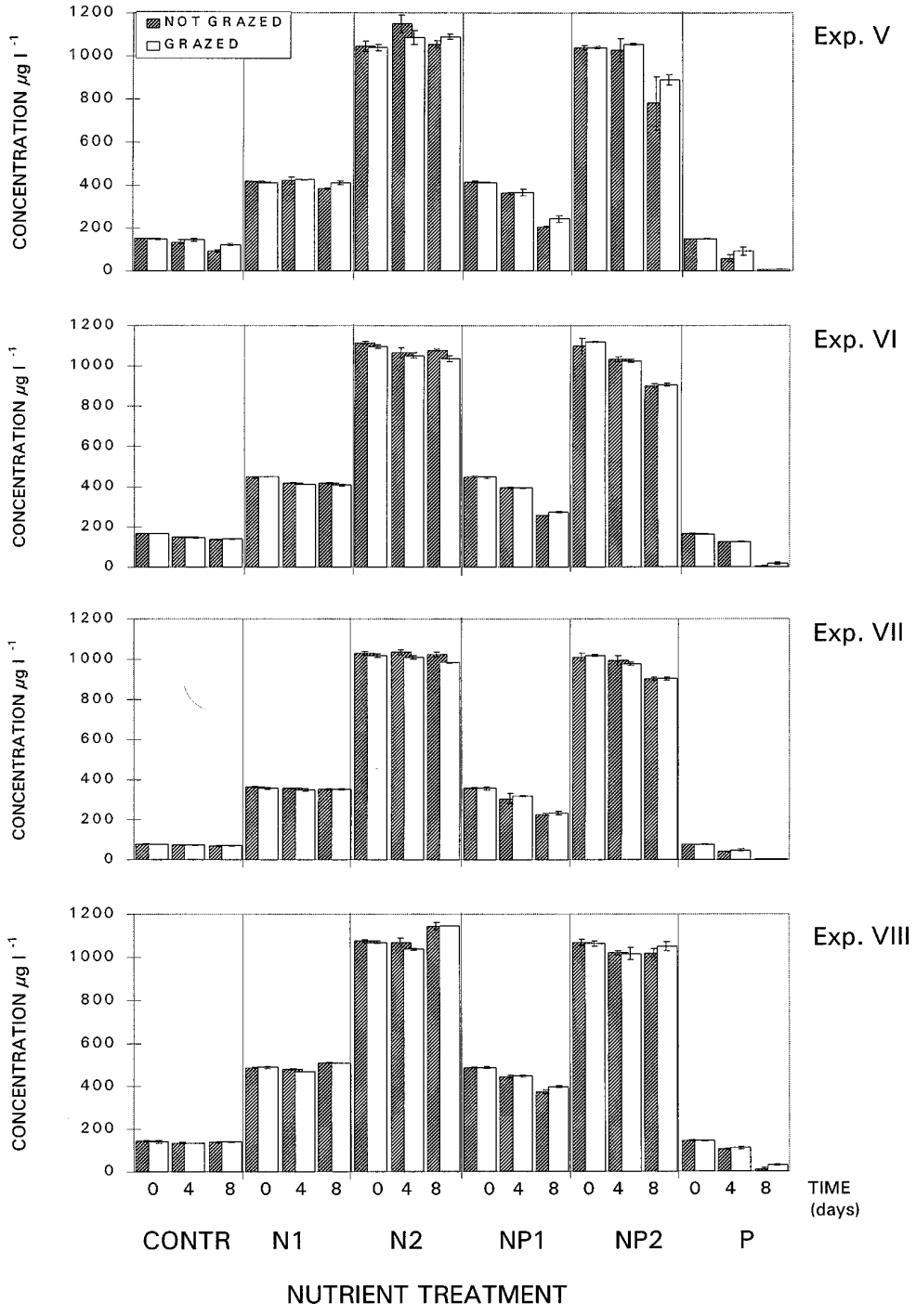

Figure 3. The mean NO_3–N concentrations (± SD) in experiments V–VIII in year 1995 in different treatments. Nutrient treatment abbreviations are explained in Table 2.

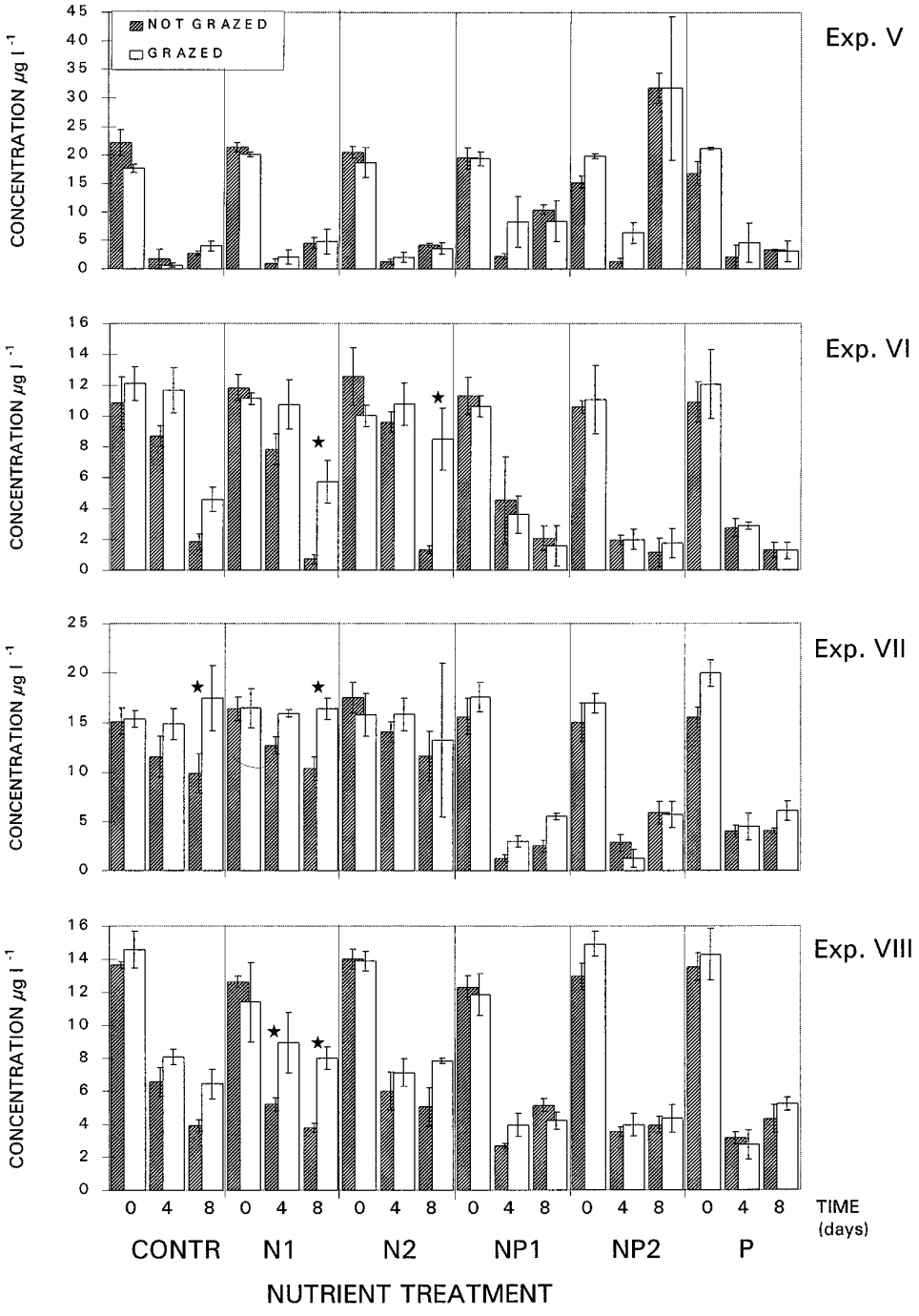

Figure 4. The mean NH_4–N concentrations (± SD) in experiments V–VIII in year 1995 in different treatments. Nutrient treatment abbreviations are explained in Table 2. Statistically significant differences between *Daphnia* grazed and non-grazed treatments (Tukey, $p < 0{,}05$) are indicated with asterisks.

Table 5. The effects of *Daphnia* grazing, incubation time and nutrient treatment on changes in total N (tot N), total P (tot P), NO_3–N ja PO_4–P in experiments V–VIII, (Kruskall-Wallis analysis of variance).

Exp	Parameter	*Daphnia* grazing			Incubation time			Nutrient treatment		
		χ^2	df	*p*	χ^2	df	*p*	χ^2	df	*p*
V	tot N change	9.326	1	0.0023	33.656	2	0.0000	9.009	5	0.1087
V	tot P change	10.566	1	0.0012	10.566	2	0.0000	5.159	5	0.3968
V	NO_3–N change	1.963	1	0.1612	24.842	2	0.0000	34.219	5	0.0000
V	PO_4–P change	1.914	1	0.1666	27.005	2	0.0000	32.037	5	0.0000
VI	tot N change	0.108	1	0.7423	65.271	2	0.0000	4.924	5	0.4253
VI	tot P change	5.212	1	0.0224	70.003	2	0.0000	6.943	5	0.2249
VI	NO_3–N change	0.131	1	0.7170	71.369	2	0.0000	16.219	5	0.0062
VI	PO_4–P change	1.837	1	0.1754	34.477	2	0.0000	33.498	5	0.0000
VII	tot N change	5.401	1	0.0201	31.553	2	0.0000	17.196	5	0.0041
VII	tot P change	8.948	1	0.0028	48.946	2	0.0000	6.718	5	0.2425
VII	NO_3–N change	0.221	1	0.6380	53.585	2	0.0000	17.825	5	0.0032
VII	PO_4–P change	0.569	1	0.4507	42.909	2	0.0000	26.539	5	0.0001
VIII	tot N change	2.533	1	0.1115	64.5396	2	0.0000	0.383	5	0.9958
VIII	tot P change	5.686	1	0.0171	62.4138	2	0.0000	8.788	5	0.1178
VIII	NO_3–N change	0.050	1	0.8225	26.7485	2	0.0000	31.094	5	0.0000
VIII	PO_4–P change	0.286	1	0.8658	58.1237	2	0.0000	21.523	5	0.0006

$p < 0{,}05$). *Daphnia* grazing did not have any effects on the concentration of PO_4–P in the water (Table 5).

Phytoplankton composition

The composition of phytoplankton changed during the incubation. Diatoms were the most common phytoplankton species in all treatments at the end of each experiment (in non-grazed treatments, 64–95%, and in grazed nutrient treatments, 45–96% of the total biomass). Diatoms increased usually proportionally more than the other phytoplankton groups (in the beginning 26–47% of the total biomass). The most common taxa were *Rhizosolenia* spp. based on number and *Acanthoceras zachariasii* measured as biomass. The biomass of chrysophytes was lower in treatments with phosphorus compared with the control and N treatments (see Figure 5 on results of exp. VII). *Daphnia* grazing decreased the biomass of diatoms (Figure 5).

Discussion

In our experiments, the increase in phytoplankton biomass (measured as chlorophyll *a* concentration) was to a large extent determined by the amount of inorganic phosphorus in the water. Inorganic nitrogen regulated biomass only in those cases where incubation had been going on already for a couple of days, and the phosphorus additions caused a high production of biomass. In cases,where phosphorus alone was added, nitrogen probably became the most limiting nutrient, which is a likely response in single nutrient enrichments (Elser et al., 1990). The nitrogen addition alone, however, did not cause any higher increase in the phytoplankton biomass compared to the controls. The amounts of inorganic nutrients, i.e. low inorganic phosphorus concentration compared to inorganic nitrogen concentration at the beginning of the experiments supports our conclusion on the importance of phosphorus in regulating phytoplankton growth.

Relatively low level of ammonium may inhibit the uptake of nitrate (7-11 NH_4-N μg l^{-1}, Dodds et al., 1991). In our experiments, the ammonium concentration decreased strongly as the phytoplankton biomass increased. Especially in treatments with high biomass production, also the amounts of nitrate lowered. Simultaneous increase of phytoplankton biomass and decrease of inorganic nutrients indicate their uptake in building algal biomass.

The amount of dissolved inorganic nutrients in waterbody is usually much smaller than the amount of nutrients bound in particles (Dodds, 1993). In short time scales, biological processes strongly determine the amounts of inorganic nutrients in the water (Dodds et al., 1991). Ammonium is the basic form of nitrogen

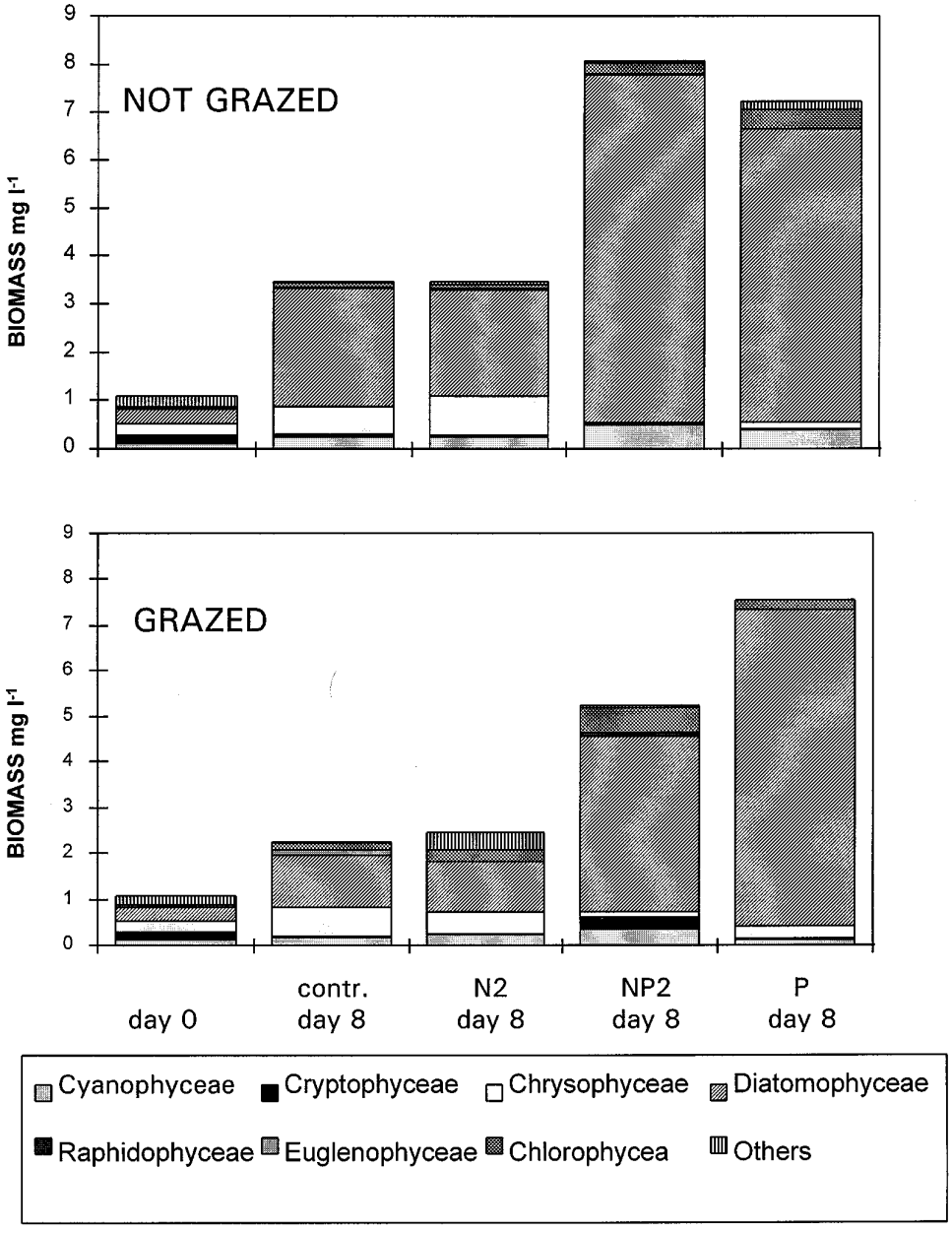

Figure 5. The group composition of phytoplankton in experiment VII in year 1995 in different treatments. Nutrient treatment abbreviations are explained in Table 2.

produced by excretion (Lehman, 1980). Therefore, in the case of low ammonium content and high nitrate content grazing may enhance significantly the availability of nitrogen for phytoplankters by excretion. In our study, the ammonium concentrations were higher in some of the grazed controls and nitrogen treatments when compared with the respective units without grazing (Figure 4). In treatments where phosphorus was added and the increase of phytoplankton biomass was high, no differences were found. It is probable that *Daphnia* grazing cycled nitrogen as ammonium and in the treatments where the production was relatively low, surplus ammonium stayed in the water column. It is possible that the effects of *Daphnia* grazing were

indirect via changes in smaller size zooplankton (i.e. protozoans, ciliates), which were not affected by the presieving of the water and were obviously present in all experiments. This was not, however, examined here. When the increase of phytoplankton biomass was high and extra phosphorus (P and NP treatments) was added, the excreted ammonium was probably utilized by phytoplankton.

The negative effects of grazing on phytoplankton biomass can be, to some extent, compensated for by the better availability of nutrients (Sterner, 1986; Sterner, 1990). The excretion of nutrients is dependent on the nutrient demands of the grazer. The ratio of nitrogen to phosphorus of *Daphnia* is relatively low (Sterner et al., 1992), with *Daphnia magna* being around 12.7 (by atoms) according to Hessen (1990). Therefore, *Daphnia* have probably acted more like phosphorus traps rather than effectively recycling phosphorus in the systems studied.

In the studied systems, the biomass of phytoplankton was generally lower in grazer treatment combinations compared with non-grazer treatments. This indicates a major impact of zooplankton grazing in addition to nutrient control (especially phosphorus) of phytoplankton. The negative effects of large *Daphnia* on phytoplankton, in spite of a nutrient load, have also been found in *in situ* experiments (Mazumder & Lean, 1994). In addition to decreasing the phytoplankton biomass, grazing may also have effects on the efficiency of phytoplankton primary production. These effects, however, remain unclear, because we did not make any direct production measurements.

The sieving rate of *Daphnia pulex*, controlled for instance by temperature (Mourelatos & Lacroix, 1990), density of food particles (Wiedner & Vareschi, 1995) and age of the individual, has been measured to be about 2 ml ind.$^{-1}$ h^{-1} in natural cell densities (Wiedner & Vareschi, 1995). Based on these estimates, eight *D. pulex* individuals may sieve three litres in eight days, which exceeds the volume of one experimental unit used in our experiment.

Different phytoplankton species have different demands of inorganic nutrients. These demands and the capability to take up nutrients differ according to abiotic conditions (Rhee & Gotham, 1981), the physiological status of phytoplankton (Lean & Pick, 1981) and the ecological strategy concerning the nutrition of the algae (autotrophy-mixotrophy) (Bird & Kalff, 1987; Nygaard & Tobiensen, 1993). Some experiments have shown that both herbivory and changes in the nutrient loading can change diversity of algae dramatically (Proulx et al., 1996). In our study, the phytoplankton composition at the group level became more homogenous during the incubation. The relatively high amounts of diatoms in all the nutrient treatments was probably partly due to our incubation conditions. Diatoms are known to be good competitors at low light levels (Willen, 1991) and may, therefore, have gained advantage due to the relatively low light levels used. Some algae, including many diatoms, have also good capability of storing phosphorus, which explain the increasing algal biomass in control and nitrogen treatments, where the amounts of available phosphorus were low.

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