Master's thesis

Impact of increased dissolved organic carbon and nutrient loading on humic lake phytoplankton

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ABSTRACT

The effects of increased dissolved organic carbon (DOC) loading on the phytoplankton of a small humic lake (Alinen Mustajärvi) were studied in a whole-lake manipulation experiment during 2007–2009. 2007 was a control year without any manipulation, cane sugar was added monthly to the lake during 2008 and 2009, and additional responses to inorganic nutrient additions were examined with small-scale field enclosures three times during the open water season of 2009. The aim was to increase the amount of easily degradable DOC for heterotrophic bacteria without affecting the light climate for phytoplankton. Differences in responses of autotrophs and mixotrophs were studied. There were no consistent responses to increased DOC loading; phytoplankton communities in the two manipulation years were very different. The most pronounced change was the dominance of the raphidophyte *Gonyostomum semen* during the second manipulation year, but this was probably not due to the manipulation. There were no differences in the responses of autotrophs and mixotrophs. In summer phytoplankton growth was co-limited by phosphorus and nitrogen, but during other times the community was not nutrient limited. Any future increase in DOC loading will likely affect the phytoplankton community only if it is accompanied by increased inorganic nutrient loading, and even then mostly during summer.

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TIIVISTELMÄ

Lisääntyneen liuenneen orgaanisen hiilen (DOC) kuormituksen vaikutuksia humusjärven (Alinen Mustajärvi) kasviplanktoniin tutkittiin koko järven manipulaatio -kokeessa 2007–2009. 2007 oli tutkimuksen kontrollivuosi ilman manipulaatiota, 2008–2009 järveen lisättiin kerran kuukaudessa ruokosokeria, ja lisäksi ravinnelisäysten vaikutuksia tutkittiin pussikokeissa kolme kertaa avovesikaudella 2009. Tarkoituksena oli lisätä heterotrofisten bakteerien ravintona käyttämän helposti hajotettavan DOC:in määrää ilman, että se vaikuttaisi kasviplanktonin kokemiin valaisuolosuhteisiin. Autotrofisten ja miksotrofisten levien reaktioita vertailtiin kokeissa keskenään. Kasviplanktonyhteisöissä oli suuria eroja manipulointivuosien kesken, ja johdonmukaisia reaktioita DOC kuormituksen lisääntymiseen ei havaittu. Suurin havaittu muutos oli limalevän *Gonyostomum semen* määrän huomattava kasvu toisena manipulointivuotena, mutta tämä ei todennäköisesti johtunut DOC-lisäyksistä. Autotrofien ja miksotrofien reaktioissa ei havaittu eroja. Kasviplanktonyhteisön kasvua rajoitti kesällä samanaikaisesti fosfori ja typpi, mutta muina aikoina kasvu ei ollut ravinnerajoitteista. Ilmastonmuutoksen myötä lisääntyvä DOC-kuormitus vaikuttaa humusjärven kasviplanktoniin todennäköisesti vain jos sen yhteydessä esiintyy lisääntynyttä ravinnekuormitusta, ja silloinkin vaikutuksia ilmenee lähinnä kesällä.

Contents

1. INTRODUCTION

Phytoplankton communities consist of both autotrophic and mixotrophic species. Phototrophic organisms use light energy to synthesize organic carbon from inorganic carbon (photosynthesis) and are therefore autotrophic. Heterotrophic organisms are not able to synthesize organic carbon on their own but use particulate and dissolved organic compounds for nutrition. Mixotrophic organisms combine these two modes of nutrition by ingesting food particles (phagotrophy) or by taking up dissolved organic molecules for nutrition (osmotrophy) in addition to photosynthesis and, amongst the phytoplankton are mostly flagellates. Their prey can consist of bacteria, cyanobacteria, algae, protists, metazoan gametes and viruses (Isaksson 1998). The ingestion rates of phagotrophic mixotrophs can be equal to ingestion rates of pure heterotrophs (Porter 1988) and, instead of ciliates and zooplankton, mixotrophic algae can be responsible for the majority of bacterial grazing in some lakes (Bird and Kalff 1987, Isaksson *et al*. 1999). Phagotrophy can also be used to obtain inorganic nutrients or other important growth factors in addition to organic nutrition (Isaksson 1998).

The traditional view has been that the pelagic food webs are driven by autotrophic production by phytoplankton (Fig. 1). Phytoplankton is grazed by zooplankton which itself is the prey of invertebrates and fishes. Some of the energy is also channelled through the microbial loop: bacteria use dissolved organic carbon (DOC) released by phytoplankton and are grazed by zooplankton or mixo- and heterotrophic flagellates and ciliates. More than half of the organic carbon in humic lakes, however, is of allochthonous origin (Meili 1992). Many studies have shown (e.g. Tranvik 1988, Tulonen *et al*. 1992) that bacteria can also use allochthonous DOC as a substrate, and the high bacterial production found in humic lakes can not be supported only by DOC released by phytoplankton (Jones & Salonen 1985, Hessen 1992). In lakes with a significant input of allochthonous DOC a large proportion of the energy can be transported via the microbial loop instead of the phytoplankton to higher trophic levels and the lake can be net heterotrophic (Salonen *et al*. 1983, del Giorgio *et al*. 1997), meaning the respiration of the plankton community exceeds the phytoplankton primary production. Allochthonous DOC can also affect lake metabolism by altering the light climate experienced by phytoplankton (Jones 1992a).

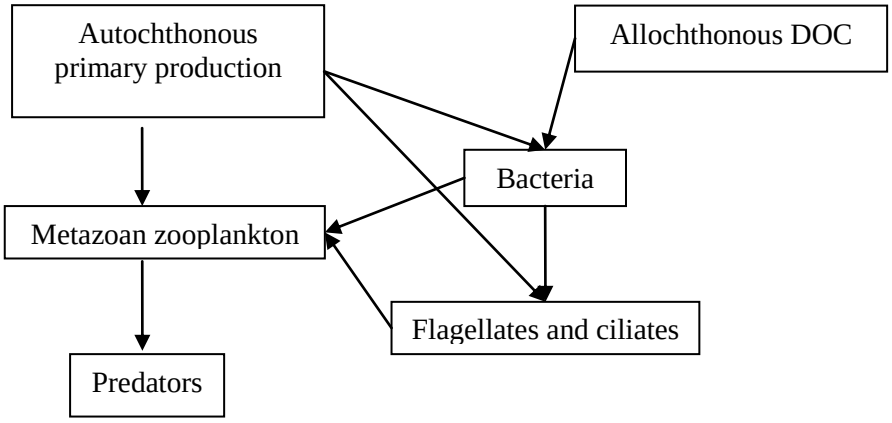

Figure 1. Pelagic food chains based on autochthonous primary production and allochthonous DOC (modified from Jones 1992a).

Global warming will affect the future climate of Finland. Winters are expected to become warmer with increased precipitation (Watson *et al*. 2001). This can lead to increased runoff and more DOC may enter aquatic ecosystems (Forsberg 1992, Tranvik & Jansson 2002). Increased DOC loading is expected to enhance the growth of pelagic bacteria. Drakare *et al.* (2002) reported that high flow episodes with input of fresh DOC

into a lake stimulated bacterial production. However, in a mesocosm study of two humic lakes Kankaala *et al.* (2010b) found that other factors apart from DOC availability may regulate the growth of bacteria. As bacterial biomass increases, the competition for inorganic nutrients between phytoplankton and bacteria is expected to intensify. Pelagic bacteria are thought to be superior competitors for nutrients over phytoplankton due to their smaller size and associated greater surface area to volume ratios (Currie & Kalff 1984). Hence if increased DOC provides more substrate for pelagic bacteria they might be expected to use more of the available inorganic nutrients at the expense of phytoplankton (Joint *et al*. 2002). As a result, mixotrophic algae could become more common, at the expense of obligate autotrophs, since (phagotrophic) mixotrophs can also gain nutrients by ingesting bacteria (Isaksson 1998). Flagellate algae are very common in humic lakes and they can also obtain nutrients in steeply stratified lakes by vertical migrations (Ilmavirta 1983, 1988, Salonen *et al*. 1984). The share of flagellate algae in the phytoplankton community may therefore increase as a result of the intensifying nutrient competition between phytoplankton and bacteria.

Carpenter *et al*. (1998) studied the impact of coloured DOC, phosphorus and grazing on phytoplankton biomass and production in whole-lake manipulation experiments. They found that DOC had a negative impact on phytoplankton biomass (measured as chlorophyll *a*) and production, probably because of the shading effect of coloured DOC. Enclosure experiments made in a large humic lake Pääjärvi showed an increase in bacterial production but not in biomass with additions of humic matter, and especially with a simultaneous addition of phosphorus (Arvola *et al.* 1996). The highest phytoplankton biomasses were also found in enclosures with phosphorus or phosphorus and humic matter additions, but only minor changes in the community composition of phytoplankton were observed. Arvola *et al*. (1996) concluded that the autotrophic production in Pääjärvi was limited by phosphorus and bacterial production was limited by DOC released by phytoplankton. Blomqvist *et al*. (2001) studied the impacts of DOC without the shading effect in pelagic food chains in a whole-lake experiment in Sweden by adding sugar (sucrose) into a clearwater lake. That study showed a significant increase in bacterial biomass with a decrease in autotrophic phytoplankton biomass due to additions of DOC. The increase of bacterial prey benefitted the mixotrophic and heterotrophic flagellates, but no effects were found on higher trophic levels. However, no corresponding whole-lake study in a boreal humic lake has been done and the results from a study of a clearwater lake can not be directly applied to humic lakes. Humic lakes are very common in Finland and their food webs can differ significantly from those of clearwater lakes' (Jones 1992a). Therefore, a whole-lake manipulation experiment (Academy of Finland funded study: A whole-lake experimental test of the impacts of increased dissolved organic carbon loading on lake metabolism and food webs) was carried out in which cane sugar was added to a humic lake, Alinen Mustajärvi in the Evo district of southern Finland, for two years to simulate a possible future increase in DOC loading. By adding cane sugar the goal was to increase the amount of easily degradable DOC available for heterotrophic bacteria without affecting the light climate for autotrophic phytoplankton, and thus to distinguish between these two potentially confounding effects.

Increased DOC loading caused by climate change might also be associated with increased nutrient loading (Kortelainen *et al*. 2006). This study tried to reveal what will happen to the planktic community with different DOC and nutrient (phosphorus and nitrogen) loading scenarios by using small-scale field enclosures in addition to the whole-lake DOC manipulation experiment. Generally, phytoplankton production in freshwater systems is thought to be limited by phosphorus (P) or nitrogen (N), P-limitation being

more common (Hecky & Kilham 1988). Humic lakes generally have high total P concentrations, but only part of this P is available to the biota (Jones *et al*. 1988, Jones 1992b). There have been several studies on humic lakes to identify limiting nutrients for phytoplankton growth in them and in some cases P (Arvola *et al*. 1996, Nürnberg & Shaw 1999) and in other cases N (Järvinen 2002, Jansson *et al*. 2001, Pålsson & Graneli 2004) has been found to be the limiting nutrient. Järvinen & Salonen (1998) also reported a change from P- to N-limitation of phytoplankton in a humic lake following a food-web manipulation. Studies of the small humic lakes in the Evo region of southern Finland have revealed that the turnover times for phosphate in these lakes are relatively long, which suggests that the plankton in these lakes probably is not P-limited (Jones *et al*. 1988). A possible reason for this is that phosphate is bound in humus-iron complexes and is slowly released from them which prevents the development of acute P-limitation. This argument is supported by findings of probable co-limitation by both P and N in these lakes, because the nutrient supply seems to be in balance with the needs of phytoplankton (Jones 1990). However, autotrophic and mixotrophic part of the phytoplankton community might be limited by different nutrients (Jansson *et al*. 1996, Lepistö & Saura 1998). Moreover, nutrient limitation may change seasonally (Elser *et al*. 1995) and therefore the nutrient addition experiments were carried out in three seasons during the ice-free period.

My thesis is a part of this larger study of impacts of increased carbon and nutrient loading on humic lake food webs and focuses on the phytoplankton community. The aim of my thesis was to study the potential changes in phytoplankton biovolume and community composition caused by the increase in DOC loading considering the possibility that carbon loading will also be accompanied by increased nutrient loading. Differences in responses of autotrophic and mixotrophic phytoplankton were studied and the share of flagellate algae in the community was also of interest.

2. METHODS

2.1. Study Lake

The study lake, Alinen Mustajärvi (Fig. 2) is a moderately coloured, headwater lake in the Evo region of southern Finland (61^{o}12'N, 25^{o}7'E) about 20 km north from Lammi Biological Station (University of Helsinki). The lake is sheltered, and the small (<0.5 km^{2}) catchment area consists of coniferous forest (>90 %) and peatland (<10 %). The lake has an area of 0.74 ha and volume of 31×10^{3} m^{3}. It has one small outlet but no inlets, and it receives most of its' inflow from groundwater. The mean depth is 4 m and the maximum depth is 6.5 m. The lake is partially meromictic since it does not always mix completely during overturns. The lake stratifies very steeply during summer when only about the upper two metres of the water column usually remains oxic. The mean (± SD) water colour in the epilimnion during the study was 106 ± 12 mg Pt l^{-1} and the natural epilimnetic DOC concentration of the lake is around 10 mg C l^{-1}.

The DOC concentration of the lake was manipulated by monthly additions of cane sugar during the ice-free season (May to October) in 2008 and 2009, and additional inorganic nutrient addition experiments were carried out in small bags in the lake during spring, summer and autumn 2009.

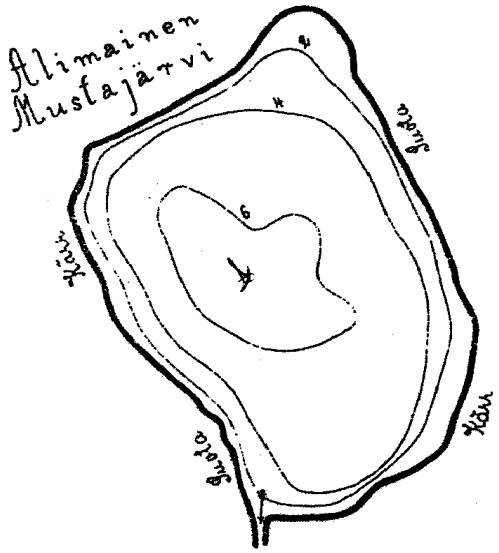

Figure 2. The bathymetric map of Alinen Mustajärvi with depth contours shown for 2 m intervals (Brofeldt 1920).

2.2. Whole-lake manipulation experiment

In the whole-lake manipulation experiment, cane sugar was added to the lake for two years to evaluate the possible impact of predicted future increase in DOC loading. The goal was to increase the amount of DOC available for heterotrophic bacteria without affecting the light climate for autotrophic phytoplankton. About 66 kg of cane sugar was added to the lake every month during the ice-free periods of 2008 and 2009. The amount was calculated to produce an approximately 2 mg l^{-1} rise in the DOC concentration in the epilimnion, intended to raise labile DOC concentration from around the first quartile level for boreal lakes to that in lakes around the third quartile level (Henriksen *et al*. 1998). To make the additions, the sugar was dissolved in lake water in buckets and the sugar-water was then pumped into the surface of the lake from a boat. The aim was to add the sugar as evenly as possible to the epilimnion of the lake.

Water samples for the study of lake phytoplankton were collected every two weeks during the ice-free periods of 2007–2009. The samples taken in 2007 were used to assess the situation in the lake before the manipulation. Integrated samples were collected from epilimnion, metalimnion and hypolimnion. The depths of these layers were judged from vertical profiles of temperature and oxygen recorded *in situ* with an automatic oxygen and temperature sensor (YSI 55 probe, Yellow Springs Instruments) before water sampling. Large volume composite samples were taken with a 30-cm-long acrylic tube sampler (Limnos, about 2 l) from each depth layer, thoroughly mixed in a bucket, and 200 ml sub-samples were taken. The phytoplankton samples were preserved with acid Lugol's solution and kept cold and dark.

Water temperature and dissolved oxygen were measured during sampling. Chlorophyll *a*, phosphorus (PO_4^--P, total P) and nitrogen (NH_4^+-N, NO_2^- + NO_3^--N, total N), DOC, pH and water colour analyses were carried out in the laboratory of Lammi Biological Station by the technical staff using standard analytical methods (http://www.sfs.fi). Weighted averages for epilimnion and metalimnion values were calculated for chlorophyll *a*, nutrients, and pH to better correspond to the phytoplankton samples (see below). Light penetration into the lake was measured *in situ* once in 2007

with an automatic sensor (LI-193, LI-COR). Also precipitation data were available from Lammi Biological Station, which is situated 20 km south from the lake.

2.3. Nutrient addition experiments

Nutrient addition experiments were carried out on three occasions (at the beginning of May, July and September) during the open water season of 2009. The spring experiment (May) was conducted right after ice-break, the summer experiment (July) during warm water and stable temperature and oxygen stratification, and the autumn experiment during autumn overturn. Experiments were initiated about 24 hours after the monthly cane sugar addition to the lake.

A large volume composite sample was collected with a Limnos sampler from depths of 0 m, 1 m and 1.5 m from the epilimnion and filtered with a plankton net (mesh size 50 µm) to exclude large zooplankton. An initial sub-sample (day 0 sample) was taken and the remaining water was then divided between four tubs. Phosphorus (as KH_2PO_4) was added into one tub, one was enriched with nitrogen (as NH_4NO_3), and one with both P and N. One tub served as a control with no nutrient additions. Nutrients were adjusted to carbon concentration according to the Redfield atomic ratio of 106C:16N:1P (Reynolds 2006), with the target rise in concentrations being 0.3 mg l^{-1} for N and 0.02 mg l^{-1} for P. Six transparent polypropylene bags were filled with 2 L of water from each of the tubs (24 bags in total). All air was excluded and the bags were sealed tightly. The bags were bound into bundles with one bag from each treatment (in total four bags in one bundle) to ensure that the environmental conditions (for example water temperature and light climate) were similar between treatments. The bundles were incubated *in situ* at a depth of 0.5 m, which approximated the effective light climate in the water column. Half of the bags (three replicates from each treatment) were collected on day four and half on day seven of the experiment. The phytoplankton samples were preserved with acid Lugol's solution and the same environmental measurements were made from the bags as from the lake samples. Phytoplankton samples were analyzed only from the day 0 and day 7 samples, assuming that it takes longer for the phytoplankton community to react to the nutrient additions than, for example, the bacteria.

2.4. Preparing and analyzing the phytoplankton samples

Samples from the nutrient addition experiments were prepared as follows. The sample bottle was shaken for 1–2 minutes and a 15–25 ml subsample was measured with a graduated cylinder. The size of the subsample depended on the density of the sample. The subsample was poured into a settling chamber and the graduated cylinder was rinsed twice. The settling chamber was then filled with water and a circular glass cover was slid onto it. The samples were left to sediment overnight. For each experiment, the sample from day 0 and three replicates of each treatment from day 7 were counted, making 39 samples in total.

Preliminary checks of lake samples indicated substantial relative proportions of phytoplankton in the metalimnion samples. Therefore, epilimnion and metalimnion samples from the lake were pooled to reduce the counting and to provide a better overview of the entire phytoplankton community in the water column. The amounts of the samples were weighted according to the lengths of the depth zones from which they had been collected. For example, in 2007 composite epilimnion samples were taken from depths of 0, 1 and 2 m, and composite metalimnion samples from 3 and 4 m, so in the mixed sample there were three parts of the epilimnion sample and two parts of metalimnion sample. The bottles were shaken and 2 x 6 ml = 12 ml (epilimnion) and 2 x 4 ml = 8 ml (metalimnion)

was measured with a Finn pipette, giving a mixed sample of 20 ml. The sample was then prepared as described for the samples from the bag experiments. Two samples were counted from each month (May-October) from the control year 2007 and one sample per month from the two manipulation years, making 24 samples in total.

The samples were counted using two different inverted microscopes, an Olympus 1X50 or Wild M40. 50–100 random fields were counted with a magnification of x400–600; at least 500 counting units (cells, colonies or filaments) in total and at least 50 units of each of the most common species were counted. Phytoplankton was identified to species if that was possible within a reasonable time; if not, the genus or a higher taxon name was recorded. Some plankters, especially the smallest ones, were recorded only as "unidentified", "unidentified flagellate" or "unidentified non-flagellate". Identification mainly followed Tikkanen (1986) although various more specialist works were also consulted. Phytoplankton taxa were divided into autotrophs and potential mixotrophs (referred as mixotrophs hereafter) according to the literature (Table 1). Some small heterotrophic flagellates, which were of similar size to phytoplankton, were also counted, but not ciliates. All the plankters were measured and divided into size classes, and the volumes were defined according to the Phytoplankton Register of the Finnish Environment Institute (SYKE). The densities (ind. l^{-1}) and biovolumes (mm^3 l^{-1}) were calculated using the formulae in Lepistö *et al*. (2006).

Table 1. The phytoplankton genera recorded in the samples that were considered capable of phagotrophy according to Isaksson (1998) and Rengefors *et al*. (2008).

CHRYSOPHYCEAE	DINOPHYCEAE
Chrysococcus	*Gymnodinium*
Dinobryon	*Peridinium*
Pseudopedinella	
Spiniferomonas	CRYPTOPHYCEAE
Uroglena	*Cryptomonas*
	RAPHIDOPHYCEAE
	Gonyostomum [1]

[1] osmotrophic

2.5. Statistical tests

Differences in phytoplankton biovolume, as well as in autotroph, mixotroph and heterotroph biovolume and in different taxonomic group biovolume, between treatments in the nutrient addition experiments were tested statistically. The day 0 sample could not be included in testing because there were no replicate samples. Differences in total phytoplankton biovolume on day 7 were also tested between the experiments. Since all the assumptions for using a parametric test (ANOVA) were not met, the Kruskal-Wallis non-parametric test of variance was used and pair-wise comparisons were made with the Mann-Whitney U-test. The results were considered to be statistically significant if $p \leq 0.05$. The lake data could not be tested statistically, as only a single count was available for each date.

The following abbreviations for taxonomic groups are used hereafter in figures and tables: chl-a=chlorophyll *a*, phyto=total phytoplankton, auto=autotrophs, mixo=mixotrophs, hetero=heterotrophs, cyano=cyanophytes, crypto=cryptophytes, dino=dinophytes, chryso=chrysophytes, raphido=raphidophytes, chloro=chlorophytes and other=unidentified phytoplankton + euglenophytes + xanthophytes.

3. RESULTS

3.1. Whole-lake manipulation experiment

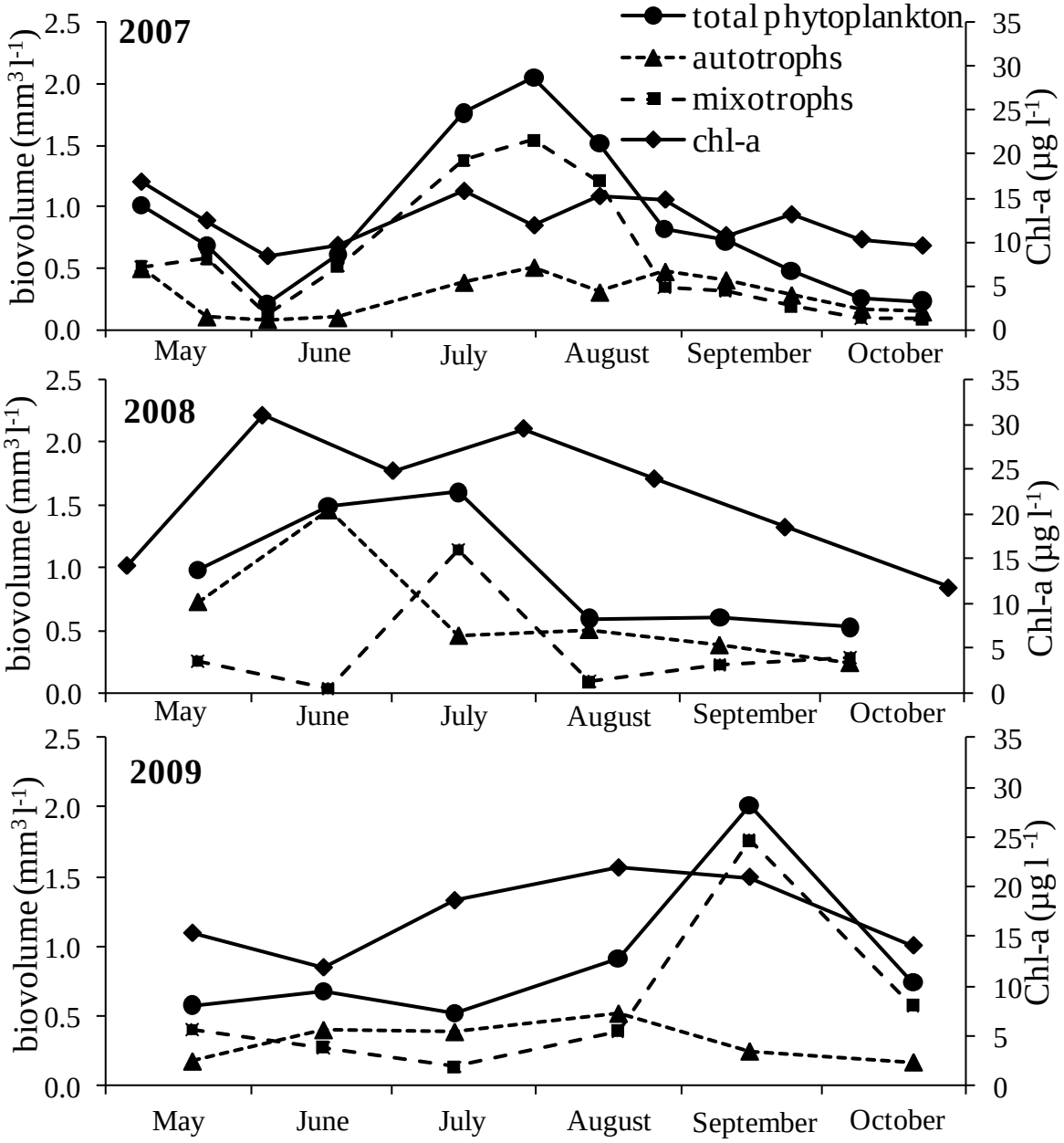

Figure 3. Total phytoplankton biovolume, and autotroph and mixotroph biovolume, and the concentration of chlorophyll *a* during the sampling period of May-October in the control year (2007) and in the two manipulation years (2008 & 2009) in Alinen Mustajärvi.

In total, 60 taxa of phytoplankton and 7 of heterotrophic flagellates were found in the samples. The most diverse groups of phytoplankton were chlorophytes (23 taxa),

chrysophytes (16) and cyanophytes (12). 24 of the phytoplankton taxa were flagellates. Complete species lists in each sample are given in the supplementary data (CD).

The phytoplankton biovolume varied tenfold between 0.21 $mm^3 l^{-1}$ and 2.05 $mm^3 l^{-1}$ during the sampling period from May to October in 2007–2009 (Fig. 3). The highest measured biovolumes were similar in all the years but the timing of the biovolume maxima differed. In the control year (2007), the first biovolume peak seemed to be in the spring and the highest point might have occurred already before the sampling started on 8 May. The second (and higher) maximum was at the end of July. In the two manipulation years (2008 and 2009) the seasonal development of biovolume was rather different and only one biovolume maximum was detected, in June-July of 2008 and in September of 2009.

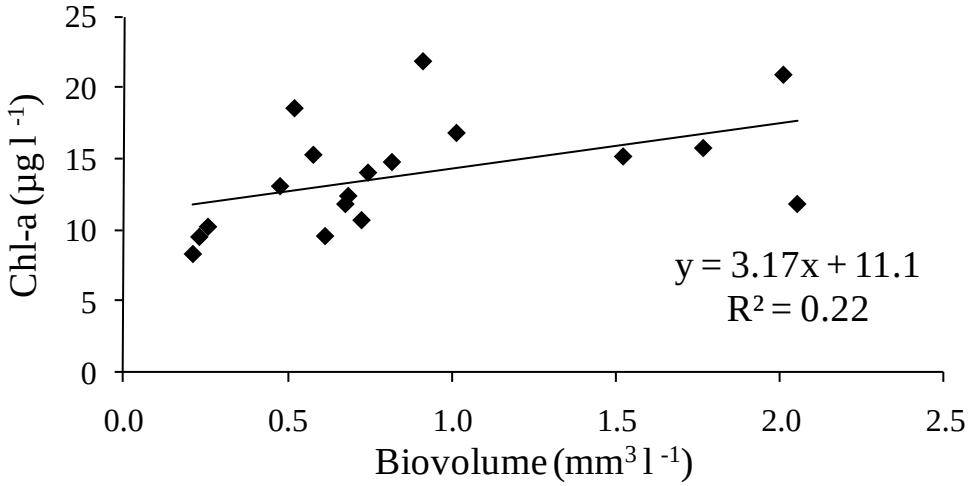

Figure 4. The correlation between total phytoplankton biovolume and chlorophyll *a* in the lake samples (excluding the year 2008).

The chlorophyll *a* concentration showed a similar seasonal variation as the phytoplankton biovolume, the range being 8–31 µg l^{-1} (Fig. 3). The chlorophyll *a* concentrations measured in 2008 were somewhat higher than in 2007 or 2009, but the biovolumes did not show a similar pattern. Very high chlorophyll *a* values have been measured in the hypolimnion of Alinen Mustajärvi (data not shown). Comparing the chlorophyll *a* concentration to the phytoplankton biovolume was not possible for all the data, because the chlorophyll results for the year 2008 were from different dates than the counted phytoplankton samples. For the rest of the data, it seems that there is a significant positive correlation (Fig. 4), but that it is not very strong, the R^2-value being only 0.22 (Linear regression: R=0.47, F=4.594, P=0.048, n=18).

Mixotrophs were the dominant component of the phytoplankton community in 2007 (Fig. 3), but their biovolume decreased in the autumn to below the biovolume of autotrophs. The biovolume of autotrophs was more constant in 2007 compared to mixotrophs. The seasonal variation of autotroph and mixotroph biovolumes was rather different between the two manipulation years. In 2008 the maximum autotroph biovolume occurred in June when the sample consisted almost entirely of autotrophs. This high autotroph biovolume in June was mainly due to high abundance of the autotrophic chrysophyte *Mallomonas lychenensis*. The biovolume of mixotrophs peaked in July, again due to high abundance of a chrysophyte species, but this time the mixotrophic *Chrysococcus* sp. In 2009 the biovolume of autotrophs was at a rather constant level, as in 2007, but the biovolume of mixotrophs varied much more. The mixotroph biovolume was low during the first months of sampling and the maximum occurred in September, mainly due to high abundance of the mixotrophic raphidophyte *Gonyostomum semen* (Fig. 5). Two

weeks prior to the sampling in September, there were algal mats of different sizes floating on the surface of the lake. The mats consisted mostly of *G. semen* and a chlorophyte *Oocystis* sp., and the phytoplankton biovolume in the mats was 15–100 times the biovolumes in the regular lake samples. These algal mats were very short-lived and disappeared within 24 hours.

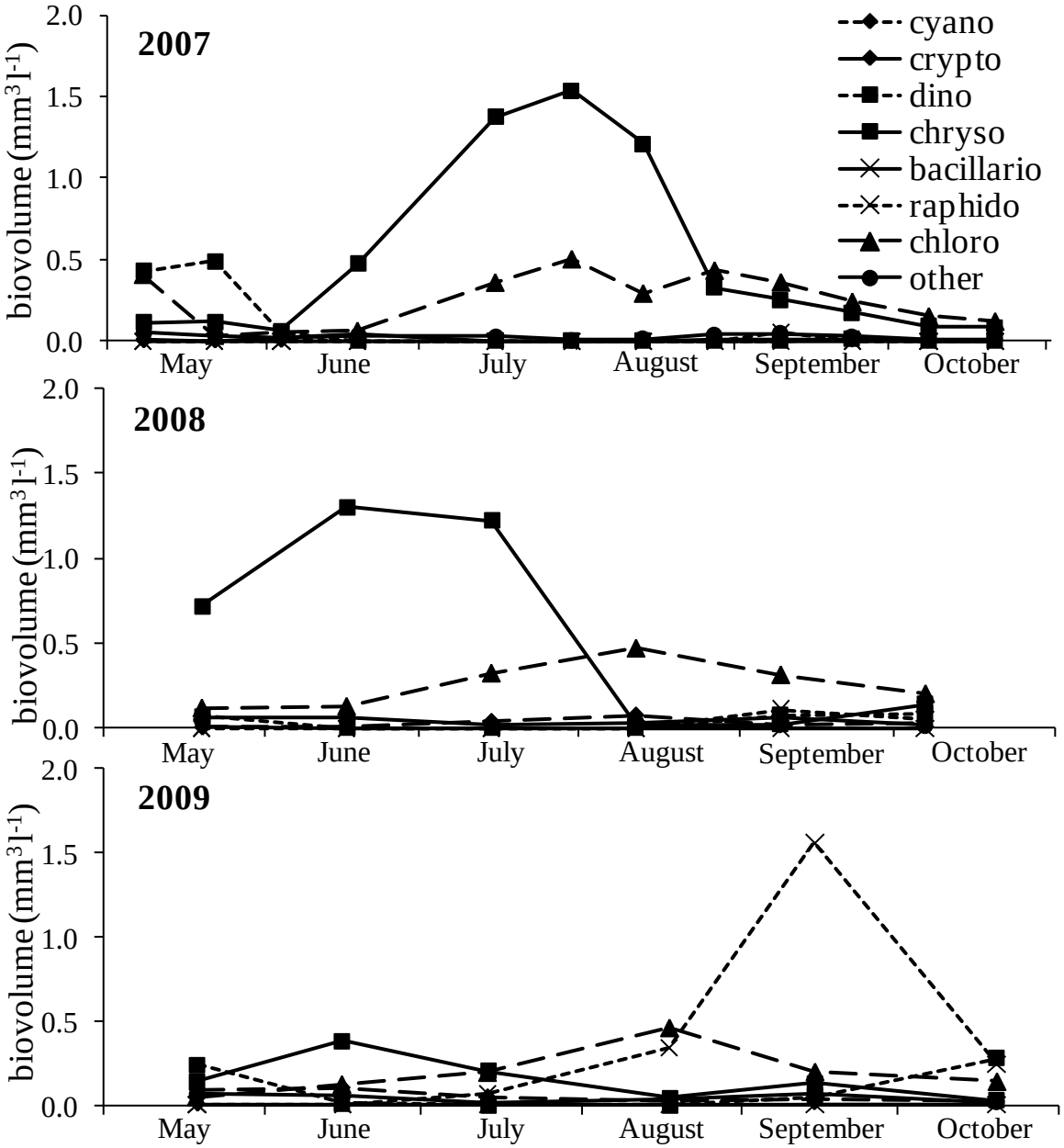

Figure 5. The biovolumes of different phytoplankton taxonomic groups during the sampling period of May-October in the control year (2007) and in the two manipulation years (2008 & 2009) in Alinen Mustajärvi.

The phytoplankton community in 2007 and 2008 was dominated by chrysophytes for most of the summer but they were less abundant in 2009 (Fig. 5). The most abundant chrysophytes were *Pseudopedinella* sp., *Chrysococcus* sp. and *Mallomonas lychenensis*. *Chrysococcus* sp. was abundant mostly in the control year and in July 2008, and *M. lychenensis* was detected only in the two manipulation years. Dinophytes were an important group in the spring of 2007 and 2009. Chlorophytes contributed significantly and constantly to the community in all the years, and the highest chlorophyte biovolumes

(about 0.5 mm^3 l^{-1}) were measured in July or August. The most abundant chlorophytes were *Chlamydomonas* sp., *Koliella longiseta*, *Scourfieldia cordiformis* and *Oocystis* sp. Diatoms, cryptophytes and cyanophytes were not abundant.

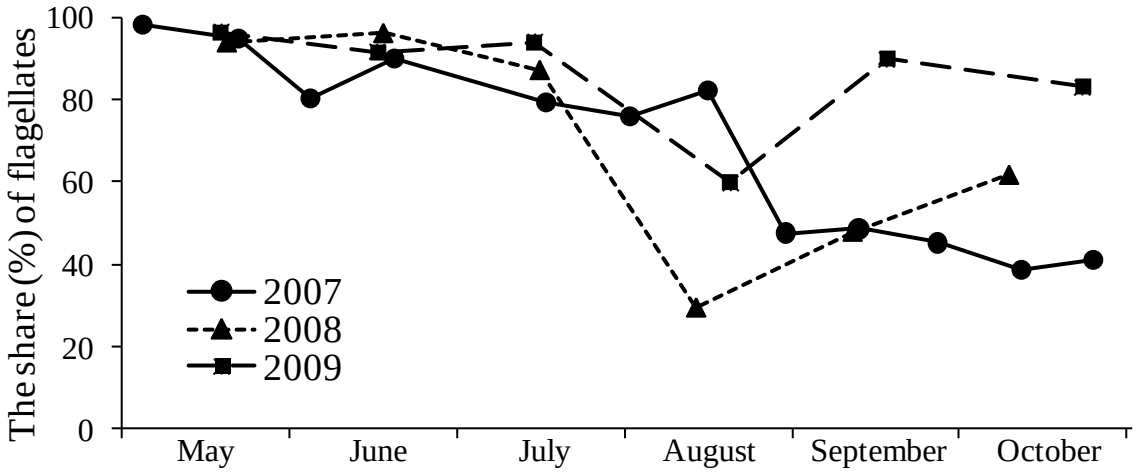

Figure 6. The relative contribution of flagellate algae to phytoplankton biovolume during May-October in the control year (2007) and in the two manipulation years (2008 and 2009) in Alinen Mustajärvi.

Less than half (24) of the phytoplankton taxa were flagellates, but the taxa which contributed most to the biovolume were flagellates. All chrysophytes, cryptophytes and dinophytes are flagellates and also some chlorophytes. The share of flagellates in phytoplankton biovolume varied from 30 to 98 %, high values being found at the beginning of the sampling period and lower values in August-October (Fig. 6). There were no notable differences in the contribution of flagellates to phytoplankton biovolume in different years, although the share of flagellates in 2009 remained high even during the autumn. Again, this reflects the abundance of *G. semen* at this time.

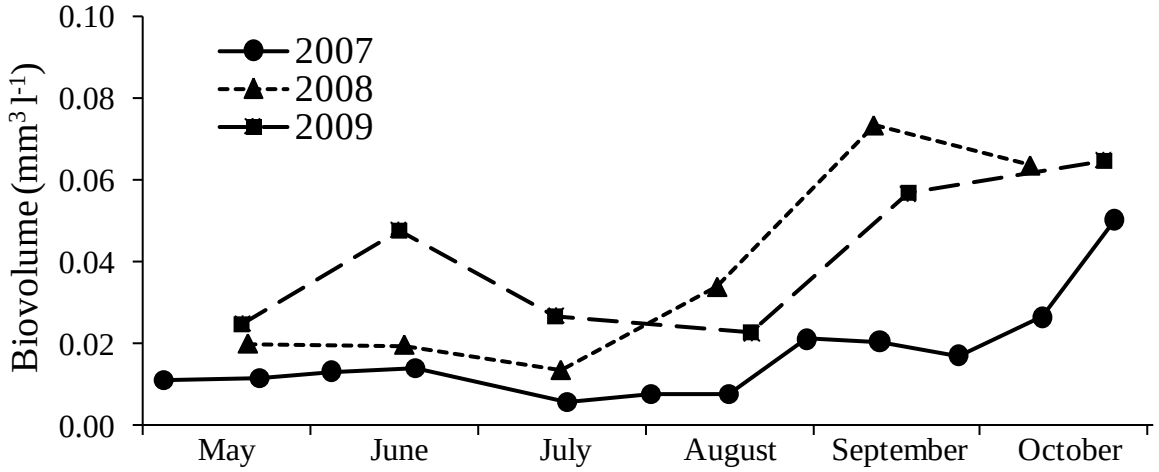

Figure 7. The biovolume of small heterotrophic flagellates during May-October in the control year (2007) and in the two manipulation years (2008 and 2009) in Alinen Mustajärvi.

The abundance of small heterotrophic flagellates ranged from 90 x 10^3 to 2000 x 10^3 cells l^{-1}. Their biovolume was much lower than the biovolume of phytoplankton and varied between 0.01 and 0.07 mm^3 l^{-1}. The most abundant taxa of the heterotrophs were *Monomastix* sp., *Bicosoeca* spp., *Petalomonas* sp. and *Katablepharis* sp. The biovolume of the heterotrophic flagellates increased during the manipulation (Fig. 7). The highest

biovolumes of heterotrophic flagellates were always measured in September or October as well as highest abundances (data not shown).

3.2. Environmental conditions

The DOC concentration in the epilimnion of the lake did not show marked seasonal variation (Fig. 8). The goal of raising the DOC concentration by 2 mg l^{-1} was attained during the manipulation. There was no change in the water colour of the epilimnion (data not shown).

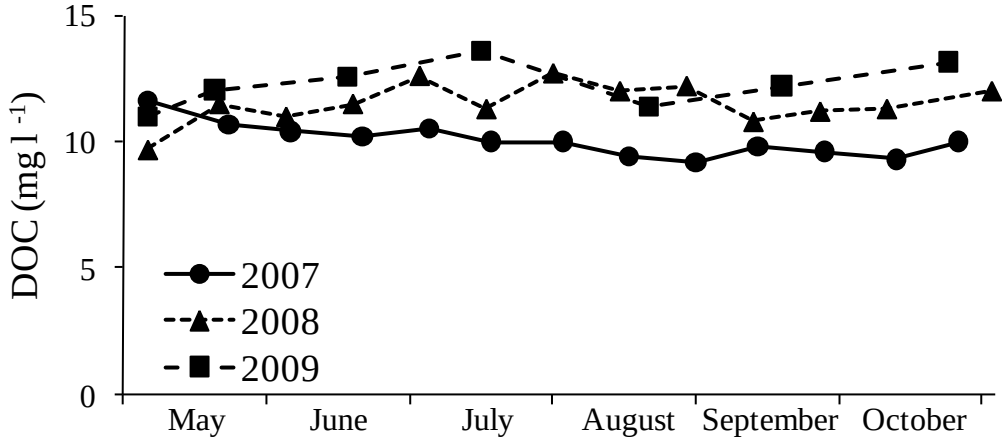

Figure 8. Dissolved organic carbon (DOC) concentration in the epilimnion of Alinen Mustajärvi in the control year (2007) and in the two manipulation years (2008 and 2009).

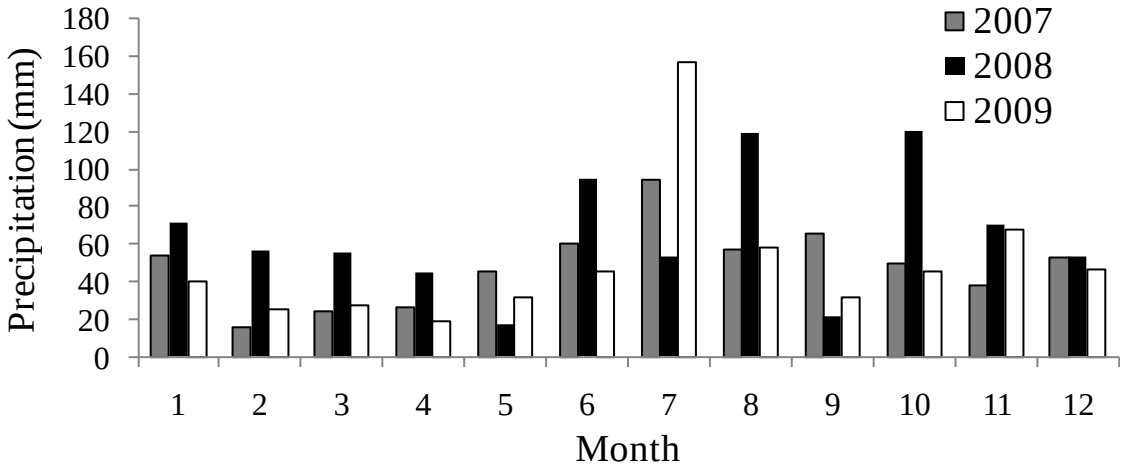

Figure 9. Monthly precipitation (mm) during 2007–2009 measured at Lammi Biological Station.

Monthly precipitation measured at nearby Lammi Biological Station varied between the years (Fig. 9). The years 2007 and 2009 were similar with respect to precipitation (total precipitation 585 and 594 mm respectively) but in 2008 the total precipitation was markedly higher (778 mm). July 2009 was a particularly rainy month and there were heavy rains just before the phytoplankton sampling on 14 July.

The lake was stratified, regarding temperature and dissolved oxygen, already at the start of sampling in May (Fig. 10a)). Highest epilimnetic water temperatures (around 20^{o}C) were measured in July or August and during summer stagnation the anoxic layer started at a depth of about 2 m (Fig. 10b)). The deepest part of the lake does not mix completely every year even during autumn overturn (Fig. 10c)). It is possible that the mixing reached the deepest part of the lake after the sampling in October, since the lake did not freeze until

November or December during 2007–2009. The stratification and mixing events seem to be similar in all the years, but some variation between years was evident during the first sampling of the year. The epilimnion of the lake was slightly shallower during the summer of 2009 than in the previous two summers.

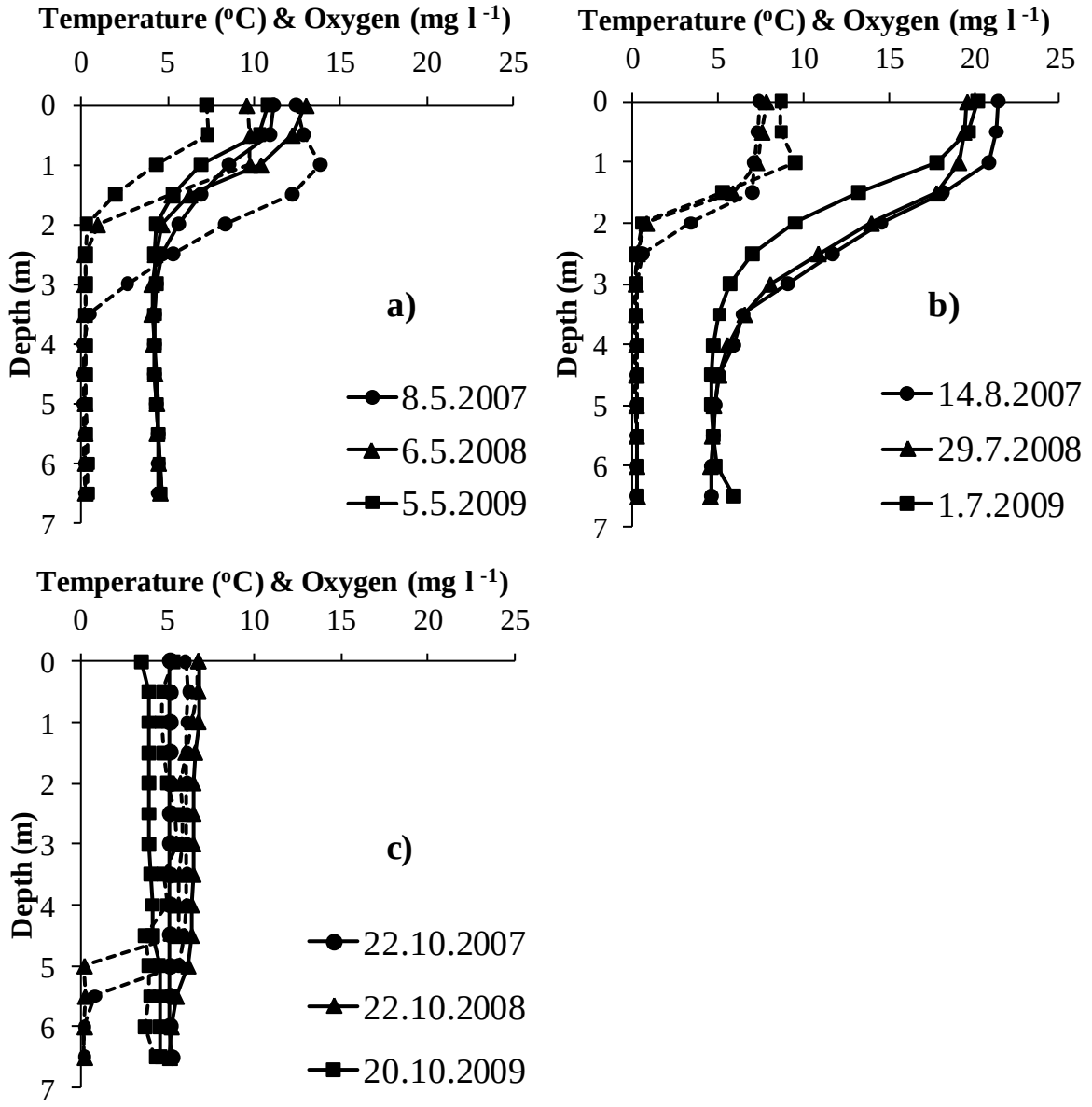

Figure 10. Vertical profiles of temperature (solid line) and dissolved oxygen (dashed line) in Alinen Mustajärvi during a) the first sampling after ice-break, b) the highest water temperature and c) the autumn overturn in 2007–2009.

Light attenuation in Alinen Mustajärvi was measured once in 2007 during summer stratification (Fig. 11). The decrease in photosynthetic photon flux (PPF) is quite rapid and below a depth of 2 m there is practically no photosynthetically active radiation. The euphotic zone depth (measured as the depth where radiation is 1 % of the surface value) is approximately 2 m, so the photic zone approximately coincides with the epilimnion during the summer stratification.

The variation in nutrient concentrations was similar in all the years (Fig. 12). Concentrations were higher in the spring and decreased during the summer. The concentrations in 2008 seemed to be higher at the beginning of the sampling period

compared to the years 2007 and 2009. There was a marked rise in the nitrite plus nitrate (NO_2^- + NO_3^--N) concentration in July or in August in all the years. The rise was seen in epilimnetic values (2009) or in both epilimnetic and metalimnetic values (data not shown).

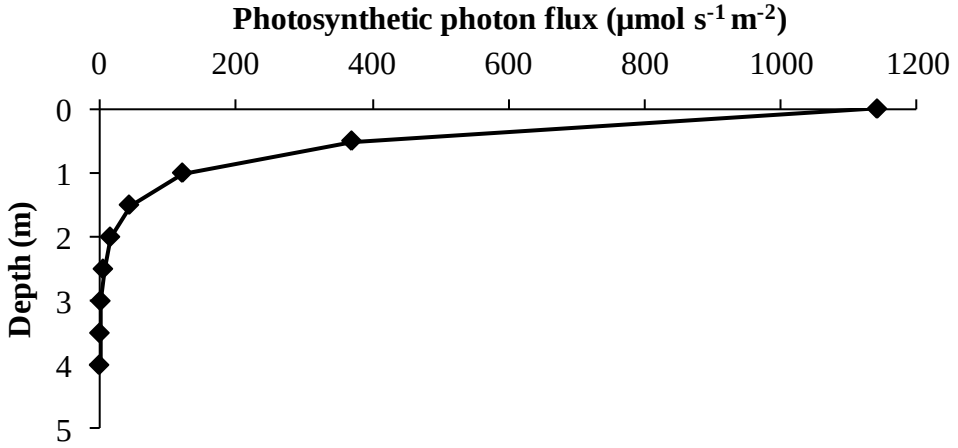

Figure 11. Photosynthetic photon flux (PPF) in Alinen Mustajärvi on 20.7.2007.

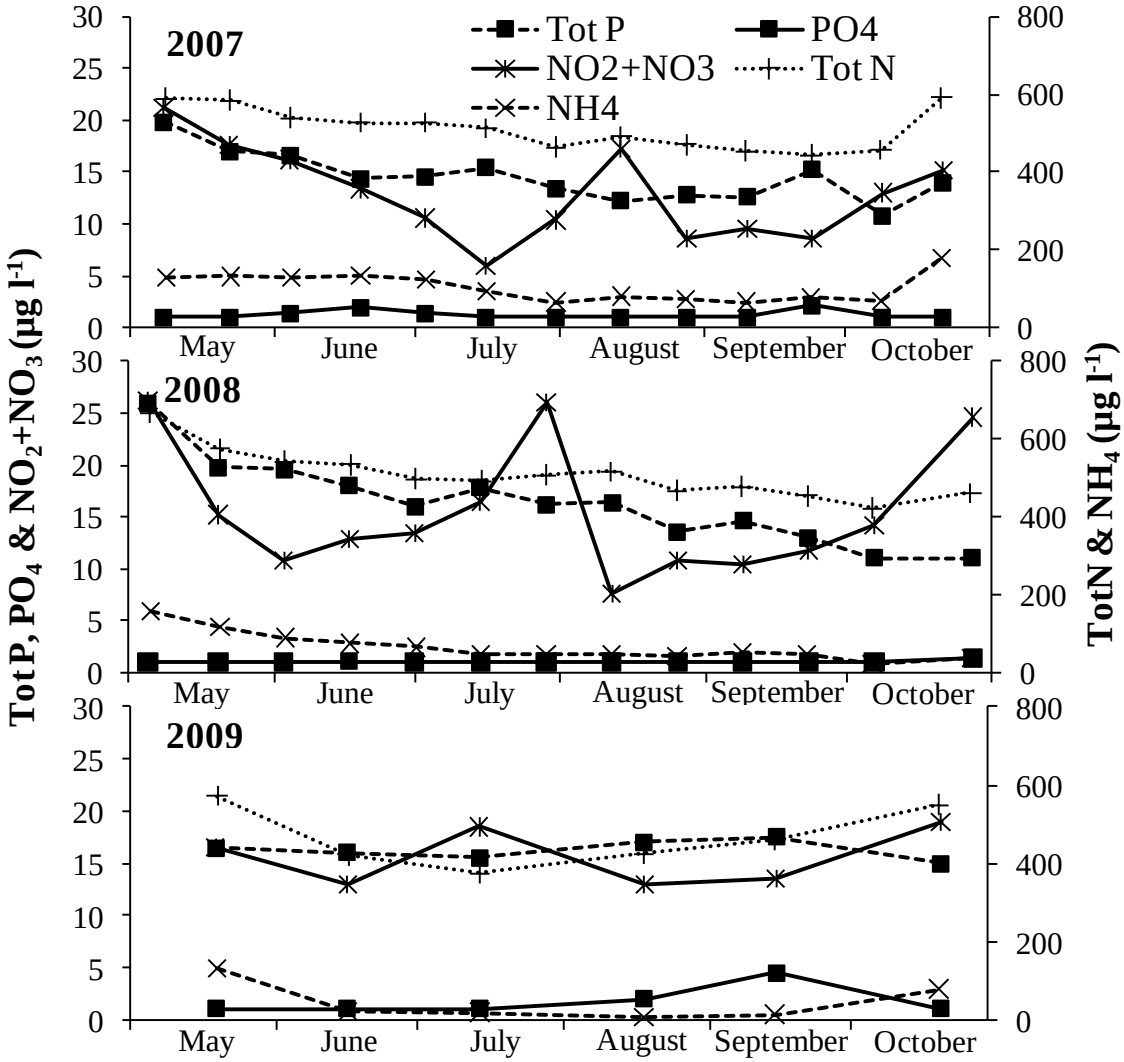

Figure 12. The weighted averages for epilimnetic and metalimnetic concentrations of phosphate phosphorus (PO_4^-), total phosphorus (TotP), ammonium nitrogen (NH_4^+), nitrate and nitrite nitrogen (NO_2^- + NO_3^-) and total nitrogen (TotN) during May-October in 2007–2009 in Alinen Mustajärvi.

3.3. Nutrient addition experiments

The concentrations of nutrients at the beginning of the summer experiment were somewhat lower than in the other experiments (see Appendix 1 for the nutrient concentrations in the experiments). In all the experiments the phosphate (PO_4^--P) concentration was low in treatments without P addition (control and the N). Phosphate was consumed by day 7 in the N+P treatment in all the experiments but only in autumn in the P treatment, while in the spring and summer experiments only part of the phosphate in the P treatment was used. Ammonium (NH_4^+-N) concentration was higher at the beginning of the spring experiment than in the summer or autumn experiments, but by day 7 it was consumed in treatments other than the N. In the summer experiment the concentration of ammonium decreased somewhat by day 7 in the N treatment and was undetectable in the N+P treatment. In the autumn experiment the ammonium concentration decreased to a low level in both the N and N+P treatments. Little nitrite and nitrate (NO_2^- + NO_3^--N) usage was found in the experiments; only in the N+P treatments and in the N treatment of the autumn experiment was the concentration much lower on day 7 than on day 0.

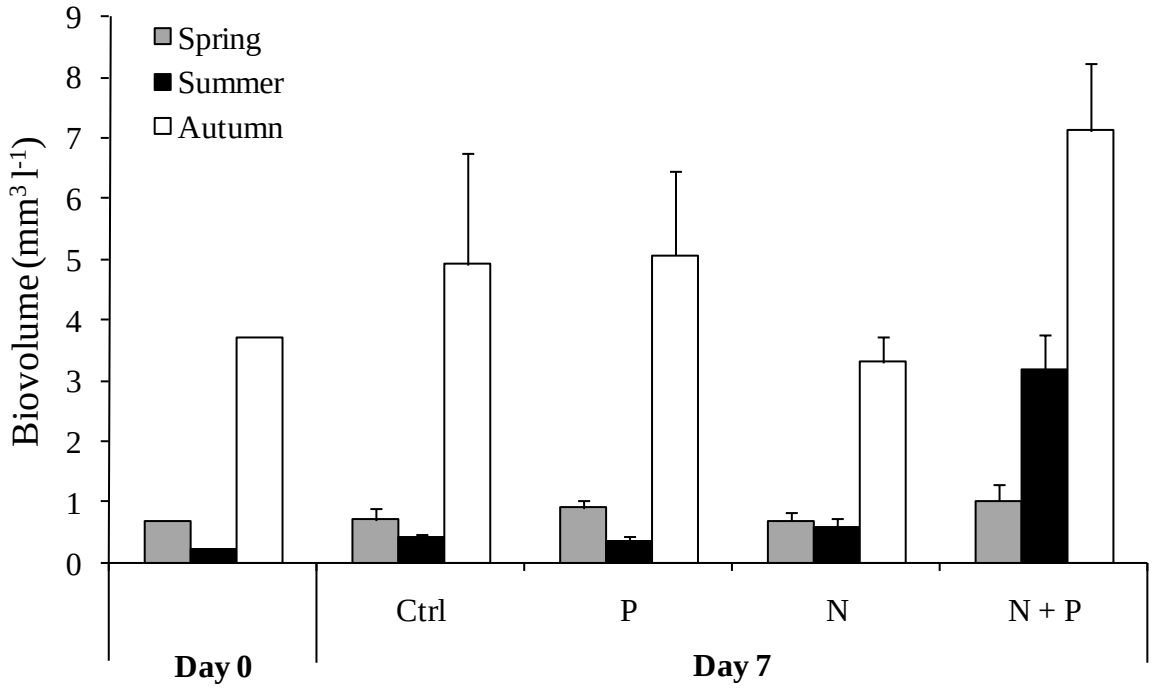

Figure 13. The biovolume of phytoplankton at the beginning of the experiment and on day 7 in different treatments. The day seven values are means of three replicates and error bars represent standard deviation.

The overall day 7 biovolume of phytoplankton varied between the three experiments (Kruskal-Wallis: $P<0.001$, $\chi 2=22.541$, $N=36$) (Fig. 13). The biovolume was higher in the autumn bag experiment than in the spring (Mann-Whitney U: $P<0.001$, $Z=-4.157$, $N=24$) or in the summer (Mann-Whitney U: $P<0.001$, $Z=-3.811$, $N=24$), mainly due to the high abundance of the raphidophyte *G. semen*. There was no difference between the spring and summer experiments.

There was a strong positive correlation between phytoplankton biovolume and chlorophyll *a* concentration (Fig. 14) in the nutrient addition experiments (Linear regression: $R=0.90$, $F=153.07$, $P<0.001$, $n=39$). The biovolume of phytoplankton explains 81 % of the variation in chlorophyll *a* values. The number of observations and the variation

in chlorophyll *a* concentration and in phytoplankton biovolume was much greater in the bag experiment data than in the lake data (Fig 4.).

No statistically significant differences between the treatments were found in phytoplankton biovolume, or in autotroph and mixotroph biovolume in the spring experiment (Table 2). Autotroph biovolume in both the P and N treatments were at almost the same level as the control, and biovolume in the N+P treatment was higher than in the other treatments (Fig. 15). It seems that the autotroph biovolume in all the treatments was higher than in the day 0 sample. The biovolume of mixotrophs was similar in all treatments and in the initial sample.

The chlorophyll *a* concentration on the other hand differed significantly between treatments in the spring experiment (Table 2, Fig. 15). No difference was found between the control and the N, but in both of them the concentration was significantly lower than in the P or the N+P treatments (see Appendix A2 for pair-wise comparisons). In the summer and autumn experiments the chlorophyll *a* concentration showed the same pattern as in the spring; the concentration in the P treatment was higher than in the control or the N and highest in the N+P treatment. The trend in the chlorophyll *a* concentration was the same in all the three experiments but the level differed. The highest chlorophyll *a* values were measured in the autumn and the lowest in the spring experiment.

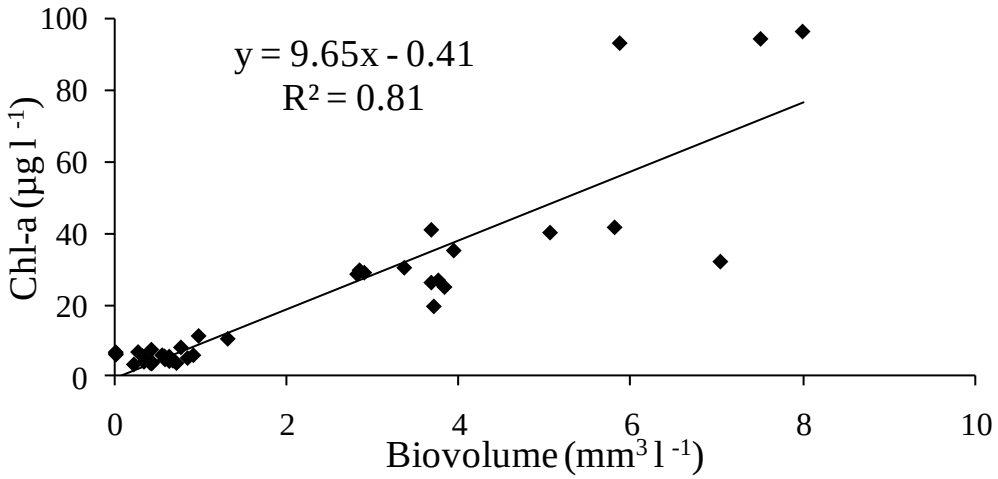

Figure 14. The correlation between total phytoplankton biovolume and the chlorophyll *a* concentration in the nutrient addition experiments.

The biovolume of cyanophytes differed significantly between the treatments in the spring experiment (Table 2). Biovolume was highest in the N+P treatment, and also significantly higher in the P treatment than in the control or N treatments (Appendix A2). There was no difference between the control and N treatments. There was a suppression of cryptophytes (mostly *Cryptomonas* sp.) during the spring experiment (Fig. 16) in all the treatments in relation to day 0. The differences between the treatments were not significant. The differences in dinophytes, chrysophyte, raphidophytes, chlorophytes and other phytoplankton were not significant. The relatively high biovolume of the group other, especially in the N+P treatment, consisted mostly of small unidentified flagellates.

In the summer experiment there was a statistically significant difference between treatments in the biovolumes of phytoplankton, autotrophs, mixotrophs, cyanophytes, chrysophytes, raphidophytes, chlorophytes and other phytoplankton, as well as in the concentration of chlorophyll *a* (Table 2). Only for cryptophytes and dinophytes were the

differences between treatments not significant but in both these cases the biovolumes were low. The general trend is that the N+P treatment differs from the other treatments.

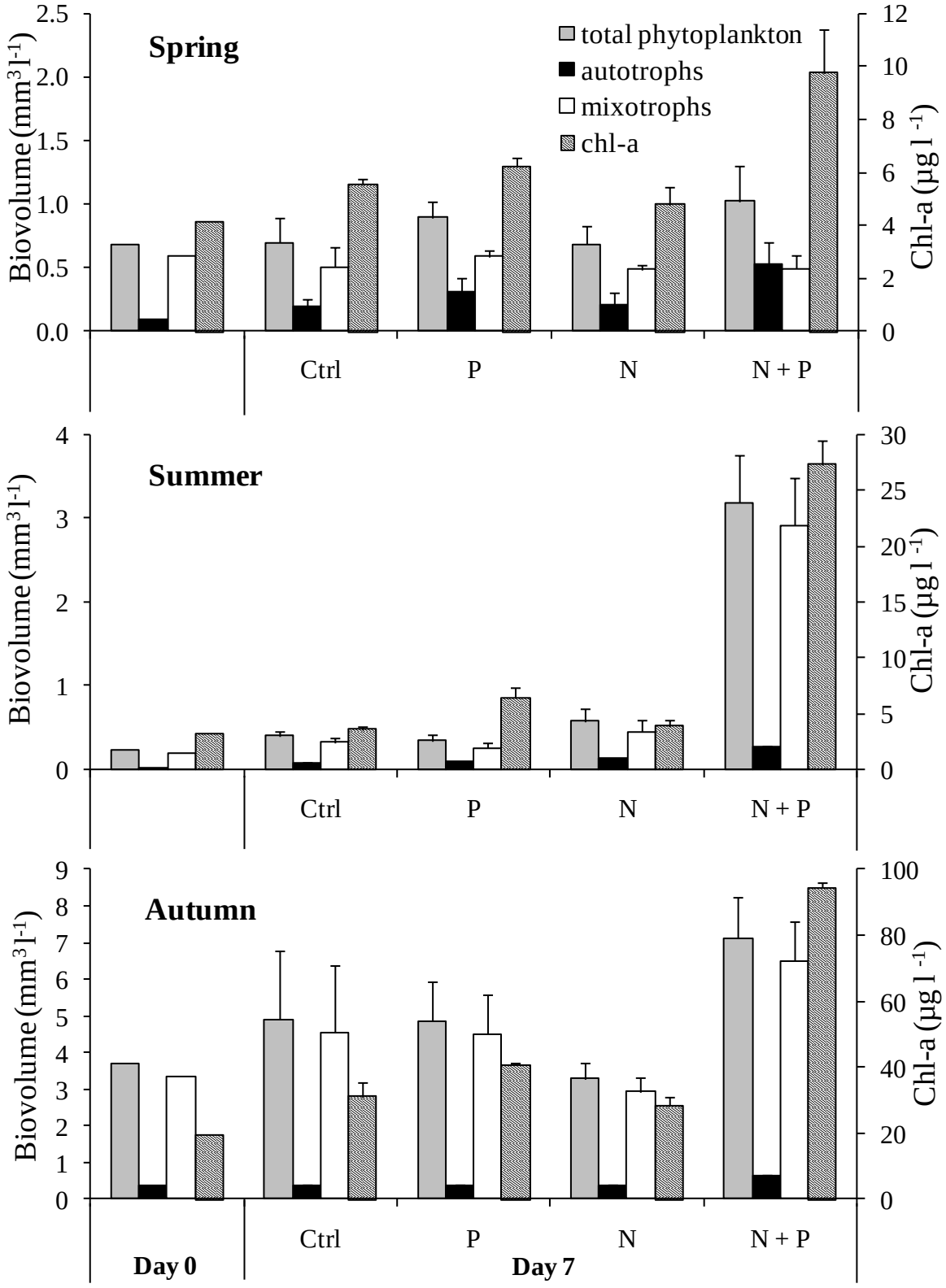

Figure 15. The biovolume of phytoplankton, autotrophs and mixotrophs as well as chlorophyll *a* concentration in the three nutrient addition experiments. The day seven values are means of three replicates and error bars represent standard deviation.

Table 2. Kruskal-Wallis tests of the biovolume for differences in the biovolumes of phytoplankton, mixotrophs, autotrophs, heterotrophs and different taxonomic groups and chlorophyll *a* concentration between treatments in the three experiments. Significant P-values denoted with *.

	Spring			Summer			Autumn		
Variable	P	χ^2	N	P	χ^2	N	P	χ^2	N
Chl-a	0.018*	10.009	12	0.025*	9.359	12	0.022*	9.667	12
Phyto	0.147	5.359	12	0.038*	8.436	12	0.059	7.462	12
Auto	0.069	7.103	12	0.025*	9.359	12	0.099	6.282	12
Mixo	0.478	2.487	12	0.038*	8.436	12	0.043*	8.128	12
Hetero	0.022*	9.667	12	0.022*	9.667	12	0.082	6.692	12
Cyano	0.025*	9.359	12	0.025*	9.359	12	0.106	6.116	12
Crypto	0.086	6.590	12	0.086	6.600	12	0.086	6.590	12
Dino	0.668	1.564	12	0.880	0.670	12	0.468	2.538	12
Chryso	0.516	2.282	12	0.033*	8.744	12	0.050*	7.821	12
Raphido	0.136	5.546	12	0.041*	8.231	12	0.072	7.000	12
Chloro	0.063	7.308	12	0.024*	9.462	12	0.060	7.423	12
Other	0.141	5.462	12	0.041*	8.231	12	0.137	5.520	12

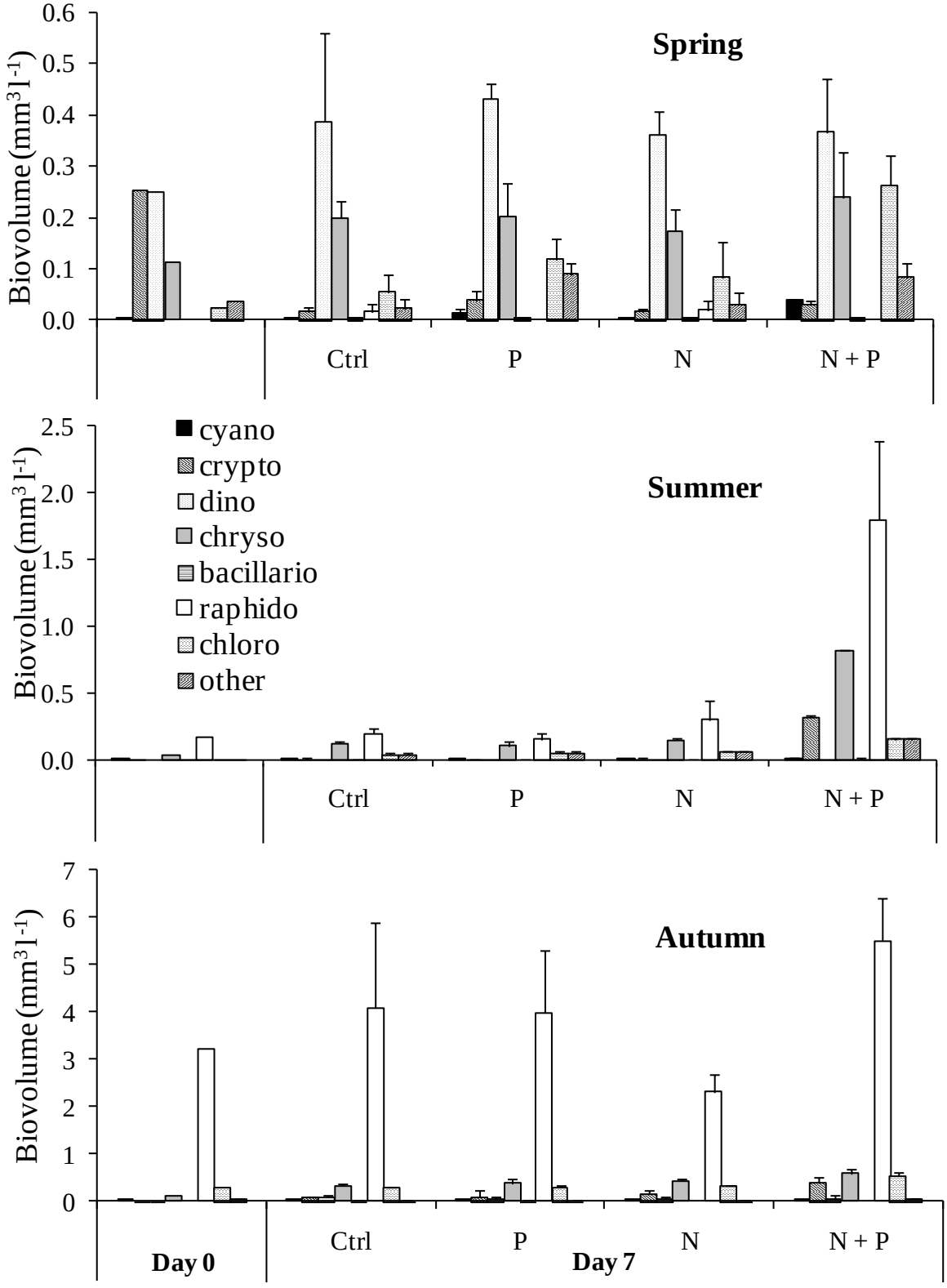

Figure 16. The biovolumes of different phytoplankton taxonomic groups in the three nutrient addition experiments. The day seven values are means of three replicates and error bars represent standard deviation.

The biovolume of autotrophs was generally lower in the summer than in the spring experiment (Fig. 15). Here again, it seems that the biovolume of autotrophs increased in all treatments in relation to the day 0 sample. In the N treatment their biovolumes differed

from other treatments and no difference was found between the control and the P treatment (Appendix A2). The addition of both P and N resulted in the highest biovolume. The mixotrophs formed the main part of the community during the summer experiment and responded strongly (and only) to the N+P addition.

The largest group of mixotrophs (and also of phytoplankton) during the summer experiment was raphidophytes (Fig. 16), with just one species: *Gonyostomum semen*. *G. semen* was also present in the lake samples at the time but was not as abundant as in the experiment (Fig. 6). Another important group was the chrysophytes. They followed the general pattern of the highest biovolume being found in the N+P treatment, but there was also a difference between the P and the N treatments, although neither of them differed from the control. The most abundant chrysophyte species during the experiment were *Dinobryon divergens* and *Pseudopedinella* sp, both of which are mixotrophic. The mean biovolume of cryptophytes also increased in the N+P treatment (biovolume 50 times of that in the control), but the increase was not statistically significantly and in other treatments it was barely detectable (Fig. 16).

The overall phytoplankton biovolume in the autumn experiment was very high in all the treatments and already in the day 0 sample (Fig. 15). The high biovolume was caused by high abundance of the raphidophyte *G. semen*; the biovolumes of other groups were similar to the spring or summer experiments. No significant differences were found between the treatments, but there seemed to be some tendency for the N+P to differ from the N treatment. The variance between the three replicates was high, which makes it harder to identify true differences.

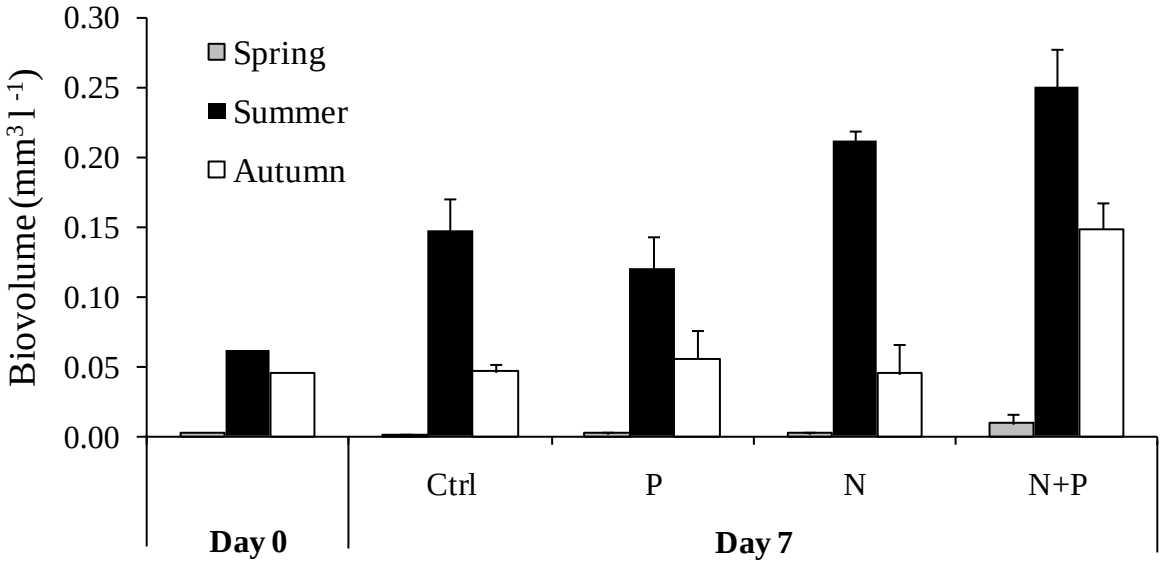

Figure 17. The biovolume of small heterotrophic flagellates in the three bag experiments. The day seven values are means of three replicates and error bars represent standard deviation.

The biovolume of autotrophs in the autumn experiment was similar as in the spring experiment and no significant differences were found between the treatments (Fig. 15). The biovolume of mixotrophs (comprising mostly *G. semen*) in the N treatment was significantly lower than in all the other treatments. The growth of chrysophytes, on the other hand, was significantly stimulated in the N treatment, and even more in the N+P (Table 1) (Fig. 16). The dominant species of chrysophytes during the autumn experiment was *Pseudopedinella* sp. As in the summer experiment, the mean biovolume of cryptophytes seemed to increase, but the increase was not statistically significantly, in the

N+P treatment (Fig. 16), although, in the autumn experiment they were more abundant across the treatments than in the summer experiment.

The highest biovolumes of small heterotrophic flagellates were measured in the summer experiment and the lowest in the spring experiment, in contrast to the lake samples where highest biovolumes were found in the autumn (Fig. 17 & Fig. 7). In the spring experiment their biovolume increased significantly in all the treatments compared to the control (Table 2). There was no difference between the N and P treatments but the biovolume in the N+P treatment was higher than in either of them. In the summer experiment the biovolume of heterotrophs increased in all the treatments in relation to the day 0 sample and the biovolumes were generally much higher than in the lake samples at the time (Fig. 7). There was no difference between the control and P treatments but all the other treatments differed: biovolume in the N treatment was significantly higher than in the control or in P treatment and biovolume in N+P treatment was higher than in all other treatments. In the autumn experiment there were no significant differences in biovolume of heterotrophs between the treatments (Table 2).

4. DISCUSSION

4.1. The phytoplankton community of Alinen Mustajärvi

Chlorophyll *a* values in this study are similar to those reported from a previous study of Alinen Mustajärvi (Arvola 1984) except for the higher values found in 2008. It is hard to tell whether these higher chlorophyll values were caused by higher algal biovolume since the results for the phytoplankton biovolume are from different dates. Nevertheless, the phytoplankton biovolumes in 2008 were generally not higher than in the two other years. Chlorophyll *a* concentration is routinely used as an index for phytoplankton biomass, although it is more a reflection of the photosynthetic capacity. With the present lake data, however, its usefulness as a biomass index is questionable, since the correlation between phytoplankton biovolume and chlorophyll *a* is not strong and phytoplankton biovolume explains only 22 % of the variation in chlorophyll *a* values. According to Ilmavirta (1982) there is often no firm correlation between phytoplankton biomass and chlorophyll *a* in humic lakes, but Lepistö & Saura (1998) did find a highly significant correlation for the polyhumic lake Kalliojärvi. The chlorophyll *a* concentration of a phytoplankton cell is not a constant but varies with, for example, temperature (Geider 1987), nutrient and light availability (Nicholls & Dillon 1978), growth rate (Hunter & Laws 1981), nutritional strategy (Sanders *et al*. 1990), cell size (Vörös & Padisák 1991), and species (Hitchcock 1982). Besides, using only chlorophyll *a* to examine the phytoplankton community provides no data, for example, on species composition and seasonal succession. High chlorophyll values were found in the hypolimnion of the lake during the study and these could have been caused by photosynthetic sulphur bacteria; hypolimnetic chlorophyll maxima caused by sulphur bacteria have been detected in Alinen Mustajärvi before (Arvola 1984) and in other lakes (Eloranta 1985, Keskitalo *et al*. 1998). Bacteriochlorophyll affects the chlorophyll *a* measurement and, therefore, the chlorophyll *a* values for hypolimnion and to some extent also for metalimnion in Alinen Mustajärvi are not entirely realistic.

The composition of the phytoplankton community in Alinen Mustajärvi was generally similar to that in other, previously studied humic lakes (Lepistö & Rosenström 1998, Keskitalo *et al*. 1998, Holopainen *et al*. 2003) except for the scarcity of cryptophytes which are frequently common in humic lakes (Eloranta 1995a, Lepistö & Rosenström

1998). Cryptophytes are favourite food items for zooplankton (Knisely & Geller 1986) and grazing can keep their numbers low, which could explain why in this study cryptophytes were more abundant in the bag experiments where large grazers were absent. Some differences arise when comparing the phytoplankton community with a previous study from Alinen Mustajärvi (Arvola 1984). In that study the raphidophyte *G. semen* was absent and cryptophytes formed at times 50 % of the phytoplankton biomass in the upper 2 metres in contrast to results of this study.

The number of phytoplankton taxa found in the samples is in accordance with some previous studies of small humic lakes; for example, Lake Pieni Hietajärvi with 77 taxa found during one year of sampling (Holopainen *et al*. 2003) and brown-water lakes in Finnish national parks (Eloranta 1995a). Most taxa in Lake Pieni Hietajärvi (Holopainen *et al*. 2003) were chrysophytes and chlorophytes as in this study of Alinen Mustajärvi. On the other hand, Peltomaa & Ojala (2010) found over twice the number of taxa (ca. 150) in Valkea-Kotinen, a small humic lake situated near Alinen Mustajärvi in the Evo region of southern Finland. Joniak (2007) found fewer taxa in a meso- (42) and a polyhumic (37) lake in Poland. In addition to the natural variation between lakes in species diversity, the number of taxa found in a study depends on, for example, duration of the study, number of samples analyzed, and the expertise of the analyzer, which makes it difficult to compare the species diversity between different studies.

Chrysophytes are a typical component of the phytoplankton community in small humic lakes (Ilmavirta 1983, Eloranta 1995a, Lepistö & Rosenström 1998) and they were an important part of the phytoplankton community in Alinen Mustajärvi as well. At times, chrysophytes formed the bulk of the phytoplankton biovolume. A chrysophyte biomass maximum has been found to occur during the warm summer months (June-August) in Finnish lakes (Eloranta 1995b) and this was also seen in Alinen Mustajärvi in 2007 and 2008 when a clear chrysophyte maximum was detected. *Pseudopedinella* sp. was one of the most abundant chrysophytes during the study both in lake samples and in nutrient addition experiments. According to Lepistö & Rosenström (1998) it is common in humic lakes, in contrast to previous findings where *Pseudopedinella* sp. has been associated with eutrophic lakes (Tikkanen 1986). *Chrysococcus* sp. has been found to sometimes dominate the phytoplankton biomass during the spring bloom of phytoplankton in small lakes (Arvola 1986), but in Alinen Mustajärvi it was more common during the summer months. Other abundant chrysophytes include *Mallomonas lychenensis,* which was present in the lake samples only in the two manipulation years and was particularly abundant in June 2008, and *Dinobryon divergens,* which was often present in the lake samples and was abundant in the summer experiment. The latter is a colony-forming species that, along with many other *Dinobryon* species, is very common in the summer phytoplankton of different types of lakes in Finland (Eloranta 1989, Lepistö & Rosenström 1998).

Diatoms, on the other hand, were barely detectable in Alinen Mustajärvi. Many diatom cells are large and heavy, and have trouble staying in the upper part of the water column in stratified conditions when there is little turbulence (Reynolds & Wiseman 1982). Therefore, they sink faster than small or flagellate cells and can rapidly sediment out of the shallow photic zone of a small, wind-sheltered lake, like Alinen Mustajärvi. Lepistö & Rosenström (1998) and Ilmavirta (1983) found some diatoms to be common in dystrophic lakes, but their data came partly from larger lakes with presumably more turbulent water than Alinen Mustajärvi.

The biovolume of cyanophytes was low in Alinen Mustajärvi, but the number of taxa was quite high. Cyanophytes are not typically very abundant in these small forest lakes

(Ilmavirta 1983, Lepistö & Rosenström 1998). According to Brock (1973) low pH can limit the growth of cyanophytes and he found no cyanophytes in natural systems with pH 4.8 or less. The pH of Alinen Mustajärvi in the epilimnion and metalimnion varied between 4.4 and 5.3 in 2007–2009, and the low pH values could have restricted the growth of cyanophytes. Dinophytes were only important in spring samples from Alinen Mustajärvi, and have been found to dominate the spring phytoplankton in some humic lakes (Keskitalo *et al*. 1998).

Chlorophytes had the most phytoplankton taxa in Alinen Mustajärvi and they contributed constantly to the total phytoplankton biovolume in all the years. According to Eloranta (1995a) chlorophytes can contribute significantly to the total number of taxa in humic lakes, even though their biomass often is low. Two of the four most abundant chlorophyte taxa in Alinen Mustajärvi were flagellates. One of the abundant flagellate chlorophytes was *Chlamydomonas* sp. *Chlamydomonas* spp. have been found to dominate the phytoplankton spring bloom of some small humic lakes (Arvola 1986, Similä 1988, Arvola & Kankaala 1989) and it was most abundant in Alinen Mustajärvi in the spring and early summer. Taxonomy and the number of species in this genus are still unclear, and probably multiple species of the genus were present in Alinen Mustajärvi also; at least many different size classes of *Chlamydomonas* were detected. Some species of this genus may utilize organic substrates in addition to photosynthesis and can, therefore, be osmotrophic (Tulonen *et al*. 1992, Laliberté & Noüe 1993). However, because of the ongoing debate on the trophic status of this genus in natural systems, *Chlamydomonas* was not classified as mixotrophic in this study.

The abundance and biovolume of small heterotrophic flagellates in Alinen Mustajärvi was of similar magnitude as in some previous studies (Auer & Arndt 2001, Boenigk & Arndt 2002), but there seem to be differences in the seasonal development of the heterotroph biovolume compared to other lakes. For example, Auer & Arndt (2001) observed a heterotroph abundance maximum in the spring or early summer in lakes of different trophic status, while in Alinen Mustajärvi the highest abundances were found in the autumn. The maximum biomass of ciliates generally occurs in lakes in the spring (Prof. Lauri Arvola, pers. comm.). On the other hand, Lepistö & Saura (1998) found that the importance of heterotrophic flagellates increased in autumn in a boreal brown-water lake. These differences can arise from, for example, the differences between the food webs in humic and clear-water lakes since the lakes that Auer & Arndt (2001) studied were not humic.

4.2. Whole-lake manipulation experiment

This study tried to illustrate the fate of the phytoplankton community in a situation of increased DOC loading by using a whole-lake manipulation experiment. Whole-lake manipulations are useful tools for studying whole community responses to certain variables and it is often easier to generalize their results to natural systems than, for example, results from a laboratory experiment. On the other hand, it is impossible to control all conditions in field experiments and to find suitable control or reference sites, which help to distinguish natural variation in the community from manipulation induced responses. Whole-lake manipulation experiments can also be laborious and expensive which can limit the number of possible replicates (Carpenter 1989).

If replicates or control/reference site are not available, the other conditions (meaning other than the intended manipulation) should ideally stay similar before, during and after the manipulation to be able to distinguish the changes in the community caused by the manipulation. However, during the three years of this study there were interannual

differences in the environmental conditions, which can affect the system in addition to the intended manipulation. For example, precipitation was higher in 2008, which might have resulted in higher runoff to the lake, even though the catchment area of the lake is small and most of the inflow to the lake is from groundwater. The higher nutrient concentrations at the beginning of summer 2008 could be caused by increased loading from the catchment as a result of the increased precipitation. Short-term weather events are one of the factors that are difficult to take into account in field experiments. For example, heavy rains can result in major wash-out of phytoplankton from the epilimnion of a lake, but in Alinen Mustajärvi it is not probable since there is very little outflow from the lake via the outlet. The stratification of the lake was similar in all the years, except for the epilimnion being slightly shallower during the summer of 2009. This small difference in stratification was taken into account during sampling and therefore, the samples from different years remained comparable.

The maximum biovolumes of phytoplankton were similar in all the years, even though the timing of the maximum and the community structure during it differed greatly between years. This suggests that the growth of the phytoplankton community was strongly controlled by some factor (e.g. nutrients or grazing) and the maximum phytoplankton biovolume in the lake was restricted to the observed ca. 2 mm^3 l^{-1}. This is supported further by the higher biovolumes found in some of the bag experiments, where large grazers were absent and more nutrients were available.

Grazing pressure by zooplankton is one factor that can influence the abundance and composition of phytoplankton community. The responses of zooplankton to DOC additions in Alinen Mustajärvi were reported for 2007 and 2008 by Ewane (2010). The zooplankton community of Alinen Mustajärvi contains high numbers of rotifers, but their contribution to biomass is small, while the bulk of biomass is formed by copepods. Cladocerans are not as abundant as copepods. As a response to DOC additions the mean density of rotifers increased, as did the carbon biomass of copepods, while the density and biomass of cladocerans decreased. Stable isotope analyses suggested that the dependence of the zooplankton community on heterotrophic bacteria increased with the increasing DOC loading.

Ewane (2010) measured the zooplankton dynamics, expressed as monthly mean densities and monthly mean carbon biomasses, during the control year and the first manipulation year. The variation in zooplankton mean carbon biomass was small in the control year (2007) while a density maximum appeared in June. This density maximum coincides with a phytoplankton biovolume minimum at the beginning of June. After the maximum the zooplankton density decreased to a low level for the rest of the summer and this was followed by the second peak in phytoplankton biovolume in July-August. Therefore, in the control year the phytoplankton biovolume seems to correlate negatively with the zooplankton mean density but not with the mean carbon biomass. In the first manipulation year (2008) both the mean density and the carbon biomass of zooplankton varied more. Density increased from May to July and then decreased, followed a second density maximum in November. The phytoplankton biovolume also increased in the spring and beginning of the summer, and decreased after that. The zooplankton density maximum in the autumn 2008 (consisting mostly of rotifers) could not have been supported by phytoplankton since phytoplankton biovolume was low at that time. The zooplankton carbon biomass, on the other hand, seems to correlate negatively with phytoplankton biovolume, with a sharp decrease in zooplankton carbon biomass in June when phytoplankton biovolume was still high, following a high biomass maximum in August when biovolume of phytoplankton was low. However, linking the zooplankton and

phytoplankton community dynamics is difficult, since the zooplankton is also regulated by, for example, predation by fish and macroinvertebrates, water temperature and food supplies other than phytoplankton. The same is true for phytoplankton dynamics; nutrients, light, water temperature, grazing by ciliates and other loss processes play a key role in controlling the phytoplankton community in addition to grazing by zooplankton. There seems to be some cases in Alinen Mustajärvi when high zooplankton density or biomass has led to low phytoplankton biovolume, and also cases, for example in the autumn of the first manipulation year, when high zooplankton abundance and biomass could not have been supported by phytoplankton growth alone. The latter supports the conclusion of Ewane (2010) that the dependence of zooplankton on phytoplankton for nutrition decreased as a result of the manipulation.

The number of phytoplankton taxa found in the samples did not change between the years and, therefore, it seems that the manipulation did not affect the diversity of phytoplankton (data not shown). Some differences, on the other hand, were seen in the community composition. Interestingly, the chrysophyte *Mallomonas lychenensis* and the raphidophyte *G. semen* were present in the lake samples only in the two manipulation years. However, marked interannual changes in phytoplankton community composition also occur naturally, especially in small lakes (e.g. Cottingham *et al*. 1998, Keskitalo *et al*. 1998).

Flagellate algae are common in humic waters and are thought to have an advantage in stratified conditions because their motility enables them to collect nutrients also from the meta- or hypolimnia (Ilmavirta 1983, 1988, Salonen *et al*. 1984). Flagellates can dominate the winter phytoplankton (Phillips & Fawley 2002), and one possible reason for the high share of flagellates in phytoplankton biovolume during spring in Alinen Mustajärvi is that they are abundant in the lake during winter. The share of flagellates in the phytoplankton biovolume was at times almost 100 %, even before the manipulation. The contribution of flagellates did not increase as a result of the addition of DOC. The raphidophyte *G. semen* is a flagellate and its' high abundance in autumn 2009 explains the high relative contribution of flagellates to phytoplankton biovolume compared to autumns 2007 and 2008.

From these data it seems unlikely that the increased DOC loading has had a major impact on the phytoplankton community. The two manipulation years seem very different from each other in regard to the phytoplankton community. There were also confounding factors, for example differences in the environmental conditions between the years (precipitation), which can affect the system in addition to the intended manipulation. The observed difference in the phytoplankton biovolume maxima between the control and manipulation years could be caused by natural variation in the phytoplankton community or even by the smaller number of samples analyzed from the years 2008 and 2009 compared to year 2007. The variation in phytoplankton community between years can also be very pronounced in small forest lakes (Keskitalo *et al*. 1998). There was no consistent increase in the biovolume of mixotrophs nor a decrease in biovolume of autotrophs. However, the division of species to autotrophs and mixotrophs was based on literature alone and not on actual observations of mixotrophic activity in Alinen Mustajärvi. Hence the division may not have been an entirely realistic reflection of the different nutritional strategies being employed by the taxa. The observation of mixotrophic activity in the lake could also have revealed changes in the degree of autotrophy of the community. It is possible that a change in the degree of autotrophy could have affected the chlorophyll *a* concentration, since changes in a cell's nutritional strategy are reflected in the chlorophyll *a* concentration of the cell. Sanders *et al*. (1990) reported that when a mixotrophic

chrysophyte *Poterioochromonas malhamensis* switched to a more heterotrophic nutritional strategy, meaning it started to graze on bacteria, its' chlorophyll *a* concentration per cell started to decline. The high chlorophyll *a* concentration in Alinen Mustajärvi in 2008 could have been caused by an increase in the degree of autotrophy of the phytoplankton community.

The largest detectable change was the emergence of the raphidophyte *G. semen*, which became very abundant during late summer and autumn of 2009 after being virtually absent in the samples from the two previous years; indeed the autumn biovolume peak of phytoplankton in 2009 was mostly due to this species. *G. semen* was not detected in a previous study in 1979–1980 by Arvola (1984) but in a later study by Kankaala *et al*. (2010a) it formed 35 % of the phytoplankton biomass in Alinen Mustajärvi in October 2006. *G. semen* has become more common in Finnish and Swedish lakes during the past few decades (Cronberg *et al*. 1988, Lepistö *et al*. 1994). This species is known to build up large biomass, and even to form blooms, in small humic lakes particularly in late summer (Keskitalo *et al*. 1998, Salonen & Rosenberg 2000). Sudden one summer blooms of *G. semen* where it has hardly been detected before have been reported for other lakes as well, for example Kalliojärvi (Lepistö & Saura 1998), whereas in some lakes, for example Valkea Kotinen, it dominates the autumn phytoplankton during most years (Keskitalo *et al*. 1998). Low light intensity has been reported to enhance the bloom formation in *G. semen* and its' biovolume has been found to correlate with total P and DOC concentrations (Eloranta & Räike 1995, Findlay *et al*. 2005). Factors that have been proposed to promote the dominance of *G. semen* include extensive vertical migrations to gain access to nutrients in the hypolimnion (Salonen & Rosenberg 2000), and forming resting cysts that help *G. semen* to survive in unfavourable environmental conditions (Figueroa & Rengefors 2006). The nutrient concentrations in the anoxic hypolimnion of Alinen Mustajärvi are much higher than in the epilimnion and a possibility to exploit these nutrient reserves would certainly be beneficial to the phytoplankton species capable of that. Therefore, even though *G. semen* is osmotrophic, meaning it can use DOC for nutrition (Rengefors *et al*. 2008), it is hard to tell whether its dominance in 2009 in Alinen Mustajärvi has anything to do with the increased DOC loading. It is uncertain if *G. semen* was behaving mixotrophically in this lake and how much mixotrophy generally benefits its growth. As it also contributed significantly to the phytoplankton biomass in 2006, there probably are other things apart from the manipulation behind its dominance in 2009.

As a response to the DOC manipulation the biovolume of the heterotrophic flagellates increased, which could be explained by the increase in bacterial prey (see below). Berninger *et al*. (1991) found that the abundance of bacteria was correlated with the abundance of heterotrophic nanoflagellates in freshwater systems. In a humic lake Pääjärvi small heterotrophic flagellates are the most important bacterial grazers (Kankaala *et al*. 1996). Chrzanowski & Simek (1990) reported heterotrophic flagellates of different sizes to prefer large bacteria as prey and suggested that in some systems, in addition to controlling the bacterial abundance, the heterotrophic flagellates can also affect the size-distribution of the bacterial community. In Alinen Mustajärvi heterotrophic flagellates are not the only bacteriovores (also ciliates, rotifers, crustacean zooplankton and mixotrophic algae can ingest bacteria) and their ability to affect the bacterial community remains uncertain. For example, the biovolume of phagotrophic mixotrophs was much higher than the biovolume of heterotrophs, and thus they had a potential to be more important bacterial grazers. On the other hand, only selected taxa of heterotrophic flagellates were counted and therefore, the heterotroph biovolume values reported here will be underestimates. It might have been interesting to also study the role of ciliates in the food web of Alinen

Mustajärvi. In Pääjärvi ciliates are important algal grazers (Kankaala *et al.* 1996). Since ciliates were not included in this study nor in the study of zooplankton in Alinen Mustajärvi (Ewane 2010) there is no information of how they might have reacted to the DOC manipulation.

It seems that even though the DOC additions had no effect on the phytoplankton community, there has been an impact on the bacterial community since the abundance of bacteria has increased while the mean cell size has decreased (MSc Sari Peura, University of Jyväskylä, pers. comm.). This is probably the reason for the increase in the biovolume of the small heterotrophic flagellates which are better able to ingest small bacterial cells. The effects of the manipulation have evidently also transferred to higher trophic levels, as according to results from stable isotope analyses part of the crustacean zooplankton community has been found to rely more on heterotrophic bacteria for nutrition (Ewane 2010) while carbon from the cane sugar has transferred to fish via zooplankton and littoral macroinvertebrates (Prof. Roger Jones, University of Jyväskylä, pers. comm.).

One possible reason for the lack of impact on the phytoplankton community is that the phytoplankton community mostly consisted of flagellate species, which are capable of vertical migrations and can obtain nutrients also from deeper in the water column (Salonen *et al*. 1984) and can, therefore, escape nutrient competition with bacteria. The phytoplankton community was not nutrient limited during most of the summer (see below). Jones (1990) found that in these small humic lakes in the Evo region phytoplankton can also successfully compete with bacteria for phosphorus and suggested phagotrophy by mixotrophic algae to be a possible reason for this. It is also possible that the induced increase in DOC concentration was not sufficient to override other processes affecting the phytoplankton community.

It would be interesting to know if the use of different DOC source, for example natural DOC, instead of cane sugar would have led to responses in the phytoplankton community. Järvinen (2002) reported that at times primary production decreased with glucose additions in bioassay experiments with water from humic lake Valkea-Kotinen even with a simultaneous addition of P or N. In contrast to glucose or cane sugar, allochtonous DOC from the catchment area of lakes is usually coloured, and the use of natural DOC could have resulted in more accurate predictions of the effects on phytoplankton community. Kankaala *et al*. (2010b) did not find direct negative impacts of humic water additions on phytoplankton biomass or primary production in earlier enclosure experiments made in Alinen Mustajärvi. The water colour did become darker but the change of light attenuation was reported to be insignificant in the 0.5 m deep enclosures. However, in the enclosure experiments performed in Pääjärvi (Arvola *et al*. 1996) the addition of humic water alone stimulated the primary production during summer, probably because the added natural humic water also contained P. The addition of humic water simultaneously worsened the light climate and, therefore the stimulation of primary production by the added P was not very pronounced. Carpenter *et al*. (1998) found that a 4 mg l^{-1} rise (twice the 2 mg l^{-1} used in this study) in DOC concentration could lead to ca. 20 % decrease in the primary production. Deterioration of the light climate could have benefitted the mixotrophs as they have been shown to supplement photosynthesis with phagotrophy and maintain growth in low light conditions (Isaksson 1998). Originally, cane sugar was chosen in order to study the food web effects of increasing the readily useable substrate to bacteria. The use of natural DOC could have led to more complicated results as it also affects the light climate. Another important reason for choosing cane sugar was that it has a different stable carbon isotope ratio than the natural DOC in the lake, which gave an opportunity to follow its' path through the food web via stable isotope analysis (SIA).

SIA could not be used to study the phytoplankton since it is difficult to separate the phytoplankton cells from other particles (mainly detritus) in the water.

4.3. Nutrient addition experiments

The growth of the phytoplankton community was found to be nutrient-limited only during summer, and then it was co-limited by N and P. This supports the findings of Jones (1990) from the small lakes (including Alinen Mustajärvi) in the Evo district. He concluded that the supply of nutrients in these lakes seems to be in balance with the need of plankton and the addition of either P or N alone would probably not benefit the plankton community. Other studies have shown that in a nearby large humic lake Pääjärvi the phytoplankton production is limited by the availability of P, but the nitrate concentration of Lake Pääjärvi is much higher than in Alinen Mustajärvi which is why N-limitation of phytoplankton in Pääjärvi is not probable (Arvola *et al*. 1996). Nürnberg & Shaw (1999) found also in their dataset of 500 clear and humic lakes P to be more likely the limiting nutrient in both types of lakes. On the other hand, Pålsson & Granéli (2004) found N limitation to be more common in lakes with high humic content. An increase in the biomass and primary production and a decrease in the diversity of phytoplankton were reported by Cottingham *et al*. (1998) for whole-lake manipulation experiments with increased loading of both P and N. No decrease in the diversity of phytoplankton was found in the present study (data not shown) however, where nutrient additions were made in small enclosures for a much shorter period of time.

There were generally no differences between the nutrient limitation of the autotrophs and mixotrophs (as they were not limited by nutrients at other times than summer), but autotrophs were stimulated also by addition of N alone in the summer experiment whereas the mixotrophs responded only to addition of both P and N. This is in accordance with the findings of Jansson *et al*. (2001) from a study of nutrient limitation in two small humic lakes in northern Sweden in which they found that autotrophic phytoplankton was stimulated by N addition. On the other hand, in Lake Örträsket (Jansson *et al*. 1996) autotrophs were stimulated only by N+P addition and mixotrophic flagellates were found to be N-limited. Jansson *et al*. (1996) suggested that the N-limitation of mixotrophs was caused by grazing on P-rich bacteria. However, no N-limitation of mixotrophs was found in Alinen Mustajärvi during the three experiments. Pålsson & Granéli (2004) also found no tendency for mixotrophs to be more likely N-limited than autotrophs.

One confounding factor in the bag experiments was that the potential vertical migrations of the flagellate phytoplankton were prohibited. If the phytoplankton were collecting nutrients from the hypolimnion, then the results from the bag experiments would not give realistic results of the nutrient limitation of phytoplankton community in the lake. Järvinen (2002) suggested that in brown-water lakes phytoplankton does not often experience nutrient-limitation *in situ*, but nutrient-limitation evolves during long incubation times in enrichment bioassays when vertical migrations are prohibited and nutrient stores run out.

There was a strong correlation between phytoplankton biovolume and chlorophyll *a* concentration in the bag experiments. The range of the values in both the biovolume and chlorophyll *a* concentration was greater than in the lake samples, which can partly explain the better correlation between the two in the nutrient addition experiments. The chlorophyll *a* values had generally less variation than the biovolume values, which may be because larger sample volume was used to measure the chlorophyll *a* concentration than to analyze the phytoplankton samples. The chlorophyll *a* concentrations differed between experiments but the differences between treatments were similar among the experiments. The

concentration increased in the P treatment (very little in the spring experiment though) and even more in the N+P treatment. This is in contrast to the phytoplankton community which generally was not limited by P, and was not nutrient limited in the spring or autumn experiment. This suggests that there were other organisms containing chlorophyll *a*, for example picophytoplankton, that were not included in the phytoplankton counting but affected the chlorophyll *a* measurements. This can also be one reason for the higher measured chlorophyll *a* concentrations in 2008 lake samples. Autotrophic picophytoplankton has been found to dominate at times the winter phytoplankton biomass in Alinen Mustajärvi (Arvola & Kankaala 1989).

The phytoplankton community, apart from cyanophytes, was not limited by nutrients during the spring experiment. The light availability could have limited the growth of phytoplankton in the spring, even though the concentrations of nutrients were higher already in the day 0 sample compared to the summer experiment. This is in accordance with the lake samples: nutrient concentrations were generally higher at the beginning of the sampling in the spring compared to summer. The temperature, on the other hand, did probably not limit the growth of phytoplankton at that time of the year since phytoplankton production and biomass maximum can occur already under ice-cover (Prof. Lauri Arvola, pers. comm.).

The higher biovolumes of autotrophs in the spring experiment in all treatments compared to the day 0 sample could be caused by exclusion of large zooplankton in the bags as most of the autotrophs were small and presumably edible (for example the chlorophytes and other phytoplankton). The mixotrophic part of the phytoplankton community was largely composed of bigger species, such as different *Peridinium* species, which are not so susceptible to grazing and therefore did not potentially benefit so much from the exclusion of the grazers. There was a suppression of cryptophytes in relation to the day 0 sample in the spring experiment, and for some reason they did not grow well in the bags in the spring experiment. Grazing is one possible reason why cryptophytes were never abundant in lake samples (see above). Large grazers were excluded from the bags by filtration but there were still rotifers and ciliates that can graze on cryptophytes. On the other hand, small and edible autotrophs did grow well in the bags. The most common cryptophyte was a *Cryptomonas* sp., which is a medium-sized, mixotrophic species with two flagella and is capable of migrating in the water column (Smolander & Arvola 1988, Salonen *et al*. 1984). Cryptophytes seemed to be co-limited by P and N in the summer and autumn experiments, in line with the findings of Jansson *et al*. (1996).

It seems that in the spring and summer experiments the cyanophytes were limited by P and when P was added, the limitation shifted to co-limitation by P and N. Some cyanophytes can fix nitrogen anaerobically in specialized cells, called heterocysts, and therefore, can more likely be limited by P (Howarth *et al*. 1988). However, no cyanophytes with heterocysts were found in this study.

The growth of the phytoplankton community was generally co-limited by N and P during the summer experiment. It is reasonable that high biovolumes were attained in the N+P treatment since the environmental conditions for phytoplankton growth during the experiment were favourable: the water temperature was high, light availability was good, algae were provided with nutrients and large grazers were absent. If the N+P addition had not had any impact, the phytoplankton community would presumably have been limited by some other factor, for example some other nutrient or trace element.

The biovolume of autotrophs in the summer experiment seemed to be limited by N, but when N was added, the limitation shifted to co-limitation by N and P. The same was

found also for chlorophytes and other phytoplankton, which are autotrophic, but the autotrophic cyanophytes showed the same pattern of P-limitation as during the spring experiment. Why the autotrophs were N-limited, even though there were detectable amounts of usable N (NH_4^+-N and NO_2^-+NO_3^--N) in the water during and after the experiment, is unclear. Järvinen (2002) suggested that N-limitation could be induced by prohibiting the vertical migrations of flagellate phytoplankton in enrichment bioassay experiments. Some of the autotrophs in the summer experiment were flagellates but most of them were small (for example *Chlamydomonas* sp.) and probably not capable of extensive vertical migrations. The N concentrations were lower in the beginning of the summer experiment than of the spring or autumn experiment. The earlier mesocosm experiments (Kankaala *et al.* 2010b) suggested that the bacterial activity in Alinen Mustajärvi was N-limited, even though N concentrations were at a similar level as in this experiment. Jansson *et al*. (1996) also reported N-limitation of phytoplankton in a humic lake with higher NH_4^+-N and NO_2^-+NO_3^--N concentrations than in Alinen Mustajärvi. Mixotrophs, on the other hand, were strongly co-limited by N and P in summer, as was their most abundant species, the raphidophyte *G. semen*. Chlorophyll *a* also increased strongly in the N+P treatment in the summer experiment, reflecting the increase of mixotrophs rather than that of autotrophs. This suggests that mixotrophic cells contained plenty of chlorophyll and their nutritional strategy was perhaps close to autotrophy.

The growth of the phytoplankton community in the autumn experiment was generally not limited by P or N. It is possible that in the autumn experiment phytoplankton biovolume at the beginning of the experiment was already at such a high level that it resulted in self-shading, in addition to the naturally lower levels of irradiance in the autumn compared to summer. *G. semen* has a number of chloroplasts in a layer just beneath the cell surface, and this has been suggested to be a useful trait in low light conditions (Coleman & Heywood 1981). Larger algae have also been found to be less affected by self-shading than small algae (Agustí 1991), which can be one reason for the dominance of the large-celled *G. semen*.

The biovolume of the mixotrophs was lower in the N treatment than in all other treatments in the autumn experiment, but it seems that only *G. semen* showed this tendency of suppression in the N treatment. The addition of N may have enhanced some other organism's competitive ability at the expense of *G. semen*. For example, the growth of chrysophytes, most of which were mixotrophs, was stimulated by the addition of N. Though, with addition of both N and P *G. semen* managed to grow at least as well as in the control and the P treatment. *G. semen* comprised approximately 65–85 % of the phytoplankton biovolume (and even more of the mixotroph biovolume) in the autumn experiment.

The community composition of phytoplankton in the experiments was generally similar to the lake samples at those times. In the spring experiment the day 0 sample included high abundance of cryptophytes but these were scarce in the lake sample from May 2009. This lake sample was actually taken two weeks after the initiation of the experiment, by when the abundance of cryptophytes in the lake had probably decreased as a result of seasonal succession. *G. semen* was also more abundant in the summer and autumn experiments than in the lake samples at those times. These lake samples were taken on different dates as in the spring, but in this case, the biovolume of *G. semen* was increasing towards autumn. Therefore, it might be expected to be more common in the lake sample from July that was taken later than the day 0 sample of the summer experiment. On the other hand, the water for all the experiments was taken from the epilimnion but the phytoplankton biovolumes in lake were weighted averages for epilimnion and

metalimnion, and they are thus not directly comparable. It might have been useful to also study the vertical distribution of the phytoplankton since Arvola (1984) found a phytoplankton biomass maximum in Alinen Mustajärvi at the depth of 3–4 m. If *G. semen* stays in the surface layer of the lake during the day (and therefore during the sampling and initiation of the experiment) it would explain why it was more common in the day 0 samples than in the lake. Findlay *et al.* (2005) found that blooms of *G. semen* developed when light intensity decreased below 100 µmol m^{-2} s^{-1} and Eloranta & Räike (1995) found light intensity to regulate the vertical migrations of *G. semen*; the population of *G. semen* migrated upwards during morning but stopped when light intensity reached ca. 75–95 µmol m^{-2} s^{-1}. This light intensity is attained at a depth of little below 1 m in Alinen Mustajärvi and if *G. semen* showed the same pattern of vertical migration as in the experiment of Eloranta & Räike (1995) a larger share of the population would have been found in the epilimnion compared to metalimnion during the day. On the other hand, the sampling in Alinen Mustajärvi was generally done in the forenoon when the vertical migration of *G. semen* might not have yet reached its' highest point. For example, in a similar near-by humic lake Valkea-Kotinen the biomass maximum of *G. semen* in the forenoon is often at a depth of 3–4 m (Prof. Lauri Arvola, pers. comm.).

The light intensity in the water is, of course, affected by the weather, and in cloudy days the light intensity is lower which would, according to Eloranta & Räike (1995), result in *G. semen* migrating nearer to the surface. This leads to difficulties in sampling phytoplankton in a representative and standardized way in lakes where they show vertical migrations since the vertical distribution of the algae can change in relation to season, weather, and time of the sampling.

Possible factors regulating the biovolume of small heterotrophic flagellates in the experiments, as well as in the lake, were water temperature, prey availability and predation. Biovolume was high during the summer experiment, when water was warm (about 20 ^{o}C) and low during the spring experiment when the water temperature was low (10 ^{o}C). In autumn both the biovolume and water temperature (15 ^{o}C) were at an intermediate level. High biovolume of heterotrophs in the N+P treatment in the spring experiment could be caused by high abundance of bacterial prey. The abundance of bacteria was lowest during the autumn experiment and highest during the spring experiment, and very high abundances were measured in the N+P treatment of the spring experiment (MSc Sari Peura, University of Jyväskylä pers. comm.). In the summer experiment the growth of the heterotrophs was stimulated in all treatments compared to day 0 and especially in the N and N+P treatments. Exclusion of grazers might have benefitted the heterotrophs and also prey availability (algae + bacteria) was enhanced in N+P treatment. In the autumn there were no significant differences between the treatments in biovolume of heterotrophs, as in the abundance of bacteria or biovolume of phytoplankton. The level and seasonal development of heterotroph biovolume was different in the bag experiments compared to the lake samples. The biovolumes were generally higher in the experiments than in the lake. The biovolumes in the nutrient addition experiments were highest in the summer but high biovolumes in lake samples were found in the autumn. These differences could be caused by the differences in sampling the lake compared to the experiments and the vertical distribution of the heterotrophs (see discussion of *G. semen* above), though light availability would hardly affect the vertical distribution of heterotrophs.

4.4. Conclusions

The phytoplankton community of Alinen Mustajärvi is typical for a small boreal humic lake, and therefore, the results obtained here can, to a certain extent, be used to assess the possible responses of similar small humic lakes to a future increase in carbon and nutrient loading. However, whether cane sugar was a good substitute for natural humic matter in assessing the responses of phytoplankton is unclear and the results reported here should be used with some caution.

The phytoplankton community in Alinen Mustajärvi did not show clear or consistent responses to the increased DOC loading. The two manipulation years seemed very different with respect to phytoplankton community, which makes it harder to interpret the results. Natural changes in phytoplankton communities occur both seasonally and interannually especially in small lakes (Cottingham *et al.* 1998, Keskitalo *et al.* 1998). The most pronounced change in the community was the dominance of the mixotrophic raphidophyte *Gonyostomum semen* during the second manipulation year, but this was probably not due to the manipulation. There were no differences in the responses of autotrophs and mixotrophs, and the share of flagellates did not change during the manipulation.

During most of the open water season the phytoplankton community was not nutrient limited. According to my results, if the predicted future increase in DOC loading will be accompanied by increased nutrient loading, it will affect the phytoplankton community mostly during summer, and then only the increased loading of both P and N will produce an effect. Whether the increase in phytoplankton biovolume during summer was caused by the combined effect of DOC and inorganic nutrients or just by nutrients is unclear. Small heterotrophic flagellates, on the other hand, clearly benefitted from the increased DOC loading and the highest biovolumes were attained in summer with P and N addition.

It seems that any future increase in DOC loading will affect more clearwater than humic lakes if we consider the phytoplankton community. Blomqvist *et al*. (2001) reported drastic changes in the phytoplankton community of a clearwater lake under DOC manipulation. In this study, however, only minor changes in community composition were observed in the humic lake Alinen Mustajärvi, and a major increase in biovolume was only observed in summer with combined loading of DOC, P and N. Similar results were reported for the enclosure experiments in a humic lake Pääjärvi where phytoplankton biomass and production responded to additions of humic matter mostly during summer and with simultaneous addition of P (Arvola *et al*. 1996). The stoichiometry of the runoff to lakes will be an important factor determining the responses of phytoplankton communities to climate change and phytoplankton in humic lakes can potentially be more affected by the increased nutrient than carbon loading.

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REFERENCES

Agustí S. 1991. Light environment within dense algal populations: cell size influences on self-shading. *J. Plankton Res*. 13: 863–871.

Arvola L. 1984. Vertical distribution of primary production and phytoplankton in two small lakes with different humus concentration in southern Finland. *Holarct. Ecol.* 7: 390–398.

Arvola L. 1986. Spring phytoplankton of 54 small lakes in southern Finland. *Hydrobiologia* 137: 125–134.

Arvola L. & Kankaala P. 1989. Winter and spring variability in phyto- and bacterioplankton in lakes with different water colour. *Aqua Fennica* 19: 29–39.

Arvola L., Kankaala P., Tulonen T. & Ojala A. 1996. Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake (Lake Pääjärvi). *Can. J. Fish. Aquat. Sci*. 53: 1646–1662.

Auer B. & Arndt H. 2001. Taxonomic composition and biomass of heterotrophic flagellates in relation to lake trophy and season. *Freshw. Biology* 46: 959–972.

Berninger U.-K., Finlay B.J. & Kuuppo-Leinikki P. 1991. Protozoan Control of Bacterial Abundances in Freshwater. *Limnol. Oceanogr*. 36: 139–147.

Bird D.F. and Kalff J. 1987. Algal phagotrophy: Regulating factors and importance relative to photosynthesis in Dinobryon (Chrysophyceae). *Limnol. Oceanogr*. 32: 277–284.

Blomqvist P., Jansson M., Drakare S., Bergström A.-K. & Brydsten L. 2001. Effects of Additions of DOC on Pelagic Biota in a Clearwater System: Results from a Whole Lake Experiment in Northern Sweden. *Microb. Ecol.* 42: 383–394.

Boenigk J. & Arndt H. 2002. Bacteriovory by heterotrophic flagellates: community structure and feeding strategies. *Antonie van Leeuwenhoek* 81: 465–480.

Brock T.D. 1973. Lower pH Limit for the Existence of Blue-green Algae: Evolutionary and Ecological Implications. *Science* 179: 480–483.

Brofeldt P. 1920. *Evon kalastuskoeasema (Suomen Kalatalous, Nide 6)*. Kalastushallitus, Helsinki, 141 p.

Carpenter S.R. 1989. Replication and treatment strength in whole-lake experiments. *Ecology* 70: 453–463.

Carpenter S.R., Cole J.J., Kitchell J.F & Pace M.L. 1998. Impact of Dissolved Organic Carbon, Phosphorus, and Grazing on Phytoplankton Biomass and Production in Experimental Lakes. *Limnol. Oceanogr*. 43: 73–80.

Chrzanowski T.H. & Simek K. 1990. Prey-Size Selection by freshwater Flagellated Protozoa. *Limnol. Oceanogr*. 35: 1429–1436.

Coleman A.W. & Heywood P. 1981. Structure of the chloroplast and its DNA in chloromonadophycean algae. *J. Cell Sci*. 49: 401–409.

Cottingham K.L., Carpenter S.R. & Amand L.St. 1998. Responses of epilimnetic phytoplankton to experimental nutrient enrichment in three small seepage lakes. *J. Plankton Res*. 20: 1889–1914.

Cronberg. G., Lindmark G. & Björk S. 1988. Mass development of the flagellate Gonyostomum semen (Raphidophyta) in Swedish forest lakes – and effect of acidification?. *Hydrobiologia* 161: 217–236.

Currie D.J. & Kalff J. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. *Limnol. Oceanogr*. 29: 298–310.

Del Giorgio P.A., Cole J.J. & Cimbleris A. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic ecosystems. *Nature* 385:148–151.

Drakare S., Blomqvist P., Bergström A.-K. & Jansson M. 2002. Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in humic Lake Östräsket. *Freshw. Biology*. 47: 41–52.

Eloranta P. 1985. Hypolimnetic chlorophyll maximum by algae and sulphur bacteria in one eutrophic pond. *Arch. Hydrobio. Suppl*. 71: 459–469.

Eloranta P. 1989. On the ecology of the genus Dinobryon in Finnish lakes. *Beiheft zur Nova Hedwigia* 95: 99–109.

Eloranta P. 1995a. Phytoplankton of the national park lakes in central and southern Finland. *Ann. Bot. Fennici* 32: 193–209.

Eloranta P. 1995b. Biogeography of chrysophytes in Finnish lakes. In Sandgren C.D., Smol J.P. & Kristiansen J. (eds.). *Chrysophyte algae: ecology, phylogeny, and development*. Cambridge University Press, Great Britain, pp. 214–231.

Eloranta P. & Räike A. 1995. Light as a factor affecting the vertical distribution of *Gonyostomum semen* (Her.) Diesing (Rhapidophyceae) in lakes. *Aqua Fennica* 25: 15–22.

Elser J.J., Lubnow F.S., Marzolf E.R., Brett M.T., Dion G. & Goldman C.R. 1995. Factors associated with interannual and intraannual variation in nutrient limitation of phytoplankton growth in Castle Lake, California. *Can. J. Fish. Aquat. Sci*. 52: 93–104.

Ewane E.B. 2010. Responses of zooplankton to cane sugar additions to a small humic lake, Alinen Mustajärvi. Master's thesis, University of Jyväskylä, Finland, 32p.

Figueroa R.I. & Rengefors K. 2006. Life cycle and sexuality of the freshwater raphidophyte *Gonyostomum semen* (Raphidophyceae). *J. Phycol*. 42: 859–871.

Findlay D.L., Paterson M.J., Hendzel L.L. & Kling H.J. 2005. Factors influencing Gonyostomum semen blooms in a small boreal reservoir lake. *Hydrobiologia* 533: 243–252.

Forsberg C. 1992. Will an increased greenhouse impact in Fennoscandia give rise to more humic and coloured lakes?. *Hydrobiologia* 229: 51–58.

Geider R.T. 1987. Light and Temperature Dependence of the Carbon to Chlorophyll *a* Ratio in Microalgae and Cyanobacteria: Implications for Physiology and Growth of Phytoplankton. *New Phytol*. 106: 1–34.

Hecky R.E. & Kilham P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence of the effects of enrichment. *Limnol. Oceanogr*. 33: 796–822.

Henriksen A., Skjelvåle B.L., Mannio J., Wilander A., Harriman R., Curtis C., Jensen J.P. Fjeld E. & Moiseenko T. 1998. Northern European Lake Survey, 1995. *Ambio* 27: 80–91.

Hessen D.O. 1992. Dissolved organic carbon in a humic lake: effects on bacterial production and respiration. *Hydrobiologia* 229: 115–123.

Hitchcock G.L. 1982. A comparative study of the size-dependent organic composition of marine diatoms and dinoflagellates. *J. Plankton Res*. 4: 363–377.

Holopainen A.-L., Niinioja R. & Rämö R. 2003. Seasonal succession, vertical distribution and long term variation of phytoplankton communities in two shallow forest lakes in eastern Finland. *Hydrobiologia* 506–509: 237–245.

Howarth R.W., Marino R., Lane J. & Cole J.T. Nitrogen Fixation in Freshwater, Estuarine, and Marine Ecosystems. 1. Rates and Importance. *Limnol. Oceanogr*. 33: 669–687.

Hunter B.L. & Laws E.A. 1981. ATP and Chlorophyll *a* as estimators of phytoplankton carbon biomass. *Limnol. Oceanogr*. 26: 944–956.

Ilmavirta V. 1982. Dynamics of phytoplankton in Finnish lakes. *Hydrobiologia* 86: 11–20.

Ilmavirta V. 1983. The role of flagellated phytoplankton in chains of small brown-water lakes in southern Finland. *Ann. Bot. Fennici*. 20: 187–195.

Ilmavirta V. 1988. Phytoflagellates and their ecology in Finnish brown-water lakes. *Hydrobiologia* 161: 255–270.

Isaksson A. 1998. Phagotrophic phytoflagellates in lakes – a literature review. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* 51: 63-90.

Isaksson A., Bergström A.-K., Blomqvist P. & Jansson M. 1999. Bacterial grazing by phagotrophic phytoflagellates in a deep humic lake in northern Sweden. *J. Plankton Res*. 21: 247–268.

Jansson M., Blomqvist P. & Jonsson A. & Bergström A.-K. 1996. Nutrient Limitation of Bacterioplankton, Autotrophic and Mixotrophic Phytoplankton, and Heterotrophic Nanoflagellates in Lake Ortrasket. *Limnol. Oceanogr*. 41: 1552–1559.

Jansson M., Bergström A.-K., Drakare S. & Blomqvist P. 2001. Nutrient limitation of bacterioplankton and phytoplankton in humic lakes in northern Sweden. *Freshw. Biology* 46: 653–666.

Joint I., Henriksen P., Fonnes G.A., Bourne D., Thingstad T. F. & Riemann B. 2002. Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. *Aquat. Microb. Ecol*. 29: 145–159.

Jones R.I. 1990. Phosphorus transformations in the epilimnion of humic lakes: biological uptake of phosphate. *Freshw. Biology* 23: 323–337.

Jones R.I. 1992a. The influence of humic substances on lacustrine planktic food chains. *Hydrobiologia* 229: 73–91.

Jones R.I. 1992b. Phorphorus transformations in the epilimnia of small humic forest lake. *Hydrobiologia*. 243/244: 105–111.

Jones R.I. & Salonen K. 1985. The importance of bacterial utilization of released phytoplankton photosynthate in two humic forest lakes in southern Finland. *Holarctic Ecol.* 8: 133–140.

Jones R.I., Salonen K. & De Haan H. 1988. Phosphorus transformations in the epilimnion of humic lakes: interactions between dissolved humic materials and phosphate. *Freshw. Biology* 19: 357–369.

Joniak T. 2007. Seasonal variations of dominant phytoplankton in humic forest lakes. *Int. J. Oceanol. Limnol.* 36: 49–59.

Järvinen M. 2002. Control of plankton and nutrient limitation in small boreal brown-water lakes: evidence from small- and large-scale manipulation experiments. PhD Dissertation, University of Helsinki, Finland, 45 p.

Järvinen M. & Salonen K. 1998. Influence of changing food web structure on nutrient limitation of phytoplankton in a highly humic lake. *Can. J. Fish. Aquat. Sci.* 55: 2562–2571.

Kankaala P., Taipale S. & Li L. 2010a. Diets of crustaceaen zooplankton, inferred from stable carbon and nitrogen isotope anlyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquat. Ecol*. 44: 781–795.

Kankaala P., Arvola L., Tulonen T. & Ojala A. 1996. Carbon budget for the pelagic food web of the euphotic zone in a boreal humic lake (Lake Pääjärvi). *Can. J. Fish. Aquat. Sci*. 53: 1663–1674.

Kankaala P., Peura S., Nykänen H., Sonninen E., Taipale S., Tiirola M. & Jones R.I. 2010b. Impacts of added dissolved organic carbon on boreal freshwater pelagic metabolism and

food webs in mesocosm experiments. *Fundam. Appl. Limnol., Arch. Hydrobiol*. 177(3): 161–176.

Keskitalo J., Salonen K. & Holopainen A.-L. 1998. Long-term fluctuations in environmental conditions, plankton, and macrophytes in a humic lake, Valkea-Kotinen. *Bor. Environ. Res*. 3: 251–262.

Knisely K. & Geller W. 1986. Selective feeding of four zooplankton species on natural lake phytoplankton. *Oceologia* 69: 86–94.

Kortelainen P., Mattson T., Finér L., Ahtiainen M., Saukkonen S. & Sallantaus T. 2006. Controls on the export of C, N, P and Fe from undisturbed boreal catchments, Finland. *Aquat. Sci*. 68: 453–468.

Laliberté G. & de la Noüe J. 1993. Auto-, hetero-, and mixotrophic growth of *Chlamydomonas humicola* (chlorophyceae) on acetate. *J. Phycol*. 29: 612–620.

Lepistö L. & Rosenström U. 1998. The most typical phytoplankton taxa in four types of boreal lakes. *Hydrobiologia* 369/370: 89–97.

Lepistö L. & Saura M. 1998. Effects of forest nutrient addition on phytoplankton in a boreal brown-water lake. *Boreal Env. Res*. 3: 33–43.

Lepistö L., Antikainen S. & Kivinen J.1994. The occurence of *Gonyostomum semen* (Ehr.) Diesing in Finnish lakes. *Hydrobiologia* 273: 1–8.

Lepistö L., Vuorio K., Holopainen A.-L. Huttunen M., Jokipii R. & Niemelä M. 2006. Kasviplanktonin tutkimusmenetelmät. *Vesitalous* 2006(1): 16–21.

Meili M. 1992. Sources, concentrations and characteristics of organic matter in softwater lakes and streams of the Swedish forest region. *Hydrobiologia* 229: 23–41.

Nicholls K.H. & Dillon P.J. 1978. An Evaluation of Phosphorus-Chlorophyll-Phytoplankton Relationships for Lakes. *Int. Rev. Gesamten Hydrobiol*. 63: 141–154.

Nürnberg G.K. & Shaw M. 1999. Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. *Hydrobiologia* 382: 97–112.

Peltomaa E. & Ojala A. 2010. Size-related photosynthesis of algae in a strongly stratified humic lake. *J. Plakton Res*. 35: 341–355.

Phillips K. A. & Fawley M.W. 2002. Winter phytoplankton community structure in three shallow temperate lakes during ice cover. *Hydrobiologia* 470: 97–113.

Porter K.G. 1988. Phagotrophic phytoflagellates in microbial food webs. *Hydrobiologia* 159: 89–97.

Pålsson C. & Granéli W. 2004. Nutrient limitation of autotrophic and mixotrophic phytoplankton in a temperate and tropical humic lake gradient. *J. Plankton Res*. 26: 1005–1014.

Rengefors K., Pålsson C., Hansson L.-A. & Heiberg L. 2008. Cell lysis of competitors and osmotrophy enhance growth of the bloom-forming alga *Gonyostomum semen*. *Aquat. Microb. Ecol*. 51: 87–96.

Reynolds C. 2006. *Ecology of phytoplankton*. Cambridge University Press, Cambridge, 535 p.

Reynolds C.S. & Wiseman S.W. 1982. Sinking losses of phytoplankton in closed limnetic systems. *J. Plankton Res*. 4: 489–522.

Salonen K. & Rosenberg M. 2000. Advantages from diel vertical migration can explain the dominance of *Gonyostomum semen* in a small, steeply-stratified humic lake. *J. Plankton Res*. 22: 1841–1853.

Salonen K., Kononen K. & Arvola L. 1983. Respiration of plankton in two small, polyhumic lakes. *Hydrobiologia* 101: 65–70.

Salonen K., Jones R.I. & Arvola L. 1984. Hypolimnetic phosphorus retrieval by diel vertical migrations of lake phytoplakton. *Freshw. Biology* 14: 431–438.

Sanders R.W., Porter K.G. & Caron D.A. 1990. Relationship Between Phototrophy and Phagotrophy in the Mixotrophic Chrysophyte Poterioochromonas malhamensis. *Microb. Ecol*. 19: 97–109.

Similä A. 1988. Spring development of a *Chlamydomonas* population in lake Nimetön, a small humic forest lake in southern Finland. *Hydrobiologia* 161: 149–157.

Smolander U. & Arvola L. 1988. Seasonal variation in the diel vertical distribution of the migratory alga *Cryptomonas marssonii* (Cryptophyceae) in a small, highly humic lake. *Hydrobiologia* 161: 98–98.

Tikkanen T. 1986. *Kasviplanktonopas*. Forssan kirjapaino Oy, Forssa, 278 p.

Tranvik L.J. 1988. Availability of Dissolved Organic Carbon for Planktonic Bacteria in Oligotrophic Lakes of Differing Humic Content. *Microb. Ecol*. 16: 31 –322.

Tranvik L.J. & Jansson M. 2002. Climate change (Communication arising): Terrestrial export of organic carbon. *Nature* 415: 861–862.

Tulonen T., Salonen K. & Arvola L. 1992. Effects of different molecular weight fractions of dissolved organic matter on the growth of bacteria, algae and protozoa from a highly humic lake. *Hydrobiologia* 229: 239–252.

Vörös L. & Padosák J. 1991. Phytoplankton biomass and Clorophyll *a* in some shallow lakes in central Europe. *Hydrobiologia* 215: 111–119.

Watson R.T. & the Core Writing Team (eds.). 2001. *Climate Change 2001: Synthesis Report. A Contribution of Working Groups I, II, and III to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom, and New York, NY, USA, 398 p.

APPENDIX 1

Table A1. The concentration of nutrients at the beginning and on day seven of the nutrient addition experiments. The day seven values are means of three replicates ± standard deviation.

			Nutrient concentration		
Experiment	Treatment	Day	PO_4-P µg l^{-1}	NO_2+NO_3-N µg l^{-1}	NH_4-N µg l^{-1}
Spring	Ctrl	0	1	26	74
	P	0	45	26	75
	N	0	1	194	260
	N+P	0	44	189	253
	Ctrl	7	1 ± 0	40 ± 16	8 ± 4
	P	7	15 ± 3	50 ± 38	5 ± 1
	N	7	1 ± 0	226 ± 65	202 ± 5
	N+P	7	3 ± 2	52 ± 28	6 ± 1
Summer	Ctrl	0	1	15	4
	P	0	45	14	4
	N	0	5	187	182
	N+P	0	44	185	181
	Ctrl	7	2 ± 1	60 ± 10	6 ± 2
	P	7	33 ± 2	70 ± 29	6 ± 2
	N	7	2 ± 1	230 ± 32	110 ± 3
	N+P	7	6 ± 1	65 ± 37	8 ± 3
Autumn	Ctrl	0	1	33	13
	P	0	34	13	5
	N	0	1	185	160
	N+P	0	30	194	165
	Ctrl	7	1 ± 0	13 ± 3	12 ± 13
	P	7	1 ± 0	10 ± 0	5 ± 1
	N	7	1 ± 0	146 ± 10	30 ± 6
	N+P	7	1 ± 0	16 ± 0	30 ± 40

APPENDIX 2

Table A2. The pair-wise comparisons (Mann-Whitney U-test) for the significant test results of the Kruskal-Wallis tests in the nutrient addition experiments.

Experiment	Variable	Test-results	Pair-wise comparisons					
			Ctrl vs. P	Ctrl vs. N	Ctrl vs. N+P	P vs. N	P vs. N+P	N vs. N+P
SPRING	Chl-a	P	0.046*	0.121	0.046*	0.050*	0.050*	0.050*
		Z	-1.55	-1.993	-1.993	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Hetero	P	0.05*	0.05*	0.05*	0.275	0.05*	0.05*
		Z	-1.964	-1.964	-1.964	-1.091	-1.964	-1.964
		N	6	6	6	6	6	6
	Cyano	P	0.050*	0.827	0.050*	0.050*	0.050*	0.050*
		Z	-1.964	-0.218	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
SUMMER	Chl-a	P	0.827	0.05*	0.05*	0.05*	0.05*	0.05*
		Z	-0.218	-1.964	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Phyto	P	0.127	0.275	0.05*	0.127	0.05*	0.05*
		Z	-1.528	-1.091	1.964	-1.528	1.964	1.964
		N	6	6	6	6	6	6
	Auto	P	0.827	0.05*	0.05*	0.05*	0.05*	0.05*
		Z	-0.218	-1.964	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Mixo	P	0.127	0.275	0.05*	0.127	0.05*	0.05*
		Z	-1.528	-1.091	-1.964	-1.528	-1.964	-1.964
		N	6	6	6	6	6	6

	Hetero	P	0.275	0.05*	0.05*	0.05*	0.05*	0.05*
		Z	-1.091	-1.964	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Cyano	P	0.05*	0.827	0.05*	0.05*	0.05*	0.05*
		Z	-1.964	-0.218	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Chryso	P	0.827	0.127	0.05*	0.05*	0.05*	0.05*
		Z	-0.218	-1.528	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Raphido	P	0.127	0.513	0.05*	0.127	0.05*	0.05*
		Z	-1.528	-0.655	-1.964	-1.528	-1.964	-1.964
		N	6	6	6	6	6	6
	Chloro	P	0.513	0.05*	0.05*	0.05*	0.05*	0.05*
		Z	-0.655	-1.964	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Other	P	0.275	0.05*	0.05*	0.827	0.05*	0.05*
		Z	-1.091	13.964	13.964	-0.218	13.964	13.964
		N	6	21.928	21.928	6	21.928	21.928
AUTUMN	Chl-a	P	0.275	0.05*	0.05*	0.05*	0.05*	0.05*
		Z	-1.091	-1.964	-1.964	-1.964	-1.964	-1.964
		N	6	6	6	6	6	6
	Mixo	P	0.827	0.05*	0.127	0.05*	0.127	0.05*
		Z	-0.218	-1.964	-1.528	-1.964	-1.528	-1.964
		N	6	6	6	6	6	6
	Chryso	P	0.827	0.05*	0.05*	0.513	0.05*	0.05*
		Z	-0.218	-1.964	-1.964	-0.655	-1.964	-1.964
		N	6	6	6	6	6	6