

## **Does Microbial Biomass Affect Pelagic Ecosystem Efficiency? An Experimental Study**

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*Received: 9 May 1993; Revised: 12 October 1993*

**Abstract.** Bacteria and other microorganisms in the pelagic zone participate in the recycling of organic matter and nutrients within the water column. The microbial loop is thought to enhance ecosystem efficiency through rapid recycling and reduced sinking rates, thus reducing the loss of nutrients contained in organisms remaining within the photic zone. We conducted experiments with lake communities in 5400-liter mesocosms, and measured the flux of materials and nutrients out of the water column. A factorial design manipulated 8 nutrient treatments: 4 phosphorus levels  $\times$  2 nitrogen levels. Total sedimentation rates were greatest in high-N mesocosms; within N-surplus communities,  $\geq 1 \mu\text{M}$  P resulted in 50% increase in total particulate losses. P additions without added N had small effects on nutrient losses from the photic zone;  $+2 \mu\text{M}$  P tanks received 334 mg P per tank, yet after 14 days lost only 69 mg more particulate-P than did control communities. Nutrient treatments resulted in marked differences in phytoplankton biomass (twofold N effect, fivefold P effect in +N mesocosms only), bacterioplankton densities (twofold N-effect, twofold P effects in -N and +N mesocosms), and the relative importance of autotrophic picoplankton (maximum in high N:P mesocosms). Multiple regression analysis found that of 8 plankton and water chemistry variables, the ratio of autotrophic picoplankton to total phytoplankton (measured as chlorophyll *a*) explained the largest portion of the total variation in sedimentation loss rates (65% of P-flux, 57% of N-flux, 26% of total flux). In each case, systems with greater relative importance of autotrophic picoplankton had significantly reduced loss rates. In contrast, greater numbers of planktonic bacteria were associated with increased sedimentation rates and lower system efficiency. We suggest that different microbial components may have contrasting effects on the presumed enhanced efficiency provided by the microbial loop.

## Introduction

The loss of planktonic materials to the sediments of lakes is one of the fundamental processes in freshwater ecosystems. These losses lead toward the immobilization of organic matter and nutrients to the sediments, and the eventual recycling of material following decomposition and turnover. Despite the fact that most of the sedimented organic matter produced by phytoplankton is decomposed [45], autochthonous sinking particles (dead phytoplankton, fecal pellets, organic detritus) represent a net loss of nutrients from the photic zone in most stratified lakes, until after turnover. Traditionally, nutrient cycling in lakes is thus thought of in terms of a phytoplankton–zooplankton–fish model, in which the return of nutrients depends on settled particles being decomposed and remixed into the water column in the spring or autumn. Factors affecting the flux rate of planktonic materials may include system productivity, plankton particle size, and basin morphometry. However, within a given productivity range, other biotic processes may enhance the regeneration of nutrients within the water column and reduce system losses to the sediments. Bacteria and other pelagic microorganisms participate in the recycling of organic matter [3, 5, 6].

It has been proposed that a microbial food web consisting of bacteria, picophytoplankton, and herbivorous flagellates and ciliates can act to shunt primary production and nutrients away from a “linear” metazoan food chain to a more rapid and efficient recycling mechanism [3, 33]. The importance of this microbial loop for pelagic ecosystem efficiency and the retention of inorganic nutrients in the photic zone needs to be quantified. Arguments have followed that predict that microorganisms, due to their high surface area to volume ratios, rapid growth rates, and slower sinking rates, will enhance the likelihood of nutrients contained in organisms remaining within the photic zone [37]. Thus, a yardstick for pelagic ecosystem efficiency may be measured according to reduced rates of downward nutrient flux (=“losses”), and may be compared with recently proposed measures of bacterial and other microbial biomass and growth.

Based on the ideas represented in microbial loop theory, rapid growth rates and efficient remineralization of nutrients can be maintained only if there is tight coupling between protist grazers and their bacterial prey [18, 37]. However, it is not clear whether this prediction considers both heterotrophic bacteria and autotrophic picoplankton in this size range. These two components, while physiologically quite distinct, compete for some of the same resources. Recent experiments indicate that the growth of heterotrophic bacteria may at times be limited by inorganic nutrients [15, 40], suggesting that these microorganisms may at times become a sink for nutrients within the microbial loop. Indeed, Caron [14] has shown that phytoplankton typically have C:N and C:P ratios higher than the Redfield ratio of 106C:16N:1P, while planktonic bacteria tend to have C:N and C:P ratios below this ratio.

Studies have shown that the relative abundance of autotrophic picoplankton tends to be greatest in oligotrophic (typically P-limited in freshwater) ecosystems [13, 35, 43], but apparently is not limited by the supply of dissolved inorganic phosphorus (DIP) in either eutrophic or oligotrophic lakes [41, 44]. Surprisingly, the growth of heterotrophic bacterioplankton was shown to be directly limited by DIP availability in the same system [40]. These experiments raise questions concerning the apparent

universal importance of P limitation in freshwaters [32]. The ecosystem impact of more efficient processing by certain microorganisms and protozoa may thus depend not only on the total production of microbial organisms, but also on the differing nutrient constraints that affect the compartments within the microbial loop.

In this study we propose that differences in total microbial biomass and the relative importance of heterotrophic and autotrophic picoplankton within the photic zone will affect system efficiency. Based on earlier results indicating differences in nutrient limitation, we predict that autotrophic picoplankton in particular will have a positive effect on the retention of nutrients in the water column (i.e., reduced nutrient sedimentation). We tested this idea by manipulating pelagic lake communities in large mesocosms, and measuring the flux of materials and nutrients out of the water column.

## Materials and Methods

### *Site Description and Design*

Experiments were conducted at the Experimental Lake Facility (ELF) at the Louis Calder Center, Fordham University (Armonk, NY, USA). The facility contains 24 fiberglass, outdoor mesocosms (1.9 m diam; 2.1 m high; filled to 5400 liters per tank) that are directly pump-fed with Calder Lake water [see 41, 42 for details on the lake] through prewashed, opaque polyethylene pipe. Mesocosms were filled in darkness during an 8h period on 1 June 1992, when Calder Lake was weakly stratified ( $\Delta T \approx 2^\circ\text{C/m}$ ). Night collection ensured that vertically migrating zooplankton (principally *Daphnia pulicaria*) occurred in all ELF tanks at similar levels. Nylon netting (1 cm mesh) was placed over each to minimize inputs of leaves and woody debris.

A factorial design consisted of 8 nutrient treatments: 4 P levels (0, 0.5, 1.0, or 2.0  $\mu\text{mol K}_2\text{HPO}_4/\text{l}$  added)  $\times$  2 N levels (0 or 10  $\mu\text{mol NH}_4\text{NO}_3/\text{l}$ ), with each block replicated three times. The intent of this design was to manipulate nutrient levels and N:P ratios such that phytoplankton production and community size structure varied over the full range observed in earlier studies [41]. Nutrients were added on day 0 during filling to allow sufficient mixing, and a second identical set was applied on day 14 following the third weekly sampling (see below); the experiment ran for 28 days. The primary goal was to examine whether losses of planktonic materials from the water column increase or decrease as a function of nutrient conditions and microbial processes. The basic models tested include (1) an ANOVA model, in which manipulated variables (N, P, N  $\times$  P) are classified as treatments, and (2) a regression model, in which observed plankton variables (levels of planktonic bacteria and "algal" picoplankton, nanoplankton, and microplankton) are classified as independent variables (see also later in Data Analysis).

### *Field Sampling*

A series of samples were collected weekly starting on day 0 (4h after filling) through day 28. Temperature and pH were measured at the ELF tanks in situ. Water and plankton samples were collected by peristaltic pump from 0.5 m depth. Pairs of water chemistry samples were filtered in line (Whatman GF/F); one set was acidified to pH 2 (with  $\text{H}_2\text{SO}_4$ ), and the other set was frozen ( $-15^\circ\text{C}$ ). Plankton chlorophyll was collected in 1-liter bottles while prefiltering microplankton through 20- $\mu\text{m}$  mesh size Nitex. The remaining filtrate was kept refrigerated ( $\approx 4^\circ\text{C}$ ) until returned to the lab.

To measure rates of plankton and other particulate losses, a free-standing, vertical sediment trap (with weighted bottom) was placed in the center-bottom of each ELF tank (retrieved by two nylon strings attached to the rim of the tanks). The cylindrical design followed general recommendations of Hargrave and Burns [20] and Bloesch and Burns [8], with a length/width ratio of about 5.5:1 (total

volume 550 ml) to minimize turbulence. Traps were constructed from 55 mm ID white PVC, with a polypropylene funnel fitted inside at the bottom to focus the settled sediment. A clamped plastic tube was attached to each funnel so that weekly samples of trapped sediment could be siphoned into sample bottles (stored at 4°C) and the traps returned to each ELF tank immediately after sampling. Following Kirchner [24], 90 ml of 5% NaCl was first placed into the bottom of each trap to create a density gradient and prevent material loss while traps were raised to the surface. Tests with saline plus methylene blue indicated little or no loss during transfer.

### Laboratory Methods

Water chemistry samples were analyzed for concentrations of soluble-reactive phosphorus (SRP) using the antimony-ascorbate-molybdate method [1, 9] and for total dissolved P as above, following persulfate digestion [29].  $\text{NH}_4^+$ -N concentrations were measured by the phenol-hypochlorite method, and  $\text{NO}_3^-$  (after reduction to  $\text{NO}_2^-$  in a Cd-Cu column) via reaction with sulfanilamide-NNED [1, 10, 11]. Soluble-reactive Si (as  $\text{SiO}_2$ ) was measured by the molybdosilicate method [1, 12]. Methods were modified for automated analysis and run on a Traacs 800 automated analyzer (Bran + Luebbe Technologies, Inc., Buffalo Grove, IL).

Sediment-trap samples (in water) were mixed thoroughly, split into four parts, and filtered onto either 25 mm (for particulate N, P) or 47 mm (total dry weight, pigments) diameter Whatman GF/F glass fiber filters (volumes recorded). Portions of filtered residue used for N, P, and pigment analysis were immediately frozen until digestion; total dry mass samples were placed in a drying oven (105°C) for 48h, then cooled in a desiccator. Particulate N and P were digested (30 min) under pressure (120°C, 15 psi) in Pyrex tubes using acid ( $\text{H}_2\text{SO}_4$ ) perchlorate [29]. Dry mass accumulation was measured to the nearest 0.1 mg. Sedimented pigments (chlorophyll *a* and pheophytin *a*) concentrations were measured following extraction in glass tissue grinders with  $\approx 4$  ml neutral 90% acetone (+ $\text{MgCO}_3$ ). Extracts were poured into graduated centrifuge tubes, made up to known volume, and further extracted in the dark (<4°C) for 18–24 h. Samples were centrifuged and measured spectrophotometrically (Shimadzu model UV-160) [1, 27]. The majority of measured pigment in the sediment traps ( $\approx 80\%$  on average) consisted of pheophytin.

Bacterioplankton in water samples ( $\approx 5$  ml) were preserved with glutaraldehyde (to 2%), stained for fluorescent microscopy using DAPI, filtered onto Irgalan black stained 25-mm polycarbonate filters (0.2  $\mu\text{m}$  poresize; Poretics Corp.), and stored at  $-15^\circ\text{C}$  until later observation [22, 31]. Counts were made at  $\times 1250$  magnification using a Nikon Labophot epifluorescence microscope, using 365 nm excitation and 520 nm barrier sets. A minimum of 10 grids and 300 cells were counted per sample replicate. Phytoplankton biomass (measured as chlorophyll *a*) was size fractionated (using polycarbonate filters) according to categories defined previously [34, 41]. Briefly, microplankton are cells or colonies 20–200  $\mu\text{m}$ , nanoplankton are 2–20  $\mu\text{m}$ , and picoplankton are 0.2–2  $\mu\text{m}$ . Filtering methods have been described previously [41, 42]; chlorophyll *a* was measured by spectrophotometer (corrected for pheophytin), as described above.

### Data Analysis

Data were compiled and analyzed using the SYSTAT 4.2 program [46]. Two types of hypothesis tests were set up to evaluate the models (nutrient and plankton effects) outlined in the experimental design section. For both, the dependent variables were particulate losses out of the water column to the sediment, measured as (1) total dry weight, (2) particulate P, (3) particulate N, and (3) pheophytin deposition. The first hypothesis tests were conducted using two-way ANOVA of manipulated variables. Treatments were (a) nitrogen (2 levels) and (b) phosphorus (4 levels) treatments, and (a  $\times$  b) an interaction term (N  $\times$  P), as categorical variables. Effects were judged to be significant if the probability ( $\alpha$ ) of an event occurring due to chance (P) was less than 0.05.

A second set of tests was used to derive predictions about how measured key water-column variables affect sedimentation rates, using stepwise multiple linear regression (MLR). In these, 8 independent

**Table 1.** Physical and chemical conditions measured in 24 experimental mesocosms at the start of the experiment, classified according to fertilization treatment. Temperature was measured in °C, and chemistry values are expressed in  $\mu\text{mol/l} \pm 1 \text{ SD}$  ( $n = 3$ ; SRP, soluble-reactive phosphorus)

	Phosphorus treatment ( $\mu\text{M}$ )			
	0 P	0.5 P	1.0 P	2.0 P
<b>NO N ADDED</b>				
Temp	18.5 $\pm$ 0.1	18.8 $\pm$ 0.9	18.4 $\pm$ 0.2	18.6 $\pm$ 0.1
pH	7.56 $\pm$ 0.12	7.56 $\pm$ 0.14	7.54 $\pm$ 0.29	7.49 $\pm$ 0.08
SRP	0.04 $\pm$ 0.01	0.38 $\pm$ 0.02	0.81 $\pm$ 0.06	1.71 $\pm$ 0.05
NH <sub>4</sub>	6.7 $\pm$ 1.9	6.7 $\pm$ 0.4	6.1 $\pm$ 0.2	6.0 $\pm$ 0.1
NO <sub>3</sub>	0.34 $\pm$ 0.01	0.31 $\pm$ 0.02	0.30 $\pm$ 0.04	0.29 $\pm$ 0.04
SiO <sub>2</sub>	11.7 $\pm$ 0.3	11.1 $\pm$ 0.1	11.1 $\pm$ 0.1	11.4 $\pm$ 0.03
<b>+ 10 <math>\mu\text{M}</math> NH<sub>4</sub>NO<sub>3</sub></b>				
Temp	18.8 $\pm$ 0.5	18.6 $\pm$ 0.1	18.3 $\pm$ 0.5	18.4 $\pm$ 0.6
pH	7.54 $\pm$ 0.27	7.54 $\pm$ 0.12	7.53 $\pm$ 0.22	7.58 $\pm$ 0.22
SRP	0.03 $\pm$ 0.01	0.34 $\pm$ 0.02	0.80 $\pm$ 0.02	1.76 $\pm$ 0.04
NH <sub>4</sub>	10.6 $\pm$ 0.5	14.8 $\pm$ 0.7	9.7 $\pm$ 0.5	14.6 $\pm$ 0.3
NO <sub>3</sub>	11.5 $\pm$ 0.3	11.4 $\pm$ 0.2	11.6 $\pm$ 0.3	11.6 $\pm$ 0.3
SiO <sub>2</sub>	10.9 $\pm$ 0.1	11.4 $\pm$ 0.1	11.3 $\pm$ 0.4	11.3 $\pm$ 0.1

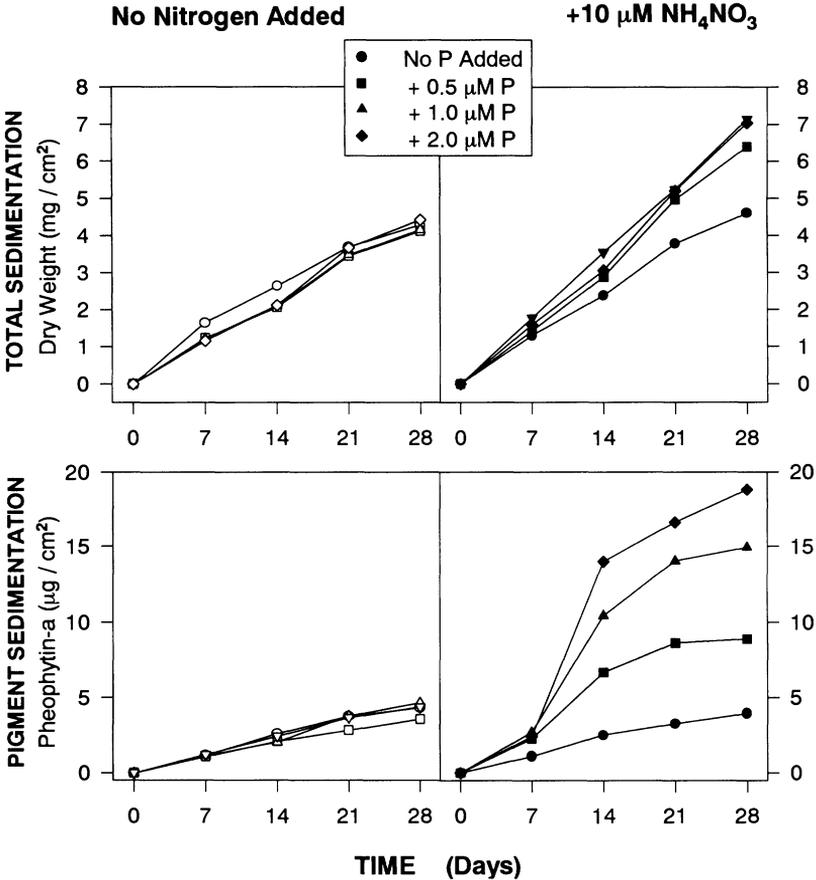
variables were tested for their effects on sedimentation losses: bacterioplankton density, autotrophic picoplankton, autotrophic nanoplankton, autotrophic microplankton, ratio of pico:  $\Sigma\text{Chla}$ , ratio of bacteria:  $\Sigma\text{Chla}$ , [SRP], and dissolved inorganic N ([DIN]). Ratios of pico:  $\Sigma\text{Chla}$  and bacteria:  $\Sigma\text{Chla}$  were included to test whether a predominance (rather than simple density) of microbial cells, both autotrophic and heterotrophic, alter systems losses and ecosystem efficiency. The maximum criterion for inclusion of independent variables into the model was  $\alpha = 0.05$ , and the minimum for exclusion was  $\alpha = 0.10$ . All data were checked for assumptions of normality and homogeneity of variances prior to analysis.

In summary, the two models make different biological assumptions about the proximate and ultimate factors leading to particulate losses to the benthos. The ANOVA models examine solely the effect of manipulations (i.e., N and P loadings), which may have direct effects on sedimentation, or indirect effects through changes in plankton biomass or shifts in community and size structure. The MLR models identify which of several measured variables in the water column, singly or collectively explain the differences in sedimentation losses over time. These factors may be thought of as having more immediate and direct effects on sedimentation rates, despite having not been directly manipulated. Each approach has its limitations, but both are useful starting points for making predictions about ecosystem efficiency under different nutrient and plankton community conditions.

## Results

### *Physical and Chemical Conditions*

The measured concentrations of SRP at the start of the experiment were somewhat below nominal treatment levels for P added (about 80%), but N treatments were close to target values (Table 1). Nutrients were nonetheless significantly different across treatments (ANOVA;  $P < 0.01$  in all cases) and not significantly different within treatments (ANOVA;  $P > 0.5$  in all cases). Actual P treatments thus represented a gradient of about  $1\times$ ,  $10\times$ ,  $25\times$ , and  $50\times$  of ambient conditions in Calder



**Fig. 1.** Cumulative, total particle flux from the water column to the sediments as a function of nitrogen and phosphorus manipulations, measured weekly in Calder Lake mesocosms. Rates are measured in terms of total dry weight (*upper panel*), and phaeophytin *a* accumulation (*lower panel*). All data are average rates from three replicate mesocosms for each nutrient treatment.

Lake. All non-manipulated variables measured, such as temperature, pH, and other chemical conditions, remained similar among all 24 tanks throughout the study.

### *Sedimentation Losses*

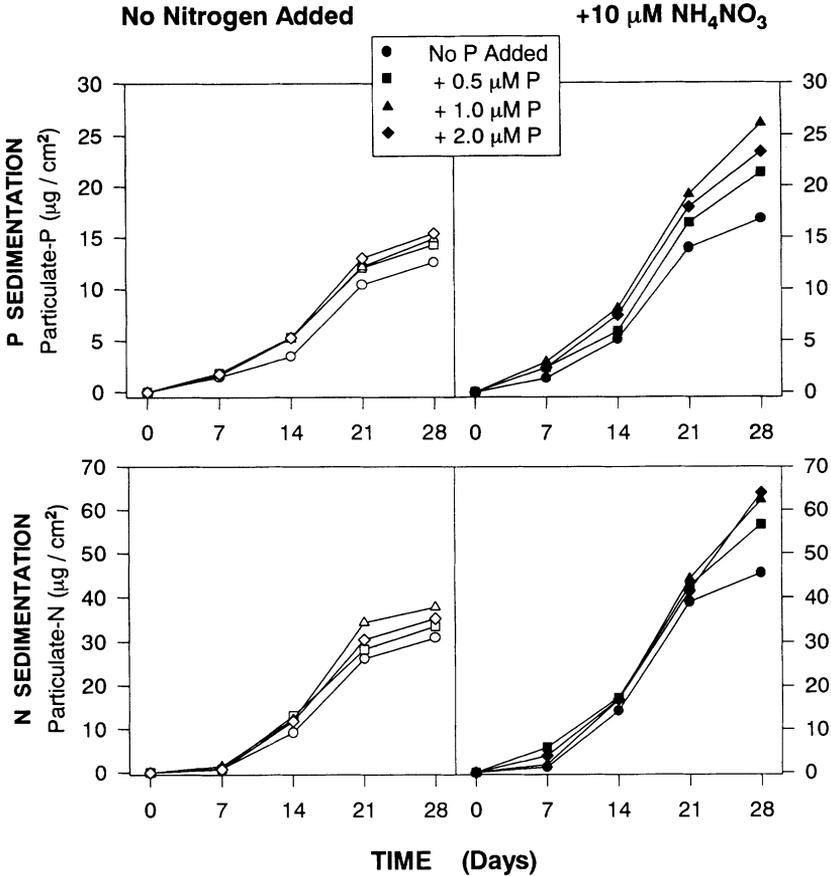
The first goal was to determine whether manipulations of nitrogen and phosphorus levels affect total and specific losses of materials out of the euphotic zone. A particular interest was whether losses observed differ from those predicted by simple increases in plankton biomass. Over 28 days, sedimentation proceeded at markedly different rates according to treatment conditions (Fig. 1). Each pair of treatment groups (no N added vs. 10  $\mu\text{mol NH}_4\text{NO}_3$  added per liter) is presented in two plots representing the effect of P additions on particulate losses to the sedi-

ments. Sedimentation of planktonic materials (dry weight) out of the water column was not different across a wide P-treatment gradient in mesocosms without added N (Fig. 1, *upper panel*). However, losses overall were nearly twice as great in systems with 10  $\mu\text{mol NH}_4\text{NO}_3$  added per liter, which correspond with a doubling in surface plankton biomass (see below). Within these N-fertilized mesocosms, phosphorus treatments  $\geq 1 \mu\text{mol/l}$  resulted in about 50% greater total plankton losses than control-P tanks. This effect was apparent by day 14 of the study.

Phytoplankton death and sinking rates, as judged by the weekly accumulation of pheophytin *a*, were dramatically affected by both aqueous N and P conditions (Fig. 1, *lower panel*). Nitrogen-fertilized mesocosms received up to three times more dead algal material than the sediments of N-limited systems, although this was not observed in communities lacking a P addition. The P treatments also elicited earlier (by day 14) and stronger responses than were observed for total or particulate nutrient sedimentation. The range of 0 to 2.0  $\mu\text{mol P}$  added per liter ( $\approx 0.4$  to 1.8  $\mu\text{M P}$  measured) resulted in an average increase in the cumulative loss rate from 3.9 to 18.8  $\mu\text{g pheophytin per cm}^2$  of sediment surface, nearly fivefold (i.e., 500%) greater. This greatly accelerated pigment accumulation corresponds with only a 50% increase in the total mass of sedimented planktonic materials.

Particulate nutrient losses were not necessarily related to increased loadings of those nutrients. In Calder Lake water, P additions alone had no apparent effect on losses of this nutrient to sediments (Fig. 2, *upper panel*). Particulate-P accumulated in the sediments of N-limited systems at similar rates across the wide range of P treatments. This means that a 50-fold increase in DIP loading had little effect on P losses in these mesocosms. Because the total volume of each mesocosm is known, differences in the retention of P within the euphotic zone can be estimated accurately. Systems supplied with 2.0  $\mu\text{mol P/l}$  received on day 0 an additional 334 mg of P (as  $\text{K}_2\text{HPO}_4$ ). After 14 days these communities lost only 69 mg more particulate-P (24.9% greater) than did control communities. However, the addition of inorganic N greatly accelerated P sedimentation, with maximum losses observed at high N and intermediate P (0.5–1.0  $\mu\text{mol P}$  added per liter) treatments. By the end of the experiment, sediment traps in phosphorus-limited mesocosms received about 28% less particulate-P ( $\approx 16.8 \mu\text{g/cm}^2$ ) than those receiving 2.0  $\mu\text{mol P/l}$  ( $\approx 23.3 \mu\text{g/cm}^2$ ). Nitrogen losses exhibited a similar pattern (Fig. 2, *lower panel*), although P treatments had apparently little effect on N retention or N loss in most systems.

The significance of direct nutrient manipulations on sedimentation rates was considered for each date by two-way analysis of variance (Table 2). The total loss of materials (as dry weight) was not significantly affected by either N or P until day 14, and by P treatments only by day 28. Nitrogen clearly was the most important treatment variable controlling total sedimentation rates. ANOVA was not able to detect any significant N  $\times$  P interaction effect (such as N:P ratio in the water column) on these rates. Losses of P, however, were affected by both N and P treatments (and N  $\times$  P interactions) in the water column by the end of the experiment. System losses of N were significantly affected by N additions throughout the study, but the analysis indicated that P additions had no significant effects. Unlike the other variables measured, pheophytin sedimentation was significantly affected by both N and P treatments, and exhibited significantly treatment interactions. From this perspective, P treatments resulted in significantly greater loss of algal material when N was also added, even on day 7. The importance of N  $\times$  P



**Fig. 2.** Cumulative, particulate nutrient flux from the water column to the sediments as a function of nitrogen and phosphorus manipulations, measured weekly in Calder Lake mesocosms. Rates are measured in terms of particulate P (*upper panel*), and particulate N accumulation (*lower panel*). All data are average rates from three replicate mesocosms for each nutrient treatment.

interaction on algal losses contrasts with the repeated nonsignificance of such an interaction effect on total sedimentation rates.

### *Microbial Biomass and Sedimentation*

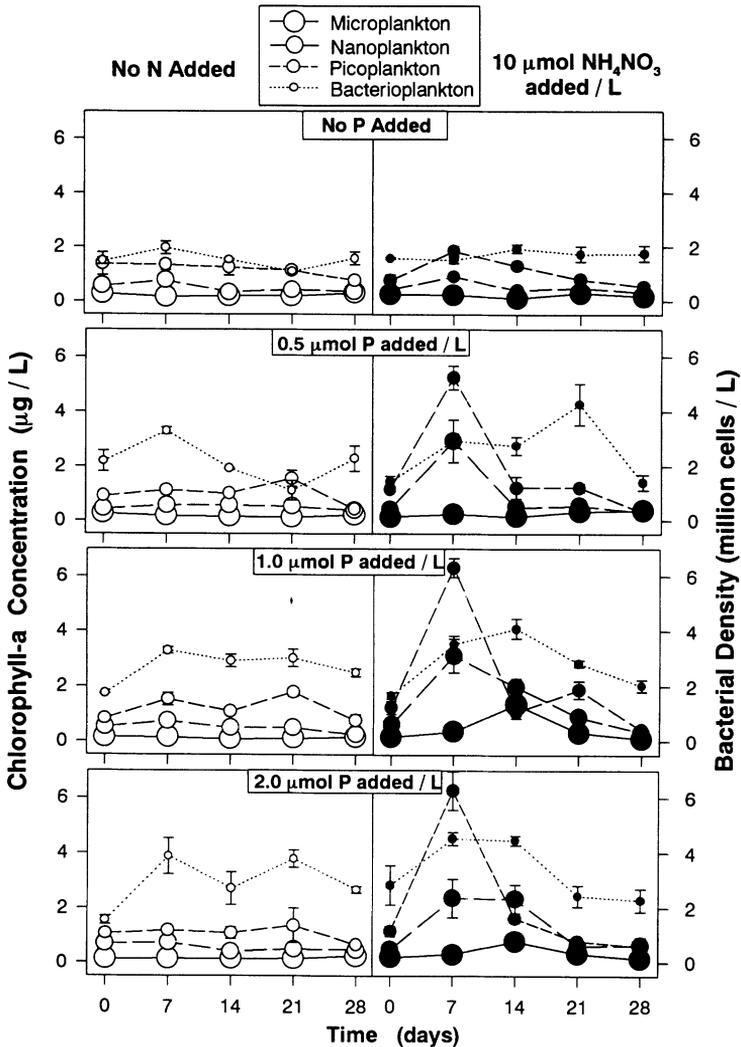
A second objective of this study was to examine the relationship between microbial plankton biomass (algal and bacterial) and system efficiency. Direct manipulations of N and P supply to the euphotic zone altered total biomass levels as well as the relative importance of various size fractions within the plankton (Fig. 3). Control (no P or N added) densities of phytoplankton and bacteria were not significantly different from levels in Calder Lake. Phytoplankton and bacterioplankton production levels were greatest in N-supplemented systems, but only bacteria increased in response to P additions without added N. Biomass responses to P additions in

**Table 2.** Effects of fertilization on plankton sedimentation in experimental mesocosms, as characterized by total (dry mass), phosphorus, nitrogen, and pheophytin loss rates (see Fig. 1 for details). Data are from a two-way analysis of variance of the effects of N addition (none or 10  $\mu\text{M}$   $\text{NH}_4\text{NO}_3$ ) and/or P addition (none, 0.5, 1, or 2  $\mu\text{M}$   $\text{K}_2\text{HPO}_4$ ) on rates of total dry mass (g dry wt/cm<sup>2</sup> per day), P, N ( $\mu\text{g}$  nutrient/cm<sup>2</sup> per day), and pheophytin ( $\mu\text{g}$  pheo *a*/cm<sup>2</sup> per day) accumulation measured at weekly intervals (a nominal *P* level of "0.000" is some unknown probability less than 0.001)

Treatment	Day 7		Day 14		Day 21		Day 28	
	<i>F</i> -ratio	<i>P</i> -value						
Total sedimentation								
N	0.327	0.576	15.346	0.001	6.867	0.019	38.607	0.000
P	0.488	0.696	0.914	0.456	2.335	0.115	4.112	0.024
N $\times$ P	0.830	0.497	1.820	0.184	0.866	0.086	2.795	0.074
Phosphorus sedimentation								
N	7.798	0.013	12.763	0.003	15.706	0.001	64.374	0.000
P	3.299	0.047	3.931	0.028	0.424	0.739	8.553	0.001
N $\times$ P	1.102	0.377	1.338	0.294	1.112	0.373	5.002	0.012
Nitrogen sedimentation								
N	6.394	0.022	9.343	0.008	9.101	0.008	46.280	0.000
P	0.676	0.580	1.272	0.318	0.319	0.812	2.630	0.086
N $\times$ P	1.434	0.270	2.356	0.110	0.466	0.710	0.014	0.014
Pheophytin sedimentation								
N	47.069	0.000	89.321	0.000	12.549	0.003	14.106	0.002
P	6.089	0.006	16.628	0.000	5.841	0.008	4.273	0.021
N $\times$ P	6.136	0.006	17.955	0.000	3.123	0.057	3.936	0.028

N-fertilized mesocosms also differed temporally among the 4 plankton size classes. Autotrophic picoplankton levels peaked on day 7, while bacterioplankton numbers tended to increase later in the experiment and remain at enhanced levels over longer periods. Differences in these biomass maxima and minima in turn altered the size spectrum of the community over time. The proportion of total chlorophyll *a* measured in picoplankton cells ranged between 70% in low-P mesocosms (high N:P ratios), to only about 30% in high-P, high-N mesocosms. Over this spectrum, total algal biomass increased twofold, mainly within the nano- and microplankton size classes. The ratio of bacterial numbers to total phytoplankton chlorophyll *a* ( $\approx$ bacterial importance) also varied with nutrient treatment. The relative importance of planktonic bacteria was greatest in high-N (50–100% increase) and high-P (80–130% increase) systems, yet the reverse trend was observed for autotrophic picoplankton.

These biomass and size shifts had apparent effects on the quantity and quality of water column materials lost to the sediments (Table 3). Simple bivariate correlations revealed greater overall sedimentation rates in all systems with greater total phytoplankton and bacterial biomass. The most important variables leading to greater total sedimentation and sedimentation of particulate P and N were autotrophic nanoplankton and microplankton, and heterotrophic bacteria. Larger algae and cyanobacteria consisted primarily of *Coelosphaerium naeglianum*, *Ceratium hirundinella*, and *Anabaena cylindricum*. Two marked exceptions to this positive feedback pattern were (1) a nonsignificant relationship with the biomass of autotrophic picoplankton, and (2) a significant negative relationship with pico-



**Fig. 3.** Effects of nitrogen (treatments = no N added vs.  $10 \mu\text{mol NH}_4\text{NO}_3$  added per liter) and phosphorus (treatments = none,  $0.5$ ,  $1.0$ , and  $2.0 \mu\text{mol PO}_4$  added per liter) manipulations on phytoplankton biomass (measured as chlorophyll *a*) in three size fractions, and bacterioplankton densities in Calder Lake mesocosms ( $n = 3$ ; error bars =  $\pm 1$  SE; error bars smaller than symbol size not shown).

plankton as a percentage of total chlorophyll *a*. As these biological (and several key chemical) variables may collectively influence ecosystem processes, and may themselves be autocorrelated, a multiple regression analysis was used to identify the most important factors (+ or -) affecting sedimentation rates, and to produce a more complete model that could describe the controls on system efficiency.

A stepwise multiple regression model evaluated the effect of 8 water-column variables: (1) bacterial density, (2) autotrophic microplankton ( $>20 \mu\text{m}$ ), (3)

**Table 3.** Relationships between microbial plankton biomass in mesocosms and particulate losses out of the water column. Data are Pearson product-moment correlations between sedimentation rates (total dry mass, particulate P, particulate N, and pheopigments) biomass levels of the autotrophic and bacterial plankton community in Calder Lake; relative microbial dominance estimated by the ratio between bacteria and total chlorophyll *a*, and the ratio of autotrophic picoplankton and total chlorophyll *a* ( $n = 24$ )

Plankton factors	Sedimentation variables			
	Total dry mass (mg/cm <sup>2</sup> per day)	Particulate P (μg/cm <sup>2</sup> per day)	Particulate N (μg/cm <sup>2</sup> per day)	Pheophytin (μg/cm <sup>2</sup> per day)
Bacteria	0.499*	0.721***	0.499*	0.744***
Autotrophic Pico	0.051	-0.096	-0.253	0.219
Autotrophic Nano	0.449*	0.734***	0.626**	0.845***
Autotrophic Micro	0.514*	0.612**	0.593**	0.653**
Auto-Pico: ΣChl <i>a</i>	-0.515*	-0.804***	-0.755***	-0.764***
Bact: ΣChl <i>a</i>	-0.099	-0.039	-0.101	-0.300

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

autotrophic nanoplankton ( $>2-20 \mu\text{m}$ ), (4) autotrophic picoplankton ( $>0.2-2 \mu\text{m}$ ), (5) the ratio of bacteria:ΣChl*a*, (6) the ratio of autotrophic picoplankton:ΣChl*a* (labeled as “% Pico”), (7) measured SRP concentration, and (8) measured dissolved inorganic N (DIN) concentration. The variables bacteria:ΣChl*a* and pico:ΣChl*a* were used to characterize communities with greater or lesser importance of microbial biomass. Total sedimentation rates were considered first (Table 4).

With a greater percentage of the phytoplankton biomass in small cells, total sedimentation rates were significantly less. The variable “% Pico” was identified as the primary variable explaining total system loss, although the complete model, with the inclusion of aqueous DIN concentration, could explain less than half of this response. In contrast, over 80% of the variation in particulate P losses could be predicted by a model including % Pico (–), bacterial density (+), and autotrophic microplankton (–); nearly 60% of the N-loss rates were explained by % Pico alone. In both instances, a greater importance of picoplankton resulted in reduced losses of nutrient elements. Planktonic bacteria are predicted to have no such effect on enhanced ecosystem efficiency. In fact, greater bacterial biomass was identified as a factor that may lead to increased P losses. Algal pigment sedimentation (measured as pheophytin) rates were strongly influenced by greater autotrophic nanoplankton levels, with the complete model predicting more than 75% of this response. All 4 sedimentation rates responded significantly to N and P manipulations (Table 2), yet their relationship to biotic factors from within the plankton were not alike. Pigment losses, unlike the other sedimentation variables, were not related to indexes of microbial processes (e.g., % Pico, or bact:ΣChl*a*).

Differences in these relationships are clearly revealed in bivariate plots of the 4 measures of sedimentation loss against the primary independent water-column variable identified by each multiple regression model (Fig. 4). In 3 out of 4 measures, the most important predictor (% Pico) exerted a negative influence on system losses. These models predict that a greater retention of materials within the euphotic zone results from a picoplankton-dominated phytoplankton community.

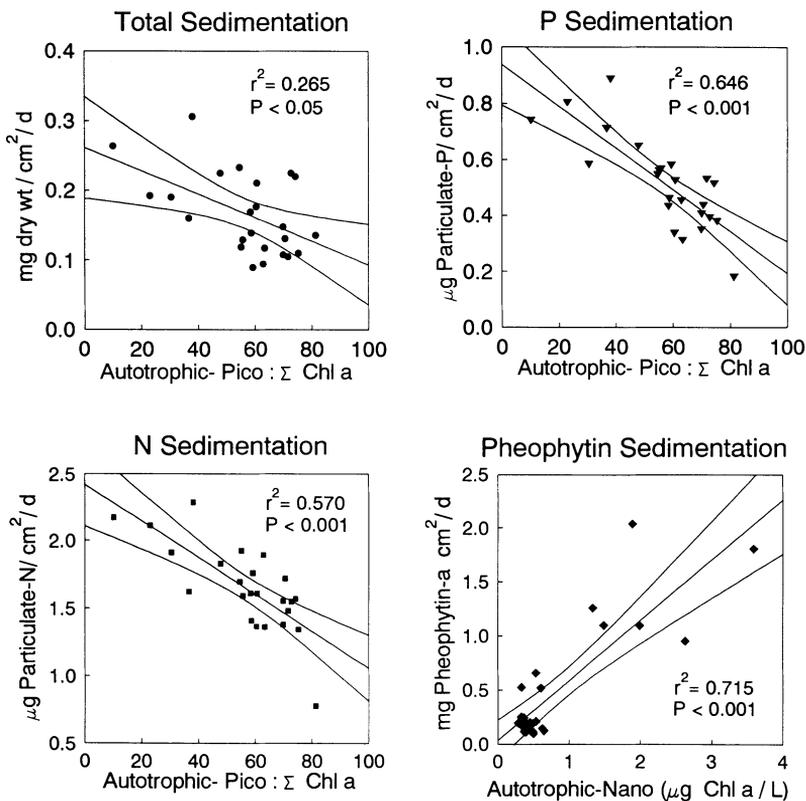
**Table 4.** Results of multiple regression analysis of the combined effects of biological and chemical characteristics in surface waters on rates of sedimentation losses. Eight independent variables tested were bacteria density, autotrophic picoplankton, autotrophic nanoplankton, autotrophic microplankton, ratio of pico: $\Sigma$ Chla, ratio of bacteria: $\Sigma$ Chla, [SRP], and [DIN]. The minimum criterion for inclusion of independent variables into the model was  $\alpha = 0.05$ , and 0.10 to remove them. Independent variables are listed in order of inclusion, with the first in each set explaining the largest proportion of the total variance ( $n = 24$ ; coef, coefficient of slope; SE, standard error; t, Student's *t*-score; *P*, probability; *F*, *F*-ratio from ANOVA;  $r^2$ , final coefficient of determination)

Step: indep var	coef	SE	t	<i>P</i>	
Dependent variable = total sedimentation					
1: % Pico	-0.002	0.0005	-3.189	0.004	
2: [DIN]	0.001	0.0002	3.031	0.006	
Y-intercept	0.232	0.032	7.291	<0.001	
complete model	<i>F</i> = 10.045		<i>P</i> = 0.001		$r^2 = 0.489$
Dependent variable = phaeo sedimentation					
1: Nano	0.412	0.085	4.827	<0.001	
2: Bacteria	0.176	0.065	2.698	0.013	
Y-intercept	-0.329	0.157	-2.099	0.048	
complete model	<i>F</i> = 39.041		<i>P</i> = <0.001		$r^2 = 0.788$
Dependent variable = P sedimentation					
1: % Pico	-0.010	0.002	-5.463	<0.001	
2: Bacteria	0.066	0.016	4.235	<0.001	
3: Auto-Micro	-0.194	0.066	-2.953	0.008	
Y-intercept	0.969	0.138	7.038	<0.001	
complete model	<i>F</i> = 34.663		<i>P</i> = <0.001		$r^2 = 0.839$
Dependent variable = N sedimentation					
1: % Pico	-0.014	0.003	-5.396	<0.001	
Y-intercept	2.421	0.149	16.202	<0.001	
complete model	<i>F</i> = 29.117		<i>P</i> = <0.001		$r^2 = 0.570$

Planktonic bacteria did not, however, exhibit this relationship. Total sedimentation rates were less effectively modeled, but more than 50% of the variation in both N and P retention or loss could be predicted by % Pico alone. In contrast, reduced ecosystem retention of phytoplankton pigments was predicted to be the result of a greater biomass of autotrophic nanoplankton.

## Discussion

Nutrient manipulations in lake mesocosms, consisting of two pulses (on day 0 and day 14) of nitrogen and/or phosphorus, affected the biomass and size structure of the plankton community, and also the flux of sediment exported out of the water column. Maximum loss rates were observed in high N (10  $\mu\text{mol NH}_4\text{NO}_3$  added per liter and P (2  $\mu\text{mol K}_2\text{HPO}_4$  added per liter) systems, with N exerting the larger effect. Because fertilization will often result in greater plankton biomass, it is expected to some degree that nutrient-surplus conditions can lead to greater rates of sedimentation [e.g., 7]. Other studies have shown that phosphorus sedimentation in lakes can be partially predicted by levels of epilimnetic P, but also by the amounts of particulate P contained within planktonic materials >20  $\mu\text{m}$  in size [28]. The



**Fig. 4.** Bivariate regression plots of the key water column predictors (independent variables) versus 4 measures of particle flux into sediment traps in Calder Lake mesocosms. The most important independent variable in each case was extracted by stepwise multiple regression ( $n = 24$ ; lines fit by least squares regression;  $P$ , probability;  $r^2$ , coefficient of determination). For details of each independent variable, see text.

mechanism behind these dynamics may not simply be a matter of greater plankton biomass, but also enhanced ecosystem efficiency.

Our experiments, which directly manipulated short-term nutrient concentrations (two discrete pulses), were designed to cause a shift in the biological community and in longer-term chemical conditions within the euphotic zone. These characteristics, particularly phytoplankton size structure and the relative importance of a pelagic microbial loop, should be given particular attention, as they are believed to play a central role in regulating ecosystem efficiency [3, 33]. The theory states that materials that pass through a microbial loop are more efficiently remineralized and fed back into soluble pools. Experimental studies support this theory. The uptake and regeneration of both N and P within surface waters has been shown to be regulated mainly by planktonic organisms  $<3 \mu\text{m}$  in size [4, 16, 17], and less by herbivorous zooplankton, such as cladocerans. Other studies have shown that smaller protist grazers, such as heterotrophic nanoflagellates and ciliates, can accelerate microbial regeneration of nutrients, particularly P, within the water

column [5, 6]. Sedimentation studies in a tropical marine system have shown that picoparticles do not tend to settle out over 24h, while significant percentages of autotrophic nanoplankton ( $\approx 15\%$ ) and microplankton ( $\approx 30\%$ ) are exported to the benthos daily [23]. For these reasons, it has been specifically suggested that nutrients cycled within the microbial loop should have a greater probability of remaining within the photic zone [37]. Hence, whole-system manipulations which lead toward greater microbial processing may be used to test whether ecosystem efficiency is truly enhanced.

This study demonstrates that changes in the size structure of a phytoplankton community, that is, a shift from predominantly large-celled to small-celled assemblages, correspond with a greater retention of materials within the photic zone, including both P and N. Sedimentation rates of particulate P regress negatively against a simple index of microbial dominance, the ratio of autotrophic picoplankton: $\Sigma$ Chla. The slope predicts that for every 10% increase in the predominance of picoplankton, the system (on an areal basis) will retain 7.4  $\mu\text{g}$  more P and 13.6  $\mu\text{g}$  more N/ $\text{m}^2$  per day. The ecosystem significance of this effect in lakes will further depend in part on protozoan grazing activity, but also the depth of the photic zone that will support autotrophic picoplankton. There are data that suggest that phycoerythrin-rich picoplankters, such as some species of *Synechococcus*, may utilize reduced light field more efficiently than other autotrophs [e.g., 19, 26], which may favor their greater relative abundance with depth [44]. Thus, low-P (high N:P ratios), deep lakes may be those ecosystems with greatest pelagic, microbial recycling and nutrient retention.

Our data support earlier studies indicating that greater N:P ratios in freshwaters not only alter phytoplankton production levels, but may result in communities dominated by bacterial-sized cyanobacteria, such as *Synechococcus* [36, 41, 44]. Suttle and Harrison [38] have shown that a high affinity for DIP in *Synechococcus* spp. may be critical in favoring smaller autotrophs in P-limited systems. Our data reveal that the ecosystem implications of this higher affinity for P are reduced losses of nutrients out of the water column, as well as significantly lower total sedimentation rates. The lack of a similar effect by planktonic, heterotrophic bacteria (Tables 3, 4), which also fall into this size range (or even smaller), is not easily explained. Recent studies have shown, however, that production rates of planktonic bacteria may be directly limited by inorganic P in freshwaters [15, 40], and this limitation may occur independently of a phytoplankton response [25]. It has been suggested that DIP uptake by planktonic bacteria may exceed demands and result in  $\text{PO}_4\text{-P}$  becoming immobilized, unless there is considerable protozoan activity [2, 39]. Similar data have led some researchers to state that planktonic bacteria may be a phosphorus sink [6]. It is still difficult to explain how in the same communities from Calder Lake, that greater numbers of autotrophic picoplankton will lead to increased P retention, while presumably more rapidly-growing (and grazed) bacteria may have the opposite effect (Table 4). Effectiveness and mechanisms of nutrient turnover by heterotrophic (and mixotrophic) flagellates may perhaps differ according to food item, with more rapid mineralization occurring with herbivory on autotrophic cells such as *Synechococcus*.

Further, the ratio of recycled C:N:P resulting from zooplankton herbivory is the result not only of the grazing rate and the nutrient composition of food items, but is also affected by different nutrient demands among various zooplankton species

[21]. For example, low P:C ratios in P-starved bacteria may lead to still lower P release rates by grazing flagellates that have high P demands. Phosphorus remineralization may even approach zero during active grazing if P:C ratios of bacteria and phytoplankton fall below critical levels [30]. Net mineralization measurements [e.g., 6] and sedimentation efflux measurements (the present study) reflect the combined effects of nutrient turnover (leakage, excretion, grazing effects) and utilization (uptake, storage, growth) by each microbial component. Our data suggest that heterotrophic bacteria and autotrophic picoplankton must differ in some critical but not fully known ways that lead to their roles as either sinks or sources for P and N within the photic zone.

*Acknowledgments.* This study was funded in part by the NSF, grant # DIR-9002145, and by the Louis Calder Center, Fordham University. We thank Ken Cavassi for helpful assistance in the field.

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