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# Modification of the N : P ratio in lakes by in situ processes

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*Abstract*

In situ mesocosms in two Canadian Shield lakes were used to evaluate the contributions of in-lake vs. external sources of nitrogen and phosphorus to nutrient budgets and N : P ratios. These mesocosms were designed to have variable exchange with sediments. Half were fertilized with N and P at a ratio great enough to ensure P limitation for most phytoplankton (atomic ratio, 33 : 1); the other half were fertilized at a ratio low enough to cause N limitation (4.4 : 1) in the absence of compensation mechanisms.

For littoral mesocosms, sediments were a major source of N, but not of P. A comparison of mesocosms having sediments with one having a plastic floor indicated that sediment N return was derived largely from decomposing material at the sediment surface, rather than from deep sediments. Disproportionate returns of N from sediments, along with lower denitrification, reduced N limitation in the low N : P mesocosms. In pelagic mesocosms, which lacked sediment contact, $N_2$ fixation and thermocline entrainment late in the experiment were the principal internal N sources at low N : P.

Biogeochemical mechanisms for reducing N : P in the water column at high N : P supply ratios were less effective than those that ameliorated N shortages at low N : P. The most important mechanism for reducing N : P was denitrification, while both $N_2$ fixation and sediment return raised N : P. We conclude that biogeochemical mechanisms allow N shortages to be more readily overcome than P shortages in Canadian Shield lakes. Due to the importance of sediments as an N source, it is important to scale mesocosms so that they have sediment : water ratios similar to those of the lakes they are designed to simulate.

Phosphorus usually limits the biomass and productivity of the phytoplankton community in temperate lakes during summer (Hecky and Kilham 1988). In general, P limitation in phytoplankton is expected when the N : P ratio of the nutrient supply is substantially larger than the mean atomic ratio of N to P in phytoplankton cells, 15 : 1 (Redfield 1958); an ambient ratio $\ll 15 : 1$ suggests N limitation. The effective supply of nutrients to phytoplankton includes both allochthonous inputs to the lake and internal inputs, such as $N_2$ fixation, releases from sediments, and recycling from heterotrophs. Because the measurement of internal inputs is difficult and often impractical, nutrient limitation usually is predicted from the lake's allochthonous N : P supply ratio alone, or from in situ nutrient concentration ratios [e.g. total N : total P (TN : TP), dissolved inorganic N : soluble reactive P (DIN : SRP), or DIN : TP] (Vollenweider and Kerekes

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1980; Morris and Lewis 1989). The use of concentration ratios instead of external supply ratios provides some representation for internal sources. Thus, in many lakes, epilimnetic TN : TP and DIN : SRP ratios are higher than allochthonous N : P supply ratios (Smith 1983; Schindler 1985). However, it has not yet been shown how the different concentration ratios relate to the total (external plus internal) N : P supply ratio.

In this study, we used mesocosm experiments to address the question of whether internal processes significantly affect the supplies of N and P (and thus the effective N : P supply ratio) to phytoplankton in Canadian Shield lakes. We wished to know in particular whether the impact of very high N : P or very low N : P allochthonous supply ratios might be mitigated through $N_2$ fixation (at low N : P), denitrification (at high N : P), or differential recycling of N and P from sediments (as suggested by Schindler 1977; Howarth 1988).

We manipulated the potential for nutrient recycling within the mesocosms by using a combination of mesocosms open to sediments, with plastic floors (which limited nutrient recycling from sediments to recently deposited particles), and open to the thermocline (where no return of nutrients from sediments was possible). This strategy also facilitated separation of the contributions to N and P pools by nutrient exchange with the atmosphere, nutrient release from newly sedimented material, and nutrient release from older sediments. Mesocosms were placed in two lakes within the Experimental Lakes Area (ELA) of northwestern Ontario and fertilized with nitrate and phosphate at two N : P ratios, one large enough to ensure P limitation (33 : 1 by moles; 15 : 1 by wt), and the other low enough to cause N limitation (4.4 : 1 by moles, 2 : 1 by wt) in the absence of in situ compensation mechanisms.

## *Materials and methods*

*Description*—The ELA is an Ontario region (93°30′–94°00′W, 49°30′–49°45′N) set aside for the study of freshwater pollution through whole-lake (and lesser scale) experimentation (Schindler 1985). The ELA lakes are typical of the hundreds of thousands of Precambrian Shield lakes present in Canada, parts of the northern United States, and Scandinavia.

Two headwater lakes, Lake 303 and the south basin of Lake 302 (Lake 302S; which is separated from the north basin by a vinyl curtain), were chosen as sites for the mesocosm experiments. Both lakes are in granitic basins with negligible groundwater flux (Newbury and Beaty 1980), a characteristic which greatly simplifies calculations of mass balance. They are naturally oligotrophic, with summer phytoplankton communities dominated by mixtures of chrysophytes, diatoms, and small green algae. Macrophytes are rare in both lakes and were not present in the mesocosms.

The sediments of the lakes are generally flocculent (water content >90%) and highly organic (organic C content, ~30% of dry wt) because of the low input of clastic materials (Brunskill et al. 1971). Only near inflows are there extensive areas of sand and gravel. The pore-water diffusion coefficients for the organic sediments are very low ($1–2 \times 10^5$ cm$^2$ s$^{-1}$, Hesslein 1980). Consequently, sediments are depleted of oxygen below a few millimeters depth, and active populations of denitrifiers and anaerobic bacteria can be found close to the sediment surface (Kelly et al. 1982). It has been shown through sediment profiles and various mass balance and radiotracer experiments both in sediment cores and in situ (Schindler et al. 1975, 1977, 1987; Levine et al. 1986) that ELA lake sediments retain P almost quantitatively under both oxic and anoxic conditions. In contrast, $NH_4$ concentrations are high in these sediments, and N release may be considerable at times (Schindler et al. 1977, 1987).

Lakes 303 and 302S are similar in area (9.9 vs. 10.9 ha,), but not in depth (mean depth, 1.5 vs. 5.1 m). Thus, Lake 302S stratifies in summer (epilimnion depth, 2–4 m) while Lake 303 is monomictic. The lakes also have different histories of experimental manipulation. At the time of our experiments, Lake 302S had never been manipulated, although the north basin (302N, downstream) had been used to study the effects of hypolimnetic fertilization (Schind-

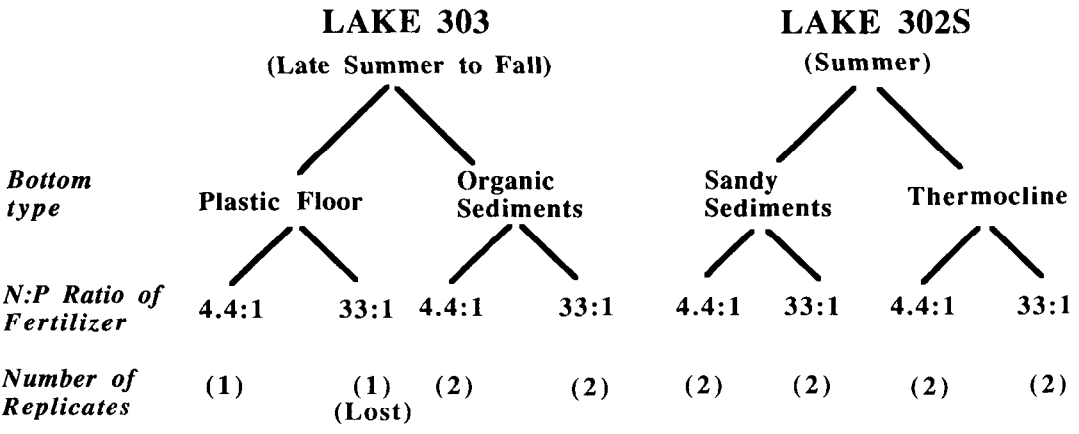


Fig. 1. Treatment scheme for the mesocosm experiments.

ler et al. 1980). Lake 303, on the other hand, was experimentally fertilized during the two summers prior to our experiments with 216 mmol N $m^{-2}$ $yr^{-1}$ as $NaNO_3$ and 6.5 mmol P $m^{-2}$ $yr^{-1}$ as $H_3PO_4$ (N : P = 33 : 1, by moles). While fertilized, the lake had algal standing crops and water chemistry typical of a eutrophic lake (Levine and Schindler 1989). By the initiation of our experiment, algal standing crops and P chemistry had returned to background. The flux of $NH_4$ from sediments, however, was still well above the baseline (Levine and Schindler 1989).

*Experimental design*—Figure 1 is a schematic of the experimental design for the mesocosm experiments. Briefly, P was added weekly to mesocosms at similar rates on an areal basis, while N was added at two different levels, yielding atomic N : P supply ratios of 33 : 1 and 4.4 : 1 (mesocosms treated under the two fertilization schemes will hereafter be called N : P = 33 and N : P = 4 mesocosms). The two treatments were repeated in duplicate at three sites: over organic sediments in shallow Lake 303, in a sandy region of the littoral zone of Lake 302S, and in pelagic Lake 302S. In addition, two mesocosms in Lake 303 were fitted with polyethylene floors and fertilized at N : P ratios of 4.4 : 1 and 33 : 1. The floors blocked nutrient inputs from the sediments, but did not prevent recycling of material sedimented during the course of the experiments. Gas accumulation under the floors caused the floored N : P = 33 mesocosm to fail early in the experiment. Destruction of the floored N : P = 4 mesocosm was prevented by a gas-venting system (Levine 1983). The pelagic mesocosms were open at the bottom, allowing settling particles to fall through the thermocline. Because the thermocline had a very low rate of vertical diffusion (Quay et al. 1980), it prevented significant inputs of nutrients from below. Two unfertilized mesocosms, one with and one without a plastic floor, were included in the experiments in Lake 303. These allowed an evaluation of the influence of enclosure on phytoplankton (Levine 1983; Levine and Schindler in prep.) and on water chemistry.

*The mesocosms*—The mesocosms used in Lake 303 were 10-m-diameter cylinders of clear cross-woven polyethylene sheeting suspended from frames at the lake surface and extending ~0.5 m into the sediments. Slack in the walls and heavy weights sewn into their hems prevented movement out of the sediments during stormy weather (*see also* Levine and Schindler 1989). The mesocosms in Lake 302S were similar, except that the walls were somewhat thicker (10 vs. 6 mil), and for the pelagic mesocosms, much longer (6 vs. 3 m). The weighted hems of the mesocosms could not be pushed into the sandy littoral sediments of Lake 302S and instead were spread out horizontally and covered with cobbles and 5–10 cm of sand. The walls of the open-bottomed pelagic mesocosms extended below the thermocline, and their hems were weighted and tied down with lines. The littoral meso-

cosms in both lakes were in 2 m of water, and the pelagic mesocosms were at 7–9 m. All of the mesocosms had temperature profiles similar to those in the surrounding lakes and had dissolved oxygen concentrations near saturation during the day (Levine 1983; Levine and Schindler unpubl. data).

*Water exchange between the mesocosms and lake*—For in situ mesocosms, there is always potential for water exchange with the surrounding lake, through seams, holes in walls, movement of water through porous sediments, or splashing from surface waves. To assess the magnitude of such exchanges, we added 555 mBq (15 mCi) of tritiated water to each mesocosm on 1–3 occasions and monitored its activity over 3–4 weeks. Initial $^3H$ activities allowed calculation of mesocosm volumes, and rates of tritium disappearance allowed calculation of water flushing rates.

The Lake 303 mesocosms had relatively long water residence times, 7–15 months, despite the presence of some tiny holes in their walls. The Lake 302S mesocosms had thicker walls, but retained water for much shorter periods of time (1–3 months in the littoral and 1–1.5 months in the pelagic mesocosms). For the littoral Lake 302S mesocosms, water loss was probably principally through the sandy sediments (sands have high hydraulic conductivities: Freeze and Cherry 1979). In the pelagic mesocosms, the continued dilution of the $^3H$ label after its initial mixing into surface waters probably was a consequence of a slow exchange of water between the epilimnion and the weakly stratified upper portion of the metalimnion.

*Fertilization*—Phosphate fertilizer was added weekly to the Lake 303 mesocosms to yield a final concentration of 0.32 μmol P liter$^{-1}$. Nitrate was added at the same time at a rate of either 1.4 or 11 μmol N liter$^{-1}$ week$^{-1}$, depending on the N:P ratio desired. The pelagic mesocosms of Lake 302S initially received the same P dose as the Lake 303 mesocosms. However, because of thermally induced increases in epilimnion depth, volumetric loading of nutrient to these mesocosms decreased over time. At the end of the experiment, our weekly nutrient additions (which did not change over time) yielded final concentrations of 0.23 μmol P liter$^{-1}$ and either 1.0 or 7.6 μmol N liter$^{-1}$.

P fertilization of the mesocosms in the littoral region of Lake 302S yielded final concentrations between 0.39 and 0.52 μmol P liter$^{-1}$. The range in final concentrations was due to a decision to fertilize at a constant value of $I_P$ $V_o$ [where $I_P$ was the P addition in μmol liter$^{-1}$ week$^{-1}$ and $V_o$ the volume (liters) of water exchanged weekly through leakage], rather than at a constant value of $I_P$, and thus create more consistent rates of productivity in the mesocosms than is possible by ignoring differences in flushing rates. For all of the Lake 302S mesocosms, the N dose was maintained at either 4.4 or 33 times the P dose by moles, to yield the desired N:P supply ratios.

For the Lake 303 mesocosms, fertilization began on 10 August 1977 and continued until 25 October 1977, when ice formed on the lake. The experiments in the Lake 302S mesocosms began earlier in the year, 10 June 1978, and ended on 5 September, when the thermocline of the lake deepened beyond 6 m and the mesocosms were flushed with outside water.

*Sampling and analysis*—Depth-integrated water samples were collected from the mesocosms biweekly with PVC pipe (length, 2 m for littoral and 3 m for pelagic mesocosms) and a rubber bung. Three or four sites within each mesocosm were sampled and the water samples pooled. Among the chemical fractions measured (procedures of Stainton et al. 1977) were suspended N and P, total dissolved N and P (TDN and TDP), $NH_4^+$-N and $NO_3^-$ + $NO_2^-$-N. $NO_2^-$-N was not measured separately, as it has rarely been detected in surface waters of ELA lakes. Chlorophyll *a* concentration and primary productivity ($H^{14}CO_3^-$ incorporation) also were measured biweekly. Phytoplankton species were identified and enumerated and are reported elsewhere (Levine 1983; Levine and Schindler in prep.).

When heterocystous cyanobacteria were present in phytoplankton samples, the acetylene reduction method of Flett et al. (1976) was used to estimate $N_2$ fixation. Samples were incubated in syringes positioned on the rotating wheels of a primary production in-

cubator (Shearer 1976). The incubator was used rather than incubations in situ because it allowed a more precise definition of the light-response function for fixation per gram of fixer biomass. Daily fixation rates in the mesocosms were estimated from the light-response functions, the rate of light extinction with depth in the mesocosms (measured biweekly with a submersible quantum sensor), biweekly records of fixer biomass, and daily records of incident solar radiation (Levine and Lewis 1987).

*Mass balance calculations*—Biweekly mass balances for P and N in the mesocosms were calculated. Briefly,

$$\Delta M = L - O$$

where $\Delta M$ is the change in nutrient concentration over 2 weeks, and $L$ and $O$ are the total inputs and outputs of the nutrient (Levine and Schindler 1989). Inputs included the external sources, fertilization plus atmospheric precipitation ($I$); sources from within the mesocosms, $N_2$ fixation ($H$), sediment return ($F$), and metalimnetic entrainment ($T$); and net nutrient gains through water exchange between the mesocosms and the surrounding lake ($E$). Outputs included sedimentation ($S$), denitrification ($D$), and net nutrient loss through water leakage ($E$).

The fertilizer input to the mesocosms was precisely known, and the nutrient supply from precipitation was easily calculated from rainfall records and chemistry. For the Lake 303 mesocosms, the net nutrient input or loss due to leakage was the product of the tritium-determined water exchange rate and the difference between total N (TN) or total P (TP) concentrations on the two sides of the mesocosm walls. For the Lake 302S mesocosms, $E$ was calculated differently, because we believe that "leakage" occurred at the bottom of these mesocosms rather than across the walls (*see above*). For littoral mesocosms, the exchanged water probably moved through sandy sediments, excluding suspended particles. Therefore, we estimated their $E$ as the product of the water flux and the *dissolved* nutrient gradient across the mesocosm walls. For pelagic mesocosms, we assumed that $E$ represented the net exchange of nutrient between the epilimnion and the upper, weakly stratified portion of the metalimnion (the uppermost meter of water with a temperature gradient $>1.0$°C) and estimated this exchange by multiplying the water loss rate by the difference in nutrient concentration between the epilimnion and the upper metalimnion. Because the metalimnion was sampled only monthly and at 1-m intervals, mean upper-metalimnion nutrient concentrations had to be calculated through interpolation. The error introduced by this procedure was inconsequential to overall mass balance because the exchange rates for these mesocosms were small compared to external sources. Another source of error to our mass balance calculations for pelagic mesocosms was lack of data on possible nutrient exchange across the thermocline due to vertical migrations of zooplankton and fish.

Late in the experiment, wind action deepened the thermocline in the pelagic mesocosms and nutrients were gained through entrainment of metalimnetic waters. These gains ($T$) were calculated by multiplying the volume of entrained water by its nutrient concentration (which also was estimated through interpolation).

P sedimentation in all mesocosms and N sedimentation from the pelagic mesocosms was calculated by difference ($S_P = L_P - E_P - T_P - \Delta M_P$; $S_N = L_N + H - E_N - T_N - \Delta M_N$), as there was no return of nutrients from sediments or the hypolimnion. For littoral mesocosms, $S_N$ was confounded with N feedback from sediments, so that the above calculation yielded only a value for net sedimentation. To estimate gross $S_N$ in these mesocosms ($S'_N$), we multiplied the mesocosm-specific $S_P$ rates by the mean ratios for suspended N : suspended P in the mesocosm. This method assumed that sedimenting particles had the same N : P ratio as particles in suspension—a reasonable assumption given the shallow depth of the mesocosms (2 m). The difference between gross and net N sedimentation ($S'_N - S_N$) yielded an estimate of N feedback from sediments plus denitrification ($F_N$). Positive values indicated a net feedback of N and negative values indicated net denitrification.

These mass balance calculations could not

Table 1. For the 12-week fertilization experiment in Lake 303, the mean Chl *a* concentration, average distribution of N and P among different fractions, and mean TN : TP and mean $L_N : L_P$ ratios (SD in parentheses) in duplicate mesocosms (R1, R2). All mesocosms were fertilized with P at a similar rate, but N fertilizer was added at two rates to yield N : P fertilization ratios of 4.4 : 1 and 33 : 1. Samples were taken biweekly. Also included are data for the water surrounding the mesocosms (lake).

| | | Mesocosm with a floor N : P = 4.4 | Mesocosms without floors | | | |
|---|---|---|---|---|---|---|
| | | | N : P = 4.4 | | N : P = 33 | |
| | Lake | R1 | R1 | R2 | R1 | R2 |
| Chl *a*, μg liter$^{-1}$ | 3(1) | 6(1) | 13(2) | 11(4) | 18(12) | 24(8) |
| TN, μmol liter$^{-1}$ | 84(16) | 42(2) | 53(9) | 68(9) | 107(34) | 91(21) |
| % suspended N | 9(4) | 26(4) | 34(7) | 28(8) | 21(6) | 25(5) |
| % DON | 36(8) | 72(4) | 65(7) | 47(6) | 37(9) | 32(9) |
| % DIN | 55(11) | 2(2) | 1(0) | 25(10) | 42(12) | 43(13) |
| % $NH_4^+$ | 46(7) | 1(0) | 1(1) | 20(9) | 14(9) | 9(5) |
| % $NO_3^- + NO_2^-$ | 9(6) | 1(1) | 0(0) | 5(2) | 28(6) | 34(9) |
| TP, μmol liter$^{-1}$ | 0.3(0.0) | 1.3(0.5) | 1.1(0.3) | 1.1(0.3) | 1.1(0.5) | 0.9(0.1) |
| % suspended P | 53(6) | 52(20) | 70(6) | 69(8) | 65(4) | 74(3) |
| % TDP | 47(6) | 48(20) | 30(6) | 31(8) | 35(4) | 26(3) |
| TN : TP | 276(8) | 38(20) | 50(10) | 64(12) | 102(9) | 106(15) |
| $L_N : L_P$ | — | 11(5) | 19(9) | 23(8) | 42(8) | 44(8) |

distinguish between losses of P and N to sedimentation and losses to periphyton uptake. Thus, for the littoral N : P = 4 mesocosms of Lake 302S, which developed thick mats of periphyton over sediments, $S_P$, $S_N$, and $S_N'$ include both loss terms. Calculation of $S'_N$ and $F_N$ assumed that periphyton incorporated N and P at the same N : P ratio as the phytoplankton (i.e. at the suspended N : suspended P ratio). Because we have no supporting data for this assumption (the taxonomic composition of the two communities was quite different), the estimates presented should be viewed only as crude approximations.

Total nutrient input ($L$) was calculated as the sum of the external inputs (atmospheric precipitation and fertilization) and the gains through thermocline entrainment, "leakage," sediment feedback and, for N, $N_2$ fixation.

*Statistical analyses*—To test the statistical significance (at $\alpha < 0.05$) of the effect of N : P fertilization ratio on the nutrient concentrations and nutrient fluxes in the mesocosms of each lake, we used two-factor repeated-measures ANOVAs (Winer 1971), with time as the repeated measure. Repeated-measures ANOVA accounts for variability within treatment subjects (mesocosms) in addition to variability due to treatment, time, treatment-time interaction, and error. Because ANOVAs cannot use data from unreplicated treatments (which have no df), the significance of differences between unreplicated treatments (the N : P = 4 plastic-bottomed mesocosm, the unfertilized control mesocosms, and the epilimnions of the two lakes) and replicated treatments was tested with *t*-tests. In these tests, we estimated population variance from the between-subjects mean squares of the repeated-measures ANOVA (using log-transformed data). Correlations between variables were sought with Pearson moment-product analysis.

## *Results*

Fertilization with nitrate and phosphate increased algal biomass in all mesocosms. In most, the increases were almost entirely as phytoplankton biomass (Table 1), but in 2 mesocosms (those over sand with N : P = 4), periphyton rather than phytoplankton responded to fertilization (Levine 1983; Levine and Schindler in prep.). Fertilization also led to changes in the cycling of N and P. Some of these changes were associated with the increase in P supply and occurred in all treated mesocosms. Others were affected by the N : P ratio of the added fertilizer and by the nature of the mesocosm bottoms.

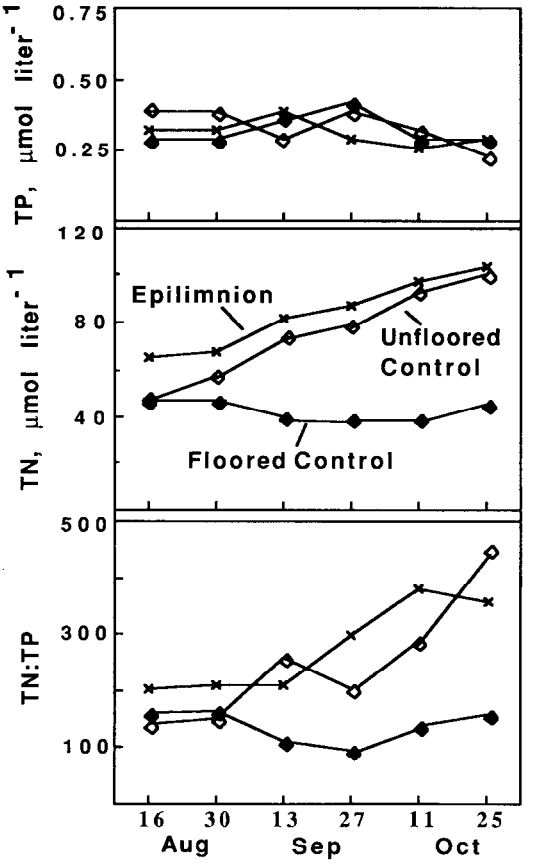


Fig. 2. TP, TN, and TN : TP ratios in two reference (i.e. unfertilized) mesocosms and in Lake 303 during the experimental period. One reference mesocosm had a plastic floor; the other did not.

*Phosphorus chemistry*—TP concentrations in the lake and unfertilized reference mesocosms were consistently low (usually 0.25–0.30 µmol liter$^{-1}$) during the experiments. Enclosure had little effect on TP. In reference mesocosms, whether floored or with sediment contact, mean TP concentrations were not significantly different ($\alpha < 0.05$, by $t$-tests) from those in the lake (Fig. 2).

Fertilization of the mesocosms resulted in an increase of TP in the water, which stabilized after 6–8 weeks at concentrations roughly 4–7 times background (Fig. 3). Neither the N : P ratio of the fertilizer nor bottom type affected the TP concentrations in the mesocosms (Tables 1, 2); repeated measures ANOVAs and $t$-tests showed no treatment effect for either variable. One mesocosm (Littoral Lake 302S, N : P = 33, R1) had TP concentrations substantially above those in other mesocosms. This mesocosm was fertilized more heavily than the others because its flushing rate was greater; however, with water losses principally through the sand, more P was retained by the mesocosm than we initially anticipated.

The partitioning of P between particles and "solution" was similar in 10 of the 13 fertilized mesocosms, roughly ⅔ was present as suspended P and ⅓ as TDP (Tables 1, 2). This distribution contrasted with a roughly equal partitioning of suspended and "dissolved" nutrient in the unfertilized lakes, where much of the P input is in organic forms rather than as phosphate (Schindler et al. 1975). Three of the mesocosms with N : P = 4 (the Lake 303 mesocosm with a floor, and those over sand in Lake 302S) differed from the other mesocosms in containing as much or more dissolved P than particulate P. These mesocosms had Chl *a* concentrations and C fixation rates substantially below those in other fertilized mesocosms (Table 2; Levine

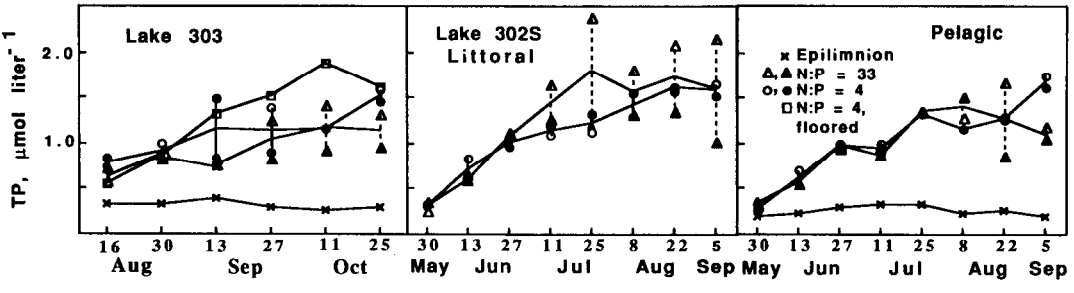


Fig. 3. TP concentrations in the experimental mesocosms over the course of the experiment. The lines connect treatment means for sampling dates; symbols show the data for individual mesocosms.

Table 2. As Table 1, but for the 14-week fertilization experiment in Lake 302S (prefertilization values excluded).

| | Lake | Littoral | | | | Pelagic | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | N:P = 4.4 | | N:P = 33 | | N:P = 4.4 | | N:P = 33 | |
| | | R1 | R2 | R1 | R2 | R1 | R2 | R1 | R2 |
| Chl *a*, μg liter$^{-1}$ | 4(1) | 6(5) | 4(2) | 79(67) | 18(15) | 14(11) | 11(11) | 19(18) | 17(12) |
| TN, μmol liter$^{-1}$ | 26(5) | 28(2) | 28(3) | 72(25) | 60(5) | 36(10) | 33(8) | 58(15) | 57(17) |
| % suspended N | 29(5) | 30(8) | 31(6) | 47(15) | 27(6) | 44(8) | 40(8) | 27(9) | 26(7) |
| % DON | 66(5) | 65(5) | 64(5) | 38(5) | 42(8) | 51(8) | 55(6) | 37(4) | 39(10) |
| % DIN | 6(7) | 5(7) | 5(7) | 20(13) | 32(8) | 5(4) | 6(8) | 36(11) | 34(10) |
| % $NH_4^+$ | 2(1) | 2(1) | 2(1) | 2(3) | 4(1) | 2(1) | 2(1) | 4(5) | 2(2) |
| % $NO_3^- + NO_2^-$ | 3(6) | 3(6) | 3(6) | 18(12) | 28(8) | 3(3) | 4(8) | 32(11) | 32(10) |
| TP, μmol liter$^{-1}$ | 0.3(0.0) | 1.2(0.3) | 1.3(0.4) | 1.7(0.6) | 1.1(0.3) | 1.2(0.3) | 1.1(0.3) | 1.1(0.3) | 1.0(0.3) |
| % suspended P | 56(4) | 35(15) | 35(7) | 66(14) | 58(9) | 67(10) | 61(9) | 66(6) | 68(5) |
| % TDP | 44(4) | 65(15) | 65(7) | 34(14) | 42(9) | 33(10) | 39(9) | 34(6) | 32(5) |
| TN:TP | 101(15) | 23(4) | 24(9) | 44(7) | 55(7) | 32(9) | 33(11) | 52(7) | 57(7) |
| $L_N:L_P$ | – | 16(6) | 17(6) | 32(6) | 30(11) | 15(7) | 14(9) | 35(3) | 36(3) |

1983). We believe that the phytoplankton in these mesocosms were N limited, while both N and P or P alone limited phytoplankton in the other mesocosms (Levine 1983; Levine and Schindler in prep.).

Mass balance calculations indicated that P inputs to fertilized mesocosms were almost entirely allochthonous (Fig. 4). The principal source (>98% of the total for littoral mesocosms; >74% for pelagic mesocosms) was fertilization. Pelagic mesocosms gained some P through the entrainment of metalimnetic waters as the lake cooled in autumn and the thermocline deepened, but this accounted for <15% of the total. Because fertilization dominated inputs, overall P loading was relatively uniform over the course of the experiments (standard deviation around the experimental mean was <1% for littoral mesocosms and <15% for pelagic mesocosms).

The principal fate of P in mesocosms was incorporation into phytoplankton followed by sedimentation or, for the mesocosms with periphyton mats, the combined action of P uptake by periphyton plus sedimentation (Fig. 4). From 54 to 90% of all incoming P was sedimented (or incorporated into periphyton) during the experiment. $S_P$ was greater in Lake 303 than in Lake 302S mesocosms, probably because the former experiments were in autumn and the latter in summer. Sedimentation of N and P is usually maximal during autumn in ELA lakes (Schindler et al. 1973). Within a lake, $S_P$ did not differ significantly (at $\alpha < 0.05$) according to the N:P fertilization ratio, however.

Little P was lost to leakage: <5% for the mesocosms in Lake 303 and in the pelagic region of Lake 302S; 10–20% for the sandy-bottomed littoral mesocosms of Lake 303. Biweekly P storage in the water column (as dissolved organics or in phytoplankton) was usually <25% of P input. Three N:P = 4 mesocosms (the floored mesocosm in Lake 303 and the two pelagic mesocosms in Lake 302S) stored ~40% of incoming P; however, these storage rates did not differ from the others at $\alpha < 0.05$.

*Nitrogen chemistry*—In pristine Lake 302S, TN concentration was relatively constant during the experiments, usually ranging between 20 and 30 μmol liter$^{-1}$ (Fig. 2).

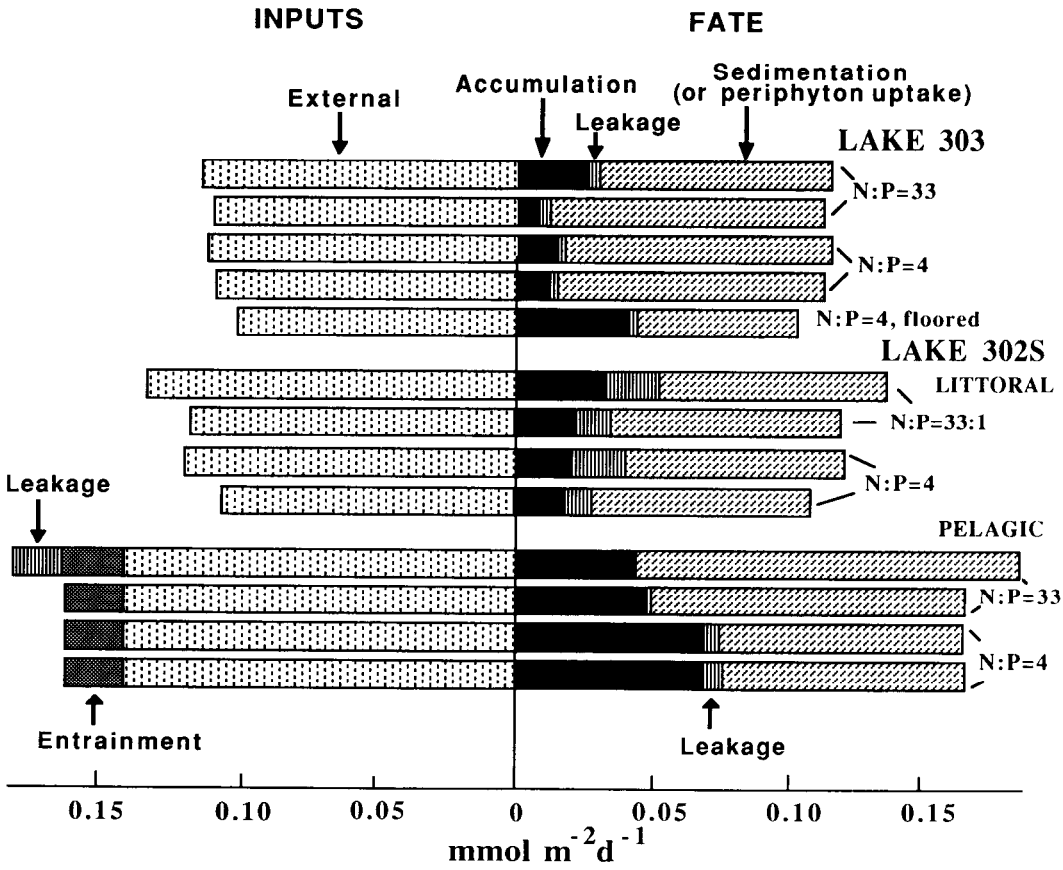


Fig. 4. Mass balance for P in the experimental mesocosms. The values represented are the means for the experiments (obtained by averaging the biweekly rates and then dividing by 14 d). The standard deviations around the means (given as percentages of the means) were: < 1% for external inputs; 10–30% for thermocline entrainment; 5–60 and 150–280% for leakage from littoral and pelagic mesocosms; 30–180% for accumulation (except in one mesocosm with a very small mean; 600%); and 10–70 and 70–105% for sedimentation in littoral and pelagic mesocosms.

About ⅔ of this N was in dissolved organic compounds, probably mostly as recalcitrant colloids (Levine and Schindler 1989), while only ~5% was present as $NO_3^-$, $NO_2^-$, or $NH_4^+$ (Table 2). The remaining 30% or so was associated with suspended particles.

Fertilization of mesocosms with $NO_3^-$ and phosphate at N : P = 4 had no effect on TN concentration (Fig. 4) in the littoral zone of Lake 302 (mean TN concentrations for these mesocosms and the lake were not significantly different; $\alpha < 0.05$). Despite $NO_3^-$ additions, $NO_3^-$ + $NO_2^-$ and $NH_4^+$ concentrations were maintained at very low levels, and >95% of the N was found in dissolved organic compounds or suspended particles (Fig. 4; Table 2). In the pelagic mesocosms with this fertilization scheme, TN concentrations were also as low as in the lake during most of the experiment, but rose when heterocystous blue-greens appeared in July and $N_2$ fixation began. At this time, suspended N concentrations increased. By contrast, fertilization of Lake 302S mesocosms at N : P = 33 resulted in an accumulation of N. Steady state TN concentrations exceeded the baseline by threefold. Thus, there was a treatment effect (at $\alpha < 0.05$) for N : P = 4 vs. N : P = 33; and DIN accounted for about a third of the nitrogen present (Table 2).

The N chemistry of Lake 303 was very different. Because the lake was responding to 2 yr of whole-lake fertilization with an

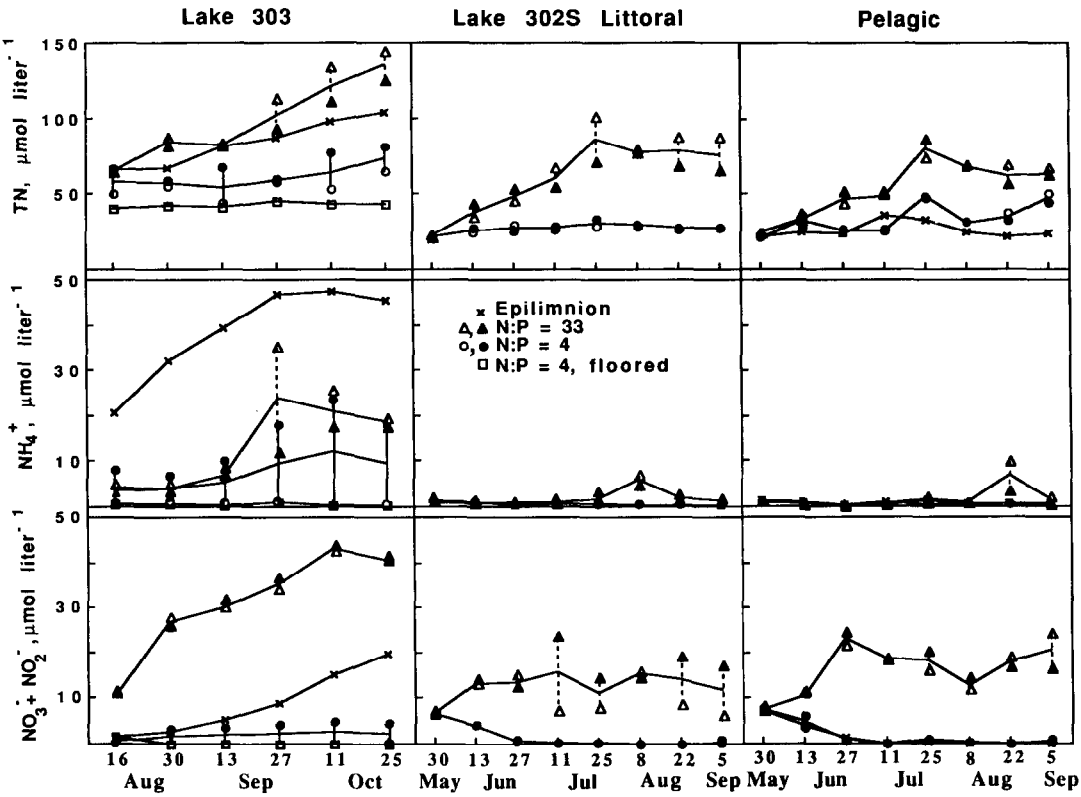


Fig. 5. TN, $(NO_3^- + NO_2^-)$-N, and $NH_4^+$-N concentrations in the experimental mesocosms over the course of the experiment. The lines connect treatment means for sampling dates; symbols show the data for individual mesocosms.

outflux of N from sediments (Levine and Schindler 1989), epilimnetic TN concentrations were much greater than in Lake 302S and increased (from ~60 to 100 µmol liter$^{-1}$) as the experiment progressed (Fig. 5). Almost half of the N in the water was present as $NH_4^+$ (Table 1), the principal form of N to diffuse from sediments. Another 10% was $NO_3^- + NO_2^-$, probably formed from the $NH_4^+$ through nitrification. Dissolved organic compounds contained about a third of the N [estimated from TDN—$(NO_3^- + NO_2^-)$- and $NH_4^+$-N], and suspended particles <10%.

TN concentration was consistently lower in the open-bottomed reference mesocosm than in Lake 303 (although only slightly so; Fig. 2), possibly because the walls of the mesocosm excluded N that entered the lake from the watershed during the experiment (Levine and Schindler 1989). Although atmospheric precipitation dominates P inputs to the ELA reference lakes, runoff contributes substantial amounts of nitrate (Armstrong and Schindler 1971). TN concentrations in the floored reference mesocosm were much lower than in either the open-bottomed reference mesocosm or the lake because this mesocosm excluded N inputs from sediments as well as those from the watershed.

The N:P = 4 mesocosms in Lake 303 had TN concentrations less than those in the lake and control mesocosms (significant at $\alpha < 0.10$ but not at $\alpha < 0.05$, by $t$-tests; Fig. 5), probably because the added P increased biological activity and thus the uptake and subsequent sedimentation of sediment-derived N. The floored N:P = 4 mesocosm had especially low TN concentrations, since its floor blocked N input from sediments. $NO_3^- + NO_2^-$ and $NH_4^+$ were

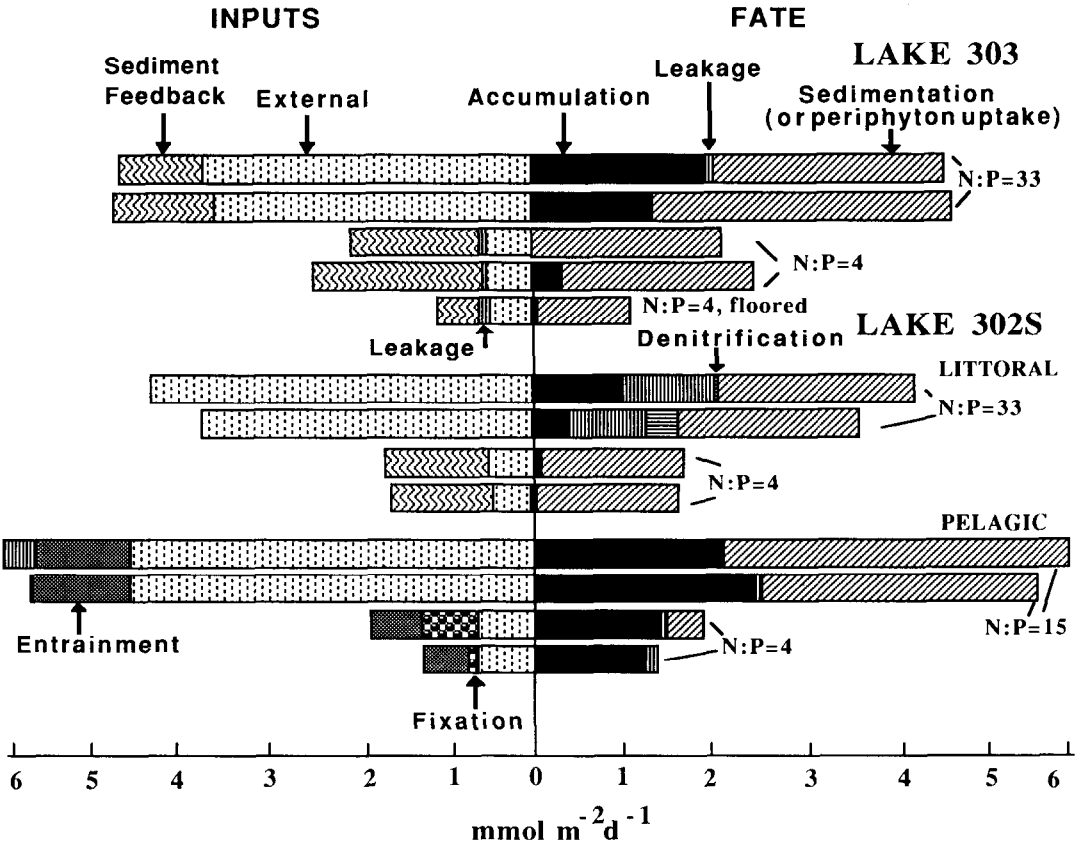


Fig. 6. Mass balance for N in the experimental mesocosms. The values represented are the means for the experiments. The standard deviations around the means (given as percentages of the means) were: 1–5% for external inputs; 110–115% for thermocline entrainment (which was seasonal); 180–200% for $N_2$ fixation (also seasonal); 45–98% for sediment feedback; 300–900% for denitrification; 30–70 and 500–1000% for leakage from littoral and pelagic mesocosms; 200–450% for accumulation (but 4,000% in a mesocosm with a very small mean); and 10–80 and 65–100% for sedimentation in littoral and pelagic mesocosms.

so depleted in this mesocosm, and also in one of the open-bottomed N : P = 4 mesocosms, that DIN usually accounted for <2% of TN (Fig. 5, Table 1). The N : P = 33 mesocosms in Lake 303 had TN concentrations similar to those in the N-rich lake (experimental means insignificantly different at $\alpha < 0.05$). The partitioning of N between particles and solution differed, however: the mesocosms contained much more suspended N and less DIN than the lake (Table 1).

The results of the mass balance calculations for N were markedly different from those for P (Fig. 6 vs. Fig. 4). While external sources (fertilizer + precipitation) dominated the N inputs to N : P = 33 mesocosms, the N : P = 4 mesocosms received more than half of their N from internal sources. For the four unfloored N : P = 4 mesocosms, sediment inputs of $NH_4^+$ were the principal source of N (70–77% of the total; 1.4–1.9 mmol N $m^{-2}$ $d^{-1}$). Unexpectedly, the floored N : P = 4 mesocosm in Lake 303 also received much of its N (42%) from the bottom, not from the sediments proper, but from the layer of newly sedimented, decomposing detritus on its floor.

The pelagic mesocosms in Lake 302S could not acquire N from sediments because they were isolated from them, and the hypolimnion, by the thermocline. Major internal sources of N for the pelagic N : P = 4 mesocosms were metalimnetic entrain-

ment (28 and 35% of the total inputs) and $N_2$ fixation (30 and 8%).

Nitrogen-fixing heterocystous blue-greens appeared in the pelagic N : P = 4 mesocosms in July, but maintained small populations until late August. Over this period $N_2$-fixation rates rose from <0.2 to as high as 3 mmol $m^{-2}$ $d^{-1}$ (areal rather than volumetric rates are given because fixation is dependent on light, and thus depth). For the last 2 weeks of the experiment, we estimated that fixation contributed 60 and 28% of incoming N. Actual fixation rates probably exceeded these values; our model assumed an even distribution of fixers throughout the epilimnion when, in fact, *Anabaena* was concentrated near the surface where higher light intensities favored greater fixation rates. Evidence for underestimation of fixation was provided by mass balance: while *Anabaena* was present in mesocosms, negative values for net $S_N$ were obtained. $S_N$ cannot be negative in a system without sediment feedback, thus, one of our mass balance estimates (most likely fixation, but possibly N inputs from deep water) must have been in error by a significant amount. For one mesocosm (R2), our estimate of $N_2$ fixation would have to be increased by 80% to bring total N loading up to a value where the estimate of $S_N$ would be zero. In addition to fixation by planktonic *Anabaena,* unmeasured fixation by algae attached to mesocosm walls and bottom sand may have added to N supplies. However, heterocystous blue-greens were poorly represented in the periphyton on strips of wall material that we hung in the mesocosms (Levine 1983).

The principal sinks for N were the same as for P, phytoplankton uptake, and sedimentation, or, in the mesocosms with periphyton mats, periphyton uptake; 52–100% of incoming N was lost to the bottom in 11 of 13 mesocosms. The two pelagic N : P = 4 mesocosms were exceptional in that they lost <20% of their N to sedimentation, perhaps because of efficient N cycling under the severe N shortages which were present, and because a substantial proportion of the total algal biomass in these mesocosms consisted of cyanobacteria with buoyant gas vesicles. In addition, we probably underestimated $N_2$ fixation. As mentioned above, such an error would cause underestimation of sedimentation, because the latter was calculated by difference.

Leakage was a minor mechanism for N exchange, except in the sandy bottomed littoral mesocosms of Lake 302S, where 12 and 14% of the N entering the N : P = 33 mesocosms was lost through this mechanism. In the N : P = 4 mesocosms, there was a small net gain of N through leakage. $F_N$ was negative in the two littoral N : P = 33 mesocosms of Lake 303, implicating denitrification as a significant N sink. Although Fig. 6 shows denitrification accounting for $\leq$10% of N loss from the mesocosms, these estimates are minimal, because the mass balance calculations confound denitrification with sediment feedback, a major N source. Below, we will attempt to separate for the Lake 303 mesocosms the relative contributions to $F_N$ of sediment feedback and denitrification.

Storage of N in the water column was minimal in littoral N : P = 4 mesocosms (0–13% of N accumulated), but was equal to 13–24% of the N input to the littoral N : P = 33 mesocosms. In the pelagic mesocosms, storage of N was much more substantial: the N : P = 4 mesocosms retained 64 and 81% of incoming N, the N : P = 33 mesocosms 40 and 33%.

Many (but not all) of the mass balance variables had high standard deviations (SD) around their experimental means (ranges given in the legends for Figs. 4, 6). This variability was largely due to temporal trends in the data (i.e. seasonality and slow initial response to loading changes). In addition, the sensitivity of mass balance to small changes in nutrient standing stock contributed to variability (of the mass balance terms, $\Delta M$ had the greatest SD). Consequently, the partitioning of nutrient inputs and outputs shown in Figs. 3 and 5 should be viewed as the average condition; the relative importance of some fluxes varied from week to week.

*N : P ratios*—Fertilization always led to a significant reduction of the TN : TP ratio in mesocosms (means significantly different from the lake's at $\alpha < 0.05$; Fig. 7, Table 1). Nevertheless, the TN : TP ratios attained were always greater than the N : P ratio of

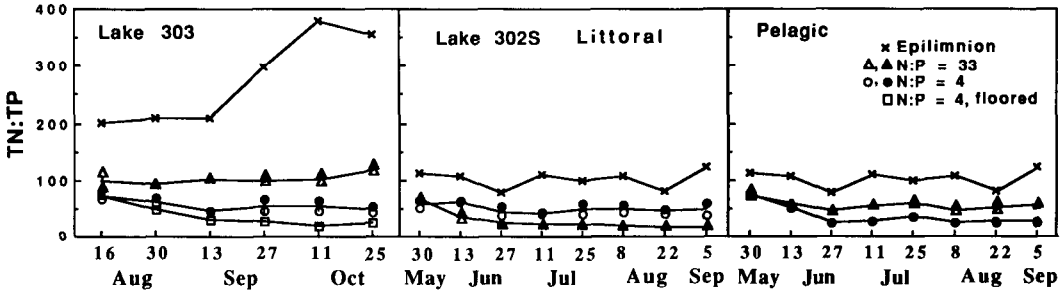


Fig. 7. TN : TP ratios in the experimental mesocosms over the course of the experiment. The lines connect treatment means for sampling dates; symbols show the data for individual mesocosms.

the added fertilizer. In Lake 302S, the baseline ratio was ~110 : 1, and the steady state (i.e. late experiment) ratios for the mesocosms fertilized at ratios of 33 : 1 and 4 : 1 were ~55 : 1 and 22 : 1, respectively. In Lake 303, the background ratio was much greater and increased during the experiment (from ~220 : 1 to >325 : 1). Here the N : P = 33 mesocosms attained a steady state TN : TP of ~110 : 1, while the steady state TN : TP for the N : P = 4 mesocosms was ~55 : 1 when sediments were present and 45 : 1 when they were not.

The N : P ratio of the total biweekly nutrient supply to plankton (external + internal sources), $L_N : L_P$, averaged 14 : 1 and 15 : 1 in the two pelagic N : P = 4 mesocosms of Lake 302S and 11 : 1 in the plastic-floored mesocosm in Lake 303 (Table 1). This ratio was greater in N : P = 4 mesocosms with sediment contact (means 19 : 1 and 23 : 1 in Lake 303, 16 : 1 and 17 : 1 in Lake 302S). In contrast, the N : P = 33 mesocosms had mean $L_N : L_P$ ratios (35 and 36 : 1) near the fertilization ratio in Lake 302S, while in Lake 303 this ratio was somewhat greater (means of 42 : 1 and 44 : 1).

## *Discussion*

Nitrogen limitation in marine bays and estuaries has been explained by the unimportance of $N_2$ fixation in the sea and the preferential recycling of P relative to N from marine sediments (Nixon et al. 1980; Howarth 1988). $NH_4^+$ is retained by these sediments largely through cation exchange (which is less important in freshwater sediments), while $NO_3^-$ is permanently lost through denitrification (Seitzinger 1988; Seitzinger et al. 1991). Fertilization experiments in ELA mesocosms clearly identified mechanisms within lakes that also contribute or consume N and P differentially, and thus cause the effective N : P supply ratio to plankton ($L_N : L_P$) to differ from the ratio due to allochthonous supply alone. For lakes, however, the net effect of internal processes is to increase, rather than lower, the N : P ratio. Internal mechanisms that affected the N : P ratios in mesocosms included: $N_2$ fixation, sedimentation of N and P at a ratio different from the allochthonous supply ratio, N release from sediments in the absence of significant P release, and denitrification.

*$N_2$ fixation as a N source*—$N_2$ fixation has received considerable attention as a natural process capable of reducing N shortages in lakes (Schindler 1977; Flett et al. 1980; Tilman et al. 1982; Howarth et al. 1988). Fixation rates of 1–7 mol N $m^{-2}$ $yr^{-1}$, equivalent to 10–80% of total annual N inputs, have been reported for a number of lakes with low allochthonous N : P supply ratios (Howarth et al. 1988). In some of these lakes (e.g. Lakes 227 and 226 within the ELA), $N_2$ fixation contributes sufficient N to permit P-proportional algal growth (Schindler 1977); whereas in others, N shortages persist despite fixation, because the frequency or the magnitude of the process is restrained by excessive turbulence (e.g. in Lake Valencia, Venezuela: Levine and Lewis 1987) or other environmental factors.

This experiment also demonstrated a reduction in N deficiency due to $N_2$ fixation. Although *Anabaena* bloomed in the two pelagic N : P = 4 mesocosms, fixation rates frequently exceeded allochthonous N inputs

(sometimes by fivefold!). Over the course of the entire experiment, fixation supplied ~8 and 30% of incoming N to these two mesocosms (Fig. 6). Fixers did not attain detectable populations in any littoral mesocosm, however. For the two open-bottomed N : P = 4 mesocosms in Lake 303, this was expected. Flett et al. (1980) showed that $N_2$ fixation in Canadian Shield lakes occurs only when N : P molar supply ratios are <22 : 1. The Lake 303 sediments provided enough N to raise the $L_N : L_P$ ratios in the two open-bottomed mesocosms above this critical value. Lack of fixers in the floored mesocosm of this lake and in the periphyton-dominated littoral mesocosms of Lake 302S, however, cannot be explained on the basis of $L_N : L_P$ alone. It is possible that the floors and algal mats in these mesocosms excluded $N_2$ fixers with resting stages in the sediments (Levine 1983; Levine and Schindler in prep.).

*Sediments as a source of nutrients*—In shallow aquatic ecosystems, most particles reach the bottom intact or only partially decomposed. Thus the sediment surface is the principal site for mineralization (Hargrave 1973). $NH_4^+$ and phosphate released during mineralization may diffuse to the sediment surface and be released into the overlying water column, or some (or all) may be adsorbed by sediments, immobilized by sediment microbes, or incorporated into minerals; in the case of $NH_4^+$, N may also be lost through the combined processes of nitrification and denitrification. Because $NH_4^+$ and phosphate have different charges and reactivities, their fate in sediments is expected to be different. Thus a constant proportional release of N and P from sediments is probably rare, regardless of sediment type.

As discussed earlier, several studies have indicated that P release from ELA sediments is negligible at all times of the year and under both oxic and anoxic conditions. The present study provided additional evidence for low P return rates from these sediments: littoral and pelagic mesocosms with the same fertilization schemes, and unfertilized mesocosms with and without plastic floors, had net $S_P$ rates that were insignificantly different at $\alpha < 0.5$ (Fig. 4; Levine and Schindler 1989). Because $S_P$ includes P release from sediments, substantial P return from this source would have caused lower $S_P$ in the mesocosms with sediment contact.

We expected some benthic recycling of N in the mesocosms with sediment contact because substantial $NH_4^+$ fluxes from ELA sediments have been measured in the past (Schindler et al. 1977, 1987). Nevertheless, we were surprised by the magnitude of this flux in Lake 303, which was recovering from two previous years of fertilization at an N : P molar ratio of 33 : 1 (Levine and Schindler 1989). For the open-bottomed N : P = 4 mesocosms of this lake, the average biweekly sediment contribution of N ($F_N$) exceeded the contribution from fertilizer and precipitation by threefold (Fig. 6). $NH_4^+$ accumulated in these mesocosms despite oxic conditions (Fig. 5); thus the flux of $NH_4^+$ from sediments must have exceeded nitrification rates. Lower estimates of net N feedback were obtained for the N : P = 33 mesocosms, which was expected because the variable $F_N$ included denitrification (which lowered its value) as well as sediment feedback. With large allochthonous $NO_3^-$ inputs (7.5× that for the N : P = 4 mesocosms) and P-limited phytoplankton, these mesocosms accumulated $NO_3^-$, which was denitrified. Actual sediment feedback of N was probably greater in the N : P = 33 than in the N : P = 4 mesocosms, because more N was sedimented in the former (Fig. 7). In the N : P = 4 mesocosms, partially or totally N-limited phytoplankton (Levine 1983) kept $NO_3^-$ concentrations low, suppressing the denitrification of $NO_3^-$ through substrate limitation. The $F_N$ value for these mesocosms probably approximated the actual sediment flux.

From the $F_N$ values for the littoral N : P = 4 mesocosms of Lake 302S, we judged that the sediments of this lake released only slightly less N than those of Lake 303. However, little of this N was available to the phytoplankton in low N : P mesocosms; instead it was incorporated into periphyton. (Both TDN and suspended N concentrations were very low in these mesocosms, while the thickness of periphyton mats suggested considerable retention of N.) $F_N$ values for the littoral N : P = 33 mesocosms

(which were relatively free of periphyton) were negative, suggesting that more N was lost to denitrification than was gained through sediment feedback.

When N and P fluxes from freshwater and marine sediments have been estimated from the accumulation of $NH_4^+$ and P in boxes placed over sediments, the values obtained have often been much larger than estimates based on pore-water gradients (Schindler et al. 1977; Callender 1982; Sweerts et al. 1986). The difference in values under the two methods suggests that a major portion of sediment nutrient feedback may be from decomposition at the sediment surface (i.e. from newly sedimented detritus), which is rarely sampled during pore-water gradient determinations. Corroborating evidence has been obtained by Sweerts et al. (1986), who measured much lower rates of respiration and sulfate reduction in exposed sandy Lake 302S sediments than in similar sediments covered with detrital floc. The mesocosm experiment in Lake 303 offered a special opportunity to compare the relative importance of new and old sediments as nutrient sources under in situ conditions. The N : P = 4 mesocosm with a plastic floor received no inputs from deep sediment, but accumulated newly sedimented material over the course of the experiment. By contrast, the two N : P = 4 mesocosms without floors received nutrient inputs from both newly sedimented material and older sediments.

From mass balance (Fig. 6), we estimated that ~45% of the N sedimented in the plastic-floored mesocosm was returned to the water during the course of the experiment (assuming that denitrification was minimal in this system due to relatively low $NO_3^-$ inputs and strong competition for $NO_3^-$ by N-stressed phytoplankton, Levine and Schindler in prep., so that $F_N$ approximated feedback from new sediments alone). We then estimated N flux from new sediments in the other Lake 303 mesocosms from the equation: $F_{N\,new} = 0.45 \times S'_N$ (thus we assumed that a similar proportion of gross $S_N$ was returned in all mesocosms).

It was expected that N feedback from sediments older than the experiment, $F_{N\,old}$, should be similar in all mesocosms, as the mesocosms were relatively close to one another and their sediments and water had the same nutrient-loading history before the experiment. Because $F_{N\,new}$ was very small in the unfertilized open-bottomed mesocosm (and thus unlikely to influence the value of $F_{N\,old}$, even if in error by 100%), we used the difference between the mean values of $F_N$ and $F_{N\,new}$ in this mesocosm to calculate $F_{N\,old}$. A value of 1.0 mmol N $m^{-2}$ $d^{-1}$ for $F_{N\,old}$ was obtained and used in subsequent calculations (*see below*). This value must have been somewhat higher than background due to the previous fertilization of Lake 303, and if we had been able to perform similar calculations in Lake 302S, lower values would probably have resulted.

A final calculation of $F_{N\,new}$ as a fraction of total feedback (new plus old) suggested that in the fertilized unfloored mesocosms about half of the N flux from the sediments was derived from algae that sedimented during the experiment and underwent decomposition (Table 3). $F_{N\,old}$ included both N generated through abiotic processes in deep sediments and N mineralized from detritus relatively near the surface but still older than the experiment. The greater importance of the latter process was suggested by comparing the value of $F_N$ for Lake 303 in summer 1977, the first year of recovery from eutrophy, with much lower values for years prior to whole-lake fertilization (Levine and Schindler 1989). Thus N feedback in ELA sediments appears to be related primarily to microbially mediated mineralization in near-surface sediments.

*Denitrification as a function of N : P supply ratio*—Denitrifiers permanently remove N from ecosystems by converting $NO_3^-$ to the gases $N_2$, NO, and $N_2O$ (Payne 1981). In the coastal ocean, where P is almost quantitatively returned from sediments to the water (Nixon et al. 1980), substantial denitrification often results in N limitation in the water column (Seitzinger 1988). The situation may be very different in freshwater, however, because denitrification generally is less intense (Seitzinger 1988) and the extent to which P (and possibly N) is returned from sediments is much more variable (Kamp-Nielsen 1974; Boström and Pettersson 1982). However, where atmospheric pollution or fertilization supplies

Table 3. Estimates of the relative inputs of N to the Lake 303 mesocosms from new (produced during the experiment) and old (produced before the experiment) sediments, and of the loss of $NO_3^-$ to denitrification. All fluxes are in mmol N $m^{-2}$ $d^{-1}$.

| Mesocosm | Feedback from new sediments ($F_{N\,new}$ = 0.45 × $S'_N$)* | Feedback from old sediments ($F_{N\,old}$)† | Total feedback ($F_{TN}$ = $F_{N\,new}$ + $F_{N\,old}$) | $F_N$ | Denitrification ($D = F_{TN} - F_N$) | TN loading ($L_N - D$) | TN load denitrified (%) |
|---|---|---|---|---|---|---|---|
| N:P = 2 | | | | | | | |
| with floor | 0.5 | 0 | 0.5 | 0.5 | 0 | 1.0 | 0 |
| open-bottomed | | | | | | | |
| R1 | 1.0 | 1.0 | 2.0 | 1.5 | 0.5 | 2.5 | 20 |
| R2 | 0.9 | 1.0 | 1.9 | 1.9 | 0.04 | 2.4 | 1.5 |
| N:P = 15 | | | | | | | |
| open-bottomed | | | | | | | |
| R1 | 1.2 | 1.0 | 2.2 | 0.9 | 1.3 | 6.0 | 21 |
| R2 | 1.5 | 1.0 | 2.5 | 1.1 | 1.4 | 6.2 | 23 |

* $S'_N$ is gross sedimentation.
† Assumed to be constant for all open-bottomed mesocosms and calculated with data from the unfertilized control mesocosm; $F_{N\,old} = F_N - 0.45\,(S'_N)$.

excess $NO_3^-$, substantial denitrification can occur (Rudd et al. 1990). For the Lake 303 experiment, we were able to obtain rough estimates of the denitrification of $NO_3^-$ (Table 3) from the difference between calculated total N feedback from sediments ($F_{N\,new} + F_{N\,old}$, where = $F_{N\,new} = 0.45 \times S'_N$ and $F_{N\,old} = 1.0$) and mass balance estimates of $F_N$ (total feedback from sediments − denitrification).

Our estimates of denitrification rates were within the range of values reported for other temperate lakes (Seitzinger 1988), but were much greater in mesocosms fertilized at an N:P ratio of 33:1 than in mesocosms fertilized at a ratio of 4:1 (1.3–1.4 vs. 0.1–0.5 mmol N $m^{-2}$ $d^{-1}$). The latter finding was not surprising given the much greater availability of $NO_3$ in the N:P = 33 mesocosms; not only were these mesocosms fertilized with 7.5× as much $NO_3^-$ as the others, but their phytoplankton were P limited and allowed $NO_3^-$ to accumulate. By contrast, essentially all water-column $NO_3^-$ was removed from the more N-stressed N:P = 4 mesocosms. Kelly et al. (1990) have shown that significant denitrification occurs in ELA lakes when $NO_3^-$-N concentrations exceed ~1 μmol liter$^{-1}$. In most of the N:P = 4 mesocosms, $NO_3^-$-N concentrations were below this threshold during half or more of the experiment. Recently, the potential of ELA lakes for denitrification under favorable conditions (high N:P, high $NO_3^-$ loading, and warm temperatures) was demonstrated by addition of $HNO_3$ to Lake 302N (Rudd et al. 1990). High $NO_3^-$ additions in the absence of P resulted in summer denitrification rates 40 times the baseline. Seitzinger (1988) also noted a relationship between denitrification rates in estuarine or freshwaters and external N inputs. The relationship has important (and heretofore unemphasized) implications for nutrient limitation dynamics: a negative feedback loop between N shortages in the water and the drain of N to denitrification diminishes the probability of N limitation among phytoplankton.

*Impact of internal processes on N:P ratios*—As a result of sediment recycling of N in mesocosms with sediment contact and of $N_2$ fixation in pelagic mesocosms without sediment contact, mesocosms fertilized at an N:P ratio of 4:1 consistently had total N:P supply ratios much above the fertilization ratio ($L_N:L_P$ = 13:1–24:1). Thus internal mechanisms greatly reduced the potential for N limitation. By contrast, mechanisms for alleviating P limitation (sediment recycling of P, denitrification) were ineffective: mesocosms fertilized at N:P 33:1 had $L_N:L_P$ ratios close to or above the fertilization ratio (31:1–44:1). TN:TP ratios in all mesocosms were consistently greater than $L_N:L_P$ ratios, although the two were highly correlated ($r$ = 0.84, $n$ = 11). The discrepancy between TN:TP and $L_N:L_P$ may reflect greater persistence (i.e. lower bioavailability) of dis-

solved organic N than of dissolved organic P in the water (Levine and Schindler 1989).

*Extrapolation of results to lakes*—Although recycling from sediments was a major N source for the littoral mesocosms of this study, its importance for ELA lakes will depend on lake morphometry. In shallow lakes that mix to the bottom, even small fluxes from sediments can contribute substantially to nutrient budgets; whereas, in large stratified lakes with most sediment area below the mixed layer, much greater fluxes from sediments may be responsible for only a minor portion of the nutrient used by phytoplankton. If release of nutrients from sediments is constant, the ratio of epilimnion sediment area : epilimnion volume ($S_e : V_e$) represents the relative contribution of return from sediments to internal cycling in a lake. For a monomictic lake, this is equal to the reciprocal of the mean depth, 0.625 in the case of Lake 303. However, for a lake with a relatively large area below the thermocline this ratio is small because much of the epilimnion is underlain by the thermocline, where nutrient returns are very low (Schindler et al. 1977), instead of by sediments. For Lake 302S, the $S_e : V_e$ ratio is only 0.112. Consequently, its epilimnion sediments are expected to be sixfold less important in internal cycling than those in Lake 303. In lakes off the Canadian Shield, the flux of N and P from sediments may differ from the estimates provided here for Lakes 303 and 302S, depending on sediment mineralogy and texture, sediment microbiology, and the nutrient input history of the lake.

Given the poor competitive abilities of N fixers under P limitation (Tilman 1982), $N_2$ fixation probably is an important N compensation mechanism in lakes only when $L_N : L_P$ ratios are so low that N limitation is the rule among nonfixing phytoplankton. As long as sediment inputs and other processes raise $L_N : L_P$ ratios to levels fostering P limitation, fixation may be avoided. The threshold between $L_N : L_P$ ratios compatible and incompatible with fixation may be well below the Redfield ratio, which represents the average, rather than the minimal, N : P ratio found in healthy phytoplankton. Many cyanobacteria and some diatoms routinely incorporate N and P at lower N : P ratios: e.g. *Microcystis* sp. has an optimal N : P supply ratio of just 7 : 1 (Tilman 1982). In this study, $N_2$ fixation occurred only in pelagic mesocosms with no sediment contact.

Although this study provided estimates of N and P inputs to the water from the atmosphere and sediments, we did not address the issue of nutrient recycling in the water itself. Studies by others (Goldman et al. 1987; Tezuka 1989; Sterner 1990) suggest that the overall impact of recycling of nutrients from heterotrophs to phytoplankton may be an accentuation of existing nutrient limitations. Bacteria, invertebrate grazers, and fish recycle nutrients at rates that are governed by the difference between the elemental content of their food and their own nutrient requirements. Hence, when P limits algal growth and algal N : P is high, proportionally more N than P is recycled from heterotrophs. The opposite discrimination, proportionally more P than N, occurs when N limits growth and algal N : P is low. Thus, the processes addressed during this study are probably the major mechanisms for internal mitigation of nutrient limitation.

*Effects of experiment duration*—Like all experiments in mesocosms, ours was conducted over a short period (10–12 weeks) relative to the time that lakes have to respond to nutrient input changes. Although the changes that we observed in N and P cycling occurred primarily during the initial 6–8 weeks of fertilization, some nutrient fractions were still slowly increasing or decreasing in concentration at the end of the experiment. These trajectories were expected because there are large nutrient pools in lakes (colloids in the water and nutrients in deep sediments) that turn over very slowly. Had we continued mesocosm fertilization into a second summer, further changes in N and P cycling might have occurred. In particular, the sediment return of N may have increased in littoral mesocosms, because the mass of undegraded organic N at the sediment surface would thicken under the continued rain of N-rich sediments. In the pelagic N : P = 4 mesocosms, DIN concentrations and N : P ratios would be lower at the beginning of a second summer of fer-

tilization than at the beginning of the first. Consequently, $N_2$ fixation may have begun earlier and been a more substantial contribution to the N budget. It is also possible that fixers would establish themselves in the N-stressed plastic-floored and sandy-bottomed mesocosms. When whole lakes in the ELA have been fertilized with N and P at a low N : P ratio, their N content has increased annually for several years after fertilization began (Schindler et al. 1977, 1987).

## *Conclusions*

Although the predominance of P limitation among Canadian Shield lakes can be explained by allochthonous nutrient supplies with generally high N : P ratios, this study suggests that P limitation is also favored by mechanisms within these lakes that ameliorate N shortages, but are ineffective at increasing P availability. Several years may be needed to observe the total response of lakes to changes in nutrient loading. However, that three N compensation devices (greater N than P feedback from sediments, $N_2$ fixation, and reduced denitrification) were initiated within 10–12 weeks of mesocosm fertilization and were increasing in intensity when the experiment ended suggests that these mechanisms deserve more attention than limnologists have given them. A major difference between marine coastal and freshwater ecosystems may be the role that internal processes play in modifying N : P. In marine systems, $N_2$ fixation is usually unimportant (Howarth et al. 1988) and sediments appear to recycle P more efficiently than N (Nixon et al. 1980; Howarth 1988); in lakes, substantial inputs of N through $N_2$ fixation are possible and sediments may retain P more efficiently than N. Thus, N limitation may occur along marine coasts and P limitation in lakes at similar allochthonous N : P supply ratios.

The findings of this study also suggest that experiments in mesocosms to predict the results of nutrient management on lakes must be carefully designed with regard to sediment area : water volume ratio. Different N : P ratios in the epilimnion, and also different phytoplankton species (Levine 1983; Levine and Schindler in prep.), were obtained when sediments were used as the bottom for mesocosms than when the thermocline was used. Still other results were obtained when sealed mesocosms were used. This difference was due largely to the difference in N return, and thus in the effective N : P supply ratio to phytoplankton.

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