Uncoupling of Bacterioplankton and Phytoplankton Production in Fresh Waters Is Affected by Inorganic Nutrient Limitation

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Pelagic bacterial production is often positively correlated, or coupled, with primary production through utilization of autotrophically produced dissolved organic carbon. Recent studies indicate that inorganic N or P can directly limit both bacterial and phytoplanktonic growth. Our mesocosm experiments, with whole communities from mesotrophic Calder Lake, test whether this apparent bacterial-algal coupling may be the result of independent responses to limiting inorganic nutrients. In systems without N additions, numbers of bacteria but not phytoplankton increased 2- to 2.5-fold in response to P fertilization (0 to 2.0 μmol of P per liter); this resulted in uncoupled production patterns. In systems supplemented with 10 μmol of NH₄NO₃ per liter, P addition resulted in up to threefold increases in bacteria and two- to fivefold increases in total phytoplankton biomass (close coupling). P limitation of pelagic bacteria occurred independently of phytoplankton dynamics, and regressions between bacterial abundance and phytoplankton chlorophyll a were nonsignificant in all systems without added N. We describe a useful and simple coupling index which predicts that shifts in phytoplankton and bacterioplankton growth will be unrelated (Δ bacteria/Δ phytoplankton → either +∞ or −∞ in systems with inorganic N/P (molar) ratios of < 40. In systems with higher N/P ratios (>40), the coupling index will approach 1.0 and close coupling between bacteria and phytoplankton is predicted to occur.

Recent investigations have shown that a significant proportion of the carbon fixed by phytoplankton is processed through a microbial food web in which planktonic bacteria assimilate and funnel extracellular dissolved organic carbon (DOC) to heterotrophic and mixotrophic protists and on to larger metazoans (2, 20, 21, 24, 38, 41). Studies of a variety of marine and freshwater ecosystems have further shown that the abundance and production of planktonic, heterotrophic bacteria are positively correlated with phytoplankton chlorophyll a levels and production (2, 6, 15, 24). The slopes of regressions between direct counts of pelagic bacteria and phytoplankton chlorophyll a levels have also been used to describe the nature of these dynamics (6, 19, 33). All of these relationships have been interpreted to be the result of a close metabolic coupling between bacterioplankton and phytoplankton. Indeed, organic carbon exudates from phytoplankton are thought to be a critical limiting resource for bacterial growth (7, 8, 15, 16, 31).

In contrast, a few studies have shown that inorganic nutrients, or nonphytoplankton DOC, may also limit the growth of heterotrophic bacteria. Some regression models describing whole communities in lakes have found a stronger relationship between bacterial production and dissolved P (DIP) than with phytoplankton (18, 28). Recent studies have also demonstrated that bacterial abundance and production in fresh water can be directly limited by levels of DIP (17, 36, 37). Marine bacterioplankton growth can apparently be limited by inorganic N deficiency; bacteria may also account for a large portion of ammonium uptake in the sea (42). In the freshwater tidal region of the Hudson River (New York State), bacterial production is apparently driven by DOC derived from macrophytes and allochthonous sources (19). These studies all suggest that specific factors which affect either bacterioplankton or phytoplankton production solely may lead to their dynamics becoming uncoupled.

Studies which have identified quantitative relationships between the abundance of pelagic bacteria and phytoplankton chlorophyll a may overlook the separate but direct influences of N- and P-limited growth on each component. Cross-system analyses in the literature are useful for identifying certain correlations (e.g., references 6 and 15), but such comparisons may be unable to identify specific causative factors, such as temperature, different nutrient requirements, or trophic dynamics (8, 18, 19). Indeed, Bird and Kalff (6) have mentioned that despite strong quantitative links between bacteria and phytoplankton in many ecosystems, a considerable amount of scatter in these relationships still needs to be explained. Covariance among several factors (studied by correlation) can further lead to misleading relationships between the abundance of bacteria and phytoplankton biomass (5). For example, an analysis of studies of many lakes revealed that bacterial abundance, the chlorophyll a level, and the aqueous total P level covary, but the correlation between bacteria and phytoplankton is not as strong as that between bacteria and the total P level (18). Because our studies have indicated that levels of DIP may regulate the production of pelagic bacteria in fresh water (36, 37), phosphorus may be one critical factor that drives the apparent coupling between bacteria and phytoplankton.

In this study, experiments were conducted with large mesocosms containing whole plankton communities from mesotrophic Calder Lake. A range of N and P concentrations and N/P ratios were tested in a factorial design (two factors tested simultaneously and in combination) to examine coupling between the biomass of planktonic bacteria and that of phytoplankton under nutrient-limited and nutrient-surplus conditions. We hypothesize that (i) the growth of both bacteria and phytoplankton can be stimulated directly and separately by N or P loading, (ii) increases in bacterial growth may occur independently of those of phytoplankton, and (iii) correlation between bacterial abundance and phytoplankton (as chloro-
phyll a) will be affected by the different abilities of these organisms to obtain P. We also aimed to develop a simple model which may be used to predict and understand patterns of coupled and uncoupled production in pelagic bacteria and phytoplankton more broadly in aquatic ecosystems.

MATERIALS AND METHODS

Site description. Calder Lake is a small (3.9-ha) mesotrophic, dimitic lake located in southern New York State; this is also the site of Fordham University's biological field station. The lake has an average depth of 2.8 m and a maximum depth of 6.7 m. DIC concentrations range from <0.03 μmol/liter to 0.84 μmol/liter (±1.0 to 26 μg/liter of P per liter), and total P concentrations range from 0.12 μmol/liter to 2.74 μmol/liter (3.7 to 85 μg/liter of P per liter [39, 40]). The DOC concentration tends to be constant (relative variation of usually <10%) on different days and among depths, with an average of 3.7 mg/liter (36, 37).

Facilities, experimental design, and sampling. Experiments were conducted in 24 large cylindrical fiberglass tanks (Experimental Lake Facility), each filled to a volume of 5,400 liters (1.9-m diameter by 2.1-m depth [41]). The tanks are located about 100 m from Calder Lake. Lake water containing zooplankton, phytoplankton, and bacteria was pumped (±1-m depth) directly into Experimental Lake Facility tanks from a site near the deepest part of the lake during the night. A 2 × 4 factorial design was used with two N levels and four P levels, each replicated three times. The two N treatments included (i) no N added and (ii) 10 μmol of NH$_4$NO$_3$ added per liter. The four P treatments included (i) no P added or (ii) 0.5, (iii) 1.0, and (iv) 2.0 μmol of K$_2$HPO$_4$ added per liter. Nutrient pulses were applied on day 0 (~4 h prior to sampling) and repeated on day 14.

Water was collected from Experimental Lake Facility tanks with a peristaltic pump from a 1-m depth at four weekly intervals (days 0, 7, 14, and 21) and stored in the dark and cold (~5°C) until returned to the laboratory (~2 h). Autotrophic microplankton (algae and cyanobacterial cells or colonies, >20 μm to 200 μm) were first filtered in situ with 47-mm Nalgene in-line filter units fitted with 20-μm Nitrile disks (stored on ice until returned to the laboratory [~2 h]). The remaining fractions were also filtered in the laboratory later (described below). Chemistry samples (collected in duplicate) were also filtered in line (Whatman GF/F filters) to peristaltic pump from ~1-m depth; half were preserved to pH <2.0 with concentrated H$_2$SO$_4$. Whole lake water was collected in an otherwise identical manner for bacterial samples.

Phytoplankton and bacteria. Smaller size fractions of phytoplankton were separated in the laboratory by using polycarbonate filters under a vacuum of ≤100 mm Hg (~13 KPa). A series of twelve 1,000-ml polycarbonate collection units were linked in series to filter 12 samples simultaneously. Nanoplankton (20 μm to >2 μm) were collected on 2.0-μm pore-size filters; picoplankton (2 μm to >0.2 μm) passed through 2.0-μm filters and were collected on 0.2-μm pore-size filters (Poretics Corp.). Filters and 20-μm Nitrile disks were placed into extraction vials containing 5 ml of neutral 90% acetone, ground with a glass pestle, and then extracted cold (4°C) in the dark for 18 h. Extracts were centrifuged and measured for chlorophyll a concentrations spectrophotometrically and corrected for phycophtin a (1, 25).

Bacteria were separated on 0.2-μm-pore-size prestained (Irgalan) black filters, preserved with 2% glutaraldehyde, stained with 4',6-diamidino-2-phenylindole (DAPI), and counted with a Nikon epifluorescence microscope with a UV-2A filter (excitation, 330 nm; barrier, 420 nm) at a magnification of ×1,250 (29). A minimum of 300 cells were counted from each sample.

Water chemistry. Soluble-reactive phosphorus (SRP) was measured via the antimony-ascorbate-molybdate method (1, 10). NH$_4$+ -N was measured by the phenol-hypochlorite method, and NO$_3$ - was determined with sulfanilamide-N-1-naphthylhexylenediamine dihydrochloride, following reduction to NO$_2$ - in a Cd-Cu column (1, 11, 12). All of the methods were modified for automated analysis and run on a TrAAce-800 analyzer (Bran + Luebbe Technologies, Inc.).

Statistical analyses. To determine nutrient effects on both bacterial abundance and phytoplankton chlorophyll a, a two-way analysis of variance was run with the SYSTAT 5.1 program (43), with factors A (N addition) and B (P addition) as independent variables. Response variables included chlorophyll a in three size fractions (micro-, nano-, and picoplankton), total chlorophyll a, and bacterial density. Variables were first tested for assumptions of normality and homogeneity of variances; a log$_{10}$ transformation was required to reduce skewness. Regressions between bacterial abundance and total chlorophyll a, with or without N addition, were used to evaluate factors affecting coupling. Regression equations were derived from log$_{10}$-transformed data in order to meet normality assumptions. This approach also enabled direct comparisons with prior studies (6, 19). In our regressions, we assume a functional relationship between bacterial numbers and phytoplankton, especially because temperature effects were minimized by using data from experiments conducted during the month of June. We recognize, however, that both variables were measured with error. Therefore, a second set of slopes (based on the geometric means) were computed according to a model II regression, as described by Rickar (30) and Sokal and Rohlf (34). For all hypothesis tests, effects were judged to be significant if the probability of an event having occurred because of chance (P) was <0.05.

RESULTS

Nutrient chemistry. Average concentrations of SRP, NH$_4$ + , and NO$_3$ - in mesocosms on four sampling days according to experimental treatments are presented in Table 1. The chemical pulses were applied twice—4 h before sampling on day 0 and 14 days after the first sampling. Two sets of samples were taken before (day 14b) and after (day 14a) the pulse on day 14. Differences between target P concentrations and measured P varied within ±10% to ±20%. Addition of 10 μmol of NH$_4$NO$_3$ per liter resulted in measured NO$_3$ -N concentrations increasing from 0.3 μmol/liter to about 11.6 μmol/liter on day 0. These higher levels were depleted within the water column to below the detection limit (<0.07 μmol/liter) by day 7 in tanks with P added (0.5 to 2.0 μmol/liter). However, no change in NO$_3$ -N concentration was observed in systems without added P. In contrast, nearly all systems experienced a large depletion of NH$_4$ + -N from the water column regardless of P treatment. Only one treatment community, those receiving no added P but with 10 μmol of NH$_4$NO$_3$ added, had large, but lower, rates of NH$_4$ + -N depletion (~50% removed by day 7).

For the purposes of this study, we define the average depletion rate of SRP as the per day rate of change in P concentration at each weekly period. The P depletion rates varied from 0.0 μmol/liter/day to 0.03 μmol/liter/day in systems without N added but were enhanced up to 0.18 μmol/liter/day (5.7 times greater) in systems with N added (Table 2). At the start and at the end of the experiment, pairwise contrasts (i.e.,
Although the table was correctly transcribed, the text contains a mix of tabular data and narrative content. Here is the table and a summary of the text:

### Table 1: Average nutrient concentrations in Calder Lake mesocosms with different N and P additions in five sampling periods

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<thead>
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<th>N</th>
<th>P</th>
<th>Variable</th>
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<th>7</th>
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<th>14a</th>
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<tbody>
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<td>0</td>
<td>SRP</td>
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<td>&lt;0.02</td>
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<td>0.81 ± 0.06</td>
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<td>1.44 ± 0.08</td>
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<td>1.70 ± 0.05</td>
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<td>0.02</td>
<td>0.14 ± 0.02</td>
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<td>1.33 ± 0.09</td>
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<td>0.0</td>
<td>NH₄⁺-N</td>
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<td>0.16 ± 0.19</td>
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<td>14.8 ± 0.66</td>
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<td>0.48 ± 0.12</td>
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<td>6.70 ± 0.65</td>
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<td>0.65 ± 0.08</td>
<td>0.61 ± 0.06</td>
<td>0.47 ± 0.11</td>
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<td></td>
<td>9.68 ± 0.54</td>
<td>0.26 ± 0.18</td>
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<td>0.62 ± 0.05</td>
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<td>8.41 ± 1.11</td>
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<td>1.86 ± 0.69</td>
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<td>1.92 ± 0.69</td>
<td>14.4 ± 1.36</td>
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<td>NO₃⁻-N</td>
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<td>&lt;0.07</td>
<td>0.14 ± 0.04</td>
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<td></td>
<td>0.31 ± 0.02</td>
<td>&lt;0.07</td>
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<td>0.31 ± 0.04</td>
<td>&lt;0.07</td>
<td>&lt;0.07</td>
<td>0.07 ± 0.01</td>
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<td>0.29 ± 0.04</td>
<td>&lt;0.07</td>
<td>&lt;0.07</td>
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<tr>
<td>20</td>
<td>0.0</td>
<td></td>
<td>11.5 ± 0.31</td>
<td>11.5 ± 0.28</td>
<td>11.6 ± 0.29</td>
<td>15.3 ± 0.04</td>
<td>15.2 ± 0.71</td>
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<td>11.4 ± 0.19</td>
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<td>0.07 ± 0.11</td>
<td>10.2 ± 0.44</td>
<td>8.67 ± 0.32</td>
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<tr>
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<td>11.6 ± 0.34</td>
<td>&lt;0.07</td>
<td>&lt;0.07</td>
<td>10.3 ± 0.10</td>
<td>10.7 ± 0.62</td>
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<tr>
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<td>2.0</td>
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<td>11.6 ± 0.34</td>
<td>&lt;0.07</td>
<td>&lt;0.07</td>
<td>10.0 ± 0.34</td>
<td>4.29 ± 0.61</td>
</tr>
</tbody>
</table>

* Treatment additions were 0 or 10 μmol of NH₄NO₃ per liter for N and 0.0, 0.5, 1.0, and 2.0 μmol of K₂HPO₄ per liter for P.

Effects of nutrients on biomass of phytoplankton and abundance of bacteria. The chlorophyll a densities measured in size-fractionated phytoplankton and the abundance of bacteria in different treatments are presented in Fig. 1. Total and size-fractionated chlorophyll a levels were not significantly different across all N and P treatments at the start of the experiment (day 0). On days 7 and 14, total and size-fractionated chlorophyll a concentrations increased in response to P additions, but only in mesocosms supplied with added NH₄NO₃. In contrast, bacterioplankton abundance increased following P addition regardless of N treatment. In all systems (with and without N addition), bacterial numbers increased by between about 2 × 10⁵ to 5 × 10⁶ cells per ml (more than a 100% increase) along the experimental P gradient. Among mesocosms supplied with 10 μmol of NH₄NO₃ per liter, the P stimulation effect on different phytoplankton size fractions differed according to day. There was a positive response to P treatment (threelfold increase) by picoplankton chlorophyll a by day 7 (coefficient of variation, ~24% among treatments). The nanoplanckton chlorophyll a level increased 2.5-fold in response to P addition by day 7 (coefficient of variation, ~14% among treatments) and increased nearly 4-fold by day 14 (coefficient of variation, ~18% among treatments). Larger cells and colonies comprising the microplankton exhibited an increase in chlorophyll a level later in the experiment, but the effect averaged roughly a fivefold increase (coefficient of variation, ~13% among treatments). On day 21, both total and size-fractionated phytoplankton chlorophyll a levels declined from previous weeks (Fig. 1 [day 21]). However, there was still a marked positive relationship between bacterial abundance and P treatments in systems without added N; the bacterial growth response to these P additions was more than threefold.

Two-way analysis of variance indicated that N, P, and combined N-P treatments had several significant and different influences on the plankton. The effects among different size classes occurred on different days and extended over different

### Table 2: Average daily depletion rate of DIP in mesocosms receiving different N and P treatment combinations for three periods during the study

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Depletion rate of DIP (μmol of P/liter/day) fromb:</th>
<th>Day 0 to day 7</th>
<th>Day 7 to day 14b</th>
<th>Day 14a to day 21</th>
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</thead>
<tbody>
<tr>
<td>No N added</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>3.1 ± 2.2</td>
<td>4.1 ± 5.7</td>
<td>Undetectablec</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>31.0 ± 2.1</td>
<td>21.6 ± 0.7</td>
<td>8.0 ± 3.7</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>30.0 ± 0.0</td>
<td>26.0 ± 0.0</td>
<td>4.6 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>32.3 ± 5.4</td>
<td>28.3 ± 1.3</td>
<td>18.4 ± 11.3</td>
<td></td>
</tr>
<tr>
<td>N added</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>4.3 ± 1.3</td>
<td>0.5 ± 0.7</td>
<td>Undetectablec</td>
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<tr>
<td>0.5</td>
<td>46.0 ± 1.1</td>
<td>0.6 ± 0.8</td>
<td>39.5 ± 1.9</td>
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</tr>
<tr>
<td>1.0</td>
<td>103.5 ± 4.6</td>
<td>9.8 ± 3.1</td>
<td>86.3 ± 8.3</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>183.7 ± 3.2</td>
<td>35.9 ± 4.9</td>
<td>171.1 ± 17.9</td>
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</tbody>
</table>

* Treatment additions were 0 or 10 μmol of NH₄NO₃ per liter of N, and 0.0, 0.5, 1.0, and 2.0 μmol of K₂HPO₄ per liter for P (n = 3 for each).

\[ a \text{ Data are averages ± standard deviation.} \]

Undetectable, concentrations below the analytical limit of detection.
periods (Table 3). For example, N fertilization elicited significant effects on microplankton chlorophyll $a$ on day 7; this effect continued through day 21. Overall, N pulses resulted in the largest significant effects on picoplankton, but these effects were observed only on day 7. Analysis of variance also indicated that all treatments ($N$, $P$, and $N \times P$ interaction) had significant effects on total phytoplankton chlorophyll $a$ on days 7 and 14. In contrast, $N$ additions had a significant effect on bacterial abundance on day 14 but no significant effects on day 0, 7, or 21. $P$ additions had sustained, significant effects on the abundance of bacteria on all days after day 0. The interaction of $N$ and $P$ had a significant effect only on day 21, a period in which phytoplankton were unaffected by the combination of $N$ and $P$.

Relationship between bacterial abundance and phytoplankton biomass. To examine relationships between bacterial abundance and phytoplankton biomass, two regressions between bacterial numbers and total chlorophyll were run separately: mesocosms with and without N addition (Fig. 2). Bacterial numbers were not significantly correlated with total phytoplankton chlorophyll $a$ level when $N$ was not added (Fig. 2A); less than 1% of the total variation in bacterial numbers was explained by algal biomass. In contrast, there was a significant positive regression between these two components ($P < 0.002$) in mesocosms supplemented with 10 $\mu$mol of NH$_4$NO$_3$ per liter (Fig. 2B). While the relationship is noisy, more than half of the variation in bacterial abundance was explained by its dependence on phytoplankton chlorophyll $a$.

These relationships were compared with the results of earlier studies (Table 4). Three of them find that the intercept of this relationship is very near 6.3 ($\sim 2 \times 10^9$ cells per ml), although the slopes appear to differ. A significant model I regression of Calder Lake bacterial density versus chlorophyll $a$ level was possible only for N-fertilized mesocosms. However, model II (geometric mean) slopes for both sets of Calder Lake communities were similar. The slope for N-fertilized communities averaged about 30% lower than was observed among several Quebec lakes (6), but these values are not significantly different ($P > 0.05$).

**DISCUSSION**

Our results clearly show that bacterial abundance in fresh waters can be stimulated by inorganic nutrients. We have found that bacterial production can be stimulated by $P$ addition alone without being coupled to increases in phytoplankton

TABLE 3. Two-way analysis of variance testing the effects of fertilization on phytoplankton chlorophyll $a$ level and bacterial abundance in Calder Lake mesocosms.

<table>
<thead>
<tr>
<th>Plankton category</th>
<th>Factor</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microphytoplankton</td>
<td>$N$</td>
<td>0.48</td>
<td>0.497</td>
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<td>&lt;0.001</td>
</tr>
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<td>$P$</td>
<td>3.38</td>
<td>0.044</td>
<td>0.001</td>
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<td></td>
<td>$N \times P$</td>
<td>1.77</td>
<td>0.193</td>
<td>0.001</td>
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</tr>
<tr>
<td>Nanophytoplankton</td>
<td>$N$</td>
<td>0.80</td>
<td>0.384</td>
<td>&lt;0.001</td>
<td>49.65</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>1.20</td>
<td>0.342</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$N \times P$</td>
<td>1.39</td>
<td>0.281</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Picophytoplankton</td>
<td>$N$</td>
<td>0.62</td>
<td>0.442</td>
<td>0.001</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.55</td>
<td>0.656</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$N \times P$</td>
<td>0.52</td>
<td>0.166</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total phytoplankton</td>
<td>$N$</td>
<td>0.08</td>
<td>0.785</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.62</td>
<td>0.012</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$N \times P$</td>
<td>1.63</td>
<td>0.222</td>
<td>0.001</td>
<td>5.56</td>
</tr>
<tr>
<td>Planktonic bacteria</td>
<td>$N$</td>
<td>0.12</td>
<td>0.733</td>
<td>0.016</td>
<td>0.692</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>0.82</td>
<td>0.504</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$N \times P$</td>
<td>2.54</td>
<td>0.093</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Results compare the effects of $N$ addition ($0$ or $10$ $\mu$mol of NH$_4$NO$_3$ per liter), $P$ addition ($0.5$, $1.0$, or $2.0$ $\mu$mol of K$_2$HPO$_4$ per liter), and $N \times P$ interaction on three size classes of phytoplankton chlorophyll $a$ (micrograms per liter) and bacterial abundance (cells per milliliter [see Materials and Methods for details regarding assumptions]).

* All $P$ values of <0.05 are given in boldface.
and without N addition. Further proof can be seen in a nonsignificant regression between bacterial numbers and phytoplankton chlorophyll a level in systems without added N (Fig. 2A). A significant, positive regression between bacterial abundance and phytoplankton chlorophyll a level in mesocosms with N added (Fig. 2B) suggests that bacterial-algal coupling may not be primarily based on DOC supplied by phytoplankton, as suggested previously by Cole and others (15). Such linkages may be, at least in part, a response to different inorganic nutrient regimes. Unexplained declines in plankton chlorophyll a levels (especially algal nanoplankton on day 21 [Fig. 1]) may be the result of grazing pressure brought on by increasing numbers of large cladocerans in the mesocosms (12a). Because many zooplankton species are known to graze size selectively, cladocerans, ciliates, and small heterotrophic protists may also affect apparent algal-bacterial coupling. Between day 14 and day 21, more than half of the phytoplankton chlorophyll a was removed from the water column in tanks supplied with higher levels of N and P, presumably via grazing and/or sedimentation (41). Despite a probable decline in potential phytoplankton DOC, bacterioplankton continued to be stimulated by N and P additions.

Most of the DOC available to planktonic bacteria in lakes and open oceans is thought to be produced by phytoplankton (3, 7, 13, 15, 16). Thus, the relatively constant, high concentrations of DOC in meso-eutrophic Calder Lake (spring to summer, 260 to 325 μmol/liter [36, 37]) and similar levels in mesotrophic Dillon Lake, Colo. (125 to 210 μmol/liter [28]), as well as oligotrophic Mirror Lake, N.H. (200 to 270 μmol/liter [23]), suggest that DOC levels may exceed the amount required for bacterial growth. In heterotrophic systems such as the Hudson River, allochthonous, rather than autochthonous, sources of DOC apparently meet bacterial demands (19). Collectively, these data and the present experimental results imply that phytoplankton-derived DOC may not be the sole cause for the quantitative link between phytoplankton and bacteria. Inorganic nutrient conditions may also play a central role in the widely observed phenomenon of algal-bacterial coupling.

It is unreasonable, however, to ignore many previous findings which show that algal exudates are important sources of carbon for pelagic, heterotrophic bacteria (3, 7, 13, 14, 16, 31). One possible explanation for data which depart from DOC-based models of bacterium-phytoplankton coupling lies in variations in substrate lability. Bacterial growth yields varied widely (8 to 60%) in eutrophic Lake Fredericksborg (Denmark) as a function of the lability of DOC seasonally (26). The possible causes for changes in concentrations of labile DOC were not clear, however, because labile DOC concentrations in that system were not significantly correlated with phytoplankton chlorophyll a concentrations. Experiments in mesotrophic Lake Schönhsee (Germany) demonstrated that total DOC levels were greatest in N-P-enriched mesocosms and that the contribution of carbohydrates and amino acids (high-quality DOC) to the DOC pool was also greater (14). In the present Calder Lake experiments, additions of N may have resulted in higher-quality DOC being excreted by phytoplankton, which would then result in the establishment of a closer coupling between phytoplankton and bacterial production.

Since both algal production and bacterial production in fresh water also respond directly to P availability (17, 37), we may also hypothesize that algal-bacterial coupling is indirect, derived from a mutual dependence on P. This hypothesis makes it necessary to examine possible common factors that may regulate both bacteria and phytoplankton simultaneously but independently. In general, phytoplankton require dissolved inorganic N and DIP approximating the Redfield ratio of 16:1, but it is not yet clear whether the inorganic requirements of bacterioplankton are similar (but see Discussion in references 37 and 41).

The first experiment we tested this idea was by running regressions between the system-level P depletion rate (per day rate of SRP decline) and (i) bacterial numbers or (ii) phytoplankton chlorophyll a concentration, separated by N treatment (Fig. 3). A steeper slope implies that more rapid P depletion may result from greater microbial (bacteria or phytoplankton) production levels. Our results show that in systems without added N, a significant regression (P < 0.01) exists between water-column P depletion rates and bacterial numbers only (Fig. 3A); there was no significant dependence on phytoplankton biomass (as chlorophyll a [Fig. 3B]). One may conclude that under low-N conditions, P dynamics are regulated more by bacterioplankton than phytoplankton activity; the two components of the microbial loop are uncoupled. However, in mesocosms with N added, P depletion rates are significantly correlated with both bacterial abundance and phytoplankton biomass (Fig. 3C and D). The results also show
that depletion of dissolved P from the water column was greatly enhanced by the addition of N. P depletion rates were roughly three times greater for equivalent bacterial densities (Fig. 3A and C).

We interpret these phenomena to be the result of differences in algal versus bacterial responses to N and P deficiencies in lake water. When N was not added, phytoplankton experienced an apparent N deficiency (day 7), but bacteria did not. Because bacteria have high affinities for inorganic N (42), they may be able to obtain enough N (perhaps as dissolved organic N) from occasional pulses (e.g., lake snow) but may be limited by adequate supplies of DIP. In our experiments, addition of P without N stimulates the growth of planktonic bacteria but does not stimulate the growth of phytoplankton. Our data suggest that bacteria are not superior competitors for low P supplies in mixed communities. We observed three consecutive weeks of apparent P limitation in bacteria, while phytoplankton groups experienced only one or two such periods (Fig. 1 and Table 3). Bacterioplankton may simply be unable to store polyphosphates as efficiently as most algal species, making them dependent on a more continuous supply of DIP. We observed that bacteria respond positively and significantly to P addition, apparently regardless of external dissolved inorganic N pools. When N is added, both bacteria and phytoplankton can respond to P addition, and so their growth appears coupled. This suggests that N and P conditions are mutual factors affecting algal-bacterial coupling, which elicit independent but parallel increases in bacterial and phytoplanktonic levels.

An analysis of data from the literature representing many lakes found a less consistent relationship between chlorophyll a concentrations and bacterial production among contrasting systems. Chlorophyll a levels could explain only about 16% of the total variability in bacterial production (18). Some longer-term studies of single lakes have found significant positive relationships between bacteria and phytoplankton (32), while other single-lake studies have found no significant bacterium-chlorophyll relationship (27, 28). Other factors clearly must be involved, which act differently on algae and bacteria (e.g., protist and metazoan grazers). We propose that inorganic N and P may be two key factors that link bacterial and phytoplankton production and may account for some of the unexplained variation (residuals) found in past studies.

Coevolution bacterial and phytoplankton production can depend on both N and P status. In systems with a low N/P ratio (=40; N limited), bacteria are more responsive to pulses of P, and hence the two components become uncoupled. In systems with higher N/P ratios, both components of the community should respond to P dynamics. The necessity of greater N in this dynamic suggests that a high N/P ratio (>40) is necessary for a close coupling between bacteria and phytoplankton in fresh water. We have proposed a simple index which describes the strength of this coupling. The coupling index (CI) is defined as the ratio of percent change in bacterial abundance or biomass to the percent change in phytoplankton biomass over the same period, expressed as follows: CI = % rate of Δ bacterial% rate of Δ phytoplankton. The range of values for CI should be $-\infty < CI < +\infty$. When bacterial and phytoplanktonic growth are closely linked, CI will be small and vary only between −1 and +1. We predict that the variation in CI will be

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**TABLE 4. Regression results describing the relationship between bacterial density and phytoplankton chlorophyll a level in Calder Lake mesocosm experiments**

<table>
<thead>
<tr>
<th>Water source</th>
<th>x</th>
<th>y</th>
<th>n</th>
<th>Intercept</th>
<th>Slope</th>
<th>$r^2$</th>
<th>Model II slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calder Lake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No N added</td>
<td></td>
<td>Chlorophyll a</td>
<td>DSDC</td>
<td>16</td>
<td>6.33 (±0.11)</td>
<td>0.14 (±0.42)</td>
<td>0.007</td>
</tr>
<tr>
<td>N added</td>
<td></td>
<td>Chlorophyll a</td>
<td>DSDC</td>
<td>16</td>
<td>6.26 (±0.05)</td>
<td>0.39 (±0.09)</td>
<td>0.539**</td>
</tr>
<tr>
<td>Quebec lakes</td>
<td></td>
<td>Chlorophyll a</td>
<td>AODC</td>
<td>13</td>
<td>6.28 (±0.19)</td>
<td>0.57 (±0.27)</td>
<td>0.66***</td>
</tr>
<tr>
<td>Hudson River</td>
<td></td>
<td>Chlorophyll a</td>
<td>AODC</td>
<td>17</td>
<td>6.78 (±0.09)</td>
<td>-0.11 (±0.09)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Results are divided into the two main N treatments (without and with addition of 10 μmol of NH₄NO₃). The variables tested were chlorophyll a concentration (micrograms per liter [μg]) and bacterial numbers counted by DAPI-stained direct counts from prior studies (AODC). Variables were log transformed to more closely meet normality assumptions. The first set of values for the y intercept and slope ($±95\%$ confidence limits are given in parentheses) were based on model I regression assumptions (on the basis of the presumed dependence of bacterial growth on phytoplankton-produced DOC). A model II regression (geometric mean) estimate of the slope is provided for comparison (on the basis of the recognition that both variables are measured with error).

a, b, **, P < 0.01; ***, P < 0.001.

Results from reference 6.

Results from reference 19.
FIG. 4. Relationship between the empirically derived bacterial-
phytoplanktonic CI (expressing closeness of bacterial and phytoplank-
tonic growth activities) and inorganic N/P ratios in experimental
mesocosms containing Calder Lake communities. CI is a rate function
expressing the ratio of daily percent changes in bacterial abundance
divided by the daily percent change in phytoplankton chlorophyll a
level at each sampling interval (see Materials and Methods for details).

large (either positive or negative) in systems with low N/P
ratios and limited in systems with high N/P ratios. We tested
this hypothesis by plotting CI against the N/P ratio in all
mesocosms over the 4-week experimental period (n = 72); the
results match our predictions fairly closely (Fig. 4). Relatively
little variation in CI (values of \(\sim 1.0\)) is observed in systems
with N/P ratios \(\sim 40\). Prior experiments indicate that these
conditions favor the dominance of smaller phytoplankton
species, particularly the cyanobacterium *Synechococcus* sp. (35,
39, 40).

Among studies which have examined the presence or lack of
algal-bacterial coupling, attention has been given to the resid-
ual (or unexplained) variation in this relationship. Some
studies suggest that nutrient levels, variations in allochthonous
carbon levels, and even methodological errors may be involved
(6, 18, 19, 22). Data from other systems would be similarly
useful to test preliminary ideas concerning our CI. Our ex-
periments demonstrate directly that N limitation can uncouple
this linkage and result in different trajectories for the growth of
bacterioplankton and phytoplankton along P gradients. It is
possible that N regeneration, as mediated by grazing zooplank-
ton and protozoans (4, 9, 20), may be the short-term stimulus
between turnover periods which links phytoplankton and bac-
terial production.

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