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Patterns of nutrient release from nutrient diffusing substrates in flowing water

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Abstract

As nutrient diffusing substrates age, the availability of nutrients to periphyton may decline with time either because of diffusion or dilution of nutrients into the water column or because of the effects of grazing by herbivores. Typically, large amounts of nutrients are added to nutrient diffusing substrates (NDS) to insure continuous enrichment throughout experimental periods of 2 to 8 weeks. This study examined the release of phosphates and nitrates from NDS exposed to three different current velocities (0.07 m s^{-1}, 0.11 m s^{-1}, 0.20 m s^{-1}) in recirculating laboratory flumes. Replicated agar samples from four treatments (control, nitrate (N), phosphate (P), and N+P) were sampled throughout 32 days (day 1, 2, 3, 6, 12, 18, 24, 32). Increasing concentrations of agar were required to solidify the P and N+P treatments.

Nutrient release rates from NDS were independent of agar concentrations (with the exception of $[PO_4]$ in the medium velocity flume). Nutrient concentrations in the agar of spiked samples declined substantially within a week when exposed to flowing water. Nitrates were retained in agar to a greater extent than phosphates particularly when NDS were exposed to low or medium flows. Although floods physically remove or abrade periphyton in natural streams, findings from this laboratory study suggest that ambient flows deplete the availability of nutrient concentrations to potential periphyton colonizers within the first week of incubation. Because of the rapid decline of nutrients from NDS, short incubation periods in natural running waters seem warranted.

Introduction

Nutrient limitation of epilithic algae has been demonstrated in many drainage basins and on different continents (Grimm & Fisher, 1986; Winterbourn, 1990). When nitrogen or phosphorus is added to streams, there is often a corresponding increase in algal biomass or productivity (Stockner & Shortreed, 1978; Elwood et al., 1981; Winterbourn, 1990).

Several *in situ* methods have been used to study direct effects of nutrient enrichment in rivers including whole stream enrichment (Huntsman, 1948; Warren et al., 1964; Elwood et al., 1981), and flow-through systems (Stockner & Shortreed, 1978; Bothwell, 1988; Mundie et al., 1991). These studies examined bulk additions of nutrients to flowing water. Other approaches (Fairchild & Lowe, 1984; Pringle & Bowers, 1984) focused on experimental manipulation of nutrients in the substrate enabling relationships to be established directly between nutrients and the attached periphyton such that periphyton can take up nutrients before the nutrients become diluted in water. Here, nutrients are continuously released from the substrate into the surrounding medium and samples are easily replicated. This technique of using nutrient diffusing substrates (NDS) mimics the nutrient decay for leaves (Hynes & Kaushik, 1969), senescing aquatic macrophytes (Landers, 1982) and other naturally decomposing substrates (Pringle & Bowers, 1984).

Typically, large amounts of nutrients have been added to substrates to insure a continuous supply of water soluble nutrients throughout experimental periods ranging from 2–8 weeks. This time period has been assumed to be appropriate because of the increased pigment concentrations on nutrient-enriched versus control substrates (Winterbourn, 1990). However, nutrient

concentrations in the agar have yet to be analysed after field incubation.

Nutrient release rates have been examined in the laboratory by placing NDS into beakers or jars of water and analysing the nutrients that entered the water column (Pringle & Bowers, 1984; Munn et al., 1989; Fairchild et al., 1989). Flow was simulated by using a shaker (Pringle & Bowers, 1984) or a magnetic stirrer (Munn et al., 1989). Fairchild et al. (1989) examined release rates from modified flower pots into still water to simulate lentic habitats.

In this study, I wanted to estimate appropriate incubation periods for NDS in rivers and to determine the effect of current velocity on nutrient release. To do this, I examined diffusion of phosphates and nitrates leaching from nutrient-treated agar in NDS exposed to three different current velocities in laboratory flumes by analysing nutrient concentrations within the agar substrate.

Materials and methods

Nutrient diffusing substrates (NDS)

To investigate the diffusion of nutrients from NDS, I modified Pringle & Bower's (1984) sand-agar nutrient mixtures to prepare large numbers of NDS that could be placed individually in a laboratory flume. Each NDS was assembled using a plastic petri plate. The bottom plate (diameter: 8.8 cm, depth: 1.3 cm) was filled with 80 mL of agar and nutrient mixtures. A hole (diameter: 7.5 cm) was cut into the centre of the cover of each petri plate to enable agar samples to be taken. The cover was secured to the bottom plate by affixing vinyl tape around the circumference of the assembled NDS. Treatment designation was coded on the top edge of each plate. The cover provided extra mass so that the NDS could be positioned on the bottom of the flume without drifting. Aluminum wire was used to secure all NDS in the flumes.

Flumes

Dechlorinated water was added to three previously acid-washed, recirculating elliptical flumes (perimeter: 3.1 m, volume: 40 L, water depth: 15 cm), each of which was powered by a motor-driven paddlewheel. In a closed system, nutrients that are released from the NDS into the water column of a flume may return to the agar. This situation is somewhat similar to field situations where nutrients within the water column are available to adhere to the NDS positioned on the streambed.

Current velocity (slow, 0.07 m s^{-1}; medium, 0.11 m s^{-1}; fast, 0.20 m s^{-1}) was altered by using different sized belts connecting the paddewheel to the motor of each flume. The current velocity was measured 30 cm downstream from the paddlewheel in each flume using an Ott-C1 current meter. Reynold's number, calculated for each flume (6 000 to 60 000), indicated that flow was turbulent, representing natural conditions in rivers.

The flumes were housed in a rooftop glasshouse during the month of August and water temperatures (diel variation: 17° to 27°C) corresponded to summertime temperatures in a reference stream. Dechlorinated water was added daily to compensate for water that evaporated or splashed from the flumes.

Nutrient treatments

The NDS used in the flumes consisted of petri plates filled with a 20 g L^{-1} agar and mixtures of nutrients (cf. Pringle & Bowers, 1984). Nutrient treatments were C (control) = agar; N (nitrate) = 0.5 M NO_3 L^{-1} + agar; P (phosphate) = 0.5 M PO_4 L^{-1} + agar; and, N+P (nitrate and phosphate) = 0.5 M NO_3 L^{-1} + 0.5 M PO_4 L^{-1} + agar. Nitrates and phosphates were added as $NaNO_3$ and K_2HPO_4, respectively.

The protocol was to sample nutrients directly from the agar of the NDS rather than to infer nutrient concentrations from samples obtained from the water column. Agar samples (5 mL) were obtained from NDS positioned in the raceways using an acid-washed, cut-off, plastic syringe (1 cm diameter). Different syringes were used for each nutrient treatment in each of the three flumes.

Concentrations of phosphates and nitrates were analysed using standard methods (APHA, 1985). The molybdenum blue technique was used to determine phosphates; the cadmium reduction technique was used for nitrates.

Laboratory experiment

The laboratory experiment was conducted to determine the diffusion of nutrients from replicated agar NDS placed randomly in each of three laboratory streams set at different current velocities for 32 d. There were three flumes used in the study and each was set at a different current velocity.

One aspect of the study was to determine if nutrient concentrations (PO_4 and NO_3) differed among control NDS (with increasing concentrations of agar) and sampling time (day 1 and day 32). In order for the agar to solidify, the C and N treatments required 2% of agar; P and N+P treatments required 4% and 8%, respectively. The three control NDS (C1, 2% agar; C2, 4% agar; C4, 8% agar) were positioned in the flumes along with other NDS that were inoculated with either PO_4 and/or NO_3. Thus, sources of nutrients within the control NDS may have originated from the agar itself or from absorption of nutrients released from NDS inoculated with nutrients and released into the water column. Samples also were obtained from N, P, and N+P nutrient treatments to be analyzed for PO_4 and NO_3 concentrations after 1 d and 32 d of incubation in each of the three flumes. Five replicates per treatment (controls and nutrients) were analyzed.

In addition, agar samples from each treatment [C (2% agar), N, P, N+P] (3 replicates per treatment per flume) were sampled eight times throughout the experiment (day 1, 2, 3, 6, 12, 18, 24, 32) to examine patterns of nutrient release.

Results and discussion

Regardless of the nutrient enrichment technique used, it is not surprising that nutrient enrichment elicits such a noticeable response by the algal community given the higher than natural levels of nutrients that are used in experimental treatments (Mulholland et al., 1991). Concentrations of nutrients used in the present study were at least six orders of magnitude higher than natural levels. Such high concentrations were used to demonstrate the process of release of water soluble nutrients from NDS in laboratory streams.

As nutrient diffusing substrates age, the availability of nutrients to periphyton decline with time either because of the diffusion or dilution of nutrients into the water column or because of the effects of herbivores grazing on the attached algae or periphyton. Herbivory may regulate the periphyton community by depleting algal standing crop (Gregory, 1983) or by returning nutrients assimilated by the algae back to the water (Newbold et al., 1982).

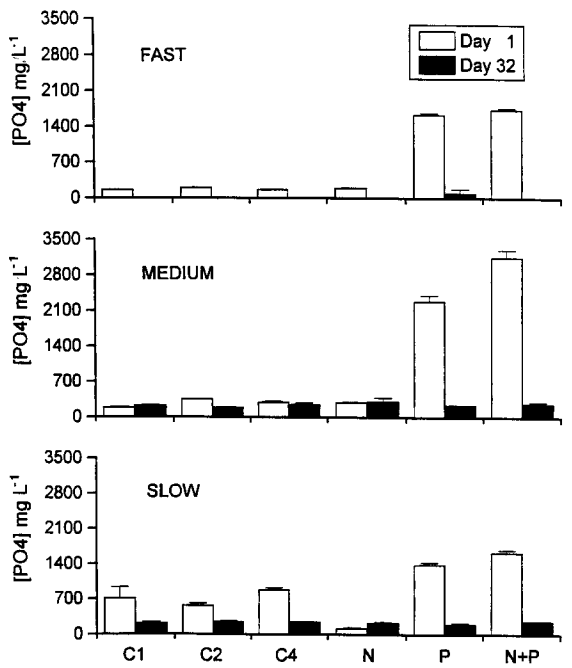

Figure 1. Phosphate concentrations (mean ± S.E., $n = 5$) at day 1 and day 32 obtained from six different nutrient treatments exposed to three different current velocities (slow, 0.07 m s^{-1}; medium, 0.11 m s^{-1}; fast, 0.20 m s^{-1}). The nutrient treatments included three controls with increasing concentrations of agar (C1, 20 g^{-L} agar; C2, 40 g^{-L} agar; C4, 80 g^{-L} agar) as well as nitrate (N) = 0.5 M NO_3 L^{-1} + agar; phosphate (P) = 0.5 M PO_4 L^{-1} + agar; and, nitrate and phosphate (N+P) = 0.5 M NO_3 L^{-1} + 0.5 M PO_4 L^{-1} + agar.

Variation of nutrients in control NDS on day 1 & day 32

Results of the two-way factorial experiment (agar, time) with repeated measures on time (days 1 and 32) are presented in Table 1. Nutrient concentrations of PO_4 and NO_3 present in the control samples decreased with increasing current velocity (Figures 1 and 2). With one exception ([PO_4] in the medium velocity flume), there were no significant differences in nutrient concentrations among the three control NDS prepared with different concentrations of agar (C1, C2, C4) (Figures 1 and 2, Table 1). Concentrations of both PO_4 and NO_3 in the control NDS decreased between day 1 and day 32 (except [NO_3] in the medium velocity flume), indicating that initial nutrient concentrations in the control NDS were washed out of the agar when exposed to flowing water (Figures 1 and 2).

These results support Pringle and Bower's (1984) findings, indicating that nutrient release rates from

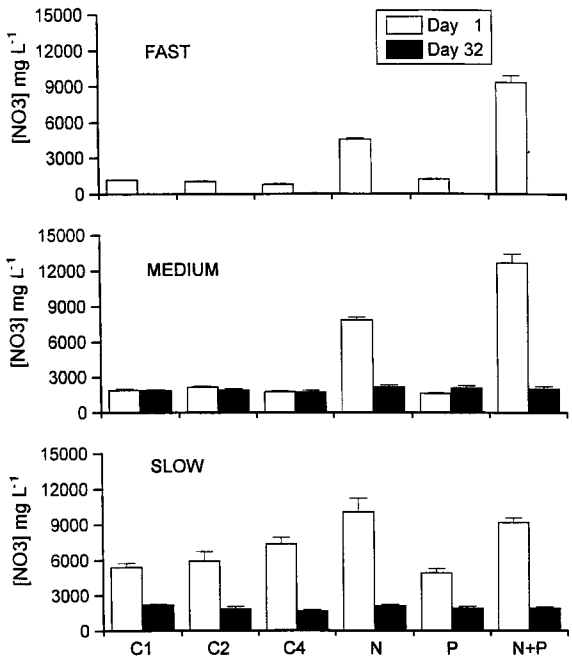

Figure 2. Nitrate concentrations (mean ± S.E., $n = 5$) at day 1 and day 32 obtained from six different nutrient treatments exposed to three different current velocities (slow, 0.07 m s^{-1}; medium, 0.11 m s^{-1}; fast, 0.20 m s^{-1}). See Figure 1 caption for the description of the nutrient treatments.

Table 1. Summary of a two factorial experiment with repeated measures to examine effects of agar concentrations (20 g L^{-1}, 40 g L^{-1}, 80 g L^{-1}) and time (repeated measures on days 1 and 32) on phosphate and nitrate concentrations in agar samples at three velocities (low, 0.07 m s^{-1}; medium, 0.11 m s^{-1}; high, 0.20 m s^{-1}). Data were ln transformed. NS: not significant (0.05 level).

Source of variation	df	Phosphate F	Phosphate P	Nitrate F	Nitrate P
LOW VELOCITY					
Agar	2	2.37	NS	0.40	NS
Time	1	159.09	<0.0001	346.23	<0.0001
Time × Agar	2	2.62	NS	6.99	0.0097
Error	12				
MEDIUM VELOCITY					
Agar	2	8.75	0.0045	3.38	NS
Time	1	28.00	0.0002	3.02	NS
Time × Agar	2	33.99	<0.0001	0.85	NS
Error	12				
HIGH VELOCITY					
Agar	2	1.77	NS	3.41	NS
Time	1	389.91	<0.0001	648.89	<0.0001
Time × Agar	2	2.48	NS	13.97	0.0007
Error	12				

NDS were generally independent of agar concentrations. Thus, if NDS treatments using N+P treatments are desired, the amount of agar may be increased to 80 g L^{-1} to solidify the colonizing medium without noticeably affecting the release of nutrients. However, it is not known if algae or bacteria differentially colonize NDS prepared with different amounts of agar.

As expected, PO_4 concentrations were higher in day 1 for those treatments (P, N+P) inoculated with K_2HPO_4 (Figure 1). Similarly, NO_3 concentrations were elevated in day 1 for those treatments (N, N+P) inoculated with $NaNO_3$ (Figure 2). Concentrations of PO_4 and NO_3 in all nutrient treatments (N, P, N+P) decreased substantially between day 1 and day 32 in each flume (Figures 1 and 2).

Patterns of nutrient diffusion throughout 32 days

Nitrates were retained in the agar to a greater extent than phosphates, particularly when NDS were exposed to low or medium flows (Figures 3 and 4). Phosphates, typically the limiting nutrient in many freshwaters (Wetzel, 1975), diffused rapidly from NDS in all

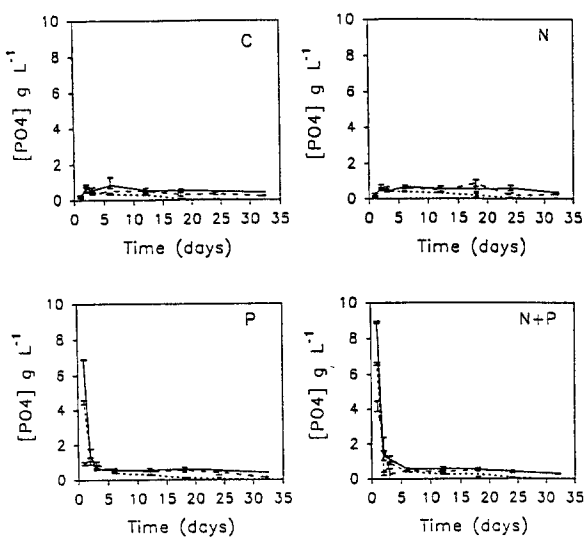

Figure 3. Phosphate concentrations (mean ± S.E., $n = 3$) at three different current velocities (solid line = 0.07 m s^{-1}; long dashes = 0.11 m s^{-1}; short dashes = 0.20 m s^{-1}) for four nutrient treatments (C, N, P, N+P).

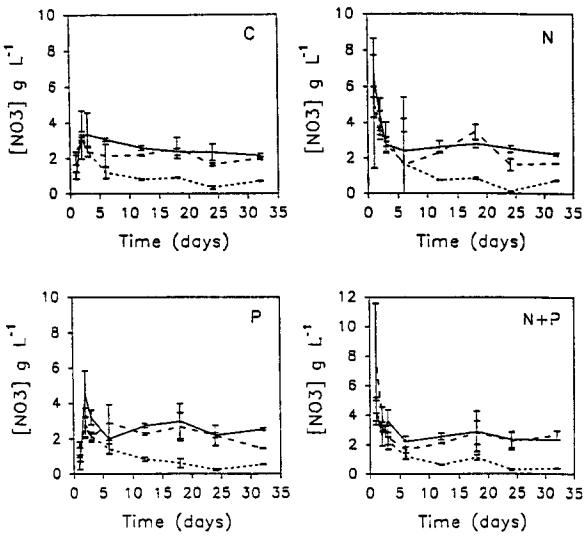

Figure 4. Nitrate concentrations (mean ± S.E., $n = 3$) at three different current velocities (solid line = 0.07 m s^{-1}; long dashes = 0.11 m s^{-1}; short dashes = 0.20 m s^{-1}) for four nutrient treatments (C, N, P, N+P).

three flow regimes. The rate of nutrient spiralling (the recycling of nutrients between the water column and streambed) may differ between nitrates and phosphates because of the ability of nitrates to remain associated with particular substrate surfaces.

Results of the three-way factorial experiment (PO_4, NO_3, time) with repeated measures (time) revealed significant differences in retention of nutrients over time. Phosphate concentrations decreased with time at all three current velocities (Figure 3, Table 2). Nitrate concentrations decreased with time in the high and medium velocity flumes, but not at low flows (Figure 4, Table 2). Moreover, values of NO_3 concentrations obtained from agar samples in the NDS were much more variable than the PO_4 concentrations (Figure 3 and 4).

Phosphate concentrations in the agar inoculated with P and N+P, declined rapidly during the first 6 days of the experiment. Concentrations of PO_4 in the other treatments (C and N) were low throughout the experiment (Figure 3). The minimal concentrations of PO_4 detected in the C and N agar samples reflected the amount of phosphate absorbed by these NDS from the flowing water in the recirculating flumes. Because nutrients had been washed from the agar, no differences in phosphates were observed among the four treatments (including controls) from day 6 to day 32 of the incubation period.

Table 2. Summary of a three factorial experiment with repeated measures to examine the influence of nutrient treatments (presence/absence of nitrate concentration and present/absence of phosphate concentration) on phosphate and nitrate concentrations in agar samples obtained from the NDS that were sampled at 8 different time periods. Analyses were conducted at three different current velocities (low, 0.07 m s^{-1}; medium, 0.11 m s^{-1}; high, 0.20 m s^{-1}) over time (32 d). NS: not significant (0.05 level).

Source of variation	df	Phosphate F	Phosphate P	Nitrate F	Nitrate P
LOW VELOCITY					
Nitrate (N)	1	0.02	NS	3.86	NS
Phosphate (P)	1	23.89	0.0012	1.23	NS
N × P	1	0.24	NS	0.01	NS
Error	8				
Time (T)	7	11.40	<0.0001	1.47	NS
T × N	7	0.46	NS	5.49	0.0001
T × P	7	5.04	0.0002	1.00	NS
T × N × P	7	0.74	NS	0.94	NS
Error	56				
MEDIUM VELOCITY					
Nitrate (N)	1	2.15	NS	0.87	NS
Phosphate (P)	1	13.79	0.0059	0.03	NS
N × P	1	0.12	NS	0.21	NS
Error	8				
Time (T)	7	18.01	<0.0001	3.16	0.0069
T × N	7	2.59	NS	2.40	0.0319
T × P	7	6.58	<0.0001	0.67	NS
T × N × P	7	2.09	NS	1.09	NS
Error	56				
HIGH VELOCITY					
Nitrate (N)	1	1.85	NS	2.30	NS
Phosphate (P)	1	7.23	0.0276	2.65	NS
N × P	1	1.88	NS	0.86	NS
Error	8				
Time (T)	7	167.15	<0.0001	97.65	<0.0001
T × N	7	3.63	0.0027	5.89	<0.0001
T × P	7	5.83	<0.0001	1.70	NS
T × N × P	7	3.23	0.0060	3.21	0.0063
Error	56				

As expected, nitrate concentrations were greater in those treatments (N, N+P) inoculated with $NaNO_3$ than in the other treatments (C, P), especially during the first week of the experiment (Figure 4). At later times (days 6 to 32), NO_3 concentrations were similar among all treatments (including controls). There was a greater loss of NO_3 concentrations from the NDS in the fast flowing flume than in the other flumes with more

moderate flows among the four treatments (Figure 4). There were significant differences in nitrate concentrations among interaction terms in the low (time × NO_3), medium (time × NO_3) and high (time × NO_3, time × NO_3 × PO_4) velocity flumes (Table 2). Nitrate concentrations in the NDS did not differ significantly among any of the main nutrient treatments.

In this study, nutrient concentrations of spiked samples declined substantially within a week when exposed to flowing water (Figures 3 and 4). Fewer nutrients were released in others' laboratory experiments that employed no water flow (Fairchild et al., 1989) or simulated flow (Pringle & Bowers, 1984; Munn et al., 1989) than in this study in which NDS were exposed to flowing water conditions. Even in simulated flow conditions, nutrient concentrations decline exponentially, with most nutrients entering the water column within the first few days (Pringle & Bowers, 1984; Munn et al., 1989). Thus, researchers designing field experiments should recognize the consequence of rapid nutrient loss from NDS in flowing water.

Researchers have incubated NDS in rivers or lakes for time periods ranging from 2 to 8 weeks (Pringle & Bowers, 1984; Lowe et al., 1986; Pringle et al., 1986; Winterbourn & Fegley, 1989; Fairchild et al., 1989). The periphyton and associated macroinvertebrates that initially colonize diffusing substrates may be affected directly by nutrients. However, the presence of late colonizers on diffusing substrates may be a function of the morphological features and/or behavioural interactions of early colonizers rather than initial concentrations of nutrients, which are likely to have become depleted. Whether NDS are exposed to flowing water or wave washed shores of lakes, flow conditions should be considered when colonization experiments using NDS are planned. Concentrations of phosphates and nitrates (except low and medium flows) in agar were depleted after 6 days of incubation.

In natural streams, floods physically remove or abrade periphyton. Biggs (1988) identified discharge regime, current velocity as well as size and stability of bed materials to be of prime importance in affecting the composition, biomass and growth of epilithic periphyton in running waters. Findings from the present study suggest that even ambient flow conditions will affect the availability of nutrient concentrations and potential periphyton colonizers. Given the rapid decline of nutrients from NDS, short field incubation periods seem warranted.

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