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Responses of chlorophyll-a, organic matter, and macroinvertebrates to nutrient additions in rivers flowing through agricultural and forested land

Lynda D. Corkum[1]

University of Windsor, Canada

With 10 figures and 5 tables in the text

Abstract: I conducted field studies to determine if chlorophyll-a, organic matter, and macroinvertebrates would respond to nutrient changes in rivers in agricultural and forested areas of the Eastern Deciduous Forest in southwestern Ontario, Canada. Nutrient manipulations were examined using nutrient diffusing substrates (NDS) modified to permit independent sampling units. Results of a 5–6 week field experiment conducted in three forested and three agricultural rivers during mid-summer showed that concentrations of chlorophyll-a colonizing NDS varied among rivers and between land use types. As expected, concentrations of chlorophyll-a were higher in rivers flowing through agricultural than forested rivers. Variation among rivers within land use type was greater than between land use types in terms of organic matter and invertebrate responses to nutrient amendments. The NDS that were spiked with phosphate contained consistently higher quantities of phosphate than control or nitrate treatments after incubation. There were no significant differences in nitrate concentrations in NDS among treatments after incubation. Chironomids were the most abundant invertebrates that colonized NDS. Of the organisms examined, only one taxon (Tipulidae) appeared to be phosphate limited in forested rivers. Changes in the macroinvertebrate community would be expected after sustained additions of nutrients. The lack of significant differences between macroinvertebrates inhabiting natural rocks and those colonizing control NDS suggests that similarly designed substrata could be used to monitor macroinvertebrates in microhabitats.

Introduction

Hydrological features, riparian vegetation, characteristic climax vegetation of biomes and land use practices within drainage basins all interact to account for

[1] **Author's address:** Department of Biological Sciences, University of Windsor, Windsor, ON N9B 3P4 Canada.

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distributional patterns of lotic macroinvertebrates (CORKUM 1989, 1992 a). However, less is known about the effects of nutrient limitation on algal biomass and invertebrate activity in rivers that flow through different land use areas. Typically, more nutrients are released into receiving waters from croplands than from forested areas (LIKENS & BORMANN 1974). Nutrient release from croplands varies with runoff, farming method, and crop type (JORDAN et al. 1986, SHARPLEY et al. 1992). Nutrient release from forests varies with age of forests, amount of disturbance and time since last disturbance (BORMANN & LIKENS 1979). Although land use within a drainage basin influences productivity of receiving waters (CORKUM 1992 a), the transfer rate of materials and nutrients can be altered by riparian vegetation (SCHLOSSER & KARR 1981), which may take up or filter nutrients from surface water running off croplands into river channels (JORDAN et al. 1986).

Few studies have compared linkages among nutrients, periphyton and productivity in forested and open channel reaches (TROELSTRUP & PERRY 1989); however, nutrient limitation of periphyton has been shown in many streams (STOCKNER & SHORTREED 1978, ELWOOD et al. 1981, BOTHWELL 1985, GRIMM & FISHER 1986, PRINGLE 1987). WINTERBOURN (1990), using nutrient diffusing substrates (NDS) in one New Zealand river, showed that periphyton biomass and chironomid density increased with added nutrients; more periphyton and grazers colonized NDS placed in open river sites than in forested sites. Chlorophyll-a, algal accumulation and biovolume are all greater in streams with an open versus closed canopy (LOWE et al. 1986). However, WINTERBOURN (1990) showed that algal assemblages were nutrient limited at both open and forested sites along a stream.

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. 1984, ELWOOD et al. 1981) and flow-through systems (STOCKNER & SHORTREED 1978, BOTHWELL 1988, MUNDIE et al. 1991). Additions of inorganic nutrients to rivers have been shown to increase chlorophyll-a concentrations tenfold; diatoms, a common food source for stream invertebrates dominated the algal biomass (PERRIN et al. 1987, MUNDIE et al. 1991). JOHNSTON et al. (1990) demonstrated a three to fivefold increase in invertebrate standing stock in whole river fertilization studies.

In this study, I randomly selected three rivers in both agricultural and forested areas of the Eastern Deciduous Forest of Ontario, Canada. Nutrient diffusing substrates, with and without nitrate and phosphate additions, were used to compare colonization of periphyton (chlorophyll-a), organic matter, and associated macroinvertebrates in rivers within each land use type. Because nutrient limitation is often greater in receiving waters of forested than agricultural areas (LIKENS & BORMANN 1974), I expected organisms to exhibit a greater response to nutrient enriched NDS in rivers flowing through forests than farm-

Table 1. Mean annual (±S.E.) levels of nutrients in agricultural (Avon, North Thames, Maitland) and forested (Beaver, Credit, Saugeen) rivers for total phosphorus (TP), soluble reactive phosphate (SRP), and the sum of nitrates and nitrites. Values were based on monthly samples obtained in 1990 from the Ontario Ministry of Environment and Energy. N.D. = no data available.

River	TP	SRP	NO_3+NO_2
	(μg/L)	(μg/L)	(mg/L)
Avon	59.3 (1.4)	21.6 (0.4)	5.761 (0.746)
North Thames	14.2 (0.5)	11.7 (1.2)	7.972 (0.918)
Maitland	37.3 (1.0)	3.9 (0.4)	3.806 (0.672)
Beaver	11.7 (1.0)	3.5 (0.3)	ND
Credit	25.8 (0.6)	2.9 (0.3)	1.121 (0.153)
Saugeen	9.3 (0.7)	2.1 (0.1)	0.340 (0.037)

lands. I also expected periphyton and their macroinvertebrate grazers to respond only to phosphate treatments since phosphate is more limiting than nitrate in rivers of this region (Ontario Ministry of Environment and Energy, unpublished data; Table 1).

Materials and methods

Study area

I conducted a field experiment to determine if macroinvertebrates would respond to nutrient additions in rivers of deforested and forested areas of the Eastern Deciduous Forest in southwestern Ontario. Because of extensive agricultural and urban development in the region, there are no drainage basins that are entirely forested. Accordingly, I classified forested land use areas as those with mature trees both in the river valleys and extending along the above plateaus. One site on each of three rivers flowing through agricultural (Avon, Maitland, North Thames) and forested (Beaver, Credit, Saugeen) areas was selected for study (Table 2). Rivers were selected randomly within each land use area of southwestern Ontario, but sites were selected on the basis of road access and water depth. I needed to be able to wade through the rivers to sample them.

Table 2. Physical description of the sample sites on each of the six rivers.

River	Depth (cm) Mean±S.E.	Current velocity ($m \cdot s^{-1}$)	River width (m)	Overhanging vegetation (%)	Dominant substrate
Agricultural					
Avon	31±1	0.10	12	0	silt/sand
Maitland	44±9	0.09	26	0	cobbles/gravel/sand
North Thames	18±1	0.17–0.27	51	0	boulder/cobble/gravel/silt
Forested					
Beaver	31±4	0.91–0.96	14	5–8	cobble/pebble/gravel/sand
Credit	34±1	0.40–0.67	18.5	20	cobble/pebble/gravel/sand
Saugeen	16±2	0.17–0.34	13	3	boulder/cobble

Nutrient diffusing substrates (NDS)

To investigate the colonization of nutrient enriched patches in rivers, I used NDS modified from PRINGLE & BOWER's (1984) sand-agar nutrient mixtures and WINTERBOURN's (1990) plastic cups with agar and netting. Each NDS was assembled using a polystyrene petri dish (diameter of dish bottom ≈ 8.7 cm, height = 1.3 cm). A single 12-cm strand of 20 gauge wire was heated in a flame and then inserted from the outside edge of the bottom dish, across and through the opposite side of the dish. A second length of wire was inserted across the bottom of the dish, perpendicular to the first wire. Needle-nose pliers were used to bend the wire into 1 cm diameter loops protruding from the outside edges.

The dish bottom was filled with a mixture of warm agar solution (2‰) and nutrients depending on the particular treatment. The four treatments examined were control (C) = agar, nitrate (N) = 0.5 M $NO_3 \cdot L^{-1}$ + agar, phosphate (P) = 0.5 M $PO_4 \cdot L^{-1}$ + agar, nitrate and phosphate (N+P) = 0.5 M $NO_3 \cdot L^{-1}$ + 0.5 M $PO_4 \cdot L^{-1}$ + agar. Nitrates and phosphates were added as $NaNO_3$ and K_2HPO_4, respectively (cf. PRINGLE & BOWERS 1984). The NDS with N+P treatment required more agar (8 %) to solidify the colonizing medium. The increasing agar concentration had no noticeable effect on nutrient release (CORKUM, unpubl. data).

Once the agar had cooled and solidified, a circle of nylon netting (250 μm mesh) was cut and placed on the agar surface. A petri dish cover (with a 50 cm^2 hole in the centre) was placed over the netting on the agar-filled bottom and secured with hot glue. The area of exposed netting served as a colonization surface once the NDS were positioned in the river.

Two 50-cm lengths of heavy (16 gauge) wire were used to secure each NDS to the riverbed. In soft bottom areas, the wires were bent into an inverted U shapes so that each end could be inserted into one of the loops of a NDS and pushed far enough into the substrate that the NDS sat on the substrate surface. In riverbeds with coarse substrates, the wire could be wrapped around large cobbles to secure NDS.

The main advantage of this NDS design was that each unit could be randomly placed and secured on the streambed. In addition, the netting used for colonization could be easily cut with scissors into sectors for analysis.

Study design

A four factor, mixed-model ANOVA design was used to examine the effect of land use, rivers within land use, nitrate, phosphate and the interaction of these factors on chlorophyll-a, macroinvertebrate density, and organic matter (measured as loss on ignition). In this model, rivers were randomly selected, but all other factors (land use, nitrate, phosphate) were fixed.

Forty NDS (10 Control, 10 N, 10 P, 10 N+P) were positioned randomly in a grid pattern (4 columns × 10 rows) in each river. The incubation period for the NDS in each of the six rivers was five-six weeks beginning 2–9 August 1990. The NDS were incubated in agricultural rivers for 37–43 d and in forested rivers for 40–45 d. Various physical features of the sample site were recorded (Table 2). Current velocity was measured at the upstream and downstream locations of the grid.

Fig. 1. Photograph of a nutrient diffusing substrate retrieved from river after 5 weeks of incubation.

On the date of removal, NDS were retrieved individually (Fig. 1) beginning at the downstream row of a grid and moving upstream. Each NDS was placed immediately in a polyethylene ("ziploc®") bag, sealed and then placed on dry ice. In addition, six natural rocks that were similar in size to the NDS were retrieved to compare the macroinvertebrate fauna that had colonized the NDS with natural substrates. In the laboratory, the surface area of each rock was calculated once organisms were removed. Individual rocks were wrapped in aluminum foil and the area of a rock was determined using a planimeter to measure a photocopied impression of the foil.

Comparisons were made between macroinvertebrate densities in rivers located in agricultural versus forested areas and between fauna that colonized natural rocks versus control NDS using Hotelling's T test. A two-way (nitrate and phosphate) MANOVA was used to determine the influence of nutrients on the macroinvertebrate community composition.

Laboratory procedures

In the laboratory, petri dishes were thawed and disassembled. The colonized 250 μm netting was removed from the surface of the agar and cut into quarter sections ($12.5\ cm^2$) so that the macroinvertebrates, chlorophyll-a, and organic matter could be analysed (a fourth section was retained in reserve). Macroinvertebrates were sorted from the periphyton and identified to the familial level using a dissecting microscope (12×). Use of a coarse taxonomic designation is justified when benthic distributional patterns are sought on a large geographic scale (Corkum 1992 a). Photosynthetic pigments were extracted in acetone to provide an indicator of algal biomass (WINTERBOURN 1990). Chlorophyll-a concentration was determined using an acetone extraction method that used intensity differences detected by spectrophotometer readings (GOLTERMAN & OHNSTAD 1978). Organic matter (loss on ignition, LOI) was determined by measuring the difference between ashed and oven-dried samples. All adhering material, organisms and detritus, was air dried for 24 h, oven dried (103°, 24 h), weighed, ashed (550 ± 25 °C, 1 h) and reweighed. A correction factor was subtracted for the mass of the netting.

The concentrations of nitrate and phosphate remaining in the agar of NDS were measured directly from subsamples of the agar (3 replicates per NDS, 4 NDS per treatment). The agar (5 mL) was sampled using an acid-washed, cut-off, polystyrene, disposable syringe (1 cm diameter). Clean syringes were used for each NDS. Concentrations of nutrients were analysed using standard methods (APHA 1985). The molybdenum blue technique was used to assay phosphate; the cadmium reduction technique was used for nitrate.

Results

Concentrations of PO_4 and NO_3 in the agar of NDS after incubation in rivers

The concentrations of PO_4 and NO_3 remaining in the agar of NDS after river incubation are presented in Figs. 2 and 3, respectively. Results of the 4-way, mixed-model ANOVA revealed that there were significant differences in phosphate concentrations remaining in the agar among rivers ($P < 0.001$) and three interactions terms (land use × phosphate, land use × nitrate × phosphate, nitrate × phosphate) (Table 3). The significant interaction effects between land use and nutrients were observed in treatments (P, N+P) that exhibited higher phosphate concentrations in agricultural than forested rivers (Fig. 2). The NDS that were spiked with phosphates in agricultural rivers contained consistently higher quantities of phosphates than either control or nitrate treatments.

Although there were significant differences in nitrate concentrations among rivers ($F_{(4,84)} = 8.59$, $P < 0.001$), there were no significant differences in nitrate concentrations for any other main effects or interactions. Apparently, agar attracts or absorbs nitrates from the water column of rivers, obscuring any differences among treatments.

Factors affecting chlorophyll-a, LOI, and total macroinvertebrate density

Results of three, 4-way mixed model ANOVA tests were used to examine the effects of land use, rivers nested within land use, nitrate, phosphate, and the

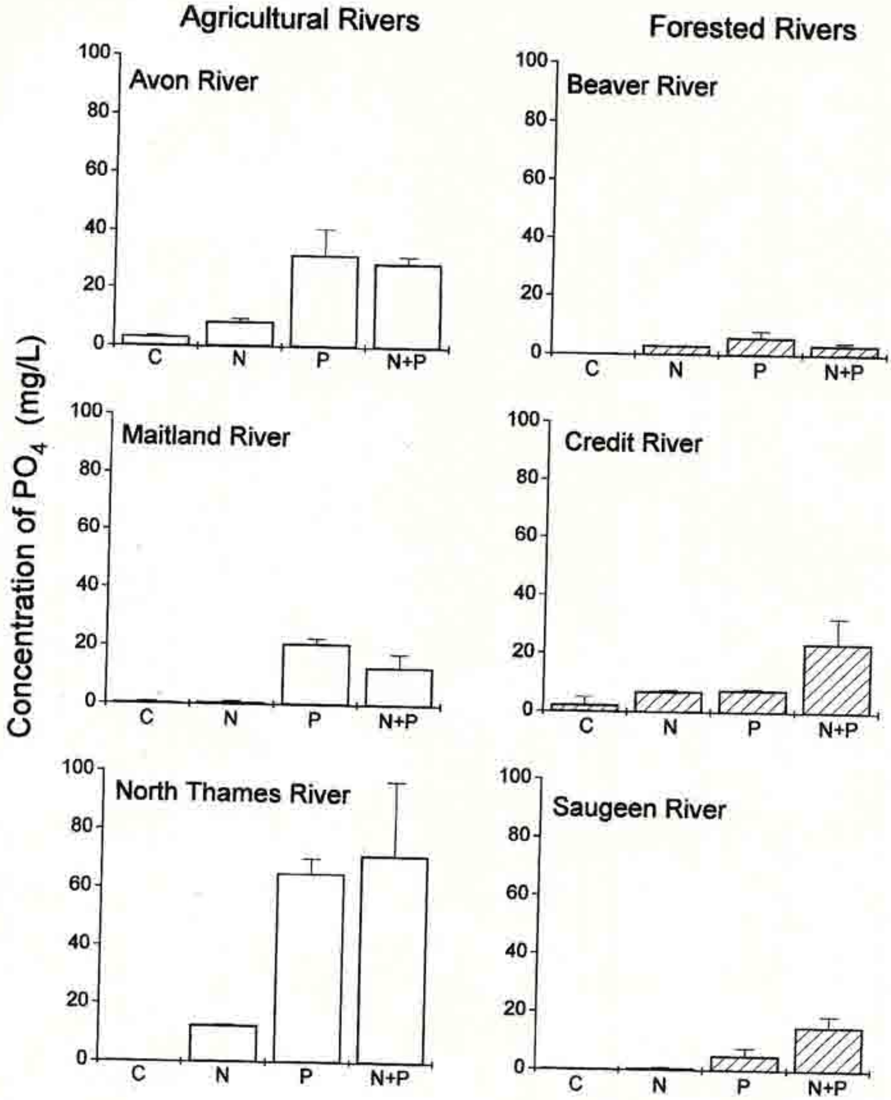

Fig. 2. Mean ± S.E. of PO_4 concentration (mg/L) in agar of the NDS after retrieval from the six rivers. Three replicates per NDS and 4 NDS per nutrient treatment (C, N, P, N+P) were analysed. Open bars represent samples from agricultural sites; hatched bars represent samples from forested sites.

interaction of these factors on 1) chlorophyll-a, 2) loss on ignition (LOI), and 3) total macroinvertebrate density obtained from the netting on the incubated NDS.

Differences between land use and among rivers significantly influenced concentrations of chlorophyll-a associated with the NDS (Table 4, Fig. 4). As

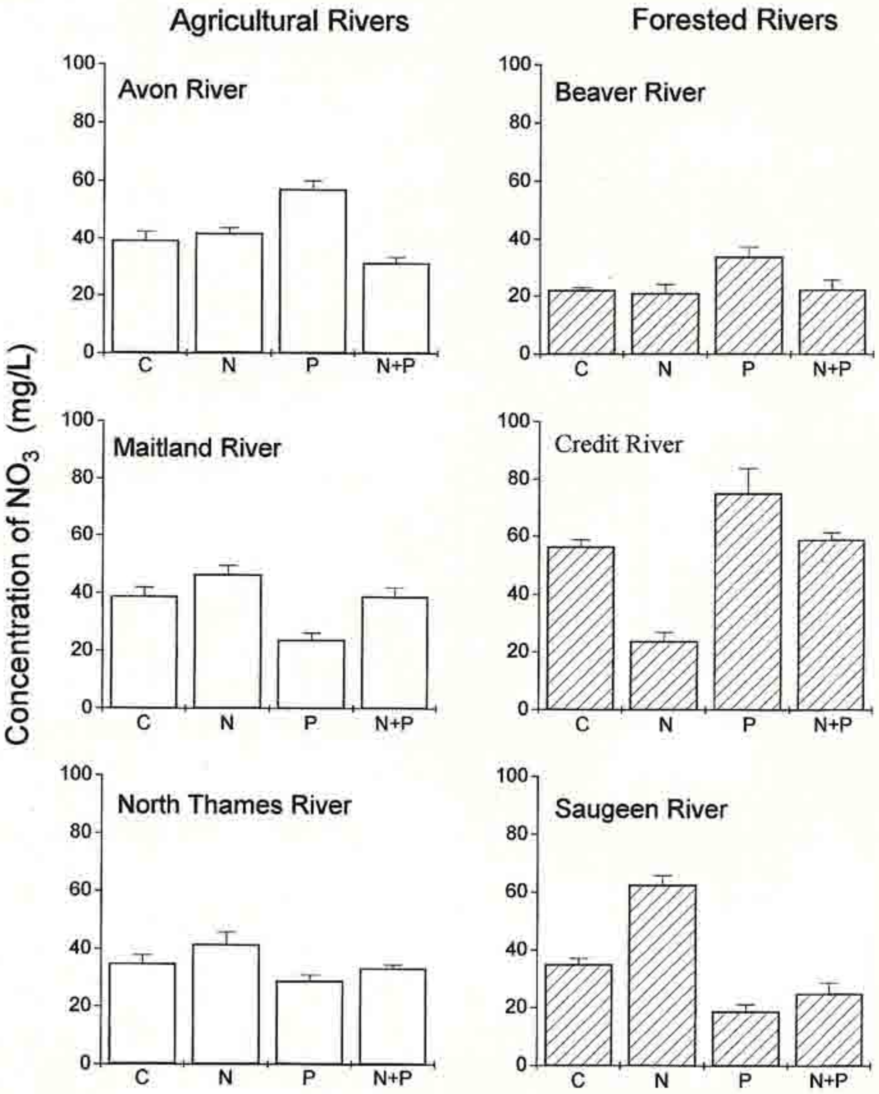

Fig. 3. Mean ± S.E. of NO_3 concentration (mg/L) in agar of the NDS after retrieval from the six rivers. Three replicates per NDS and 4 NDS per nutrient treatment (C, N, P, N+P) were analysed. Open bars represent samples from agricultural sites; hatched bars represent samples from forested sites.

expected, concentrations of chlorophyll-a were higher in rivers flowing through agricultural than forested areas. In agricultural areas, chlorophyll-a concentrations were higher in the Avon and North Thames rivers than in the Maitland River. In forested areas, chlorophyll-a concentrations were higher in the Credit River than in the Beaver or Saugeen rivers.

Table 3. Summary results of the mixed model ANOVA sources of variation on phosphate concentrations remaining in the agar of the NDS after a 5–6 week incubation period in rivers. Rivers were randomly selected within each land use type. Other main sources of variation were fixed. F_s (land use) = MS (land use)/MS (rivers); F_s Nadd = MS (Nadd)/MS (Nadd × Padd); F_s Padd = MS(Padd)/MS (Nadd × Padd); F_s (other variables) = MS (group)/MS (error). NS = not significant at the 0.05 level.

Source of variation	df	MS	F	P
Land use	1,4	15.8994	3.44	NS
Rivers	4,84	4.6196	13.35	<0.001
Nitrates added (Nadd)	1,1	7.2306	2.12	NS
Phosphates added (Padd)	1,1	65.6459	19.25	NS
Land use × Nadd	1,84	0.2603	0.75	NS
Land use × Padd	1,84	6.4908	18.76	<0.001
Nadd × Padd	1,84	3.3410	9.86	<0.005
Land use × Nadd × Padd	1,84	1.7104	4.94	<0.05
Error	84	0.34599		

Table 4. Summary of significant effects of land use, rivers nested within land use, nitrates, phosphates and their interaction on chlorophyll-a, loss on ignition (LOI), and total macroinvertebrate density.

Dependent variable	Source of variation	df	F	P
Chlorophyll-a	Land use	1,4	14.79	<0.025
	Rivers	4,185	97.18	<0.001
LOI	Rivers	4,185	19.70	<0.001
	Phosphates	1,1	331.94	<0.05
Total density	Rivers	4,185	61.44	<0.001

There were significant differences in amounts of organic matter as indicated by LOI among rivers and nutrient treatments with phosphate (Table 4). There also were significant differences in total macroinvertebrate densities among rivers (Table 4). Within each river, only Maitland and Saugeen showed evidence of nutrient limitation on organic matter (Fig. 5) and almost no indication of nutrient effects on macroinvertebrate density (Fig. 6). There is little difference in macroinvertebrate density among rivers with the exception of the Avon, which is high (Fig. 6).

Chironomidae

Chironomid larvae dominated the benthic invertebrate fauna on both natural substrates (80.6 ‰ of 10,250 organisms per 10^3 cm^2) and NDS (68.6 % of 27,468 organisms per 10^3 cm^2). Although chironomid density did not vary between land use types, among nutrient treatments or interactions, density of the midges differed significantly among rivers ($F_{4,12}$ = 64.83, $P<0.001$). Highest

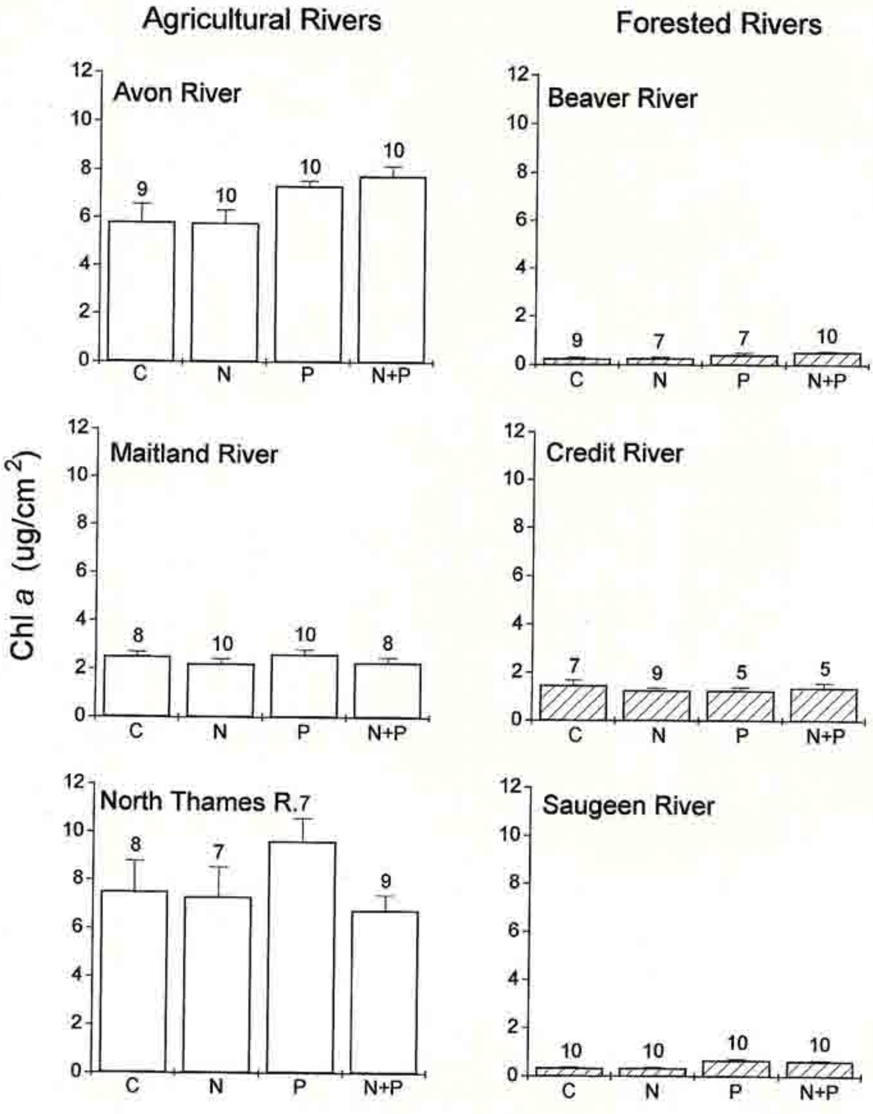

Fig. 4. Mean ± S.E. of chlorophyll-a (μg/cm^2) obtained from the netting of NDS after retrieval from the six rivers. Numbers above the error bars of each nutrient treatment (C, N, P, N+P) indicate the number of NDS retrieved from each river. Open bars represent samples from agricultural sites; hatched bars represent samples from forested sites.

chironomid density on control NDS (3,020 per 10^3 cm^2) occurred in the Avon River (Table 5). The fine substrate of the Avon River matched the colonizing surface of the NDS and was more suitable to grazing chironomid habits than

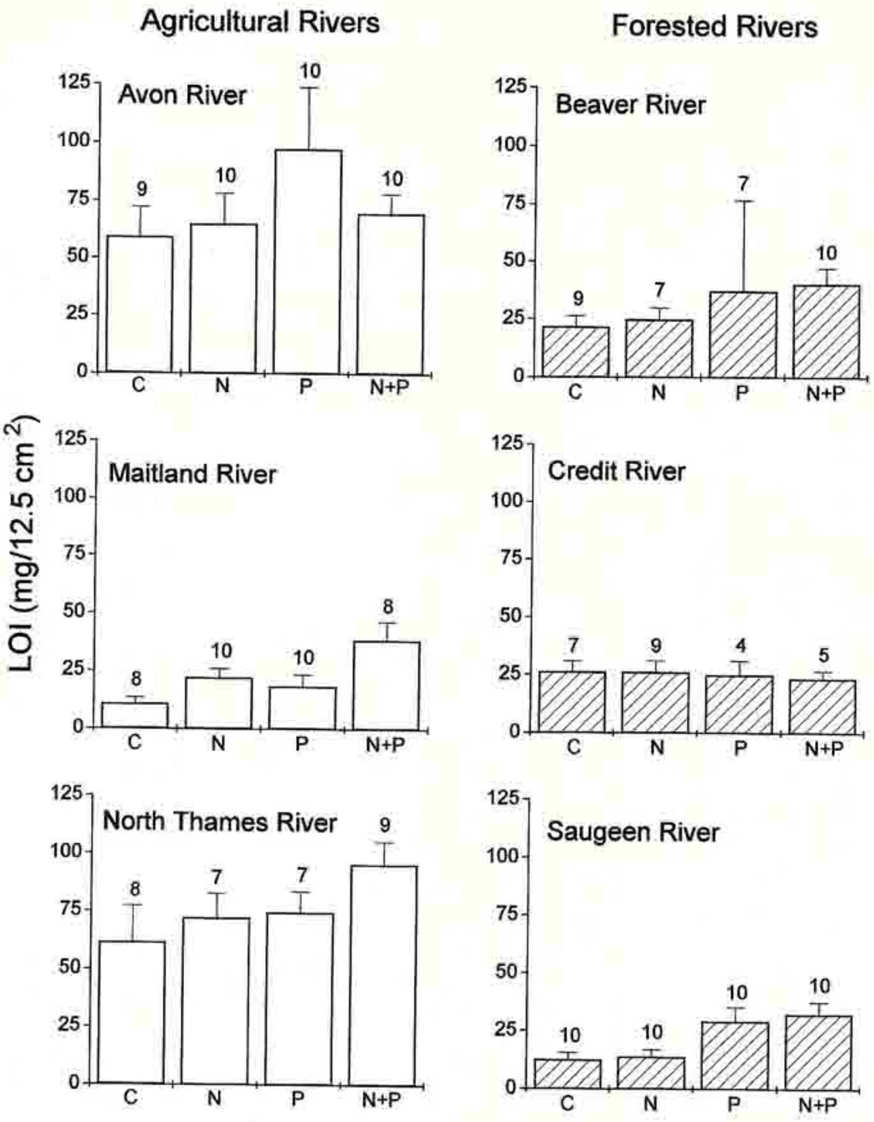

Fig. 5. Mean ± S.E. of organic matter [loss on ignition (LOI) (mg/12.5 cm^2)] obtained from the netting of the NDS after retrieval from the six rivers. The numbers above the error bars of each nutrient treatment (C, N, P, N+P) indicate the number of NDS retrieved from each river. Open bars represent samples from agricultural sites; hatched bars represent samples from forested sites.

the coarse, heterogeneous substrates in other rivers. Higher chironomid density retrieved from natural substrates occurred in the Avon, Credit, and Saugeen rivers than in the other three rivers (Table 5).

Table 5. Mean and (standard error) of chironomid density (number/10^3 cm^2) retrieved from natural substrates (rocks) and NDS (control, C; nitrate, N; phosphate, P; nitrates+phosphates, N+P) in agricultural (Avon, Maitland, North Thames) and forested (Beaver, Credit, Saugeen) rivers. n = number of samples retrieved.

		Substrate type				
Rivers		Rocks	C	N	P	N+P
Avon	Mean	1800	3020	3470	2830	2844
	S.E.	(662)	(615)	(484)	(600)	(646)
	n	6	9	10	10	10
Maitland	Mean	982	140	216	120	328
	S.E.	(260)	(41)	(52)	(41)	(93)
	n	6	8	10	10	8
North Thames	Mean	808	415	271	391	354
	S.E.	(179)	(104)	(67)	(84)	(75)
	n	6	8	7	7	9
Beaver	Mean	488	484	366	469	402
	S.E.	(128)	(71)	(73)	(24)	(55)
	n	6	9	7	7	10
Credit	Mean	2100	463	405	612	332
	S.E.	(358)	(86)	(74)	(48)	(101)
	n	6	7	9	4	5
Saugeen	Mean	2086	216	156	246	284
	S.E.	(255)	(38)	(24)	(40)	(30)
	n	6	10	10	10	10

Comparison of macroinvertebrates between natural rocks and control NDS

Macroinvertebrates, excluding chironomids, that comprised the most abundant 10 taxa from each of the six rivers were compared between natural rocks and control NDS in forested and agricultural land use areas. Twenty-one dependent variables (taxa) were examined (Figs. 7 and 8). I used the multivariate Hotelling's T^2 procedure to test whether both groups (natural rocks and control NDS) had equal means for all variables.

In forested areas, there was no overall significant difference between the density of macroinvertebrates that occupied rocks and those that occupied control NDS (Hotelling's $T^2 = 6.57$, $P = 0.806$, Fig. 7). However, there were significant differences for taxa that occurred only on rocks (Tricorythidae, $P = 0.016$; Caenidae, $P = 0.016$; Hydroptilidae, $P = 0.029$; Psephenidae, $P = 0.028$; Ceratopogonidae, $P = 0.016$) and only on control NDS (Turbellaria, $P = 0.16$; Oligochaeta, $P = 0.031$). Hydracarina (mites) occurred more often on rocks than the control NDS ($P = 0.034$); whereas Helicopsychidae occurred more often on control NDS than rocks.

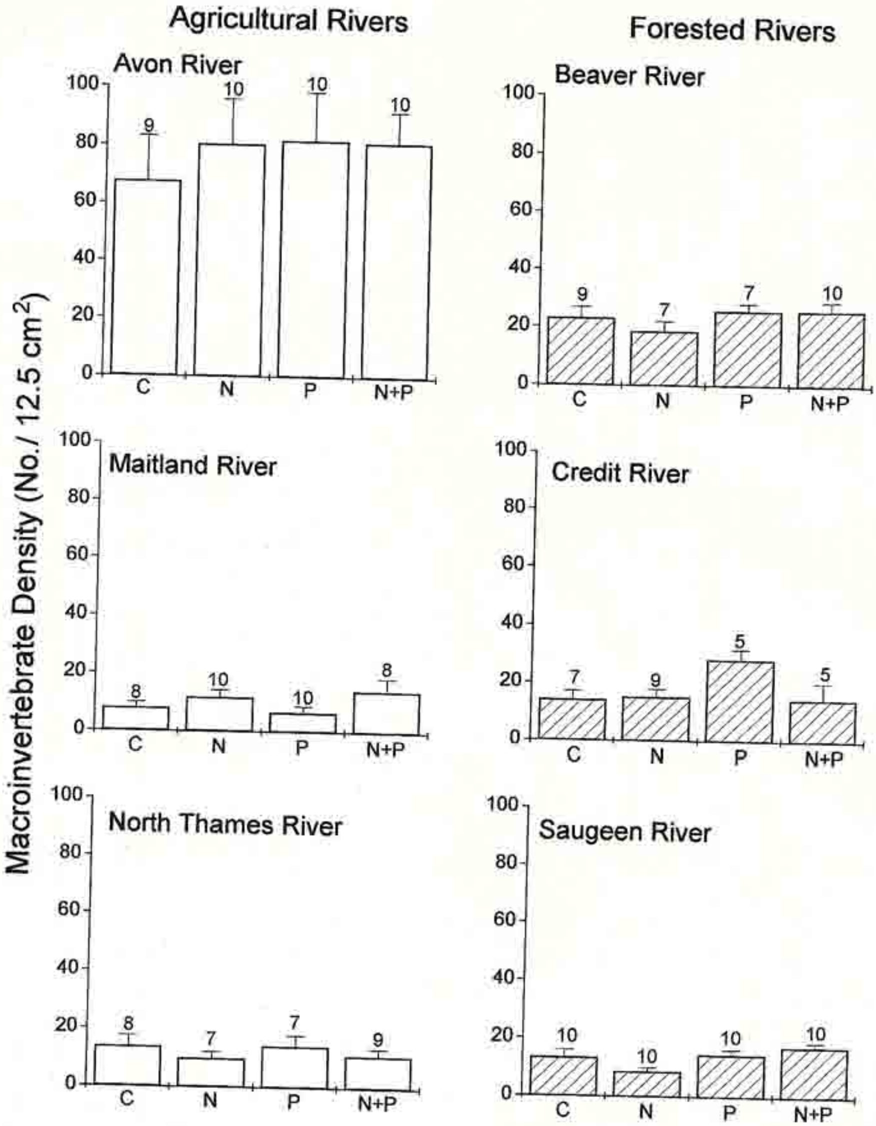

Fig. 6. Mean ± S.E. of macroinvertebrate density (number per 12.5 cm^2) obtained from the netting of NDS after retrieval from the six rivers. Numbers above the error bars of each treatment (C, N, P, N+P) indicate the number of NDS retrieved from each river. Open bars represent samples from agricultural sites; hatched bars represent samples from forested sites.

In agricultural areas, there was no overall significant differences between numbers of macroinvertebrates occurring on rocks and control NDS (Hotelling's $T^2 = 7.54$, $P = 0.781$, Fig. 8). Significant differences occurred between

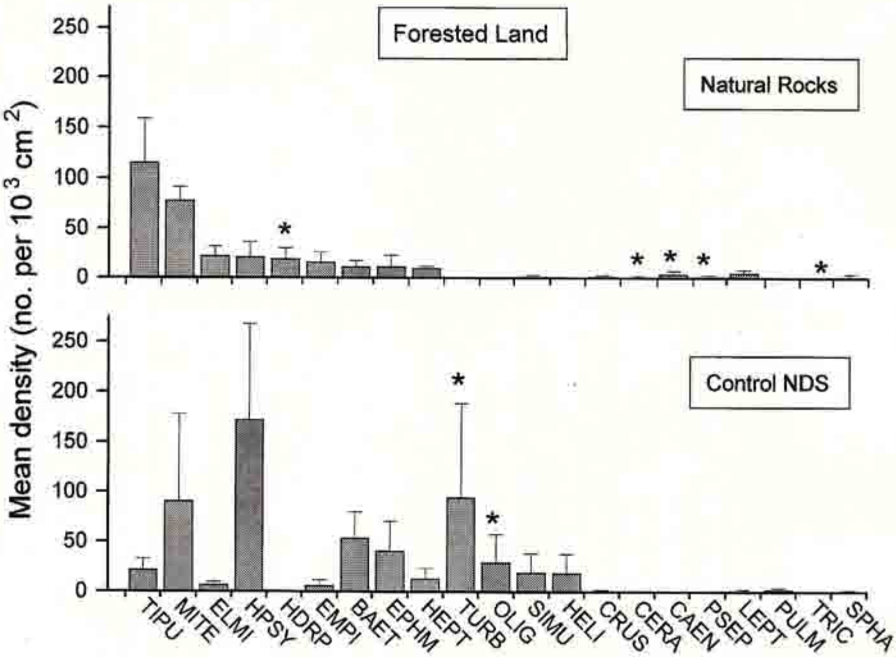

Fig. 7. Mean (± S.E.) densities of macroinvertebrates (excluding chironomids) retrieved from the control NDS and natural rocks from rivers that flowed through forested land. * Indicates those taxa that occurred only on one of the two substrate types.

these two substrate types for four taxa. Heptageniidae ($P = 0.016$), Helicopsychidae ($P = 0.017$) and Tipulidae ($P = 0.017$) occurred only on rocks; Sphaeriidae ($P = 0.045$) occurred only on control NDS.

There was no overall significant difference in numbers of organisms that inhabited rocks in the two land use areas (Hotelling's $T^2 = 2.08$, $P = 0.950$, Figs. 7 and 8). When individual taxa were considered, Sphaeriidae ($P = 0.016$) inhabited rocks only in forested rivers and mites occurred on rocks in forested rivers significantly more often than in agricultural rivers ($P = 0.040$). Pulmonata ($P = 0.016$) and Turbellaria ($P = 0.016$) occurred on rocks only in agricultural rivers. As expected, burrowing Oligochaeta were not found on rocks.

There was no overall significant difference between numbers of macroinvertebrates that occupied control NDS in the agricultural and forested land use types (Hotelling's $T^2 = 81.60$, $P = 0.319$, Figs. 7 and 8). Significant differences were observed for some taxa. Helicopsychidae ($P = 0.016$) and Tipulidae ($P = 0.042$) occupied the control NDS only in forested areas. Three taxa (Tricorythidae, $P = 0.016$; Psephenidae, $P = 0.016$; Ceratopogonidae, $P = 0.016$) inhabited only those control NDS that were incubated in agricultural areas. The microcaddisfly, Hydroptilidae, was never found on control NDS.

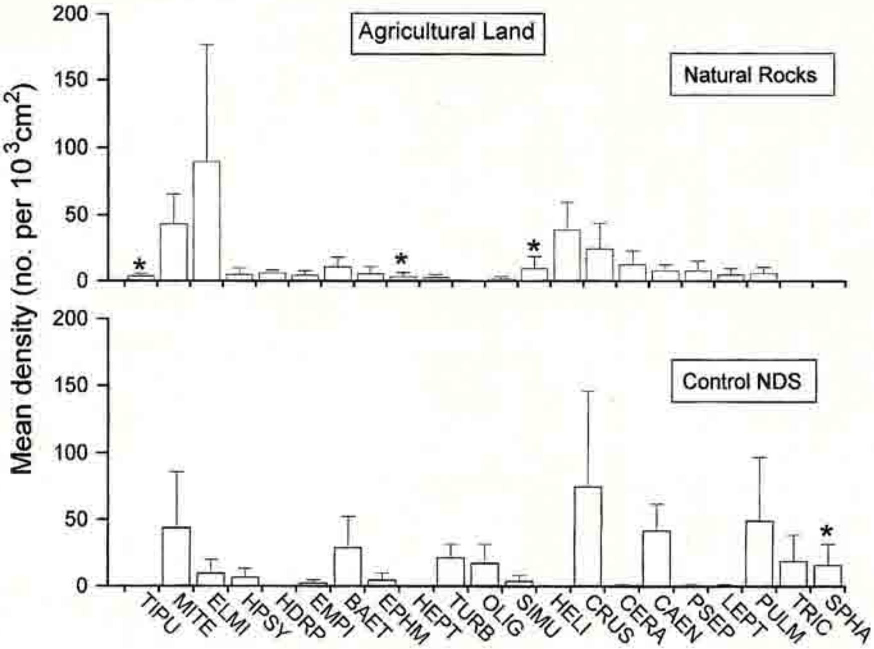

Fig. 8. Mean (± S.E.) densities of macroinvertebrates (excluding chironomids) retrieved from the control NDS and natural rocks from rivers that flowed through agricultural land. * Indicates those taxa that occurred only on one of the two substrate types.

Comparison of macroinvertebrate taxa among nutrient treatments within each land use type

A MANOVA was used to determine if macroinvertebrate composition differed among nutrient treatments. For example, did a taxon (or taxa) colonize any one treatment more than another? Twenty-one taxa (dependent variables) were compared simultaneously. The data were analysed separately for each land use type.

Overall, macroinvertebrate composition did not differ among nutrient treatments flowing through forested areas (Fig. 9). Neither the mayfly, Caenidae, nor the caddisfly, Hydroptilidae, which were common on natural stones, colonized any of the NDS. The density of one taxon, Tipulidae (the cranefly, *Antocha*), was significantly greater ($P = 0.051$) on NDS that were spiked with phosphate (P, N+P) than on other treatments (C, N).

In agricultural rivers, there were no overall significant differences in macroinvertebrate composition among treatments when all taxa (dependent variables) were combined (Fig. 10). Tipulidae (craneflies), however, were absent from all NDS in rivers flowing through farmlands. Also, there were no signifi-

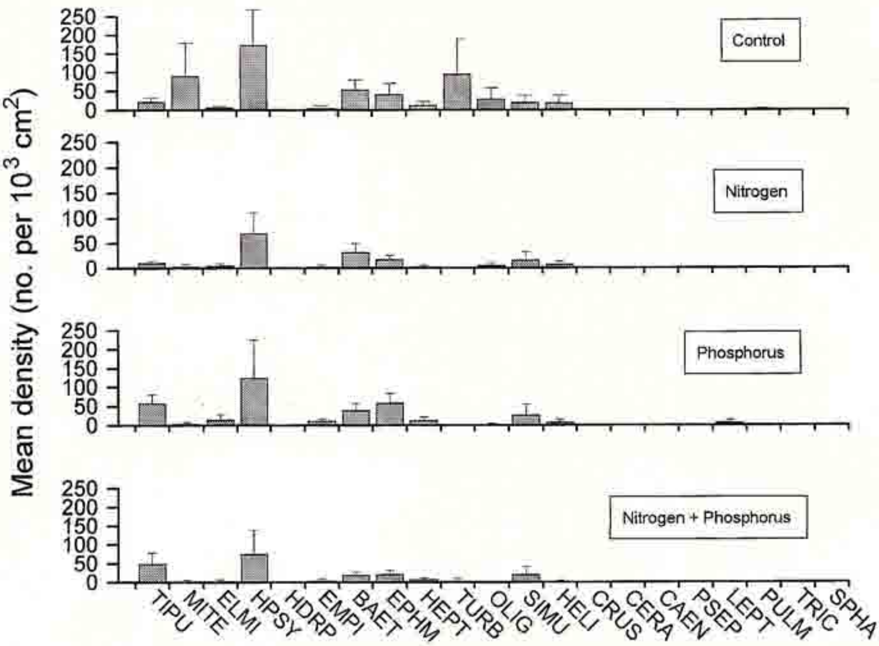

Fig. 9. Mean (± S.E.) density of macroinvertebrates (excluding chironomids) retrieved from the four NDS treatments incubated in rivers that flowed through forested land.

cant differences in any single taxon occurring on NDS in which PO_4 or NO_3 were added either alone or in combination.

Discussion

In this study, I examined the concentrations of both phosphate and nitrate remaining in the agar of the NDS that were incubated in agricultural and forested rivers for 5–6 weeks. Although there were significant differences among rivers in both phosphate and nitrate concentrations remaining in the agar of NDS, no other main effects altered these nutrient concentrations significantly. However, significant interaction effects of land use and nutrients were observed. There were higher concentrations of phosphate in NDS retrieved from agricultural than forested rivers (Fig. 2). This difference in phosphate concentrations may be attributed to flow conditions between the different land use areas (Table 2). Specifically, the elevated current velocities in the forested rivers may have "washed" phosphates from the NDS. Pringle (1987) noted that the nature of the algal composition and gelling of agar may interfere with nutrient treatments. In this study, the NDS attracted nitrate from the water col-

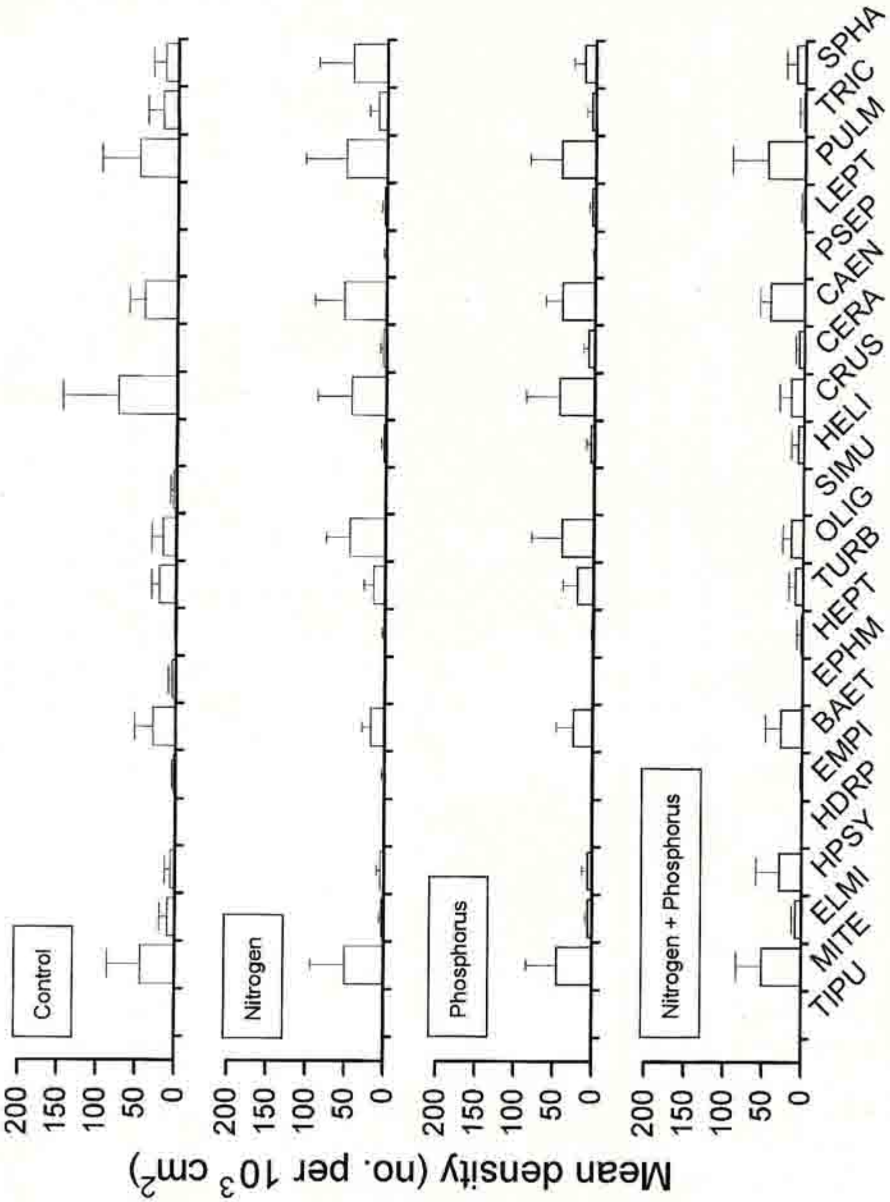

Fig. 10. Mean (± S.E.) density of macroinvertebrates (excluding chironomids) retrieved from the four NDS treatments incubated in rivers that flowed through agricultural land.

umn such that by the end of the study, there were no significant differences among the nutrient treatments.

Chlorophyll-a is an estimate of living algal biomass; LOI is a measure of living and dead organic matter (i.e., algal biomass and detritus) from a variety of sources. Detritus may originate from vegetation within the drainage basin, bank erosion and inputs from upstream and instream algal or macrophyte production (CORKUM 1992b). CORKUM (1992b) showed that detritus was significantly correlated with total macroinvertebrate density in rivers within the Eastern Deciduous Forest.

Since chlorophyll-a and LOI represent different measures, the responses by these variables to nutrient enrichment and landscape features were expected to differ. Study results revealed that concentrations of chlorophyll-a differed among rivers and between land use types. Total organic matter associated with NDS was phosphate limited and differed among rivers. Since the river channels of agricultural areas were open (i.e. unshaded), it was not surprising that chlorophyll-a concentrations were higher in agricultural rivers than in forested rivers (Fig. 4). In this study, nutrient treatments had no effect on chlorophyll-a associated with the NDS. Although nutrient limitation of epilithic algae has been shown in many watersheds and on different continents (GRIMM & FISHER 1986; WINTERBOURN 1990), this was not the case in the present study.

Regardless of the nutrient enrichment technique used, it is not surprising that nutrient enrichment elicits noticeable responses by the benthic community owing to the higher than natural levels used in the experimental treatments (MULHOLLAND et al. 1991). The effect of nutrients may decline with time because of the diffusion and subsequent dilution of nutrients into the surrounding medium or to the effects of herbivores grazing on the attached algae or periphyton. Herbivory may regulate the periphyton community by depleting algal standing crop (GREGORY 1983, MUNDIE et al. 1991) or by returning nutrients assimilated by the algae back to the water (NEWBOLD et al. 1982).

There were no differences in macroinvertebrate density in rivers flowing through different land use areas, yet total macroinvertebrate density varied significantly among rivers. Chironomids were the most abundant invertebrates that colonized the netting of the NDS, but their numbers did not differ among nutrient treatments. However, increased abundance of chironomids in response to enrichment has been observed by others (WINTERBOURN 1990, MUNDIE et al. 1991). WINTERBOURN (1990) suggested that the netting of NDS trapped particles, facilitating the tube making habits of midges. Enhanced populations of chironomids having short life cycles could contribute to trophic relationships uniformly.

There were few significant differences in density of specific taxa among nutrient treatments in rivers flowing through either agricultural or forested areas. Only one taxon, Tipulidae, was apparently phosphorus limited (P =

0.051) in forested rivers (Fig. 9). MUNDIE et al. (1991) compared the responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus over seven weeks. Numbers of individuals emerging from treatments were more than twice those from controls and most (77 %) were chironomids. These authors suggested that changes in benthic insects would be most apparent after sustained levels of available food (3–4 months of nutrient additions). Similarly, if levels of nutrients had been sustained throughout the incubation period in the present study, a more significant response by benthos to nutrient enrichment would have been anticipated.

Study results showed that there was no overall significant difference between numbers of macroinvertebrates that occurred on natural rocks and numbers colonizing control NDS in either agricultural or forested rivers. Since significant differences did occur between substrate types for selected taxa and given the relatively low level of replication (n = three rivers per land use), additional studies with continuous nutrient enhancement are warranted. Nevertheless, an advantage of the particular type of NDS that I used is that the control NDS (or similarly designed substrata) could be used as artificial substrates in habitats that may be difficult to sample using either natural or standardized artificial substrates (e.g., multiple-plate or basket-type samplers). The small size of the NDS relative to other artificial substrate samplers would be advantageous in many microhabitats.

In this study, rivers were selected randomly within each land use type. If one wished to maximize the chances of finding differences between land use, one could have selected rivers in completely wooded basins for comparison with rivers flowing through farmlands exhibiting the same agricultural crop. In selecting rivers randomly, I wanted to establish a more rigorous test of land use affinity, but in doing so, the variability exhibited among rivers may have masked differences in taxonomic composition. Regardless, it is clear that chlorophyll-a concentrations on NDS were higher in agricultural rivers than in forested rivers. Although macroinvertebrates did not discriminate between land forms, one taxon (Tipulidae) was phosphate limited in forested rivers.

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References

American Public Health Association (APHA) (1985): Standard methods for the examination of water and waste water. 16th ed. – APHA.

BORMANN, F. H. & LIKENS, G. E. (1979): Pattern and process in a forested ecosystem. – Springer-Verlag.

BOTHWELL, M. L. (1985): Phosphorus limitation of lotic periphyton growth rates: an intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). – Limnol. Oceanogr. **30:** 527–542.

– (1988): Growth rate responses of lotic periphytic diatoms to experimental phosphorus enrichment: the influence of temperature and light. – Can. J. Fish. Aquat. Sci. **45:** 261–270.

CORKUM, L. D. (1989): Patterns of benthic invertebrate assemblages in rivers of northwestern North America. – Freshwat. Biol. **21:** 191–205.

– (1992 a): Spatial distributional patterns of macroinvertebrates along rivers within and among biomes. – Hydrobiologia **239:** 101–114.

– (1992 b): Relationships between density of macroinvertebrates and detritus in rivers. – Arch. Hydrobiol. **125:** 149–166.

ELWOOD, J. W., NEWBOLD, J. D., TRIMBLE, A. F. & STARK, R. W. (1981): The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. – Ecology **62:** 146–158.

GOLTERMAN, H. L. & OHNSTAD, M. A. M. (1978): Methods for physical and chemical analysis of fresh waters. 2nd ed. – McGraw Hill.

GREGORY, S. V. (1983): Plant-herbivore interactions in stream systems. – In: BARNES, J. R. & MINSHALL, G. W. (eds.): Stream ecology: application and testing of general ecological theory. – Plenum, pp. 159–189.

GRIMM, N. B. & FISHER, S. G. (1986): Nitrogen limitation in a Sonoran desert stream. – J. N. Amer. Benthol. Soc. **5:** 2–15.

HUNTSMAN, A. G. (1948): Fertility and fertilization of streams. – J. Fish. Res. Bd. Can. **7:** 248–253.

JOHNSTON, N. T., PERRIN, C. J., SLANEY, P. A. & WARD, B. R. (1990): Increased juvenile salmonid growth by whole-river fertilization. – Can. J. Fish. Aquat. Sci. **47:** 862–872.

JORDAN, T. E., CORRELL, D. L., PETERJOHN, W. T. & WELLER, D. E. (1986): Nutrient flux in a landscape: the Rhode River watershed and receiving waters. – In: CORREL, D. L. (ed.): Watershed Research Perspectives. – Smithsonian Institution Press, Washington, D.C., pp. 57–76.

LIKENS, G. E. & BORMANN, F. H. (1974): Linkages between terrestrial and aquatic systems. – BioScience **24:** 447–456.

LOWE, R. L., GOLLADAY, S. W. & WEBSTER, J. R. (1986): Periphyton response to nutrient manipulation in streams draining clearcut and forested watersheds. – J. N. Amer. Benthol. Soc. **5:** 221–229.

MULHOLLAND, P. J., STEINMAN, A. D., PALUMBO, A. V. & ELWOOD, J. W. (1991): Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. – Ecology **72:** 966–982.

MUNDIE, J. H., SIMPSON, K. S. & PERRIN, C. J. (1991): Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm. – Can. J. Fish. Aquat. Sci. **48:** 2061–2072.

NEWBOLD, J. D., O'NEILL, R. V., ELWOOD, J. W. & VAN WINKLE, W. (1982): Nutrient spiralling in streams: implications for nutrient limitation and invertebrate activity. – Amer. Nat. **120:** 628–652.

PERRIN, C. J., BOTHWELL, M. L. & SLANEY, P. A. (1987): Experimental enrichment of a coastal stream in British Columbia: effects of organic and inorganic additions on autotrophic periphyton production. – Can. J. Fish. Aquat. Sci. **44:** 1247–1256.

PRINGLE, C. M. (1987): Effects of water and substratum nutrient supplies on lotic periphyton growth: an integrated bioassay. – Can. J. Fish. Aquat. Sci. **44:** 619–629.

PRINGLE, C. M. & BOWERS, J. A. (1984): An in situ substratum fertilization technique: diatom colonization on nutrient-enriched, sand substrata. – Can. J. Fish. Aquat. Sci. **41:** 1247–1251.

SCHLOSSER, I. J. & KARR, J. R. (1981): Riparian vegetation and channel morphology impact on spatial patterns of water quality in agricultural watersheds. - Environ. Managem. **5:** 233–243.

SHARPLEY, A. N., SMITH, S. J., JONES, O. R., BERG, W. A. & COLEMAN, G. A. (1992): The transport of bioavailable phosphorus in agricultural runoff. – J. Environ. Qual. **21:** 30–35.

STOCKNER, J. G. & SHORTREED, K. R. S. (1978): Enhancement of autotrophic production by nutrient addition in a coastal rainforest stream on Vancouver Island. – J. Fish. Res. Bd. Can. **35:** 28–34.

TROELSTRUP Jr., N. H. & J. A. PERRY (1989): Water quality in southeastern Minnesota streams: observations along a gradient of land use and geology. – J. Minnesota Acad. Sci. **55:** 6–13.

WARREN, C. E., WALES, J. H., DAVIS, G. E. & DOUDOROFF, P. (1984): Trout production in an experimental stream enriched with sucrose. – J. Wildl. Manage. **28:** 617–660.

WINTERBOURN, M. J. (1990): Interactions among nutrients, algae and invertebrates in a New Zealand mountain stream. – Freshwat. Biol. **23:** 463–474.

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