Benthic algae as bioindicators of agricultural pollution in the streams and rivers of southern Québec (Canada)

Isabelle Lavoie,[1,2*] Warwick F. Vincent,[1,2] Reinhard Pienitz,[2,3] and Jean Painchaud[4]

[1]*Département de biologie, Université Laval, Québec, G1K 7P4 Canada*
[2]*Centre d'Études Nordiques, Université Laval, Québec, G1K 7P4 Canada*
[3]*Département de géographie, Université Laval, Québec, G1K 7P4 Canada*
[4]*Direction du suivi de l'état de l'environnement, ministère de l'Environnement du Québec, 675 René-Lévesque est, Québec, G1R 5V7 Canada*
**Corresponding author: E-mail: ilavoie@trentu.ca*

The objective of this study was to evaluate the effect of agricultural pollution on periphyton in streams and rivers of southern Québec. We sampled benthic algae incubated from mid-July to mid-August on artificial substrates at 29 sites and analysed the variations in community structure and total community biomass. Diatom community structure as well as total benthic algae community were analysed. Water samples were taken to provide background chemical information, and land use data were also obtained. Preliminary tests showed that colonisation of the artificial substrates (unglazed ceramic tiles) resulted in biomass levels (Chlorophyll a *and ash-free dry weight) and species composition that were not statistically different from those on natural rock substrates. The canonical correspondence analyses showed that pH, conductivity and suspended solids were the most significant environmental variables accounting for variations among sites and diatom community structure. No additional resolving power was obtained by including cyanobacteria, green algae and flagellates. This total community analysis substantially increased variance and sample processing time while reducing the relationship with environmental variables. These results indicate that an analysis based exclusively on diatoms provided the optimal approach. Traditional nutrient measurements (phosphorus and nitrogen) did not explain a significant part of the variance in the species composition among sites. The ordination analyses clearly separated agriculturally-impacted streams from reference sites, but no significant grouping was observed related to the intensity and type of agriculture, indicating the greater importance of local farming practices. The use of periphyton as a bioindicator provides an integrated measurement of water quality as experienced by the aquatic biota, and therefore offers a useful addition to physico-chemical water quality monitoring strategies.*

Keywords: artificial substrates, land use, multivariate analyses, nutrients, periphyton, water quality

Introduction

Intense farming has led to severe disturbance of watersheds throughout the world, resulting in fundamental changes in the structure and functioning of stream ecosystems. Modern intensive agriculture is responsible for chemical and physical alterations such as increased contaminant and nutrient runoff, an increase in suspended solids due to erosion, and changes in discharge and channel morphology (Skinner et al., 1997). The traditional physico-chemical measurements used in water quality monitoring programs such as total phosphorus and suspended sediment load are an important guide to environmental change. However, they are

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only representative of short-term conditions found at the instant of sampling and do not provide information about the effects of these changes on biological communities. The need for a better comprehension of interactions between environmental quality and ecosystem integrity has increased the interest in finding biological indicators that provide a more accurate guide to changes in ecological conditions.

From the earliest years of the last century, periphytic (benthic) algae have been identified as a valuable option for the biomonitoring of stream and river ecosystems (Kolkwitz and Marsson, 1908 cited by Hill et al., 2000). More recently, this approach has been applied with success to evaluate a variety of water quality problems (e.g., Kutka and Richards, 1996; Mattila and Räisänen, 1998; Rott et al., 1998; Hill et al., 2000; Winter and Duthie, 2000a; Munn et al., 2002; Potapova and Charles, 2003). Periphytic communities provide an integrated measurement of water quality as experienced by the aquatic biota and have many biological attributes that make them ideal organisms for biological monitoring. Algae lie at the base of aquatic food webs and therefore occupy a pivotal position at the interface between biological communities and their physico-chemical environment (Lowe and Pan, 1996). Furthermore, benthic algae have short life cycles and can therefore be expected to respond quickly to changes in the environment (McCormick and Stevenson, 1998). However, few studies to date have examined the potential for algal bio-monitoring across a gradient of agriculturally impacted streams.

The present study was undertaken to evaluate the application of periphyton bio-monitoring to enriched streams within agricultural landscapes as a tool to assess water quality. We hypothesised that periphytic algal community structure would be strongly influenced by the presence, intensity and type of farming activity in the surrounding watershed. We evaluated this hypothesis by examining the colonisation of ceramic substrates incubated in 29 streams and rivers in southern Québec, Canada, across a gradient of agricultural impacts. By applying multivariate analysis to the resultant patterns of benthic algal community structure, we identified the potential controlling variables and relationships with farming activities. As secondary objectives, we evaluated to what extent the community biomass and structure on our artificial substrates represented natural communities and whether a total algal community analysis provided additional bio-monitoring information beyond that provided by an analysis restricted to diatoms.

Materials and methods

Study sites

The substrate comparison was carried out in the Boyer River (watershed area, 217 km^2) situated on the south shore of the St. Lawrence River, Québec (site 1 in Figure 1). The Boyer River discharges into the St. Lawrence approximately 30 km east of Québec City. The land use in the watershed is 60% farmland and 40% broadleaf-conifer forest. Our sampling site was within a 10 meter section of the river just downstream of small riffles. The stream bed was mostly gravel and rocks with some sandy areas.

The main part of the study was conducted at 29 sites in southern Québec (Figure 1). While the objective of the study was to evaluate the diatom community structure across a gradient of agriculturally impacted sites, four unimpacted sites were also sampled in order to have information at the lower boundary of the enrichment gradient. The sites were chosen from a network of approximately 400 sites that have been routinely monitored for water quality for more than 20 years by the Québec Ministry of the Environment (MENV) (Painchaud, 1997). We selected the sites according to the availability of physico-chemical data and on the basis of land use information with the aim of sampling across a gradient of farm types and intensities.

Physico-chemical measurements

Water samples were taken from the 29 sites at weekly intervals from mid-July to mid-August 1999 and were analysed by the MENV for the following variables: pH, conductivity, temperature, suspended solids (SS), turbidity (TUR), dissolved total-N (TN), ammonium ($NH_4{}^+$-N), nitrate (NO_3-N), total phosphorus (TP), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC). The P and N variables were analysed by standard colorimetric assays using a Technicon Autoanalyzer. Total nitrogen (TN) was analysed after Kjeldahl digestion and TP after acid digestion at 550°C. Conductivity and pH were measured with appropriate meters in the laboratory within several hours of collection. Turbidity was measured by nephelometry, SS were measured by dry weight analysis and temperature was measured on site. The methods for all analyses and detection limits are given in Hébert (1999). Land use information upstream of each site was provided by the MENV and included: population in 1996 (pop. 96), municipal area in hectare (M.A.), % cropped area (% C.A.), % corn

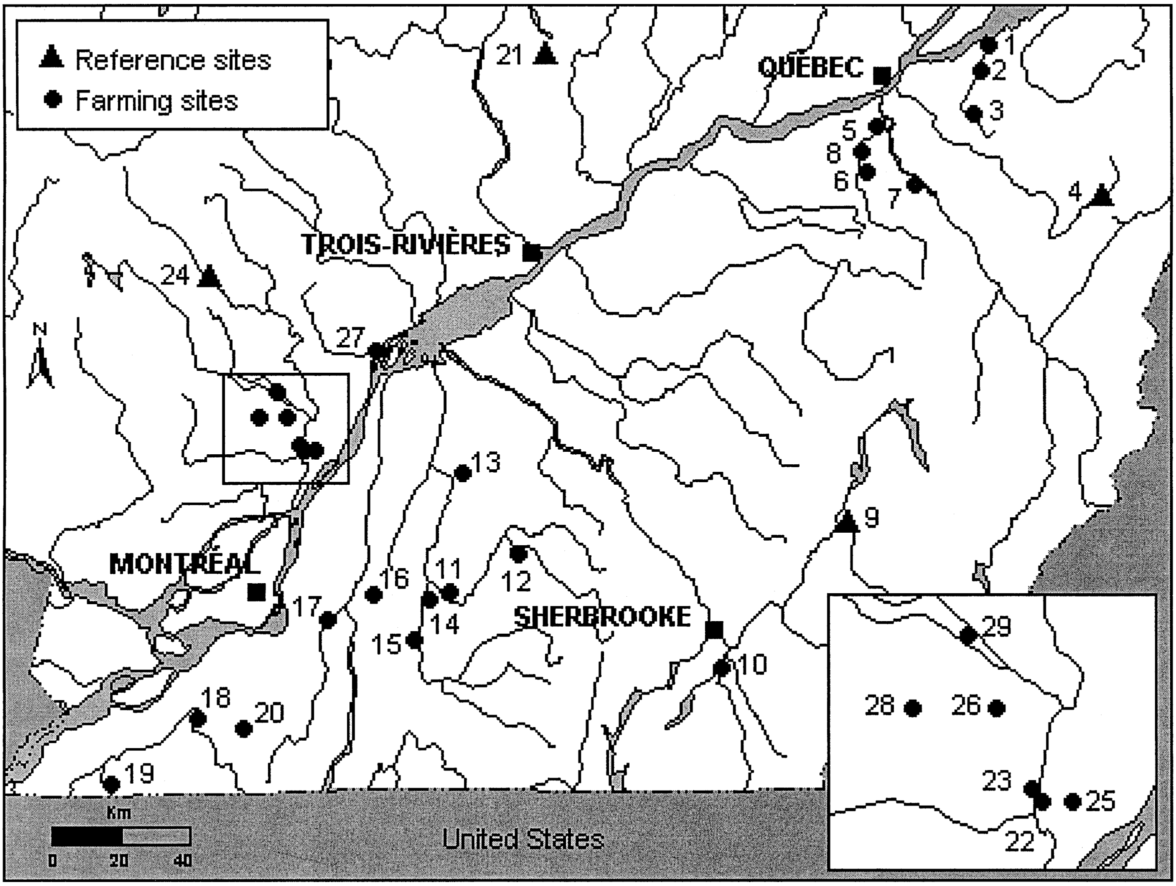


Figure 1. Distribution of sites analysed in the present study. Key to sites (*indicates unimpacted reference sites): 1 = Boyer River; 2 = Du Portage Stream; 3 = Honfleur Stream; 4 = *Etchemin River; 5 = Beaurivage River; 6 = Bras d'Henri River; 7 = Des Iles-Brulées River; 8 = Bélair River; 9 = *Au Saumon River; 10 = Coaticook River; 11 = Noire River; 12 = Runnels Stream; 13 = Chibouet River; 14 = A la Barbue River; 15 = Du Sud-Ouest River; 16 = Des Hurons River; 17 = L'Acadie River; 18 = Des Anglais River; 19 = Châteauguay River; 20 = Norton Stream; 21 = *Des Envies River; 22 = De l'Achigan River; 23 = Saint-Esprit River; 24 = *L'Assomption River; 25 = Point du Jour Stream; 26 = Vacher Stream; 27 = Bayonne River; 28 = Saint-Esprit Stream; 29 = Desrochers Stream. The substrate comparison was conducted at site 1.

crop (% C.C.), % forage (% F.), % row crops (% R.C.), % small grains (% S.G.), animal density in animal units per hectare (A.D.), % beef cattle (% B.C.), % hog (% H.) and % poultry (% P.).

Artificial substrates

The substrates selected for this study were grey, non-glazed ceramic tiles of 23 cm^2, fixed to concrete blocks with plastic-coated wire. They provided a homogeneous, near-natural surface for colonisation. Nine ceramic tiles were fixed on each concrete block in order to have triplicate samples for each type of analysis (chlorophyll a (Chl *a*), ash-free-dry-weight (AFDW), and taxonomic analysis). The blocks were placed in the stream bed in unshaded areas where water was flowing with the ceramic tiles oriented horizontally. Excavation was necessary at some sites to insure a minimum of water above each substrate.

For the experiment on artificial substrates in the Boyer River, we sampled periphytic algae on natural rocks, sterile substrates and artificial substrates to evaluate the temporal evolution of biomass, assessed as AFDW and Chl *a*, and diatom community succession on different substrate types. The sterile substrates were natural rocks taken from the adjacent field and placed on the river bed. The periphytic community on the substrates was scraped every two weeks from May 27 to August 8, 1999 using a template

($13\ cm^2$), blade and brush. Known areas of $13\ cm^2$ were scraped from three separate tiles, sterile rocks or natural rocks for each analysis (Chl *a*, AFDW and taxonomy).

Biomass analysis and community structure on different substrates

Samples for Chl a and AFDW analyses were filtered on to GF/C glass fiber filters and additional samples were preserved with a solution of 10% paraformaldehyde and glutaraldehyde (Lovejoy et al., 1993) for taxonomic analysis. Chlorophyll *a* was extracted in 95% ethanol at 60°C (Nusch, 1980) and quantified by spectrophotometry at 480, 663 and 750 nm. Samples were then acidified for phaeophytin correction. Pigment concentrations were calculated using Goltermann's (1971) equation. Ash-free-dry-weight was determined by drying the samples for 24 h at 80°C followed by combustion in a muffle furnace at 500°C for 2 h (see review by Aloi, 1990).

Samples for diatom analysis were cleaned using a mixture of 1:1 sulphuric and nitric acid and mounted on slides with Naphrax (Pienitz et al., 1995). Diatoms were then identified and counted with a Zeiss Axiovert 10 inverted microscope at 1000× magnification. A minimum of 400 valves were enumerated for each sample (Prygiel and Coste, 1993). Diatom identifications were based mainly on Krammer and Lange-Bertalot (1986, 1988, 1991a, b).

Analysis of variance (ANOVA; SIGMASTAT version 2.03) was used to assess differences in periphytic biomass between the three types of substrates studied in the Boyer River from May to August 1999. Data were tested for deviations from normality and homogeneity of variance, and transformations were made if necessary to fulfil the assumptions for ANOVA.

Effects of agricultural development

For the main study, artificial substrates were scraped for biomass and taxonomic analyses after a 4 wk incubation (mid-July to mid-August 1999). Chlorophyll *a*, AFDW and diatom community structure were analysed following the above methods. The total algal community structure (diatoms and non-diatom taxa) was also analysed in order to evaluate if this broader analysis of all algal components would add information beyond that provided by the observations on the diatom community. The overall benthic algal community was analysed by FNU microscopy (Lovejoy et al., 1993) and by calculating the biovolume (Kirschtel, 1993; Hillebrand et al., 1999) of each taxon. Non-diatom algae identifications were based mainly on Smith (1950), Bourrelly (1966a, 1966b, 1970), Prescott (1970) and Findlay and Kling (1979a, b).

Multivariate statistical analyses for the evaluation of benthic algal community structure at each site were conducted using CANOCO version 4.0 (ter Braak and Ŝmilauer, 1998). Data were tested for deviations from normality and transformations were made if necessary. Diatom species were included in ordinations if they made up >1% in at least 2 sites. Taxa for the overall benthic community were included in the analyses if the biovolume was >1% in at least one site.

Detrended correspondence analysis (DCA) was first used to determine the maximum amount of variation in the diatom species data and the overall benthic algal data. The results (3.0 SD and 4.1 SD respectively for the first axis) suggested that a test based on a unimodal response model was most appropriate. Canonical correspondence analysis (CCA) was therefore used to observe relationships between diatom community structure and water quality variables. All diatom taxa were square-root transformed in order to reduce the influence of the most abundant species, whereas rare species were downweighted. Environmental variables with variance inflation factors >5 (as in Winter and Duthie, 2000a) were not used in the analysis because of their multicolinearity. A forward selection (based on *t*-tests) was then conducted to identify the variables that each explained significant directions of variance in the distribution of the taxa. The statistical significance of the relationship between algal taxa and environmental variables was evaluated using Monte Carlo permutation tests (199 random permutations; $p < 0.05$).

Results

Substrate comparison

Periphyton biomass measured as Chl *a* and AFDW fluctuated greatly during the sampling season, ranging from $0.77\ \mu g\ cm^{-2}$ to $26\ \mu g\ cm^{-2}$ Chl *a* and from 3 to $79\ g\ m^{-2}$ AFDW on all substrates (Figure 2). Two-way ANOVA of Chl *a* and AFDW showed a highly significant influence of the sampling date on biomass variation (Chl *a*: $F_{(5,36)} = 151.42$, $p < 0.001$ and AFDW: $F_{(5,36)} = 98.67$, $p < 0.001$) and showed that there were no significant differences between the three types of substrates (Chl *a*: $F_{(2,36)} = 2.08$, $p = 0.14$ and AFDW: $F_{(2,36)} = 1.32$, $p = 0.28$). However, the interaction term was significant (Chl *a*: $F_{(10,36)} = 6.52$, $p < 0.001$ and AFDW: $F_{(10,36)} = 3.04$, $p = 0.007$), indicating that

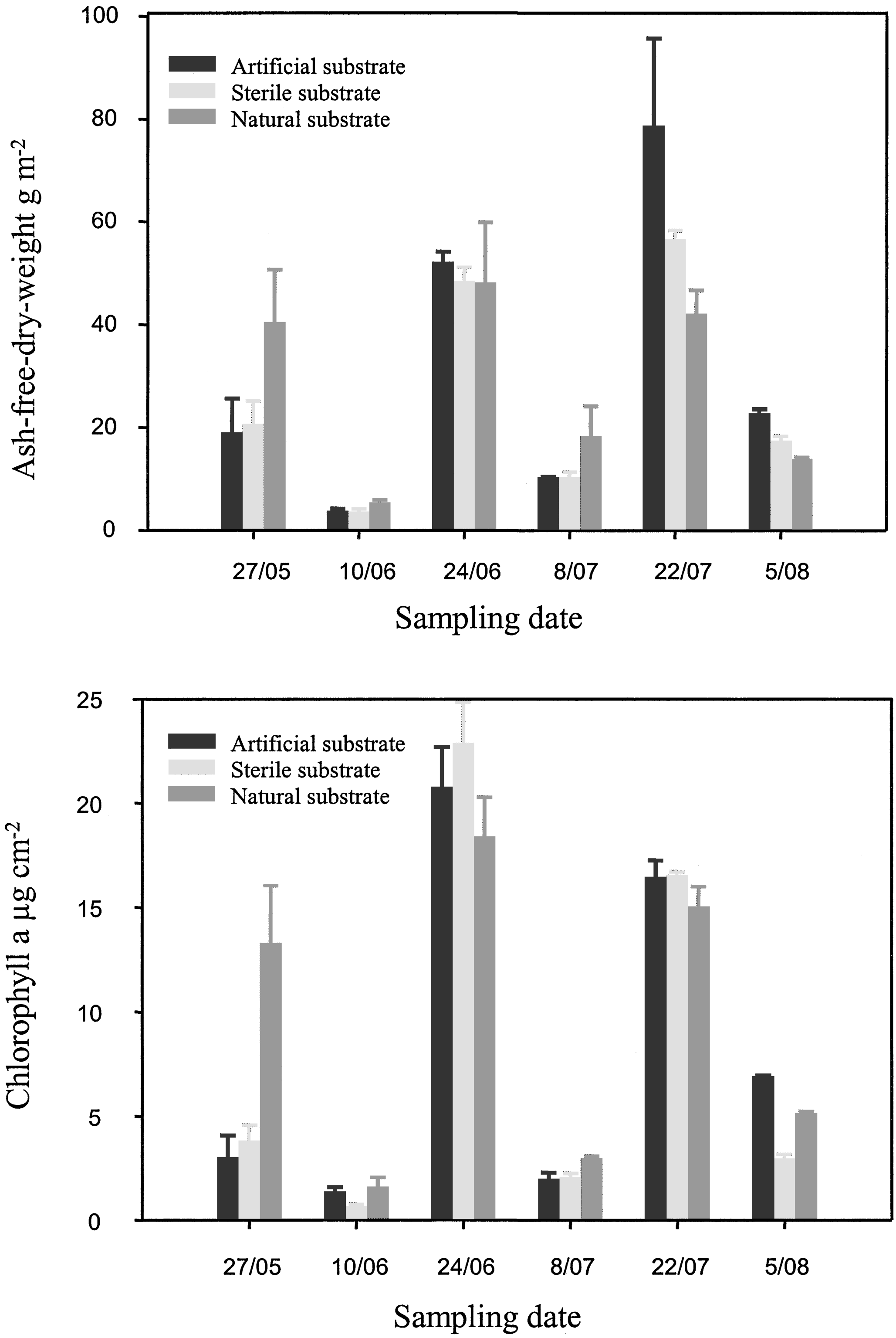


Figure 2. Periphytic biomass expressed as ash-free-dry-weight (upper graph) and Chl *a* (lower graph) on natural, sterile and artificial substrates in the Boyer River, 1999.

substrate type did influence the strength of the temporal variation. Some data did not respect normality after being transformed. However, as noted by Scheffé (1959) and Montgomery (2001), ANOVAs are relatively insensitive to moderate deviations from normality and this deviation is unlikely to affect the major effects observed here. Previous studies on lake epiphytic algae have shown that 5 to 6 independent replicates may be necessary for periphyton biomass estimation to address certain questions (Cattaneo et al., 1993). Our analysis of triplicate variability in the present study showed that the coefficients of variation for natural, sterile and artificial substrates were 21%, 17% and 23%, respectively, for Chl *a* analysis and 30%, 17% and 23%, respectively, for AFDW, giving an adequate degree of resolution for enrichment effects.

Diatom community structure also fluctuated markedly throughout the course of the 3 mo of sampling (Lavoie et al., 2003). The ANOVA conducted on diatom community structure (percent total number of valves for the six dominant species) showed the major influence of sampling date and the minor influence of substrate type. Different treatments explained, on average, less than 2% of the total variance while the contribution of sampling date averaged more than 42% of the total variance (Table 1). Log 10 or $\sqrt{}$arcsin transformations were necessary in order to respect normality.

Land use analyses

Mean values for the physico-chemical variables at each site are shown in Table 2 and land use information is shown in Table 3. Conductivity, TN, NH_4^+-N, NO_3^--N, TP, TDP, SRP, pH, SS and turbidity were all markedly and significantly lower at the reference sites (Table 4). Total phosphorus and TN values ranged from 0.02 to 0.53 mg l^{-1} and from 0.21 to 4.75 mg l^{-1} respectively. The mean TP was 0.02 mg l^{-1} (at the detection limit) for the reference sites and 0.19 mg l^{-1} for the agricultural sites and the mean TN was 0.275 mg l^{-1} for the reference sites and 1.56 mg l^{-1} for the agricultural sites. The waters were typically alkaline with pH values up to 8.7 and conductivity ranging from 24.6 to 1120 μS cm^{-1}. A Pearson correlation matrix showed that there were only a few significant relationships between land use and water quality, notably conductivity (Table 5). All forms of P were highly correlated with conductivity. Total nitrogen and $NH_4{}^+$-N were also correlated with conductivity. Animal density was positively correlated with conductivity, TN, $NH_4{}^+$-N, TP, suspended solids and turbidity while % beef cattle, % hog and % poultry had no significant relationship with physico-chemical variables. Percent cropped area, % row crop, % small grains and % corn crop were positively correlated with nutrients and conductivity.

Table 1. Summary of ANOVA statistics for the evaluation of substrate and date of sampling effect in the Boyer River.

Taxa	Substrate Effect	Sampling Date Effect	Interaction Term
Cymbella sinuata	$F = 0.5$	$F = 38.6$	$F = 3.07$
	$p = 0.63$	$p < 0.001$	$p = 0.006$
	0.4% of total variance	74% of total variance	Interaction
Nitzschia spp.	$F = 2.3$	$F = 76.6$	$F = 3.27$
	$p = 0.12$	$p < 0.001$	$p = 0.004$
	0.99% of total variance	84% of total variance	Interaction
Navicula seminulum	$F = 0.4$	$F = 22.9$	$F = 1.47$
	$p = 0.65$	$p < 0.001$	$p = 0.191$
	0.5% of total variance	69% of total variance	No interaction
Navicula cryptocephala	$F = 2.1$	$F = 34.6$	$F = 1.76$
	$p = 0.14$	$p < 0.001$	$p = 0.104$
	1.8% of total variance	75% of total variance	No interaction
Navicula saprophila	$F = 17.4$	$F = 76.0$	$F = 2.02$
	$p < 0.001$	$p < 0.001$	$p = 0.06$
	7.4% of total variance	81% of total variance	No interaction
Navicula subminuscula	$F = 3.09$	$F = 8.88$	$F = 1.92$
	$p = 0.06$	$p < 0.001$	$p = 0.075$
	5.8% of total variance	42% of total variance	No interaction

Table 2. Mean physico-chemical values and mean Chl *a* and AFDW concentrations at the 29 sites during the period of sampling (mid-July to mid-August 1999).

Site	DOC (mg C l^{-1})	COND (μS cm^{-1})	Total-N (mg N l^{-1})	NH_3 (mg N l^{-1})	NO_3-N (mg N l^{-1})	SRP (mg P l^{-1})	Total-P (mg P l^{-1})	TDP (mg P l^{-1})	pH	SS (mg l^{-1})	TEMP (°C)	TUR (NTU)	AFDW (g m^{-2})	Chl *a* (μg cm^{-2})
1	9.2	293.5	1.95	0.04	1.67	0.07	0.15	0.09	8.1	39.5	20.1	23.7	25.7	3.2
2	12.3	235.0	1.16	0.06	0.85	0.07	0.15	0.11	8.2	14.8	18.2	7.3	3.6	3.5
3	8.7	304.0	3.04	0.02	2.85	0.08	0.13	0.10	8.2	8.3	17.3	3.8	33.6	8.8
4*	6.3	40.9	0.36	0.02	0.14	0.01	0.02	0.01	7.2	2.8	15.0	0.8	1.1	5.6
5	10.0	217.0	0.52	0.05	0.13	0.02	0.06	0.03	8.2	7.0	21.8	4.9	14.1	2.2
6	10.9	350.2	1.35	0.06	0.95	0.10	0.19	0.14	8.7	9.8	25.5	4.6	16.1	0.2
7	12.4	525.0	1.00	0.04	0.56	0.06	0.14	0.08	8.5	13.3	24.3	4.1	6.8	1.6
8	5.3	480.0	4.75	0.04	4.50	0.28	0.34	0.33	8.5	5.0	15.9	1.6	23.0	36.9
9*	8.8	99.0	0.31	0.02	0.05	0.01	0.02	0.01	7.6	3.2	21.0	1.3	10.1	0.5
10	5.0	252.6	0.61	0.04	0.38	0.01	0.04	0.01	8.1	7.8	23.7	3.0	4.6	0.9
11	11.2	211.0	0.87	0.05	0.39	0.03	0.09	0.06	8.1	5.5	23.0	2.4	23.8	4.0
12	11.1	212.0	0.84	0.07	0.26	0.06	0.13	0.09	8.1	8.3	23.2	4.2	17.1	1.7
13	10.8	682.5	2.12	0.05	1.62	0.13	0.19	0.16	8.4	11.8	26.5	4.8	4.8	0.7
14	8.3	577.5	1.50	0.07	0.99	0.03	0.17	0.05	8.2	45.0	24.3	20.6	23.4	2.8
15	11.3	640.0	1.65	0.08	1.03	0.21	0.30	0.26	8.3	23.0	23.3	12.3	9.8	3.7
16	8.3	1045.0	3.38	0.96	1.68	0.31	0.53	0.38	8.1	33.3	24.0	21.3	3.9	3.6
17	7.9	967.5	0.48	0.05	0.03	0.09	0.14	0.13	8.3	3.5	23.5	2.3	13.7	0.8
18	9.5	401.5	0.51	0.02	0.04	0.14	0.23	0.18	8.0	6.8	24.6	2.5	12.3	2.3
19	4.0	145.5	0.32	0.02	0.13	0.08	0.10	0.09	8.7	8.0	22.1	0.7	28.5	1.6
20	14.0	717.5	0.86	0.12	0.48	0.37	0.51	0.46	8.3	18.0	23.8	10.3	9.0	1.8
21*	6.4	24.6	0.21	0.02	0.02	0.01	0.02	0.01	7.0	1.8	22.4	0.8	9.3	0.6
22	6.1	288.6	0.91	0.10	0.44	0.04	0.10	0.05	8.0	26.3	21.0	14.5	17.0	12.4
23	5.3	380.6	1.00	0.05	0.70	0.03	0.09	0.04	7.9	25.0	19.0	14.7	1.1	0.4
24*	4.8	33.7	0.22	0.02	0.02	0.01	0.02	0.01	7.1	2.0	20.3	0.4	4.6	0.4
25	10.3	535.0	3.00	0.11	2.54	0.03	0.09	0.04	7.8	31.0	20.3	23.5	28.9	10.6
26	4.4	725.0	4.20	0.74	2.56	0.25	0.36	0.29	7.8	16.5	18.5	5.4	27.6	8.0
27	5.7	1120.0	1.34	0.05	1.03	0.09	0.20	0.12	8.1	21.8	19.3	9.0	12.3	2.3
28	3.8	477.5	1.06	0.03	0.80	0.04	0.07	0.05	8.0	4.8	18.7	2.0	16.4	5.2
29	5.1	550.0	0.55	0.03	0.17	0.14	0.16	0.16	8.2	4.4	19.4	2.1	8.3	3.2

*Reference sites.

Table 3. Land use information for the catchments upstream of each sampling site.

Site	Pop. 1996	M.A. (ha)	C.A. %	R.C. %	S.G. %	C.C. %	F. %	A.D. a.u. ha^{-1}	B.C. %	H. %	P. %
1	6550	21049	52	5	11	4	35	1.58	54	41	4
2	454	2089	43	2	11	1	29	1.23	72	22	4
3	415	2410	67	7	16	6	44	1.98	43	53	2
4*	426	9974	2	0	0	0	1	1.19	57	39	2
5	15500	70029	26	3	3	3	20	3.06	36	59	4
6	2969	15717	38	7	3	7	27	4.57	27	67	6
7	504	2147	66	21	4	21	41	5.56	27	61	12
8	432	3771	23	1	2	1	20	2.48	41	51	8
9*	3450	99605	4	0	1	0	3	1.10	83	9	0
10	11535	34646	37	7	4	6	25	1.23	79	18	0
11	43213	144923	35	14	3	12	18	2.05	34	58	6
12	1591	10856	32	13	2	11	17	2.79	25	70	4
13	3015	16313	66	41	8	34	17	1.80	27	60	12
14	4252	12861	60	39	5	30	13	3.48	10	71	18
15	2587	8588	67	44	7	35	16	1.63	57	39	3
16	18602	26387	64	44	6	29	12	0.75	48	42	7
17	21423	36583	71	55	5	35	11	0.32	71	11	14
18	10112	51244	40	24	3	12	12	0.58	83	7	7
19	58	425	42	17	3	11	21	0.77	83	6	3
20	4969	21632	40	27	3	9	9	0.44	74	5	15
21*	821	6227	11	0	3	0	7	0.67	90	0	5
22	40993	63673	22	13	3	7	6	1.34	21	66	9
23	9837	21917	48	28	5	19	14	1.21	27	62	8
24*	436	66694	0	0	0	0	0	1.34	43	16	33
25	6676	7055	38	18	8	7	11	0.46	73	13	9
26	2997	2481	69	49	6	32	14	0.66	39	45	4
27	12804	35851	38	16	6	11	15	1.76	29	15	55
28	795	2699	83	47	10	32	25	0.87	37	58	4
29	529	1721	71	47	6	29	17	0.69	38	48	4

*Reference sites. Pop = total human resident population in the catchment in 1996; (M.A.) = municipal area in hectare; (% C.A.) = % cropped area; (% C.C.) = % corn crop; (% F.) = % forage; (% R.C.) = % row crops; (% S.G.) = % small grains; (A.D.) = animal density in animal units per hectare; (% B.C.) = % beef cattle; (% H.) = % hog and (% P.) = % poultry.

Benthic algal colonisation varied markedly from site to site, with biomass ranging from 1.1 g m^{-2} to 33.6 g m^{-2} AFDW and from 0.2 μg cm^{-2} to 36.9 μg cm^{-2} Chl *a*. The agriculturally impacted sites had a mean of 15.4 g m^{-2} AFDW (SD = 9.3) and 4.9 μg cm^{-2} Chl *a* (SD = 7.4) compared with 6.3 g m^{-2} AFDW (SD = 4.2) and 1.8 μg cm^{-2} Chl *a* (SD = 2.5) for the reference sites. Chl *a* was correlated with temperature, NO_3-N, TN and % cropped area ($r = -0.515$, $p < 0.01$; $r = 0.765$, $p < 0.005$; $r = 0.684$, $p < 0.005$ and $r = 0.667$, $p < 0.005$, respectively). Ash free dry weight was correlated with NO_3-N ($r = 0.495$, $p < 0.01$).

Community composition was also very different among the sampling sites, with diatom biovolume ranging from 5% to 98% (mean = 55.6%; SD = 30.6) of the total benthic algal community. The most abundant non-diatom taxa expressed as biovolume were *Scenedesmus* spp., cf. *Serratus* sp., filamentous chlorophytes and a pigmented 5 μm flagellate. In terms of cell concentrations, the cyanobacterium *Leptolyngbya* was abundant. The most abundant species of diatoms at the agriculturally influenced sites were *Cocconeis placentula*, *Cocconeis pediculus, Cyclotella meneghiniana*, *Navicula cryptocephala*, *Navicula lanceolata* and *Surirella brebissonii*. At the

Table 4. Water quality conditions at the reference (unimpacted) and agricultural sites. Nutrient values are in mg l^{-1}, conductivity in $\mu S\ cm^{-1}$ and suspended sediments in mg l^{-1}. The significance of differences between the two types of sites was determined by Mann-Whitney Rank Sum Test.

	Unimpacted Sites		Agricultural Sites		Rank Test
	Mean	Range	Mean	Range	p-value
Conductivity	49.55	74.4	493.36	974.5	***
pH	7.23	0.6	8.19	0.9	***
Suspended solids	2.45	1.4	15.94	41.5	***
Total-N	0.275	0.15	1.559	4.43	***
NO_3^--N	0.058	0.12	1.071	4.47	**
NH_4^+-N	0.02*	0.0	0.118	0.94	**
Total-P	0.02*	0.0	0.186	0.49	***
SRP	0.01*	0.0	0.11	0.36	***

*detection limit, **$p < 0.01$, ***$p < 0.005$.

reference sites, *Achnanthes minutissima* sp.1, *Fragilaria capucina* and *Brachysira neoexilis* were more common. Deleting rare species reduced the number in the subsequent multivariate analyses from 171 to 61 for diatom data and from 151 to 93 for the overall community data. All samples were used in the CCAs. A list of species seen in this study may be obtained by application to I. Lavoie.

The first CCA analysis was performed using only water quality data. The CCA identified pH, conductivity and suspended solids as variables that each explained significant ($p < 0.05$) and independent directions of variance in the diatom data. The eigenvalues of CCA axis 1 (0.48) and axis 2 (0.23) were similar to those for DCA (0.57 and 0.27), indicating that the physico-chemical variables used accounted for most of the diatom species variance. The first two axes for environmental variable ordination explained 82.7% of the variance in diatom community structure, indicating that pH, conductivity, and suspended solids accounted for the major gradients in the diatom community structure. The cumulative percentage of variance in species distribution was 28%. These values of variance explained by environmental variables or species distribution are slightly higher than the range commonly found in the literature (e.g., Fallu and Pienitz, 1999; Winter and Duthie, 2000a). The first axis was highly correlated with pH, indicating that this variable is important for site and species distribution (Figure 3). Site and species distribution showed a clear separation between the four reference sites and the rest of the agriculturally impacted sites.

Another CCA analysis was performed to include all water quality variables as well as land use data for each sampling site. Adding land use characteristics did not increase the percentage of variance explained and pH, conductivity, and suspended solids were still the only variables that each explained significant ($p < 0.05$) and independent directions of variance in the diatom data (not shown).

Since reference sites and farming sites were principally separated by the pH gradient, we conducted another CCA without the reference sites in an attempt to obtain a better distribution among the farming sites as a function of land use and water quality. The only significant variable that remained in the ordination was suspended solids. The cumulative percentage of variance in species distribution was 8.7% (not shown). The site ordination excluding the reference sites showed a more even distribution, but grouping as a function of agriculture type or intensity was still not evident.

The results obtained by conducting a CCA on the overall benthic algal community were similar to those obtained for the benthic diatom data. The four reference sites were clearly separated from the agriculturally impacted sites and no grouping as a function of farming type was observed (Figure 4). The only variable that explained significant variance ($p < 0.05$) in the data was conductivity. The variance explained by the taxa distribution was 6%.

Discussion

Substrate comparison

Previous studies using artificial substrates for periphyton colonisation have led to divergent views on their

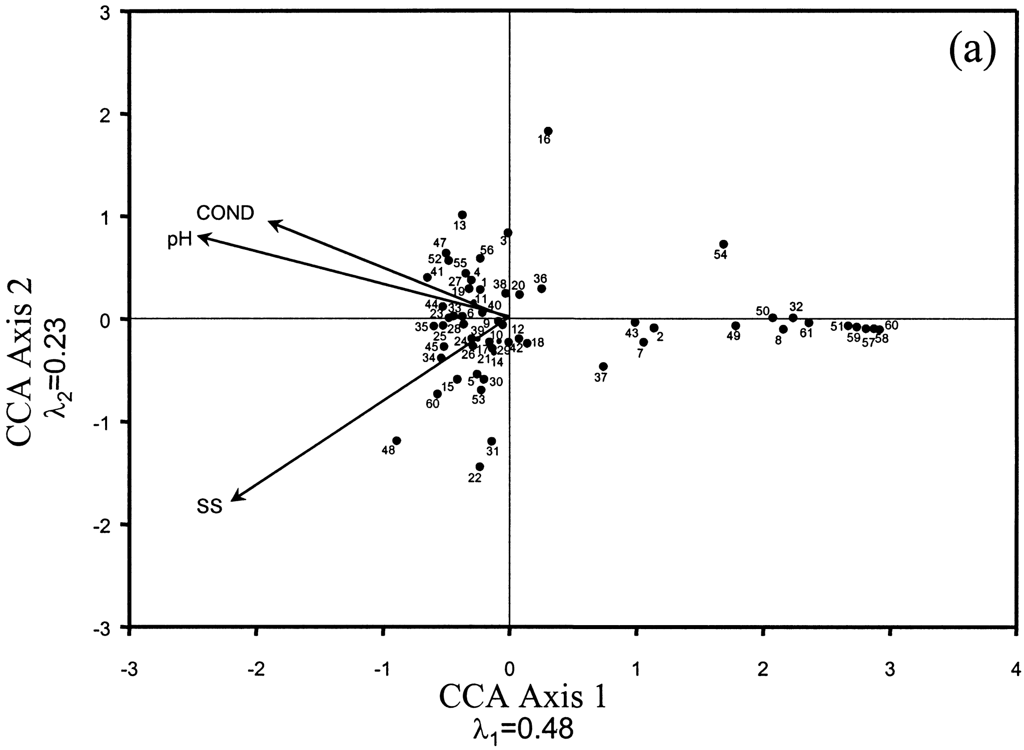
Table 5. Pearson correlation matrix for the relationships among physico-chemical variables and land use data. See Table 3 for the definition of abbreviations.

	Pop.	M.A.	A.D.	% C.A.	% R.C.	% S.G.	% C.C.	% F.	% B.C.
Pop.	1								
M.A.	0.704***	1							
A.D.	0.331	−0.11	1						
% C.A.	−0.073	−0.425*	0.445*	1					
% R.C.	0.056	−0.254	0.542***	0.812***	1				
% S.G.	−0.176	−0.421*	0.277	0.667***	0.252	1			
% C.C.	0.031	−0.251	0.452*	0.834***	0.965***	0.262	1		
% F.	−0.199	−0.327	−0.08	0.515***	−0.068	0.653***	0.038	1	
% B.C.	−0.177	−0.002	−0.155	−0.267	−0.209	−0.066	−0.326	−0.195	1
% H.	0.155	−0.051	0.056	0.313	0.162	0.137	0.302	0.348	−0.883***
% P.	0.086	0.117	0.03	−0.1	0.033	−0.118	0.016	−0.233	−0.287
DOC	0.067	0.126	−0.133	0.219	−0.074	0.105	−0.047	0.238	−0.001
COND	0.090	−0.278	0.596***	0.211	0.83***	0.264	0.774***	0.034	−0.137
Total-N	−0.108	−0.341	0.521***	0.339	0.196	0.388	0.194	0.245	−0.227
NH_3	0.162	−0.073	0.638***	0.328	0.406	0.067	0.375*	−0.136	−0.128
NO_3-N	−0.191	−0.365	0.35	0.349	0.032	0.442*	0.039	0.342	−0.201
SRP	−0.177	−0.333	0.254	0.274	0.33	0.075	0.249	0.081	0.063
Total-P	−0.019	−0.261	0.41*	0.11	0.484**	0.177	0.401*	0.041	−0.113
TDP	−0.088	−0.227	0.217	0.392*	0.452*	0.058	0.379*	0.007	0.026
pH	0.054	−0.202	0.044	0.148	0.29	0.262	0.334	0.592***	−0.254
SS	0.159	−0.173	0.462*	0.098	0.244	0.332	0.233	0.059	−0.299
TEMP	0.233	0.206	−0.03	0.962***	0.273	−0.195	0.326	−0.018	−0.045
TUR	0.209	−0.117	0.484**	0.147	0.184	0.344	0.144	0.001	−0.223

	% H.	% P.	DOC	COND	Total-N	NH_3	NO_3-N	RSP	Total-P
% H.	1								
% P.	−0.018	1							
DOC	0.64	−0.015	1						
COND	0.121	0.2	0.173	1					
Total-N	0.259	−0.079	−0.036	0.491**	1				
NH3	0.102	−0.017	−0.018	0.557**	0.546	1			
NO_3-N	0.237	−0.066	−0.044	0.334	0.958***	0.301	1		
RSP	−0.089	0.012	0.22	0.611***	0.573***	0.535***	0.47**	1	
Total-P	0.68	0.065	0.273	0.717***	0.582***	0.664***	0.435*	0.898***	1
TDP	−0.048	0.01	0.301	0.674***	0.491**	0.537***	0.366	0.945***	0.93***
pH	0.329	−0.086	0.329	0.434*	0.238	0.006	0.252	0.387*	0.414*
SS	0.198	0.255	0.186	0.359	0.34	0.365	0.277	0.142	0.43*
TEMP	−0.045	0.25	0.46*	0.292	−0.282	0.11	−0.373*	0.045	0.159
TUR	0.105	0.296	0.211	0.339	0.328	0.349	0.274	0.11	0.392*

	TDP	pH	SS	TEMP	TUR
TDP	1				
pH	0.397*	1			
SS	0.193	0.175	1		
TEMP	0.193	0.353	0.181	1	
TUR	0.148	0.098	0.962***	0.147	1

*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.



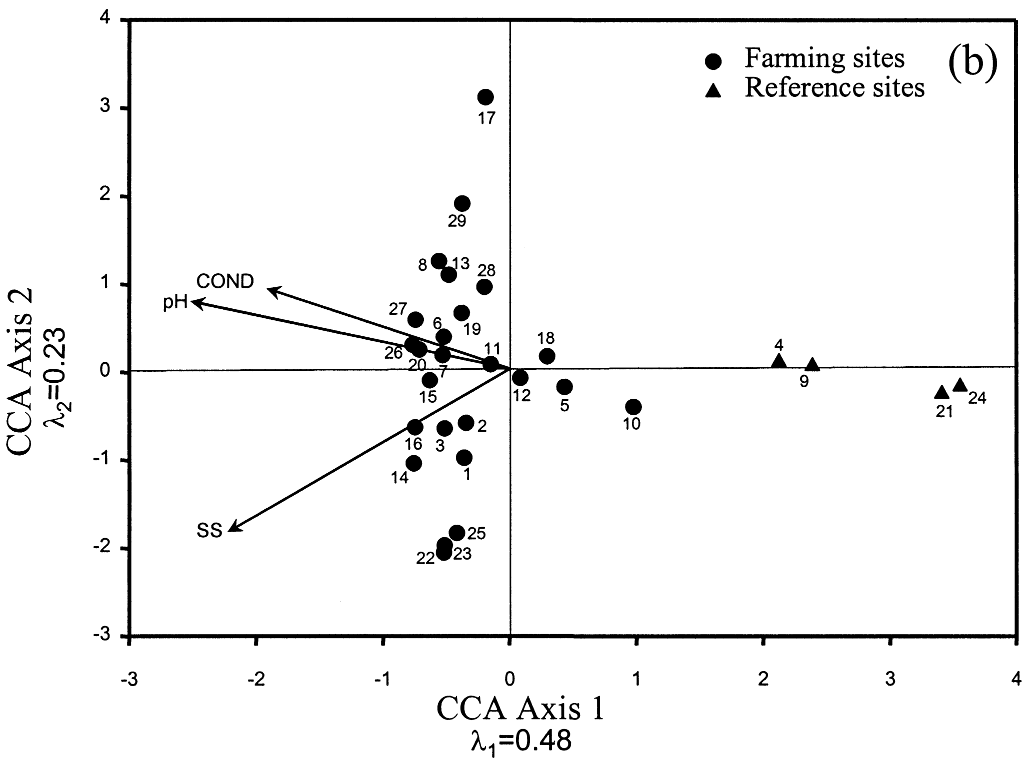


Figure 3. Canonical correspondence analysis biplots showing diatom species scores (a) and sample scores (b) as well as significant ($p < 0.05$) and independent (variance inflation factor <5) environmental variables.

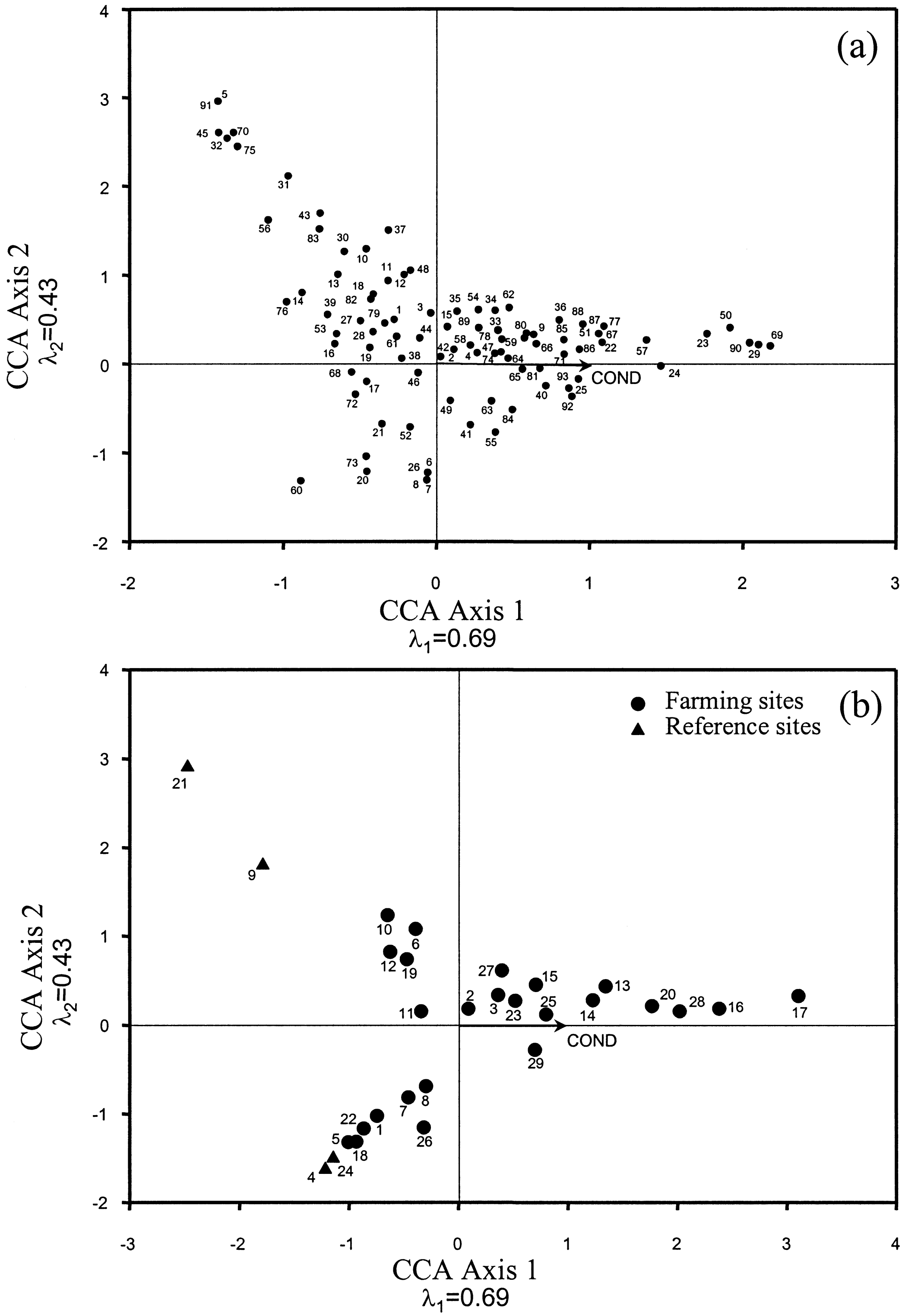


Figure 4. Canonical correspondence analysis biplots showing the overall taxa scores (a) and sample scores (b) as well as significant ($p < 0.05$) and independent (variance inflation factor <5) environmental variables.

ability to reproduce natural conditions. Tuchman and Stevenson (1980) found that sterilised rocks and clay tiles represented the natural community poorly and in a comparative study of lakes of differing trophic status, Ellis et al. (2001) found that the nature of the substrate (glass, wood, plastic) considerably affected the patterns of colonisation of periphytic algae. Similarly, Leland and Porter (2000) and Hill et al. (2000) have shown the influence of natural substrate type on benthic algae assemblages. These results contrast with a review on the use of artificial substrates for benthic algal studies which suggests that choice of material is not crucial and that any substrate-induced variations are less important than those introduced by trophy, temperature and the time available for colonisation (Cattaneo and Amireault, 1992). Eulin and Le Cohu (1998) compared periphytic algae on natural and artificial (mica schist) substrates and also concluded that the specific composition of communities did not show significant differences between the two substrates studied.

The experiment in the Boyer River showed that the type of ceramic tiles chosen for the present study (unglazed with good surface rugosity) provided a reasonable analogue to rocky substrates. Since the purpose of the experiment was to evaluate the use of algae as an indicator of farming activity, the use of artificial substrates eliminated any potential effect of different surfaces or substrate geochemistry. These effects, however, may be small relative to those associated with differences in water quality. The use of artificial substrates substantially increased the logistic difficulties of sampling and it may be preferable to select sites where there are natural rocky substrates for sampling.

Land use effects on benthic algal biomass and community structure

Benthic algal Chl *a* was correlated with TN, NO_3-N and temperature while AFDW was correlated with NO_3-N. However, the correlation analyses suggest that the periphyton community structure was primarily influenced by pH, conductivity and suspended solids. This result is consistent with that of Mosisch et al. (1999) who found that periphyton biomass accrual under unshaded conditions was N-limited (most agriculturally impacted sites in this study were unshaded). In our experiment, benthic algal biomass was uncorrelated with P, again consistent with the study of Mosisch et al. (2001) who found that P-enrichment appeared to have no positive effect on periphyton accrual. The lack of any biomass-phosphorus relationship suggests that P is not a limiting element for benthic algal growth in the streams and rivers sampled in this study and that the ecological impact of P- and N-loading from agricultural sources is very different. Phosphorus-loading results in higher biomass of planktonic algae in fresh water (Correll, 1998), while our data suggest a greater influence of N-loading (or a correlate of TN and nitrate) on benthic agal biomass. This would imply that excess N cycles mostly through the benthic foodweb, while excess P may have a greater influence on the plankton. The ecological impact of N- and P-loading is also likely to differ in terms of spatial scale, since excess production of planktonic algae will be exported further and faster in lotic ecosystems than excess production of benthic algae. Thus, the impact of N- versus P-loading on the structure and function of freshwater ecosystems will differ markedly. It is also possible that the turnover rates for N and P differ substantially, with much faster recycling rates (shorter nutrient spiral length sensu Wetzel, 2002) for P. This suggests that analyses of N rather than P would provide a more accurate guide to the overall nutrient status of the stream.

The results obtained from CCA showed that pH, conductivity and suspended solids were the most significant environmental variables explaining species composition and the ordination of sites. Canonical correspondence analyses clearly separated reference sites from the overall farming sites indicating that the specific composition of diatoms and total algal community responded strongly to these two disparate sets of conditions. Diatom community structure in farming sites and reference sites was principally distributed along the pH gradient. Excluding the reference sites from the CCA analysis showed that pH was no longer a significant variable and that suspended solids was the most important variable explaining the farming site distribution. The pH values in agriculturally influenced streams had a tendency to be higher, possibly related to farming practices but also to the soil type in which agricultural activities are localised.

Our more detailed studies on the overall benthic algal community added substantially to the total analysis time due to difficulties in identifying all algal groups and differentiating viable diatom frustules, that is, those with cellular content. It did not, however, add any significant information beyond our analyses restricted to the total diatom community. The large additional effort required for a full community analysis does not therefore seem justified in future work, except where there are specific water quality issues such as unsightly *Cladophora* growth or geosmin production by benthic cyanobacteria that can taint water supplies.

Other studies have shown the importance of conductivity (Biggs, 1990; Leland and Porter, 2000; Munn et al., 2002) and pH (Pan et al., 1996) for algal community composition in streams and rivers. The results of Hill et al. (2000) provide another example where N and P were not significant environmental variables for evaluating the use of periphyton assemblage data as an index of biotic integrity. As hypothesised by Pan et al. (1996), regression and calibration models based on P and diatoms may not be as robust and predictable for P-enriched rivers and streams as they are for lakes. However, reliable models evaluating diatom response to TN and TP have been developed (e.g., Pan et al., 1996; Leland and Porter, 2000; Winter and Duthie, 2000a; Munn et al., 2002). The lack of a relationship between diatom species and N and P observed in this study could also reflect our distribution of sites. We found that almost all our sites clustered together at the highly enriched end of the gradient, and were far separated from the four unimpacted sites. The inclusion of intermediate levels of enrichment would likely have allowed a more sensitive analysis of nutrient effects on diatom community structure.

In our multivariate analyses, traditional nutrient measurements (P and N concentrations) did not explain a significant part of the variance in the species-specific composition among different sites. However, TN, NH_4-N and all forms of P as well as pH were correlated to conductivity (Table 5). An increase in conductivity can be associated with erosion and runoff loaded with major ions. Phosphorus and nitrate are also affected by the extent of runoff and soil erosion. Turbidity (suspended solids) could also be linked to P and nitrate since erosion (responsible for a higher turbidity) leads to a loss in soil and nutrient-rich organic matter. We thus conclude that pH, conductivity and SS measurements were better integrative guides of water quality in these agriculturally impacted ecosystems than specific nutrient variables.

The results showed a clear difference in diatom community structure between the farming and reference sites (Figures 3 and 4). However, contrary to our hypothesis and even with the ordination of sites according to species composition along conductivity and SS gradients, no grouping was observed as a function of farming type or intensity. For example, sites 8 and 28 were very close to each other in the site ordination as a function of diatom community (Figure 3), but land use for those 2 sites was very different (Table 3). Site 8 was characterised by a low percentage of cultivated area (23%) and a high animal density (2.48 a.u. ha^{-1}) while site 28 had a high percentage of cultivated area (83%) and a low animal density (0.9 a.u. ha^{-1}). The dominant crop and livestock production also differed between sites 8 and 28. Moreover, some sites that had similar land use characteristics also had very different diatom community structure, such as sites 19 and 25 (Figure 3 and Table 3). These results suggest that local farming practices such as soil tillage, presence of a buffer zone, ecological agriculture and crop type as well as geological properties of each site have a strong over-riding influence on water quality properties and periphyton community structure. Discharge is another major physical variable (not measured in this study) that might have influenced the community composition of the benthic algae (Biggs et al., 1998a, b, 1999; Lavoie et al., 2003). The rivers varied in size from meters to tens of meters in width. However, despite this variability all of the agricultural sites clustered within a single highly impacted group. This substantial separation of all farming sites from the reference sites draws attention to the strong impact of agriculture in this region irrespective of intensity and farm type. In part this may reflect differences in geology given that agriculture in this region is primarily within regions on sedimentary bedrock and flood plain soils, while our unimpacted sites included two on the Canadian Shield. However, the magnitude of this separation implies that there is a need for substantial improvements in environmental management in the agricultural catchments of this region to achieve any shift in water quality towards natural baseline conditions.

In many European countries, water quality monitoring is routinely and effectively achieved using biological indices based on benthic diatoms. For example, the Czech SLA diatom index was developed by Sládeček (1986) to evaluate saprobity levels (degree of organic enrichment). The French Polluo-Sensitivity-Index (Coste, 1982) and the French Biotic Diatom Index (Lenoir and Coste, 1996) were developed to evaluate general stream water quality. Similarly, the English Trophic Diatom Index (Kelly and Whitton, 1995) and the German trophic index (SHE; Steinberg and Schiefele, 1998) were developed and applied to assess stream trophic status. The present study and other work in Canada also show the potential of diatom communities as an indicator of water quality (this study; Reavie and Smol, 1998; Vis et al., 1998; Winter and Duthie, 2000a, b, c; Winter and Duthie, 2001; Belore et al., 2002; Wunsam et al., 2002). However, there are no quantitative indices currently in use in water quality monitoring programs in Canada. This study suggests that conductivity, pH and suspended solids are major variables that separate community structures across environmental gradients. Further work is required to study the potential of diatoms as biological indicators of water quality on a broader array of impacted streams that are more evenly distributed across gradients of nutrient enrichment, and to develop indices that would be easily integrated within routine water management and monitoring strategies.

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