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ORIGINAL ARTICLE

Influence of Algal Community Structure on Denitrification Rates in Periphyton Cultivated on Artificial Substrata

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Abstract We conducted a field survey of periphyton cultivated on benthic mesh installations in freshwater aquatic systems, including two constructed wetlands and a pond, and also studied periphyton grown on a benthic mesh in laboratory mesocosms. The objectives of this study were to (1) determine if periphyton cultivated on benthic mesh denitrifies at higher rates than the underlying sediments and (2) determine if denitrification rates within periphyton vary with characteristics such as algal and bacterial community structure and biomass. We measured denitrification potential rates of field and laboratory periphyton by the acetylene inhibition method. We characterized algal community composition by algal identification and bacterial community composition by terminal restriction fragment length polymorphisms. Periphyton collected on benthic mesh from our field sites denitrified at significantly higher rates than the underlying sediments, regardless of sampling site or season. Results from both our field survey and laboratory studies indicated a significant, positive correlation between diatom presence and denitrification rate. In our laboratory studies, we found that periphyton with the highest diatom abundance showed the highest denitrification rates as well as a distinct bacterial community composition. These results suggest a synergistic relationship between diatoms and denitrifying bacteria that warrants further study.

Introduction

Wetlands are increasingly recognized as instrumental environments in mitigating nutrient-enriched surface waters, as they are hot spots for denitrification [29, 53]. Traditionally, wetlands research has focused on sediments as the primary site for denitrification [45, 65], identifying maximum denitrification rates in the upper 3–5 cm of wetland sediment [11, 32, 34, 57]. Other research has shown, however, that denitrification activity is not limited to sediments. Significant denitrification rates have also been measured in periphyton (also "biofilms") attached to natural substrata [15, 35] and on artificial substrata [56], including benthic, polyethylene mesh [55]. Sirivedhin and Gray [55] reported that the denitrification potential (DNP) normalized to cell density within periphyton growing on benthic, polyethylene mesh (used to stabilize sediments) in constructed wetlands greatly exceeded that measured in the underlying wetland sediments. Fleming-Singer and Horne [18] reported that denitrification rates in microcosms were enhanced significantly when wetland sediments were overlain by a loose aggregation of plant litter. These results suggest that habitat manipulation of natural systems may enhance DNP because of biotic interactions between denitrifying bacteria and autotrophic microorganisms residing above the sediment surface.

Periphyton is a mixture of algae, fungi, microbes, and organic and inorganic detrital material attached to various substrata in photic zones of aquatic systems [66]. The

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metabolic activity of the microorganisms within these communities increases periphyton thickness and consumes oxygen, thereby, slowing diffusion and creating solute gradients across its depth. Anoxic microzones that result from these processes allow for anaerobic processes such as denitrification [48, 63]. Accrual of periphytic biomass and the establishment of solute gradients may limit the supply of carbon and dissolved inorganic nutrients from the overlying water to deeper strata of biofilms [13, 61]. Thus, heterotrophic bacteria within biofilms depend on autotrophic microorganisms (algae and cyanobacteria) for much of their fixed carbon, supplied either by release of extracellular organic products or by algal decomposition [23, 41, 42]. Bacterial growth and metabolism within photoautotrophic biofilms are sensitive to variation in the activity of algal biofilm residents [16, 24, 52]. It is also probable that the taxonomic structure of bacterial assemblages within biofilms vary with algal species representation, as indicated by observations in non-biofilm systems [17, 22, 54]. The chemical nature of the algal exudates differs among algal species [21, 25, 40], a fact that may be instrumental in dictating the composition of bacterial consortia that exploit such carbon sources [48]. Because bacterial taxa within a functional guild (e.g., denitrifiers) vary phylogenetically [28, 62, 69], it follows that denitrification within periphytic biofilms may be sensitive to differences in carbon exudates produced by periphyton of differing algal taxonomic composition. Although periphyton can be highly variable in composition and coverage in natural systems [44, 47], the use of uniform artificial substrata greatly reduces such variability [36, 64]. The placement of artificial substrata (benthic mesh), then, may serve as a uniform substratum for the cultivation of high biomass periphyton communities that support high denitrification rates.

The specific objectives of this study were to (1) determine if periphyton cultivated on a benthic mesh under a variety of field conditions denitrifies at significantly higher rates than the underlying sediments and (2) determine if denitrification rates in periphyton communities vary as a function of algal and bacterial assemblage structure and biomass.

Methods

Field Site Descriptions and Sample Collection

We conducted a field survey of periphyton communities cultivated on a benthic mesh installed in three freshwater systems. Two of the sites, Middle Quarry (MQ) and Oak Grove (OG), were constructed wetlands located at the Des Plaines River Wetland Demonstration Project (DPRWDP) in Wadsworth, IL, USA. The third was a pond located at the Chicago Botanic Garden (BG) in Glencoe, IL, USA. The benthic mesh was made from flexible, durable polyethylene and had a thickness of 4 mm with 7×7 mm^2 mesh size [26]. The primary purpose of the mesh was to stabilize the sediments after pond construction and to protect new plantings by preventing invasive fish (e.g., carp) from foraging in sediments. When field conditions permitted, we collected samples from benthic mesh, underlying sediment, and the overlying water at selected sampling locations within each system. Field samples were collected in the fall of 2002 (FA), summer of 2003 (SU), and spring of 2004 (SP). Adverse field conditions (drought in 2002 and flood in 2004) prevented sample collection from all sites for certain sampling seasons.

At each field site, four non-shaded sampling locations were chosen for the collection of water, sediment, and mesh samples. The sampling locations were selected such that they differed in water depth (Table 1). At each location and on each sampling date, three square mesh samples measuring 10 cm^2 were cut from the colonized mesh, and sample volumes of approximately 1.5 l were collected from the overlying water. Three sediment cores (upper 3–5 cm) were drawn with a hand corer from directly beneath each of the three mesh cuttings (nine cores per sampling location). Water depth, temperature, and dissolved oxygen concentrations were measured at the time of sampling (Hydrolab Minisonde Multiprobe, HACH, Loveland, CO, USA).

Laboratory Mesocosms Setup and Sampling

The model laboratory mesocosms consisted of three identical Plexiglas channels (243 cm long, 63 cm wide, and 44 cm high) that were packed with 4 cm of clean natural silica sand (Ottawa #12 flint silica sand, mean diameter 486 μm) to form a flat surface. Baffles were installed at the inlet and outlet of each mesocosm to maintain the water column depth at 10 cm [2, 33]. A layer of benthic mesh was laid over the sand surface. Water was recirculated within the experimental system using a centrifugal pump at a flow rate of 350 l/min and distributed among the three mesocosms at three different flow rates with excess water recycled back into a common reservoir to ensure that the microbial communities in the mesocosms were not isolated from one another. The mesocosms were each seeded with 2 l of dense homogenized algal-bacterial slurries, derived from periphyton collected during November 2004 from Mill Creek near Wadsworth, IL, USA. After allowing 24 h for settling under stagnant water conditions, lights were turned on and flow was initiated in each mesocosm and maintained at a constant average velocity of 0.05, 0.5, or 5 cm/s for 4 months to allow for periphyton development. Water temperature was maintained at 15±2°C, and irradiance was supplied at 140±10 μmol m^{-2} s^{-1} via

Table 1 Physical and chemical characteristics of field sites

Field characteristic	NO_3^- (mg N/L)		PO_4^{-3} (*mgPL*)		Alkalinity (mg $CaCO_3$/L)		DOC (mg C/L)		DO (mg O_2/L)		Water depth (cm)		Water temperature (°C)	
Site and season	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
FAMQ	49–135	81	0.8–1	1	180–187	183	13–13	13	11–15	13	14–20	17	8.4–10	9
FAOG	24–49	40	0.7–1.3	1	285–298	294	8.4–8.9	9	14–16	15	10–35	24	7.3–9	8
SUBG	13–19	16	68–113	86	124–221	172	12–13	13	9.2–9.6	9	11–25	18	23–24	24
SUMQ	4.2–10	7	15–30	25	156–181	168	15–16	15	7.9–8.2	8	7.5–30	14	20–22	21
SUOG	7.6–10	9	24–29	27	232–241	237	17–18	17	8.4–8.6	8	10–16	13	22–23	23
SPBG	134–196	152	52–97	69	111–278	156	5.6–7.4	7	3.1–5.8	5	8–32	21	18–20	20
SPMQ	3.8–7	5	55–59	57	181–206	192	7.4–11	8	6.5–7.9	7	30–53	38	25–25	25

400 W metal halide lights (Hubbell, Spartanburg, SC, USA) at a light/dark cycle of 8:16 h. Minimal nutrients solution was added every 3 weeks as described by Arnon et al. [2]. Samples were collected from four locations in each mesocosm after 4 months of cultivation (three mesh samples per location, 10 cm^2 each).

Sample Processing

For both laboratory and field periphyton, the three mesh cuttings sampled from each location on a given date were processed as follows: Each cutting was separated into two equal halves, and one half from each of the three cuttings per location was combined to form a composite sample from which subsamples for determination of chlorophyll a, ash-free dry mass (AFDM), total bacterial counts, and algal and bacterial community structure were drawn. The other half of each cutting was placed into individual, presterilized serum jars for DNP rate measurement (three jars per location). All nine sediment cores per sampling location were combined in a laboratory blender (Waring Commercial, Torrington, CT, USA) to form a sediment slurry that was divided into three presterilized serum jars for DNP rate measurement (approximately 30 g [wet weight] per jar). Twenty milliliters of periphyton composite samples were preserved in a solution containing 0.25–0.50% of glutaraldehyde and sent for determination of algal abundances and identification (to genus level) via epifluorescent and light microscopy. Field periphyton samples were sent to Phycotech, Inc. (South Bend, IN, USA) for algal identification, and periphyton cultivated in the laboratory were sent to Loyola University Chicago (Chicago, IL, USA) for algal identification.

Analytical Methods

Filtered water samples were analyzed for NO_3^- (Apha Standard Method 4500-NO_3 H. [1]), PO_4^{-3} (Apha Standard Method 4500-P E. [1]) and dissolved organic carbon (Apha Standard Method 5310 C.[1]). Unfiltered water samples were analyzed for alkalinity (Apha Standard Method 2320 B. [1]).

The DNP rates of periphyton and underlying sediments were measured by the acetylene inhibition method as described by Sirivedhin and Gray [55]. Measured amounts of each sample (wet mass for sediment or benthic mesh area for periphyton) were placed in presterilized, airtight jars, and 100 ml solution of 721 ppm KNO_3, 1,800 ppm glucose, and 225 ppm chloramphenicol were added to each sample. The jars were purged with high purity nitrogen gas before acetylene gas addition, and nitrous oxide production was measured over time. Direct bacterial cell counts were performed on sediment and periphyton samples by epifluorescence microscopy (Zeiss Axiophot, mercury bulb) of 4′, 6-diamidino-2-phenylindole-stained material [12, 19, 27, 43]. Cyanobacteria were not included in these counts but were captured in the algal taxa identification described below.

Periphyton mass was determined by measuring AFDM via procedures outlined by Hauer [25] and modified by Kostel et al. [33]. Algal communities were characterized by chlorophyll a content and algal taxa identification. Chlorophyll a measurements and pigment abundance calculations were made according to the procedures described by Jeffrey and Humphrey [31]. The algal identification methods were described by Kostel et al. [33].

Bacterial community structure for periphyton cultivated in laboratory systems was characterized using terminal restriction fragment length polymorphisms (T-RFLP). DNA was extracted from periphyton samples with a Mo Bio™ soil-DNA extraction kit (Mo Bio, Carlsbad, CA, USA). However, DNA was extracted with an alternative lysis method (hot detergent/vortex) [5] using the Mo Bio™ kit that entailed two cycles of heating the samples to 70°C for 5 min and vortexing samples with DNA extraction beads for 5 s. Isolated DNA from periphyton samples was amplified via polymerase chain reaction (PCR) using universal eubacteria primers 8F (IR800, LI-COR, Lincoln, Nebraska), a fluorescently labeled forward primer (5′ AGAGTTTGATCCTGGCTCAG, [37]) and 926R (Operon Technologies, Huntsville, AL, USA), a non-labeled reverse primer (5′CCGTCAATTCCTTTRAGTTT, [37]). The PCR and T-RFLP protocols followed were as described by Janus et al. [30].

Ordination Analyses

To assess variation in physical and chemical characteristics of the aquatic systems as well as algal and bacterial community structures, non-metric multiple dimensional scaling (MDS) [9] was employed using the Primer V.5 software package (Primer-E Ltd., Plymouth, UK). All physical and chemical data were normalized to one, and a similarity matrix was calculated based on Euclidean distances [9]. The T-RFLP presence/absence data sets were reduced to include only TRF that were present in two or more samples. Algal community structural data sets (percent biovolume of each taxon) were not reduced or transformed for MDS analyses. Both the algal and bacterial community data sets were imported into Primer V.5, and similarity matrices were calculated based on the Bray-Curtis coefficient [6]. MDS was then used to ordinate the similarity data (ordination was computed after 100 random restarts). The analysis of similarity (ANOSIM) routine in Primer V.5 was used to examine the statistical significance of differences between groups of samples for each set of data. Significance data are reported as *p* values and *R* statistics. Possible *R* statistics range from −1 to 1, with a value close to 1, indicating a significant difference between two groups of samples, and the *p* value representing the statistical significance of the *R* statistic [6].

Results

Field Survey

Physical and Chemical Characteristics

The physical and chemical characteristics of the field sites are shown in Table 1. MDS ordination of these data suggests that the site characteristics show distinct seasonal differences and that the summer samples are more closely grouped than the spring or fall samples (Fig. 1). These observations are confirmed by ANOSIM analysis (two-way crossed) of site characteristic data, which shows significant differences by season (global R=0.913, p<0.001; fall vs summer, R=1.000, p<0.001; summer vs spring, R=0.828, p<0.001; fall vs spring, R=1.000, p<0.001). As expected, the site characteristics across seasons differed in nutrient concentrations and water temperature (Table 1). ANOSIM analysis showed no significant differences between the sites sampled during the same season or between sites across all seasons.

Algal Community Composition

A total of 74 algal genera were identified in the periphyton samples collected from benthic mesh in the field over all seasons, 33 of which comprised ≥3% of the mean algal biovolume among replicates collected on at least one sampling date in at least one wetland (Table 2). Representa-

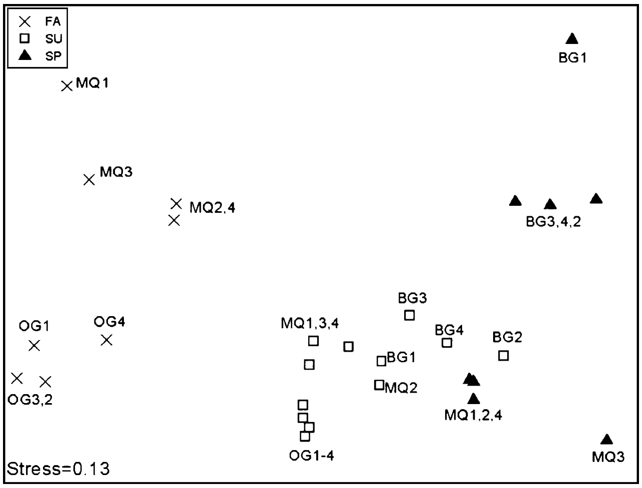

Figure 1 MDS ordination of physical and chemical characteristics of field sites: Middle Quarry (*MQ*), Oak Grove (*OG*), and Chicago Botanic Garden (*BG*). Samples were collected in fall 2002 (*FA*), summer 2003 (*SU*), and spring 2004 (*SP*)

tives of the algal divisions Chlorophyta (green algae), Bacillariophyta (diatoms), and the Cyanobacteria (blue-green algae) comprised 99% by biovolume (Fig. 2a,c,e). For all sampling locations and sampling times, cyanobacteria were dominant based on cell numbers (Fig. 2b,d,f), but algal taxonomic representation in periphyton based on biovolume varied considerably both temporally and among sites (Fig. 2a,c,e).

Despite significantly different physical and chemical characteristics across seasons, algal communities in periphyton cultivated on a benthic mesh were only marginally different (data not shown). ANOSIM of algal community structural data only showed significant differences between MQ and BG ($R=0.688$, $p<0.05$).

Denitrification Potential

Rates of DNP measured in periphyton communities on benthic mesh and in sediments were normalized to the number of bacterial cells. Periphyton and sediment core samples were grouped by season. In most cases, the DNP rates of the periphyton exceeded those of the underlying sediments, with

Table 2 Mean relative biovolume (%) of taxa comprising at least 3.0% of average algal biovolume within periphyton samples taken on a least one sampling date and at least one site

Taxon	Fall		Summer			Spring	
	MQ	OG	BG	MQ	OG	BG	MQ
Bacillariophyta							
Achnanthidium	1.3	0.2	1.5	3.0	2.7	0.4	0.3
Amphipleura	0.9	3.5		.	.	.	.
Amphora	13.0	0.4	0.1	1.9	5.4	.	1.4
Bacillaria	5.0	.	.	.	.	.	.
Caloneis	.	.	.	0.1	.	0.3	4.6
Cocconeis	10.3	0.1	0.3	.	0.2	3.9	1.9
Epithemia	.	.	8.9	.	.	17.9	.
Eunotia	0.4	.	.	.	9.0	.	.
Fragilaria	<0.1	0.1	<0.1	.	0.1	0.3	0.5
Mastogloia	.	5.6	.	.	.	.	.
Navicula	7.1	9.8	1.4	1.2	0.6	1.4	1.6
Nitzschia	9.5	1.1	0.9	26.5	9.8	4.7	7.6
Rhopalodia	.	.	0.3	.	4.2	.	.
Surirella	.	4.0	.	.	.	.	.
Synedra	.	<0.1	<0.1	.	5.4	.	0.8
Chlorophyta							
Chlorococcum	3.1	2.4	20.2	0.6	1.4	3.9	0.3
Cladophora	.	.	20.0	.	.	.	38.6
Coleochaetae	.	.	.	.	.	.	8.4
Mougeotia	.	10.6	.	1.8	.	8.0	.
Oedogonium	25.8	31.4	14.6	42.3	26.5	18.3	14.0
Protoderma	.	0.6	.	0.7	<0.1	0.7	5.5
Pyramichlamys	.	13.6	.	.	.	.	.
Rhizoclonium	.	.	.	.	8.4	9.7	.
Spirogyra	.	.	.	.	.	5.6	.
Stigeoclonium	6.3	0.5	0.5	3.7	0.2	0.3	0.5
Ulothrix	.	12.3	.	1.7	.	.	.
Zygnema	.	.	.	.	.	0.2	.
Cyanobacteria							
Aphanocapsa	0.2	<0.1	0.2	0.2	.	4.0	.
Calothrix	.	<0.1	19.1	.	10.4	1.9	0.5
Unidentified *Chroococcaceae*	5.5	0.7	0.8	4.7	0.6	0.1	3.2
Lyngbya	2.2	0.1	9.7	0.5	3.6	3.0	1.4
Oscillatoria	3.4	<0.1	0.3	0.4	8.5	0.6	1.4
Synechoccus	1.8	0.9	0.5	5	0.5	1.2	5.1
Xanthophyta							
Goniochloris	.	.	.	3.1	.	.	.

The period within the cells indicates that a taxon was not encountered in the sample.

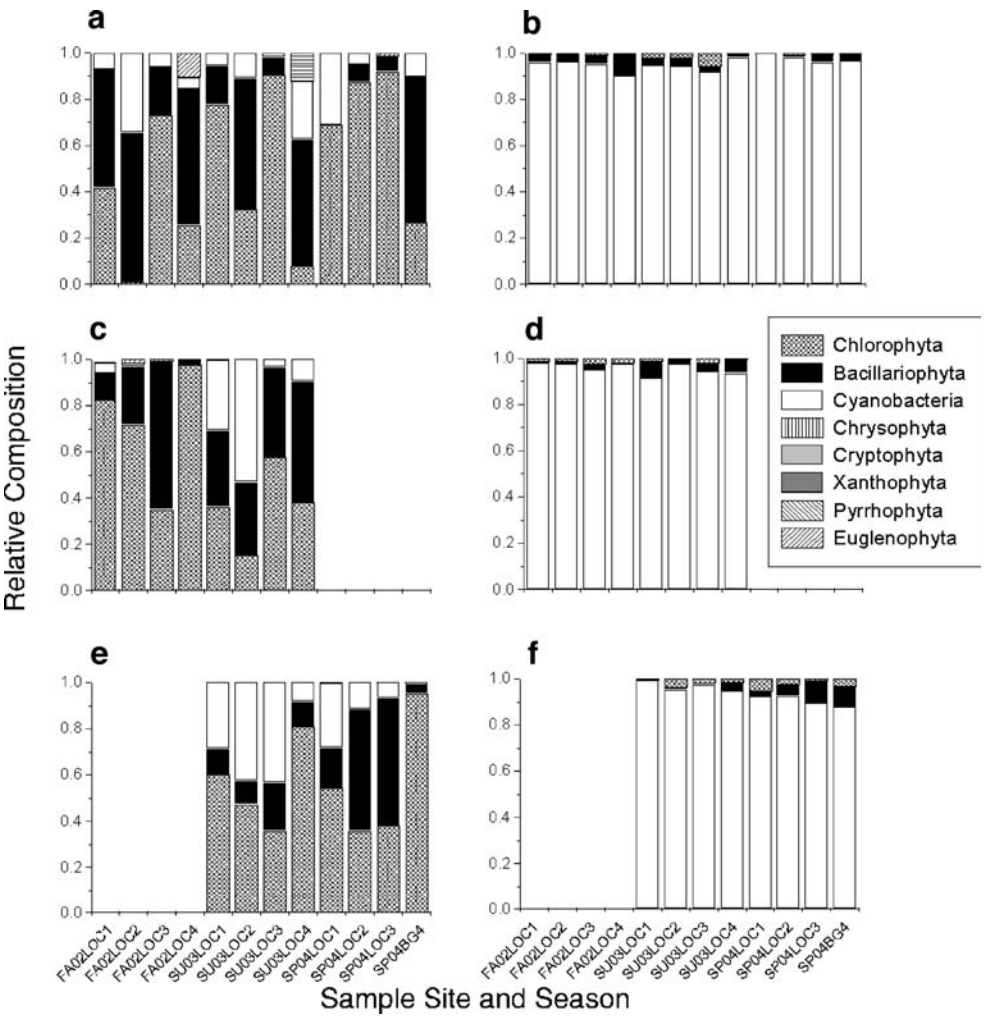

Figure 2 a–f Relative abundance of algae based on biovolume (**a**, **c**, **e**) and cell numbers (**b**, **d**, **f**) in periphyton samples from MQ (**a** and **b**) and OG wetlands (**c** and **d**), and the BG pond (**e** and **f**)

the exception of spring MQ samples (Fig. 3a). An extreme, natural flood event at the MQ site in May 2004 significantly altered sediment characteristics and water depth. Therefore, data from MQ in Spring 2004 (Fig. 3a) were omitted from further analyses. One-way analysis of variance (ANOVA) of natural-log transformed DNP-rate data from all wetlands and seasons (*sans* MQ Spring) revealed significantly ($p<0.0001$) higher DNP in periphyton than in sediments. Two-factor (sample type × season) ANOVA of DNP data from individual wetlands revealed significantly higher DNP in periphyton samples than in sediments from all wetlands [$p<0.0001$ (MQ and BG), $p=0.0005$ (OG)]. Significant effects of season ($p=0.022$) were noted in OG, with higher DNP in summer than fall (Fig. 3b). In BG, a significant sample type × season interaction ($p=0.032$) illustrated DNP in sediment samples decreased between Spring and Summer to a greater extent than in periphyton (Fig. 3).

Periphyton Community Attributes and Denitrification

Regression analyses of DNP rates (averaged by sampling location) vs biomass metrics showed that periphyton with greater AFDM supported higher bacterial numbers ($R=0.712$, $p<0.002$). However, there were no significant relationships between algal biomass parameters (total biovolume, and chlorophyll a content) and DNP rates (data not shown). ANOSIM of algal community structural data showed no significant differences in algal community structure with respect to DNP rate.

However, when DNP rates were regressed against the relative biovolume of the dominant algal divisions individually, a significant, positive relationship was observed between DNP and diatom biovolume (Fig. 4a) but not between green algae (Fig. 4b) or cyanobacteria (Fig. 4c). In addition, samples containing higher percentages of diatoms by biovolume also supported significantly higher bacterial densities ($R=0.776$, $p<0.0001$), whereas no significant relationships were evident between bacterial densities and green algal or cyanobacterial abundance (data not shown). Results of partial correlation analysis illustrated that the positive relationship between DNP rates (per area) and percent diatom biovolume remained strong even after controlling for variation in bacterial numbers (total correlation, $R=0.448$, $p<0.03$; partial correlation, $R=0.475$, $p<0.025$).

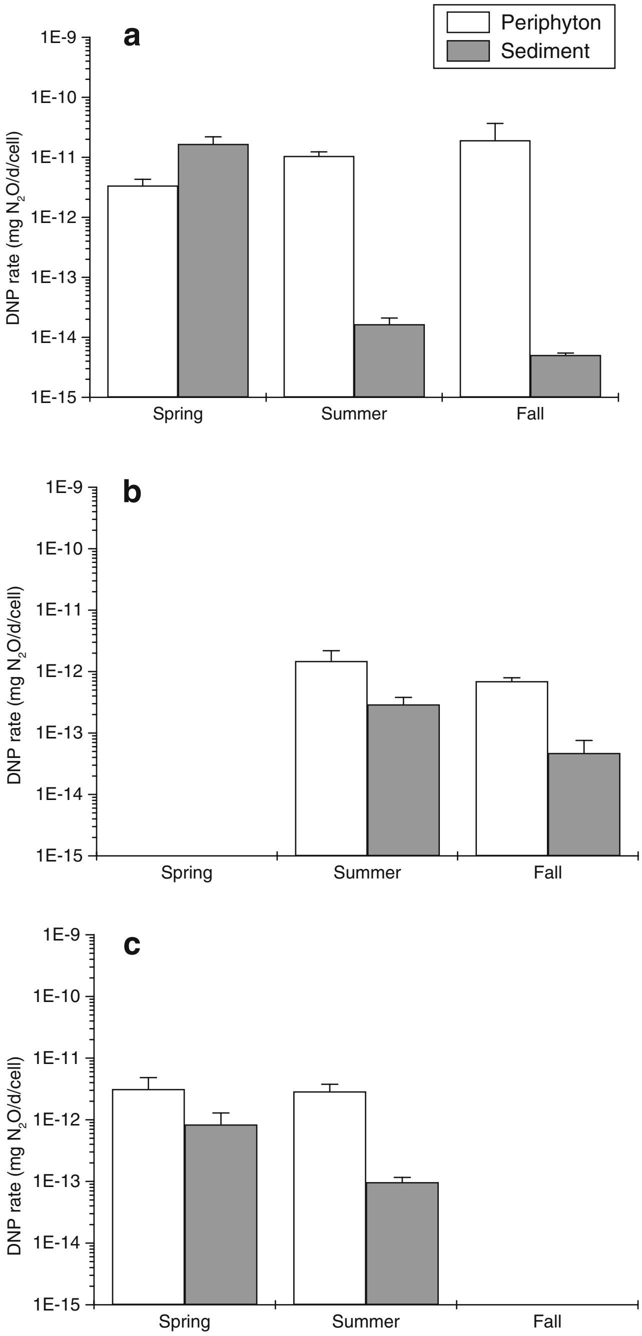

Figure 3 Average DNP rates measured on a per cell basis in periphyton and underlying sediments for samples collected from **a** MQ, **b** OG, and **c** BG all grouped by season and sample type. The *error bars* are based on the standard deviation of all samples collected by season

The most prevalent diatom genera found in field periphyton samples were *Achnanthidium* (present in 26:28 samples), *Navicula* (27:28 samples), and *Nitzschia* (25:28 samples). Of these, *Nitzschia* comprised the greater relative biovolume. A significant logarithmic relationship was observed between DNP rates (per area) and *Nitzschia* biovolume concentrations (Fig. 4d). The relationship between DNP rates and *Achnanthidium* biovolume was marginally significant (Fig. 4e), whereas no relationship was evident between *Navicula* (Fig. 4f) presence and DNP rates.

Laboratory Mesocosms

Algal Community Composition

Only 12 algal taxa were identified in periphyton collected from benthic mesh in laboratory mesocosms, 5 of which averaged >5% of the biovolume of algal assemblages from at least one flow regime (Table 3). Periphyton grown at the lowest flow velocity (0.05 cm/s) was homogeneous, loosely attached to the benthic mesh and bright green in color. Under medium flow (0.50 cm/s), biofilms were more adherent and also predominantly bright green, whereas periphyton cultivated at high-flow velocities (5.0 cm/s) were firmly attached and heterogeneous in color (green and brown patches) [2]. Diatoms, predominantly *Achnanthidium minutissimum* (Kütz.) Czarnecki, a monoraphid species that can attach to substrata via short mucilaginous stalks or form more loose associations [7, 38], comprised an increasing proportion of algal biovolume within periphyton progressing from lowest to highest velocity flow regimes. In contrast, the loosely attached chlorophyte, *Oocystis* sp., exhibited the opposite pattern, contributing over 50% of algal biovolume in the low-flow model wetland and just over 20% under a 5 cm/s flow regime (Table 3).

The highest average diatom dominance (56%) was observed under the highest flow conditions, whereas periphyton developed under medium (27%) and low-flow (19%) conditions supported lower average diatom concentrations (Table 3).

Periphyton Community Attributes and Denitrification

The highest DNP rates were observed in periphyton with the greatest diatom dominance. On average, the DNP rates of the high flow per 56% diatom relative biovolume measured 0.36 mg N_2O d^{-1} m^{-2}, medium flow per 27% diatom relative biovolume measured 0.08 mg N_2O d^{-1} m^{-2}, and low flow per 19% diatom relative biovolume measured 0.005 mg N_2O d^{-1} m^{-2} [2].

ANOSIM of MDS ordination of the algal consortia in the laboratory mesocosm (Fig. 5) clearly separated the algal communities developed in the highest and lowest velocity flow regimes (H vs M, R=0.963, p<0.005; H vs L, R=1.000, p<0.010; M vs L, R=−0.111, p=0.886). Samples labeled “HGR” and “HBR” in Fig. 5 were drawn from discrete locations in the high-flow mesocosm where periphyton color, either green (HGR) or brown (HBR), differed from that of the surrounding periphyton. Separa-

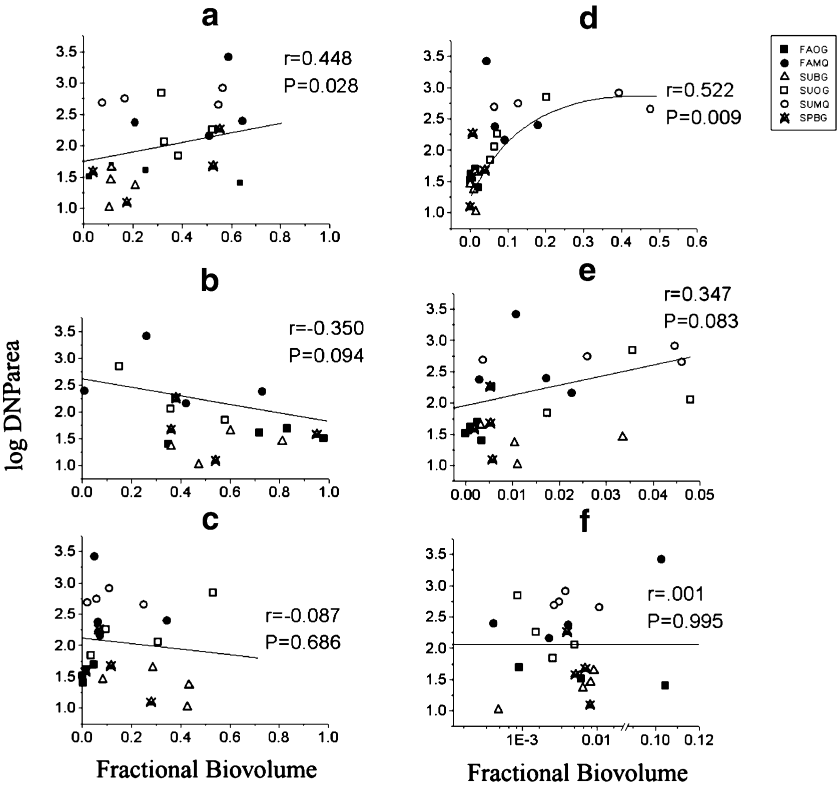

Figure 4 Scatter plots of DNP rates (mg N_2O m^{-2} d^{-1}, log normalized) and relative biovolume of **a** diatoms, **b** Chlorophyta, **c** Cyanobacteria, **d** *Nitzschia*, **e** *Achnanthidium*, and **f** *Navicula*. The *closed symbols* represent the fall 2002 data, *open symbols* represent the summer 2003 data, and the *crossed symbols* represent the spring 2004

tion of HGR and HBR samples from others taken from the 5.0 cm/s flow treatment stemmed from different proportional representation of *Oocystis* sp. (HGR/HBR=29.9/45.4% vs 56.4–66.9% in other samples).

MDS analyses of bacterial T-RFLP data showed distinct bacterial communities associated with periphyton differing in degree of diatom dominance (Fig. 6). ANOSIM results show that the global comparison (56 vs 27 vs 19% diatoms) of bacterial communities is statistically significant (R= 0.822, p<0.03) and that the bacterial communities with 56 vs 27% diatoms are marginally different (R=0.963, p< 0.10). There were no significant differences in bacterial communities with 56 vs 19% or 27 vs 19% diatoms.

Discussion

Periphyton Cultivated on Artificial Substrates in the Field

Despite seasonably variable conditions (Fig. 1, Table 1), algal community structures and DNP rates did not differ significantly across field systems or seasons. Our results are consistent with those of Sirivedhin and Gray [55], suggesting that, unlike periphyton attached to natural substrata, in which denitrification rates can vary considerably among seasons [58], the benthic mesh provides surface for uniform periphyton attachment and growth that show consistently high rates of denitrification across seasons and at a variety of field sites. The DNP rates that we measured in field samples are in the high range of those reported by other researchers [61, 63]. Furthermore, our results suggest that the benthic mesh not only encourages growth of periphyton exhibiting consistent denitrifying activity under variable conditions but also promotes development of algal communities of uniform biomass and community structure (Figs. 2 and 3). These findings indicate potential for benthic modification, such as the introduction of benthic mesh to encourage periphyton colonization in the abatement of nitrate loading in aquatic systems via enhanced denitrification.

Further investigation of the potential links between the algal community structure and bacterial cell densities, and function (i.e., DNP rates) revealed elevated DNP rates in periphyton with high relative diatom biovolumes (Fig. 4a), a result that remained robust after effects of variation in bacterial numbers were controlled for via partial correlation analysis. Because these comparisons are based upon equal bacterial densities, they indicate that there may be a specific relationship between algal and bacterial community structure. In periphyton with higher diatom concentrations (by biovolume), bacterial communities denitrified at higher rates than those in periphyton with lower diatom concen-

Table 3 Taxa comprising at least 3.0% of average algal biovolume within a least one flow regime in laboratory periphyton cultivated on benthic mesh under variable hydrologic conditions

Taxon	Low (0.05 cm/s) Mean (SD), n=4	Medium (0.50 cm/s) Mean (SD), n=4	High (5.0 cm/s) Mean (SD), n=6
Achnanthidium minutissimum (diatom)	19.3 (8.0)	26.5 (11.5)	55.6 (8.3)
Chroococcus sp. (cyanobacteria)	3.0 (4.1)	2.2 (1.6)	6.2 (6.1)
Oocystis sp. 1 (green algae)	52.7 (17.5)	51.8 (20.8)	21.9 (15.2)
*Oocystis** (green algae)	16.3 (12.2)	9.5 (5.6)	1.2 (1.3)
Stichococcus bacillaris (green algae)	0.9 (0.3)	3.0 (3.0)	8.2 (7.4)

*These cells had the same size, shape, and external morphological features as *Oocystis* sp. 1, but cell contents differed considerably, with indistinct chloroplasts and what appear to be oil vesicles.

trations. These results suggest that specific attributes of diatom dominated algal assemblages within periphyton selected for resident bacteria and stimulate those bacteria to elevated rates of denitrification.

The inflection point of the exponential regression line at 15% relative biovolume of *Nitzschia* (Fig. 4d) suggests the existence of a threshold above which increasing dominance of a particular taxon has no additional stimulatory effects on bacterial metabolism. White and Reddy [67], in a study of denitrification in wetland soils in the Everglades, showed that the activity of denitrifying enzymes fit an exponential function of change over time and exhibited a threshold similar to that which we observed. They reported significant correlations between microbial biomass and soil P content [67], suggesting that phosphorus limited bacterial growth. Our results relating denitrification to diatom presence also implicated resource limitation as an underlying cause of the observed DNP threshold. Because the addition of phosphorus was not part of our DNP rate measurement protocol and carbon and nitrogen were in excess, it is possible that phosphorus, or perhaps silica, may have been limiting.

The clear differences we measured in DNP rates between sediment and periphyton samples (Fig. 3) suggest that biogeochemical conditions within periphyton attached to the benthic mesh substrata favored a distinct bacterial consortium that possessed a higher capacity for denitrification than that associated with the underlying sediments.

Periphyton Cultivated in Laboratory Mesocosms

Periphyton cultivated in laboratory mesocosms had a lower number of algal species than those found in field samples (Table 3). This is often the case in mesocosm studies, particularly in later stages of algal succession [39, 46, 59], but it does provide an opportunity to assess bacterial/algal associations in a much less complex system. Two of the dominant diatom genera from our field survey (*Nitzschia*

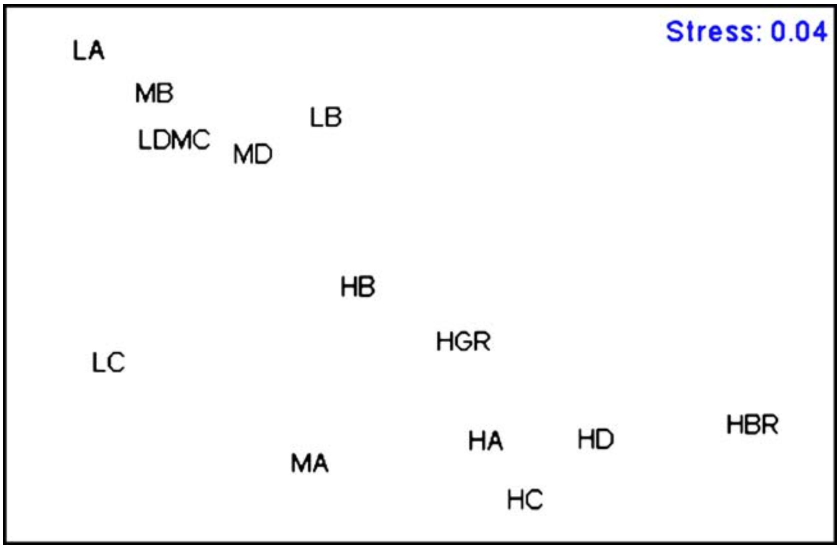

Figure 5 MDS of algal community composition in periphyton cultivated in a laboratory mesocosms under different flow regimes. The prefix stands for the flow rate through the mesocosm (*L* low, *M* medium, and *H* high), and the suffix stands for the sample replicate. The "HGR" and "HBR" samples were collected from the high flow mesocosm from two areas that were homogeneous in color (*brown* or *green*). The suffixes *A–D* indicate various sampling locations within the mesocosms

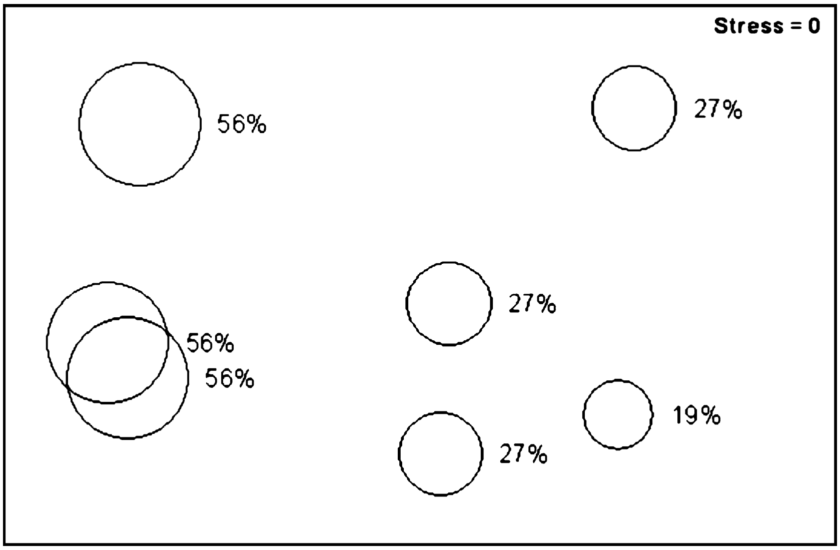

Figure 6 MDS of bacterial community T-RFLP data from periphyton cultivated in laboratory mesocosms under low flow (0.05 cm/s, 19% diatoms by biovolume), medium flow (0.5 cm/s, 27% diatoms by biovolume), and high flow (5cm/s, 56% diatoms by biovolume) conditions. These data are shown as a *bubble plot* in which bubble size corresponds to the percentage of diatoms in the periphyton based on biovolume

and *Navicula*) were not found in laboratory periphyton, but *Achnanthidium* was the dominant diatom present in laboratory periphyton.

Clear differences in dominance patterns were evident among laboratory assemblages developed under the three different flow-regime treatments (Table 3). Primary among these was increased dominance of the diatom, *Achnanthidium minutissimum*, with increasing flow velocity and a significant reduction in contribution of the chlorophyte, *Oocystis* sp., in the higher flow regime. The higher degree of macroscopically observable spatial heterogeneity in the highest-flow treatment suggests a higher degree of flow-related influence in this treatment relative to slower velocity treatments.

The influence of flow regime on generation of spatial heterogeneity in both taxonomic structure and physiological activity of attached algal communities is well documented and involves both physical constraints, such as shear stress, and physiological stimulation [60]. Under the low flow velocities employed in our laboratory mesocosms, physical constraints were likely less influential than inter-treatment variation in nutrient supply rates. Increased water movement in the 5 cm/s wetland mesocosm likely enhanced transport rates of dissolved nutrients to a larger percentage of the cells residing in attached biofilms. Water movement reduces thickness of the diffusive boundary layer through which dissolved nutrients must pass [68] and increases the depth to which these molecules can penetrate into biofilms [60], potentially relieving nutrient-depleted conditions in the lower strata of thick biofilms and stimulating algal growth [3, 4, 46]. Increases in current velocity, even within the low range of velocities we employed, can enhance delivery and uptake of dissolved nutrients by biofilm residents [50]. The apparent increase in nutrient availability associated with the higher flow velocities may have favored proliferation of *Achnanthidium minutissimum*, a diatom taxon that favors nutrient-rich environments [3, 4, 38].

The DNP rates measured in samples taken from the mesocosms were within the low range of those measured by other researchers [58, 61, 63]. However, the significant relationship between diatom presence and DNP rates supports our field data. The significant relationship between DNP rates and *Achnanthidium minutissimum* parallels the marginally significant relationships in field periphyton (Figs. 4e and 6). Denitrification rates in natural systems are influenced by a number of abiotic and biotic factors, which also can modify bacterial [14, 49, 51] and algal community structures [8, 10, 20, 48]. Sirivedhin and Gray [55] suggested that the increased denitrification rates observed in periphyton communities vs underlying sediments arose because of the secretions of easily degradable, algal-derived organic carbon in periphyton. Other research has also shown that taxonomically distinct bacterial communities developed after incubation with different marine diatom species, although the diatoms were phylogenetically similar [21]. Researchers have also found that the soluble carbohydrates secreted by diatoms during stationary and growth phases in culture vary in molecular weight and differ as a function of diatom species [57]. Based on these observations, together with results of our field and lab studies, we hypothesize that there are specific relationships between the algae and bacteria in periphyton grown on a benthic mesh that promote high rates of denitrification.

Bacterial community structure data support our field observations and suggest a relationship between diatom presence and denitrification rates. We have documented that distinct bacterial consortia associated with biofilms developed under 5.0 cm/s flow conditions, and in association with algal biovolume dominated by diatoms, exhibited significantly higher rates of denitrification than consortia developed in diatom-poor periphytic biofilms under slower flow conditions. As the primers used in this study for T-RFLP analysis were universal 16S ribosomal RNA (rRNA) primers, it is possible that 16S rRNA from algal chloroplasts and cyanobacteria could have been amplified and, thus, could have contributed to the T-RFLP profiles. However, based on microscopic direct counts, the numbers of bacterial cells (excluding cyanobacteria) were three to five orders of magnitude higher than the numbers of algae and cyanobacteria (10^5–10^7 bacterial cells/cm^2 mesh compared to 10^2 algal and cyanobacterial cells/cm^2 mesh). Therefore, it is unlikely that the relatively small numbers of algae and cyanobacteria would have made a significant contribution to the T-RFLP profiles.

Overall, our study demonstrated that the use of a benthic mesh in restored aquatic systems, such as riparian areas, constructed wetlands and ponds, holds promise as a feasible way to boost nitrate removal by the cultivation of periphyton communities that promote bacterial consortia of consistently high denitrifying capacity. This strategy for enhancing denitrification rates may be best suited to newly constructed wetlands as well as ponds or lakes that have minimal vegetation and, therefore, minimal shading and detrital deposition. Our laboratory studies illustrated that periphyton cultivated under flow velocities of 5.0 cm/s had higher biomass and higher *potential* rates of denitrification than periphyton developed at lower flow velocities. We attribute these differences in DNP rates to flow-related variation in periphyton community structure. Further study, however, is required to probe the exact nature of this relationship (e.g., secretion of preferential organic carbon source by diatoms for enhanced denitrification). These results indicate that a benthic mesh may also have a potential application in creating periphyton habitat in shallow streams and drainage ditches (high flow velocities), where high nitrate loads often occur.

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