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Epilithic bacterial responses to variations in algal biomass and labile dissolved organic carbon during biofilm colonization

WILLIAM V. SOBCZAK[1]

Department of Zoology and Kellogg Biological Station, Michigan State University, East Lansing, Michigan 48824 USA

Abstract. This study experimentally examines potential shifts in epilithic bacterial biomass and productivity in response to variations in epilithic algal biomass and labile dissolved organic carbon (DOC) during stream biofilm colonization. I predicted that epilithic bacteria would respond positively to allochthonous DOC early in biofilm colonization and respond positively to increased algal biomass late in biofilm colonization. Using once-through, experimental-stream channels, a 2 × 2 factorial design was employed in which light (shaded vs. non-shaded) and labile DOC (glucose-amended vs. ambient) were manipulated. Ceramic tiles were used as substrates for biofilm colonization and were sampled at different colonization stages. Shading significantly reduced chlorophyll *a*, live-algal biovolume, and ash-free dry mass throughout colonization. Bacterial biomass increased significantly during biofilm colonization, but was not significantly different among treatments. Incorporation of [^{3}H]thymidine into bacterial DNA, which was measured as a surrogate for bacterial productivity, was significantly greater in the glucose-amended channels throughout colonization, but it increased in the unshaded, ambient treatment in late colonization as well. These results suggest that labile DOC in the water column can potentially function as a control for epilithic bacteria throughout biofilm colonization, whereas epilithic algae can stimulate bacteria late in biofilm colonization in productive stream ecosystems.

Key words: epilithic bacteria, epilithon, stream biofilm, bacterial productivity, stream ecosystem, dissolved organic carbon.

Interactions between bacteria and algae have been well documented in planktonic aquatic ecosystems (see Cole 1982, Cole et al. 1988, White et al. 1991). However, the strength of the link between epilithic bacteria and algae in lotic systems continues to be debated (Haack and McFeters 1982a, Goulder 1988, Kaplan and Bott 1989, Stock and Ward 1989, Hudson et al. 1992, Findlay et al. 1993). A stream biofilm (i.e., epilithon) is a diverse assemblage of autotrophic and heterotrophic microorganisms embedded in a polysaccharide and glyco-protein matrix attached to stream substrates (see Rounick and Winterbourn 1983, Lock et al. 1984). In many streams, biofilms play important, if not central roles, in stream ecosystem function; they can greatly influence stream primary production (Minshall 1978, Lock 1981), secondary production (Fuller et al. 1986, Mayer and Likens 1987, Winterbourn 1990), decomposition (Ladd et al. 1982, Ford and Lock 1987), and nutrient retention (Paul and Duthie 1988, Mulholland et al. 1991).

Although the importance of biofilms in stream-ecosystem function is well established, research on microbial controls within stream biofilms is less developed, and often ambiguous (see review by Lock 1993). Several studies have suggested that epilithic bacteria can rely on exudates from epilithic algae (Haack and McFeters 1982a, 1982b, Goulder 1988, Kaplan and Bott 1989). Mechanisms postulated for a bacterial–algal linkage include: 1) the presence of a nutrient-rich microzone or phycosphere surrounding live algal cells (Cole 1982), 2) algal senescence within the epilithon (Haack and McFeters 1982b), 3) the formation of a diffusional barrier in thick biofilms which excludes organic matter from the water column (Lock 1981, Peterson and Grimm 1992), and 4) algal-generated diel changes in the availability of dissolved organic carbon (DOC) (Kaplan and Bott 1989). Although several studies have shown a bacterial–algal linkage, others (Bott et al. 1984, Ford and Lock 1987, Findlay et al. 1993) have demonstrated that benthic bacteria can be supported by allochthonous DOC.

Although past studies have examined the covariation of epilithic bacteria and algae, few have used a field-based experimental approach

[1] Present address: Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545 USA.

(Kaplan and Bott 1989, Steinman and Parker 1990, Findlay et al. 1993) and adequately assessed the role of biofilm colonization. Thickness of a biofilm can influence storage product deposition (e.g., glycogen and poly-beta-hydroxyalkanoate production) by prokaryotes within the biofilm, regardless of stream water chemistry (Blenkinsopp et al. 1991). In addition, a thick polysaccharide matrix may exclude exogenous DOC and serve as a potential carbon source for bacteria, even in the absence of endogenous DOC production (Freeman and Lock 1995). In a study that investigated the role of nutrient cycling and grazing in regulating periphyton, Mulholland et al. (1991) proposed a conceptual model which suggests that under nutrient-limited conditions a thin, disturbed biofilm should rely primarily on nutrients from the water column, whereas a thick, mature biofilm should rely primarily on nutrients recycled within the biofilm. Collectively, these papers (Blenkinsopp et al. 1991, Mulholland et al. 1991, Freeman and Lock 1995) suggest that the degree to which epilithic bacteria and algae covary in stream biofilms may be influenced by a biofilm's successional stage in colonization.

I hypothesized that epilithic bacteria readily respond, as measured by increased bacterial biomass and productivity, to labile DOC early in biofilm colonization when algal biomass and productivity (areal) are low. Increased algal productivity in later successional stages of biofilm colonization should lead to a greater effect on epilithic bacteria. Thus, the strength of a possible algal–bacterial linkage should increase with the maturity of the biofilm. To test this hypothesis I compared the responses of epilithic bacteria in biofilms containing low and high algal biomass to pulses of glucose during different stages in biofilm colonization in a controlled, replicated experiment.

Methods

Study site and experimental streams

I constructed once-through, experimental-stream channels beside a 3rd-order section of Augusta Creek in the Kellogg Biological Station's Experimental Forest in southwest Michigan. Augusta Creek contains moderate to high concentrations of nitrate, sulfate, carbonate, bicarbonate, and base cations (Wetzel and Manny 1977, Hedin and Brown 1994). The watershed contains natural wetlands, deciduous forests, and agricultural fields. DOC in the 3rd-order section of the creek is ~3 mg/L and shows low diel variability (Hedin and Brown 1994).

I used experimental-stream channels to provide replication and to assure the development of mature biofilms. Water from Augusta Creek was pumped with a high volume, centrifugal pump (Teel Industrial Series, model 1P551A, 2HP) through PVC piping into a 1700-L opaque, fiberglass reservoir. The reservoir enabled water to be gravity fed from a constant head into the experimental channels via PVC pipes mounted to the base of the reservoir (Fig. 1A). Adjustable plastic ball valves regulated flow into channels. Vinyl rain-gutters (3 m long, 10 cm wide, 10 cm high) mounted on elevated supports served as experimental channels. Long channels were used to assure homogeneous flow in sampling sections. Channels were lined with white (non-glazed) ceramic tiles (tile area = 6.5 cm^2), which served as the substrate for biofilm colonization. Periphyton that colonizes clay tiles has been shown to be representative of communities that colonize natural substrates (Tuchman and Stevenson 1980, Lamberti and Resh 1985). In addition, clay tiles aid in quantifying the biofilm community. The channel bottoms were completely covered with tiles to minimize boundary layer variation. Fluorescent, inert dye injections prior to experimentation demonstrated even flow across tiles. Channel discharge was monitored twice daily throughout the experiment by measuring the rate at which the effluent from each channel filled a 19-L bucket. White channels and white tiles were used to minimize heat absorption during the initial stages of epilithon colonization.

Experimental design

A completely randomized block design (3 replicate blocks), with a 2 × 2 factorial design (12 channels total) in which light (shade vs. non-shade) and labile DOC (glucose-amended vs. ambient) were manipulated, was employed during 2 separate colonization periods in August and September 1992. Experimental channels served as experimental units and individual tiles within channels served as samples. Light was manipulated by shading select channels with hardware cloth throughout biofilm colo-

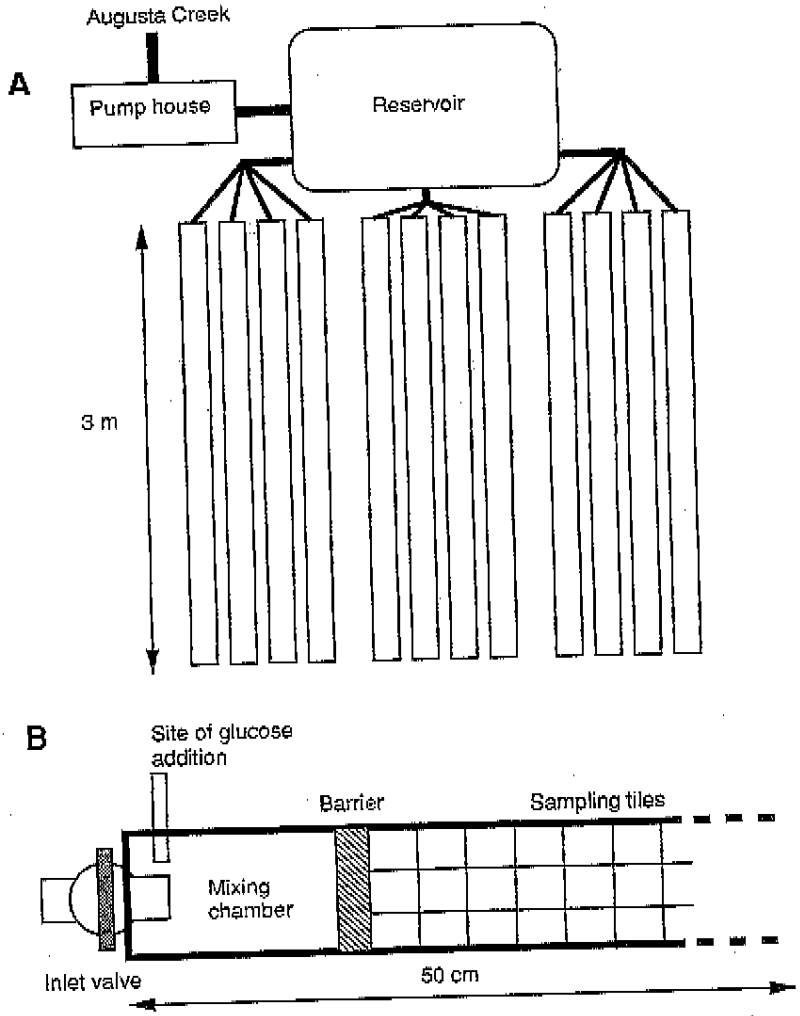

FIG. 1. A.—Schematic diagram of the experimental-stream channel arrangement. B.—Schematic diagram of the upstream section of a channel.

nization (~90% of photosynthetically active radiation was blocked). The shading treatment was designed to establish contrasting amounts of epilithic-algal biomass. DOC was manipulated with periodic glucose additions during biofilm colonization. Stream-water DOC was elevated from ~3 mg/L to 10 mg/L with the addition of glucose. Each channel contained a mixing chamber upstream of the sampling section (see Fig. 1B).

Glucose was selected as the additional DOC source for several reasons: 1) glucose is extremely labile and used by microbes to synthesize many other compounds necessary for growth (see Chapelle 1993, Neidhardt et al. 1990), 2) it is found naturally in streams (albeit, in low concentrations) (see Thurman 1985), 3) it ensures contrasting amounts of bioavailable DOC, 4) it is less likely to stimulate algal growth than other possible labile carbon sources which can also include substantial amounts of inorganic nutrients (e.g., leaf leachates, algal exudates, soil pore-water), and 5) unlike other potential carbon sources, it does not provide an exogenous source of thymidine that could potentially confound bacterial productivity estimates (see *Lab-*

oratory protocol below). I added glucose for 72 h before each biofilm sampling to provide ample time for changes in physiology or composition (or both) in the epilithic-bacterial community. Glucose was not added throughout the entire experiment in an attempt to avoid the formation of a monoculture or unnatural bacterial community. Further, the experiment aimed to evaluate the response, rather than the acclimation, of the biofilm's bacterial assemblage to elevated DOC.

The sampling regime was designed to establish biofilms at different colonization stages. Biofilm communities were sampled on days 3, 10, and 25; these dates were representative of early, intermediate, and late (mature community) colonizational stages. On each sampling day, I randomly removed 20 tiles from the same section of each experimental channel. The sampled tiles were immediately replaced to avoid flow disturbance (Stevenson 1983) and their placement was marked to prevent re-sampling. Sampled tiles were randomly placed in individual, pre-labelled sterilized bags and placed in the dark on ice. For each of the 12 channels, tiles were sampled to estimate ash-free dry mass (AFDM) (n = 5), chlorophyll *a* (n = 5), live-diatom biovolume (n = 3), bacterial biomass (n = 3), and [^{3}H]thymidine uptake (n = 4).

Laboratory protocol

AFDM.—Samples (i.e., tiles) were stored frozen. Tiles were scraped with a razor blade and rinsed with distilled water into individual aluminum weighing-pans. The difference between dry weight (24 h at 60°C) and ashed weight (1 h at 450°C) was measured. AFDM was not measured on the first sampling date.

Chlorophyll a.—Samples were frozen for 24 h to lyse cells. Tiles were scrubbed in buffered 90% acetone to extract pigments and filtered for fluorometric analysis. Chlorophyll *a* was then determined by the procedures outlined in APHA (1985: Methods 1003C and 1002G) and converted to $\mu g/cm^2$.

Live-algal biovolume.—Samples were fixed with a 2% M3 solution (0.05% KI, 1% iodine, 5% glacial acetic acid, 25% formalin), scraped from the tile with a razor blade, and sonicated for 5 min. Sub-samples were pipetted onto 22-mm^2 glass coverslips. The coverslips were air dried and permanently mounted on glass slides using Hyrax medium. The algal assemblage was dominated by diatoms. Diatom counts were done at 1250× magnification on a Zeiss microscope equipped with phase contrast illumination. Frustules in which internal protoplasm was readily apparent were designated as "live". For each sample, >100 live diatoms were counted. Cell volumes were estimated for the 10 most common species based on simple geometric shapes. Live-algal biovolume was quantified by summing each species' live density multiplied by its cell volume.

Epilithic bacterial biomass.—Samples were preserved with filtered (pore size = 0.22 μm) 5% formalin, scraped from tiles with a sterile razor blade, and sonicated to further disrupt aggregates. Sub-samples were stained for 20 min in the dark on ice with DAPI (4'6-diamidino-2-phenylindole, 20 μg/mL, Sigma) diluted to yield a final concentration of 2 μg/mL. This concentration has been found to maximize direct cell counts, but avoids over-staining in stream benthic samples (Haack et al. 1988). Portions (0.25–3.0 mL) of stained sub-samples were filtered onto pre-stained black, 25-mm diameter, 0.2-μm pore size, polycarbonate membrane filters which were backed with 25-mm, 0.45-μm Millipore filters. Blanks were routinely prepared to assure that only the samples were contributing to the bacteria being counted. Bacteria were observed with a Leitz Laborlux II microscope, equipped with an HBO 50-W mercury light source, Leitz wide-band UV filter set A (excitation filter 340–380 nm, mirror 400 nm, and barrier filter 430 nm), a 100/1.25 oil immersion objective, and 15× oculars. At least 20 cells were counted/field; 10 fields were counted for each sample. Past work has shown that 80% of the technique's variance is caused by variance in microscope fields as opposed to variance in filters or sub-samples (Kirchman et al. 1982); hence, emphasis was placed on multiple field counts per filter per sample. Bacterial cells were divided into 10 arbitrary size classes (2 cocci, 4 narrow rods, and 4 wide rods) to account for size differences among morphologies (see Haack et al. 1988). Cell volumes were calculated using simple geometric shapes. Volumes of cocci were calculated as spheres, and rods were assumed to be cylinders capped with half spheres. Bacterial biomass as μg C/cm^2 was estimated for each of the 10 size classes as mean cells/cm^2 × cell volume × (5.6×10^{-13} g C)/μm^3 of bacterial

biovolume (Bratbak 1985, Haack et al. 1988). The summation of bacterial biomass for the 10 size classes represents bacterial biomass for the sample.

Most studies report bacterial abundance (instead of converting to bacterial biomass) or simply use an average cell size for converting bacterial abundance into biomass. These approaches may not detect a bacterial response attributed to changes in cell size. Few studies report biomass estimates derived from cell counts in which all cells were measured, because of the time required to obtain an adequate sample size. An alternative approach is to devise a set of size classes that represents the range in cell size encountered so that bacteria can be quickly segregated into these size classes while counting a large number of samples with large sample sizes. This approach is a logical compromise between the 2 methods described above for studies in which a large number of samples must be processed.

Epilithic [^{3}H]thymidine uptake.—Incorporation of [^{3}H]thymidine (TdR) into bacterial DNA was measured as a surrogate for bacterial productivity (Findlay et al. 1984). On each sampling day, 4 tiles from each experimental channel were taken to the laboratory; each tile was in 20 mL of channel water on ice to minimize changes in biofilm chemistry and reduce cellular activity before incubation. To initiate incubation, 1 nmole of TdR (20 μCi/nmole) was added to each sample. Two of the 4 samples from each channel were "killed" with 5% filtered formalin immediately after the addition of TdR. The remaining 2 samples were "killed" following a 1-h incubation. Samples were incubated at stream temperature (17°C) on a mechanical shaker. Following incubation, the naturally sloughed portion of the biofilm from each tile was collected on a 0.45-μm filter. Each tile and filter were rinsed 3 times with 5% formalin to remove unincorporated TdR. Each filter was frozen with its representative tile until extraction.

Alkaline extract (20 mL of 0.3N NaOH + 1% SDS + 25 mM EDTA) was added to frozen samples which were then mechanically shaken at 25°C for 18 h (Findlay et al. 1984). A 5-mL aliquot of the supernatant was chilled to 0°C, neutralized with 1 mL of 3N HCL, and acidified with TCA to a final concentration of 5%. Carrier DNA (0.1 mg) was added to aid the precipitation of DNA during centrifugation (15,000 × *g* for 15 min at 4°C). The supernatant, which contained the hydrolyzed RNA, was aspirated. Additional cold 5% TCA was added to the pellet, the sample was re-centrifuged, and the supernatant was aspirated again. DNA was hydrolyzed from the pellet in 5% TCA for 30 min at 95°C. A 1-mL aliquot of the supernatant was radioassayed (to obtain dpm in DNA). Differences in dpm between the samples that were immediately "killed" and those incubated for 1 h ("live") were used as the dpm (corrected dpm) in the DNA for each experimental channel. Live samples routinely incorporated >10× the TdR incorporated by the killed controls. In addition, past work has demonstrated that uptake of TdR is linear for short incubations ranging from several minutes to several hours (Findlay et al. 1984, Sobczak, unpublished data). Isotope dilution was examined using tiles that had been colonized for 10 d in ambient stream conditions. Five levels (at least 3 replicate tiles each) of cold thymidine ranging from 0.5× to 10× labelled TdR concentration were used (see Findlay et al. 1984). Exogenous thymidine was estimated as 225 nmol/L. Isotope dilutions were not estimated for additional colonization conditions or dates because of time and expense considerations. Bacterial productivity is directly related to TdR uptake by means of two conversion factors: 1) bacterial cells per nmole TdR incorporated into DNA and 2) isotopic dilution. Because isotopic dilution among treatments and sampling dates was not estimated independently, TdR uptake (dpm cm^{-2} h^{-1}) is not converted to bacterial productivity.

Statistical analysis

Analysis of variance with repeated measures (ANOVAR) was conducted to test main effects of 2 levels of light (shaded vs. non-shaded), main effects of 2 levels of DOC (glucose-amended vs. ambient), change over time (3 sampling dates), and interactions among the 3 for each of the 5 dependent variables of interest: chlorophyll *a*, AFDM, live-algal biovolume, bacterial biomass, and TdR uptake (SYSTAT 5.2.1, Evanston, Illinois). All variables were natural-log transformed to eliminate correlations of means and variances associated with comparing samples from different colonization times. Because the experimental channels are the experimental units, the means of samples within a channel

TABLE 1. Summary of ANOVARs (p values) for the following dependent variables: AFDM, chlorophyll *a* (Chl. *a*), LABV, bacterial biomass (BB), and [^{3}H]thymidine uptake (TdR). All data were natural-log transformed. Statistics designated by * = $p \leq 0.05$; ** = $p \leq 0.01$.

Source of variation (df)	AFDM	Chl. *a*	LABV	BB	TdR
Between subjects					
Block (2)	0.158	0.398	0.352	0.027*	0.062
Glucose (1)	0.084	0.274	0.723	0.491	<0.001**
Shade (1)	<0.001**	<0.001**	<0.001**	0.930	0.437
Shade × Glucose (1)	0.105	0.636	0.777	0.593	0.047*
Error (6)					
Within subjects					
Time (1)	<0.001**	<0.001**	0.092	<0.001**	<0.001**
Block × Time (1)	0.756	0.543	0.124	0.009**	0.034*
Glucose × Time (1)	0.758	0.067	0.960	0.399	0.002**
Shade × Time (1)	<0.001**	<0.001**	0.013*	0.522	0.072
Shade × Glucose × Time (1)	0.474	0.183	0.989	0.140	0.240
Error (6)					

were used in the analyses. Power analyses were conducted for each repeated measure (i.e., each sampling day) for ANOVARs in which no significant main effects were detected. Power is a function of the pre-set alpha value, effect size (magnitude of differences between means), sample size, and sample variance. If power is low, then the beta error is high. Thus, when power is low, negative results (non-rejection of null hypothesis) are ambiguous.

Results

AFDM

AFDM was significantly reduced ($p < 0.001$) in the shaded vs. non-shaded treatments overall (Table 1, Fig. 2A). A nearly 10-fold difference was observed between the shaded and non-shaded treatments on day 25. A significant ($p < 0.001$) shade × time effect occurred as well. Overall, AFDM was not significantly affected ($p = 0.08$) by the glucose treatment.

Chlorophyll a

Throughout colonization, chlorophyll *a* was significantly reduced ($p < 0.001$) in the shaded vs. non-shaded treatments (Table 1, Fig. 2B). A significant shade × time effect ($p < 0.001$) occurred as well, indicating that the effect of ambient light increased throughout biofilm colonization. Differences were evident on all sampling days, regardless of colonization time. A nearly 10-fold difference was observed between the shaded and non-shaded treatments on day 25. Chlorophyll *a* was not significantly affected ($p = 0.27$) by the glucose treatment when all sampling dates were analyzed together (Table 1), however, if day 25 is analyzed independently, chlorophyll *a* significantly decreased ($p < 0.05$) in response to the glucose treatment. Overall, chlorophyll *a* levels increased throughout colonization time in the non-shaded channels (Fig. 2B). These results are consistent with those for AFDM.

Live-algal biovolume (LABV)

Consistent with the AFDM and chlorophyll *a* results, LABV was significantly reduced ($p < 0.001$) in the shaded treatments (Table 1, Fig. 2C). However, LABV did not increase significantly through colonization time ($p = 0.09$) (Table 1, Fig. 2C). Initial colonists had less chlorophyll *a*/LABV than the diatom assemblages that were sampled later in biofilm colonization. A nearly 10-fold difference between shaded and non-shaded treatments was observed on days 10 and 25 for LABV. The benthic algal assemblage was dominated by diatoms. *Synedra ulna* and *Nitzschia linearis*, both large diatoms, dominated LABV in all treatments on all 3 sampling dates (Fig. 3). *Synedra ulna* represented ~50% of LABV, regardless of treatment or sampling date, whereas *Nitzschia linearis* represented between

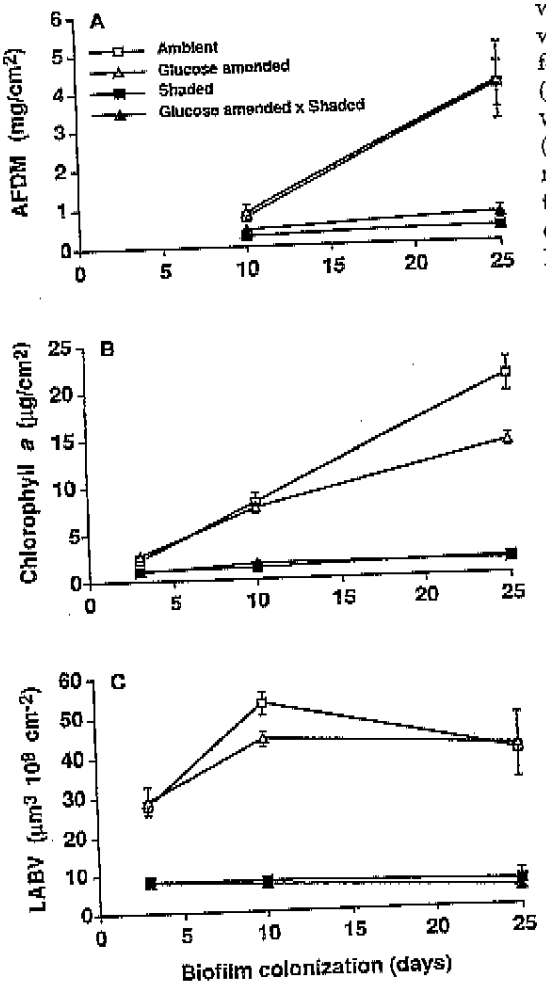

FIG. 2. AFDM (A), chlorophyll *a* (B), and LABV (C) through biofilm colonization. Each point is the mean (±1 SE, $n = 3$) for each treatment.

~50% (day 3) and ~20% (day 25) of LABV. The relative abundance of additional species increased on day 25.

Epilithic bacterial biomass

Epilithic bacterial biomass did not differ significantly among treatments (Table 1, Fig. 4A), yet increased significantly ($p < 0.001$) throughout colonization, reaching an overall average of ~10 µg C/cm² on day 25 (Fig. 4A). Similar results were found when the data were analyzed as cells/cm². Because no significant main effects were detected for the ANOVAR, power analyses were conducted for each repeated measure (i.e., for each sampling day). The power was low (~0.14–0.28) primarily because the effect sizes were low compared to the standard deviations (Cohen 1988). However, if differences between means had been larger (e.g., effect sizes > 0.80), then power would have been considerably higher, even with the reported variance within replicates.

Epilithic TdR uptake

TdR uptake increased significantly ($p < 0.001$) throughout colonization; especially between days 10 and 25. TdR uptake by bacteria

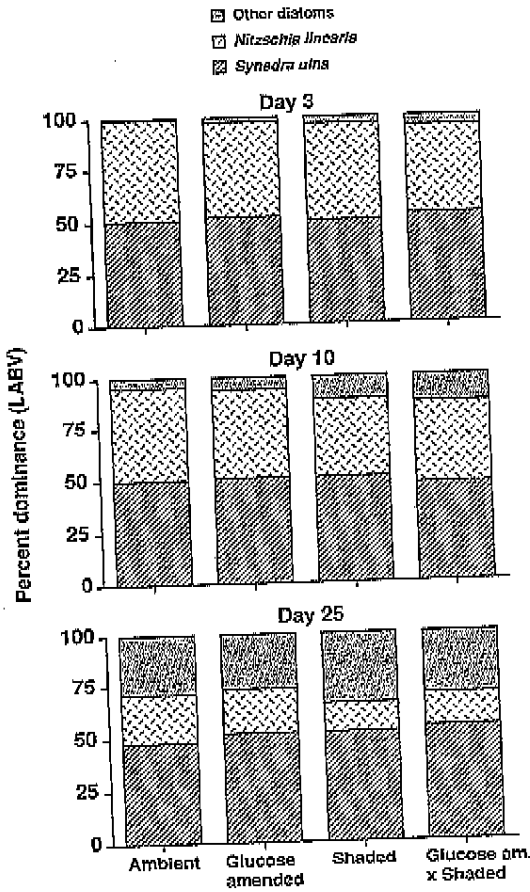

FIG. 3. Percent dominance in terms of LABV for dominant diatom species (*Synedra ulna* and *Nitzschia linearis*) on biofilm colonization days 3, 10, and 25.

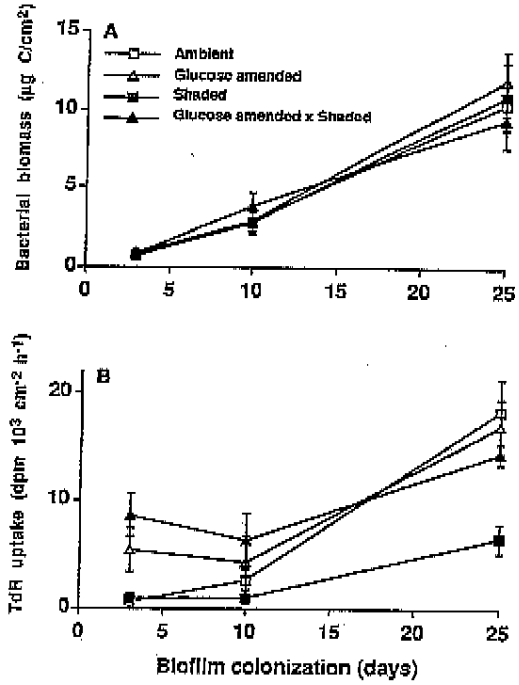

FIG. 4. Epilithic bacterial biomass (A) and [^{3}H]thymidine uptake (TdR uptake) (B) through biofilm colonization. Each point is the mean (±1 SE, n = 3) for each treatment.

showed a significant glucose × time interaction as well ($p < 0.01$) (Table 1, Fig. 4B). A glucose effect is evident early in colonization, especially on day 3 (Fig. 4B). On day 3, treatments receiving labile DOC showed more than 5-fold increases in TdR uptake. The glucose effect is less evident on day 10 when TdR uptake in the glucose amended channels decreases slightly. On day 25, the channels that were shaded and not amended with DOC had a TdR uptake one-third that of channels with either high algal abundance or labile DOC (Fig. 4B).

Discussion

Algal response

The manipulation of light and labile DOC resulted in contrasting environments for biofilm development. The shade treatment reduced algal biomass as was evident from estimates of AFDM, chlorophyll *a*, and LABV (Fig. 2). Conversely, the labile DOC treatment did not significantly alter these parameters, except for chlorophyll *a* on day 25. A possible mechanism for the modest chlorophyll *a* inhibition by glucose on day 25 is not apparent, and the difference between the unshaded and shaded treatments is large. Hence, the discrepancy in chlorophyll *a* between the two unshaded treatments on day 25 does not appear to confound treatments within the factorial experimental design.

LABV was dominated by *Synedra ulna* and *Nitzschia linearis* on all 3 sampling dates; however this dominance is less conspicuous on day 25 when additional species contributed ~25% of the LABV (Fig. 3). *Synedra ulna* and *Nitzschia linearis* are relatively large diatoms and may have settled into the boundary layer and become embedded in the biofilm matrix more readily than smaller diatom species (Oemke and Burton 1986, Steinman and McIntire 1986). Once embedded into the biofilm matrix, *Synedra ulna* and *Nitzschia linearis* may out-compete other diatom species for light because of their size, and motility in the case of *Nitzschia linearis*. Although these dominant species constitute most of the live-algal biovolume and most likely are responsible for a large portion of the chlorophyll *a*, their large individual size may not be as conducive to supporting benthic bacteria as smaller diatoms because of their relatively small surface area/volume ratio. Further, diatom assemblages consisting of smaller diatoms may have larger chlorophyll *a*/LABV ratios. This effect of size may explain the absence of an increase in LABV in the unshaded treatments between day 10 and day 25, even though AFDM and chlorophyll *a* increased more than 2-fold.

Overall, the algal response established the desired conditions necessary to test the experiment's hypothesis.

Bacterial response

TdR uptake responded to increased labile DOC early in colonization, yet responded to both the presence of algae (non-shade treatment) and increased DOC in late colonization. Glucose additions prior to the first sampling date had a greater effect on TdR uptake than algal biomass, which was low at the time. The modest decline in TdR uptake between days 3 and 10 in the glucose-amended treatments is difficult to explain; however TdR uptake in these treatments still exceeded TdR uptake in

the treatments that were not amended with glucose. As algal abundance increased by day 25, the TdR uptake supported by the epilithic algae equalled TdR uptake supported by glucose additions (Fig. 4). These results support the hypothesis that allochthonous DOC has a maximum effect on bacteria early in biofilm development but can be equalled by algal carbon once the biofilm approaches maturity. The fact that high epilithic algae plus glucose did not lead to further increases in TdR uptake suggests that a maximum productivity was reached for the given environments. It is possible that bioavailable carbon was not limiting bacterial production in these treatments by the final date. Factors other than bioavailable carbon (e.g., inorganic nutrients) may limit, or at least influence, bacterial production in stream biofilms, but most of the literature on stream biofilms suggests that stream bacteria are carbon limited (see Lock 1993).

Although increased bacterial biomass was predicted in the labile-DOC-amended channels early in colonization and in the non-shaded (increased algae) channels late in colonization, significant treatment differences were not detected. These results were surprising, especially given the TdR uptake results. Obvious discrepancies in treatment effects between bacterial biomass and productivity were not anticipated; other studies have demonstrated a relationship (Cole et al. 1988, White et al. 1991, Findlay et al. 1993). Three possible reasons for the discrepancy between bacterial biomass and TdR uptake can be postulated: 1) variation in isotopic dilution among treatments and sampling dates, 2) accumulation of non-viable cells from the water column throughout colonization, and 3) density-dependent grazing of bacteria by microorganisms.

Although spatial and temporal variations in exogenous thymidine can influence estimates of labelled thymidine incorporation into bacterial DNA (Kaplan et al. 1992, Chrzanowski et al. 1993, Findlay 1993), significant variation among treatments and sampling dates is unlikely in this experiment because of the following reasons: 1) the same water source (Augusta Creek) was used for all treatments and productivity incubations, 2) Augusta Creek was at baseflow throughout the 25-d experiment, 3) DOC additions were void of thymidine, and 4) biofilms in all treatments were dominated by similar diatom assemblages. Significant variation in isotopic dilution is more likely to influence estimates of bacterial productivity in studies that compare disparate streams and sampling dates that span seasons; conversely, several studies have reported only modest variation in epilithic bacterial productivity among streams and sampling dates (Palumbo et al. 1989, Hudson et al. 1990, Findlay et al. 1993).

An alternative explanation for the discrepancies between estimates of bacterial biomass and TdR uptake is the accumulation of non-viable bacteria in the biofilm matrix. Rapid accumulation of bacterial cells from the water column may, in some cases, mask actual differences in bacterial production within biofilms. Bacterial biomass increased during colonization in all treatments, hence non-viable bacteria may have accumulated in biofilms over time. Biomass estimates based on direct microscopic counts do not readily differentiate between viable and non-viable cells; hence, only a portion of the bacteria counted may actually have been contributing to productivity (Palumbo et al. 1989). If only a small percentage of the total bacteria counted (e.g., 10%) was viable, then even statistically significant differences in productivity might not result in discernible differences in biomass. However, rapid turnover of bacterial biomass would make this explanation unlikely. If TdR uptake (i.e., dpm cm^{-2} h^{-1}) is converted into bacterial carbon production (i.e., μg C cm^{-2} h^{-1}) (see Findlay et al. 1984 and Findlay 1993), I can estimate a bacterial biomass turnover time (standing stock bacterial carbon/bacterial carbon production). For example, on colonization day 10 in the ambient channels (from which isotopic dilution was estimated), I estimate bacterial productivity to be 0.05 μg C cm^{-2} h^{-1} and bacterial biomass to be 2.5 $\mu g/cm^2$; hence bacterial biomass turnover is ~2 d (50 h). In other words, bacterial production of new biomass would equal the standing stock of bacterial biomass after ~2 d. Assuming that bacterial biomass turnover requires only a few days, changes in biomass should have been detected after a 25-d colonization period.

Another explanation for the absence of treatment differences in bacterial biomass is grazing by microorganisms within the biofilm. Grazers can crop bacterial biomass such that differences among treatments are not apparent when bacterial biomass is viewed as a series of "snap-

shots" in colonization time. Ciliates and microflagellates can significantly reduce bacterial biomass in streambed sediments (Bott and Kaplan 1990) and copepods can significantly reduce detritally associated bacteria (Perlmutter and Meyer 1991). Bott and Kaplan (1990) estimated that as much as 80% of annual benthic bacterial productivity may be consumed by grazing meiofauna. The possible importance of grazers in my study is suggested by the difference between potential and realized accumulation of bacterial carbon. Density-dependent grazing may have led to similar bacterial biomass among treatments, despite differences in bacterial productivity. Overall, our current understanding of the role that meiofaunal grazers play in structuring biofilm microbial communities is rudimentary.

Epilithic bacterial–algal relationships across stream ecosystems

Bacterial controls in most aquatic ecosystems have been well documented (see reviews by Cole et al. 1988, White et al. 1991, Sander and Kalff 1993). In both freshwater and marine planktonic environments, bacterial activity and abundance is generally thought to be regulated by planktonic algal productivity and abundance. Such a bacterial–algal relationship is less apparent in lake sediments where bacterial abundance appears to be regulated by allochthonous carbon, while most autochthonous carbon is used in planktonic microbial cycling (see cross-system comparison by Schallenberg and Kalff 1993). Hence, benthic bacteria in lakes are regulated primarily by inputs of more refractory allochthonous carbon. Although our knowledge of bacterial controls in stream ecosystems has grown considerably in the last decade (see reviews by Kaplan and Newbold 1993 and Lock 1993), an analogous conceptual model is lacking.

My study suggests that DOC in the water column may be controlling bacteria throughout biofilm colonization, with epilithic algae becoming increasingly important in late stages of biofilm development. Hence, epilithic bacteria in biofilms, which have colonization trajectories continually disturbed or "re-set" by scouring, grazing, or fluctuating discharge, may rely primarily on allochthonous DOC, whereas communities within a well-developed biofilm that supports high algal biomass may rely on both allochthonous and autochthonous carbon. In more oligotrophic streams, in which even mature biofilms are relatively thin and algal-poor, bacterial productivity may never be affected by benthic algae (Findlay et al. 1993). An appreciation of shifts in biofilm structure during colonization, limitations to algal productivity, and natural disturbance events will lead to a greater understanding of temporal and spatial variation in epilithic bacterial activity. Future work that examines how variations in DOC quality and quantity regulate epilithic bacteria among streams that span a range of algal productivity and stability is necessary to develop a general model of bacterial–algal linkages in stream ecosystems.

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