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Does nutrient enrichment decouple algal–bacterial production in periphyton?

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Abstract. Coupled production between algae and bacteria in stream epilithon was assessed along a nutrient-enrichment gradient in 8 Texas streams with open canopies. Photosynthesis (PS) and bacterial biomass production (BBP) were measured simultaneously using a dual-label radioassay (^{14}C-HCO_3^- uptake and ^3H-L-leucine incorporation into protein) on multiple samples within a stream reach. PS and BBP were measured after light (1200–1500 μmol m^{-2} s^{-1}) and dark incubations. The degree of coupled production between algae and bacteria within a stream was estimated as the covariation (i.e., correlation or covariance) between PS and BBP derived from unshaded replicates in each stream. Streamwater nutrients ranged from 0.18 to 8.1 mg/L total N and 0.009 to 2.0 mg/L total P. Epilithon N and P content (as % dry mass) and C:N:P ratios varied widely among streams and were positively correlated with streamwater nutrient concentrations. Mean BBP measured in light incubations (BBP_L) was greater than mean BBP measured in dark incubations (BBP_D), and the difference between the 2 means (BBP_L – BBP_D) was positively correlated with mean PS among streams ($R^2 = 0.53$). Covariance between PS and BBP_L within streams ($COV_{PS–BBP}$) decreased as epilithon nutrient content increased. $COV_{PS–BBP}$ was positively correlated with both epilithon C:N ($R^2 = 0.78$) and C:P ($R^2 = 0.77$) among streams. These results suggest that algal and bacterial production are decoupled by nutrient enrichment, and that algae might rely more heavily on bacterial-regenerated nutrients than on streamwater nutrients to support production in nutrient-poor streams.

Key words: algal–bacterial interaction, bacterial production, ecological stoichiometry, eutrophication, microbial interactions, nutrient criteria, nutrient ratios, nutrient regeneration, periphyton, photosynthesis, streams, water quality.

The inherent link between autotrophic and heterotrophic microbial production has been a recurring topic in aquatic ecology. In planktonic systems, the covariation between phytoplankton and bacterioplankton abundance and production often is a function of bacterial metabolism of phytoplankton-derived extracellular organic C (EOC) (Cole et al. 1982, Coveney 1982, Coveney and Wetzel 1989). However, bacterioplankton growth also can be limited by P (Toolan et al. 1991), and inorganic P is the preferred form of P for both phytoplankton and bacterioplankton (Cotner and Wetzel 1992). Therefore, 2 competing models of covariation have been adopted for pelagic systems. The 1st model suggests that phytoplankton production varies with P concentration, and bacterioplankton vary in response to phytoplankton. The 2nd model suggests that both phytoplankton and bacterioplankton respond simultaneously to P. However, patterns in P dynamics and phytoplankton and bacterioplankton production observed at multiple scales have not conformed consistently to either model. As a result, some researchers have speculated that the observed covariation depends on interactions involving multiple resources (i.e., P and C) used by, or pressures (i.e., grazing) experienced by, both algae and bacteria (Currie 1990, Coveney and Wetzel 1995).

Most microbial production in low- to mid-order stream ecosystems generally occurs in benthic biofilms (Vannote et al. 1980). As in planktonic systems, bacterial production in stream biofilms (namely epilithon) often is coupled to algal production and has been attributed to bacterial metabolism of algal EOC (Haack and McFeters 1982, Murray et al. 1987). However, streams with significant canopy cover, such

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as forested systems, often have heterotrophic biofilms that rely on allochthonous organic matter rather than algal-derived EOC (Findlay et al. 1993). Nevertheless, bacterial production usually is coupled with algal production in epilithon when light intensities are sufficient to stimulate benthic photosynthesis (Kaplan and Bott 1989, Jones and Lock 1993).

The involvement of nutrients in the interplay between autotrophic and heterotrophic production in stream epilithon has been debated, but it has been studied less than in planktonic systems. Rier and Stevenson (2001) found that algal biomass was the best predictor of bacterial cell densities and surmised that nutrients indirectly influenced bacteria by controlling algal biomass. They also found no quantitative relationship between algal biomass and bacterial density when algal biomass was <5 μg chlorophyll a/cm^2. Low algal biomass occurred primarily in nutrient-poor streams, suggesting a potential competitive interaction between algae and bacteria for nutrients. In a subsequent experimental study, Rier and Stevenson (2002) demonstrated that epilithic bacteria were colimited by organic C and inorganic nutrients, but they also found that competition for nutrients did not decrease algal and bacterial covariation. Carr et al. (2005) also found no evidence of competition between algae and bacteria for nutrients. Rier and Stevenson (2002) and Carr et al. (2005) suggested that the coupling between algae and bacteria in stream biofilms is related to the construction of the polysaccharide matrix, which provides ample buffering to EOC supply (Freeman and Lock 1995) and is an area of intense nutrient retention and recycling (Wetzel 1993, Scott et al. 2007). Rier and Stevenson (2002) went one step farther to suggest that nutrient-poor biofilms might exhibit stronger algal–bacterial coupling than nutrient-rich biofilms.

Based on the results of these studies, we present 2 hypotheses: 1) Algae and bacteria do not compete for nutrients in stream biofilms, but algal and bacterial production are tightly coupled through simultaneous cycling of C, N, and P. Increased nutrient availability decouples algal and bacterial production as algae become less reliant on bacterial remineralization of nutrients and bacteria become less stressed for photosynthetically derived EOC (Fig. 1A). 2) Algal–bacterial coupling decreases with increasing nutrient availability (as in hypothesis 1), but decoupling also occurs in very-low-nutrient environments because of algal–bacterial competition for nutrients (Fig. 1B). To test these hypotheses, we measured algal and bacterial production along a nutrient-enrichment gradient in several streams in Texas. We quantified the covariation between algal and bacterial production within each stream and assessed whether this covariation was correlated across streams with several metrics of nutrient availability to periphyton. Based on evidence from other systems (i.e., King and Richardson 2007), we suspected that epilithon nutrient content would track the gradient of nutrient enrichment in surface water. In particular, we were interested in relating the degree of coupling between epilithic algal and bacterial production to the nutrient content of the epilithon itself.

Methods

Study area

The study was conducted in the Cross Timbers Ecoregion within the Brazos River Basin in central Texas (Fig. 2). Eight wadeable and historically perennial streams were selected as candidates based on surface-water soluble reactive P (SRP) and total P (TP) concentrations as measured by the Texas Commission on Environmental Quality within the previous 5 y (TCEQ 2007). The candidate streams were intended to represent a continuum of nutrient enrichment. Reconnaissance sampling in June 2006 revealed that these streams were distributed along a steep nutrient gradient, spanning <10 μg/L to >1 mg/L TP. In a 3-wk period from July to August 2006, one 300- to 500-m study site was sampled in each stream to assess coupling between algal photosynthesis (PS) and heterotrophic bacterial biomass production (BBP), and C, N, and P content of epilithon. Surface-water total N (TN) and TP concentrations and discharge were measured in each site.

Field sampling

In each site, 5 rocks (~50 cm^2) were collected for simultaneous measurements of PS and BBP under high-light (1400–1700 μmol m^{-2} s^{-1}) conditions, 5 rocks were collected for PS and BBP measurements in dark incubations, and 2 rocks were collected to serve as killed (4% buffered formalin) controls. Rocks were collected from shallow pools (<0.3 m) and from areas of open canopy with high light exposure. Epilithon growing in unshaded environments does not generally experience photoinhibition (Boston and Hill 1991). The rocks in all streams were colonized by thin biofilms. Some small patches of macroalgae occurred sporadically in all streams, and these growth forms were intentionally avoided.

Twenty-five rocks were collected from each site (5 rocks × 5 locations/site) for measurement of epilithon ash-free dry mass (AFDM) and C, N, and P content. Epilithon was removed from rocks in the field. Rocks

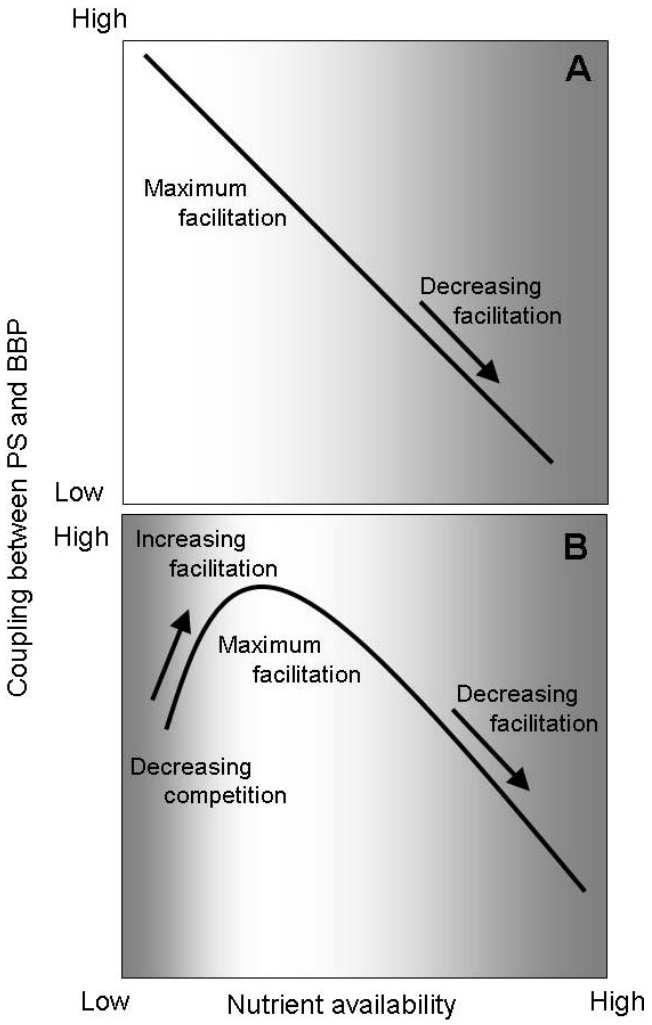

FIG. 1. Two alternative models (hypotheses) of photosynthesis (PS) and bacterial biomass production (BBP) coupling in stream epilithon along a nutrient-enrichment gradient. Areas of no shading within each graph indicate the zone of maximum facilitation (greatest coupling) along a nutrient gradient. A.—Coupling is highest in nutrient-poor streams and diminishes as nutrient availability increases. B.—Algae and bacteria are slightly decoupled in lowest-nutrient streams because of competition for nutrients, coupled under moderate-nutrient availability, and decreasingly coupled as nutrient availability increases.

were scraped with a knife, scrubbed with a toothbrush, and rinsed into a pan with stream water. Epilithon from the 25 rocks was pooled into a single sample, diluted to a known volume in a 1-L dark bottle, and returned to the laboratory. Rock surface area was measured by pressing aluminum foil over each rock and cutting the foil margin to match the size of the scraped area. We weighed the cleaned and dried foil in the laboratory and estimated rock surface area based on a foil mass–area relationship.

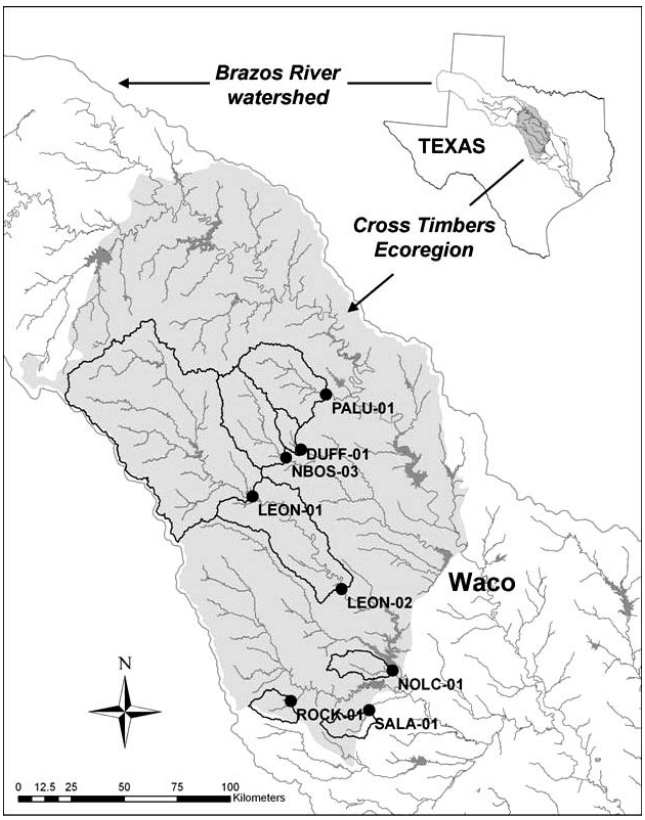

FIG. 2. Study sites in 8 wadeable streams in the Cross Timbers Ecoregion within the Brazos River watershed in central Texas. Watershed boundaries for each stream sampled are indicated with dark outline.

PS and BBP measurements

PS and BBP were estimated simultaneously on individual rocks using ^{14}C-HCO_3^- uptake and ^3H-L-leucine incorporation into protein, respectively. Rocks were put top-down into 60-mL wide-mouth jars. Jars were filled with filtered (0.2-μm pore size) stream water until no air space remained, and they were sealed with open-top caps lined with silicon septa. Rocks were inverted so that the jars could be placed upside down in streams for incubation. Five jars were left unwrapped for light incubation, and the other 5 were wrapped in aluminum foil for dark incubation. Killed controls were incubated in light.

Each jar was injected with 50 μL of ^{14}C-labeled $NaHCO_3$ solution (54 μCi/mL) and placed in the stream at a depth of 7 to 10 cm for 1.5 h. After that time, 50 μL of ^3H-labeled L-leucine were injected into each jar, and the incubation was continued for 30 min. Concentrations of leucine ranged from 500 to 580 nmol/L (specific activity = 0.166 μCi/nmol leucine, assuming negligible background leucine concentration), depending on the volume of rock being

incubated. Leucine concentrations >400 to 500 nmol/L were necessary to saturate leucine incorporation into protein in similar microbial communities (Thomaz and Wetzel 1995, Gillies et al. 2006, JTS, unpublished data). Incubations were stopped by adding buffered formalin to a final concentration of 4%. Samples were returned to the laboratory and stored at 4°C until processing.

Proteins in intact epilithon were precipitated with trichloroacetic acid (TCA). A previous study on metaphyton showed that TCA protein precipitation was necessary to maximize ^{3}H-L-leucine recovery and had little effect on ^{14}C retention by photoautotrophs (Scott and Doyle 2006). Incubation water was removed from each jar and replaced with 5% TCA. Jars were placed on ice for 1 h, after which samples were removed from TCA. Epilithon was scraped from rocks with a toothbrush, rinsed into a slurry with 5% TCA, and diluted to a known volume (30–50 mL) with 5% TCA. Samples were homogenized with a vortex mixer. A subsample was filtered onto a precombusted and weighed glass-fiber filter (GF/F; pore size = 0.7 μm) for determination of AFDM. A 2nd subsample was filtered onto a 0.2-μm-pore-size polycarbonate filter and washed twice with 5% TCA, once with 80% ethanol, and once with deionized (DI) water. These filters were placed in scintillation vials with 5 mL of alkaline solution (0.5 mol/L NaOH, 25 mmol/L ethylenediamine-tetra-acetic acid [EDTA], and 0.1% sodium dodecylsulfate) and agitated for 1 h at 85°C. Material attached to polycarbonate filters dissolved in the alkaline solution (Buesing and Gessner 2003). A 2.5-mL aliquot of this solution was radioassayed for ^{14}C and ^{3}H activity on a Beckman LS 6500 liquid scintillation counter (Beckman Coulter, Fullerton, California). Activities of both isotopes were corrected for quench with external standards and then converted from radioactivity estimates to HCO_3^- and leucine uptake rates based on the specific activity of each isotope used in incubations.

The specific activity of inorganic C in each sample was determined by combining the quantity of ^{14}C-labeled HCO_3^- with the quantity of unlabeled dissolved inorganic C (DIC) in incubation. DIC concentration in filtered incubation water was measured with a Shimadzu TOC-Vcsh C analyzer (Shimadzu Scientific Instruments, Columbia, Maryland). DIC concentration was multiplied by the volume of water in incubation jars to estimate the total quantity of inorganic C available. The volume of each individual rock was measured via liquid displacement and subtracted from 60 mL to arrive at the final water volume in incubation. The measured ^{14}C radioactivity of samples was converted into PS using the specific activity of inorganic C for all incubations. PS was normalized to the surface area of the rock from which the epilithon originated (see *Field sampling* section) or to measured AFDM and expressed as μg C cm^{-2} h^{-1} and μg C mg^{-1} AFDM h^{-1}, respectively (Wetzel and Likens 2000).

Background leucine concentration in incubation water was assumed to be negligible. Therefore, the specific activity of leucine was constant, and measured ^{3}H radioactivity was converted directly to leucine uptake. BBP was calculated from leucine uptake (nmol leucine/h) by assuming that the fraction of leucine in protein = 7.3%, cellular C/protein = 86%, and isotope dilution was negligible (Kirchman 2001). BBP was normalized to the surface area of the rock from which the epilithon originated (see *Field sampling* section) or to measured AFDM and expressed as μg C cm^{-2} h^{-1} or μg C mg^{-1} AFDM h^{-1}, respectively.

AFDM and nutrients

Epilithon from samples used in PS and BBP incubations was processed for AFDM only. Epilithon from the 25-rock composite sample collected from each stream was processed for determination of AFDM and C, N, and P content. The epilithon–streamwater slurry was homogenized using a blender and diluted to a known volume with DI water, and 3 subsamples were removed. While the epilithon slurry was mixed vigorously, a 2- to 5-mL subsample was transferred onto a precombusted (500°C, 4 h) and weighed GF/F for AFDM measurement. A 2nd 100- to 160-mL subsample was transferred into a large weighing boat. The water was allowed to evaporate, and the sample was dried at 60°C. The bulk sample was then homogenized into a powder for C, N, and P analysis. A 3rd subsample was transferred into a 50-mL centrifuge tube. Epilithon in these tubes was separated from inorganic material by centrifugation in colloidal Si using the method of Hamilton et al. (2005). Following separation and several rinsing steps to remove Si, organic matter (OM) samples were dried and homogenized for C, N, and P determination. C and N content in bulk epilithon and epilithon OM were measured simultaneously using a Thermo Finnigan FlashEA 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, Massachusetts). P content was measured colorimetrically on a Lachat Quickchem 8500 (Hach, Loveland, Colorado) following a 3-h digestion in concentrated H_2SO_4 at 350°C (APHA 1998). Total N and P content in water samples collected from each stream was measured colorimetrically on a Lachat Quickchem 8500 following either alkaline persulfate (N) or acid persulfate (P) digestions (APHA 1998).

TABLE 1. Water chemistry and flow at sampling locations. DIC = dissolved inorganic C.

Stream	Site code	DIC (mg/L)	Total N (μg/L)	Total P (μg/L)	Discharge(m^3/s)
Paluxy River	PALU-01	26.6	215.0	9.89	0.001
Rocky Creek	ROCK-01	19.9	176.3	10.0	0.009
Salado Creek	SALA-01	17.2	296.0	12.3	0.005
Duffau Creek	DUFF-01	16.7	507.3	24.4	0.0
North Bosque River	NBOS-03	8.92	1686	45.6	0.0
Leon River upstream	LEON-01	22.2	1197	62.7	0.0
Nolan Creek	NOLC-01	23.4	6925	1860	0.556
Leon River downstream	LEON-02	25.9	8110	2030	0.046

Data analyses

The amount of BBP stimulated by PS in each stream was calculated by subtracting the mean BBP rate measured in dark incubations (BBP_D) from the mean BBP rate measured in light incubations (BBP_L) (mean BBP_L – mean BBP_D). The respective errors were propagated additively. Model II major-axis regression was used to assess the relationship between (mean BBP_L – mean BBP_D) and mean PS because our predictor (mean PS) and response (mean BBP_L – mean BBP_D) variables were random estimates with error (Legendre and Legendre 1998). Statistical tests on slope and *y*-intercept were conducted using 999 random permutations of our data. A FORTRAN program available online (Casgrain and Legendre 2007) was used for this analysis because model II regression is not available in most standard statistical software packages. The relationship between PS and BBP_L across all streams was assessed using ordinary least squares (OLS) regression (Proc GLM, SAS version 9.1; SAS Institute, Cary, North Carolina). The covariance value between PS and BBP_L within each stream reach was calculated as

$$\mathrm{COV}_{\mathrm{PS-BBP}} = \sum_{i=1}^{n} \frac{(\mathrm{PS}_i - \mathrm{PS})(\mathrm{BBP}_{\mathrm{L}i} - \mathrm{BBP}_{\mathrm{L}})}{n},$$

where *i* is the incubation replicate. The correlation coefficient ($CORR_{PS–BBP}$) was the covariance divided by the product of individual standard deviations (SD) of PS and BBP_L. OLS regression was used to determine how strongly each metric of PS–BBP_L coupling (i.e., correlation and covariance) was related to streamwater nutrient concentrations and epilithon (bulk and OM) nutrient content. Negative relationships were expected between PS–BBP_L coupling metrics and streamwater and epilithon nutrient concentrations. Positive relationships, indicative of decreasing covariance between PS and BBP_L with increasing nutrient content in epilithon, were expected between PS–BBP_L coupling and epilithon C:N or C:P.

Results

Habitat and epilithon elemental composition

Streamwater nutrient concentrations confirmed that a strong nutrient-enrichment gradient existed among sites. TP levels ranged from <10 μg/L to >2 mg/L, and TN levels ranged from <200 μg/L to >8 mg/L among sites (Table 1). DIC concentrations varied little among sites, with the exception of NBOS-03, where DIC was 2× to 3× lower than in other sites. Three sites, DUFF-01, LEON-01, and NBOS-03, had substantial standing water but no detectable surface flow during sampling events. Surface flow was only slightly above detection levels at PALU-01, ROCK-01, and SALA-01, but it was relatively high in sites below wastewater discharges (LEON-02 and NOLC-01) (Table 1).

Epilithon elemental composition also confirmed the existence of a nutrient-enrichment gradient. Epilithon bulk P content ranged from 0.028% at ROCK-01 to 0.224% at NOLC-01, and bulk N content ranged from 0.28% at PALU-01 to 1.4% at NOLC-01 (Table 2). Epilithon OM P content ranged from 0.030% at ROCK-01 to 0.235% at NOLC-01, and OM N content ranged from 0.76% at ROCK-01 to 1.98% at LEON-02. Both bulk and OM C content were highly variable among sites but did not appear to be related to the nutrient-enrichment gradient (Table 2). Epilithon OM nutrient content was more similar among streams than were streamwater nutrient concentrations. For instance, sites with very similar streamwater P (PALU-01, ROCK-01, and SALA-01; Table 1) had decidedly different OM P contents (Table 2). Epilithon AFDM was variable among sites, but this variation was not explicitly related to differences in nutrient levels among sites (Table 2).

Epilithon PS and BBP_L

PS and BBP_L were highly variable among sites (Table 3). BBP_L rates were always greater than BBP_D rates, and mean BBP_L – mean BBP_D was positively correlated with mean PS across all sites (Fig. 3).

TABLE 2. C, N, and P content (% of dry mass) measured from bulk epilithon and epilithon organic matter (OM) separated by centrifugation. Values represent a single composite sample from 25 rocks sampled in each stream reach. Mean (±1 SD) ash-free dry mass (AFDM) is of epilithon from 12 individual rocks used in ^{14}C and ^{3}H assays (5 replicate light incubation, 5 replicate dark incubation, 2 killed controls). IS = insufficient amount of sample available for analysis.

Site code	% bulk C	% bulk N	% bulk P	% OM C	% OM N	% OM P	AFDM (mg/cm^2)
PALU-01	8.61	0.28	0.036	IS	IS	0.080	0.99 ± 0.32
ROCK-01	13.57	0.55	0.028	14.99	0.76	0.030	0.67 ± 0.19
SALA-01	18.53	0.75	0.030	18.11	0.98	0.050	1.39 ± 0.27
DUFF-01	7.83	0.45	0.050	12.38	0.82	0.073	1.39 ± 0.40
NBOS-03	12.42	0.77	0.117	18.19	1.45	0.142	1.87 ± 0.65
LEON-01	8.42	0.62	0.068	12.71	1.10	0.090	1.35 ± 0.59
NOLC-01	16.14	1.38	0.224	19.19	1.68	0.235	0.85 ± 0.21
LEON-02	11.25	0.86	0.100	33.00	1.98	0.189	1.21 ± 0.33

Epilithon PS and BBP_L values in individual replicates were positively correlated across streams sites regardless of whether surface area (Fig. 4A) or AFDM (Fig. 4B) was used to normalize PS and BBP_L. However, the relatively large amount of epilithon biomass at NBOS-03 (Table 2) exerted disproportional influence on areal production rates. When this site was excluded from the regression analysis of area-normalized rates, the slope of the linear fit decreased from 0.4 to 0.16, and the amount of BBP_L variation explained by PS increased by 23% (Fig. 4A). In a similar analysis using the AFDM-normalized rates, slope did not change, but the amount of explained variation increased by 20% (Fig. 4B).

Replicate data within sites indicated that PS and BBP_L were positively correlated in all streams, with the exception of area-normalized rates at DUFF-01 (Table 3). Mean correlation coefficients for PS and BBP_L among sites were similar for area-normalized ($r = 0.78 \pm 0.42$ SD) and AFDM-normalized ($r = 0.79 \pm 0.26$ SD) rates. PS–BBP_L covariance was much more variable, and covariance of area- and AFDM-normalized rates ranged across 4 orders of magnitude (Table 3). Average area-normalized PS–BBP_L covariance was $1.6 \times 10^{-3} \pm 1.8 \times 10^{-3}$, whereas average AFDM-normalized PS–BBP_L covariance was $2.3 \times 10^{-3} \pm 4.7 \times 10^{-3}$.

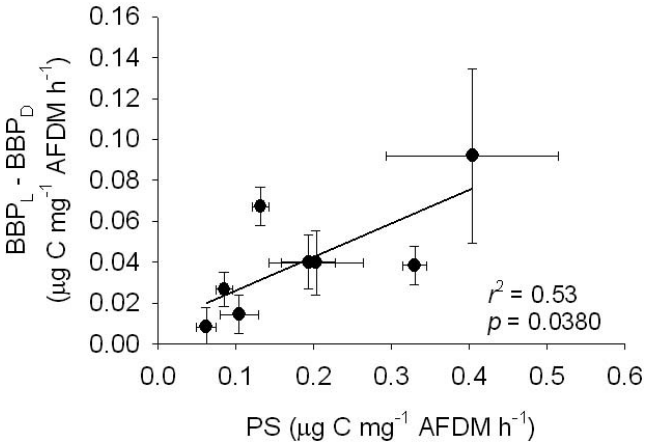

FIG. 3. Major-axis regression of mean (±1 SE) difference between bacterial biomass production in light (BBP_L) and in dark (BBP_D) incubations ($BBP_L - BBP_D$) versus photosynthesis (PS) of epilithon at sites in 8 Texas streams along a nutrient-enrichment gradient. Data were normalized relative to AFDM of the epilithon; $n = 5$ in each 8 streams.

PS and BBP_L covariation along nutrient-enrichment gradient

Correlation coefficients ($CORR_{PS–BBP}$) and covariance values ($COV_{PS–BBP}$) for area-normalized PS and BBP_L rates were not correlated with any measure of nutrient concentration. $CORR_{PS–BBP}$ for AFDM-normalized PS and BBP_L rates also were not correlated with any measure of nutrient concentration. However, $COV_{PS–BBP}$ values for AFDM-normalized PS and BBP_L ($COV_{PS–BBP,AFDM}$) were correlated with multiple metrics of nutrient concentration across sites.

$COV_{PS–BBP,AFDM}$ was not significantly related to streamwater TN (Fig. 5A) or TP (Fig. 5B), epilithon bulk % N (Fig. 5C) or bulk % P (Fig. 5D), or to epilithon bulk C:N (Fig. 5E). In 2 streams, study sites were directly downstream of wastewater inputs. Data from these 2 sites were particularly influential in several of these regressions (white circles; Fig. 5A–F). Exclusion of these streams from the regression analyses improved the relationship between $COV_{PS–BBP,AFDM}$ and streamwater TN and TP (Fig. 5A, B), and epilithon bulk % P and bulk C:P (Fig. 5D, E, respectively), but only the relationship between $COV_{PS–BBP,AFDM}$ and bulk % P was statistically significant after excluding these streams (Fig. 5D). The relationship between $COV_{PS–BBP,AFDM}$ and bulk C:P was statistically significant regardless of whether wastewater-influenced streams were includ-

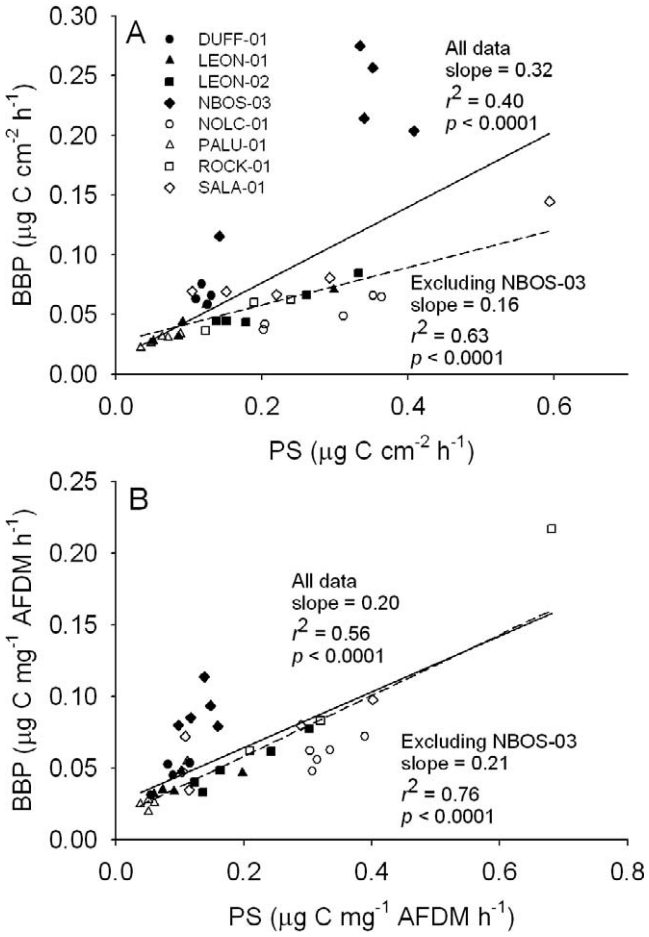

FIG. 4. Linear regression of bacterial biomass production in the light (BBP_L) versus photosynthesis (PS) normalized relative to surface area (A) and ash-free dry mass (AFDM) (B) of epilithon on 5 individual rocks at sites in 8 Texas streams along a nutrient-enrichment gradient. Solid lines are regressions based on data from all streams. Dashed lines are regressions based on data that excluded site NBOS-03. Site codes are given in Table 1.

ed, but the relationship was much stronger when wastewater-influenced streams were excluded (Fig. 5F).

$COV_{PS–BBP,AFDM}$ was not significantly related to OM % N regardless of whether the wastewater-influenced streams were included (Fig. 6A). $COV_{PS–BBP,AFDM}$ was not significantly related to OM % P when all sites were included, but it was significantly negatively related to OM % P when wastewater-impacted streams were excluded (Fig. 6B). $COV_{PS–BBP,AFDM}$ was significantly positively related to OM C:N and C:P (Fig. 6C, D). Exclusion of wastewater-influenced sites had little effect on the relationship between $COV_{PS–BBP,AFDM}$ and OM C:N (Fig. 6C) but strengthened the relationship between $COV_{PS-BBP,AFDM}$ and OM C:P (Fig. 6D).

Discussion

The objective of our study was to assess how nutrient availability was related to coupling between algal and bacterial production in stream epilithon. We hypothesized that: 1) nutrient enrichment would decrease the covariation between algal and bacterial production, and 2) covariation between algal and bacterial production would diminish in nutrient-poor epilithon because of competition for nutrients. Our results support hypothesis 1 but not hypothesis 2. $COV_{PS–BBP,AFDM}$ decreased with increasing nutrient concentration, and the relationship was particularly strong when comparing $COV_{PS–BBP,AFDM}$ with epilithon OM C:N and C:P (Fig. 6C, D). This result suggests that algae (and perhaps both algae and bacteria) rely on regenerated nutrients to support production in nutrient-poor streams, but they rely less on regenerated nutrients for production as nutrient availability increases. This paradigm has been well established in oceanic plankton communities (sensu Dugdale and Goering 1967), but it has not been well

TABLE 3. Mean (±1 SD) photosynthesis (PS) and bacterial biomass production in the light (BBP_L) for all streams and multiple metrics for estimating the relationship between PS and BBP_L at each site. $CORR_{PS–BBP}$ is the correlation coefficient between PS and BBP_L in light incubations in each stream. $COV_{PS–BBP}$ is the covariance between these variables in each stream. Metrics were calculated for rates normalized to surface area of rocks and epilithon ash-free dry mass (AFDM).

Site code	PS (μg C cm^{-2} h^{-1})	BBP_L (μg C cm^{-2} h^{-1})	$CORR_{PS–BBP,area}$	$COV_{PS–BBP,area}$	$CORR_{PS–BBP,AFDM}$	$COV_{PS–BBP,AFDM}$
PALU-01	0.061 ± 0.021	0.029 ± 0.005	0.96	7.45×10^{-5}	0.96	3.03×10^{-4}
ROCK-01	0.184 ± 0.059	0.053 ± 0.014	1.0	5.24×10^{-4}	1.0	1.37×10^{-2}
SALA-01	0.272 ± 0.193	0.086 ± 0.033	0.97	4.92×10^{-3}	0.88	2.30×10^{-3}
DUFF-01	0.121 ± 0.009	0.066 ± 0.007	–0.24	-7.52×10^{-6}	0.86	1.64×10^{-4}
NBOS-03	0.315 ± 0.101	0.213 ± 0.062	0.76	3.77×10^{-3}	0.21	6.70×10^{-5}
LEON-01	0.130 ± 0.010	0.046 ± 0.018	0.86	1.25×10^{-3}	0.64	2.44×10^{-4}
NOLC-01	0.287 ± 0.079	0.052 ± 0.013	0.92	7.81×10^{-4}	0.79	2.02×10^{-4}
LEON-02	0.212 ± 0.082	0.057 ± 0.018	0.99	1.19×10^{-3}	0.99	1.06×10^{-3}

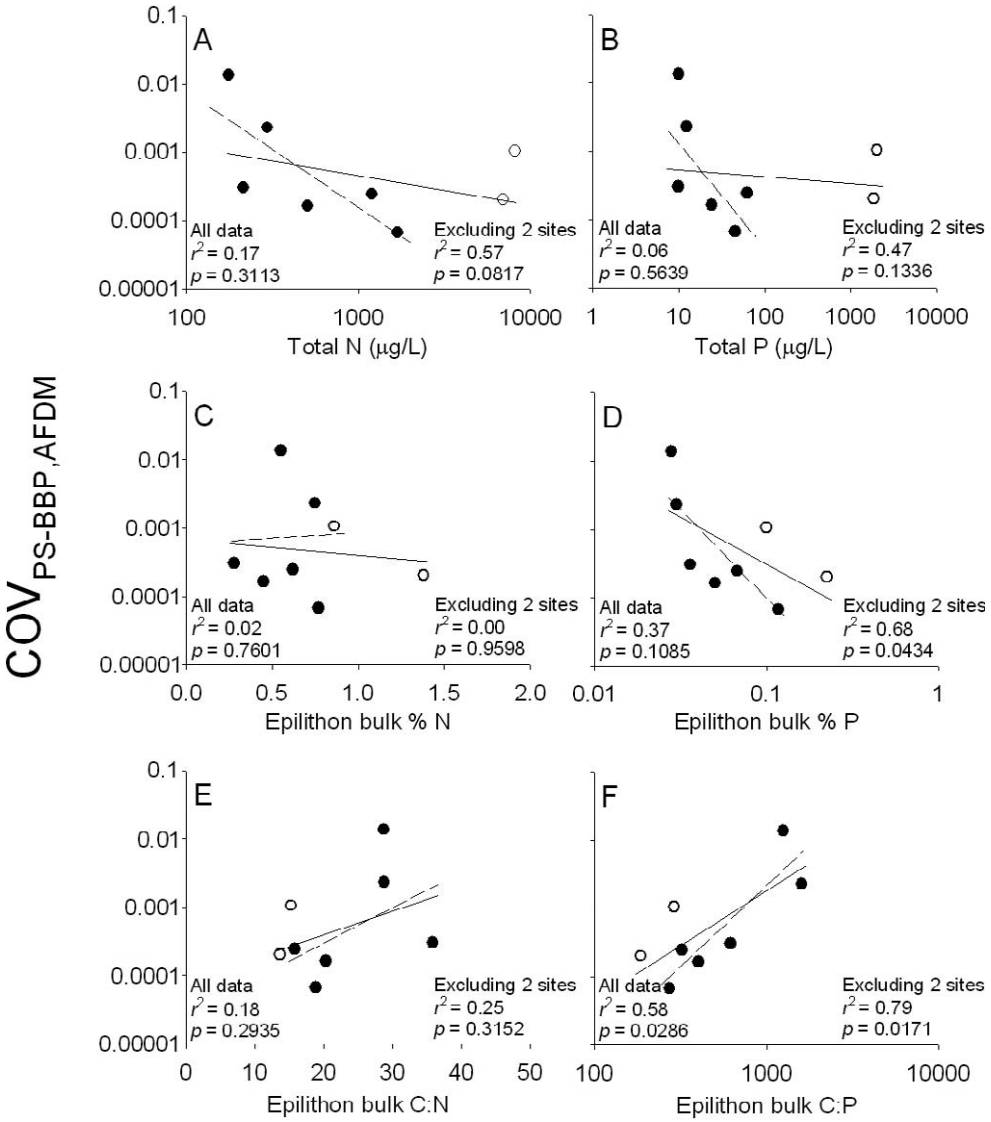

FIG. 5. Linear regression of the covariance of photosynthesis (PS) and bacterial biomass production in the light (BBP_L) normalized to epilithon ash-free dry mass (AFDM) ($COV_{PS–BBP,AFDM}$) vs streamwater total N (TN) (A) and total P (TP) (B), epilithon bulk % N (C) and % P (D) content, and epilithon bulk C:N (E) and C:P (F) in 8 Texas streams along a nutrient-enrichment gradient. Solid lines are regressions based on data from all streams. Dashed lines are regressions based on data excluding 2 streams from which sites were directly below wastewater discharges (open circles; NOLC-01 and LEON-02). Site codes are given in Table 1; $n = 5$ rocks/stream for $COV_{PS\text{-}BBP,AFDM}$.

demonstrated in attached microbial communities, such as epilithon.

We found no evidence that competitive interactions decoupled algal and bacterial production in nutrient-poor streams. In fact, $COV_{PS–BBP,AFDM}$ was greatest in the most nutrient-poor streams, and this result is similar to a model proposed for planktonic microbial communities (Cotner and Biddanda 2002). The lack of competitive decoupling between epiphytic algae and bacteria also is consistent with results of other studies on epilithic stream biofilms (Rier and Stevenson 2002, Carr et al. 2005), which provide support for the existence of mutually beneficial interactions between microbial autotrophs and heterotrophs. Efficient recycling of C, N, and P within biofilm matrices has long been suspected as an important driver of community function in biofilms (Wetzel 1993). A recent stable-isotope study of metaphyton N cycling provides support for this idea (Scott et al. 2007). Larned et al. (2004) demonstrated that mass transfer of nutrients into epilithon is the most likely rate-limiting step in nutrient transfer from stream water into epilithic algal cells. Therefore, nutrient regeneration in biofilms might be the most significant process affecting nutrient

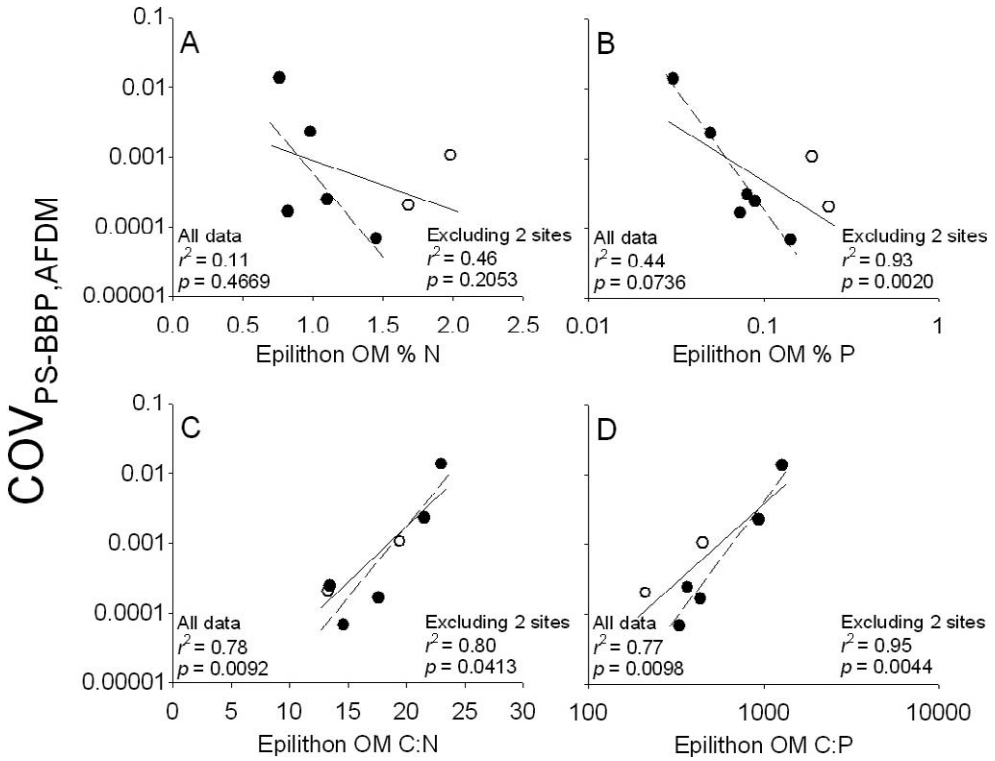

FIG. 6. Linear regression of the covariance of photosynthesis (PS) and bacterial biomass production in the light (BBP_L) normalized to epilithon ash-free dry mass (AFDM) ($COV_{PS–BBP,AFDM}$) vs epilithon organic matter (OM) % N content (A), % P content (B), C:N (C), and C:P (D) in 8 Texas streams along a nutrient-enrichment gradient. Solid lines are regressions based on data from all streams. Dashed lines are regressions based on data excluding 2 streams from which sites were directly below wastewater discharges (open circles; NOLC-01 and LEON-02). Site codes are given in Table 1; $n = 5$ rocks/stream for $COV_{PS-BBP,AFDM}$.

availability to cells growing within its polysaccharide matrix.

Influences of streamwater nutrients vs epilithon nutrients on algal–bacterial coupling

$COV_{PS–BBP,AFDM}$ was more strongly related to epilithon OM nutrient content than to epilithon bulk nutrient content or streamwater nutrient concentrations. Previous studies exploring algal–bacterial covariation along nutrient gradients have used primarily streamwater nutrient concentrations as metrics of nutrient availability (Carr et al. 2005). However, epilithon nutrient content, including extracellular nutrients within the biofilm matrix, integrate short-term temporal changes in streamwater nutrient availability. Therefore, epilithon nutrient content might be a more representative indicator of average stream conditions than a single water sample. In our study, epilithon nutrient content, and OM nutrient content in particular, differed more among streams than did streamwater nutrient concentrations. This contrast was particularly evident in streams with low streamwater nutrient concentrations. Thus, our study indicates that epilithon nutrient content is a better indicator of nutrient availability to biofilm microorganisms than are streamwater nutrient concentrations.

The strongest correlations with $COV_{PS–BBP,AFDM}$ were revealed when epilithon OM was explicitly isolated and elemental ratios of this organic fraction (e.g., C:N and C:P) were used. Epilithon from wastewater-impacted streams had the highest % N and % P, but also the highest % C. Therefore, C:N and C:P values in epilithon of wastewater-impacted streams were higher than in other streams, even though wastewater-impacted streams had the most nutrient-rich epilithon (as a percentage of dry mass). This result suggests that epilithon C content in wastewater-impacted streams might be influenced by the dissolved organic C available in effluent. For example, $COV_{PS–BBP,AFDM}$ was higher than expected for wastewater-impacted streams when evaluated using streamwater nutrient (Fig. 5A, B), bulk epilithon nutrient (Fig. 5C, D), or epilithon OM nutrient (Fig. 6A, B) models. However, elemental ratios accurately predicted $COV_{PS–BBP,AFDM}$ across all streams, particularly when derived from epilithon OM (Fig. 6C, D). Thus, a stoichiometrically explicit approach (sensu

Sterner and Elser 2002) might be particularly useful for understanding trophic dynamics in stream microbial communities (Frost et al. 2002, 2005).

Nutrient regeneration as a basis for trophic coupling

Based on evidence gathered in this and other studies (Cotner and Biddanda 2002, Rier and Stevenson 2001, 2002, Carr et al. 2005), we suggest that nutrient deficiency strengthens trophic coupling between algae and bacteria in the epilithon of oligotrophic streams through the interdependence of C, N, and P cycles within the biofilm matrix. For example, bacterial metabolism of algae-derived EOC is supported by studies that show increased bacterial extracellular enzyme activity associated with algal photosynthesis (Espeland et al. 2001, Francoeur and Wetzel 2003). Furthermore, the decomposition of P-containing compounds at night, or during periods of low photosynthetic activity, is supported by observations of concomitant increases in bacterial phosphatase activity (Espeland and Wetzel 2001). Rier et al. (2007) found that bulk phosphatase (derived from algae, bacteria, and fungi) activity remained high at night in stream periphyton growing on inert substrata and on leaf detritus in a high-light environment. Therefore, sustained bulk phosphatase (Rier et al. 2007) or increased bacterial phosphatase (Espeland and Wetzel 2001) activity coupled with decreased algal uptake of P at night (Reshkin and Knauer 1979) would result in substantially higher bacterial P uptake or P accumulation in the biofilm matrix. P accumulation at night could fuel P-limited photosynthesis and bacterial EOC decomposition in the subsequent daylight hours. Other studies have suggested that bacteria specifically control P recycling within biofilm communities. Sharma et al. (2005) found that phosphatase activity was spatially segregated from chlorophyll-containing microorganisms in metaphyton from the strongly P-limited Florida Everglades. They hypothesized that bacteria provided the primary mechanism for P remineralization, which directly increased algal photosynthesis and subsequently EOC production within metaphyton.

Recent studies have indicated that dissolved biochemicals such as deoxyribonucleic acid (DNA) can be an important source of P in marine sediments (Dell'Anno and Danavaro 2005) and that bacteria might use extracellular DNA specifically as a nutritional supplement (Palchevskiy and Finkel 2006). Furthermore, decomposition of biochemical aggregates, which are likely to include P-rich lipids, might provide another important source of P recycling in biofilms (Wotton 2007).

The same argument might be presented for N cycling. However, fewer studies on N recycling in periphyton exist, and results of those studies are inconsistent. For instance, Francoeur and Wetzel (2003) found that experimental periphyton communities exhibited higher leucine-aminopeptidase (LAMP) activity when grown in dark conditions with low dissolved inorganic N (DIN) availability. However, the addition of glucose resulted in higher LAMP activity in DIN-deficient periphyton grown under lighted conditions. Furthermore, LAMP activity in natural periphyton communities was affected inconsistently by light, glucose, and DIN additions (Francoeur and Wetzel 2003). However, Scott and Doyle (2006) demonstrated that bacterial production and algal production were strongly coupled in nutrient-deficient metaphyton, but they became decoupled following N enrichment.

Interactions between microbial autotrophs and heterotrophs no doubt exist, and growing evidence from photosynthesis, microbial enzyme linkages (Romaní and Sabater 1999, Espeland et al. 2001, Francoeur and Wetzel 2003, Francoeur et al. 2006, Rier et al. 2007), and coupled algal–bacterial production studies (Scott and Doyle 2006, our study) suggest that mutually beneficial interactions might be common. In mutualistic interactions, bacteria facilitate increased algal production via nutrient regeneration, and algae in turn, facilitate increased bacterial production via increased EOC generation. Mutualism in this form might increase production efficiencies, support the relatively high and sustained production observed in nutrient-poor systems (Wetzel 1993), and exert substantial influence on the energy budgets of oligotrophic ecosystems (Vadeboncoeur et al. 2002).

Spatial heterogeneity within streams and sources of variation among streams

Our results support the idea that epilithon functions as microbial landscapes (Battin et al. 2007). Both PS and BBP_L were highly variable within and among sites. In our study, the standard deviation of epilithic biomass on rocks within the same stream (determined from samples used in radioassays) was usually 30% to 50% of mean biomass value (Table 2). Furthermore, biomass substantially influenced both PS and BBP_L on individual rocks (Fig. 4A). However, AFDM-normalized PS and BBP_L still showed considerable variation within sites (possible sources of variation discussed next). We exploited this spatial variation to better understand the coupling between PS and BBP along the nutrient gradient. However, many studies overlook microscale variability and struggle to control the

substantial noise generated by spatial heterogeneity in ecological data. We echo the sentiments of Battin et al. (2007) and urge benthic microbial ecologists to exploit microscale heterogeneity in biofilm communities, which is undoubtedly important to ecological processes.

Our study was designed to identify only correlative relationships across a stream nutrient-enrichment gradient. Therefore, we could not control other factors that might have contributed to differences in epilithon metabolic activity and coupling between algal and bacterial production. We attempted to minimize differences in stream habitat conditions, but some variables exhibited substantial variation among streams. For instance, our intention was to sample open-canopy stream habitats with similar flow conditions, but an extended drought in Texas during the summer of 2006 resulted in diminished stream flow in most of our study streams and no measurable flow in some (Table 1). The 2 streams with greatest current velocity were those in which wastewater effluent provided most of the flow. Current velocity and discharge were not correlated directly with any measurements of algal and bacterial metabolism or epilithon nutrient content in our study. However, indirect effects of reduced flow, which might have contributed to measured variations between streams, such as boundary-layer conditions, periphyton thickness, and periphyton community composition (Stevenson 1996), were not explicitly examined.

The intentional focus of our study was on stream epilithon growing in open-canopy, high-light conditions. Similar patterns seem unlikely for periphyton growing in shaded habitats. Findlay et al. (1993) found that algae and bacteria were not strongly coupled in heterotrophic biofilms of forested streams. Nutrient cycling might be governed by similar mechanisms in shaded and unshaded periphyton communities, but the links between autotrophs and heterotrophs probably are damped by the reliance of heterotrophs on allochthonous organic matter.

Concluding remarks

Evidence from our study suggests that the degree of coupling between autotrophs and heterotrophs in periphyton decreases along a nutrient-enrichment gradient (Fig. 1A). However, decoupling does not appear to occur as a result of competition for nutrients in nutrient-poor streams (Fig. 1B). Because our study was correlative by design, these results should be confirmed with reach-scale or stream-mesocosm manipulative experiments. Our results support the idea of mutual facilitation between photoautotrophs and heterotrophic bacteria in aquatic microbial communities. Microbial mutualisms of this sort might support high sustained production in attached communities and heavily influence the energetic budgets of oligotrophic ecosystems. Nutrient enrichment of surface waters and accelerated eutrophication of aquatic ecosystems undoubtedly alter ecosystem functions. Our study provides another example of the effect of anthropogenic nutrient enrichment on aquatic ecosystem function by demonstrating that stream-nutrient enrichment can decouple algal and bacterial production in stream epilithon.

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