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Coupled photosynthesis and heterotrophic bacterial biomass production in a nutrient-limited wetland periphyton mat

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ABSTRACT: Simultaneous measurements of photosynthesis (PS) and bacterial biomass production (BBP) were made on floating periphyton mats using a dual-isotope radioassay (^{14}C-bicarbonate and ^{3}H-L-leucine). Measurements were made across a gradient of light intensity in nutrient-limited periphyton, and under saturating light in a nutrient enrichment experiment. Mean PS in nutrient-limited periphyton followed standard light-saturation kinetics, but mean BBP showed little or no relationship to light. However, individual measurements of PS and BBP were correlated when data from all light levels were combined. Laboratory experiments revealed that both N and P enrichment altered the relationship between PS and BBP. N enrichment increased average PS and BBP, but weakened the correlation between these variables. P enrichment did not statistically increase PS or BBP, but did increase the dispersion of these data in bivariate space. Although the relationship between PS and BBP in the P enrichment was weaker than the relationship observed in the control, PS and BBP remained more tightly coupled during P enrichment than in N enrichment. Results of the present study suggest that N enrichment (i.e. induced P-limitation) may have decoupled PS and BBP by causing a competitive interaction for inorganic P. P enrichment, however, may have further facilitated a cooperative relationship, whereby bacterial production depended on the supply of either photosynthetically derived extracellular organic carbon or perhaps N derived from light-dependent N_2 fixation.

KEY WORDS: Metaphyton · Algal–bacterial interaction · Nitrogen limitation · Phosphorus limitation

INTRODUCTION

Floating periphyton mats, also termed microbial mats (Paerl & Pinckney 1996), metaphyton (Stevenson 1996), or cyanobacterial mats (Rejmánková et al. 2004), are often conspicuous features of wetland and shallow lake environments. These consortia can provide key ecosystem services such as fixing large quantities of atmospheric N_2 (Rejmánková & Komárková 2000, Inglett et al. 2004), seasonal nutrient storage and retention (Scinto & Reddy 2003), and contributing to basal resources in the food web (Lamberti 1996). However, many aspects of the functioning of floating periphyton mats, and biofilms in general, have received little attention relative to the functioning of their pelagic counterparts (Vadeboncoeur et al. 2002).

Most studies on photosynthetic–bacterial interactions in aquatic systems have focused attention on bacterioplankton use of extracellular organic carbon (EOC) generated by phytoplankton during photosynthesis (PS) (i.e. Cole et al. 1982, Coveney & Wetzel 1989, Medina-Sánchez et al. 2004). Few studies have explored similar relationships in periphyton. Murray et al. (1986) were among the first to demonstrate increased rates of bacterial DNA synthesis in response to photosynthetically derived EOC in periphyton. In another study, Neely & Wetzel (1995) demonstrated a positive correlation between PS and bacterial biomass production (BBP) in periphyton using short-term incubations (2 h) across a range of photosynthetically active irradiance (20 to 400 µmol m^{-2} s^{-1}). Espeland et al. (2001) found that even low rates of PS under low light

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intensities (<100 µmol m^{-2} s^{-1}) could substantially increase the rate of BBP in periphyton after long-term exposure (≥8 h). Other studies have focused on the influence of periphytic PS on bacterial enzyme activity (Espeland et al. 2001, Francouer & Wetzel 2003), providing further evidence for the coupling of these processes. The majority of these studies, however, were carried out in the laboratory in nutrient-rich media and therefore did not consider any community interactions (commensal, mutual, or competitive) relative to nutrient stress.

Some studies on photosynthetic–bacterial interactions in periphyton have focused on periods when nutrient resources for periphyton were high as a result of decaying macrophyte biomass (Neely 1994, Neely & Wetzel 1997), or have focused on attachment substrate without regard to nutrients supplied by the substrate (Stanley et al. 2003). Furthermore, studies that have tested the effect of nutrient supply to periphyton communities have assumed that interactions between photoautotrophs and bacteria occur, but did not explicitly examine any relationships between PS and BBP (Haglund & Hillebrand 2005). Although it has been established that phytoplankton and bacterioplankton may compete for important nutrient resources in oligotrophic lakes (i.e. Chrzanowski et al. 1996), further research is needed to assess the type and degree of interaction (if any) displayed by photoautotrophs and bacteria in nutrient-limited periphyton communities.

With respect to nutrient supply, any competitive, commensalistic, or mutualistic interaction between periphytic photoautotrophs and bacteria is poorly understood. Recently, Sharma et al. (2005) used microscopic evidence on the spatial segregation of chlorophyll and phosphatase in Everglades periphyton mats to propose a cooperative interaction between photoautotrophs and bacteria in that system. They suggested that bacterial phosphatase activity might increase the availability of inorganic phosphorus to photoautotrophs, thereby increasing the rate of P-limited PS and subsequently the supply of 'photosynthetically fixed carbon' to bacteria. However, this hypothesis contrasts the findings and interpretations of other researchers. Specifically, Espeland & Wetzel (2001) suggested that periphytic bacteria regulated their phosphatase activity in response to photosynthetic production of EOC in order to maximize their competitive ability for phosphates.

In this study, we attempted to identify the degree of coupling between PS and heterotrophic BBP in a nutrient-limited periphyton mat. To that end, we measured PS and BBP simultaneously in a series of light and nutrient enrichment experiments. The objectives of the study were (1) to identify the degree of correlation between PS and BBP across a range of photosynthetically active irradiance in a nutrient-limited periphyton mat, and (2) to determine the simultaneous response of PS and BBP to increased supply of N and P.

MATERIALS AND METHODS

Study location and sampling. Periphyton samples were collected at the Lake Waco Wetland (LWW) complex, near Waco, Texas, USA. The floating periphyton community in this system generally comprises green algae (*Hydrodiction* sp. or *Cladophora* sp.) and a consortium of attached microbiota including diatoms, cyanobacteria, and heterotrophic bacteria (J. T. Scott unpubl.). Scott et al. (2005) demonstrated that the periphyton community in the downstream areas of the LWW was strongly nitrogen-limited and exhibited high rates of N_2 fixation. Furthermore, periphytic N_2 fixation in the LWW was light-dependent and generally carried out by heterocystous cyanobacteria or diatoms with cyanobacterial endosymbionts such as *Epithemia adnata* and *Rhopalodia gibba*. In this study, periphyton mat samples from the N-limited region of the LWW were collected by cutting approximately 100 cm^2 of material from the center of an intact mat which was then returned to the laboratory for experiments and radioassays. Samples were collected on 27 June 2005, 9 July 2005, and 13 July 2005 for use in a methodological experiment (^{14}C retention by photoautotrophs), a light saturation, and a nutrient enrichment experiment, respectively. Details of each of these experiments are provided below, following a brief description of the dual-labeled radioassay.

Dual-labeled radioassay. We used a dual-labeled radioassay similar to that of Neely & Wetzel (1995) to simultaneously measure PS and BBP in periphyton mats. PS was measured by ^{14}C-bicarbonate uptake and BBP was measured by ^{3}H-L-leucine incorporation into protein. Assays were conducted under *in situ* temperature conditions in the laboratory. Periphyton samples were subdivided into replicate samples by cutting small portions (~10 mg dry weight, DW) from the surface of a mat and transferring the material into borosilicate scintillation vials with Teflon open-top caps. Each vial was filled to capacity (~23.5 ml) with filtered site water. Replicate samples were acclimated to incubation conditions for 1 h prior to the addition of any isotope to establish experimental conditions (details of incubation conditions are provided in the experiment descriptions below). At the end of this acclimation period, 50 µl of ^{14}C-bicarbonate (final activity 0.0125 µCi ml^{-1}) were injected into each vial and incubations were continued for a period of 1.5 h. After that time, 20 µl of 500 nmol l^{-1} ^{3}H-L-leucine (3.4 nmol l^{-1} labeled, 496.6 nmol l^{-1} unlabeled; specific activity =

0.85 µCi nmol^{-1} leucine) were injected into each vial and incubations were continued for 30 min. Previous experiments on periphyton mats from the LLW indicated 500 nmol l^{-1} was sufficient to saturate leucine incorporation into protein (J. T. Scott unpubl.). Both formalin-killed controls and dark incubations were used in all experiments to account for background retention of both isotopes. At the end of the incubation period, assays were stopped in all samples by the addition of formalin to a final concentration of 2%. The precipitation of proteins for bacterial production measurements followed the method of Buesing & Gessner (2003). Trichloroacetic acid (TCA) was added to each vial to a final concentration of 5% by volume and samples were homogenized. Samples were placed on ice for 1 h to allow protein precipitation by cold TCA. A 10 ml aliquot of each sample was then filtered onto a pre-washed, dried, and weighed glass fiber filter. Filters were then dried at 60°C for 1 h and re-weighed to determine the DW of material used in the radioassay. A second 10 ml aliquot of the TCA-precipitated sample was filtered onto a polycarbonate filter (0.2 µm pore size) and washed, twice with 5% TCA, once with 80% ethanol, and once with deionized water. Polycarbonate filters were then placed in scintillation vials with an alkaline solution (0.5 mol l^{-1} NaOH, 25 mmol l^{-1} EDTA, and 0.1% sodium dodecyl sulfate) and shaken for 1 h at 85°C. Material attached to polycarbonate filters dissolved in the alkaline solution and a 5 ml aliquot of this solution was subsequently radioassayed for both ^{14}C and ^{3}H activity on a Beckman LS 6500 liquid scintillation counter. Measured activities of both isotopes were corrected for quench with external standards then converted to carbon and leucine uptake rates based on the specific activity of each isotope in the incubations. The rate of leucine uptake (nmol leucine h^{-1}) was converted to BBP by assuming the fraction of leucine in protein to be 7.3%, the cellular carbon per protein to be 86%, and that isotope dilution was negligible (Kirchman 2001). BBP was normalized to DW and expressed as µg C g^{-1} DW h^{-1}. The ratio of labeled to unlabeled bicarbonate in samples was calculated using the concentration of dissolved inorganic carbon (DIC) in filtered incubation water. DIC concentration of incubation water was estimated from measures of temperature, pH (Orion model 720 pH meter), and alkalinity (gran titration; Wetzel & Likens 2000). The rate of carbon uptake was normalized to DW and expressed as photosynthetic production in µg C g^{-1} DW h^{-1}.

Effect of TCA precipitation on autotrophic ^{14}C retention. TCA protein precipitation is needed to accurately determine the rate of ^{3}H-L-leucine incorporation into bacterial protein. However, this step is not generally used in ^{14}C-bicarbonate uptake assays for measuring PS. In a previous study on the use of dual-radioassays, Neely & Wetzel (1995) found that 44 to 66% of ^{14}C taken up by periphytic photoautotrophs was lost when samples were taken through a hot TCA precipitation step. To account for this potential loss in our assays, we tested the effect of the cold TCA precipitation/alkaline dissolution method (Buesing & Gessner 2003) on ^{14}C retention by photoautotrophs in periphyton mats. A ^{14}C-bicarbonate uptake radioassay was carried out on floating periphyton mat samples as previously described, but excluding ^{3}H-L-leucine additions. After samples were killed with formalin and homogenized, three 10 ml aliquots were subsampled from each vial. The first aliquot was used to measure the DW of radioassayed material and the second was taken through the TCA precipitation/alkaline dissolution method then radioassayed. The third aliquot was filtered onto a nitrocellulose filter (0.45 µm pore size), placed in a scintillation vial and allowed to dry overnight. After drying, 1.0 ml ethyl acetate was added to each vial to dissolve filters, samples were mixed thoroughly and radioassayed as described previously. Radioactivity in disintegrations per minute from paired samples were normalized to DW then compared using a paired *t*-test in SAS 9.1 (SAS 1999).

Light saturation of PS and relationship of PS to BBP. We tested the effect of increasing irradiance on PS and BBP using the dual-labeled radioassay method. Freshly collected periphyton samples were incubated under increasing photosynthetically active radiation (photon flux density [PFD] levels were 0, 19, 44, 94, 202, and 480 µmol m^{-2} s^{-1}) under *in situ* temperature conditions in the laboratory. The relationship PFD and PS in periphyton was modeled with Michaelis-Menten kinetics using nonlinear regression analysis in SigmaPlot 9.0 (SigmaPlot 2004). The effect of each light level and the effect of dark vs. pooled light levels on BBP were tested using a Kruskal-Wallis test in SAS 9.1 (SAS 1999). Finally, the correlation of PS and BBP across all light levels was tested using linear regression analysis on the paired samples in SigmaPlot 9.0 (SigmaPlot 2004).

Effect of nutrient enrichment on PS and BBP. The effect of nutrient enrichment on PS and BBP was tested using a completely randomized-nested design on laboratory enrichments of nitrogen (N) and phosphorus (P) in periphyton samples. Equal portions of a periphyton mat (ca. 2 g DW) were divided into 15 separate glass jars with 300 ml filtered site water (background DIN and PO_4-P were approximately 13 and 5 µg l^{-1}, respectively; see Scott et al. 2005). At random, 5 jars were enriched with N (664 µg l^{-1} NO_3-N), 5 more were enriched with P (20 µg l^{-1} PO_4-P), and 5 were left untreated to serve as a control. The enrichment concentrations were based on the average concentration of dissolved N and P in waters flowing into the wetland

(J. T. Scott unpubl.). Jars were randomly placed under artificial lights in a water bath in the laboratory and incubated at 26°C for 48 h under a 13.5:10.5 h light:dark cycle (PFD was 250 µmol m^{-2} s^{-1} during light period). At the end of 48 h, 3 replicate samples (~10 mg DW) were taken from each jar (nesting factor), put into borosilicate scintillation vials, and filled with newly prepared incubation water for each respective treatment (control, N, P). Samples were placed under artificial lights (PFD = 250 µmol m^{-2} s^{-1}) at 26°C and allowed to adjust to incubation conditions for a period of 1 h. Additionally, a single sample was randomly taken from 3 of the 5 experimental jars, enriched with the appropriate nutrient, and incubated at 26°C in the dark for 1 h. These samples were used to assess any dark uptake of ^{14}C and the rate of BBP without the influence of PS. At the end of the 1 h acclimation period, the dual-radioassay method was used to determine PS and BBP rates in all samples as previously described.

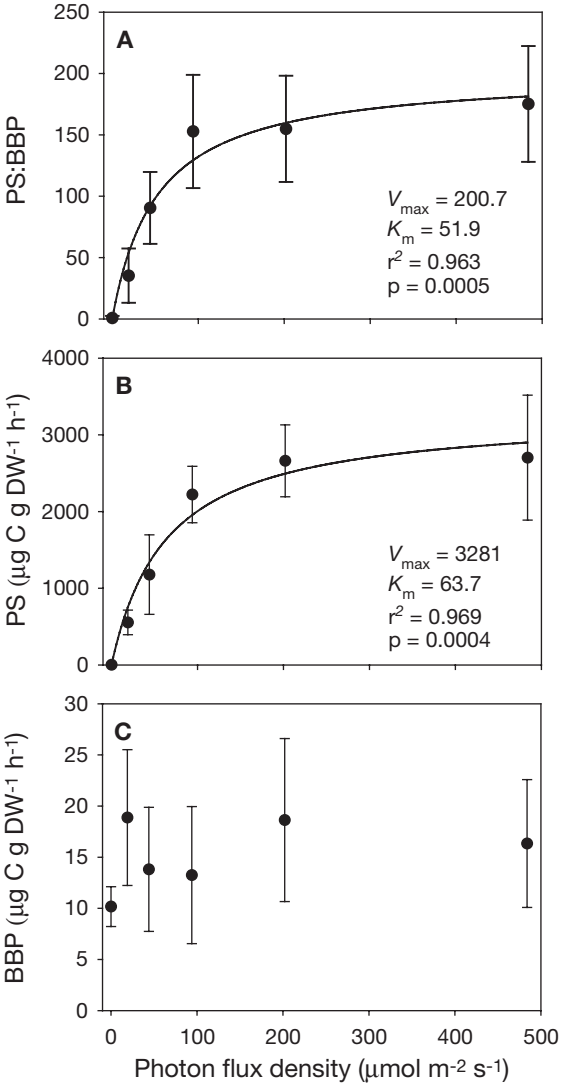

Fig. 1. Response of photosynthesis (PS) and bacterial biomass production (BBP) to varying light. (A) Ratio of PS:BBP observed with increasing irradiance; (B) Response of mean PS to increasing irradiance; (C) Response of BBP to increasing irradiance. Error bars are ±SD

The effect of nutrient enrichment on BBP measured in dark bottles was tested using a Kruskal-Wallis test in SAS 9.1 (SAS 1999). For samples incubated under light, the correlation between PS on BBP within each treatment was tested using linear regression analysis in SigmaPlot 9.0 (SigmaPlot 2004). Further, because we were interested in the coupled response of PS and BBP of periphyton, we chose a multivariate method to test the simultaneous response of PS and BBP to nutrient enrichment. We used the nonparametric multiple analysis of variance (NPMANOVA) method developed by Anderson (2001). NPMANOVA is superior to other multivariate procedures because it is capable of handling complex experimental designs, including nesting. In this method, inter-point distances in bivariate (or multivariate) space are used to derive the sums of squares for a statistical test (F). The use of inter-point distances allows additive partitioning of sums of squares, thereby enabling crossed or nested factors in a design to be partitioned out of the total sums of squares.

Specifically, we used the permutational multivariate analysis of variance (PERMANOVA) software (Anderson 2005a) to test for differences in the bivariate location of PS and BBP data among treatments and the permutational analysis of multivariate dispersion (PERMDISP) software (Anderson 2005b) to test for differences in the bivariate dispersions of PS and BBP data among treatments. Data were standardized to z scores in both analyses to account for the large difference in scale between PS and BBP rates. Euclidean distance was used to calculate inter-point distances, and sample nesting within each level of the nutrient treatment was included as a factor in both analyses. Post-hoc multiple comparisons with Bonferroni error correction were used to identify differences between individual treatments when overall statistical tests were significant at $\alpha = 0.05$.

RESULTS

Effect of TCA precipitation on autotrophic ^{14}C retention

Cold TCA protein precipitation had no effect on the degree of ^{14}C retention by photosynthetic organisms in periphyton mats (t = 0.01, p = 0.9915, df = 20). In all subsequent assays, we assumed no loss of ^{14}C due to cold TCA treatment.

Light saturation of PS and relationship of PS to BBP

The mean rate of PS in nutrient-limited periphyton mats followed normal Michaelis-Menten kinetics when incubated under increasing light intensities (Fig. 1B). Although the mean rate of BBP in the dark incubations was statistically lower than the mean BBP in the pooled data from light incubations (Kruskal-Wallis test, $\chi^2 = 5.3$, df = 1, p = 0.0209), a statistical difference was not observed between discrete light levels (Kruskal-Wallis test, $\chi^2 = 7.32$, df = 5, p = 0.1947). Further, no pattern was apparent between increasing light intensity and mean BBP (Fig. 1C). Because mean BBP remained relatively constant across the increasing light levels, the ratio of PS to BBP (PS:BBP) exhibited a pattern similar to PS when examined across light intensities (Fig. 1A).

Although the mean rates of PS and BBP did not appear coupled in the light saturation experiments, regression analysis of the individual observations indicated a weak positive correlation between PS and BBP across the range of all light intensities (Fig. 2).

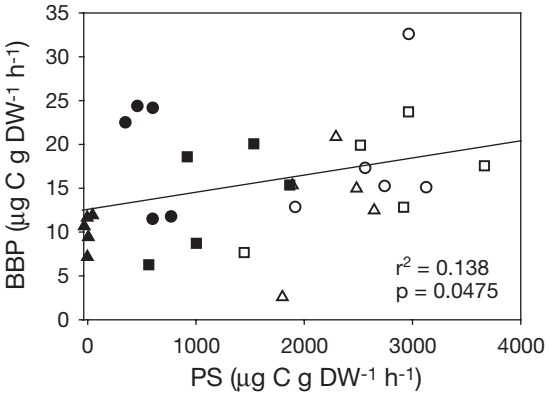

Fig. 2. Periphyton photosynthesis (PS) and bacterial biomass production (BBP) in light saturation experiment; samples incubated across a range of photon flux density. Respective light levels are represented as: ▲ = 0 µmol m^{-2} s^{-1}; ● = 19 µmol m^{-2} s^{-1}; ■ = 44 µmol m^{-2} s^{-1}; △ = 94 µmol m^{-2} s^{-1}; ○ = 202 µmol m^{-2} s^{-1}; □ = 484 µmol m^{-2} s^{-1}

Effect of nutrient enrichment on PS and BBP

Results from the PERMANOVA and PERMDISP tests, along with the results of the corresponding post-hoc tests are provided in Table 1. Nutrient enrichment had a statistically significant effect on the location (PERMANOVA) and dispersion (PERMDISP) of PS and BBP data in bivariate space. Although the effect of nesting was statistically significant in the bivariate location test (PERMANOVA), the magnitude of this effect did not appear to dampen the nutrient effect. The nesting factor was not important in the bivariate dispersion test (PERMDISP). In the multiple comparison tests within PERMANOVA, we found that the location of PS and BBP data in bivariate space was only different between the N enrichment and the control. Multiple comparisons from PERMDISP revealed that the dispersion of PS and BBP data was not different between the N enrichment and control. Although mean PS in the P enrichment was 3.6 times greater than mean PS in the control group, the variance of PS data from the P enrichment was 2.5 times greater than the control (Table 1). Because of this large variation in PS and BBP data, the bivariate location of data was not statistically different between the P enrichment and control. However, the dispersion of PS and BBP data in the P enrichment was statistically greater than the dispersion in both the control and N enrichment (Table 1).

Table 1. ANOVA results for bivariate test of location (PERMANOVA), bivariate test of dispersion (PERMDISP), and the results of post-hoc multiple comparisons for both statistical tests. Mean photosynthesis (PS) and bacterial biomass production (BBP) rates and the average ratio of PS:BBP in the nutrient enrichment experiment are reported with the results of the post-hoc comparisons

	df	SS	MS	*F*	p
PERMANOVA					
Nutrient enrichment	2	34.9	17.9	7.93	0.0060
Nesting	12	26.4	2.2	2.46	0.0136
Residual	30	26.8	0.9		
Total	44	88.0			
PERMDISP					
Nutrient enrichment	2	6.2	3.1	17.7	0.0008
Nesting	12	2.1	0.2	1.4	0.2418
Residual	30	3.7	0.1		
Total	44	12.0			

Post-hoc comparisons

Treatment	Mean PS ± SE (µg C g^{-1} DW h^{-1})	Mean BBP ± SE (µg C g^{-1} DW h^{-1})	Mean PS:BBP ± SE
Control	8673 ± 2210	5.79 ± 1.77	1852 ± 213
Nitrogen	34909 ± 1767[a]	15.67 ± 0.54[a]	2236 ± 96.1
Phosphorus	31235 ± 5453[b]	12.25 ± 1.74[b]	2586 ± 212

[a] Statistically different from the control group ($t = 4.05$, p = 0.0082) in the bivariate location test

[b] Statistically different from the control group ($t = 4.93$, p = 0.0064) and nitrogen group ($t = 5.12$, p = 0.0078) in the bivariate dispersion test

In addition to the differences observed in bivariate location and dispersion of PS and BBP data, the relationship between PS and BBP was different among the 3 experimental groups. A strong positive correlation between PS and BBP was evident in the control group (Fig. 3C) but was weaker in each of the nutrient enrichments (Fig. 3A,B). In the N enrichment, PS rates were never lower than 20 mg C g^{-1} DW h^{-1} and BBP rates were never lower than 10 µg C g^{-1} DW h^{-1}. However, the range of PS and BBP data observed in the P enrichment spanned the minimum and maximum of all observed values, as indicated by the statistically significant outcome in the bivariate dispersion test. The slope of the best fit line in the control group was 4 times steeper than the slope of the best fit lines in each of the nutrient enrichments, indicating that PS in the nutrient enrichments changed with greater magnitude than did BBP (Fig. 3). This trend was also illustrated in the mean PS:BBP for each of the treatments. Mean PS:BBP in the N and P enrichments was 20 and 40% greater than the mean PS:BBP of the control, respectively (Table 1). These data indicate a larger increase in PS relative to BBP by both N and P enrichment.

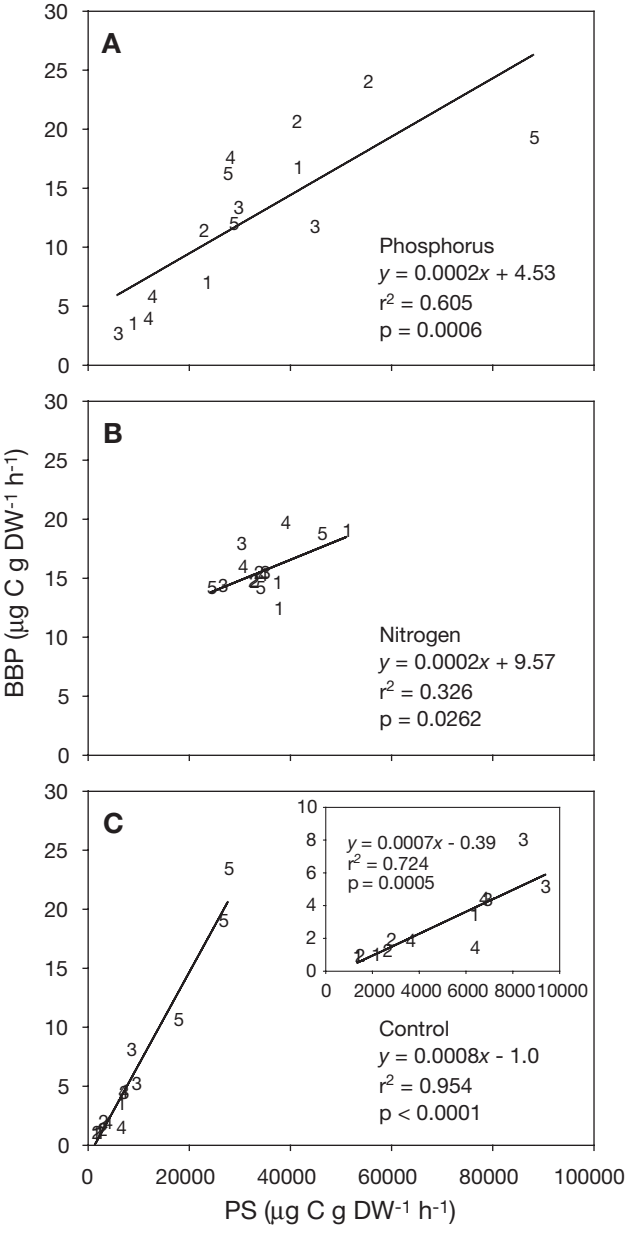

Fig. 3. Periphyton photosynthesis (PS) and bacterial biomass production (BBP) across each nutrient enrichment where all samples were incubated under equal photon flux density (250 µmol m^{-2} s^{-1}): (A) phosphorus enrichment, (B) nitrogen enrichment, (C) control (inset in panel C shows the relationship of data when Jar 5 is excluded). Numbers represent jars (nesting factor in PERMANOVA and PERMDISP) from which triplicate measures were made

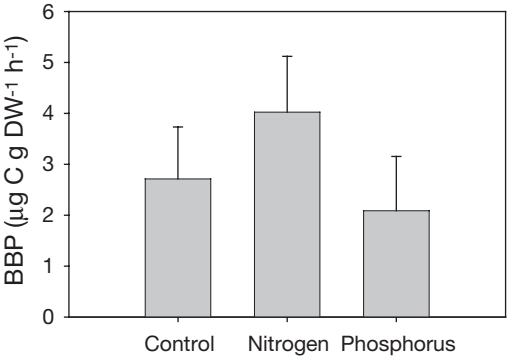

Fig. 4. Bacterial biomass production (BBP) rates for samples incubated under dark conditions in the nutrient enrichment experiment (mean ± SD; n = 3)

There were no statistically significant differences in dark BBP (independent from PS) rates among the control and nutrient enrichments (Kruskal-Wallis test, χ^2 = 3.2, df = 2, p = 0.2019). Although a general pattern of higher dark BBP was observed in the N enrichment (Fig. 4), the low sample size (n = 3) and subsequent reduced statistical power were not sufficient for detecting statistical differences.

DISCUSSION

The results of this study indicated that PS and heterotrophic BBP were generally coupled in nutrient-limited wetland periphyton mats under light intensities greater than ~20 µmol m^{-2} s^{-1}. In the light saturation experiment, mean PS rates increased in a predictable pattern with increased light intensity (Fig. 1B). Conversely, mean BBP rates did not follow any pattern across increasing light intensity (Fig. 1C). However, when individual PS and BBP measurements were combined in a scatterplot, a positive linear re-

lationship became apparent in the data across all light intensities (Fig. 2). Furthermore, the relationship between PS and BBP was stronger under saturating light (≥90 µmol m^{-2} s^{-1}).

Although the correlation between PS and BBP data across all light intensities was weak (r^2 = 0.14) compared to another published value (r^2 = 0.79 in Neely & Wetzel 1995), assays in that study were conducted on nutrient-rich media as opposed to the nutrient-limited community used in this study. We believe that the strength of coupling between PS and BBP will be diminished in an environment where both photoautotrophs and bacteria may be experiencing nutrient limitation. Further, samples for the light saturation experiment in this study were generally collected during early morning and only provided 1 h acclimation and 2 h of incubation. Espeland et al. (2001) found that BBP tended to be higher in periphyton that received ≥8 h of relatively low irradiance (<100 µmol m^{-2} s^{-1}). Therefore, the short-term experiments used in this study probably provide a conservative estimate of the degree of coupling between PS and BBP in nutrient-limited periphyton mats.

As shown in the multivariate statistical tests, both the location and dispersion of PS and BBP data were affected by nutrient enrichment. In particular, N (but not P) enrichment resulted in a statistically significant increase in both PS and BBP rates relative to the control. These findings suggest that photoautotrophs in floating periphyton mats sampled in this study were N-limited, which supports the findings of previous studies on periphyton in this ecosystem (Scott et al. 2005). The response of BBP, however, was more difficult to interpret. Had the increased BBP measured in dark incubations during N enrichment been statistically greater than BBP in control, we might have concluded with confidence that bacteria were also N-limited. Although the trend in the data suggests that N might have had some influence on dark BBP (Fig. 4), it is difficult to know whether increased BBP observed under light-saturated N enrichment was caused by increased N supply or simply increased EOC supplied at higher rates of PS. However, because light-saturated N enrichment decreased the strength of correlation between PS and BBP (Fig. 3), we suspect that bacteria were responding more to inorganic N than they were increased EOC supplied by PS.

The bivariate test of location was not significant when comparing the P enrichment to the control; however, the bivariate dispersion of data was statistically different between these treatments (Table 1). The dispersion of data in the P enrichment was also statistically greater than that observed in the N treatment. We conclude that PS and BBP were responding in some fashion to P enrichment; however, the mechanism at work is difficult to interpret using results of the statistical tests only.

PS in the control group was assumed to be N-limited (see Scott et al. 2005). Therefore, the addition of P alone would only have increased the degree of N-limitation. The N treatment however, likely shifted PS from N- to P-limitation. A shift to P-limitation may explain the apparent decoupling of PS and BBP observed in the N treatment. P-limitation may have induced a competitive interaction for P between photoautotrophs and bacteria (Currie & Kalff 1984).

Because PS and BBP remained more strongly coupled during P enrichment (i.e. strengthened N-limitation), a commensalistic or perhaps mutualistic interaction may be occurring during N deficiency. It is possible that a mutualistic relationship exists whereby bacteria regulate P availability to photoautotrophs, and photoautotrophs supply fixed N to bacteria (rather than simply EOC as suggested by Sharma et al. 2005). A mutualistic relationship of this sort would help explain the ecological necessity of periphytic N_2 fixation in strongly P-limited environments such as tropical and sub-tropical marshes (Rejmánková & Komárková 2000, Inglett et al. 2004). However, more research is needed to identify the nature of photoautotrophic and heterotrophic bacterial interaction across a diverse range of nutrient deficiencies and elemental imbalances.

In the present study, we attempted to elucidate the effect of nutrient enrichment on PS and BBP in floating periphyton mats using laboratory experiments. It should be noted that the timing of experiments relative to sampling and laboratory conditions of the experiments appeared to change the proportion of measured PS:BBP in floating periphyton mats. For instance, in the light saturation experiment where periphyton samples were collected and radioassayed within a matter of hours, PS:BBP was in the range of typical values found in the literature (Table 2). However, PS rates increased slightly and BBP rates decreased substantially when samples were taken over a 48 h acclimation period. This resulted in an increase in PS:BBP by an order of magnitude (see Table 2). Further, when periphyton mats were provided a 48 h acclimation period, the strength of correlation between PS and BBP appeared to increase substantially (cf. Fig. 3C with Fig. 2). Although the strength of correlation observed in the nutrient enrichment controls was to some degree driven by high PS and BBP rates measured in Jar 5, samples from Jars 1 to 4 only (Fig. 3C, inset) still exhibited stronger correlation than did PS and BBP in freshly collected samples under saturating light (Fig. 2; r^2 = 0.25, p = 0.1463, for light levels 202 and 494 µmol m^{-2} s^{-1}). The cause of this change remains unknown; however, we suspect that exposing the photoautotrophs only to

Table 2. Ratio of photosynthesis to bacterial biomass production (PS:BBP) observed in this study and others

Source	Community/ growth substrate	Incubation light (μmol m^{-2} s^{-1})	Incubation temp. (°C)	Nutrient amendments	PS:BBP	Notes
Present	Periphyton floating mat	202 – 484	26	None	165	Measured immediately after sampling
Present	Periphyton floating mat	250	26	None	1852	Measured following a 48 h experiment acclimation period, in which samples were exposed only to artificial PAR
Neely & Wetzel (1995)	Periphyton on glass slides	400	20	Agar	29.4	
Neely & Wetzel (1997)	Epiphyton on Typha	500	20	None	14.3 22.5 128.5	Following 20 d in direct sunlight Following 40 d in direct sunlight Following 60 d in direct sunlight

the favorable conditions of photosynthetically active radiation may have increased their ability to compete with bacteria for inorganic nutrients. Futhermore, because all treatments in the nutrient enrichment experiment were exposed to the same laboratory conditions, this 'bottle effect' does not alter the interpretation of the experimental results.

We used leucine incorporation into protein for heterotrophic bacterial production estimates in order to account for production in cell maintenance as opposed to production via cell division (e.g. using thymidine incorporation into DNA). It should be noted that some studies have shown that leucine can be taken up by photosynthetic cyanobacteria such as *Microcystis aeruginosa* (Kamjunke & Jähnichen 2000) and *Nodularia* spp. (Hietanen et al. 2002). It remains unclear whether N_2-fixing cyanobacteria such as *Anabaena* and *Aphanizomenon*, which are common taxa in floating periphyton mats, exhibit similar capabilities.

The results of this study also support the usefulness of a dual-isotope technique for simultaneous measurements of PS and BBP in microbial communities. The major strength of the dual-isotope technique is in generating a measurement of both PS and BBP on a sample incubated in a single vial. Because periphyton communities are notoriously heterogeneous (Stevenson 1996), comparing measured rates of biological processes between samples is problematic at best, and using mean rates from a small number of replicates limits the power of detecting statistical differences. Simultaneous measures from a single vial provide increased accuracy and comparability for biological processes in heterogeneous periphyton communities. One drawback of the dual-isotope method is the difference in sample preparation between ^{14}C-bicarbonate radioassays for PS and ^{3}H-L-leucine radioassays for BBP. In a previous study, Neely & Wetzel (1995) reported that as much as 66% of ^{14}C incorporated by periphytic photoautotrophs growing on glass slides was lost during hot TCA precipitation of proteins. However, in our study, the cold TCA protein precipitation/alkaline dissolution method of Buesing & Gessner (2003) had no effect on the degree of ^{14}C retention by photosynthetic organisms in floating periphyton mats. It is difficult to know whether the differences observed between the studies were a function of the different communities assayed, or perhaps a function of using hot versus cold TCA precipitation. What is clear from Neely & Wetzel (1995) and the present study is that the power to detect coupling between PS and BBP increases when measurements originate from the same vial. In our study, the importance of simultaneous measurements was critical for examining PS and BBP coupling in both the light saturation and nutrient enrichment experiments.

CONCLUSIONS

The results of the present study indicate that although PS and BBP in floating periphyton mats remain coupled under all environmental conditions examined, the magnitude of these rates and the strength of their relationship is influenced by the degree and type of nutrient limitation. N enrichment weakened the relationship between PS and BBP possibly by inducing a competitive interaction between bacteria and photoautotrophs for P. In contrast, coupling of PS and BBP remained high under P enrichment. BBP increased in P enrichments either due to an increased supply of EOC from PS or perhaps due to an increased N supply from light-dependent N_2 fixation. The possibility of linking photoautotrophs and bacteria in floating periphyton mats through multiple elemental interactions provides an important new line of questioning about the ecological functioning of these communities.

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LITERATURE CITED

Anderson MJ (2001) A new method for non-parametric multivariate analysis. Austral Ecol 26:32–46

Anderson MJ (2005a) PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland

Anderson MJ (2005b) PERMDISP: a FORTRAN computer program for permutational analysis of multivariate dispersions (for any two-factor ANOVA design) using permutation tests. Department of Statistics, University of Auckland

Buesing N, Gessner MO (2003) Incorporation of radiolabeled leucine into protein to estimate bacterial production in plant litter, sediment, epiphytic biofilms, and water samples. Microb Ecol 45:291–301

Chrzanowski TH, Kyle M, Elser JJ, Sterner RW (1996) Element ratios and growth dynamics of bacteria in an oligotrophic Canadian shield lake. Aquat Microb Ecol 11: 119–125

Cole JJ, Likens GE, Stayer DL (1982) Photosynthetically produced dissolved organic carbon: an important source for planktonic bacteria. Limnol Oceanogr 27:1080–1090

Coveney MF, Wetzel RG (1989) Bacterial metabolism of algal extracellular carbon. Hydrobiologia 79:61–71

Currie DJ, Kalff J (1984) Can bacteria outcompete phytoplankton for phosphorus? A chemostat test. Microb Ecol 10:205–216

Espeland EM, Wetzel RG (2001) Effects of photosynthesis on bacterial phosphatase production in biofilms. Microb Ecol 42:328–337

Espeland EM, Francoeur SN, Wetzel RG (2001) Influence of algal photosynthesis on biofilm bacterial production and associated glucosidase and xylosidase activities. Microb Ecol 42:524–530

Francoeur SN, Wetzel RG (2003) Regulation of periphytic leucine-aminopeptidase activity. Aquat Microb Ecol 31:249–258

Haglund A, Hillebrand H (2005) The effect of grazing and nutrient supply on periphyton associated bacteria. FEMS Microbiol Ecol 52:31–41

Hietanen S, Lehtimäki JM, Tuominen L, Sivonen K, Kuparinen J (2002) *Nodularia* spp. (Cyanobacteria) incorporate leucine but not thymidine: importance for bacterial-production measurements. Aquat Microb Ecol 28:99–104

Inglett PW, Reddy KR, McCormick PV (2004) Periphyton chemistry and nitrogenase activity in a northern Everglades ecosystem. Biogeochemistry 67:213–233

Kamjunke N, Jähnichen S (2000) Leucine incorporation by *Microcystis aeruginosa*. Limnol Oceanogr 45:741–743

Kirchman D (2001) Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. In: Paul JH (ed) Methods in microbiology, Vol 30. Academic Press, San Diego, CA, p 227–236

Lamberti GA (1996) The role of periphyton in benthic food webs. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology—freshwater benthic habitats. Academic Press, San Diego, CA, p 533–564

Medina-Sánchez JM, Villar-Argaiz M, Carrillo P (2004) Neither with nor without you: a complex algal control on bacterioplankton in a high mountain lake. Limnol Oceanogr 49:1722–1733

Murray RE, Cooksey KE, Priscu JC (1986) Stimulation of bacterial DNA synthesis by algal exudates in attached bacterial consortia. Appl Environ Microbiol 41:1378–1382

Neely RK (1994) Evidence for positive interactions between epiphytic algae and heterotrophic decomposers during decomposition of *Typha latifolia*. Arch Hydrobiol 129:443–457

Neely RK, Wetzel RG (1995) Simultaneous use of ^{14}C and ^{3}H to determine autotrophic production and bacterial protein production in periphyton. Microb Ecol 30:227–237

Neely RK, Wetzel RG (1997) Autumnal production by bacteria and autotrophs attached to *Typha latifolia* L. detritus. J Freshw Ecol 12:253–267

Paerl HW, Pinckney JL (1996) A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. Microb Ecol 31:225–247

Rejmánková E, Komárková J (2000) A function of cyanobacterial mats in phosphorus-limited tropical wetlands. Hydrobiologia 431:135–153

Rejmánková E, Komárek J, Komárková J (2004) Cyanobacteria—a neglected component of biodiversity: patterns of species diversity in inland marshes of northern Belize (Central America). Divers Distrib 10:189–199

SAS Institute (1999) SAS/STAT® user's guide, Version 8. SAS, Cary, NC

Scinto LJ, Reddy KR (2003) Biotic and abiotic uptake of phosphorus by periphyton in a subtropical freshwater wetland. Aquat Bot 77:203–222

Scott, JT, Doyle RD, Filstrup CT (2005) Periphyton nutrient limitation and nitrogen fixation potential along a wetland nutrient-depletion gradient. Wetlands 25:439–448

Sharma K, Inglett PW, Reddy KR, Ogram AV (2005) Microscopic examination of photoautotrophic and phosphatase-producing organisms in phosphorus-limited Everglades periphyton mats. Limnol Oceanogr 50:2057–2062

SigmaPlot (2004) SigmaPlot® 9.0 user's manual. Systat Software, Point Richmond, CA

Stanley EH, Johnson MD, Ward AK (2003) Evaluating the influence of macrophytes on algal and bacterial production in multiple habitats of a freshwater wetland. Limnol Oceanogr 48:1101–1111

Stevenson RJ (1996) An introduction to algal ecology in freshwater benthic habitats. In: Stevenson RJ, Bothwell ML, Lowe RL (eds) Algal ecology—freshwater benthic habitats. Academic Press, San Diego, CA, p 3–30

Vadeboncoeur Y, Vander Zanden MJ, Lodge DM (2002) Putting the lake back together: reintegrating benthic pathways into lake food web models. BioScience 52:44–54

Wetzel RG, Likens GE (2000) Limnological analyses, 3rd edn. Springer, New York

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