

## Algal-bacterial co-variation in streams: A cross-stream comparison

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With 3 figures

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**Abstract:** Algal-bacterial co-variation has been frequently observed in lentic and marine environments, but the existence of such relationships in lotic ecosystems is not well established. To examine possible co-variation, bacterial number and chlorophyll-a concentration in water and sediments of nine streams from different regions in the USA were examined. In the water, a strong relationship was found between chlorophyll concentration and bacterial abundance. There was not a significant linear relationship between the abundance of sediment bacteria and sediment or water chlorophyll concentration. The linear regression results obtained between bacterial numbers and chlorophyll concentration in water were generally similar to those reported in other studies on lentic and marine systems suggesting that factors that cause this co-variation may be similar.

**Key words:** algae, bacteria, chlorophyll, DOC, stream.

### Introduction

Algal-bacterial co-variation is frequently observed in lentic and marine ecosystems (e.g., AIZAKI et al. 1981, BIRD & KALFF 1984). Although there is limited direct evidence, co-variation between algae and bacteria is often thought to reflect the reliance of bacteria on algae for their organic carbon requirements (GASOL & DUARTE 2000) given that up to 50% of algal primary production is released as DOC (LYCHE et al. 1996) and algal-released DOC may

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support up to 95 % of bacterial production (e.g., COVENEY 1982, LYCHE et al. 1996). Algal-bacterial co-variation could also stem from similar responses of both groups of organisms to common regulating factors (COVENEY & WETZEL 1995), such as the supply of inorganic nutrients (e.g., LE et al. 1994, RIER & STEVENSON 2001).

Although many studies have reported strong algal-bacteria coupling, some studies have found that algal-bacterial co-variation is weak or undetectable (e.g., COFFIN & SHARP 1987, FINDLAY et al. 1991). Because most of these studies were conducted in systems that receive a large amount of allochthonous DOC, the lack of coupling between algae and bacteria may be caused by reliance of bacteria on allochthonous DOC and reduced relative importance of algal-released DOC. Also, most of these studies were conducted in single systems and thus the range of algal biomass and bacterial abundance examined was smaller than if several systems were examined. For example, in the Hudson River estuary, FINDLAY et al. (1991) found no statistically significant correlation between bacterial abundance and chlorophyll concentration. Chlorophyll concentrations in their study ranged from about 4–15  $\mu\text{g} \cdot \text{L}^{-1}$ . In contrast, in several cross-system studies that detected significant correlations between algal and bacterial abundance, chlorophyll concentration ranged from 0.2–189  $\mu\text{g} \cdot \text{L}^{-1}$  (AIZAKI et al. 1981), 0.05 to 120  $\mu\text{g} \cdot \text{L}^{-1}$  (BIRD & KALFF 1984), and less than 1  $\mu\text{g} \cdot \text{L}^{-1}$  to more than 100  $\mu\text{g} \cdot \text{L}^{-1}$  (COLE et al. 1988). Overall, most cross-system studies on algal-bacterial co-variation have been conducted in lentic and marine systems (BIRD & KALFF 1984, COLE et al. 1988). Few cross-system studies have been conducted in lotic systems (RIER & STEVENSON 2001, ROSENFELD & HUDSON 1997).

Studies of algal-bacterial coupling in lotic systems have produced mixed results. For example, FINDLAY et al. (1993) found that alteration of chlorophyll concentrations did not affect bacterial growth or abundance. In contrast, HEPINSTALL & FULLER (1994) and RIER & STEVENSON (2002) found a positive relationship between algae and bacteria in streams. In a comparison of 69 streams, RIER & STEVENSON (2001) found that epilithic bacterial numbers were related to chlorophyll concentration over a broad range (<1 to >10  $\mu\text{g}/\text{cm}^2$ ) but that such a relationship was not apparent when only streams with low concentrations of chlorophyll were included (<5  $\mu\text{g}/\text{cm}^2$ ). GEESEY et al. (1978) found that there was a positive relationship between chlorophyll concentrations and epilithic bacteria number in one stream and a negative relationship in another stream in the same watershed.

Overall, although the presence of algal-bacterial co-variation in marine systems and lakes is rather well established, evidence of this phenomenon in lotic systems is more limited and results are mixed. To shed additional light on this phenomenon, we examined co-variation between algae and bacteria in the water and sediments of nine low order streams from various locations in the USA by comparing chlorophyll-a concentrations with total bacterial counts.

## Methods

### Study sites

Water and sediment samples were collected from 9 streams including Meyers Branch in South Carolina (MB-SC), Black Creek in Georgia (BC-GA), Hugh White Creek in North Carolina (HWC-NC), Buzzard's Branch in Virginia (BB-VA), Allequash Creek in Wisconsin (AC-WI), Augusta Creek in Michigan (AuC-MI), Buffalo Creek in Iowa (BuC-IA), King's Creek in Kansas (KC-KS), and the West Branch of Mahoning River in Ohio (WB-OH). For some of the streams sampled there is detailed information about organic matter budgets (WEBSTER & MEYER 1997). Specifically, BB-VA and HWC-NC are dominated by allochthonous inputs (SMOCK 1997 and WEBSTER et al. 1997), whereas in KC-KS the importance of allochthonous inputs is reduced in up-stream reaches (GRAY 1997).

### Sample collection and processing

Samples were collected during summer 2001 (between June 11 and July 17) from three different sites in each stream (sites A, B, and C for a given stream). These sites were 100–1000 m apart and were either selected based on sampling sites from prior studies and accessibility. In some streams, there were notable intra-stream differences among the three sites as reflected in the results; for example, in King's Creek (KS) the role of autochthonous and allochthonous inputs changes along the stream (GRAY 1997). Five replicate water samples (300 mL) were collected from each of the three sites in each stream (total number of sites = 27) from the mid-channel surface. Also from each site, 5 replicate sediment samples were collected from the top 2 cm of sediment. Samples for bacterial enumeration were preserved (phosphate-buffered saline + 8% paraformaldehyde) and kept on ice during transport to the laboratory.

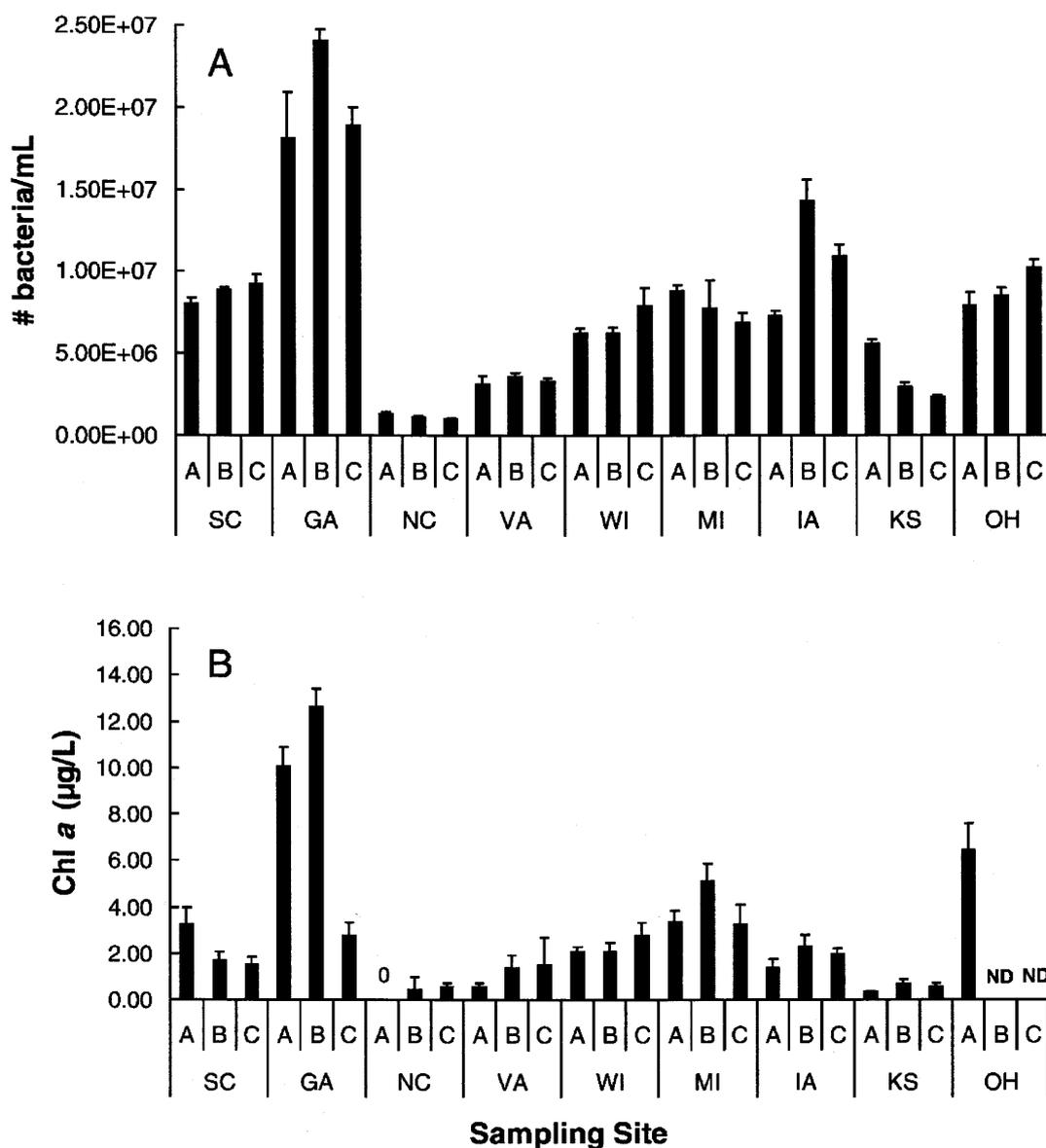
To determine chlorophyll-a concentration, samples were filtered through a Whatman GF/F filter (Whatman International Ltd, Maidstone, England) and the filter was then ground and chlorophyll-a extracted with 90% acetone for 2 hours at 4 °C in the dark. Chlorophyll-a concentration was determined using the standard spectrophotometric method (APHA 1995). Chlorophyll data from sites B and C of WB-OH were not obtained.

The total number of bacteria was determined using the DAPI (4',6-diamidino-2-phenylindole) direct count method (PORTER & FEIG 1980). Sediment samples were treated with sodium pyrophosphate (0.1%) and sonicated (5 minutes in a Branson Model 2210 ultrasonic bath, Ultrasonics Corporation, Danbury, CT) prior to analysis. Bacteria were concentrated by vacuum (15 kPa) onto 0.2 µm-pore black polycarbonate filters (Osmonics, Minnetonka, MN), rinsed with deionized water, stained with DAPI (1 µg mL<sup>-1</sup> final concentration) for 5 min in the dark, and mounted on a glass slide with non-fluorescent immersion oil. Stained cells enumerated using epifluorescence microscopy.

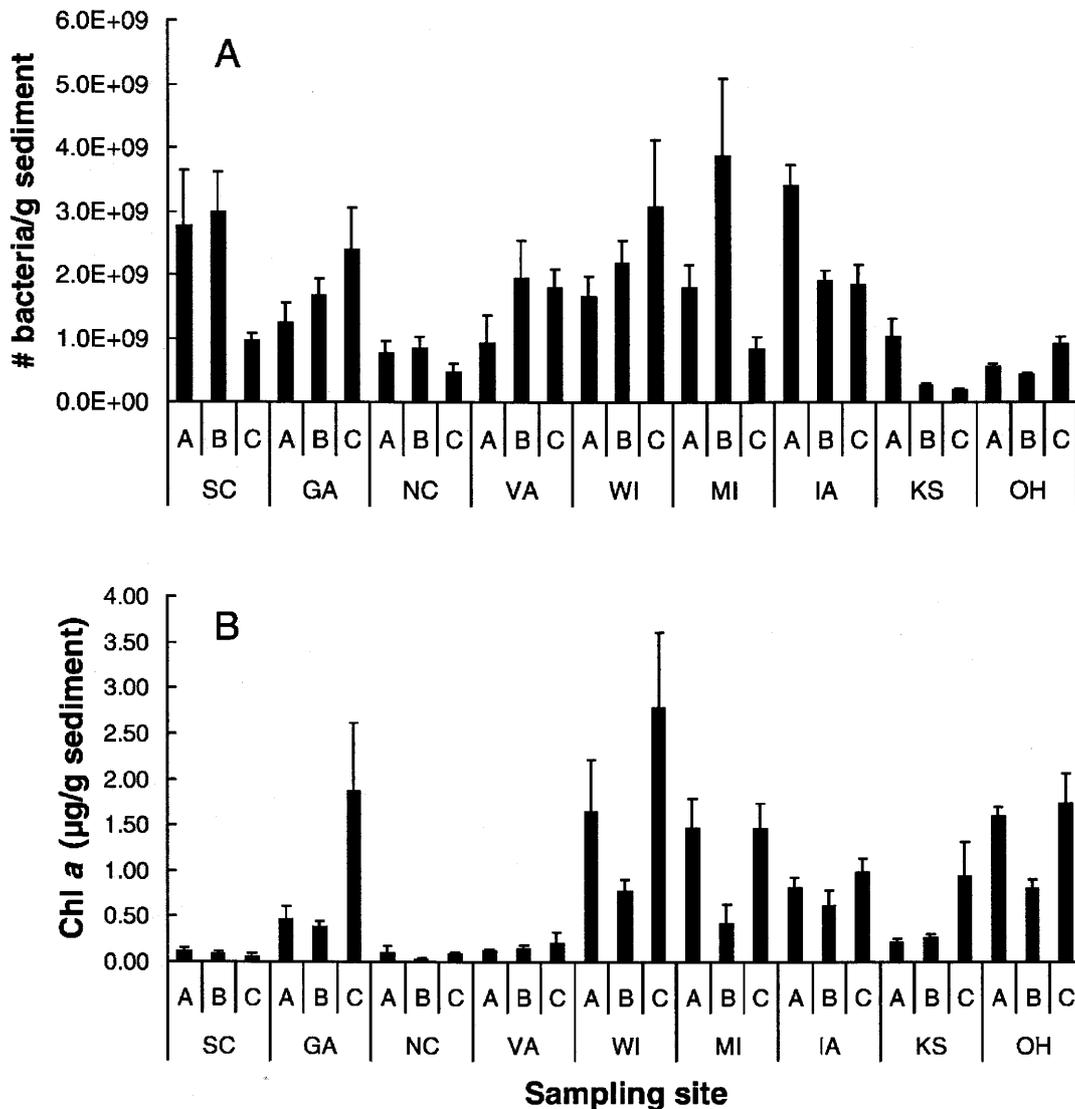
Statistical differences among streams and among sites within streams were determined using nested ANOVAs. Relationships between algal and bacterial abundance was examined using linear regression.

## Results

Total bacterial abundance in water ranged from  $1.0 \times 10^6$  cells  $\cdot$  mL<sup>-1</sup> at HWC-NC (Site A) to  $2.4 \times 10^7$  cells  $\cdot$  mL<sup>-1</sup> at BC-GA (Site B) and differed significantly among streams ( $P < 0.0001$ ) and sites within a stream ( $P < 0.0001$ ) (Fig. 1 A). In general, bacterial abundance at the three sites within a given stream was relatively similar. However, intra-stream variation was also observed, with the largest difference occurring between sites A and B in BuC-IA. Mean bacterial abundance at these two sites was  $7.3 \times 10^6$  and  $14.3 \times 10^6$  cells  $\cdot$  mL<sup>-1</sup>, respectively, approximately a two-fold difference (Fig. 1 A). Sig-



**Fig. 1.** Total bacterial counts (DAPI) and chlorophyll-a concentration in water. Values represent mean  $\pm$  1 SE ( $n = 5$ ). ND represents “no data”.



**Fig. 2.** Total bacterial counts (DAPI) and chlorophyll-a concentration in sediments. Values represent mean  $\pm$  1 SE ( $n=5$ ).

nificant intra-stream difference was also observed among sites A, B, and C of KC-KS; sources of organic matter also vary among these sites (GRAY 1997).

Chlorophyll-a concentration in water followed a trend similar to that observed for bacterial abundance, ranging from  $0.0 \mu\text{g} \cdot \text{L}^{-1}$  at HWC-NC (Site A) to  $12.7 \mu\text{g} \cdot \text{L}^{-1}$  at BC-GA (Site B; Fig. 1 B), and differed significantly among streams ( $P < 0.0001$ ) and sites within a stream ( $P < 0.0001$ ). The largest intra-stream difference was found in BC-GA, where chlorophyll-a concentration varied  $\sim 4.5$ -fold between sites B and C (Fig. 1 B).

Total bacterial abundance in the sediments also differed significantly among streams ( $P < 0.0001$ ) and sites within a stream ( $P = 0.0004$ ; Fig. 2 A), ranging from  $1.9 \times 10^8 \text{ cells} \cdot \text{g sediment}^{-1}$  at KC-KS (Site C) to  $3.9 \times 10^9 \text{ cells} \cdot \text{g sedi}$

ment<sup>-1</sup> at AuC-MI (Site B). Compared with the water samples, bacterial abundance in the sediment was more variable among replicates and among sites (Fig. 2 A).

Sediment chlorophyll-a concentration differed significantly among streams ( $P < 0.0001$ ) and sites within a stream ( $P = 0.0002$ ; Fig. 2 B), ranging from  $0.04 \mu\text{g} \cdot \text{g sediment}^{-1}$  at HWC-NC (Site B) to  $2.79 \mu\text{g} \cdot \text{g sediment}^{-1}$  at AC-WI (Site C). As with the water samples (Fig. 1 B), chlorophyll-a concentrations showed significant intra-stream variability (Fig. 2 B).

### Algal-bacterial co-variation

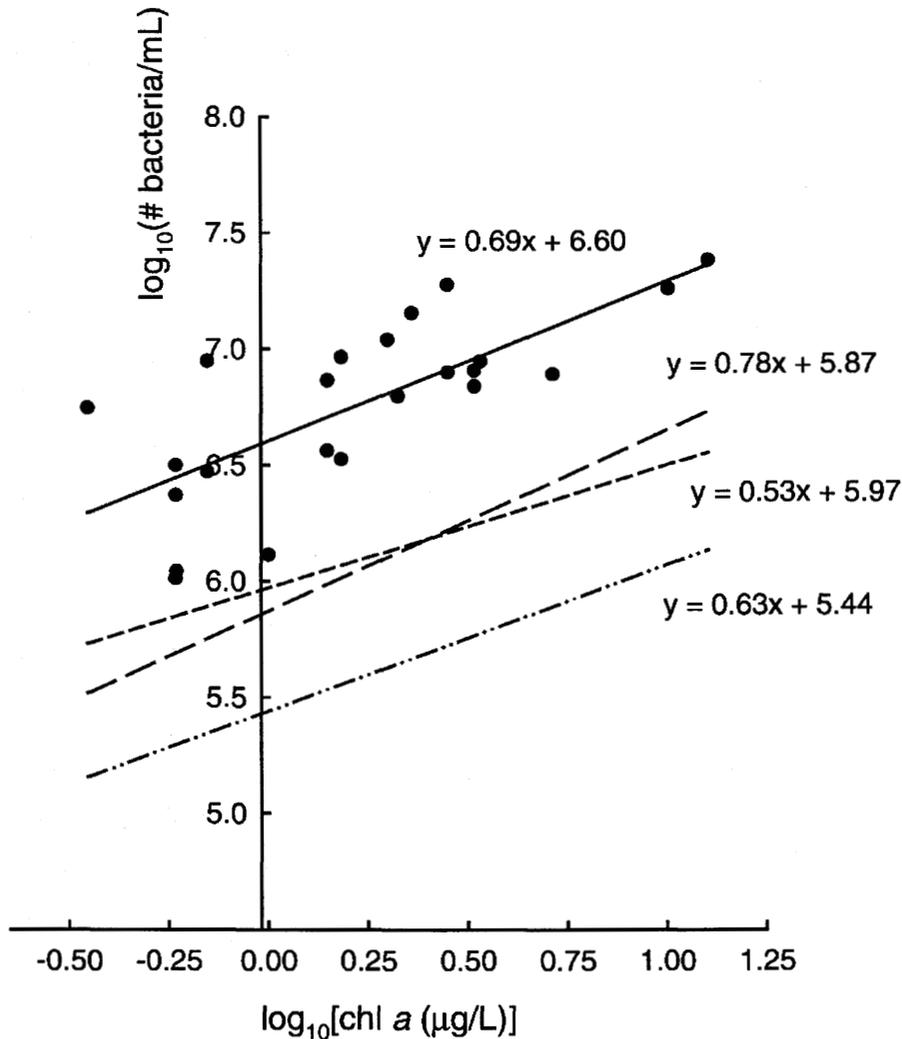
Total bacterial abundance in water was significantly related to chlorophyll-a concentration in water ( $P < 0.0001$ ,  $R^2 = 0.60$ ). The slope of the linear regression between bacterial abundance and chlorophyll-a concentration in water was  $1.5 \times 10^6 \text{ cells} \cdot \mu\text{g}^{-1}$  and the y-intercept was  $3.8 \times 10^6 \text{ cells}$ . In order to compare our findings to previously published studies, values are expressed on a log-log basis in Fig. 3.

In contrast with the water samples, there was not a significant linear relationship between benthic bacterial number and sediment chlorophyll-a ( $P = 0.37$ ,  $R^2 = 0.04$ ). Also, there were no statistically significant linear regressions between chlorophyll concentrations or bacterial numbers in sediments compared to the water column ( $P > 0.05$ ).

## Discussion

In comparison with cross-system studies conducted in oceans and lakes (AIZAKI et al. 1981, BIRD & KALFF 1984, COLE et al. 1988), the gradient of chlorophyll-a concentration in water in this study was relatively narrow ( $0.0$  to  $12.7 \mu\text{g} \cdot \text{L}^{-1}$ ). In spite of this narrow range, and even though the streams studied derive a significant portion of organic matter from allochthonous sources (GRAY 1997, SMOCK 1997, WEBSTER et al. 1997), a significant linear relationship between algal and bacterial abundance in the water was detected. There were not significant relationships between sediment bacterial number and sediment or water chlorophyll-a concentration.

In cross-system studies of algal-bacterial co-variation in lentic and marine ecosystems, a strong linear relationship has been found between the log of chlorophyll-a concentration and the log of bacterial abundance (AIZAKI et al. 1981, BIRD & KALFF 1984, COLE et al. 1988). When our results are expressed in this same manner, a strong Log-Log relationship was also observed between water chlorophyll-a concentration and bacterial abundance in the water (Fig. 3).



**Fig. 3.** Log-Log correlations between bacterial abundance and chlorophyll concentration obtained in this study and by other researchers. Equation obtained from: (—) this study; (---) BIRD & KALFF (1984); (· · · ·) COLE et al. (1988); (-·-·-·) AIZAIKI et al. (1981).

The slope of our equation (0.69) falls in the range of other slopes observed (range = 0.53–0.78, Fig. 3), indicating that the relationship between algae and bacteria in streams follows the same pattern as in lakes and oceans. In streams, factors that control bacterial number in the water column (like discharge, nutrient concentration, temperature, etc.) may also influence chlorophyll concentration. Alternatively, perhaps the relationships observed reflect an underlying trophic connection between algae and bacteria; this study does not provide information that allows us to distinguish between these possibilities.

The  $R^2$  value for the log-log relationship from our study (0.54) is lower than the  $R^2$  value obtained in lentic and marine environments (0.75–0.92), suggesting that algal-bacterial co-variation in lotic environments tends to be

influenced more significantly by other factors, such as allochthonous DOC, than in lentic and marine environments. In many of the streams we sampled, allochthonous organic matter was a dominant component of the organic matter budget (GRAY 1997, SMOCK 1997, WEBSTER et al. 1997). The influence of allochthonous DOC might explain why the y-intercept of the linear regression equation obtained in our study (6.60) is higher than that obtained in lentic environments (5.44–5.97, Fig. 3).

In this study, there was no significant linear relationship between benthic bacterial abundance and chlorophyll. Likewise, FINDLAY et al. (1993) did not find a correlation between algal and bacterial abundance in epilithon. In contrast, RIER & STEVENSON (2001) did find a significant linear relationship between epilithic bacterial number and chlorophyll concentration. Differences in the importance of algal-bacterial co-variation among studies may relate to differences in the importance of allochthonous DOC and/or trophic status of the stream (FINDLAY et al. 1993). Specifically, more oligotrophic streams may tend to have weak algal-bacterial coupling while more eutrophic streams may have stronger algal-bacteria coupling (FINDLAY et al. 1993). In our study, the streams varied in their trophic status and perhaps if only eutrophic streams were sampled, a correlation between benthic bacterial number and chlorophyll might be observed. Alternatively, perhaps epilithic algal-bacterial co-variation is stronger than in sediments as a whole, because of the direct co-existence of algae and bacteria in biofilms (i.e., periphyton).

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