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Relationship of bacterial growth efficiency to spatial variation in bacterial activity in the Hudson River

Paul A. del Giorgio[1,*], M. L. Pace[2], D. Fischer[2]

[1]Département des sciences biologiques, Université du Québec à Montréal (UQAM), CP 8888, succursale Centre Ville, Montréal (Québec) H3C 3P8, Canada

[2]Institute of Ecosystem Studies, PO Box AB, Millbrook, New York 12545, USA

ABSTRACT: Variation in bacterial production (BP) is used as an indicator of bacterial metabolism and carbon processing in the analysis of aquatic ecosystems. The allocation of carbon by bacteria to either BP or respiration (BR), however, is variable and may potentially influence the assessment of carbon cycling by bacteria in ecosystems. We studied 10 transects in the Hudson River estuary where there is a gradient in BP and BR along the flow path of the estuary. We measured BP and BR in filtered samples to derive an estimate of bacterial growth efficiency (BGE) that we could compare with independent measurements of total BP (BP_T) from unfiltered samples to evaluate the relationship of BGE to BP. We further tested the assumption that BGE derived from filtered samples is a good estimator of the ambient BGE on a subset of transects in the upriver section where bacteria dominate respiration. There was good agreement (near 1:1) between respiration measured in unfiltered samples and total BR estimated from BP_T and BGE in the filtered fraction (total BR = $[BP_T/BGE] - BP_T$). BGE averaged 0.29 but varied from 0.07 to 0.61, and did not explain the general decline in BP_T along the river. Rather, BGE was strongly correlated to the residuals of the BP_T and specific BP_T (i.e. growth) vs. flow path (= river kilometer) relationship, indicating that shifts in bacterial carbon allocation explained local variations in bacterial metabolic activity, and these shifts were superimposed on the larger scale decline in carbon consumption and BP. The pattern in BP along the Hudson River is clearly a combination of changes in consumption as well as in BGE, to the point that the pattern in BP_T or growth would be impossible to recreate from any one of these 2 components. We conclude that BGE indicates changes in carbon allocation of bacteria that reflect shifts in relative BR and BP at shorter time and space scales that are distinct from larger overall patterns in consumption and BP.

KEY WORDS: Bacterial production · Respiration · Growth efficiency · Growth · River · Organic carbon

INTRODUCTION

Like all organisms, bacteria partition energy into growth and respiration. Growth includes increase in cell mass as well as cell division leading to new bacteria. These 2 processes are referred to as bacterial production (BP). Bacterial respiration (BR) represents the end result of cellular metabolism and is the largest energy expenditure. The ratio of BP to total energy utilization, including production and respiration, is referred to as bacterial growth efficiency (BGE). Measurements of BP, BR, and BGE are central to ecological and biogeochemical studies of bacterial activity in ecosystems. Together with bacterial abundance, BP has been the most common measurement performed in aquatic microbial ecology for the past 3 decades. These data provide the primary basis for current understanding of bacterial growth and biomass turnover in aquatic microbial communities (Ducklow & Carlson 1992).

One of the central applications of BP has been as an indication of bacterial carbon consumption (BCC) and thus as a proxy for organic matter availability to bacteria. The measurements of BP made in this context have led, for example, to (1) the notion that 30% or more of

*Email: del_giorgio.paul@uqam.ca

total marine primary production is eventually processed by bacteria (Cole et al. 1988), (2) the realization that there is significant microbial metabolism in mesopelagic and deep ocean waters (Ducklow & Carlson 1992), and (3) the notion that bacteria consume significant amounts of organic matter of terrestrial origin in lakes and estuaries (Findlay et al. 1996, Tranvik 1998, Jansson et al. 2000, Wetzel 2003) among other patterns. As a proxy of BCC, BP has also been frequently used as the endpoint in experiments that have assessed the effect of UV radiation, nutrients and other factors on the availability of organic matter (e.g. Amon & Benner 1996, Obernosterer & Herndl 2000), in such a way that increases in BP have been interpreted as increases in the availability and supply of organic matter.

The robustness of BP as an index of BCC was questioned almost a decade ago by Jahnke & Craven (1995). The connection between BP and consumption is mediated by BGE, and Jahnke & Craven (1995) argued that BGE might be neither as high nor as constant as was generally assumed at the time. Since their paper, there has been a renewed interest in BGE and a large increase in the number of measurements reported for a wide range of aquatic ecosystems. It is now clear that BGE is, as Jahnke & Craven (1995) argued, variable, and that most values are generally well below 40% (del Giorgio & Cole 1998, Toolan 2001, Pradeep Ram et al. 2003, Reinthaler & Herndl 2005). In spite of these advances, the fundamental problem posed by Jahnke & Craven (1995) has never been adequately answered: 'To what extent are the spatial and temporal variations in the magnitude of BP in aquatic ecosystems driven by environmental processes that control overall carbon availability and consumption by bacteria, versus processes that control how this carbon is processed, i.e. by BGE?' Large-scale patterns in BP (e.g. Cole et al. 1988) are clearly related to the variation in organic matter supply and critical nutrients. Variations in BGE that may result from internal allocation controls, however, could modulate or influence relative variation in BP and BR, especially at smaller spatial and temporal scales and within narrower environmental gradients of individual ecosystems often studied in microbial ecology.

In the present study, we explore the connection between BP, growth, BGE and total respiration, and in particular we test whether spatial patterns in total and cell-specific BP are influenced by BGE in the Hudson River (New York, USA), a system where temporal and spatial patterns of BP have been well characterized (Findlay et al. 1991, 1996, Findlay 2003, Maranger et al. 2005). These prior studies document that (1) the system is dominated by heterotrophic metabolism, mostly bacterial (Findlay et al. 1991, Howarth et al. 1996), and (2) rates of BP consistently decline along the north to south flow path (Maranger et al. 2005). We present simultaneous measurements of BP and BR based on short incubations of filtered samples and total BP (BP_T) measured in unfiltered samples from high resolution spatial transects in the Hudson River estuary. Because of filtration effects, the filtered measurements (filtered BP and BR) are only weakly correlated to BP_T, and thus the resulting BGE estimates are relatively independent of total activity. We relate estimates of BGE derived from filtered samples to these measurements made in unfiltered samples to assess how much of the spatial variance in BP_T and cell-specific BP_T (i.e. bacterial growth rate) along the river can be explained by BGE. We further assess the robustness of the estimates of BGE by combining BGE and measurements of BP_T to derive total BR, and comparing these estimates of total BR to independent measurements of respiration in a portion of the Hudson River where BR is dominant. We show that estimates of total BR derived from combining BP_T and BGE measurements agree well with other independent estimates of total BR. These results illustrate that BGE measured in filtered samples is robust in the Hudson River, and that these estimates help explain ecologically significant patterns of variation in bacterial community metabolism.

MATERIALS AND METHODS

Study site and sampling scheme. The Hudson River estuary extends from the southern tip of Manhattan (river kilometer, hereafter rkm 0) to a dam that blocks further tidal flow north of Troy, New York (rkm 238). The average depth of the river is 8 to 9 m, with a greater percentage of shallow area (<3 m depth) in the northern reach (Maranger et al. 2005). The river is completely mixed vertically because of extensive tidal and wind action (Cole et al. 1992, Raymond et al. 1997). Much of the Hudson River estuary is freshwater, although the salt front (>1 ppt) intrudes northward during summer as freshwater flow declines.

The Hudson River estuary receives organic matter and nutrients (nitrogen and phosphorus) primarily from the upper Hudson and Mohawk drainages (Howarth et al. 1996). High loadings of sewage organic matter were substantially reduced during the last several decades of the 20th century. Nevertheless, discharges from wastewater treatment plants especially in the Albany and New York City regions contribute significant nutrient inputs (Garside et al. 1976, Clark et al. 1992, Lampman et al. 1999), and combined sewer overflows in some urban reaches result in untreated sewage entering the river under high flows.

Hudson River waters are turbid and relatively rich in dissolved ions and nutrients. High suspended sediment concentrations are maintained by vigorous mix-

ing of the water column. Consequently, light transmission is limited, and Secchi depths are typically 50 to 100 cm. Oxygen is typically undersaturated and carbon dioxide oversaturated with respect to atmospheric concentrations (Raymond et al. 1997, Caraco et al. 2000). These gases reflect the net heterotrophic condition of the ecosystem. Respiration exceeds primary production as the result of substantial degradation of allochthonous organic matter within the Hudson River (Howarth et al. 1996, Raymond et al. 1997).

We sampled from a series of transects along the north to south axis of the river (see map in Maranger et al. 2005). We initially sampled 6 transects at 24 stations from rkm 45 (Tappan Zee Bridge) to 232 (Albany, NY) in September 2000, October 2000, May 2001, July 2001, October 2001, and August 2002. Each transect was visited over 2 consecutive days, so that 12 samples were processed each day. In 2003, we visited an additional 4 transects (June, July, September, and October), each consisting of 9 sampling sites spread evenly along a 53 km stretch on the upper portion of the river from rkm 211 to 264 (Castleton to Stillwater [above the head of the estuary], NY). These transects were included to better characterize bacterial carbon metabolism in the headwaters of the estuary, which is dominated by allochthonous inputs and where algal biomass and authochthonous production are typically low. All 4 transects were visited and samples processed on a single day. The data from the 4 additional transects were not combined with the data from the 6 transects sampled during 2000 to 2002, but rather were used for different purposes, as described below.

Water samples were taken underway from a boat in the center channel using an on-board pump. The only exception was the uppermost sampling station at Stillwater during 2003, which was sampled from the shore. Water samples were dispensed into either acid-washed 20 l carboys (bacterial metabolism) or clean 1 l sample bottles and kept in the dark in coolers during transit to the laboratory for processing. The transit time of samples was always less than 4 h. We collected a total of 180 samples for measurements of microbial metabolism and other properties (e.g. seston concentration).

Metabolic measurements. The protocol used to determine BR and BGE has been described in detail before (del Giorgio & Bouvier 2002). In short, in the laboratory, a portion of the sampled water was set aside for analysis of chlorophyll, dissolved organic carbon (DOC), and seston and to measure BP and abundance in the unfiltered water. The remainder was filtered to separate bacteria from other planktonic components to perform measurements of BR and BP in order to estimate the BGE. In preliminary experiments we tested a wide variety of filters for separating bacteria, including cellulose and polycarbonate membranes, glass fiber filters and prefilters with pore sizes ranging from 0.6 to over 5 µm. Glass fiber Millipore AP 25 filters were the most effective in reducing other plankton while maintaining most of the original free-bacterial community (del Giorgio & Bouvier 2002).

Approximately 10 l of water from each site were gently filtered through Millipore AP 25 (15 cm diameter) filters using a peristaltic pump and acid-washed tubing. Filtrate was used to fill one 4 l acid-washed Erlenmeyer flask, and a 4 l acid-washed cubitainer bag. The cubitainer bag was placed on a stand and connected by acid-washed silicone tubing to below the Erlenmeyer flask, so that a flow could be established. The Erlenmeyer was sealed with an acid-washed silicone stopper fitted with 2 glass tubes: one connected via Teflon tubing to the cubitainer reservoir, the other connected also by a length of Teflon tubing and closed with a pinch-valve at the end, which was used as a sampling port. All the samples collected in a single day, up to 18 samples, could be set up and processed simultaneously. In 2003, we also measured respiration rates in unfiltered water samples in parallel to the determinations of BR. These parallel determinations of total plankton respiration were carried out using the same approach described above, with the exception that the flow-through systems were filled with the unfiltered water sample. For each of the 4 sampling dates of 2003, we thus processed the 9 filtered samples plus the 9 unfiltered samples simultaneously.

Samples for oxygen concentration and leucine incorporation were retrieved from the lower flask by opening the valve of the outlet and allowing 5 ml of water to overflow through a sampling port before collecting the samples. Each flow-through system was sampled every 2 h for 6 to 8 h in 2000 to 2001 and for 6 h in 2002 to 2003. Diffusion through the cubitainers is minimal within the length of these incubations (E. Smith pers. comm.) and insufficient to measurably affect the O_2 concentrations in the bags. In addition, a total of less than 40 ml was withdrawn from the flask at each sampling time, so less than 1 % of the flask volume was renewed with water from the connected cubitainer at each time point. The samples thus reflect the internal oxygen concentrations in the flasks during incubations (6 to 8 h).

Bacterial and total respiration. For bacterial (as measured in filtered water) and total respiration, water samples taken every 2 h from the incubation flasks were used to determine the time course of oxygen consumption. The water samples for the determination of oxygen concentration were taken directly from the flasks by inserting the outflow tube into the bottom of a 5 ml glass tube and allowing the water to slightly overflow. Triplicate tubes were filled this way for every

time point during the incubation. Tubes were poisoned with 8 µl saturated $HgCl_2$ solution and capped with a ground glass stopper. The tubes were kept immersed in water at 10°C for subsequent gas analysis in the laboratory. Previous work has shown that gas concentrations in the tubes remain stable for weeks (Sampou & Kemp 1994). Oxygen concentration in the samples was measured using membrane-inlet mass spectrometry within 1 wk of collection. Briefly, the method is based on the spectrometric determination of the ratio of argon to oxygen in the sample, after the gases in the sample have been allowed to diffuse through a permeable membrane into a high vacuum system connected to the mass spectrometer (Kana et al. 1994). The oxygen concentration was derived from this ratio by determining the solubility of argon corrected for salinity and temperature. The average standard error of triplicate oxygen determinations performed this way was less than 1 µg O_2 l^{-1}, far below the changes in oxygen concentration occurring in all of the samples during the incubation. Rates of oxygen consumption were calculated from the slope of the O_2 vs. time relationship fitted to an ordinary least square regression. Oxygen consumption was converted to CO_2 production assuming a respiratory quotient (RQ) of 1. In a parallel study, we determined the actual CO_2 production together with the O_2 consumption in a subset of 28 samples from these and other similar systems, and found that the RQ ranged from 0.65 to 1.40, but with an average bacterial RQ of almost 1 (P. A. del Giorgio unpubl. data).

Bacterial production, abundance and growth efficiency. BP was determined from water prior to filtration and in each of the time points used to determine respiration during the incubation of the filtered water. Rates of BP were estimated from the uptake of ^{3}H-leucine following the centrifugation method of Smith & Azam (1992). The final tritiated leucine concentration in all experiments was 40 to 60 nM, based on preliminary experiments that showed that uptake rates were maximal in this range. At least 3 replicate microfuge tubes plus a killed control were incubated for 1 h for each production measurement. During time courses of oxygen consumption in the filtered samples, we carried out at least 3 individual measurements of leucine uptake, which were averaged to obtain a mean rate of leucine uptake for the incubation period. Thus, the leucine measurements were compared to respiration measured over the same time interval. Leucine uptake was converted to rates of C production assuming a conversion factor of 3.1 kg C mol^{-1} leucine (Kirchman 1993). All the leucine incorporation data were corrected for isotope retention of the microcentrifuge tubes as suggested by Pace et al. (2004). Bacterial abundance in the bulk and filtered water samples was determined using flow cytometry. BGE was calculated as BP/(BP + BR) measured in the filtered samples. The standard error for respiration rates (the error of the slope of the regression of O_2 vs. time) was consistently below 10% of the slope value. Incubations were not replicated but preliminary experiments have shown that slopes of oxygen change from triplicate incubations were within 10%. The average coefficient of variation (CV) of individual measurements of leucine incorporation tubes was 7%, and the CV of leucine incorporation from repeated samples taken during incubations was 32%.

Other analysis. DOC was measured on filtered samples (pre-combusted GF/F) with a Shimadzu high-temperature carbon analyzer using a non-purgable organic carbon method (i.e. DOC samples are acidified and sparged prior to injection into the analyzer; Kaplan 1992). Chlorophyll was measured from filter extracts using a fluorometer (Findlay et al. 1991, data kindly provided by N. Caraco and J. Cole). Seston was measured by weighing dried filters before and after filtration of a measured volume of water.

RESULTS

The 10 transects resulted in a total of 180 samples, but because of various types of sampling or analytical problems the final data consisted of 171 measurements of BP_T and 157 measurements of BR and BP in the filtered fraction (used for determinations of BGE). These data are split into 2 sets: (1) data from the 6 detailed transects sampled during 2000 to 2002 and consisting of 24 stations each, distributed along a 20 km stretch of the river; and (2) data from the 4 transects sampled during 2003, focused only on 9 stations in the upper 50 km of the river, where we made a total of 26 simultaneous measurements of total and filtered respiration and BP. Because the sites for the 2003 sampling did not coincide with the prior transects, we have not combined the 2 data sets, and rather we use them for different purposes: (1) data from the 6 transects were used to describe the average spatial patterns in microbial metabolism along the river, while (2) data from the 4 transects visited in 2003 were used to further constrain and validate the estimates of BGE and of total BR. The present study focuses on the average spatial patterns in microbial carbon metabolism and associated environmental factors along the Hudson River. Thus, we do not present individual transect data but rather have averaged all the data available for each of the 24 sites sampled during 2000 to 2002. At 7 of the 24 sites, some measurements were lost, reducing the number of observations to 5 for some of the variables compared to the 6 observations made at each of the other sites.

Spatial distribution of seston, chlorophyll and DOC

Seston, chlorophyll, and DOC concentrations in the river varied between sampling dates related to freshwater inputs and other features of the river system, but the average spatial patterns described here were recurrent and were observed in most individual transects. On average, seston concentrations tended to increase downstream from the headwaters toward the oligohaline portion of the estuary, and there were local peaks (>12 mg l^{-1}) of seston (i.e. between rkm 80 to 100), which correspond to shallow areas of reduced water depth and with increased sediment resuspension (Fig. 1A). The mean chlorophyll concentration also varied spatially, with consistently low concentrations in the upper reaches (average of 1 to 2 µg l^{-1}), a peak downstream near rkm 150 (average of 8 to 10 µg l^{-1}), and a sharp decline below this point (Fig. 1B). These spatial patterns of chlorophyll have been extensively described and are linked to the massive development of zebra mussels in the estuary (Caraco et al. 2000). DOC concentration was relatively constant seasonally, with a consistent downstream decline of (on average) 0.3 to 0.4 mg l^{-1} (Fig. 1C).

Seasonal variability in bulk bacterial abundance and metabolism

For the 2000 to 2002 data, the spatial variation of BP, BR and BGE within a given transect was in general larger than the variation between average values for transects at different times of the year. For example, there was only a 2-fold difference in the average BP_T among the 6 transects, and the average BGE per transect varied even less among all transects (range from 0.25 and 0.32). Temperature did not explain any of the variance in bacterial variables, possibly because we only sampled in late spring, summer and early autumn, with a modest transect average temperature range of <7°C. Overall, the differences in average microbial metabolism among transects at different times of the year were more strongly related to hydrology, with generally higher rates during periods of higher discharge.

Spatial patterns in total bacterial abundance, production and specific production

For the analysis of spatial variability we averaged measurements (6 per site) taken at each site to derive a general pattern of microbial metabolism along the river transect. We thus present 24 average (±SE) values for each of the variables. The average bacterial abundance declined systematically along the river, from 6 to 3×10^6 cells ml^{-1} (Fig. 2A). BP_T, measured in the unfiltered water samples, varied from 0.4 to 8 µg C l^{-1} h^{-1}, but the average BP_T per site was considerably less, ranging from 1 to 4 µg C l^{-1} h^{-1}, and declined continuously along the river, except for a sharp increase at the southernmost, oligohaline stations (below rkm 70, Fig. 2B). Specific BP was calculated by dividing each individual measurement of BP_T by its corresponding measurement of bacterial abundance and is thus an index of bacterial growth rate (ranging from 0.2 to 0.8 fg C $cell^{-1}$ h^{-1}), and also declined along the river, albeit less steeply than BP_T. Specific BP_T also showed a marked increase in the oligohaline portion of the river (Fig. 2C). Although the downriver decline in bacterial abundance, BP_T, and specific BP_T is the dominant

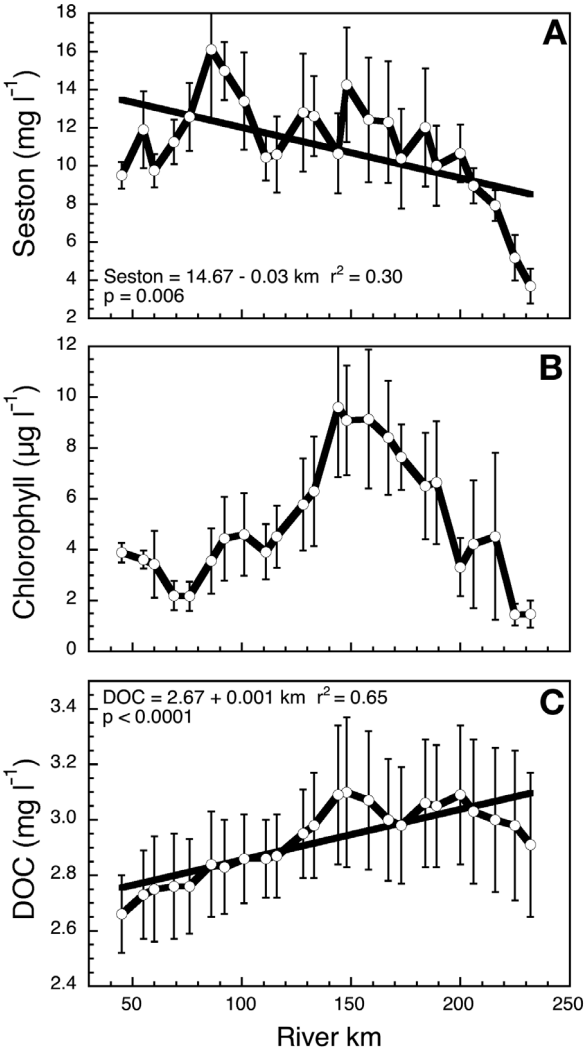

Fig. 1. Average concentration of (A) seston, (B) chlorophyll, and (C) dissolved organic carbon (DOC) for transect stations. Bars represent ±SE and reflect seasonal variability at each site, for the 6 transects covered during 2000 to 2002

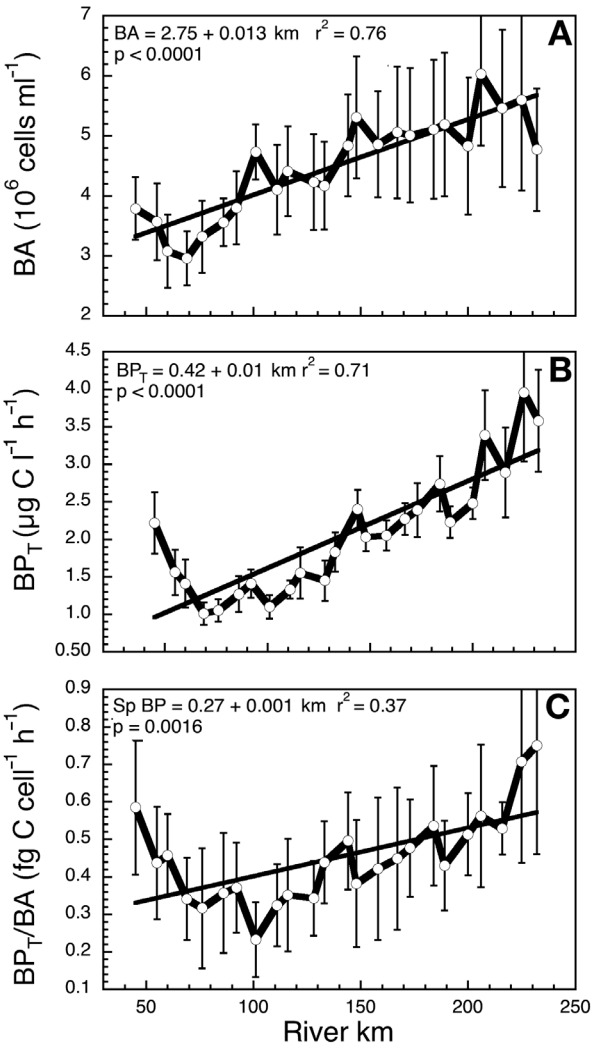

Fig. 2. (A) Total bacterial abundance (BA), (B) total bacterial production (BP_T), and (C) specific (Sp) bacterial production (BP_T/BA); numbers are means (±SE) of 6 measurements for each of the 24 stations. Solid line is the linear regression of each variable vs. river km. Residuals of this general spatial relationship are used in later analyses

spatial feature in the Hudson River, there are strong secondary features in the spatial pattern, in the form of recurrent peaks and troughs.

BP, BR and BGE in filtered samples

BP in the filtered samples varied from 0.2 to 5 µg C l^{-1} h^{-1}, but the average filtered BP per site varied only 3-fold along the transect, from 0.52 to 1.72 µg C l^{-1} h^{-1} (individual data not shown here), with a consistent decline from the upper reaches along the transect and a subsequent increase in the lower, oligohaline portion of the transect. Filtration removed a variable, and often large, fraction of the BP_T measured in the unfiltered samples, and the relationship between total (= unfiltered) and filtered BP was weak (filtered BP = 0.23 + 0.30 × BP_T, r^2 = 0.49, $p < 0.0001$; data not shown). Filtered BP was on average 62% of BP_T.

Respiration in the filtered fraction, hereafter referred to as BR, varied from 0.2 to 10 µg C l^{-1} h^{-1}, and was generally high upstream. BR also had a pronounced peak between rkm 70 and 100, but in contrast to filtered BP and BP_T, BR hardly increased in the lower oligohaline section of the river. There was a significant positive relationship between the rates of BP and BR (BR = 1.56 + 0.76 × BP, r^2 = 0.22, p = 0.02; data not shown), but this relationship explained only a small portion of the variability in BR. For any value of BP, there was an order of magnitude range in the corresponding measured respiration.

The large degree of uncoupling between the simultaneous measurements of BP and BR resulted in a wide range of calculated BGE, from 0.07 to over 0.6. Most of this variation was spatial within transects rather than seasonal, as mentioned above, and there were no significant differences between the average BGE for the 6 transects (ANOVA, $p < 0.05$). In Fig. 3, the BGE values obtained in 2003 in the upper reaches of the river have been superimposed to show that there is both spatial as well as inter-annual coherence in these measurements. Mean BGE varied from 0.17 to 0.42 along the river transect, and this spatial variation was not random. There were sections of the river with systematically low or high BGE relative to the overall transect mean. The most upstream sites all had high BGE throughout the study (Fig. 3). In the stations directly downstream of the city of Albany there was a sharp drop in BGE, and the lowest BGE was consistently recorded

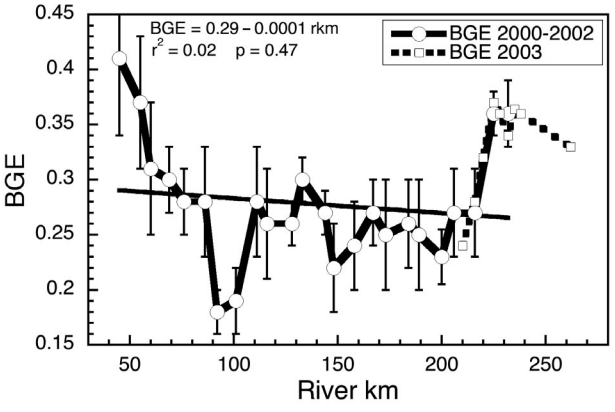

Fig. 3. Bacterial growth efficiency (BGE) at each site along the river transect. Each point represents the average (±SE) of 6 measurements at each site during 2000 to 2002. Because the sampling scheme was different in 2003, these data are presented separately, each point being the average (±SE) of 4 samples taken in 2003

between rkm 80 and 120. Finally, there was a sharp increase in BGE in the southern portion of the estuary, and the highest values were usually recorded below rkm 70.

None of the major environmental variables (chlorophyll, DOC, seston, nutrients), individually or in combination helped explain the overall spatial pattern in BGE. As described above, seston, chlorophyll and DOC each had a strong spatial pattern along the river, and none resembled that of BGE. While the overall patterns did not match, local peaks and troughs in both seston and DOC appeared to match those of BGE. To further explore this possible link, we first removed the overall spatial pattern by calculating the residuals of the seston vs. rkm and DOC vs. rkm linear relationships (shown in Fig. 1A and C, respectively), and we then regressed these to the residuals of the BGE vs. rkm relationship (shown in Fig. 3). The residual variation in BGE was strongly correlated to the residual variation of the seston vs. rkm and DOC vs. rkm relationships (Fig. 4A and B, respectively). These relationships between residuals suggest that BGE responds to local variations in DOC, seston and probably other factors that are related to carbon supply.

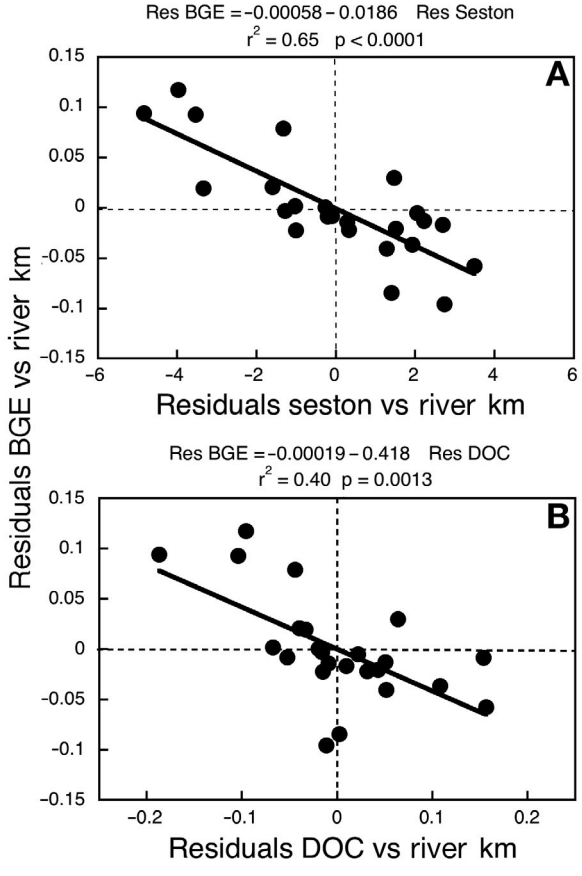

Fig. 4. Relationship between the residuals (res) of the relationship between BGE and river km as a function of the residuals of the relationship between (A) seston vs. river km and (B) DOC vs. river km. Each point represents the average for the 24 stations sampled during 2000 to 2002

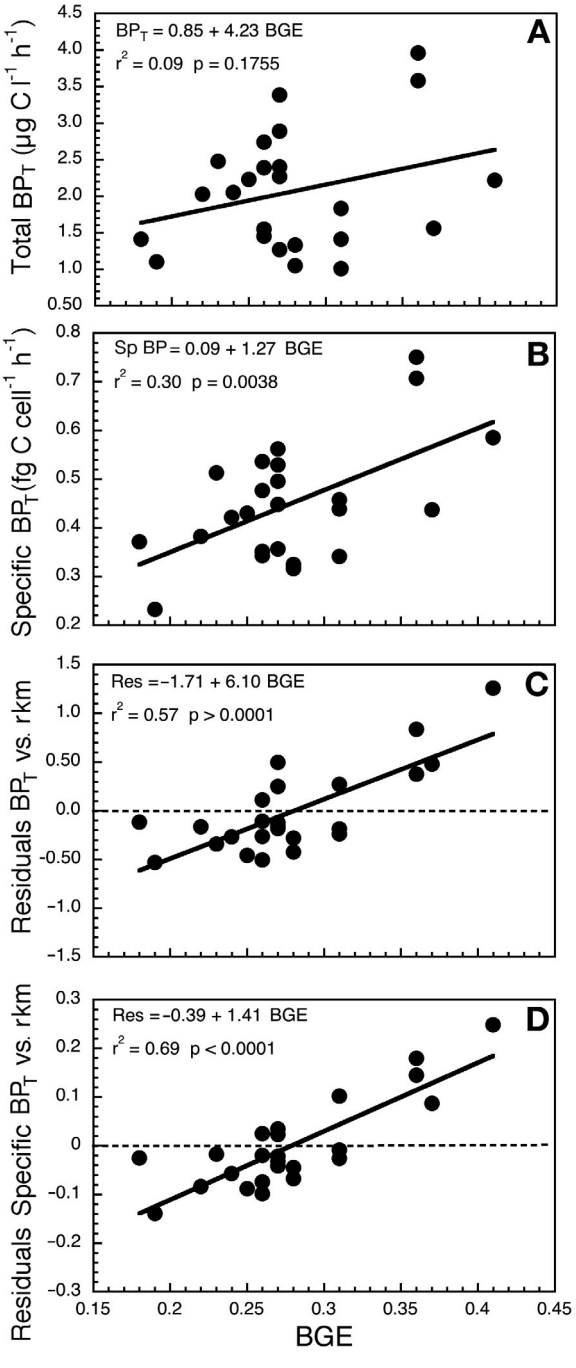

Fig. 5. Relationship between (A) BP_T and (B) specific (Sp) BP_T with BGE. (C) and (D) represent the relationship between the residuals of the BP_T and specific BP_T vs. river km (rkm) and BGE, respectively. Each point represents the average for the 6 data points for the 24 stations sampled during 2000 to 2002

Relationship between BP_T, specific BP and BGE

BGE explained only a small fraction of the overall spatial variability in BP_T (Fig. 5A, $r^2 = 0.09$) and of specific BP_T (Fig. 5B, $r^2 = 0.30$). To explore the finer features of the spatial pattern, we calculated the residuals of the BP_T vs. rkm and of specific BP_T vs. rkm relationships (shown in Fig. 2B and C, respectively). These residuals indicate local departures from the general declining pattern in BP_T and specific BP_T. BGE explained a much greater fraction of the residual variability of both BP_T (Fig. 5C, $r^2 = 0.57$) and of specific BP_T (Fig. 5D, $r^2 = 0.69$), suggesting that a significant portion of the local variation in both total BP and growth may be linked to local shifts in the processing of the carbon. Note also that the relationships depicted in Fig. 5 are based on independent measurements as BGEs are derived from the filtered samples while BP_T and specific BP_T are separately measured in unfiltered samples.

Estimating total BR in the Hudson River based on BP_T and BGE

BGE is related to local variations in BP_T and growth rate but does not explain the overall declining pattern along the river, which is presumably due to changes in bacterial carbon resources and other ecological factors. In order to test this hypothesis we require an estimate of total bacterial consumption. BR, being by far the largest component of bacterial carbon metabolism, provides a good indication of total consumption, but our measurements of filtered BR underestimate total BR, potentially by a large factor, as described for BP. One way to estimate total BR (BR_T) from our data is by combining the measured BP_T rates with the measured BGE (in the filtered samples) at each site (total BR = $[BP_T/BGE] - BP_T$). This approach assumes that BGE measured in the filtered sample applies to the entire bacterial community.

We assessed the approach of estimating BR_T from BP_T and filtered BGE with the samples taken in 2003, where we measured total plankton respiration in addition to all the other parameters. Total plankton respiration sets an upper limit to bacterioplankton respiration, and the latter can be approximated from total plankton respiration by subtracting the estimated contribution of algae and zooplankton. We used the values of chlorophyll concentration for each station sampled in 2003 to estimate primary production using the empirical equation in del Giorgio & Peters (1993), and assumed that algal respiration was 25% of the estimated rates of primary production. This approach probably overestimates primary production in the turbid Hudson River and thus results in an upper limit of algal respiration in the river. We further assumed that micro- and macrozooplankton respiration equalled that of algae, so we subtracted 2× algal respiration from the measurements of total plankton respiration to derive an estimate of BR_T. Total BR estimated from BP_T and BGE was not significantly different ($p < 0.05$) from the total BR estimated from total plankton respiration (mean of 3.81 and 3.33 µg C l^{-1} h^{-1}, respectively), and the two were significantly related (Fig. 6). We conclude that scaling BP_T measurements using empirically determined BGE results in reasonable estimates of total BR in this system.

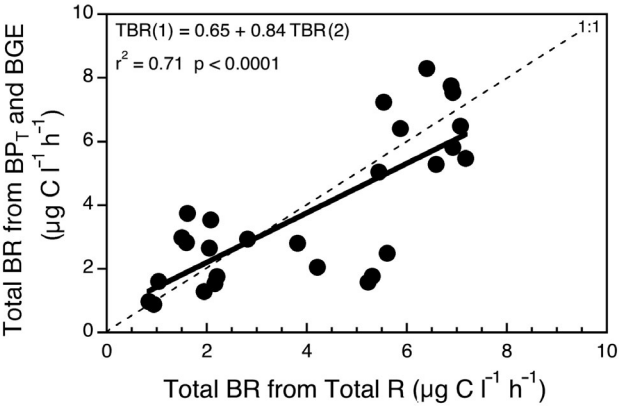

Fig. 6. Relationship between total bacterial respiration (Total BR) estimated by combining BP_T and BGE (TBR[1]), and total BR estimated from the measured total plankton respiration minus the assumed plankton respiration (Total R) (TBR[2]). See 'Results' for details

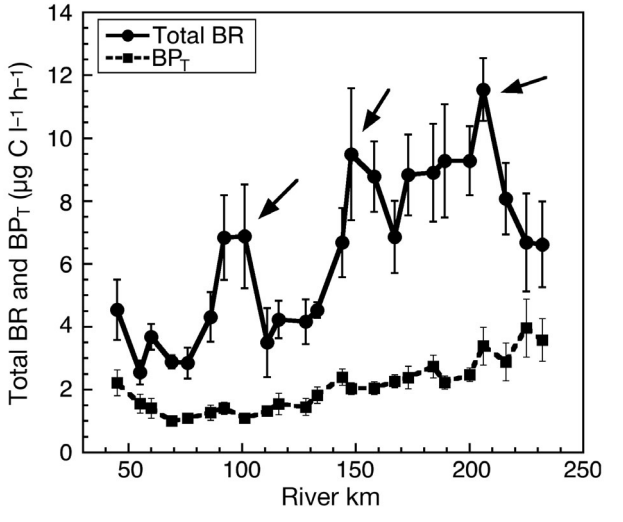

Fig. 7. Average total BR, estimated for each of the sites by combining BP_T and BGE, as a function of river km. Each bar represents the average (±SE) for the 6 samplings carried out at each site during 2000 to 2002. Average (±SE) BP_T taken from Fig. 2B. Arrows indicate examples of peaks in BR_T that did not have a counterpart in BP_T

Spatial patterns in BP_T and BR of the Hudson River

We applied the approach described above (BR_T = [BP_T/BGE] – BP_T) to calculate the pattern in total BR in the Hudson River using the 2000 to 2002 data (from Figs. 2B & 3). The resulting average spatial pattern in BR_T was substantially different from that of BP_T (see Fig. 7). There was an overall decline in BR_T along the north–south flow path of the river, suggesting a decline in overall organic matter availability and BCC along the river. There were also strikingly different features in the patterns of BP and BR: BR_T (and thus total carbon consumption) was highest in the mid-reaches of river, as opposed to BP_T which peaked in the upper portions, and in general there were peaks in BR at specific locations (marked with arrows in the Fig. 7) that did not have a counterpart in BP_T.

DISCUSSION

Jahnke & Craven (1995) posed a fundamental question that, while conceptually simple, has been difficult to address, and in practice the underlying assumption of most studies of aquatic microbial metabolism still is that patterns in BP reflect changes in BCC. Studies designed to assess the magnitude and importance of BGE rely primarily on simultaneous measurements of BP and BR (BGE = BP/BP + BR) in water samples that have been prefiltered to remove other planktonic components that could interfere with the respiration measurements. Likewise, short-term BCC cannot at present be measured independently and must be estimated from the sum of the measured BP and BR (BCC = BP + BR) (e.g. Apple 2005). Thus, no measurements of BGE and BCC are truly independent of BP, posing statistical as well as conceptual problems.

In addition to the above issues, we have shown that filtration removes significant portions of the total bacterial metabolism. Most studies of BR to date have assumed that the filtered fraction represents the majority of microbial metabolism (i.e. Biddanda et al. 1994, 2001, Pomeroy et al. 1994, Obernosterer et al. 2003, Reinthaler & Herndl 2005), but our results show that the approach tends to significantly underestimate BR. Further, because retention of bacterial activity in filters is not uniform and probably depends on the particulate load, size and attachment of bacteria, DOC and colloidal matrix, and type of filter used, key aspects of microbial carbon dynamics *in situ* might be masked or entirely changed by filtration artifacts. The problems associated with size-fractionation have been previously noted (Hopkinson et al. 1989, Obernosterer et al. 2003). For example, Hopkinson et al. (1989) measured increased respiration in <1 µm size fractions relative to more coarsely screened samples, while Obernosterer et al. (2003) reported a disproportional loss of BR after filtration. Both studies concluded that changes in respiration were due to the uncoupling of bacteria from nutrient recycling. Our results indicate that a variable and often large fraction of bacterial metabolism is simply lost to filtration, and that respiration rates measured in the filtered samples cannot be used to estimate total BCC directly because they underestimate the total rates of BR.

In our study, we attempted to circumvent these problems by combining our measurements in a different manner. We derived BGE estimates from filtered samples, and related these to the spatial patterns in BP_T and cell-specific BP_T (i.e. growth rate). The BP_T measurements were not used in the calculation of BGE, and are only weakly correlated to the filtered BP measurements used to calculate BGE. BGE explained a significant portion of the residual spatial variability in BP_T. In turn, combining BP_T measurements with the estimated BGE from the filtered fraction results in reasonable rates of total BR, based on our independent estimates derived from total plankton respiration in areas overwhelmingly dominated by bacterial activity. This gives us further confidence that the estimates of BGE derived from filtered samples are realistic and representative of bulk bacterial carbon metabolism. This in turn allowed us to overcome the problem of estimating total BR on the basis of filtered samples, and to reconstruct the spatial pattern in BR_T.

The main conclusion of our study is that the overall decline in the rates of BP along the Hudson River flow path is mainly driven by a decline in BCC and thus availability, but that BGE plays an important role in shaping both BP and growth at the local scale in different stretches of the river. The pattern in BP along the Hudson River is clearly a combination of changes in consumption as well as changes in BGE, to the point that the pattern in BP_T or bacterial growth would be impossible to recreate from any one of these 2 components individually.

These results provide insight on the spatial and temporal scales at which these different processes influencing bacterial metabolism operate in a large and variable ecosystem such as the Hudson River. There was a remarkable consistency in the average BGE obtained for the different transects, with mean values ranging from 0.25 to 0.32, and the general spatial pattern in BGE was similar among transects. In previous studies of other estuarine systems, we found a negative effect of temperature on BGE, but that these temperature effects are modest in the 15 to 25°C range (Apple et al. 2006), which may explain the lack of a significant influence of temperature in our data

set. In contrast, mean BP_T and BR_T varied by 2- to 3-fold among transects, primarily following changes in hydrology and, to some extent, temperature. Thus, a combination of hydrology and temperature seems to influence the average level of BCC along the river, whereas these factors appear to have less influence on BGE, at least during the seasons we sampled. We infer from these observations that the factors affecting total BCC (BR + BP) are not the same as those that influence BGE, indicating that these 2 aspects of carbon metabolism are regulated independently. Total organic carbon availability and thus consumption sets the average level of BP_T and BR_T along the river, whereas BGE is related to dynamic variations around these mean levels, and thus BGE influences the finer features of the spatial pattern.

Spatial patterns in BGE

The average BGE values we obtained are well within the range expected based on the average productivity of the system (del Giorgio & Cole 1998), and are in agreement with previous measurements of BGE in the Hudson River using different approaches (Findlay et al. 1992, Roland & Cole 1999, Maranger et al. 2005). The spatial patterns in BGE might be related to a number of factors that interact, including the supply and quality of the organic matter consumed, nutrient availability, and bacterial community composition. We found that BGE peaked both in the uppermost reaches and in the lower oligohaline area, and this pattern does not coincide with the general declining trend in nutrient concentrations along the river (Lampman et al. 1999, Maranger et al. 2005). The residual analysis (Fig. 4) indicates that departures in BGE from the overall mean are linked to local variations in seston and DOC, suggesting that bacteria are quickly reacting to changes in local organic matter inputs. As pointed out above, BGE appears to be independent of the rates of carbon consumption (BP_T + BR_T), so the local differences observed in BGE may more closely reflect varying qualities of the DOC rather than its rate of supply. Eiler et al. (2003) recently concluded that BGE and growth rate are generally not constrained by substrate availability in most natural waters, and previous studies have linked BGE to the nature and lability of the organic substrates in freshwater samples (Middelboe & Søndergaard 1993). In this regard, Kirchman et al. (2004) have recently concluded that there must be substantial shifts in the quality of DOC inputs along the Hudson River, on the basis of drastic shifts in composition of local bacterial assemblages and the resulting exoenzymatic activity in different sections of the river.

Links between BP, BR and BGE

Total BR estimated using filtered fractioned BGE and BP_T averaged 156 µg C l^{-1} d^{-1}, in agreement with previous estimates of respiration in the river based on different approaches (Findlay et al. 1992, Howarth et al. 1996, Maranger et al. 2005). The declining trend in BP from the headwaters southward through the estuary has been described before (Findlay et al. 1992, Maranger et al. 2005), and has been interpreted to indicate a proportional decline in carbon consumption, and thus of carbon input, availability and transport along the river. This interpretation agrees with the systematic decline in DOC concentration that occurs along the same transect (Fig. 1), and with the notion that much (>75%) of the organic carbon loading to the river occurs in the upper reaches, and that this carbon is progressively decomposed and utilized during transit, fueling ecosystem metabolism along the flow path (Howarth et al. 1996, Taylor et al. 2003). Our results are consistent with these prior studies, but further suggest that the underlying patterns in carbon consumption and processing are considerably more complex and dynamic than previously thought.

Our estimates of total BR show that areas with the highest BP are not necessarily those with the highest total BCC (Fig. 7). Likewise, the highest apparent rates of bacterial carbon metabolism occur in the mid section of the river, between rkm 140 and 200, whereas the highest average rates of BP consistently occur above rkm 200 (Fig. 7). Further, there seem to be very localized areas that are characterized by extremely high rates of carbon metabolism. Examples of such hot spots, which are not apparent for total BP but are notable for total BR, are marked with arrows in Fig. 7. Interestingly, the peaks of chlorophyll that are recurrent between rkm 150 and 190 do not necessarily coincide with the areas of highest total BR. Rather, there appear to be hot spots of metabolic activity that may reflect areas of enhanced interaction between the pelagic and benthic components as well as zones of particularly active exchange between the river and adjacent wetland/terrestrial areas. The existence of these hot spots would suggest that the interaction between the river and the terrestrial ecosystem may not be continuous but rather that there may be ecotones that are critical to the functioning of the river system, as suggested by McClain et al. (2003). These 'hot spots' are not just areas with local increases of total system metabolism, but also with substantially different patterns of carbon (and possibly nutrient) processing by bacteria, as evidenced from our BGE data.

The plasticity in bacterial carbon metabolism that we report for the Hudson River is probably a feature of many aquatic ecosystems, although there are very few

previous studies that have assessed BR and BGE with sufficient detail to effectively detect and describe such patterns. For example, Lemée et al. (2002) followed BP and BR at an open-water station in the Mediterranean Sea, where rates of BP and BR greatly diverged both with depth and in different seasons. Likewise, Pradeep Ram et al. (2003) reported substantial seasonal variability in BGE and systematic differences in BR and BGE between 2 nearby estuarine sites. Sherry et al. (1999) also report a relatively high degree of uncoupling between BP and BR in the water column of the North Pacific. In one of the largest studies of the type to date, Reinthaler & Herndl (2005) found a high degree of uncoupling between BR and BP and a clear link between BGE and primary production in the North Sea. This is in stark contrast to our own results, which show that some of the lowest average BGE values occurred precisely in the areas of peak chlorophyll development. These contrasting results are not necessarily conflicting, since the reliance of bacteria on the supply of algal carbon is very different between these systems, and it is clear that in the Hudson River, algal-derived carbon represents only a small fraction of the total BCC (Findlay et al. 1991). There is clearly no single factor that can capture the complexity of the metabolic response of bacteria to changing carbon resources.

Our analysis confirms the general assumption that planktonic bacteria are metabolically versatile and sensitive to local changes not only in the amount but also in the nature of the organic inputs, and quickly react by adjusting the balance between consumption, respiration and biosynthesis. This metabolic flexibility has been predicted from physiological models (Vallino et al. 1996) as a means of maximizing growth in the context of limiting and heterogenous resources, and has been observed in single bacterial cultures (Egli 1996); however, it has seldom been empirically described in natural communities, most likely because these measurements require a level of resolution that is difficult to achieve. We have also shown that growth rate and growth efficiency are positively linked in the Hudson River. This link has been often hypothesized (Vallino et al. 1996, del Giorgio & Cole 1998), and has been empirically established in cultures and chemostat experiments (Egli 1996, Søndergaard & Theil-Nielsen 1997) but has been difficult to observe under ambient conditions.

There has been increased interest in recent years regarding the magnitude and regulation of BGE in natural aquatic ecosystems. Recent studies suggest that there is a positive curvilinear relationship between BGE and BP (i.e. del Giorgio & Cole 1998, Roland & Cole 1999, Reinthaler & Herndl 2005, Kritzberg et al. 2005). Interestingly, most studies to date have treated BGE as the dependent variable and BP as the independent variable, perhaps reflecting the fact that BGE estimates are most often derived from BP measurements. The positive correlation between BGE and BP may simply reflect the fact that both carbon consumption and BGE tend to increase with overall system productivity, and therefore so does BP, when large gradients are considered. From a physiological standpoint, however, the sequence is different, because BGE modulates the amount of production that results from a given rate of carbon consumption, and not vice versa. Our results are consistent with this sequence and further support the key role that BGE plays in modulating BP, a role that appears largely independent of the rates and controls of carbon consumption. We have shown that BGE explains a significant fraction of the local variability in BP_T and bacterial growth, and it is clear that shifts in the allocation of carbon play a significant role in shaping the overall patterns in BP and growth in this ecosystem.

The range of metabolic versatility of heterotrophic bacterial metabolism that we have described here has often been assumed to occur as the response to large shifts in resources across major ecosystems or along extremely broad environmental gradients (i.e. coastal vs. open ocean, oligotrophic vs. eutrophic lakes, polluted vs. pristine rivers). Here we show that the same versatility exists within much narrower environmental gradients and reduced spatial and temporal scales. These observations indicate that it is conceptually and practically incorrect to equate organic matter input, BCC and BP and growth, even within a single ecosystem.

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