Nutrient Constraints on Metabolism Affect the Temperature Regulation of Aquatic Bacterial Growth Efficiency

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Abstract Inorganic nutrient availability and temperature are recognized as major regulators of organic carbon processing by aquatic bacteria, but little is known about how these two factors interact to control bacterial metabolic processes. We manipulated the temperature of boreal humic stream water samples within 0–25°C and measured bacterial production (BP) and respiration (BR) with and without inorganic nitrogen+phosphorus addition. Both BP and BR increased exponentially with temperature in all experiments, with Q_{10} values varying between 1.2 and 2.4. The bacterial growth efficiency (BGE) showed strong negative relationships with temperature in nutrient-enriched samples and in natural stream water where community-level BP and BR were not limited by nutrients. However, there were no relationships between BGE and temperature in samples where BP and BR were significantly constrained by the inorganic nutrient availability. The results suggest that metabolic responses of aquatic bacterial communities to temperature variations can be strongly dependent on whether the bacterial metabolism is limited by inorganic nutrients or not. Such responses can have consequences for both the carbon flux through aquatic food webs and for the flux of CO_2 from aquatic systems to the atmosphere.

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Introduction

Bacterial metabolism plays a key role for cycling of dissolved organic carbon (DOC) in marine and freshwater systems [1, 2]. Bacterial use of DOC for growth represents a point of entry for organic carbon into aquatic food webs [1, 3] and bacterial respiration results in production of CO_2 which is largely returned to the atmosphere [4, 5]. Bacterial processing of DOC is therefore important both for the function of aquatic food webs and for the exchange of CO_2 between aquatic systems and the atmosphere. The relationship between bacterial production (BP) and respiration (BR) is defined by the bacterial growth efficiency (BGE), expressed as BP divided by the sum of BP and BR [6].

Aquatic systems, especially freshwaters, are subject to large seasonal and spatial temperature variations. In temperate, boreal, and subarctic lakes, the annual temperature amplitude can be >25°C and vertical temperature differences due to stratification are often >10°C. In spite of the fundamental role of temperature as a regulator of biological process rates, surprisingly little is known about how the metabolism of aquatic bacteria responds to changing temperatures. If bacterial taxa are living well below their temperature optima, which is often the case in cold waters [7], metabolic processes seems to be regulated by temperature in the same way as kinetically enhanced chemical and enzymatic reactions, i.e., with exponentially increasing activities in response to rising temperature [8]. Such temperature dependence can be characterized by the Q_{10} factor which denotes the relative process rate increase for a temperature raise of 10°C. Metabolic rates of pure cultures of aquatic bacteria often have a temperature dependence that can be described by Q_{10} values of 2–3 [9].

The temperature dependence of BP and BR in natural bacterial communities is often difficult to describe by a

parameterized Q_{10} value [10]. Patterns inconsistent with a common Q_{10} dependence have been observed together with limited supply of DOC [9, 11], at extreme temperatures [9], or as a result of shifts in bacterial community structures [12]. A few recent studies show a tendency of decreased BGE with increased temperature [10, 11, 13], indicating systematically higher Q_{10} for BR, relative to the Q_{10} for BP, possibly due to increased respiratory costs for cell maintenance at higher temperatures [14, 15]. However, field data show a very large variation around the regression values of BGE inferred from temperature, especially in cold water [13]. Such observations and the fact that the most extensive review on BGE so far [6] did not find a consistent relationship between BGE and temperature show that we still do not fully understand how temperature regulate BP, BR, and BGE.

A reason why clear patterns of, e.g., BGE, in response to temperature are difficult to derive from field data is that bacterial metabolic rates are largely determined by other factors than temperature. Previous studies have focused on how the supply of resources in general [11, 16], and particularly labile DOC [9], may affect the relationships between bacterial metabolic processes and temperature. Less attention has been given to the role of inorganic nutrient limitation. In a variety of aquatic systems, low concentrations of nitrogen (N) [6] and, especially, phosphorus (P) [17, 18] limit the metabolic rates of bacterial communities, while carbon uptake appear to be in excess relative to bacterial physiological needs [19]. On a community level, nutrient limitation slows down both BP and BR, to varying degrees, but it also tends to decrease BGE [20]. If bacterial metabolic rates depend on both temperature and nutrient limitation, plus possible interaction effects between these two factors [9], it is logical that field data of BGE as a function of temperature show a great variation [13, 16] caused by varying degrees of nutrient limitation. For a better understanding of the variation of BGE with temperature, experimental work is needed in which bacteria are exposed to limiting and excess concentrations of inorganic nutrients.

In this study, we tested the hypothesis that the availability of inorganic nutrients, relative to bacterial needs, controls bacterial metabolic responses to variation in temperature. We selected two well-studied boreal forest streams representing natural states of nutrient-limiting versus non-nutrient-limiting conditions. We measured BP, BR, and BGE in stream water incubated at different temperatures (0–25°C) with and without nutrient (N+P) addition. The study was performed on boreal systems with a high input of allochthonous carbon, supporting most of the bacterial metabolism. Inland waters have recently been recognized as a significant component in the global carbon cycle [5, 21, 22]. Boreal freshwaters are of particular interest as they serve as recipients for DOC drainage from northern soil systems, which hold one of the largest pools of organic carbon on the surface of the earth [23–25]. Moreover, brownwater systems are often subject to very rapid temperature changes, due to light absorption by DOC, and stable thermal stratifications in lakes cause large temperature differences between different strata [26]. Variations in BGE in such systems are crucial for the fate of allochthonous carbon. A high BGE can result in a high degree of allochthony (share of allochthonous carbon in food web members) [3] and low BGE can cause efficient recycling of terrestrial organic carbon back to the atmosphere [5].

Methods

The study was carried out in the Krycklan catchment at the Vindeln Experimental Forests (64°14′N, 19°46′E) in northern Sweden [27]. Water samples with naturally inorganic nutrient-limited bacteria were obtained from the small headwater stream Västrabäcken, where the bacterial metabolism is nutrient (N+P) limited [28]. Samples with naturally non-inorganic nutrient-limited bacterial assemblages were taken from the adjacent stream Kallkällsmyren [28]. We defined nutrient limitation as the state where BP and BR of a bacterial community responds positively to addition of inorganic nutrients (N+P) in laboratory dark incubations.

Addition of labile DOC (glucose), N (NH_4NO_3), and P (KH_2PO_4) to stream samples 2005 and 2008 (Berggren, unpublished) showed that BP in Kallkällsmyren was stimulated by addition of labile DOC only. BP in Västrabäcken was significantly affected by DOC addition only after an initial addition of N+P. Although the ratios between the elements C, N, and P are similar in the two streams (Table 1), the differences in DOC quality are pronounced. Both streams are likely to be almost totally dominated by allochthonous organic carbon [29]. However, compared to Kallkällsmyren, the DOC in Västrabäcken is to a much larger extent constituted by labile low molecular weight organic carbon compounds that have a potential of contributing to efficient bacterial growth [28, 29]. In order to make full use of this potential, bacteria are in need of more inorganic nutrients than what is available in Västrabäcken [28].

No other nutrients than N or P were considered for addition. Combined additions of C, N, and P in 2008 to the study streams (Berggren, unpublished) resulted in increases in BP and BR in several hundreds of percent. Thus, we assumed no elements or compounds other than organic C, or inorganic N and P, were limiting the metabolism in this

Table 1 In situ concentrations of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP), together with the in situ temperature (T), of the study streams

Sample	DOC (mgL^{-1})	TDN (mgL^{-1})	TDP (µgL^{-1})	T (°C)
Västrabäcken (experiment 1)	13.2	0.31	14	8.6
Västrabäcken (experiment 2)	14.5	0.29	15	0.0
Kallkällsmyren	23.4	0.55	31	8.1

study. Limitation of bacterial metabolism by micronutrients such as iron (Fe) has rarely been reported in humic freshwaters, possibly because freshwater bacteria can produce siderophores that react with the large pool of refractory organically bound Fe, forming bioavailable siderophore–Fe complexes [30].

Streams in this region are of general interest because they exert a major influence on the input of DOC, nutrients, and bacterial cells to downstream aquatic systems [31]. Moreover, in water bodies that have a short water turnover time (<100 days), including most Swedish lakes, the bacterial community structure is similar to (and controlled by) the community structure of inflowing streams [32]. The catchment areas (0.14–0.19 km^2) were dominated by Norway spruce (*Picea abies*), Scots pine (*Pinus sylvestris*), and, in the case of Kallkällsmyren, by patches of *Sphagnum* peat mire. The climate of the region is characterized by short summers and long winters, with snow usually covering the ground from the end of October to the beginning of May. The temperature of both study stream is normally <10°C during the whole year. For detailed descriptions of catchment characteristics, see Ågren et al. [33]. Spatial and temporal patterns of aquatic bacterial metabolism in the Krycklan catchment have previously been described [28, 29, 34, 35].

Sampling was conducted on June 2 (both streams) and November 24 (Västrabäcken only) 2008. Catchment discharge was close to baseflow conditions on both sampling dates (ca. 3 Ls^{-1}km^{-2}; the streams do not normally dry out at any time of the year). Water was collected in 10-L carefully washed and stream water-rinsed plastic cans that were kept dark and cool until arrival at the laboratory. The samples were thereafter (maximum 2 h after collection) equilibrated with standard air (78% N$_2$, 21% O$_2$, and 0.03% CO$_2$) and then subsampled into 22-mL glass bottles, sealed with gas-tight septa [28]. The subsampling procedure was carried out using both non-enriched control water and water added 1 mgNL^{-1} in the form of NH$_4$NO$_3$ and 100 µgPL^{-1} in the form of KH$_2$PO$_4$. This generated DOC/N/P ratios between 116:11:1 and 179:12:1. Considering that only ca. 10% of the DOC is available for bacterial uptake on a relevant time scale [28, 29, 34], these ratios should represent situations of strong carbon limitation [17–19]. Subsets of the bottles were incubated in 0, 5, 10, 15, 20,

and 25°C using cooling incubators (SANYO MIR-153) for the temperature range 5–25°C and ice baths for the 0°C treatment. All samples were incubated in the dark without shaking (photosynthetic ability was negligible). Samples inside the cooling incubators had stable temperatures ±0.5°C around the targeted values. Most ice bath samples were close to the freezing point all of the time (new ice was added each time the temperature rose to ca. 1.5°C). However, one ice bath with samples from Kallkällsmyren was unstable (sample temperature up to >5°C) and, therefore, Kallkällsmyren BP data from 0°C were lost.

BP and BR were analyzed on triplicate samples, repeatedly on six to eight occasions distributed within 16 days from sealing of the incubation bottles. In the first experiment (water sampled June 2), BR was measured as dissolved inorganic carbon (DIC) production using a GC–FID (Perkin-Elmer), with a headspace auto-sampler that operated directly on the incubation bottles. The incubation bottles that were intended for gas chromatography were filled with 15 mL of sample water and 7 mL of a standard air gas mix (78% N$_2$, 21% O$_2$, and 0.03% CO$_2$) at the incubation start. Before analysis, the samples were acidified (converting all HCO$_3^-$ and CO$_3^{2-}$ to CO$_2$ and H$_2$CO$_3$) and shaken to achieve equilibrium. Separation was carried out on an Elite-PLOT Q column using N$_2$ as carrier gas. Each incubation bottle was only used once and then discarded. In the second experiment (water sampled November 24), BR was measured as DIC production estimated from oxygen consumption assuming a respiratory quotient of 1. This method gives results comparable to direct CO$_2$ production measurements [36]. Oxygen concentrations were measured using a FIBOX 3 (PreSens) by optical readings from the outside of top-filled incubation bottles (without headspace) with sensor spots attached to the inside wall. Recordings were repeatedly performed on each sensor. Oxygen concentrations by the end of the experiments were never <5 mgL^{-1}. It was assumed that potential respiration of protozoa and metazoa was negligible, as the total biomass in humic "unfiltered" water during dark incubations has been shown to be vastly dominated (ca. 90%) by bacteria [37].

BP was measured with the leucine incorporation method described by Simon and Azam [38]. Aliquots of 1.2 mL of the samples (15 mL sample, 7 mL gas) were exposed to

50 nM [^{3}H]-leucine during 1 h in treatment temperature (0–25°C). Blanks were pretreated with 5% w/v of trichloroacetic acid (TCA). Leucine incorporation into protein was determined by precipitation with TCA and centrifugation, followed by scintillation counting (Beckman LS 6,500). The leucine incorporation was converted into carbon units according to Simon and Azam [38]. It was assumed that the leucine that was taken up was incorporated into protein and not to a significant extent respired. Del Giorgio et al. [39] found that leucine respiration was strongly negatively correlated to BGE and that little leucine respiration (10–20%) occurred when BGE was 0.20 or higher (the range of BGE previously observed in the study streams is 0.19–0.46 [28, 34]). The added concentration of leucine (0.0036 mg L^{-1} of DOC) should not have significantly changed the availability of DOC for bacteria. In both study streams, natural concentrations of highly labile DOC in the form of free amino acids, simple carbohydrates, and low molecular weight organic acids are, collectively, at least two orders of magnitude higher [29]. Similarly, reported concentrations of naturally bioavailable N (inorganic or organic) in headwater streams of the region [40] are two orders of magnitude higher than the content of N in the added leucine (50 nm leu=0.0007 mg L^{-1} of N).

Average BP during the incubations was calculated by integrating the area under the BP curve over time and dividing the integrated value with the number of days. For this purpose, BP was linearly interpolated between measurements over time. BR was calculated from the slope of linear regression lines for DIC as a function of incubation time. Values generated from the triplicate BP and BR readings were used to calculate triplicates of BGE values by the equation BGE = BP/(BP + BR).

Using filtered samples (0.45 μm Millipore), DOC and TDN (total dissolved nitrogen) were measured on a Hach IL 500 TOC/TN analyzer and TDP (total dissolved phosphorus) after persulfate oxidation followed by the standard molybdenum blue method. The standard deviation of the between-replicate variations was <3% for DOC and TDN.

All statistical analyses were performed in PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA). The significance of exponential (Q_{10}) temperature dependencies was tested by analyzing bivariate correlations between temperature and the logarithms of BP, BR, and BGE. Q_{10} values for significant relationships were calculated as $e^{b \cdot 10}$, where e is the base of the natural logarithm and b is one of the two constants (b and c) in the standard exponential trendline equation $y = c \cdot e^{b \cdot x}$. Difference in Q_{10} between BP and BR were tested using a two-tailed paired t test. Deviations from the exponential temperature dependence were tested by performing residual analyses, where BP, BR, and BGE values suggested by the exponential trendline equations were subtracted from observed values. Residuals were tested for significant patterns using "Curve Estimation" with ANOVA table (under "Analyze" and then "Regression") in PASW 18.

Results

The bacterial production (BP), calculated from leucine uptake, generally increased during the incubation period, when temperature was 0–5°C, and decreased during incubation at 20–25°C. At intermediate temperatures (10–15°C), BP increased during the first part of the incubation while showing decreases during the later part. Hereafter, BP is referred to as the integrated average for the whole incubation period (see Methods). BR was stable during incubation at all temperatures.

Average BP in water from Västrabäcken was 6–35 μg C L^{-1} day^{-1} (Fig. 1a, b) and the BR was 7–63 μg C L^{-1} day^{-1} (Fig. 1d, e). Inorganic nutrient (N+P) addition stimulated BP of Västrabäcken samples at all temperatures. BR was consistently stimulated only at the higher temperatures 20–25°C, while showing no stimulation at 0°C and mixed responses at intermediate temperatures. BP and BR in nutrient-enriched Västrabäcken samples were 13–54 and 8–94 μg C L^{-1} day^{-1}, respectively. The values from Kallkällsmyren were 20–30 (BP) and 16–96 μg C L^{-1} day^{-1} (BR) in both natural and nutrient-enriched samples (Fig. 1c, f).

Both BP and BR showed significant ($R^2 > 0.66$, $n=6$, $p < 0.05$) positive exponential responses to increased temperature in all experiments (Fig. 1a–f), with Q_{10} varying between 1.2 and 2.4. In nutrient-enriched samples, the Q_{10} of both BP and BR (and the slope of BGE as function of temperature) showed considerably less variation between streams and experiments compared to non-enriched samples. Overall, Q_{10} was significantly higher for BR, compared to BP (two-tail $p < 0.01$, paired $t=-4.0$, $df=5$). However, the difference in Q_{10} between BP and BR was much larger in nutrient-enriched Västrabäcken samples and in samples from Kallkällsmyren (0.62–0.91 units of difference) compared to the inorganic nutrient-limited natural samples from Västrabäcken (0.04–0.25 units of difference).

In contrast to BP and BR, BGE in naturally inorganic nutrient-limited samples from Västrabäcken (varied between 0.29 and 0.45) was not significantly related to temperature (Fig. 1g, h). However, in nutrient-enriched (Fig. 1g, h) and naturally non-nutrient-limited samples (Fig. 1i), where BGE was 0.24–0.62, there were strong negative exponential relationships between BGE and temperature ($R^2 > 0.92$, $n=6$, $p < 0.01$). The average response in BGE to nutrient addition in Västrabäcken samples was 0.21 units at 0°C, but this response decreased linearly ($R^2 = 0.91$, $n=6$, $p < 0.01$) to about 0 units at 25°C (Fig. 2). The
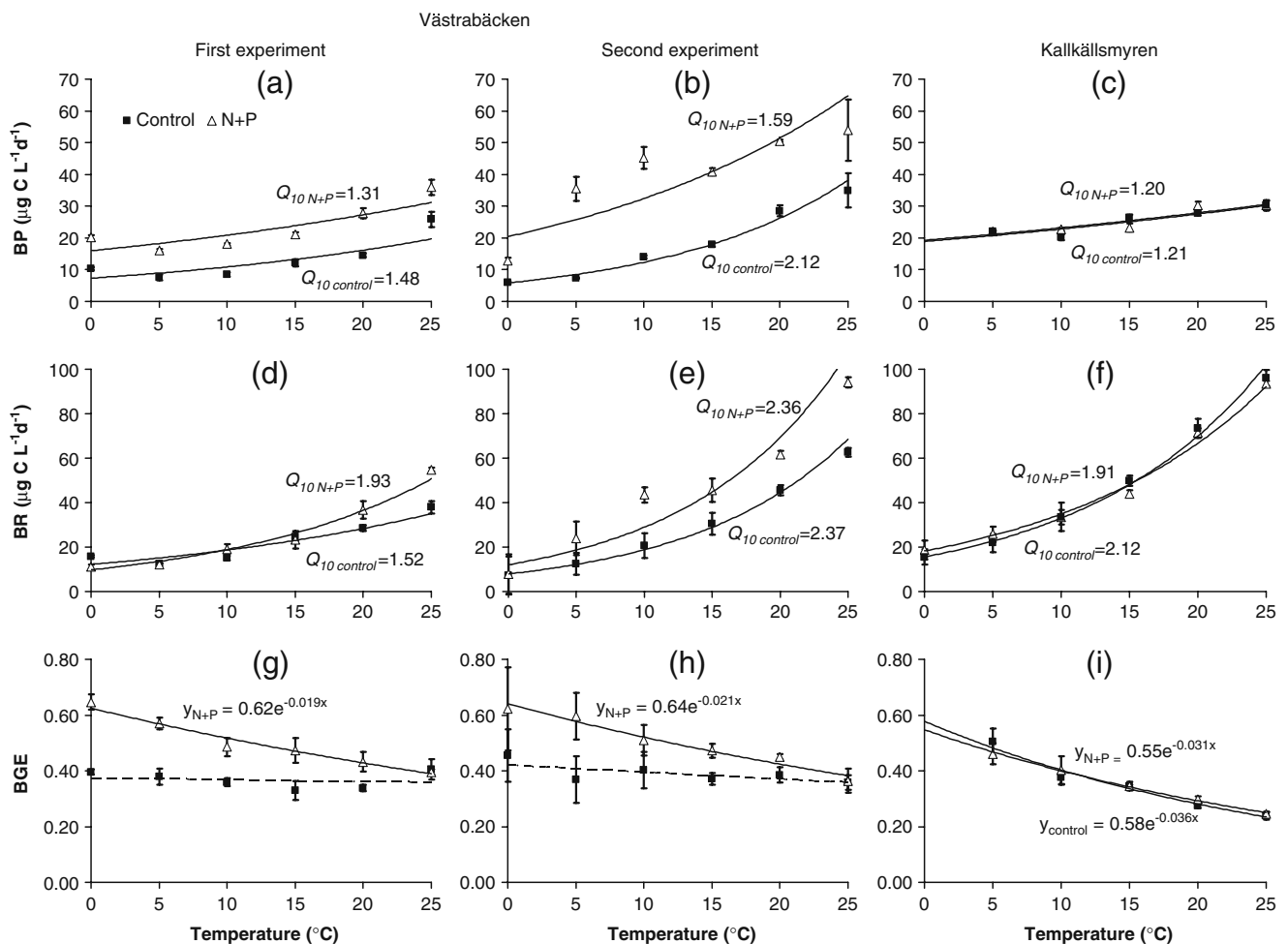
Figure 1 Average bacterial production (**a**–**c**), bacterial respiration (**d**–**f**), and bacterial growth efficiency (**g**–**i**) during 16-day bioassays as functions of incubation temperature in non-addition (control) and in nutrient-enriched (N+P) samples. *Solid regression lines* denote signif-icant ($p < 0.05$) exponential relationships and *dashed regression lines* denote non-significant relationships. *Error bars* show SD of triplicate incubation series

relationships between BGE and temperature in nutrient-enriched and naturally non-nutrient-limited samples (Fig. 1g–i) suggested that BGE decreased exponentially by 17–30% for each 10°C increase in temperature.
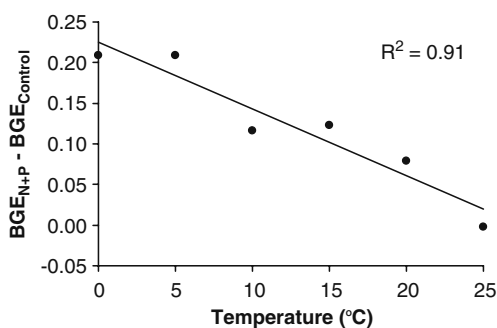

Figure 2 Response in bacterial growth efficiency to inorganic nutrient addition at variable temperatures. *Dots* denote mean response of two experiments with naturally nutrient-limited stream bacteria from Västrabäcken

Although the temperature dependencies of BP, BR, and BGE were best described by exponential regression lines in all experiments (Fig. 1), the residuals in these regressions were random only for BGE. Residuals for both BP ($R^2 = 0.35$, $F_{2,\,31} = 7.9$, $p < 0.01$) and BR ($R^2 = 0.43$, $F_{2,\,33} = 12.6$, $p < 0.001$) showed significant quadratic relationships with the absolute difference between experiment temperature and in situ temperature (Fig. 3). Negative BP and BR residuals occurred at low or high degrees of temperature manipulation, and positive residuals peaked at intermediate degrees of manipulation (Fig. 3). This pattern was independent of whether bacterial metabolism was limited by inorganic nutrients or not.

Discussion

Inorganic N and P are critical building blocks of biomolecules, such as peptides and RNA, involved in protein
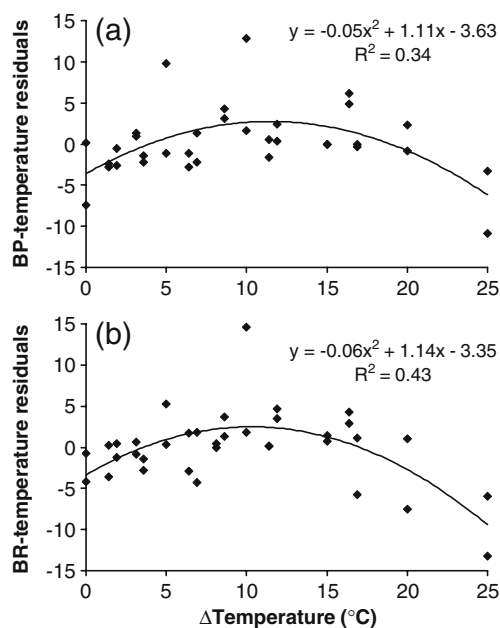
Figure 3 Residuals from the BP–temperature regression (**a**) and the BR–temperature regression (**b**) as functions of ΔTemperature. The residuals represent observed values of metabolic rates (BP and BR) minus values suggested by the exponential trendline equations in Fig. 1. *ΔTemperature* denotes absolute difference between experiment and in situ temperature

synthesis and the growth of bacteria [19]. Low concentrations of available N and P often constrain BGE, i.e., the degree to which bacteria can allocate assimilated organic carbon to production of biomass [6]. Previous studies have reported that BGE may [10, 11, 13] or may not [6, 16] respond negatively to increased temperature. Results from this study (Fig. 1g–i) clearly demonstrate that temperature did not affect BGE when inorganic nutrients were scarce, suggesting that the inorganic nutrient limitation of BGE can override the temperature regulation. With sufficient supply of N and P, however, there were strong negative relationships between BGE and temperature (Fig. 1g–i), caused by a higher increase in BR, relative to the increase in BP (Fig. 1).

Decrease in BGE of non-nutrient-limited bacteria with higher temperature is likely triggered by higher respiratory costs for compensating increased leakage of solutes across cell membranes, and for maintaining the function of macromolecules that may not be adapted to high temperatures and, therefore, become increasingly unstable [14]. Nutrient (N+P) limited communities represent a bioenergetically different situation, where the availability of carbon and energy is, relative to bacterial needs for growth, higher than the availability of inorganic nutrients. Under such conditions, BR of the single active cells tends to be high (although community BR is relatively low). Maximizing BR makes it possible for bacterial cells to activate

metabolic pathways that increase nutrient use efficiency, i.e., growth per unit of limiting nutrient [20]. It has also been hypothesized that increased BR, together with the production of organic storage products and exudates, can be a mean for disposing surplus uptake of carbon [19], e.g., in energy spilling respiratory reaction cycles [41]. The high rate of BR in inorganic nutrient-limited bacterial cells should cause low and stable BGE at all temperatures, as observed in samples from Västrabäcken (Fig. 1g, h). If energy is in excess, the increased energy demand for cell maintenance at higher temperatures seems to be met without the need for re-allocation of carbon use between BP and BR, and hence BGE can be decoupled from temperature.

Both BP and BR were in the range of values previously observed in incubation studies on the same streams at a given temperature [28, 34, 35]. In this study, water was collected at natural streams temperatures of 0–8.6°C and then exposed to temperatures from 0 to 25°C. Contrary to previous studies [11, 42], we found no major deviations from the exponential (Q_{10}) dependence of BP and BR, e.g., caused by manipulation of temperatures outside of the range that bacteria physiologically can adapt to. Nonetheless, significant quadratic relationships ("U" upside down) were found between the BP and BP residuals (i.e., the observed rates minus the rates suggested by exponential trend line equations) and the degree of temperature manipulation (Fig. 3). These relationships were driven by (1) negative residuals at the highest levels of temperature elevation and (2) positive residuals at intermediate levels of manipulation towards either warmer or colder temperatures. The first is expected from theory as a temperature-induced increase in any metabolic rate eventually will need to depart from the exponential course and level off at a temperature optimum [7, 10]. What is more surprising is the positive residuals at intermediate changes, especially since adaptation to a new temperature is associated with energetic costs, e.g., for changing of membrane lipid composition to maximize function at the new temperature [43–45]. One could speculate that a certain degree of temperature change poses a "positive stress" that triggers physiological responses and might force dormant cells [46] to increase their activity in order to adapt. A more concrete explanation for a similar pattern was recently found by Adams et al. [47] who observed that bacterial communities can have dual or multiple temperature "sub-optima", each represented by a different group of taxa within the bacterial community. If the in situ water temperature is between two such sub-optima, both increased and decreased temperature can have positive marginal effects on bacterial metabolic rates.

This study was conducted in freshwaters of the boreal region where spatial and temporal variations in temperature

are high. In Sweden, the annual snow melt flood in spring [48] replaces the entire water volume of most lakes [32] with ca. 0°C water. Soon after this event, light absorption by DOC leads to a rapid warming, with stratified lake temperatures from 4°C at the bottom to 20°C or more at the surface [26]. Thus, all temperatures included in this experimental study (0–25°C) can be expressed by a single boreal lake within a short period of transition between spring and summer. In line with several previous studies [9, 10, 45], BP and BR increased with increasing water temperatures at all temperature intervals with Q_{10} between 1.2 and 2.4 (Fig. 1a–f), showing that the rates of bacterial use of DOC is much higher in warmer than in colder water temperatures. However, the metabolic response to temperature was also clearly dependent on whether bacterial community-level metabolic rates were nutrient limited or not, and our results suggest that the potential for highest BGE is found in nutrient-saturated bacteria at low temperatures.

The response in BGE to addition of inorganic nutrients (N or P) has previously been measured mainly at higher temperatures (≥ 20°C) where BGE is generally not stimulated more than with ca. 0.10 units [28, 45, 49–51]. The potential for nutrient-induced boosts in BGE should be much larger at lower temperatures, suggested by the strong negative correlation between temperature and BGE response in samples from Västrabäcken (Fig. 2). Too few multi-temperature addition experiments have been performed to confirm that this pattern applies to in situ natural systems. However, our results fit the large-scale patterns of BGE distribution across different temperatures, e.g., as presented by Rivkin and Legendre [13]. In their data set, BGE showed a relatively narrow range (<0.28) at high temperatures (>20°C), regardless of natural between-site differences in nutrient availability. In contrast, there was a large scatter of BGE at low temperatures (<10°C), from 0.09 to 0.73. High and variable BGE at low temperatures can be caused by the influence of inorganic nutrients. However, large-scale variations in BGE across temperatures have, so far, mainly been studied in relation to productivity (chlorophyll concentration) [16], and the role of inorganic nutrients remains to be specifically addressed.

In natural ecosystems, there may be additional factors that interact with nutrient (N+P) limitation and temperature in the control of bacterial metabolism, thus making the interpretation of how our results apply to nature less straightforward. For example, the degree of BGE stimulation by P addition has been shown to decrease with increasing viral-induced bacterial mortality [52]. The pattern of decreased nutrient stimulation of BGE with higher temperature could hypothetically reflect the activities of viruses, given that viruses affected a larger share of the bacterial cells in warm water compared to cold water. On the contrary, reports indicate that viral particles per bacterium rather tend to decrease with

increasing temperature in both laboratory [53] and empirical [54] studies, and markedly high infection rates have been reported in Antarctic and Arctic bacterioplankton [55]. Therefore, the possibility that viruses mediated patterns of BGE responses in this study seems unlikely.

Another interacting feature in the regulation of bacterial metabolism is the community structure. As a response to changed temperature, shifts in dominant community members (taxa) can occur within a few days [47]. Lakes that have a long water turnover time (>100 days) start to develop their own bacterial communities that are fundamentally different from the communities of the input streams [32]. It is not known if headwater stream communities and communities of lakes with long turnover time respond differently to changes in temperature. However, in boreal lakes with short turnover times and high inputs of DOC and bacterial cells from headwater sources, it is reasonable to assume that the responses in community metabolism and structure to changing temperature can be qualitatively similar to the community responses in the present study.

When bacterial metabolic rates are not limited by inorganic nutrients, the availability of labile (and energy-rich) DOC is often a crucial regulator of BGE [6]. It should be pointed out that each of our relationships between BGE and temperature represents a pattern at a similar level of carbon availability. In environments with low availability of labile carbon, e.g., deep oceanic waters [56] and hypolimnia of large lakes [57], BGE is very low in spite of high inorganic nutrient levels and low temperatures. This suggests that the relationship between BGE and temperature is different at different levels of carbon availabilities, and that the relationships obtained in this study, therefore, cannot be used to quantitatively predict BGE in situations of extreme exhaustion of labile DOC.

Most lakes in the study region have short water turnover times, regular mixing events, and high loadings of labile allochthonous DOC, which makes the entire water column strongly influenced by terrestrial drainage from upstream headwaters [31, 58] such as the streams in this study. If our experimental results apply to natural boreal lake conditions, the bacterial carbon uptake is to a large extent used for BP during winter, and in cold hypolimnic water during summer, provided that inorganic nutrients are supplied in sufficient concentrations. BR and CO_2 production are maximized by low BGE in warm epilimnetic waters where there is continuous input of labile DOC from terrestrial sources and from autochthonous primary production. Thus, the nutrient-temperature control of BGE could cause increased support for aquatic food webs via efficient BP in cold water, when overall basal production is low, and enhance the role of lake epilimnia as CO_2 sources via increased BR during summer when lakes receive large amounts of terrestrial carbon.

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