

Bottom-up controls on bacterial production in tropical lowland rivers

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Abstract

The importance of organic carbon and inorganic nutrients in controlling bacterial production was investigated in two tropical lowland rivers draining undisturbed forested catchments. Glucose (C), ammonium (N), phosphate (P), leaf leachate, and algal leachate were added alone or combined to water collected from one clear-water (Cataniapo) and one black-water (Autana) river of the Middle Orinoco basin, and bacterial production (BP) was measured at 0, 8, 24, and 36 h of incubation. The rivers have low pH (3.8–5.9) and conductivity (6.3–9.1 $\mu\text{S cm}^{-1}$) and abundant nitrogen (total N, 273–314 $\mu\text{g L}^{-1}$) compared to phosphorus (total P, 3.6–5.5 $\mu\text{g L}^{-1}$). BP was significantly stimulated by additions of P, CP, NP, and CNP in both river waters. N or C alone or combined did not stimulate BP, suggesting that P rather than carbon is the primary limiting nutrient in these rivers. Higher responses to CNP and CP amendments (3–7 times over controls), compared to P and NP (1.3–4 times over controls), indicate that carbon is an important secondary constraint to bacterial production. Responses to NP and P were more frequent in the Autana than in the Cataniapo, suggesting that bacteria in the clear-water river were less resource limited than those in the black-water river. Responses to added leaf leachate, which had high concentrations of P and dissolved organic carbon (DOC), were generally greater than responses to added algal leachate. Seasonal patterns in response to nutrient addition suggest that the size of the labile fraction of DOC may increase during periods of low and rising water.

Resource availability is recognized as an important factor in the regulation of heterotrophic bacteria. However, there is uncertainty concerning the relative importance of organic carbon versus inorganic nutrients in limiting bacterial production. Evidence that bacterial production is regulated by carbon availability derives primarily from studies conducted in the ocean (Kirchman 1990; Kirchman and Rich 1997; Rivkin and Anderson 1997), where dissolved organic carbon (DOC) is less abundant than in continental waters (Williams 2000). Nevertheless, C limitation has also been reported in temperate lakes (Kristiansen et al. 1992; Schweitzer and Simon 1995) and in temperate and tropical rivers (Benner et

al. 1995; Foreman et al. 1998). Based on the high affinity of bacteria for phosphorus, relative to phytoplankton, it has been argued (Currie and Kalff 1984; Kirchman 1994) that bacteria can outcompete algae to obtain all necessary inorganic nutrients and instead are limited by the supply of labile carbon from algae. Therefore, most evidence supporting carbon limitation of bacterial production (BP) involves a dependency of bacteria on algal carbon and assumes that bacteria and phytoplankton are competing for inorganic nutrients.

The evidence for bacterial limitation by inorganic nutrients comes from studies in freshwater and marine systems. BP stimulation by experimental addition of ammonium (Morris and Lewis 1992) and phosphate (Toolan et al. 1991; Coveney and Wetzel 1992; Morris and Lewis 1992; Schweitzer and Simon 1995) has been observed in temperate lakes and in some marine systems (Rivkin and Anderson 1997; Thingstad et al. 1998). Most results suggest that although bacteria may depend upon algal carbon, both phytoplankton and bacteria are limited by the low availability of inorganic nutrients (Morris and Lewis 1992; Thingstad and Rassoulzadegan 1995; Rivkin and Anderson 1997). Bacterial limitation by inorganic nutrients may explain in part why labile DOC accumulates in the ocean (Rivkin and Anderson 1997) and in lakes (Morris and Lewis 1992).

To date, most evidence suggests that carbon or nutrient limitation can occur in both freshwater and marine systems; however, we do not fully understand what system conditions result in carbon or nutrient limitation or which limitation to

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expect in oligotrophic or eutrophic systems (Thingstad 2000; Williams 2000). In some freshwater and marine systems, nutrient limitation of BP occurs under N or P deficiency (Toolan et al. 1991; Morris and Lewis 1992; Thingstad et al. 1998). However, nutrient limitation has been observed in coastal areas where riverine inputs of nutrients are presumably high (Williams 2000). It is possible that resource levels alone are not as important as the balance between the supply rates of labile carbon and inorganic nutrients (Rivkin and Anderson 1997; Tranvik 1998; Thingstad 2000). In addition, limiting factors may show seasonal and spatial variation across different aquatic systems (Morris and Lewis 1992; Schweitzer and Simon 1995; Rivkin and Anderson 1997; Skoog et al. 1999).

Bacterial production and its limiting factors in large rivers have received little study despite the likely importance of riverine bacterial activity to the amount and quality of carbon transported to the oceans (Richey et al. 1990; Findlay et al. 1992; Benner et al. 1995). Rivers typically receive large amounts of carbon from allochthonous sources, and so bacteria may depend less on algal carbon than is reported for the open ocean or large lakes (Findlay et al. 1991), except when rivers show high levels of primary production (Berger et al. 1995; Sinsabaugh et al. 1997). However, bacteria could be carbon limited in rivers draining highly agricultural watersheds, like Ohio's Maumee River, where nutrients are very abundant (Foreman et al. 1998). Less disturbed rivers like the Amazon may be carbon limited because transported organic substrates could be refractory (Amon and Benner 1996). In the Rio Negro, a black-water tributary of the Amazon River, bacterial production was only stimulated by the simultaneous addition of glucose, ammonium, and phosphate, suggesting that inorganic nutrients could be colimiting BP (Benner et al. 1995). In relatively undisturbed black-water and clear-water rivers of the middle Orinoco basin, bacterial production is low, as are phosphorus concentrations, and although DOC is abundant it is believed to be highly refractory due to its high humic content (Villaró 1997; Castillo 2000a). In these rivers, however, temporal variation in BP is influenced by hydrological seasonality, with higher BP levels during periods of high chlorophyll and P concentrations or during periods of increasing discharge and DOC concentrations; this suggests that seasonal changes in inputs of nutrients and algae-derived or land-derived organic matter could stimulate bacterial production (Castillo 2000a,b). These observations also suggest that phosphorus or organic carbon could play an important role in controlling bacterial production in these tropical rivers, and their influence could change seasonally.

To investigate the role of labile carbon and inorganic nutrients in the regulation of riverine bacterial production, inorganic nutrients and different sources of organic carbon were added to water samples from the black-water Autana and clear-water Cataniapo rivers in the Orinoco basin. The experiments were performed several times to determine seasonal variation in these pristine ecosystems.

Materials and methods

Study rivers—Bioassays were conducted with surface water from the Autana, a black-water river, and the Cataniapo,

Table 1. Range of values of physical, chemical, and biological characteristics for the Autana and Cataniapo river water during the study period (this study and Castillo 2000a), except for concentrations of humic and fulvic acids reported by Villaró (1997).

	Autana	Cataniapo
Temperature (°C)	24–27	26–27
pH	4.1–5.4	5.4–6.6
Specific conductance ($\mu\text{S cm}^{-1}$)	7.3–10.5	5.0–7.2
DOC (mg L^{-1})	7.3–11.4	1.2–2.4
Humic and fulvic acids (mg L^{-1})	6–8	1–2
Nitrate ($\mu\text{g L}^{-1}$)	3.6–66.4	25–137
Ammonium ($\mu\text{g L}^{-1}$)	0	0
Total N ($\mu\text{g L}^{-1}$)	238–355	235–380
SRP ($\mu\text{g L}^{-1}$)	0.0–2.5	0.0–3.2
Total P ($\mu\text{g L}^{-1}$)	1.0–5.4	3.3–8.0

a clear-water river. These sixth-order rivers are located in the middle Orinoco Basin south of Puerto Ayacucho in Amazonas State, Venezuela. The Cataniapo River sampling station was located approximately 3 km above its confluence with the Orinoco River. Autana River water was collected approximately 1 km above its confluence with the Sipapo River. The Autana and the Cataniapo rivers showed contrasting water chemistry (Table 1). The Autana River has a lower pH and higher concentrations of DOC and humics relative to the Cataniapo. Nitrate and total P concentrations were higher in Cataniapo than in Autana, while soluble reactive phosphorus (SRP) showed similar concentrations.

Bioassay design—Surface water samples from the Autana and the Cataniapo rivers were collected in 10-liter plastic carboys and were kept dark and at constant temperature in a cooler during transport to the laboratory in Puerto Ayacucho. Experiment setup began upon arrival, approximately 6–8 h after collection in the Autana and within 2 h after collection in the Cataniapo. Experiments consisted of the addition of carbon (glucose) and inorganic nutrients (N and P) to river water to investigate their role in limiting bacterial production. Various concentrations of inorganic nutrients were used in these experiments, as well as different combinations of inorganic nutrients and glucose. Because bacterial production in the study rivers was greater during times when chlorophyll *a* (Chl *a*) was high or DOC concentrations were increasing (Castillo 2000a), and because sources of DOC in the rivers included terrestrial and algal sources, leaf and algal leachate amendments were used in separate experiments to study responses to natural sources of C, N, and P. Bioassays were performed between October 1997 and July 1999 (Fig. 1). These dates included the periods of low, rising, high, and falling water to assess potential variation in BP response associated with hydrological seasonality.

Each treatment used two replicate bottles with a final volume of 0.5 liters. Incubations were conducted in the dark at ambient river temperatures (26–28°C). Subsamples (10 ml) were removed from each bottle to conduct bacterial production assays at the beginning and after 8, 24, and 36 h of incubation. BP was determined by the leucine incorporation method (Kirchman 1993) in one control and two subsamples taken from each incubation bottle. Controls consisted of wa-

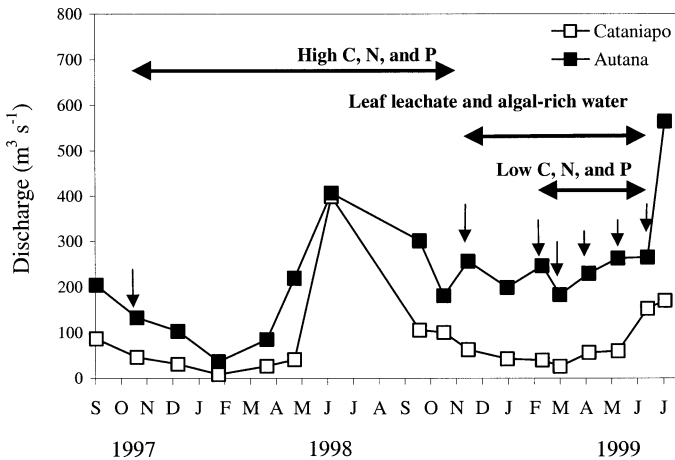


Fig. 1. Discharge and timing of bioassays in the Autana and Cataniapo rivers. Arrows denote date of experiment. Discharge data from Castillo (2000a).

ter samples with buffered formalin (2% final concentration) added prior to the addition of radio-labeled leucine. A conversion factor of 3.1 kg of carbon per mole of leucine was used to convert the leucine incorporation into carbon units (Kirchman 1993).

Carbon and inorganic nutrient amendments—Carbon, nitrogen, and phosphorus were added, alone or combined, to

unfiltered river water in the form of glucose, ammonium (as NH_4Cl), and phosphate (as KH_2PO_4), respectively (Table 2). River water DOC was augmented by 1 mg L^{-1} over background concentrations by glucose addition for all bioassays. Final concentrations of nitrogen at $200 \text{ } \mu\text{g L}^{-1}$ and of phosphorus at $100 \text{ } \mu\text{g L}^{-1}$ over background were used for all experiments through February 1999 (Fig. 1). Experiments with these concentration levels will be referred to hereafter as “high.” Because these initial nutrient amendments yielded strong responses, experiments after February 1999 used lower levels of N and P to more closely resemble the concentrations observed in the rivers and to test whether bacteria would respond to these lower levels of N and P. In February and March 1999 the responses to different levels of nutrients were compared.

Leaf leachate and algal leachate—Leaf leachate and algal leachate additions were performed on six occasions between November 1998 and July 1999 as part of the carbon and inorganic nutrient bioassay experiments. To prepare fresh substrate before each experiment, leaves collected from the floor of local forests were placed in distilled water for 3 d. Leachate was filtered through a Whatman GF/C filter to remove large particles and then through a $0.2\text{-}\mu\text{m}$ membrane to remove bacteria. Algal leachate was obtained from a local pond (Chl *a* between 65 and $409 \text{ } \mu\text{g L}^{-1}$) and filtered as above. In July 1999, algal leachate was obtained from a laboratory culture of *Scenedesmus* sp. Additions of leaf leachate

Table 2. Concentrations of glucose and inorganic nutrient amendments added to river water for bioassay experiments. For the leaf and algal leachates, final concentration of leachate ammonium, SRP, and DOC are shown.

	Date	Ammonium N ($\mu\text{g L}^{-1}$)	Phosphate P or SRP ($\mu\text{g L}^{-1}$)	Glucose or DOC (mg L^{-1})	
Glucose	Oct 1997			1	
	Nov 1998				
	Mar, May 1999				
Low N	Apr, May 1999	10			
Low P	Apr, May 1999		5		
Very low NP	Mar 1999	5	2		
Low NP	Feb, Mar, Apr, May, Jul 1999	10	5		
Med NP	Feb 1999	20	10		
High NP	Oct 1997	200	100		
Low CN	Nov 1998				
	Feb 1999				
	Jul 1999	10		1	
	Low CP	Jul 1999		5	1
	Very low CNP	Mar 1999	5	2	1
	Low CNP	Mar, May, Jul 1999	10	5	1
	High CNP	Oct 1997	200	100	1
Leaf (algal) leachates	Nov 1998				
	Nov 1998				
	Feb 1999	1.3 (1.7)	3 (13)	4.5 (0.7)	
	Mar 1999	0 (0)	27 (0)	7.6 (1.0)	
	Apr 1999	0 (0)	17 (0)	2.7 (1.1)	
	May 1999	1 (0)	41 (0)	0.9 (0.2)	
Jul 1999	0 (1.6)	30 (0)	1.5 (0.3)		
Jul 1999	0 (1.8)	30 (5)	1.5 (14.3)		

usually comprised between 1% and 8% of the final volume, while algal leachate was usually 10% of final volume. After substrates were added to river water, pH and conductivity were determined on each experimental bottle. DOC, phosphorus (total P and SRP), and ammonium concentrations were determined for leaf leachate and algal leachate.

Additions of leaf leachate increased DOC concentrations from 0.9 to 7.6 mg L⁻¹ over background concentrations (Table 2). Algal leachate generally resulted in smaller increases in DOC concentrations compared to leaf leachate amendments (0.2–1.1 mg L⁻¹), except in July 1999 when DOC was increased by 14.3 mg L⁻¹ over background concentrations (Table 2). Although higher additions of algal carbon would have been preferred, this would require a greater volume of water (~20% of final volume), affecting the pH and conductivity of the river water and diluting bacterial density compared with other treatments (Castillo 2000a). SRP concentrations were increased over background (Table 2) by the addition of leaf leachate (3–41 µg L⁻¹) and algal leachate (5–13 µg L⁻¹); however, increases in ammonium concentrations over background (nondetectable in both rivers) levels were small (<2 µg L⁻¹) in both leaf leachate and algal leachate amendments.

Data analyses—BP responses were analyzed using integrated responses over time. Integrated responses (as µg C L⁻¹ produced) were calculated by estimating the areas under the time curves of BP for each amendment. Differences in the integrated BP responses between rivers and among treatments at each experiment date were tested by two-way analyses of variance (ANOVA). When the interaction was non-significant in two-way ANOVAs (occurred in two of seven two-way ANOVAs), Tukey's test was used to compare the treatment marginal means when main effects were significant. When the interaction was significant (occurred in five of seven ANOVAs), Bonferroni multiple comparisons were used to compare treatments with controls for each river and to compare responses to treatments between rivers. The level of statistical significance used was $\alpha = 0.05$. For all data, Levene's statistic and plots of the residuals against predicted values were used to test for homogeneity of variance, while normality was tested by normal plots and the Lilliefors test using the residuals. When serious departures from normality or homogeneity were observed, logarithmic transformations were applied to the data. Because BP in the controls generally was higher in clear-water than in black-water rivers, relative responses were calculated as the ratio of treatment to average control (treatment/control) to compare the magnitude of the response in BP between rivers for each treatment. Relative responses were also compared between rivers by two-way ANOVA. Because low NP, leaf leachate, and algal leachate amendments were repeated at several times of the year, BP relative responses (as treatment/control) were compared among dates and between rivers by two-way ANOVA using a Bonferroni correction for the full set of ANOVAs.

Results

Responses to carbon and inorganic nutrient amendments—BP was stimulated by additions of phosphate (P);

Table 3. Frequency of significant responses ($\alpha = 0.05$) to all amendments using Autana (black water) and Cataniapo (clear water) river water. Numbers of significant integrated responses over the entire incubation time are shown. The No. experiments column indicates the number of times an amendment was tested.

Amendment	Autana		Cataniapo	
	No. significant responses	No. experiments	No. significant responses	No. experiments
Glucose	0	4	0	4
Low N	0	2	0	2
Low P	2	2	1	2
Very low NP	1	1	0	1
Low NP	4	5	3	5
Med NP	1	1	0	1
High NP	2	3	2	3
Low CN	0	1	0	1
Low CP	1	1	1	1
Very low CNP	1	1	1	1
Low CNP	3	3	3	3
High CNP	2	2	2	2
Leaf leachate	6	6	6	6
Algal leachate	2	6	3	6

glucose and phosphate (CP); ammonium and phosphate (NP); and glucose, phosphate, and ammonium combined (CNP) based on the number of significant ANOVA results for the experiments (Table 3; Fig. 2A). Bacteria did not respond to additions of glucose and ammonium, either alone or combined (CN), in either river water (Table 3).

Phosphorus was the only single-nutrient amendment that yielded significant responses in BP (Table 3; Fig. 2A). Phosphate alone at the low concentration of 5 µg L⁻¹ was tested on two occasions (during rising and high water), and in both cases it stimulated BP in the Autana, compared to only once in the Cataniapo (during rising water) (Table 3).

Responses to NP amendments were significant on most dates in the Autana, while in the Cataniapo the frequency of significant absolute responses was lower (Table 3). Because P additions stimulated bacterial production and no significant responses were obtained with ammonium additions (alone or combined with glucose) it appears that bacteria responded primarily or at least initially to P in the NP amendments. In addition, no significant differences were observed between the responses to low P and low NP in April (Bonferroni $p > 0.98$) or May 1999 (Bonferroni $p > 0.17$), suggesting that the role of N was negligible. Amendments that included the two inorganic nutrients combined (NP) or one inorganic nutrient combined with glucose (CP or CN) were compared in July 1999, and results indicated greater responses for those additions including P (Fig. 2B). Although this comparison was only conducted in July 1999 during the period of high water, results are consistent with responses to P addition at other experimental dates.

Responses to low, medium, and high levels of NP were statistically similar when compared in February 1999 (Fig. 2C), suggesting that low concentrations of P (5 µg L⁻¹) were sufficient to stimulate BP and that further increments of P

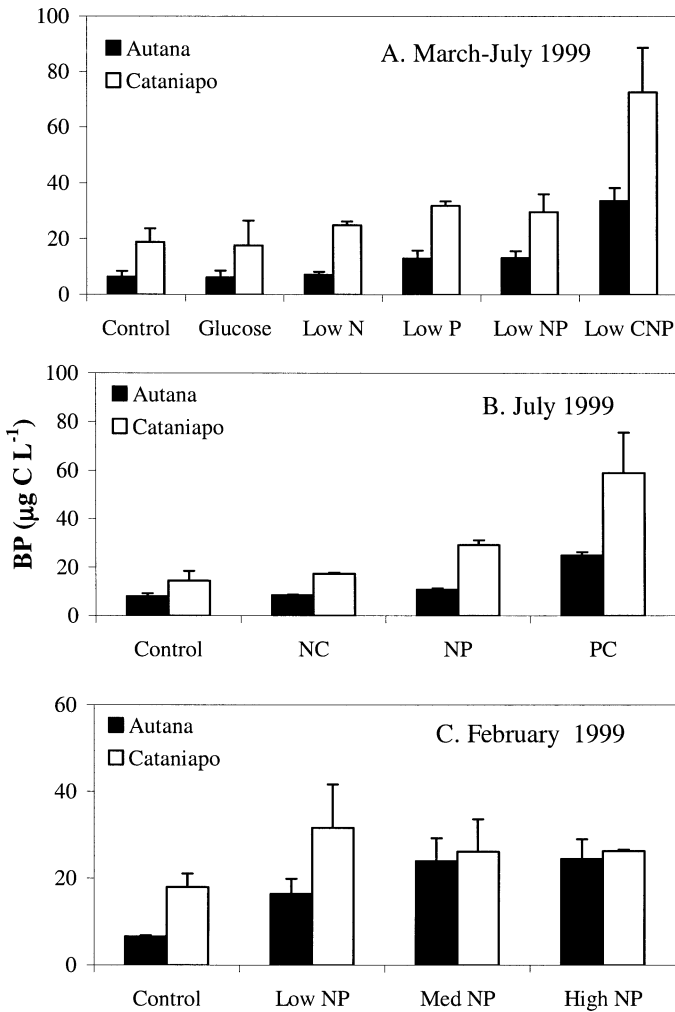


Fig. 2. Responses to selected inorganic nutrient and glucose amendments in the study rivers. Integrated BP response curves are shown. (A) Responses to glucose and inorganic nutrients added alone or combined in the study rivers. Responses to experiments conducted in March, April, May, and July 1999 were average and standard deviations are shown. (B) Responses to NP, NC, and PC amendments in July 1999 in the study rivers. Average and standard deviations of two replicates are shown. (C) Responses to low, medium, and high NP amendments in February 1999 in the study rivers were averaged. Standard deviations are shown.

had little additional effect on bacterial production, at least on that date. Even lower NP additions ($5 \mu\text{g NH}_4\text{-N L}^{-1}$ and $2 \mu\text{g PO}_4\text{-P L}^{-1}$) produced a significant increase in the integrated BP response in the Autana (but not in the Cataniapo) in March 1999, which did not differ statistically from the BP responses to the low NP addition (Bonferroni $p > 0.05$; data not shown). These results suggest that nutrient limitation in the Autana was stronger than in the Cataniapo.

CNP amendments always produced a significant increase in BP in both Autana and Cataniapo river water, and these responses were generally higher than responses to other amendments (Table 3; Fig. 2A). CNP amendments produced the greatest relative increase (3–7 times) over control values (Fig. 3). These relative responses were more than twofold

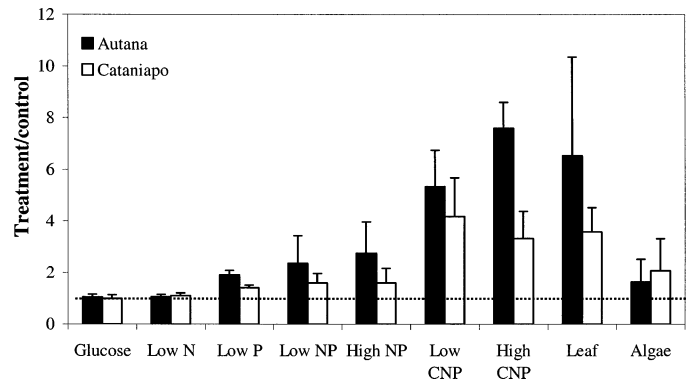


Fig. 3. Relative responses (treatment/control) to most amendments in the Autana and Cataniapo rivers. Average and standard deviations for all experimental dates are shown. The dotted line indicates a treatment/control response equal to one.

higher than the average relative responses to NP in both river waters, which on average increased BP between 1.5 and 4 times over the controls (Fig. 3). CNP amendments in either low or high concentrations were tested on five dates, and responses were significant across all dates and incubation times. Very low CNP amendments also produced a significant integrated response in March 1999 in both rivers. It is likely that bacteria were responding to the combination of C and P in the CNP amendments because nitrogen was available in the rivers (Table 1), and at least in July 1999 the response to CP additions in both rivers was high compared to the controls and not statistically different from the response to CNP (Bonferroni $p > 0.094$).

Relative responses that enhanced bacterial production often were more pronounced in the black-water compared with the clear-water river. BP relative responses in the Autana were significantly higher ($p < 0.05$) than in the Cataniapo for six out of ten NP and P amendments, and similar relative responses were observed in the rest of the experiments; this suggests that inorganic nutrient limitation of bacterial production could be stronger in the black-water Autana. Combining all dates and amendments, BP relative responses were higher in Autana than in Cataniapo river water in 14 of 38 trials, while Cataniapo relative responses were never higher than the Autana, despite the higher absolute responses observed in the Cataniapo.

Temporal variation in responses to low NP (February through July 1999) was analyzed using relative responses because background BP is known to vary seasonally (Castillo 2000a,b). In the Autana, relative responses to NP amendments in February, March, and April 1999 were significantly higher than in May and July 1999 (Bonferroni $p < 0.005$) (Fig. 4). This suggests that responses to NP during low and rising water (February, March, and April 1999, Fig. 1) were greater than during high water (July 1999), which implies that the magnitude of nutrient limitation varied seasonally. In contrast, no significant statistical differences in the responses among dates were observed in the Cataniapo (Fig. 4), which is consistent with the lower frequency (Table 3) and magnitude of relative responses to NP amendments

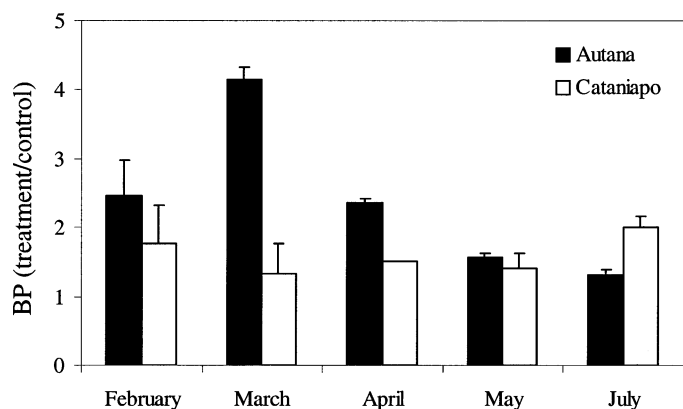


Fig. 4. Seasonal variation in the relative response of BP (treatment/control) to NP amendment during 1999 in the Autana and Cataniapo rivers. Values shown are relative responses for each date (average of two replicates). Error bars represent 1 SD.

(Fig. 3) exhibited by the Cataniapo compared with the Autana.

Responses to leaf and algal leachates—Leaf leachate stimulated BP on all occasions, whereas BP responded to algal leachate amendments in fewer than half of the trials (Table 3). BP responses to leaf leachate were similar in magnitude to BP responses to CNP (Fig. 5) and therefore greater than the responses to other amendments such as NP and algal leachate on most dates. Only in November 1998 was the response to algal leachate greater than the response to leaf leachate in either river. Although responses to algal leachate only produced statistically significant responses in November 1998 and July 1999, this amendment stimulated BP on average more than twofold over background (Fig. 5). On these dates the addition of algal leachate increased the background phosphorus concentrations above $5 \mu\text{g L}^{-1}$, which coincides with the amount of P that stimulated BP in the P and NP amendments in both rivers. Because an even lower P amendment stimulated BP in the Autana, the threshold for P stimulation appears to be in the $2\text{--}5 \mu\text{g L}^{-1}$ range. In addition, the relative response to algal leachate showed a strong, significant Pearson correlation with the added total dissolved phosphorus concentrations in the amendments in both rivers (Autana $r = 0.88$, $p < 0.05$; Cataniapo $r = 0.95$, $p < 0.05$) (Fig. 6). In these experiments, P again appeared to be an important factor controlling BP.

Discussion

Nutrient limitation—Amendment experiments suggest that nitrogen and organic carbon are sufficiently available to bacteria in the study rivers because glucose or ammonium alone and together were insufficient to stimulate BP (Table 3; Fig. 2A). Although ammonium concentrations were below detection in the Autana and Cataniapo rivers, nitrate and total nitrogen were abundant compared to phosphorus (Table 1), suggesting that bacteria could obtain enough N for growth. Morris and Lewis (1992) observed a small BP response to ammonium addition only when nitrate concentra-

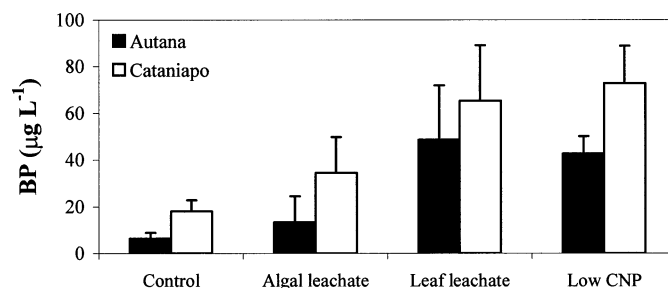


Fig. 5. Responses to leaf leachate and algal leachate in the study rivers. Average and standard deviations for all experimental dates are shown.

tions in Lake Dillon were below $1 \mu\text{g L}^{-1}$. In the subarctic Pacific Ocean, where ammonium did not stimulate BP, nitrate concentrations were usually above $84 \mu\text{g L}^{-1}$ (Kirchman 1990). Therefore, the availability of nitrogen in these Orinoco tributaries is consistent with nonsignificant BP responses to ammonium additions in other systems.

Although DOC is abundant in the Autana and Cataniapo rivers (Table 1), humic compounds comprise a high proportion of the DOC (Villaró 1997), which suggests that the bulk of this organic carbon might be refractory (Moran and Hodson 1990). However, the lack of response to glucose addition (Table 3; Fig. 2A) in the study rivers indicates either that some fraction of DOC is already available to bacteria or another factor is colimiting bacterial growth. BP has responded to glucose addition in systems where the availability of DOC is low and nutrient concentrations are high. For example, in the Equatorial Pacific Ocean, where phosphorus levels are high, glucose alone or combined with ammonium or free amino acids stimulated thymidine incorporation (Kirchman and Rich 1997). BP was stimulated by glucose addition in Lake Constance during the winter when algal production, and thus presumably the supply of DOC, was low (Schweitzer and Simon 1995). In the Amazon River

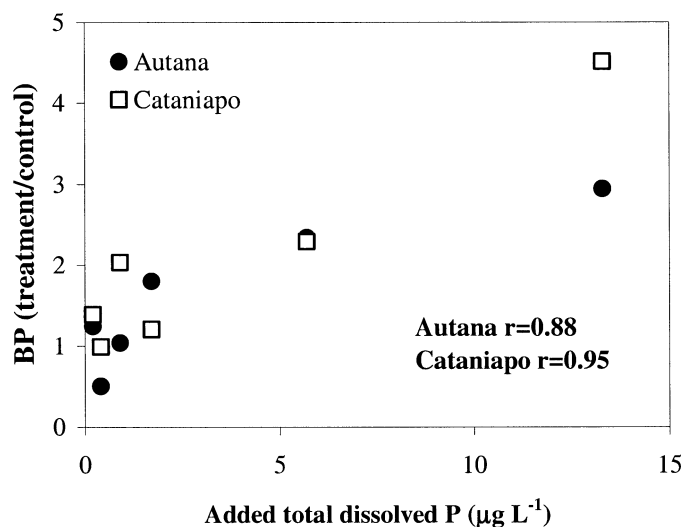


Fig. 6. Correlation between added phosphorus concentrations and relative BP responses to algal leachate amendments in the Autana and the Cataniapo rivers.

main stem, bacterial production responded to glucose but not to P additions, while BP in the Rio Negro was only stimulated by the simultaneous addition of glucose, phosphate, and ammonium, suggesting that bacteria are colimited by these three elements (Benner et al. 1995). In the Orinoco study rivers, in contrast, responses to P and NP additions (Table 3; Fig. 2) suggest that some carbon is available to bacteria, and P rather than carbon may be the primary limiting nutrient at most times.

BP responses to P amendments occurred in a background of low P concentrations in the study rivers. Average total P concentrations were $5 \mu\text{g L}^{-1}$ in the Cataniapo and $3.6 \mu\text{g L}^{-1}$ in the Autana, whereas total N concentrations exceeded $230 \mu\text{g L}^{-1}$ in both rivers and resulted in very high N:P ratios ($>100:1$) compared with the molar C:N:P ratio of bacteria ($50:10:1$) as determined by Fagerbakke et al. (1996). SRP concentrations averaged $0.9 \mu\text{g L}^{-1}$ in both rivers, and so the ratio of nitrate:phosphate also was very high. According to Fagerbakke et al. (1996), bacteria have a molar C:P ratio between 37 and 65, which suggests that bacteria have high P requirements relative to algae (C:N:P 106:16:1, Redfield et al. 1963). The molar DOC:total P ratio in the study rivers was on average over 1,000, indicating that the organic substrate for bacteria was poor in P. Therefore, low P concentrations and high N:P and C:P ratios in these Orinoco rivers are consistent with the results of the bioassays and corroborate other evidence that phosphorus can play an important role in regulating BP.

Inorganic nutrient limitation of BP has not been reported previously for tropical rivers, although it has been reported in some oligotrophic temperate lakes and marine systems (Toolan et al. 1991; Coveney and Wetzel 1992; Morris and Lewis 1992). In the tropics, high temperatures and precipitation enhance chemical weathering of phosphorus (Lewis 1996), and high denitrification rates could represent a significant loss of N from aquatic systems, so nitrogen limitation is presumably more widespread than phosphorus limitation (Downing et al. 1999). However, this study suggests that P limitation can occur in tropical rivers draining highly weathered terrain and where terrestrial ecosystems are also likely to be P limited (Jordan and Herrera 1981; Bruijnzeel 1991) and thus release only low amounts of P to aquatic systems.

These results are at odds with reports that bacterial production is regulated by the availability of carbon and that bacteria can readily obtain the inorganic nutrients required for growth (Kirchman 1994; Kirchman and Rich 1997). However, other studies report stimulation of BP by nutrients, particularly P, in several marine (Rivkin and Anderson 1997; Thingstad et al. 1998) and freshwater systems (Coveney and Wetzel 1992; Chrzanowski et al. 1995; Jansson et al. 1996). In Calder Lake, New York, phosphate additions as low as $1.6 \mu\text{g L}^{-1}$ stimulated BP by threefold to eightfold over the control (Toolan et al. 1991). In humic Lake Ötråsket, in northern Sweden, bacterioplankton is P limited since the abundant humic solutes represent a source of carbon for bacteria (Jansson et al. 1996). In Lake Dillon, Colorado, where total P concentrations ($5.3\text{--}7.1 \mu\text{g L}^{-1}$) are similar to those observed in our study rivers, amendments including P alone or combined with N or C produced greater increases in BP

than did amendments lacking P (Morris and Lewis 1992). The results of this study suggest that P is a limiting factor throughout the year, even considering that this study was conducted during a La Niña year when rainfall was higher than normal and the inputs of P and the flushing of DOC from land were enhanced (Castillo 2000a). It is unknown whether P limitation will be replaced by C limitation during El Niño years when inputs of DOC from land are reduced due to lower rainfall, chlorophyll levels increase, and P levels are slightly lower than during La Niña years.

Low concentrations of P in the environment do not ensure that bacterial growth is limited by P (Thingstad and Rasmuzadegan 1995; Thingstad et al. 1998). Bacteria have a high affinity for P (Vadstein and Olsen 1989; Currie 1990) and so may assimilate P at concentrations well below detection levels (Thingstad et al. 1998). Vadstein et al. (1988) observed no correlation between bacterial growth rates and SRP concentrations but found a good correlation between growth rates and decreasing bacterial C:P ratios. Although the cellular P content of bacteria in the study rivers is unknown, low P concentrations in the river water (Table 1) were accompanied by P stimulation of BP (Table 3; Fig. 2A), which suggests that availability of P to bacteria was quite low.

DOC availability—In these rivers of the Middle Orinoco basin, responses of bacteria to P or NP suggest that some fraction of the DOC is labile and can be used by bacteria once nutrients are provided. Three lines of evidence support the hypothesis that some labile carbon is present but only in small concentrations. First, bacterial growth occurred in the controls, although BP values are low compared to other freshwater systems (Castillo 2000a). Second, BP responded to P and NP amendments combined; however, P and NP responses were smaller than responses to CNP (Fig. 2A), suggesting that some labile carbon was present, or that naturally occurring carbon was less labile than amended glucose, or both. Third, BP stimulation was similar across the range of phosphate concentrations tested ($5, 10, \text{ and } 100 \mu\text{g L}^{-1}$) in the NP amendments conducted in February 1999 (Fig. 2C), suggesting that on that date another factor, probably carbon availability, constrained BP once the initial P limitation was overcome. Similar results were obtained by Toolan et al. (1991) in Calder Lake, where P additions above $1.6 \mu\text{g L}^{-1}$ did not increase BP, suggesting that either the labile carbon pool was very small or a secondary limiting nutrient, probably N in that system, was influencing the response. In this study, the experimental results suggest that carbon rather than nitrogen is the secondary limiting nutrient.

Although this study does not provide direct evidence of a draw-down of the C pool due to nutrient inputs, we estimated the use of DOC by assuming a 10% growth efficiency over the extrapolated time period of growth in the bioassays where inorganic nutrients were added. Based on the linear extrapolation of the decline in BP at the end of the bioassays (e.g., from the 24 to 36 h measurements), we estimated that all the labile carbon would be consumed on average in 61 h and 59 h for the Autana and Cataniapo, respectively. Given this, the average fraction of the total DOC pool that was

used by bacteria in the NP and P amendments was 2.9% (0.25 mg L^{-1}) in the Autana and 24.5% (0.42 mg L^{-1}) in the Cataniapo. Similar calculations for the controls yielded lower values of 1.2% and 13% of the labile carbon in the Autana and Cataniapo, respectively, confirming that inorganic nutrient addition allowed the bacteria to use more of the labile DOC pool. On the basis of these calculations, the amount of DOC available (without nutrient enrichment) in the study rivers was roughly similar to that found in the Amazon River (1.4%–7.5%) (Amon and Benner 1996) and in the black-water Ogeechee River (<1%) (Leff and Meyer 1991; Søndergaard and Middleboe 1995) but lower than the average proportion of labile DOC found in other rivers (19%, Søndergaard and Middleboe 1995). Given these estimates of the labile DOC pool and the rate of DOC use by bacteria in the control situations, we calculate that bacteria could use approximately 0.9% of DOC during its transport along the Autana River where average water residence time is 40 h from the headwaters to the confluence with Orinoco River (Castillo 2000a). In the Cataniapo the average residence time is longer (72 h from the headwaters to the confluence with the Sipapo River) and the impact of bacteria on DOC could be higher (~15% of the labile DOC pool could be used). The impact of bacteria on DOC would likely increase under conditions of low flow, lower bacterial growth efficiency, or increased concentrations of inorganic nutrients, particularly phosphorus, which would stimulate bacterial growth.

More frequent responses to P and NP amendments in Autana river water (Table 3) could indicate either that labile carbon is more frequently available in the black-water river or that nutrient limitation is stronger in the Autana than in the Cataniapo. These responses probably are related to differences in background P concentrations. Mean annual total P in the Cataniapo ($4.9 \mu\text{g L}^{-1}$; SD, $1.7 \mu\text{g L}^{-1}$) was significantly higher than in the Autana ($3.6 \mu\text{g L}^{-1}$; SD, $1.7 \mu\text{g L}^{-1}$) ($t = 2.10$, $df = 32$, $p = 0.04$) (Castillo, 2000a), although no significant differences were found for SRP concentrations. Lower P availability in the Autana River could allow for the accumulation of labile carbon, which would explain why P amendments result in a higher response in Autana than in Cataniapo, where P is more available.

Differences in the extent of phosphorus limitation between black-water and clear-water rivers could influence the processing of carbon along a river network. In the Orinoco system, clear-water rivers can flow into black-water rivers, and eventually black-water and clear-water rivers flow into the Orinoco main stem, where the influence of white water tributaries increases the levels of nutrients (Weibezahn 1990). In situations where phosphorus limits the bacterial processing of carbon, the confluence of river waters with different P content could increase the processing of labile DOC, thereby affecting the quality and quantity of carbon transported by the Orinoco River.

Sources of the labile carbon found in the rivers include in situ algal production, terrestrial inputs, or photochemical production of labile DOC in the river. Bacterial responses to amendments of leaf leachate and algal leachate in this study (Table 3; Fig. 5) suggest that bacteria can use both sources of carbon. Because chlorophyll was below $0.5 \mu\text{g L}^{-1}$ in the

study rivers (Castillo 2000a), most DOC probably was of terrestrial origin. Although presumably highly refractory, some fraction of land-derived DOC must still be useable by aquatic bacteria due to their different metabolic capabilities and different chemical and physical conditions in the water column versus in the soil waters where these compounds originate. Exposure of DOC to sunlight during the movement of DOC from soils to surface waters could result in the phototransformation of DOC and the production of labile carbon for bacteria (Münster and De Haan 1998). Land-derived DOC may also include carbon recently leached from leaf litter (Schiff et al. 1997), and several studies have reported that leaf leachate additions stimulate BP (Kaplan and Bott 1983; Meyer et al. 1987; Findlay et al. 1992; Kling 1995; Sun et al. 1997). For leaf-litter DOC to reach the rivers, the flowpath needs to be mainly across the surface of the soils, as occurs during storms, to avoid DOC sorption in the soil (Kaplan and Newbold 1994; Schiff et al. 1997) or DOC consumption by soil microbes. Thus, meteorological factors such as irradiance and rainfall regimes and catchment properties such as soil type and landforms could influence seasonal variation in DOC availability.

Temporal variation in relative responses of BP to NP additions in black-water rivers (Fig. 4) suggests seasonal variation in the availability of labile carbon to bacteria throughout the year. Higher responses observed in February, March, and April 1999 could be partly due to increased availability of DOC during low water due to higher irradiance (fewer clouds) and enhanced phototransformation through the shallower water column, and during rising water due to the flushing of fresh, land-derived DOC. Seasonal variation in the composition or activity of the bacterial assemblage may also play a role, but it is unclear at present how this would influence BP in the study rivers.

In conclusion, bacterial production in the Autana and Cataniapo Rivers is influenced by the availability of both phosphorus and carbon. Phosphorus appears to be the primary constraint at most times, but the greater response of BP to amendments of phosphorus and glucose together suggests that carbon is an important secondary constraint for BP. Thus, the results of this study support the view that limitation of bacterial production by inorganic phosphorus could be characteristic of systems where P is deficient. The time scale over which this P-limited condition persists will depend on seasonal and interannual variation in the supply of phosphorus and labile carbon to the rivers.

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