Kent State University
Digital Commons @ Kent State University Libraries

Biological Sciences Publications | Department of Biological Sciences

2005

Abundance of Three Bacterial Populations in Selected Streams

Ola A. Olapade

Xueqing Gao

Laura G. Leff
Kent State University - Kent Campus, lleff@kent.edu

Follow this and additional works at: http://digitalcommons.kent.edu/bscipubs

 Part of the Biology Commons

Recommended Citation

Olapade, Ola A.; Gao, Xueqing; and Leff, Laura G. (2005). Abundance of Three Bacterial Populations in Selected Streams. *Microbial Ecology* 49(3), 461-467. doi: 10.1007/s00248-004-0030-x Retrieved from http://digitalcommons.kent.edu/bscipubs/73

This Article is brought to you for free and open access by the Department of Biological Sciences at Digital Commons @ Kent State University Libraries. It has been accepted for inclusion in Biological Sciences Publications by an authorized administrator of Digital Commons @ Kent State University Libraries. For more information, please contact earicha1@kent.edu, tk@kent.edu.

Microbial Ecology

Abundance of Three Bacterial Populations in Selected Streams

O.A. Olapade, X. Gao and L.G. Leff

Department of Biological Sciences, Kent State University, Kent, OH, 44242, USA

Received: 6 February 2004 / Accepted: 1 June 2004 / Online publication: 7 July 2005

Abstract

The population sizes of three bacterial species, *Acinetobacter calcoaceticus*, *Burkholderia cepacia*, and *Pseudomonas putida*, were examined in water and sediment from nine streams in different parts of the United States using fluorescent *in situ* hybridization (FISH). Population sizes were determined from three sites (upstream, midstream, and downstream) in each stream to compare differences in the occurrence and distribution of the species within each stream and among streams. Physical and chemical variables measured reflected differences in environmental conditions among the streams. In the water, *B. cepacia* numbers were highest in the agricultural, Iowa stream. *P. putida* numbers were highest in the southern coastal plain streams, Black Creek (GA) and Meyers Branch (SC). Compared to the other two species, the abundance of *A. calcoaceticus* was similar in all the streams. In sediment, the greatest abundance of all three species was found in the Iowa stream, while the lowest was in Hugh White Creek (NC). Detrended correspondence analysis (DCA) explained 95.8% and 83.9% of the total variation in bacterial numbers in water and sediment of the streams, respectively. In sediments and water, *B. cepacia* numbers were related to nitrate concentrations. *A. calcoaceticus* in water clustered with several environmental variables (i.e., SRP, pH, and conductivity) but benthic populations were less well correlated with these variables. This study reveals the potential influence of various environmental conditions on different bacterial populations in stream communities.

Present address for X. Gao: Florida Department of Environmental Protection, 2600 Blair Stone Road, Tallahassee, FL 32399, USA
Correspondence to: O.A. Olapade at present address: Great Lakes WATER Institute, Wisconsin Aquatic Technology and Environmental Research, University of Wisconsin, Milwaukee, WI 53204, USA; E-mail: olapade@uwm.edu

Introduction

Several studies have reported dynamic changes, both spatially, within a watershed, and temporally, in the abundance and distribution of selected stream bacterial populations [11, 12, 14–17, 19]. However, differences in stream bacterial populations among watersheds from diverse regions exhibiting differing environmental conditions have not been examined [10]. Factors such as nutrient and organic matter availability, anthropogenic disturbance, turbidity, and precipitation [12, 16, 20, 30] are thought to influence bacteria in streams. These effects are likely to vary among ecosystems and thus, responses of the bacterial populations to these factors are predicted to differ among streams.

In this study, abundance of three bacterial species, *Acinetobacter calcoaceticus*, *Burkholderia cepacia*, and *Pseudomonas putida*, in the water and sediment of nine streams in midwestern, eastern, and southern parts of the United States were examined using fluorescent *in situ* hybridization (FISH). Population sizes of the species (defined as the abundance of a given species at a particular location, given that the spatial boundaries of bacterial populations have not been defined in lotic systems) were also compared among sites (upstream, midstream, and downstream) within each stream. Each of these species is a Gram-negative member of the proteobacteria group. *P. putida* had been described as a cosmopolitan opportunist *par excellence* and is commonly found in soil and water [27]. *B. cepacia* is common in soil, water, and in association with plants and is an opportunist pathogen of cystic fibrosis patients; this taxon contains a complex of genomovars and recently described species, such as *B. cenocepacia* [5, 28]. *A. calcoaceticus* is a strict aerobe that is nonmotile whose high frequency of transformation [23] and degradation of hydrocarbons have been well studied (reviewed by [1]).

These three species vary spatially and temporally in abundance and distribution; specifically, *P. putida* numbers changed sporadically in the water of the Cuyahoga River (OH, USA), while changes in *A. calcoaceticus*

population size correlated with water temperature [19]. In the Ogeechee River (GA, USA), *P. putida* numbers were higher and more consistent spatially than *A. calcoaceticus* numbers [11]. Likewise, on decomposing leaves in a stream, numbers of *B. cepacia*, *A. calcoaceticus*, and *P. putida* exhibited different responses [20]. *A. calcoaceticus* was not affected by leaf leachate, whereas phenolic compounds (e.g., tannic acids) appeared to limit *P. putida* and enhance *B. cepacia* populations. In contrast, in sediments of Four Mile Creek (SC, USA) population sizes of these three species were similar to each other and did not vary greatly temporally or spatially [21]. Overall, prior studies suggest that *A. calcoaceticus* is relatively well suited for the water column and benthic habitats and that *P. putida* is best suited for terrestrial environments, perhaps entering the streams from allochthonous sources, based on abundance in floodplain soil [14] and correlations of temporal changes in abundance with turbidity [19]. *B. cepacia* is an exceptionally versatile species in terms of DOM use and seems particularly responsive to leaf leachate [13, 20], and thus its abundance may relate to DOM pool composition.

This study focused on comparing the abundance of the three target bacterial species among nine streams located in the southeastern and midwestern United States. Streams were selected based on differences in their organic matter dynamics and use in prior studies [3, 7, 9, 13, 15, 22, 26, 29]. Streams examined included coastal plain blackwater streams (with high amounts of DOM, in VA, GA, and SC), well-canopied streams (Appalachian Mountain, NC, and northeast OH), more open canopied streams (prairie, KS, and agricultural, IA), a stream with large populations of macrophytes (WI), and a stream in a watershed with forests, agricultural fields, and wetlands (MI). Differences among the streams in DOM sources, inorganic nutrient concentrations, and other environmental features were hypothesized to likely influence the abundance of the bacterial species examined. In addition, to provide a frame of reference for looking at interstream differences, intrastream variation was assessed by collecting samples from three sites within each stream.

Methods

Study Sites and Sample Collection. The nine streams examined in this study were selected because of their use in other studies and differences in their sources and quantities of organic matter [6, 7, 9, 13, 29]. They are second- to third-order streams with different vegetation types, located in different regions of the USA. The streams are as follows: Meyers Branch, located on the Savannah River Site near Aiken, South Carolina a blackwater stream with dense vegetation dominated by cypress, tupelo, maple, hickory, willow, and birch [9]; Black Creek, another blackwater stream, on the lower coastal plain of Georgia with cypress-dominated floodplains [15]; Hugh White Creek, a higher gradient stream in the southern Appalachian Mountains at the Coweeta Hydrologic Laboratory (an NSF Long Term Ecological Research [LTER] site) in North Carolina with a watershed dominated by oak, maple, birch, poplar, hickory, and rhododendron [29]; Buzzard's Branch, a blackwater stream on Virginia's coastal plain with a watershed dominated by gum, maple, ash, and oak [26]; Allequash Creek, located at the North Temperate Lakes (NSF LTER) in Wisconsin, with large amounts of macrophytes, including *Sparganium* and *Elodea* [22]; Augusta Creek, located at the Kellogg Biological Station (NSF LTER) in Michigan and tranversing hardwood forests, wetlands and agricultural fields [3]; Buffalo Creek, in Iowa, which drains an agricultural watershed dominated by corn farming with limited riparian vegetation and an open canopy; King's Creek, located within the Konza Prairie Research Natural Area (also an NSF LTER site) in Kansas with vegetation types ranging from prairie (open canopy) to shrubs, oak, ash, and elm in the gallery forest region [7]; and the West Branch of the Mahoning River located within Jenning's Woods in Portage County, Ohio [13], a highly shaded stream with riparian vegetation that includes oak, maple, sycamore, and poplar.

The nine streams were sampled under base-flow conditions during June and July 2001 at three sites (referred to here as upstream, A, midstream, B, and downstream, C) along each stream. Three sites were examined per stream to assess intrastream variation and enhance our ability to compare the different streams. The three sites, 100 to 1000 m apart, were selected based on their use in prior studies, accessibility, and recommendations by researchers familiar with a particular stream (see acknowledgments). When the samples were collected the average water depth (in cm) at the study sites was 22 (GA), 25 (IA), 29 (KS), 35 (MI), 5 (NC), 15 (OH) 16 (SC), 13 (VA), and 35 (WI). Flow rate (in cm/s) was 2 (GA), 5 (IA), 33 (MI), 12 (NC), 5 (SC), 10 (VA), and 10 (WI); there are no flow data from KS and OH.

Three replicate water samples were collected from the middle of the channel at each of the three sites in a stream and preserved with 8% paraformaldehyde in 1× PBS (7.6 g NaCl, 1.9 g $Na_2HPO_4{\cdot}7H_2O$, 0.7 g $NaH_2PO_4{\cdot}2H_2O$ per liter of 0.2 μm-filtered dH2O, pH 7.2). Five replicate sediment samples were collected from the top 2 cm of sediment in the middle of the channel with a scoop sampler. Fifteen g of sediment was measured into sterile Whirlpak bags and preservative was added. Then, 30 ml of 0.1% sodium pyrophosphate was added prior to sonication (Branson 2210 Sonicator, Danbury, CT) for 5 min to detach bacteria as described in McNamara et al. [21].

Physical and chemical properties of the streams were measured in triplicate at the time of sampling. These included temperature and pH (Oakton WD 35615 pH/mV/Temperature Meter, Singapore). Also determined were turbidity (2100P Hach Turbidimeter, Loveland, CO), conductivity (Hach 44600 Conductivity/TDS meter), soluble reactive phosphorus (SRP, Hach DR100 Loveland, CO), and nitrate/nitrite (Hach colorimeter DR100, Loveland, CO). Dissolved organic carbon (DOC) concentration was determined using a Shimazu TDC-5000 carbon analyzer. Organic matter content of sediment samples was determined by drying samples at 103°C for 24 h, and then ashing for 12 h at 550°C.

Chlorophyll *a* was determined by filtering samples through a Whatman GF/F filter (Whatman International, Maidstone, England). The filter was then ground and chlorophyll *a* extracted using 90% acetone for 2 h at 4°C in the dark. Chlorophyll *a* concentration was determined using the standard spectrophotometric method [2].

In Situ Hybridization. Populations of the three bacterial species were emamerated by *in situ* hybridization with fluorescently labeled oligonucleotides, using the method of Lemke et al. [18]. Samples were concentrated under 15 kPa vacuum onto a 0.2 μm-pore Anodisc 25 filter (Whatman, Maidstone, UK) and rinsed with deionized water and then 1 mL 0.1% Nonidet P-40. Filters were placed in a Petri dish before addition of 40 μL of 5 ng/μL Texas Red–labeled probe, and then incubated at the appropriate temperature for 4 h.

The three species-specific probe sequences and hybridization temperatures used were *A. calcoaceticus* 5′-AGCATCCTATCGCTAGGTA-3′ at 49°C [3]; *B. cepacia* 5′-CCCATCGCAATCTAACAAT-3′ at 47°C [25]; and *P. putida* 5′-GCTGGCCTAACCTTC-3′ at 50°C [25]. These same probes and methods were used in Lemke et al. [16], Lemke and Leff [17], and McNamara et al. [21]. Controls used in the hybridizations were American Type Culture Collection (ATCC Manassas, VA) strains preserved with PBS/paraformaldehyde. Cultures used were *A. calcoaceticus* (ATCC#23055), *B. cepacia* (ATCC#25416), and *P. putida* (ATCC#12633).

After the 4 h incubation, the filters were rinsed twice with 400 μL of wash buffer to remove excess probe followed by incubation at the respective hybridization temperature for 10 min. This step was repeated and followed by two 400-μL rinses with deionized water. Filters were mounted on glass slides with immersion oil and hybridized cells counted using epifluorescence microscopy.

Statistical Analyses. Differences among streams and sampling sites within a stream were analyzed using nested ANOVAs. Correlations between bacterial populations in the biofilms and environmental variables were calculated using Pearson's correlation test. Detrended correspondence analysis (DCA, rotated with Kaiser normalization), a form of ordination, was used to examine the relationships between spatial patterns in bacterial numbers and environmental variables. DCA was selected because it does not suffer from the same major problems as correspondence analysis (e.g., detrending reduces the arch effect [8, 24]) and was performed using MVSP (Multivariate Statistics Package) version 3.8 (Kovach Computing Services).

Results

The streams differed in the physical and chemical variables measured (Table 1). Water temperature differed significantly among streams ($P < 0.05$), with values ranging from as low as 16.9°C in HWC-NC, a stream with a relatively closed canopy, to 26.5°C in King's Creek, a prairie stream. pH varied significantly among the streams ($P < 0.01$); the lowest pH (5.57) was recorded in Black Creek (a blackwater stream) while Augusta Creek was 7.80. Significant differences were also detected in turbidity ($P < 0.01$) and nitrate ($P < 0.05$) concentrations among the streams. Black Creek had the most turbid water, while Buffalo Creek (in a highly agricultural area) had the highest nitrate concentration (5.30 mg/L). DOC concentrations were higher in Buffalo Creek (IA) and Black Creek, GA (27.98 and 21.71 mg/L, respectively) than the rest of the streams. The organic matter content of the sediments was generally similar.

A. calcoaceticus populations in water ranged from 3.6×10^2 cells mL^{-1} at Hugh White Creek (NC, site A) to 4.9×10^3 cells mL^{-1} at Black Creek (GA, site B) and differed significantly among streams ($F = 9.83$, $P < 0.0001$) and sites within a stream ($F = 4.64$, $P < 0.0001$) (Fig. 1A). In general, differences among the three sites in a given stream were of lower magnitude than differences among streams. However, there were a few streams with relatively large intrastream variation, including Black Creek (GA) and the West Branch of the Mahoning River (OH). In both of these streams there was a downstream increase in turbidity (Black Creek: site A, 7.4; site B, 43.5, and site C, 33.4; West Branch: site A, 5.9; site B, 12.7, and site C, 12.5), but none of the measured environmental variables followed the same pattern of change as *A. calcoaceticus* numbers (data not shown).

When *B. cepacia* populations were examined in the water, the numbers ranged from 2.5×10^3 cells mL^{-1} at Meyers Branch (SC, site C) to 2.5×10^4 cells mL^{-1} at Buffalo Creek (IA, site C) and differed significantly among streams ($F = 143.25$, $P < 0.0001$) and sites within a stream ($F = 37.51$, $P < 0.0001$) (Fig. 1B). As for *A. calcoaceticus*, in many streams the variation among three sites was relatively low compared to differences among streams. For *B. cepacia*, the largest intrastream differences

Table 1. Environmental variables measured at the study sites

Streams	*Temperature (°C)*	pH	*Conductivity (μS/cm)*	*Turbidity (NTU)*	*SRP (mgP/L)*	*Nitrate/Nitrite (mgN/L)*	*DOC (mgC/L)*	%OM	*Chl a W (μg/L)*	*Chl a S (M-g/g)*
Meyer Branch	22.91	6.72	0.06	7.44	0.09	0.06	8.67	0.81	1.84	0.09
(SC)	[0.13]	[0.08]	[0.00]	[1.05]	[0.00]	[0.00]	[0.55]	[0.30]	[0.76]	[0.02]
Black Creek	22.64	5.57	0.07	28.09	0.31	0.00	21.71	0.85	8.53	0.91
(GA)	[0.03]	[0.05]	[0.00]	[1.91]	[0.06]	[0.00]	[0.77]	[0.14]	[2.95]	[0.48]
Hugh White	16.94	6.39	0.03	11.15	0.24	0.03	0.92	1.62	0.39	0.08
Creek (NC)	[0.11]	[0.16]	[0.00]	[2.53]	[0.04]	[0.00]	[0.04]	[0.08]	[0.20]	[0.02]
Buzzard's	21.61	6.28	0.08	19.72	0.29	0.95	6.07	0.99	1.17	0.16
Branch (VA)	[0.01]	[0.12]	[0.00]	[0.95]	[0.06]	[0.03]	[0.27]	[0.40]	[0.30]	[0.02]
Allequash	19.57	6.92	0.04	1.59	0.27	0.02	7.45	1.70	2.35	1.74
Creek (WI)	[0.09]	[0.08]	[0.00]	[0.09]	[0.02]	[0.00]	[0.33]	[0.59]	[0.24]	[0.58]
Augusta Creek	18.15	7.80	0.18	5.82	0.39	0.48	9.40	1.98	3.95	1.12
(MI)	[0.05]	[0.06]	[0.00]	[0.54]	[0.03]	[0.02]	[0.51]	[0.66]	[0.61]	[0.35]
Buffalo Creek	22.41	7.19	0.18	13.77	0.33	5.30	27.98	1.94	1.64	0.81
(IA)	[0.18]	[0.03]	[0.00]	[0.82]	[0.01]	[0.45]	[1.07]	[0.38]	[0.18]	[0.11]
King's Creek	26.49	7.60	0.21	4.12	0.16	0.12	16.09	1.62	0.55	0.48
(KS)	[0.25]	[0.03]	[0.00]	[0.65]	[0.01]	[0.03]	[1.16]	[0.29]	[0.10]	[0.24]
West Branch	22.39	7.62	0.24	10.37	0.16	0.07	8.03	1.48	2.15	1.39
(OH)	[0.07]	[0.03]	[0.00]	[0.84]	[0.02]	[0.01]	[0.13]	[0.26]	[0.00]	[0.29]

Values are means of nine samples: three replicate samples each from three sites per stream. Standard errors are in brackets. SRP: Soluble reactive phosphorus; %OM: percent organic matter in sediments; Chl*a* W: water chlorophyll; Chl*a* S: sediment chlorophyll.

were found in the Iowa stream where numbers were higher at the two downstream sites than at the upstream site. There were noticeable differences in temperature (site A, 20.4, site B, 23.9, and site C, 23.0°C) and turbidity (site A, 8.4, site B; 19.8, and site C, 13.2 NTU) among the three sites in the Iowa stream. SRP also increased along the length of this stream (site A, 0.20, site B, 0.31, and site C, 0.47 mg P/L).

The numbers of *P. putida* in water ranged from 6.9×10^2 cells mL^{-1} at Allequash Creek (WI, site C) to 7.3×10^3 cells mL^{-1} in the Southern Carolina stream (Meyers Branch, Fig. 1C). The numbers also differed significantly among streams ($F = 31.03$, $P < 0.0001$), but in contrast to the other species, no significant differences were found among sites within a stream ($F = 1.49$, $P = 0.13$).

Numbers of *A. calcoaceticus* in sediment ranged from 1.4×10^5 cells g^{-1} DW at King's Creek in Kansas (site C) to 9.2×10^6 cells g^{-1} DW at the Iowa stream (site A) and differed significantly among streams ($F = 34.69$, $P < 0.0001$) and sites within a stream ($F = 16.50$, $P < 0.0001$) (Fig. 2A). The pattern of differences in the benthos differed from those observed in the water. Within a stream, benthic numbers at the three sites were often similar; a notable exception was King's Creek (KS), where the numbers at site A were much higher than at sites B and C. DOC concentration was higher at site A of King's Creek than at sites B and C (22.37, 11.73, and 14.17 mg/L, respectively). In contrast, chlorophyll *a* and nitrate concentrations were lower at site A than the other two sites (chlorophyll: 0.35, 0.70, 0.59 μg/L and nitrate: 0.03, 0.02, 0.31 mg-N/L, respectively). In the Iowa stream, there was also a downstream decrease in abundance of *A. calcoaceticus* in the sediments, and turbidity, temperature, and SRP concentration varied among these sites (see above). As in the KS stream, chlorophyll concentration in the water also varied from 1.41 μg/L at site A to 2.29 μg/L and 1.99 μg/L at sites B and C, respectively.

B. cepacia populations in sediments differed significantly among streams ($F = 42.82$, $P < 0.0001$) and sites within a stream ($F = 14.20$, $P < 0.0001$) and ranged from 9.3×10^4 cells g^{-1} DW at Hugh White Creek (NC, Site B) to 1.42×10^7 cells g^{-1} DW in the Iowa stream (site A, Fig. 2B). Like *A. calcoaceticus*, large differences among sites were observed in King's Creek (KS) and in the Iowa stream.

In sediment, *P. putida* numbers ranged from 5.7×10^4 cells g^{-1} DW at Hugh White Creek (NC, site B) to 1.06×10^7 cells g^{-1} DW at Buffalo Creek (IA, site A) and differed significantly among streams ($F = 16.38$, $P < 0.0001$) and sites within a stream ($F = 7.78$, $P < 0.0001$) (Fig. 2C). Numbers were also high at site A of King's Creek and site C of the OH stream. As for the other two species, there were large differences among sites within the KS and IA streams in numbers of benthic *P. putida*.

DCA was carried out to further examine the main environmental variables related to the abundance of the three species in water and sediment of the streams. When DCA was performed on the water data set, two axes were extracted. Together these axes explained 95.8% of the observed variance, and *A. calcoaceticus*, *B. cepacia*, and all the environmental variables measured with the exception of nitrate were clustered together (Fig. 3). *P. putida* was on the negative side of axis 1, indicating that abundance of this particular species was negatively correlated with the measured environmental variables. Based on the correlation analysis, there was significant correlation between *B. cepacia* numbers and nitrate and DOC concentration ($R = 0.50$ and 0.78, respectively). There were

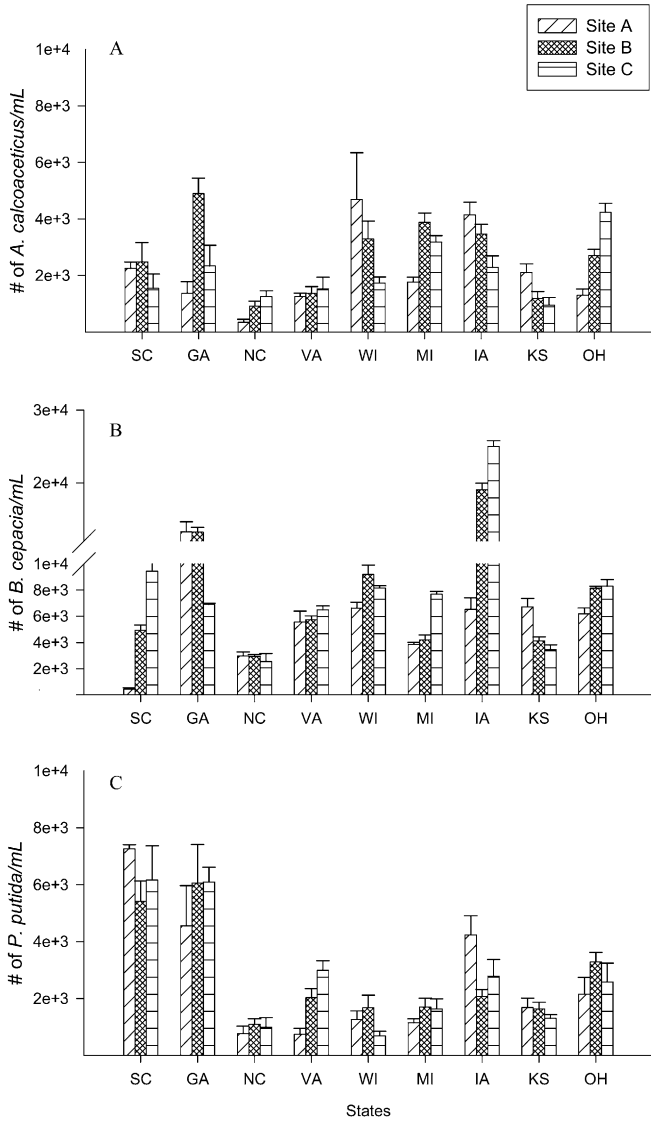

Figure 1. Number of cells detected by fluorescent *in situ* hybridization using *A. calcoaceticus*, *B. cepacia*, and *P. putida* probes (panel A, B, and C, respectively) in water. Values are means of three replicates ± SE.

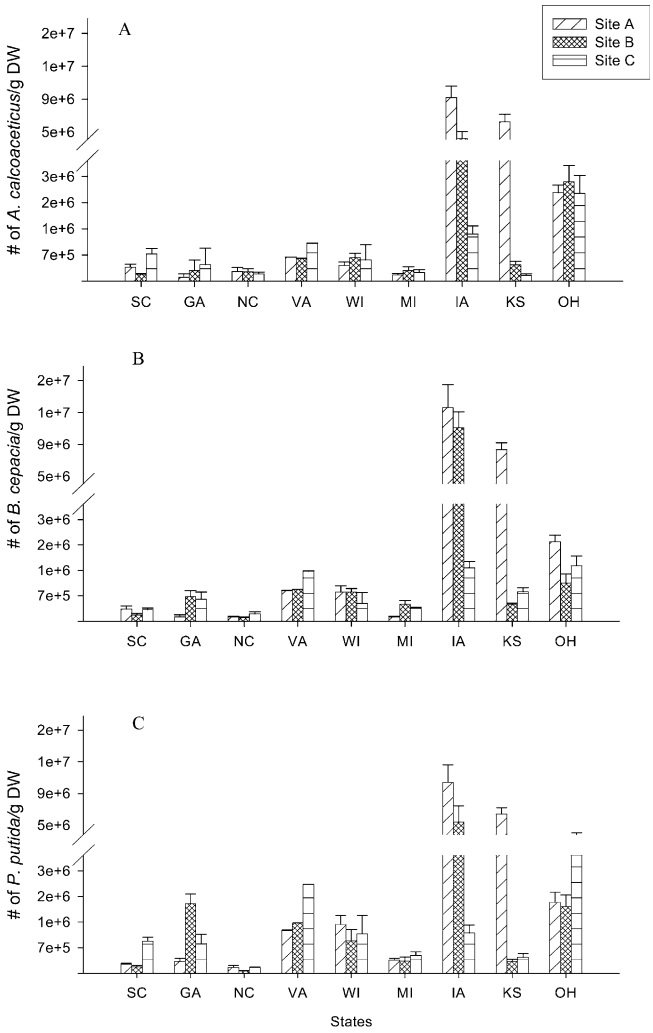

Figure 2. Number of cells detected by fluorescent *in situ* hybridization using *A. calcoaceticus*, *B. cepacia*, and *P. putida* probes (panel A, B, and C, respectively) in sediment. Values are means of five replicates ± SE.

no other significant correlations between bacterial numbers and any other environmental variables.

Two axes were also extracted when DCA was performed on the sediment data set (Fig. 4). The two axes explained in total 83.9% of the observed variance. *B. cepacia* and *P. putida* abundance was most closely related to nitrate concentrations ($R = 0.69$ and 0.53, respectively).

Discussion

The streams examined differ in environmental conditions, watershed features, and riparian vegetation types, and these inherent dissimilarities were predicted to affect the abundance and distribution of the bacterial populations examined. The populations of the three bacterial species have been reported in earlier investigation to differ relative to changing environmental conditions [14, 16, 17, 19, 21] and in their ability to utilize leaf leachate [20].

Differences among streams varied among the three species, suggesting that factors that influence their populations differ. For *B. cepacia*, highest abundance was achieved at sites with high DOC and nitrate concentrations. In contrast, *P. putida* numbers were highest in the two most southern coastal plain streams (GA and SC), perhaps as a result of input of cells from the extensive floodplains of these two streams [14], whereas *A. calcoaceticus* numbers were similar among several streams. For all three species, numbers were lowest in the mountain stream in NC (Hugh White Creek). The abundance of

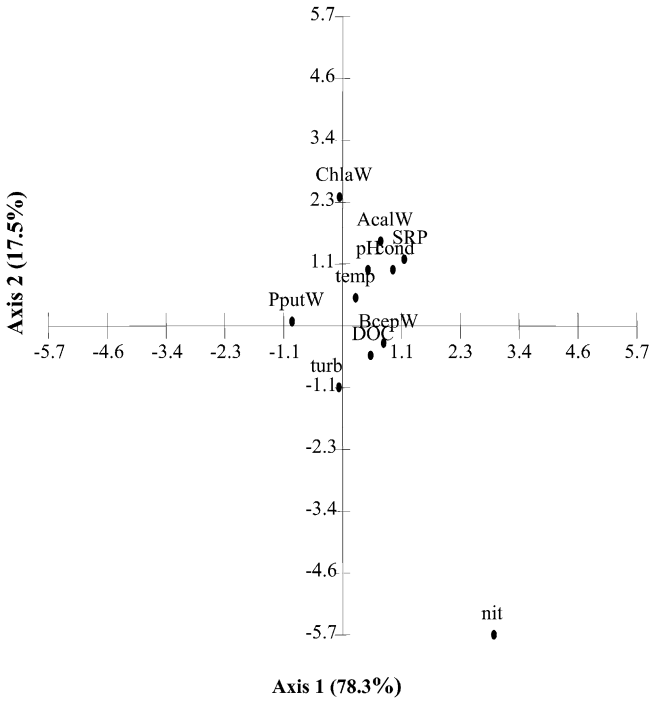

Figure 3. Two-dimensional plot of detrended correspondence analysis (DCA) performed for the three bacterial species in the water and the environmental variables. AcalW: *A. calcoaceticus*; BcepW: *B. cepacia*; PputW: *P. putida*; ChlaW: water chlorophyll *a* concentration; DOC: dissolved organic carbon; SRP: soluble reactive phosphorus; cond: conductivity; temp: temperature; nit: nitrate; turb: turbidity.

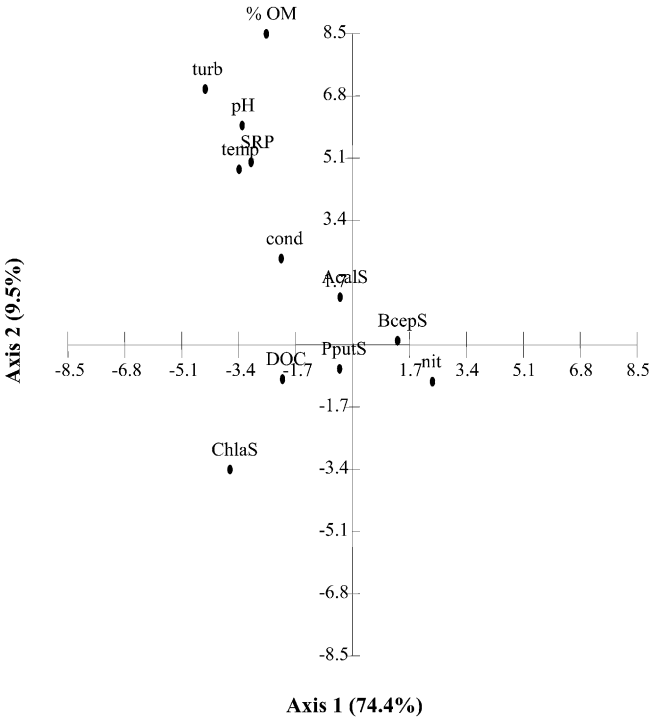

Figure 4. Two-dimensional plot of detrended correspondence analysis (DCA) performed for the three bacterial species in the sediment and the environmental variables. AcalS: *A. calcoaceticus*; BcepS: *B. cepacia*; PputS: *P. putida*; ChlaS: sediment chlorophyll *a* concentration; DOC: dissolved organic carbon; SRP: soluble reactive phosphorus; cond: conductivity; temp: temperature; %OM: percent organic matter; nit: nitrate; turb: turbidity. Water chemistry analyses are from water column samples.

two of the bacterial species (*A. calcoaceticus* and *B. cepacia*) in water was related to several of the environmental variables (particularly DOC concentration for *B. cepacia*); in contrast, *P. putida* abundance did not appear to be related to any of the measured environmental variables. This trend perhaps results from a dependence of *A. calcoaceticus* and *B. cepacia* on resources and conditions in the stream channels, whereas *P. putida* may be influenced by some other factor not directly measured in this study, such as input of allochthonous cells.

Another potentially important component of the environment that we did not assess directly is the source and composition of the DOM pool that likely differs greatly between streams. The blackwater streams (VA, GA, SC) have large concentrations of recalcitrant compounds, and streams with higher in-channel primary production (such as in the WI stream) are more dominated by autochthonous DOM pools. Thus, for example, although high DOC concentrations were observed in both the GA and LA stream, the quality of this DOC and corresponding bacterial response are likely very different.

In sediments, the three bacterial species exhibited similar numbers that differed in comparable manners among the nine streams. For all species, numbers in the Iowa stream at site A were consistently higher than those in the other streams. The Iowa stream is in a heavily agricultural area and had much higher nitrate concentrations than the other streams and supported greater bacterial populations. The Kansas and Ohio streams also had large benthic populations. For site A of the Kansas stream, large population sizes for all three species in the sediments may be related to the high DOC concentration in the water at this site. However, for the Ohio stream, the large population sizes of *A. calcoaceticus* did not appear to be related to the environmental variables measured. Lowest numbers were found in the North Carolina stream; this is likely attributable to the low nutrients and DOC concentration in this stream. The similarity among the three species and consistency among the three sites in a given stream are comparable to those reported by McNamara et al. [21], who observed few differences in benthic abundance of the three species spatially within a stream (Four Mile Creek, SC, USA) and that the population sizes of the species at a given site were similar to each other.

This study suggests environmental conditions potentially favor different bacterial populations,

depending on both the characteristics of the bacterial species and stream location [17, 19]. Prior studies have suggested that *P. putida* abundance in streams is related to inputs of allochthonous cells [14]. In sediments, *B. cepacia* numbers were related to nitrate concentrations but not to chlorophyll, DOC, or benthic organic matter, whereas in water, DOC appeared more important. Other studies have suggested that *B. cepacia* populations are related to the concentration and type of DOM [13, 20]. *A. calcoaceticus* in water clustered with several environmental variables (pH, conductivity, SRP), including temperature as previously demonstrated [19], but benthic populations were less well correlated with these variables.

Acknowledgments

This research was supported by a National Science Foundation grant (DEB-0087051). We thank Adam Leff, Mark Crissman, Mandy Brothers, Angela Lindell, Brian Kloeppel, Leonard Smock, Anne Wright, Tim Kratz, Stephen Hamilton, and Walter Dodds for field assistance. We thank the Savannah River Ecology Laboratory, Coweeta Hydrological Laboratory, Department of Biology at Virginia Commonwealth University, Trout Lake Station at University of Wisconsin, Kellogg Biological Station at Michigan State University, and Konza Prairie Biological Station at Kansas State University for providing access to study sites.

References

1. Abdel El-Haleem, D (2003) *Acinetobacter*: environmental and biotechnological applications. African J Biotechnol 2: 71–74
2. APHA (1995) Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC
3. Bott, TL, Brock, JT, Dunn, CS, Naiman, RJ, Ovink, RW, Petersen, RC (1985) Benthic community metabolism in four temperate stream systems: an interbiome comparison and evaluation of the river continuum concept. Hydrobiologia 123: 3–45
4. Braun-Howland, EB, Vescio, PA, Nierzwicki-Bauer, SA (1993) Use of simplified cell blot technique and 16S rRNA-directed probes for identification of common environmental isolates. Appl Environ Microbiol 59: 3219–3224
5. Dalmastri, C, Fiore, A, Alisi, C, Bevivino, A, Tabacchioni, S, Giuliano, G, Sprocati, AR, Segre, L, Mahenthiralingam, E, Chiarini, L, Vandamme, P (2003) A rhizospheric *Burkholderia cepacia* complex population: genotypic and phenotypic diversity of *Burkholderia cenocepacia* and *Burkholderia ambifaria*. FEMS Microbiol Ecol 46: 179–187
6. Gao, X, Olapade, OA, Kershner, MW, Leff, LG (2004) Algal-bacterial co-variation in streams: a cross-stream comparison. Arch Hydrobiol 159: 253–261
7. Gray, IJ (1997) Organic matter dynamics in Kings Creek, Konza Prairie, Kansas, USA. J North Am Benthol Soc 16: 50–53
8. Hill, MO, Gauch, HG Jr (1980) Detrended Correspondence Analysis: an improved ordination technique. Vegetation 42: 47–58
9. Koetsier, P, McArthur, JV, Leff, LG (1997) Spatial and temporal response of stream bacteria to sources of dissolved organic carbon in a blackwater stream system. Freshwat Biol 37: 79–89
10. Leff, LG (1994) Stream bacterial ecology: a neglected field? Am Soc Microbiol News 60: 135–138
11. Leff, LG (2000) Longitudinal changes in microbial assemblages of the Ogeechee River. Freshwat Biol 43: 605–615
12. Leff, LG, Brown, BJ, Lemke, MJ (1999) Spatial and temporal changes in bacterial assemblages of the Cuyahoga River. Ohio J Sci 99: 44–48
13. Leff, LG, Leff, AA, Lemke, MJ (1998) Seasonal changes in planktonic bacterial assemblages of two Ohio streams. Freshwat Biol 39: 129–134
14. Leff, LG, McNamara, CJ, Lemke, MJ (2003) Bacterial populations of the floodplain of a South Carolina (USA) stream: a comparison of two species. Arch Hydrobiol 156: 255–270
15. Leff, LG, Meyer, JL (1991) Biological availability of dissolved organic carbon along the Ogeechee River. Limnol Oceanogr 36: 315–323
16. Lemke, MJ, Brown, B, Leff, LG (1997) The response of stream bacterial populations to pollution. Microb Ecol 34: 224–231
17. Lemke, MJ, Leff, LG (1999) Bacterial populations in an anthropogenically disturbed stream: comparison of different seasons. Microb Ecol 38: 234–243
18. Lemke, MJ, McNamara, C, Leff, LG (1997) Comparison of methods for the concentration of bacterioplankton for *in situ* hybridization. J Microbiol Methods 29: 23–29
19. Liu, J, Leff, LG (2003) Temporal changes in the bacterioplankton of a northeastern Ohio (USA) river. Hydrobiologia 489: 151–159
20. McNamara, CJ, Leff, LG (2004) Response of biofilm bacteria to dissolved organic matter from decomposing maple leaves. Microb Ecol 48(3): 324–330
21. McNamara, CJ, Lemke, MJ, Leff, LG (2002) Cultivable and nonculturable fractions of bacterial populations in sediments of a South Carolina stream. Hydrobiologia 482: 151–159
22. Meyer JL, Crocker T, D'Angelo D, Dodds W, Findlay S, Oswood M, Repert D, Toetz D (1993) Stream research in the long-term ecological research network. LTER Publication No. 15
23. Palmen, R, Hellingwerf, KJ (1997) Uptake and processing of DNA by *Acinetobacter calcoaceticus*—a review. Gene 192: 179–190
24. Peet, RK, Knox, RG, Case, JS, Allen, RB (1988) Putting things in order: the advantages of detrended correspondence analysis. Am Naturalist 131: 924–34
25. Schleifer, KH, Amman, R, Ludwig, W, Rothemund, C, Springer, N, Dorn, S (1992) Nucleic acid probes for the identification and *in situ* detection of *Pseudomonas*. In: Galli, E, Silver, S, Witholt, B (Eds.) *Pseudomonas*: Molecular Biology and Biotechnology, American Society for Microbiology, Washington, DC
26. Smock, LA (1997) Organic matter dynamics of Buzzards Branch, a blackwater stream in Virginia, USA. J North Am Benthol Soc 16: 54–57
27. Timmis, KN (2002) *Pseudomonas putida*: a cosmopolitan opportunist par excellence. Environ Microbiol 4: 779–781
28. Vermis, K, VanDamme, PAR, Nelis, HJ (2003) *Burkholderia cepacia* complex genomovars: utilization of carbon sources, susceptibility to antimicrobial agents and growth on selective media. J Appl Microbiol 6: 1191–1199
29. Webster, JR, Meyer, JL, Wallace, JB, Benfield, EF (1997) Organic matter dynamics in Hugh White Creek, Coweeta Hydrologic Laboratory, North Carolina, USA. J North Am Benthol Soc 16: 74–77
30. Xu, P, Leff, LG (2004) Longitudinal changes in the bacterial community of the Mahoning River (Ohio, USA). Hydrobiologia 522: 329–335