

REPORT

Bacterial diversity patterns along a gradient of primary productivity

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Abstract

Primary productivity is a key determinant of biodiversity patterns in plants and animals but has not previously been shown to affect bacterial diversity. We examined the relationship between productivity and bacterial richness in aquatic mesocosms designed to mimic small ponds. We observed that productivity could influence the composition and richness of bacterial communities. We showed that, even within the same system, different bacterial taxonomic groups could exhibit different responses to changes in productivity. The richness of members of the Cytophaga-Flavobacteria-Bacteroides group exhibited a significant hump-shaped relationship with productivity, as is often observed for plant and animal richness in aquatic systems. In contrast, we observed a significant U-shaped relationship between richness and productivity for α -proteobacteria and no discernable relationship for β -proteobacteria. We show, for the first time, that bacterial diversity varies along a gradient of primary productivity and thus make an important step towards understanding processes responsible for the maintenance of bacterial biodiversity.

Keywords

Bacterial diversity, biodiversity, community composition, eutrophication, freshwater ecology, microbial ecology, primary productivity, ribosomal DNA, taxonomic diversity.

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INTRODUCTION

A primary goal of ecology is to understand the distribution of organisms. Investigating patterns of biodiversity is a crucial step towards achieving this goal (Lubchenco *et al.* 1991). While many factors likely affect the biodiversity of a region, primary productivity (the rate of energy capture and carbon fixation by primary producers) is emerging as a key determinant of plant and animal biodiversity, especially species richness (i.e. the number of species present; Rosenzweig 1995; Mittelbach *et al.* 2001). However, it is unknown how, or even if, bacterial diversity varies with primary productivity. Understanding patterns of bacterial diversity is of particular importance because bacteria may well comprise the majority of the earth's biodiversity and they mediate critical ecosystem processes (Cavigelli & Robertson 2000; Torsvik *et al.* 2002).

The relationship between productivity and diversity has been of long-standing interest to ecologists. Many studies of plants and animals have reported a hump-shaped relationship between productivity and diversity, where diversity peaks at intermediate productivity (Rosenzweig 1995; Leibold 1999;

Dodson *et al.* 2000; Mittelbach *et al.* 2001). Other studies have reported a monotonic increase of diversity with productivity, a decrease, a U-shaped relationship or no discernible pattern (Abrams 1995; Mittelbach *et al.* 2001).

Variation in observed productivity–diversity patterns may result both from differences in study design and differential responses of organisms at various spatial and temporal scales (Mittelbach *et al.* 2001). For example, the geographical scale (e.g. local vs. regional) and ecological scale (e.g. within vs. among communities) of studies often influences patterns even within the same taxonomic group (Waide *et al.* 1999; Gross *et al.* 2000). It is also possible that some studies lump taxa or guilds that differ in their responses to productivity and thus mask patterns at different taxonomic scales (e.g. Haddad *et al.* 2000; Torsvik *et al.* 2002). In addition, our understanding of productivity–diversity relationships is influenced strongly by particular well-studied taxonomic groups such as terrestrial plants and, therefore, may be biased by these taxa (Mittelbach *et al.* 2001). Other taxa (especially microorganisms) remain relatively unstudied.

Our ignorance regarding patterns of bacterial diversity is primarily due to significant theoretical and practical problems

that have, until recently, hindered the quantification of bacterial diversity. These problems include the very small proportion of microbial species that can be cultured (Amann *et al.* 1995), the very large number of individuals that may be present per sample, the high diversity that may be present at a small scale and the difficulty of defining a microbial species (Goodfellow & O'Donnell 1993). However, solutions to many of these problems have recently been developed (O'Donnell *et al.* 1994; Ovreas 2000; Hughes *et al.* 2001). For example, a number of techniques have been developed that assess bacterial diversity without requiring growth. The most promising of these techniques use ribosomal gene sequences (obtained from DNA isolated from the environment) as the indicators of bacterial phylogenetic richness (O'Donnell *et al.* 1994; Stackebrandt & Rainey 1995).

Evidence from laboratory studies suggests that productivity may influence microbial diversity, and such studies offer insight into possible mechanisms responsible for observed productivity–diversity relationships (Kaunzinger & Morin 1998; Bohannan & Lenski 2000; Kassen *et al.* 2000). For example, Kassen *et al.* (2000) found that bacterial diversity peaked at intermediate productivity in heterogeneous lab microcosms. They suggested that this was likely due to niche specialization in a heterogeneous environment. This work, however, considered only strains of one bacterial species and, like most terrestrial plant studies, contained only one trophic level. In contrast, Kaunzinger & Morin (1998) used multi-trophic level laboratory microcosms comprised of aquatic bacteria and protists, to demonstrate that increasing productivity resulted in increased food chain length. Furthermore, they observed that food chain length determined the numerical response of a given trophic level to productivity; for example, bacteria increased in abundance in response to productivity only in food chains with an odd number of trophic levels. Finally, Bohannan & Lenski (2000) observed that increasing productivity resulted in an increase in the relative importance of competition (“bottom–up” effects) and predation (“top–down” effects) as determinants of bacterial community composition in laboratory microcosms. At low productivity levels, superior competitors were favoured, while at high levels of productivity more predator-resistant types were favoured. These observations are consistent with the keystone-predation model (Leibold 1996), which predicts a unimodal relationship between productivity and diversity.

Observations from several field studies suggest that increased nutrients (and associated increased primary productivity) can alter bacterial community composition (Broughton & Gross 2000; Fisher *et al.* 2000; Lindstrom 2000; Schafer *et al.* 2001; Ovreas *et al.* 2003). For example, Fisher *et al.* (2000) assessed the composition of microbial communities in aquatic mesocosms that varied in nutrient inputs. They observed that community composition,

assessed by using length heterogeneities in the intergenic region between 16S and 23S ribosomal genes, changed in response to additions of nitrogen and phosphorous.

We know of only three field studies that have attempted to document the relationship between primary productivity and bacterial diversity (Benlloch *et al.* 1995; Torsvik *et al.* 1998; Schafer *et al.* 2001). Over a period of 13 days, Schafer *et al.* (2001) found that nutrient addition first decreased bacterial diversity (measured as the number of DNA derived DGGE bands). A subsequent increase in the abundance of protists, and thus possible increased grazing, was accompanied by increased bacterial diversity. In the post-grazing stage, bacterial diversity once again decreased. These results suggest that both bottom–up and top–down processes might control bacterial diversity. They also observed that community composition varied with nutrient addition. Benlloch *et al.* (1995) used the richness of 16S rDNA sequences as an estimate of bacterial diversity in two coastal lagoons that differed in primary productivity. They observed a greater number of unique ribosomal gene sequences in the more productive lagoon than in the less productive lagoon, suggesting that bacterial richness may increase with primary productivity. In addition, the sample from the more productive lagoon contained individuals related to 10 major phylogenetic groups while the less productive lagoon contained representatives from only five. However, due to the time and effort necessary to clone and sequence, only 50 clones per lagoon were assessed, and 70% of these clones were unique. This suggests that the lagoons were likely very undersampled (Benlloch *et al.* 1995; Hughes *et al.* 2001). Torsvik *et al.* (1998) observed that pristine aquatic sediments had much higher prokaryotic diversity than sediments below fish farms (which receive a substantial input of nutrients via fish feed), suggesting that increased nutrients may decrease diversity. In all three studies discussed above, only two productivity levels were sampled, and thus the authors could not differentiate between a linear and quadratic trend.

To determine the relationship between both bacterial and algal richness and primary productivity, we estimated bacterial taxonomic richness along a gradient of primary productivity in freshwater mesocosms. Small ponds and, similarly, mesocosms that mimic small ponds have proven to be excellent model systems with which to study productivity–diversity relationships (Wilbur 1997; Leibold 1999; Chase & Leibold 2002; Downing & Leibold 2002). In particular, small, fishless ponds represent a relatively simple natural system comprised of viruses, bacteria, algae and zooplankton. Productivity is easily manipulated via the addition of inorganic nutrients, and eukaryotic organisms in such systems tend to have short generation times and thus can respond quickly to such manipulations. In addition, substances (such as humic acids) that interfere with

molecular techniques used to estimate bacterial diversity are usually found in low concentrations. Both natural ponds (e.g. Dodson *et al.* 2000; Leibold 1999) and mesocosm studies (e.g. Leibold unpublished) have shown unimodal relationships between primary productivity and richness of planktonic plants. We manipulated the primary productivity of aquatic mesocosms by altering the input of inorganic nutrients. We used culture-independent techniques coupled with statistical extrapolation methods to estimate bacterial taxonomic richness as a measure of bacterial diversity in the water column of mesocosms that varied in primary productivity. We compare these bacterial results with patterns observed for algal richness from the same system. Our results suggest that, like algal richness, bacterial richness can vary with primary productivity and that the nature of this relationship may vary among taxonomic groups of bacteria.

MATERIAL AND METHODS

Mesocosms

We established 16 mesocosms at the Kellogg Biological Station (KBS) in southern Michigan as part of a larger experiment to assess the relationships among productivity, diversity and food web structure. Each mesocosm consisted of a 2 m-diameter polyethylene cattle tank with a screen cover, filled with well water. Mesocosms were evenly spaced in a random block design in a field at KBS (Downing & Leibold 2002). Each mesocosm was inoculated from the same pooled sample collected from six ponds in southern Michigan that spanned a natural gradient in primary productivity. The samples were filtered through a 30 µm mesh and flushed with CO₂ to kill macro-zooplankton. A gradient of primary productivity was established across the mesocosms by maintaining otherwise identical mesocosms with different input concentrations of nitrogen and phosphorus. Four levels of nutrient input were used: high (27 × baseline), intermediate (9 × baseline and 3 × baseline) and low (1 × baseline) with four replicates at each level. We added sand to each mesocosm as a bottom substrate and maintained a constant water level by adding nutrient-poor well water when necessary. Although microorganisms could enter the mesocosms through the well water or through the air, we have no reason to believe that microbes from these sources differentially colonized the mesocosms. The mesocosms were sampled at the end of the 4-month summer growing season. Fourteen samples were collected from the water column of each mesocosm, from just below the pond surface and to a depth of one foot. To minimize sampling effects due to spatial heterogeneity within the mesocosms, samples were pooled to form one

large sample for each mesocosm. Replicate subsamples were taken from the pooled sample for analysis.

Productivity and nutrients

We estimated primary productivity as chlorophyll *a*. Chlorophyll *a* has been shown to be a highly significant correlate of primary productivity in aquatic systems (Lambou *et al.* 1982) and especially in aquatic mesocosms (Downing & Leibold 2002). Samples from 16 mesocosms were analysed for total nitrogen, phosphorous and chlorophyll *a* within an hour of sampling. We measured chlorophyll *a* using fluorometric methods, and total nitrogen and phosphorous using spectrophotometry after persulfate digestion at the end of the growing season.

Algae

Algal richness was determined morphologically to genus by microscopic inspection of subsamples preserved in 5% glutaraldehyde immediately after sampling. We examined similarity in algal composition among ponds by calculating Jaccard similarity coefficients for each pair of ponds (Magurran 1988). The Jaccard index is the ratio of species or unique types shared by two sites to the total number of species or types in the two sites combined. We then used a multidimensional scaling algorithm (MDS with a Kruskal loss function, SYSTAT 8.0, Chicago, IL, USA) to reveal clustering by treatment. We tested the significance of this clustering with a two-way analysis of similarity (ANOSIM; Carr 1997). ANOSIM is a permutation-based test, i.e. an analogue of the commonly used univariate ANOVA, and tests for differences between groups of multivariate samples from different treatments.

Bacteria

In order to maximize the proportion of bacterial diversity represented in our samples, we chose to sample intensively a subset of five of 16 mesocosms for bacterial diversity. The five mesocosms were selected to represent the range of primary productivity available. These five samples represent, to date, the largest collection of bacterial sequences ever sampled along an environmental gradient (760 individual sequences) and thus offer a fuller picture of bacterial diversity than currently exists. Of these mesocosms, two received low inputs of nitrogen and phosphorus, one received intermediate inputs, and two received high. Bacterial abundance was determined by direct microbial counts of DAPI (4',6-Diamidino-2-phenylindole) stained subsamples preserved in 5% glutaraldehyde immediately after sampling. Samples for bacterial richness analysis were pre-filtered through a 45 µm filter and then concentrated on a 0.2 µm

cylindrical filter (Somerville *et al.* 1989). We flushed the filters with SET buffer and stored them at -20°C until analysis.

Extraction, amplification, cloning and sequencing of bacterial rDNA

We used cloning and sequencing of 16S rDNA to assess bacterial richness in the five mesocosms. Community DNA was extracted from the concentrated cells using a combination of chemical lysis and physical disruption (cycles of freezing and thawing). Bacterial rDNA was amplified using the PCR, with primer sequences corresponding to *Escherichia coli* nucleotides 1392–1406 (1392r) and 8–26 (8f). This primer set is known to amplify only sequences within the domain Bacteria (Amann *et al.* 1995). Twenty PCR cycles were used, with each cycle consisting of 30 s at 94°C , 30 s at 57°C , and 90 s at 72°C . The amplicons were ligated into plasmid pCR2.1 and cloned using the TOPO-TA cloning system (Invitrogen, Carlsbad, CA, USA). We used an ABI 377 automated DNA sequencer to determine the sequence of the 5' terminal 500 nucleotides of 760 of the cloned rDNA amplicons (Stackebrandt & Rainey 1995).

PCR-based methods for assessing bacterial diversity are less biased than culture-based techniques, but potential sources of bias remain (Wintzingerode *et al.* 1997; Speksnijder *et al.* 2001). We selected PCR conditions to reduce these potential biases. For example, we limited the PCR to 20 cycles, we used DMSO in our PCR reactions to reduce biases due to GC content, and we pooled five replicate PCR reactions prior to cloning (Devereux & Willis 1995; Stackebrandt & Rainey 1995; Qiu *et al.* 2001; Speksnijder *et al.* 2001). In addition, we minimized changes in the relative abundance of bacteria in the samples by chilling immediately after sampling and storing the samples at -20°C within an hour of sampling. We also used both chemical lysis and physical disruption to extract nucleic acids from a broad array of possible cell types.

Phylogenetic analysis of rDNA

We used the RDP database (Maidak *et al.* 2001) and ARB software (Ludwig & Strunk 1999) to initially align the rDNA sequences from our five clone libraries. Ambiguously and incorrectly aligned positions were aligned manually based on conserved primary sequence and secondary structure. We identified and excluded potential chimeras by using the Chimera_Check program (Maidak *et al.* 2001) and by using ARB to create and compare trees generated from the 5'-end vs. the 3'-end sequences separated at the break point suggested by Chimera_Check. We repeated this process for a break point in the middle of the sequence. Clones were identified as chimeric if their two ends showed affiliation with different reference species.

Similarity matrices were generated using 334–450 unambiguously aligned positions. We used the Felsenstein correction and the neighbor joining method to build a phylogenetic tree using the ARB software program (Ludwig & Strunk 1999). We grouped sequences into operational taxonomic units (OTUs) based on rDNA sequence similarity (Stackebrandt & Rainey 1995). There is no common standard for defining operational taxonomic units (OTUs) based on rDNA sequences. Therefore we used the three most commonly used definitions to group our sequences into OTUs: 95% sequence similarity (Speksnijder *et al.* 2001), 97% sequence similarity (Stackebrandt & Rainey 1995) and 99% sequence similarity (Kroes *et al.* 1999). When it was not possible to separate sequences into OTUs unambiguously, we used complete-linkage clustering to resolve the OTUs, starting with the largest group of most closely related sequences (Dunn & Everitt 1982).

Bacterial diversity estimation

We used OTU richness as our estimate of bacterial phylogenetic richness. OTU richness was estimated via extrapolation from observed OTU richness patterns using the Chao1 approach (Hughes *et al.* 2001). These calculations were performed using EstimateS software (Colwell 1997).

In addition to richness, we examined similarity in bacterial composition among mesocosms in two ways. First, we used the Jaccard index to examine clustering in OTU composition (as described above for algae). Second, we used a Mantel test (MANTEL 2.0) to test whether the composition of algae and bacteria were correlated by determining the significance between similarity matrices for the two groups (Sokal & Rohlf 1995). We tested the correlation between Jaccard similarity coefficients for bacteria and algae for pairs of ponds to examine similarity in species turnover or associations among algal and bacterial types. We used the RDP (Maidak *et al.* 2001) and Bergey's Manual of Systematic Bacteriology (Garrity 2001) to determine the putative taxonomic affiliation of each clone. We focused additional analyses on the three most commonly observed taxonomic groups in our study: the Cytophaga-Flavobacterium-Bacteroides (CFB) group, the α -proteobacteria, and the β -proteobacteria.

Statistical analysis

We used a generalized linear model and quadratic regression models (GLM regression, SYSTAT 8.0) to examine the relationship between productivity and richness (Mittelbach *et al.* 2001). Values were log-transformed when necessary. We applied the test of Mitchell-Olds and Shaw (hereafter, MOS test; Mitchell-Olds & Shaw 1997; Leibold 1999; Mittelbach *et al.* 2001) to determine if a curvilinear

Table 1 The effect of productivity on algal and bacterial richness. GLM results in conjunction with the test of Mitchell-Olds and Shaw (1997) show that algal richness exhibits a unimodal trend with productivity as measured by chlorophyll *a*. Overall bacterial richness does not show a significant relationship with productivity

		Coeff.	SE (Coeff.)	<i>t</i>	<i>P</i>	Multiple <i>r</i> ²	d.f.	
Algae	Overall linear model				0.136	0.152	1	
	Constant	24.763	6.528	3.793	0.002			
	Prod	-5.737	3.622	-1.584	0.136			
	Overall quadratic model					0.079	0.323	2
		Constant	-25.156	28.228	-0.891	0.389		
		Prod	51.262	31.661	3.483	1.619		
		Prod ²	-15.450	8.534	-1.811	0.093		
		Prod* < Prod _{max}			-2.209	0.046		
Prod* < Prod _{min}				1.283	0.222			
Overall bacteria	Overall linear model				0.190	0.487	1	
	Constant	26.497	17.387	1.524	0.225			
	Prod	15.371	9.104	1.688	0.190			

Abbreviations: Prod = Log chlorophyll *a*, Prod* = observed Log chlorophyll *a*, Coeff. = regression coefficient of each term, SE (Coeff.) = standard error of the coefficient, *t* = value of the *t*-test, *P* = probability value for each term and d.f. = degrees of freedom for the overall model. Also shown are *P*-values for the MOS-test of the hypothesis that the function maximum or minimum is located at productivity levels less than the greatest (Prod* < Prod_{max}) and greater than the least (Prod* < Prod_{min}) observed productivity level. Significant terms for both denote an internal minimum or maximum. The MOS test for algae shows that the internal maximum is significantly less than the observed maximum productivity. We are less confident that an extreme minimum value of productivity does not possess maximum species richness. Thus we characterize the productivity-diversity relationship for algae only as a unimodal trend.

Table 2 The effect of productivity on bacterial richness. GLM results in conjunction with the test of Mitchell-Olds and Shaw (1997) show that α -proteobacterial richness exhibits a unimodal relationship with productivity as measured by chlorophyll *a*. CFB richness shows a significant U-shaped relationship with productivity. Abbreviations are as in Table 1

		Coeff.	SE (Coeff.)	<i>t</i>	<i>P</i>	Multiple <i>r</i> ²	d.f.	
α -proteobacteria	Overall linear model				0.469	0.186	1	
	Constant	4.966	9.828	0.505	0.648			
	Prod	4.255	5.146	0.827	0.469			
	Overall quadratic model					0.090	0.910	2
		Constant	88.308	21.091	4.187	0.053		
		Prod	-87.409	22.873	-3.821	0.062		
		Prod ²	24.123	5.994	4.024	0.057		
		Prod* < Prod _{max}			4.420	0.048		
Prod* < Prod _{min}				-3.466	0.074			
CFBs	Overall linear model				0.712	0.052	1	
	Constant	17.838	11.372	1.569	0.215			
	Prod	-2.413	5.954	-0.405	0.712			
	Overall quadratic model					0.095	0.905	2
		Constant	-79.118	23.311	-3.394	0.077		
		Prod	104.224	25.282	4.123	0.054		
		Prod ²	-28.064	6.626	-4.236	0.051		
		Prod* < Prod _{max}			-4.361	0.049		
Prod* < Prod _{min}				3.894	0.060			
β -proteobacteria	Overall model				0.172	0.515	1	
	Constant	12.023	3.500	3.435	0.041			
	Prod	-3.271	1.832	-1.785	0.172			

relationship reaches a maximum or minimum with the observed range of productivities. Curvilinear relationships that show an internal maximum or minimum are considered unimodal or U-shaped, respectively. Values for the MOS test are reported in Tables 1 and 2. Due to the constraints inherent in sampling and analysing bacterial richness, the number of mesocosms sampled was low (5), thus limiting statistical power. We assumed *a priori* a 10% probability of Type I error in our statistical analyses (as discussed by Gill 1978), rather than the more common assumption of 5%. We thus deemed relationships as significant trends if $P \leq 0.10$.

RESULTS

Increasing inorganic nutrients increased primary productivity, estimated as chlorophyll *a*, as anticipated. In addition, bacterial abundance increased with increasing primary productivity ($r^2 = 0.709$, $P = 0.07$; Fig. 1).

The 760 bacterial 16S sequences sampled grouped into 150 OTUs, using the broadest OTU definition (95% similarity). Almost half the OTUs were singletons ($n = 74$), and 15 OTUs were represented by more than 10 sequences. The most abundant OTU across all mesocosms contained 112 clones and was most similar to a member of the β -proteobacteria belonging to the family Alcaligenaceae. The number of OTUs and the number of singletons tended to increase as the OTU definition was narrowed.

Both bacterial and algal taxonomic composition differed according to productivity level (ANOSIM: $P = 0.067$ for both; Fig. 2). Algal composition was more similar between pairs of high and low productivity ponds than it was between the intermediate productivity pond and others. All three productivity levels clustered independently for bacteria. Mantel tests showed that pairs of sites that were similar for algae were also similar for β -proteobacteria ($r = 0.73$, 100 permutations, $P < 0.025$) but not for CFBs ($r = 0.42$, 100 permutations, $P > 0.10$) or α -proteobacteria ($r = 0.18$, 100 permutations, $P > 0.10$).

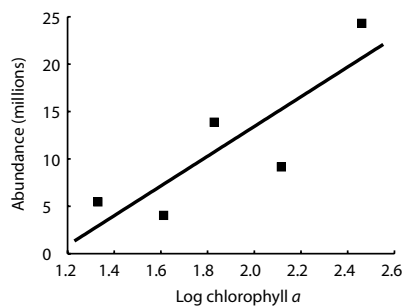


Figure 1 Total bacterial abundance increased with primary productivity ($r^2 = 0.709$, $P = 0.07$).

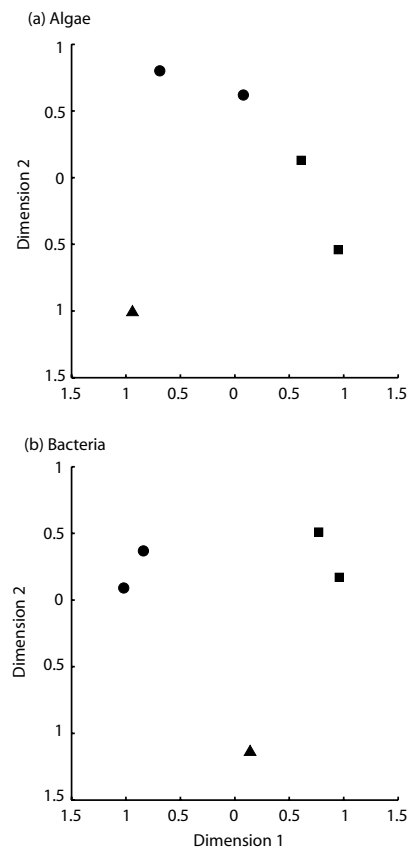


Figure 2 The relationship between species composition and nutrient input level for both (a) algae and (b) bacteria. These multidimensional scaling algorithm plots show values of Jaccard similarity coefficients among productivity levels. Distance between two points is inversely proportional to the Jaccard similarity value for a given pair such that sites positioned close together share more species than sites further apart. The triangle represents the intermediate productivity mesocosm, the squares represent the high productivity mesocosms, and the circles represent the low productivity treatments. Both algal and bacterial composition differs significantly with primary productivity (ANOSIM, $P = 0.067$ for both).

Overall bacterial and algal richness exhibited different trends with productivity. Algal richness exhibited a hump-shaped trend with increasing productivity ($r^2 = 0.323$, $P = 0.079$; Fig. 3a, Table 1). In contrast, overall bacterial richness showed no trend with increasing productivity ($r^2 = 0.487$, $P = 0.190$, Fig. 3b, Table 1), although bacterial richness did decrease significantly with increasing algal richness ($r^2 = 0.713$, $P = 0.072$). Changing the OTU definition from 95% similarity to 97 or 99% did not qualitatively alter these results.

Our estimates of overall bacterial richness included sequences from many different phylogenetic groups.

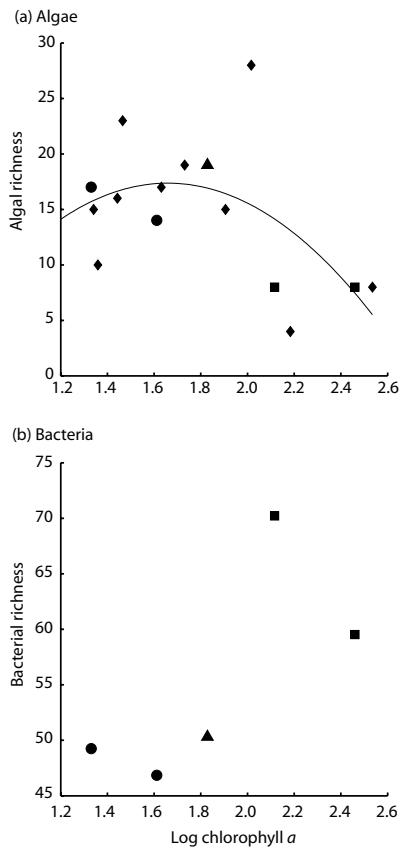


Figure 3 The relationship between primary productivity and taxonomic richness. (a) Algal richness and primary productivity. Large symbols represent mesocosms sampled for bacteria across a productivity gradient. Algal richness exhibits a unimodal trend with productivity ($r^2 = 0.323$, $P = 0.079$). (b) Overall bacterial richness and primary productivity. There is no significant trend between bacterial richness and productivity ($r^2 = 0.487$, $P = 0.190$).

Combining diverse groups can potentially mask patterns present in individual groups (Peet & Christensen 1988; Haddad *et al.* 2000). In an attempt to increase the resolution of our analysis, we reanalysed our data, focusing on the three most numerically dominant taxonomic groups in our sample: the CFB group (representing 20.1% of sequences sampled), the α -proteobacteria (29.9%), and the β -proteobacteria (29.7%). We observed that these three groups exhibited very different responses to primary productivity (Fig. 4, Table 2). α -proteobacterial richness exhibited a significant U-shaped relationship with productivity ($r^2 = 0.91$, $P = 0.09$), and CFB richness exhibited a significant hump-shaped relationship with productivity ($r^2 = 0.90$, $P = 0.095$). In contrast, β -proteobacterial richness showed no discernible response to increasing productivity ($r^2 = 0.533$, $P = 0.172$). Again, changing the OTU definition from 95 to 97 or 99% did not qualitatively alter these relationships.

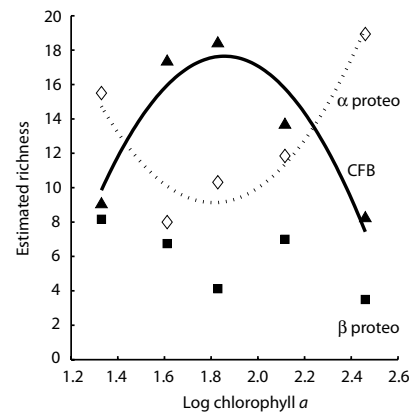


Figure 4 Richness of three major groups of bacteria and primary productivity. α -proteobacterial richness exhibited a significant U-shaped relationship with productivity ($r^2 = 0.91$, $P = 0.090$). CFB richness has a unimodal relationship with productivity ($r^2 = 0.905$, $P = 0.095$), and β -proteobacterial richness shows no discernible response to increasing productivity ($r^2 = 0.515$, $P = 0.172$).

DISCUSSION

We observed that bacterial abundance increased with increasing primary productivity in our system, as reported in studies of other aquatic systems (Kauzinger & Morin 1998, and reviewed in Gasol & Duarte 2000). These observations suggest that, despite the presence of bacteriophages, top-down effects are, in general, less important than bottom-up effects in determining the overall abundance of bacteria in aquatic systems.

Previous studies also observed an effect of primary productivity or nutrient status on bacterial community composition (e.g. Broughton & Gross 2000; Fisher *et al.* 2000; Lindstrom 2000; Schafer *et al.* 2001; Ovreas *et al.* 2003). Our results are consistent with these observations. The communities at each productivity level were more similar to each other in species composition than to communities at either of the other two productivity levels. Furthermore, we found a statistical association between particular β -proteobacteria and algae but not between algae and other bacterial types.

Bacterial and algal diversity had different responses to primary productivity. Our observation that algal diversity exhibited a hump-shaped relationship with increasing productivity is consistent with observations in other aquatic ecosystems (e.g. Leibold 1999; Waide *et al.* 1999; Dodson *et al.* 2000; Mittelbach *et al.* 2001; Chase & Leibold 2002). In contrast, overall bacterial richness showed no trend. However, when we examined the most common taxonomic groups within the broad group of bacteria sampled by our methods, we found that different taxonomic groups of bacteria exhibited different responses to changes in primary

productivity. The CFBs exhibited a hump-shaped relationship, the α -proteobacteria exhibited a U-shaped relationship, and the β -proteobacteria showed no relationship between primary productivity and richness.

Due to the high richness of these systems, it was necessary to sample a subset of five mesocosms intensively rather than sample additional mesocosms less thoroughly. This limited number of mesocosms, of course, limits statistical power. As the P values for the trends we report range between 0.05 and 0.1, our results may be viewed more as suggestive than definitive. Regardless, to our knowledge these are the first observations that suggest that significant nonlinear relationships between bacterial richness and primary productivity may exist, and that these relationships may vary by taxonomic group. Further studies are necessary to demonstrate the generality of these patterns.

The CFBs, β -proteobacteria and α -proteobacteria are broad taxonomic groups that each includes members with very different metabolic lifestyles. It is thus remarkable that significant patterns in richness would be evident at this taxonomic scale. However, others have reported shifts in the relative abundances of these three groups in response to environmental change. For example, changes in the relative abundances of these three groups has been observed in response to increased predation (e.g. Jurgens *et al.* 1999) and increased inorganic nutrients (e.g. Lebaron *et al.* 2001). There is also evidence for differences at this taxonomic level in important ecological traits. For example, α -proteobacteria and β -proteobacteria have been observed to respond to predation differently (Pernthaler *et al.* 1997). Differences in competitive ability for resources have also been observed; for example, members of the CFB group are distinctive in their ability to degrade high molecular weight polymers, although they can degrade low molecular weight compounds with reduced efficiency (Kirchman 2002). Ecological traits such as these (i.e. response to predators and competitive ability for resources) have been suggested as underlying the relationships between diversity and productivity in plants and animals (e.g. Leibold 1996) and could be responsible for the patterns we observed for bacteria as well.

The hump shaped relationship observed for algae and CFBs has also been observed for a number of other taxa. This relationship appears to be especially common in plant and animal studies that sample diversity across communities (as opposed to within a single community) and studies that use plant biomass as a measure of primary productivity (Mittelbach *et al.* 2001). It is also the predominant pattern observed in aquatic communities (Dodson *et al.* 2000; Mittelbach *et al.* 2001). Explaining this pattern has been the focus of a growing body of theory (e.g. Tilman 1982; Abrams 1995; Rosenzweig 1995; Leibold 1999). For example, theory predicts that hump-shaped relationships between productivity and diversity can result from trade-

offs between competitive ability and resistance to predators (Leibold 1999), trade-offs between competitive ability for nitrogen and for phosphorus (Tilman 1982), from changes in the spatial distribution of resources that occur as productivity increases, and from a number of other ecological processes (reviewed in Rosenzweig 1995). Frequent observations of hump-shaped patterns have led some to claim that this is the "true" or most fundamental relationship between productivity and diversity (e.g. Tilman & Pacala 1993; Rosenzweig 1995). However, recent surveys have demonstrated that other patterns occur commonly as well, although they are not as well studied or understood as hump-shaped relationships (Waide *et al.* 1999; Mittelbach *et al.* 2001). Our observations of bacteria are consistent with these surveys; significant hump-shaped patterns were observed for algae and the CFB group of bacteria, but not for α - or β -proteobacteria.

The U-shaped relationship exhibited by the α -proteobacteria in our study has also been observed in other taxa and systems. A recent review of 171 published studies found that U-shaped relationships comprised 36% of the data with quadratic responses to productivity (Mittelbach *et al.* 2001). However, to our knowledge, there is currently no theory proposed to explain a U-shaped pattern (but see Scheiner & Jones 2002). It is possible that competition between α -proteobacteria and CFBs might result in the observed respective U-shaped and hump-shaped patterns. It would be possible to assess this hypothesis in future work by measuring the concentration of different resources (i.e. different carbon sources) as well as predation intensity due to both viral and protistan grazers.

Our results also have implications for understanding how aquatic systems respond to anthropogenic additions of nitrogen and phosphorous (eutrophication). Inputs of nitrogen and phosphorous, the growth limiting nutrients for most aquatic primary producers, have increased in aquatic systems due to such sources as domestic and industrial wastes, and runoff from fertilized agricultural areas. This anthropogenic influx of nutrients often results in a decrease in plant and animal species diversity (Gough *et al.* 2000). The response of bacteria to eutrophication may be of particular importance as bacteria play significant roles in biogeochemical cycling (Torsvik *et al.* 2002). As suggested here, eutrophication could have a significant influence on bacterial taxonomic composition and richness, and changes in composition and richness have been shown to result in differences in the rates and controls of biogeochemical processes in some studies (Bruns *et al.* 1999; Cavigelli & Robertson 2000).

Finally, perhaps the most fundamental biotic attribute of a region, ecosystem or community is the number of species it supports. While many factors are likely to affect species richness in a region, the supply of available energy (the

primary productivity of a region) has emerged as a major determinant of observed variation in species richness (Rosenzweig 1995). Our study is consistent with this observation. Using culture-independent molecular methods to characterize bacterial richness, we observed that overall bacterial richness did not have a significant relationship with primary productivity. However, when the three numerically dominant taxonomic groups of bacteria were analysed separately, patterns emerged. Understanding observed variation in productivity-richness relationships is central to understanding the underlying mechanisms responsible for the generation and maintenance of biodiversity; such an effort is in progress for plants and animals (Mittelbach *et al.* 2001) and is an important goal for future work on microorganisms.

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REFERENCES

- Abrams, P.A. (1995). Monotonic or unimodal diversity-productivity gradients: what does competition theory predict? *Ecology*, 76, 2019–2027.
- Amann, R.L., Ludwig, W., Schleifer, K.H., Torsvik, V.L. & Goksoyr, J. (1995). Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. *Microbiolog. Rev.*, 59, 143–169.
- Benlloch, S., Rodriguez-Valera, F. & Martinez-Murcia, A.J. (1995). Bacterial diversity in two coastal lagoons deduced from 16S rDNA PCR amplification and partial sequencing. *FEMS Microbiol. Ecol.*, 18, 267–279.
- Bohannon, B.J.M. & Lenski, R.E. (2000). Linking genetic change to community evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.*, 3, 362–377.
- Broughton, L.C. & Gross, K.L. (2000). Patterns of diversity in plant and soil microbial communities along a productivity gradient in a Michigan old-field. *Oecologia*, 125, 420–427.
- Bruns, M.A., Stephen, J.R., Kowalchuk, G.A., Prosser, J.I. & Paul, E.A. (1999). Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled, and successional soils. *Appl. Environ. Microbiol.*, 65, 2994–3000.
- Carr, M.R. (1997). *PRIMER user manual (Plymouth Routines in Multivariate Ecological Research)*. Plymouth Marine Laboratory, Plymouth, UK.
- Cavigelli, M.A. & Robertson, G.P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, 81, 1402–1414.
- Chase, J.M. & Leibold, M.A. (2002). Spatial scale dictates the productivity-biodiversity relationship. *Nature*, 416, 427–430.
- Colwell, R.K. (1997). EstimateS: Statistical estimation of species richness and shared species from samples. User's guide and application published at: <http://viceroy.eeb.uconn.edu/estimates>.
- Devereux, R. & Willis, S.G. (1995). Amplification of ribosomal RNA sequences. In: *Molecular Microbial Ecology Manual* (eds Akkermans, A.D.L., Van Elsas, J.D. & De Bruijn, F.J.). Kluwer Academic Publishers, Dordrecht, Netherlands.
- Dodson, S.I., Arnott, S.E. & Cottingham, K.L. (2000). The relationship in lake communities between primary productivity and species richness. *Ecology*, 81, 2662–2679.
- Downing, A.L. & Leibold, M.A. (2002). Ecosystem consequences or species richness and composition in pond food webs. *Nature*, 25, 837–841.
- Dunn, G. & Everitt, B.S. (1982). *An Introduction to Mathematical Taxonomy*. Cambridge University Press, Cambridge, UK.
- Fisher, M.M., Klug, J.L., Lauster, G., Newton, M. & Triplett, E.W. (2000). Effects of resources and trophic interactions on freshwater bacterioplankton diversity. *Microbiol. Ecol.*, 40, 125–138.
- Garrity, G.M. (2001). *Bergey's manual of systematic bacteriology*, 2nd edn. Springer, New York.
- Gasol, J.M. & Duarte, C.M. (2000). Comparative analyses in aquatic microbial ecology: How far do they go? *FEMS Microbiol. Ecol.*, 31, 99–106.
- Gill, J.L. (1978). *Design and Analysis of Experiments*. Iowa State University Press, Ames, IA.
- Goodfellow, M. & O'Donnell, A.G. (1993). Roots of bacterial systematics. In: *Handbook of New Bacterial Systematics* (eds Goodfellow, M. & O'Donnell, A.G.). Academic Press, London, pp. 2–54.
- Gough, L., Osenberg, C.W., Gross, K.L. & Collins, S.L. (2000). Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos*, 89, 428–439.
- Gross, K.L., Willig, M.R., Gough, L., Inouye, R. & Cox, S.B. (2000). Patterns of species density and productivity at different spatial scales in herbaceous plant communities. *Oikos*, 89, 417–427.
- Haddad, N.M., Haarstad, J. & Tilman, D. (2000). The effects of long-term nitrogen loading on grassland insect communities. *Oecologia*, 124, 73–84.
- Hughes, J.B., Hellmann, J.J., Ricketts, T.H. & Bohannon, B.J.M. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl. Environ. Microbiol.*, 67, 4399–4406.
- Jurgens, K., Pernthaler, J., Schalla, S. & Amann, R. (1999). Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Appl. Environ. Microbiol.*, 65, 1241–1250.
- Kassen, R., Buckling, A., Bell, G. & Rainey, P.B. (2000). Diversity peaks at intermediate productivity in a laboratory microcosm. *Nature*, 406, 508–512.
- Kaunzinger, C.M.K. & Morin, P.J. (1998). Productivity controls food-chain properties in microbial communities. *Nature*, 395, 495–497.

- Kirchman, D.L. (2002). The ecology of *Cytophaga-Flavobacteria* in aquatic environments. *FEMS Microbiol. Ecol.*, 39, 91–100.
- Kroes, I., Lepp, P.W. & Relman, D.A. (1999). Bacterial diversity within the human subgingival crevice. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 14547–14552.
- Lambou, V.W., Hern, S.C., Taylor, W.D. & Williams, L.R. (1982). Chlorophyll, phosphorus, secchi disk, and trophic state. *Wat. Resour. Bull.*, 807–813.
- Lebaron, P., Servais, P., Troussellier, M., Courties, C., Muyzer, G., Bernard, L. et al. (2001). Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in abundances, activity and composition. *FEMS Microbiol. Ecol.*, 34, 255–266.
- Leibold, M.A. (1996). A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. *Am. Natural.*, 147, 784–812.
- Leibold, M.A. (1999). Biodiversity and nutrient enrichment in pond plankton communities. *Evolutionary Ecol. Res.*, 1, 73–95.
- Lindstrom, E.S. (2000). Bacterioplankton community composition in five lakes differing in trophic status and humic content. *Microbiol. Ecol.*, 40, 104–113.
- Lubchenco, J., Olson, A.M., Brubaker, L.B., Carpenter, S.R., Holland, M.M., Hubbell, S.P. et al. (1991). The Sustainable Biosphere Initiative: an ecological research agenda: a report from the Ecological-Society-of-America. *Ecology*, 72, 371–412.
- Ludwig, W. & Strunk, O. (1999). ARB: a software environment for sequence data. Technische Universitat Munchen, Munchen, Germany.
- Magurran, A.E. (1988). *Ecological Diversity and Its Measurement*. Princeton University Press, Princeton, NJ.
- Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T.J., Saxman, P.R., Farris, R.J. et al. (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res.*, 29, 173–174.
- Mitchell-Olds, T. & Shaw, R.G. (1997). Regression-analysis of natural-selection: statistical-inference and biological interpretation. *Evolution*, 1149–1161.
- Mittelbach, G.G., Steiner, C.F., Scheiner, S.M., Gross, K.L., Reynolds, H.L., Waide, R.B. et al. (2001). What is the observed relationship between species richness and productivity? *Ecology*, 82, 2381–2396.
- O'Donnell, A.G., Goodfellow, M. & Hawksworth, D.L. (1994). Theoretical and practical aspects of the quantification of biodiversity among microorganisms. *Philosophical Transactions of The Royal Society of London Series B – Biological Sciences*, 345, 65–73.
- Ovreas, L. (2000). Population and community level approaches for analysing microbial diversity in natural environments. *Ecol. Lett.*, 3, 236–251.
- Ovreas, L., Bourne, D., Sandaa, R.A., Casamayor, E.O., Benlloch, S., Goddard, V. et al. (2003). Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. *Aquatic Microbiol. Ecol.*, 31, 109–121.
- Peet, R.K. & Christensen, N.L. (1988). *Changes in Species Diversity During Secondary Forest Succession on the North Carolina Piedmont*. Academic Publishing, The Hague, The Netherlands.
- Pernthaler, J., Posch, T., Simek, K., Vrba, J., Amann, R. & Psenner, R. (1997). Contrasting bacterial strategies to coexist with a flagellate predator in an experimental microbial assemblage. *Appl. Environ. Microbiol.*, 63, 596–601.
- Qiu, X., Wu, L., Huang, H., McDonel, P.E., Palumbo, A.V., Tiedje, J.M. et al. (2001). Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl. Environ. Microbiol.*, 67, 880–887.
- Rosenzweig, M.L. (1995). *Species Diversity in Space and Time*. Cambridge University Press, Cambridge, England.
- Schafer, H., Bernard, L., Courties, C., Lebaron, P., Servais, P., Pukall, R. et al. (2001). Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. *FEMS Microbiol. Ecol.*, 34, 243–253.
- Scheiner, S.M. & Jones, S. (2002). Diversity, productivity and scale in Wisconsin vegetation. *Evolutionary Ecol. Res.*, 4, 1097–1117.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W. H. Freeman and Company, New York.
- Somerville, C.C., Knight, I.T., Straube, W.L. & Colwell, R.R. (1989). Simple, rapid method for direct isolation of nucleic acids from aquatic environments. *Appl. Environ. Microbiol.*, 55, 548–554.
- Speksnijder, A., Kowalchuk, G.A., De Jong, S., Kline, E., Stephen, J.R. & Laanbroek, H.J. (2001). Microvariation artifacts introduced by PCR and cloning of closely related 16S rRNA gene sequences. *Appl. Environ. Microbiol.*, 67, 469–472.
- Stackebrandt, E. & Rainey, F.A. (1995). Partial and complete 16S rDNA sequences, their use in generation of 16S rDNA phylogenetic trees and their implications in molecular ecological studies. In: *Molecular Microbial Ecology Manual* (eds Akkermans, A.D.L., Van Elsas, J.D. & De Bruijn, F.J.). Kluwer Academic Publishers, Dordrecht, Netherlands.
- Tilman, D. (1982). *Resource Competition and Community Structure*. Princeton University Press, Princeton, NJ.
- Tilman, D. & Pacala, S. (1993). The maintenance of species richness in plant communities. In: *Species Diversity in Ecological Communities: Historical and Geographical Perspectives* (eds Ricklefs, R.E. & Schluter, D.). University of Chicago Press, Chicago, pp. 13–25.
- Torsvik, V., Daae, F.L., Sandaa, R.A. & Ovreas, L. (1998). Novel techniques for analysing microbial diversity in natural and perturbed environments. *J. Biotechnol.*, 64, 53–62.
- Torsvik, V., Ovreas, L. & Thingstad, T.F. (2002). Prokaryotic diversity – magnitude, dynamics, and controlling factors. *Science*, 296, 1064–1065.
- Waide, R.B., Willig, M.R., Steiner, C.F., Mittelbach, G., Gough, L., Dodson, S.I. et al. (1999). The relationship between productivity and species richness. *Annu. Rev. Ecol. Systemat.*, 30, 257–300.
- Wilbur, H.M. (1997). Experimental ecology of food webs: complex systems in temporary ponds. *Ecology*, 78, 2279–2302.
- Wintzingerode, F.V., Goebel, U.B. & Stackebrandt, E. (1997). Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol. Rev.*, 21, 213–229.

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