Microbial Ecology

Annual Patterns in Bacterioplankton Community Variability in a Humic Lake

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Abstract

Bacterioplankton community composition (BCC) was monitored in a shallow humic lake in northern Wisconsin, USA, over 3 years using automated ribosomal intergenic spacer analysis (ARISA). Comparison of ARISA profiles of bacterial communities over time indicated that BCC was highly variable on a seasonal and annual scale. Nonmetric multidimensional scaling (MDS) analysis indicated little similarity in BCC from year to year. Nevertheless, annual patterns in bacterioplankton community diversity were observed. Trends in bacterioplankton community diversity were correlated to annual patterns in community succession observed for phytoplankton and zooplankton populations, consistent with the notion that food web interactions affect bacterioplankton community structure in this humic lake. Bacterioplankton communities experience a dramatic drop in richness and abundance each year in early summer, concurrent with an increase in the abundance of both mixotrophic and heterotrophic flagellates. A second drop in richness, but not abundance, is observed each year in late summer, coinciding with an intense bloom of the nonphagotrophic dinoflagellate *Peridinium limbatum*. A relationship between bacterial community composition, size, and abundance and the population dynamics of *Daphnia* was also observed. The noted synchrony between these major population and species shifts suggests that linkages across trophic levels play a role in determining the annual time course of events for the microbial and metazoan components of the plankton.

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Introduction

Microbes are important for ecosystem function [14, 37, 65], but microbial activity levels and population dynamics are embedded in a web of changing ecological and biological relationships. Whereas the seasonal dynamics of phytoplankton and zooplankton populations in pelagic ecosystems have been well characterized [62], the diversity and population dynamics of bacterioplankton are poorly understood, as are the factors that contribute to changes in bacterial community composition (BCC). Mesocosm studies have shown that individual bacterial populations are highly dynamic and can differ strongly in their response to resource availability (in particular, organic carbon and inorganic nutrients such as nitrogen and phosphorus) and to food web structure [20, 40, 41, 56]. Previous work by Crump et al. [15] demonstrated that shifts in BCC were related to seasonal cycles in the source and lability of dissolved organic matter. Similarly, succession in marine bacterioplankton assemblages occurred in response to seasonal shifts in water column stability and water temperature, suggesting that BCC may demonstrate an annual pattern of variability [48]. Other studies have demonstrated relationships between BCC and seasonal dynamics of other members of the aquatic food web [2, 18, 27, 30]. None of these studies examined BCC dynamics over multiple years, however, making it unclear whether the observed patterns of BCC change were repeated on an annual basis.

Repeating patterns in BCC could be due to a variety of factors. Aquatic systems undergo fairly predictable variations in physical parameters and resource availability on an annual scale [63], and changes in BCC could be due to the evolving resource base (i.e., "bot-

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tom up'' influences [4, 7, 8, 20, 25, 42]). In addition, the seasonal dynamics of phytoplankton and zooplankton communities have been well characterized in temperate lake ecosystems [61], and bacterioplankton communities could be influenced by interactions with these planktonic communities. These interactions could include predation by protozoan, metazoan, and flagellate grazers [1, 3, 9, 10, 27, 35] and mutualisms with phytoplankton [2, 67]. Algal and bacterial competition, physical factors, and life cycle adaptations have also been shown to induce changes in bacterial communities [25, 27, 50, 59, 60, 68]. Humic lakes represent good systems in which to study these mechanisms, as the general absence of planktivorous and piscivorous fish in these lakes results in trophic interactions that are less complex than in eutrophic or oligotrophic lakes. Several studies have reported on the abundance and productivity of bacterial communities in humic lakes [3, 19, 55, 57], but few studies have examined BCC or explored the relationship between changes in BCC and changes in other biological and ecological parts of the lake system [43, 44, 70].

A recent study conducted in three temperate lakes, including a humic lake, suggested that lake bacterial communities undergo seasonal patterns of change that repeat on a yearly basis [70]. BCC in the humic lake changed dramatically over the course of the summer, and the summer communities displayed remarkable and periodic drops in bacterial richness. However, the factors related to this pattern of summer change were not explored. Here the chemical and biological factors associated with BCC in a humic lake over the course of three consecutive summers are explored. Particular focus is on the two annual summer bacterial richness minima. Specifically, lines of evidence related to four potential explanations for the observed changes are examined. These include the following: (1) patterns of change are due to species interactions within the bacterial community (i.e., classical succession); (2) patterns of change are due to temporal shifts in the availability of organic and inorganic resources; (3) patterns of change are due to the top-down influence of grazing organisms; and (4) patterns of change are due to succession in the phytoplankton community [24]. Consideration of these potential explanations should help shed light on the important biological and ecological interactions that shape bacterial communities in lakes.

Materials and Methods

Study Sites and Sample Collection. Crystal Bog Lake is a shallow humic lake located in the Northern Highlands State Forest in Vilas County, Wisconsin (89° 36′ W long, 46° N lat). This lake is surrounded by an extensive *Sphagnum* mat, from which it receives large inputs of DOC. Crystal Bog Lake is part of the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) program [45] (http://lter.limnology.wisc.edu). Detailed chemical and physical limnological data for this lake were made available by the NTL-LTER. Data used for this study include monthly measures of total nitrogen, nitrate, nitrite, ammonia, and total phosphorus, and biweekly measures of chlorophyll. Complete methodology and data may be accessed at http://lterquery.limnology.wisc.edu.

Sample collection for 2000 and 2001 was done as described by Yannarell et al. [70]. Briefly, integrated epilimnetic samples were collected biweekly during the ice-free season from a station at maximum depth using an integrated water column sampler. Bacteria present in these samples were concentrated in aliquots of 250–500 mL onto 0.2-μm filters (Supor-200; Gelman). Filters were frozen immediately in liquid nitrogen and stored at −80°C, awaiting DNA extraction using the FastPrep DNA purification kit (BIO101). In addition, 250 mL of unfiltered water was preserved in 2% glutaraldehyde for the determination of phytoplankton identity and abundance, and also for enumeration of bacterioplankton and heterotrophic nanoflagellates.

Sampling effort increased in 2002. Samples were collected approximately every other day throughout the summer (20 May–15 Aug). During periods of rapid changes in planktonic communities (early June), samples were collected daily.

Planktonic Community Composition

Bacterioplankton. Bacterial community composition and diversity was assessed using automated ribosomal intergenic spacer analysis (ARISA) [21]. PCR reactions contained PCR buffer consisting of 50 mM Tris (pH 8.0), 250 μg of bovine serum albumin per mL, and 3.0 mM $MgC1_2$ (Idaho Tech); 250 μM of each dNTP, 10 pmol of each primer, 1.25 U of *Taq* polymerase (Promega), and 1 μL of lake-extracted DNA in a final volume of 25 μL. The primers used for ARISA were 1406f, 5′ TGYACACCGCCCGT 3′ (universal, 16S rRNA gene), and 23Sr, 5′ GGGTTBCCCCATTCRG 3′ (bacteria-specific, 23S rRNA gene). The 1460f primer was labeled at the 5′ end with the phosphoramidite dye 6-FAM. PCR was carried out in an Eppendorf MasterCycler Gradient (Eppendorf) with an initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 35 s, 55°C for 45 s, and 72°C for 2 min, with a final extension carried out at 72°C for 2 min.

Denaturing capillary electrophoresis was carried out for each PCR reaction using an ABI 310 Genetic Analyzer (PE Biosystems). Electrophoresis conditions were 60°C and 15 kV with a run time of 50 min using the POP-4 polymer. A custom 100- to 2000-bp Rhodamine X-la-

beled size standard (Bioventures) was used as the internal size standard for each sample. ARISA profiles were analyzed using GeneScan 3.1.2 (Applied Biosystems) and aligned using Genotyper 2.5 (Applied Biosystems). To include the maximum number of peaks while excluding background fluorescence, a fluorescence cutoff of 500 fluorescence units was used.

To determine bacterial abundance, cells were stained with 4′, 6′-diamidino-2-phenylindole (DAPI) and counted on black 0.2-μm PCTE filters using a Nikon Diaphot epifluorescence microscope [52]. A subset of the Whipple grid that contains ~30 cells was selected for counting. Ten random Whipple grids were counted per slide on two perpendicular transects. Detailed information on bacterial enumeration is contained in the online methods manual for the Microbial Observatory for the North Temperate Lakes Long Term Ecological Research (NTL-LTER) site (http://microbes.limnology.wisc.edu/methods.htm). In 2001 and 2002, more detailed data on bacterial abundance were collected. In addition to total abundance, filamentous bacteria (defined as elongated single cells >5 μm in length; colonies of cells arranged end-to-end were not observed in the counts and thus were not enumerated as filamentous bacteria) were counted. Bacteria classified as filamentous are presumed to be less accessible to flagellate grazing [16, 40].

Phytoplankton. Volumes ranging from 10 to 25 mL of preserved sample were settled in chambers for at least 24 h prior to counting. Counting was performed on an Olympus IX-50 inverted microscope at 200× and 400×. Actual volume counted varied from 5 to 25 mL depending on the density of algae and protozoa. Identifications of dominant phytoplankton species were determined by established methods [53, 57, 65]. Identifications were made to species where possible. For samples collected in 2000, ~10 individuals were measured for size with a calibrated ocular micrometer. Volumes were calculated based on standard geometric formulas [28]. A mean cell, colony, or filament volume was calculated for each species. Biovolume values calculated for each species in 2000 were applied to samples collected in subsequent years. Abundance of each species was expressed as cells per liter and biovolume per liter.

Zooplankton and Heterotrophic Nanoflagellates. Zooplankton samples were collected biweekly with a modified Schindler-Patalas trap (53-μm mesh) at a station of maximum depth, preserved in 80% ethanol, identified, and enumerated (complete methodology and data available at http://lterquery.limnology.wisc.edu). Heterotrophic nanoflagellates were visualized with DAPI and enumerated on black 0.8-μm PCTE filters as described in the on-line methods manual for the Microbial Observatory of the NTL-LTER (http://microbes.limnology.wisc.edu/methods.htm).

Data Analysis. ARISA profiles were transformed to a binary array, indicating the presence or absence of each fragment in individual samples. ARISA profiles were compared using the Sorenson index of similarity (C_s) [20, 46]. A similarity matrix was generated in which the Sorenson's index was determined for all possible pairs of sample dates for all ARISA profiles generated in 2000, 2001, and 2002. This matrix was used to generate nonmetric multidimensional scaling (MDS) plots for the 3-year dataset and for individual years. The MDS method attempts to place the samples in a plot so that the rank order of the distances between the samples in the plot exactly agrees with the rank order of the samples from the similarity matrix. A stress coefficient is calculated for each plot to reflect any lack of agreement between the plot and the actual rank order of each sample [70]. This ordination results in a visual representation of the similarity of species composition over the three-year sampling period.

Analysis of similarity (ANOSIM), as described by Clarke and Green [11, 12], was used previously to distinguish lake samples [70] and to determine the annual differences in lake bacterial communities. Sample ARISA profiles were grouped according to the year in which they were collected, and ANOSIM was used to test the hypothesis that communities from samples collected in the same year were more similar to each other than to communities in different years. ANOSIM generates a test statistic, R, with a value between -1 and 1. The magnitude of R is indicative of the degree of separation between groups, with a score of 1 indicating complete separation and 0 indicating no separation; negative numbers (which would indicate that samples from different groups were more similar than those in the same groups) are rare [11, 12]. Monte-Carlo randomization of group labels was used to generate null distributions in order to test the hypothesis that within-year similarities were higher than would be expected if sample ARISA profiles were grouped at random.

Time series plots were constructed showing trajectories of change for ARISA fragment richness (a proxy here for bacterial species richness), bacterial abundance, *Daphnia* abundance, nanoflagellate abundance, abundances of various important algal species, and the concentrations of chlorophyll *a* and various organic and inorganic nutrients. These plots were compared to determine if nutrient concentration, algal abundance, or grazer abundance changed in tandem with bacterial richness and/or abundance, and to determine if the relationships or the patterns of change were consistent in all 3 years.

Results

Bacterioplankton Community Dynamics

Bacterioplankton Community Composition. The ARISA profiles generated from Crystal Bog Lake bacterioplankton communities exhibited a great deal of variability within each year (Fig. 1). The MDS plot of 2002 (Fig. 1c), showed a clear picture of bacterioplankton community succession over the summer months. In 2002, the bacterioplankton communities were sampled on a finer temporal scale than in earlier years. In each year, however, BCC was quite variable during the summer, while the communities observed in spring and fall showed less dramatic changes (indicated as clusters of sample dates).

Bacterial community composition showed a great deal of variability over the three-year period of observation. Of the 127 ARISA fragments detected in this lake over 3 years, 42% were detected in all 3 years. In addition, most of the peaks present in all years did not show the same seasonal distribution from year to year. Thus, although a subset of fragments persisted from year to year, there was no annual pattern that was apparent in the succession of bacterial species assemblages (Fig. 2). ANOSIM showed a higher similarity for samples within any given year ($R = 0.583$, $p < 0.001$) than for samples among different years. Pairwise comparisons were all significant ($p < 0.001$), and R statistics for communities in 2000 vs 2001, 2000 vs 2002, and 2001 vs 2002 were 0.268, 0.521, and 0.727, respectively.

Bacterioplankton Species Richness. Bacterioplankton species richness showed annual patterns of variation. Within each year, ARISA fragment richness was generally high in spring, but exhibited a great deal of variability throughout the summer months. The summer period was marked by dramatic drops in fragment richness in mid- to late June and again in late summer (generally August, although the richness minimum occurred in early September in 2001) (Fig. 3). The most dramatic of these declines in richness was observed on 28 June 2000, where ARISA detected no dominant populations of bacterioplankton (Fig. 3A). This result was confirmed by generation of multiple ARISA profiles from replicate filters collected on this sample date. This spring decline in richness was observed again in 2001 (14 June) and in 2002 (10 June). The annual late summer drop in ARISA fragment richness was first observed on 8 August 2000, 5 September 2001, and 8 August 2002.

Bacterioplankton Abundance. An annual minimum in bacterioplankton abundance was observed each year in June (Fig. 3B). This decline in abundance coincided with the early summer decline in ARISA fragment richness. In

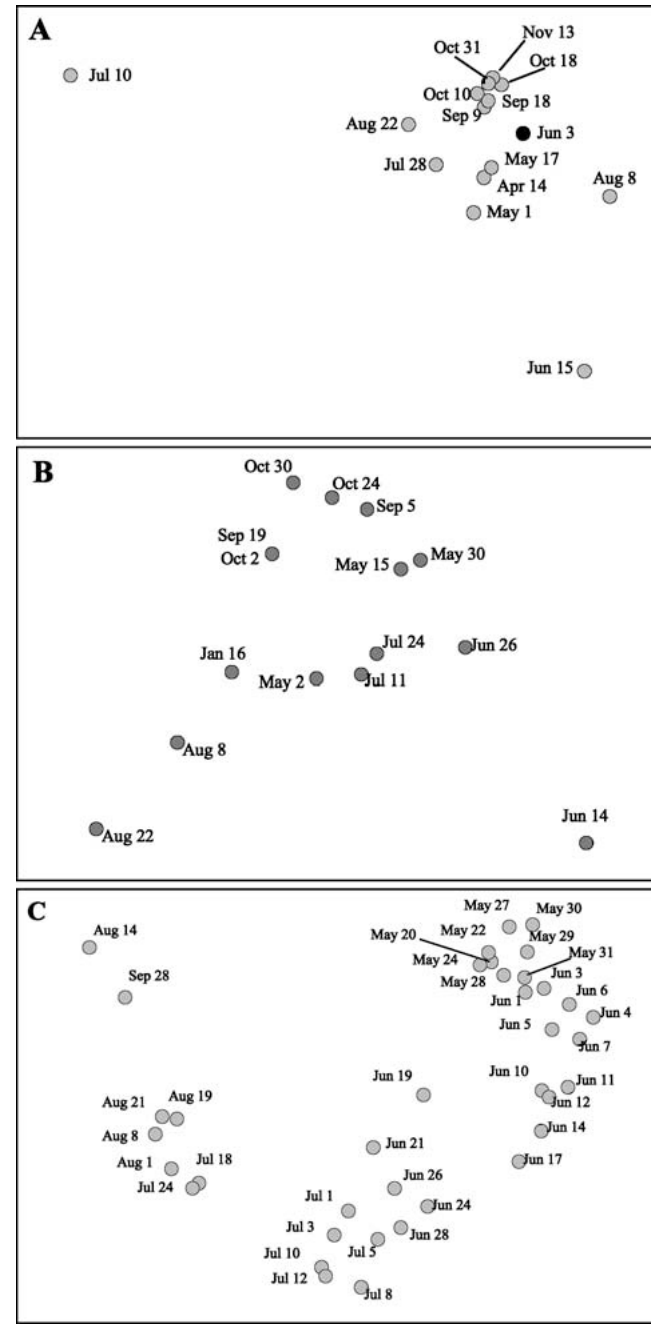

Figure 1. MDS plots for ARISA profiles collected from Crystal Bog each year. Because plots were generated from separate ordinations, absolute distances on different plots cannot be considered equal. Sample dates are indicated next to each point. (A) Profiles for the year 2000. Stress = 0.09. The point representing the June 28 sample was excluded from this plot because it was dramatically different from other time points (Sorenson's index is 0 for all comparisons). MDS would have plotted this point at an infinite distance from the other points in a random direction. Omission of this point allows better visualization of variability among the other ARISA profiles. (B) Profiles for the year 2001. Stress = 0.1. (C) Profiles for the year 2002. Stress = 0.1.

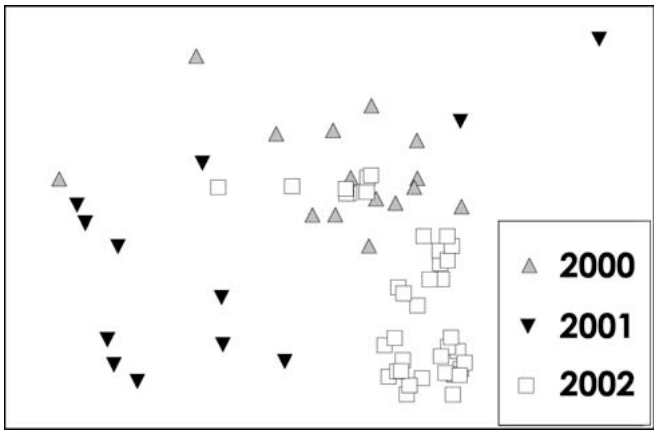

Figure 2. MDS plots for ARISA profiles collected from Crystal Bog over 3 years. Samples collected in 2000 are represented by gray triangles, 2001 samples are black triangles, and 2002 samples are white squares. Stress = 0.15. In order to better visualize the variability among ARISA profiles, the profile generated from the 28 June 2000 sample was omitted from this ordination.

2001 and 2002, data were also gathered on the abundance of filamentous bacteria (cells > 5 μm). In both of these years, a peak in the percentage of filamentous bacteria was observed in early summer: 31 May in 2001, and 27 May in 2002 (with another peak on 7 June). Abundance of filamentous bacteria (both absolute numbers and as a percentage of total bacteria) was much lower for sample dates later in the summer and fall (Fig. 3B).

Phytoplankton Community Dynamics. Although annual patterns in bacterial community composition were not apparent from the ARISA profiles, examination of the phytoplankton community did reveal annual trends. In general, the spring and early summer phytoplankton communities were dominated by the nonphagotrophic dinoflagellate species *Peridiniopsis quadridens.* A brief bloom of the mixotrophic chrysophyte, *Dinobryon*, was also a repeated feature of the phytoplankton community each summer, peaking on 28 June in 2000, 30 May in 2001, and on 27 May and 6 June in 2002 (Fig. 3D). *Dinobryon seratularia* was the dominant *Dinobryon* species each year, but *Dinobryon bavaricum* and *Dinobryon divergens* were also present.

The phytoplankton communities observed at midsummer were more variable when comparing composition among years, but often a bloom of *Cryptomonas* was observed. Dinoflagellates *Gymnodinium fuscum* and *Peridinium limbatum* dominated late summer and fall phytoplankton communities each year. *Peridinium limbatum* alone accounted for nearly 90% of the total phytoplankton biovolume each August in this humic lake (Fig. 3E).

The spring bloom of *Dinobryon* coincides with the spring drop in bacterial species richness and total abundance observed in each year (Fig. 3). The abundance of filamentous bacteria is also positively correlated (Pearson $r = 0.69$) with the abundance of this mixotrophic flagellate (Fig. 3B,D). The late summer drop in bacterial community richness observed each year occurs during the annual *Peridinium* bloom (Fig. 3A,E).

Chlorophyll. Chlorophyll measures are included as an approximation of total phytoplankton abundance and also as an indicator of energy inputs into the system through primary productivity. Chlorophyll levels in all three years peak early each summer in late May or June, and again in August (Fig. 3F). These peaks represent the early summer bloom of dinoflagellates and chrysophytes, and the intense late summer bloom of *Peridinium limbatum*. A midsummer chlorophyll peak was also observed in 2002.

Zooplankton Community Dynamics. As in other temperate lakes, an annual pattern of *Daphnia* abundance is observed in Crystal Bog Lake. *Daphnia* populations experience an annual peak in abundance early summer: 14 June in 2000, 11 July in 2001, and 18 June in 2002 (Fig. 3C).

Daphnia maxima are associated with increased bacterial abundance (Fig. 3), as well as a decrease in filamentous bacteria (Figs. 3B and 3C) in 2001 and 2002. The relationship with bacterial abundance is less clear in 2000 where the *Daphnia* peak preceded the *Dinobryon* peak. In 2001 and 2002, the peak in *Daphnia* abundance also coincided with a drop in *Dinobryon* abundance (Figs. 3C,D).

Nutrient Dynamics

Nitrogen. Nitrate, ammonia, and total filtered nitrogen all declined through April and May 2000, reaching a minimum in early June (Fig. 4A,B). The early summer minimum in bacterial community richness occurred during a peak in nitrate and ammonia measures in late June (Figs. 3A and 4A). After this point, the nitrogen measures returned to stable, low values for the remainder of the summer. In 2001, nitrate and ammonia both declined throughout the spring, along with the bacterial community richness (Figs. 3A and 4A). Ammonia levels remained low throughout the summer, but nitrate levels were variable. Nitrate (along with bacterial community richness) increased in midsummer and experienced a late summer minimum. Total nitrate was high during both the early and later summer declines in bacterial community richness (Figs. 3A and 4B). In 2002, all nitrogen measures were low during the early summer decline in bacterial diversity. Ammonia remained low throughout the summer, but nitrate and total filtered nitrogen were high during the late summer diversity minimum (Figs. 3A and 4A,B).

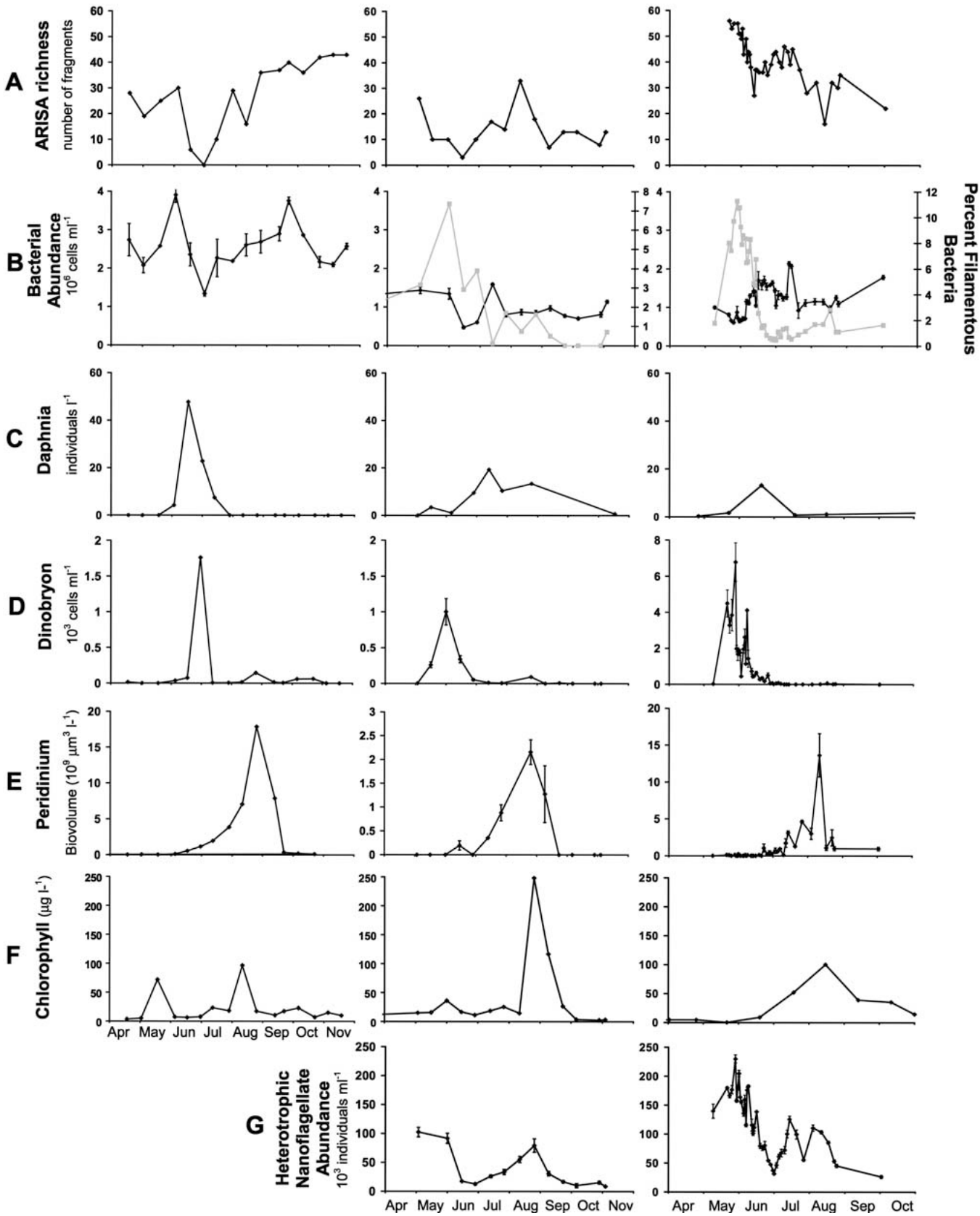

Figure 3. Time series of planktonic population density and diversity in Crystal Bog over 3 years. Time series of ARISA fragment richness (A); total bacterial abundance (black line) and percent of total bacteria classified as filamentous bacteria (gray line) (B); number of *Daphnia* (C); number of *Dinobryon* (D); biovolume of *Peridinium* (E); concentration of chlorophyll (F); and abundance of heterotrophic nanoflagellates (G). The left, middle, and right columns represent data collected in 2000, 2001, and 2002, respectively. Error bars indicate the standard error for each count.

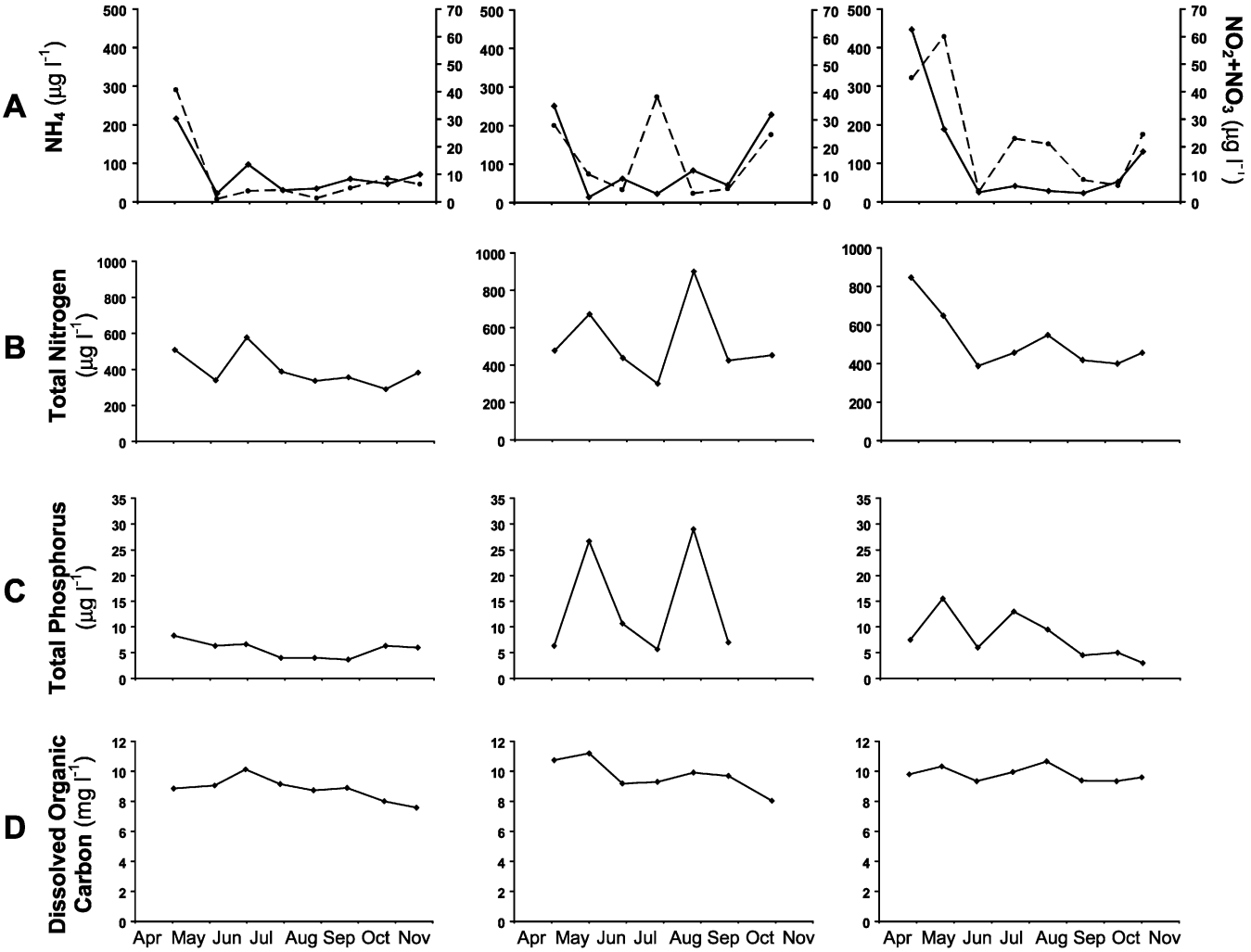

Figure 4. Time series of nutrients in Crystal Bog. Ammonia (solid line) and combined nitrate and nitrite (dashed line) (A), total nitrogen (B), total phosphorus (C), and dissolved organic carbon (D).

Phosphorus. Phosphorus (P) levels in 2000 were generally low and did not fluctuate greatly, but decreased somewhat in August and September (Fig. 4C). There are no patterns that correspond to the dynamics of planktonic communities in Crystal Bog Lake for this year (Fig. 3). Total phosphorus is high during the declines in bacterial community richness in 2001. Phosphorus levels are strongly correlated with total nitrogen ($r = 0.93$), and both of these measures are correlated with chlorophyll measures in 2001 ($r = 0.85$ for total nitrogen, and $r = 0.72$ for total phosphorus) (Figs. 3F and 4A,C). No strong correlations between phosphorus and total nitrogen dynamics are observed in 2002 ($r = 0.38$), though all measures are low during the early summer clear water phase (18 June 2002) and higher in midsummer. A midsummer peak in these measures corresponds to the midsummer chlorophyll peak. In 2002, the phosphorus levels are declining as bacterial community diversity reaches a minimum in both early and late summer (Figs. 3A and 4C).

Dissolved Organic Carbon. Crystal Bog Lake receives large inputs of dissolved organic carbon (DOC) from the extensive *Sphagnum* mat that surrounds the lake. The terrestrial inputs of DOC are supplemented by autochthonous production of organic matter in the ice-free season. As a result, this humic lake has high levels of dissolved organic carbon (DOC). DOC concentrations are stable for each of the 3 years (Fig. 4D), and DOC concentration does not appear to correspond to dynamics of planktonic populations (Fig. 3).

Discussion

Annual Patterns in BCC. There were repeated annual patterns in the seasonal dynamics of bacterial communities observed in this humic lake, despite the interannual differences observed in the composition of bacterial communities. Each year bacterial community composition was relatively stable in the early spring and fall, while

summer bacterial communities experienced rapid changes in species richness and abundance. Bacterial communities experienced two minima in species richness each year. The timing of these periods of low diversity was consistent from year to year, occurring in early summer (typically June), and again in late summer (August or early September). Minima in bacterial abundance and shifts in the proportion of filamentous bacteria each summer also showed an annual pattern. The observation that the bacterial communities in this lake are experiencing similar patterns of change each year even though the community composition differs greatly suggests that interactions among bacterial species are not the primary factor in determining seasonal changes in bacterial community structure. Drivers external to the bacterial community may be acting to structure the bacterial community.

Biological and Ecological Factors That Influence BCC on an Annual Basis. The aquatic food web has many linkages to the bacterioplankton community. Grazing by protozoan, metazoan, and flagellate populations provides "top-down" control of bacterioplankton community abundance [3, 9, 10, 27, 35]. Through selective grazing on particular size fractions or actively growing populations of bacteria, these grazer populations are also able to influence bacterioplankton community composition [26, 27, 40]. At the same time, the quantity and quality of available resources affects bacterioplankton abundance and growth rates ("bottom-up" control) [15, 23, 49, 50]. Previous studies have suggested that DOC composition (which changes as a result of phytoplankton community succession) may influence BCC [2, 15, 67]. Much of this evidence has been generated in mesocosm studies [42, 67], or in marine [2] or eutrophic freshwater [30, 32, 49] systems. Fewer studies have examined BCC in relation to factors that influence bacterial community dynamics in humic lake ecosystems, and multiyear studies of BCC in any aquatic system are rare. Our 3 year study of microbial communities in a humic lake explores the relationship between the patterns of change in BCC and the dynamics of other planktonic communities and environmental factors.

Resource Availability. Changes in resource availability influence aggregate measures for assessing bacterioplankton communities, such as abundance and activity [41, 50]. Since individual bacterial populations differ in their response to nutrient fluctuations [20], resource availability affects BCC as well. Nitrogen (N) and phosphorus (P) levels in Crystal Bog Lake can vary substantially within each year (Fig. 4), and while levels of these nutrients may exert an influence on BCC and bacterioplankton dynamics, there are no consistent annual patterns in N and P concentrations that can be correlated with the observed annual patterns in bacterial community dynamics. DOC levels in this lake are stable and are not correlated to changes in bacterial community richness. However, the stability in DOC concentration over time may mask seasonal changes in DOC lability due to succession in the phytoplankton community [2, 15, 67]. There may be seasonal or annual patterns in DOC quality that correspond to bacterioplankton dynamics. In addition, the observed DOC values may merely reflect levels of recalcitrant DOC that are stable over time and may not be a good measure of the dynamics of more labile forms that are readily utilized by bacterial populations. Changing quantity and composition of this labile dissolved organic matter over time may play an important role in determining the structure of bacterioplankton communities [13, 15, 19, 69].

Grazing. The annual drop in bacterial abundance in early summer, concurrent with the shifts in ARISA fragment richness and diversity in Crystal Bog Lake, is consistent with the idea that grazing may strongly influence bacterioplankton community dynamics in this humic lake. Changes in the proportion of filamentous bacteria, presumed to be less vulnerable to flagellate grazing [16, 40], coincide with rapid changes in BCC. This observation offers further evidence that grazing may be a mechanism that influences BCC dynamics and community structure in this environment. An annual bloom of the mixotrophic flagellate *Dinobryon* coincides with or closely precedes the drop in bacterial community richness and abundance each year (Fig. 3B,D), along with a peak in the abundance of heterotrophic nanoflagellates (Fig. 3G). The abundance of potential bacterivores coinciding with the drop in bacterial abundance suggests a causal relationship [5, 6, 17, 29, 31, 33, 35, 40, 53, 66]. The increase in the proportion of filamentous bacteria may also indicate predation by flagellates. The abundance of *Dinobryon* and heterotrophic nanoflagellates is positively correlated with the proportion of filamentous bacteria for 2001 and 2002 ($r = 0.77$ for *Dinobryon*, and $r = 0.82$ for heterotrophic nanoflagellates). The data collected in 2002 suggest that changes in grazing pressure can rapidly induce compensatory changes in bacterial morphology that result in the formation of a size refuge from grazing (Fig. 3B,D). Shifts in cell morphology have been suggested as a mechanism to buffer bacterioplankton populations against large fluctuations in abundance [34]. The dramatic reduction in bacterial abundance in early summer each year may indicate that the flagellate grazer dynamics in this humic lake are able to outpace the ability of the bacterioplankton to shift to a grazing-resistant morphology.

This rapid change in bacterioplankton community abundance has consequences for bacterial community diversity. The number of ARISA fragments detected

during these drops in bacterioplankton abundance is also reduced (Fig. 3A), and the bacterial community composition observed during these periods is distinct from that observed on other dates (Fig. 1). BCC profiles observed during the early summer minima in richness and abundance each year do not show a great deal of similarity to each other [Sorenson's index (C_s)= 0 for comparisons of communities observed in 2000 with either 2001 or 2002; C_s = 0.15 for 2001 compared to 2002], even though the bacterial community richness shows a consistent annual pattern with respect to flagellate grazer population dynamics.

Predation is a major force in structuring the organization of aquatic food webs and determining the species composition of the different trophic levels. The absence of planktivorous fish in this humic lake allows large zooplankton to play a dominant role in the food web. The cladoceran genus *Daphnia* in particular can have a major impact on the microbial food web [9, 35, 40]. Both mixotrophic and heterotrophic flagellate abundance declines as *Daphnia* populations increase in 2001 and 2002, likely due to *Daphnia* predation on the flagellate populations [1, 8, 9, 35, 40]. This suggests that bacterial communities may be released from flagellate grazing by the increase in cladoceran abundance [40, 58]. In these years, increased bacterioplankton abundance was also correlated with an increase in the *Daphnia* population. In addition, the proportion of filamentous bacteria declined as the *Daphnia* population increased. Results from previous studies suggest that such a shift in bacterial cell size correlated to *Daphnia* population dynamics may be an indirect effect due to the suppression of populations of flagellate grazers or a direct effect of cladocerans grazing more efficiently on bacterial aggregates [35, 36], or both. Similarly, the increase in bacterial abundance that coincides with increased *Daphnia* abundance may represent a combination of direct and indirect effects, including release from flagellate grazing [22, 27, 34–36] and regeneration of nutrients after consumption of phytoplankton [39, 47]. Bacterial community richness also recovers as the *Daphnia* population peaks, though the community composition observed during this period is distinct from the BCC observed preceding the flagellate grazing (Fig. 1). In addition, the bacterioplankton communities present as the *Daphnia* population peaks are not similar from year to year (C_s = 0.43 for communities observed during the peak in *Daphnia* abundance in 2000 compared with 2001, C_s = 0.17 for 2000 compared to 2002, and C_s = 0.42 for 2001 compared to 2002).

Phytoplankton Succession. The humic lake that is the focus of this study experiences an intense dinoflagellate bloom in late summer each year. A dramatic drop in bacterioplankton community richness coincides with this bloom each summer. This is in contrast to BCC dynamics reported in freshwater eutrophic or marine systems [2, 30], where bacterioplankton communities were more diverse during phytoplankton blooms. The phytoplankton community diversity in Crystal Bog Lake is very low at this time as the biomass of these late summer blooms overwhelmingly consists of a single dinoflagellate species [24]. Previous studies indicate that a more diverse phytoplankton community may support a more diverse bacterioplankton community [2, 30, 67]. The low richness observed in the bacterioplankton community each summer may reflect the subset of the bacterioplankton population that is best adapted to take advantage of the specific substrate offered by *Peridinium limbatum.* It is interesting to note, however, that the bacterioplankton communities present during this late summer dinoflagellate bloom are different each year (C_s = 0 for BCC observed during the peak of the *Peridinium* bloom in 2000 compared with 2001, C_s = 0.31 for 2000 compared to 2002, and C_s = 0.49 for 2001 compared to 2002).

Mixotrophy has also been reported for some dinoflagellate species, including some *Peridinium* species [64]. Although mixotrophy may contribute to the decline in bacterioplankton community richness observed in early summer in this lake, those shifts are accompanied by shifts in bacterioplankton abundance and morphology. Such changes are not observed during the fall drop in ARISA fragment richness, discounting the likelihood of mixotrophy by this particular *Peridinium* species.

Conclusions

The current study explored a number of factors that may potentially influence bacterioplankton community structure and population dynamics across years. No repeated patterns in bacterial community succession were observed, suggesting that species interactions within the bacterioplankton were not an important driver of bacterioplankton dynamics. While availability of resources affects the growth rate and abundance of individual bacterial populations [50, 60], there were no consistent annual patterns in concentrations of N and P that would suggest an important role for resource availability in the determination of bacterioplankton dynamics on an annual basis. The strongest correspondence was observed between bacterioplankton dynamics and biological factors such as the abundance of flagellate grazers and annual recurrence of intense dinoflagellate blooms. Correlations between the dramatic changes observed for the bacterioplankton community and the dynamics of other planktonic populations were consistent over 3 years. These data are consistent with the interpretation that trophic interactions are the dominant force influencing bacterioplankton dynamics in this humic lake. Degradation of organic matter and remineralization of

nutrients are related to bacterioplankton community composition [49, 51, 54]. Thus, dramatic shifts in bacterial community richness and abundance may result in the loss of taxa responsible for specific processes. Consequently, the ecosystem may experience shifts in resource utilization patterns that can affect communities at higher trophic levels [38].

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References

1. Adrian, R, Schneider-Olt, B (1999) Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. J Plankton Res 21: 2175–2190
2. Arrieta, J, Herndl, G (2002) Changes in bacterial β-glucosidase diversity during a coastal phytoplankton bloom. Limnol Oceanogr 47: 594–599
3. Arvola, L, Salonen, K (2001) Plankton community of a polyhumic lake with and without *Daphnia longispina* (Cladocera). Hydrobiologia 445: 141–150
4. Arvola, L, Tulonen, T (1998) Effects of allochthonous dissolved organic matter and inorganic nutrients on the growth of bacteria and algae from a highly humic lake. Environ Int 24: 509–520
5. Bird, D, Kalff, J (1986) Bacterial grazing by planktonic lake algae. Science 231: 493–495
6. Bird, DF, Kalff, J (1987) Algal phagotrophy—regulating factors and importance relative to photosynthesis in *Dinobryon* (Chrysophyceae). Limnol Oceanogr 32: 277–284
7. Burns, CW, Schallenberg, M (1998) Impacts of nutrients and zooplankton on the microbial food web of an ultra-oligotrophic lake. J Plankton Res 20: 1501–1525
8. Burns, CW, Schallenberg, M (2001) Short-term impacts of nutrients, *Daphnia*, and copepods on microbial food-webs of an oligotrophic and eutrophic lake. N Z J Mar Freshwat Res 35: 695–710
9. Callieri, C, Bertoni, R, Corno, G (2002) Dynamics of bacteria and mixotrophic flagellates in an Alpine lake in relation to *Daphnia* population development. J Limnol 61: 177–182
10. Chrzanowski, TH, Simek, K (1993) Bacterial-growth and losses due to bacterivory in a mesotrophic lake. J Plankton Res 15: 771–785
11. Clarke, KR (1993) Non-parametric multivariate analyses of changes in community structure. Aust J Ecol 18: 117–143
12. Clarke, KR, Green, RH (1988) Statistical design and analysis for a "biological effects" study. Mar Ecol Prog Ser 46: 213–226
13. Cole, JJ (1999) Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. Ecosystems 2: 215–225
14. Cotner, JB, Biddanda, BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5: 105–121
15. Crump, BC, Kling, GW, Bahr, M, Hobbie, JE (2003) Bacterioplankton community shifts in an Arctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69: 2253–2268
16. Degans, H, Zollner, E, Van der Gucht, K, De Meester, L, Jurgens, K (2002) Rapid *Daphnia*-mediated changes in microbial community structure: an experimental study. FEMS Microbiol Ecol 42: 137–149
17. Domaizon, I, Viboud, S, Fontvieille, D (2003) Taxon-specific and seasonal variations in flagellates grazing on heterotrophic bacteria in the oligotrophic Lake Annecy—importance of mixotrophy. FEMS Microbiol Ecol 46: 317–329
18. Fandino, LB, Riemann, L, Steward, GF, Long, RA, Azam, F (2001) Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. Aquat Microb Ecol 23: 119–130
19. Fisher, MM, Graham, JM, Graham, LE (1998) Bacterial abundance and activity across sites within two northern Wisconsin *Sphagnum* bogs. Microb Ecol 36: 259–269
20. Fisher, MM, Klug, JL, Lauster, G, Newton, M, Triplett, EW (2000) Effects of resources and trophic interactions on freshwater bacterioplankton diversity. Microb Ecol 40: 125–138
21. Fisher, MM, Triplett, EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65: 4630–4636
22. Gasol, JM, Vaque, D (1993) Lack of coupling between heterotrophic nanoflagellates and bacteria—a general phenomenon across aquatic systems. Limnol Oceanogr 38: 657–665
23. Glibert, PM (1998) Interactions of top-down and bottom-up control in planktonic nitrogen cycling. Hydrobiologia 363: 1–12
24. Graham, JM, Kent, AD, Lauster, GH, Yannarell, AC, Graham, LE, Kratz, TK, Triplett, EW (2003) Seasonal dynamics of phytoplankton and protoplankton communities in a northern temperate humic lake: diversity in a dinoflagellate dominated system. Microb Ecol 48(4): 528–540
25. Grover, JP (2000) Resource competition and community structure in aquatic microorganisms: experimental studies of algae and bacteria along a gradient of organic carbon to inorganic phosphorus supply. J Plankton Res 22: 1591–1610
26. Hahn, MW, Höfle, MG (1999) Flagellate predation on a bacterial model community: interplay of size-selective grazing, specific bacterial cell size, and bacterial community composition. Appl Environ Microbiol 65: 4863–4872
27. Hahn, MW, Höfle, MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 35: 113–121
28. Hillebrand, H, Durselen, CD, Kirschtel, D, Pollingher, U, Zohary, T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35: 403–424
29. Hitchman, RB, Jones, HLJ (2000) The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. Freshwat Biol 43: 231–241
30. Höfle, MG, Haas, H, Dominik, K (1999) Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. Appl Environ Microbiol 65: 3164–3174
31. Isaksson, A, Bergstrom, AK, Blomqvist, P, Jansson, M (1999) Bacterial grazing by phagotrophic phytoflagellates in a deep humic lake in northern Sweden. J Plankton Res 21: 247–268
32. Jeppesen, E, Erlandsen, M, Sondergaard, M (1997) Can simple empirical equations describe the seasonal dynamics of bacterioplankton in lakes: an eight-year study in shallow hypertrophic and biologically highly dynamic Lake Sobygard, Denmark. Microb Ecol 34: 11–26

33. Jones, RI, Rees, S (1994) Influence of temperature and light on particle ingestion by the freshwater phytoflagellate Dinobryon. Archiv für Hydrobiologie 132: 203–211
34. Jurgens, K, Gude, H (1994) The potential importance of grazing-resistant bacteria in planktonic systems. Mar Ecol Prog Ser 112: 169–188
35. Jurgens, K, Jeppesen, E (2000) The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. J Plankton Res 22: 1047–1070
36. Jurgens, K, Sala, MM (2000) Predation-mediated shifts in size distribution of microbial biomass and activity during detritus decomposition. Oikos 91: 29–40
37. Kato, K (1996) Bacteria—a link among ecosystem constituents. Res Popul Ecol 38: 185–190
38. Kinzig, AP, Pacala, SW (Eds.) (2001) The Functional Consequences of Biodiversity: Empirical Progress and Theoretical Extensions. Princeton University Press, Princeton, NJ
39. Lampert, W (1978) Release of dissolved organic carbon by zooplankton grazing. Limnol Oceanogr 23: 831–843
40. Langenheder, S, Jurgens, K (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol Oceanogr 46: 121–134
41. Lebaron, P, Servais, P, Troussellier, M, Courties, C, Muyzer, G, Bernard, L, Schafer, H, Pukall, R, Stackebrandt, E, Guindulain, T, Vives-Rego, J (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in abundances, activity and composition. FEMS Microbiol Ecol 34: 255–266
42. Lebaron, P, Servais, P, Troussellier, M, Courties, C, Vives-Rego, J, Muyzer, G, Bernard, L, Guindulain, T, Schafer, H, Stackebrandt, E (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. Aquat Microb Ecol 19: 255–267
43. Lindstrom, ES (2000) Bacterioplankton community composition in five lakes differing in trophic status and humic content. Microb Ecol 40: 104–113
44. Lindstrom, ES (2001) Investigating influential factors on bacterioplankton community composition: results from a field study of five mesotrophic lakes. Microb Ecol 42: 598–605
45. Magnuson, JJ, Kratz, TK, Allen, TF, Armstrong, DE, Benson, BJ, Bowser, CJ, Bolgrien, DW, Carpenter, SR, Frost, TF, Gower, ST, Lillesand, TM, Pike, JA, Turner, MG (1997) Regionalization of long-term ecological research (LTER) on north temperate lakes. Verh Internal Verein Limnol 26: 522–528
46. Magurran, AE (1988) Ecological Diversity and Its Measurement. Princeton University Press, Princeton, NJ
47. Moegenburg, SM, Vanni, MJ (1991) Nutrient regeneration by zooplankton—effects on nutrient limitation of phytoplankton in a eutrophic lake. J Plankton Res 13: 573–588
48. Murray, AE, Preston, CM, Massana, R, Taylor, LT, Blakis, A, Wu, K, DeLong, EF (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl Environ Microbiol 64: 2585–2595
49. Muylaert, K, Van der Gucht, K, Vloemans, N, De Meester, L, Gillis, M, Vyverman, W (2002) Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. Appl Environ Microbiol 68: 4740–4750
50. Pace, ML, Cole, JJ (1996) Regulation of bacteria by resources and predation tested in whole-lake experiments. Limnol Oceanogr 41: 1448–1460
51. Pinhassi, J, Hagstrom, A (2000) Seasonal succession in marine bacterioplankton. Aquat Microb Ecol 21: 245–256
52. Porter, K, Feig, Y (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25: 943–948
53. Posch, T, Simek, K, Vrba, J, Pernthaler, S, Nedoma, J, Sattler, B, Sonntag, B, Psenner, R (1999) Predator-induced changes of bacterial size–structure and productivity studied on an experimental microbial community. Aquat Microb Ecol 18: 235–246
54. Riemann, L, Steward, GF, Azam, F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. AppI Environ Microbiol 66: 578–587
55. Salonen, K, Kankaala, P, Tulonen, T, Hammar, T, James, M, Metsala, TR, Arvola, L (1992) Planktonic food-chains of a highly humic lake. 2. A mesocosm experiment in summer during dominance of heterotrophic processes. Hydrobiologia 229: 143–157
56. Schafer, H, Bernard, L, Courties, C, Lebaron, P, Servais, P, Pukall, R, Stackebrandt, E, Troussellier, M, Guindulain, T, Vives-Rego, J, Muyzer, G (2001) Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. FEMS Microbiol Ecol 34: 243–253
57. Simek, K, Babenzien, D, Bittl, T, Koschel, R, Macek, M, Nedoma, J, Vrba, J (1998) Microbial food webs in an artificially divided acidic bog lake. Int Rev Hydrobiol 83: 3–18
58. Simek, K, Hartman, P, Nedoma, J, Pernthaler, J, Springmann, D, Vrba, J, Psenner, R (1997) Community structure, picoplankton grazing and zooplankton control of heterotrophic nanoflagellates in a eutrophic reservoir during the summer phytoplankton maximum. Aquat Microb Ecol 12: 49–63
59. Simek, K, Hornak, K, Masin, M, Christaki, U, Nedoma, J, Weinbauer, MG, Dolan, JR (2003) Comparing the effects of resource enrichment and grazing on a bacterioplankton community of a meso-eutrophic reservoir. Aquat Microb Ecol 31: 123–135
60. Smith, VH (2002) Effects of resource supplies on the structure and function of microbial communities. Ant Van Leeuwen 81: 99–106
61. Sommer, U (1989) Plankton Ecology: Succession in Plankton Communities. Springer-Verlag, New York
62. Sommer, U, Gliwicz, ZM, Lampert, W, Duncan, A (1986) The PEG-model of seasonal succession of planktonic events in fresh waters. Arch Hydrobiol 106: 433–471
63. Chemical Limnology of North Temperate Lakes Primary Study Lakes, North Temperate Lakes Long Term Ecological Research program, NSF (http://lter.limnology.wisc.edu), Stanley EH, Center for Limnology, University of Wisconsin–Madison
64. Stoecker, DK (1999) Mixotrophy among dinoflagellates. J Eukaryot Microbiol 46: 397–401
65. Stone, L, Weisburd, RSJ (1992) Positive feedback in aquatic ecosystems. Trends Ecol Evol 7: 263–267
66. Tadonleke, RD, Jugnia, LB, Sime-Ngando, T, Devaux, J, Romagoux, JC (2002) Food web structure in the recently flooded Sep Reservoir as inferred from phytoplankton population dynamics and living microbial biomass. Microb Ecol 43: 67–81
67. van Hannen, EJ, Mooij, W, van Agterveld, MP, Gons, HJ, Laanbroek, HJ (1999) Detritus-dependent development of the microbial community in an experimental system: qualitative analysis by denaturing gradient gel electrophoresis. Appl Environ Microbiol 65: 2478–2484
68. Weinbauer, MG, Hofle, MG (1998) Distribution and life strategies of two bacterial populations in a eutrophic lake. Appl Environ Microbiol 64: 3776–3783
69. Weiss, M, Simon, M (1999) Consumption of labile dissolved organic matter by limnetic bacterioplankton: the relative significance of amino acids and carbohydrates. Aquat Microbiol Ecol 17: 1–12
70. Yannarell, AC, Kent, AD, Lauster, GH, Kratz, TK, Triplett, EW (2003) Temporal patterns in bacterial communities in three temperate lakes of different trophic status. Microb Ecol 46: 391–405