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Species-sorting may explain an apparent minimal effect of immigration on freshwater bacterial community dynamics

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Summary

Long distance atmospheric transport of bacterial cells is often implied as a driver of the apparent cosmopolitan distribution of bacterial taxa. Surprisingly, efforts to measure immigration in bacterial communities are rare. An 8-week time series of within-lake bacterial community composition and atmospheric deposition rates and composition were used to estimate the influence of immigration on bacterial community dynamics in two north temperate lakes. Characterization of bacterial community dynamics using automated ribosomal intergenic spacer analysis suggested moderate overlap in composition between the lakes and atmospherically deposited cells. However, taxa that appeared to be delivered by atmospheric deposition had a relatively minor influence on lake bacterial community dynamics. The weak influence of immigrating bacterial taxa suggests that a species-sorting concept best describes aquatic bacterial metacommunity dynamics.

Introduction

In 1934, Baas Becking (1934) coined the phrase 'Everything is everywhere, but the environment selects' while discussing microbial species distributions. The first half of the statement evokes thoughts of biogeographical patterns (or the lack thereof) in microbial populations. Microbial biogeography has been the subject of several recent publications (e.g. Fenchel and Finlay, 2004a; Martiny *et al*., 2006) and the concept of 'everything is everywhere' continues to be the subject of much study and debate. Here we focus instead on the portion of Becking's statement that is less often explicitly evaluated, 'but the environment selects'.

Becking's recognition of environmental selection likely grew out of his training in the Delft school of microbiology (e.g. De Wit and Bouvier, 2006), and is based on the observation that bacterial taxa are confronted by abiotic and biotic conditions that select for or against their proliferation. This idea highlights the importance of trade-offs in ecological traits among bacterial species and is reminiscent of the niche concept (Grinnell, 1904; 1917). The incorporation of the niche concept into an environmentally heterogeneous or patchy landscape forms one of four metacommunity paradigms outlined by Leibold and others (Leibold *et al*., 2004). Termed 'species-sorting', this paradigm emphasizes spatial niche partitioning and de-emphasizes dispersal dynamics. It assumes that local environmental gradients create patches that are occupied by species with a distinct specialized set of environmental tolerances. However, dispersal among patches allows local communities to track changes in environmental features. This perspective may appear to be in conflict with neutral assembly theory, but in fact could explain local spatial variation that at a large scale has emergent properties that appear neutral (Holt, 2006).

Community dynamics within a species-sorting framework are likely to correlate strongly with abiotic conditions or biotic interactions and have little relationship with the composition or number of immigrants entering the system (Chase and Leibold, 2003). Previous research in aquatic microbial ecology has demonstrated strong links between bacterial community dynamics and local environmental conditions or interactions with other members of the microbial food web (e.g. Simek *et al*., 2003; Kent *et al*., 2004; 2007; Yannarell and Triplett, 2005; Shade *et al*., 2007). However, few have studied the rate at which immigrants enter a lake or the taxonomic identity of those immigrants. Lindstrom and colleagues demonstrated that inlet streams may be vectors of immigration into lakes (Lindstrom and Bergstrom, 2004; Lindstrom *et al*., 2006). However, many of the world's lakes have no surface water inputs (i.e. they are seepage lakes) and would not be influenced by stream or riverine inputs of immigrant bacteria (Downing *et al*., 2006).

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Long-range transport of bacteria through the atmosphere has long been assumed to contribute to the cosmopolitan nature of bacteria (Baas Becking, 1934; Fenchel and Finlay, 2004a,b). If bacteria are constantly transported around the globe, atmospheric deposition of bacterial cells may be another key route for immigration, especially in seepage lakes. But previous research suggests that immigration rates were virtually negligible in comparison to within-lake sources and sinks, and the overlap between small subunit ribosomal RNA genes (16S rRNA) observed between rain and freshwater systems was limited (Jones *et al*., 2008). However, these estimates were made using measurements of atmospheric composition from a single 8-day period (Jones *et al*., 2008) and a database of 16S rRNA genes previously recovered from freshwater systems worldwide (Zwart *et al*., 2002; Newton *et al*., 2006). The comparison did not allow for a direct assessment of the influence of atmospheric cell deposition on aquatic bacterial community dynamics, but did suggest that deposition may have a weak impact.

Based upon previously observed correlations between bacterial community dynamics and environmental characteristics in lakes, and the apparent lack of taxonomic overlap between freshwater aquatic bacteria and bacteria observed in the atmosphere, we hypothesized that immigrants to seepage lakes do not have a significant influence on bacterial community dynamics. We believe that the species-sorting paradigm (Chase and Leibold, 2003) holds true for lake bacterial communities.

Results

Cell concentrations and deposition rates

We measured bacterial cell concentrations in two lakes in northern Wisconsin, USA (Crystal Bog and Little Rock) three times per week over a period of 2 months, while also measuring bacterial cell deposition rates. Cell deposition rates were calculated by enumerating bacteria in dust collectors floating on the water surface at stations in the littoral and pelagic zones of the lakes. Both the lake bacterial concentrations and the atmospheric cell deposition rates varied over 1–2 orders of magnitude during the study period (Fig. 1). No significant differences in cell concentrations or deposition rates were seen between littoral and pelagic stations or between the two lakes (paired *t*-tests, all $P > 0.1$). The proportion of the within-lake standing stock of bacteria represented by an hour of atmospheric deposition was never greater than 0.1% (Fig. 1). We also observed a weak, but statistically significant relationship between precipitation and rate of bacterial cell deposition (repeated measures analysis of variance, $R^2 = 0.18$, $P < 0.01$, Fig. 2). Average wind speed never explained a significant portion of variation in the deposition rate. No significant difference in cell concentration was observed between dust collectors with and without fixative deployed side-by-side (paired *t*-test, $P > 0.5$).

Lake community and dust collector composition

The composition of bacterial communities in the lake water and of assemblages captured in the dust collectors

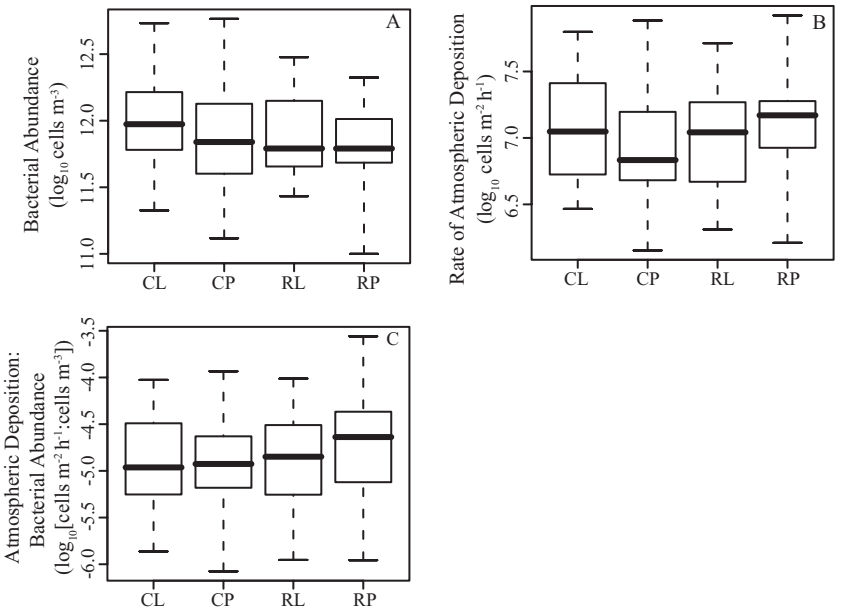

Fig. 1. Box plots of $\log_{10}$ bacterial cell concentration (A), rate of atmospheric deposition (B) and proportion of bacterial abundance that could be accounted for by atmospheric deposition (C) for pelagic and littoral stations in Crystal Bog and Little Rock Lake. Dark bars represent mean values, boxes are the interquartile range, and whiskers are data extremes. CL, Crystal Bog littoral; CP, Crystal Bog pelagic; RL, Little Rock littoral; RP, Little Rock pelagic.

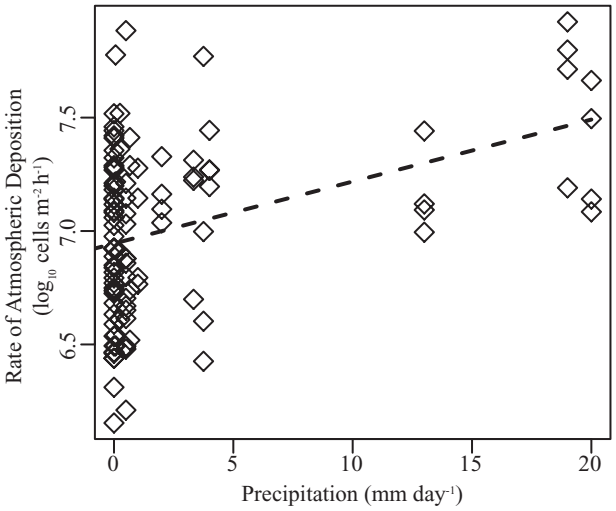

Fig. 2. Precipitation (mm day^{-1}) plotted against the $\log_{10}$ rate of atmospheric deposition of bacterial cells at littoral and pelagic stations on Crystal Bog and Little Rock Lake. The dashed line is the sum of squared errors best fit linear relationship between precipitation and bacterial deposition (repeated measures analysis of variance, $n = 104$, $R^2 = 0.18$, $P < 0.01$).

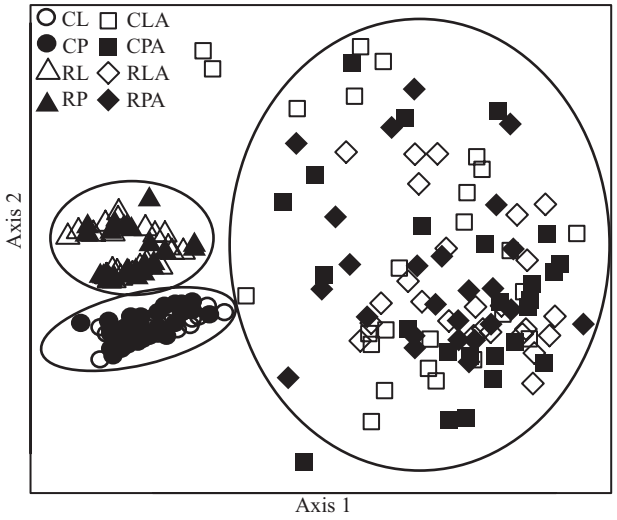

Fig. 3. A non-metric multidimensional scaling ordination of the bacterial composition of lake and dust collector samples across the sampling period. The ordination is based on a Bray–Curtis dissimilarity matrix. Stress = 0.18. C, Crystal Bog; R, Little Rock Lake; L, littoral stations; P, pelagic stations; A, dust collector contents.

were assessed using automated ribosomal intergenic spacer analysis (ARISA). We compared the operational taxonomic unit (OTU) composition among lakes, among stations within lakes, and between lake and dust collectors. There were strong distinctions in community composition among samples collected from Crystal Bog, Little Rock Lake and dust collectors (Table 1). However, no differences could be detected between littoral and pelagic stations within Crystal Bog or Little Rock Lake (Fig. 3). Dust collector assemblage composition was as variable between stations within a lake as was community composition in stations across lakes, and was generally much more variable than lake samples collected during the study (Fig. 3 and Table 1). Spatial and temporal variation of the taxa detected in dust collectors were comparable, but were always greater than variation within a lake across time or space (Fig. 4). In general, lakes appeared to vary more with time than between littoral and pelagic stations on a given date (Fig. 4).

Table 1. Analysis of similarity results for comparison of community composition among sites across the 8-week time series.

Comparison	ANOSIM *R*	Significance
C versus R	0.98	< 0.001
CL versus CP	0.001	0.4
RL versus RP	–0.04	1.0
Across dust collectors	0.048	0.002
CA versus RA	0.041	0.006
C versus CA	0.70	< 0.001
R versus RA	0.88	< 0.001

Significance is attributed to the ANOSIM R statistic based on 1000 randomizations.
C, Crystal Bog; R, Little Rock Lake; L, littoral stations; P, pelagic stations; A, dust collector contents.

Some overlap occurred between ARISA fragments observed in lake samples and dust collectors. Around 200 of the defined 270 ARISA OTUs were observed at a given site across the time series. Approximately 110 of these 200 OTUs were exclusively observed in the dust collector samples and 24 were only observed in lake samples. The remaining 66 OTUs were observed in both dust collector and lake samples. Two-dimensional hierarchical clustering of OTUs and samples, combined with a heatmap of occurrence frequency, revealed groups of samples and OTUs that had similar occurrence patterns (Fig. 5). The distribution of OTUs among sample types was uneven; the majority of observed OTUs were predominantly detected in either lake samples or dust collectors (groups A–D and groups E and G, Fig. 5). The OTUs in cluster F of Fig. 5 were most commonly found in both environments.

We found no relationship between deposition rates and rate of change in community composition (data not shown). In order to evaluate whether deposition contributed to community change, we created a data set comprised of lake community members only (OTUs observed in dust collectors were removed). There were moderate to strong correlations between whole community dynamics and the dynamics of communities with potential immigrants removed (Table 2). These correlations were much stronger than those observed between the composition of dust collectors and lakes across the time series (Mantel test, mean $R^2 = 0.38$, $P < 0.01$).

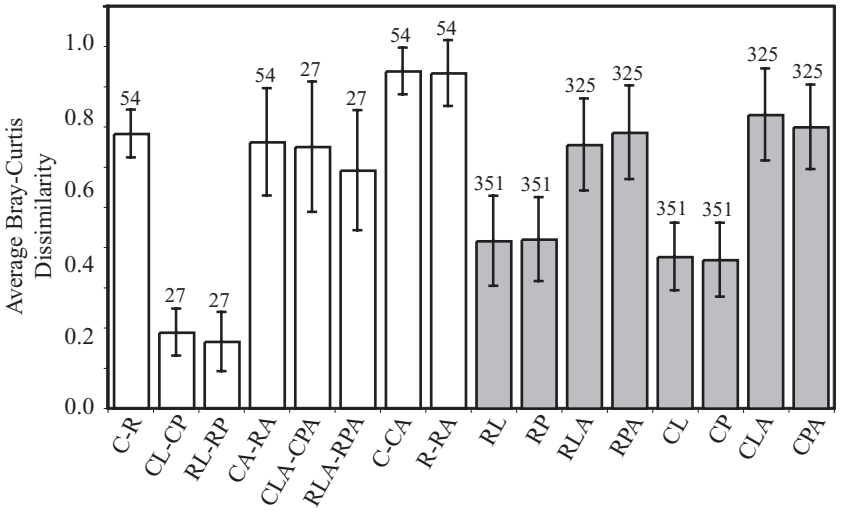

Fig. 4. Average Bray–Curtis dissimilarity between samples distributed in space or time. Open bars represent spatial differences across the time series and shaded bars represent temporal variability at a single site. Numbers above the bars indicate the number of pairwise comparisons used to calculate the dissimilarity. For example, CPA corresponds to the average Bray–Curtis dissimilarity at the Crystal Bog pelagic station dust collector, for 325 pairwise comparisons. CLA-CPA corresponds to the average Bray–Curtis dissimilarity between Crystal Bog littoral and pelagic site dust collectors for 27 pairwise comparisons. Error bars represent one standard deviation. C, Crystal Bog; R, Little Rock Lake; L, littoral stations; P, pelagic stations; A, dust collector contents.

Discussion

Previous work with freshwater planktonic communities, especially zooplankton, has suggested that species-sorting or habitat-filtering play a significant role in regulating the influence of immigrants (Leibold *et al*., 2004). However, a perception of a constant and widespread transport of bacteria around the globe seems to be common among aquatic microbial ecologists (e.g. Baas Becking, 1934; Fenchel, 2003). Recent work emphasizing the role of random events (including immigration, death, cellular division and extirpation from a system) seemed to explain some patterns in aquatic bacterial communities (Sloan *et al*., 2006; Woodcock *et al*., 2007). Although assuming ecological equality among all community members has allowed some interesting inferences to be made (Sloan *et al*., 2006; Woodcock *et al*., 2007), it is more likely that random events (such as immigration) and deterministic ecological interactions [such as predation (Simek *et al*., 2003; Kent *et al*., 2004) and competition (Horner-Devine *et al*., 2003)] combine to drive change in bacterial community composition. Our data set provides a unique opportunity to empirically approximate the importance of immigration in forcing bacterial community change within otherwise closed systems such as seepage lakes.

The taxonomic composition of atmospherically deposited cells was significantly more variable than the composition of lake bacterial communities (Figs 3 and 4). A

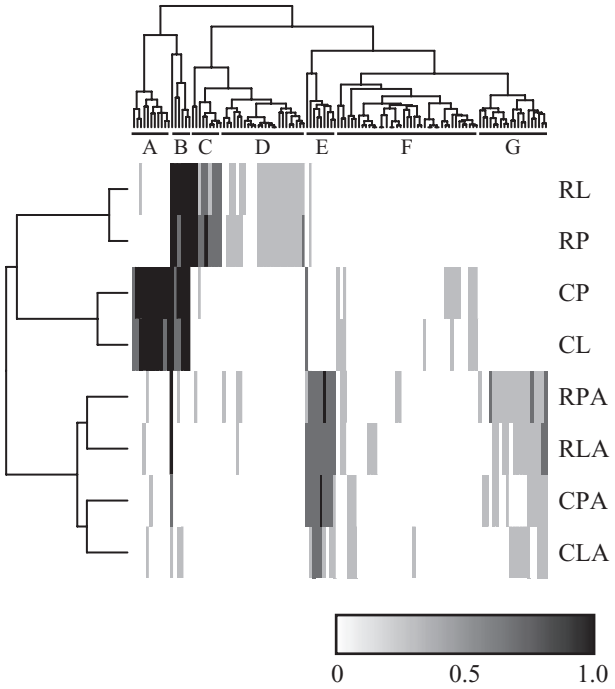

Fig. 5. A heatmap of ARISA fragment (columns) occurrences at each sample location (rows). Darker boxes indicate that a given ARISA fragment occurred in a larger proportion of samples from that location. Dendrograms represent hierarchical clustering of locations or ARISA fragments. The lettered (A–G) clusters in the ARISA fragment dendrogram are discussed further in the text. C, Crystal Bog; R, Little Rock Lake; L, littoral stations; P, pelagic stations; A, dust collector contents.

Table 2. Mantel *R* statistics for unmanipulated and 'immigration adjusted' Jaccard distance matrices.

	Number of days over which atmospherically deposited taxa were assumed to have contributed to dynamics		
Station	2–3 days	4–5 days	7 days
CL	0.91	0.83	0.82
CP	0.85	0.80	0.78
RL	0.95	0.91	0.92
RP	0.78	0.69	0.70

All *R* statistics are significant at alpha = 0.001.
CL, Crystal Bog littoral; CP, Crystal Bog pelagic; RL, Little Rock Lake littoral; RP, Little Rock Lake pelagic.

strong influence of habitat filtering may explain this observation. It appears that the physical and chemical features of lakes act as a strong set of filters for bacteria that arrive via the atmosphere. The majority of taxa consistently observed in Crystal Bog, Little Rock Lake or dust collectors did not overlap (Fig. 5). This suggests that dominant lake taxa may rarely be dispersed via the atmosphere (clusters A–D, Fig. 5) and consistently dispersed bacterial taxa are strongly selected against by environmental features of lakes (clusters E and G, Fig. 5). Likewise, previous work has suggested that the atmosphere serves as a very weak vector of planktonic zooplankton dispersal (Jenkins and Underwood, 1998). Limited work that has characterized the composition of the atmosphere, although not collected over aquatic environments, suggests soil microbes are more commonly dispersed relative to characteristically aquatic taxa (Fierer *et al*., 2008; Jones *et al*., 2008).

Our results also indicate that the epilimnia of small lakes are relatively well mixed in terms of bacterial community composition while the atmosphere is more spatially heterogeneous. Perhaps the large heterogeneity in deposited bacterial cells is a result of local sources dominating the composition of the atmosphere. However, the spatially distinct composition of deposited cells contradicts trends observed by Fierer and colleagues (2008). Fierer and colleagues (2008) characterized the bacterial composition of the atmosphere from five temporally dispersed samples in Boulder, Colorado and two other spatially dispersed sites across the USA, and suggested that differences in composition among samples in the time series were greater than among the three geographic locations. Differences in conclusions about spatial differences in atmospheric composition may result from differences in methodology of sample collection, the number of samples used to make inference about spatial variability, and taxonomic level of resolution used when making comparisons. Fierer and colleagues (2008) sought to characterize the complete composition of the atmosphere, while we intended to characterize only the fraction of bacteria in the atmosphere that are deposited on lakes. In addition, Fierer and colleagues (2008) surveyed a limited number of samples that were spatially dispersed and made compositional comparisons at family to genus-level taxonomic assignments. Our study had a large number of spatially dispersed samples (~100) and the taxonomic resolution of ARISA likely approaches the genus or species level.

Our results suggest that immigration may drive ~20% (average remaining variation from Table 2) of bacterial dynamics in seepage lakes. However, this is likely an overestimation. When creating 'immigration adjusted' data sets we assumed that the entire contribution of an ARISA peak was due to immigration if the peak was present in the corresponding dust collector in a preceding time step. It is possible that immigration events may only contribute a small portion of a previously existing lake population, and therefore our treatment of the data would be overly stringent. It is also difficult to select an appropriate time scale over which an immigrant could be expected to appear in the community. A simple magnitude estimate can suggest lower and upper bounds for the amount of time necessary for an immigrant to represent a significant member of the lake bacterial community (here defined as 1% of ARISA profile fluorescence). If all cells deposited on the surface of 1 m^3 of epilimnion were a single taxon and lake cell concentrations were held constant at 1×10^{12} cells m^{-3}, the taxon would be 1% of the community after 9–10 doubling times. This figure increases to 33–34 doubling times if a single cell were deposited in 1 m^3 of lake water. The paucity of published estimates for mean doubling time of bacteria residing in north temperate lakes makes this estimate even more uncertain. Assuming the mean doubling time is somewhere between 1 h and 3 days, it would take between 10 h and 100 days for an immigrant to constitute 1% of the lake's bacterial community. This range of potential doubling times is slightly larger than previous estimates (8–60 h) made using fluorescent *in situ* hybridization (Simek *et al*., 2005). In any case, immigration explains much less of the bacterial community variation than do potential drivers acting within the lake such as abiotic environmental parameters (Crump *et al*., 2003; Yannarell *et al*., 2003; Shade *et al*., 2007) and food web interactions (Simek *et al*., 2003; 2005; Kent *et al*., 2004; 2007; Newton *et al*., 2006).

The above calculations emphasize the need for further estimates of bacterial growth rates in natural settings and experimental exploration of the fate of bacterial immigrants once they enter a system of interest. Such experiments have been conducted on much simpler systems. Ives and colleagues (2004) and Woody and colleagues (2007) used fluorescently labelled immigrants to explore the influence of immigration on the dynamics of fungal populations residing on apple tree leaves. Mesocosm experiments in lakes with a similar approach would provide a better understanding of interactions between immigrating individuals and nascent community members. Research in this area would lead to a more complete understanding of bacterial community dynamics and metacommunity theory for bacteria (Leibold *et al*., 2004). An additional approach to measure the influence of immigrants would be to use phylogenetic tools on immigrant and community samples dispersed in time to quantify the influence of immigrants on population genetics of the recipient community. Such methodologies have been applied to marine macrofaunal communities to make inferences about dispersal among marine protected areas (Palumbi, 2003) and would be ideal for the

Table 3. Table of lake characteristics for Crystal Bog and Little Rock Lake south basin.

Lake characteristic	Crystal Bog (C)	Little Rock Lake (R)
Latitude and longitude (N, W)	46.008, −89.606	45.999, −89.704
Area (ha)	0.5	8.3
Maximum depth (m)	2.5	6.5
Dissolved organic carbon (mg l^{-1})	8.7	2.9
Total phosphorus (μg P l^{-1})	18.2	8.5
pH	5.2	6.0
Cholorphyll a (μg l^{-1})	9.9	2.5

Characteristics for Crystal Bog from the North Temperate Lakes-Long-term Ecological Research site (Magnuson *et al*., 2006; http://www.limnology.wisc.edu) and for Little Rock Lake from Brezonik and colleagues (1986). Data are seasonal averages.

genetic sequence-based data collected by microbial ecologists.

Experimental procedures

Site descriptions

Crystal Bog is a small, humic lake located in Vilas County, Wisconsin, USA. This polymictic, shallow lake is stained and acidic as a result of the surrounding *Sphagnum* bog mat (Table 3). Little Rock Lake is an oligotrophic, kettle lake approximately 3 km east of Crystal Bog. Little Rock Lake is not humic, but is shallow and polymictic (Table 3). Both lakes lack an inlet or outlet stream and therefore receive feeding water from precipitation and groundwater.

Sample collection

Samples for enumeration and compositional characterization of lake and atmospherically deposited bacteria were collected at littoral and pelagic stations three times weekly from 28 May 2007 to 27 July 2007. Atmospherically deposited bacteria were captured using 0.2 m diameter dust collectors containing reverse osmosis (RO) purified water. Pelagic lake samples were collected using a 2 m integrated sampler at approximately the geographic centre of the lake. Littoral lake samples were collected from a single near-shore sampling point (~2 m from shore) at the lake surface as to avoid sampling of sediments. Samples were transported to the nearby University of Wisconsin Trout Lake Station, and planktonic communities were captured on 0.2 μm filters (Supor 200 membrane filters, Pall). Filters were stored at −20°C until further processing. Dust collector contents were also captured onto 0.2 μm filters following estimation of dust collector volume. Subsamples (25 ml) from dust collector and lake samples were preserved with formaldehyde (final concentration 3%) and stored at room temperature until bacterial cells could be enumerated. Twelve side-by-side comparisons of dust collectors deployed with RO water or 5% final concentration formaldehyde in RO water were made across the 8-week sampling period to check for bacterial growth in dust collectors.

Bacterial enumeration

Enumeration of bacterial cells was conducted using the DNA stain SybrGreen (Invitrogen) and flow cytometry. The method for bacterial enumeration by flow cytometry developed by Del Giorgio *et al*., 1996) using SYTO 13 was adapted for SybrGreen according to Marie and colleagues (1997). Briefly, formaldehyde preserved samples were incubated with potassium citrate (25 mM final concentration) and SybrGreen (1× final concentration) for 10 min. Following incubation, an internal bead standard (Fluoresbrite Microspheres, Polysciences) of a known concentration was added, and samples were run on the low flow rate (~1 μl s^{-1}) of a BD FACSCalibur flow cytometer (Becton Dickinson). Gates for SybrGreen positive cells were determined based on comparison with a subset of 10 dust collector and 10 lake samples enumerated using SybrGreen and epifluorescence microscopy (Zeiss Axioplan2). All flow cytometry results were analysed using BD CellQuest Pro (Becton Dickinson). The reported volumetric concentration of cells in lake samples corresponded to in-lake concentrations. The volumetric concentration of cells in dust collector samples was used to calculate an areal deposition rate across each deployment. Concentration measurements were multiplied by the volume contained in the dust collector at the time of collection, divided by the area of the dust collector, and divided by the number of days over which deposited particles were collected.

Characterization of bacterial composition

We used ARISA (Fisher and Triplett, 1999) to characterize bacterial community composition of all lake and dust collector samples. ARISA exploits length heterogeneity of the spacer region between the 23S and 16S ribosomal RNA subunit genes. The 1406f (5′-TGYACACACCGCCCGT-3′, 5′ labelled with the phosphoramidite dye 6-FAM) and 23Sr (5′-GGGTTBCCCCATTCRG-3′) primers were used for ARISA PCR; conditions for ARISA PCR are described elsewhere (Yannarell *et al*., 2003). Denaturing capillary electrophoresis was carried out for each PCR reaction using an ABI 3730 (Applied Biosystems). A custom 100–2000 bp Rhodamine X-labelled size standard (Bioventures) was used as the internal size standard for each sample.

ARISA profiles were analysed using Genemarker v 1.5 software (SoftGenetics LLC) and a script developed in the R Statistics Package (R Development Core Team, 2007) to aid in high-throughput 'binning' of peaks. Candidate peaks were selected based upon a switch from positive to negative slope in ARISA electropherograms. A baseline was determined for each sample using the distributional method developed by Abdo and colleagues (2006). Briefly, candidate peaks with a height greater than two standard deviations above the mean of candidate peak heights were removed from the candidate peak pool and designated 'signal' peaks, and this process was repeated until no candidate peak heights were greater than two standard deviations above the mean height. The remaining candidate peaks were assumed to be 'noise' and the baseline was set at the maximum height of the 'noise' peaks.

Definition of ARISA fragment length bins is required to account for slight run-to-run variation in ARISA fragment

length (Hewson and Fuhrman, 2006). We used the overlay feature of Genemarker v 1.5 to visually define 322 ARISA fragment bins. The 'signal' peaks identified for each ARISA profile as described above were placed in the appropriate bin and total profile fluorescence was normalized to one. Finally, any peak with a relative fluorescence below 1% was removed in order to reduce the complexity of the data set. Profiles were relativized again to achieve a total profile fluorescence of 1. The reduction of ARISA fragment bins from 322 to 270 had little influence on the overall structure of the data set (Mantel test, $R^2 = 0.99$, $P < 0.001$). Raw and processed ARISA data are available for download in the Microbial Observatory section of the North Temperate Lakes Long-term Ecological Research website (http://www.limnology.wisc.edu).

Data analyses and statistics

Pairwise comparisons of community composition were conducted using the Bray–Curtis dissimilarity metric according to the equation: sum[abs($x_{ij} - x_{ik}$)]/sum[($x_{ij} + x_{ik}$)]. Rates of bacterial community change were calculated using pairwise Bray–Curtis dissimilarities calculated between two successive samples and then dividing by the number of days between sampling events.

Paired *t*-tests were used to make comparisons among univariate measures of sample sets, such as cell concentrations and deposition rates. Analysis of similarity [ANOSIM (Clarke, 1993)] was used to make multivariate comparisons. Hierarchical clustering and non-metric multidimensional scaling (NMDS) were used for visual representation and to analyse multivariate patterns among samples and ARISA fragments. Both were conducted using relativized peak height calculated as described above. All statistical analyses were conducted in the R Statistics Package (R Development Core Team, 2007). In addition, the proportion of samples in which a fragment was present relative to the total number of samples collected from a given site was used in the heatmap function in the R Statistics Package (R Development Core Team, 2007) to highlight differences among ARISA fragment occurrence patterns.

To assess the potential influence of atmospherically deposited cells on lake bacterial community dynamics, we used a scenario that assumed the presence of an ARISA fragment in a dust collector completely explained the presence of that fragment in the lake community. We used Mantel tests to determine the correlation between lake time series of bacterial community composition and time series data with dust collector contents removed. First, we transformed ARISA fragment-by-sample matrices for each lake region [CL (Crystal Bog littoral), CP (Crystal Bog pelagic), RL (Little Rock littoral), RP (Little Rock pelagic)] from relative fluorescence to presence–absence and then removed any taxa on a given date that were also present in the dust collector on that date. Jaccard (Legendre and Legendre, 1998) distance matrices were calculated for the unmanipulated and 'immigration adjusted' species-sample matrices covering the entire time series. We consider this Jaccard distance matrix to summarize the structure or inter-sample differences across the time series. We then used a Mantel test (Legendre and Legendre, 1998) to determine how much the act of removing deposited taxa from the data set influenced the structure of the data set. In this way, we were attempting to estimate the influence of atmospherically deposited bacteria on bacterial community dynamics through time, as summarized by the Jaccard distance matrix. If no ARISA fragments in a lake sample were observed in the preceding dust collector sample, no lake fragments would be removed when creating the 'immigration adjusted' ARISA profile, and the correlation between the two profiles would be 1.0. Conversely, if the exact same profile was obtained for a lake sample and the preceding dust collector sample, all peaks would be removed when creating the 'immigration adjusted' profile, and the correlation between the two profiles would be zero. Therefore, a Mantel *R* statistic near one would suggest a lack of influence by immigrating bacterial taxa and a Mantel *R* statistic near zero would suggest strong control of lake bacterial community dynamics by immigration. Significance of the Mantel *R* statistic was evaluated using randomized permutations. The influence of a temporal lag of up to one week, or three samples, was also explored by considering the contents of the current dust collector (2–3 days), current and previous dust collector (4–5 days), or current and two previous dust collector samples (7 days) as immigrant bacterial taxa when creating the 'immigration adjusted' species-sample matrix. All matrix manipulation and Mantel tests were conducted using the R Statistics Package (R Development Core Team, 2007).

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