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Spatial synchrony in microbial community dynamics: testing among-year and lake patterns

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Introduction

Quantifying the relative influence of forces that determine population and community dynamics is essential to our understanding of microbial function in lakes. Both intrinsic (site-specific) and extrinsic (regional) factors have the potential to influence ecosystems, but the relative importance of each is currently the subject of considerable discussion (Hudson & Cattadori 1999, Rusak et al. 1999, Bjørnstad & Grenfell 2001, Liebhold et al. 2004, Hessen et al. 2006). Lakes are model systems for examining the role of intrinsic and extrinsic drivers of microbial communities; distinct shoreline boundaries allow us to easily partition forces acting from within and outside the system. Regional extrinsic factors can impart synchrony to the dynamics of various ecosystem parameters (Liebhold et al. 2004), and variables such as temperature and water chemistry have strong interannual synchrony across lakes in a region (Magnuson et al. 1990, Kratz et al. 1998). Lake-specific intrinsic drivers, such as food-web interactions and stochastic population dynamics, typically dampen such patterns in plankton (Rusak et al. 1999, Baines et al. 2000, Magnuson et al. 2005).

Earlier work exploring the relative importance of extrinsic and intrinsic factors in ecology has typically examined population synchrony (Grenfell et al. 1998, Hudson & Cattadori 1999, Rusak et al. 1999). In this context, spatial synchrony (or temporal coherence; Magnuson et al. 1990) refers to correlated temporal variability in the abundance of a particular taxon among sites, usually within regions (Liebhold et al. 2004). In the absence of dispersal, synchrony among populations is often attributed to the "Moran effect," synchrony that is plausibly correlated with extrinsic climatic drivers (Moran 1953) or to trophic interactions with populations that exhibit spatial synchrony (Grenfell et al. 1998, Hudson & Cattadori 1999, Liebhold et al. 2004). From a community perspective, concordance is an analogous concept that quantifies the degree to which spatial patterns in community structure are similar among locations or co-occurring taxonomic groups (Paszkowski & Tonn 2000, Peres-Neto & Jackson 2001). Applied across time rather than space, 'temporal concordance' implies that changes in community structure occur at the roughly the same time and pace among different communities, thus providing a very useful approach to quantifying synchrony from a community perspective (Kent et al. 2007).

Kent et al. (2007) documented strong synchrony in the seasonal dynamics of microbial communities among 6 lakes in northern Wisconsin over the course of a single year using unaggregated intra-annual "species" level data. With generation times on the scale of days and the ability to adapt to environmental change over the course of a season, intra-annual time intervals for microbes may be somewhat analogous to annual dynamics for longer-lived organisms. We now have the opportunity to revisit this question of synchrony with another year of bacterial community composition (BCC) data in 2 of the same 6 lakes using a highly aggregated data set. Thus, using these data, we (1) test for ongoing synchrony in BCC among lakes with an additional year of data, and (2) compare patterns among years to explore both similarities and differences in the predictability and consistency of BCC and dynamics.

Key words: bacterial succession, concordance, north-temperate lakes, temporal coherence

Study site

Our study lakes are located in the Northern Highland Lake District (NHLD) in northern Wisconsin, an area dominated by second-growth mixed forests growing on a large overburden of glacial till, thus making groundwater an important component of water budgets for most lakes. This area has more than 7500 lakes, approximately half of which are the size of the 2 waterbodies we study (~1 ha) or smaller (Hanson et al. 2007). Although small, such systems clearly make up and important component of the landscape. Crystal Bog (CB: N 46°00'N; 89°36'W) and Trout Bog (TB: 46°02'N; 89°41'W) are hydrologically-isolated dystrophic lakes with low pH, high dissolved oxygen content (DOC) concentrations and elevated nutrient concentrations (Table 1). Trout Bog is dimictic, but because of the differences in depth, CB is essentially polymictic although it does stratify weakly at times during the summer. Because of this difference in mixis,

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Table 1. Selected limnological characteristics for Crystal Bog and Trout Bog (WI, USA). Morphometric data are from the NTL-LTER website (http://lter.limnology.wisc.edu/), while mean water chemistry data were derived from water samples collected over the course of the study period. Numbers in brackets are standard deviations.

Lake	Area (ha)	zmax (m)	DOC (mg/L)	pH	TP (µg/L)	TN (µg/L)	Chl-*a* (µg/L)
Crystal	0.56	2.5	9.5 (1.1)	5.1 (0.2)	22.5 (6.4)	598 (123)	22.0 (14.8)
Trout	1.01	7.9	21.0 (3.6)	4.8 (0.1)	33.9 (9.4)	808 (133)	31.5 (14.4)

only the epilimnetic community from each lake was used in this study. The extensive *Sphagnum* mat surrounding both lakes is responsible for their acidity as well as the high concentration of humic substances.

Methods

Integrated epilimnion (as determined by temperature and dissolved oxygen profiles) water chemistry and microbial samples were collected twice weekly from CB and TB during the ice-free period in 2003 (Kent et al. 2004) and approximately once weekly in 2005 at the deepest point in each lake using an integrated water column sampler. Bacteria present in these samples were concentrated onto 0.2-mm filters (Supor-200, Pall Gelman, East Hills, NY). Filters were frozen immediately and stored at −80 °C for DNA extraction using the FastPrep DNA purification kit (MPBiomedicals, Solon, OH). Detailed information on bacterial methodology is also contained in the Microbial Observatory on-line methods manual at the of the North Temperate Lakes Long Term Ecological Research (NTL-LTER) site (http://microbes.limnology.wisc.edu).

Bacterial community composition and diversity were assessed using automated ribosomal intergenic spacer analysis (ARISA; Fisher & Triplett 1999, Kent et al. 2004, Yannarell & Triplett 2005). Polymerase chain reactions (PCRs) contained 1 µl of lake-extracted DNA and primers typically used for ARISA 1406f, 5'-TGYACACACCGCCCGT- 3' (universal. 16S rRNA gene), and 23Sr, 5'-GGGTTBCCCCATTCRG-3' (bacteriaspecific, 23S rRNA gene). The 1406f primer was labeled at the 5' end with the phosphoramidite dye 6-FAM. The PCRs were carried out in an Eppendorf MasterCycler Gradient (Eppendorf AG, Hamburg, Germany) 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 using an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA) as described previously (Kent et al. 2004, Yannarell & Triplett 2004). Size-calling was carried out using GeneScan 3.1.2 (Applied Biosystems) in 2003 and GenoSpectrum v2.2.0 (SpectruMedix LLC) in 2005. Capillary electrophoresis results in minor run-to-run variations in observed versus actual fragment length were resolved using the allele-calling features in Genotyper 2.5 (Applied Biosystems) in 2003 and GenoSpectrum v2.2.0 (SpectruMedix LLC) in 2005, before analysis. To include the maximum number of peaks while excluding background fluorescence, a threshold of 100 fluorescence units was used. The signal strength (i.e., peak area) of each peak was normalized to account for run-to-run variations in signal detection by dividing the area of individual peaks by the total fluorescence (area) detected in each profile, expressing each peak as a proportion of the observed community (Rees et al. 2004, Yannarell & Triplett 2005). Fragments from ARISA were then aggregated into 9 large taxonomic groups based on classifications determined by Newton et al. (2006): Actinobacteria, Bacteroidetes, Firmicutes, Alpha/Beta/Delta/Gamma Proteobacteria, TM7, Verrucomicrobia. This level of taxonomic resolution is similar to that achieved by fluorescent *in situ* hybridization (FISH; Gloeckner et al. 1996).

The above changes in software used for analysis of ARISA profiles between years could have introduced biases as a result of the different proprietary algorithms used by each software package. However, we believe that at this highly aggregated level of taxonomic resolution these differences should be minimized, particularly for our comparison of community synchrony between lakes for a given year, which relies on datasets derived using the same software. This may influence our conclusions concerning the differences in the predictability of BCC observed between 2003 and 2005 for a given lake. Future analyses revisiting the issue of predictability of BCC among years are planned once a common dataset is derived.

Statistically, we employed a correspondence analysis (CA) of $\log_{10}(x + 1)$ aggregate abundances to derive visual estimates of concordance among lakes and years and provide the first 2 axis scores that formed the basis of further analyses (StatSoft 2005). A Procrustes analysis was used to assess the degree of association, or concordance, of BCC among years within each lake, as well as among lakes for a given year, using the first 2 CA axes (Jackson 1995, Peres-Neto & Jackson 2001). The metric of association (m^2) measures the degree of concordance between 2 matrices and varies between 0 and 1, where smaller values of m^2 indicate stronger concordance between data sets. Significance was assessed by permutation tests (999 permutations) using the PROTEST package (Jackson 1995). Because the sampling frequency differed somewhat among years, we rarified the 2003 data to match the timing and resolution of the 2005 data prior to running the CA.

Results

Our CA plot of individual sample dates for both lakes and years together revealed striking differences among

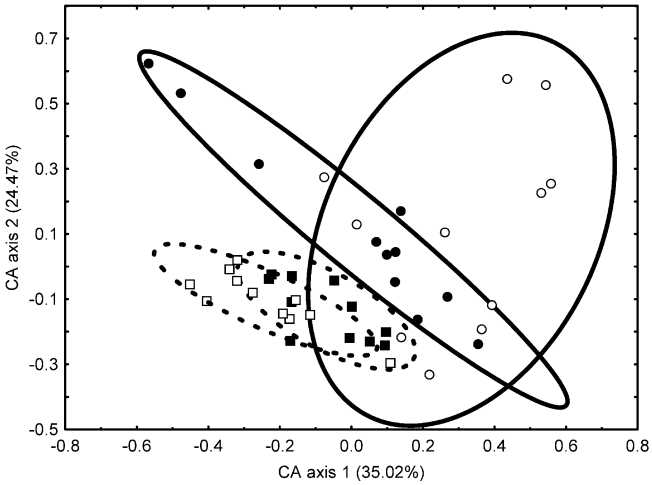

Fig. 1. Plot of sample coordinates on the first two axes of a correspondence analysis that included both lakes over the two years of monitoring. Circles represent samples from 2003 and square symbols are from 2005 (CB = filled / TB = open). Each ellipse contains 75 % of the variation in ice-free samples for a particular lake-year.

lakes and years in overall community variability and structure (Fig. 1). First, although the sizes of the prediction ellipse (capturing 75 % of variation in the data) for 2003 are quite similar, the underlying community structure is quite different, as suggested by ellipse orientation and the scatter of points within. In 2005, however, community structure is much more similar in the 2 lakes. Second, the variability among years is quite different. Ellipses are much smaller in 2005 than 2003, suggesting that community structure was much more constrained in this year. Further, among years, community structure is much more similar in CB than in TB.

Visually, the individual CA summaries of BCC for each lake-year suggest that the pattern and rate of change is similar and predictable in 2003 (implying intra-annual temporal concordance or synchrony) and perhaps somewhat similar, but far less predictable, in 2005 (Fig. 2).

Procrustes analysis revealed that the synchrony of BCC (among lakes for a given year) was marginally significant in 2003 (m^2 = 0.63; p = 0.05), suggesting that extrinsic forces were primarily responsible for determining bacterial community composition in that year, but not in 2005 (m^2 = 0.92; p = 0.71). This result substantiates the patterns documented in 2003 by Kent et al. (2007), but suggests that community synchrony among lakes may not be present in all years. We also examined whether patterns were repeated among years in a given lake. Although this analysis does not quantify the defined "temporal concordance" of communities *per se*, it does speak to the repeatability of BCC in different years for a particular lake. The BCC was not concordant among years in either CB (m^2 = 0.89; p = 0.47) or TB (m^2 = 0.88; p = 0.51).

Discussion

Although the spatial synchrony of population dynamics is becoming a fairly well-studied phenomenon, the spa-

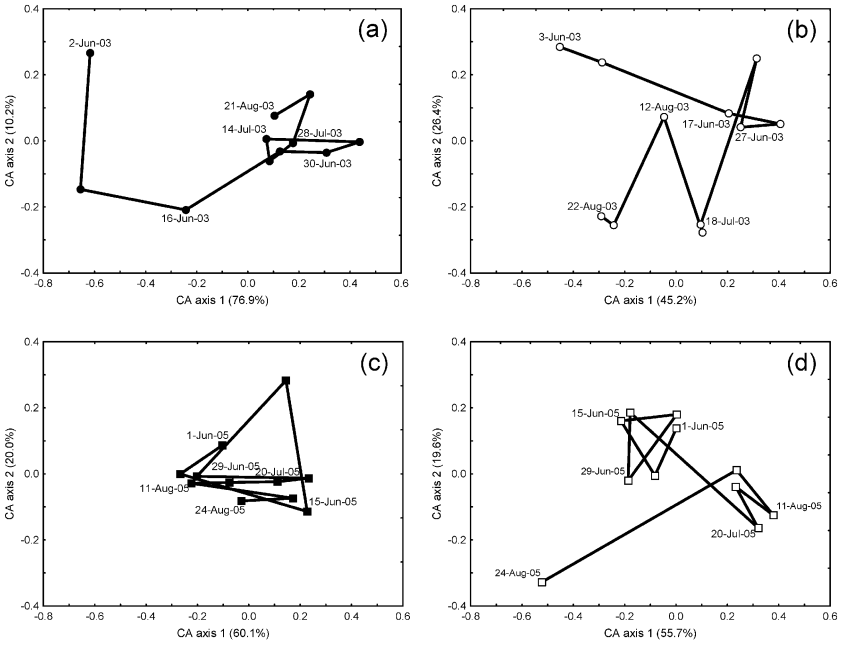

Fig. 2. Plot of sample coordinates on the first two axes of a correspondence analysis for a) CB in 2003, b) TB in 2003, c) CB in 2005, and d) TB in 2005. Every other sample is labeled with its date.

tial synchrony of community dynamics is virtually unexplored (Kent et al. 2007). Our study corroborates the documentation of community-level synchrony among lakes in 2003 (Kent et al. 2007), this time using a highly aggregated dataset to represent microbial community dynamics. However, although some similarity in patterns of BCC were evident, synchrony was largely absent in intra-annual dynamics between lakes in 2005, suggesting that the role played by extrinsic factors may not always override lake-specific, intrinsic variables. Interannual differences in BCC dynamics for a given lake suggest that the predictability of intra-annual community dynamics may be more difficult than initially thought, particularly in years that are not synchronous.

We documented significant among-lake community synchrony in 2003 but not in 2005. Despite this lack of significance for 2005, the patterns of community dynamics appear visually similar, particularly in the early part of the ice-free period. However, in 2005, there seems to be far less predictability to changes in community structure, whereas in 2003 the microbial community follows a similar successional trajectory over the course of the season. In many respects this result is not as anomalous as it might seem; synchronizing forces are unlikely to always be dominant when comparing potential regional climatic influences among years. If a year is climatically "normal" for the factors that are most influential in determining synchronous dynamics, lake-specific intrinsic forces may indeed dominate BCC. We examined a diverse set of meteorological factors with the potential to act as synchronizing agents (annual means [May–Sep] for air temperature, photosynthetically active radiation [PAR], wind speed, precipitation, and ice-off date) for "unusual" values from 1990 through 2006. If we restrict our definition of unusual to the 3 highest or lowest values for this period, PAR is the only variable identified in 2003 (second highest year in record), while wind speed and temperature were both unusual in 2005 (both the third highest year in record). Interestingly, Kent et al. (2007) found that extrinsic forcing acted to synchronize BCC, primarily via changes in phytoplankton community dynamics, which are also known to display seasonality (Sommer 1989, Anneville et al. 2002). Clearly, PAR has the potential to influence primary production and may also alter phytoplankton community structure and dynamics. Further, lake mixing has been identified as a mechanism that can reset BCC (Jones et al. 2008), thus the higher than average wind speeds documented in 2005 may plausibly be contributing to enhanced epilimnetic mixing that subsequently disrupts any synchronous patterns from developing in this year.

Future research into the determinants of synchronous and asynchronous community dynamics among lakes will be of primary importance in advancing our understanding of microbial community structure and function. This study provides insights into these issues, but also raises numerous additional questions in this important field of research. The dominant patterns, their predictability and synchrony, as well as the mechanisms (both intrinsic and extrinsic) that govern intra and interannual differences, remain important issues for microbial researchers.

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