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The influence of habitat heterogeneity on freshwater bacterial community composition and dynamics

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Summary

Multiple forces structure natural microbial communities, but the relative roles and interactions of these drivers are poorly understood. Gradients of physical and chemical parameters can be especially influential. In traditional ecological theory, variability in environmental conditions across space and time represents habitat heterogeneity, which may shape communities. Here we used aquatic microbial communities as a model to investigate the relationship between habitat heterogeneity and community composition and dynamics. We defined spatial habitat heterogeneity as vertical temperature and dissolved oxygen (DO) gradients in the water column, and temporal habitat heterogeneity as variation throughout the open-water season in these environmental parameters. Seasonal lake mixing events contribute to temporal habitat heterogeneity by destroying and re-creating these gradients. Because of this, we selected three lakes along a range of annual mixing frequency (polymictic, dimictic, meromictic) for our study. We found that bacterial community composition (BCC) was distinct between the epilimnion and hypolimnion within stratified lakes, and also more variable within the epilimnia through time. We found stark differences in patterns of epilimnion and hypolimnion dynamics over time and across lakes, suggesting that specific drivers have distinct relative importance for each community.

Introduction

Multiple interacting drivers structure natural microbial communities. In lake ecosystems specifically, it has been shown that biotic interactions (i.e. food–web or predator–prey dynamics) often play a significant role in shaping bacterial communities (Kent *et al*., 2004; 2006), as do lake geography, landscape position (Yannarell and Triplett, 2005), trophic status (Yannarell *et al*., 2003), pH and other abiotic water chemistry parameters (Lindstrom *et al*., 2005; Newton *et al*., 2007). However, aquatic microbial ecologists are yet a long way from developing a conceptual framework for community assembly that accounts for the relative importance of, and interactions among, these potentially hierarchical factors. This is partially due to the difficulty in determining specific responses of the community to a single driver, as often the effects of separate drivers cannot be isolated from each other. Also challenging is the isolation or manipulation of a bacterial community outside of its natural setting, or the experimental control of the community within its environment. General theories that incorporate hierarchical forces are needed to explain bacterial community assembly.

Habitat heterogeneity is a measure of the number of niches available in an ecosystem. Because it accounts for multiple factors influencing microbial communities, this traditional ecological concept is potentially a useful framework for considering community structure (Wu and Loucks, 1995; Konopka *et al*., 2007). Habitat heterogeneity is thought to determine species diversity and composition in communities (Cornell and Lawton, 1992; Tilman, 1999). As an example, the niche diversification hypothesis (Hutchinson, 1961) predicts that highest diversity will occur at maximum habitat heterogeneity because each species will specialize to a unique niche, minimizing competition (Schoener, 1974; Magnuson *et al*., 1979; Yang *et al*., 2005). Multiple 'dimensions' contribute to heterogeneity, such as habitat, food and time dimensions (Schoener, 1974). Schoener proposed that habitat dimensions (physical availability/use of space) play the largest role in determining community structure. Likewise, temporal habitat heterogeneity and temporal scale of observation can contribute to community patterns (e.g. Menge and Sutherland, 1976); these studies often suggest an overlap of spatial and temporal heterogeneity, and an influence of history on community dynamics (e.g. Poff and Ward, 1990; Fuhrman *et al*., 2006; Shade *et al*., 2007).

Lakes that thermally stratify present an interesting system with which to investigate proximate physical and

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chemical drivers of aquatic bacterial communities in the ecological framework of habitat heterogeneity. There are demonstrably stark distinctions in physical and chemical conditions across thermal layers of stratified lakes (epilimnia and hypolimnia) and each layer is known to harbour different bacterial communities (Selig *et al*., 2004; Tammert *et al*., 2005; Tonno *et al*., 2005; Boucher *et al*., 2006). During stratification, the cooler hypolimnion generally is nutrient-replete, thermally stable and potentially anoxic, while the warmer epilimnion typically is characterized by a scarcity of available nutrients, relatively oxygen-rich conditions, the potential to be influenced by weather events and exposure to higher solar radiation. Lake mixing quickly and drastically alters the chemical and physical conditions within the water column (Lehours *et al*., 2005; C.-Y. Chiu, S.E. Jones, T.K. Kratz, J.-T. Wu, A. Shade, and K.D. McMahon, submitted), and creates a largely homogeneous water column (except with respect to light) until stratification is re-established.

Lake mixing is an ultimate driver of community change because it destroys vertical habitat heterogeneity in environmental parameters such as dissolved oxygen (DO) and temperature, which in turn affect the bacterial community structure. Subsequent stratification of the water column then re-establishes these gradients. Because lake mixing is a phenomenon driven by seasonal changes, it is also coupled with temporal habitat heterogeneity. In this way, lake mixing provides a unique opportunity to explore bacterial community response to two dimensions (spatial and temporal) of habitat heterogeneity.

Our objectives were as follows: (i) to characterize environmental heterogeneity in the vertical water column over space and time, (ii) to relate this heterogeneity to differences in bacterial community composition (BCC) and dynamics (richness, composition and variability) between thermal layers within a lake, and (iii) to search for general cross-lake relationships between bacterial community dynamics and environmental heterogeneity. To accomplish these objectives, we surveyed three humic lakes in Northern Wisconsin, USA, along a gradient of mixing frequencies: polymictic Crystal Bog (CB), dimictic Trout Bog (TB) and meromictic Mary Lake (MA). These lakes were comparable in other environmental characteristics, such as surface area and pH (Table 1). We measured DO and temperature gradients across time and space (depth) in the water column as a proxy for habitat heterogeneity. We defined our experimental unit as a lake thermal layer (epilimnion or hypolimnion), and habitat heterogeneity as the distribution and abundance of habitats in the vertical water column of each layer. Weekly bacterial community fingerprints were used to observe high-resolution dynamics, because this temporal scale captures much of the variation in BCC in these systems (Kent *et al*., 2006; Newton *et al*., 2006). Our results show that bacterial community structure and dynamics in humic lakes are affected largely by proximate environmental drivers that play unique roles in each thermal layer.

Table 1. Physical and chemical lake characteristics, including difference in temperature and mean dissolved oxygen (DO) between thermal layers of each lake.

	CB	TB	MA
Epilimnion mean temperature (°C)	20.1	19.4	19.0
Hypolimnion mean temperature (°C)	16.0	6.6	5.5
Temperature mean difference (°C)	4.0	12.7	13.5
Epilimnion mean DO (mg l^{-1})	6.8	6.6	7.6
Hypolimnion mean DO (mg l^{-1})	4.0	1.0	1.2
DO mean difference (mg l^{-1})	2.9	5.6	6.4
pH	5.1	4.8	5.5–6.0
Dissolved organic carbon (mg l^{-1})	19.2	25.0	26.4
Epilimnion total nitrogen (μg l^{-1})	725	810	892
Hypolimnion total nitrogen (μg l^{-1})	566	1086	3086
Epilimnion total phosphorus (μg l^{-1})	28	38	30
Hypolimnion total phosphorus (μg l^{-1})	22	43	452
Surface area (km^2)	0.005	0.011	0.012
Maximum depth (m)	2.5	7	21.5

Dissolved oxygen and temperature differences are based on an average of measurements taken between late May and early November. Polymictic CB had a smaller difference between layers in both temperature and DO than the two stratified lakes, indicating less vertical habitat heterogeneity.

Results

Spatial and temporal environmental heterogeneity in the study system

Temporal and spatial environmental heterogeneity in the water column were observed weekly using vertical DO and temperature profiles through the open-water period (Fig. 1). Average spatial differences with depth in epilimnion and hypolimnion DO (mg l^{-1}) and temperature (°C) over the sampling period for each lake were greater in the stratified lakes than in polymictic CB (Table 1). Taken together, these environmental data were used to characterize habitat heterogeneity through time and space in our study system.

Comparison of within-lake BCC and dynamics

We investigated whether there were significant differences in taxonomic richness between lakes of different mixing regimes, or between thermal layers within one lake. We observed differences in both richness [defined as number of unique automated ribosomal intergenic spacer analysis (ARISA) fragments] and BCC between the study lakes (Fig. 2A and B). A total of 399 operational taxonomic units (OTUs) were observed, across all three lakes. Both MA and TB were significantly richer than CB (Bonferroni-corrected *t*-test, alpha = 0.05, $P < 0.0001$ for all). Meromictic MA had the most unique OTUs (as measured by ARISA fragment presence/absence

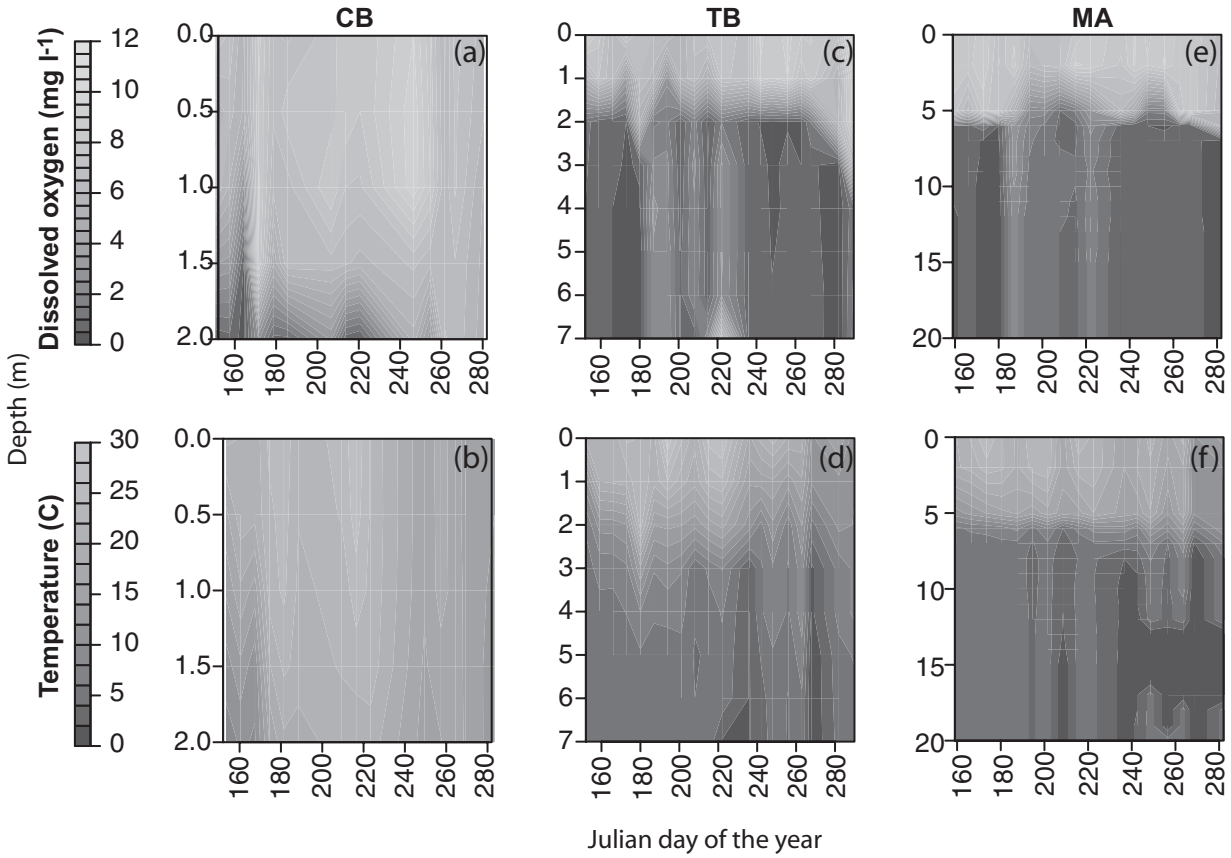

Fig. 1. Dissolved oxygen (DO) (mg l^{-1}) and thermal (°C) gradient maps of the lakes over the study period, 2005. The maps are labelled as follows: (A) CB DO, (B) CB temperature, (C) TB DO, (D) TB temperature, (E) MA DO, (F) MA temperature. As proxied by DO and temperature profiles through time, polymictic CB has lower habitat heterogeneity in the vertical water column than the two stratified lakes, TB and MA.

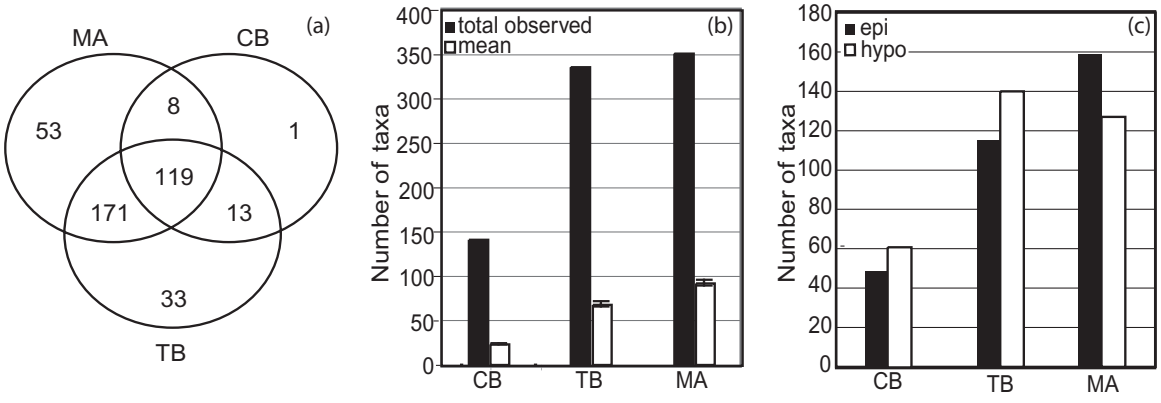

Fig. 2. Summary of bacterial community richness assessed using ARISA.
A. Venn diagram representation of total observed OTUs common to bacterial communities in TB, CB and MA. Crystal Bog had one unique OTU, as compared with 33 and 53 unique OTUs observed in TB and MA respectively.
B. Histogram of average and total observed OTUs per lake. There were 399 total OTUs observed from May to November 2005. Crystal Bog was the least rich of the three study lakes, followed by TB, and then MA.
C. Histogram of total observed OTUs per thermal layer. Polymictic CB had the least observed OTUs, in each layer; the epilimnia and hypolimnia of stratified MA and TB were comparable in their total observed OTUs.

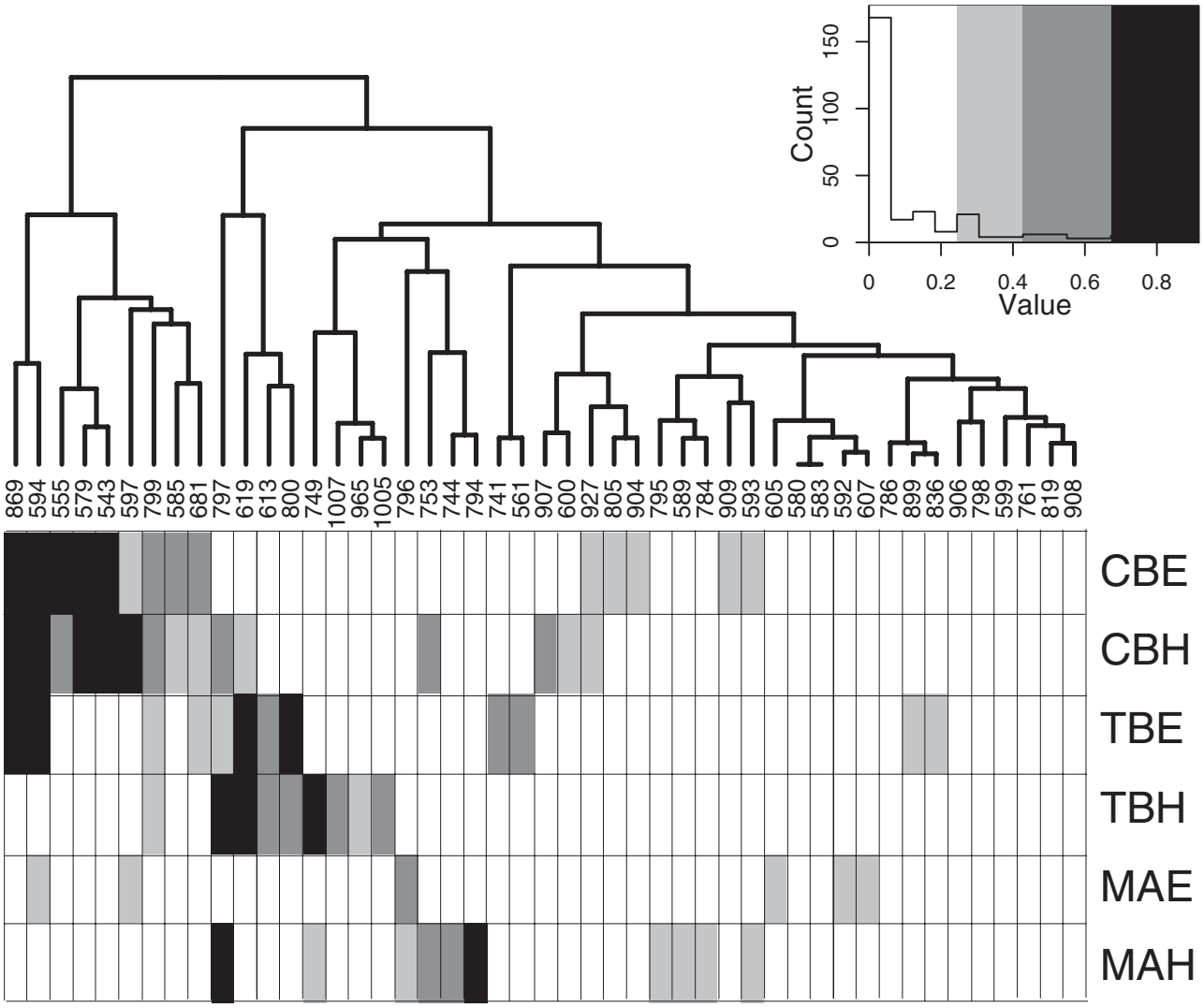

Fig. 3. Operational taxonomic unit (OTU) persistence in each lake layer through time. Each column represents a different OTU (defined by ARISA fragment size), and each row represents a lake layer. The gradient represents persistence of the OTU in that particular lake layer, as calculated by the proportion of OTU occurrences (based on presence/absence) per total observations for that lake layer. To reduce complexity in the data set, OTUs were only included if they were detected with a relative peak area of 0.025 or greater, and in at least five observations. Some OTUs were more persistent than others, and some of these were unique to a lake layer.

observations), followed by TB and CB. Polymictic CB had only one exclusive OTU. We also observed differences in richness between thermal layers within and across lakes (Fig. 2C). Mary Lake and TB were similar in richness for the epilimnion and hypolimnion, with more observed OTUs than CB. To reveal patterns in OTU persistence through time, we calculated occurrences of each OTU per number of samples collected for each lake layer (Fig. 3). Each lake contained a distinct subset of persistent OTUs. Mary Lake epilimnion and hypolimnion were least comparable in their overlap of persistent OTUs, while CB epilimnion and hypolimnion were most comparable.

Correspondence analysis (CA) and analysis of similarity (ANOSIM) indicated that epilimnion and hypolimnion BCC were distinct within stratified lakes, but not within polymictic CB (Fig. 4, Table 2). Communities in the stratified lakes were strongly separated by thermal layer along axis 1 in the CA plots. To explore whether environmental drivers were linked to variation in our bacterial communities, we correlated DO, water temperature and time to the axis scores of our CA ordinations (CANOCO CorE). As expected, mean DO concentration and water temperature were strongly correlated with axis 1 (Table 3), indicating an influence of these environmental gradients on the distinction between epilimnion and hypolimnion communities. In polymictic CB, time was most correlated with axis 1 (Table 3), indicating a stronger influence of season or community history than of DO or temperature.

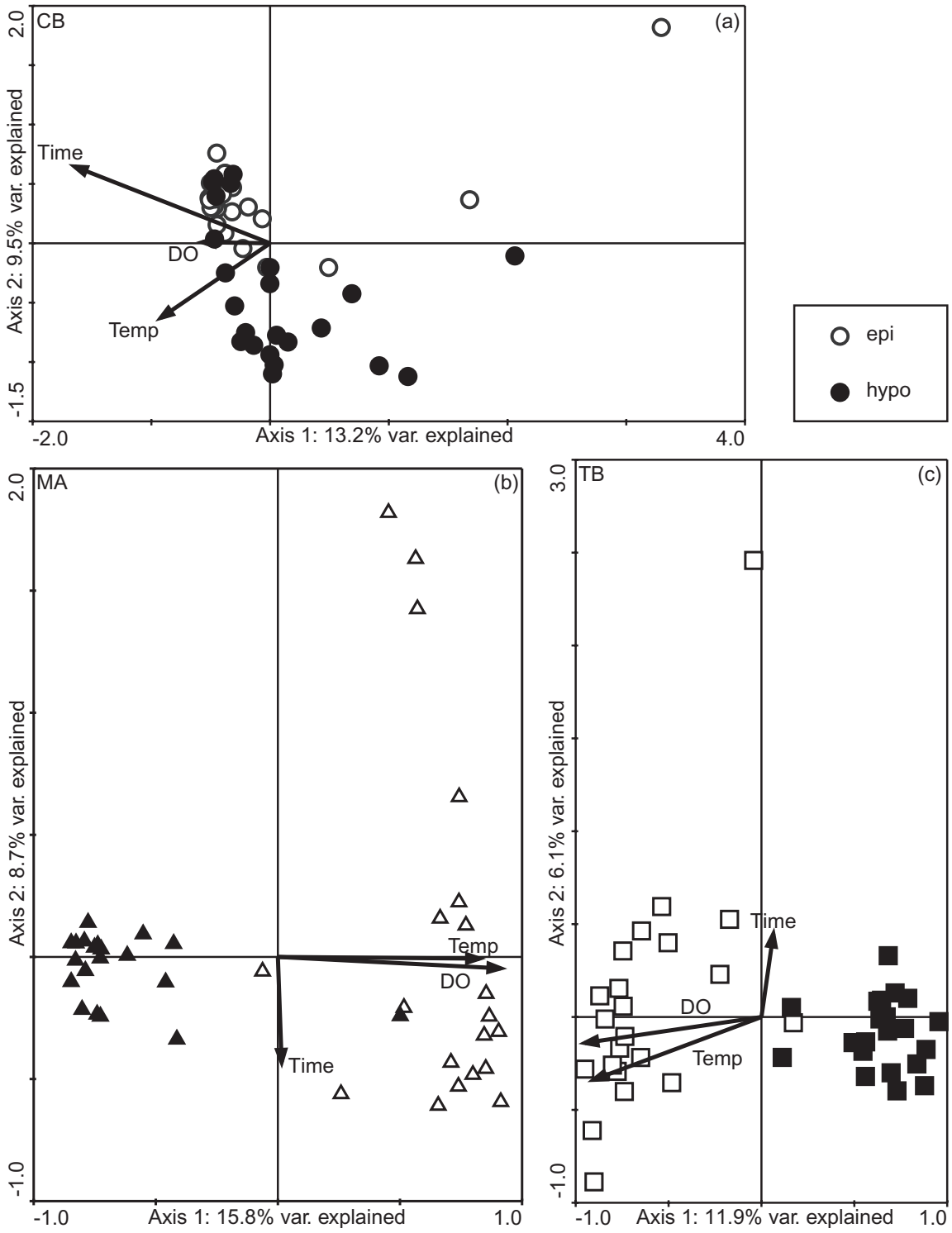

Fig. 4. Correspondence analyses (CAs) to explore differences between bacterial communities from different thermal layers within the same lake. Epilimnion communities are symbolized by open circles, and hypolimnion by filled circles.
A. Crystal Bog (CB) epilimnion and hypolimnion communities were not distinct, were highly correlated to time (CANOCO CorE $r = -0.68$ to axis 1) and followed a similar trajectory through time.
B. Mary Lake (MA) thermal layers harboured distinct bacterial communities.
C. Trout Bog (TB) thermal layers harboured distinct bacterial communities.
Both MA and TB epilimnion communities are correlated to high DO ($r = 0.81$ and -0.79 respectively) and temperature ($r = 0.73$ and -0.75 respectively) along axis 1, and to time along axis 2 ($r = -0.51$ and 0.53 respectively).

Table 2. Bacterial communities within lakes across thermal layers are different in composition and variation.

Comparison	ANOSIM	PERMDISP2
CBE, CBH	$R = 0.19$, $P < 0.002$	$P < 0.156$
MAE, MAH	$R = 0.83$, $P < 0.001$	$P < 0.005$
TBE, TBH	$R = 0.82$, $P < 0.001$	$P < 0.018$

CBE, Crystal Bog epilimnion; CBH, Crystal Bog hypolimnion; MAE, Mary Lake epilimnion; MAH, Mary Lake hypolimnion; TBE, Trout Bog epilimnion; TBH, Trout Bog hypolimnion. Analysis of similarity (ANOSIM) *R* statistic and permutated analysis of multivariate dispersion (PERMDISP2) *P*-values are reported for pair-wise within-lake thermal layer comparisons. Permutated *P*-values are given to indicate significance of the degree of distinction between groups.

Time was correlated to the second axes of the MA and TB ordinations; these axes represented the most variation in the epilimnion communities for these two lakes (Fig. 4).

To explore and compare the temporal variability of bacterial communities in each lake layer, we used the permutated distance-based test for homogeneity of multivariate dispersion (PERMDISP2). While ANOSIM tests whether there is a distinction between groups, PERMDISP2 specifically can attribute this distinction to a difference in dispersion around group centroids in multidimensional space. Significant differences between two groups of community samples would suggest that BCC was more variable within one of the groups. Pair-wise comparisons of the BCC in each lake layer resulted in significant differences in variability between stratified lake epilimnia and hypolimnia (Table 2). There were no significant differences in variability between any two epilimnia communities (data not shown), or between CB epilimnion and hypolimnion communities. Interestingly, the difference in variation between the meromictic (MA) thermal layers was the strongest among all within-lake comparisons. To corroborate the PERMDISP2 results, we used mean centroid analysis based on CA axis scores. By this analysis, the CB epilimnion and hypolimnion had comparable variation

Table 3. Correlations of DO, temperature and time with the first and second axes of individual lake correspondence analyses (CANOCO CorE values, see Fig. 3 for ordination plots).

		CB	MA	TB
DO (mg l^{-1})	Axis 1	–0.24	0.81	–0.79
	Axis 2	0.00	–0.06	–0.17
Temperature (°C)	Axis 1	–0.39	0.73	–0.75
	Axis 2	–0.31	–0.01	–0.40
Time (day number)	Axis 1	–0.68	0.01	0.05
	Axis 2	0.32	–0.51	0.53

Mean layer DO and temperature (calculated as an average across the layer for each sample date) were strongly correlated with axis 1 in the stratified lakes (MA and TB) while time was more strongly correlated with variation along axis 2 in these lakes. In polymictic CB, time was most strongly correlated with axis 1.

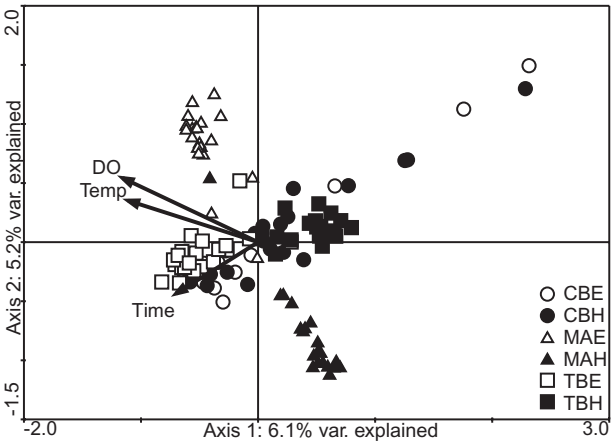

Fig. 5. Partial correspondence analysis (pCA; lake as covariable) ordination of all study lakes. Underlying variation among bacterial communities after accounting for lake-to-lake differences suggests that there are general patterns in spatial habitat heterogeneity, as all hypolimnia and epilimnia separate along axis 1. Mean dissolved oxygen (DO) and temperature per layer were highly correlated to the first axis while time was less correlated (Table 3).

(0.26 ± 0.03 and 0.31 ± 0.15 respectively), but within TB and MA, epilimnia were more variable than hypolimnia (TB: 0.28 ± 0.02 epilimnia versus 0.18 ± 0.02 hypolimnia; MA: 0.19 ± 0.02 epilimnia versus 0.14 ± 0.01 hypolimnia).

Across-lake patterns of bacterial community dynamics and habitat heterogeneity

After observing differences in richness, composition and variability of lake bacterial communities, we conducted a partial correspondence analysis (pCA) to search for more general patterns across all lakes and thermal layers. Because it is known that different lakes harbour different bacterial communities (Yannarell and Triplett, 2004) we used pCA to block for the variation explained by interlake differences by incorporating lake as a covariable. The resulting variation was what remained after accounting for interlake differences. In the pCA ordination, bacterial communities from epilimnia and hypolimnia separated along axis 1 (Fig. 5). Axis 1 scores were correlated with DO and temperature ($r = -0.55$ and -0.54 respectively). Time was also correlated with axis 1, but to a lesser extent ($r = -0.33$).

To examine temporal community dynamics, we used CA and a mean squared successive differences test (MSSD) to observe a significant trajectory of change in the epilimnion communities of MA and TB (MSSD, $P < 0.0025$, for both). This pattern was also observed in both layers of polymictic CB (MSSD, $P < 0.0025$). No pattern was detected in the hypolimnion communities of the stratified lakes in the CA ordinations, but the TB

hypolimnion demonstrated a moderately significant succession (MSSD, $P < 0.05$), while the MA hypolimnion had no significant succession.

Differences in variation across lake layers may indicate either that potentially separate drivers were acting on each lake layer, or that each community responded to the same driver differentially. Communities that change at a comparable pace through time can also be indicative of a common driver (Kent *et al*., 2007). We conducted Procrustean superimposition analysis to determine if the epilimnion successions occurred at the same pace, thus signifying the potential of a common extrinsic driver. Mary Lake and CB epilimnia, as well as MA and TB epilimnia, were concordant ($r = 0.609$ and 0.763, $P < 0.001$ respectively); TB and CB epilimnia were not concordant ($r = 0.422$, $P < 0.067$). Polymictic CB hypolimnion communities were also concordant with MA and TB epilimnia ($r = 0.842$ and 0.654, $P < 0.001$ respectively). No significant concordance was detected between epilimnion and hypolimnion within or across the two stratified lakes, MA and TB. However, CB epilimnion and hypolimnion were concordant ($r = 0.619$, $P < 0.001$).

Discussion

Lakes provide an interesting study system with which to examine the influence of habitat heterogeneity on microbial community assembly because of lake mixing and stratification dynamics. However, as with all molecular studies reliant on amplification of nucleic acids with polymerase chain reaction (PCR), our microbial community fingerprinting method, ARISA, may be biased by the primers chosen and differential amplification efficiency. Previous work has shown that ecological patterns observed using ARISA fingerprints are robust to primer set choices and PCR conditions (Jones *et al*., 2007). But, because of the inherent biases of PCR methods, we caution readers that specific differences in richness should not be interpreted for absolute values, but rather considered for cross-lake and cross-lake layer comparative purposes only.

Our study supports the traditional niche diversification hypothesis, as our most spatially homogeneous (polymictic) lake maintained the lowest observed number of OTUs, while our stratified lakes were more comparable in their richness. It is interesting to consider our results in the context of Schoener's (1974) classic perception of the importance of spatial over temporal dimensions, and to compare and contrast microbial responses with those of traditional macro-scale studies. Our epilimnion bacterial communities are variable through time (Table 2) and this variation may be higher through temporal than spatial dimensions (Yannarell and Triplett, 2004). However, if a more resolved spatial scale (e.g. cm or mm) were to be considered, it is possible that the hypolimnion bacterial communities would vary more with water column depth than through a season. Both 'macro' ecologists and microbial ecologists struggle with appropriate observation scale; temporal scale is often more difficult to observe for macro-ecologists, while spatial scale is challenging for microbial ecologists (e.g. Levin, 1992; Jessup *et al*., 2004). Furthermore, bacterial communities may not always follow the same theoretical rules as traditional macro-scale communities (Prosser *et al*., 2007). Despite these possible caveats, our results advocate using microbial communities as model systems to explore traditional habitat heterogeneity theory at novel and relevant spatial and temporal scales of response.

The observed difference in community dynamics and variation between epilimnia and hypolimnia is interesting and, given prior work, fits well into our conceptual framework of hierarchical drivers. For example, Kent and colleagues (2007) suggest that epilimnion bacterial communities are structured most immediately by interactions with phytoplankton, which in turn may be responsive to meteorological drivers. As observed previously (Kent *et al*., 2004; Newton *et al*., 2006; Shade *et al*., 2007), the epilimnion communities in our study followed a clear trajectory of change through time (Fig. 4, MSSD results). Interestingly, this same temporal succession was not observed in the hypolimnion communities, where we would expect limited phytoplankton interactions.

Furthermore, epilimnion communities were more variable through time than their hypolimnion counterparts at our temporal observation scale (PERMDISP2 results). We observed evidence for epilimnion response to seasonal drivers (Fig. 4, Table 3), but the temporal scale required to observe the hypolimnion communities' responses to lake stratification in spring was probably not captured by our sampling effort. We predict that the most extensive changes in the hypolimnion communities occur immediately following seasonal overturn while stratification becomes re-established. It is reasonable to expect that hypolimnion communities are more strongly driven by vertical gradients in electron acceptor availability, while epilimnion communities are more strongly driven by biotic intra-layer competition and/or extrinsic weather-related drivers. Future work will explore the possibility of hypolimnion community succession during and after seasonal lake mixing.

As previously observed (Newton *et al*., 2006), certain OTUs persisted in each lake layer through time, but the majority of observed OTUs were present only on a few sample dates (Fig. 3). This type of analysis does not capture changes in the relative abundance of OTUs over time. For example, it is possible that some OTUs were in high abundance in early spring, and then low abundance by autumn, but persisted throughout the sampling period.

One of the OTUs that most consistently occurred in the CB and TB epilimnia had an ARISA fragment length of approximately 594 bp. This length is highly conserved in *Actinobacteria*, which are common in freshwater systems and speculated to be ubiquitous (Newton *et al*., 2007). Interestingly, a fragment of length 797 bp was observed consistently in the hypolimnia of TB and MA. Relatives of the cosmopolitan *Polynucleobacter* clade share this ribosomal intergenic spacer size (Newton *et al*., 2006), implying that *Polynucleobacter*-like populations normally observed in the upper mixed layer of humic lakes (Hahn *et al*., 2005) may have been persisting in these lake layers even during periods of hypoxia. Further study using 16S rRNA gene sequencing is needed to confirm these speculations and to address the contribution of individual population dynamics to overall successional trends in the community.

Because of PCR biases and the constraints associated with using relative abundance data for analyses, it is difficult to calculate the more common metrics of diversity using ARISA BCC data. However, it has been suggested that multivariate dispersion tests, such as PERMDISP2, can be used to measure beta diversity of a community (Anderson *et al*., 2006). Beta diversity, which is the difference or change in community composition between sampling units, is perhaps the most appropriate type of diversity to discuss in the context of our study. For example, our results show that in stratified lakes, epilimnion bacterial communities are more variable in their composition than those of hypolimnion communities, which implies that epilimnion communities have higher beta diversity. In community assembly theory, high beta diversity is suggestive of multiple stable-state community equilibria within a system (Chase, 2003). This is in contrast to theory suggesting that environmental filters select for a single unique assemblage among a ubiquitous dispersal pool. High beta diversity is thought to result from relatively large regional species pools, small connectivity between community patches (thus inhibiting interpatch dispersal), high productivity and low disturbance frequency (see (Chase, 2003) for a review).

Beta diversity in the context of community assembly could be interesting to explore in epilimnia and hypolimnia. In lake epilimnia, bacterial communities may experience relatively large immigrant species pools because of weather events (precipitation or wind) and terrestrial connectivity (i.e. run-off). However, epilimnion communities may arguably experience a higher frequency of disturbance because of the transient physical response of the epilimnion to weather-related variables. All of this suggests that multiple stable states may exist for epilimnion bacterial communities. In contrast, hypolimnion communities are buffered from external drivers and may also experience a relatively smaller rate of immigration. Also, hypolimnia have relatively low productivity, and relatively low disturbance frequency in stratified lakes. If hypolimnion bacterial community assembly is driven more strongly by vertical gradients in temperature and DO, this suggests a stringent environmental filter that may constrain the communities to a single stable equilibrium. Few studies have explored patch dynamics among free-living aquatic bacterial communities (e.g. Blackburn *et al*., 1998), likely because of the difficulty in defining patch boundaries, and so little can be stated concerning the role of patch connectivity in determining beta diversity in aquatic BCC. Future work should be directed in this area to unravel community assembly rules for each thermal layer and across lake systems.

In conclusion, our results show differences in bacterial community richness, composition and variation between lake thermal layers of varying environmental gradients, and generally to spatial habitat heterogeneity across lakes. From our results, vertical water column habitat heterogeneity may contribute to bacterial community dynamics by mediating the influence of more immediate BCC drivers (DO, temperature, electron acceptors, nutrient availability). We observed a succession through the open-water phase in the epilimnion bacterial communities, but not in the hypolimnion communities. This suggests that epilimnion bacterial community response to temporal heterogeneity is observed on a daily to weekly time scale, whereas response of the hypolimnion community may be more directly observed during re-stratification of lakes directly after mixing, which occurs seasonally. The response of the aquatic bacterial communities to spatial and temporal habitat heterogeneity observed here suggests that both are interacting drivers in the hierarchical network influencing bacterial community assembly and dynamics.

Experimental procedures

Sample collection

The BCC of three small northern Wisconsin lakes, CB (polymictic, maximum depth 2.5 m), TB (dimictic, maximum depth 7 m) and MA (meromictic, maximum depth 21.5 m), were sampled weekly during the open-water period of 2005 (late May to early November). These lakes were chosen because of their shared dystrophy, acidity, similar surface area (range 0.6–1.2 ha), comparable physical/chemical characteristics, proximity to one another and range of annual of mixing frequencies. Samples were collected over the deepest point of each lake. All lakes were sampled on the same day each week.

Temperature and DO measurements were observed for every sample date using YSI model 58 dissolved oxygen meter (Yellow Springs, OH) calibrated at each sample date as per manufacturer's instruction. Trout Bog and MA profiles were observed at every metre and CB profiles were observed at every half metre. Integrated water column samples were

collected for each thermal layer. Thermal layer boundaries were determined based on temperature profiles. The top of the hypolimnion was determined to be where the temperature decreased by 1.5–2.0°C over 0.5 m or less. In polymictic CB, the epilimnion was consistently sampled at 0.0–1.0 m, and the hypolimnion at 1.0–2.0 m; these depths were based on thermal profiles of CB's weak stratification (rate of change of 0.5°C m^{-1} or greater), as observed in instrumented buoy temperature profiles available from the Global Lakes Ecological Observatory Network database (GLEON, http://www.gleon.org). According to buoy data temperature profiles, CB mixed a minimum of nine times over the sampling season in 2005.

Bacterial cells were immediately recovered from 100 to 250 ml of lake sample water by filtration onto a 0.2 μm polyestersulfone filter (Pall, New York, NY), and stored at −80°C until further processing.

Mean difference in DO and temperature for each layer was calculated by averaging the profile observations included in the layer of interest for each sample date, and then subtracting the average of the hypolimnion profile from the epilimnion. These differences were then averaged over the entire sampling period when a global mean was desired.

Additional physical and chemical data for TB and CB were provided by the North Temperate Lakes Long-term Ecological Research (NTL-LTER) and the Center for Limnology at University of Wisconsin-Madison.

Sample processing and bacterial community fingerprinting

Total DNA was extracted from replicate filters as previously described using the Bio 101 FastDNA kit (QBiogene, Carlsbad, CA), as per manufacturers' instructions with published minor modifications (Yannarell *et al*., 2003; Yannarell and Triplett, 2005). The intergenic spacer region between the 16S and 23S rRNA genes was amplified from the total extracted DNA using 6-FAM-labelled universal 1406F primer (5′-TGYACACACCGCCCGT-3′) and bacterial specific primer 23Sr (5′-GGGTTBCCCCATTCRG-3′) (Fisher and Triplett, 1999; Yannarell *et al*., 2003). Polymerase chain reaction conditions were as follows: 2 min denaturation at 94°C, 30 cycles of 35 s denaturation at 94°C, 45 s annealing at 55°C, 2 min elongation at 72°, and a final extension for 2 min at 72°C. Polymerase chain reaction was conducted on an Mastercycler gradient thermocyler (Eppendorf, New York, New York), and 5 ng of extracted DNA was used as template.

ARISA was used to examine bacterial community fingerprints (Fisher and Triplett, 1999) with published minor modifications (Yannarell *et al*., 2003; Yannarell and Triplett, 2005). Denaturing capillary electrophoresis was conducted on the amplified DNA on a SpectruMedix RVL 9612 (State College, Pennsylvania). Profiles were analysed and aligned using GenoSpectrum V 2.2.1 (State College, Pennsylvania). ARISA fragments were grouped into OTUs based on profile alignments. Normalized peak area was used as a proxy for relative abundance of each OTU in a profile (Sestanovic *et al*., 2004; Yannarell and Triplett, 2005). Individual relative OTU peak areas from replicate samples were averaged prior to statistical analysis.

Statistical analyses

Operational taxonomic unit persistence through time was represented as the proportion of samples in which a particular OTU was present within a lake layer. The 'heatmap.2' function in the R software for statistical computing (Iacus and Urbanek, 2005) was used to visualize this proportion as a gradient grid. To reduce complexity in the data set, we only included OTUs in this analysis if they were detected with a relative peak area of 0.025 or greater, and in at least five observations (i.e. in more than ~3% of samples).

Correspondence analysis was conducted to search for patterns in BCC by lake and layer using the CANOCO for Windows software package 4.5.1 (ter Braak and Smilauer, 2002). Relativized abundance matrices were used for these analyses, and biplot scaling was used to calculate intersample distances. Partial correspondence analysis (with a covariable included) was also conducted. CorE values were reported for correlation of environmental variables to axis scores.

To test for significant differences between sets of communities, ANOSIM (Clarke and Gorley, 2001) between classified communities was conducted using Bray-Curtis coefficient similarity matrices created from pair-wise comparisons of normalized bacterial community fingerprints (Legendre and Legendre, 1998). ANOSIM generates an *R* test statistic to indicate the degree of separation between groups, where an *R*-value of 1 indicates complete separation and 0 indicates no separation.

A PERMDISP2 test (Anderson, 2006) was used to compare the temporal variability of communities collected from different thermal layers. PERMDISP2 is a multivariate analogue to Levene's test (Levene, 1960), which is essentially an analysis of variance on the absolute values of deviations of observations from their group mean. PERMDISP2 specifically tests for differences in the dispersion around a mean centroid in multivariate space. It uses any pair-wise distance matrix to conduct principle coordinates analysis and measure spread around the mean centroid of groups of samples (i.e. communities), and tests the null hypothesis that there is no difference in dispersion between groups. Bray-Curtis dissimilarities calculated from relativized ARISA profiles were used for this analysis, and *P*-values reported are based on least-squares residual permutations around centroids.

Mean centroid distance based on CA is a measure of variation in a cluster of points around the centre of a cloud; lower mean centroid distance is representative of less dispersion within a classification cluster (Legendre and Legendre, 1998). Both epilimnion and hypolimnion layers were used to generate individual lake CAs, and sample scores from the first and second axes of these CAs (Fig. 4) were used. All confidence intervals were computed as standard error.

Mean square successive difference (von Neumann *et al*., 1941; Zar, 1998) was used to test for a successional pattern in the bacterial communities through time. The null hypothesis in this test states that consecutive measurements have random variability, and the alternative hypothesis is that consecutive measurements are serially correlated. Axis 1 scores from individual lake layer CAs were used in this test.

Procrustean superimposition analysis is a symmetric permutation-based analysis, which we used to calculate an *r*

correlation statistic of community concordance between and among lakes (Jackson, 1995; Peres-Neto and Jackson 2001). The R software for statistical computing (Iacus and Urbanek, 2005), and the PROTEST function in the vegan package (Oksanen *et al*., 2006) were used to conduct this analysis. Axis 1 and 2 scores from individual lake layer CAs were used to determine whether groups (i.e. CB epilimnion and MA epilimnion) were moving at the same pace. The resulting *r* correlation ranges from 0 to 1, where groups perfectly concordant in their dynamics receive a score of 1. One thousand permutations were used for each pair-wise analysis.

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