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Resistance, resilience and recovery: aquatic bacterial dynamics after water column disturbance

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Summary

For lake microbes, water column mixing acts as a disturbance because it homogenizes thermal and chemical gradients known to define the distributions of microbial taxa. Our first objective was to isolate hypothesized drivers of lake bacterial response to water column mixing. To accomplish this, we designed an enclosure experiment with three treatments to independently test key biogeochemical changes induced by mixing: oxygen addition to the hypolimnion, nutrient addition to the epilimnion, and full water column mixing. We used molecular fingerprinting to observe bacterial community dynamics in the treatment and control enclosures, and in ambient lake water. We found that oxygen and nutrient amendments simulated the physical-chemical water column environment following mixing and resulted in similar bacterial communities to the mixing treatment, affirming that these were important drivers of community change. These results demonstrate that specific environmental changes can replicate broad disturbance effects on microbial communities. Our second objective was to characterize bacterial community stability by quantifying community resistance, recovery and resilience to an episodic disturbance. The communities in the nutrient and oxygen amendments changed quickly (had low resistance), but generally matched the control composition by the 10th day after treatment, exhibiting resilience. These results imply that aquatic bacterial assemblages are generally stable in the face of disturbance.

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Introduction

Lake mixing and the associated environmental conditions of a mixed water column are important disturbances for freshwater plankton communities. Both experimental and observational evidence support this (Hambright and Zohary, 2000; Weithoff *et al*., 2000; Diehl *et al*., 2002; Huisman *et al*., 2004; Pannard *et al*., 2007; 2008). Lake mixing studies have focused primarily on phytoplankton and zooplankton responses rather than bacterioplankton responses. Because bacterioplankton play an important role in lake metabolism (i.e. respiration and production) by mediating carbon and nutrient transformations, bacterioplankton response to mixing is important for understanding emergent properties of the lake 'microbial loop,' including seasonal dynamics and food–web interactions.

Many northern temperate lakes mix twice annually, once in the spring after ice-off, and once in the autumn before freezing. The hypolimnion environment is generally hypoxic to anoxic because of microbial respiration, high in nutrients because of debris from the epilimnion falling downward and decomposing, and low in temperature. In contrast, the epilimnion environment is generally high in dissolved oxygen (DO) because of photosynthesis and atmospheric exchange, limited in nutrients because of uptake by heterotrophs and phototrophs, and high in temperature because of proximity to sunlight. When lakes mix, these gradients are disrupted, leading to the redistribution of oxygen from the epilimnion and nutrients from the hypolimnion throughout the vertical water column. Central to the current model of zooplankton and phytoplankton community assembly, nutrient upwelling from seasonal spring mixing initiates summer plankton succession by inducing a spring bloom dominated by phototrophs, followed by a clear-water phase dominated by grazers (Wetzel, 2001). This suggests that spring mixing initiates ice-off succession for plankton communities, and has implications for their seasonality.

Mixing is also an important structuring event for freshwater bacterial communities. Similar to its role in initiating spring plankton trajectories, mixing has been linked to starting seasonal trajectories for bacterial assemblages (Shade *et al*., 2007; Nelson, 2009). Also, water column stability metrics, which are derived from temperature measurements and serve as indicators of lake mixing status, have shown to be good predictors of bacterial community composition (BCC) (Nelson, 2009; Shade *et al*., 2010a). Furthermore, bacterioplankton response to mixing may be linked to various lake characteristics, such as annual mixing regimes, trophic status and thermal layer (epilimnion or hypolimnion) (e.g. Hollibaugh *et al*., 2001; Lehours *et al*., 2005; Shade *et al*., 2008). All of this suggests that mixing plays an important role in lake bacterial dynamics.

Previous work showed that epilimnion and hypolimnion bacterial communities differ in how they change after mixing, with respect to both pace and direction (Shade *et al*., 2008; 2010a,b). One reason for this is that the initial community composition in each layer is distinct (Jones *et al*., 2008; Shade *et al*., 2008). However, there are other factors besides the initial composition that likely influence post-mixing dynamics. For example, after lake mixing, the post-mixing community may be structured by competition among new 'neighbours' in the mixed condition, as well as environmental filtering. Therefore, organisms capable of surviving in both the epilimnion and hypolimnion environments ('generalists') may be key to community re-assembly following mixing (Shade *et al*., 2010b).

Bacterial community response to lake mixing is additionally relevant for understanding microbial ecology of disturbance and stability. Stability is the general capacity to return to equilibrium after perturbation, and includes components of resistance, recovery and resilience (Pimm, 1984). Resistance is a community's ability to remain unchanged when challenged with disturbance. Recovery is a community's ability to return to its pre-disturbance composition or function, and resilience is the rate at which this return occurs. Lake mixing provides an opportunity to understand stability of naturally occurring microbial communities after a typical disturbance.

In aquatic systems, a few natural experiments have provided evidence of microbial community stability. Low resistance and rapid resilience were observed in two separate studies of aquatic microbial communities challenged with sudden storm-mediated disturbance. The first study focused on composition, where multiple pulse typhoon disturbances in a small lake in Taiwan 're-set' the bacterial community in the epilimnion and hypolimnion of the lake, initiating a repeatable community trajectory (Jones *et al*., 2008). The second study focused on function, where a hurricane disturbance to a cyanobacterial mat resulted in maintenance of normal nitrogen fixation levels despite a shift in community composition, as measured by clone-library analyses (Yannarell *et al*., 2007). These observed responses to natural disturbances suggested that aquatic microbial communities are predictable in their response to mixing, and may serve as useful models for microbial disturbance ecology.

In the current study, our first objective was to separate the hypothesized environmental drivers of bacterial communities during mixing. We hypothesized that DO drives hypolimnion bacterial dynamics after mixing, while nutrient upwelling drives epilimnion dynamics. Although many other environmental changes occur during lake overturn that could influence bacterial dynamics (such as temperature, alternative electron acceptor and donor availabilities, and the increased exposure of hypolimnion bacteria to light), we chose to manipulate nutrients and DO because previous studies underscored their general importance for aquatic communities (e.g. Lehours *et al*., 2005; 2009; Fenchel and Finlay, 2008; Jones *et al*., 2008). We hypothesized that nutrients up-welled from the hypolimnion promote blooms of quick-growing microbes, thereby inducing change in the epilimnion community. We further hypothesized that oxygen enables success of microaerophiles or facultative anaerobes, thereby inducing hypolimnion community changes.

Our second objective was to characterize aquatic bacterial community stability by quantifying resistance, recovery and resilience to an episodic disturbance. We hypothesized that epilimnion and hypolimnion communities are sensitive to disturbance but have different rates and extents of recovery. Though both oxygen and nutrients disrupt the aquatic environment, nutrients are not toxic to epilimnion bacteria in the way that oxygen is toxic to some hypolimnion bacteria. Therefore, we expected hypolimnion resilience would be synchronous with the depletion of oxygen, but that epilimnion communities may not recover as directly because of the added complexity of competition and absence of a strong environmental filter.

To accomplish these objectives, we designed an *in situ* experiment with 4 treatments $\times$ 3 replicates $\times$ 7 time points. We enclosed a portion of the water column from the sediment to the surface, and for each treatment perturbed one key biogeochemical effect of mixing; oxygen in the hypolimnion and nutrients (nitrogen and phosphorus) in the epilimnion. We included a physically mixed treatment and an unmixed control. We used automated ribosomal intergenic spacer analysis (ARISA) to profile the bacterial communities and observe changes through time and with respect to treatment.

Results

Our experiment included three treatments, an untreated Control and ambient lake water observations. The 'Mix'

Table 1. Treatments and hypothesized response groups for the experiment.

Treatment name (description)	Stratum (abbreviation)	Hypothesized response group
Oxygen (*Hypolimnion aeration*)	Epilimnion (OE)	Stratified epilimnion
	Hypolimnion (OH)	Overturn hypolimnion
Nutrient (*Epilimnion nutrient addition*)	Epilimnion (NE)	Overturn epilimnion
	Hypolimnion (NH)	Stratified hypolimnion
Mix (*Water column mixing*)	Epilimnion (ME)	Overturn epilimnion
	Hypolimnion (MH)	Overturn hypolimnion
Control (*No treatment*)	Epilimnion (CE)	Stratified epilimnion
	Hypolimnion (CH)	Stratified hypolimnion
Ambient	Epilimnion (AE)	Stratified epilimnion
	Hypolimnion (AH)	Stratified hypolimnion

Four response groups were expected: epilimnion and hypolimnion that resembled the ambient stratified lake ('Stratified'), and epilimnion and hypolimnion that resembled those strata in a lake that was mixing ('Overturn'). It was hypothesized that 'Overturn' chemical and biological responses would be observed in the Oxygen hypolimnion, Nutrient epilimnion and both layers of the Mix treatment. All else was expected to resemble stratified conditions.

treatment was imposed by physically mixing the water column. The 'Oxygen' treatment was an oxygen amendment to the hypolimnion using bubble diffusers that injected compressed air without disrupting thermal stratification. The 'Nutrient' treatment was an addition of nitrogen (N) and phosphorus (P) to the epilimnion to reach the target concentration of a volumetrically mixed water column given contemporary lake conditions. Each treatment was executed in three replicate enclosures (limnocorrals). Day 0 refers to the day of treatment, and each day number was a 'recovery' day post treatment. Our experimental units were unique thermal layer and treatment combinations (for example, 'Mix Epilimnion'). Finally, for analyses, we classified our experimental units into hypothesized response groups to designate which treatments should resemble a stratified lake versus a lake during overturn. Table 1 depicts the experimental design and Fig. S1 shows a schematic of a limnocorral.

Success of the manipulation in mimicking mixed or stratified conditions

Controls resembled ambient conditions. Generally, there were high correlations between the Control and ambient environmental conditions, suggesting a limited 'bag effect' in our experiment. Most measured water chemistry variables were highly correlated ($0.76 < r < 1.00$, all $P < 0.05$) between ambient and control. Exceptions were dissolved organic carbon (DOC, $r = 0.69$, $P = 0.03$) and dissolved inorganic carbon (DIC, $P = 0.36$). This is likely due to sediment resuspension from mesocosm deployment that had settled by day 2 (Fig. S2). Analysis of similarity (ANOSIM) of the suite of environmental measurements showed little difference in the environmental conditions of the control versus the ambient epilimnion or hypolimnion (ANOSIM control versus ambient epilimnion $R = 0.28$, $P = 0.07$; hypolimnion $R = 0.15$, $P = 0.17$).

Treatments simulated mixed environmental conditions. When compared with controls, the Nutrient and Oxygen treatments intended to resemble mixed conditions generally mimicked the Mix treatment. The Mix treatment disrupted thermal stratification within the limnocorrals (Fig. 1A), increased the DO in the hypolimnion (Fig. 1E), and increased total N and P levels in the epilimnion (Fig. 2A and E). The Oxygen treatment maintained thermal stratification (Fig. 1C) and low nutrient concentrations (Fig. 2C and G), but increased DO in the hypolimnion (Fig. 1G). The Nutrient treatment maintained thermal stratification (Fig. 1B) and low DO in the hypolimnion (Fig. 1F), and had elevated epilimnion nutrient concentrations (Fig. 2A and E).

The total P and N concentrations in Nutrient and Mix treatments over the time-course were not significantly different by a *t*-test ($P = 0.67$ and 0.35), while both were different from the Oxygen treatment (N and P *t*-tests all $P < 0.005$). Across treatments, diel surface (0.5 m depth) temperature fluctuations are apparent (Fig. 1A, C and D).

Epilimnion and hypolimnion environmental parameters were distinct (ANOSIM $R = 0.64$, $P < 0.001$, see also principle components analysis, Fig. S2). ANOSIM was used to test for differences in the chemistry of hypothesized response groups (Table 2, upper diagonal). The Control and Oxygen epilimnia, expected to resemble a stratified epilimnion, were distinct from those expected to resemble epilimnion during overturn (the Mix and Nutrient epilimnia, ANOSIM $R = 0.29$, $P < 0.001$). The same distinctions were observed between 'stratified' and 'overturn' hypolimnion (ANOSIM $R = 0.16$, $P < 0.001$).

Temporal changes in environmental conditions. To determine on what day of the experiment the carbon and nutrient concentrations in each treatment returned to match that of the control, we performed pairwise *t*-tests for each

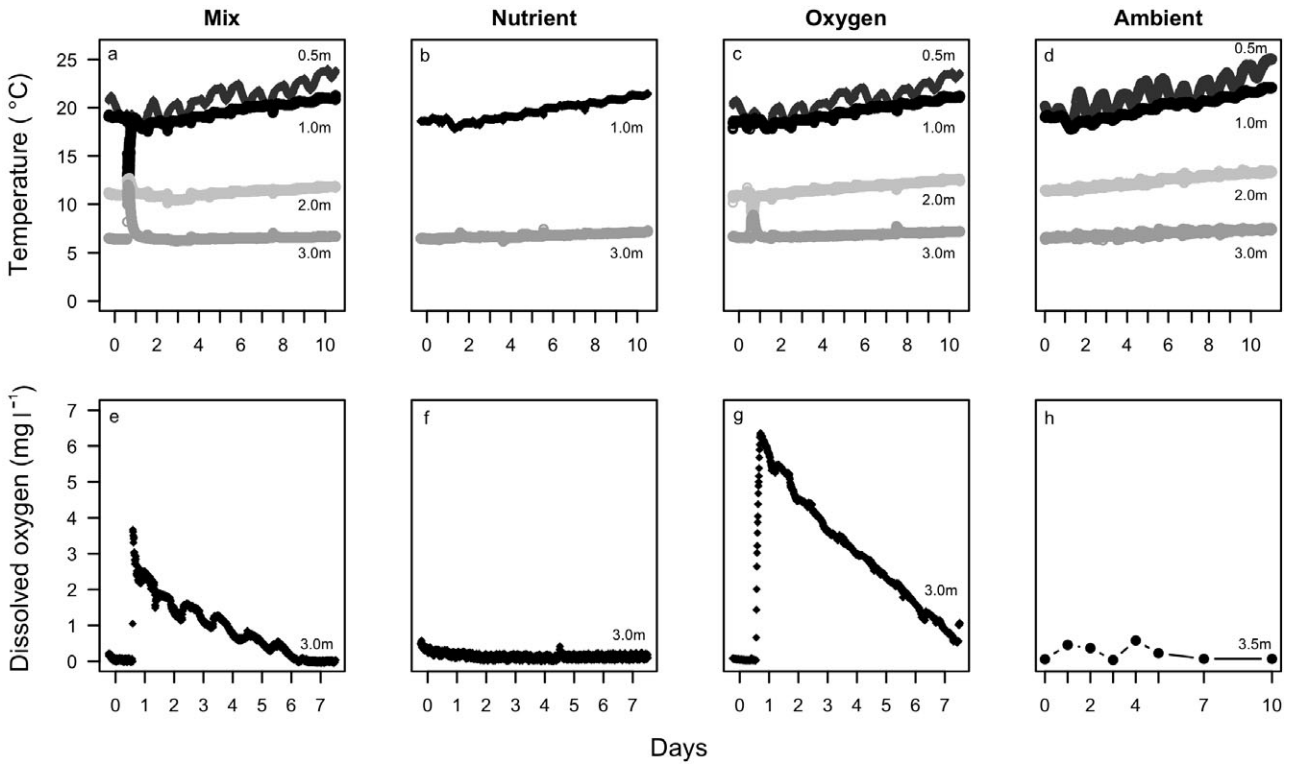

Fig. 1. Water column temperature and oxygen concentrations. Each column is a different treatment (labelled at the top).
A–C. Thermal profiles as measured by thermistors that logged temperature every three minutes (at 0.5, 1, 2 and 3 m depth below the surface) in the Mix, Oxygen and Ambient treatments, and at 1 and 3 m in the Nutrient treatment. Day 0 is the day of treatment, 16 June 2008.
D. The ambient water temperature at the same depths, as observed at 10 min intervals by an instrumented buoy.
E–G. Dissolved oxygen (DO) measured by sondes deployed at 3 m from the lake surface to observe hypolimnion oxygen dynamics, logged every 10 min.
H. DO measured using a hand-held probe in the ambient lake at 3.5 m depth.

day. For the Mix treatment in the epilimnion, dissolved N and DIC matched the control by day 2, total P returned by day 4, and total N and dissolved P returned by day 7. For the Nutrient treatment in the epilimnion, the impact of treatment on carbon and nutrients was slightly more sustained: DIC and dissolved P matched the control by day 4, and total and dissolved N returned by day 7, and total P returned by day 10 (all $P > 0.05$, Fig. 2, Fig. S3). DOC was not different from the control in the epilimnion in any treatment. In the hypolimnia, no measurements of nutrients or carbon were significantly different from the control.

Objective 1: Isolating hypothesized drivers of bacterial communities after lake mixing

Nutrient epilimnion and Oxygen hypolimnion bacterial communities were similar to Mix. We compared BCC across treatments, and hypothesized response groups (see Table 1) using non-metric multidimensional scaling analysis (Fig. 3A) and ANOSIM (Table 2, lower diagonal, Fig. 4). As expected, there was a strong BCC distinction between epilimnion and hypolimnion across all treatments (ANOSIM $R = 0.78$, $P < 0.001$), as shown in other work (e.g. Shade *et al.*, 2008). Those communities hypothesized to be more compositionally different, such as the Stratified epilimnion and hypolimnion, were different; those expected to less different, such as the Overturn epilimnion and hypolimnion, were less different (Table 2, lower diagonal). However, communities observed immediately after Nutrient epilimnion, Oxygen hypolimnion and Mix treatment clustered together in the NMDS, outside of their epilimnion or hypolimnion groups (circle labelled 'Mix' in Fig. 3A). The higher similarity between Mix communities relative to the other groups was supported by the treatment ANOSIMs (Fig. 4). The ANOSIMs also showed the Oxygen treatment in the hypolimnion (OH:CH) resulted in a greater change than the Nutrient treatment in the epilimnion (NE:CE), relative to the Mix treatment in each (ME:CE and MH:CH). Therefore, the Oxygen treatment was the most disruptive in the hypolimnion, while the Mix treatment was the most disruptive in the epilimnion. Finally, Mix and Nutrient epilimnion were not as similar to each other as Mix and Oxygen hypolimnion, suggesting

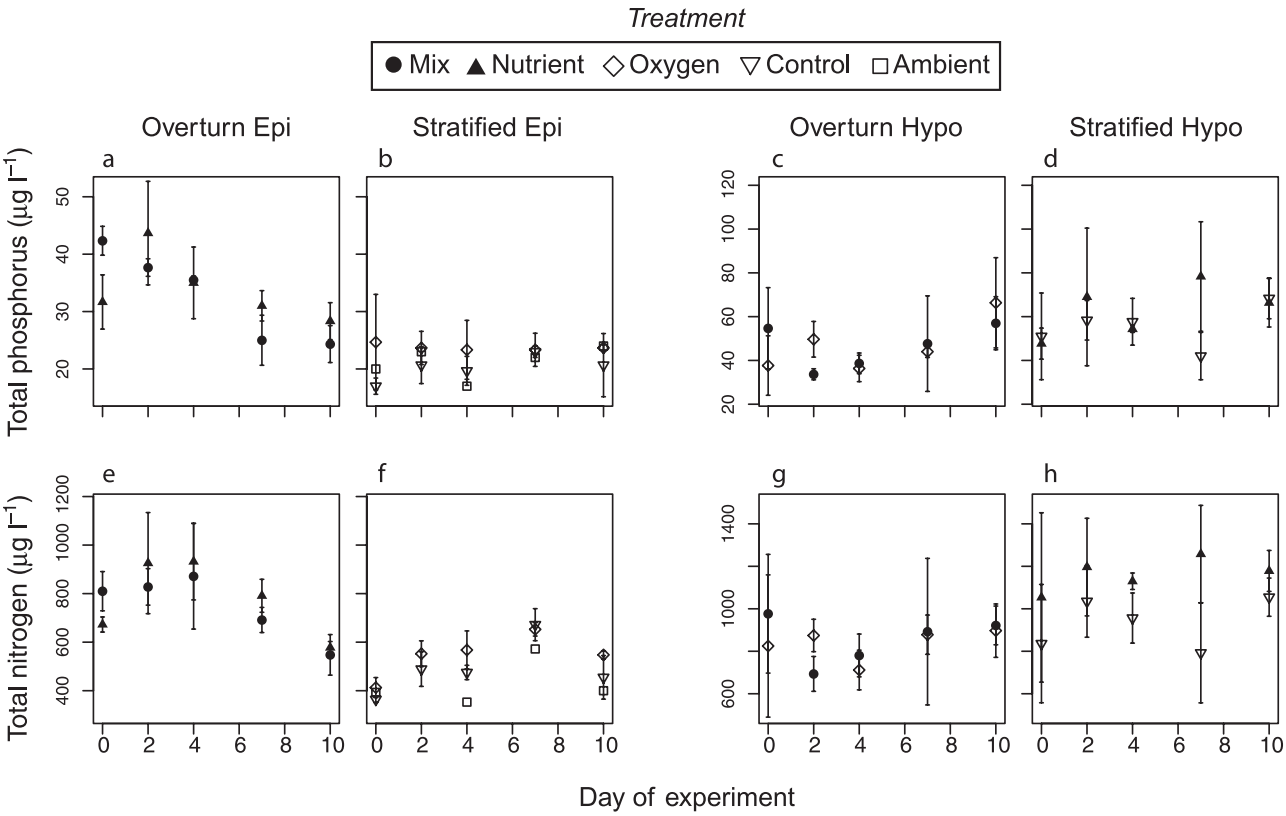

Fig. 2. Mean nitrogen and phosphorus concentrations for each treatment. A-d are phosphorus concentrations; e-h are nitrogen. Each column is a hypothesized response group (Table 1, Overturn or Stratified). The two left columns are epilimnion, and the two right columns are hypolimnion. Error bars are standard deviation. Error bars are not given for ambient because only one observation was collected per time point. Note differences in *y*-axis scale for hypolimnion and epilimnion observations.

again that nutrient treatment did not replicate bacterial response to mixing as well as oxygen.

To better observe the temporal changes in each treatment, the epilimnion and hypolimnion communities were analysed separately using NMDS. The Mix epilimnion experienced the largest change between day 0 and 1, and changed the most relative to the other treatments (Fig. 3B). The hypolimnion Mix and Oxygen treatments both experienced large changes over the time-course (Fig. 3C). However, an analysis of each treatment with its control revealed that all generally resembled the control by day 10 (Fig. S4). Finally, a Mantel test between the environmental measurements and the BCC associated with each observation revealed a moderate but significant correlation ($r = 0.38$, $P < 0.001$), suggesting that the environmental conditions contributed in part to community recovery.

Table 2. Analysis of similarity (ANOSIM) between hypothesized response groups (see Table 1).

	Stratified E	Stratified H	Overturn E	Overturn H
Stratified E		0.85	0.29	0.70
Stratified H	1.00		0.69	0.16
Overturn E	0.13	0.84		0.50
Overturn H	0.79	0.34	0.43	

A higher *R*-value indicates a stronger compositional distinction between groups. All $P < 0.001$. 'E' is epilimnion, 'H' is hypolimnion. The lower half of the diagonal is ANOSIM *R* for differences in bacterial community compositions based on Bray–Curtis similarity, and the upper half is differences in environmental conditions based on Euclidean distance.

Bacterial OTUs associated with treatments. To determine if population-level changes played a role in the emergent community response, we next asked which bacterial operational taxonomic units (OTUs; defined by ARISA amplicon length) were associated with particular treatments. We created a heatmap and performed a cluster analysis of the relative abundances of OTUs across the time series (Fig. 5). We classified observations by their hypothesized response group (Overturn or Stratified) following treatment. Not surprisingly, most of the observed OTUs were rare (observed in low relative abundance or infrequently in the dataset). Among the more abundant OTUs, the absence of particular OTUs (with 'absence' implying that an OTU was below our detection limit) defined overall BCC differences between hypothesized

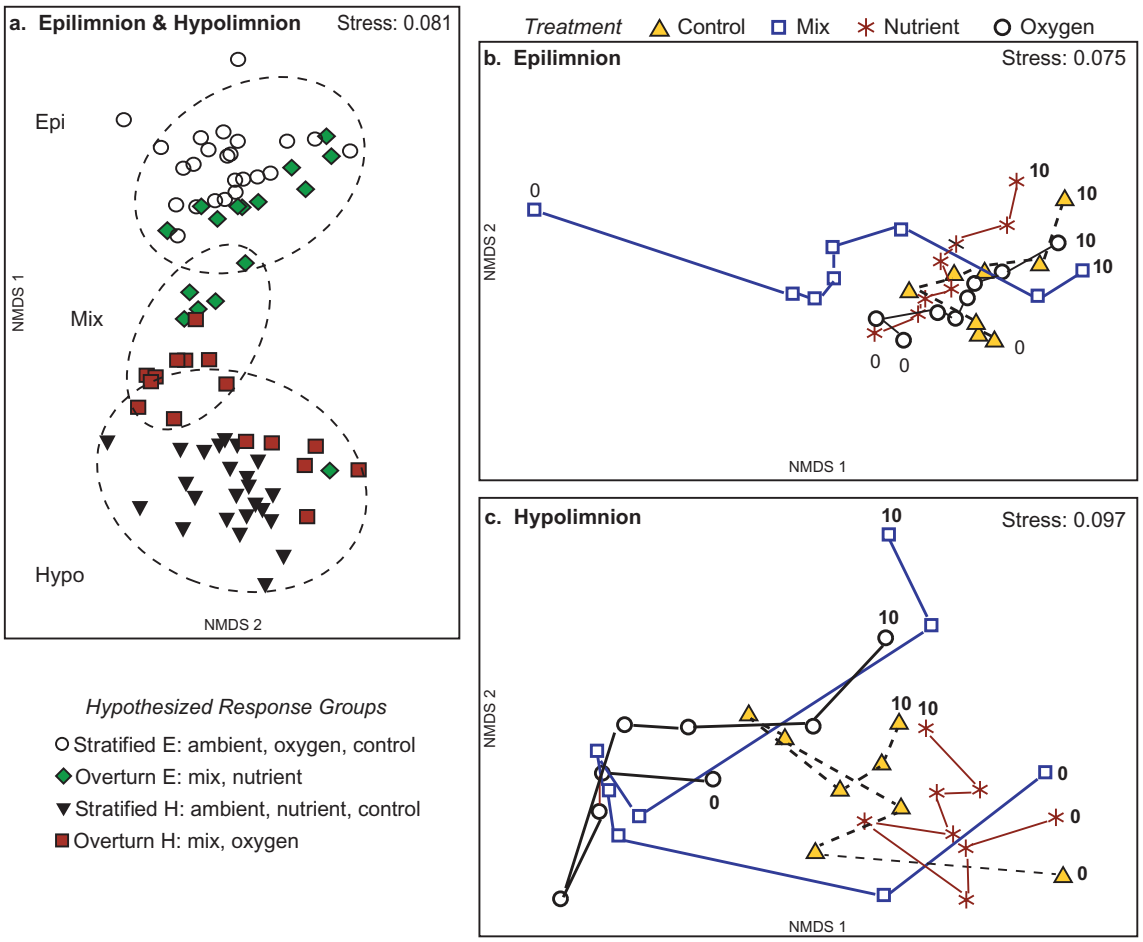

Fig. 3. Non-metric multidimensional scaling analysis of bacterial communities based on Bray–Curtis similarities and averaged across replicates.
A. NMDS of epilimnion and hypolimnion communities and classified by hypothesized response groups. The circles indicate 70% Bray–Curtis similarity shared among communities within the area of the circle, which corresponds to epilimnion, mix and hypolimnion samples.
B. NMDS of epilimnion communities classified by treatment. Connecting lines are trajectories within each treatment through days 0–5, 7 and 10. Communities observed on days 0 and 10 are labelled.
C. NMDS of hypolimnion communities classified by treatment. Connecting lines are trajectories. See Fig. S4 for a breakdown of each treatment trajectory as compared with the control.

response groups. For example, many of the Stratified epilimnion observations generally lacked OTUs from cluster A. Because these OTUs were present in Overturn epilimnion and most of the hypolimnion observations, they may be hypolimnion-associated taxa, or taxa capable of growth in environments where nutrients are not limiting. Similarly, Stratified hypolimnion observations generally lacked many of the OTUs in the cluster B of Fig. 5, while most of these were detected in the Overturn hypolimnion observations (with the exceptions of OTUs 583 and 797). The OTUs in this cluster may be epilimnion-associated OTUs or OTUs capable of aerobic growth in a mixed hypolimnion. Finally, the cluster analysis reveals candidate OTUs that may be capable of serving as post-mixing pioneers in community assembly, such as OTUs 596, 559 and 598 (cluster C in Fig. 5). These OTUs defined a cluster of treatments that simulated overturn epilimnion and hypolimnion conditions, but also occurred in the stratified conditions of each layer.

Objective 2: Characterizing components of bacterial community stability after disturbance

Resistance and resilience of bacterial communities. To measure the resistance of the bacterial communities to each treatment, we compared average daily Bray–Curtis similarity between each treatment and its Control (Table 3). We also compared the minimum daily Bray–Curtis similarity between each treatment and its Control; this minimum indicated the largest treatment divergence between the treatment and the Control. The community least resistant to treatment (changed the most given the

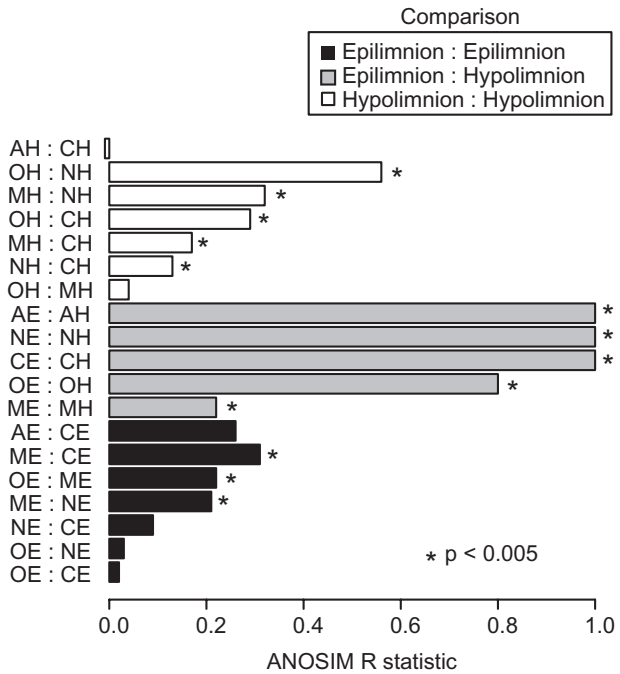

Fig. 4. Analyses of similarity (ANOSIM) to determine differences in BCC across treatments. For each group label, the first letter is the treatment (Oxygen, Nutrient, Mix, Control or Ambient), and the second letter is the thermal stratum (epilimnion or hypolimnion). High *R* statistics indicate greater compositional differences, where 1 is complete separation. Negative *R* statistics indicate communities are more similar across groups than within groups. All analyses with asterisks were significant at $P < 0.005$.

disturbance) was the Mix epilimnion, followed by the Oxygen hypolimnion. The Nutrient epilimnion was the most resistant. Notably, the Oxygen epilimnion and Nutrient hypolimnion were not necessarily expected to change because they were not directly manipulated, and served as additional references.

Next, we measured resilience and assessed bacterial community recovery by comparing the number of days for each treatment to become indistinguishable from the control. To account for succession that may have occurred over 10 days (as expected, given the documented seasonal progression of temperate lake bacterial communities, e.g. Kent *et al.*, 2007; Shade *et al.*, 2007; Nelson, 2009), we used the control dynamics as reference. We compared the similarities over time within the control to the similarities over time between each the treatment and the control. Control Day 0 was the reference point, and the differences between the control and treatment were calculated for all replicates for each day, and then tested for differences using Welch's *t*-test (Fig. 6, also see Fig. S4). The hypolimnion communities were less stable in their recovery than the epilimnion. Where the epilimnion treatments recovered and then remained similar to the control through the rest of the time-course, the hypolimnion treatments were less consistent and moved in and out of statistical similarity with the control.

Recovery was computed as average Bray–Curtis similarity to Control day 10. All communities were above 70% similarity to the Control by day 10, suggesting that all treatments ultimately achieved a degree of recovery by the end of the time-course (Table 3, Fig. S4).

Because hypolimnion community dynamics were generally less directional than in the epilimnion, we tested whether this was a significant trend using the RELATE test for seriation (evidence of observations in a sequence, such as spatially or temporally related observations). All epilimnion communities had strong evidence of seriation, as indicated by high Rho values above 0.70 and all $P < 0.001$. Hypolimnion community results were more variable and had weaker evidence of seriation (Control Rho = 0.27, $P < 0.09$; Mix = 0.44, $P < 0.02$; Nutrient = 0.55, $P < 0.001$; Oxygen = 0.49, $P < 0.01$). This supports that the hypolimnion communities were less directional than epilimnion communities after perturbation.

We next asked if there was also a difference in the rate of community change across treatments. To calculate the rate of change, we fit linear models to Bray–Curtis similarities regressed against time between observation pairs (Fig. S5, Table S1). The slope of the regression was the rate of change, or resilience, of the bacterial community. A comparison of all slopes suggested that hypolimnion communities changed less rapidly than their epilimnion counterparts, and that all treated communities changed more rapidly than their respective control (Fig. 7). The Mix epilimnion community changed most quickly. In summary, the Mix epilimnion community was least resistant but most resilient. Both epilimnion and hypolimnion Mix communities recovered less completely than their Oxygen and Nutrient counterparts (Fig. 8).

Discussion

In this experiment, we simulated lake mixing disturbance in controlled, *in situ* limnocorrals by manipulating the hypothesized drivers of BCC after mixing: oxygen in the hypolimnion and nutrients in the epilimnion. We hypothesized that nutrients up-welled from the hypolimnion to the epilimnion were a main epilimnion driver because they promote growth of *r*-strategists that quickly utilize resources in otherwise limiting environments. We further hypothesized that oxygen increase was a main hypolimnion driver because it disrupts vertical oxidation–reduction niches and permits growth of organisms efficient at using oxygen as their terminal electron acceptor. The results of the experiment supported our basic predictions: both manipulations provoked an overall bacterial community response similar to the fully mixed treat-

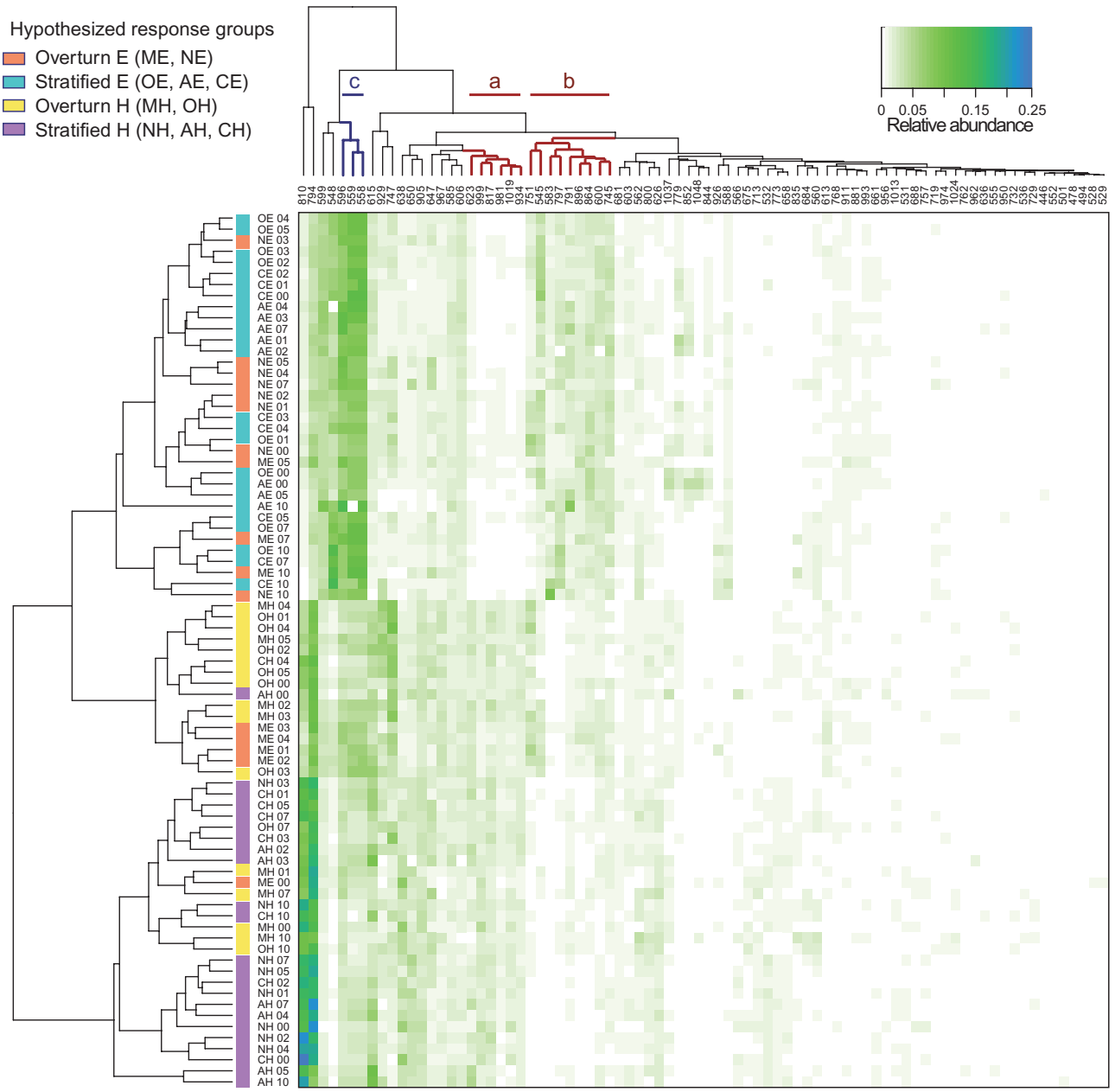

Fig. 5. Heatmap and cluster analysis of OTUs observed during the experiment. The *x*-axis is different OTUs by ARISA fragment length, and the *y*-axis is community observations. For each observation label, the first letter is the treatment (Oxygen, Nutrient, Mix, Control or Ambient), the second letter is the thermal stratum (epilimnion or hypolimnion), and the number is the experimental day. Observations are colour coded by their hypothesized response group (see Table 1). The least abundant 36 OTUs were trimmed from the figure for clarity.

ment. However, the oxygen treatment was a more intense disturbance than mixing in the hypolimnion, while the nutrient treatment was less intense than mixing in the epilimnion.

We further hypothesized that both epilimnion and hypolimnion communities would be sensitive to disturbance, but recover at different rates and extents. The epilimnion was less sensitive (more resistant) than the hypolimnion and, likely as a result, recovered more quickly. In contrast, the hypolimnion changed more drastically. Hypolimnion recovery was less stable but generally followed the recovery of oxygen depletion in the hypolimnion, whereas the epilimnion recovery was not aligned with the depletion of excess nutrients. This suggests that strong environmental filtering based on oxygen was more important in structuring the hypolimnion response and recovery, while other factors (potentially biological interactions) played a more important role in the epilimnion response.

Table 3. Resistance and recovery of bacterial communities for each treatment.

	Resistance: Minimum similarity to Control	Resistance: Average similarity to Control	Recovery: Day 10 average similarity to Control
Mix E	40.6	69.4	77.9
Mix H	67.4	75.3	72.7
Nutrient E	69.3	81.4	80.5
Nutrient H*	72.7	79.7	85.4
Oxygen E*	74.7	83.7	86.4
Oxygen H	58.4	72.5	78.6

E or H designates the thermal layer (epilimnion or hypolimnion). Resistance was the average daily Bray–Curtis similarity between the treatment and the Control. An additional metric of resistance was the minimum Bray–Curtis similarity between the Control and treatment in the time series, giving the highest degree of change observed. Lower Bray–Curtis similarities suggest less resistant bacterial communities. Recovery was the Bray–Curtis similarity between the treatment and the Control on day 10, the final time point in the experiment. Asterisks indicate that the environment was not manipulated directly, and should resemble the control.

The success of nutrients and oxygen in reproducing bacterial community patterns after mixing

A key component in testing our hypotheses was to successfully mimic mixing with oxygen and nutrient amendments to the hypolimnion and epilimnion. Overall, as indicated by the ANOSIMs of treatment and hypothesized response groups, these two treatments reproduced effects of water column mixing on the bacterial communities. However, when time is considered by incorporating day-to-day comparisons between Mix and Control, this was not the case. Notably, the Mix epilimnion was more divergent from its Control than the Nutrient, and recovered less quickly. This is likely because the nature of the disturbance for each layer is very different: oxygen can kill obligate anaerobes, but nutrients stimulate uptake and competition. Though both nutrients and oxygen are disturbances because the microbial environment was altered, Oxygen was of higher intensity because it likely resulted in mortality.

Objective 1: Isolating hypothesized drivers of bacterial communities after lake mixing

Mixing and nutrient amendment to the epilimnion. Excess nutrients are typically thought to promote bacterial growth during temperate lake mixing. A previous study examined food–web interactions between plankton communities in a similar mixing manipulation (Weithoff *et al.*, 2000). The authors observed a general increase in phytoplankton and bacterial abundance, and a differential response among zooplankton populations. They attributed the increase in bacterial abundance to the higher concentrations of nutrients up-welled from the hypolimnion (as measured by ammonium, nitrate, and soluble reactive P concentrations), and possibly to increased phytoplankton

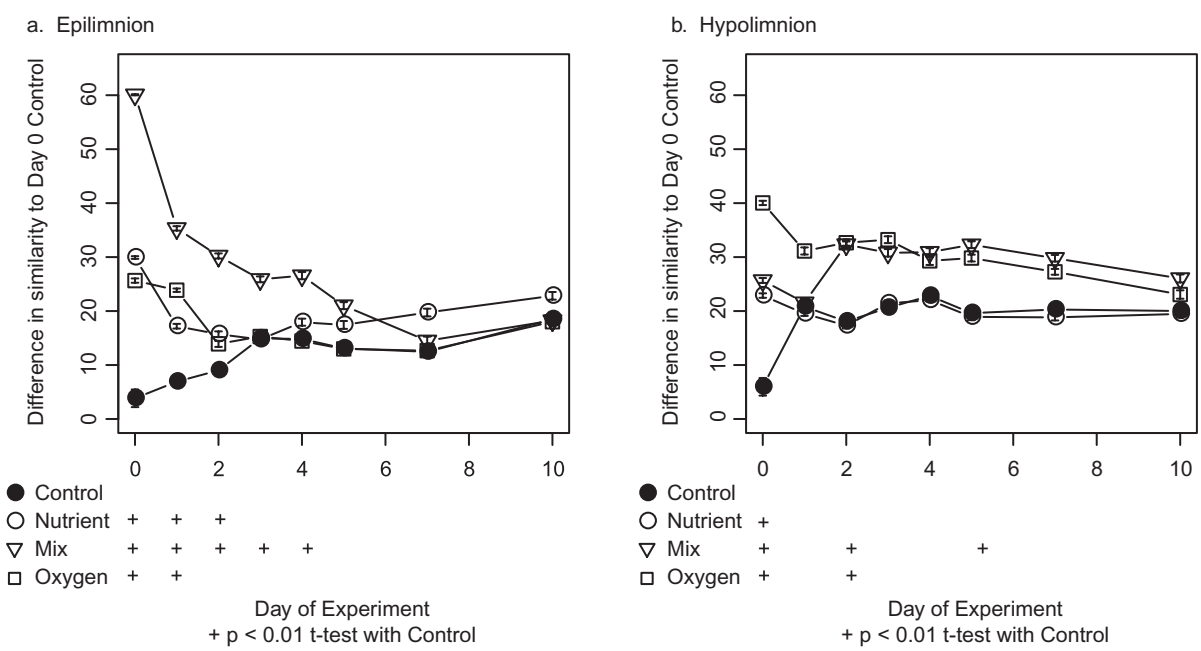

Fig. 6. Recovery was calculated as the difference in Bray–Curtis similarity for each treatment (open symbols) to the Control on day 0. The dynamics in the control (filled circle) was used as a reference for how much community change was expected without any perturbation. Plus signs (+) indicate points where significant differences between the control and the treatment were observed by a *t*-test ($P < 0.01$). When the difference between the treatment and the control on day 0 was not statistically distinct from the difference between the control to day 0, the community was considered to be recovered. Error bars are standard error.

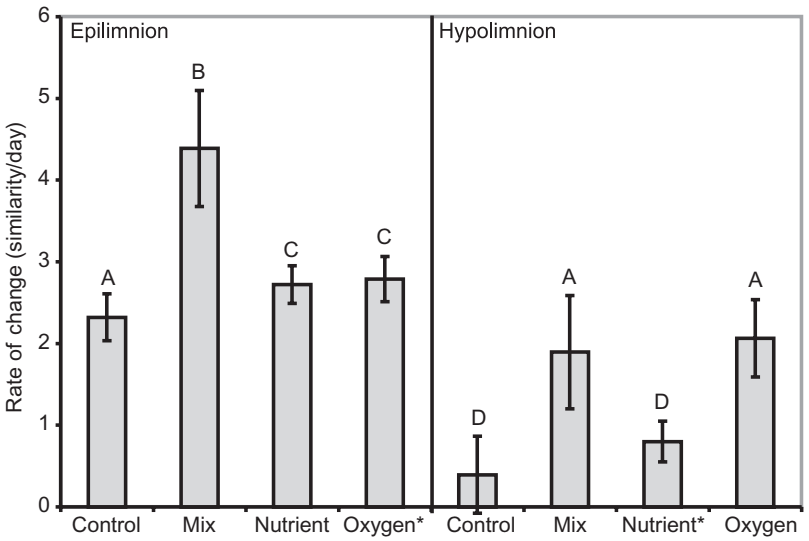

Fig. 7. Epilimnion and hypolimnion communities rates of change (resilience), calculated by the slope of the Bray–Curtis similarities over the time between observations for each treatment. The letters above the bars indicate statistically distinct groups, as determined by standard error. Asterisks indicate that the environment was not manipulated directly, and should resemble the control.

exudates (not measured). We observed decreases in dissolved P and N through time in the Nutrient epilimnion. Because hypolimnion dissolved nutrient concentrations did not increase at the same pace as the epilimnion concentrations decreased, we deduce that the nutrients were not simply sinking into the hypolimnion, but were used by the epilimnion plankton. However, we cannot determine if the nutrients were consumed by bacteria or phytoplankton, or (most likely) both.

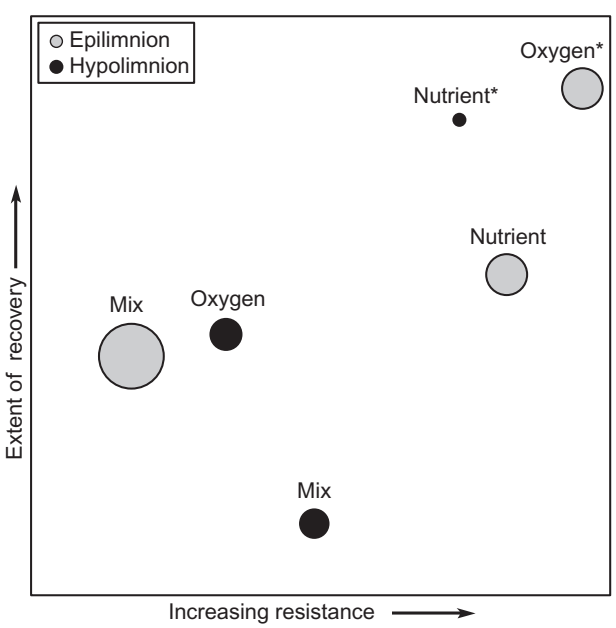

Fig. 8. Summary of resistance, recovery and resilience across treatments. Grey circles are epilimnion communities, black are hypolimnion. Resistance was the average Bray–Curtis similarity between the treatment and control (Table 3). Recovery was the Bray–Curtis similarity between the treatment and control on the final observation of the time series. Resilience was the community rate of change, calculated as the slope of all within-treatment Bray–Curtis similarities against time between observations. Each community's resilience is proportional to size of its symbol. Asterisks indicate that the environment was not manipulated directly, and should resemble the control.

The number of trophic levels in the epilimnion plankton food web (including zooplankton predators, phytoplankton, and bacteria phototrophs and bacteria heterotrophs) presents a challenge for interpreting the direct impacts of the nutrient addition on BCC. This is important because both top-down (grazing) effects and bottom-up (phytoplankton exudates) effects are known to strongly influence BCC (Kent *et al*., 2006; Grossart *et al*., 2008; Simek *et al*., 2008). For example, an indirect effect of mixing would be if the nutrients had stimulated phytoplankton growth, resulting in increased exudates that were then consumed by heterotrophic bacteria. The added nutrients could have also been consumed directly by heterotrophic bacteria. Despite not knowing the mechanism, we could attribute changes in BCC to nutrient amendment (though its rapid recovery likely caused the aggregate ANOSIM between the two groups to not be overall significant). This suggests that whether by direct or indirect mechanisms, mixing causes changes in BCC because of nutrient upwelling. Further work is needed to determine if phytoplankton responded first and triggered a change in BCC. We hypothesize that both mechanisms are important; some bacterial OTUs will be directly linked to phytoplankton responses (Kent *et al*., 2007), while others will behave independently.

Mixing and oxygen amendment to the hypolimnion. In a stratified water column with high hypolimnion respiration rates, the availability of DO and other respiration substrates commonly structures bacterial communities (e.g. Finlay and Esteban, 2004; Lehours *et al*., 2009). When hypolimnion microbial respiration is high, DO is often

depleted and is replaced by other respiration substrates in order of their energetic favourability. Physiological requirements restrict certain bacterial populations to specific DO concentrations, such as obligate anaerobic or microaerobic species (Fenchel, 2005). In our experiment, the increased hypolimnion oxygen may have stressed or killed oxygen-intolerant bacterial populations. Alternatively, facultative populations tolerant of a range of DO concentrations may have been granted a competitive advantage given even a briefly aerobic environment. Additionally, integrating epilimnion water in the Mix treatment likely introduced aerobes primed to rapidly consume oxygen. Taking these points together, the relatively quick response and recovery of the hypolimnion bacterial communities may be explained by strong oxygen constraints.

The clear overlap in bacterial populations shared across the Mix and Oxygen treatments in the cluster analysis suggests that oxygen addition to the hypolimnion directly changed the bacterial community. This may be because the hypolimnion harbours fewer trophic levels (e.g. no aerobic phototrophs) that allowed for a straightforward resource-driven response. The hypolimnion Oxygen treatment, where BCC is thought to be structured primarily by respiration substrate availability rather than the trophic interactions as observed in the epilimnion, appeared to have a more direct response (though, also a less stable recovery) than the epilimnion had to nutrient treatment. From these observations, we hypothesize that a more complex network of trophic interactions (e.g. more linkages among taxa and potential redundancy in those linkages) may buffer response to environmental changes.

Towards a mechanistic framework for bacterioplankton responses to lake mixing. Previous experimental research had begun to decipher plankton responses to lake mixing though bacterioplankton were not highlighted in these studies. Pannard and colleagues (2007) and Diehl and colleagues (2002) both focused on the response of phytoplankton communities, and Weithoff and colleagues (2000) observed changes in zooplankton and phytoplankton communities that were coupled with bulk bacterioplankton biomass. By observing the response of the bacterial communities to mixing and mixing-associated environmental conditions in controlled enclosures, we sought to fill the gaps in our understanding of lake mixing implications for the bacterial components of plankton food webs.

The results of the cluster analysis revealed that OTUs were characteristic of both Overturn and Stratified conditions within each layer, suggesting that composition plays a fundamental role in the post-mixing dynamics. This supports the findings of a previous experiment that proposed the existence of layer-specific OTUs and 'generalist' OTUs capable of existing in either the epilimnion or hypolimnion (Shade *et al.*, 2010b). In the previous study, epilimnion, hypolimnion and mixed water were incubated in dialysis tubing at discrete depths within either the epilimnion or hypolimnion. This was to understand the influence of the layer-specific environmental conditions on the bacterial community, excluding competition with or re-seeding by species from the ambient water. Some OTUs detected throughout the five-day series were present in the source water from both the epilimnion and hypolimnion, and these 'generalists' were hypothesized to initiate post-mixing bacterial community assembly. In the current study, the OTUs shared by the Overturn epilimnion and Overturn hypolimnion groups (but excluded from the Stratified groups) could be considered pioneer OTUs, responsible for initiating post-mixing succession, similar to the generalists of the previous transplant experiment. Because the current experiment did not restrict bacteria to a layer using dialysis tubing, as in Shade and colleagues (2010b), it is unlikely that OTUs persisted in a layer because they could not return to conditions more favourable for growth (e.g. via chemotaxis). Given the volume of the limnocorrals, it is also unlikely that interactions among co-occurring OTUs were constrained because of a restricted spatial environment. Therefore, the results of this experiment built on the previous one by permitting the interplay of environmental filtering and meta-community as well as community-level interactions (e.g. immigration, competition).

Objective 2: Characterizing components of bacterial community stability after disturbance

Aquatic bacterial community stability. We know little about the components of stability (resistance, recovery and resilience) in microbial systems (Botton *et al.*, 2006). This is in part because few studies follow a time-course of the microbial community after disturbance, and instead focus on the community sensitivity or immediate response to perturbation (as previously noted by Allison and Martiny, 2008). Our understanding of microbial community stability hinges on our ability to (i) design appropriate longitudinal studies that make observations of the community at a scale relevant to growth and community turnover, and (ii) develop a direct method for comparing results across various ecosystems and community profiling techniques.

In our experiment, bacterial communities were not resistant to any treatment. The changes observed in Nutrient epilimnion, Oxygen hypolimnion, and Mix treatments were greater than the typical community succession observed in control and ambient communities. But all communities recovered rapidly, within 10 days. This is relative to the previously observed community turnover in these lakes, which gradually proceeded on a 'stepping-

stone' trajectory through the ice-off season, as shown from twice or once weekly observations (Kent *et al*., 2007; Shade *et al*., 2008). Thus, our definition of rapid is referenced to our system. All this agrees with previous observational work that suggested aquatic bacterial communities are stable, not because they are resistant, but because they are highly resilient to disturbances (Yannarell *et al*., 2007; Jones *et al*., 2008; Shade *et al*., 2010a).

The Oxygen treatment was less resistant than the Mix treatment in the hypolimnion, likely because the oxygen concentration in the Oxygen treatment was nearly double that of the Mix treatment. However, the Mix and the Oxygen hypolimnion communities recovered at identical rates, suggesting that ~ 3.5 mg l^{-1} oxygen was of a similar disturbance intensity as ~ 6 mg l^{-1}, and given the same temperature, nutrient availability and initial BCC, both resulted in similar resilience among the hypolimnion bacterial communities in the Mix and Oxygen treatments. This indicates that there may be a 'threshold' of oxygen concentration that elicits this response, and that hypolimnion communities may be predictable in recovery after similar disturbances above this threshold.

Stability and temporal-versus-spatial niche partitioning. Our results highlight possible implications of temporal and spatial habitat heterogeneity for microbial responses to disturbance. First, consider the separate influences of temporal and spatial variation in this study system. In thermally stratified lakes, the epilimnion is thought to be generally homogeneous in space because of mixing surface waters and external inputs, like precipitation and allochthonous material. In contrast, the hypolimnion contains many 'layers' of different respiration substrates, even at depths below where the temperature remains constant and oxygen is depleted. But over time, the epilimnion environment is variable, as evidenced by the known seasonal drivers of temperate lakes and the diel temperature changes. Yet, over the same ice-off season that the epilimnion experiences temporal variability, the hypolimnion environment remains relatively isolated and unchanging. Therefore, the epilimnion environment is homogeneous in space but variable in time, and the hypolimnion environment is heterogeneous in space but stable in time.

Following this, our study has focused on two communities at the complementary intersection of these spatial and temporal niche spaces. Perhaps one reason that both epilimnion and hypolimnion communities were resilient is because of the ecological trade-offs required to exist at this intersection. We hypothesize that communities living in environments highly heterogeneous in both space and time, or highly homogenous in both space and time, are not as robust to disturbance. Two principles in ecology, niche diversification (e.g. Hutchinson, 1961; Huisman and Weissing, 1999) and the storage effect (Warner and Chesson, 1985; Miller and Chesson, 2009) address the concepts of spatial and temporal niche partitioning, and may provide a useful framework for determining mechanisms of microbial community resilience. Our results show that the hypolimnion bacterial community recovered at a similar temporal scale as the re-establishment of the oxygen gradient (7 days post treatment), which supports spatial niche partitioning as one possible mechanism of observed community response. Our results also show that epilimnion bacterial communities were more directional in time (as shown by the RELATE evidence of seriation) than those of the hypolimnion, hinting that temporal partitioning may play a role in recovery. Further work is needed to address these hypotheses, especially across different study systems, but such work would contribute greatly to building a general ecological framework for microbial communities.

Conclusions

Our results provide clear evidence that specific environmental changes can replicate broad disturbance effects on microbial communities. Our results also provide quantitative evidence of stability in aquatic bacterial communities, with low resistance but rapid resilience after perturbation.

Experimental procedures

Study site

North Sparkling Bog is a dimictic, dystrophic bog lake in northern Wisconsin, USA (46°00′17.36″N, 89°42′18.11″W, maximum depth 4.5 m, surface area 0.46 ha). During the experiment, the epilimnion extended to 2 m depth, and the hypolimnion was below 2 m. An instrumented buoy was deployed on North Sparkling Bog during the experiment. Temperature, DO and meteorological data observed every 10 min at the buoy are available online (http://www.gleon.org).

Experimental design

The experiment was conducted from 16 to 26 June 2008. In the first treatment, oxygen was added to the hypolimnion. In the second, nutrients were added to the epilimnion. The third treatment simulated a mixing event (overturn). There also was a control enclosure with no treatment and sampling of the ambient lake water. Throughout this manuscript, we refer to these as 'Oxygen', 'Nutrient', 'Mix', 'Control' and 'Ambient'.

Twelve 'limnocorrals' were constructed as enclosures for the experiment (Weithoff *et al*., 2000; Frost *et al*., 2006) (Fig. S1). Each limnocorral was cylindrical and extended vertically from the surface of the lake to the sediment (approxi-

mately 4 m). The total volume was approximately 5050 l. Details of limnocorral construction are provided in online Supporting Information.

The limnocorrals were deployed on 15 June 2008 to allow the sediment and water column to stabilize before treatment on 16 June 2008. The limnocorrals were deployed in a random spatial arrangement throughout the lake, at a maximum depth of 3.25 to 3.5 m. Replicates from each treatment were instrumented with a chain of HOBO temperature sensors (Onset), and one replicate from each had a self-logging DO sonde (Yellow Springs Incorporated) in the hypolimnion (3 m depth). More thermistors were deployed in the Oxygen and Mix treatments because thermal stratification was important for evaluating success of these treatments.

For the Mix treatment, a 60 cm flat disk was raised and lowered between 3.5 m depth and the lake surface. Holes were drilled through the disk surface to increase turbulence (Sanford, 1997; Regel *et al*., 2004). A brick was tied underneath the disk to maintain stability. We manually oscillated the disk every 10 min for 1 h and then, after a 1 h break, continued for an additional hour. Temperature and DO profiles were monitored within the limnocorral with a hand-held probe to track mixing progress.

The goal of the Oxygen treatment was to aerate the hypolimnion water without allowing it to mix with the epilimnion. This treatment was achieved by pumping hypolimnion water from the bottom of the limnocorral into an external cooler where the water was aerated with bubble diffusers, and then returned to the bottom of the limnocorral (Fig. S1). Valves on a compressed air cylinder were used to control the delivery of air to the coolers. One cooler was maintained for each replicate limnocorral. Thermally insulated tubing was used to transport water. A thermistor was deployed in each cooler to ensure ambient hypolimnion temperature was maintained. The water was removed and returned using two linear diffusers that were 0.6 m in length, spanning a depth range of approximately 2.5–3.1 m within the hypolimnion. The diffusers faced inward with 0.5 m fixed distance between them, retained by a plastic divider. This treatment was applied continuously over 3 h, until DO concentrations increased.

The Nutrient treatment was achieved by adding ammonium chloride (NH_4Cl) and potassium phosphate monobasic (KH_2PO_4) as N and P sources. These compounds were chosen because they are commonly bioavailable sources of nutrients (e.g. Smith and Prairie, 2004). P was added to the epilimnion to achieve a final concentration of 3 μg P l^{-1}, which was approximately the average concentration expected in the mixed water column. This value was based on nutrient analyses from integrated water collected on 9 June 2008 in North Sparkling Bog, a week prior to experiment start. Similarly, N was added to achieve a final concentration of 70 μg N l^{-1}. The limnocorral's epilimnion volume (0–2 m integrated depth) was calculated to be 2520 l, and we used the molar mass to determine the amount of each nutrient added to the epilimnion to achieve the expected mixed concentration. Dry chemicals were dissolved into to 500 ml of surface water from each limnocorral, and then added into each separately. Rationale for directly manipulating only one layer in the Oxygen and Nutrient treatments is given in the online Supporting Information.

The Control limnocorrals were left undisturbed. To prevent mixing during equipment removal, all tubing was left inside the limnocorrals until the experiment ended.

Sample collection

Integrated epilimnion (0–2 m) and integrated hypolimnion (2 m to just above sediment) water samples were collected for each limnocorral and ambient water. A 3.18 cm diameter polyvinyl chloride pipe with valves was used for water collection. The pipe was rinsed with ambient water between samples. Samples were collected immediately after treatment was ended on 16 June 2008 (day 0, approximately 17:00 hours), and on June 17 (day 1), 18 (day 2), 19 (day 3), 20 (day 4), 21 (day 5), 23 (day 7), and 26 (day 10). Manual temperature and DO profiles were observed using a hand-held probe (YSI 550A and 55, Yellow Springs Instruments). Water for bacterial community analyses was immediately filtered onto 0.22 μm polyethersulfone filters (Pall) and frozen at −20°C until further processing. Additional water was preserved for water chemistry analyses on days 0, 2, 4, 7 and 10. Water chemistry was analysed according to North Temperate Lakes Long Term Ecological Research standard protocols at the University of Wisconsin-Madison Center for Limnology (http://www.lter.limnology.wisc.edu).

Data analyses

Methods for quality control and transformation of environmental variables are given in online Supporting Information. Average stratum temperature and DO values were calculated from manual profile observations in each limnocorral. A Euclidean distance resemblance matrix was calculated for ANOSIM. ANOSIM was performed to determine if there were statistically significant distinctions between treatments. ANOSIM returns a permutation-derived *P*-value as well as an *R* statistic, where a higher *R* indicates a stronger distinction between the mean centroid and/or dispersion of the groups. These analyses were performed using the PRIMER v6 software (Clarke and Gorley, 2006) and the *R* environment for statistical computing (R Development Core Team, 2005). To determine on what day in the experiment nutrient and carbon measurements from the Oxygen, Nutrient and Mix treatments matched the control, pairwise *t*-tests were performed for each day. A cut-off of *P*-value less than 0.05 was used for group means to be distinct.

Bacterial community composition was determined using ARISA, which was performed as described previously (Shade *et al*., 2010b). Details of ARISA and caveats to the method are provided in online Supporting Information. ANOSIM (based on Bray–Curtis similarities) was used to test for differences in BCC among treatments. We classified hypothesized response groups (Table 1) based on our hypotheses that DO drove bacterial community dynamics in the hypolimnion during mixing, and that nutrients drove dynamics in the epilimnion. If these hypotheses were true, the hypolimnion communities in the Mix and Oxygen treatments should respond similarly and be representative of the hypolimnion community in a mixed lake ('Overturn' hypolimnion hypothesized response group), and the same for the Mix and Nutrient treatments for the epilimnion communities

('Overturn' epilimnion hypothesized response group). We also would expect the untreated strata to resemble communities in a stratified lake ('Stratified' hypolimnion and epilimnion hypothesized response groups). To observe BCC patterns across treatments, replicates were averaged and non-metric multidimensional scaling was performed using Bray–Curtis similarities in PRIMER. A heatmap was constructed to explore the patterns of OTU occurrences. This was constructed in the *R* environment for statistical computing (R Development Core Team, 2005). The RELATE test in PRIMER was used to determine if there was evidence of temporal seriation (Clarke and Warwick, 2001; Clarke and Gorley, 2006). RELATE is a permutation-based test that returns a Rho statistic and a *P*-value, where high Rho indicates stronger evidence of data in a series.

To determine the influence of the environmental variables on the BCC, a Mantel test was performed in the *R* environment for statistical computing using the vegan package (R Development Core Team, 2005; Oksanen *et al*., 2008). The input matrices were Bray–Curtis similarity for the BCC, and Euclidean distance for the water chemistry.

Resistance is the degree to which a community remains unchanged when challenged with a disturbance (i.e. Allison, 2004; Allison and Martiny, 2008). The Bray–Curtis distance between the untreated Control communities and the treatment communities on day 0 was our metric for bacterial community resistance. We used the dynamics in the Control as a reference. For each treatment, we calculated the difference in similarity between control on day 0 and every other day. We next compared the Control and treatment means of these differences using Welch's *t*-tests, with a *P*-value of 0.01 to determine the days that the treatment was not any more different from the control day 0 as the control was to itself on day 0. To summarize overall recovery at the end of the experiment, we compared the average Bray–Curtis similarity of the treatment to its control on day 10.

To determine community rate of change (resilience), a linear model was fit to all pairwise Bray–Curtis similarity indices regressed against the days between observations. The slope of the regression was the rate of change for that treatment, and a 'steeper' slope (larger absolute value) was indicative of a more rapidly changing community. The slope was our rate of change metric of bacterial community resilience. All linear models were fit in the *R* environment for statistical computing.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Schematic of the general limnocorral design, and of the Oxygen treatments' water (blue thick arrows) and air movement (black thin arrows). All limnocorrals had the three structural support hoops, plastic lay-flat sheet tubing, surface floats and weighted tubes. In the Oxygen treatment, we aimed to aerate the hypolimnion water without disrupting vertical thermal stratification. To accomplish this, hypolimnion water integrated between 2 and 3.5 m was pumped from the limnocorral in insulated tubing, and aerated in an external cooler. The air was delivered to each cooler via a pressurized tubing line from a SCUBA tank. The water was then returned gently to the hypolimnion using a second diffuser. Inset photo is a top view of one of the Oxygen limnocorrals.
Fig. S2. Principal components analysis of water chemistry associated with observations on days 0, 2, 4, 6 and 10 of the experiment. Water chemistry was measured from integrated epilimnion and integrated hypolimnion water, collected from each limnocorral. Observations were classified by hypothesized response groups (Table 1). Environmental variables included in this PCA were average stratum temperature and DO calculated from manual profiles, dissolved organic (DOC) and inorganic carbon (DIC), total phosphorus (TP) and nitrogen (TN), dissolved total phosphorus (DTP) and nitrogen (DTN), and water colour.
Fig. S3. Mean dissolved nutrient and carbon concentrations for each treatment. Each column is a different hypothesized response group (Table 1). The two left hand columns are epilimnion observations, and the two rightmost columns are

hypolimnion. Data are broken down by hypothesized response group (e.g. Overturn or Stratified). Error bars are standard deviation. Error bars are not given for ambient because only one observation was collected per time point. Note differences in *y*-axis scale for hypolimnion and epilimnion observations.

Fig. S4. NMDS of epilimnion and hypolimnion bacterial communities trajectories bacterial communities when challenged with mixing, nutrient and oxygen disturbances. NMDS are calculated from Bray–Curtis similarities. Epilimnion community analyses are on the right, and hypolimnion on the left. Control communities are closed triangles, and treatment communities are open circles, and trajectories are solid and dashed lines respectively. Communities are labelled by day of the experiment, where day 0 was collected immediately after treatment ceased.

Fig. S5. Bacterial community rate of change (resilience) for epilimnion and hypolimnion communities. For all observations within a treatment, pairwise Bray–Curtis similarity was plotted against the time between comparisons. Linear regression models were fit to these data, and the rate of change was the slope of the regression. For each group label, the first letter indicates the treatment (Oxygen, Nutrient, Mix or Control), and the second letter indicates the thermal stratum (epilimnion or hypolimnion). Open symbols are epilimnion, closed are hypolimnion. The number of asterisks indicates the significance of the slope. A larger absolute slope is evidence of a faster rate of change. In addition to the controls, the Oxygen Epilimnion (OE) and Nutrient Hypolimnion (NH) environments were not manipulated directly.

Table S1. Regression coefficients and details for the community rate of change linear models (Fig. S5). 'E' is for epilimnion, 'H' is for hypolimnion. DF is degrees of freedom. Asterisks indicate that the environment was not manipulated directly, and should therefore resemble the control.