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Effect of short-term, diel changes in environmental conditions on active microbial communities in a salt marsh pond

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ABSTRACT: Microbial communities play key roles in biogeochemical cycles across the planet and their composition and the factors that structure them are well documented. However, how changes in abiotic conditions can affect the active proportion of the microbial community that is responsible for the delivery of key ecosystem services is poorly understood. Salt marshes, in particular salt marsh ponds, are highly dynamic habitats with abiotic conditions in the pond water that fluctuate on daily cycles. To determine how diurnally driven changes in abiotic conditions affect active microbial communities, we sampled a single salt marsh pond every 4 h over 2 diel cycles, sampling 2 dynamically different habitats: the pond sediment and overlying water. We assessed abiotic conditions and the total and active microbial communities using high-throughput sequencing of the 16S rRNA gene and 16S rRNA. Sediments displayed no discernable pattern and low variation in abiotic conditions, leading to stable active microbial communities. However, the cyclical, rapidly changing abiotic conditions in the overlying water resulted in large changes in the active microbial community and in the percent of inactive taxa. Our data suggest that changes in environmental condition over short time periods alter the structure of active microbial communities. Further, our data show that the most abundant taxa in the active communities in the overlying water were rare (<1% total abundance), suggesting that under environmental change, rare taxa can disproportionally contribute to the activity of microbial communities.

KEY WORDS: Microbial activity · Dormancy · Rare biosphere · Salt marsh · Marsh pond

INTRODUCTION

Microbial communities face considerable physiological challenges from changing abiotic conditions, and the way microbes respond to these changes can have profound effects on ecosystem function. Broad scale spatial and temporal drivers often control microbial composition (Lozupone & Knight 2007, Gilbert et al. 2012); however, variation in local environmental conditions can confer changes in microbial community composition, activity, and geochemical output (Allison & Martiny 2008, Shade et al. 2012, Griffiths & Philippot 2013, Reed & Martiny 2013). Under some circumstances, however, microbial community composition may remain resistant to perturbations (Bowen et al. 2011, Kearns et al. 2016), despite differences in microbial activity or geochemistry (Koop-Jakobsen & Giblin 2010, Bowen et al. 2011, Kearns et al. 2016). While variation in community composition can lead to changes in function (Reed & Martiny 2013), the correlation between composition and function can also be disconnected (Balser and Firestone 2005, Strickland et al. 2009, Frossard et al. 2012, Bowen et al. 2014) due to offsets in timing between gene expression and as-

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sociated function, or because the end product of microbial activity can be consumed prior to being detected (i.e. cryptic cycling). Functional redundancy, where multiple organisms perform the same function, can also result in a change in community structure with no net change in function (Allison & Martiny 2008). Finally, inactive or dormant microbes may decrease our ability to determine microbial responses to changing environmental conditions (e.g. Bowen et al. 2011, Kearns et al. 2016).

Dormancy is a strategy some microorganisms use to persist in their environment during unfavorable conditions (Lennon & Jones 2011). Dormant microbes exist in a wide range of ecosystems; however, their proportion of the total bacterial community differs (Lennon & Jones 2011), and this can limit our ability to detect community responses to environmental change. Ecosystems that have high proportions of dormant taxa, such as soils and sediments, possess a large degree of spatial and temporal heterogeneity (Lennon & Jones 2011, Kearns et al. 2016). Additional controls on the proportion of inactive or dormant cells include changes in abiotic conditions such as salinity (Aanderud et al. 2016) and excess nutrients (Kearns et al. 2016); however, nutrient limitation can also increase dormancy in some systems (Jones & Lennon 2010, Aanderud et al. 2016). Ultimately, the controls over dormancy and the role of environmental change on the proportion of dormant cells remain unclear, resulting in a pressing need to understand the role of the environment in maintenance and resuscitation of dormant taxa.

Salt marshes provide a dynamic natural experiment to test hypotheses regarding how varying environmental conditions influence the activity of microorganisms. Salt marshes are highly productive, tidally flooded, coastal grassland ecosystems that provide numerous services to both the land and sea (Teal 1962, Deegan 1993, Mcleod et al. 2011). Due to their low elevation relative to mean tidal height (Nicholls et al. 1999), salt marshes are highly susceptible to changes in sea level. Increases in sea levels can cause net shifts in marsh plant communities as well as increases in the numbers of ponds, which are unvegetated areas that have lower elevation than the surrounding marsh platform (Redfield 1972, Reed 2002, Day et al. 2011). Salt marsh ponds can exchange water with the coastal ocean on very high flooding tides; however, ponds often experience extended periods of disconnect from tidal flooding. During their isolation, the water in marsh ponds can experience oscillations of oxygen saturation and other constituents (e.g. pH, temperature) over short periods of time (Smith & Able 2003, Day et al. 2011). Conversely, the sediments underlying ponds remain relatively unperturbed on short time scales. Salt marsh ponds are very shallow (often <20 cm water depth), so sediments and overlying water represent highly connected, yet distinct systems that are ideal for studying how environmental change alters microbial activity over short time scales.

The activity of microbial cells has often been assessed using transcripts of the small subunit of prokaryotic ribosomes (16S rRNA) due to its correlation with protein synthesis (Kerkhof & Kemp 1999, Deutscher et al. 2006), and it has also been used to assess dormancy in environmental samples (Jones & Lennon 2010). Despite the caveats to this approach (see Blazewicz et al. 2013), 16S rRNA can be useful as a relative measure of active microbial community structure and how it varies over time or as a result of experimental manipulation (Jones & Lennon 2010, Campbell et al. 2011, Aanderud et al. 2016). The aim of this study was to assess the effect of cyclical diurnal changes in environmental conditions on total and active microbial community composition. We tested the hypothesis that cyclically dynamic abiotic conditions in the overlying water (temperature, oxygen, salinity, pH, reactive nitrogen species) caused by high daytime primary productivity, would drive strong changes in active community composition, while static conditions in the sediment would promote stable active communities.

MATERIALS AND METHODS

Study site and sample collection

We sampled the sediment and overlying water in a salt marsh pond located at the Plum Island Ecosystems Long Term Ecological Research (LTER) site in Rowley, MA, USA (42.759° N, 70.891° W) every 4 h over 2 diel cycles (48 h). The pond (located at 42.741° N, 70.831° W) was ~20 cm in depth and approximately 7120 m^2 in area. During the course of the experiment and 2 d prior to our sampling, the pond was isolated from tidal flooding due to low tidal amplitude. Sampling began at 10:00 h on June 30, 2014 and ended at 10:00 h on July 2, 2014, capturing 2 full diel cycles. On the days sampled, the sun rose between 05:09 and 05:15 h and set between 20:25 and 20:30 h. The weather was clear with nominal wind speeds (<1 m s^{-1}) and the air temperature ranged from a low of 17°C to a high of 31°C (mean 24°C). To account for spatial variability within the pond, we sampled 3 dif-

ferent locations that were approximately 15 m apart, and at each site, we sampled within 1 m of a central point.

Every 2 h over the course of 2 d, we collected environmental parameters (dissolved oxygen, salinity, pH, temperature) with a Hanna HI9828 multiparameter meter (Geoscientific) and we collected and filtered 50 ml of water from each location for nutrient analysis. The water was passed through a 0.46 µm Whatman GF/F filter into sterile 50 ml centrifuge tubes and stored on dry ice. Additionally, every 4 h, 1 l water was filtered onto a 0.22 µm Millipore Sterivex filter from each site and flash frozen in liquid nitrogen, giving a total of 39 water samples. We collected 39 surface sediment (top 2 cm) samples from all 3 locations within the pond using a 15 cm inner-diameter PVC corer. Sediments from each site were individually homogenized in a sterile centrifuge tube and subsamples were placed into cryovials for storage on liquid nitrogen. The remaining sediment was stored on dry ice for determination of carbon and nitrogen content.

Nutrient analyses

Nitrate, ammonium, and phosphate concentrations in the overlying water column were analyzed on a Lachat Flow 118 injection analyzer (Hach) based on QuikChem methods 31-107-04-1-E 119, 31-107-06-1-B, and E31-115-01-1-W, respectively at the University of Massachusetts Boston Environmental Analytical Facility. The lower limit of detection for the methods was 0.60, 1.45 and 0.25 µM, respectively. Ammonium and phosphate concentrations were measured in sediment porewater following the methods outlined above; however, due to the low volume of sediment pore water, nitrate concentrations were measured by chemiluminescence after vanadium reduction (Garside 1982, Braman & Hendrix 1989) on a Teledyne NOx analyzer. Elemental composition (percent nitrogen and carbon) was measured on sediments dried at 50°C for 3 d, using a Perkin Elmer 2400 Series elemental analyzer following standard procedures. To compare the variability of abiotic conditions within the pond water and porewater, we calculated the coefficient of variation for each parameter in both habitats.

Nucleic acid extraction and reverse transcription

Nucleic acids from sediments were extracted with the MoBio PowerSoil Total RNA Isolation Kit from ~1.5 g of sediment following manufacturer's instructions. Nucleic acids were extracted from water filters using the MoBio PowerWater Kit following manufacturer's instructions. RNA was checked for DNA contamination with general bacterial primers and any DNA contamination was removed using DNAse I (New England BioLabs) following the manufacturer's instructions. RNA was reverse transcribed to cDNA using the Invitrogen Superscript RT III cDNA synthesis kit following manufacturer's instructions for random hexamers. cDNA synthesis was confirmed with PCR using general bacterial primers and verified on a 1.5% agarose gel stained with ethidium bromide.

PCR and sequencing

DNA and RNA were quantified with Picogreen and Ribogreen (Invitrogen) kits respectively, and nucleic acids were normalized to 1 ng μl^{-1} for all PCR reactions. Samples were prepared for sequencing on an Illumina MiSeq following the protocol outlined in Caporaso et al. (2011). We used general bacterial primers 515F and 806R (Caporaso et al. 2011), with Illumina adaptors and individual 12-bp GoLay barcodes attached to reverse primers, to amplify a section of the V4 hypervariable region of the 16S rRNA gene. PCR reactions were performed in triplicate, verified with gel electrophoresis, and fragments were excised and purified using the Qiagen QIAquick gel extraction kit. Samples were quantified fluorometrically on a Qubit (ThermoFisher) and pooled in equal molar concentrations for sequencing on the Illumina MiSeq platform for paired-end 151 bp sequencing. All sequencing was performed at the University of Massachusetts Boston using V2 chemistry.

Quantitative PCR

Quantitative PCR (qPCR) was performed to assess 16S rRNA copy number for both the active and total microbial communities. All DNA, cDNA, and standards prepared from purified PCR product were quantified with a Qubit. DNA from each sample was normalized to 3 ng μl^{-1} and serial dilutions of standards were prepared. DNA and cDNA were amplified in triplicate reactions, along with standards and internal controls on a Stratagene MX-3005p quantitative thermocycler with the primer pair 357F and 519R (Biddle et al. 2008) using conditions described previously (Bowen et al. 2011). All qPCR reactions were verified for proper product formation with gel

electrophoresis and melt curves. All standard curves had $R^2 > 0.99$ and qPCR efficiency ranged from 90 to 103%. To test significant differences in abundance of 16S rRNA we used an ANOVA in R (R Core Team 2012). Homogeneity of variances was evaluated with a Bartlett test, and outliers and normality were evaluated with a Q-Q plot.

Sequence and data analysis

Paired-end reads were joined with fastq-join (Aronesty 2011) using default parameters, and sequences were demultiplexed and quality-filtered in QIIME (version 1.9; Caporaso et al. 2010b) following the guidelines recommended by Bokulich et al. (2013). Sequences were checked for chimeras using USEARCH in de novo mode, and chimeric sequences were subsequently removed (Edgar 2010). Following quality filtering, we retained a total of 3.87×10^6 sequences (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/a080p029_supp1.xls). Operational taxonomic units (OTUs) were clustered at 97% sequence identity with Swarm (Mahé et al. 2014) in QIIME, and OTUs appearing only once across the dataset were discarded. Taxonomy was assigned using uclust (Edgar et al. 2011) against the GreenGenes database (version 13.5). All sequences matching *Archaea* and chloroplasts were filtered from the dataset. Sequences were aligned with PyNast (Caporaso et al. 2010a), and a phylogenetic tree was constructed with fasttree (Price et al. 2010).

We calculated beta diversity on normalized OTU tables using weighted UniFrac (Lozupone & Knight 2005) in QIIME and visualized the results with a principal coordinate analysis. Significant differences in groups were assessed with a non-parametric MANOVA (Anderson 2001) with 10 000 permutations. Alpha diversity was calculated on a rarefied OTU table (10 000 restarts, steps of 50) normalized to the lowest sampling depth (11 596 sequences). To assess heterogeneity in the sediment communities and determine if overdispersion was impeding our ability to detect changes in sediment communities, we performed a regression-based overdispersion test (Cameron & Trivedi 1990) in R. We calculated Shannon diversity and assessed significant differences in diversity with a 1-way ANOVA in R (R Core Team 2012, with a Tukey HSD test for multiple comparisons). To assess the degree of inactivity among taxa in our samples, we calculated the 16S rRNA:16S rRNA gene ratio (hereafter '16S ratio') for each OTU in each sample and defined a taxon as active with a ratio >1 (Jones & Lennon 2010). To assess the contribution of the rare biosphere to the active communities of the sediment and water, we used a Chi-squared test to determine the differences in abundance between OTUs in the total (expected) and active (observed) microbial communities using taxa with average abundance in the total microbial community of <1%. Further, we calculated non-parametric Spearman correlations between 16S rRNA gene and 16S rRNA profiles to examine how the abundance of taxa in the total communities correlates to their abundance in the active community. Sequences from this data set can be found in the Sequence Read Archive under accession number SRR4419663.

RESULTS

Environmental conditions

Abiotic conditions in the marsh pond sediment porewater (Fig. 1) did not vary appreciably over the time course of our experiment. The coefficient of variation (the ratio of the standard deviation to the mean) was typically low for environmental parameters (Fig. 1), ranging from 0.01 to 0.03, suggesting low variability in environmental conditions in the sediment over the 48 h experiment. While there was slight variation in abiotic conditions, this variability was not due to systematic temporal effects. Abiotic parameters in the overlying water column, however, did vary with the time of day (Fig. 1). Temperature, pH, and oxygen had high levels during the day and decreased levels at night. Ammonium and nitrate displayed the inverse pattern, with high concentrations at night and low or undetectable concentrations during the day. Water column salinity, while variable, only changed by ~0.5 ppt, and phosphate was stable over time. The coefficient of variation of environmental conditions in the water (Fig. 1) ranged from 0.9 to 1.97 and was significantly higher than the variation in the sediments ($t = 14.90$, $p < 0.002$), providing evidence that environmental conditions in the water were relatively more temporally dynamic than in the sediment.

Community composition and abundance

The total (16S rRNA gene) and active (16S rRNA) communities in the pond sediment were significantly different from each other (PERMANOVA, $F_{1,78}$ =

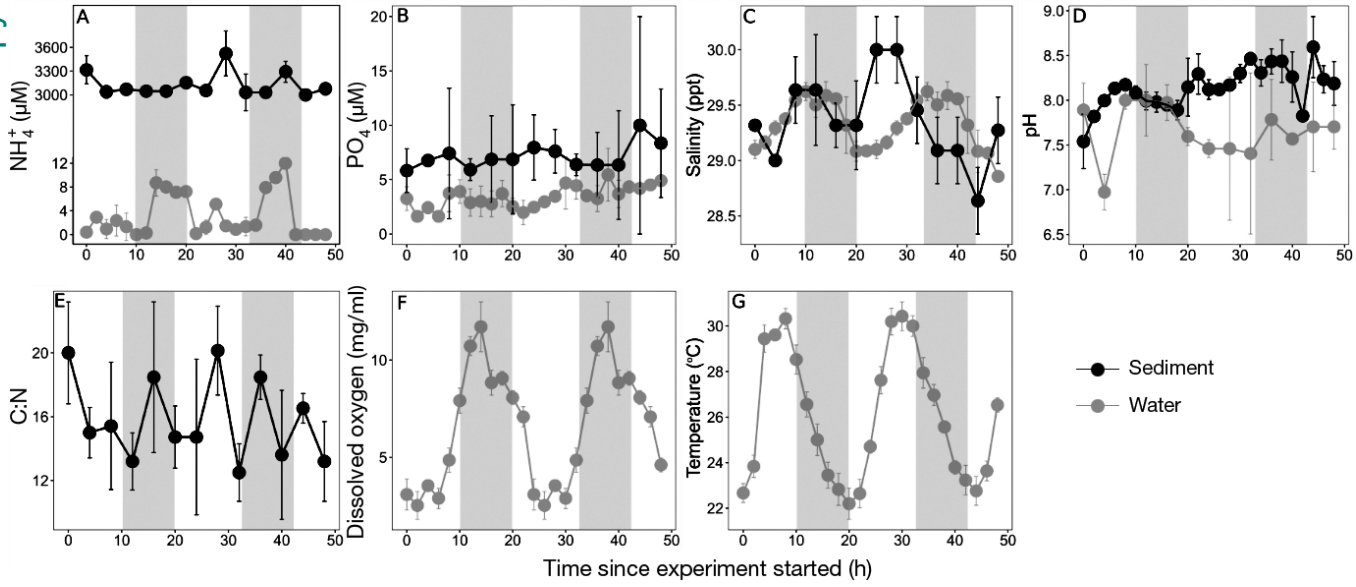

Fig. 1. Environmental parameters in sediment pore water (black) and overlying water (grey) in a salt marsh pond in Massachusetts, USA: (A) ammonium; (B) phosphate; (C) salinity; (D) pH; (E) C:N ratio; (F) dissolved oxygen; (G) temperature. Grey vertical bars indicate night time hours. Data are means ± SEM (n = 3 sites)

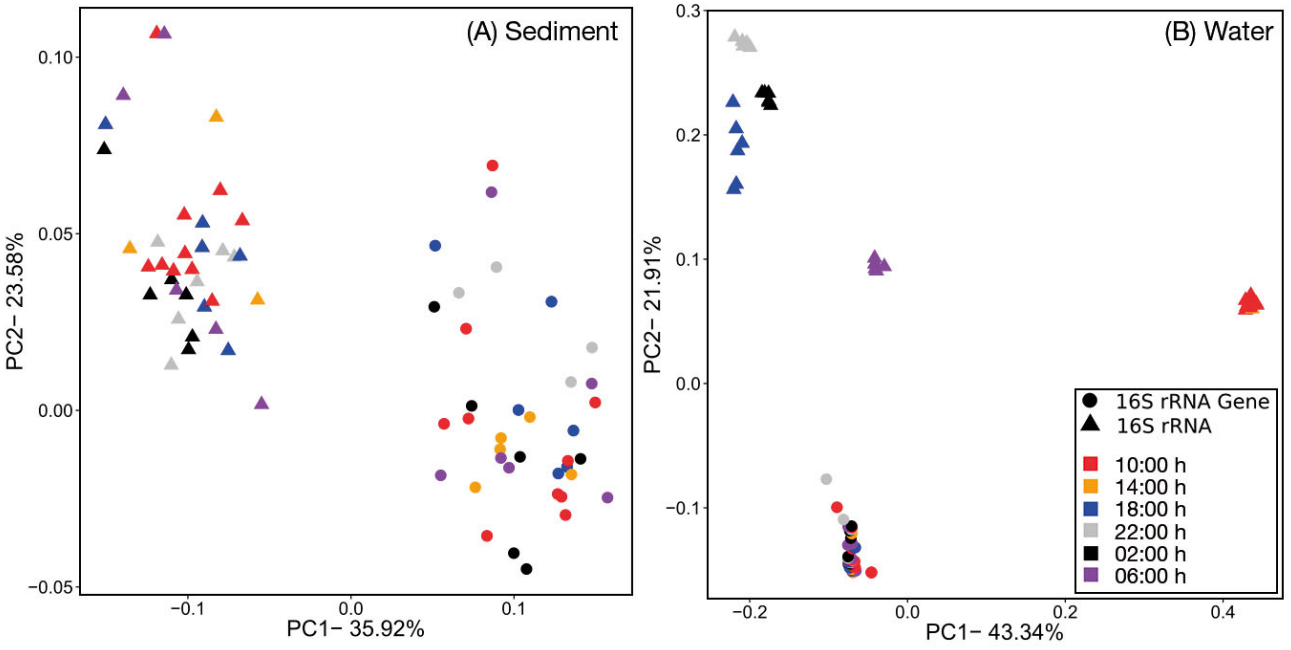

Fig. 2. Principal coordinate analysis based on weighted UniFrac similarity for (A) sediment and (B) overlying water of a salt marsh pond in Massachusetts, USA, sampled over a 48 h period

12.97, $p < 0.001$; Fig. 2A), but neither the total ($F_{4,35}$ = 4.45, p = 0.60) nor potentially active ($F_{4,35}$ = 6.04, p = 0.56) sediment communities displayed significant short-term temporal ($F_{5,34}$ = 2.43, p = 0.91) or spatial ($F_{2,37}$ = 0.90, p = 0.64) shifts in composition and the variation that exists was minimal for total and active communities, with mean weighted UniFrac similarity values of 96.04 and 94.56 % respectively. Further, an overdispersion test indicated no significant ($p > 0.56$) effect of variance resulting from time or site on sediment composition. As with sediment communities, water column active and total microbial communities were significantly different from each other ($F_{1,78}$ = 19.33, $p < 0.001$; Fig. 2B). The total community in the

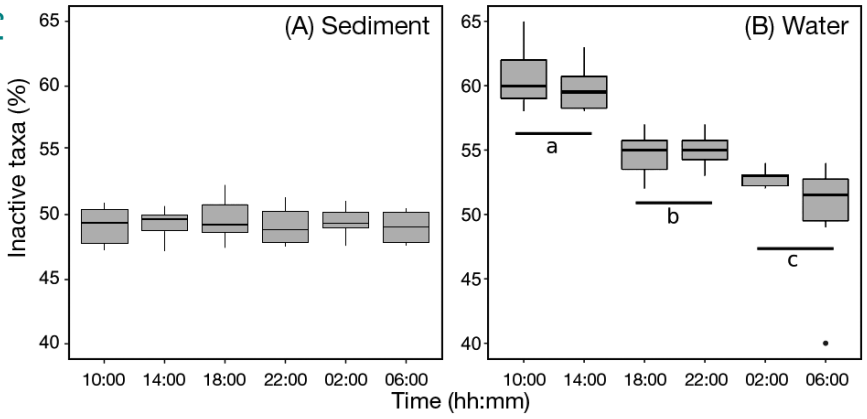

Fig. 3. Box-and-whisker plot of the percentage of inactive taxa in microbial communities in the (A) sediment and (B) overlying water of a salt marsh pond. Taxa are defined as inactive if their 16S rRNA:16S rRNA gene ratio was less than 1. Boxes represent 25 to 75% quartiles, the solid black line is the median value and whiskers are the upper and lower extremes. Differences among categories in (B) are significant based on a Tukey HSD test corrected for multiple comparisons. There were no significant differences in (A)

overlying water also remained stable over the 48 h period, displaying no significant spatial ($F_{3,36}$ = 9.45, p = 0.90) or temporal effects ($F_{4,34}$ = 11.43, p = 0.80) and with an average weighted UniFrac similarity of 97.5%. The potentially active community, however, displayed consistent and recurring diurnal changes over the 48 h of the experiment, displaying 5 significantly different clusters ($F_{4,34}$ = 19.21, p < 0.001) corresponding to time of day, with no evidence of spatial effects, and with average similarity of 52.43%. A plot of the 16S ratio, as assessed by qPCR (Fig. S1 in Supplement 2 at www.int-res.com/articles/suppl/a080p029_supp2.pdf) revealed a significantly higher ratio in sediment than water communities (ANOVA, $F_{4,24}$ = 29.45, p < 0.001), but neither ratio varied significantly over the course of the experiment in the sediment or the water.

Diversity and activity

Shannon diversity in the active and total communities remained constant in the sediment over the 48 h period (Fig. S2 in Supplement 2). In both the sediment and water column, Shannon diversity was higher in the total community than in the active community. The water column active community, however, had significantly different Shannon diversity values at different times of day. Patterns in the percentage of inactive taxa (16S ratio < 1) varied between sediment and water (Fig. 3). The percentage of inactive taxa in the sediment ranged from 45 to 55% of the community (Fig. 3A) and did not vary significantly ($F_{4,24}$ = 0.98, p > 0.05) during the duration of this experiment or between sampling locations. Inactivity in the water column, by contrast, ranged from 50 to 65% of the community and was temporally variable (ANOVA, $F_{4,24}$ = 12.43, p < 0.001,). The percentage of inactive taxa was significantly higher in the water than the sediment ($F_{1,76}$ = 12.43, p < 0.01).

Taxonomic composition

The taxonomic composition of the total sediment community did not vary over the 48 h experiment (Fig. S3 in Supplement 2). This community was comprised of largely anaerobic or facultatively anaerobic orders including Chromatiales (~17%), *Alteromonadales* (12%), *Myxococcales* (9%), *Bacteroidales* (8%), *Cytophagales* (7.5%) and *Desulfobacterales* (7%). The potentially active sediment community (Fig. 4A) also did not vary in its structure over time and was largely composed of several largely anaerobic orders such as *Chromatiales* (~19%), *Desulfobacterales* (~9%), *Myxococcales* (8%) and *Alteromonadales* (~7%). Phototrophic taxa such as *Cyanobacteria* (~15%) were present in the potentially active sediment microbial community throughout the course of the experiment.

The taxonomic composition of the total water community (Fig. S4 in Supplement 2) was consistent through time and was largely composed of the orders *Fusobacterales* (~12%), *Altermonadales* (11%), *Thiotrichales* (10%), *Myxococcales* (9%), and *Actinomycetales* (~7%). A stacked bar plot of the active communities in the water column displayed dynamic shifts in taxonomic composition (Fig. 4B). Peak photosynthetic hours (10:00 to 14:00 h) were dominated by an unclassified cyanobacterial order (~60%) in addition to the alphaproteobacterial order *Rickettsiales* (~13%). Four hours later at 18:00 h, the water column active microbial community was dominated by the gammaproteobacterial orders *Vibrionales* (~70%) and *Pseudomonadales* (~10%). At 22:00 h, the first time point after sunset, the composition of the active community was primarily comprised of the orders *Actinomycetales* (~22%) *Vibrionales* (~15%), *Flavobacterales* (~10%), and *Pseudomondales* (~7%). When oxygen concentration

was lowest at 02:00 h, the active water column community was dominated by the order *Bacillales* (~50 %), with a smaller contribution from the order *Vibrionales* (~15 %). Finally, at 06:00 h, the time point when the largest diversity of active taxa was observed, the community was comprised of the orders *Burkholderiales* (~17 %) *Flavobacterales* (~13 %), and unclassified *Cyanobacteria* (~7 %).

Activity of dominant taxonomic groups

We further explored patterns among active taxa by calculating the 16S ratio for the most active taxa (16S ratio >15) in the overlying water (Fig. 5), and aggregating the data at the order level. A heatmap of this ratio revealed numerous taxa with activity that was restricted to few times of the day (unclassified *Cyano-*

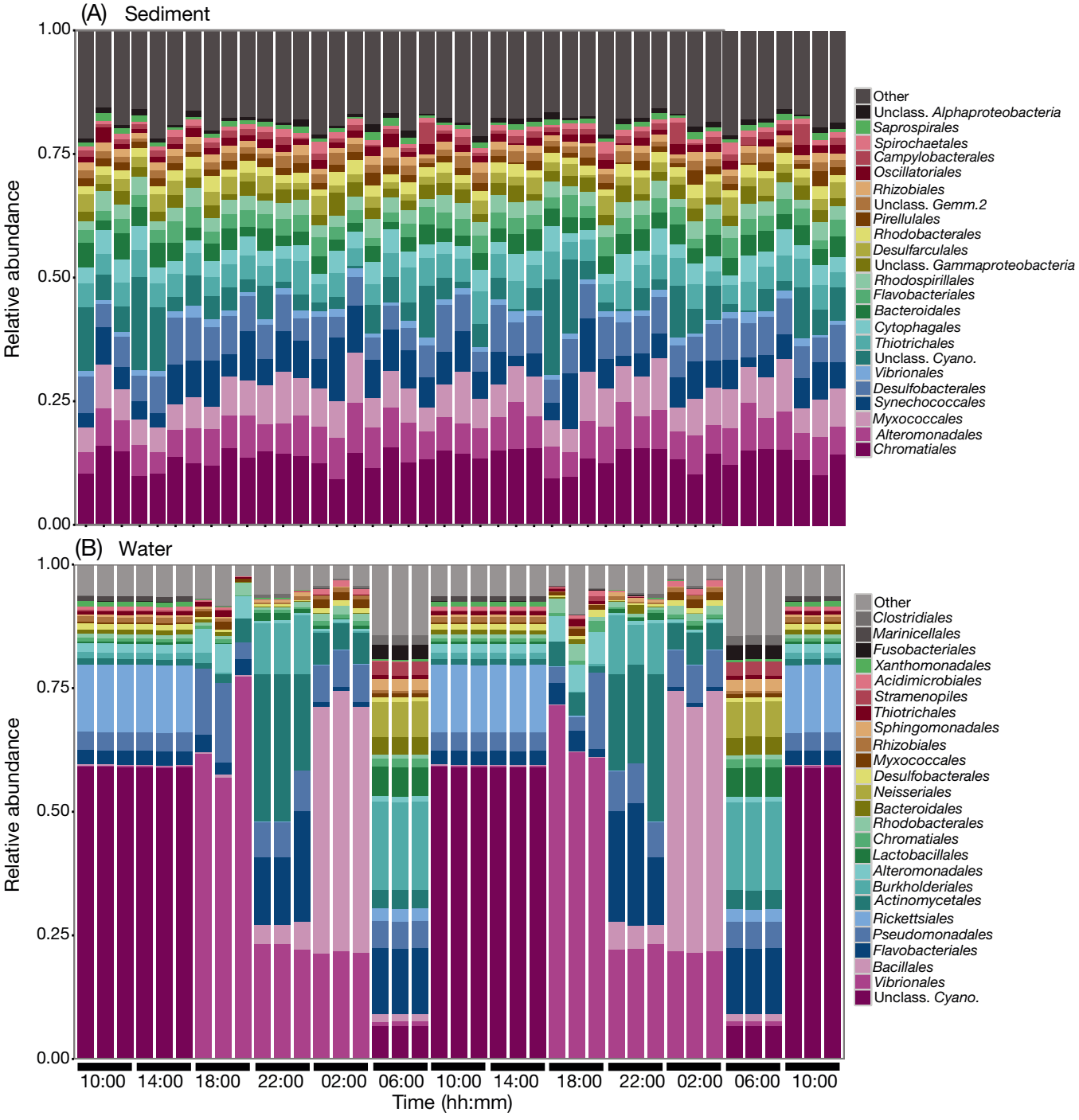

Fig. 4. Top 25 most abundant active microbial orders in the (A) sediment and (B) water of a salt marsh pond. The category 'other' is the sum of the remaining microbial orders

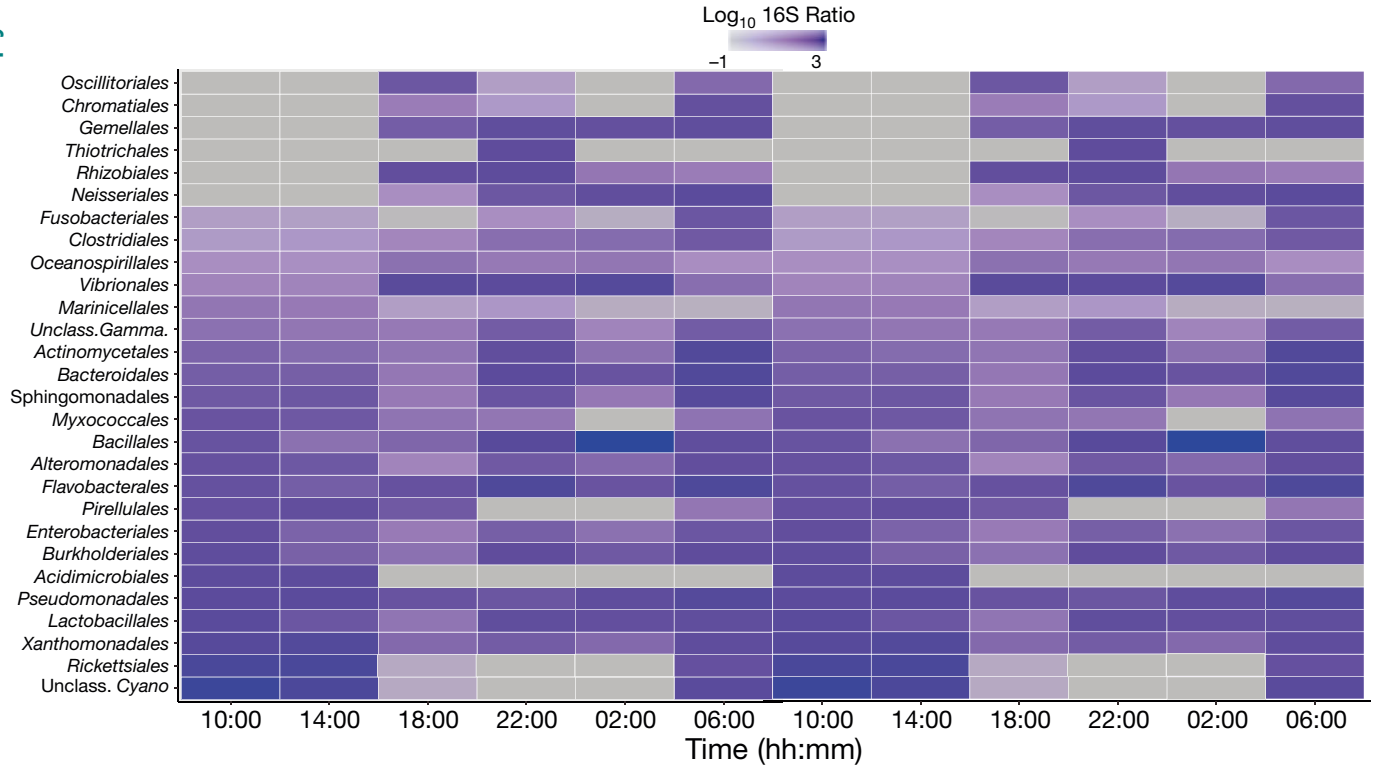

Fig. 5. Heat map showing the sum of the 16S rRNA:16S rRNA gene ratio (16S ratio) for the 28 most active microbial orders in the water column of a salt marsh pond

bacteria, *Rickettsiales*, *Acidimicrobiales*, *Bacillales*) suggesting their activity was limited by environmental conditions or competition with other taxa. Other taxa were active more frequently (*Vibrionales*, *Burkholderiales*, *Actinomycetales*, *Marinicellales*) suggesting less stringent growth requirements. Further, certain taxa appear to be active/inactive in concert with one another (unclassified *Cyanobacteria* and *Rickettsiales*), possibly suggesting a symbiotic relationship, niche overlap, or a similar response to environmental cues. A heat map of the most active sediment taxa (Fig. S5 in Supplement 2) generally displayed a consistent 16S ratio for the most active taxa over the course of the 48 h experiment.

Rank abundance and the rare biosphere

To assess the role that abundance of taxa in the total community plays in their concomitant abundance in sediment and water active communities we created bi-plots comparing the abundance of OTUs in the 16S rRNA to their abundance in the 16S rRNA gene (Fig. 6). The abundance of sediment taxa in the 16S rRNA was strongly correlated (Spearman's $\rho = 0.85$) to their abundance in the 16S rRNA gene (Fig. 7A), as highlighted by the highly abundant OTUs from the order *Chromatiales* (0.15 to 0.20 relative abundance), which was similar in abundance in the total and active communities. Water communities, however, displayed a disconnect in the abundance in the 16S rRNA and 16S rRNA gene (Spearman's $\rho = 0.31$). The disconnect was largely due to several taxa, including those from the order *Vibrionales*, *Bacillales*, *Rickettsiales*, and unclassified *Cyanobacteria*, that were in low abundance in the 16S rRNA gene compared to 16S rRNA, suggesting a role for rare taxa. We next explored the role of the rare biosphere by constructing rank–abundance plots comparing 16S rRNA gene rank of OTUs versus the corresponding abundance in the 16S rRNA (Fig. 7). Rank–abundance plots allowed us to both explore the correlation in abundance in the total and active communities and to determine the contribution of rare taxa to active community composition. The sediment community rank–abundance curve (Fig. 7A) indicated that the most abundant taxa in the total community were also the most active taxa. Indeed, no taxa in the sediment rare biosphere (<1% total abundance) significantly varied in abundance between the total and active microbial communities (Chi-squared test, $p > 0.5$). Communities in the overlying water, however, had vastly

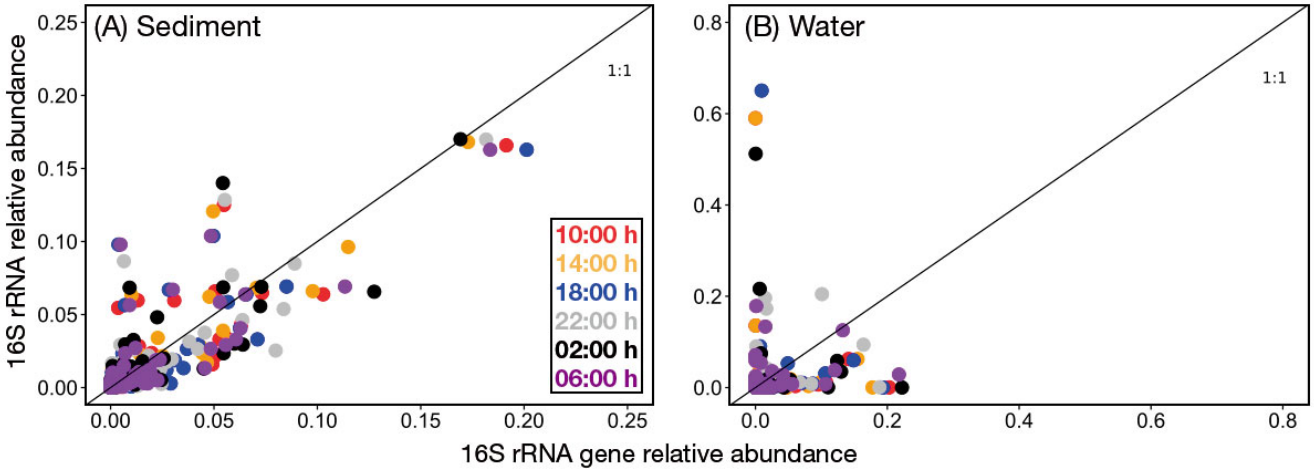

Fig. 6. Plot of the relative abundance of the top 1000 OTUs (comprising >90% of the data) in 16S rRNA relative to the 16S rRNA gene for microbial communities in (A) sediment and (B) water of a salt marsh pond. Points are colored by the time point when they were most abundant. Black lines in (A) and (B) are 1:1 lines

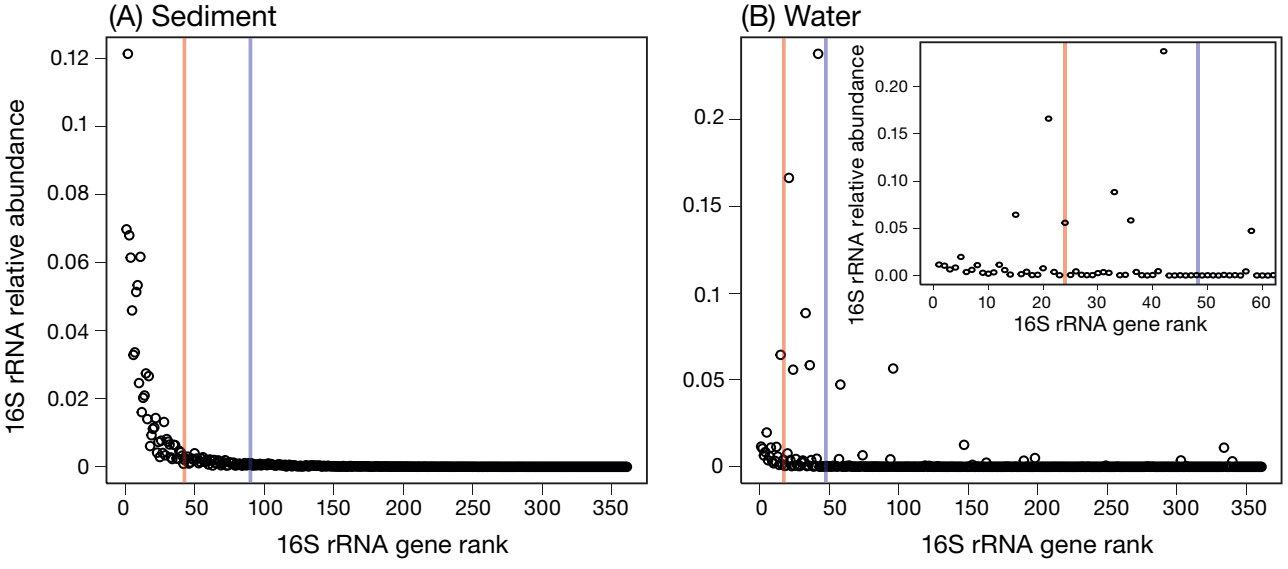

Fig. 7. 16S rRNA gene rank of the top 380 OTUs (comprising ~90% of the data) versus the relative abundance in 16S rRNA for microbial communities in (A) sediment and (B) water of a salt marsh pond. Inset of (B) highlights the top 60 OTUs in the water. Vertical red lines indicate taxa whose abundance in the total community is <1% and vertical blue lines indicate taxa whose abundance in the total community is <0.001%

different rank–abundance curves relative to the sediment (Fig. 7B). Typically, the most abundant taxa in the active water column community were in very low abundance in the total community (<1% total abundance) and of the rare taxa, 15% displayed significantly higher abundance in the active community relative to the total community (Chi-squared test, $p < 0.05$) suggesting a greater contribution of the rare biosphere to the structure of the active water community than to that of the sediment.

DISCUSSION

Our study examined the effect of short-term changes in environmental conditions on the total and active microbial communities in a salt marsh pond. Our analysis revealed differential responses of sediment and water column microbial communities to diel changes in environmental conditions. Sediments, while having significantly different total and active communities (Fig. 2A), displayed no temporal

changes in active microbial community composition or diversity. The lack of change in the active sediment communities may be due to the short duration of this study (48 h), which may be out of step with the duration of time it takes abiotic conditions in the sediments to significantly vary. In addition, stochastic assembly processes (Stegen et al. 2012, Nemergut et al. 2013) may decrease our ability to detect changes in sediment communities. However, the striking stability of the taxonomic composition of the total and active sediment communities (Figs. 4 & S3) and high degree of similarity between samples suggests this is not the case. Coastal sediments display shallow gradients in numerous abiotic parameters (Canfield et al. 1993) that can give rise to gradual changes in microbial community composition (Armitage et al. 2012). Our sampling method (top 2 cm) integrates over these gradients and may mask potential fine-scale changes that occur over shallow spatial scales. However, the sampling technique employed here (top 2 cm of surface sediment) was previously (Kearns et al. 2016) used to elucidate the dramatic effect of nitrogen enrichment on active salt marsh sediment bacterial communities, suggesting that this method should be robust to detect integrated large-scale changes in the active bacterial community within the pond. Finally, we documented highly abundant taxa from the *Chromatiales* in the sediment (~20% relative abundance) and water (~1.5% relative abundance) suggesting that as in vegetated marsh areas (Bolhuis & Stal 2011, Kearns et al. 2016), anoxygenic photosynthesizers from the order *Chromatiales* play an important role in the ecology of salt marsh ponds.

Conversely, the water column displayed highly variable active microbial diversity (Fig. S2B) and community composition (Fig. 2B). Our results suggest that cyclically changing environmental conditions can lead to rapid shifts in the active microbial community of the water column, while the relatively unchanging conditions found in the pond sediments promote more consistent structure across time for both active and total bacterial communities there. Changes in active community composition can be correlated with changes in transcriptional activity (Gilbert et al. 2010) suggesting that relatively static habitats, like pond sediments, likely promote consistent metabolic processes, while the microbial communities in dynamic habitats likely display variable metabolic output due to environmental changes.

There were large variations in the percentage of inactive taxa (Fig. 3B; 16S ratio < 1) in the overlying water. Of the 352 microbial orders present within the active community of the overlying water, 62% (n = 217) never achieved a 16S ratio greater than one, suggesting these taxa were likely dormant within this system. Several orders (n = 52) had 16S ratios that oscillated between <1 and >10 suggesting a respective increase and decrease in their activity over time in response to environmental conditions. These taxa were likely not in a true state of dormancy during times when their ratio was less than one since the cellular investment dormancy requires (Lennon & Jones 2011) is a longer-term ecological strategy. A final group of taxa (n = 82), had 16S ratios that cycled between <1 and 5 and their status as inactive (short durations of time with ratios less than one) or dormant (longer durations of time with ratios less than one) remains unclear because several taxa have been shown to need a 16S ratio greater than one to divide (Blazewicz et al. 2013). However, cell division is not the only form of activity a microbe can undertake; instead microbes may be performing biogeochemical processes or other cellular processes such as repair rather than dividing (Kirchman 2016). Our results highlight the difficulty in interpreting 16S ratios from metagenomic data, in particular for taxa with ratios that change rapidly. Further work on microbial dormancy is needed to better understand what the 16S ratio means for phylogenetically widespread taxa and to determine how the 16S ratio varies in different stages of growth such as division, metabolic activity, inactivity, and dormancy. This will allow us to better interpret not only the 16S ratio but also how changes in 16S rRNA community profiles correlate to physical measurements of microbial activity such as geochemical rates or enzyme-based assays.

Our results indicate that the active microbial community in the overlying water in marsh ponds was tightly controlled by diel cycles. The transcriptional activity of autotrophic and heterotrophic microbial taxa due to diel changes, typically driven by light, has been previously observed (Winter et al. 2004, Ito et al. 2009, Zinser et al. 2009, Tomasch et al. 2011, Ottesen et al. 2014) and their metabolic activity can be tightly coupled in aquatic systems (Gasol et al. 1998). The changes in the taxonomic composition of the active community observed in our experiment corresponded with changes in environmental conditions, which are, in turn, driven by the activity of the microbes themselves. In addition to responding to changes in the environment, active taxa in our study often appeared to respond to the activity of other taxa. For example, the activity of taxa highly abundant in the active community from the *Vibrionales* order closely followed the activity of *Cyanobacteria*

(Figs. 4 & 5), suggesting members of the *Vibrionales* may be involved with either predation of *Cyanobacteria* or consumption of cyanobacterial metabolic products, something also documented for seasonal cycles (Turner et al. 2009). Further, taxa from the order *Bacillales* were only active when oxygen concentration was lowest (22:00 to 06:00 h), suggesting that their oxygen requirements as anaerobes restricts their activity. The tight control of diel cycles on the active community composition in the overlying water highlights the importance of both abiotic conditions and bacterial interactions in determining microbial community structure and function.

We demonstrate that taxa from the rare biosphere (<1% total abundance) disproportionately contributed to the composition of the active community in the overlying water relative to the sediment (Fig. 7). Abiotic conditions within the overlying pond water did not appear to promote the activity of the most abundant taxa; rather, many of the most abundant taxa in the active community (*Cyanobacteria*, *Vibrionales*, *Bacillales*) were relatively rare in the total community. The rare biosphere is often thought of as a reservoir of low-abundance taxa that provides resilience in the face of environmental perturbations (Sogin et al. 2006, Pedrós-Alió 2012). Rare taxa can display dynamic patterns of occurrence along environmental gradients (Shade & Gilbert 2015) as well as dynamic abundances and activities on seasonal timescales (Campbell et al. 2011, Shade et al. 2014). Shade et al. (2014) recently demonstrated that rare taxa can significantly contribute to community turnover in many different ecosystems. Further, rare taxa can display transitory or persistent patterns within and between ecosystems (Shade & Gilbert 2015), which may allow them to respond to novel abiotic conditions and lead to pulses in their activity and changes in total community composition (Aanderud et al. 2015). Our results demonstrate that despite the enhanced activity of rare taxa, there was no net change in the total community, indicating that either these taxa were not producing biomass through their activity or not enough time had elapsed to alter total community composition. In addition, the high percentage of inactive taxa (Fig. 3), which was likely driven by the dynamic nature of the system, maintained a reserve of taxa (rare and abundant), allowing for future response to additional changes within the system.

In conclusion, our results highlight the rapid pace at which microbial communities can respond to changes in their environment. The dynamic environmental conditions observed in the overlying water promote changes in active taxa, while the relatively static conditions in the sediment promote stable communities, highlighting the importance of temporal sampling of active microbial communities in dynamic environments. Our results suggest that in communities that are in static environments or that have had time to adapt to a perturbation, the most abundant taxa are also the most active taxa. However, under dynamic conditions or perhaps during a transition, there is a disconnect between the presence and activity of taxa leading to high levels of inactivity of abundant taxa and an increase in activity of rare taxa. While the pond water column community and the abiotic conditions changed at a rapid pace, the mechanisms that control activity of the sediment community likely acted over longer time periods, and those static conditions promoted a stable active microbial community.

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