





The Effect of Organic Carbon Addition on the Community Structure and Kinetics of Microcystin-Degrading Bacterial Consortia

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Abstract: Microcystin (MC), a hepatotoxin that is associated with cyanobacterial blooms in freshwater lakes, threatens the quality of drinking water resources. Biodegradation of MC using biofiltration is emerging as a cost-effective solution for drinking water treatment. This study reports isolation of five MC-degrading microbial consortia and investigation of their community structure and kinetics in the presence or absence of a readily-bioavailable organic carbon source. The results indicated that the presence of a bioavailable organic carbon source caused: (1) the proliferation of community members previously unobserved in each consortium cultured without ethanol; (2) a shift in abundance of representative taxa; (3) a fluctuation in genera affiliated with MC-biodegradation; and, (4) a unique response in simulated diversity among consortia. These changes to each microbial consortium were paralleled by a significant decline in MC degradation kinetics. Overall, this study highlights the importance of integrating environmental conditions into the design and operation of biofiltration systems for MC biodegradation.

Keywords: microcystin; biodegradation; microbial interactions; community structure; biodegradation kinetics

1. Introduction

Harmful cyanobacterial blooms have increased in both frequency and severity worldwide as a result of climate change, population growth, and rapid urbanization [1]. These blooms are termed "harmful" in that many cyanobacteria are responsible for the production and release of toxins that are harmful to humans and existing aquatic ecosystems [2]. The most common freshwater genera of harmful cyanobacteria include *Anabaena*, *Nostoc*, *Oscillatoria*, *Planktothrix*, and *Microcystis*, which produce a suite of biotoxins, including microcystin (MC) [2]. MCs are a class of heptapeptides, which are formed from seven amino acids, in which their mono-cyclical structure imparts a high stability in the environment [3]. Although approximately 100 structural congeners have been identified, MC-LR (*L* and *R* standing for Leucine and Arginine, respectively, for two of the variable amino acids in the cyclical structure) is the most common and toxic MC [4,5]. Due to its acute toxicity, both the WHO (World Health Organization) and US EPA (United States Environmental Protection Agency) have adopted a guideline of 1 μ g/L of dissolved MC for drinking water [5].

MC-LR, along with other common structural variants observed in the environment (i.e., LA, RR), is water soluble, chemically-stable, and low in molecular weight, which renders it resistant to most conventional drinking water treatment processes [6]. Biological filtration (biofiltration), which relies on the development of biofilm communities on immobilized media (i.e., sand) to degrade cyanotoxins, has been proposed as an alternative, cost-effective, and sustainable drinking water treatment technology to target MC removal [7–13].

Previous studies have demonstrated the complete biodegradation of MC-LR within laboratory scale biofiltration systems following a preliminary lag phase of removal [14–17]. However, the toxin removal efficiency and extent of this lag phase can vary significantly with bacterial composition, nutrient concentration, and other environmental parameters that are associated with the source water. Efforts to evolve biofiltration from a passive process into a more standardized, controlled, and perhaps "engineered", biological treatment process for targeted removal of pollutants will require a better understanding of the physiology and genetics of MC-degrading bacteria [12–14,18–20].

Significant efforts have been made to isolate and characterize the specific MC-degrading bacterial populations during algal bloom events in the source water and sediments, and from full scale biological treatment units in drinking water treatment facilities [7–9,17,21–24]. However, these studies have not fully explored how these isolates function in mixed bacterial communities, nor the influences of mutualistic or antagonistic interactions on biodegradation kinetics. Several previous studies, however, have considered the effects of environmental stimuli (i.e., varying organic carbon concentrations) on MC-degrading bacterial consortia under aerobic conditions [7,8,23,25–29]. Research showed that addition of organic carbon sources (i.e., glucose, acetate, or uncharacterized dissolved organic carbon (DOC)) significantly inhibits MC degradation kinetics of bacterial consortia [7,23,25,29]. Catabolite repression is postulated as a mechanism underlying this inhibition, where MC-degrading populations may prefer more energetically-favorable (easily metabolized) over more energetically-intensive substrates, such as MC [30]. Other (fewer) studies have demonstrated that the addition of an alternative organic carbon source facilitates MC degradation rates, where the organic carbon was postulated to stimulate the growth and energy availability for production of the necessary enzymes required for MC-degradation by these bacterial populations [31,32] In addition, several studies have reported a net neutral effect with the addition of alternative organic carbon sources [7,8,26,27]. The community structure of the microbial consortia that was involved in MC biodegradation was shown to change with environmental conditions [8,27,33,34]. For example, Mou and co-workers [33] reported that Methylophilales (Methylotenera genus) and Burkholderiales (Bortadella, Burkholderia, Cupriavidus, Ralstonia genera) were the most significant taxa in microcosms containing MC. There was significant enhancement of the broad class of metabolic functions when these MC-degrading populations were exposed to MC [33].

Consequently, insight into the changes in community structure in response to a shift in environmental condition, and the influence of such change on MC degradation kinetics can better inform engineered strategies to improve MC removal under various environmental conditions. In this study, we report the comparison of community structure of five MC-degrading consortia in the presence or absence of organic carbon besides MC. We asked, "how and to what extent does the MC-degrading community structure and degradation kinetics change in response to an organic carbon addition?" We expect that the MC degradation kinetics of the consortia will be strongly driven by changes in the community structure with the addition of an exogenous carbon source, as changes to the presence or absence of community members should directly correlate with changes in representative specific metabolic pathways that are involved in MC degradation.

2. Materials and Methods

2.1. Isolation of Microcystin-Degrading Bacterial Consortia

One lake surface water sample (LSB) and four bed sediment samples (10B, 11B, 12B, 14A) were collected from a drinking water reservoir in Southern California that experiences periodic cyanobacterial blooms. Bed sediment samples were centrifuged (10,000 g for 10 min) to remove coarse sands. Both the surface water and supernatant from the sediment samples were filtered through a 1 μ m pore size glass fiber filter to remove large protozoa and zooplankton potentially present in the samples.

The initial isolation of each consortium (i.e., Generation A) involved the addition of 5 mL of the filtrate from each environmental sample into a flask containing 20 mL of M9 minimal media (per liter

media contains: 12.8 g of Na₂HPO₄-7H₂O, 3.0 g of KH₂PO₄, 0.5 g of NaCl, 1.0 g of NH₄Cl, 0.25 g of NaNO₃, 0.002 g of MgSO₄, 0.001 g of CaCl₂, pH of 7.3) with 200 μ g/L of MC-LR, and 316 mg/L of ethanol. The composition of the initial medium used for isolation was kept consistent with the composition used for enrichment to effectively acclimatize each consortium to the conditions expected during laboratory culturing. This isolation period, which was maintained for seven days prior to further enrichment, also allowed for each consortium to consume any residual organic carbon present in the initial samples that may have affected batch biodegradation results that were acquired during future experimentation.

To enrich for MC degradation consortia, two parallel tracks of experimentation were conducted (Generation B) (Figure 1). The first track involved the addition of 5 mL of the previous culture (Generation A) into a flask containing 20 mL of M9 minimal with 200 μ g/L of MC-LR (a congener common to Southern California lakes) [35]. The seeding concentration of MC (200 μ g/L) represented a higher range than that typically detected in the environment but it was within the range of commonly used concentrations in enrichment studies to elicit a detectable response from the isolated consortia. The second parallel track of enrichment cultures (again with 5 mL of previous culture) was set up to include 316 mg/L of ethanol in addition to the M9 media and MC-LR, to serve as the comparison of MC biodegradation kinetics and community composition of each MC-degrading consortia. We expect that predicted MC-degrading half-lives and changes in the composition and abundance of the bacterial consortia will be the most effective and practical means to compare changes among MC-LR-degrading communities in the presence and absence of ethanol.



Figure 1. Flow-chart of the experimental design employed in this study. Following initial isolation of 5 consortia from different locations within a reservoir, a parallel track of enrichment was set up to investigate the effect of ethanol addition on MC-LR biodegradation kinetics. The letters (A-U) indicate the relative generation age of each consortium during each track of enrichment (1–2).

The selection of ethanol as an organic carbon substrate was two-fold: (1) MC-LR was supplied in ethanol from the manufacturer (Cayman Chemical), thus it requires no further purification to include ethanol as an organic supplement; (2) ethanol is an energy-excess substrate (compared to glucose). Ethanol is readily metabolized by most bacteria in the environment and potentially serves as a preferential substrate, as indicated in multiple studies [36,37]. The use of ethanol represents the availability of labile and simple forms of dissolved organic carbon that become readily available in freshwater ecosystems during harmful algal bloom (HAB) periods [38,39]. As labile, dissolved organic carbon does not solely originate from cyanobacteria during these bloom periods, the concentration of ethanol (316 mg/L) was set to a similar order of magnitude to resemble the higher range of total dissolved organic carbon concentrations that were reported by the USA EPA's National Lake Assessment survey (ranging from 2-516 mg/L) [40]. This concentration of ethanol is reflective of dissolved organic carbon concentrations expected during worst case bloom conditions in lakes or reservoirs throughout the USA. In addition, this concentration of ethanol was considered to be non-inhibitory based on a thorough comparison of ethanol tolerances across common bacteria in the environment (i.e., E. coli), which indicated that the selected concentration was approximately 79–132 times lower than the threshold of growth inhibition [41–44].

Sub-culturing of the isolated bacterial consortia involved transferring 5 mL of the previous culture into a new sterile tissue flask with 20 mL of M9 minimal media spiked with 200 μ g/L of MC-LR with or without ethanol. As MC was initially supplied in ethanol, a temperature controlled (4 °C), rotary evaporator was used to obtain ethanol free suspensions of MC (for Track 1 enrichment cultures only). Sub-culturing frequency was intentionally kept to a minimum (every two weeks) to prevent potential shifts in microbial community composition prior to performing batch degradation experiments; thus, each consortium was transferred approximately twenty times (Generation U) (Figure 1). It is important to note that, although five consortia were initially isolated (Generation A: 10B, 11B, 12B, 14A, LSB), ten distinct consortia were ultimately obtained with the experimental enrichment conditions employed. We make the important distinction between consortia enriched with (i.e., 10B-WE) or without ethanol addition (10B-NE) to avoid any further confusion.

MC concentrations in the flasks were monitored using an ADDA-ELISA kit (ABRAXIS, Warminster, PA, USA) to evaluate the MC degradation potential of the enriched samples. UPLC-MS/MS (Waters Quattro Premier QqQ) was also used initially for the quantification of MC. The correlation coefficients for all ELISA and UPLC-MS/MS tests ranged from 0.99 to 1, indicating that the results for each assay were reproducible (Figures S1 and S2). In addition, ELISA and UPLC-MS/MS measurements for initial experiments indicated good agreement (data not shown). Only the ELISA kit was used later for MC detection and quantification due to the high sensitivity at the low MC concentration range employed (0.1 μ g/L detection limit). The flasks that showed a significant removal of MC (at or below the detection limit) within the seven-day enrichment period were deemed as MC-degrading consortia and used in later investigations.

2.2. Batch Degradation Experiments

Batch degradation experiments were carried out to quantify the MC degradation kinetics in each consortium using three experimental replicates. Culture conditions during batch degradation tests were identical to sub-culturing conditions, except for an additional washing step, in which the 5-mL consortium culture was pelleted and washed three times to remove any residual MC or ethanol present in the transfer culture. The washed cells were finally re-suspended in 5 mL of sterile M9 media and were transferred to the testing flasks. The initial optical density (OD at 600 nm) for experiments with and without the presence of ethanol was 0.002 and 0.0002, respectively. Although there was a detectable change in OD for the consortia amended with ethanol, the consortia without the addition of ethanol had a negligible change in OD. Flow cytometry was used to verify the growth of bacterial cells for each consortium cultured without the addition of ethanol (see Supplementary Materials, Section 2 for details).

All of the flasks were shaken at 120 rpm in the dark at ambient temperature (24 $^{\circ}$ C), where 500 μ L of sample was taken every 24 h for an eight-day period to quantify the concentration of MC-LR using an ADDA-ELISA kit following the manufacturer-recommended protocols.

2.3. Kinetic Model of Microcystin Biodegradation

Visual examination of the MC degradation kinetics revealed a 24-hour lag phase for all batch experiments, which indicated that simple zero or first order kinetics were not adequate to predict MC degradation kinetics. A bi-phasic kinetic model that was proposed by Ouiroga and co-workers [45] for the biodegradation of surfactants was adopted to model the removal of MC by each consortium as a function of time because it directly accounted for bacterial growth on MC along with substrate (MC) consumption (Equation (1)). In this model, the substrate utilization kinetics depend on the concentrations of degrading microorganisms (*X*), the substrate available (*C*), and the rate of substrate consumption (*K*) by degrading microbes to produce new biomass and other products.

$$\frac{dC}{dt} = -KCX \tag{1}$$

The analytical solution to Equation (1) results in (see Supplementary Materials, Section 4 for derivation):

$$C = \frac{h(S_0 - q) - q(S_0 - h)e^{pt}}{(S_0 - q) - (S_0 - h)e^{pt}}$$
(2)

where the substrate MC concentration (*C*) is related to its initial concentration (S_0), the maximum MC available for biodegradation (h, in μ g/L), the non-biodegradable portion of MC (q, in μ g/L), and the maximum specific growth rate of the degrading microorganisms (p, in 1/day).

The fitting of the experimental data to the bi-phasic kinetic model was carried out using the $DREAM_{ZS}$ (Differential Evolution Adaptive Metropolis, sampling from past states) (v1.0) software package [46,47]. Details of this approach are presented in the Supplementary Materials, Section 5. The goodness of the model-data fit was compared through the assessment of two quantitative metrics, the r^2 value and the RMSE (root mean square error), as calculated during the fitting process. The distribution in posterior parameters derived from $DREAM_{ZS}$ was used to estimate the MC degradation half-lives for comparison among different consortia and under different conditions.

2.4. Analysis of Bacterial Community Structure

The bacterial community structure of each bacterial consortia was analyzed using the 16S rRNA gene amplicon on a Roche 454 genome sequencer FLX+ (454 GS-FLX+, RTL Genomics, Lubbock, TX, USA). The pyrosequencing analysis was conducted immediately following one particular replicate of the eight-day batch biodegradation experiments. A 1 mL sample volume of the batch degradation experiment was pelleted (10,000 g for 15 min), supernatant discarded, and shipped on dry ice to the RTL laboratory for further analysis. Both the DNA extraction and amplicon sequencing were performed by RTL Genomics (Lubbock, TX, USA). The 16S rRNA gene universal eubacterial primers 939F (5'-TTGACGGGGGCCCGCACAAG-3') and 1492R (5'-TACCTTGTTACGACTT-3') were used to amplify approximately 550 bp of the variable regions V6 to V8. The pyrosequencing reads were analyzed using Quantitative Insights into Microbial Ecology (QIIME) (v. 1.9.1) [48], following the program's instruction. The bacterial community structure and taxonomic abundance for samples with and without the presence of ethanol were compared using the statistical analysis of taxonomic and functional profiles (STAMP) bioinformatics software (v.2.1.3) [49]. The statistical significance of the taxonomic abundance on the order level between the samples was determined using a two-sided G-test with Yates and Fisher's correction and Bonferroni multiple test correction. The difference in taxonomic abundance was deemed to be significant if the calculated *p*-value between the samples was below 0.05. Rarefaction plots, Beta diversity metrics (unweighted/weighted Unifrac distances), and

jackknifed Beta diversity (principal coordinate analysis) were carried out using scripts provided by the QIIME module.

"True" as opposed to "raw" Alpha diversity indices were adopted in this study to facilitate comparisons of the abundance and presence or absence of species within the MC degrading communities with or without ethanol addition [50–54]. Common "raw" Alpha diversity measures, such as Shannon entropy, evenness, or Simpson's dominance suffer from difficult interpretation and comparison, since they do not share a common mathematical grounding (i.e., common units, derivation) or favorable mathematical properties (i.e., the "replication" principle) [55]. In a seminal study, Hill [56] introduced a unified basis of calculation to transform the existing "raw" measures to "true" Alpha diversity measures with the advent of a single variable: the diversity order, q (Equation (3), where S is the total number of species and p_i is the relative proportion of the *i*th species within a given community). Lower diversity orders (q < 0) place more emphasis on the frequency of rarer species within a given population, whereas higher diversity orders (q > 1) weigh the frequency of dominant species more heavily [50,55,56]. When *q* is equal to unity (corresponding to Shannon entropy), an equal weight is placed on the frequency of rare and dominant species [50,55,56]. Intuitively, a zero-order (q = 0) diversity value corresponds to the species richness within a given community [50,55,56]. It is important to note that the units of all true Alpha diversity orders are in number of species (or genera, depending on the taxonomic level), also termed "effective number of species", which provides a unified basis for comparison across diversity orders [55].

$$D^{q} = \left(\sum_{i=1}^{S} p_{i}^{q}\right)^{\frac{1}{(1-q)}}$$
(3)

As biological or technical replicates of the metagenomic analyses were not conducted in this study, a series of Monte Carlo (MCA) simulations was conducted to simulate the statistical variability in "true" Alpha diversity indices. These simulations were based on an empirical, non-linear relationship that was developed between the taxon mean relative abundance and standard deviation of replicate measurements that were reported across similar metagenomic analyses in the scientific literature (see Supplementary Materials, Section 6, Table S5, Figures S7 and S8). After running a sufficiently large number of MCA simulations (N = 20,000), normal distributions in true Alpha diversity metrics across several diversity orders (q = -1, 0, 1, 2, 3) were reached (Figures S9 and S10). For each MC degrading community, the significance of changes in true Alpha diversity metrics (across all diversity orders) with the addition of ethanol were simultaneously assessed using a one-way analysis of variance (ANOVA) with Bonferroni multiple comparison test correction [57]. In addition, both the direction and magnitude of the differences between the resulting statistical distributions of the Alpha diversity metrics were quantified using an effect size (ES) calculation (Equation (4), where $\mu_{we/ne}$ and $\sigma_{we/ne}$ refer to the mean and standard deviation of normal distributions with or without ethanol addition) [58].

$$ES = \left(\frac{\mu_{we} - \mu_{ne}}{\sqrt{\frac{\sigma_{we}^2 + \sigma_{ne}^2}{2}}}\right) \tag{4}$$

Metagenomic stability, which is defined as the persistence of individual operational taxonomic units (OTUs) across different MC degrading communities profiled, of the microbial communities was evaluated using the QIIME generated output (OTU vs. abundance) from the genera level of analysis (after removing OTUs that comprised less than 0.1% of the total communities) [59,60]. Stability values were simply the coefficient of variation (CV) ((σ/μ) × 100%) of the abundance across each microbial community with or without ethanol addition, where the values for OTUs less than 200% are considered to be stable [60]. In this study, we report an aggregate stability value for MC degrading consortia

(with or without ethanol) by calculating the proportion of OTUs that are statistically "stable" out of the total number of OTUs present.

3. Results

3.1. Microcystin Biodegradation Kinetics

As shown in Figure 2, all five isolated consortia possess the ability to degrade MC within three to seven days in the presence or absence of ethanol with a one-day lag phase. This degradation was clearly biologically mediated as control experiments (without bacterial inoculum) demonstrated slow and relatively insignificant MC removal over the eight-day period investigated (Figure S4). The shape of the MC-LR degradation curves was nearly identical for each isolated consortium across three experimental replicates (shown by the small standard deviation for each point of measurement), indicating that the degradation kinetics were consistent and repeatable. Although multiple measurements were taken within the first 24 h, only measurements that were taken at evenly spaced 24-hour intervals were included in Figure 2 to reduce the noise in the graphs. For consortia 12B and 14A without ethanol, a sharp decline in MC concentrations at day 2 was followed by a rapid rebound at day 3. Notably, this trend was apparent for all experimental replicates and was it not considered an error in measurements. It is also important to mention that although most of the MC concentrations appear in Figure 2 to be near zero after four days, the substrate concentrations rarely approached the limit of detection $(0.1 \ \mu g/L)$. These results suggest that a portion of the MC substrate was potentially non-biodegradable. Similar results were presented by Eleuterio and Batista [23] while using an ELISA quantification method, with measured MC residuals ranging up to $2 \mu g/L$.

OD600 and flow cytometry results confirmed cell growth during the degradation experiments for MC consortia with and without ethanol addition, respectively. Flow cytometry results indicated that initial cell concentrations were on the order of 10^6 – 10^7 for consortia without ethanol addition (cells/mL). However, growth rates were highly variable across individual replicates for consortia without ethanol addition, with final cell concentrations ranging from 10^8 – 10^9 cells/mL (Figure S3).

The bi-phasic model accurately predicted the experimental results, as noted by the narrow widths of the 95% total predictive uncertainty intervals and the close proximity of the experimental data points to the best fitting solutions for all consortia analyzed (Figure 2). The r^2 is approaching 1 in 8 of the 10 degradation experiments, indicating a good fit of the model to the experimental data (Figure 2, Table S2). Wider uncertainty intervals were observed for consortia 12B and 14A under some of the testing conditions, with r^2 ranging from 0.68 to 0.79, indicating less accurate model predictions. The wider predictive uncertainty intervals were attributed to unknown factors that were not currently considered in the model structure. The parameter estimability was relatively high across all experiments (as indicated by the defined shape of posterior parameter distributions); however, significant correlations were observed between the *h* (maximum available MC for degradation) and *p* (maximum specific growth rate) parameters, suggesting non-unique values of these parameters for these cases (Table S4, Figure S6).



Figure 2. MC-LR biodegradation by microbial consortia isolated from five environmental samples (10B, 11B, 12B, 14A, and LSB) in the absence (NE—no ethanol) (**A**) or presence (WE—with ethanol) of ethanol (**B**). Error bars are standard deviations for three experimental replicates at each measurement point (excluding t_0 and t_{24}). Light grey shading indicates the 95% total predictive uncertainty intervals of the kinetic model, while the red line indicates the best fit of the model to the experimental data.

The presence of ethanol slowed the degradation kinetics of MC-LR in all five consortia, as indicated by the longer respective mean half-lives (Table 1). This decline in MC biodegradation kinetics was statistically-significant for all consortia (p-value $< 1 \times 10^{-8}$) based on the distribution of half-lives that was generated by the DREAM_{ZS} model-data fitting procedure (N = 25,000 samples). Greater variations in predicted degradation kinetics were observed in some cases (i.e., 12B and 14A without ethanol), as indicated by the moderately high standard deviation of predicted half-lives (Table 1).

Compartie ID	Half-Li	t-Test		
Consortia ID	NE	WE	<i>p</i> -Value	
10B	1.98 ± 0.003	3.28 ± 0.371	$< 1 \times 10^{-8}$	
11B	2.14 ± 0.017	2.30 ± 0.016	$< 1 \times 10^{-8}$	
12B	2.74 ± 0.765	3.37 ± 0.367	$< 1 \times 10^{-8}$	
14A	2.14 ± 0.835	2.47 ± 0.046	$< 1 \times 10^{-8}$	
LSB	2.16 ± 0.016	4.41 ± 0.307	$<1 \times 10^{-8}$	

Table 1. Summary of half-lives and *t*-test significance for MC-LR degrading consortia without ethanol (NE) and with (WE) ethanol addition. The mean and standard deviation of the posterior distributions of half-lives are provided for reference.

3.2. Community Analysis of Microcystin-Degrading Consortia with and without Ethanol Addition

Pyrosequencing yielded, on average, greater than 2000 reads per sample (Table S9). Rarefaction curves indicated that the sequencing depth was adequate to accurately characterize the microbial communities in each consortium (Figure S12). As shown in Figure 3A, the taxonomic composition and relative abundance of each MC-degrading community varied by the sampling location. In descending order, *Pseudomonodales, Burkholderiales, Xanthomonadales,* and *Rhizobiales* were the most representative bacterial orders in the degrading consortia in the absence of ethanol (Figure 3A). *Rhizobiales* was a more representative order in the consortia isolated from bed sediment (10B, 11B, 12B, 14A), as compared to the lake water (LSB). However, *Xanthomonadales* was more representative in the lake water consortium as compared to the sediment consortia (Figure 3A). In addition, *Rhodospirillales* dominated the community composition of the 12B sediment consortia as compared to other sediment consortia.

In the presence of ethanol, the general taxonomic composition and relative abundance of each MC-degrading community changed noticeably, especially for the 10B and 12B consortia (Figure 3B). Specifically, the presence of bacterial populations within the *Rhizobiales* order declined greatly in the 10B, 11B, and 12B consortia. Moreover, the *Rhodospirillales* order declined to a great extent in the 12B consortium cultured with ethanol (Figure 3B). The relative abundance of the order *Burkholderiales* was noticeably depressed (and replaced by either *Xanthomonadales* or *Pseudomonadales*) in the 10B and LSB consortia cultured with ethanol. Relative abundances of *Xanthomonadales, Burkholderiales, Sphingomonadales*, and *Caulobacterales* increased considerably in the 14A consortium that was cultured with ethanol (Figure 3B). In addition, previously unobserved taxa within the orders of *Actinomycetales, Flavobacteriales, Cytophagales, Sphingobacteriales, Rhodobacterales*, and *Euglenozoa* were detected in the 12B consortium treated with ethanol (Figure 3B). Comparably, previously unobserved bacterial populations within the orders *Chlamydiales, Cytophagales, Rickettsiales, Legionellales,* and *Sphingobacteriales* were primarily detected in the 10B consortium treated with ethanol (Figure 3B).



Figure 3. General taxonomic differences in MC-LR degrading consortia isolated from lake sediment (10B, 11B, 12B, 14A) or surface water (LSB) analyzed on the order level in the (**A**) absence (NE) or (**B**) presence (WE) of ethanol. The pyrosequencing analysis was conducted immediately following the eight-day batch biodegradation experiments.

Statistically-significant differences in taxonomic composition and abundance were observed when comparing the consortia cultured in the presence or absence of ethanol (Figure 4). For most of the MC-LR degrading consortia (three out of five consortia or above), the dominant community shifts in the presence of ethanol included an increment in bacterial orders of *Xanthomonadales*, *Pseudomonadales*, and *Burkholderiales*, and a decrement in *Sphingomonadales*, *Rhodospirillales*, and *Rhizobiales* (Figure 4).



In addition, consortia with ethanol had many new community members not found in the absence of ethanol, including the bacterial orders *Cytophagales*, *Sphingobacteriales*, *Flavobacteriales*, *Saprospirales*, *Chlamydiales*, *Rhodobacterales*, *Rhodocyclales*, *Methylophilales*, *Legionellales*, and *Rickettsiales* (Figure 4).

Figure 4. Statistically-significant differences in relative abundance of the phylogenetic orders for all MC-LR-degrading consortia treated with (WE) and without ethanol (NE) (*p*-value < 0.05, Bonferroni corrected). Error bars represent 95% confidence intervals for relative abundances. X-axes are split to compare differences in relative abundances among isolated consortia. Y-axes are split for the 11B and 14A consortia to better visualize changes of underrepresented taxa.

Simulated distributions in Alpha diversity metrics achieved from the MCA analysis were used to interpret changes in the within consortium bacterial community taxonomic composition and abundances in the presence of ethanol (Table 2). The range in *p*-values resulting from the multiple comparison test ($<1 \times 10^{-8}$ to 4.80×10^{-8}) demonstrated a statistically significant difference in simulated true Alpha diversity metrics across all diversity orders for the 10B, 12B, 14A, and LSB consortia in the presence of ethanol (Table 2). However, differences in simulated true Alpha diversity metrics were not statistically significant for the higher diversity orders (*q* = 2 and 3) when comparing the 11B consortium (Table 2). In addition, the aggregate metagenomic stability of consortia, or the persistence of different OTUs across communities, increased for MC degrading communities in the presence of ethanol (from 53.7% to 65.7%, not depicted in Table 2).

Diversity Order	Consortia ID				
	10 B	11B	12B	14A	LSB
D ⁻¹	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$	$<1 \times 10^{-8}$	$< 1 \times 10^{-8}$	$<1 \times 10^{-8}$
D^0	$< 1 \times 10^{-8}$	$4.80 imes10^{-8}$			
D^1	$<1 \times 10^{-8}$	$5.04 imes10^{-5}$	$< 1 \times 10^{-8}$	$<1 \times 10^{-8}$	$< 1 \times 10^{-8}$
D^2	$<1 \times 10^{-8}$	1	$< 1 imes 10^{-8}$	$< 1 \times 10^{-8}$	$<1 imes10^{-8}$
D^3	$<1 \times 10^{-8}$	1	$< 1 imes 10^{-8}$	$< 1 imes 10^{-8}$	$<1 imes10^{-8}$

Table 2. Summary of significant differences (*p*-values) in Alpha diversity metrics between MC-LR degrading bacterial consortia with and without ethanol addition as a function of diversity order.

To address how and to what extent the true Alpha diversity indices were shifting with ethanol treatment, both the direction and magnitude of changes in the simulated distributions of true Alpha diversity metrics were investigated by incorporating an effect size calculation. Figure 5 compares

the direction and magnitude of the effect sizes calculated between distributions in simulated Alpha diversity metrics with and without ethanol addition. The diversity orders are grouped into classes that give heavier (q = -1, 0), equal (q = 1), and lesser (q = 2, 3) weight to the frequencies of rare over abundant genera in each calculation.

The results of the effect size calculations indicated that, in the presence of ethanol, there was an increase in simulated Alpha diversity for the sediment consortia (10B, 11B, 12B, 14A) and a decrease in simulated Alpha diversity for the lake water consortia (LSB) when both rare and abundant genera were weighted equally (q = 1, "neutral"), which is equivalent to Shannon's entropy value [55]. The magnitude of this shift in Alpha diversity (q = 1) varied across each consortium, with the lake water consortium (LSB) demonstrating the largest change in magnitude (and 10B/11B the smallest change) (Figure 5). For the sediment consortia (10B-14A), there was a mixed response in the direction and magnitude of the shifts in lower (q = -1, 0) and higher order (q = 2, 3) diversity indices. Lower order diversity indices for both the 10B and 12B consortia increased by a similar level of magnitude, whereas higher order diversity indices increased for the 11B, 12B, and 14A consortia, albeit by different magnitudes (Figure 5). Comparably, a large depression in higher order diversity indices was observed for the lake water consortia (LSB) (Figure 5). These results signified that changes to the overall Alpha diversity of each consortium were attributed to: (a) more rare phyla for 10B and 12B consortia, as well as (b) more abundant phyla for 14A and LSB consortia. Due to the lack of statistical significance in higher order indices (Table 2) and similar magnitude changes among low and high diversity orders, inconclusive results were obtained for the 11B consortium (Figure 5).



Figure 5. Differences in effect size (direction and magnitude) as a function of true Alpha diversity order comparing MC-LR-degrading consortia treated with (WE) and without ethanol (NE). To clarify, the effect size is the difference in means of the distributions in diversity orders calculated for communities with ethanol as compared to without, normalized by the "average" standard deviations

from both distributions $\left(\frac{\mu_{we}-\mu_{ne}}{\sqrt{\frac{\sigma_{we}^2+\sigma_{ne}^2}{2}}}\right)$. Error bars represent 95% confidence intervals calculated for the effect sizes [58]. The diversity orders are grouped (and color coded) according to the weight placed on the frequency of rare versus abundant species during calculation. For example, diversity orders that place heavier weight on the frequencies of rare over abundant genera in each calculation (q = -1, 0) are colored in green.

Beta diversity metrics were analyzed to compare differences in the bacterial phylogenetic composition between consortia degrading MC-LR with and without ethanol (Table 3). Statistically significant differences were observed in the phylogenetic composition for 10B, 12B, and LSB-isolated consortia that were treated with and without ethanol (p-value < 1 × 10⁻⁸). However, the phylogenetic composition was not statistically significant when comparing the 11B and 14A consortia with and without the presence of ethanol (p-value = 1) (Table 3). These consortia demonstrated the highest phylogenetic similarity (p-value = 1) out of all the pairwise comparisons, indicating that the taxonomic composition of some MC-degrading consortia might be less sensitive to changes in the availability of alternative organic carbon sources (Table 3). Overall, the majority of consortia that were treated with ethanol (3/5) demonstrated statistically significant changes in phylogeny, which suggests that the composition of MC-degrading communities in the environment will be affected by the availability of alternative organic carbon sources.

Table 3. Summary of Beta diversity metrics and significance testing for MC-LR degrading bacterial consortia without (NE) and with (WE) ethanol addition. Beta diversity metrics were computed between each consortium (i.e., 10B) with and without the presence of ethanol. The corrected Bonferroni p-value was used to ascertain whether the phylogenetics of each consortium were in fact statistically significant.

Consortia ID	Unweighted Unifrac Distance	Weighted Unifrac Distance	Bonferroni Corrected <i>p</i> -Value
10B	0.957	0.218	$< 1 \times 10^{-8}$
11B	0.425	0.075	1
12B	0.921	0.382	$< 1 \times 10^{-8}$
14A	0.775	0.218	1
LSB	0.561	0.112	$< 1 \times 10^{-8}$

4. Discussion

This study has successfully isolated five MC-degrading bacterial consortia from lake surface water and bed sediments in the absence of a cyanobacterial bloom. The metagenomic analyses indicated highly diverse and distinct bacterial populations in each consortium, suggesting that many different MC-degrading and non-degrading populations may co-exist in each consortium. A comparison of common genera observed in the pyrosequencing results (in the absence of ethanol) to MC-degrading isolates reported in the literature indicated that species of the genera *Sphingopyxis, Sphingomonas, Acinetobacter, Aeromonas, Novosphingobium Pseudomonas, Stenotrophomonas, Ochrobactrum, Rhodococcus,* and *Steroidobacter* in the consortia may potentially comprise the MC-degrading populations (Figures S13–S16, Table S10) (see [26] for an updated summary). Of these genera identified, *Sphingopyxis, Sphingomonas, Stenotrophomonas,* and *Novosphingobium* have been affiliated with MC degrading species utilizing the well-known *mlr* gene pathway (Table S10). However, this identification of degrading populations is only tentative because MC-degradation by alternative pathways (other than *mlr*) may also exist, but are currently unknown [33,61]. As these alternative pathways are still yet to be determined, MC-degradation may be performed by a much greater number of bacterial genera than those that were identified in past research.

A comparison of the MC-degradation rates observed in this study with a compilation of half-lives reported for both MC-degrading bacterial isolates (n = 146) and microbial consortia (n = 167) indicated comparable degradation rates with those reported for consortia (Figure S18). However, the range of half-lives observed in all consortia including the present study are longer than those of MC-degrading bacterial isolates reported previously (Figure S18). In comparison with previous study designs that were focused on MC degrading microbial consortia [23,34,61], this study is significantly different in which we have isolated the MC-degrading consortia by sub-culturing in minimal media enriched with MC. We have demonstrated the stability and unique individual characteristics of each isolated consortia for MC-degradation. These isolated consortia provide the opportunity to identify the effect

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of specific environmental conditions on MC-degradation kinetics without other interfering factors that could skew the results (i.e., the presence of uncharacterized dissolved organic matter from field samples or algal extracts). This approach was advantageous, because it allowed a parallel track of manipulation of consortia, where we could simultaneously enrich consortia with and without the presence of an organic carbon source that was supplemented with MC as the sole carbon/energy source from the onset of isolation to the batch degradation experiments performed. The large range of degradation rates reported in the literature likely reflects the complexity of environmental conditions and degradation community responses.

As the complexity of the environmental conditions was tightly controlled in this study, the main driver of observed differences in MC degradation kinetics was the initial diversity of each microbial community. High diversity, of both potential MC degrading and non-degrading populations alike, ultimately led to the isolation of distinct bacterial communities with rather unique MC biodegradation kinetic profiles. Ultimately, the trophic status of the lake of origin (i.e., productivity and nutrient availability) is an integral factor affecting bacterial community diversity [62–66]. Although true diversity indices were presented in the results, the "raw" Shannon H values that were calculated from microbial communities in this study for both lake and sediment samples were on the same order of those reported in meso to fully eutrophic lake systems (i.e., 3–4) (Table S8) [62–66]. These results may suggest that MC-degrading bacterioplankton community diversity may increase with trophic status (from oligo to fully eutrophic) of freshwater lakes. However, as indicated by [67–69], the relationship between trophic status and community diversity of bacterioplankton is not so clear-cut, as other studies have reported contradictory trends [68,69]. Furthermore, it is still unclear how diversity may influence MC biodegradation kinetics in the environment as the temporal stability of these bacterioplankton communities is relatively unknown (i.e., do they achieve a relative steady state?). It is more likely that in an open system, such as the environment, the influence of community diversity on MC biodegradation may be diminished as highly variable environmental factors (such as zooplankton predation or hydrodynamics) result in rapid succession, growth, and elimination of different populations comprising the MC-degrading community.

Ethanol addition resulted in a statistically significant increase in the simulated first order true Alpha diversity metrics for all sediment consortia along with a statistically significant decrease in first order metrics for the lake water consortium (LSB). As this first order index equally accounts for the frequency of rare and abundant genera in its calculation, it is often viewed as a surrogate of overall changes in community Alpha diversity (similar to Shannon entropy) [55]. More detailed examination of changes in the lower and higher order indices indicated that changes in the overall diversity were more affiliated with either changes in the abundance and/or presence of rare genera (for 10B and 12B) or dominant genera (for LSB and 14A). Notably, differences in true diversity orders also provide evidence as to the relative importance of species evenness and richness components to the overall diversity of each community [55]. For example, as the order of diversity approaches positive infinity, the richness component of diversity in the mathematical calculation becomes completely insignificant (and vice versa when approaching negative infinity) [55]. This interpretation suggests that changes in the overall diversity of LSB and 14A consortia were likely more influenced by changes in community evenness as opposed to richness (and vice versa for 10B and 12B consortia). In the case of the LSB and 14A consortia, the observed change in diversity (attributed to evenness over richness) either resulted in an increase in the dominance of certain community members or an increase in equitability among certain community members, respectively (which is supported by diversity profiles presented in Figure S11).

This wide variety of simulated responses in Alpha diversity metrics across each consortium was unique, as it defined several potential scenarios that organic carbon addition could trigger within MC degrading communities, which may include: (a) an increase in equitability of certain members within the community (as in 14A); (b) an enrichment (increasing richness) of previously unobserved community members (as in 10B or 12B); (c) the dominance of a few or more representative

taxa within a given community (LSB); or, (d) resilience to changes in community composition (11B). Statistically significant differences in Beta diversities for 10B and 12B consortia further support the notion that the richness increased for these consortia, resulting in MC-degrading communities that were phylogenetically dissimilar with and without the addition of ethanol. It is also important to mention that, as the metagenomic stability increased with the addition of ethanol, the proportion of OTUs shared across each consortium also increased, suggesting that some of these previously unobserved community members (comprising this increase in richness) were of similar phylogenetic identity among both the 10B and 12B consortia. Moreover, the lack of a significant change in higher order Alpha diversity metrics (q = 2, 3) and Beta diversity metrics for 11B confirms some form of resiliency for the 11B consortium to changes in supplemental organic carbon concentrations.

Changes to the Alpha diversity metrics of each community have critical implications in terms of the sustenance, decline, or enhancement of community member interactions involving MC and carbon source metabolism in the environment. If, for example, scenario (c) defined above occurs for a given community, the average variety of interactions among individuals could decline, as the rare species that may be responsible for some more obscure metabolic functions (i.e., microcystin biodegradation) might be replaced by an array of fast growing, superabundant species with more generalized metabolic functions (i.e., organic carbon metabolism) [55]. It was unclear in this study, however, whether these changes in Alpha diversity metrics consisted of primarily non-degrading or MC-degrading community members. Although we did not specifically differentiate between non-degrading or degrading community members in this study (by measuring total 16S rRNA and *mlrA* gene copy numbers), similar competitive interactions between potentially non-degrading and MC-degrading populations within the greater community in the presence of additional organic carbon have been proposed and documented by Li and co-workers [7,25].

The MCA analysis provided a valuable technique to analyze the statistical significance of changes in the true Alpha diversity of each community with and without ethanol addition. Even though the results generated significant differences in true Alpha diversity metrics, it is important to recognize that these are simulated distributions, with some given uncertainty, that must be assessed accordingly. To reduce the uncertainty that is associated with these simulations, we selected studies from the literature solely analyzing the 16S rRNA of bacterial communities, with an emphasis being placed on soil and water samples only (see Table S5 for a complete summary). Most of these studies used identical platforms (454 pyrosequencing) and analysis pipelines (QIIME), which makes the extrapolation of their uncertainty and measurement error to our results very credible. In addition, these studies incorporated both biological and technical replicates in their experimental designs, which allowed our simulations to account for potential heterogeneity in community composition expected from the sampling location in addition to uncertainty that is associated with the combined measurement and analysis processes. The main uncertainty in our simulations was derived from the relative lack of abundance data (with replication) exceeding 30% mean relative abundance (on the genera level) that was reported in the literature. This lack of data resulted in high predictive uncertainty for the expected standard deviation among replicates at mean relative abundances exceeding this value (>30%). However, since most of the abundance data on the genera level of analysis observed in our study was below this threshold, many of the simulated distributions in diversity orders had low variability (COVs < 22%, Tables S6 and S7). These results confirm that the MCA simulations were indeed valid and adequately reflected the uncertainty that is associated with actual 16S rRNA experimental replications.

Another contribution of the study was the introduction of a bi-phasic model that accounts for coupled bacterial growth and substrate degradation to describe MC biodegradation kinetics. Our results showed a more accurate estimation of MC-biodegradation kinetics, as compared to simple zero or first order models previously used to describe MC-biodegradation kinetics. This kinetic model also accounted for portions of non-biodegradable substrate observed experimentally and resulted in relevant ranges in maximum specific growth rates [70]. We observed a sudden drop of MC and a

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rapid rebound of MC concentration in two degradation experiments (12B and 14A consortia without ethanol), which cannot be fully explained by the current model. These degradation kinetic profiles may be attributed to biosorption or passive uptake (facilitated diffusion) of MC either onto or into non-degrading cells, both of which have been reported for polycyclic aromatic hydrocarbons (PAHs) in the environment [71]. This explanation is similar to that proposed by Jones and co-workers [30], attributing some initial removal of MC to non-specific absorption of fast growing, non-MC-degrading bacterial populations. Future investigations should be directed to adequately explain the deviation from the bi-phasic kinetics that was observed in this study and to comprehend why a portion of the MC substrate was non-biodegradable.

The sampling times and frequency for MC quantification during batch experiments, which was limited to uniform, one-day intervals, was a potential drawback of the experimental design that should be adequately discussed. As observed in Figure 2, the degradation kinetic profiles were quite similar for the 11B consortium (with and without ethanol) and very few data points were collected during the most dynamic portions of the experiment (i.e., few intermediate concentrations between $0-200 \ \mu g/L$ were measured). With few of these intermediate data points available for calibration, there is some uncertainty in the degradation response during critical time periods of each experiment. To review this uncertainty, another MCA was conducted by simulating experimental datasets with a greater number of time points included during model calibration as compared to the original dataset (Supplementary Materials, Section 12). We found that including a high number of sampling points during the most dynamic time of experimentation did not significantly alter our main conclusions (i.e., ethanol still significantly slows MC biodegradation). This result was attributed to the inability of the model to mechanistically account for highly variable MC-degradation kinetics (similar to results that are presented for 12B-NE and 14A-NE in Figure 2). This uncertainty can be greatly reduced in future experiments by properly designing the sampling times and frequencies of each experiment a priori. Ultimately, application of optimal experimental design techniques with global sensitivity analysis can improve parameter identification and the validity of statistical hypothesis tests [72–74].

The isolation of MC-degrading populations in the absence of an on-going or proceeding a HAB implies that lake bacteria harbor the degradation capability in the absence of MC. These bacteria likely possess diverse metabolic pathways that allow for quick adaption to changing environmental conditions through effective, sometimes simultaneous, consumption of different sources of organic carbon. Egli [75,76] has summarized the diversity of bacteria able to consume multiple carbon sources simultaneously under simulated oligotrophic conditions. Jones and co-workers [30] detailed the isolation of a *Sphingomonas* strain (ACM-3962) that was capable of degrading both complex carbon sources present in a peptone, yeast extract media as well as MC, supporting that MC degrading bacteria may exhibit simultaneous consumption patterns. Overall, the spatial and temporal persistence of MC-degrading bacterial populations within Southern California lakes, afforded by their diverse metabolic pathways, supports the potential application of biofiltration technologies. Shotgun metagenomic sequencing, as compared to 16S rRNA alone conducted in this study, could provide a more in depth understanding of the diverse array of functional genes that are present in these isolated MC-degrading consortia and offer some insight as to how this functional profile changes in the presence or absence of certain environmental stimuli.

However, the high sensitivity of each isolated consortia to the presence of an alternative, bioavailable carbon source, poses a significant challenge to the advent of biofiltration technology for MC removal from drinking water. Biofiltration systems targeting MC removal rely on the formation of stable bacterial communities that can rapidly assimilate MC toxins along with other bioavailable nutrients. The results of this study provided initial evidence that the presence of alternative carbon sources (other than MC) offsets the stability of the degrading communities and retards MC biodegradation kinetics, thereby increasing MC treatment variability. In the presence of an alternative organic carbon source, there was a statistically-significant shift to the bacterial community composition, which was paralleled by a statistically-significant decline in MC-LR degradation kinetics. These results

confirm previous findings by Giaramida and co-workers [77], Li and co-workers [8,25], and Eleuterio and Batista [23]. In addition, the pyrosequencing results identified the previously unobserved members in each community (i.e., *Cytophagales, Flavobacteriales,* and *Rickettsiales*) and changes in the abundance of representative taxa (i.e., *Pseudomonadales, Burkholderiales,* or *Xanthomonadales*). The sequencing data also detected both a statistically significant increase and a decline in existing bacterial genera that were previously affiliated with MC degradation (i.e., *Stenotrophomonas* and *Sphingopyxis,* respectively) in the presence of an organic carbon source across most consortia (Figure S17). These results may suggest different, more complex patterns of substrate utilization or preference among various individual MC-degrading populations within each consortium. Given these relatively unique community responses to ethanol addition that were observed among several isolated MC-degrading consortia, site and season specific biofiltration strategies, such as nutrient amendment (biostimulation), could be tailored to both (1) stabilize the natural MC-degrading community structure and (2) enhance metabolic functions that are related to individual MC-degrading populations comprising the greater community.

Importantly, the disparity in initial seeding concentrations between MC and ethanol employed in this study was targeted to reflect realistic nutrient conditions in most lake and reservoir environments. In these freshwater environments (throughout a range in trophic levels), MC will not be the primary carbon and energy source being metabolized by degrading bacteria, since it is often present in low background concentrations (ng/L to ug/L) and not secreted until algal bloom senescence or collapse [78,79]. Instead, the presence of other bioavailable dissolved organic carbon (DOC), including other non-toxic cyanobacterial oligopeptides or exudates, represents the primary carbon and energy source for both competing degrading and non-degrading bacterial populations [23].

Furthermore, the relatively high magnitude of MC (200 μ g/L) and ethanol (~300 mg/L) concentrations that were employed in this study were selected to represent worst case bloom conditions. Past sampling of lakes and reservoirs throughout the United States (U.S.) has indicated that concentrations of total MC rarely exceed 200 μ g/L [78–84], whereas total dissolved organic carbon (DOC) concentrations ranged from 0.2 to 516 mg/L [40]. It is important to note a considerable portion of the readily bioavailable fraction of the total DOC pool may be attributed to secreted algal organic matter, especially in HAB impacted environments [38,39]. Thus, the shifts in community composition and kinetics of MC degrading consortia when exposed to labile organic carbon observed in this study are reflective of shifts in composition and kinetics of degrading communities during collapse or senescence of a significant bloom event in the environment.

We further recognize the limitations of the current experimental approach because isolation based-studies have the tendency to alter MC degradation kinetics from that in the environmental setting. Culturing these isolated communities on MC as the sole carbon and energy source may have had the most significant impact on changing the original physiological state and structure of the natural community [33]. Moreover, sub-culturing, which is necessary for maintaining the viability of each consortium, may have led to unintended shifts in the community composition and kinetics of substrate metabolism from initial isolation [85]. However, regardless of these unintended effects from enrichment and culture maintenance, in the absence of an in-situ tracking mechanism to follow the dynamics of different MC-degrading populations in the natural environment, isolating a consortium and studying its degradation kinetics under controlled laboratory conditions is necessary.

Improvement in the experimental design can be achieved by quantifying the total *mlr* MC-degrading population in each consortium or natural environment through the tracking of the known MC-degradation gene, i.e., *mlrA* gene, by quantitative PCR (qPCR). The *mlrA* gene copy number may be correlated with the period of lag phase in the degradation curve, such that MC-degradation can only be observed after the *mlrA* gene level reaches a certain threshold concentration. Determining the ratio of *mlrA* to 16S rRNA gene copy in each consortium or the natural environment may elucidate the difference in the degradation kinetics that was observed among different consortia. Confidence in the results would have been greatly improved if the presence of these MC-degrading populations could be directly evaluated through tracking *mlrA* gene expression by reverse transcription

quantitative PCR (TR-qPCR). These analyses would have provided crucial quantitative indications as to the active degradation bacteria. Lastly, tracking the *mlrA* and 16S rRNA gene copy number in each consortium could provide some explanation as to the high variability in growth rates experimentally observed using OD600 and flow cytometry. It is important to acknowledge here that the *mlrA* gene detection protocols that are described above will not quantify the total abundance of degrading populations within each consortium. Many previous studies have indicated that alternative degradation pathways (i.e., xenobiotic degrading) may exist and contribute to the removal of MCs in addition to the *mlr* pathway identified [33,61]. Despite these limitations in quantifying total degrader abundances, the initial consortia composition (i.e., the proportion of MC-degrading vs. non-degrading populations) was a primary factor governing observed differences in cell growth kinetics across replicate experiments.

5. Conclusions

The results that are presented in this study exemplify the intimate link between MC-biodegradation and alternative organic carbon sources in the environment. In the presence of a readily available organic carbon source (ethanol), a statistically significant change to the taxonomic composition of the MC-degrading communities was observed. The following specific, statistically significant changes in taxonomic composition were detected with the addition of an organic carbon source: (1) an increase in the abundance of members that were previously unobserved in each community without ethanol; (2) a shift in the abundance of representative taxa (including *Rhizobiales, Pseudomonadales, Xanthomonadales,* and *Burkholderiales*); (3) a variation in the abundance of genera previously affiliated with MC biodegradation (i.e., *Sphingopyxis* and *Stenotrophomonas*); and, (4) an increase or decline in simulated Alpha diversity for sediment and lake water consortia, respectively. Changes in simulated Alpha diversity metrics. These changes to the community structure in the presence of an organic carbon source were paralleled by a statistically significant decline in MC biodegradation half-lives, as predicted through the application of a bi-phasic kinetic model.

The instability of the MC degrading communities in the presence of an organic carbon source presents a significant challenge to the advent of MC remediation technologies, such as biofiltration. Depending on the environmental conditions of the lake or reservoir (i.e., the trophic status), among many factors, high MC treatment variability will be expected in practice as other available DOC, as opposed to MC, may be considered as the primary carbon/energy source for many degrading populations within these greater bacterial communities. However, before biofiltration strategies to address these issues can be fully considered, the underlying mechanisms driving these changes must be further elucidated and disentangled. In addition to potential substrate competition between MC and available DOC, the potential higher order interactions between community members may change significantly in the presence of an alternative organic carbon source. Future studies should consider the isolated study of these potential mechanisms that are indicated above to more clearly identify why changes in MC-degrading bacterial community structure and degradation kinetics occurred in the presence of an alternative organic carbon source.

In addition to temperature and pH, changes in nutrient availability, such as the concentration of alternative organic carbon sources, is likely one of the many environmental factors that govern MC-biodegradation kinetics in the environment. The outcome from this study highlights the challenges in advancing biofiltration into a truly "engineered" treatment system, as MC removal kinetics may be highly variable under different environmental conditions. As of current, biofiltration may serve as a pretreatment or post treatment system to reduce dissolved MC concentrations in drinking water. Additional treatment barriers, such as ozonation or activated carbon, are necessary to protect the safety and quality of drinking water resources.

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