



Niche Separation Increases With Genetic Distance Among Bloom-Forming Cyanobacteria

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Bacterial communities are composed of distinct groups of potentially interacting lineages, each thought to occupy a distinct ecological niche. It remains unclear, however, how quickly niche preference evolves and whether more closely related lineages are more likely to share ecological niches. We addressed these questions by following the dynamics of two bloom-forming cyanobacterial genera over an 8-year time-course in Lake Champlain, Canada, using 16S amplicon sequencing and measurements of several environmental parameters. The two genera, *Microcystis* (*M*) and *Dolichospermum* (*D*), are frequently observed simultaneously during bloom events and thus have partially overlapping niches. However, the extent of their niche overlap is debated, and it is also unclear to what extent niche partitioning occurs among strains within each genus. To identify strains within each genus, we applied minimum entropy decomposition (MED) to 16S rRNA gene sequences. We confirmed that at a genus level, *M* and *D* have different preferences for nitrogen and phosphorus concentrations. Within each genus, we also identified strains differentially associated with temperature, precipitation, and concentrations of nutrients and toxins. In general, niche similarity between strains (as measured by co-occurrence over time) declined with genetic distance. This pattern is consistent with habitat filtering – in which closely related taxa are ecologically similar, and therefore tend to co-occur under similar environmental conditions. In contrast with this general pattern, similarity in certain niche dimensions (notably particulate nitrogen and phosphorus) did not decline linearly with genetic distance, and instead showed a complex polynomial relationship. This observation suggests the importance of processes other than habitat filtering – such as competition between closely related taxa, or convergent trait evolution in distantly related taxa – in shaping particular traits in microbial communities.

Keywords: cyanobacteria, ecological niche, niche partitioning, habitat filtering, competition, *Microcystis*, *Dolichospermum*

INTRODUCTION

Microbial communities are composed of potentially diverse groups of lineages, which must be sufficiently similar to survive in the same habitat, yet sufficiently dissimilar to occupy distinct ecological niches and avoid competition. This tension between selection for common traits to survive in a common environment (habitat filtering) and selection for divergent traits to reduce competition (niche partitioning among closely related species) was recognized by Darwin, and the relative impacts of the two processes on communities are still debated (Cavender-Bares et al., 2009). A pioneering study of microbial communities using phylogenetic marker genes found evidence for phylogenetic clustering, suggesting the importance of habitat filtering in selecting for closely related taxa sharing specific traits allowing them to survive in a given habitat (Horner-Devine and Bohannan, 2006). However, phylogenetic overdispersion (the opposite pattern as phylogenetic clustering) has also been observed, suggesting that competition between closely related taxa can lead to niche partitioning (Koeppel and Wu, 2014). Importantly, the power to detect phylogenetic overdispersion depends on the phylogenetic resolution (e.g., whether operational taxonomic units are defined at 97, 98, or 99% identity in a marker gene) (Koeppel and Wu, 2014).

Beyond searching for phylogenetic patterns of clustering or overdispersion, explicitly considering the associations between microbial traits and niches can help understand the selective pressures shaping microbial communities on different evolutionary time scales. It is known that certain traits (e.g., salinity preference, methanogenesis) are relatively slow-evolving and thus restricted to only certain lineages, whereas other traits (e.g., phage resistance, organic phosphate utilization) can be acquired by a single point mutation or gene acquisition, thus evolving rapidly in response to ecological selection and competition (Martiny et al., 2015). Therefore, habitat filtering might be stronger for slow-evolving traits, while niche partitioning will be more likely for fast-evolving traits.

In this study, we use Hutchinson's definition of a fundamental niche as the set of abiotic conditions under which an organism can survive and reproduce, and a realized niche as the conditions under which it is actually observed in nature, accounting for both abiotic and biotic (e.g., competition, predation, cooperation) interactions (Hutchinson, 1957). If two species have identical ecological niches, one should competitively exclude the other (Gauze, 1934; Tilman, 1982) unless competition is weak due to abundant resources. In practice, closely related taxa often compete for space and resources (Cavender-Bares et al., 2009), favoring specialization to reduce overlap in niche space. For example, coexisting (sympatric) taxa within the same genus tend to have different realized niches, experiencing different seasonal growth patterns or responding differently to environmental parameters (Gray et al., 2004; Jaspers and Overmann, 2004; Hunt et al., 2008; Šimek et al., 2010; Jezbera et al., 2011; Neuenschwander et al., 2015). Pairs of taxa with similar realized niches can be identified when they co-occur in repeated sampling over space and time, and such co-occurrence networks are readily inferred from deep amplicon sequencing datasets

(Friedman and Alm, 2012). Typically, niches are considered as features of species. However, when niches are considered as collections of traits or environmental associations, the Hutchinsonian niche concept can be extended to taxonomic groupings more inclusive than species, even if the biological "reality" of such groups is doubtful. Here, we apply the niche concept to both fine-grained (i.e., sub-genus level) and coarse-grained (i.e., genus level) taxonomic units. We focus mainly on niche specialization within genera, showing that specialization is extensive and that lumping bacterial diversity at the genus level obscures finer-scale niche preferences.

Cyanobacteria are widely and naturally present in freshwater ecosystems, and some lineages form blooms under appropriate conditions of temperature and nutrients (Konopka and Brock, 1978; Harke et al., 2016). Several studies have shown that different cyanobacterial genera can co-occur during blooms, thus sharing at least some dimensions of their realized niches (Paerl et al., 2001; Yamamoto and Tsukada, 2009). Nitrogen (N) and phosphorus (P) can both be limiting for bloom formation, and different cyanobacterial taxa apparently have different preferences for N and P concentrations (Dolman et al., 2012). For example, cyanobacteria capable of N-fixation (such as *Dolichospermum*) are associated with more efficient P-utilization at the community level, suggesting P-limitation when N-fixers are abundant (Andersson et al., 2015; Olli et al., 2015). Clearly, N and P utilization are ecologically important traits for cyanobacteria, and may be important for niche partitioning among closely related strains. In the marine cyanobacterium *Prochlorococcus*, P uptake and metabolism appears to be relatively fast-evolving perhaps due to horizontal gene transfer of P-related genes (Coleman and Chisholm, 2010) while light preferences are slow-evolving, and temperature preferences are intermediate (Martiny et al., 2015).

We investigated niche partitioning within and between *Microcystis* and *Dolichospermum*, two genera of potentially toxigenic cyanobacteria that bloom nearly every year in a large eutrophic North American lake, Lake Champlain. In a previously described 8-year time-course analysis (2006–2013) spanning multiple bloom events, we used 16S amplicon sequencing to broadly survey changes in the lake microbial community over time, generally at the genus level (Tromas et al., 2017). Here, we use Minimum Entropy Decomposition (MED) of amplicon sequences (Eren et al., 2014) to identify sub-genus strains (MED nodes; here used interchangeably with "strains") within each of the two dominant cyanobacterial genera, *Microcystis* and *Dolichospermum*, at single nucleotide resolution (i.e., each MED node is an exact sequence variant, distinguishable from other variants that differ by at least one nucleotide substitution). MED discards low-entropy nucleotide positions, which effectively filters out sequencing errors at the expense of possibly also removing true polymorphism at very low frequency. Therefore, although MED outputs exact sequences that are actually present in the sample (after denoising), it is possible the MED nodes contain finer-scale genetic variation which could be captured using additional marker genes or whole genome sequencing. A previous study demonstrated that oligotypes (similar to MED nodes) lacked the resolution to distinguish

toxic and non-toxic *Microcystis* lineages, but could potentially be informative about other, more phylogenetically conserved niches such as eutrophic (nutrient-rich) vs. oligotrophic (nutrient-poor) lake preferences (Berry et al., 2017). Toxin production is thought to be fast-evolving because toxin biosynthesis genes are widely distributed across cyanobacterial genera, suggesting rapid gain and loss. While horizontal gene transfer is a likely explanation, transfer is probably more frequent among closely related lineages because toxin gene trees are congruent with ribosomal phylogenies of distantly related cyanobacteria genera (Rantala et al., 2004).

We used a combination of genetic data (diversity of MED nodes) and matched environmental data (e.g., temperature, nutrient concentrations, precipitation) to address three specific questions. First, how similar are the niches of the two dominant cyanobacterial genera *Microcystis* and *Dolichospermum*? Second, how similar are the niches of strains within each genus? Third, how does niche similarity change with genetic relatedness? We confirm that *Microcystis* and *Dolichospermum* are broadly co-occurring during blooms, but have distinct nutrient preferences. We also identified niche partitioning at the sub-genus level, and observe a general decline in realized niche similarity with genetic distance, consistent with habitat filtering. However, certain niche dimensions (particulate nutrient concentrations) show a complex polynomial relationship with genetic distance, suggesting that a combination of habitat filtering and competitive interactions shapes the evolution of these traits.

MATERIALS AND METHODS

Sampling, DNA Extraction, Purification, and Sequencing

Open-water season samples (April to November) collected over 8 years (2006–2013) from the photic zone of Missisquoi Bay at two different sites (littoral and pelagic) of Lake Champlain, QC, Canada (45°02'45"N, 73°07'58"W) were filtered and extracted for DNA sequencing as described in Tromas et al. (2017).

Sequence Analysis

A total of 7,949,602 sequences of the 16S rRNA gene V4 region were obtained from 150 lake samples, with a median of 41,982 per sample as previously described (Tromas et al., 2017). These sequences were processed with the default parameters of the SmileTrain pipeline¹ that includes reads quality filtering, chimera filtering, and merging using USEARCH (version 7.0.1090², default parameter) (Edgar, 2010), Mothur (version 1.33.3) (Schloss et al., 2009), Biopython (version 2.7) and custom scripts. MED was then applied to the filtered and merged reads to partition sequence reads into MED nodes (Eren et al., 2014). MED was performed using the following parameters – M noise filter set to 500 resulting in ~7% of reads filtered and 941 MED nodes (Supplementary Data Sheet 2). Samples with

less than 1,000 reads were removed, yielding a final dataset of 135 samples. Finally, taxonomy was assigned using the assign_taxonomy.py QIIME script (default parameters), and a combination of GreenGenes and a freshwater-specific database (Freshwater database 2016 August 18 release; Newton et al., 2011), using TaxAss³, installation date: September 13th 2016; Rohwer et al., unpublished). After assignment, nodes that belong to Eukaryotes but still present in the database (Cryptophyta, Streptophyta, Chlorophyta, and Stramenopiles orders) were removed, leading to a total of 891 nodes.

Diversity Analysis

Comparing changes in the diversity of *Microcystis* strains to the diversity of *Dolichospermum* strains was performed using betta (Willis et al., 2017). Betta accounts for strains that are present in the environment but not observed in the samples due to incomplete sampling. The number of unobserved strains is estimated based on the number of strains that are observed and their abundances. The total strain diversity was estimated using breakaway (R package v4) which accounts for ecological interactions between strains (Willis and Bunge, 2015).

Conditionally Rare Taxa Analysis

We investigated the temporal dynamics of *Microcystis* and *Dolichospermum* nodes by measuring the composition for each genus in conditionally rare taxa. The matrix of node absolute abundances was used as input for the R script CRT_Functions_v1.1.R⁴ (Shade et al., 2014) using the default parameters. Conditionally rare taxa are defined as usually rare taxa that occasionally become very abundant, without showing rhythmic or seasonal patterns.

Node–Environment Relationships Analysis

To investigate node–environment relationships, we used an environmental data matrix that included: particulate phosphorus in µg/L (PP, the difference between TP and DP), particulate nitrogen in mg/L (PN, the difference between TN and DN), total dissolved phosphorus in µg/L (DP), total dissolved nitrogen in mg/L (DN), 1-week-cumulative precipitation in mm and 1-week-average air temperature in Celsius. Total nutrients were measured directly from collected lake water and the dissolved nutrients were measured in filtered water (Glass microfiber Filters grade GF/F, 0.7 microns). The detailed measurements of each environment variable are described in Tromas et al. (2017). In this previous study, we showed that these environmental variables, over the 8 years, were not correlated with one another.

Response to Abiotic Factors

To analyze the response of each node to abiotic environmental data, we used a Latent Variable Model (LVM) framework, which combines Generalized Linear Models with Bayesian Markov Chain Monte Carlo (MCMC) methods (boral package in R;

¹<https://github.com/almlab/SmileTrain/wiki>

²<http://www.drive5.com/usearch/>

³<https://github.com/McMahonLab/TaxAss>

⁴<https://github.com/ShadeLab/ConditionallyRareTaxa>

Hui et al., 2015; Warton et al., 2015). LVM is a model-based approach for analyzing multivariate data (e.g., numerous taxa within the response matrix) that partitions the different drivers of taxa co-occurrence patterns into two components: the first is a regression component, which models the taxon-specific environmental responses, and the second is a latent variable component, which is used to identify residual patterns of co-occurrence resulting from unmeasured factors and/or biotic interactions (Letten et al., 2015). In this study, we used LVMs to examine how taxa co-respond to abiotic gradients, and used these co-responses as a proxy for niche similarity. To do so, we extracted the *environmental correlation matrix* from the regression model, which, for any two taxa, corresponds to the correlation between their fitted values ($x_i\beta_j$). In particular, for each node we calculated the predicted probability of mean abundance for each site on linear and non-linear (i.e., $Y \sim X + X^2$) response scales, providing a vector of fitted values. Correlations among the fitted responses of any two taxa were then calculated based upon these vectors (correlating the vector of taxon A with the vector of taxon B, for instance).

We first tested LVMs using a response matrix of two columns, corresponding to the relative abundances of the *Dolichospermum* and *Microcystis* genera, relative to the rest of the bacterial community. For these models, *Dolichospermum* and *Microcystis* abundances were normalized by the total counts of all bacteria using the centered log ratio to correct for data compositionality (clr-inter genus) (Aitchison, 1986; Paliy and Shankar, 2016) using a zero-replacement procedure as suggested in Gloor and Reid (2016). To then examine dynamics within these genera, we ran separate LVMs for *Dolichospermum* and *Microcystis* nodes (i.e., response matrix consisted of the *Dolichospermum* and *Microcystis* nodes, respectively). For this second set of LVMs, node abundances were normalized by the total counts of *Dolichospermum* and *Microcystis*, respectively, to obtain an intra-genus relative abundance (clr-intra genus) for each node. Environmental variables were standardized to mean zero and unit variance to reduce the correlation between the linear (X) and non-linear (X^2) fit, which also simplified model interpretation and stabilized MCMC sampling. As most of the *Dolichospermum* nodes were conditionally rare, we included only the *Dolichospermum* nodes that were present in at least 70% of samples, to avoid overfitting based on too little data. To complement this multivariate LVM analysis and help visualize the univariate response of each genus and node to the suite of environmental variables, we also tested linear (degree-1 polynomial) and non-linear (degree-2 polynomial) relationships between each response and explanatory variable. We used AIC to find the single best-fit model (either linear or non-linear) for each relationship and plotted the best-fit model.

Lastly, to test whether co-responses or niche separations were stronger for more closely or distantly related nodes, we extracted the correlations between fitted responses of any two nodes from the *environmental correlation matrix* of each best-fit LVM and examined the relationship between each significant correlation and the pairwise genetic distances of the two respective taxa (percent identity in the V4 region of the 16S gene). Here, significant positive correlations represent co-response

between any two taxa whereas significant negative correlations represent the degree of niche separation. The genetic distance between nodes was measured within each genus *Microcystis* and *Dolichospermum* using the software MEGA (version 7.0.18) using the p-distance (the proportion of nucleotide sites at which two sequences differ), calculated by dividing the number of sites with nucleotide differences by the total number of sites compared (excluding sites with gaps). R code to reproduce these analyses and relevant figures is provided in Supplementary Data Sheet 3.

Co-occurrence Analysis

Co-occurrences between *Microcystis* or *Dolichospermum* and other taxa were calculated with SparCC (Friedman and Alm, 2012), with 20 iterations to estimate the median correlation of each pair of MED nodes, and 500 bootstraps to assess the statistical significance. Correlations were then filtered for statistical significance ($P < 0.01$) and correlations with $R > \pm 0.6$, were selected to build networks using Cytoscape (version 3.1.0).

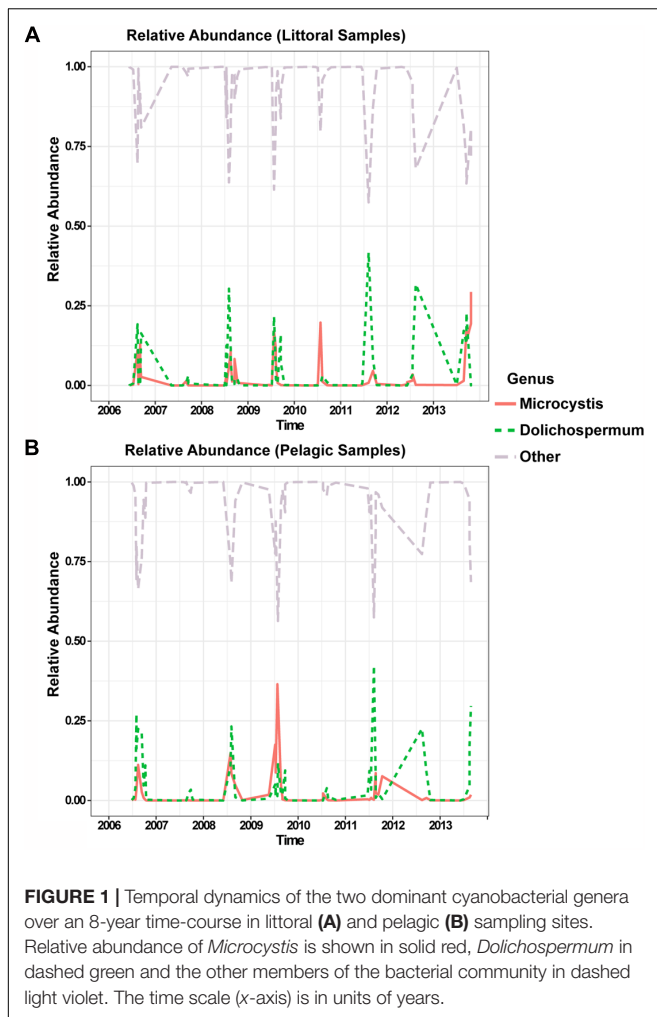
RESULTS

Do *Microcystis* and *Dolichospermum* Co-occur Temporally?

We have previously shown that *Microcystis* and *Dolichospermum* were the two most dominant bloom forming cyanobacteria in Lake Champlain's Missisquoi Bay (Tromas et al., 2017). Both genera were present every year at both littoral and pelagic sampling sites, between 2006 and 2013, with a relative abundance of at least 15% on average during summer (max = 24.8%), with the exception of summer 2007 (0.7%) when no substantial blooms occurred (Figure 1). *Dolichospermum* was generally the most dominant genus except for 2009 and 2010 when *Microcystis* dominated (Supplementary Figure S1). *Dolichospermum* and *Microcystis* were both present every year (Supplementary Figure S1) suggesting that they share similar physico-temporal niche during a bloom, although other aspects of their niches likely differ. For example, *Dolichospermum* can fix nitrogen but *Microcystis* cannot (Hajdu et al., 2007).

Are *Microcystis* and *Dolichospermum* Equally Diverse and Dynamic?

To explore the temporal dynamics of the finer-scale taxa within each genus, we partitioned each genus into MED nodes, which we call "strains" (Methods). We obtained 25 *Dolichospermum* strains and 6 *Microcystis* strains, and after accounting for low-abundance strains that may be missing from the samples using Breakaway (Methods), we conclude that diversity is significantly lower within *Microcystis* than within *Dolichospermum* ($P < 0.001$). The difference in the number of nodes is unlikely to be an artifact of sequencing depth, because there is no correlation between the number of sequence reads and the number of nodes per genus (Supplementary Figure S2). *Dolichospermum* thus appears to be more diverse than *Microcystis*.



We then examined whether nodes within the *Microcystis* and *Dolichospermum* genera followed the same temporal dynamics. We observed that 15/25 *Dolichospermum* nodes were conditionally rare, meaning that they are usually rare but occasionally become relatively abundant, without following any apparent rhythm (Table 1).

The proportion of rare *Dolichospermum* nodes was significantly higher than what is observed in other nodes in the lake community (Fisher's exact test, $P < 0.001$). Several conditionally rare *Dolichospermum* nodes seemed

to dominate several times without showing seasonal patterns (Supplementary Figure S3). Furthermore, we noticed a shift in node composition after 2011, where node D5505 decreased while D2282 increased in relative abundance. In contrast, we only found one conditionally rare *Microcystis* node. However, only two *Microcystis* nodes (M5732 and M5733) were consistently dominant over time (Supplementary Figure S4). Similarly, only two *Dolichospermum* nodes, but not always the same two, dominated at any given time (Supplementary Figure S3). Overall, these results suggest that *Dolichospermum* is more genetically diverse, and that this genetic diversity varies over time. In contrast, *Microcystis* is less diverse and more stable over time.

Do *Microcystis* and *Dolichospermum* Share the Same Realized Niche Within the Community?

To investigate the niche separation between *Dolichospermum* and *Microcystis*, we analyzed their relationships with several environmental conditions measured at the time of sampling (temperature, nutrient concentrations, precipitation and toxin concentrations). We observed that these two cyanobacterial genera have different responses to nutrients (Supplementary Figure S5) as previously observed (Andersson et al., 2015; Harke et al., 2016). *Microcystis* relative abundance was positively correlated with DP (Supplementary Figure S5; $R^2 = 7\%$), in agreement with previous observations (Homma et al., 2008). In contrast, *Dolichospermum* was not significantly correlated with DP, and instead was positively correlated with PP and PN (Supplementary Figure S5). *Dolichospermum* also responded negatively to dissolved nitrogen, in agreement with previous studies demonstrating that Nostocales (the Order containing *Dolichospermum*) are favored under conditions of low dissolved inorganic nitrogen, due to their ability to utilize atmospheric nitrogen for growth (Suikkanen et al., 2013; Andersson et al., 2015). Overall, these results confirmed that *Microcystis* and *Dolichospermum* share a spatio-temporal niche during a bloom, but have distinct nutrient preferences.

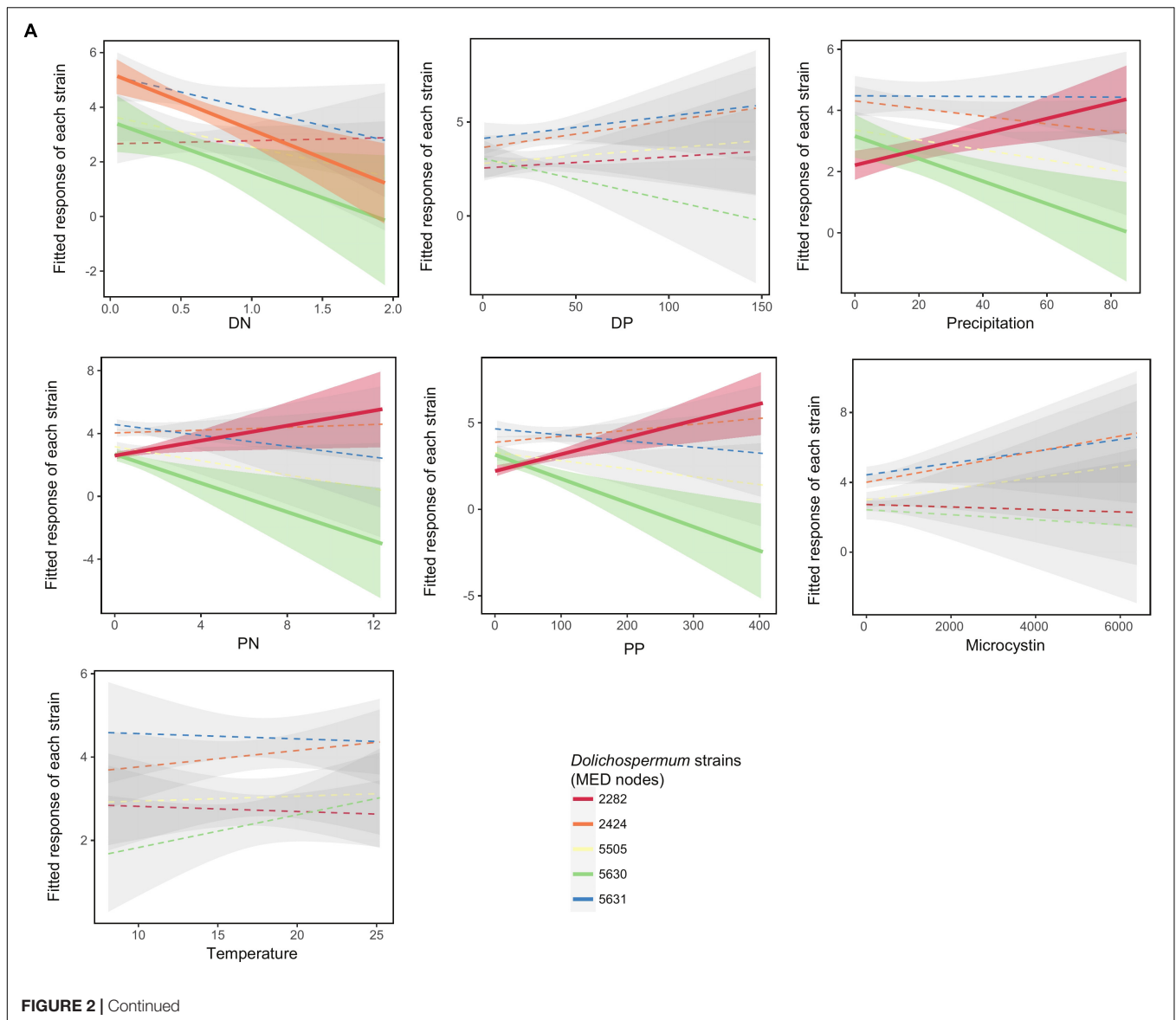
How Similar Are the Niches of Strains Within a Genus?

Numerous studies have focused on environmental conditions that favor cyanobacterial blooms, but few studies have examined how different cyanobacterial strains might respond differently to environmental conditions due to niche partitioning. We

TABLE 1 | Conditionally rarity analysis.

	Number of conditionally rare nodes	Number of total nodes	Proportion conditional rare/total nodes	Fisher's exact test P -value
Whole bacterial community	95	891	0.10	
<i>Dolichospermum</i>	15	25	0.60	$P < 0.001$
<i>Microcystis</i>	1	6	0.16	$P > 0.1$

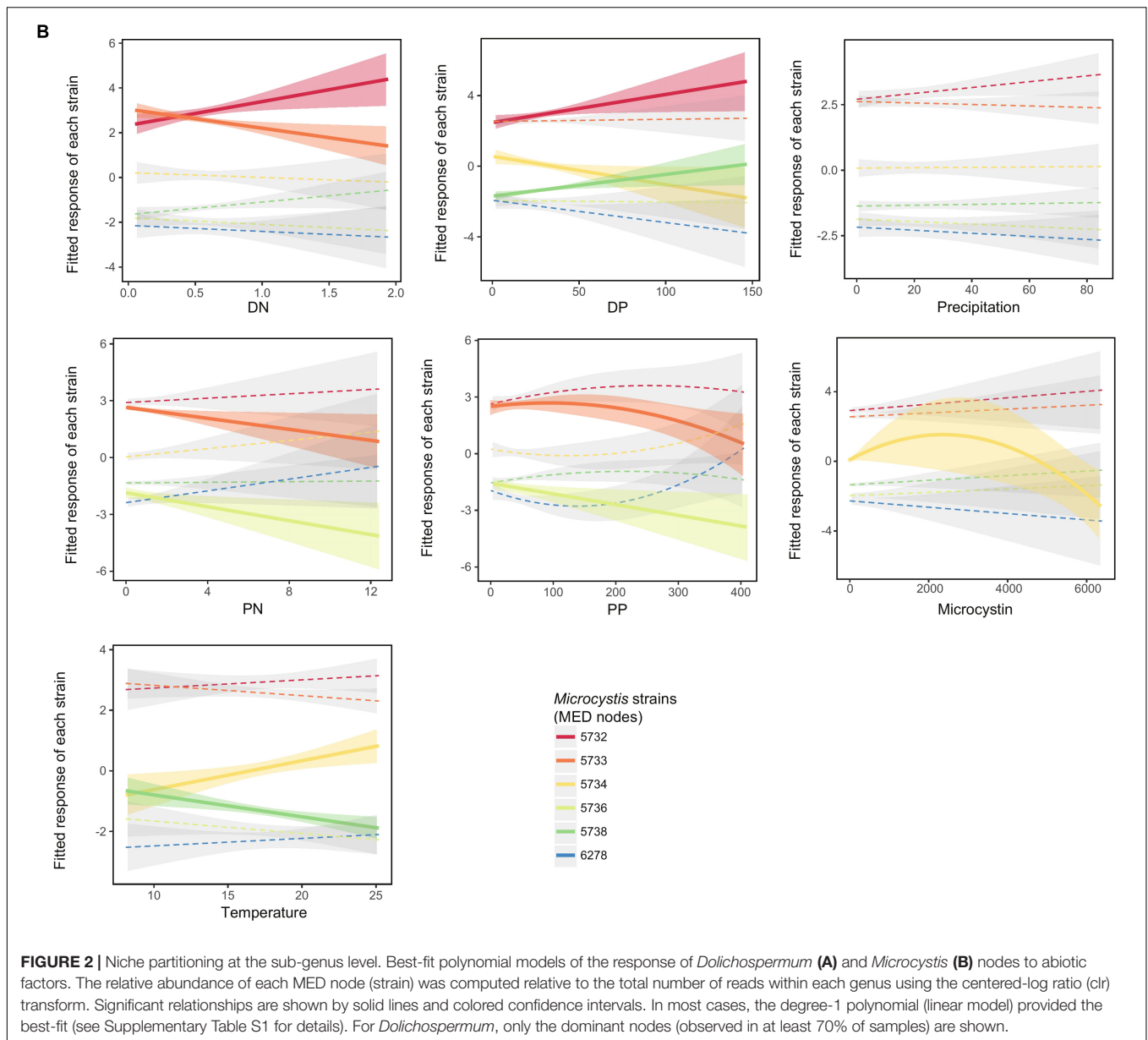
We compared the proportion of conditionally rare *Microcystis* or *Dolichospermum* MED nodes to the proportion expected among MED nodes in the entire lake bacterial community using Fisher's exact test.



observed that *Dolichospermum* and *Microcystis* strains, within each genus, appear to have qualitatively different dynamics (Supplementary Figures S3, S4). To test whether these different dynamics were due to niche partitioning, we first analyzed how the different strains within each genus were related to environmental variables, and then used LVMs to determine the co-responses (niche similarity) as well as niche separation between strains.

We found that nearly all (19/20) of the significant relationships between strain relative abundances (within each genus) and environmental conditions were linear (Figure 2 and Supplementary Table S2). *Dolichospermum* nodes D2282 (red) and D5630 (green) showed opposite responses to nutrients (PP and PN) and precipitation (Figure 2A). The LVMs confirmed a significant niche separation for these niche dimensions (Supplementary Figure S6 and Supplementary

Table S3). In contrast, nodes D2424 and D5630 both displayed a similar negative relationship (Figure 2A), resulting in a significant co-response (niche similarity) to DN (Supplementary Figure S6 and Supplementary Table S3). Within the *Microcystis* genus, we observed several nodes with similar responses to PP, PN, and DP (Figure 2B), and significant co-responses between nodes were detected for these niches (Supplementary Figure S7). We also found significant niche separations for DN (involving M5732, M5733, and M5738) and temperature (involving M5734 and M5738) (Supplementary Figure S7). Overall, the LVM analysis showed that niche separation occurs among nodes within a genus (Supplementary Figure S8). In *Dolichospermum*, niche separation occurred mainly in the niche dimensions of particulate nutrients (PP and PN) and precipitation (Supplementary Figure S6), whereas in *Microcystis* niche separation occurred mainly



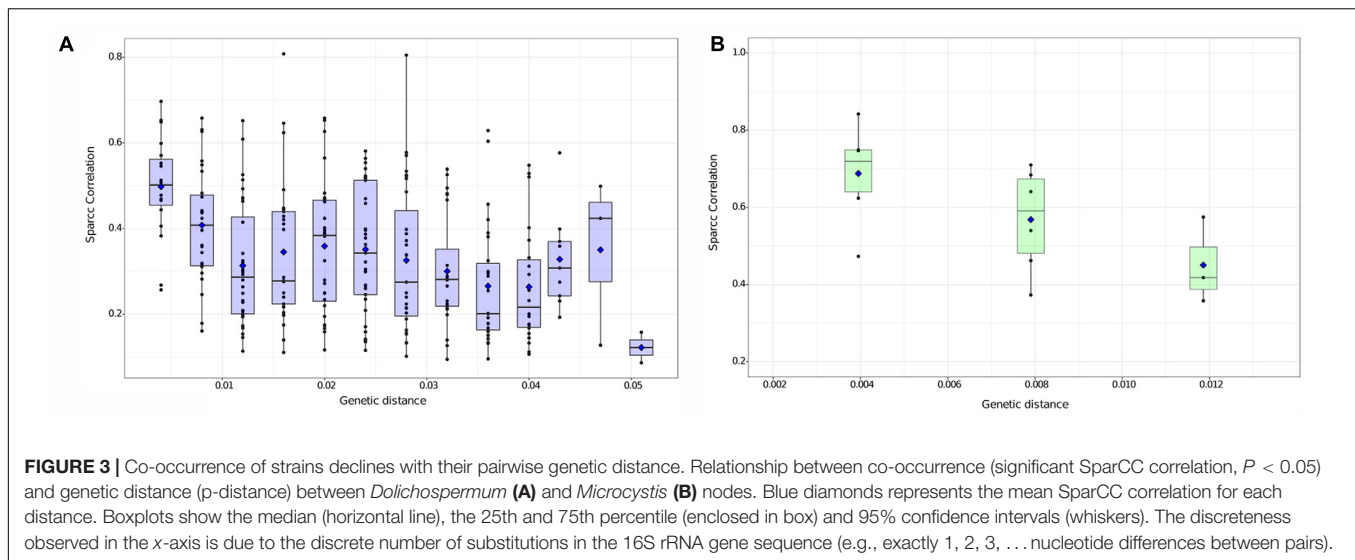
according to temperature and DN preferences (Supplementary Figure S7).

How Do Niche Preferences Vary With Genetic Distance?

When two taxa share the same realized niche (i.e., where they are actually able to survive in the wild, including biotic and abiotic niches), they are more likely to be observed together, and thus to be correlated in survey data. We therefore used co-occurrence patterns between pairs of *Dolichospermum* or *Microcystis* nodes as a proxy for similarity in their realized niches, and asked if more genetically similar nodes are more likely to have similar realized niches. Indeed, we found that pairwise SparCC correlation coefficients between nodes tends to decline with genetic distance (Figure 3).

This pattern was significant within both *Dolichospermum* [linear regression, $F(1,279) = 28.3$, $P < 0.001$, adjusted $R^2 = 8.9\%$] and *Microcystis* [linear regression, $F(1,13) = 7.9$, $P < 0.05$, adjusted $R^2 = 33.0\%$]. The higher R^2 observed for *Microcystis* might be explained by its more limited genetic diversity (maximum pairwise genetic distance ~ 0.01) compared to *Dolichospermum* (maximum distance ~ 0.05). There appears to be a rapid decline in niche similarity as genetic distance goes from 0 to ~ 0.01 (adjusted $R^2 = 23.0\%$ in *Dolichospermum* when considering only this distance range) followed by a flatter relationship for genetic distance > 0.01 .

Ecologically distinct lineages are expected to be associated with distinct surrounding communities, due to a combination of direct microbe-microbe (biotic) interactions and shared preferences for abiotic conditions (Cohan and Koepfel, 2008).



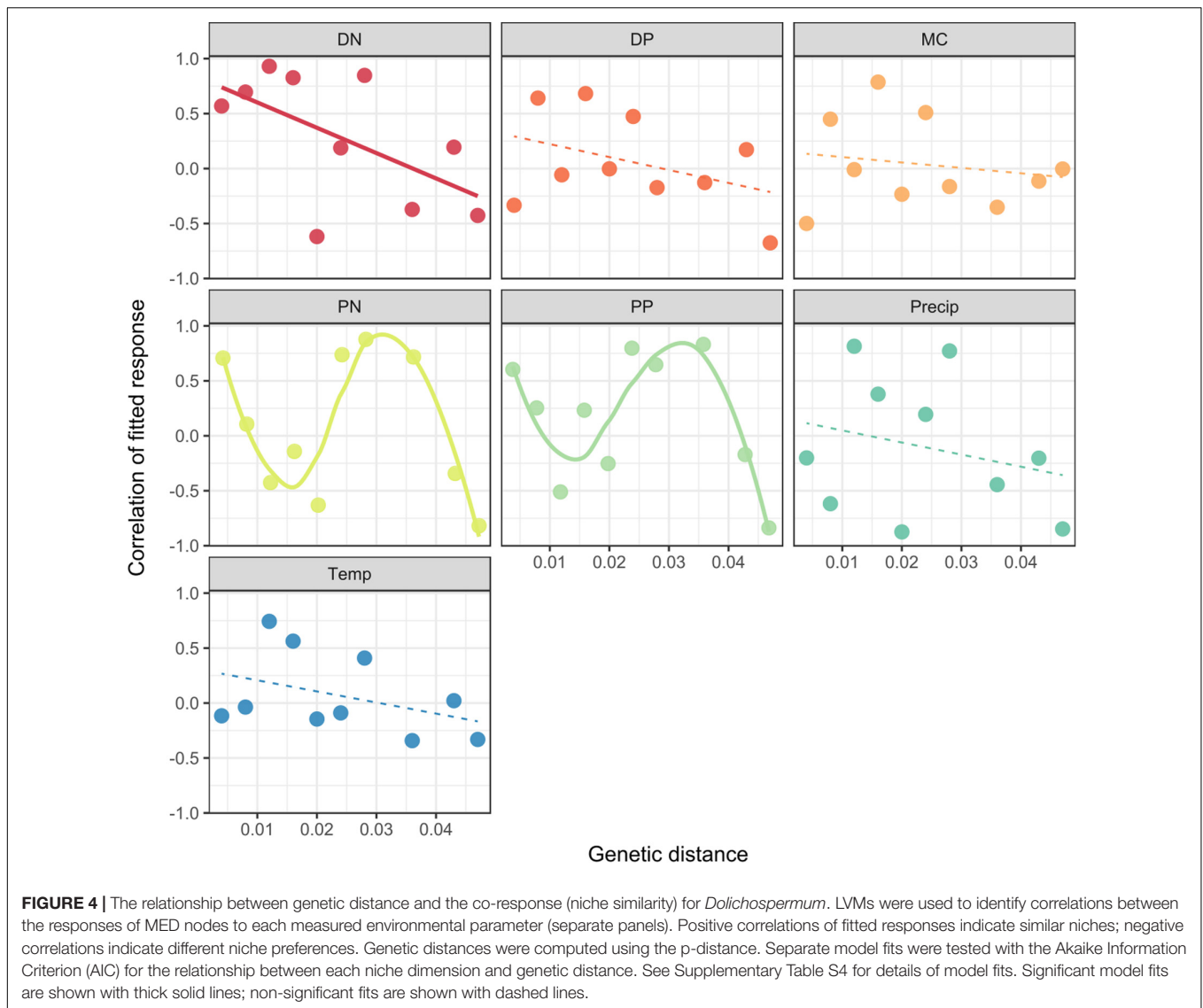
We therefore analyzed the co-occurrence patterns between each *Dolichospermum* or *Microcystis* node and other bacterial taxa in the lake community. We identified non-cyanobacterial MED nodes that co-occurred with each *Dolichospermum* and *Microcystis* node and found that *Microcystis* nodes were generally more connected with other members of the bacterial community (Supplementary Figure S9). We also found that relatively few taxa (4 out of 26; Supplementary Table S5) are significantly correlated with both *Microcystis* and *Dolichospermum*, suggesting that *Microcystis* and *Dolichospermum* strains co-occur with distinct sets of other bacteria, and thus have distinct realized niches.

We further investigated whether more closely related *Microcystis* or *Dolichospermum* nodes have more similar correlations with potentially interacting community members. We focused on members of the *Cytophagaceae* family (MED nodes 3667, 5983, and 5984), which are potential predators of cyanobacteria (Rashidan and Bird, 2001), members of the *Rhizobiales* order (nodes 4737, 3705, and 3726), which are potential N-fixers that could provide nitrogen for non-N-fixing *Microcystis* (Louati et al., 2015), and all the taxa that co-occurred with both *Dolichospermum* and *Microcystis* nodes (nodes 1061, 4674, 7272, and 4756). For each pair of *Dolichospermum* or *Microcystis* nodes, we correlated their genetic distance (as in Figure 3) with the absolute difference in the correlations (r) with each potentially interacting community member (Supplementary Figure S11). We found that closely related *Dolichospermum* nodes tend to have more similar correlations with *Cytophagaceae* node 3667, whereas more distantly related *Dolichospermum* nodes have more different correlations with node 3667 (correlation between $|\Delta r|$ and genetic distance, adjusted $R^2 = 0.059$, $P < 0.001$). Similar but not statistically significant patterns were observed for several *Microcystis* nodes. It is difficult to generalize from these results, but at least in some cases, more phylogenetically similar strains may share conserved interactions with other community members.

Finally, we asked whether the decline in niche similarity with genetic distance (Figure 3) was a common feature of all niche dimensions, or if different abiotic parameters showed different patterns. We considered all significant models, both linear and non-linear as shown in Figure 4 (for *Dolichospermum*) and Supplementary Figure S10 (for *Microcystis*). We found a negative linear relationship between DN niche similarity and genetic distance (adjusted $R^2 = 25\%$, $P = 0.0791$). Qualitatively, most other niche dimensions showed a similar pattern for *Dolichospermum* (Figure 4), and among *Microcystis* nodes we found a significant negative linear relationship between the correlated fitted response to microcystin concentrations and genetic distance (Supplementary Figure S10). However, we also identified non-linear relationships between *Dolichospermum* niche similarity and genetic distance for PP (adjusted $R^2 = 69\%$, $P = 0.0184$) and PN (adjusted $R^2 = 63\%$, $P = 0.0280$). For these niche dimensions, the correlation of fitted responses declines from genetic distances of 0 to ~ 0.01 , then rises to another peak around ~ 0.03 before declining again (Figure 4). While these non-linear models provided significantly better fits to the data compared to linear models, we cannot exclude the possibility that the fits were driven by a few outlying points particular to our data set. Replicating these findings in additional data sets (e.g., from different lakes, over different time scales) is therefore essential.

DISCUSSION

Overall, our results show how *Microcystis* and *Dolichospermum* can achieve similar levels of dominance during cyanobacterial blooms by partitioning niche space within and between genera. Over 8 years of sampling, we observed that *Dolichospermum* and *Microcystis* generally co-occurred during blooms, suggesting a broadly similar physico-temporal niche (Figure 1 and Supplementary Figure S1). However, we confirmed that *Dolichospermum* was associated with lower concentrations



of dissolved nitrogen, consistent with its known ability to fix nitrogen (Andersson et al., 2015; Harke et al., 2016) while *Microcystis* was associated with higher concentrations of dissolved phosphorus (Supplementary Figure S5). These genus-level traits could mask niche differentiation among strains within each genus.

To dissect niche preferences at finer taxonomic resolution, below the genus level, we used MED, allowing single-nucleotide resolution of 16S amplicon sequences, i.e., each MED node is an exact sequence variant. Finer taxonomic resolution has been shown to increase the power to correctly identify phylogenetic overdispersion, a signature of competitive interactions being more important than habitat filtering (Koeppel and Wu, 2014). However, even at high resolution, the 16S marker gene may be too slow-evolving to be a good marker for fast-evolving traits, such as toxin production (Berry et al., 2017) – a trait which likely evolves rapidly by horizontal gene transfer (Moffit and Neilan, 2004).

Despite these limitations, MED analysis provided additional insights into the distinct niches of *Microcystis* and *Dolichospermum*. *Dolichospermum* diversity was higher and most of the MED nodes (strains) were conditionally rare, some of them being bloom-associated in some years but not in others (e.g., D2282, Supplementary Figure S3). *Microcystis* nodes, on the other hand, were less diverse but more consistently bloom-associated (nodes M5732 and M5733; Supplementary Figure S4) and more correlated with other taxa (Supplementary Figure S9), suggesting that the two dominant bloom-forming genera might use different ecological strategies, beyond what is already known about nitrogen and phosphorus utilization.

Examining niche partitioning below the genus level also allowed us to detect patterns not evident at the genus level. For example, *Dolichospermum* is associated with higher concentrations of PP and PN at the genus level (Supplementary Figure S5), but contains a strain (D5630) that shows the

opposite pattern (**Figure 2** and Supplementary Figure S6). Within *Microcystis*, niche partitioning occurred mainly for temperature and DN, and the two most dominant *Microcystis* nodes had a significant niche separation for DN (**Figure 2** and Supplementary Figure S7). It is known that *Microcystis* does not fix atmospheric nitrogen, but is able to use refractory N-containing compounds such as urea or amino acids (Dai et al., 2009; Moisaner et al., 2009), and DN is likely needed for toxin production (Monchamp et al., 2014). Therefore, different *Microcystis* strains could specialize in their preference for different forms of nitrogen.

The relative importance of habitat filtering and competition in shaping microbial communities is widely debated, and distinguishing between the two processes can be technically challenging (Koeppel and Wu, 2014; Cadotte and Tucker, 2017). Consistent with a general effect of habitat filtering in selecting for genetically similar cyanobacteria under similar conditions, we found a negative relationship between MED node co-occurrence and pairwise genetic distance (**Figure 3**). This result is in agreement with an early study showing the importance of habitat filtering in microbial communities (Horner-Devine and Bohannan, 2006) and is also consistent with what was observed by Silverman et al. (2017) using human microbiome data. Here, we considered co-occurrence as a proxy for shared realized niches, including both biotic and abiotic factors.

Consistent with the general importance of habitat filtering in shaping cyanobacterial communities, we observed that closely related strains tended to have similar co-responses to several measured abiotic environmental parameters. For example, we found negative linear relationships between niche similarity and *Dolichospermum* genetic distance for most niche dimensions, particularly DN (**Figure 4**). This observation is in agreement with a previous study that showed that bacterial and fungal responses to N fertilization tend to be more similar among close relatives, with a decline in similarity between genetic distances of 0 and 0.05 (Martiny et al., 2015; Amend et al., 2016). In *Microcystis*, we found that more genetically similar strains tended to be observed at more similar concentrations of the toxin microcystin (Supplementary Figure S10). Although 16S is a poor marker for microcystin production (Berry et al., 2017), our results suggest that phylogenetic relatedness is nevertheless somewhat predictive of microcystin concentrations. This means that microcystin production or tolerance is more likely to be shared by close relatives.

In contrast to the general pattern of declining niche similarity with genetic distance, PN and PP both had non-linear relationships with genetic distance in *Dolichospermum* (**Figure 4**). After an initial decline in co-responses to PN and PP to a genetic distance of ~ 0.01 , co-response rose to another peak at ~ 0.03 before declining again. This response is consistent with near-identical MED nodes sharing the same preferences for PN and PP concentrations, and that competition between close-relatives (up to genetic distance of ~ 0.01) imposes divergent selection for distinct niche preferences. The similarity in PN and PP niches for

more distant relatives (distance ~ 0.03) can be explained if distant relatives have diverged to reduce competition in other niche dimensions, allowing them to converge in PN and PP preferences. It is unclear why this non-linear relationship is observed for PN and PP, but not for other niche dimensions. One possibility is that P uptake and metabolism genes are easily acquired by horizontal gene transfer, as observed in marine cyanobacteria (Coleman and Chisholm, 2010) and may thus be more rapidly evolving, resulting in non-linear relationship with phylogenetic distance. Yet it is unclear why the non-linear pattern is observed for particulate but not dissolved N and P. Particulate nutrients could be a marker for biomass (e.g., bloom density), but it is equally unclear how rapidly “bloom preference” would be expected to evolve. The same non-linear pattern might be expected for microcystin, due to frequent gain/loss of the underlying biosynthetic genes. However, we observe a linear relationship (Supplementary Figure S10) between microcystin concentrations and genetic distance among *Microcystis* strains – but only in a very narrow range of genetic distance (99–100% identity). Therefore, non-linear relationships could become apparent among more distantly related strains (e.g., 95–99% identity).

To investigate how biotic niches change over evolutionary time, we studied how cyanobacterial interactions (SparCC correlations) with non-cyanobacterial community members varied with genetic distance among cyanobacteria. We found that genetically similar *Dolichospermum* strains tended to have similar interactions with a strain of *Cytophagaceae*, a potential cyanobacterial predator, whereas more distantly related *Dolichospermum* strains diverged in terms of their interactions (Supplementary Figure S11). We limited our analyses to only the most common interacting partners (common to *Microcystis* and *Dolichospermum*) or those previously suspected to interact with cyanobacteria via predation or cross-feeding. Future studies could expand on these analyses in a more comprehensive fashion to quantify how biotic interactions evolve over different time scales and in different lineages.

Overall, our results show how different traits may have different relationships with genetic distance, highlighting the importance of considering each niche dimension separately, because adaptation to different niche dimensions can occur at dramatically different rates (Martiny et al., 2015). Our results also suggest that the same trait could evolve at different rates in different cyanobacterial lineages. For example, temperature adaptation evolves at an intermediate rate in *Prochlorococcus* (Martiny et al., 2015) and perhaps at a slower rate in hot spring *Synechococcus* (Becraft et al., 2011, 2015). Using our comparative framework of two broadly sympatric genera, we identify a general decline in ecological similarity with genetic distance – although certain traits (e.g., PN and PP in *Dolichospermum* but not in *Microcystis*) go against this trend. A future challenge in microbial ecology and evolution will be to determine which traits are generally fast- or slow-evolving, and most interestingly, which lineages provide exceptions. For example, particularly rapid evolution of a generally slow-evolving trait in a particular lineage

could provide evidence for an unusual genetic architecture or strong selective pressure on that particular trait in that particular lineage.

DATA AVAILABILITY

Raw sequence data have been deposited NCBI GenBank under BioProject number PRJNA353865: SRP099259.

AUTHOR CONTRIBUTIONS

BS and NT conceived the study. NT, ZT, BM, and AW analyzed the data and made figures. NT, NF, and CG collected the samples and data. NT, ZT, and BS wrote the manuscript. All authors have edited and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2018.00438/full#supplementary-material>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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