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Research Article

Contrasting alpha, beta, and gamma diversity in the littoral zones of mountain lakes: effects of habitat size and within-lake community structuring on bacterial biogeography

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Abstract

Research on microbial biogeography has revealed key patterns like the diversity–area relationship and distance–decay of similarity. However, how habitat size affects bacterial diversity in freshwater environments remains largely unclear. Here, we characterize bacterial communities in the littoral zones of 10 mountain lakes in the Sierra Nevada, CA, ranging in surface area from 0.92 to 71.72 ha. Despite significant habitat size effects on community composition, dominant bacterial phyla were shared across lakes. We found no evidence for diversity–area relationships, either in single samples (alpha diversity) or cumulative lake-level samples (within-lake gamma diversity), when accounting for environmental variation. Moreover, within-lake beta diversity showed little spatial structuring, with similar bacterial community composition across samples regardless of geographic distance. Gamma diversity did not reach saturation with our sample size, and lake size had no effect on the predicted sample size necessary to reach gamma diversity saturation. Our findings offer new insights into diversity–area dynamics and spatial structuring by investigating alpha, beta, and gamma diversity in freshwater environments. Notably, individual water samples captured much of the bacterial community, with strong correlations between alpha and gamma diversity. These results advance our understanding of microbial biogeography and inform sampling designs for characterizing bacterial diversity in freshwater ecosystems.

Keywords: Sierra Nevada; freshwater; bacterioplankton; 16S rRNA gene sequencing; diversity; metabarcoding

Introduction

Determining the factors that shape the geographic distribution of biodiversity is a major goal across ecology and evolution and can also have crucial implications for conservation (Hutchinson 1959, MacArthur and Wilson 1967, Hillebrand 2004, Losos and Ricklefs 2009, Brown 2014). While there is a plethora of research describing biogeographic patterns for plants and animals and the underlying biological processes structuring these patterns (Rahbek 1997, Hillebrand 2004, Peters et al. 2016, Gutierrez et al. 2018, Ji et al. 2019), much less is known about microbial biogeography. However, recent technological advances in DNA sequencing allow investigating microbial diversity at an unprecedented scale (Thompson et al. 2017). For example, in accordance with classic biogeographic patterns described for macroorganisms, geographic structuring of microbial diversity has been detected across latitudes (latitudinal diversity gradient) (Andam et al. 2016, Thompson et al. 2017, Neu et al. 2021), elevations (elevational diversity gradient) (Singh et al. 2012, Wang et al. 2017, Aivelo et al. 2021), and habitat size (diversity–area relationship) (Horner-Devine et al. 2004, Reche et al. 2005). However, patterns differ across microorganisms and environment types, between free-living microbial communities and those associated with plant or animal hosts, and depending on the spatial scale investigated, among others (reviewed in Martiny et al. 2006, Dickey et al. 2021, Härer and Rennison 2023). While these initial attempts to characterize the geographic distribution of microbial diversity provided valuable insights, many open questions remain regarding the factors that structure microbial biogeography.

The species–area relationship (SAR), which was recently extended with the diversity–area relationship (DAR) to incorporate species abundance, is a classic biogeographic pattern stating that richness (SAR) and diversity (DAR) of biological communities are positively associated with habitat size (Arrhenius 1921, Lomolino 2000, Ma 2018). Neutral (greater population sizes) and deterministic (greater environmental heterogeneity) processes underlie these patterns at varying scales (Nilsson et al. 1988). Evidence for the DAR has been found in a broad range of organisms including animals, plants, and fungi (Ricklefs and Lovette 1999, Peay et al. 2007, Harte et al. 2009, Helmus et al. 2014), but also in bacteria (Horner-Devine et al. 2004, Noguez et al. 2005, Reche et al. 2005). However, the slope of bacterial DAR curves appears to differ from those of macroorganisms and varies at different spatial scales (Green and Bohannan 2006, Martiny et al. 2011), raising the question of what drives the observed variation and whether mi-

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crobes and macroorganisms fundamentally differ in their biogeographic patterns. Due to this variability of observed patterns and small number of prior studies investigating bacterial DAR, more studies across various environment types (e.g. marine, freshwater, and terrestrial) are necessary before drawing general inferences.

Our current knowledge regarding bacterial DAR is particularly limited for freshwater environments because observed patterns are often conflicting and come with key limitations. For example, evidence for a positive bacterial DAR has been detected for high-mountain lakes from the Sierra Nevada, Spain (Reche et al. 2005). In contrast, a negative bacterial DAR was observed across lakes in Sweden (Logue et al. 2012). Another study in Nicaraguan lakes found evidence for higher bacterial diversity in larger lakes (Härer et al. 2022), although inferences are complicated by covariance with lake age. Lake size might also covary with elevation or latitude, which might partially explain the discrepancies across studies (Fuhrman et al. 2008, Wang et al. 2017). The inconsistency among these previous results suggests that the DAR does not universally apply to bacterial freshwater communities, warranting further exploration of the factors that might contribute to variation in these patterns. One open question is whether observed DAR patterns might differ across broader spatial scales. The lakes surveyed by Reche et al. (2005) are all of very small size, ranging from 0.01 to 2.10 ha; Logue et al. (2012) overcame this limitation by surveying a broader range of lake sizes. Yet, we do not have a good understanding of when, or how generally, the DAR applies to bacterial diversity patterns in freshwater environments.

Another key limitation to our current knowledge is that the aforementioned studies used only one sample (or multiple replicates from the same sampling site) per lake which may not be sufficient to accurately capture bacterial diversity due to within-lake spatial variation. Indeed, there is limited data on the extent of spatial variation of free-living bacterial communities (Yannarell and Triplett 2004, Jones et al. 2012, Lear et al. 2014, Gu et al. 2023) for freshwater environments. This raises two major questions regarding the spatial distribution of bacterial diversity within environments. First, do the DAR patterns observed from individual samples (i.e. alpha diversity) reflect patterns observed for the cumulative diversity across multiple samples (i.e. within-environment gamma diversity)? To the best of our knowledge, only one prior study tested for an effect of habitat size on the cumulative bacterial diversity calculated across multiple samples of the same saline ponds, but no positive association between gamma diversity and habitat size was found (Kavazos et al. 2018).

Second, does the extent of within-habitat spatial variation in bacterial diversity vary with habitat size? Spatial turnover and, thus, decrease in community composition similarity with geographic distance has been detected at a small scale of around 20 m within small ponds (Lear et al. 2014) and at a larger scale of hundreds of meters within and across lakes (Yannarell and Triplett 2004). Such decrease in compositional similarity (beta diversity) among communities with increasing geographic distance, known as the distance–decay of similarity, are a well-documented pattern (Nekola and White 1999). Distance–decay patterns commonly vary across habitat types and spatial scales and can be produced by a range of factors, including dispersal limitation and environmental heterogeneity, and extensive work has investigated abiotic and biotic factors influencing distance–decay patterns in bacterial communities (Martiny et al. 2006, 2011, Clark et al. 2021). While previous studies on freshwater bacterial diversity clearly show that heterogeneity in community composition exists even across small spatial scales, it remains to be tested whether spatial structuring of bacterial diversity varies with habitat size.

To determine the effects of habitat size on the spatial structuring of bacterial diversity in the littoral zone, we surveyed 10 freshwater mountain lakes of glacial origin in the Sierra Nevada, CA, that vary in surface area from 0.92 to 71.72 ha (Table 1). These lakes are nestled within granite and granitoid substrates (Sickman et al. 2001), are oligotrophic, and lie along an elevational gradient (2321–3279 m; Table 1), classifying them as upper montane (2100–2700 m), subalpine (2700–3200 m), and alpine (above 3200 m). This elevational gradient is associated with climate and dissolved organic carbon that shapes community structure and ecosystem functioning (Symons and Shurin 2016). Past work has revealed strong heterogeneity in microbial community structure among lakes as a function of geography, nutrients, and climate (Schulhof et al. 2020), however, variation within lakes has yet to be examined. We employed environmental DNA sampling coupled with 16S rRNA sequencing, which facilitates standardized sampling and taxonomic identification of bacterial communities (Taberlet et al. 2012, Deiner et al. 2017, Huerlimann et al. 2020, Nguyen et al. 2020). We tested three hypotheses regarding the bacterial diversity of single water samples (alpha diversity), the cumulative diversity across all water samples of a lake (within-lake gamma diversity, which in our study refers to the bacterial diversity detected in littoral surface water) as well as the dissimilarity among water samples of a lake (within-lake beta diversity). Based on previous work, we predicted higher within-lake beta diversity in larger lakes due to greater geographic distances among samples and, potentially, stronger environmental heterogeneity (Horner-Devine et al. 2004, Martiny et al. 2011). Next, we predicted a positive association between lake size and bacterial alpha and gamma diversity in accordance with the DAR (Reche et al. 2005). Lastly, we sought to determine the number of water samples necessary to capture within-lake gamma diversity and we predicted that gamma diversity saturation would be achieved with fewer samples in smaller lakes due to lower spatial variation of bacterial communities (Yannarell and Triplett 2004, Martiny et al. 2011). This data will improve our understanding of the geographic distribution of bacterial diversity within and across habitats of varying size and can have direct implications for designing studies investigating bacterial biogeography in freshwater environments.

Materials and methods

Sampling

Water samples were collected across 10 glacial freshwater lakes during the summer of 2023 (Fig. 1 and Table 1). For each lake, 10 water samples were collected from the littoral zone (except for Upper Granite for which only seven samples were collected due to severe weather conditions). To obtain information on the spatial distribution of bacterial diversity within lakes, samples were collected at approximately equal distances along the shore and GPS coordinates were recorded for each sampling site (Table S1). To reduce the risk of contamination across lakes, precautionary steps were implemented. Prior to our field trip, all sampling equipment (i.e. forceps, syringes, filter housings, and tubes) was sterilized using UV light. Additionally, sampling kits were prepared for each lake, ensuring they remained sealed until we reached the specific location. Water samples were collected without direct contact with the water, using disposable gloves to prevent human-induced contamination. Each sample, measuring 120 ml, underwent manual filtration through a cellulose nitrate filter (Whatman plc, Maidstone, UK; ø 25 mm, pore size 0.2 µm) using a 50 ml sterile syringe equipped with a Luer lock. After filtration, the fil-

Table 1. Sample sizes, surface area, elevation, as well as environmental parameters of the 10 lakes included in this study. The environmental data shown represent the means and SD (in parentheses) of three replicates collected at three sites (temperature, conductivity, and pH) or of two replicate measurements collected at two sites (chl-*a*) along each lake's shore. Note that microbial community data was collected in the summer of 2023 whereas environmental data was collected in the summer of 2024.

Lake	Sample size	Surface area (ha)	Elevation (m)	Temperature (F)	Conductivity (µS/cm)	pH	chl-*a* (µg/l)
Cascade	10	2.04	3147	54.18 (0.32)	2.82 (0.07)	6.16 (0.05)	0.45 (0.18)
Convict	10	71.72	2321	63.8 (0.4)	105.54 (0.38)	7.86 (0.03)	0.53 (0.05)
L. Gaylor	10	8.71	3150	62.07 (0.13)	8.3 (0)	7.01 (0.03)	0.42 (0.11)
U. Gaylor	10	3.86	3204	59.31 (0.29)	10.24 (0.17)	7.01 (0.04)	0.56 (0.17)
L. Granite	10	7.48	3167	60.33 (0.05)	8.5 (0)	7.45 (0.05)	0.53 (0.05)
U. Granite	7	5.79	3177	58.19 (0.11)	6.23 (0.05)	6.8 (0.05)	0.33 (0.02)
Long	10	14.00	3279	59.07 (0.1)	6.93 (0.05)	7.02 (0.06)	0.48 (0.03)
Mary	10	43.26	2719	65.17 (0.1)	26.69 (0.52)	7.17 (0.03)	0.76 (0.05)
May	10	20.52	2845	63.89 (0.31)	2.9 (0)	6.38 (0.55)	0.33 (0.01)
Wasco	10	0.92	3141	65.79 (0.33)	7.39 (0.12)	6.47 (0.1)	0.39 (0.11)

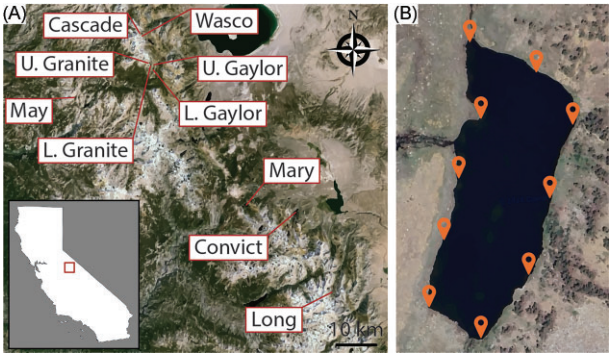

Figure 1. Modified satellite image of the sampling region including all 10 lakes, map of California, with a highlighted square, indicates the geographic location of the study area (A). Example of our sampling strategy to collect 10 samples along the shoreline of each lake with approximately equal distance among them (B).

ters were carefully extracted from the housing using sterile disposable forceps and placed into 1.5 ml tubes. These tubes were immediately placed on ice and transferred to −80°C the day of collection. Between sampling sites of the same lake, syringes were rinsed multiple times with lake water before collecting the next sample. Water samples were collected roughly at the same time of day (late morning to early afternoon).

In the summer of 2024, we measured an array of physicochemical and ecosystem parameters to examine how variation in environmental conditions within and across lakes may shape microbial diversity (Table 1). Two replicates of shoreline chlorophyll-a (chl-*a*) concentration were measured as a proxy for primary production in each lake by filtering a known volume of lake water (500–800 ml) through a 0.45-um glass fiber filter (Whatman GFF). After water filtration, filters were preserved at −20°C for downstream processing. A 24-h chl-*a* extraction was performed using 90% acetone at 4°C and a fluorometer (Turner Designs, San Jose, CA) was used to measure chl-*a* concentrations. Moreover, we used a YSI Professional Plus device (YSI Incorporated, Yellow Springs, OH) to collect physicochemical parameters of the lakes (temperature, conductivity, and pH). For each lake, we collected three replicate YSI measurements at three sites, and we then averaged values since we found very little variation across sites of the same lake.

DNA extraction and amplification

DNA was extracted using the QIAGEN DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) with slight modifications. First, filters were immersed in 450 µl buffer ATL and 50 µl proteinase K and incubated at 65°C for 1 h. Next, 500 µl buffer AL and 500 µl 100% ethanol were added and the tube was thoroughly vortexed. Then, the mixture was applied to a DNeasy Mini spin and the extraction was done according to the manufacturer's instructions from this point. Subsequently, a 291-bp fragment from the V4 region of the 16S rRNA gene was amplified employing barcoded 515F and 806R primers (obtained from https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP.md). Polymerase chain reactions (PCRs) were conducted in 20 µl reaction volume using the Platinum II Hot Start PCR master mix (Thermo Fisher Scientific, Waltham, MA). Negative controls were included during DNA extraction and PCR amplification; none of these controls exhibited detectable DNA concentrations before or after PCR amplification. The PCR procedure comprised an initial denaturation step at 98°C for 60 s, followed by 35 amplification cycles comprising 10 s at 98°C, 20 s at 56°C, and 60 s at 72°C, concluding with a final elongation step at 72°C for 10 min. Gel electrophoresis (2% agarose) confirmed amplification specificity. DNA concentrations of amplicons were measured using a Qubit 4 Fluorometer (Thermo Fisher Scientific) and libraries were constructed by equimolar pooling of barcoded samples. Subsequent purification of libraries, quality assessment using a Bioanalyzer (Agilent Technologies, Santa Clara, CA) and sequencing on the Illumina MiSeq 500 (PE250) platform were performed at the UC Davis Genome Center. The raw sequencing data (https://doi.org/10.6084/m9.figshare.25404235.v1), and data files and R scripts (https://doi.org/10.6084/m9.figshare.25411975.v1) are accessible from the figshare repository.

Data processing

The sequencing run produced 3 792 804 raw reads across our 97 water samples (mean: 39 101 reads/sample; Table S1). Data were imported into QIIME2 (Bolyen et al. 2019), quality of sequencing data was checked, reads were merged and corrected, and chimeric sequences were removed to obtain amplicon sequencing variants (ASVs) using the *dada2* denoise-paired plug-in (Callahan et al. 2016). We then produced a bacterial phylogeny across all ASVs using FastTree 2.1.3 (Price et al. 2010) and assigned taxonomy to ASVs based on the SILVA 138 ribosomal RNA (rRNA) database with a 99% similarity threshold (Quast et al. 2013). As a final filter-

ing step, ASVs with low abundance (<10 reads) that were only present in one sample, that could not be assigned below the phylum level, or were chloroplasts, mitochondria, or archaea were removed. These filtering steps left us with a total of 2 390 171 reads (mean: 24 641 reads/sample; Table S1). ASV counts were normalized using scaling with ranked subsampling with C_{min} values of 15 675 reads on the sample level (alpha diversity) and 169 183 reads on the lake level (gamma diversity) (Beule and Karlovsky 2020), which represent sufficient sequencing depths to accurately capture bacterial diversity in our system (Fig. S1).

Data analysis

Effects of lake size were tested for different metrics of alpha diversity (i.e. bacterial diversity of individual water samples; ASV richness, Faith's phylogenetic diversity, and Shannon diversity), beta diversity (i.e. dissimilarity of microbial communities between water samples; nonphylogenetic: Bray–Curtis dissimilarity, phylogenetic: unweighted and weighted UniFrac) (Lozupone and Knight 2005, Lozupone et al. 2011), and gamma diversity (i.e. cumulative bacterial diversity of lakes; ASV richness, Faith's phylogenetic diversity). Using a combination of abundance-based (Bray–Curtis) and phylogeny-based (UniFrac) beta diversity metrics allowed us to obtain a comprehensive picture of the turnover in abundance and phylogenetic diversity of bacterial lineages across the investigated communities. For alpha diversity, linear mixed effect-models (*lmer* function in the "lme4" package v1.1–31) (Bates et al. 2015) were used for Faith's phylogenetic diversity and Shannon diversity. Negative binomial generalized linear mixed-effects models (*glmer.NB* function in the "lme4" package v1.1–31), which are suitable for analyzing count data by accounting for skewness and overdispersion of the data, were used for ASV richness. All models included lake as random effect and log surface area, elevation, temperature, conductivity, pH, and chl-*a* concentration as fixed effects. For gamma diversity, linear models (*lm* function in the "stats" package v4.2.1) were used for Faith's phylogenetic diversity and Shannon diversity and negative binomial generalized linear models (*glm.NB* function in the "MASS" package v7.3–57) (Venables and Ripley 2002) were used for ASV richness with the same fixed effects as in the alpha diversity models. Including elevation and other abiotic and biotic factors that have been shown to affect microbial communities (Yannarell and Triplett 2004, Lear et al. 2014, Wang et al. 2017) in our models allowed us to test for effects of lake size on bacterial diversity (i.e. DAR) after accounting for environmental variation. To account for the fact that environmental data was collected 1 year after bacterial communities were sampled, we conducted all analyses regarding the DAR for alpha and gamma diversity, as well as PERMANOVAs for beta diversity, both with and without environmental variables, finding that results were qualitatively similar in both cases. Further, Pearson correlation coefficients (except for Shannon diversity for which we used the Spearman's correlation coefficient because data was not normally distributed based on a Shapiro–Wilk test) between mean alpha diversity and gamma diversity on the lake level were calculated using the *cor.test* function in the "stats" package v4.2.1.

To test for differences in dissimilarity of bacterial communities across lakes, PERMANOVA (*adonis2* function in the "vegan" package v2.6–2) (Anderson 2001, Oksanen et al. 2019) was used with either lake or log surface area, elevation, temperature, conductivity, pH, and chl-*a* concentration as fixed effects. Within-lake beta diversity dispersion was further calculated by determining the distance of each water sample from the centroid of its respective lake (*betadisper* function in the "vegan" package v2.6–2). Linear mixed effect-models with lake as random effect and log surface area as well as the environmental variables mentioned above as fixed effects were then used to test for differences in within-lake beta diversity dispersion. Ranked partial Mantel tests using Spearman correlations (*mantel.partial* function in the "vegan" package v2.6–2), accounting for geographic distance among lakes, were used to determine whether across-lake bacterial community dissimilarity is correlated with difference in surface area. To investigate spatial structuring of bacterial diversity within lakes, ranked Mantel tests using Spearman correlations (*mantel* function in the "vegan" package v v2.6–2) were used whereas geographic distances between sampling sites were calculated with the *geoDist* function in the "geosphere" package v1.5–18. Using ranked Mantel tests allowed us to determine the strength of distance–decay gradients of similarity of bacterial communities within each lake (Graco-Roza et al. 2022). Then, we used linear models (*lm* function in the "stats" package v4.2.1) to test for associations between log surface area and within-lake correlation coefficients taking into account environmental variables.

To study potential effects of lake size on gamma diversity saturation, random subsets of water samples from each lake were selected, with sample sizes ranging from 2 to 10 (or 7 in the case of Upper Granite) and gamma diversity was calculated for each sample size and lake. Ten iterations at each sample size were run and mean gamma diversity values per sample size and lake were calculated. For each lake, linear and quadratic models with sample size as fixed effect were applied to compare, which model represented the better fit for explaining changes in mean gamma diversity across sample sizes. Model selection was done based on ANOVA *F*-tests as well as AIC scores and the proportion of variance explained by each model. Since quadratic models represented the better fit for all lakes, the sample size at which gamma diversity is expected to saturate for each lake was predicted based on those models using the *predict* function in the "stats" package v4.2.1. Next, it was tested whether the sample size associated with predicted gamma diversity saturation differs with log surface area using linear models. Bacterial community composition across lakes was visualized on the phylum level, and only phyla with a mean relative abundance of >1% are shown. All statistical analyses were done in R v4.2.1 (R Core Team 2022).

Results

Bacterial community composition varies with lake size

Across the 10 mountain lakes, we found that bacterial communities were dominated by Proteobacteria (26.9%–52.1%), Bacteroidota (6.7%–42%), and Actinobacteriota (10.3%–61.7%); these phyla were highly abundant in all lakes with some variation in relative abundance across lakes (Fig. 2A). At a higher taxonomic resolution, May Lake differed from all other lakes since an uncultured bacterial taxa of the family Sporichthyaceae constituted 57.5% of the bacterial community while this taxon was far less common across the other lakes (0%–7.3%). Despite the major bacterial phyla being in large part shared across lakes, bacterial community composition strongly differed (Bray–Curtis dissimilarity: $F_{1,96} = 104.34$, $R^2 = 0.915$, $P = .001$; unweighted UniFrac: $F_{1,96} = 11.84$, $R^2 = 0.55$, $P = .001$; and weighted UniFrac: $F_{1,96} = 80.98$, $R^2 = 0.893$, $P = .001$), with 55%–91.5% of beta diversity captured across lakes (Fig. 2B). Moreover, surface area affected bacterial communities (Bray–Curtis dissimilarity: $F_{1,96} = 27.36$, $R^2 = 0.082$, $P = .001$; unweighted UniFrac: $F_{1,96} = 5.47$, $R^2 = 0.035$, $P = .001$; and weighted

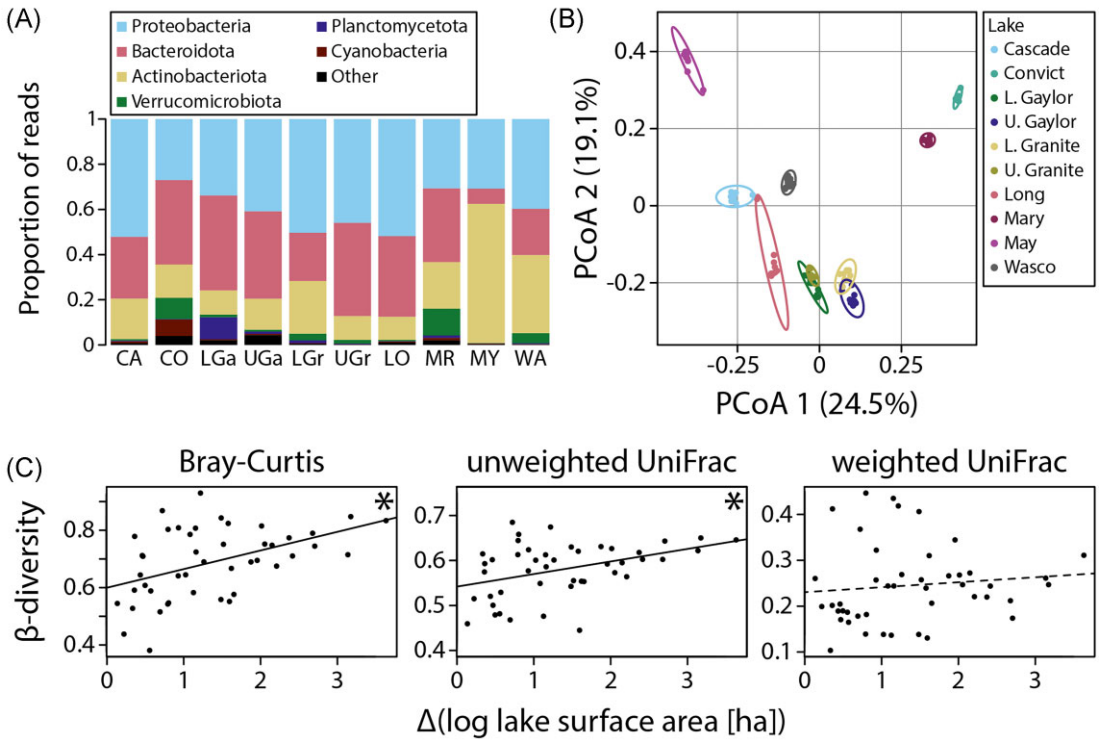

Figure 2. Taxonomic bar plots showing the relative abundances of the six major phyla (A), PCoA plot based on Bray–Curtis dissimilarity (B), and correlations between across-lake beta diversity and differences in lake size based on Bray–Curtis dissimilarity, unweighted UniFrac, and weighted UniFrac (C). CA: Cascade; CO: Convict; LGa: Lower Gaylor; Uga: Upper Gaylor; LGr: Lower Granite; UGr: Upper Granite; LO: Long; MR: Mary; MY: May; and WA: Wasco. $*P < .05$.

UniFrac: $F_{1,96} = 9.71$, $R^2 = 0.038$, $P = .001$) after accounting for effects of elevation, temperature, conductivity, pH, and chl-*a* concentration (Table S2). While surface area had the strongest effect on beta diversity based on Bray–Curtis dissimilarity, the other environmental variables were also significant predictors and some of them had stronger effects on beta diversity than lake size based on the two UniFrac metrics (Table S2). Further, after accounting for geographic distance between lakes, there was a positive correlation between surface area difference and mean beta diversity among lakes based on Bray–Curtis dissimilarity (partial Mantel test; $r = 0.347$, $P = .044$) and unweighted UniFrac ($r = 0.335$, $P = .031$), but not for weighted UniFrac: ($r = 0.223$, $P = .156$) (Fig. 2C). In sum, these results highlight that community composition varies with lake size, after controlling for environmental variables, allowing us to investigate the effects of habitat size on the geographic distribution of bacterial alpha, beta, and gamma diversity in these freshwater environments.

Within-lake spatial structuring of bacterial community composition

The extent of dissimilarity of bacterial communities (beta diversity) calculated among the samples from each lake did not vary with surface area based on Bray–Curtis dissimilarity ($\beta = 0.042$, $P = .110$), unweighted UniFrac ($\beta = 0.02$, $P = .557$) or weighted UniFrac ($\beta = 0.001$, $P = .408$) after accounting for environmental variables (Fig. 3A). Geographic distance among sampling sites within a lake was positively correlated with beta diversity only in Wasco Lake for Bray–Curtis dissimilarity ($r = 0.295$, $P = .038$) and Long Lake for weighted UniFrac ($r = 0.225$, $P = .044$). There was no effect of log surface area on correlation coefficients calculated between within-lake geographic distances and beta diversity (Bray–Curtis dissimilarity: $\beta = 0.045$, $P = .447$; unweighted UniFrac: $\beta = 0.086$, $P = .134$; and weighted UniFrac: $\beta = 0.096$, $P = .328$) (Fig. 3B). The proportion of shared bacterial ASVs was not correlated with geographic distance among sampling sites in any lake and again, there was no association between log surface area and the strength of correlation ($\beta = 0.017$, $P = .793$).

DAR for alpha and within-lake gamma diversity of bacterial communities

Across all lakes, we did not find evidence for associations between log surface area and alpha diversity (ASV richness: $\beta = 0.043$, $P = .641$; Shannon diversity: $\beta = -0.136$, $P = .693$; and Faith's phylogenetic diversity: $\beta = -0.002$, $P = .999$) of bacterial communities after accounting for effects of elevation, temperature, conductivity, pH, and chl-*a* concentration. (Fig. S2A). Of these factors, only chl-*a* concentration had an effect on alpha diversity based on ASV richness ($\beta = 0.185$, $P < .001$). Regarding the effect of lake size, a similar pattern of no evidence for the DAR was observed when investigating gamma diversity on the lake level (ASV richness: $\beta = -0.175$, $P = .118$; Shannon diversity: $\beta = -0.073$, $P = .819$; and Faith's phylogenetic diversity: $\beta = -2.122$, $P = .663$) (Fig. S2B). All other environmental factors were nonsignificant based on Shannon diversity and Faith's phylogenetic diversity, but elevation ($\beta = 0.002$, $P = .039$), pH ($\beta = -0.559$, $P = .048$), and chl-*a* concentration ($\beta = 2.547$, $P < .001$) had significant effects on within-lake gamma diversity based on ASV richness. The high abundance of an uncultured bacterium from the family Sporichthyaceae that was far less abundant in all other lakes potentially led to a reduced bacterial diversity within May Lake (Fig. S2). Excluding May Lake from our dataset highlighted trends of higher alpha and gamma diversity based on Shannon diversity (Fig. 4), yet we did not detect sta-

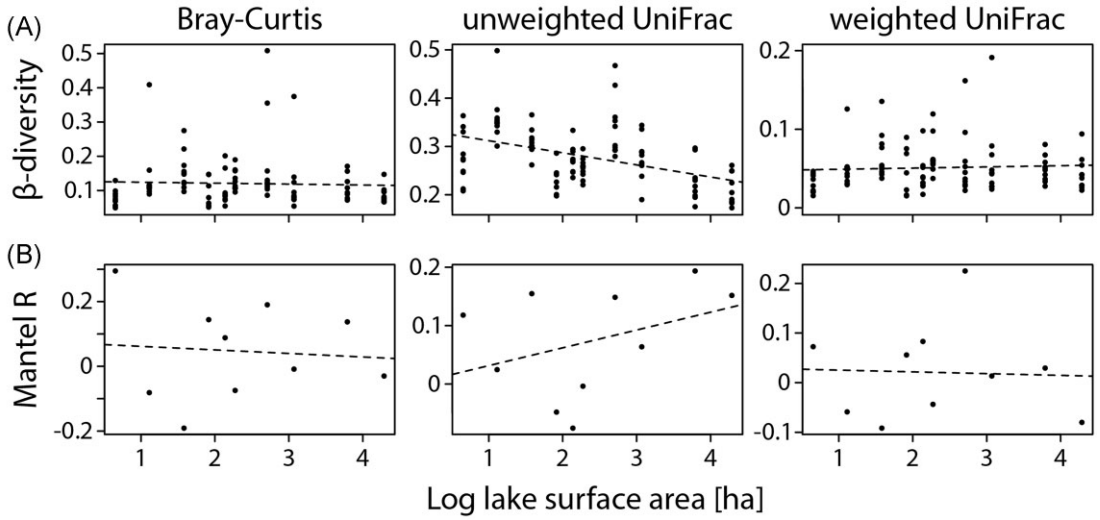

Figure 3. Within-lake beta diversity did not differ with lakes size for all three beta diversity metrics (A). Correlation coefficients (Mantel R) of within-lake beta diversity and within-lake geographic distances also did not differ with lake size (B).

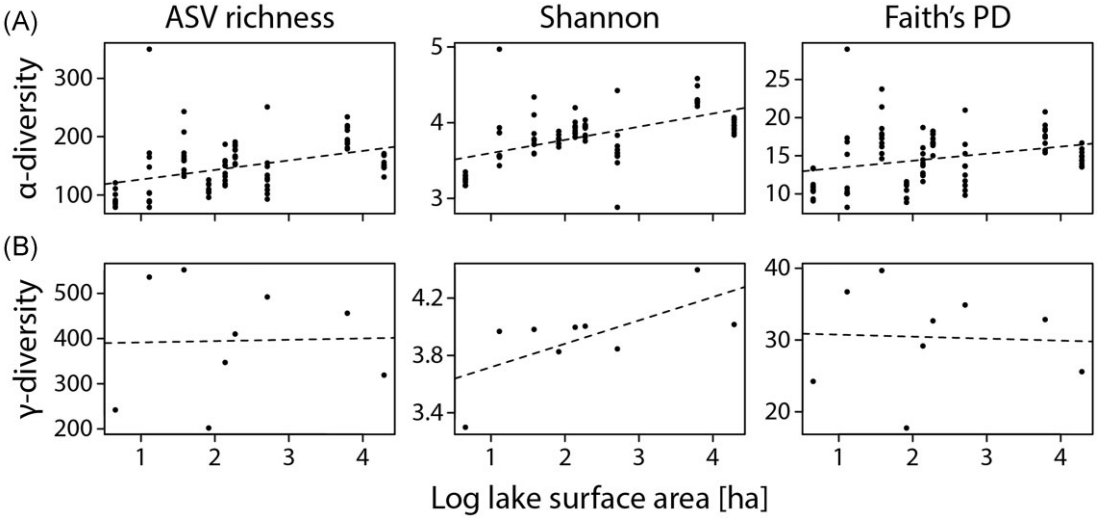

Figure 4. No evidence for DAR was detected for alpha diversity (A) or within-lake gamma diversity (B) based on ASV richness, Shannon diversity, or Faith's phylogenetic diversity.

tistical evidence for an effect of lake size on the measures after accounting for variation in environmental factors. Notably, mean alpha diversity and gamma diversity measures calculated on the lake level were strongly correlated for ASV richness ($r = 0.668$, $P = .035$), Shannon diversity ($\rho = 0.976$, $P < .001$), and Faith's phylogenetic diversity ($r = 0.792$, $P = .006$).

Association between lake size and within-lake gamma diversity saturation of bacterial communities

We found that the within-lake gamma diversity of bacterial communities increased with sample size across lakes but did not reach saturation with our sample size of 10 sites per lake based on ASV richness (Fig. 5A) and Faith's phylogenetic diversity (Fig. 5B). We then compared linear and quadratic regression models to determine if there are indications for within-lake gamma diversity saturation, i.e. the quadratic model represents a better fit than the linear model. For both ASV richness (Table S3) and Faith's phylogenetic diversity (Table S4), quadratic models represented a significantly better fit in all lakes (except for Upper Granite, where no difference between models was detected), and they explained a larger proportion of the variance and AIC scores were lower compared to linear models in all lakes. Next, we predicted the sample size at which gamma diversity would saturate within each lake, assuming a quadratic distribution, and tested whether within-lake gamma diversity would be reached with lower sample sizes in smaller lakes. Based on our predictions, ASV richness saturated at 9–28 samples (Table S3) whereas Faith's phylogenetic diversity saturated at 8–16 samples across lakes (Table S4). We detected no association between the sample size of saturated within-lake gamma diversity and lake size, neither for ASV richness ($\beta = 0.084$, $P = .958$) nor for Faith's phylogenetic diversity ($\beta = 0.679$, $P = .330$).

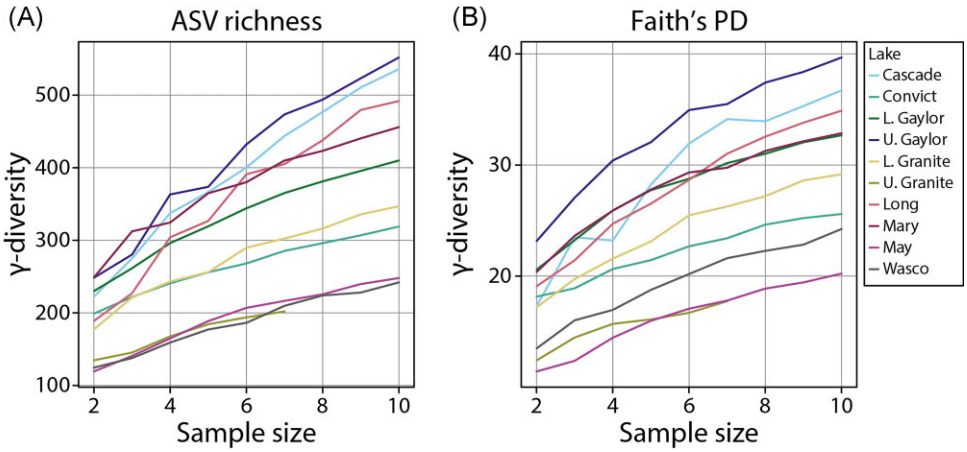

Figure 5. Gamma diversity saturation with increasing sample size for ASV richness (A) and Faith's phylogenetic diversity (B) across the 10 lakes.

Discussion

While there is substantial evidence that microbial diversity is geographically structured, we still lack a comprehensive understanding of bacterial biogeography within aquatic environments despite some initial attempts to address this topic (Yannarell and Triplett 2004, Lear et al. 2014, Dickey et al. 2021). Many open questions remain and our study specifically explores how habitat size affects the spatial distribution of bacterial diversity of the littoral zone, within and across mountain lakes of the Sierra Nevada, CA. Bacterial communities were consistently dominated by three phyla; Proteobacteria, Bacteroidota, and Actinobacteriota, which is in agreement with previous studies of aquatic habitats in different geographic regions and climate zones (Sevellec et al. 2018, Härer et al. 2020, Tandon et al. 2020, Rojas-Jimenez et al. 2021). Although these phyla dominated bacterial communities across the 10 mountain lakes, we detected a strong effect of lake size on bacterial communities, even when controlling for variation in elevation and environmental factors such as temperature, conductivity, pH, and chl-*a* concentration. This allowed us to explore the effects of habitat size on the spatial distribution of bacterial diversity, but we detected no evidence for the DAR across lakes or for distance–decay gradients of similarity within lakes (Arrhenius 1921, Nekola and White 1999).

Distance–decay patterns are a common feature characterizing the geographic distribution of biodiversity and they can be produced by two key mechanisms (Nekola and White 1999). First, as geographic distance increases the environmental conditions may change and such heterogeneity can lead to divergence in locally adapted biological communities. Second, dispersal limitation can result in distinct biological communities with low similarity between distant geographic locations (Bell 2010). Distance–decay patterns have been detected previously in a variety of microbial communities (e.g. Langenheder and Ragnarsson 2007, Martiny et al. 2011), including bacteria in freshwater habitats (Yannarell and Triplett 2004, Jones et al. 2012, Lear et al. 2014, Gu et al. 2023). Yet, the spatial scale at which such patterns are observed can vary; substantial within-lake turnover of bacterial community composition has been shown to occur at a scale of <20 m (Lear et al. 2014) to hundreds of meters (Yannarell and Triplett 2004). Notably, we found no evidence for within-lake distance–decay patterns of bacterial communities (except for Wasco Lake and Long Lake) with distances between sampling sites ranging from 22.2 to 260.7 m in the smallest lake and from 182.2 to 1497.7 m in the largest lake. This is clearly larger than the scale at which distance–decay patterns were found in previous studies (e.g. Lear et al. 2014), suggesting very little spatial structuring of bacterial communities in the mountain lakes we investigated. This was underscored by the observation that a large proportion of the variation in bacterial communities (55%–91.5%) was explained across, rather than, within lakes. Further support was provided by the observation that the abundance of major bacterial phyla was largely similar across sampling sites within a lake (Fig. S3), despite clear separation of bacterial communities among lakes (Fig. 2).

The strength of distance–decay patterns, measured by the correlation coefficients of within-lake microbial community dissimilarity and geographic distance among sampling site of the same lake, did not differ with lake size. Hence, the extent of within-lake spatial variation of bacterial communities appears not to be a function of habitat size in our system. The absence of distance–decay gradients in all but two lakes, independent of their surface area, could be explained by a lack of substantial within-lake environmental heterogeneity, at least within the littoral zone, and/or a lack of dispersal limitation within these lakes ranging in surface area from 0.92 to 71.72 ha. Since our sampling was limited to the littoral zone, a more comprehensive sampling of the pelagic zone may have uncovered stronger distance–decay gradients driven by environmental heterogeneity between these habitat types. Yet, a previous study did not detect consistent differences between littoral and pelagic habitats (Jones et al. 2012), which leaves open the question of how much these habitats differ in bacterial community composition. Surface water sampled in the littoral zone might be enriched in soil microbes and distance–decay patterns and spatial turnover might differ for aquatic and soil bacteria, which is not possible to disentangle with our current dataset. Yet, we took precautionary measures to reduce the likelihood of sampling soil bacteria (i.e. not entering the water, carefully taking water samples without disturbing the water, and dispensing water on the shore after filtering). We are confident that our data largely consists of aquatic bacteria; however, future studies investigating within-lake variation of bacterial communities could conduct systematic sampling across littoral and pelagic zones and, ideally, also collect soil samples along the shore to contrast microbial biogeography patterns for aquatic and soil bacteria. Importantly, the area of the littoral zone scales with lake size, and the geographic distances among sampling sites also increase in larger lakes, meaning that our sampling approach is well-suited to addressing the relationship between lake size and the diversity

and turnover of bacterial communities in this habitat. Overall, our findings illustrate that spatial structuring of bacterial diversity along the shorelines is not affected by lake size, even when considering the greater geographic distance between sampling sites in larger lakes. Yet, the exact mechanisms that lead to the absence of distance–decay patterns remain to be investigated and assessing the extent of within-lake environmental heterogeneity will be instrumental.

Next, we tested for associations between lake size and bacterial alpha and gamma diversity, i.e. the DAR (Ma 2018). After accounting for elevation, which has been shown to affect diversity of microbial communities (Wang et al. 2011, Singh et al. 2012, Aivelo et al. 2021), and a range of environmental factors, there was no evidence for DAR patterns based on alpha and within-lake gamma diversity, both when using the full dataset or when excluding one intermediate-sized lake (May Lake) with particularly low bacterial diversity and anomalously high abundance of a single uncultured bacterial taxa of the family Sporichthyaceae. This taxon was abundant across multiple samples from May Lake, suggesting that its dominance was not an error or due to contamination. For ASV richness, we found that chl-*a* concentration affected alpha diversity and that elevation, pH and chl-*a* concentration affected within-lake gamma diversity. These results suggest that other environmental factors, besides lake size, have a stronger effect on the richness of bacterial communities in our system and highlight the importance of including a range of environmental variables when studying diversity patterns of microbial communities. Different explanations have been brought forward to explain the DAR, such as heterogeneity of habitats and resources, variation in immigration and extinction rates as well as geographic isolation (reviewed in Lomolino 2001). Yet, we currently do not have a good understanding of how these factors might contribute to shaping the DAR of microorganisms in aquatic environments. Previous work investigating bacterial DAR in aquatic environments found varying patterns, including positive (Reche et al. 2005), negative (Logue et al. 2012), or no relationships (Kavazos et al. 2018) between bacterial diversity and habitat size. It is unclear why such contrasting patterns have been observed, but technical differences among studies such as sampling effort (one vs multiple samples per lake), sample type (water vs biofilm), and method used for characterizing bacterial communities (community profiling via denaturing-gradient gel electrophoresis versus high-throughput sequencing) might play a role. Further, one study found that nutrient availability, rather than habitat size, was the main driver of bacterial diversity (Logue et al. 2012). While we did not detect evidence for the DAR, our study represents one of the very few to test DAR patterns for bacterial communities in aquatic environments and, to the best of our knowledge, we are the first to contrast alpha and gamma diversity measures. In line with previous studies, our results highlight that abiotic and biotic characteristics can affect bacterial diversity of the investigated environments and should be considered when investigating microbial biogeography.

Notably, we detected significant effects of temperature, conductivity, pH, and chl-*a* concentration on across-lake bacterial community composition (beta diversity), but not for alpha or gamma diversity (except for some effects on ASV richness, see above). The beta diversity results are in line with numerous previous studies showing strong effects of environmental factors on aquatic bacterial communities (Lindstrom and Leskinen 2002, Yannarell and Triplett 2004, Logue et al. 2012, Lear et al. 2014). Notably, a previous study on these mountain lakes found strong evidence for the effect of nutrients and climate structuring beta diversity, which in combination with our results demonstrates the importance of local environmental selection in shaping bacterial diversity in this system (Schulhof et al. 2020). Two of the factors we measured, pH and temperature, are known to affect bacterial communities and we also found them to be strong predictors of beta diversity across lakes. Regarding alpha and gamma diversity, our results contrast previous studies that found a positive correlation between water temperature and alpha diversity across lakes (Yannarell and Triplett 2004), and where water chemistry explained a significant proportion of variation in gamma diversity across saline ponds (Kavazos et al. 2018). While we currently do not know why we found different patterns for alpha and gamma diversity compared to previous studies, the discrepancy could be explained by the fact that environmental data was collected 1 year after the bacterial communities were sampled, yet all samples were collected in summer. Thus, temperature, conductivity, pH, and chl-*a* do not necessarily reflect the conditions at the time when the microbial communities were sampled. However, we argue that this environmental data is biologically relevant and meaningful since the ranking of lakes based on these characteristics is not expected to change strongly, i.e. the coldest lake in the summer of 2023 is likely the coldest in the summer of 2024 as well. Yet, this limitation should be taken into account when interpreting our results. Importantly, we ran statistical analyses with and without the environmental variables and found that the results remained qualitatively similar, further supporting the robustness of our findings. Thus, our results provide crucial first insights into associations between habitat size and bacterial diversity but additional work will be necessary to better understand to what extent such associations are affected by environmental factors.

Most studies on bacterial diversity in aquatic environments have been limited to one or a few sampling sites per habitat and, thus, the relationship between alpha and gamma diversity remains largely unknown. One crucial question in our effort to better understand bacterial biogeography is how well individual samples represent the cumulative diversity of a given habitat, in our case the littoral zones of mountain lakes. Our results revealed that 10 samples per lake were not sufficient to reach gamma diversity saturation and statistical models indicate that between 8 and 28 samples, depending on lake and the chosen diversity metric, would be necessary to reach saturation (Tables S3 and S4). Note that predicted saturation at eight samples is for Upper Granite Lake for which we were only able to collect seven water samples. This prediction is based on the observation that gamma diversity saturates rather than increasing linearly with sampling effort as indicated by the better fit of quadratic models compared to linear models for explaining gamma diversity (Tables S3 and S4). Importantly, our sampling was restricted to the littoral zone, which may underestimate the true gamma diversity of these lakes. Sampling additional habitats, such as the pelagic zone or deeper water layers, could further increase gamma diversity and potentially shift the sample size required for reaching saturation. This may be particularly relevant in lakes that are vertically stratified and show environmental heterogeneity associated with depth, such as primary productivity and consumer communities. Exploring how saturation curves differ when integrating varying habitats and depths within a lake would provide additional valuable insights into the factors shaping bacterial biogeography. Notably, the sample size at which bacterial gamma diversity might saturate has not been explicitly tested in aquatic habitats. We found no effect of lake size on the sample size necessary to reach gamma diversity saturation, which could be explained by the observation that

we generally detected large overlap of bacterial community composition (Fig. 2A) and observed no distance–decay patterns within lakes (Fig. 3B). In contrast, distance–decay patterns of bacterial communities have been found in other aquatic systems and, thus, we predict that gamma diversity would be saturated with larger sample sizes in such environments with stronger spatial structuring of bacterial diversity (Yannarell and Triplett 2004, Lear et al. 2014, Gu et al. 2023).

The results of our study also have practical implications for sampling design when characterizing bacterial diversity in aquatic environments. While we found support for gamma diversity saturation (i.e. quadratic models represented a better fit than linear models), linear models still represented a good fit for explaining gamma diversity in relation to sample size. Moreover, mean alpha diversity and within-lake gamma diversity were strongly correlated and bacterial community composition showed high similarity across samples of the same lake (Fig. S3), suggesting that alpha diversity measures obtained from one or a few samples appear to be a good indicator for within-lake gamma diversity. However, DAR patterns appeared to vary to some extent between alpha and gamma diversity, suggesting it is necessary to carefully explore the relationships between these two diversity measures when studying bacterial biogeography. In sum, our results indicate that low sample sizes can offer a useful indicator for the bacterial diversity of aquatic environments, yet depending on the research question more exhaustive sampling might be necessary for comprehensively describing and comparing bacterial diversity. We further highlight that in our study "gamma diversity" refers solely to the bacterial diversity detected in littoral surface water in the summer of 2023, and patterns would most likely differ if water samples were taken at multiple timepoints, from the littoral and pelagic zones or from various depths. Thus, the spatial and temporal magnitude of sampling efforts needs to be considered when describing gamma diversity, and in particular when comparing results across studies to infer general patterns. Overall, our results show that most spatial turnover in lake bacterioplankton communities occurs between rather than within lakes, with different sites of the same lake showing highly similar composition. This suggests that high connectivity and low environmental heterogeneity within Sierra Nevada mountain lakes leads to much more homogenous bacteria assemblages than those found in different lakes.

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Author contributions

Andreas Härer (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing), Joshua Dominguez (Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing), Jonathan B. Shurin (Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing), and Diana J. Rennison (Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing).

Supplementary data

Supplementary data is available at *FEMSEC Journal* online.

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