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RESEARCH

Environment and Host Genetics Influence the Biogeography of Plant Microbiome Structure

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Abstract

To understand how microbiota influence plant populations in nature, it is important to examine the biogeographic distribution of plant-associated microbiomes and the underlying mechanisms. However, we currently lack a fundamental understanding of the biogeography of plant microbiomes across populations and the environmental and host genetic factors that shape their distribution. Leveraging the broad distribution and extensive genetic variation in duckweeds (the *Lemna* species complex), we identified key factors that governed plant microbiome diversity and compositional variation geographically. In line with the microbial biogeography of free-living microbiomes, we observed higher bacterial richness in temperate regions relative to lower latitudes in duckweed microbiomes (with 10% higher in temperate populations). Our analyses revealed that higher temperature and sodium concentration in aquatic environments showed a negative impact on duckweed bacterial richness, whereas temperature, precipitation, pH, and concentrations of phosphorus and calcium, along with duckweed genetic variation, influenced the biogeographic variation of duckweed bacterial community composition. Analyses of plant microbiome assembly processes further revealed that niche-based selection played an important role (26%) in driving the biogeographic variation of duckweed bacterial communities, alongside the contributions of dispersal limitation (33%) and drift (39%). These findings add significantly to our understanding of host-associated microbial biogeography and provide important insights for predicting plant microbiome vulnerability and resilience under changing climates and intensifying anthropogenic activities.

Keywords Biogeography · Duckweeds · Freshwater ecosystem · Host genetics · Microbiome · Water chemistry

Introduction

Plants host diverse microorganisms, and these microbial symbionts are important for the functioning of plants within ecosystems [1, 2]. To better understand the influence of microbiomes on plant populations across geographic ranges in nature, it is important to examine the biogeographic patterns of plant-associated microbiomes and the mechanisms that drive these patterns [3]. While our knowledge of microbial biogeography has advanced greatly through investigating free-living microbiomes across terrestrial, marine, and atmospheric ecosystems [4–8], significant knowledge gaps exist as to what drives the distribution of local microbiome diversity and compositional variation in host-associated microbiomes [3, 9]. As a result, it remains largely unclear whether the principles of microbial biogeography derived from free-living microbiomes can be generalized to host-associated microbiomes not only at the broad biome level (representing distinct community types consisting of different flora) [10–13] but especially at the individual host organism level across populations [3].

While various biogeography theories have been proposed to explain the distribution of diversity in plants and animals [14, 15], microbial diversity often does not follow the same patterns as observed in their macroscopic counterparts despite notable exceptions [3, 9, 16]. For instance, in contrast to the latitudinal diversity gradient that decreases from lower to higher latitudes in plants and animals [14, 15], global bacterial diversity peaks in temperate regions across free-living soil, marine, and airborne microbiomes [4–7]. In host-associated microbiomes, ectomycorrhizal fungal

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richness associated with roots, for instance, also peaks in midlatitude regions from tropical to subarctic biomes [12]. The frequent deviations of microbial biogeography from macroorganisms and complex distribution patterns of diversity [9] suggest that ecological factors that may or may not follow latitudinal gradients can drive the biogeographic distribution of microbial diversity. Factors that covary with latitude such as temperature and precipitation have been found to influence the distribution of bacterial and fungal richness in free-living microbiomes [4–6], whereas factors that do not exhibit such a correlation (e.g., soil pH, nutrient concentration) may weaken the patterns and lead to a distinct biogeographic distribution [7, 17]. Compared to free-living microbiomes, symbiotic microbiomes are subject to host-imposed niche filtering [3, 9–12, 18–20], which has the potential to modify the role of environmental factors in driving microbial biogeography. The extent to which host plants, such as their genetic variation, affect the distribution of microbial diversity may depend on whether hosts have adapted to the same or different environmental factors that influence microbial diversity. If hosts exhibit adaptation to the same environmental factors as microbes, host genetic variation may reinforce the patterns of microbial diversity caused by environments, whereas dissimilar adaptations may weaken the patterns. This has yet to be examined for plant microbial biogeography.

Different from the complexity in microbial diversity patterns, decay in microbial community similarity over geographic distance is ubiquitously detected in free-living [5–7] and host-associated microbiomes [11, 13, 21, 22]. Such distance decay can arise due to a combination of processes including selection, dispersal, and drift [23–26]. In free-living soil, marine, and airborne microbiomes, dispersal limitation and drift that promote stochasticity play a major role (55–87% and 3–25%, respectively) in driving the biogeographic variation of microbial community composition, whereas niche-based selection by environments accounts for 11–26% [6]. Compared to free-living microbiomes in nature, the relative importance of selection, dispersal, and drift in host-associated microbiomes has rarely been quantified. In host-associated microbiomes, apart from selection by environments, selection by host genetic variation may also contribute to the biogeographic variation of microbial community composition. The respective and collective roles of host genetic and environmental variation will depend on the extent to which host genetic variation is shaped by similar or different environmental factors.

To enhance our understanding of the biogeography of microbiome diversity and compositional variation in plant microbiomes and the underlying mechanisms, we leveraged the broad distribution and extensive genetic variation of the duckweed, *Lemna* species complex [27]. *Lemna* are floating aquatic plants commonly found in slow-moving freshwater ecosystems worldwide [28], and play an important role in ecosystem functions and services, such as carbon sequestration, phytoremediation, biofuel production, and animal feedstock [29, 30]. While *Lemna* are morphologically similar, hybridization has led to extensive genetic variation [27]. In this study, we examined *Lemna* microbiomes across 34 different populations in the United States, covering both the cool temperate and hot humid subtropical regions. Our purposes were twofold. First, we sought to test the hypothesis that bacterial richness is higher in temperate regions relative to lower latitudes and uncover the environmental and host genetic factors driving the observed diversity pattern. Second, we aimed to quantify the respective impact of different processes (selection, dispersal, and drift) and identify the environmental and host genetic factors driving the biogeographic variation of bacterial community composition.

Materials and Methods

Field Collection

We collected *Lemna* (referred to as duckweeds for simplicity) and associated microbiomes from 34 populations in the northern and southern range of its distribution in the USA (Fig. 1a and Table S1): Ohio (OH, Cleveland, $N=8$; Columbus, $N=5$), New Hampshire (NH, $N=2$), Massachusetts (MA, $N=2$), Rhode Island (RI, $N=2$), Louisiana (LA, $N=7$), Georgia (GA, $N=4$), and South Carolina (SC, $N=4$). The field sampling was conducted during the fast-growing season of duckweeds during June–August 2022. In addition, we collected samples from the same two Massachusetts populations during the late growing season in October 2022 to confirm the negligible influence of temporal dynamics on duckweed microbiomes, relative to the other factors we investigated in this study. It is worth noting that temporal dynamics is expected to be stronger in northern populations due to stronger seasonality compared to southern populations. At each population, we collected duckweeds using ethanol-sterilized forks into sterile plastic bags and stored them at 4°C until microbiome isolation within five days. We also measured the pH, conductivity (EC), and total dissolved solids (TDS) of the aquatic environment at each population using an Ohaus ST20M-B meter (Ohaus Corporation, Parsippany, New Jersey). Additionally, we collected 100 mL surface water in sterile centrifuge tubes and sent to the Wetland Biochemistry Analytical Services at Louisiana State University for additional water chemistry analysis (total organic carbon, TOC; total nitrogen, TN; total phosphorus, TP; major and trace elements including Na, Ca, Mg, Fe, Si, Cu, Zn, Mn, Pb, Cd; Table S1).

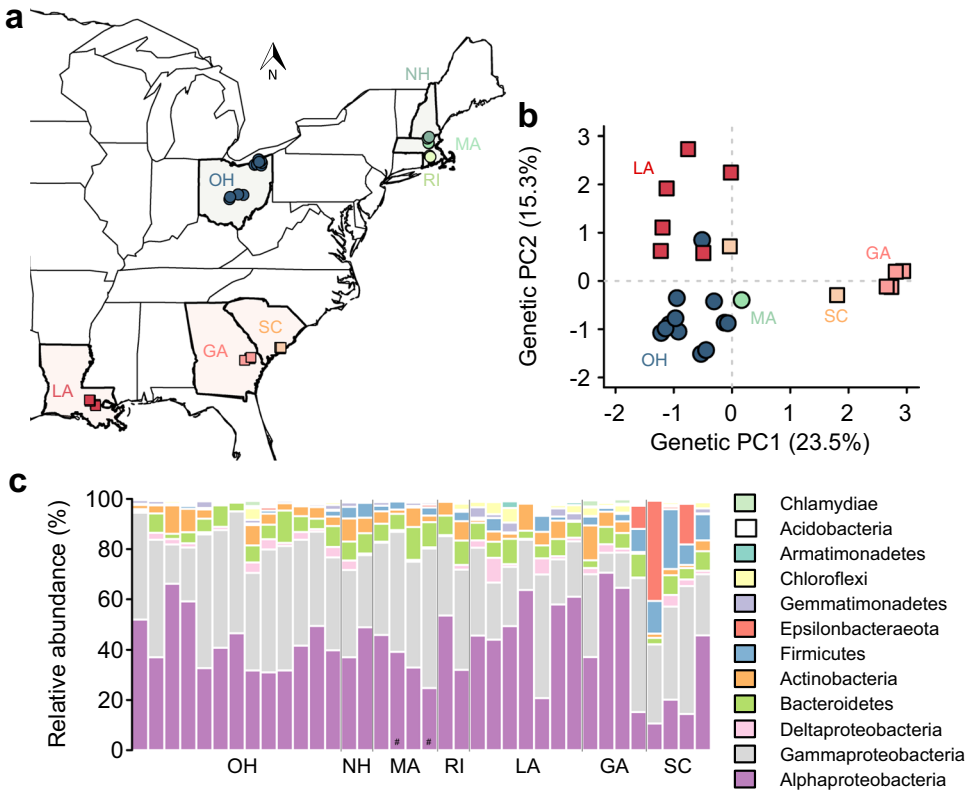

Fig. 1 *Lemna* populations and microbiomes. (**a**) We collected the *Lemna* species complex from the northern and southern range of its distribution in the USA (34 total populations: OH, 13; NH, 2; MA, 2; RI, 2; LA, 7; GA, 4; SC, 4). (**b**) *Lemna* genetic variation was examined among 25 out of the 34 populations based on ISSR markers using a principal component analysis (PCA). Populations with missing genetic information were due to the unsuccess in generating axenic genetic lines. (**c**) For *Lemna* bacterial microbiomes, the top 10 most abundant phyla (class level for Proteobacteria) are shown. The two MA populations (referred to as MA.1 and MA.2) were sampled at two separate times during the peak (June–August) and the end of the growing reason (October, denoted with "#") in 2022. The order of the four MA samples in the plot follows MA.1 (peak and end season) and then MA.2 (peak and end season)

Microbiome Isolation and Sequencing

Duckweed microbiome isolation was conducted aseptically under a laminar flow hood. For each population, we used sterilized forceps to remove debris from duckweed tissues, and rinsed *c*. 500 individuals in 20 mL sterile water to remove environmental microbes from their aquatic habitats. These individual plants were then transferred to 20-mL sterile 0.25 × phosphate-buffered saline. We collected epiphytic microbiomes by vortexing for 20 min, sonicating at 40 kHz for 5 min, and centrifuging at 13,200 rpm for 10 min. Microbial cells (from 5 mL out of the 20 mL epiphytic microbiome wash) were used for DNA extraction using cetyltrimethylammonium bromide (CTAB) and purified using polyethylene glycol (PEG) 8000. Briefly, microbial pellets were lysed with 500 µL sterile CTAB buffer (2% w/v CTAB, 100 mM Tris–HCl, 20 mM EDTA, 1.4 M NaCl, 5 mM ascorbic acid, and 10 mM dithiothreitol) and two autoclaved 4 mm stainless steel beads on a Vortex Genie 2 (Scientific Industries, Bohemia, New York) for 40 min. An equal volume (500 µL) of chloroform:isoamyl alcohol (24:1) was then added for phase separation at 13,200 rpm for 5 min. DNA was then recovered by adding the upper phase to 1 mL of cold pure ethanol overnight at -20°C and centrifuging at 13,200 rpm for 5 min. Pelleted DNA was washed with 500 µL of cold 70% ethanol and eluted in sterile TE buffer. We further purified the eluted DNA by conducting an additional round of chloroform: isoamyl alcohol phase separation, and then DNA was recovered by adding the upper phase to an equal volume of autoclaved PEG 8000 (20% w/v PEG 8000, 2.5 M NaCl), incubating at 37°C for 30 min, and centrifuging at 13,200 rpm for 5 min. Purified DNA pellet was washed with cold 70% ethanol and eluted in 60 µL sterile TE buffer and sent to the Argonne National Laboratory for bacterial library preparation (16S rRNA V5–V6 region, 799f–1115r primer pair: AACMGGATTAGATACCCKG, AGGGTTGCGCTC GTTG) and sequencing using Illumina MiSeq (paired-end 250 bp).

The paired-end (PE) reads were used for detecting bacterial amplicon sequence variants (ASVs) using the package

DADA2 v1.20.0 [31] in R v4.1.0 [32]. Following previous pipelines [19, 33], the PE reads were trimmed and quality filtered [truncLen = c(240, 230), trimLeft = c(10, 0), maxN = 0, truncQ = 2, maxEE = c(2,2)] and then used for unique sequence identification that took into account sequence errors. The PE reads were then end joined (minOverlap = 20, maxMismatch = 4) for ASV detection and chimera removal. The ASVs were assigned with taxonomic identification based on the SILVA reference database (132 release NR 99) implemented in DADA2. The ASVs were further filtered before conversion into a bacterial community matrix using the package phyloseq [34]. First, we removed non-focal ASVs (Archaea, chloroplasts, and mitochondria). Second, we conducted rarefaction analysis using the package iNEXT [35] to confirm that the sequencing effort was sufficient to capture duckweed bacterial richness (Fig. S1). We further normalized per-sample reads (median = 20,192 reads) by rarefying to 10,000 reads. Three populations that had fewer reads (one from OH: 9787 reads; two from GA: 5775 and 9484 reads, respectively) but plateaued in the rarefaction analysis (Fig. S1) were normalized to 10,000 reads following the previous pipeline [33]. Lastly, we removed low-frequency ASVs (< 0.001% of total observations). The final bacterial community matrix consisted of 4880 ASVs across the 36 samples from 34 different populations and was used for all downstream analyses.

Duckweed Genotyping

After microbiome isolation, duckweeds were bleached to create axenic plants. Briefly, *c*. 30 clusters (100 plants) per population were bleached in 15 mL 1% sodium hypochlorite until clusters turned white, and then washed in 15 mL sterile water three times. Individual clusters were then grown in 0.5 × Hoagland salt (PhytoTech Labs, Lenexa, Kansas) with 0.5% sucrose under 24°C and 16-h light for contamination check. A single axenic cluster was selected from a population (referred to as one genetic line) for further propagation in the same media for DNA extraction. Fresh duckweeds (*c*. 60 clonal plants) of each genetic line were used for DNA extraction using E.Z.N.A. SP Plant DNA Kit (Omega Bio-Tek Inc., Norcross, Georgia) and eluted in 100 µL sterile TE buffer. To examine duckweed genetic variation, we genotyped the genetic lines ($N = 25$, due to the unsuccess in generating some of the axenic genetic lines), using three polymorphic ISSR markers (UBC827, UBC855, UBC856) that generated a total of 46 polymorphic bands across the genetic lines (Table S2). PCRs were carried out in 10 µL reactions that contained 1.5 µL of extracted DNA, 0.5 µM primer, 4 mM $MgCl_2$, 0.5 mg/mL BSA, 5 µL GoTaq Colorless Master Mix (Promega Corporation, Madison, Wisconsin) including 200 µM of each dNTP and 1 unit Taq DNA polymerase, and H_2O. PCRs followed a standard protocol: 94°C for 5 min; 40 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 5 min. PCR amplicons were quantified with GeneRuler 100 bp plus DNA Ladder (Thermo Fisher Scientific Inc., Waltham, Massachusetts) on 1.5% agarose gels in 1 × TBE buffer under 95 V for 1:40 h.

Alleles were scored as presence or absence (1 or 0) using GelJ v2.0 [36]. Population genetic structure was analyzed using STRUCTURE v2.3.4 [37] and the package pophelper [38]. Genetic variation among populations was examined using a principal component analysis (PCA) in R.

Statistical Analyses

Microbiome Richness and Environmental and Genetic Correlates

To test whether northern duckweed populations harbor more bacterial richness than southern populations, we conducted a general linear mixed model (LMM) with region (northern vs. southern) as the predictor and a nested random effect (states nested within regions) using the package lme4 [39]. We conducted the LMM for both observed ASV richness (i.e., the number of ASVs) and asymptotic ASV richness (Chao estimator) using iNEXT. To identify which environmental factors might influence the biogeographic distribution of bacterial richness, we focused on 19 climatic and 13 water chemistry variables. We extracted the 19 climatic variables from WorldClim v2.1 [40] at 30 arc second resolution for all the populations. For water chemistry variables, we focused on pH, EC, TDS, nutrients (TOC, TN, TP, and C/N carbon to nitrogen ratio), and major and trace elements (Na, Ca, Mg, Si, Fe, and Mn). We did not consider some trace elements (Cd, Cu, Pb, and Zn) that showed little variation among populations or below the detection level (0.001 mg/L; Table S1). The water chemistry variables (except pH) were natural log transformed (log (x + 0.01)) for analyses. For the climatic or water chemistry variables, we first conducted univariate regressions (general linear models, LMs) to select for potential candidate predictors to be included in multiple regressions. We then used stepwise model selection (i.e., both forward and backward selections) of the multiple regressions based on the Akaike Information Criterion (AIC) to select for the most parsimonious model and identify significant predictors. The lack of collinearity was confirmed using the variance inflation factor (VIF). Duckweed genetic variation, represented by the first two axes of the genetic PCA (genetic PC1 and genetic PC2; Fig. 1b), was identified as non-significant predictors of bacterial richness by univariate regressions.

Microbiome Compositional Variation and Environmental and Genetic Correlates

To examine how diverse processes, such as niche-based selection (by environments and host genetics), dispersal, and drift, shaped the biogeographic variation of bacterial community composition, we conducted four analyses. First, to assess the degree of distance decay in bacterial community similarity, we conducted a Mantel test between bacterial community distance (the Bray–Curtis distance) and geographic distance using the package vegan [41]. We further examined whether such distance decay was explained by geographic distance alone or environments. To do so, we conducted partial Mantel tests between bacterial community distance and climatic distance (all 19 climatic variables) and between bacterial community distance and water chemistry distance (all 13 water chemistry variables), while controlling for geographic distance. The climatic and water chemistry variables were standardized (zero mean and unit variance for individual variables) prior to the estimation of their Euclidean distance among populations to avoid variable biases caused by scale differences. The geographic distance was estimated based on the latitudes and longitudes of the populations (Table S1) using the package geodist [42]. Second, to quantify the relative importance of selection, dispersal, and drift in driving microbiome assembly among populations, we used a phylogenetic binning based null model analysis (iCAMP) [26]. As iCAMP considers the possibility that different processes may affect individual microbial lineages differently [26] rather than uniformly [25], the relative importance of selection, dispersal, and drift was abundance-weighted average across microbial lineages (i.e., phylogenetic bins) in a group of communities of interest in iCAMP. Third, to further identify which environmental variables contributed to selection, we conducted univariate constrained principal coordinates analysis (cPCoA) to select for potential predictors that may influence bacterial community composition. For the climatic variables, univariate cPCoAs revealed the significant impact of all 19 climatic variables, and thus we used the first two axes of the PCA of these climatic variables (climatic PC1 and PC2, accounting for 72.4% and 17.6% of total variation, respectively; Fig. S2). For water chemistry, univariate cPCoAs identified the impact of seven variables (TN, TP, C/N, Ca, Mg, Fe, and pH), and we further used multivariate cPCoAs and stepwise model selection to reduce the number of potential water chemistry predictors to be included together with climatic PC1 and climatic PC2 for final model selection. The lack of collinearity was confirmed using VIFs. Fourth, to examine the influence of duckweed genetic variation, which can be potentially shaped by environmental selection (see analysis below), on bacterial community composition, we conducted variation partitioning of bacterial communities using the package vegan among duckweed genetic variation (genetic PC1 and genetic PC2), climate, and water chemistry (with predictors identified by model selections described above).

Duckweed Genetic Variation and Environmental Correlates

To examine how duckweed genetic variation was influenced by environments, we used univariate and multiple regressions with stepwise model selection to identify the significant environmental predictors of genetic PC1 and genetic PC2. As univariate regressions revealed the significant impact of many climatic variables on genetic PC1 and genetic PC2, we used climatic PC1 and climatic PC2 as potential predictors, along with the water chemistry predictors identified by univariate regressions, in multiple regressions for model selection.

Results

Duckweed Microbiomes and Populations

Similar to terrestrial plants [18, 43], duckweed microbiomes were dominated by Proteobacteria (79% of the ASVs), especially Alphaproteobacteria (42%) and Gammaproteobacteria (36%), followed by Bacteroidetes (7%), Actinobacteria (5%), Firmicutes (3%), and others (Fig. 1c). The microbiomes of duckweeds collected from the same populations (MA, Fig. 1c) were similar regardless of the sampling time (either during the peak or at the end of the growing season).

The analysis of duckweed genetic data revealed evidence of admixture (Fig. S3). We observed genetic differentiation between northern and southern populations along both the genetic PC1 and PC2 (Fig. 1b). We further found that genetic variation among duckweed populations was influenced by climate and water chemistry (Table S3). Specifically, duckweed genetic PC1 was influenced by precipitations (climatic PC2; multiple regression, LM: $t = 3.57$, $P = 0.002$) and water TN ($t = 2.26$, $P = 0.035$), and marginally by pH ($t = -1.96$, $P = 0.063$; Table S3). Duckweed genetic PC2 was primarily influenced by temperatures (climatic PC1, $t = 5.80$, $P < 0.001$; Table S3).

Biogeographic Variation of Duckweed Microbiome Richness

To test whether bacterial richness is higher in northern duckweed populations compared to southern populations, we used a LMM and found that the northern populations hosted 10% more bacterial ASVs than the southern populations (LS mean; observed richness: northern = 350 ± 30, southern = 321 ± 28, Fig. 2a; asymptotic richness: northern = 428 ± 44; southern = 388 ± 39; Fig. S4), while the mean

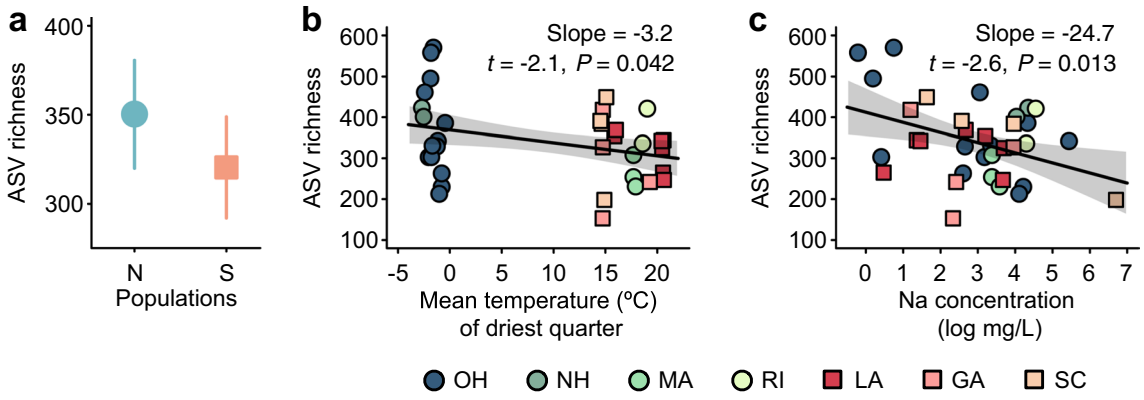

Fig. 2 Biogeographic variation of *Lemna* microbiome richness. (**a**) The least-squares mean (LS mean) ± SE of bacterial ASV richness (the number of ASVs) are plotted for the northern populations ('N': OH, NH, MA, RI) and southern populations ('S': LA, GA, SC) using a general linear mixed model with region (northern vs. southern) as the predictor and states nested within regions as the random effect. (**b**) The mean temeprature of the driest quarter (BIO9) and (**c**) the (natural log transformed) Na concentration of aquatic environments were identified as the important factors driving the distribution of bacterial richness of *Lemna* microbiomes after model selection of multiple regressions. Slopes with shaded 95% confidence intervals are shown. For statistical details, see Table S4

difference between northern and southern populations was not statistically significant ($P > 0.05$; Fig. 2a and Fig. S4).

Among the 19 climatic variables, only the mean temperature of the driest quarter (BIO9) showed a significant impact on bacterial richness, with a negative association observed between BIO9 and bacterial richness (multiple regression, LM: $t = -2.12$, $P = 0.042$; Fig. 2c and Fig. S4; Table S4). For water chemistry, while both concentrations of Na and TP were identified as potential factors influencing duckweed bacterial richness by univariate regressions, the multiple regression revealed that only Na concentration had a significant impact on bacterial richness, with lower richness associated with higher Na concentrations (LM: $t = -2.63$, $P = 0.013$; Fig. 2c and Fig. S4; Table S4). Unlike climate and water chemistry, the genetic variation of duckweed populations (genetic PC1 and PC2) did not influence bacterial richness ($P > 0.05$; Table S4).

Biogeographic Variation of Duckweed Microbiome Composition

Duckweed bacterial communities exhibited distance decay in similarity (Mantel test, $r = 0.46$, $P = 0.001$; Fig. 3a). Such distance decay was not solely driven by geographic distance, but also by environmental factors ($r_{\text{ClimatelGeo}} = 0.27$, $P = 0.001$; $r_{\text{Water chemistrylGeo}} = 0.29$, $P = 0.001$). This result indicated that both selection and dispersal as well as drift influenced duckweed microbiome assembly. We further found that selection played an important role in driving the biogeographic variation of duckweed bacterial community composition (28% in northern populations, 25% in southern populations, and 26% in all populations; Fig. 3b), in addition to dispersal limitation (northern, 25%; southern, 42%; all, 33%), homogenizing dispersal (1%, 2%, 2%), and drift (45%, 31%, 39%). A similar pattern of the relative importance of selection, dispersal, and drift was also found among populations within states (Fig. S5).

Among the environmental factors, climatic PC1 (temperatures) and PC2 (precipitations) together with water pH, TP, and Ca were the most important variables driving the biogeographic variation of duckweed bacterial community composition (cPCoA: climatic PC1, 7.2% of variation, $F = 2.9$, $P = 0.001$; climatic PC2, 4.3%, $F = 1.7$, $P = 0.006$; pH, 5.7%, $F = 2.3$, $P = 0.001$; TP, 3.6%, $F = 1.4$, $P = 0.048$; Ca, 3.9%, $F = 1.6$, $P = 0.012$; Fig. 3c and Table S5). Climatic PC1 (temperatures) and TP were found to influence bacterial community cPCoA 1, which was dominated by *Arcobacter* on the positive axis and *Rhizobacter* and *Rhodobacter* on the negative axis (Table S6). Climatic PC2 (precipitations), pH, and Ca were found to influence cPCoA 2 (Fig. 3c), which was dominated by *Variovorax* and *Ideonella* on the positive axis and *Rhizobacter* and *Porphyrobacter* on the negative axis (Table S6). Based on the subset of populations ($N = 25$) with duckweed genetic data, we found that duckweed genetic variation affected bacterial community composition (cPCoA: genetic PC1, 7.7%, $F = 2.0$, $P = 0.001$; genetic PC2, 9.9%, $F = 2.6$, $P = 0.001$; Table S5). Variation partitioning analysis further pointed out the collective roles of climate, water chemistry, and host genetic variation on duckweed bacterial community composition (Fig. 3d), with the unexplained variation reflecting other processes (e.g., drift and dispersal limitation) operating in these subset populations.

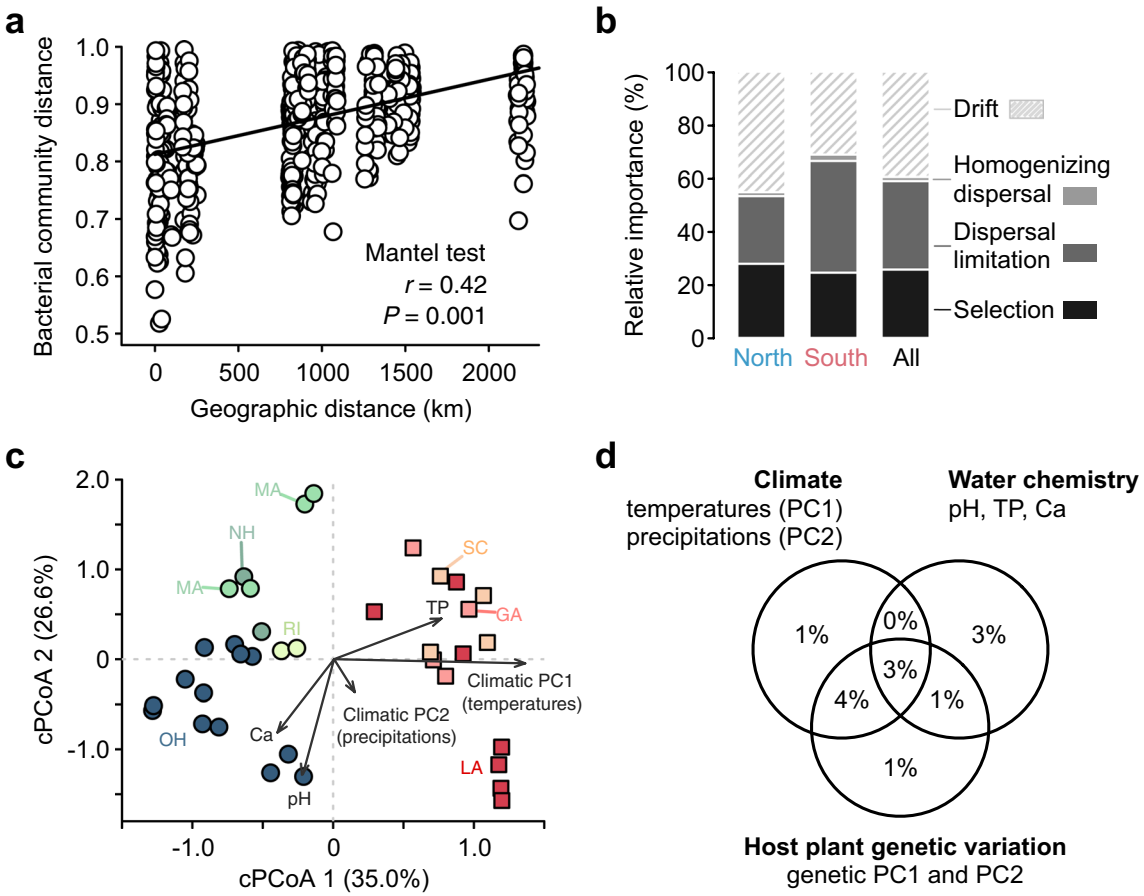

Fig. 3 Biogeographic variation of *Lemna* microbiome composition. (**a**) The Mantel test indicates a significant correlation between the Bray–Curtis distance of *Lemna* bacterial communities and geographic distance. (**b**) The relative importance of different processes driving *Lemna* bacterial community assembly was quantified using the package iCAMP among the northern (OH, NH, MA, RI) and southern populations (LA, GA, SC) as well as all populations together. (**c**) The first two axes of the principal component analysis (PCA) of the 19 climatic variables (climatic PC1 and climatic PC2), pH, and concentrations of total phosphorus (TP) and calcium (Ca) were identified as the most important factors driving the biogeographic variation of *Lemna* bacterial community composition after model selection of constrained principal coordinates analyses (cPCoAs). (**d**) Variation partitioning indicates the collective roles of duckweed genetic variation, climate, and water chemistry in explaining the biogeographic variation of *Lemna* bacterial community composition. For statistical details, see Table S5

Discussion

Our study on the microbiomes of wide-ranging duckweeds revealed that the distribution of plant microbiome diversity supported the standing hypothesis of microbial biogeography, with bacterial richness higher in temperate regions relative to lower latitudes as observed in free-living microbiomes [4–7]. We found that environmental factors that follow latitude (the temperature of the driest quarter, BIO9) or not (Na concentration) were negatively associated with duckweed bacterial richness, whereas host genetic variation showed no strong impact. In contrast to bacterial richness, the biogeographic variation of duckweed bacterial community composition was influenced by all 19 climatic variables, including temperatures (climatic PC1) and precipitations (climatic PC2), and water chemistry variables such as pH and concentrations of TP and Ca. Our results further underscored the collective roles of host genetic variation, climate, and water chemistry in driving duckweed bacterial community composition. Together, selection played an important role (26%) in microbiome assembly across duckweed populations, alongside dispersal limitation (33%) and drift (39%).

Bacterial Richness of Plant Microbiomes Is Higher in Temperate Populations

Our findings of higher bacterial richness in temperate relative to subtropical duckweed populations were consistent with the diversity patterns of microbial biogeography in free-living microbiomes across ecosystems, including soil, marine, and airborne microbiomes [4–7]. In host-associated microbiomes, several studies have examined microbial

symbionts, primarily fungi, associated with representative plant lineages from different biomes [10–13], and revealed inconsistent diversity patterns. For instance, some studies have reported a decrease in richness with latitude in leaf fungal communities [10, 13], while others have found richness to peak in midlatitude regions in root ectomycorrhizal fungal communities [12]. As plant lineages differ in associated microbiomes [10], the assessment of biogeographic patterns of plant microbiome richness at the broad biome level is susceptible to potential confounding factors, such as variation in plant lineages across different biomes [3]. Research on plant microbiomes at the population level has been limited in terms of evaluating the richness hypotheses of microbial biogeography [21, 44–47]. Yet, there have been some exceptions [48, 49]. For instance, research on root bacterial microbiomes associated with soybean has detected an increase in richness from tropical to temperate regions [49], whereas research on root fungal microbiomes associated with five grass species in the plains of the USA has revealed complex diversity patterns (e.g., decrease, unimodal, or no significant change) from subtropical to temperate regions [48]. In our study, we observed 10% higher bacterial richness in temperate duckweed populations compared to subtropical populations, while the mean difference between the two regions was not statistically significant. These studies and ours suggest that ecological factors that do not follow latitude, might influence the biogeographic distribution of plant microbiome richness, such as soil pH in grasslands [48] and Na concentration in freshwaters here (Fig. 2c).

In this study, we found that Na concentration negatively impacted bacterial richness in these natural duckweed populations. While it is unclear whether the observed negative association between Na concentration and duckweed bacterial richness was driven by the independent or joint effects of plants or environmental microbiomes in this study, Na concentration has been found to negatively impact the bacterial richness of free-living aquatic microbiomes [50, 51] and the growth of bacteria associated with duckweeds [52] as well as duckweeds themselves [52, 53]. Interestingly, we observed high Na concentration in populations from both temperate and subtropical regions (Table S1), potentially reflecting road salt use in the north and proximity to seawater in the south. This suggests that factors such as increased salinity in freshwater ecosystems due to, for instance, road salt flux [54, 55] and sea level rise [56, 57], as well as increased temperature [58], under global change may have negative impacts on plant microbiome richness and their distribution patterns.

Environmental Factors Influence the Biogeographic Variation of Plant Microbiome Composition

The biogeographic variation of duckweed bacterial community composition exhibited distance decay consistent with the prediction of microbial biogeography [3, 9], and was driven by diverse processes. Among these processes, dispersal limitation and drift played a major role (together 72%), similar to the observations (70–80%) in the global distributions of free-living soil and marine microbiomes [6]. Consistent with global soil microbiomes [6], selection accounted for 26% of the processes driving the biogeographic variation of duckweed bacterial community composition. Specifically, environmental pH, which has been identified as a dominant driver of the compositional variation in global soil bacterial communities [7, 17], as well as root fungal communities in grasses [48] and root bacterial communities in *Arabidopsis* [47] and soybean [49], was also found to influence the compositional variation of bacterial communities associated with duckweeds in aquatic environments. Similar to marine microbiomes [5], temperatures strongly impacted duckweed bacterial community composition. Such effects of temperature and pH on bacterial community composition have been demonstrated experimentally in duckweeds [59]. One should note, however, the detected strong effect of temperatures on the biogeographic variation of duckweed bacterial community composition reflected historical processes that have shaped the adaptation and genetic variation of host populations and their associated microbiomes. As such, the extent to which the pattern changes under future climates merits further investigation. Moreover, we found that phosphorus, one of the most important limiting factors in freshwater ecosystems [60], influenced duckweed bacterial community composition, similar to the observations in bacterial communities associated with marine algae [61]. Additionally, calcium concentration, reflecting the hardness of aquatic environments, was also found to drive the compositional variation of duckweed bacterial communities, independent from the strong impact of pH (after model selection). Our study, together with previous research, point to some general principles of microbial biogeography regarding the influence of selection by environments and the underlying drivers, and also call for the need of additional research to experimentally verify the causality of these drivers. Together, the findings will provide predictive insights into the potential impacts on the biogeographic variation of microbiome composition of climate change and anthropogenic activities, particularly nutrient deposition and discharge into ecosystems [62, 63] and the overall quality of aquatic environments.

Host Genetic Variation Plays a Role in Plant Microbial Biogeography

Accumulating evidence has suggested that plant genetic factors can exert a crucial role in mediating microbial community assembly by influencing plant morphology, physiology, metabolic pathways, and immune systems [20]. Different from free-living microbiomes, our findings pointed out the

joint role of plant genetic variation and environmental variation, which has been largely overlooked in plant microbial biogeography [21, 44–49]. Our study showed that plant genetic variation influenced duckweed bacterial community composition, not bacterial richness, via its joint effect with climate and water chemistry, rather than their independent effects (Fig. 3d). This was primarily because the genetic variation of duckweeds was strongly influenced by the same factors that influenced their microbiome composition, such as temperatures, precipitations, nitrogen concentration (which was correlated with phosphorus concentration), and pH. The strong coupling of host genetic variation and microbiomes with environmental factors made it challenging to separate the effects of host genetic and environmental variation on microbiome composition in natural populations without manipulative experiments (e.g., reciprocal common garden experiments, [47]). This observation should not be unique to duckweeds but is expected to be common in plant microbiomes as seen, for instance, in seaweed microbiomes [22], as local adaptation to environments is a widespread phenomenon in plants [64]. Even though duckweeds undergo clonal reproduction, we should acknowledge that by genotyping a single genetic line from each duckweed population we may have underestimated host genetic variation within populations. While the genetic variation within populations is yet to be determined in nature, it is expected to be smaller than the genetic variation among populations due to the local adaptation of duckweeds. Nevertheless, the observation of strong coupling of host genetic variation and microbiomes with environmental factors across populations underscores the potential for even stronger impacts on the distribution, structure, and function of plant microbiomes in the cases of misaligned responses between plants and microbes to climate change and anthropogenic activities.

Conclusions

Our study elucidates the biogeographic distribution of plant microbiome structure and the underlying mechanisms, highlighting both the commonalities and differences in microbial biogeography relative to free-living microbiomes. While our findings are based on a geographically broad sampling of the *Lemna* species complex [27], it is important to acknowledge that further research that incorporates representative populations across continents is needed to provide a comprehensive global perspective on plant microbial biogeography in duckweeds as well as in other plant species. Such studies together will further advance our understanding of the principles of microbial biogeography across diverse plant lineages and ecosystems. The key drivers identified in our study, including temperatures, precipitations, pH, and concentrations of sodium, phosphorus, and calcium, along with host genetic variation, provide important insights into predicting the vulnerability and resilience of plant microbiomes and their impacts on ecosystem functioning under changing climates and intensifying anthropogenic activities.

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Author Contribution Na Wei and Jiaqi Tan administered the research. Na Wei conceived the conceptual development of the research. Na Wei and Jiaqi Tan collected the data. Na Wei conducted data analyses and visualization and wrote the manuscript. Na Wei and Jiaqi Tan contributed to manuscript revision.

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Data Availability Microbial community matrix and ASV data are available on Mendeley Data (https://doi.org/10.17632/fgfjpn86bb.1).

Declarations

Competing Interests The authors declare no competing interests.

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