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Seasonal Assembly of Nectar Microbial Communities Across Angiosperm Plant Species: Assessing Contributions of Climate and Plant Traits

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Keywords: bacteria | coexistence | community assembly | microbiome | nectar | plant–microbe | pollination syndrome | species interactions | synthetic community | yeasts

ABSTRACT

Plant–microbe associations are ubiquitous, but parsing contributions of dispersal, host filtering, competition and temperature on microbial community composition is challenging. Floral nectar-inhabiting microbes, which can influence flowering plant health and pollination, offer a tractable system to disentangle community assembly processes. We inoculated a synthetic community of yeasts and bacteria into nectars of 31 plant species while excluding pollinators. We monitored weather and, after 24 h, collected and cultured communities. We found a strong signature of plant species on resulting microbial abundance and community composition, in part explained by plant phylogeny and nectar peroxide content, but not floral morphology. Increasing temperature reduced microbial diversity, while higher minimum temperatures increased growth, suggesting complex ecological effects of temperature. Consistent nectar microbial communities within plant species could enable plant or pollinator adaptation. Our work supports the roles of host identity, traits and temperature in microbial community assembly, and indicates diversity–productivity relationships within host-associated microbiomes.

1 | Introduction

Plant–microbe associations vary widely in community composition, ecological relationships formed and microbial abundance (Pineda et al. 2015) with consequences for plant phenology and fitness (O'Brien et al. 2021). Community surveys link variation in microbial community composition in plant tissues to microbial effects on plant traits, such as maximum photosynthesis rate or abiotic stress tolerance (Friesen et al. 2011), and experimental studies confirm these trends (Fitzpatrick et al. 2018; Harrison and Griffin 2020). Numerous factors contribute to observed variation in microbial community composition among plants, including neighbourhood effects, microbe immigration and dispersal and microbe–microbe interactions (Trivedi et al. 2020). Yet, surveys that characterise plant microbial communities cannot disentangle the simultaneous effects of dispersal, species interactions and plant traits on microbial assembly (Francis, Mueller, and Vannette 2023; Leopold and Busby 2020).

Experimental manipulations using synthetic (artificially assembled) microbe communities (Vorholt et al. 2017) have increased our knowledge of the role of plant traits and microbe arrival order in determining microbial community composition (Carlström et al. 2019). However, synthetic community studies

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are often limited to one or a few model plant species. Increasing the phylogenetic breadth of experimentally inoculated plants may shed light on determinants of microbe community assembly, paving the way for inoculation strategies in agriculture that target crop growth, pathogen resistance or stress tolerance (Vishwakarma et al. 2020).

The composition of plant-associated microbe communities can also be affected by variation in geographic location and climatic conditions (Aizenberg-Gershtein, Izhaki, and Halpern 2017; Sharaby et al. 2020; Trivedi et al. 2022) which impact plant physiology, microbe species pools, growth dynamics and microbe–microbe interactions. However, studies assessing how climate variation affects the microbiome of aboveground plant tissues remain scarce (Zhu et al. 2022). Uncoupling geographical and phenological variation from climate effects across plant species complicates efforts to examine how changing temperatures impact plant–microbe interactions on large scales (Sharma et al. 2022). Leveraging synthetic community inoculations over seasons and changes in temperatures would allow for insights on the effects of climate and plant traits on microbial establishment and growth, particularly for plant communities in thermally variable habitats.

The microbiome of flower nectar has proven a tractable model system for addressing questions in microbial ecology and plant–microbe interactions due to characteristically species-poor, highly filtered and short-lived microbial communities (Chappell and Fukami 2018). Flowers mediate plant reproduction via interactions with flower-visiting animals (hereafter, 'pollinators'), which also disperse microbes to nectar, leading to complex three-way interactions (Vannette 2020). Pollinators, as well as abiotic forces like wind, introduce microbes, including plant and pollinator pathogens (McArt et al. 2014), to flowers which may then subsequently affect floral traits or pollinator preference (Herrera, Pozo, and Medrano 2013; Vannette, Gauthier, and Fukami 2013) and plant fitness (Francis et al. 2021).

Nectar microbiomes vary in composition across plant species (Canto, Herrera, and Rodriguez 2017; Francis, Mueller, and Vannette 2023; Mittelbach et al. 2015; de Vega et al. 2021), but most previous work characterises microbiomes of open flowers visited by pollinators (Herrera et al. 2009; Rering et al. 2024; Zemenick, Vannette, and Rosenheim 2021). Pollinators introduce characteristic microbial assemblages (Brysch-Herzberg 2004; Herrera et al. 2009; de Vega, Herrera, and Johnson 2009), and consequently, studies cannot directly compare how plant species themselves vary in their effects on the establishment and growth of microbes introduced to their nectar, as the effects of dispersal by pollinators can mask any effects of microbe filtering by host plants (Francis, Mueller, and Vannette 2023). This hampers our knowledge of whether different plant nectars deterministically select for specific nectar microbes outside of pollinator dispersal, representing a major knowledge gap in our understanding of widespread plant–microbe–pollinator interactions.

Empirical plant–pollinator–microbe networks suggest biotic and abiotic filtering mechanisms play a role alongside dispersal in nectar microbe community assembly (Zemenick, Vannette, and Rosenheim 2021). Host plant-related filtering mechanisms may be mediated by floral morphology, as microbial communities can vary even across organs within a single flower (Junker and Keller 2015; Rebolleda Gómez and Ashman 2019). Additionally, chemical constituents of nectar, including sugars, proteins, secondary metabolites and peroxides, have been found to influence microbial growth (Aizenberg-Gershtein et al. 2015; Mueller, Francis, and Vannette 2023; Schmitt et al. 2018). Yet, prior studies have not assessed the effects of floral traits per se or plant relatedness on microbial community assembly across diverse plant species (Félix et al. 2021; Mittelbach et al. 2015; Morris et al. 2020) and have been limited to a single plant or microbial taxon (Francis, Mueller, and Vannette 2023; Herrera 2014; Marre, Ushio, and Sakai 2022; Russell et al. 2019; Tsuji and Fukami 2018). Experimentally assessing the assembly of microbe communities across a wide variety of plant species in roots (Fitzpatrick et al. 2018; Koyama, Maherali, and Antunes 2019) and phyllospheres (Gaube et al. 2023; Leopold and Busby 2020; Meyer et al. 2022) has yielded significant insights into the processes and traits which shape plant–microbe interactions. Studies including a broader diversity of plant taxa are crucial to better understand the evolution and ecology of how nectar traits mediate plant–microbe interactions.

In this study, we leverage a synthetic community of nectar-inhabiting bacteria and yeasts, inoculating the floral nectar of 31 plant species. The focal microbes are representative of Northern California flowers and include both nectar specialists and generalists (i.e., phyllosphere and/or pollinator-associated species; Table S1). Our experimental approach provides unique perspectives of nectar microbiome assembly across plant hosts. Using a single uniform community in bagged flowers controls for variation from priority effects and removes pollinator dispersal. Additionally, we control for geography by conducting inoculations in plant species growing within the same general area. By comparing the growth of an initially uniform microbe community across plant species and across seasons due to variation in species' flowering phenology, we test the following hypotheses: (1) nectar microbes differ in their establishment and growth in nectars of different plant species in the absence of pollinator-mediated dispersal; (2) within a plant species, microbial assembly is deterministic and predicted by floral traits (e.g., nectar volume, nectar peroxide content, floral morphology) and/or plant phylogenetic relatedness (phylosymbiosis) and (3) higher temperatures alter microbial community composition by increasing abundance and favouring growth of certain taxa over others. We found that microbial community assembly in nectar diverged among plant species, in part explained by nectar defence traits, but also with seasonal variation in temperature maxima and minima. Divergence in the trajectory of microbial communities may hold relevance for pollinator visitation and plant reproductive success.

2 | Materials and Methods

2.1 | Creating Nectar Microbe Inoculum

We selected five microbe species (Table S1) that are common, widely distributed representatives of nectar microbiomes in various plant species, including those in Northern

California (Vannette 2020; Vannette et al. 2021): the yeasts *Metschnikowia reukaufii* and *Aureobasidium pullulans*, and the bacteria *Neokomagataea thailandica*, *Acinetobacter pollinis* and *Apilactobacillus micheneri*. We created our microbial inoculum (Figure 1A; Supporting Information: Inoculum preparation) as described in Cecala and Vannette (2024). The inoculum contained ~10^4 cells μL^{-1} of each species (5×10^4 total cells μL^{-1}).

2.2 | Floral Bagging and Inoculation

We conducted 11 rounds of floral inoculation on the University of California, Davis campus (38.540°N, 121.756°W) (USA: California: Yolo County) from 22 March to 29 June 2023. In the morning the day before inoculations, we bagged ~10 unopened flower buds on each of 5–8 species of flowering plants (Figure S1) to prevent the transfer of microbes by pollinators. We secured organza bags (7×8.5 cm, 10×13 cm or 13×18 cm) around flowers, removing all open flowers prior to sealing the bag. Each time we handled flowers, we inspected for any breaches by ants or thrips.

Flowers that opened within bags were inoculated between 09:00 to 11:00 h. To inoculate a flower, 1 μL inoculum, carried into the field on ice, was delivered onto the nectary using a micropipette and autoclaved tips (Figure 1B), then flowers were tagged with a unique identifier, and re-bagged. Each week, we inoculated roughly five to eight flowers per plant species (~40 flowers per week). Over the course of the study, we recorded temperature extrema (afternoon highs and overnight lows) for all inoculation days from a local weather station (Figure 1C; Figure S2).

2.3 | Nectar Extraction and Plating

Roughly 24 h after inoculation, we excised flowers from plants, sealed them in containers and transported them to the laboratory. Inside a laminar flow hood, we used glass microcapillary tubes (VWR, Drummond) to extract and measure the volume of total nectar in each flower (Figure 1D). We quantified microbes in nectar as in Cecala and Vannette (2024). Briefly, we diluted pure nectar in Dulbecco's phosphate-buffered saline, plated aliquots on each of three agar media types and incubated for 6 days, after which CFUs were identified and tallied (Supporting Information: Quantifying microbes in nectar). Microbial growth from four bagged flowers breached by crawling insects did not differ markedly from that of other flowers and remained in analyses.

For each flower, we calculated: (1) the density of CFUs per μL nectar, by dividing the number of CFUs per plate by the actual volume of pure nectar in the aliquot; and (2) the estimated total abundance of CFUs per flower, by multiplying our calculated density (1) by the total volume of nectar originally extracted from that flower. The above values (1 and 2) were calculated for each inoculated microbe individually and for all five species collectively.

For comparison with real nectars and to test our inoculum in artificial solutions, we also added 1 μL of inoculum to 10 μL of 30% m/m sucrose and an artificial nectar containing sugars and peptone ('experimental controls'; $n = 6$ replicates each; Supporting Information: Media recipes) in strip tubes. Tubes were sealed and incubated at 25°C for 24 h, then processed identically to actual nectar samples.

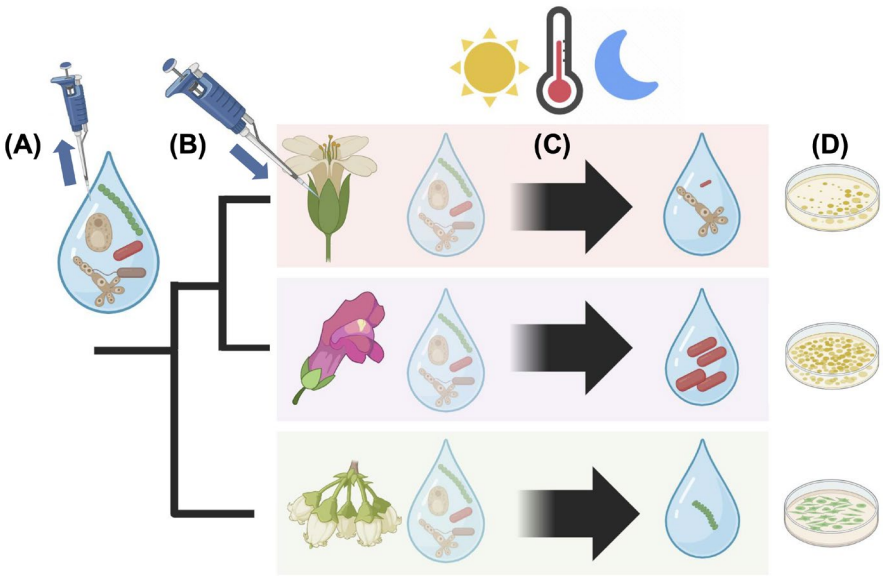

FIGURE 1 | Schematic overview of the experiment. (A) We prepared a standardised, synthetic community of five microbe species—two yeasts and three bacteria—which are common representatives of floral nectar communities. (B) We inoculated 1 μL of this community (containing roughly 10^4 cells of each species) suspended in a sucrose-glycerol solution into the standing nectar of flowers of various species of plants which were bagged prior to opening to prevent microbial deposition by pollinators. Small blue arrows indicate direction of pipetting. (C) We left inoculated flowers bagged on plants for 24 h, recording afternoon high and overnight low temperatures. (D) After 24 h, we extracted nectar and plated aliquots on agar media and incubated them for a week. Afterwards, all CFUs were identified, tallied and used to calculate microbial abundance and density in the original nectar sample. Figure was prepared using BioRender (biorender.com).

2.4 | Determination of Floral Traits

We estimated concentrations of hydrogen peroxide (H_2O_2), a known antimicrobial reactive oxygen species found in some nectars (Carter and Thornburg 2004; Mueller, Francis, and Vannette 2023), in the nectar of separate, noninoculated flowers of most sampled plant species (Supporting Information: Peroxide quantification; Table S2). Peroxide values from noninoculated nectar represent initial conditions which would be experienced by microbes arriving in flowers. To assess the contribution of floral morphology, we scored floral phenotypes of all plant species on the basis of 28 binary traits used in past studies (Faegri and van der Pijl 1979; Ollerton et al. 2009) to represent pollination syndromes in multivariate space using Bray–Curtis dissimilarity. We determined trait states through a combination of observation and reference with the Jepson eFlora (ucjeps.berkeley.edu/eflora). We also encoded other traits of particular interest such as inflorescence density and corolla fusion.

2.5 | Scope of Collected Data

We excluded from analysis five plant species for which we had few, low-quality samples (Table S2). In total, we inoculated 398 flowers across 31 species of plants, 372 of which (93.5%) contained nectar after 24 h (range: 7–16 flowers per species; mean = 12 flowers per species). The absence of nectar in flowers did not coincide with any recorded variables. These species comprised 29 genera in 21 families. From the 372 nectar samples, we tallied 1,016,048 CFUs on agar media, of which 99.94% were our inoculated species: 72,242 *Metschnikowia*; 16,640 *Aureobasidium*; 20,121 *Neokomagataea*; 795,332 *Acinetobacter*; 111,149 *Apilactobacillus*. We classified 564 CFUs as noninoculated bacteria or fungi (0.056% of all CFUs), likely originating from other plant tissues or the environment, and excluded these from analyses.

2.6 | Statistical Analyses

We conducted analyses in R (R Core Team 2024). Using package *lme4* (Bates et al. 2015), we constructed linear mixed-effect models with nectar volume, total and by-species CFU density and CFU Shannon–Wiener diversity index as dependent variables. As independent variables, we included nectar volume and temperature extrema, and plant species as a random intercept effect. We obtained type III sums of squares, *F*- and *p*-values and Kenward-Roger degrees of freedom using function 'Anova' in package *car* (Fox and Weisberg 2019). We inspected model residuals for normality and variance inflation factors to assess multicollinearity. We also created separate linear models with either plant species or nectar peroxide concentration as a fixed effect, as peroxide data were not collected for three species (Table S2). For linear models in which we included a quadratic predictor, we conducted a likelihood ratio test comparing the goodness of fit of the models with and without the quadratic term.

To test if microbial community composition (as Bray–Curtis dissimilarity) differed by plant species and temperature extrema, we used function 'adonis' in package *vegan* (Oksanen et al. 2020) to perform a permutational multivariate analysis of variance. We used function 'betadisper' to examine multivariate homogeneity of dispersions across plant species. Community composition was visualised using nonmetric multidimensional scaling (NMDS) ordination, and we tested for significant microbe species vectors using function 'envfit'. As above, a separate analysis was conducted with peroxide concentration as a predictor variable. To test for co-occurrence between microbe species, we generated Pearson correlation matrices on CFU densities, for both our entire dataset and for each plant species individually, and visualised matrices using package 'corrplot' (Wei and Simko 2021).

To estimate plant phylogenetic relationships among sampled plant species, we used the function 'phylo.maker' in package *V.PhyloMaker2* (Jin and Qian 2022) using the reference plant phylogeny GBOTB.extended.TPL. Using this tree, we tested for a phylogenetic signal of nectar volume, CFU densities and Shannon diversity using function 'multiPhylosignal' in package *picante* (Kembel et al. 2010) with 10,000 simulations.

To test for relationships between plant phylogenetic relatedness and multivariate microbe community composition, we created a pairwise distance matrix of plant phylogenetic relatedness using function 'cophenetic.phylo' in package *ape* (Paradis and Schliep 2019). We compared this distance matrix to a Bray–Curtis dissimilarity matrix of the mean CFU densities of each microbe by plant species using a Mantel test via function 'mantel' in package *vegan*, calculating Spearman's ρ with 10,000 permutations. We also created a Bray–Curtis dissimilarity matrix of plant species based on floral trait data and compared this to the two aforementioned matrices. We controlled for the effect of plant phylogenetic distance on pollination syndrome using a partial Mantel test via function 'mantel.partial'. We generated correlograms for all Mantel tests using the function 'mgram' in package *ecodist* (Goslee and Urban 2007). Figures were created using package *ggplot2* (Wickham 2016) and tree plots using ggtree (Yu et al. 2017) and custom function 'ggtreeplot' (Hackl 2018).

3 | Results

3.1 | Microbial Community Assembly Varies Across Plant Species

Total CFU density in nectar the day after inoculation varied over three orders of magnitude across the 31 plant species ($F_{30,338} = 8.34$, $p < 0.0001$), ranging from (mean ± SE) $9.0 \pm 6.8\,\mu L^{-1}$ in *Arbutus unedo* to $3.2 \pm 1.7 \times 10^4\,\mu L^{-1}$ in *Hesperaloe parviflora*, averaging $2.95 \pm 0.52 \times 10^3\,\mu L^{-1}$ (Figure 2). Comparing CFU densities with the inoculum initial cell density suggests some die-off of microbes between inoculation and harvesting in certain plant species, but growth in others. Total CFU density was not correlated with nectar volume ($F_{1,219} = 0.92$, $p = 0.34$), but total estimated CFUs per flower was ($F_{1,216} = 65.62$, $p < 0.0001$) (Figure S4), ranging from 25.79 ± 13.96 CFUs in *Arbutus unedo* to $1.91 \pm 0.35 \times 10^6$ CFUs in *Erythrina crista-galli*. Nectar volume varied over two orders of magnitude across plant species ($F_{30,341} = 43.54$,

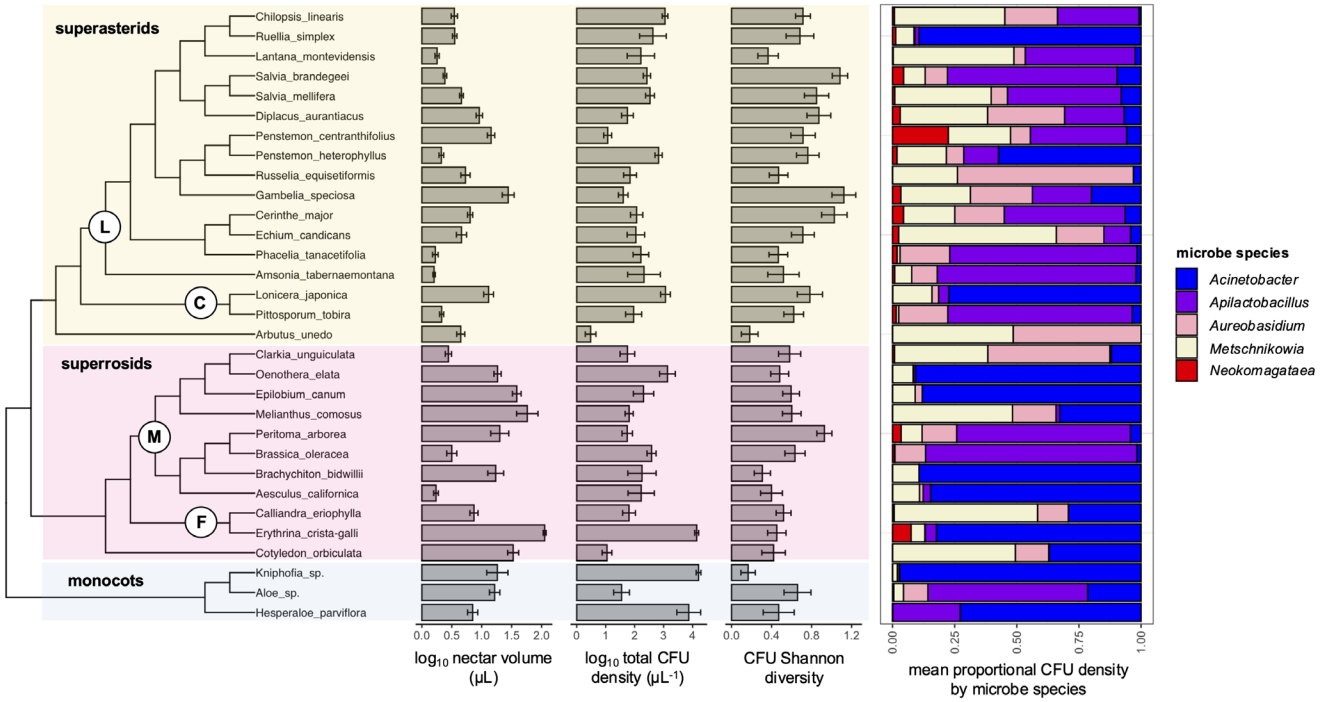

FIGURE 2 | Phylogenetic relationships among plant species (left) inoculated in this study, with plant species aligned to their respective nectar volumes, microbe CFU densities, CFU Shannon diversities (middle), and mean proportional composition of microbe species (right). For simplicity, the cladogram represents only branching order, not divergence times, among plant species. Coloured, transparent boxes indicate three major clades of angiosperms: Monocots in blue, and the eudicot superasterids in yellow and superrosids in pink. Subclades are indicated with circles positioned at their ancestral node: The asterid subclades the lamiids (L) and campanulids (C), along with the rosid subclades the malvids (M) and fabids (F).

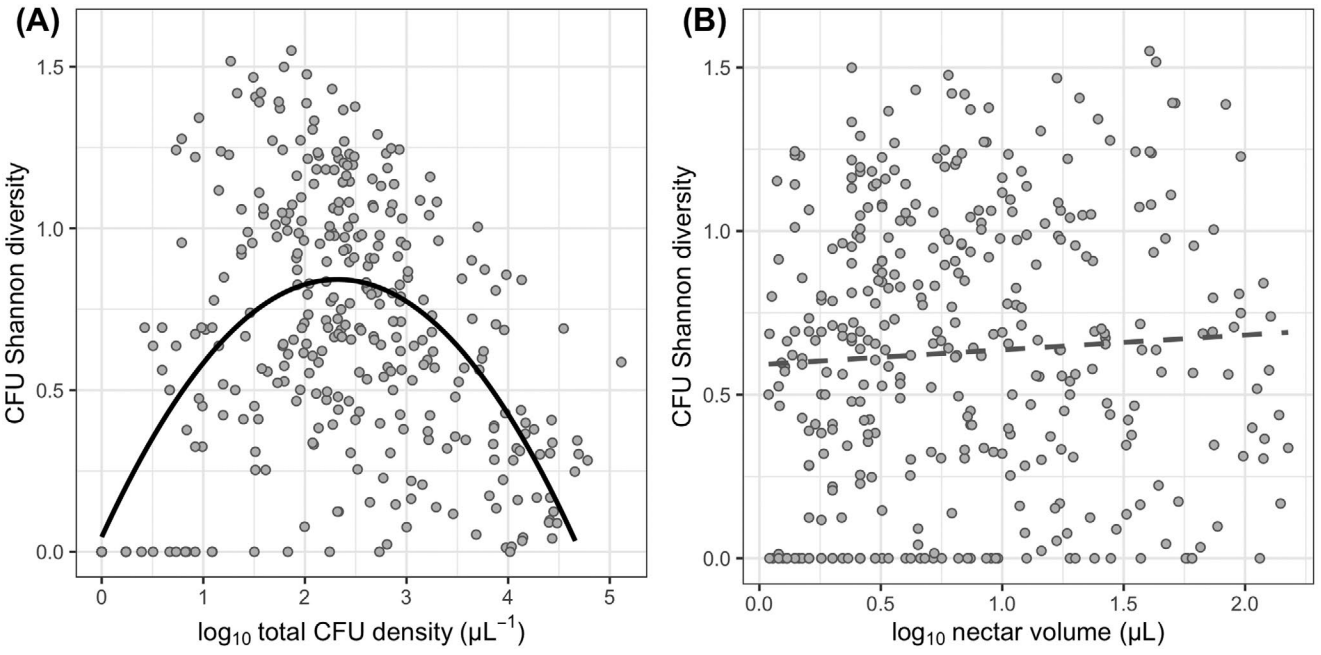

FIGURE 3 | Shannon diversity of inoculated microbe CFUs in relation to (A) total CFU density and (B) nectar volume across all plant species. In (A), the curved black solid line indicates a significant quadratic relationship, while in (B), the dashed grey line represents a nonsignificant linear trend line. Each point represents one inoculated flower, and all plant species are included.

$p < 0.0001$), ranging from 0.1 to 149.5 μL, averaging 15.8 ± 1.4 μL (Figure 2).

CFU densities of individual microbe species varied over four orders of magnitude across plant species (Figure S3). CFU Shannon diversity varied across plant species ($F_{30,338} = 4.71$, $p < 0.0001$) (Figure 2) and was not related to nectar volume ($F_{1,90} = 0.86$, $p = 0.36$), but displayed a unimodal relationship with increasing CFU density ($F_{1,326} = 172.31$, $p < 0.0001$), peaking at roughly 10^2 CFU μL^{-1} (Figure 3). Plant species identity

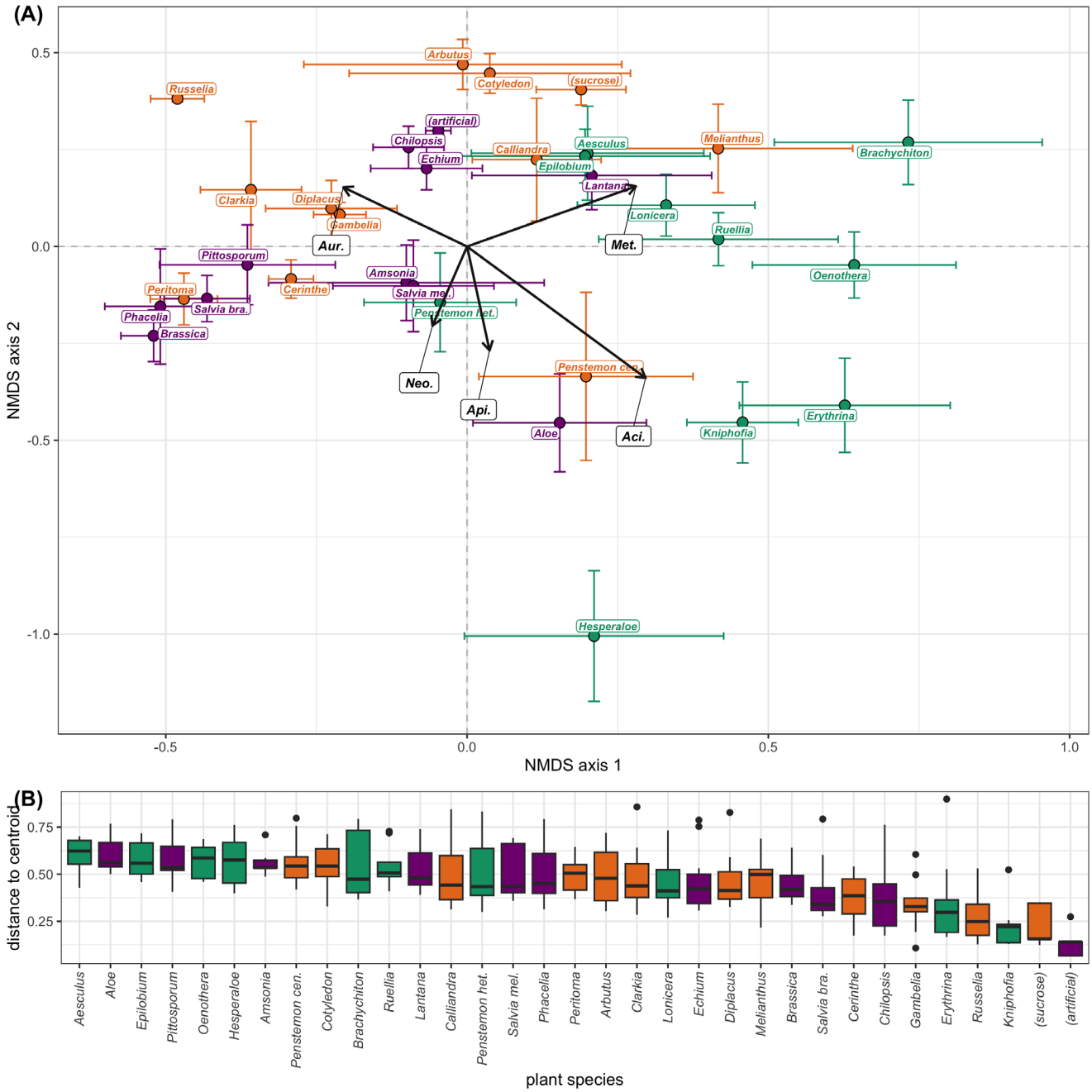

FIGURE 4 | (A) Nonmetric multidimensional scaling (NMDS) ordination of microbe community composition by plant species based on mean CFU densities. Each circular point represents the centroid of all observations for a given plant species, with whiskers representing the standard error of the mean for replicates in vertical and horizontal dimensions. Plants further apart in two-dimensional space exhibited more dissimilar microbe communities. Plants are additionally coloured orange, green or purple based on the three clusters generated via hierarchical clustering analysis of microbe community composition (Figure S5). Statistically significant ($p < 0.05$) vectors for microbe species are shown as labelled black arrows (Met. = *Metschnikowia*, Aur. = *Aureobasidium*, etc.). The size and direction of vectors indicate the strength of the correlation and direction of increase for the variable in NMDS space respectively. (B) Boxplot showing median and interquartile range (with outliers as points) of multivariate homogeneity of group dispersions (equivalent to beta diversity) for the 31 plant species and two experimental control solutions. The *y*-axis represents the distance of replicates to their respective group centroids, or equivalently the variability in community composition within plant species. Boxes are arranged in order of descending mean distance, with the three colours indicating the respective hierarchical cluster.

alone explained 38.1% of variation in multivariate CFU community composition ($F_{32,312} = 6.10$, $p < 0.0001$, Figure 4A), with multivariate group dispersions of microbe communities (beta diversity) also varying by plant species ($F_{32,314} = 6.17$, $p < 0.0001$, Figure 4B). In an NMDS ordination of microbe community composition, plotted vectors for all five microbial species were found to be significant (all $p < 0.01$). Hierarchical clustering analysis (Figure S5) of plant species based on mean CFU densities yielded three major clusters: one comprising plant species with high proportional densities of the bacterium *Acinetobacter*, another with high densities of the bacterium *Apilactobacillus* and another with plants that either

exhibited high densities of the yeast *Metschnikowia* or had all five microbe species present.

Pearson correlations of CFU densities across all plant species revealed that, of the 10 possible pairwise relationships between our five microbe species, four were significantly positive, with the remaining six being null (Figure S6). The bacterium *Neokomagataea* was the only microbe whose growth was not associated with that of any other microbe across plant species. Within-plant species correlation matrices indicated that co-occurrence patterns differed depending on plant species (Table S3).

3.2 | Phylogenetic Relatedness and Peroxide Content, but Not Floral Morphology, Correlate With Variation in Microbe Community Across Plant Species

We detected a plant phylogenetic signal in nectar volume across plant species ($K=0.80$, $p=0.025$), but no phylogenetic signal was detected in the CFU densities of any individual microbe species (all $p>0.11$), total CFU density ($K=0.77$, $p=0.080$) or CFU Shannon diversity ($K=0.37$, $p=0.32$). However, the Mantel test showed a positive correlation between microbe community dissimilarity and plant phylogenetic distance ($r=0.16$, $p=0.024$, Figure S7A). Floral morphological traits (Figure S8) were correlated with plant phylogenetic distance ($r=0.19$, $p=0.0013$, Figure S7B), but were not significantly associated with microbe community dissimilarity ($r=0.074$, $p=0.076$) even when accounting for variation due to plant phylogeny ($r=0.045$, $p=0.17$).

Higher mean nectar peroxide concentrations were associated with lower total CFU densities ($F_{1,330}=6.42$, $p=0.012$; Figure 5) across plant species. Microbe species differed in their association with peroxide: peroxide concentration was negatively associated with CFU density of the yeast *Aureobasidium* ($F_{1,332}=9.50$, $p=0.0022$), positively with that of the bacterium *Apilactobacillus* ($F_{1,332}=4.29$, $p=0.039$) and showed no association with densities of the yeast *Metschnikowia* ($F_{1,333}=1.70$, $p=0.19$) or the bacteria *Neokomagataea* ($F_{1,333}=0.47$, $p=0.49$) or *Acinetobacter* ($F_{1,331}=1.31$, $p=0.25$). Mean peroxide concentration was associated with microbe community composition ($F_{1,305}=1.98$, $p=0.032$) but explained only 0.65% of total variation.

3.3 | Temperature Correlates With Microbe Density and Community Composition

Overnight low temperatures ($F_{1,361}=12.83$, $p<0.0001$), but not afternoon high temperatures ($F_{1,349}=2.96$, $p=0.09$, Figure 6A,B), were positively correlated with total CFU densities across plant species. CFU Shannon diversity declined with higher afternoon high temperatures ($F_{1,362}=6.38$, $p=0.012$), but was not related to overnight low temperatures ($F_{1,349}=0.00$, $p=0.997$, Figure 6C,D). Microbe community composition also varied with afternoon high ($F_{1,312}=1.89$, $p=0.042$) and overnight low temperatures ($F_{1,312}=3.21$, $p=0.0014$, Figure 4A). Temperature effects on the microbiome were not driven by changes in nectar volume, as volume was not associated with either afternoon high ($F_{1,342}=3.73$, $p=0.054$) or overnight low temperatures ($F_{1,347}=1.14$, $p=0.29$).

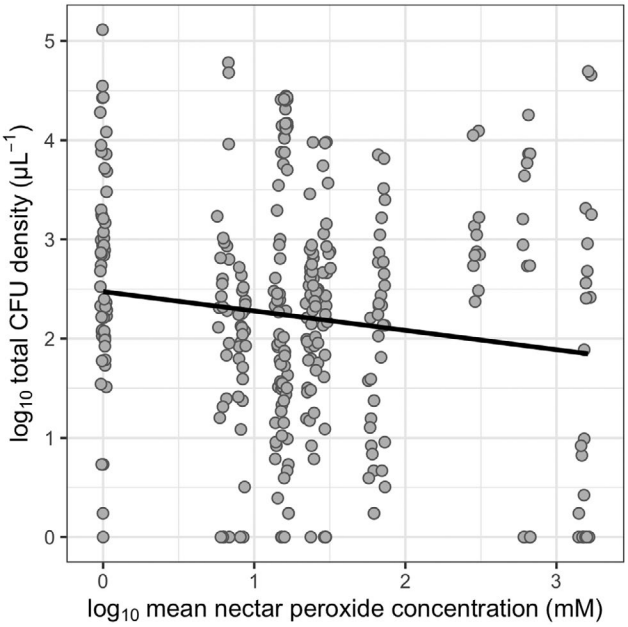

FIGURE 5 | Relationship between microbial total CFU density in nectar of inoculated flowers and mean nectar peroxide concentration by plant species. Each point represents one inoculated flower and the respective species mean peroxide value. Points are jittered slightly horizontally to better visualise density.

Individual microbe taxa responded to temperatures differently (Figure S9). CFU densities of both the yeast *Metschnikowia* and the bacterium *Acinetobacter* were positively associated with overnight low (*Metschnikowia*: $F_{1,367}=10.62$, $p=0.0012$; *Acinetobacter*: $F_{1,361}=5.48$, $p=0.020$), but not with afternoon high temperatures (*Metschnikowia*: $F_{1,355}=0.54$, $p=0.46$; *Acinetobacter*: $F_{1,349}=1.06$, $p=0.30$). In contrast, CFU densities of the bacteria *Apilactobacillus* and *Neokomagataea* both decreased with afternoon high temperatures (*Apilactobacillus*: $F_{1,348}=6.73$, $p=0.0099$; *Neokomagataea*: $F_{1,362}=19.96$, $p<0.0001$). *Aureobasidium* yeast CFU density increased with afternoon high temperatures ($F_{1,359}=6.21$, $p=0.013$), but decreased with overnight low temperatures ($F_{1,367}=8.98$, $p=0.0029$).

4 | Discussion

In this study, we observed shifts in the composition of a synthetic microbe community inoculated into the floral nectar of 31 flowering plant species, mainly predicted by plant species and temperature. Host species-dependency of plant microbiomes is consistent with previous observational studies of nectar (Brysch-Herzberg 2004), pollen (Ambika Manirajan et al. 2016), phyllospheres (Gaube et al. 2023) and roots (Fitzpatrick et al. 2018). Our manipulative study complements this work by leveraging a phylogenetically diverse array of plant taxa, highlighting the role of plant host identity as a driver of microbe community assembly outside of dispersal and priority effects. Specifically, we provide experimental evidence that nectar microbiomes become distinct across plants even when initial community composition is the same.

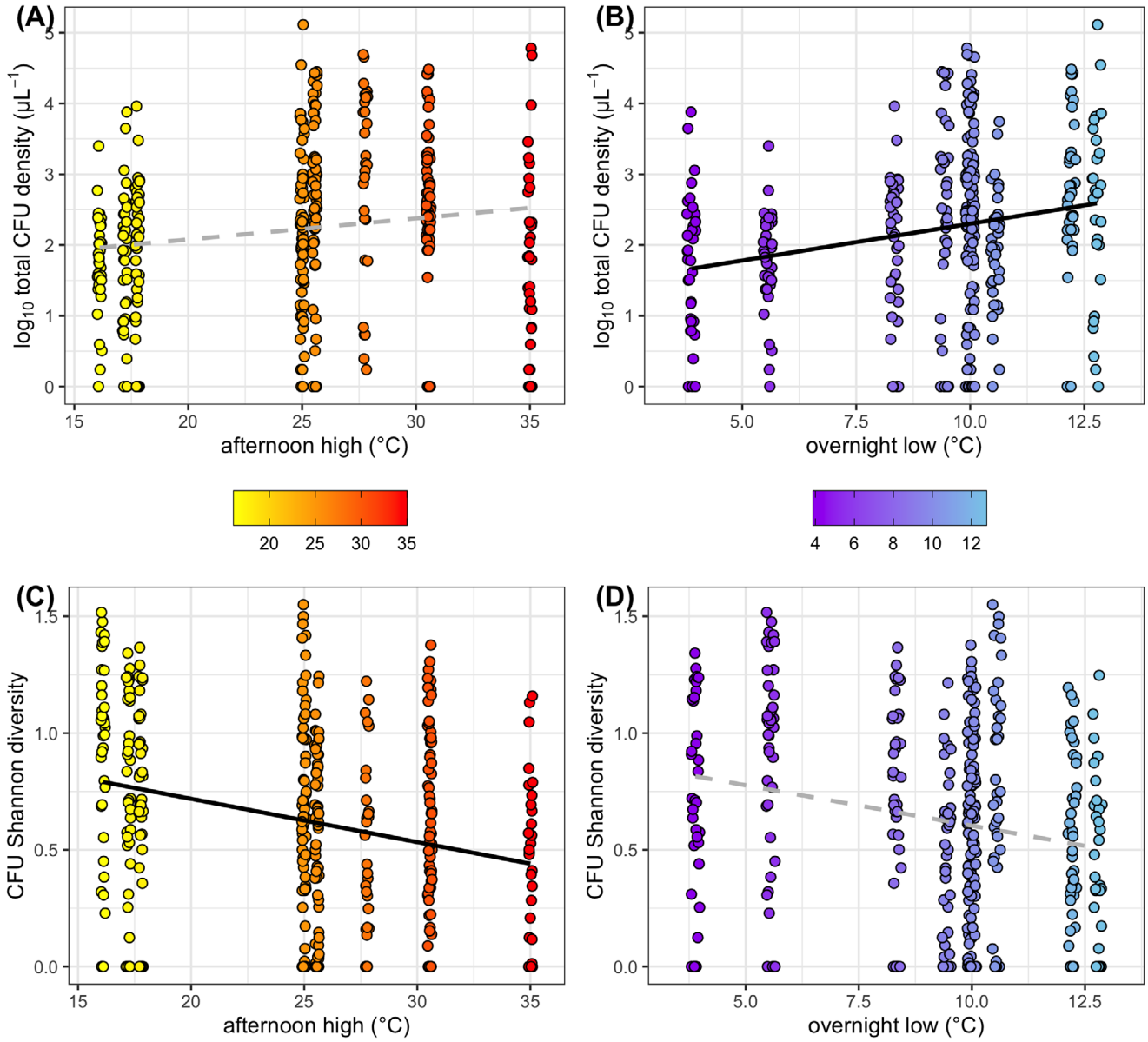

FIGURE 6 | Relationships between temperature extrema and microbe community metrics: (A, B) total CFU density and (C, D) CFU Shannon diversity. Statistically significant ($p < 0.05$) relationships with temperature variables (afternoon high of day of inoculation, and overnight low of night following inoculation) according to linear mixed models are indicated with solid black lines in panels (B, C), while nonsignificant trend lines are indicated with grey-dashed lines in panels (A, D). Points are jittered slightly horizontally to better visualise density.

4.1 | Biotic Factors: Plants

Several factors can impact nectar microbe community assembly even when controlling for dispersal (Sharaby et al. 2020), including filtering by host plants and interactions among microbe species (Chappell and Fukami 2018). Our study shows support for both processes. In certain plant species (e.g., *Arbutus unedo*), few microbe taxa established and were at low densities. Furthermore, community assembly in certain plant species was more stochastic than in others (Figure 4B); it may be that environmental stress generates stronger selection and more uniform communities (Chase 2007; Tripathi et al. 2018; Zhou and Ning 2017). Interestingly, the highest uniformity in community composition we documented was in our two experimental control solutions (30% sucrose and artificial nectar). This suggests community stochasticity is higher in actual flowers, likely due to variation in nectar properties across plants. Previous work in a single plant species (Vannette and Fukami 2017) suggests dispersal positively contributes to nectar microbe beta diversity via priority effects. Our study suggests that even in the absence of dispersal, nectar microbe beta diversity may be more constrained in some plant species than in others.

Plant-level mediation of microbe community assembly outside of pollinator vectoring is consistent with the hypothesis that physical or chemical properties of flowers and nectars (Mueller, Francis, and Vannette 2023) differentially inhibit microbe

growth. This phenomenon may serve as an adaptive defence against nectar spoilage ('antimicrobial hypothesis'; Canto and Herrera 2012), but it is also possible that some nectars could facilitate the growth of particular microbes. Several plants we inoculated belong to genera (e.g., *Aesculus*, *Brassica*, *Echium*) containing species known to produce antimicrobial nectar metabolites: for example, alkaloids, phenolics, and terpenoids (Palmer-Young et al. 2019). We suspect that the occurrence of secondary metabolites or other nectar constituents (Roy et al. 2017) might explain the distinct differences in community structure we observed across plant species. This is supported by our finding that nectar peroxide concentration, which is regulated via nectarin proteins in *Nicotiana* (Carter and Thornburg 2004), was negatively associated with total microbe density across plant species. The effects of peroxide concentration differed depending on microbe species, mirroring trends from in vitro assays (Mueller, Francis, and Vannette 2023), perhaps due to differences in microbial detoxification mechanisms. However, mean peroxide concentration on its own explained very little variation in the dataset. Future work incorporating a much broader diversity of nectar chemicals and compounds in a similarly diverse array of plant species is needed to determine if such a predictive framework exists.

Similarity in nectar chemistry among species can be associated with phylogenetic relatedness in certain plant clades (Pozo, Lievens, and Jacquemyn 2015). We found plant relatedness was weakly positively associated with similarity in microbe community composition, but not with the densities of any individual microbes. Plant relatedness alone was not sufficient to explain the similarity in microbe community assembly however as this relationship was not monotonic. Within major plant clades, plant species in our study hosted similarly composed microbe communities (Figure 2), but several exceptions are clear. Hierarchical clustering analysis reflected this pattern as some, but not all, plant species of major clades clustered together and congeneric plant species did not necessarily cluster closely. In other plant microbiomes, host plant phylogeny can be a predictor of microbial communities (Fitzpatrick et al. 2018), vary between bacteria versus fungi (Calvert et al. 2023) or show little predictive power (Kembel and Mueller 2014; Tellez et al. 2022; Vincent, Weiblen, and May 2016). In the latter cases, microbe communities were better predicted by plant traits, implying a weak relationship between plant phylogeny and traits (Schroeder et al. 2019).

We found that floral morphological traits were correlated with plant relatedness, but were not predictive of nectar microbe communities (Francis, Mueller, and Vannette 2023), suggesting that key host traits mediating microbial growth were not measured in the current experiment. Floral trait similarity, here approximating pollination syndromes (Ollerton et al. 2009), not predicting variation in microbial composition is contrary to predictions based on floral surveys of open flowers in which pollinator identity or pollination syndrome is a key predictor of nectar microbial communities (Félix et al. 2021; Mittelbach et al. 2015; Morris et al. 2020; de Vega, Herrera, and Johnson 2009). Nevertheless, the microbes used here are common in most geographical regions sampled to date, and we expect that pollinator movement will homogenise microbial populations to some extent within co-flowering communities.

4.2 | Biotic Factors: Microbe–Microbe Interactions

Interactions among microbes likely influenced community assembly within flowers, and we detected signatures of both facilitation and competition depending on analytical approach. All five species in our synthetic community were capable of coexisting after 24 h at varying densities in artificial nectar in vitro. We detected only positive or neutral correlations (none negative) between microbe species pairs, similar to Francis, Mueller, and Vannette (2023), in both our pooled dataset and separately within each plant species. In the pooled dataset, *Neokomagataea* was the only species showing no positive correlations with any other microbe, perhaps due to unknown specificities in its nutrient requirements. At first, this all seems to suggest facilitation among some species pairs (Mueller, Francis, and Vannette 2023), or that competition between microbes at 24 h was insignificant.

However, we also observed a unimodal, 'hump-shaped' relationship between CFU Shannon diversity and increasing total CFU density across plant species. Shannon diversity increased with CFU density until roughly 10^2 CFU μL^{-1}, after which diversity declined as density increased. Similar unimodal relationships between microbe diversity and productivity (here analogous to density) have been documented in both artificial (Kassen et al. 2000) and natural aquatic environments (Smith 2007). Several underlying mechanisms have been proposed for this relationship, including a shift from abiotic (habitat tolerance) to biotic (competitive exclusion) pressures along the gradient of increasing productivity (Geyer and Barrett 2019). We suggest that extreme resource limitation or antimicrobial conditions in some nectars may limit the growth of all microbes in some nectars, whereas the availability of pollen (Christensen, Munkres, and Vannette 2021) or other nutrients may enable dominance of specific microbes in other nectars.

Co-occurrence networks reflect the combined influence of biotic interactions and the environment and may underrepresent negative, nontrophic interactions (e.g., intra-guild competition) relative to empirically observed interactions (Freilich et al. 2018). Additionally, only 24 h post inoculation may represent an early to intermediate time point in community progression, perhaps preceding manifestation of antagonistic interactions (Mittelbach et al. 2016). Conversely, the unimodal relationship across plant species suggests that the growth of specific microbes in highly productive environments (reflected by high CFU density) can effectively reduce community diversity, resulting in competitive exclusion of other microbes. We hypothesise that such competitive dynamics will be more apparent in longer persisting or senescing flowers (Morris et al. 2020; Tucker and Fukami 2014), but interaction outcomes can also depend on microbes' phylogenetic relatedness (Dhami, Hartwig, and Fukami 2016; Maherali and Klironomos 2007; Peay, Belisle, and Fukami 2011) or their local adaptations to flower environments (Herrera, Pozo, and Bazaga 2014).

4.3 | Abiotic Factors

Consistent with our expectations, microbial growth was correlated with seasonal temperature shifts. Maximum and minimum ambient temperatures over 24 h of growth were differentially associated with components of community assembly and species individual densities, further supporting that nectar microbe species differ in their temperature ranges for optimal growth (Russell and McFrederick 2022a, 2022b). Notably, increases in daily minimum temperature increased densities of the nectar yeast *Metschnikowia* and bacterium *Acinetobacter*, suggesting that their population densities are limited by growth rate. In contrast, high maximum daily temperatures decreased *Lactobacillus* and *Neokomagatea* densities, suggesting temperature thresholds for these microbes. These patterns are consistent with previous observations that nectar yeast prevalence was found to be positively correlated with temperature (Pozo, Herrera, and Alonso 2014), while high temperatures can negatively impact nectar bacterial diversity (Sharaby et al. 2020). Although we did not detect a significant effect of temperature on nectar volume in the current study (using bagged flowers), open flowers likely experience increased evaporation affecting nectar composition and secretion (Freeman and Head 1990).

In any case, our observations indicate, similar to other plant–microbe systems (Keeler, Rose-Person, and Rafferty 2021; Rasmussen, Bennett, and Tack 2020), that shifts in temperature extrema over time may alter baseline effects of plant host filtering on nectar microbial communities in predictable ways, such as favouring certain microbe species over others or limiting maximum achievable levels of diversity. The implications of these shifts for plant–pollinator interactions deserve further attention. We also emphasise that in our study, different plant species were necessarily sampled at different times of year due to flowering phenology, so temperature was confounded with other variables like plant host identity, humidity and solar radiation, all of which affect microbial assembly in flowers (Aleklett, Hart, and Shade 2014). Nevertheless, our results suggest that differential response to temperature minima and maxima mediates microbial growth and interactions.

We show that plant species host consistent microbial communities, suggesting plant populations could potentially adapt to the presence of specific microbes (Vannette 2020). Insect populations could also adapt to plant-specific microbial growth, such as perceived volatile cues (Cusumano and Lievens 2023) or acquired microbes (Adler et al. 2021). Our results also carry implications for microbes which respond predictably to changing temperatures. For example, *Apilactobacillus* is thought to benefit pollinators (but see Brar et al. 2024), thus increasing high temperatures may inhibit the growth of this beneficial microbe. Similarly, it will be pertinent to explore the effects of heat-tolerant microbes like *Acinetobacter* (Sharaby et al. 2020) on pollinator health and behaviour in the face of climate warming.

Our results are a product of the microbe taxa we chose for our synthetic community and the plant species inoculated. However, our findings are likely generalisable to other bioregions, given the widespread geographic distributions of our focal microbes (de Vega et al. 2021) and the inclusion of both native and non-native plant species. Regardless, future research will benefit from incorporating additional plant and nectar traits and increasing replication within clades of plant taxa (Agrawal and Weber 2015) to clarify mechanisms influencing nectar microbe ecology.

5 | Conclusions

In summary, we found a strong signature of host species and temperature extrema on the trajectory of a symbiotic microbe community. Our findings provide insights into the roles of host filtering, microbial interactions and abiotic conditions on the assembly of nectar specialist and generalist microbe communities outside the confounding influence of pollinator dispersal. Our results suggest that general scaling hypotheses, including diversity–productivity relationships, may apply to some host-associated microbiomes. Our study shows how species-specific responses to temperature extrema can alter communities under changing temperature regimes, and highlights roles of both high-temperature thresholds and increasing temperature minima. We suspect many other microbial communities in thermally variable ecosystems may be subject to these effects under changing climate scenarios, including many host-associated systems.

Author Contributions

Jacob M. Cecala and Rachel L. Vannette designed the study and collected data. Jacob M. Cecala performed analyses and wrote the first draft of the manuscript. Leta Landucci performed laboratory and field work to collect data on nectar hydrogen peroxide concentrations. Jacob M. Cecala and Rachel L. Vannette contributed substantially to revisions with input from Leta Landucci.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data supporting the results are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.xsj3tx9q2.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.