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Environment–host–microbial interactions shape the *Sarracenia purpurea* microbiome at the continental scale

ZACHARY B. FREEDMAN ^{1,8}, ALICIA MCGREW,^{2,3} BENJAMIN BAISER,³ MATHILDE BESSON,⁴ DOMINIQUE GRAVEL,⁵ TIMOTHÉE POISOT ⁴, SYDNE RECORD ⁶, LAUREN B. TROTTA,² AND NICHOLAS J. GOTELLI⁷

¹Department of Soil Science, University of Wisconsin-Madison, Madison, Wisconsin 53706 USA

²School of Natural Resources and Environment, University of Florida, Gainesville, Florida 32603 USA

³Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida 32603 USA

⁴Département de Sciences Biologiques, Université de Montréal, Montréal, Québec H2V 0B3 Canada

⁵Département de Biologie, Faculté des Sciences, Université de Sherbrooke, Sherbrooke, Québec J1K 2R1 Canada

⁶Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010 USA

⁷Department of Biology, University of Vermont, Burlington, Vermont 05405 USA

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Abstract. The importance of climate, habitat structure, and higher trophic levels on microbial diversity is only beginning to be understood. Here, we examined the influence of climate variables, plant morphology, and the abundance of aquatic invertebrates on the microbial biodiversity of the northern pitcher plant *Sarracenia purpurea*. The plant's cup-shaped leaves fill with rainwater and support a miniature, yet full-fledged, ecosystem with a diverse microbiome that decomposes captured prey and a small network of shredding and filter-feeding aquatic invertebrates that feed on microbes. We characterized pitcher microbiomes of 108 plants sampled at 36 sites from Florida to Quebec. Structural equation models revealed that annual precipitation and temperature, plant size, and midge abundance had direct effects on microbiome taxonomic and phylogenetic diversity. Climate variables also exerted indirect effects through plant size and midge abundance. Further, spatial structure and climate influenced taxonomic composition, but not phylogenetic composition. Our results suggest that direct effects of midge abundance and climate and indirect effects of climate through its effect on plant-associated factors lead to greater richness of microbial phylotypes in warmer, wetter sites.

Key words: bacteria; biogeography; food webs; phylogenetic diversity; *Sarracenia purpurea*.

INTRODUCTION

Complex interactions between ecological communities and their environment can influence the assembly and maintenance of biodiversity across space and time. While major drivers of plant and animal distributions are well understood (Cox and Moore 2016, Rice et al. 2019), drivers of microbial distributions are less resolved (Hendershot et al. 2017). It has been established that microbial diversity often exhibits different and weaker biogeographic patterns from those of plant and animal communities (Meyer et al. 2018). Further, evidence is

building that food web dynamics can exert a strong influence on microbial diversity (Koltz et al. 2018, Gralka et al. 2020), that may or may not elicit functional consequences (Peschel et al. 2015, Cline et al. 2017, Beier et al. 2020). Though the extent to which these dynamics affect microbial diversity across large spatial scales is uncertain.

Phytotelmata, water-filled cavities in plants, provide an ideal system for investigating the influence of higher trophic levels on microbial diversity across large spatial scales. These unique environments are discrete units that provide similar habitats across large scales, reducing the environmental variation prevalent in studies of microbial biogeography (Sul et al. 2013, Freedman and Zak 2015). One particularly well-studied phytotelmata ecosystem is the carnivorous pitcher plant *Sarracenia purpurea*, which traps and digests

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⁸E-mail: zfreedman@wisc.edu

insect prey in its rain-filled, pitcher-shaped leaves and is a model system in ecology (Addicott 1974, Ellison and Gotelli 2003, Gotelli and Ellison 2006). To decompose prey, *S. purpurea* relies on a food web that resides in the pitcher composed of microorganisms, protozoa, rotifers, and dipteran larvae, among other organisms. This micro-ecosystem has been used to study keystone predation, community assembly, tipping points, and evolution, among other community dynamics (e.g., Addicott 1974, terHorst et al. 2010, Miller 2012, Sirota et al. 2013). Furthermore, *S. purpurea* has a wide distribution across North America (Fig. 1), which provides the opportunity to explore the biogeography of an entire food web (Buckley et al. 2010, Baiser et al. 2012, Gray et al. 2012).

Although the microbial community is responsible for the main ecosystem function, decomposition, in the *S. purpurea* system (Butler et al. 2008), current understanding of the degree to which climatic-, pitcher-habitat-, and food-web-associated factors influence the *S. purpurea* microbiome across continental spatial scales is nascent. Macroecological theory has different predictions regarding the relative importance of direct and indirect drivers of microbial diversity. For example, the metabolic theory of ecology (Brown et al. 2004) predicts that climatic conditions should directly constrain microbial diversity as warmer environments are expected to support greater turnover due to increasing metabolic demand, regardless of local species composition (Fig. 2A; Gray et al. 2016). This theory has been used as support for macroecological patterns like the latitudinal diversity gradient, where species richness generally increases from high to low latitudes (Pianka 1966). Conversely, the temperature-dependent consumer–resource theory (Vasseur and McCann 2005) predicts an increased top-down influence of predators on producers in warmer environments. Indeed, the presence of a keystone predator (*Wyeomyia smithii*) can increase bacterial richness in *S. purpurea* pitchers (Peterson et al. 2008), and this response may be dependent on climate as bacterial thermal tolerances can differ from that of bacterivorous consumers (Parain et al. 2016). As a result, this theory would support an indirect impact of climate through food web-associated factors on microbial diversity (Fig. 2B). Alternatively, the Habitat Amount Hypothesis (Fahrig 2013) predicts that microbial diversity will increase with the amount of habitat type (e.g., pitcher size and morphology). As warmer, wetter climates select for larger pitchers (Ellison et al. 2004), and variation in the pitcher habitat can influence members of its captive ecosystem (Gotelli and Ellison 2006), this hypothesis would predict an indirect effect of climate through plant-associated factors influencing microbial diversity (Fig. 2C). Last, niche theory (Hutchinson 1957) predicts a sequential turnover of species along the climatic gradient that can be attributed to multidimensional changes in environmental

conditions and resources. This would result in climatic-, plant-, and food-web-associated factors together influencing the *S. purpurea* microbiome (Fig. 2D).

In this study, we investigated the influence of climatic-, pitcher-habitat-, and food-web-associated factors in shaping the bacterial communities inhabiting *S. purpurea* pitchers along its North American latitudinal range. Specifically, we draw on macroecological theory to develop and test four hypotheses (Fig. 2) where microbial diversity is directly affected by climate (H1; Fig. 2A), or indirectly affected by climate's interactions with characteristics of the food web (H2; Fig. 2B), pitcher-habitat-associated factors (e.g., plant morphology, water volume, and pH; H3; Fig. 2C), or both pitcher-habitat- and food-web-associated factors (H4; Fig. 2D). We tested these hypotheses with a survey of microbial assemblages collected from 108 pitchers from 36 sites across 23.53° of latitude, from Florida, USA to northern Quebec, Canada.

MATERIALS AND METHODS

Site selection and sample processing

We collected fluid from 108 pitchers at 36 sites across *S. purpurea*'s North American latitudinal range (30.197° N–53.722° N; Appendix S1: Section S1; Fig. 1). Selected sites had a minimum of seven individuals and maximized latitudinal coverage of *S. purpurea*'s geographic distribution. Sampling of each site was timed to ensure that plants were sampled between four and six weeks after new pitchers opened, which is sufficient time for pitchers to capture prey and be colonized by their aquatic food web (Buckley et al. 2010).

At each site, we marked plants at 5-m intervals along a 120-m transect and selected three pitchers randomly for further analysis. For each selected plant, we measured the diameter of each plant, pitcher liquid was collected from the largest new pitcher, and the leaf was collected for morphometry measurements (Ellison and Gotelli 2003). We then homogenized the pitcher fluid, transferred it to a sterile 50-mL Falcon tube and immediately recorded water volume and pH. Captured prey and pitcher invertebrates (flesh flies, midges, and mosquitoes) were visually counted. Samples were stored in the field at –20°C in an electric chest freezer. Upon return to the lab, we stored subsamples of liquid at –20°C and at –80°C.

Microbiome analysis

We extracted total DNA from 300 µL of homogenized pitcher fluid and the V4 region of the 16S rRNA gene was amplified and sequenced (Appendix S1: Section S1). Bacterial phylotypes were delineated by clustering operational taxonomic units (OTUs) at the ≥97% sequence similarity in QIIME (Version 1.9.1; Caporaso et al.

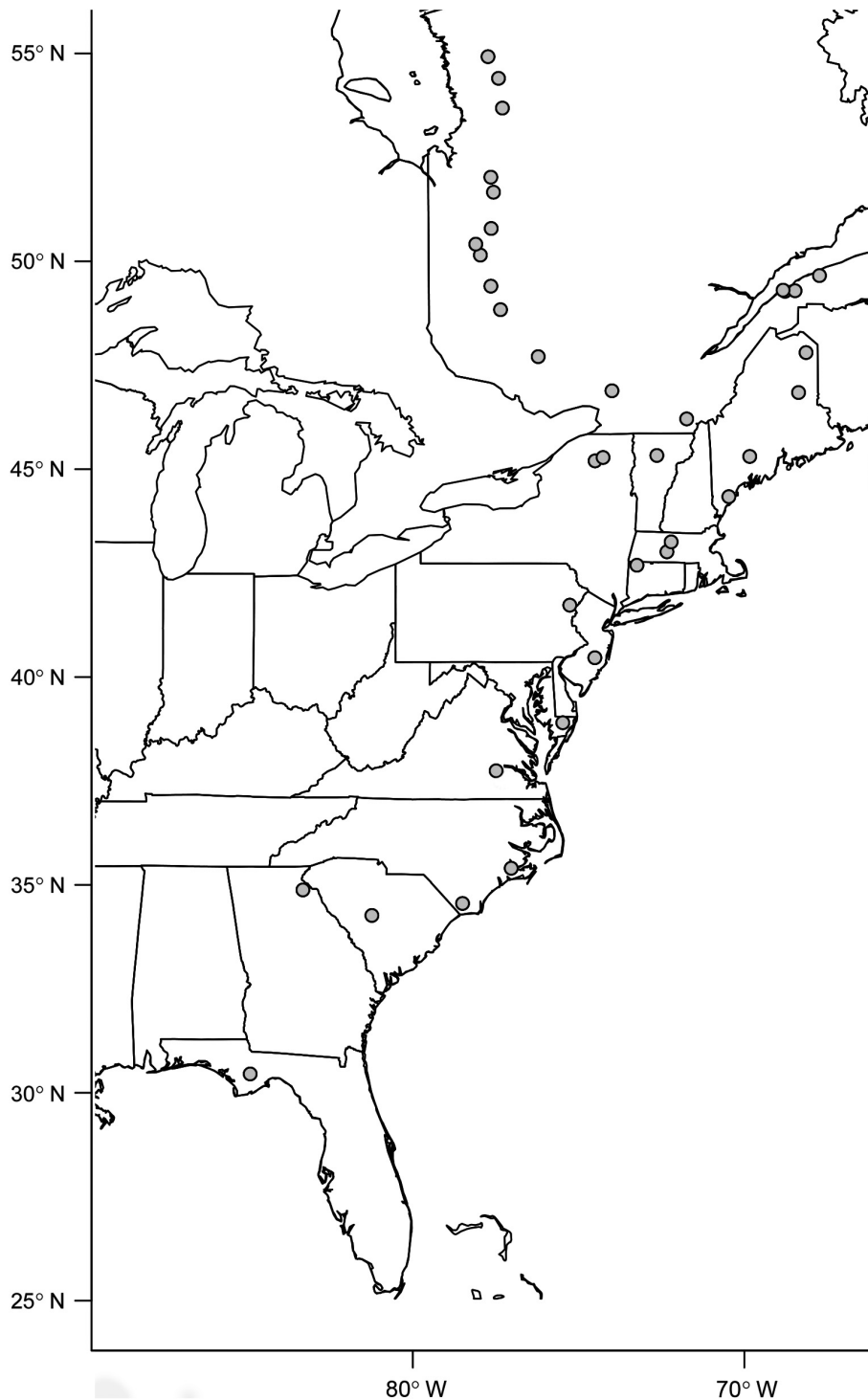


FIG. 1. Map detailing the location of the 36 sampling sites in this study. Circles represent sampling sites. Three microbial samples were analyzed from each location. One site (in the southeastern coastal plain of Georgia, USA) was omitted from the map by landowner request.

2010), from which α and β taxonomic and phylogenetic diversity were determined. We rarefied the data set to 10,000 sequences per sample prior to downstream α - and β -diversity analyses.

Statistical analysis

We employed linear mixed models with site as a random effect to test for associations between bacterial

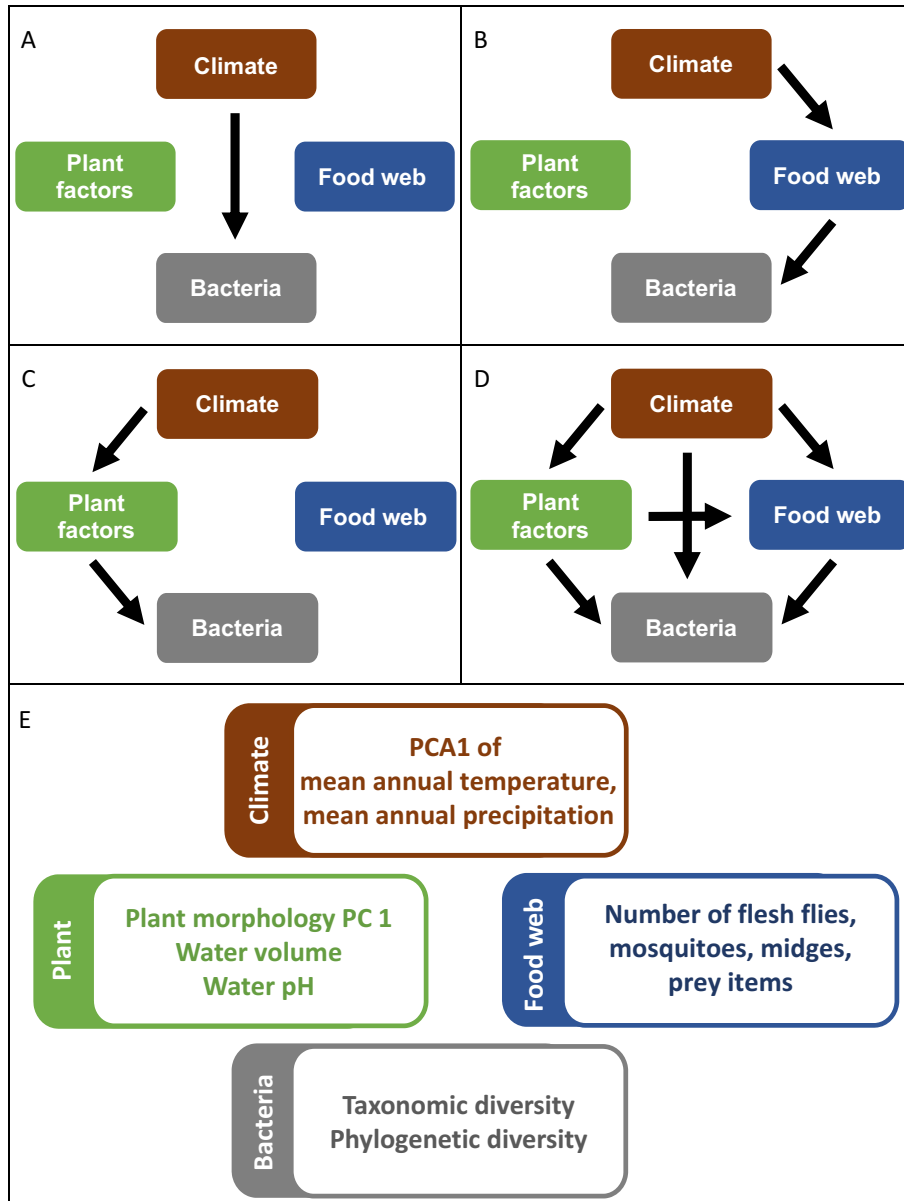


FIG. 2. (A) Direct and (B–D) indirect hypotheses by which climate impacts the bacterial diversity within *Sarracenia purpurea* pitchers. (E) The climatic-, pitcher-habitat-, and food-web-associated factors measured in this study. Plant morphology metrics included pitcher length and width, keel width, mouth diameter, and lip width.

taxonomic (Chao1) and phylogenetic (MPD, SES.MPD) α -diversity and latitude. Briefly, Chao1 is a nonparametric richness estimator, MPD is the mean phylogenetic distance among all pairs of phylotypes within a community, and SES.MPD is the standardized effect size of MPD compared to a null model (Appendix S1: Section S1). We then performed structural equation modeling (SEM) to test hypotheses of how climate influences pitcher-habitat- and food-web-associated factors as well as their collective influence on the taxonomic and phylogenetic α -diversity of the

S. purpurea microbiome (Fig 2; Appendix S1: Section S1). We assessed each model's goodness-of-fit using tests of directed separation (Shiple 2000). Partial redundancy analysis (RDA) and variance partitioning were then conducted to test the role of climatic-, pitcher-habitat-, and food-web-associated factors on bacterial taxonomic and phylogenetic composition (Appendix S1: Sections S2 and S3). Partial RDAs and distance-based RDAs (dbRDA) were conditioned on distance-based Moran's Eigenvector Maps (dbMEM) to partition out the role of spatial structure.

RESULTS

Diversity of the S. purpurea microbiome across its North American latitudinal range

In total, 16,157,518 high-quality sequences were obtained from 108 pitchers across the 36 sites. After rarefaction, sites averaged 336–1,141 OTUs, and there were 22,307 total OTUs across all sites (Good's coverage of 0.986 ± 0.003 [mean \pm SE]). The most abundant OTUs at the individual pitcher level were also the most widely distributed across the latitudinal range, as there was a positive correlation between OTU abundance and number of pitchers occupied ($F_{1,105,029} = 2.2 \times 10^5$; $R^2 = 0.68$, $P < 0.01$; Appendix S1: Section S4 and Fig. S4). Across all sites, *S. purpurea* pitcher microbiomes were dominated by members of the phyla Proteobacteria (54–79% of community; Appendix S1: Table S3), Bacteroidetes (6–21%), and Actinobacteria (4–20%), while members of the Firmicutes (1–7%) and Acidobacteria (1–2%) were less abundant. Among the proteobacterial phylum, the most dominant taxa were members of the class beta-proteobacteria (14–39%), alpha-proteobacteria (12–35%), and gamma-proteobacteria (11–39%).

S. purpurea microbiome taxonomic α -diversity (i.e., Chao1 diversity) decreased with latitude toward the north (coefficient = -17.77 ; $t_{1,34} = -3.30$; $P < 0.005$; $R^2 = 0.17$; Fig. 3). Phylogenetic α -diversity measured as MPD showed no relationship with latitude ($P = 0.16$) but standardized MPD (SES.MPD) increased slightly with latitude (coefficient = 0.10 ; $t_{1,34} = 2.18$; $P < 0.05$; $R^2 = 0.06$).

Drivers of the S. purpurea microbiome biogeography

While SEMs tested all hypothesized relationships (Fig. 2), results and subsequent discussion will focus on variables that directly or indirectly influenced microbial diversity. Full SEM results may be found in Appendix S1: Section S5. Climate and food web variables directly influenced bacterial taxonomic α -diversity (i.e., Chao1 diversity). Chao1 increased in warmer, wetter climates (Fig 3; standardized coefficient (std. coef.) = 0.32 ; $P = 0.02$) and increased with midge abundance (std. coef. = 0.17 ; $P = 0.04$). The direct effect of climate was almost twice as large the direct effect of midges. Climate also indirectly influenced Chao1 through its effect on pitcher morphology and water volume (Fig. 3C and E). Warmer, rainier climates resulted in greater pitcher water volumes directly (std. coef. = 0.32 , $P < 0.005$) and indirectly through plant morphology (std. coef. = 0.16 ; P values unavailable for indirect effects, which are the product of the standardized coefficients along the path; Appendix S1: Section S1). Pitchers with larger volumes of water harbored greater abundances of midges (unstandardized coef. = 1.14 , $P < 0.001$; standardized coefficients unavailable for non-Gaussian error

distributions) and, as noted above, midges increased bacterial taxonomic α -diversity. Overall, 26% of the variation in the *S. purpurea* microbiome taxonomic α -diversity was described by the SEM model (Fisher's $C = 20.10$, $df = 14$, $P = 0.13$).

Both measures of phylogenetic α -diversity (i.e., MPD and SES.MPD) responded similarly (i.e., same significant paths and signs of coefficients) to climatic-, pitcher-habitat-, and food-web-associated variables. For brevity, only MPD results will be presented (SES.MPD results can be found in Appendix S1: Section S5 and Table S7). *Sarracenia purpurea* microbiome phylogenetic α -diversity was directly influenced by climatic-, pitcher-habitat-, and food-web-associated factors. MPD decreased in warmer, rainier sites (Fig. 3D and F; std. coef. = -0.37 ; $P < 0.005$) and increased in larger pitchers (std. coef. = 0.36 ; $P < 0.005$) and pitchers with greater midge abundances (std. coef. = 0.23 ; $P < 0.05$). Climate indirectly influenced MPD along the same paths as it did for Chao1 diversity (i.e., through volume and midge abundance), and it also indirectly affected MPD through plant morphology. Note that the direct effect of climate on MPD is negative, while the indirect effects of climate on MPD are positive. Overall, 21% of the variation in MPD was explained by our model (Fisher's $C = 16.01$, $df = 14$, $P = 0.31$). Coefficient tables for all SEM equations can be found in Appendix S1: Tables S5–S7.

The constraints in partial RDA model described only 2% of the variation in taxonomic composition (Appendix S1: Section S6) with water volume ($P = 0.05$) showing significance. Variance partitioning indicated that the food web, plant morphology, climate, and spatial structure (i.e., dbMEMs) together accounted for 14% of the variation in community composition. The variance partitions for spatial structure (5%), shared spatial structure and climate (4%) explained most of the variation. For phylogenetic composition, the constraints and conditions (i.e., dbMEMs) in the partial dbRDA described $< 1\%$ of the variation.

DISCUSSION

Bacterial communities inhabiting *S. purpurea* pitchers exhibit biogeographic structure across the plant's North American range, driven by the direct and indirect effect of climate through pitcher-habitat- and food-web-associated factors. The empirical data from this study supports hypothesis H4: the *S. purpurea* microbiome is directly affected by climate and indirectly affected by climate through interactions with both pitcher-habitat- and food-web-associated factors (Figs. 2 and 3). In this way, our findings are also consistent with the latitudinal diversity gradient and provide support for the metabolic theory of ecology at the continental scale. Conversely, there was no support for the temperature-dependent consumer–resource theory, as the detritus-shredding midge, but not mosquito larvae (i.e., a keystone predator), emerged as the food web-associated factor most

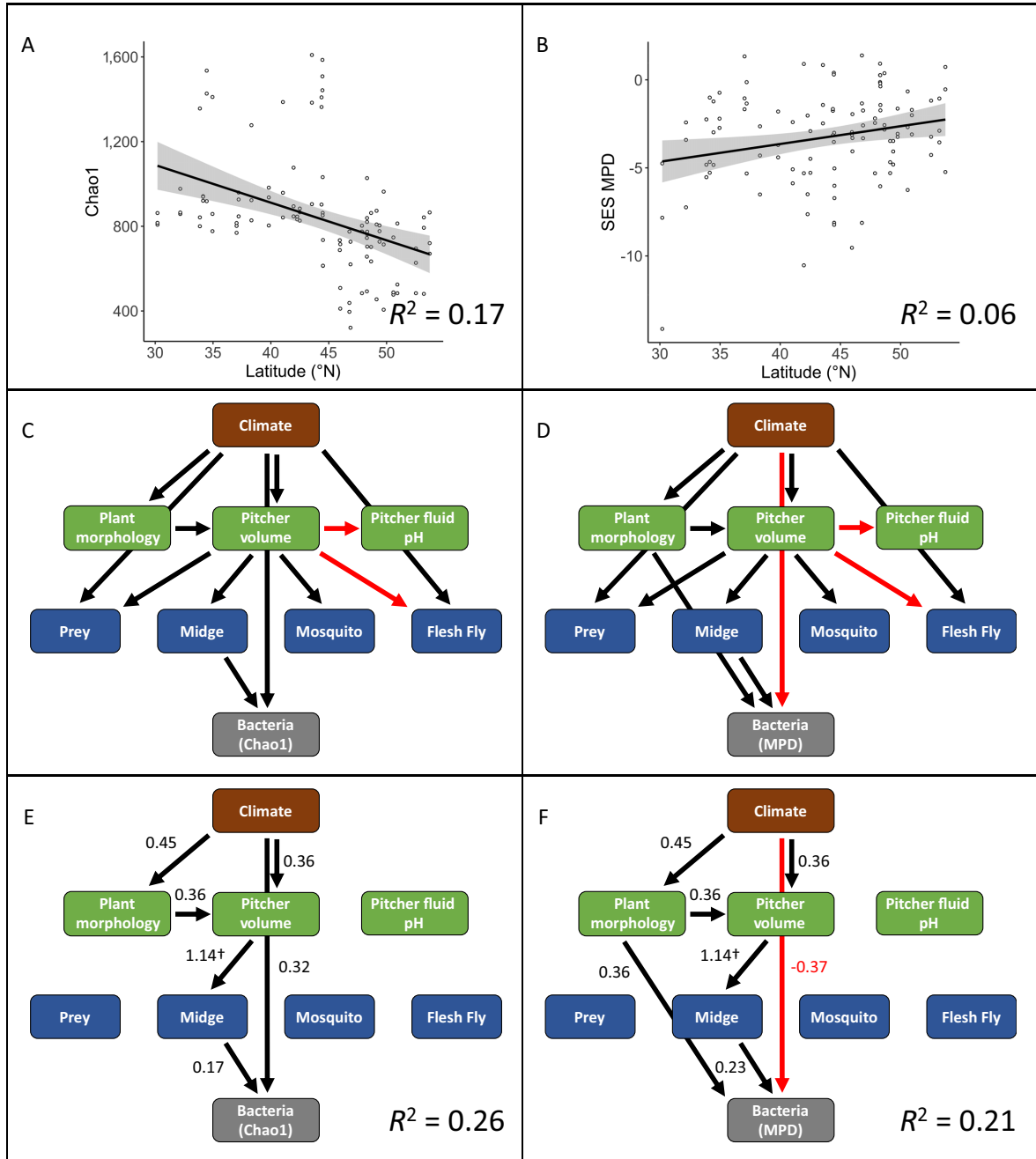


FIG. 3. (A, B) Biplots of diversity vs. latitude and (C–F) results of structural equation models (SEM) linking climate-, pitcher-habitat-, and food-web-associated factors to bacterial taxonomic (C, E) and phylogenetic (D, F) diversity. All significant paths are shown in panels C and D whereas only significant paths leading to bacterial Chao1 (model $R^2 = 0.26$) and mean phylogenetic distance (MPD; model $R^2 = 0.21$) are shown in panels E and F. Black and red arrows denote positive and negative coefficients, respectively. Standardized coefficients are presented unless marked by a dagger (†).

influential to pitcher microbiome diversity. Taxonomic α -diversity was greatest in pitchers that experienced warm temperatures and high levels of precipitation, generally located at low latitudes, and it decreased with increasing latitude where sites are relatively cooler and drier. The inverse relationship between taxonomic

diversity and latitude is consistent with observations of marine and aquatic bacterial diversity (Fuhrman et al. 2008, Ladau et al. 2013) and diversity for many other organisms (i.e., the latitudinal diversity gradient; Fischer 1960). Conversely, bacterial communities exhibited a weak negative latitudinal–phylogenetic α -diversity

relationship (i.e., greater phylogenetic clustering at lower latitudes), a trend that has been previously observed with bacteria (Andam et al. 2016). Given that higher temperatures are associated with high rates of speciation (Allen et al. 2006), it is plausible that in southern *S. purpurea* pitcher ecosystems the high taxonomic and low phylogenetic α -diversity may be maintained by higher diversification rates that generate rich assemblages of closely related phylotypes as compared to northern *S. purpurea* ecosystems.

The relationship between climate and *S. purpurea* morphology has consequences for pitcher bacterial diversity. For example, wetter climates generally have larger pitchers (Ellison and Gotelli 2003) that can hold a greater volume of rainwater, which in turn can influence members of the *S. purpurea* food web (Gotelli and Ellison 2006). Here, our results show that climatic-driven changes in pitcher morphology increase phylogenetic α -diversity of pitcher microbial communities. Larger pitchers potentially provide more habitat heterogeneity (i.e., sub-habitats) and total potential energy (i.e., prey), which together can support distinct microbial communities (Krieger and Kourtev 2012). The observation that the *S. purpurea* microbiome is partially constrained by pitcher-associated factors has been previously noted (Peterson et al. 2008) and is not surprising, as the *S. purpurea* microbiome has had the opportunity to coevolve and adapt since *Sarracenia* diverged from its congeners ~ 23 million years ago (Ellison et al. 2012).

Pitcher plant morphology also interacts with members of higher trophic levels in the food web, particularly the shredding midge *Metriocnemus knabi*, to influence bacterial diversity. Midge abundance has a positive relationship with pitcher size (Buckley et al. 2010), and our results suggest that this pitcher-morphology–midge relationship leads to greater bacterial taxonomic and phylogenetic α -diversity. *Metriocnemus knabi* is the only shredder in the system and is part of a commensal “processing chain” where it shreds detritus into smaller pieces, thereby facilitating bacterial growth by increasing surface area (Heard 1994) and increasing decomposition rates of detritus (Butler et al. 2008, Baiser et al. 2011). Increased bacterial density and smaller detrital particle sizes can increase the growth rate of the mosquito, *W. smithii*, the top-level consumer in the food web (Heard 1994). Whereas it has been proposed that *W. smithii* is a keystone predator that constrains *S. purpurea* microbiome diversity at the local scale (Peterson et al. 2008), results presented here suggest that the shredding midge is more influential in driving taxonomic and phylogenetic diversity of the *S. purpurea* microbiome at the continental scale. While *M. knabi* can facilitate bacterial diversity by providing resources through detrital shredding (Heard 1994, Butler et al. 2008) we did not assess the role of mosquito instar and thus could not directly test the role of the full “processing chain” described by Heard (1994).

The relatively similar conditions that the *S. purpurea* microbiome experiences (e.g., same plant species and food web) across a large spatial scale provide an ideal standardized system to sample the microbiome and explore external sources of variation in composition. Taxonomic composition was mainly driven by spatial factors and climate, indicating that microbiomes that are geographically closer to one another and are more alike in climatic conditions have similar microbial composition. On the other hand, phylogenetic composition was not driven by any of the variables in our study. This suggests that while dispersal limitation and filtering due to climate may influence taxonomic composition, most microbial clades are distributed across the range of climate, plant habitat conditions, and food web composition in our study.

Overall, we show that climatic conditions shape *S. purpurea* microbiome diversity at the continental scale both directly and indirectly through their effect on local variables related to the host pitcher habitat and members of higher trophic levels. In this study, we considered food-web-associated influences on *S. purpurea* bacterial diversity by quantifying the abundance of higher trophic-level organisms. To advance the study of microbial diversity in a food web context, future studies should aim to more closely tie trophic dynamics with microbial diversity through methods such as stable isotope tracing. Altogether, considering the direct and indirect effects of climate, food web structure and microbiome host conditions across broad spatial scales can lead to greater insights into the macroecology of microbes.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3308/supinfo>

DATA AVAILABILITY

Raw sequence reads for the pitcher plant microbiome are available from the National Center for Biotechnology Information (NCBI) under accession no. PRJNA641293.