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Heterogeneous landscape promotes distinct microbial communities in an imperiled scrub ecosystem

Aaron S. David ^a, Damian J. Hernandez ^b, Eric S. Menges ^a, Vivienne L. Sclater^a, Michelle E. Afkhami ^b, and Christopher A. Searcy ^b

^aArchbold Biological Station, 123 Main Drive, Venus, Florida 33960; ^bDepartment of Biology, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146

ABSTRACT

Habitat heterogeneity is a key driver of biodiversity of macroorganisms, yet how heterogeneity structures belowground microbial communities is not well understood. Importantly, belowground microbial communities may respond to any number of abiotic, biotic, and spatial drivers found in heterogeneous environments. Here, we examine potential drivers of prokaryotic and fungal communities in soils across the heterogeneous landscape of the imperiled Florida scrub, a pyrogenic ecosystem where slight differences in elevation lead to large changes in water and nutrient availability and vegetation composition. We employ a comprehensive, large-scale sampling design to characterize the communities of prokaryotes and fungi associated with three habitat types and two soil depths (crust and subterranean) to evaluate (i) differences in microbial communities across these heterogeneous habitats, (ii) the relative roles of abiotic, biotic, and spatial drivers in shaping community structure, and (iii) the distribution of fungal guilds across these habitats. We sequenced soils from 40 complete replicates of habitat × soil depth combinations and sequenced the prokaryotic 16S and fungal internal transcribed spacer (ITS) regions using Illumina MiSeq. Habitat heterogeneity generated distinct communities of soil prokaryotes and fungi. Spatial distance played a role in structuring crust communities, whereas subterranean microbial communities were primarily structured by the shrub community, whose roots they presumably interacted with. This result helps to explain the unexpected transition we observed between arbuscular mycorrhiza-dominated soils at low-elevation habitats to ectomycorrhiza-dominated soils at high-elevation habitats. Our results challenge previous notions of environmental determinism of microbial communities and generate new hypotheses regarding symbiotic relationships across heterogeneous environments.

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mycorrhizae; shrubland;
spatial drivers

INTRODUCTION

Habitat heterogeneity is a key driver of biodiversity of plants and animals (Allouche et al. 2012; MacArthur and MacArthur 1961; Ricklefs 1977; Stein et al. 2014). Heterogeneity is often generated by environmental gradients (e.g., soil fertility or soil moisture) or disturbances (e.g., fire) across a landscape that correspond to variable primary productivity. Such environmental factors, coupled with biotic interactions and species dispersal limitation, lead to distinct plant and animal communities and ultimately increase diversity. For example, a meta-analysis demonstrated that heterogeneity generated by a variety of abiotic, biotic, and spatial sources is positively associated with biodiversity across a wide range of plant and animal communities (Stein et al. 2014). Yet, for belowground microbial communities, we are still learning how taxa are distributed across heterogeneous environments, and

whether the patterns observed for plant and animal communities also apply to communities of prokaryotes and fungi.

Importantly, belowground microbial communities may respond to any number of environmental, biotic, and spatial drivers found in heterogeneous environments (e.g., Nemergut et al. 2013; Philippot et al. 2013; Walters and Martiny 2020). First, they may respond to and assemble based on the same environmental drivers as the plant community. For example, stress related to soil fertility, soil moisture, temperature, and pollutants can have variable effects on microbial composition and diversity (e.g., Collins et al. 2018; O'Brien et al. 2022) and destabilize species networks (de Vries et al. 2018; Hernandez et al. 2021). Second, microbial communities may respond to the plant community themselves, particularly if the microbial taxa are reliant on or prefer to associate with

CONTACT Aaron S. David  adavid@archbold-station.org

Aaron S. David and Damian J. Hernandez are joint first authors.

Michelle E. Afkhami and Christopher A. Searcy are joint senior authors.

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particular plant species (e.g., Bever et al. 2012; van der Putten et al. 2016). For example, plant species often cultivate specific microbial taxa, which can, in turn, favor or disfavor the plant species in the future (i.e., plant-soil feedbacks). Third, dispersal of microbial taxa may result in spatial patterns of (dis)similarity across a landscape (Bever et al. 2012; Eppinga et al. 2022; Tedersoo et al. 2020). In some cases, populations of microbial taxa may function as metapopulations that undergo colonization and extinction in patches of particular habitat types across the heterogeneous landscape. These potential drivers of heterogeneity, when coupled with direct competitive or facilitative interactions among microbial taxa themselves (Goldford et al. 2018; Hernandez et al. 2021; Romdhane et al. 2022), may generate the patterns of diversity found across the landscape. Unraveling the linkages among microbial communities and their potential drivers will improve our understanding of how heterogeneous environments might promote microbial biodiversity.

Here, we examine the potential drivers of prokaryotic and fungal communities in soils across the heterogeneous landscape of the imperiled Florida scrub. This endemic ecosystem contains several pyrogenic habitat types in which slight differences in elevation result in large changes in water and nutrient availability and ultimately vegetation composition (Abrahamson et al. 1984; David et al. 2019; Weekley et al. 2007). Furthermore, the patchy nature of habitats has made it an important model ecosystem for studying the intersection of metapopulation dynamics, population viability, and community ecology in macroorganisms (Menges and Quintana-Ascencio 2004; Menges et al. 2017a; Quintana-Ascencio et al. 2018, 2019). To extend this same detailed understanding to the co-occurring soil microbiome, we employ a comprehensive, large-scale sampling design to characterize the communities of prokaryotes and fungi associated with three distinct habitat types along the elevational gradient—flatwoods, scrubby flatwoods, and rosemary scrub. Next, we evaluate the relative roles of abiotic, biotic, and spatial drivers in shaping community structure across the heterogeneous landscape at two biologically important soil depths. Finally, to extend our understanding of community function, we evaluate the distribution of fungal guilds across these habitats.

MATERIALS AND METHODS

Study system.—The study was conducted at Archbold Biological Station (27°11'N, 81°21'W) located at the southern end of the Lake Wales Ridges in central peninsular Florida, USA (Swain 1998), where elevations range from 36 to 67 m above sea level (Abrahamson

et al. 1984). The Lake Wales Ridge is composed of relict sand dunes that form a present-day ridge in the central region of the state and is home to dozens of endemic plant and animal species. The climate is subtropical and characterized by hot, wet summers and mild, dry winters (Abrahamson et al. 1984). The natural landscape is dominated by a mosaic of various scrub habitats that vary along elevational gradients and by soil type (Abrahamson et al. 1984).

Here, we focused on three habitats that commonly occur along an elevational stress gradient: flatwoods, scrubby flatwoods, and rosemary scrub. All three habitat types are dominated by shrubs of several species, particularly oaks (*Quercus* spp.), heaths (family Ericaceae), and palmettos (*Sabal etonia* and *Serenoa repens*) (Abrahamson et al. 1984). Flatwoods are characterized by their relatively mesic soils and dense shrub cover, whereas scrubby flatwoods and rosemary scrub contain progressively xeric, well-drained soils and decreasing shrub cover. In particular, rosemary scrub, which typically occurs as relatively higher elevation patches only a few meters above that of the adjacent habitats, is home to an exceptionally large number of endemic, endangered herbs.

Data collection.—We characterized prokaryotic and fungal communities in the soil across habitats using environmental sequencing. The full methods have been previously described in Hernandez et al. (2021). Briefly, we collected soil cores from 71 rosemary scrub patches and their neighboring scrubby flatwood and flatwood habitats along their elevational gradients in July 2017 (3 habitats $\times$ 71 replicated gradients = 213 sites). The mean distance between any two sites in the study was 2.8 km (range: 0.08–9.1 km). At each site, soil was collected from two depths—crust (0.0–2.5 cm) and subterranean (33.0–35.5 cm). Therefore, a complete “block” consisted of six collected soils (3 habitats $\times$ 2 soil depths)

Of the collected soils, we successfully sequenced the prokaryotic 16S (V4) and fungal internal transcribed spacer ITS1 regions of samples from 40 blocks corresponding to 120 sites, as well as samples (crust and subterranean) from an additional 20 sites from rosemary scrub habitat only. DNA was extracted and amplified from the soil samples before sequencing on Illumina MiSeq (Illumina, San Diego, California) at the University of Minnesota Genomics Center. Sequence reads were clustered into operational taxonomic units (OTUs) at 97% similarity to Greengenes (DeSantis et al. 2006) or the UNITE database (Nilsson et al. 2019) for prokaryotes and fungi, respectively. Both the 16S and ITS community data sets were each rarefied

to 2000 sequences. Fungal OTUs were further assigned to one of five guilds using FUNGuild (Nguyen et al. 2015) confidence rankings of “Highly probable” or “Probable” using the unrarefied data set. All demultiplexed sequences and metadata are available at the National Center for Biotechnology Information (NCBI; BioProject: PRJNA559142).

We collected additional environmental and biotic data for each sampling point. Two types of environmental data—relative elevation and time-since-fire—were determined by querying Archbold’s land management database (Menges et al. 2017b; Quintana-Ascencio et al. 2018). Relative elevation was calculated as the difference in actual elevation between the sampling point and the shoreline of the nearest wetland and represents the water availability that plants or microbes experience (Quintana-Ascencio et al. 2018). We also recorded the composition of the surrounding shrub community; at each sampling point, we laid a 3-m transect centered at the sampling point and oriented in a random direction and recorded the presence of all shrub species that intersected the transect. Finally, for the rosemary scrub sites, we also collected data on the area of the patch and its isolation from other patches (Quintana-Ascencio et al. 2018).

Analysis.—All analyses were conducted with R 4.1.1 (R Core Team 2021).

Site and vegetation analyses. These analyses were used to test a priori assumptions about the plant communities. First, we checked our assumption that the relative elevation, but not the time-since-fire, would differ across the three habitats using mixed-effects models that included site as a random effect and habitat as a fixed effect. Next, we analyzed whether the shrub community differed across habitats with a permutational analysis of variance using the *adonis2()* function in the *VEGAN* package (Oksanen et al. 2020), including “block” (i.e., set of six habitat–soil depth combinations) as a strata. We conducted a principal coordinates analysis (PCoA) of the shrub community and extracted the first two axes of variation in shrub community composition for use in the analyses of the microbial communities described below. Finally, we tested for differences in shrub species richness using a mixed-effects model as described above.

Communities of prokaryotes and fungi. The first set of analyses of the microbial communities focused on the whole communities of prokaryotes and fungi in the different habitat and soil depths. For both taxonomic groups, we conducted a permutational analysis of variance to test the effects of habitat and soil depth on community composition using a permutation structure

that accounted for the nested sampling of soil depth within habitat within block that was implemented with the *how()* function in the *PERMUTE* package (Simpson 2022) and *adonis2()*. We conducted post hoc, pairwise tests between each habitat–soil depth combination and used a Bonferroni correction to account for the 15 pairwise comparisons. We visualized both prokaryote and fungal communities found across all habitat–soil depth combinations using PCoA. We analyzed differences in within-group beta diversity (i.e., the amount of species turnover within a habitat–soil depth combination) as the mean distance to the centroid in the respective PCoAs among these habitat × soil depth groups using the *betadisper()* function (Oksanen et al. 2020) and further analyzed group differences using Tukey’s honest significant difference tests.

Next, we used distance-based redundancy analysis (db-RDA) to test which factors (soil depth, environmental, biotic, or spatial distance) were primarily responsible for structuring the soil microbial community across the landscape. In these analyses, we did not include habitat as an explanatory variable and instead used elevation and shrub community (see below) as proxies for the respective environmental and biotic components of habitat. The environmental variables included in the analysis were relative elevation and time-since-fire (hereafter, “fire”), as these have been shown to be the primary factors structuring the plant community. These variables were standardized prior to analysis to have a mean of 0 and a standard deviation of 1. Spatial configuration was incorporated into the db-RDAs using principle coordinates of neighborhood matrices (PCNMs), and we initially included all 51 axes with positive eigenvalues in our models. The biotic factors included in the analysis were the first two axes of a PCoA of the shrub community composition (SUPPLEMENTARY FIG. 1) and shrub species richness, as shrubs are the dominant vegetation in these scrub habitats. For each db-RDA (prokaryotic and fungal communities), we first tested a global model for overall significance, then, if significant, proceeded to perform forward selection (Blanchet et al. 2008) with 99 999 permutations. We partitioned explained variance in the final model into sources of variation using the *varpart()* function. We conducted two sets of db-RDAs. First, we analyzed all sites together to quantify the variation explained by all sources of variation—soil depth, elevation, fire, shrub, and spatial distance. Second, we analyzed how communities located in the crust and subterranean depths might be differentially structured by the remaining factors.

Fungal guilds. We selected the six most relevant guilds to our study of soil fungi—saprotrophs, plant pathogens, lichens, arbuscular mycorrhizal (AM)

fungi, ectomycorrhizal (EcM) fungi, and ericoid mycorrhizal (ErM) fungi—and restricted our analyses to taxa that were members of a single guild. Because several guilds had low representation in the sequenced samples, we used the unrarefied number of sequences from each guild as a response variable. Generalized linear mixed models (GLMMTMB package; Brooks et al. 2017) were constructed for the number of sequences from each guild using the fixed effects of habitat, depth, and the habitat $\times$ depth interaction, the random effects of block (i.e., set of six habitat $\times$ depth combinations) and habitat nested within block, an offset of the number of ITS sequences per sample (natural log-transformed), and a negative binomial error distribution. Because we did not detect lichens in the subterranean samples and this prevented the model from running, we analyzed lichen data as the logit-transformed proportion of sequences per sample with the same model structure as above. We evaluated the significance of the model terms using analysis of deviance with the CAR package (Fox and Weisberg 2019) and post hoc, pairwise differences among groups using the Tukey method with the EMMEANS package (Lenth 2021). We also evaluated how the relative abundances of each guild at the family level varied across habitats using multivariate linear models with the MVBUND package (Wang et al. 2012).

Because our results (see below) revealed a transition in the subterranean communities between AM fungus-dominated flatwoods and EcM fungus-dominated scrubby flatwoods and rosemary scrub, we conducted two additional analyses. First, we tested whether there was indeed a negative association between AM and EcM abundances using a Pearson correlation test across all subterranean samples. Second, we investigated whether the ectomycorrhizal status of the shrubs present corroborated our finding of the AM-EcM transition. We determined the presence of likely EcM-associating plant species in the associated shrub data set. Of the shrubs in our data set, we assumed that oaks (*Quercus* spp.) and pines (*Pinus* spp.) were the only genera capable of forming EcM associations (van der Heijden et al. 2015). We used a generalized linear mixed model to analyze the presence of at least one EcM-associated shrub species as a function of habitat type, with “block” as a random effect and a binomial error distribution.

Finally, we focused on rosemary scrub, where the patchy nature of the habitat allowed for further investigation of dispersal-related predictor variables. For each guild within a soil depth, we constructed generalized linear models with the same random effects, error distribution, and offset as above that tested the effects of the following predictor variables: shrub community PC1 and PC2, relative elevation, time-since-fire, patch area,

and patch isolation. We used backward stepwise regression to determine the most important predictors.

RESULTS

Site and vegetation.—Elevation relative to the nearest wetland differed significantly ($\chi^2 = 216.7$, d.f. = 2, $P < 0.001$) among the three habitats—flatwoods ($0.5 \text{ m} \pm 0.1 \text{ m SE}$), scrubby flatwoods ($0.9 \text{ m} \pm 0.08 \text{ SE}$), and rosemary scrub ($1.4 \text{ m} \pm 0.07 \text{ SE}$). Time-since-fire did not differ across habitats ($\chi^2 = 4.2$, d.f. = 2, $P = 0.117$). Shrub communities significantly differed among the three habitats ($R^2 = 0.22$; $P < 0.001$), and pairwise comparisons between shrub communities of each habitat were also significant (all comparisons $P < 0.001$). In a PCoA of the shrub community (SUPPLEMENTARY FIG. 1), the first axis (PC1, variance explained = 10.3%) was correlated with relative elevation (Pearson correlation coefficient = 0.47, $P < 0.001$), but not the second axis (PC2, variance explained = 7.9%; Pearson correlation coefficient = 0.06, $P = 0.507$). Using analysis of variance (ANOVA), habitat explained 58% of the variation in PC1 ($P < 0.001$) but did not significantly explain any of the variation in PC2 ($P = 0.219$), indicating that PC1 approximated the across-habitat shrub community differences, whereas PC2 approximated within-habitat type differences. Shrub species richness did not differ by habitat ($\chi^2 = 4.0$, d.f. = 2, $P = 0.132$) and averaged $4.6 \pm 0.2 \text{ SE}$ species per site.

Communities of prokaryotes and fungi.—

Permutational ANOVA revealed effects of habitat type and soil depth on both prokaryotic and fungal communities (FIG. 1A, B). Prokaryotic communities significantly differed by habitat ($R^2 = 0.05$; $P < 0.001$), soil depth ($R^2 = 0.39$; $P < 0.001$), and the habitat $\times$ soil depth interaction ($R^2 = 0.04$; $P < 0.001$). Post hoc, pairwise comparisons of prokaryotic communities revealed that all combinations of habitat–soil depth differed significantly from one another (Bonferroni-corrected threshold of $P = 0.003$). Similarly, fungal communities differed by habitat ($R^2 = 0.05$; $P < 0.001$), soil depth ($R^2 = 0.17$; $P < 0.001$), and the habitat $\times$ soil depth interaction ($R^2 = 0.05$; $P < 0.001$), with all habitat–soil depth combinations differing significantly from one another ($P \leq 0.003$), except subterranean communities from scrubby flatwoods and rosemary scrub ($P = 0.131$).

Beta diversity (measured as mean distance to the centroid in the PCoA) significantly varied among habitat–soil depth combinations for both prokaryotes ($P < 0.001$; FIG. 1C) and fungi ($P < 0.001$; FIG. 1D).

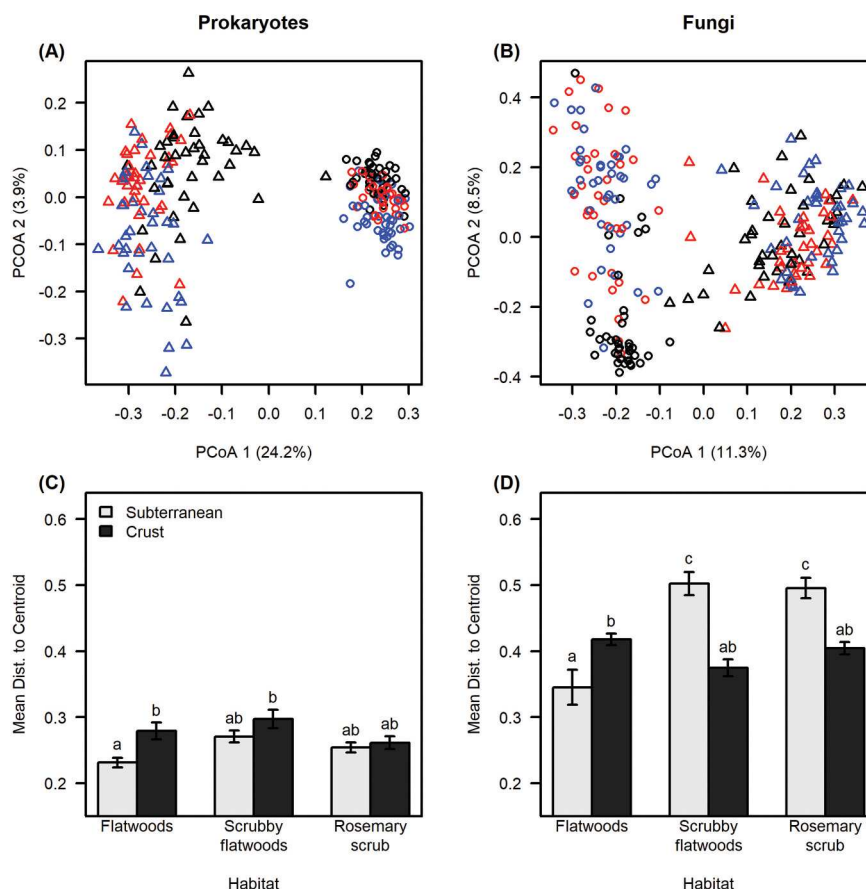


Figure 1. Prokaryote and fungal communities differ across habitats and soil depths. Principal coordinates analysis of (A) prokaryotic and (B) fungal community composition based on operational taxonomic units with 97% sequence similarity. Percent of the variance explained by each PCoA axis shown in parentheses. Colors denote habitats: flatwoods (black), scrubby flatwoods (blue), and rosemary scrub (red). Shapes denote depth: crust (circles) and subterranean (triangles). Beta diversity for (C) prokaryote and (D) fungal communities measured as mean distance to the centroid (± 1 SE). Letters denote significant differences among groups using Tukey's honest significant differences test.

For prokaryotes, beta diversity was significantly higher in the flatwoods and scrubby flatwoods crust compared with the flatwood subterranean communities. For fungi, beta diversity in subterranean samples increased along the stress gradient from flatwoods to rosemary scrub. In flatwoods, fungal beta diversity was higher in the crust than subterranean communities. However, the reverse was found for scrubby flatwoods and rosemary scrub in that beta diversity was higher in subterranean than the crust communities.

Variance partitioning of the db-RDAs across all sites confirmed the importance of soil depth compared with other predictor variables (FIG. 2A). Soil depth explained substantially more variation in both prokaryotic and fungal communities (39% and 17%, respectively) than spatial distance (prokaryotes 2.8%, fungi 0.8%) and shrub community composition (prokaryotes 1.9%, fungi 3.3%). Neither elevation nor time-since-fire was included in the best models for either prokaryotic or fungal communities.

Analyses conducted within soil depths showed a stronger role of spatial distance in shaping crust communities than subterranean communities. Crust communities were best explained by spatial distance (15.1% and 10.3% of variance explained for prokaryotes and fungi, respectively), followed by the biotic factors (prokaryotes 5.5%, fungi 6.1%) and the abiotic factor of elevation (prokaryotes 3.7%, fungi 2.8%) (FIG. 2B). In contrast, the subterranean communities were best explained by biotic factors (prokaryotes 11.8%, fungi 13.4%), followed by spatial distance (prokaryotes 4.7%, fungi 6.6%) and abiotic factors (prokaryotes 6.0%, fungi 4.6%) (FIG. 2C). Abiotic factors were primarily attributable to elevation (prokaryotes 4.4%, fungi 4.6%) rather than fire (prokaryotes 1.6%, fungi 0.0%).

Predictors of fungal guilds.—We analyzed how the abiotic, biotic, and spatial drivers underlied each of six fungal guilds. Overall, crusts contained higher

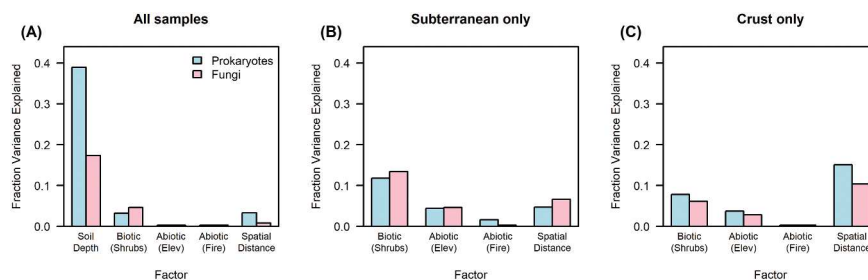


Figure 2. Variance explained for prokaryote and fungal communities in (A) all soil samples, (B) crust samples only, and (C) subterranean samples only. Variance explained was calculated using distance-based redundancy analyses (db-RDAs) that were first fitted using a global model, followed by forward selection (see text for details).

proportions of saprotrophs and plant pathogens than subterranean soils, and all lichens were located in crusts (SUPPLEMENTARY TABLE 1; FIG. 3). Subterranean soil contained higher proportions of AM, EcM, and ErM fungi than crusts. Within the subterranean soils, AM fungi were most abundant in flatwoods, whereas EM fungi were most abundant in scrubby flatwoods and rosemary scrub. Within guilds, we observed significant variation in the relative abundances of common fungal families across habitat types (SUPPLEMENTARY TABLE 2), with notable higher relative abundances of the saprotrophic Trichosphaeriaceae, the plant pathogen Pleosporaceae, and the EcM Russulaceae in higher-elevation rosemary scrub, and higher relative abundances of AM Glomeraceae in the lower-elevation flatwoods. ErM sequences were rarely observed (0.03% of all ITS sequences), and all belonged to the genus *Oidiodendron* (family: Myxotrichaceae).

Further analysis of the association between EcM and AM fungi revealed a strong negative correlation within the subterranean samples (Pearson correlation coefficient = -0.58 , $P < 0.001$). Additionally, habitat types significantly varied in the occurrences of EcM-associating plant genera ($\chi^2 = 8.1$, d.f. = 2, $P = 0.017$). As predicted, flatwoods had fewer plots with at least one EcM-associating plant species ($60\% \pm 8\%$ SE) compared with scrubby flatwoods (100%) and rosemary scrub ($90\% \pm 5\%$ SE).

Within rosemary scrub, the shrub community PC1 (associated with the elevational gradient) was a strong, positive predictor of EcM fungi (crust and subterranean) and plant pathogens (subterranean only), and a negative predictor of crust AM and lichenized fungi (SUPPLEMENTARY TABLE 3). Shrub PC2, which was associated with within-habitat differences, was positively associated with subterranean plant pathogens and ErM fungi and negatively associated with crust lichens.

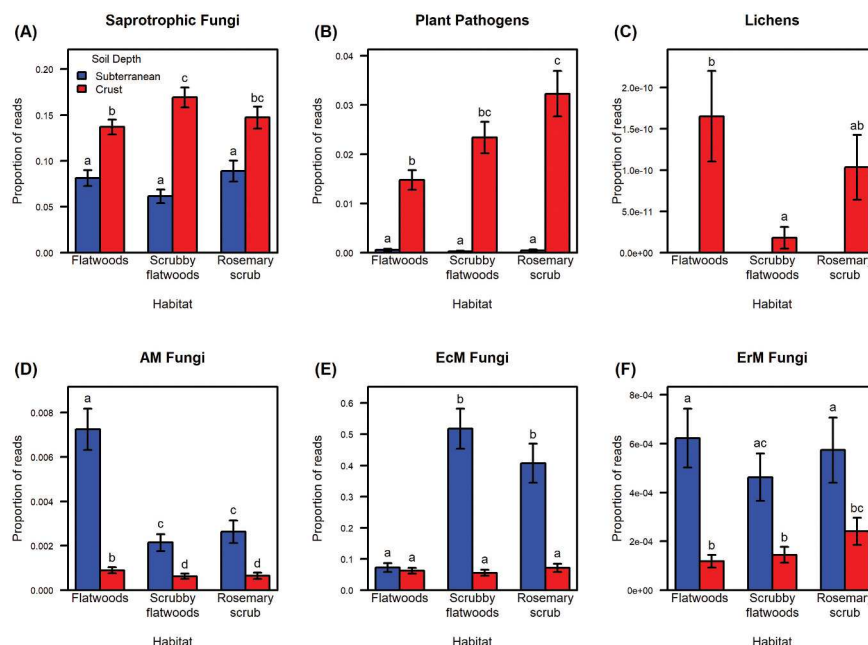


Figure 3. Relative abundances of fungal guilds. (A) Saprotrophic fungi. (B) Plant pathogens. (C) Lichens. (D) Arbuscular mycorrhizal fungi. (E) Ectomycorrhizal fungi. (F) Ericoid mycorrhizal fungi. Letters denote significant pairwise differences using the Tukey method.

Shrub richness was weakly positively associated with both AM and EcM fungi. Elevation was strongly, negatively associated with crust AM fungi (abundance of crust AM fungi was generally low) and weakly associated with several other guilds. Patch area was present in several models and was positively associated with AM fungi, particularly in the crusts. Time-since-fire and patch isolation appeared in several fitted models, but their effects were generally weak.

DISCUSSION

Habitat heterogeneity is known to promote diversity in macroorganisms (e.g., plants and animals) (Stein et al. 2014), but the effects on belowground microbial communities are less clear. Here, we conducted an exceptionally large, replicated study of prokaryote and fungal communities in soils collected across a heterogeneous landscape, and we report three major findings. First, habitat heterogeneity generated distinct communities of soil prokaryotes and fungi across the three habitat types and two soil depths. Second, the role of spatial distance in community composition was limited to crust communities of both prokaryotes and fungi. Third, the shrub community was a better predictor of microbial communities than the abiotic factor of elevation. This last result helps to explain the unexpected transition we observed between arbuscular mycorrhiza-dominated soils at low-elevation habitats (dominated by AM-associating shrubs) to ectomycorrhiza-dominated soils at high-elevation habitats (dominated by EcM-associating oaks and pines). Our results indicate that patterns of microbial communities at a given soil depth across a heterogeneous landscape are better predicted by the surrounding plant community than by the environmental factors thought to structure that plant community. Our findings challenge previous notions of environmental determinism of microbial communities and generate new hypotheses regarding symbiotic relationships across heterogeneous environments.

Habitat heterogeneity promotes distinct microbial communities.—Similar to communities of macroorganisms, communities of microbes are unique to specific habitats, and a heterogeneous landscape of several habitat types is associated with a range of microbial community compositions. In many cases, plant diversity and microbial diversity are coupled, particularly at local scales (Fei et al. 2022). Although our study of prokaryote and fungal communities in the soils of the Florida scrub demonstrated this finding across the three habitat types,

the underlying mechanisms for this finding varied between taxonomic groups.

Soil depth proved to be the most critical component of heterogeneity for both prokaryote and fungal communities. However, within soil depths, subterranean microbes tended to be relatively influenced by the plant community, whereas crust microbes were influenced by spatial distance. Interestingly, the primary driver of plant community heterogeneity, elevation (Abrahamson et al. 1984; Boughton et al. 2006), had only minor effects on both prokaryotic and, especially, fungal communities.

In contrast to the abiotic and biotic drivers, spatial drivers of microbial communities were relatively less pronounced in our study and limited to crusts. One potential reason that we did not find similar results for the subterranean community could be that we conducted this study over a relatively small landscape scale (maximum distance between points = 9.1 km), and spatial processes for subterranean communities could operate at larger spatial scales. Bacterial taxa that occur near the soil surface can utilize wind for aerial dispersal (Elliott et al. 2019), and, if such is the case in the Florida scrub, this dispersal mechanism could explain the difference in explanatory power of spatial distance between crust and subterranean communities. Interestingly, AM fungi in the crusts were positively affected by patch area, which could suggest that larger patches of rosemary scrub are more conducive to the development of AM networks in the crust. Although we report that AM fungi abundance in the crusts was generally low, these AM networks in the crusts can supply nutrients to rosemary scrub herbs (Hawkes and Casper 2002) and may be critical for plant persistence. Our study adds to the growing body of work over the past decades that has demonstrated the role of spatial processes in determining microbial communities (e.g., Bever et al. 2012; Eppinga et al. 2022; Peay et al. 2010).

Importance of mycorrhizal types.—One unexpected finding was the transition between relatively arbuscular mycorrhiza-dominated soils in low-elevation flatwood habitats to ectomycorrhiza-dominated soils in high-elevation scrubby flatwood and rosemary scrub habitats. This finding was attributable to the higher prevalence of EcM-associating shrubs (oaks and pines) in the higher-elevation habitats. The dominant type of mycorrhizal association within a habitat has lasting consequences for both the resident plant community (Tedersoo et al. 2020) and ecosystem processes (Phillips and Fahey 2006).

Importantly, differential mycorrhizal associations across species can lead to various outcomes of

coexistence or competitive dominance (van Nuland et al. 2021). For example, common mycorrhizal networks among species of the same mycorrhizal types can act as equalizing mechanisms that reduce fitness differences, whereas negative plant-soil feedbacks, which are relatively more common for AM-associating plants (Bennett et al. 2017), increase niche differentiation and thus act as stabilizing mechanisms (Tedersoo et al. 2020). In the Florida scrub, the common shrub genera fall into three general mycorrhizal categories: EcM (shrubs in the genera *Quercus*, *Pinus*, and *Carya*), AM (palmettos in the genera *Sabal* and *Serenoa*), and ericoid mycorrhizae (Ericaceae shrubs in the genera *Lyonia*, *Bejaria*, *Vaccinium*, and *Ceratiola*), with the region's dominant vegetation being classified as EcM-associating (Jo et al. 2019). These shrub species coexist with one another in distinct assemblages across the landscape, suggesting that both equalizing and stabilizing mechanisms are likely at play. Our present study suggests that EcM fungi are common in scrub habitats, and we hypothesize that EcMs may play important roles for providing nutrients to the dominant shrubs. Importantly, given the abundance of ericaceous shrubs, it was surprising how few ErM fungi were observed. It is possible that relatively few propagules were in the soil, that the group did not sequence well, or simply that ErM fungi are lacking in the scrub. Going forward, how these three mycorrhizal associations may help shape these assemblages through equalizing and stabilizing mechanisms remains an open question for future research.

Finally, the relatively high abundance of plant pathogens in the crusts of the high-elevation rosemary scrub habitat could contribute to the “specialized” nature of this habitat. Rosemary scrub is home to several endemic, specialist herbaceous species, and relatively few nonendemic species are able to persist there (Abrahamson et al. 1984; David et al. 2020; Menges 2007). The crust community, which consists of cyanobacteria, diatoms, fungi, and algae, can be highly heterogeneous within small (<1 m) spatial scales (Hawkes and Flechtner 2002), and crusts in rosemary scrub are known to benefit several of the endemic plant species by fixing nitrogen (Hawkes 2003) or harboring AM networks (Hawkes and Casper 2002). Fungal pathogens in the crusts could limit plant recruitment via seed germination and thus reduce colonization by non-native plants or native plants that are typically excluded from rosemary scrub.

A limited role for fire in microbial communities.—

Fire is a critical ecosystem process for maintaining plant and animal diversity in the Florida scrub (e.g.,

Fitzpatrick and Bowman 2016; Menges 2007; Quintana-Ascencio et al. 2018). Despite the well-documented effects of fire on microbial communities in other systems (e.g., Enright et al. 2022; Glassman et al. 2016), fire played little to no role in shaping the soil microbial communities in our study. Although experimental research in this system has shown that fire can have an immediate “pulse” effect that alters microbial communities with a subsequent negative feedback on plant germination and seedling biomass (Revillini et al. 2022), there are several explanations for why we did not detect an effect of time-since-fire here. First, fire in the Florida scrub typically penetrates less than 2 cm into the ground (Carrington 2010), suggesting that fire could have directly affected crust samples (samples were collected to a depth of 2.5 cm), but not subterranean samples. Second, any effects of fire would likely be indirectly mediated via changes in the plant community. Because most shrub species in the Florida scrub resprout following fire (Menges and Kohfeldt 1995), such indirect changes belowground might be unlikely. However, since shrubs in this system can take several years to recover following fire (Dole et al. 2023), changes in the landscape (e.g., additional sunlight, reduced aboveground plant biomass) or reduced production of allelopathic chemicals (Revillini et al. 2023) by a dominant shrub (*Ceratiola ericoides*) could, in theory, have had indirect effects on microbial communities.

CONCLUSIONS

In this study, we demonstrated that habitat heterogeneity can generate distinct patterns of prokaryotic and fungal communities in soils. Contrary to predictions, patterns within soil depths are primarily linked not to environmental drivers, but rather to the dominant shrub vegetation and, in some cases, spatial distance. Further research is needed to better understand how plant-microbe interactions, particularly those involved with symbiosis, shape plant assemblages across heterogeneous landscapes.

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ORCID

Aaron S. David  <http://orcid.org/0000-0002-0802-7799>
 Damian J. Hernandez  <http://orcid.org/0000-0001-9833-1155>
 Eric S. Menges  <http://orcid.org/0000-0002-9486-4189>
 Michelle E. Afkhami  <http://orcid.org/0000-0001-7207-6256>
 Christopher A. Searcy  <http://orcid.org/0000-0003-4497-0992>

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