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Habitat specialization and edge effects of soil microbial communities in a fragmented landscape

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Abstract

Soil microorganisms play outsized roles in nutrient cycling, plant health, and climate regulation. Despite their importance, we have a limited understanding of how soil microbes are affected by habitat fragmentation, including their responses to conditions at fragment edges, or "edge effects." To understand the responses of soil communities to edge effects, we analyzed the distributions of soil bacteria, archaea, and fungi in an experimentally fragmented system of open patches embedded within a forest matrix. In addition, we identified taxa that consistently differed among patch, edge, or matrix habitats ("specialists") and taxa that showed no habitat preference ("nonspecialists"). We hypothesized that microbial community turnover would be most pronounced at the edge between habitats. We also hypothesized that specialist fungi would be more likely to be mycorrhizal than nonspecialist fungi because mycorrhizae should be affected more by different plant hosts among habitats, whereas specialist prokaryotes would have smaller genomes (indicating reduced metabolic versatility) and be less likely to be able to sporulate than nonspecialist prokaryotes. Across all replicate sites, the matrix and patch soils harbored distinct microbial communities. However, sites where the contrasts in vegetation and pH between the patch and matrix were most pronounced exhibited larger differences between patch and matrix communities and tended to have edge communities that differed from those in the patch and forest. There were similar numbers of patch and matrix specialists, but very few edge specialist taxa. Acidobacteria and ectomycorrhizae were more likely to be forest specialists, while Chloroflexi, Ascomycota, and Glomeromycota (i.e., arbuscular mycorrhizae) were more likely to be patch specialists. Contrary to our hypotheses, nonspecialist bacteria were not more likely than specialist bacteria to have larger genomes or to be spore-formers. We found partial support for our mycorrhizal hypothesis: arbuscular mycorrhizae, but not ectomycorrhizae, were more likely to be specialists. Overall, our results indicate that soil microbial communities are sensitive to edges, but not all taxa are equally affected, with arbuscular mycorrhizae in particular showing a strong response to habitat edges. In the context of increasing habitat fragmentation worldwide, our results can help inform efforts to maintain the structure and functioning of the soil microbiome.

KEYWORDS
edge effects, habitat fragmentation, landscape ecology, mycorrhizal fungi, soil bacteria, soil fungi, soil microbiome

INTRODUCTION

Habitat fragmentation is an urgent threat to biodiversity worldwide (Haddad et al., 2015; Pimm & Raven, 2000). By creating sharp contrasts in environmental conditions between remnant and matrix ecosystems, habitat fragmentation causes edge effects, that is, changes in biotic and abiotic conditions at the boundary between adjacent habitat types (Ries et al., 2004). Edge effects have been extensively documented in macro-organisms and are of paramount importance to conservation managers seeking to preserve original communities and ecosystem functioning in limited amounts of remnant habitat (Murcia, 1995; Resasco et al., 2017; Ries et al., 2004). However, most research to date on edge effects has focused exclusively on aboveground plants and animals. We know far less about how soil microorganisms, including bacteria, archaea, and fungi, respond to habitat fragmentation. Soil microbes mediate important ecosystem services, such as carbon storage, nutrient cycling, and soil fertility (Fierer, 2017) and can influence plant health and plant community structure (e.g., Bennett et al., 2017). However, due to their small size, large populations, varied dispersal capacities, and diverse strategies to cope with unfavorable environmental conditions, microbial distributions may be governed by processes operating at different scales than those of larger organisms.

As with plant and animal communities, we would also expect habitat edges to affect the distributions and abundances of soil microorganisms for several reasons. First, environmental conditions such as moisture, temperature, light, and resource availability change at habitat boundaries (Ries et al., 2004, 2017), and these differential environmental conditions between adjoining habitat types are likely to select for certain microbial taxa or lineages (Crockatt, 2012; Lauber et al., 2009; Nordén et al., 2013). For example, Tatsumi et al. (2023) found that nitrifying bacteria increased in soils near the urban forest edge in tandem with increased nitrification rates at these edges. Second, differences in plant and animal communities across habitats should lead to differences in the distributions of their associated microbial communities across habitats (Mony et al., 2022). Most notably, with changes in plant community composition, we would expect corresponding changes in soil bacterial and fungal communities given that some microbial taxa, including plant symbionts, can exhibit a high degree of host specificity.

Not all soil microbial taxa are expected to share similar responses to changes in biotic and abiotic conditions associated with habitat fragmentation. For example, some bacteria and fungi are relatively insensitive to changes in soil pH (Kawahara et al., 2016; Lauber et al., 2009) or can associate with a broad range of different plant taxa (Davison et al., 2015; Semchenko et al., 2022; Vieira et al., 2020). Likewise, microbes can exist in a dormant or near-dormant state in soil and may persist in soil even if the environmental conditions are not ideal for their growth (Blagodatskaya & Kuzyakov, 2013; Lennon & Jones, 2011). Microbes also differ in their dispersal capacities and their ability to move from one habitat type to another. For example, a recent study comparing the fungal communities in harvest gaps and adjoining intact forest found evidence that wind-dispersed fungi, including many wood saprotrophs, are more limited in their dispersal capacity than many mycorrhizal taxa that rely on small mammals for dispersal (Borgmann-Winter et al., 2023). In short, some taxa will be more sensitive to environmental changes along habitat edges than others. Finally, we do not expect the transition between microbial community types to occur exactly at the fragment edge as there is likely "spillover" or "mass effects" that lead to some taxa moving across habitat boundaries more readily than others (e.g., Bell & Tylianakis, 2016; Ries & Sisk, 2004). Habitat boundaries evident from changes in vegetation structure may not necessarily equate with observed changes in the composition of belowground microbial communities.

The impacts of habitat fragmentation on soil microbes and the spatial patterns in community changes across habitat boundaries will likely differ depending on the microbial taxa in question. We expect mycorrhizal fungi to be more sensitive to habitat differences than non-mycorrhizal fungi given that many mycorrhizal fungi exhibit some degree of specificity for particular host plants (Semchenko et al., 2022; Tatsumi et al., 2023; van Der Heijden et al., 2015). For prokaryotes (bacteria and archaea), we would expect that taxa with larger genomes should be more ubiquitous across habitat types because the increase in metabolic versatility associated with larger genomes should provide these taxa with the flexibility to withstand differing environmental conditions (Barberán et al., 2014; Bell & Tylianakis, 2016; Bentkowski et al., 2015; Cobo-Simón & Tamames, 2017; Konstantinidis & Tiedje, 2004). Likewise, bacteria that form spores should exhibit an attenuated response to habitat fragmentation

because they can persist under conditions that may not match their particular environmental preferences (Blagodatskaya & Kuzyakov, 2013; Lennon & Jones, 2011) and because spore-forming bacteria may be more likely to be dispersed across distinct habitat types via aerial transport (Aalismail et al., 2019). Determining the traits that help predict soil microbial responses to fragmentation-driven environmental change is a prerequisite for designing evidence-based conservation strategies to preserve the ecosystem services they provide.

With this study, we investigated how and why the distributions of soil prokaryotes and fungi varied across two adjacent habitat types in experimentally created habitat fragments. Specifically, we collected soils across six replicate 1.375-ha, rectangular open patches surrounded by a forested matrix. In each patch, we sampled along four 100-m transects spanning the patch/matrix boundary, which allowed us to quantify spatial turnover in belowground communities across the open patch, the forested matrix, and the edge habitat between these two habitat types. We then used marker gene sequencing to characterize the prokaryotic and fungal communities in these 240 soil samples, using indicator species analyses to identify taxa that specialized in either the patch, the forest matrix, or the boundary (edge) between these two habitat types. To complement the taxonomic analyses, we also compared the distributions of inferred traits, including mycorrhizal status, genome size, and sporulation ability, between soil microbes identified as being habitat specialists or generalists.

We used the collected samples and corresponding data on the soil microbial communities to ask three questions. First, what is the spatial organization of soil microbial communities across the boundary between open patches and the surrounding forest matrix? Second, which particular soil microbial taxa are more sensitive to habitat fragmentation and edge effects? Third, what microbial traits are the most important predictors of habitat specialization in fragmented landscapes? We hypothesized that the open patch and the forested matrix would be distinct, but that the rate of community turnover would be most pronounced near the edge where these two habitats meet, indicating an edge effect. We also expected that taxa that were not sensitive to the edge, that is, those that did not consistently prefer patch, edge, or matrix habitat, would have distinct life history traits from habitat specialists. Namely, we hypothesized that these nonspecialists would be less likely to be mycorrhizal, given the close ties between many mycorrhizal fungi and specific types of plants. We also expected that nonspecialist taxa would have larger genomes and be more likely to sporulate, because these traits should confer broader environmental tolerances, allowing these taxa to persist in the different conditions characterizing the two different habitat types considered here.

METHODS

Soil sampling and environmental data collection

For this study, we leveraged the long-term, landscape-scale experimental design at the Savannah River Site (SRS) Corridor Project near New Ellenton, South Carolina, USA. The SRS is a 1240-km^2 Department of Energy (DOE) National Environmental Research Park. The sandy upland sites where we sampled have been managed as loblolly (*Pinus taeda*) and longleaf (*Pinus palustris*) pine plantations, which contain mixed hardwoods, by the US Department of Agriculture-Forest Service since the founding of the SRS in 1951 (Kilgo & Blake, 2005). In 1999, 2000, and 2007, researchers cleared open patches for the SRS Corridor Project (see Tewksbury et al., 2002 for additional details on the experimental design). Briefly, the Corridor Project design consists of seven experimental units (EUs) which are randomly oriented on the landscape. Each EU consists of open patches separated by a forested matrix. Importantly, in this study, we considered only six of the 137.5 m × 100 m rectangular-shaped patches. Each one of our six rectangular patches is in a separate EU. The open patches are undergoing restoration to longleaf pine savanna, the historical ecosystem of uplands at the SRS which was characterized by low density longleaf pines and a high diversity of native grasses and forbs (see Damschen et al., 2019a, 2019b for details on the plant communities in the open patches). See Figure 1 for a map showing the locations of the six EUs sampled across the SRS.

In May 2021, we established four 100-m transects spanning the boundary between open patch and forested matrix in each of the six replicate rectangular patches (Figure 1). We placed our transects such that the 50-m point corresponded to where the boundary between the open patch and forested matrix was created (Tewksbury et al., 2002). To explore possible edge effects and because the exact patch/matrix boundary would be difficult to precisely delineate on the ground, we considered the edge in this study to be from meters 40 to 60. Meters 10–30 of each transect were in the open patch and meters 70–100 were in the forested matrix. Since earlier research has established the importance of edge orientation in driving edge effects (Ries et al., 2017), we established four perpendicular transects per patch to better control for aspect in our sampling design (Figure 1). At each 10-m point along each transect, we used a 2-m^2 quadrat and collected eight

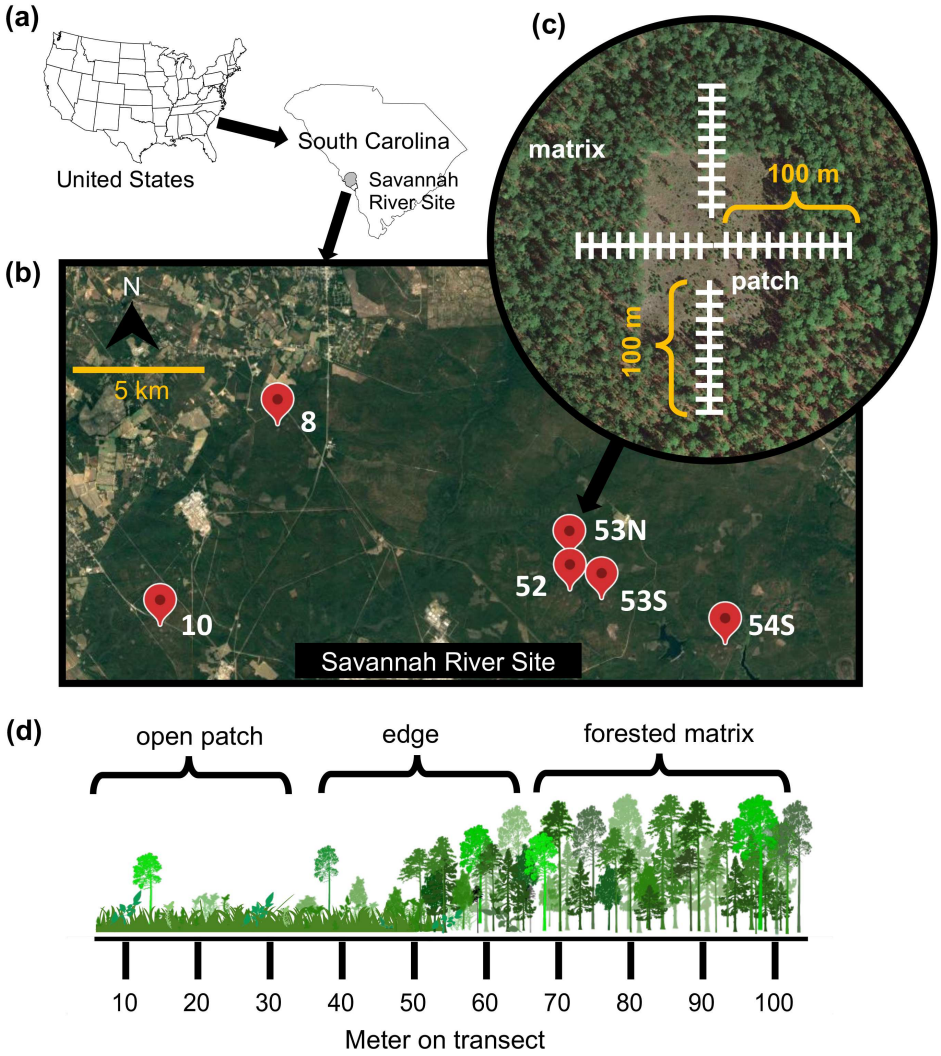


FIGURE 1 Location of the Savannah River Site (SRS) in South Carolina, USA (a), an aerial view of the SRS with the 6 replicate experimental units (EUs) shown as red placemarks (b), an aerial view of one EU with the study's transect design imposed on top (c), and an illustration of transect sampling design divided into open patch, edge, and forested matrix habitats (d). Meters 10–30 were in the patch, meters 40–60 were classified as edge habitat, and meters 70–100 were in the matrix. Aerial photographs sourced from Google Earth. Plant silhouettes are adapted from images obtained from phylopic.org and include images in the public domain or available to share non-commercially with credit. *Pinus taeda* is by Michele M. Tobias, adapted from an image by Dcrjsr (CC BY 3.0) via Wikipedia at https://commons.wikimedia.org/w/index.php?curid=18059686 under a Attribution-NonCommercial-ShareAlike 3.0 Unported license. Public domain images include the following: *Solidago missouriensis* and *Betula pendula* uploaded by G. Dera; *Pinus sect. Trifoliae* by G. Midolo; *Vaccinium erythrocarpum* uploaded by T. M. Keesey; *Pinus palustris* by M. McNair. Plant images are for illustrative purposes only and not all species shown are present at the SRS.

soil subsamples (0–5 cm deep, mineral soil only). We chose to sample to 5 cm, a common depth for sampling surface soils, because surface soils tend to harbor the greatest microbial biomass and richness (Baldrian et al., 2012; Blume et al., 2002; Fierer et al., 2003). Furthermore, we focused on surface soil taxa because they are better represented than taxa from lower soil depths in publicly available genome databases (Brewer et al., 2019), which we drew on for our trait-based analyses. We combined the 8 soil subsamples, sieved to 2.0 mm, and homogenized the 8 soil subsamples from each 10-m point along each transect, resulting in 10 samples per transect, 40 samples from each of the 6 replicate EUs, and a total of 240 soil samples. We then stored about half of each soil sample at −20°C until DNA extraction. The remaining portion of each soil sample was used for the measurement of soil edaphic properties, as described below.

At each of the 10 sampling points per transect, we measured percent ground vegetation cover and canopy

cover. To estimate percent ground vegetation cover, we took pictures of each quadrat from 1.5 m above the soil surface. We later superimposed a 10 × 10 grid on top of the ground vegetation cover photographs and counted the number of boxes in the grid that were covered with vegetation. We used a convex spherical densiometer to measure canopy cover at each of the 240 sampling points. For pH measurements, we created soil slurries consisting of 1 g of soil and 10-mL deionized water, vortexed the slurries at maximum speed for 20 s, and then let them equilibrate for 1 h, obtaining 2–3 replicate measurements per sample. Finally, to determine how basic soil properties differ between the more interior portions of each patch versus the end of the transect in the forested matrix, we sent a portion of each of the 48 soil samples collected from the 10 and 100 m positions on each transect to the Soil, Water and Plant Testing Laboratory at Colorado State University. Specifically, we obtained measurements of soil texture, percent organic matter, and the concentrations of various soil nutrients (NO_3-N, P, and K), following standard protocols (Sparks et al., 2020).

DNA extraction and marker gene sequencing

To extract DNA from our 240 soil samples, we created soil slurries consisting of 1 g of soil and 2-mL DNA-free water. After vortexing these mixtures for 10 s, we added 400 μL of each slurry to a well in the DNeasy PowerSoil HTP 96 Kit (Qiagen, Germantown, MD). We followed the manufacturer's standard protocol, but added a 5 min room temperature incubation before the final elution step. To test for any potential contamination introduced during DNA extractions, we prepared a total of 22 extraction blanks (400-μL DNA-free water) across the three 96-well extraction plates. The extracted DNA was stored at −20°C until the amplicon libraries were prepared.

We targeted the V4 region of the 16S ribosomal RNA (rRNA) gene using universal primers 515 forward (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806 reverse (5′-GGACTACHVGGGTWTCTAAT-3′) to characterize the bacterial and archaeal communities in our soils (Walters et al., 2016). To determine the fungal communities, we amplified the internal transcribed spacer of the fungal rRNA operon using primers ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2-R (5′-GCTGCGTTCTTCATCGATGC-3′) (Bellemain et al., 2010). Both the 16S and ITS rRNA primer sets included 12-bp barcodes and Illumina adapters to enable multiplexed sequencing (Caporaso et al., 2012). We performed all polymerase chain reactions (PCRs) in duplicate, pooling the amplicons from the duplicate reactions and sequencing a total of 240 soil samples, 22 extraction blanks, and 3 no-template controls. Each 25-μL reaction consisted of 12.5-μL Platinum II Hot-Start Master Mix (Invitrogen, Waltham, MA, USA), 10.5-μL PCR grade water, 1 μL of both forward and reverse primer, and 1-μL template DNA. Our thermocycler conditions for both the prokaryotic and fungal-targeting PCRs were 94°C for 2 min followed by 35 cycles of 94°C for 15 s, 60°C for 15 s, and 68°C for 1 min. The final elongation step was performed at 72°C for 10 min. We pooled, cleaned, and normalized amplicons using SequelPrep Normalization Plate Kits (Applied Biosystems, Waltham, MA, USA). Finally, we sequenced the 16S and ITS rRNA gene libraries separately on an Illumina MiSeq, using the 2 × 150 bp paired-end sequencing chemistry for 16S rRNA gene sequencing and the 2 × 250 bp paired-end sequencing chemistry for the ITS sequencing.

Bioinformatics

We performed all bioinformatics and statistical analyses separately for prokaryote (16S) and fungal (ITS) datasets in the R statistical language (version 4.3.0, R Core Team, 2023). Visualizations were created using the R package ggplot2 (version 3.4.4, Wickham, 2016). For demultiplexing reads and primer trimming, we used the programs idemp (https://github.com/yhwu/idemp) and cutadapt (version 1.8.1, Martin, 2011) with their default parameters. We followed the DADA2 bioinformatics pipeline (Callahan et al., 2016, dada2 R package version 1.14.1) and the associated tutorial (https://benjjneb.github.io/dada2/) for quality filtering, denoising, and the dereplicating and merging of paired-end reads, using default parameters unless otherwise noted. For 16S rRNA gene data, we truncated the forward reads at position 150 and the reverse reads at position 140. For our ITS data, we removed reads that were less than 50 bases long. When performing sample inference using the "dada" function, we set pool = TRUE in both ITS and 16S pipelines.

We assigned taxonomy to the amplicon sequence variants (ASVs, clustered at 100% sequence similarity) by training a naïve Bayesian classifier algorithm (Wang et al., 2007) on the SILVA reference database version 132 for prokaryotes (Quast et al., 2013; Yilmaz et al., 2014) and on the UNITE reference database version 8.3 for our fungal reads (Abarenkov et al., 2010; Nilsson et al., 2019). To do so, we implemented the "assignTaxonomy" function from the dada2 package, using all default parameters with the exception of setting "tryRC = TRUE." Next, we used the phyloseq package (version 1.44.0, McMurdie & Holmes, 2013) to combine sample environmental metadata, ASV tables, and the

taxonomy tables into separate prokaryotic and fungal phyloseq objects for use in downstream analyses. We removed all chloroplast and mitochondrial ASVs from the 16S dataset, as well as any ASVs not assigned to at least the phylum level of resolution from both the 16S and ITS datasets. To account for an uneven number of reads across samples, we rarefied to a depth of 14,973 reads and 9,717 reads per sample for prokaryotic and fungal analyses, respectively. As rare taxa were not the focus of this study, we removed ASVs represented by fewer than 50 reads in total across all samples as well as those ASVs that were not detected in at least 40 samples.

Statistical analyses

To test if patch, edge, and matrix samples differed in bacterial/archaeal and fungal community composition across all samples and within EUs, we performed permutational multivariate analyses of variance (PERMANOVAs) on Bray–Curtis dissimilarity matrices (Anderson, 2001; McArdle & Anderson, 2001) using the function "adonis2" with 9999 permutations (from the vegan R package, version 2.6.4, Oksanen et al., 2022) and then applied Bonferroni corrections. To account for the influence of EU in our PERMANOVAs conducted across all samples, we included EU as the first term in the model and used sequential sums of squares so that as much variation as possible was accounted for by EU before considering habitat type, following the suggestions in Bakker (2024a). Importantly, we set the permutations to only occur within the EU blocks using the "how" function within the permute package version 0.9-7 (Simpson, 2022), as in Bakker (2024b). To achieve the balanced dataset required for restricting permutations in our across-EU PERMANOVAs, we randomly sampled 10 and 11 samples, respectively, from the fungal and bacterial/archaeal datasets from each combination of EU and habitat type (10 or 11 samples was the minimum number of samples in an EU and habitat type combination). We created NMDS plots to visualize differences among habitat types in our "across-EU" models. In these plots, we drew ellipses that show one SD around each habitat type's spatial centroid. Ellipses were plotted with the function "gg_ordiplot" (R package ggordiplots version 0.4.3, Quensen et al., 2024), which employs the vegan function "ordiellipse" to calculate spatial centroids and their SDs.

To assess our prediction that the rate of change in community composition would be greatest across the edge, we examined turnover across the transect by EU. More specifically, for each EU, we derived Bray–Curtis dissimilarity values from the median abundance of each ASV (out of the four transects per EU) at each 10-m point along the transect. We examined where rates of turnover were greatest by visualizing the turnover for each meter sampling point across the transect relative to the 10-m and the 100-m points, which are the points farthest away from the edge in the patch and matrix, respectively.

Next, for prokaryotic and fungal communities separately, we created distance-based redundancy analysis models (db-RDAs, Legendre & Anderson, 1999) to identify the factors driving sample differences among samples across all EUs, using the same smaller, balanced dataset used in the "across-EU" PERMANOVAs described above. The explanatory variables we considered for all models were habitat type (open patch, edge, or forested matrix), pH, ground vegetation cover, canopy cover, and meter along the transect, while the response variable was a matrix of Bray–Curtis dissimilarities among samples. We also conditioned on the variable EU to remove its effect before testing the other variables. To test the significance of the db-RDA models' constraints and terms, we used permutational tests (vegan function "anova.cca" with 9999 permutations, setting model = "reduced" and by = "margin"), restricting permutations to within EU as described above. We then performed forward, stepwise model selection with the vegan function "ordiR2step" on each model to see if we could improve it, using the same permutational scheme described above. Based on the result of model selection, we then revised the db-RDAs to maximize the adjusted R^2.

To identify ASVs that differed in abundance among the open patch, the edge, and the forested matrix habitats, that is, those taxa identified as being "habitat specialists," we performed indicator species analyses (De Cáceres & Legendre, 2009). Specifically, we used the function "multipatt" in the R package indicspecies (version 1.7.13, De Cáceres & Legendre, 2009) to estimate group-equalized, point-biserial correlation coefficients, r_g, between each ASV and the different habitat types, testing the significance of these correlations by performing 9999 permutations.

Traits of specialist and nonspecialist microbes

To explore why microbes may vary in their habitat specificities (open patch, edge, forested matrix, or no discernible preference for habitat type), we searched publicly available databases for information on the ecological attributes of our ASVs. To estimate bacterial and archaeal genome sizes, we used the program vsearch (version 2.15.2, Rognes et al., 2016) to match the 16S rRNA representative gene sequences of our ASVs to genomes in the Genome Taxonomy Database (GTDB, release 207; Parks et al., 2018, 2020, 2022; Rinke et al., 2021). We only

considered genomes that were at least 90% complete and allowed up to two mismatches with our 16S rRNA gene reads (>99% sequence similarity). If one of our ASVs matched equally to multiple GTDB entries, we calculated the mean genome size of the matches. In such cases, we retained the ASV in our analyses only if this mean calculated genome size was less than 10% of the range between the smallest and the largest matched genomes. To obtain information on the possible spore-forming capabilities of our taxa, we matched the NCBI Taxon IDs of our taxa (obtained from GTDB as described above) to taxa in the curated bacterial and archaeal trait databases in Madin et al. (2020). Since we were only able to match about 3% of our ASVs to the approximately 17,000 taxa in the Madin et al. (2020) databases for which spore formation information was available, we inferred sporulation ability at the family level. Specifically, we categorized each family for which sporulation data were available as either spore-formers (all taxa within family are known to form spores), "potential" spore-formers (some, but not all members form spores), or non-spore-formers (all tested members of family cannot form spores). We then classified the possible spore-forming ability of each ASV in our dataset based on the sporulation ability of the assigned family. To determine which of our fungal ASVs were likely ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM), we matched our fungal ASVs to those in FUNGuild (version 1.1, Nguyen et al., 2016). We considered only ecological guild classifications that were "highly probable" or "probable."

To test differences in genome size between "specialist" ASVs (i.e., those identified as indicators of open patch, edge, or forested matrix habitats) and "nonspecialist" ASVs (those that did not exhibit preferences for particular habitat types), we performed one-way ANOVAs. We performed three chi-squared tests for equality of proportions to test if specialist and nonspecialist ASVs differed in their proportions of non-sporulating taxa or in their classifications as AM or ECM fungi relative to non-mycorrhizal fungi (i.e., all other non-mycorrhizal guilds returned from FUNGuild).

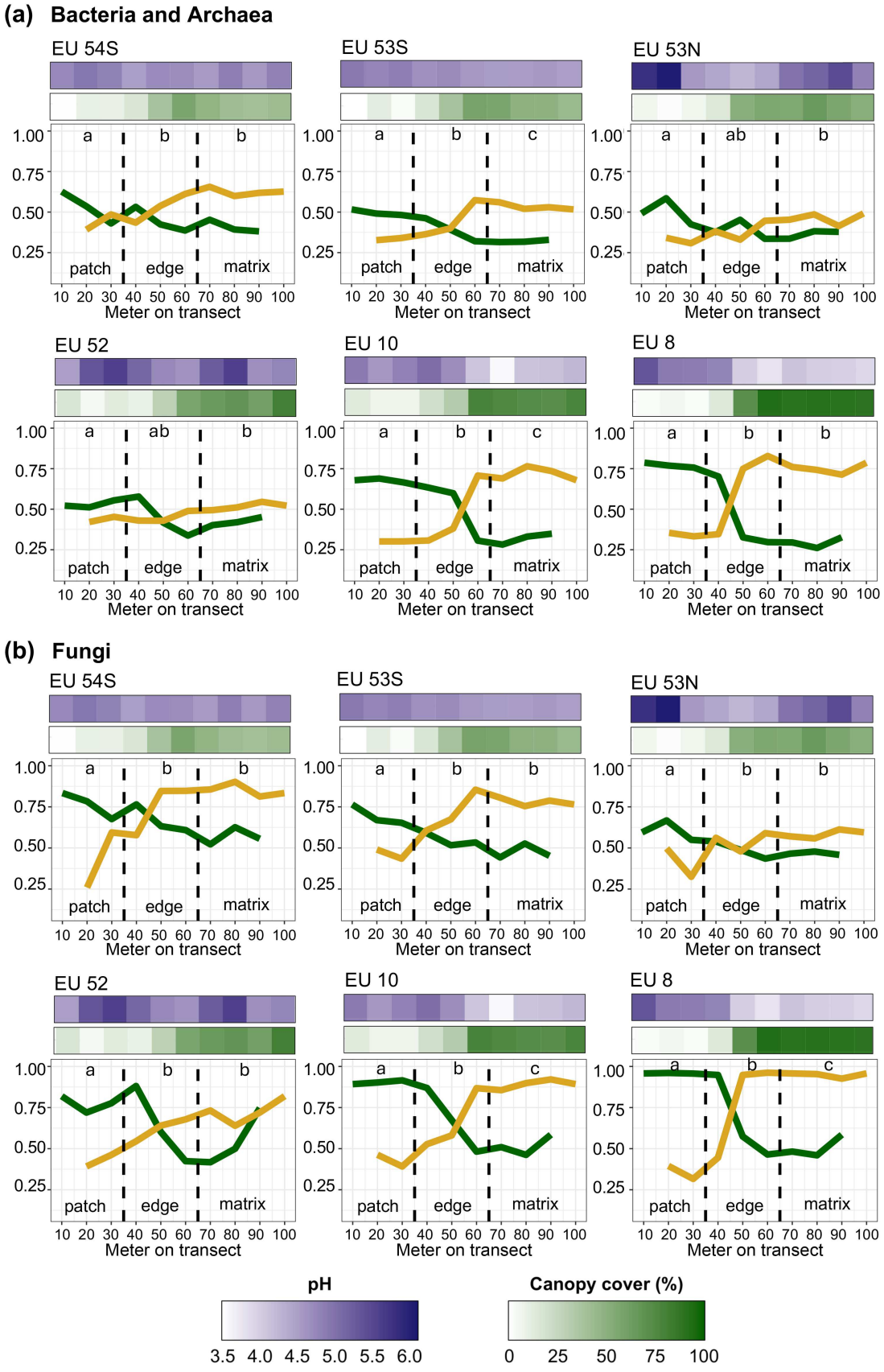
RESULTS AND DISCUSSION

Overview of environmental conditions

The forested matrix and the open patch habitats were distinct in most measured environmental characteristics, but the magnitude of these differences varied across the six replicate EUs sampled for this study (Figure 2; Appendix S1: Figure S1, Table S1). Based on our analyses of soils collected at the transect endpoints, the soils in the forested matrix and the patch did not differ in the concentrations of extractable organic matter, NO_3-N, P, or K (t test on transect median values, paired by EU: $p > 0.05$ in all cases, Appendix S1: Table S1). Percent ground vegetation cover was generally higher in the open patch (mean = 69.4%) than in the forested matrix (mean = 48.8%), while canopy cover was higher in the matrix (canopy cover: patch mean = 6.5%, matrix mean = 64.0%, Figure 2. Also see Appendix S1: Figure S1). Importantly, however, EUs differed in the degree of contrast in canopy cover and ground vegetation cover between the matrix and the patch (Figure 2, Appendix S1: Figure S1). One reason for this may be that the encroachment of shrubs and small hardwood trees into the pine-dominated matrix varied across EUs. The soils collected from the forested matrix tended to be more acidic than those from the patch, but the patterns in soil pH across the 100-m transects were often inconsistent (Appendix S1: Figure S1). This variation in soil pH may be a product of historical land-use patterns, as the SRS was once a mosaic of agricultural plots and remnant forest prior to the 1951 establishment of the pine plantation (Brudvig et al., 2013; Kilgo & Blake, 2005; Turley et al., 2020).

Community-level responses to habitat type and environmental heterogeneity

Nearly all of the collected soils were dominated by the bacterial phyla Acidobacteria, Verrucomicrobia, and Proteobacteria, with the dominant fungal phyla consisting of Basidiomycota, Ascomycota, and Mortierellomycota (Appendix S1: Figures S2 and S3). Although relatively rare, archaea (predominantly members of Thaumarchaeota), were also detected in these soils (Appendix S1: Figure S2). Despite similar dominant phyla, the overall composition of soil fungal and prokaryotic communities differed depending on whether they were collected in the open patch versus the forested matrix regardless of whether analyses were conducted across all EUs combined or within individual EUs (Figures 2 and 3, $p < 0.01$ in all PERMANOVAs, Appendix S1: Table S2). In contrast, although the "edge" habitat (the boundary between the forested matrix and patch, between 40 and 60 m on our transects, Figure 1) differed compositionally from patch and matrix communities on average across all EUs (Figure 3, $p < 0.001$ in all PERMANOVAs), within individual EUs this was not always the case (Appendix S1: Table S2). This variability was likely due to environmental differences across EUs, which we discuss below. Finally, the differences in community composition between the patch and the other two habitat types were larger for the fungal communities than for the prokaryotic communities



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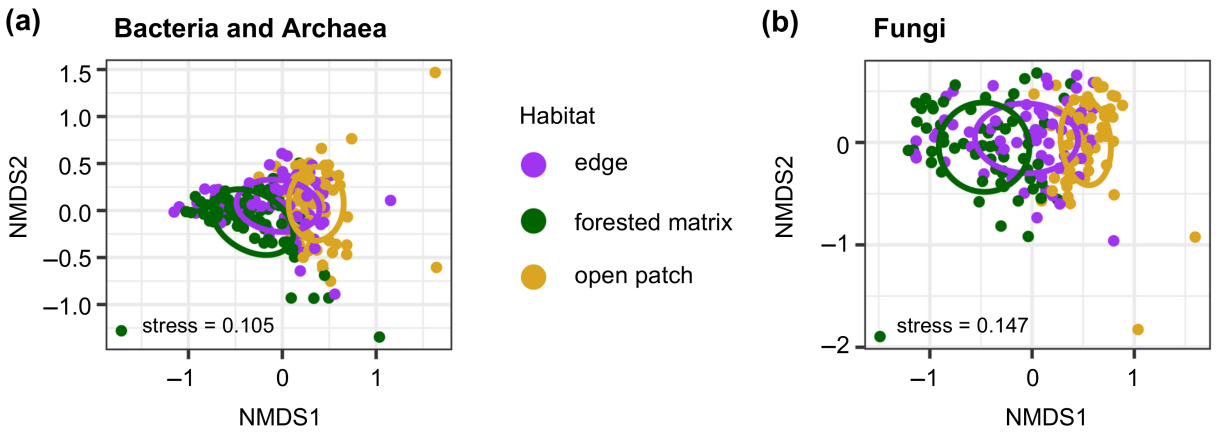

FIGURE 3 Nonmetric multidimensional scaling ordinations (NMDS) based on Bray–Curtis dissimilarities among samples for bacteria and archaea (prokaryotes, a) and fungi (b). Ellipses are colored by habitat type and show one SD around each habitat type's spatial centroid. Soil prokaryotic and fungal communities are distinct among habitat types (open patch, forested matrix, and edge) based on PERMANOVAs with Bonferroni post hoc corrections ($p < 0.001$ for all comparisons for both prokaryotes and fungi, see text and Appendix S1: Table S2 for more details).

(see sum of squares in the across-EU models above, Appendix S1: Table S2). This pattern may be a product of fungal communities being more sensitive to changes in vegetation type than prokaryotic communities (e.g., Chen et al., 2022; Urbanová et al., 2015).

We note that the observed differences in prokaryotic and fungal communities between habitat types are likely driven by a myriad of biotic and abiotic factors that differ among these habitats, including plant community composition, soil carbon availability, moisture availability, temperature, and other soil edaphic variables (e.g., Fierer, 2017; Tedersoo et al., 2014). Of those factors we measured directly across each of the sampled 100-m transects, the observed differences in fungal and bacterial/archaeal community composition were most strongly associated with canopy cover and soil pH, respectively. Specifically, after performing model selection on the initial db-RDAs (Appendix S1: Tables S3 and S4), canopy cover emerged as the best variable explaining fungal community composition (10.9% of variation explained in final db-RDA; permutational ANOVA: $F(1, 173) = 25.50$, $p < 0.001$), whereas differences among bacterial/archaeal communities were best explained by only pH (7.3% of variation explained in final db-RDA; permutational ANOVA: $F(1, 191) = 18.44$, $p < 0.001$). Notably, meter along the transect was not a significant predictor for prokaryote or fungal community dissimilarity, likely because of its strong correlation with canopy cover (Pearson correlation coefficient = 0.80), as well as variation in environmental conditions at the same meter among transects. Our results agree with other studies reporting that pH is often a primary factor associated with differences in soil bacterial community composition at local to global scales (Fierer et al., 2007; Fierer & Jackson, 2006; Nielsen et al., 2010). Likewise, our observation that fungal community composition was most strongly associated with differences in canopy cover (and to a lesser degree, ground vegetation cover, see Appendix S1: Tables S3 and S4) is consistent with previous work showing that the biogeographical patterns in fungal community composition are often closely associated with vegetation type (Tedersoo et al., 2014).

Across all EUs, the rate of microbial community turnover was greatest across the edge (Figure 2), supporting our hypothesis of edge effects in soil microbial communities.

FIGURE 2 Changes in the overall composition of the prokaryotic (a) and fungal communities (b), measured using Bray–Curtis dissimilarity values, across the transects located in each experimental unit (EU). Bray–Curtis dissimilarity values were derived from the median abundance (out of the four transects per EU) of each amplicon sequence variant at each location along the transect within an EU. Green lines show the Bray–Curtis dissimilarity value of each point along the transect meter compared with 100 m, which is the sampling point furthest into the forested matrix. Yellow lines represent the Bray–Curtis dissimilarity value of each point along the transect meter compared with 10 m, which is the sampling point farthest away from the edge in the open patch. Letters denote which habitat types (patch, edge, and matrix) within each EU were significantly different ($p < 0.05$) based on PERMANOVAs with Bonferroni post hoc corrections. Above each of the plots, the purple and green color scale bars respectively show the mean pH value and the mean canopy cover value for each 10-m point along the transect.

However, EUs varied in the strength of their edge effect responses. The greater an EU's contrast in vegetation structure between the open patch and the forested matrix, the more pronounced the turnover across the edge, the greater the magnitude of community turnover across the transect, and the greater the likelihood that edge communities would

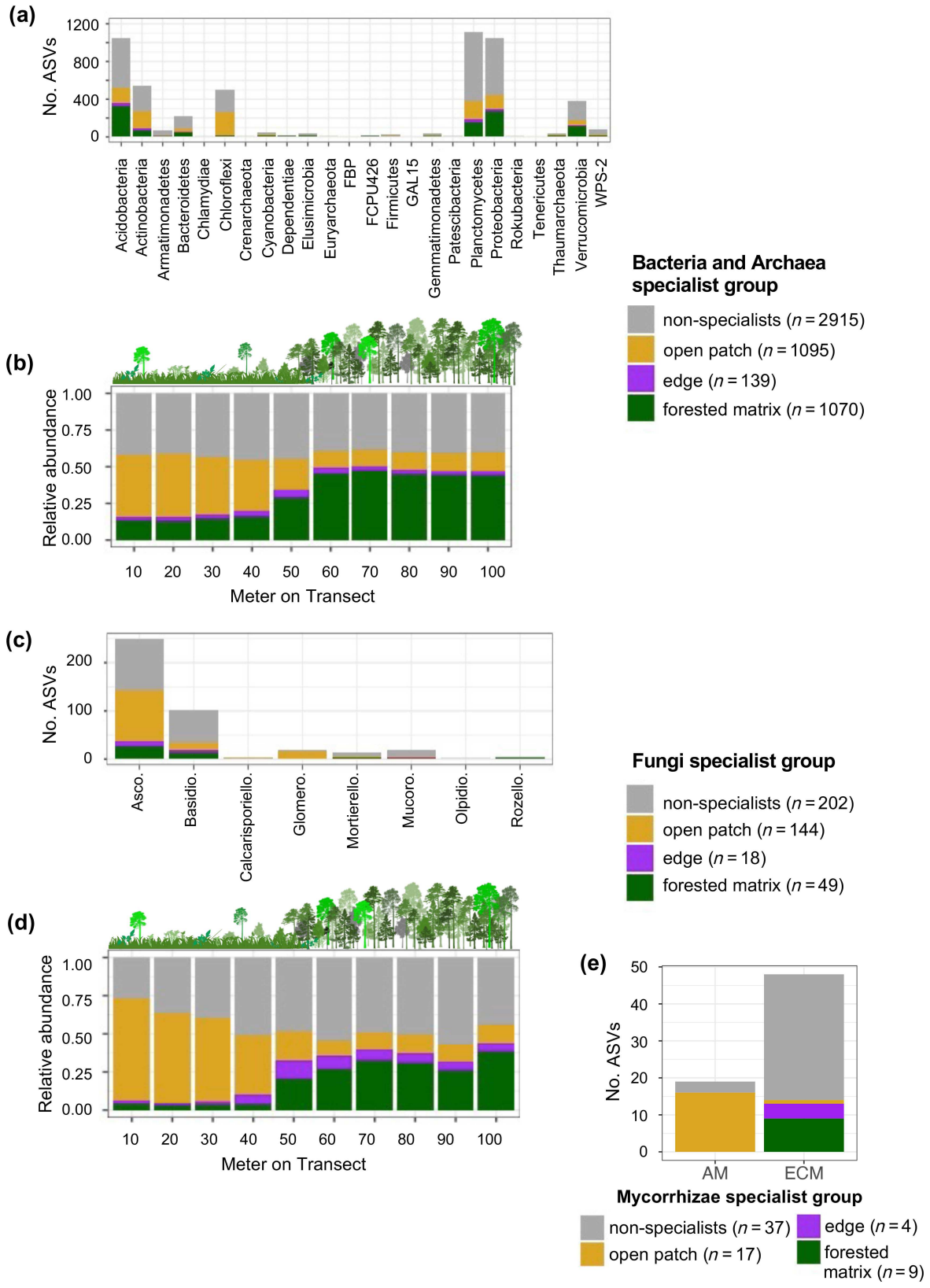

FIGURE 4 Legend on next page.

differ from those in the patch or matrix (Figure 2, Appendix S1: Table S2). The varying degrees of turnover across the edge among EUs appear to be driven by turnover in patch specialist taxa in particular; in the high vegetation-contrast EUs 8 and 10 (Figure 2), there were much fewer patch specialists in the matrix than in EUs with lower vegetation contrast (Appendix S1: Figure S4). Our findings are consistent with previous work on macro-organisms which often find that the contrast between matrix and patch mediates the strength of a species' responses to the edge (Laurance, 2008; Ries et al., 2004), and that accounting for environmental heterogeneity in the matrix leads to better predictions of species abundances (Brudvig et al., 2017).

Our results suggest that the observed edge effects were mostly driven by taxa that tended to associate with either the patch or the matrix, not edge specialists. Specifically, there were relatively few edge specialist taxa, and they comprised a lower proportion of reads as compared with the patch and matrix specialists (Figure 4). This suggests that even though edge communities were often distinct from patch and matrix (Figure 2, Appendix S1: Table S2), these compositional differences were mostly due to changes in the abundance of taxa that are also relatively common in the patch or matrix. Patch soils had higher relative abundances of taxa assigned to the Chloroflexi, Ascomycota, and Glomeromycota phyla as compared with the forested matrix soils where members of the Acidobacteria, Proteobacteria, and Basidiomycota phyla were more dominant (Figure 4, Appendix S1: Figures S2 and S3). Many members of Chloroflexi are photosynthetic, and thus likely prefer the greater light conditions of the open patch over the forested matrix. Chloroflexi are also dominant in biocrusts (e.g., Pombubpa et al., 2020), which were common in the patch but not in the matrix (personal observation). The vast majority of Glomeromycota are AM which are likely to associate with the angiosperms that dominate in the open, savanna-like patch, but not in the conifer-dominated matrix (Schüßler et al., 2001). Accordingly, nearly all AM taxa were patch specialists while ECM taxa were more often associated with the matrix and, to a lesser degree, with the edge (Figure 4e). Taken together, we show that soil microbes are similar to macro-organisms in that most taxa that show a response to fragmentation prefer one of the two dominant habitat types over the edge habitat (Ries et al., 2004); however, the taxon-specific responses to the edge depend on the characteristics of the edge in question (Ries & Sisk, 2010).

Traits of specialist versus nonspecialist taxa

Although we observed consistent differences between patch and matrix soil communities, many of the taxa were equally abundant across these habitats or had no identifiable habitat preference as their distributions were so variable (Figure 4). Thus, we next set out to identify which taxa were "specialists" versus "nonspecialists" and whether there were specific traits or life history strategies that differentiated the "specialist" and "nonspecialist" taxa. We focused our analyses on prokaryote genome size, bacterial sporulation ability, and fungal mycorrhizal status. We acknowledge that these traits show a phylogenetic signal. For example, all arbuscular mycorrhizae are in the phylum Glomeromycota (Schüßler et al., 2001), and some bacterial phyla tend to have larger genomes than others (Martinez-Gutierrez & Aylward, 2022). Importantly, however, our goal was not to test if these traits are selected for independent of phylogeny; rather we aimed to provide another perspective to complement our taxonomy-based assessment of specialists and nonspecialists. Identifying unifying traits of taxa that are sensitive to habitat turnover in fragmented landscapes may increase our ability to predict the responses of taxa to edges a priori (Ries & Sisk, 2010).

We hypothesized that specialist ASVs would feature a greater proportion of mycorrhizal fungi than nonspecialist ASVs because the associations between mycorrhizal fungi and their plant partners might make mycorrhizal fungi more sensitive to vegetation change across the edge. We found mixed support for this hypothesis. As shown in Figure 4e, there was a larger proportion of AM taxa among specialists than among nonspecialists, as we hypothesized

FIGURE 4 Taxonomic identification (phylum level) of the bacterial and archaeal taxa identified as being "nonspecialists" (no clear habitat preference) versus open patch, edge, and forest "specialists" (panel a). Panel b shows the changes in the summed relative abundances of those amplicon sequence variants (ASVs) assigned to the four categories along the open patch to forested matrix transects with values combined across six replicate sites (EUs). Panel c shows the taxonomic affiliation (phylum) of the fungal ASVs designated as specialists versus nonspecialists with the changes in their summed relative abundances across the transects (panel d). Note that phylum names in c have been abbreviated by removing the suffix "-mycota." Panel e shows the proportional representation of ecological guilds (arbuscular and ectomycorrhizal fungi) across ASVs designated as "nonspecialists" versus the three categories of habitat "specialists." Plant silhouettes are adapted from images from phylopic.org, all of which were either in the public domain or that are available to share non-commercially with credit. See Figure 1 caption for more detailed plant picture attributions.

(χ^2 (1, $N = 183$) = 6.95, $p < 0.01$). Conversely, ECM fungi were more likely to be non-specialists than specialists (χ^2 (1, $N = 212$) = 5.35, $p < 0.05$). While our results suggest that some ECM are found across habitat types, AM fungi seem to be largely excluded from the forested matrix (Appendix S1: Figure S5). As pines nearly always associate with ECM fungi (Smith & Read, 2010), AM fungi may not be able to establish in the pine-dominated matrix. It is possible that the matrix in our system represents a significant barrier to the spread of AM fungi among patches in our system. An important next step would be to investigate the capacity for AM fungal dispersal across this landscape. However, some previous work suggests that fungi could face dispersal challenges in fragmented landscapes (Nordén et al., 2013), especially if the small mammals that AM fungi often rely on for dispersal (Borgmann-Winter et al., 2023) tend to be found mostly in the center of the patch, as has been observed at our study site (Orrock & Danielson, 2005). Partnerships with AM fungi are an integral way that plants deal with abiotic stressors (Chen et al., 2018). If dispersal of AM fungi is reduced in fragmented landscapes, this would likely compound the stressors plants are known to face in fragmented landscapes.

Larger prokaryotic genomes have been associated with more metabolic pathways to survive in more varied environments (Barberán et al., 2014; Bentkowski et al., 2015; Cobo-Simón & Tamames, 2017; Konstantinidis & Tiedje, 2004), whereas sporulation can allow prokaryotes to persist in dormant states when conditions are unfavorable for growth (e.g., Lennon & Jones, 2011). For these reasons, we hypothesized that larger genomes and the ability to sporulate would be more common among bacteria and archaea identified as nonspecialists. However, we did not find support for either of these hypotheses. Specialists and nonspecialists did not differ in their genome sizes ($F(1, 303) = 0.68$, $p = 0.41$, Appendix S1: Figure S6a). In addition, there were proportionally more non-spore-formers among nonspecialist ASVs than among specialist ASVs (χ^2 (1, $N = 2$, 839) = 9.08, $p < 0.01$, Appendix S1: Figure S7), a pattern opposite to our predictions. One potential explanation for these findings is that the movement of most bacteria may be so high between the open patch and the forested matrix (i.e., mass effects, Mouquet & Loreau, 2002; Shmida & Wilson, 1985) that the advantage of a larger genome or sporulation ability does not translate into changes in habitat specificity across this landscape. Another possibility is that although our habitat types were different in terms of vegetation characteristics and pH (Figure 2, Appendix S1: Figure S1), the soil edaphic variables (Appendix S1: Table S1) and climate characteristics are similar enough among habitats that there is little selection pressure on nonspecialists to have the expanded metabolic capabilities conferred by a larger genome. We know of no other study that has directly examined whether sporulation capacity is important for understanding bacterial distributions on a scale similar to that considered here. However, it is possible that the ability to form spores is less important for soil taxa than assumed, which aligns with work by Choudoir et al. (2018) which found that bacteria likely capable of spore formation had smaller range sizes across the continental United States than non-spore-formers.

In addition to possible ecological explanations for the lack of support for our hypotheses on trait differences between prokaryotic habitat specialists and nonspecialists, our study may also be limited by the information available in reference databases. As soil taxa are particularly underrepresented in preexisting genome databases (Fierer, 2017), it was not surprising that we were able to match only 5.8% of our taxa (305/5219 ASVs), all of them bacteria, to genomes in GTDB for genome size comparisons. Likewise, our ability to assess the full ecological importance of sporulation is likely hampered by cultivation biases, since nearly all of our information on which taxa sporulate comes from cultivated taxa (e.g., Madin et al., 2020). There is accumulating evidence that the diversity of bacteria capable of spore formation is larger than recognized from cultivation-dependent studies (e.g., Brewer et al., 2019; Browne et al., 2016; Kearney et al., 2018), so it is possible that there are more soil bacterial taxa capable of spore formation than inferred here.

Conclusions

A clearer understanding of edge effects and habitat specialization among soil microbes may help guide the conservation of soil microbes and their contributions to ecosystem functioning in the context of habitat fragmentation. We found that the strength of the edge effects and the magnitude of differences in patch and matrix communities increased with canopy cover contrast between patch and matrix. This suggests that soil microbes may vary less from the interior of the patch to the edge if the patch is surrounded by a matrix that is similar in vegetation structure. We also found evidence that patch and matrix communities differed, despite their close proximity, suggesting strong effects of environmental filtering in our system. In contrast, we found only a few edge-specializing prokaryotes or fungi, indicating that the observed edge effects were driven mostly by taxa that tended to prefer the patch or matrix. Future work should also consider additional edge types to explore the generality of our work. Overall, by investigating edge effects and habitat specialization in a fragmented system, our work represents

an important step to better incorporate microbes into the broader conversation about biodiversity loss in our increasingly fragmented world.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequencing data are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession number PRJNA898410 at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA898410.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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