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RESEARCH ARTICLE

DNA metabarcoding uncovers dispersal-constrained arthropods in a highly fragmented restoration setting

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Degraded areas are often restored through active revegetation; however, recolonization by animals is rarely engineered. Recolonization may be rapid for species with strong dispersal abilities. However, poor dispersers, such as many flightless arthropods, may struggle to recolonize newly restored sites. Actively reintroducing or "rewilding" arthropods may therefore be necessary to facilitate recolonization and restoration of arthropod communities and the ecological functions they perform. However, active interventions are rare. The purpose of this study was twofold. First, we asked whether potential source remnant arthropod communities were dispersal-constrained and struggling to recolonize restoration sites. Second, we tested whether reintroducing entire arthropod communities from remnant populations would help dispersal-constrained species establish during farmland ecological restoration in southern Australia. Rewilding was conducted in summer 2018 by transplanting leaf litter, soil, and entire communities contained within it from remnant source populations into geographically isolated restoration sites, which were paired with untreated controls ($n = 6$ remnant, rewilding transplant, and control sites). We collected leaf litter and extracted arthropod communities 19 months after the initial rewilding event, then sequenced mite, springtail, and insect communities using a metabarcoding approach. Within all groups, community similarity decreased with spatial distance between sites, suggesting significant dispersal barriers. However, only mite communities showed a strong response to rewilding, which was expressed as increased compositional similarity toward remnant sites and greater species richness relative to controls. Our results demonstrate that many arthropod species may struggle to recolonize geographically isolated restoration sites and that full community restoration requires active interventions via rewilding.

Key words: arthropods, dispersal, ecological restoration, litter transplants, recolonization, rewilding, soil transplants

Implications for Practice

- Arthropods can be poor dispersers and our metabarcoding approach confirmed limited movement between remnant source sites and revegetated farmland.
- In addition, we reintroduced arthropod communities via remnant leaf litter transplants and found dispersal-constrained groups established successfully, whereas establishment of other groups was limited by environmental differences between sites.
- Our methods present a novel, cost-effective, and practical way to restore entire communities during ecological restoration.
- We suggest litter transplants should be implemented in conjunction with replanting to assist recolonization for dispersal-constrained species and thus increase the overall effectiveness of restoration.

Introduction

Species dispersal is a fundamental process for community assembly. Understanding how species move through space and time not only teases apart complex community patterns but allows a prediction of which species are likely to recolonize an area (Jønsson et al. 2016). Nowhere is this more relevant than in ecological restoration, where new habitat is created, and we rely largely on species dispersal to build the new community, that is, the "field of dreams" hypothesis (Sudduth et al. 2011). Ecological restoration is often touted as the "acid test" of ecological theory and allows empirical tests of concepts such as assembly theory and diversity–function relationships (Young et al. 2005). Restoration efforts can, however, be negatively affected when they ignore principles of ecological theory and community assembly (Lake et al. 2007). For example, large areas of degraded land require restoration, but constrained funding and habitat fragmentation can result in localized restoration efforts that fail to connect metacommunities across the wider

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landscape (Tambosi et al. 2014). This approach to restoration can therefore accentuate inherent dispersal limitations present within species in the regional species pool, leading to incomplete community reassembly during restoration (Tonkin et al. 2014).

Arthropods make up the bulk of terrestrial diversity and facilitate restoration through their keystone effects on ecosystem processes (de Almeida et al. 2020). Thus, understanding the ability of arthropods to disperse to and colonize restored habitats should be at the forefront of restoration activities. Many species of arthropods are dispersal constrained and can struggle to recolonize newly restored habitat (Magura et al. 2015). Even in the absence of obvious dispersal barriers, such as in undisturbed forests, soil arthropods show dispersal constraints at distances less than a few kilometers, resulting in high levels of genetic differentiation between spatially similar populations (Arribas et al. 2021). Highly fragmented areas that fail to connect metacommunities can therefore accentuate inherent dispersal limitations and further restrict successful arthropod recolonization of newly restored habitat. However, this is not the case for all species, as highly mobile arthropods may rapidly recolonize fragmented restoration sites (Griffin et al. 2017). Abiotic differences between restoration and source sites are another important consideration for arthropod community reassembly. For example, reduced soil moisture can filter which arthropod species successfully recolonize rainforest restoration sites (Nakamura et al. 2003). There is therefore a significant knowledge gap surrounding which groups can easily disperse to restoration sites, which will struggle, and which are filtered by abiotic differences between restoration and source sites. Addressing this gap will entail accurately assessing arthropod species movement across landscapes and thus quantifying recolonization potential.

A decline in the availability of taxonomists, the prevalence of cryptic species complexes, and the often overwhelming levels of diversity of arthropod communities make traditional morphological assessment time and cost intensive (Watts et al. 2019). Metabarcoding has recently emerged as an alternative way to assess arthropod community composition. Bulk community DNA amplification and high-throughput sequencing of small barcoding regions can accurately classify arthropod communities and is rapidly becoming cheaper and faster than taxonomic sorting (Watts et al. 2019; Meyer et al. 2021). Metabarcoding data consist of multiple genetic hierarchical levels from within species (e.g., haplotypes) to higher taxonomic levels. These datasets can be used to quantify the dispersal capabilities of entire communities and thus indicate whether niche (e.g., abiotic filtering of species traits) or neutral (e.g., dispersal) processes drive community assembly and macroecological patterns (Arribas et al. 2021; Baselga et al. 2022). For example, metabarcoding datasets are not only suitable for species identification, but also include data on distinct genetic haplotypes within species that can be analyzed independently to assess dispersal constraints (Arribas et al. 2021).

Understanding the relative importance of neutral verse niche processes in driving community reassembly is paramount for a successful restoration effort (Conradi et al. 2017). Neutral community theory predicts that dispersal limitation by physical distance, rather than environmental constraints, is the dominant processes dictating species distributions (Chave 2004). This process is akin to the neutral theory of evolution, where neutral within-species haplotypes are not selected upon by the environment and instead evolve via dispersal and genetic drift (see Galtier et al. 2009 for an argument against haplotype neutrality). When neutral processes dictate both community assembly and within species population structure there should be a uniform decay in community similarity with spatial distance at both the haplotype and species levels (Baselga et al. 2013, 2015). In contrast, niche theory predicts that environmental filtering of species traits is the dominant process driving community assembly (Thompson & Townsend 2006). Assemblage turnover will therefore be driven by abiotic differences between sites, rather than spatial distance, and thus differs from the neutral processes acting on haplotypes, resulting in differences in the distance–decay rate between species and haplotypes (Arribas et al. 2021). Metabarcoding analysis that includes both haplotype- and species-level genetic data therefore has significant applications in a restoration setting as it can empirically test whether species are struggling to recolonize via dispersal (neutral processes) or whether the habitat of the restoration site is inadequate for recolonizing species (niche processes). Recently, metabarcoding has been implemented in a restoration context to track community recovery (Fernandes et al. 2019; van der Heyde et al. 2022); however, its use in determining the underlying processes dictating community assembly in restored sites has not been tested.

Here, we employ metabarcoding to test whether arthropods are dispersal constrained in a highly fragmented matrix consisting of undisturbed remnant sites (containing source populations) and revegetated farmland sites. We focused on litter-dwelling mesoarthropods (<5 mm) which includes mites, springtails, and many small insect species (George et al. 2017). The smaller size of mesoarthropods results in high levels of passive aerial rafting, with some species able to disperse great distances (Lehmitz et al. 2011) and obtain almost cosmopolitan distributions. However, recent metabarcoding analyses has shown many soil mesoarthropods are highly dispersal-constrained, even over relatively short distances (Arribas et al. 2021). This discrepancy in findings suggests more work is needed to understand mesoarthropod dispersal in the context of habitat restoration and fragmentation.

The aims of this study were twofold; first, we aimed to determine the dispersal capability of mesoarthropods moving from source remnant sites into restoration sites and whether this was driven by niche or neutral processes. Second, we tested whether reintroducing entire communities of mesoarthropods from remnant sites into revegetated farmland sites would assist establishment for less dispersive species. Reintroducing or "rewilding" species can be used when restoration is exceedingly slow or fails to restore communities to pre-degradation composition (Contos et al. 2021). Rewilding was conducted by transplanting leaf litter and soil with entire communities of mesoarthropods in situ from remnant sites into geographically isolated revegetated sites.

We employed metabarcoding analyses on mite (Acari), springtail (Collembola), and insect (Insecta) communities 19 months after the initial rewilding event in remnant sites, control revegetation sites, and rewilding revegetation sites. We hypothesized that where community assembly was driven by neutral processes (e.g., dispersal), rewilding would reintroduce dispersal-constrained species and thus increase compositional similarity toward remnant communities (Fig. 1). Rewilding was predicted to have minimal effect on species establishment for communities where niche processes (e.g., environmental filtering) were driving assembly.

Methods

Study Sites and Experimental Design

We tested the dispersal capacity of mesoarthropods and efficacy of litter transplants in southeastern Australia (37°0′20.76″S, 145°28′6.05″E). We conducted our study in a highly fragmented system consisting of undisturbed remnant sites and geographically isolated revegetated sections of farmland. We sampled 18 sites within this region with 6 blocked replicates of three treatments: "remnant" (uncleared conservation area, source of litter transplant), "control" (revegetated pasture with no transplant), and "rewilding transplant" (revegetated pasture with a litter transplant). Within each block, the remnant site was the source of the transplant and was grouped with spatially paired revegetation sites (remnant sites were, however, geographically distant) (Fig. 2). Revegetated sites were paired spatially to rule out landscape factors such as habitat corridors influencing community assembly, but also paired based on age, size, aspect, and elevation. Remnant vegetation was typified by *Eucalyptus* woodlands with a canopy consisting of *E. ovata* and *E. viminalis*, with a shrub layer dominated by *Acacia* spp. and *Bursaria spinosa*. Revegetated sites were historically cleared for pasture and then replanted using native tree and shrub tubestock 14–22 years prior to the commencement of this study and fenced to exclude livestock. Sites within this age range had a developed leaf litter layer, providing critical habitat for transplanted litter-dwelling species. We constructed a 10 × 10 m experimental grid within each of the 18 sites, around which we centered our sampling.

Rewilding Using Litter Transplants

Litter transplants were sourced from paired remnant sites as these were considered "target" states, that is, sites with a community of species that may have existed in the restoration sites pre-degradation (Mcdonald et al. 2016). Our approach allowed us to steer community composition of rewilding transplant sites toward that of remnant sites and ensured that we transplanted species with appropriate functional and life history traits. Each litter transplant sample was 80 cm litter × 80 cm litter × 5 mm soil depth. To ensure viability of transplanted populations, we transplanted 10 of these litter samples 1 m apart from each other into the experimental grid at the rewilding transplant sites (Contos et al. 2023). We included the thin layer of topsoil during each event as mesoarthropods move into this superficial layer during dry conditions. Furthermore, by only sampling 5 mm deep we avoided species that were adapted to deeper subsurface environments as these were not the focus of our transplant efforts (Ponge et al. 1993; Salmon et al. 2014).

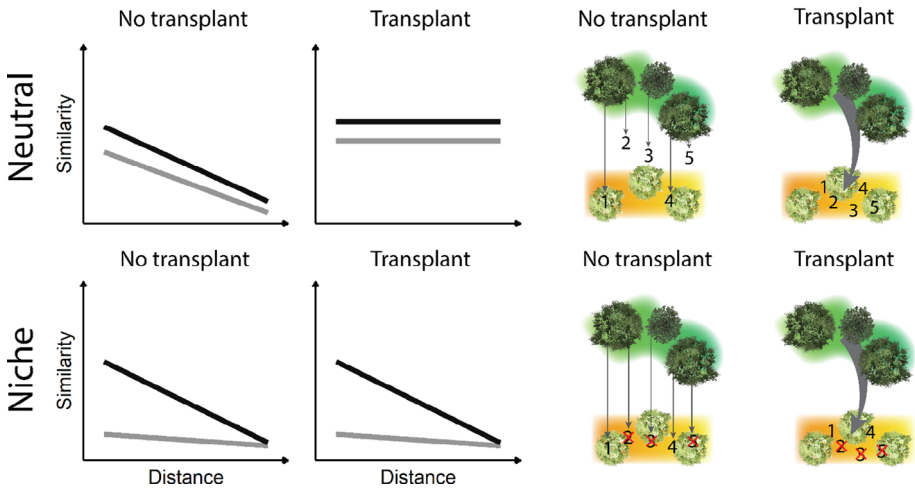

Figure 1. Conceptual diagram with predicted effects of rewilding via litter transplants under differing community assembly processes. Dispersal-constrained groups were identified when community similarity negatively declined with distance between sites. Where dispersal is driving community assembly (neutral processes) community similarity is predicted to decay with distance between revegetated and remnant sites at similar rates between haplotype-level (gray) and species-level (black) thresholds in areas with no litter transplants. Under neutral processes, litter transplants were expected to remove all distance–decay relationships. Where environmental filtering of species traits is driving community assembly (niche processes), distance–decay relationships were expected to differ between haplotype and species levels (Arribas et al. 2021). Litter transplants were not predicted to affect distance–decay patterns under niche processes. Our predicted difference between haplotype and species distance–decay rates under niche scenarios fits best with modeled predictions of communities where haplotypes are locally limited (Baselga et al. 2013). The success of five hypothetical species (numbered) is shown as they move from remnant areas (green) into restoration sites (yellow) under differing community assembly scenarios.

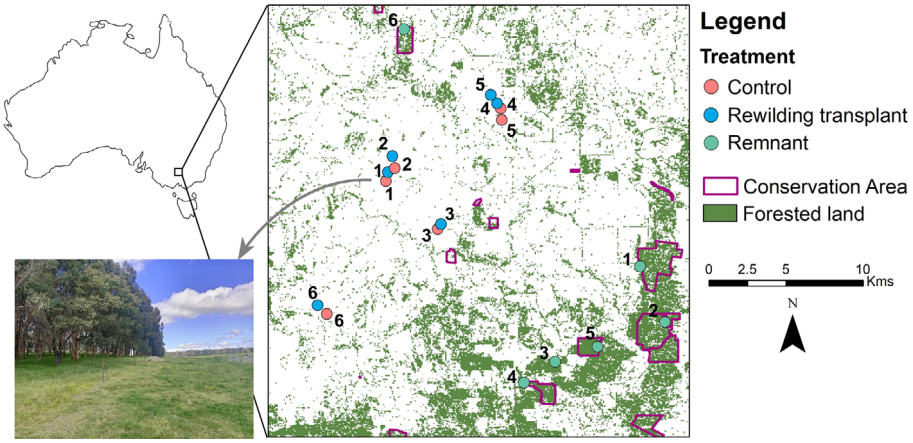

Figure 2. From clockwise top left: location of study sites in Australia; distribution of sites and treatments within the study region, showing forest cover (trees >2 m in height); and a typical revegetation site used in the experiment (bottom left).

We conducted the first leaf litter transplant in November 2018 with a subsequent transplant event on June 2019. Repeat events over different seasons increased the likelihood of capturing all community components as mesoarthropods express high turnover rates (Fagan et al. 2006). Litter samples were collected and transplanted on the same day and were kept cool during transport to reduce mortality, which was likely <5% (Contos et al. 2023). Transplanting leaf litter likely increased the habitat availability in rewilding transplant sites by ∼11%. However, this is well within the range of similar studies that transplant habitat (see table 1 in Contos et al. 2021) and likely did not attract additional species to the experimental grid (Contos et al. 2023).

Mesoarthropod Collection

We sampled communities 19 months after the initial rewilding event (May 2020) by scraping 25 cm × 25 cm leaf litter samples with 5 mm of the underlying soil into zip-locked bags. Three of these sub-samples were taken 2 m from the experimental grid at each of the 18 sites (54 sub-samples in total). Sampling outside of the experimental grid ensured potential microclimatic changes and the minor increase in litter volume inside the experimental grid did not bias communities sampled. We used Tullgren funnels with 25 W lamps to extract live animals from leaf litter into 100% ethanol. Each sub-sample was placed into a single Tullgren funnel and left for 7 days to ensure all animals were expelled into the ethanol. Mesoarthropod communities were stored at 4°C prior to DNA extraction.

Environmental Variables

In conjunction with the mesoarthropod survey, we measured a suite of environmental variables at each site. We included the following variables: elevation (meters above sea level), weed cover (%), tree canopy cover (%), tree height (m), litter depth (mm), litter cover (%), and diameter breast height (DBH, cm) of trees. Surveys were conducted in a 4 m × 4 m quadrat every 10 m along a 50 m transect. In addition, we used a Fieldscout TR150 soil probe to measure soil temperature (°C) and soil volumetric water content (VWC, %) at each site. For these two variables, an average measurement was taken based on four probe readings at the corner of each experimental grid.

DNA Extraction, Amplification, Sequencing, and Bioinformatics

We employed a non-destructive DNA extraction method on whole mesoarthropod communities as described by Batovska et al. (2021). We did not standardize the size of larger mesoarthropods (e.g., by only using a single removed leg) as the smaller size of our community (<5 mm in length) meant that most individuals were similar in size. Briefly, we first evaporated residual ethanol in each 1.5 mL Eppendorf tube containing a sub-sample community before adding 100 µL of QuickExtract DNA Extraction Solution (Lucigen) to each tube, ensuring all specimens were immersed in the buffer. As per the manufacturers protocols, we then vortexed each tube for 30 s, followed by a 6-minute incubation at 65°C, vortexed for 15 s, followed by a final 2-minute incubation at 95°C. We then transferred the buffer to a new 1.5 mL tube ensuring no specimens were removed in the process. We quantified the DNA concentration of each sample using a NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) before standardizing each sample to 10 ng/µL and storing at −20°C before Polymerase Chain Reaction (PCR) amplification. Preliminary tests on varying concentrations indicated samples containing 10 ng/µL DNA improved amplification efficiency relative to PCR inhibitors. The remaining mesoarthropod specimens were resuspended in 100% ethanol and counted to quantify whether physical abundances were reflective of sequencing counts (Table S1).

We amplified a ∼300 bp fragment of mitochondrial *Cytochrome Oxidase 1* for our metabarcoding analyses. PCR reactions were carried out in 25 µL solutions consisting of 12.5 µL of MyTaq DNA polymerase (Bioline), 8.5 µL of Milli Q PCR grade water, 1.25 µL each of 10 µM forward (mlCOIintF; Leray

et al. 2013) and reverse (Fol-degen-rev; Yang et al. 2014) primers and 2.5 μL of DNA template. Initial PCR cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 30 cycles of 95°C for 15 s, 46°C for 30 s, 72°C for 15 s, followed by a final extension at 72°C for 1 min. We then ran a secondary PCR amplification that attached a unique combination of multiplex identifier tags to each sample. Negative extraction controls were run in parallel with community samples. We purified each sample using AMPure beads (Beckman Coulter) to remove non-target size sequences and primer–dimer. PCR samples were then quantified using a Qubit dsDNA HS kit (Thermo Fisher Scientific), standardized to 4 nM using Milli Q PCR grade water, and finally pooled into equimolar concentrations to produce a PCR amplicon library. The final pooled library was sequenced with 20% phiX control on the Illumina MiSeq platform using a v3 kit 2 × 300 cycle as per the manufacturer's instructions.

Raw sequencing reads for both the forward and reverse target amplicon sequences were trimmed using the "cutadapt" package (Martin 2011) such that reads that were not between 150 and 300 nucleotides were removed. We further filtered sequences in R using the "dada2" package (Callahan et al. 2016) by filtering out sequences with a minimum nucleotide size of 75, truncating 200 and 160 nucleotides on the forward and reverse reads respectively, and removing phiX. The forward and reverse reads were then dereplicated and the error rates were estimated using the "derepFastq" and "learnError" function, respectively, and used to remove likely sequence errors. We merged the forward and reverse reads to create unique Amplicon Sequence Variants (ASVs) before a final filtering step which removed chimeras. We determined the lowest possible ASV taxonomy by subjecting them to BLAST searches against the nucleotide database NCBI GenBank and using MEGAN (Huson et al. 2007). Finally, as many ASVs could not be accurately assigned to species, we aligned the sequences in Geneious Prime (v2022.2.2) and conservatively determined species-level units by clustering ASVs at a 95% lineage similarity threshold (Saitoh et al. 2016).

Statistical analyses

All statistical analyses were performed in R (R Core Team 2021). No ASVs that were found in the negative extraction control were present in other samples. Rare ASVs were removed by excluding those that contributed <0.005% of reads to the total read count (which was 314,334 sequence reads). This removed 389 ASVs from the original total of 1535. Of the remaining total, we removed all ASVs that were not identified as mites, springtails, or insects, leaving 930 on-target ASVs. We combined the three sub-samples taken at each site to create a single community sample per site (18 in total across three treatments, $n = 6$) and constructed community matrices for each group at both the ASV (i.e., every unique haplotype) and species (ASVs clustered at 95% similarity) level. We further filtered the dataset to reduce issues with inflation and over-estimating richness by removing haplotypes/species that contributed <0.005% of reads to the total read count within each community matrix. This removed all haplotypes/species that contributed <2 reads within mite communities, <3 for insect communities, and <4 for springtail communities. We lowered the threshold to 0.0025% for springtails as 4 out of 425 springtail haplotypes contributed ∼53% to the total read count, therefore reducing the risk of false negatives. Read counts were then transformed to presence/absence for further analyses. We constructed rarefaction curves for each taxon using the "iNEXT" package (Hsieh et al. 2016) to ensure that our sampling effort captured the bulk of haplotype/species diversity.

We tested dispersal capabilities of our three mesoarthropod groups by examining distance–decay relationships across treatment pairings. We constructed community similarity matrices using the Jaccard similarity coefficient in the "vegan" package (Oksanen et al. 2020) for both haplotype- and species-level identification. As we were interested in both dispersal across a highly fragmented landscape and the effect of rewilding on overcoming dispersal barriers, we looked at multiple treatment pairings. The first set of comparisons informed us of the overall dispersal across the landscape. It involved pairwise comparisons between remnant versus control revegetation sites, and between control revegetation sites separately. The second set of comparisons allowed us to infer the efficacy of litter transplants. It involved pairwise comparisons between remnant sites and rewilding revegetation sites, and between rewilding revegetation sites separately. If dispersal constraints were found within the first set, but not in the second set, this would suggest that rewilding has effectively reintroduced a suite of dispersal-constrained species (Fig. 1). We matched the Euclidean distance (km) between sites with the site pairings community similarity to construct a distance–decay relationship. We analyzed this data using generalized mixed effects models (GLMMs) in the "lme4" package (Bates et al. 2015) with Gaussian log link functions, "pairwise similarity" (1 – Jaccard values) for either haplotype- or species-level similarity as the response variable, and "distance" (km) as the predictor. Differences or similarities in decay slopes between haplotype- and species-level responses were examined both visually, and statistically via the GLMM results. We fitted models with Gaussian log link-functions as the "pairwise similarity" data were normally distributed and non-discrete (Zuur et al. 2007). In addition, we made pairwise comparisons between remnant vs. remnant sites to assess background dispersal rates (Fig. S1). However, biases in past land clearing locations within Australia (Simmonds et al. 2017) have resulted in inherent spatial correlation between remnant sites which limited our ability to infer background dispersal.

We characterized abiotic differences between sites using a principal component analysis (PCA) with scaled means for each habitat variable at each site in the base "stats" package (R Core Team 2021). Prior to analysis, we checked whether our PCA respected assumptions by determining whether there were any outliers or missing values in our dataset, and by checking for linear relationships between environmental variables using Bartlett's test of sphericity (Zuur et al. 2007). A biplot was created in the package "ggfortify" (Tang et al. 2016) and the contribution of each habitat variable to the first two principal components that explained the most variance was recorded. In addition, we calculated the "environmental distance" between

sites by determining the distance between site points on the PCA graph. We matched environmental distance between sites with community similarity between sites to determine whether environmental differences were driving community assembly. Environmental distance–decay relationships were analyzed using GLMMs with Gaussian log link functions, "pairwise similarity" (1 – Jaccard values) for either haplotype- or species-level similarity as the response variable, and "environmental distance" as the predictor.

We tested whether rewilding transplant communities had deviated from controls and become more similar to remnant communities using permutational multivariate analysis of variance (PERMANOVAs) on community matrices in the "vegan" package (Oksanen et al. 2020). We ran PERMANOVA models with 999 permutations using "treatment" as a fixed effect, "site" as a random factor, and with Jaccard similarity. We used post hoc tests in the "adonisPairwise" package to provide greater insight into differences between levels of a main effect (Arbizu 2020). Additional non-metric multidimensional scaling plots (nMDS) were constructed to visualize community differences between treatments. To observe finer-scale differences in species between sites, we ran a multipattern analysis to determine whether species were significantly associated with any of the treatments using the R package "indicspecies" (de Caceres & Legendre 2009).

To test whether haplotype/species richness responded to the treatments, we used GLMMs with fixed effects of "treatment", "threshold" (haplotype or species), an interaction between these two, "sequencing depth", and included "site" as a random effect. The inclusion of sequencing depth from each taxon in the model (i.e., the number of sequencing hits per taxa) ensured that perceived differences in richness were not due to different sequencing depths between samples. We fitted models using Poisson log link functions. Where main effects were significant, we used post hoc estimated marginal means tests in the "emmeans" package (Lenth 2021) to test for differences between levels within main effects.

Results

There was a total of 293,689 sequences distributed across springtails, mites, and insects, across 18 community samples with an average of 16,316 (SE ± 724) sequences per sample (Supplement S1). Springtails (Collembola) were the bulk of sequencing reads, totaling 192,999 sequences across 409 haplotypes (ASVs). When analyzed at the 95% lineage threshold level for species-units, we found a total of 122 springtail species units (Fig. S2). Of these springtail species units, 74 were represented by only a single haplotype (Fig. S3). Insects were the next most abundant group by sequencing reads, with 63,740 sequences from 362 insect haplotypes. When combined at the species level, these haplotypes formed 175 species units, of which 118 were present as only a single haplotype. Finally, there was a total of 36,950 sequences across 143 mite haplotypes, which when combined into species units, equaled 81 species. Of these mite species, 61 were represented by only a single haplotype.

Distance–Decay Relationships

For communities moving between control revegetation and remnant sites, mite similarity significantly decreased with spatial distance at both the species and haplotype levels (Table S2; Fig. 3A). Springtail community similarity between control and remnant sites significantly decreased with distance at the species level but not at the haplotype level (Fig. 3B). There was also a strong disconnect in the decay rate between haplotype and species levels for springtails. Insect community similarity between control and remnant sites significantly decreased with distance at the haplotype level, but not at the species level, despite similarities in their slope of decay (Fig. 3C).

In the rewilding transplant–remnant comparison, all distance–decay relationships were removed for mite communities. There was no distance–decay relationship for springtail community similarity in the rewilding transplant–remnant comparison at the species level; however, there was an increase in similarity with distance at the haplotype level. There were strong negative distance–decay relationships for insects communities at both the species and haplotype levels for the rewilding transplant–remnant comparison.

For communities moving between control revegetation patches, we found that mite community similarity at the species level significantly decreased with distance but did not at the haplotype level despite similarities in their slope of decay. The sharp cut-off in genetic similarity suggests that revegetation sites >10 km apart may restrict mite gene flow significantly (Fig. 3A). Similar, albeit weaker, patterns were found for springtail communities moving between control revegetation sites, whereas no significant patterns were found for insect communities.

For communities moving between rewilding transplant sites, we found the complete removal of distance–decay relationships for both mite and springtail communities. However, insect community similarity did significantly decrease with distance at the haplotype, but not species, level.

Habitat Differences

The first two principal components explained 39.00 and 18.77% of habitat variation among sites respectively (Fig. 4). PC1 split remnant sites from revegetation sites. Remnant sites were typified by taller trees, higher elevation, larger tree DBH values, cooler soil temperatures, and soil with a higher moisture content (Table S3). When comparing environmental distance between sites with pairwise community similarity, we found no evidence for significant environmental distance–decay relationships among taxa (Fig. S4).

Compositional Similarity

PERMANOVAs showed a significant effect of 'treatment' on community similarity within each taxon across both haplotype- and species-level communities (Fig. 5). In each case, pairwise comparisons revealed that the remnant was significantly different from both the control and rewilding transplant (Table S4). Mite communities showed the greatest convergence

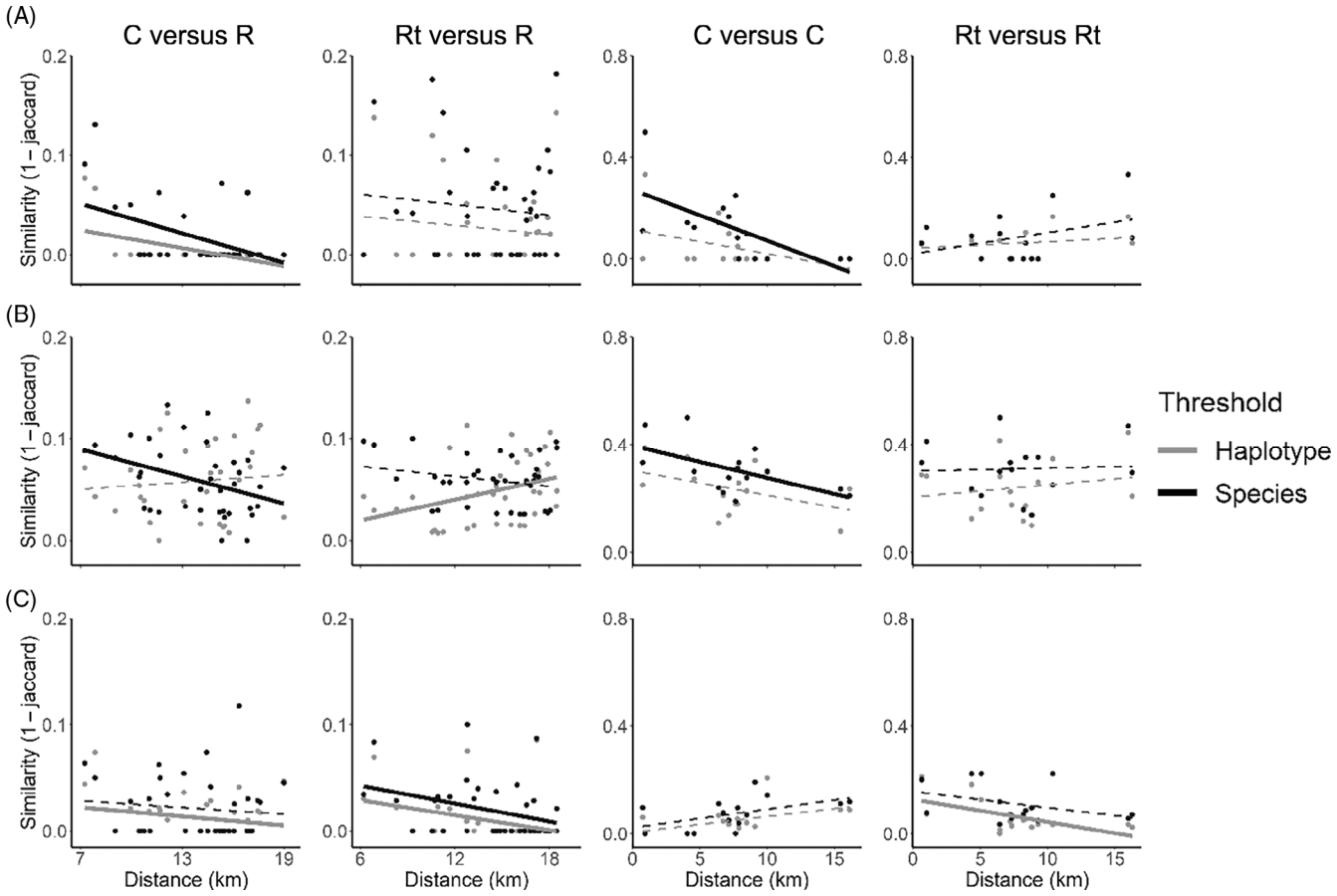

Figure 3. Distance–decay relationships of mite (A), springtail (B), and insect (C) community similarity (1 – Jaccard) with spatial distance within different treatment pairings ("C" = Control, "Rt" = Rewilding transplant, "R" = Remnant). Solid lines are significant relationships whereas dashed lines are non-significant relationships.

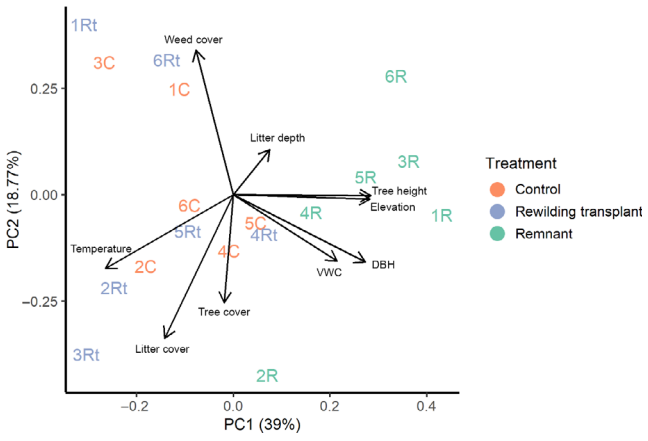

Figure 4. PCA analysis of habitat variables across sites with site number overlaid. "C" = Control, "Rt" = Rewilding transplant, "R" = Remnant.

between rewilding transplant and remnant sites. Post hoc analyses showed that rewilding transplant sites tended to deviate from control sites at both the haplotype and species levels. nMDS plots suggested that mite communities at both the haplotype and species levels in rewilding transplant sites were intermediate between control and remnant sites (Fig. 5A). Remnant springtail communities remained strongly distinct from both control and rewilding transplant sites (Fig. 5B). Similarly, remnant insect communities differed from control and rewilding communities (Fig. 5C).

Indicator Analyses

Fifteen species showed significant association ($p < 0.05$) with a treatment or combined treatment pairing. Springtail species showed an especially high fidelity to remnant sites, accounting for all species that were significantly associated with this treatment (Table S5). Six out of nine species that were associated with the remnant sites were from the family Isotomidae (mostly from the *Subistoma* genus). Two species were significantly associated with the rewilding transplant sites, with one species a beetle (Coleoptera: Staphylinidae) and the other species an ant from the genus *Solenopsis* (Hymenoptera: Formicidae). Three species were significantly associated with the rewilding transplant + control sites and all were springtails. One Hypogastrurid springtail species was associated with control

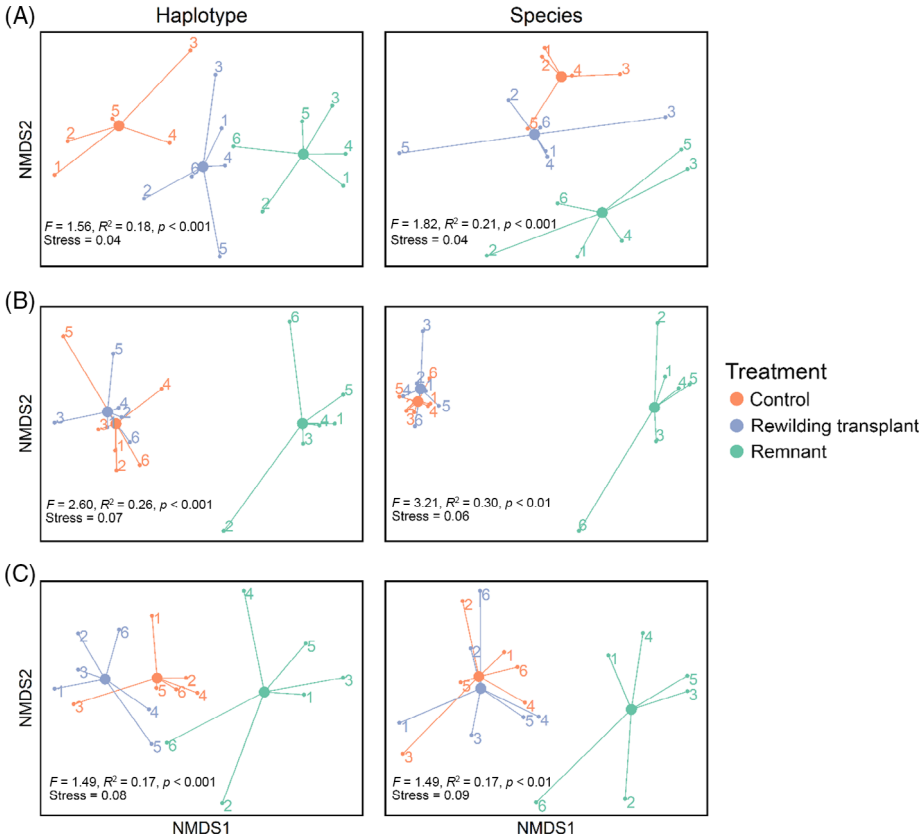

Figure 5. nMDS spider plots with PERMANOVA results for main treatment effects for both haplotype- and species-level mite (A), springtail (B), and insect (C) communities. Site number is displayed with each community point.

revegetation sites. Analysis of haplotype indicators showed a similar pattern in results (Table S6).

Richness Responses

Across all communities, there was a significant main effect of "treatment" on richness (Table S7). Within mite haplotype richness responses, post hoc analyses showed that there were significantly more mite haplotypes in remnant areas as opposed to both control and rewilding transplant areas (Table S8; Fig. 6A). Mite haplotype richness was comparable between control and rewilding transplant areas. Similar patterns were found for springtail haplotype richness (Fig. 6B). Within insect communities, there were significantly more insect haplotypes found within rewilding transplant areas as compared to control sites, with both having significantly fewer haplotypes than remnant areas (Fig. 6C).

Within species richness responses, post hoc analyses showed that there were significantly more mite species in rewilding transplant sites relative to control sites (Fig. 6A) whereas rewilding transplant sites had comparable richness relative to remnant sites. Insect species richness showed similar, albeit weaker, pattern to mite richness. Insect species richness in remnant sites was significantly greater than control sites, whereas remnant and rewilding sites had comparable richness. This indicates that insect species richness in rewilding sites was intermediate in comparison to control and remnant areas (Fig. 6C). Springtail species richness in remnant areas was significantly greater than both control and rewilding transplant richness (Fig. 6B).

Discussion

Species recolonization is paramount for successful restoration, but the dispersal capacity of species in the regional species pool can limit recolonization (Baur 2014). Using DNA metabarcoding, we have shown that movement of most mesoarthropods from both source remnant patches into restoration sites and among restoration patches is limited. In support of our hypothesis that rewilding would reintroduce dispersal constrained species, litter transplants had a pronounced effect on mite assemblages. Litter transplants removed any distance–decay relationships, increased compositional similarity to remnant sites, and increased mite species richness, suggesting transplants introduced a suite of dispersal constrained species. However, transplants had minimal effect on springtail and insect assemblages, suggesting that environmental or methodological constraints restricted their establishment post-rewilding. Our metabarcoding approach effectively quantified mesoarthropod dispersal in a highly fragmented restoration setting and provided

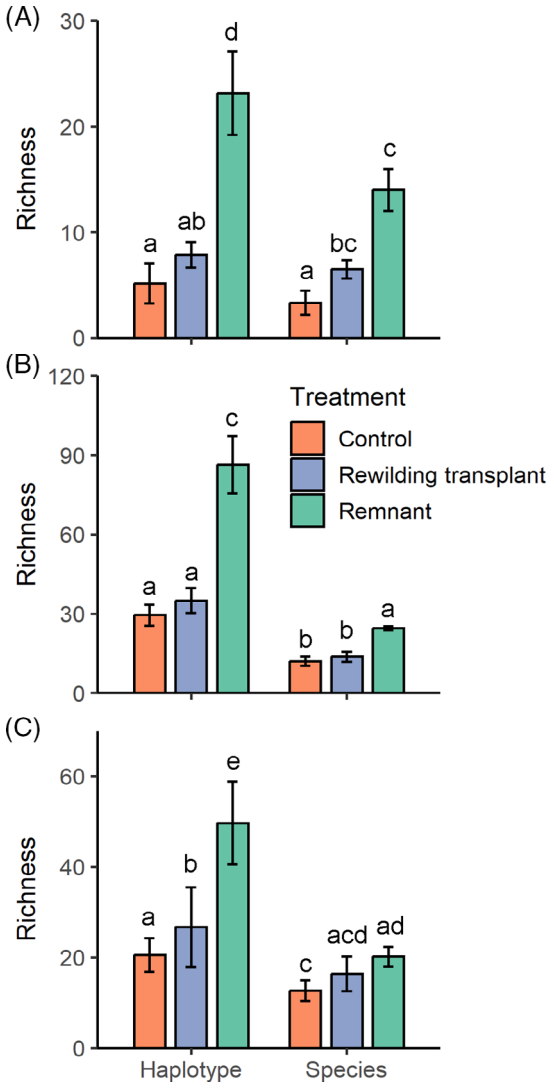

Figure 6. Mean richness (± SE) at both genetic thresholds for mite (A), springtail (B), and insect (C) communities. Different letters denote statistically significant comparisons ($p < 0.05$).

experimental evidence that rewilding overcomes dispersal barriers for poor dispersing species.

Evidence for Dispersal Constraints

Mite communities showed strong evidence for dispersal constraints within a highly fragmented landscape. This was true for mites both dispersing from remnants to restoration sites and among revegetation patches without transplants. Our results suggest that distances >10 km between revegetation sites may restrict gene flow significantly. This is an important consideration for future restoration planning and highlights the need for greater connectivity among restoration sites. Mite community dispersal can be facilitated by connecting geographically isolated habitats, although this understanding largely stems from small-scale mesocosm experiments (Rantalainen et al. 2006; Starzomski & Srivastava 2007). The similarity in decay slopes between mite haplotype- and species-level analyses suggests that neutral processes (e.g., dispersal), rather than environmental filtering of species traits, are primarily driving community assembly in this system (Baselga et al. 2015). Mites are a diverse group with a plethora of life strategies, and therefore vary in their ability to disperse between habitats. Some groups, such as spider mites (Acari: Tetranychidae), balloon on silk threads and can raft aerially over several kilometers (Bell et al. 2005). However, our remnant sites were dominated by Oribatida, with one haplotype from the Genus *Oribatella* (Acari: Oribatellidae) (ASV 47) showing strong affinity to remnant sites and accounting for ~10% of mite sequencing abundance. Oribatids are generally considered poor dispersers, although some species can passively disperse via wind or water (Schuppenhauer et al. 2019). Genetic methods have confirmed deep phylogeographic structuring, indicative of low dispersal capacity, within some Oribatid species (Schäffer et al. 2010). Rewilding via litter transplants may have therefore assisted the recolonization of this less dispersive group of mites.

We found evidence that springtails moving between control revegetation sites were significantly dispersal constrained. This suggests that for species that typify restoration sites (e.g., Hypogastruridae), distance among sites and stochastic events such as aerial rafting may be important in dictating community assembly (Roberts & Weeks 2011). Increasing connectivity among restoration sites may facilitate species movement (Halme et al. 2013). However, many of the springtail species sampled in restoration sites were invasive species with European ancestry (P. Greenslade 2022, Federation University, personal communication; Greenslade & Ireson 1986). Practitioners will therefore need to consider the trade-offs between increasing restoration site connectivity and facilitating the spread of invasive species.

Analyses of insect communities indicated limited levels of movement from remnant sites into both control and rewilding revegetation sites. Insect orders such as Hymenoptera and Diptera include many strong dispersers (Leitch et al. 2021). However, similar studies focused on soil insects in a similar size range (0.1–2 mm) have revealed dispersal constraints over short distances, potentially due to small size (Arribas et al. 2021). In a restoration context, Jellinek et al. (2013) found that long-term habitat fragmentation in agricultural landscapes results in little difference in beetle communities between remnant and revegetated farmland sites. Results for the within treatment effects of control comparisons and rewilding transplant comparisons were idiosyncratic. Community similarity tended to increase with distance in control sites and decreased with distance in rewilding transplant sites. These results may have been affected by the high turnover rates evident from the insect rarefaction curves, which made it difficult to compare overlap between communities for some sites. Metabarcoding studies may therefore be improved by increasing replicates when sampling broad taxonomic groups, thus increasing the likelihood of capturing all community components. Our findings should also be considered in the context of the high turnover rates in our dataset, which limited our ability to compare multiple haplotypes within the same species across sites.

Litter Transplants Can Overcome Dispersal Limitations

Whole-community transplants are a complex successional event which always comprise of failed and successful establishments. For example, Benetková et al. (2020) found that transplanting remnant soil and litter communities increased nematode diversity but failed to reintroduce mesoarthropods. Others have, however, demonstrated broad establishment of taxa such as mites and springtails post-remnant soil inoculation (Wubs et al. 2016). Similarly in our instance, mites were clear benefactors post-rewilding. This conclusion is supported at multiple levels of analyses. We not only saw the removal of any distance–decay relationships across the landscape, but also observed an increase in mite species richness and the emergence of compositional similarities between transplant and remnant communities. Rewilding transplant sites therefore consist of a mix of in situ revegetation and dispersal-constrained remnant mite haplotypes/species. Our results suggest restoration sites have appropriate habitat to support remnant mite species. Active reintroductions of mite communities may therefore be needed where restoration sites are geographically isolated from source remnant populations.

Although our results suggest that springtails moving between control revegetation patches were dispersal-constrained, we note highly different decay rates between haplotype and species levels when observing remnant springtails moving into restoration sites. This suggests niche processes are dictating springtail community reassembly and that there is strong environmental filtering of recolonizing species from remnant sites—which may be linked to habitat differences between remnant and revegetation sites (Baselga et al. 2013). However, we found no correlation between environmental and assemblage composition distances for springtails. Although remnant sites were distinct from revegetation sites in terms of larger and older trees, we may have missed habitat components that facilitate successful remnant springtail establishment. For example, below-ground variables, such as bulk density and soil pH, can drive springtail community assembly and distributional patterns (Errington et al. 2018). Our surveys included some below ground variables (VWC, Temperature) and above-ground variables that are important for soil-dwelling species (Litter depth/cover) (Nakamura et al. 2003). However, these variables may have not adequately captured the specific drivers behind springtail establishment in our study system. Failed establishment of springtails may also be partly explained by potential missing biotic interactions. For example, springtails are mostly microbial grazers (Wang et al. 2009), and if our methods failed to reintroduce remnant fungi and bacteria, then species with specialist diets would be unlikely to persist in their new environment. The hypothesis that remnant springtail community reassembly was driven by niche processes was also supported from the nMDS graphs where remnant assemblages were highly distinct from restoration sites, and the indicator analyses where all species associated with remnant sites were springtails. Springtails that typify remnant areas can show deep affinity to local environments, with severely restricted gene flow between populations and a consequently high degree of phylogeographic structuring (Cicconardi et al. 2010). We suggest restoration sites may therefore need greater focus on below-ground habitat reconstruction prior to transplanting in order to better facilitate establishment of remnant springtail mature-forest specialists.

Although there was evidence for dispersal constraints within insect communities moving between remnant and revegetation sites, we did not observe an effect of litter transplants. There was not a strong increase in compositional similarity for insects in rewilding transplant sites relative to remnant sites; however, species and haplotype richness did improve post-rewilding. This suggests that we may have reintroduced some dispersal constrained insect species. However, the high turnover rates found within this group may have limited our ability to detect broad changes at the community level or changes in distance–decay relationships. Furthermore, we found one insect species significantly associated with rewilding transplant sites (Coleoptera: Staphylinidae) which interestingly aligns with morphological results in our previous study that showed staphylinid beetles were most likely to establish during rewilding (Contos et al. 2023). This further demonstrates the potential of metabarcoding to accurately assess community composition.

Our study revealed that arthropods were dispersal constrained within a highly fragmented agricultural landscape. This is an important consideration for future revegetation projects as a lack of connectivity between restored and remnant source populations may limit the overall effectiveness of restoration activities (Halme et al. 2013). We have also shown that metabarcoding is effective in assessing both the dispersal capability of communities and whether niche or neutral processes dictate community assembly. We suggest that some groups (mites) will need to be assisted via active reintroductions, whereas other groups (springtails) will need improved habitat in revegetation sites pre-rewilding. Overall, we have shown that our interventions can facilitate arthropod recolonization during restoration and that litter transplants are an effective method for the reintroduction of some dispersal-constrained groups.

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Data Availability Statement

Data is available in the Dryad repository at: https://doi.org/10.5061/dryad.83bk3j9zx.

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Supporting Information

The following information may be found in the online version of this article:

Supplement S1. Sequencing counts for each Amplicon Sequence Variant.
Figure S1. Distance–decay relationships of mite.
Figure S2. Rarefaction curves for haplotype and species richness.
Figure S3. Rank abundance curves that rank species units by the number of haplotypes (log + 1) found within each species.
Figure S4. Distance–decay relationships of mite, springtail, and insect community similarity.
Table S1. Physical abundance counts for each group used in analysis.
Table S2. Distance–decay relationships as determined by generalized linear mixed-effects models.
Table S3. Percentage contribution of each habitat variable to the first two principal components.
Table S4. Post hoc analyses of PERMANOVA community similarity results.
Table S5. Indicator species analyses for species-level communities.
Table S6. Indicator species analyses results for haplotype-level communities.
Table S7. Outputs for main effects considered in the richness GLMMs.
Table S8. Post hoc comparisons for the main effect of "treatment" on richness.

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