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Patch size, isolation, and matrix effects on biodiversity and ecosystem functioning in a landscape microcosm

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Abstract. The amount and arrangement of habitat is a fundamental determinant of biodiversity and ecosystem processes in a landscape. Biodiversity is expected to decline following habitat loss and isolation, potentially impeding ecosystem function. But because greater isolation usually accompanies habitat loss, the effects of habitat amount and isolation can be confounded. Moreover, the type or quality of the intervening matrix habitat can mediate amount and isolation effects on biodiversity. We used a landscape microcosm of oak leaf litter patches to examine the responses of bacterial communities and the ecosystem function of oak leaf decomposition to patch size, degree of isolation, and matrix habitat type (pine litter or bare ground). We found that oak patch size had no significant effect on bacterial communities or decomposition rates. However, bacterial richness increased with greater patch isolation and when oak litter patches were surrounded by a matrix of pine litter, rather than bare ground. The benefit of patch isolation for biodiversity runs counter to that expected by island biogeography theory, suggesting that spatially dependent interspecific interactions, such as predation or competition, may override direct dispersal effects. Higher bacterial richness in oak litter patches surrounded by a pine litter matrix indicated that spillover from neighboring matrix habitat can increase local richness. Instead of greater bacterial richness enhancing ecosystem functioning, leaf litter decomposition was negatively correlated with bacterial richness: Decomposition was slower in isolated oak litter patches and patches surrounded by a pine litter matrix. This negative relationship may be a result of spatial dynamics that can promote the persistence of bacteria from pine litter habitats that are not well suited to oak litter decomposition. Overall, our experiment indicates that effects of habitat loss, isolation, and matrix quality on richness and composition may depend on spatially constrained interspecific interactions, which can determine the functional ability of communities.

Key words: biodiversity; fragmentation; leaf litter decomposition; metacommunity; microbial community; soil; spatial; spillover; terminal restriction fragment length polymorphism.

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INTRODUCTION

The earth's ecosystems are being transformed by human land-use and land cover change (Haddad et al. 2015). The resultant loss and degradation of natural habitat is a principal threat to global biodiversity and ecosystem function (Gibson et al. 2013, Pimm et al. 2014, Newbold et al. 2015). The premise that a greater amount of less-isolated habitat enhances biodiversity is rooted in early theory such as the species–area relationship (Preston 1962) and island biogeography (MacArthur and Wilson 1967) and has become central to landscape ecology and conservation biology. Yet there is ongoing debate as to the importance of habitat amount and isolation

for biodiversity (Didham et al. 2012, Fahrig 2013, Haddad et al. 2017). The debate continues in part because of the inherent challenge of disentangling the effects of habitat amount and isolation on biodiversity (Fahrig 2003), but also because the quality of intervening habitat (the matrix) can mediate these effects. For example, matrix habitat type or quality can affect the ease of dispersal between patches (Castellón and Sieving 2006, Haynes and Cronin 2006), or act as a colonization source for habitat generalists (Cook et al. 2002, Spiesman and Cumming 2008). Moreover, because biodiversity can affect ecosystem functioning (Hättenschwiler et al. 2005, Tilman et al. 2014), landscape structure should affect both biodiversity and ecosystem function.

In habitat loss and fragmentation studies, habitat amount often has a stronger (positive) effect on communities than the spatial arrangement of habitat (reviewed in Fahrig 2003, but see Haddad et al. 2017). When habitat arrangement does have an effect, a common expectation is that increased isolation will reduce biodiversity. However, Fahrig's (2017) review of the literature suggests the opposite is true: When the effect of habitat amount is accounted for, a significant effect of habitat isolation on biodiversity is usually positive. Isolation effects on functional connectivity or interspecific interactions, as opposed to a pure dispersal effect, may help explain positive isolation effects. For example, isolated patches could serve as refuges from predation or competition, thereby increasing persistence if predators (Huffaker 1958, Holyoak and Lawler 1996, Cooper et al. 2012) or dominant competitors (Levins and Culver 1971, Calcagno et al. 2006, Livingston et al. 2012) cannot reach isolated patches.

The type or quality of matrix habitat can affect the connectivity of local communities (Ricketts 2001, Jules and Shahani 2003, Kennedy et al. 2011) and thus affect local biodiversity in at least three ways. One is by altering the functional connectivity among local communities. For example, a matrix habitat that presents harsh abiotic conditions or is difficult to penetrate may be a barrier to dispersal (Gonzalez et al. 1998, Kuefler et al. 2010). Second, matrix habitats can supplement local resources. Temporary excursions into the matrix to forage for alternative resources (Brotons et al. 2003, Rand et al. 2006), or spillover of resources from the matrix into focal habitat patches (spatial subsidies) can have a range of effects on local communities (reviewed in Polis et al. 1997). Spillover of detritus, for instance, may alter local biogeochemistry or nutrient composition, thereby affecting local habitat suitability or ecosystem process rates (Rousk et al. 2010). Third, the matrix can act as a source of species. Habitat generalists that colonize a focal habitat from the matrix may increase diversity in the focal habitat (Cook et al. 2002). However, the effect of colonizers on competitive dynamics and/or trophic interactions could have variable effects on local diversity (reviewed in Holt 1993, Leibold et al. 2004, Rand et al. 2006).

Landscape structure effects on biodiversity will have functional consequences. A large body of research has shown that increasing biodiversity generally enhances the functioning of ecosystems because diverse communities are more likely to include highly functional species and/or because of the greater potential for functional complementarity (Vandermeer 1989, Hättenschwiler et al. 2005, Bardgett and van der Putten 2014, Tilman et al. 2014). Leaf litter decomposition, for example, is a vital function that links biotic and abiotic components of the ecosystem by recycling nutrients for plants, such as nitrogen, and phosphorous (Coleman et al. 2004, Van Der Heijden et al. 2008). Both the composition and richness of microbial decomposer species can influence decomposition rates (Coûteaux et al. 1995, Strickland et al. 2009), and the benefit of microbial diversity for decomposition may result from increased functional complementarity among decomposer groups (Heemsbergen et al. 2004). Consequently, if the amount and isolation of habitat in a landscape affects the diversity of decomposer communities, decomposition rates are likely to be affected. Moreover, some microbes are better adapted for decomposing particular material (Strickland et al. 2009). Therefore, if emigration of bacteria from a different habitat (e.g., the matrix) increases local richness, effects on decomposition may not follow biodiversity-ecosystem functioning predictions if these emigrants are poorly adapted for decomposing the litter in their new patch. Understanding how land cover change affects the richness and composition of local communities may therefore strengthen our understanding of the relationship between biodiversity and ecosystem

functioning (Loreau et al. 2003, Hooper et al. 2005, Van Der Heijden et al. 2008).

Here, we present the results of a microcosm experiment in which we simultaneously manipulated habitat area, isolation, and matrix habitat type to determine their effects on the structure and function (i.e., leaf litter decomposition rate) of ecological communities. We focused on the response of natural bacteria communities inhabiting and moving among experimentally arranged patches of oak (*Quercus geminata*) and pine (*Pinus clausa*) leaf litter and soil in 1 × 1 m landscapes (Fig. 1). Similar to other microcosm studies that use microbes or small arthropods, we use a small-scale system to mimic the effects of land cover change on community and ecosystem dynamics that could occur over broader spatial and temporal scales in human-dominated landscapes (Gonzalez et al. 1998, Srivastava and Lawton 1998, Holyoak 2000, Kneitel et al. 2003). We use these replicated miniature landscapes to ask three questions: (1) How do structural features of a landscape (i.e., patch size, isolation, and matrix habitat type) affect the local richness and composition of bacterial communities; (2) How does patch size, isolation, and matrix type affect the local rate of leaf litter decomposition; and (3) How is decomposition associated with landscape-dependent effects on bacterial communities? We expected that bacterial richness would be greater in larger and more connected patches, but that patch size and arrangement effects would depend on matrix type (i.e., as indicated by a statistical interaction). We also expected that landscape-dependent benefits to bacterial richness and composition would result in greater rates of oak leaf decomposition.

Methods

Experimental design

Using microcosm landscapes, we examined how patch size, isolation, and matrix habitat type affect bacterial richness and composition, and leaf litter decomposition rates. Large and small focal patches of oak leaf litter and soil, along with their associated natural communities of microorganisms and arthropods, were arranged in a connected or isolated pattern and set within a matrix of either bare ground or pine litter (Fig. 1). Patch size, arrangement, and matrix type were thus manipulated in a 2 × 2 × 2 fully

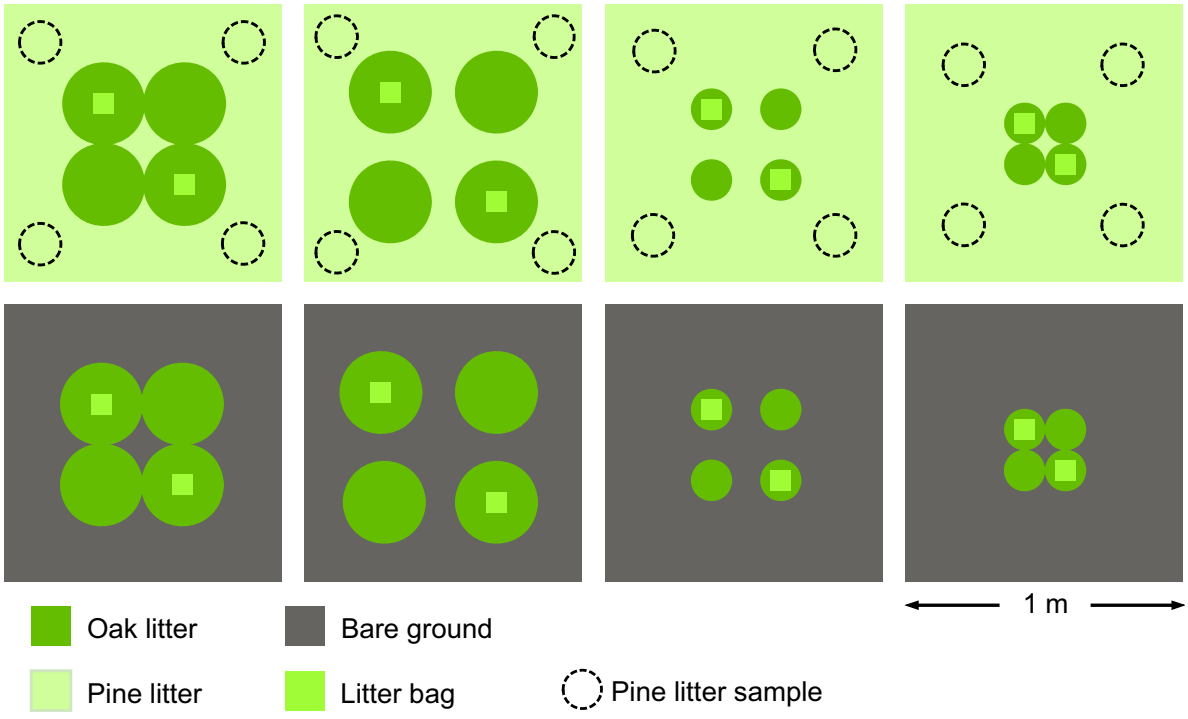

Fig. 1. Layout of experimental landscapes (to scale).

factorial design. Each 1-m^2 landscape contained four focal patches of oak litter that were either large (30 cm d) or small (15 cm d) and arranged in either a connected (patches barely touched) or isolated (patches separated by 10 cm) pattern.

Although bacteria may seem an abstract representation and removed from the plants, vertebrates, and invertebrates typically studied at a landscape scale, microorganisms are often used to test hypotheses in spatial ecology (Holyoak and Lawler 1996, Holyoak 2000, Kneitel et al. 2003). Moreover, the size of our microcosm landscapes and the bacteria that inhabit them scale approximately proportionally to macroorganisms inhabiting larger landscapes. For example, the length of a soil bacterium typically ranges between approximately 1 and 5 μm (Portillo et al. 2013), which results in bacterium to landscape size ratios ranging from 1 to 5 μm/m. By comparison, the size ratio of larger organisms, such as understory forb seeds, insects, or birds (0.25–10 cm) inhabiting 2-km landscapes, is 0.125–10.0 cm/km. Converting to the same units, the ratios span overlapping ranges (bacteria: 1.0×10^{-4} to 5.0×10^{-4} cm/m, larger organisms: 1.25×10^{-4} to 5.0×10^{-3} cm/m). We assume that most of the movement of bacteria among our experimental patches occurs passively through the flow of rain water and/or through the movement of soil microarthropods (e.g., collembola or oribatid mites). This type of dispersal is conceptually similar to dispersal in plants, for example, that rely on wind or animals for seed dispersal. Although we designed our microcosms to provide insight into larger-scale processes, some aspects of bacterial biology and/or ecology may not scale up accordingly. For example, because the generation time of bacteria is relatively short, the results we show here may take longer to materialize in landscapes of longer-lived plants and animals.

The experiment was established at the Florida State University Mission Road Research Facility in Tallahassee, Florida, USA, in July 2008. Experimental landscapes were constructed on an open lawn by first laying down black landscaping fabric. Oak litter and topsoil were collected from a homogeneous stand of *Quercus geminata* (sand live oak) in the nearby Apalachicola National Forest (ANF). Pine litter and topsoil were collected from a homogeneous stand of *Pinus clausa* (sand pine) on a managed pine plantation near Tallahassee, Florida. Both leaf litter types were collected along with the top 3 cm of soil below the leaves. Leaves and soil of each type were then separately mixed in large containers to homogenize their respective communities. Landscapes were constructed using templates to ensure patch size and arrangement for each replicate. For each leaf litter type, a 3-cm bed of matching top soil was laid down on the fabric and then covered with another 3-cm layer of the corresponding leaf litter. In the "bare ground-matrix" treatment, the bare landscaping fabric served as an alternative matrix habitat type. Leaf litter patches were held in place using 1.5-cm mesh plastic bird netting, which maintained the physical structure of landscapes over the course of the experiment. Shade cloth with 30% light transmission was suspended approximately 1.5 m above the landscapes to mimic the effects of natural tree canopy cover on microclimate conditions and limit temperature extremes. Experimental landscapes were separated by 1.5 m and divided into three spatial blocks to account for a slight slope that may have introduced a moisture gradient. Treatments were watered during longer periods without rain.

Mass loss from litter bags was used to quantify differences in decomposition rates of oak litter among landscape treatments. Litter bags were made of two 10 × 10 cm pieces of 2-mm polyester mesh. Freshly fallen *Q. geminata* leaves were collected from two locations in ANF and then oven-dried at 65°C for 48 h, and approximately 2 g of whole leaves was placed in each litter bag. After initial masses were recorded, litter bags were placed on the surface of two oak patches in each landscape (Fig. 1).

Experimental landscapes were destructively sampled, and litter bags were collected in July 2009. For the focal oak habitat, each entire patch of oak litter and soil was collected. From landscapes containing a pine litter matrix, we also collected four 15 cm d samples of pine litter and soil halfway between each oak patch and the landscape corner (Fig. 1). Samples for microbial community analyses were then preserved at −20°C. Litter bags were dried, and the contents were reweighed to determine the percent dry mass loss.

We measured soil pH and temperature to determine how potential landscape-dependent

differences in abiotic factors affect decomposition rates. Soil temperature was measured approximately 2 cm below the surface of each oak leaf litter patch, and soil pH was measured at the end of the experiment from collected samples.

A molecular characterization of leaf litter microbial communities

We used terminal restriction fragment length polymorphism (T-RFLP) to characterize and quantify differences among leaf litter microbial communities (Liu et al. 1997). The T-RFLP method relies on differences among species in the binding site position of specific restriction enzymes on a target gene to generate a fingerprint representing the unique combination of species in a sample. To perform T-RFLP analysis, we first homogenized preserved leaf litter and soil samples from experimental patches, and then isolated genomic DNA from 0.25 g of the homogenized samples using the PowerSoil DNA Isolation Kit following the manufacturer's instructions (Mo Bio Laboratories, Carlsbad, California, USA). We then amplified the 16S rRNA gene using the universal forward primer 27F (5′-AGA GTT TGAT CCT GGC TCA G-3′) and the universal reverse primer 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′). The 5′ end of the forward primer was labeled with the dye 6-FAM (Integrated DNA Technologies, Coralville, Iowa, USA). The PCR reaction included 1 μL template DNA (~30 ng/μL), 45 μL platinum PCR Supermix High Fidelity (Life Technologies, Carlsbad, California, USA), and 0.2 μmol/L of each of the forward and reverse primers. PCR amplification proceeded with initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 20 s, 53°C for 15 s, and 68°C for 105 s. Amplified DNA was then purified using a magnetic bead process (SprintPrep; Agencourt Bioscience, Beverly, Massachusetts, USA) following the manufacturer's guidelines. Each sample was then prepared for T-RFLP analysis by combining 1 μL of the purified PCR product with 11.72 μL of formamide and 0.28 μL of size standard (GeneScan 500 LIZ; Applied Biosystems, Foster City, California, USA) on 96-well plates. Samples were processed on two plates in the Florida State University Department of Biology Bioanalytical and Sequencing Facility using an Applied Biosystems 3730 Genetic Analyzer. This automated system first digests samples with restriction enzymes, then separates fragments by capillary electrophoresis, followed by laser detection of the dye-labeled terminal restriction fragments (T-RFs). Peaks in dye intensity represent the set of T-RFs (or operational taxonomic units) present in the sample. We aligned, noise-filtered, and then converted T-RFs to a sample by T-RF presence–absence matrix for statistical analysis using the program T-REX (Culman et al. 2009).

Terminal restriction fragment length polymorphism is a robust and reproducible method (Osborn et al. 2000) that has been effectively used to assess dissimilarity among bacterial community samples (Fierer and Jackson 2006, Jesus et al. 2009) and changes over space and time (Lukow et al. 2000). A principle limitation of T-RFLP is that multiple species can be represented by the same T-RF if they share the same restriction enzyme binding sites. Thus, using T-RFLP to identify taxonomic species or estimate the actual number of species is difficult. Another potential limitation is that additional minor T-RF peaks have been detected within some fungal species near a dominant peak (Avis et al. 2006). However, we have no reason to believe our bacterial samples were systematically biased in this regard. That said, T-RFLP is widely regarded as an effective method for comparing relative microbial richness and dissimilarity between samples and assessing change over time when carefully applied (Lukow et al. 2000, Lueders and Friedrich 2003, Orcutt et al. 2009). Although we recognize that each T-RF does not necessarily represent a unique species, we use the number of T-RFs in each sample as an estimate of relative bacterial richness.

Statistical analysis

We used linear mixed-effects models in R v3.3.3 (R Development Core Team 2017) with the package lme4 v1.1-13 (Bates et al. 2015) to examine fixed effects of oak leaf litter patch size (large or small), arrangement (connected or isolated), and matrix type (pine litter or bare ground) on bacterial richness. Soil pH and temperature were included as covariates to examine how potential changes in abiotic conditions affect richness. Spatial block (A, B, or C) and T-RFLP analysis block (plate A or B) were also included as fixed effects. Because multiple samples (patches) were taken of each landscape, landscape ID was included as

a random effect. We initially included two-way interactions between the three landscape treatments and removed non-significant interactions and covariates from the analysis to simplify our statistical models. *P*-values were calculated based on Satterthwaite approximations of degrees of freedom using the R package afex v0.17-8 (Singmann et al. 2017).

Permutational multivariate analysis of variance (PERMANOVA) was conducted using the R package vegan v2.4-0 (Oksanen et al. 2016) to examine the effect of patch size, arrangement, and matrix quality on microbial community composition. We used Jaccard dissimilarity based on presence–absence data. Soil pH, temperature, spatial block, and T-RFLP analysis block were included in the analysis as covariates. Because four oak litter patches occur within each landscape, we included landscape ID as a grouping factor (strata) in which to constrain permutations. In order to assess the potential for movement of habitat generalists originating from the pine litter matrix, we also used PERMANOVA to assess compositional differences between oak and pine litter habitats. The same covariates and grouping factor described above were included in this analysis. For both PERMANOVAs, we removed non-significant covariates and *P*-values were based on marginal effects of the terms, estimated over 9999 permutations.

Using linear mixed-effects models, we performed three separate analyses to test for effects of the landscape treatments, bacterial richness, and composition on litter mass loss. For each analysis, soil pH, temperature, spatial block, T-RFLP analysis block, and litter origin were included as covariates and landscape ID was included as a random effect. Species composition was represented by a one-axis non-metric multidimensional scaling solution based on presence–absence data and Jaccard distances. The rate of leaf litter decomposition can be affected by abiotic conditions, such as soil pH and temperature (Coleman et al. 2004). We therefore used linear mixed-effects models to assess the effect of the landscape treatments on soil pH and temperature (a potential mechanism of a landscape structure effect on leaf litter decomposition). Spatial block, patch size, arrangement, and matrix type were included as fixed effects, and landscape ID was included as a random effect. For all analyses, multicollinearity among predictor variables was low (variance inflation factor < 2) and residuals were normally distributed.

Results

Terminal restriction fragment length polymorphism analysis of 16S rRNA gene samples showed that each 0.25 g sample of litter and soil contained on average 34.2 (±14.7 SD) T-RFs, each representing at least one (but likely multiple) taxonomic species. Although relative bacterial (T-RF) richness was, on average, lower in small compared to large patches, there was no significant effect of patch size (Fig. 2A; $t_{24,88} = -1.4$, $P = 0.185$). Bacterial richness was, however, significantly greater in isolated compared to connected patches (Fig. 2B; $t_{24,88} = 2.7$, $P = 0.012$) and significantly greater when the focal oak litter was surrounded by a pine litter matrix compared to a matrix of bare ground (Fig. 2C; $t_{24,88} = 2.1$, $P = 0.044$). There were no significant two-way interactions among landscape treatments and no significant main effects of soil pH, temperature, or T-RFLP analysis block (plate) on bacterial richness. We therefore removed these factors from the final model (full statistics are shown in Appendix S1: Table S1). Permutational multivariate analysis of variance showed that community composition was also significantly affected by arrangement ($F_{1,90} = 2.7$, $P = 0.007$) and matrix type ($F_{1,90} = 1.9$, $P = 0.045$), but not by patch size ($F_{1,90} = 1.2$, $P = 0.250$; Fig. 3; Appendix S1: Table S2). Like the effects of arrangement and matrix type on bacterial richness, connected and bare ground-matrix patches had similar effects on community composition. The covariates soil pH, temperature, and T-RFLP analysis block had no significant effects and were removed from the final statistical model.

Permutational multivariate analysis of variance also revealed a significant difference in bacterial composition between oak and pine litter habitats ($F_{1,\ 140} = 2.4$, $P = 0.004$; Appendix S1: Table S3). The covariates soil temperature, and spatial block had no significant effects and were removed from the final statistical model.

The rate of oak leaf litter decomposition within oak patches depended on matrix type and patch arrangement (Fig. 4; Appendix S1: Table S4). Matrix type had the strongest effect on decomposition, with mass loss after one year 5.8% greater in

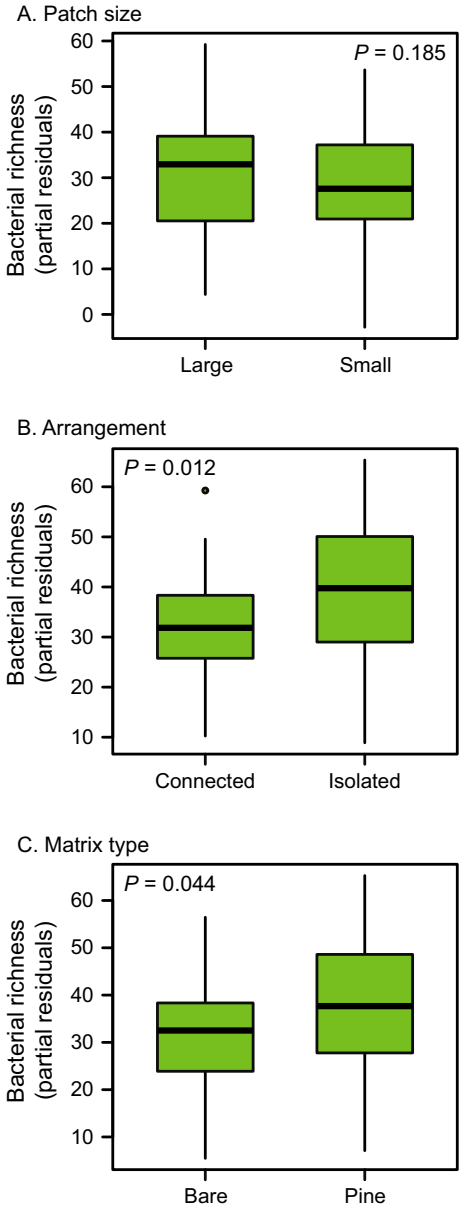

Fig. 2. Landscape treatment effects on bacterial richness (number of terminal restriction fragments) in oak patches. Bars indicate the median, and whiskers extend to the first and third quartiles. Partial residuals are shown after accounting for all other covariates. N = 48 for each treatment.

patches surrounded by bare ground compared to a pine litter matrix ($t_{40,31} = -5.3$, $P < 0.001$). Connected patches had 3.5% greater leaf mass loss than isolated patches ($t_{40,31} = -3.3$, $P = 0.002$). The effects of matrix type and patch arrangement on decomposition were therefore opposite that for bacterial richness, with greater decomposition in treatments with lower bacterial richness. Patch size had no significant effect on leaf mass loss ($t_{40,31} = 0.1$, $P = 0.929$). There were no significant two-way interactions between landscape treatments in their effect on decomposition, which were therefore removed from the final statistical model.

Neither soil pH nor temperature had a significant effect on decomposition. We therefore removed these factors from the final model. Moreover, there was no significant effect of the landscape treatments on soil temperature (patch size: $t_{40,32} = 0.4$, $P = 0.679$; arrangement: $t_{40,32} = 1.6$, $P = 0.130$; matrix: $t_{40,32} = 0.5$, $P = 0.651$). There was no significant effect of arrangement ($t_{40,32} = -1.1$, $P = 0.280$) or matrix type ($t_{40,32} = -1.8$, $P = 0.075$) on soil pH. Although there was a significant patch size effect on soil pH ($t_{40,32} = 2.5$, $P = 0.015$), patch size had no effect on leaf litter decomposition. Together, these results indicate that landscape structure effects on litter decomposition were not a result of effects on the abiotic environment. Instead, the effect of landscape structure on decomposition may be a result of the landscape effect on local community structure. There was a significant negative relationship between bacterial richness and the rate of oak litter decomposition ($t_{35,33} = -2.4$, $P = 0.024$;

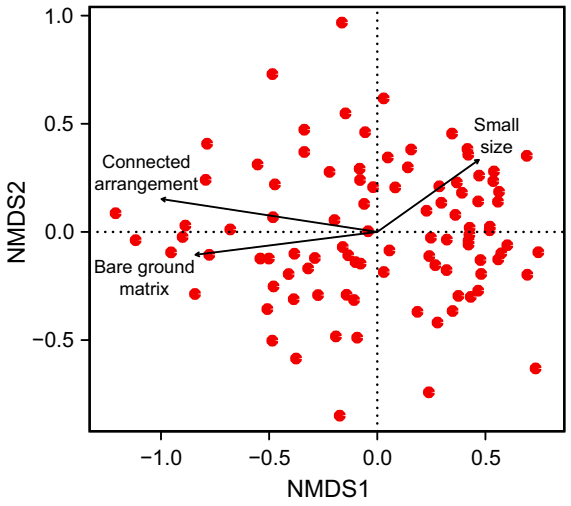

Fig. 3. Non-metric multidimensional scaling visualization of permutational multivariate analysis of variance results. Vector overlay length and direction are proportional to their correlation with the two axes.

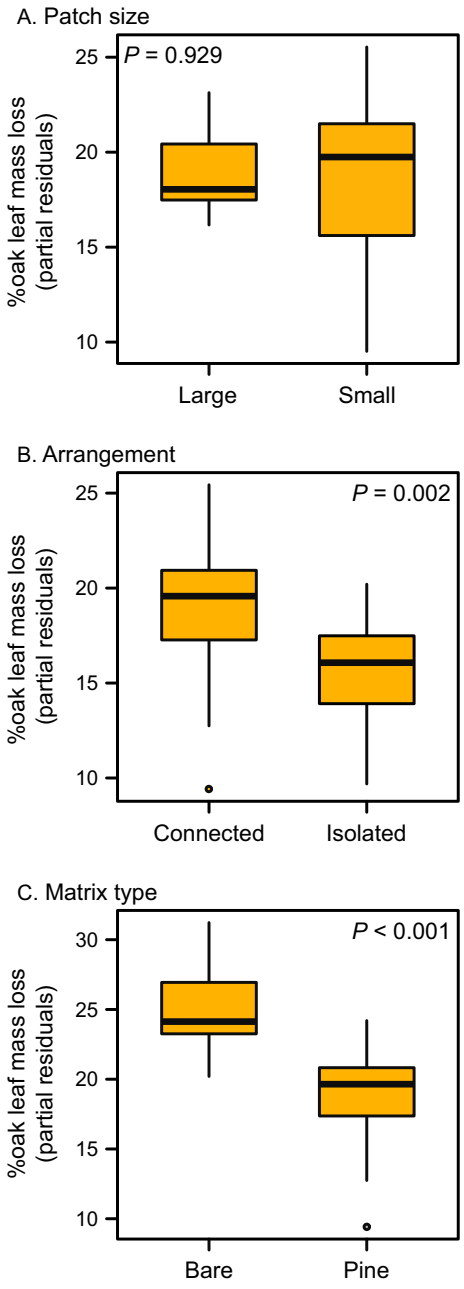

Fig. 4. Landscape treatment effects on percentage of oak litter mass loss. Bars indicate the median, and whiskers extend to the first and third quartiles. Partial residuals are shown after accounting for all other covariates. N = 24 for each treatment.

Fig. 5A; Appendix S1: Table S5), which is consistent with the effects of landscape structure on decomposition. The local composition of bacteria in oak litter patches was also correlated with the rate of litter decomposition ($t_{39,33}$ = 3.4, P = 0.002; Fig. 5B; Appendix S1: Table S6).

Discussion

We manipulated focal patch size, isolation, and matrix type in landscape microcosms to examine how landscape structure affects the biodiversity and functioning of bacterial communities. Our

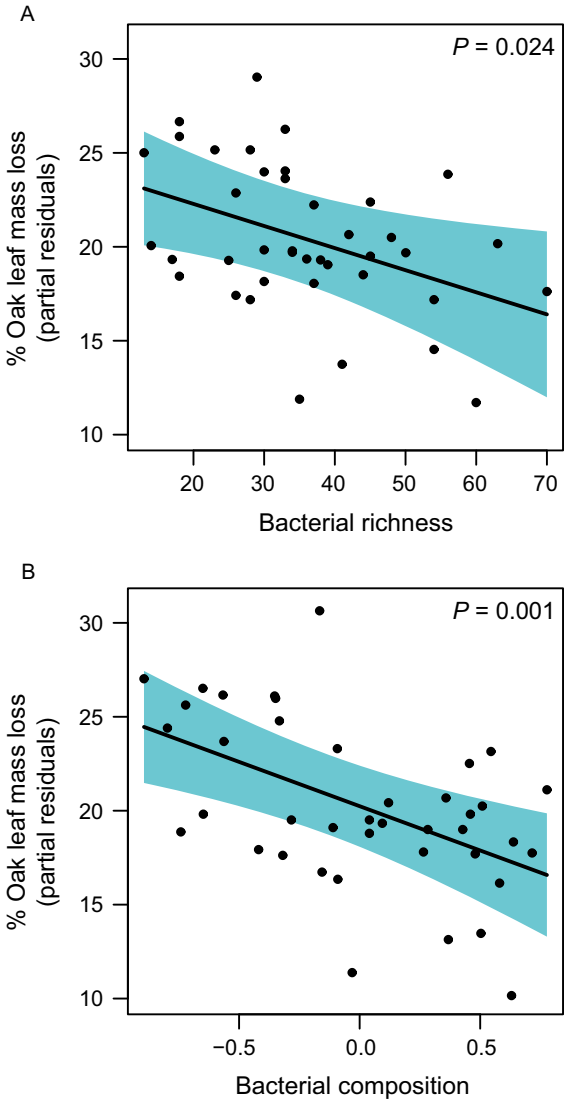

Fig. 5. Relationships between oak leaf litter mass loss and (A) bacterial richness or (B) composition (i.e., single-axis non-metric multidimensional scaling scores). Partial residuals are shown after accounting for all other covariates.

results showed that bacterial (T-RF) richness increased in more isolated oak litter patches and patches surrounded by a matrix of pine leaf litter, as opposed to bare ground. Bacterial community composition, which was correlated with richness, responded similarly. The functioning of leaf litter communities, quantified as the rate of oak leaf litter decomposition, was also affected by patch isolation and matrix type, with faster litter decomposition in connected patches and patches surrounded by bare ground. Decomposition rates were therefore lower in communities with more species, counter to expectation. We found no significant effects of patch size on community structure or decomposition. Moreover, we found no interacting effects of patch size, isolation, and matrix type, suggesting that these factors have independent effects on bacterial communities and decomposition rates in this system.

Patch isolation had the strongest effect on bacterial richness and composition. However, contrary to the negative effect of isolation predicted by island biogeography theory (MacArthur and Wilson 1967), bacterial richness was greater in isolated patches, compared to connected patches. Increasing isolation is typically thought to decrease species richness by reducing the ease of dispersal among patches (Gonzalez et al. 1998, Peay et al. 2010), limiting access to resources (Steffan-Dewenter 2003), and precluding rescue effects (Brown and Kodric-Brown 1977). However, as Fahrig's (2017) review has shown, when isolation (or fragmentation per se) has an effect, it is more often positive than negative for biodiversity. For example, isolated patches have greater edge area that can be more structurally complex and resource-rich, which can promote diversity (Macreadie et al. 2010, Caruso et al. 2011). Functional connectivity can also be enhanced with greater edge area (Healey and Hovel 2004). In our system, greater edge area may increase the capability of patches to intercept flows from other patches, thus increasing local richness through rescue effects or immigration of novel species. Alternatively, some of the limits on movement that isolation places on species may serve to promote diversity by affecting interspecific interactions. For example, moderately isolated patches could allow for competition–colonization trade-offs (Livingston et al. 2012) or repeated immigration of inferior competitors that enhance their persistence in isolated patches (Dufour et al. 2006). Isolation may also provide refuge from predation (Huffaker 1958, Holyoak 2000). Many microarthropods and other invertebrates consume bacteria, which can affect microbial composition, size structure, and abundance (Hahn and Höfle 2001, Rønn et al. 2002). Because microarthropods can require high connectivity among patches for persistence (Gonzalez et al. 1998), bacteria may escape predation in isolated patches. However, isolation can span a continuum (as opposed to our binary levels of isolation) with biodiversity peaking at intermediate levels of isolation; very high levels of isolation that prevent interpatch dispersal (potentially higher than we examined) can reduce biodiversity (Tilman et al. 1994, Holyoak and Lawler 1996, Kneitel et al. 2003). Furthermore, because our study encompassed many more generations than typical of studies conducted in larger-scale landscapes, we cannot rule out the possibility that our results would only be seen on timescales longer than most management activity. Nevertheless, our experimental results provide additional support for the idea that conservation programs should consider the potential for positive effects of habitat isolation on biodiversity (Fahrig 2017).

There was a significant difference in bacterial composition and greater richness in oak litter patches surrounded by a pine litter matrix compared to bare ground. This, in combination with the significant compositional difference between oak and pine litter habitats, suggests that colonization by generalist matrix species was important for oak litter communities. Flows of organisms across habitat boundaries can play critical roles in determining the abundance and composition of species (Holt 1993, Leibold et al. 2004, Tscharntke et al. 2012). For example, a mass effect (Shmida and Wilson 1985) or source-sink dynamics (Pulliam 1988) may allow for a flow of individuals from the pine litter matrix to oak patches that enhances persistence and local bacterial richness. Although netting maintained the physical structure of our landscapes throughout the experiment, frequent and heavy north Florida rains may have allowed for movement of bacteria among patches within surface flows of water. A matrix effect on richness may also result from more complex trophic dynamics. For instance, movement of mobile predators from the pine litter matrix may enhance coexistence of competitors (Caswell 1978). It is

possible that the presence of a pine litter matrix affected connectivity among oak patches; however, if that were the case, we would have expected to find an interaction between isolation and matrix type, which we did not. An increased flow of organisms from the pine litter matrix would imply that variability in matrix habitat quality can affect a different form of connectivity than discussed above—connectivity among different habitats. It will be difficult to separate matrix effects via spillover from connectivity of heterogeneous habitats.

As expected, larger patches had greater mean bacterial richness; however, this increase in richness was not significant. In typical landscapes, larger areas tend to include greater habitat heterogeneity, which, up to a point, supports more different species (Rosenzweig 1995). It may be that our small oak litter patches, which were intentionally mixed to be relatively homogeneous within treatments and replicates, were sufficiently large to include a saturating level of habitat heterogeneity for local microbial communities. However, the greater bacterial richness in oak litter patches surrounded by pine litter matrix suggests a somewhat different area effect, which is that increasing habitat heterogeneity at a spatial scale beyond the focal patch can increase focal patch richness (i.e., more pine litter vs. inhospitable bare ground; Tscharntke et al. 2012).

The effects of landscape structure on oak leaf litter decomposition appear to have been mediated by changes in bacterial composition and richness rather than landscape-dependent changes in temperature or soil pH. Like bacterial communities, local decomposition rates were affected by matrix type and patch arrangement, with decomposition being lower in oak patches surrounded by pine litter and in isolated patches. Thus, there was a negative correlation between decomposition rate and bacterial richness. Although a positive effect of richness on ecosystem functioning is generally expected (Tilman et al. 2014), Jiang et al. (2008) point out that negative effects should be likely for some functions, such as decomposition. For example, variation in bacterial community composition may explain the negative effect we see if there is variation among bacteria in their ability to break down oak leaf litter (Strickland et al. 2009). In our study, matrix type had the strongest effect on decomposition, and although bacterial spillover from the pine litter matrix may have resulted in greater richness, it may have also resulted in greater abundances of bacteria that are poorly adapted to breaking down oak litter. Similarly, or in combination, dispersal from the pine litter matrix resulting in strong source-sink dynamics may override habitat filtering effects, allowing for the persistence of species that are poor decomposers of oak litter (Leibold et al. 2017).

Conclusions

We found that patch isolation and matrix habitat type affected the bacterial richness and composition in a landscape microcosm. However, contrary to expectations from island biogeography theory (MacArthur and Wilson 1967), which assumes no interspecific interactions, richness was greater in isolated, compared to connected patches. This unexpected result supports the idea that the details of interspecific interactions may be important for understanding how land cover change affects biodiversity (Robertson et al. 2013). Therefore, a more thorough integration of the holistic mechanisms of landscape ecology with the more reductionist mechanisms of metacommunity ecology might provide deeper insight into how landscape structure and interspecific interactions combine to affect biodiversity. This may also apply to the functional consequences of land cover change effects on biodiversity. For example, it is possible that the unexpected negative correlation between bacterial richness and decomposition results from a combination of bacterial functional traits and spatial dynamics. An interesting follow-up study would also include fungi or microarthropods, which can be important decomposers (Setälä and McLean 2004, Santonja et al. 2017) that might show different responses to microcosm landscape structure and explain the bacterial responses. Furthermore, our study should be corroborated by larger-scale experimentation on landscapes of plants and/or animals, which may help address any lingering questions of the utility of microcosms as a model for landscapes of larger, charismatic species (Srivastava et al. 2004). For example, what is the timescale of landscape structure effects on ecosystems relative to generation time? Nevertheless, our results support the case for greater inclusion of spatial dynamics into studies of ecosystem functioning (Loreau et al. 2003, Bardgett and van der Putten 2014).

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