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Fragmentation disrupts microbial effects on native plant community productivity

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Abstract

1. Anthropogenic habitat fragmentation – the breaking up of natural landscapes – is a pervasive threat to biodiversity and ecosystem function worldwide. Fragmentation results in a mosaic of remnant native habitat patches embedded in human-modified habitat known as the "matrix". By introducing novel environmental conditions in matrix habitats and reducing connectivity of native habitats, fragmentation can dramatically change how organisms experience their environment. The effects of fragmentation can be especially important in urban landscapes, which are expanding across the globe. Despite this surging threat and the importance of microbiomes for ecosystem services, we know very little about how fragmentation affects microbiomes and even less about their consequences for plant-microbe interactions in urban landscapes.
2. By combining field surveys, microbiome sequencing, and experimental mesocosms, we (1) investigated how microbial community diversity, composition, and functional profiles differed between 15 native pine rockland fragments and the adjacent urban matrix habitat, (2) identified habitat attributes that explained significant variation in microbial diversity of native core habitat compared to urban matrix, and (3) tested how changes in urbanized and low connectivity microbiomes affected plant community productivity.
3. We found urban and native microbiomes differed substantively in diversity, composition, and functional profiles, including symbiotic fungi decreasing 81% and pathogens increasing 327% in the urban matrix compared to native habitat. Further, fungal diversity rapidly declined as native habitats became

increasingly isolated, with ~50% of variation across the landscape explained by habitat connectivity alone. Interestingly, microbiomes from native habitats increased plant productivity by ~300% while urban matrix microbiomes had no effect, suggesting that urbanization may decouple beneficial plant-microbe interactions. In addition, microbial diversity within native habitats explained significant variation in plant community productivity, with higher productivity linked to more diverse microbiomes from more connected, larger fragments.

4. *Synthesis*. Taken together, our study not only documents significant changes in microbial diversity, composition, and functions in the urban matrix, but also supports that two aspects of habitat fragmentation — the introduction of a novel urban matrix and reduced habitat connectivity — disrupt microbial effects on plant community productivity, highlighting preservation of native microbiomes as critical for productivity in remnant fragments.

Introduction

Land conversion and anthropogenic habitat fragmentation - the breaking up of natural landscapes - are widespread threats to biodiversity (Fletcher et al. 2018; Haddad et al. 2015). An estimated 75% of Earth's terrestrial surface has been subjected to substantive human impacts, such as habitat fragmentation and loss (Venter et al. 2016). Fragmentation can have meaningful consequences for biodiversity and native communities through changes to remaining habitat patches (e.g., degradation in habitat quality, decrease in habitat area) and their connectivity (e.g., increased patch isolation) (Laurance et al. 2002; Haddad et al. 2015). Anthropogenic fragmentation can also affect communities through

93 introduction of novel "matrix" habitat(s), which is non-natural habitat interspersed
94 between native fragments (Kupfer et al. 2006). Historically matrix habitats were viewed as
95 uniform and ecologically less important than remaining fragments. However,
96 contemporary habitat fragmentation research has found that novel matrix habitats are also
97 critical component to understanding fragmentation's effects on organisms and
98 communities persisting in human-modified landscapes (Driscoll et al. 2013; Jules &
99 Shahani 2003; Öckinger et al. 2012; Ricketts 2001; Williams et al. 2006;).
100
101 The introduction of novel matrix habitat into landscapes can have consequences for native
102 communities through several pathways. The type and characteristics of matrix habitats can
103 affect organisms' dispersal between patches. At one extreme, matrix habitats can form
104 impassable barriers to species, decreasing dispersal to and from fragments and isolating
105 populations on individual remnants (Kupfer et al. 2006; Ricketts, 2001). At the other
106 extreme, matrices can act as conduits between native patches, providing additional
107 resources and transportation that increase dispersal (Bridgman et al. 2012; Stasek et al.
108 2008). Moreover, the introduction of novel matrix habitats surrounding fragments can also
109 affect organisms by changing availability and/or quality of habitat in the landscape
110 (Driscoll et al. 2013). For instance, the matrix can be uninhabitable for certain species, such
111 that they can only utilize remaining native habitat. However, in some cases, the matrix can
112 provide a new, preferred habitat or mitigate negative effects of habitat loss by providing
113 suitable, but lower quality habitat or additional resources (Ewers and Didham 2006; Evans
114 et al. 2016). The matrix habitat may also affect native communities by altering biotic
115 interactions that organisms usually experience in their native habitats. Matrix habitat can

have different environmental conditions than the native habitats they supplant (e.g., urban environments with higher temperatures and increased pollutants; Grimm et al. 2008; Johnson & Munshi-South 2017). These environmental changes can filter which organisms occur in the area thereby changing the pool of possible competitors, predators, prey, and mutualists available to interact with (Barros et al. 2019; Grimm et al. 2008; Miles et al. 2019; Moreira et al. 2019; Parsons et al. 2019). These changes in interactions can be as important for organismal and community responses to the introduction of matrix habitats as its abiotic features (Grimm et al. 2008; Johnson & Munshi-South 2017; Zarnetske et al. 2017). Through all of these ecological mechanisms, the matrix habitat can shape how individuals and communities interact within fragmented landscapes.

In recent years, it has become increasingly clear that matrix habitats are vital to understanding how communities are impacted by fragmented landscapes (Ewers & Didham 2006; Jules & Shahani 2003; Kupfer et al. 2006). As a result, investigating how communities differ in composition and productivity between matrix habitats and the cores of native fragments is garnering more attention in the recent habitat fragmentation literature (Evans et al. 2016; Ewers & Didham 2006; Jules & Shahani 2003; Kupfer et al. 2006; Lasmer et al. 2021; Matthews et al. 2021; Schlapfer et al. 2018). Studies examining consequences of matrix type for plant species diversity and richness have found their effects can be on par or even stronger than effects of habitat isolation and patch area (e.g., Guirado et al. 2006; Metzger 2000; Ockinger et al. 2012; Williams et al. 2006). For example, Ockinger et al. 2012 found variation in plant richness of grassland fragments was explained solely by matrix type. Fragments surrounded by a natural forest had higher richness than

fragments surrounded by agricultural matrix. However, these studies mainly focus on macro-organisms despite microorganisms' importance to many communities and ecosystem processes (e.g., nutrient cycling) as well as plant and animal health and functioning (Christian et al. 2015; Fierer 2017; Mony et al 2022; Wagg et al. 2019; Wardle 2004).

Microbiomes are also likely to be affected by fragmentation of natural ecosystems. For instance, microbial dispersal across landscapes, which depends on dispersal limitation and environmental filtering, can be impacted by fragmentation through both reducing habitat connectivity (e.g., increasing distance between suitable habitat/hosts) as well as shifts in abiotic/biotic conditions on remanent fragments (e.g., reducing habitat quality) (Martiny et al. 2006; Mony et al. 2022). Recent work has demonstrated that at least some microbial taxa experience dispersal limitation as well as provided support for changes in the environmental that are often associated with fragmentation affecting microbial communities (Boeraeve et al. 2018; Flores-Rentería et al. 2016; Grilli et al. 2014; Kiesewetter & Afkhami 2021; Peay et al. 2010; 2012; Vannette et al. 2016; Xiao et al. 2018). Further, Kiesewetter & Afkhami (2021) demonstrated microbial communities responded to fragmentation-driven changes in habitat connectivity, which in turn affected native plant performance/allocation (at the individual plant level). However, it remains unclear how fragmentation-driven connectivity and matrix effects on the microbiome scale up to impact plant community-level dynamics and plant productivity in human-modified landscapes.

Here, we aim to improve our understanding of how urbanization and consequent habitat fragmentation shape microbiomes and how these changes in microbial communities scale up to affect plant community composition and productivity. We do this by integrating field surveys, microbiome sequencing, and experimental mesocosm studies in the highly-fragmented pine rockland habitat of South Florida using microbiomes from native core habitats and urban matrix habitats. Microbiomes from native core habitat (hereafter referred to as "native microbiomes") are microbial communities collected from near the center of pine rockland fragments, while urban matrix microbiomes (hereafter "urban microbiomes") are microbiomes collected from adjacent urban environments in which the native pine rockland remnant habitat are embedded. Specifically, we (1) assessed diversity, composition, and functional profiles of microbiomes from 15 habitat pairs of native fragment core habitats and adjacent urban matrix habitats, (2) identified habitat attributes that explained variation in microbial diversity within native versus urban habitats, and (3) experimentally tested how these 15 native and 15 urban microbiomes affect plant community productivity and composition. Given the strong contrast in the habitat characteristics of native and urban habitats (Nugent & Allison, 2022), we hypothesized that urban microbiomes will significantly differ in community composition, diversity, and functional guilds from the native microbiomes. We also predicted that habitat connectivity will play an important role in explaining variation in native microbiomes due to the increased isolation native habitats experience in a fragmented landscape, while unique stresses and frequent disturbances of the urban matrix (Nugent & Allison, 2022) may lead to more stochastic urban microbial communities. Finally, we hypothesized that native plant community productivity in our experimental mesocosms

would be greater when microbes from either habitat type are present compared to sterile treatments, but plant community productivity will benefit more from native habitat microbiomes than their urban counterparts.

Methods

Study system

The pine rocklands is a critically imperiled ecosystem occurring exclusively in South Florida and the Florida Keys with limited distribution on some Caribbean islands (FNAI 2010). This habitat is characterized by frequent fire, oolitic limestone outcroppings, open pine canopy, patchy subcanopy of palms and shrubs, and a rich herbaceous layer (Snyder et al. 1990). Urbanization of Miami-Dade County introduced a novel matrix habitat that highly fragmented the pine rocklands along the Miami rock ridge, an ~80km oolitic limestone ridge where pine rocklands historically existed (Possley et al. 2008; Snyder et al. 1990). The remaining habitat (~2% of the historic range; Figure 1) occurs along this ridge in 1-300 acre fragments.

Habitat classifications, soil collections, and habitat attributes

To assess how microbial communities differed between native habitat and urban, we identified 15 pine rockland fragments (Figure 1) spanning the historic pine rockland range and whose adjacent matrix habitats (i.e., those within 500m buffer zones around the fragments' centroids) included high-intensity urban areas as classified by Kawula & Redner (2018). To determine each native habitat fragment's core, we created a 10m buffer around

each fragment's centroid (ArcMap 10.5; ESRI, Redlands, CA, USA). For each of the 15 native-urban fragment pairs, we randomly selected three points within the native core buffer zone and three points within the urban matrix buffer zone. Soils were aseptically collected from each point, homogenized within fragment-habitat type combination (totaling 30 microbiomes), and transported to the University of Miami (Coral Gables, FL) on ice. We also collected data on ten habitat attributes at each site, including three common metrics of habitat connectivity to quantify the degree of fragmentation (i.e., distance to nearest neighboring patch, patch-based weighted sum, number of fragments in 10km buffer; Calabrese & Fagan, 2004; Kindlmann & Burel, 2008), three patch characteristics (fragment area, time since most recent fire, plant richness), two urban matrix attributes (percent transportation, evenness of the surrounding matrix habitat types), and two soil properties (pH, Total Kjeldahl Nitrogen). We also determined phosphorus availability for a subset of site pairs. See SI Methods on "Habitat attributes" for more details on these metrics. Field work was permitted by Miami-Dade County Parks and Recreation (permit #309).

Microbial DNA extractions, library preparations, processing, and characterization

We extracted microbial DNA from 0.3g of soil for all sites using the DNeasy 96 PowerSoil Pro QIAcube HT kit (Qiagen; 47021; Revillini et al. 2022). We checked DNA concentrations and performed initial quality control using endpoint PCR, gel electrophoresis, and Qubit 4 Fluorometer (Invitrogen; Q33327) measurements. We prepared 60 prokaryotic and fungal libraries using a two-step dual indexing protocol with *16S 515F/806R* (forward/reverse) and *ITS ITS1/ITS2* amplicons and magnetic bead cleanups (Gohl et al., 2016). Libraries were subsequently sequenced at University of Miami's Genomics Core with the Illumina

MiSeq platform (v3, 300bp paired end). Lack of contamination was confirmed with negative controls (Ultrapure water replaced soil during extractions). After sequencing, reads were processed using *QIIME2* (v2020.1) and denoised and grouped into exact sequence variants (i.e., ESVs; 100% sequence similarity) using *dada2*. All samples reached saturation (i.e., refraction curve plateaued) by ~2000 reads. We normalized samples, calculated Shannon diversity, species richness, and Pielou's evenness (*vegan*; Oksanen 2020), and classified ESVs into microbial "species" using microbial databases (McDonald et al. 2012; Werner et al. 2012; Nilsson et al. 2019). Details are available in SI Methods: "DNA extractions, library preparation, and processing" and "Microbial DNA characterization"*)*.

Understory community experiment

We grew eight pine rockland native, understory plant species (ranging in life histories and habitat specialization; Table S2) in a factorial mesocosm experiment with microbiomes from 15 fragment origins x 2 habitat types (urban/native) x 2 microbial treatment (live/sterilized) x 3 replicates (total=180 mesocosms). Sterile 1.6L pots were inoculated with either *live* (microbial active) or *sterilized* soils from each of the 30 fragment-habitat type combinations (i.e., 15 pairs of urban and native habitat microbiomes). Each pot contained 30% (by volume) sterilized Miami rock ridge limestone, 50% sterile pine rocklands background soil, a 15% inoculum layer of microbially active or sterile soil from the relevant fragment-habitat type combination, and a 5% sterile soil cap to avoid microbial desiccation (David et al. 2020; Kiesewetter & Afkhami 2021; SI Methods: "Experimental setup"). To minimize site-specific abiotic effects of soils, we used a standardized sterile background soil collected from a pine rockland site that is roughly

central to all fragments used in our study (Porter Russell Pine Rockland). This was done to ensure that the majority of soil in each mesocosm had identical abiotic properties, with only biotic components of the soil being able to disperse from the much smaller amount of treatment soil (inoculum) to colonize the rest of the pot. Sterilized limestone/soils were autoclaved three times at 121°C (2 hours/cycle) to remove the soil biota, which includes fungi and prokaryotes (the focus of this study) as well as other soil fauna. All seeds were collected from Miami-Dade pine rocklands by the authors and collaborators from pine rockland restoration groups (Table S2), surface sterilized, and planted into prepared mesocosms (1 seed/species/pot). Germination and survival were recorded weekly. After 7 months (December 2019-July 2020), plants were harvested, dried, and weighed. Aboveground biomass was collected separately for individual plants within each mesocosm, while belowground biomass was collected for each mesocosm.

Data analysis

All statistical analyses were performed with R v3.6.1 (R Core Team, 2021). To determine if microbial diversity differed between native and urban habitats, we performed mixed linear models (*lme4*; Bates, 2019) with habitat type as the explanatory variable, fragment-urban pair identity as a random effect, and either fungal or prokaryotic diversity as the response variable. Upon finding significant diversity effects, we investigated which components of Shannon diversity (species richness or Pielou's evenness) were responsive to habitat type using mixed linear models with fungal/prokaryotic diversity components as new response variables and the same explanatory and random variables (Table S3). We then performed PERMANOVA to test if microbial community composition differed between habitat types

using adonis (*vegan,* Oksanen 2020), with a Bray-Curtis distance matrix of prokaryotic or fungal taxa, and a random effect of fragment identity. To investigate which microbial families are particularly important for distinguishing habitat type, we performed random forest models with Boruta feature selection, accounting for multiple comparisons (*Boruta*, Kursa & Rudnicki 2020). We used FUNGuild (Nguyen et al. 2016) to classify fungal taxa into trophic modes – pathotrophs, saprotrophs, and symbiotrophs – and permutational ANOVA (aovp in *lmPerm;* Wheeler & Torchiano 2016) to determine if the relative abundance of trophic modes differed between habitat types (Table S4). After determining microbiomes diverged significantly between habitat types, we evaluated what habitat attributes had main effects that explained variation in microbial diversity in each habitat (Table S5). We conducted model selection using *dredge* (MuMIn package, Bartón 2022) with diversity as the response variable and habitat attributes as explanatory variables. The best regression model was selected based on corrected Akaike information criterion (Akaike 1974). We then calculated relative importance of each habitat attribute selected by the best model using calc.relimp (*relaimpo*; Grömping, 2006).

To investigate plant community productivity, we used ANOVA with the mesocosms' total biomass (overall productivity) as the response and microbial treatment, habitat type, and their interaction as explanatory variables, and planned contrasts between live and sterile inoculum treatments within each habitat type. After finding significant effects on overall productivity, we conducted these analyses separately for shoot and root biomass (summed across all species for each mesocosm) to determine whether changes in productivity aboveground, belowground, or both were responsible for changes in overall productivity

(Table S6). For each habitat type, we also investigated how shifts in microbial diversity, richness, and evenness may affect plant community properties with a linear regression where the response variable was a plant community metric (e.g., mesocosm total biomass) and the explanatory variable was a prokaryotic or fungal diversity metric and its interaction with microbial treatment (Table S7). Since we could preserve plant species identity for shoot biomass, we tested whether the effect of urban and native microbiomes on shoot mass was similar across plant species within the community for the four species with the highest germination using permutational ANOVAs (aovp function) and planned contrasts between the live and sterile treatments within habitat type (Table S8). We additionally summed the shoot biomass for the other four plant species with lower germination and repeated the same analysis. Finally, to determine if plant community composition was affected by microbial treatment, habitat type and their interaction, we performed a PERMANOVA (*adonis*) with a response matrix of the presence/absence of each plant species in each mesocosm (SI Methods: "Data analysis").

Results

Native and urban habitats significantly differed in microbial diversity, composition, and functional guilds.

Prokaryotic diversity in native habitat fragments was ~12% greater than in the adjacent urban habitat (χ^2(1,N=15)=3.94,P=0.047; Figure 2**a**). This change in diversity was driven primarily by a 47% increase in prokaryotic richness in native habitat (χ^2(1,N=15)=5.54, P=0.019; Figure 2**b**). In contrast, fungal communities from native habitats were ~18% less

diverse than those from urban habitats (χ^2(1,N=15)=9.13,P=0.003; Figure 2**a**). Interestingly, the difference in fungal diversity was largely driven by a 21% increase in Pielou's evenness in the urban habitat (χ^2(1,N=15)=22.0, P<0.001; Figure 2**c**) rather than changes in richness (χ^2(1,N=15)=0.032, P=0.858; Figure 2**b**).

Additionally, both prokaryotic and fungal community composition differed significantly between native and urban habitats (prokaryotic: $F_{1,29}$=1.11, P=0.020; fungi: $F_{1,29}$=2.54, P=0.001; Figure 3). Using random forest models, we identified seven prokaryotic families and 14 fungal families that were important for distinguishing between habitat types (Table 1). Interestingly, all eight prokaryotic families had higher relative abundances in native habitats than in urban, while the 15 fungal families' associations with urban versus native habitat were more equally distributed (Table 1). Grouping these fungal families into putative trophic modes (i.e., saprotrophs, pathotrophs, symbiotrophs; Table 1) provided additional insight. We found that plant-associated guilds -- pathotrophs and symbiotrophs -- had higher relative abundance in urban and native habitats, respectively. These results were additionally supported by a FUNGuild analysis of the broader fungal community, which found fungal pathogens were 327% more abundant in urban than native core ($F_{1,28}$=16.4, P<0.001) and symbiotrophs were 420% more abundant in native habitat, although the latter trend was non-significant ($F_{1,28}$=1.96, P=0.172).

Habitat attributes, including connectivity, were better able to explain variation in native microbial diversity than urban diversity.

In native habitats, 81% of the variation in fungal diversity (R^2=0.811, $F_{3,10}$=14.32, P=0.001) and ~40% of the variation in prokaryotic diversity (R^2=0.432, $F_{2,11}$=4.19, P=0.044) were explained by habitat attributes. Specifically, 46% of landscape-wide variation in fungal diversity was explained by habitat connectivity (patch-based weighted sum: $F_{1,10}$=25.98, P<0.001; Figure 4**a**), and another 35% was explained by the fragment's plant richness ($F_{1,10}$=8.11, P=0.017) and the adjacent urban habitat's percent transportation ($F_{1,10}$=6.08, P=0.033; Figure 4**a**). Matrix evenness ($F_{1,11}$=6.22, P=0.030) and soil pH ($F_{1,11}$=5.97, P=0.033) also each explained ~20% of the variation in prokaryotic diversity (Figure 4**a**). In contrast, in urban microbiomes prokaryotic diversity was not explained by any of the ten habitat attributes, while transportation, time-since-fire, and number of fragments in a 10km buffer together explained ~70% of the variation in fungal diversity (R^2=0.701, $F_{2,11}$=7.82, P=0.006; Figure 4**c**), with the habitat connectivity only explaining 17% of the total variation (number of fragments within a 10km buffer: $F_{1,11}$=6.00, P=0.034; Figure 4**c**). We also found that in native habitats ~80% and 41% of the variation in fungal richness and prokaryotic richness, respectively, were explained by habitat attributes (fungal: $F_{4,9}$=8.96, P=0.003; bacterial: $F_{2,11}$=3.82, P=0.055; Figure 4**b**). Specifically, ~15% of the variation in fungal richness was explained by fragment area ($F_{1,9}$=13.72, P=0.005), while the other 65% was explained by matrix evenness (i.e. the evenness of the habitat types in the matrix surrounding the fragment) (R^2=0.088, $F_{1,9}$=5.42, P=0.045), plant richness (R^2=0.472, $F_{1,9}$=31.96, P<0.001), and soil pH (R^2=0.094, $F_{1,9}$=11.28, P=0.008). Prokaryotic richness was explained equally by two attributes – % transportation (R^2=0.181, $F_{1,11}$=3.94, P=0.073) and soil pH (R^2=0.229, $F_{1,11}$=4.84, P=0.050; Figure 4**b**). Similar to diversity, the habitat attributes explained none of the variation in prokaryotic richness and a smaller amount of

the variation in fungal richness (60%) in the urban habitats ($F_{2,11}$=8.21, P=0.007; Figure 4**d**). Overall, we found that habitat attributes, including measures of habitat connectivity, are explaining less, if any, variation in urban microbiomes compared to native microbiomes.

Microbiomes from highly-connected native habitats increase plant community productivity unlike urban microbiomes.

Native habitat microbiomes increased overall plant community productivity by ~290% (contrast of native live vs. sterilized treatments: $F_{1,177}$=17.5, P<0.001; Figure 5**a**), while urban microbiomes had a weakly positive, nonsignificant effect on plant community productivity (contrast of urban live vs. sterilized treatments: $F_{1,177}$=3.25, P=0.073; Figure 5**a**). When we investigated the components of overall productivity -- shoot and root biomass -- to determine if microbiome effects on above- or below-ground productivity were driving microbial effects on overall productivity, we found that both components were impacted in similar ways to overall productivity. Aboveground productivity of communities grown with native habitat microbiomes increased ~319% and belowground productivity increased ~244% compared to the sterile treatment (shoot: $F_{1,177}$=17.5, P<0.001; root: $F_{1,177}$=12.0, P<0.001; Figure 5**b-c**). Urban microbiomes had weakly positive, nonsignificant effects on communities' aboveground and belowground biomass (shoot: $F_{1,177}$=2.44, P=0.120; root: $F_{1,177}$=3.60, P=0.059; Figure 5**b-c**). These results indicate that native microbiomes play key roles in plant productivity and urbanization of the microbiome likely reduces beneficial effects of and plant reliance on the soil microbiome. Despite a decreased plant reliance on the urban soil microbiomes, these communities had

an overall higher productivity than communities grown with native soil microbiomes. While our experiment used a design aimed at minimizing the differences in abiotic conditions (i.e. by using the same soil/limestone rock for 85% of the soil structure in all mesocosms), the inoculum soils from urban habitats had ~58% higher nitrogen (3943.7 +/- 414.5 sem) and ~280% higher phosphorus (9.19 +/- 1.41 sem) compared to the inoculum soils from native habitats (nitrogen: 2517.3 +/- 623.2 sem; phosphorus: 2.43 +/- 0.17 sem) (nitrogen: $F_{1,26}$=3.63, P=0.068; phosphorus: $F_{1,12}$=22.72, P<0.001), which likely explains the overall higher plant community productivity in urban mesocosms. Further, plant community richness was higher in urban than native habitats ($F_{1,157}$=6.95, P=0.005) and increased when microbes were present ($F_{1,157}$=10.2, P=0.001); however, unlike productivity, the strength of microbial effects was consistent between habitat types (i.e., no significant interactive effect).

To determine whether aboveground biomass responses to native and urban microbiomes were driven by particular plant community members, we evaluated individual responses of the four highest germinating species (Fig S5). Three of four species - *Bidens alba*, *Passiflora suberosa*, and *Psychotria nervosa* -- grew significantly better with native habitat microbiomes, increasing productivity from double to ~25 times more compared to sterilized treatment (Fig S5**a, c, d;** P=0.020, P=0.008, P=0.019). The fourth, *Callicarpa americana*, also followed this trend (~19.5 times more biomass with native microbiomes; Fig S5**b;** P=0.111). The consistent beneficial effects of native microbiome on plant biomass across these dominant species indicates the community-level productivity response to native microbiomes was not driven by an outlier species but rather may represent a

general importance of native habitat microbiomes to native plant productivity. Conversely, urban microbiomes significantly increased productivity of only one species, *P. suberosa* ($P<0.001$; Fig S3***c***), with the other three dominant species unresponsive to urban microbiomes. Additionally, when we evaluated the combined aboveground biomass of the four lower-germinating species (*Chamaecrista fasciculata, Senna ligustrina, Sorghastrum secundum, Vernonia blodgettii*), we found that shoot biomass was higher in microbially live treatments ($P<0.001$), regardless of habitat type, indicating that the difference between urban and native microbiomes is less important for productivity of these low germinating taxa.

To further understand how fragmentation-associated changes in microbiomes affect plant communities, we evaluated the relationship between microbial community properties (i.e. diversity, richness and evenness) and plant productivity and richness in our understory mesocosm. For instance, native microbiome diversity explained significant variation in plant community productivity and richness (Figure 4). Specifically, shifts towards greater fungal diversity – which occurred at sites with higher habitat connectivity ($F_{1,10}=25.98$, $P<0.001$) – were associated with increased shoot biomass in our mesocosm experiment ($F_{1,86}=4.43$, $P=0.038$; Figure 4**a**). Similarly, high fungal richness – which occurred in larger fragments ($F_{1,9}=13.72$, $P=0.005$)– was associated with increased total biomass ($F_{1,86}=8.53$, $P=0.004$), shoot biomass ($F_{1,86}=6.98$, $P=0.010$), root biomass ($F_{1,86}=6.92$, $P=0.010$) and richness ($F_{1,86}=5.47$, $P=0.022$) (Figure 4**b**). Importantly, these positive relationships between microbial diversity/richness and plant productivity/richness were only found when the microbiome was present (live soil inoculations). In sterile soil treatments

(microbes absent), soils from sites with higher fungal diversity and richness were associated with decreased plant community productivity and richness. Together, these findings indicate the positive effects on productivity are likely caused by changes in microbial community properties rather than changes in soil properties. Plant communities grown with urban microbiomes only responded to changes in prokaryotic richness – where increases in richness were linked to increased shoot biomass ($F_{1,86}$=6.83, P=0.011). However, changes in urban prokaryotic richness were not explained by any of our fragmentation metrics, suggesting these shifts in prokaryotic richness affecting shoot biomass were driven by non-fragmentation factors.

Discussion

Our study demonstrates how habitat fragmentation-associated changes in microbiomes of both native and urban habitats can alter plant-microbe interactions and plant community productivity. Specifically, urban microbiomes had lower prokaryotic diversity, increased fungal pathogens, and weakened relationships between microbial properties and commonly-important habitat attributes. The urban microbiomes, in turn, showed reduced microbial benefits for plant community productivity and decoupling of the strong positive relationships between microbial diversity and plant community productivity. In contrast, native core microbiomes had greater prokaryotic diversity, fewer pathogens and possibly more symbiotic microbes, and these microbiomes significantly benefited both community-wide and species-specific plant productivity (~300% greater productivity). The dichotomy between the native and urban microbiomes' makeup and effects on plant productivity

illustrates how introduction of novel, nonnative habitats into fragmented landscapes could disrupt natural plant-microbiome interactions. Interestingly, the beneficial effects of native habitat microbiomes may also be weakened by fragmentation through changes in habitat connectivity, as compositional shift in the microbiomes from low connectivity sites were associated with declines in plant productivity. Microbial diversity in the native habitat was well explained by common habitat attributes (e.g., 80% of variation in fungal diversity), and importantly, this included a significant decline in diversity in patches with lower habitat connectivity. The positive effects of native microbiomes were also sensitive to these fragmentation-associated changes in microbial diversity and richness, with microbiomes from low connectivity sites providing reduced growth benefits to our understory plant communities. Overall, these results support fragmentation affecting plant productivity through two microbial pathways: (1) changes in urban matrix microbiomes decoupling of plant-microbial interactions and (2) isolation-associated shifts in native microbiome diversity underlying negative effects on plant productivity in native remnant habitats. Below we further explore these novel results and highlight future directions.

Fragmentation's role in shaping soil microbiomes through introduction of a novel habitat matrix and reduced habitat connectivity.

Urban microbiomes diverged in community composition, diversity, and putative functional guilds, which suggests urbanization's filtering/selection on microbial communities can occur in consistent ways across large swaths of a fragmented landscape. Other studies have also found compositional differences between native and matrix habitats (Brinkmann et al. 2019; Chen et al. 2020; Mendes et al. 2015; Nakayama et al. 2019), but fewer found effects

on microbial diversity (but see Schneider et al. 2015; Tin et al. 2018). Native remnants in our study had higher prokaryotic diversity due to much higher richness, suggesting habitat heterogeneity may generate more microniches for prokaryotes on native fragments (Groffman et al. 2014). In contrast, fungal diversity was lower in native habitats and was primarily driven by decreased fungal evenness, suggesting native habitat supports more dominant fungal taxa. The stronger difference in microbial diversity between native and urban habitats that we detected compared to other studies may result from differences in the type of matrix habitat(s) and the matrix contrast (i.e., how greatly the matrix habitat differs from the natural habitat; Kupfer et al. 2016) investigated. The high-intensity urban matrix surrounding native forests in our study represents substantial matrix contrast, which could explain why we found stronger changes in diversity of urban microbiomes than has been found in other systems where contrast is lower (e.g., agricultural fields: Mendes et al. 2015; Nakayama et al. 2019).

Further, changes in urban microbiome composition and diversity appear to cause functional guild shifts that can negatively impact primary producers. For instance, all five putative pathogenic fungal families identified by random forest models as differentiating urban and native habitats were higher in urban matrix, while putative symbiotic fungi were higher in native habitats. This shift from symbiotrophs to pathotrophs in the urban matrix was further supported by FUNGuild functional assignments. One other study found similar guild trends when comparing tropical forests and agricultural matrix; however, effect sizes were smaller, possibly due to lower matrix contrast (Brinkmann et al. 2019). Taken together, our results emphasize how the introduction of novel matrix habitats across

505 fragmented landscapes could shape microbiomes with potential to impact plant-
506 microbiome interactions.
507
508 Our study also highlighted how microbiomes inside remaining native habitats change with
509 increasing habitat fragmentation. In fact, ~50% of variation in native fungal diversity was
510 explained by habitat connectivity alone, suggesting dispersal limitation (a key mechanism
511 for understanding microbial biogeography; Martiny et al. 2006; Mony et al. 2022) is
512 important in structuring microbial diversity across the landscape. While historically the
513 paradigm 'everything is everywhere' has been applied to microbes (Baas-Becking 1934),
514 increasingly evidence supports dispersal limitation as important for fungi (Kiesewetter &
515 Afkhami 2021; Vannette et al. 2016). For instance, Peay et al. 2010 found in a naturally-
516 fragmented landscape ectomycorrhizal fungi were dispersal limited, with species richness
517 decreasing ~50% at ~1km. Further, environmental filtering (another key component to
518 microbial biogeography; Martiny et al. 2006; Mony et al., 2022) is also contributing to
519 microbiome structure. For instance, despite dispersal limitation being unlikely within
520 fragment-urban pairs, there were significant differences in microbial diversity,
521 composition, and function between native habitat and adjacent urban matrix. Due to the
522 overwhelming amount of urban matrix in which native fragments are embedded (similar to
523 many urban environments; Angel et al. 2011), microbial inundation from the urban matrix
524 into native habitats is expected to be especially strong. Yet, we still find clear differentiation
525 of native habitat microbiomes from nearby urban matrix as well as less pathogenic/more
526 symbiotic microbes on native habitat fragments, supporting filtering-mediated resistance
527 of native microbiomes to the introduction of matrix habitats. The importance of

environmental filtering across the landscape was also emphasized by the large amount of variation in microbial diversity explained by habitat attributes in native habitats. In contrast, habitat attributes explained much less variation in urban microbiomes (~30%-43% less than for native microbiomes), possibly due to urban matrix habitats being more stochastic and/or disturbed (Nugent & Allison 2022). Our findings underscore the importance of improving habitat connectivity and preserving abiotic and biotic conditions that underpin environmental filtering in native fragments.

Urbanization and disruption of beneficial plant-microbial interactions in the urban matrix and remnant native habitats

Given the rapidly increasing fragmentation of landscapes and the importance of microbiomes for plant community health and dynamics (Berendsen et al. 2012; Fierer 2017), there is growing interest in how this relationship plays out in fragmented landscapes. Observational studies have found microbial diversity is correlated with shifts in plant community composition of revegetated/restored matrix habitat (Gellie et al. 2017; Turley et al. 2020) and tied land conversion to microbial shifts in plant rhizospheres (Estendorfer et al, 2017; McGee et al. 2020). For instance, tree rhizosphere communities significantly differed for forests adjacent to rural/suburban versus urban matrices (Rosier et al. 2021). By evaluating rhizosphere microbiomes, these studies infer which microbes are likely interacting with which plants under different contexts, contributing towards understanding microbe-plant interactions in fragmented landscapes. However, correlative approaches cannot test whether changes in plant microbiomes are responsible for changes in performance/composition of plant communities nor can they decouple microbial-

mediated effects and effects of other biotic and abiotic differences between habitat types. Our study takes a step toward addressing this gap by experimentally manipulating microbial communities from native and urban matrix habitats and assessing impacts on plant community productivity reaching two main conclusions. First, urban microbiomes, which are shifted in composition, diversity, and functions, did not confer productivity benefits provided by native habitat microbiomes, suggesting that urban matrix disrupts natural plant-microbial associations. Second, native microbiomes, which were shaped by fragmentation-associated habitat attributes such as connectivity, play crucial beneficial roles in native plant community productivity (here increasing it by nearly 300%). Therefore, our results support that in addition to fragmentation shaping plant-microbiome interactions through introduction of a novel urban matrix, fragmentation also shapes microbiome effects on plant productivity through changes in habitat connectivity. In line with this result, we recently demonstrated that connectivity-associated changes to microbiomes can impact individual-level plant performance (Kiesewetter & Afkhami 2021), but this is the first experimental study showing that these changes in the microbiome cascade up to impact plant community level properties. Taken together, our results revealed how urbanization and habitat fragmentation are likely shaping hidden microbial players in several ways that affect plant communities.

Despite plant communities' decreased reliance on urban microbiomes, it is important to note that the consequences of introduced matrix habitats for plants can be complex, altering many aspects of the abiotic and biotic environment organisms experience (Driscoll et al. 2013; Kupfer et al. 2006). In our study, plant communities grown in urban soils,

574 regardless of microbial treatment, did better than those grown with native soils. Increased
575 productivity in urban mesocosms likely resulted from higher nutrients in urban matrix
576 soils (nitrogen: $F_{1,26}$=3.63, P=0.068; phosphorus: $F_{1,12}$=22.72, P<0.001), as commonly
577 reported for other urban environments (Grimm et al. 2008; Johnson & Munshi-South
578 2017). While the shift in urban microbiomes may itself have reduced microbially-conferred
579 productivity benefits (recall: increased pathotrophs and fewer symbiotrophs in urban
580 microbiomes), an alternative hypothesis is urban matrix-specific changes in context (i.e.,
581 increased nutrient availability) reduced the value of the microbiome to the plant
582 community, decreasing plant community reliance on urban microbiomes compared to
583 native microbiomes. In both cases, the urban matrix would be affecting plant-microbiome
584 interactions (i.e., by shifting microbiome makeup or by shifting its value to plant
585 communities). In reality, these 'alternatives' are not mutually exclusive and likely both
586 contribute to plant community productivity. In addition to differences in nutrient
587 availability, urban matrices are characterized by increased impervious land area (Johnson
588 & Munshi-South 2017), higher heavy metal concentration (Rodríguez Martin et al. 2015),
589 and higher temperatures (Grimm et al. 2008), which can reduce physical space for plant
590 communities and increase stress they experience. Additional stress in urban matrix may
591 generate greater reliance of the plant community on beneficial microbial partners
592 (Chandrasekaran et al. 2014; Meena et al. 2014) or could make the plant community more
593 susceptible to the negative effects of microbial pathogens (Pandey & Senthil-Kumar 2019).
594 Future work testing how key abiotic and biotic changes in the urban matrix modulate the
595 relationship between microbiomes and plant community dynamics (e.g., nutrient additions,

596 changes in other soil properties and sunlight environment, etc in native and urban soils)
597 will be an important next step.
598
599 *Conclusions and future directions*
600 In conclusion, our study not only illustrates how matrix introduction and habitat isolation
601 could drive meaningful differences in diversity, composition, and functions of microbiomes,
602 but also experimentally demonstrated for the first time how these changes can scale up to
603 strongly impact plant community productivity. From this work, we also identified four
604 areas for future research to further our understanding of plant-microbial interactions in
605 urbanized and fragmented environments. First, we postulate that matrix type and contrast
606 are important factors for determining fragmentation's consequences for microbial
607 diversity and composition with cascading effects on plants. Therefore, we advocate for
608 future work aimed at how multiple matrix types and contrasts (ideally within the same
609 system) impact microbiomes and their interactions with plant communities. Second, plant-
610 associating functional fungal guilds -- symbiotrophs and pathrotrophs – differed in relative
611 abundance in urban and native habitats in our study, such that microbial communities in
612 the urban matrix appear to be functionally shifted towards less plant-beneficial
613 microbiomes. Therefore, detailed studies of microbial functional traits in matrix versus
614 native habitats would be especially valuable. In addition to functional assays,
615 metatranscriptomic approaches would be useful to gain community-wide profiles of
616 microbial functional shifts via changes in expression of functional genes in native and
617 urban matrix environments. Third, while our work focused on soil microbiomes, soils
618 contain complex communities, which also include other important and understudied soil

fauna (e.g., nematodes). As urbanization presents unique challenges for soils and their communities, an important next step is to study how other members of the soil fauna and their interactions with microbial communities respond to urbanized landscapes. Finally, our work highlights how fragmentation can disrupt beneficial microbiome-plant interactions through multiple pathways, spotlighting the need to study whether this change will affect the resiliency of fragmented communities to other global change stressors, such as climate change, as urbanization continues to increase globally (Seto et al. 2012).

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Kasey N. Kiesewetter and Michelle E. Afkhami developed the conceptual framework for the project and experimental design. Kasey N. Kiesewetter performed field collections, lab work, and sample processing with supervision from Michelle E. Afkhami. Leydiana Otano performed lab work and experimental setup, with supervision from Michelle E. Afkhami and Kasey N. Kiesewetter. Writing, editing, and reviewing were done by Kasey N. Kiesewetter and Michelle E. Afkhami.

Data Availability

Demultiplexed sequencing data are available in the NCBI Sequence Read Archive (Bio-Project ID: PRJNA938951; accession numbers: SAMN33450569 - SAMN33450598). All experimental data script and data from the experiment and processed microbial data are available on Zenodo (10.5281/zenodo.7699344).

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Figure legends

Figure 1. Map of historic Miami Rock Ridge pine rocklands (white layer) overlaid with current pine rockland habitat (red layer) showing the 15 pine rockland fragments where pairs of soil microbiomes were collected. Soils were collected in the core of each pine rockland fragment and in the adjacent urban matrix. See Fig. S1 for more details.

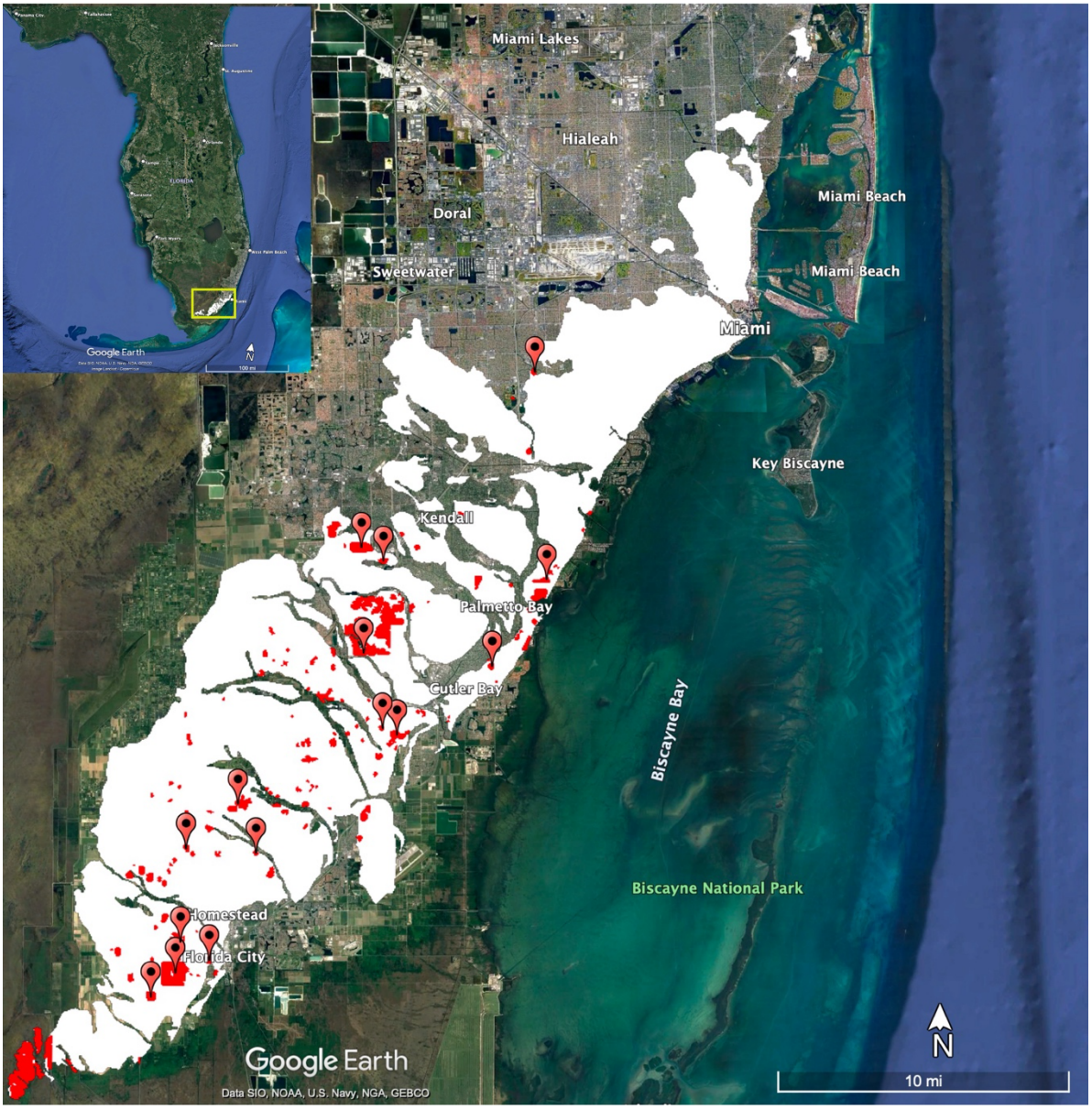
Figure 2. Shannon diversity **(a,b)** and its components (species richness **(c,d)** and Pielou's evenness **(e,f)**) in native and urban matrix habitat types. Prokaryotic diversity was higher (χ^2(1,N=15)=3.94; P=0.047; **a**) and fungal diversity was lower (χ^2(1,N=15)=9.13,P=0.003; **b**) in native habitats compared to urban matrix. Prokaryotic diversity was mainly driven by differences in species richness (χ^2(1,N=15)=5.54,P=0.019; **c**) rather than evenness (χ^2(1,N=15)=1.16,P=0.281; **e**). Fungal diversity was not driven by species richness (χ^2(1,N=15)=0.032,P=0.858; **d**) but rather fungal evenness (χ^2(1,N=15)=22.0,P<0.001; **f**). 'n.s.' denotes a nonsignificant p-value, *****denotes a p-value <0.05, **denotes a p-value < 0.01, and *** denotes a p-value <0.001. Error bars represent mean +/- standard error.

Figure 3. Bacterial (**a, b**) and fungal (**c, d**) community compositions differed in native core habitats versus urban matrix habitats. The principle coordinate analyses (**a, c**) show native core sites as green points and urban matrix sites as purple points, and points are labeled

954 with fragment identity abbreviations that correspond to the fragment identities listed in
955 Table S1. F statistics and p-values are reported from a PERMANOVA with a Bray-Curtis
956 distance matrix for either bacteria (**a**) or fungi (**c**) and the explanatory variables of habitat
957 type (i.e. native core versus urban matrix) and fragment identity. Bolded values indicate
958 significant p-value. For the bar graphs (**b, d**), each color represents the relative abundance
959 of a microbial class present in our data. Microbial taxa that could not be grouped as far
960 down as class, were grouped together into "unclassified" phylum or kingdom based on the
961 next most specific taxonomic order known for each OTU.
962
963 **Figure 4.** Habitat-associated shifts in fungal diversity and richness of the native
964 microbiomes explain variation in plant community productivity and richness. First,
965 variation in native microbiome diversity and composition (**a, b**) were explained by habitat
966 attributes of the landscape, including habitat connectivity and fragment area, while urban
967 microbiomes were not (**c, d**). This result is shown in graphs a-d, which show the strength of
968 habitat attributes' relationships with diversity and richness of prokaryotes (blue bars) and
969 fungi (red bars) based on the attributes' standardized regression slopes from best fit
970 models. Bars below zero represent a negative relationship, while bars above zero represent
971 positive relationships. Second, native fungal diversity and richness explained significant
972 variation in multiple plant performance metrics (solid red arrows) while urban diversity
973 did not. Arrows represent significant relationships between shifting microbial
974 diversity/richness and plant community productivity metrics (i.e., total biomass, shoot
975 biomass, and root biomass) and mesocosm richness. Solid arrows represent the positive
976 relationships we detected for live treatments (i.e., microbes were present) and faded

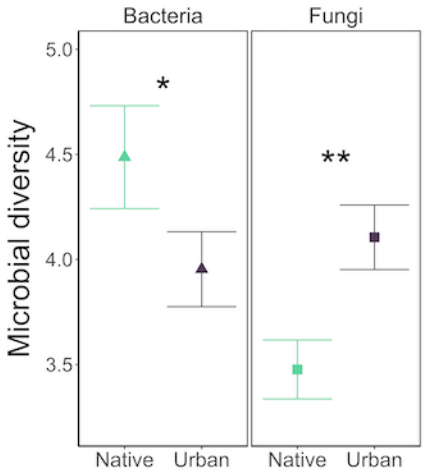
977 arrows represent the negative relationships we detected for sterile treatments (i.e.,
978 microbes were absent).
979
980 **Figure 5.** Native microbiomes increased community productivity, while urban
981 microbiomes did not significantly affect productivity. Overall productivity **(a)** was
982 significantly greater in mesocosms grown with the native habitat microbiomes compared
983 to mesocosms grown with sterilized native inoculum (total biomass: $F_{1,177}$=17.5, P<0.001),
984 while microbial effects of urban microbiomes on total biomass were weak ($F_{1,177}$=3.25,
985 P=0.073). Aboveground **(b)** and belowground **(c)** productivity showed the same outcome
986 with native microbiomes increasing both components of productivity (shoot mass:
987 $F_{1,177}$=17.5, P<0.001; root biomass: $F_{1,177}$=12.0, P<0.001) and urban microbiomes having
988 nonsignificant effects on both components (shoot mass: $F_{1,177}$=2.44, P=0.120; root mass:
989 $F_{1,177}$=3.60, P=0.059). Significance between microbial treatments within habitat type were
990 determined using planned contrasts. 'n.s.' denotes a nonsignificant p-value, 'n.s.[1]', denotes a
991 marginally significant, p-value (0.05-0.1), *denotes a p-value <0.05, **denotes a p-value <
992 0.01, and *** denotes a p-value <0.001. Error bars represent mean +/- standard error. M+
993 indicates microbial active/live treatment and M- indicates microbial sterile treatments.
994
995 **Table 1.** Microbial families selected as important for distinguishing between native and
996 urban matrix habitat types by the random forest models with Boruta feature selection.
997
998
999

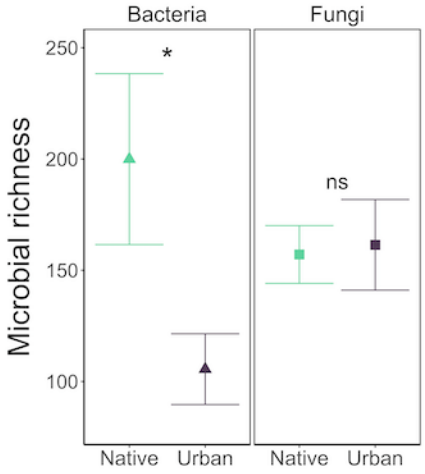
1000 Figure 1.



1001

Figure 2.


Bacteria
Fungi
Microbial diversity
5.0
4.5
4.0
3.5
*
**
Native
Urban
Native
Urban


Bacteria
Fungi
Microbial richness
250
200
150
100
*
ns
Native
Urban
Native
Urban

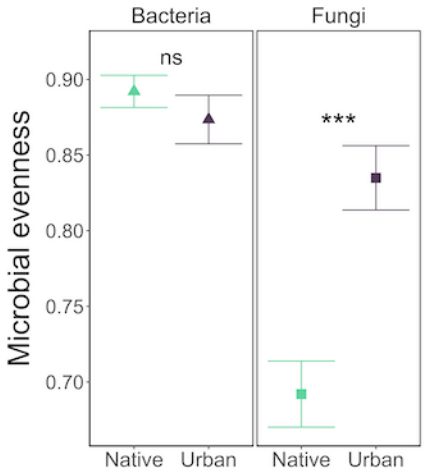

Bacteria
Fungi
Microbial evenness
0.90
0.85
0.80
0.75
0.70
ns
***
Native
Urban
Native
Urban

Figure 3.

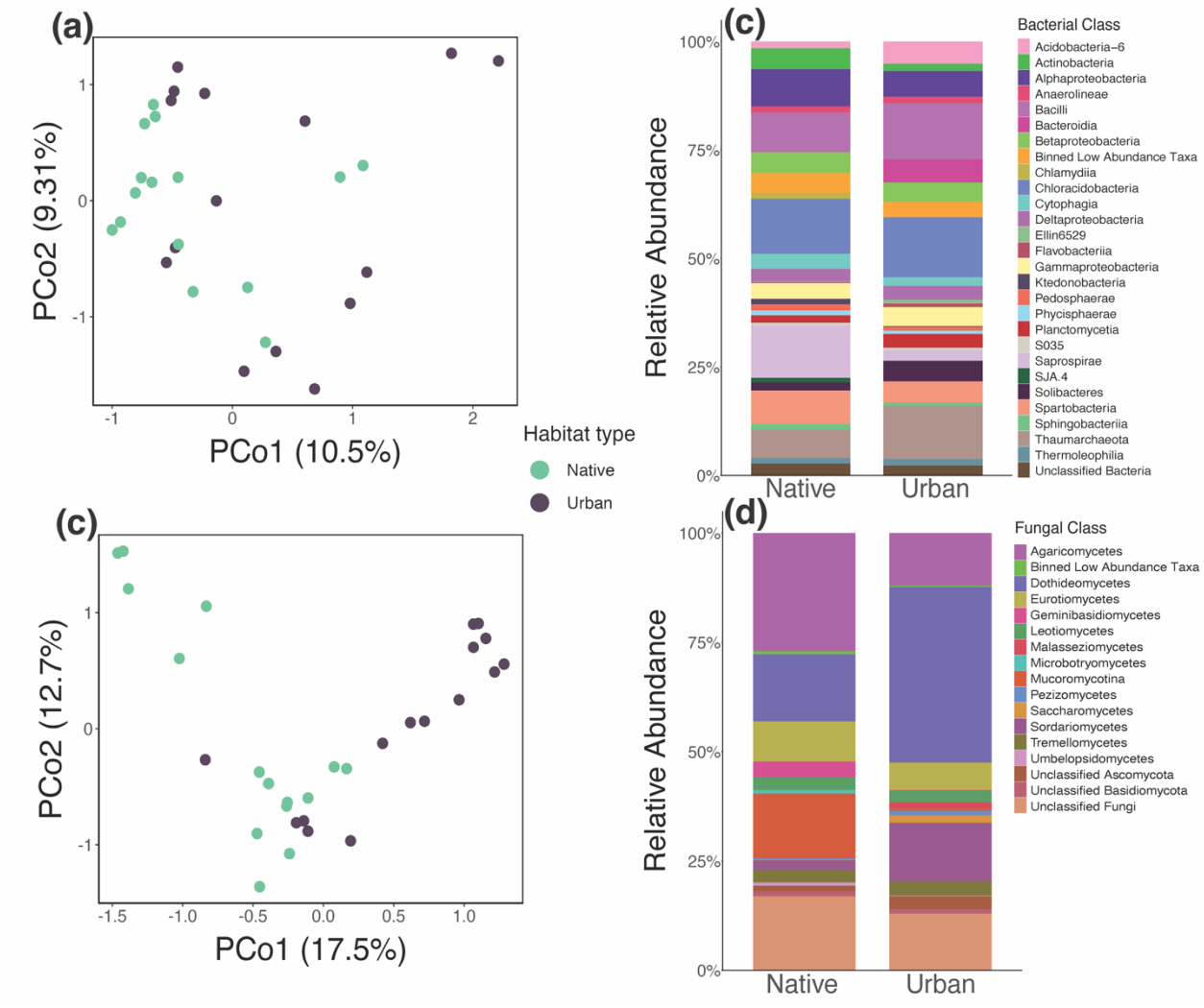
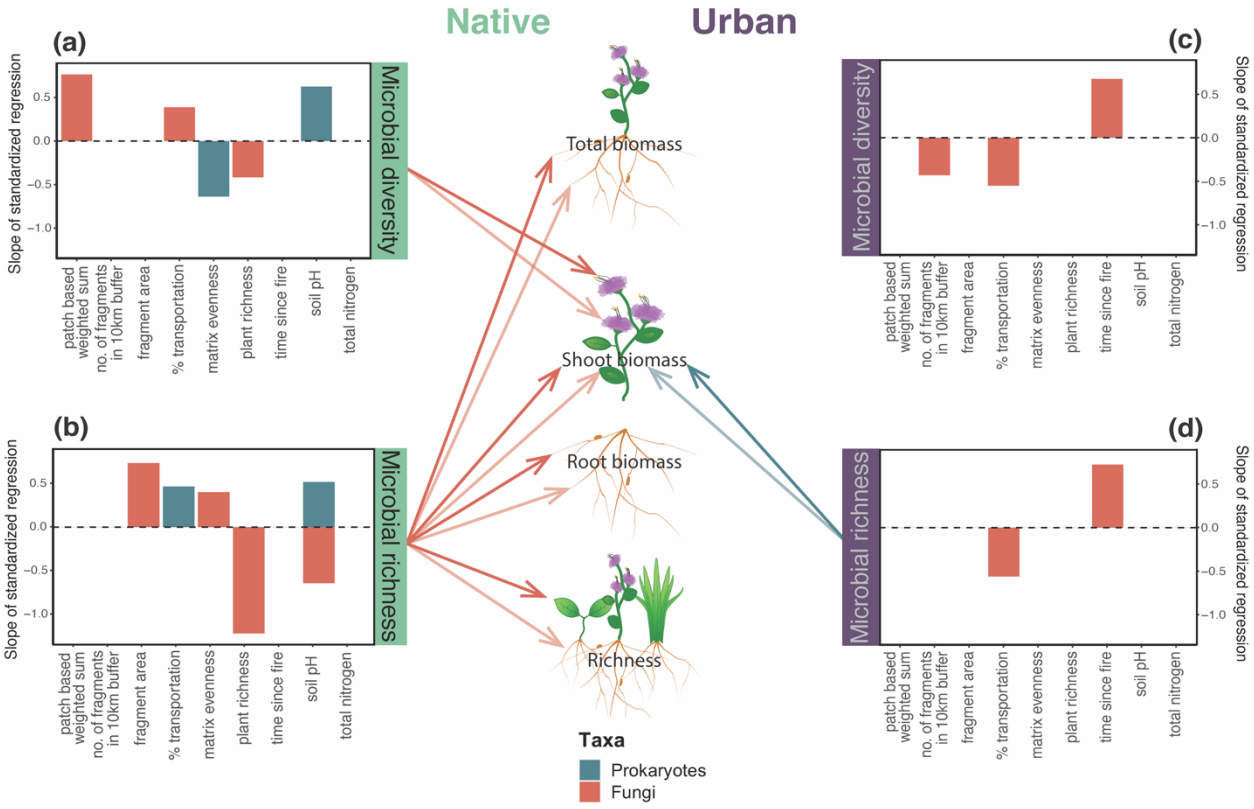

(a)
PCo2 (9.31%)
PCo1 (10.5%)
Habitat type
Native
Urban
(c)
PCo2 (12.7%)
PCo1 (17.5%)
(c)
Relative Abundance
Native
Urban
Bacterial Class
Acidobacteria–6
Actinobacteria
Alphaproteobacteria
Anaerolineae
Bacilli
Bacteroidia
Betaproteobacteria
Binned Low Abundance Taxa
Chlamydiia
Chloracidobacteria
Cytophagia
Deltaproteobacteria
Ellin6529
Flavobacteriia
Gammaproteobacteria
Ktedonobacteria
Pedosphaerae
Phycisphaerae
Planctomycetia
S035
Saprospirae
SJA.4
Solibacteres
Spartobacteria
Sphingobacteriia
Thaumarchaeota
Thermoleophilia
Unclassified Bacteria
(d)
Relative Abundance
Native
Urban
Fungal Class
Agaricomycetes
Binned Low Abundance Taxa
Dothideomycetes
Eurotiomycetes
Geminibasidiomycetes
Leotiomycetes
Malasseziomycetes
Microbotryomycetes
Mucoromycotina
Pezizomycetes
Saccharomycetes
Sordariomycetes
Tremellomycetes
Umbelopsidomycetes
Unclassified Ascomycota
Unclassified Basidiomycota
Unclassified Fungi

Figure 4.


Native
Urban
(a)
Microbial diversity
(b)
Microbial richness
(c)
Microbial diversity
(d)
Microbial richness
Slope of standardized regression
Slope of standardized regression
patch based weighted sum
no. of fragments in 10km buffer
fragment area
% transportation
matrix evenness
plant richness
time since fire
soil pH
total nitrogen
Total biomass
Shoot biomass
Root biomass
Richness
Taxa
Prokaryotes
Fungi

1046 Figure 5.