Bacterial communities in the natural and supplemental nests of an endangered ecosystem engineer

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Abstract. Supplemental nests are often used to restore habitats for a variety of rare and endangered taxa. However, though they mimic the function of natural nests, they vary in design and construction material. We know from previous research on human buildings that these differences in architecture can alter the types of microbes to which inhabitants are exposed, and these shifts in microbial interactions can be detrimental for individual health and well-being. Yet, no one has tested whether bacterial communities in supplemental structures are distinct from those found in natural nests. Here, we sampled the bacteria from inside supplemental nests of the endangered Key Largo woodrat (Neotoma floridana smalli). We then compared the diversity and composition of those bacteria to the bacteria collected from natural stick-nests and the surrounding forest environment in Key Largo, Florida. In addition, we sampled woodrat bodies to assess the microbiota of nest inhabitants. We observed distinct bacterial communities in Key Largo woodrat nests, relative to the forest environment; however, we could not differentiate between the bacterial communities collected from supplemental and natural nests. Furthermore, when we considered the potential accumulation of rodent-associated bacterial pathogens, we found no evidence of their presence in supplemental nests, in natural nests, or on the forest floor. Where we expected to see an accumulation of pathogens, we instead observed high relative abundances of bacteria from antimicrobial-producing groups (i.e., Pseudonocardiales and Streptomycetales). The bacteria on Key Largo woodrat individuals resembled those of their nests, with a low relative abundance of potential pathogens (0.3% of sequence reads) and a high relative abundance of bacteria from antimicrobial-producing groups. Our results suggest that, although there is some microbial interaction between nests and nest inhabitants, there are no detectable differences in the types of bacteria to which Key Largo woodrats are exposed in supplemental and natural nest structures.

Key words: antimicrobial bacteria; built environment; conservation; Key Largo woodrat; microbiome; Neotoma floridana smalli; nest supplementation.

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INTRODUCTION

Supplemental nests are often used in the conservation and management of threatened and endangered species to increase and restore available nesting habitat and to provide protection from predators, competitors, and the environment (Newton 1994, Spring et al. 2001, Libois et al. 2012). However, though supplemental nests model the function of their natural counterparts,
they are commonly built with relatively little attention to mimicking the intricate details of natural nest design and are constructed from manufactured materials, such as metal and plastic. For example, supplemental nests of the Scarlet Macaw (*Ara macao*), a tree cavity nesting species, are constructed from wood, poly-vinyl chloride tubes, and 55-gallon poly-acryl amide barrels (Vaughan et al. 2003). Despite these differences in construction design and nesting substrate, it has not been previously considered whether species interactions are altered in supplemental nest environments. For instance, alternative materials (e.g., plastic) could potentially limit the dispersal of environmental species into nests and alter microclimate conditions, affecting the overall diversity and succession of the microbial communities that are able to colonize and persist inside. These shifts in species interactions could then lead to negative health outcomes for individuals, ultimately hindering conservation efforts.

We already know that captivity can result in a loss of body-associated bacterial diversity, resulting in adverse health consequences—a trend that has been observed among terrestrial mammals, aquatic mammals, and amphibians (Becker et al. 2014, Loudon et al. 2014, Cheng et al. 2015, Wan et al. 2016). One example of this comes from research on the critically endangered Panamanian golden frog (*Atelopus zeteki*), in which it has been shown that captivity reduces the species richness and phylogenetic diversity of bacteria on the skin (Becker et al. 2014). This frog is now found only in captive environments, and the observed changes in body-associated communities have been linked to an increase in the risk of infection. Further, the effects of captivity are not restricted to the skin or discreet external body sites. The Tasmanian devil (*Sarcophilus harrisi*) gut, skin, pouch, and mouth microbiomes all exhibit compositional differences, based on whether an animal is captive or wild (Cheng et al. 2015). However, though we know that the environment is an important determinant of the microbes that live on animals (and hence individual health), no one has characterized the microbial communities in the supplemental nests themselves or compared how those communities vary to those found in the natural environment. Therefore, to better predict how the design and use of supplemental nests might alter microbial species interactions, we pull from the literature on human dwellings.

We know from the study of human-built structures (e.g., homes and office buildings) that building material and architectural design strongly influence the diversity and types of microbes found on interior surfaces. In recent years, there have been a number of studies suggesting that as we have moved into more modified homes, we have lost exposures to diverse environmental species (e.g., Haahntela et al. 2015, Stein et al. 2016, Thoemmes et al. 2018). For example, contemporary houses have far fewer environmental microbes than do more open traditional homes, such as thatched houses in the Amazon (Ruiz-Calderon et al. 2016). While the absence of some bacteria in our daily lives is beneficial, the absence of others is associated with negative health outcomes. For example, a decrease in the abundance of soil bacteria on the skin is directly linked to an increase in the prevalence of atopic sensitization and autoimmune disorders in humans (Fyhrquist et al. 2014, Ruokolainen et al. 2015). Additionally, as indoor microbial diversity decreases there is a subsequent increase in the abundance of bodily microbes, such as those from feces and skin (Dunn et al. 2013, Lax et al. 2014), and pathogens found in both homes (e.g., *Staphylococcus aureus*; Gandara et al. 2006) and hospitals (Kembel et al. 2014). These findings highlight the importance of understanding how human-built supplemental nests might alter the microbial communities to which species of concern are exposed. A loss in microbial diversity or the accumulation of pathogens in supplemental nests could have detrimental effects, particularly for species at a high risk of extinction, such as the Key Largo woodrat (*Neotoma floridana smallii*)

The Key Largo woodrat is a federally endangered subspecies endemic to Key Largo, Florida (US Department of the Interior 1984). Once ranging throughout the tropical hardwood hammock, historical habitat loss and land alterations during the agricultural era have limited their distribution to North Key Largo and reduced the availability of natural nesting substrate in the environment (Winchester et al. 2009, Cove et al. 2017). This loss of habitat and nesting sites has been detrimental to the survival of these
ecosystem engineers, as they build substantial stick-nests by layering forest debris at the bases of trees (Fig. 1a), in fallen tree throws, or in solution holes (Cove and Maurer 2019). Additionally, recent evidence suggests that woodrat distributions have been further limited by the presence of feral and free-ranging cats (*Felis catus*)—resulting in a shift away from their natural stick-nest building behavior (Cove et al. 2019). Once estimated to number fewer than 100 individuals (McCleery et al. 2005), Key Largo woodrats have benefitted greatly from conservation management practices, including nest supplementation and exotic predator removal (Cove et al. 2019), and there are now more than 2000 supplemental Key Largo woodrat nests located in their protected habitats (Cove et al. 2017). These nests are constructed from large plastic culvert pipes and covered with rocks or chunks of fossilized coral (Cove et al. 2017). On the exterior, Key Largo woodrats maintain supplemental and natural nests in the same way (i.e., stick-stacking behavior; Cove et al. 2017), but supplemental nest interiors are more enclosed with comparatively little air flow and moisture penetration (Barth 2014).

Here, we examine the diversity and composition of bacteria in natural and supplemental Key Largo woodrat nests to assess whether there are differences in bacterial communities associated with nest supplementation. Based on what we know from contemporary human homes, we might expect supplemental nests to have less bacterial diversity than natural nests. Similarly, since supplemental nests are composed from materials that could potentially restrict the colonization of environmental microbes, we might expect there to be a difference in which bacterial taxa are most abundant, including an increase in the accumulation of body associates and pathogenic bacteria. Finally, as we know there is an interaction between the microbiota of the body and the built environment (Hospodsky et al. 2012, Dunn et al. 2013, Becker et al. 2014, Loudon et al. 2014, Meadow et al. 2014, Lax et al. 2014, Cheng et al. 2015, Gibbons et al. 2015, Wan et al. 2016), we characterize the bacteria found on the bodies of Key Largo woodrat individuals.

**METHODS**

The Crocodile Lake National Wildlife Refuge is located in North Key Largo, Florida, USA. This refuge is composed of mangroves, coastal wetlands, and part of the last remaining large tract of tropical hardwood hammock habitat. When combined with the Dagny Johnson Key Largo Hammock Botanical State Park, this forest type covers <1000 ha (Frank et al. 1997, US Fish and Wildlife Service 1999). However, despite its limited area, the tropical hardwood hammock is home to a variety of endemic and endangered species, including the Key Largo woodrat, the Key Largo cotton mouse (*Peromyscus gossypinus allapaticola*), and the Stock Island tree snail.
Here, our study focused on Key Largo woodrat nests and individuals in December 2017. This research was conducted, and all data were collected in accordance with federal, state, and institutional animal ethics guidelines and approved under the following permits: US Fish and Wildlife Service Permit [TE 697819-4], Florida Department of Environmental Protection [01171715], and the North Carolina State University Institutional Animal Care and Use Committee (IACUC) [#13-003-O].

Natural nests that have been previously identified and all supplemental nests are individually marked as part of a long-term monitoring project. From these, we visited 10 natural (Fig. 1a) and 10 supplemental nests (Fig. 1b, \( n = 20 \)), focusing on the area of the refuge that has the highest Key Largo woodrat population density (Cove et al. 2019). We determined nest occupancy based on visual surveys of active stick-stacking behavior (Balcom and Yahner 1996, Cove et al. 2017), camera trap surveys, and/or Sherman live traps baited with a mixture of peanut butter powder and rolled oats. Once occupancy was confirmed, we swabbed each nest with dual-tipped sterile rayon BBL CultureSwabs (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). To standardize the distance into each nest, as well as to avoid contamination of sample swabs on exterior building material, we inserted a PVC pipe (approximately 0.5 m in length; seen in Fig. 1a) into each nest prior to sample collection. We targeted entrances that appeared to be used most frequently by the nest inhabitant(s), placed the pipe into the nest interior, and threaded each swab through to the sample location. To lengthen each swab, we wedged a wooden dowel into the cross hatches found on the exterior of the swab cap, avoiding any contact between the dowel and the sample swab itself. After sampling, we removed the pipe and swab from the nest at the same time and sterilized the interior and exterior of the pipe between each sampling event. We then swabbed the forest floor approximately 0.5–0.75 m from the outer edge of all natural nests (\( n = 10 \)), targeting an area that did not appear to be trafficked by humans or wildlife. Finally, we collected bacteria from the flank and ventral side of Key Largo woodrat individuals to investigate the microbial interaction between nests and nest inhabitants (\( n = 10 \)). All individuals were captured near sampled nests with Sherman live traps, and to prevent repeated sampling of individuals, we verified identity with double-marked monel 1005 ear (National Band and Tag Company, Newport, Kentucky, USA) and subcutaneous PIT (Biomark, Boise, Idaho, USA) tags. All environmental and animal samples were collected for approximately 15 s and subsequently stored at \(-20^\circ\text{C}\) until processing for DNA analyses.

**Molecular methods and analyses**

We performed DNA extractions with a DNeasy PowerSoil Kit (Qiagen, product #12888-100), with the modifications described in Fierer et al. (2008). PCRs were performed in triplicate and all amplicons were pooled in equimolar concentrations prior to sequencing on the Illumina MiSeq platform at the University of Boulder, Colorado, using the 515f/806r primer pair to amplify the V4-V5 region of the 16S rRNA gene (Flores et al. 2012).

We demultiplexed and quality-filtered the resulting sequence data using default parameters in the QIIME2 pipeline (version 2019.7.10; Bolyen et al. 2019). We identified amplicon sequence variants (ASVs) with Deblur (via deblur denoise-16S; Amir et al. 2017) and assigned taxonomy with the naive Bayes classifier (Bokulich et al. 2018), trained on the Greengenes 13_8 99% OTUs reference database (version 8.15.13; McDonald et al. 2012). When compared to other methods (e.g., OTU clustering algorithms), ASV assignment provides a more accurate characterization of bacterial communities (Caruso et al. 2019) and accurately identifies microbes to the species or even subspecies level of taxonomic resolution (Callahan et al. 2017). We then rarefied our data to 4000 sequences per sample and analyzed all data in R (version 3.4.4) with the mtoolsr, vegan, PMCMRplus, and FSA packages (Oksanen et al. 2008). PCRs were performed in triplicate and all amplicons were pooled in equimolar concentrations prior to sequencing on the Illumina MiSeq platform at the University of Boulder, Colorado, using the 515f/806r primer pair to amplify the V4-V5 region of the 16S rRNA gene (Flores et al. 2012).

We compared differences in ASV richness and Shannon diversity between nest and forest floor samples with Kruskal-Wallis, using Dunnett’s test and Benjamini-Hochberg method for multiple comparisons (\( n = 29 \); Dunnett 1955;
Benjamini and Hochberg 1995). We then quantified differences in the composition of bacterial communities among samples with Bray-Curtis dissimilarity, weighted by ASV abundance (Bray and Curtis 1957). We examined differences in bacterial community composition between natural and supplemental nests with a permutational multivariate analysis of variance (PERMANOVA), where we compared differences between nest type (i.e., natural and supplemental nests) and between natural nest and forest samples separately.

We then calculated the percent relative abundance of previously described bacterial pathogens in rodents, excluding zoonotic species that are known only to be infectious in humans (Table 1). Since we do not know the physical condition of the individuals that use and/or live in the woodrat nests sampled in this study, we included opportunistic species that have been shown to have increased virulence potential when rodents are immunocompromised (e.g., *Pasteurella pneumotropica*; Heyl 1963, Towne et al. 2014). Additionally, since the bacteria associated with Key Largo woodrats have not been previously characterized, we included all bacterial pathogens described from rodents, regardless of host species. Though there is likely to be some variation in the pathogens found on Key Largo woodrats compared to other rodents, the bacterial taxa included in our analyses encompass 55 species across 29 genera, including those of well-known rodent-associated pathogens (e.g., *Yersinia pestis*; Butler et al. 1982). We also included 5 genera for which all (or nearly all) mammal-associated species studied to date have been described as pathogenic (e.g., *Leptospira*; Picardeau 2017). Though we may have missed fine-scale interactions (e.g., previously undescribed pathogens or species-specific associations), we believe this representative dataset has captured generalized patterns in the accumulation of potentially pathogenic bacteria in Key Largo woodrat nests. Differences in the relative abundance of all bacterial taxa of interest were compared with Kruskal-Wallis tests.

Finally, we characterized the bacteria found on Key Largo woodrats and compared bacterial community composition between nests and woodrat bodies with Bray-Curtis dissimilarity (Bray and Curtis 1957). We visualized community data with

| Genus | Species | References |
|-------|---------|------------|
| Anaplasma | phagocytophilum | Foley et al. (2008) |
| Bacillus | anthracis | Marchette et al. (1957) |
| Bartonella | spp. | Firth et al. (2014) |
| Bordetella | bronchiseptica | Baker (1998)† |
| Bordetella | hinzii | Whary et al. (2015)† |
| Bordetella | tularensis | Whary et al. (2015)† |
| Brucella | spp. | Tiller et al. (2010) |
| Burkholderia | humptydooensis | Angus et al. (2014) |
| Burkholderia | mallei | Angus et al. (2014) |
| Burkholderia | oklahomensis | Angus et al. (2014) |
| Burkholderia | pseudomallei | Angus et al. (2014) |
| Burkholderia | thailandensis | Angus et al. (2014) |
| CAR | bacillus | Baker (1998)† |
| Chlamydial | muridarum | Whary et al. (2015)† |
| Chlamydia | psittaci | Whary et al. (2015)† |
| Chlamydia | trachomatis | Whary et al. (2015)† |
| Citrobacter | rodentium | Baker (1998)† |
| Clostridium | difficile | Baker (1998)† |
| Clostridium | perfingens | Whary et al. (2015)† |
| Clostridium | piliforme | Baker (1998)† |
| Corynebacterium | kutscheri | Baker (1998)† |
| Corynebacterium | pseudodiphtheriticum | Baker (1998)† |
| Ehrlichia | muris | Kawahara et al. (1999) |
| Eimeria | spp. | Baker (1998)† |
| Enterococcus | faecalis | Goh et al. (2017) |
| Enterococcus | faecium | Goh et al. (2017) |
| Escherichia | coli | Whary et al. (2015)† |
| Flexipina | rappini | Baker (1998)† |
| Francisella | tularensis | Ellis et al. (2002)† |
| Helicobacter | bilis | Baker (1998)† |
| Helicobacter | hepaticus | Baker (1998)† |
| Helicobacter | muridarum | Baker (1998)† |
| Helicobacter | rodentium | Baker (1998)† |
| Helicobacter | trosontium | Baker (1998)† |
| Helicobacter | typhlonius | Whary et al. (2015)† |
| Klebsiella | oxytoca | Whary et al. (2015)† |
| Klebsiella | pneumoniae | Baker (1998)† |
| Lawsonia | intracellularis | Whary et al. (2015)† |
| Leptospira | spp. | Boey et al. (2019) |
| Mycobacterium | avium-intracellularis | Whary et al. (2015)† |
| Mycobacterium | leprae-murium | Whary et al. (2015)† |
| Mycobacterium | microti | Whary et al. (2015)† |
| Mycoplasma | coccoides | Whary et al. (2015)† |
| Mycoplasma | hominis | Whary et al. (2015)† |
| Mycoplasma | neurolyticum | Whary et al. (2015)† |
| Mycoplasma | pulmonis | Baker (1998)† |
| Pasteurella | pneumotropica | Baker (1998)† |
| Pasteurella | pneumotropica | Heyl 1963; Towne (2014) |
| Proteus | mirabilis | Whary et al. (2015)† |
| Pseudomonas | aeruginosa | Baker (1998)† |
| Salmonella | enterica | Firth et al. (2014) |

*Table 1. Bacterial taxa that have been previously described as rodent-associated pathogens.*
non-metric multidimensional scaling (NMDS) ordination plots and quantified observed differences with PERMANOVA, using an FDR correction for multiple comparisons. We then calculated the percent relative abundance of potential rodent-associated pathogens and other bacterial taxa of interest recovered from Key Largo woodrat individuals (n = 9).

RESULTS

After rarefaction, we observed a total of 714 ASVs among natural Key Largo woodrat nests (n = 10), with an average of 254 ASVs represented per individual nest. ASV richness in natural nests did not differ significantly from supplemental nests (average of 278 ASVs per individual nest; P = 0.21) or from the forest floor (average of 275 ASVs per sample location; P = 0.39; Fig. 2a). We found a similar pattern for Shannon diversity, with no significant difference between natural and supplemental nests (n = 20; P = 0.65) or between natural nests and the forest floor outside of those nests (n = 19; P = 0.62; Fig. 2b). In natural and supplemental nests, the three most abundant phyla were Proteobacteria (natural 31%, supplemental 32%), Actinobacteria (natural 30%, supplemental 33%), and Bacteroidetes (natural 11%, supplemental 13%). At the genus level of identification, Candidatus Nitrososphaera, a group of ammonia-oxidizing archaea (Zhalnina et al. 2014), was the most abundant taxa in natural nests (3.8% of bacterial sequence reads) and accounted for 2.4% of bacterial sequences in supplemental nests. In supplemental nests, the most abundant genus was Streptomyces (5% of bacterial sequence reads), a common source of antibiotic medications (de Lima Procópio et al. 2012), and Streptomyces was the second most abundant genus recovered from natural nests. Additionally, we

| Genus       | Species       | References                  |
|-------------|---------------|-----------------------------|
| Salmonella  | enteritidis   | Baker (1998)†               |
| Spirillum   | minus         | Firth et al. (2014)         |
| Spiroplasma | mirum         | Bastian et al. (1984)       |
| Staphylococcus | aureus     | Baker (1998); (Schulz et al. 2017) |
| Streptobacillus | moniliformis | Whary et al. (2015)†        |
| Streptococcus | pneumoniae   | Baker (1998)†               |
| Streptococcus | dyegalactiae | Whary et al. (2015)†        |
| Treponema   | spp.          | Baker (1998)†               |
| Yersinia    | pestis        | Butler et al. (1982)        |

Note: † Denotes reviewed in citation.

Fig. 2. Diversity of bacteria collected from natural nests, supplemental nests, and the forest floor. (a) There were no observed differences in amplicon sequence variant (ASV) richness between natural and supplemental nests (P = 0.21) or between nests and the forest floor (P = 0.39). (b) As with richness, there was no significant differences in Shannon diversity between natural and supplemental nests (P = 0.65) or between nests and the forest floor (P = 0.62).
observed no significant clustering of the bacterial communities for natural and supplemental nests \((n = 20; \text{PERMANOVA: } P = 0.495; \text{Fig. 3})\); however, we did detect differences between Key Largo woodrat nests overall compared to the surrounding forest environment \((n = 19; \text{PERMANOVA: } P = 0.004; \text{Fig. 3})\).

Differences between Key Largo woodrat nests and the forest floor were not driven by an accumulation of rodent-associated pathogens. Of the 60 taxa considered (Table 1), we did not detect any in natural nests, supplemental nests, or from forest floor samples. However, while we saw no accumulation of potential pathogens, we saw a high relative abundance of Pseudonocardiaceae and Streptomycetaceae in Key Largo woodrat nests (Fig. 4). These bacterial families contain important antimicrobial-producing groups (Platas et al. 1998, Kämpfer et al. 2014) and include bacteria that produce many of our common commercial antibiotics, such as erythromycin and vancomycin (Sakoulas et al. 2004, Jafari et al. 2014, Kämpfer et al. 2014). We might have expected to detect these bacteria in nest and forest samples, as they are found in diverse environments and are abundant in soils, globally. However, it is the high abundance of these taxa that is notable. Relative to all other taxa, Pseudonocardiaceae and Streptomycetaceae were the most abundant bacterial families in both natural and supplemental nests, accounting for 10.5% of all sequence reads from natural nests and 13.3% of all sequence reads from supplemental nests \((n = 20; \text{Fig. 4})\). Further, they were significantly more abundant in nests than on the forest floor, where they represented only 3.6% of all sequence reads \((\chi^2 = 9.68, P = 0.002)\). Both Pseudonocardiaceae and Streptomycetaceae were recovered from all nest and forest samples collected in our study \((n = 29)\).

When we examined Key Largo woodrat body microbiota \((n = 9)\), the three most abundant phyla were Bacteroidetes (33%), Firmicutes (24%), and Proteobacteria (19%), and the most abundant taxa were S24-7 (28.1% of bacterial sequences) and Lactobacillus (8.2% of bacterial sequences; Fig. 4). S24-7 bacteria are almost exclusively found (and are highly abundant) in the guts of mammals (Ormerod et al. 2016), and Lactobacillus is commonly found in mammalian guts and vaginas (Hartemink et al. 1997). Woodrat bacterial communities were distinctly different from those found in nests (PERMANOVA: \(P < 0.001\)), even more so than were nests from the forest environment (PERMANOVA: \(P < 0.002; \text{Fig. 3})\), and they exhibited the greatest amount of variation among samples compared to all other sample types (Fig. 3).

Overall, the relative accumulation of potential pathogens on Key Largo woodrat bodies was very low. We observed only two of the 60...
taxa examined, accounting for just 0.3% of all bacterial sequences. These included *Treponema* spp. and *Streptobacillus moniliformis*. Additionally, due to the high relative abundance of Pseudonocardiaceae and Streptomycetaceae bacteria in nests, we tested for their presence on Key Largo woodrat bodies. Pseudonocardiaceae was the third most abundant family (average of 6% of bacterial sequences; range of 1–16.5%). Streptomycetaceae was less abundant, however, representing an average of 1.2% of bacterial sequences among individuals (Fig. 4). Despite the range in abundance between these taxa and among samples, Pseudonocardiaceae and Streptomycetaceae were found on all individuals.
DISCUSSION

We found the bacterial communities in Key Largo woodrat nests to be distinct from the forest environment, but there were no significant differences in bacterial diversity (Fig. 2) or community composition (Fig. 3) between natural and supplemental nests. Additionally, we did not detect any potential pathogens from nest or forest environments. Key Largo woodrats were host to potential pathogens, but we only detected two of the 60 taxa considered, which comprised a small portion of the total bacteria recovered. Instead, we found a high abundance of bacteria from common antimicrobial-producing bacterial groups (Pseudonocardiaceae and Streptomycetaceae), both in nests and on individuals. However, though Key Largo woodrat body bacteria differ significantly from those of their nests overall (Fig. 3), the high prevalence of these groups suggests some sharing of microbes between individuals and their environment. This microbial exchange might account for some of the similarity among natural and supplemental nests (Fig. 3), despite their differences in construction design and materials.

We expected supplemental nests to be similar to other structures built by humans, in that they might have a less diverse, unique assemblage of bacteria compared to natural nests (Dunn et al. 2013, Lax et al. 2014). If supplemental nests alter bacterial species interactions, this could have detrimental effects on woodrat health. For example, exposure to a greater diversity of microbes increases immune response and the ability to fight off infectious disease in rodents (Beura et al. 2016), other mammals (Becker et al. 2014, Fyhrquist et al. 2014, Ruokolainen et al. 2015), and even in amphibians (Loudon et al. 2014). However, we found no evidence of such an effect. Relative to the forest environment, nests had a similar diversity of bacteria, regardless of whether they were natural or supplemental (Fig. 2). Nests did diverge from the forest environment in the composition of those communities—hosting distinct assemblages of bacteria that were not significantly different from one another, based on nest type (Fig. 3). This suggests that, likely through some combination of nest design or pattern of use, supplemental nests maintain a bacterial community that is no different from their natural counterparts. One explanation might be that the culvert pipes used in supplemental nest construction have open ends. These openings could act in a similar way to the gaps in natural nests or to open windows in human homes, in which bacterial diversity increases on surfaces compared to those that are more closed from the outdoor environment (Kembel et al. 2012, Barberán et al. 2015).

We also found no evidence of potential pathogens in natural or supplemental nests. High bacterial diversity and a high relative abundance of Pseudonocardiaeae and Streptomycetaceae in nests are likely contributing factors, as we know that high bacterial diversity and the application of antimicrobials in human buildings are associated with a decrease in the abundance of pathogenic microbes and a reduction in exposure risk (Lax et al. 2014, Ruokolainen et al. 2017). On the other hand, the application of antimicrobials in homes has favored antibiotic-resistant strains (Hartmann et al. 2016), and therefore, we might expect to find antibiotic-resistant bacteria in Key Largo woodrat nests, particularly since they are typically used for several generations and persist over long periods of time (Rainey 1956).

To understand whether there were shared bacterial taxa between nests and nest inhabitants, we also characterized the bacteria found on Key Largo woodrat bodies. The high abundance of gut-associated bacteria suggests that they are in close contact with their feces. Overall, Key Largo woodrat bacterial communities were distinctly different from those of their nest environments \((P < 0.001; \text{Fig. 3})\). Additionally, the variation in bacterial community composition among individuals was much greater than what we observed between and among nest and forest samples (Fig. 3). One likely explanation is that woodrats harbor bacteria unique to the body compared to environmental bacteria. However, this does not account for the lack of variation we would also expect to see among soil communities. Therefore, another potential explanation might be the convergence of soil-associated and soil-adjacent bacterial communities in response to the landfall of Hurricane Irma in September 2017 (3 months prior to sample collection). Catastrophic weather events can homogenize biological communities (Savage et al. 2018), and therefore, this hurricane event might account for
the similarity between and among nest and forest communities.

Individuals were host to potential pathogens, including Treponema spp. and Streptobacillus moniliformis. However, though these taxa were detected, they only accounted for 0.3% of total sequences. As with nests, the low abundance of pathogens on bodies might be due, in part, to the high abundances of Pseudonocardiaceae and Streptomycetaceae. Further, although these taxa are described as infectious agents among rodents (Baker 1998, Whary et al. 2015), we are unable to confirm whether the presence of these specific bacteria increases infection risk for the Key Largo woodrat. Without further investigation, it would be imprudent for us to directly attribute the bacteria recovered from the Key Largo woodrats (or from their nests) to pathogenesis, but rather, we can use these results as a proxy for understanding the broad-scale patterns of pathogen accumulation.

One of our more unusual findings was the high prevalence of bacteria from Pseudonocardiaceae and Streptomycetaceae in Key Largo woodrat nests. Associations between animals and antimicrobial-producing bacteria have been described in social insects (e.g., from ants and wasps; Currie et al. 1999, Cafaro and Currie 2005, Madden et al. 2013), but to our knowledge, such a relationship has never been observed among non-human mammals. Based on our study design, we cannot ascribe a causative relationship between the Key Largo woodrats and the presence of these bacteria. However, due to their high relative abundance and ubiquity among natural and supplemental nests, we propose the possibility that the bodies and/or behaviors of Key Largo woodrats promote the colonization and accumulation of these bacteria.

Unlike other human-built structures, such as homes and hospitals, supplemental nests do not appear to alter bacterial species interactions in the ways we would predict to be detrimental for individual health. This includes the loss of diverse species interactions that are important for immune development in rodents and other animals, as well as the subsequent accumulation of noxious organisms (Kembel et al. 2012, Becker et al. 2014, Loudon et al. 2014, Cheng et al. 2015, Beura et al. 2016, Stein et al. 2016, Wan et al. 2016). However, due to the variation in the types of supplemental nests and nest boxes used in threatened and endangered species conservation, we recommend that more research is needed prior to the extrapolation of results to the nests constructed for other species of concern. As animals increasingly inhabit supplemental structures that are different from those in which they evolved to live, breed, or seek refuge, it is important that we more fully incorporate microbiome research into conservation biology (Trevelline et al. 2019, West et al. 2019) and consider the comprehensive implications of conservation management practices on animal health.

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**DATA AVAILABILITY**

All 16S rRNA sequences and metadata tables are publicly available in the NCBI Sequence Read Archive (BioProject ID PRJNA632923) and in Qiita (study ID 13137; https://qiita.ucsd.edu/study/description/13137). Additionally, QIIME2 and R code are available on GitHub at https://github.com/hillms/KLwoodrat_bacteria.