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Dog introduction alters the home dust microbiota

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Abstract

Research has largely reported that dog exposure is associated with reduced allergic disease risk. Responsible mechanism(s) are not understood. The goal was to investigate whether introducing a dog into the home changes the home's dust microbiota. Families without dogs or cats planning to adopt a dog and those who were not were recruited. Dust samples were collected from the homes at recruitment and 12 months later. Microbiota composition and taxa (V4 region of the 16S rRNA gene) were compared between homes that did and did not adopt a dog. A total of 91 dust samples from 54 families (27 each, dog and no dog; 17 dog and 20 no dog homes with paired samples) were analyzed. A significant dog effect was seen across time in both unweighted UniFrac and Canberra metrics (both p=0.008), indicating dog introduction may result in rapid establishment of rarer and phylogenetically related taxa. A significant dog-time interaction was seen in both weighted UniFrac (p<0.001) and Bray-Curtis (p=0.002) metrics, suggesting that while there may not initially be large relative abundance shifts following dog introduction, differences can be seen within a year. Therefore, dog introduction into the home has both immediate effects and effects that emerge over time.

Keywords

microbiome; dog; allergy; asthma; microbiota hypothesis; hygiene hypothesis

BACKGROUND

Similar to how growing up on a livestock farm has been shown to be protective against the development of allergies and asthma in European children, living with a pet in early life has been investigated for its relationship with the subsequent development of allergies and asthma in numerous studies in the United States.[1–9] Recent meta-analyses and additional studies have generally reported that pet exposure, particularly to dogs, decreases the risk of

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allergic diseases[9–11], though some have reported an increased risk.[12] With the reasonably consistent findings across many cohorts and populations, animal exposure—specifically to dogs—remains a promising avenue for identifying a prevention strategy for childhood allergic diseases.

The responsible mechanism(s), either wholly or in part, for the effects of dogs on allergic disease development, are not completely understood. Recent hypotheses posit that dogs change the environmental microbiome, which in turns alters the gut microbiome and subsequent immune system development of young children residing in homes with or in proximity to dogs. We and others have found that house dust microbiota communities vary with the presence of dogs and cats in the home (versus homes without dogs and cats).[13–15] As this prior work was cross-sectional, there has been little evidence examining whether a dog changes the home's dust microbiome. Additionally, dust sample collection in previous studies has typically not been targeted to coincide with or carefully measure timing of dog introduction. The goal of this work was to test the hypothesis that introducing a dog into the home has the capacity to change that home's dust microbiota – both in terms of its overall composition and its relative abundance of specific taxa.

METHODS

Study Design

Participants were recruited from the metro-Detroit area. Eligible households had to have lived in their current home for at least one year, planned to live there for the next year and have not kept dogs or cats in their home for one week or more in the previous year. Keeping fish, birds, and other small caged/contained pets like snakes, turtles, hamsters, or insects was not an exclusion criterion. Other exclusions included smokers and household residents who work with, handle, or interact with pets or animals on a daily, weekly, or monthly basis. We recruited 2 groups of households: those that were adopting a new dog ("dog homes"), and those who were not planning to add a dog or other furred pet to their household over the subsequent year ("dog-free homes"). New dog owners were to have their newly added dog inside of their home for at least 12 hours per day with the dog permitted to roam freely about the home, and to not own another dog or cat for the duration of the study (12 months). Once a dog household was recruited, a dog-free household was recruited within 1 month. Participants were recruited from September 21, 2013 – July 09, 2014. This work was approved by the Henry Ford Health System IRB and written informed consent was provided by the study participants.

While participants were recruited through targeted radio broadcasts and Henry Ford Health System's electronic communications and website, most "dog" households were recruited through the Michigan Humane Society's local adoption center and their semi-annual adoption events at the local zoo. Trained interviewing staff identified participants that were eligible. Participants completed a baseline visit within several days of the dog's introduction into their new home. Interviews about household characteristics, residents, and dog activities were conducted at recruitment, and six and twelve months later.

Dust Sample Collection

Dust samples were collected from participating dog homes by study staff within a few days of the dog's introduction into their new home and again at 12 months after the introduction of the new dog. Dust samples were collected from dog-free homes at the time of recruitment and 12 months later. For families with a dog, the main floor area where the family and dog would spend time was vacuumed. For families without a pet, the main floor area where the family reported spending their time was vacuumed. Two areas of the same room were vacuumed. The first vacuuming was in a prefabricated rectangle measuring 1 meter by 2 meters for 2 minutes. Vacuum sample collection "socks" were then switched. The field staff then vacuumed all of the areas outside of that rectangle in the same room, including areas around baseboards, in corners and around and under furniture for 5 minutes. Participants were asked to maintain their typical cleaning practices prior to sampling.

Dust samples were stored at −80°C until initial processing. Dust socks were cut open in a sterile petri dish using sterile ethanol-flamed scissors and forceps. Dust was transferred from the sock into Lysing Matrix E tubes (MP Biomedicals, Santa Ana, CA) using a sterile single-use spatula. These tubes were again stored at −80°C until shipment on dry ice to our collaborating lab at the University of California – San Francisco (UCSF) for sequencing.

Microbiota Sequencing

The analyses of dust samples collected over 5 minutes are presented here. These sample volumes were generally larger than those collected from the premeasured rectangles and therefore provided a higher likelihood of having sufficient sample volume for microbial DNA sequencing. The methods for sequencing the microbial DNA are provided in the Supplemental Text.

Statistical Analysis

Differences in household characteristics between dog and dog-free homes were calculated using ANOVA for numerical covariates and the chi-square test for categorical covariates. Alpha diversity metrics of bacterial richness (number of unique OTUs present), Pielou's evenness (relative distribution of OTUs in a community), Faith's phylogenetic diversity, and Shannon's (non-phylogenetic) diversity were estimated using QIIME[16] and R vegan[17]. Differences in these metrics at each time point by group (dog versus dog-free homes) were calculated using ANOVA, while longitudinal effects were assessed using mixed effect models. Between-subject similarity (i.e., beta diversity) was defined using both weighted and unweighted UniFrac[18], Canberra, and Bray-Curtis distance matrices. UniFrac matrices weight bacterial phylogenetic relationships in the calculation of between-sample distances, while Canberra and Bray-Curtis matrices are the non-phylogenetic correlates to the unweighted and weighted UniFrac matrices, respectively. Relationships between dust microbiota composition and variables measured cross-sectionally and for within-subject effects with repeated measures data (time effects, group*time interaction effects) were assessed using PERMANOVA[19] as implemented in the R package vegan. Compositional tests for between-subject effects with repeated measures data (group effects) was performed using nested PERMANOVA in the BiodiversityR package[20]. Visual representations of compositional differences were plotted using non-metric multidimensional scaling (nMDS)

in the R vegan package. Differences in OTU relative abundance (dog versus dog-free homes) were tested using a two-part zero-inflated beta regression model with the R package ZIBR,[21] which tests for differences in presence/absence, as well as relative abundance. To avoid testing overly sparse OTUs, only OTUs observed in 10% or more of homes were tested. OTUs were considered significant if the false discovery rate adjusted p-value for the joint test (i.e., test of both presence/absence and relative abundance) was less than 0.05.[22]

RESULTS

Sample Size and Description

At least one sample was included in the analysis from the homes of all 54 families (27 each, dog and no dog). These homes yielded 100 total 5-minute dust samples, with 99 successfully amplified for microbial sequencing. One of the 12-month samples was from a family that moved between baseline and 12-months—a protocol deviation—and was therefore excluded. Of the remaining 98 samples that met inclusion criteria, 7 samples were excluded due to unusually low sequencing depth (six with <11 sequence reads; one with <5,000 sequence reads). The OTU table was then rarefied to the minimum depth among the remaining 91 (minimum depth: 34,714 sequence reads).

Among dog homes, 17 (63%) had both baseline and 12-month dust samples, 9 (33%) only had a baseline dust sample included, and 1 (4%) family had only a 12-month dust sample included. Among dog-free homes, 20 (74%) homes had results from samples from both time points, 6 (22%) had dust sample results from baseline only, and 1 (4%) had only a 12-month sample included. Of the 26 dog home baseline dust samples included, 7 were collected on the same day or prior to the dog arriving (“early dog homes”, average number of days from collection of home dust until dog’s arrival into the home=2.1, median=0, range=0 to 9), 18 were collected a day or more after dog arrival (“late dog homes”, average number of days dog was in the home prior to dust collection=3.5, median=2.0, range 1 to 29), and one sample was missing information on timing of dog introduction.

Comparing Households

The dog and dog-free homes were very similar with respect to the household compositions and characteristics, including, but not limited to: number of residents in the home, the number of residents in the home under the age of 18 years, whether the home was a single-family dwelling, and the home’s square footage and room count (all p>0.05; Supplemental Table 1). Four homes without dogs did not have forced air heat while all other homes did (p=0.038; Supplemental Table 1). Homes without dogs also tended to have more children under the age of 5 years (p=0.002; Supplemental Table 1). Though there were differences in the month baseline dust was collected (p=0.003), this was strongly driven by May/June (large dog adoption event in May, followed by recruiting dog-free homes in June), which did not have an impact on seasonal differences between the two groups (p=1.00).

Alpha Diversity Measures

None of the alpha diversity metrics (richness, Pielou’s evenness, Faith’s phylogenetic diversity, and Shannon diversity) differed significantly between the dog homes and dog-free

homes at baseline or at 12 months (Supplemental Figure 1). The results were unchanged after further separating the homes that adopted dogs by early and late dog introduction with respect to sample collection timing (Supplemental Table 2).

Dust microbiota compositional differences

Compositional differences between the homes with and without dogs were noted for the unweighted UniFrac and Canberra metrics at baseline and at 12-months, as well as only at 12-months for weighted UniFrac and Bray-Curtis (Table 1). At 12-months, having a dog in the home consistently explained a higher percentage of variation in bacterial dust composition (approximately 1.3-3.3 percent of variation explained), versus baseline. An nMDS plot of unweighted UniFrac distances visually confirmed the compositional distinction between dog and no dog homes (Figure 1).

Whether these results were affected by the timing of the dust sample collection compared to the introduction of the dog into the home (early vs. late dog homes) was investigated using pairwise comparisons between the 3 groups (Table 2). When dog-free homes were compared to late dog homes at baseline, a significant difference was found using both unweighted metrics (unweighted UniFrac p=0.046, R^2=0.031; Canberra p=0.056, R^2=0.026). These effects persisted at 12-months (unweighted UniFrac p=0.025, R^2=0.025; Canberra p=0.025, R^2=0.033). Further, there were significant differences at 12-months in the weighted metrics for early dog vs. dog-free homes (weighted UniFrac p=0.004, R^2=0.143; Bray-Curtis p=0.015, R^2=0.072).

These cross-sectional results were consistent with repeated measures analyses performed to test if the effect of dog varied over time (Table 3). For each metric, the dog-by-time multiplicative interaction term was not statistically significant. However, the main effect of dog was significant in 3 of the 4 metrics: unweighted UniFrac (p=0.008), Canberra (p=0.008), and Bray-Curtis (p=0.045), meaning dog and dog-free homes were compositionally distinct across the study period. From this, we hypothesized that the lack of a dog-by-time interaction may be due to the fact that many baseline dust samples from dog homes were collected shortly after the dog was introduced (late dog homes), which would result in baseline and 12-month dog dust samples being more similar to one another. Therefore, we subsequently recomputed this model after removing all baseline samples from late dog homes (Table 3). In this case, the interaction term was statistically significant for the weighted UniFrac (p<0.001) and Bray-Curtis metrics (p=0.002), while the main effect of dog remained statistically significant for unweighted UniFrac (p=0.047) and approached, but did not reach, statistical significance for Canberra dissimilarity (p=0.081).

Taxonomic Differences

Dust OTU differences between dog and dog-free homes at the 12-month time point were then tested. Figure 2 shows a total of 109 OTUs at 12-months that were associated with differences between dog and dog-free homes (joint FDR-adjusted p-value<0.05; N=48 where presence/absence differed significantly, N=51 where relative abundance differed significantly, and N=10 where both significantly differed; Supplemental Table 3). The majority of differential OTUs were more likely to be detectable in dog homes or have greater

relative abundance in dog homes, compared to dog-free homes. Specifically, of the 58 OTUs where presence/absence significantly differed, 51 (88%) were more likely to be present in dog homes compared to dog-free homes. Similarly, of the 61 OTUs with significant differential relative abundance, 46 (75%) had higher relative abundance in dog homes compared to dog-free homes. OTUs that were more likely to be detected in dog homes at 12-months included specific members of the genera *Moraxella*, *Porphyromonas*, *Capnocytophaga*, *Fusobacterium*, *Streptococcus*, and *Treponema*. OTUs that had higher relative abundance in dog homes at 12-months included specific members of the genera *Anaerococcus* and *Prevotella*.

DISCUSSION

These data suggest that introducing a dog into the home has both immediate effects on dust bacterial composition as well as effects that emerge over time. The effects seen in both unweighted UniFrac and Canberra—which do not take into account relative abundance, and therefore have increased power to detect shifts in rare lineages[23]—indicate that the introduction of a dog in the home may result in the rapid establishment of rarer and phylogenetically related taxa into the dust microbiota. The significant dog-by-time interaction seen in both the weighted UniFrac and Bray-Curtis metrics—which take relative abundance into account, and therefore have increased power to detect shifts in more common lineages—further suggests that while there may not initially be large relative abundance shifts following dog introduction, these differences may be seen with time. Given that this dog-by-time interaction is only significant in the early but not the late dog homes, this suggests that the time course for this relative abundance shift may begin within the first week following dog introduction, dampening the ability to detect any effect in the late dog homes.

The immediate observed changes in dust microbiota after dog introduction may have been expected given other studies showing rapid shifts in the built environment. For example, Lax et al. recently examined changes in microbial colonization patterns in a newly opened hospital, and found that several hospital surfaces began to resemble human skin microbiota immediately after opening.[24] In a study of house dust embedded in carpet coupons, bacteria growth appeared after a week (with 100% equilibrium relative humidity levels).[25] Similar quick shifts have also been observed in gut microbial communities in response to new dietary exposures [26–27], and in vaginal microbial communities in response to bacterial vaginosis antibiotic treatment.[28]

Our results appear to be consistent with what we and others have previously shown regarding the effect of dog ownership on house dust microbiota. A previous study from our group examined a small set of house dust samples drawn from a birth cohort and revealed that dust from dog-keeping homes had higher relative abundances of specific *Treponema*, *Capnocytophagta*, and *Moraxella* taxa compared to dust from no-pet homes[13]. Additionally, Barberán et al. recently demonstrated in a sample of approximately 1,200 homes across the US that house dust in homes with dogs had higher relative abundances of *Porphyromonas* and *Moraxella* compared to house dust in homes without dogs.[15]

While the current study indicates that dogs alter dust microbiota composition, understanding the specific mechanisms of this effect require further investigation. A limitation of this study is that we did not measure the microbiota of dogs themselves (e.g., oral, gut, skin) nor of their outdoor environments (e.g., soil microbiota). Therefore, it is unclear if the bacteria introduced into dog homes was sourced from the dogs directly or, alternatively, if they were outdoor environment-associated "bacterial hitchhikers" brought into the homes by dogs. However, many of the taxa enriched in dog homes have previously been identified as common members of the canine oral microbiota (*Porphyromonas*, *Fusobacterium, Capnocytophaga, Moraxella*) [29–30], as well as the canine gastrointestinal tract microbiota (*Fusobacterium*, *Prevotella*, *Streptococcus*)[31–32]. The microbiota of the skin of the dog has also been investigated in cross-sectional studies.[33–35] While between dog variability of microbiota is high, with sampling site, sex of the dog, and skin health status contributing to the variability, all skin samples from a series of pure-breed dogs included the following families: *Corynebateriaceae* (Actinobacteria), *Streptococcaceae* and *Lachnospiraceae* (Firmicutes); Fusobacteriaceae (Fusobacteria); and *Comamonadaceae*, *Oxalobacteraceae* and *Neisseriaceae* (Proteobacteria).[35] Another cross-sectional study of dogs of varying breeds reported *Oxalobacteraceae* and Proteobacteria were the most abundant phylum and family identified, respectively.[33]

Many of the key taxa found to be significantly different by dog (*Moraxella*, *Porphyromonas*, *Capnocytophaga*, *Fusobacterium*, *Streptococcus*, and *Treponema*) [36–39] have also emerged in recent studies of the microbiota and its associations with allergies and asthma.[36, 38, 40–42] However, the evidence from these reports is conflicting when comparing results across studies – possibly because the data were derived from various types of samples (e.g., stool, sputum, airway, etc.). It should be noted that though these specific taxa were found to differentiate by dog ownership in house dust, they may not necessarily also differentiate by dog ownership in the gut microbiota of at-risk children. Alternatively, it is possible that they alter the gut environment in such a way that other microbes may thrive or decline, and these other microbes could directly affect the risk of allergies and asthma. Additional studies are needed to understand how dog-exposed house dust alters gut microbiota in early life.

These results should be viewed as further evidence in support of the evolving "Microbiota Hypothesis" related to allergy and asthma risk. [43] The hypothesis is that the environmental microbial diversity influences the assemblage process of an infant's gut microbiota ecosystem which subsequently, in partnership with exposure to allergens and microbes, influences the child's immune development and risk for allergies and asthma.[43–45] Evidence supporting this hypothesis continues to increase. Children with dogs in homes in early life have been shown to have less allergy and asthma.[6, 9–11, 46–47]

Other work has shown that children with dogs in the home in the first year of life have greater microbial diversity in their stool, [48] while other studies have shown that children with greater microbial diversity in their stool in early life have less allergy and asthma. [43, 49–53] The present work demonstrates that dogs change the home dust microbiota by increasing the types and relative abundances of specific genera. Home dust bacterial composition and allergen levels have been found to be associated with subsequent allergic sensitization and wheezing[54]. However, the precise mechanisms by which a child's gut

microbiota can be influenced by their home dust microbiota are not specifically known. Additionally, it is not known if a specific species or a network of species are necessary to impact the immune system's development. It is possible that many different combinations of bacteria in early life could yield better health in the child, but perhaps the optimal combinations depend on to what the child has already been exposed. It is also not known if gut microbial composition and immune function changes can be induced after the immune system has been educated in early life.

A limitation of the study is that we did not conduct repeated sampling over shorter intervals that would have allowed us to more precisely identify when significant changes in taxa presence and relative abundance occurred. Further, due to the difficulty in identifying and recruiting dog families prior to receiving their new dog, many of the baseline dog home samples were collected after the dog was introduced, adding variation to dog home samples at baseline. However, given that we observed differences by dog at baseline among those who had only been resident for a short time—which were not merely explained by dog-associated demographic or household characteristics—dogs may affect bacterial house dust composition more rapidly than anticipated.

Further investigation is required to determine if these particular taxonomic changes are generalizable or are specific to metropolitan Detroit. Additionally, specific dog characteristics such as size, fur type or indoor/outdoor activity were not examined due to the small sample size. However, by including in this project homes that had not been keeping furred animals for a period of approximately one year or more, as well as a comparison population similar in terms of household characteristics and demographics, we think the strengths of this work reinforce its importance.

In summary, this work demonstrates that dogs can rapidly alter the home dust microbiota, primarily by increasing the presence and relative abundance of specific bacteria and that this alteration is more easily seen after one year. This evidence, partnered with epidemiological studies relating early dog exposure to the diminished occurrence of allergic disorders, supports the Microbiota Hypothesis for impacting asthma and allergies. Future studies must identify which taxon or combinations of taxa will be most beneficial for the developing gut microbiota for the long-term health of children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Practical Implications

In many studies, living with dogs has been shown to decrease the subsequent risk of allergies and asthma; however, the mechanisms are not well understood. Recent and current investigations are examining the role of the gut microbiome in allergic disease development. As part of this hypothesis, dogs could alter the environmental microbiome which could, in turn, modify the gut microbiome. This study was conducted to investigate how the introduction of a dog into a home changes the home's environmental microbiota. Dog introduction into the home appears to immediately introduce low abundant bacterial taxa, and by 12-months post-introduction, results in large relative abundance shifts. Operation taxonomic units (OTUs) that were more likely to be detected in dog homes at 12-months included specific members of the genera *Moraxella*, *Porphyromonas*, *Capnocytophaga*, *Fusobacterium*, *Streptococcus*, and *Treponema*. OTUs that had higher relative abundance in dog homes at 12-months included specific members of the genera *Anaerococcus* and *Prevotella*.

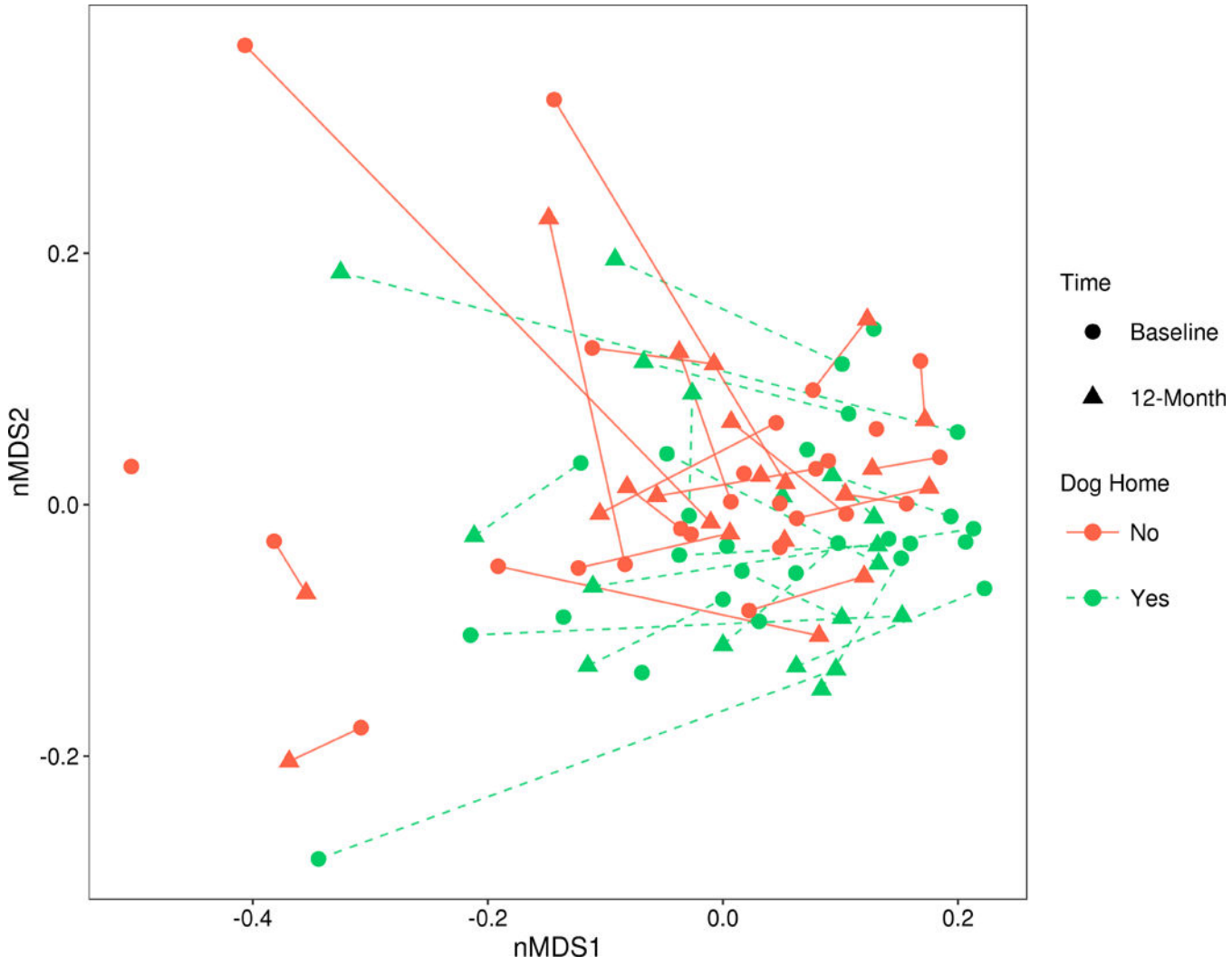

Figure 1.

Non-metric multidimensional scaling (nMDS) plot of unweighted UniFrac distances, by dog introduction status and timing of sample collection. Samples from the same home are connected by a line (solid for dog-free homes and dashed for dog homes).

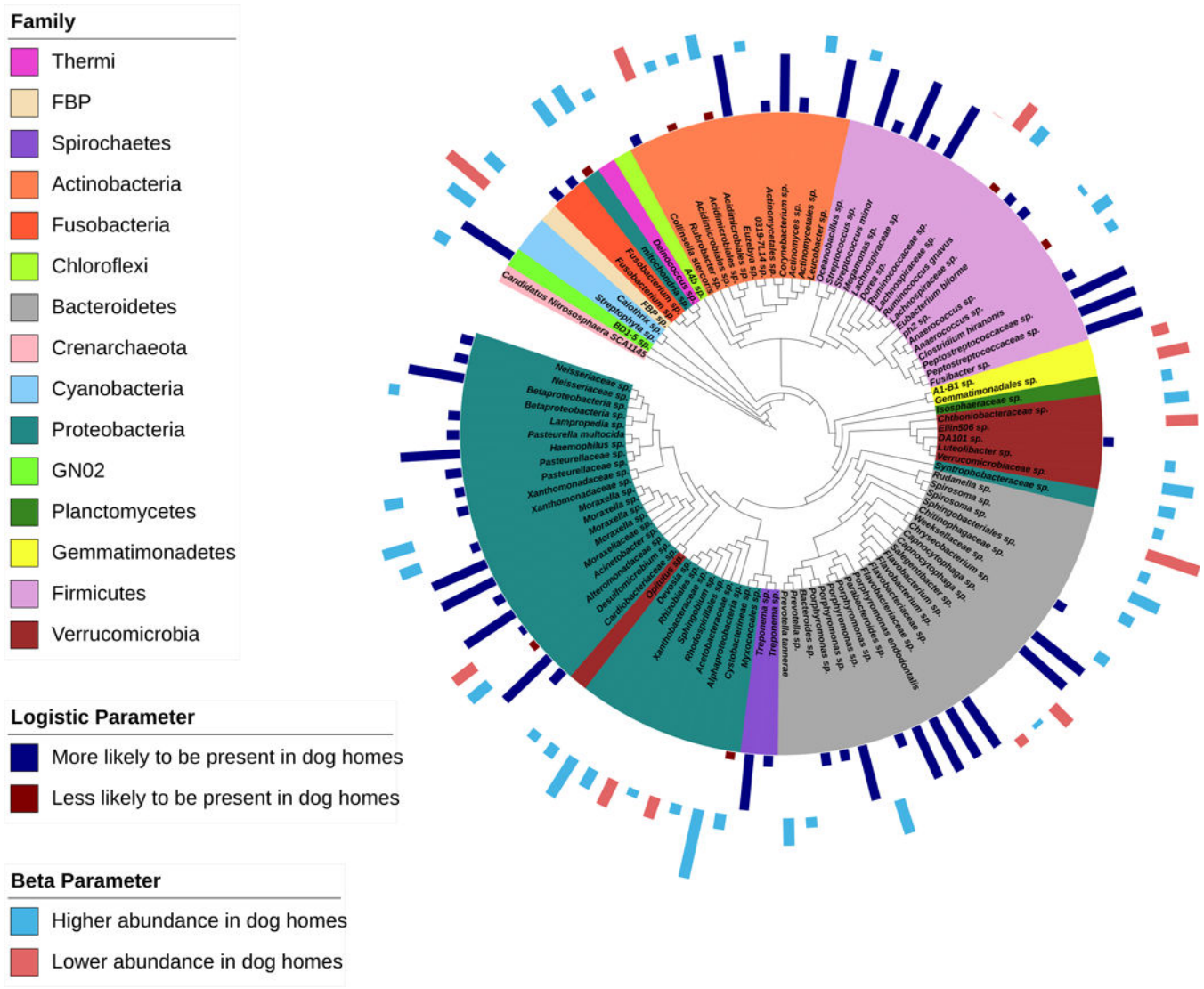

Figure 2.

12-month OTUs associated with differences between dog and no dog homes, (joint FDR-adjusted p-value<0.05), identified by a two-part zero-inflated beta regression model.

Table 1

Compositional differences between dog and dog-free homes, stratified by timing of sample collection.

Metric	Baseline*	12-Month†
	p-value (R^2)	p-value (R^2)
Unweighted UniFrac	0.025 (0.028)	0.021 (0.037)
Weighted UniFrac	0.52 (0.017)	0.049 (0.056)
Canberra	0.043 (0.023)	0.024 (0.031)
Bray-Curtis	0.20 (0.023)	0.015 (0.044)

*At baseline: N=26 dog-free samples; N=26 dog samples

†At 12-months: N=21 dog-free samples; N=18 dog samples

Table 2

Compositional differences by dog introduction status and timing of sample collection.

Metric	Overall*	Dog-free vs. Early Dog Home	Dog-free vs. Late Dog Home	Early Dog Home vs. Late Dog Home
	p-value (R^2)	p-value (R^2)	p-value (R^2)	p-value (R^2)
Baseline†				
Unweighted UniFrac	0.12 (0.05)	0.25 (0.03)	0.05 (0.03)	0.92 (0.04)
Weighted UniFrac	0.69 (0.03)	0.27 (0.04)	0.79 (0.01)	0.78 (0.02)
Canberra	0.20 (0.04)	0.39 (0.03)	0.06 (0.03)	0.92 (0.04)
Bray-Curtis	0.30 (0.04)	0.15 (0.04)	0.33 (0.03)	0.62 (0.04)
12-Months‡				
Unweighted UniFrac	0.04 (0.06)	0.14 (0.05)	0.03 (0.04)	0.30 (0.06)
Weighted UniFrac	0.02 (0.11)	0.004 (0.14)	0.17 (0.04)	0.14 (0.10)
Canberra	0.02 (0.06)	0.07 (0.05)	0.03 (0.03)	0.11 (0.06)
Bray-Curtis	0.02 (0.08)	0.02 (0.07)	0.05 (0.04)	0.17 (0.07)

*These p-values correspond to the overall test of the three group comparison (dog-free vs. early dog vs. late dog).

†At baseline: N=26 dog-free samples; N=7 early dog samples; N=18 late dog samples (one baseline dog sample with unknown timing of dog introduction, excluded)

‡At 12-months: N=21 dog-free samples; N=3 early dog samples; N=15 late dog samples

Table 3

Compositional differences by dog introduction status, across time using repeated measures analysis.*

Metric	p-value for Dog Effect	p-value for Time Effect	p-value for Interaction Term between Dog and Time
Overall (N=91)			
Unweighted UniFrac	0.01	0.23	0.27
Weighted UniFrac	0.11	0.07	0.55
Canberra	0.01	0.15	0.32
Bray-Curtis	0.05	0.13	0.56
After removing baseline dust samples collected in late dog homes from the analyses (N=73)			
Unweighted UniFrac	0.05	0.21	0.34
Weighted UniFrac	0.16	0.03	<0.001
Canberra	0.08	0.24	0.39
Bray-Curtis	0.25	0.31	0.002

*Nested PERMANOVA models were used for the repeated measures analyses.