

Sequencing the Microbiome of Agricultural Household  
Dust: Characterizing Community Structure

Natalie Soto

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Committee:

Elaine Faustman (chair)

Edmund Seto

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Department of Environmental and Occupational Health Sciences

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Natalie Soto

University of Washington

**Abstract**

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Natalie Soto

Chair of the Supervisory Committee:

Elaine Faustman PhD DABT

Department of Environmental and Occupational Health Sciences

**Intro:** Understanding how indoor environments can not only affect but also shape human health is a new area of emerging research. Considering that we spend a large portion of our daily lives indoors, dust is a major complex environmental mixture we are exposed to every day. While we already know from previous studies that several factors can influence the indoor dust microbial community, few studies have examined the effects of agricultural activities on the indoor dust microbiome, especially through the perspective of occupational pesticide exposures.

**Methods:** We performed shotgun metagenomic sequencing on 104 household dust samples collected from a longitudinal agricultural cohort from the Lower Yakima Valley of Washington. After successfully sequencing our dust samples, not only did we compare the indoor dust microbial community structure between farmworker (FW) and nonfarmworker (NFW) households, but we also evaluated how the indoor dust microbiome changed over time since we collected these samples throughout the course of two time periods (2005 and 2011) and across two agricultural seasons (Thinning and Nonspray Season). Additionally, since we also had total dust organophosphates (OPs) concentrations for each of our household dust samples, we also examined how the indoor dust microbial community changed in relation to organophosphates exposures.

**Results:** We observed significant longitudinal and occupational effects on the indoor dust microbiome. We found that for both Alpha and Beta diversity the indoor dust microbial community structure significantly changed between CHC2 and CHC3, with the difference in total dust OP concentrations between the two cohort years playing a significant role in the observed Shannon diversity differences. Additionally, when assessing differential taxa abundance using ANCOM-BC we found that 7 Orders and 40 Genera were significantly impacted by total dust OP concentrations as some taxa decreased when total dust OP

concentrations increased and other taxa showed the inverse relationship. Furthermore, we also observed significant Occupational effects on individual taxa abundances at the Order, Family and Genus level. Although only one Order had significantly higher abundances in NFW households compared to FW households, both of our significant Family members as well as our significant Genera each had higher abundances within FW households.

**Conclusion:** These results have demonstrated that the indoor dust microbial community is influenced by agricultural activities. While we not only provided evidence of the occupational take-home pathway by showing that in addition to pesticide residues being brought into the home, environment bacteria can also be introduced, we also showed that organophosphates found within household dust can directly affect the indoor dust microbiome. While researchers are still uncovering the many functions of all these unique and different bacteria, considering that household dust is a significant reservoir and route for indoor microbial exposures, understanding whether these microbes can positively or negatively influence human health will be an important next step for future public health, especially for the many families and children residing within these farm exposed households.

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## **Introduction**

Over the years there has been a growing interest in understanding how indoor environments can affect and shape human health. Considering the increase in urbanization trends (Kelley & Gilbert, 2013; Seto et al., 2013) and the fact that we spend greater than 90% of our time indoors through activities within homes, workplaces, vehicles, and other recreational buildings (Klepeis et al., 2001), the indoor environment has been recognized as an important contributor to potential environmental exposures over these past decades. With the onset of the 2020 Covid-19 pandemic caused by the novel SARS-CoV-2 coronavirus, local and state governments mandated stay at home orders as well as non-essential business closures to mitigate viral transmission (Abouk & Heydari, 2021; Weerakoon et al., 2021). Although some of the short-term lifestyle changes like not going to gyms, bars, and restaurants during the peak of the pandemic have eased up as social distancing orders and case counts go down (CDC, 2020), there still has been long-term changes in daily life as many businesses and employees continue to operate remotely from home. With this new era of “work from home” and more time these days being spent at personal residences, studies investigating the impact of indoor environments on human health are warranted more than ever before.

While various pollutants in indoor air have been measured and quantified throughout the latter half of the 20<sup>th</sup> century with the advancement of analytical tools and technology (Samet & Spengler, 2003; Tran et al., 2020), in recent years scientist have begun to look at another important reservoir of indoor environmental exposures: house dust. Considering that humans spend a large portion of their daily lives indoors with the majority of that time at home (Weerakoon et al., 2021), dust is a major complex environmental mixture we are exposed to everyday. Since household dust is comprised of various physical, chemical, and biological materials, dust can come in a variety of shapes and sizes ranging from >2 mm to <63 µm with approximately one third of dust being smaller than 500 µm (Lanzerstorfer, 2017). This is not only concerning due to the fact that these finer fractions are small enough to be inhaled or ingested (Butte & Heinzow, 2002), but it has also been suggested that particles <150 µm can more easily adhere to skin and hands (HC et al., 2018; Vicente et al., 2019). Given that the average home collects around 18kg of dust each year (Prussin & Marr, 2015), exposures to household dust are likely to occur, especially for young children who have greater hand to mouth activity and are crawling in spaces close to dust in carpets and furnishings (Black et al., 2005). While dust might have traditionally been viewed as a benign everyday nuisance, many studies have shown this is not the case as toxic environmental contaminants have been found within this medium such as radionuclides, asbestos, persistent organic pollutants, metals, microplastics, phthalates, allergens, tobacco smoke residues, and even more (Bennett et al., 2019; Curl et al., 2002; Isley et al., 2022; Lanzerstorfer, 2017; Liroy et al., 2002; Mitro et al., 2016, 2016; Smith et al., 2017; B. Thompson et al., 2003, 2014; Whitehead et al., 2011; J. Zhang et al., 2020). This not only suggests that household dust is an important route and a significant source of toxic environmental exposures, but also shows that dust is a valuable resource for assessing human exposures since multiple contaminants can be found within this single medium (Butte & Heinzow, 2002; Liroy et al., 2002; Rintala et al., 2012; Whitehead et al., 2011).

In addition to the various toxic environmental contaminants found within dust, dust is a large heterogenous mixture of organic and inorganic materials including hair, dead skin cells, dander,

pollen, spores, mites, plant debris, insect parts, particulate matter, dirt, building materials, fibers from clothing/furniture, and an abundance of microbes (Macher, 2001; Morawska & Salthammer, 2003; J. R. Thompson et al., 2021). In fact, it has been estimated that the concentration of bacteria in household dust can range all the ways up to  $10^9$  bacterial colony forming units (cfu) per gram of dust (Rintala et al., 2012), making dust a significant reservoir for human microbial exposures. Although we have known for a long time that microbes are ubiquitous in nature (Horve et al., 2020), up until the 21st century, before the discovery of next generation sequencing (NGS) technologies, indoor microbial compositions remained poorly understood (Barba et al., 2014; Gilbert & Stephens, 2018). While previous studies relied on culturing techniques as well as other assays to quantify bacteria and their components (endotoxins, metabolites, etc.) (Carnes et al., 2017; Macher, 2001; Peltola et al., 2001; Rennie et al., 2012; Täubel et al., 2011), these methods were limited due to the fact that they could only detect a small portion of the microbial flora present in the environment (Gupta et al., 2017; Rintala et al., 2008). With the development and application of NGS technologies, scientist have now been able to characterize previously unculturable bacteria, gaining valuable insights into the complex dynamic nature of microbiome communities (Kelley & Gilbert, 2013). This technology has not only provided researchers the opportunity to explore the diversity of microbes within our indoor environments and better quantify microbial exposures, but has also allowed us to further elucidate the complicated relationship between bacterial interactions and human health.

Although early indoor bacterial investigations primarily focused on disease transmission and pathogenesis of a few select taxa (Gilbert & Stephens, 2018; Samet & Spengler, 2003), through utilization of NGS technology there has been a shift in terms of how we think of indoor microbial exposures as we are now able to detect the many unknown bacteria within our surroundings. Considering that there around 500-1000 different bacterial species within household dust (Rintala et al., 2012), humans interact with a wide variety of different microbes at home every day. Although some of these bacteria may be harmful (Fan et al., 2010; Haysom & Sharp, 2003; Martinez et al., 2019; B. Y. Zhang et al., 2015), the majority of these bacteria are not infectious and their roles within human health remain unknown. While there has been a lot of progress over the years in characterizing the complex bacterial diversity within household dust (Adams et al., 2014; Barberán et al., 2015; Gangneux et al., 2020; Hanson et al., 2016; Mäki et al., 2021; Nygaard et al., 2020; Shan et al., 2020; Sitarik et al., 2018; Täubel et al., 2009; J. R. Thompson et al., 2021), there has also been increasing evidence that some of these indoor bacterial exposures may be beneficial. For example, many epidemiology studies have shown a low prevalence of allergic diseases throughout farming communities (Adler et al., 2005; Douwes et al., 2007; House et al., 2017; Riedler et al., 2001; Von Ehrenstein et al., 2000; von Mutius & Vercelli, 2010). Considering that farmworkers are exposed to a wide variety of biodiverse agents (plants, animals, soils, etc.), evidence suggests that the high microbial diversity found within a farming environment may be the main driver for allergy protection (Birzele et al., 2017; Debarry et al., 2007; Ege et al., 2011; Hagner et al., 2013; Kirjavainen et al., 2019; M. K. Lee et al., 2018; Valkonen et al., 2015; von Mutius & Vercelli, 2010). In fact, several studies have shown that not only do farms harbor a rich and diverse microbial community (Deckers et al., 2019; Ege et al., 2011; Vestergaard et al., 2018; White et al., 2019), but also these microbes can be found within the dust of farmworker homes (Birzele et al., 2017; Ege et al., 2011; Korthals et al., 2008; M. K. Lee et al., 2018; Normand et al., 2011; Vestergaard et al., 2018). Additionally, dust from farmworker households has been shown to have a higher richness and diversity compared to non-

farmworker households (Ege et al., 2011; Kärkkäinen et al., 2010; Kirjavainen et al., 2019; Vestergaard et al., 2018). While this not only indicates that the outdoor environment can influence the indoor environment, this may also help us understand why allergic diseases are on the rise throughout Westernized nations as modern urbanization has reduced the microbial diversity within both our outdoor and indoor environments through contemporary building/city design and improved sanitation practices (Böbel et al., 2018; Flies et al., 2020; Haahtela, 2019; Hanski et al., 2012; Liu, 2015; Parajuli et al., 2018; Rook et al., 2013; Schröder et al., 2015; Shan et al., 2020). Furthermore, asthma risk in children of non-farm homes was shown to decrease as the composition of their dust microbiomes increased in similarity to that of farm homes (Kirjavainen et al., 2019). With all of this, evidence suggests that exposures to the indoor dust microbiome can influence human health, and while this not only emphasizes the importance of characterizing and understanding the diversity of household dust microbiota, we should also investigate the factors that determine their indoor compositions.

Several factors are known to impact the indoor dust microbiome such as the indoor environment including ventilation, lighting, and moisture as well as the outdoor environment including things like geography, local climate, plants and soils (Dunn et al., 2013; Fahimipour et al., 2018; Gilbert & Stephens, 2018; Gupta et al., 2020; Leung & Lee, 2016; Rai et al., 2021a). However, anthropogenic factors like occupancy number, demographics, pets, and even lifestyle have also been shown to be some of the most significant contributors to indoor dust bacteria (Adams et al., 2014; Barberán et al., 2015; Dunn et al., 2013; Gangneux et al., 2020; Gupta et al., 2020; Hanson et al., 2016; Lax et al., 2014; Leung & Lee, 2016; Rai et al., 2021a; Täubel et al., 2009). While we already know from previous studies that farmworker households contain many unique and diverse microbiota (Ege et al., 2011; Kirjavainen et al., 2019; Normand et al., 2011; Vestergaard et al., 2018), our understanding of how certain agricultural activities shape the indoor dust microbiome remains limited as the majority of microbial studies within farming environments have primarily focused on livestock work (Birzele et al., 2017; Böbel et al., 2018; Debarry et al., 2007; Kirjavainen et al., 2019; Korthals et al., 2008; Normand et al., 2011; Vestergaard et al., 2018; White et al., 2019). Considering that there are different types of farms and farming involves a wide variety of different tasks, knowing how these different agricultural activities determine protective or deleterious microbiota within indoor environments could have important implications for human health especially among family and children residing within farm exposed households. On top of this, although many agricultural activities involve the use of pesticides, it is surprising to see how little we know about how occupational pesticide exposures can influence the indoor dust microbiome, since we already know from previous studies looking at household dust that farmworkers can carry pesticides into the home environment through the occupational take home pathway (Bennett et al., 2019; Coronado et al., 2006; Curl et al., 2002; Smith et al., 2017; B. Thompson et al., 2003, 2014).

By utilizing a well-characterized longitudinal agricultural cohort from the lower Yakima Valley of Washington we hope to further our understanding of how certain occupational farming activities impact the indoor dust microbiome by comparing household dust from farmworkers (defined as agricultural workers working with pome fruits) and non-farmworkers (occupations other than farmworkers, which in this cohort included positions in factories, dairies, stores, schools, and daycares) using shotgun metagenomic sequencing. This cohort not only gives us an exciting opportunity to explore how farmworkers with known elevated occupational pesticide exposures can influence the indoor dust microbiome, but also because our household dust

samples were collected from farmworker and non-farmworker households between 2005/2006 and 2011 across two agricultural seasons, we can also investigate how the indoor dust microbiome varies longitudinally throughout agricultural seasons. Although Lee et al. (2018) was the first to define an “agricultural dust microbiome” using a large-scale agricultural study which examined how different farming activities affect household dust bacteria, this study was limited as the researchers only had one time point for each household dust sample and did not include occupational pesticide exposure information. In contrast, for our study, we not only have detailed pesticide concentrations for each household dust sample, along with previous analysis showing that farmworker households had higher overall dust pesticide concentrations than non-farmworker households (Bennett et al., 2019; B. Thompson et al., 2014), but also given that different agricultural activities happen during different seasonal time points, we cannot assume that a single dust sample will accurately reflect the household dust microbiome over time, which is why this study includes both longitudinal and seasonal time points. Furthermore, although there have been a few studies in the literature exploring seasonal effects on indoor dust microbiota (Adams et al., 2014; Frankel et al., 2012; Gupta et al., 2020; M. K. Lee et al., 2018; Rai et al., 2021b; Rintala, Pitkäranta, et al., 2008), none of these studies have explored this concept through the lens of seasonal agricultural activities and pesticide applications. We know from previous studies that pesticide residues in household dust are highest during the thinning season when pesticides application occurs (Smith et al., 2017; Tamaro et al., 2018; B. Thompson et al., 2014), but the impact of seasonal pesticide sprays on the indoor dust microbiome remains poorly understood.

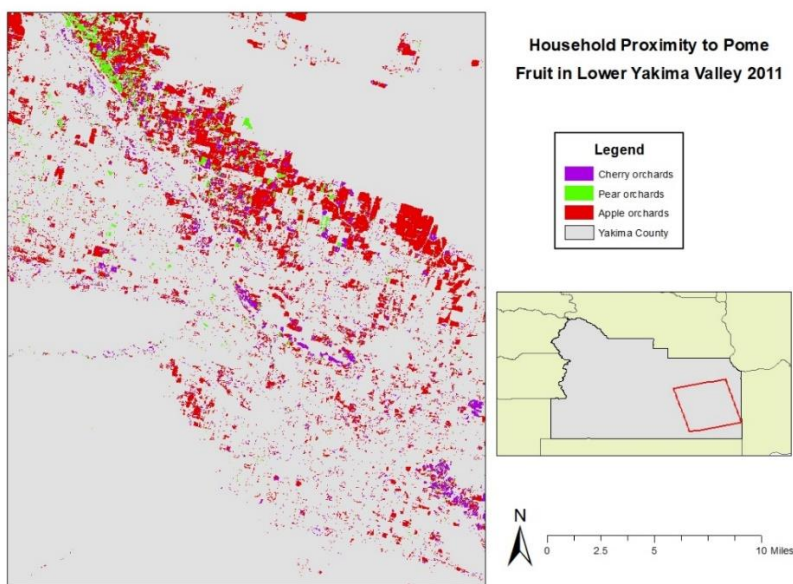
Thompson et al. (J. R. Thompson et al., 2021) previously described the concept of a common core indoor dust microbiome, where all households regardless of geolocation, occupancy, and environmental/indoor factors share common dust microbiota. Based off this concept, although our study may differ from previous studies, especially the ones focusing on nonfarming populations, we would still expect to find some similarities between the indoor dust microbiota in our samples with other study samples. Additionally, given that our entire cohort is from the same agricultural region sharing the same geography and bioclimatic environment, building further on this concept of a common core indoor dust microbiome, regardless of occupational status, we expect to find similar farm associated bacteria throughout all of our dust samples. So, while this project is designed to investigate how agricultural activities can influence the indoor dust microbiome, this study will also characterize a common core indoor dust microbiome within an agricultural setting. Overall, we hypothesize that within our cohort not only will the indoor dust microbiome community structure change with occupational status, time, and season, but we also hypothesize that we will observe a high abundance of agricultural associated bacteria.

## **Methods**

### ***Setting***

The setting has already been described previously (Bennett et al., 2019; Plascak et al., 2019; Smith et al., 2017; B. Thompson et al., 2014), but briefly; this study took place in the Lower Yakima Valley of Washington State where the population is approximately 50.2% Latino, while only 13% of Latinos make up Washington’s overall population (U.S. Census Bureau QuickFacts, 2021), and the agricultural industry is the leading employment sector in the county providing around 27.8% of jobs (Meseck, 2022). Yakima County cultivates a wide variety of fruits and

vegetables and is well known for being a top producer of apples, pears, sweet cherries, and hops in Washington state (“Crop Production Yakima County, Washington State University,” 2022). Many pesticides have been used in the production of these crops over the years, especially organophosphates such as Azinphos-methyl and Phosmet back in 2005 when this longitudinal study began, however Azinphos-methyl is no longer used today (Goldberger et al., 2011). The majority of the agricultural workers in Yakima County are Latino and perform a wide variety of agricultural tasks some of which involve the cultivation, harvesting, and care of crops which often results in pesticide exposures. **Figures 1** and **Supplementary Figure 1** show the Lower Yakima Valley study area along with the distribution of pome fruit orchards during 2005/2006 and 2011.



**Figure 1. ArcGIS map of study area in 2011.** Map of Washington’s Lower Yakima Valley showing distribution of pome fruit orchards in 2011 during CHC3

### ***Cohort Description***

The University of Washington Center for Child Environmental Health Risks Research (CHC) Cohort was designed as a community based participatory research study to examine the pathways by which children are exposed to pesticides. A number of reports have been published detailing the work performed within these communities (Coronado et al., 2004, 2006, 2009, 2011; Curl et al., 2002; B. Thompson et al., 2003, 2008, 2014) including detailed longitudinal exposure monitoring to capture changes in agricultural and non-agricultural pesticide use over time (Bennett et al. 2019). 100 families of the 2011 (CHC3) cohort were mostly drawn (N=91) from the prior 2005 (CHC2) cohort with the other eight families newly recruited and one from a previous cohort (CHC1). The initial CHC2 cohort was predominantly Latino (>95%) and included 200 households (100 FW and 100 NFW households), each represented by an adult ( $\geq 18$  years old) and child pair between the ages of 2-6. All farmworkers included in this study worked with pome fruit (apples and pears) whereas non-farmworkers were defined as people with occupations in factories, dairies, stores, schools, and daycares. The reason why we limited farmworker recruitment to those who only worked with pome fruit was due to previous analysis showing that pome fruit workers had significantly higher concentrations of dimethyl pesticide

metabolites in their urine along with higher household and vehicle dust pesticide concentrations compared to farmworkers who did not work with these crops (Thompson et al., 2003). So, by including only pome fruit workers in this cohort, we not only had a farming population with similar pesticide exposure patterns, since apples and pears are treated with similar types of pesticides as well as similar growing/harvesting cycles, but we also wanted to achieve high exposure variability when comparing farmworkers to nonfarm workers in order to assess occupational pesticide exposure impacts. In 2005 and 2011, dust samples and survey questionnaires from each household were collected as well as urine, blood, and oral buccal specimens from adults and children, and if study participants changed residence between 2005 and 2011, samples were collected from their new homes in 2011. The participating adults in both groups were largely female (80%) and the average age in both groups (FW and NFW) of adults was 31 years. Marital status and housing type were similar between both sampling years with most study participants living in single family homes; however, income was higher in 2011 than in 2005 for both FWs and NFWs.

### ***Dust Collection***

Cohort Dust samples were previously collected during the thinning and non-spray seasons of 2005/2006 (CHC2) and 2011 (CHC3). The thinning season is when buds and small fruit are removed from trees to promote the growth of larger fruit and during this time organophosphate pesticides (OPs) are heavily used. Previous studies support this as they found that OP exposure is the highest in household dust and urine during the thinning season (Bennett et al., 2019; Smith et al., 2017; Tamaro et al., 2018; Thompson et al., 2014). The non-spray season is the time of year when crops are dormant and when pesticide application is not occurring. Between both cohorts and seasons the same dust collection protocol was followed, however the collection period for the thinning season differed between the time periods to match agricultural activities. In 2005 sampling occurred for the thinning season from April-July whereas in 2011 sampling occurred from June-August. In addition to this, different vacuum cleaners were used to collect dust between CHC2 and CHC3. In 2005/2006, household dust samples were collected using a Nilfisk GS-80 vacuum cleaner unit (model GS-80; Nilfisk of America, Malvern, PA), whereas in 2011 a Metropolitan VM-500 High-Powered hand-led vacuum (VM500; Metropolitan Vacuum Cleaner Company, Inc, Suffern, NY) was used. Bennett et al., 2019 previously explained that although the Metropolitan vacuum collected less dust than the Nilfisk vacuum, both machines were able to collect sufficient dust for pesticide analysis based on the level of detection. Additionally, 200mg of dust for each sample was used for DNA extraction in this study, further minimizing this difference.

The dust collection protocol has previously been described in detail (Bennett et al., 2019; Coronado, Holte, Vigoren, Griffith, Barr, et al., 2011; Plascak et al., 2019, 2019; Smith et al., 2017; Tamaro et al., 2018; Thompson et al., 2014), but briefly; dust collection began with a parent participant identifying the area of the household where the child played the most. Using a  $0.5 \times 0.5$  m<sup>2</sup> square template, dust collection was standardized based on the floor surface: 4 templates for plush carpets, 6 templates for thin/flat carpets, and 8 templates for hard floors. Templates were traversed vertically and horizontally subsequently with the hose held upright while the vacuum ran for 10s to ensure that all material passed into the bag. In CHC2 the hose, nozzle, and lower container of the vacuum were cleaned between each use, and a new polyliner and vacuum bag were used for each sample collection. In CHC3 one Metropolitan vacuum was

used per household and was cleaned after each collection with soap and water and rinsed with methanol. After dust collection, samples were stored on ice and transported to the University of Washington where they were frozen at -10°C. All participants provided informed consent, and all sample collection procedures were reviewed and approved by the Fred Hutchinson Cancer Research Center’s Institutional Review Board (File IR 5946). Samples were analyzed under the University of Washington Institutional Review Board (Files 40794 and 40570).

### ***Dust Sample Preparation***

Dust samples were transferred to 150-µm metal sieves (VWR, West Chester, PA, USA) and sieved for 10 min in a sieve shaker (Model RX-24; WS Tyler Inc, Mentor, OH, USA). Dust passed through the sieve was weighed and partitioned into two 1-g aliquots, and the remaining was saved in a separate container. All dust samples were then stored at -10°C until analysis.

House Dust	2005/2006				2011				Total Samples
	Thinning Season		Non-spray Season		Thinning Season		Non-spray Season		
Occupation	FW	NFW	FW	NFW	FW	NFW	FW	NFW	104
# of Households	18	19	24	16	11	5	8	3	
# of Households/Season	37		40		16		11		
Total Sample/Year	77				27				

**Table 1. Breakdown of household dust samples used.** Between CHC2 and CHC3 104 household dust samples were used in total with each household categorized as either FW or NFW households and dust samples were collected during the thinning or Nonspray season.

### ***DNA Extraction, Sequencing Prep, and Metagenome assembly of Illumina fastq reads***

**Table 1** shows the breakdown of our house dust samples between cohort Year, Occupation, and Season. Total genomic DNA was manually extracted from 104 dust samples (27 dust samples were from CHC3 and 77 dust samples were from CHC2) using the Mag Max Microbiome Ultra Nucleic Acid Extraction Kit with an input of 200 mg of dust. The concentration of these DNA extracts was determined using the Qubit dsDNA HS Kit. These samples were then prepped for whole genome sequencing using the Illumina DNA prep kit. The sample input varied based on the concentration measured. Prepared libraries were pooled to a final concentration of 21.74 nM. Pooled samples were subjected to 150 base paired-end sequencing with an average insert size of 350 base pairs using the Illumina NovaSeq platform at SeqMatic (Emeryville, CA). Generated raw FASTQ format files were assembled into DNA contigs using the MEGAHIT v.1.2.9 software with default parameters and the “mega large” option (Li et al., 2015). The taxonomy was assigned using the Kraken2 pipeline (Wood et al., 2019). Proper quality control assessment was performed for all samples before data analysis. The average sequencing Phred score was 35 (a Phred score is used to evaluate the quality of sequenced DNA, and a Phred score greater than 30 is considered good), and only one sample was removed (sample 3T-75\_S32) due to a low count of 10 reads, whereas the rest of our samples had an average of 18,912,289 reads (48,112 reads was our minimum and 139,325,114 reads was our maximum).

### ***Data and Statistical Analysis***

All data analysis was performed in R studio (R Development Core Team, 2013). The vegan package (Oksanen et al., 2022) was used to calculate alpha (Richness, Shannon, and Inverse

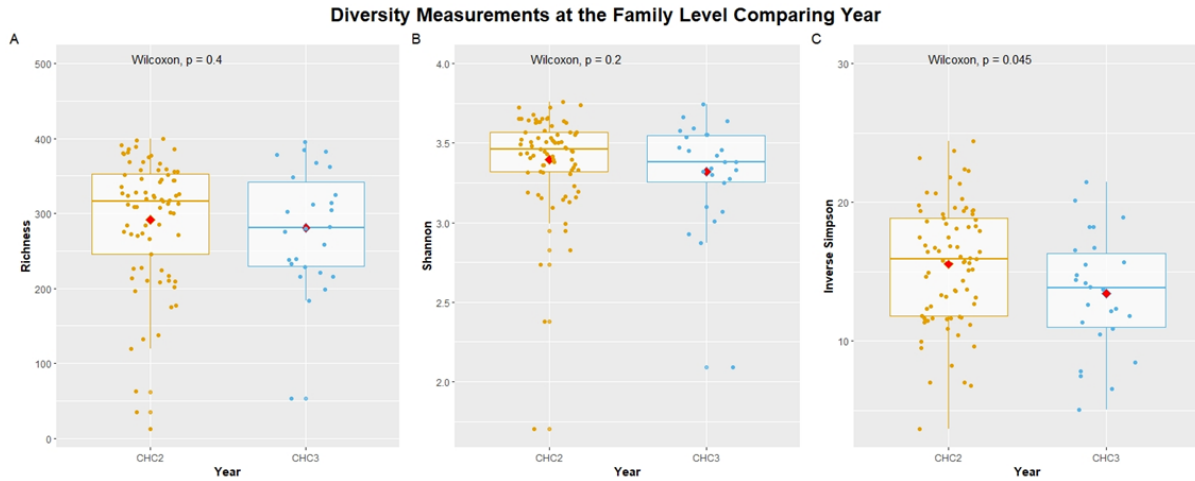
Simpson) diversity at the Phylum, Family, and genus level and Wilcoxon rank sum tests were used to assess statistical significance through the GGPubr package (Kassambara, 2020). Beta diversity was also calculated via the vegan package using Bray Curtis Dissimilarity, a common beta diversity metric that takes into account both the presence and abundance of taxa. While alpha diversity measures within sample diversity, beta diversity measures the dissimilarity between samples. Using Principle Coordinate Analysis we plotted an ordination of our Bray Curtis distance matrix and tested whether beta diversity between our samples differed across Year, Occupation, and Season with permutational multivariate analysis of variance (PERMANOVA) set at a 1000 permutations via the adonis function in the vegan package (Oksanen et al., 2022). Additionally, due to the inherent nature of varying library sizes (different read numbers) across sequenced samples, we utilized the ANCOM-BC method to normalize our sample counts throughout all taxonomic ranks before assessing differential taxa abundance between samples. Unlike rarefying where samples are normalized solely on the basis of library size, the ANCOM-BC method takes into account the compositionality of microbiome data and normalizes by sampling fraction, which is defined as the ratio of the expected absolute abundance of a taxon in a random sample (in our case dust sample) to its absolute abundance in a unit volume of the ecosystem (in our case household) where the sample was derived from (Lin & Peddada, 2020). While rarefying has been shown to result in high rates of false positives when testing for differentially abundant taxa between samples (McMurdie & Holmes, 2014), in contrast the ANCOM-BC method was shown to control false discovery rate very well (Lin & Peddada, 2020), and unlike rarefying using the ANCOM-BC method does not require researchers to discard perfectly valid data. In order to correct for multiple hypothesis testing, we used the Holm method.

## **Results**

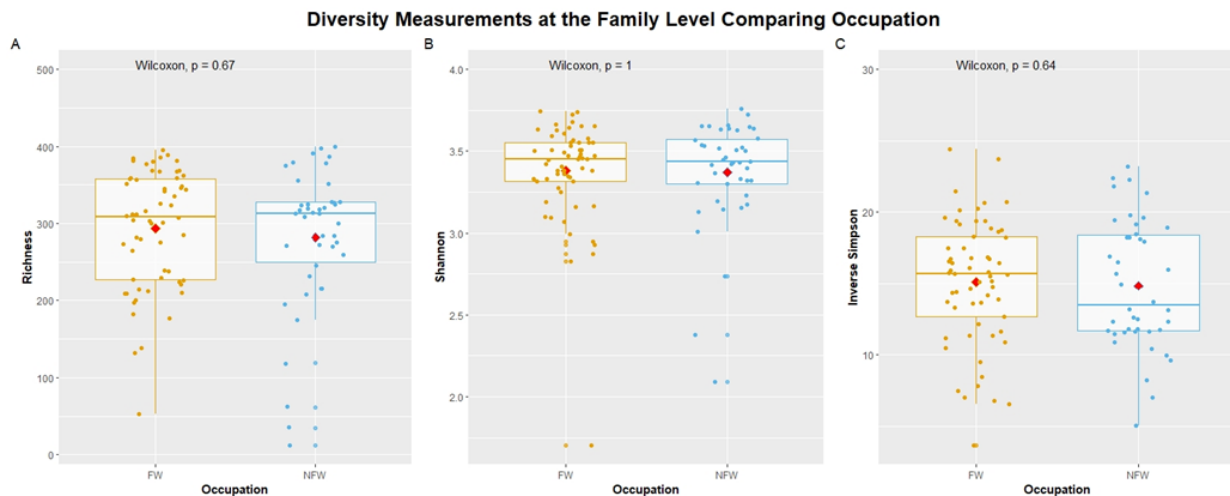
### ***Alpha and Beta Diversity***

Three diversity metrics were used to calculate alpha diversity; Richness, Shannon, and Inverse Simpson at both the Family and Genus level for all samples. **Figures 2-4** and **Supplementary Figures 2-4** show whether our alpha diversity metrics differed between Year, Occupation, and Season using Wilcoxon rank sum tests. While Richness simply measures the number of species within a community, both Shannon and Inverse Simpson consider Richness and Evenness where Evenness is defined as the distribution of species within a community. While Shannon and Inverse Simpson are similar in this respect, their formulas are quite different. The Inverse Simpson gives more weight to dominant/common species which is in contrast to Shannon diversity (Moore, 2013). For both Shannon and Inverse Simpson, the greater the diversity metric the more diverse a community is. A community is considered to have high diversity when it not only contains a lot of different species, but also when these species are evenly distributed. Looking at **Figures 3-5**, we can see that at the Family level only Inverse Simpson was significant when comparing Year, where CHC2 had a higher Inverse Simpson diversity than CHC3 (**Figure 3**,  $p=0.045$ ). When looking at the Genus level (**Supplementary Figures 2-4**) we can see that for all three of our variables (Year, Occupation, and Season) none of them were statistically significant for changes in alpha diversity across all three-diversity metrics. However, Inverse Simpson when comparing year at the Genus level (**Supplementary Figure 2**) was similar to the Family level (**Figure 3**), but did not cross statistical significance ( $p=0.071$ ). To further investigate whether our three variables were simultaneously having an impact on alpha diversity, we first divided all our samples by Year, Occupation, and Season and then made Violin plots to

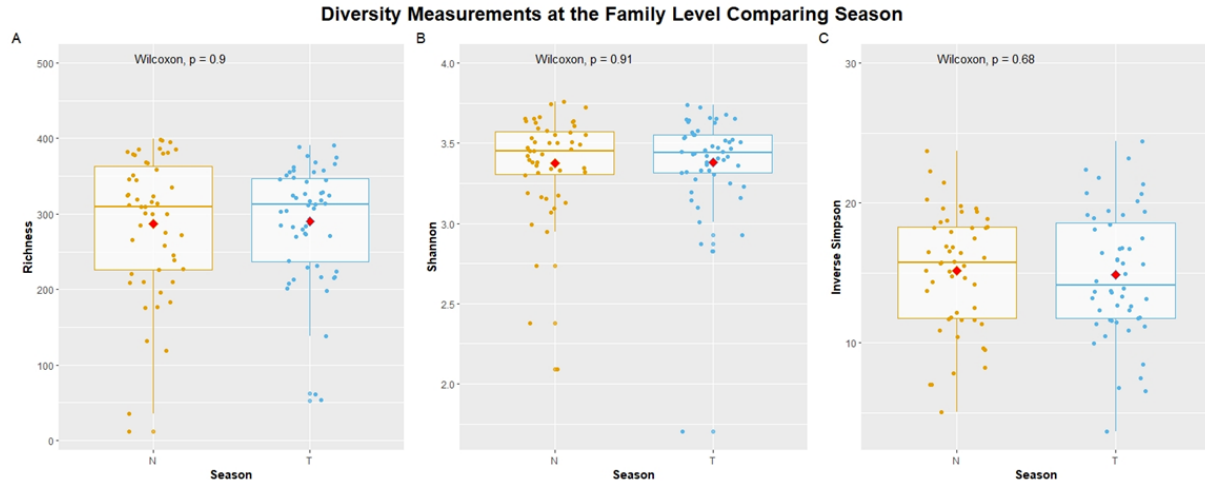
compare them across our three alpha diversity metrics (Richness, Shannon, and Inverse Simpson) at the Genus level. **Supplementary Figures 5-7** show that when dividing our samples by all three of our categorical variables, none of them showed statically significant changes in alpha diversity. However, when performing a three-way ANOVA to test whether Year, Occupation, and Season were influencing alpha diversity together, only Year was shown to have a significant effect on the Inverse Simpson at the Family level (**Table 2**,  $p=0.038$ ) and borderline significance at the genus level (**Table 3**,  $p=0.055$ ).



**Figure 2. Boxplots comparing three alpha diversity metrics against CHC dust collection year at the Family level.** The red diamond in boxplots represents the mean. **(A)** There was no significant change in Richness between CHC2 and CHC3. **(B)** There was no significant change in Shannon diversity between CHC2 and CHC3. **(C)** There was a significant change in the Inverse Simpson between CHC2 and CHC3 project years (Wilcoxon test  $p=0.045$ ).



**Figure 3. Boxplots comparing three alpha diversity metrics against Occupation at the Family level.** The red diamond in boxplots represents the mean. **(A)** There was no significant change in Richness between FW and NFW households. **(B)** There was no significant change in Shannon diversity between FW and NFW households. **(C)** There was no significant change in the Inverse Simpson between FW and NFW households.



**Figure 4. Boxplots comparing three alpha diversity metrics against Season at the Family level.** The red diamond in boxplots represents the mean. **(A)** There was no significant change in Richness between Thinning (T) and Nonspray Season (N). **(B)** There was no significant change in Shannon diversity between T and N. **(C)** There was no significant change in the Inverse Simpson between T and N.

Three Way ANOVA Results for Inverse Simpson Diversity at the Family Level					
Variable	df	Sum sq	Mean sq	Statistic	p.value
Year	1	85.88331	85.88331	4.445592	<b>*0.037623</b>
Occupation	1	8.667208	8.667208	0.448642	0.504605
Season	1	0.105835	0.105835	0.005478	0.941153
Year: Occupation	1	0.299236	0.299236	0.015489	0.901217
Year: Season	1	11.7569	11.7569	0.608575	0.437264
Occupation: Season	1	37.26579	37.26579	1.928995	0.168116
Year: Occupation: Season	1	5.913747	5.913747	0.306114	0.581374
Residuals	95	1835.282	19.31876		

**Table 2. Three-way ANOVA results for Inverse Simpson Diversity at the Family Level.** Three-way ANOVA results showing that for alpha diversity measure Inverse Simpson, only Year (CHC2 vs. CHC3) is significant ( $p=0.038$ ), and there are no significant interactions between our three variables of interest (Year, Occupation, Season).

Three Way ANOVA Results for Inverse Simpson Diversity at the Genus Level					
Variable	df	Sum sq	Mean sq	statistic	p.value
Year	1	261.0277	261.0277	3.783891	<b>0.054705</b>
Occupation	1	55.00215	55.00215	0.797318	0.374153
Season	1	76.55603	76.55603	1.109766	0.294805
Year: Occupation	1	9.391301	9.391301	0.136138	0.712972
Year: Season	1	0.454287	0.454287	0.006585	0.935493
Occupation: Season	1	8.135198	8.135198	0.117929	0.732051
Year: Occupation: Season	1	59.96866	59.96866	0.869313	0.353509
Residuals	95	6553.473	68.98393		

**Table 3. Three-way ANOVA results for Inverse Simpson Diversity at the Genus Level.** Three-way ANOVA results showing that for alpha diversity measure Inverse Simpson, Year (CHC2 vs. CHC3) is borderline significant ( $p=0.054$ ), and there are no significant interactions between our three variables of interest (Year, Occupation, Season)

To explore beta diversity, we used Principal Coordinate Analysis to visualize any clustering between Year, Occupation, and Season with Bray Curtis distance at the Family and Genus level. Looking at **Supplementary Figures 8 and 9** we can see that out of our three variables only Year showed distinct clustering between CHC2 and CHC3, but CHC3 appears to lie within CHC2 as CHC2 has greater dispersion. To test whether our samples significantly differed in Bray Curtis beta diversity between Year, Occupation, and Season we used permutational multivariate analysis of variance (PERMANOVA) at the Family and Genus levels. Looking at **Tables 4 and 5** we can see that only Year came out as statistically significant for beta diversity at the Family level (**Table 4**,  $p=0.038$ ) and the Genus level (**Table 5**,  $p=0.029$ ) with no significant interactions between Year, Occupation, and Season. However, looking at the  $R^2$  value we can see that Year only explained 2.1% of the variability at the Family level (**Table 4**), and only 2.0% of the variability at the Genus level (**Table 5**).

PERMANOVA Results for Bray Cutis Distance at the Family Level						
Variable	Df	Sums Of Sqs	Mean Sqs	F. Model	R <sup>2</sup>	p.value
Year	1	0.19429603	0.19429603	2.256367	0.02141	<b>*0.037962</b>
Occupation	1	0.14640705	0.14640705	1.70023	0.016133	0.091908
Season	1	0.12240167	0.12240167	1.421455	0.013488	0.13986
Year: Occupation	1	0.15552379	0.15552379	1.806103	0.017138	0.082917
Year: Season	1	0.04260612	0.04260612	0.494786	0.004695	0.91009
Occupation: Season	1	0.12470782	0.12470782	1.448236	0.013742	0.143856
Year: Occupation: Season	1	0.10861981	0.10861981	1.261406	0.011969	0.21978
Residuals	95	8.18046295	0.08611014		0.901426	
Total	102	9.07502524				1

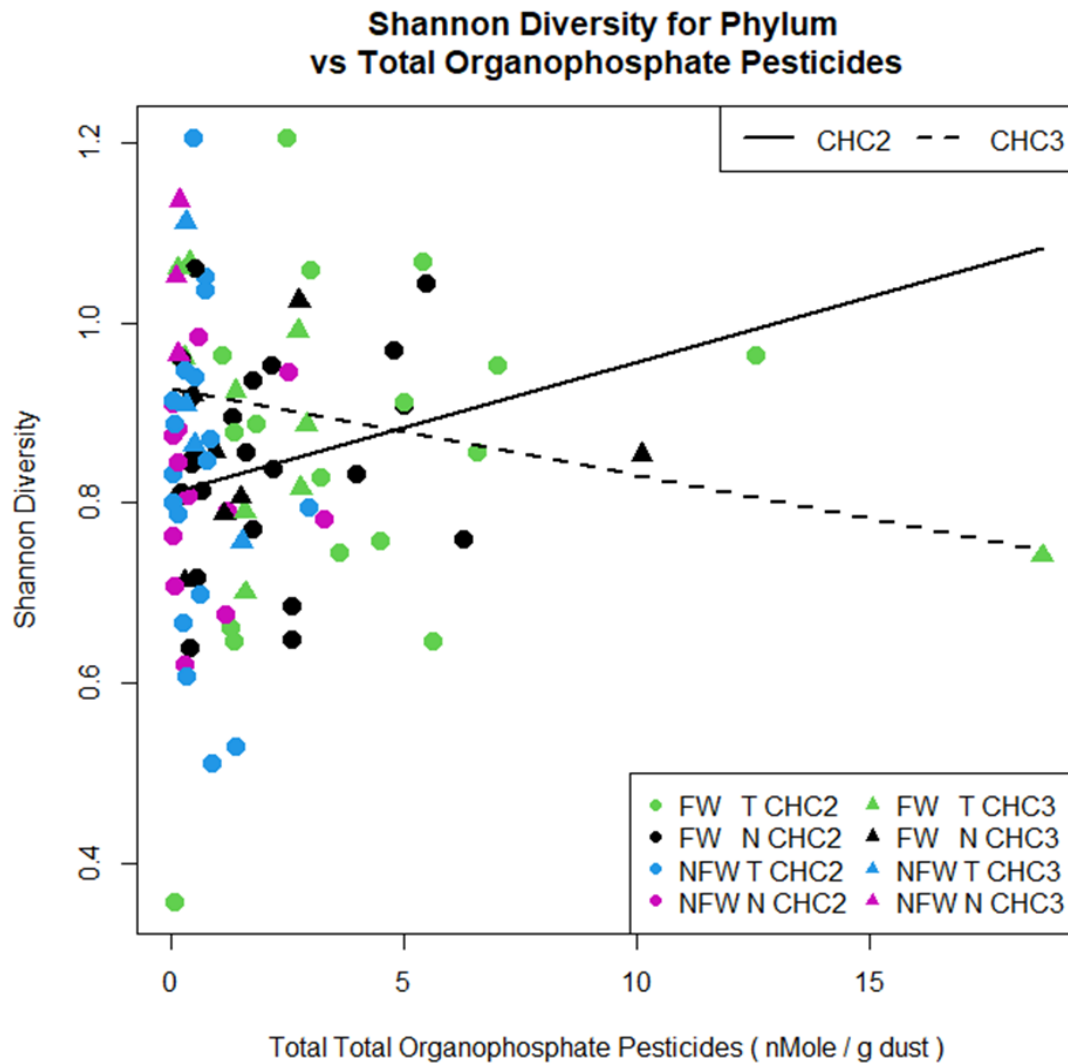
**Table 4. Permutational multivariate analysis of variance (PERMANOVA) results for Bray Curtis Distance at the Family level.** There were no significant interactions between our three variables (Year, Occupation, Season), and only Year (CHC2 vs. CHC3) was significant for beta diversity ( $p=0.038$ ) (1000 permutations).

PERMANOVA Results for Bray Curtis Distance at the Genus Level						
Variable	Df	Sums Of Sqs	Mean Sqs	F. Model	R^2	p.value
Year	1	0.224401	0.224401	2.131647	0.020319	<b>*0.028971</b>
Occupation	1	0.177491	0.177491	1.686033	0.016072	0.070929
Season	1	0.138827	0.138827	1.318762	0.012571	0.185814
Year: Occupation	1	0.168424	0.168424	1.599909	0.015251	0.095904
Year: Season	1	0.052186	0.052186	0.495725	0.004725	0.946054
Occupation: Season	1	0.174434	0.174434	1.657001	0.015795	0.082917
Year: Occupation: Season	1	0.107129	0.107129	1.017645	0.0097	0.396603
Residuals	95	10.00075	0.105271		0.905566	
Total	102	11.04365			1	

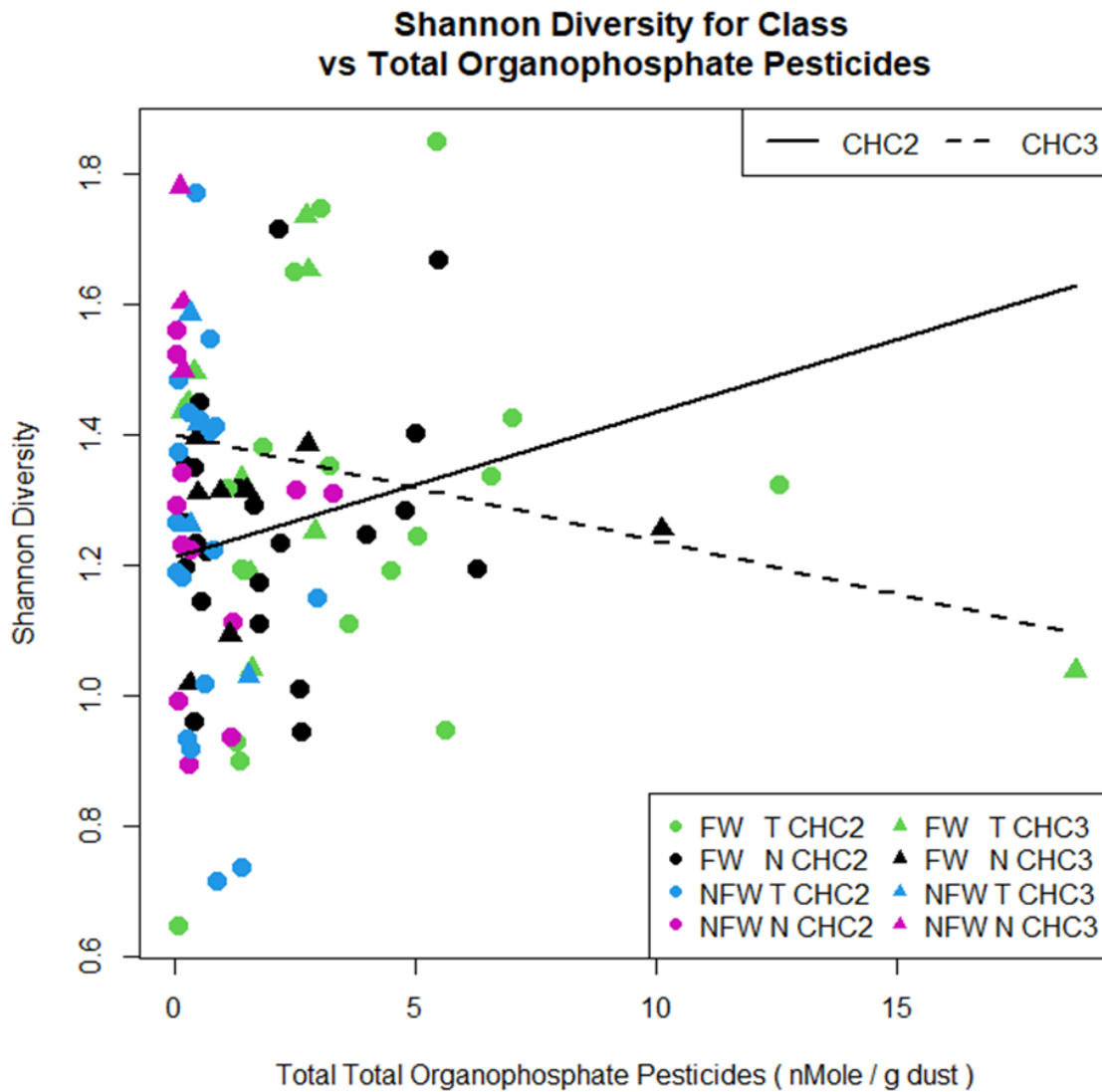
**Table 5. Permutational multivariate analysis of variance (PERMANOVA) results for Bray Curtis Distance at the Genus level.** There were no significant interactions between our three variables (Year, Occupation, Season), and only Year (CHC2 vs. CHC3) was significant for beta diversity ( $p=0.029$ ).

### Organophosphates and the Dust Microbiome

Because we know household organophosphate (OP) dust concentrations greatly differed between CHC2 and CHC3, we wanted to examine whether this difference was driving changes in the indoor dust microbiome between the two cohort years since we observed significant changes in both alpha and beta diversity between CHC2 and CHC3. Using total organophosphate (OP) dust concentrations from previous analysis (Bennett et al., 2019; Smith et al., 2017; B. Thompson et al., 2014) we explored how diversity and individual taxa abundance differed in relation to total dust organophosphates, Year, Occupation, and Season with a mixed effects model. Looking at **Figure 5** we can see that Shannon diversity significantly changed according to total OP dust concentrations ( $p=0.018$ ) with a significant difference in the slope observed between the two cohort years ( $p=0.031$ ) at the Phylum level. This regression was also adjusted for the non-significant factors of occupation ( $p=0.53$ ) and season ( $p=0.79$ ). For the taxonomic rank Class (**Figure 6**) it was also found that Shannon diversity significantly differed in relation to total OP dust concentrations ( $p=0.021$ ), which we also observed a significant difference in slope for the two years ( $p=0.035$ ). Out of all our taxonomic ranks, these were the only two that exhibited significant changes in Shannon Diversity in relation to total dust OPs.



**Figure 5. Plot showing the relationship between Shannon Diversity, Total Organophosphate dust concentrations, Cohort year, Occupation, and Season at the Phylum level.** Shannon diversity significantly changed in relation to total OP dust concentrations ( $p=0.018$ ) with significant differences in the slopes of CHC2 and CHC3 ( $p=0.031$ ) such that an increase in total dust OPs in CHC2 lead to an increase in Shannon Diversity, and an increased in total dust OPs in CHC3 lead to a decrease in Shannon Diversity. This regression was also adjusted for the non-significant factors of occupation ( $p=0.53$ ) and season ( $p=0.79$ ).



**Figure 6. Plot showing the relationship between Shannon Diversity, Total Organophosphate dust concentrations, Cohort year, Occupation, and Season at the Class level.** Shannon diversity significantly changed in relation to total OP dust concentrations ( $p=0.021$ ) with significant differences in the slopes of CHC2 and CHC3 ( $p=0.035$ ) such that an increase in total dust OPs in CHC2 lead to an increase in Shannon Diversity, and an increased in total dust OPs in CHC3 lead to a decrease in Shannon Diversity. This regression was also adjusted for the non-significant factors of occupation and season.

Following Shannon Diversity, we next evaluated whether total OPs in dust along with Year, Occupation, and Season were correlated with any taxa abundance changes by using a mixed effects model. Looking at **Table 6** and **7** and **Supplementary Figures 10** and **11** we can see that several abundances were significantly altered by total dust OPs at the Order and Genus level. Starting with Order, the first four Orders Pseudomonadales, Chromatiales, Catenulisporales, and Spirochaetales significantly (adjusted p value < 0.5) decreased as total dust OPs increased whereas the Orders Erysipelotrichales, Saprospirales, and Dehalococcoidales had the inverse effect. For the Genus level, all 40 of the genera whose abundances significantly changed in relation to total dust OPs were all shown to decrease as total dust OPs increased.

Order	Total Dust OPs						
	abundance	rank	q	beta	se	p	W
<i>Pseudomonadales</i>	6.69E-02	3	4.47E-02	-0.352	0.0967	2.71E-04	-3.64
<i>Chromatiales</i>	1.50E-03	37	2.45E-02	-0.283	0.0746	1.46E-04	-3.8
<i>Catenulisporales</i>	2.38E-04	64	4.05E-02	-0.249	0.0679	2.44E-04	-3.67
<i>Spirochaetales</i>	9.21E-05	85	2.17E-06	-0.281	0.0493	1.27E-08	-5.69
<i>Erysipelotrichales</i>	7.77E-05	90	1.13E-02	0.382	0.0957	6.67E-05	3.99
<i>Saprospirales</i>	1.35E-05	132	1.55E-03	0.218	0.0492	9.14E-06	4.44
<i>Dehalococcoidales</i>	6.42E-06	148	2.67E-02	0.352	0.0933	1.60E-04	3.77

**Table 6. Orders significantly affected by Total Dust OP Concentrations.** Using ANCOM-BC differential taxa abundance was performed. The first column list the Orders significantly affected by Total Dust OP concentrations. The adjusted p-values are shown in the column labeled "q" and the unadjusted p-values are in the column labeled "p". The column labeled "W" is the statistic used in ANCOM-BC to calculate adjusted p-values. The column labeled "se" is the standard error for the beta coefficient. The column labeled "abundance" is the median relative abundance across all samples, and "rank" is the rank from largest to smallest of median relative abundances. The negative beta sign indicates a decrease in abundance as total dust OP concentrations increased, whereas a positive beta sign indicates the inverse relationship. All p values were adjusted for multiple hypothesis testing using the Holm method.

Genera	Total Dust OPs						
	abundance	rank	q	beta	se	p	W
<i>Brachybacterium</i>	1.04E-02	18	1.42E-02	-0.111	0.0252	9.57E-06	-4.43
<i>Janibacter</i>	8.00E-03	22	1.51E-03	-0.114	0.0233	1.01E-06	-4.89
<i>Phycococcus</i>	4.49E-03	44	3.44E-02	-0.0929	0.022	2.33E-05	-4.23
<i>Brevibacterium</i>	2.51E-03	73	9.63E-03	-0.0891	0.0198	6.47E-06	-4.51
<i>Glutamicibacter</i>	1.51E-03	94	3.76E-04	-0.138	0.0268	2.50E-07	-5.16
<i>Intrasporangium</i>	1.34E-03	104	1.08E-03	-0.109	0.022	7.18E-07	-4.96
<i>Ruania</i>	1.18E-03	110	2.33E-02	-0.0871	0.0202	1.58E-05	-4.32
<i>Saccharopolyspora</i>	1.08E-03	121	4.78E-02	-0.089	0.0214	3.25E-05	-4.16
<i>Saccharomonospora</i>	8.75E-04	137	1.03E-02	-0.0924	0.0206	6.91E-06	-4.5
<i>Miniimonas</i>	7.75E-04	146	2.02E-02	-0.0935	0.0215	1.37E-05	-4.35
<i>Ilumatobacter</i>	6.85E-04	159	1.48E-08	-0.155	0.0228	9.81E-12	-6.81
<i>Oerskovia</i>	6.31E-04	171	1.73E-03	-0.104	0.0214	1.16E-06	-4.86
<i>Xylanimonas</i>	5.65E-04	184	5.43E-04	-0.104	0.0205	3.61E-07	-5.09
<i>Mumia</i>	4.81E-04	204	3.65E-02	-0.0766	0.0182	2.47E-05	-4.22
<i>Luteitalea</i>	4.77E-04	205	1.21E-02	-0.141	0.0317	8.16E-06	-4.46
<i>Paraerskovia</i>	2.82E-04	259	2.78E-03	-0.109	0.0229	1.86E-06	-4.77
<i>Pseudactinotalea</i>	2.72E-04	262	1.52E-02	-0.0971	0.022	1.02E-05	-4.41
<i>Epidermidibacterium</i>	2.18E-04	299	3.03E-05	-0.107	0.0191	2.00E-08	-5.61
<i>Pyxidicoccus</i>	1.85E-04	329	9.09E-03	-0.123	0.0272	6.10E-06	-4.52
<i>Nesterenkonia</i>	1.55E-04	349	1.28E-03	-0.115	0.0235	8.51E-07	-4.92
<i>Usitatibacter</i>	1.45E-04	361	7.09E-03	-0.0945	0.0207	4.76E-06	-4.58
<i>Pseudodesulfovibrio</i>	1.03E-04	426	1.61E-03	-0.111	0.0228	1.08E-06	-4.88
<i>Verrucomicrobium</i>	8.19E-05	466	3.36E-05	-0.118	0.0211	2.23E-08	-5.59
<i>Solimonas</i>	7.09E-05	494	7.90E-03	-0.0959	0.0211	5.30E-06	-4.55
<i>Pirellulimonas</i>	6.83E-05	502	3.90E-02	-0.0975	0.0232	2.64E-05	-4.2
<i>Candidatus.Promineofilum</i>	6.80E-05	503	4.48E-02	-0.0882	0.0211	3.04E-05	-4.17
<i>Neomicrococcus</i>	6.25E-05	519	2.77E-02	-0.0992	0.0232	1.88E-05	-4.28
<i>Methyloligella</i>	5.89E-05	541	1.82E-03	-0.103	0.0212	1.22E-06	-4.85
<i>Symbiobacterium</i>	5.21E-05	574	2.48E-04	-0.0966	0.0184	1.64E-07	-5.24
<i>Meiothermus</i>	5.03E-05	587	3.09E-02	-0.0798	0.0187	2.09E-05	-4.25
<i>Cohnella</i>	4.87E-05	594	2.95E-03	-0.0799	0.0168	1.97E-06	-4.76
<i>Candidatus.Accumulibacter</i>	4.72E-05	601	1.45E-03	-0.088	0.018	9.64E-07	-4.9
<i>Niveibacterium</i>	3.71E-05	650	1.04E-02	-0.068	0.0151	7.01E-06	-4.49
<i>Tumebacillus</i>	3.50E-05	669	2.32E-07	-0.0781	0.0122	1.54E-10	-6.4
<i>Rhodomicrobium</i>	3.09E-05	704	2.84E-02	-0.0692	0.0162	1.92E-05	-4.27
<i>Winkia</i>	3.01E-05	711	3.60E-03	-0.0765	0.0162	2.42E-06	-4.72
<i>Chlorobaculum</i>	2.86E-05	725	3.69E-04	-0.067	0.013	2.45E-07	-5.16
<i>Pararhodospirillum</i>	2.49E-05	766	1.40E-02	-0.0695	0.0157	9.40E-06	-4.43
<i>Devriesea</i>	2.24E-05	796	1.31E-03	-0.0654	0.0133	8.72E-07	-4.92
<i>Ferrimonas</i>	1.68E-05	871	8.66E-04	-0.0477	0.00954	5.76E-07	-5

**Table 7. Genera significantly affected by Total Dust OP Concentrations.** Using ANCOM-BC differential taxa abundance was performed. The first column list the Orders significantly affected by Total Dust OP concentrations. The adjusted p-values are shown in the column labeled "q" and the unadjusted p-values are in the column labeled "p". The column labeled "W" is the statistic used in ANCOM-BC to calculate adjusted p-values. The column labeled "se" is the standard error for the beta coefficient. The column labeled "abundance" is the median relative abundance across all samples, and "rank" is the rank from largest to smallest of median relative abundances. The negative beta sign indicates a decrease in abundance as total dust OP concentrations increased, whereas a positive beta sign indicates the inverse relationship. All p values were adjusted for multiple hypothesis testing using the Holm method.

In addition to abundances changing in relation to total dust OPs, we also observed significant Occupational effects across several taxonomic ranks. Starting with Order (**Table 8** and **Supplementary Figure 12**), Burkholderiales was one Order that had a significantly higher abundance in NFW households compared to FW households. However opposite of this, the following two Families Rhodobacteraceae and Dermabacteraceae had lower abundances in NFW households compared to FW households (**Table 9** and **Supplementary Figure 13**). Additionally, the last taxonomic rank we observed significant changes in differential taxa abundance occurred at the Genus Level. For all four of the following genera Paracoccus, Defluviimonas, Tabrizicola, and Yoonia, NFW households had significantly lower abundances than FW households (**Table 10** and **Supplementary Figure 14**).

Occupation							
Order	abundance	rank	q	beta	se	p	W
<i>Burkholderiales</i>	0.0478	6	0.0114	1.26	0.317	6.67E-05	3.99

**Table 8. Orders significantly affected by Occupational Status.** Using ANCOM-BC differential taxa abundance was performed. The first column list the Orders significantly affected by Occupation. The adjusted p-values are shown in the column labeled "q" and the unadjusted p-values are in the column labeled "p". The column labeled "W" is the statistic used in ANCOM-BC to calculate adjusted p-values. The column labeled "se" is the standard error for the beta coefficient. The column labeled "abundance" is the median relative abundance across all samples, and "rank" is the rank from largest to smallest of median relative abundances. The positive beta sign indicates that Burkholderiales had a higher abundance in NFW households compared to FW households. All p values were adjusted for multiple hypothesis testing using the Holm method.

Occupation							
Family	abundance	rank	q	beta	se	p	W
<i>Rhodobacteraceae</i>	0.0337	8	7.53E-05	-1.08	0.208	1.89E-07	-5.21
<i>Dermabacteraceae</i>	0.0104	25	3.38E-02	-1.19	0.304	8.52E-05	-3.93

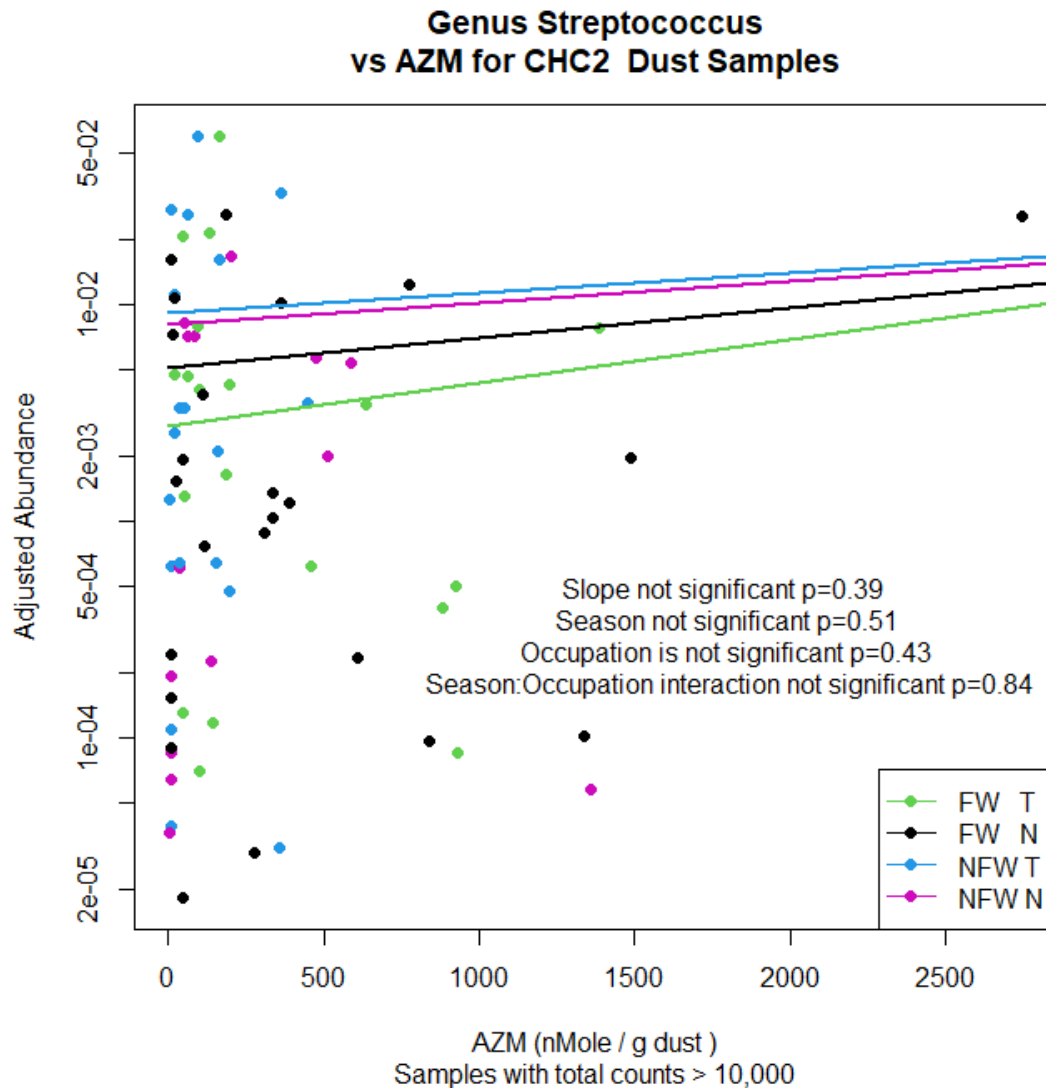
**Table 9. Families significantly affected by Occupational Status.** Using ANCOM-BC differential taxa abundance was performed. The first column list the Families significantly

affected by Occupation. The adjusted p-values are shown in the column labeled "q" and the unadjusted p-values are in the column labeled "p". The column labeled "W" is the statistic used in ANCOM-BC to calculate adjusted p-values. The column labeled "se" is the standard error for the beta coefficient. The column labeled "abundance" is the median relative abundance across all samples, and "rank" is the rank from largest to smallest of median relative abundances. The negative beta sign indicates that both of these Families had a lower abundance in NFW households compared to FW households. All p values were adjusted for multiple hypothesis testing using the Holm method.

Genera	Occupation						
	abundance	rank	q	beta	se	p	W
<i>Paracoccus</i>	2.59E-02	7	0.000021	-1.45	0.255	1.39E-08	-5.68
<i>Defluviimonas</i>	2.20E-04	296	0.0207	-0.966	0.222	1.37E-05	-4.35
<i>Tabrizicola</i>	1.05E-04	424	0.00392	-1.01	0.215	2.60E-06	-4.7
<i>Yoonia</i>	4.98E-05	589	0.0261	-0.744	0.173	1.73E-05	-4.3

**Table 10. Genera significantly affected by Occupational Status.** Using ANCOM-BC differential taxa abundance was performed. The first column list the Genera significantly affected by Occupation. The adjusted p-values are shown in the column labeled "q" and the unadjusted p-values are in the column labeled "p". The column labeled "W" is the statistic used in ANCOM-BC to calculate adjusted p-values. The column labeled "se" is the standard error for the beta coefficient. The column labeled "abundance" is the median relative abundance across all samples, and "rank" is the rank from largest to smallest of median relative abundances. The negative beta sign indicates that all of these Genera had lower abundances in NFW households compared to FW households. All p values were adjusted for multiple hypothesis testing using the Holm method.

Lastly, since we know through previous work with CHC2 that the oral microbiome significantly changed among individuals exposed to Azinphos-Methyl (AZM) with Streptococcus having a lower abundance among individuals exposed to AZM compared to unexposed individuals (Stanaway et al., 2017), we wanted to further analyze whether AZM in household dust significantly altered Streptococcus abundance. Looking at **Figure 7**, we can see that when using a mixed effects model that neither AZM, Occupation, or Season significantly affected Streptococcus abundance within CHC2 household dust.

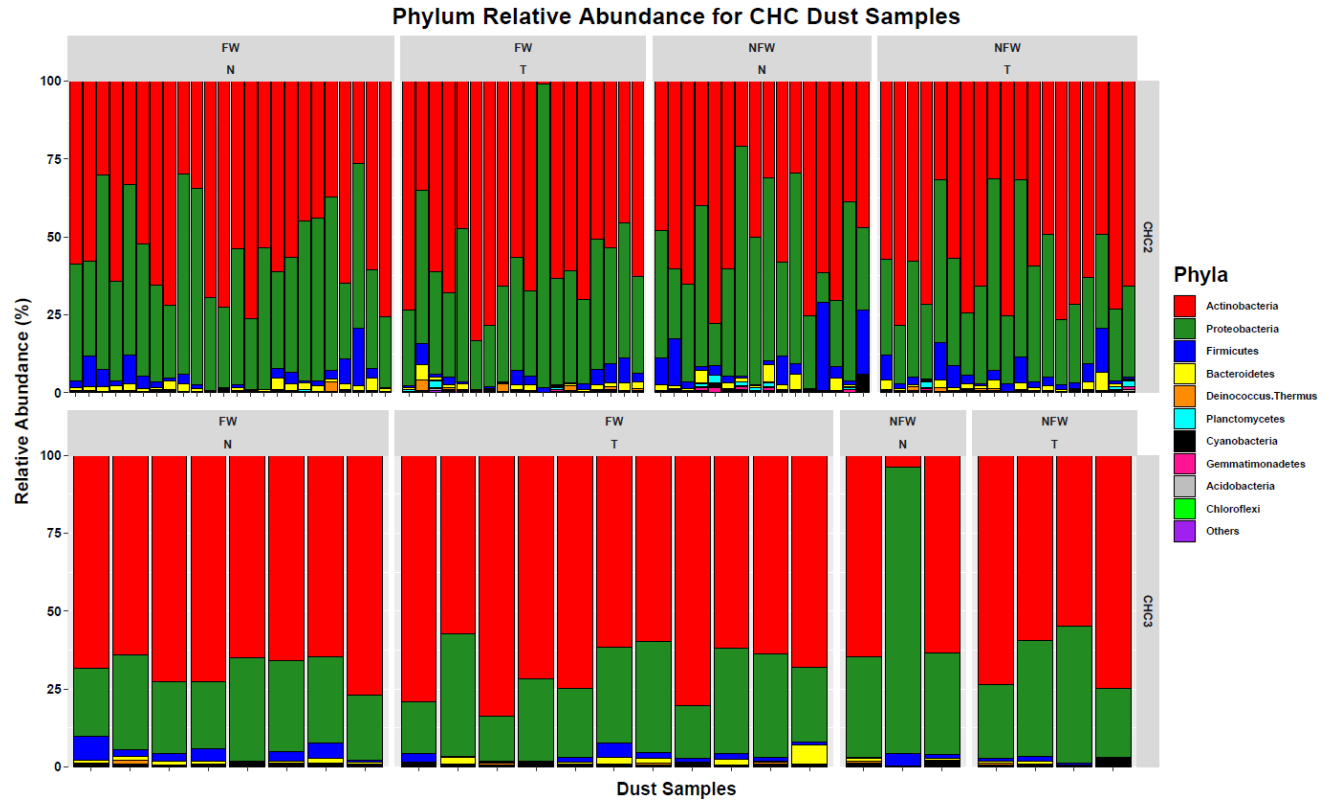


**Figure 7. Plot assessing the relationship between AZM, Occupation, and Season with Streptococcus abundance in CHC2 using a mixed effects model at the Genus level.** Using a mixed effects model with a random effect for household and fixed effects for the concentration of dust AZM, Occupation, and Season we did not observe any significant relationship between Streptococcus abundance, AZM dust concentrations, Occupation, and Season in CHC2.

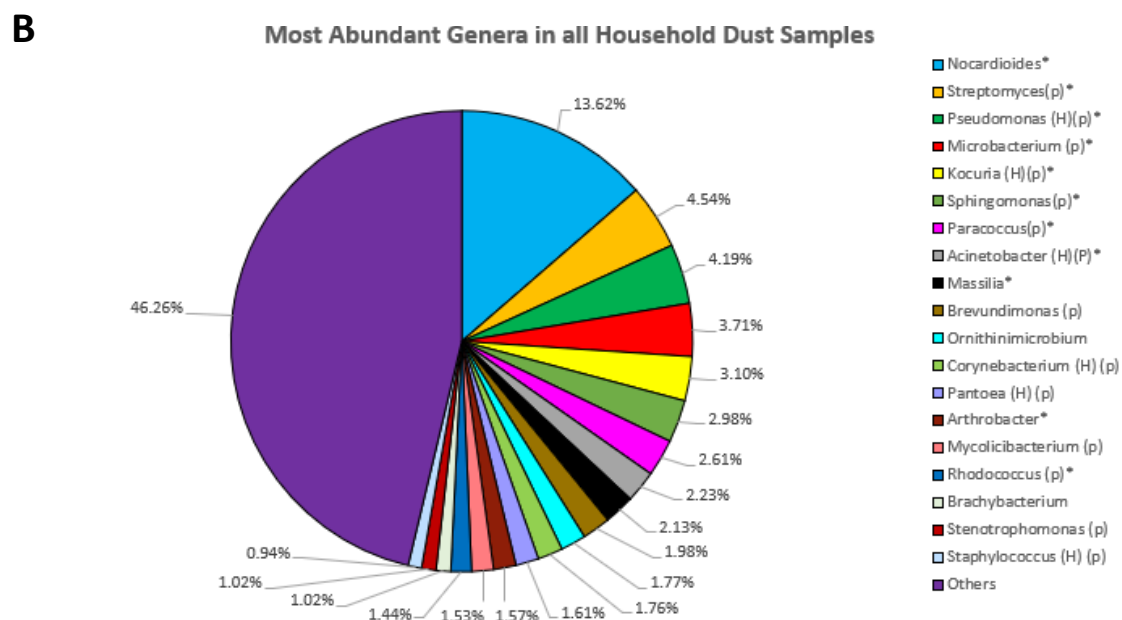
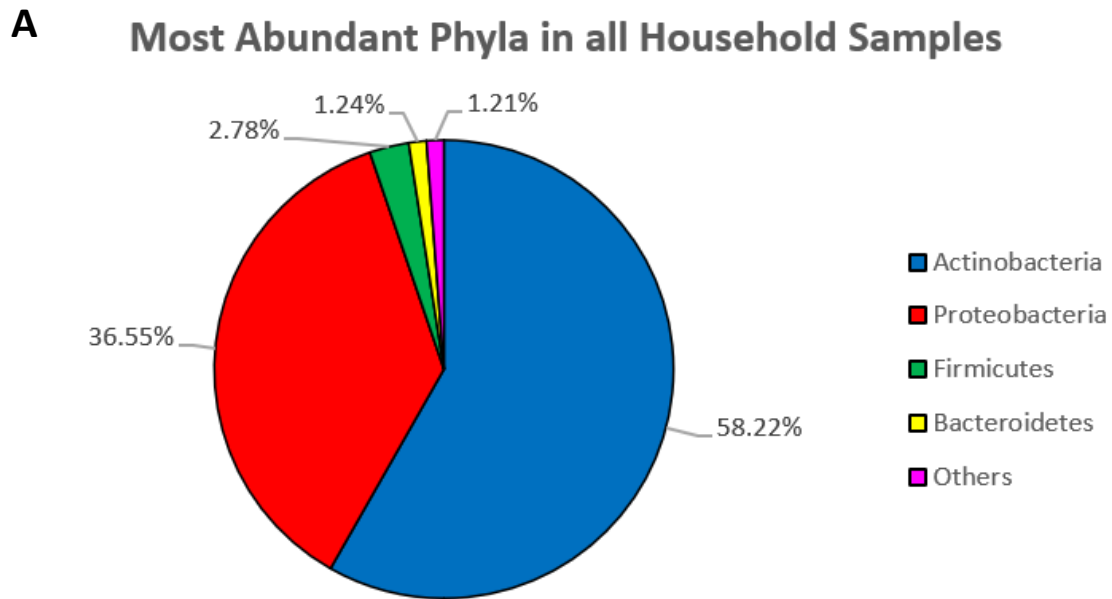
### Overall Taxa Abundance

Starting at the Phylum level, **Figure 8** illustrates the dominant phyla present throughout all of our dust samples. We can see that Actinobacteria and Proteobacteria are the two phyla that dominate the majority of our samples, making up 58.2% and 36.5% of our overall phylum abundance, followed by Firmicutes (2.8%) and Bacteroidetes (1.2%) (**Figure 9A**). When zooming down to the Genus level (**Figure 9B**) we can see that Nocardioidees is our most abundant genera making up 13.62%, followed by Streptomyces (4.54%), Pseudomonas (4.19%), Microbacterium (3.71%), Kocuria (3.1%) and then 13 other genera each with individual

abundances less than 3% but greater than 1%, followed by the remaining genera in the “other” category making up the rest of the overall abundance (46.26%).



**Figure 8. Phylum stacked bar chart showing Phylum relative abundance in CHC dust samples.** Relative abundance (%) for 103 (one sample removed for low sequencing reads) of our sequenced dust samples were organized by CHC2 vs. CHC3, Farmworker (FW) vs. nonfarmworker (NFW), and Thinning (T) vs. Nonspray (N) season. Looking at the relative abundances we can see that the top four phyla in all of our samples include Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes.



**Figure 9. Pie charts showing most abundant Phyla and Genera in CHC dust samples.** Using the total counts of the taxonomic ranks Phylum and Genus we calculated which Phyla and Genera were the most dominant in all of our samples. **(A)** Dust samples were dominated by four phyla groups: Actinobacteria (58.2%), Proteobacteria (36.5%), Firmicutes (2.8%), and Bacteroidetes (1.2%). **(B)** Overall, the top genera (making up >3% of overall samples abundance) were Nocardioiodes (13.6%), Streptomyces (4.5%), Pseudomonas (4.2%), Microbacterium (3.71%), and Kocuria (3.1%), with several following genera making up for >1% abundance. All the genera in the other category accounted for less than 1% of total sample abundance. Genera in legend denoted with an “(H)” indicate human commensals, “\*” means the genera has been reported to break down pesticides, and “(p)” means some species within a genera group are opportunistic pathogens.

## **Discussion**

To the best of our knowledge, this is the first study to examine the household dust microbiome among an agricultural cohort of pome fruit workers using shotgun metagenomic sequencing. While other farm microbial studies have primarily focused on livestock work (Birzele et al., 2017; Böbel et al., 2018; Debarry et al., 2007; Kirjavainen et al., 2019; Korthals et al., 2008; Normand et al., 2011; Vestergaard et al., 2018; White et al., 2019) this study looked at farm activities involving crop production, specifically pome fruits, with an emphasis on occupational pesticide exposures. We already know from previous work that pome fruit workers are exposed to a wide variety of different pesticides and these pesticides can be brought into the home environment through the occupational take-home pathway (Bennett et al., 2019; Coronado et al., 2006; Curl et al., 2002; Smith et al., 2017; B. Thompson et al., 2003, 2014). However, unlike other studies where researchers only had one time point for each household dust sample this study included both longitudinal and seasonal collection periods to accurately depict the indoor dust microbiota over time.

Looking over these results not only did we observe a significant change in the Inverse Simpson alpha diversity between CHC2 and CHC3 at the Family level with CHC2 having a higher alpha diversity than CHC3 (**Figure 2C**), but we also observed a significant difference in Bray Curtis beta diversity between the cohort years at both the Family and Genus levels (**Tables 4 and 5**). With these significant longitudinal changes in alpha and beta diversity, we couldn't help but wonder what exactly changed between CHC2 and CHC3 since the majority of households from CHC3 came from CHC2. Since we already know from previous work that CHC2 had higher organophosphate dust concentrations than CHC3, and this was consistent with Washington's reported agricultural use of OPs during 2005 and 2011 (Bennett et al., 2019), we decided to include total OP dust concentrations in our mixed effect models to assess whether this impacted indoor dust microbial abundance. Consistent with our longitudinal diversity trends, our results showed that not only was Shannon Diversity (at the Phylum and Class level) significantly affected by total dust OP concentrations (**Figure 5 and 6**) but also several taxa abundances significantly changed across the taxonomic ranks Order and Genus (**Tables 6 and 7**). While it is unclear why the slopes of CHC2 and CHC3 differed in relation to total dust OP concentrations, with CHC2 indicating that an increase in total dust OPs lead to a higher Shannon Diversity and CHC3 the opposite effect, it's important to note that because we are using total OPs for this analysis along with the fact that there are a wide variety of different OPs used in agriculture, it is possible that the overall mixture of OPs in our dust samples changed between the cohort years where some OPs were present at higher concentrations in one year compared to the other, but using total OPs masks this difference. This could explain why the Shannon diversity is going in opposite directions when dust OPs are increasing between the two years, as different OPs might have different effects on individual taxa abundances.

While there has been a lot of growing evidence over the years showing that some individual bacteria can break down organophosphate pesticides (Sethunathan & Yoshida, 1973; Siddaramappa et al., 1973; Singh, 2002), our understanding of whether OPs can impact the entire microbial community remains unclear. Given that previous studies have shown that organophosphates can alter the gut microbiome in rats and mice (Fang et al., 2018; B. Gao et al., 2017; Roman et al., 2019), it is surprising how little we know about OP microbial affects within

indoor environments especially since these compounds are still widely used today and found within household dust where many bacteria reside (Kuiper et al., 2022; Rintala et al., 2012). While there have been numerous studies examining how farming environments/activities can influence the indoor dust microbiome, none of these studies have investigated this through the lens of agricultural pesticide exposures. And although we know through previous work that *Streptococcus* was significantly deterred in the oral microbiomes of AZM exposed individuals (majority of them being farmworkers) (Stanaway et al., 2017), our results did not line up with these findings as we did not find a significant relationship between AZM and *Streptococcus* within our household dust samples (**Figure 7**). While this not only suggest that organophosphates may have variable effects on microbial communities between different environments, especially since the human body is very different than a household structure, this also highlights why we need more studies to differentiate OP bacterial effects within variable settings as the effects seen in one environment might not translate to another. And although there haven't been any other studies examining whether pesticides damper indoor dust microbiota, our results have shown that 4 Orders as well as 40 Genera can significantly be inhibited by these indoor dust OPs (**Tables 6 and 7**). Additionally, since our results also showed that some bacteria may thrive within OP contaminated dust, as we observed 3 Orders with significant increases in overall abundance as total OP concentrations went up (**Table 6**), understanding why some of these bacteria may be inhibited or promoted by OPs and whether this contributes negatively or positively to human health will be an important next step for public health. Considering that some microbes can breakdown and utilize organophosphates as a potential resource (Abraham et al., 2013; S. Fan et al., 2018; Yadav et al., 2021), this could explain why some bacteria are able to survive in such heavily contaminated environments as the high concentrations of OPs in our household dust samples could drive the selection for these specific microbes.

In addition to total dust OPs altering the indoor dust microbiome we also observed significant occupational effects within our mixed effects model at the Order, Family, and Genus level. Given that our study took place in an agricultural region where FWs work with crops and soils, the significant Genera and Families observed within our household dust samples agree with this setting. Not only did four of our genera *Paracoccus*, *Defluviimonas*, *Tabrizicola*, and *Yoonia* have significantly higher abundances in FW households compared to NFW households (**Table 10**), but also all of them can be found within the *Rhodobacteraceae* Family, which is one of our two Families that had significantly higher abundances within FW households as well (**Table 9**). Considering that *Paracoccus* has been previously isolated within soils of farming environments (Heo et al., 2019) and this is one of our most abundant genera present within our dust samples (**Figure 9B**) it makes sense that we observed such high abundances of *Paracoccus* within our occupational FW homes. Additionally, although *Rhodobacteraceae* are often associated with aquatic environments as the genera *Defluviimonas*, *Tabrizicola*, and *Yoonia* have all been previously isolated in water (Jung et al., 2014; Pohlner et al., 2019; Ren et al., 2022; Tarhriz et al., 2019), this isn't the first-time water associated bacteria have been found within terrestrial environments as cyanobacteria is another common aquatic Genus that has been observed within agricultural soils more and more as NGS technologies have become more widely available (Chamizo et al., 2019).

While we expected to see more overlap between our significant occupational taxa and our taxa that were significantly affected by total dust OPs, since we know FW households have higher

overall dust OP concentrations than NFW households, it is important to note that this difference in pesticide concentrations between the two occupations was not always clear cut, as several NFW households within our cohort also had high concentrations of dust OPs as well. Additionally, because our analysis included both CHC2 and CHC3 and agricultural use of OPs decreased in 2011 compared to 2005 (Bennett et al., 2019), there may have been too great of a difference in occupational pesticide exposures and OP dust concentrations between the two cohorts to observe significant overlap in the taxa affected by Occupation and total dust OP when looking at the data all together. So, while FWs had higher overall dust OPs compared to NFW households in both CHC2 and CHC3, FW households had significantly lower dust OPs in 2011 compared to 2005/2006 indicating a large difference in occupational organophosphate exposures between the two cohorts. In addition to this, It should also be noted that although NFW households tended to have lower dust OPs than FW households, compared to a similar NHANES population in the corresponding years, NFWs had higher urinary OP metabolites than the average NHANES participant (B. Thompson et al., 2014). So although NFWs may not directly work with organophosphates, due to all participants in this cohort being from the same agricultural region surrounded by many crops and orchards treated with pesticides, NFWs still had much greater OP exposures compared to the average population (Bennett et al., 2019).

However, since we did observe a significant occupational effect at the Order, Family, and Genus level, this finding does support our original hypothesis that the indoor dust microbiome is influenced by occupation. And while this not only agrees with many other studies showing that farming activities can alter the indoor dust microbiome (Birzele et al., 2017; Böbel et al., 2018; Debarry et al., 2007; Ege et al., 2011; Kirjavainen et al., 2019; Korthals et al., 2008; Normand et al., 2011; Vestergaard et al., 2018; White et al., 2019), our findings also support the concept of the occupational take-home pathway where, in addition to pesticide residues being brought into the home environment, farmworkers can also introduce agricultural microbes. Considering that farmworkers work with a variety of biodiverse agents such as soils, fertilizers, and plants, it makes sense that FW households would have somewhat different microbial compositions compared to NFW households as FWs are more likely to track in these biodiverse agents. And although we are now just beginning to uncover the many diverse bacteria associated with agriculture, our understanding of how certain farming activities select and determine specific bacteria in household dust still needs to be further investigated. For example, Lee et al. 2018 showed that household dust from crop farmworkers differed from animal farmworkers as certain bacteria associated with crop farming were not associated with animal farming, and this was seen vice versa (M. K. Lee et al., 2018). So, given that different farming activities like working with cows versus working with pigs can determine distinct bacterial communities in the home environment, understanding which specific agricultural activities lead to beneficial or deleterious bacteria will have important implications for human health as this information could contribute to better occupational safety interventions to reduce potential harmful bacterial exposures in the household environment where children and families reside.

Although season was not significant in any of our models, this finding is consistent with many other indoor dust bacterial studies (Adams et al., 2014; M. K. Lee et al., 2018; Rintala, Pitkaranta, et al., 2008; Shan et al., 2019). While we initially expected to see a difference in microbiome composition between the thinning and nonspray seasons due to their difference in agricultural pesticide use, since we don't know exactly how long these pesticides can persist in

indoor dust, the timing between the thinning and nonspray season might not have been long enough for these pesticides to break down and show a significant difference in their effect on the indoor dust microbiome. In fact, one study looking at agricultural communities within California found no seasonal differences in OP house dust concentrations, suggesting that within indoor environments OPs can potentially persist long-term (Kuiper et al., 2022). Additionally, since the immediate surrounding environment as well as the participant's activities likely did not change dramatically between the thinning and nonspray seasons, this may also explain why we didn't see any dramatic seasonal effects as previous studies have established the important role anthropogenic factors and the surrounding environment play in shaping the indoor dust microbiome (Dunn et al., 2013; Gilbert & Stephens, 2018; Gupta et al., 2020; Leung & Lee, 2016; Rai et al., 2021a).

However, in line with the concept of a core indoor dust microbiome previously described by Thompson et al 2021, we did find similarities in the composition of our household dust microbiota compared to other farm studies confirming a unique agricultural signature. For example, in agreement with other agricultural studies our samples were also dominated by the same four phylum groups (**Figure 9A**): Actinobacteria (58.2%), Proteobacteria (36.5%), Firmicutes (2.8%), and Bacteroidetes (1.2%). While our samples had higher levels of Actinobacteria, other agricultural studies reported either greater abundances of Proteobacteria followed by Firmicutes, Actinobacteria, and Bacteroidetes (Kirjavainen et al., 2019; M. K. Lee et al., 2018, 2021) or higher levels of Firmicutes with subsequently abundant Proteobacteria, Actinobacteria, and Bacteroidetes (Birzele et al., 2017; Vestergaard et al., 2018). While Proteobacteria contain many bacteria that are associated with the human microbiome some of which are well known opportunistic pathogens like Salmonella, Vibrio, Helicobacter, Yersinia, Legionella, and Escherichia coli (M. K. Lee et al., 2018; Moon et al., 2018), Actinobacteria contain a wide variety of metabolically diverse bacteria that are commonly found in soils. Given that Actinobacteria and Proteobacteria make up 94.7% of our total sample abundance and there have been reports of opportunistic pathogens like Corynebacterium, Mycobacterium, Nocardia, Propionibacterium, and Tropheryma within the Actinobacteria phylum (Barka et al., 2016), both of these phyla in our dust samples may pose significant health risks for our agricultural cohort.

Additionally, in agreement with previous studies showing that humans make up a large portion of the indoor dust microbiome (Adams et al., 2014; Barberán et al., 2015; Dunn et al., 2013; Gangneux et al., 2020; Gupta et al., 2020; Hanson et al., 2016; Lax et al., 2014; Leung & Lee, 2016; Rai et al., 2021a; Täubel et al., 2009), looking at our top Genera (**Figure 9B**) we can see that several of them are commonly associated with the human body (denoted with an H in the **Figure 9B**). And while this is not only consistent with both farming and nonfarming studies showing high abundances of human associated Acinetobacter, Staphylococcus, and Corynebacterium (Birzele et al., 2017; J. R. Thompson et al., 2021), this also supports the idea of a common core indoor dust microbiome because even when comparing studies with distinct populations and regions, we still can observe similar bacterial signatures often related to human commensals. Furthermore, since all of our household dust samples came from the same agricultural region, regardless of occupational status, when reviewing our most abundant genera (**Figure 9B**) we find that that majority of these organisms are not only metabolically and ecologically diverse, but also are associated with soils, plants, and agriculture. For example, Nocardioideae, Paracoccus, Massilia, Streptomyces, Sphingomonas, Microbacterium,

*Pseudomonas*, *Arthrobacter*, *Stenotrophomonas*, and *Pantoea*, have all been previously isolated from plants/soils within farming environments (Briceño et al., 2018; Dutkiewicz et al., 2016; Fernández et al., 2013; M. Gao et al., 2013; Green et al., 1974; Hayward et al., 2010; Heo et al., 2019; J. M. Lee et al., 2022; Rath et al., 2020; Sørensen et al., 2001). And while both *Rhodococcus* and *Pseudomonas* have been known to cause serious infections in farm animals, with *Pseudomonas* being shown to cause mastitis in cows and *Rhodococcus* leading to pneumonia in foals (Kawai et al., 2017; Rakowska et al., 2020), many of our top genera can also break down several classes of pesticides (Briceño et al., 2018; Fernández et al., 2013; Gilani et al., 2016, 2016; Lu et al., 2019; Mawang et al., 2021a, 2021b; Pan et al., 2016; Peng et al., 2008; Xie et al., 2009). So, given that our study is centered around an agricultural area where many pesticides are used to aid in the production of crops, which is evident from the high concentrations of pesticide residues measured in both our FWs and NFW households, it makes sense that we observed such large abundances of agricultural associated bacteria throughout all of our samples. And while some of these bacteria may be harmless, over the years there has been growing evidence that some of them may cause human bacterial infections. So, with all of this, these results not only suggest that the outdoor environment can influence the indoor environment as we found that the indoor dust microbiome is sensitive to nearby agricultural activities, but these findings also provide evidence of a strong agricultural signal.

Lastly, it is important to note the strengths and weaknesses of this study. As stated previously, while we know through prior studies that several factors impact the indoor dust microbiome such as lighting, ventilation, humidity, occupancy number, and pets, we did not account for any of these factors in this study. And although each of our households contain either an adult FW or NFW child pair, because we do not know the number, occupations, and activities of other members within each household, this may affect our result interpretations. Additionally, since this cohort was initially designed to investigate the effects of occupational pesticide exposures, one advantage of this study is that we were able to explore how agricultural pesticide exposures can influence the indoor dust microbiome as we had extensive OP pesticide concentrations for each of our household dust samples. However, because our classification for NFW households also included adults working in dairies (with the other occupations being factories, stores, schools, and daycares), some of our NFW households likely had agricultural occupational effects in their indoor dust microbiomes as well. But even with these limitations, this study also includes a number of strengths. One strength that is important to highlight is the fact that we utilized shotgun metagenomic sequencing. In contrast to targeted 16S rRNA sequencing, shotgun sequencing is not only able to characterize bacteria with higher resolution down to the species level, but also this method is not subject to primer bias since all the DNA within a sample is being sequenced (Durazzi et al., 2021). Additionally, because we sequenced all the DNA within our samples, we can also perform functional gene analysis in the future. And as we stated previously, unlike other microbial studies that only utilized one dust sample for each household, this study included both seasonal and longitudinal collection periods to gain a more accurate depiction of the indoor dust microbiome over time. And finally, while many other studies often rely on rarefying their samples, another strength of this study is that we decided to use the newly developed robust ANCOM-BC method. While ANCOM-BC has not only been shown to control false discovery rate very well, it is also, in comparison to other methods, the only procedure currently out there that can provide valid p-values and confidence intervals for each individual taxon when performing differential taxa abundance (Lin & Peddada, 2020).

## **Conclusion**

In this study, we investigated the relationship between agricultural activities and the indoor dust microbiome by performing shotgun metagenomic sequencing on household dust samples from a well-characterized longitudinal agricultural cohort from the lower Yakima Valley of Washington. While our results not only indicate the presence of a strong agricultural signal, as we observed many farm associated bacteria throughout all of our samples, our findings also showed significant longitudinal and occupational effects on the indoor dust microbiome. Given that organophosphate dust concentrations significantly changed between CHC2 and CHC3, we found that this difference drove substantial changes in the abundance of several taxa at the Order and Genus level. And while our results not only demonstrate that organophosphate pesticides and farming activities can impact the indoor dust microbiota, these findings also highlight the important role statewide agricultural pesticide regulations play in shaping indoor dust, as these policies can directly affect the concentration of pesticide residues within household dust and therefore influence the indoor dust microbiome. So, given that household dust is a significant reservoir and route for indoor microbial exposures, understanding how these microbial interactions influence health will be crucial for public health. And so, while this study provides evidence that the indoor dust microbiome is influenced by agricultural activities, whether these agricultural associated bacteria are beneficial or harmful to human health needs to be further investigated.

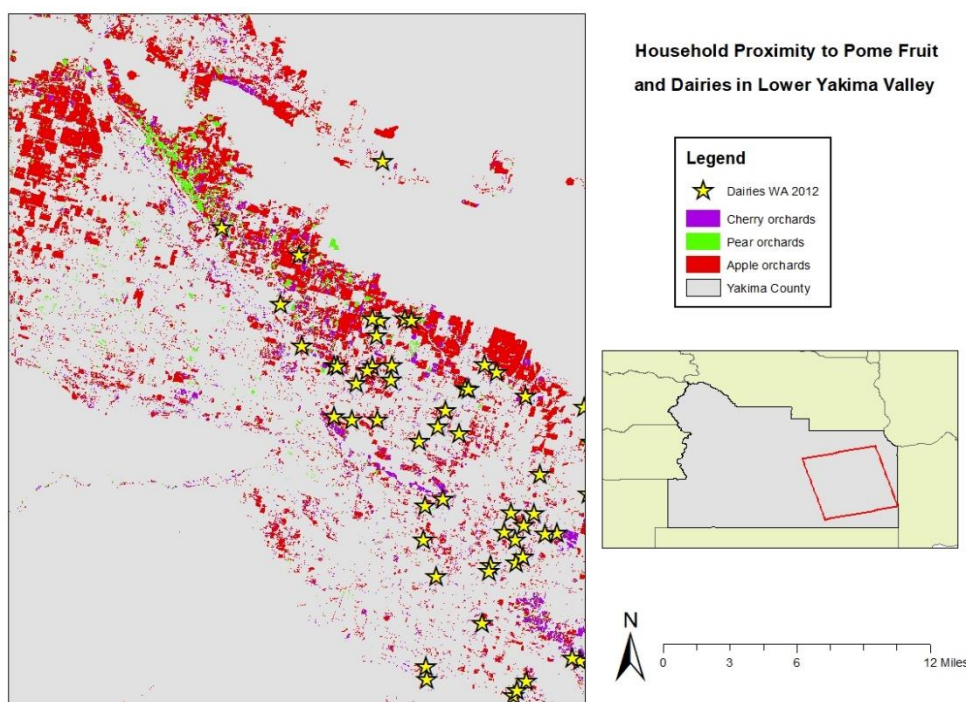
## **Future Directions**

It is possible that there are a number of other factors influencing the household dust microbiome within our samples. While our preliminary results included Occupational, Seasonal, and longitudinal effects along with an initial examination of how total OPs in household dust impact the indoor dust microbiome, given that we have extensive pesticide analysis for each of our household dust samples from a prior study (Bennett et al., 2019), in our next steps we will take a deeper look at how the indoor dust microbiome changes when exposed to different classes of pesticides. Additionally, because we know that antimicrobial compounds within our dust samples greatly differed between CHC2 and CHC3, with CHC3 having higher overall microbicide compounds than CHC2 (Bennett et al., 2019), it will be interesting to investigate whether this difference lead to significant changes in the indoor dust microbiota as well. And because this cohort also has respiratory health information for some of our study participants, this information will also allow us to connect the indoor dust microbiome with a human health outcome.

Additionally, one advantage of this study compared to other microbial studies is that we used shotgun metagenomic sequencing, a method that sequences all the DNA present within a sample. And so, because we did this not only were we able to characterize the many different bacteria present within our samples down to the species level, but we can also investigate their functional genes. By looking at functional genes, we can potentially further our understanding of the metabolic capabilities of these bacteria as well as identify specific antimicrobial resistant (AMR) genes. Given that these AMR genes have been shown to increase bacterial resistance to antibiotic treatment and therefore decrease our ability to manage and prevent bacterial infections,

characterizing and understanding how these AMR genes are formed and passed down between different bacteria within the household environment will be crucial for public health.

Lastly, because we have geocoordinates for each of our household dust samples, we have the opportunity to investigate how the indoor dust microbiome is affected by nearby agricultural activities. While we not only will spatially link the density of surrounding pome orchards to the indoor dust microbiome, we will also examine this relationship with nearby dairies as there are a number of them within our study area (**Figure 10**). Additionally, because antibiotics are often utilized in commercial dairy production (Oliver et al., 2011; Sawant et al., 2005), we also have the exciting opportunity to explore whether the antimicrobial genes present within our dust samples correlate with nearby operating dairies.



**Figure 10. ArcGis map of study area in 2011.** Map of Washington’s Lower Yakima Valley showing distribution of pome fruit orchards in 2011 and the distribution of Dairies in 2012.

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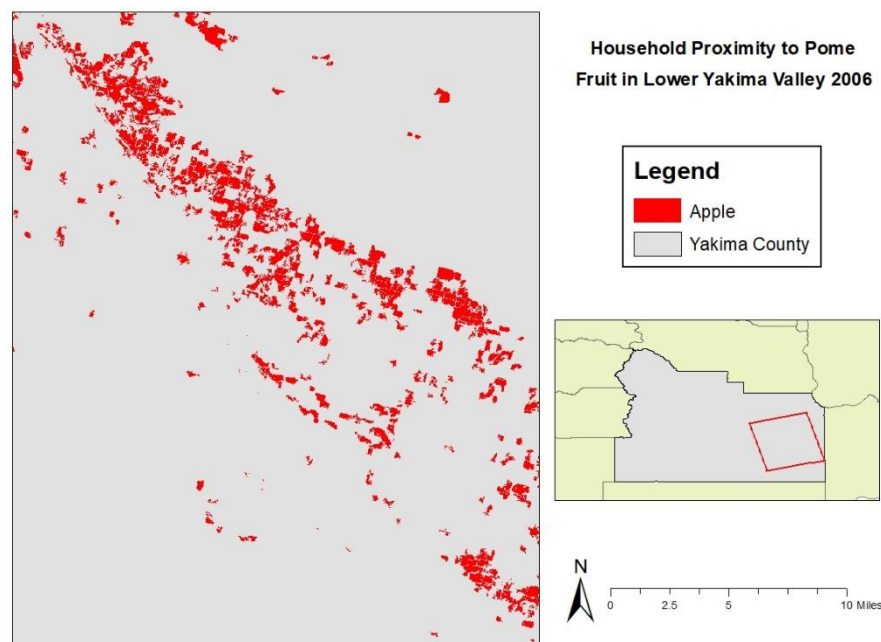
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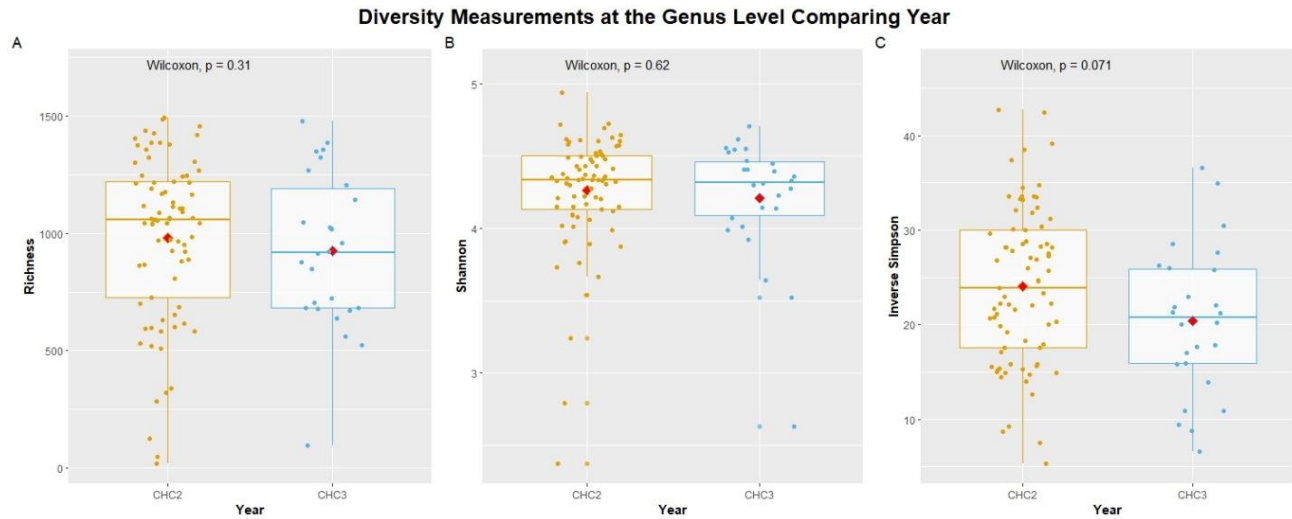
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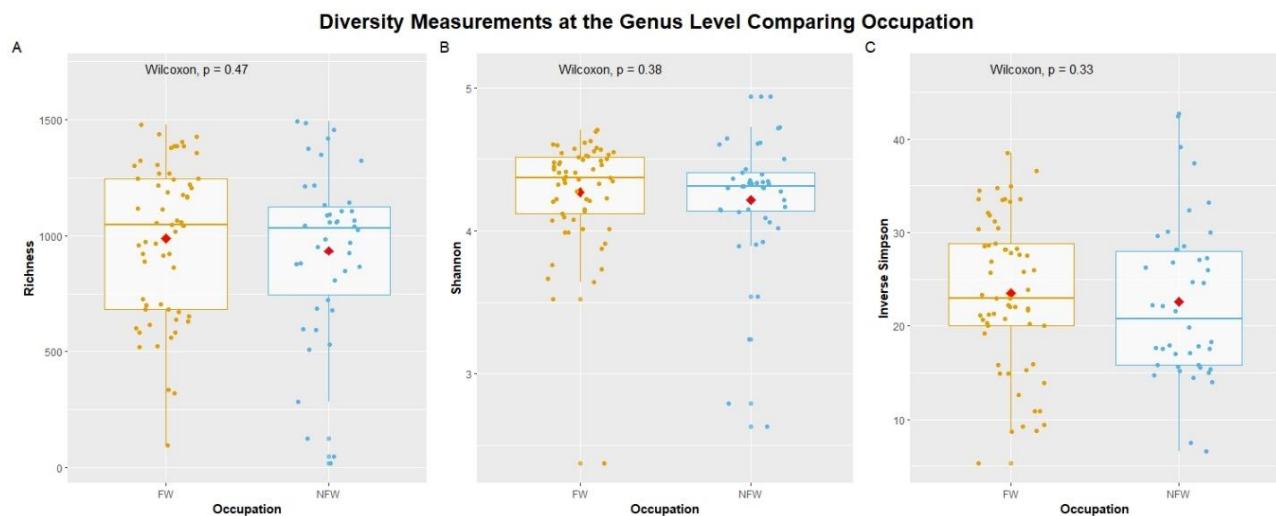
### **Supplemental Figures and Tables**



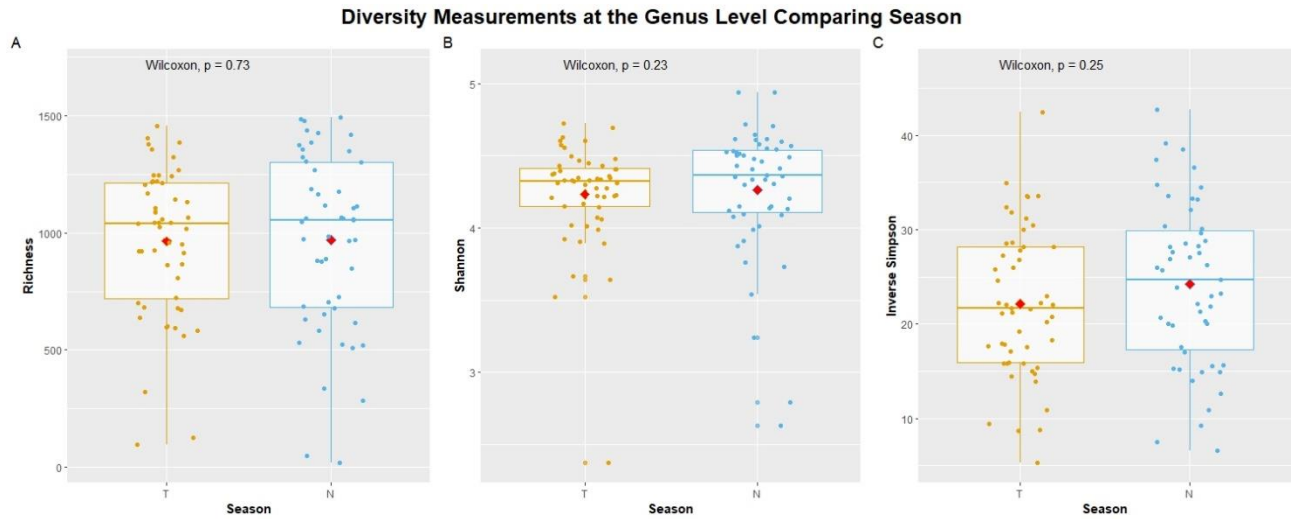
**Supplementary Figure 1. ArcGIS map of study area in 2006.** Map of Washington’s Lower Yakima Valley showing distribution of pome fruit orchards (Cropscape only had apple orchards available in 2006 data set) in 2006 during CHC2.



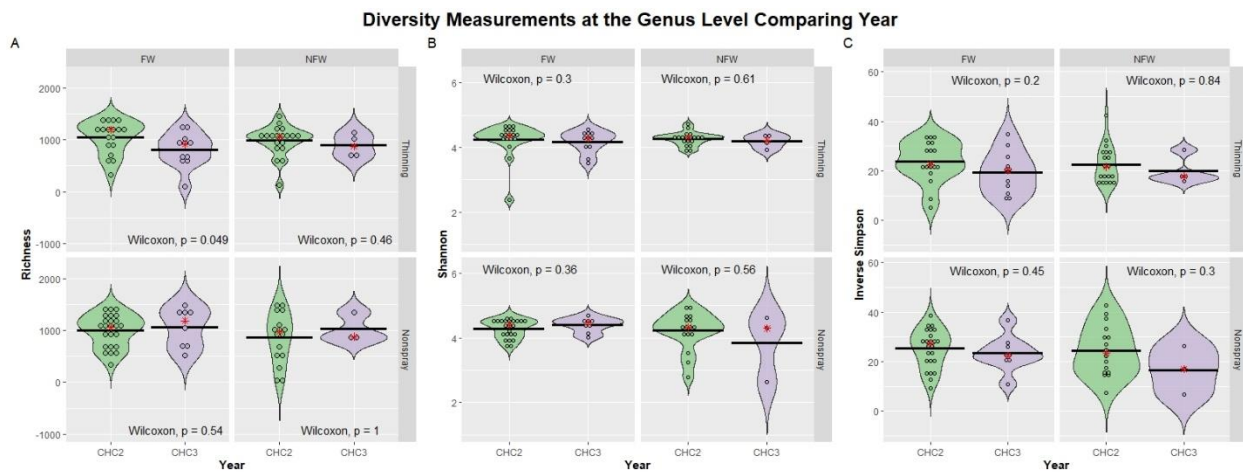
**Supplementary Figure 2. Boxplots comparing three alpha diversity metrics against CHC dust collection year at the Genus level.** The red diamond in boxplots represents the mean. **(A)** There was no significant change in Richness between CHC2 and CHC3. **(B)** There was no significant change in Shannon diversity between CHC2 and CHC3. **(C)** There was a slight change in the Inverse Simpson diversity between CHC2 and CHC3, but the p value was not significant (Wilcoxon,  $p=0.071$ ).



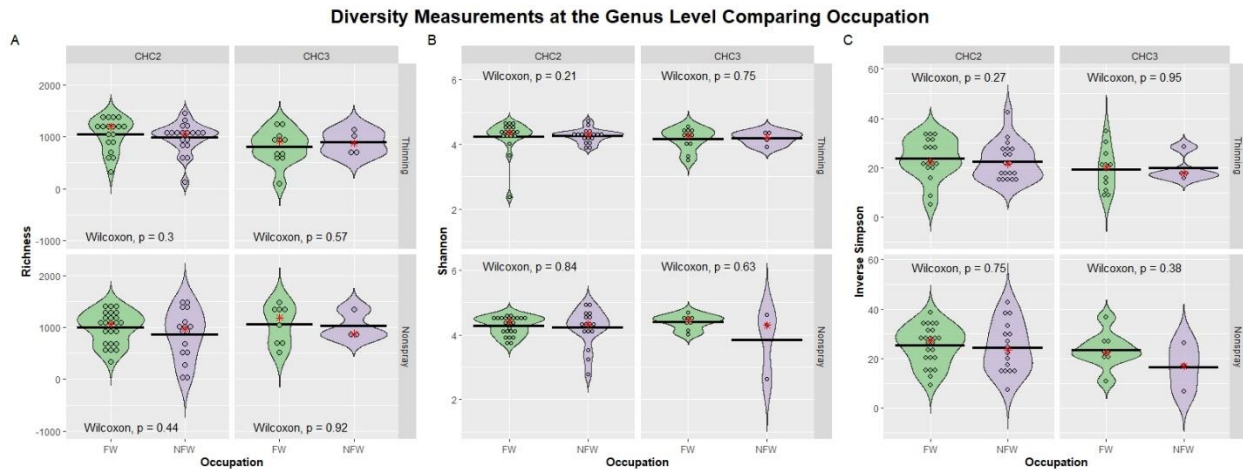
**Supplementary Figure 3. Boxplots comparing three alpha diversity metrics against Occupation at the Genus level.** The red diamond in boxplots represents the mean. **(A)** There was no significant change in Richness between FW and NFW households. **(B)** There was no significant change in Shannon diversity between FW and NFW households. **(C)** There was no significant change in the Inverse Simpson between FW and NFW households.



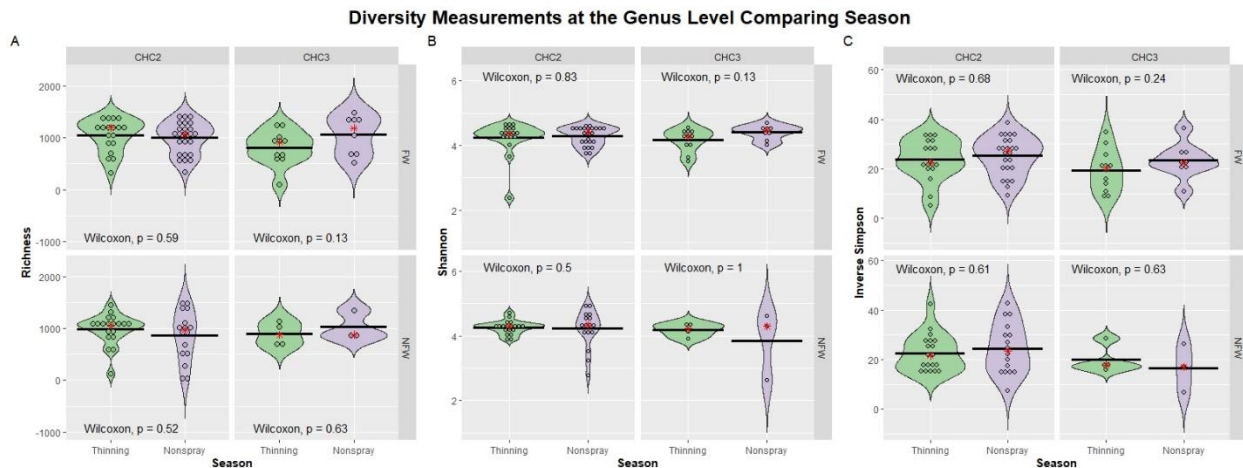
**Supplementary Figure 4. Boxplots comparing three alpha diversity metrics against Season at the Genus level.** The red diamond in boxplots represents the mean. **(A)** There was no significant change in Richness between Thinning (T) and Nonspray Season (N). **(B)** There was no significant change in Shannon diversity between T and N. **(C)** There was no significant change in the Inverse Simpson between T and N.



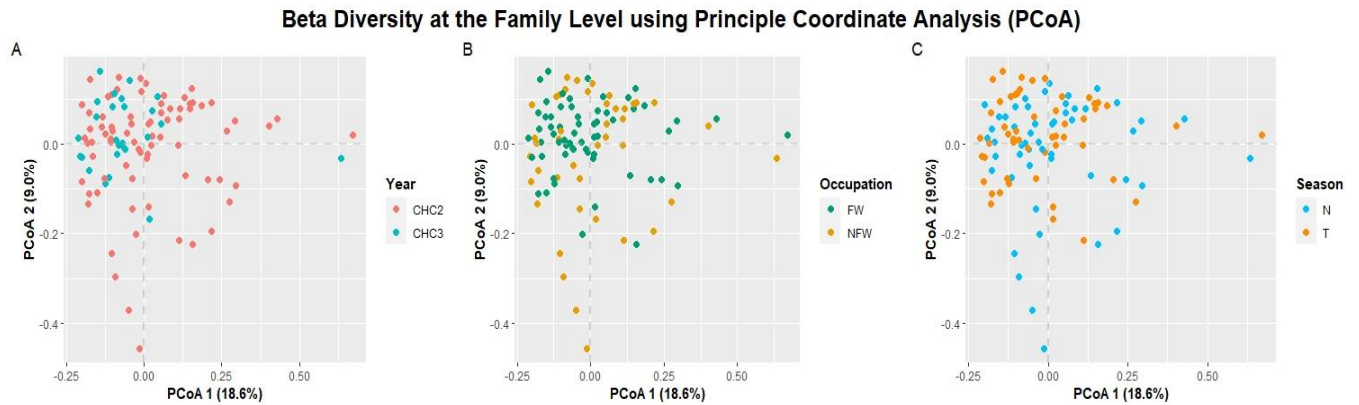
**Supplementary Figure 5. Violin plots comparing three alpha diversity metrics against all of our categorical variables (Year, Occupation, and Season) directly comparing Year at the Genus level.** The red asterisk in the violin plots represents the median and the black crossbar represents the mean. The dots within each violin shows the distribution of the data. **(A)** There was no significant change in Richness between CHC2 and CHC3 across our categorical variables Season and Occupation **(B)** There was no significant change in Shannon Diversity between CHC2 and CHC3 across our categorical variables Season and Occupation. **(C)** There was no significant change in the Inverse Simpson between CHC2 and CHC3 across our categorical variables Season and Occupation.



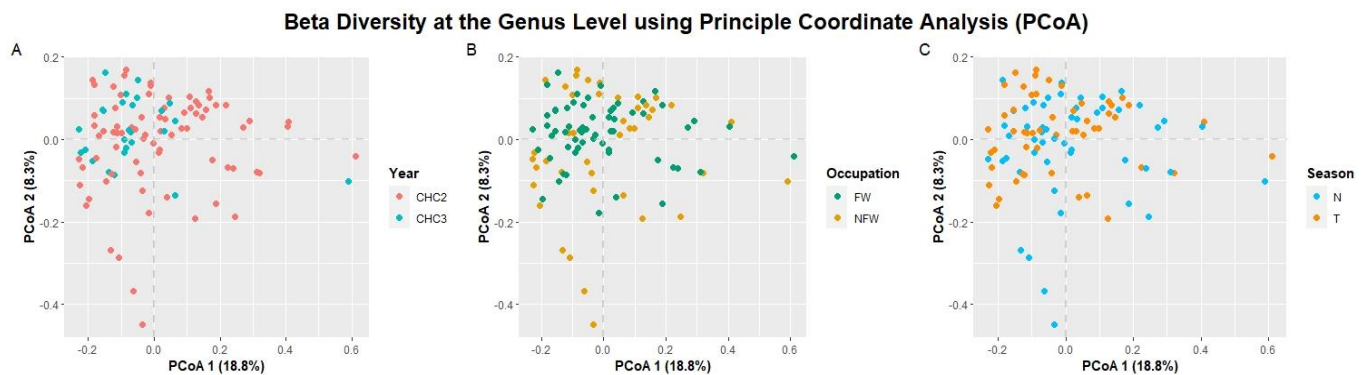
**Supplementary Figure 6. Violin plots comparing three alpha diversity metrics against all of our categorical variables (Year, Occupation, and Season) directly comparing Occupation at the Genus level.** The red asterisk in the violin plots represents the median and the black crossbar represents the mean. The dots within each violin shows the distribution of the data. **(A)** There was no significant change in Richness between FW and NFW households across our categorical variables Season and Year **(B)** There was no significant change in Shannon Diversity between FW and NFW households across our categorical variables Season and Year. **(C)** There was no significant change in the Inverse Simpson between FW and NFW households across our categorical variables Season and Year.



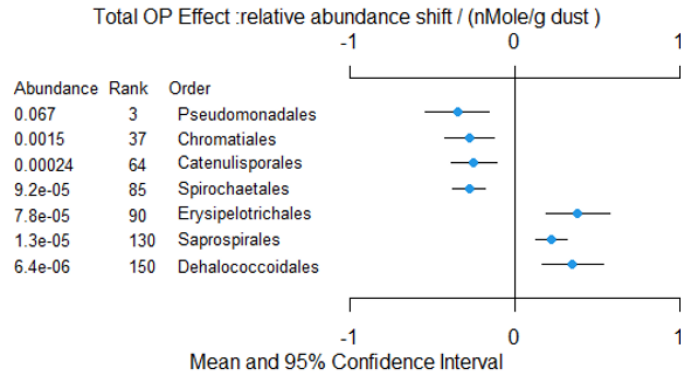
**Supplementary Figure 7. Violin plots comparing three alpha diversity metrics against all of our categorical variables (Year, Occupation, and Season) directly comparing Season at the Genus level.** The red asterisk in the violin plots represents the median and the black crossbar represents the mean. The dots within each violin shows the distribution of the data. **(A)** There was no significant change in Richness between T and N season across our categorical variables Year and Occupation **(B)** There was no significant change in Shannon Diversity between T and N season across our categorical variables Year and Occupation. **(C)** There was no significant change in the Inverse Simpson between T and N season across our categorical variables Year and Occupation.



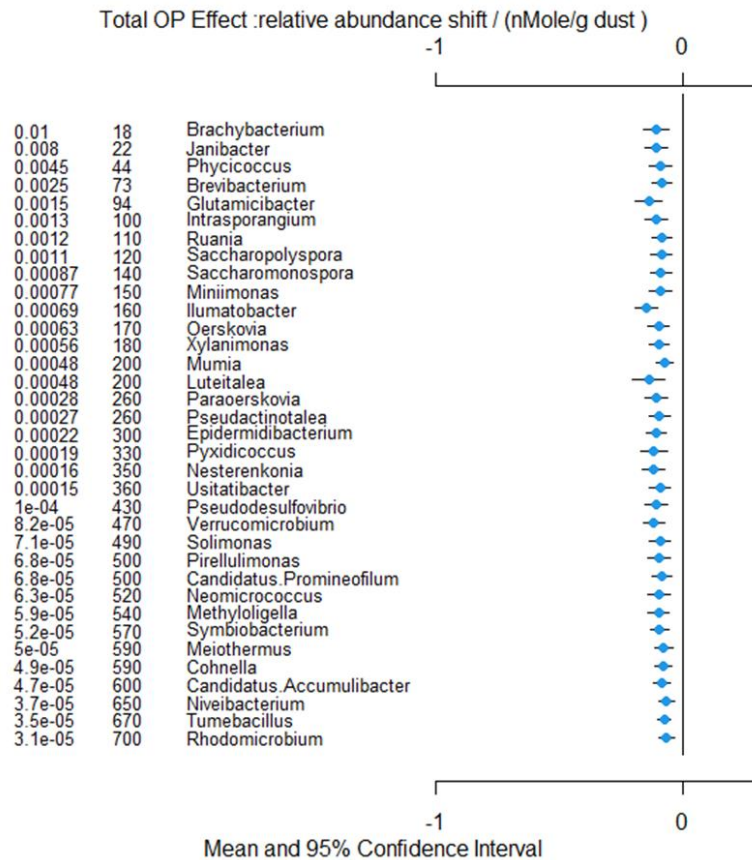
**Supplementary Figure 8. Principle Coordinate Analysis using Bray Curtis distance at the Family Level.** Each dot represents a dust sample and plots A, B, and C, show which specific variable we are looking at. **(A)** We can see distinct clustering between CHC2 vs. CHC3, but the dispersion of CHC2 is greater and overlapping with CHC3. **(B)** There is not distinct clustering between FW and NFW households. **(C)** There is no distinct clustering between T and N seasons.



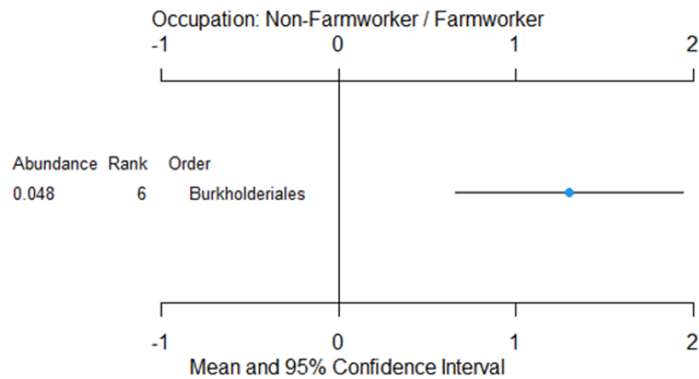
**Supplementary Figure 9. Principle Coordinate Analysis using Bray Curtis distance at the Genus Level.** Each dot represents a dust sample and plots A, B, and C, show which specific variable we are looking at. **(A)** We can see distinct clustering between CHC2 vs. CHC3, but the dispersion of CHC2 is greater and overlapping with CHC3. **(B)** There is not distinct clustering separation between FW and NFW households. **(C)** There is no distinct clustering separation between T and N seasons.



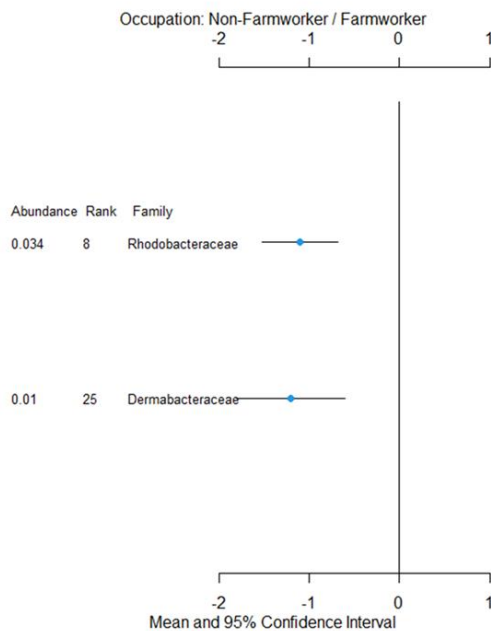
**Supplementary Figure 10. Orders significantly affected by Total Dust Organophosphate Concentrations.** this plot shows the 95% confidence intervals for the Orders significantly affected by total dust OPs using ANCOM-BC. The Orders below zero all had abundances that significantly decreased as total dust OPs increased, and the Order above zero had the inverse relationship.



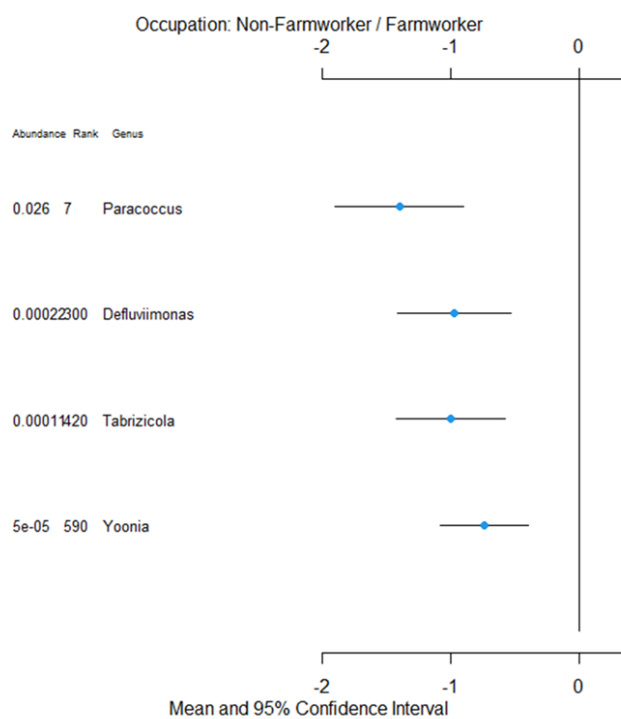
**Supplementary Figure 11. Genera significantly affected by Total Dust Organophosphate Concentrations.** this plot shows the 95% confidence intervals for the Genera significantly affected by total dust OPs using ANCOM-BC. The Orders below zero all had abundances that significantly decreased as total dust OPs increased, and the Order above zero had the inverse relationship.



**Supplementary Figure 12. Orders significantly affected by Occupational Status.** this plot shows the 95% confidence interval for the Order significantly affected by Occupation using ANCOM-BC. Burkholderiales had a higher abundance in NFW households compared to FW households.



**Supplementary Figure 13. Families significantly affected by Occupational Status.** this plot shows the 95% confidence interval for the Families significantly affected by Occupation using ANCOM-BC. Both Rhodobacteraceae and Dermabacteraceae had higher abundances in FW households compared to NFW households.



**Supplementary Figure 14. Genera significantly affected by Occupational Status.** this plot shows the 95% confidence interval for the Genera significantly affected by Occupation using ANCOM-BC. All four of our Genera had higher abundances in FW households compared to NFW households.

Sample ID	Year	Occupation	Season	Total Reads	Total bp	phred_forward	phred_reverse
2N-23_S102	CHC2	NFW	N	175518	26448192	35	35
2N-95_S37	CHC2	NFW	N	48112	7251190	35	35
3T-172_S42	CHC3	FW	T	422254	63626044	35	35
2T-37_S13	CHC2	NFW	T	370708	55864414	35	35
2N-80_S103	CHC2	NFW	N	1255902	189262087	35	35
2N-106_S56	CHC2	FW	N	2105026	317229241	35	34
3N-84_S49	CHC3	FW	N	2981048	449238869	35	35
2N-185_S63	CHC2	NFW	N	2516518	379246230	35	35
2N-51_S53	CHC2	FW	N	2951588	444803678	35	35
3T-177_S66	CHC3	FW	T	2904976	437789031	34	35
2N-84_S67	CHC2	FW	N	3494896	526682851	35	35
2N-140_S58	CHC2	FW	N	3245286	489054434	35	35
3T-84_S48	CHC3	FW	T	4111286	619565303	35	34
2T-161_S14	CHC2	FW	T	1590718	239716789	35	35
2T-24_S65	CHC2	FW	T	3519986	530465391	35	34
2T-141_S20	CHC2	FW	T	3513168	529399365	35	34
2T-53_S52	CHC2	NFW	T	3861474	581898293	35	35
3N-139_S69	CHC3	FW	N	3442364	518755086	35	35
3T-13_S73	CHC3	FW	T	4031834	607573572	35	35
2N-69_S41	CHC2	NFW	N	4064282	612466009	35	35
2N-149_S50	CHC2	FW	N	3349412	504781521	35	35
3T-143_S55	CHC3	FW	T	4471718	673866694	35	35
3N-143_S60	CHC3	FW	N	4659586	702208011	35	35
3T-35_S62	CHC3	NFW	T	2690854	405514047	35	35
3T-123_S57	CHC3	NFW	T	4782668	720705467	35	35
2N-123_S90	CHC2	NFW	N	4273570	644035023	35	35
2T-61_S44	CHC2	NFW	T	4519744	681129902	35	34
2T-75_S51	CHC2	NFW	T	5559064	830202371	35	35
2T-179_S23	CHC2	FW	T	4692624	707138787	35	35
2T-51_S72	CHC2	FW	T	5646818	850974163	35	35
2N-126_S34	CHC2	FW	N	5184928	781351251	35	35
2T-30_S26	CHC2	NFW	T	6445074	971280311	35	35
2N-139_S70	CHC2	FW	N	4463836	672701089	35	35
3T-141_S17	CHC3	FW	T	6670040	1005191017	35	35
3N-123_S47	CHC3	NFW	N	5681740	856223180	35	35
3T-106_S77	CHC3	FW	T	9476906	1428158876	35	34
2N-75_S36	CHC2	NFW	N	7552724	1138233891	35	35
2N-53_S35	CHC2	NFW	N	7483408	1127741526	35	34
2N-35_S74	CHC2	NFW	N	7912652	1192438526	35	35
2T-123_S88	CHC2	NFW	T	8628210	1300206866	35	35
3N-26_S18	CHC3	NFW	N	5760982	868192009	35	34
2N-61_S46	CHC2	NFW	N	6224178	937977645	35	35
2N-52_S38	CHC2	NFW	N	10270136	1547672972	35	34
2N-143_S24	CHC2	FW	N	10939832	1648528101	35	35
2T-149_S29	CHC2	FW	T	8466440	1275842811	35	35
2T-80_S25	CHC2	NFW	T	10157774	1530726226	35	35
2T-2_S19	CHC2	NFW	T	9705980	1462670627	35	35
3T-161_S15	CHC3	FW	T	11442418	1724381975	35	34
2N-147_S39	CHC2	FW	N	9994536	1506221390	35	35
2T-36_S30	CHC2	NFW	T	11751400	1770862052	35	34
2T-69_S33	CHC2	NFW	T	12413042	1870621550	35	35
2N-127_S85	CHC2	FW	N	14015888	2112211669	35	35
2T-26_S8	CHC2	NFW	T	11798218	1777912109	35	35
2T-52_S27	CHC2	NFW	T	12830346	1933501590	35	35
2T-23_S12	CHC2	NFW	T	13128242	1978381738	35	34
2N-172_S6	CHC2	FW	N	12822310	1932245964	35	35
3T-26_S28	CHC3	NFW	T	14221306	2143124142	35	35
2T-172_S61	CHC2	FW	T	14903560	2245900812	35	35
2N-157_S16	CHC2	FW	N	16407678	2472594757	35	35
2N-177_S40	CHC2	FW	N	15191110	2289359317	35	35
3N-177_S22	CHC3	FW	N	16955036	2554991232	35	35
3T-132_S31	CHC3	FW	T	12210300	1840002882	35	35
2T-35_S10	CHC2	NFW	T	15073750	2271543465	35	35
2N-15_S105	CHC2	FW	N	32206216	158871596	35	35
2T-157_S5	CHC2	FW	T	17710604	2668957184	35	35
2T-108_S89	CHC2	NFW	T	18457150	2781550036	34	35
3N-106_S80	CHC3	FW	N	19129440	2882719949	35	34
2N-101_S86	CHC2	FW	N	17766562	2677299096	35	34
3T-37_S79	CHC3	NFW	T	16483752	2484049075	35	34
2N-108_S87	CHC2	NFW	N	15387778	2318952283	35	34
2T-147_S11	CHC2	FW	T	20169702	3039602105	35	35
2N-179_S3	CHC2	FW	N	14137824	2130511817	35	34
2N-132_S54	CHC2	FW	N	21407476	3226133686	35	34
2T-19_S91	CHC2	FW	T	69825588	195084852	34	34
2T-95_S76	CHC2	NFW	T	17981088	2709731895	35	35
2T-101_S68	CHC2	FW	T	22911448	3452606777	35	35
2T-8_S83	CHC2	FW	T	21502718	3240319216	35	35
2T-39_S4	CHC2	NFW	T	18741028	2824122639	35	34
2N-124_S78	CHC2	FW	N	27724134	4177907566	35	35
2N-117_S93	CHC2	FW	N	49894464	7522248050	34	34
2T-119_S84	CHC2	FW	T	22326498	3364583119	35	34
2N-24_S96	CHC2	FW	N	29635096	4466297465	35	34
3N-13_S81	CHC3	FW	N	29228050	4404609886	35	35
2T-140_S7	CHC2	FW	T	25998492	3917738779	35	35
2N-119_S71	CHC2	FW	N	28263782	4259246797	35	35
2N-13_S45	CHC2	FW	N	26993716	4067819133	35	35
2N-39_S75	CHC2	NFW	N	23608300	3557589015	34	34
3T-117_S82	CHC3	FW	T	26872464	4049428430	35	35
3T-24_S98	CHC3	FW	T	98368168	410742002	35	35
2T-126_S59	CHC2	FW	T	26763742	4033209812	35	35
2N-141_S64	CHC2	FW	N	27957904	4197961775	35	35
2N-19_S100	CHC2	FW	N	52867542	7967134250	35	35
2T-94_S104	CHC2	NFW	T	29946412	4512831553	35	35
2T-15_S101	CHC2	FW	T	49877768	7516861721	33	35
2T-177_S43	CHC2	FW	T	37963604	5720822556	35	35
2N-2_S2	CHC2	NFW	N	28500020	4294783524	35	35
2T-127_S94	CHC2	FW	T	71787956	1.0818E+10	34	35
3N-24_S97	CHC3	FW	N	85538358	1.289E+10	35	35
2N-30_S21	CHC2	NFW	N	46465760	7002177603	35	35
2N-36_S9	CHC2	NFW	N	32559114	4906396818	35	35
3N-94_S92	CHC3	NFW	N	52622482	7929786200	35	35
3N-8_S99	CHC3	FW	N	91771462	1.3829E+10	35	35
2T-185_S95	CHC2	NFW	T	139325114	2.0995E+10	35	35

**Supplementary Table 1. Sample read numbers and quality control.** Table shows total number of reads and average phred score for each of our dust samples.

Sample ID	Year	Occupation	Season	Total Contigs	Total Contig bp	Mean Contig bp	Contig_N50
2N-23_S102	CHC2	NFW	N	177	63377		358
2N-95_S37	CHC2	NFW	N	206	84546		382
3T-172_S42	CHC3	FW	T	638	232772		352
2T-37_S13	CHC2	NFW	T	928	347443		358
2N-80_S103	CHC2	NFW	N	3256	1201980		356
2N-106_S56	CHC2	FW	N	14092	6045073		413
3N-84_S49	CHC3	FW	N	18697	7351194		374
2N-185_S63	CHC2	NFW	N	19300	7714104		380
2N-51_S53	CHC2	FW	N	21030	8119567		368
3T-177_S66	CHC3	FW	T	22424	9131857		388
2N-84_S67	CHC2	FW	N	29824	11521301		372
2N-140_S58	CHC2	FW	N	31769	13321949		402
3T-84_S48	CHC3	FW	T	32525	14195701		410
2T-161_S14	CHC2	FW	T	33486	16009787		457
2T-24_S65	CHC2	FW	T	34499	14434222		401
2T-141_S20	CHC2	FW	T	35620	15404795		416
2T-53_S52	CHC2	NFW	T	36316	15421431		402
3N-139_S69	CHC3	FW	N	40358	16572675		392
3T-13_S73	CHC3	FW	T	40612	18153487		426
2N-69_S41	CHC2	NFW	N	41139	17688306		407
2N-149_S50	CHC2	FW	N	44470	18239276		392
3T-143_S55	CHC3	FW	T	44557	17840752		384
3N-143_S60	CHC3	FW	N	46281	18691333		387
3T-35_S62	CHC3	NFW	T	46747	29219782		623
3T-123_S57	CHC3	NFW	T	47762	19999980		398
2N-123_S90	CHC2	NFW	N	48779	21612140		440
2T-61_S44	CHC2	NFW	T	50594	20933855		397
2T-75_S51	CHC2	NFW	T	54955	22881906		395
2T-179_S23	CHC2	FW	T	56700	24883922		411
2T-51_S72	CHC2	FW	T	61649	25280918		390
2N-126_S34	CHC2	FW	N	61873	25031391		388
2T-30_S26	CHC2	NFW	T	63825	26037012		391
2N-139_S70	CHC2	FW	N	63880	28212073		417
3T-141_S17	CHC3	FW	T	98363	43706220		419
3N-123_S47	CHC3	NFW	N	100638	44132034		421
3T-106_S77	CHC3	FW	T	106869	47469036		428
2N-75_S36	CHC2	NFW	N	108632	47605156		417
2N-53_S35	CHC2	NFW	N	110119	46626044		409
2N-35_S74	CHC2	NFW	N	112879	47225850		394
2T-123_S88	CHC2	NFW	T	115307	49738421		411
3N-26_S18	CHC3	NFW	N	138676	94136250		778
2N-61_S46	CHC2	NFW	N	143804	65335865		434
2N-52_S38	CHC2	NFW	N	157338	67058030		409
2N-143_S24	CHC2	FW	N	164384	72066577		416
2T-149_S29	CHC2	FW	T	169288	75108644		424
2T-80_S25	CHC2	NFW	T	172514	74176662		414
2T-2_S19	CHC2	NFW	T	190054	78660259		398
3T-161_S15	CHC3	FW	T	194200	81653951		403
2N-147_S39	CHC2	FW	N	205041	95514866		440
2T-36_S30	CHC2	NFW	T	206981	88119111		405
2T-69_S33	CHC2	NFW	T	209524	91038127		413
2N-127_S85	CHC2	FW	N	212336	91590101		410
2T-26_S8	CHC2	NFW	T	234232	95565922		392
2T-52_S27	CHC2	NFW	T	234250	99925095		408
2T-23_S12	CHC2	NFW	T	235292	105122267		423
2N-172_S6	CHC2	FW	N	243285	101935535		401
3T-26_S28	CHC3	NFW	T	263291	130587056		456
2T-172_S61	CHC2	FW	T	268440	122261196		431
2N-157_S16	CHC2	FW	N	269964	117520704		420
2N-177_S40	CHC2	FW	N	274802	139294759		466
3N-177_S22	CHC3	FW	N	296589	127736612		411
3T-132_S31	CHC3	FW	T	298267	139583689		449
2T-35_S10	CHC2	NFW	T	303514	134675453		425
2N-15_S105	CHC2	FW	N	304301	158871596		502
2T-157_S5	CHC2	FW	T	304318	127963108		403
2T-108_S89	CHC2	NFW	T	309370	139052832		417
3N-106_S80	CHC3	FW	N	310803	139074172		429
2N-101_S86	CHC2	FW	N	321125	136911310		408
3T-37_S79	CHC3	NFW	T	328768	147448487		430
2N-108_S87	CHC2	NFW	N	330996	139509956		407
2T-147_S11	CHC2	FW	T	343464	161190142		451
2N-179_S3	CHC2	FW	N	345156	150471642		411
2N-132_S54	CHC2	FW	N	395575	219817690		515
2T-19_S91	CHC2	FW	T	404089	195084852		461
2T-95_S76	CHC2	NFW	T	425940	188835669		429
2T-101_S68	CHC2	FW	T	427640	189961232		417
2T-8_S83	CHC2	FW	T	427878	198354343		438
2T-39_S4	CHC2	NFW	T	489730	208756871		406
2N-124_S78	CHC2	FW	N	496212	283013177		547
2N-117_S93	CHC2	FW	N	527527	247179970		432
2T-119_S84	CHC2	FW	T	553668	261237320		437
2N-24_S96	CHC2	FW	N	555490	253825443		431
3N-13_S81	CHC3	FW	N	587623	275511585		443
2T-140_S7	CHC2	FW	T	596992	283807660		475
2N-119_S71	CHC2	FW	N	615420	269551464		437
2N-13_S45	CHC2	FW	N	677717	368535381		530
2N-39_S75	CHC2	NFW	N	685551	351147654		497
3T-117_S82	CHC3	FW	T	686116	341017393		497
3T-24_S98	CHC3	FW	T	695912	410742002		573
2T-126_S59	CHC2	FW	T	707307	357864219		487
2N-141_S64	CHC2	FW	N	803873	395913187		474
2N-19_S100	CHC2	FW	N	814780	385000976		450
2T-94_S104	CHC2	NFW	T	834459	429349108		502
2T-15_S101	CHC2	FW	T	853735	362485467		408
2T-177_S43	CHC2	FW	T	882777	462903939		509
2N-2_S2	CHC2	NFW	N	934230	483684117		517
2T-127_S94	CHC2	FW	T	974441	429598508		420
3N-24_S97	CHC3	FW	N	976661	467757973		449
2N-30_S21	CHC2	NFW	N	987267	431405306		417
2N-36_S9	CHC2	NFW	N	1036764	565331467		537
3N-94_S92	CHC3	NFW	N	1126003	522433408		446
3N-8_S99	CHC3	FW	N	1339397	636766162		456
2T-185_S95	CHC2	NFW	T	2341840	1210933883		497

**Supplementary Table 2. Sample Contig numbers and quality control.** Table shows total number of Contigs and N50 for each of our dust samples.