The Environmental and Infant Gut Microbiomes and Allergic Disorders: Human Studies

New Perspectives: Addressing the Asthma & Allergy Epidemics

Detroit, October 2015

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&
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No relevant financial relationships.

Personal financial interests in commercial entities that are relevant to my presentation: None

No discussion of off label drug use

Research Support: National Institutes of Health, Fund for Henry Ford Hospital

Legal Fees: None

Gifts: None

Other potential conflicts: None

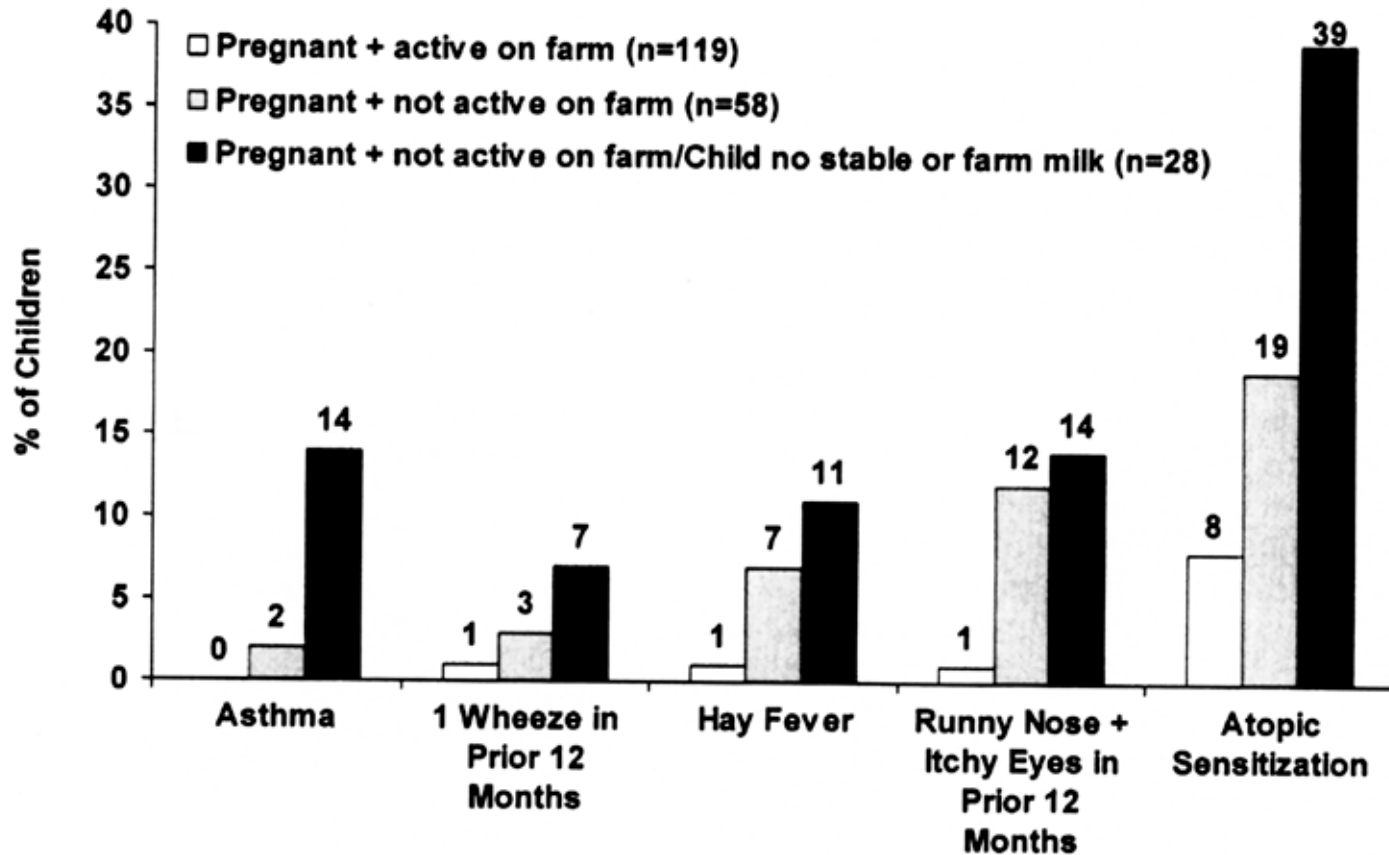# Risk of Hay Fever Inversely Related to Number of Older Siblings

The Beginning of the "Hygiene Hypothesis"

Hygiene Factors

- Decreased family size
- Increased standard of living
- Suburbanization
- Less exposure to animals
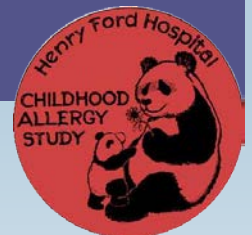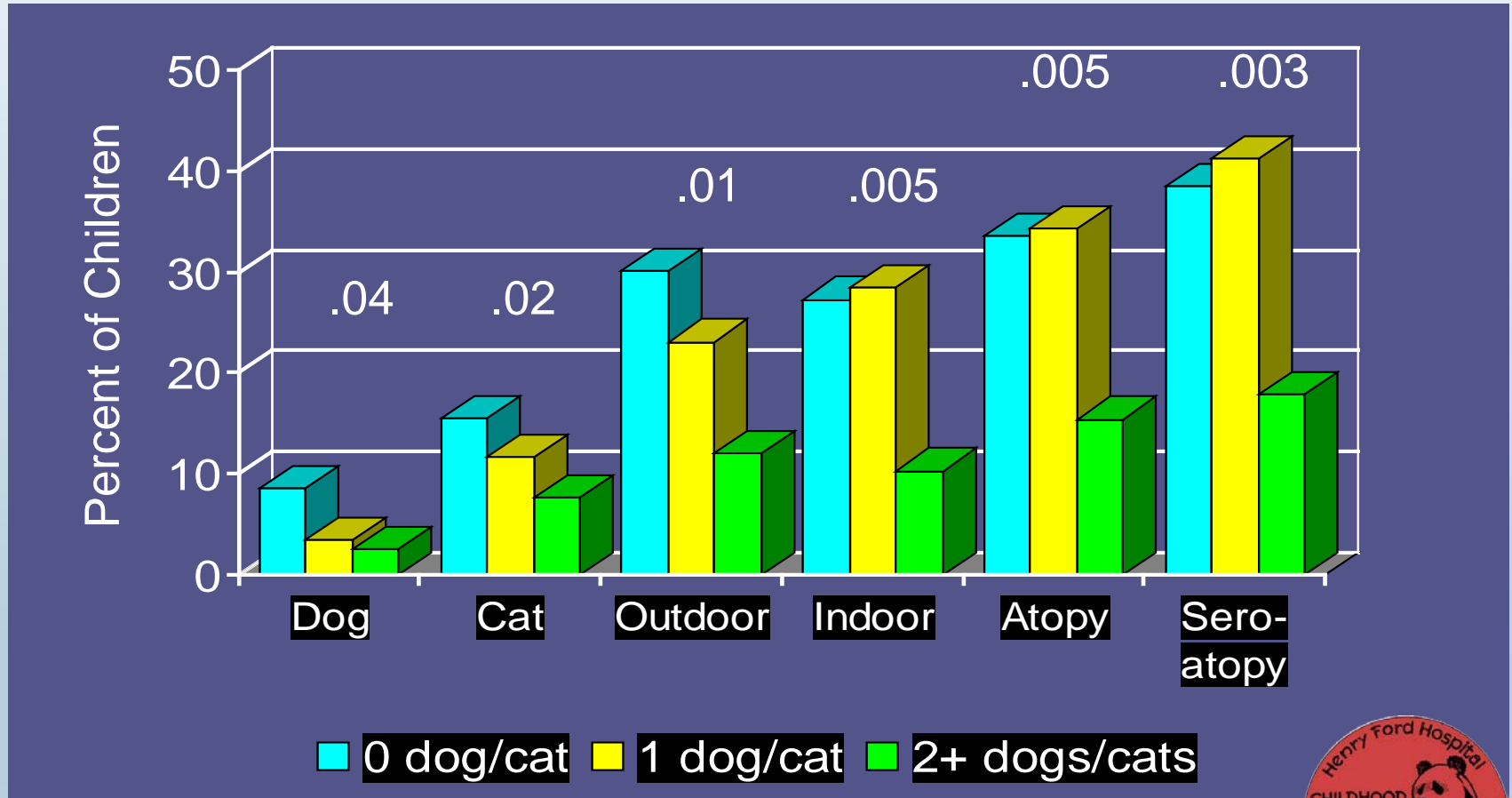- More immunizations
- More antibiotics use

Pregnant Mothers Living on Bavarian Farms (ALEX Study, n=901)

Riedler J, et al. Lancet 2001;358:1129-33

Farming, Bavarian Style

Pets in First Year of Life Inversely Associated with Allergic Sensitivity at age 6 yrs

Ownby et al. JAMA 2002

Evolution of the HYGIENE HYPOTHESIS

1989:   DECREASED INFECTIONS?

2000: DECREASED BACTERIA EXPOSURE?

NIH HUMAN
MICROBIOME
PROJECT

2007: CULTURE **INDEPENDENT** TECHNOLOGY

THE MICROBIAL DYSBIOSIS HYPOTHESIS

Sue Lynch

Homer Boushey

Kei Fujimura

Nick Lukacs

Haejin Kim

Kevin Bobbitt
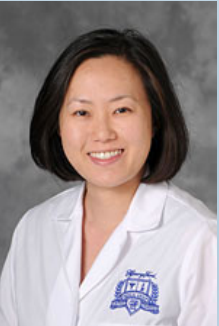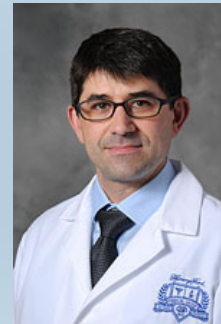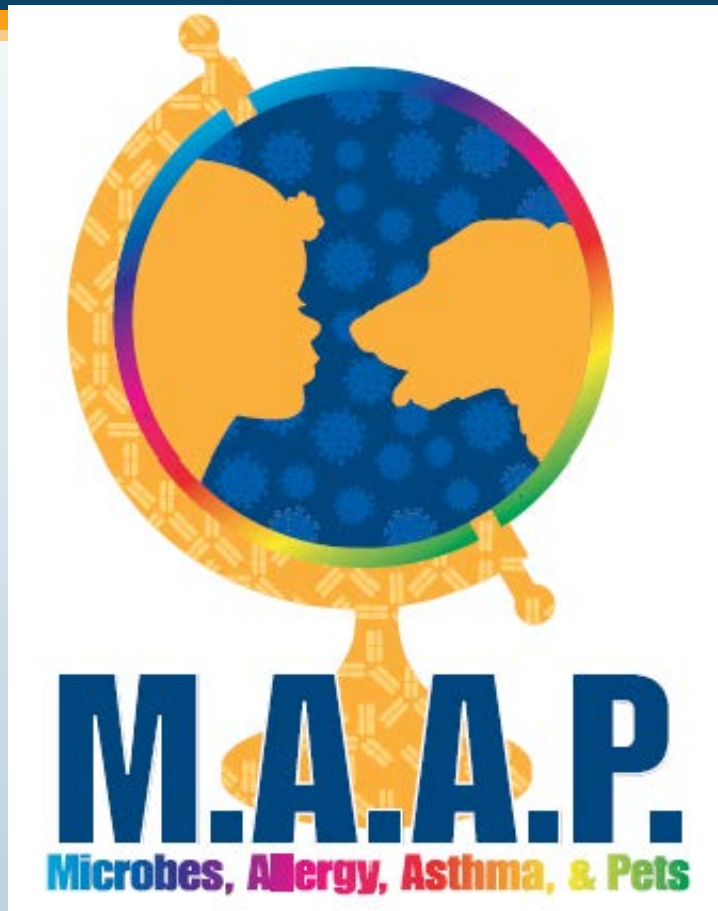
Ganesa Wegienka

Suzanne Havstad

Alex Sitarik

Al Levin

Andrea Casssidy-Bushrow

Chris Johnson
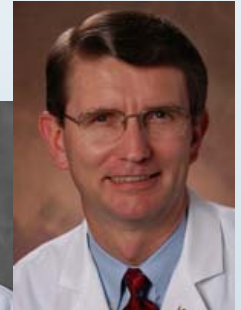
Kim Woodcroft

Dennis Ownby

Ed Zoratti

Kyra Jones
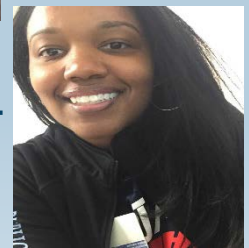
M.A.A.P.
Microbes, Allergy, Asthma, & Pets
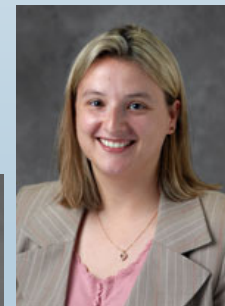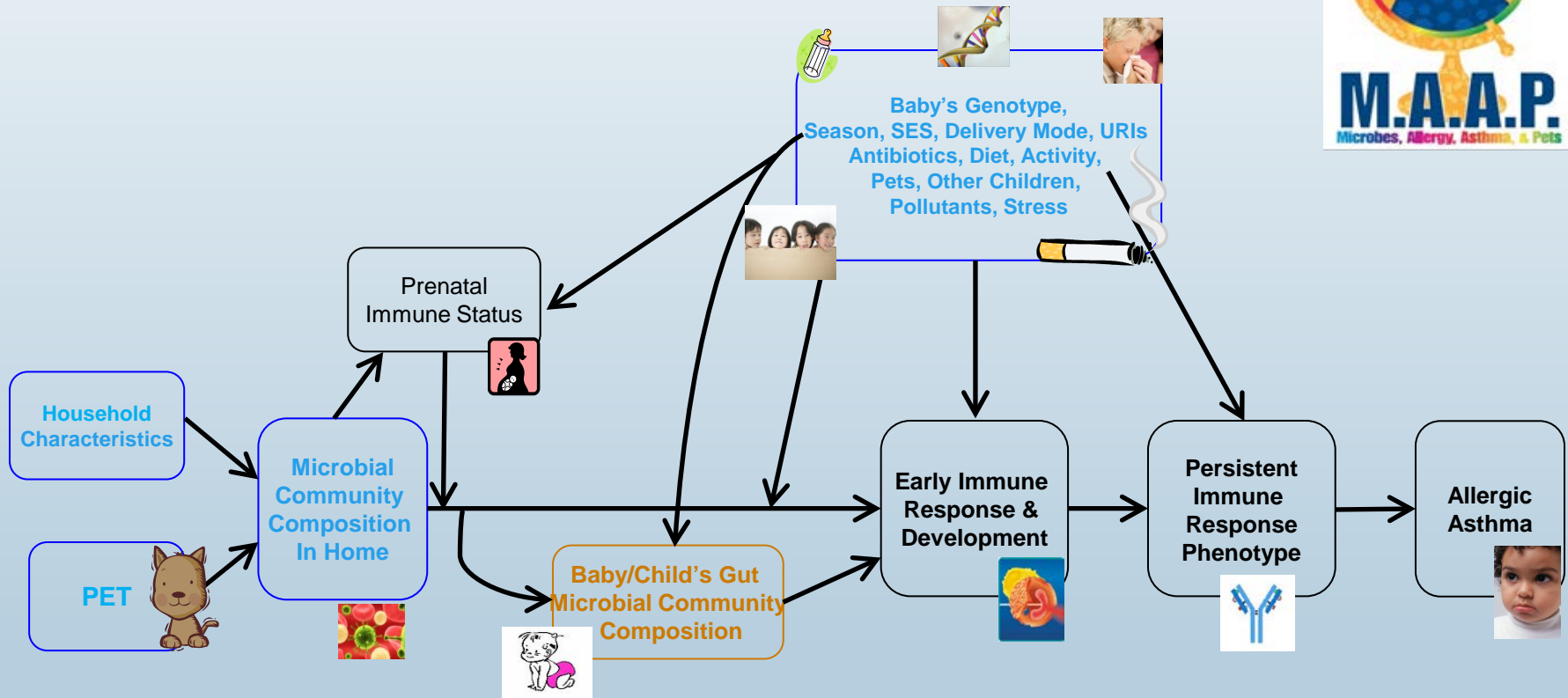
MAAP Causal Model

Questions…

- What environmental and social characteristics are related to the environmental microbiome?

- What social and environmental characteristics are related to the infant gut microbiome?

- How does the infant gut microbiome relate to atopic conditions?

Wayne County Health, Environment, Allergy & Asthma Longitudinal Study (WHEALS) Birth Cohort

- Pregnant mothers recruited 2003-2007, from
  Henry Ford Health System OB clinics in metropolitan
  Detroit Michigan USA (urban/suburban)

- Racially diverse (50% minority)

- Diverse socio-economic status

- Population-based (n=1258)

- Conducted interviews with mothers at prenatal and approximately
  1 month (neonate) and 6 month (infant) home visits

- Dust and Stool samples collected at same home visits

The Indoor Microbiome: What does the dust tell us?
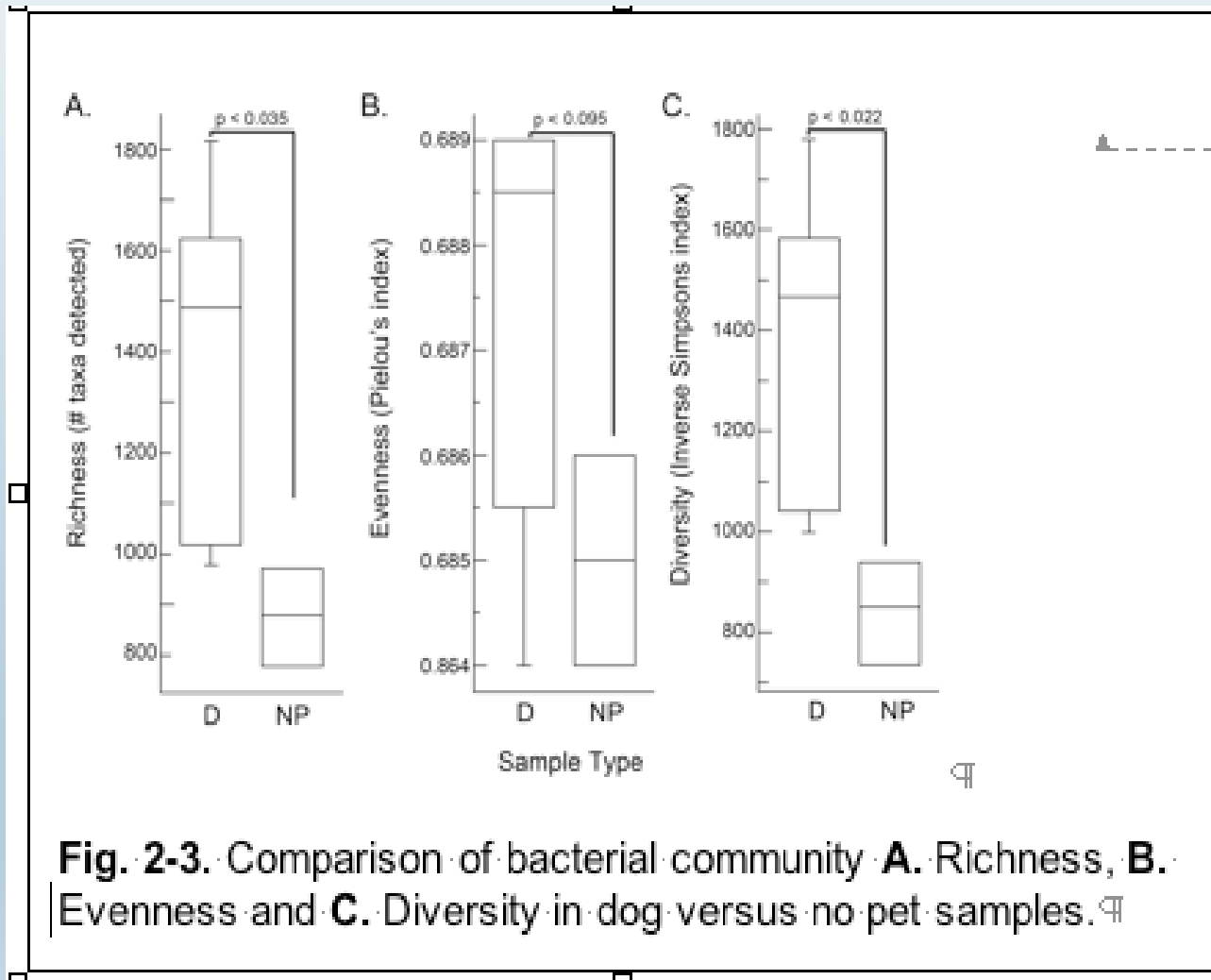
Are Babies exposed to House Dust?

- Hand-to-mouth activity in all children

- Well studied by toxicologists

- Average dust ingestion is 30–100 mg/day
  (20 – 70 million bacteria) for children 6 months – 11 yrs of age.

*U.S. EPA. Child Specific Exposure Factors Handbook 2008*

Bacterial Communities* in House Dust from Dog vs No-Pet Households

Fig. 2-3. Comparison of bacterial community **A.** Richness, **B.** Evenness and **C.** Diversity in dog versus no pet samples.

*measured by PhyloChip

Fujimura KE, JACI 2010;126:410-412

Atopic Wheezers Associated with Lowest Allergen & Bacterial Exposures In House Dust, URECA Cohort, age 3 yrs

Lynch, JACI 2014
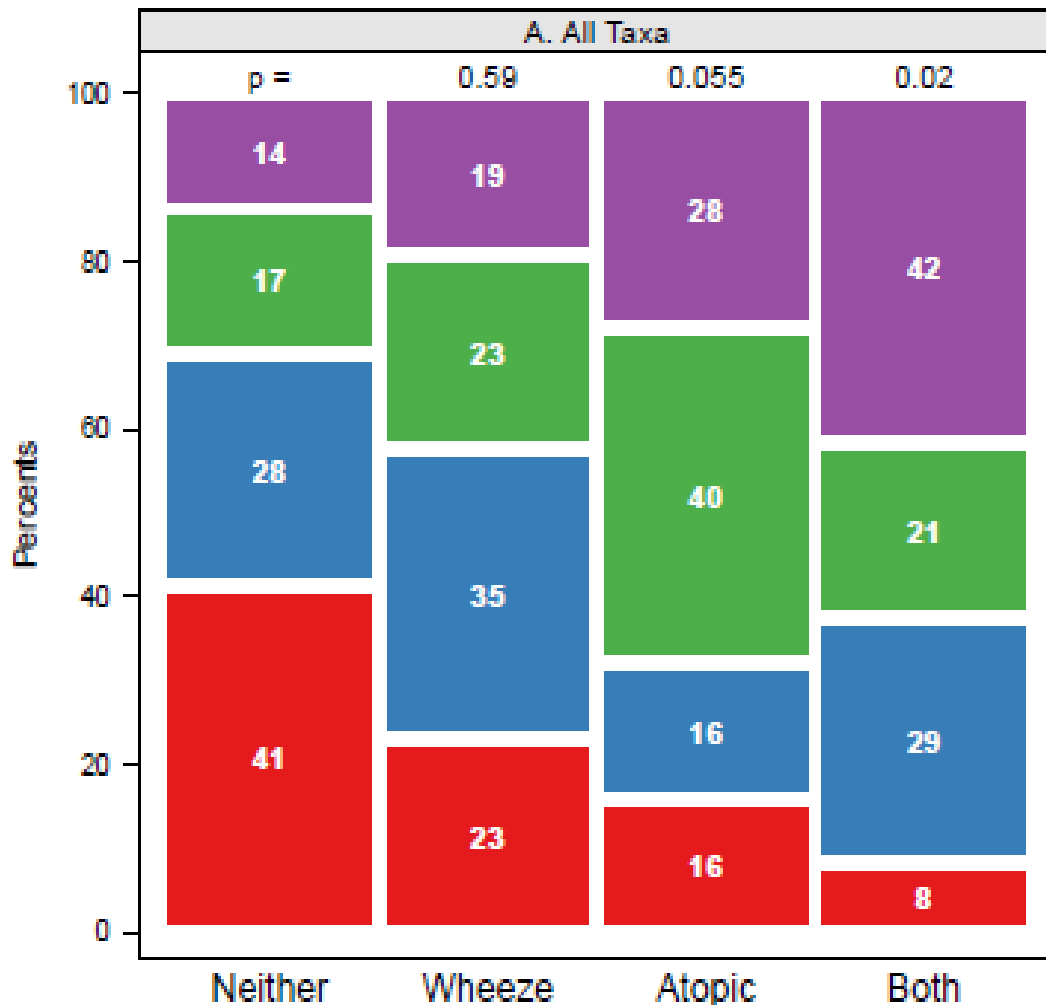
How Do Environment and Social Factors affect the Environmental Microbiome?

- Studies show that dogs, cats and number of children affect the microbiome composition of the home.

- WHEALS 1 month and 6 month dust samples being measured

- Ongoing "Dog Adoption" Study

- Ongoing study funded by Sloan Foundation measuring home characteristics and microbial and fungal content of dust

The Infant's Microbiome: What Do the Stools Tell Us?

Analytic Sample

- Drawn from WHEALS
- 298 stool samples met inclusion criteria and had sufficient DNA load for sequencing
  - 1 month study visit: N=130, Median=35 days ,IQR=17 days
  - 6 month study visit: N=168, Median=201 days, IQR=37 days
- MAAP Sample representative of WHEALS in terms of:
  - Race
  - Pet ownership
  - Gender
  - Family history of allergic disease
  - Mode of Delivery
- Tended to be higher income participants

Data Collection and Measurement

Environmental, Social & Clinical

- <u>What:</u> Maternal, Birth, and Household Early Life Characteristics
  - <u>When:</u> Prenatal Period, 1 (neonatal) and 6 (infant) Month Home Study Visits
- <u>How:</u> Questionnaires, Chart Abstraction, Dust Samples, Medical Records, Clinical Exams

Microbiome

- <u>What:</u> Infant Gut Microbiome
- <u>When:</u> Neonatal and Infant Home Study Visit
- <u>How:</u> Illumina MiSeq Sequencing platform -tag sequencing of the 16S rRNA gene (v4 region) to identify bacteria present (Operational Taxonomic Units or OTUs)

Analytic Approach

- Differences in stool bacterial indices of richness, evenness and diversity: Wilcoxon Rank Sum tests

- PERMANOVA: tests compositional differences in microbiomes using Operational Taxonomic Units (OTUs)

- Principal Coordinates Analysis: PCoA – graphical depiction of distances (weighted or unweighted Unifrac) between subjects based on multidimensional data (thousands of OTUs):

    ➡️ visual display of bacterial community composition differences by subject

- Dirichlet Mixture Model: identifies distinct microbiome profiles based on OTUs
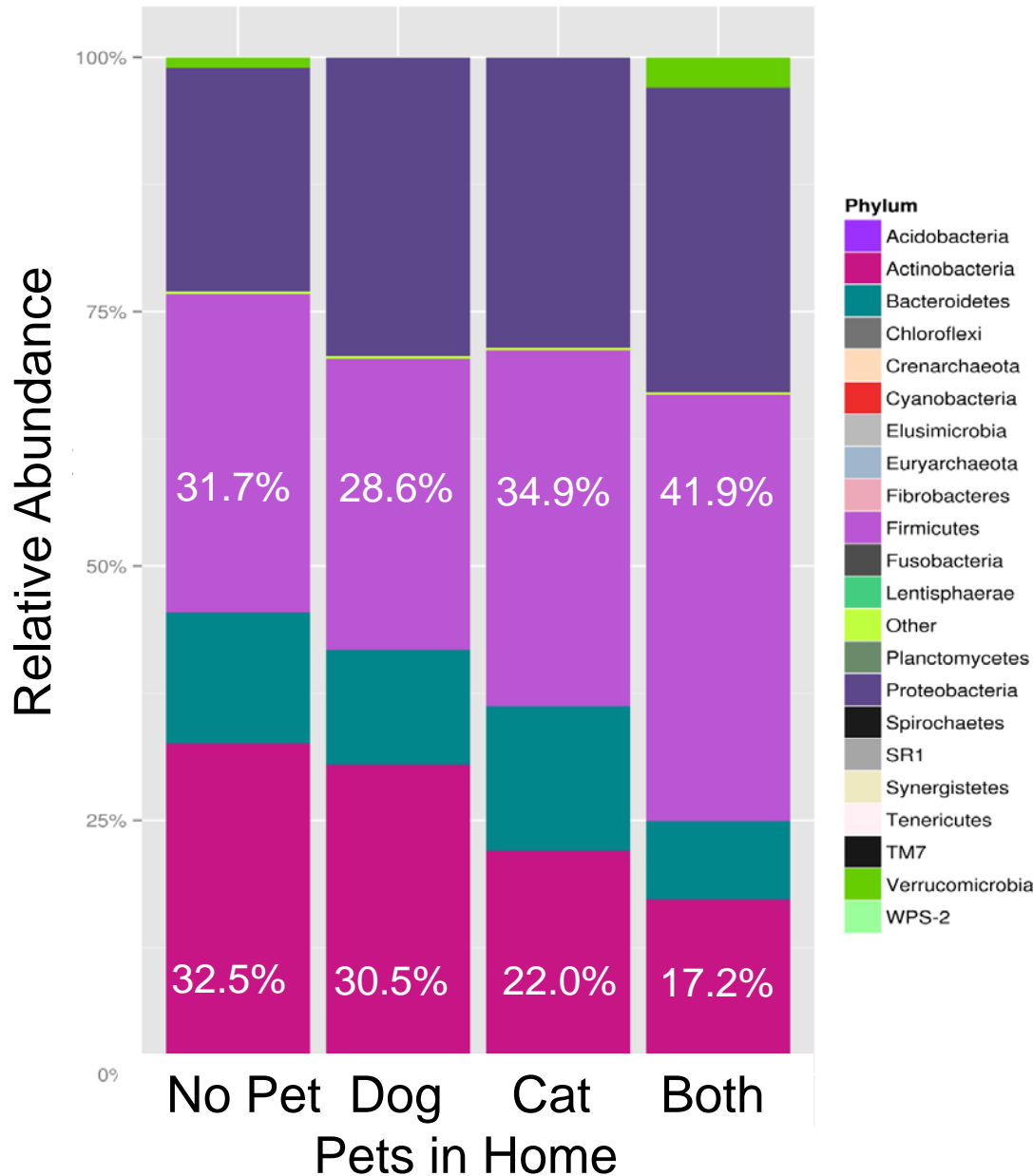
Bacterial Family Relative Abundance by Sample Time in WHEALS Children
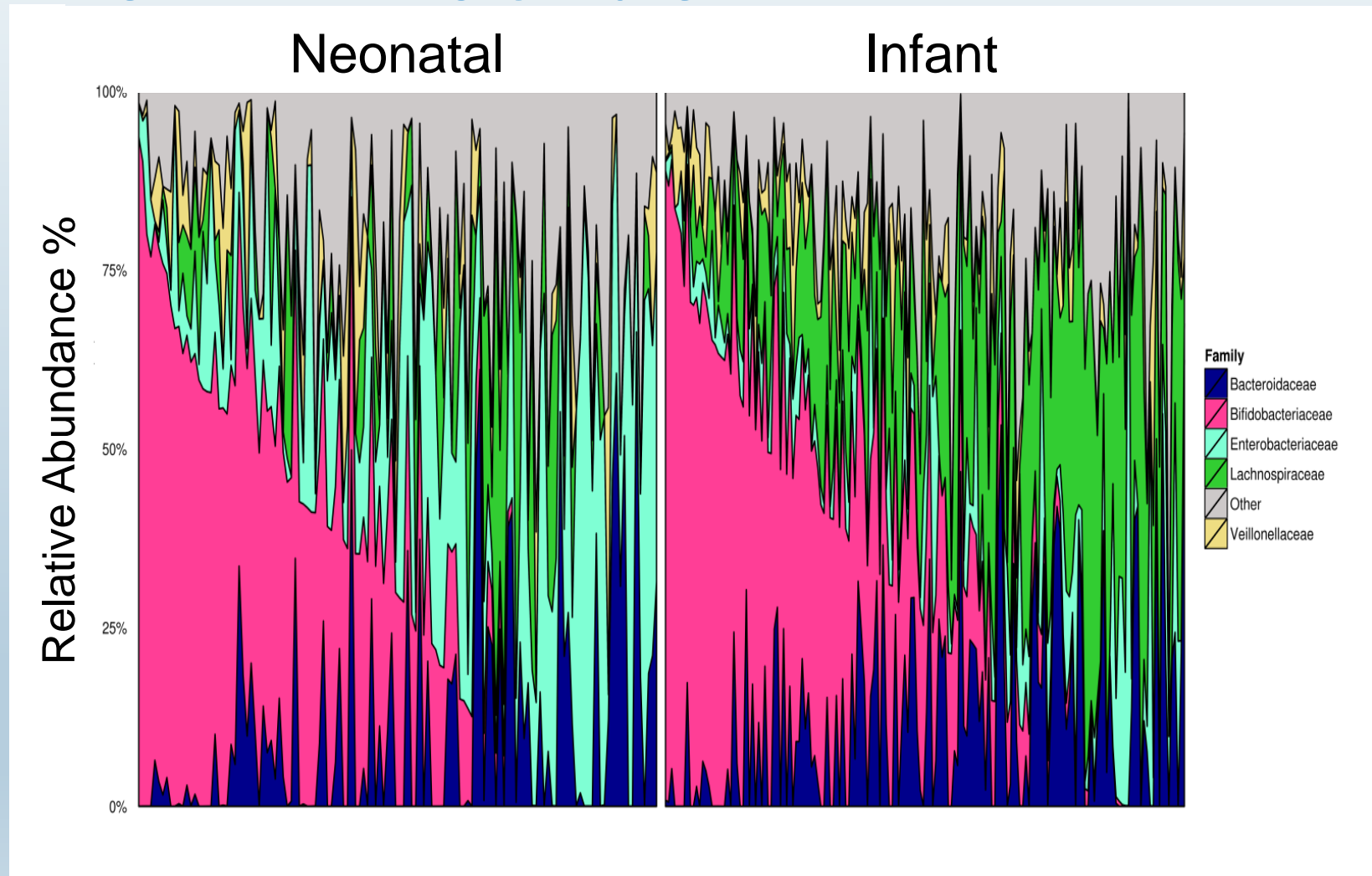
Baby Stool Bacterial Family Compositional Differences: by Mode of Delivery
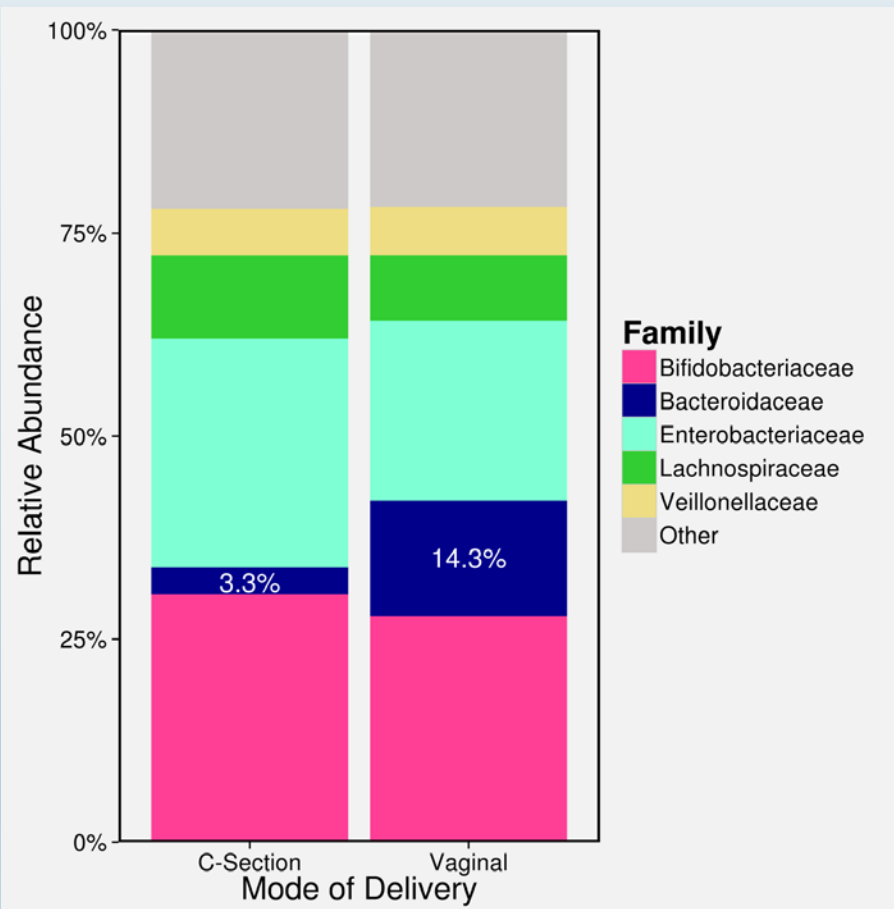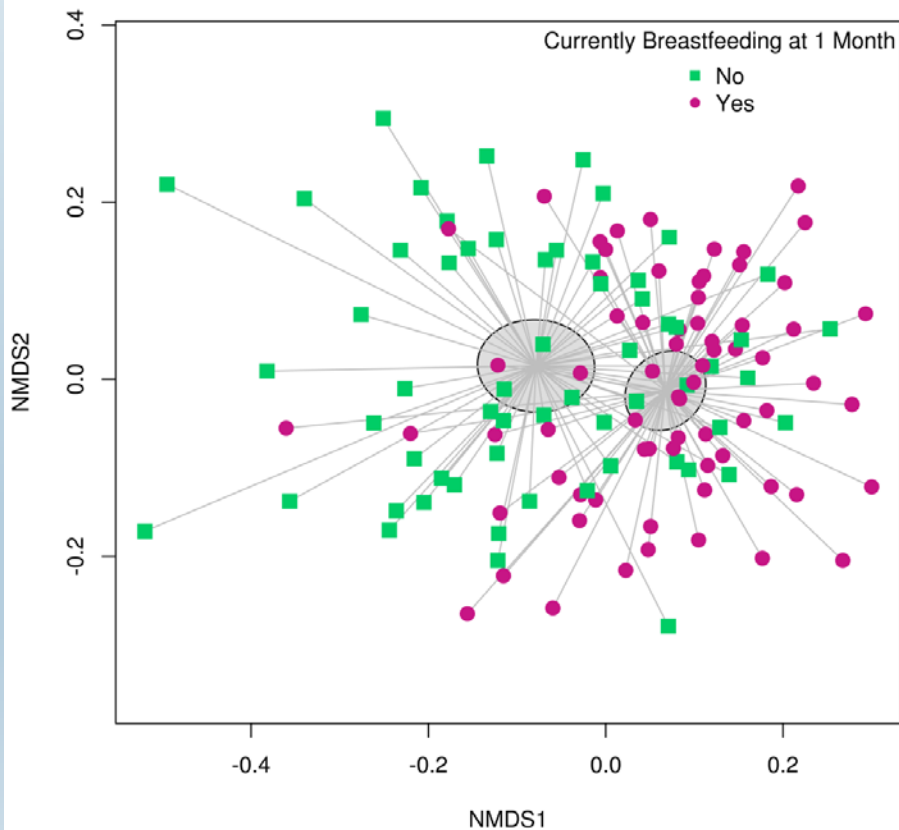

- 1 month stools
- p-value<0.001
- R^2 = 1.9%

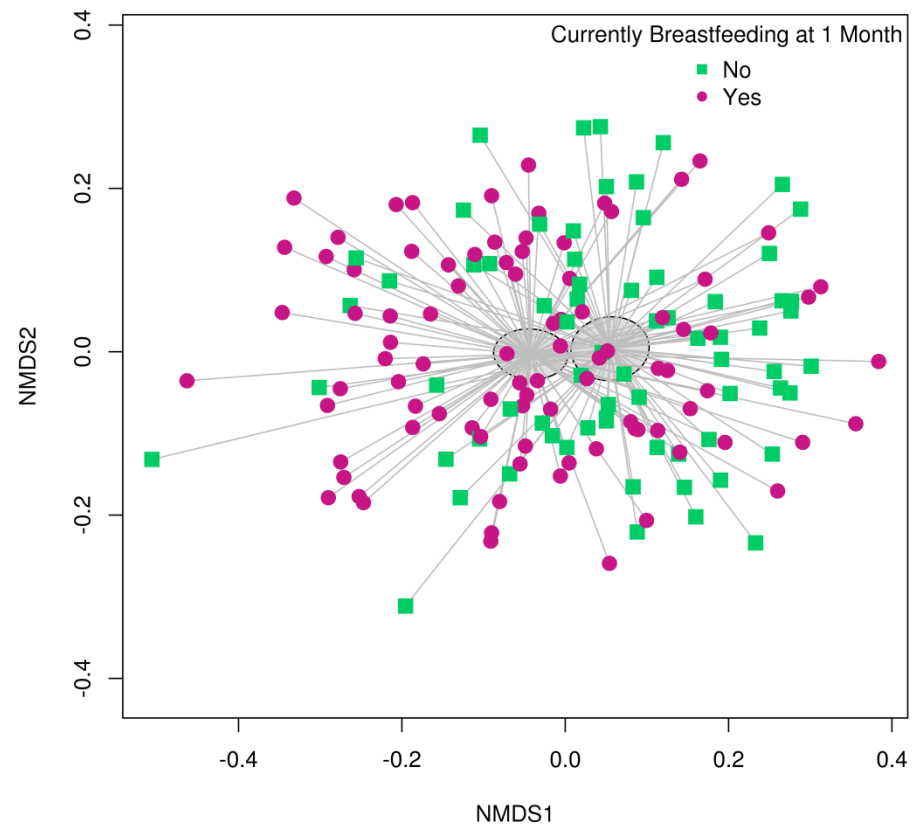Pet-Keeping associated with bacterial Phyla Composition at 1 month visit; p-value=0.026

Bacterial Community Composition Differs by Breastfeeding

Both p-values<0.001
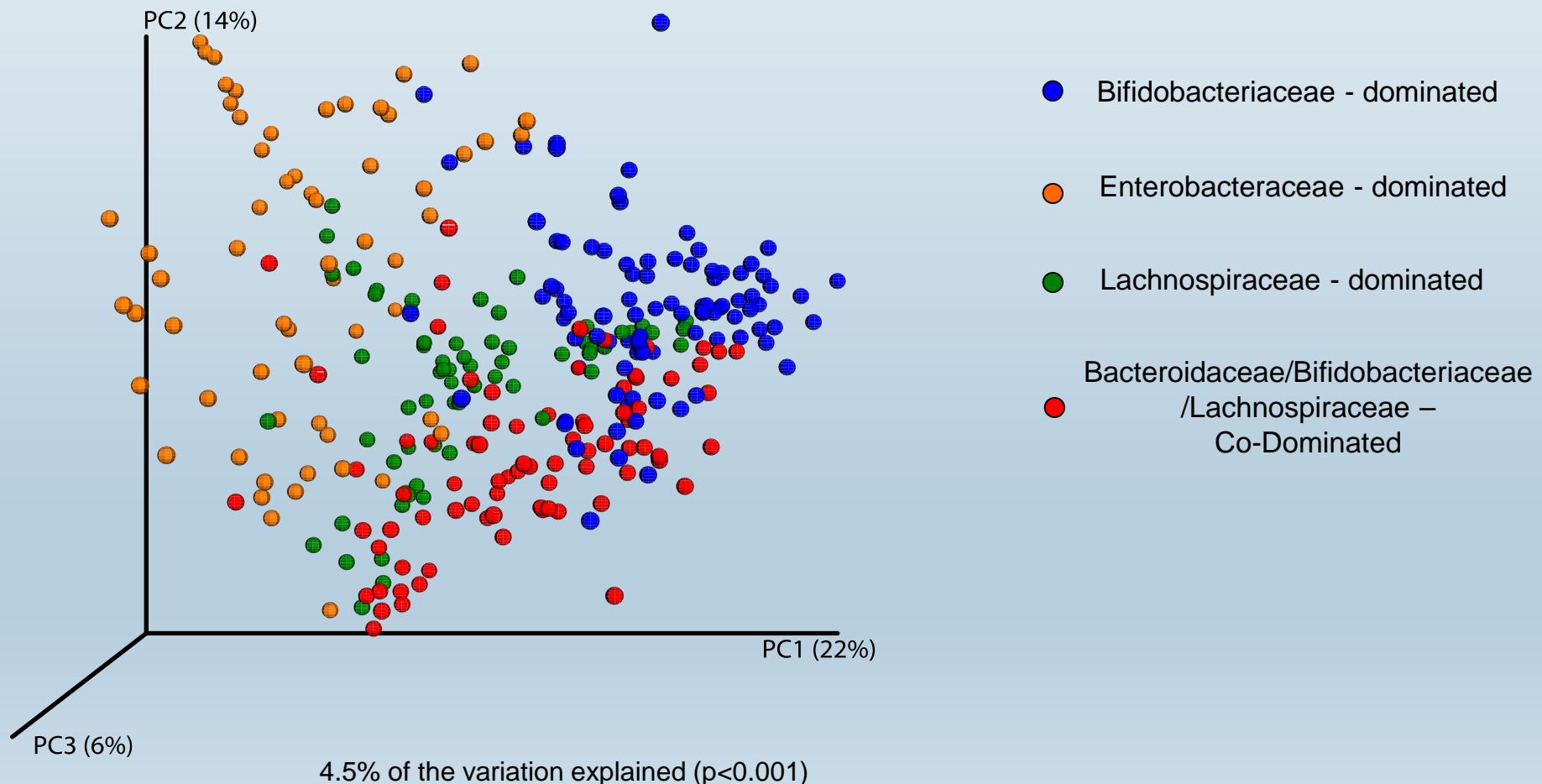

Neonatal Visit

Infant Visit

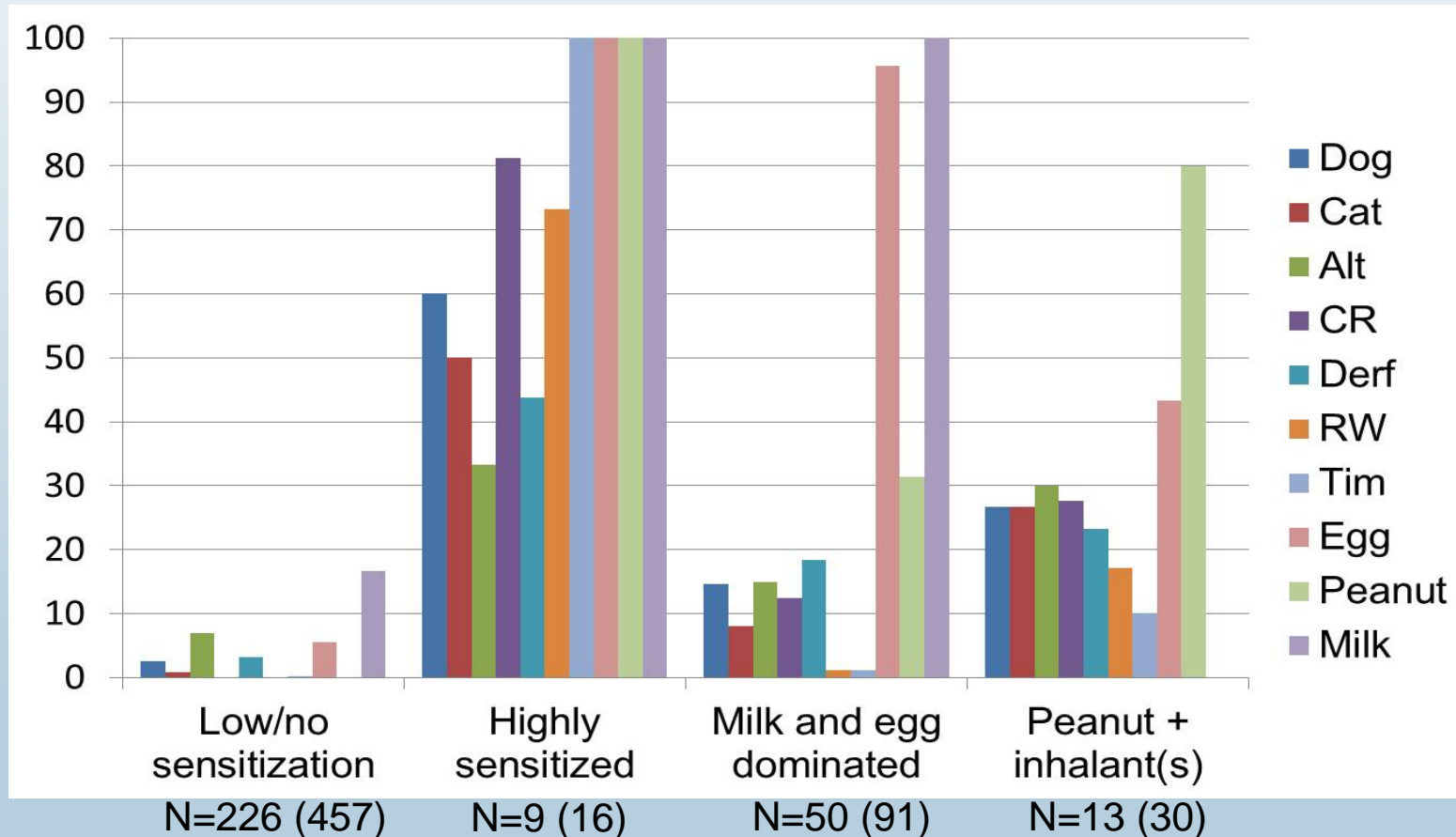# How does the Infant's Microbiome relate to Disease Outcomes?

First Year Gut Microbiome Stratifies into Four Distinct Enterotypes

Dirichlet Mixture Model to statistically define infant sub-populations based on microbiome composition

PC2 (14%)

PC1 (22%)

PC3 (6%)

● Bifidobacteriaceae - dominated

● Enterobacteraceae - dominated

● Lachnospiraceae - dominated

● Bacteroidaceae/Bifidobacteriaceae /Lachnospiraceae – Co-Dominated

4.5% of the variation explained (p<0.001)

Prevalence of Sensitization at 2 yrs (allergen sIgE >0.35) for 10 Allergens within each Latent Class

Havstad et al. 2014

Predominantly Multi-sensitized group (PM group)

Infants with Co-Dominant Neonatal Enterotype had Higher Risk of Developing Multi-Sensitization

	NEONATES Risk Ratio (95% CI)			
Outcome	**E to B**	**C to B**	**C to E**	p-value
Multiple sensitization	1.43 (0.73-2.81)	2.94 (1.42-6.09)	2.06 (1.01-4.19)	0.034

	INFANTS Risk Ratio (95% CI)	
Outcome	**B to L**	p-value
Multiple sensitization	1.02 (0.59-1.75)	0.94

Key

E = Enterobacteriaceae B = Bifidobacteriaceae C = Co-Dominant   L = Lachnospiraceae
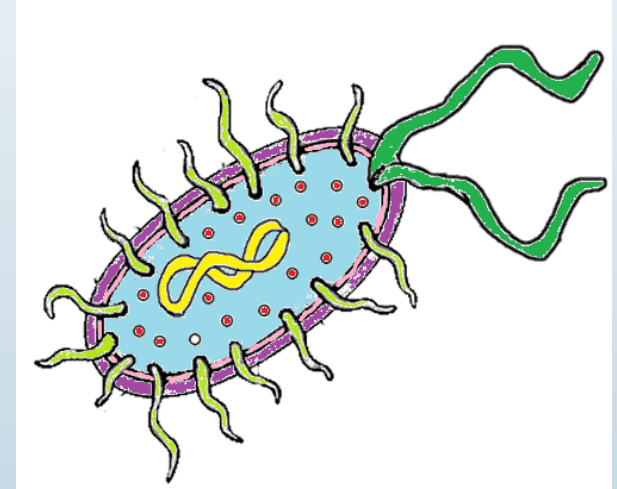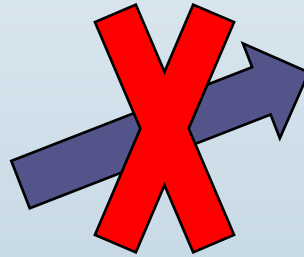
Conclusions & Hypotheses

The development of allergy and asthma is:

- mainly influenced by gut **microbes** to which a child is exposed in the **first year** of life

- the composition of these gut microbes is determined by maternal and environmental factors.

Our Cities:
Microbial Deserts?
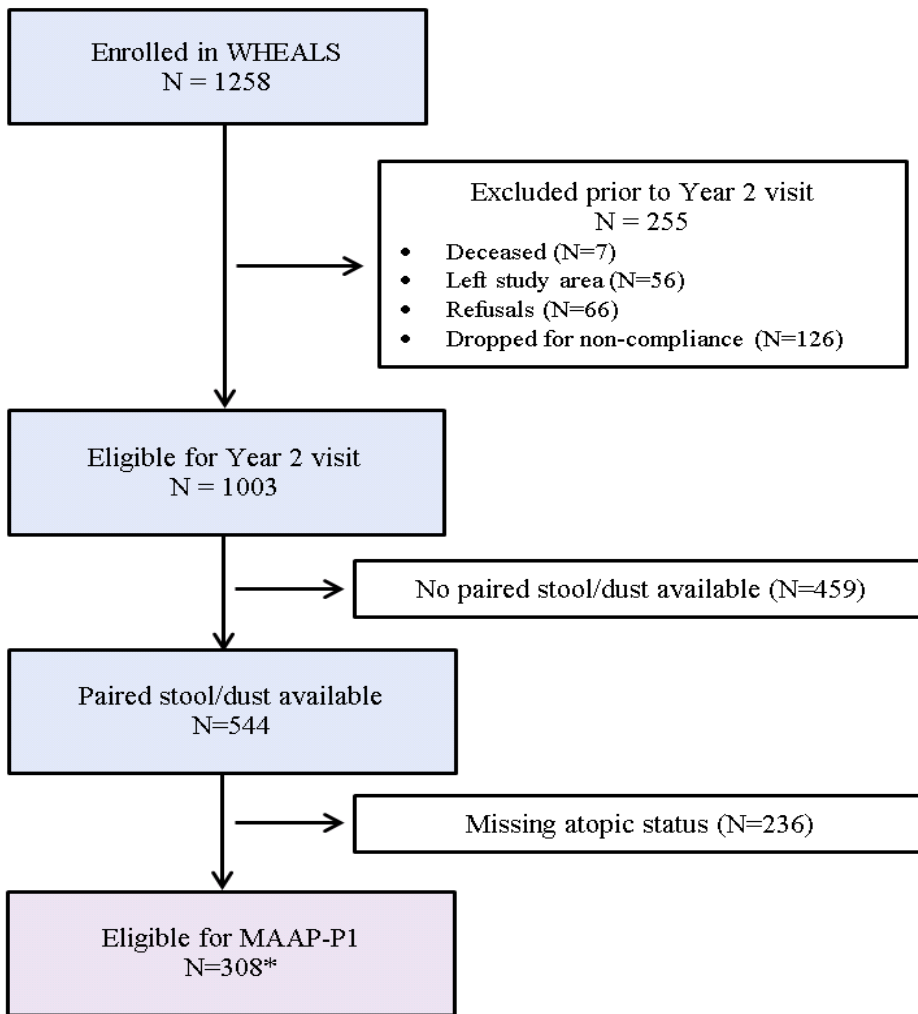
Acknowledgments

Participants

We thank the families and children who have participated in the WHEALS birth cohort and other P01 and HFHS birth cohort studies.
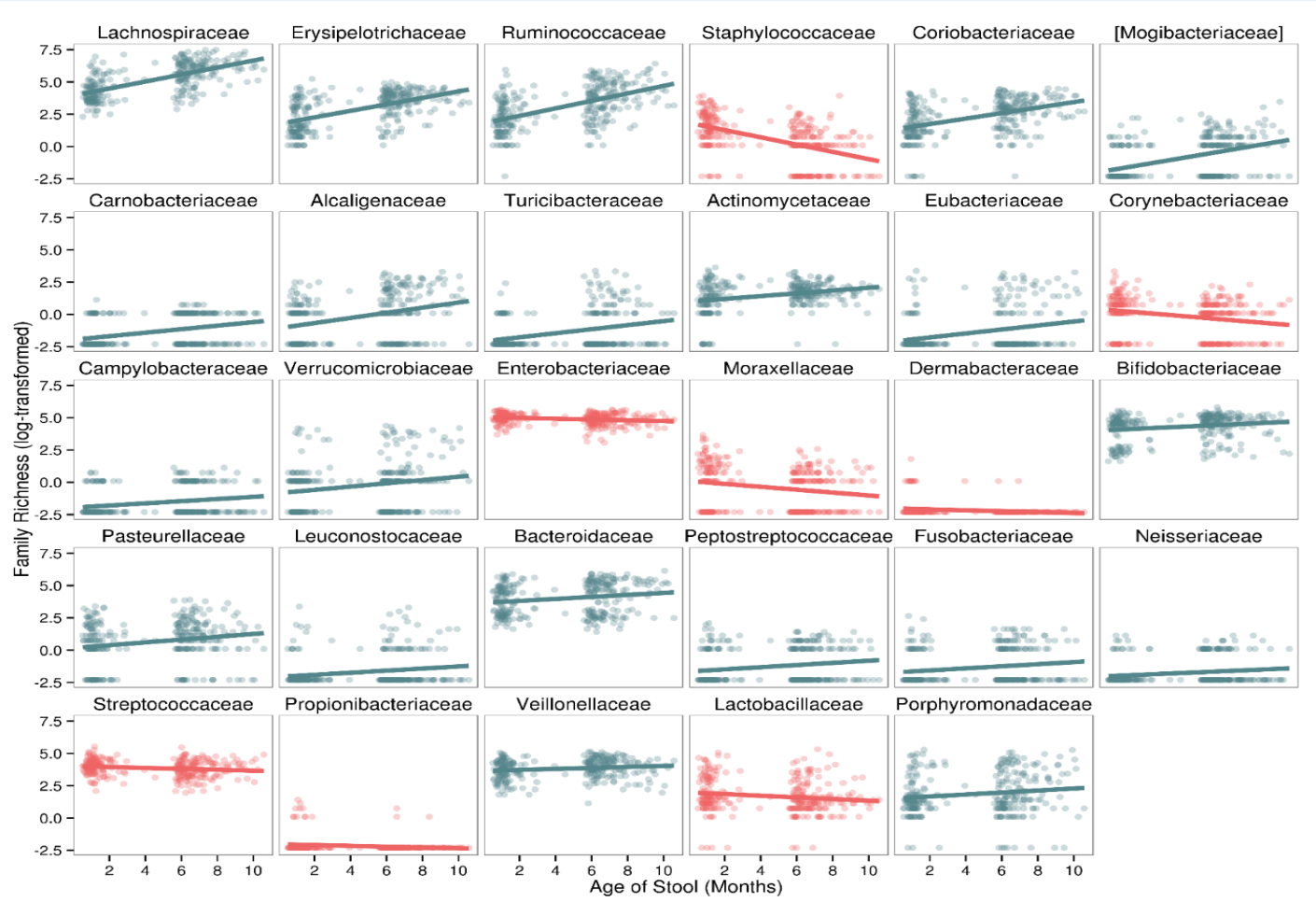
QUESTIONS?

Supplementary Figure 1: MAAP Sample Selection

Figure 2 . Within-family richness by stool sample collection time. Figure displays all families with a significant trend (FDR adjusted p-value<0.05) and is ordered by significance (e.g., Lachnospiraceae is most significant). Color indicates direction of association.

MAAP – Stool Samples

Selection criteria:

- Needed to have 2 year outcome data

- Needed a "paired" dust and stool sample available in our repository for microbiome analyses at either the 1 month or 6 month visit

- Family still in study so eligible for future visits

    N=308 stool/dust pairs sent to Univ California-San Francisco laboratory (Susan Lynch's lab) for processing

Conclusions

- Breastfeeding may protect against colonization of specific Lachnospiraceae bacteria at 1 month of age
  - Associated with increased risk of allergic-like response to pets at age 4
  - Demonstrated significant functional differences that may contribute to differential immune response

- Lachnospiraceae: common adult gut colonizers
  - Newborns (1%) → Infants (10%) → Adults (17%)
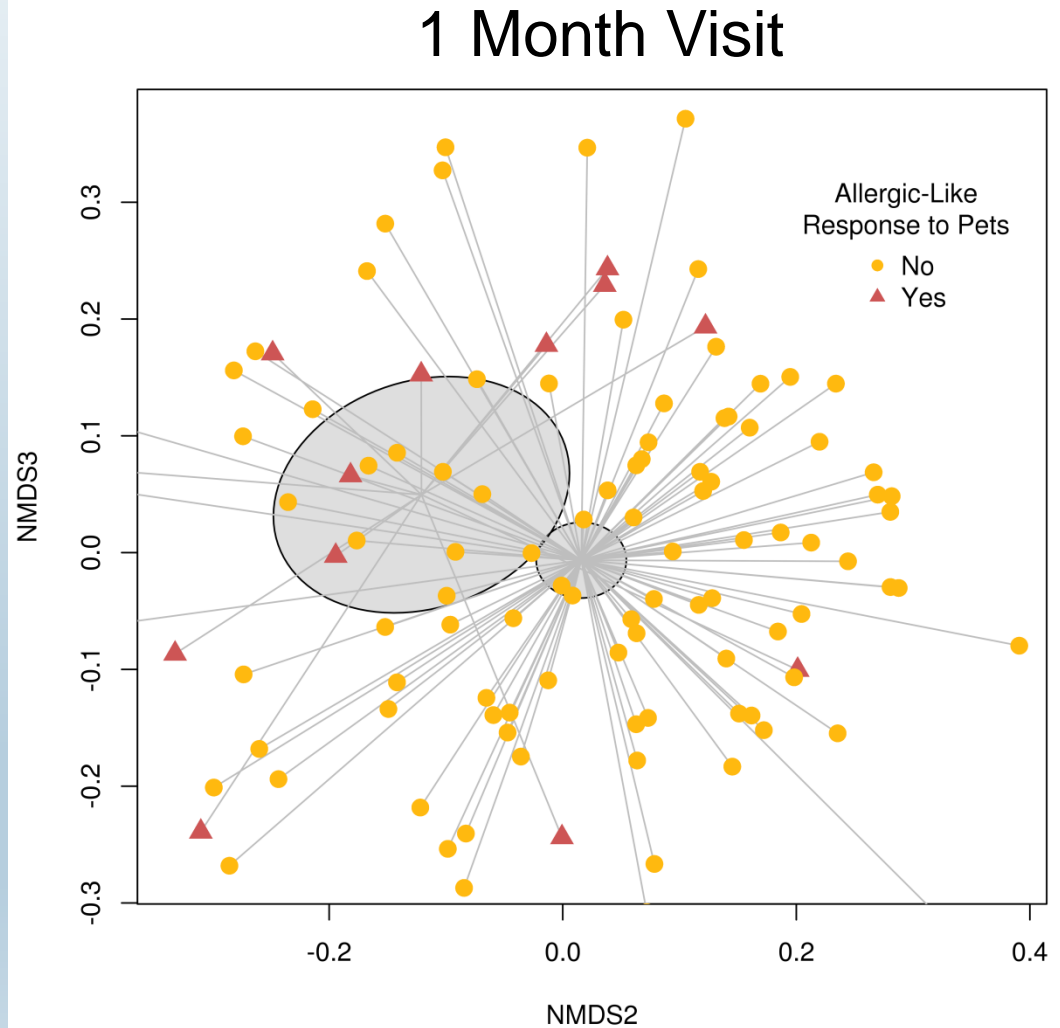 - In terms of gut microbiome, does breastfeeding prevent a premature shift to adulthood?

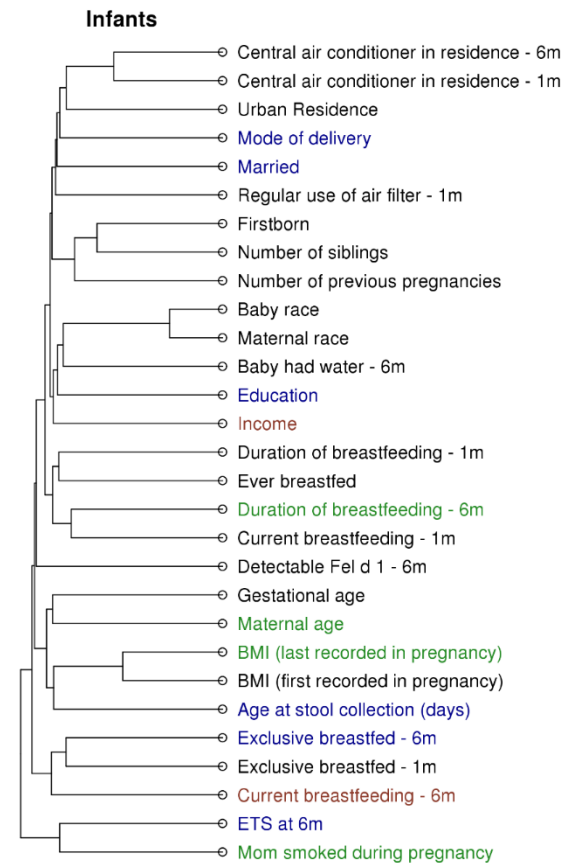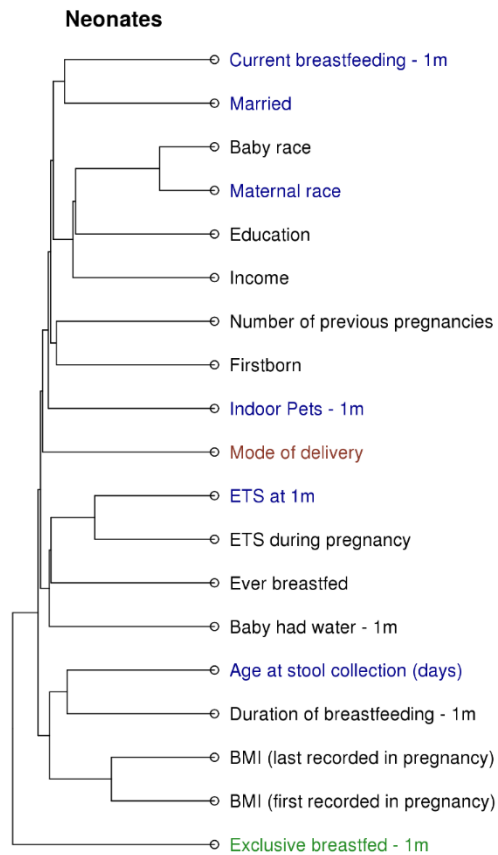# Compositional Differences by Allergic-Like Response to Pets

1 Month Visit:
- p-value = 0.023

6 Month Visit:
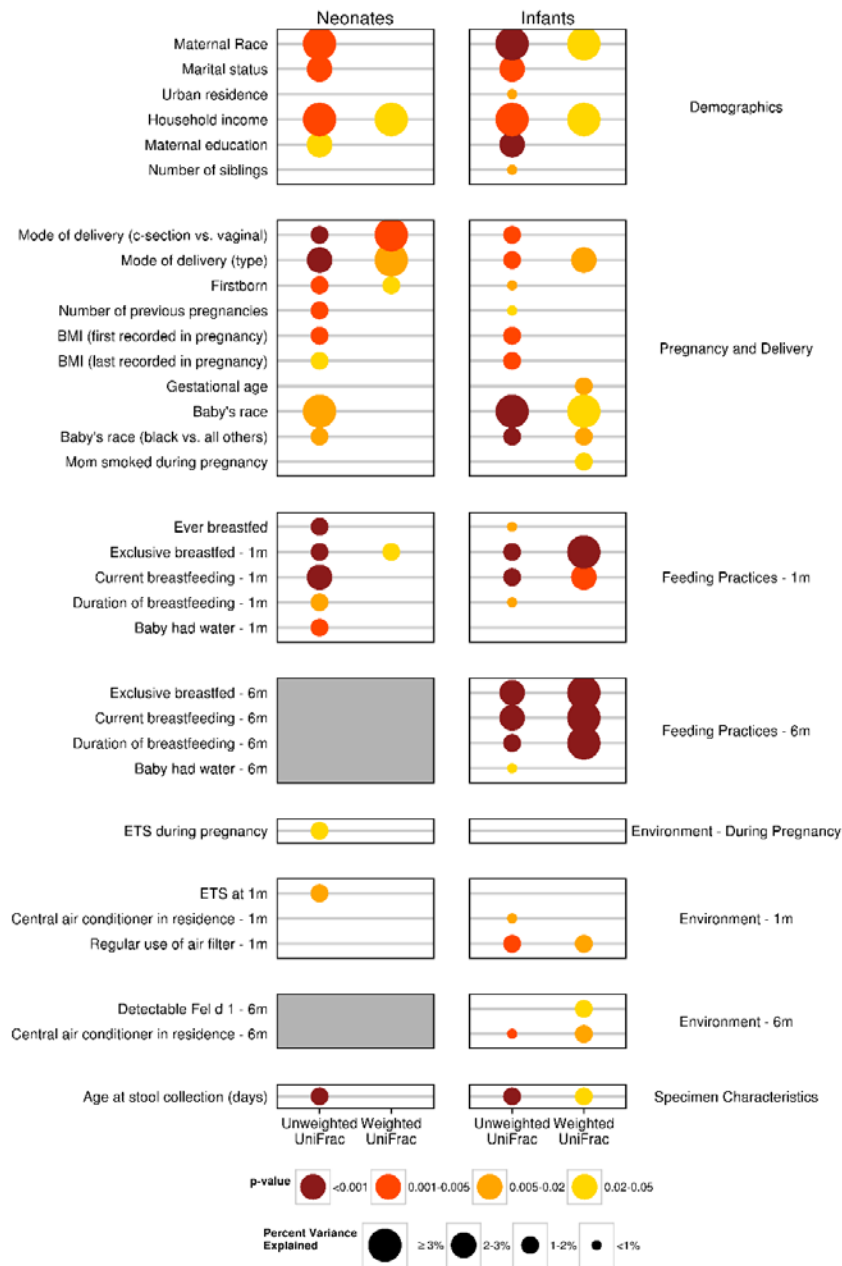- p-value=0.60

1 Month Visit

Clustering of factors univariately or multivariately associated with compositional differences in the neonatal and infant gut microbiome. Uses Unweighted UniFrac to define between-factor dissimilarity. Factors are colored if they were included in a multivariate model: 1.) Blue = retained in unweighted UniFrac model only, 2.) Green = retained in weighted UniFrac model only, and 3.) Red = retained in both models.

Significant Associations between Dust and Stool Mycobiomes in Late Infancy
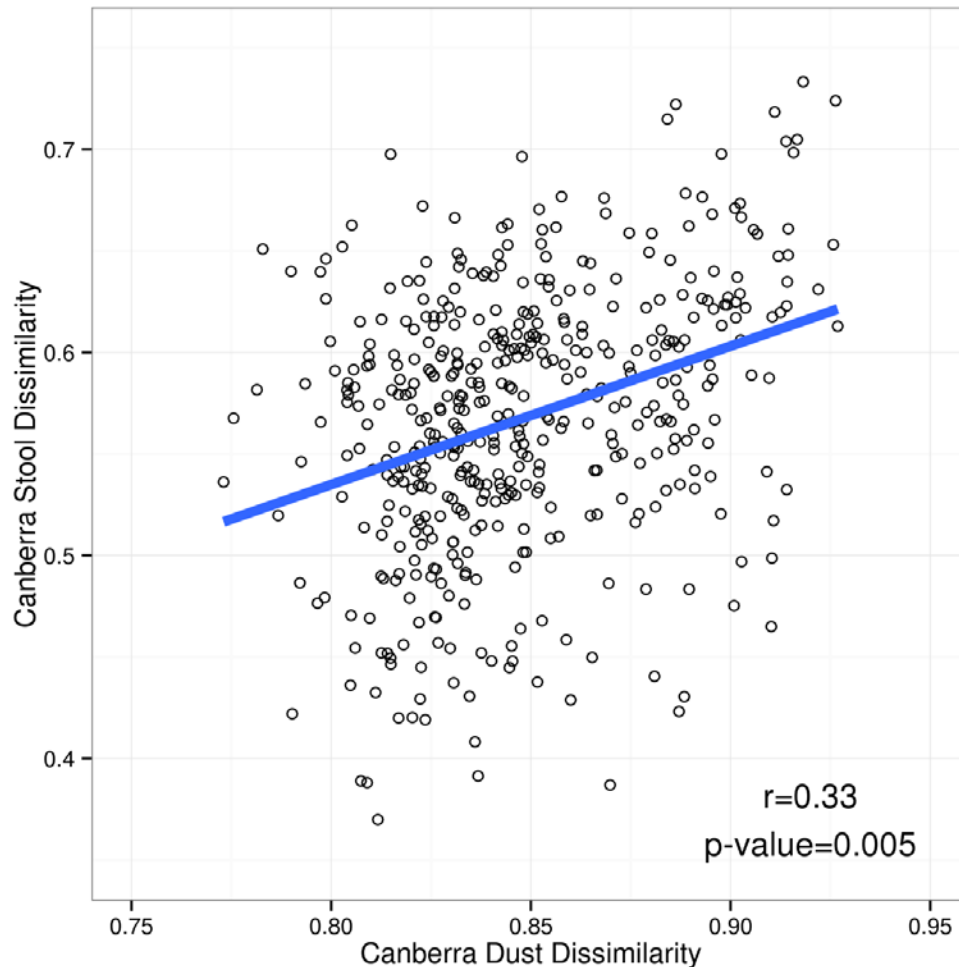
- Mantel test using Canberra dissimilarity measures
- Significant positive association between the fungal dust and fungal stool communities in **infancy** Implies that samples with similar fungal dust microbiome also have similar fungal stool microbiome composition
- **Demonstrates a significant link between household fungal exposure and the infant gut mycobiome**

Univariate gut microbiome compositional analyses.

Only displays factors significantly associated with composition (p value<0.05)

Three Microbe Defined Social Cultural Clusters