Effects of siblings and pets on the developmental trajectory of human infant gut microbiomes, after antibiotic exposure

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Background

The environment shapes our microbiota

The evolutionary link - the old friend hypothesis

Antibiotics influence how the microbiota is shaped - perturbs the natural equilibria

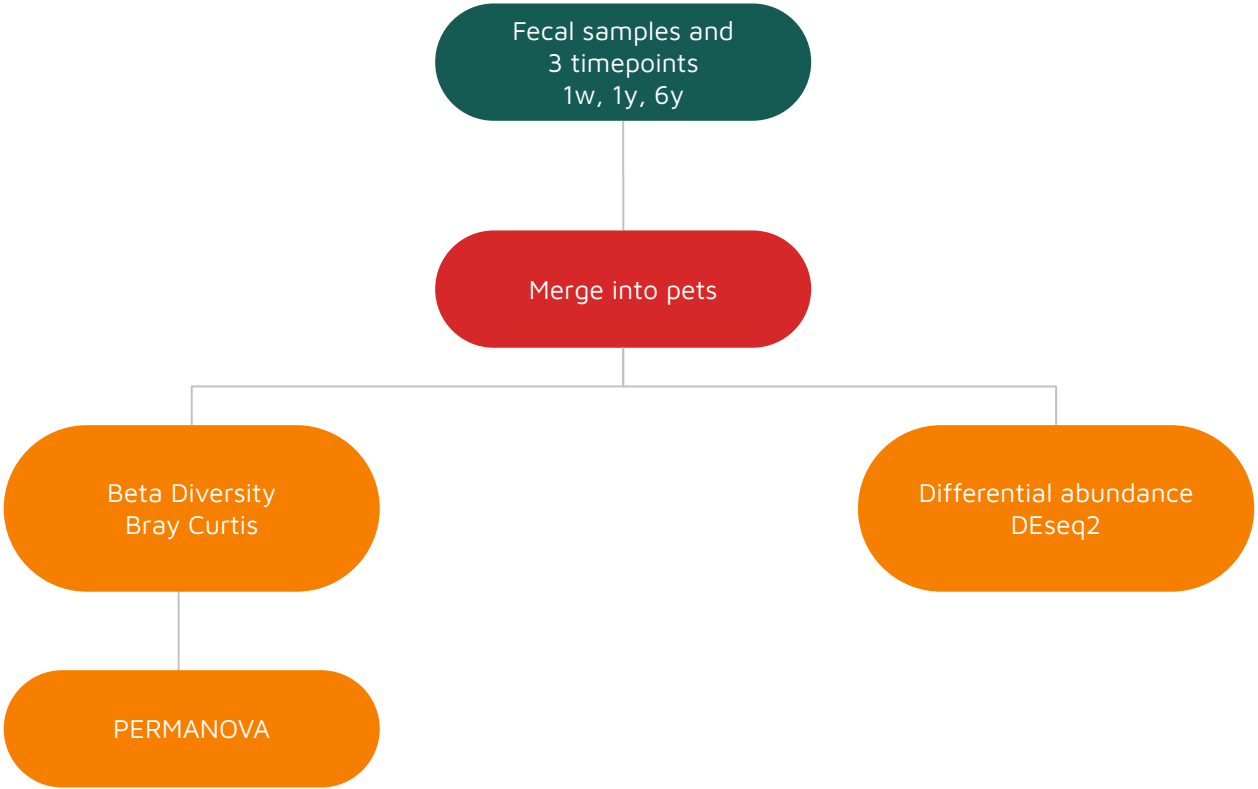
Critical window of opportunity

Pets and siblings introduce infants to microbes.

Hypothesis

Aims to investigate the effect that siblings or pets have on the developmental trajectory of the gut microbiota in a cohort of children, from the Copenhagen Prospective Studies on Asthma in Childhood$_{2010}$ (COPSAC$_{2010}$), who were exposed to antibiotics at birth.

Methods


Fecal samples and
3 timepoints
1w, 1y, 6y
Merge into pets
Beta Diversity
Bray Curtis
Differential abundance
DEseq2
PERMANOVA

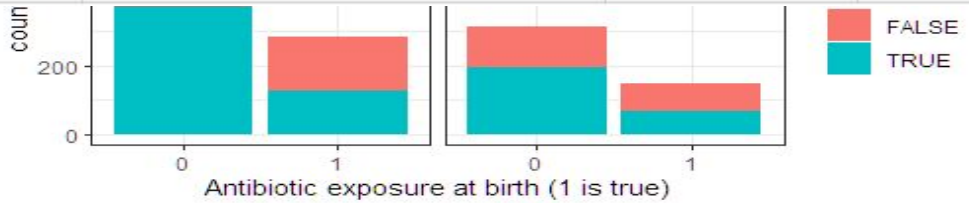
Methods

Samples' group division

A Samples characteristics before merge(Feces)

Cat_birth: 0 | Cat_birth: 1

		Time Period		
	Overall	1 week/No antibotics at birth/ With antibiotics	1 year/No AB/With AB	6 year/No AB/With AB
	1363	683/330/153	560/383/177	320/221/99
Have any pets				
Yes	457	169/115/54	193/131/62	95/64/31
No	906	314/215/99	367/252/115	225/157/68
Have any sibling				
Yes	758	267/197/70	313/231/82	178/136/42
No	605	216/133/83	247/152/95	142/85/57

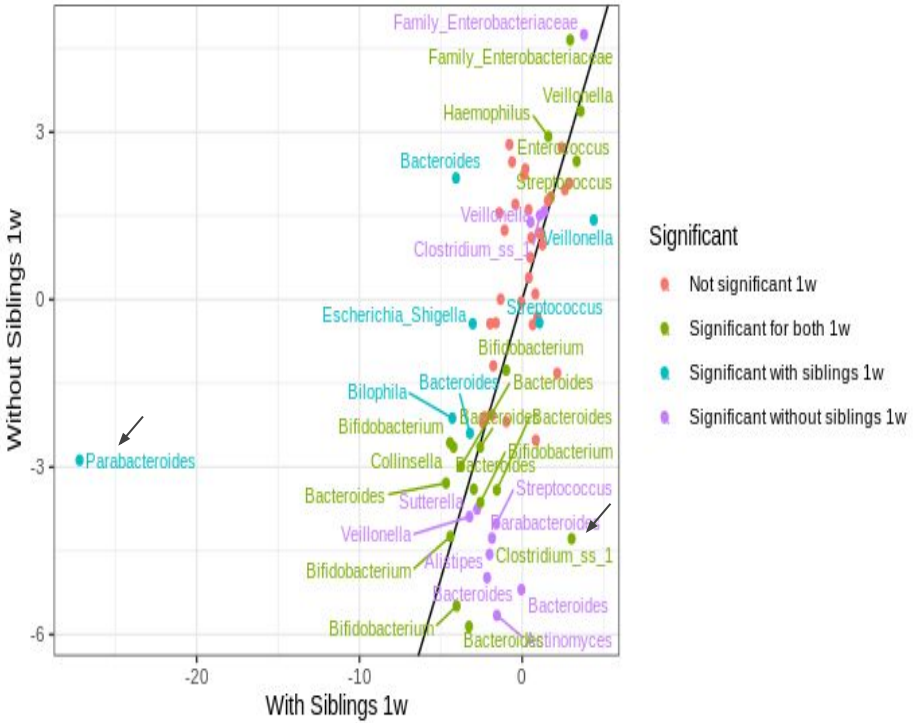


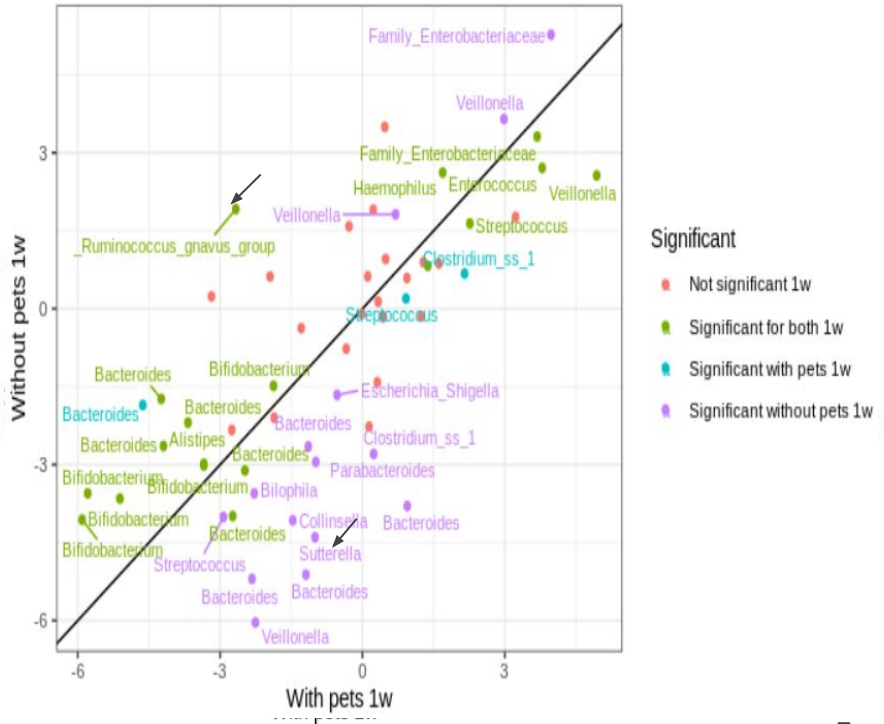
Results

1. PERMANOVA of Bray-Curtis dissimilarity

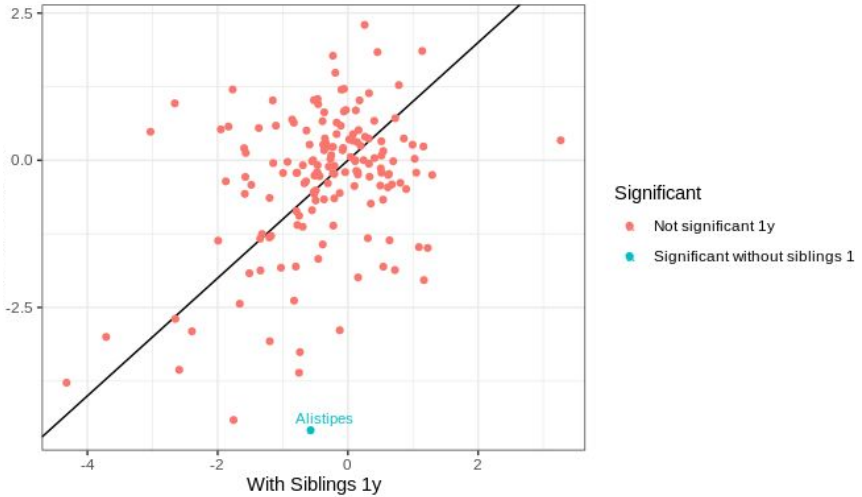
Factor	Time point: Week 1		Year 1		Year 6	
	R^2	P-value	R^2	P-value	R^2	P-value
AB_birth	0.02921	0.001*	0.00397	0.007*	0.00387	0.164
Sibling	0.00635	0.001*	0.00832	0.001*	0.00490	0.025*
Pets	0.00350	0.047*	0.00133	0.801	0.00247	0.812
AB:Sibling	0.00141	0.832	0.00170	0.510	0.00335	0.332
AB:Pets	0.00155	0.715	0.00089	0.984	0.00207	0.958

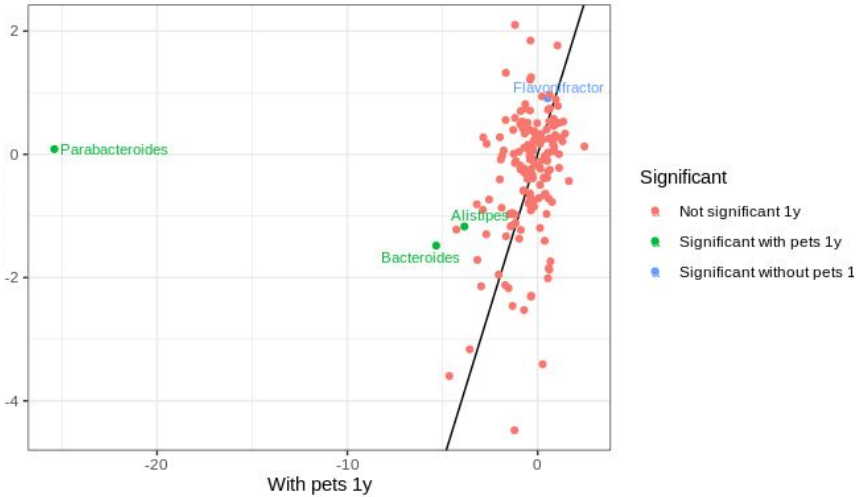
2. Differential abundance Week 1



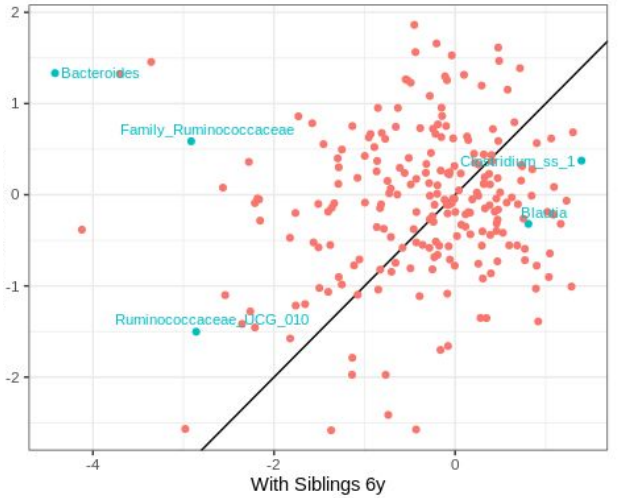


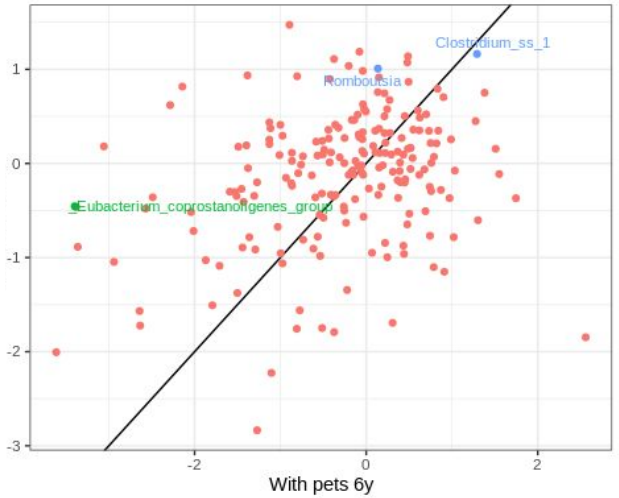
Differential abundance year 1

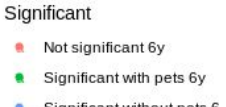




Differential abundance year 6







Discussion

- **Beta diversity:** Factors are independent but have individual effect.
    - The effect of the factors decrease over time. → gut maturation (around two years)
    - Siblings vs pets: Harbour more relevant and closely related bacterial species to export
    - How the pets are kept?
- **Differential abundance**: Few species, with certain well described features, that were significantly different between the groups.
- Week one, large variation in species that are significantly associated with the presence or absence of either pets or siblings + compared groups have a lot of bacteria in common.
- Decrease over time.

Considerations

- Study design
- Subdividing pet/siblings
- Delivery mode
- Optimal if more timepoints

Conclusion

- **Beta diversity:** Antibiotic exposure at birth, showed significant impact on Bray-Curtis dissimilarity on week 1.
- Siblings sustained such effect significantly until year 6.
- Effects of antibiotics and pets fade.
- Need more information to test if the factors have an actual impact.
- **Differential abundance:** Several species or genus could be altered by the existence of siblings or pets; *Ruminococcus gnavus*, *Alistepes*, *Parabacteroides.* Those species are found to have relationships to certain disease or other physiological responses.
- Neither pets, siblings nor the interaction between those variables significantly changed the beta diversity of gut microbiome or last during infants' life (1 week, 1 year, 6 years), but this research revealed that certain species could be affected by those factors in terms of abundance.

Permanova 1w normal delivery and correct pets/sib

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
AB_birth	1	1.271	1.27149	3.4809	0.00894	0.001	***
Sib	1	1.048	1.04804	2.8692	0.00737	0.001	***
Pets	1	0.365	0.36503	0.9993	0.00257	0.426	
AB_birth:Sib	1	0.396	0.39608	1.0843	0.00279	0.328	
AB_birth:Pets	1	0.308	0.30802	0.8432	0.00217	0.619	
Residuals	380	138.805	0.36528		0.97617		
Total	385	142.194			1.00000		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Permanova 1y normal delivery and correct pets/sib

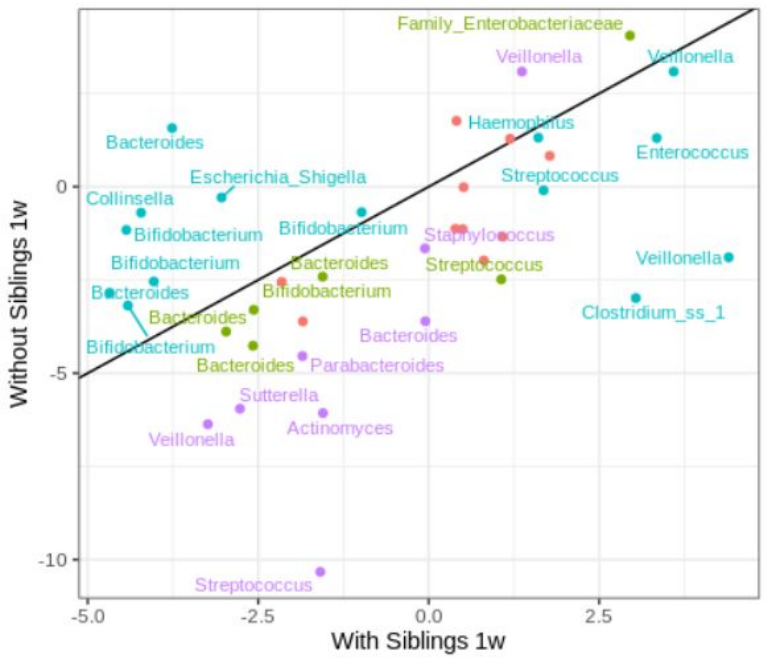
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
AB_birth	1	0.542	0.54189	1.5002	0.00333	0.071	.
Sib	1	1.289	1.28904	3.5686	0.00792	0.001	***
Pets	1	0.434	0.43406	1.2017	0.00267	0.213	
AB_birth:Sib	1	0.468	0.46770	1.2948	0.00287	0.141	
AB_birth:Pets	1	0.374	0.37370	1.0346	0.00230	0.393	
Residuals	442	159.660	0.36122		0.98092		
Total	447	162.766			1.00000		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Permanova 6y normal delivery and correct pets/sib

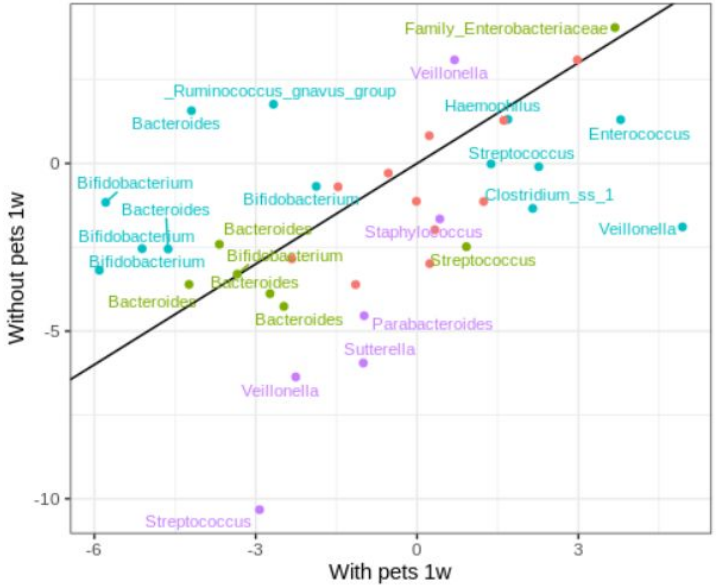
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
AB_birth	1	0.300	0.30021	1.06669	0.00415	0.320	
Sib	1	0.397	0.39705	1.41078	0.00548	0.063	.
Pets	1	0.192	0.19247	0.68387	0.00266	0.946	
AB_birth:Sib	1	0.294	0.29391	1.04430	0.00406	0.372	
AB_birth:Pets	1	0.288	0.28759	1.02185	0.00397	0.424	
Residuals	252	70.923	0.28144		0.97968		
Total	257	72.394			1.00000		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



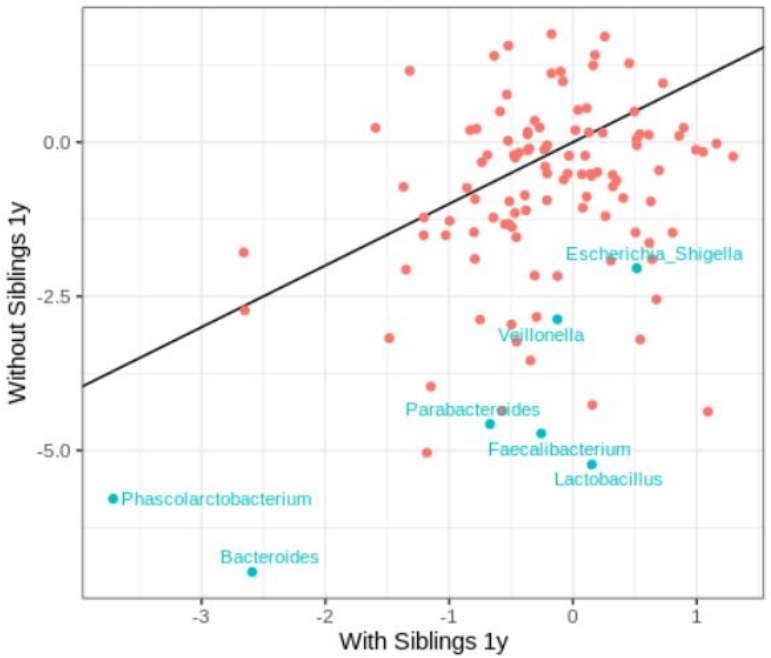
Significant

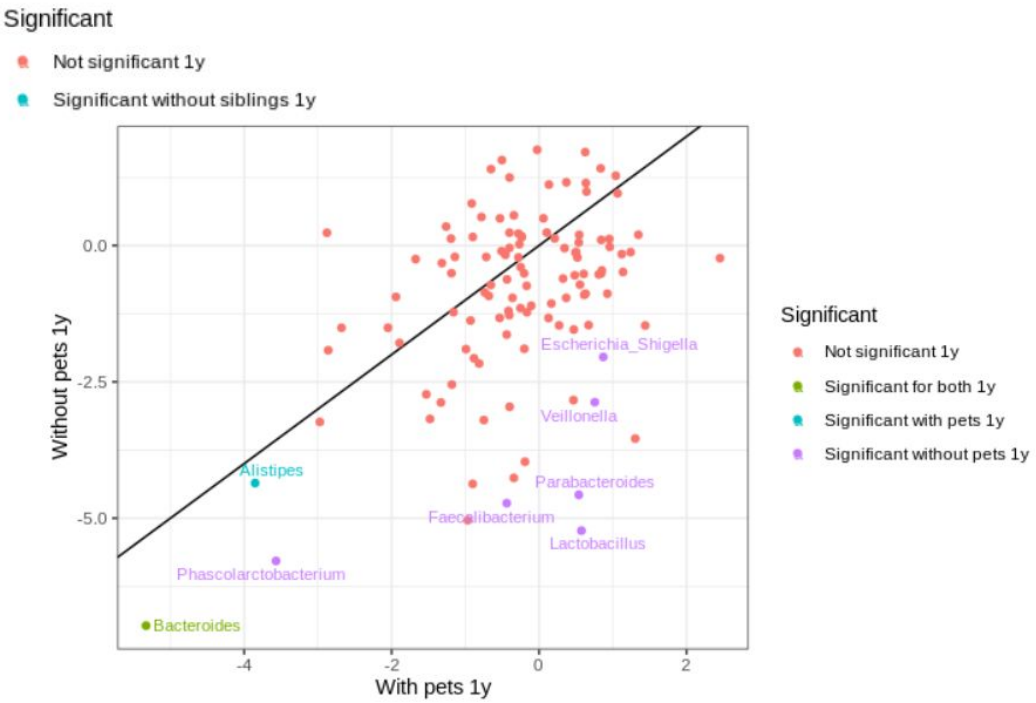
- Not significant 1w
- Significant for both 1w
- Significant with siblings 1w
- Significant without siblings 1w

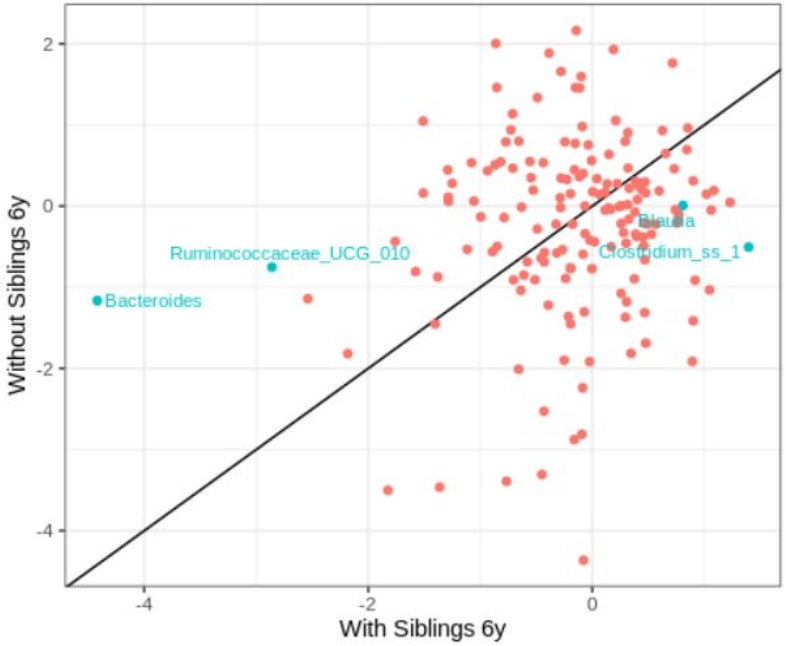


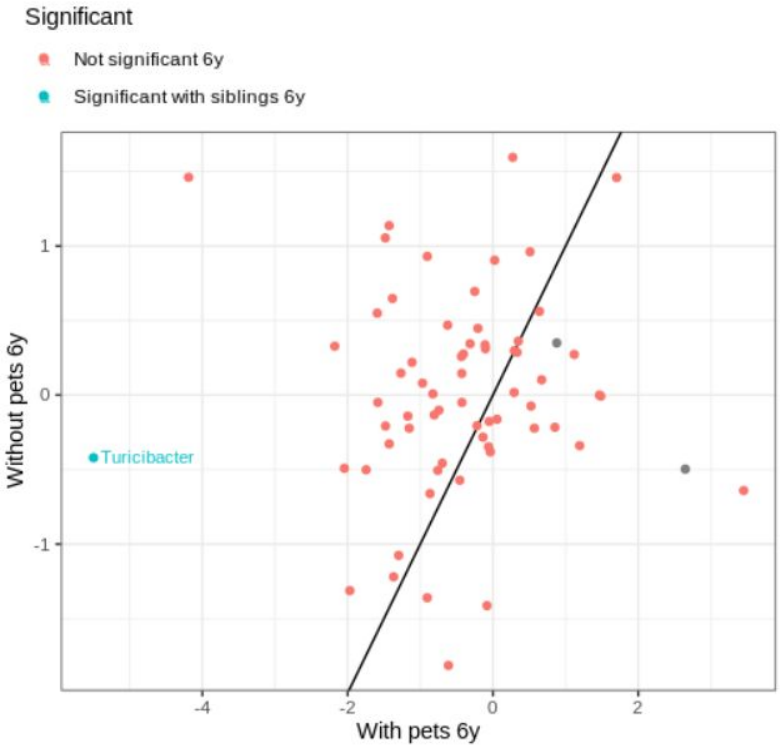
Significant

- Not significant 1w
- Significant for both 1w
- Significant with pets 1w
- Significant without pets 1w


Without Siblings 1y
With Siblings 1y
0.0
-2.5
-5.0
-3
-2
-1
0
1
Escherichia_Shigella
Veillonella
Parabacteroides
Faecalibacterium
Lactobacillus
Phascolarctobacterium
Bacteroides


Significant
Not significant 1y
Significant without siblings 1y
Without pets 1y
With pets 1y
0.0
-2.5
-5.0
-4
-2
0
2
Escherichia_Shigella
Veillonella
Alistipes
Parabacteroides
Faecalibacterium
Lactobacillus
Phascolarctobacterium
Bacteroides
Significant
Not significant 1y
Significant for both 1y
Significant with pets 1y
Significant without pets 1y


Without Siblings 6y
With Siblings 6y
2
0
-2
-4
-4
-2
0
Ruminococcaceae_UCG_010
Bacteroides
Blautia
Clostridium_ss_1


Significant
Not significant 6y
Significant with siblings 6y
Without pets 6y
With pets 6y
1
0
-1
-4
-2
0
2
Turicibacter
Significant
Not significant 6y
Significant with pets 6y
NA

Highlights

- ↓***Parabacteroides*** week with 1 sibling. Increased abundance linked to eczema
- ↑***Clostridium_ss_1*** + sibling, ↓ - sib.
    - Mediate the accumulation of Treg cells in mouse models, alleviating symptoms of allergies and Inflamed Bowel Disease (IBD)
    - Low levels of Clostridium correlated with the persistent atopic dermatitis
- ↓ ***Ruminococcus gnavus*** + pets, ↑ - pets: High abundance *R. gnavus* at an early age, correlated with the risk of developing respiratory allergies
- ↓ ***Sutterella*** - pets: The presence of sutterella can increase risk of developing environmental enteric dysfunction (EED).
- ↓ ***Alistepes*** + pets/sibling year 1. Significant for both sibling and pets: Lower abundance of Alistipes at 1 year samples were associated with development of asthma age 5, if the children were born to asthmatic mothers

Shaping of the gut microbiota

Which factors (environment, siblings, animals etc) have the largest influence on the shaping of the microbiome. / what bacteria do we lack and does it correlate with the results we see in the old friend hypothesis.

do we see an effect on the sibling … ASK if we have data on the sibling.

Data from feces and from the lungs.

The variants or the evenness… Shannon for evenness.

How to separate the factors and we have to have a big cohort.

Check pcoa plot on environment and animals → how much does it vary from a “normal” microbiota.

environment include the animals and the other stuff to see how much of a difference it makes.

what bacteria are good for things. people in rural areas vs city has other bacteria??

Atopic bacteria due to lack of bacteria.. too clean!!! pathogens can stimulate immune system so you get less

Birth season

How does the season shape the microbiome?

(pollen and other allergens → people less allergic, when are they born.)

inside/winter and animals, does it do something.

file:///C:/Users/MAHEM/Downloads/children-07-00045-v2%20(3).pdf

Summer vitamin D. Does that have an effect on your microbiota.

siblings vs pets

Is there a difference between siblings animals after exposure to antibiotics. Also check born in different environments.

Do the long evolutionary relationship, and yet larger variance in microbiome in dogs and cats, make them more ideal for recovery of ab-altered microbiomes in human infants, relative to their siblings?

- What is a healthy microbiome for children?
- How do they recover after treatment of antibiotics. With and without pets/siblings.
-

Background

The environment shapes our microbiota

The evolutionary link - the old friend hypothesis

Antibiotics influence how the microbiota is shaped - perturbs the natural equilibria

Critical window of opportunity

Pets and siblings introduce infants to microbes.

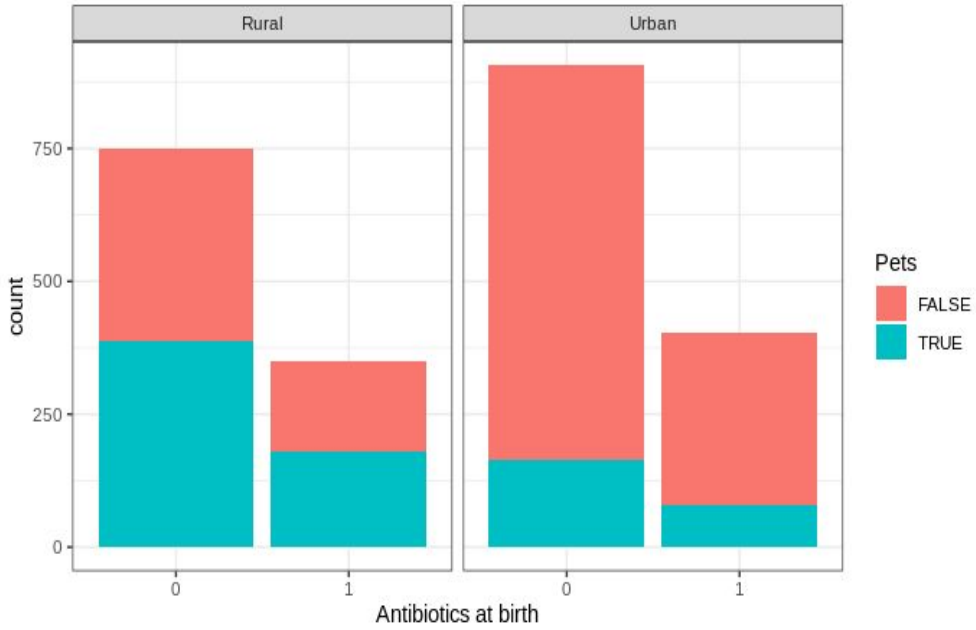
Hypothesis

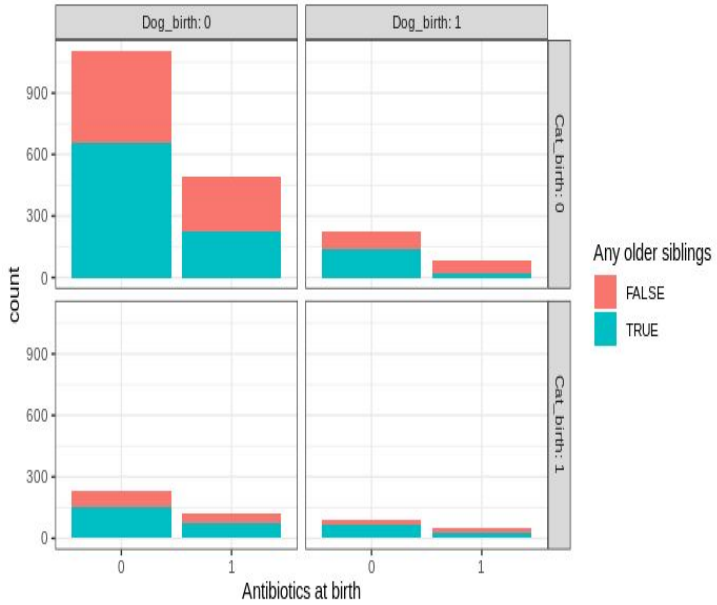
Are pets (dog and cats) associated with re-establishment of a “healthy” microbiome in children who have been exposed to antibiotics at birth? - if so, is the effect of having pets stronger compared to having siblings (hygiene regimen, evolutionary relationship and microbiome diversity)?

Process

How are we going to do it?

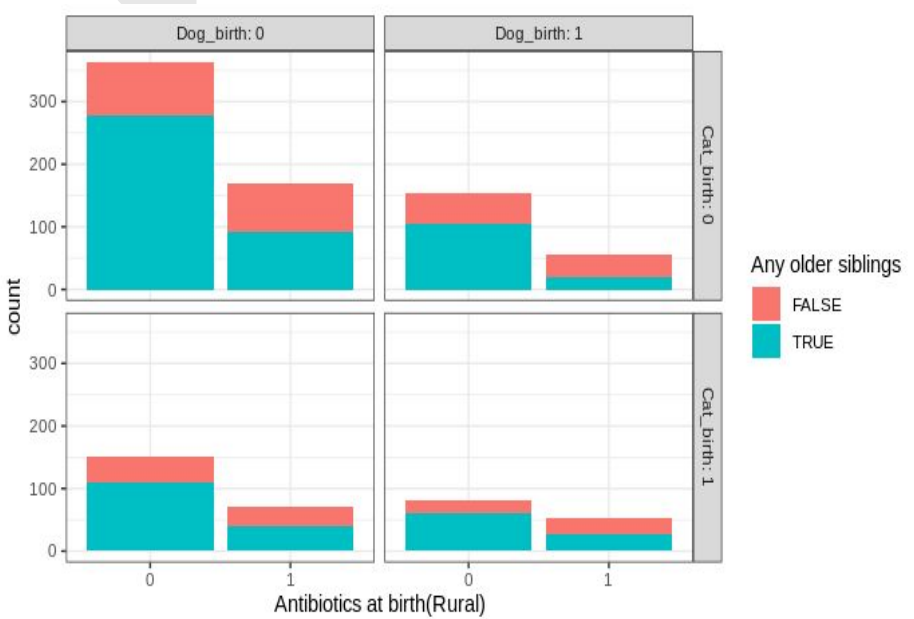
- Is there a significant taxonomic difference between rural and urban areas in our “0 pets / siblings” control group?
- If yes: do the analysis based on each environment (rural/urban)
- If no: concatenate data from each environment
- Analyse the temporal development of the microbiota (phylogeny) in the cohort who received antibiotics and had pets/no pets, and compare it to the control group.
- Figure out which parameter (sibling, dog or cat) have the greatest effect in the re-establishment of a normal microbiota.

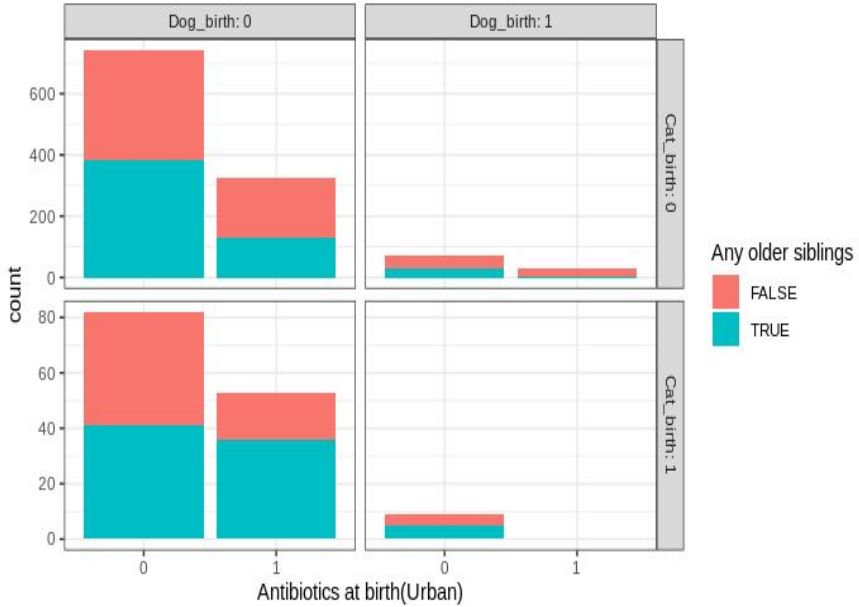


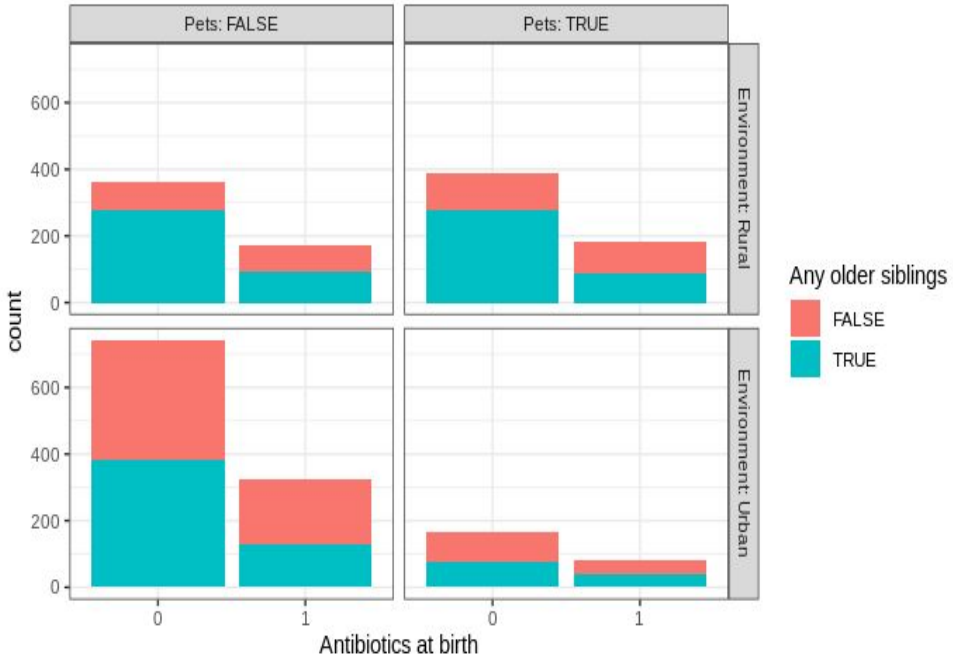


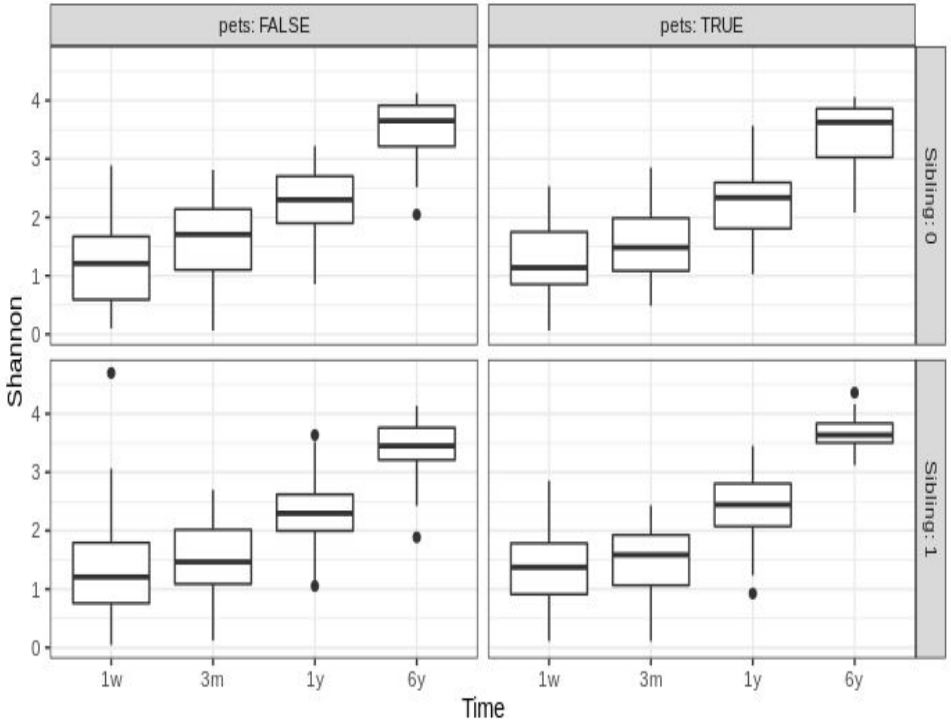
#1 Do we see a significant phylogenetic difference in the two environments

Merge data for cats and dogs

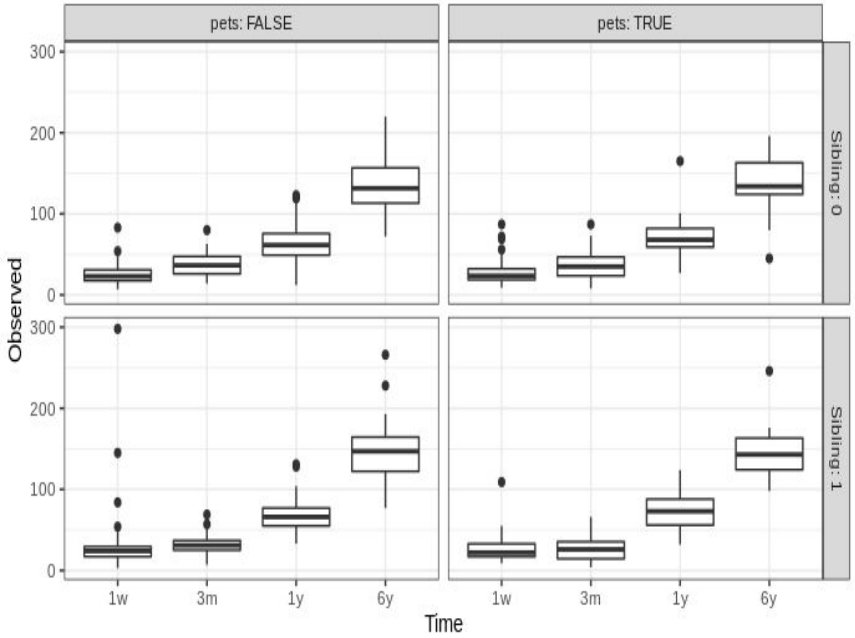




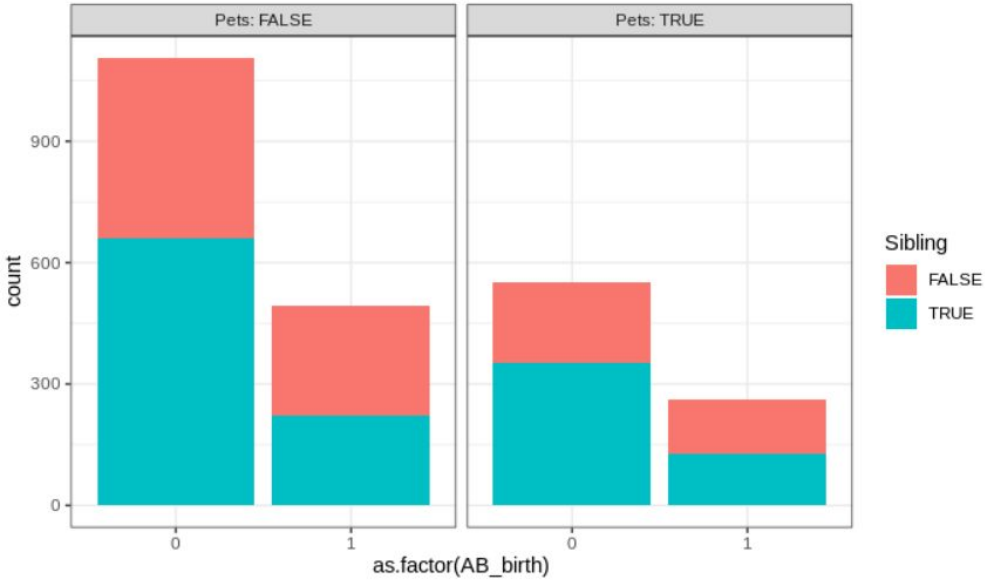

Pets: FALSE
Pets: TRUE
Environment: Rural
Environment: Urban
count
600
400
200
0
0
1
Antibiotics at birth
Any older siblings
FALSE
TRUE

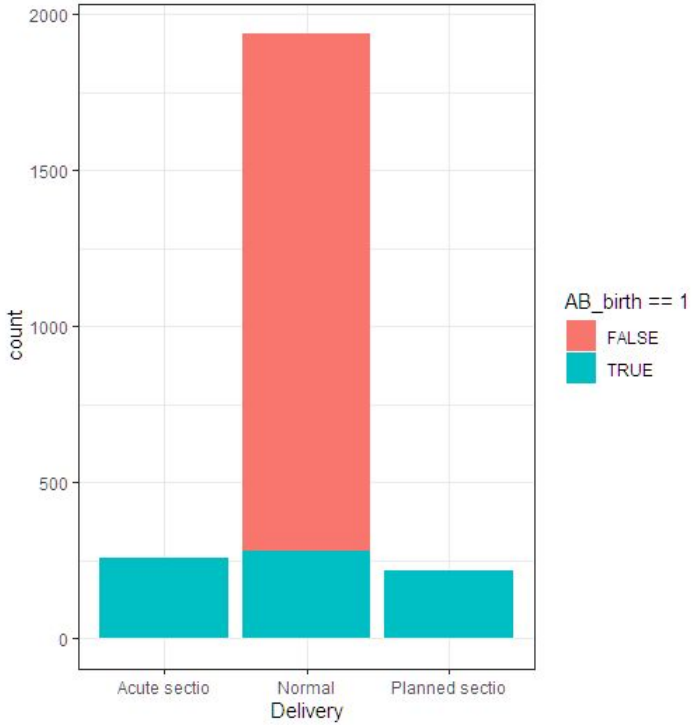


shannon alpha for antibiotic-treated only



observed alpha for antibiotic-treated only





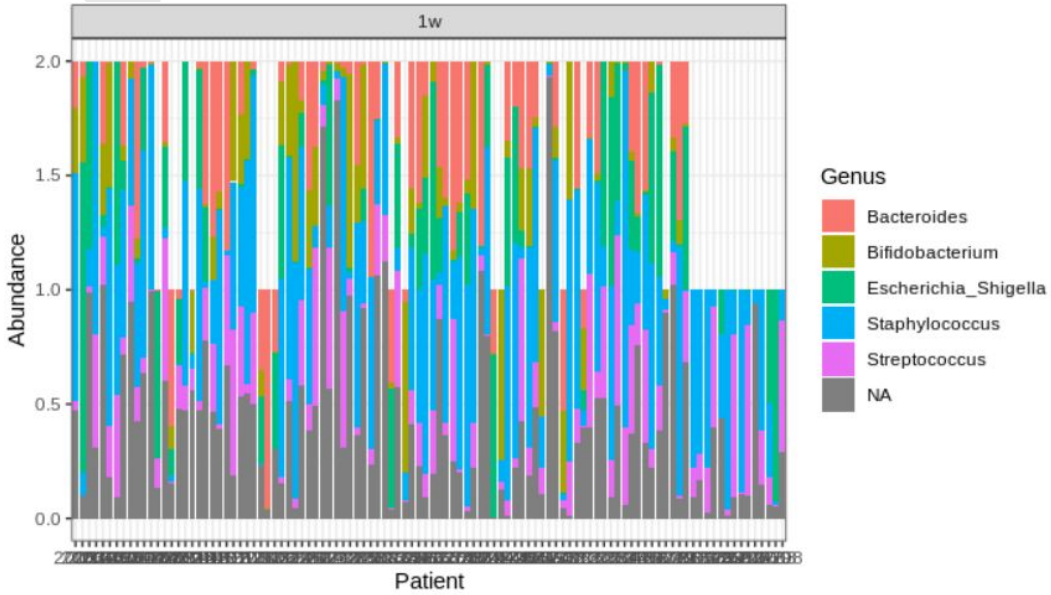
-pet/-sib/0ab = control group
-pet/+sib/0ab
-pet/-sib/1ab = ab control group
-pet/+sib/1ab = Does siblings matter?

+pet/-sib/0ab
+pet/+sib/0ab
+pet/-sib/1ab = does pets matter?
+pet/+sib/1ab = combined effect of pets/sib

Temporal difference in microbiota (control vs ab control)

Extract taxonomic data from each group - make beta diversity analysis. At which point do we stop seeing a significant difference in the two control groups?

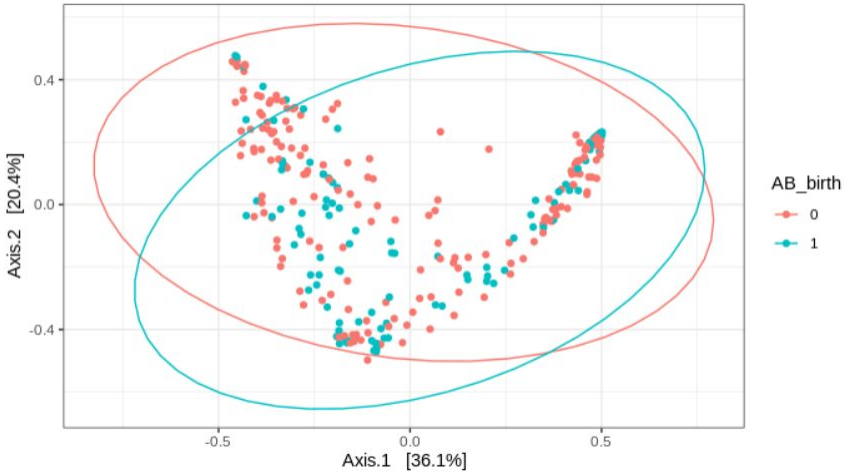
relative abundance -antibiotic -sib



Week 1 - difference?

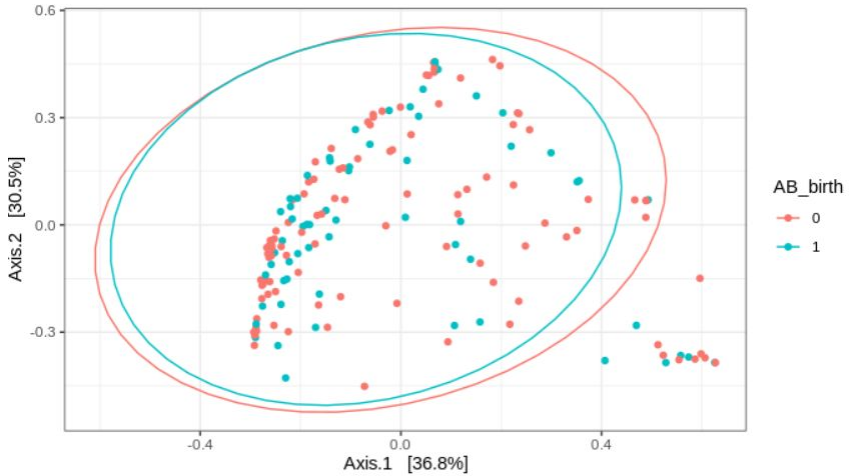
```
Terms added sequentially (first to last)

           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
AB_birth    1     1.881 1.88138  6.4264 0.02213  0.001 ***
Residuals 284    83.144 0.29276         0.97787
Total     285    85.025                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’
```

permanova 1w



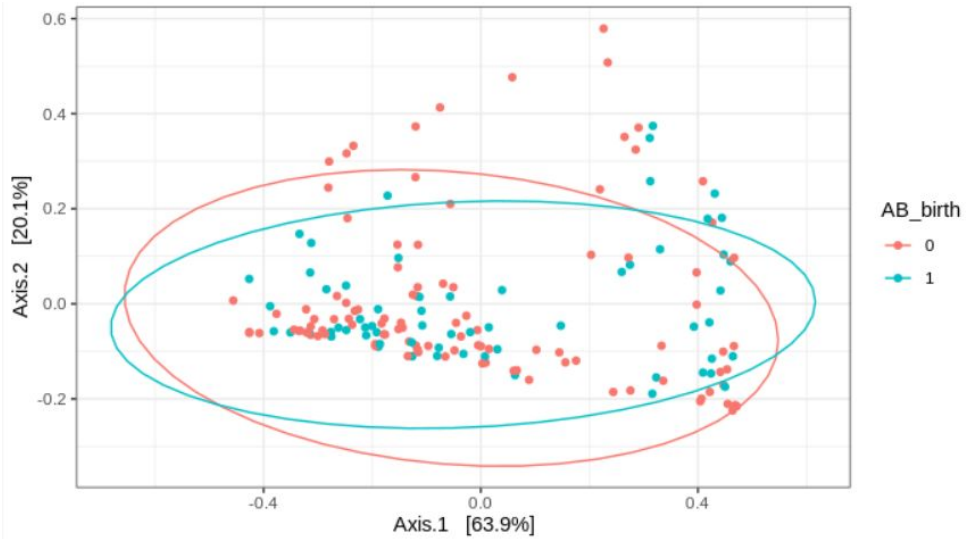
permanova 3m

	Df	SumsOfSqs	MeanSqs	F.Model
AB_birth	1	0.2137	0.21365	1.1469
Residuals	163	30.3643	0.18628	
Total	164	30.5779		



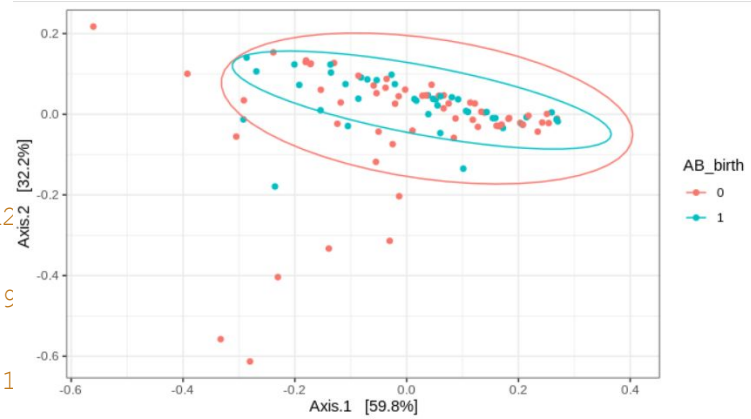
permanova 1y

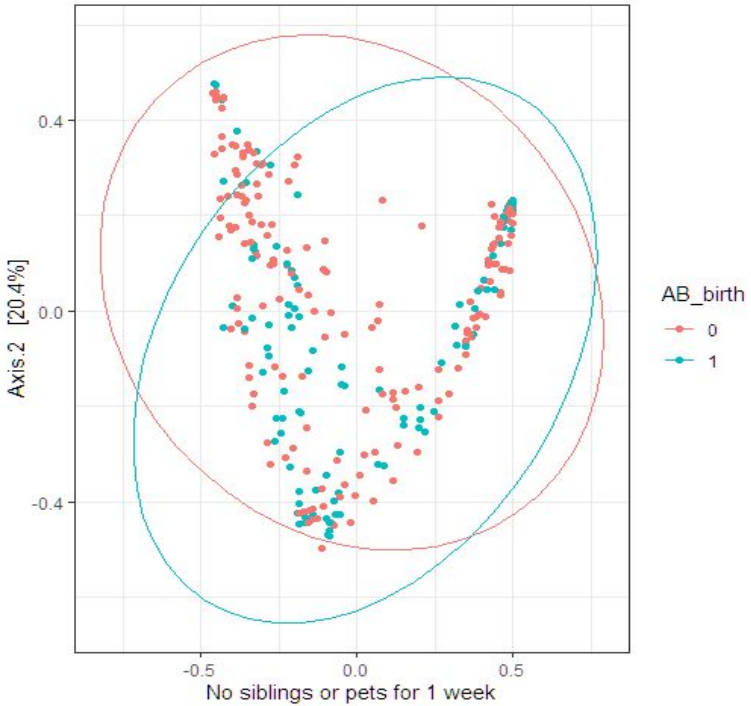
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
AB_birth	1	0.0625	0.062472			
Residuals	164	19.8094	0.120789			
Total	165	19.8719				

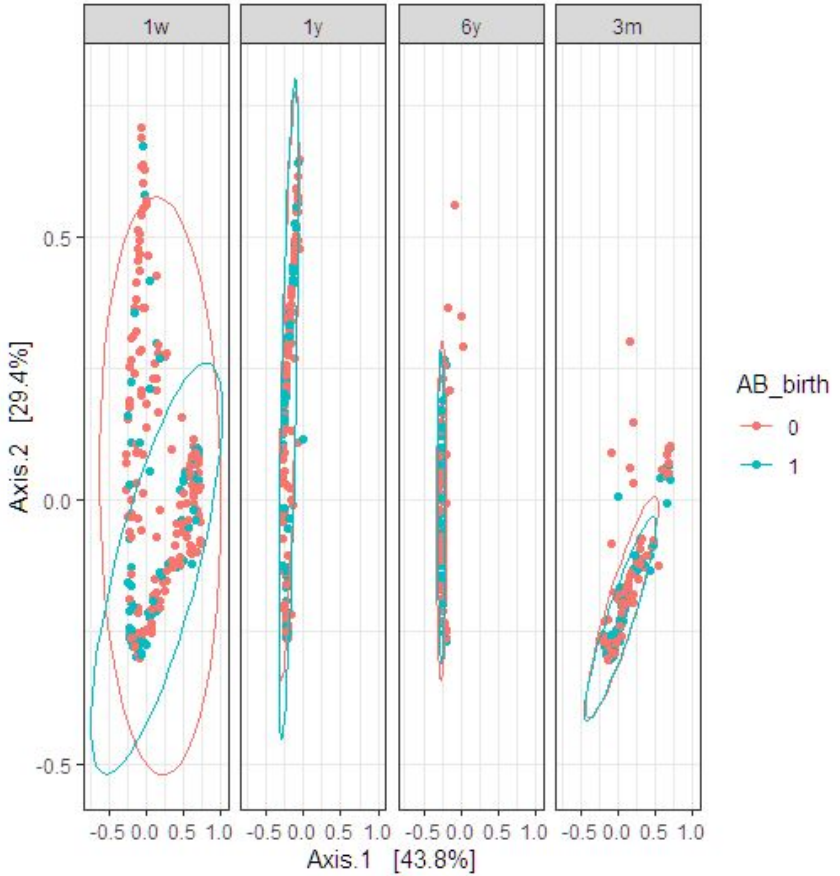


permanova 6y

	Df	SumsOfSqs	MeanSqs	F.Model	R2
AB_birth	1	0.0511	0.051068	1.0492	0.01059
Residuals	98	4.7702	0.048676		0.98941
Total	99	4.8213			1.00000




0.4
0.0
-0.4
Axis.2 [20.4%]
-0.5
0.0
0.5
No siblings or pets for 1 week
AB_birth
0
1

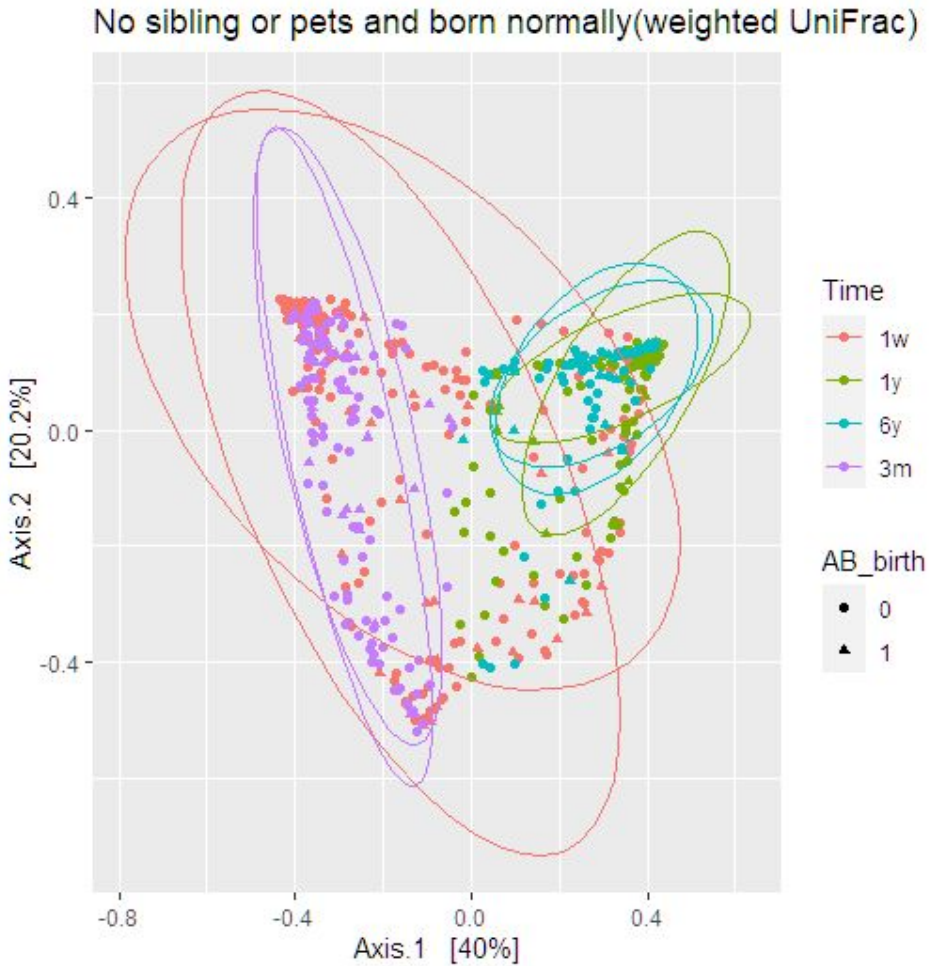

1w
1y
6y
3m
0.5
0.0
-0.5
Axis.2 [29.4%]
-0.5 0.0 0.5 1.0
Axis.1 [43.8%]
AB_birth
0
1

noter

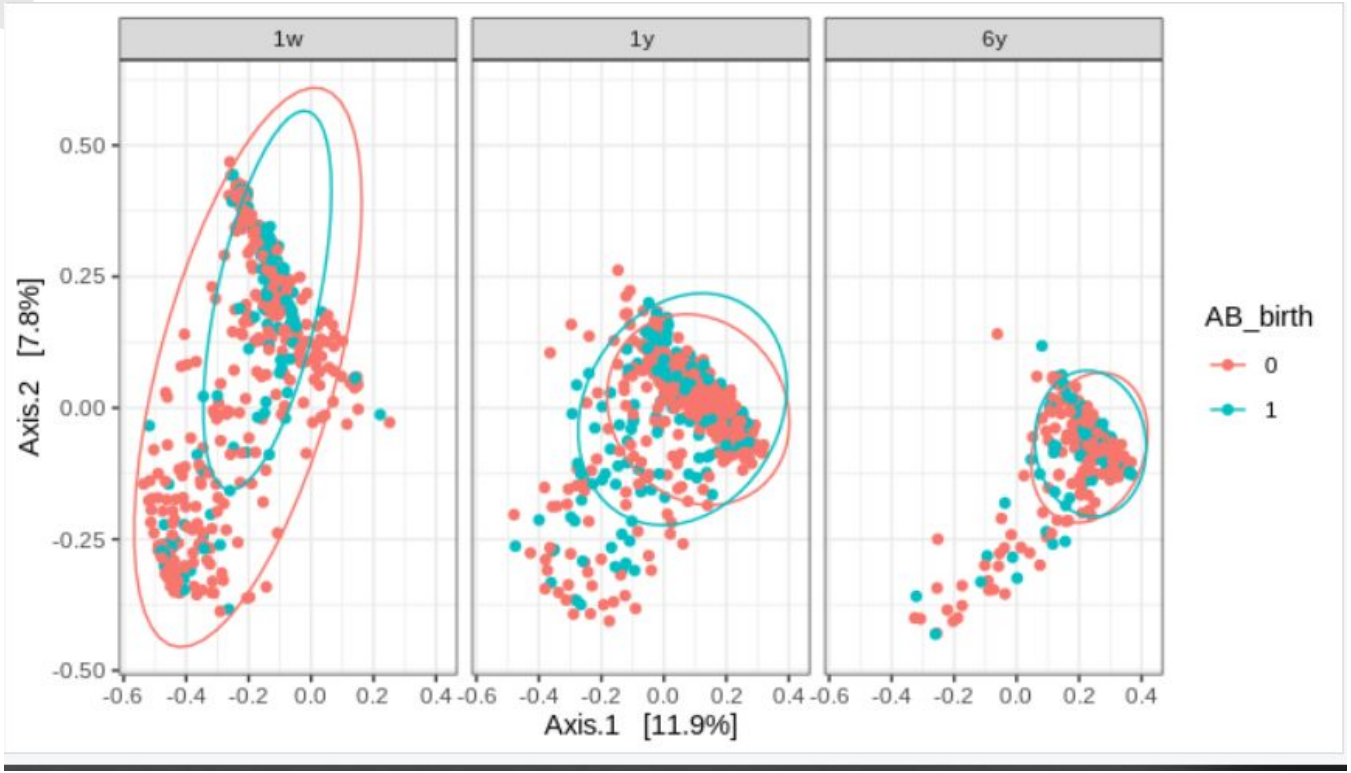
different distance methods can give the different results. That is because the different distance metrics consider different things. For example, bray cutis considers only the bacterial community abundance but weighted unifrac consider both the bacterial community abundance and phylogenetic distances between the different bacteria. So please do not worry about the "negative" results which are actually reasonable.

- do we have to take into consideration the abundance for each time point (it is up to the questions you want to answer. but your analysis results make sense and usually antibiotics only have a short term role on bacteria).

need to make a two paired test too.


No sibling or pets and born normally(weighted UniFrac)
0.4
0.0
-0.4
Axis.2 [20.2%]
-0.8
-0.4
0.0
0.4
Axis.1 [40%]
Time
1w
1y
6y
3m
AB_birth
0
1

Bray only with feces



Bray permanova 1w

```
adonis(formula = BC_sample_1w ~ AB_birth, data =
data.frame(sample_data(sample_1w_trans)))

Permutation: free
Number of permutations: 999

Terms added sequentially (first to last)

           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
AB_birth    1     5.413  5.4129  14.474 0.02921  0.001 ***
Residuals 481   179.887  0.3740         0.97079
Total     482   185.300                1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Bray permanova 1y

```
Call:

adonis(formula = BC_sample_1y ~ AB_birth, data =
data.frame(sample_data(sample_1y_trans)))



Permutation: free

Number of permutations: 999



Terms added sequentially (first to last)
```

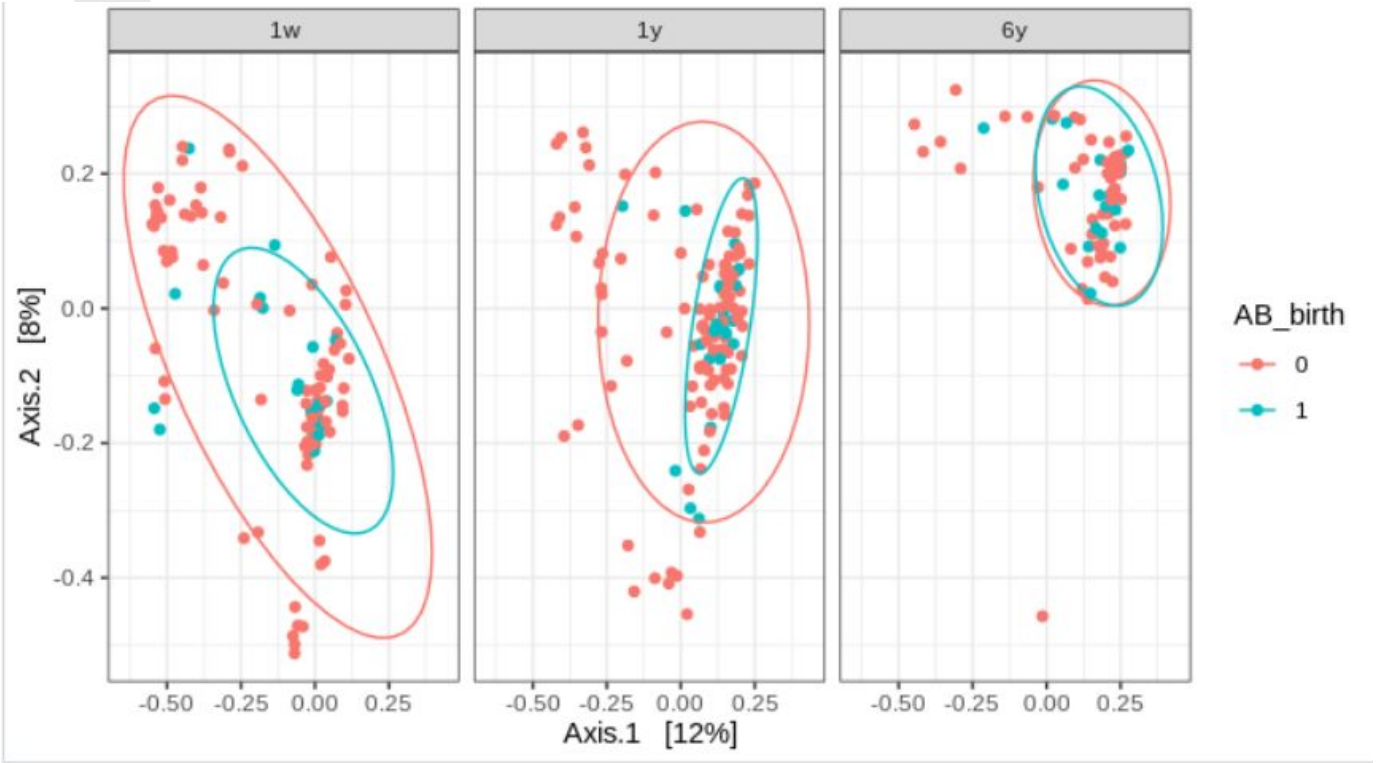
Permanova all

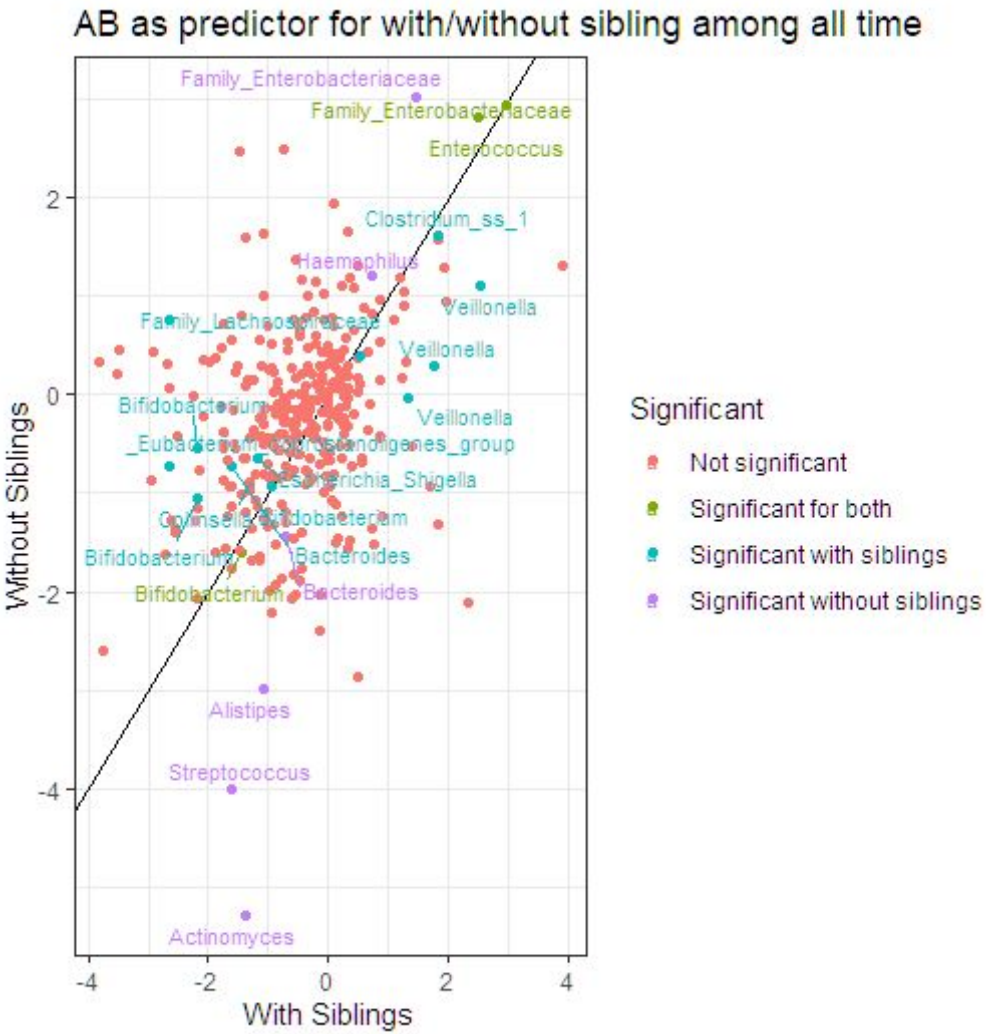
```
Call:

adonis(formula = BC_sample_all ~ AB_birth * Time, data =
data.frame(sample_data(sample_trans)))



Permutation: free

Number of permutations: 999



Terms added sequentially (first to last)
```

permanova 6 years

```
Call:

adonis(formula = BC_sample_6y ~ AB_birth, data =
data.frame(sample_data(sample_6y_trans)))



Permutation: free

Number of permutations: 999



Terms added sequentially (first to last)
```

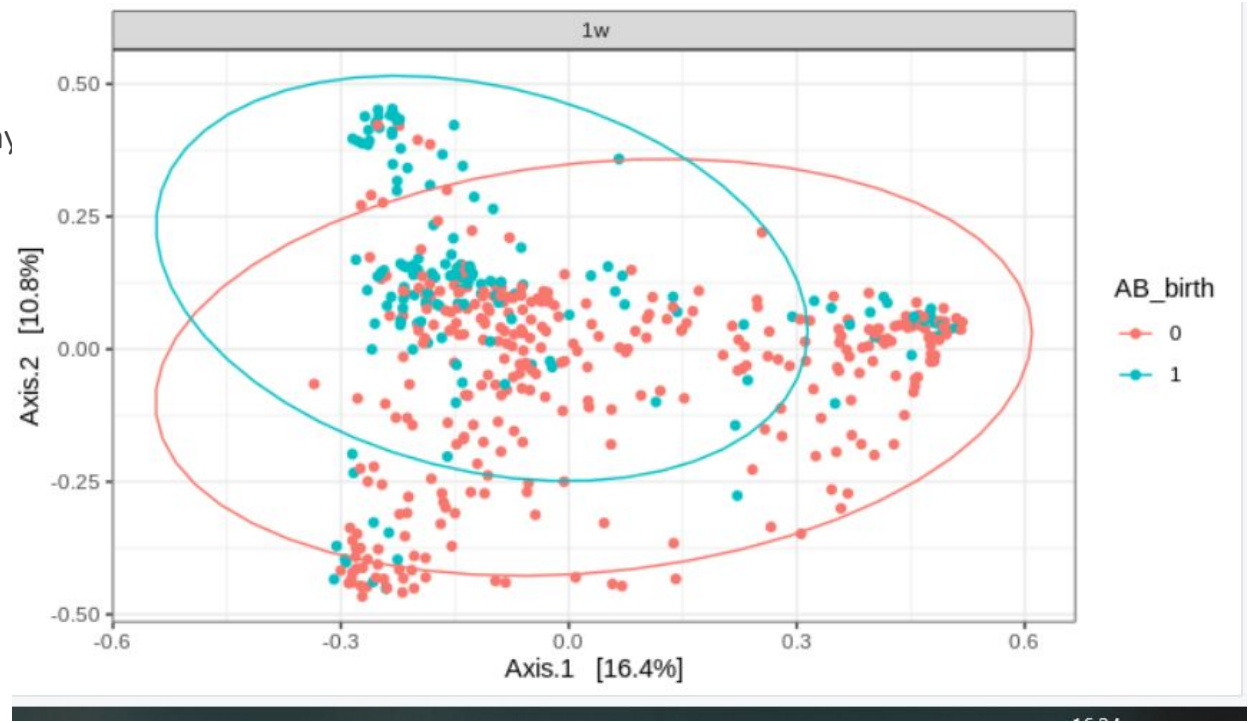
Pcoa Feces, normal delivery, - pets -




AB as predictor for with/without sibling among all time
Family_Enterobacteriaceae
Family_Enterobacteriaceae
Enterococcus
Clostridium_ss_1
Haemophilus
Veillonella
Family_Lachnospiraceae
Veillonella
Bifidobacterium
Veillonella
Bacteroides
Bifidobacterium
Bacteroides
Alistipes
Streptococcus
Actinomyces
Without Siblings
With Siblings
2
0
-2
-4
-4
-2
0
2
4
Significant
Not significant
Significant for both
Significant with siblings
Significant without siblings

New results . obs the ordination plots are only feces

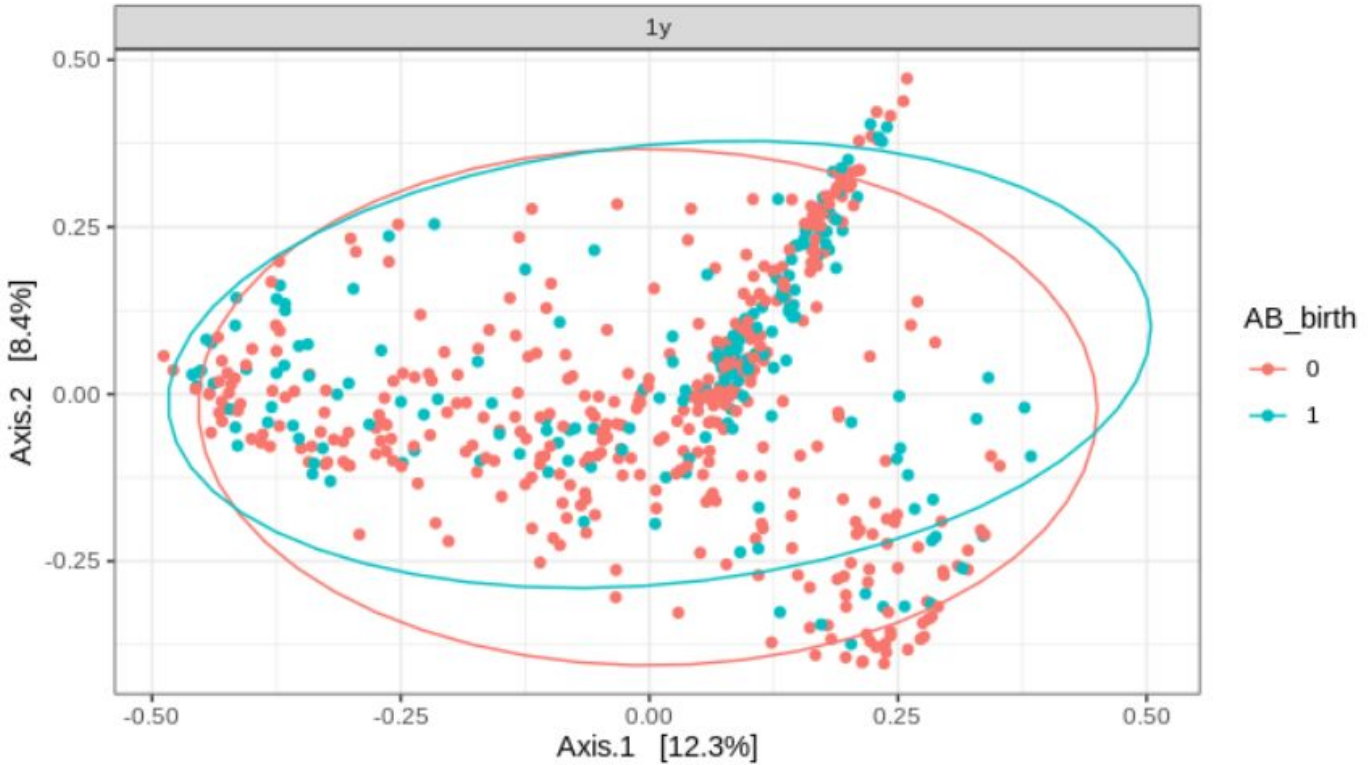
ordination plot bray



adonis/bray results 1w

```
Call:

adonis(formula = BC_sample_1w ~ AB_birth + Sibling + Pets + AB_birth:Sibling +
AB_birth:Pets, data = data.frame(sample_data(sample_1w_trans)))



Permutation: free

Number of permutations: 999



Terms added sequentially (first to last)
```

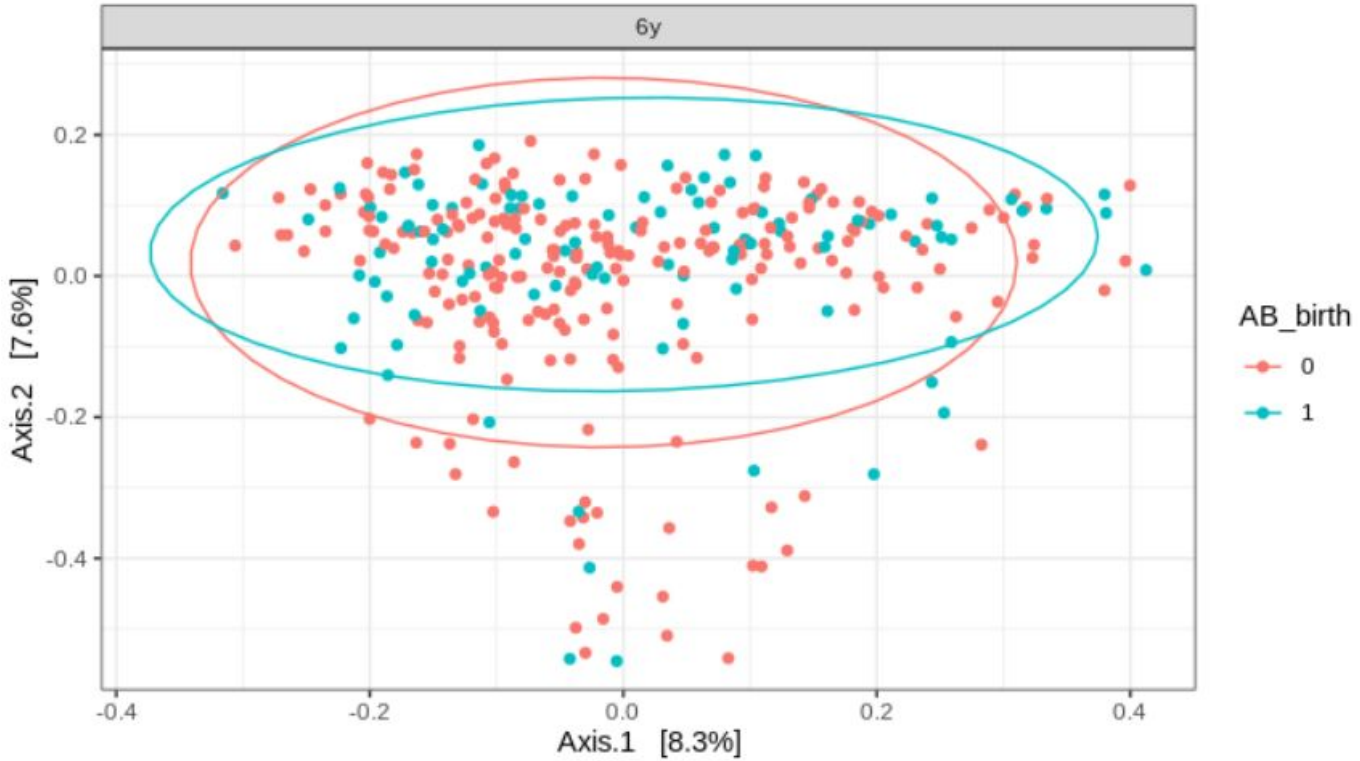
ordinationplot 1y bray



adonis/bray 1y

```
Call:

adonis(formula = BC_sample_1y ~ AB_birth + Sibling + Pets + AB_birth:Sibling +
AB_birth:Pets, data = data.frame(sample_data(sample_1y_trans)))



Permutation: free

Number of permutations: 999



Terms added sequentially (first to last)
```

ordiantion plot bray 6y



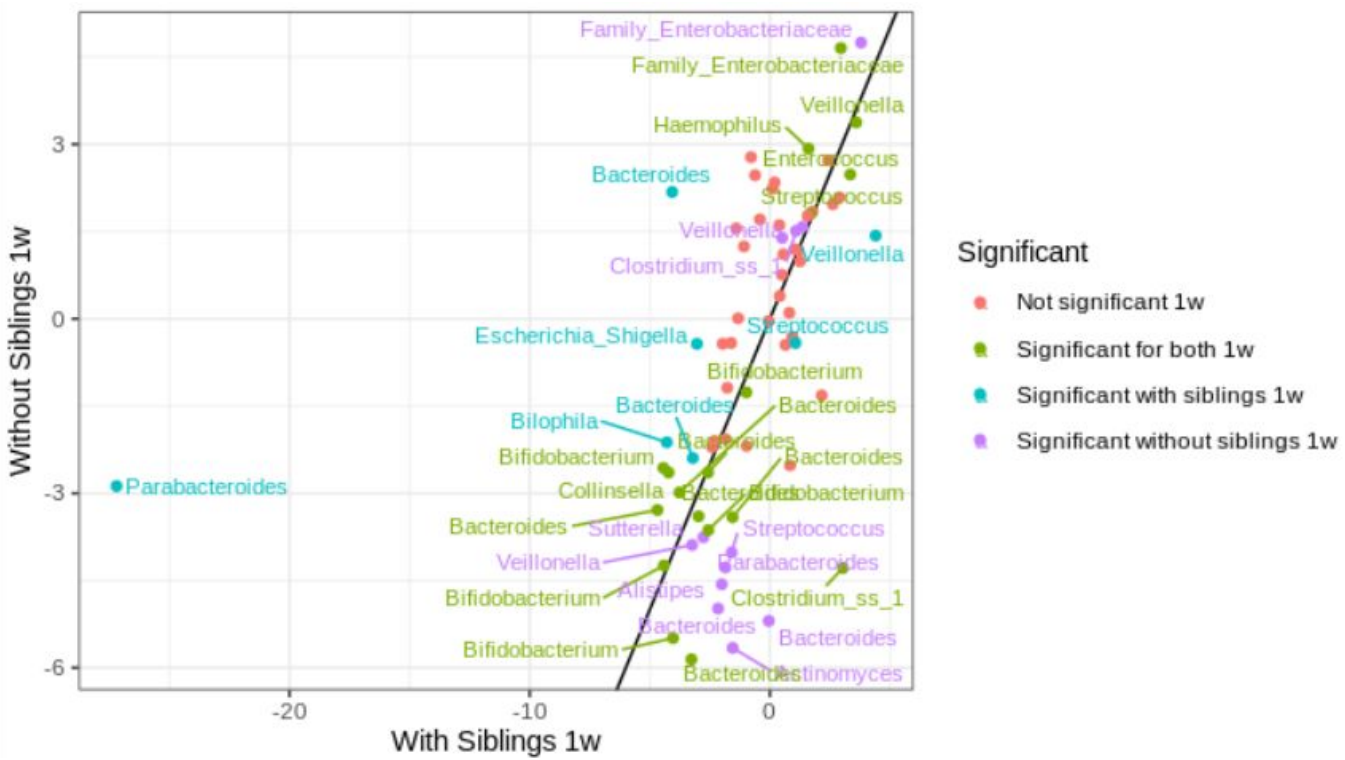
bray 6y

```
Call:

adonis(formula = BC_sample_6y ~ AB_birth + Sibling + Pets + AB_birth:Sibling +
AB_birth:Pets, data = data.frame(sample_data(sample_6y_trans)))



Permutation: free

Number of permutations: 999



Terms added sequentially (first to last)
```

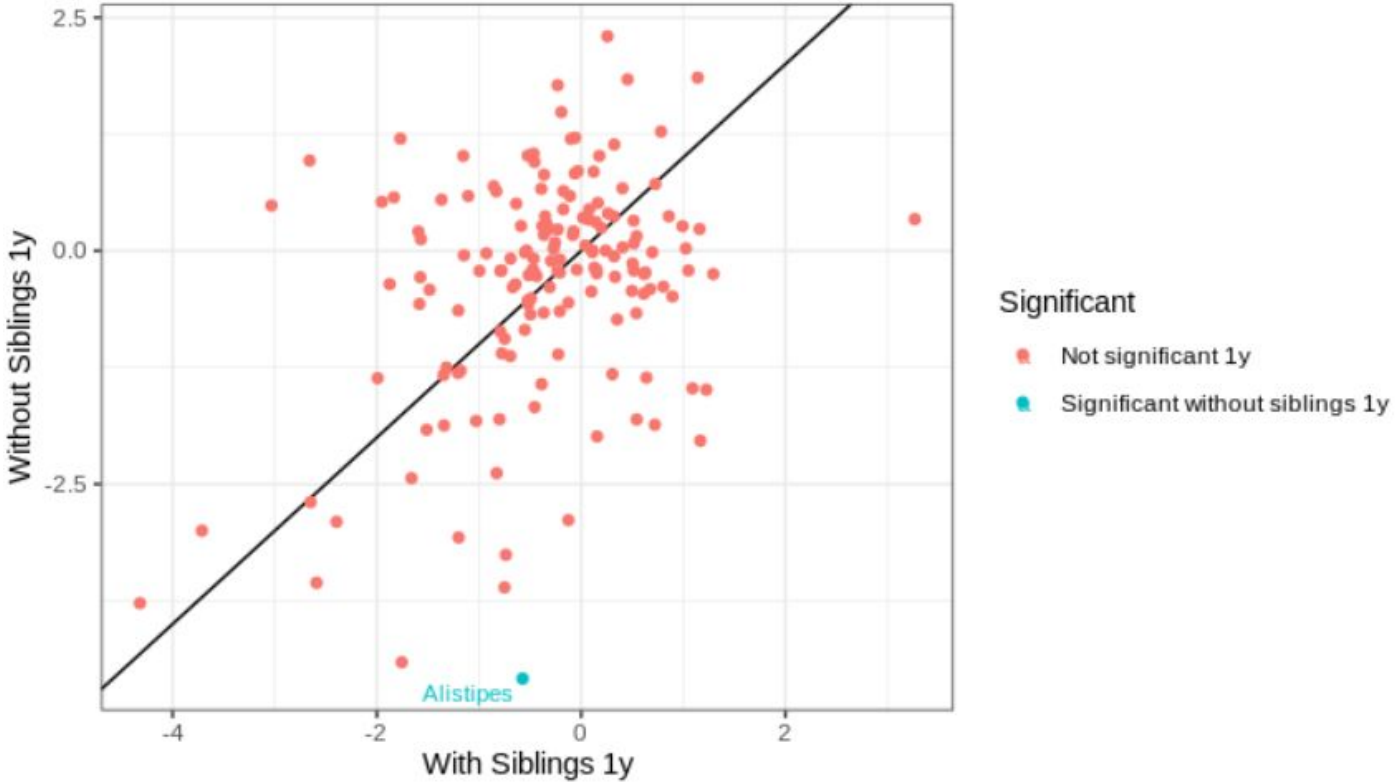
differential abundance deseq 1w w/wo sibli

log fold

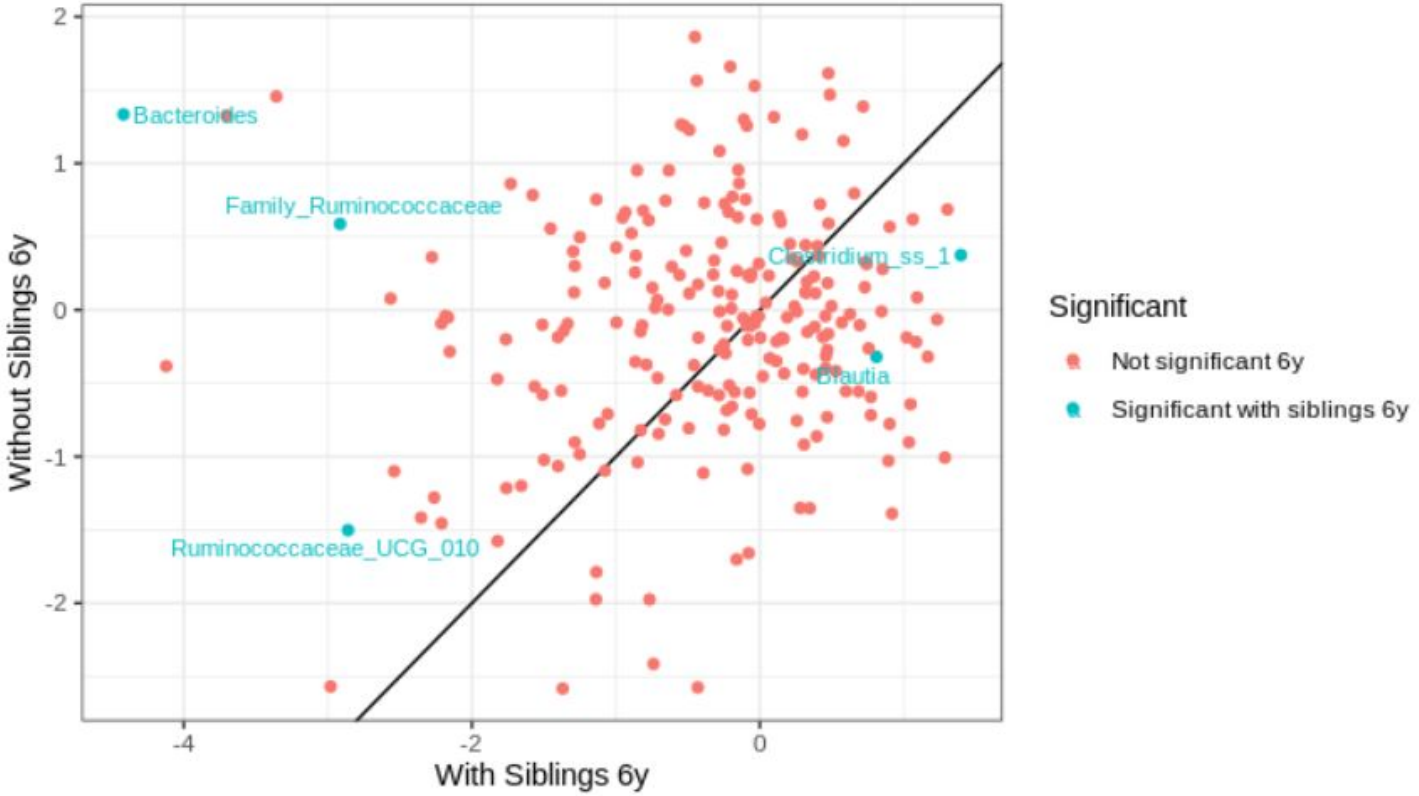
changes



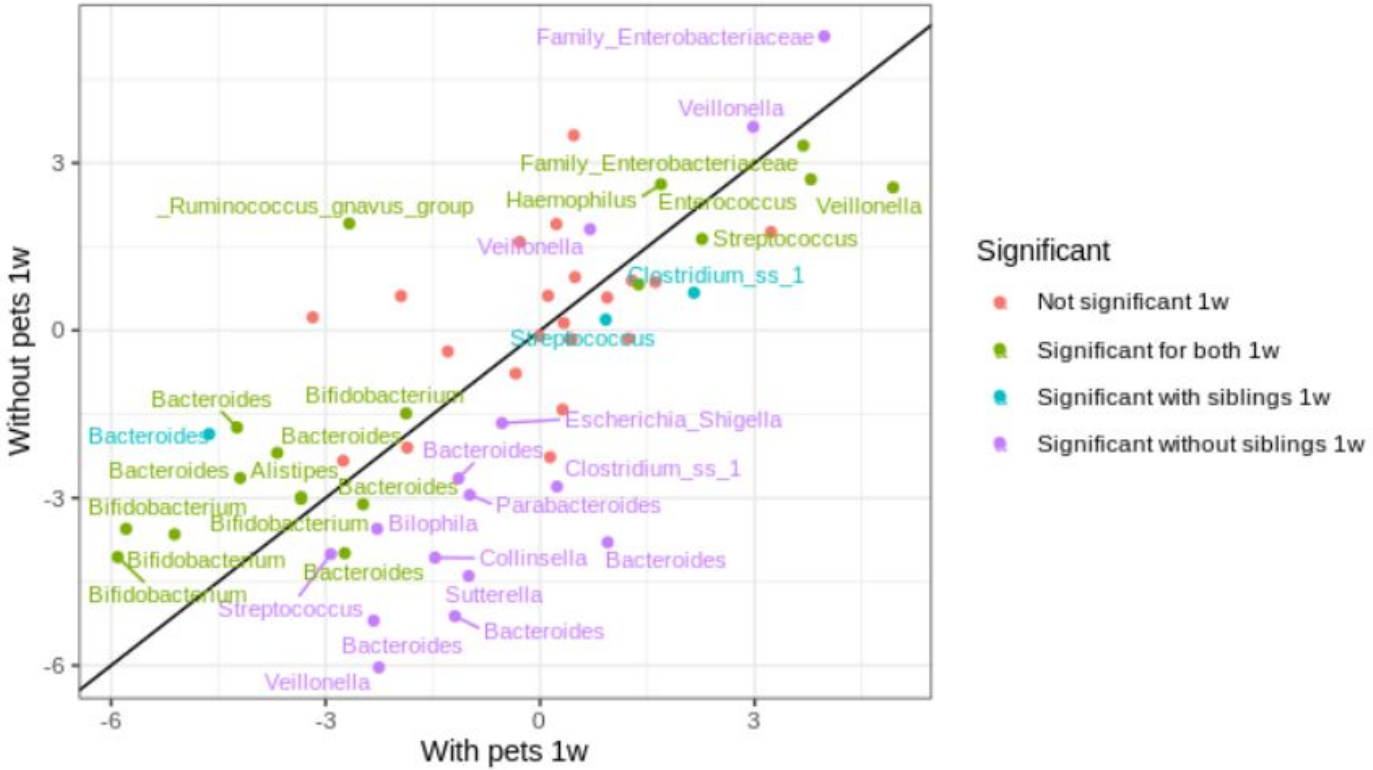
differential abundance 1y sibling



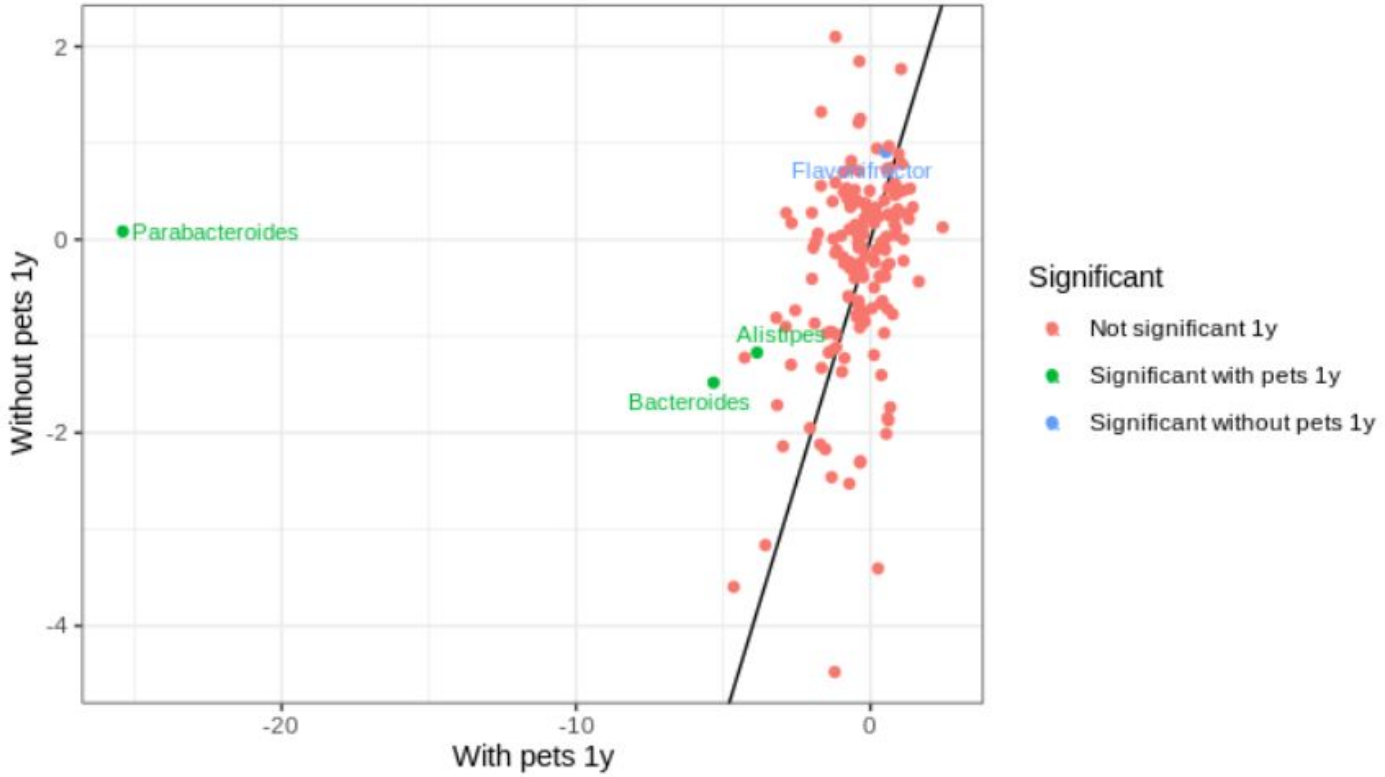
differential abundance 6y sibling



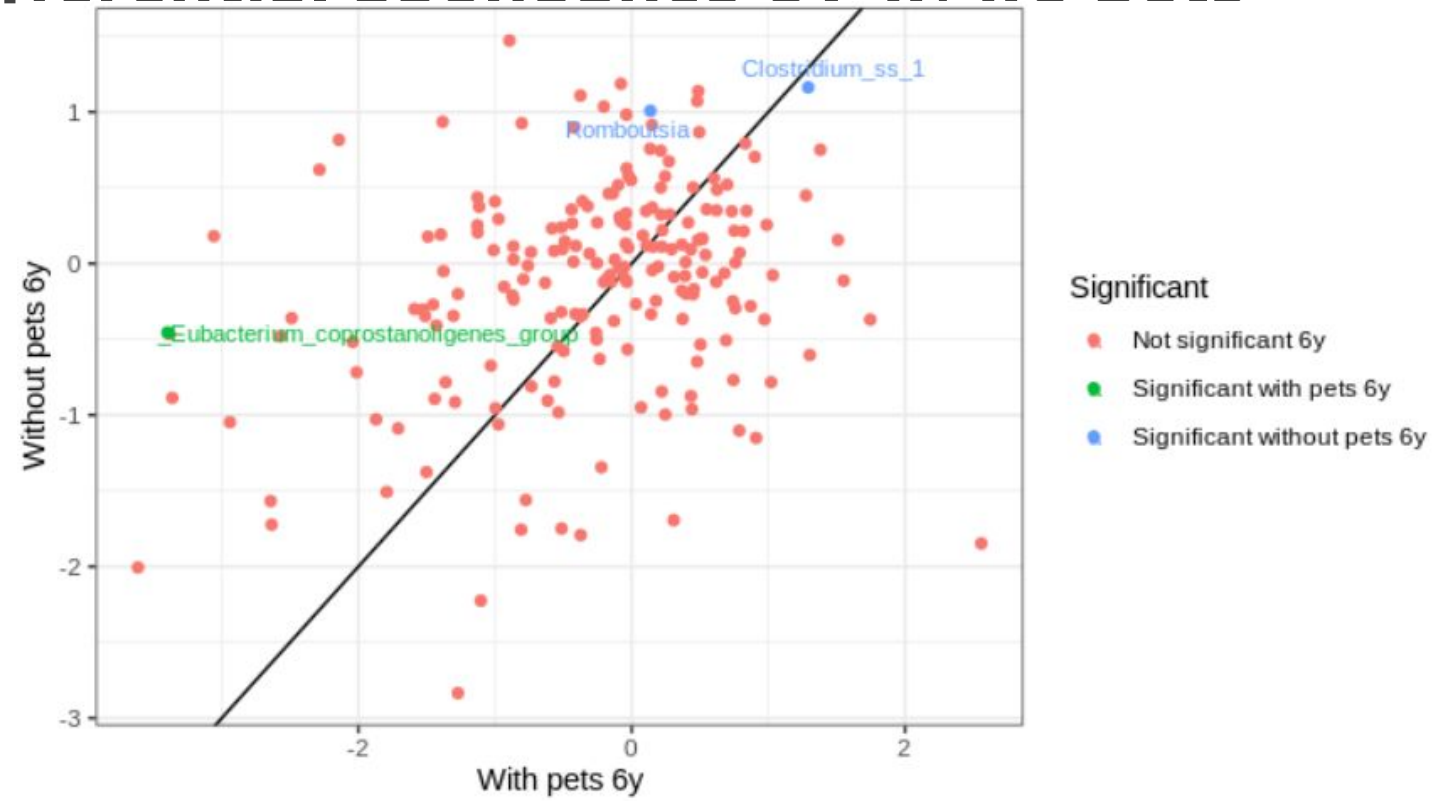
differential abundance 1w w and wo pets



differential abundance 1y w/wo pets



differential abundance 6y w/wo pets



code for the things

```
#get things ready

library(phyloseq)

library(ggplot2)

library(tidyverse)

library(DAtest)

library(vegan)

library(ggrepel)
```