

# qiime usage

All stages of the analysis were done in qiime2-2021.2

## Preprocessing + taxonomical analysis

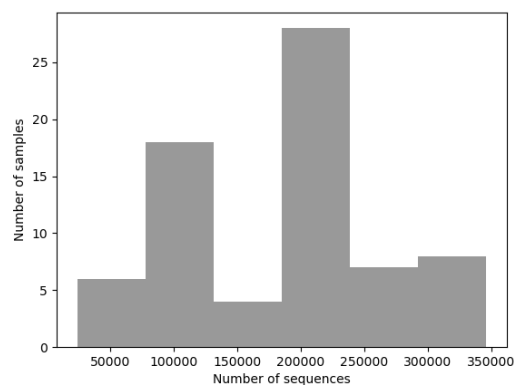
```
qiime tools import --type 'SampleData[PairedEndSequencesWithQuality]' --input-path ./ --input-format CasavaOneEightSingleLanePerSampleDirFmt --output-path demux-paired-end.qza
```

```
qiime demux summarize --i-data demux-paired-end.qza --o-visualization demux-summary-1.qzv
```

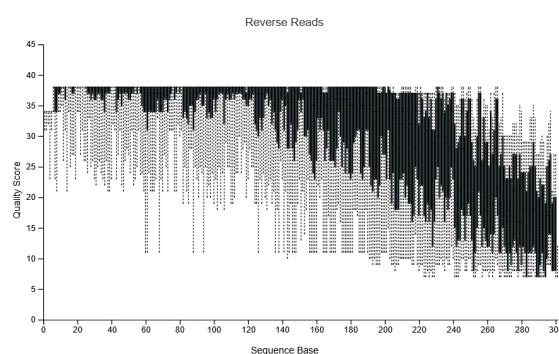
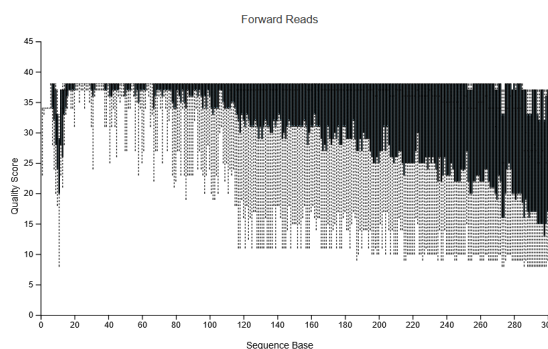
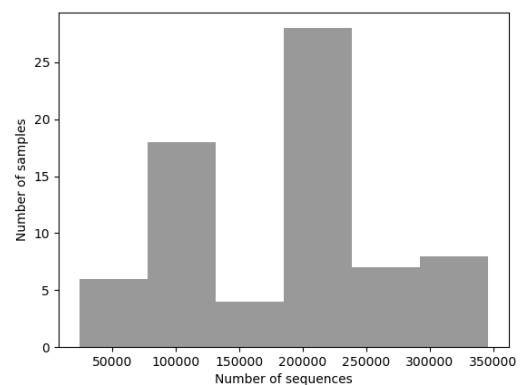
### Demultiplexed sequence counts summary

Property	# forward reads	# reverse reads
Minimum	23988	23988
Median	206576	206576
Mean	185457	185457
Maximum	346110	346110
Total	13167447	13167447

### Forward Reads Frequency Histogram



### Reverse Reads Frequency Histogram



### Parametric seven-number summary for position 68

Box plot feature	Percentile	Quality
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### Parametric seven-number summary for position 85

Box plot feature	Percentile	Quality score
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Aa Box plot feature	≡ Percentile	# Quality
(Not shown in box plot)	2nd	11
Lower Whisker	9th	27
Bottom of Box	25th	36
Middle of Box	50th (Median)	38
Top of Box	75th	38
Upper Whisker	91st	38
(Not shown in box plot)	98th	38

Aa Box plot feature	≡ Percentile	# Quality score
(Not shown in box plot)	2nd	11
Lower Whisker	9th	19
Bottom of Box	25th	32
Middle of Box	50th (Median)	38
Top of Box	75th	38
Upper Whisker	91st	38
(Not shown in box plot)	98th	38

## Demultiplexed sequence length summary

### Reverse Reads

Aa Total Sequences Sampled	♥ 10000
2%	300 nts
9%	300 nts
25%	300 nts
50% (Median)	300 nts
75%	300 nts
91%	301 nts
98%	301 nts

### Forward Reads

Aa Total Sequences Sampled	≡ 10000
2%	297 nts
9%	300 nts
25%	301 nts
50% (Median)	301 nts
75%	301 nts
91%	301 nts
98%	301 nts

```
qiime dada2 denoise-single --i-demultiplexed-seqs demux-paired-end.qza --p-trim-left 15 --o-representative-sequences rep-seqs-dada2.qza --o-table table-dada2.qza --o-denoising-stats stats-dada2.qz --p-trunc-len 300
```

```
qiime metadata tabulate --m-input-file stats-dada2.qz.qza --o-visualization stats-dada2.qz
```

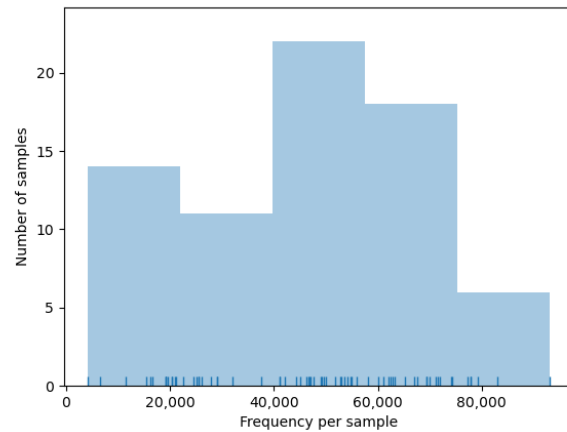
```
qiime feature-table summarize --i-table table-dada2.qza --o-visualization table-dada2.qzv
```

### Table summary

Aa Metric	≡ Sample
Number of samples	71
Number of features	10,652
Total frequency	3,301,944

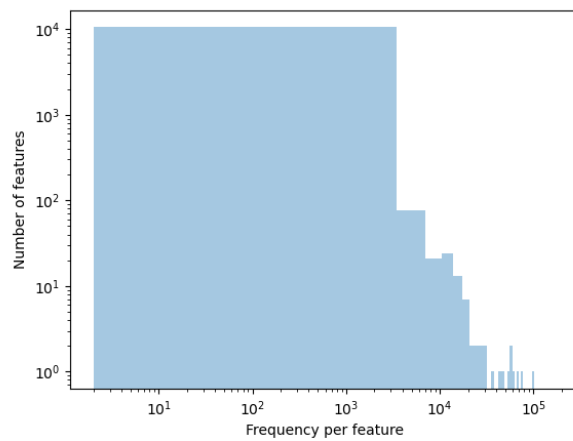
### Frequency per sample

Aa Property	≡ Frequency
Minimum frequency	4,056.0
1st quartile	25,501.0
Median frequency	49,051.0
3rd quartile	62,992.5
Maximum frequency	93,183.0
Mean frequency	46,506.25352112676



### Frequency per feature

Property	Frequency
<u>Minimum frequency.</u>	2.0
<u>1st quartile</u>	6.0
<u>Median frequency.</u>	19.0
<u>3rd quartile</u>	69.0
<u>Maximum frequency.</u>	175,016.0
<u>Mean frequency.</u>	309.98347728126174



```
qiime feature-table tabulate-seqs --i-data rep-seqs-dada2.qza --o-visualization rep-seqs.qzv
```

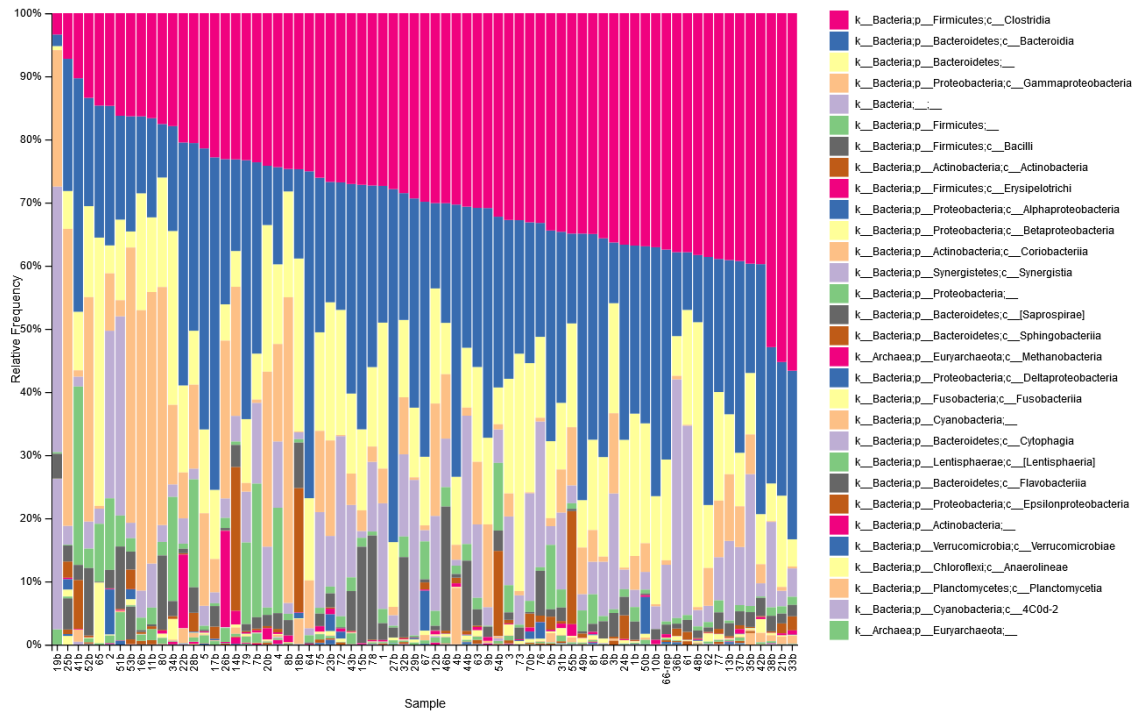
### Taxonomy classification

```
qiime feature-classifier classify-sklearn --i-classifier gg-13-8-99-515-806-nb-classifier.qza --i-reads rep-seqs-dada2.qza --m-metadata-file metadata_old.tsv --o-classification taxonomy.qza
```

```
qiime metadata tabulate --m-input-file taxonomy.qza --o-visualization taxonomy.qzv
```

```
qiime taxa barplot --i-table table-dada2.qza --i-taxonomy taxonomy.qza --m-metadata-file metadata_old.tsv --o-visualization taxa-bar-plots.qzv
```

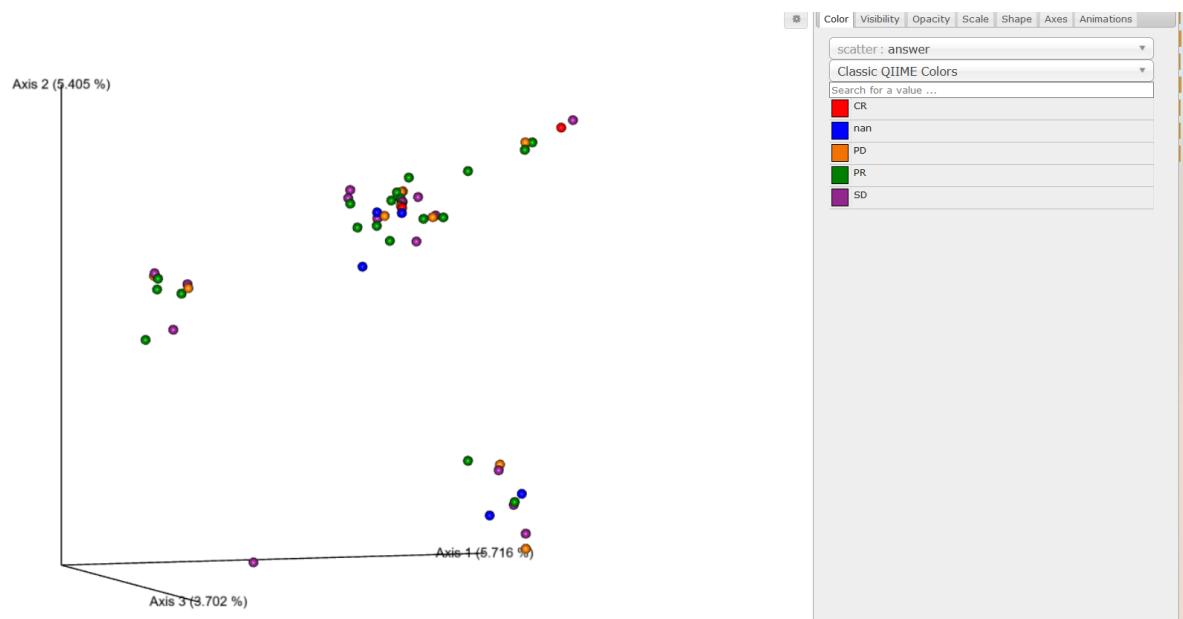
Example of barplot in level 3



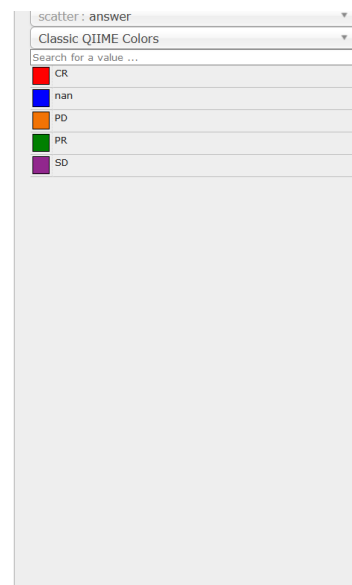
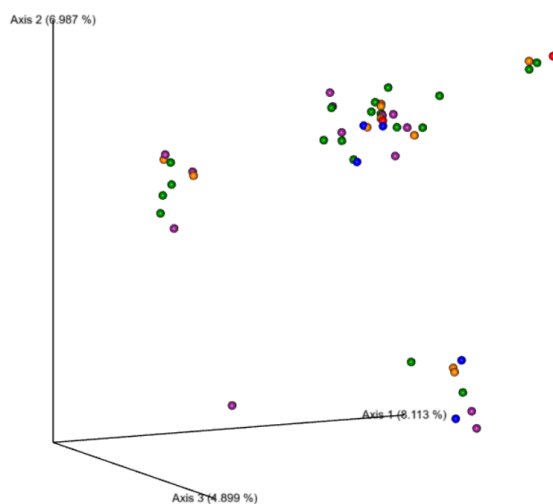
## Diversity analysis

```
qiime diversity core-metrics-phylogenetic \
  --i-phylogeny rooted-tree.qz.qza \
  --i-table table-dada2.qza \
  --p-sampling-depth 4 \
  --m-metadata-file metadata.csv \
  --output-dir ./phylo
```

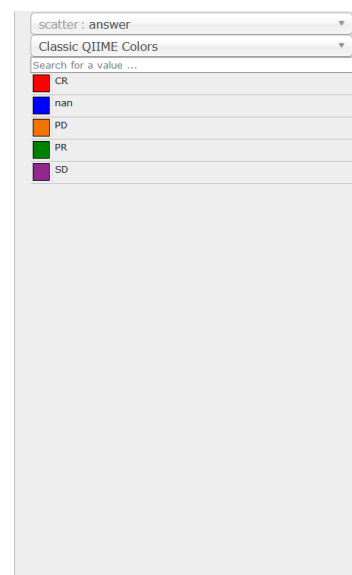
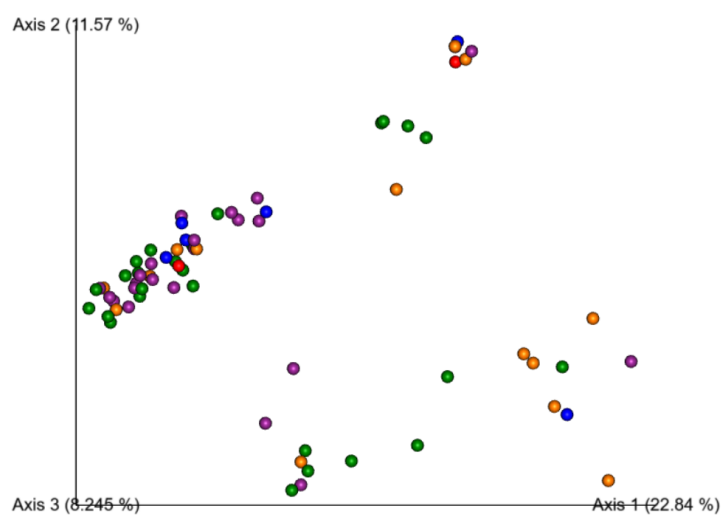
Clusters by jaccard distances:



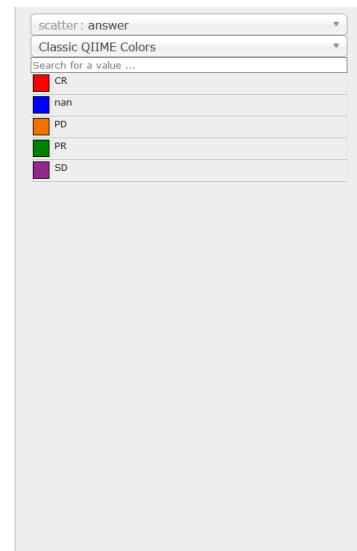
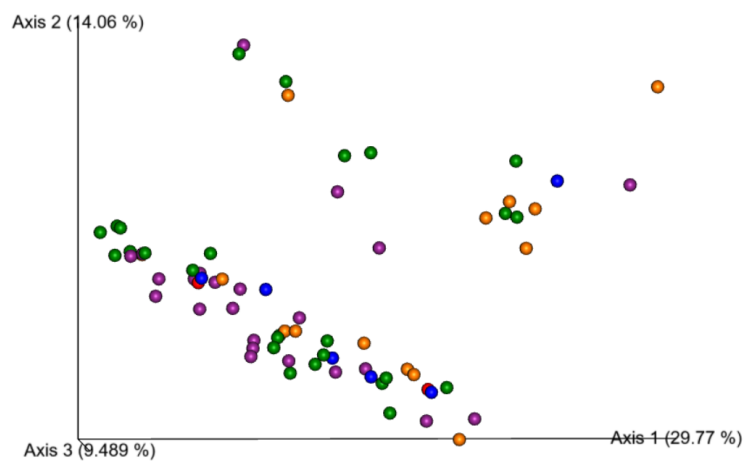
Clusters by bray-curtis distances:



Clusters by unweighted unifracs distances:

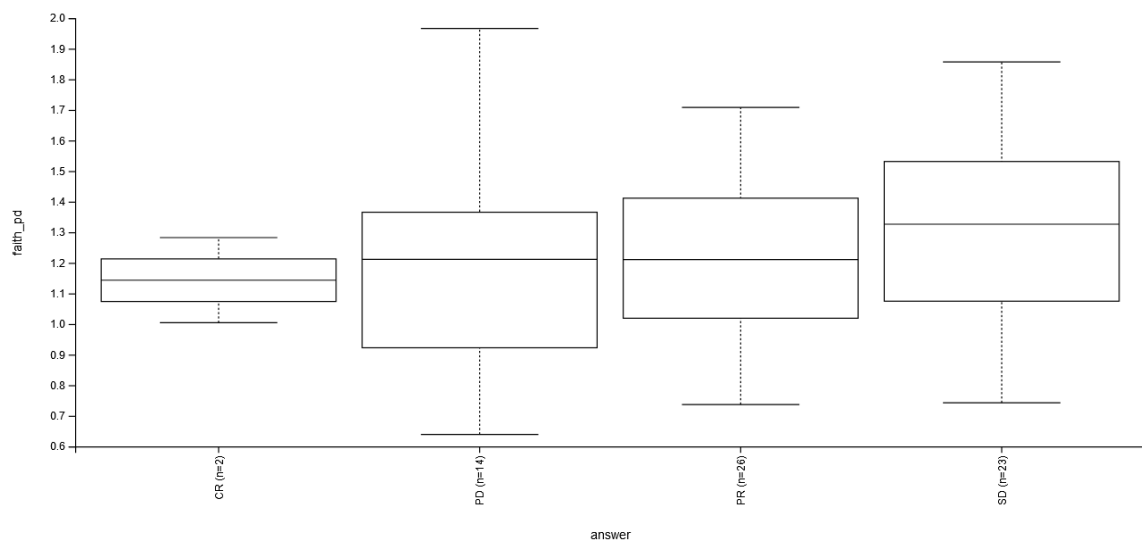


Clusters by weighted unifracs distances:



## Alpha diversity analysis

```
qiime diversity alpha-group-significance \
  --i-alpha-diversity ./phylo3/faith_pd_vector.qza \
  --m-metadata-file metadata.tsv \
  --o-visualization ./phylo3/faith-pd-group-significance.qzv
```

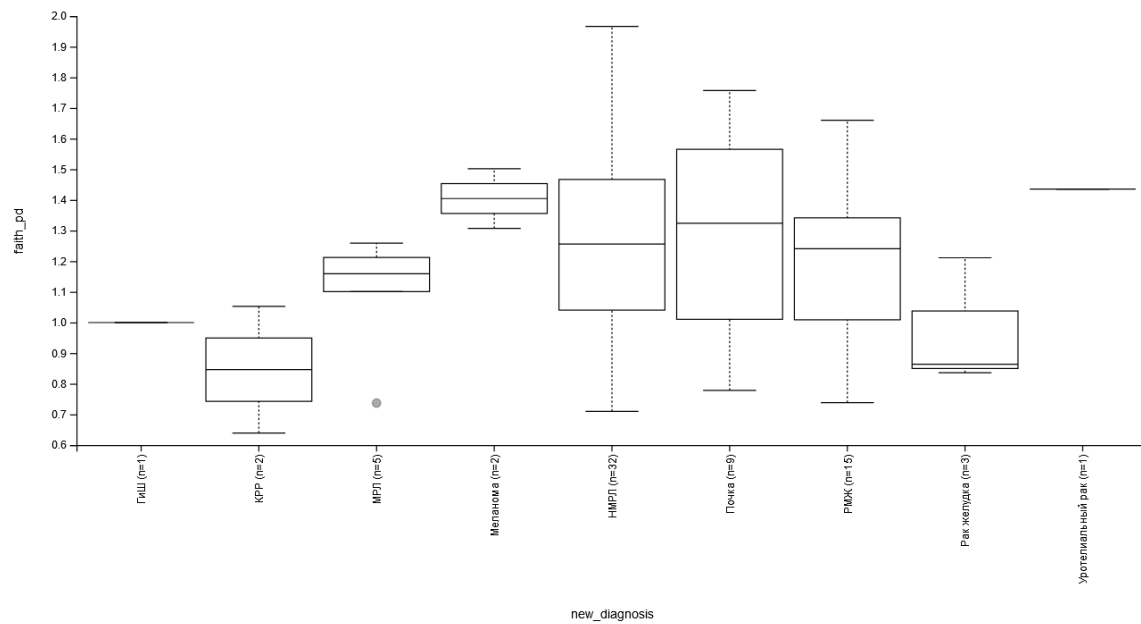


### Kruskal-Wallis (all groups)

Aa Property	# Result
<u>H</u>	1.598
<u>p-value</u>	0.66

### Kruskal-Wallis (pairwise)

Aa Group 1	≡ Group 2	≡ H	≡ p-value	# q-value
<u>CR (n=2)</u>	PD (n=14)	0,025	0,874	0.874
<u>CR (n=2)</u>	PR (n=26)	0,286	0,592	0.874
<u>CR (n=2)</u>	SD (n=23)	0,642	0,423	0.846
<u>PD (n=14)</u>	PR (n=26)	0,065	0,799	0.874
<u>PD (n=14)</u>	SD (n=23)	0,715	0,398	0.846
<u>PR (n=26)</u>	SD (n=23)	1,044	0,307	0.846



#### Kruskal-Wallis (all groups)

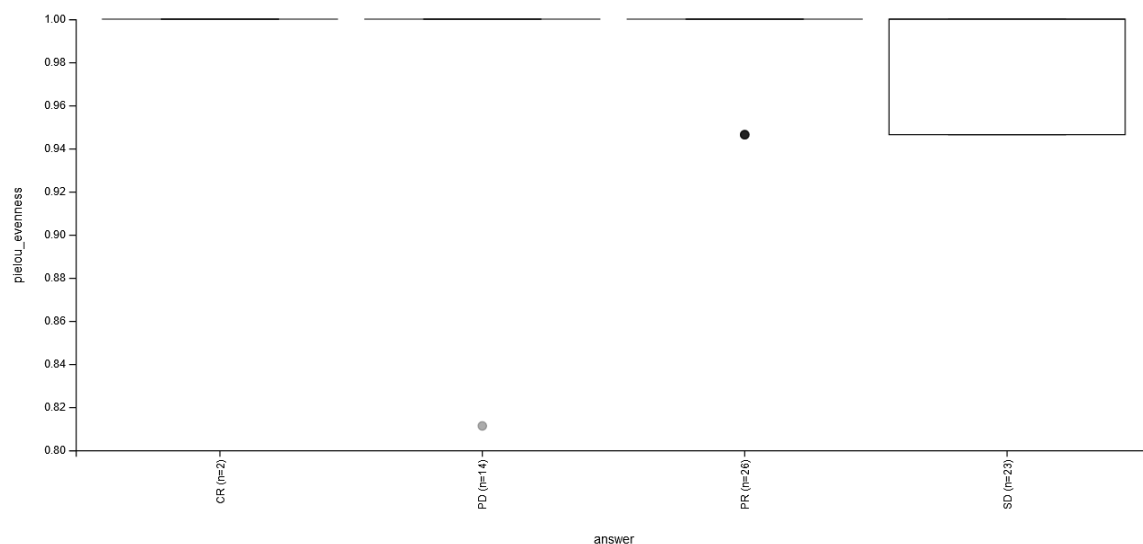
Aa Property	# Result
H	9.839793762575425
p-value	0.2764508938127179

#### Kruskal-Wallis (pairwise)

Aa Property	1	H	p-value	q-value
Group 1	Group 2			
ГиШ (n=1).	КРР (n=2)	0	1	1
ГиШ (n=1).	МРЛ (n=5)	0,7714	0,38	0,5761
ГиШ (n=1).	Меланома (n=2)	1,5	0,221	0,5383
ГиШ (n=1).	НМРЛ (n=32)	1,1029	0,294	0,5383
ГиШ (n=1).	Почка (n=9)	0,7576	0,384	0,5761
ГиШ (n=1).	РМЖ (n=15)	0,9529	0,329	0,5383
ГиШ (n=1).	Рак желудка (n=3)	0,2	0,655	0,7366
ГиШ (n=1).	Уротелиальный рак (n=1)	1	0,317	0,5383
КРР (n=2).	МРЛ (n=5)	2,4	0,121	0,5383
КРР (n=2).	Меланома (n=2)	2,4	0,121	0,5383
КРР (n=2).	НМРЛ (n=32)	2,8339	0,092	0,5383
КРР (n=2).	Почка (n=9)	2	0,157	0,5383
КРР (n=2).	РМЖ (n=15)	2,2222	0,136	0,5383
КРР (n=2).	Рак желудка (n=3)	0,3333	0,564	0,6764
КРР (n=2).	Уротелиальный рак (n=1)	1,5	0,221	0,5383
МРЛ (n=5).	Меланома (n=2)	3,75	0,053	0,5383
МРЛ (n=5).	НМРЛ (n=32)	1,0441	0,307	0,5383
МРЛ (n=5).	Почка (n=9)	1,2844	0,257	0,5383
МРЛ (n=5).	РМЖ (n=15)	1,1905	0,275	0,5383
МРЛ (n=5).	Рак желудка (n=3)	0,5556	0,456	0,5968
МРЛ (n=5).	Уротелиальный рак (n=1)	2,1429	0,143	0,5383
Меланома (n=2).	НМРЛ (n=32)	0,5357	0,464	0,5968

Aa Property	1	H	p-value	q-value
<u>Меланома (n=2)</u>	Почка (n=9)	0,0556	0,814	0,8876
<u>Меланома (n=2)</u>	РМЖ (n=15)	1,4222	0,233	0,5383
<u>Меланома (n=2)</u>	Рак желудка (n=3)	3	0,083	0,5383
<u>Меланома (n=2)</u>	Уротелиальный рак (n=1)	0	1	1
<u>НМРЛ (n=32)</u>	Почка (n=9)	0,004	0,95	1
<u>НМРЛ (n=32)</u>	РМЖ (n=15)	0,638	0,424	0,5968
<u>НМРЛ (n=32)</u>	Рак желудка (n=3)	3,125	0,077	0,5383
<u>НМРЛ (n=32)</u>	Уротелиальный рак (n=1)	0,3971	0,529	0,6562
<u>Почка (n=9)</u>	РМЖ (n=15)	0,5556	0,456	0,5968
<u>Почка (n=9)</u>	Рак желудка (n=3)	1,4444	0,229	0,5383
<u>Почка (n=9)</u>	Уротелиальный рак (n=1)	0,2727	0,602	0,6985
<u>РМЖ (n=15)</u>	Рак желудка (n=3)	1,5474	0,214	0,5383
<u>РМЖ (n=15)</u>	Уротелиальный рак (n=1)	0,9529	0,329	0,5383
<u>Рак желудка (n=3)</u>	Уротелиальный рак (n=1)	1,8	0,18	0,5383

```
qiime diversity alpha-group-significance \
--i-alpha-diversity ./phylo3/evenness_vector.qza \
--m-metadata-file metadata.tsv \
--o-visualization ./phylo3/evenness-group-significance.qzv
```



#### Kruskal-Wallis (all groups)

Aa Property	Result
<u>H</u>	4,163
<u>p-value</u>	0,244

#### Kruskal-Wallis (pairwise)

Aa Property	1	H	p-value	q-value
<u>Group 1</u>	Group 2			
<u>CR (n=2)</u>	PD (n=14)	0,143	0,705	0,705
<u>CR (n=2)</u>	PR (n=26)	0,452	0,502	0,602
<u>CR (n=2)</u>	SD (n=23)	0,982	0,322	0,558
<u>PD (n=14)</u>	PR (n=26)	0,796	0,372	0,558
<u>PD (n=14)</u>	SD (n=23)	2,889	0,089	0,535
<u>PR (n=26)</u>	SD (n=23)	1,483	0,223	0,558

### Beta diversity analysis



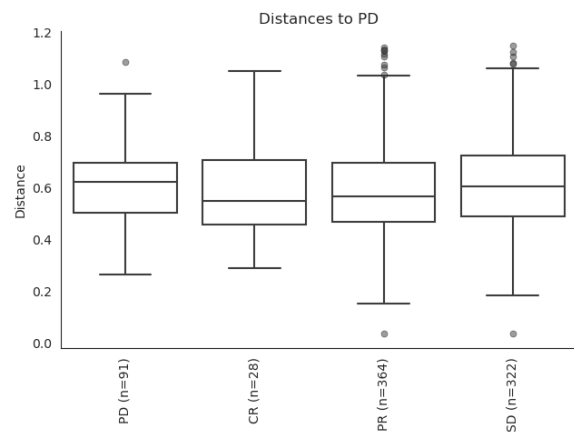
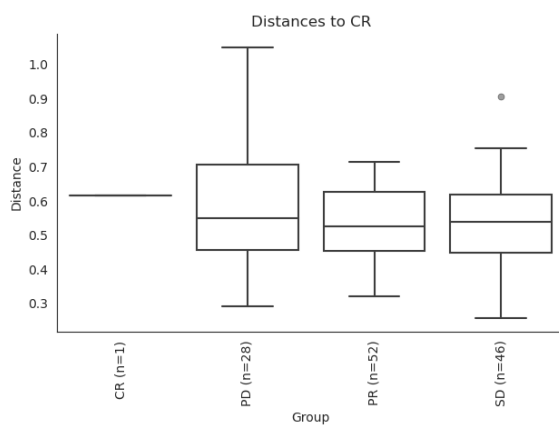
```
qiime diversity beta-group-significance \
--i-distance-matrix ./phylo3/weighted_unifrac_distance_matrix.qza \
--m-metadata-file metadata.tsv \
--m-metadata-column answer \
--o-visualization ./phylo3/weighted-unifrac-answer-significance.qzv \
--p-pairwise
```

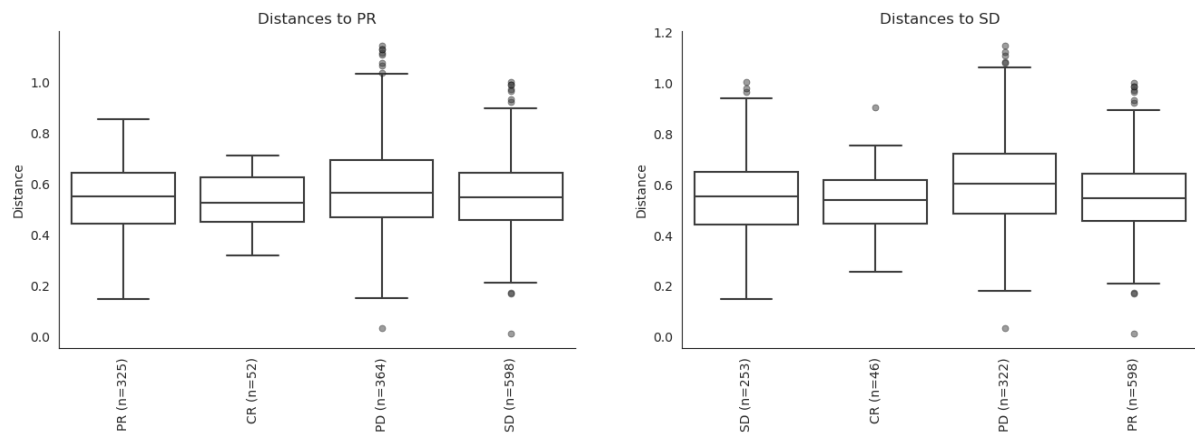
## Overview

Property	PERMANOVA results
method name	PERMANOVA
test statistic name	pseudo-F
sample size	65
number of groups	4
test statistic	1.4614
p-value	0.082
number of permutations	999

## Pairwise permanova results

Property	1	Sample size	Permutations	pseudo-F	p-value	q-value
Group 1	Group 2	0	0	0	0	0
CR	PD	16	999	0.789842	0.625	0.883
CR	PR	28	999	0.599877	0.775	0.883
CR	SD	25	999	0.524779	0.883	0.883
PD	PR	40	999	2.11232	0.058	0.174
PD	SD	37	999	2.991727	0.011	0.066
PR	SD	49	999	0.855005	0.512	0.883

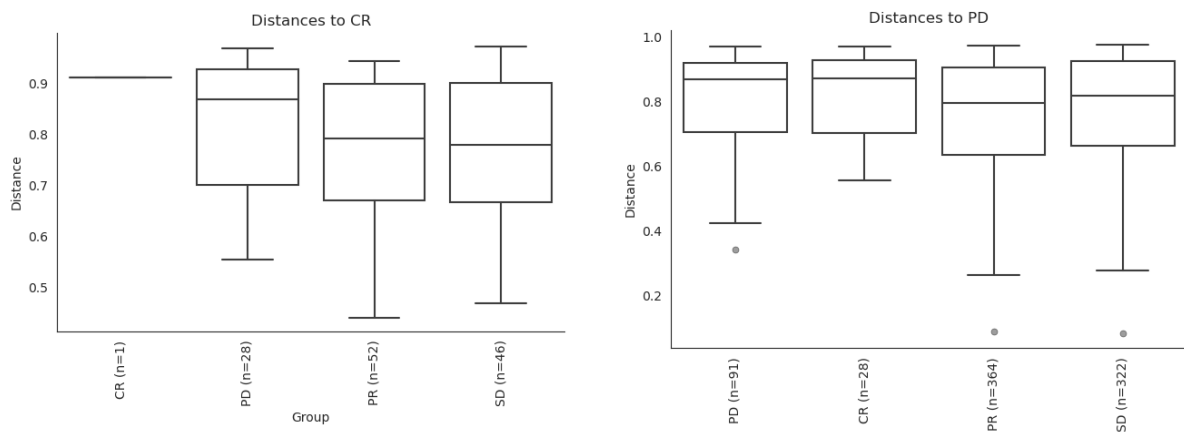


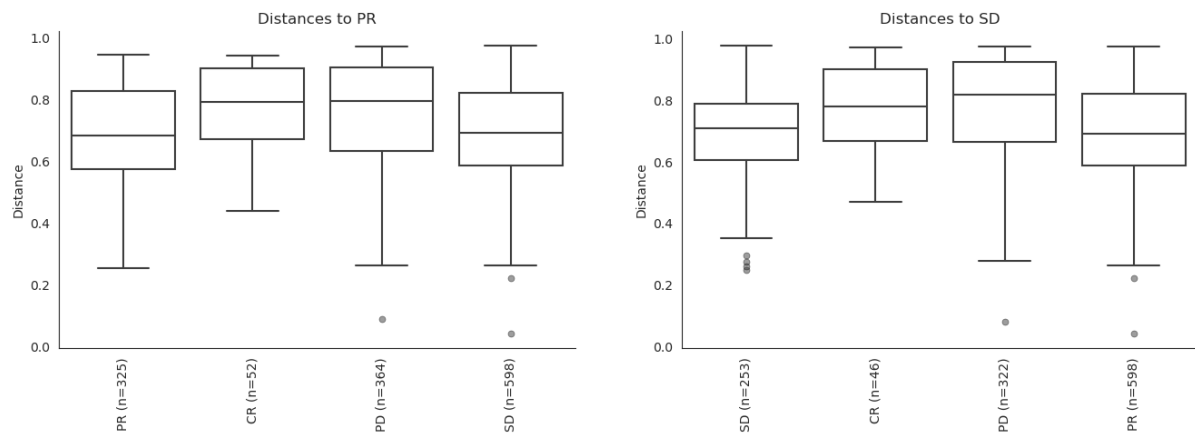


```
qiime diversity beta-group-significance --i-distance-matrix ./phylo/unweighted_unifrac_distance_matrix.qza --m-metadata-file metadata.tsv --m-metadata-column answer --o-visualization ./phylo/unweighted-unifrac-answer-significance.qzv --p-pairwise
```

## Overview

Property	PERMANOVA results
method name	PERMANOVA
test statistic name	pseudo-F
sample size	65
number of groups	4
test statistic	1.46927
p-value	0.032
number of permutations	999





### Pairwise permanova results

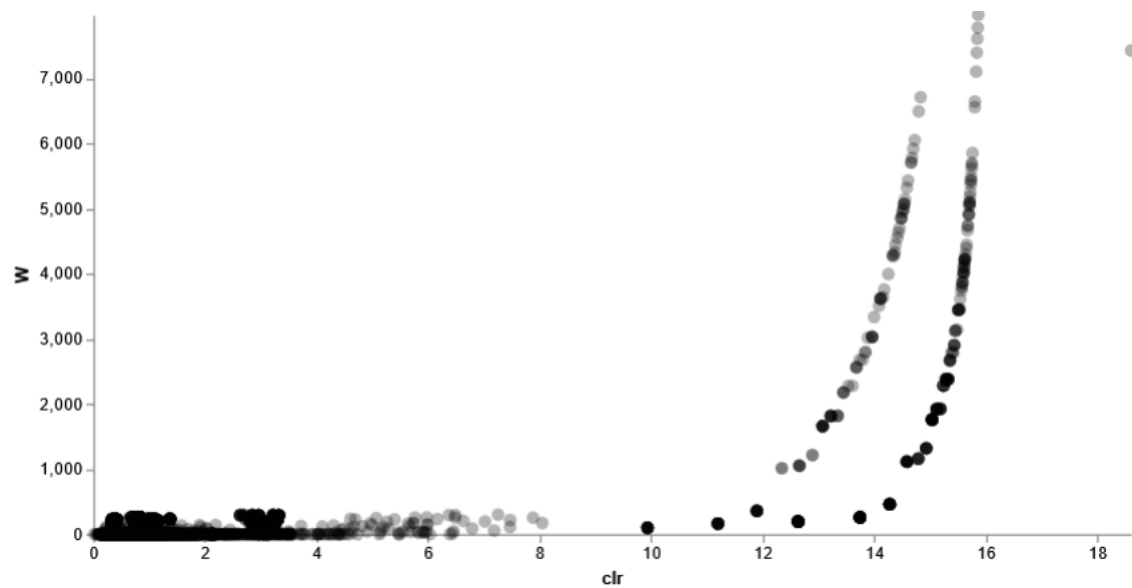
Aa Property	≡ 1	# Sample size	# Permutations	# pseudo-F	# p-value	# q-value
<u>Group 1</u>	Group 2	0	0	0	0	0
<u>CR</u>	PD	16	999	0.957013	0.503	0.503
<u>CR</u>	PR	28	999	1.212632	0.199	0.3165
<u>CR</u>	SD	25	999	1.146111	0.211	0.3165
<u>PD</u>	PR	40	999	1.723926	0.06	0.18
<u>PD</u>	SD	37	999	2.28916	0.015	0.09
<u>PR</u>	SD	49	999	1.08105	0.322	0.3864

### ANCOM diversity analysis

```
qiime feature-table filter-samples --i-table table-dada2.qza --m-metadata-file metadata.tsv --o-filtered-table nmrl-table.qza
```

```
qiime composition add-pseudocount --i-table nmrl-table.qza --o-composition-table comp-nmrl-table.qza
```

```
qiime composition ancom --i-table comp-nmrl-table.qza --m-metadata-file metadata.tsv --m-metadata-column answer --o-visualization ancom-answer.qzv
```



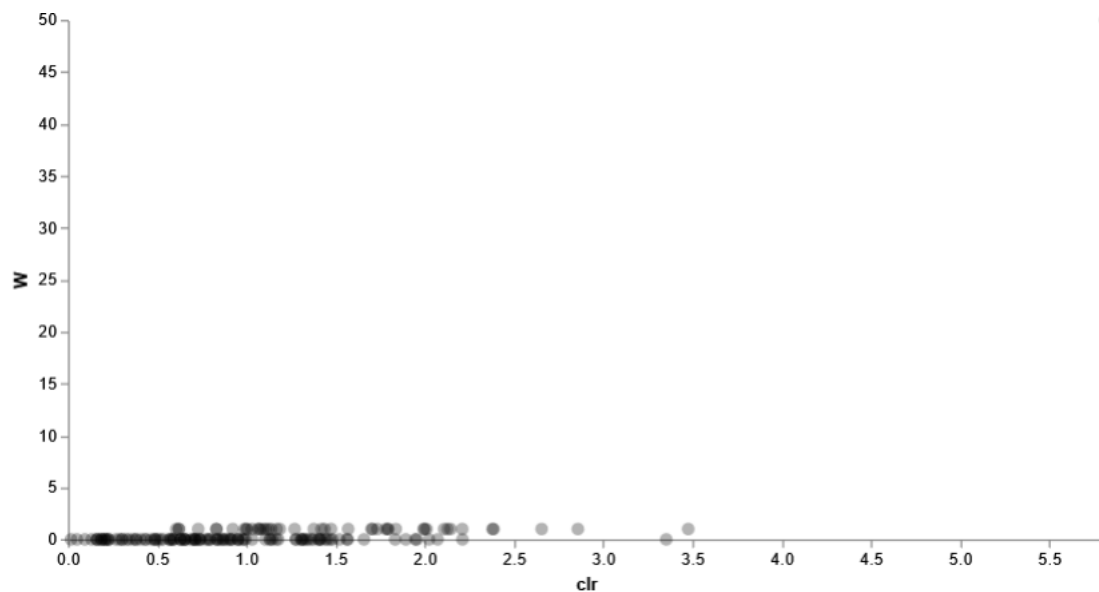
#### ANCOM statistical results

Aa Property	# W
<a href="#">8cfe521992ddb4e0f6135d565177430</a>	7977
<a href="#">31edfa54e028429a52fdc8493a10b7c1</a>	7778
<a href="#">cd348c9798781ea702c36ca56b53981f</a>	7609
<a href="#">2caff55d1333461efc2ba3148d9be5c4</a>	7427
<a href="#">26c8d6ad6e8d7a24c495a82b4fd20867</a>	7395
<a href="#">fb669f41e001ec53df6a80d55696757e</a>	7103

```
qiime taxa collapse --i-table ./metadata/nmrl-table.qza --i-taxonomy taxonomy.qza --p-level 6 --o-collapsed-table ./metadata/nmrl-table-l6.qza
```

```
qiime composition add-pseudocount --i-table ./metadata/nmrl-table-l6.qza --o-composition-table ./metadata/comp-nmrl-table-l6.qza
```

```
qiime composition ancom --i-table ./metadata/comp-nmrl-table-l6.qza --m-metadata-file ./metadata/metadata.tsv --m-metadata-column answer --o-visualization ./metadata/l6-ancom-nmrl.qzv
```



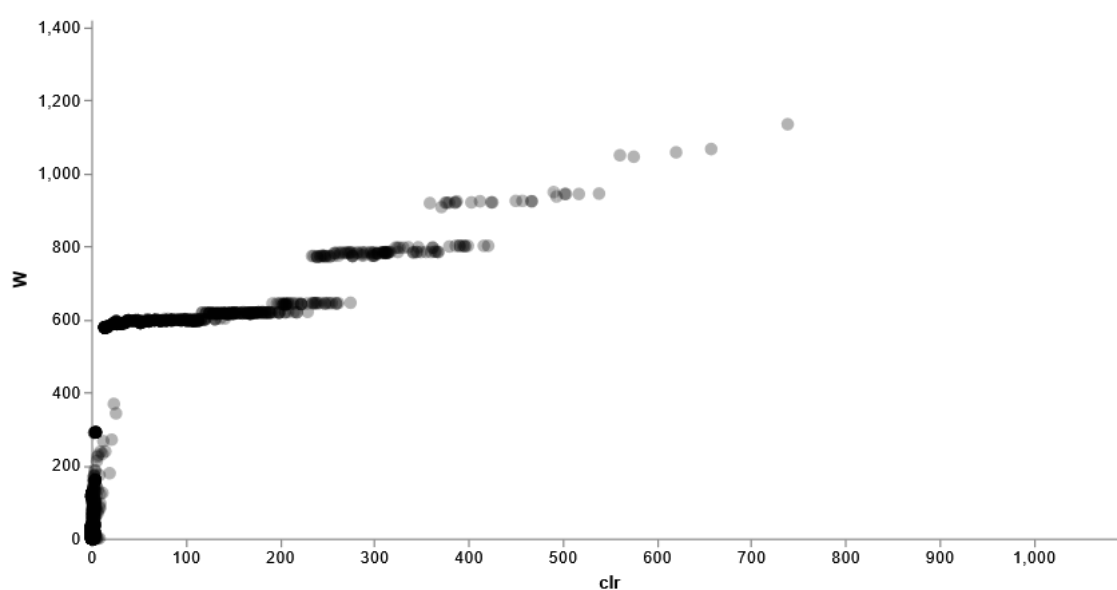
#### ANCOM statistical results

Property	# W
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae;g_Anaerosinus	50

```
qiime feature-table filter-samples --i-table table-dada2.qza --m-metadata-file metadata.tsv --p-where "answer='PD'" --o-filtered-table gut-table.qza
```

```
qiime composition add-pseudocount --i-table gut-table.qza --o-composition-table comp-gut-table.qza
```

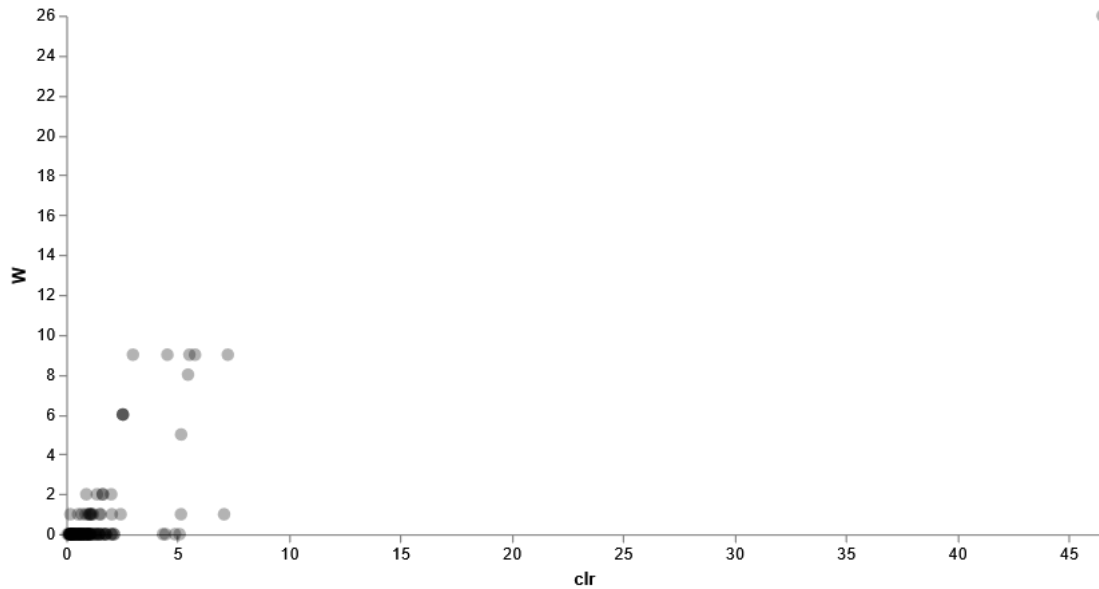
```
qiime composition ancom --i-table comp-gut-table.qza --m-metadata-file metadata.tsv --m-metadata-column new_diagnosis --o-visualization ancom-Subject.qzv
```



```
qiime taxa collapse --i-table ./metadata/gut-table.qza --i-taxonomy taxonomy.qza --p-level 6 --o-collapsed-table ./metadata/gut-table-l6.qza
```

```
qiime composition add-pseudocount --i-table gut-table-l6.qza --o-composition-table comp-gut-table-l6.qza
```

```
qiime composition ancom --i-table ./metadata/comp-gut-table-l6.qza --m-metadata-file ./metadata/metadata.tsv --m-metadata-column new_diagnosis --o-visualization ./metadata/l6-ancom-Subject.qzv
```



Aa Property	# W	<input checked="" type="checkbox"/> Reject null hypothesis
<a href="#">k_Bacteria;p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae;g_Eggerthella</a>	26	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae;g_Alloscardovia</a>	9	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Leuconostocaceae;g_Weissella</a>	9	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Fusobacteria;c_Fusobacteriia;o_Fusobacteriales;f_Fusobacteriaceae;g_</a>	9	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Leuconostocaceae;g_Oenococcus</a>	9	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Fusobacteria;c_Fusobacteriia;o_Fusobacteriales;f_Leptotrichiaceae;_</a>	9	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Bacteroidetes;c_Cytophagia;o_Cytophagales;f_Cyclobacteriaceae;_</a>	8	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae;_</a>	6	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rickettsiales;f_mitochondria;_</a>	6	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Corynebacteriaceae;g_Corynebacterium</a>	6	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;_</a>	5	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;_</a>	2	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae;g_Pseudomonas</a>	2	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;_;</a>	2	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales;_;</a>	2	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Planctomycetes;c_Planctomycetia;o_Pirellulales;f_Pirellulaceae;_</a>	2	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae;g_Leptolinea</a>	1	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Bejerinckiaciaceae;_</a>	1	<input checked="" type="checkbox"/>
<a href="#">k_Bacteria;p_Bacteroidetes;c_Cytophagia;o_Cytophagales;f_Cyclobacteriaceae;g_Echinicola</a>	1	<input checked="" type="checkbox"/>

Aa Property	# W	<input checked="" type="checkbox"/> Reject null hypothesis
<u>k_Bacteria;p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_cc_115</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae;g_Clostridium</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae;g_Alistipes</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae;g_Petrimonas</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Carnobacteriaceae;g_Isobaculum</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Marinilabiaceae;g_Cytophaga</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_g__</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Actinobacteria;_i;_i;_i</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Anaerotruncus</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Oxalobacteraceae;_g</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pasteurellales;f_Pasteurellaceae;g_Mannheimia</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae;g_Oscillospira</u>	1	<input checked="" type="checkbox"/>
<u>k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae;g_Robinsoniella</u>	1	<input checked="" type="checkbox"/>

## Diversity analysis for dada2 pipeline

```
biom convert -i dada_normalized.tsv -o dada.biom --to-hdf5
```

```
qiime tools import \
  --input-path dada.biom \
  --type 'FeatureTable[Frequency]' \
  --input-format BIOMV210Format \
  --output-path dada.qza
```

```
qiime feature-table summarize \
  --i-table dada.qza \
  --o-visualization dada_feature.qzv \
  --m-sample-metadata-file metadata.tsv
```

### Table summary

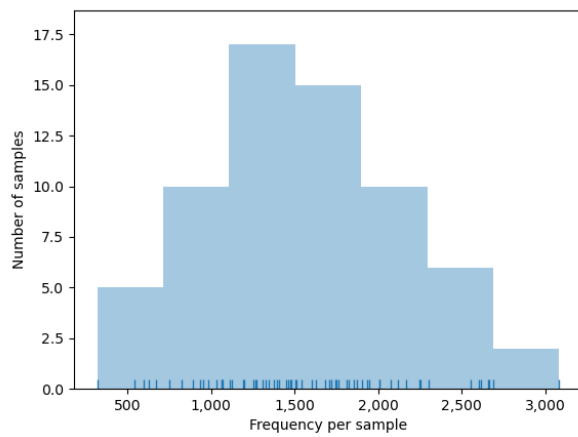
Aa Metric	Sample
<u>Number of samples</u>	65
<u>Number of features</u>	89
<u>Total frequency</u>	102,120

### Frequency per sample

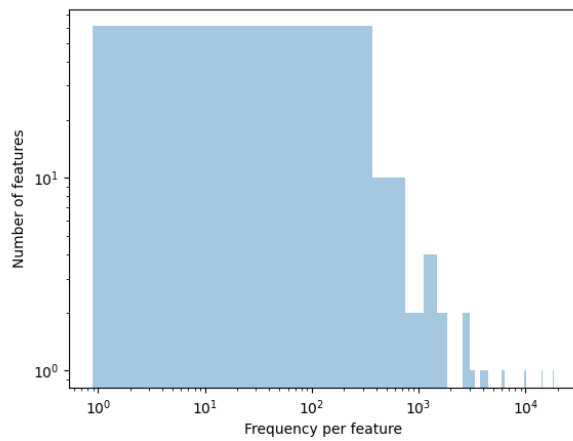
Aa Property	Frequency
<u>Minimum frequency</u>	318.3618967111167
<u>1st quartile</u>	1,127.1998461509056
<u>Median frequency</u>	1,506.7266086264692
<u>3rd quartile</u>	1,932.1853385551474
<u>Maximum frequency</u>	3,084.4565670690517
<u>Mean frequency</u>	1,571.0828567184328

### Frequency per feature

Aa Property	Frequency
-------------	-----------



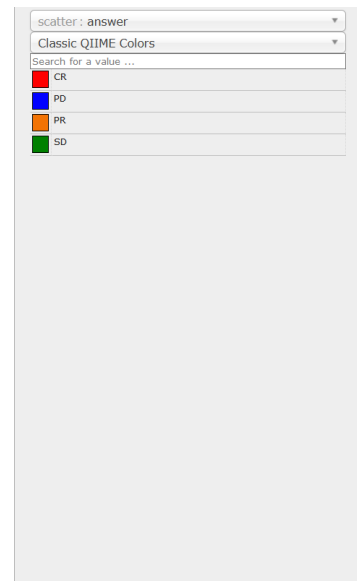
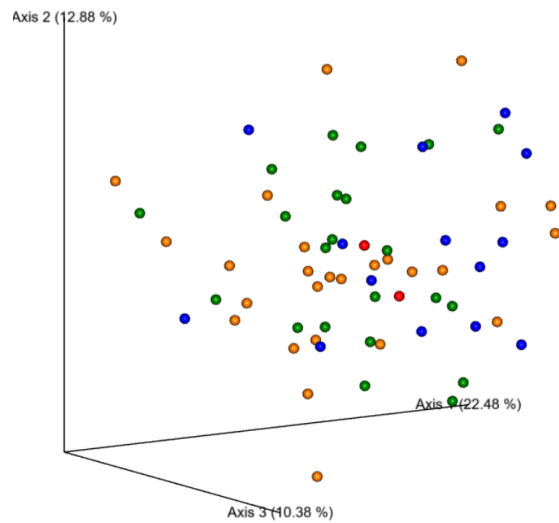
Property	Frequency
<u>Minimum frequency.</u>	0.879084641372472
<u>1st quartile</u>	9.897050753357469
<u>Median frequency.</u>	51.59282314049115
<u>3rd quartile</u>	536.4770776069847
<u>Maximum frequency.</u>	18,620.901263924585
<u>Mean frequency.</u>	1,147.4200638954844



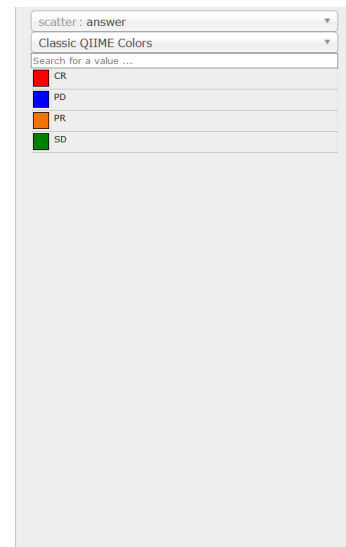
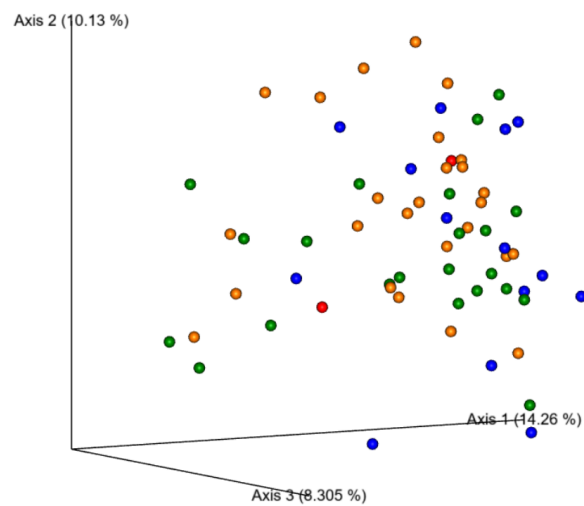
```
qiime diversity core-metrics \
  --i-table dada.qza \
  --m-metadata-file metadata.tsv \
  --output-dir phylo \
  --p-sampling-depth 500
```

Clusters by bray-curtis distances:

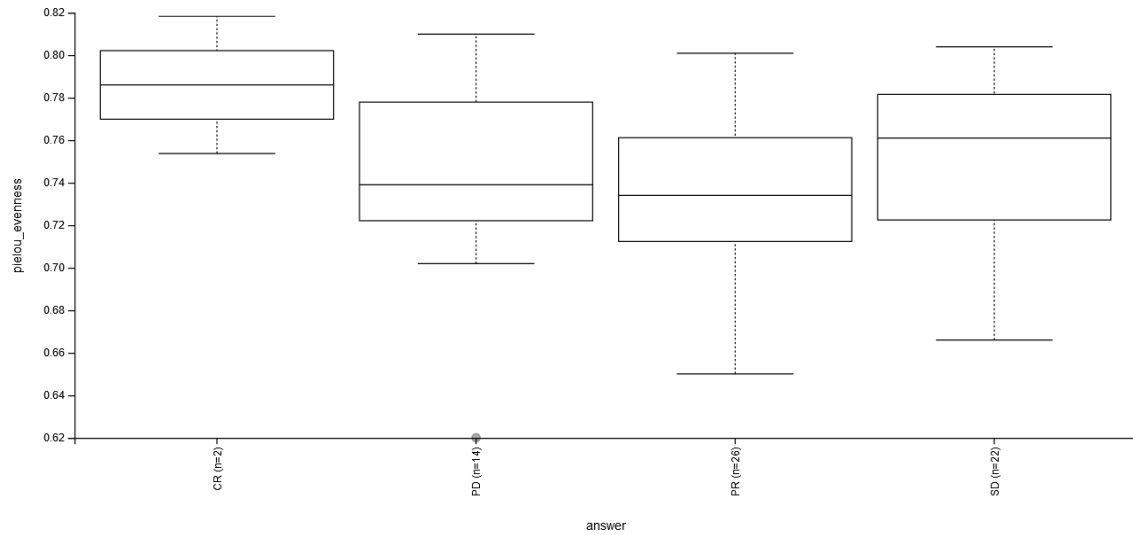




Clusters by jacard distances:



```
qiime diversity alpha-group-significance \
  --i-alpha-diversity ./phylo/evenness_vector.qza \
  --m-metadata-file metadata.tsv \
  --o-visualization ./phylo/evenness-group-significance.qzv
```



#### Kruskal-Wallis (all groups)

Aa Property	# Result
H	4.98217455621301
p-value	0.17310707394219432

#### Kruskal-Wallis (pairwise)

Aa Property	1	H	p-value	q-value
Group 1	Group 2			
CR (n=2)	PD (n=14)	1,613445	0,204008	0,408016
CR (n=2)	PR (n=26)	2,578249	0,108341	0,325024
CR (n=2)	SD (n=22)	1,090909	0,29627	0,444405
PD (n=14)	PR (n=26)	0,463147	0,496157	0,496157
PD (n=14)	SD (n=22)	0,557038	0,455456	0,496157
PR (n=26)	SD (n=22)	3,166548	0,075161	0,325024