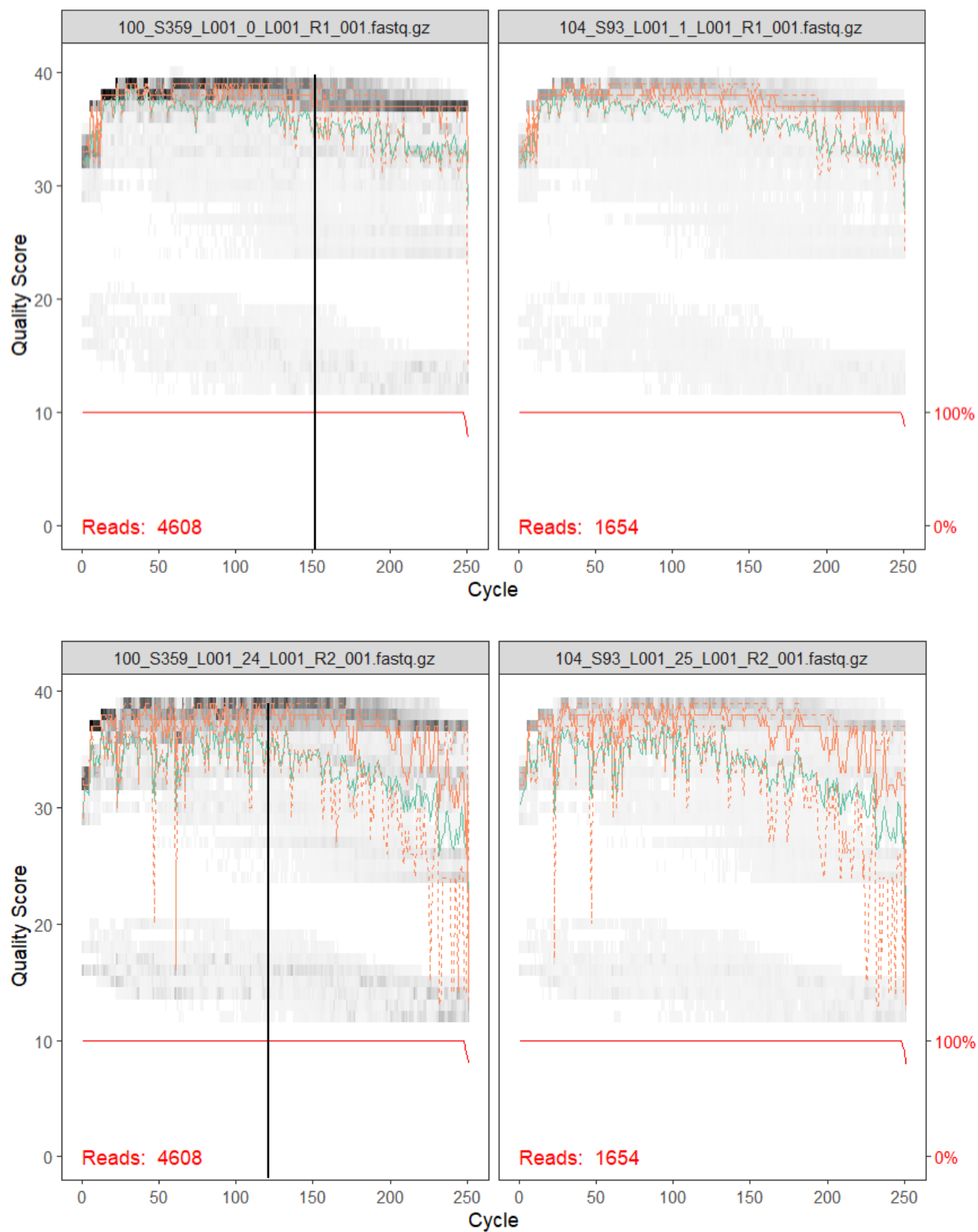


Group 2 - Microbiome Section Report

1. Include a screenshot of your interactive quality plot.



Based on the plots above, what values would you choose for `--p-trunc-len` and `--p-trim-left` for both the forward and reverse reads? Why have you chosen those numbers?

For forward (R1) reads, the truncated length would be approximately 150, while the trim length would be approximately 15. This is because the quality begins dropping after 150 cycles.

For reverse (R2) reads, the truncated length would be approximately 120, while the trim length would be 0. Although the quality on the reverse reads are bad from observation, the quality seems to drop at 120.

2. How would you modify the code above to truncate and trim in your desired way?

```
qiime dada2 denoise-single \
qiime dada2 denoise-paired --i-demultiplexed-seqs demux.qza --p-trim-left-f 15 --
p-trunc-len-f 150 --p-trim-left-r 0 --p-trunc-len-r 120 --o-representative-sequences
rep-seqs.qza --o-table table.qza --o-denoising-stats stats.qza
```

3. In the tutorial, you had to `mv` the files to rename them to just `rep-seqs.qza`, `table.qza`, and `stats.qza`. How could you modify the above code to skip that step? How do you need to modify `qiime metadata tabulate` in order to account for the renamed files being generated?

I would modify the code by simply excluding the 'dada2' portion from the tutorial.

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

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

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

4. Your metadata file has a different name than that in the tutorial. How do you adjust your code in order to use the metadata file you have been given?

I adjusted the following code by adjusting the names of the files I personalized
qiime feature-table summarize \

--i-table table.qza \

--o-visualization table.qzv \

--m-sample-metadata-file **metadata.tsv**

qiime feature-table tabulate-seqs \

--i-data rep-seqs.qza \

--o-visualization rep-seqs.qzv

5. Table summary screenshot:

sample-id	input	filtered	percentage of input passed filter	denoised	merged	percentage of input merged	non-chimeric	percentage of input non-chimeric
id	numeric	numeric	numeric	numeric	numeric	numeric	numeric	numeric
100_S359_L001	4608	4537	98.46	4474	4365	94.73	4315	93.64
104_S93_L001	1654	1625	98.25	1584	1474	89.12	1441	87.12
106_S98_L001	72586	71814	98.94	71247	69609	95.9	67666	93.22
125_S13_L001	4058	4007	98.5	3963	3798	93.36	3759	92.4
128_S36_L001	36568	36196	98.98	36051	35433	96.9	34644	94.74
13_S95_L001	56433	55818	98.91	55226	53684	94.95	52396	92.83
163_S60_L001	20712	20512	99.03	20446	20118	97.13	19924	96.2
168_S37_L001	10109	9916	98.09	9795	9587	94.84	9356	92.55
174_S146_L001	52670	51964	98.66	51529	49816	94.58	48598	92.27
189_S23_L001	575	556	96.7	519	497	86.43	497	86.43
18_S71_L001	44037	43465	98.7	43090	42316	96.09	39890	90.58
212_S94_L001	34796	34426	98.94	34281	33707	96.87	33199	95.41
245_S122_L001	21098	20867	98.91	20525	19929	94.46	19588	92.84
254_S69_L001	11041	10886	98.6	10809	10617	96.16	10552	95.57
265_S133_L001	7976	7858	98.52	7665	7390	92.65	7124	89.32
307_S70_L001	39942	39516	98.93	39352	38485	96.35	37934	94.97
309_S47_L001	42840	42298	96.73	41467	39503	92.21	38397	89.63
364_S22_L001	49724	49227	99	48861	47591	95.71	46518	93.55
366_S45_L001	31030	30656	98.79	30299	29395	94.73	28483	91.79
385_S170_L001	22038	21766	98.77	21484	20771	94.25	20393	92.54

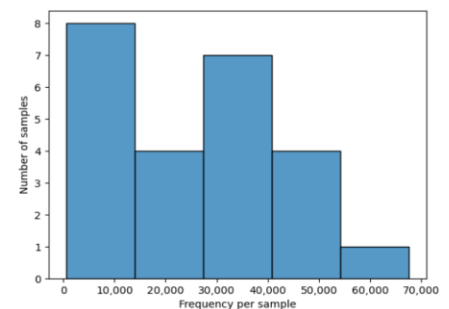
Screenshot of the sequence length statistics from the rep-seqs file:

Table summary

Metric	Sample
Number of samples	24
Number of features	6,233
Total frequency	641,599

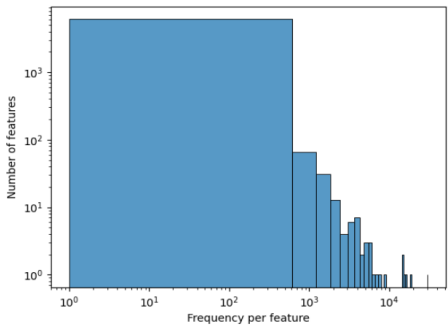
Frequency per sample

	Frequency
Minimum frequency	497.0
1st quartile	10,253.0
Median frequency	24,873.0
3rd quartile	38,770.2
Maximum frequency	67,666.0
Mean frequency	26,733.3



Frequency per feature

	Frequency
Minimum frequency	1.0
1st quartile	10.0
Median frequency	22.0
3rd quartile	52.0
Maximum frequency	30,368.0
Mean frequency	102.9



6. Taxonomic Visualizations: Top hits (top) versus Low hits (bottom)

Feature ID	Taxon	Confidence
9a29paca	Ecological	Ecological
366bcc4b44c2de8220e6a78715e8b1a	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	1.00000000000000069
c53c3145bccb762a8796bd0c51d7826	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	1.00000000000000058
94c59a44ac7a6a72467ac54c79724b1b	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	1.00000000000000056
07a45fc2ab5a10a9a91614a890c95b7d	K__Bacteria; p__Fibrobacteres; c__Fibrobacteria; o__258ds10; f__g__; s__	1.0
4e8bc72b3ed5b44e334189271e6759a	K__Bacteria; p__Fibrobacteres; c__Fibrobacteria; o__258ds10; f__g__; s__	1.0
5875f3188620ae1628c89d139e5f07	K__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cytophagaceae; g__Emticia; s__	1.0
773872719af2c8615c155061e828aca3	K__Bacteria; p__Ammatimonadetes; c__SHA-37; o__; f__; g__; s__	1.0
774599bc0996b81a9b687bcc8b4d592	K__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cytophagaceae; g__Runella; s__	1.0
85c390a58e207a8f8267d739d9ece8b	K__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Tissierellaceae]; g__Anaerococcus; s__	1.0
c9716c5ca8a62b72954c8a2230b5d	K__Bacteria; p__Fusobacteria; c__Fusobacteria; o__Fusobacteriales; f__Leptotrichiaceae; g__Leptotrichia; s__	1.0
a7edad8154bdfc47530582f5e43db7	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999991
k769f3fc3ceac3b81f75a0a388b4947	K__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatales; f__Isosphaeraceae	0.99999999999999982
f0612c9c5700cfc302af74ca675b6a	K__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatales; f__Isosphaeraceae	0.99999999999999974
d5dc1378129c121610007e11251b087	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999997
e3361e92968a84a17abe66b5b03e1c	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999969
e791b5b510b9d939c351b8b394503d	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999963
62f8da0f0e3fc5b54c65a168bde9882	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999953
902700416102a8a9a26810a5972c433c	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999955
998ff38e735f9330e7694b971066e87	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	0.99999999999999942
0738980877107517c189c4ac57c16	K__Bacteria; p__Verrucomicrobia; c__[Methylacidiphilae]; o__Methylacidiphilae; f__; g__; s__	0.9999999999999858

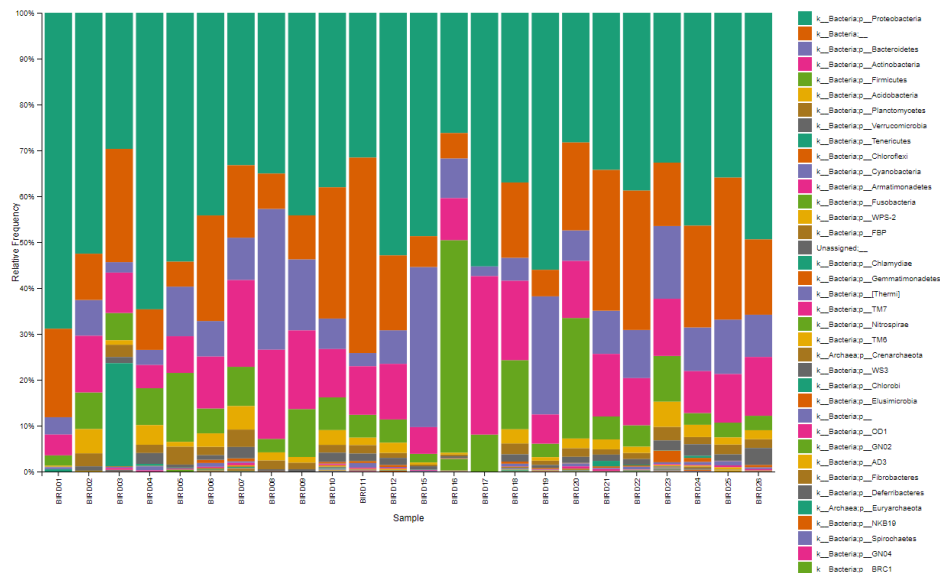
Feature ID	Taxon	Confidence
9a29paca	Ecological	Ecological
469bbc18d2696d04d5dbcf3b7d07e2	Unassigned	0.31745631200841207
17500807795ccba2aa948d7a283ce46	Unassigned	0.6785684019700632
a8efcd9d4756807c8686744d8085a23	K__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__	0.7001559384314862
7a5dadc1910e5dc4e42a567062cc3eb	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Phyllobacteriaceae; g__; s__	0.7004449528203128
8a302966694826dbc6675ef0344d192	K__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Comamonas	0.700599231696644
5ee2c452832047494e70002ac028bd1e	K__Bacteria; p__Bacteroidetes	0.7010311331521333
713e1636aa9d89772445965057b4529	K__Bacteria	0.7015512368943739
878fbda8a63e687c1b07757c4174a32	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Methylobacteriaceae; g__Methylobacterium; s__komagatae	0.701669099513035
41c0ca6a52a8dfeca2c1834860faa4	K__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Dokdonella; s__	0.7017565878987908
e51551ed35f9640025902661611324	K__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__Myxococcaceae; g__Corallococcus; s__exiguus	0.7018131346471717
326a8c7d4762fe120e432535278b96ba	K__Bacteria; p__Chlamydiae; c__Chlamydia; o__Chlamydiales; f__; g__; s__	0.7020696771361354
5689d067573279f02f56059f5c2270	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__; g__; s__	0.7021326438073767
b4c1ed7221087979c0b5b97c1e04eea	K__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardoidaceae; g__Nocardioides	0.7021560488979466
a2bb4bcca355ab70cc70ff7e435d4f	K__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacteriales]; f__[Chthoniobacteraceae]; g__Candidatus Xiphinematobacter; s__	0.7022612361017069
e022a56289e996365f6c334547e7126	K__Bacteria; p__Proteobacteria	0.702264385433073
44c04eba35b2c8aa73fe40e58e719	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Novosphingobium	0.7024998033544733
38bf71056228a3e78c3f95e2810723a	K__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus	0.7027697847171561
7c645243c70e20cc98a1a3eaddf04	K__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__; s__	0.7029684576291164
0af451ca6c9eb9e057c322b00dd026	K__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Chlorophyta	0.7030227542834645
e84428170c094f20d4a2aa07e8b0f02b	K__Bacteria; p__; c__; o__; f__; g__; s__	0.7030961222213472

7. The parameters of the code excluded mitochondrial and chloroplast genetic information. This is integral because the specimen are prokaryotes, and the mitochondria and chloroplast contain their own genetic material, because they're endosymbionts. Mitochondrial and chloroplast genetic material may act as contaminants in the sample data.
8. Taxonomic Visualizations – Updated. There are differences in the hit, because the code eliminated contaminants of eukaryotic genetic material by excluding mitochondria and chloroplast genes. Compared to the previous output, mitochondria are no longer listed in the taxon.

Feature ID #Seq-type	Taxon Category	Confidence Category
0015850ab7998535e34b6417f303cb	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Alteromonadales; f__Alteromonadaeae; g__Cellvibrio; s__	0.9999997165794074
001bac90cc4b188a67ab821d8a51f0	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Burkholderiaceae; g__Burkholderia; s__	0.9598987797134925
001ec447b7301455b84ae4933028a5e4	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__SC-I-84; f__g__; s__	0.9912247820583873
003833e6957b2394ee6a208460159fc	k__Bacteria; p__Fusobacteria; c__Fusobacteria; o__Fusobacteriales; f__Leptotrichiaceae; g__Leptotrichia; s__	0.9999999999991773
00390869269b08281ade39574ba8403	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Corynebacteriaceae; g__Corynebacterium; s__	0.9999039191499205
00428e767c96d901b84b63759da8f30	k__Bacteria; p__Acidobacteria; c__Acidobacteria; o__Acidobacteriales; f__Acidobacteriaceae	0.9999999999678981
005922123231cf1a00524e728cfa03e	k__Bacteria; p__Actinobacteria; c__Thermoleophila; o__Solirubrobacterales	0.999999991811199
005c8e6c3b12cb1f0cf9eafca7c62e	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardioidaceae; g__Nocardioides	0.7863254520905945
005f6ab7d9d1cb57dfc7e1517e7fa01	k__Bacteria; p__Planctomycetes; c__Phycisphaerae; o__WD2101; f__g__; s__	0.99999999997276916
0063547c822e895efb27539a202732	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Paenibacillaceae; g__Paenibacillus; s__	0.9860732396232434
006495c43a9c8185073737db769e4	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Sinobacteraceae; g__s__	0.99999992408582766
006957bc02761ae38f85feabaf864	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria	0.9958309249614337
0079521da298637a27f35691d35787	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Ellin329; f__g__; s__	0.9999986373263043
008067eaa159bce92a2a70743cabbce	k__Bacteria; p__Actinobacteria; c__Acidimicrobia; o__Acidimicrobiales; f__g__; s__	0.9999321582693513
00820c5cc02ea194d3c57c8e7482285	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Natronobacillus; s__	0.9160214152297208
008c1596f6a6138f5395dfbccc494a6	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatiales; f__Gemmataceae; g__s__	0.9976574667031609
0092fa5cc36c996b2282e040b7f4cf	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria	0.9889229423052268
0095a18cd04ab403c41771f036ee76d8	k__Bacteria	0.9797421602573498
009fb56356576bd3c5bad880748d14c	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coproccoccus; s__	0.7416541802540727
00a3fa780a09050c43ebcd1ae1506e28	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Kineosporiaceae; g__s__	0.9484528493982899
Feature ID #Seq-type	Taxon Category	Confidence Category
469bbc818d2696d046d0bc7b7d07e2	Unassigned	0.31745631200841207
775008077955cca2aa948d7a283ce46	Unassigned	0.6785684019700632
a8efcd9d4756807c686674448085a23	k__Bacteria; p__Bacteroidetes; c__[Saprosirae]; o__[Saprosirales]; f__Chitinophagaceae; g__s__	0.7001559384314862
7a5dacc1910e5dc4e4f2a587082cc3eb	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiaceae; g__s__	0.7004449528203128
8a302966694826db5c6578c344d192	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Comamonas	0.700599231696644
5ee2c452832d47494e70002acd28bd1e	k__Bacteria; p__Bacteroidetes	0.7010311331521333
713e1636aa9d89772445985057b4529	k__Bacteria	0.7015512368943739
878fbda8a3e5e87c1b07757c4174a32	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Methylobacteriaceae; g__Methylobacterium; s__komagatae	0.701669099513035
41c0ca625a8dfca2c18349600faa4	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Dakdonella; s__	0.7017565878987908
e51551ed35fb96400259002661611324	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__Myxococcaceae; g__Coralococcus; s__exiguus	0.7018131346471717
326a8c7d4762fe120e432535278b96ba	k__Bacteria; p__Chlamydiae; c__Chlamydia; o__Chlamydiales; f__g__; s__	0.7020696771561354
5689ad675732799d2f56059f575c270	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__g__; s__	0.7021326438073767
b4c1ed7221087979c5b97c1e04eea	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardioidaceae; g__Nocardioides	0.7021560488979466
a27b4bcbca3554b70cc77d7e43504f	k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacteriales]; f__[Chthoniobacteraceae]; g__Candidatus Xiphinematobacter; s__	0.7022612361017069
e022a5628e9b963658f3c34547e7126	k__Bacteria; p__Proteobacteria	0.702264385433073
44c04eba35b2cfaa73f640e58e719	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Novosphingobium	0.702499803544733
380f7105622a32e78c3f95e2810723a	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Bacillus	0.7027698747171561
7c645243c70c29c98a1a3efad9b4	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__s__	0.7029684576291164
0af5451ca6c9eb9e057c322000db26	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Chlorophyta	0.7030227542834645
e84428170c9492064a2aa07e8b0f2b	k__Bacteria; p__c__; o__c__; f__g__; s__	0.7030961222213472

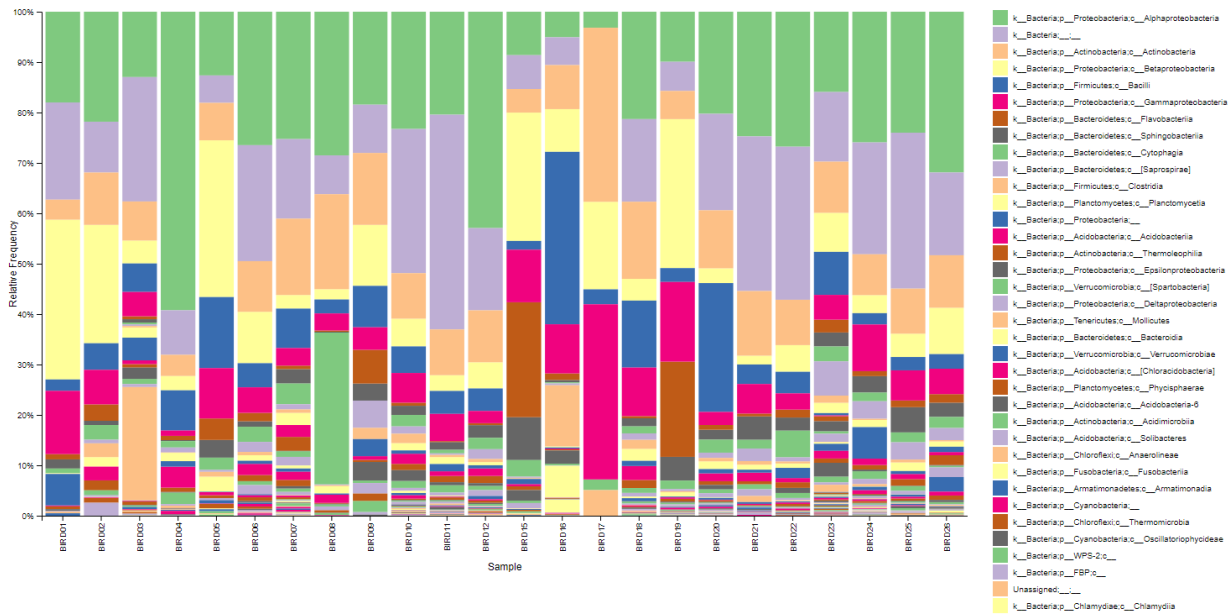
9. Looking at taxa bar plots, what are your top 2 phyla?

The top 2 phyla are Proteobacteria and technically, an unknown phylum, but the third highest frequency are Bacteroidetes.



The top 5 most abundant classes are Alphaproteobacteria, Actinobacteria, Betaproteobacteria, Bacilli, and Gammaproteobacteria.

BIRD26 | k__Bacteria.p__Cyanobacteria.c__4C0d-2 | 0.106%



10. What is the difference between alpha and beta diversity? You will have to read outside resources to answer this question. Your response should be in your own words.

- Alpha diversity is an analysis of the species in a community and their distributions. On the other hand, beta diversity is the analysis of how similar the different species samples are.

11. Before you calculate your diversity metrics, you have to choose a sampling depth. What file previously generated will you use to help you determine what to choose? Defend your choice of sampling depth. How many samples do you retain and how many do you lose?
- The file that helped determine the sampling depth would be the 'table.qzv' file.
 - Based on the table.qzv file that was generated, I concluded that the sampling depth for this data set would be 6741. Though I originally wanted a retention rate of approximately 50%. Unfortunately, though it's possible to have a retention rate of 50%, these features would've represented 10 out of the 24 samples, less than half the samples, so that wasn't ideal. At least with a sampling depth of 6741, although retention isn't high, at least 20 of the 24 samples would have been represented.
 - With the sampling depth of 6741, the results Retained 134,820 (22.34%) features in 20 (83.33%) samples. This means that 77.66% features in 20 samples were lost.
12. For alpha diversity, you need to create visualizations for Shannon diversity and Observed features. This will require you to modify the `alpha-group-significance` code. For which metadata values were graphs generated? Were any of those comparisons significant? How do you know whether they were or were not significant? Briefly describe what Shannon diversity and Observed features are measuring (less than 1 paragraph).

Of the metadata values, graphs were generated for the following: population, sex, and flock. Though the comparisons between the populations (migratory and resident) and sexes (female versus male) metadata displayed no significance, the comparison between the flock displayed significance in both Shannon Diversity and Observed features. The significance is measured by p-value, values less than 0.05 are considered significant.

Between migratory and resident samples from both Shannon and Observed, the p-value was about 0.18367, displaying no significant comparisons between the samples; between the female versus male samples, the p-value was 1.0 in Shannon, and 0.289918 in Observed, displaying no significant comparisons between the samples; however, between the different flocks, the most notable significant value in both Shannon and Observed lied between the migratory females and resident females with a p-value of 0.033006. Interestingly though, Observed additionally displayed a significant difference between migratory females and

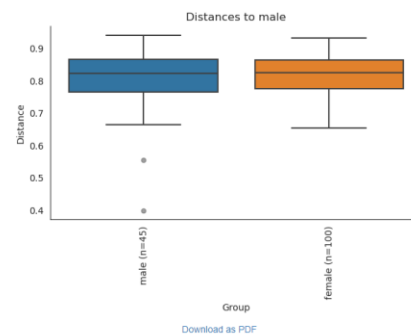
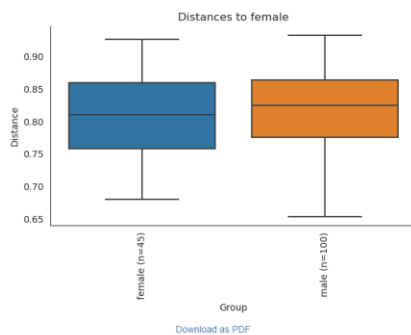
resident males with a p-value of 0.027486 (Shannon had an output of a p-value of 0.086411 for this comparison).

13. For beta diversity, you will need to create visualizations for Bray Curtis dissimilarity. This will require your to modify the `beta-group-significance` code. You should have one visualization for sex, one for population, and one for flock. Include a screenshot of each visualization. Is there any significance? Regardless of significance, how can you interpret these results (hint: what is beta diversity looking at?)

Sex:

Group significance plots

[Download raw data as TSV](#)

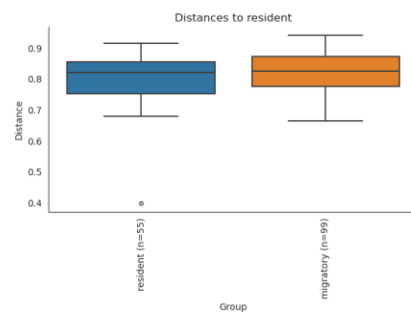
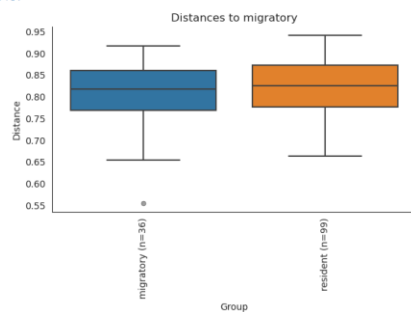


There is no significant difference between female and male samples, considering the p-value of 0.168. This implies that the microbiome of females and males are similar.

Population:

Group significance plots

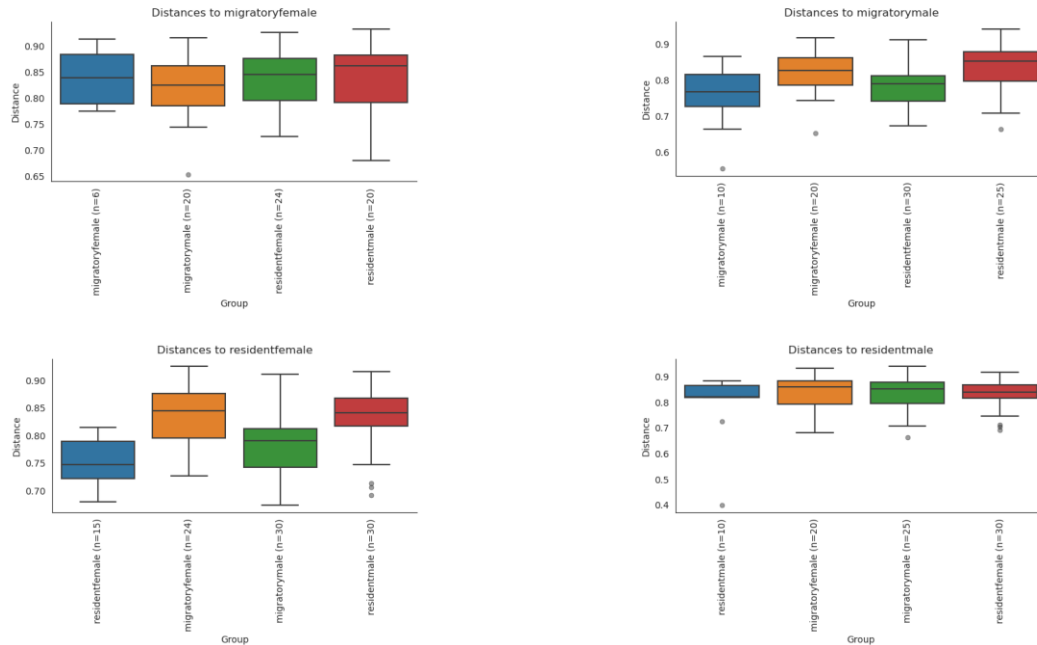
[Download raw data as TSV](#)



There is a significant difference between the migratory and resident populations, considering the p-value of 0.025. This implies that the microbiome between migratory and resident populations are different.

Flock:

Group significance plots

[Download raw data as TSV](#)


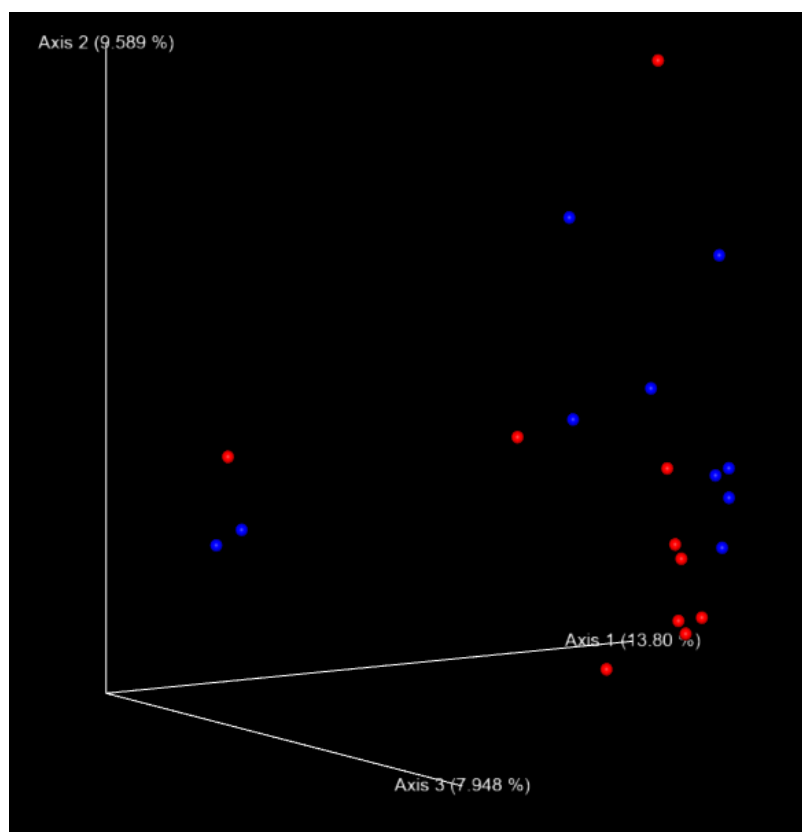
There is a significant difference between migratory females versus resident females considering the p-value of 0.006. This implies that the microbiome between migratory females, migratory males, and resident males are similar, while the microbiome between migratory females and resident females are different.

There is a significant difference between migratory males versus resident females AND males, considering the respective p-values of 0.005 and 0.015. This implies that the microbiome between migratory males and resident females and males are different.

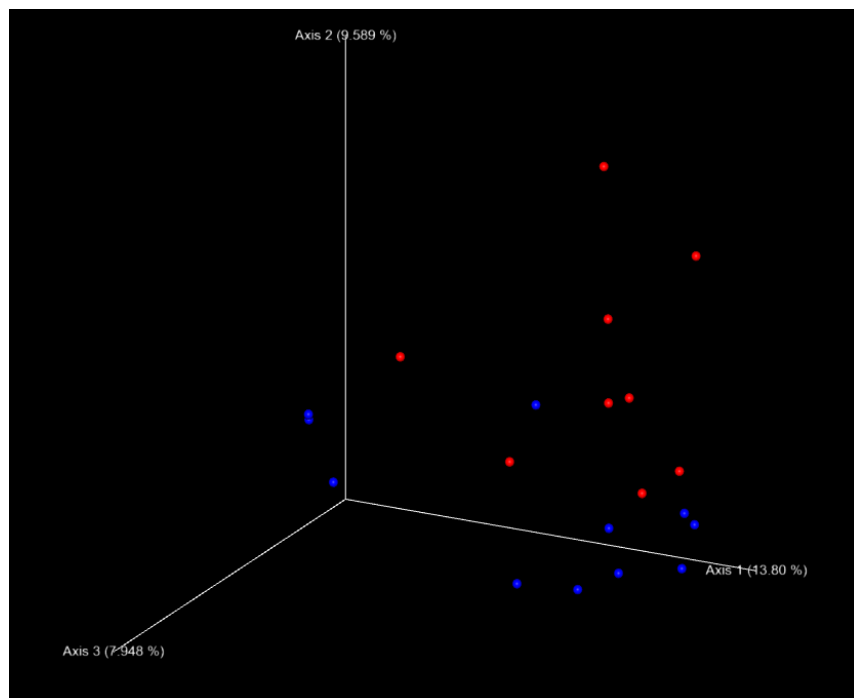
There is a significant difference between resident females and resident males, considering the p-value of 0.008. This implies that the microbiome of the resident females versus resident males are drastically different.

- The `core-metrics-phylogeny` command generates a file called `bray-curtis-empower.qzv`. Include 3 screenshots total (1 where the points are colored based on sex, one on population, one on flock).

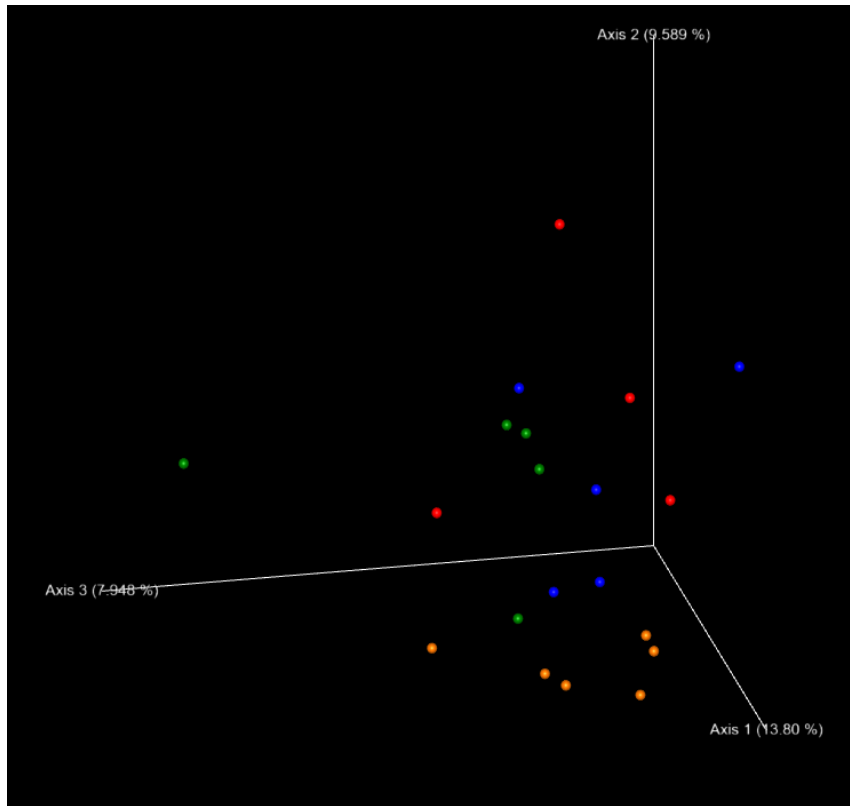
On sex - Female; Male



On population – Migratory; Resident



On flock – migratory female, migratory male, resident female, resident male



How do these results help you make sense of the results you got from question 13?

These results help me make sense of the results I achieved from Q13 because EMPeror provides a visual aid on the distance between the specific samples of interest.