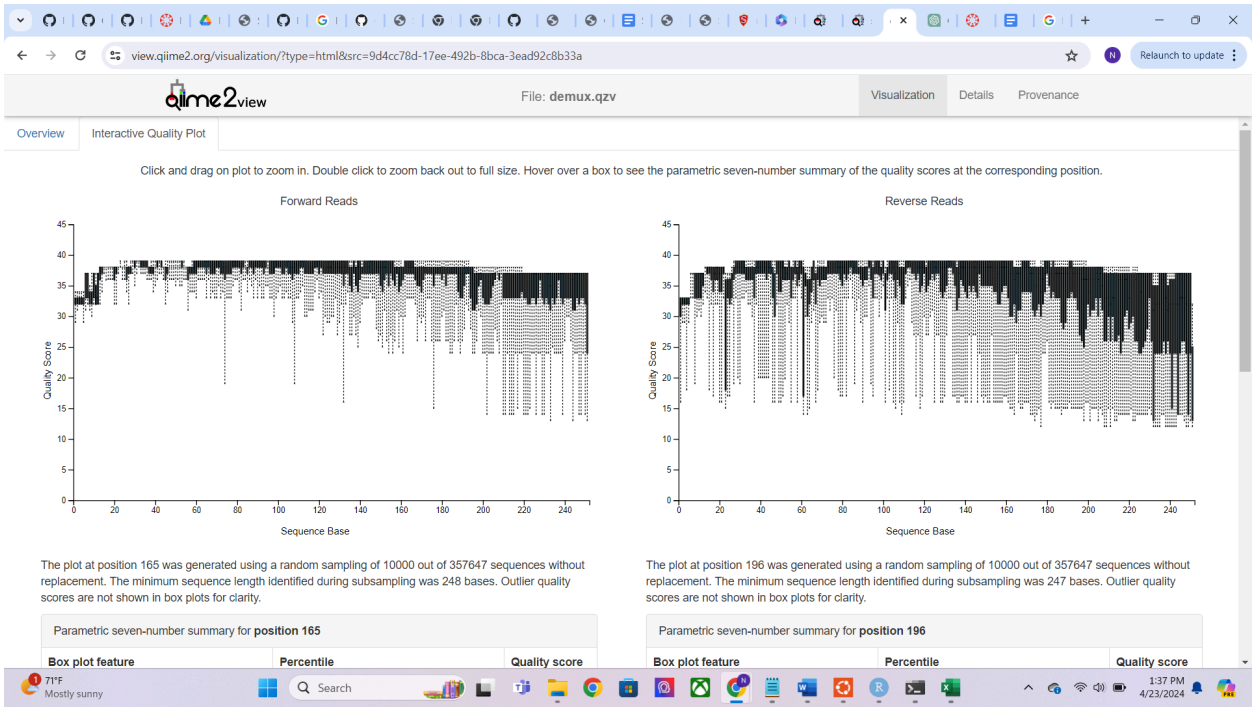
1) Include a screenshot of your interactive quality plot. Based on this plot, what values would you choose for `--p-trunc-len` and `--p-trim-left` for both the forward and reverse reads? Why have you chosen those numbers? **10-240 on the forward read and 10-220.**



2) How would you modify the code above to truncate and trim in your desired way? By adding in the numbers you want. (This is how, below)

```
qiime dada2 denoise-paired \
  --i-demultiplexed-seqs demux.qza \
  --p-trim-left-f 10 \
  --p-trunc-len-f 240 \
  --p-trim-left-r 10 \
  --p-trunc-len-r 220 \
  --o-representative-sequences rep-seqs-dada2.qza \
  --o-table table-dada2.qza \
  --o-denoising-stats stats-dada2.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?

**qiime metadata tabulate **
**--m-input-file stats-dada2.qza **
--o-visualization stats-dada2.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?

**qiime quality-filter q-score **

**--i-demux demux.qza **

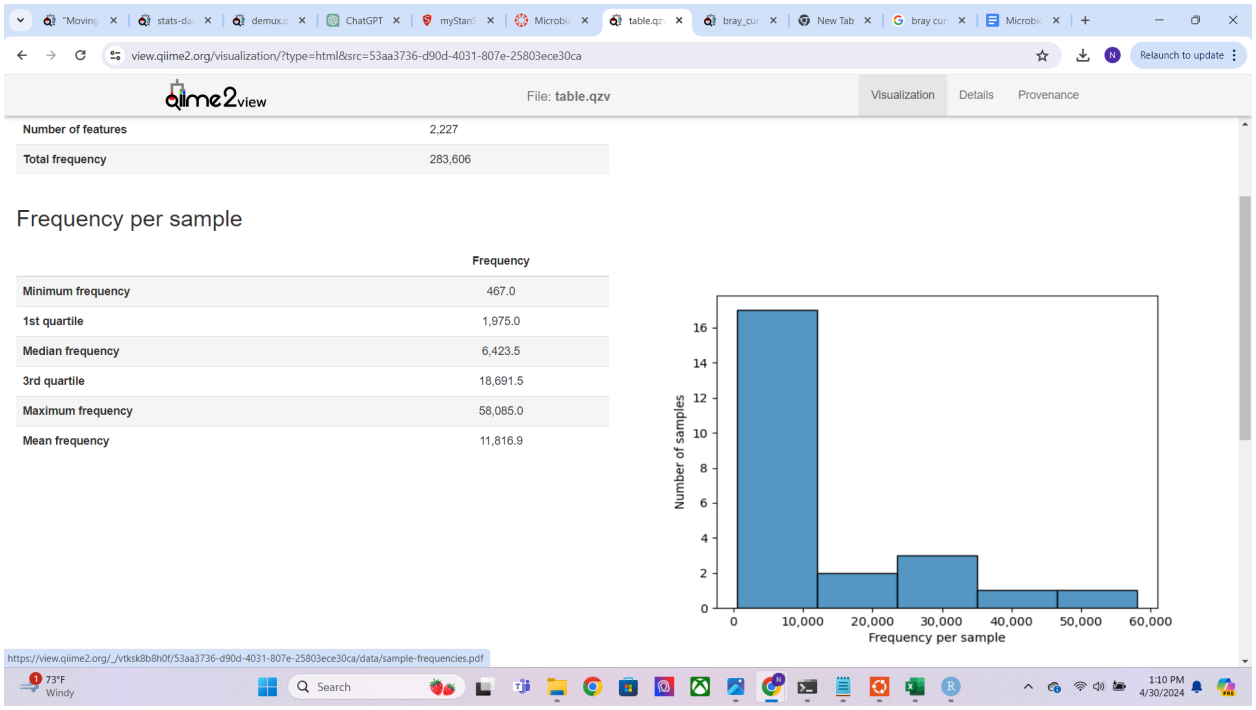
**--o-filtered-sequences demux-filtered.qza **

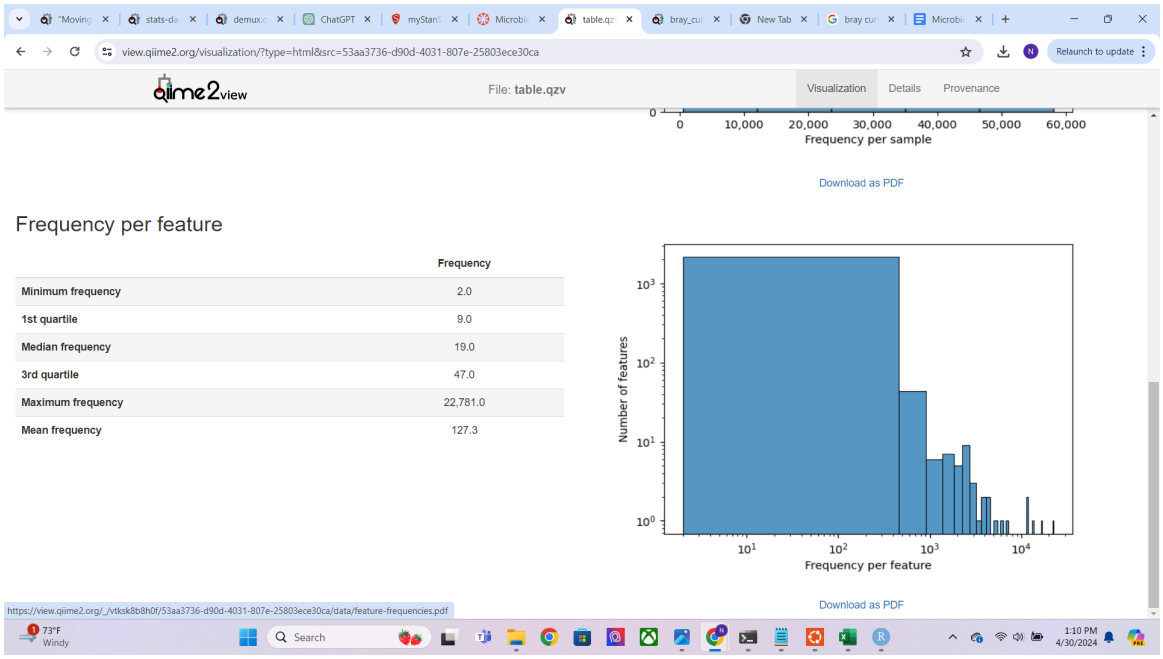
--o-filter-stats demux-filter-stats.qza

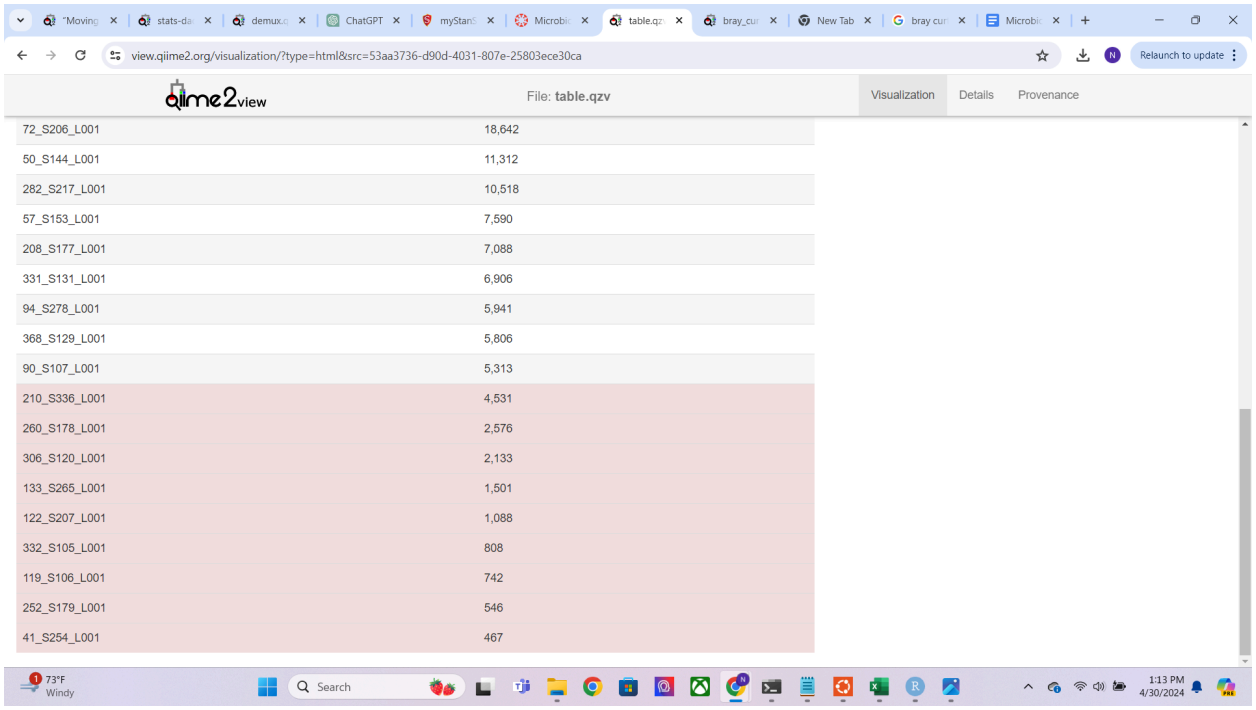
5) Include a screenshot of the table summary from visualizing your table and a screenshot of the sequence length statistics from the rep-seqs file.

210_S336_L001	4,531
260_S178_L001	2,576
306_S120_L001	2,133
133_S265_L001	1,501
122_S207_L001	1,088

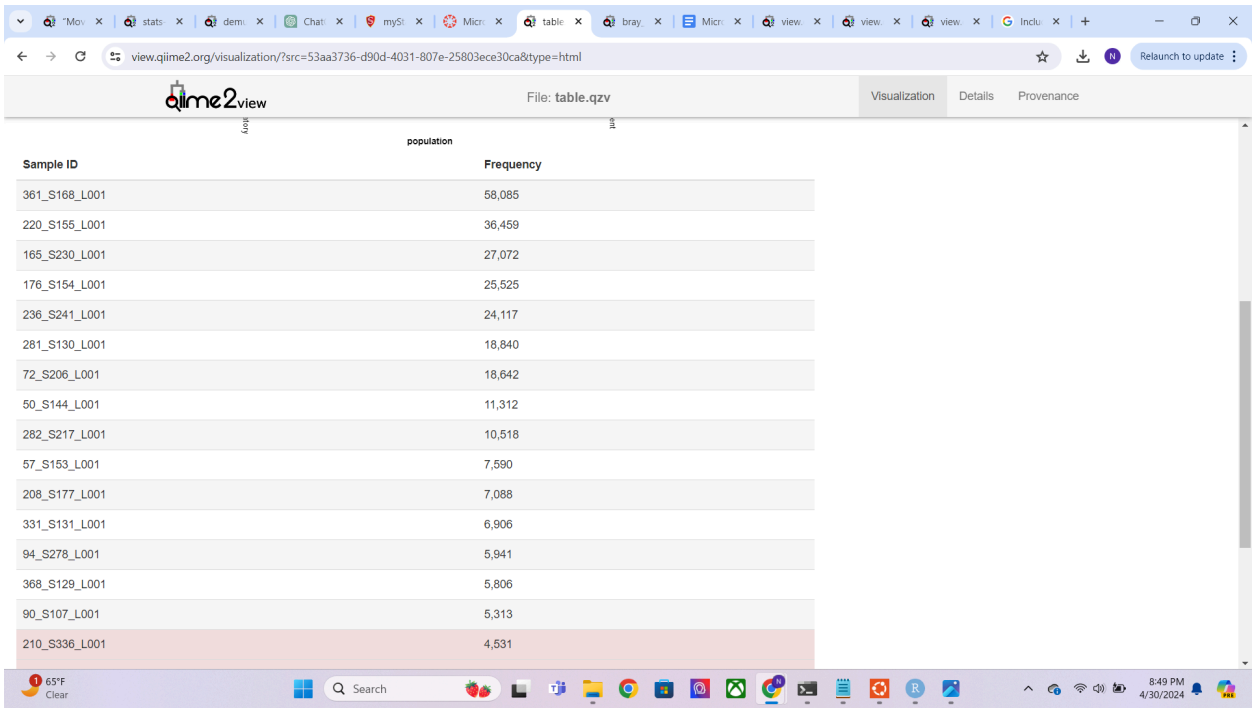
332_S105_L001	808
119_S106_L001	742
252_S179_L001	546
41_S254_L001	467


view.qiime2.org/visualization/?type=html&src=53aa3736-d90d-4031-807e-25803ece30ca
Relaunch to update
qiime2view
File: table.qzv
Visualization
Details
Provenance
Number of features 2,227
Total frequency 283,606
Frequency per sample
Frequency
Minimum frequency 467.0
1st quartile 1,975.0
Median frequency 6,423.5
3rd quartile 18,691.5
Maximum frequency 58,085.0
Mean frequency 11,816.9
Number of samples
Frequency per sample
https://view.qiime2.org/_/vtksk8b8h0f/53aa3736-d90d-4031-807e-25803ece30ca/data/sample-frequencies.pdf


view.qiime2.org/visualization/?type=html&src=53aa3736-d90d-4031-807e-25803ece30ca
Relaunch to update
qiime2view
File: table.qzv
Visualization
Details
Provenance
Frequency per sample
Download as PDF
Frequency per feature
Frequency
Minimum frequency 2.0
1st quartile 9.0
Median frequency 19.0
3rd quartile 47.0
Maximum frequency 22,781.0
Mean frequency 127.3
Number of features
Frequency per feature
Download as PDF
https://view.qiime2.org/_/vtksk8b8h0f/53aa3736-d90d-4031-807e-25803ece30ca/data/feature-frequencies.pdf



6) Jump down to taxonomy. Once you have generated your taxonomy visualization, sort it by confidence. What are your top hits? **Top hits Frequency from 58,085. Cut everything below 4,532.**



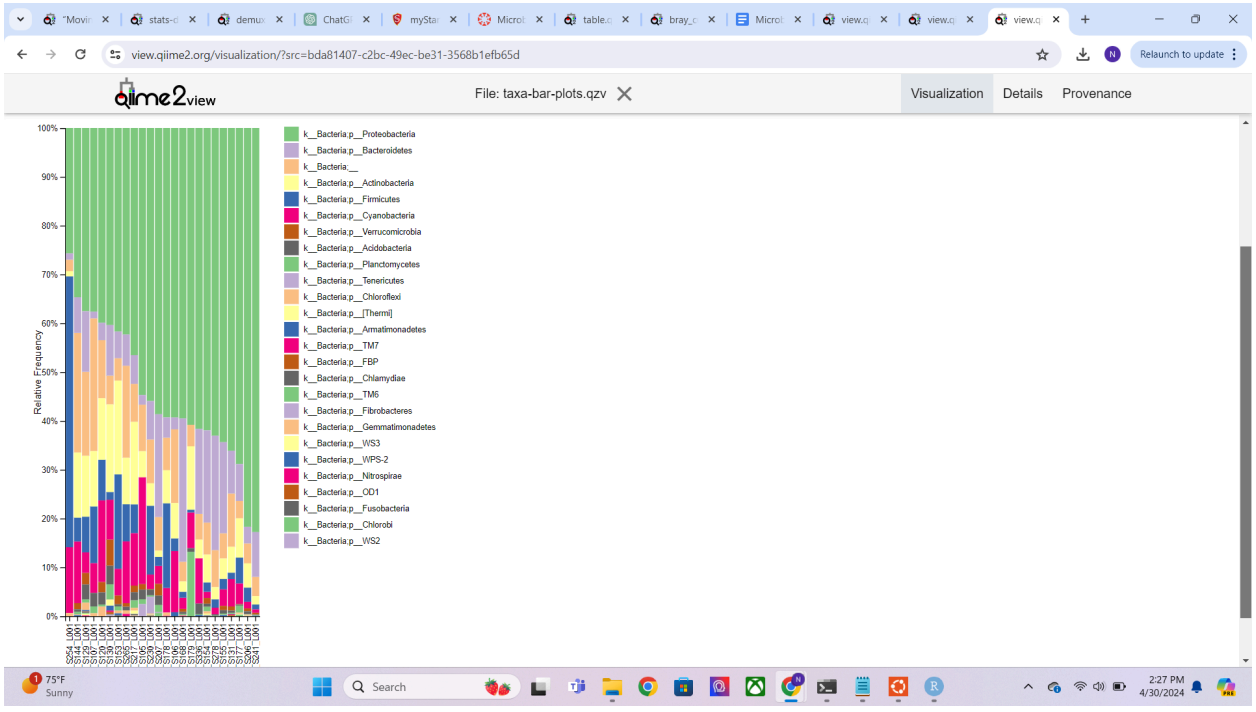
For question 7: Run this code

```
qiime taxa filter-table \
  --i-table table.qza \     = i=input abundance data for the taxa
  --i-taxonomy taxonomy.qza \ =i=input taxonomy's annotations
  --p-exclude mitochondria,chloroplast \ =exclude taxons that
include mitochondria and chloroplast from our data table (I am
assuming plants)
  --o-filtered-table table.qza =location of the filtered table.
```

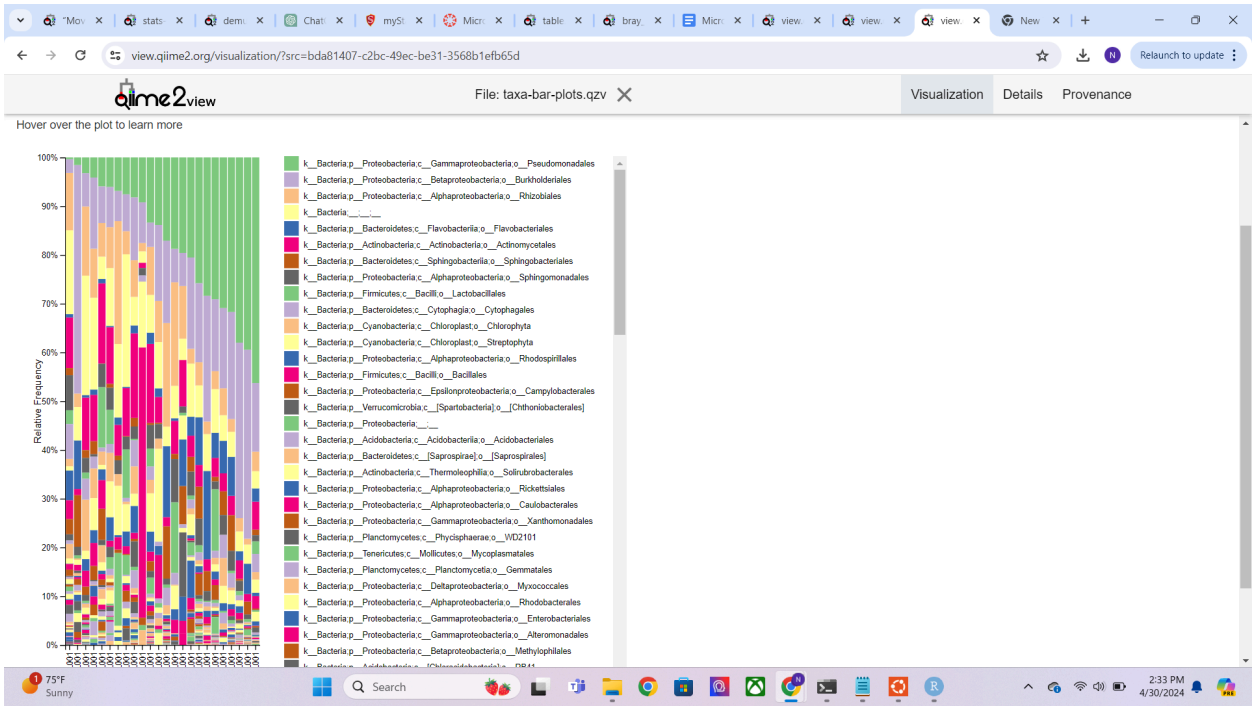
7) What do you think this code is doing? Why do you think this is a necessary or important step? **Removing plant taxons so we can focus on the bacteria. Giving us better, more accurate results, the stuff we want.**

8) Re-do your table visualization and re-do your taxonomy commands. Do you have any differences now in the hits with the highest confidence? Why or why not? Really think about what the code is doing. **The code is filtering out non bacteria sequences. Before filtering it would have included non bacteria sequences skewing our results. After filtering it wouldn't include non bacteria which would of course change the hits with the highest confidence.**

9) Looking at taxa bar plots, what are your top 2 phyla? Include a screenshot. What are the top 5 most abundant classes? Include a screenshot.



Top 2 phyla are: Firmicutesk, Actinobacteriak



The top 5 most abundant classes:Pseudomonadales, Burkholderiales, Rhizobiales,k__Bacteria;__;__;__(Blank?),Flavobacteriales

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.

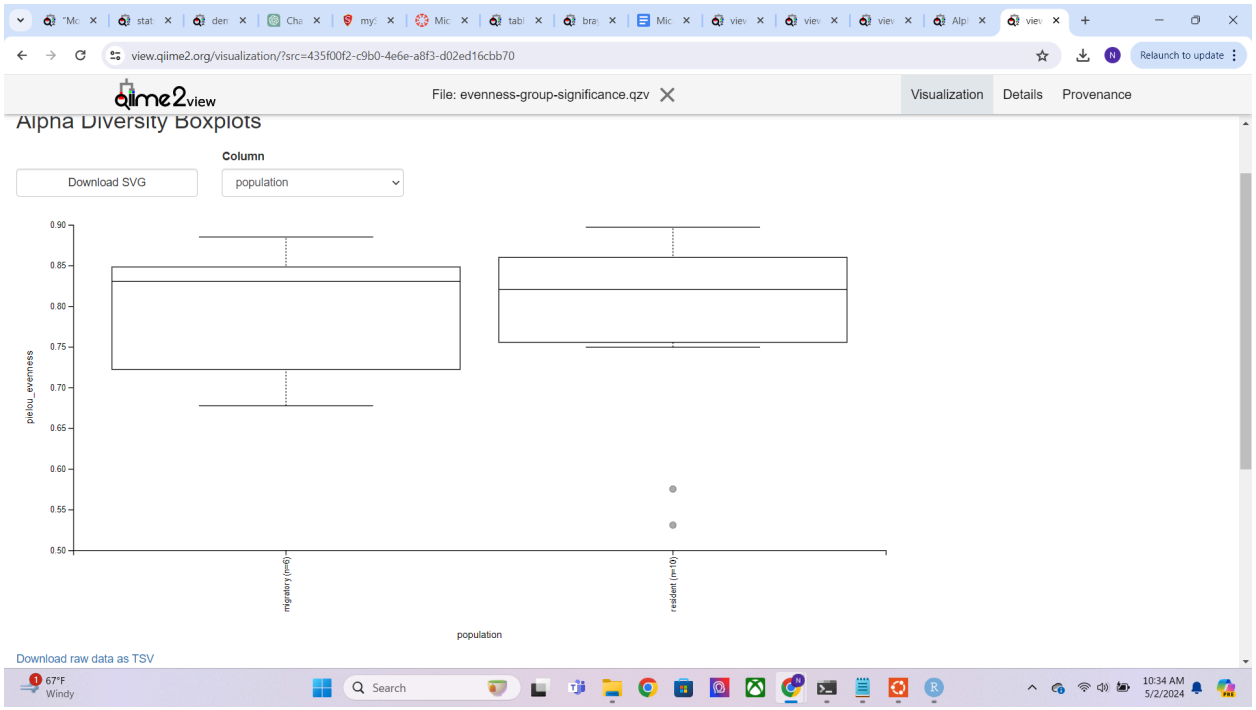
Alpha diversity= compares the contents of one community via species richness, uses shannon diversity index(accounts for richness and evenness),

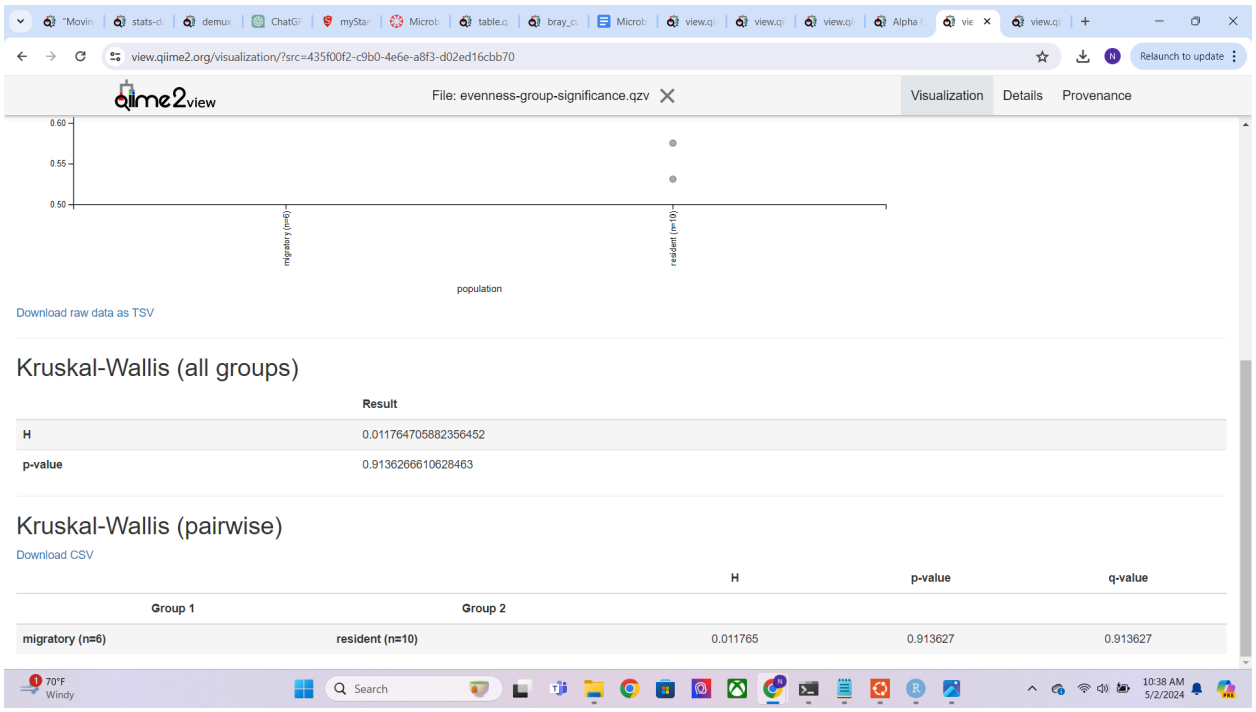
Beta diversity=compares several communities/ similarity between communities, uses bray curtis (emperor),

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? **I chose 58,085-5,313 and I cut 4,531-467 (keeping 15 samples and "losing" 9 samples)**

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).





Kruskal-Wallis (all groups)

	Result
H	0.011764705882356452
p-value	0.9136266610628463

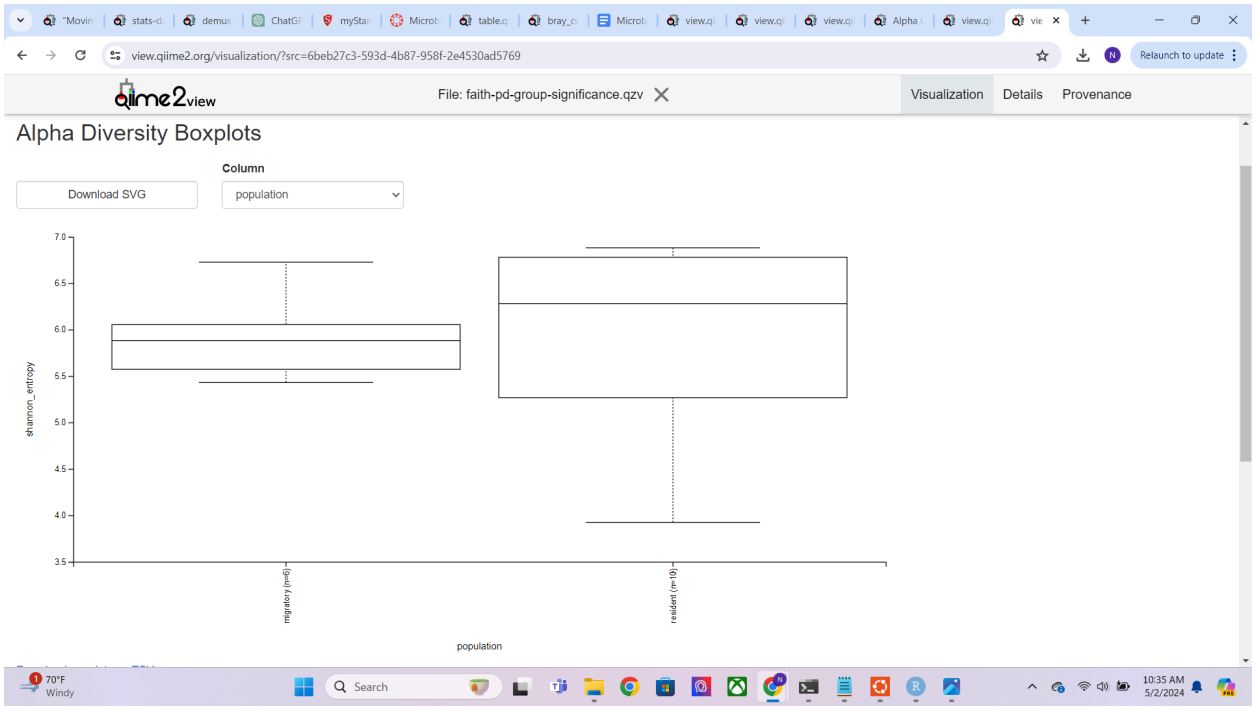
Kruskal-Wallis (pairwise)

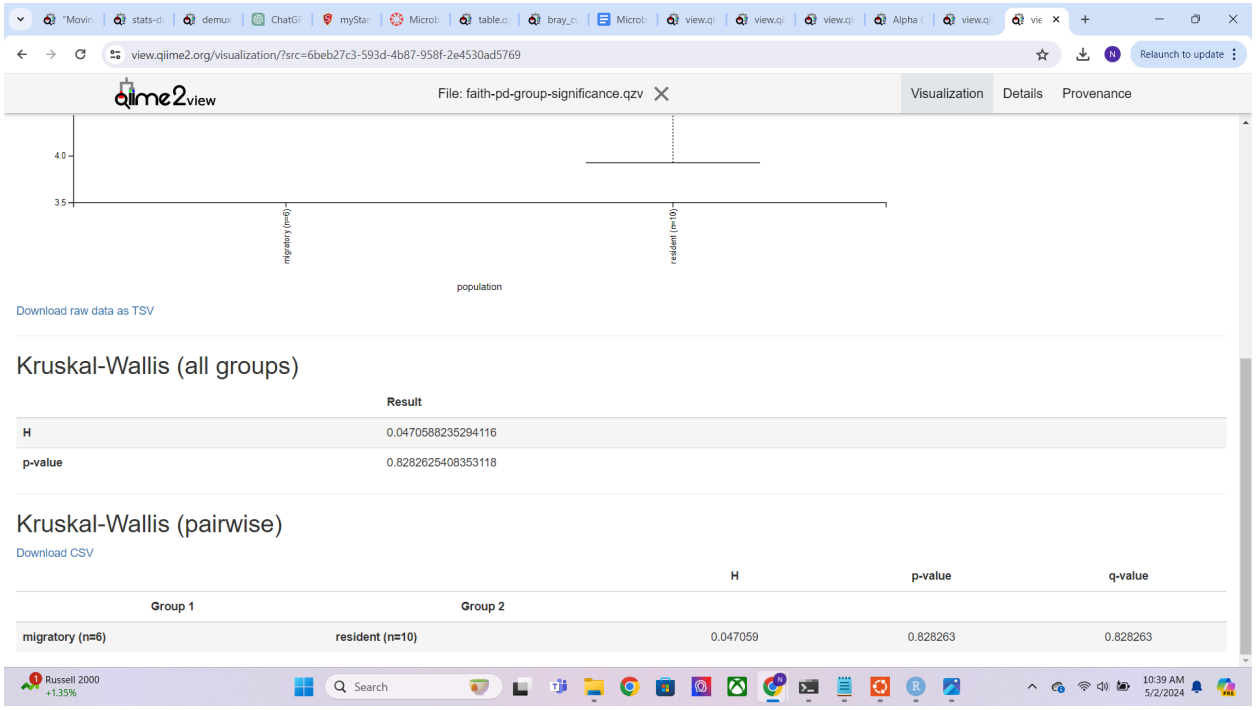
Download CSV

		H	p-value	q-value
Group 1	**Group 2**			
migratory (n=6)	**resident (n=10)**	0.011765	0.913627	0.913627

For the “Kruskal-Wallis (all groups)” the p-value is not less than .05, it is .91 so we fail to reject the null hypothesis.

For the “Kruskal-Wallis (all groups)” the p-value is also not less than .05, it is .91 there is no significant difference in alpha diversity between the 2 groups.

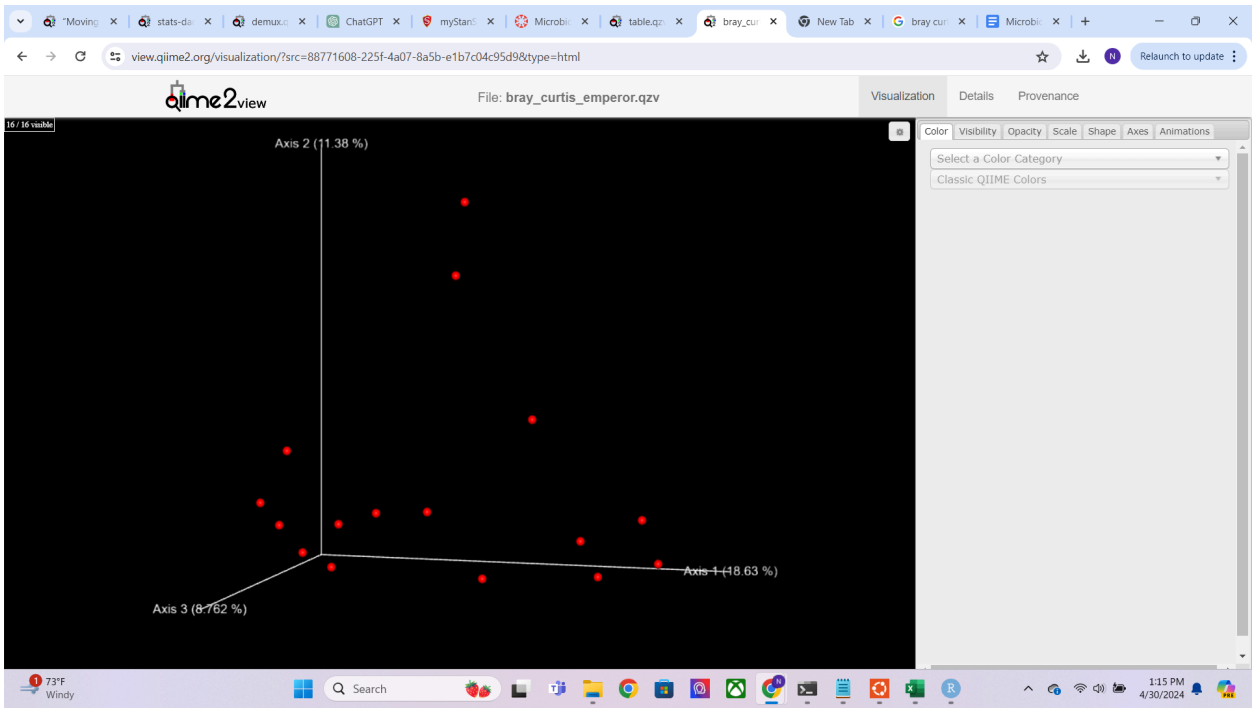




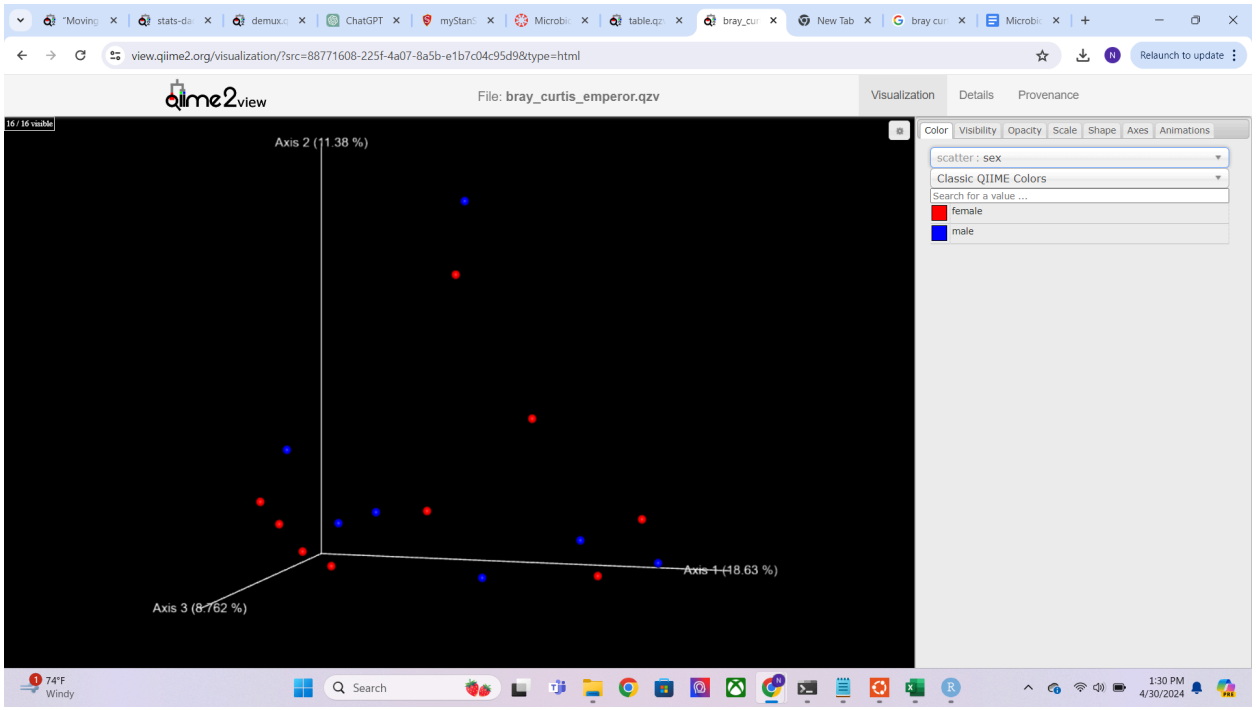
For the “Kruskal-Wallis (all groups)” the p-value is not less than .05, it is .82 so we fail to reject the null hypothesis.

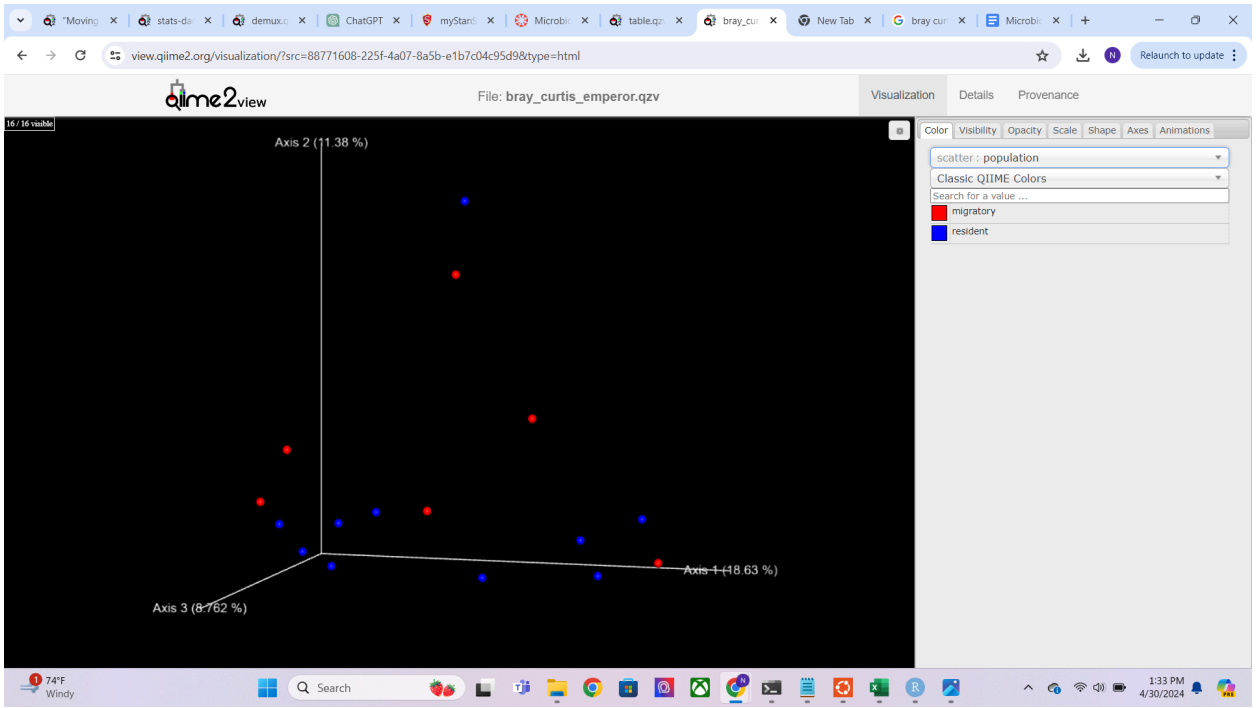
For the “Kruskal-Wallis (all groups)” the p-value is also not less than .05, it is .82 there is no significant difference in alpha diversity between the 2 groups.

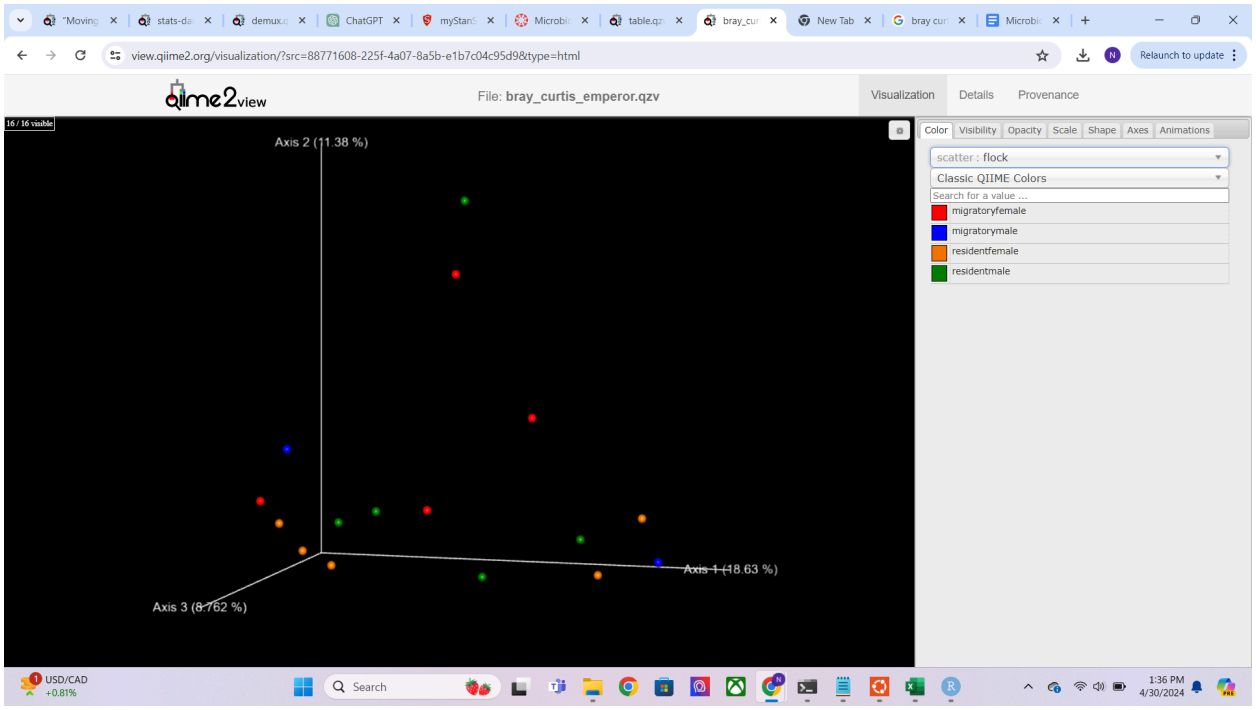
13) For beta diversity, you will need to create visualizations for Bray Curtis dissimilarity. This will require you to modify the `beta-group-significance` code. You should have one visualization for sex, one for population, and one for flock. Include a screenshot of each visualization. Is there any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



14) The `core-metrics-phylogeny` command generates a file called `bray-curtis-emporer.qzv.` Include 3 screenshots total (1 where the points are colored based on sex, one on population, one on flock). How do these results help you make sense of the results you got from question 13?







How to format your code:

Please submit a document of all the code you used. You do not need to include the output of the code, just the code itself. For each chunk of code, you should include an explanation of what that code is doing.

Here is an example:

```
#the function of this code is to generate a visualization of my table file.

#it uses my metadata and the table artifact I generated earlier to create that visualization.

#the visualization will include information that will allow me to calculate my sampling depth.

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

To run a command as administrator (user "root"), use "sudo <command>".
See "man sudo_root" for details.

My Code:

```
To run a command as administrator (user "root"), use "sudo <command>".
See "man sudo_root" for details.

(base) nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$
conda activate qiime2-amplicon-2024.2
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
demux summarize \
  --i-data demux.qza \
  --o-visualization demux.qzv

Saved Visualization to: demux.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
tools view demux.qzv
Viewing visualization failed while attempting to open
/tmp/qiime2/nick/data/9d4cc78d-17ee-492b-8bca-3ead92c8b33a/data/index.html
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ mv
rep-seqs-dada2.qza rep-seqs.qza
mv table-dada2.qza table.qza
mv: cannot stat 'rep-seqs-dada2.qza': No such file or directory
mv: cannot stat 'table-dada2.qza': No such file or directory
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
quality-filter q-score \
 --i-demux demux.qza \
 --o-filtered-sequences demux-filtered.qza \
 --o-filter-stats demux-filter-stats.qza

Saved SampleData[SequencesWithQuality] to: demux-filtered.qza
Saved QualityFilterStats to: demux-filter-stats.qza
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$
```

```
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
deblur denoise-16S \
  --i-demultiplexed-seqs demux-filtered.qza \
  --p-trim-length 120 \
  --o-representative-sequences rep-seqs-deblur.qza \
  --o-table table-deblur.qza \
  --p-sample-stats \
  --o-stats deblur-stats.qza

Plugin error from deblur:

  Deblur cannot operate on sample IDs that contain underscores. The following ID(s)
contain one or more underscores: 119_S106_L001, 122_S207_L001, 133_S265_L001,
165_S230_L001, 176_S154_L001, 208_S177_L001, 210_S336_L001,
220_S155_L001, 236_S241_L001, 252_S179_L001, 260_S178_L001,
281_S130_L001, 282_S217_L001, 306_S120_L001, 331_S131_L001,
332_S105_L001, 361_S168_L001, 368_S129_L001, 41_S254_L001, 50_S144_L001,
57_S153_L001, 72_S206_L001, 90_S107_L001, 94_S278_L001.

Debug info has been saved to /tmp/qiime2-q2cli-err-l4u5_9w_.log
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
deblur denoise-16S \
  --i-demultiplexed-seqs demux-filtered.qza \
  --p-trim-length 120 \
  --o-representative-sequences rep-seqs-deblur.qza \
  --o-table table-deblur.qza \
  --p-sample-stats \
  --o-stats deblur-stats.qza
Plugin error from deblur:

  Deblur cannot operate on sample IDs that contain underscores. The following ID(s)
contain one or more underscores: 119_S106_L001, 122_S207_L001, 133_S265_L001,
165_S230_L001, 176_S154_L001, 208_S177_L001, 210_S336_L001,
220_S155_L001, 236_S241_L001, 252_S179_L001, 260_S178_L001,
281_S130_L001, 282_S217_L001, 306_S120_L001, 331_S131_L001,
```

332_S105_L001, 361_S168_L001, 368_S129_L001, 41_S254_L001, 50_S144_L001, 57_S153_L001, 72_S206_L001, 90_S107_L001, 94_S278_L001.

Debug info has been saved to /tmp/qiime2-q2cli-err-dbw4kyg4.log
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime metadata tabulate \
  --m-input-file demux-filter-stats.qza \
  --o-visualization demux-filter-stats.qzv
qiime deblur visualize-stats \
  --i-deblur-stats deblur-stats.qza \
  --o-visualization deblur-stats.qzv
Saved Visualization to: demux-filter-stats.qzv
Usage: qiime deblur visualize-stats [OPTIONS]

  Display Deblur statistics per sample

Inputs:
  --i-deblur-stats ARTIFACT
    DeblurStats   Summary statistics of the Deblur process.   [required]
Outputs:
  --o-visualization VISUALIZATION
                                                    [required]
Miscellaneous:
  --output-dir PATH   Output unspecified results to a directory
  --verbose / --quiet Display verbose output to stdout and/or stderr during
                       execution of this action. Or silence output if
                       execution is successful (silence is golden).
  --example-data PATH Write example data and exit.
  --citations   Show citations and exit.
  --help   Show this message and exit.

Examples:
  # ### example: visualize stats
  qiime deblur visualize-stats \
    --i-deblur-stats deblur-stats.qza \
    --o-visualization deblur-stats-viz.qzv

There was a problem with the command:

(1/1) Invalid value for '--i-deblur-stats': deblur-stats.qza does not exist.
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime deblur denoise-16S \
  --i-demultiplexed-seqs demux-filtered.qza \
  --p-trim-length 120 \
  --o-representative-sequences rep-seqs-deblur.qza \
  --o-table table-deblur.qza \
  --p-sample-stats \
  --o-stats deblur-stats.qza
Plugin error from deblur:

  Deblur cannot operate on sample IDs that contain underscores. The following ID(s) contain one or more underscores: 119_S106_L001, 122_S207_L001, 133_S265_L001, 165_S230_L001, 176_S154_L001, 208_S177_L001, 210_S336_L001, 220_S155_L001, 236_S241_L001, 252_S179_L001, 260_S178_L001, 281_S130_L001, 282_S217_L001, 306_S120_L001, 331_S131_L001, 332_S105_L001, 361_S168_L001, 368_S129_L001, 41_S254_L001, 50_S144_L001, 57_S153_L001, 72_S206_L001, 90_S107_L001, 94_S278_L001.

Debug info has been saved to /tmp/qiime2-q2cli-err-b6xopuq0.log
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime deblur denoise-16S \
  --i-demultiplexed-seqs demux-filtered.qza \
  --p-trim-length 120 \
  --o-representative-sequences rep-seqs-deblur.qza \
  --o-table table-deblur.qza \
  --p-sample-stats \
  --o-stats deblur-stats.qza

Plugin error from deblur:

  Deblur cannot operate on sample IDs that contain underscores. The following ID(s) contain one or more underscores: 119_S106_L001, 122_S207_L001, 133_S265_L001, 165_S230_L001, 176_S154_L001, 208_S177_L001, 210_S336_L001, 220_S155_L001, 236_S241_L001, 252_S179_L001, 260_S178_L001, 281_S130_L001, 282_S217_L001, 306_S120_L001, 331_S131_L001, 332_S105_L001, 361_S168_L001, 368_S129_L001, 41_S254_L001, 50_S144_L001, 57_S153_L001, 72_S206_L001, 90_S107_L001, 94_S278_L001.

```
Debug info has been saved to /tmp/qiime2-q2cli-err-u9brp9_1.log
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ with
open('demux-filtered.qza') as f:
    lines = f.readlines()
-bash: syntax error near unexpected token `('
-bash: syntax error near unexpected token `('
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ python
replace_underscores.py
python: can't open file 'replace_underscores.py': [Errno 2] No such file or directory
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ line =
line.replace('_', '')
        f.write(line)
-bash: syntax error near unexpected token `('
-bash: syntax error near unexpected token `line'
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
dada2 denoise-paired \
  --i-demultiplexed-seqs demux.qza \
  --p-trim-left-f 10 \
  --p-trunc-len-f 240 \
  --p-trim-left-r 10 \
  --p-trunc-len-r 220 \
  --o-representative-sequences rep-seqs-dada2.qza \
  --o-table table-dada2.qza \
  --o-denoising-stats stats-dada2.qza
Saved FeatureTable[Frequency] to: table-dada2.qza
Saved FeatureData[Sequence] to: rep-seqs-dada2.qza
Saved SampleData[DADA2Stats] to: stats-dada2.qza
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
metadata tabulate \
  --m-input-file stats-dada2.qza \
  --o-visualization stats-dada2.qzv
Saved Visualization to: stats-dada2.qzv
```

```
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ mv
rep-seqs.qza, table.qza, and stats.qza.
mv: target 'stats.qza.' is not a directory
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ ^C
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ mv
rep-seqs-dada2.qza rep-seqs.qza
mv table-dada2.qza table.qza
mv stats-dada2.qza stats.qza
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file sample-metadata.tsv
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzv
There was an issue with loading the file sample-metadata.tsv as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.tsv

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

Saved Visualization to: rep-seqs.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file sample-metadata.tsv
qiime feature-table tabulate-seqs \
```

```
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzvqiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file sample-metadata.tsv
qiime feature-table tabulate-seqs \
  -qiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzvqiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzv
There was an issue with loading the file sample-metadata.tsv as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.tsv

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

Usage: qiime feature-table tabulate-seqs [OPTIONS]

  Generate tabular view of feature identifier to sequence mapping, including
  links to BLAST each sequence against the NCBI nt database.

Inputs:
  --i-data ARTIFACT FeatureData[Sequence | AlignedSequence]
                        The feature sequences to be tabulated.     [required]
  --i-taxonomy ARTIFACTS... Collection[FeatureData[Taxonomy]]
```

```
                    The taxonomic classifications of the tabulated
                    features.                              [optional]
Parameters:
  --m-metadata-file METADATA...
    (multiple           Any additional metadata for the tabulated features.
     arguments will be
     merged)                                               [optional]
  --p-merge-method TEXT Choices('strict', 'union', 'intersect')
                    Method that joins data sets       [default: 'strict']
Outputs:
  --o-visualization VISUALIZATION
                                                        [required]
Miscellaneous:
  --output-dir PATH      Output unspecified results to a directory
  --verbose / --quiet    Display verbose output to stdout and/or stderr
                    during execution of this action. Or silence output if
                    execution is successful (silence is golden).
  --example-data PATH    Write example data and exit.
  --citations            Show citations and exit.
  --help                 Show this message and exit.

Examples:
  # ### example: feature table tabulate seqs
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs single taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-single-taxon.qza \
    --i-taxonomy single-taxonomy.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs multi taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-multi-taxon.qza \
    --i-taxonomy multi-taxonomy/ \
    --o-visualization rep-seqs.qzv
```

There were some problems with the command:
(1/2?) No such option: --i-table
(2/2?) No such option: --m-sample-metadata-file Did you mean --m-metadata-file?
Usage: qiime feature-table tabulate-seqs [OPTIONS]

Generate tabular view of feature identifier to sequence mapping, including links to BLAST each sequence against the NCBI nt database.

Inputs:
--i-data ARTIFACT FeatureData[Sequence | AlignedSequence]
The feature sequences to be tabulated. [required]
--i-taxonomy ARTIFACTS... Collection[FeatureData[Taxonomy]]
The taxonomic classifications of the tabulated features. [optional]
Parameters:
--m-metadata-file METADATA...
(multiple arguments will be merged) Any additional metadata for the tabulated features. [optional]
--p-merge-method TEXT Choices('strict', 'union', 'intersect')
Method that joins data sets [default: 'strict']
Outputs:
--o-visualization VISUALIZATION
[required]
Miscellaneous:
--output-dir PATH Output unspecified results to a directory
--verbose / --quiet Display verbose output to stdout and/or stderr during execution of this action. Or silence output if execution is successful (silence is golden).
--example-data PATH Write example data and exit.
--citations Show citations and exit.
--help Show this message and exit.

Examples:
example: feature table tabulate seqs
qiime feature-table tabulate-seqs \
--i-data rep-seqs.qza \
--o-visualization rep-seqs.qzv

```
  # ### example: feature table tabulate seqs single taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-single-taxon.qza \
    --i-taxonomy single-taxonomy.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs multi taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-multi-taxon.qza \
    --i-taxonomy multi-taxonomy/ \
    --o-visualization rep-seqs.qzv


                There were some problems with the command:
 (1/3?) No such option: -q
 (2/3?) No such option: --i-table
 (3/3?) No such option: --m-sample-metadata-file Did you mean --m-metadata-
  file?
Usage: qiime feature-table tabulate-seqs [OPTIONS]

  Generate tabular view of feature identifier to sequence mapping, including
  links to BLAST each sequence against the NCBI nt database.

Inputs:
  --i-data ARTIFACT FeatureData[Sequence | AlignedSequence]
                   The feature sequences to be tabulated.     [required]
  --i-taxonomy ARTIFACTS... Collection[FeatureData[Taxonomy]]
                   The taxonomic classifications of the tabulated
                   features.                                  [optional]
Parameters:
  --m-metadata-file METADATA...
    (multiple          Any additional metadata for the tabulated features.
     arguments will be
     merged)                                                  [optional]
  --p-merge-method TEXT Choices('strict', 'union', 'intersect')
                   Method that joins data sets       [default: 'strict']
Outputs:
  --o-visualization VISUALIZATION
                                                          [required]
Miscellaneous:
```

```
  --output-dir PATH      Output unspecified results to a directory
  --verbose / --quiet    Display verbose output to stdout and/or stderr
                         during execution of this action. Or silence output if
                         execution is successful (silence is golden).
  --example-data PATH    Write example data and exit.
  --citations            Show citations and exit.
  --help                 Show this message and exit.

Examples:
  # ### example: feature table tabulate seqs
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs single taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-single-taxon.qza \
    --i-taxonomy single-taxonomy.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs multi taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-multi-taxon.qza \
    --i-taxonomy multi-taxonomy/ \
    --o-visualization rep-seqs.qzv


                There were some problems with the command:
 (1/2?) No such option: --i-table
 (2/2?) No such option: --m-sample-metadata-file Did you mean --m-metadata-
  file?
Saved Visualization to: rep-seqs.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
```

```
  --o-visualization rep-seqs.qzvqiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzv

There was an issue with loading the file metadata.txt as metadata:

  Metadata file must be encoded as UTF-8 or ASCII, found UTF-16. If this file is from
Microsoft Excel, save as a plain text file, not 'UTF-16 Unicode'

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

Usage: qiime feature-table tabulate-seqs [OPTIONS]

  Generate tabular view of feature identifier to sequence mapping, including
  links to BLAST each sequence against the NCBI nt database.

Inputs:
  --i-data ARTIFACT FeatureData[Sequence | AlignedSequence]
                   The feature sequences to be tabulated.     [required]
  --i-taxonomy ARTIFACTS... Collection[FeatureData[Taxonomy]]
                   The taxonomic classifications of the tabulated
                   features.                                  [optional]
Parameters:
  --m-metadata-file METADATA...
    (multiple           Any additional metadata for the tabulated features.
     arguments will be
     merged)                                                  [optional]
  --p-merge-method TEXT Choices('strict', 'union', 'intersect')
                   Method that joins data sets       [default: 'strict']
Outputs:
  --o-visualization VISUALIZATION
                                                          [required]
```

```
Miscellaneous:
  --output-dir PATH      Output unspecified results to a directory
  --verbose / --quiet    Display verbose output to stdout and/or stderr
                         during execution of this action. Or silence output if
                         execution is successful (silence is golden).
  --example-data PATH    Write example data and exit.
  --citations            Show citations and exit.
  --help                 Show this message and exit.

Examples:
  # ### example: feature table tabulate seqs
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs single taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-single-taxon.qza \
    --i-taxonomy single-taxonomy.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs multi taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-multi-taxon.qza \
    --i-taxonomy multi-taxonomy/ \
    --o-visualization rep-seqs.qzv


                There were some problems with the command:
 (1/2?) No such option: --i-table
 (2/2?) No such option: --m-sample-metadata-file Did you mean --m-metadata-
  file?
Saved Visualization to: rep-seqs.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt
qiime feature-table tabulate-seqs \
```

```
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzvqiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzv

There was an issue with loading the file metadata.txt as metadata:

  Metadata file must be encoded as UTF-8 or ASCII, found UTF-16. If this file is from
Microsoft Excel, save as a plain text file, not 'UTF-16 Unicode'

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

Usage: qiime feature-table tabulate-seqs [OPTIONS]

  Generate tabular view of feature identifier to sequence mapping, including
  links to BLAST each sequence against the NCBI nt database.

Inputs:
  --i-data ARTIFACT FeatureData[Sequence | AlignedSequence]
                   The feature sequences to be tabulated.     [required]
  --i-taxonomy ARTIFACTS... Collection[FeatureData[Taxonomy]]
                   The taxonomic classifications of the tabulated
                   features.                                  [optional]
Parameters:
  --m-metadata-file METADATA...
    (multiple          Any additional metadata for the tabulated features.
     arguments will be
     merged)                                                  [optional]
  --p-merge-method TEXT Choices('strict', 'union', 'intersect')
                   Method that joins data sets       [default: 'strict']
Outputs:
  --o-visualization VISUALIZATION
```

[required]

Miscellaneous:
  --output-dir PATH   Output unspecified results to a directory
  --verbose / --quiet   Display verbose output to stdout and/or stderr
                         during execution of this action. Or silence output if
                         execution is successful (silence is golden).
  --example-data PATH   Write example data and exit.
  --citations   Show citations and exit.
  --help   Show this message and exit.

Examples:
  # ### example: feature table tabulate seqs
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs single taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-single-taxon.qza \
    --i-taxonomy single-taxonomy.qza \
    --o-visualization rep-seqs.qzv

  # ### example: feature table tabulate seqs multi taxon
  qiime feature-table tabulate-seqs \
    --i-data rep-seqs-multi-taxon.qza \
    --i-taxonomy multi-taxonomy/ \
    --o-visualization rep-seqs.qzv

There were some problems with the command:
 (1/2?) No such option: --i-table
 (2/2?) No such option: --m-sample-metadata-file Did you mean --m-metadata-
  file?
Saved Visualization to: rep-seqs.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file metadata.txt

```
qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzv
Saved Visualization to: table.qzv
Saved Visualization to: rep-seqs.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
phylogeny align-to-tree-mafft-fasttree \
  --i-sequences rep-seqs.qza \
  --o-alignment aligned-rep-seqs.qza \
  --o-masked-alignment masked-aligned-rep-seqs.qza \
  --o-tree unrooted-tree.qza \
  --o-rooted-tree rooted-tree.qza
Saved FeatureData[AlignedSequence] to: aligned-rep-seqs.qza
Saved FeatureData[AlignedSequence] to: masked-aligned-rep-seqs.qza
Saved Phylogeny[Unrooted] to: unrooted-tree.qza
Saved Phylogeny[Rooted] to: rooted-tree.qza
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity core-metrics-phylogenetic \
  --i-phylogeny rooted-tree.qza \
  --i-table table.qza \
  --p-sampling-depth 1103 \
  --m-metadata-file sample-metadata.tsv \
  --output-dir core-metrics-results
There was an issue with loading the file sample-metadata.tsv as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.tsv

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity core-metrics-phylogenetic \
```

```
  --i-phylogeny rooted-tree.qza \
  --i-table table.qza \
  --p-sampling-depth 4530 \
  --m-metadata-file metadata.txt \
  --output-dir core-metrics-results
Saved FeatureTable[Frequency] to: core-metrics-results/rarefied_table.qza
Saved SampleData[AlphaDiversity] to: core-metrics-results/faith_pd_vector.qza
Saved SampleData[AlphaDiversity] to:
core-metrics-results/observed_features_vector.qza
Saved SampleData[AlphaDiversity] to: core-metrics-results/shannon_vector.qza
Saved SampleData[AlphaDiversity] to: core-metrics-results/evenness_vector.qza
Saved DistanceMatrix to: core-metrics-results/unweighted_unifrac_distance_matrix.qza
Saved DistanceMatrix to: core-metrics-results/weighted_unifrac_distance_matrix.qza
Saved DistanceMatrix to: core-metrics-results/jaccard_distance_matrix.qza
Saved DistanceMatrix to: core-metrics-results/bray_curtis_distance_matrix.qza
Saved PCoAResults to: core-metrics-results/unweighted_unifrac_pcoa_results.qza
Saved PCoAResults to: core-metrics-results/weighted_unifrac_pcoa_results.qza
Saved PCoAResults to: core-metrics-results/jaccard_pcoa_results.qza
Saved PCoAResults to: core-metrics-results/bray_curtis_pcoa_results.qza
Saved Visualization to: core-metrics-results/unweighted_unifrac_emperor.qzv
Saved Visualization to: core-metrics-results/weighted_unifrac_emperor.qzv
Saved Visualization to: core-metrics-results/jaccard_emperor.qzv
Saved Visualization to: core-metrics-results/bray_curtis_emperor.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-results/faith_pd_vector.qza \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization core-metrics-results/faith-pd-group-significance.qzv

qiime diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-results/evenness_vector.qza \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization core-metrics-results/evenness-group-significance.qzv
There was an issue with loading the file sample-metadata.tsv as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.tsv
```

```
  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

There was an issue with loading the file sample-metadata.tsv as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.tsv

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity alpha-group-significance \
  --i-alpha-diversity core-metrics-results/evenness_vector.qza \
  --m-metadata-file metadata.txt \
  --o-visualization core-metrics-results/evenness-group-significance.qzv
Saved Visualization to: core-metrics-results/evenness-group-significance.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file metadata.txt \
  --m-metadata-column subject \
  --o-visualization
core-metrics-results/unweighted-unifrac-subject-group-significance.qzv \
  --p-pairwise
Usage: qiime diversity beta-group-significance [OPTIONS]

  Determine whether groups of samples are significantly different from one
  another using a permutation-based statistical test.

Inputs:
```

```
  --i-distance-matrix ARTIFACT
    DistanceMatrix      Matrix of distances between pairs of samples.
                                                          [required]
Parameters:
  --m-metadata-file METADATA
  --m-metadata-column COLUMN  MetadataColumn[Categorical]
                      Categorical sample metadata column.         [required]
  --p-method TEXT Choices('permanova', 'anosim', 'permdisp')
                      The group significance test to be applied.
                                              [default: 'permanova']
  --p-pairwise / --p-no-pairwise
                      Perform pairwise tests between all pairs of groups in
                      addition to the test across all groups. This can be
                      very slow if there are a lot of groups in the metadata
                      column.                          [default: False]
  --p-permutations INTEGER
                      The number of permutations to be run when computing
                      p-values.                         [default: 999]
Outputs:
  --o-visualization VISUALIZATION
                                                          [required]
Miscellaneous:
  --output-dir PATH    Output unspecified results to a directory
  --verbose / --quiet  Display verbose output to stdout and/or stderr during
                      execution of this action. Or silence output if
                      execution is successful (silence is golden).
  --example-data PATH  Write example data and exit.
  --citations          Show citations and exit.
  --help               Show this message and exit.

                  There was a problem with the command:
 (1/1) Invalid value for '--m-metadata-file': There was an issue with
  retrieving column 'subject' from the metadata.
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file metadata.txt \
  --m-metadata-column subject \
```

```
  --o-visualization
core-metrics-results/unweighted-unifrac-subject-group-significance.qzv \
  --p-pairwise
Usage: qiime diversity beta-group-significance [OPTIONS]

  Determine whether groups of samples are significantly different from one
  another using a permutation-based statistical test.

Inputs:
  --i-distance-matrix ARTIFACT
    DistanceMatrix      Matrix of distances between pairs of samples.
                                                        [required]
Parameters:
  --m-metadata-file METADATA
  --m-metadata-column COLUMN  MetadataColumn[Categorical]
                      Categorical sample metadata column.          [required]
  --p-method TEXT Choices('permanova', 'anosim', 'permdisp')
                      The group significance test to be applied.
                                           [default: 'permanova']
  --p-pairwise / --p-no-pairwise
                      Perform pairwise tests between all pairs of groups in
                      addition to the test across all groups. This can be
                      very slow if there are a lot of groups in the metadata
                      column.                          [default: False]
  --p-permutations INTEGER
                      The number of permutations to be run when computing
                      p-values.                         [default: 999]
Outputs:
  --o-visualization VISUALIZATION
                                                        [required]
Miscellaneous:
  --output-dir PATH     Output unspecified results to a directory
  --verbose / --quiet  Display verbose output to stdout and/or stderr during
                      execution of this action. Or silence output if
                      execution is successful (silence is golden).
  --example-data PATH  Write example data and exit.
  --citations          Show citations and exit.
  --help               Show this message and exit.

                There was a problem with the command:
```

```
 (1/1) Invalid value for '--m-metadata-file': There was an issue with
  retrieving column 'subject' from the metadata.
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file metadata.txt \
  --m-metadata-column body-site \
  --o-visualization core-metrics-results/unweighted-unifrac-body-site-significance.qzv \
  --p-pairwise

qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file metadata.txt \
  --m-metadata-column subject \
  --o-visualization
core-metrics-results/unweighted-unifrac-subject-group-significance.qzv \
  --p-pairwise
Usage: qiime diversity beta-group-significance [OPTIONS]

  Determine whether groups of samples are significantly different from one
  another using a permutation-based statistical test.

Inputs:
  --i-distance-matrix ARTIFACT
    DistanceMatrix     Matrix of distances between pairs of samples.
                                                        [required]
Parameters:
  --m-metadata-file METADATA
  --m-metadata-column COLUMN  MetadataColumn[Categorical]
                      Categorical sample metadata column.         [required]
  --p-method TEXT Choices('permanova', 'anosim', 'permdisp')
                      The group significance test to be applied.
                                          [default: 'permanova']
  --p-pairwise / --p-no-pairwise
                      Perform pairwise tests between all pairs of groups in
                      addition to the test across all groups. This can be
                      very slow if there are a lot of groups in the metadata
                      column.                         [default: False]
  --p-permutations INTEGER
```

```
                    The number of permutations to be run when computing
                    p-values.                          [default: 999]
Outputs:
  --o-visualization VISUALIZATION
                                                       [required]
Miscellaneous:
  --output-dir PATH     Output unspecified results to a directory
  --verbose / --quiet   Display verbose output to stdout and/or stderr during
                        execution of this action. Or silence output if
                        execution is successful (silence is golden).
  --example-data PATH   Write example data and exit.
  --citations           Show citations and exit.
  --help                Show this message and exit.

                  There was a problem with the command:
 (1/1) Invalid value for '--m-metadata-file': There was an issue with
  retrieving column 'body-site' from the metadata.
Usage: qiime diversity beta-group-significance [OPTIONS]

  Determine whether groups of samples are significantly different from one
  another using a permutation-based statistical test.

Inputs:
  --i-distance-matrix ARTIFACT
    DistanceMatrix      Matrix of distances between pairs of samples.
                                                       [required]
Parameters:
  --m-metadata-file METADATA
  --m-metadata-column COLUMN  MetadataColumn[Categorical]
                        Categorical sample metadata column.          [required]
  --p-method TEXT Choices('permanova', 'anosim', 'permdisp')
                        The group significance test to be applied.
                                             [default: 'permanova']
  --p-pairwise / --p-no-pairwise
                        Perform pairwise tests between all pairs of groups in
                        addition to the test across all groups. This can be
                        very slow if there are a lot of groups in the metadata
                        column.                          [default: False]
  --p-permutations INTEGER
                        The number of permutations to be run when computing
```

```
                    p-values.                          [default: 999]
Outputs:
  --o-visualization VISUALIZATION
                                                       [required]
Miscellaneous:
  --output-dir PATH    Output unspecified results to a directory
  --verbose / --quiet  Display verbose output to stdout and/or stderr during
                       execution of this action. Or silence output if
                       execution is successful (silence is golden).
  --example-data PATH  Write example data and exit.
  --citations          Show citations and exit.
  --help               Show this message and exit.

                  There was a problem with the command:
 (1/1) Invalid value for '--m-metadata-file': There was an issue with
  retrieving column 'subject' from the metadata.
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file metadata.txt \
  --m-metadata-column population \
  --o-visualization core-metrics-results/unweighted-unifrac-body-site-significance.qzv \
  --p-pairwise

qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file metadata.txt \
  --m-metadata-column sex \
  --o-visualization
core-metrics-results/unweighted-unifrac-subject-group-significance.qzv \
  --p-pairwise
Saved Visualization to:
core-metrics-results/unweighted-unifrac-body-site-significance.qzv
Saved Visualization to:
core-metrics-results/unweighted-unifrac-subject-group-significance.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
taxa barplot \
  --i-table table.qza \
```

```
  --i-taxonomy taxonomy.qza \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization taxa-bar-plots.qzv
There was an issue with loading the file sample-metadata.tsv as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.tsv

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
taxa barplot \
  --i-table table.qza \
  --i-taxonomy taxonomy.qza \
  --m-metadata-file sample-metadata.txt \
  --o-visualization taxa-bar-plots.qzv
There was an issue with loading the file sample-metadata.txt as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.txt

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
taxa barplot \
  --i-table table.qza \
  --i-taxonomy taxonomy.qza \
  --m-metadata-file sample-metadata.txt \
```

```
  --o-visualization taxa-bar-plots.qzv
There was an issue with loading the file sample-metadata.txt as metadata:

  Metadata file path doesn't exist, or the path points to something other than a file.
Please check that the path exists, has read permissions, and points to a regular file (not
a directory): sample-metadata.txt

  There may be more errors present in the metadata file. To get a full report,
sample/feature metadata files can be validated with Keemei: https://keemei.qiime2.org

  Find details on QIIME 2 metadata requirements here:
https://docs.qiime2.org/2024.2/tutorials/metadata/

(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$  qiime
taxa barplot \
  --i-table table.qza \
  --i-taxonomy taxonomy.qza \
  --m-metadata-file metadata1.txt \
  --o-visualization taxa-bar-plots.qzv
Usage: qiime taxa barplot [OPTIONS]

  This visualizer produces an interactive barplot visualization of taxonomies.
  Interactive features include multi-level sorting, plot recoloring, sample
  relabeling, and SVG figure export.

Inputs:
  --i-table ARTIFACT FeatureTable[Frequency | PresenceAbsence]
                    Feature table to visualize at various taxonomic
                    levels.                              [required]
  --i-taxonomy ARTIFACT FeatureData[Taxonomy]
                    Taxonomic annotations for features in the provided
                    feature table. All features in the feature table must
                    have a corresponding taxonomic annotation. Taxonomic
                    annotations that are not present in the feature table
                    will be ignored. If no taxonomy is provided, the
                    feature IDs will be used as labels.        [optional]
Parameters:
  --m-metadata-file METADATA...
    (multiple           The sample metadata.
```

```
    arguments will be
    merged)                                          [optional]
  --p-level-delimiter TEXT
                    Attempt to parse hierarchical taxonomic information
                    from feature IDs by separating levels with this
                    character. This parameter is ignored if a taxonomy is
                    provided as input.                [optional]
Outputs:
  --o-visualization VISUALIZATION
                                                     [required]
Miscellaneous:
  --output-dir PATH      Output unspecified results to a directory
  --verbose / --quiet    Display verbose output to stdout and/or stderr
                    during execution of this action. Or silence output if
                    execution is successful (silence is golden).
  --example-data PATH    Write example data and exit.
  --citations            Show citations and exit.
  --help                 Show this message and exit.

Examples:
  # ### example: barplot
  qiime taxa barplot \
    --i-table table.qza \
    --i-taxonomy taxonomy.qza \
    --m-metadata-file sample-metadata.tsv \
    --o-visualization taxa-bar-plots.qzv


                There was a problem with the command:
 (1/1) Invalid value for '--i-taxonomy': taxonomy.qza does not exist.
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ wget \
  -O "gg-13-8-99-515-806-nb-classifier.qza" \
  "https://data.qiime2.org/2024.2/common/gg-13-8-99-515-806-nb-classifier.qza"
--2024-04-30 14:23:48--
https://data.qiime2.org/2024.2/common/gg-13-8-99-515-806-nb-classifier.qza
Resolving data.qiime2.org (data.qiime2.org)... 54.200.1.12
Connecting to data.qiime2.org (data.qiime2.org)|54.200.1.12|:443... connected.
HTTP request sent, awaiting response... 302 FOUND
```

```
Location:
https://s3-us-west-2.amazonaws.com/qiime2-data/2024.2/common/gg-13-8-99-515-806-
nb-classifier.qza [following]
--2024-04-30 14:23:48--
https://s3-us-west-2.amazonaws.com/qiime2-data/2024.2/common/gg-13-8-99-515-806-
nb-classifier.qza
Resolving s3-us-west-2.amazonaws.com (s3-us-west-2.amazonaws.com)...
52.92.204.24, 52.92.178.200, 52.92.184.0, ...
Connecting to s3-us-west-2.amazonaws.com
(s3-us-west-2.amazonaws.com)|52.92.204.24|:443... connected.
HTTP request sent, awaiting response... 200 OK
Length: 28289645 (27M) [binary/octet-stream]
Saving to: ‘gg-13-8-99-515-806-nb-classifier.qza’

gg-13-8-99-515-806-nb-classif
100%[=============================================>]  26.98M
8.20MB/s    in 3.3s

2024-04-30 14:23:51 (8.20 MB/s) - ‘gg-13-8-99-515-806-nb-classifier.qza’ saved
[28289645/28289645]

(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
feature-classifier classify-sklearn \
  --i-classifier gg-13-8-99-515-806-nb-classifier.qza \
  --i-reads rep-seqs.qza \
  --o-classification taxonomy.qza

qiime metadata tabulate \
  --m-input-file taxonomy.qza \
  --o-visualization taxonomy.qzv
Saved FeatureData[Taxonomy] to: taxonomy.qza
Saved Visualization to: taxonomy.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$ qiime
taxa barplot \
  --i-table table.qza \
  --i-taxonomy taxonomy.qza \
  --m-metadata-file metadata1.txt \
  --o-visualization taxa-bar-plots.qzv
```

Saved Visualization to: taxa-bar-plots.qzv
(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionrepo(qiime(qiime2-(qiime2-amplicon-2024.2)
nick@MSI:/mnt/c/Users/Nick/Desktop/bioinformatics/microbiomesectionreport$