

Overview

Almost every issue that can be encountered when using AOViz will be related to initial data formatting. I have compiled a list of common formatting errors that cause most issues:

Column names:

AOViz requires specific column names to function properly and to allow all visualizations to be generated from just two files. The column names are spelling and case-sensitive:

- Feature.ID
- Consensus.Lineage
- ReprSequence
- SampleName

AOViz generates several data columns during data processing, and so the following column names are *blacklisted* and should not be included in your metadata table, in any form:

- rowID
- Order
- Taxonomy
- TaxaName
- Input or input

Sample column names:

These column names are less sensitive to issues because they are expected to be varied. However, the R environment is sensitive to specific characters, and so all special (and some non-special) characters should be avoided:

- Avoid “.” and “-” as separators and do not use spaces because they both get modified to periods when imported into R. Instead, use underscores (“_”).
- Do not include forward or back slashes (“/” or “\”) (e.g., g/L). Instead, use g_L or gL.
- Do not include brackets or parentheses.
- In general, avoid special characters (e.g., !, @, \$, #...)

Collapsed data:

AOViz will accept collapsed data that most commonly comes in the form of taxa-collapsed tables from QIIME2 or DADA2. However, it will accept any data that follows the collapsed table formatting, which is to say a “Feature.ID” column containing some kind of unique identifier and sample columns with associated numeric count data (e.g., functional tables like FAPROTAX). **NOTE:** AOViz was not

initially designed to process anything but ASV tables, so your mileage may vary. That said, it is capable of processing any correctly formatted count data:

- Collapsed tables only require a “Feature.ID” column and sample columns (no Consensus.Lineage or ReprSequence columns).
- For non-ASV data, the Feature.ID column will accept any type of text, although special characters should be avoided, and final figure labels might not work as intended.

Metadata files:

Metadata formatting is critical to AOViz. Only a single column is required, the “SampleName” column, and it *must* be named appropriately and contain sample names that exactly match the column names in your ASV table. Any samples absent from the metadata file will be removed from the ASV table, and vice versa, and will not be represented in any analyses or figures. Otherwise, there are no restrictions on the number of additional columns nor the type of data, so long as it is text or numeric and avoids the special characters and blacklisted names mentioned previously.

Rooted-tree (UniFrac):

A rooted phylogenetic tree is required to generate UniFrac analyses, presented in Newick format. It does not matter how or where this tree is generated from (e.g., QIIME2, IQtree). However, the taxon names *must* be the ASV ids from your original ASV table and *all* ASVs *must* be presented in the tree, otherwise UniFrac computations are not possible. However, not all ASVs in the tree must be included in the ASV table. Practically, this means that any rarefying or filtering of your ASV table that removes ASVs will still allow UniFrac metrics to be calculated. If you’re encountering issues with the UniFrac triplot, it is likely a tree-specific issue.

Q: The screen goes grey and says “disconnected” when I load my data!

A: AOViz requires specific data formatting so that each visualization can be generated from two standard files: your ASV table and metadata table. These tables *must* contain specific case-sensitive column names, and sample names should not contain special characters. If you encounter a greyed screen and “disconnected” message, AOViz has likely encountered an issue related to formatting issues. Verify that you’ve followed the correct formatting, and pay special attention to the following:

1. If using a collapsed table, make sure you’ve selected the “is my data collapsed?” button on the “Data upload” tab.
2. Check the spelling and casing of your critical columns.