Training on Galaxy: Metabarcoding

October 2023 - Webinar

FROGS Practice on ITS data and Workflow creation – *correction* and *checkpoints*

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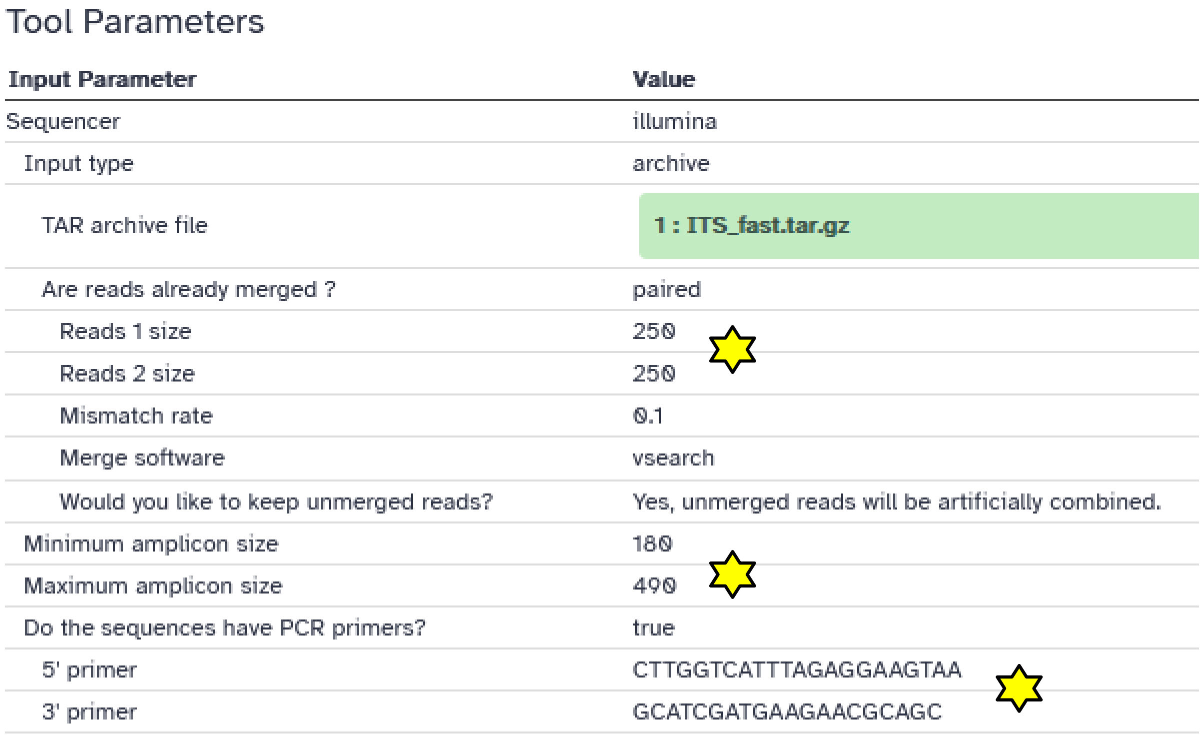
Correction ITS

HISTORY : ITS_FORMATION_2023_PREV50

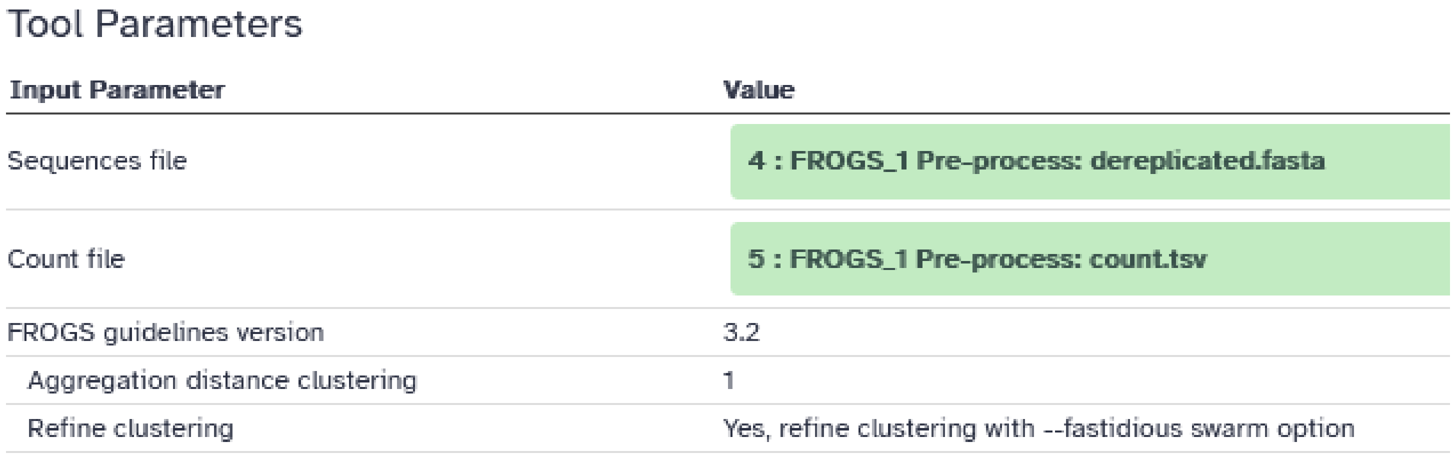
FROGS_1 Pre-process



Tool Parameters

Input Parameter	Value
Sequencer	illumina
Input type	archive
TAR archive file	1 : ITS_fast.tar.gz
Are reads already merged ?	paired
Reads 1 size	250
Reads 2 size	250
Mismatch rate	0.1
Merge software	vsearch
Would you like to keep unmerged reads?	Yes, unmerged reads will be artificially combined.
Minimum amplicon size	180
Maximum amplicon size	490
Do the sequences have PCR primers?	true
5' primer	CTTGGTCATTTAGAGGAAGTAA
3' primer	GCATCGATGAAGAACGCAGC

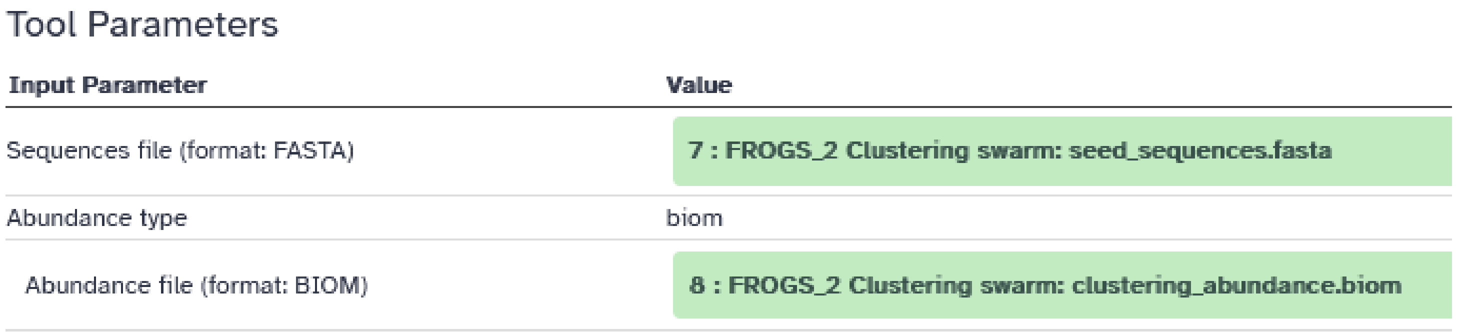
FROGS_2 Clustering swarm



Tool Parameters

Input Parameter	Value
Sequences file	**4 : FROGS_1 Pre-process: dereplicated.fasta**
Count file	**5 : FROGS_1 Pre-process: count.tsv**
FROGS guidelines version	3.2
Aggregation distance clustering	1
Refine clustering	Yes, refine clustering with --fastidious swarm option

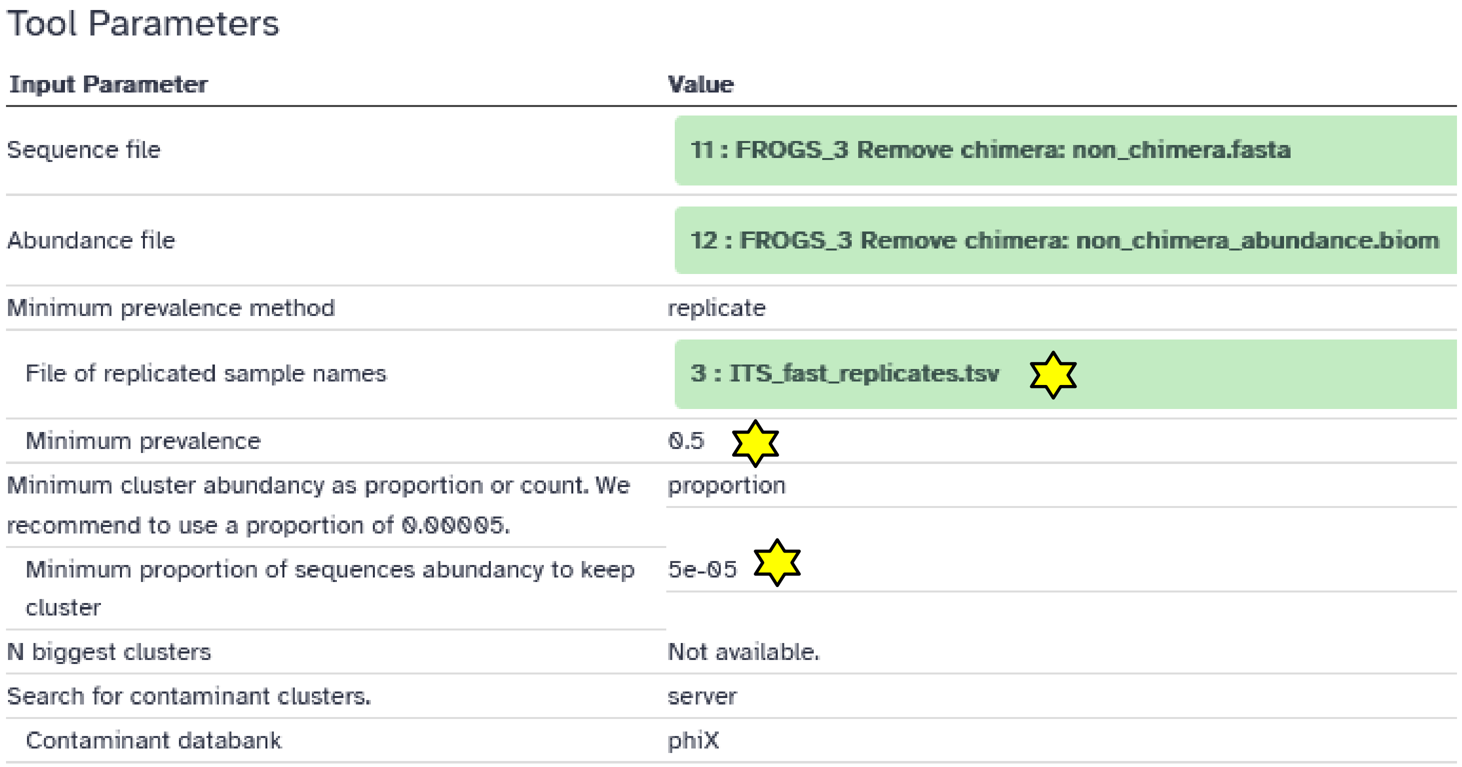
FROGS_3 Remove chimera



Tool Parameters

Input Parameter	Value
Sequences file (format: FASTA)	**7 : FROGS_2 Clustering swarm: seed_sequences.fasta**
Abundance type	biom
Abundance file (format: BIOM)	**8 : FROGS_2 Clustering swarm: clustering_abundance.biom**

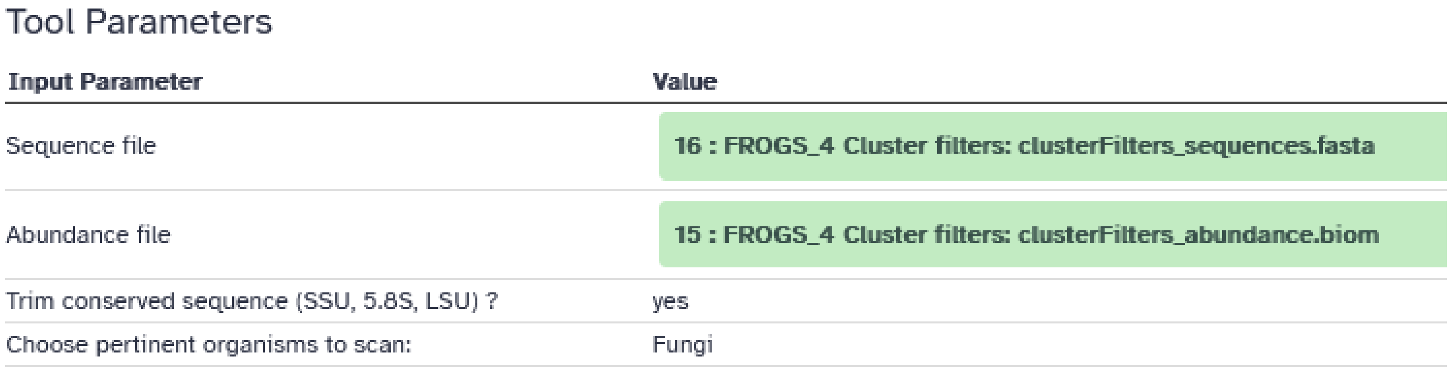
FROGS_4 Cluster filters



Tool Parameters

Input Parameter	Value
Sequence file	**11 : FROGS_3 Remove chimera: non_chimera.fasta**
Abundance file	**12 : FROGS_3 Remove chimera: non_chimera_abundance.biom**
Minimum prevalence method	replicate
File of replicated sample names	**3 : ITS_fast_replicates.tsv** ✡
Minimum prevalence	0.5 ✡
Minimum cluster abundancy as proportion or count. We recommend to use a proportion of 0.00005.	proportion
Minimum proportion of sequences abundancy to keep cluster	5e-05 ✡
N biggest clusters	Not available.
Search for contaminant clusters.	server
Contaminant databank	phiX

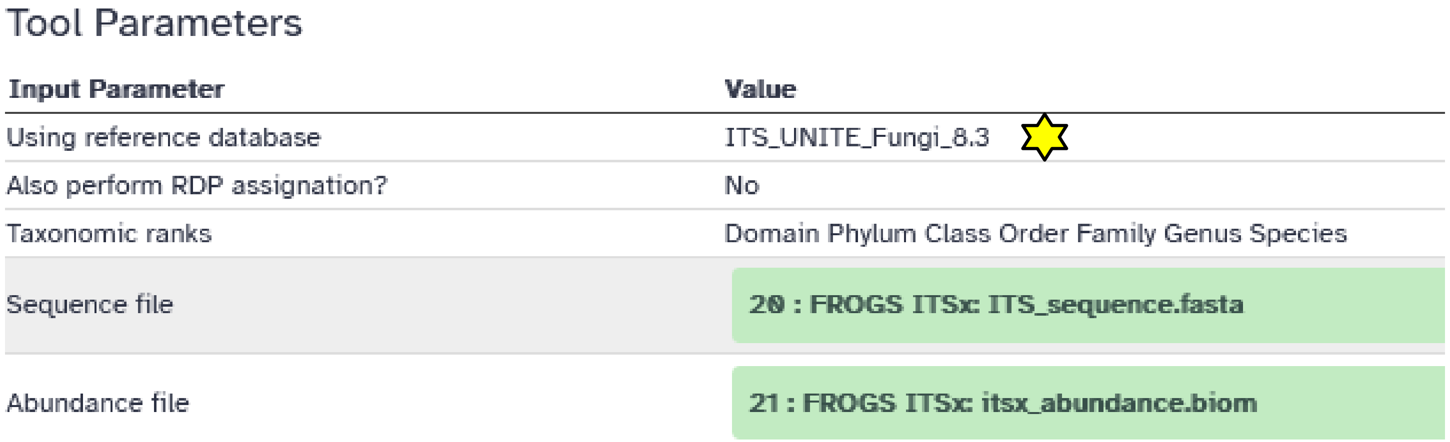
FROGS ITSx



Tool Parameters

Input Parameter	Value
Sequence file	**16 : FROGS_4 Cluster filters: clusterFilters_sequences.fasta**
Abundance file	**15 : FROGS_4 Cluster filters: clusterFilters_abundance.biom**
Trim conserved sequence (SSU, 5.8S, LSU) ?	yes
Choose pertinent organisms to scan:	Fungi

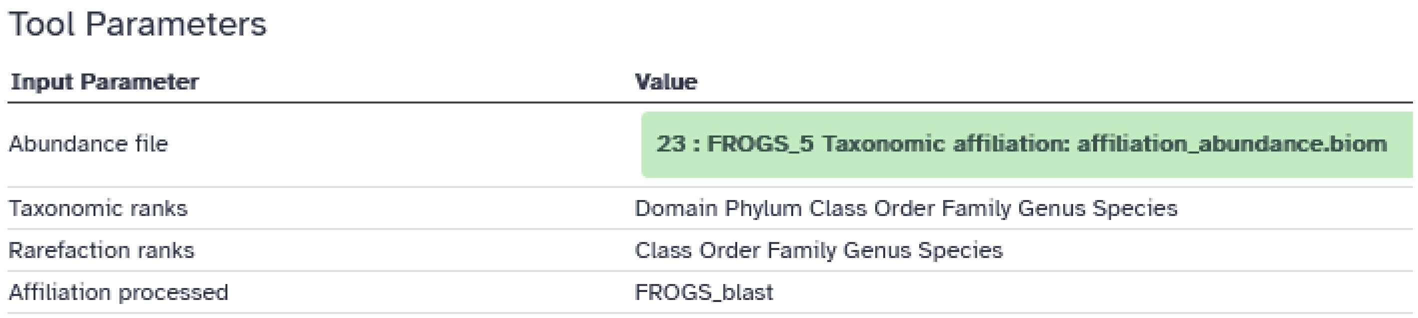
FROGS_5 Taxonomic affiliation



Tool Parameters

Input Parameter	Value
Using reference database	ITS_UNITE_Fungi_8.3
Also perform RDP assignation?	No
Taxonomic ranks	Domain Phylum Class Order Family Genus Species
Sequence file	**20 : FROGS ITSx: ITS_sequence.fasta**
Abundance file	**21 : FROGS ITSx: itsx_abundance.biom**

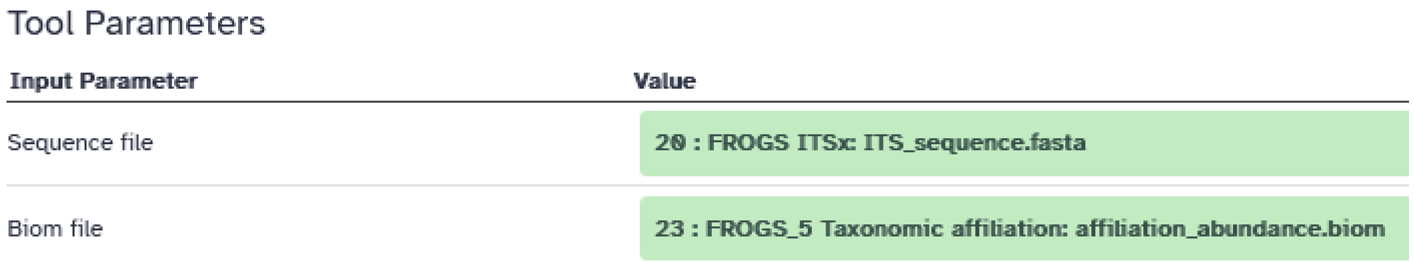
FROGS_6_Affiliation_Stat



Tool Parameters

Input Parameter	Value
Abundance file	23 : FROGS_5 Taxonomic affiliation: affiliation_abundance.biom
Taxonomic ranks	Domain Phylum Class Order Family Genus Species
Rarefaction ranks	Class Order Family Genus Species
Affiliation processed	FROGS_blast

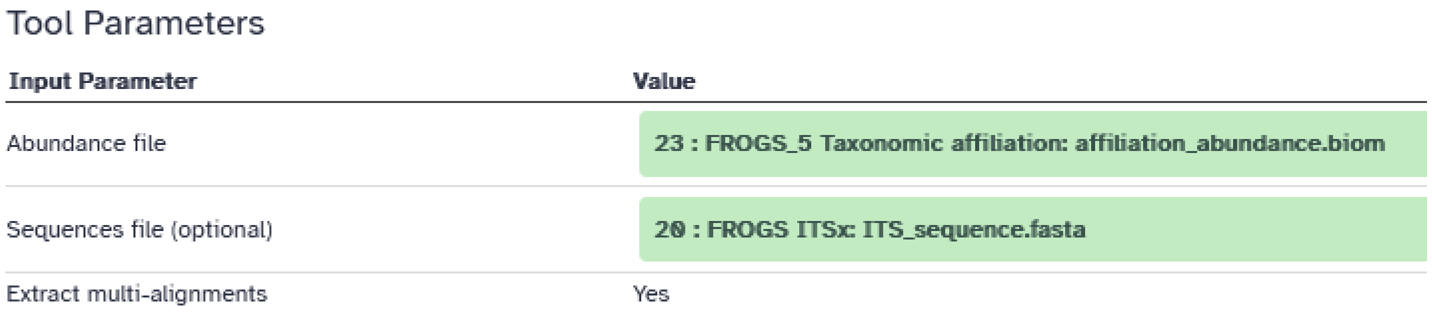
FROGS Tree



Tool Parameters

Input Parameter	Value
Sequence file	20 : FROGS ITSx: ITS_sequence.fasta
Biom file	23 : FROGS_5 Taxonomic affiliation: affiliation_abundance.biom

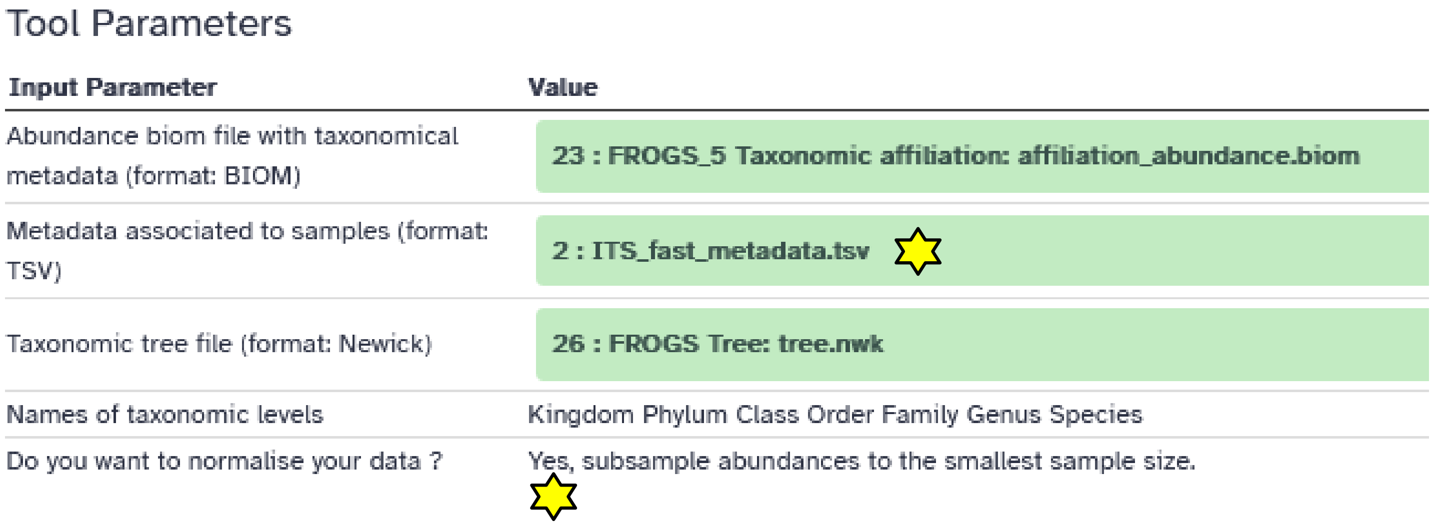
FROGS BIOM to TSV



Tool Parameters

Input Parameter	Value
Abundance file	23 : FROGS_5 Taxonomic affiliation: affiliation_abundance.biom
Sequences file (optional)	20 : FROGS ITSx: ITS_sequence.fasta
Extract multi-alignments	Yes

FROGSSTAT Phyloseq Import Data



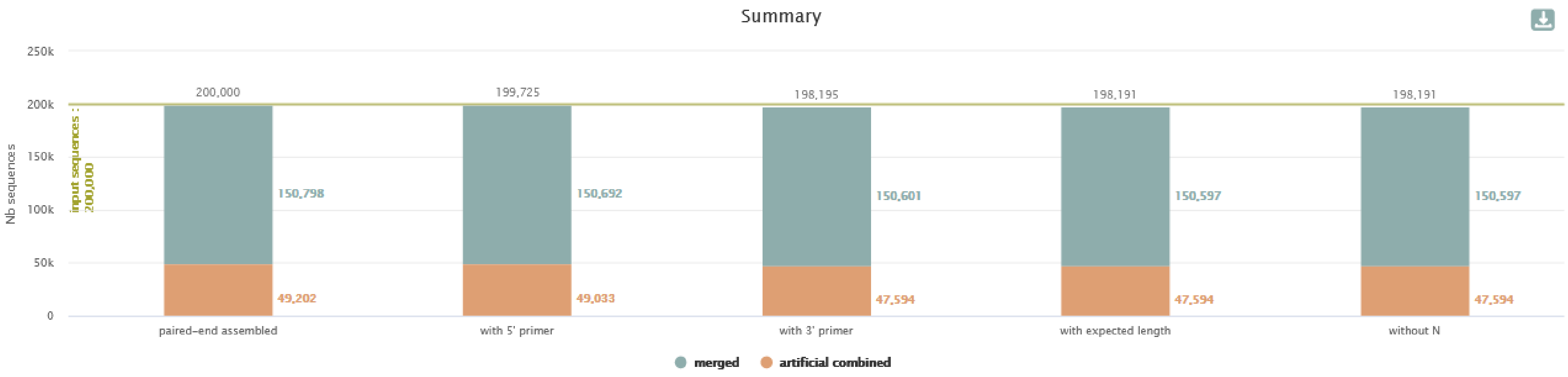
Tool Parameters

Input Parameter	Value
Abundance biom file with taxonomical metadata (format: BIOM)	23 : FROGS_5 Taxonomic affiliation: affiliation_abundance.biom
Metadata associated to samples (format: TSV)	2 : ITS_fast_metadata.tsv ★
Taxonomic tree file (format: Newick)	26 : FROGS Tree: tree.nwk
Names of taxonomic levels	Kingdom Phylum Class Order Family Genus Species
Do you want to normalise your data ?	Yes, subsample abundances to the smallest sample size. ★

Points de vigilance

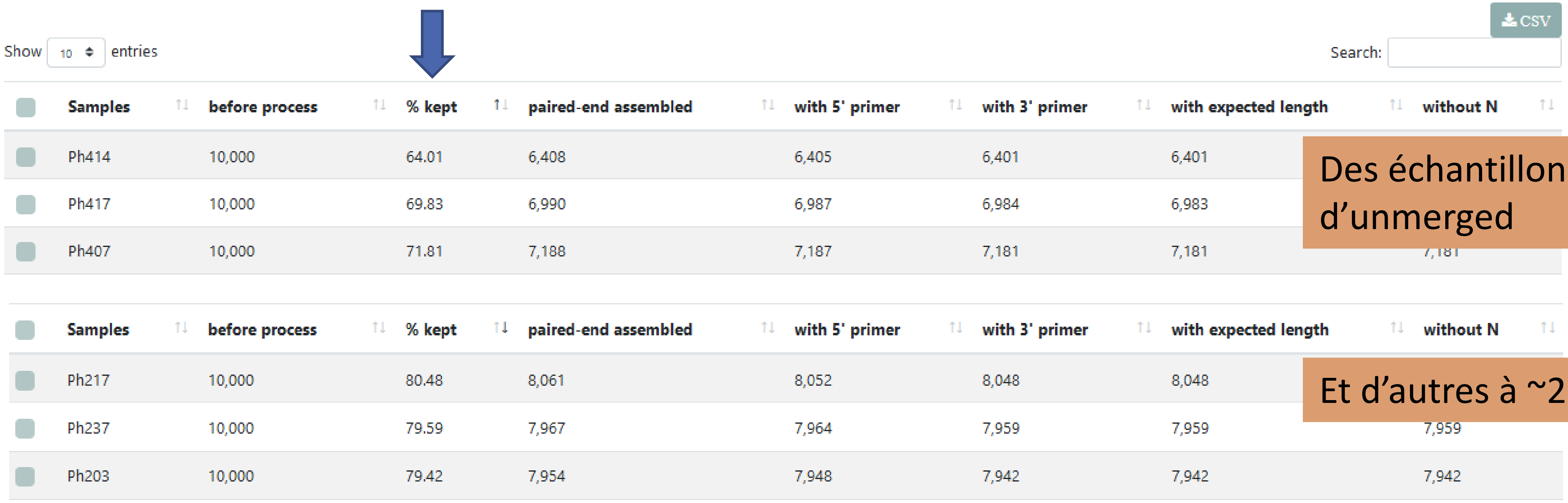
FROGS_1 Pre-process

Preprocess summary



Une quantité importante de artificial combined

Details on merged sequences



☐	Samples	before process	% kept	paired-end assembled	with 5' primer	with 3' primer	with expected length	without N
☐	Ph414	10,000	64.01	6,408	6,405	6,401	6,401	
☐	Ph417	10,000	69.83	6,990	6,987	6,984	6,983	
☐	Ph407	10,000	71.81	7,188	7,187	7,181	7,181	7,181

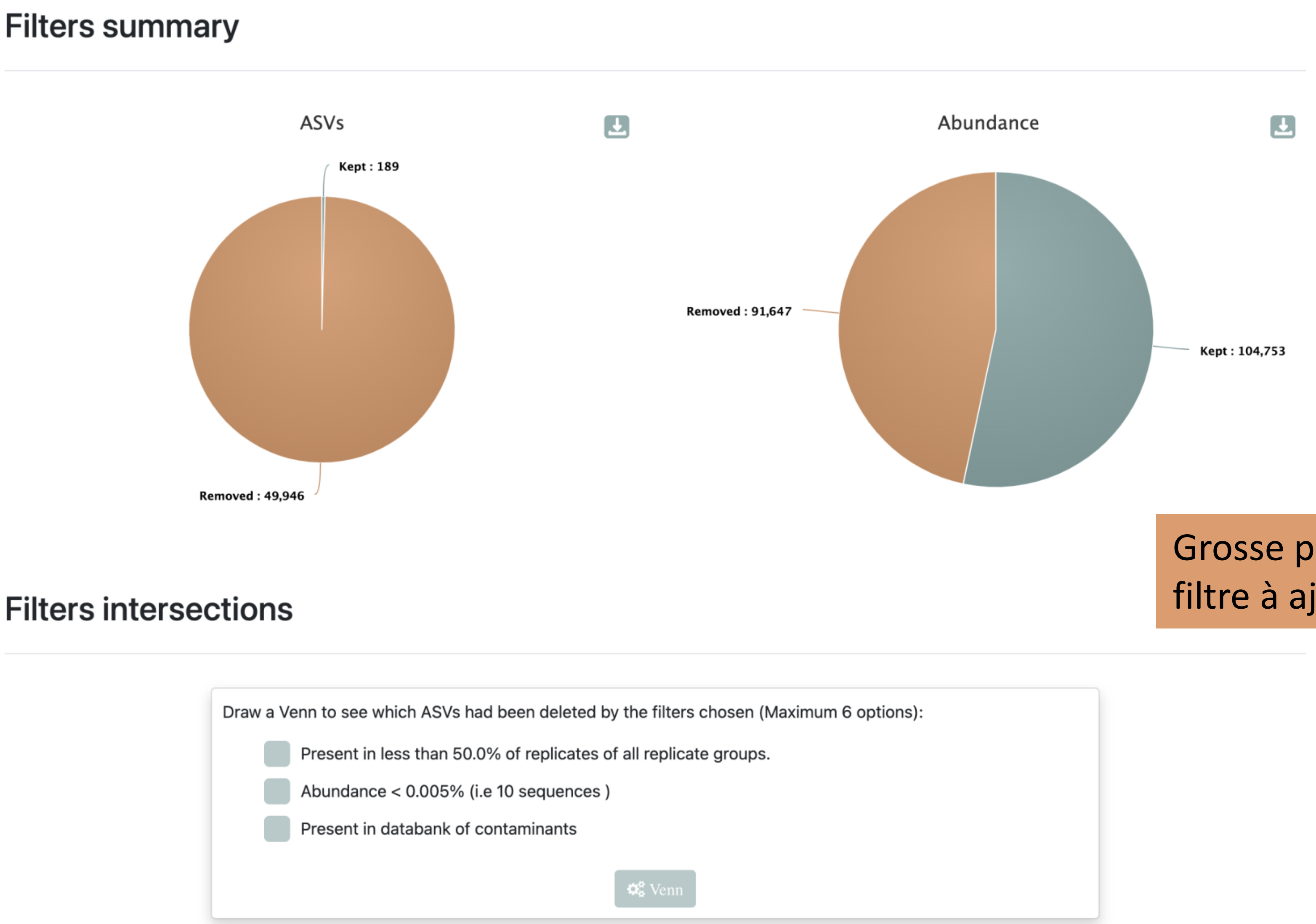
Des échantillons avec >30% d'unmerged

☐	Samples	before process	% kept	paired-end assembled	with 5' primer	with 3' primer	with expected length	without N
☐	Ph217	10,000	80.48	8,061	8,052	8,048	8,048	
☐	Ph237	10,000	79.59	7,967	7,964	7,959	7,959	7,959
☐	Ph203	10,000	79.42	7,954	7,948	7,942	7,942	7,942

Et d'autres à ~20%

FROGS_4 Cluster filters

Filtres à 50% de prévalence par groupe de réplicats :



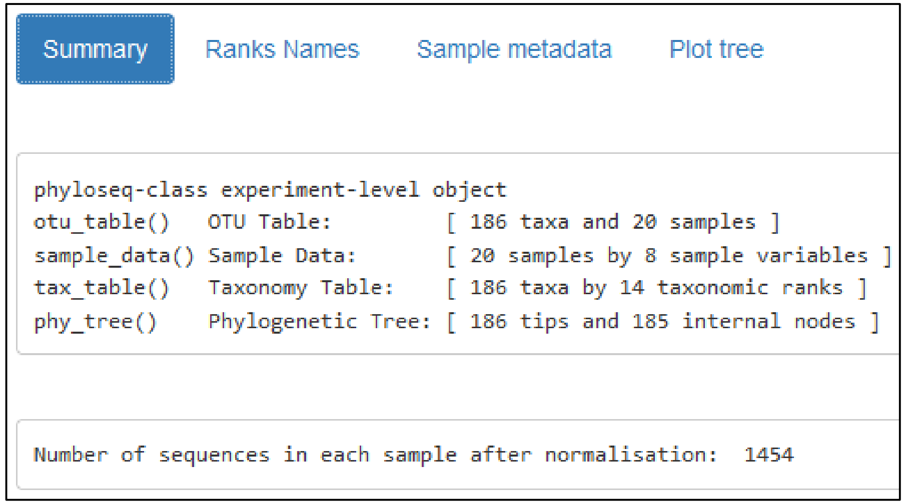
Draw a Venn to see which ASVs had been deleted by the filters chosen (Maximum 6 options):

- ☐ Present in less than 50.0% of replicates of all replicate groups.
- ☐ Abundance < 0.005% (i.e 10 sequences)
- ☐ Present in databank of contaminants

Grosse perte de séquences, filtre à ajuster ou modifier?

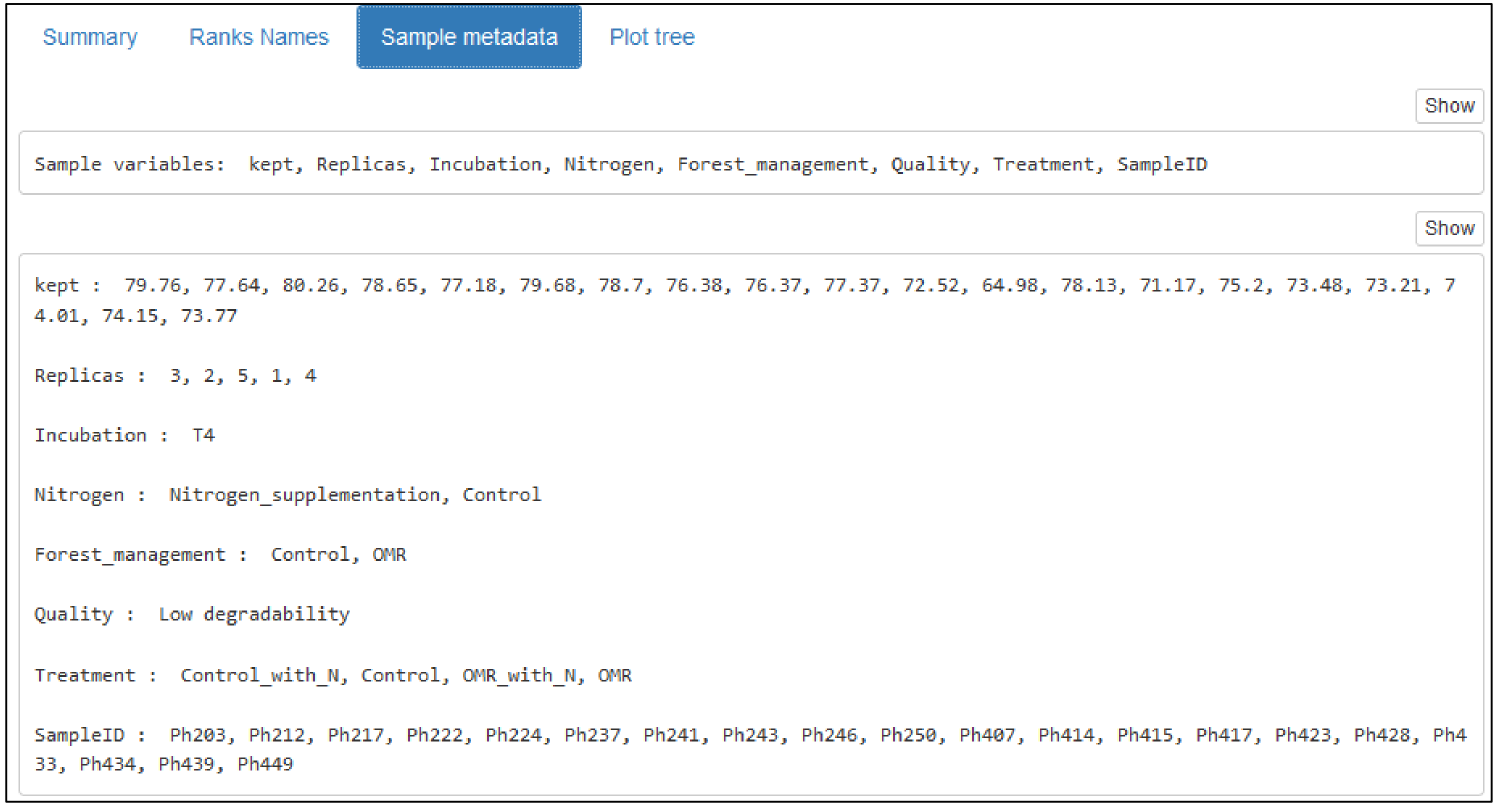
FROGSSTAT Phyloseq Import Data

FROGSSTAT Phyloseq Import Data



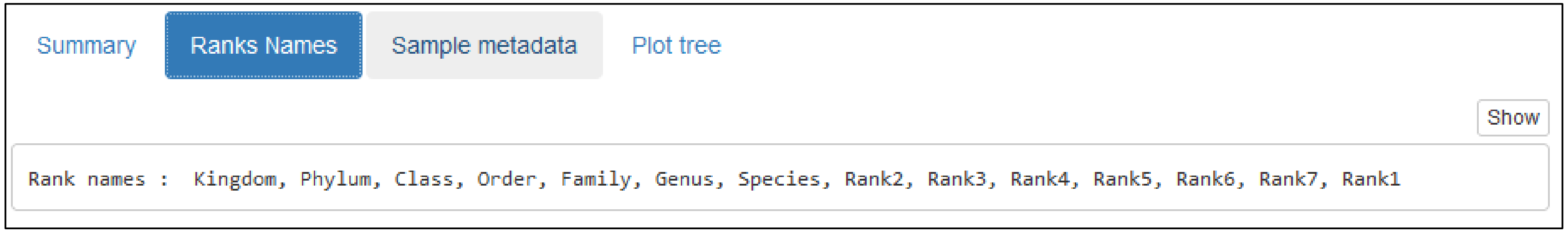
```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 186 taxa and 20 samples ]
sample_data() Sample Data:       [ 20 samples by 8 sample variables ]
tax_table()   Taxonomy Table:    [ 186 taxa by 14 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 186 tips and 185 internal nodes ]
```

```
Number of sequences in each sample after normalisation:  1454
```

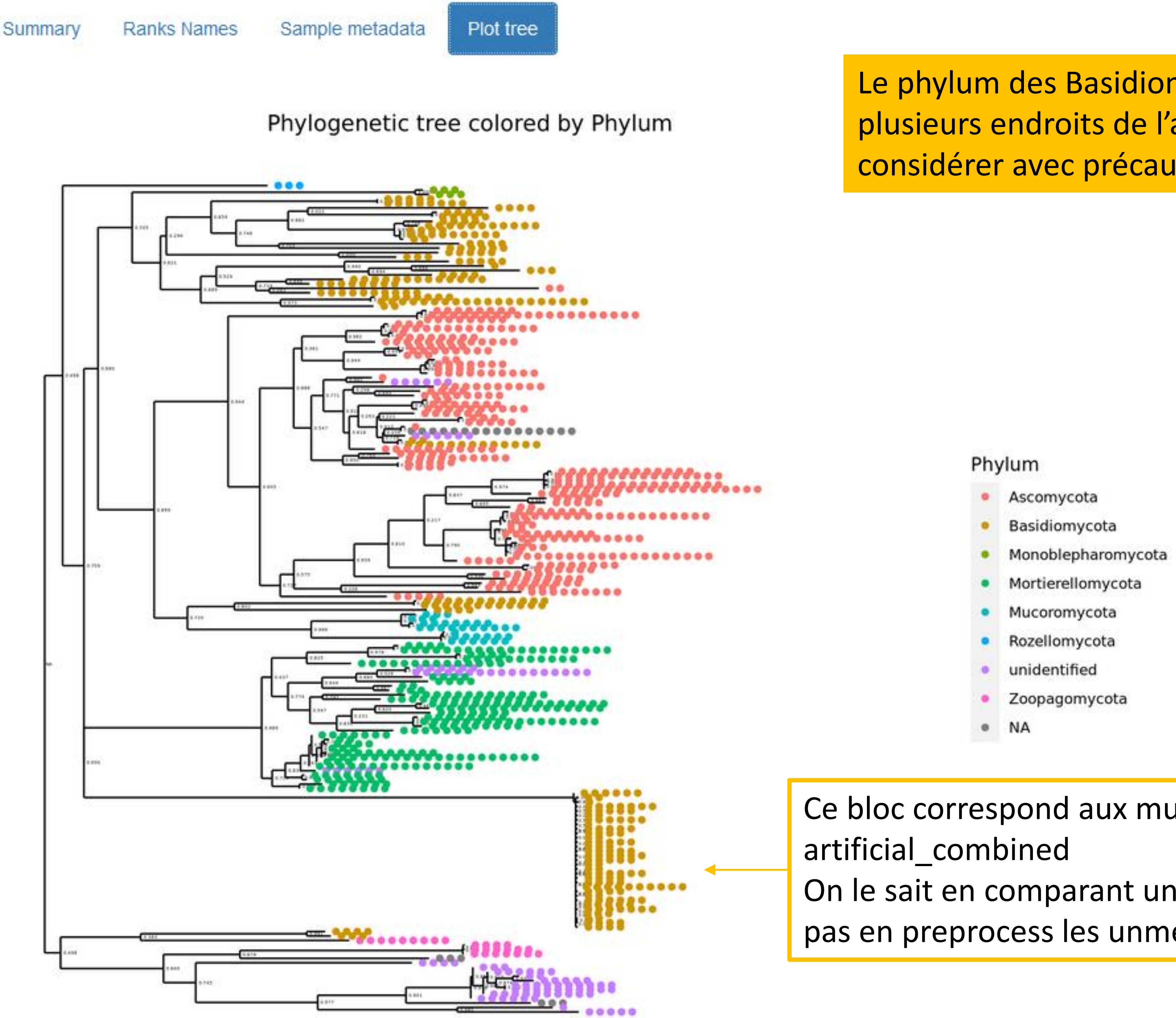


```
Sample variables:  kept, Replicas, Incubation, Nitrogen, Forest_management, Quality, Treatment, SampleID
```

```
kept :  79.76, 77.64, 80.26, 78.65, 77.18, 79.68, 78.7, 76.38, 76.37, 77.37, 72.52, 64.98, 78.13, 71.17, 75.2, 73.48, 73.21, 7
4.01, 74.15, 73.77

Replicas :  3, 2, 5, 1, 4

Incubation :  T4

Nitrogen :  Nitrogen_supplementation, Control

Forest_management :  Control, OMR

Quality :  Low degradability

Treatment :  Control_with_N, Control, OMR_with_N, OMR

SampleID :  Ph203, Ph212, Ph217, Ph222, Ph224, Ph237, Ph241, Ph243, Ph246, Ph250, Ph407, Ph414, Ph415, Ph417, Ph423, Ph428, Ph4
33, Ph434, Ph439, Ph449
```



```
Rank names :  Kingdom, Phylum, Class, Order, Family, Genus, Species, Rank2, Rank3, Rank4, Rank5, Rank6, Rank7, Rank1
```



Le phylum des Basidiomycota est éclaté en plusieurs endroits de l’arbre, données Unifrac à considérer avec précautions

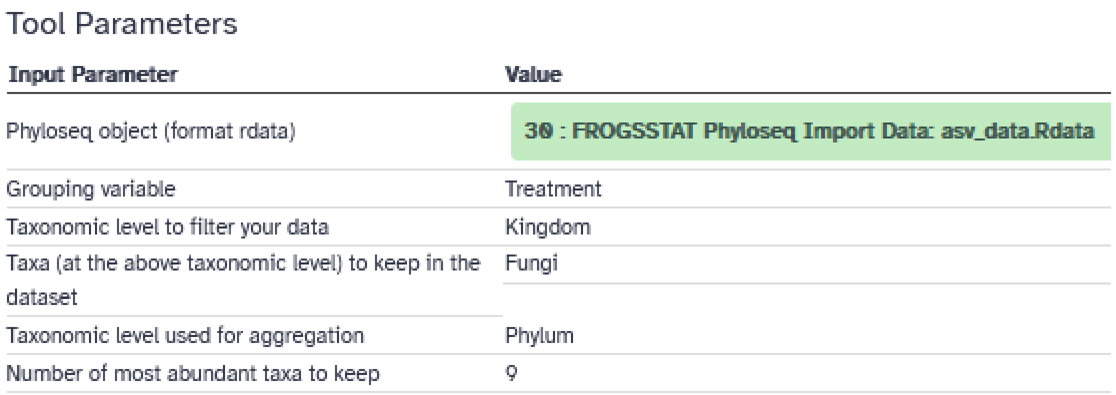
Ce bloc correspond aux multiples clusters artificial_combined
On le sait en comparant un historique n’acceptant pas en preprocess les unmerged.

Pistes stat pour ITS

SANS POST-AFFILIATION (ET DONC SANS REGROUPEMENT DE CERTAINS ARTIFICIAL_COMBINED)

HISTORY ITS_FORMATION_2023_PREV50

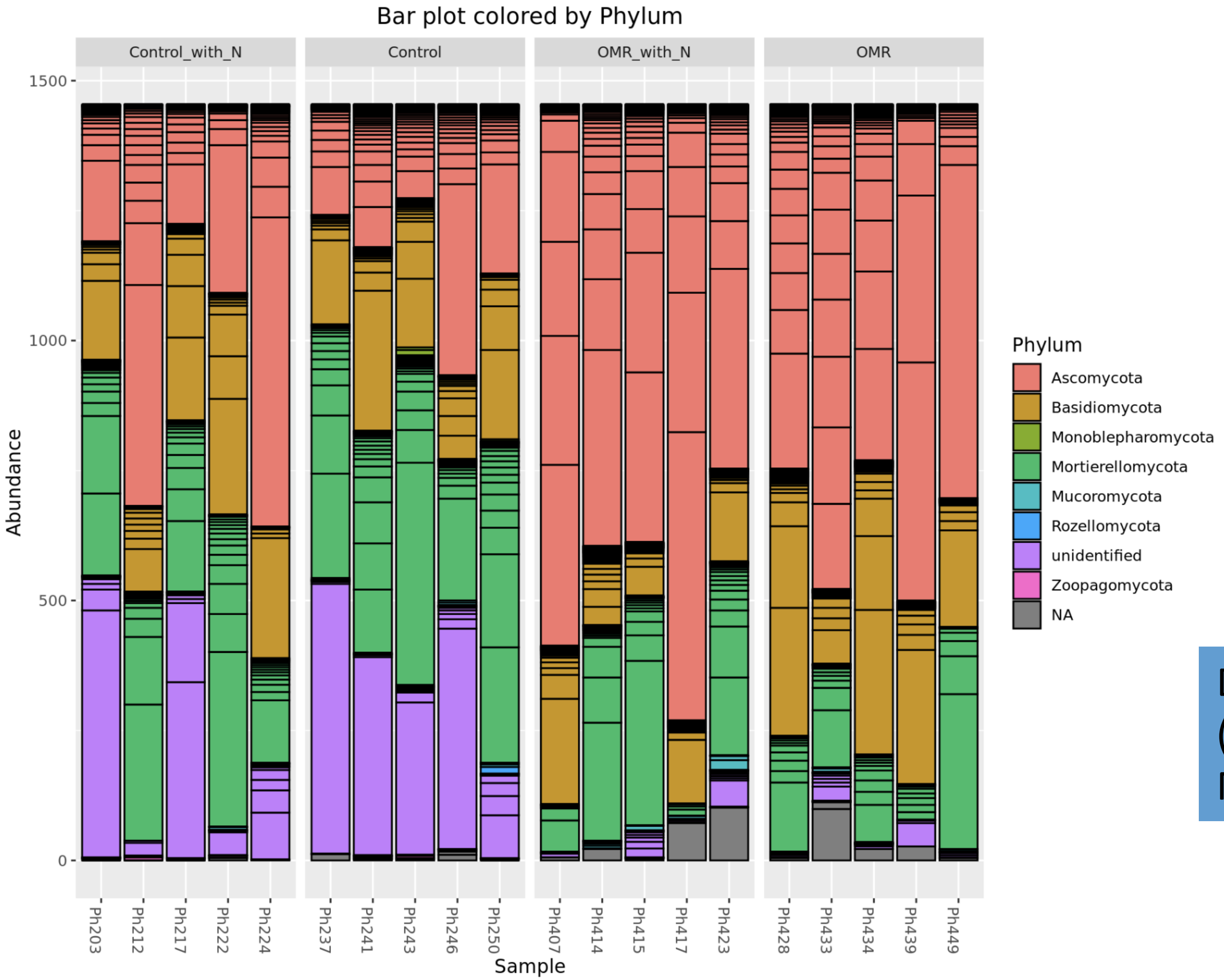
FROGSSTAT Phyloseq Composition Visualisation



Tool Parameters

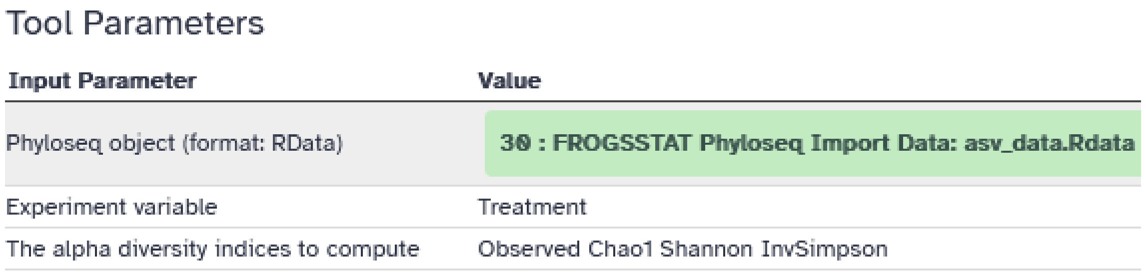
Input Parameter	**Value**
Phyloseq object (format rdata)	**30 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata**
Grouping variable	Treatment
Taxonomic level to filter your data	Kingdom
Taxa (at the above taxonomic level) to keep in the dataset	Fungi
Taxonomic level used for aggregation	Phylum
Number of most abundant taxa to keep	9

Interprétation des barplots :
La plupart des outils de visualisation FROGS n'autorisent qu'une seule variable de tri.
Utiliser la variable Treatment (qui combine les deux variables Forest_management et Nitrogen) permet de « tricher » pour afficher deux variables dans les outils de visualisation



Différences évidentes entre Control et OMR (augmentation des Ascomycota)
Moins évident entre with N et sans

FROGSSTAT Phyloseq Alpha Diversity

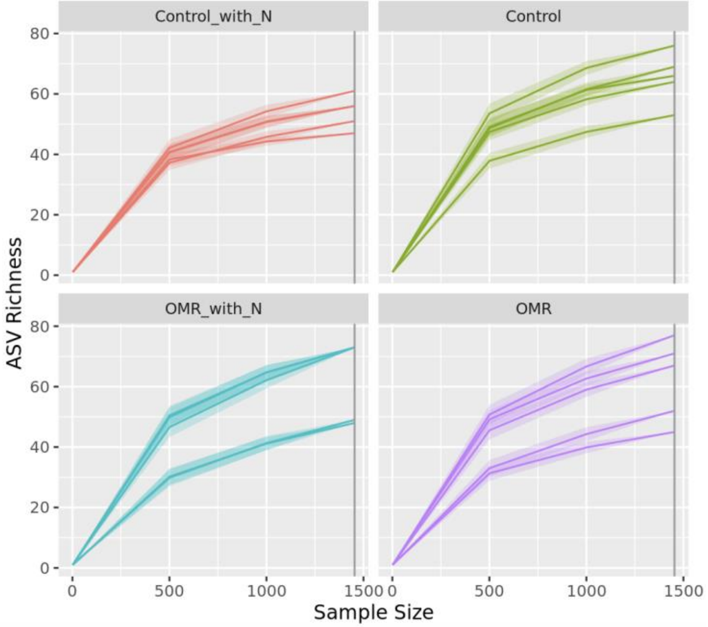


Tool Parameters

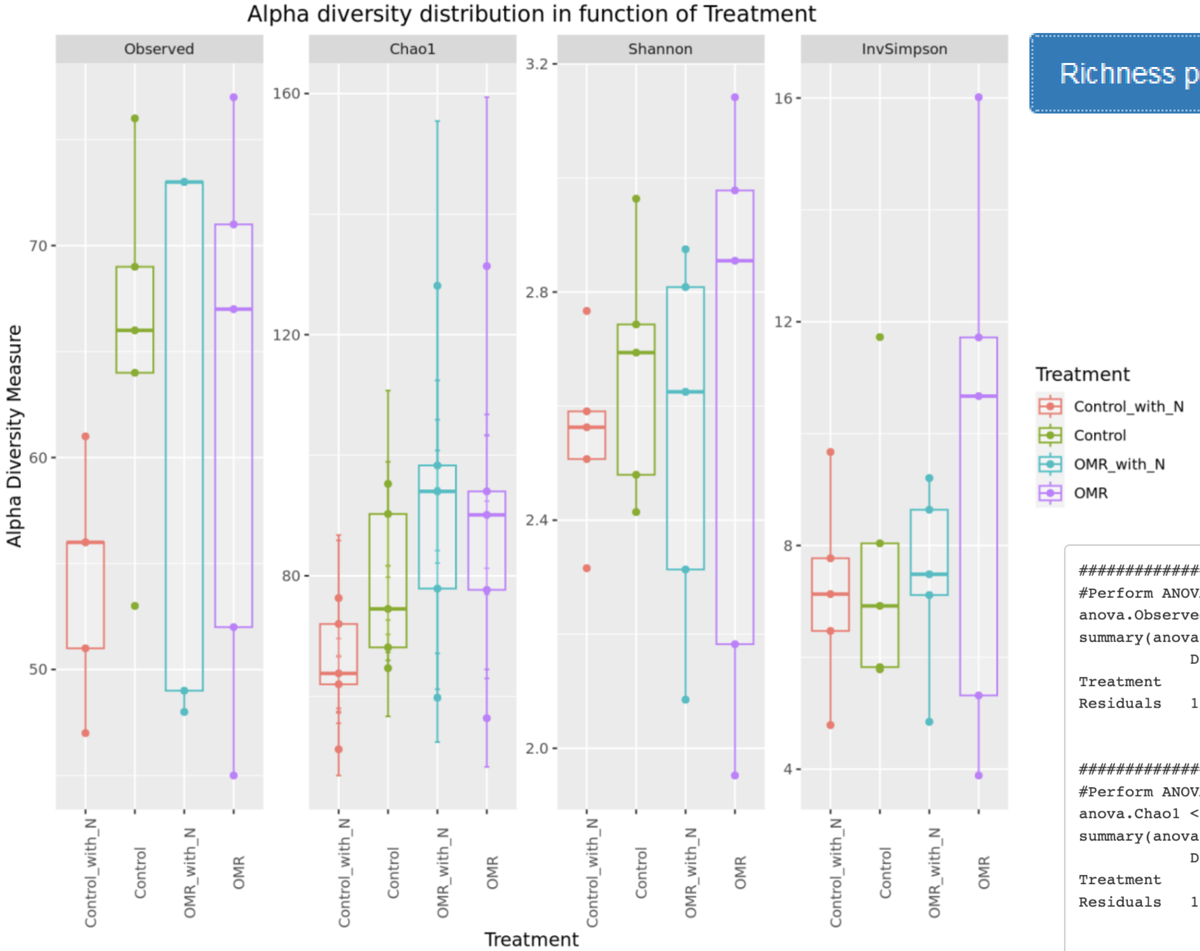
Input Parameter	Value
Phyloseq object (format: RData)	**30 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata**
Experiment variable	Treatment
The alpha diversity indices to compute	Observed Chao1 Shannon InvSimpson

Show

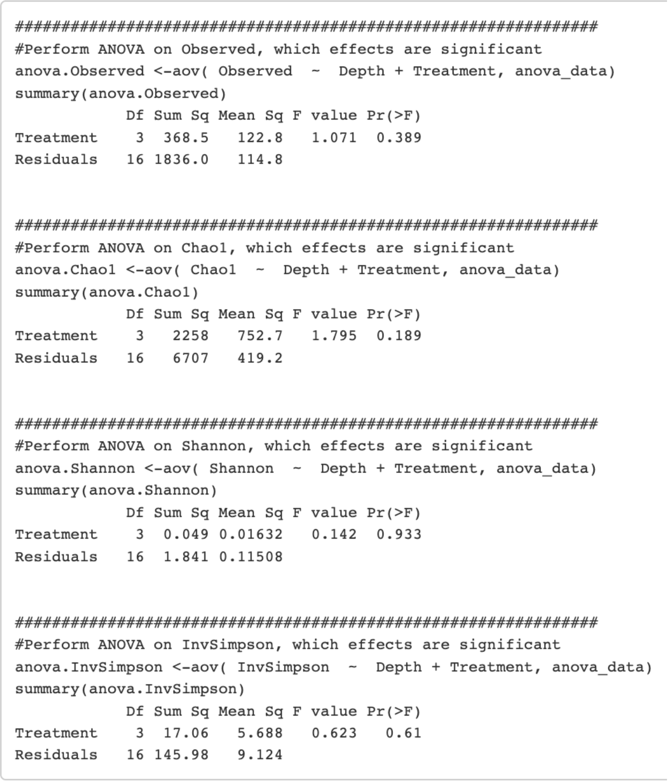
Rarefaction curves



Richness plot with boxplot



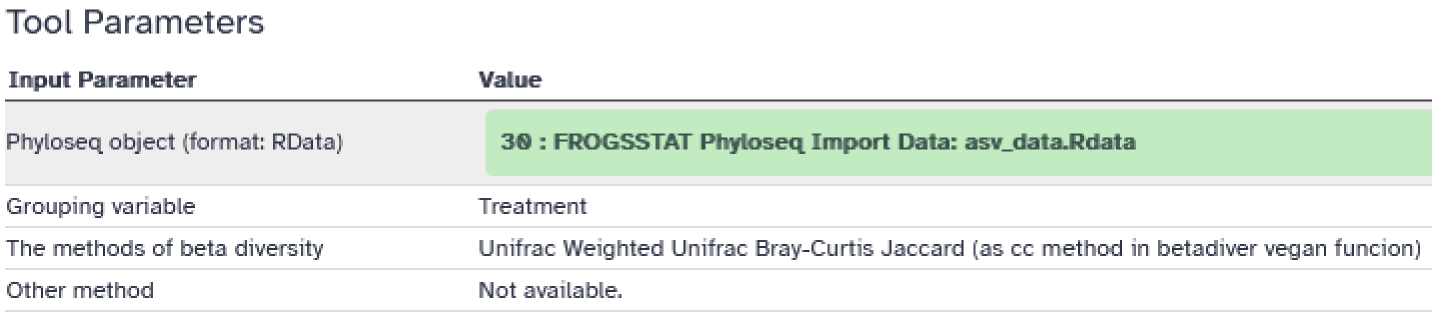
Alpha Diversity Indice Anova Analysis



```
##############################################################
#Perform ANOVA on Observed, which effects are significant
anova.Observed <-aov( Observed  ~  Depth + Treatment, anova_data)
summary(anova.Observed)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3  368.5   122.8   1.071  0.389
Residuals   16 1836.0   114.8


##############################################################
#Perform ANOVA on Chao1, which effects are significant
anova.Chao1 <-aov( Chao1  ~  Depth + Treatment, anova_data)
summary(anova.Chao1)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3   2258   752.7   1.795  0.189
Residuals   16   6707   419.2


##############################################################
#Perform ANOVA on Shannon, which effects are significant
anova.Shannon <-aov( Shannon  ~  Depth + Treatment, anova_data)
summary(anova.Shannon)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3  0.049 0.01632   0.142  0.933
Residuals   16  1.841 0.11508


##############################################################
#Perform ANOVA on InvSimpson, which effects are significant
anova.InvSimpson <-aov( InvSimpson  ~  Depth + Treatment, anova_data)
summary(anova.InvSimpson)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3  17.06   5.688   0.623   0.61
Residuals   16 145.98   9.124
```

Pas d’effet significatif du traitement sur aucun indice.
Profondeur de séquençage très faible (sous-échantillon pour le TP et effets des filtres à 50%)

FROGSSTAT Phyloseq Beta Diversity

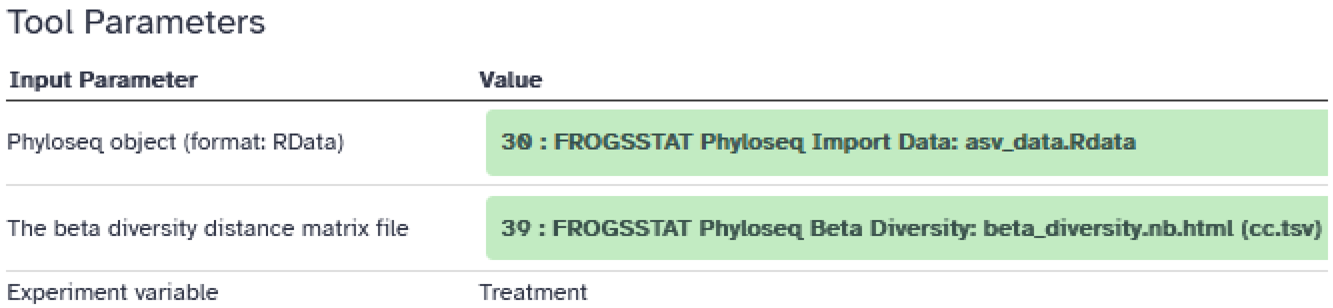


Tool Parameters

Input Parameter	Value
Phyloseq object (format: RData)	30 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata
Grouping variable	Treatment
The methods of beta diversity	Unifrac Weighted Unifrac Bray-Curtis Jaccard (as cc method in betadiver vegan funcion)
Other method	Not available.

Cet outil sert à produire des matrices pour ensuite faire la clusterisation et la visualisation

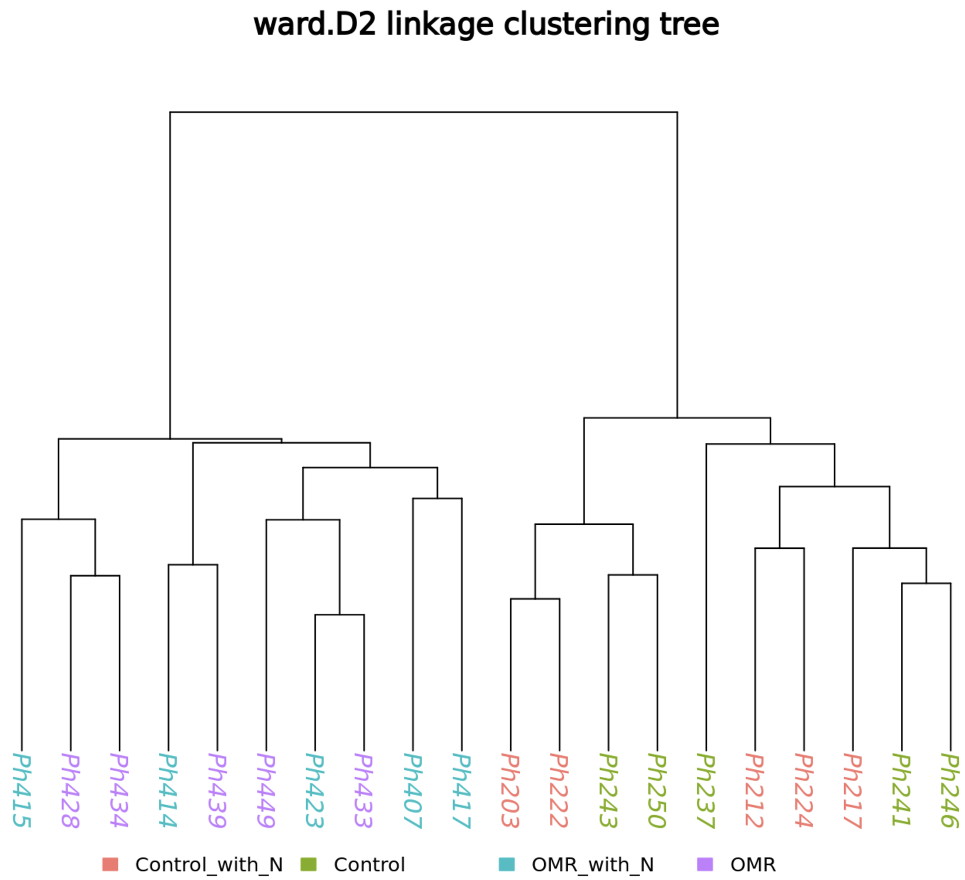
FROGSSTAT Phyloseq Sample Clustering



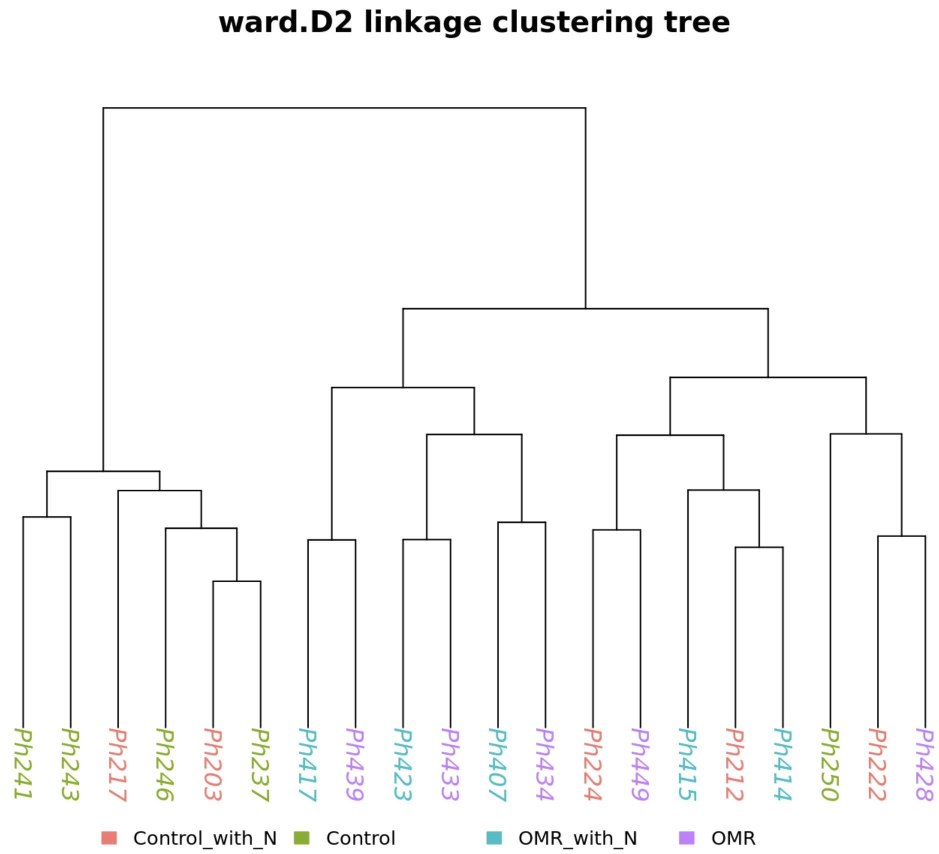
Tool Parameters

Input Parameter	Value
Phyloseq object (format: RData)	30 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata
The beta diversity distance matrix file	39 : FROGSSTAT Phyloseq Beta Diversity: beta_diversity.nb.html (cc.tsv)
Experiment variable	Treatment

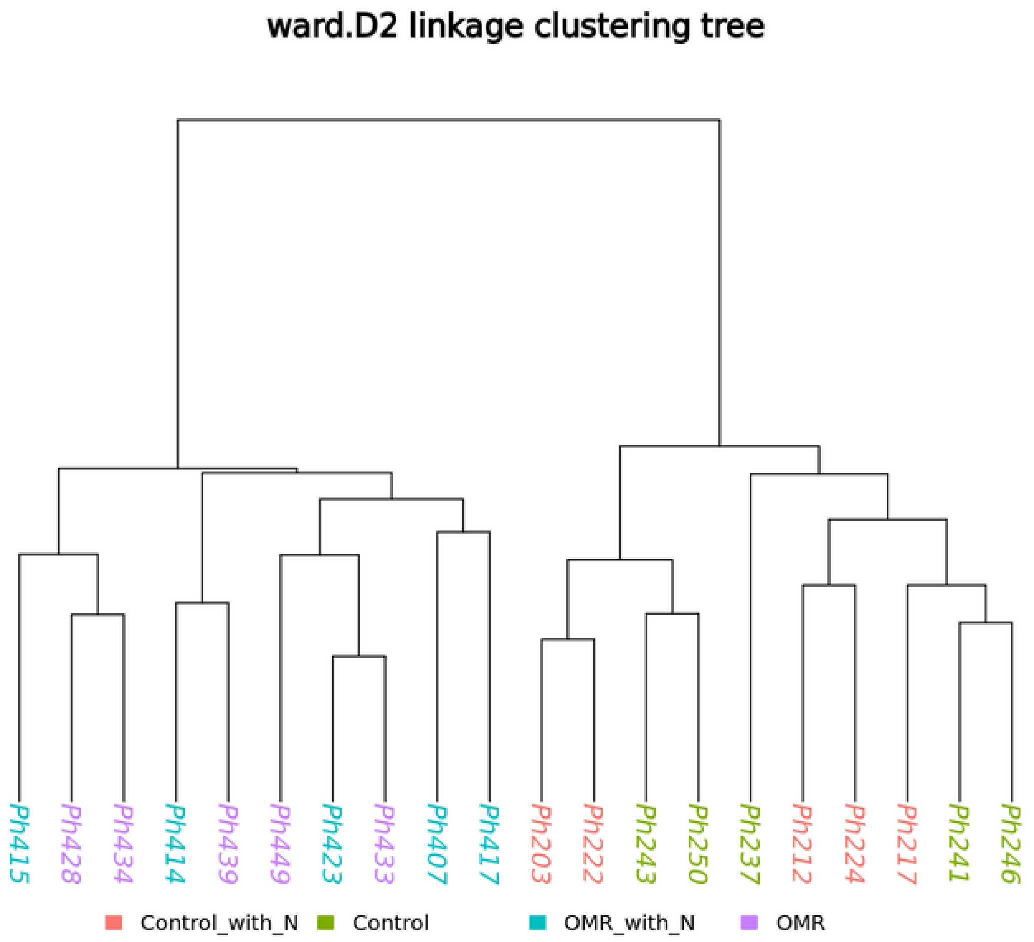
Diversité bêta – clustering :



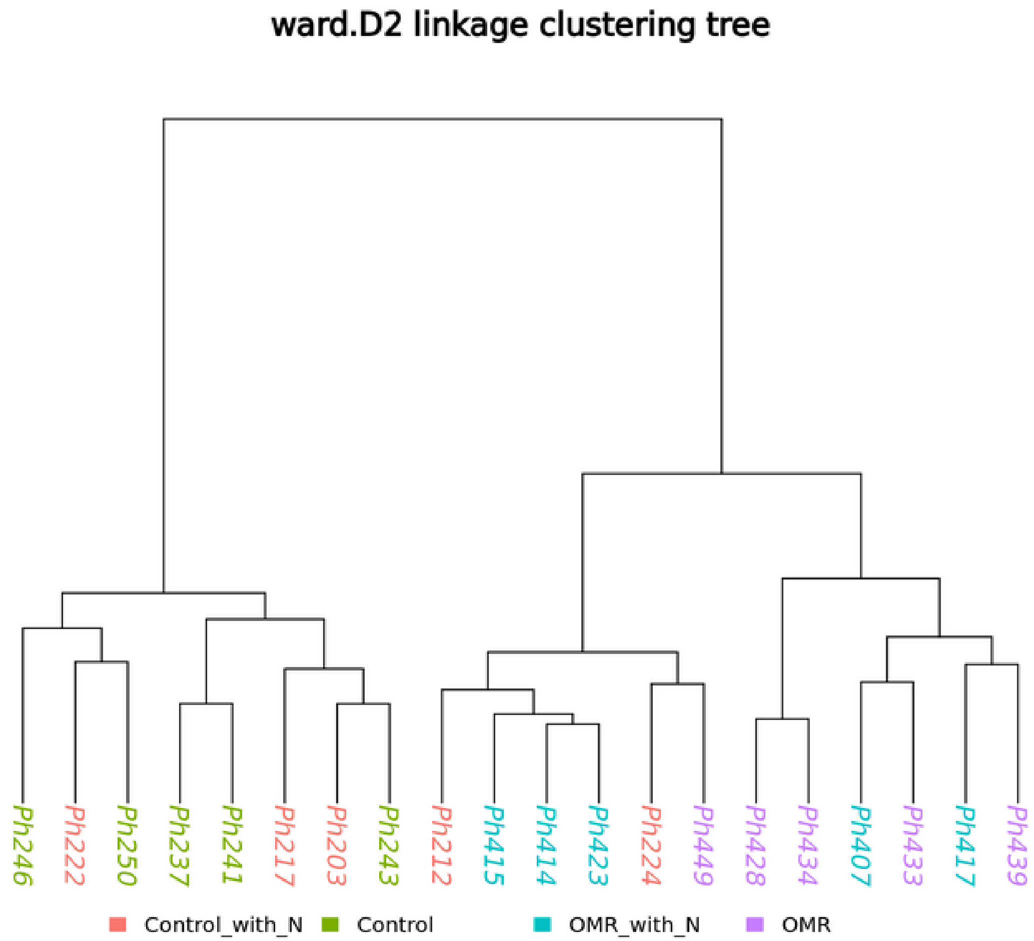
Jaccard



Bray



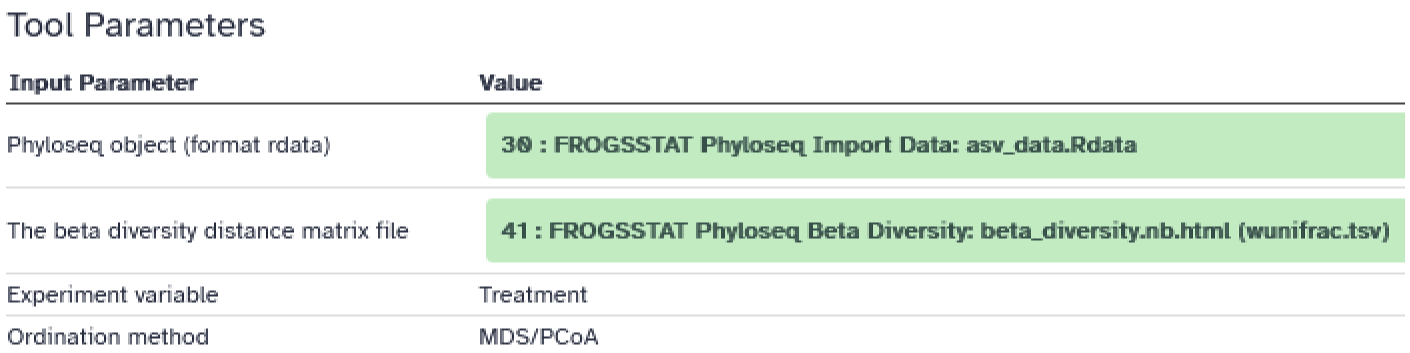
Unifrac



wUnifrac

Différences entre Control et OMR mais pas avec l'ajout d'azote.
Meilleur clustering avec les méthodes qualitatives qu'avec les méthodes quantitatives :
les différences sont plutôt dues à des ASV peu abondants
(quand on prend en compte les abondances les échantillons se ressemblent davantage)

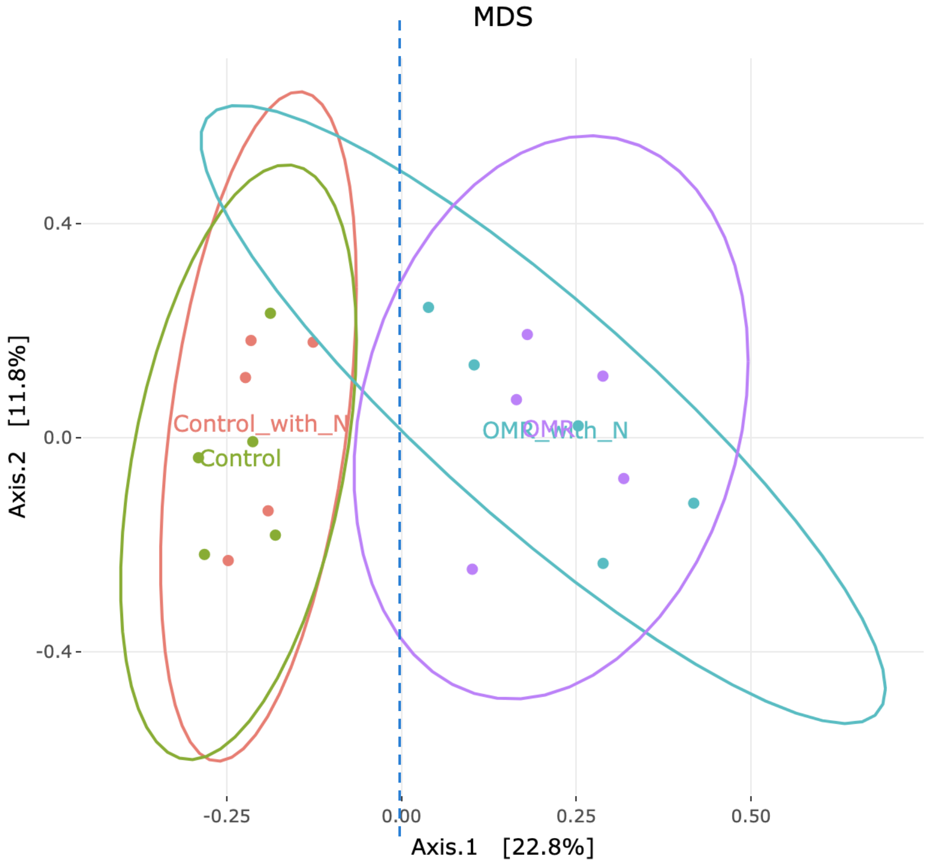
FROGSSTAT Phyloseq Structure Visualisation



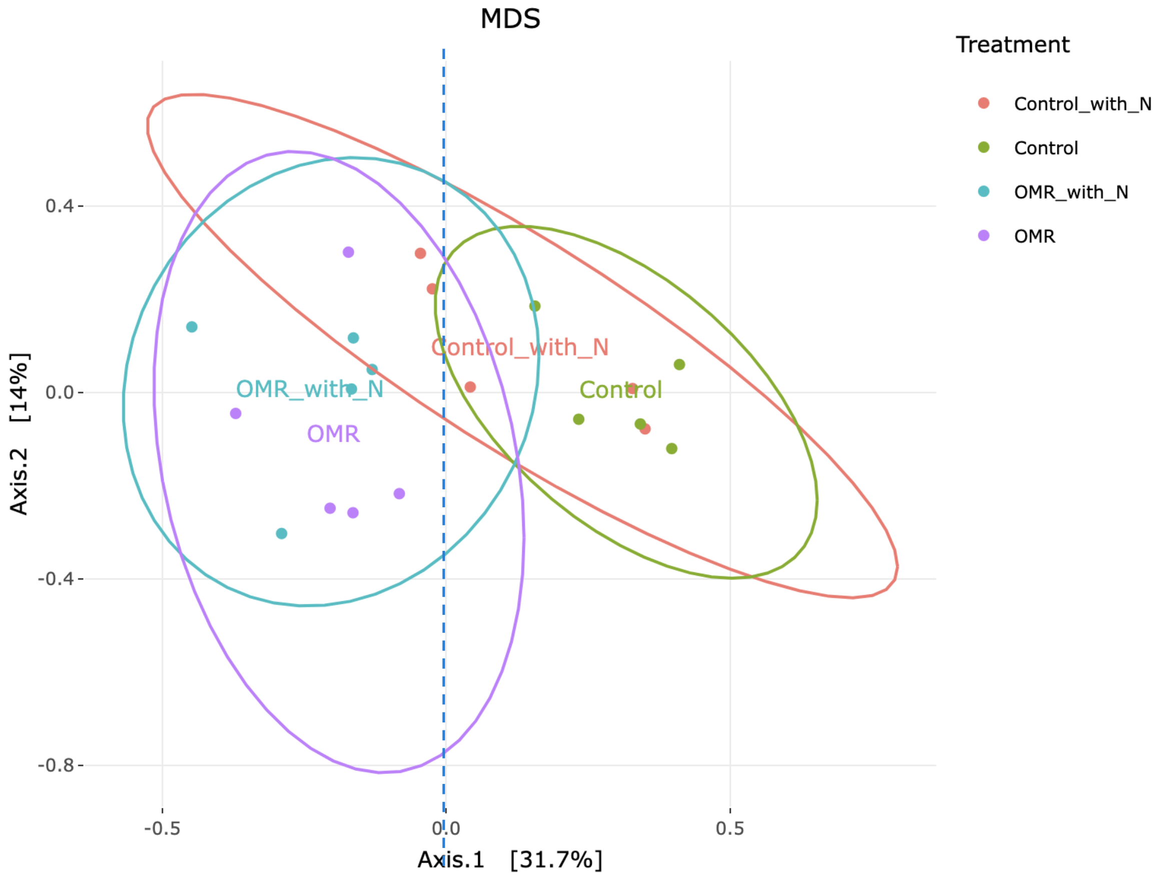
Tool Parameters

Input Parameter	Value
Phyloseq object (format rdata)	**30 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata**
The beta diversity distance matrix file	**41 : FROGSSTAT Phyloseq Beta Diversity: beta_diversity.nb.html (wunifrac.tsv)**
Experiment variable	Treatment
Ordination method	MDS/PCoA

Diversité bêta – ordination :



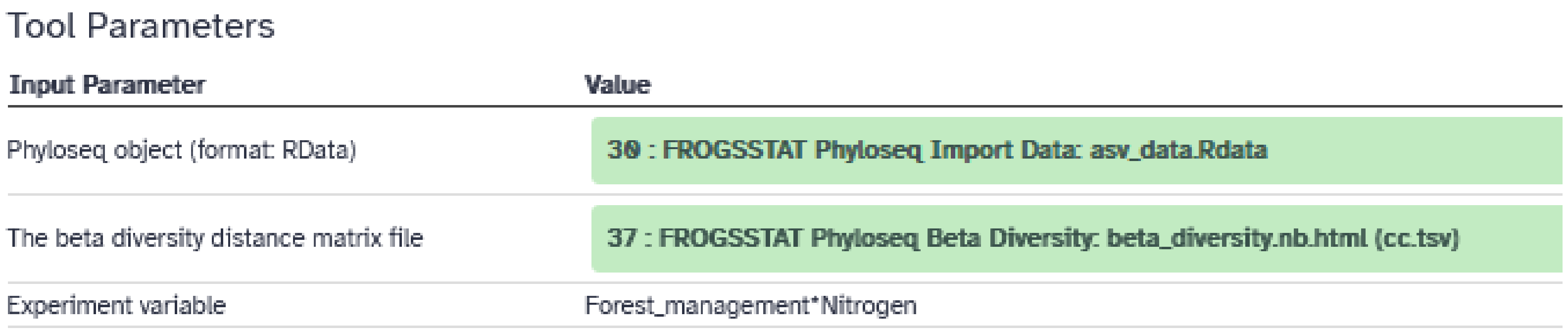
Jaccard



Bray

Différences entre Control et OMR mais pas avec l’ajout d’azote.
Meilleure séparation (sur l’axe1) avec les méthodes qualitatives qu’avec les méthodes quantitatives :
les différences sont plutôt dues à des ASV peu abondants
(quand on prend en compte les abondances les échantillons se ressemblent davantage)

FROGSSTAT Phyloseq Multivariate Analysis Of Variance



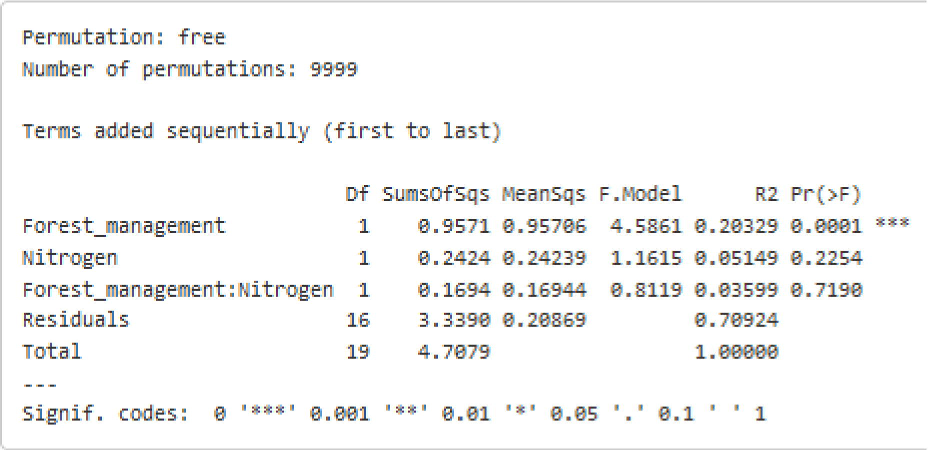
Tool Parameters

Input Parameter	**Value**
Phyloseq object (format: RData)	**30 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata**
The beta diversity distance matrix file	**37 : FROGSSTAT Phyloseq Beta Diversity: beta_diversity.nb.html (cc.tsv)**
Experiment variable	Forest_management*Nitrogen

Diversité bêta – PERMANOVA :

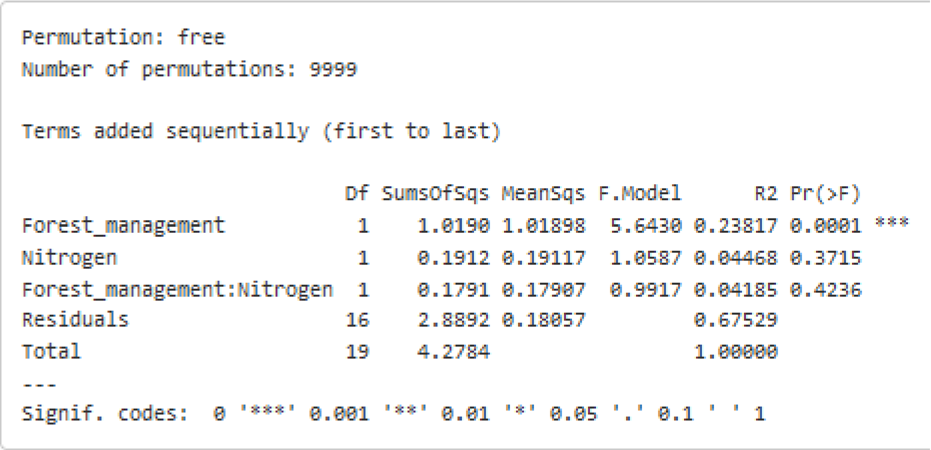
Il est ici possible de donner plusieurs variables séparées par des + ou * (effets additifs ou avec interaction), et donc plus pertinent d'utiliser Nitrogen et Forest_management plutôt que Treatment

```
adonis(formula = dist ~ Forest_management * Nitrogen, data = metadata,
    permutations = 9999)
```



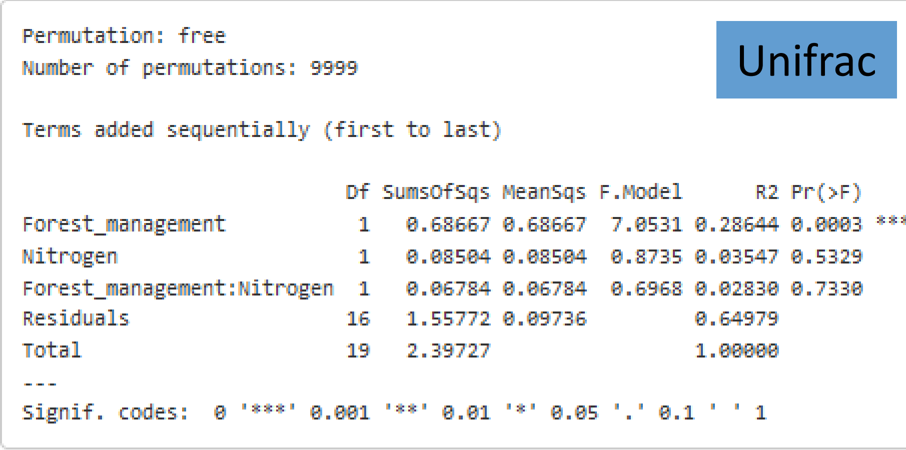
```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1    0.9571 0.95706  4.5861 0.20329 0.0001 ***
Nitrogen                    1    0.2424 0.24239  1.1615 0.05149 0.2254    
Forest_management:Nitrogen  1    0.1694 0.16944  0.8119 0.03599 0.7190    
Residuals                  16    3.3390 0.20869         0.70924           
Total                      19    4.7079                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Jaccard



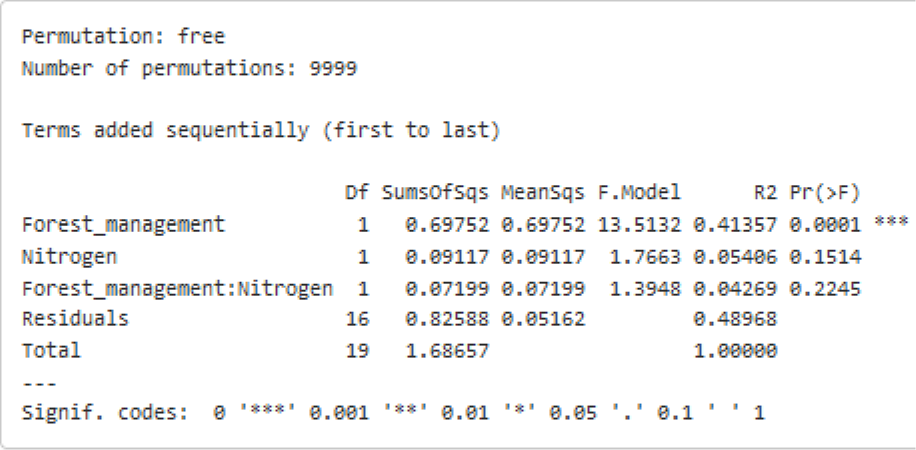
```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1    1.0190 1.01898  5.6430 0.23817 0.0001 ***
Nitrogen                    1    0.1912 0.19117  1.0587 0.04468 0.3715    
Forest_management:Nitrogen  1    0.1791 0.17907  0.9917 0.04185 0.4236    
Residuals                  16    2.8892 0.18057         0.67529           
Total                      19    4.2784                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Bray



Unifrac

```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1   0.68667 0.68667  7.0531 0.28644 0.0003 ***
Nitrogen                    1   0.08504 0.08504  0.8735 0.03547 0.5329    
Forest_management:Nitrogen  1   0.06784 0.06784  0.6968 0.02830 0.7330    
Residuals                  16   1.55772 0.09736         0.64979           
Total                      19   2.39727                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

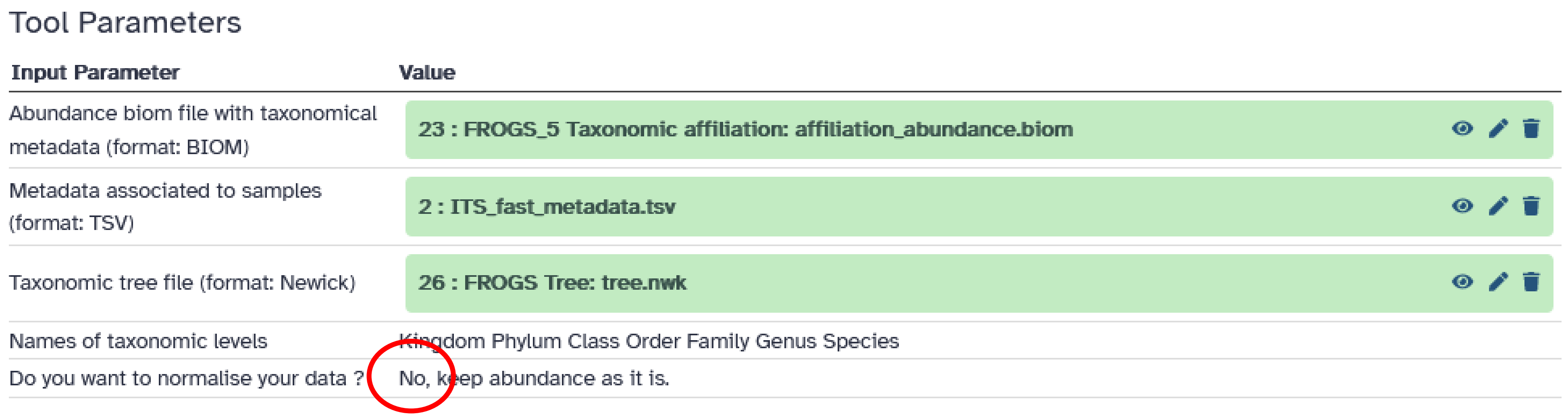


```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1   0.69752 0.69752 13.5132 0.41357 0.0001 ***
Nitrogen                    1   0.09117 0.09117  1.7663 0.05406 0.1514    
Forest_management:Nitrogen  1   0.07199 0.07199  1.3948 0.04269 0.2245    
Residuals                  16   0.82588 0.05162         0.48968           
Total                      19   1.68657                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

wUnifrac

Les PERMANOVA confirment avec tous les indices un effet significatif de la variable Forest_management mais pas de la variable Nitrogen.

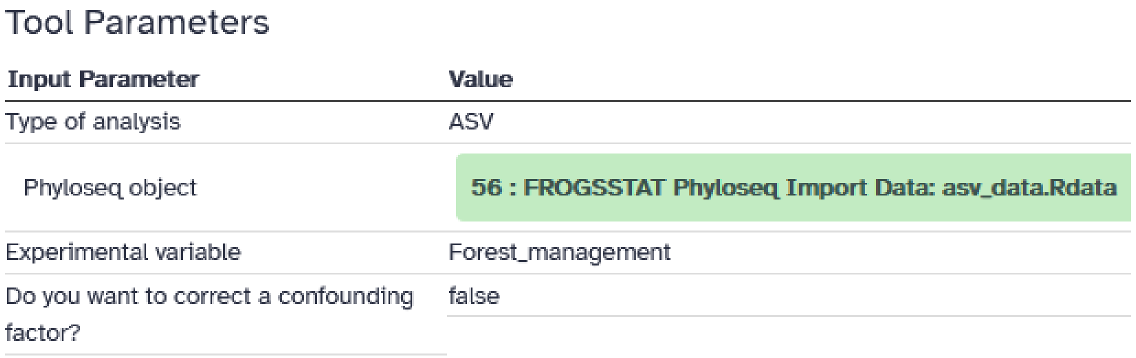
FROGSSTAT Phyloseq Import Data



Tool Parameters

Input Parameter	Value
Abundance biom file with taxonomical metadata (format: BIOM)	23 : FROGS_5 Taxonomic affiliation: affiliation_abundance.biom
Metadata associated to samples (format: TSV)	2 : ITS_fast_metadata.tsv
Taxonomic tree file (format: Newick)	26 : FROGS Tree: tree.nwk
Names of taxonomic levels	Kingdom Phylum Class Order Family Genus Species
Do you want to normalise your data ?	No, keep abundance as it is.

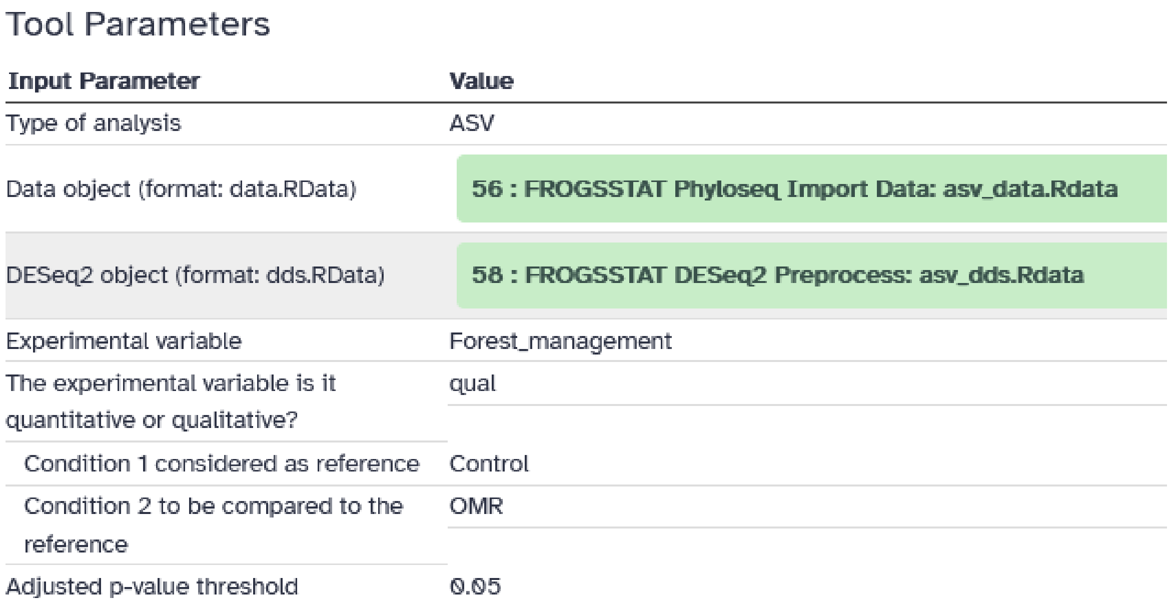
FROGSSTAT DESeq2 Preprocess



Tool Parameters

Input Parameter	Value
Type of analysis	ASV
Phyloseq object	56 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata
Experimental variable	Forest_management
Do you want to correct a confounding factor?	false

FROGSSTAT DESeq2 Visualisation



Tool Parameters

Input Parameter	Value
Type of analysis	ASV
Data object (format: data.RData)	56 : FROGSSTAT Phyloseq Import Data: asv_data.Rdata
DESeq2 object (format: dds.RData)	58 : FROGSSTAT DESeq2 Preprocess: asv_dds.Rdata
Experimental variable	Forest_management
The experimental variable is it quantitative or qualitative?	qual
Condition 1 considered as reference	Control
Condition 2 to be compared to the reference	OMR
Adjusted p-value threshold	0.05

Seule la variable Forest_management est pertinente à analyser puisque Nitrogen n'a pas d'effet significatif.

On pourrait aussi utiliser Treatment et faire des comparaisons entre groupes pertinents, mais les effectifs (en nombre d'échantillons dans chaque groupe) seraient plus petits.

Plusieurs clusters différentiellement abondants, dans un sens ou dans l'autre. **Et beaucoup de FROGS_combined !!!**

Rq: ces FROGS_combined ont le même comportement = peut-être la même espèce = peut-être utile de faire une post-affiliation

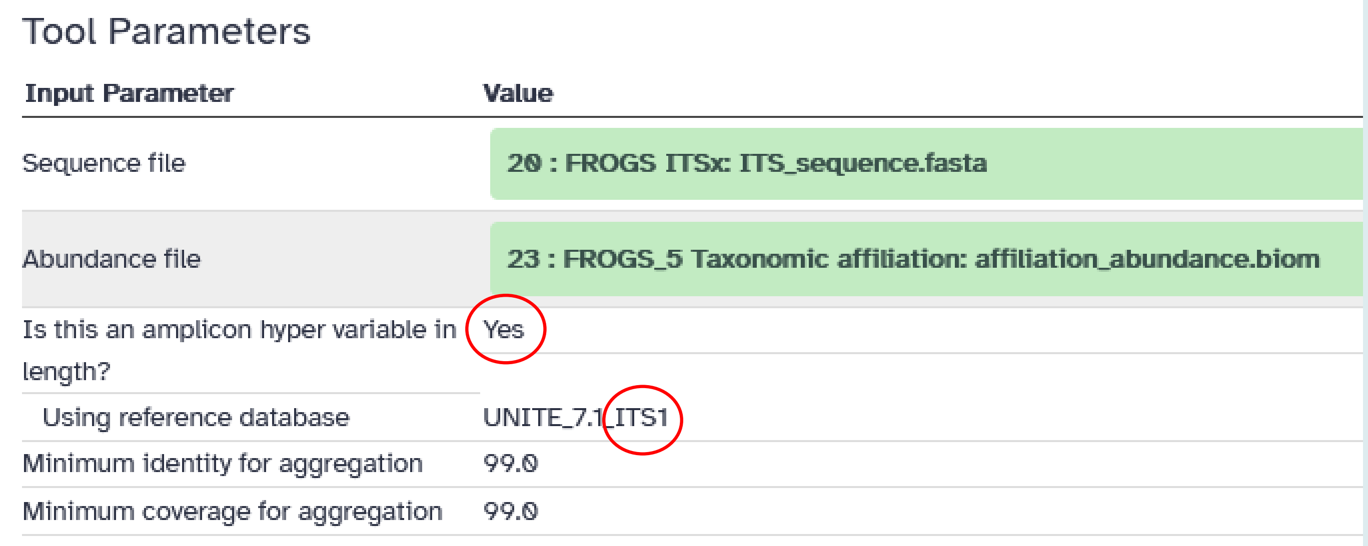
Sans un paramétrage adapté ITS lors du FROGS_1_preprocess ces clusters aurait été éliminés pendant le traitement et n'auraient donc pas été identifiés

Pistes stat pour ITS

AVEC POST-AFFILIATION (ET DONC AVEC REGROUPEMENT DE CERTAINS ARTIFICIAL_COMBINED)

HISTORY : ITS_FORMATION_2023_WITH_AFFI_POSTPROCESS

A la suite de FROGS ITSx et FROGS_5 Taxonomic affiliation FROGS_Affiliation_Stat lancement de FROGS Affiliation postprocess



Tool Parameters

Input Parameter	Value
Sequence file	20 : FROGS ITSx: ITS_sequence.fasta
Abundance file	23 : FROGS_5 Taxonomic affiliation: affiliation_abundance.biom
Is this an amplicon hyper variable in length?	Yes
Using reference database	UNITE_7.1_ITS1
Minimum identity for aggregation	99.0
Minimum coverage for aggregation	99.0

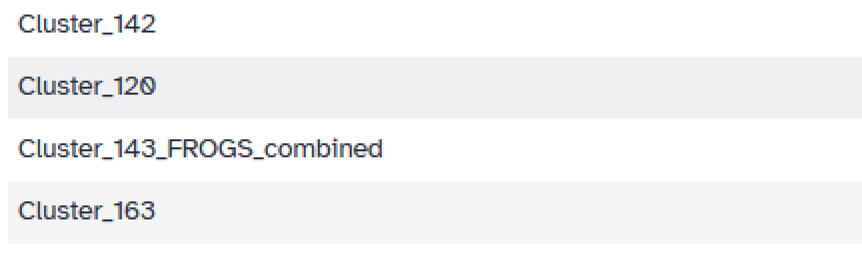
Cluster_40

Cluster_75_FROGS_combined Cluster_121_FROGS_combined Cluster_137_FROGS_combined Cluster_144_FROGS_combined Cluster_149_FROGS_combined Cluster_150_FROGS_combined Cluster_155_FROGS_combined Cluster_156_FROGS_combined Cluster_167_FROGS_combined Cluster_168_FROGS_combined Cluster_169_FROGS_combined Cluster_185_FROGS_combined Cluster_186_FROGS_combined Cluster_187_FROGS_combined Cluster_188_FROGS_combined Cluster_190_FROGS_combined Cluster_191_FROGS_combined Cluster_192_FROGS_combined Cluster_193_FROGS_combined Cluster_198_FROGS_combined Cluster_212_FROGS_combined Cluster_236_FROGS_combined Cluster_249_FROGS_combined Cluster_261_FROGS_combined Cluster_262_FROGS_combined Cluster_263_FROGS_combined Cluster_264_FROGS_combined Cluster_265_FROGS_combined Cluster_266_FROGS_combined Cluster_267_FROGS_combined Cluster_268_FROGS_combined Cluster_269_FROGS_combined

Cluster_38

Cluster_55

Cluster_43

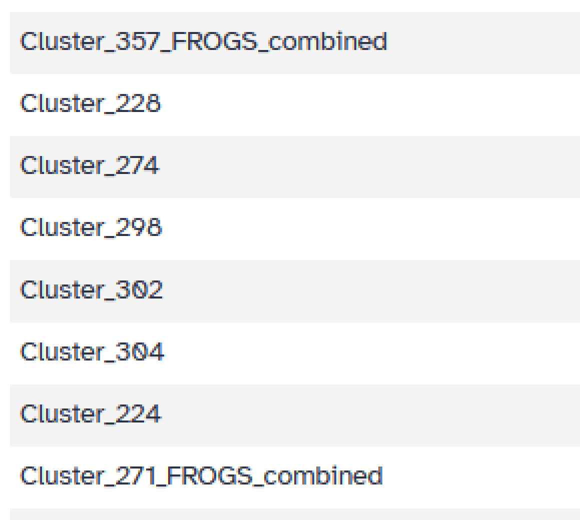


Cluster_142

Cluster_120

Cluster_143_FROGS_combined

Cluster_163



Cluster_357_FROGS_combined

Cluster_228

Cluster_274

Cluster_298

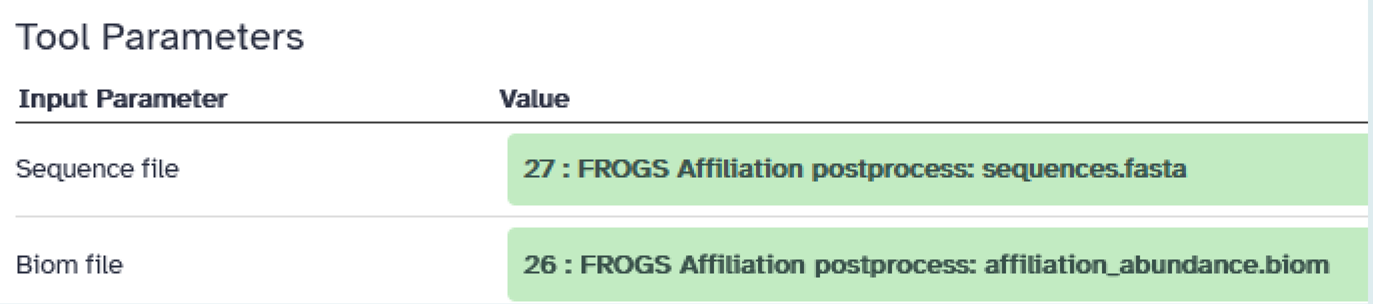
Cluster_302

Cluster_304

Cluster_224

Cluster_271_FROGS_combined

FROGS Tree



Tool Parameters

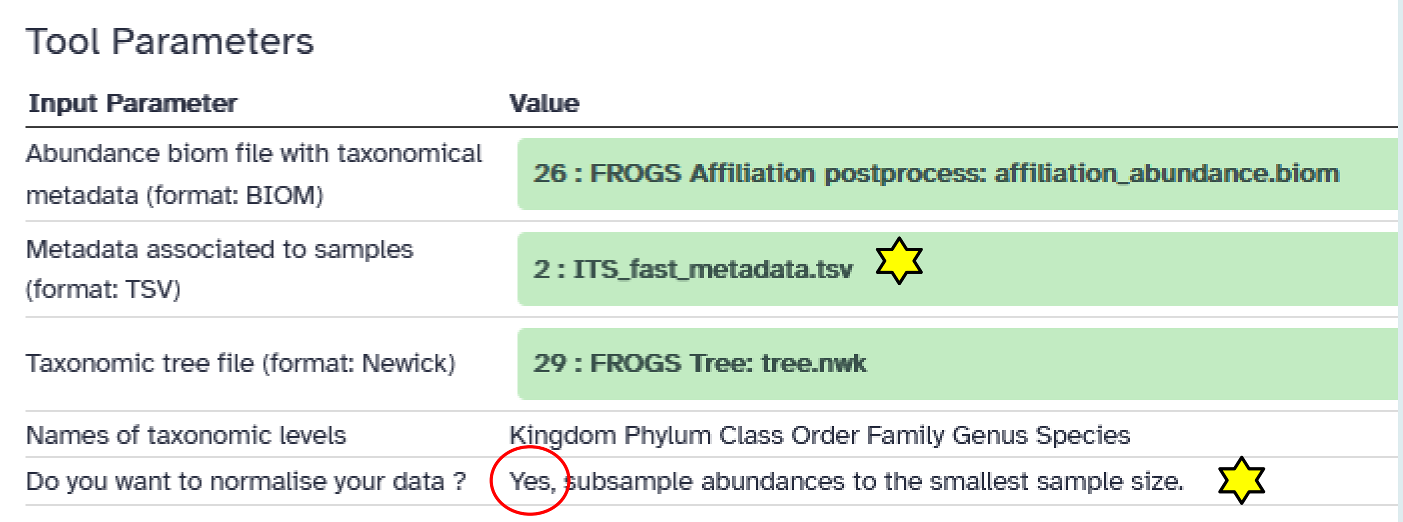
Input Parameter	Value
Sequence file	**27 : FROGS Affiliation postprocess: sequences.fasta**
Biom file	**26 : FROGS Affiliation postprocess: affiliation_abundance.biom**

FROGS BIOM to TSV

Tool Parameters

Input Parameter	Value
Abundance file	26 : FROGS Affiliation postprocess: affiliation_abundance.biom
Sequences file (optional)	27 : FROGS Affiliation postprocess: sequences.fasta
Extract multi-alignments	Yes

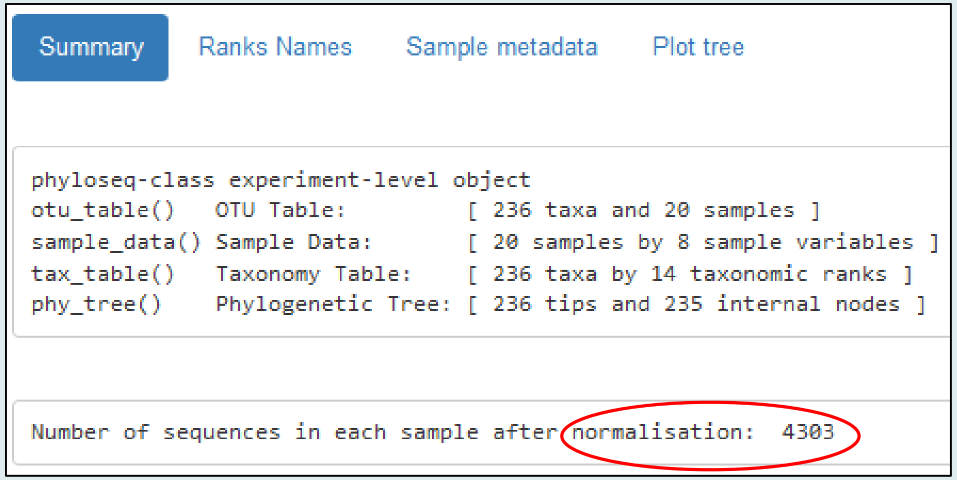
FROGSSTAT Phyloseq Import Data



Tool Parameters

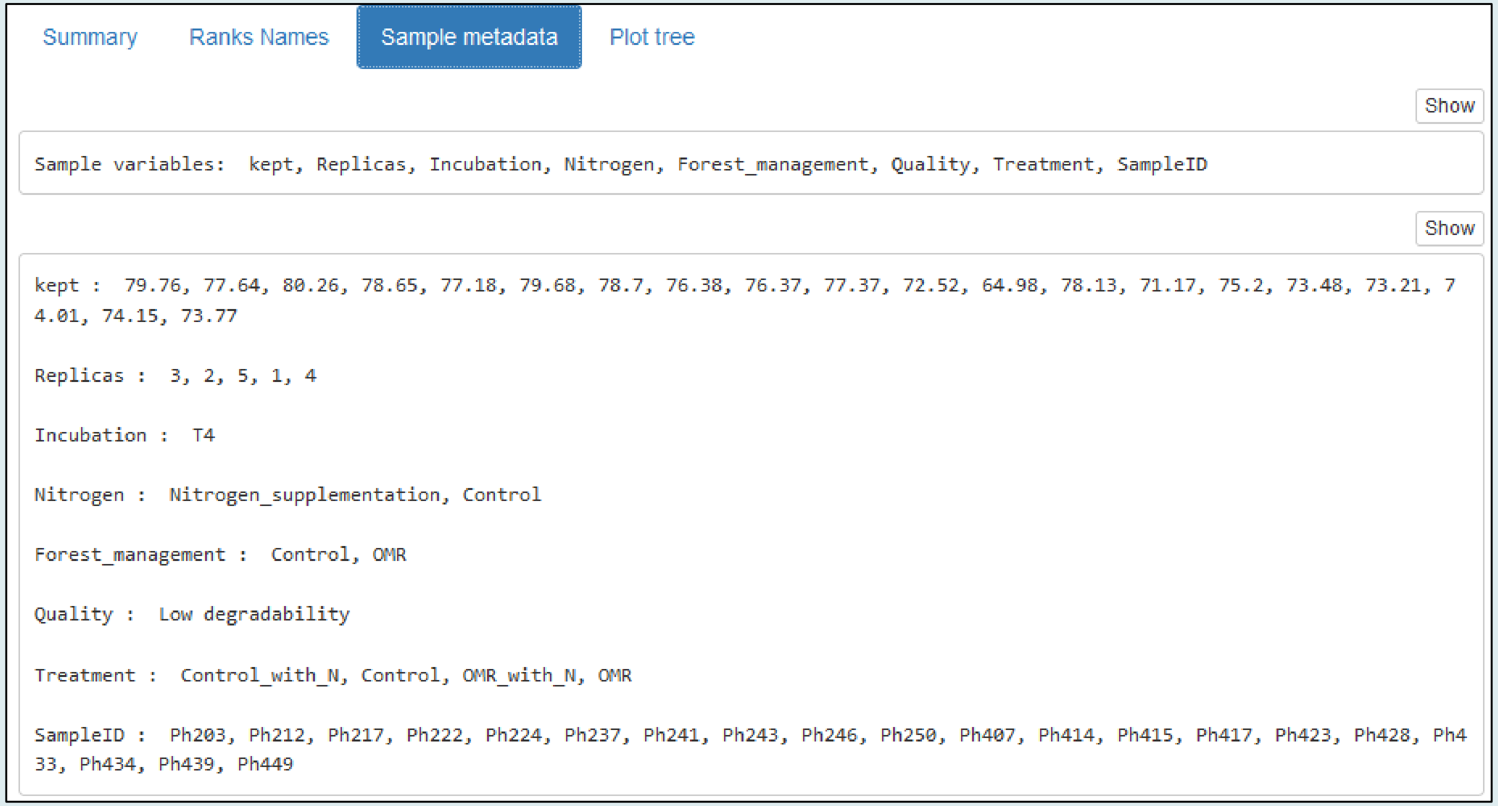
Input Parameter	Value
Abundance biom file with taxonomical metadata (format: BIOM)	26 : FROGS Affiliation postprocess: affiliation_abundance.biom
Metadata associated to samples (format: TSV)	2 : ITS_fast_metadata.tsv
Taxonomic tree file (format: Newick)	29 : FROGS Tree: tree.nwk
Names of taxonomic levels	Kingdom Phylum Class Order Family Genus Species
Do you want to normalise your data ?	Yes, subsample abundances to the smallest sample size.

FROGSSTAT Phyloseq Import Data



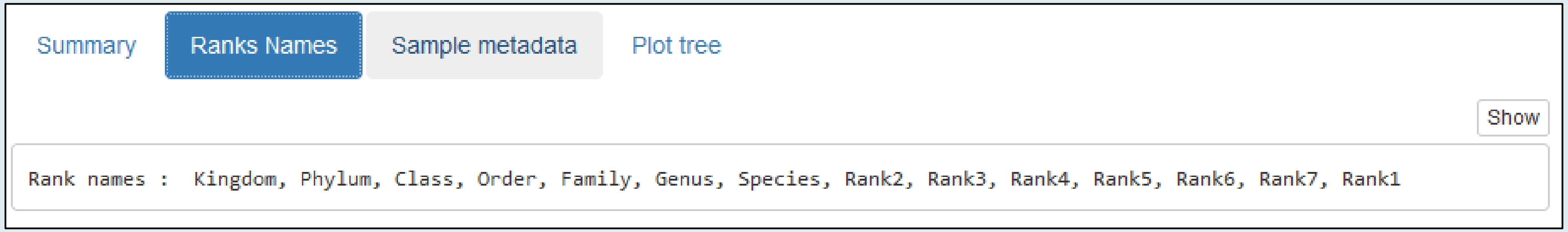
```
phyloseq-class experiment-level object
otu_table()   OTU Table:         [ 236 taxa and 20 samples ]
sample_data() Sample Data:       [ 20 samples by 8 sample variables ]
tax_table()   Taxonomy Table:    [ 236 taxa by 14 taxonomic ranks ]
phy_tree()    Phylogenetic Tree: [ 236 tips and 235 internal nodes ]
```

```
Number of sequences in each sample after normalisation:  4303
```



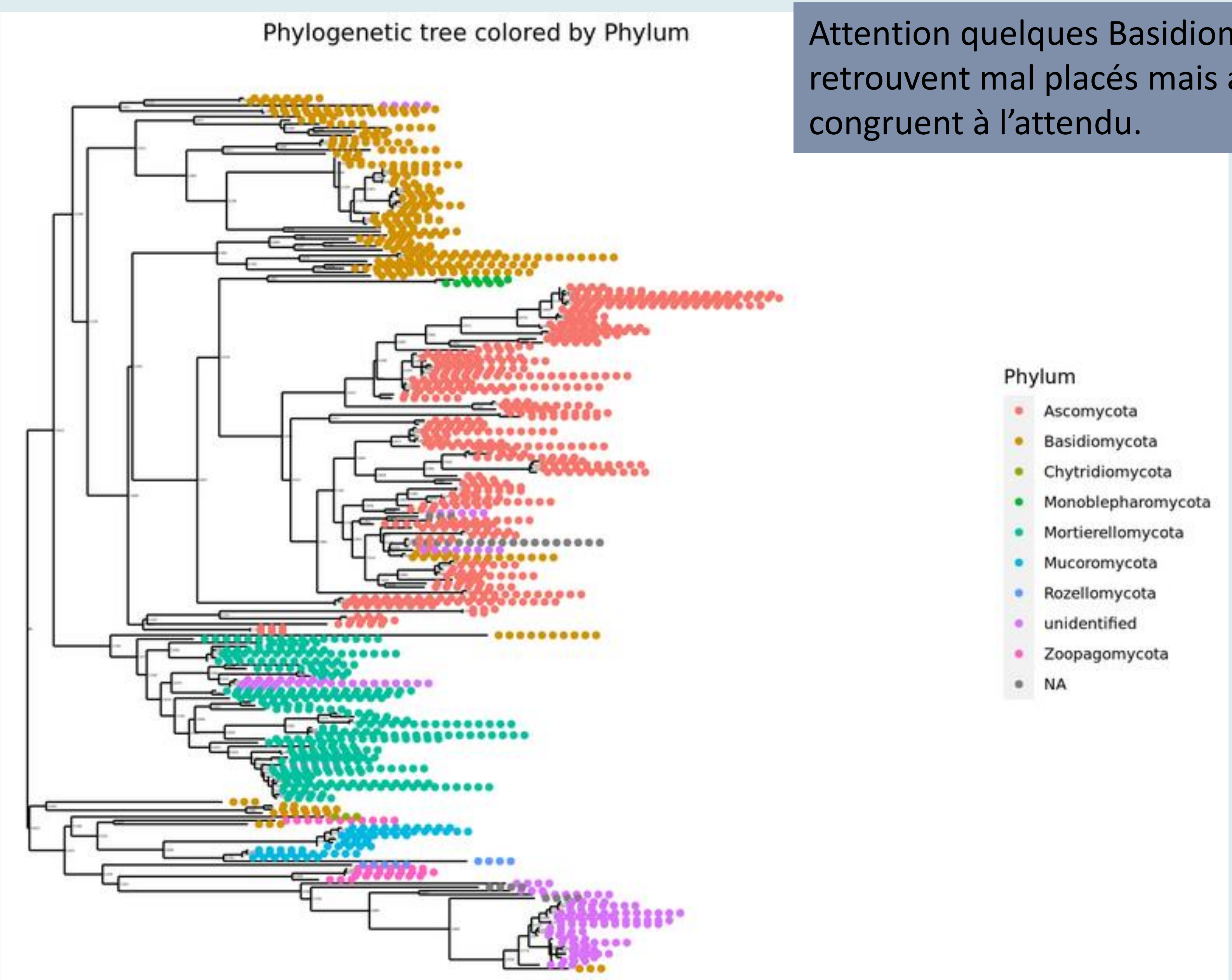
```
Sample variables:  kept, Replicas, Incubation, Nitrogen, Forest_management, Quality, Treatment, SampleID
```

```
kept :  79.76, 77.64, 80.26, 78.65, 77.18, 79.68, 78.7, 76.38, 76.37, 77.37, 72.52, 64.98, 78.13, 71.17, 75.2, 73.48, 73.21, 7
4.01, 74.15, 73.77

Replicas :  3, 2, 5, 1, 4

Incubation :  T4

Nitrogen :  Nitrogen_supplementation, Control

Forest_management :  Control, OMR

Quality :  Low degradability

Treatment :  Control_with_N, Control, OMR_with_N, OMR

SampleID :  Ph203, Ph212, Ph217, Ph222, Ph224, Ph237, Ph241, Ph243, Ph246, Ph250, Ph407, Ph414, Ph415, Ph417, Ph423, Ph428, Ph4
33, Ph434, Ph439, Ph449
```

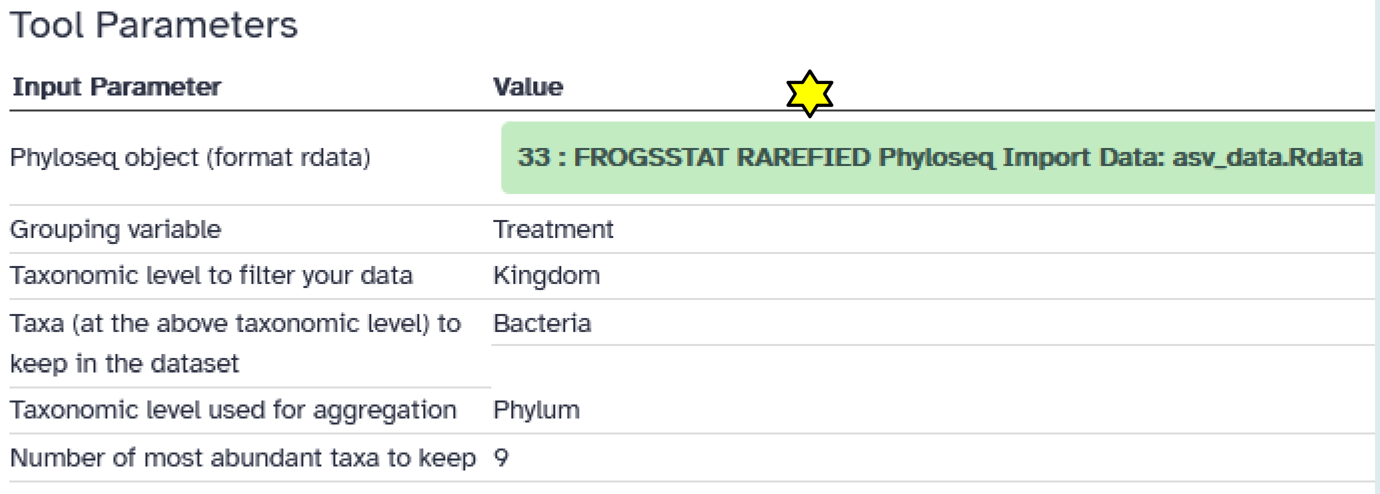


```
Rank names :  Kingdom, Phylum, Class, Order, Family, Genus, Species, Rank2, Rank3, Rank4, Rank5, Rank6, Rank7, Rank1
```

Attention quelques Basidiomycota qui se retrouvent mal placés mais arbre globalement congruent à l’attendu.



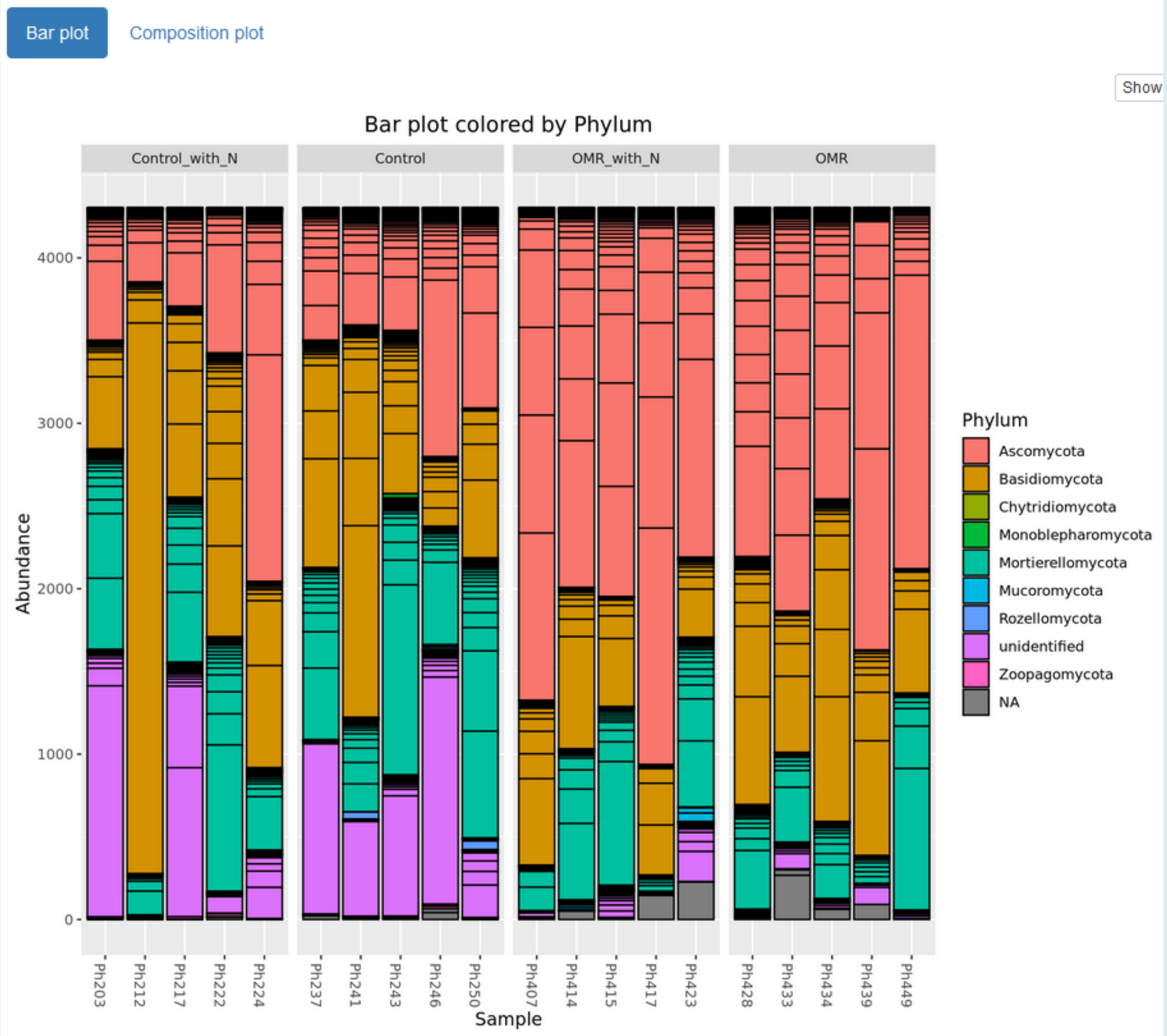
FROGSSTAT Phyloseq Composition Visualisation



Tool Parameters

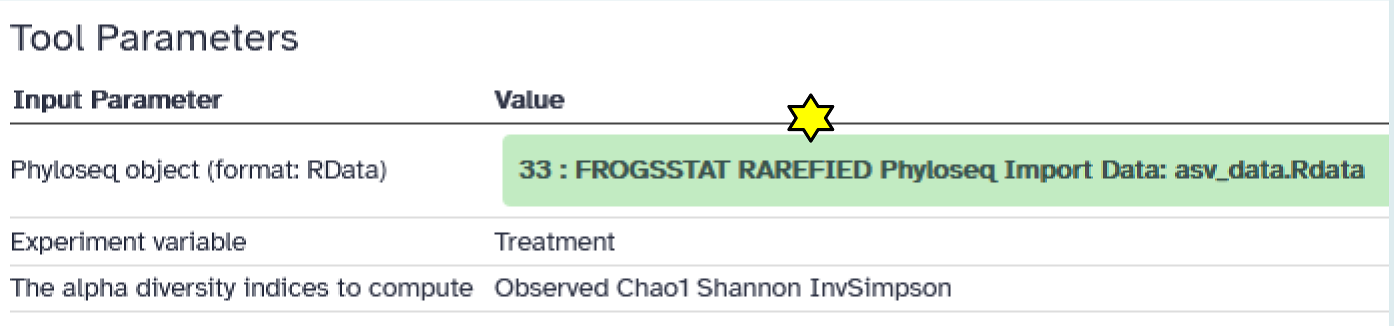
Input Parameter	Value
Phyloseq object (format rdata)	33 : FROGSSTAT RAREFIED Phyloseq Import Data: asv_data.Rdata
Grouping variable	Treatment
Taxonomic level to filter your data	Kingdom
Taxa (at the above taxonomic level) to keep in the dataset	Bacteria
Taxonomic level used for aggregation	Phylum
Number of most abundant taxa to keep	9

Interprétation des barplots :
La plupart des outils de visualisation FROGS n’autorisent qu’une seule variable de tri.
Utiliser la variable Treatment (qui combine les deux variables Forest_management et Nitrogen) permet de « tricher » pour afficher deux variables dans les outils de visualisation



Différences évidentes entre Control et OMR (augmentation des Ascomycota)
Moins évident entre with N et sans

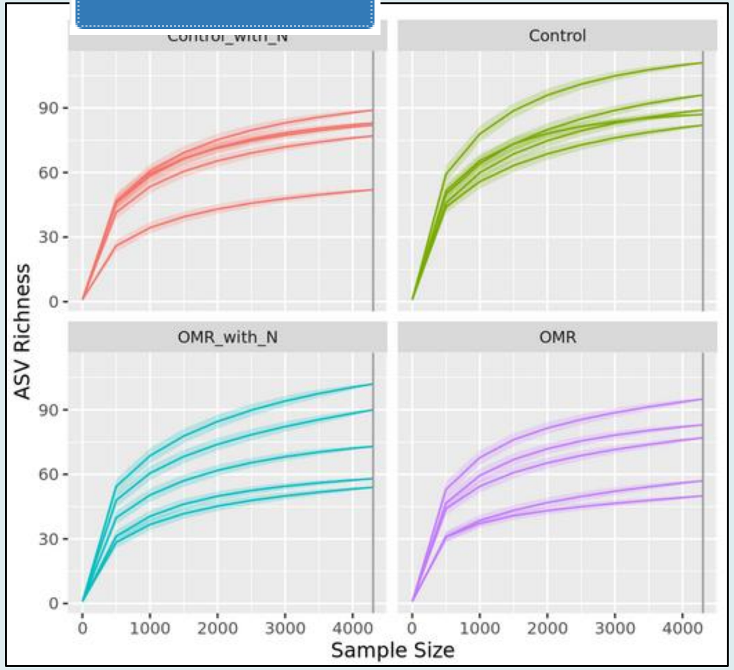
FROGSSTAT Phyloseq Alpha Diversity

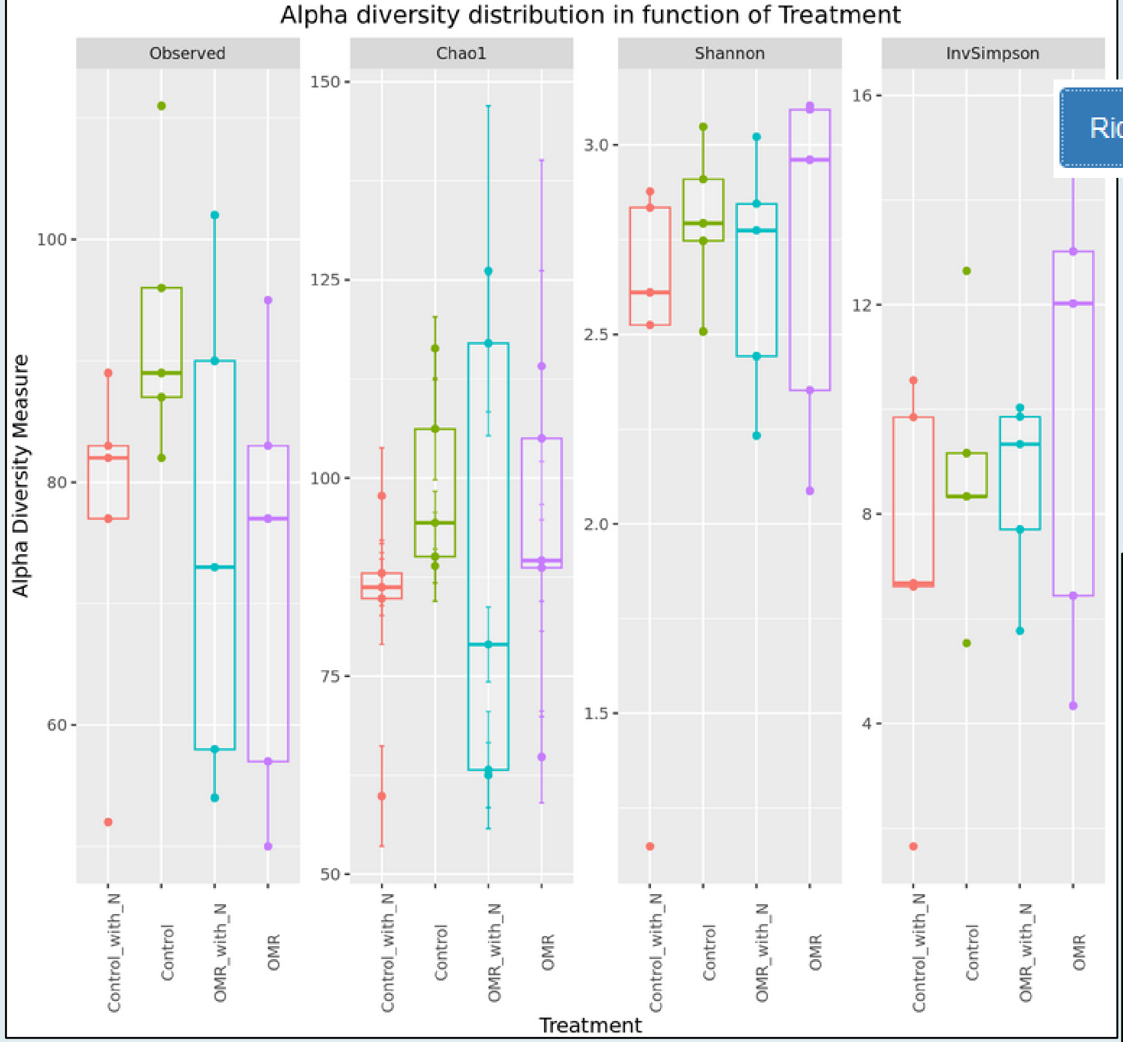


Tool Parameters

Input Parameter	Value
Phyloseq object (format: RData)	33 : FROGSSTAT RAREFIED Phyloseq Import Data: asv_data.Rdata
Experiment variable	Treatment
The alpha diversity indices to compute	Observed Chao1 Shannon InvSimpson

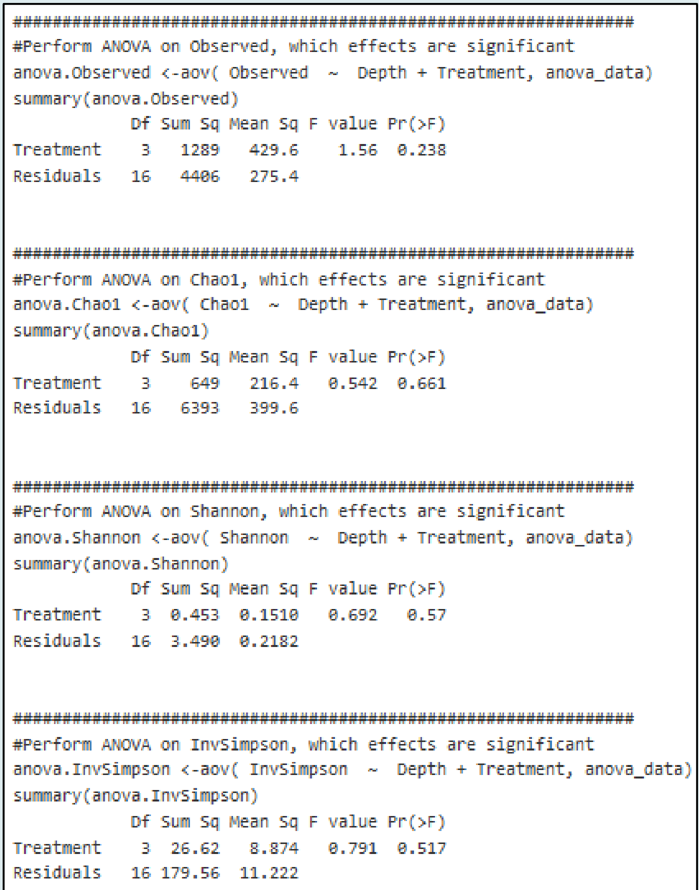
Rarefaction curves





Richness plot with boxplot

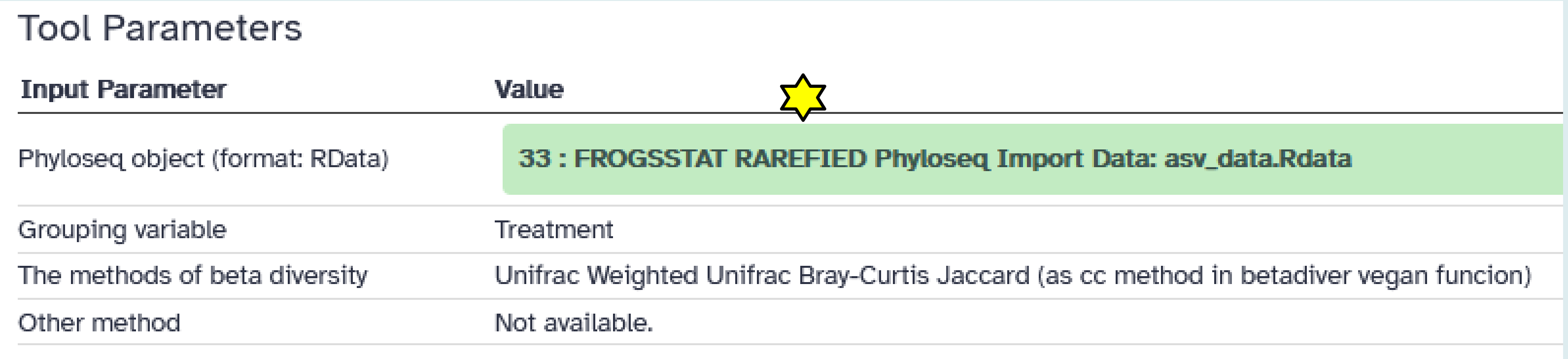
Alpha Diversity Indice Anova Analysis



```
###############################################################
#Perform ANOVA on Observed, which effects are significant
anova.Observed <-aov( Observed  ~  Depth + Treatment, anova_data)
summary(anova.Observed)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3   1289   429.6    1.56  0.238
Residuals   16   4406   275.4


###############################################################
#Perform ANOVA on Chao1, which effects are significant
anova.Chao1 <-aov( Chao1  ~  Depth + Treatment, anova_data)
summary(anova.Chao1)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3    649   216.4   0.542  0.661
Residuals   16   6393   399.6


###############################################################
#Perform ANOVA on Shannon, which effects are significant
anova.Shannon <-aov( Shannon  ~  Depth + Treatment, anova_data)
summary(anova.Shannon)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3  0.453  0.1510   0.692   0.57
Residuals   16  3.490  0.2182


###############################################################
#Perform ANOVA on InvSimpson, which effects are significant
anova.InvSimpson <-aov( InvSimpson  ~  Depth + Treatment, anova_data)
summary(anova.InvSimpson)
            Df Sum Sq Mean Sq F value Pr(>F)
Treatment    3  26.62   8.874   0.791  0.517
Residuals   16 179.56  11.222
```

Pas d’effet significatif du traitement sur aucun indice.
Profondeur de séquençage très faible (sous-échantillon pour le TP et effets des filtres à 50%)

FROGSSTAT Phyloseq Beta Diversity

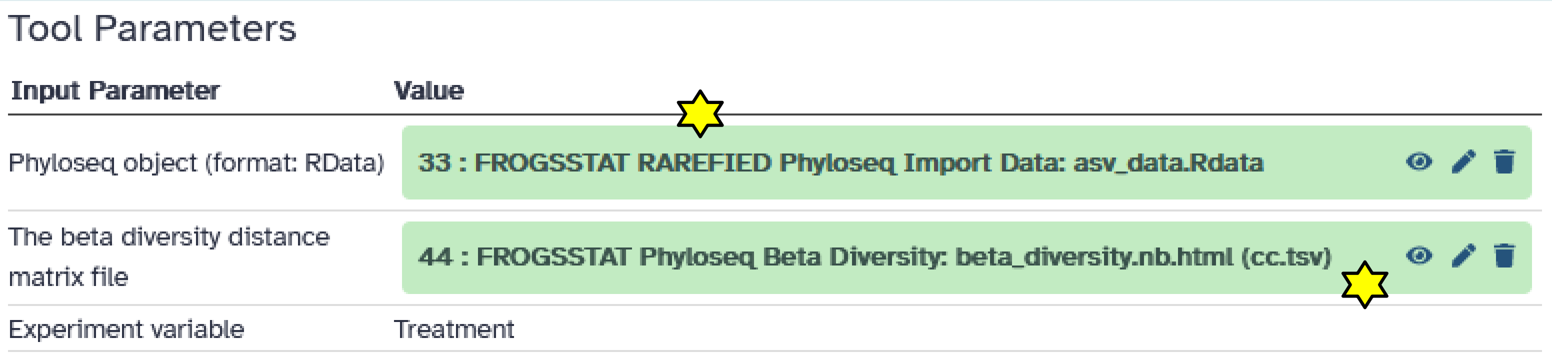


Tool Parameters

Input Parameter	Value
Phyloseq object (format: RData)	33 : FROGSSTAT RAREFIED Phyloseq Import Data: asv_data.Rdata
Grouping variable	Treatment
The methods of beta diversity	Unifrac Weighted Unifrac Bray-Curtis Jaccard (as cc method in betadiver vegan funcion)
Other method	Not available.

Cet outil sert à produire des matrices pour ensuite faire la clusterisation et la visualisation

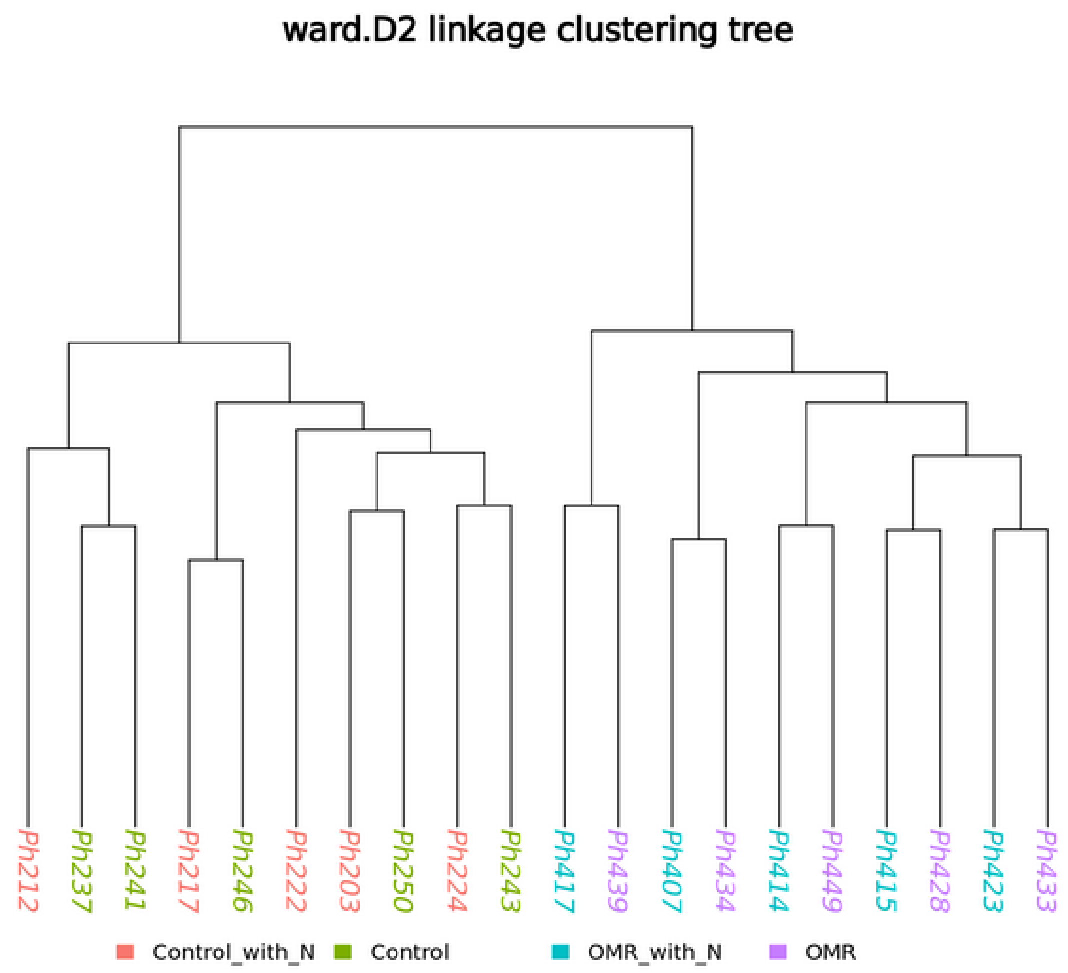
FROGSSTAT Phyloseq Sample Clustering



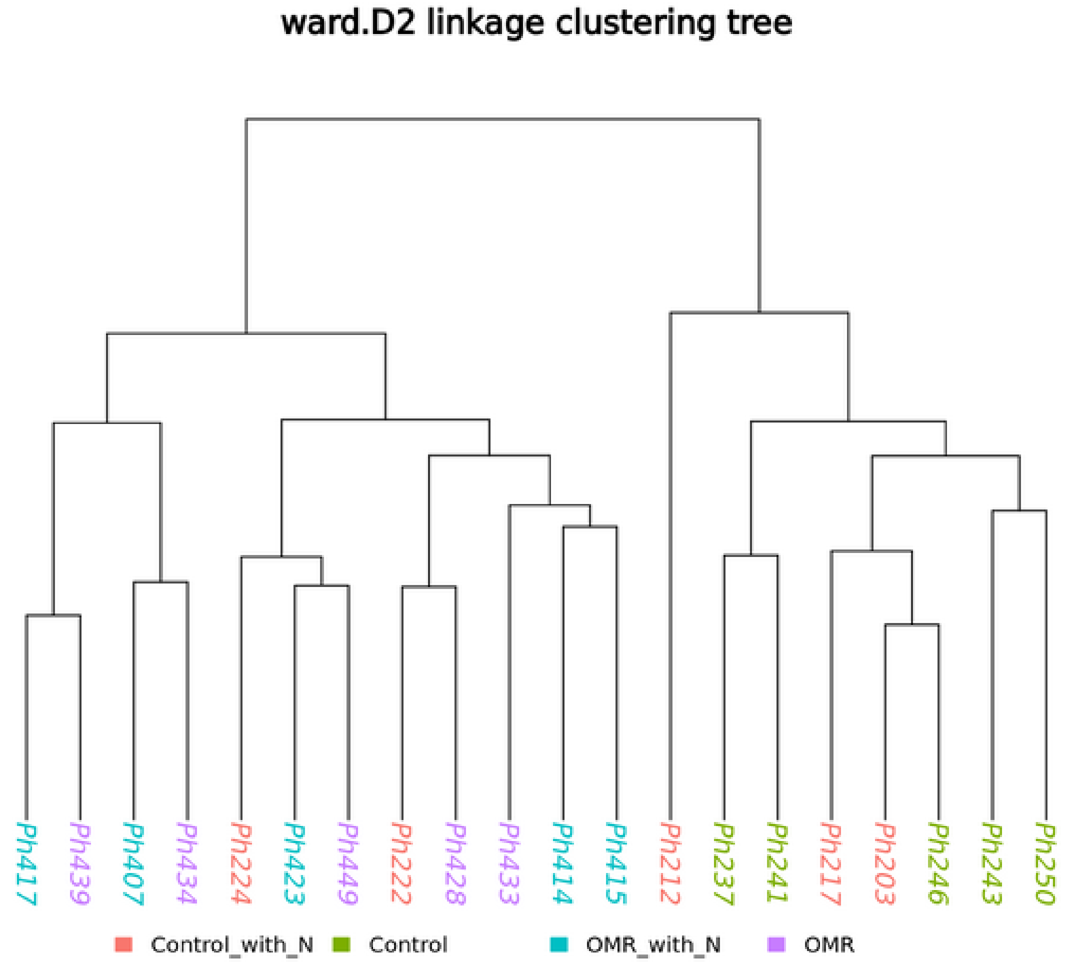
Tool Parameters

Input Parameter	Value
Phyloseq object (format: RData)	33 : FROGSSTAT RAREFIED Phyloseq Import Data: asv_data.Rdata
The beta diversity distance matrix file	44 : FROGSSTAT Phyloseq Beta Diversity: beta_diversity.nb.html (cc.tsv)
Experiment variable	Treatment

Pour chacune des tables: cc, bray, unirac et wunifrac



Jaccard



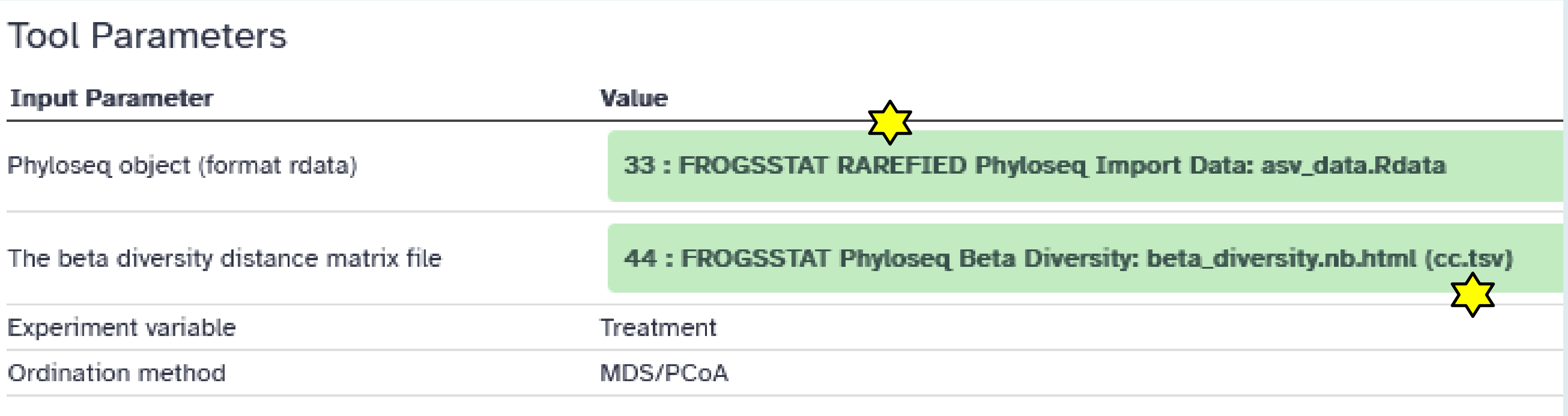
Bray

Unifrac

wUnifrac

Différences entre Control et OMR mais pas avec l'ajout d'azote.
Meilleur clustering avec les méthodes qualitatives qu'avec les méthodes quantitatives :
les différences sont plutôt dues à des ASV peu abondants
(quand on prend en compte les abondances les échantillons se ressemblent davantage)

FROGSSTAT Phyloseq Structure Visualisation

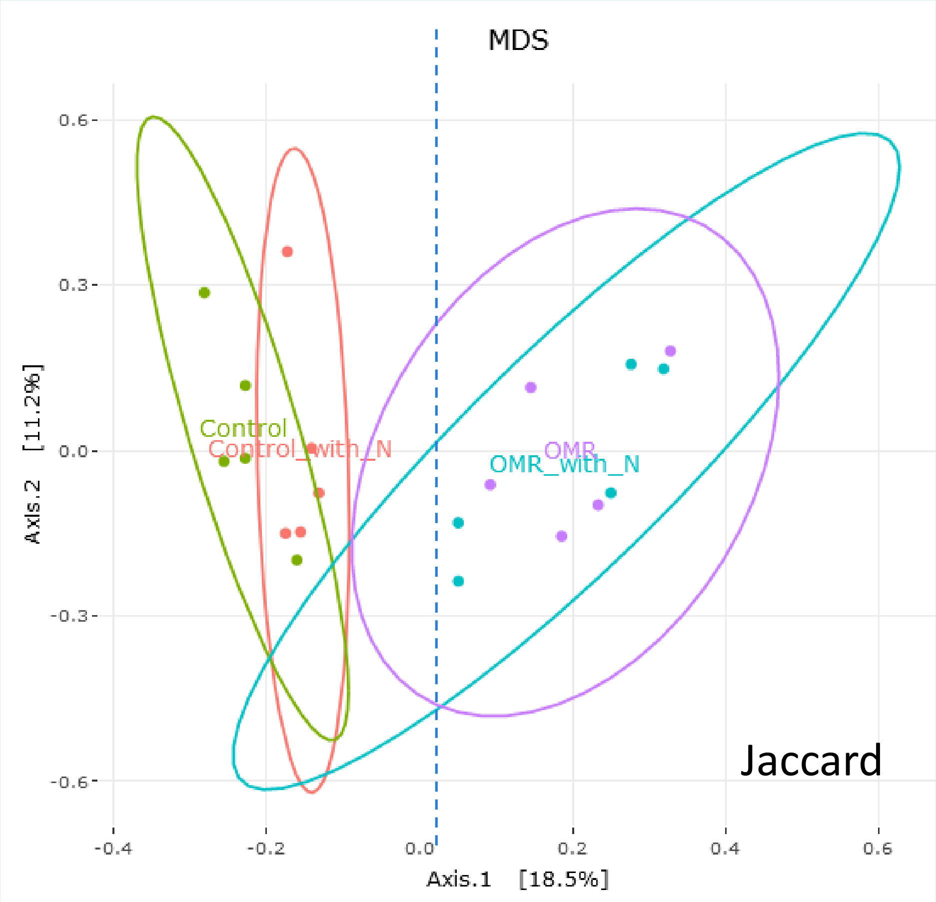


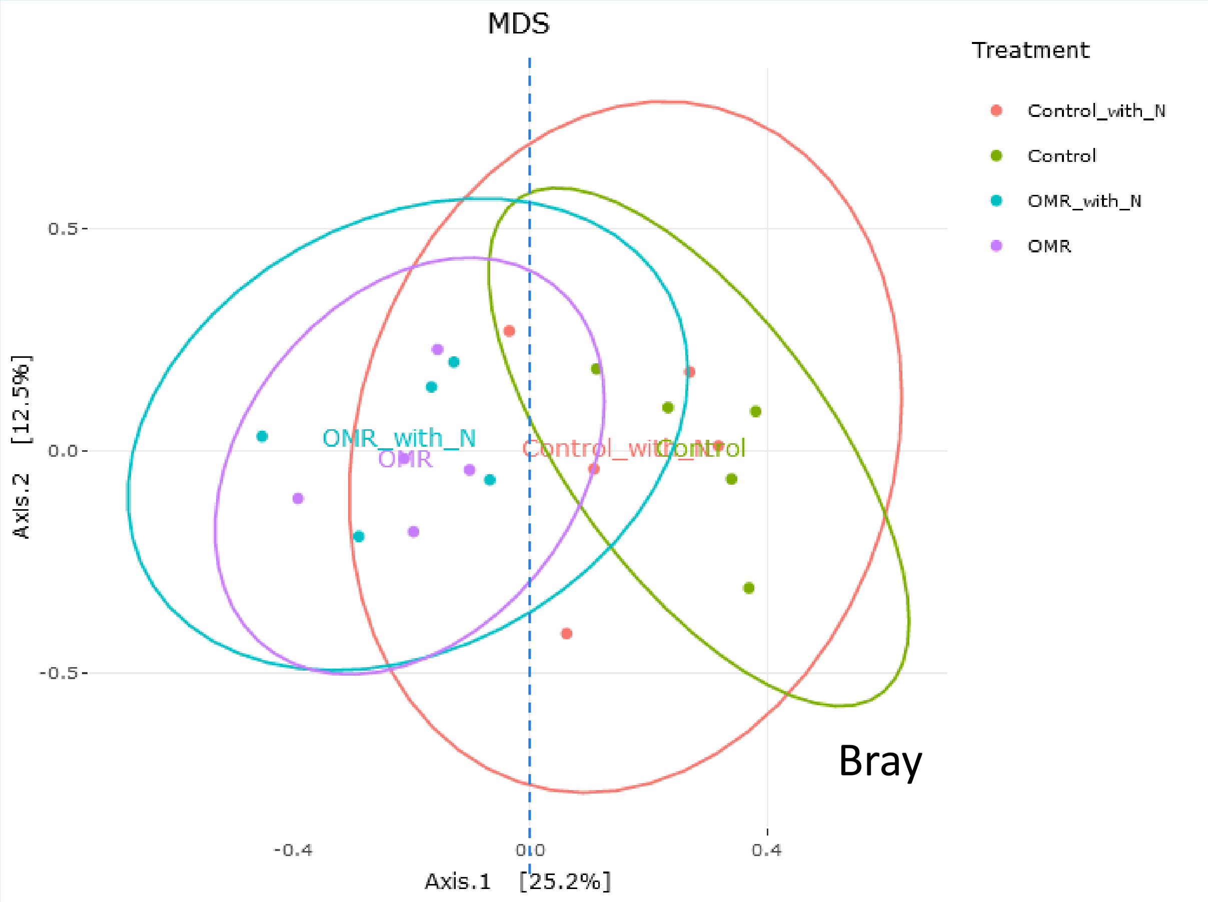
Tool Parameters

Input Parameter	Value
Phyloseq object (format rdata)	33 : FROGSSTAT RAREFIED Phyloseq Import Data: asv_data.Rdata
The beta diversity distance matrix file	44 : FROGSSTAT Phyloseq Beta Diversity: beta_diversity.nb.html (cc.tsv)
Experiment variable	Treatment
Ordination method	MDS/PCoA

Pour chacune des tables: cc, bray, unirac et wunifrac

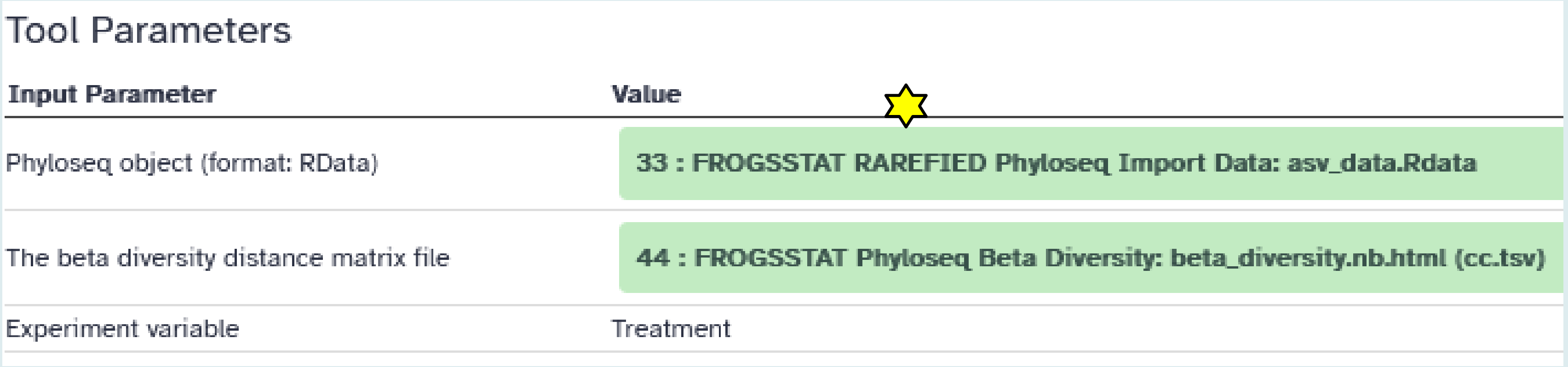
Diversité bêta – ordination :





Différences entre Control et OMR mais pas avec l'ajout d'azote.
Meilleure séparation (sur l'axe1) avec les méthodes qualitatives qu'avec les méthodes quantitatives :
les différences sont plutôt dues à des ASV peu abondants
(quand on prend en compte les abondances les échantillons se ressemblent davantage)

FROGSSTAT Phyloseq Multivariate Analysis Of Variance

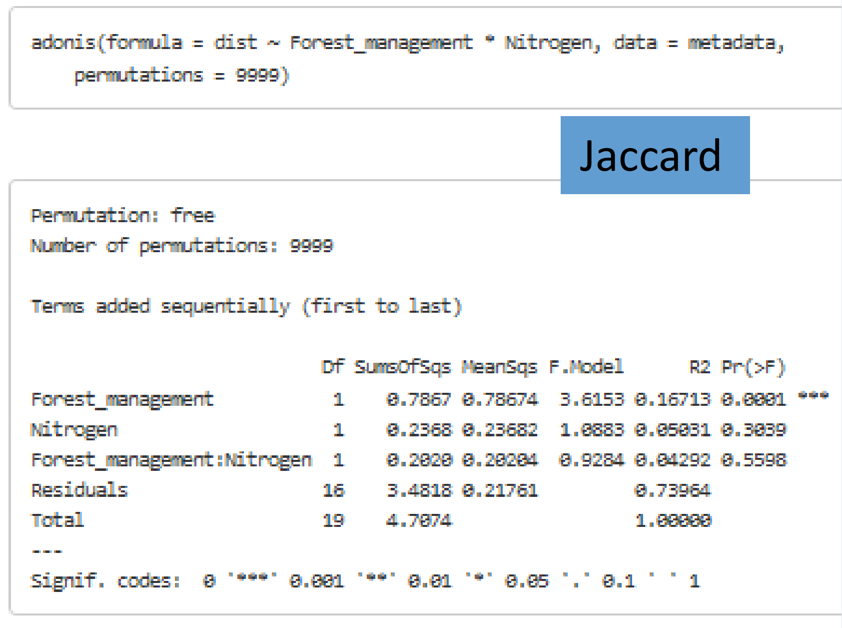


Tool Parameters

Input Parameter	**Value**
Phyloseq object (format: RData)	**33 : FROGSSTAT RAREFIED Phyloseq Import Data: asv_data.Rdata**
The beta diversity distance matrix file	**44 : FROGSSTAT Phyloseq Beta Diversity: beta_diversity.nb.html (cc.tsv)**
Experiment variable	Treatment

Diversité bêta – PERMANOVA :

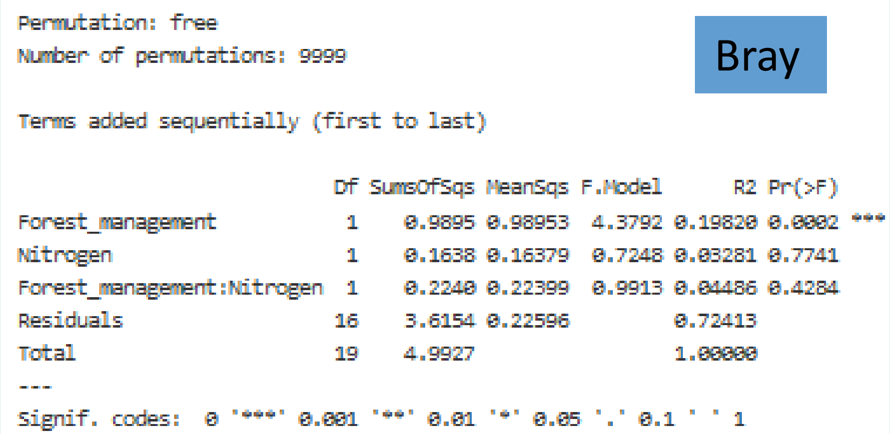
Il est ici possible de donner plusieurs variables séparées par des + ou * (effets additifs ou avec interaction), et donc plus pertinent d’utiliser Nitrogen et Forest_management plutôt que Treatment



Jaccard

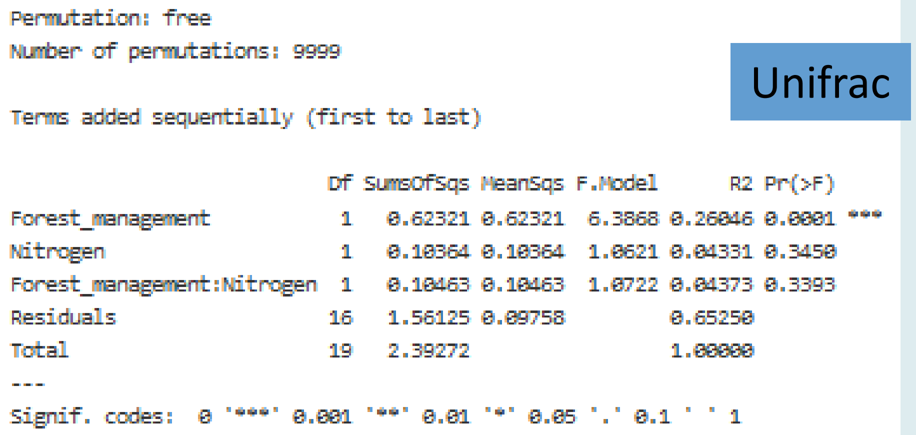
```
adonis(formula = dist ~ Forest_management * Nitrogen, data = metadata,
    permutations = 9999)
```

```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1    0.7867 0.78674  3.6153 0.16713 0.0001 ***
Nitrogen                    1    0.2368 0.23682  1.0883 0.05031 0.3039    
Forest_management:Nitrogen  1    0.2020 0.20204  0.9284 0.04292 0.5598    
Residuals                  16    3.4818 0.21761         0.73964           
Total                      19    4.7074                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



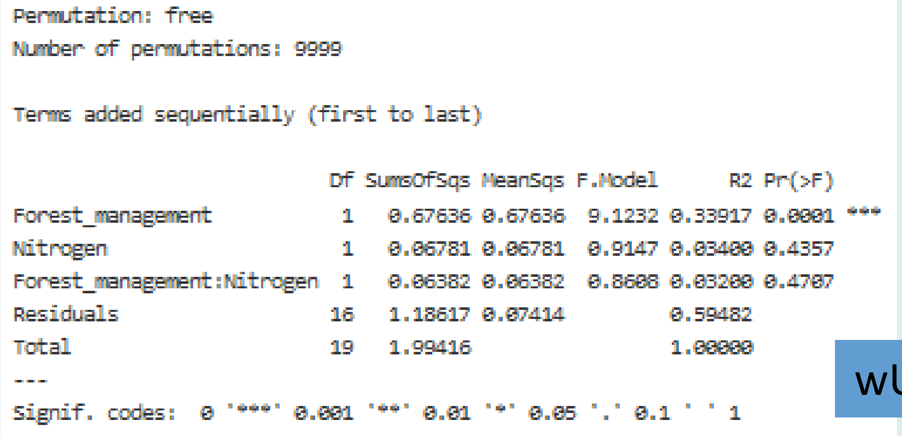
Bray

```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1    0.9895 0.98953  4.3792 0.19820 0.0002 ***
Nitrogen                    1    0.1638 0.16379  0.7248 0.03281 0.7741    
Forest_management:Nitrogen  1    0.2240 0.22399  0.9913 0.04486 0.4284    
Residuals                  16    3.6154 0.22596         0.72413           
Total                      19    4.9927                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



Unifrac

```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1   0.62321 0.62321  6.3868 0.26046 0.0001 ***
Nitrogen                    1   0.10364 0.10364  1.0621 0.04331 0.3450    
Forest_management:Nitrogen  1   0.10463 0.10463  1.0722 0.04373 0.3393    
Residuals                  16   1.56125 0.09758         0.65250           
Total                      19   2.39272                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

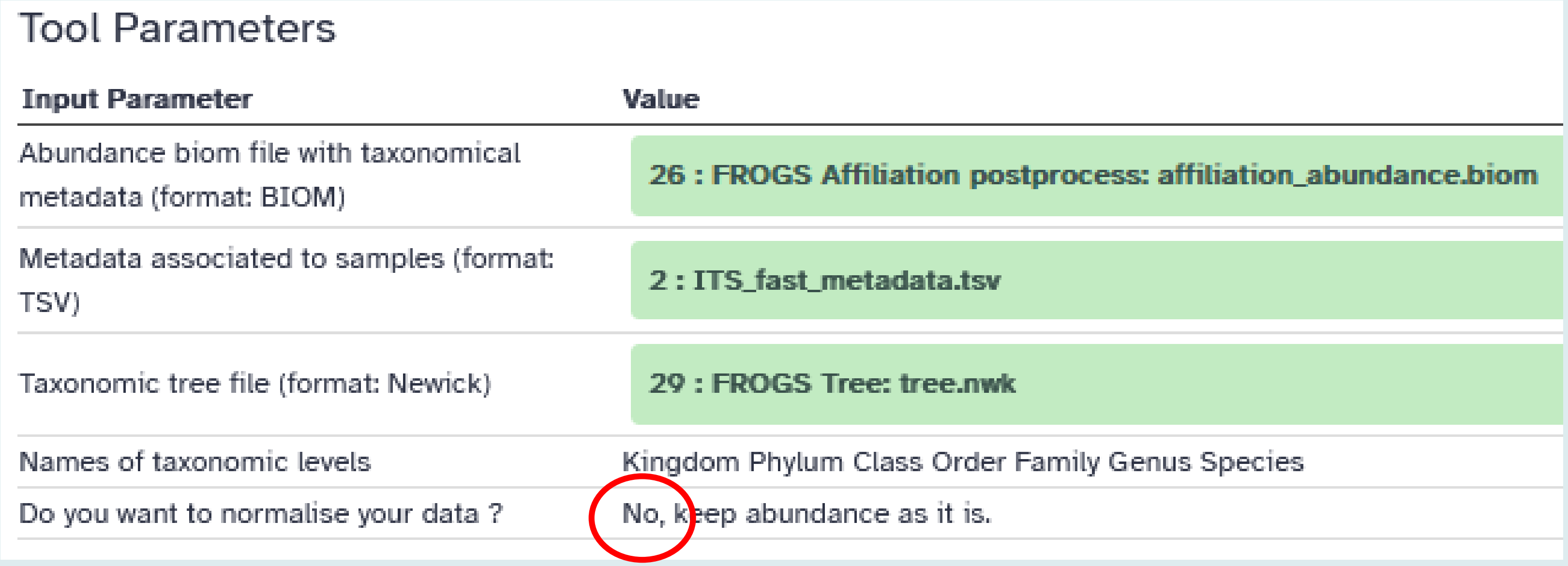


wUnifrac

```
Permutation: free
Number of permutations: 9999

Terms added sequentially (first to last)

                           Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
Forest_management           1   0.67636 0.67636  9.1232 0.33917 0.0001 ***
Nitrogen                    1   0.06781 0.06781  0.9147 0.03400 0.4357    
Forest_management:Nitrogen  1   0.06382 0.06382  0.8608 0.03200 0.4707    
Residuals                  16   1.18617 0.07414         0.59482           
Total                      19   1.99416                 1.00000           
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Les PERMANOVA confirment avec tous les indices un effet significatif de la variable Forest_management mais pas de la variable Nitrogen.

FROGSSTAT Phyloseq Import Data



Tool Parameters

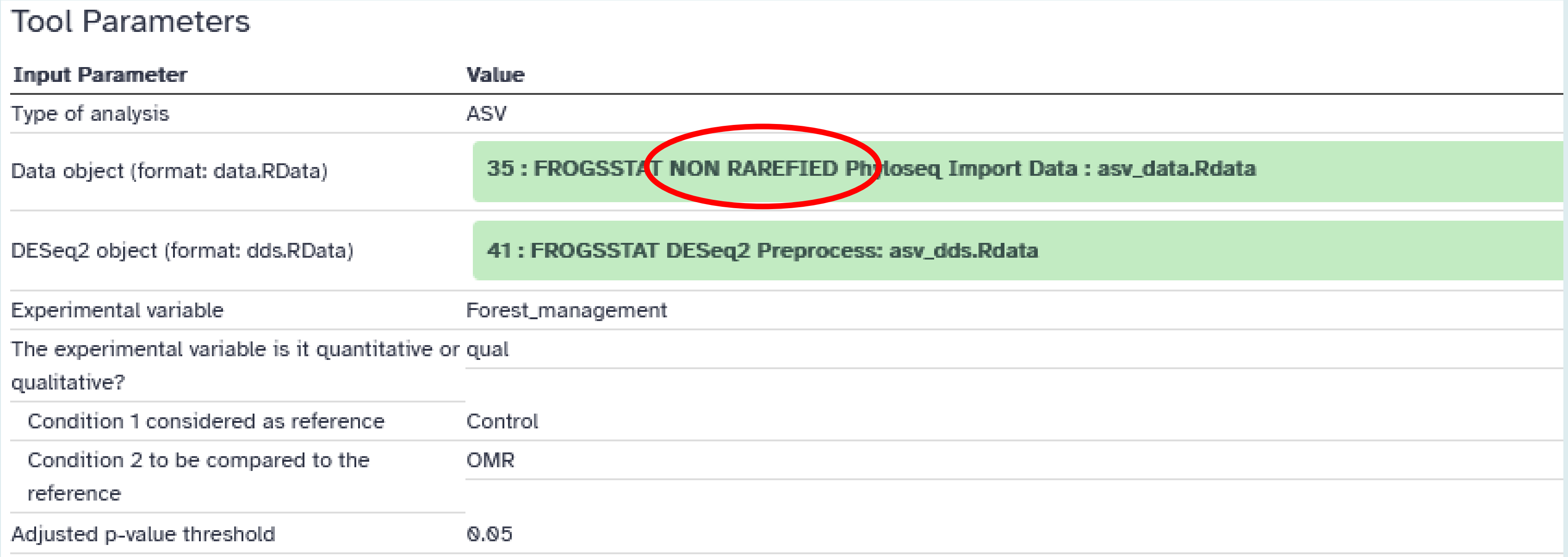
Input Parameter	Value
Abundance biom file with taxonomical metadata (format: BIOM)	**26 : FROGS Affiliation postprocess: affiliation_abundance.biom**
Metadata associated to samples (format: TSV)	**2 : ITS_fast_metadata.tsv**
Taxonomic tree file (format: Newick)	**29 : FROGS Tree: tree.nwk**
Names of taxonomic levels	Kingdom Phylum Class Order Family Genus Species
Do you want to normalise your data ?	No, keep abundance as it is.

FROGSSTAT DESeq2 Preprocess

Tool Parameters

Input Parameter	Value
Type of analysis	ASV
Phyloseq object	35 : FROGSSTAT NON RAREFIED Phyloseq Import Data : asv_data.Rdata
Experimental variable	Forest_management
Do you want to correct a confounding factor?	false

FROGSSTAT DESeq2 Visualisation



Tool Parameters

Input Parameter	Value
Type of analysis	ASV
Data object (format: data.RData)	35 : FROGSSTAT NON RAREFIED Phyloseq Import Data : asv_data.Rdata
DESeq2 object (format: dds.RData)	41 : FROGSSTAT DESeq2 Preprocess: asv_dds.Rdata
Experimental variable	Forest_management
The experimental variable is it quantitative or qualitative?	qual
Condition 1 considered as reference	Control
Condition 2 to be compared to the reference	OMR
Adjusted p-value threshold	0.05

Seule la variable Forest_management est pertinente à analyser puisque Nitrogen n'a pas d'effet significatif.

On pourrait aussi utiliser Treatment et faire des comparaisons entre groupes pertinents, mais les effectifs (en nombre d'échantillons dans chaque groupe) seraient plus petits.

Plusieurs clusters différentiellement abondants, dans un sens ou dans l'autre.
Et 2 ASV FROGS_combined !!!

Sans un paramétrage adapté ITS lors du FROGS_1_preprocess ces clusters aurait été éliminés pendant le traitement et n'auraient donc pas été identifiés