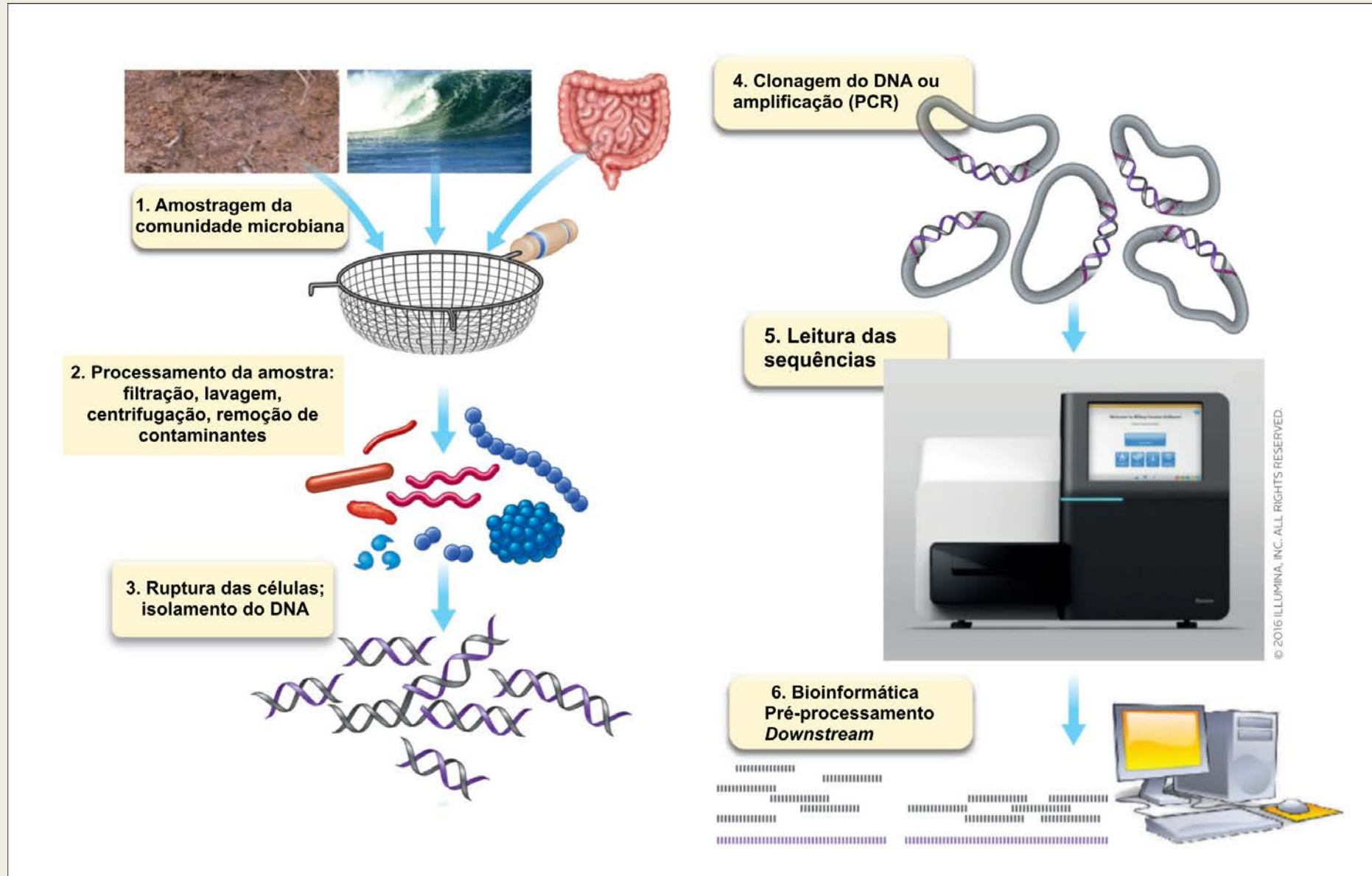


Metataxonômica com qiime2!

Rafael Correia da Silva
Laboratório de Bioinformática - FCAV/UNESP



Um rápido olhar...



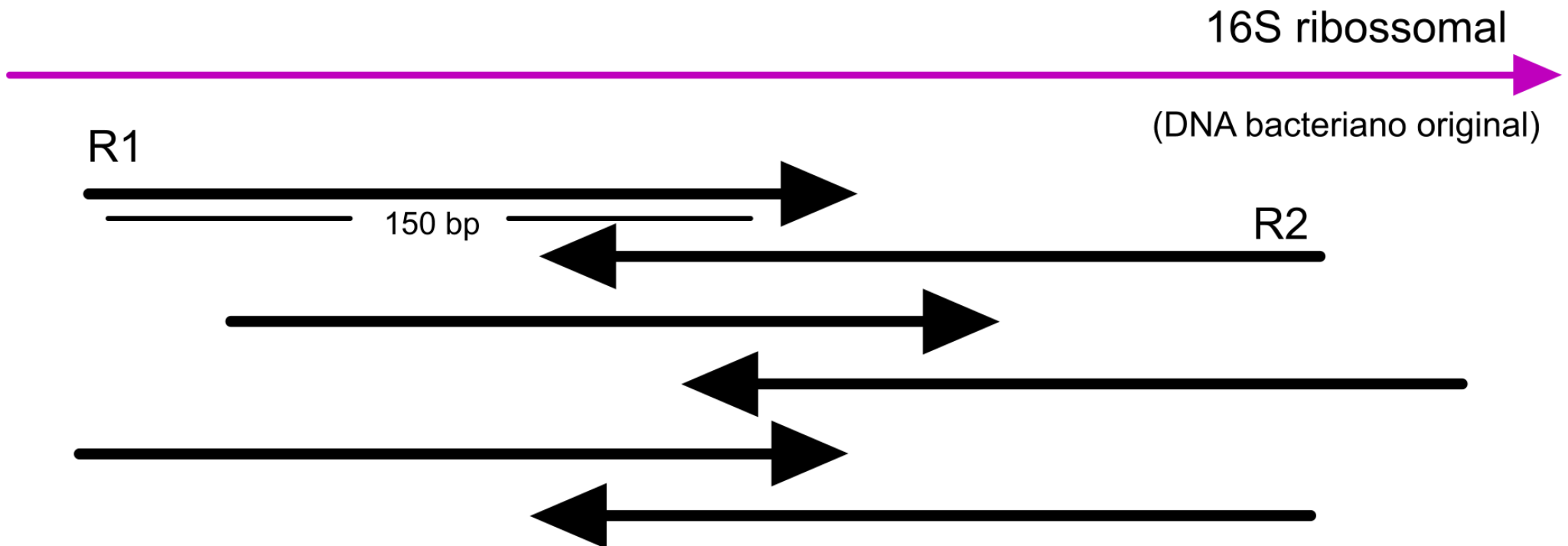
Transformar sequências em informações úteis

1. Limpeza das sequências.

```
@M03081:98:000000000-BVYJC:1:1101:16345:1685 1:N:0:CTAGAGGA+CTCTCTA
CCTACGGGTGGCTGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGT
AAGAACATTTGTGTAAGTAAGTATGCACGTCTTGACGTTACCTATTCAGAAAGCCACGGCTAACTAC
CCGGTCTTATTGGGCGTAAAGCGCGCGTAGGCGGTTT
+
AAAAA1A>A1BBAABBF31E0EBAGH2A0EAAEBA0BA///BFHG00///?E/00>EEEEGGG/
00?00<011?1@0?111?11?<1?111<.<<<F11<..</.=000=0000//;/:--::.0;0/
/9-9--;B//;///---;---/9-----9---/---;9
@M03081:98:000000000-BVYJC:1:1101:16133:1691 1:N:0:CTAGAGGA+CTCTCTA
CCTACGGGCGGCAGCAGTGGGGAATATTGCACAATGGGCGGAAGCCTGATGCAGCCACGCCGCGTGT
ACGAAGCGTTTGTGACTGTTCTCTCAAGAAGCACCGTCTCACTACGTGCCAGCCGCCGCCCTTCT
TCGCTCTTACTCGGTTTGTACGTCGTCTGTGTATTT
+
AAAAA?3A?A2>2AABBBFFGCEEG3FG555G51BGGFEE0>/1?FGG1B3B3FG111>?FEEGGG/
.<C--.<-:.-.;/0000:0;000///0000///99-.../0090/./9C//;.-----/./
///.9...;////...;.99/9..9--...9////:/
@M03081:98:000000000-BVYJC:1:1101:14283:1706 1:N:0:CTAGAGGA+CTCTCTA
CCTACGGGGGGCAGCAGTGGGGGATATTGCACAATGGGGGGAACCCTGATGCAGCAACGCCGCGTGA
AAGAAACAATTGTCTCTACCATAACAAGCTCCGCCTAACTACGTGCCAGCACCCGCGCTATTAC
GTGCGTAGGCGGTTAGTTAAGTCGAGGTTAAAAGG
```

Transformar sequências em informações úteis

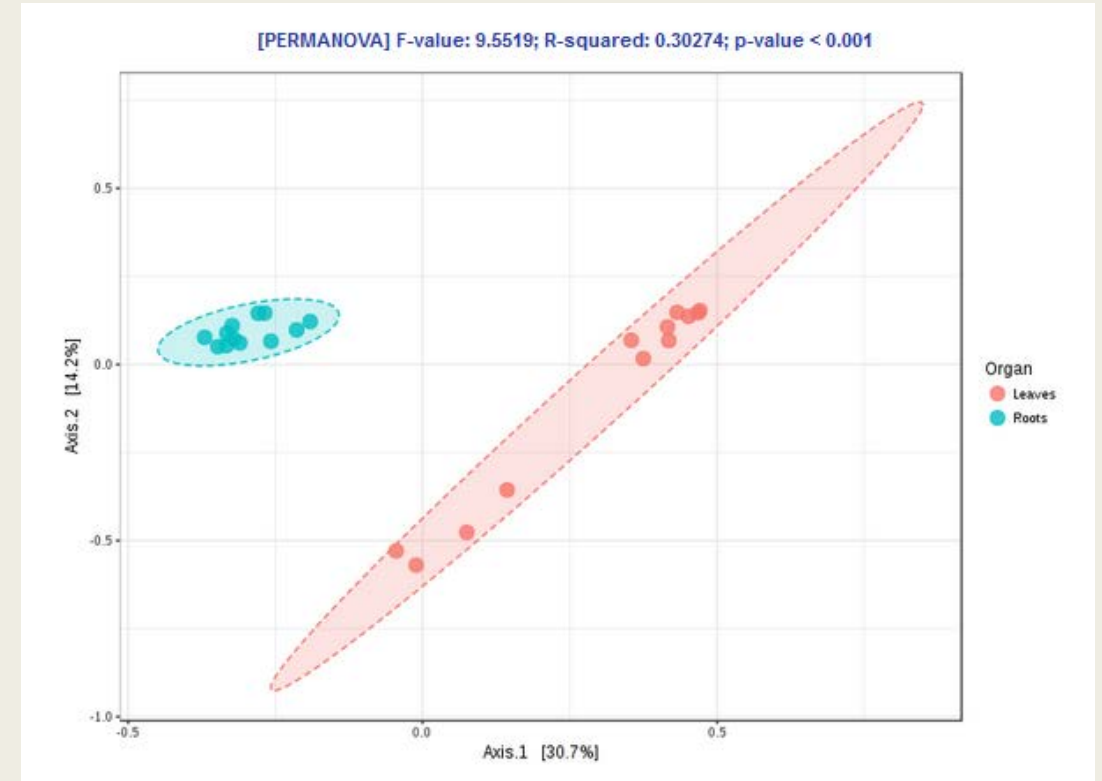
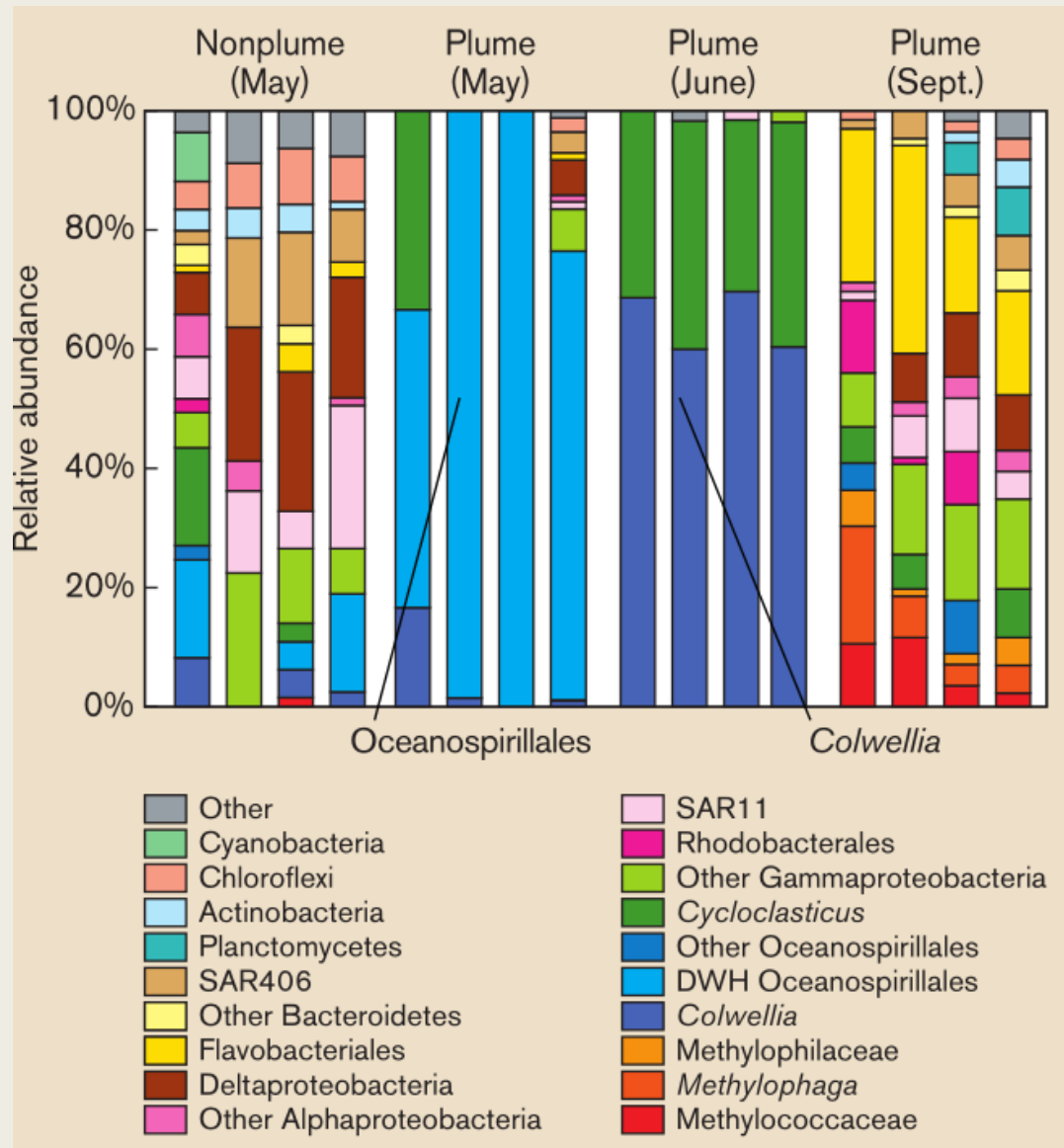
1. Limpeza das sequências.



2. Tabulação das comunidades

Crenarchaeota	4275	3369	6417	6578	7114	7992
Euryarchaeota	123040	80922	180651	184134	178571	226787
Nanoarchaeota	44	14	30	25	26	41
Thaumarchaeota	4612	9478	12599	17515	12659	16358
Abditibacteriota	929	587	1413	1189	1368	1687
Acidobacteria	244963	147992	310906	248757	317332	393379
Actinobacteria	5228062	3311546	7836146	6931200	7420686	9393100
Aquificae	1658	878	1884	1563	1862	2252
Armatimonadetes	4781	3093	6941	5576	6732	8122
Bacteroidetes	586296	234335	613992	471940	520083	569975
Balneolaeota	2217	1194	2739	2279	2718	3043
Caldiserica	60	38	82	47	71	79
Calditrichaeota	287	222	443	336	425	545

3. Análises ecológicas e quantitativas (diagnóstico)



Análise do microbioma de raízes e folhas de Guaraná
(*Paullinia cupana*, não-publicado)



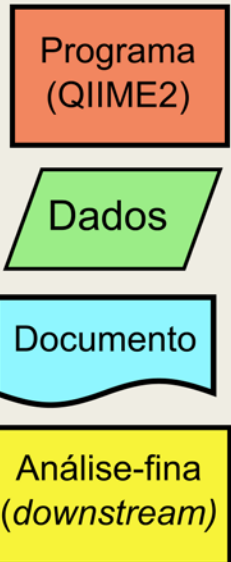
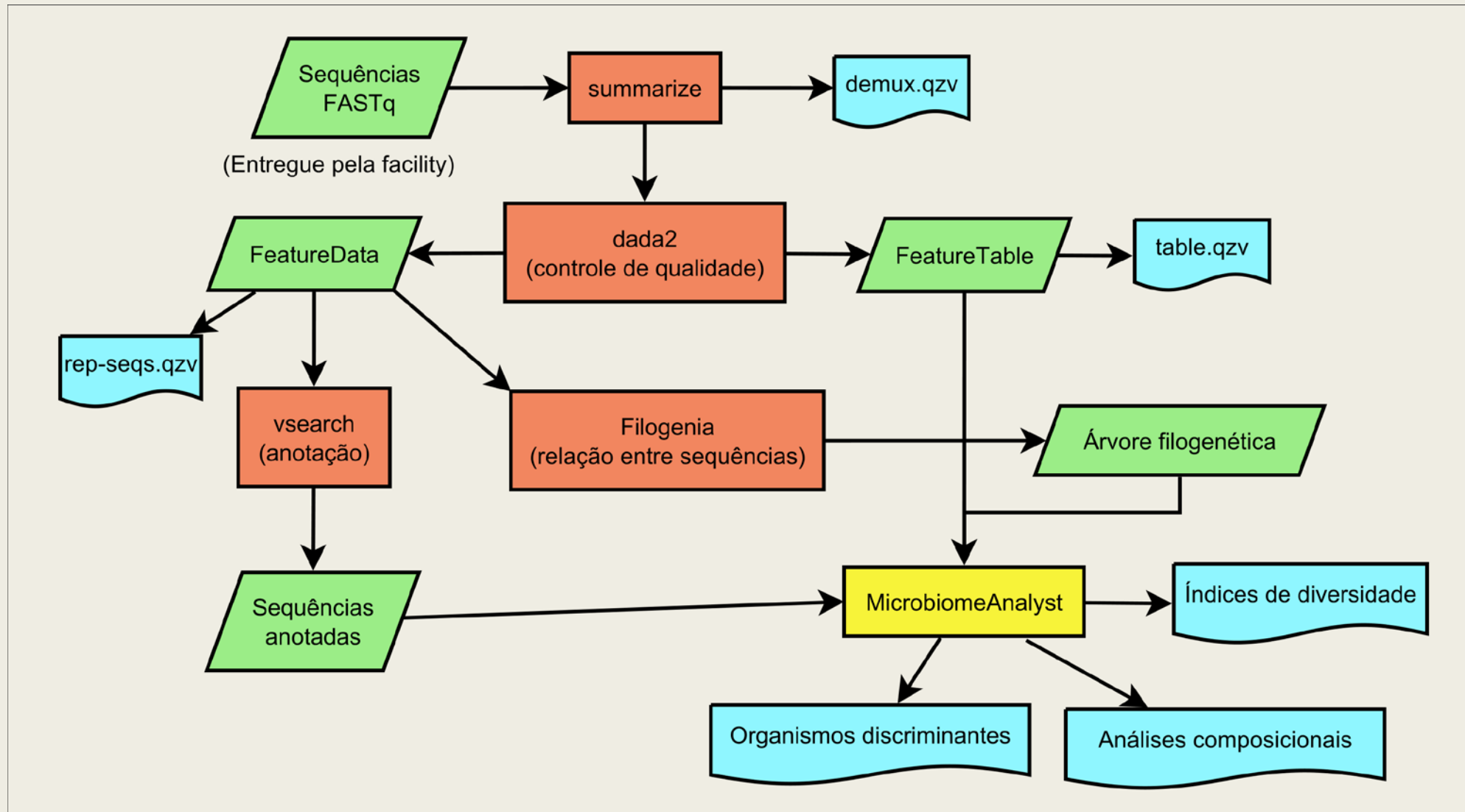
Vantagens

1. Rastreabilidade completa da análise (*provenance*)
2. Dicionário analítico controlado
3. Sistema de *plugins*
4. Suporte a diversas interfaces
5. Parcialmente *open source*
6. Instalação relativamente fácil

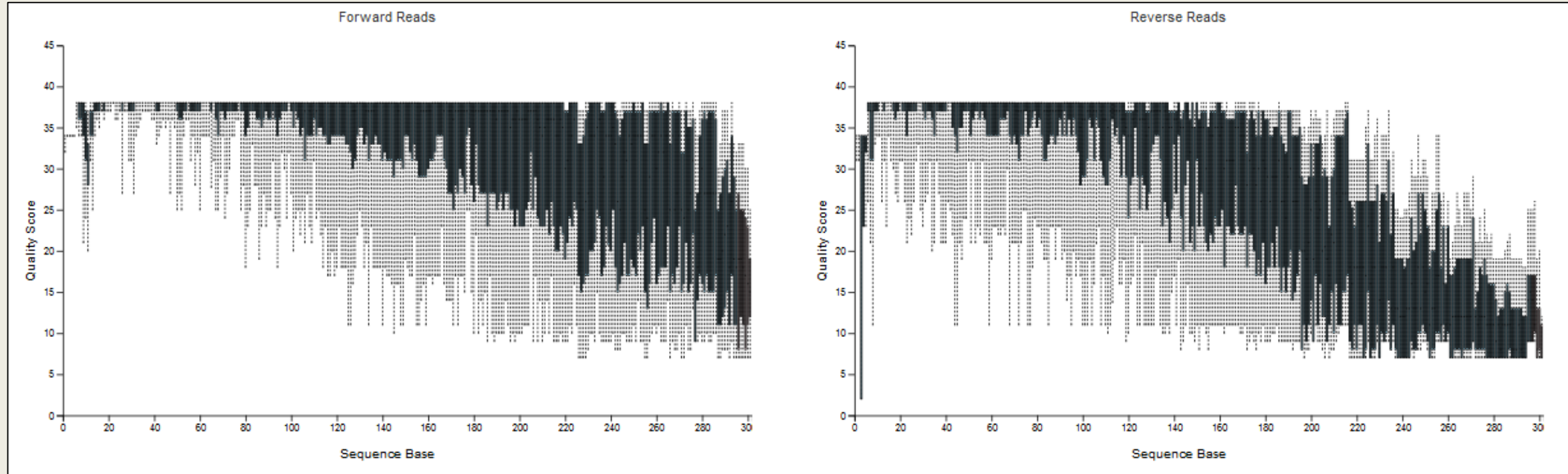
Desvantagens

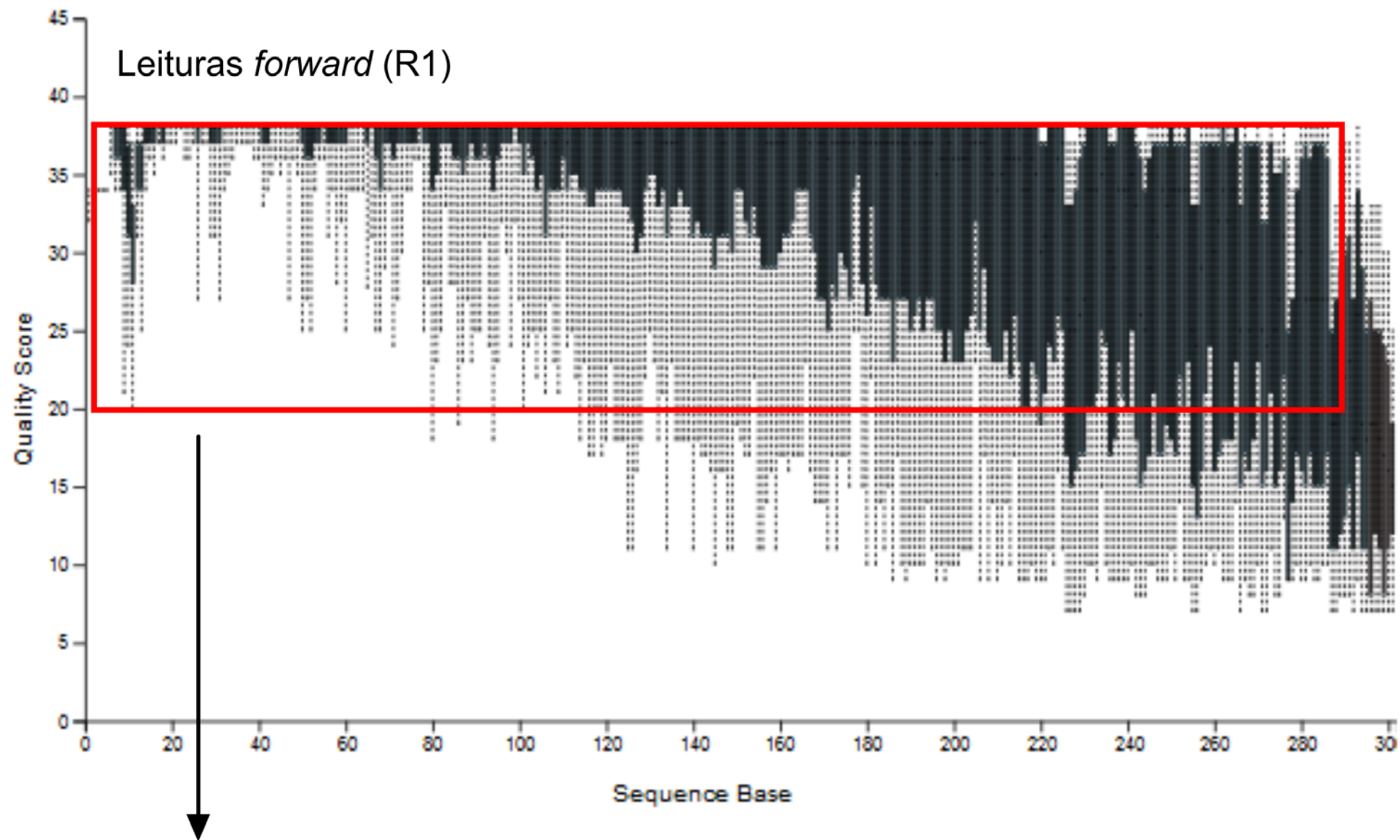
1. Pouco ajuste *fino* sobre parâmetros (*black box*)
2. Curva de aprendizado sobre os métodos e termos muito íngreme
3. Pouco estável: termos e métodos mudam de nome a cada versão
4. Pouca integração com os métodos legado de análise de microbiomas

QIIME2 reduzido às suas mínimas partes

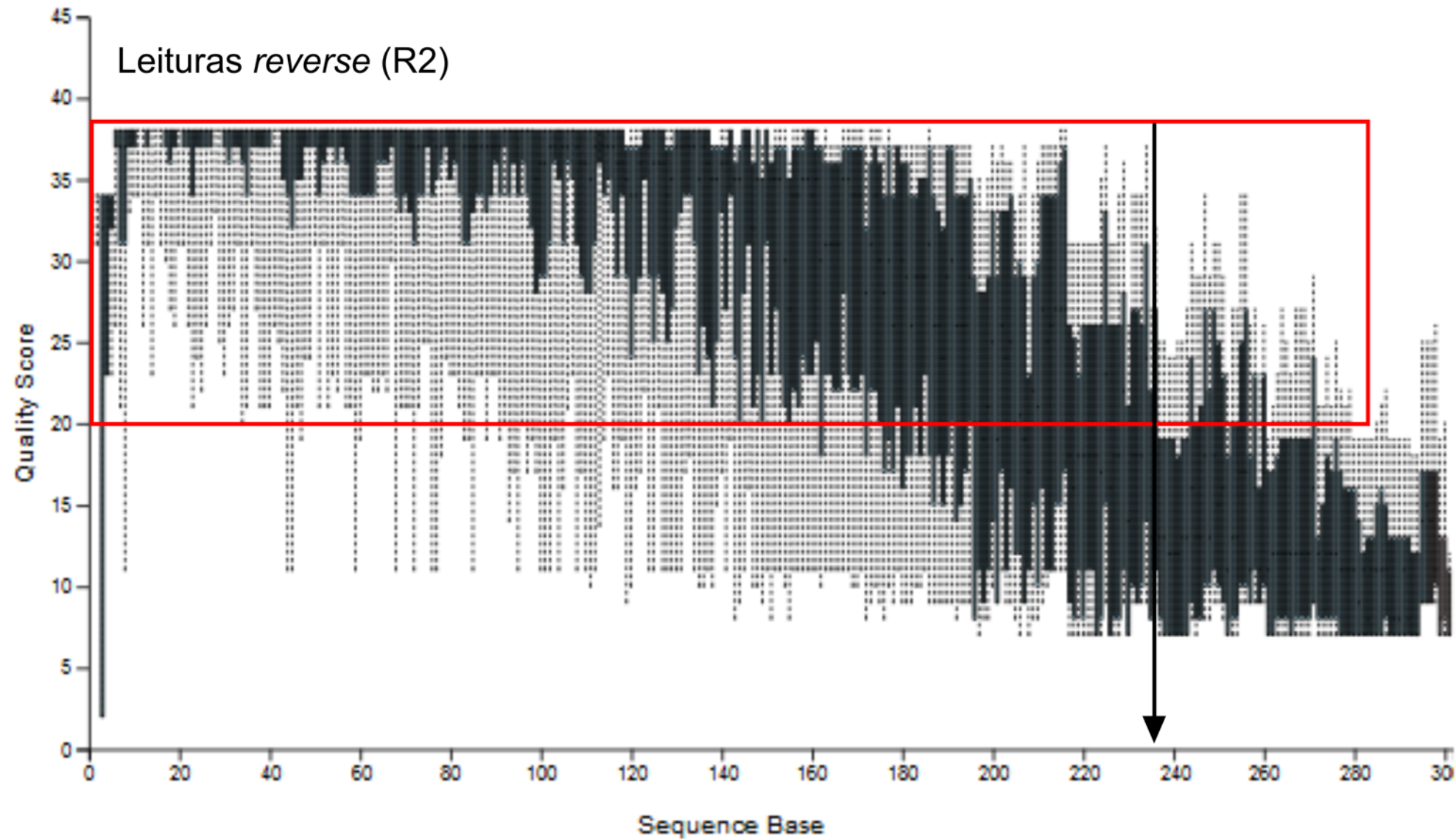


Pré-processamento: relatório da qualidade



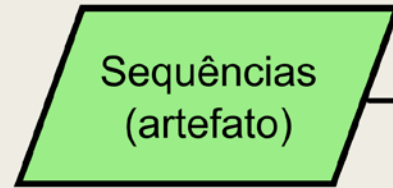


Truncar em 280 pares de bases
Admitir qualidade de 2.8 erros por sequência (1/100)



Truncar em 220-230 pares de bases
Admitir qualidade de 2.3 erros por sequência (1/100)

Parâmetros de pré-processamento



Descoberta do perfil de erros

Filtragem e trimagem

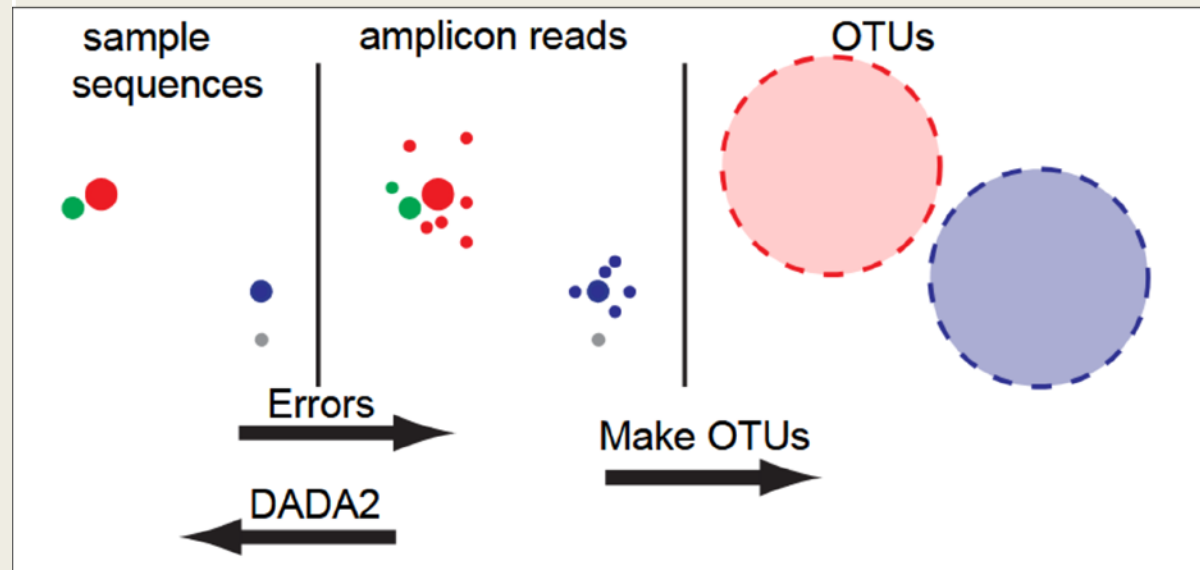
Algoritmo de remoção de ruídos

Merge das leituras

Remoção de quimeras

FeatureTable

FeatureData




```
graph TD
    FeatureData[FeatureData] --> FeatureData
    FeatureData --> FeatureData
    FeatureData --> FeatureData
```



```

graph TD
    FeatureTable[FeatureTable] --> FeatureTable
  
```

[illegible]

n = número de sequências verdadeiras únicas inferidas do sequenciamento (DADA2)

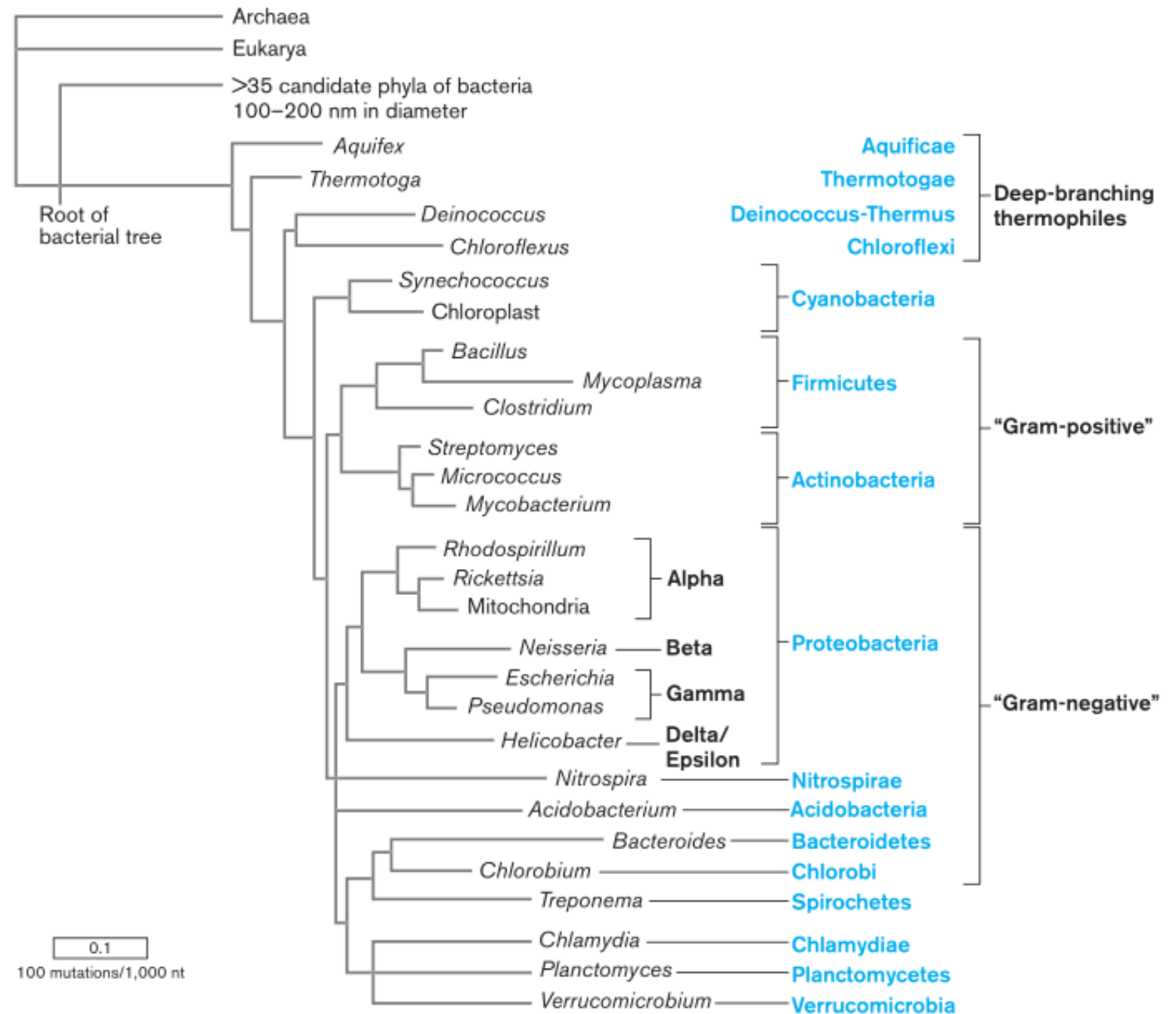
VSEARCH

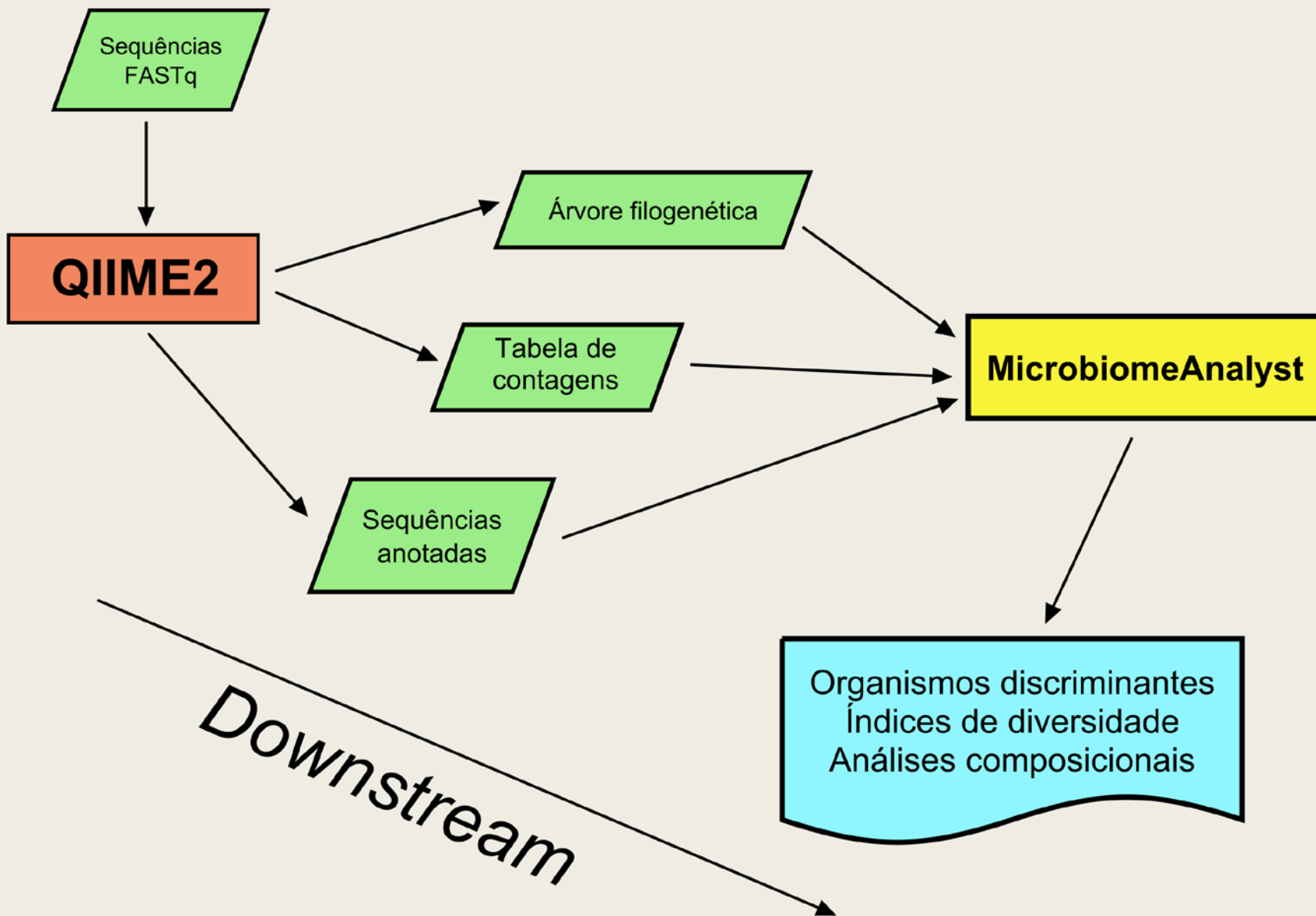


SEQ3 >>> <<< 97, 99, 100%
Neisseria ●—————●

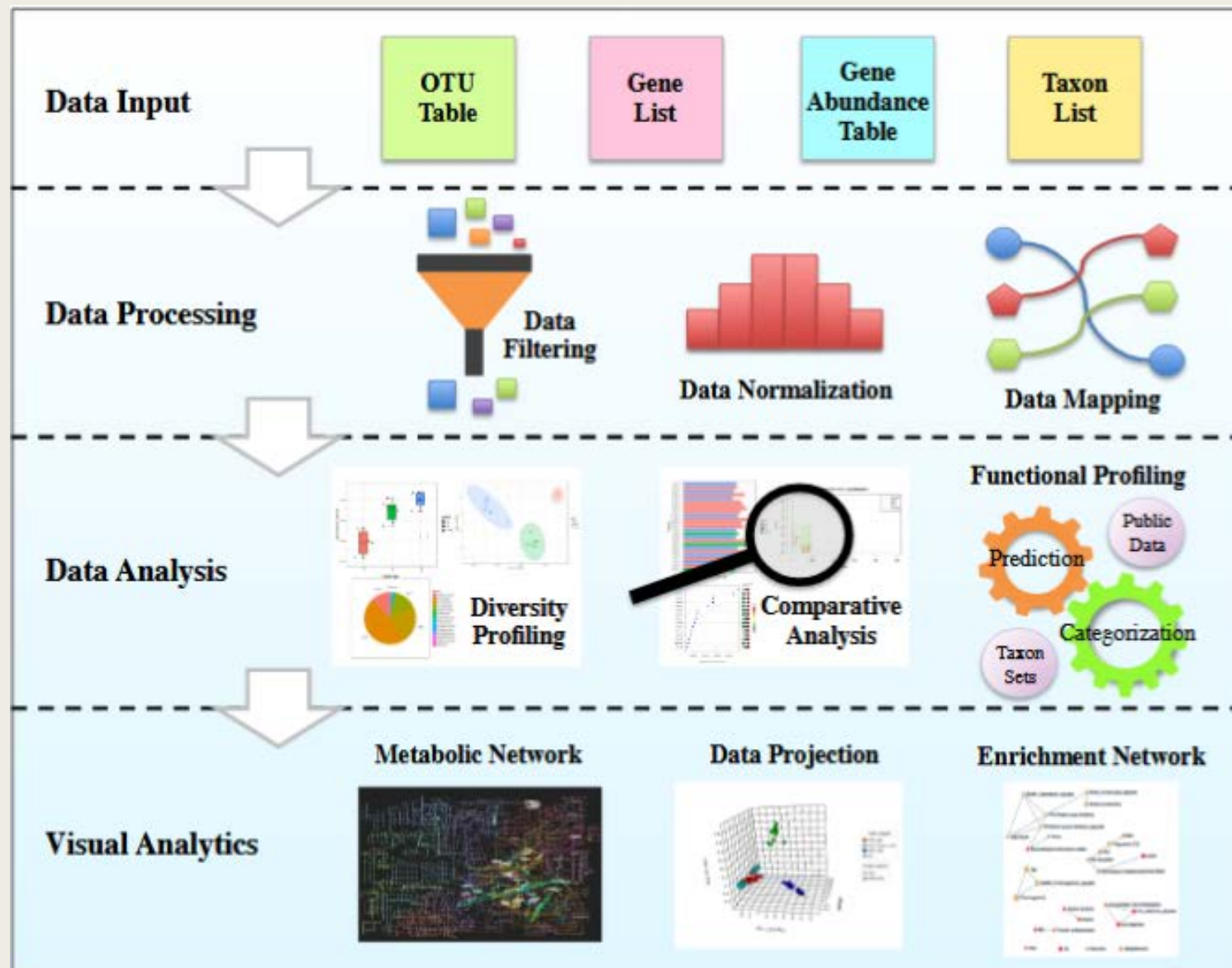
Sequência	Classificação
SEQ1	<i>Neisseria</i>
SEQ2	<i>Staphylococcus</i>
SEQ3	<i>Neisseria</i>
SEQ4	<i>Unclassified...</i>

Filogenia

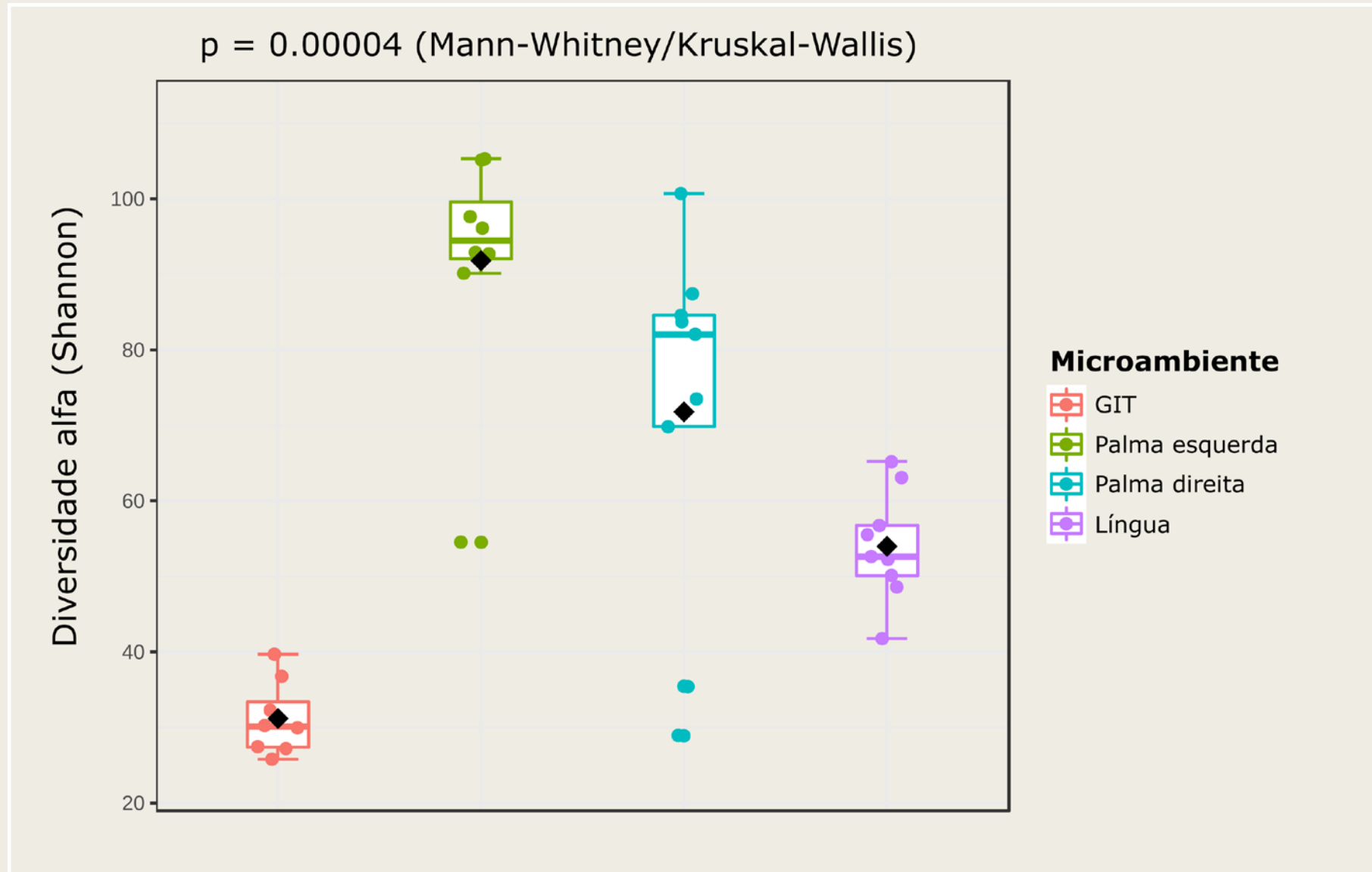




MicrobiomeAnalyst

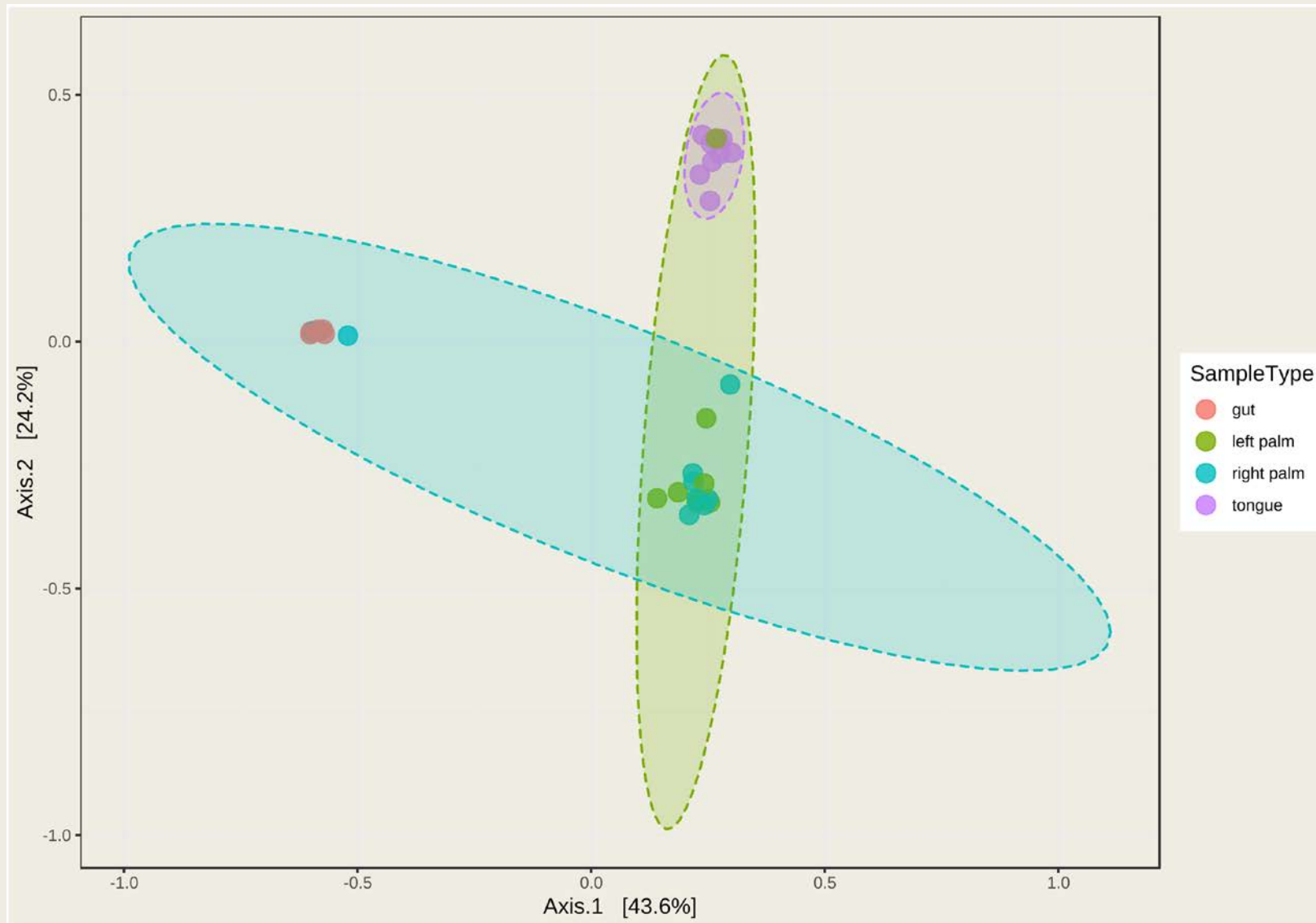


Alfa-diversidade



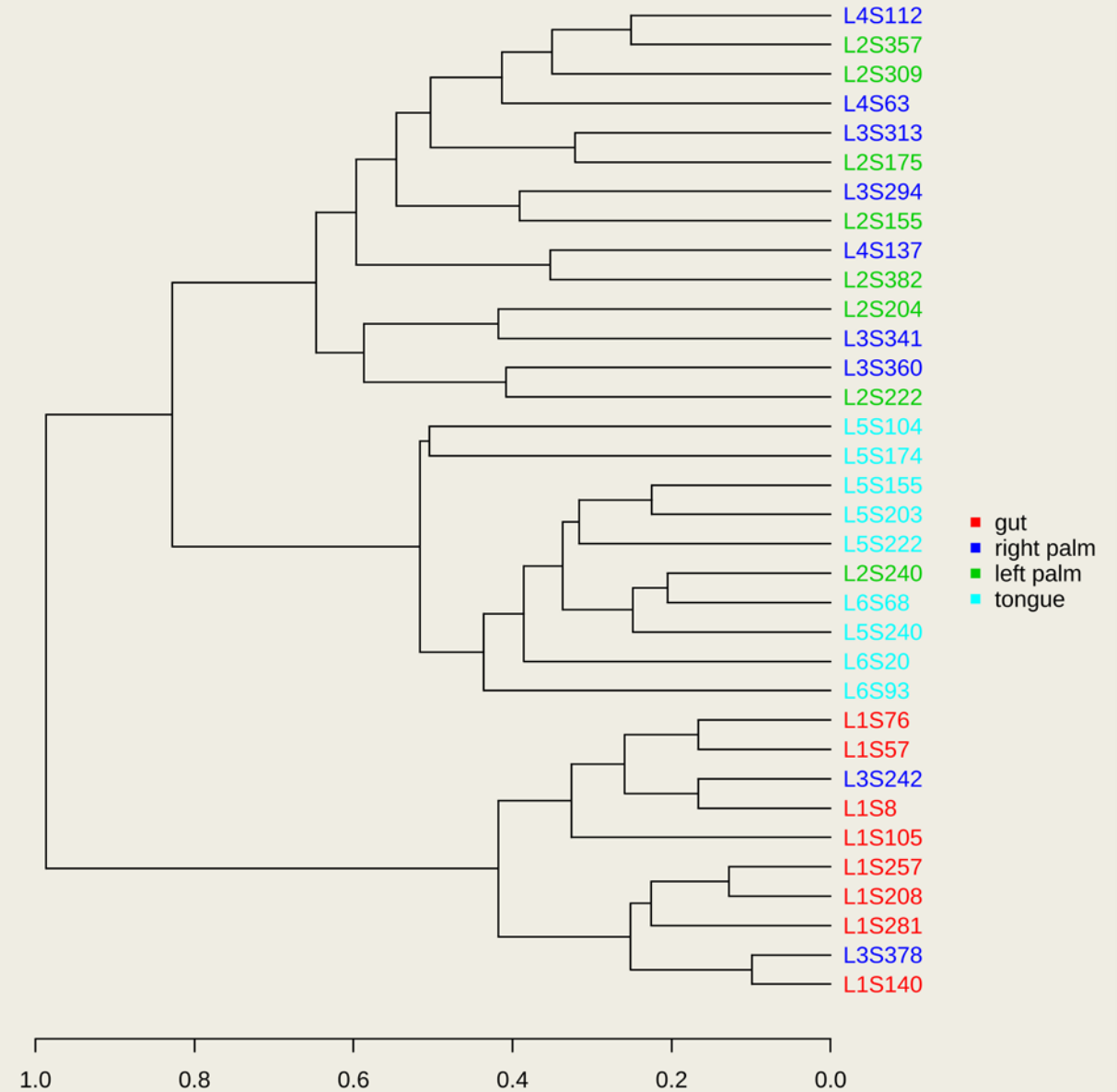
Beta-diversidade

Comparação entre comunidades



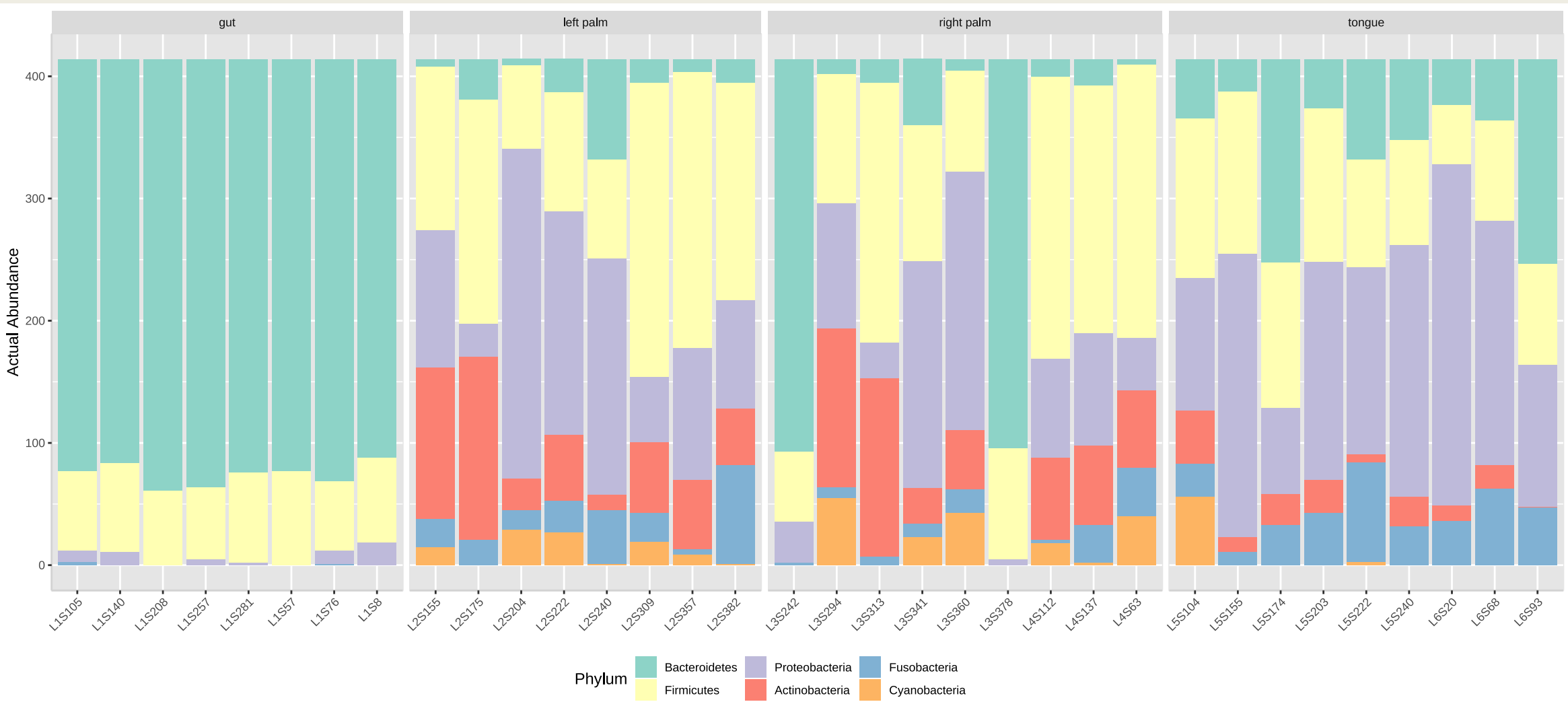
Beta-diversidade

Comparação entre comunidades



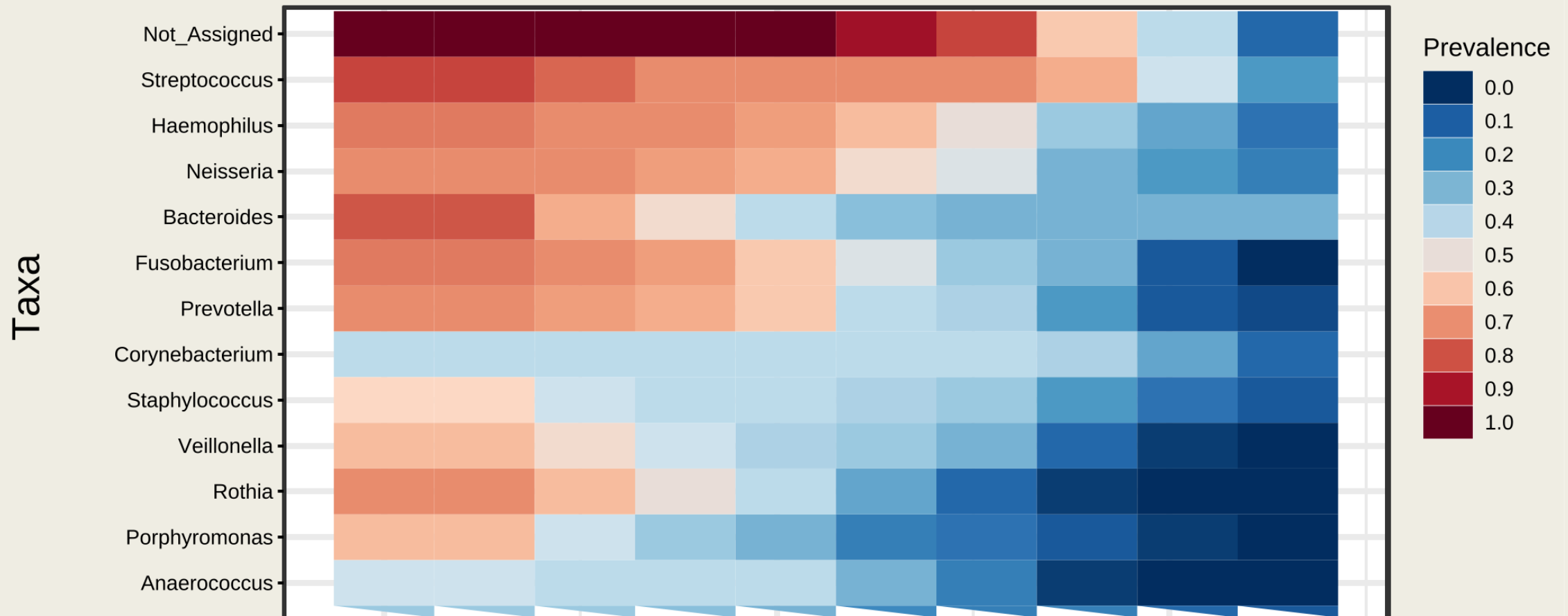
Análise composicional

(Ideal para taxa mais alta na hierarquia)



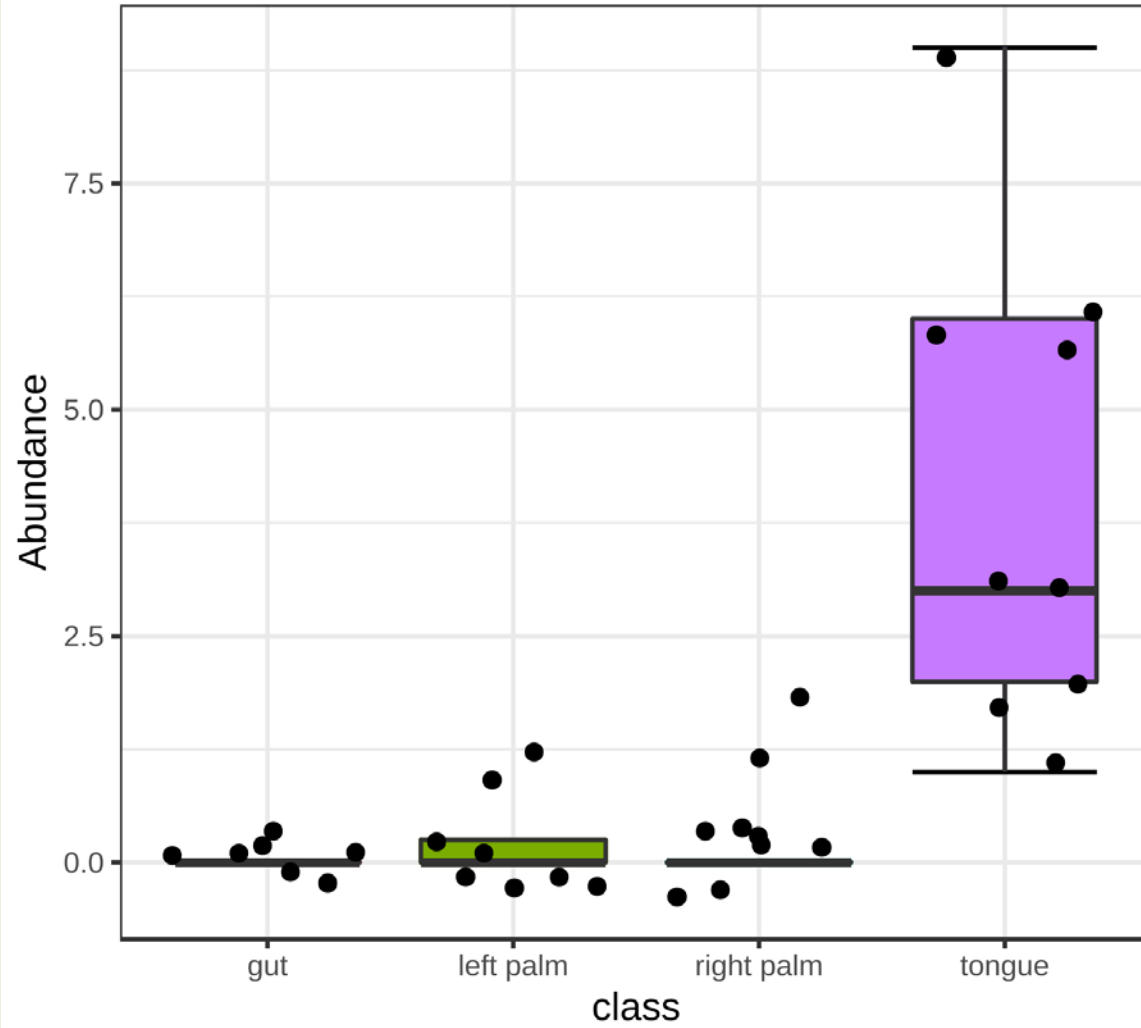
Microbioma fundamental

(Quem são os microrganismos mais prevalentes a um ambiente?)

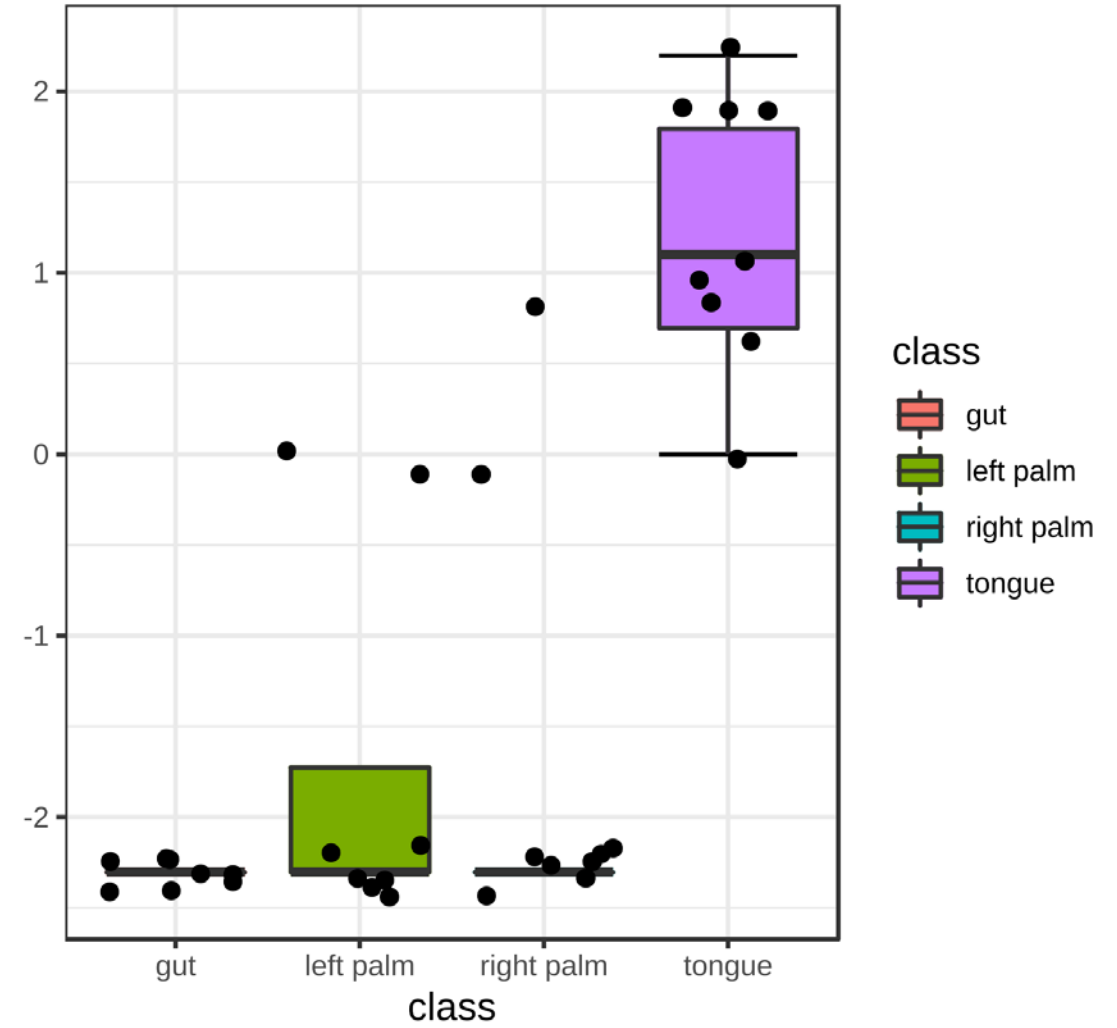


Campylobacter

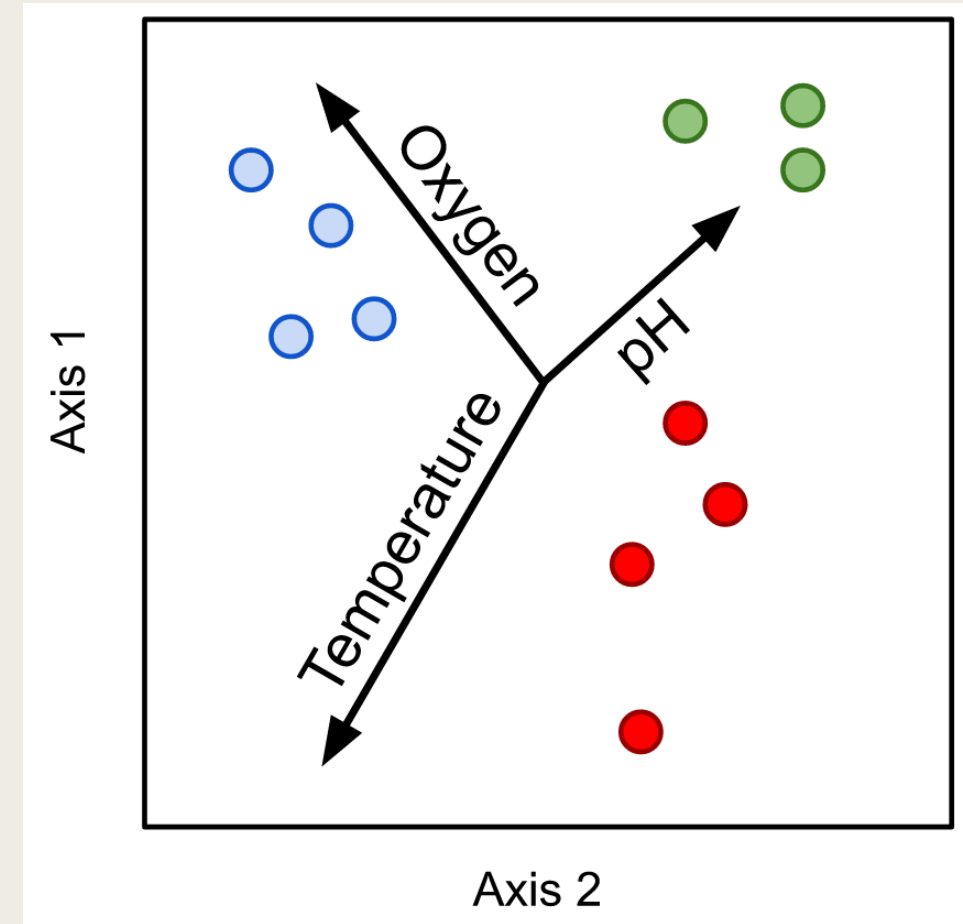
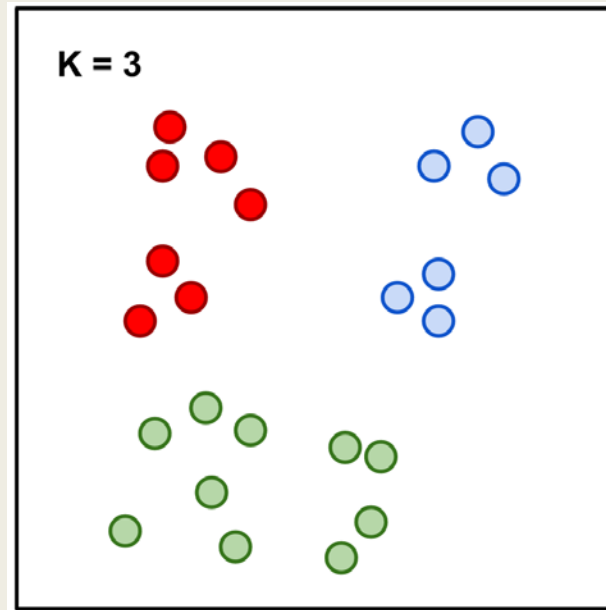
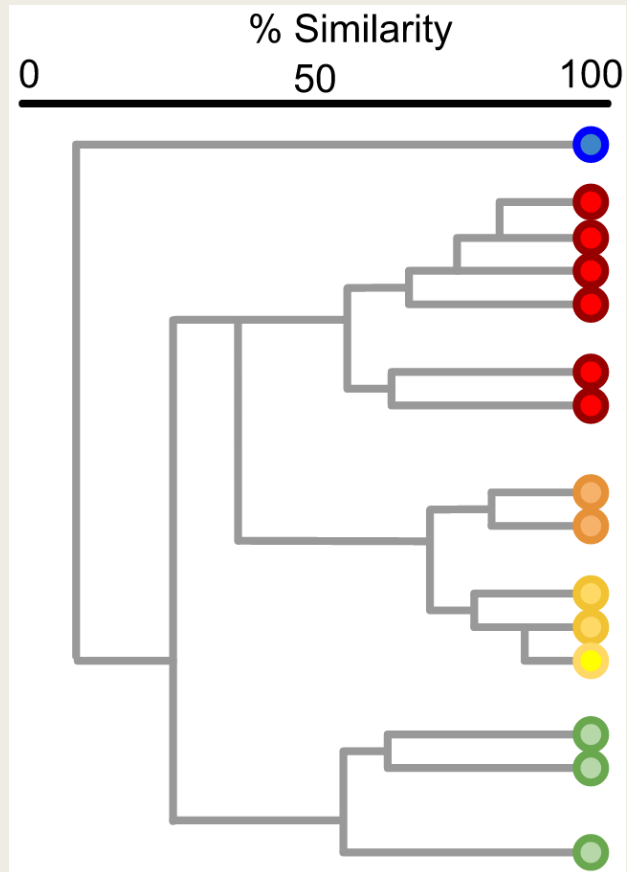
Original count



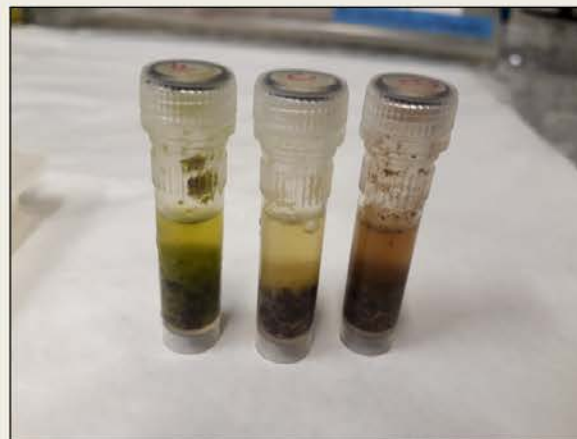
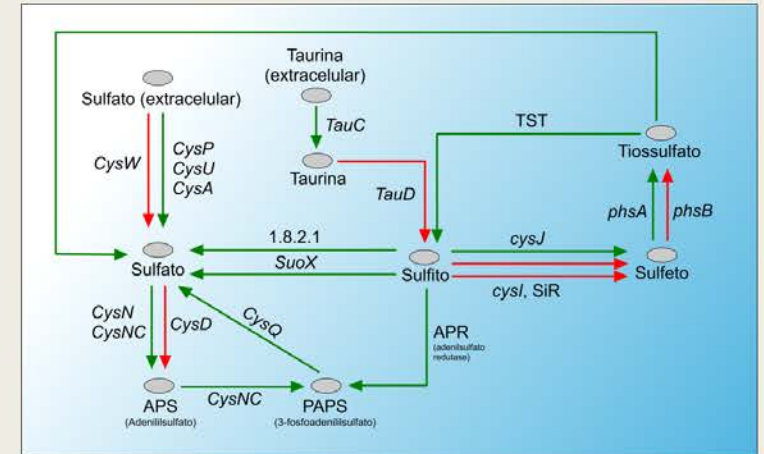
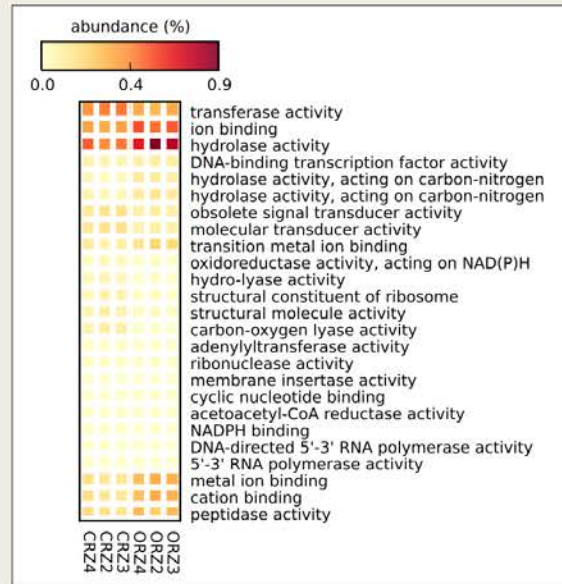
Log-transformed Count



Após o MicrobiomeAnalyst...



Sobre o nosso lab..



FIM