

# Sulfate-reducing bacteria in Mediterranean lagoons: a comparison between 16S rRNA- and dsr- based analyses

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# Introduction

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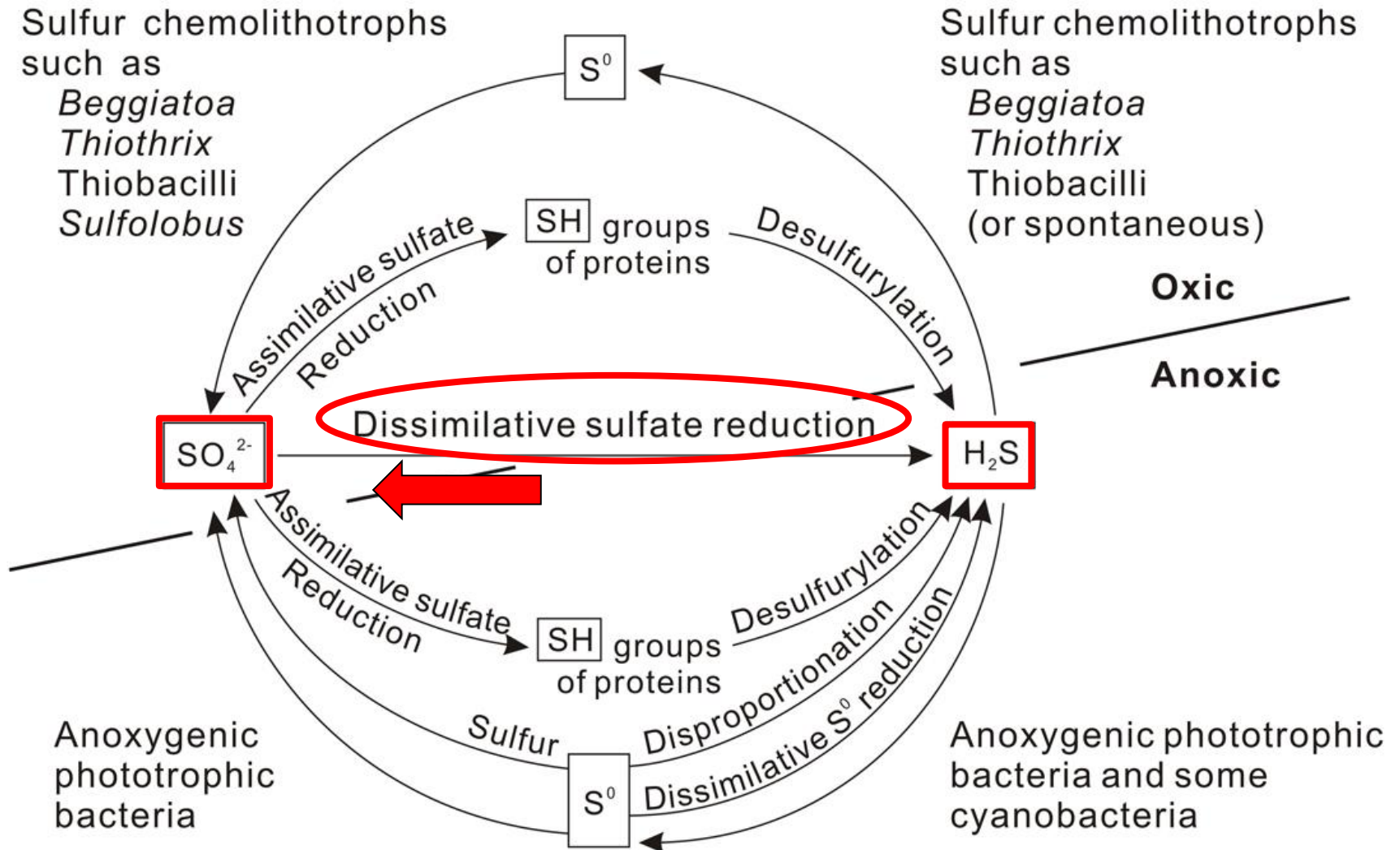
## Lagoons:

- naturally enriched habitats
- unstable environmental conditions
- increased hypoxia
- high concentrations of hydrogen sulfide

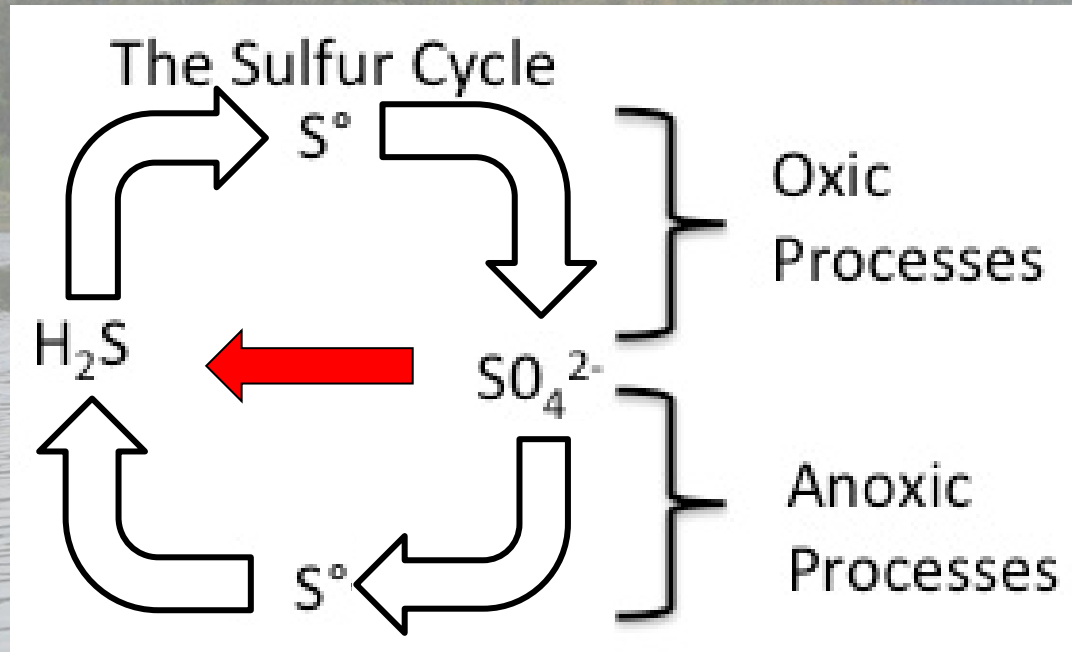




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Based on comparative analysis of 16S rRNA sequences, the known SRB can be grouped into seven phylogenetic lineages (Muyzer & Stams, 2008):

- Deltaproteobacteria,
- Clostridia (*Desulfotomaculum*, *Desulfosporosinus*, *Desulfosporomusa*)
- Nitrospirae (*Thermodesulfovibrio*)
- Thermodesulfobacteria (*Thermodesulfobacterium*)
- Thermodesulfobiaceae (*Thermodesulfobium*)
- Euryarchaeota (*Archaeoglobus*)
- Crenarchaeota (*Thermocladium*, *Calditerrivirga*)



# Aims

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- Identification of sulfate reducing communities in hypoxic ecosystems (who?)

## ***16S rRNA:***

gold standard for estimating phylogenetic diversity in microbial communities

*multiple-copy*

*little resolution below the species level*

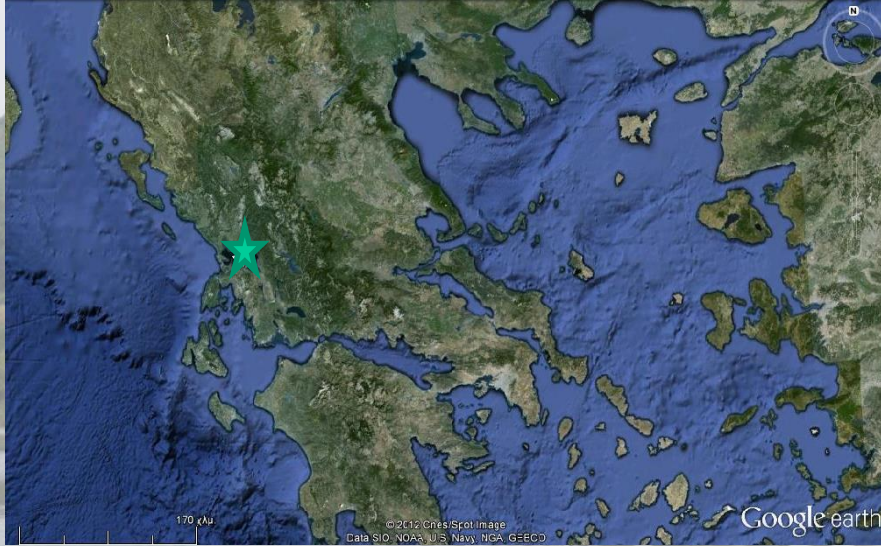
## ***dsr (dissimilatory sulfite reductase):***

single-copy

protein-coding gene

# Methodology - Sampling stations

Lagoonal complex of the Amvrakikos Gulf (Ionian Sea, Western Greece)





# Methodology - Sampling stations

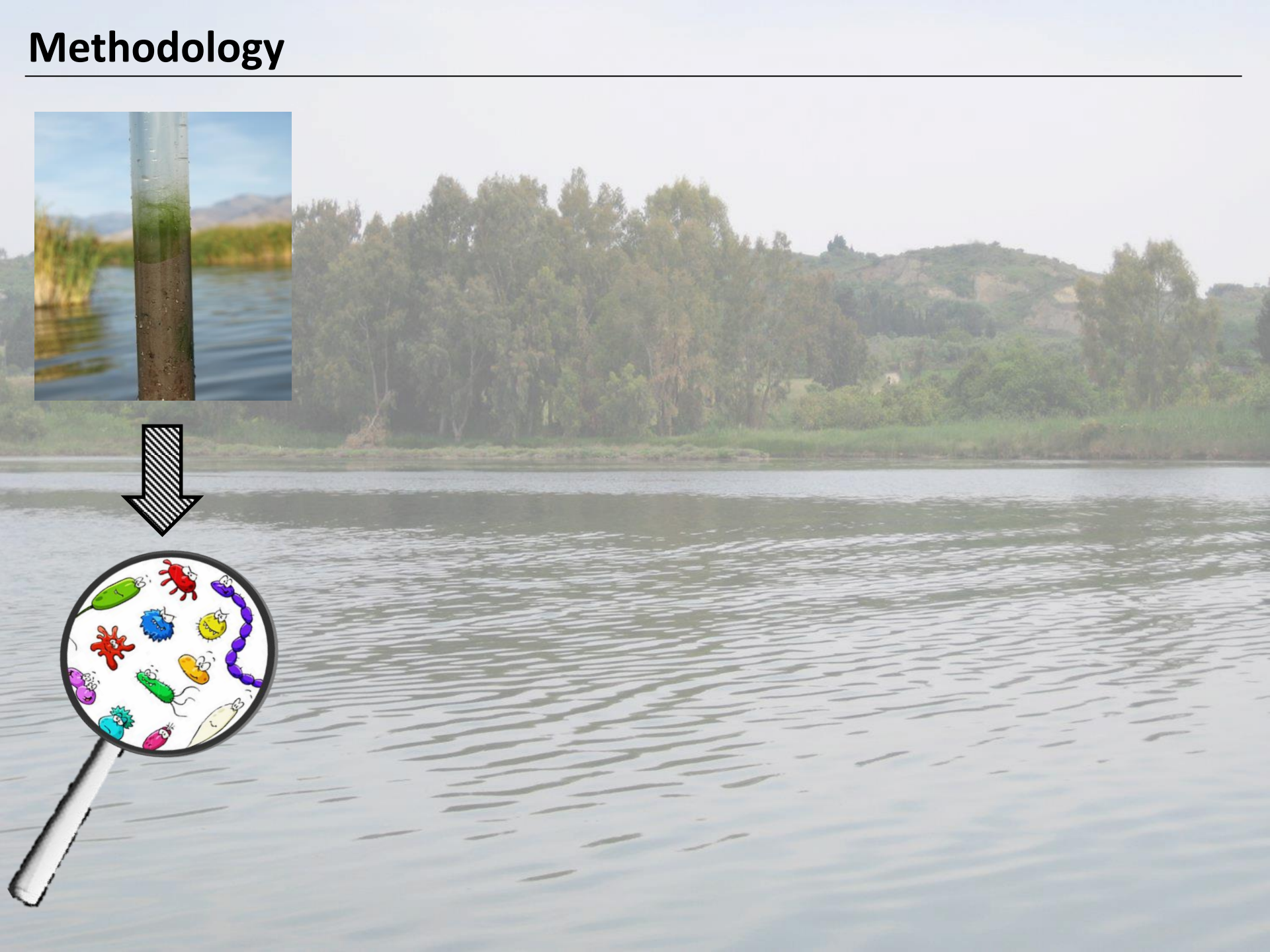
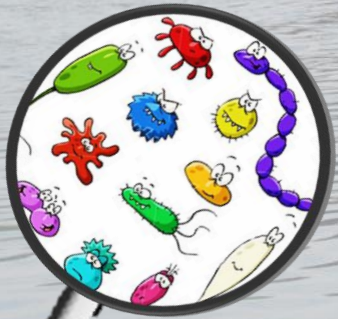
Lagoonal complex of the Amvrakikos Gulf (Ionian Sea, Western Greece)





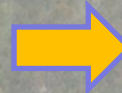
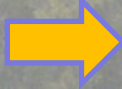
# Methodology

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# Methodology

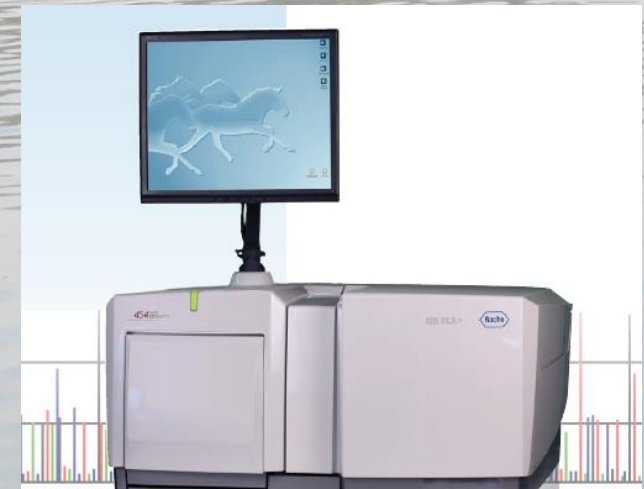
## Collection of sediment samples



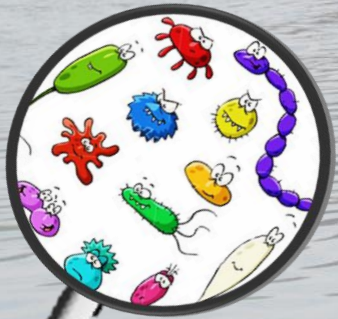
## DNA extraction



- PCR amplification
- Next generation sequencing



454 GS FLX Titanium Series



## ampliconnoise

Noise removal from pyrosequenced amplicons

Removal of:

- PCR errors
- Sequencing errors
- Chimeras

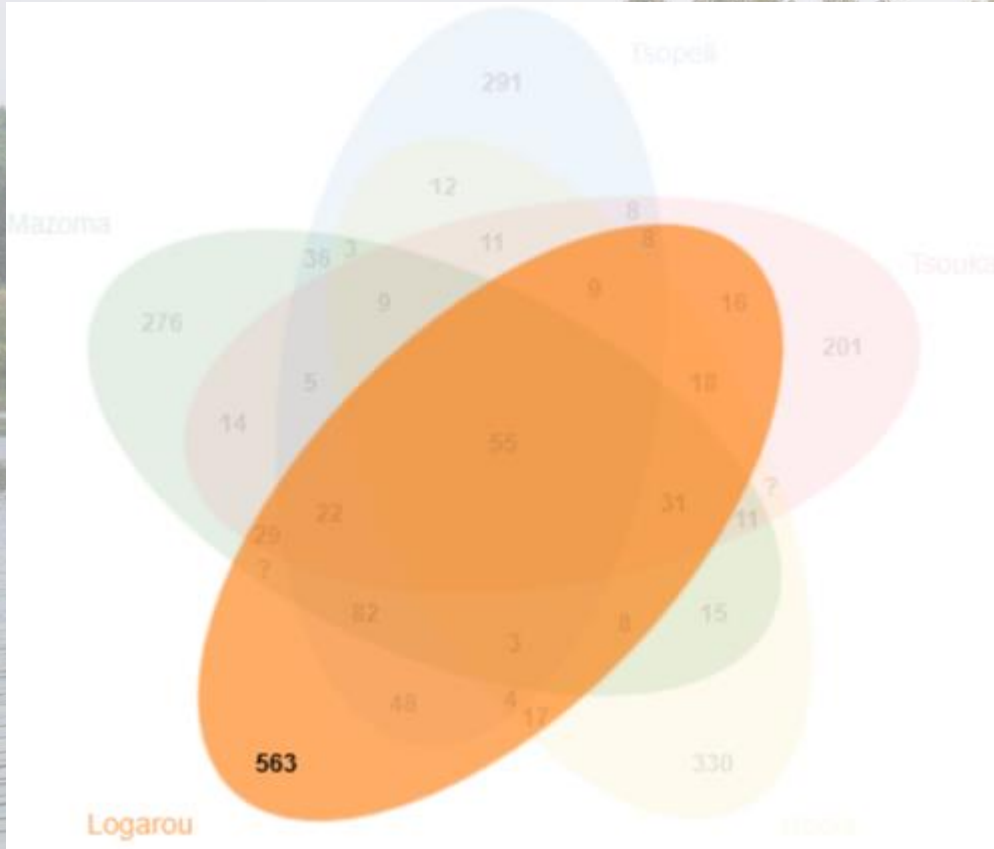




# Results - *dsr*

6,323 OTUs (using the 3% dissimilarity level)

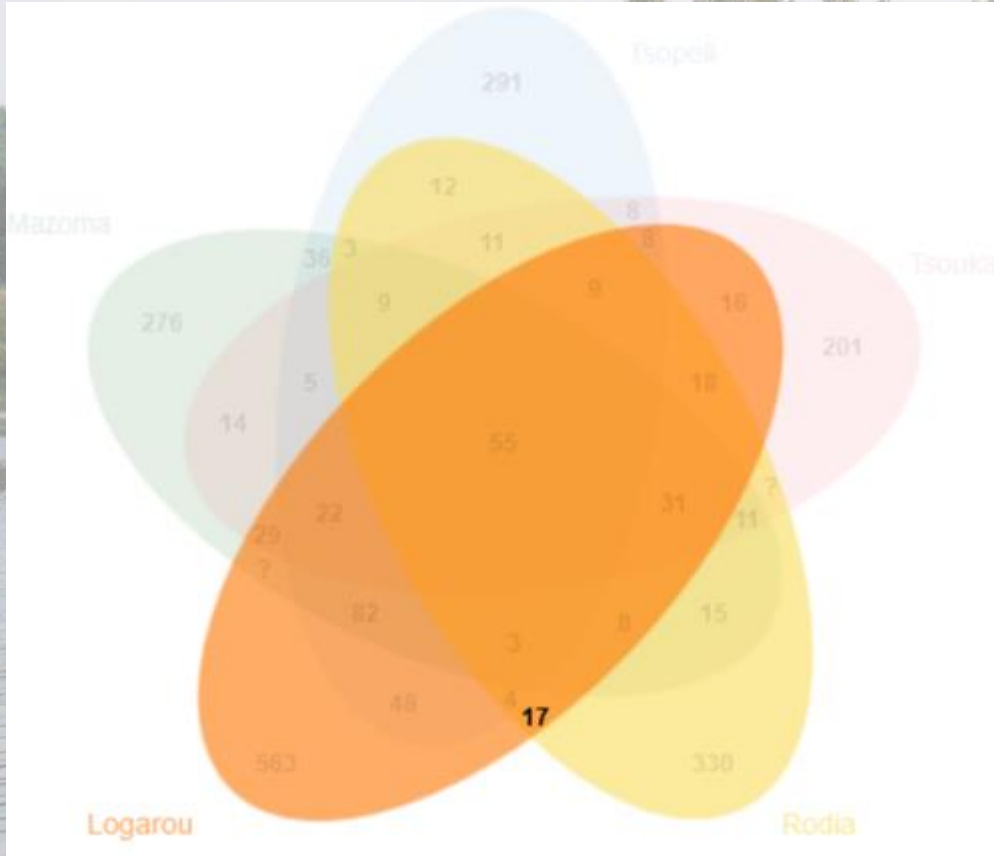
→ 4,172 OTUs correspond to Uncultured Sulfate Reducing Prokaryotes



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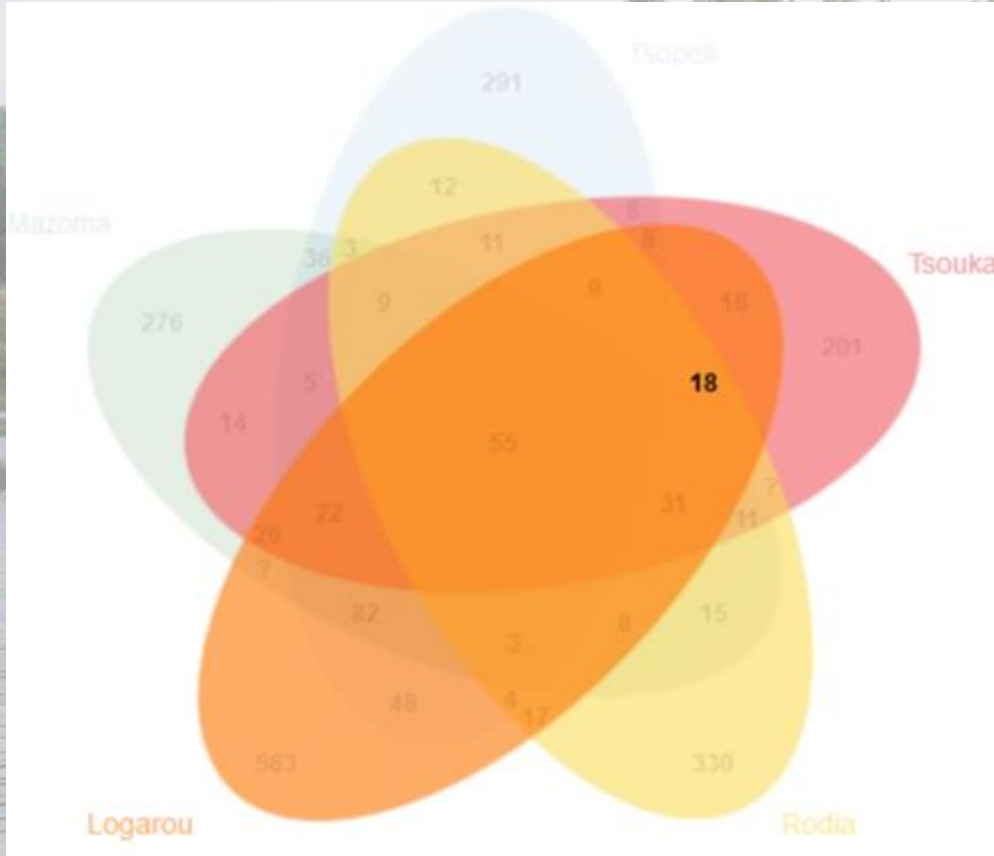




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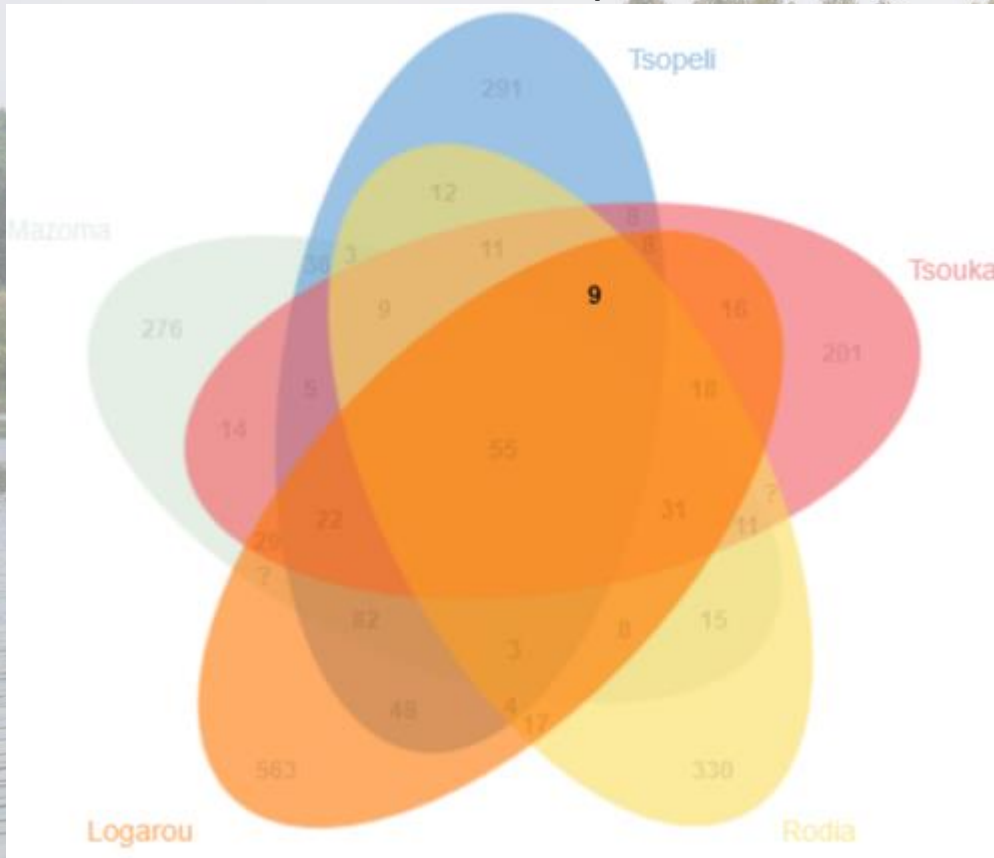
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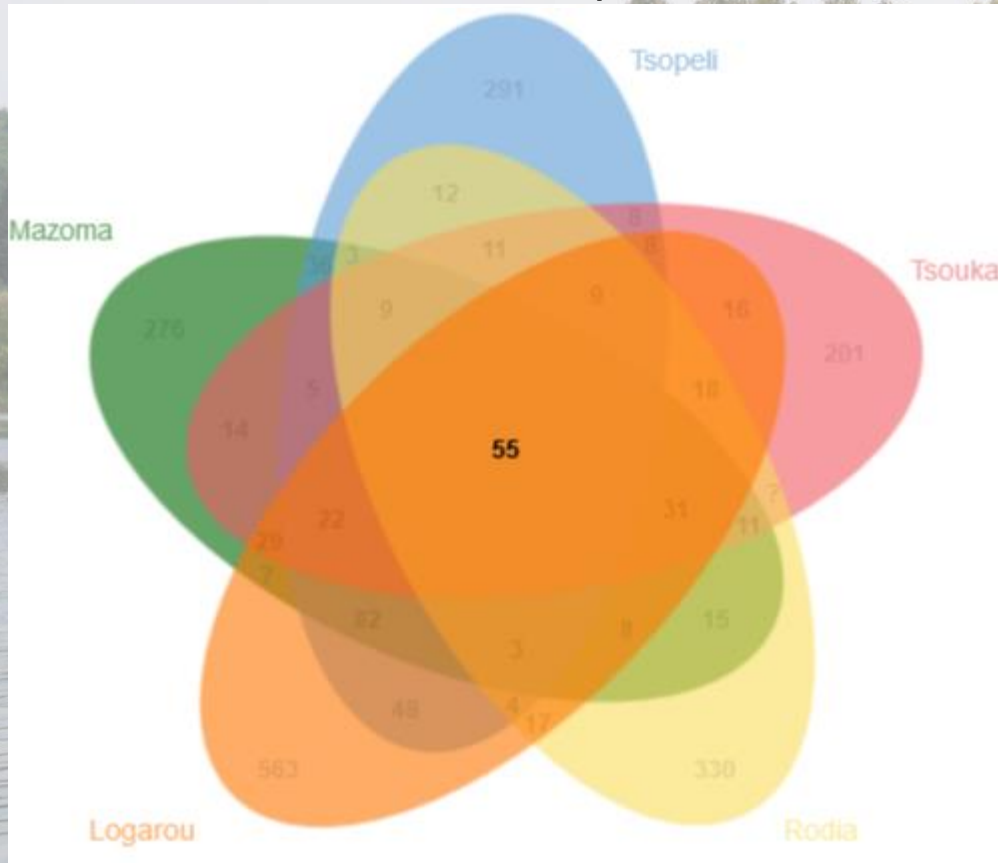




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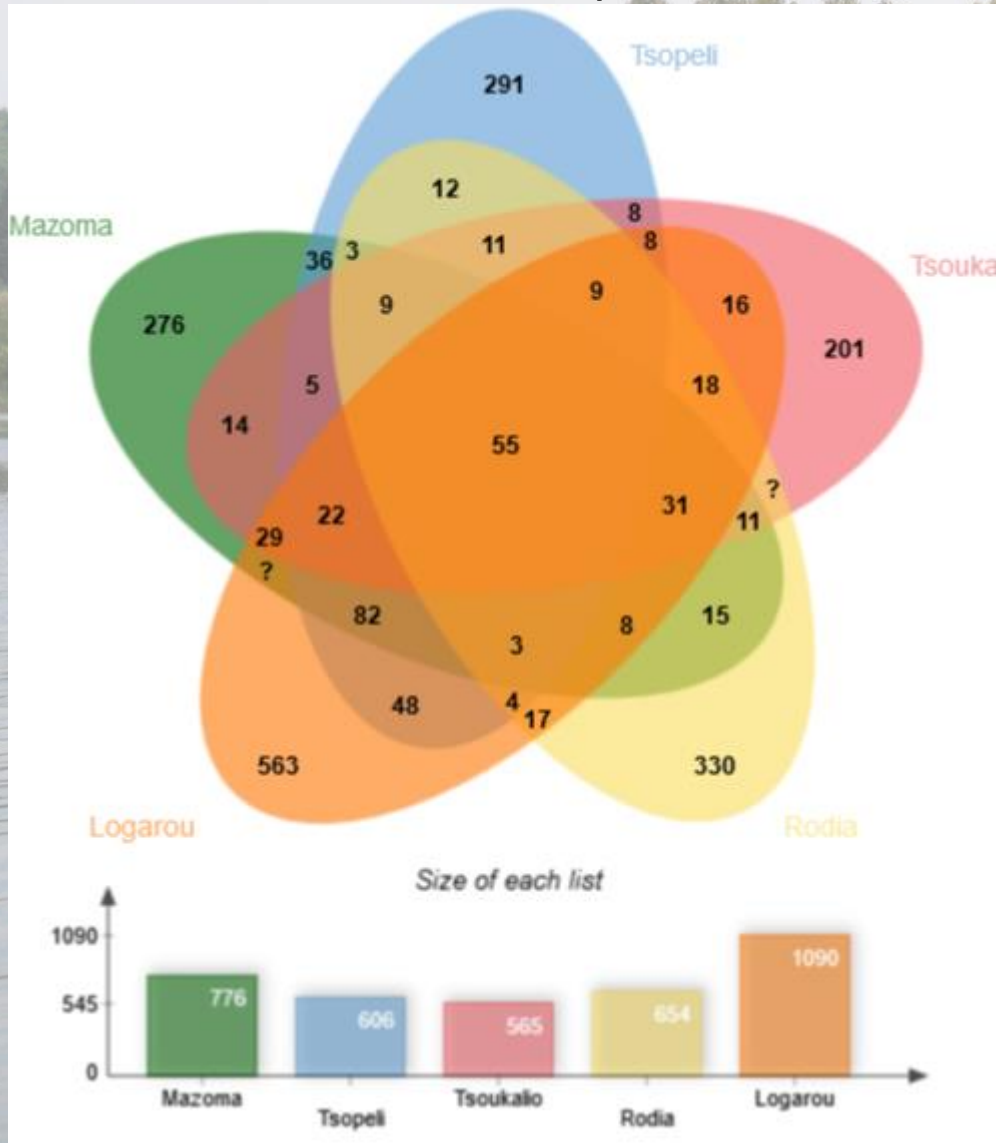
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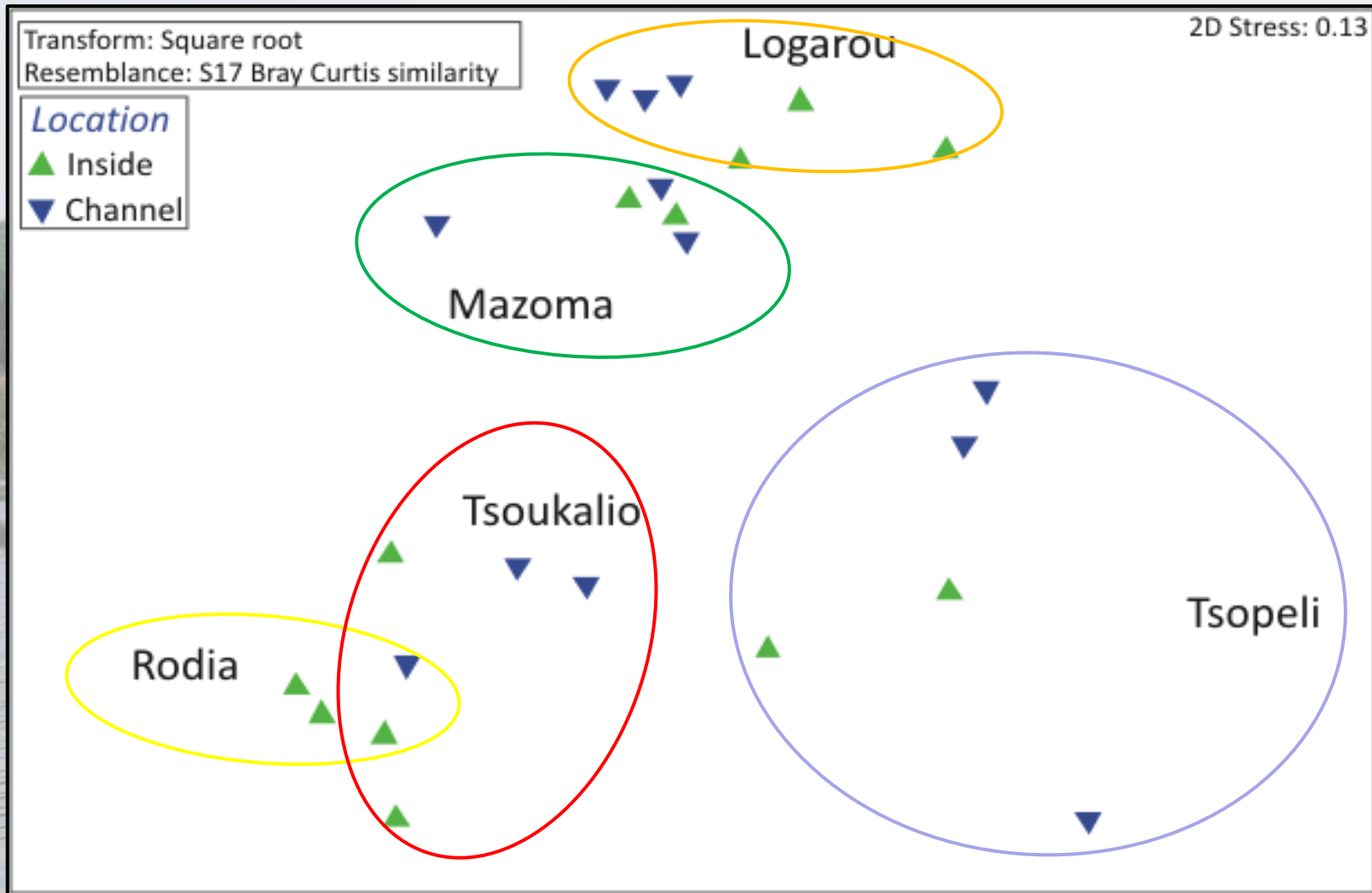
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# Results - *dsr*



*PERMANOVA:*

Factors: **Lagoon** ( $p = 0,001$ ), **Location** ( $p = 0,043$ ),  
**Lagoon x Location** ( $p = 0,001$ )

# Results - *dsr*

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## *BIOENV*

Ammonium (uM)	Salinity (psu)	Median Diameter	Total Reduced Inorganic Sulfur (uM/g)	Phaeopigments (ug/g)	$\rho_w$
+	+	+			0.585 **
+	+	+	+	+	0.466 **



# Results - *dsr*

BIOENV

Ammonium (uM)	Salinity (psu)	Median Diameter	Total Reduced Inorganic Sulfur (uM/g)	Phaeopigments (ug/g)	$\rho_w$
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+	+	+	+	+	0.466 **



## Acid Volatile Sulfides:

H<sub>2</sub>S

FeS

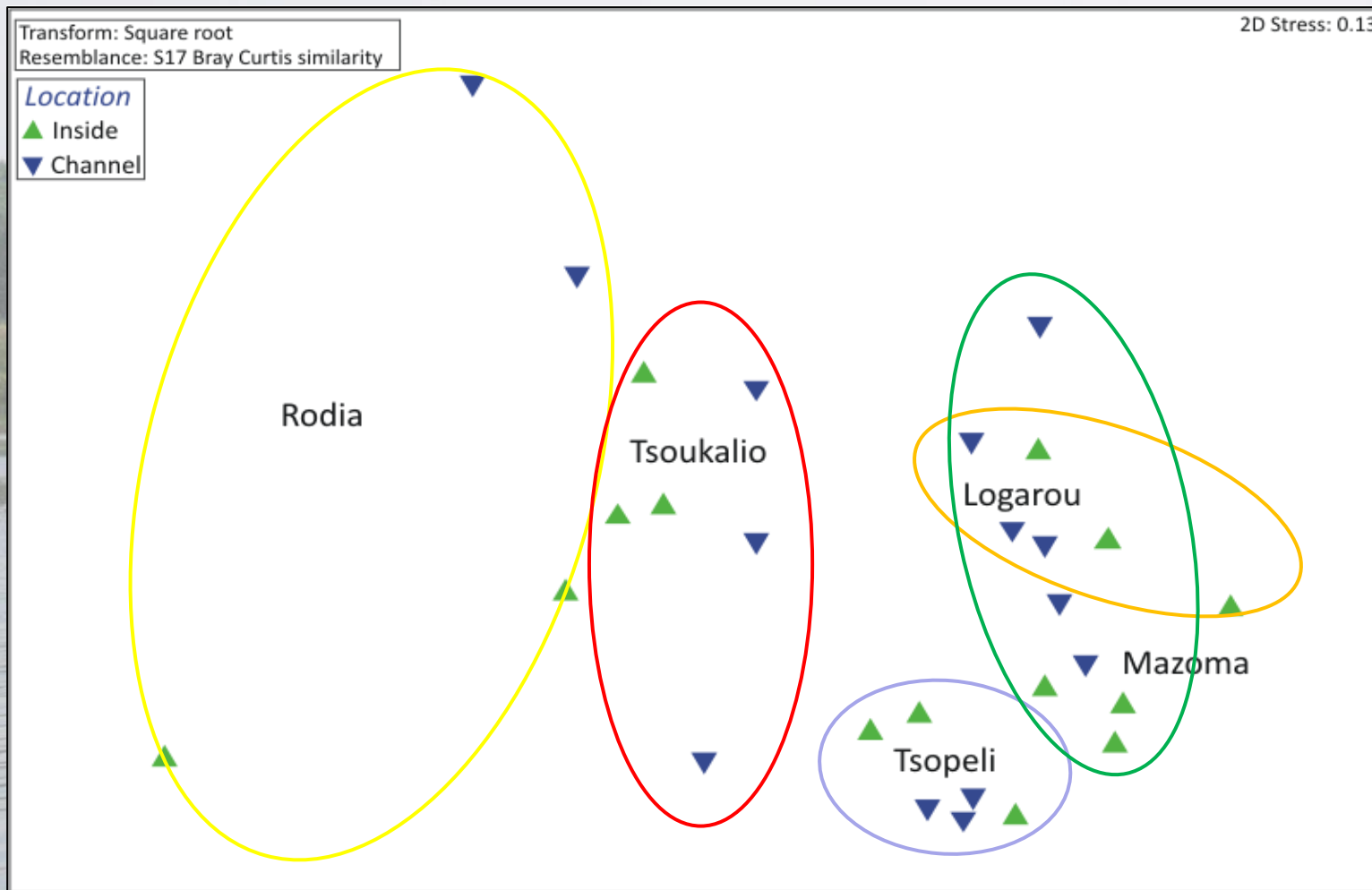
## Chromium Reducible Sulfur:

S<sup>0</sup>

FeS<sub>2</sub>

# Results - 16S rRNA

14,789 OTUs (using the 3% dissimilarity level)



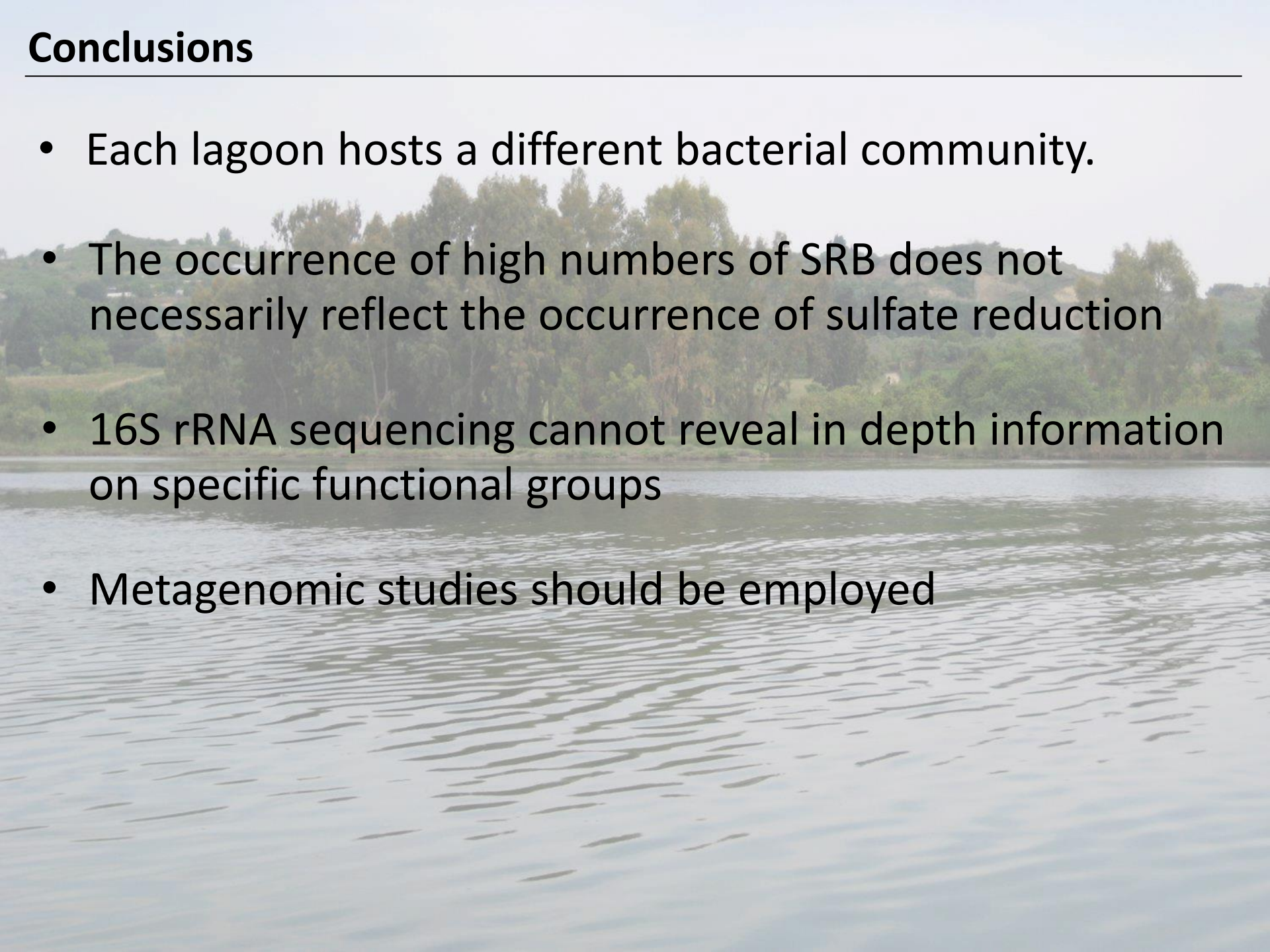
**PERMANOVA:**

Factors: **Lagoon** ( $p = 0,001$ ), **Location** ( $p = 0,001$ ),  
**Lagoon x Location** ( $p = 0,001$ )



# Conclusions

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- Each lagoon hosts a different bacterial community.
  - The occurrence of high numbers of SRB does not necessarily reflect the occurrence of sulfate reduction
  - 16S rRNA sequencing cannot reveal in depth information on specific functional groups
  - Metagenomic studies should be employed
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*it's the only culture some people have*