

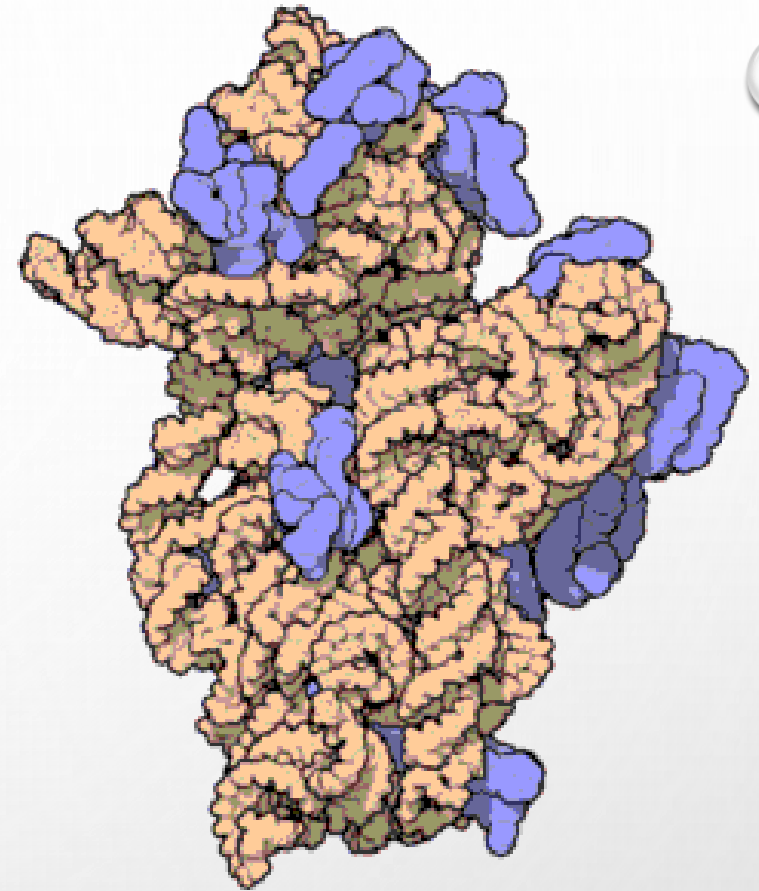


# 16S RIBOSOMAL DNA SEQUENCING IN WATER RESEARCH AND AGRICULTURAL SCIENCES

an overview

**16S ribosomal RNA** (or 16S rRNA) is the component of the small subunit of a prokaryotic ribosome

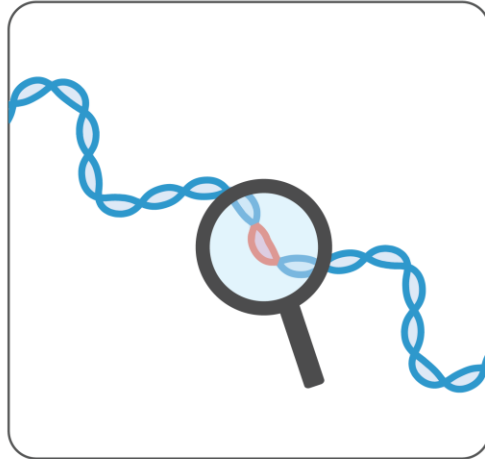
- It is **highly conserved** between different species of bacteria and archaea
- Contains **nine** hypervariable regions (V1-V9) ranging from about 30-100 base pairs
- These regions could **distinguish** bacterial taxons: phyla, families, genera and even species



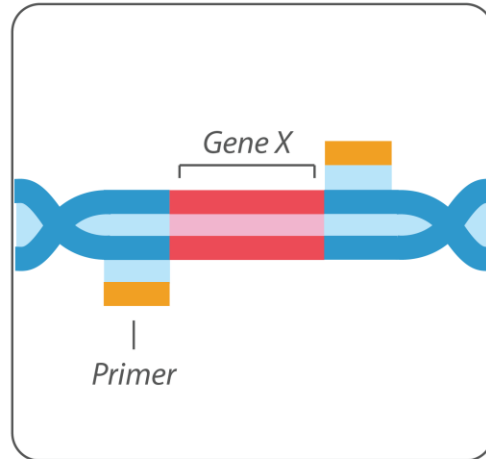
Small subunit (30S) of prokaryotic ribosome.

orange - rRNA  
(*Thermus thermophilus*)

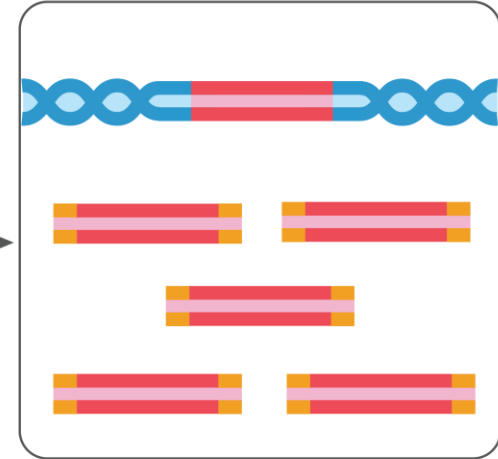
# Amplicon Sequencing



**Genomic or Template DNA**



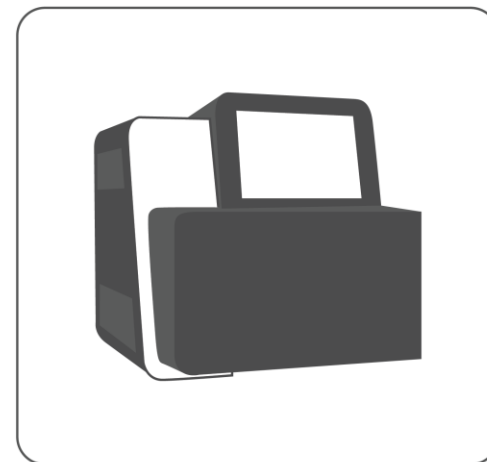
**Gene-specific Primer**



**PCR Amplification**



**Alignment**



**Sequencing**

# 16S rRNA databases

- **Ribosomal Database Project (RDP)** – the optimal choice for the analysis of **intestinal microbiota**
- **SILVA** – the best choice for the analysis of **soil bacterial** communities
- **GreenGenes** – outdated now (not supported)

# 16S taxonomic profiling results example (taxon abundance table)

soil samples →

← bacterial phyla

Taxon	Average	PMB_11	PMB_13	PMR_11	PMR_12	POB_11	POB_12	POB_13	POR_11	POR_12	POR_13	WMB_11	WMB_12	WMB_13	WMR_11
<b>Plant</b>		Potato	Potato	Potato	Potato	Potato	Potato	Potato	Potato	Potato	Potato	Whitemus	Whitemus	Whitemus	Whitemus
<b>Locus</b>		Bulksoil	Bulksoil	Rhizosphe	Rhizosphe	Bulksoil	Bulksoil	Bulksoil	Rhizosphe	Rhizosphe	Rhizosphe	Bulksoil	Bulksoil	Bulksoil	Rhizosphe
<b>Fertilizer</b>		Mineral	Mineral	Mineral	Mineral	Organic	Organic	Organic	Organic	Organic	Organic	Mineral	Mineral	Mineral	Mineral
<i>Proteobacteria</i>	18696.85	17036.5	15566.6	20570.2	19467.3	13528.0	10368.1	12732.6	13283.4	14238.6	13463.2	17880.0	19287.8	17930.0	28284.0
<i>Acidobacteria</i>	5369.079	4925.4	4248.5	3750.1	3546.0	7469.3	9735.3	8389.1	7584.1	7209.0	7604.3	6897.0	3828.6	3171.2	2322.9
<i>Verrucomicrobia</i>	3864.611	4908.4	4335.3	3511.2	3677.5	4822.1	5052.3	5424.9	6235.0	5699.0	5089.2	2861.6	2744.4	2845.6	2636.8
<i>Actinobacteria</i>	3546.558	5706.7	5631.7	4757.2	5545.3	2649.5	2450.5	2499.0	1907.2	2284.2	2676.1	5706.7	7154.2	7231.9	3492.3
<i>Bacteroidetes</i>	3059.884	1463.5	1348.4	1730.8	1752.7	3950.6	2281.6	3262.0	6072.4	3798.3	3599.8	2844.0	2258.0	1737.5	1925.9
<i>Thaumarchaeota</i>	2852.37	4642.3	5328.3	3529.9	3690.1	2190.6	2429.1	2191.9	2213.4	2028.0	2088.5	1480.8	2202.8	1765.9	2945.1
<i>Planctomycetes</i>	2067.071	1487.5	2396.2	2575.8	2395.2	2082.1	2503.5	2102.0	2376.0	2369.1	2458.8	1561.9	2285.1	2584.4	1738.2
<i>Chloroflexi</i>	1737.008	2108.9	2440.3	1985.2	2232.0	2096.8	2533.7	1923.1	1505.3	2071.3	2136.8	2368.8	2059.1	2377.9	1129.6
<i>Firmicutes</i>	382.3118	861.9	538.6	518.9	417.3	489.4	359.3	464.4	389.1	397.1	326.4	977.4	748.9	582.4	246.8
<i>Gemmatimonadetes</i>	336.908	535.0	808.5	646.9	648.7	209.1	192.9	179.8	57.4	193.0	220.8	779.8	1145.7	1166.0	390.2
<i>Rokubacteria</i>	326.8566	259.0	197.8	111.0	89.8	279.2	621.5	385.8	232.8	409.9	430.2	188.2	164.6	40.4	71.7
<i>Cyanobacteria</i>	215.3412	76.4	133.6	180.9	140.4	260.0	192.9	246.2	277.5	233.0	138.1	23.5	145.8	52.5	89.9
<i>Armatimonadetes</i>	214.0984	212.3	264.6	126.3	218.8	81.4	230.7	165.7	223.2	100.9	154.0	58.8	103.1	268.8	250.3
<i>Nitrospirae</i>	94.79081	51.0	52.1	17.1	40.5	165.0	210.5	201.3	133.9	168.9	177.7	29.4	47.9	12.0	18.2
<i>Elusimicrobia</i>	46.89505	55.2	10.7	52.9	50.6	67.8	94.5	84.3	73.4	52.8	64.2	11.8	13.5	22.9	54.6
<i>Patescibacteria</i>	45.9225	53.8	65.5	52.9	37.9	58.8	167.7	71.2	44.6	30.4	54.5	14.1	7.3	47.0	35.3
<i>Fibrobacteres</i>	29.01703	0.0	12.0	0.0	0.0	111.9	31.5	74.0	44.6	15.2	82.7	24.7	0.0	27.3	3.4
<i>Euryarchaeota</i>	20.57839	0.0	0.0	0.0	0.0	55.4	30.3	43.1	41.5	64.9	46.6	61.2	4.2	6.6	0.0
<i>Dependentiae</i>	16.24939	15.6	10.7	10.2	3.8	19.2	21.4	23.4	6.4	30.4	25.5	11.8	9.4	0.0	9.1
<i>Deinococcus-Thermus</i>	13.96904	0.0	4.0	6.8	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	3.1	0.0	6.8

The slide features a light gray background with a subtle gradient. In the top-left and bottom-right corners, there are clusters of realistic, 3D-rendered water droplets of various sizes, some overlapping. The text is centered horizontally and vertically on the slide.

# APPLICATIONS OF 16S rRNA SEQUENCING





# **AGRICULTURAL SCIENCE**

## **APPLICATIONS OF 16S rRNA SEQUENCING**

- Detection and analysis of **adverse factors** (pathogenic bacteria and fungi)
- Monitoring of the **microbial response** to fertilizers
- Bacterial community structure as biomarker of soil conditions, properties, assessment of biogeochemical processes (presence of nitrifying bacteria, etc.)

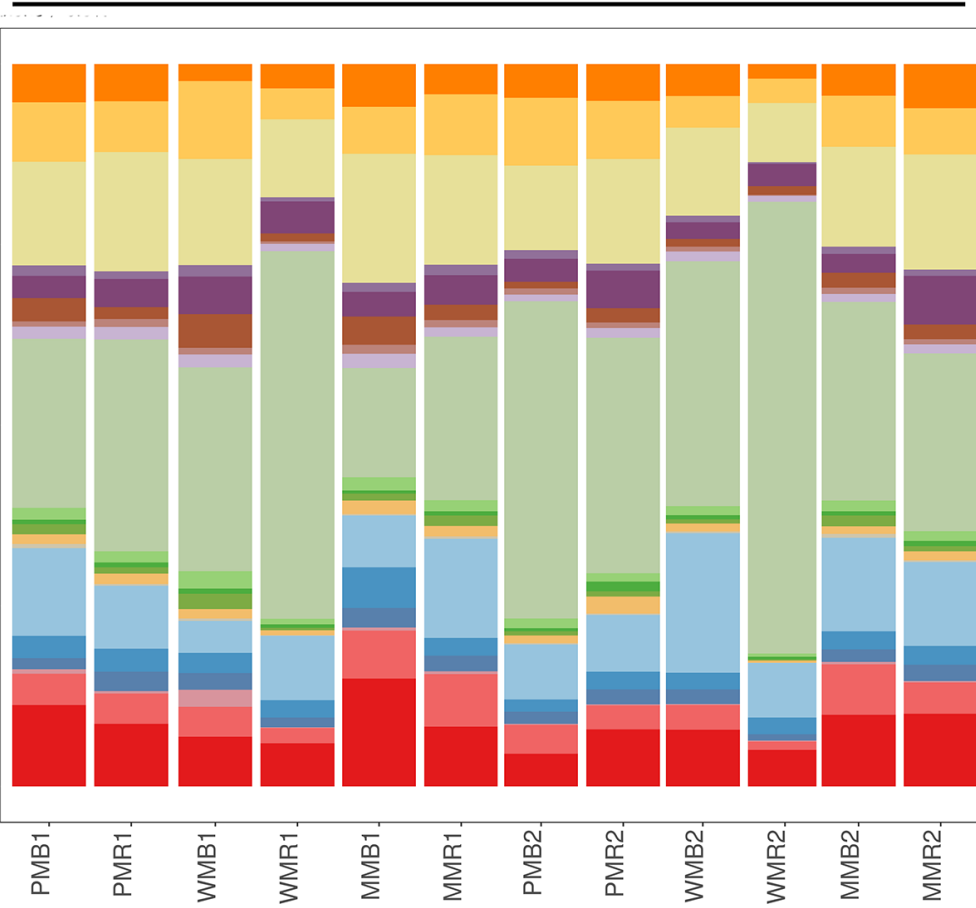




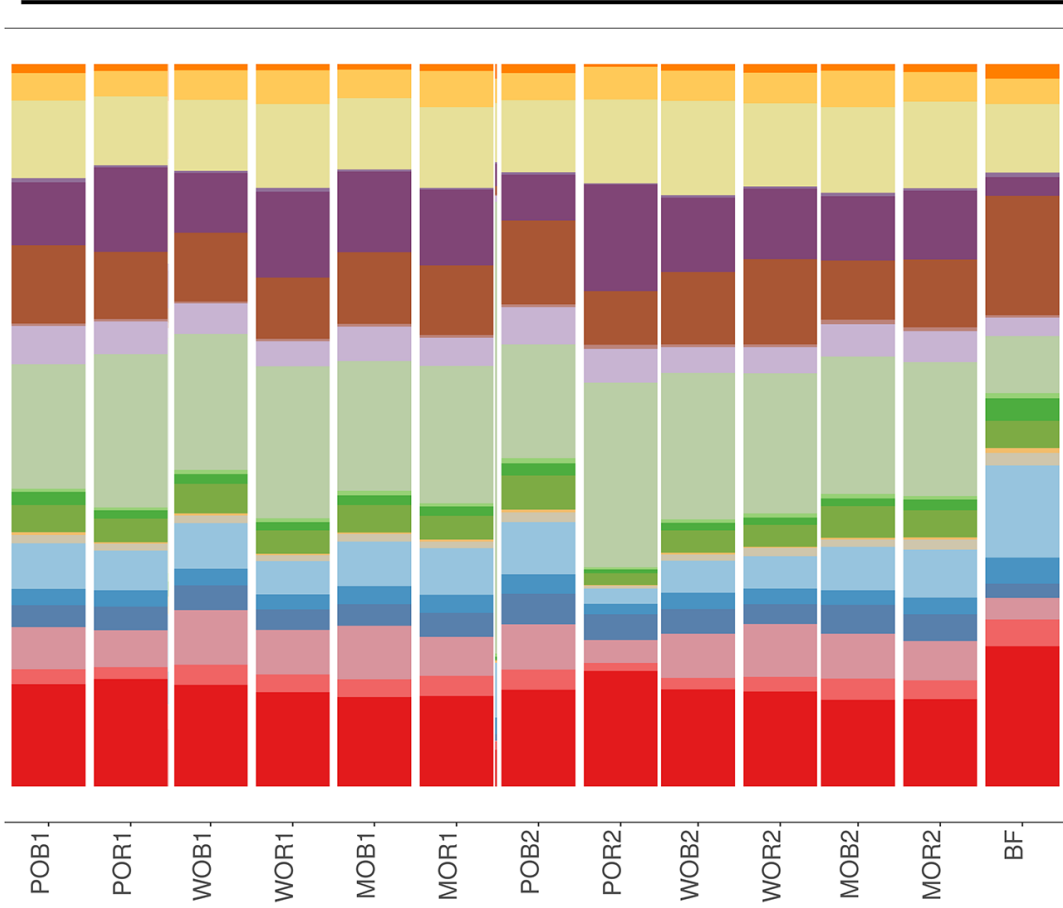
# Impact of fertilizer on soil microbial community

Data representation: barplots

### Mineral fertilizer



### Organic fertilizer



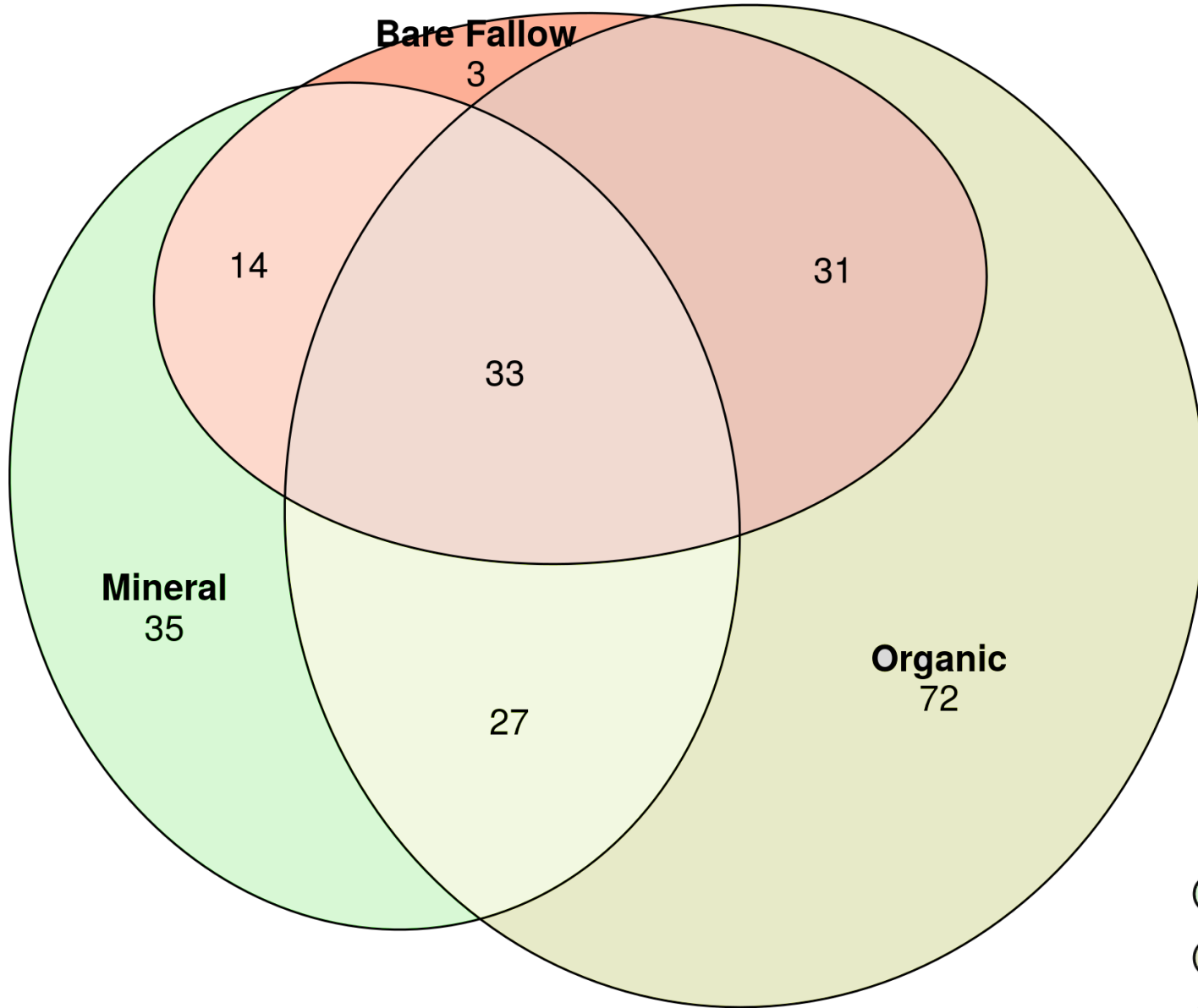
- Taxon
- Acidobacteria
  - Actinobacteria
  - Alphaproteobacteria
  - Bacilli
  - Bacteroidia
  - Blastocatellia (Subgroup 4)
  - Chloroflexia
  - Deltaproteobacteria
  - Gammaproteobacteria
  - Gemmatimonadetes
  - Holophagae
  - KD4-96
  - Ktedonobacteria
  - NC10
  - Nitrososphaeria
  - Phycisphaerae
  - Planctomycetacia
  - Subgroup 6
  - Thermoleophilia
  - Verrucomicrobiae

# Impact of fertilizer on soil microbial community

Taxon	LogFC	Log.Abandance Mineral	Log.Abandance Organic	Min [Rhiz] mean	Org [Rhiz] mean	t-test p-value	Mann-Wh. p-value	Spearman r
KD4-96	2.01			277.0	1144.9	3.131E-12	1.815E-09	0.86
Clostridia	3.52			13.4	258.2	1.253E-09	7.318E-07	0.87
Acidobacteriia	-2.38			1828.5	344.3	2.033E-07	9.075E-10	-0.86
Deltaproteobacteria	1.54			523.0	1545.1	1.759E-10	9.075E-10	0.86
NC10	2.02			79.0	351.1	8.388E-07	4.084E-08	0.82
Bacteroidia	1.12			1992.0	4331.5	2.677E-08	1.77E-07	0.80
Nitrospira	2.07			28.1	150.5	1.978E-08	7.361E-07	0.86
Ktedonobacteria	-2.61			513.2	75.5	0.0009	1.887E-06	-0.75
Nitrososphaeria	-1.13			4107.7	1870.3	4.534E-06	3.63E-09	-0.85
OM190	2.80			4.4	90.6	1.044E-09	3.598E-06	0.81
Gemmatimonadetes	-1.39			457.1	168.4	5.558E-06	1.446E-06	-0.76
Thermoanaerobaculia	2.08			20.4	118.3	2.477E-07	7.318E-07	0.87
Verrucomicrobiae	0.67			3240.7	5171.7	2.324E-08	1.089E-08	0.84
Acidimicrobiia	1.02			120.2	254.8	1.774E-07	1.887E-06	0.75
Gammaproteobacteria	-1.08			16791.6	7925.0	0.0003	4.61E-07	-0.78
AD3	-2.05			115.3	20.3	7.129E-06	1.752E-06	-0.84
Bacilli	-1.49			337.2	113.8	0.0001	0.0004	-0.59
Planctomycetacia	0.67			768.3	1230.4	4.006E-06	3.152E-06	0.74
Chthonomonadetes	-0.87			228.1	120.7	1.887E-05	1.97E-05	-0.69
AT-s3-28	2.63			0.0	51.8	2.329E-09	1.692E-07	0.91
MB-A2-108	1.47			38.5	124.4	0.0003	7.16E-05	0.69
Fibrobacteria	2.65			3.2	72.7	0.0003	1.167E-06	0.85



# Euler-Venn diagrams



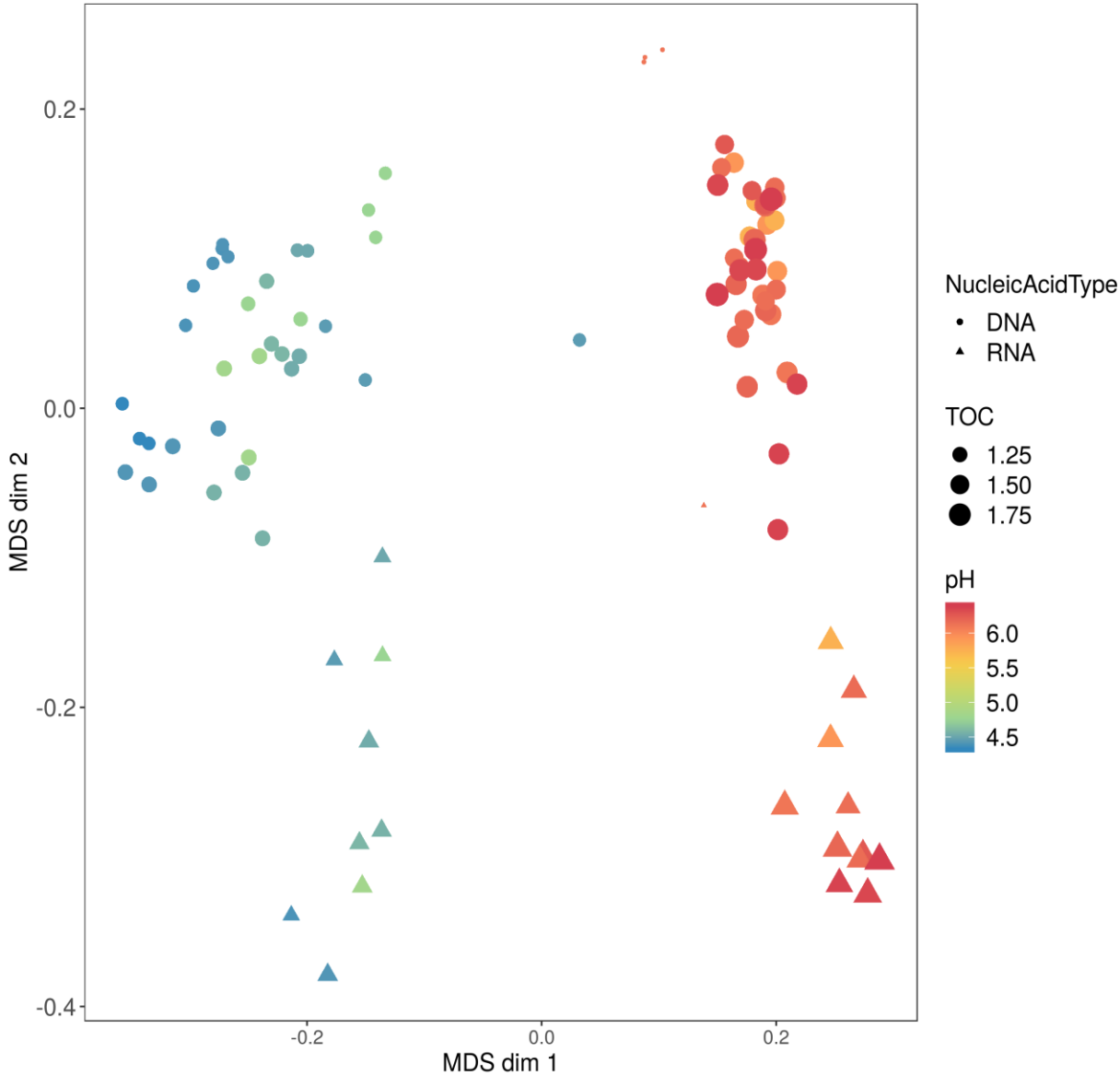
- Mineral
- Organic
- Bare Fallow

- Mineral and organic fertilizers contribute to microbial diversity, but in different ways
- Organic fertilizers increase the diversity more effectively than mineral ones.
- The optimal way is to combine both organic and mineral fertilizers

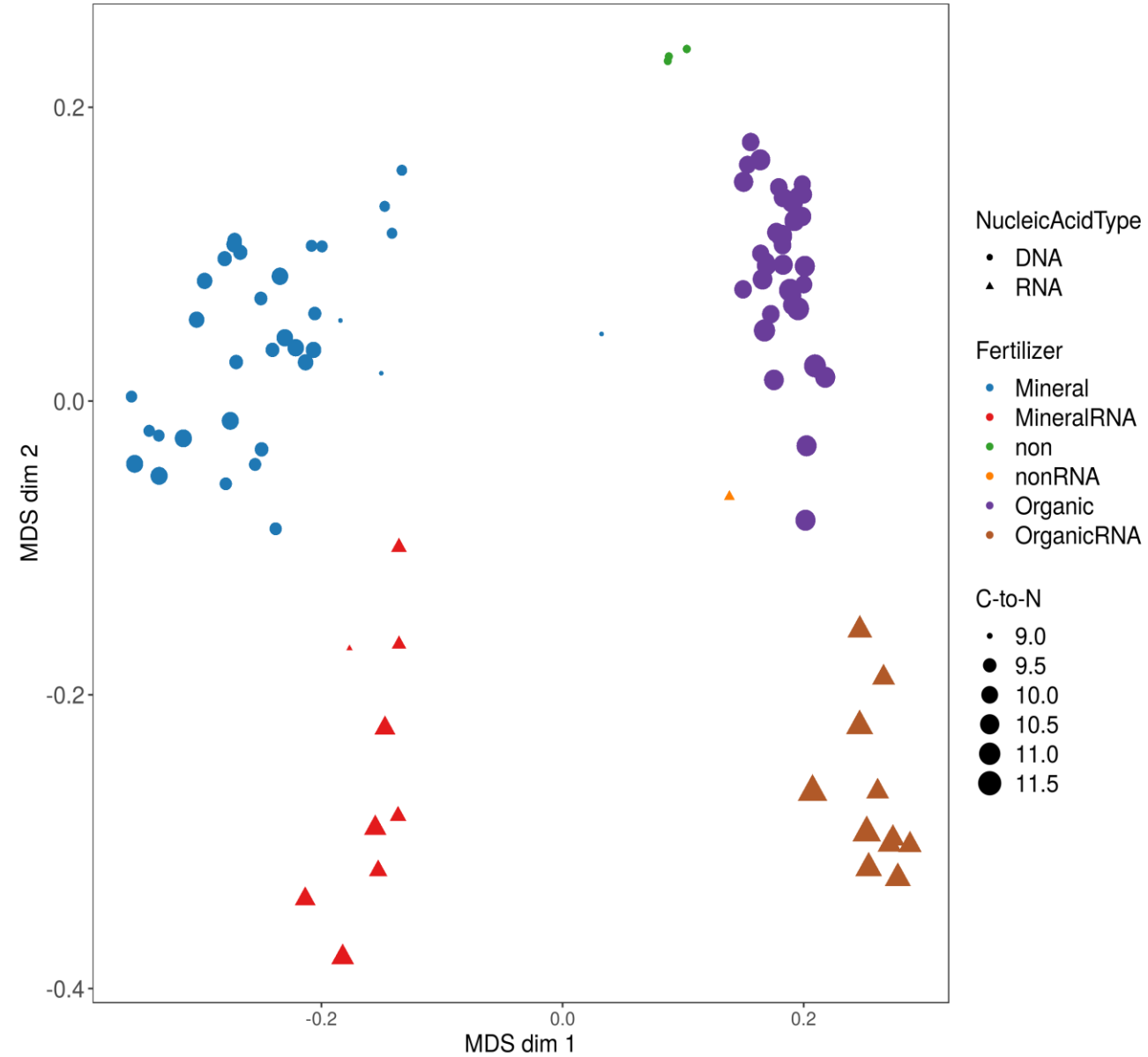


# MDS analysis

MDS plot, family



MDS plot, family





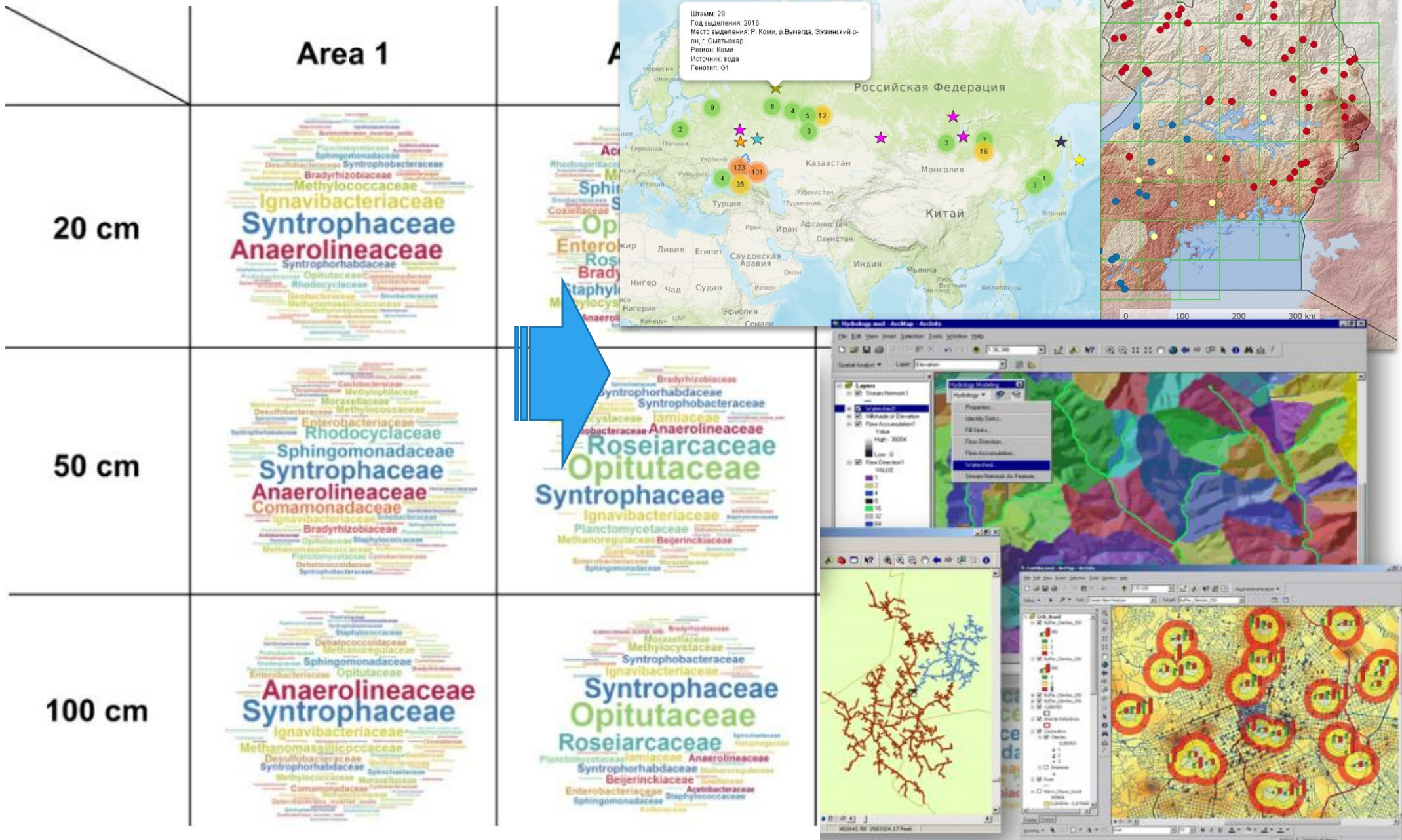
# **ECOLOGY AND WATER RESEARCH**

## **APPLICATIONS OF 16S rRNA SEQUENCING**

- **Microbial diversity** analysis of soils and natural water reservoirs, incl. GIS-supported
- Monitoring the response of the environment to external **harmful factors**: discharge from plants, wastewater discharge, intensive application of fertilizers
- Evaluating stability of an ecosystem
- Evaluating capability of an ecosystem to degrade pollutants



# Using GIS in Molecular Biology







# **WASTEWATER TREATMENT**

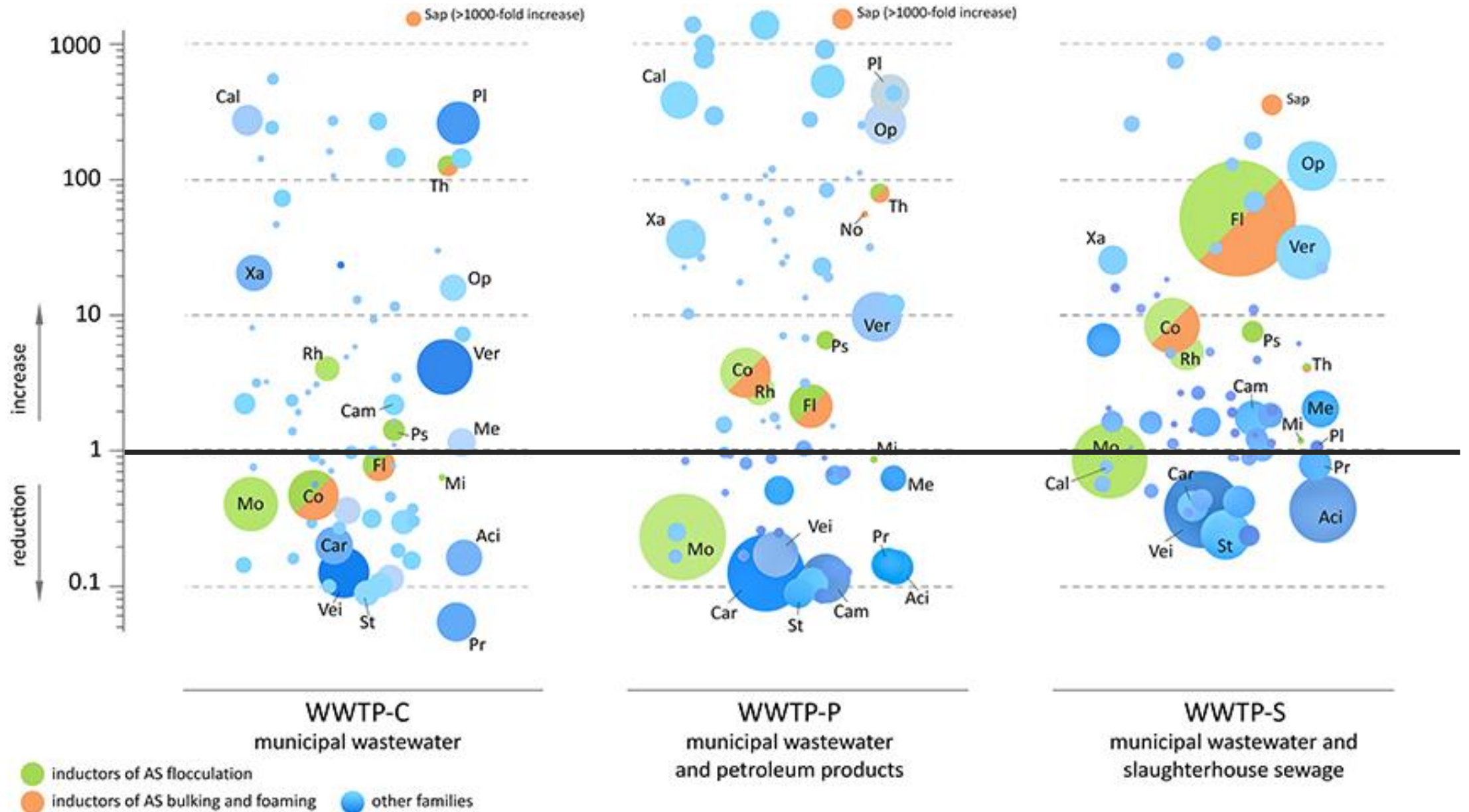
## **APPLICATIONS OF 16S rRNA SEQUENCING**

- Monitoring of the **'health status'** of activated sludge or constructed wetlands (CW)
- Analysis of the **impact of input wastewater** (chemical composition; presence of xenobiotics) on the **'health status'** of activated sludge or CW
- Evaluating the efficacy of elimination of pathogenic bacteria
- Monitoring of the seasonal dependence of microbial structure of activated sludge or CW



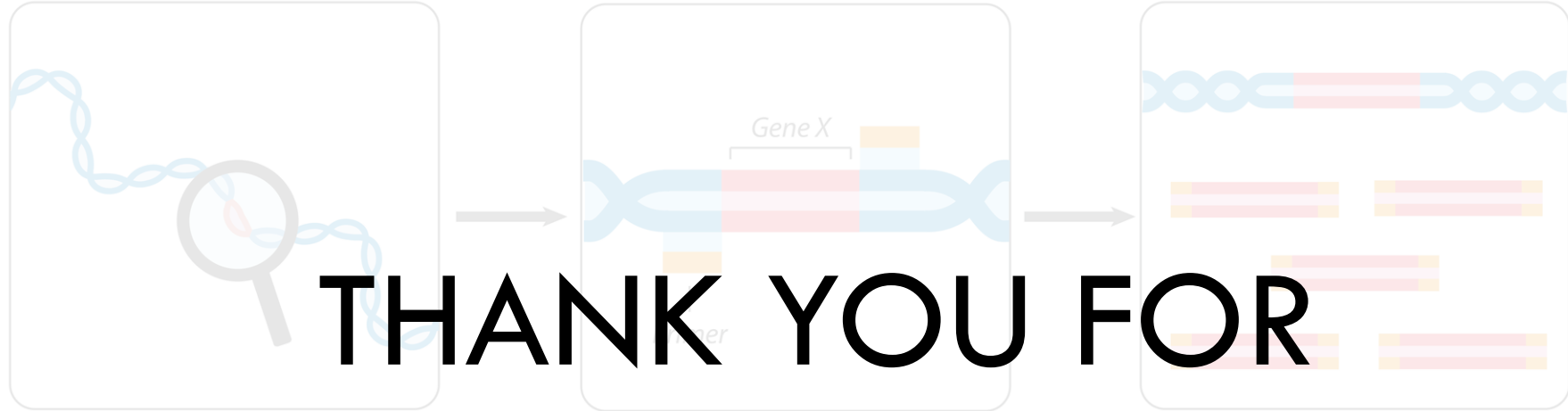


# Comparison of microbial community structure of activated sludge and incoming sewage





# Amplicon Sequencing



Genomic or Template DNA

Gene-specific Primers

PCR Amplification

**THANK YOU FOR  
ATTENTION**



Alignment



Sequencing