

Biostimulators Meditated Dynamic Changes in Microbial Community During Soil Remediation

Presenter: Dr. Peter (Yunliang) Li[☆]

Supervisor: Dr. Steven D Siciliano

Department of Soil Science, University of Saskatchewan

Oct. 18. 2024

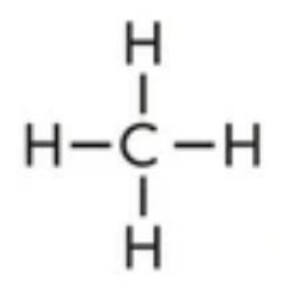
[☆]Current Institution:
Department of Biological Sciences, University of Calgary

Petroleum Hydrocarbon (PHC)

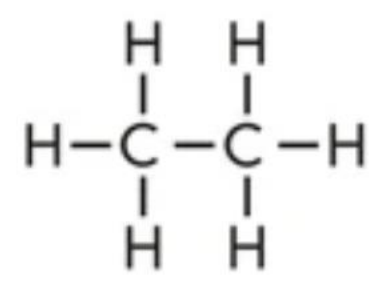
Organic compounds primarily made up of carbon and hydrogen atoms

- Alkanes (Paraffins), saturated hydrocarbons

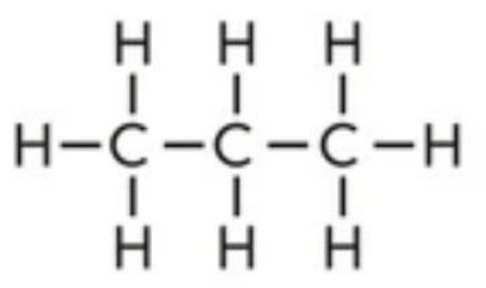
methane



ethane

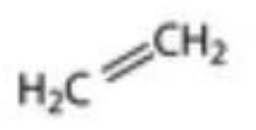


propane



- Alkenes, Alkynes, unsaturated hydrocarbons

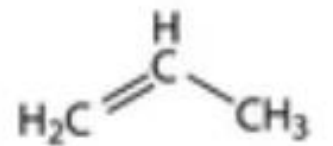
Ethene (ethylene)



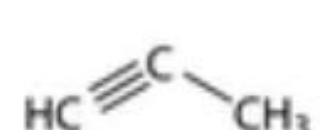
Ethyne (acetylene)



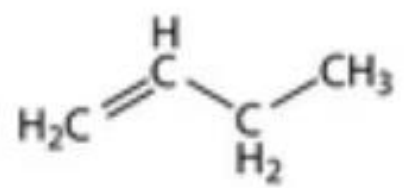
Propene (propylene)



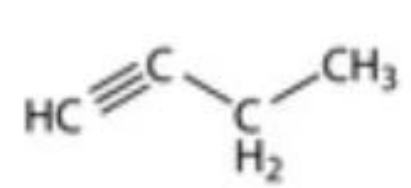
Propyne (allylene)



Butene-1 (butylene-1)



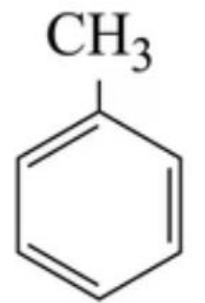
Butyne-1



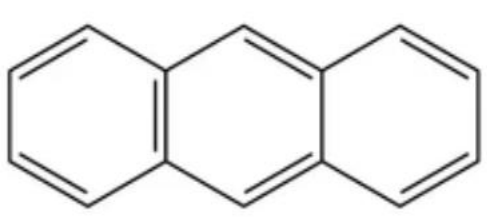
- Aromatic Hydrocarbons, one or more benzene rings



Benzene

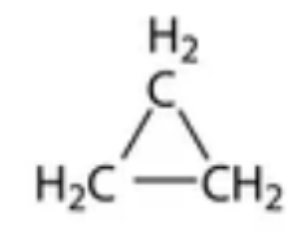


Toluene

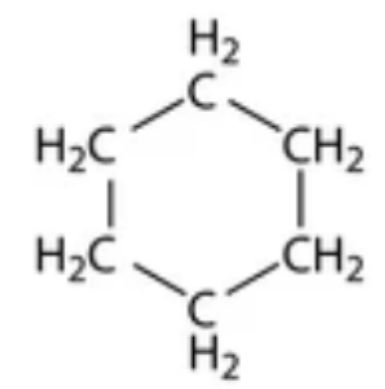


Anthracene

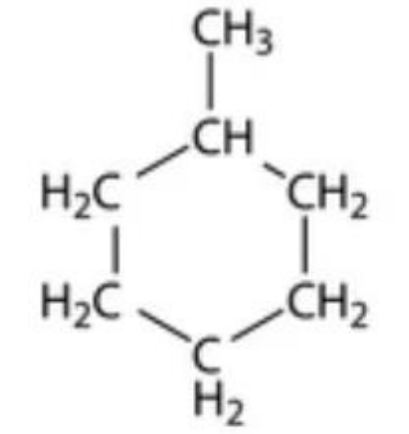
- Cycloalkanes, saturated hydrocarbons with ring structures



Cyclopropane

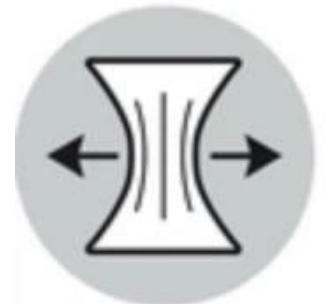
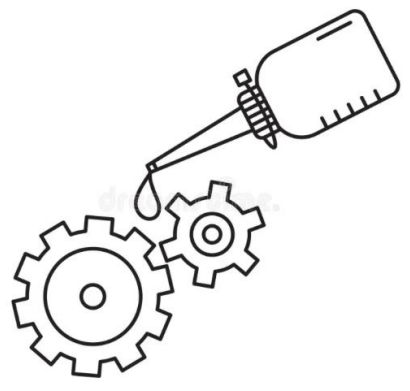


Cyclohexane



Methylcyclohexane

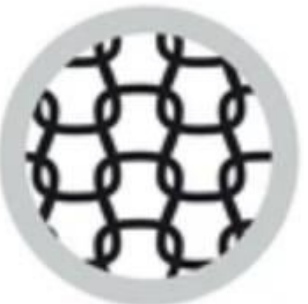
Widely applied across various industries



ELASTANE



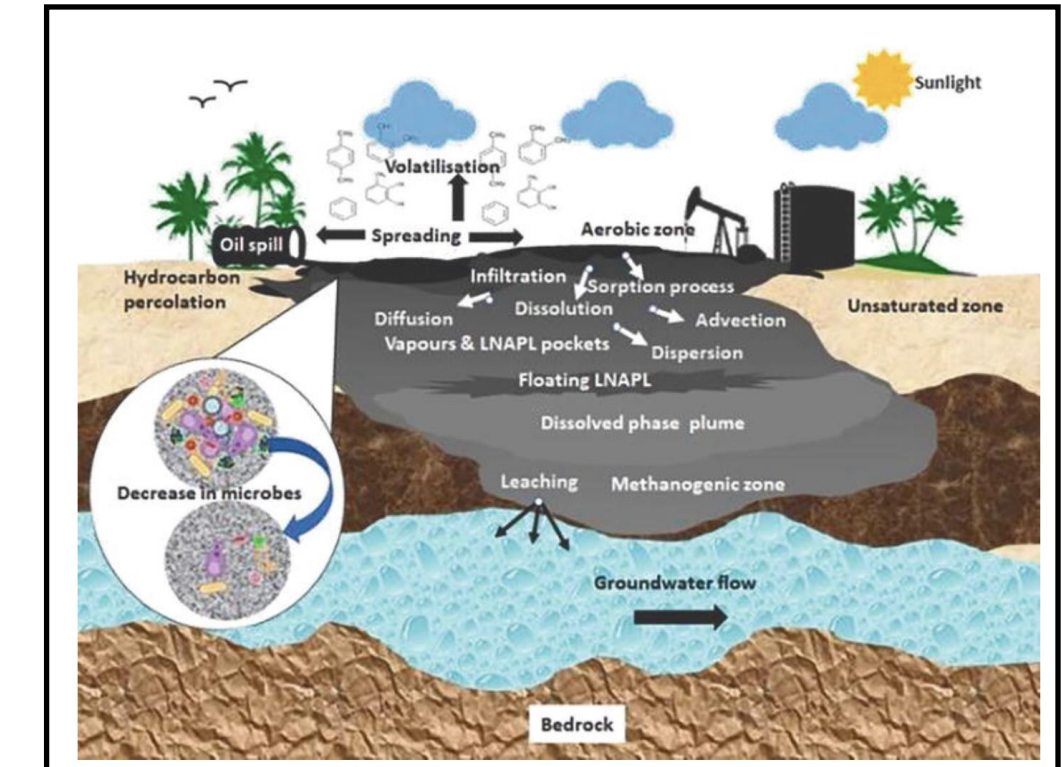
ARAMID



NYLON

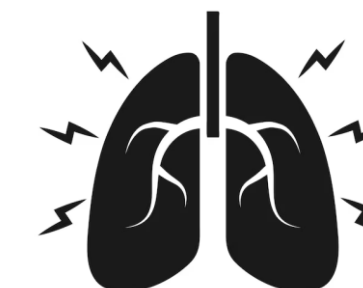
1 Environmental risks

- **Soil and Water Contamination:** Hydrocarbons can seep into soil and groundwater, leading to long-term contamination. This can render soil infertile and make water sources unsafe for consumption or use.
- **Impact on Wildlife:** Contaminated water bodies and soils can harm aquatic life, birds, and terrestrial animals.
- **Ecosystem Disruption:** Affect entire ecosystem, for example: Disrupting food chains and leading to loss of biodiversity.



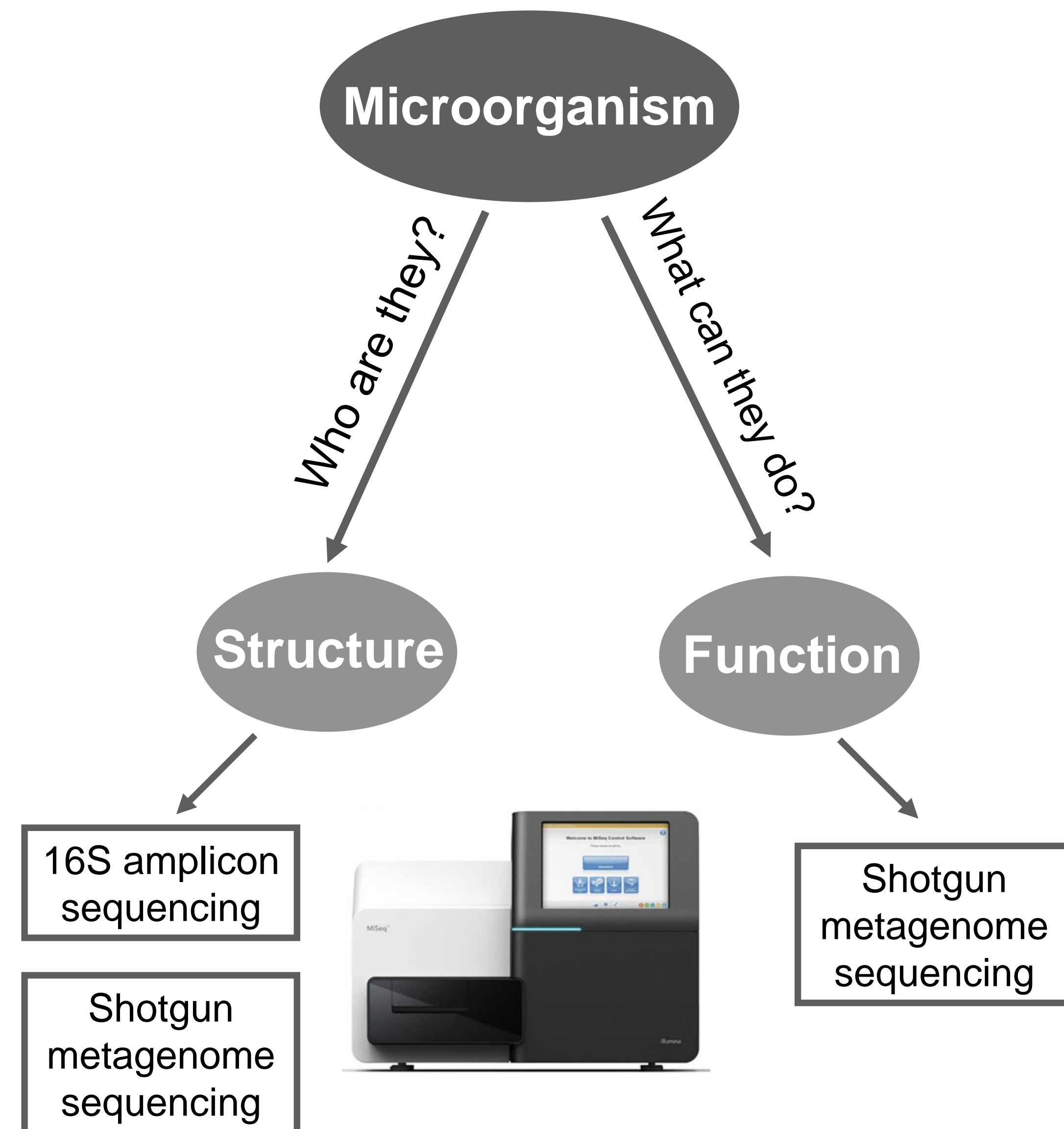
2 Health risks

- **Toxicity:** cause a range of health problems if ingested, inhaled, or absorbed through the skin.
- **Respiratory Issues:** Inhalation of hydrocarbon vapors can irritate the respiratory system
- **Carcinogenicity:** hydrocarbons like benzene, are known carcinogens, increasing the risk of cancer with prolonged exposure.

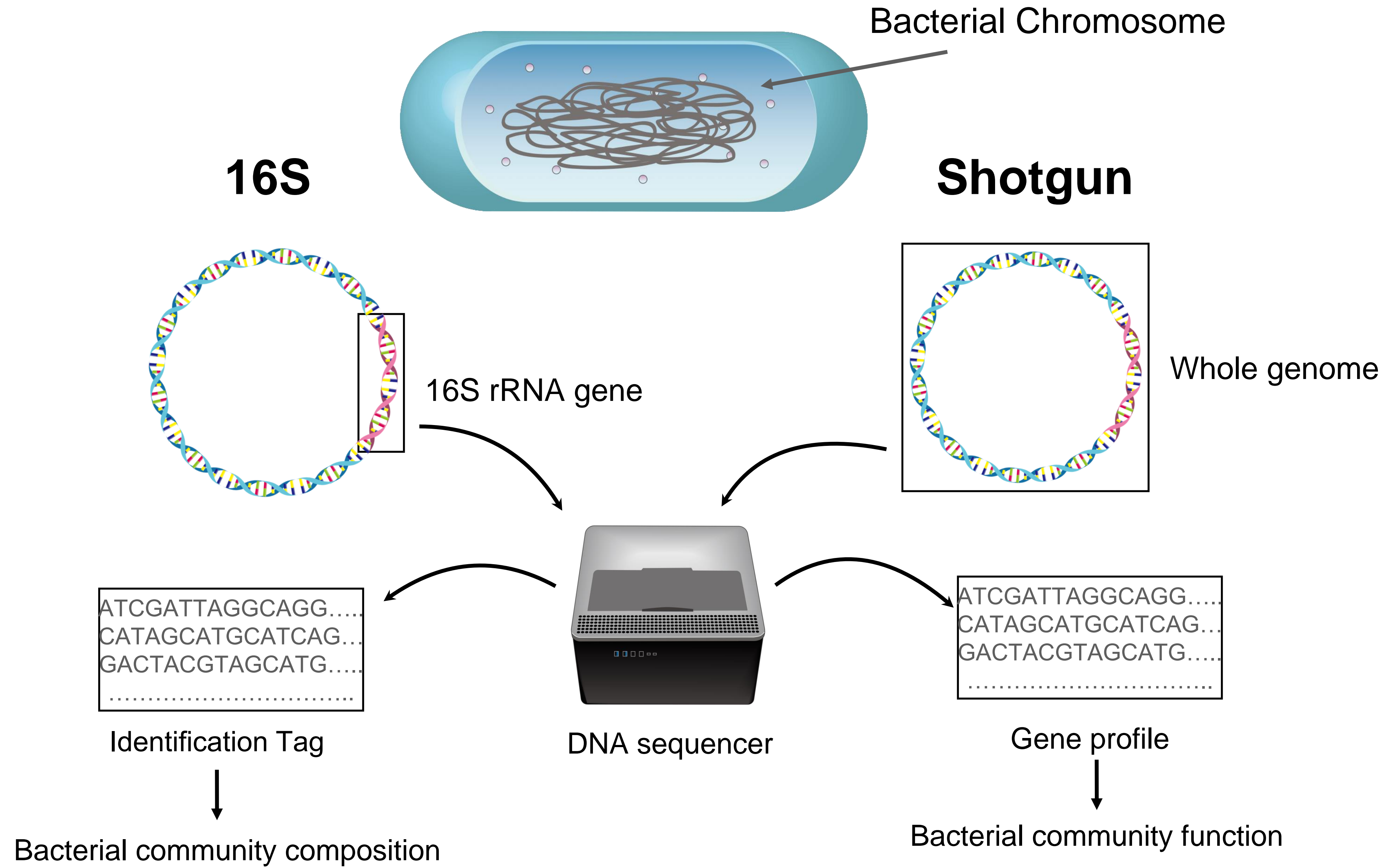


Methods for PHC Bioremediation in Soil

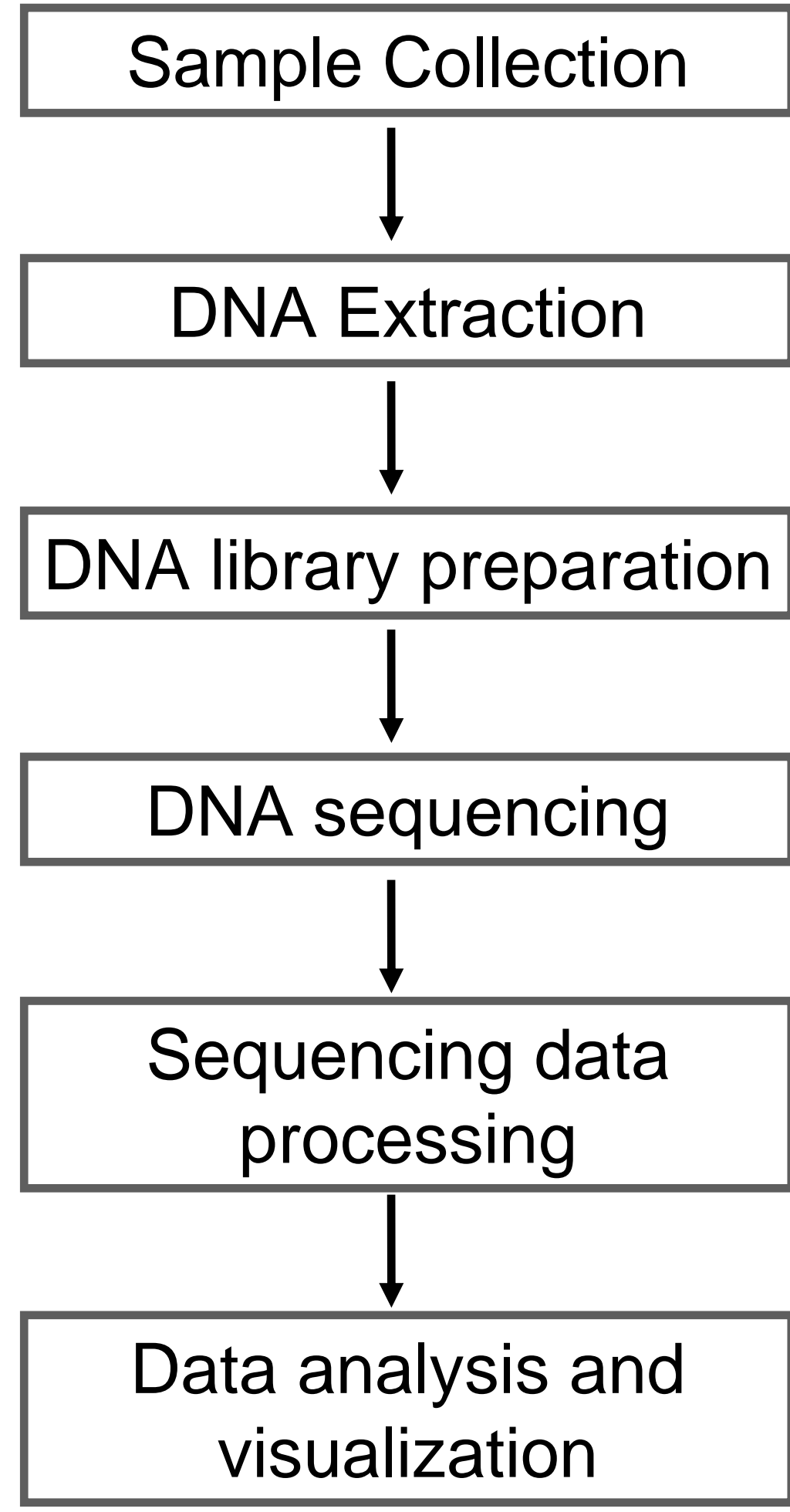
- 1 **Biostimulation** modification of the contaminated environment to enhance the ability of existing bacteria to degrade PAHs, including water, nutrients, and electron donors in anaerobic processes
- 2 **Composting** is a biological process where petroleum-contaminated soil are mixed with organic waste to enhance the breakdown of hydrocarbons by microorganisms. The composting process provides an optimized environment for microbial activity.
- 3 **Bioaugmentation** the introduction of specific strains or consortia of microorganisms that are more efficient at breaking down the PHC
- 4 **Soil-Slurry Bioreactors** contaminated soil is mixed with water and sometimes additional nutrients or microorganisms to create a slurry, which is then treated under controlled environmental conditions to enhance microbial degradation of pollutants.



Introduction of 16S Amplicon and Shotgun Metagenome Sequencing



Work flow

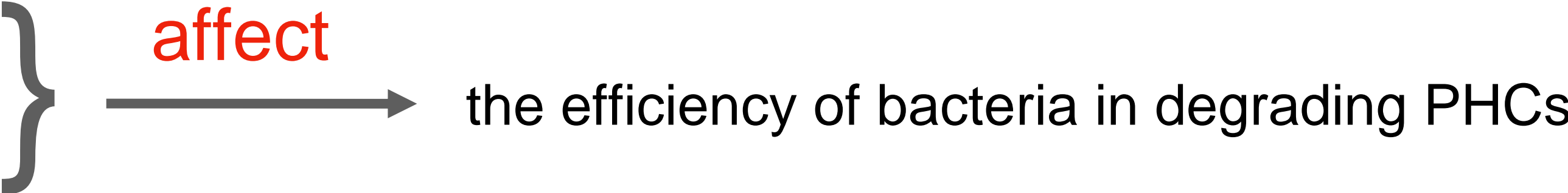


Effects of biostimulator applied in PHC-contaminated soil

Background

Nutrients: like N and P

Electron acceptors: like NO_3^- , SO_4^{2-} , Fe^{3+}



Experiment Design

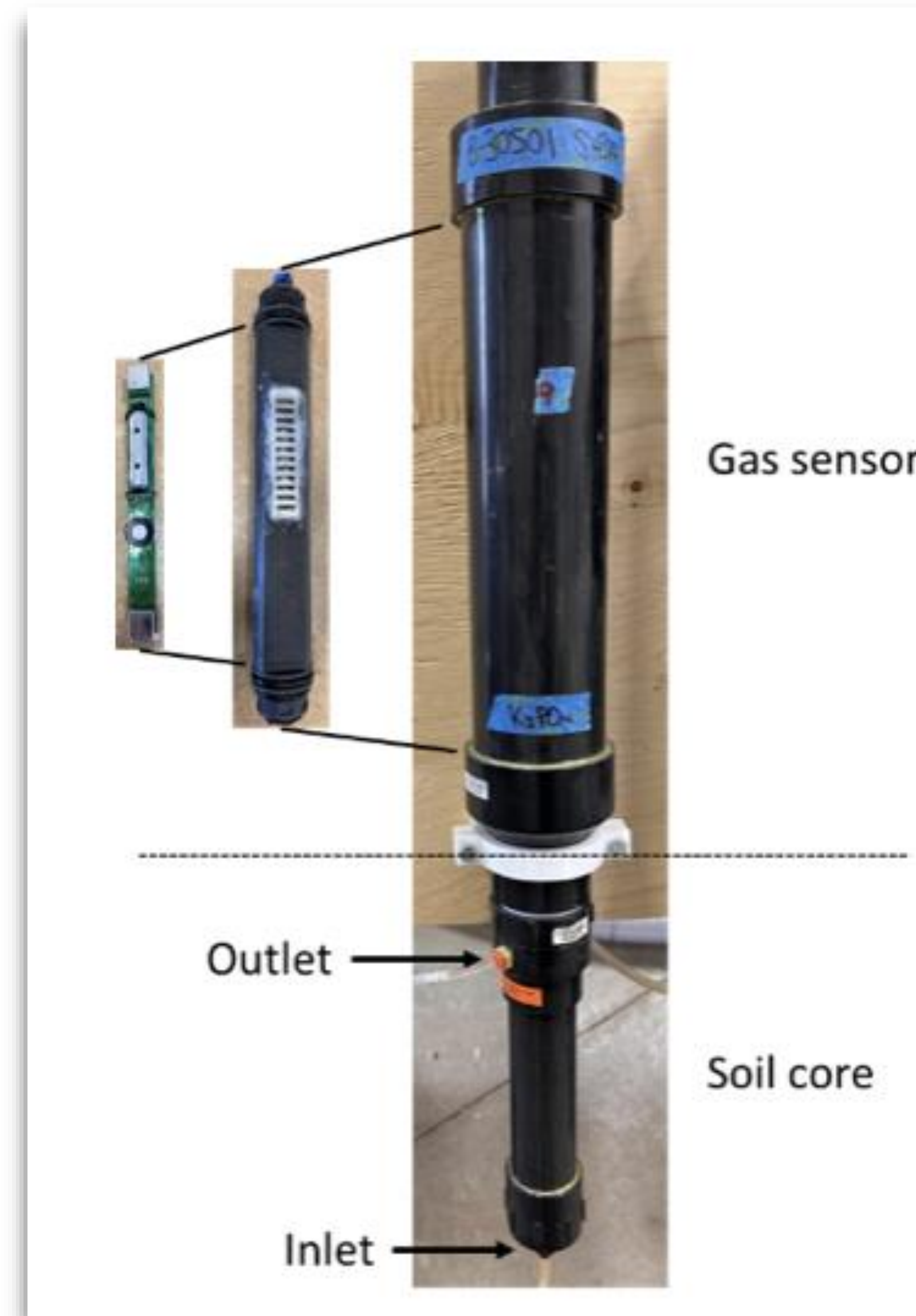
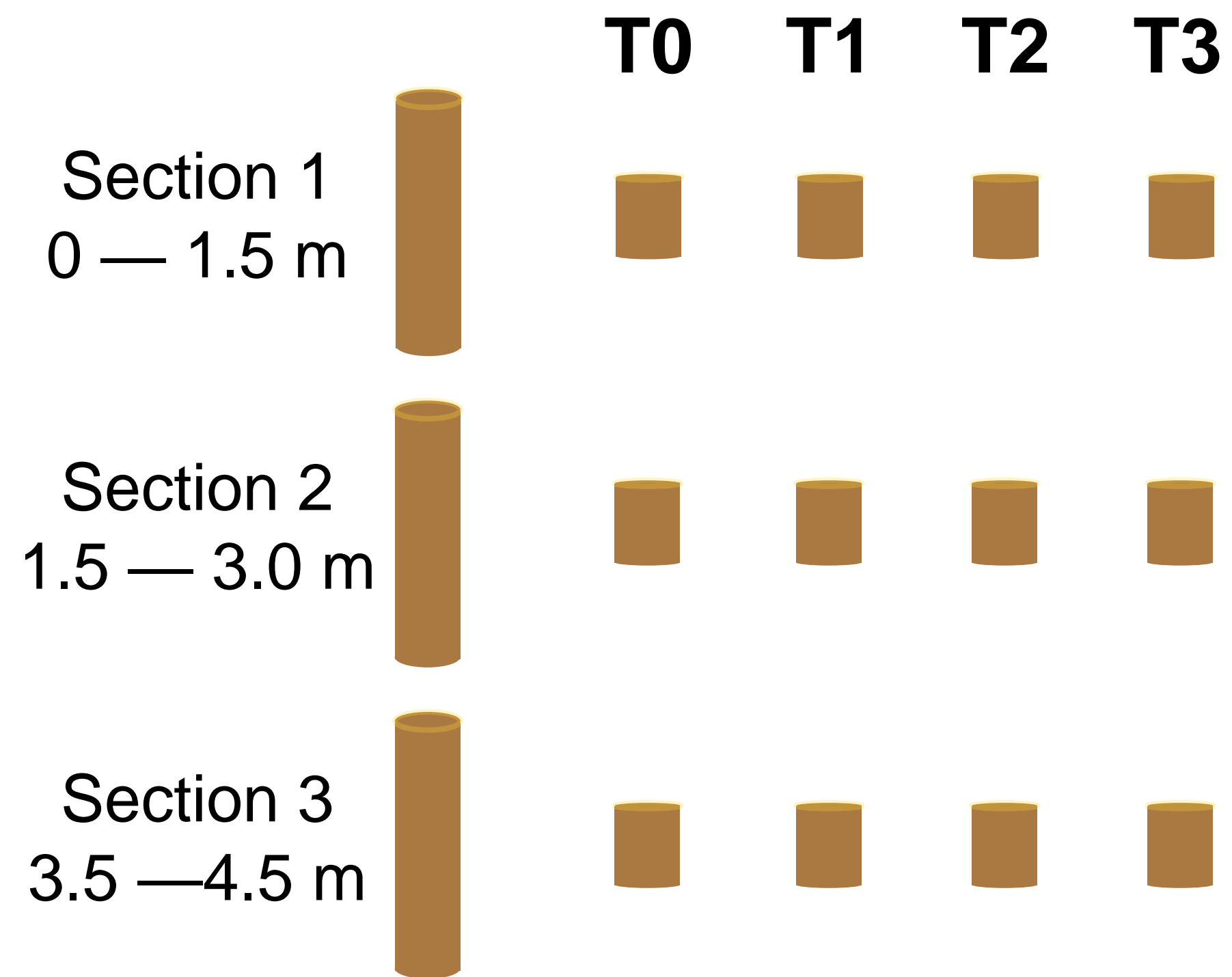
Treatment	HNO_3	TPP ^a	Fe (III)NH ₄ -citrate	MgSO ₄	K ₂ SO ₄
Base (T0)	0.00024	0.0001	0.00024	0.0013	—
High citrate (T1)	0.00024	0.0001	0.00991	0.0013	—
High K, low Mg (T2)	0.00024	0.0001	0.00024	—	0.0013
No citrate (T3)	0.00024	0.0001	—	0.0013	—

^aTPP= tripolyphosphate.

Unit: mol L⁻¹

- 1 If the soil has high buffering capacity, citrate addition can chelate calcium or magnesium released due to high soil buffering capacity, reducing phosphate precipitation (high iron citrate).
- 2 Soils with low calcium require the addition of potassium sulfate (K_2SO_4) instead of magnesium sulfate to prevent the formation of newberyite ($MgHPO_4 \cdot 3H_2O$) that causes phosphate unavailability (high potassium).
- 3 In iron rich soils, citrate should be excluded (no iron citrate).

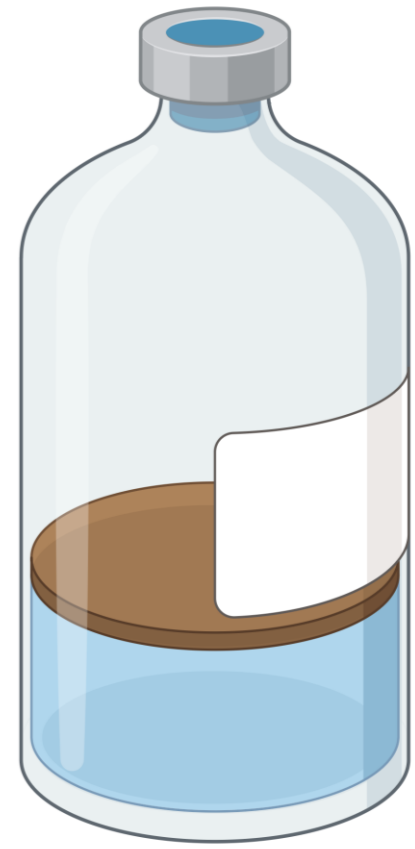
PHC-contaminated soil sample



Soil samples were treated with biostimulator solutions for 1 month

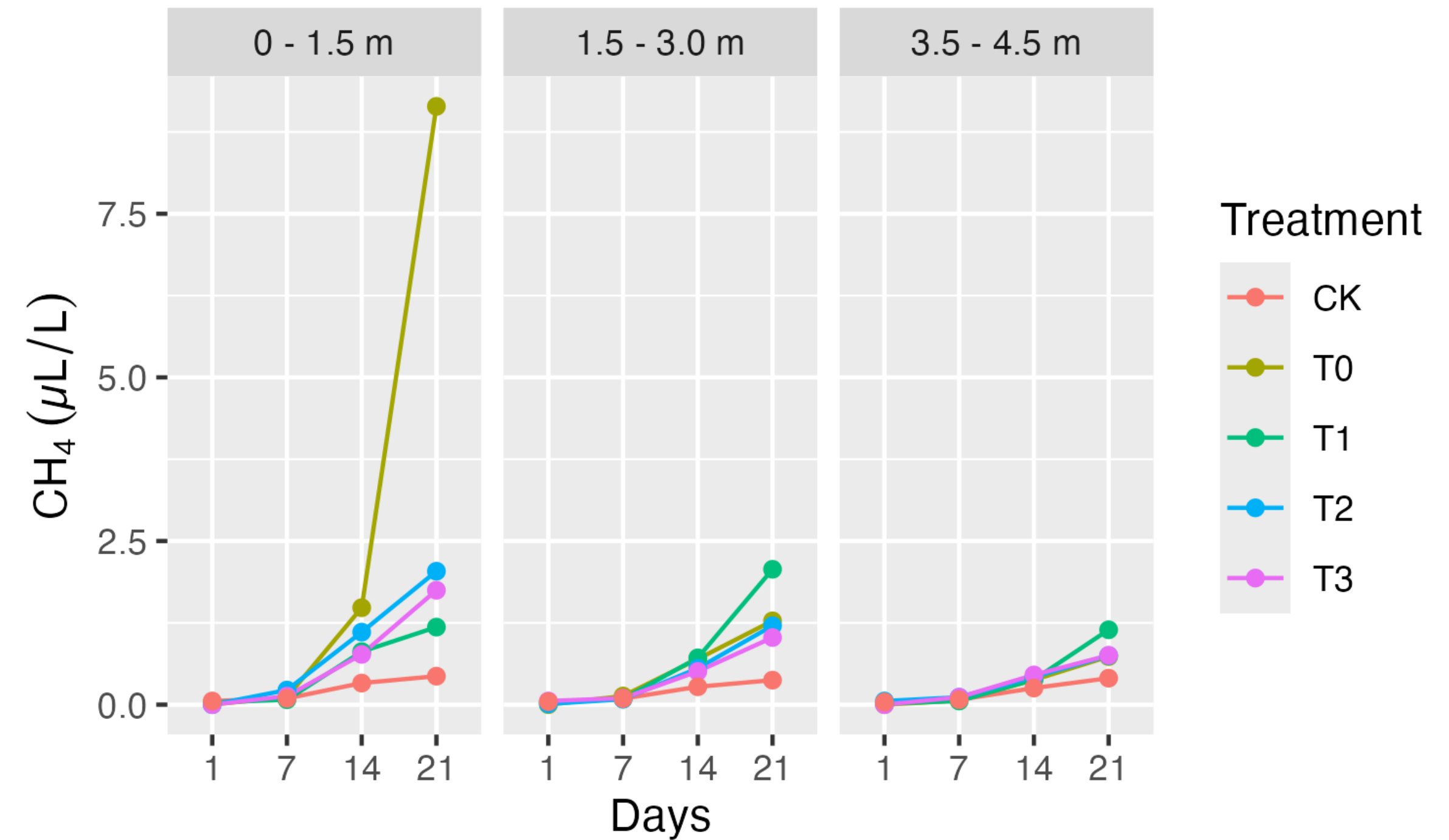
Result

Methanogen activity



Caption

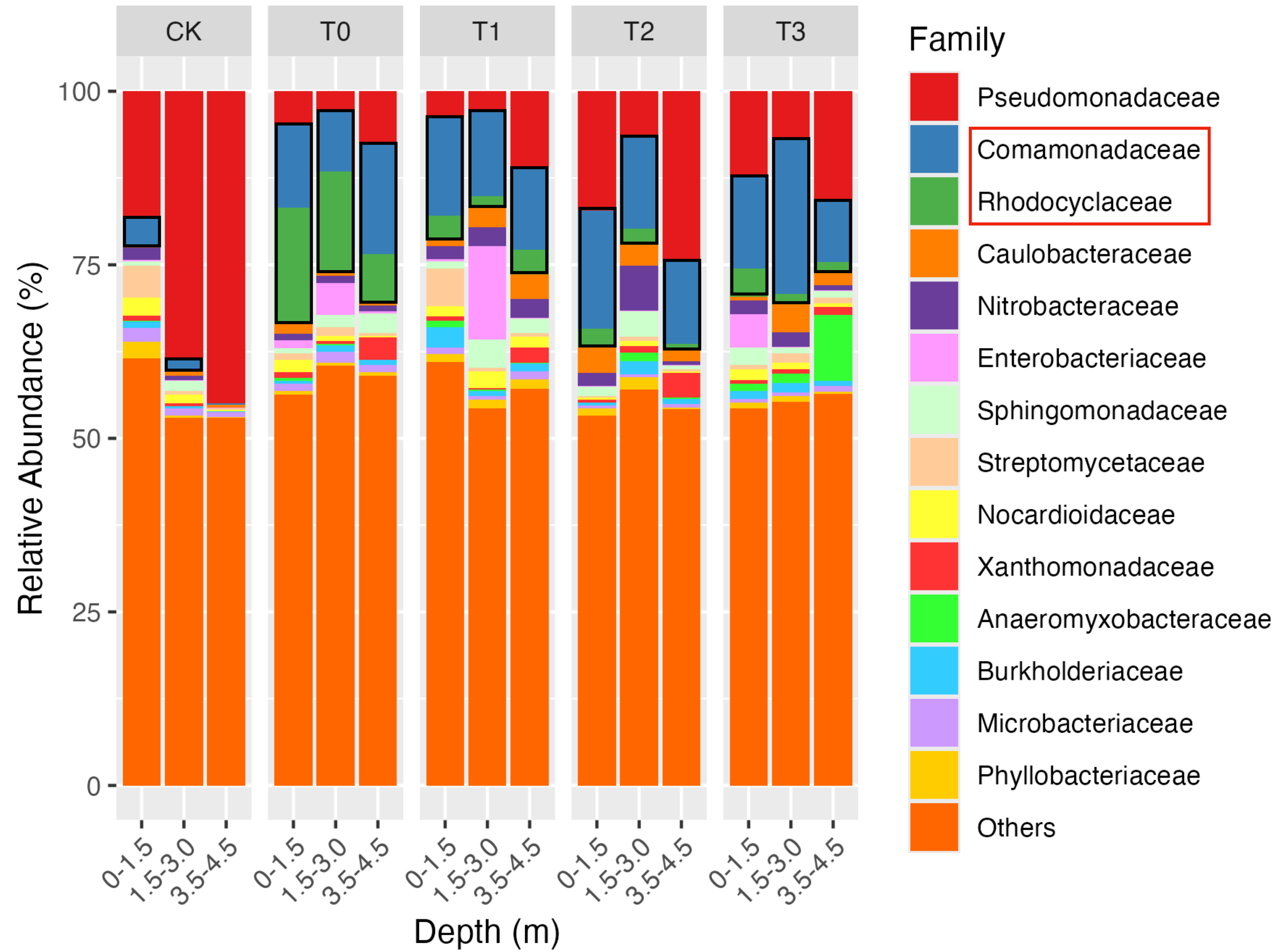
- Flush the headspace with a gas mixture of $N_2:CO_2:H_2$ (80:10:10) to maintain an anaerobic environment
- Collect gas samples using syringe for GC analysis



Caption

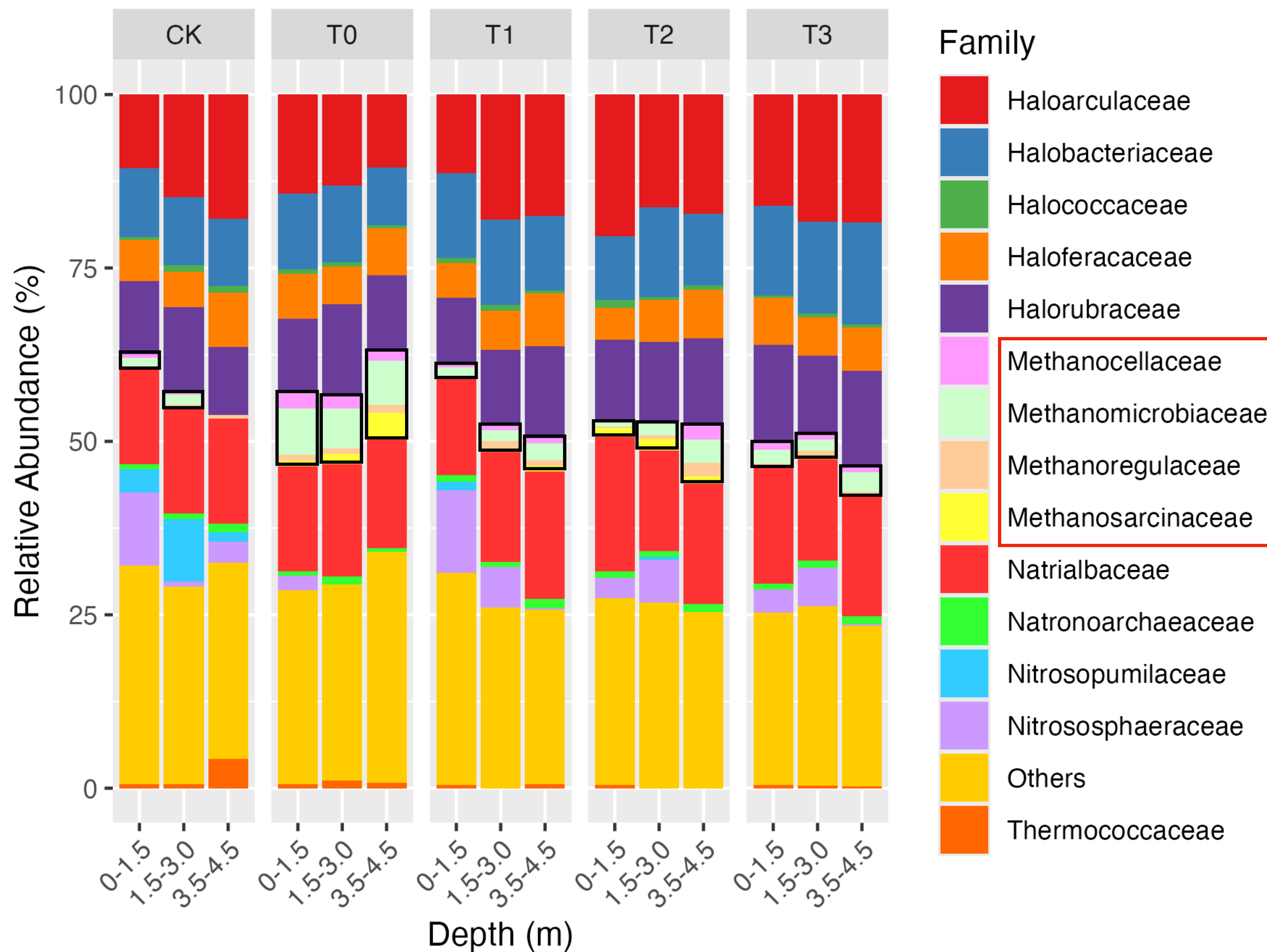
- All treatments increased the methanogenesis activity
- The methanogenesis activity after treatment was decreased following depth

Bacterial Composition



Many bacteria in Comamonadaceae and Rhodocyclaceae were reported to play important role in hydrocarbon degradation

Archaeal Composition



The relative abundance of methanogens increased under all treatments



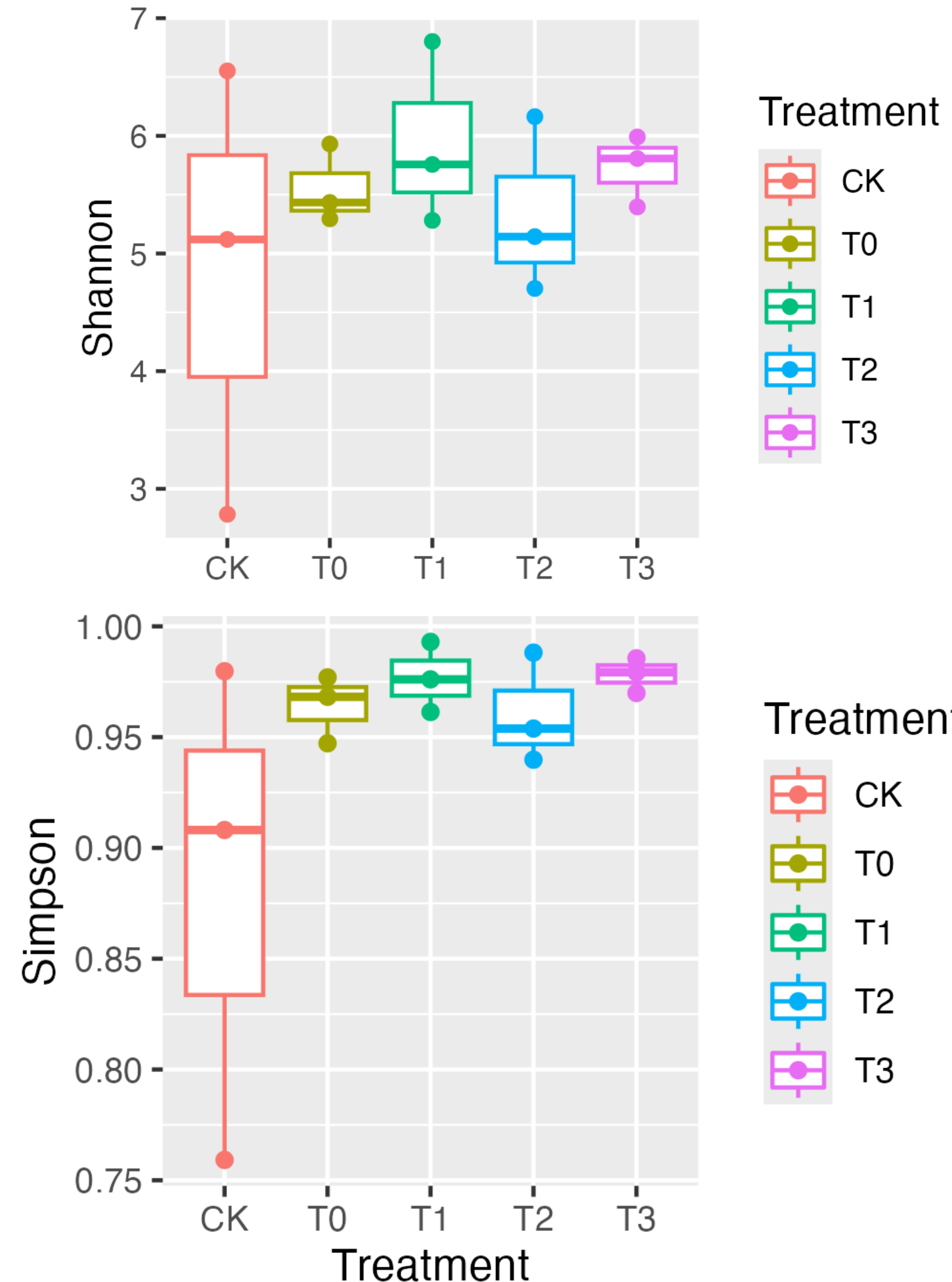
1 Shannon and Simpson Indices

Shannon and **Simpson** indices help quantify the **diversity** within a microbial community, and both indices take into account **species richness** (the number of different species present) and **evenness** (the relative abundance of each species), but they emphasize different aspects of diversity

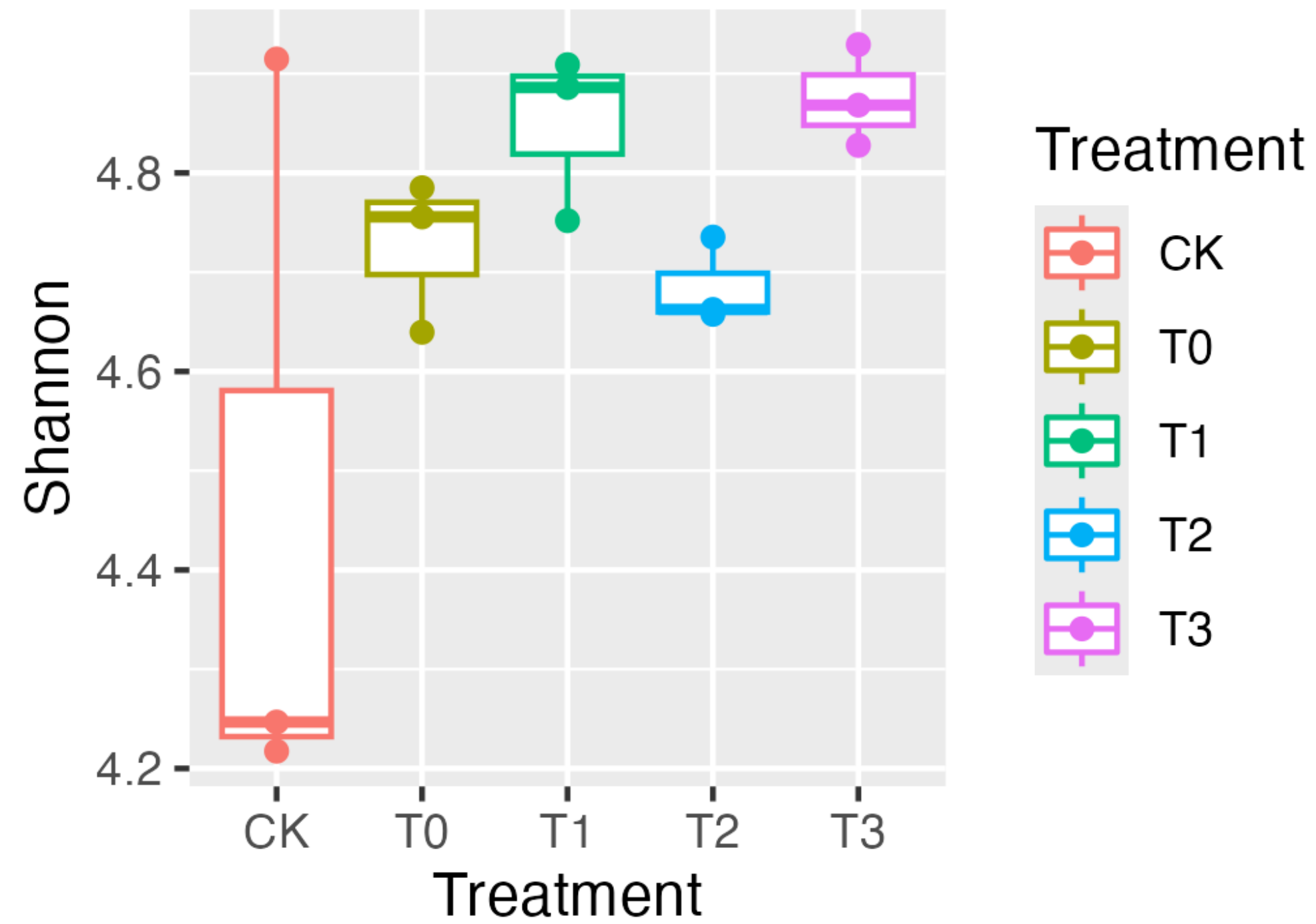
2 Differences

Shannon emphasizes **richness** and accounts more for the presence of **rare** species.

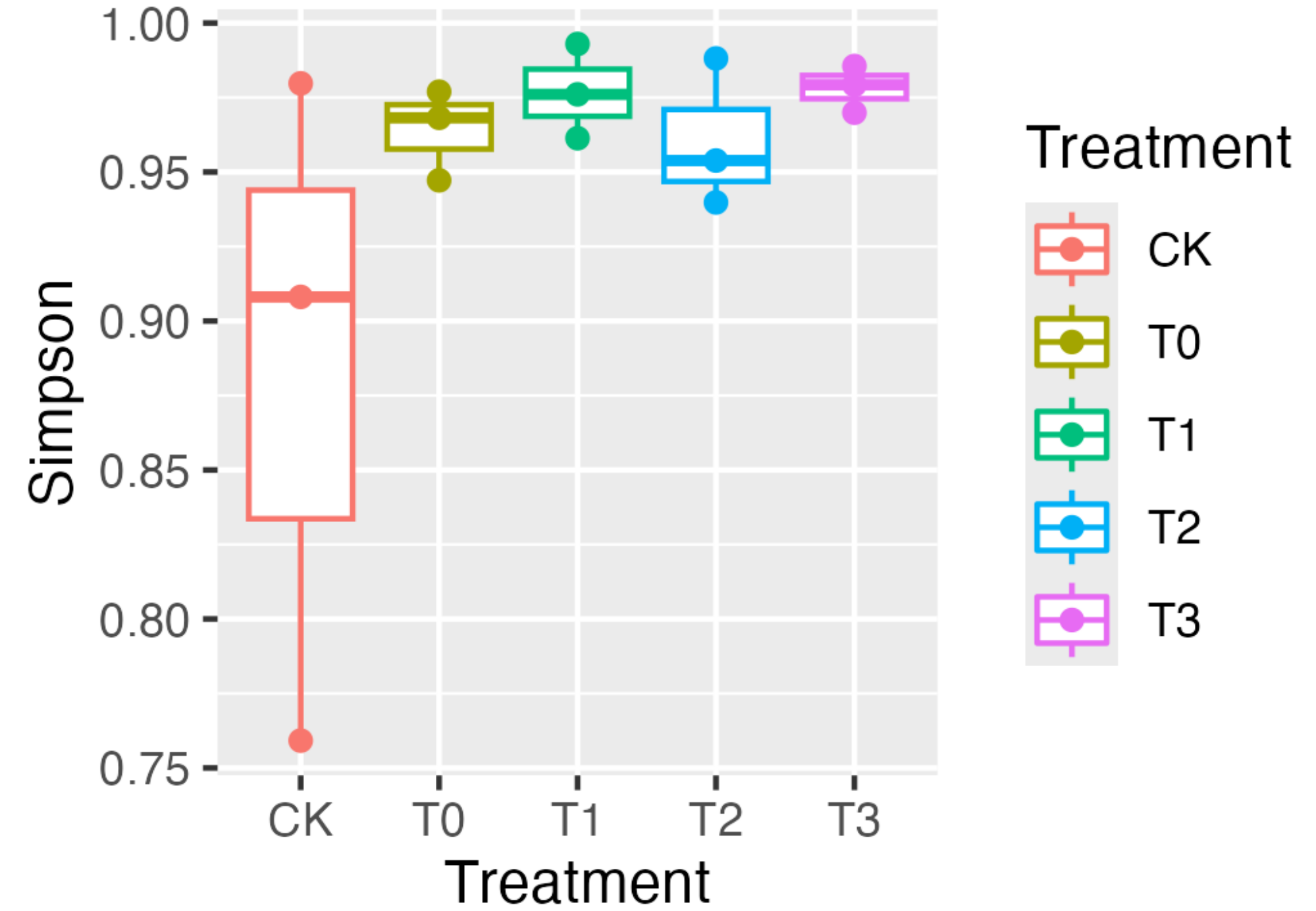
Simpson emphasizes **evenness** and is more influenced by **dominant** species.



alpha-Diversity (Archaea)



Caption



Caption

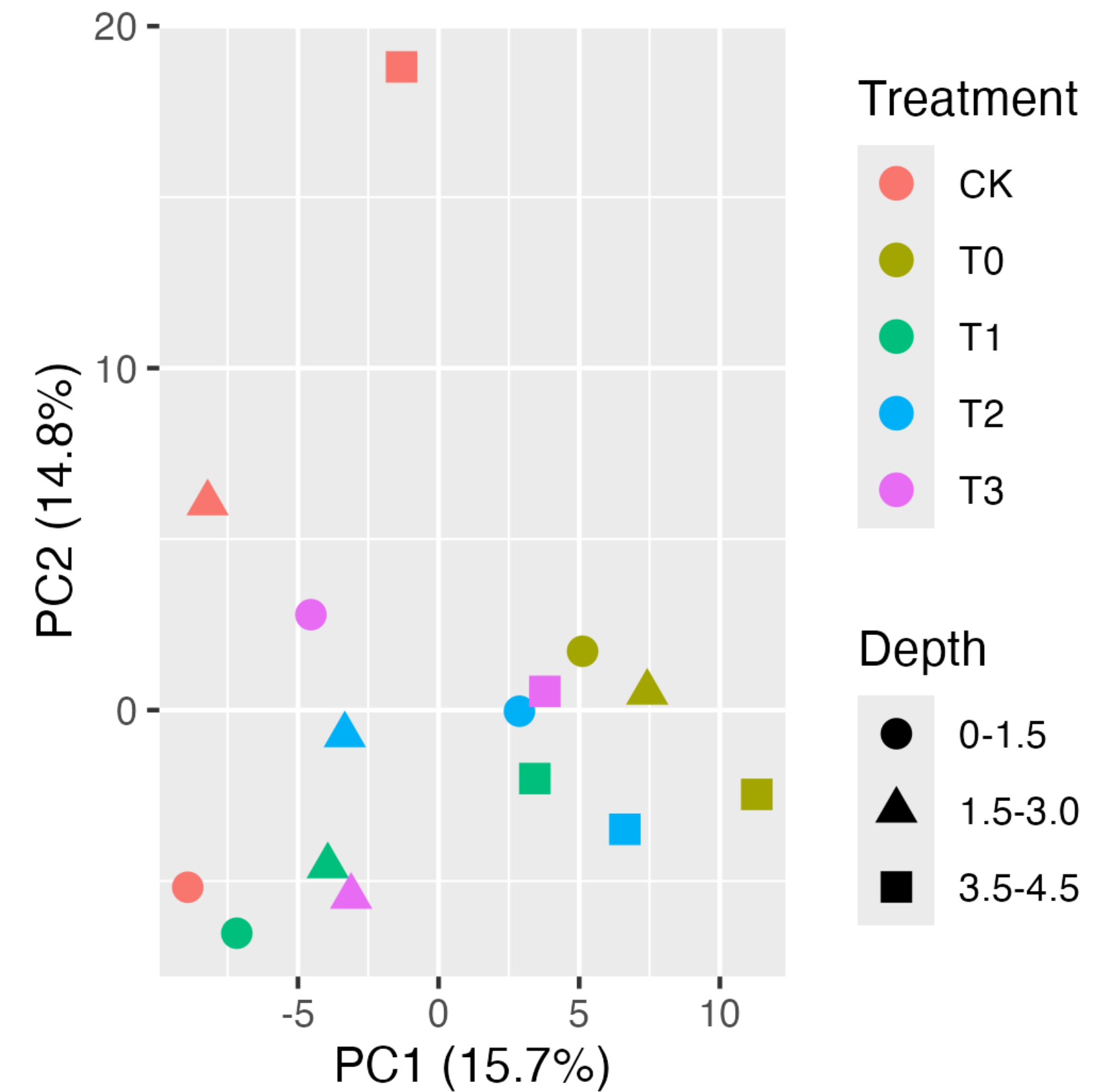
Both bacterial and archaeal alpha-diversity were generally increased by biostimulators

beta-Diversity (Bacteria)

Microbial beta-diversity

It refers to the variation in microbial communities across different habitats, environments, or conditions.

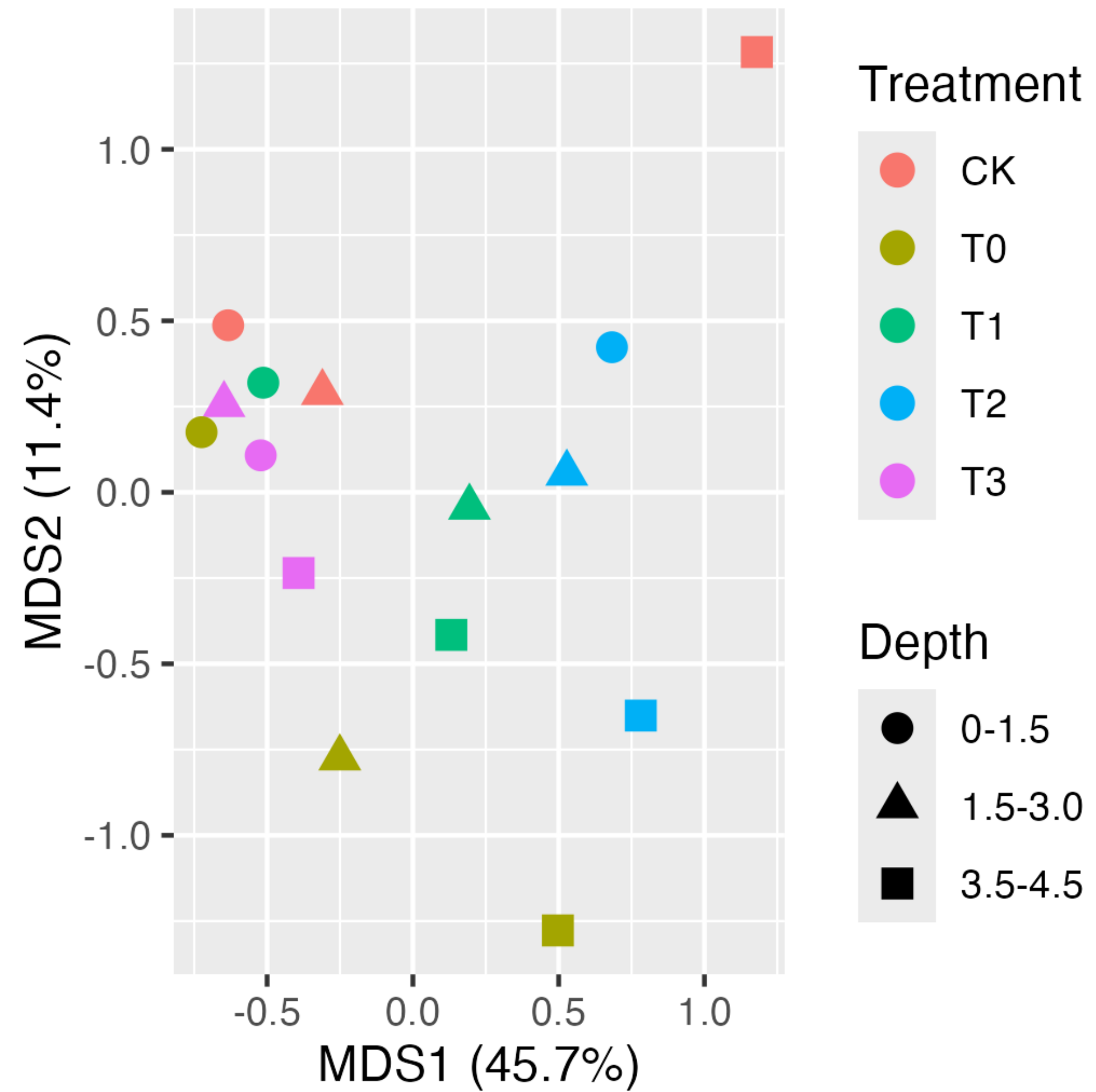
It is a measure of how dissimilar microbial communities are from one another in terms of their composition and abundance of species



Caption

Factor	R ²	F	P-value
Stimulators	0.34	1.27	0.028
Depth	0.17	1.22	0.077

beta-Diversity (Archaea)

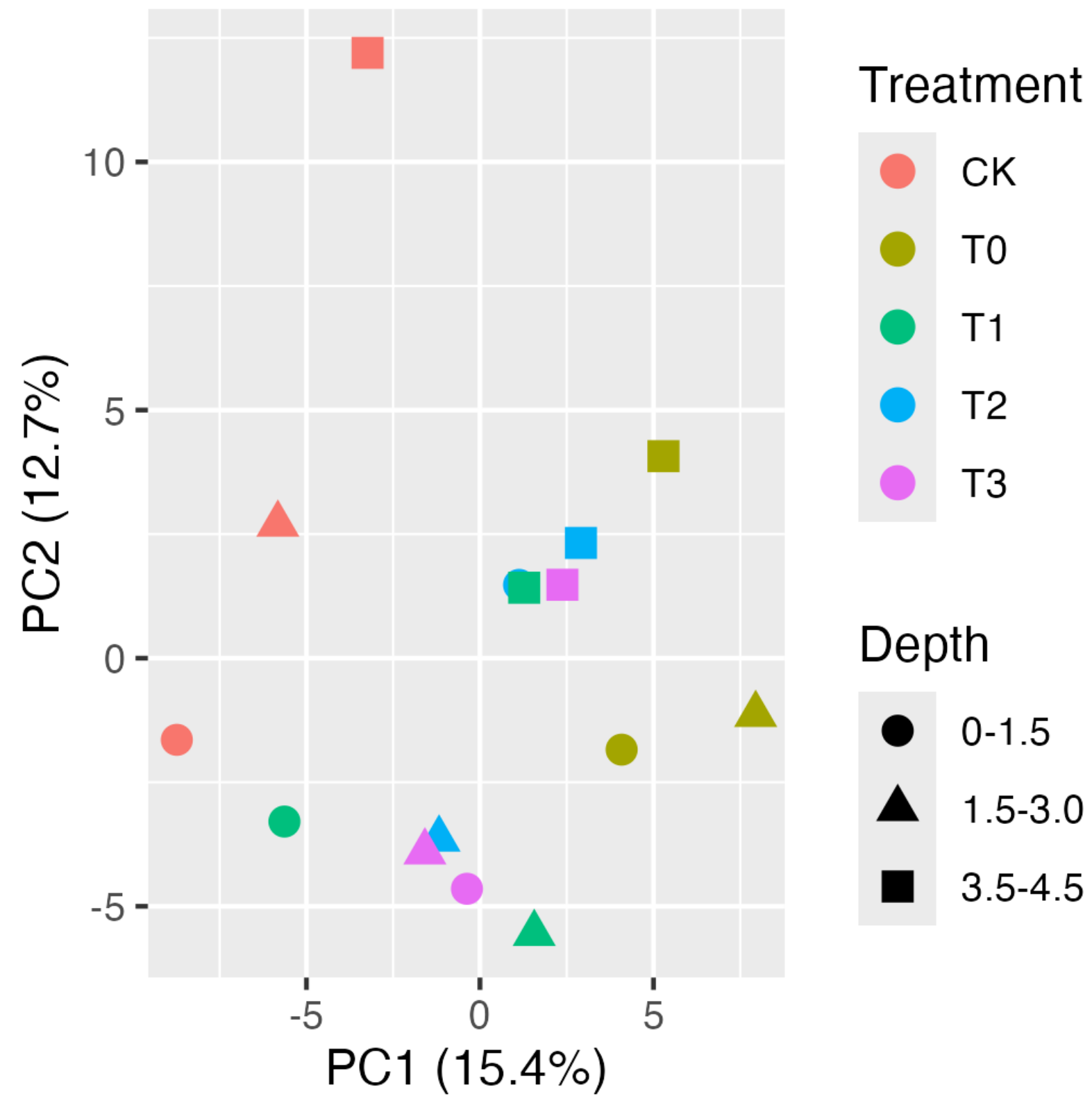


Caption

Factor	R ²	F	P-value
Stimulators	0.39	1.61	0.007
Depth	0.17	1.24	0.144

Dissimilarity at Function Level

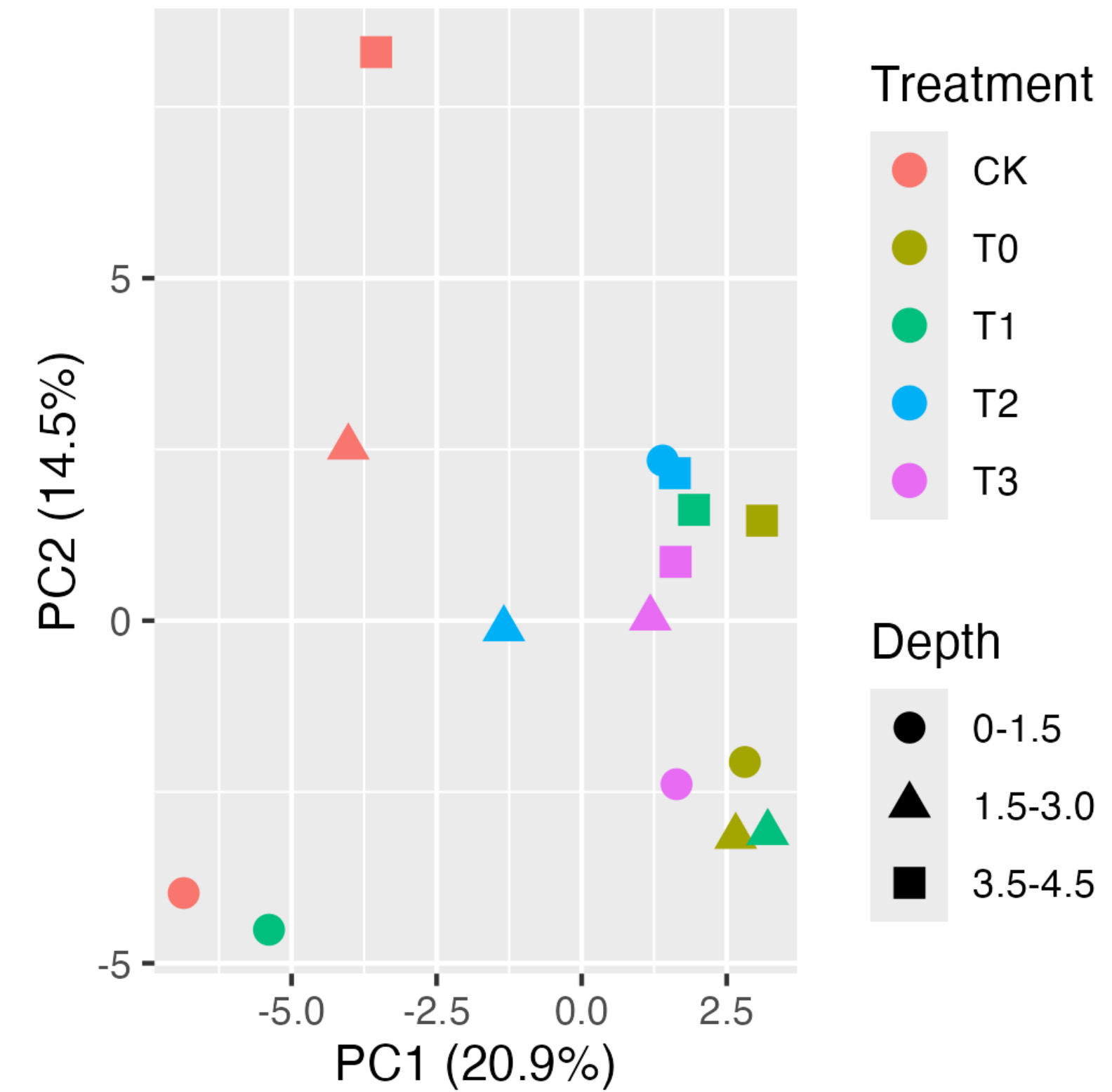
Bacteria



Caption

Factor	R ²	F	P-value
Stimulators	0.35	1.33	0.01
Depth	0.17	1.26	0.053

Archaea



Caption

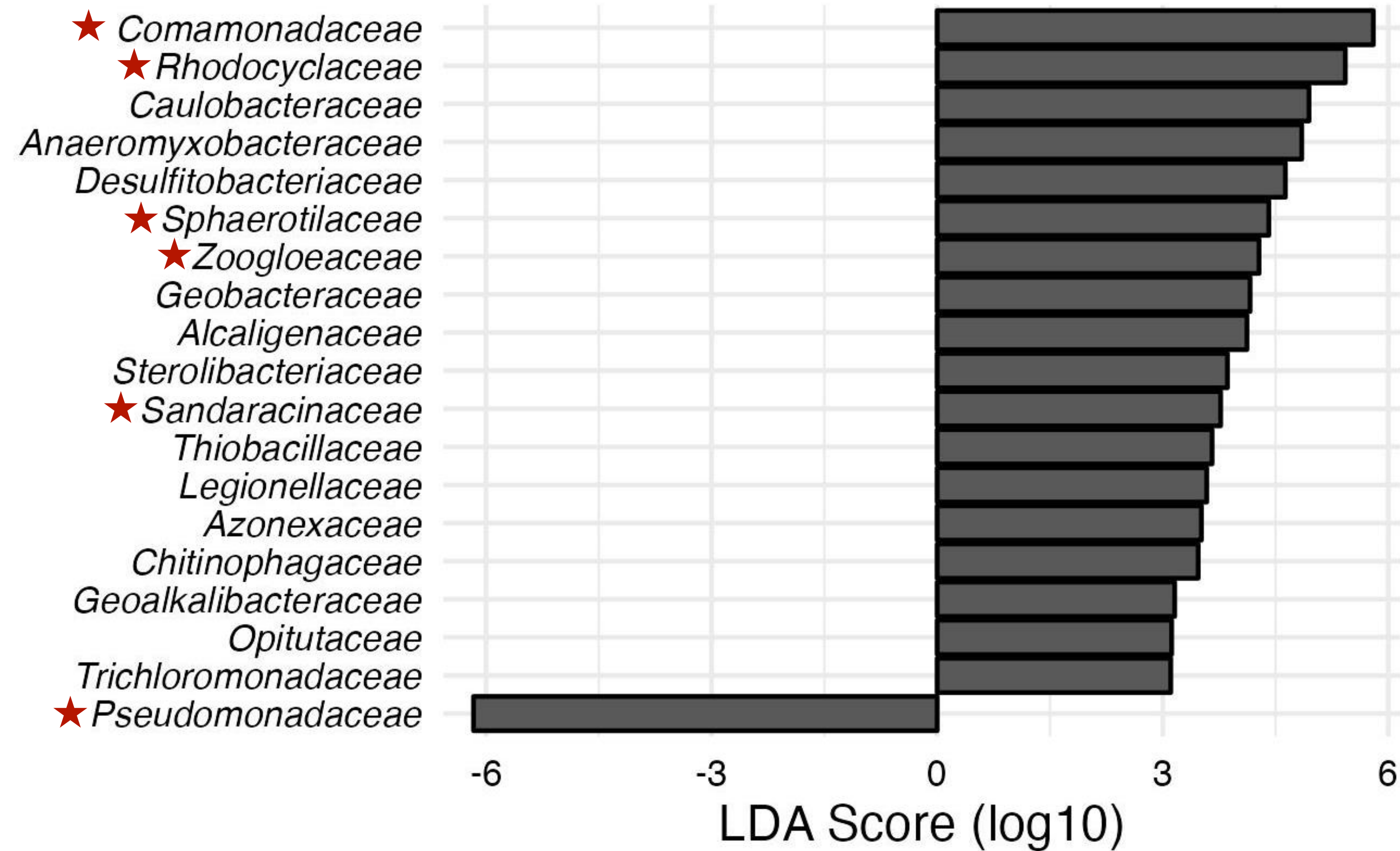
Factor	R ²	F	P-value
Stimulators	0.36	1.38	0.013
Depth	0.16	1.13	0.247



Differentially-Abundant Bacteria

Family level

Treatment V.S. CK

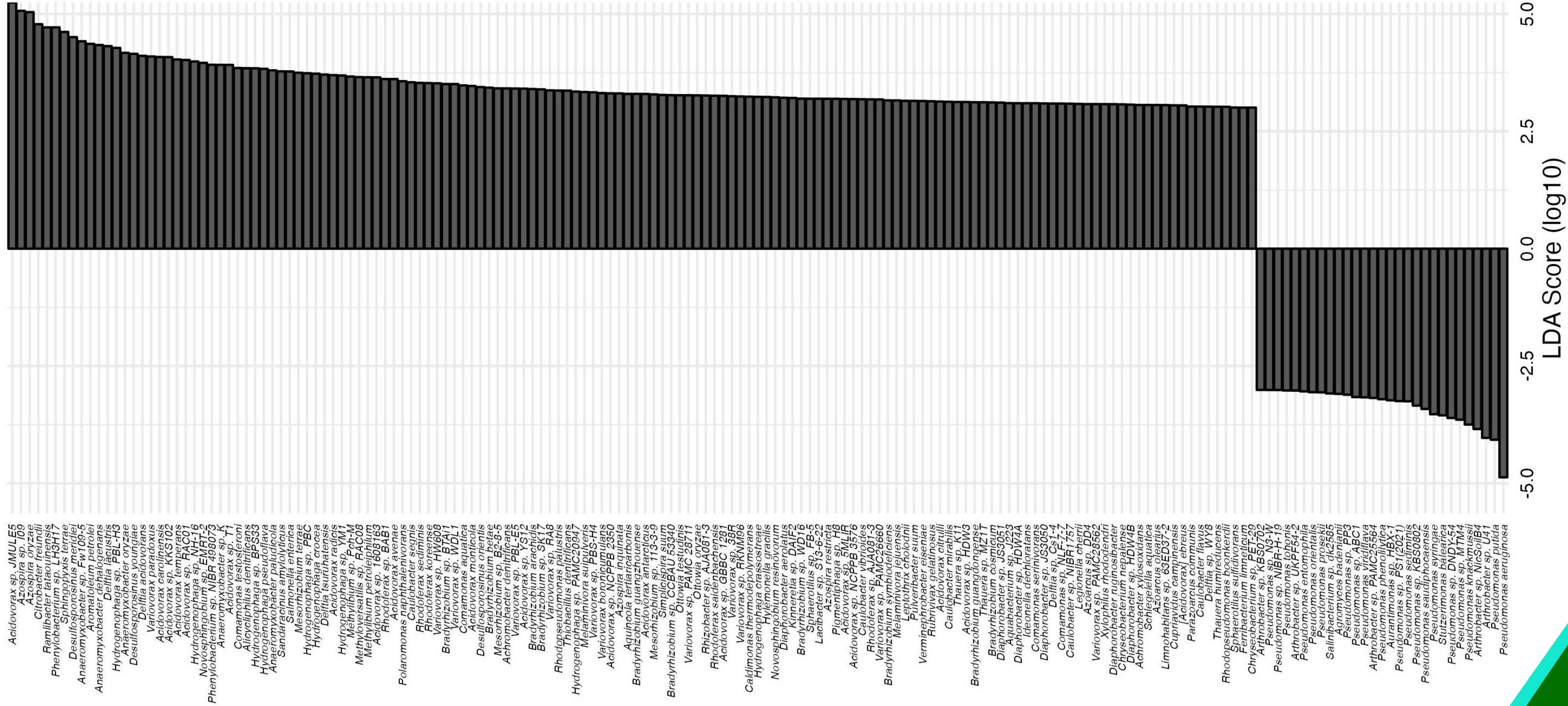


★ Hydrocarbon degradation

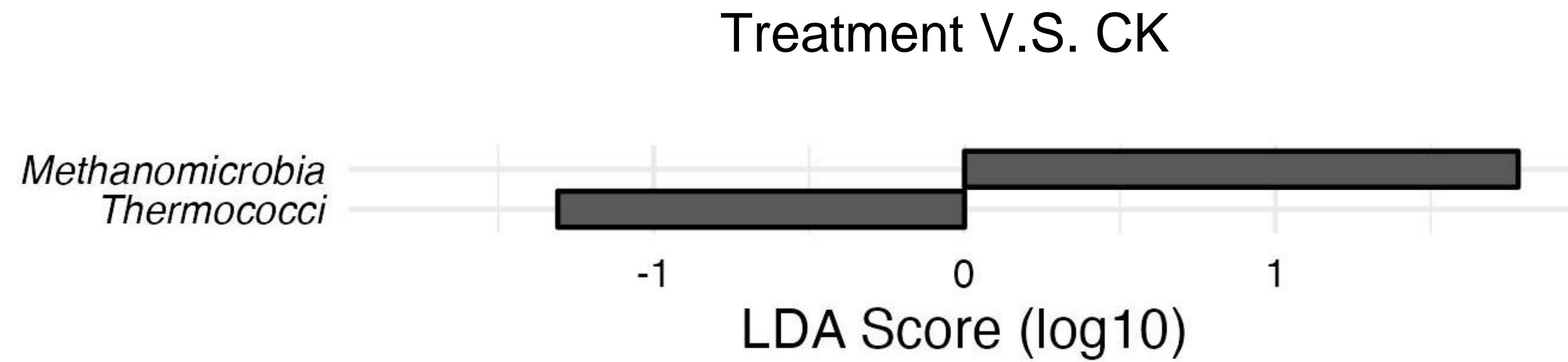
Caption

Differentially-Abundant Bacteria

Species level



Differentially-Abundant Archaea at Class level

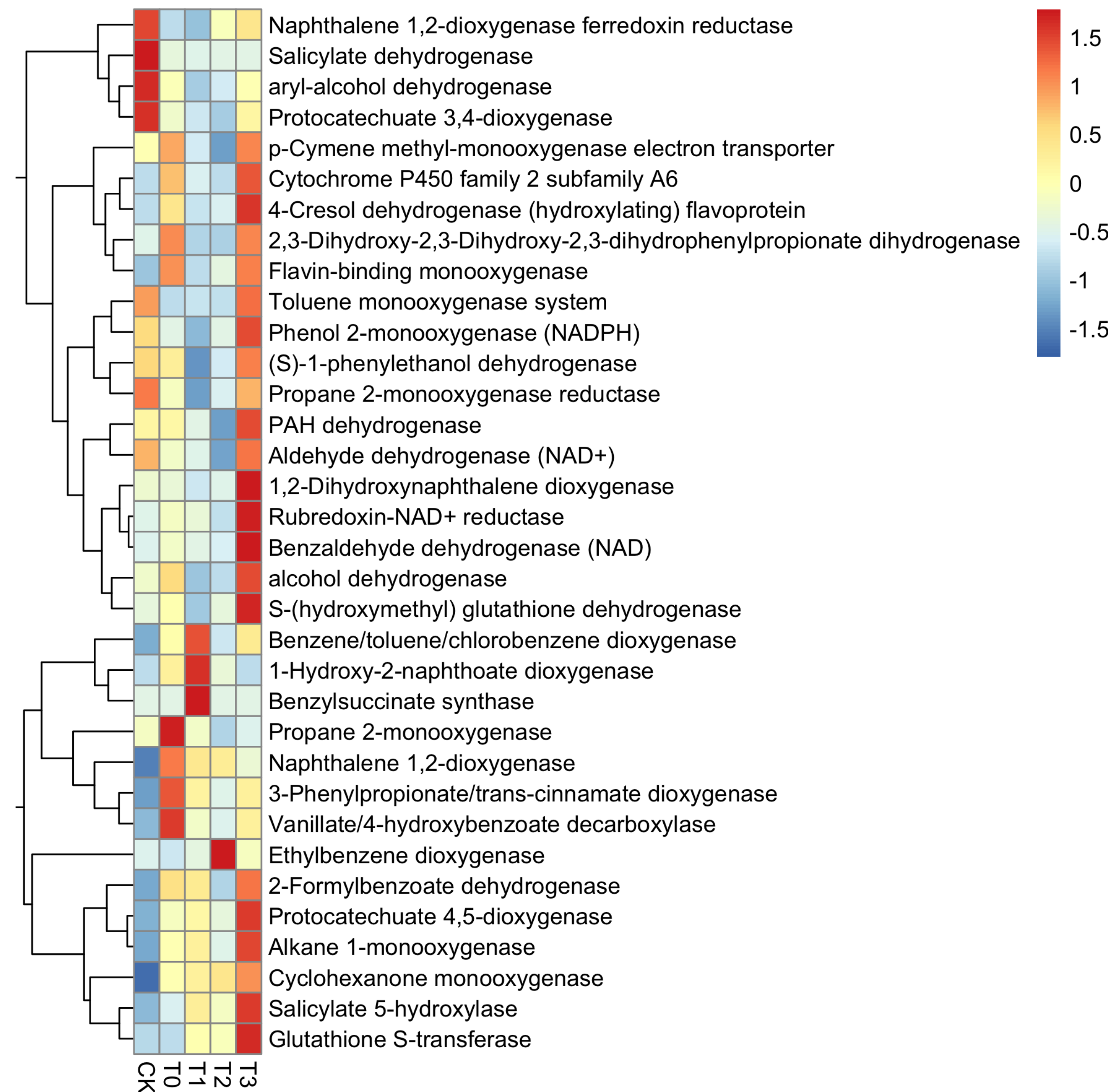


Caption

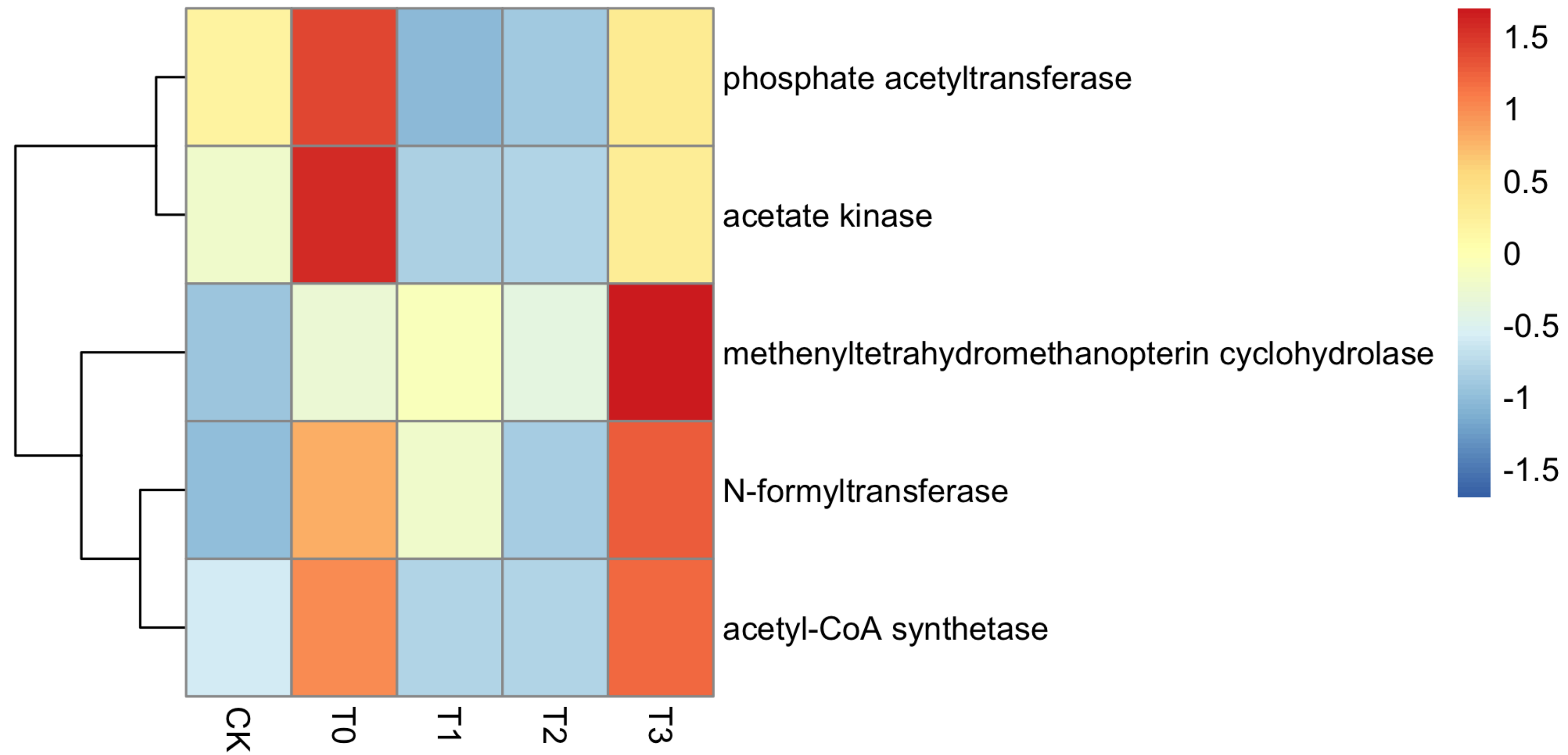


Dynamics of Hydrocarbon-Degradation Related Enzymes

Hydrocarbon-degradation
related enzymes



Dynamics of Methanogenesis Related Enzymes



Caption



- 1 Microorganisms play a central role in soil remediation, and shotgun metagenomic sequencing is a powerful tool to uncover both their composition and functional potential
- 2 Biostimulators application increased microbial activity in PHC-contaminated soil
- 3 Biostimulators impacted bacterial and archaeal alpha and beta diversity, and a specific subset of bacteria and archaea showed a significant response, including PHC degradation related microbes
- 4 Hydrocarbon degradation and methanogenesis-related enzymes exhibited differential responses to the varying compositions of biostimulators.

Acknowledgement

Prof. Steven D Siciliano

And Colleagues in the Lab



Federated Co-operatives Ltd.



Caption

Thank You