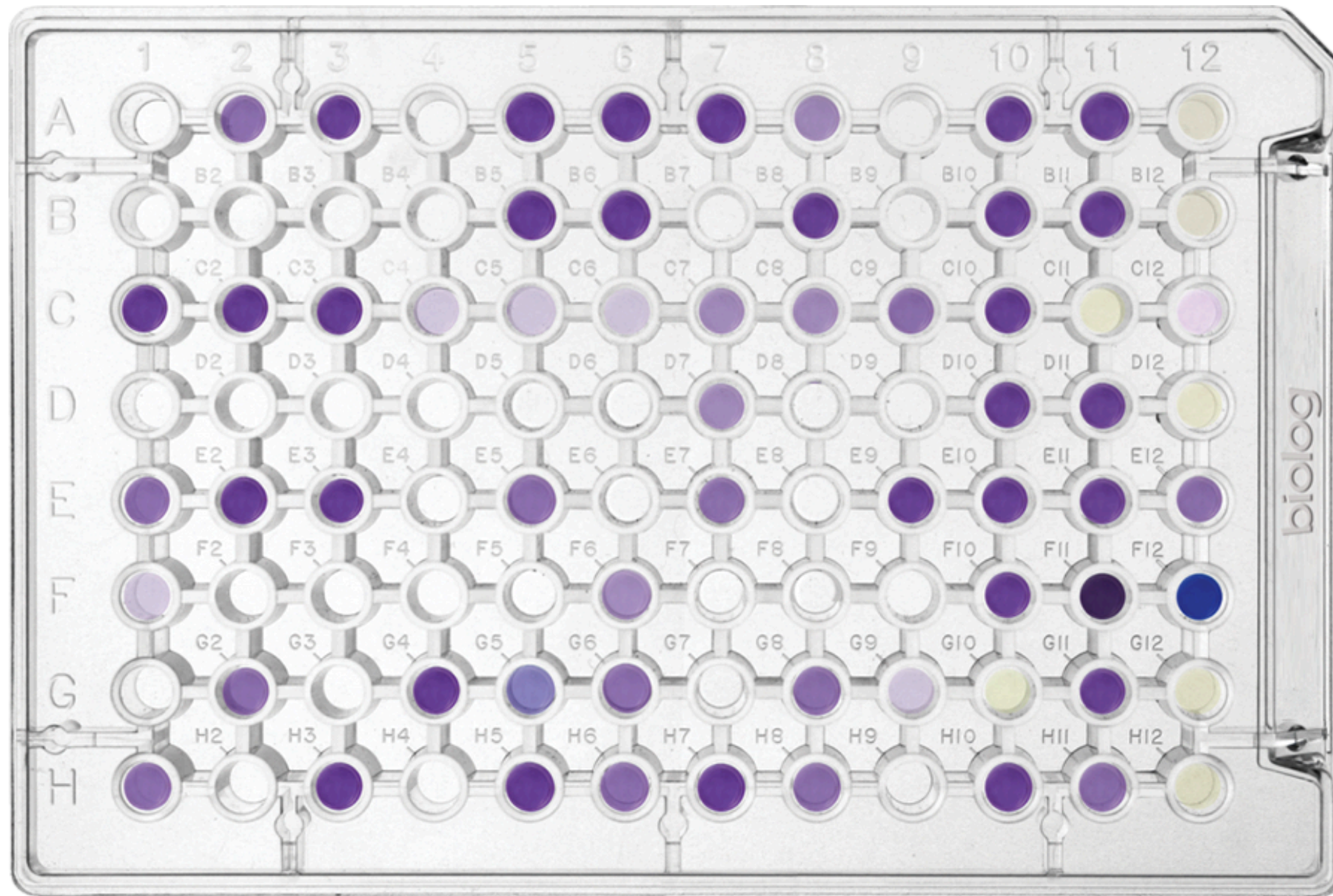


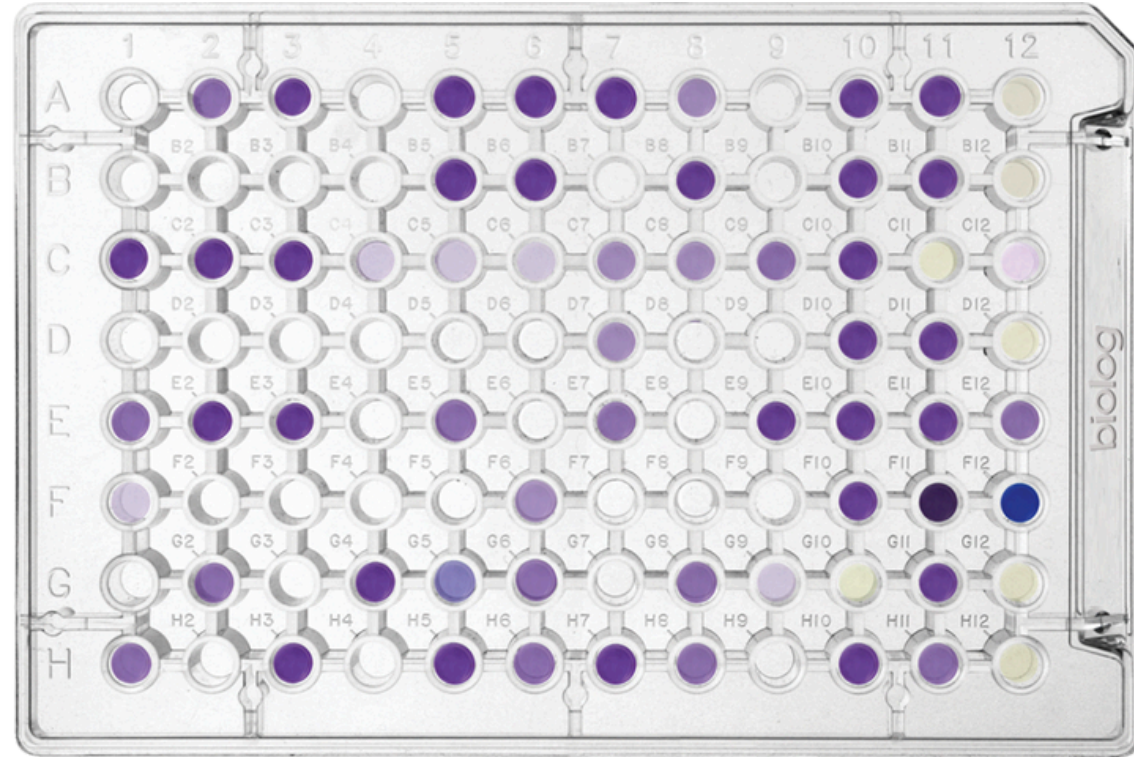


Biolog EcoPlates

Microbial Ecology

Fanny Bitoun & Miyuka Laurenson

General Presentation :



- Assessment of bacterial substrate use and metabolic potential in the environment
- 31 environmentally relevant organic carbon substrates + 1 control
- 3 sets of 32 wells : data replicates
- Dye present in each well

- Carbon sources classed in 6 categories
- 9/31 are reported plant exudates

Carbon Sources

Polymers

Carbohydrates

Carboxylic Acids

Amino-acids

Amines

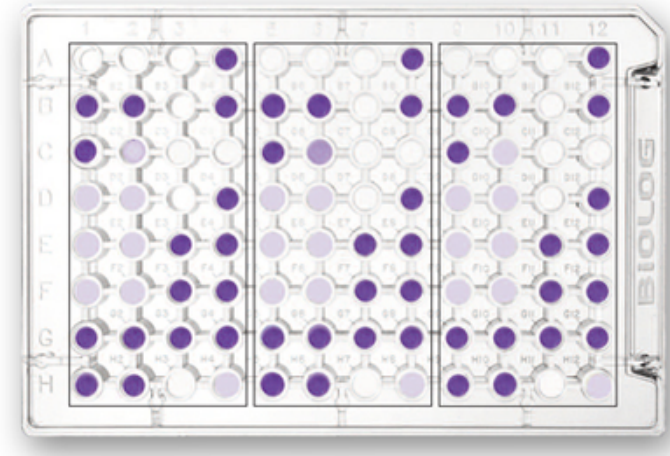
Phenolic compounds

Science behind the method



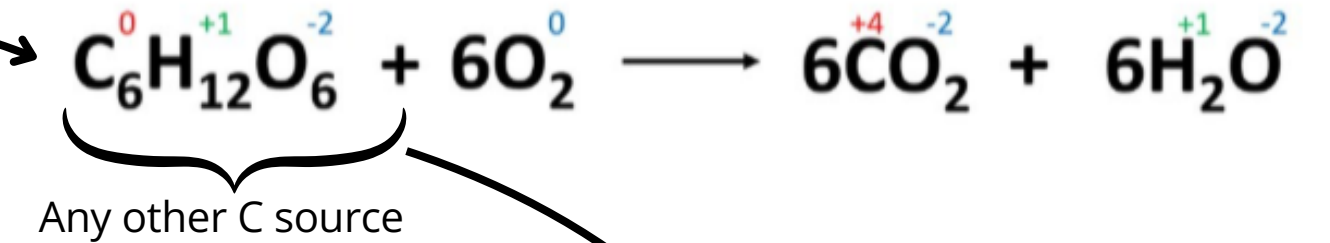
Soil or water sample

Dilution and inoculation



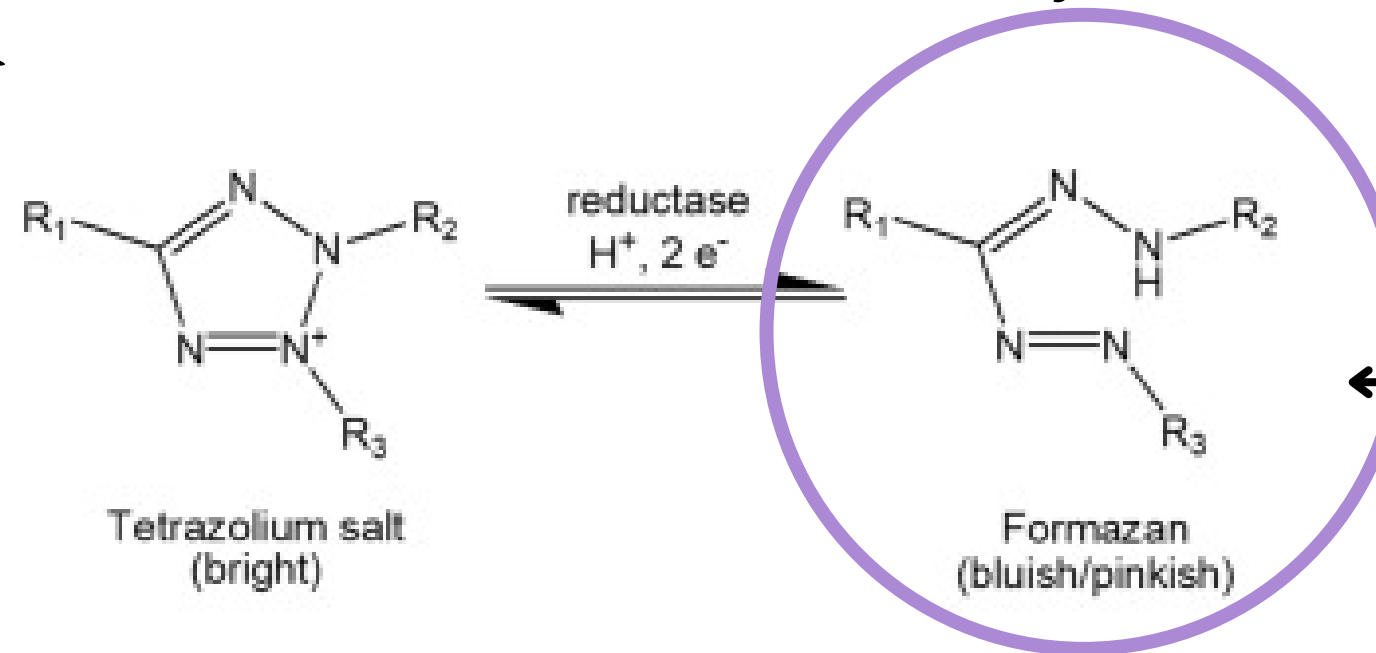
Incubation

Respiration occurs

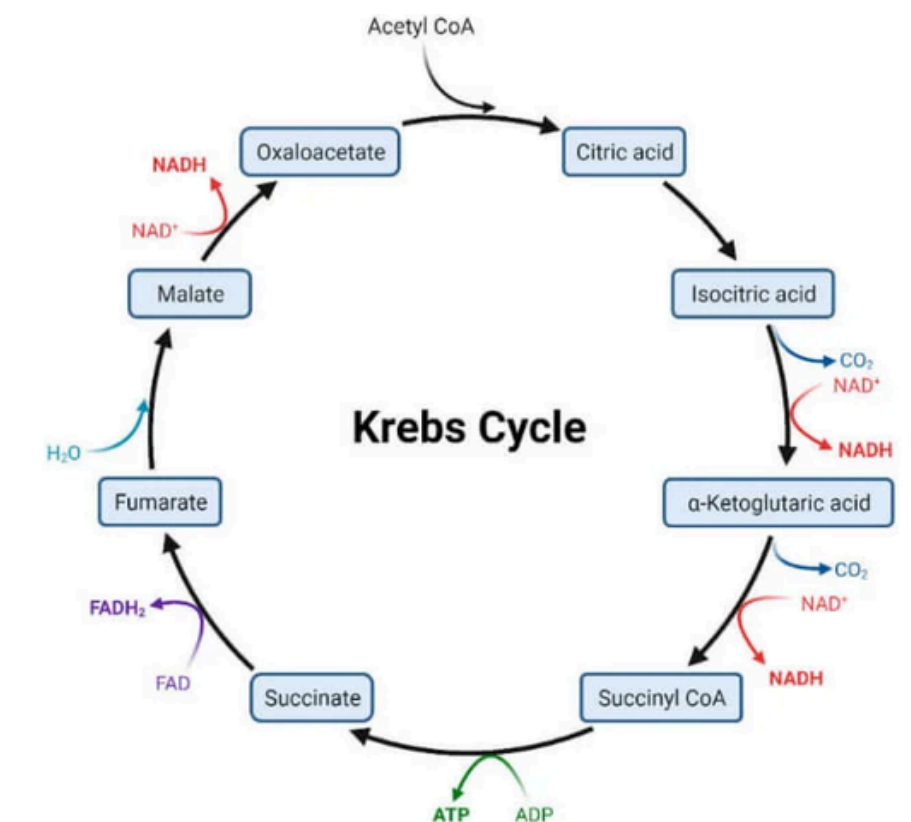


Krebs Cycle : formation of NADH

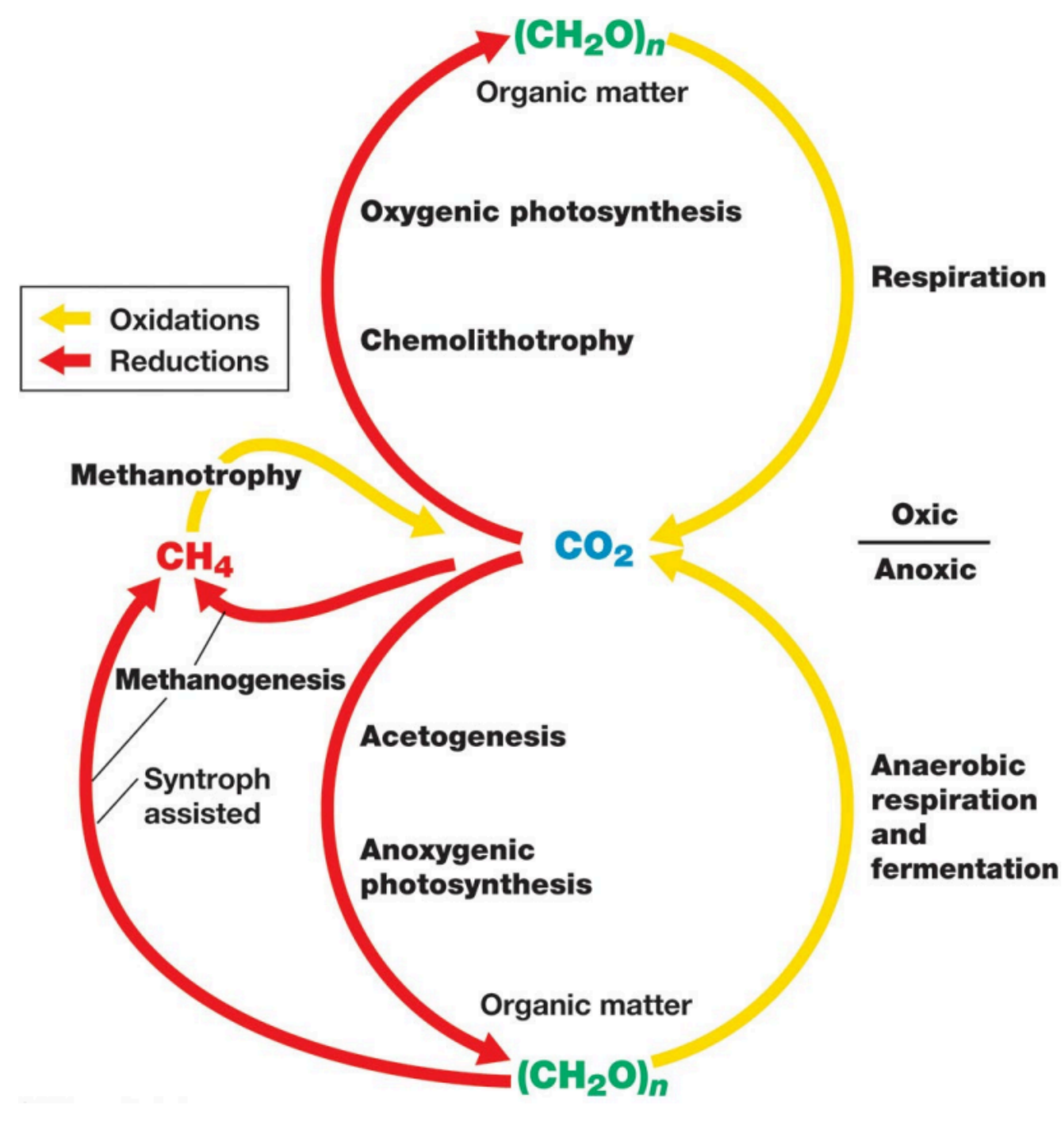
Reaction between NADH and the dye :



The dye is reduced
NADH oxidized to NAD⁺

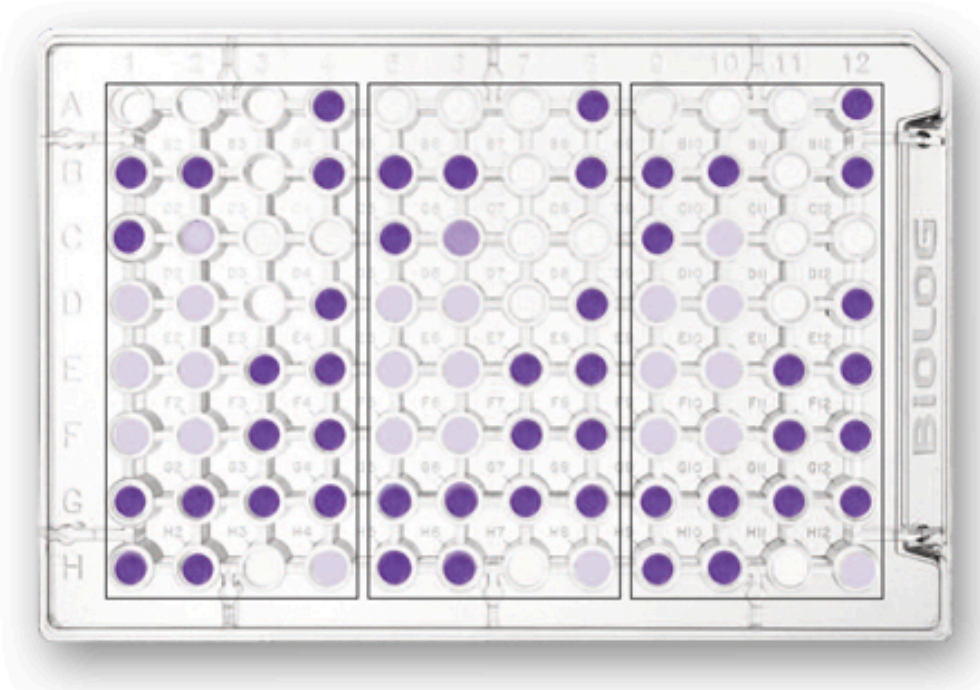


Reminder : Carbon cycling



- Carbon : essential growth & metabolic component
- Ecoplates target specific growth strategies :
 - Organic C source : Heterotrophy
 - Organic e- and H+ donors : Chemo organotrophy
 - Mostly used in aerobic conditions

Key Parameters to Analyze :



- Pattern development : **similarity** between replicates
- Rate of color change in each well : **microbial activity**
- Richness of well response : **diversity**

In class :

- Isolated microorganism
- No replicates
- Activity and diversity **qualitatively** assessed

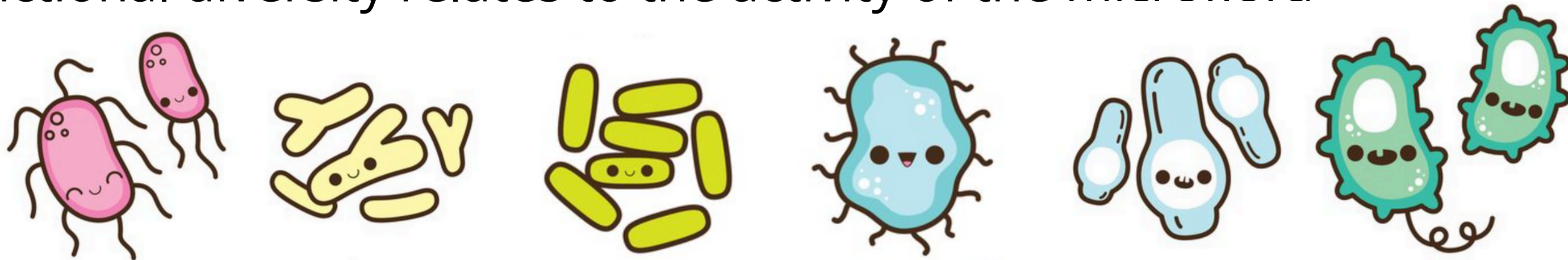
In literature :

- Community level
- Absorbance directly measured over time → **quantitative** assessment

Community-Level Physiological Profiling (CLPP) :

Characterization and classification of heterotrophic microbial communities based on **sole carbon source utilization patterns**

→ Functional diversity relates to the activity of the microflora



→ Quickly evaluate impact of practices (pollutant, fertilizer etc) on soil microflora



Community-Level Physiological Profiling (CLPP) :

Metrics derived :

- **AWCD** (Average Well Color Development) :

High AWCD : High **microbial activity + diversity**

$$AWCD = \frac{\sum_{i=1}^N OD_i}{N}$$

Optical Density

- **Shannon-Weaver Index (H) :**

Considers both Richness and Evenness

High Shannon-Weaver Index = the community uses many carbon sources equally well -> **greater diversity**

$$H = - \sum_{i=1}^N P_i \cdot \ln P_i , \quad P_i = \frac{OD_i}{\sum_{i=1}^N OD_i}$$

Limitations of Ecoplates

Microbial Growth {

- Only cultivable bacteria
- Bias toward fast growing bacteria

Incubation {

- High substrate concentrations
- No combination of substrate

Inoculation {

- Measurements should be done as fast as possible after sampling
- Choice of the part of the soil to use

The Biolog EcoPlate™ Technique for Assessing the Effect of Metal Oxide Nanoparticles on Freshwater Microbial Communities

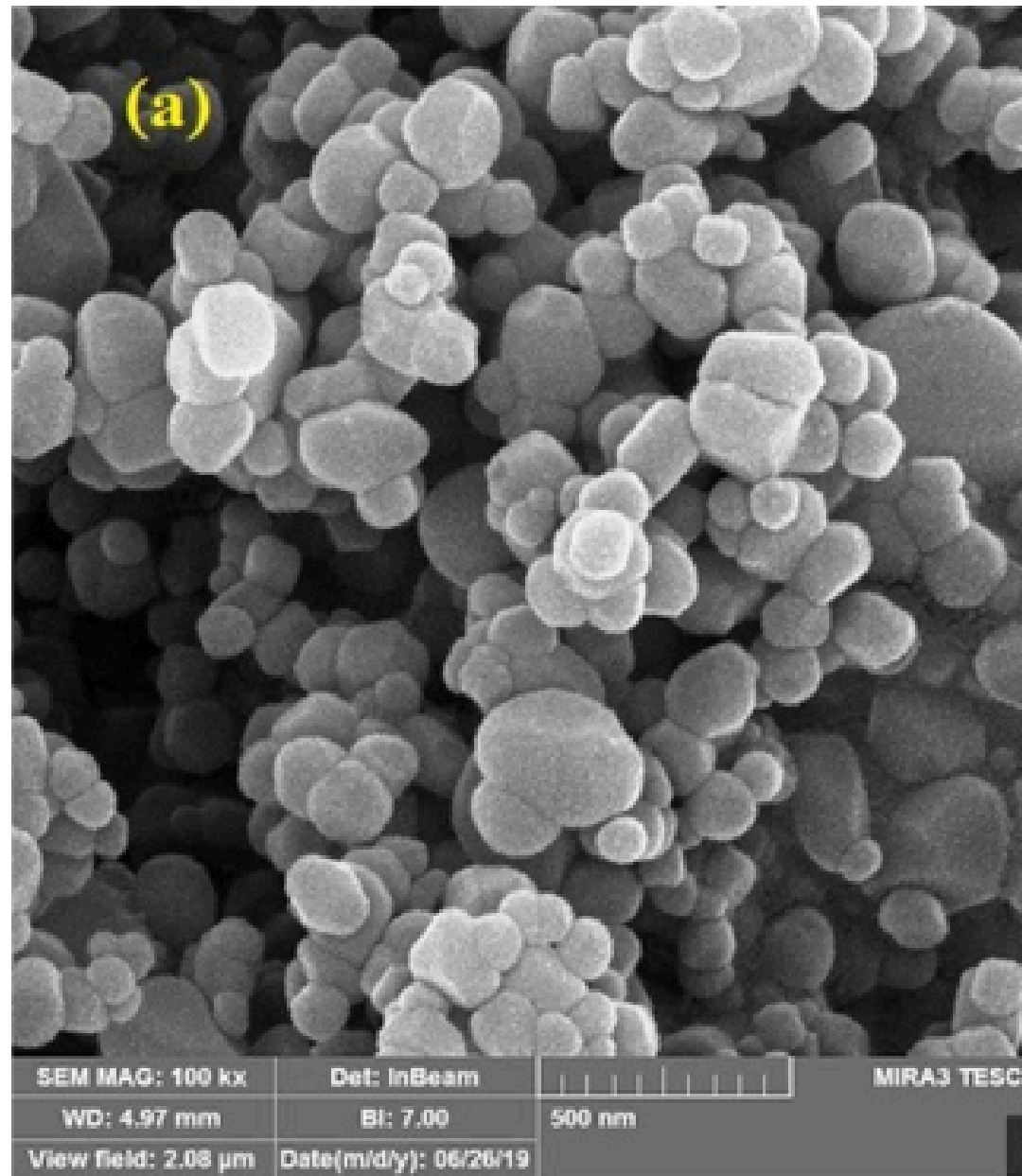
[Imre Németh](#)¹, [Szabina Molnár](#)¹, [Emese Vaszita](#)¹, [Mónika Molnár](#)^{1,*}



Research Question :

Assessing the impact of nTiO₂ and nZnO released in freshwater ecosystems on local microbial communities

What are nTiO₂ and nZnO?



<https://www.nanoshel.com/product/tio2-nanoparticles>

Nano titanium dioxide (nTiO₂) and **nano zinc oxide** (nZnO) :

- Anthropogenic engineered metal oxide nanoparticles
- Used in medicine, electronics, cosmetic and textile industry
- Widely produced and transported
→ risk of release in the environment

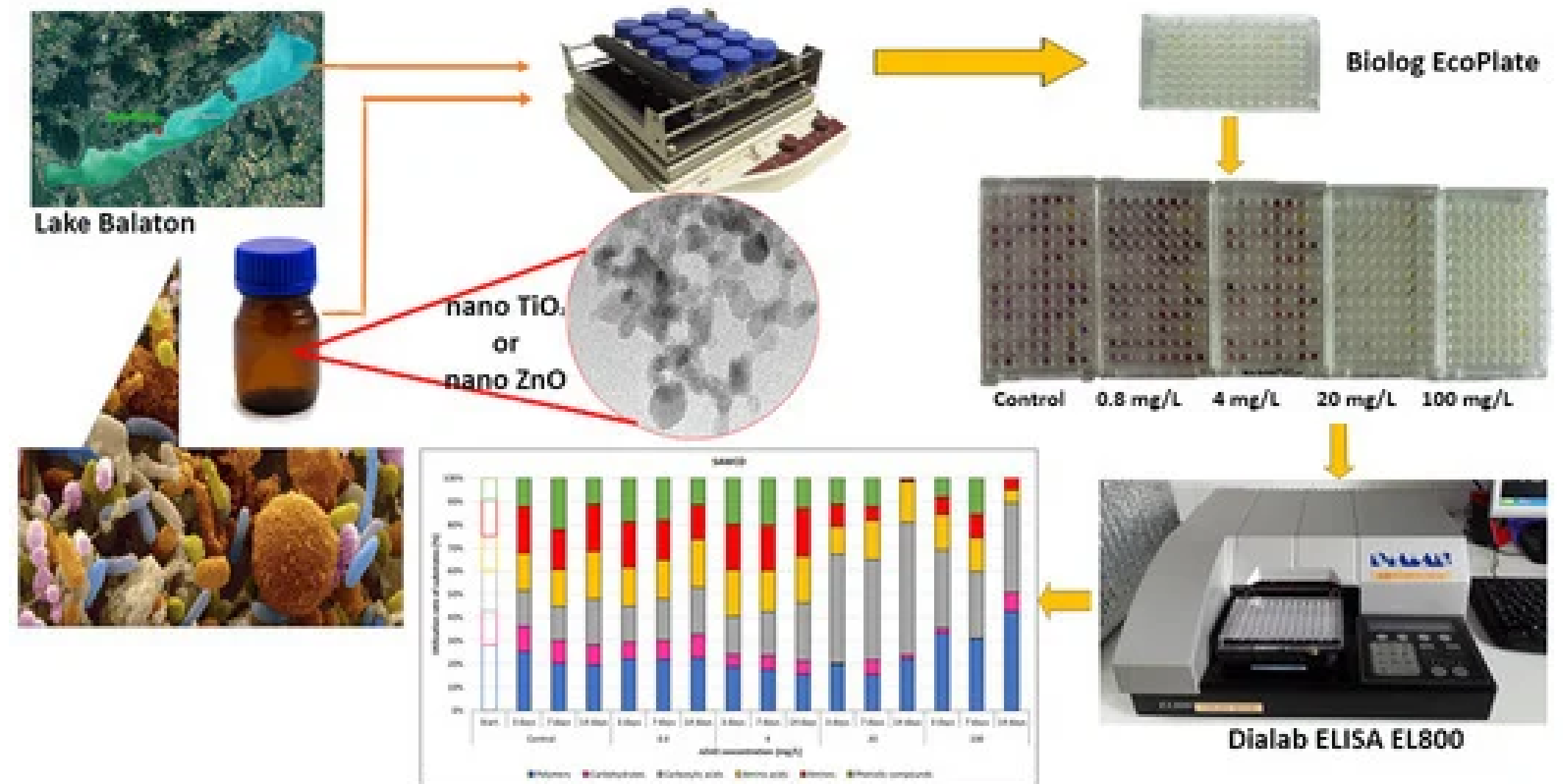


Impact of nanoparticles on bacteria :

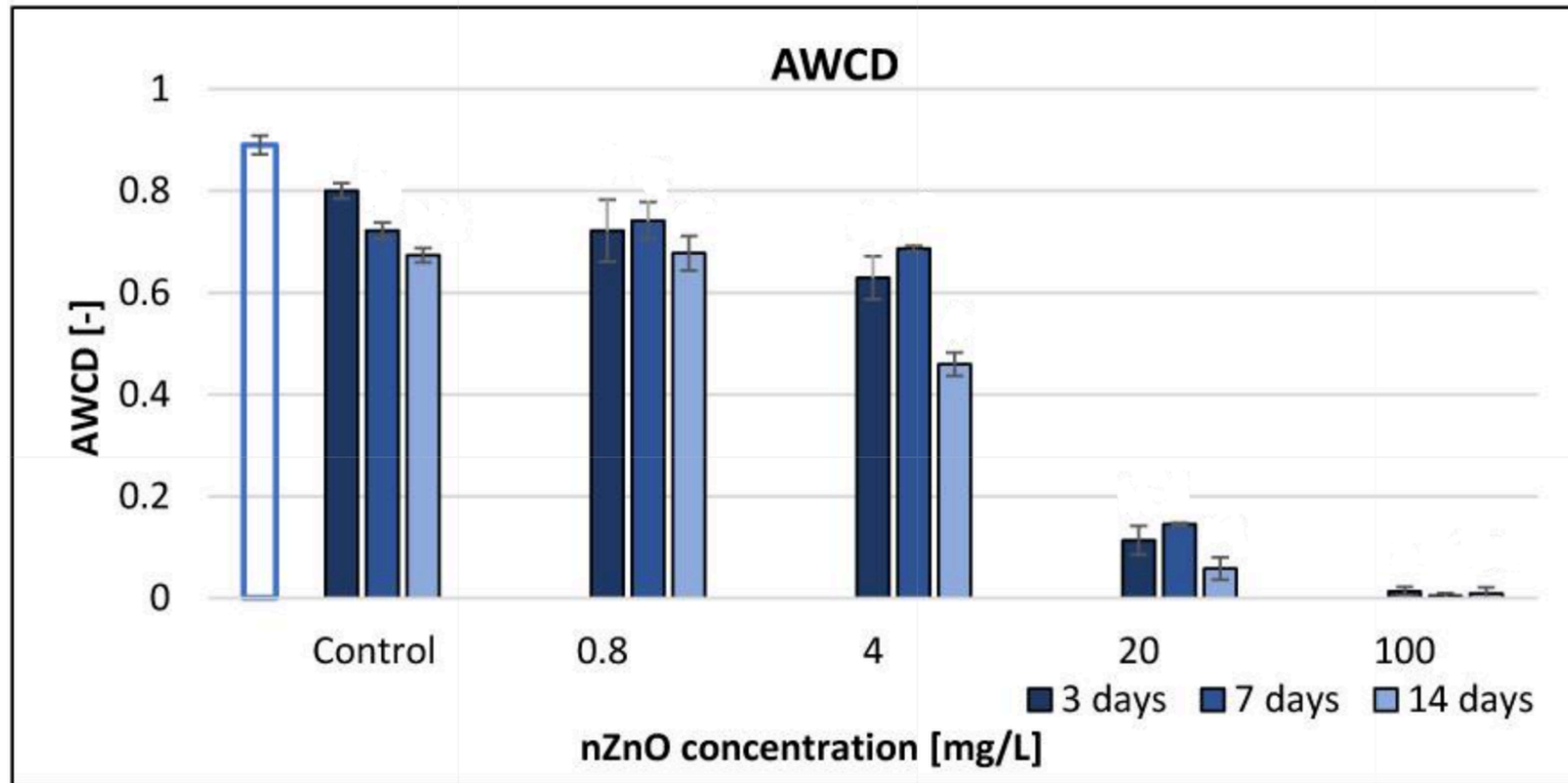
Can interact with various component in the cells (membrane, proteins, genes expression etc)
→ reduction of enzymatic activity, reproduction, or increase morbidity

Methods

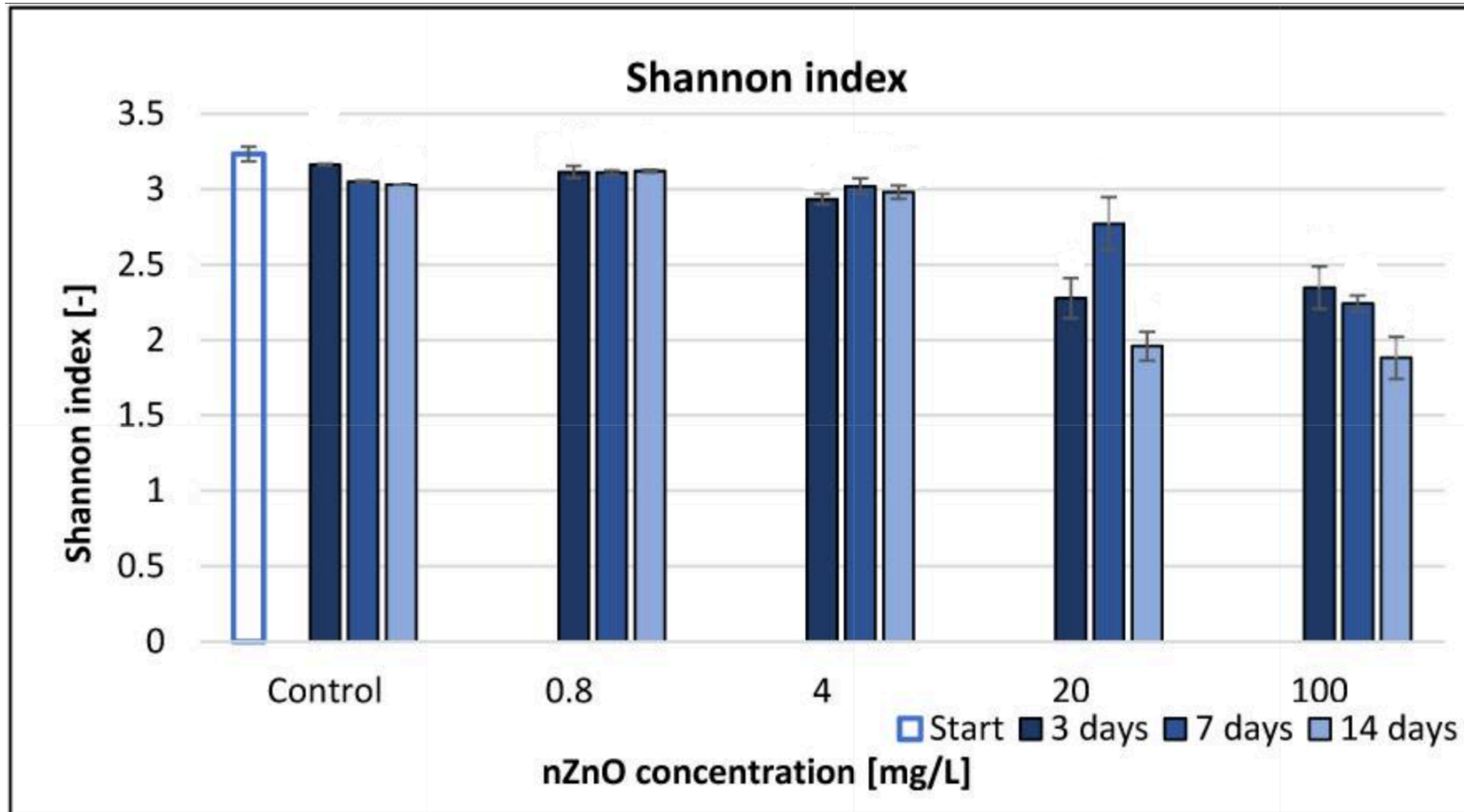
- **Water samples** collected in Lake Balaton, Hungary in June and October 2020
- **Inoculation** with the two nanoparticles suspension
- **Community-level physiological profiling** using Biolog Ecoplates → Substrate utilization over time
- **Metrics** analyzed : AWCD and Shannon diversity index



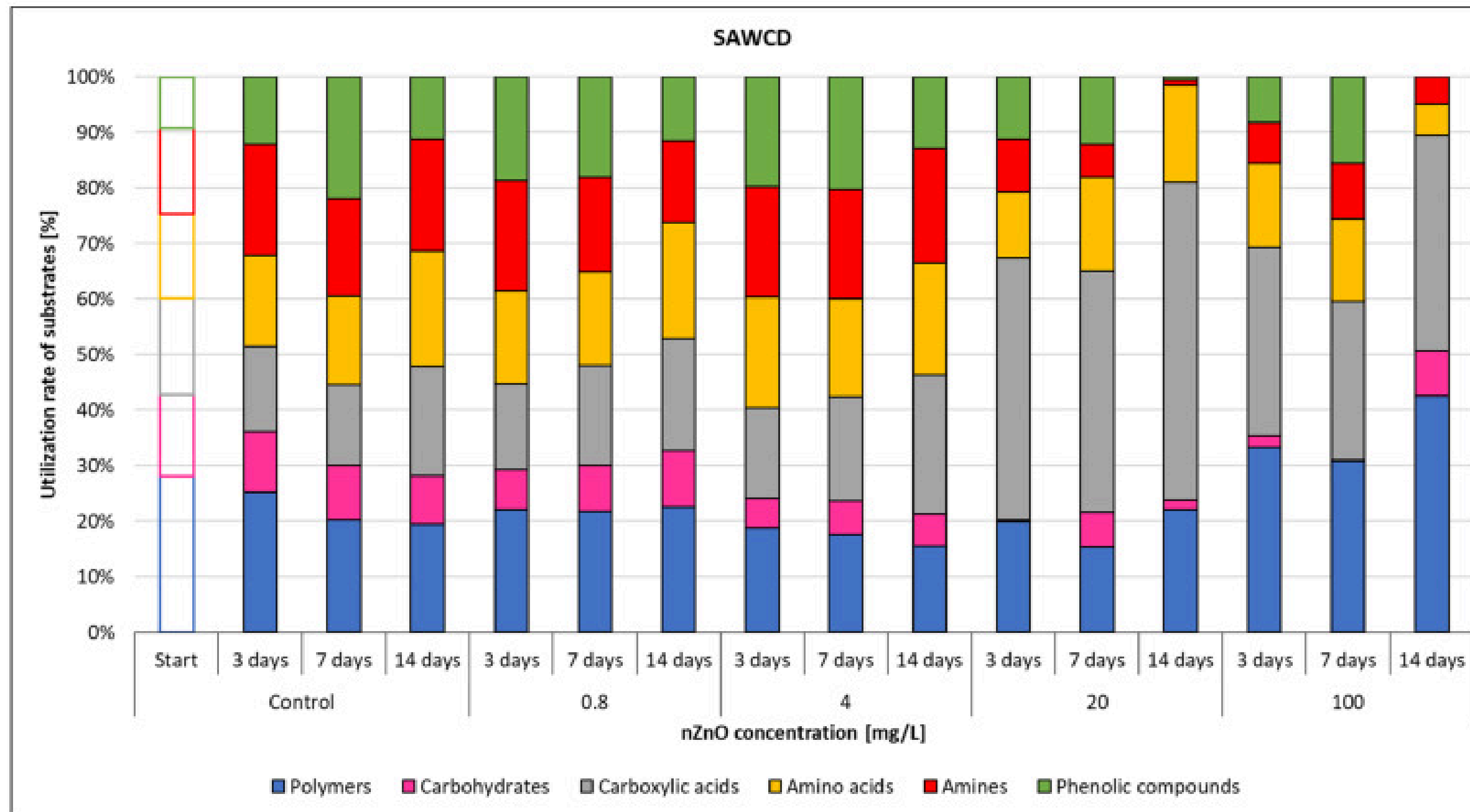
Results : Average Well Color Development



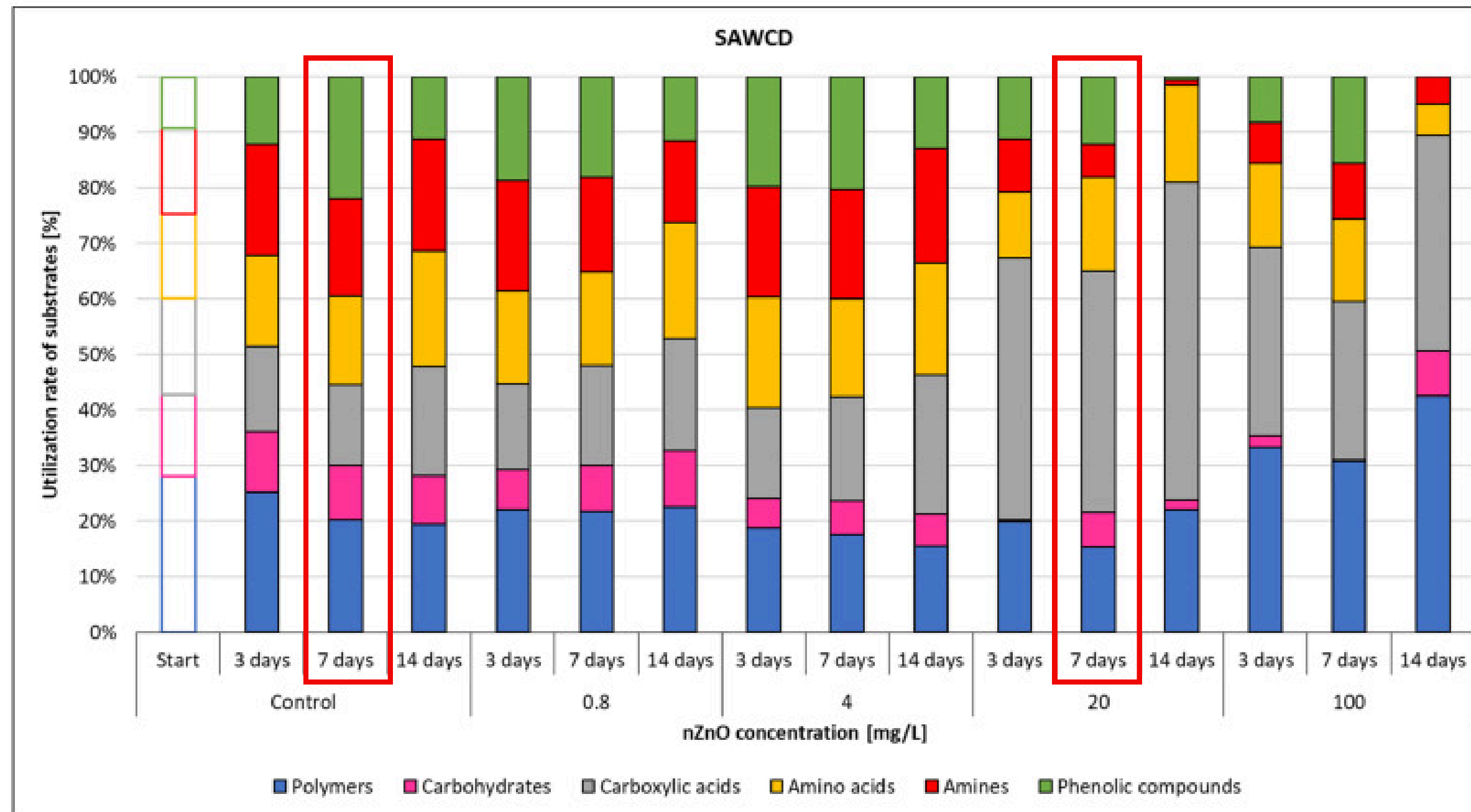
Results : Shannon diversity index



Results : AWCD by substrate category



Results : AWCD by substrate category



Conclusion

Main advantages :

- Simple, rapid and cost-effective method
- Allows for the comparison of community level response against changing/disturbed environments

One step further :

- Ecoplates give only a first insight on the community profile
- Pair its utilization with enzymatic activity measurement or genomics methods