

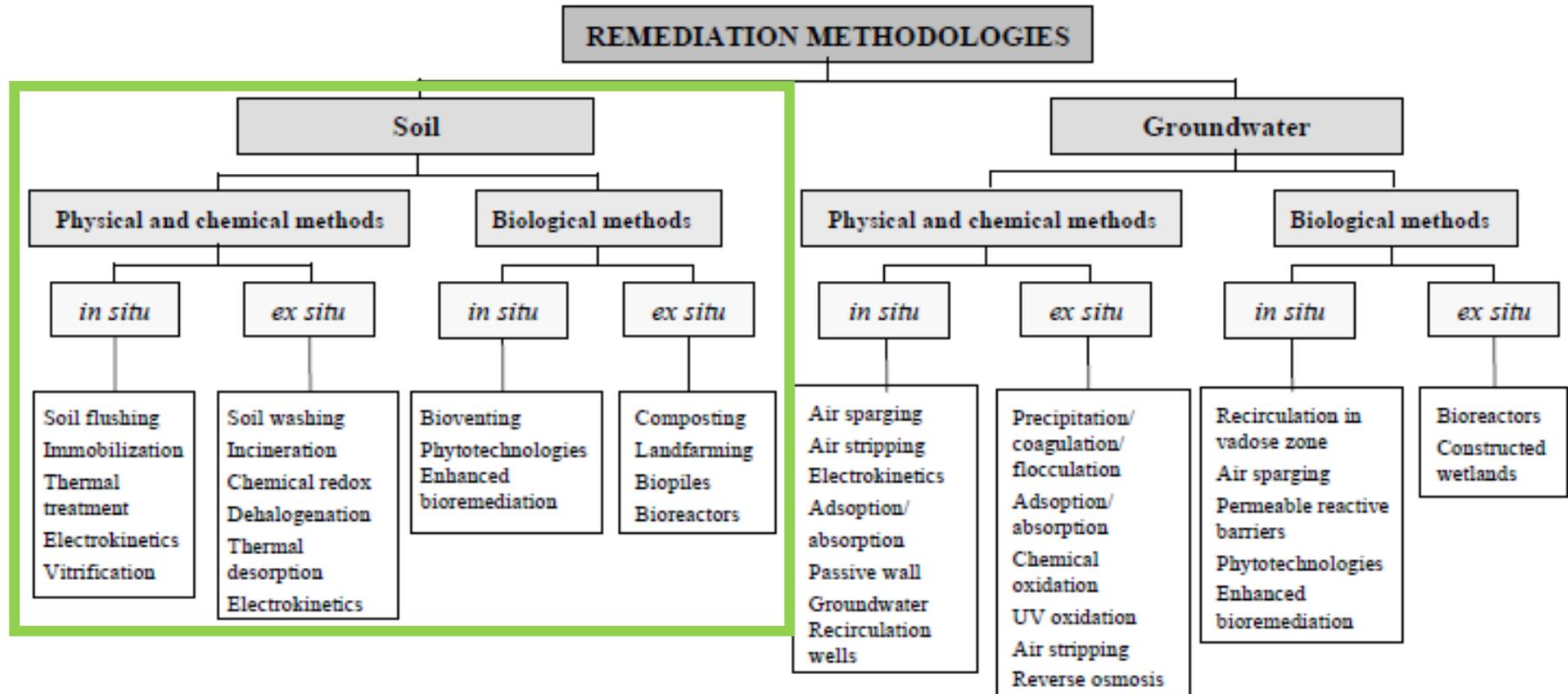


# Biochemical indicators of soil under bioremediation

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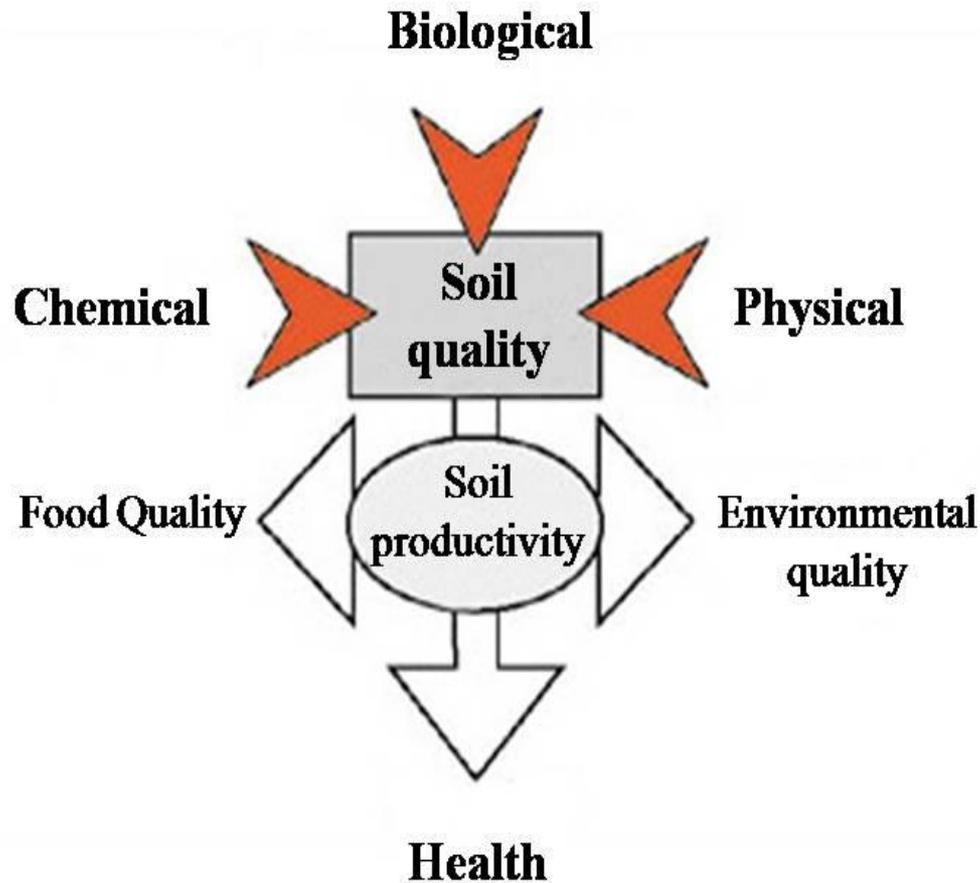
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# Soil bioremediation



# Indicators of soil quality

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“The capacity of soil to interact with the ecosystem in order to maintain the biological productivity, the quality of other environmental compartments, thus promoting the health of plants and animals including humans”

*(Doran and Parkin, 1994)*

## Direct analysis of contaminant vs. indicators

Monitoring the types and quantities of toxic substances that are entering into the terrestrial environment



an exhaustive and problematic task



the complexity and cost resulting from the identification of the chemical substances involved.

# Ideal bioindicator

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“The ideal soil microbiological and biochemical indicator to determine soil quality would be simple to measure, should work equally well in all environments and reliably reveal which problems existed where”.

*Schlöter et al. (2003)*



# What is an indicator?

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Bioindicator is a species or group of species that reflects biotic and abiotic levels of contamination of an environment, presenting alterations that enables the generation of information about the quality of the environment, for example, accumulating substances in concentrations higher than those considered normal or essential for its body metabolism.

*(Hodkinson and Jackson 2005)*

# Indicators of soil quality

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A good indicators should be:

- correlate well with ecosystem processes
- integrate soil physical, chemical, and biological properties and processes
- be accessible to many users
- be sensitive to management and climate
- be components of existing database
- be interpretable

*Doran and Parkin, 1996*

# Types of indicators

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## Soil physico-chemical properties



Traditionally used as indicators of the impact of anthropic activity on soil fertility and health.

## Soil biological properties



In the last years, increasingly used as biological indicators of the functioning of the soil ecosystem

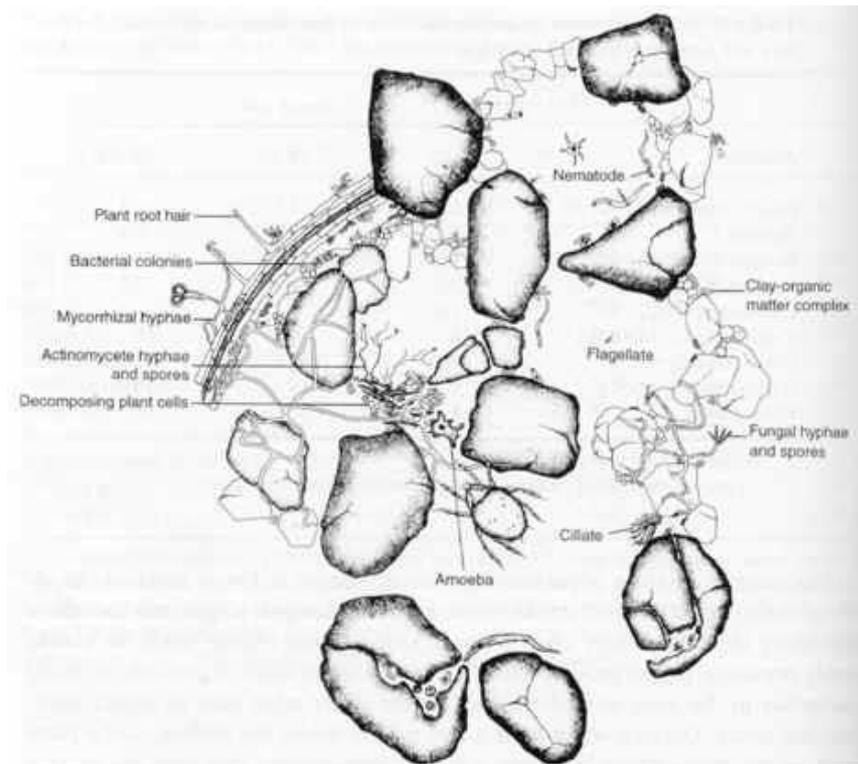


**high sensitivity, rapid response and capacity to provide information that integrates many environmental factors**

# Function of a bioindicator

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- To monitor biological and biochemical changes of a soil as the result of anthropic and environmental effects.
- To make these changes evident.
- To provide a quick response to the occurring disturb.



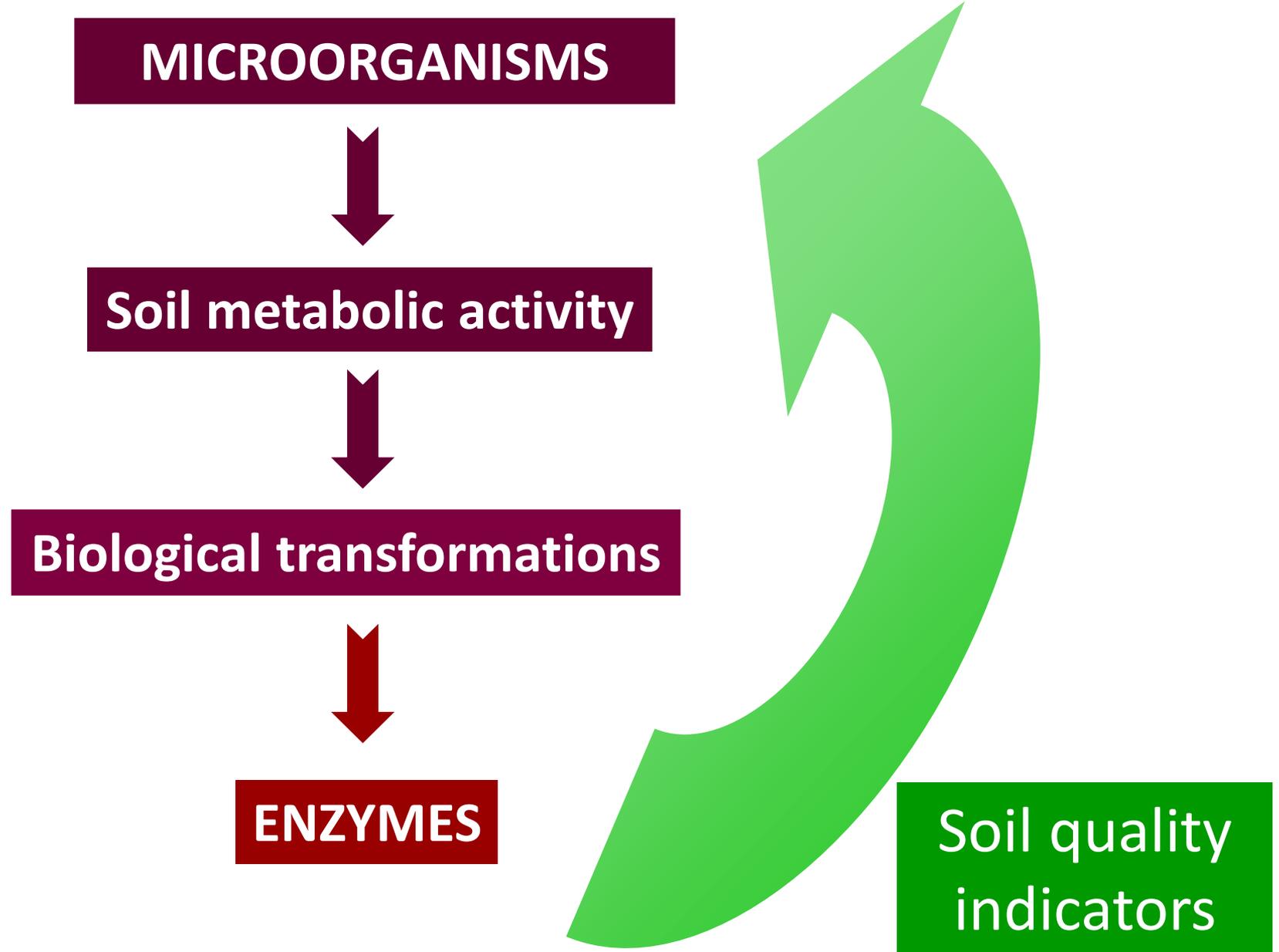
# Microorganisms and enzymatic activities

They may act as bioindicators because:

- They respond quickly to environmental stress and to anthropic changes such as:
  - Climate changes
  - Addition of fertilizers
  - Agricultural practices
  - Presence of pollutants



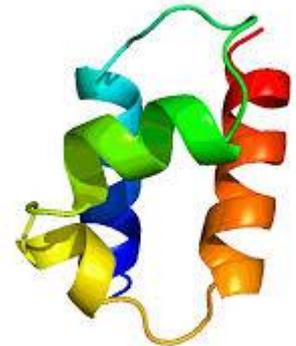
# Microorganisms vs. enzymatic activities



# Enzyme Activities in Soil

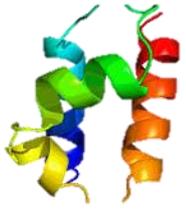
Soil enzymes activities have been suggested as suitable indicators of soil quality because they:

- are a measure of the soil microbial activity and therefore they are strictly related to the nutrient cycles and transformations
- may rapidly respond to the changes caused by both natural and anthropogenic factors
- are easy to measure



# Advantages of enzymatic assays

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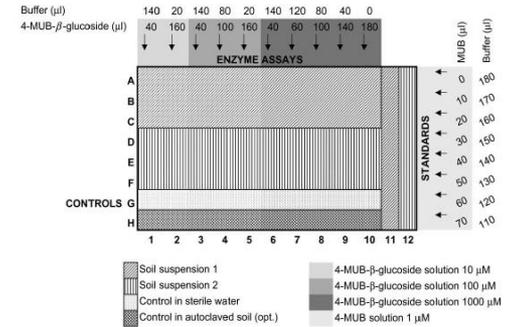
- Enzymatic assays are short-term laboratory procedures that are usually carried under standardizing environmental factors (use of sieved samples, standardized temperature, water content and pH value).
- These defined experimental conditions may allow that changes in biomass structure during the experiments may be minimized
- Soils from different geographical locations and environmental conditions, and also results from different laboratories, could be compared.

# Soil enzyme assays



**Tube assay**

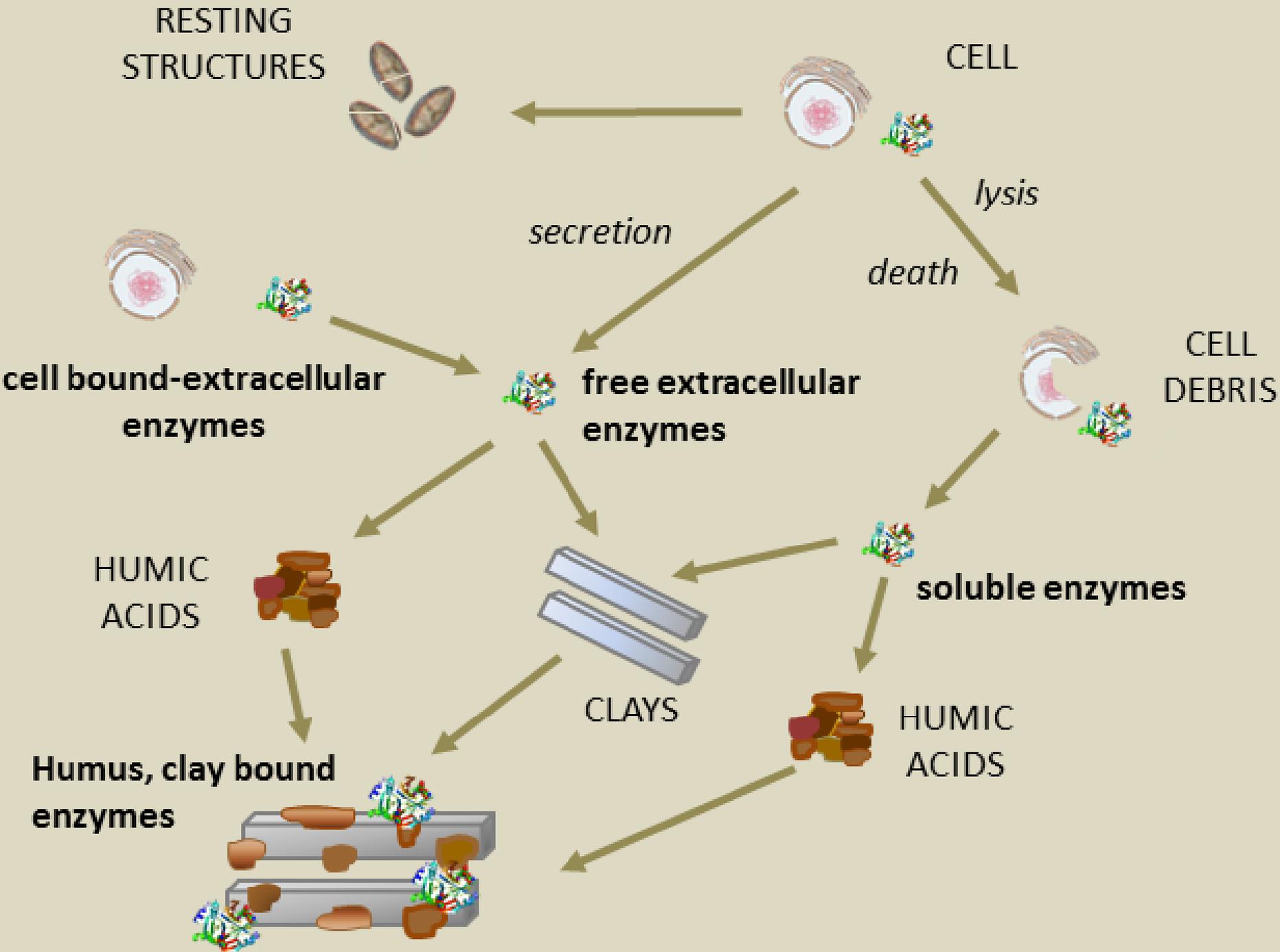
or



**Microplate assay**

Long-term experiments have demonstrated the potential of microplate fluorimetric soil enzyme assays to differentiate plots under contrasting organic and inorganic fertilization regimes on multiple soil enzyme activities

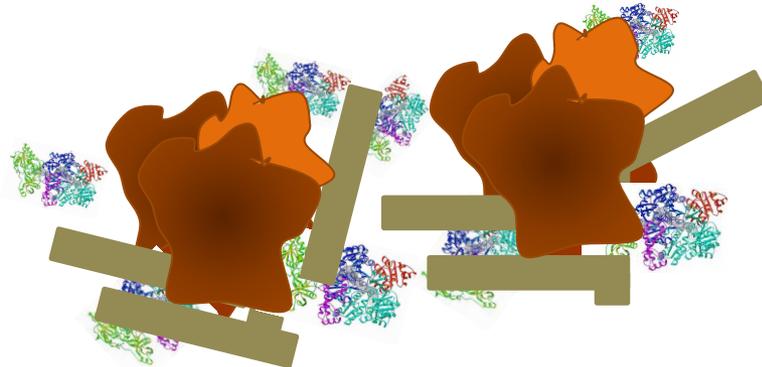
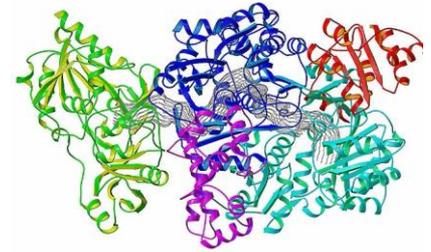
Microplate-scale fluorimetry is a fast throughput tool for the assessment of soil functional diversity and soil quality.



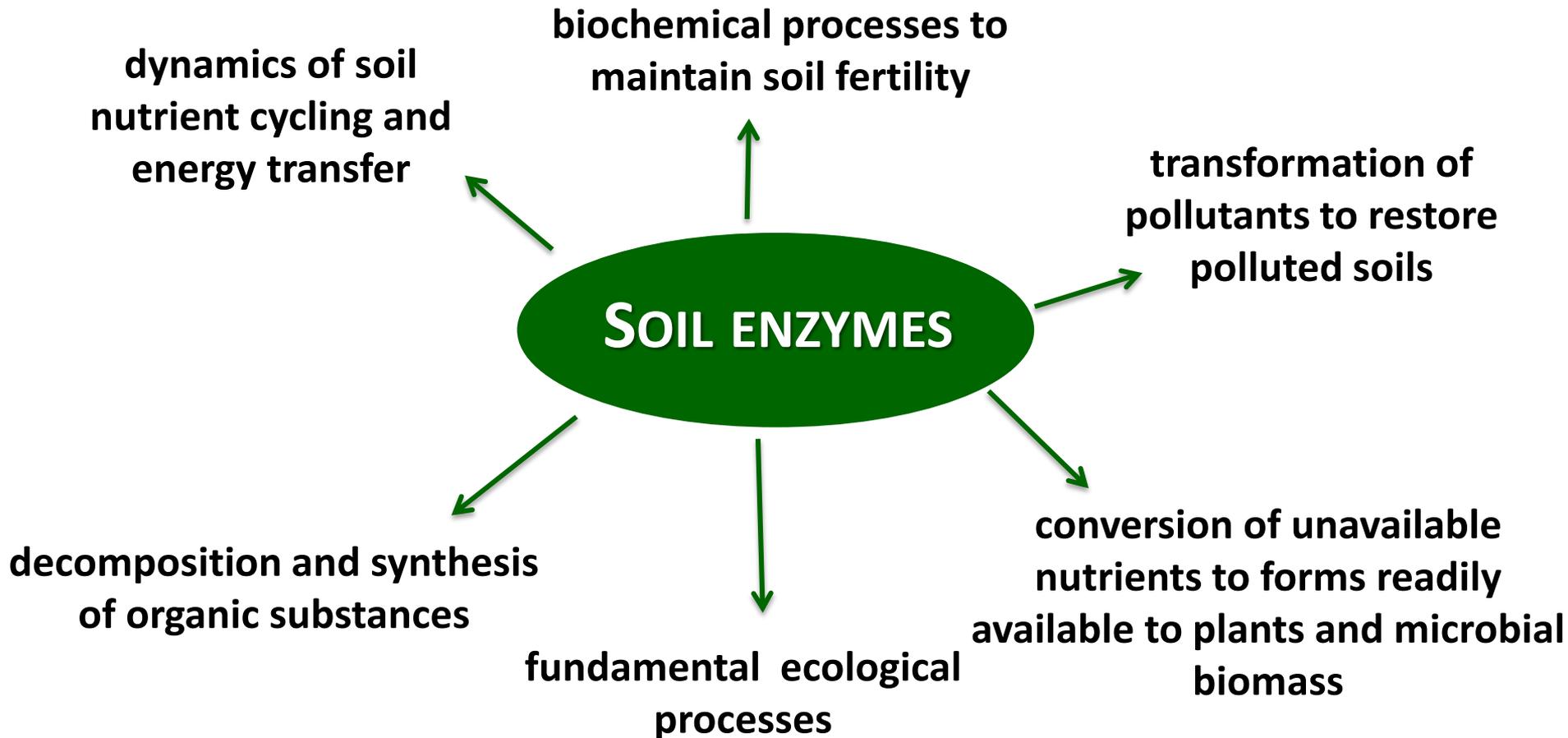
# Soil enzymes

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- ❖ Hydrolases
- ❖ Oxidoreductases
- ❖ Transferases
- ❖ Lyases



# Enzymes in the soil-plant-environment system



# Bioindicators in monitoring hydrocarbons removal

Bioindicator	Pollutant specificity	Sensitivity and range tested	References
<i>Enzymes</i>			
Soil lipase	Diesel oil, mineral oil	Sensitive; up to 1 mg g <sup>-1</sup> soil	Margesin et al. (1999, 2000)
Soil dehydrogenase	Crude oil and refined petroleum products	Moderately sensitive; 20–60% (w/w) oil/dry soil.	Frankenberger and Johanson (1982)
Urease and catalase	Mineral oil	Less sensitive; detectable at high TPH concentration (5000 mg kg <sup>-1</sup> soil)	Margesin et al. (2000)
<i>Seed germination</i>			
Prairie grass (Canada blue grass and slender wheatgrass)	Aromatics (Halogenated)	Sensitive; 13–133 µg kg <sup>-1</sup> soil	Wang and Freemark (1995), Siciliano et al. (1997)
<i>L. sativum</i>	PAHs	Moderately sensitive, 50–1000 mg kg <sup>-1</sup> soil	Maila and Cloete (2002)
<i>Microbial biomass</i>			
	Oil contaminated soil	Moderately sensitive	Kandeler et al. (1994)
<i>Batteries of bioindicators</i>			
Microbial bioluminescence, earthworm and seed germination	Creosote, heavy, medium and light crude oils.	Moderately sensitive. Earthworm > seed germination > bioluminescence 25–17, 400 µg g <sup>-1</sup> soil.	Wang and Freemark (1995), Dorn et al. (1998), Marwood et al. (1998), Phillips et al. (2000), Shakir et al. (2002)

*Maila and Clote, 2005*

# Case study

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Combined biostimulation and bioaugmentation of  
a PCP contaminated soil

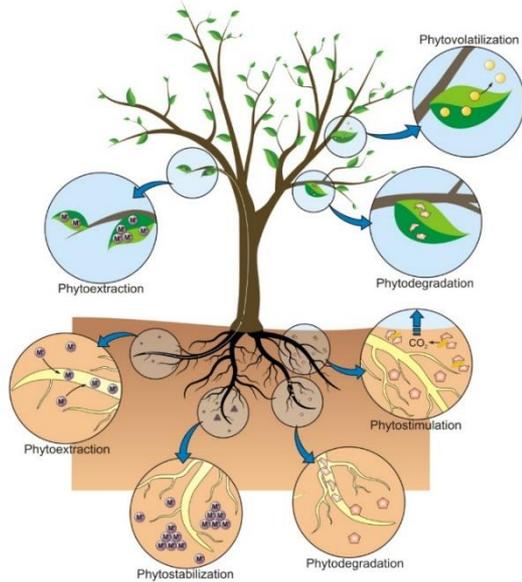
*Waremi et al. submitted*

## Case study – Main remarks

- Natural attenuation occurred in contaminated soil.
- Biostimulation treatments were efficient and successful in remediation process, though differences among composts appeared; conversely bioaugmentation alone led to no significant reduction in PCP concentration.
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- The combined biostimulation + bioaugmentation process achieved more efficient results.
- Ageing phenomenon plays an important role in the complex remediation process.

# Concluding remarks

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- The assessment of the soil quality recovery after bioremediation process could be carried out through the biochemical indicators.
- Besides microbial biomass C and respiration, soil enzymes are useful bioindicator of soil quality.
- However an holistic approach can lead to wide assessment and help to understand the complex processes occurring in soil.

**Thank you for your attention!**

