CNR
INSTITUTE OF ECOSYSTEM STUDY
PISA-ITALY

Research group

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Cristina Macci (permanent researcher)
Serena Doni (research contract)
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Technician: Fernando Di Giovanni
Students: PhD, master degree thesis, stages

Collaborations

Nationals:

- University of Pisa, Viterbo, Roma, Napoli, Firenze, Padova, Milano, Cagliari
- Acque S.p.A. (Pisa)
- San Giuliano Terme Municiality (Pisa)

Internationals:

- CSIC. Consejo Superior de Investigaciones Cientificas of Murcia, Madrid, Granada, Salamanca (Spain)
- Warwick University (United Kingdom)
- China University of Geoscience (China)
- Università BIOTERRA Bucarest (Romania)
- Colpos-Colegio de post graduados of Veracruz, Mexico

MAIN RESEARCH TOPICS

Soil quality and functionality and ecological techniques to recover stressed soil

2005-2008 European project ALMOND PRO-SOIL LIFE05/ENV "Soil protection in Mediterranean areas through cultivation of new varieties of almond tree"

Bioindicators to evaluate soil degradation and desertification

2004-2006 European Project INDEX. STREP n° 505450 "Indicators and Thresholds for Desertification, Soil Quality, and Remediation"

Bioremediation of polluted soils and sediments through ecological methodologies

2009-2012 European project AGRIPORT ECO/08/239065/SI2.532262 "Agricultural Reuse of Polluted Dredged Sediments"

2006-2012 National project financed by San Giuliano Terme Municipality "Ecological approach to remediate polluted soil located in Madonna dell'Acqua (San Giuliano Terme municipality) through natural technologies"

Valorization of organic residue (organic fraction of waste residues, Olive residues, biological sewage sludges) through biological techniques

2004-2012 **National project** financed by Acque S.p.A. (Pisa) "Phytomineralization of sewage sludge"

2000-2002 **National project** financed by San Giuliano Terme Municipality "Valorization of olive residues through vermicomposting process (*Eisenia foetida*)"

INNOVATIVE SYSTEM FOR THE BIOCHEMICAL RESTORATION AND MONITORING OF DEGRADED SOILS

BIOREM

LIFE11 ENV/IT/000113

Beneficiaries:

Coordinator Institute for Ecosystem Studies of the National Research Council
Dr. **Grazia Masciandaro**, e-mail: grazia.masciandaro@ise.cnr.it

Partners Azienda Pantanello-ALSIA, Italy
AMEK S.c.r.l., Italy
Consejo Superior De Investigaciones Científicas–Centro de Edafología y Biología Aplicada del Segura, Spain
Abonos Orgánicos Pedrín, Spain

Expected start date: 01/01/2013

Total budget	1 320 092,00 €
EU contribution	619 170,00 €

Expected end date: 31/03/2015

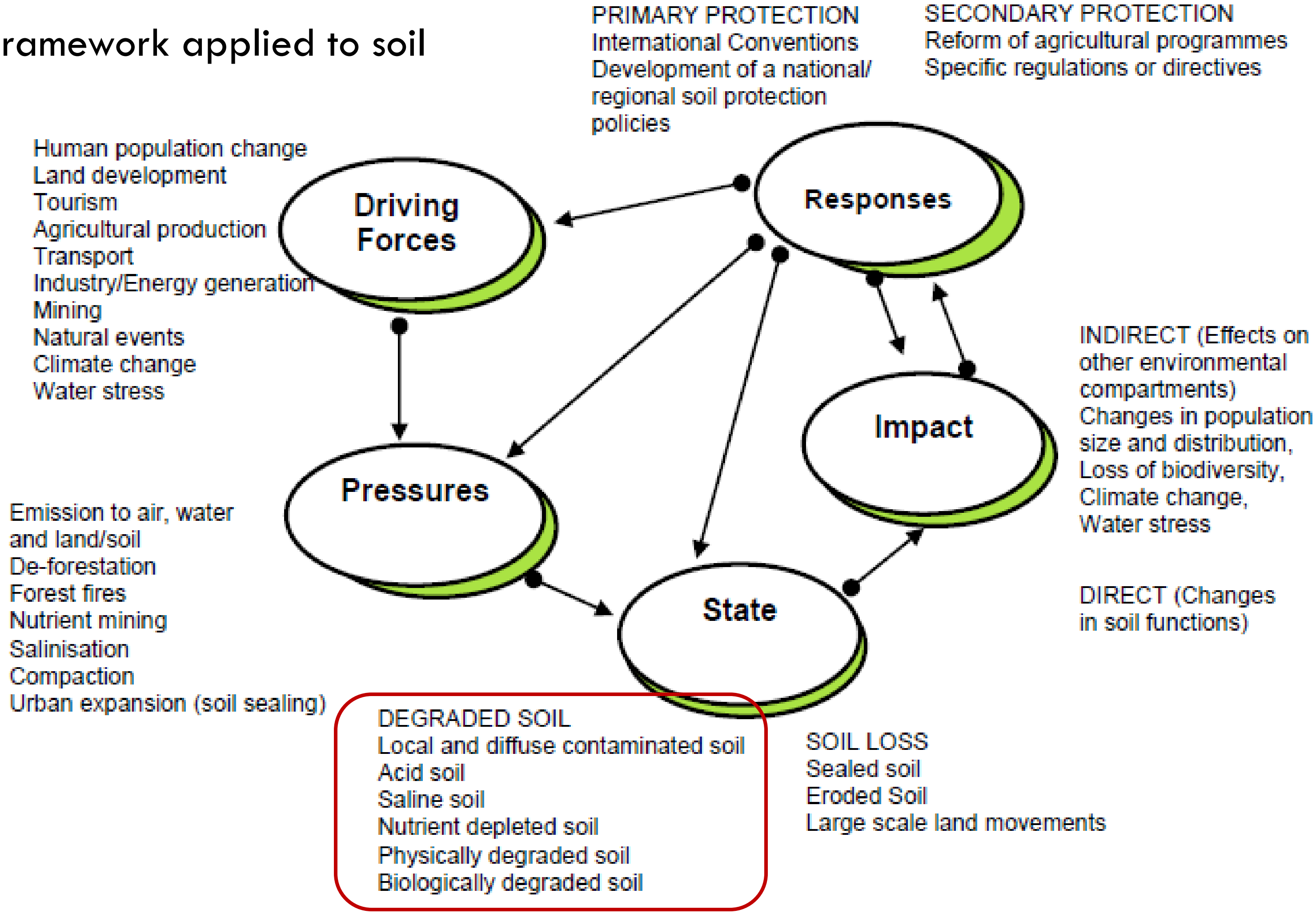
Soil degradation

- In the Mediterranean countries about 45% of the European soils have low organic-matter content, thus causing a decline in soil quality.

- Current methods for the assessment of soil conditions are mainly able to provide "static" physical, chemical, and biological reports about the status of soil in a given moment and, consequently, only short-term interventions can be planned

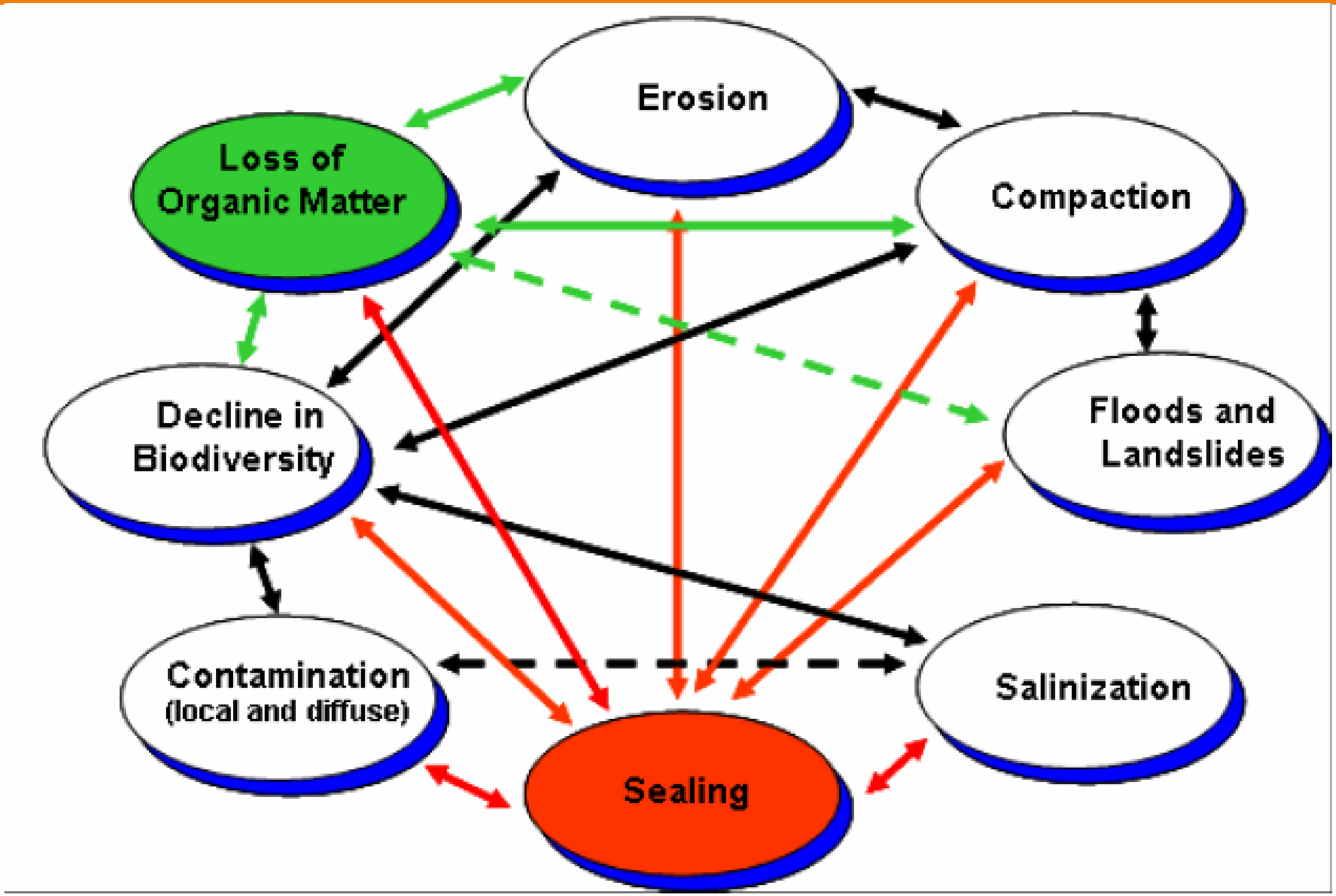
European soil thematic strategy for soil protection

DPSIR framework applied to soil



W. Blum Urban soils and the European soil thematic strategy. Symposium, Suitma Cairo 2005

Threats to soil and their functional interdependencies



W. Blum Urban soils and the European soil thematic strategy. Symposium, Suitma Cairo 2005

Project main objective

To demonstrate an innovative, **integrated methodology** for the restoration and biochemical monitoring of degraded soils.

- In terms of soil remediation, the project will demonstrate that the BIOREM strategy can successfully restore degraded soils, enhancing their physical-chemical properties and biochemical activity, and increasing fertility.

- In terms of soil monitoring, the project promises faster and dynamic monitoring of soil conditions.

Project specific objectives

- Restoration of degraded soils, taking into account current and planned uses, along with the provision of strategies and good practices for cost-effective restoration;

- Prevention of further soil degradation;

- Furthering of the ability of policy-makers and land managers in all sectors to devise and implement new and effective soil use and management plans.

SELECTED SOILS

Italy (North and South):

1) Imola (BO): *overexploited clayey* soil: **Imola**

2) *Overexploited sandy* and weakly developed soil: **Tebano**

3) Matera: soil characterized by *high erosion* and low water input: **Fusetto**

4) Matera: overexploited soil with *poor vegetable* cover: **Fontana**

5) Matera: poorly developed *semi-arid* soil; **Albicocco**

Spain (South):

6) Santomera: soil with *50% vegetation cover*: **Santomera Canas**

7) Santomera: soil with *25% vegetation cover*: **Santomera Entrada**

8) Abanilla: xeric soil with scarce content in organic matter and *vegetation (5-10%):* **Abaran**

9) Los Cuadros, Cieza: *stressed* soil: **Boqueron**

10) Cartagena: *sandy* and stony soil with a low and *fragile vegetable cover*: **Cartagena**

Italian sites

Imola

Tebano

Albicocco

Fontana

Fusetto

Spanish sites

Santomera Canas

Santomera Entrada

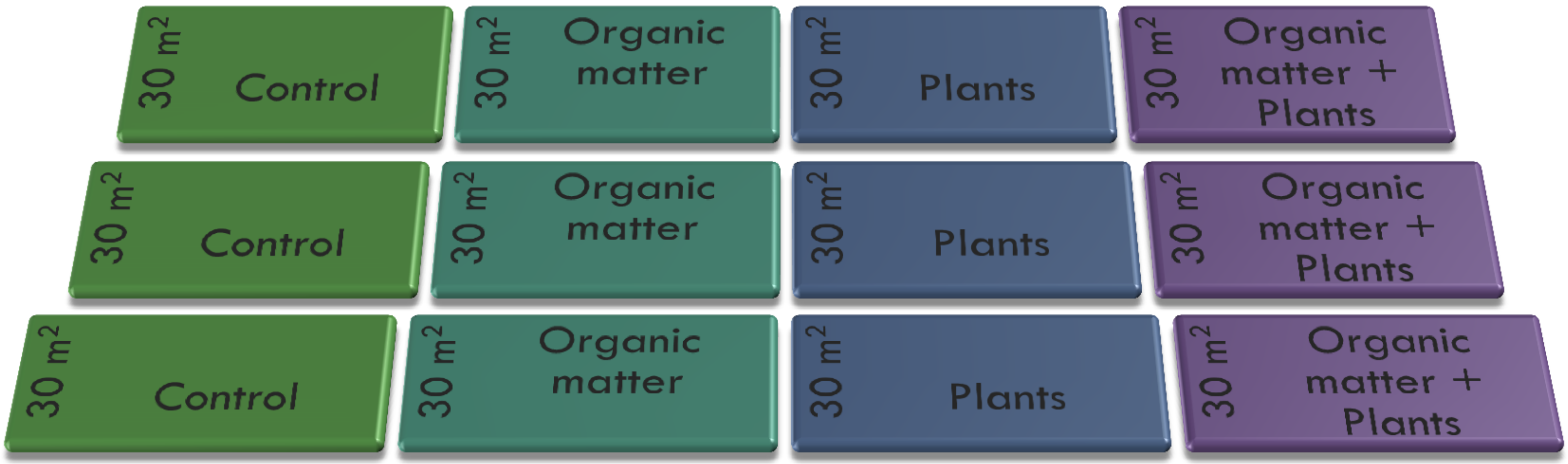
Boqueron

Abaran

Cartagena

TREATMENTS

Demostration plots (360 m^2 per site)



The following plants have been selected and planted: *Pino halepensis* and *Pistacia lentiscus*

The organic amendment (COMPOST) at the dose of 12 kg m^2 has been incorporated into the soil top layer (0-20 cm depth)

Why Plants?

- Vegetation holds soil in place

-Root exudates generate an environment that promote bacterial activity

Why Organic Matter?

STRATEGIES TO IMPROVE SOIL QUALITY

Preserve existing organic matter:
- ***Avoiding excessive tillage***
- ***Retaining crop residues***
- ***Preserving vegetation cover***

Add an adequate source of organic matter

Adding organic matter to soil
Types

FRESH ORGANIC MATTER
(e.g. **sewage sludge, organic fraction of urban residues**)

Short-term functionality:
Rich in available nutrients and substrates;
Rich in active microorganisms

Long-term functionality:
promotion of *in situ* formation of humic substances specific for that soil

STABILIZED ORGANIC MATTER
HUMUS
(compost, vermicompost)

Long-term effects on soil properties
Stabilized OM steadily sustains soil fertility, promotes C sequestration, preserves soil biodiversity

↓

Improvement of soil **resistance** to environmental impacts
Contribution to soil **resilience** during regeneration practices

BIOREM - Monitoring

Soil samples from all the 40 sub-plots will be analyzed **every six months:**

-immediately after the application of the remediation strategies

-6 months later

-12 months later

-18 months later

Each soil sample will consist of three subsamples collected at **0-20 and 20-40 cm**, mixed, homogenised, sieved (2 mm) and stored dried at room temperature until chemical analysis, and stored at 4°C until biological analysis.

During these months, we will perform the ordinary management and empirical monitoring of the involved soils.

Analysis of 960 composite soil samples

(10 sites x 4 treatments x 2 depth x 4 sampling times x three replicates)

BIOREM – Innovative diagnostic methodology

Plant Monitoring

- Superoxide dismutase enzyme expression and activity (biomarker of stress conditions)

Soil Monitoring

- **Physical-chemical parameters** (e.g. stability of aggregates, total organic carbon, etc.) → change slowly (static conditions)

- **Biological parameters:**

  Microbial biomass quantity and biodiversity (genomic analysis)

  Protein expressed by microorganisms (metaproteomic analysis)

- **Biochemical parameters:**

  Enzyme activities (β-glucosidase, phosphatase, dehydrogenase)

  Humic-bound enzymes

BIOINDICATORS AT ECOSYSTEM LEVEL

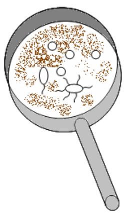
Very sensitive (dynamic conditions)

Soils remain **lifeless** without enzymes.

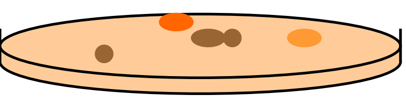
Microbial biomass quantity and biodiversity

Metagenomics is the study of genomic material obtained directly from the environment, instead of from culture.

Traditional approach



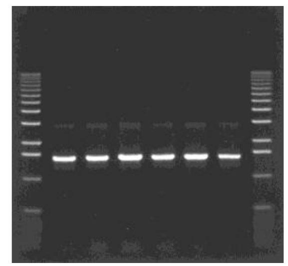
Colture *in vitro*



Each gram of soil contains about 10^9 microrganisms; ***most of these are NOT coltivable***

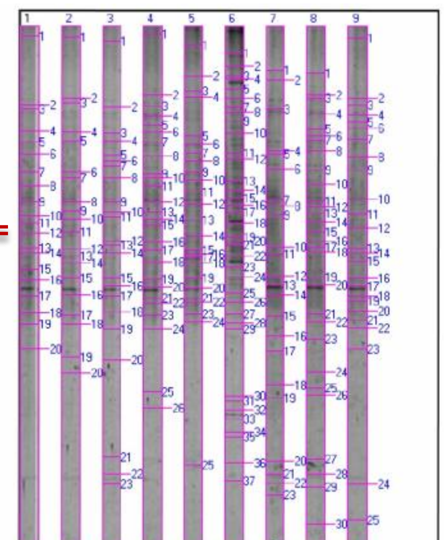
BIOMOLECULAR APPROACH

Direct DNA extraction

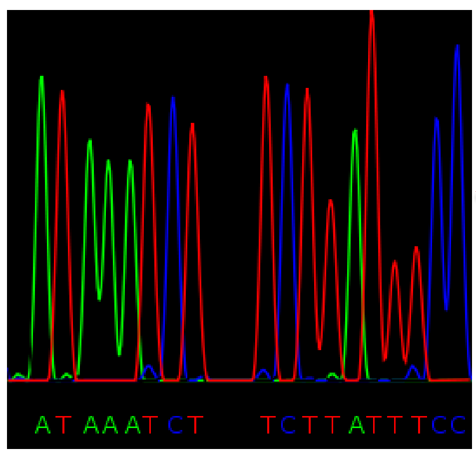


PCR - 16S rDNA Amplification

T-RFLP, DGGE



Evaluation of microbial diversity



DNA sequencing
Bioinformatic analysis

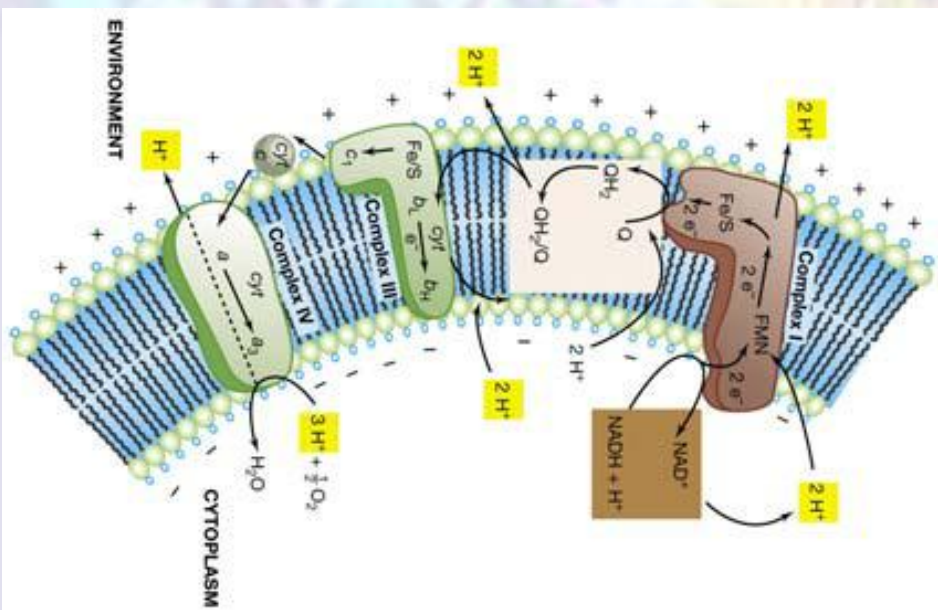
ENZYMES

Microbial biomass activity

Dehydrogenase activity

Indicator of global microbial metabolism

$$R\text{-}H_2 + NAD^+ \rightarrow NADH + R + H^+$$



Dehydrogenase catalyzes the oxidation of organic compounds with the removal of two hydrogen atoms that are transferred to the molecule of NAD^+

ENZYMES
Microbial biomass activity

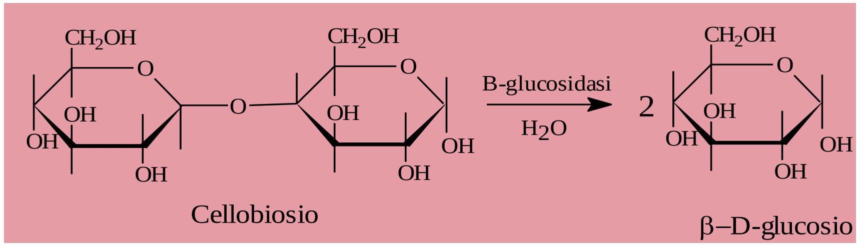
β-glucosidase

- **Carbon mineralization:**

β-Glucosidase catalyzes the final limiting step of cellulose degradation to glucose

- **Carbon sequestration/condensation:**

The decomposition stage is followed by condensation and polymerization reactions that create new larger molecules (humic substances) from the small molecules released during decomposition.



Phosphatase

- **Phosphatase catalyzes the hydrolysis of various organic phosphate esters to phosphate**

$$R\text{-}O\text{-}PO_3^{2-} + H_2O \longrightarrow R\text{-}O\text{-}H + HO\text{-}PO_3^{2-}$$

PHOSPHORUS CYCLE

- **P requirement is ensured by phosphatase activity which has, therefore, a main role in soil fertility**

ENZYMES

Enzymes are produced by microrganisms and are inside (intracellular enzymes) or released outside (extracellular enzymes) the cells

Extracellular enzymes can be found free or stabilised by mineral and organic compounds (humo-enzyme complexes

humic substances bind extracellular enzymes that (**humo-enzymes**) are **more stable** to some environmental stresses **than free enzymes** and can persist longer than microbial cells that produced them.

Humo-enzymes represent a ***sink*** of biochemical energy and slow-release nutrients capable to sustain the ecosystem functionality even in stressed situations; they are considered the last barrier against irreversible soil degradation (desertification) and are co-responsible of ***soil resilience***.

How to study humo-enzyme complexes

Sodium pyrophosphate is the favoured solution used to extract organic matter from soil, and, under neutral conditions, to study ***stabilized humic extracellular enzymes complexes***

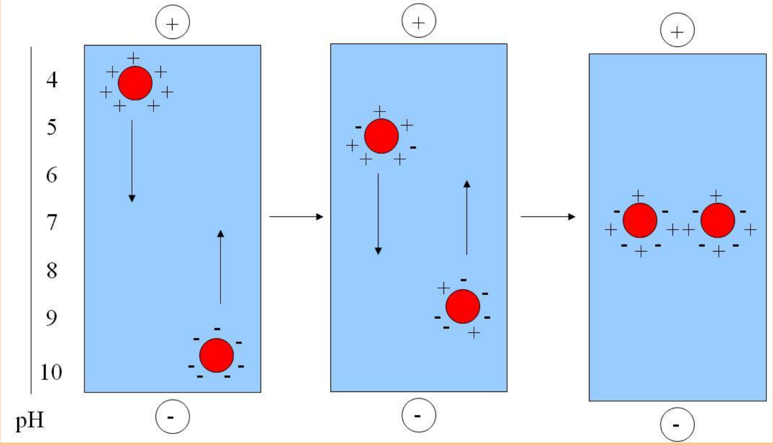
Efficient method to isolate, purify and characterise the enzymatically active fractions of SOM (extracellular humic–enzyme complexes) is based on three steps:
(1) **neutral pyrophosphate extraction** of humic matter,

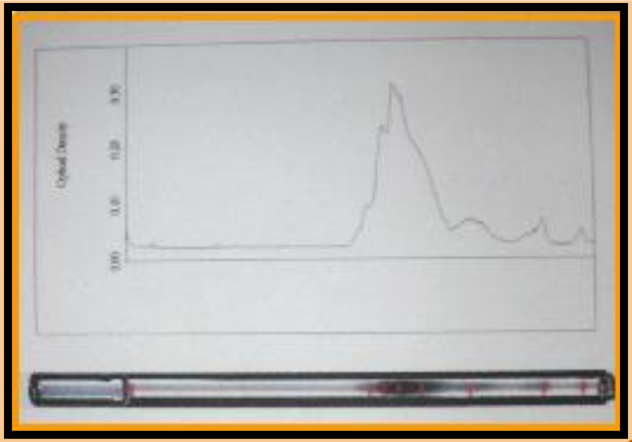
(2) **ultrafiltration** (UF) of the various components of the organic extracts on molecular mass exclusion membranes, followed by

(3)the analytical **isoelectric focussing** technique (IEF)

The IEF on polyacrylamide rod gels with a restricted pH gradient ranging between 6.0 and 4.0 gave different humic bands on the basis of the little differences of their electric charges (pI).

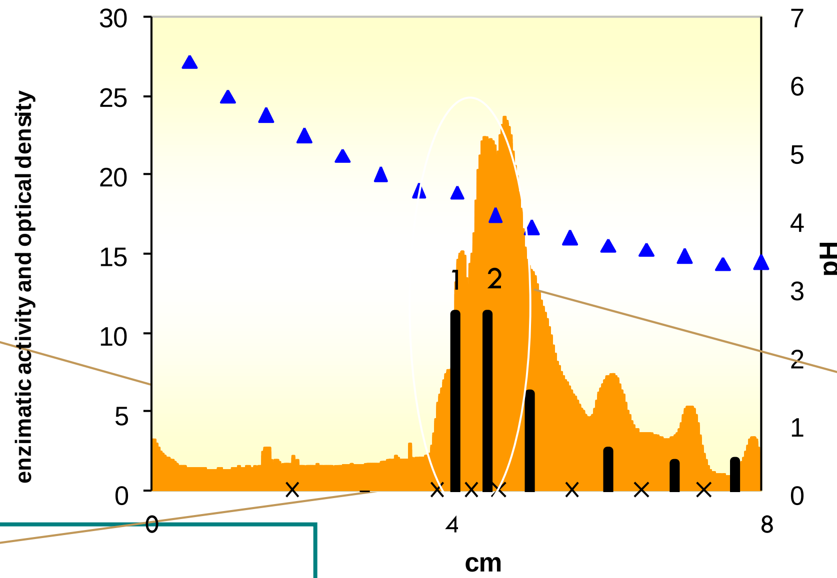
Analytical IEF separates humic–enzyme complexes on the basis of their surface electrical net charge





Enzyme activities in the IEF bands

Humic β-glucosidase activity:
β-glucosidase activity in the stable humic complex (bands 1 and 2) after IEF



Active humic carbon:
carbon amount calculated from the IEF peak areas focused in the pH range 4.5-4.0 (bands 1 and 2) after IEF

Specific Humic β-glucosidase activity:
Enzyme activity of the stable humic complex (bands 1 and 2) with respect to the focalised carbon content in the same bands

Expected results

Demonstration of the effectiveness of BIOREM's innovative restoration and monitoring strategy

Environmental benefits:

a) improvement of soil fertility and productivity:

25% increase in carbon content;

95% plant cover

b) reducing environmental impact such as water contamination and atmospheric emissions

annual carbon sequestration of 130 g cm^{-2}

adsorption of water and nutrients from the soil by the tree roots, reducing potentially dangerous leaching (eg. nitrate) into groundwater

c) protecting habitats or ecosystems

120% increase in microbial content and biodiversity

100% reduction in organic matter loss

95% reduction of water-caused erosion

Positive technical and socio-economical impact deriving from BIOREM strategy