Soil Biology and Fertility (NRM-213)

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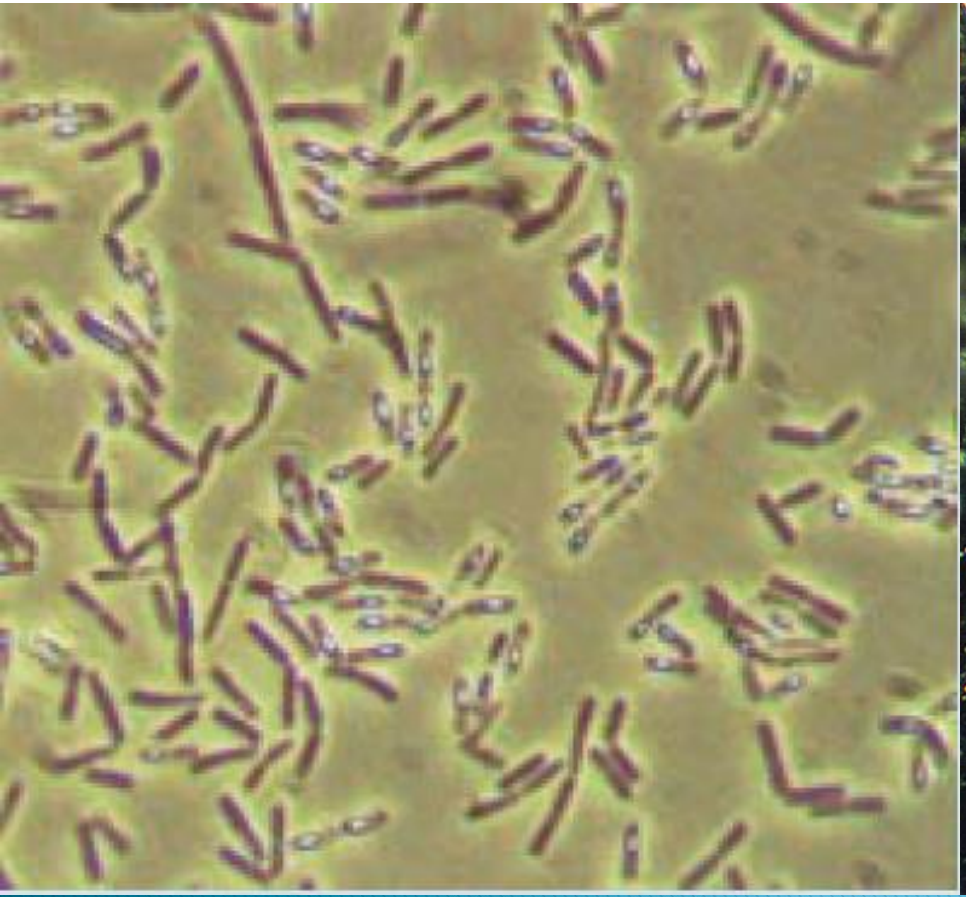
Microorganisms as indicators of soil health



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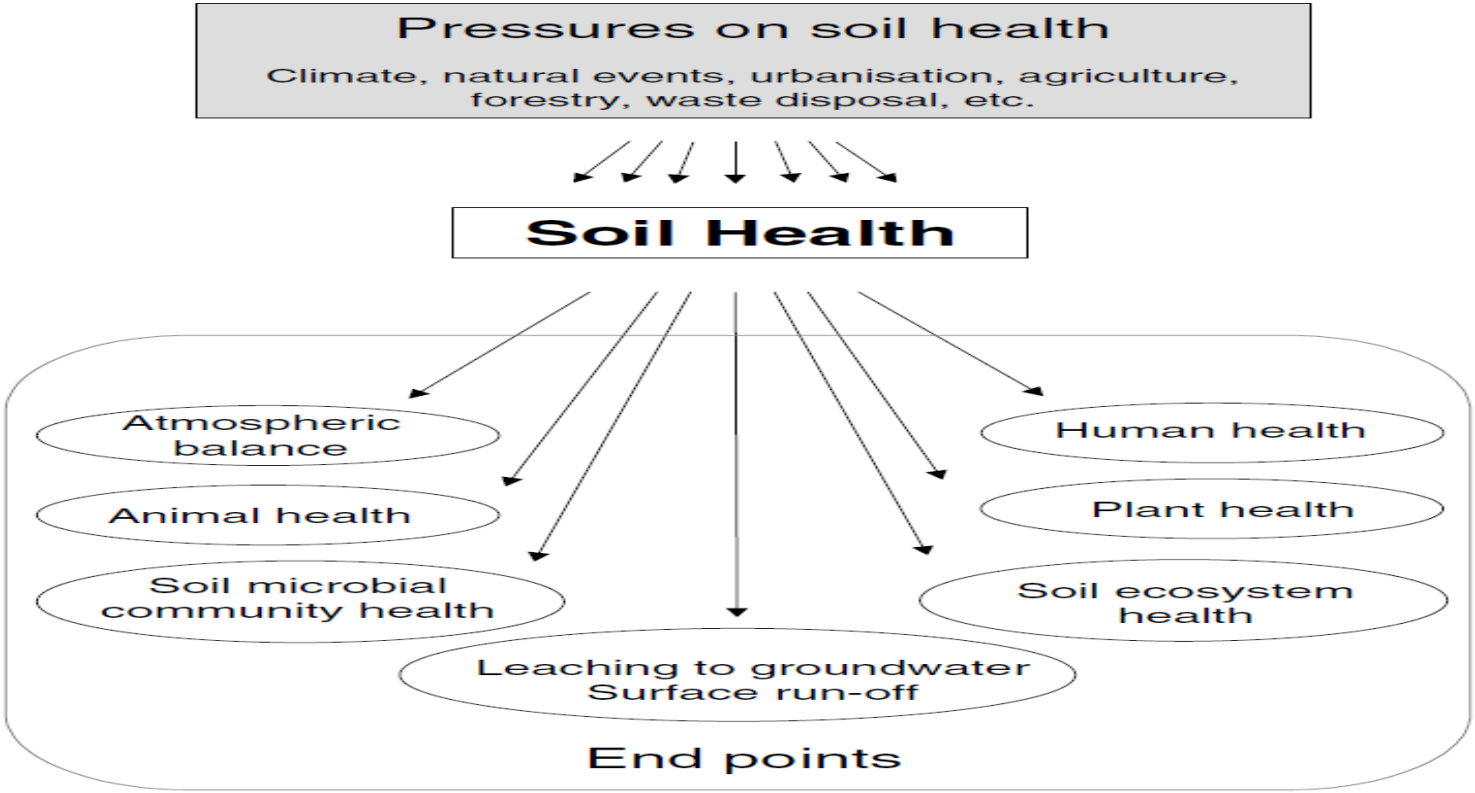
Introduction

- Soil health is the result of continuous conservation and degradation processes and represents the continued capacity of soil to function as a vital living ecosystem.
- A unique balance of chemical, physical and biological components contribute to maintaining soil health.



- **Microbial indicator-** A microbial parameter that represents properties of the environment which can be interpreted beyond the information that the measured or observed parameter represents by itself
- Environmental indicators must fulfil the following three basic criteria.
  - Policy relevance and utility for users
  - Analytical soundness
  - Measurability
- **Criteria specific for soil health indicators**

1. Correlated with ecosystem processes
2. Integrated with soil physical, chemical, and biological properties
3. Cost effectiveness
4. Responsive to variations in management and climate at an appropriate time scale
5. Compatible with existing soil data bases .

End point of soil health	Soil ecosystem parameter	Proposed microbial indicator
Atmospheric balance	C-cycling	Methane oxidation
Soil ecosystem health	Biomass	Microbial biomass (direct method)
	C-cycling	Decomposition of organic matter
	N-cycling	N-mineralisation
	Biodiversity	Genetic diversity Functional diversity Structural diversity
	Key species	Mycorrhiza
Soil microbial community health	C-cycling	Decomposition of organic matter
	Microbial activity	Bacterial DNA / protein synthesis
	Biodiversity	Genetic diversity Functional diversity Structural diversity
	Bioavailability	Biosensor bacteria
Leaching to groundwater or surface run-off	N-cycling	N-mineralisation
	Bioavailability	Biosensor bacteria
Plant health	N-cycling	N-mineralisation
	Key species	Mycorrhiza
Animal health	Biomass	Protozoa biomass
	Bioavailability	Antibiotic-resistant bacteria
Human health	Bioavailability	Antibiotic-resistant bacteria
	Key species	Human pathogens

Methods of measurement

1.***in vitro* measurements**

Example-**soil respiration, chloroform fumigation incubation method(CFI) ,chloroform fumigation extraction method (CFE), Substrate induced respiration (SIR), N-mineralisation, nitrification, denitrification, MPN and other growth-based methods**

2. ***In situ measurements***

- *based either on direct measurements* in the field or fixed samples analysed in the laboratory

Example- ***gas emissions*, phospholipid fatty acid (PLFA) , organic matter decomposition, thymidine and leucine incorporation, short-term enzyme assays and most molecular methods**

- **phospholipid fatty acid (PLFA)** analysis provides information about **soil microbial biomass fungal-bacterial ratio, biodiversity and occurrence of key species**

- **Substrate induced respiration (SIR)** provides measurement of **basal respiration and soil biomass.**

- **carbon utilisation pattern (BIOLOGTM)** provides a **profile of the microbial community** and information on **potential metabolic capacity**, which together comprise functional diversity.

Indicators of biodiversity

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Soil microbial community health	**Biodiversity**	Genetic diversity Functional diversity Structural diversity

- Diversity of a microbial community is often described by the **Shannon-Weaver index (H')**
- The genetic diversity of soil microorganisms is an indicator of the genetic resource.

Microbial genetic diversity	Methods of study
Bacterial genetic diversity	Diversity of the **16S rDNA** genes by - **PCR-DGGE** - **T-RFLP** - **Terminal Restriction Fragment Length Polymorphism (T-RFLP)**
Fungal genetic diversity	1. Number and morphology of fruiting bodies. 2. Molecular methods based on 18S rDNA by - **PCR-DGGE** **- PCRTGGE**
Protozoan genetic diversity	1. Taxonomic features 2. Molecular methods- by **PCR-DGGE** targeting an **18S rDNA** fragment (specific to ***Kinetoplastida***)

- Functional diversity of microbial populations in soil may be determined by either expression of different enzymes or diversity of nucleic acids within cells .

Microbial functional diversity	Methods of study	
Carbon utilisation patterns	(BIOLOG™ assay)	- Result of the assay is a **qualitative physiological profile** of the potential functions within the microbial community.
Enzyme pattern	Incubation of the soil extract with commercial **fluorogenic enzyme substrates (4-methylumbelliferin (MUF) and 4-methylcoumarinyl-7-amide (MC)** or **colometric substrates (remazol brilliant blue, p-nitrophenol or tetrazolium salt)** coupled with **specific compounds of interest (e.g. cellulose or phosphate).**	
Diversity of mRNA	**Reverse transcription PCR(RT-PCR) method**	nucleotide sequences of mRNA molecules reflect the type of enzymes synthesised

Structural diversity

- fingerprint of the relative Phospholipid fatty acids (PLFAs) composition of the resident microbial community.
- PLFAs are extracted from soil samples and subsequently analysed by gas chromatography
- ratio of oligotrophs to copiotrophs reflect the nutrient stress tolerance of the species present in soil.
  - **High ratio**, e.g. dominance of oligotrophs indicate stable environmental conditions with low substrate availability.
  - **low ratio**, e.g. dominance of copiotrophs indicate an environment regularly receiving input of organic rich substrate, e.g. addition of sewage sludge or pesticides.
- The ratio of oligotrophs to copiotrophs can be determined by **colony appearance on agar substrates or rRNA-expression in bacterial microcolonies.**

Indicators of carbon cycling

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Soil microbial community health Atmospheric balance	**Carbon cycling**	Soil respiration Organic matter decomposition Soil enzymes Methane oxidation

Indicators	Methods of study	
Soil respiration	**CO2 production** **O2 consumption**	indicator of pesticide and heavy metal toxicity.
	metabolic quotient (qCO2)	microbial respiration rate (measured as **evolution of CO2**) **per unit microbial biomass**
Organic matter decomposition	**Field incubation** of different types of **plant litter** or more standardised pieces such as **cotton strips** and **wood sticks** .	
Soil enzymes	**1.Enzyme specific assays** **2.Hydrolysis of the fluorescent fluorescein diacetate** (protease, lipase, esterase) **3.dye reaction** followed by a **spectrophotometric measurement**.	Early indicators of changes in soil health
Methane oxidation	1. **fluorescent *in situ hybridisation (FISH)*** 2.**standardgrowth-dependent MPN counts** 3.**PCR-DGGE using methanotrophs-specific 16S rDNA primers**	indicator of potential greenhouse gas consumption.

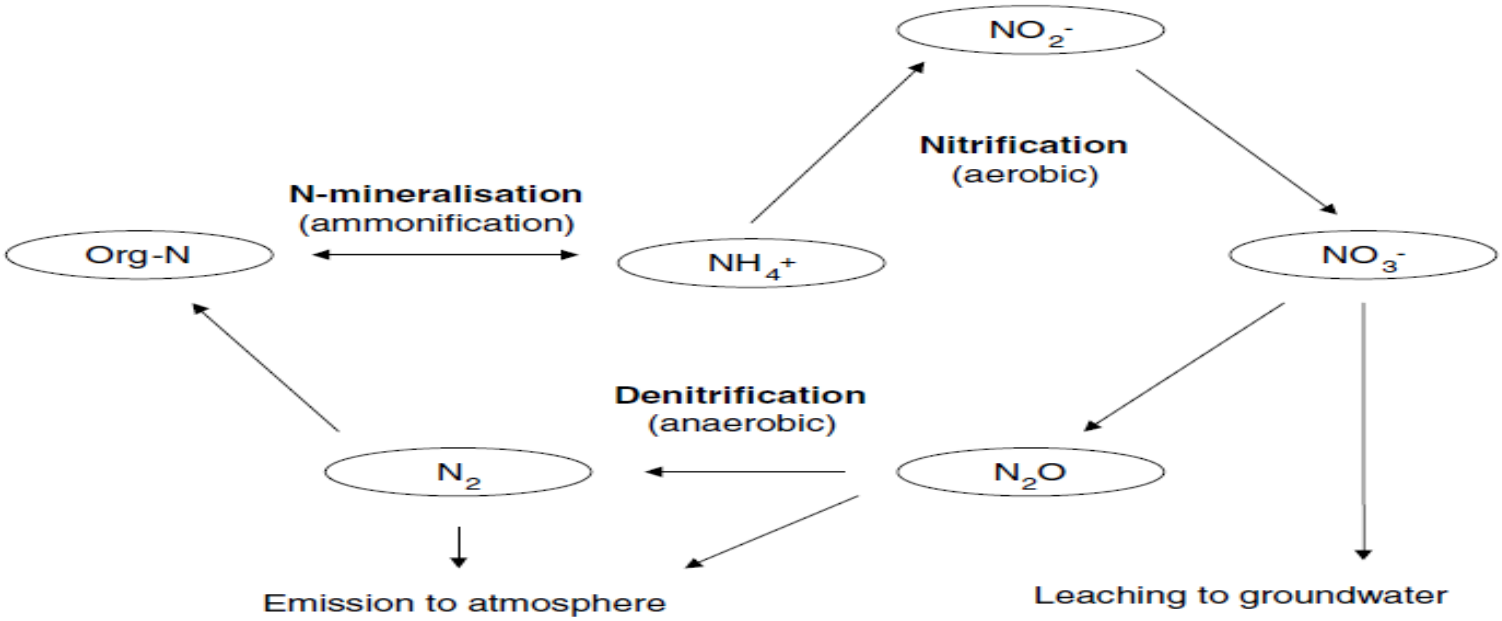
Soil enzymes as indicators of soil health.

Soil enzyme	Enzyme reaction	Indicator of
Dehydrogenase	Electron transport system	Microbial activity
Beta-glucosidase	Cellobiose hydrolysis	C-cycling
Cellulase	Cellulose hydrolysis	C- cycling
Phenol oxidase	Lignin hydrolysis	C- cycling
Urease	Urea hydrolysis	N- cycling
Amidase	N-mineralisation	N- cycling
Phosphatase	Release of PO_4^-	P- cycling
Arylsulphatase	Release of SO_4^-	S- cycling
Soil enzymes	Hydrolysis	General OM* degradative enzyme activities

Indicators of nitrogen cycling

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Plant health Leaching to groundwa-ter Surface run-off Atmospheric balance	Nitrogen cycling	N-mineralisation Nitrification Denitrification N-fixation

Global cycling of nitrogen



Indicators	Methods of study	
N-mineralisation	accumulation of NH4 + in soil slurry under aerobic conditions	reflects the **potential N-mineralisation in soil**
Nitrification	**Ammonium oxidising assay (**soil slurry is incubated with **excess ammonium** and **chlorate**)	reflect the population size of the **nitrifiers**
Dentrification	Acetylene inhibition (**nitrous oxide** is measured by **gas chromatography**.)	1.Indicate deposition of ammonia in N-limited habitats 2. historical anaerobic situations
N-fixation		
1.Rhizobium	**1.Simple pot test** 2. **Molecular method**- plasmid profiles and insertion sequence fingerprints, 16S-23S rDNA spacer sequences, PCR detection of specific genes ,*colony hybridisation , RFLP and RAPD.*	symbiotic
2. Cyanobacteria	1. **MPN methods** 2. **nitrogenase activity** by **acetylene reduction assay**	-non-symbiotic - indicators of heavy metal contamination

Indicators of soil biomass

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health	Soil biomass	Microbial biomass Protozoan biomass

Indicators	Methods of study	
Microbial biomass	**1.Direct** (a) **Microscopy** **(** b)determinations of **specific membrane phospholipid fatty acids (PLFAs))** **2. indirect** **(a) chloroform fumigation incubation method(CFI)** , (b)**chloroform fumigation extraction method (CFE)** (c)**substrate induced respiration (SIR)**.	-fraction of the soil responsible for the **energy and nutrient cycling** and the **regulation of organic matter transformation.** **-** The release of CO2 after fumigation is the result of germinating microbial spores utilising the C substrate provided by the killed microbial cells. - substrate induced respiration measures only the metabolically active portion of the microbial biomass - The **fungal-bacterial biomass ratio** can also be determined directly from measurements of **fungal-specific and bacterial-specific PLFAs** - higher **fungal-bacterial biomass ratio** is typical of long-term unfertilised soil.
fungal biomass	(a)quantification of fungal specific membrane molecules such as **ergosterol** *or* specific phospholipids (PLFAs) except oomycetous fungi (b)total hyphal length (c)Quantification of enzyme activities such as **fluorescein diacetate hydrolytic activity (FDA)** or **N-acetyl-beta-glucosaminidase (Nag) activity**	
Protozoan biomass	(a)counting directly by use of an inverted microscope (b) extraction followed by a MPN counting based on a growth medium	- applied to **heavy metal toxicity testing.**

Indicators of microbial activity

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Soil microbial community health	Microbial activity	Bacterial DNA synthesis Bacterial protein synthesis RNA measurements Bacteriophages

Indicators	Methods of study	
Bacterial DNA synthesis	incorporation of **3H-** or **14C-thymidine into bacterial DNA**	extensively used in **aquatic environments**
Bacterial protein synthesis	incorporation of **3H or 14C leucine**	More accurate than that of DNA synthesis due to higher protein contents in cell.
RNA measurements	1. **fluorescent *in situ hybridisation* (FISH)** 2. **Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)**	
Bacteriophages	standard method of extraction followed by a **plaque-assay** with specific host bacteria, e.g. *Pseudomonas* , *Bacillus* ,*Rhizobium* , *Azospirillum brasilense* , *Serratia liquefaciens*	-indicator of the activity of specific soil bacteria.

Key species

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil ecosystem health Plant health Animal health Human health	**Key species**	Mycorrhiza Suppressive soil Human pathogens

Indicators	Methods of study	
Mycorrhiza	1.extraction of spores from soil samples and subsequent counting in a microscope 2. direct detection- **18S rDNA PCR, nested PCR and AM-specific PLFAs**	1.**ectomycorrhizal** (mainly forest trees), 2.**arbuscular mycorrhizal** (terrestrial plants) 3.**ericoid mycorrhizal** (heather) fungi. - Helps in **increased P availability** due to **extra-radical mycelium.**
Suppressive soil	inoculation of target-plant seeds directly into the test soil or into a pathogen-infested test soil	able to suppress specific plant diseases by inherent biotic and abiotic factors which acts as indicator of plant health.
Human pathogens	1.**Cultivation** - **XLD agar** for ***Salmonella*** *and* ***Shigella*** *and* ***MacConkey agar*** *for* ***isolation of coliform.*** *2.**Molecular***- **quantitative PCR** *and* **specific fluorescent oligo-nucleotide probes**.	-**Source** -amendment with manure and sewage sludge. - indicator of **human health** e.g - ***Escherichia coli***

Indicators of bioavailability

End points	*Soil ecosystem parameter*	*Microbial indicators*
Soil microbial community health Leaching to groundwater Surface run-off	**Bioavailability**	Biosensor bacteria Plasmid-containing bacteria Antibiotic-resistant bacteria Catabolic genes

Indicators	Methods of study	
Biosensor bacteria	fibre optic linked membrane bound biosensor probes facilitate *in situ ecotoxicity monitoring of soil*	designed to respond to certain **stress situations** (e.g. toxicity) through the use of **reporter genes** e.g- **mercury ,chromate , *zink*** *toxicity*
Plasmid-containing bacteria	1. **endogenous approach**- plasmids extraction from soil bacteria on agar plates followed by a visualisation of the plasmids on agarose gels **2.exogenous approach.** plasmid free bacteria are mixed with a soil sample and allowed time to conjugate naturally occurring plasmids from the indigenous bacteria. Then Plasmids are extracted and visualised as in the endogenous approach.	**higher in polluted soils compared to agricultural soils**
Antibiotic resistant bacteria	1.**Cultivation** on selective growth media containing antibiotics (tetracycline ,kanamycin, etc.) 2.**Molecular methods**- **PCR** and **molecular gene probe analysis**	More in Urban effluents - indicator of industrial and urban pollution
Incidence and expression of catabolic genes	1.**Conventional culturing of degradative microorganisms** 2.**activity measurements of specific degradative key enzymes** 3.**molecular methods for detection of catabolic genes**	gives information on the ability of a soil to modify or degrade xenobiotic compounds.

Conclusion

- Soil microorganisms appear to be very suitable and sensitive early warning indicators or predictive tools in soil health monitoring.

- soil microorganisms respond rapidly to stress by adjusting
  (i) activity rates
  (ii) biomass
  (iii) community structure.

Reference

- **Microorganisms as indicators of soil health *NERI Technical Report No. 388***
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Thank you