

Sampling and testing protocols for soil health and biodiversityinitial version

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² PU=Public, CO=Confidential, only for members of the consortium (including the Commission Services), CI=Classified



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¹ R=Document, report; DEM=Demonstrator, pilot, prototype; DEC=website, patent fillings, videos, etc.; OTHER=other



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1 Introduction

NBSoil project is aimed at developing an effective pathway of learning for, so called, "soil advisors" – specialists equipped with a knowledge on how to manage soils to achieve a better soil health, as it is assumed in the Soil Mission of the European Union. The soil advisory wil lbe dedicated to a range of land use types: agricultural, forest, urban and post(industrial).

The impact of the innovative soil advisory proces will be based on the effective advisory tools and provision of up-to-date knowledge on the use of nature based solutions (NBS) in soil management. One the goals of NBSoil is, therefore, to integrate and update the knowledge on the effects of NBS on soil, especially on implementation of NBS for improving the soil health.

The WP2 of NBSoil is aimed at integration of the knowledge base on appropriate soil management in order to transfer this knowledge to soil advisors. The knowledge will be integrated through review of the existing information, based on literature and other projects but also through additinal data collection, using research sites of the NBSoil.

Task 1.2 starts with developing a plan for soil field and laboratory measurements enabling a full picture of the NBS effects on soil health, with the special emphasis given to soil biodiversity. Soil samples collected within research sites, representing all groups of NBS, will be tested for the range of soil parameters, enabling translation of NBS effects to soil status and the related ecosystem services.



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2 Soil health and soil health indicators

According the Soil Mission, the soil health is defined as "the continued capacity of soils to support ecosystem services" and is assessed through a set of proposed, measurable indicators.

The categories of Soil Mission indicators used to characterise a status of soil health are as described below. These categories will be a basis for defining a list of indicators to be used in the NBSoil research sites to characterise and demonstrate NBS effects for soil.

Presence of soil pollutants, excess nutrients and salts.

This category groups indicators expressing the content of compounds that can be treated as soil contamination: potentially toxic trace elements (PTTE), organic compunds (e.g. polycyclic aromatic hydrocarbons – PAHs, pesticide residues), nutrients or salts, if exist in too high amounts in soil. Too high content of contaminants inhibits soil biological activity and creates a risk of contaminant transfer to food, feed and water, reducing soil ecosystem services.

Soil organic carbon.

Organic carbon affects water retention in soil, crop resistance to water deficit, improves soil structure and soil biodiversity. Increase in soil organic carbon is a process of CO2 sequestration. Therefore such indicators as soil organic content or soil carbon stock belong to major soil health indicators.

Soil structure including bulk density and the absence of soil sealing and erosion.

This is a broad group of indicators that can describe physical condition of soil across a range of land use types. They refer to agricultural (soil structure, erosion resistance) and urban land (absence of soil sealing). Good status of these types of indicators enables appropriate biomass growth and infiltration of rainwater to prevent runoff and soil loss.

Soil biodiversity.

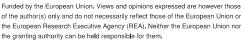
Numerous indicators can be used to characterise soil biodiversity – total abundance of organisms or abundance of microorganisms with certain functions in soil metabolism, functional diversity, genetic diversity, acticity of microbially driven processes, etc. Soil biodiversity is important for such soil functions as soil structure (and related water infiltration), liter decomposition, organic carbon storage and nutrients cycling.

Soil nutrients and pH.

This group includes essential nutrients for plant umer (N, P, K, S, Ca and micronutrients - e.g. boron (B), chlorine (Cl), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn)). Their



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deficiencies in soil might limit plant growth and negatively affect crop quality. Soil pH affects many chemical and biological processes, including plant nutrients availability and the balance and functions of soil microbial communities. Soil acidity reduces nutrient use efficiency, accelerates N leaching, reduces soil carbon accumulation, hampers soil structure and, thus, water retention in soil.

Vegetation cover.

Different type of indicators related to the coverage of soil soil surface. The duration and diversity of the vegetation cover and its net primary productivity is essential for soil health since it reduces erosion and loss of nutrients, provides additional nutrients and carbon input to soil. It is also beneficial for soil biodiversity. In urban settings vegetation cover counteracts heat islands and is beneficial for air quality.

Landscape heterogeneity.

It refers to diversity of land in agriculture (field size, fragmentation, presence of natural green elements), forestry (diversity of species, clear-cuts with bare land) and urban space (diversity and density of green infrastructures). The diversity of landscape elements affects overall biodiversity and resistance of the ecosystem to pressures, the water cycle and soil water and wind erosion.

Forest and other wooded lands area.

This category can be expressed by the umer of species, the share of nonnative tree species, and the proportion of natural and artificial regeneration. In forests, soil health is affected by naturalness in terms of species composition and management practices, and disturbance by forest clearance.



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Nature Based Solutions tested in NBSoil 3

NBSoil project addresses a wide range of NBS that can be important for sustaining or imroving soil health.

Production of organic fertilisers from locally available biowastes

Biowaste based biofiertilisers have the potential to reduce the GHG and carbon footprint of the processes, reducing the need for chemical fertilisation and increasing crop resistance to drought, thanks to improved water retention in soil. The produced biofertilizers range from common materials derived from anaerobic digestion or composting to the newer biostimulants as hydrolysates of plant and animal proteins, humic and fulvic substances, seaweed extracts, vegetal extracts, and beneficial microorganisms.

Cover crops for agricultural soils

This group of NBS includes cover crops but also intercropping, crop rotation, mixed farming, rotational grazing, shift from arable to grassland and agroforestry. All these practices can be beneficial for SOC stock, erosion resistance, soil biodoversity and nutrient balance.

Paludiculture for organic soils

Loss of SOC in organic soil is one of major threats to organic carbon stock and environmental stability of landscape. The principle of paludiculture is to develop a model for peatland rewetting and restoration, including grazing by breeds adapted to wet conditions. This model of paludiculture is expected to revert the following threats: changes in water level, mineralisation of soil C stocks, unexpected changes in plant succession, natural habitats loss, inland and sea water eutrophication.

Management for age and species diversification for forest soils

Forest management based on harvesting, thinning, and replanting methods leading to age and species diversification in both commercial tree plantations and natural secondary forests, are practices aimed at reducing erosion, risk of fires and landslides, and at larger scale promoting water quality and reducing flood risk in case of heavy rains.

Bioremediation for urban and industrial soils

Application of NBS in remediating polluted soils in-situ is aimed at restoring soil health and ecosystem services, especially in indutrial and urban land. In general, the practices shall reduce the risk related to contamination through inactivation or decomposition of contaminants, combined with improvement of soil capability to provide ecosystem services (biomass growth, water retention, erosion resistance). The use of green technologies to restore soils polluted with PTTE (phytoremediation through stimulation of contaminant decay or phytostabilisation via addition of organic amendments and revegetation) will be implemented.

Blue - green infrastructure in urban areas



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This category of NBS refers to urban zones and covers the integration of vegetation in urban development, to reduce soil sealing, improve soil permeability, and water cycle management and prevent disasters such floods, in urban and periurban areas. This will also cover urban agriculture.



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NBS and the soil challenges addressed 4

NBSoil project assumes that NBS tested would contribute to the Soil Mission objectives. This contribution can be evaluated and demonstrated through a range of indicators. The major indicators of the contribution to the Soil Mission objectives are presented in Table 1. Intensity of green color presents a contribution of particular NBS to the Mission objectives.

Those indicators shall be accompanied by additional indicators characterising soil health since appropriate agricultural or urban soil management shall ensure overall soil health (soil ability to support ecosystem services).

TABLE 1

Soil Mission Objectives	Major direct indicators considered	OF*	сс	PAL	FD	BREM	BGI
reduce desertification	Erosion rate, SOC content and stock, soil structure, water retention						
conserve soil organic carbon stocks	SOC content and stock, bulk density						
stop soil sealing and increase re-use of urban soils							
reduce soil pollution and enhance restoration	Contaminant total content and mobility						
prevent erosion	Erosion rate or its surrogate (soil stability)						
improve soil structure to enhance soil biodiversity	Water infiltration, soil functional diversity, activity of microbial processes, earthworm abundance, soil compaction						

*OF – organic fertilisers

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CC – cover crops PAL - paludiculture FD – forest diversification BREM - bioremediation BGI – blue and green infrastructure.



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5 Test areas and soil sampling

Research sites of NBSoil will address all the NBS categories considered in the project (organic fertilisers from locally available biowastes, cover crops, paludiculture, forest diversification, bioremediation, and blue - green infrastructure). Some NBS tesearch sites will be represented in several locations, depending on the partner involvement. This will ensure representation of various pedoclinatic conditions.

Taking variability of NBS, the tailor made approach will be designed for soil sampling, in collaboration with the research site providers. The approach develped will take the following criteria into account:

- · size of the site
- number of plots and practices tested
- · dynamics of soil health indicators
- access characteristics.

The final list and characterisation of the Research Sites will be delivered in Month 9 of the project (end of August 2023) which is a milestone of NBSoil. At that stage all the research/demo sites will be already operational.

In order to harmonize and unify the process of soil sampling, the following standard on soil sampling will be considered: ISO 18400 (2018). The standard consists with the following parts, relevant to NBSoil:

Soil quality — Sampling — Part 102: Selection and application of sampling techniques

Soil quality — Sampling — Part 104: Strategies

- Soil quality Sampling Part 105: Packaging, transport, storage and preservation of samples
- Soil quality Sampling Part 202: Preliminary investigations
- Soil quality Sampling Part 203: Investigation of potentially contaminated sites

Soil quality — Sampling — Part 205: Guidance on the procedure for investigation of natural, nearnatural and cultivated sites

Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory

NBSoil will also follow the developments of GLOSOLAN on harmonisation of soil sampling protocols. GLOSOLAN is a Global Soil Laboratory Network that was initiated by Global Soil Partnership.



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6 Major and additional indicators in test areas

As planned and described in the Grant Agreement, evaluation of Research sites will rely on soil sampling and laboratory analysis of chemical and physical parameters of soil samples including soil carbon contents and pool, soil texture, pH, salinity, nutrient availability and dynamics, surrogates of soil erosion risk and soil stability, soil compaction. Soil biodiversity indicators will involve soil genetic diversity measurements, protocols for evaluating changes in the metabolic potential of microbial communities (Biolog EcoPlates), biochemical parameters (soil enzymes) describing intensity of microbially-driven soil processes, abundance of earthworms. Nutrient use efficiency (NUE) indices will be calculated for each of the agricultural research sites, in order to learn how NBS contribute to potential reduction of synthetic fertilisers.

At current stage of the project the following soil characteristics are considered for evaluation of NBS effects for soil health and reaching Soil MIssion objectives (Table 2). Major indicators refer to challenges addressed by particular NBS while the additional indicators refer to overall soil health and its ecosystem services.

TABLE 2

NBS	Organic biofertilisers
Major indicators	SOC content and stock, water retention
Additional indicators	Soil biodiversity indices, soil stability, soil nutrients availability, pH, soil compaction, NUE, mineral N reduction, soil texture
NBS	Cover crops
Major indicators	SOC content and stock, soil nutrients availability, soil erosion rate (soil stability)
Additional indicators	pH, soil biodiversity, NUE, N leaching, soil texture
NBS	Paludiculture
Major indicators	SOC content and stock
Additional indicators	Nutrients availability, nitrogen dynamics, soil biodiversity indices, soil
	texture
NBS	Forest diversification
Major indicators	SOC content and stock, erosion rate
Additional indicators	Water infiltration, soil biodiversity indices, soil texture, pH
NBS	Bioremediation
Major indicators	Pollutant total content, pollutant bioavailability (mobility, leaching))
Additional indicators	Pollutant fractionation, pH, texture, SOC content and stock, nutrient
	availability, soil biodiversity indices
NBS	Green and Blue infrastructure



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Major indicators	Soil sealing density, green area connectivity, water infiltration
Additional indicators	Temperature spatial distribution, erosion rate, SOC stock,

Soil biodiversity testing

NBSoil appreciates the role of soil biodiversity to delivery of soil ecosystem services, including cycling of nutrients and organic carbon, soil structure, crop resistance and crop yields. On the other hand capacity of scientific society and advisory services to valorise soil biodiversity is still limited. Therefore in NBSoil sepcial emphasis will be dedicated to soil biodiversity characterisation across research sites. This data will constitute a basis for learning materials on the role of soil biodiversity for soil health. Therefore a wide range of soil biodiversity indicators is planned to be used for the research sites samples. They might include:

• Evaluation of the functional diversity of soil microbial communities was performed using the Biolog EcoPlate system.

• Soil genetic diversity analysis via DNA extraction followed by the 16S rRNA gene sequencing.

• Determination of the soil enzyme activities: alkaline phosphatase and acid phosphatase activity by the colorimetric method using PNP (sodium p-nitrophenyl phosphate), dehydrogenase activity was by colorimetric method with the use of TTC (triphenyl-tetrazole chloride) as a substrate.

• Total abundance of bacteria and abundance of groups of bacteria, capable of certain biological processes, for example N fixation or phosphorus solubilisation.

• Acticity of soil biological processes, such as nitrification.

• Abundance of nematodes.



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7 Synergy with EU project and initiatives

Within the NBSoil progress, we will work on synergy of the NBSoil soil samopling and analysis with other EU projects and international initiatives. They will include:

- developments of EU Soil Observatory in order to folow the data formats expected by EUSO
- LUCAS soil monitoring to enable compatibility of data and extrapolation of potentail NBS effects across Europe
- projects funded under the topic HORIZON-MISS-2021-SOIL-02-02: Validating and further developing indicators for soil health and functions
- EJP Soil project (WP6 activities and EJP Soil internal project SIREN (Stocktaking for Agricultural Soil Quality and Ecosystem Services Indicators and their Reference Values)
- PROPSOIL project dealing with harmonisation of approaches for monitoring of soil health
- GLOSOLAN developments
- National projects in countries of NBSoil Partners.



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8 Next steps

Next steps in Task 1.2 will involve:

- Final list and characteristics of research sites (month 9 August 2023)
- Synergy with projects PREPSOIL, EJP Soil, ISLANDR, new Soil Mission project
- Following EU soil initiatives Soil Health Law
- Sampling strategy and provisional indicator list for each the Research Site (month 11 October 2023).



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