



icosHELLs

D3.1 CATALOGUE OF SOIL HEALTH INDICATORS

CETENMA - Cristiano Pisani, José M Soriano Disla



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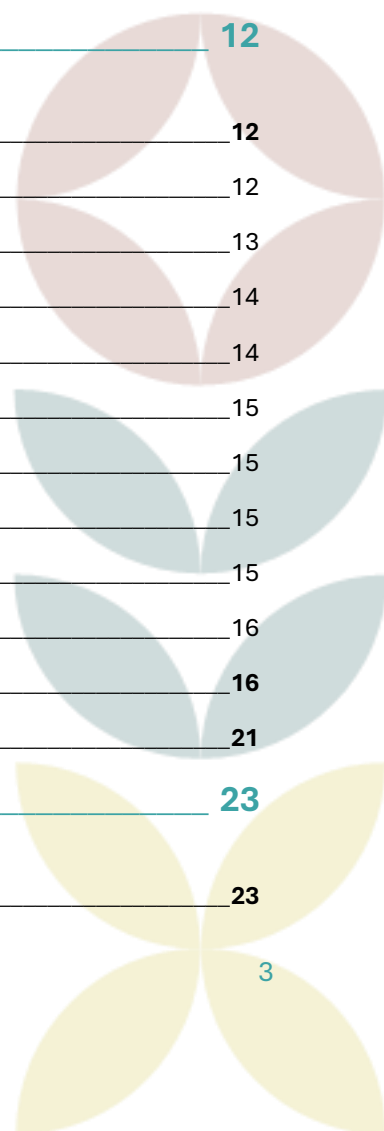
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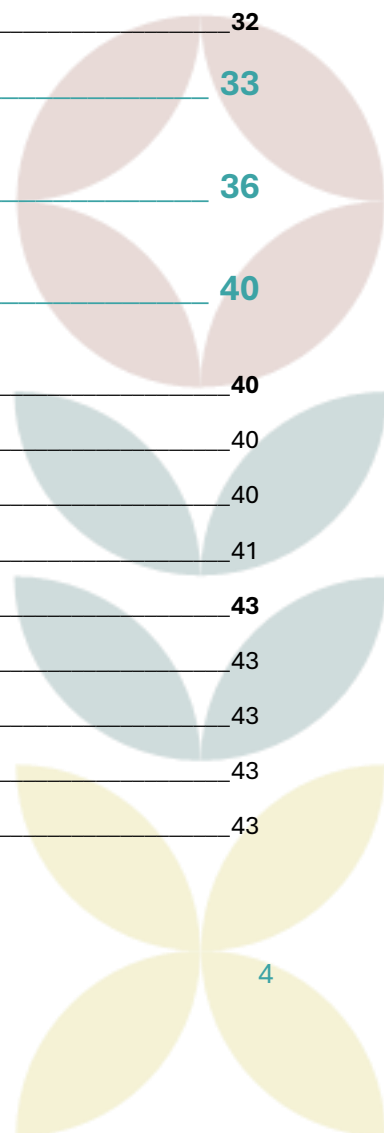
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Executive summary

Through a systemic approach, the EU project iCOSHELLS will develop, test, and validate potential solutions to improve soil health through the establishment of six soil Health Living Labs (LLs) across diverse EU edaphoclimatic zones. In order to assess the performance of those innovative solutions or practices tested across LLs on soil health, there is a need to define a set of indicators which are reliable, sensitive, informative and robust. Thus, the main aim of this report is to propose a set of indicators that can effectively monitor soil health at the LLs experimental sites.

To achieve this goal, a comprehensive review of soil health indicators, relevant policies, and related projects was conducted. Literature shows a diverse array of soil health indicators listed and tested across several projects, evidencing that a universal set of indicators is not applicable to all conditions and locations. Thus, to assess the impact of the proposed solutions, this report proposes a comprehensive set of soil health indicators, including both general for a broad overview of soil health across all LLs, and specific indicators tailored to address the specific challenges of each LL. Moreover, and to ensure consistency in data collection and soil analysis across different LLs, the document includes a detailed sampling methodology and a Standard Operating Procedure (SOP) for laboratory analysis. To guarantee efficacious and replicable results, the proposed set of indicators, the sampling methodology and the SOP were developed collaboratively by all LLs, also considering feedback from other related projects.

The general proposed indicators cover physical, chemical, and biological soil properties, while the specific ones focus on the scope and direct impact of the proposed solutions at each site. The document explains each indicator, its significance, assessment methods, and reference threshold values to facilitate the interpretation of baseline soil health and its subsequent evolution. The collected data will be then used to ensure a consistent and effective evaluation of the final best-performing solutions within and across the different LLs and create predictive models that can be utilized by both internal and external stakeholders.

In summary, the authors envisage the content of this deliverable to be useful at both project internal and external levels, assisting in the selection of the best performing indicators for the selection of solutions to improve soil health and guiding soil sampling strategies.

Introduction

Soil health, roles and threats

The EU defines soils as a vital, non-renewable resource that underpins our health, wealth, and the very fabric of life on Earth. They store, filter, and transform essential substances like water, nutrients, and carbon, supporting our cultural heritage, landscapes, economy, and overall prosperity. Soils that can sustain these functions are considered to be healthy. Therefore, and although soil health definition, concept and operationalization keep evolving over time, it can be defined as “the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans. Healthy soil gives us clean air and water, bountiful crops and forests, productive grazing lands, diverse wildlife, and beautiful landscapes¹ (Doran et al., 1996; Doran et al., 2000). Soil health in Europe has undergone significant degradation and it is currently estimated that 60% of EU soils are unhealthy (Arias-Navarro et al., 2024). This poses a serious threat to climate change mitigation and adaptation, agricultural production, food security, and the preservation of nature and biodiversity.

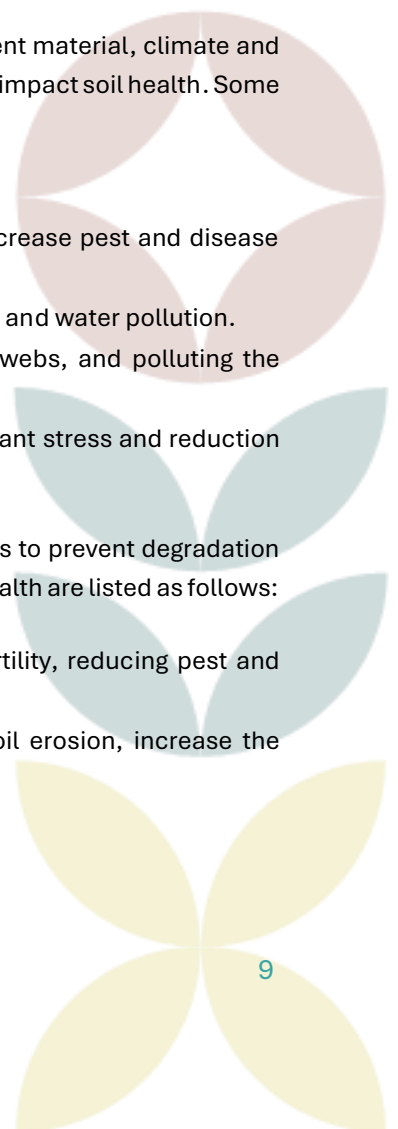
Soils are diverse and are classified in different classes depending on factors such as parent material, climate and previous management (Orgiazzi et al., 2016). Soil management practices can substantially impact soil health. Some of the common practices which negatively affect soil health are listed as follows:

- **Over-Tilling:** leading to soil erosion, loss of soil organic matter, and compaction.
- **Monocropping:** the continuous grow of the same crop can deplete soil nutrients, increase pest and disease problems, and degrade soil structure.
- **Excessive Use of Chemical Fertilizers:** causing nutrient imbalances, soil acidification, and water pollution.
- **Excessive Use of Pesticides:** killing beneficial soil organisms, disrupting soil food webs, and polluting the environment.
- **Poor Irrigation Practices:** leading to impacts such as waterlogging and salinization, plant stress and reduction of soil fertility.

It is then imperative to change these practices, adopting sustainable agricultural methods to prevent degradation and sustain soil health. Some soil management practices proven to be beneficial for soil health are listed as follows:

- **Crop Diversification:** for example, rotating different crops helps maintaining soil fertility, reducing pest and disease pressure, and improving soil structure.
- **Cover Cropping:** planting cover crops during off-seasons contributes to prevent soil erosion, increase the organic matter pool, and suppress weeds.

¹ <https://www.nrcs.usda.gov/wps/porta/nrcs/main/soils/health/>



- Sustainable tillage: for example, minimizing tillage practices reduces soil disturbance, preserves soil structure, and reduces soil erosion. Conservation tillage involves minimal soil disturbance, leaving crop residues on the soil surface to protect it from erosion and improve water infiltration.
- Low input agriculture: minimizing the consumption of water, chemicals and energy.

These practices are well described by the Food Agriculture Organization (FAO) document “Voluntary Guidelines for Sustainable Soil Management”² proposing sustainable soil management practices that enhance soil health. The document provides a general frame of action, encouraging the adaptation of the practices to the specific local conditions.

To understand soil health condition, and how they are affected by soil management practices, scientists have been employing a range of physical, chemical, and biological indicators. While traditional indicators provide valuable insights, recent advancements have led to the inclusion of more specific indicators such as: particle aggregation, infiltration, earthworm abundance, organic carbon (C) and nitrogen (N) fractions, N-mineralizing enzyme activity, pathogens, parasites, biodiversity, bioavailable and mobile toxins and compound and pore-size diversity. There are also soil health indicators related to the climate-change functions of soils, such as greenhouse gas emissions and carbon sequestration. Despite the efforts, there is no agreement on indicators that can universally assess soil health across all conditions, as those conditions are strongly affected by edaphoclimatic characteristics and management. Selecting the right set of indicators is thus crucial for accurately evaluating the effect of management solutions on soil health, this being the main challenge of the iCOSHells project.

Content and purpose of this report

The main objective of this report is to propose a set of indicators that can effectively monitor the impact of innovative practices or solutions on soil health at the LLs experimental sites. In addition, the document proposes a standard methodology to monitor and assess the effects of the developed experimental innovative practices on soil health and related ecosystem services within each of the six LLs. The data obtained will be a powerful tool for monitoring the performance of the tested solutions, and the base for the development of predictive models for the performance of future ones. To achieve these objectives and those related to the project Task 3.1 “Set of indicators and measurement methodology”, a comprehensive review of existing soil health indicators was carried out, drawing on 1) current EU policies and strategies, 2) related EU soil health projects, and 3) existing literature. The review includes an evaluation of the relevance and applicability of the indicators for them to effectively measure and monitor soil health. Aspects such as robustness, practicality to measure and sensitivity to changes were taken into account. An additional evaluation targeting targets/thresholds for each indicator was conducted. In addition to these, so-called, general indicators, it is also the aim of this document to outline a specific set of soil health indicators co-selected by the LLs to address their specific challenges and evaluate the impact of the tested solutions on soil health.

² <https://openknowledge.fao.org/server/api/core/bitstreams/9a5b9373-3558-43b3-b732-f69326a7314d/content>

Apart from proposing indicators, it is the aim of this report to propose a common and standard soil sampling methodology (Annex 1) with a standard operating procedure (SOP) for laboratory analysis (Annex 2), leading to analysis consistency, data harmonization and enabling data comparison and replicability.



Setting the Scene

In order to select global indicators which can be used across different situations and contexts, it is necessary to set the scene and explore what is already available across 1) EU Policies, Strategies and Initiatives, 2) related EU funded projects, and 3) existing bibliography. This Section highlights the importance of soil health and the efforts at EU level to address this issue. It explores key EU initiatives such as the European Soil Observatory (EUSO), the LUCAS survey, and the European Soil Data Centre (ESDAC). It also touches on projects aimed at improving soil health, including the development of soil health indicators and the promotion of sustainable soil management practices. Additionally, there is a brief mention of promising techniques that may contribute to further advancements in soil health monitoring and management.

EU Policies, Strategies and Initiatives

EU Soil Observatory

The EUSO is a Europe-wide soil health monitoring and long-term data and knowledge Hub. The main aim of this observatory is to provide reference data and knowledge at EU level for all matters related to soil. And for achieving this, the following initiatives have been developed: EU-wide soil monitoring system (LUCAS soil survey), EUSO Soil Health Dashboard, the European Soil Data Centre (ESDAC), support to soil research and innovation, support citizen engagement and policy support.

One of the most important and ambitious initiatives is related to the LUCAS Soil Survey. LUCAS is an EU-wide harmonised and regular soil survey aiming to sample and analyse the main properties of topsoil in EU Member States. Results are available for [2009](#), [2015](#) and [2018](#). The 2022 survey included a target of 41,000 soil samples. Physical samples of the soils are archived at the Joint Research Centre (JRC) premises in Istituto Superiore per la Protezione e la Ricerca Ambientale Ispra (Italy). In terms of measured properties, as shown in Jones et al (2021), the initial surveys targeted physical and chemical properties, such as particle size distribution (texture), pH, organic C concentrations, nutrient concentrations (N, phosphorus (P), K, S), metal content, salinity and cation exchange capacity (CEC). In addition, visible and near-infrared spectra data were collected on some campaigns. In the 2018 survey, bulk density, specific measurements for organic-rich soil and soil erosion assessments were carried out in the field while the core laboratory analysis was expanded to measure metal content, genetic composition (DNA), residues of plant protection products and antibiotics, and the presence of antimicrobial resistance genes. A full description of soil properties analyzed across the different campaigns can be found in the European Journal of Soil Science (Orgiazzi et al., 2018).

LUCAS is very important for iCOSHells; firstly, from a point of view of providing a baseline reference for a number of soil properties. It also proposes a methodology for soil sampling, manipulation and storage. In addition, it features a validated system for data collection and sharing.

Using information from the LUCAS survey, the EUSO features a comprehensive dashboard containing indicators and thresholds describing the following soil degradation processes: soil erosion, soil pollution, soil nutrients, loss of soil organic C, loss of soil biodiversity, soil compaction, salinization, loss of soil organic soils and soil consumption³.

All the information is centralized at the ESDAC, hosted by the European Commission's Joint Research Centre (JRC), which can be considered as the focal point for soil data for EU level, supporting policy making and awareness raising (Panagos et al., 2022). It contains a number of resources organized in the following topics: datasets, services/applications, maps, documents, events, projects and external links.

Last but not least, through the EUSO, the JRC supports research and innovation by:

- Developing further the JRC in-house research and development capacity on soils through publications.
- Co-hosting and mentoring PhD candidates.
- Supporting the implementation of Soil Mission (novel approaches on soil monitoring, new methods for data acquisition, metrics for measuring indicators, etc.).
- Collaborating with Soil Mission Projects.
- EUSO as a beneficiary of research activities in Soil Mission Projects.

Soil strategy and Mission Soil

The EU Soil Strategy for 2030 outlines a long-term vision in which, by 2050, all soil ecosystems within the Union are in a healthy state and therefore more resilient. Therefore, the Strategy sets out a basis and concrete actions for protecting, restoring and sustainably using soils and that stimulates the necessary societal engagement, financial resources, shared knowledge, sustainable practices and monitoring to reach common purposes. This is why the Soil Strategy represents a key deliverable of the EU biodiversity strategy for 2030 and will contribute to the purpose of European Green Deal.

Assuming that 60-70% of soils in Europe are in an unhealthy condition, the Mission Soil aims to significantly increase the share of currently 30-40% of healthy soils in Europe to levels that are in line with Green Deal commitments and targets by 2030. And in order to achieve this, the ambition is to create 100 LLs and Lighthouses by 2030, having the following objectives: reduce soil degradation, conserve soil organic C stocks, stop soil sealing and increase re-use of urban soils, reduce soil pollution and enhance restoration, prevent erosion, improve soil structure to enhance soil biodiversity, reduce the EU global footprint on soils and improve soil literacy in society. The Mission is expected to contribute with **soil health data**, and the necessary capacity building, assistance and support by eliminating existing data gaps and workflow bottlenecks by sharing expertise (i.e. **knowledge**).

³ <https://esdac.jrc.ec.europa.eu/euso/euso-dashboard-sources>

In order to achieve the mission objectives, there is an urgent need to define indicators and for the assessment of soil health. Soil health indicators, together with the range of values to consider soils as “healthy”, should be defined, assessed and agreed in the coming years.

Cluster on soil monitoring

The Mission Soil has established clusters for related projects to work together, exchange information and develop timely and concrete actions to increase their impact. Up to now, five clusters have been set up: 1) Data and Knowledge Management, 2) Communication and Stakeholder Engagement, 3) Soil Indicators and Monitoring, 4) Business models and investments, and 5) Soil pollution and remediation. The most relevant to this report is the third one whose main goal is to advance in the development of a roadmap for the identification of meaningful descriptors/indicators and methods to monitor different soil characteristics at the local scale. This will contribute to supporting local-level activities, particularly the implementation of the 100 LLs that are foreseen to be established by the Mission by 2030. Dedicated and specific discussions have been maintained on indicators grouped in three categories organic C, biodiversity and contamination.

Since the commencement of the iCOSHells project, and even before, the WP3 leader, CETENMA, has been regularly attending the cluster events which have been organized, both online and in-person.

Soil monitoring law

This law represents the first-ever EU legislation on soils providing a harmonized definition of soil health, proposing a monitoring framework and fostering sustainable soil management and remediation of contaminated sites. The text was finally adopted 12/11/25. Soil monitoring law defines soil as vital, limited, non-renewable and irreplaceable resource. Healthy soils form the essential basis for our economy, society and environment as they produce food, increase our resilience to climate change, to extreme weather events, drought and floods and support our well-being. Healthy soils have many other services to offer such as: storing carbon, increased capacity to absorb, store and filter water and provide vital services such as safe and nutritious food and biomass for non-food bioeconomy sectors. Due to all of these reasons, information and monitoring about soils status is badly needed. In order to do that, there is an urgent need to develop a new catalogue with different types of health soil indicators.

The Soil Monitoring Law proposes (Annex 1) a set of soil descriptors to address Mission Soil objectives (Bünemann et al. 2018) for the main soil degradation processes described above, accompanied by criteria for healthy soil condition. They come as follows: **electrical conductivity (EC), soil organic carbon (SOC), bulk density, extractable P, soil erosion rate, concentration of heavy metals and organic contaminants in soils, soil water holding capacity, saturated hydraulic conductivity, air capacity and soil organic C stocks**. In addition, other soil descriptors are proposed without criteria: **Total nitrogen in soil, SOC to N ratio, pH, bulk density in topsoil, DNA metabarcoding for fungi and bacteria, concentrations of PFAS-21 or concentrations of PFAS-43 or selected PFAS set by Member States in accordance with Article 7, concentrations of selected active substances in pesticides and their metabolites set by Member States in accordance with Article 7**. Last but not least, the Soil monitoring law also proposes descriptors for the assessment of soil sealing and soil removal:

total sealed soils and areas that underwent soil removal, soil sealing and soil removal, de-sealing and net-sealing, total settlement area.

Carbon removal certification

This certification incentivizes activities like carbon farming to capture C from the atmosphere and store it in soil, with standards to ensure sustainability and prevent fraud. It aims to contribute to achieve climate neutrality by 2050, with certification standards for various carbon storage methods and carbon farming.

Nature restoration law

This ambitious law aims to restore 20% of EU land and sea by 2030, with increasing targets for key habitats, and includes measures for agricultural ecosystems, forests, and peatlands. The law includes a list of biodiversity indicators for agricultural ecosystems such as: i) The European Grassland Butterfly Indicator, ii) Stock of organic C in cropland mineral soils, iii) High-diversity landscape features, and iv) The Common farmland bird index. The law also includes a list of biodiversity indicators for forest ecosystems such as: i) Standing deadwood, ii) Lying deadwood iv) Share of forests with uneven-aged structure, v) Forest connectivity, vi) Common Forest birds index, vii) Stock of organic C, viii) Share of forest dominated by native tree species, ix) Tree species diversity.

Clean soil outlook

This report analyzes synergies and conflicts between EU policies affecting soil, identifies emerging pollutants, and proposes recommendations for better soil protection. It highlights the existence of serious knowledge gaps regarding soil pollution due to a lack of investment in monitoring, research, lack of a systematic inventory, and reporting obligations. It also includes a common EU framework and definitions to better characterise both contaminated sites and diffuse sources.

CAP Performance indicators

Soil health is seen as an environmental priority for the proposal of the next EU's Common Agricultural Policy (CAP) 2028-2034, for which Member States shall provide support to farmers. The CAP proposes indicators like soil organic C (C.41) and soil erosion by water (C.42) to monitor the impact of agricultural practices on soil health. 1) Changes in soil organic C are monitored according to both total estimated organic C content in arable soils and their mean organic C content; These show that the annual rate of change in organic matter can vary greatly, depending on cultivation practices, the type of plant/crop cover, drainage status of the soil and weather conditions; 2) Soil erosion is monitored by estimating the rate of soil loss by water erosion as tons ha⁻¹ yr⁻¹ as well as the area (ha) of agricultural soils that are affected by that certain rate of erosion. The two soil erosion indicators have been proposed by the JRC, on the basis of an empirical computer model.

Farm to Fork Strategy

The Farm to Fork Strategy represents a comprehensive approach on how Europeans value food sustainability, representing a unique opportunity to improve lifestyles, health, and the environment. This strategy tackles pesticide and nutrient pollution from agriculture, aiming to reduce overall pesticide use and promote sustainable nutrient management practices. The overuse of pesticides is detrimental for soil health, induces water and air pollution, biodiversity loss and can harm non-target plants, insects, birds, mammals and amphibians. For these reasons, the Commission has already established a Harmonised Risk Indicator to quantify the progress in reducing the risks linked to pesticides. This indicator demonstrates a 20% decrease in the risk from pesticide use in the past five years. The UE will take additional action to reduce by 50% the overall use and risk of chemical pesticides by 2030.

Related projects

This Section reviews what other related funded projects⁴ are doing or have already done (Table 1). Under the TITLE&FUNDING column, there is information about the main focus of the projects: a) Selection and monitoring of indicators, b) Development of practices, solution or remediation techniques to improve soil health, c) Increasing awareness and soil literacy. Four projects of this table, GOV4ALL, SOILCRATES, LivingSoiLL and LILAS4SOILS, together with iCOSHells, represent the first wave of projects funded under the Mission Soil whose objective is to contribute to set up the 100 LL target.

Table 1. iCOSHells' related projects.

TITLE&FUNDING	KEY ACTIVITIES	DATE	EXPECTED OUTCOME
AI4SoilHealth^{a,b} (Mission Soil, Horizon Europe)	Collaborating with EU Soil Observatory and scientists from the University of Bucharest, developing a land degradation assessment model with 12 indicators to work with the 100 LLs and lighthouses to implement good measurement practices	Jan 2023 to Dec 2026	Improved understanding of soil health, development of effective monitoring tools, and informed decision-making
ARAGORN^b	Mapping and monitoring tools to identify pollution sources, innovative remediation techniques to restore contaminated land, planning and decision-making tools for	Oct 2023 to Sept 2027	Contribute to long-term environmental and societal benefits

⁴ [Mission Soil funded projects](#) | [Mission Soil Platform](#)

(Mission Soil, Horizon Europe)	effective land management and co-creation to promote sustainable land use		
BENCHMARKS^a (Mission Soil, Horizon Europe)	Co-developing an integrated soil health monitoring framework, testing and validating indicators, developing a European broad sampling framework, Co-developing a Soil Health Dashboard	Jan 2023 to Dec 2027	Provide a clear soil health index for benchmarking, using pertinent indicators, applicable to the land use and logistically feasible
BIN2BEAN^b (Mission Soil, Horizon Europe)	Mapping local contexts and solutions, evaluating the safety and environmental impact of soil improvers, developing a toolbox for City-Region LLs	Sept 2023 to August 2026	Improved urban soil health, enhanced urban green spaces, and increased citizen participation in urban sustainability initiatives
BIOservisES^a (Mission Soil, Horizon Europe)	Assessing soil biodiversity, identifying factors affecting soil biodiversity and developing indicators to assess ecosystem services	Sept 2023 to August 2028	Improved understanding of soil biodiversity, enhanced monitoring capabilities, and informed decision-making for sustainable land management
bioSOILUTION^b (Mission Soil, Horizon Europe)	Establishing LLs, developing soil improvers, testing soil improvers in real-world settings	June 2023 to May 2026	Improved soil health, increased agricultural productivity, and enhanced environmental sustainability
DeliSoil^b (Mission Soil, Horizon Europe)	Developing innovative technologies and practices, establishing Regional LLs and testing solutions in real-world settings	June 2023 to May 2027	Improved soil health, reduced waste, and enhanced resource efficiency
ECHO^c	Building citizen skills and knowledge, collecting citizen science data and	June 2023 to May 2027	Increased public awareness of soil health, citizen participation in soil monitoring, and improved

(Mission Soil, Horizon Europe)	establishing a long-term open-access repository		data availability for decision-making
EDAPHOS^b (Mission Soil, Horizon Europe)	Collecting soil samples for analysis, developing remediation strategies and implementing nature-based solutions	Sept 2023 to August 2027	Improved soil health, accelerated land restoration, and enhanced ecosystem services.
EJPSOIL^b (Societal Challenges, EJP Soil)	Harmonizing methods and indicators, developing databases and models and supporting research in various areas of soil science	Feb 2020 to Feb 2024	Improved understanding of soil processes, enhanced data sharing and collaboration, and informed policy decisions
FENIX^b (Mission Soil, Horizon Europe)	Developing a soil amendment formulation, testing the effectiveness of the formulation, assessing the impact on soil quality and climate change mitigation	June 2023 to July 2027	Improved soil quality, reduced greenhouse gas emissions, and enhanced sustainable biowaste management
GOV4ALL^{a,b} (Mission Soil, Horizon Europe)	Establishing five LLs in France, Greece and Spain to address soil erosion, desertification and soil biodiversity	June 2024 to Nov 2028	Increase awareness of soil health issues, improve soil health practices, increased water quality, resilience and biodiversity
HuMUS^c (Mission Soil, Horizon Europe)	Raising awareness of soil health issues, engaging municipalities and regions and promoting sustainable soil management practices	Jan 2023 to Dec 2025	Increased awareness of soil health, enhanced municipal and regional action on soil protection, and the adoption of sustainable soil management practices

InBestSoil^b (Mission Soil, Horizon Europe)	Establishing lighthouses and pilot sites, developing a multi-actor approach and applying responsible research and innovation principles	Jan 2023 to Dec 2026	Increased understanding of the economic and environmental benefits of soil health investments, the development of effective tools and models, and the promotion of sustainable land use practices
ISLANDR^{b,c} (Mission Soil, Horizon Europe)	Identifying pollution sources assessing environmental and health risks, implementing risk management strategies, developing a valuation approach	May 2023 to April 2026	Improved understanding of soil pollution, reduced risks, and informed decision-making for soil remediation and protection
LILAS4SOIL^{b,c} (Mission Soil, Horizon Europe)	Establish 5 LLs in 6 countries to co-create the adoption of Carbon Farming solutions within farmers, agri-food businesses, researchers and local authorities, and implement Carbon Farming Practices	Sept 2024 to August 2029	Reduce GHG emission, increase agricultural productivity, soil health and C sequestration, knowledge transfer and capacity building
LivingSoiLL^b (Mission Soil, Horizon Europe)	Establish five LLs in France, Italy, Poland, Portugal and Spain and involve more than 2000 actors working on permanent crops to address soil erosion, pollution and biodiversity	June 2024 to Nov 2028	Reduce soil erosion, pesticides and fertilizers use, increase resilience to climate change and biodiversity
MARVIC^b (Mission Soil, Horizon Europe)	Developing monitoring, reporting and verification (MRV) systems for different land use types, testing MRV systems in various agricultural settings and supporting the EU Carbon Removal Certification Framework	June 2023 to May 2027	A robust framework for measuring and verifying carbon sequestration in agriculture, supporting the transition to low-carbon agriculture
NBSOIL^b (Mission Soil, Horizon Europe)	Developing a blended learning program, training soil advisors on NBS, promoting collaboration between soil advisors	Dec 2022 to Nov 2026	Improved knowledge and skills of soil advisors, increased

			adoption of NBS, and enhanced soil health
NOVASOIL^{b,c} (Mission Soil, Horizon Europe)	Developing a toolbox for analyzing business cases, identifying good examples of soil health investments and categorizing models and business cases	Nov 2022 to Oct 2025	Increased awareness of the economic benefits of soil health, a practical tool for assessing investment opportunities, and the promotion of sustainable soil management practices
Prepsoil^c (Mission Soil, Horizon Europe)	Widening understanding of LLs, engaging stakeholders in soil improvement and establishing a one-stop-shop for soil literacy and communication	July 2022 to June 2025	Increased awareness of soil health issues, enhanced stakeholder engagement, and the establishment of effective Living Labs
SOB4ES^c (Mission Soil, Horizon Europe)	Comprehensive assessment of soil biodiversity, examining factors like soil composition, spatial and temporal dynamics, and interactions with aboveground biodiversity	June 2023 to May 2028	Identify the impact of various land-use practices on soil biodiversity and develop a framework to incorporate soil biodiversity into EU policies
Soil Values^{b,c} (Mission Soil, Horizon Europe)	Developing a comprehensive assessment framework, setting up testing grounds for business models, establishing communities of practice and developing a toolbox of incentives and policy recommendations	Jan 2023 to Dec 2026	A robust framework for assessing soil health investments, the promotion of innovative business models, and the development of effective policies to support soil health
SOILCRATES^b (Mission Soil, Horizon Europe)	Establish four LLs in France, Ireland, Netherlands and Spain to co-implement solutions to enhance soil structure and health.	Oct 2024 to Set 2028	Improve soil health, increased soil carbon sequestration, resilience to climate change and

			collaboration among stakeholders
SOILL-Startup^b (Mission Soil, Horizon Europe)	Fostering a network of LLs, providing dedicated support to LLs and facilitate collaboration between stakeholders	Jan 2024 to Dec 2025	A strong network of Living Labs, enhanced capacity, and accelerated adoption of sustainable soil practices
Soil-Olive^c (Mission Soil, Horizon Europe)	Analyzing the impact of pollution and land degradation, investigating the relationship between soil health and olive oil quality and implementing multidisciplinary and interdisciplinary projects	Jan 2023 to Dec 2027	Improved understanding of olive grove soil health, informed management practices, and enhanced olive oil quality
Waste4Soil^b (Mission Soil, Horizon Europe)	Developing recycling technologies and methods establishing LLs in different EU regions and testing solutions in real-world settings	June 2023 to May 2027	Improved soil health, reduced waste, and enhanced resource efficiency
WorldSoils^c (European Space Agency ESA)	Increased public awareness of soil health, citizen participation in soil monitoring, and improved data availability for decision-making	2020 to 2023	Improved understanding of global soil carbon stocks, enhanced monitoring capabilities, and informed decision-making for sustainable land management

^aSelection and monitoring of indicators; ^bDevelopment of practices, solutions or remediation techniques to improve soil health; ^cIncreasing awareness and soil literacy.

State-of-the-art indicators and monitoring technologies

In order to complete the setting of the scene, it is necessary to include a brief state-of-the-art based on information extracted from scientific articles and reports. The main takeaways message is that there is no agreement in the definition of universal indicators that reflect soil health. It really depends on many factors such as the local context,

purpose, challenge, soil type, previous management, and also funding available (as some determinations are very expensive). In this context, a project such as iCOSHells is highly needed to shed light on those potential universal indicators. In the summary that comes as follows, authors review specific promising indicators and technologies which have shown interesting results to better and more specifically evaluate soil health (Lehmann et al., 2020).

- Biological parameters are highly attractive to monitor soil health due to the key role of soil organisms and their sensitivity to changes. A number of methods have been proposed to describe soil biodiversity. One of the interesting approaches relies on the identification of soil microbial communities by Next-Generation Sequencing; for example, advanced DNA sequencing techniques in metagenomic allow for a more detailed analysis of soil health. This can reveal the diversity, abundance, and functional potential of microorganisms (Roesch et al., 2007). Other soil biodiversity descriptors as proposed in the Soil Monitoring Law are: Phospholipid fatty acid analysis (PLFA), abundance and diversity of nematodes, earthworms, springtails or native ants, soil biological quality based on arthropods (QBS-ar), presence of invasive alien species and plant pests and soil basal respiration. Also related to soil biodiversity is the presence of antimicrobial resistance genes. Soil biological activity, for example through enzyme activity (e.g. N-mineralizing enzyme activity) also provides key insights into microbial processes and soil health (Sinsabaugh et al., 2014).
- Spectroscopy techniques using different spectral ranges and techniques. Among them, infrared spectroscopy, using the mid-infrared and regions, coupled with multivariate modelling, is well-suited for the quick, unexpensive, reliable, sensitive and on-the-go proximal prediction of soil properties and assessment of soil health (Soriano-Disla et al., 2014b, Irving et al., 2024). The advance in remote sensing (e.g. Copernicus Sentinel) will be another key contributor.
- Other sensing techniques are electrochemical, bio and chemical sensors, and passive samplers.
- Stable Isotope analysis offers highly interesting information on factors influencing key processes such as carbon storage. For example, stable C isotopes are a powerful tool for studying fluxes and processes by tracking the movement of carbon isotopes through the soil-plant system, moreover can help to quantify carbon sequestration rates (Neupane et al., 2022).
- Carbon and N fractions.
- Soil greenhouse gas emissions and nutrient lixiviation.
- Pollutants: monitoring technologies have also been developed to identify and quantify pollutants which negatively affect soil health. Apart from the traditionally analysed metals and pesticides/herbicides, methods have been developed to detect specific fractions (e.g. metal solid-solution partitioning), other organic contaminants (e.g. pharmaceuticals, PCB, PAHs, PFAS), microplastics, toxins, etc.

Catalogue of key soil health indicators

After reviewing the state-of-the-art, a set of indicators that can effectively measure, and monitor soil health is proposed. For the selection, attributes such as robustness, practicality to measure, sensitivity to changes, efficiency on soil health assessment, parameter standardization, comparability and cost effectiveness, were considered. The indicators selected directly relate to the intended use or purpose of the assessment targeting the most critical aspects of soil health, such as fertility, structure, chemical proprieties and biological activity. In terms of robustness, the indicators intend to provide consistent and reliable results over time and across different locations, and they should be minimally affected by factors like weather conditions, human intervention, or laboratory errors (providing those standard guidelines, such as the one proposed in this report, are followed). Indicators have been selected to be suitable for both large-scale monitoring programs and small-scale farm assessments, besides being simple, efficient, and cost-effective. The indicators' sensitivity to changes guarantees them to reflect the impact of different management practices (such as crop rotation, cover cropping, and alternative fertilization etc.). Their efficiency on soil health can be directly measured thanks to their close link to the processes that maintain soil health, such as nutrient cycling, water infiltration, carbon sequestration, etc. Last but not least, standardized protocols will be used to ensure that data collected from different sources are comparable, using common units and scales to facilitate analysis and interpretation.

General (across living labs) soil health indicators

To assess soil health, a set of general soil indicators has been selected to provide information about the physical, chemical, and biological properties of soils. They also serve to identify potential problems and evaluate the effectiveness of tested solutions. The selected indicators are accompanied by a brief description and definition of target/thresholds, for most of them measurement methodologies (Lehmann et al., 2020) and reference values from USDA Natural Resources Conservation Service⁵ and European Soil Data Centre ESDAC⁶. The general set of soil health indicators described below and summarized on a Table in the Conclusions Section, includes soil quality parameters grouped in three categories: Physical (**bulk density, particle size distribution and soil water holding capacity**), Chemical (**pH, electrical conductivity, plant available macronutrients, cation exchange capacity, total N, total C, organic C and total heavy metals**) and Biological (**microbial biodiversity and respiration**) characteristics. Each category is related to specific soil functions: 1) Physical: water retention, transmission, filtration of pollutants, water cycling and renewability, and gaseous exchange; 2) Chemical: cycling of elements,

⁵ <https://www.nrcs.usda.gov/conservation-basics/natural-resource-concerns/soils/soil-health/soil-health-assessment>

⁶ <https://esdac.jrc.ec.europa.eu/>

elemental transformation, buffering, and leaching; and 3) Biological: waste decomposition, denaturing of pollutants, climate regulation, carbon sequestration, habitat for biota, and energy for soil organisms (Lal, 2016). Authors acknowledge the fact that the selected indicators do not provide a complete overview of the soil functions and mechanisms, but the selection has to be seen as a general compromise taking into account the fact that there are many living labs involved, and there is a specific budget allocated for this task. In addition, the general list includes indicators that are slow to react but are needed to map the baseline status, potential or capability.

As described above, and despite the lack of consensus regarding valid regionalized critical limits used as thresholds for specific soil functions or variations in those functions which are considered to be significant, each proposed general soil indicator is accompanied by reference range values according to specific guidelines and bibliography. General provisions have been already indicated above as proposed by the Soil Monitoring Law and the EUSO dashboard sources. As discussed, such lack of consensus is related to the huge diversity of local edaphoclimatic conditions and soil management practices, as well as different legal requirements. It is also conditioned by the common lack of local baseline values (in this project, initial baseline values will be determined for each experimental site).

In terms of measurement methodology for the selected indicators, and as reflected in Annex II of the Soil monitoring law, it is important to use well-established, validated and common reference methodologies expressed in the same units. Under each consideration, each indicator is accompanied by the reference preferred methodology and unit choice.

Physical

- **Bulk density** is typically measured with core method⁷ providing key information to understand soil compaction, soil porosity, available water capacity and infiltration (Panagos et al., 2024). The results are to be expressed in g/cm^3 achieved by dry soil mass (g) divided by volume (cm^3) of a standard cylinder 5 x 5.1 cm ($\sim 100 \text{ cm}^3$; without lids). It is typically determined at different depths, but soil compaction at e.g. 10-20 cm is more evident at subsurface soil, then determinations at this depth are useful for future comparison (e.g. with LUCAS samples; Panagos et al., 2024) and allow to obtain useful information on soil structure, aeration, potential root penetration beyond the surface. The USDA proposes a range of ideal values for plant growth: Sandy soil $< 1.60 \text{ (g}/\text{cm}^3)$, Silty soil $< 1.40 \text{ (g}/\text{cm}^3)$, Clayey soil $< 1.10 \text{ (g}/\text{cm}^3)$. Values of bulk density that restrict root growth are: Sandy soil $> 1.80 \text{ (g}/\text{cm}^3)$, Silty soil $> 1.65 \text{ (g}/\text{cm}^3)$, and Clayey soil $> 1.47 \text{ (g}/\text{cm}^3)$ ⁸.
- **Particle size distribution** is typically expressed as the percentage of sand, silt and clay and the result is represented on the textural triangle. The sieving and sedimentation method is the recommended measurement methodology for the determination of particle size distribution in mineral soils (ISO11277)⁹. Other methods for determining particle size distribution typically include pipette, visible imaging and infiltrometry. Soil texture as

⁷ <https://www.iso.org/standard/68255.html>

⁸ <https://www.nrcs.usda.gov/sites/default/files/2022-10/Soil%20Bulk%20Density%20Moisture%20Aeration.pdf>

⁹ <https://www.iso.org/standard/69496.html>

well as the above-mentioned bulk density condition soil structure that influences soil behaviour in many ways. These parameters, together with other such as porosity and aggregate stability, significantly influence various soil properties and processes, including water infiltration, root growth, available water capacity, soil aeration and soil microbial activity. During soil sampling for physical parameters, the presence of soil sealing and erosion (indicating the type of erosion), together with an estimate of the number of rills or gullies observed (Reynolds et al., 2002) are typically reported. Depending on the relative presence of the different textural classes, soils can be allocated to specific textural groups using the textural triangle as shown below (Figure 1).

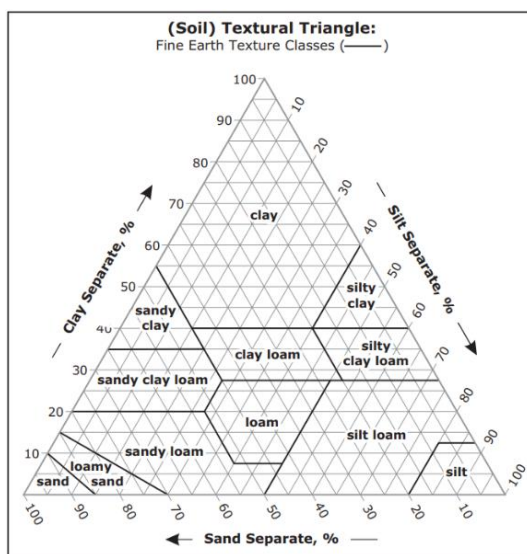


Figure 1. USDA textural triangle.

- Soil water holding capacity (WHC)** is usually expressed in percentage, indicating the max quantity of water (g) contained in a dry soil sample (100g). The methodology suggested to determine WHC is the same proposed by the Soil Monitoring Law (ISO 11274:2019)¹⁰. An alternative method for estimating WHC is based on soil texture (or particle size distribution) and is expressed in mm/cm (Tóth et al., 2015). Soil WHC provides information on soil water retention and infiltration, which are very important attributes for plant growth, water/nutrient demand, mobility of nutrients/contaminants, potential proliferation of pathogens... FAO has categorized WHC into 7 classes and created a dynamic map¹¹.

¹⁰ <https://www.iso.org/standard/68256.html>

¹¹ <https://data.apps.fao.org/catalog/dataset/cc0ebca9-0df9-4061-a338-6ffec9e9acb4>

Chemical

- Soil pH** measurement will be achieved in a 1:5 volume fraction (in water) of soil (ISO10390)¹². Other methods to assess soil pH are: extractions, passive samplers, colorimetry and electrochemistry. Soil pH is a measure of the amount of acidity or alkalinity (basicity) that is present in the soil. Optimum pH values in agricultural systems vary regarding species cultivated. But, in general, its importance relies on its ability to determine the availability of nutrients for plant uptake and contaminants such as heavy metals. A pH of 7 is considered neutral (neither acidic nor alkaline) and can be difficult to achieve. A pH range between 5.5 to 7.5 allows for an extensive nutrient availability in most of the crops (FAO, 2020). Furthermore, pH influences soil microbial presence (bacteria, fungi, protozoa, etc.) and activity. The National Soil Survey Handbook (USDA) provides general reference pH ranges for key microbial groups as follows: Bacteria (5-9), Actinomycetes (6.5-9.5), Fungi (2-7), Blue green bacteria (6-9) and Protozoa (5-8), corresponding with a pH optimum of: 7, 8, 5, >7, and >7 for these microbial groups, respectively.
- Electrical Conductivity** values (expressed in dS/m) will be determined in an aqueous 1:5 volume fraction of soil (ISO11265)¹³. Other methods to assess EC use different extractions, passive samplers, colorimetry and electrochemistry¹⁴. Electrical conductivity is a measure of the ability of a solution to carry an electric current or the concentration of soluble salts. High salinity can result in soil degradation, so it is crucial to provide spatial EC information for soil quality evaluation (Corwin and Lesch, 2005). Different plants have varying optimal soil EC values. Generally, an EC range of 0.8 to 1.8 (dS/m) is considered ideal for many plants, while values exceeding 2.5 may negatively impact plant health. Electrical conductivity has been used as a surrogate measure of soil salinity classification. Salinity classes reported in the National Soil Survey Handbook (USDA)¹⁵ are: non-saline (EC 0<2 dS/m), very slightly saline (EC 2<4 dS/m), slightly saline (EC 4<8 dS/m), moderately saline (EC 8<16 dS/m) and strongly saline (EC ≥16 dS/m).
- Plant available macronutrients** are divided into two categories: 1) primary macronutrients, Nitrogen (N), Phosphorus (P), and Potassium (K) and 2) secondary macronutrients: Calcium (Ca), Magnesium (Mg) and Sulfur (S) (Maathuis, 2009). The recommended methodologies for their determination (with results expressed in mg/kg) are: saline extraction with potassium chloride (KCl) for N (ISO/TS 14256-1:2003)¹⁶, the Olsen method for P (ISO11263:1994)¹⁷, and ammonium acetate extraction for K (USDA, 2004)¹⁸. Moreover, it is also suggested even, although not strictly needed, to assess the availability of the remaining nutrients: Ca and Mg in the same extract as the one used for CEC (ISO23470); in the case of S, as for N, it is recommended to use KCl extraction (Rayment, G. E., & Lyons, D. J. 2011). Alternatives methods to assess plant available nutrients are passive

¹² <https://www.iso.org/standard/75243.html>

¹³ <https://www.iso.org/standard/19243.html>

¹⁴ <https://www.nrcs.usda.gov/sites/default/files/2022-10/Soil%20Electrical%20Conductivity.pdf>

¹⁵ <https://www.nrcs.usda.gov/resources/guides-and-instructions/national-soil-survey-handbook>

¹⁶ <https://www.iso.org/obp/ui/en/#iso:std:iso:ts:14256:-1:ed-1:v1:en>

¹⁷ <https://www.iso.org/obp/ui/en/#iso:std:iso:11263:ed-1:v1:en>

¹⁸ https://swcst.or.th/Report/cooperative/Soil%20Survey%20Lab%20Methods%20-%20SSIR%2042/SSIR_42_v4.pdf

samplers and electrochemistry. Although minimum concentrations for plant growth vary between plant species, it is important to bear in mind general plant requirements¹⁹ to avoid a deficit (but also an excess).

- **Total soil nitrogen (TN)**, refers to the entire amount of N present in soil, comprising organic and inorganic forms. The preferred analysis method is dry combustion (ISO 13878)²⁰, although the Kjeldahl method is also generally used (ISO 11261:1995)²¹, with values expressed in percentage. Limits values are reported by ESDAC²² and represented in a map of TN distribution all around Europe. The analysis of total N influences the amount of fertiliser inputs to be used in agriculture, providing also valuable information on microbiological activity, organic matter decomposition, and it is necessary to calculate the C/N ratio.
- **Cation exchange capacity** analytical recommended method is the ISO23470²³ and alternately ISO 11260:2018²⁴. Other methods have been reported (Ross et al.,1995). Cation exchange capacity is a measure of the soil's ability to hold onto positively charged ions (cations). These cations, such as calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺), and sodium (Na⁺), are essential plant nutrients. It is to be reported in cmol(+)/kg (centimoles of charge per kilogram of dry soil), but it is also typically expressed as meq/100 g (milliequivalents of charge per 100 g of dry soil). The cation exchanges values in soil depend especially on: clay (percentage and mineralogy), soil pH and the amount of organic matter. The optimum range according to the soil texture can be resumed in seven classes: sand (1-5 meq/100g), sandy loam (2-15 meq/100g), silt loam (10-25 meq/100g), clay loam/silty clay loam (15-35 meq/100g), clay (25-100 meq/100g) organic matter (40-200 meq/100g) and humified organic matter 250-400 meq/100g)²⁵.
- **Heavy metals** results are to be reported in mg/kg, and analysed after digestion with aqua regia by ICP-MS/AES/SFMS (ISO 54321)²⁶, this methodology aligns with the used in 2018 LUCAS²⁷ Topsoil Survey, and the one with dilute nitric acid (ISO17586:2016)²⁸ with the proposed by the EU Soil Monitoring Law²⁹. Both of them include the analysis of the following heavy metals: Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb), Antimony (Sb), Thallium (Tl), Vanadium (V) and Zinc (Zn). Other methods comprise different extractions, passive samplers; bioassays; lab- on- a- chip, biosensors and electrochemistry. Heavy metal contamination represents a significant threat to soil and living organisms. Therefore, assessing heavy metal concentrations is crucial for evaluating soil health (Soriano-Disla et al., 2014a). The availability of these metals in soil is influenced by factors such as pH, EC, and organic matter content. At European level, threshold values related to the application of sewage sludge in agricultural soils have been defined (EU Directive 86/278/EC). Other threshold and guideline values for metals in soils based on

¹⁹ <https://soilsfacstaff.cals.wisc.edu/facstaff/barak/soilscience326/macronut.htm>

²⁰ <https://www.iso.org/standard/23117.html>

²¹ <https://www.iso.org/standard/19239.html>

²² <https://esdac.jrc.ec.europa.eu/themes/npk-european-soils>

²³ <https://www.iso.org/obp/ui/en/#iso:std:iso:23470:ed-2:v1:en>

²⁴ <https://www.iso.org/obp/ui/en/#iso:std:iso:11260:ed-2:v1:en>

²⁵ <https://soilqualityknowledgebase.org.au/cation-exchange-capacity/>

²⁶ <https://www.iso.org/standard/75441.html>

²⁷ https://esdac.jrc.ec.europa.eu/public_path/shared_folder/dataset/75-LUCAS-SOIL-2018/JRC_Report_2018%20LUCAS_Soil_Final-v2.pdf

²⁸ <https://www.iso.org/obp/ui/en/#iso:std:iso:17586:ed-1:v1:en>

²⁹ <https://eur-lex.europa.eu/eli/dir/2025/2360/oj/eng>

either ecological or health risk have been reported in literature (Tóth et al.,2016b). Derived from the LUCAS survey, maps of heavy metals in EU topsoils are available on ESDAC (Tóth et al.,2016a).

It is important to bear in mind that, in the iCOSHells project, heavy metals for the experimental sites not dealing with contamination issues (total amount) will be only conducted at the initial baseline characterization.

- **Soil organic matter** (SOM) content expressed in % is typically calculated from the amount of **organic carbon** after dry combustion (ISO 10694)³⁰ where also Total Carbon (TC) gets determined, Loss on ignition is also used (Roper et al.,2019). Other methods include NIR/MIR spectroscopy and ultrasound. Soil organic matter is a complex mixture of substances derived from plant and animal residues. It plays a crucial role in soil health, significantly influencing its biological, chemical, and physical properties by promoting infiltration and retention of water, and enhancing soil structure. SOM concentration depends on a number of factors (Weil and Magdoff, 2004). According to the literature (Rusco et al., 2001), SOM content can be divided into four classes: High: > 6.0%, Medium: 2.1-6.0%, Low: 1.1-2.0%, Very Low: < 1.0%.

Biological

- **Microbial biodiversity** assessment will be carried out by metagenomics, by using high-throughput sequencing of DNA barcodes for measuring taxonomical and functional diversity, showing the evolution of selected group of organisms as shown literature (Labouyrie et al.,2023). The reads obtained are generally organized in operational taxonomic units (OTUs) and then classified by taxon using a fitted classifier. For DNA extraction methodology and primers selection, it is suggested to refer to Earth Microbiome Project (EMP)³¹. Assessment should include data from at least three groups of organisms in line with EU/JRC soil biodiversity initiatives , to ensure broad coverage of soil biodiversity. Using DNA metabarcoding approach target regions recommended are: 16S ribosomal DNA for bacteria and archaea, the internal transcribed spacer for fungi (ITS), and 18S ribosomal DNA for eukaryotes (Orgiazzi et al., 2018). . Metagenomics is a powerful technique to evaluate soil biodiversity which involves analyzing the collective genetic material (DNA) of all organisms present in our soils. Moreover, by sequencing and analyzing this DNA, it is possible to have a clear idea of the composition and function of soil microbial communities (Hozzein, 2020; Roesch et al., 2007). Metagenomics allows to obtain, for example, bacterial and fungal diversity indexes which are often correlated with other soil indicators (Bahram et al., 2018). By analyzing selected functional group DNA sequences, as already achieved with LUCAS survey samples (Labouyrie et al.,2023), it is possible to obtain an estimation of soil biodiversity and monitoring the evolution through microorganisms quantification by qPCR (ISO 17601:2016)³² across the different land-use soil types of the LLs. Alternative methods to evaluate soil biodiversity include extractions, bioassays, PLFA and lab-on-a-chip.
- **Microbial respiration** will be assessed by the incubation method (ISO 16072:2002)³³ where the CO₂ production or O₂ consumption is measured, with results expressed in mg per time unit from a mass unit of soil, mg of CO₂

³⁰ <https://www.iso.org/standard/18782.html>

³¹ <https://earthmicrobiome.org/protocols-and-standards/>

³² <https://www.iso.org/obp/ui/en/#iso:std:iso:17601:ed-1:v1:en>

³³ <https://www.iso.org/obp/ui/en/#iso:std:iso:16072:ed-1:v1:en>

(or O₂)/g of dry soil*hour. Additionally, the carbon content of the CO₂ released can be quantified, with results expressed in mg of CO₂-C/kg dry soil (FAO, 2023)³⁴. Other methods which soil respiration can be measured are lab-on-a-chip, electrochemistry and/or biosensors. Soil respiration is the process by which carbon dioxide (CO₂) is released from the soil into the atmosphere, and provides information about soil biological activity. Respiration occurs primarily through the decomposition of SOM and plant litter by soil microorganisms, as well as through the respiratory activities of plant roots and soil fauna. It is a highly useful indicator that can be used to assess the effectiveness of soil management practices (Oyonarte et al., 2012; Miralles et al., 2012; Irving et al., 2024). Table 2 shows an example of soil classification based on microbial activity expressed in mg CO₂-C/kg soil/week (Doran et al.,2001).

Table 2. Showing soil quality correlation of basal respiration under optimal temperature and moisture in the lab.

SOIL RESPIRATION ACTIVITY				
Very low	Moderately low	Medium	Ideal	Unusually high
Dry, sandy soils that have little or no organic matter	Soils with marginal biological activity and organic matter	Soils that have a moderately balanced condition and to which organic matter has been added	Soils with sufficient organic matter content and populations of active micro-organisms	Soils with excessive organic matter content
APPROXIMATE LEVEL OF CO ₂ - RESPIRATION				
<300 mg CO ₂ -C/kg soil/week	300 to 500 mg CO ₂ -C/kg soil/week	500 to 1000 mg CO ₂ -C/kg soil/week	1,000 to 2,000 mg CO ₂ -C/kg soil/week	>2,000 mg CO ₂ -C/kg soil/week

Specific indicators for each living lab

Apart from the general indicators outlined in the previous Section, each LL will analyse specific indicators which are tailored to their needs, challenges and solutions tested. To minimize error, increase reproducibility and ensure consistency and coherence in the results, samples from all experimental sites within a LL will be analyzed by the

³⁴ <https://openknowledge.fao.org/server/api/core/bitstreams/e9f3525e-d101-4fee-9330-ba166eafa90d/content>

same accredited laboratory for a particular analysis, and each LL has nominated a coordinator, this information is also provided in this Section.

Swedish LL

- Scope: The Swedish LL tackles issues related to soil health on farms, such as compaction, poor structure, biodiversity loss, and nutrient imbalance especially Phosphorus pollution.
- Specific indicators: Hydraulic conductivity.
- Soil analysis laboratory: The laboratories designed for analysis are SLU and ALS Scandinavia (Sweden).
- Living lab coordinator: Nargish Parvin and Tora Raberg (RISE Research Institutes of Sweden).

Southeast Spain LL (GREENNOMED LL)

- Scope: The Spanish LL is focused on agriculture land use, and improving soil health in regions with intensive crop and tree cultivation. Their main goals are to reverse soil degradation, strengthen the resilience of ecosystems, and encourage sustainable farming practices.
- Specific indicators: Galls index (by IMIDA), fertilizers inputs analysis (by CAJAMAR) and water irrigation analysis, crop values and production analysis, N efficiency in agricultural system by isotope measure in water irrigation, fertilizers, soil, plant (by CEBAS-CSIC).
- Soil analysis laboratory: the laboratories designed for the analysis are CEBAS-CSIC (chemical), CAJAMAR (physical and soil respiration), and CETENMA (metagenomics).
- Living lab coordinator: José M. Soriano Disla (CETENMA).

Basque LL

- Scope: Basque LLs tackle soil health issues in wetland and wooded urban areas. They focus on issues like flooding, erosion, pollution, poor soil structure, and the effects of climate change. The goal is to improve soil health and urban ecosystems through collaborative research and community involvement.
- Specific indicators: related to ecosystem services.
- Soil analysis laboratory: external laboratory to be determined.
- Living lab coordinator: Begoña Benito (GAIA).

Italian LL

- Scope: The Italian LL aims to solve various soil health problems in different environments, including farms, orchards, and urban areas. Their research covers issues like soil carbon loss, urban soil pollution and compaction, poor soil structure, and declining biodiversity.



- Specific indicators: Cohex extraction, Multi-enzyme test, Microbial biomass test (dsDNA), Soil Biological Quality-arthropod (QBS-ar), Grape, must and wine analysis (sugar, total acidity, malic, tartaric, APA, K), Microvinification. The Italian LL is also considering the possibility to analyse soil organic contaminants and, for the sites involved in hydro-thermal carbonization (HTC) issues, to characterize analytically selected residual biomasses before and after their transformation.
- Soil analysis laboratory: The laboratory conducting all the Italian LL analyses will be the Soil Chemistry Laboratory of the Università Cattolica del Sacro Cuore (UCSC; Department for Sustainable Food Process). Biological analyses (microbial activity and metagenomics) will be conducted by external laboratories. Specifically, metagenomics analyses will be performed by the Biotechnology and Environmental Microbiology Laboratory of the University of Milan (Department of Food, Environmental and Nutritional Sciences).
- Living lab coordinator: Mario Gualdi (ISINNOVA).

Greek LL

- Scope: The Greek LL objective is to restoring and reclaiming land previously used for mining, which often has high levels of contaminants elements. In this project different soils require specific remediation methods and the objective is to find the best approach for restoring these former mining sites.
- Specific indicators and conduct analyses: there will be no further analysis outside of the general plan.
- Soil analysis laboratory: University of Thessaly (Greece).
- Living lab coordinator: George Chaitidis (CLUBE).

Bulgarian LL

- Scope: The Bulgarian LL aims to make vineyard soils more resilient to climate change, especially for grape and wine production. They will use ten experimental sites for collaborative research and development to achieve this goal, using remote sensing for irrigation systems, satellite data for precision farming and new decision support system.
- Specific indicators: weeds, pests and diseases analysis.
- Soil analysis laboratory: Agricultural University Plovdiv.
- Living lab coordinator: Vladislav Popov (Agricultural University Plovdiv).



Sampling and monitoring methodology

Measures and recommendations to ensure excellence in monitoring results and to decrease sampling and analysis variability are provided in this Section. These guidelines comprise key considerations provided below and a sampling protocol included in the Annex Section to achieve reliable results. For the preparation of this protocol, the methodology described in LUCAS (Tóth et al., 2013; Fernandez et al., 2017) and Soil Monitoring Law have been used.

Key considerations

In order to ensure excellence in soil monitoring results, and decrease sampling and analysis variability, the following key considerations are to be taken into account:

- A **standardized sampling protocol** has to be followed to collect soil samples from designated locations (Annex 1). Sampling will occur at three key time points: before the experiment, during the experiment (mid-season or mid-time), and at the end of the experiment. It is important to avoid sampling immediately after significant weather events to ensure accurate data collection.
- **Represent variability:** in order to represent site variability, composite samples (defined as samples comprised of a mixture of 5 subsamples) according to LUCAS soil sampling methodology will be taken. In general, and providing that large sampling plots are not expected, treatment variability will be accounted by taking a minimum of three composite samples per treatment. These sites will be revisited at the different moments of sampling as described above.
- These three minimum samples representing each treatment or solution proposed, are to be used for the performance of **robust statistical analysis** (e.g., ANOVA).
- At each LL, apart from the LL leader, a **sampling coordinator** will be nominated to ensure coherence and consistency across sampling which, whenever possible, will always be carried out by the same person.
- Follow **standard operation procedures** (SOP) for soil sample handling, storage and analysis (Annex 2).
- Samples from all experimental sites within a given LL will be analyzed by the **same laboratory** for a particular analysis to eliminate inter-laboratory bias within a LL, and using certified analytical methods.
- **Round Robin Tests** are to be followed to ensure there are no significant differences between the results from the different LLs. Therefore, interlaboratory comparisons will be conducted using two standard soil sample sent anonymously to all LL laboratories. At least, two tests will be done, one at the beginning, and one during the course of the project.
- **Cleanliness:** Ensure all tools, containers, bags... used to manipulate soil samples are clean to avoid cross-contamination.

Conclusions

The report proposes a catalogue of general and LL specific soil health indicators. As summarized in Table 3, the following general soil health indicators will be analysed across all the LLs: Physical (bulk density, particle size distribution and water holding capacity), Chemical (pH, EC, plant available macronutrients, cation exchange capacity, total N, total C, organic C and total heavy metals) and Biological (microbial biodiversity and respiration),. The information generated will provide a general picture of soil health conditions across the LLs and allow for the selection of the best-performing solutions.

Table 3. Soil Health Indicators: Measurement Units, Methods, and Reference Standards.

Soil health indicator	Units	Methods	Reference
Bulk density	g/cm ³	Core method, calculated from the mass and the volume	ISO 11272:2017
Particle size distribution	%	Sieving and sedimentation	ISO 11277:2020
Water holding capacity	%	Determination of matric pressures: using sand, kaolin or ceramic suction tables	ISO 11274:2019
pH	pH units	Aqueous extract of soil in a 1:5 (volume fraction)	ISO 10390:2021
Electrical conductivity	dS/m	Aqueous extract of soil in a 1:5 (volume fraction)	ISO 11265:1994
Plant available macronutrients (N,P,K,Ca,Mg,S)	g/kg	Saline extraction with potassium chloride for N, the Olsen method for P, and ammonium acetate extraction for K. The CEC extraction for Ca and Mg, and potassium chloride for S determination	ISO 22036:2024

Cation exchange capacity	cmol(+)/kg	Using a hexamminecobalt(III)chloride solution as extractant (or a barium chloride solution)	ISO 23470:2018
Total nitrogen	g/kg	Dumas by dry combustion	ISO 13878:1998
Total carbon & Organic carbon	%	Dry combustion (elementary analysis)	ISO 10694:1995
Total heavy metals	mg/kg	Digestion of aqua regia soluble fractions of elements (or using dilute nitric acid)	ISO 54321:2020
Microbial biodiversity	Reads/ OTUs	Metagenomic analysis through barcoding study of selected regions	Orgiazzi - 2022
Soil respiration	mg of CO ₂ (or O ₂)/kg*h	O ₂ consumption and/or CO ₂ release	ISO 16072:2002

In addition to this general set of indicators, the following specific indicators, tailored to each specific case to evaluate the performance of the prototypes, have been proposed to be measured in each of the LL:

- Swedish LL: Hydraulic conductivity.
- Southeastern Spain LL: Galls index, fertilizers inputs analysis, water irrigation analysis, crop related indicators, productivity analysis and N efficiency in agricultural system.
- Basque LL where the main interests are related to the environmental impact and nature-based solutions. Therefore, the specific indicators are related to ecosystem services.
- Italian LL: Cohex extraction, Multi-enzyme activity, and Microbial biomass activity (dsDNA), QBS-ar (Soil Biological Quality-arthropod), VSA parameters, grape, must and wine analysis (sugar, total acidity, malic, tartaric, APA, K), microvinification.
- Greek LL: There are no specific indicators, so they will follow only the general set but with a special focus on heavy metal values.
- Bulgarian LL: dedicated to viticulture and looking for new sustainable agricultural solutions, it will study specific indicators such as weeds, pests and diseases analysis.

A soil monitoring sampling protocol and a standard operating procedure have been also proposed in teamwork with all the LL participants in this project in an attempt to minimize variability in laboratory and sampling procedures across all LLs, ensuring consistent data collection despite the diverse solutions, areas of interest, challenges, and overall contexts of each LLs. It comprises some key considerations, a sampling protocol and a SOP provided in the Annex Section.

Both the proposal of a set of common indicators and a standard sampling methodology will be key for effectively monitoring and assessing the impact of the experimental innovative solutions on soil health within each of the six LLs. This approach will also guarantee the provision of homogeneous and harmonized data, which will be key for future model development. Therefore, LLs will be able to monitor the potential improvement of their initial soil health conditions at local scale, and contribute to the predictive models which will help inside and outside the project to identify solutions that can be applied in different areas with similar characteristics.

In terms of follow up work, the solutions implemented in the LL are going to be carefully monitored and new solutions will be included through cascade funding, which will imply the selection of new indicators. Based upon the work presented on this deliverable on the definition of a set of indicators, guidelines for monitoring methodologies of each indicator will be developed and presented in D 3.2. “Recommendation Paper: Guidelines for standardised monitoring methodologies” (M45, May 2028). The guidelines will include the best methods for capturing information for the measurement of indicators (sampling techniques, frequency of data collection, etc.), as well as different ways of measuring indicators in each experimental site such as visual assessment in the field, soil sampling with professional laboratory analysis, remote sensing, etc.



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Annex Section

Annex 1. Sampling protocol

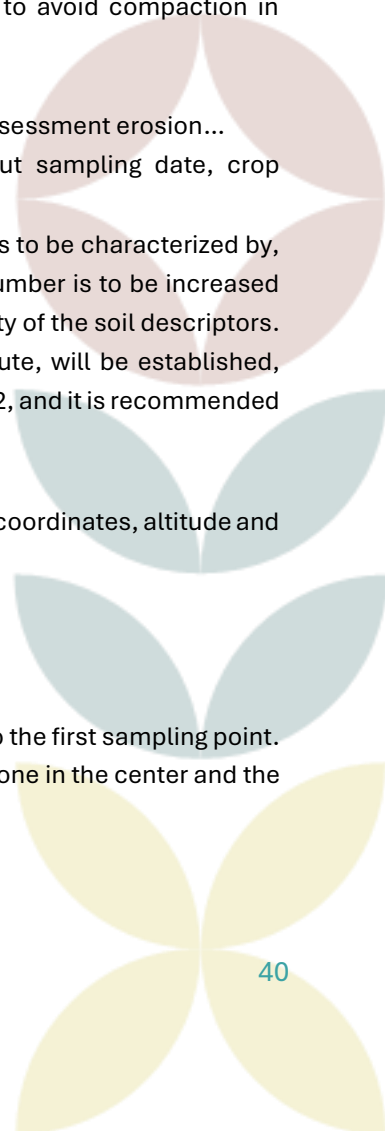
Before sampling

- **Revise and record previous site information** (if available) including previous crop/management, soil type, genetic soil horizons, etc., referring to the **FAO Guidelines for Soil Descriptions**³⁵
- **Checklist material before going to the field:** equipment for taking the samples (spade, auge, shovels), equipment for bulk density sampling (metallic ring, rubber mallet, block of wood, knife), protective equipment (gloves, glasses...), bucket for mixing subsamples, plastic bags, rubber bands, labels, pens, permanent marker, GPS device and camera (or mobile phone), wood or metal sticks, tape meter, plastic jar, scale, tape measure.
- **Check weather conditions** as sampling should be avoided in too wet conditions to avoid compaction in samples.
- **Metadata to be recorded:**
 - **General visual soil observation of the site:** slope, presence of visual assessment erosion...
 - Take **appropriate general and treatment specific information** about sampling date, crop type/condition, any specific details of area...
- **Design the sampling strategy and mark the sampling points:** each treatment needs to be characterized by, at least, 3 representative random samples (here called as “sampling points”; this number is to be increased depending on the area of sampling and expected variability) representing the variability of the soil descriptors. For selecting the sampling points, a site-representative pattern, such as zig-zag route, will be established, ignoring the initial and last (i.e. non-representative extreme) points as shown in Figure 2, and it is recommended to signpost each sampling point with a wood stick (or similar).
- **Take pictures** showing the area of sampling and the surroundings.
- **Georeferencing** sampling points with precise GIS (geo-graphical information system) coordinates, altitude and exposure.

During sampling

- Following the proposed **zig-zag route** (or alternative site-representative pattern), go to the first sampling point. As it has been discussed, each sample will comprise 5 subsamples taken as follows: one in the center and the other four at 2 m of distance, oriented to each cardinal point (N, S, E, W).

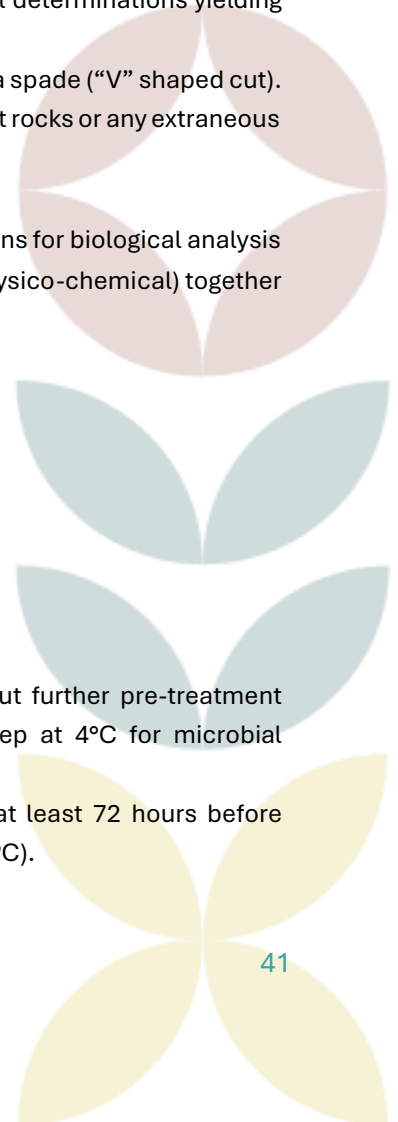
³⁵ [Guidelines for soil description](#)



- Before start digging, **remove residues, organic debris, vegetation and stones** from the surface.
- For each sampling point, **two sets of samples** are to be taken for the determination of bulk density and the biological/physico-chemical properties.
- The **first set**, to be called as “bulk” is to be taken within 10-20 cm depth following these steps:
 - Dig a hole 10 cm in depth, insert the standard metallic ring 5 x 5.1 cm (~100 cm³; without lids) reaching at least 15 cm depth, bevel edge down into the soil using the mallet and the block of wood to protect the ring and prevent soil compaction.
 - Dig a hole 2-3 centimeters away from the metallic ring. Make four cuts with the spade to create the hole. Be careful not to step on or near the ring while digging.
 - Recollect the soil core (i.e., all the soil contained inside the metallic ring) by removing the clump of soil with the ring intact.
 - Remove the excess soil from the bottom of the ring and around the outside edge of the ring with the knife
 - Place the soil core into a single plastic bag.
 - Repeat the above-described operation for the 5 subsamples (labelled as center, N, E, S and W) and place them into a bigger plastic bag labelled as “Bulk 10-20” and including the sample’s name together with the experimental site/treatment identification.
- The **other set** of samples will be used for the rest of physical, chemical and biological determinations yielding two subsets of samples as explained as follows:
 - For each sampling point, take the 5 subsamples (500 g) to a depth of 20 cm using a spade (“V” shaped cut). Try to respect the depth of sampling as previously mentioned and avoid to recollect rocks or any extraneous material.
 - Place the 5 subsamples (2.5 kg in total) in a bucket and mix thoroughly.
 - Take a representative sample of 500 g, labelled as “Bio” and store in cold conditions for biological analysis
 - The rest (2 kg) will be placed in another bag labelled as “PC” (which stands for physico-chemical) together with the experimental site/treatment identification.
- **Repeat the procedure for the remaining sampling points.**

After sampling

- **Take the samples as soon as possible to the laboratory** for analysis.
- **Clean all the material** which has been used.
- **Sample management**
 - **“Bulk” samples** will be used for the determination of bulk density.
 - **Samples for biological analysis (“Bio”)** will be analysed fresh without further pre-treatment immediately upon reception. If need to be store before analysis, keep at 4°C for microbial respiration and -20°C for DNA extraction.
 - **Samples for physico-chemical analysis (“PC”)** will be air dried for at least 72 hours before analysis. They should be kept under appropriate conditions (ideally, at 4°C).



- After completing the analysis of PC and bio samples, it is recommended to **store a backup sample** under appropriate conditions (PC at 4°C; bio, at least, at -20°C, and -80°C for longer backup needs).

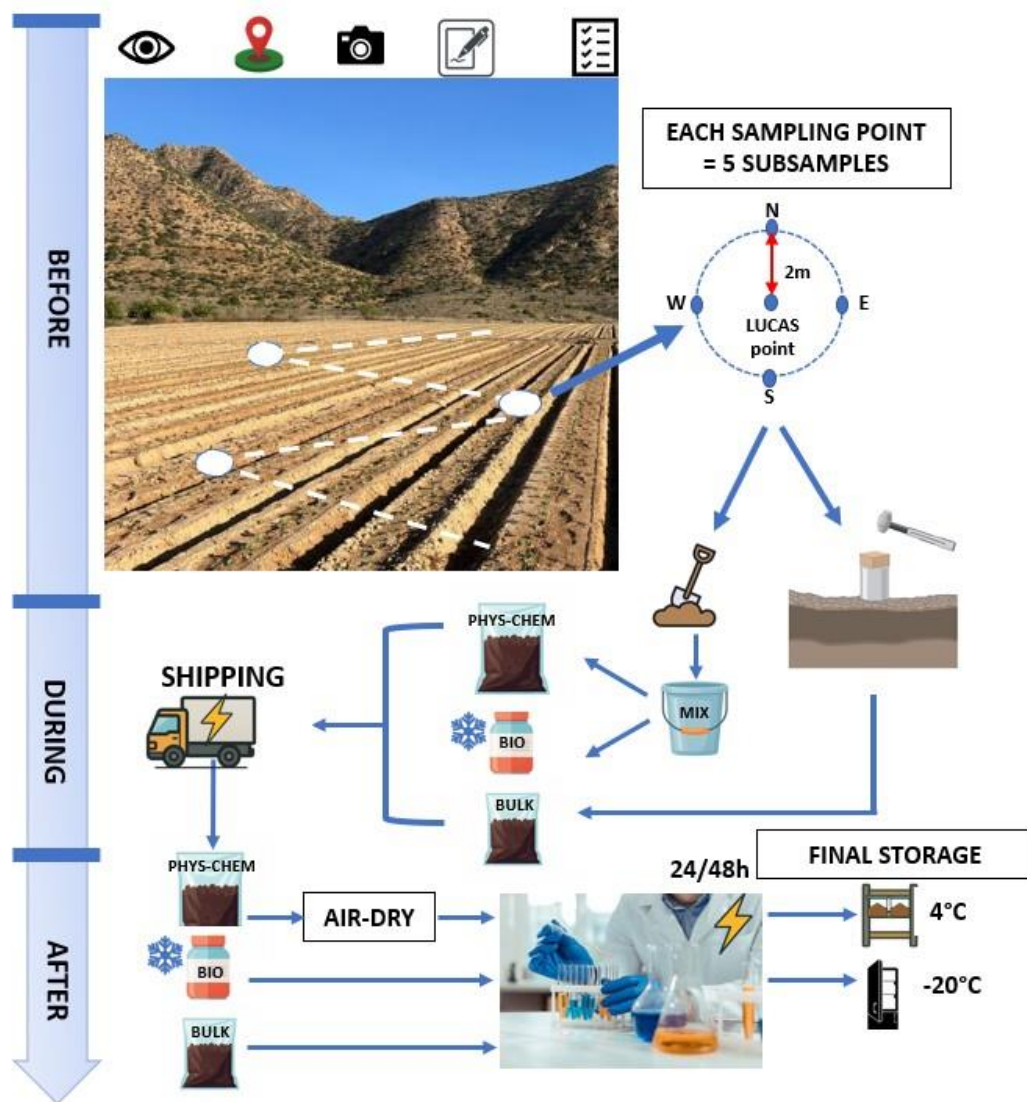


Figure 2. Sampling protocol scheme.

Annex 2. Standard Operating Procedure for soil analysis

Purpose

This SOP outlines a standardized framework for laboratory personnel across the iCOSHELL's LL to ensure harmonized analysis of soil health indicators, reduce inter-laboratory bias, and enable high-quality, comparable data. It provides a clear, consistent, and documented set of instructions for laboratory analysis (in a checklist format), complementing the procedures for sample collection, handling, and storage already outlined in this Deliverable. In addition, it is intended as a common framework for all LL, but it should be adapted as required to the specific context and capabilities of each laboratory. To support this, a dedicated section is included in the checklist below for reporting any adaptation, deviation, challenge, or proposed modification to the outlined requirements.

Applicability

Applies to all the laboratories involved in the analysis of soil samples within the iCOSHELLs project.

Summary of the method

This SOP is designed to support standardized implementation of the methodologies and ISO standards described in this Deliverable. It provides clear, simplified, and specific laboratory instructions to be followed before and during soil sample analysis. The procedures described are intended to align with Good Laboratory Practices and are based on foundational laboratory specifications detailed in the literature (Van Reeuwijk, L. P., 1998). The structure and content of this SOP were developed through desk research (Hollmann et al., 2020), tailored to meet the specific purpose of the document, and refined through internal laboratory consultations.

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SOP Code: [LL short name-Lab in charge of the analysis-progression number]

Revision Date:

Responsible: [Name of Laboratory Manager]

1. Laboratory personnel:

- 1.1 Adherence to SOP from the laboratory technicians
- 1.2 Designation of a soil sample expert laboratory technician
- 1.3 Ensure that each defined soil analysis is performed consistently by the same technician. If more than one technician is involved, specify their roles clearly.
- 1.4 Maintaining confidentiality of sensitive data information and avoiding conflict of interest
- 1.5 It is essential that all laboratory personnel are adequately trained and qualified.

2. Reagents

- 2.1 Use specific and indicated reagents and materials (ISO compliance).
- 2.2 Perform necessary quality controls (dead line, container integrity, etc.).
- 2.3 Check storage conditions, must be adequate as reported in fabricants indication of each reagent (temperature, light, humidity, etc.).
- 2.4 Record reagent lot numbers and expiry dates for traceability.

3. Equipment:

- 3.1 Allow instruments to warm up with sufficient time before use.
- 3.2 Review instrument calibration and follow manufacturer recommendations or ISO standards.
- 3.3 Verify instrument cleanliness and maintenance.
- 3.4 Record calibration and maintenance data in the instrument logbook.

4. Safety & security precaution:

- 4.1 Wear necessary personal protective equipment (PPE) (gloves, lab coat, safety glasses etc.)
- 4.2 Laboratory safety must always be the priority

5. Code of conduct

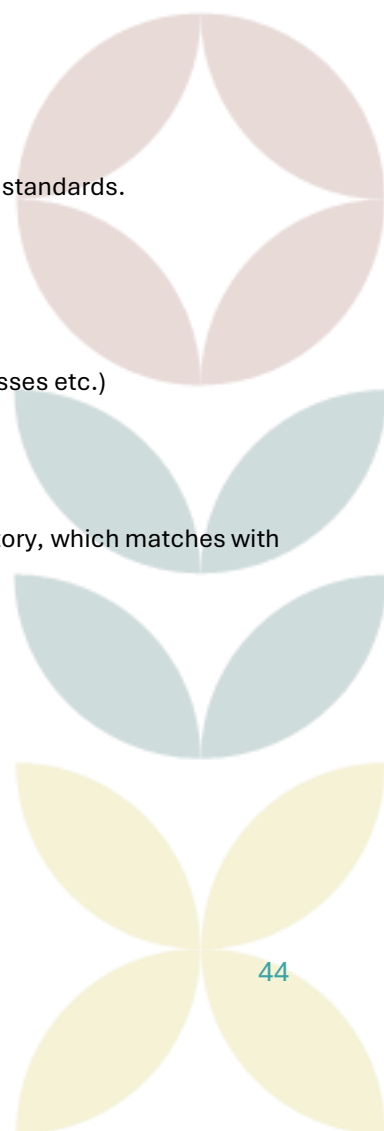
- 5.1 The Code of Conduct can be referred to the internal behavior rules of each laboratory, which matches with common professional behavior rules widely shared and also reported in literature³⁶.

6. Soil sample preparation:

- 6.1 Verify sample identity and analysis request.
- 6.2 Prepare the sample according to specific instructions (ISO11464)³⁷
- 6.3 Record sample preparation details in the laboratory logbook.

³⁶ https://www.mcgill.ca/bioengineering/files/bioengineering/20191230_code_of_conduct_1.7_web_0.pdf

³⁷ [ISO 11464:2006 - Soil quality — Pretreatment of samples for physico-chemical analysis](#)



6.4 For microbial analyses, ensure that soil is not oven-dried. Use fresh or air-dried soil stored at $\leq 4^{\circ}\text{C}$, or frozen if required.

7. Quality Control:

- 7.1 Perform periodic checks on the precision and accuracy of the analysis method.
- 7.2 Participate in intra laboratory comparison programs (e.g. round robin test) with soil standard samples
- 7.3 Record the results of quality controls and round robin test.
- 7.4 Implement corrective actions in case of out-of-specification results.

8. Analysis Performance:

- 8.1 Follow specific instructions of the analysis method for each soil indicator as reported in D3.1 Catalogue of soil health indicators.
- 8.2 Transcript raw data analysis and reference to relevant ISO standard numbers (where possible) in the laboratory logbook.
- 8.3 Document and justify any methodological deviations.
- 8.4 Accomplish with Good Laboratory Practices (GLP).

9. Data management:

- 9.1 Upload analysis results using raw data in the Excel ([Input data LL.xlsx](#)) provided in the SharePoint by the WP3 Leader.
- 9.2 Apply necessary correction factors and conversions.
- 9.3 Compare results with acceptance limits.
- 9.4 Evaluate the precision and accuracy of the results.
- 9.5 Record final results in the laboratory logbook and the Laboratory Information Management System (LIMS)

10. Waste Management and Cleanup:

- 10.1 Dispose of laboratory waste according to national or EU lab waste handling regulations.
- 10.2 Decontaminate and clean instruments and work surfaces.
- 10.3 Report any malfunctions or anomalies during all the process.
- 10.4 Record cleaning and maintenance operations in the laboratory logbook.

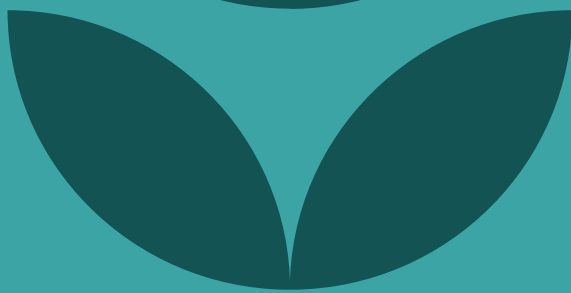
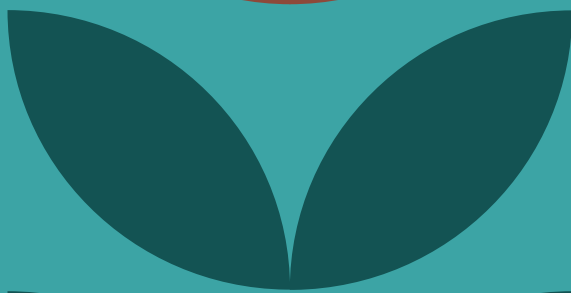
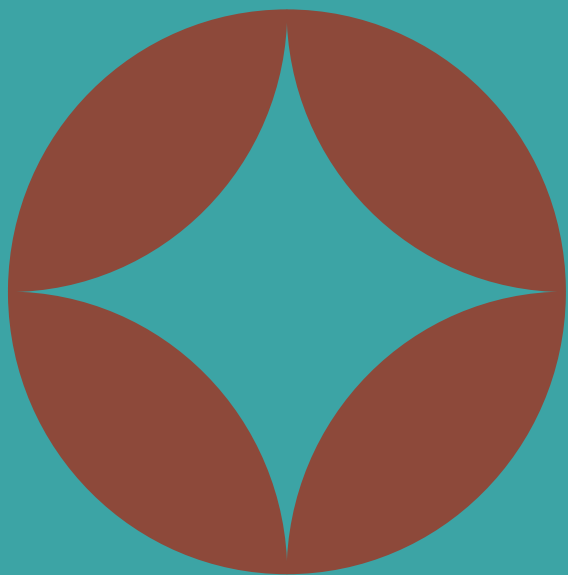
11. Documentation and Archiving:

- 11.1 Validate the completeness and accuracy of laboratory records.
- 11.2 Generate the analysis report and send it to the requester.
- 11.3 Store samples according to specific instructions in D3.1.

12. Observations

Use as many pages as needed for any required observation.





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