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AGROECOLOGICAL PRACTICES FOR SUSTAINABLE TRANSITION

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Table 1. List of Terms and Definitions

Abbreviation	Definition
ALLs	Agroecology living labs
AEPs	Agroecological practices
SOC	Soil Organic Carbon
TN	Total Nitrogen
AvP	Available phosphorus
exchK	Exchangeable Potassium
Ca	Calcium
Mg	Magnesium
ANOVA	Analysis of variance
MIR	Mid-Infrared Spectroscopy
PCA	Principal component analysis
RDA	Redundancy analysis
N_2O	Nitrous oxide
$CO_{2,}$	Carbon dioxide gas
CH_4	Methane gas
PLSR	Partial Least Square Regression
qPCR	Quantitative polymerase chain reaction
DNA	Deoxyribonucleic acid
GC	Gas chromatography
FAO	Food and Agriculture Organization of the United Nations
GHGs	Greenhouse Gases
ICRAF	World Agroforestry
ICARDA	International Centre for Agricultural Research in the Dry Areas

Executive Summary

A systematic assessment of the ecological and environmental impacts of agroecological practices (AEPs) across Agroecology Living Labs (ALLs) of CANALLS project under Task 4.1 is described in this document. This task establishes a robust, holistic assessment that quantifies ecological tradeoffs and establishes a quality and consistent database to support the agroecological practices. The evaluation framework uses a multi-dimensional monitoring approach that includes assessment of soil physicochemical properties and indicators of biological activity and diversity. The measurement of changes to soil health will involve physical (texture and bulk density), and chemical properties (SOC, TN, AvP, exchK, Ca, Mg). The abundance and diversity of the soil microbial community will be assessed through molecular techniques (i.e. metabarcoding). Climate impact of agroecological practices will be assessed through the measurement of GHG emissions (CO2, N2O, CH4). Assessment of pests and diseases will be conducted in addition to determining crop productivity and quality (yield). Monitoring of the variables is conducted within the experimental fields implemented within the ALLs of the project. In addition, soil survey campaigns were conducted in farmers' fields implementing agroecological practices within specific living labs. In the Kabare-Biega ALLs where we focus on coffee-based systems, factors included biopesticides, compost, cover crops, and hedgerow tested against the control treatments. A soil survey established the variability of soil fertility between coffee farms in the Kabare-Biega and Giheta ALL. In the Uvira ALL testing of manure and biopesticides to be used in a rice-based system was implemented. In Bujumbura, different dosages of urine-based fertilizer and biopesticides were tested in the maize-based system. In Kamonyi, Rwanda, the Mbili system is tested on cassava-beans intercropping. In the Ntui living lab, we have conducted a soil survey along the age and shade gradients in cacao systems. In these agroforestry systems, determination of biomass and species diversity is also one consideration. All the soil properties mentioned will be measured at ETHz. As the initial version of D4.1, this documentation describes the methods and tools to be used in relation to task 4.1 within the CANALLS project.

1. Introduction

Agricultural management is known to have a big impact on soil properties, primary production, microbial diversity and composition (Trivedi et al., 2016). Soil health is the state of a soil to respond to agricultural interventions in order to continue to provide yield and other ecosystem services (Shahane & Shivay, 2023). Thus, sustaining and enhancing soil health through agroecological management practices is important to sustain the health of soils, ecosystems, and hence people (Ţopa et al., 2023). To fully assess the environmental and ecological consequences of agroecological practices, we are using a multi-pronged approach that incorporates field experiments and surveys of farmer fields implementing agroecological practices versus not. The primary goal of this task is to evaluate the effect of agroecological practices on crop yield, yield components, biomass, species diversity, soil properties, and greenhouse gas emissions. Utilizing these various approaches allows us to comprise a well-rounded assessment of the potential benefits and trade-offs of agroecological practices in field, to make evidence-based recommendations for sustainable agricultural management (Garbach et al. 2014).

The assessment started with a coordinated series of field experiments where total crop yield and crop yield components are periodically and systematically measured. In the coffee and cacao agroforestry systems of some of our living labs (ALLs), trees species diversity that provide shade is measured, while various campaigns of soil surveys were also carried out to assess the impact of selected biophysical features on fertility status. In the experimental plots, soils were sampled to develop baseline data. After two years of experimental cycles, a second sampling will be conducted in order to measure the impact of agroecological practices on soil nutrient changes.

Agroecological practices represent the combination of possible practices, adapted to the unique environment and socioeconomic conditions of a given farming system. That can enhance the resilience of crops to climate extremes, improve soil fertility, and increase water use efficiency. Therefore, it is important to generate evidence regarding the benefits of these practices (Zenda & Rudolph, 2024). To fully understand the contribution of these practices to soil fertility status, it is critical to analyze physico-chemical and biological properties of the soils. Physical characteristics such as bulk density and texture are being considered in our work plan. For chemical analyses, soil organic carbon (SOC), total nitrogen (TN), available phosphorus (AvP), soil pH, and analysis of cation exchange (Potassium, calcium and magnesium). The analyses are being carried out at the Soil laboratory at ETHz using both wet chemistry and Mid-Infrared Spectroscopy techniques to reach appropriate accuracy and reliability (Fahad et al., 2022). Mid-Infrared Spectroscopy is economic by being less resource intensive and time consuming than other approaches for determining soil properties (Maia et al., 2007).

Microbial communities are vital to nutrient cycling, decomposition, and biotic disease suppression and are therefore good indicators of soil health and ecosystem function (Ţopa et al., 2023). Thus, for the biological analyses, abundance and diversity of procaryotic and eucaryotic groups will be determined. Soil enzymes that are involved in carbon and nitrogen cycles will also be analyzed to measure the functionality of the microbial community.

In order to evaluate the climatic effect of agroecological practices, greenhouse gas emissions are determined in coffee systems with different levels of shade trees. In the field, static chambers are deployed in order to capture gas samples over time and the changes in concentrations of greenhouse gases (N2O, CO2, CH4) are determined with a gas chromatograph (Yang et al., 2020). Using static chambers and gas chromatography is a widely accepted practice to quantify greenhouse gas fluxes

in agricultural systems. Assessing changes in soil carbon is important since the stabilization of soil carbon has the potential to offset GHG emissions of N2O and CH4 (Pallasser et al., 2013). The relationship between photosynthesis and GHG emissions is worth considering, especially when looking at soil (Ozlu et al., 2022).

In short, the monitoring and evaluation framework is in place to conduct a holistic evaluation of environmental impacts of agroecological practices, while supporting the development of sustainable agricultural systems which embrace productivity and good environmental stewardship (Ţopa et al., 2023; Naylor et al., 2022; Ogwu & Kosoe, 2023). Our methodology integrates field experiments, soil surveys and greenhouse gas emission monitoring to allow for a broad and holistic analysis of the impact of agroecological practices, while providing an avenue for evaluating sustainable agriculture (Sanz-Coben, et al., 2015).

1.1 Background

The pressing global imperative to transition towards sustainable agricultural systems necessitates comprehensive monitoring and evaluation of the environmental and ecological impacts of agroecological practices (Lupp & Zingraff-Hamed, 2021). Our planet faces the dual challenges of climate change and a rapidly expanding global population, demanding an agricultural paradigm shift. This shift requires adopting practices that not only foster food security but also actively reduce or reverse environmental and biodiversity degradation (Masocha et al., 2024; Warner, 2006). Conventional agricultural methods, often characterized by the excessive reliance on synthetic agrochemicals, have demonstrably depleted soil nutrients, polluted waterways, and ultimately compromised the delicate balance of agroecosystems (Enagbonma et al., 2023). Agroecology emerges as a viable and critical alternative, blending ecological principles into agricultural management to promote inherent sustainability and resilience (Petraki et al., 2025). Fundamentally, agroecological food systems strive to minimize external inputs, maximize the recycling of internal resources, and improve overall system resilience (Dubbeling et al., 2017). Therefore, a deep understanding of the complex environmental consequences of different agricultural management approaches is essential for effective decision-making and robust policymaking (Terán-Samaniego et al., 2025).

To address this crucial need, Task 4.1 aims to provide an overall assessment of the environmental and ecological impacts of agroecological practices and strategies. This assessment is conducted within multiple Living Labs, which serve as dynamic, real-life agricultural settings where agroecological approaches are co-created. A significant component of Task 4.1 involves determining the key parameters essential for accurately measuring the performance of these and strategies. It is imperative to identify indicators that sufficiently capture the multifaceted environmental (both biotic and abiotic) impacts of these efforts, thereby quantifying their effects. This measurement will also enable the identification and quantification of trade-offs among various performance parameters. Understanding these trade-offs is vital for pursuing agroecological improvements through practices that offer "win-win" scenarios, ensuring progress without inadvertently causing detrimental effects on environmental or ecological performance elsewhere (Jeanneret et al., 2021).

Task 4.1, under the leadership of ETH Zurich, has already initiated concrete measures to comprehensively evaluate and monitor the environmental and ecological implications of these agroecological practices and strategies directly within our Living Labs. The core mandate of this task

is to pinpoint key performance parameters, analyze the inherent trade-offs among numerous indicators, and assess the overall performance of the agroecological practices. This ongoing work directly supports our goal of promoting a transition towards more sustainable and resilient agricultural systems. As part of this effort, the team is actively undertaking detailed soil surveys and sampling across various experiments, with samples subsequently processed for precise laboratory measurements. These critical parameters are currently being measured, and the generated datasets will provide invaluable insights into the specific environmental and ecological impacts of the agroecological interventions.

A central pillar of this evaluation involves comparing the sustainability and efficiencies of the current agroecological practices against conventional and/or control practices within field experiments across all Living Labs. These direct comparisons are designed to definitively ascertain the environmental and ecological advantages that agroecological practices offer over their conventional counterparts. For instance, in the operational Kabare and Biega ALLs, specific treatments involving biopesticides, manure application, cover cropping, and hedgerows are being compared to a control group and against each other in a structured, stepwise approach. These comparative experimental treatments are built on shared assumptions and pose fundamental questions regarding the effective implementation of alternative practices for pest control and soil management. Similarly, in the ongoing Bujumbura ALL, the efficacy of biopesticides and varying dosages of urine fertilizers are being tested on maize crops. The overarching objective here is to reduce dependency on synthetic pesticides and mineral fertilizers while concurrently increasing maize crop yield. Furthermore, in Kamonyi ALL, cassava-legume intercropping systems utilizing the Mbili intercropping systems are being implemented to evaluate their performance against monocropping systems specifically in terms of overall crop productivity. Through these detailed comparisons, the project aims to provide compelling, evidence-based insights into the environmental and ecological benefits of agroecology.

1.2 Objectives

The following objectives have been defined for task 4.1:

- ✓ Define the necessary parameters to evaluate the performance of the agroecological practices and strategies implemented in our ALLS
- ✓ Assessing the environmental and ecological effects of the agroecological practices and strategies implemented in our ALLs
- ✓ Assessing the trade-offs between different performance parameters

2. Existing frameworks/ tools

2.1 Introduction

Our framework allows for the selection of indicators that capture the environmental and ecological dimensions of agroecological systems. It emphasizes relevance, feasibility and scientific robustness

to enable evidence-based decision making in relation to sustainable land management. Thus, depending on the outlined evaluation objectives, indicators can be grouped in a number of ways, including:

Indicators of soil health

- **Physical**: soil texture, bulk density, porosity, water holding capacity
- **Chemical**: pH, organic carbon, total nitrogen, available phosphorus, potassium, calcium, Magnesium
- **Biological**: microbial biomass, microbial abundance and diversity and enzyme activity

Indicators of ecosystem biodiversity

- **Plant biodiversity**: species richness (abundance) within crop and non-crop vegetation
- **Soil biodiversity**: diversity of soil microbial communities

Indicators of climate impacts

- **GHGs fluxes**: soil emissions of N_2O, CO_2, CH_4
- **Carbon sequestration**: changes in soil organic carbon over time

Indicators of crop productivity

- **Crop yield** (for example, bean weight, fruit size)
- **Biomass production**, harvest index

2.2 Documentation on the existing framework and tools

Soil health assessment is a complex field that requires in-depth comprehension of physical, chemical, and biological soil properties. These soil properties are directly related to agricultural productivity, environmental quality, and ecosystem functions. The following table summarizes key indicators and describes fool for measurements.

Table 2. Summary of soil health assessment framework and indicators

Category	Component/Indicator	Description/Measurement Method	Reference
Assessment Tools	General Tools	Includes on-site observations, laboratory tests and experiments, and data analytics for a holistic understanding.	(Topa et al., 2025)
Physical Indicators	Soil Texture	Influences water holding capacity and drainage.	(Shahane & Shivay, 2021)

	Soil Structure	Affects aeration, root growth, and erosion resistance. Assessed visually.	(Shahane & Shivay, 2021)
	Bulk Density	Indicates soil compaction and porosity.	(Shahane & Shivay, 2021)
	Water Infiltration Rate	Measures the soil's ability to absorb water.	(Shahane & Shivay, 2021)
	Soil Compaction	Measured with penetrometers.	(Shahane & Shivay, 2021)
Chemical Indicators	pH	A prime indicator influencing nutrient availability and microbial activity. Measured using electrochemical methods.	(Yadav et al., 2020)
	Macro & Micronutrients	Measured with various extraction and analytical techniques.	(Yadav et al., 2020)
	Soil Organic Matter (SOM)	Indicates soil fertility, structure, and water holding capacity. Measured by loss of ignition, wet oxidation, or dry-combustion.	(Yadav et al., 2020)
	Electrical Conductivity (EC)	Indicates general nutrient status and is primarily used to determine salinity.	(Yadav et al., 2020)
	Cation Exchange Capacity (CEC)	Measures the soil's potential to store nutrients.	(Yadav et al., 2020)
Biological Indicators	Microbial Biomass	Total size of the microbial community. Measured by chloroform fumigation, phospholipid fatty acid (PLFA) extraction, or total DNA levels.	(Gil-Sotres et al., 2004)
	Soil Respiration	Provides insight into the metabolic activity of soil organisms.	(Gil-Sotres et al., 2004)
	Enzyme Activity	Measures the functional capacity of the soil microbiome by targeting specific enzymes in nutrient cycling.	(Gil-Sotres et al., 2004)
	Microbial Diversity	Assesses the variety of prokaryotic and eukaryotic groups through	(Gil-Sotres et al., 2004)

		metagenomics and sequencing.	

3. Methodology description

3.1 Definition of methodology and approach

The selected methodologies and tools are specified in relation to the study to optimize the assessment of ecological, productivity, and climate impact of agroecological practices. Methodologies and tools are selected based on scientific relevance and adaptation to the local context. The purpose of taking into account local context is to generate an integrated framework capable of addressing the complex biophysical aspects of agroecological systems.

Justification for methodology and tools for selection

The selection of methodologies and tools in this study is based on several key criteria. These include scientific robustness and credibility, relevance to the objectives, feasibility of implementation in each context, and congruency with international standardization for monitoring and evaluating agroecological systems. The following elucidate criteria and justification for chosen methodology and tools:

1. Holistic and integrated approach

The objectives of this task include assessing soil fertility, biodiversity, productivity, ecological impacts and climate effects; therefore, a multidisciplinary approach is needed. The effective integration of ecological, chemical, physical, and biological methods allows thorough assessments that are necessary for understanding the complex dynamics within agroecological systems (Giller et al., 2011).

2. Use of standardized and validated protocols

Tools and methods are selected based on scientific acceptance, reproducibility, and comparability, with development following the best international practice protocols (FAO, ICARDA, etc.) (FAO, 2004; ICRAF, 2013). Standardization provides data quality, facilitates the development of benchmarks, and improves the credibility of the task's findings.

3. Design and Analysis of Soil and Microbial Protocols

Physicochemical soil analysis (pH, organic carbon and nutrients) are the core indicators of soil fertility, which greatly influence crop productivity and resilience (Brady & Weil, 2008).

Microbial community analysis using DNA methods (qPCR, Next-Generation Sequencing). Soil microbial diversity and functional potential are sensitive indicators of soil ecosystem health and management practices (Lauber et al., 2008). Molecular techniques are more informative about

community structure and functional genes than traditional microbiological methods, such as plating, chloroform fumigation, and PLFA.

4. Selection of Greenhouse Gas Measurement Methods

For greenhouse gas emissions measurement, static chamber methods and gas chromatography were chosen for the following reasons:

a) Quantifies temporal greenhouse gas fluxes under different management regimes.

b) Provides reliable and low-cost methods that are appropriate for the Central African context and standard protocol has been established.

5. Approaches to Ecosystem Services and Biodiversity Assessments

a) Species inventories and diversity indices inform on ecological resilience.

b) Soil enzyme activities provide functional indicators of microbial activity and soil biological health, which are sensitive to changes in land management (Tabatabai, 1990).

6. Data Analysis Methods

Statistical data analysis methods such as ANOVA, multivariate analyses (PCA, RDA) will be used because of:

1) the ability to handle large volume, complicated datasets with many variables.

2) the ability to expound causal relationships amongst a set of variables, thereby allowing comprehensive interpretation and understanding of results (Grace et al., 2010).

7. Feasibility and Adaptation to Local Context

Local context is considered in designing soil sampling frames to better capture variability across the landscapes in our Living Labs. Logistical aspects during sampling (technical expertise) and shipping soil are country specific and is where science meets regulations. Local laboratories (African countries partners) assist in sample preparation, while analysis is being conducted at ETH Zurich lab facility in order to assure data quality.

8. Participatory and Socioeconomic methods

Soil sampling is always backed-up with farmer surveys to acquire information on past management history of the plot. This aspect provides perspectives and considerations that increases the relevance and applicability of findings for local stakeholders and policymakers.

3.2 Techniques and tools for assessing soil Health

1. General soil assessment of physico-chemical properties

Assessment of soil physico-chemical properties calls for relevance and appropriate methods and tools (Carter et al., 2000). The output provides an important baseline of soil fertility in response to various biophysical, land management and socioeconomic factors (Lehmann, et al., 2020). The chosen methods implemented in this task have acceptable accuracy and standards, and can provide data that is reproducible across space and time. For instance, soil pH is one of the key soil attributes that indicates nutrient availability, it has important implications for microbial activity, and determines soil chemical conditions (Nigussie, 2024). Soil organic carbon is the main component of soil organic matter which is a excellent indicator for soil fertility because it positively influences soil structure, CEC, and microbial activity and thus ultimately plant growth and productivity (Kačergius et al., 2025). Importantly, soil organic carbon and nitrogen are critical requirements for soil health, sustainable biological productivity, and environmental quality (Ghimire et al., 2023). Nitrogen is needed for plant growth as well as catalyzing microbial processes; whereas the availability of phosphorus is also crucial for ensuring crops are correctly nourished as well as for maintaining soil productivity (Sudrajat et al., 2019). Finally, cation exchange capacity is a determinant of the soil's inherent ability to retain and supply cations which are essential for plant growth and functioning. Soil texture gives predictability to water retention, aeration, and nutrient interactions (Yadav et al., 2020). Soil bulk density is a physical characteristic that indicates soil compaction, soil porosity, soil structure, and basic architecture, and represents an important component of basic soil function that facilitates water infiltration, aeration, root penetration, and microbial activity. The overall physical assessment provide is important in sustaining productivity and soil health (Oklo et al., 2021). In addition, it has been shown that combining these physico-chemical analyses does generate a comprehensive picture of fertility trends, and potential restrictions under different land management practices (Ţopa et al., 2025).

2. Microbial abundance and community assessment

Microbial communities of the soil are important to soil fertility and soil health, nutrient cycling, and overall ecosystem resilience. To derive information on the functional health of soils under different agroecological practices, a comprehensive analysis of their abundance and diversity is needed (Sudrajat et al., 2019). Any assessment of soil health without taking into consideration the diversity of microbes and microbial community composition results in an incomplete perspective of soil ecosystem functioning (Trivedi et al., 2016). Microorganisms are responsible for decomposition of soil organic matter, regulate carbon storage, enable nutrient cycling, and increase nutrient uptake by plants (Chen et al., 2024). Thus, soil microorganisms are a critical component of the soil ecosystem and form the foundation for soil functioning (Zhan, 2024). Therefore, investigating microbial communities (including bacteria, fungi, archaea, and other microorganisms) provides crucial information regarding the effect of different agroecological practices.

3. Advance in molecular techniques for soil ecology

Advanced molecular methods, such as metagenomics, metatranscriptomics, and amplicon sequencing, provide high resolution characterization of soil microbial communities. Metagenomics is a way to look at the genetic material from environmental samples, which helps understand the potential functions and diversity of microorganisms, whereas metatranscriptomics shows the genes that are expressed by those communities, which allows us to see their functions at a single moment (Nwachukwu & Babalola, 2022). Amplicon sequencing provides information about the taxa (group or

lineage) and the number of microbes from a sample by targeting certain genes (such as the 16S rRNA gene for bacteria and archaea or the ITS region for fungi) (Trivedi et al., 2013). Synthesizing information about microbial diversity and soil functions requires data from a variety of methods including physico-chemical soil properties, microbial community, and functional measurements data. These multiple lines of evidence provide insight into a complete soil ecosystem and improve the ability to design sustainable land management decisions that benefit soil quality and productivity (Maron et al., 2018; Wu et al., 2011). The important point is that there is no silver bullet for soil metagenomics, but there are experimental avenues that can help to quantify the degree of methodologically derived bias, define theoretical ecology perspectives, and provide a more robust starting point for future research (Nesme et al., 2016).

The development of molecular methods, in particular PCR, has helped towards a better understanding of microbial diversity due to their ability to amplify target microbial DNA sequences from complex environmental samples (Santos et al., 2019). Culture independent methods are essential for understanding the genetic diversity, population structure, metabolic activity and environmental role of microbes, and particularly that uncultured microorganisms usually represent the vast majority of all biodiversity on the planet (Michán et al., 2021). The combination of molecular techniques and bioinformatics is key to revealing the complexities of soil microbial communities, their activities, and how they contribute to ecosystem functioning (Thomas et al., 2012).

There has been an increase in applying molecular technologies to examine the structure of microbial communities and their functional activity due to environmental change and disturbances (Michán et al., 2021). This has allowed us to obtain enormous information about the composition of soil microbial communities (Baldrián et al., 2011). The success of any land management will depend on the composition, activities and resilience of the microbial communities that reside in the soil (Amrani, 2022). Molecular tools allow researchers to address a range of diversity-related questions and offer new ecological insights (Clark et al., 2018).

As land use becomes more intense, rapid and low-cost approaches for determining soil biodiversity health status are increasingly needed (State of Knowledge of Soil Biodiversity - Status, Challenges and Potentialities, 2020). The integration of molecular techniques with conventional soil health assessments could provide a more complete picture of soil health and resilience (Shibata & Rose, 2007). Experimental designs that allow repeated observations of soil microbial communities, including the activities associated with those communities, are required to measure both resistance and resilience (Bardgett & Caruso, 2020). Next-generation sequencing technologies have brought about a revolution in the study of microbiomes, making microbiome investigations relatively easier and thus allowing to appreciate the various roles of microbiomes in a range of ecological and clinical studies (Fachrul et al., 2022). The analysis of microbiome data means applying hypothesis-testing procedures that determine the significance of differences among the groups of interest (Kers & Saccenti, 2022).

3.3 Rational for selection of specific methodology and tools

1. Wet chemistry

Soil pH is measured in calcium chloride ($Cacl_2$) at a ratio of 1:2.5 because it is based on soil moisture conditions that closely approximate in-the field scenario. Therefore, it is a well-accepted, easy, and fast method for assessing soil pH and makes it more amenable to full-scale surveys as well as for repeated measures over time (Thomas, 1982). Soil organic carbon (SOC) and total nitrogen (TN) will be quantified using dry combustion with a LECO TruMac® Series CNS Analyzer. This method, considered a gold standard for total carbon and nitrogen determination, offers accuracy, speed, and reliability (Matejovič, 1997). A weighted soil sample is combusted at 1350 °C in an oxygen-rich environment, converting all carbon and nitrogen into CO2 and N2O. Infrared detectors measure the concentrations of these gases, which the instrument then converts to total carbon and nitrogen content (Mäkinen & Smolander, 2025). LECO's rapid, automated process provides consistent results and high sample throughput, minimizing manual handling and ensuring analytical quality, making it suitable for assessing soil health indicators across diverse soils to understand the impact of agroecological practices on soil fertility and nutrient cycling. This method has the advantage of assessing a large number of soil samples within a short time frame. The Olsen method will be applied to assess available phosphorus. It involves extracting phosphorus from soil using sodium bicarbonate (pH=8.5) and colorimetry to quantify available P (Olsen et al., 1954). Soil texture will be determined by dispersing the sample and measuring particle size by diffractometer. In more detail, soil samples will be treated with sodium hexametaphosphate to disperse soil aggregates and subject to vigorous shaking and sonicating before analysis. A laser diffraction particle size analyzer (LS 13 320, Beckman Coulter) will be used to assess soil particle size distribution (Bezuglova et al., 2021). The data is classified based on the USDA classification system to define soil category (sand, silt, clay proportions) and using a textural triangle. This comprehensive approach uses chemical, mechanical and advanced analyses by providing reproducible results for a variety of applications in agriculture and environment ecosystems (Fan et al., 2025). Soil bulk density can be defined as the most basic physical property of soil indicating the degree of compactness, porosity, and overall structure of the soil. Furthermore, soil bulk density affects fundamental soil functions of water infiltration and storage, gas exchange and aeration, root penetration (growth), and microbial activity (influence by minimum management). All of which are strongly linked to measures of productivity and soil health. For the core method, a known volume of soil, extracted using a typical standard metal core sampler of volume 98 cm^3, and the dry weight was determined following oven-dry at 105 ^oC.

2. Mid-Infrared Spectroscopy (MIRS) coupled with Partial Least Squares Regression (PLSR)

Mid-Infrared Spectroscopy (MIR) with Partial Least Squares Regression (PLSR) provides a rapid, non-destructive and affordable approach for analyzing soil properties. This emerging methodology is becoming more popular in soil science due to its ability to predict soil properties with speed and accuracy. Unlike chemical analysis, MIR spectroscopic assessment requires little sample preparation ahead of analysis and provides results practically instantly. As an advantage, this method decreases labor and cost in comparison to wet chemistry (Angst et al., 2014). During MIR analysis, consistency and repeatability is maintained between each reading and the MIR captures complex vibrational

signatures produced from soil organic matter, inorganic minerals, and other components of the soil environment. The MIR internal algorithm enables prediction of many soil properties indirectly with a single scan. The spectral readings record information related to soil organic carbon, mineralogy, functional groups related to soil pH, nutrients and more. From the environmental viewpoint, MIR promotes a more sustainable practice of avoiding chemicals and energy and aligns with responsible research.

Moreover, with a multivariate calibration model (PLSR), MIR will predict a range of physical-chemical properties such as soil organic carbon, soil pH, nitrogen, phosphorus, texture and aligns with various data needs from this task. PLSR is suited for analyzing spectral data with high collinearity, it extracts latent variables of variance to explain and correlate spectral variations to soil property measurements. The calibration models built using PLSR require a subset of sample "training set" mostly 30% of the sample size measured through wet chemistry to efficiently predict properties of unknown samples (Wold et al., 2001). Incorporating the ETHZ spectral library built from the Central Africa Soils into the calibration will enhance precision (Summerauer et al., 2021). Robust predictions with high predictive performance are ensured with cross-validation and external validation. With several sites, time points, and land management regimes to evaluate, MIR with PLSR allows researchers to screen large soil datasets rapidly. Calibration models created with representative samples can be deployed to a new dataset, which is a development in continual ongoing soil monitoring that added consecutively little extra cost. In brief, MIR with PLSR provides a faster, more credible, more environmentally sound alternative to previous testing methods, laboratory testing or conventional soil testing methods. MIR-PLSR works well in tandem with previous laboratory analysis and enables rapid high-throughput, multi-variable analysis of soils and is vital to monitor agroecological systems at multiple scales for long-term soil health assessment.

3. Soil microbial analysis

Soil Microbial Abundance studies involve extracting total soil DNA and using an optimized commercial kit to ensure high yield and purification. Quantification of microbial abundance is achieved through quantitative PCR (qPCR) assays aimed at functional genes for example, 16S rRNA (bacteria), archaeal 16S rRNA, and 18S rRNA (fungi). qPCR is used to provide sensitive quantification of microbial gene copies that support tracking shifts in microbial populations in response to treatments. qPCR is also a very well-established method with high reproducibility and standardized protocols to allow comparability (Fierer et al., 2005). Microbial community diversity can be well identified by sequencing. The method and type of sequencing will vary by discipline, marker sites or genes included in amplification sequencing (e.g., 16S rRNA for bacteria and archaea; 18S rRNA or ITS regions for fungi). Sequencing allows for a great taxonomic resolution and identification of group members that detail community composition, community structure, and relative abundance, and when paired with predictive tools, sequencing can help to determine functional potentials, inform the processes of nitrogen fixation, organic matter decomposition, etc. (Douglas et al., 2020). It can identify the most common and rare taxa, inform community change that occurs with different management changes, environmental aspects and/or shifts in response to time (Lauber et al., 2008). For this task 4.1, additional indicators of soil enzymatic activities will be evaluated. Enzymatic activities involved in nutrient cycling are the most reliable indicators of microbial functional potential and soil biological activity. Enzymes involved in the decomposition of carbon include 1) β-Glucosidase, which hydrolyzes cellulose and oligosaccharides, signaling the capacity of the microbial community to decompose carbohydrates-based substrates (Sinsabaugh et al, 1991); 2) Cellobiohydrolase, which hydrolyzes cellulose. Whereas for nitrogen cycling, enzymes that hydrolyze urea into ammonia and carbon

dioxide (urease), are useful to indicate nitrogen mineralization potential. Nitrate Reductase and Nitrite Reductase that convert nitrate/nitrite into various forms of nitrogen and indicative of denitrification patterns and processes, and nitrogen removal (Tabatabai, 1990). Enzyme activities are typically analyzed using colorimetric or fluorometric substrates in an assay condition. Enzymes as functional indicators signal the capacity of the soil to cycle carbon and nitrogen, which are critical to maintaining soil fertility. The assays also respond to management changes, intensity of land use, and environmental conditions that make them ideal indicators of living soil (Sinsabaugh et al., 1998). Once correlated with microbial biomass, the result provides information on ecosystems functioning. The approaches to assess enzyme activity are standardized, which allows for comparisons across different treatments and sites. It also provides answers and evidence to explain some biological basis for learning more on the environmental processes that drive soil health and fertility and ecosystem resilience and responses to management practices that are important components for considering how sustainable agroecological systems are.

4. Greenhouse Gas Analysis using gas chromatography.

Quantifying emissions of greenhouse gases from agricultural systems is fundamental to understanding how various anthropogenic activities affect the atmosphere on Earth. Quantifying these emissions requires a robust analytical process which includes rigorous sample collection, preparation, and analysis (Ferrari et al., 2025; Hume & Cattle, 1990). Our analysis of gas samples collected in the field are shipped to ETH Zurich for analysis on a gas chromatograph, which is a routine analytical instrument that separates and quantifies the relevant volatile compounds in a gas mixture (Buckingham et al., 2023).The 456-GC apparatus from Scion Instruments is being used to quantify the mole fractions of CO_2, nitrous oxide (N_2O), and methane (CH_4) in the collected samples (Rosenstock et al., 2013). Before the samples are analyzed, the gas chromatograph is calibrated. The calibration consisted of a series of standard gas mixtures that contain a known concentration of CO_2, N_2O, and CH_4 that would be expected to be in the range of concentrations in our environmental samples (Veen et al., 2006).

Gas chromatography is based on the separation of a gas mixture into the individual components according to their physical and chemical properties (boiling point, polarity) as it passes through a chromatographic column. The gas sample is injected into the GC system through an inlet and is carried in the GC system through a chromatographic column by an inert carrier gas (helium or argon). The column is packed with a stationary phase (polar stationary phase), which interacts differently based on each gas component allowing the gases to elute from the column at different times. As each gas elutes from the column, it passes through a detector which measures the concentration of that gas, giving a signal that is proportional to the amount of gas present in the sample. The data acquired through the gas chromatography is subsequently processed to provide the mole fraction concentration of CO_2, N_2O, and CH_4 in our samples (Cassidy & Schuerch, 1976). Gas chromatography, especially with calibration and quality assurance for the process, gives a clear and easy to use analytical methodology for quantifying greenhouse gas concentrations in environmental samples (Shezi & Kiambi, 2025).

4. Conclusion and outlook

The initial version of the delivery 4.1 outlines a systematic and robust methodology for assessing the ecological and environmental impacts of agroecological practices (AEPs) within the CANALLS project's Living Labs (ALLs) across the Democratic Republic of Congo, Burundi, Cameroon, and Rwanda. Our approach goes beyond conventional evaluations by establishing a holistic assessment framework that quantifies ecological trade-offs and generates a high-quality, consistent database to support agroecological practices.

The evaluation employs a multi-dimensional monitoring strategy that comprehensively assesses soil physicochemical properties, indicators of biological activity and diversity, and climate impact. Specifically, we measure changes in soil health through physical characteristics (texture, bulk density) and critical chemical properties (SOC, TN, AvP, exch K, Ca, Mg, pH). The functionality of the soil ecosystem is further elucidated by assessing the abundance and diversity of soil microbial communities using advanced molecular techniques like metabarcoding, alongside measurements of key soil enzymes involved in nutrient cycling. The climate impact of AEPs is quantified by measuring greenhouse gas (GHG) emissions CO2, N2O and NH4 using gas chromatography. Furthermore, the study includes comprehensive assessments of pests and diseases, coupled with precise determinations of crop productivity (yield) and biomass.

Our methodology is applied across diverse agroecosystems within the ALLs, including coffee-based systems in Kabare-Biega and Giheta, rice-based systems in Uvira, maize-based systems in Bujumbura, cassava-bean intercropping in Kamonyi, and cacao agroforestry in Ntui. These interventions, which involve practices like biopesticides, compost, cover crops, hedgerows, and urine fertilizers, are compared against control or conventional treatments to determine their ecological and environmental advantages. Laboratory analyses, particularly for soil properties, are conducted at ETH Zurich to ensure data quality and adherence to international standards. By integrating field experiments, comprehensive soil surveys (including farmer fields), and GHG emission monitoring, our framework will provide a holistic understanding of agroecological impacts, offering crucial evidence for sustainable agricultural management.

By providing quantifiable evidence of the benefits and trade-offs of agroecological practices on soil health, biodiversity, climate mitigation, and crop productivity in the humid tropics of Central and Eastern Africa, task 4.1 will directly inform decision-making at multiple levels. The robust datasets on soil health (physical, chemical, biological), biodiversity changes, and GHG emissions will demonstrate the positive ecological footprint of agroecological practices compared to conventional methods. This scientific backing is crucial for driving out myths and showcasing agroecology as a viable and superior pathway for environmental protection. The detailed assessments within the diverse ALLs will yield context-specific insights into the most effective agroecological practices for different farming systems (e.g., coffee, cacao, maize, rice, cassava). This will enable the formulation of tailored, evidence-based recommendations that account for the unique biophysical conditions and farmer realities in Central and Eastern Africa. Ultimately, the comprehensive evaluation described above will demonstrate that agroecology is not just a concept but a practical, environmentally sound for transforming food systems.

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6. ANNEXES

Soil sampling protocol guide to be used for cacao system in Cameroon

Background

Soil health surveys in cacao systems are crucial for optimizing cacao production. These surveys identify site suitability, nutrient deficiencies, and inform sustainable management practices. Understanding the influence of biophysical and socioeconomic factors on soil quality, this data will facilitate the development of effective soil management strategies for cacao cultivation. Specifically, detailed soil mapping helps delineate areas with varying physical and chemical properties, allowing for targeted interventions such as fertilizer application and organic matter amendment. The analysis of soil physico-chemical parameters provides a comprehensive picture of soil fertility and its capacity to support healthy cacao growth. Ultimately, the insights gained from these soil surveys are indispensable for fostering resilient and productive cacao farming systems.

Table 3. Biophysical characteristics of the site

	Ntui
Agroecological zones	Forest-Savannah transition zone
Soil types	Acrisoils
Soil texture	Sandy clay soils
Elevation	100 – 600 m
Average rainfall (mm/year)	1232
Average temperature (°C)	25

Factors being considered

Forest-Savannah transition zone (Ntui),

1) Shade with 3 levels : Monoculture, 40-50% and > 60 % cover
2) Age with 3 levels (10-20 years, 35-45years and >55 years)
3) Soil depth 2 levels (topsoil 0-10 cm, subsoil 10-30 cm)

Sample size = (3 Shades X 3 Age class) X 5 Replications = 45 Fields/plots X 2 soil depth = **90 soil samples**

Plot size:

1) For soil sampling:
   40 long X 20 large = 800 m^2, the number of cacao trees will range from (……..-…..)/2 cocoa plants per plot.
2) Biodiversity assessment: under the 2400 m^2

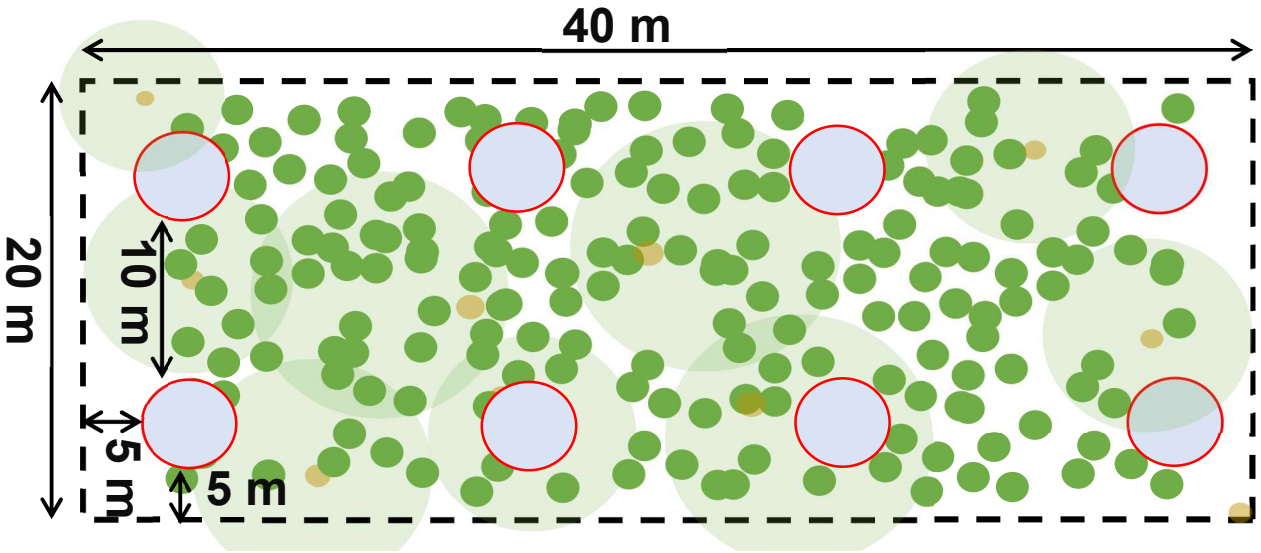

Figure 1. Sketch of the sampling points in the field

Process

1) Place the auger soil and begin to auger straight down. Use the same auger for the entire depth (0-10 cm). Changing augers may change the volume of the auger hole.

2) Be careful not to overfill the auger as this will distort the volume of the hole. To avoid this, empty the soil from the auger after every ~3 full turns.

3) Auger down to 10 cm, collecting all of the soil from the auger into the bucket1. Be sure to collect any soil that has fallen onto the sampling plate.

4) Then transfer all the soil to a clearly labelled plastic bag. The next samples to be collected are from 10-30 cm.

5) Collect **topsoil** (0-10 cm) from the center of each subplot using an auger and put the sample in a labelled bucket1.

6) Collect subsoil (10-30 cm) samples from the center of each subplot using an auger and put the sample in a labelled bucket. When auguring the subsoil, ensure that no topsoil falls into the auger hole.

7) Pool (composite) all the topsoil samples from each subplot into bucket1 and mix the soil thoroughly. At least 500 g of soil is needed for the topsoil.

8) Pool (composite) all the subsoil samples from each subplot into one bucket2 and mix the soil thoroughly. At least 500 g of soil is needed for the subsoil.

9) Note: There should be one bag of topsoil and one bag of subsoil for each plot. Auger depth restrictions are recorded (in cm) for each subplot, if they occur during sampling.

Figure 2. Soil sampling process using auger

After the field sampling: Air drying process

After getting back from the field, the samples should be air-dried as follows:

- Air-dry soil samples by spreading a sample out as a thin layer into a shallow tray or by placing in shallow plastic bowls or by opening the plastic bag at the maximum to let air circulation. If possible, break up clods as far as possible to accelerate air drying.

- It is important to ensure that no material from a sample is lost or discarded as weights of soil fractions are to be recorded on processing. Contamination from dust or other potential material should be avoided.
- Drying time depends on the samples and ambient conditions, but the samples should be thoroughly dry (i.e. constant weight)
-

Figure 3. Air drying of soil samples

- Drying soil samples will be passed through 2mm sieve, packed in a Ziplock bag with labeling ID. At least 500g of soil is needed from each composite soil sample

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Protocol Soil sampling guide Kabare Biega and Giheta

- **Each field plot consists of 4 subplots,**
- **Each subplots have r = 5.64 m**
- **The distance between subplots =12.2m**

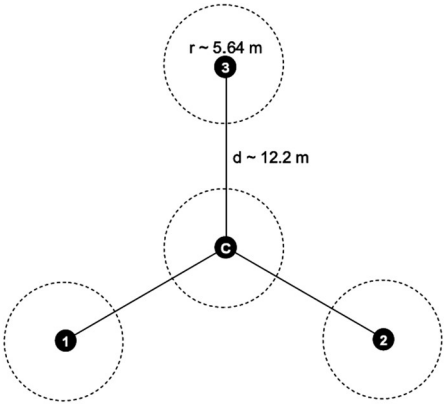

Figure 4.Y sampling frame; four composite soils

Process

1) Place the auger soil and begin to auger straight down. Use the same auger for the entire depth (0-20 cm). Changing augers may change the volume of the auger hole.

2) Be careful not to overfill the auger as this will distort the volume of the hole. To avoid this, empty the soil from the auger after every ~3 full turns.

3) Auger down to 20 cm, collecting all of the soil from the auger into the bucket. Be sure to collect any soil that has fallen onto the sampling plate.

4) Then transfer all the soil to a clearly labelled plastic bag. The next samples to be collected are from 20-50 cm.

5) Collect **topsoil** (0-20 cm) from the center of each subplot using an auger and put the sample in a labelled bucket.

Figure 5. Soil sampling process using auger

6) Collect subsoil (20-50 cm) samples from the center of each subplot using an auger and put the sample in a labelled bucket. When auguring the subsoil, ensure that no topsoil falls into the auger hole.

7) Pool (composite) all the topsoil samples from each subplot into one bucket and mix the soil thoroughly. At least 1 kg of soil is needed for the topsoil.

8) Pool (composite) all the subsoil samples from each subplot into one bucket and mix the soil thoroughly. At least 1 kg of soil is needed for the subsoil.

9) Note: There should be one bag of topsoil and one bag of subsoil for each plot. Auger depth restrictions are recorded (in cm) for each subplot, if they occur during sampling.

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After getting back from the field, the samples should be air-dried as follows:

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- Drying time depends on the samples and ambient conditions, but the samples should be thoroughly dry (i.e. constant weight).

Figure 6. Air drying of soil samples

- Drying soil samples will be passed into 2mm sieve, packed in a Ziplock bag with labeling ID. At least 500g of soil is needed from each composite soil sample

References

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