ISO TS 18721

English Title:

Assessment of ecological soil functions: indicators and methods

French title

Evaluation des fonctions écologiques des sols : indicateurs et méthodes

Scope definition

This standard provides a generic description of the methods available for measuring soil characteristics and indicators of core Ecological Soil Functions (ESF). No distinction of context is made; i.e. no differentiation of land use and management (e.g agricultural, forest, urban, natural or contaminated lands). For each ESF, the standard specifically suggests biotic and abiotic characteristics to be measured. It focuses on characteristics and indicators that are either available as ISO standards or published in peer-reviewed papers.

All terms, concepts and definitions are fully described in ISO 18718: Assessment of soil functions and related-ecosystem services: concepts and definitions.

This document applies to ESF and is not applied to soil functions such as geotechnical functions (foundation support for buildings, tunnels, etc.) or geothermal functions. Indeed, ecosystem capital does not address soils without a topsoil, or with a covered topsoil (buildings, infrastructure, greenhouse farming, solar panel parks).

The assessment of ESF can help the assessment of soil-related ecosystem services but the overall assessment of ecosystem services is not covered in the document.

Other methods based on proxy indicators (e.g. soil occupation, hydrography parameters) can also be used for land planning at large scale. These indicators are not included in this technical standard.

Introduction

In recent years the increasing concern for soil sustainability has considerably risen. Many countries are setting targets to reduce soil artificialisation and reinforce ecosystem rehabilitation and biodiversity conservation. In Europe the recent proposal for a Directive on Soil monitoring and resilience will set foundations for member states to consider.

In this context concepts of soil health and soil quality are being discussed (Kibblewhite et al., 2008; Jenzen et al., 2021; Bonfante et al., 2020) and ecological soil functions are being proposed (Bünemann et al., 2018 ; Creamer et al., 2022). However, although several promising propositions have been made to define lists of indicators of soil health and soil quality in different contexts (e.g. de Haan et al., 2021), there is presently no consensus.

Based on the definitions of soil health and quality presented in ISO/TS 18718, the present document aims to provide an overview of the existing indicators and characteristics that are linked to soil functions and the available methods to assess them whether they are normalized, standardized or exist as peer-reviewed work.

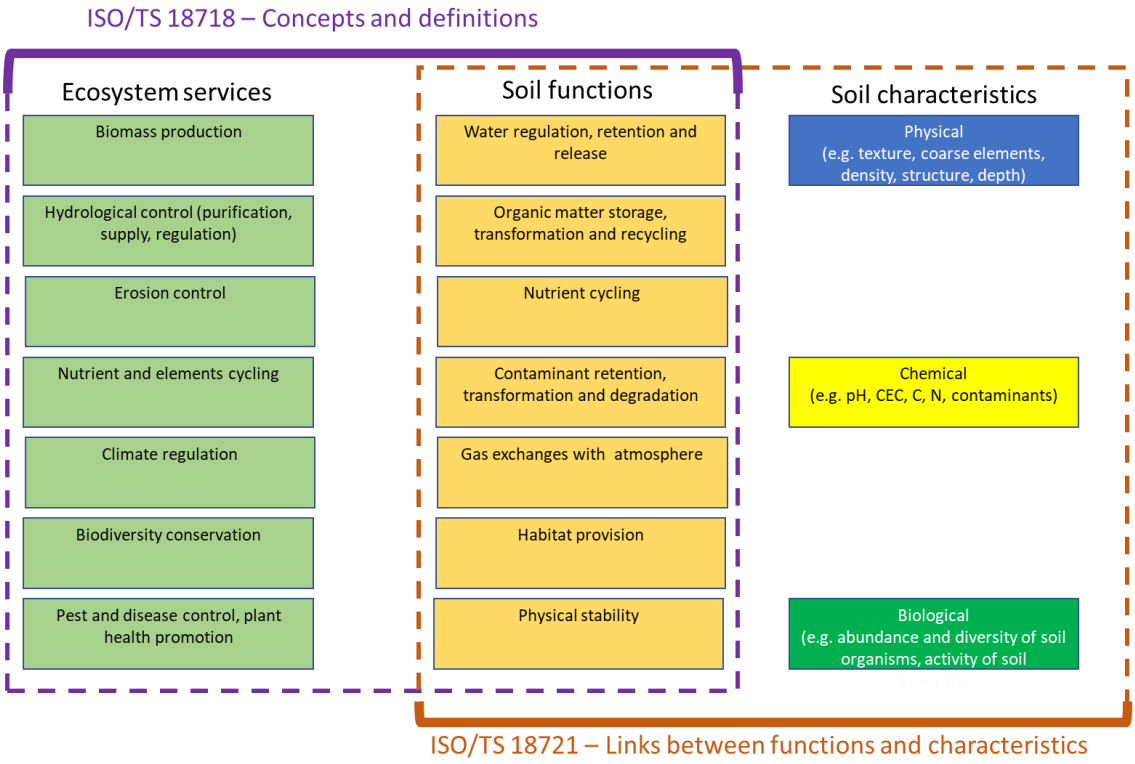

Figure 1. Links between ISO/TS 18718 and 18721 on the assessment of soil functions and related-ecosystem services

Normative references

Normative references are provided according to the different methods.

3 Terms and definitions

Proposed general text to start with, followed by specific terms and definitions of this standard:

For the purposes of this document, the terms and definitions given in ISO 11074 and the following apply.
ISO and IEC maintain terminological databases for use in standardization at the following addresses:
— ISO Online browsing platform: available at https: //www .iso .org/obp
— IEC Electropedia: available at: http: //www .electropedia .org

All other terms used in this document are defined in ISO 18718.

4 Indicators of ecological soil functions

4.1 Introduction

In ISO 18718 ecological soil functions, sub-functions and processes are described. Soil is a very complex ecosystem of which the functioning and thus ecosystem service provision relies on interactions between the abiotic and biotic components. Here we propose to separate general soil characteristics/indicators, which need to be measured as they influence soil processes, from specific ones, which directly reflect soil processes. This organisation is presented in figure 2.

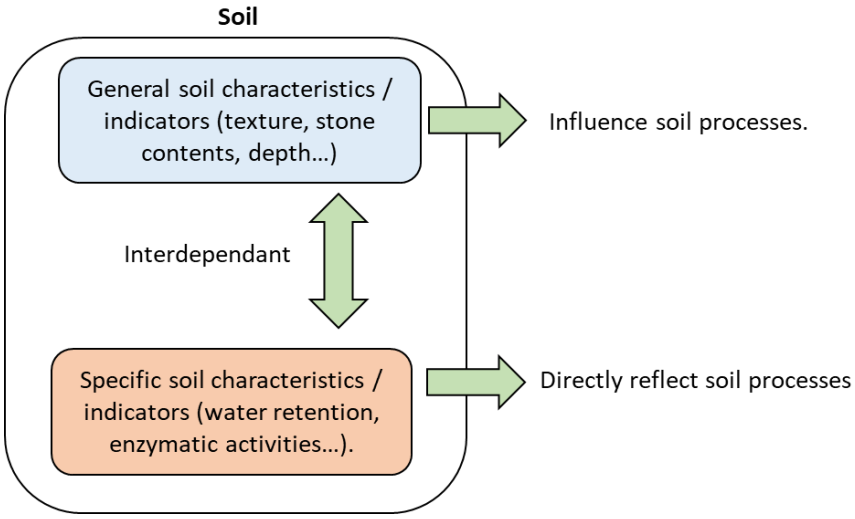

Figure 2. Differentiation between general soil characteristics and specific soil characteristics.

Many methods have been developed to measure these characteristics and indicators. Among these methods, some are normalized and applicable worldwide while others are based on normalized methods but applicable or normalized only at a sub-worldwide scale. Finally, emerging approaches are available and used in the scientific literature but are not yet normalized or do not yet dispose of an interpretation referential. These three levels of standardization are considered here and are so after named:

- Level 1: normalized methods applicable worldwide
- Level 2: normalized methods applicable or normalized at a sub-worldwide scale (proxies or modelling)
- Level 3: emerging methods not yet normalized or without an interpretation reference

4.2. Methods for measuring general soil characteristics/indicators, sampling and soil preparation.

General soil characteristics reflect the physico-chemical composition of soils and give a first assessment of a sample. Some are required to interpret specific soil characteristics/indicators. General soil characteristics/indicators include texture, soil depth, soil density, pH (acidity), CEC, cations (Ca, K, Mg, Na), total carbon, nitrogen and phosphorus, the amount of coarse elements, $CaCO_3$, and the presence of contaminants. The assessment of all or part of these characteristics is a pre-requisite for assessing soil processes and is strongly recommended.

Moreover, assessing general soil characteristics or specific characteristics requires representative sampling (see ISO 18400-104:2018 - Soil quality — Sampling) and soil preparation before analysis (see NF ISO 18 400 – 101 à 1008 Soil quality — Preparation of laboratory samples from large samples).

The existing methods for measuring general soil characteristics are presented in table 1 according to their level of standardisation.

Table 1. Existing methods to measure general soil characteristics.

General soil characteristics / indicators	Level 1	Level 2	Level 3
a - Soil texture (particle size distribution)	ISO 11277:2020: Method by sieving and sedimentation		
b - Bulk density	ISO 11272:2017 Determination of dry bulk density (three methods: core, clod and excavation)	NF X 31-501 Determination of bulk density: cylinder method	"Pedotransfer functions" (Hollis et al., 2012)
c - pH (acidity)	ISO 10390:2005: pH in H2O, KCl, and CaCl2		
d - Cation Exchange Capacity (CEC)	ISO 23470:2018; Soil quality — Determination of effective cation exchange capacity (CEC) and exchangeable cations using cobaltihexammine trichloride solution ISO 11260:2018; Soil quality — Determination of the cation exchange capacity and the saturation rate of exchangeable bases using a barium chloride solution		
e - Soil structure & stability	ISO 10930:2012 Soil quality — Measurement of the stability of soil aggregates subjected to the action of water		Spade-test: Visual Evaluation of Soil Structure (Guimaraes et al., 2011) «Mini 3D soil profile» Tomis et al. 2019 Slake test (Fajardo et al., 2016) Aggregate stability (Herrick et al., 2001) «Visual Soil Assessment» (Sheperd 2009)

			« Profil cultural » (Boizard et al., 2017)
f - Exchangeable cations (Ca, K, Mg, Na)	ISO 23470:2018; Soil quality — Determination of effective cation exchange capacity (CEC) and exchangeable cations using cobaltihexammine trichloride solution ISO 11260:2018; Soil quality — Determination of the cation exchange capacity and the saturation rate of exchangeable bases using a barium chloride solution		
g **- Organic carbon (organic matter)**	ISO 10694:1995; Dry combustion NF EN ISO 14 235; sulfochromique oxydation	NF X31-516; Soil quality - Granulodensimetric fractionation of particulate organic matter from soil in water	Rock-Eval pyrolysis combined with PartySOC model (Cécillon et al., 2021) for assessing stable and active fractions of total organic carbon in temperate ecosystems. (ISO in prep.)
h - Carbonates	ISO 10693:2014: Total carbonates		
i - Contaminants **(depending on the context)**	ISO 11466:1995 - Total metal trace element (aqua regia extraction) ISO 13 877: 2006 - Determination of PAH – gas chromatographic method with GC-MS ISO 11 504:2017- Assessment of impact from soil contaminated with petroleum hydrocarbons ISO 21268 1-2-3-4 Leaching procedure for subsequent chemical and		Pesticide Multi-residue method (Rösch et al 2023)

	ecotoxicological testing of soil and soil-like materials ISO 17 402 Guidelines for the selection and application of methods for assessing the bioavailability of contaminants in soil and soil materials ISO 16198 Plant-based test to assess the environmental bioavailability of trace elements to plants		
j - Dry matter	ISO 11465:1993 - Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method Required for the physico-chemical analysis -> allows calculation of element concentration in the dry matter		
k - Total N	ISO 13878:1998 Soil quality — Determination of total nitrogen content by dry combustion ("elemental analysis")		
l - Total P	ISO 11263:1994 Soil quality — Determination of phosphorus — Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution		

4.2. **Methods for measuring specific soil characteristics/indicators.**

Soil processes are linked to soil sub-functions and functions as described in ISO 18718. To assess soil processes, many methods exist to measure specific soil characteristics/indicators. As for general soil characteristics, these are standardised at different levels.

The following tables are organised as described in figure 3.

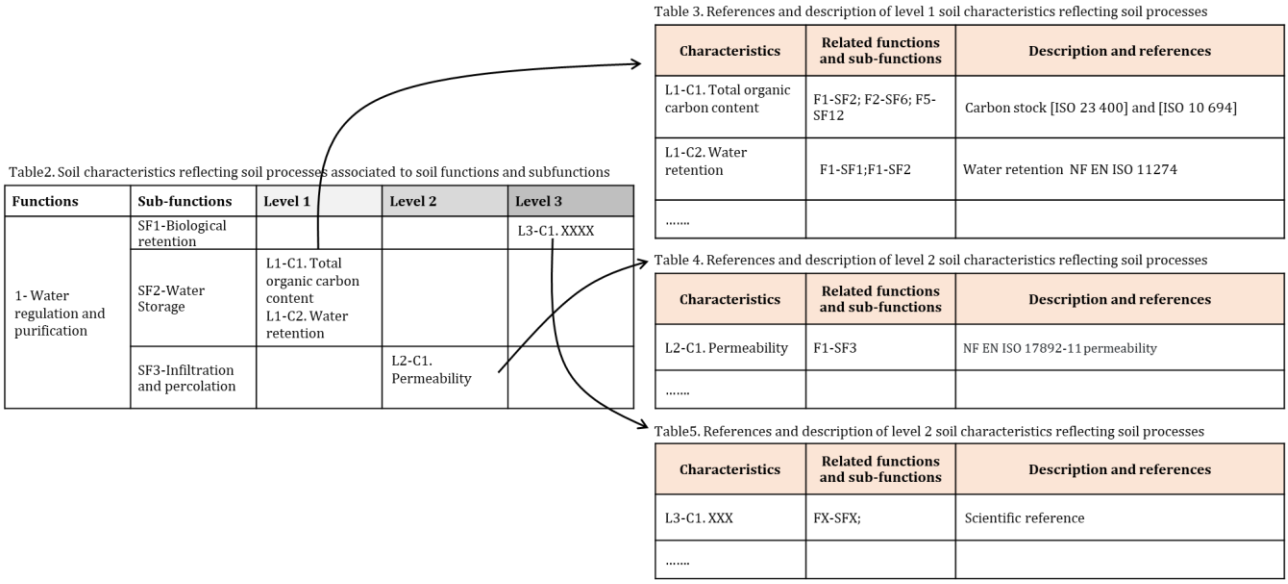

Table2. Soil characteristics reflecting soil processes associated to soil functions and subfunctions

Functions	Sub-functions	Level 1	Level 2	Level 3
1- Water regulation and purification	SF1-Biological retention			L3-C1. XXXX
	SF2-Water Storage	L1-C1. Total organic carbon content L1-C2. Water retention		
	SF3-Infiltration and percolation		L2-C1. Permeability	

Table 3. References and description of level 1 soil characteristics reflecting soil processes

Characteristics	Related functions and sub-functions	Description and references
L1-C1. Total organic carbon content	F1-SF2; F2-SF6; F5-SF12	Carbon stock [ISO 23 400] and [ISO 10 694]
L1-C2. Water retention	F1-SF1;F1-SF2	Water retention NF EN ISO 11274
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Table 4. References and description of level 2 soil characteristics reflecting soil processes

Characteristics	Related functions and sub-functions	Description and references
L2-C1. Permeability	F1-SF3	NF EN ISO 17892-11 permeability
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Table5. References and description of level 2 soil characteristics reflecting soil processes

Characteristics	Related functions and sub-functions	Description and references
L3-C1. XXX	FX-SFX;	Scientific reference
.....		

Figure 3. Organisation of the following tables which link first functions and subfunctions to soil characteristics reflecting soil processes (table 2) and then give a description for each characteristic and the references at each level and link them to soil functions and sub-functions (tables 3, 4 and 5).

Table 2. Specific soil characteristics / indicators reflecting soil processes associated to soil functions and sub-functions

Functions	Sub-functions	Level 1 (ISO)	Level 2 (standard)	Level 3 (Scientific reference)
F1- Water regulation, retention and release	SF1-Biological retention by plants			
	SF2-Water retention	L1-C1. Total organic carbon content L1-C2. Water retention		
	SF3-Infiltration and percolation	L1-C3. Permeability		L3-C1. Permeability L3-C2. Soil structure assessment via X-ray Computed Tomography
F2- Organic matter storage, transformation and recycling	SF4-Decomposition	L1-C4. Microbial soil biomass L1-C5. Feeding activity	L2-C1. Organic matter mineralisation in soil L2-C2. Litter bag	
	SF5-Resource reallocation	L1-C1. Total organic carbon content		
	SF6-Biochemical transformation	L1-C1. Total organic carbon content L1-C6. Microbial respiration (carbon mineralization)		L3-C3. Carbon quality – active and stable organic carbon L3-C4. Functional diversity: Genes coding analysis L3-C5. Microbial catabolic activities
F3- Nutrient cycling	SF7-Nutrient transformation	L1-C4. Microbial soil biomass L1-C7. Enzymatic activities L1-C8. Total nitrogen content L1-C9. Assimilable phosphorus content		L3-C5. Microbial catabolic activities

	SF8-Nutrient reallocation	**L1-C10. CEC and exchangeable cations** **L1-C7. Enzymatic activities**		
	SF9-Nutrient assimilation	**L1-C11. Available NO_2 and NO_3**		**L3-C6. Microbial activities (denitrification)** **L3-C4. Functional diversity: Genes coding analysis**
F4- Contaminants retention, transformation and degradation	**SF10-Retention**	**L1-C12. Bioavailability of metal contaminants.** **L1-C13. Total and extractable metal trace element**		
	SF11-Transformation			**L3-C5. Microbial catabolic activities**
	SF12-Degradation	**L1-C7. Enzymatic activities** **L1-C14. Soil microbial diversity** **L1-C15. Degradation of organic chemicals**		**L3-C5. Microbial catabolic activities**
F5- Gas exchanges with the atmosphere	**SF13-Emission**			**L3-C5. Microbial catabolic activities**
	SF14-Captation			**L3-C3. Carbon quality – active and stable organic carbon**
F6- Habitat provision	**SF15-Habitat quality**	**L1-C1. Total organic carbon content** **L1-C4. Microbial soil biomass** **L1-C16. Ecotoxicological quality** **L1-C17. Effect of chemicals macrofauna**		**L3-C3. Carbon quality – active and stable organic carbon** **L3-C2. Soil structure assessment via X-ray Computed Tomography**

	SF16-Harboring biodiversity	**L1-C14. Soil microbial diversity** **L1-C18. Soil invertebrates**		**L3-C7. Taxonomic diversity (bacteria & fungi** **L3-C8. Fungal, bacterial ratio**
F7- Physical Stability	**SF17-Inherent soil stability**			**L3-C2. Soil structure assessment via X-ray Computed Tomography**
	SF18-Stability evolution	**Salinisation requires:** **L1-C16. Electrical conductivity** **L1-C10. CEC and exchangeable cations**		**L3-C2. Soil structure assessment via X-ray Computed Tomography**

Table 3: References and description of level 1 specific soil characteristics

Indicator/characteristics	Related functions	Description and references
L1-C1. Total organic carbon content	**F1-SF2; F2-SF6; F5-SF12**	ISO 23 400 Carbon stock ISO 10 694 Determination of organic and total carbon after dry combustion (elementary analysis)
L1-C2. Water retention	**F1-SF2;**	NF EN ISO 11274 Soil quality — Determination of the water-retention characteristic — Laboratory methods
L1-C3. Permeability	**F1-SF3;**	ISO 17892-11 – Geotechnical investigation and testing: laboratory testing of soil – part 11: permeability tests Norme X30-418 Soil infiltration kinetics in relation to soil texture (test de Beerkan, BEST or double ring method)

		ISO 17892-11 – Geotechnical investigation and testing: laboratory testing of soil – part 11: permeability tests
L1-C4. Microbial soil biomass	**F2-SF4; F3-SF7; F6-SF15;**	ISO 14240-1 - Determination of soil microbial biomass – part 1 substrate –induced respiration method ISO/TS 29843:2021 PLFA & PLEL ISO 14240-2 - Determination of soil microbial biomass – Part 2: Fumigation – extraction method ISO 16072 - Laboratory method for determination of microbial soil respiration ISO 17155 - Determination of the activity of the soil microflora using respiration curves ISO 11063 - Direct soil DNA extraction ISO 17601:2016 - Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil
L1 – C5. Feeding activity	**F2-SF4**	ISO 18311:2016 Soil quality — Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test
L1-C6. Microbial respiration (carbon mineralization)	**F2-SF6;**	ISO 16072 Laboratory methods for determination of microbial respiration
L1-C7. Enzymatic activities	**F3-SF7; F3-SF8;**	ISO 20130 Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates ISO 23753 1-2-3-4 – Determination of dehydrogenases activity in soils
L1-C8. Total nitrogen content	**F3-SF7&SF8**	ISO 13878:1998 Soil quality — Determination of total nitrogen content by dry combustion ("elemental analysis")

L1-C9. Assimilable phosphorus content	**F3-SF7&SF8**	ISO 11263:1995 Soil quality — Determination of phosphorus — Spectrometric determination of soluble phosphorus in a sodium hydrogen carbonate solution
L1-C10. CEC and exchangeable cations (to have salinisation)	**F3-SF8;**	ISO 13878: 1998 : Total nitrogen content ISO11263:1995 – Assimilable phosphorus content ISO 11265:1994 - Electrical conductivity (to assess salinisation) NF X 31-130 or ISO 23470-2018 or ISO 11260:2018 CEC and exchangeable cations
L1-C11. Available NO_2 and NO_3	**F3-SF9;**	ISO/DIS 20951 - Guidance on methods for measuring greenhouse gases (CO2, N2O, CH4) and ammonia (NH3) fluxes between soils and the atmosphere ISO TS 20131-1 Easy laboratory assessments of soil denitrification, a process source of N2O emissions -- Part 1: Soil denitrifying enzymes activities ISO/TS TS 20131-2 - Easy laboratory assessments of soil denitrification, a process source of N2O emissions -- Part 2: Assessment of the capacity of soils to reduce N2O
L1-C12. Bioavailability of contaminants.	**F4-SF10**	ISO 17 402 - Guidelines for the selection and application of methods for assessing the bioavailability of contaminants in soil and soil materials ISO 16198 - Plant-based test to assess the environmental bioavailability of trace elements to plants ISO 21268 1-2-3-4 - Leaching procedure for subsequent chemical and ecotoxicological testing of soil and soil-like materials ISO 17 586 - Extractable metal trace element (Na NO3 0,43 M) ISO 19730 - Extractable metal trace element (NH_4NO_3, 1 M) ISO 15 952, - Effects of pollutants on juvenile land snails (Helicidae) - Determination of the effects on growth by soil contamination ISO 24 032 In-situ caging of snails to assess bioaccumulation of contaminants
L1-C13. Total and extractable metal trace element	**F4-SF10;**	ISO 11466:1995 and EDTA-BCR protocol
L1-C14. Soil microbial diversity	**F4-SF12; F6-C16;**	ISO/TS 29843-2 Determination of soil microbial diversity – part 2 : method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method

		ISO 17601 – Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil
L1-C15. Degradation of organic chemicals	**F4-SF12;**	ISO 11266 - Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions ISO 14239 - Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions ISO 15473 - Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions
L1-C16. Ecotoxicological quality	**F6-SF15**	ISO 15799:2019 Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials ISO 17616:2019 Soil quality — Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials ISO 11268-1:2012 Soil quality — Effects of pollutants on earthworms — Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei ISO 11268-2:2023 Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction of Eisenia fetida/Eisenia andrei and other earthworm species ISO 17512-1:2008 Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour — Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei ISO 16387:2014 Soil quality — Effects of contaminants on Enchytraeidae (Enchytraeus sp.) — Determination of effects on reproduction ISO 11267:2014 Soil quality — Inhibition of reproduction of Collembola (Folsomia candida) by soil contaminants ISO 21285:2019 Soil quality — Inhibition of reproduction of the soil mite (Hypoaspis aculeifer) by soil contaminants ISO 23266:2020 Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (Oppia nitens)

		ISO 10872:2020 Water and soil quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda) ISO 11269-1:2012 Soil quality — Determination of the effects of pollutants on soil flora — Part 1: Method for the measurement of inhibition of root growth ISO 11269-2:2012 Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants ISO 18763:2016 Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants ISO 22030:2005 Soil quality — Biological methods — Chronic toxicity in higher plants ISO 29200:2013 Soil quality — Assessment of genotoxic effects on higher plants — Vicia faba micronucleus test ISO 17512-1:2008 - Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour — Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei) ISO 17512-2:2011 - Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour — Part 2: Test with collembolans (Folsomia candida)
L1-C17. Effect of chemicals macrofauna	**F6-SF15**	ISO 17512-1:2008 - Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour — Part 1: Test with earthworms (Eisenia fetida and Eisenia andrei) ISO 17512-2:2011 - Soil quality — Avoidance test for determining the quality of soils and effects of chemicals on behaviour — Part 2: Test with collembolans (Folsomia candida)
L1-C18. Soil invertebrates	**L6-C16;**	ISO 23 611-1-2-3-4: - Sampling of soil invertebrates Part 1 : hand-sorting and AITC extraction of earthworms Part 2: sampling and extraction of micro-arthropods (Collembola and Acarina) Part 3 : sampling and extraction of enchytraeids Part 4: sampling extraction and identification of soil-inhabiting nematodes Part 5- sampling and extraction of macro-invertebrates

L1-C19. Electrical conductivity (to have salinisation)	**F7-SF18**	ISO 11265:1994 Soil quality — Determination of specific electrical conductivity

Table 4: References and description of level 2 specific soil characteristics

Indicator/parameter	Related functions	Description and references
L2-C1. Organic matter mineralisation in soil	**F2-SF4;**	
L2-C2. Litter bag	**F2-SF4 ;**	

Table 5: Level 3 emerging methods not yet normalized or without an interpretation reference

Indicator/parameter	Related functions	Description and references
L3-C1. Permeability	**F1-SF2;**	
L3-C2. Soil structure assessment via X-ray Computed Tomography	**F1;SF3; F6-SF15; F7-SF17&SF18;**	Assessment of soil porosity, pore size distribution to assess the soil structure (Vogel et al. 2010; Bacq-Labreuil et al. 2018)

L3-C3. Carbon quality – active and stable organic carbon	**F2-SF6; F5-SF14; F6-SF15;**	Rock-Eval – ISO in prep
L3-C4. Functional diversity: Gene coding analysis	**F2-SF6; F3-SF9;**	DNA analysis (shotgun analysis or metabarcoding)
L3-C5. Microbial catabolic activities	**F2-SF6; F3-SF7; F4-SF12; F5-SF13**	Assessment of soil microbial catabolic activities for the determination of soil community-level physiological profiles (CLPP) e.g. by measurement of Multi-substrate-induced respiration (MSIR) (Campbell et al. 2003, Rutgers et al. 2016), Gene coding analysis (L3-C4) or Enzymatic activities (L1-C7). (General characteristics from Table 1 needed for interpretation: a, b, c, g, h and i)
L3-C6. Microbial activities (denitrification)	**F3-SF9;**	
L3-C7. Taxonomic diversity (bacteria & fungi)	**F6-SF16;**	DNA analysis (shotgun analysis or metabarcoding)
L3-C8. Fungal, bacterial ratio	**F6-SF16;**	DNA analysis (qPCR) PLFA, ergosterol analysis

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