



Supplementary Materials for

Tracking, targeting, and conserving soil biodiversity

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Table S1*

Policy Sector	Strategic Goal	Target/Policy/Assessment	SoilBON priority EBV											SoilBON Indicator				
			Intraspecific genetic diversity	Population abundance	Community traits of roots	habitat extent	Functional diversity	Taxonomic diversity	Soil biomass	Litter decomposition	Soil respiration	Enzymatic activity	Soil aggregate stability		Nutrient cycling			
Convention on Biological Diversity (Aichi Targets)	A.4	By 2020, at the latest, Governments, business and stakeholders at all levels have taken steps to achieve or have implemented plans for sustainable production and consumption and have kept the impacts of use of natural resources well within safe ecological limits.		○													Soil Health Nutrient Cycling and Fertility	
	B.5	By 2020, the rate of loss of all natural habitats, including forests, is at least halved and where feasible brought close to zero, and degradation and fragmentation is significantly reduced.				●	●	●	○	○	○	○	○	○			Soil Conservation Value Ecological Vulnerability of Soils	
	B.7	By 2020 areas under agriculture, aquaculture and forestry are managed sustainably, ensuring conservation of biodiversity.		○													Soil Health Nutrient Cycling and Fertility	
	B.8	By 2020, pollution, including from excess nutrients, has been brought to levels that are not detrimental to ecosystem function and biodiversity.		○													Soil Health Nutrient Cycling and Fertility	
	B.9	By 2020, invasive alien species and pathways are identified and prioritized, priority species are controlled or eradicated, and measures are in place to manage pathways to prevent their introduction and establishment.		○	○				○	○								
	C.11	By 2020, at least 17 per cent of terrestrial and inland water, and 10 per cent of coastal and marine areas, especially areas of particular importance for biodiversity and ecosystem services, are conserved through effectively and equitably managed, ecologically representative and well connected systems of protected areas and other effective area-based conservation measures, and integrated into the wider landscapes and seascapes.		○			●	●	●									Soil Conservation Value
	C.12	By 2020 the extinction of known threatened species has been prevented and their conservation status, particularly of those most in decline, has been improved and sustained.		○					○	○								
	C.13	By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity.		○														
	D.14	By 2020, ecosystems that provide essential services, including services related to water, and contribute to health, livelihoods and well-being, are restored and safeguarded, taking into account the needs of women, indigenous and local communities, and the poor and vulnerable.		●	●	●	●	●	●	●	●	●	●	●	●	●	●	Soil Conservation Value Ecological Vulnerability of Soils Soil Biodiversity Soil Health
D.15	By 2020, ecosystem resilience and the contribution of biodiversity to carbon stocks has been enhanced, through conservation and restoration, including restoration of at least 15 per cent of degraded ecosystems, thereby contributing to climate change mitigation and adaptation and to combating desertification.		●	●	●	●	●	●	●	●	●	●	●	●	●	●	Soil Conservation Value Ecological Vulnerability of Soils Soil Biodiversity Soil Health Soil Carbon Stocks	

SoilBON priority EBV

Policy Sector	Strategic Goal	Target/Policy/Assessment	SoilBON priority EBV											SoilBON Indicator				
			Intraspecific genetic diversity	Population abundance	Community traits of roots	habitat extent	Functional diversity	Taxonomic diversity	Soil biomass	Litter decomposition	Soil respiration	Enzymatic activity	Soil aggregate stability		Nutrient cycling			
Food and Agriculture Organization		Voluntary Guidelines for Sustainable Soil Management								●	●	●	●	●	●	●	Soil Health	
		World Soil Charter	●	●	●	●	●	●	●	●	●	●	●	●	●	●	Soil Conservation value Soil Biodiversity Soil Health Nutrient Cycling and Fertility	
		The international code of conduct for the sustainable use and management of fertilizers																
		Global Soil Health Indicators and Assessment								●	●	●	●	●	●	●	Soil Health Nutrient Cycling and Fertility	
Convention to Combat Desertification		Cross cutting Initiative on conservation and sustainable use of soil biodiversity	●	●	●	●	●	●	●	●	●	●	●	●	●	●	Soil Conservation value Soil Biodiversity Soil Health	
		Global Land Outlook	●	●	●	●	●	●	●	●	●	●	●	●	●	●	Soil Conservation value Soil Biodiversity Soil Health	
		Land Degradation Neutrality				●	●	●	○	○	○	○	○	○	○	○	Soil Conservation Value Ecological Vulnerability of Soils	
Paris Agreement	Art. 5.2	Parties are encouraged to take action to implement and support, including through results-based payments, the existing framework as set out in related guidance and decisions already agreed under the Convention for: policy approaches and positive incentives for activities relating to reducing emissions from deforestation and forest degradation, and the role of conservation, sustainable management of forests and enhancement of forest carbon stocks in developing countries; and alternative policy approaches, such as joint mitigation and adaptation approaches for the integral and sustainable management of forests, while reaffirming the importance of incentivizing, as appropriate, non-carbon benefits associated with such approaches								●	●	●	●	●	●	Soil Carbon Stocks		

- direct link
- potential link (without a current SoilBON indicator)
- direct link (mentioned in the target/policy/assessment)

* although the current coverage of policy targets is limited to the one identified here, further extension of both the essential biodiversity variables and of the related indicators (by expanding the number of involved partners or by the development of new technologies) can improve the future coverage of conservation goals.

Table S2

The selection of variables was done based on a system approach (1) that focuses on the holistic representation of the soil system. With this representation four dimensions were considered: soil physics, soil chemistry, soil biodiversity, and soil functions. Together, these four dimensions provide a complementary view of the global soil systems and allow to identify general patterns, track changes in critical ecological aspects, and observe the interdependencies of biodiversity and ecosystem functioning. Starting from soil physics, we aim to characterize the main aspects of soil systems, including texture, soil aggregates, and bulk density. These relate to the chemical properties of soils (e.g., carbon, nitrogen and phosphorus content) and create an intricate network of soil habitats and specific soil environmental conditions determining, together with soil biodiversity, a plethora of soil functions (including nutrient cycling, soil respiration, litter decomposition, among others).

Essential Biodiversity Variable (EBV)	EBV Class	Description	References	Soil dimension
Intraspecific genetic diversity	Genetic composition	DNA extraction is performed from 0.2 g of entirely homogenized soil by use of the Qiagen (MoBio) DNeasy PowerSoil HTP 96 Kit (Qiagen Inc., Valencia, CA, USA). A single aliquot per sample is extracted. Furthermore, we use negative controls and positive controls during extraction to locate any external contamination and cross-contamination, should this occur. We also re-analyze a random selection of 1% of the samples and another at least 1% of the samples with extreme values for repeated analysis to validate the quality and understand variation. DNA purification is required to secure sufficient quality and concentration for metagenomics analysis. We use column-based purification FavorPrep Gel/PCR Purification kit (Favorgen Biotech Corp., Vienna, Austria). Quality check and quantification of DNA is performed fluorimetrically using the Invitrogen Qubit or any equivalent method. The results are used for deciding DNA re-extraction and re-measurement of DNA quantity. We have selected the primers based on the best available knowledge considering maximum taxonomic coverage and resolution for sequencing in Illumina (Illumina Inc., San Diego, CA, USA) and PacBio (Pacific Biosciences, Menlo Park, CA, USA) platforms. To identify fungi and other eukaryotes, the primers ITS9MUNngs (TACACACCGCCGTCG) + ITS4ngsUni (CCTSCSCTTANTDATAT GC) will be used. The amplicon of 750-850 bp covers the 3' end of 18S (V9 region), ITS1, 5.8S and ITS2 regions. This long amplicon lowers the proportion of clustering artefacts and greatly adds to taxonomic resolution and identification precision. These primers also cover >99% of all eukaryotes (except some Microsporidea and Foraminifera) and provide species-level resolution for these groups. Bacteria are identified by the commonly used Microbiome projects' 16S rRNA gene primers 515fB (GTGYCAGCMGCCGCGGTAA; Parada et al. 2016) + 806rB (GGACTACNVGGGTWTCTAAT; Apprill et al. 2015), following the protocols outlined in the Earth Microbiome Project to be able to match these data on previous and future projects. Archaea are identified using 16S Primers SSU1ArF (TCCGTTGATCCYGCBRG) + SSU1000ArR (GGCCATGCAMYW CCTCTC). The 1000-bp product provides excellent resolution and covers all major and minor groups in this domain. Alternatively, the non-fungal eukaryotes will be sequenced for 18S rRNA geneV4 variable region (310-330 bp) using specifically designed forward (Euk575Fngs; ASCYGYGGTAAWCCAGC) and reverse (Euk895Rngs; TCHNHGNATTTACCNCT) primers that have <2 mismatches to any known eukaryote taxa. Considering the length of individual amplicons, we use either Illumina MiSeq/HiSeq (bacteria and eukaryote 16S and 18S rRNA genes) or PacBio Sequel II (fungal/eukaryote ITS and Archaea 16S rRNA gene) platforms for sequencing. In spite of lower sequencing depth, the additional taxonomic resolution and quality of PacBio sequences makes it comparable to high-quality Sanger sequences. To assess genome-encoded functions of all soil organisms (active, dormant and dead). Preparation is performed using Nextera XT v2 and Nextera XT INDEX v2 kits (Illumina Inc.) that enables preparing a sequencing library from up to 384 samples simultaneously. The intended output per sample is 5,000,000 raw reads. Initial demultiplexing and quality filtering of amplicon and metagenome sequences are performed using PipeCraft and LotuS, respectively.	(2–9)	Soil biodiversity

Population abundance	Species populations	The total abundances of total bacteria (using the 16S rRNA gene; primer set Eub338/Eub518) and fungi (using the Internal transcribed spacer region (ITS); primer set ITS1-5.8S) will be quantified on a CFX-96 thermocycler (Bio-Rad, USA; see Intraspecific Genetic Diversity for more details). In parallel, nematodes will be extracted from fresh soil samples (25 g of sieved soil) using Baermann funnels. Nematodes will be preserved with DESS and the total numbers will be counted for each sample using an inverted light microscope (50-100X magnification). The first 100 nematode individuals encountered in the counting dish will be assigned to functional groups (bacterivores, fungivores, root feeders-plant parasites, omnivores and predators) based on their morphology. Total numbers per functional group will be extrapolated based on full sample counts. After extraction, the soil from funnels will be oven-dried and weighed to obtain nematode densities per g soil dry weight.	(10–17)	Soil biodiversity
Community traits of roots	Species traits	Root traits are known to affect ecosystem processes such as C and N cycling and soil stability, as well as to interact with soil biota. These include architectural traits, such as root length density, which determine the spatial configuration of the root system, but also morphological traits, such as specific root length, or more physiological traits, such as nutrient content which might relate to nutrient capture from the soil and to variations in soil biodiversity communities. Community roots will be washed in water for 10 min over a 0.63 mm sieve. Cleaned roots will be separated into coarse (> 2 mm) and fine (< 2 mm) roots. Fine roots are weighted fresh, scanned using a flatbed scanner, oven-dried (70°C, 48 h) and weighted again dry. Dry roots will be analyzed for total N. Root scans will be analyzed using Image J or WinRhizo to assess root length and diameter distribution. Specific community root traits will be assessed: root length density, specific root length, mean root diameter, variation in root diameter, root dry matter content.	(18)	Soil biodiversity Soil functions
Taxonomic diversity	Community composition	The diversity and community composition of soil archaea, bacteria, fungi, protists and invertebrates will be measured via amplicon sequencing using the Illumina MiSeq platform. Soil DNA will be extracted using the Powersoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. A portion of the bacterial 16S and eukaryotic 18S rRNA genes will be sequenced using the 515F/806R, ITS and Euk1391f/EukBr primer sets, respectively, following the EMP protocol: https://earthmicrobiome.org/protocols-and-standards/ . In parallel, nematodes, preserved in DESS (a solution containing dimethyl sulphoxide, disodium EDTA, and saturated NaCl), will be counted and identified using an inverted light microscope (50- 100X magnification): the first 100 nematode individuals encountered in the counting dish will be identified based on their morphology to a level needed for assigning trophic groups.	(10–16, 19)	Soil biodiversity
Functional diversity	Community composition	Functional diversity is an important link between community composition and multiple ecosystem functions as well as an indicator of the capacity of the community to be resistant to change in the environment. This analysis can take a molecular approach or, like in the case of nematodes, a functional group approach, as nematodes are assigned to different functional groups according to their diet, which is identifiable with a microscope from the morphology of the stoma and esophagus. The functional diversity and community of bacteria and Archaea will be analyzed using shotgun sequencing. Sequencing will be performed using an Illumina HiSeq (Illumina Inc., USA). The functional diversity of fungi will be analyzed using ITS amplicon sequencing and FunGuild. In the case of nematodes, these will be extracted from fresh soil samples (25 g of sieved soil) using Baermann funnels. Nematodes will be preserved with DESS (a solution containing dimethyl sulphoxide, disodium EDTA, and saturated NaCl) that allows for combined morphological and molecular analyses. Total nematode numbers will be counted for each sample using an inverted light microscope (50-100X magnification), and the first 100 nematode individuals encountered in the counting dish will be assigned to functional groups (bacterivores, fungivores, root feeders-plant parasites, omnivores and predators) based on their morphology. Total numbers per functional group will be extrapolated based on full sample counts. After extraction, the soil from funnels will be oven-dried and weighed to obtain nematode densities per g soil dry weight.	(10–15, 19)	Soil biodiversity Soil functions

Soil biomass	Community composition	Soil biomass corresponds to the combined measurement of soil microbial biomass, animal biomass and roots biomass. Soil microbial biomass is a powerful proxy for many ecosystem functions including belowground secondary production, soil enzyme and phosphorus dynamics, and soil nitrogen leaching (Eisenhauer et al. 2018). This measurement requires soil samples to be sieved (2 mm) to remove roots, stones, and large animals. Soil microbial biomass will be assessed using the substrate-induced respiration method of approximately 5 g soil (fresh weight) with an O ₂ -microcompensation apparatus. Substrate-induced respiration will be calculated from the respiratory response to D-glucose at 20°C for 10 h. Glucose will be added according to preliminary studies to saturate the catabolic enzymes of microorganisms (e.g., 4 mg g ⁻¹ dry weight dissolved in 400 µl deionized water for grasslands; 8 mg g ⁻¹ dry weight dissolved in 400 µl deionized water for forests). The mean of the lowest three readings within the first 10 h (between the initial peak caused by disturbing the soil and the peak caused by microbial growth) will be taken as maximum initial respiratory response (MIRR; µl O ₂ g ⁻¹ soil dry weight h ⁻¹), and microbial biomass (µg C g ⁻¹ soil dry weight) will be calculated as 38 × MIRR. In the case of animal biomass, after extraction and counting of soil nematodes (see Taxonomic Diversity for more details), the soil from funnels will be oven-dried and weighed to obtain nematode densities per g soil dry weight. We will assess taxon-specific data on nematode fresh body mass from the Nemaplex database (http://plpnmweb.ucdavis.edu/nemaplex/Ecology/nematode_weights.htm) to calculate total nematode biomass. In the case of roots, community roots will be washed in water for 10 min over a 0.63 mm sieve and oven-dried (70°C) for 48 hours (see Community Root Traits for more details).	(10, 20–23)	Soil biodiversity Soil functions
Litter decomposition	Ecosystem Function	During litter decomposition processes, CO ₂ is released back to the atmosphere while carbon and nutrients are transferred to the soil biosphere. Hence, recorded litter mass loss is used as a proxy for ecosystem functioning. For this measurement, commercially available teabags will be used as a pre-made “litterbag” with standardized litter (green tea and rooibos tea). Before the incubation, teabags are dried at 50°C until constant weight. The initial weight (bag+tag+string+tea) is noted and the teabags labeled. In the field, teabags are buried into the upper soil layer (0-5 cm) and incubated for the period of one year. After incubation, tea bags are collected, cleaned, and dried (at 50°C) and the remaining tea mass (without bag, tag, and string) recorded.	(24, 25)	Soil functions
Soil respiration	Ecosystem Function	Soil respiration refers to the process by which available soil carbon is respired into CO ₂ and forms microbial products that contribute to long-term soil carbon storage. Specifically, soil microbial respiration (µl O ₂ h ⁻¹ g ⁻¹ soil dry weight) will be measured on approximately 5 g soil (fresh weight) without addition of substrate using an O ₂ -microcompensation apparatus at hourly intervals for 24 h at 20°C. Soil respiration will be determined as mean of the O ₂ consumption rates of hours 14 to 24 after the start of the measurements.	(21, 22, 26)	Soil functions
Enzymatic activity	Ecosystem Function	Quantification of enzymatic activity potentials of acid phosphatase (EC 3.13.2), N-acetylglucosaminidase (EC 3.2.1.50), xylosidase (EC 3.2.1.37), cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) will be determined using 4-methylumbelliferone (MUB/MUF)-coupled substrates. The initially frozen soil samples will be thawed slowly overnight in the fridge. Approximately 0.35 gram of soil will be dispersed into 50 ml of 50 mM Na-Acetate Buffer (pH 5) through sonication for 5 min. The soil suspensions will be added to respective MUB-coupled substrates in a microtiter plate with eight technical replicates and incubated for 1 hour at 25 ± 1°C in the dark. Shortly before measurement, NaOH will be added to all wells to enhance fluorescence of MUB, which was excited at 360 nm and measured at 465 nm using a TECAN Infinite® F200 PRO plate reader (TECAN, Crailsheim, Germany). Fluorescence values in the assay and control wells will be corrected with auto-fluorescence values of soil suspension and buffer, respectively. MUB standards (1.25 and 2.5 µM) dissolved in buffer and soil suspensions will be used to determine emission and quench coefficients. Enzyme activities (nmol · h ⁻¹ · g ⁻¹ dry soil) and turnover rates (nmol · h ⁻¹) will be related to the amount of dry soil.	(27–29)	Soil functions
Soil aggregation	Ecosystem Function	Soil aggregation is often used as a measure to assess soil aggregate stability and their relation to specific soil biodiversity (e.g., fungi) and ecosystem functions (e.g. plant productivity). This measure will be reported as water-stable soil aggregates assessed by determining the	(30)	Soil physics Soil functions

		resistance of soil aggregates against water as a disintegrating force, by applying an approach modified from Kemper and Rosenau (1986). The resulting index represents the percentage of water-stable aggregates with a diameter smaller than 4 mm. Additionally, debris (i.e., coarse matter) will be separated from the water-stable fraction to correctly determine the water-stable aggregates (WSA) fraction of the sample: $\%WSA = (\text{water stable fraction} - \text{coarse matter}) / (4 \text{ g-coarse matter})$.		
Nutrient cycling	Ecosystem Function	Nutrient cycling is an important component of soil systems that affects not only their productive potential, but also their ecological functions and processes. This EBV will aggregate data from several nutrients regarding their presence, magnitude and availability. We will focus on nitrogen, carbon and phosphorus as target elements for which we will calculate: i) nitrogen mineralization, availability and total; ii) total and organic carbon; iii) available phosphorus. For nitrogen mineralization, air-dried soil samples are re-wetted to reach 80% of their water holding capacity and incubated in the laboratory for 14 days at 30°C. The potential net N mineralization rate is estimated as the difference between initial and final inorganic N. The availability of soil nutrients will be calculated using root simulators, available N will be colorimetrically analyzed. Available P will be determined using a colorimetric determination based on the reaction with ammonium molybdate and development of the 'Molybdenum Blue' color.	(31–35)	Soil chemistry Soil functions
Habitat extent	Ecosystem Structure	Bulk density will be measured at each site following the Cylindrical Core Method, where three sampling points will be randomly placed within the plot and sampled with a core cylinder. Next, the soil cores will be dried in the oven at 100°C for 24 h and weighted. For pH, 10 mL of CaCl ₂ (0.01 M) will be added to 4.00 g of air-dried and sieved (at 2 mm) soil and homogenized for 5 min at 300rev/min. After 1 h, pH will be measured at least three times, and the average will be recorded. For soil structure, soils are going to be separated into size classes (sand, silt, clay) and reported as proportions.	(25, 36)	Soil physics Soil chemistry

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Table S3

Linked EBVs	Indicator	Description
Litter decomposition Soil respiration Soil biomass Enzymatic activity Nutrient cycling	Soil Carbon Stocks	Soil carbon stocks are measured to show if soils are building up or losing soil organic matter (SOM). It refers to the change (calculated as a rate) of soil carbon at a given location. Significant losses can refer to increased impacts to the carbon cycling mechanisms, while significant gains can be dependent on both external inputs (e.g., fertilization) or improvements in soil conditions. This indicator should not be seen as a stand-alone indicator but rather in context with other indicators and EBVs.
Litter decomposition Soil respiration Soil biomass Enzymatic activity Soil aggregate stability Nutrient cycling	Soil Health	Soil health indicates a living dynamic functioning system supporting life, i.e. microorganisms, animals, and plant production aboveground. It refers to the overall soil functional state when compared to a local or regional (e.g., biome) reference condition. A lower soil health indicates a lower functional performance based on the respective EBVs. By being calculated based on functionally related Essential Biodiversity Variables, this indicator is sensitive to land disturbance, effects of climate change (e.g., drought), or variations in land management, being suitable for a early-warning indicator.
Litter decomposition Enzymatic activity Nutrient cycling	Nutrient Cycling and Fertility	Nutrient cycling corresponds to a critical aspect of soil functioning with clear implications for human wellbeing, agriculture/forest productivity, and groundwater quality. It refers to the overall functional response related to the carbon, nitrogen, and phosphorous cycling mechanisms, and it will be calculated as the deviation from a previously known state. Variations in this indicator will show the dominance of particular nutrient cycling mechanisms and it will allow to inform on potential ecological stress situations derived from land degradation impacts.
Habitat extent Taxonomic diversity	Ecological Vulnerability of Soils	Soil ecological vulnerability is directly related to the contraction of soil habitat extent and taxonomic diversity in a given location. This indicator aims to illustrate this contraction by combining two key soil Essential Biodiversity Variables on habitat extent and taxonomic diversity in a composite index that reflects the balance of these two EBVs across time. While habitat extent will reflect the changes in soil properties and land-use type, taxonomic diversity will reflect changes related to other global change drivers (e.g., climate change, pollution). This indicator will be calculated as the proportion of land considered to be ecologically vulnerable.
Taxonomic diversity Functional diversity Habitat extent	Soil Conservation Value	The conservation value of soils is here given by the combination of highly diverse areas, with suitable habitats, in combination with highly functional communities, presence of endemic communities or particular functional types. This indicator will combine data on all of these features to create a soil conservation hotspot indicator that can inform about the spatial location of highly relevant soil conservation areas. It refers to changes in the presence of soil conservation hotspots when compared to a previous known state, indicating the expansion or contraction (e.g., due to global change drivers) of areas with high soil ecological conservation value.
Intraspecific genetic diversity Population abundance Functional diversity Taxonomic diversity Community traits of roots	Soil Biodiversity	Soil biodiversity corresponds to a composite indicator that combines community composition, diversity, and functional trait variables. This holistic view of soil biodiversity allows to differentiate between more short-term changes (compositional approach) and more long-term changes (diversity and functional trait approach). Therefore, this indicator refers to the overall change (direction and intensity) in each of the three mentioned biodiversity facets, when compared to a previously known state. Variations in this indicator can inform on the specific impacts of land degradation, land-use change, climate change, among others.
Intraspecific genetic diversity Functional diversity Taxonomic diversity	Plant Pathogens	An important percentage of the global crop production is lost to biological threats with direct implications for food security and for the productivity and health of terrestrial plant communities worldwide. This indicator combines information related to the presence of specific plant pathogens (i.e., taxonomic and intraspecific genetic diversity) and of functional (genetic) traits that might increase the pathogenicity of a given soil community. It refers to the change (calculated as a rate) in the overall presence of soil-borne plant pathogens. Significant gains in this indicator relate to an increased potential for crop losses and higher management inputs.