# Supplementary Materials for

#### Tracking, targeting, and conserving soil biodiversity

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**This PDF file includes:** Author affiliations Tables S1 to S3 Carlos A, Guerra<sup>12</sup>, Richard D Bardgett<sup>3</sup>, Lucrezia Caon<sup>4</sup>, Thomas W, Crowther<sup>6</sup>, Manuel Delgado-Baguerizo<sup>6</sup>, Luca Montanarella<sup>7</sup>, Laetitia Navarro<sup>12</sup>, Alberto Orgiazzi<sup>7</sup>, Brajesh K. Singh<sup>10</sup>, Leho Tedersoo<sup>10,11</sup>, Ronald Vargas-Rojas<sup>4</sup>, Maria J.I. Briones<sup>12</sup>, François Buscot<sup>13,4</sup>, Erin K. Cameron<sup>14</sup>, Simone Cesarz<sup>1,4</sup>, Antonis Chatzinotas<sup>1,15,16</sup>, Don A. Cowan<sup>17</sup>, Ika Djukic<sup>18</sup>, Johan van den Hoogen<sup>5</sup>, Anika Lehmann<sup>19</sup>, Fernando T. Maestre<sup>30,21</sup>, César Marín<sup>22,23</sup>, Thomas Reitz<sup>13,1</sup>, Matthias C. Rillig<sup>19,24</sup>, Linnea C. Smith<sup>12</sup>, Franciska T. de Vries<sup>25</sup>, Alexandra Weigelt<sup>1,15</sup>, Diana H. Wall<sup>26+</sup>, Nico Eisenhauer<sup>1,15+</sup>

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### Table S1\*

	ie 21					Soi	IBO	Nр	rior	ity E	BV				
Policy Sector	Strategic Goal	Target/Policy/Assessment	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	SoilBON Indicator
	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.		0			0	0	•	•	•	•	•	•	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.				•	•	•	0	0	0	0	0	0	Soil Conservation Value Ecological Vulnerability of Soils
	B.7	By 2020 areas under agriculture, aquaculture and forestry are managed sustainably, ensuring conservation of biodiversity.		0			0	0	•	•	•	•	•	•	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.		0			0	0	•	•	•	•	•	•	Soil Health Nutrient Cycling and Fertility
hi Targets)	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.	0	0			0	0							
Convention on Biological Diversity (Aichi Targets)	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.		0		•	•	•							Soil Conservation Value
ention on E	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.		0			0	0							
Conv	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.	0												
	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

						Soi	во	Nр	riori	ity E	BV				
Policy Sector	Strategic Goal	Target/Policy/Assessment	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	SoilBON Indicator
	A.1	Increased extent of natural ecosystems (terrestrial,			-	•	•	•					•	_	Soil Conservation Value
	A.2	freshwater and marine ecosystems) Ecosystem integrity and connectivity (terrestrial, freshwater and marine ecosystems) Prevent extinction and improve the conservation	0	0	0	•	•	•	•	•	•	•	•	•	Soil Conservation Value Soil Health Ecological Vulnerability of Soils Soil Conservation Value
	A.3	status of species	•	•	٠	•	•	•							Soil Biodiversity
	A.4	Increase the population and health of species		0											
	A.5	Maintain Genetic diversity	0	0			0	0							
	A.6	Protection of critical ecosystems				٠	•	٠	0	0	0	0	0	0	Soil Conservation Value Ecological Vulnerability of Soils
0) - Document open for review for SBSTTA24 consulted July 8th 2020	B.1	Nature's regulating contributions including climate regulation, disaster prevention and other							•	•	•	•		•	Soil Carbon Stocks Nutrient Cycling and Fertility
/ 8tl	B.2	Nature's material contributions including food,													
Int		water and others Nature's non-material contributions including													
Itec	B.3	cultural													
nsu	C.1	Access to Genetic resources	0									_			
5	C.2 D.1	Sharing of the benefits Availability of sufficient financial resources	0												
IA2		Sufficient capacity building, technology transfer and													
ES	D.2	scientific cooperation													
I S	D.3	Access to technology Increase in area of terrestrial, freshwater and													
۴ ۴	T.1.1	marine ecosystems under spatial planning				•	•	•							Soil Conservation Value
svie	T.1.2	Prevention of reduction and fragmentation of				•	0	•	0	0	0	0	0	0	Ecological Vulnerability of Soils
r E	T.1.3	natural habitats due to land/sea use change Priority retention of intact / wilderness areas				0	0	0	0	0	0	0	0	0	
sn fo									_					_	Soil Health
ď	T.1.4	Restoration of degraded ecosystems				•	0	•	•	•	•	•	•	•	Ecological Vulnerability of Soils
ment	T.1.5	Maintenance and restoration of connectivity of natural ecosystems				•	•	•							Soil Conservation Value
Doc	T.2.1	Area of terrestrial, freshwater and marine ecosystem under protection and conservation				•	•	•							Soil Conservation Value
<del>;</del>	T.2.2	Areas of particular importance for biodiversity are protected and conserved as priority				•	•	•							Soil Conservation Value
st-202	T.2.3	Representative system of protected areas and other effective area-based conservation measures	•	•	•	•	•	•							Soil biodiversity Soil Conservation Value
Convention on Biological Diversity (Post-202	T.2.4	Effective management and equitable governance of the system of protected areas and other effective area- based conservation measures	•	•	•	•	•	•	•	•	•	•	•	•	Soil biodiversity Soil Conservation Value Soil Health
ogical Div	T.2.5	Connectivity within the system of protected areas and other effective area-based conservation measures				•	•	•							Soil Conservation Value
i on Biolo	T.2.6	Increased protection and conservation effectiveness	•	•	•	•	•	•	•	•	•	•	•	•	Soil biodiversity Soil Conservation Value Soil Health
tio	T.2.7	Integration into landscape and seascape context													
Conven	T.3.1	Active recovery and conservation management actions	•	•	•	•	•	•	•	•	•	•	•	•	Soil biodiversity Soil Conservation Value Soil Health
	T.3.2	Reduced human-wildlife conflicts			_										
	T.4.1	Harvest is legal, sustainable and safe for human health and biodiversity													
	T.4.2	Trade is legal, sustainable and safe for human health and biodiversity													
	T.4.3	Use is legal, sustainable and safe for human health and biodiversity													
T.5.1		Identification, control and management of pathways	0	ο			0	0							
	T.5.2	for introduction of invasive alien species Effective detection, identification, prioritization and monitoring of invasive alien species	0	0	0		0	0							

			SoilBON priority EBV												
Policy Sector	Strategic Goal	Target/Policy/Assessment	Intraspecific genetic diversity	Population abundance	Community traits of roots	habitat extent	<b>Functional diversity</b>	Taxonomic diversity	Soil biomass	Litter decomposition	Soil respiration	Enzymatic activity	Soil aggregate stability	Nutrient cycling	SoilBON Indicator
	A.1	Establishment of measures for eradication and control of invasive alien species		0		0		0							
	T.5.4	Eliminated or reduced impacts of invasive alien species	0	0	0	0	0	0	0	0	0	0	0	0	
	T.5.5	Eradication, control or management of invasive alien species in priority sites		0		0	0	0							
	T.6.1	Reduction of pollution from excess nutrients							•	•	•	•	•	•	Soil Health Nutrient Cycling and Fertility
	T.6.2	Reduction of pollution from biocides													
~	T.6.3 T.6.4	Reduction of pollution from plastic Reduction of pollution from other sources													
502(	1.0.4	Increased biodiversity contribution to climate													
y 8th 2	T.7.1	change mitigation, adaptation and disaster risk reduction							•	•	•	•		•	Soil Carbon Stocks
- Document open for review for SBSTTA24 consulted July 8th 2020	T.7.2	Minimised negative impacts on biodiversity from any mitigation, adaptation and disaster risk reduction measures	•	•	•	•	•	•	•	•	•	•	•	•	Soil Biodiversity Soil Conservation Value Ecological Vulnerability of Soils Soil Health
A24 cc	T.8.1	Sustainable management of aquatic wild species of fauna and flora, including fisheries													
BSTT	T.8.2	Sustainable management of terrestrial wild species of fauna and flora	•	٠	٠		•	•							Soil Biodiversity
eview for S	T.9.1	Sustainable management of agricultural biodiversity, including soil biodiversity, cultivated plants and farmed and domesticated animals and of wild relatives	•	•	•		•	•							Soil Biodiversity Ecological Vulnerability of Soils
r v	T.9.2	Sustainable management of aquaculture													Soil Conconvotion Value
pen f		Sustainable management of all types of forests				•	•	•							Soil Conservation Value Ecological Vulnerability of Soils
r o		Regulation of air quality										_			
ue u		Regulation of hazards and extreme events Regulation of freshwater quantity, quality, location										_			
noo	T.10.3	and timing													
	T.11.1	Access to green/blue spaces													
020)	T.11.2	Contributions of biodiversity to human health and well-being	٠				•	•							Plant Pathogens
st-2	T.12.1	Access to genetic resources	0	0			0	0							
(Po	T.12.2	Benefit shared from the use of genetic resources													
sity	T.12.3	Benefits resulting from use of traditional knowledge associated with genetic resources													
iver:	T.13.1	Biodiversity reflected in policies and planning at all													
		levels	_												
gic	T.13.2	Biodiversity reflected in national and other accounts Biodiversity values are reflected in policies and													
Convention on Biological Diversity (Post-202	T.13.3	regulations, including on biodiversity inclusive environmental impact assessments and strategic environmental assessments													
ention	T.14.1	Reduction of at least [50%] in negative impacts on biodiversity	•	•	•	•	•	•	0	0	0	0	0	0	Soil Biodiversity
Conve		Sustainable production practices, including circular economy and waste management and sustainable supply chains at national and international levels Sustainable supply chains at national and													
	T.14.3	international levels													
	T.15.1	Sustainable consumption patterns													
	T.15.2	New vision of good quality of life based on sustainability and new social norms for sustainability													
I	T.15.3	Peoples' responsibility for their choices Measures to prevent potential adverse impacts of													
	T.16.1	biotechnology on biodiversity and human health													
	T.16.2	Measures to manage adverse impacts of biotechnology on biodiversity and human health													

			SoilBON priority EBV												
Policy Sector	Strategic Goal	Target/Policy/Assessment	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	SoilBON Indicator
	A.1	Measures to control adverse impacts of biotechnology on biodiversity and human health													
	T.16.4	Restoration and compensation for damage to biodiversity caused by LMOs													
20	T.17.1	Increase in positive public and private economic and regulatory incentives													
th 20	T.17.2	Elimination, phasing out or reform of incentives and subsidies the most harmful to biodiversity													
:0) Ily 8t	T.18.1	Identification of funding needs to meet ambition of													
t-202 ed Ju	T.18.2	the goals and targets of the Framework Increase in financial resources from international													
(Posi nsult		sources Increase in financial resources from domestic													
rsity 4 col	T.18.3	sources Implementation of the strategy for capacity-building													
Dive TTA2	T.18.4 T.18.5	Implementation of the strategy for technology													
gical SBS		transfer and scientific cooperation Availability of reliable and up-to-date biodiversity	~	•	-	_	•	•	•	_	•	•	•	~	
tiolog v for	T.19.1 T.19.2	related information Promotion of awareness of values of biodiversity	0 0	0	0	0	0	0	0	0	0	0	0	0	
on B evie		Promotion of biodiversity in education	0	0	0	0	0	0	0	0	0	0	0	0	
Convention on Biological Diversity (Post-2020) t open for review for SBSTTA24 consulted July	T 10 4	Availability of research and knowledge, including traditional knowledge, innovations and practices of	~	~	~		~	~	~		~	~	~	~	
nver	T.19.4	indigenous peoples and local communities with their free, prior and informed consent	0	0	0	0	0	0	0	0	0	0	0	0	
Convention on Biological Diversity (Post-2020) Document open for review for SBSTTA24 consulted July 8th 2020	T.20.1	Equitable participation of IPLCs in decision-making related to biodiversity and rights over relevant resources													
Õ	T.20.2	Equitable participation of women and girls in decision-making related to biodiversity and rights over relevant resources													
	T.20.3	Equitable participation of youth in decision-making related to biodiversity and rights over relevant resources													
	SDG2.4	By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality.	•				•	•	•	•	•	•	•	•	Soil Health Nutrient Cycling and Fertility Plant Pathogens
	SDG12.2	By 2030, achieve the sustainable management and efficient use of natural resources.	•	•	•		•	•	•	•	•	•	•	•	Soil Health Soil Biodiversity
Sustainable Development Goals	SDG15.1	By 2020, ensure the conservation, restoration and sustainable use of terrestrial and inland freshwater ecosystems and their services, in particular forests, wetlands, mountains and drylands, in line with obligations under international agreements.		0		•	•	•	•	•	•	•	•	•	Soil Conservation Value Ecological Vulnerability of Soils Soil Health
able Develo	SDG15.3	By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world.				•		•	•	•	•	•	•	•	Soil Health Ecological Vulnerability of Soils
Sustainé	SDG15.4	By 2030, ensure the conservation of mountain ecosystems, including their biodiversity, in order to enhance their capacity to provide benefits that are essential for sustainable development.		0		•	•	•	0	0	0	0	0	0	Soil Conservation Value
	SDG15.5	Take urgent and significant action to reduce the degradation of natural habitats, halt the loss of biodiversity and, by 2020, protect and prevent the extinction of threatened species.	•	•	•	•	•	•	0	0	0	0	0	0	Soil Biodiversity Soil Conservation Value Ecological Vulnerability of Soils
<u>.</u>	SDG15.8	By 2020, introduce measures to prevent the introduction and significantly reduce the impact of invasive alien species on land and water ecosystems and control or eradicate the priority species.	0	0				0							

						301		ΝP							-
Policy Sector	Target/Policy/Assessment Voluntary Guidelines for Sustainable Soil		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	SoilBON Indicator
		Voluntary Guidelines for Sustainable Soil Management							•	•	•	•	•	•	Soil Health
Food and Agriculture Organization		World Soil Charter	•	•	•	•	•	•	•	•	•	•	•	•	Soil Conservation value Soil Biodiversity Soil Health Nutrient Cycling and Fertility
d an Org		The international code of conduct for the													
Food		sustainable use and management of fertilizers Global Soil Health Indicators and Assessment							•	•	•	•	•	•	Soil Health Nutrient Cycling and Fertility
Combat tion		Cross cutting Initiative on conservation and sustainable use of soil biodiversity	•	•	•	•	•	•	•	•	•	•	•	•	Soil Conservation value Soil Biodiversity Soil Health
Convention to Combat Desertification		Global Land Outlook	•	•	•	•	•	•	•	•	•	•	•	•	Soil Conservation value Soil Biodiversity Soil Health
Conve		Land Degradation Neutrality				•	•	•	0	0	0	0	0	0	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

SoilBON priority EBV

o 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 enegative 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) platforms. To identify fungi and other eukaryotes, the primers ITS9MUNngs (TACACACCGCCCGCTCG) + 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 515/B (GTGYCAGCMGCGCGGGTAA; Parada et al. 2016) + 806/B (GGACTACNVGGGTWTCTAAT; Apprill et al. 2015), following the protocls outlined in the Earth Microbiome Project to be able to match these data on previous and future projects. Archaea are identified using 16S Primers SU14/F	(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 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 biotiversity 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: <u>https://earthmicrobiome.org</u> / <u>protocols-and-standards/</u> . In parallel, nematodes, preserved in DESS (a solution containing dimethyl sulphoxide, disodium EDTA, and saturated NaCI), 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 <sub>2</sub> -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 <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg g <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg g <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l deionized water for grasslands; 8 mg d <sup>-1</sup> dry weight dissolved in 400 $\mu$ l 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; $\mu$ l O <sub>2</sub> g <sup>-1</sup> soil dry weight h <sup>-1</sup> ), and microbial biomass ( $\mu$ g C <sup>-1</sup> soil dry weight distabase	(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 (µI $O_2$ h <sup>-1</sup> g <sup>-1</sup> 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 $\beta$ -glucosidase (EC 3.2.1.37), cellobiohydrolase (EC 3.2.1.91), and $\beta$ -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 ml 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 $\pm$ 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 of soil suspension and buffer, respectively. MUB standards (1.25 and 2.5 $\mu$ M) dissolved in buffer and soil suspensions will be used to determine emission and quench coefficients. Enzyme activities (nmol $\cdot$ h <sup>-1</sup> g <sup>-1</sup> dry soil) and turnover rates (nmol $\cdot$ 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=(water stable fraction-coarse matter)/(4 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 CaCl2 (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 overal 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 higly 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 differenciate between more short-term changes (compositional approach) and more long-term changes (diversity and functional trait approach). Therefore, this indicator refers to the overal 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.