S©ILGUARD POLICY RECOMMENDATIONS





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Key Policy Recommendations





Soil monitoring methodologies and protocols should be standardized and harmonized whenever possible, but EU Member States (MSs) should retain some flexibility in their choice of soil biodiversity descriptors.



Measuring four indicators can account for over 70% of the variation in soil biodiversity.



We propose the following indicators for soil biodiversity: soil fungal biomass, prokaryotes richness, mites abundance, and total microbial storage biomass. These should be measured in a standardized manner by all MSs, while allowing the inclusion of other indicators depending on the context of each region.



Considering previous research and recommendations, earthworm abundance can be included as a potential fifth indicator. However, this indicator is not a result of SOILGUARD's research, and the project has not produced enough evidence to support or discard it.



Monitoring efforts should address not only soil biodiversity but also ecosystem services or Nature's Contributions to People, aligning with broader EU strategies such as the European Green Deal.



Including a clear definition of soil biodiversity in the Soil Monitoring and Resilience Directive proposal would enhance the tracking of targeted measures linked with soil-specific organisms and establish baseline parameters for consistent monitoring.

Introduction

The Soil Monitoring and Resilience Directive (SMRD) is a key legislative initiative under the European Green Deal and its Soil Strategy for 2030, aiming to ensure that all soils in the EU are in healthy condition by 2050.

Proposed by the European Commission in July 2023, the SMRD represents an attempt to establish a comprehensive legal framework for monitoring and protecting soil health, addressing transboundary impacts of soil degradation and fostering sustainable management.

The SMRD introduces measures for regular soil monitoring, assessment and remediation. It outlines specific mechanisms for achieving its objectives, including the establishment of 'soil districts' which will serve as the primary units for soil monitoring and management. MSs are required to perform regular soil health assessments and maintain national registers of contaminated sites, with monitoring data informing soil health improvement measures.

This brochure aims to contribute to policy discussions by presenting evidence-based recommendations informed by SOILGUARD research, highlighting how the SMRD can better integrate scientifically validated soil biodiversity indicators to enhance its implementation and effectiveness. The recommendations aim to create a harmonized yet adaptable monitoring framework, ensuring that the SMRD effectively supports the EU's environmental and agricultural goals.

Soilguard Overview

SOILGUARD is an H2020 project which aims to boost the sustainable use of soil biodiversity to protect soil multifunctionality from land degradation, unsustainable soil management and climate change, and thus increase economic, social and environmental well-being.

To reach this aim, SOILGUARD has developed an experimental design focused on understanding the region-specific benefits of sustainable soil management (SSM) for the conservation of soil biodiversity and the delivery of soil-mediated ecosystem services. The SSM practices considered in SOILGUARD and linked to the results presented below are: (i) no use of phytochemicals (pesticides and chemical fertilizers) in agricultural soils, (ii) plant species-rich grasslands, and (iii) continuous-cover forestry. This was achieved by combining a cross-biome network of sites across four continents, with on-field climate simulations to compare different management practices.

Evidence and Research

SOILGUARD's extensive research into soil biodiversity provides critical insights into the use of biodiversity descriptors and their application within the soil health monitoring framework of the SMRD.

In the SOILGUARD project, we assessed soil biodiversity (from bacteria to earthworms) at 233 selected sites across eight biogeographical regions and three biomes (cropland, grassland and forest), conducting one of the most extensive and comprehensive evaluations of soil biodiversity to date (Table 1). Further details on the network of sites can be found in SOILGUARD'S public deliverable D2.1 Map of the cross-biome network of sites and land degradation gradients.



Table 1. Geographical distribution of sampling sites of the cross-biome network of sites. The table shows the number of sites sampled in each region, plus its biome and biogeographical region.

REGION AND COUNTRY	N° OF SITES	BIOME	BIOGEOGRAPHICAL REGION
West Flanders (Belgium)	20	Croplands	Atlantic
Murcia (Spain)	20	Croplands	Mediterranean
Middle Jutland (Denmark)	30	Croplands	Continental
Buenos Aires (Argentina)	20	Croplands	Temperate oceanic
Latvia	20	Croplands	Boreal
South Transdanubia (Hungary)	20	Croplands	Pannonian
West Cameroon (Cameroon)	20	Croplands	Tropical humid
Chiangrai (Thailand)	29	Croplands	Tropical savannah
Southern Ireland (Ireland)	30	Grasslands	Atlantic
West Finland (Finland)	24	Forests	Boreal

SOILGUARD'S WORK, CARRIED OUT IN THE CROSS-BIOME NETWORK OF SITES, WAS FOCUSED ON VALIDATING THE FOLLOWING HYPOTHESIS:



 i) land degradation and unsustainable soil management promote biodiversity loss



 ii) SSM promotes soil biodiversity and soil multifunctionality



 iii) the impacts of land degradation and unsustainable soil management are region-specific

Soil biodiversity (bacteria, archaea, fungi, protists, nematodes, micro-arthropods and annelids) was analysed using DNA sequencing with three primer pairs recommended by the SoilBON global soil biodiversity initiative, targeting the 16S rRNA gene (region V3-V4), ITS (region ITS2), and the 18S rRNA gene (region V4). Moreover, we measured the abundance of different soil microbial groups using phospholipid fatty acids and neutral fatty acids (PLFAs and NLFAs), as well as direct counting and identification of soil faunal groups (nematodes, mites and springtails-collembola).

This set of biodiversity variables was measured at cropland sites across Europe (our main database), as well as in croplands outside Europe, and in grasslands in Ireland and forests in Finland. The data from forests and grasslands, as well as that from non-EU croplands, was not used for the main analyses presented here, but rather to validate the applicability (i.e. out-of-sample tests) of our soil biodiversity indicators to different environmental contexts (non-EU croplands) or biomes (forests and grasslands). With the results of the measures, we then searched for indicators that might represent a very high percentage of the variation in soil biodiversity (species richness or equivalent measures across the diverse soil microorganisms and faunal groups assessed) and their overall change. This is expressed as soil multidiversity, the average of all measures of species richness, standardized to a common range of values (between 0-1) so that all metrics are equally weighted, regardless of species richness or abundance of each group. In other words, with this metric we give the same importance to all soil organisms, regardless of how species-rich they are (e.g. bacteria are more speciesrich than any other soil group, and would thus drive any other way of measuring biodiversity changes, regardless of how other soil taxonimic groups change). This metric has been extensively studied and is found to positively correlate with soil functioning, and these correlations have been observed in both experimental studies (controlled conditions, minimized environmental variation) and observational studies (performed under natural, more realistic conditions, where detecting causality is more challenging). Further details on the sampling protocols and the network of sites can be found in SOILGUARD's Deliverable 2.1 – Map of the cross-biome network of sites and land degradation gradients and Deliverable 2.2 -<u>Report on the soil biodiversity status in European and</u> international biogeographical regions.

Policy Recommendations

HOW SHOULD THE SMRD ADDRESS SOIL BIODIVERSITY LOSS MONITORING?

We strongly recommend standardizing and harmonizing soil sampling protocols and laboratory methodologies as far as possible, while allowing at least some flexibility of choice of indicators for MSs.

Standardizing the set of soil biodiversity descriptors in the SMRD would enable comparisons between MSs and soil districts, facilitating the establishment of homogeneous baselines. In this regard, the SOILGUARD analysis shows that the four indicators proposed in the following sections are highly correlated with 20 biodiversity metrics, even across contrasting biomes such as grasslands and forests.

Given the significant variability of soil organisms at regional, local and site-specific scales, MSs should have at least some flexibility in selecting soil biodiversity indicators. This flexibility will ensure that the chosen indicators are representative of the context-specific soil biodiversity, in light of the fact that no single set of indicators can address all scenarios. However, it is essential to standardize soil sampling protocols and laboratory methodologies and ensure the consistency and coherence of soil biodiversity indicators over time, to allow the assessment of soil biodiversity trends at each site and the evaluation of the impact of implemented practices.

Soil biodiversity indicators are a critical yet underdeveloped component within the SMRD. Under the Commission's proposal and the Council's general approach, soil biodiversity indicators are not considered in the soil health assessment (Part C of Annex I), which risks sidelining this crucial aspect of soil health, weakening the Directive's alignment with the soil health definition included. To address this, soil biodiversity indicators should be a focus in the coming years to develop generally agreed criteria and potentially move those descriptors from Part C to Part B of Annex I, to ensure that this element has an impact on the final soil health evaluation. Research findings from SOILGUARD highlight the potential of biodiversity descriptors to provide robust knowledge on soil health and establish a scientifically grounded monitoring framework aligned with EU sustainability goals.



HOW MANY INDICATORS ARE NECESSARY TO EFFECTIVELY ASSESS SOIL BIODIVERSITY LOSS?

Our preliminary results show that measuring four indicators can account for over 70% of the variation in soil biodiversity. These findings are based on principal components analyses (PCAs) made using our extensive and comprehensive soil biodiversity database, which allowed us to identify the main axes of variation in soil biodiversity. The results were also validated through cluster analysis of the correlation patterns among the various diversity and abundance measures we assessed (Figure 1). Further details on the identification of key indicators to assess soil biodiversity can be found in <u>Deliverable 2.3 – Report on the region and</u> <u>biome-specific impact of soil degradation and management</u> on soil biodiversity status and cascading effects on soil <u>multifunctionality.</u>



Figure 1: Four soil biodiversity indicators.

Correlation (Pearson's) coefficients and hubs (black boxes) across the 20 soil biodiversity indicators evaluated (A), and their dimensionality (B), as represented with a principal component analysis. Match between the hubs (A) and axes (B) is provided with the white number in A. The best candidates for specific soil biodiversity indicators, according to (i) their loadings in each PCA axis, (ii) their degree of complementarity, and (iii) the proportion of soil biodiversity they represent, are shown within the red quadrants. From top-down/left-right in A: arbuscular mycorrhizal fungi – AMF (NLFAs); total microbial storage biomass – TMSB (NLFAs); prokaryotes biomass (16S qPCR); prokaryotes richness (16S V3V4 sequencing); eukaryotes richness 1 (18SV4 sequencing); eukaryotes richness 2 (COI sequencing); fungi richness (ITS2 sequencing); eukaryotes richness 3 (18SV4V5 sequencing); fungi biomass; bacteria biomass (PLFAs); total microbial biomass – TMB (PLFAs); nematodes abundance (microscope); protists biomass (PLFAs); nematodes richness 4 (16S mitochondrial); collembola richness (microscope); eukaryotes richness 5 (18SV6V8 sequencing); mites richness (microscope); collembola abundance (microscope); mites abundance (microscope).

WHICH INDICATORS SHOULD BE SELECTED TO EFFECTIVELY ASSESS SOIL BIODIVERSITY LOSS?

We propose the following indicators:

- Soil fungal biomass (through PLFAs)
- Prokaryotes richness (through 16s gene sequencing)
- Mites abundance (through Tullgren funnel extraction and microscope detection)
- Total microbial storage biomass (through NLFAs)

These four biodiversity indicators represent over 70% of the variation in our database. They cover contrasting environmental responses and consequences for soil functioning (Figure 2) and perform well across environments and biomes different from those used for their selection (Figure 3).

Figure 2: Environmental responses (left) and functional consequences (right).

nrae cald) O	0.24*	0 42*	0.20*	0.44*	0.00*	0.00		prok	fungi	collem	mites	amf	tmsb
prec_cold)_Q	0.54	0.42	0.29	0.44	-0.20	0.09	cropyeild	-0.14	0.18				0.15
prec_wallin_Q	0.35	0.10	0.35	0.16*	-0.3	0.04	ecosystem stability	-0.37		0.21	-0.34		0.15
prec_ury_Q	0.58*	0.26*	0.38*	0.19	-0.39*	-0.02	ecosystem_stability	0.14	0.00	0.21	-0.04		0.13
prec S	-0.37*	-0.46*	-0.35*	-0.48*	0.36*	-0.16	CSTOCK	0.14	0.32		0.29		0.11
prec A	0.54*	36*	0.35*	0.33*	-0.45*	0.05	litter_decomposition	0.39	-0.29	-0.18		0.18	-0.19
temp_cold_Q	-0.62*	-0.46*	-0.57*	-0.29*	-0.03	-0.1	available_p	0.47	0.17		-0.19	0.20	0.35
temp_warm_Q	-0.67*	-0.76*	-0.70	-0.59*	-0.08	-0.24*	tan		0.76	0.45	0.34		0.43
temp_dry_Q	-0.59*	-0.39*	-0.52*	-0.24*	-0.08	-0.08	ntr	0.27	0.54	0.32	0.40	0.13	0.47
temp_wet_Q	-0.11	-0.26*	-0.17	-0.31*	0	-0.1	1111	0.27	0.04	0.52	0.40	0.15	0.47
o temp_S	0.11	-0.18	-0.04	-0.2*	0.17	-0.08	bg	-0.16	0.32	0.33	0.29		0.34
temp_A	-0.76*	-0.72*	-0.7*	-0.52*	-0.05	-0.19*	xyl	0.11	0.54	0.26	0.41	-0.15	0.14
aridity	-0.7*	-0.65*-	0.57*	-0.52*	0.12	-0.22*	phos	0.12	0.16		0.24	-0.11	
evapotransp	-0.7*	-0.76*	-0.71*	-0.6*	-0.1	-0.26*	nag	0.15	0.46		0.20		0.30
conductivity	-0.41*	-0.34*	-0.38*	-0.25*	-0.27*	-0.14	nirKS nos71711 ratio	0.11	0.50	0.28	0.46		0.26
bulk density	0.32	-0.05	0.1	-0.08	-0.33*	-0.14		0.11	0.50	0.20	0.40		0.20
100	0.22*	0.51*	0.28*	0.5*	0.11	0.3*	aggregates	0.48		-0.19	0.10	-0.22	
PH	0.4*	-0.33*	-0.42*	-0.24*	0.28*	-0.06	whc	-0.15	0.13	0.22	0.25	0.14	0.20
day	-0.30*	-0.43^	-0.481	-0.41^	0 11	-0.10	infiltration	0.41	0.21		0.32		0.11
siit sand	-0.33*	-0.3*	-0.20*	-0.10	-0.11	-0.24*	n retained soil	-0.28	0.26	0.26		-0.14	0.19
type crop	0.42	0.38*	0.36*	0.25	0.07	-0.02	n retained soil	-0.26			0.17		
management	0.08	0.15	0.02	0.1	0.07	0.2*		0.10	0.17		0.17	0.1/	0.10
5	Ś	_	m	S	~		roof_herbivores	0.12	0.17			0.16	0.10
	ote:	olfa		ite	lfa	lfa	leaf_damage	0.15	0.36	-0.25	0.12		0.23
	ary	i D	me	Σ	<u>г</u>	с Г	NCP_food	-0.39	0.17	0.11	-0.29	-0.02	0.23
	rok	ßun	Colle		AM	ISM'	NCP_climate	0.12	0.24		0.28	0.57	0.36
	S	ш	0			н	NCP_water	0.17	0.21	0.16	0.35		0.19
	16						NCP_pests	0.17	035	-0.21		0.16	0.22
							NCP_soil	0.30	0.61	0.26	0.45		0.39
							multifunctionalty	0.23	0.60	0.18	0.39	0.13	0.44

Correlations (Spearman's) between the best candidates for soil biodiversity indicators and climate, soil attributes (texture: silt and sand content, pH, bulk density, electric conductivity) and management (type of crop, organic vs conventional). Climatic variables were obtained from Worldclim 2.0 and cover mean annual temperature (temp_A), temperature seasonality (temp_S), aridity levels, temperature during the coldest, warmest, driest and wettest quarter (temp_cold_Q, temp_warm_Q, temp_dry_Q, temp_wet_Q, respectively), and similar measures for rainfall (prec). In the panel on the right, we show partial correlation coefficients (after filtering by the effect of the environmental variation) showing the relationships between our best candidates for soil biodiversity indicators and multiple ecosystem functions of interest in croplands. Bold and shaded numbers indicate the strongest relationships detected.

Figure 3: Out-of-sample test.



Correlations (Pearson's) between the multiversity using the four indicators suggested (richness prokaryotes, abundance of fungi, mites and total microbial storage biomass) vs the multidiversity index calculated with all our 20 soil biodiversity indicators in croplands (A), grasslands (B) and forests (C).

To detect those indicators, we used three criteria:

- i) How well they represented variation across the four different PCA axes detected as relevant (Figure 4).
- ii) How complementary they were to one another.

If multiple candidates met criteria i and ii, we prioritized those that:

iii) Included a higher number of 'species' (Amplicon Sequence Variants). This is why we selected prokaryote (16S) richness over eukaryote (18S) richness – 16S not only encompassed a broader range of soil organisms but also complemented fungal abundance better than 18S.

Last but not least, we selected those indicators that:

iv) Showed the strongest correlations both with environmental conditions and our soil functioning indicators.

Further details on the criteria for identifying soil biodiversity indicators can be found in <u>Deliverable 2.3</u> <u>— Report on the region and biome-specific impact of soil</u> <u>degradation and management on soil biodiversity status</u> <u>and cascading effects on soil multifunctionality.</u>







The strength of the relationships between each soil biodiversity measure used and each of the four PCA axes (equivalent to our four clusters) is shown. This strength is represented with the squared cosine values for all diversity metrics of each of the components (axes). Red arrows indicate our best candidates for soil biodiversity indicators, other than the ones best representing this particular axis (largest bars in each panel).

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It should be noted that the four selected soil biodiversity indicators consider both richness and abundance measures, as well as indicators of soil microorganisms and fauna. In this regard, our results are generally consistent with previous recommendations for soil biodiversity and soil health indicators.^{1,2,3}

However, we did not have robust information on earthworms, which have been consistently suggested as an effective soil biodiversity indicator. Although we obtained diversity data for these organisms using a specific DNA primer, their very low representation and prevalence across the dataset prevented us from adequately assessing the usefulness of this group.

Therefore, given previous research,⁴ and following the precautionary principle, we recommend including soil earthworm abundance as a potential fifth soil biodiversity indicator. Moreover, it is important to consider that monitoring higher trophic levels such as invertebrates (e.g. earthworms) is crucial for understanding soil ecosystem resilience and function, as these organisms play a key role in nutrient cycling and maintaining soil structure. Although we have carefully selected the recommended soil biodiversity indicators based on the criteria detailed above, it must be noted that further adjustments may be necessary beyond SOILGUARD's research. Thus, the exact identity of these indicators is subject to change in the future. In any case, the selected indicators provide a broader range of information at no additional cost, which could complement future soil biodiversity assessments. For instance, 16S sequencing not only provides information about the 'richness' of the soil prokaryote community, but it can also provide information about its composition at different taxonomic resolutions, with the same cost but with higher expertise required on computational analyses. Thus, indicators based on the presence of specific taxa could also be developed from this measure without extra efforts. Similarly, PLFAs offer much more information than fungal abundance as they also provide information about the abundance of other groups of soil microorganisms. Therefore, other measurements, such as the bacterial-to-fungal abundance ratio, a well-known indicator of soil functioning, could also be obtained from this indicator.

MONITORING SOIL FUNCTIONS AND NATURE'S CONTRIBUTIONS TO PEOPLE

The four proposed indicators provide information not only about changes in soil biodiversity, but also about how soil biodiversity responds to environmental conditions and its relationship with ecosystem functioning. Specifically, Table 2 illustrates which soil biodiversity indicators are linked to the response of soil organisms to changes in climate, soil carbon, management practices, pH, and texture and compaction. It also details how these indicators reflect changes in water infiltration capacity, phosphorus availability, soil aggregate stability, food production (crop yield and stability in primary productivity), overall multifunctionality, soil organic carbon, water availability, and nutrient cycling (Figure 2).

SOIL BIODIVERSITY INDICATORS	RESPONSE OF SOIL ORGANISMS	CHANGES IN ECOSYSTEM FUNCTIONING
Prokaryotes richness	Climate, soil C, pH and texture, compaction	Water infiltration capacity, phosphorous availability, soil aggregate stability, food production
Fungal biomass	Climate, management	Overall multifunctionality, soil organic carbon
Mites abundance	Climate, soil C, pH and texture	Water availability, nutrient cycling
Total microbial storage biomass	Management, compaction	Soil C stocks, nutrient cycling

Table 2. Link between soil biodiversity indicators, environmental responses and functional consequences. This table is based on the results presented in Figure 2.

Monitoring should focus not only on soil biodiversity loss but also on its effects on soil functions and ecosystem services (or Nature's Contributions to People), such as food and feed provision, carbon sequestration, nutrient cycling and water regulation, aligning with broader EU strategies such as the European Green Deal. SOILGUARD'S Deliverable 4.2 – Region-specific economic and socio-cultural values provides further information about this aspect. Including Nature's Contributions to People in descriptors would demonstrate the relevance of soil biodiversity for public good provision and reinforce the alignment of the SMRD with EU sustainability and climate objectives. To establish recommendations in this regard, we applied a similar approach to the one described above (PCAs and indicator selection for each axis) on multifunctional indicators across SOILGUARD and other EU-funded projects (FunDivEUROPE and BIOCOM), and national-funded projects (Biodiversity Exploratories). Preliminary results suggest that five functioning indicators would suffice to represent above 60% of variation in soil-related Nature's Contributions to People. These are soil organic C, available phosphorous, soil enzymatic activities (beta-glucosidase and phosphatase), biomass production, and litter decomposition (tea bag index). In addition, potential nitrogen transformation rates could provide a good estimate of both nitrogen availability and the biological potential to transform their different sources (including denitrification processes, of particular interest for the EU Farm-to-Fork Strategy). These indicators collectively cover both nutrient stocks and transformation rates, and biologicallyand biogeochemically-driven soil elements, and should be integrated in the SMRD. Further details on the relationship between soil biodiversity indicators, soil functions and Nature's Contributions to People can be found in SOILGUARD's Deliverable 2.3 – Report on the region and biome-specific impact of soil degradation and management on soil biodiversity status and cascading effects on soil multifunctionality.

DEFINING SOIL BIOTA AND SOIL BIODIVERSITY

Including a clear definition of soil biodiversity in the SMRD would be useful for proposing and monitoring targeted measures related to soil-specific species and establishing baseline parameters for monitoring. An official and common definition of soil biodiversity is still lacking. There is an urgent need for clarity as different interpretations of soil biodiversity could lead to completely different actions.⁵ There are several definitions of soil biota and soil biodiversity (Table 3). Each definition highlights different aspects and may serve various purposes, but all acknowledge the importance of diverse life forms within the soil. An inclusive, general and broad definition of what constitutes a soil species will be a significant step toward enhancing soil biodiversity conservation.

Table 3. Soil biodiversity and soil biota definitions

SOIL BIODIVERSITY DEFINITION	REFERENCE		
Soil biodiversity is the variety of life below ground, from genes and species to the communities they form, as well as the ecological complexes to which they contribute and to which they belong, from soil micro-habitats to landscapes.	FAO ⁶		
Soil biodiversity considers the abundance, biomass and diversity of soil organisms, targeting prokaryotes (encompassing bacteria and archaea) as well as eukaryotes (including fungi, protists, nematodes, arthropods and earthworms).	SOILGUARD		
SOIL BIOTA DEFINITION	REFERENCE		
Soil biota include bacteria, fungi, algae, protists, viruses, nematodes, acari (including mites), collembola (springtails), annelids (primarily earthworms), macroarthropods (such as spiders, ants and woodlice) and vertebrates (like voles, moles and shrews), and also the plants whose root exudates provide food for soil organisms in a zone around the roots known as the 'rhizosphere'.	IUCN Common Ground report ⁷		
Soil species are those organisms that spend a key part of their life cycle within a soil profile, or predominantly inhabit the soil-litter interface. This includes soil megafauna, macrofauna, mesofauna, microfauna/flora, fungi, and micro-organisms. Although we recognize that most plants play an important role in maintaining fertility, structure, drainage and aeration of soil, these are not tagged as soil species for the IUCN Red List.	IUCN definition for soil biota for IUCN Red List of Threatened Species		

The definition from FAO is broad and inclusive, covering genetic diversity, species, communities and ecological complexes across all scales, while the SOILGUARD definition focuses on specific quantitative research measures (abundance, biomass, diversity). Further details about soil biodiversity and soil biota definitions can be found in SOILGUARD'S <u>Deliverable 6.4 – Policy and</u> <u>conservation brochure for wider dissemination</u>.

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SOILGUARD: Sustainable soil management to unleash soil biodiversity potential and increase environmental, economic and social wellbeing.

Website: https://soilguard-h2020.eu/

Join our Soilguardian Network of Knowledge to help ensure healthy soils for generations to come: https://soilguard-h2020.eu/become-a-soilguardian

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