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Establishing assessment criteria for soil bioindicators: insights from case studies in Europe

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ABSTRACT

The new EU Soil Strategy aims to restore soil health across Europe by 2050, requiring policy-relevant indicators with robust assessment criteria to control and monitor its achievement. Despite the importance of biological conditions for soil health, few EU Member States have currently established criteria (e.g., target or threshold values) for evaluation of monitoring data. Our study examines the challenges and opportunities in developing such criteria based on the normal operating ranges of bioindicators in the context of soil units (i.e., homogenous soil texture and land use). Leveraging on national case studies across Europe, we find that for most bioindicators criteria could not be defined, due to data insufficiency and environmental bias. Only in France could normal operating ranges be established for earthworms, for some soil units. We identify priorities for the development of robust assessment criteria: (1) Harmonization of evaluation units and definitions, to minimize disparities in current soil texture and land use classifications between Member States, that complicate comparability of evaluation across Europe; (2) Inclusion of climatic-region specific thresholds/targets in addition to land use and soil texture; and (3) Standardization of protocols to reduce the observed variability in methods for sampling and data aggregation. As a temporary solution to optimize monitoring sampling efforts, guidelines for dealing with data deficiencies and pseudo-replication are proposed. On the longer term, our findings highlight the importance of integrated data systems and standardised frameworks for monitoring bioindicators to evaluate progress towards achievement of the EU soil health targets.

1. Introduction

It is estimated that 60–70 % of the soils in the European Union (EU) are degraded and continue to deteriorate (EC, 2024; Efthimiou, 2025; ESDAC, 2024). Yet, healthy soils are essential to ensure resilient ecosystems, climate regulation, and food production. This recognition has

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put soil restoration and sustainable management at the heart of European environmental policy. The new Soil Strategy (EC, 2021) has the objective that all soils should be healthy by 2050. Healthy soils have been defined as soils "*that are in good chemical, biological and physical condition so that they can provide ecosystem services that are vital to humans and the environment, such as safe, nutritious and sufficient food, biomass, clean water, nutrients cycling, carbon storage and a habitat for biodiversity*" (Soil Mission, 2024). To monitor and assess soil health status, a newly proposed Soil Monitoring Law (EC, 2023) will require Member States to establish national monitoring and promote the adoption of sustainable soil management practices. The Soil Monitoring Law requires the use of a suite of chemical, physical and biological indicators for soil health, i.e., measurable soil properties or functions that indicate the degree to which soils can fulfil expected ecosystem services (Lehmann et al., 2020). While in the past soil health monitoring in Europe largely focused on abiotic properties, the recognition of the essential role of soil organisms in maintaining ecological functions (Baritz et al., 2008; Faber et al., 2022; Griffiths et al., 2016; Pulleman et al., 2012; Ritz et al., 2009) has led to the inclusion of biological indicators (or bioindicators) in recent soil monitoring programs (Bispo et al., 2009; Gardi et al., 2009; Bünemann et al., 2018; Orgiazzi et al., 2018; Hoek et al., 2019; Faber et al., 2022). In national monitoring across the EU, however, bioindicators have sparsely been implemented, and associated assessment criteria are currently largely absent (Faber et al., 2022). With the introduction of the proposed EU Soil Monitoring Law, implementation of soil bioindicators in national and EU-wide monitoring frameworks is expected to significantly increase. In order to assess soil health based on future monitoring data, the development of assessment criteria is urgently needed.

A crucial step in the implementation of soil health bioindicators in monitoring programs is the definition of assessment criteria reflecting normal operating ranges tailored in the context of specific soil texture, land use and climate combinations (Faber et al., 2022; Matson et al., 2024). Assessment criteria are critical in the evaluation of soil health because they provide baselines against which actual soil condition can be evaluated, and thus help to identify areas at risk of degradation and inform targeted management interventions. Matson et al. (2024) highlighted four approaches to determine indicator criteria (i.e., fixed values, reference values, distribution values, or simple relative change). Lacking quantitative knowledge of the relationship between indicator and service provision, and in the absence of reference values, a description of data distributions provides an elementary insight in the normal operating ranges of indicators (Matson et al., 2024). In this distribution approach, critical percentiles may be used tentatively for assessment. For example, threshold values may be used to set an acceptable limit for deviation from a healthy soil state, while target values may be specifically aimed at a particular land use or policy objective (Matson et al., 2024).

Currently national assessment criteria for soil health indicators remain limited in scope and often focus on soil structure (Cousin et al., 2024), contaminants (Chen et al., 1999), or nutrient levels (Steinfurth et al., 2022), rather than on bioindicators relevant for soil functions (Faber et al., 2022). Notable exceptions include efforts to establish soil bioindicators reference values in the Netherlands, where 23 indicators were combined into a single reference for ten typical contexts of land use and soil type (Rutgers et al., 2009, 2008). Similarly, the French monitoring soil quality network ('*Réseau de Mesures de la Qualité des Sols*', RMQS), predicted microbiological indicators thresholds based on land use, soil characteristics and elevation (Horrigue et al., 2016; Terrat et al., 2017). While some flexibility is desirable in the development of assessment criteria for soil health, in order to implement locally relevant soil management interventions, the use of different assessment approaches can lead to contrasting conclusions regarding the state of soil health, as demonstrated for soil carbon content (Matson et al., 2024). In addition, different monitoring schemes for similar indicators might promote disparity in data distribution and may ultimately limit comparability and rigorous assessment across studies and Member States. Given their pivotal role, an evaluation of assessment criteria for soil health bioindicators across soil units (defined as specific soil texture and land use or land cover combinations) and Member States in Europe is urgently needed to provide a framework for data comparability.

The aim of this paper is to investigate the feasibility of developing assessment criteria for a variety of established biological indicators for soil health, and to highlight opportunities and challenges that may arise at national and EU levels in view of implementation of the proposed EU Soil Monitoring Law. The work was based on the EU EJP SOIL project 'MINOTAUR' (EJP Soil, 2024), and the datasets included in this study represent a subset of EU Member States as well as Switzerland. We do not attempt to define or develop assessment criteria for bioindicators, as this should be undertaken using more complete datasets. Building on national datasets available from the EU EJP SOIL project 'MINOTAUR' (EJP Soil, 2024) on soil bioindicators (microorganism, soil enzymes, nematodes, microarthropods and earthworms), we first describe bioindicator metrics ranges, including possible target and threshold values, following a data distribution approach (Matson et al., 2024), per soil unit. The aim was to evaluate the current potential of the investigated bioindicators to provide assessment criteria for soil health evaluation at national level and, where feasible, across Member States. Secondly, datasets were specifically assessed to investigate how differences in sampling protocols and soil texture categorization may impact assessment criteria characterization. Building on the national case studies we identify current challenges, future research and policy requirements at national and EU level for the development of assessment criteria for soil bioindicators.

2. Material & methods

2.1. Soil bioindicators investigated

A range of metrics were evaluated for five soil bioindicators: microorganisms, soil enzymes, nematodes, microarthropods and earthworms (Table 1). These bioindicators have been applied in national monitoring in the EU (Faber et al., 2022) and have been shortlisted by the MINOTAUR consortium for harmonised monitoring across EU (EJP Soil, 2024). Criteria for indicator inclusion focused on usability for national monitoring and the availability of national datasets. As a result, some relevant and widely used bioindicators, such as soil microbial respiration, could not be included due to the lack of available national datasets within the consortium. Current amendments to the proposal for a Soil Monitoring Law also include selected bioindicators (CEU, 2025).

Microorganisms and soil enzymes: microbes are ubiquitous in soils and regulate for instance nutrient availability, aggregate stability, carbon sequestration, pollutant degradation, plant disease prevalence, and plant growth. Numerous microbial-based indicators of soil health have been proposed, and some are already routinely used (e.g., microbial biomass, fungi:bacteria ratio, (Fierer et al., 2021). Methods such as PLFA analysis, qPCR or high-throughput marker gene (typically 16S, 18S, ITS) sequencing, are widely used to assess the biomass as well as the taxonomic and functional composition of soil microbial communities (Orgiazzi et al., 2018; Siles et al., 2024). However, these methods represent an under-utilized metric of soil health compared to indirect proxies, such as basal soil respiration (but see LUCAS soil monitoring program, Orgiazzi et al., 2018). Next to microbial biomass and community composition, soil enzymes are also indicators of various decomposition and nutrient cycling processes (Burns, 1982; Leirós et al., 2000). Here we focus on enzymes involved in the phosphorus (acid phosphomonoesterase, phosphodiesterase), sulphur (arylsulphatase) and carbon (ß-glucosidase) cycles (Burns et al., 2013) (Table 1).

Nematodes: Soil nematodes, which are part of the mesofauna (<2 mm body width), play an important role in the soil food-web and hence affect nutrient flows (Powell, 2007; Young and Unc, 2023). In addition to community-based metrics such as abundance and richness, several indices can be calculated based on their feeding habits and life-history

Table 1
Summary of indicators and their corresponding metrics and assessment methods used in this study. Formulas are given in Appendix Table A.2.

Indicator	Metric	Protocol
Microorganisms	Microbial biomass (nmol PLFA g^{-1})	PLFA (ISO/TS 29843-2 (25/08/2021))
	Fungi:bacteria ratio	
	AMF biomass (nmol NLFA g^{-1}) and relative abundance	NLFA (Vestberg et al., 2012) 16S/ITS metabarcoding (Gschwend et al., 2021a; Romero et al., 2024)
	Saprotrophic fungi relative abundance	PLFA (biomass) (ISO/TS 29843-2 (25/08/2021)), 16S/ITS metabarcoding (Gschwend et al., 2021a; Romero et al., 2024)
	Plant-pathogenic fungi relative abundance	16S/ITS metabarcoding (Gschwend et al., 2021a; Romero et al., 2024)
	Nitrogen fixing, nitrifying and denitrifying bacteria	16S/ITS metabarcoding
Soil enzymes*	Acid phosphomonoesterase (μmol $g^{-1}h^{-1}$)	Fornasier and Margon, 2007; Tabatabai and Bremner, 1969; Trasar-Cepeda et al., 2008
	Phosphodiesterase (μmol $g^{-1}h^{-1}$)	Browman and Tabatabai, 1978; Trasar-Cepeda et al., 2008
	ß-Glucosidase (μmol $g^{-1}h^{-1}$)	Eivazi and Tabatabai, 1988; Trasar-Cepeda et al., 2008
	Arylsulphatase (μmol $g^{-1}h^{-1}$)	Fornasier and Margon, 2007; Tabatabai and Bremner, 1969; Trasar-Cepeda et al., 2008
Nematodes	Abundance (ind. 100 g^{-1})	Sampling, extraction and identification of soil-inhabiting nematodes (ISO 23611–4:2022)
	Abundance of plant parasites (ind. 100 g^{-1})	
	Richness (genera. 100 g^{-1})	
	Enrichment index (EI)	
	Structure index (SI)	
Micro-arthropods	Abundance (ind. m^{-2})	Sampling and extraction of micro-arthropods (ISO 23611-2:2024)
	Acari:Collembola ratio	Bachelier, 1963
	BF Richness	D'Avino et al., 2024
	(N° euedaphic BF)/(BF richness)	
	QBS-ar	Parisi et al., 2005
Earthworms	Abundance (ind. m^{-2})	Hand-sorting and extraction of earthworms (ISO 23611–1:2011, ISO 23611–1:2018; ISO 11268–3)
	Biomass (g m^{-2})	
	Species richness	

*Note difference in dimensional standard:μmol *p*-nitrophenol $g^{-1}h^{-1}$ in Spain and μmol 4-methylumbelliferone $g^{-1}h^{-1}$ in Italy.

traits (Du Preez et al., 2022). The enrichment index (EI) is an indicator metric of the presence of bacterivorous and fungivorous species that respond quickly to increased amounts of prey (Ferris et al., 2001). A high EI therefore also indicates high amounts of bacteria and fungi. The structure index (SI) is a measure of complexity, structure and interaction in the food-web (Ferris et al., 2001). Low values indicate a food-web with mostly fast-growing bacterivores and fungivores, while high values indicate a more complex community including predators, which are usually more sensitive to disturbance. Plant parasitic nematode species can have negative effects on plant growth and quality and their abundance can also be related to soil health. In this paper, we evaluate data ranges for total nematode abundance and generic richness, abundance of plant parasitic nematodes, as well as indices for community structure (Table 1).

Microarthropods: Microarthropods, such as Collembola and Acari, make up a very diverse part of the mesofauna. They are an important reservoir of soil biodiversity and support decomposition and nutrient cycling processes (George et al., 2017), and thus are suitable bioindicators of soil health (Menta and Remelli, 2020; Tóth et al., 2025). Studies of microarthropods as bioindicators have been conducted in several large-scale soil assessment and ecosystem monitoring programs across Europe (Cluzeau et al., 2012; Keith et al., 2012; Menta et al., 2018; Rutgers et al., 2009). However, species identification of soil microarthropods requires highly specialized taxonomic skills. Composite metrics, such as the Soil Biological Quality-arthropod index (QBS-ar) (Parisi et al., 2005; D'Avino et al., 2023; Menta et al., 2018; Tóth et al., 2025) and the Acari:Collembola ratio (Bachelier, 1963), are based on classification of biological forms (BF) derived from morphological traits and soil adaptation characteristics (Sacchi and Testard, 1971), which does not require species identification. The QBS-ar index is based on the notion that the number of morphological groups is higher in healthy soils than in unhealthy soils (D'Avino et al., 2024; Menta et al., 2018). In the present work, we consider five metrics based on BF classification, including Acari:Collembola ratio and QBS-ar (Table 1).

Earthworms: Earthworms belong to the macrofauna (>2 mm in diameter) and represent the highest biomass of soil fauna in temperate climates. They are a keystone species group, that have a large effect on the soil ecosystem (Paine, 1995). Their foraging and casting activities contribute to organic matter fragmentation and burial, and the regulation of essential ecosystem services such as soil structure maintenance, water regulation, erosion mitigation and plant growth (Blouin et al., 2013; Fonte et al., 2023; van Groenigen et al., 2014). Earthworm abundance and functional group composition reflect soil conditions and management, making them a preferred indicator for soil biological health monitoring programs (Fusaro et al., 2018; Pérès et al., 2011). In this paper, we evaluate data ranges for earthworm abundance, biomass and species richness (Table 1).

2.2. European and national case studies

To allow generalizable comparison of national datasets, each dataset was categorized per land use using the CORINE Land Cover categories, and by soil texture using the World Reference Base (WRB) classification where possible (WRB, 2024). In some cases, national soil texture classifications (e.g., clay or clayic) were used, as it was not feasible to translate to the WRB system, as percentages of sand, clay, silt and loam were not available (Table 2). The consequences of using different soil texture classification systems on the evaluation of targets and thresholds were assessed using the micro-arthropod dataset as a case study. Furthermore, the consequences of using different sampling methods for the same indicator were evaluated using the French earthworm dataset, for which different sampling protocols had been used to collect earthworms. Distinction between management practices (e.g., tillage, fertilization) was not considered in this analysis, as the aim was to evaluate normal operating ranges for bioindicators per soil unit across management practices. The implications of this simplification are considered in the discussion. Regarding the selection of datasets, all the datasets that were available from the EU EJP SOIL project 'MINOTAUR' (EJP Soil, 2024) research team and that were not under an embargo were used. All datasets had to include associated environmental data (land use and texture).

Microorganisms: Two national datasets were assessed for soil microbes. In the Netherlands, 151 sites were surveyed for microbial biomass using PLFAs, as part of various projects in cropland, forest and grassland between 2014–2021 (Fig. 1a, Table 2). In Switzerland, as part of the Swiss Soil Monitoring Network (NABO), 30 sites were yearly assessed over 5 years for microbial communities, using amplicon sequencing, across arable land, grassland, and forest (Gschwend et al., 2021b) (Fig. 1b, Table 2).

Soil enzymes. The case study datasets presented correspond to two thorough sampling surveys across land uses of a region in Spain (Galicia) and Italy (Marche) (Fig. 1c-d, Table 2). The enzyme activity data presented come from a series of projects aimed at characterizing the soils of Galicia (Leirós et al., 2000; Miguéns et al., 2007; Paz-Ferreiro et al., 2009; Paz-Ferreiro et al., 2007; Trasar-Cepeda et al., 2008, 2000) and of Marche (Table 2), respectively. Several hydrolytic enzyme activities were assessed in each case study, 6 in Marche and 11 in Galicia, with 4

Table 2
Summary of the datasets per indicator showing the country, years and number of sampling locations across soil units. References to the original studies are provided (Supplementary material Table A.1). * Soil texture classification used: Rutgers et al. (Rutgers et al., 2008), otherwise WRB. For some studies no soil texture was available (na).

Indicator	Country	Years	Samples	Land-use	Soil texture	References
Microorganism	Netherlands*	2014, 2018, 2020, 2021	82	Annual crops	sand, clay	Asperen et al., 2023; Bloem et al., 2022, 2024; de Vries et al., 2006; Deru et al., 2023; De Haan et al., 2021; Kurm et al., 2023; Westerink et al., 2024, Bloem et al. unpublished data
		2018, 2021	23	Forest	sand, clay	
		2005, 2020, 2021	46	Grassland	sand, clay	
	Switzerland	2012, 2013, 2014, 2015, 2016	50	Annual crops	clayic, loamic, siltic	Gschwend et al., 2021b
		2012, 2013, 2014, 2015, 2016	49	Grassland	loamic	
		2012, 2013, 2014, 2015, 2016	35	Forest	loamic, siltic	
Soil Enzymes	Spain	1993–1995, 2001–2004	118	Forests	loamic	Leirós et al., 2000; Trasar-Cepeda et al., 2000, 2008
		2001–2004	59	Grasslands	loamic	Paz-Ferreiro et al., 2009; Paz-Ferreiro et al., 2007; Trasar-Cepeda et al., 2008
		2001–2004	60	Annual crops	arenic, loamic	Miguéns et al., 2007; Trasar-Cepeda et al., 2008
	Italy	2020–2022	573	Annual crops	clayic, loamic	EJP Soil, 2024, Bragato et al. unpublished
Nematodes	Netherlands*	2007, 2009, 2011, 2019, 2021, 2022, 2023	1020	Annual crops	sand, clay	De Haan et al., 2021; Korthals et al., 2014; Nijhuis et al., 2024; Spijker et al., 2022, 2023
	Sweden*	2014, 2017, 2018, 2022	136	Annual crops	sand, clay	Viketoft et al., 2021 and unpublished data
Micro-arthropods	Italy	2001, 2003, 2005, 2006, 2012, 2018, 2021, 2022	64	Annual crops	arenic, clayic, loamic, siltic	Arpa FVG, 2024; D'Avino, 2005; Excalibur, 2022; Landi et al., 2018; Soil4Life, 2022, ERSAF unpublished
		2005, 2006, 2021	21	Permanent crops	clayic, loamic, siltic	Arpa FVG, 2024; Excalibur, 2022, ERSAF unpublished
		2005, 2006, 2019, 2022	24	Grassland	arenic, clayic, loamic, siltic	Arpa FVG, 2024; Soil4Life, 2022, ERSAF unpublished
Earthworms	Austria	1993, 1999, 2002, 2010, 2014, 2016, 2017, 2022	497	Annual crops	siltic, loamic	Burkhard et al., 2012; EJP Soil, 2024; Euteneuer et al., 2019; Lüscher et al., 2016; Phillips et al., 2021; Spiegel et al., 2018
		1977, 1991, 1992, 1996, 1998–2004, 2008–2010, 2012–2014, 2016, 2018	459	Grassland	na	Burkhardt et al., 2014; Jernej et al., 2019; Kerschbaumer et al., 2024; Lüscher et al., 2016; Seeber et al., 2022
		2015, 2019	107	Permanent crops	siltic, loamic	SECBIVIT, 2019; VineDivers, 2022
	Bulgaria	2010	119	Grassland	na	Lüscher et al., 2016
	Finland	1999, 2004–2005, 2011–2012, 2019–2023	210	Annual crops	arenic, siltic, loamic, clayic	Lavelle et al., 2021; MULTA, 2022; Phillips et al., 2021
		2004–2005; 2019–2023	45	Grassland	arenic, siltic, loamic, clayic	Lavelle et al., 2021; MULTA, 2022; Phillips et al., 2021
	France	1998, 2000, 2003, 2006–2007, 2009–2011, 2013–2014, 2017, 2018, 2019–2022, 2023, 2024	1953	Annual crops	arenic, siltic, loamic, clayic, na	Brami et al., 2021; Cluzeau et al., 2012; EcoBioSoil, 2024; EJP Soil, 2024; Hoeffner et al., 2021; Lavelle et al., 2021; Lüscher et al., 2016; Pérès et al., 2011; Phillips et al., 2021
		2000, 2006–2007, 2010–2012, 2017, 2018, 2019, 2021–2022, 2023, 2024	854	Grassland	arenic, siltic, loamic, clayic, na	Brami et al., 2021; Burkhardt et al., 2014; Cluzeau et al., 2012; EcoBioSoil, 2024; Hoeffner et al., 2024, 2021; Lavelle et al., 2021; Lüscher et al., 2016; Phillips et al., 2021
		1990–2002, 2004–2005, 2007, 2010–2011, 2013–2014, 2016, 2019, 2022, 2023, 2024	971	Permanent crops	arenic, siltic, loamic, clayic, na	EcoBioSoil, 2024; Lüscher et al., 2016; Phillips et al., 2021; SECBIVIT, 2019
		2010–2016, 2021–2023	210	Forest	arenic, siltic, loamic, clayic, na	EcoBioSoil, 2024
	Germany	1952–1953, 1980, −1982, 1985, 1987–2017	848	Annual crops	arenic, siltic, loamic, clayic, na	Burkhardt et al., 2014; Lüscher et al., 2016; Phillips et al., 2021
		1948, 1950–1954, 1967, 1971–1973, 1977–1982, 1984–2017, 2021	975	Grassland	arenic, siltic, loamic, clayic, na	Burkhardt et al., 2014; Herwig et al., 2023; Lüscher et al., 2016; Phillips et al., 2021
		1979, 1980, 2010–2014, 2019	186	Permanent crops	arenic, siltic, loamic, clayic, na	Burkhardt et al., 2014; Herwig et al., 2023; SECBIVIT, 2019
	Hungary	1993, 2010	73	Annual crops	loamic, na	Lavelle et al., 2021; Lüscher et al., 2016; Phillips et al., 2021
		2010	127	Grassland	na	Lavelle et al., 2021; Lüscher et al., 2016)
	Ireland	1994–1997, 1999, 2004, 2006–2011	202	Annual crops	siltic, loamic, na	Brown and Keith, 2022; Lavelle et al., 2021; Phillips et al., 2021
		1972, 1976–1978, 1983, 1994, 1999–2001, 2003–2004, 2006–2009, 2011, 2012	139	Grassland	siltic, loamic, na	Brown and Keith, 2022; Lavelle et al., 2021; Phillips et al., 2021
	Italy	2010, 2011, 2014, 2016, 2018–2019, 2022	163	Annual crops	loamic, na	EJP Soil, 2024; ERSAF, n.d.; Lavelle et al., 2021; Lüscher et al., 2016; Seeber et al., 2022
		2010, 2011, 2014, 2016, 2018–2020	413	Grassland	na	ERSAF, n.d.; Lüscher et al., 2016; Seeber et al., 2022

(*continued on next page*)

Table 2 (*continued*)

Indicator	Country	Years	Samples	Land-use	Soil texture	References
		2010, 2011, 2017–2020	209	Permanent crops	na	Lüscher et al., 2016; Seeber et al., 2022
	Netherlands	2010, 2017, 2019, 2022	89	Annual crops	arenic, loamic, na	(Abalos et al., 2021; EJP Soil, 2024; Lüscher et al., 2016; Velilla et al., 2021)
		2001, 2004–2006, 2009–2015, 2017	724	Grassland	arenic, loamic, clayic, na	Abalos et al., 2021; Deru et al., 2023; Lüscher et al., 2016; Morriën et al., 2017; Onrust et al., 2019; Phillips et al., 2021; Verhoeven et al., 2022
	Norway	2010	55	Grassland	na	Lüscher et al., 2016
	Portugal	1984–1985	35	Annual crops	arenic, loamic, na	Lavelle et al., 2021; Phillips et al., 2021
		1984–1985	30	Grassland	arenic, loamic, na	Lavelle et al., 2021; Phillips et al., 2021
	Romania	2015, 2019	97	Permanent crops	na	SECBIVIT, 2019; VineDivers, 2022
	Slovenia	2012–2014, 2022	31	Annual crops	clayic, loamic	EcoFINDERS, 2014; EJP Soil, 2024
		1992, 1996–1997, 2005–2009, 2013	35	Grassland	siltic, loamic, na	Phillips et al., 2021
		2012	22	Permanent crops	siltic, loamic	
	Spain	2004, 2007, 2010	64	Annual crops	loamic, clayic, na	Lavelle et al., 2021; Lüscher et al., 2016; Phillips et al., 2021
		2002–2003, 2010	174	Grassland	loamic, na	Lavelle et al., 2021; Lüscher et al., 2016; Phillips et al., 2021
		2009, 2010	62	Permanent crops	loamic, na	Lüscher et al., 2016; Phillips et al., 2021
	Sweden	1997, 2014, 2017, 2022	101	Annual crops	clayic, siltic, na	EJP Soil, 2024; Phillips et al., 2021; Torppa and Taylor, 2022; Viketoft et al., 2021
	Switzerland	1994, 2011, 2022	23	Annual crops	loamic, na	EJP Soil, 2024; Phillips et al., 2021
		1996, 2010, 2011	333	Grassland	loamic, na	Lavelle et al., 2021; Lüscher et al., 2016; Phillips et al., 2021; Zaller and Arnone III, 1999
		2010	18	Permanent crops	na	Lüscher et al., 2016

enzymes overlapping between the datasets (acid phosphatase, arylsulphatase, phosphodiesterase and ß-glucosidase). Therefore, we focused on these four enzymes involved in the phosphorus (acid phosphomonoesterase, phosphodiesterase), sulphur (arylsulphatase) and carbon (ß-glucosidase) cycles, as they are considered indicative of the metabolic capacity of the soil and, thus allow the assessment of the transformation potential of specific energy or nutrient sources (Burns et al., 2013).

Nematodes. Two datasets were used, one from the Netherlands (Haan et al., 2021; Korthals et al., 2014; Nijhuis et al., 2024; Spijker et al., 2023, 2022) and one from Sweden (Viketoft, unpublished). The Dutch data were collected between 2007 and 2023 in two long-term experiments (LTEs) on arable land and in 16 farms across the country (Fig. 1e, Table 2). The Swedish data originated from three LTEs and 66 farm fields between 2014 and 2022 (Fig. 1f, Table 2). LTEs featured a range of soil management practices (such as tillage, crop rotation, fertilization) that are known to affect soil nematodes (Korthals et al., 2014; Nijhuis et al., 2024); however, data were pooled irrespective of these practices, as the focus here was to assess the range of indicator values per soil unit only. Notably, differences in extraction methods existed between the two countries, and numbers were expressed per unit of fresh soil in the Netherlands whilst based on dry soil in Sweden. The implications of such protocolary differences in are considered in the discussion section.

Microarthropods. The microarthropod dataset comprises microarthropods sampled from 109 sites (327 replicates) across Italy between 2001 and 2020 (Fig. 1g, Table 2). In addition to abundances of soil microarthropods and the Acari:Collembola metric, biological forms (BFs) were recorded to calculate the richness, the relative presence of euedaphic BFs, and QBS-ar index (Table 1). The microarthropod dataset was used to evaluate how the use of different soil texture reference systems impacts the evaluation of indicators ranges. To do this, we compared the distribution of microarthropods data from Italy using different texture criteria conform the WRB (Schad, 2023; WRB, 2024), Hypres (Carriero et al., 2007), USDA (as reported in Corral-Pazos-de-Provens et al., 2022) and IUSS (IUSS, n.d.).

Earthworms. Two datasets were investigated, a combined European scale dataset from the MINOTAUR project (EJP Soil, 2024, Fig. 1i), and a national dataset for France (EcoBioSoil, University Rennes, UMR EcoBio, 2024, Fig. 1h) (Table 2). The European data were compiled from the literature, from LTEs, and from several projects in 16 European countries. This large dataset allowed to assess the variability of the evaluation criteria across Europe considering the heterogeneity of climate and soil contexts (Table 2). The French national dataset contains data collected across France by the University of Rennes since 1980 (Fig. 1h). A substantial sub-dataset (3472 data) allowed to assess how different sampling protocols (chemical extraction followed by hand sorting or only hand sorting (ISO 23611–1: 2011) may affect the indicator data and thus the subsequently derived assessment criteria.

2.3. Quantification of indicator value ranges

Distribution-based thresholds were calculated as the lower quantile (lowest 12.5 %) and targets as the upper quantile (above 87.5 %) for the distribution in comparable soil units (Fig. A.1). While, setting critical percentiles is a policy decision, rather than a scientific matter, here, the critical percentiles were chosen to arbitrarily exclude 25 % of extreme values (Drexler et al., 2022; Matson et al., 2024). Comparable soils units were defined as distributions stratified by soil texture and land use (annual crops, perennial crops, grassland > 5 yrs old, or forest). A threshold for sample size of a minimum of five replicates per land use and soil texture combinations was applied. Data ranges (lowest and upper quantiles, and median) for each indicator are presented in the Supplementary material Table A.1.

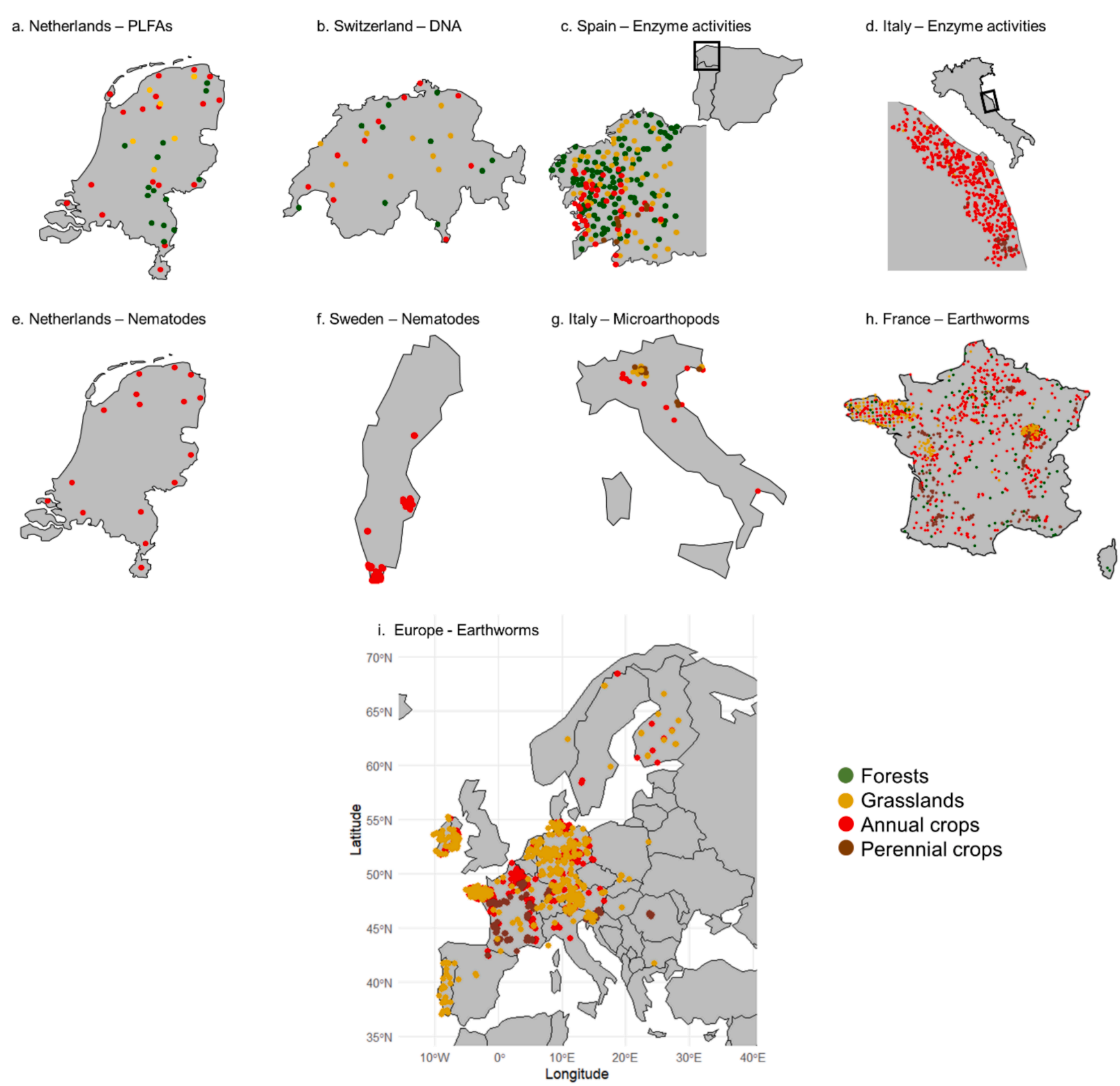

Fig. 1. Data origin locations and land uses per case-study and country. For details about each case study see Table 2.

3. Results & discussion

3.1. Current state and potential of bioindicators for setting evaluation criteria

3.1.1. Microbial biomass and relative abundance

Data coverage: The current datasets suffer from low replication, with most metrics having less than 50 replications, and no data for specific soil units (Fig. 2). For implementation in national or EU soil health assessment, additional sampling will be necessary.

Protocols comparability: The data currently used for assessing microorganism are not directly comparable between countries as Switzerland used metabarcoding-based metrics while the Netherlands used PLFAs-based metrics (Fig. 2). DNA and PLFAs based metrics might be used complementarily, as they provide information on microbial community richness and biomass respectively and therefore fulfil different monitoring scopes (biodiversity *versus* quantitative survey). Furthermore, different laboratory protocols for assessing DNA metabarcoding and PLFA analysis likely affect data quantification and value ranges within metrics, further limiting comparison among studies. For PLFAs analysis, while following the ISO protocol (ISO/TS 29843-2 (25/08/2021)), different results can still be obtained from non-standardised interpretation of the gas chromatography (manual *versus* machine automated), number of markers used, and age of the chromatography column, which can ultimately affect assessment criteria ranges (Fig. A.2).

Metrics: Our results suggest that the ranges for some of the functional groups evaluated, such as AMF biomass (PLFAs) and the relative abundance of plant pathogenic fungi (DNA metabarcoding), differ across land use types (Fig. 2). The relevance of these functional groups for crop production (Rillig et al., 2019) potentially makes them good

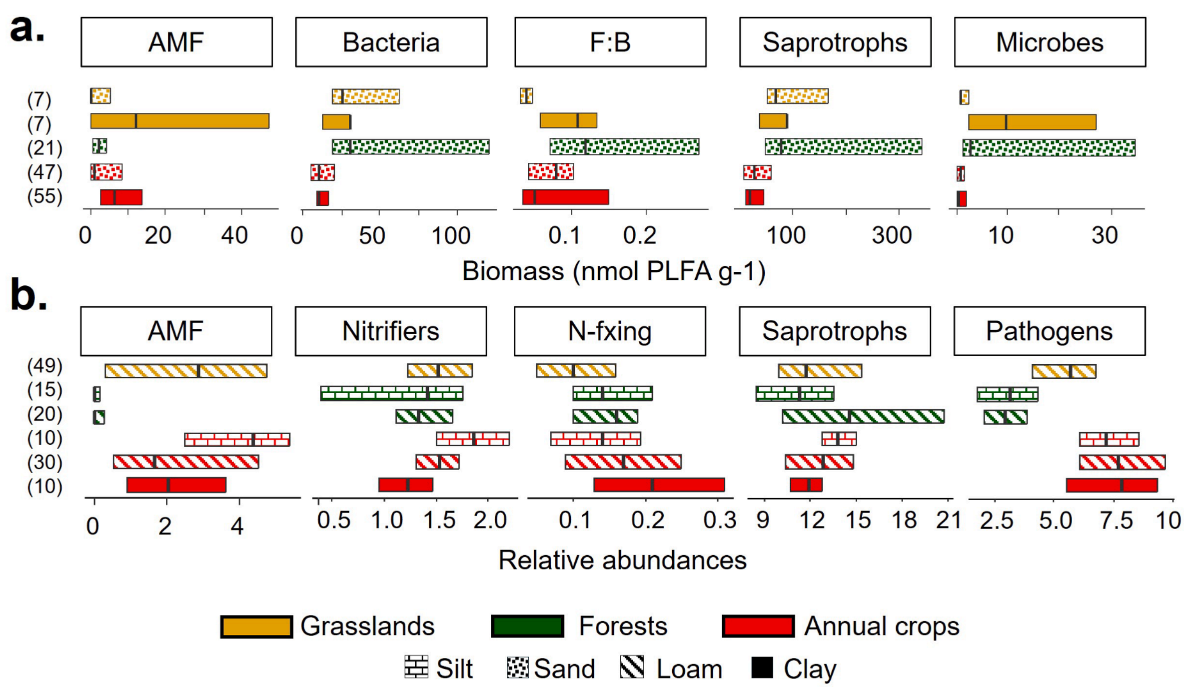

Fig. 2. Lower and upper 12.5 % quantiles and median per land use, and soil texture for: **(a)** Microbial biomass (nmol PLFA g^{-1}) (PLFAs, The Netherlands), and **(b)** Microbial relative abundance (DNA metabarcoding Switzerland) metrics. Note that for (a) local Dutch soil texture references were used, while for (b) the WRB classification was used (Clay = Clayic, Loam = Loamic, Silt = Siltic) (Table 2). Sample sizes are shown in brackets (Supplementary material Table A.1). Abbreviations: F:B, Fungi:bacteria ratio; Pathogens, Fungi plant pathogens. Note that for Pathogens the lower 12.5% quantile represents the suggested target, while the upper 12.5% quantile represents the suggested threshold.

metrics for monitoring soil health in arable lands. Comparing and linking DNA and PLFAs bioindicator metrics to soil functions is a priority. For example, assessing how relative abundances of functionally relevant microbial groups link to actual functions will be necessary to evaluate the sensitivity of DNA metrics to soil management before implementing these on a large-scale (Runnel et al., 2024).

3.1.2. Soil enzymes

Data coverage: Extensive data exists for some specific soil units, such as for annual crops on loamic soils in Italy where 400 data were collected (Fig. 3). However, as the case studies come from specific regions of Spain and Italy (Fig. 1), the derivation to national-scale evaluation criteria from these data is questionable.

Protocols comparability: Between the two datasets different laboratory protocols have been used, particularly with respect to the use of substrates (Table 1), rendering different measurement units and challenging data comparison. Additionally, differences in the collection, storage and pretreatment of soil samples, as well as a lack of standardization in protocols for determination of enzyme activities (e.g., regarding temperature, pH, substrate concentration, incubation time) hinder a comparison of data.

Metrics: Despite the potential of enzymes as bioindicators of soil health, there is no agreed set of enzyme activities for soil health assessment. This is because enzymes reflect specific substrate reactions that do not necessarily indicate broader soil functions, which depend on numerous reactions and properties. Simple indices, such as the ratio of

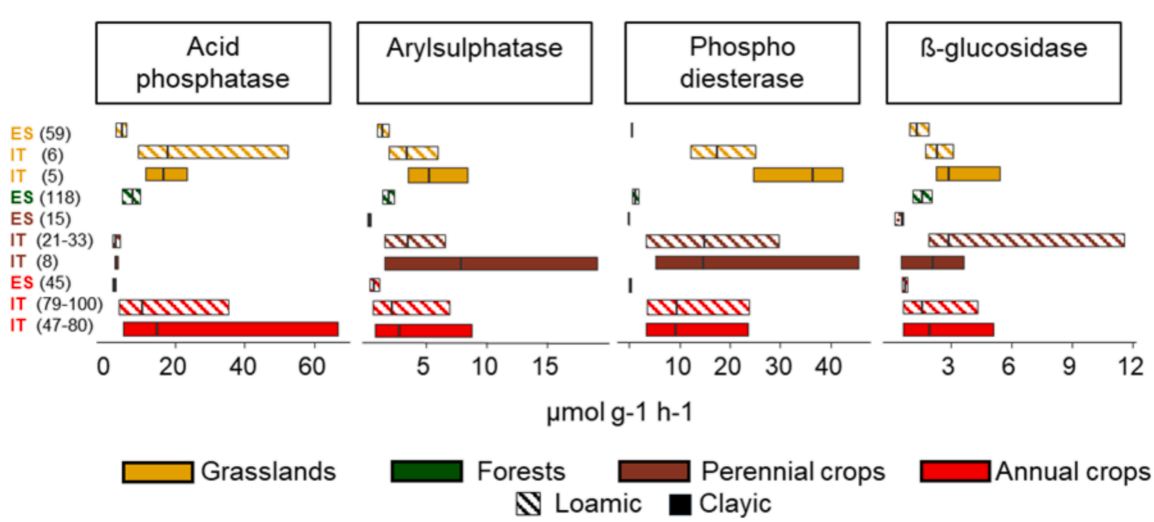

Fig. 3. Lower and upper 12.5 % quantiles and median for metrics of enzyme activities (μmol $g^{-1}h^{-1}$, SP, Spain; IT, Italy) by land use and soil texture. Sample sizes shown in brackets (Supplementary material Table A.1).

enzyme activity to total soil carbon and nitrogen content or microbial biomass, have been proposed, but exhibit similar limitations to individual enzymes, such as lack of inter-laboratory consistency (Bastida et al., 2008; Gil-Sotres et al., 2005), and lack of criteria linking the index variations to soil functions (Gil-Sotres et al., 2005). The use of complex indices combining different enzymes with soil properties might better reflect the complexity of the soil system and, therefore, be more adequate to evaluate soil health (Gil-Sotres et al., 2005; Trasar-Cepeda et al., 1997). Therefore, efforts should focus on developing composite indices that better reflect soil complexity, testing them in different regions and conditions to assess their validity, and standardising methods for microbial enzyme activity assays (Nannipieri et al., 2018).

3.1.3. Nematodes

Data coverage: The data are heavily biased towards experimental sites rather than real-life farms (Table 2), and, for the Netherlands, towards sandy soils (Fig. 4). As a result, a wide range of soil textures and land uses are not represented in the data. This is not surprising, as the application of soil nematode indicators has mostly focused on the assessment of crop health. Moreover, the data stems from experimental sites in which farm management treatments were applied, such as tillage and fertilization, known to affect nematode metrics (Puissant et al., 2021). While target or threshold values cannot be defined for many soil units due to limited data, they could be further assessed for specific soil units, such as arable sandy soils in the Netherlands, by, for example comparing normal operating ranges with those from the BISQ project (Rutgers et al., 2008). Also, for plant parasitic nematodes, damage thresholds have been identified for some species and some crops (Ravichandra, 2014) and values ranges could be further compared.

Protocols comparability: The observed effects of soil texture on nematode metrics are not consistent between countries, particularly not for nematode abundance (Fig. 4). These effects are partially due to differences in sampling and extraction protocols (abundances reported per 100 g fresh soil in the Dutch dataset *versus* 100 g dry soil for Swedish data). Different nematode extraction protocols likely have different extraction efficiencies (McSorley and Frederick, 2004; Whitehead and Hemming, 1965), while the soil moisture content of the sample determines how large the difference is between the units, with a smaller difference in dried soils.

Metrics: Nematode diversity and EI and SI indices are independent of the unit of abundance and therefore would be possible to use irrespective of data being based on fresh or dry soil, but these might be less responsive than abundances to soil health (Fig. 4, Du Preez et al., 2022). Furthermore, it will be challenging to generate broad assessment criteria for plant parasitic nematodes across soil units as the effect of the number of plant parasitic nematodes on crop yield is highly dependent on crop species and cultivar. Therefore, priority for nematodes criteria development should be on a broader functional group metrics.

3.1.4. Micro-arthropods

Data coverage: The reported dataset is mostly restricted to northern Italy and suffers from low replication (< 50 samples per land use and soil combinations) and unbalanced replication per soil unit, making it unfeasible to apply the value ranges for assessing soil health at national or European scales (Fig. 5).

Metrics: Despite low background data coverage, the present results show that BF-based indices such as BF richness, euedaphic forms:richness ratio, and QBS-ar, are sensitive to the intensity of land use, with grassland and perennial cropping rendering higher values than annual cropping (Fig. 5). Community index values tend to have higher value distribution ranges in soils with finer textures (Fig. A.6). While the QBS-ar was designed to be sensitive to land-use changes and soil degradation (Fiorini et al., 2020; Menta et al., 2017, 2020) its use is currently limited to a few countries (Menta et al., 2018; Galli et al., 2021; Kurniawan et al., 2023; Lakshmi et al., 2017). On the other hand, metrics such as abundance and Acari:Collembola ratio are generally more variable, due to aggregated distribution patterns of soil fauna (Gutierrez-Lopez et al 2010; Briones 2014). The present data suggest that the use of indices based on ecomorphological traits can be effective in the evaluation of soil health. Use of morphological trait indices for microarthropods could be performed with basic entomological knowledge, and, in the future, artificial intelligence tools may further streamline this process. Such tools could facilitate harmonization of dataset creation and promote consistency in data collection.

Protocol standardization: The effects of sampling method and sampling season have not been studied here. However, it is well-known that aberrations from a standard can affect the resulting data and therefore subsequently also affect the development of evaluation criteria. Micro-arthropods sampling and extraction has been standardized by ISO 23611–2:2024, but this standard is not always followed and aberrations in sampling technique, sample replication, sample size and depth, and extraction methods can be observed in the literature.

Combining datasets in order to generate generically valid criteria come with its own challenges, one primary is combining abundance data with presence/absence data. Additionally, inconsistencies arise when the abundance of each BF form is recorded in some cases, while in other datasets only the BF with the highest eco-morphological index within each group is documented. Indeed, this is the minimum requirement in the QBS-ar protocol. Consequently, it is imperative to reduce the variability due to each of these aspects, harmonizing protocols and data collection procedures. A first step has been accomplished with a template for calculation of QBS-ar and related metrics (D'Avino et al., 2024). Also, QBS-ar will be enlisted as a standard operating procedure by the Global Soil Laboratory Network (FAO, 2025).

3.1.5. Earthworms

Data coverage: The European earthworm dataset, which covers 16

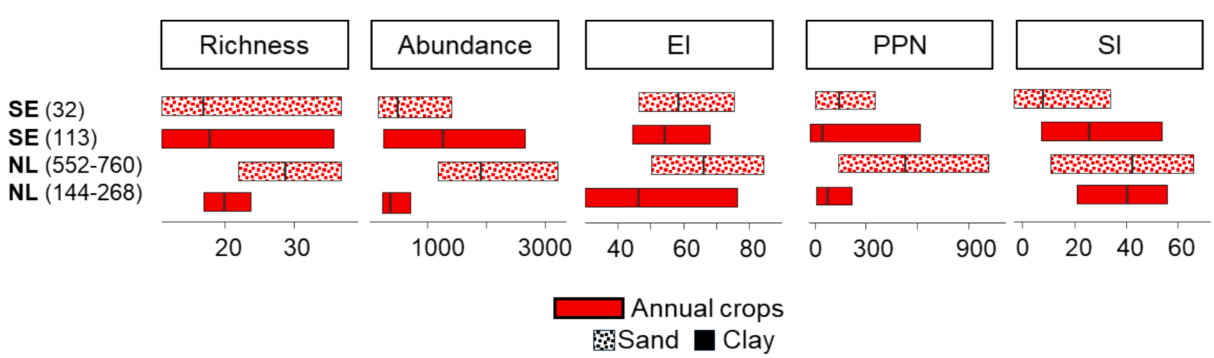

Fig. 4. Lower and upper 12.5 % quantiles and median for nematode metrics (per 100 g of fresh (NL) or dry (SE) soil) and soil texture (per country, SE – Sweden, NL – Netherlands). Abbreviations: EI, enrichment index; PPN, plant-pathogenic nematodes; SI, structure index. Note that national Dutch soil texture references were used to define soil classes for Sweden and Netherlands datasets (Table 2). Sample sizes shown in brackets (Supplementary material Table A.1). Note that for PPN (Plant parasitic nematodes) the lower 12.5 % quantile represents the suggested target, while the upper 12.5 % quantile represents the suggested threshold.

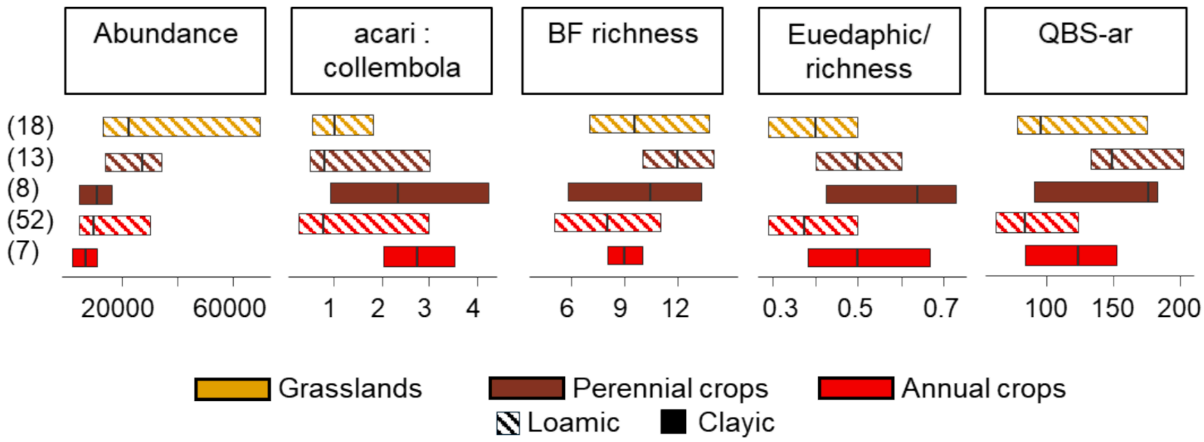

Fig. 5. Lower and upper 12.5% quantiles and median across land use, and soil texture for microarthropods metrics (per m^2) in Italy. Abbreviations (see text for explanations): BF, biological forms; QBS-ar, Soil Biological Quality-arthropod index. Note that WRB soil texture references were used (Table 2). Sample sizes shown in brackets (Supplementary material Table A.1).

European countries, shows a huge variability between countries in assessment criteria ranges for earthworm abundance, biomass and species richness (Fig. 6). We also find high heterogeneity in the value estimated of the lower 12.5 % threshold (Fig. A.4): these values ranged from less than 5 individual/m^2 to 50 individual/m^2. These differences between countries are likely due to differences in climatic zones (Nuutinen et al., 2025). Surprisingly, for countries such as Italy, threshold values are higher in annual crops than in grasslands, which is contradictory to the general notion that grasslands are better habitat for earthworms (Cluzeau et al., 2012). This is likely due to (i) management

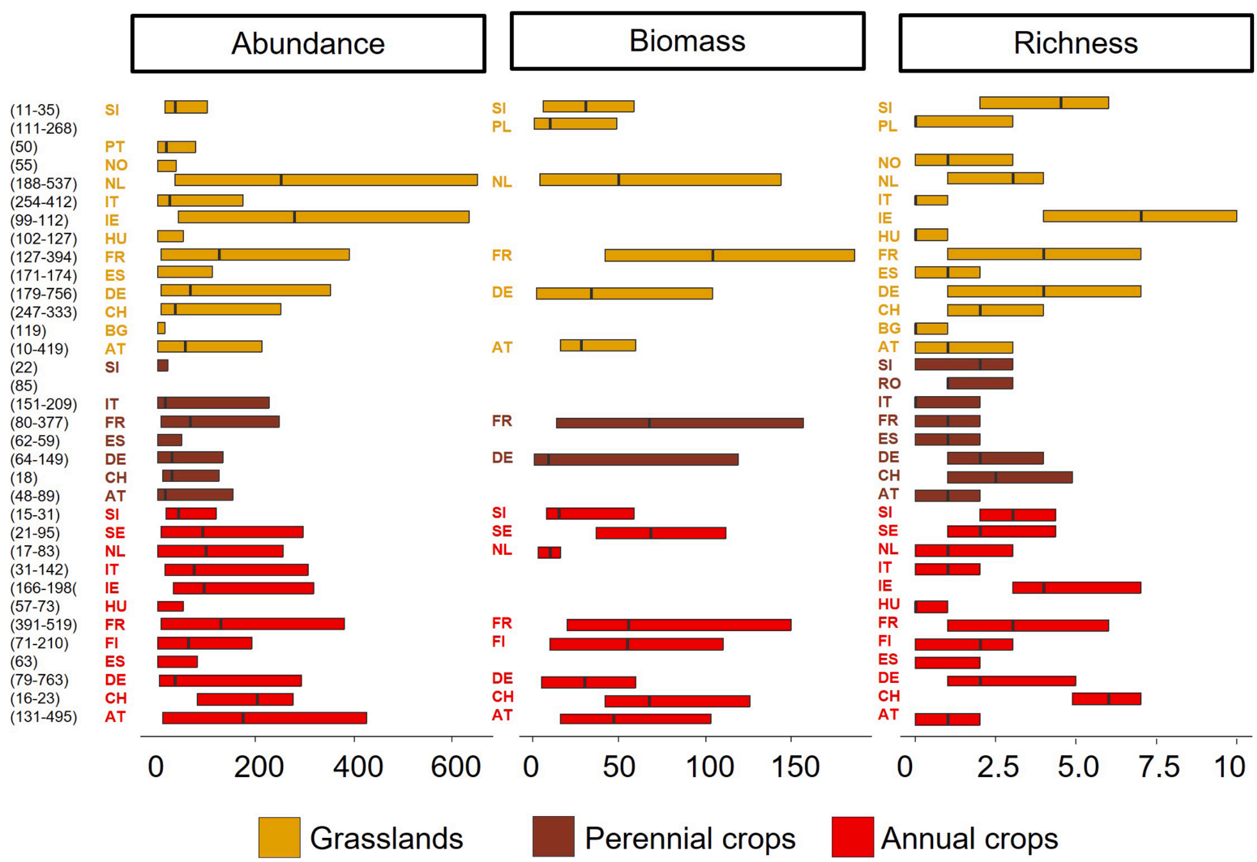

Fig. 6. Lower and upper 12.5% quantiles and median for earthworm metrics (abundance, biomass per m^2 and species richness) per land use, and country. Abbreviations: SI – Slovenia, SE – Sweden, PL – Poland, PT – Portugal, NO – Norway, NL – Netherlands, IT – Italy, IE – Ireland, HU – Hungary, FI – Finland, FR – France, ES – Spain, DE – Germany, CH – Switzerland, BG – Bulgaria, AT – Austria, RO – Romania. Multiple land uses have been sampled at the same sites. Sample sizes are shown in brackets (Supplementary material Table A.1).

practices, such as reduced tillage and organic fertilization, in annual crops, which support earthworm communities, whereas intensive grazing in grasslands reduces their populations (e.g., due to trampling), and (ii) soil characteristics, including texture and depth, which may be lower in grasslands, particularly in mountainous regions. Despite limitations for cross-national comparisons, range values could be derived for several countries where sufficient data is available (Table 2), as for example for France (Fig. 7) and for Germany (Fig. A.3). Environmental thresholds and optimal earthworm densities for different land uses have recently been modelled for Germany using soil and climate data (Salako et al., 2024). While there are some hotspots of earthworm data in Central and Western Europe (Fig. 1), data coverage could be improved in Southern, Eastern, and Northern Europe (Figs. 1 and 6). Moreover, forest and natural areas need to be assessed in more detail. The next step in countries where many data is available, i.e., France (Fig. 7) is to relate data to climate, site elevation, land use and other aspects of soil unit classification to contextualise evaluation criteria for better site-specific assessment.

Protocols comparability: Despite hand-sorting being the most used earthworm sampling method (Cousin et al., 2025), another source of variation between and within country is the use of different sampling protocols. Using different sampling protocols can modify the results for criteria assessment as shown by analyses of the national French dataset (Fig. A.5). Relying solely on hand-sorting, rather than using chemical extraction solutions and hand-sorting, underestimated the lower and upper ranges for earthworm richness (Fig. A.5). Earthworm chemical extraction, using for example mustard solution or AITC, is essential for capturing larger, deep-burrowing anecic earthworms, which are underestimated by hand-sorting alone (Cousin et al., 2025).

Metrics: Earthworm abundance and richness are the most frequently reported metrics in Europe, whereas biomass is less recorded (Fig. 6). However, biomass has been found to be a more robust and less variable metric (Cousin et al., 2025), due to lower risk of collector bias. In addition, earthworm biomass can be used to estimate soil functions important for soil health, such as bioturbation and water infiltration (Bouché and Al-Addan, 1997; Torppa and Taylor, 2022). While abundance remains an easy-to-learn metric, it should be complemented by weighing individuals at community level.

3.2. *Considerations on assessment criteria for soil bioindicators*

One of the objectives of the EU New Soil Strategy is to halt soil degradation, including loss of soil biodiversity, and ensure full soil health recovery by 2050. Thus, soil health monitoring will need to provide integrated empirical evidence for progress towards achievement of this policy objective and evaluate effectiveness and impact of the required transition towards more sustainable soil management practices. To meet these ambitions, the EU needs adequate soil health data across spatial and temporal scales, as well as robust assessment criteria. While many EU projects are currently defining which indicators, metrics and functions should be evaluated in future soil health monitoring schemes (BIOservicES, 2024; EJP Soil, 2024; Faber et al., 2022; "Soil Health Benchmarks", 2022), our work has revealed some challenges, opportunities and workflow bottlenecks in the development of assessment criteria for such bioindicators (Table 3). Below we identify two major priorities for research and policy implementation for successful establishment of assessment criteria using a distribution approach (Matson et al., 2024) (Fig. 8).

3.2.1. *Harmonization of evaluation units*

Effective policy implementation of assessment criteria based on bioindicator data distributions requires harmonization of classification systems for soil units (soil texture and land use). Discrepancies in categorization systems can complicate data interpretation and hinder coordinated action. In this context, we explore key recommendations for unified use of soil texture and land use classification systems, as well as the integration of climate in the definition of soil units.

As illustrated by the microarthropod case study, the use of different systems for categorizing soil texture can affect the data range distributions for several metrics (Fig. A.6) and ultimately the determination of assessment criteria. To avoid such limitations, we recommend that the same systems be used in all EU Member States. While more detailed analyses of soil type including pH, organic matter and percentage of sand or clay may be helpful for a mechanistic understanding and predictive context, for ease of policy implementation we suggest using soil texture classes proposed by the World Reference Base for Soil Resources: arenic, siltic, clayic, loamic (WRB, 2022), in accordance with the soil technical guidelines defined in the infrastructure for spatial information in Europe (INSPIRE, 2025). Similarly, it is also important to harmonize land use classes across Europe. For example, the classification of land uses provided by the CORINE Land Cover and IPCC systems differ in their definition of grassland/pasture. The CORINE Land Cover system subdivides into pasture (<5 years) and semi-natural habitats, but this distinction is not made in the IPCC system. Using the CORINE system as

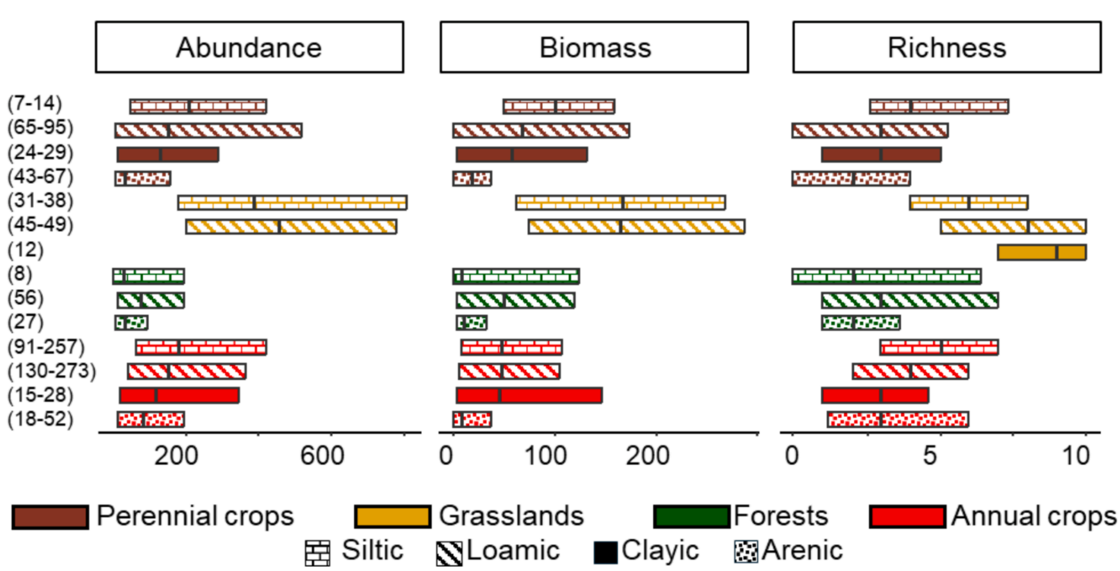

Fig. 7. Lower and upper 12.5% quantiles and median for earthworm metrics (abundance and biomass per m^2, and species richness) across different land uses and soil textures (WRB classification) in France. Earthworms were assessed by hand-sorting. Sample sizes shown in brackets (Supplementary material Table A.1).

Table 3
Summary of major challenges for development of criteria assessment for soil bioindicators in terms of data coverage, metrics and protocol standardization.

Indicator	Data coverage	Metric	Protocol standardization
Microorganisms	Limited and difficult to aggregate data between studies due to laboratory variability.	PLFAs (biomass) and DNA (biodiversity) metrics offer complementary approaches rather than comparable methods.AMF biomass and relative abundance of plant pathogens seem to be sensitive to land use and soil contexts.	DNA: Inter-laboratory variability needs to be assessed, and ISO protocols developed. PLFAs: ISO protocols need to be more detailed to reduce inter-laboratory variability (Fig. A.2)
Soil enzymes	Limited to regional scales; national scale data analysis is ongoing in France	Choice of metric depends on the functions of interest. No defined list of enzymes for assessment criteria.	Inter-laboratory comparison is problematic, particularly because of different extraction methods and measurement units. Standardisation needed, but development of ISO protocols is considered challenging. ISO 20130:2018 using colorimetric substrates is available, has been ring-tested (Cheviron et al. 2022), but is rarely used.
Nematodes	Biased towards arable land, and in some countries limited to plant parasitic nematodes.	Species level identification are knowledge intensive, but most indices operate also with genus or family level, and as such have been widely used in soil monitoring.	Inter-laboratory variability exists (i.e., different extraction methods and assessment for dry or wet soil).ISO protocol exists; consequent application needed.
Micro-arthropods	Regionally limited and biased toward arable land; integration of data across studies is possible by use of standardized protocols.	Use of metrics based on morphological traits rather than species might enhance wider implementation. BF-based metrics were more sensitive than quantitative indices (e.g., total abundance).	ISO protocols available only for sampling and some extraction methods; consequent application needed. Parisi et al 2005 reports QBS-ar protocol, the FAO-SOP is in publication.
Earthworms	Large dataset available in some countries (FR, DE) which could be used for assessment criteria evaluation.	Biomass should be more systematically assessed as it is a robust indicator	ISO protocols available. New ISO standards for chemical extraction and hand sorting are both proposed, but can lead to different assessment criteria.

a standard would not only facilitate application, as land-cover data can be easily extracted for Europe, but also allows separation between production land (e.g., pastures) and semi-natural habitats, which is relevant when setting assessment criteria for soil health. Ultimately, accounting for variability in land use management practices (e.g., intensive, extensive) will be essential to develop assessment criteria that can help evaluate the improvement of soil health resulting from sustainable soil management practices. The Soil Monitoring Law has identified a range of sustainable soil management practices (vegetation cover and diversity, soil disturbance, pesticide use, fertilization practices; Annex III, SML, 2023).

Furthermore, the earthworm case study across Europe, shows that there is high variability in assessment criteria values across latitudes. This reinforces the need to set climatic-region specific thresholds and targets in addition to land use, or land cover, and soil texture, as planned in the current Soil Monitoring Law. Integration of topography in the concept of homogeneous soil units, could also be considered as different soil characteristics occur at fine scales as a result of changes in topographic features such as site elevation (Seibert et al., 2007). These considerations are important for the establishment of robust assessment criteria for soil bioindicators, just as for soil physical and chemical indicators (Chen et al., 2023; Gobin et al., 2004). The EU Soil Monitoring Law may promote soil health evaluation across Europe by building on existing soil monitoring networks such as LUCAS and the ICP Forests Program, and on national soil inventories (e.g., RMQS in France), as well as by standardising definitions for soil units (Wellbrock et al., 2024).

3.2.2. Protocol standardization

The distribution of data values for biological indicators across EU is affected by protocols for sampling (earthworms), extraction (nematodes, micro-arthropods), and laboratory measurements (soil enzymes, DNA metabarcoding and PLFAs (Fig. A.2)). At present, this could lead to inconsistent assessment criteria in terms of reference ranges, targets or threshold values. This is not a flaw specific for bioindicators, however, as lack of international protocol harmonization is also observed in chemical and physical indicators, where different method standards may exist for the same parameter (Bispo et al., 2021). As different protocols are used for the same indicator metric in different countries and laboratories, there is probably no "best method". To ensure comparability among laboratories and countries, standardised protocols should be developed for sampling and extraction, or at least translation between protocols and units should be assessed. The EU Soil monitoring legislation requires the use of International Organization for Standardization (ISO) standards when available, or a transfer function for any alternative peer-reviewed method to be converted to the ISO protocols (Article 8, SML, 2023). Methodological challenges may still arise when (i) multiple ISO standard protocols exist for assessing the same indicator (e.g., earthworm sampling using chemical extraction, either directly on soil or after extracting a soil block [ISO 11268–3: 2014, ISO 23611–1:2011 or ISO 23611–1:2018]), (ii) an ISO standard permits different methods (e.g., nematode extraction via Oostenbrink elutriator or Baermann funnel [ISO 23611–4:2022]; earthworm sampling through chemical or physical extraction [ISO 23611–1:2018]), or (iii) non-ISO standard protocols are preferred (e.g., electric extraction for earthworms when soil block extraction is impractical, to minimize chemical and physical soil disturbance (Pelosi et al., 2021)). It may be challenging to standardise protocols across the EU, but adoption of standards by ISO, Glosolan or FAO-SOP for soil bioindicator should be prioritized where available. If unavailable, the priority should be to establish such protocols. If the use of common protocols is not feasible, national protocols should be standardised and peer-reviewed, and transfer functions should be developed to the respective ISO standards or between the different methods proposed by the ISO standards (e.g. nematodes, earthworms). In absence of standardized national protocol and pending the transfer function development, the applied protocols have to be specified. Since the development of transfer functions between differing protocols will

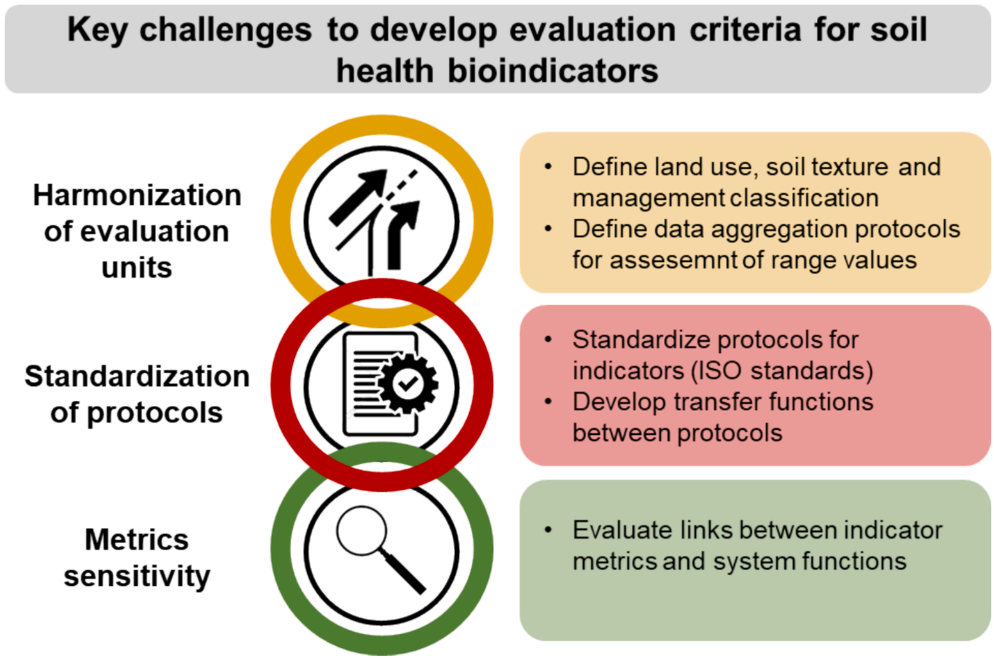

Fig. 8. Improvements in the application of bioindicators in soil health monitoring necessary for to better facilitate the development of robust evaluation criteria.

require significant resources, priority may be given to more widely implemented indicators (e.g. earthworms), or indicators that are likely to be developed as part of future national sampling schemes (e.g. DNA metabarcoding (Orgiazzi et al., 2018), or QBS-ar (Parisi et al. 2005)). Ultimately, ring-testing would be necessary to compensate and mitigate for inter-laboratory variability, as already done for some microbial methods (Cheviron et al., 2022; Petric et al., 2011). Since the assessment of soil biodiversity is currently not yet implemented in monitoring networks, with the proposed Soil Monitoring Law there is now a good chance to start with protocols harmonized across EU.

Once the data have been collected, the approaches used to aggregate the data for estimating the criteria can affect the distribution of the data and ultimately lead to different results, targets and thresholds (Davies and Gray, 2015; Ramage et al., 2013). Several of the investigated datasets did not come from national monitoring schemes, but rather from experiments, where the same sites were sampled over time or the same treatments were sampled in space (e.g. PLFA and nematode case studies). This means that for some datasets there are issues with pseudo-replications. For establishment of robust assessment criteria, we recommend handling pseudo-replication with appropriate modelling (e.g. via random effects) and avoiding spatial and temporal pseudo-replicates: i) spatially, by averaging over samples collected within the same site, field or over blocks receiving the same treatment within LTEs, ii) temporally, by averaging over a certain time period, or by focusing on a specific time point. To get a representative distribution of indicator values in a given context, it is important that the samples are independent from each other, i.e., that they are not pseudo-replicates, else the data distribution will be biased. However, in parallel, technical and biological factors inducing variability are important to understand, and to unravel from short-term oscillations caused by, e.g., different weather conditions, so as to improve the usefulness of indicators for soil health assessment (Lehmann et al., 2020).

3.2.3. Data coverage and metrics sensitivity

A main challenge to current setting of assessment criteria for soil health bioindicators in Europe is related to a lack of sufficient data. For many indicators, such as microbial biomass (PLFAs), nematodes, microarthropods, and soil enzymes, national datasets show data deficiencies, particularly limited coverage across different soil units. While this hampers an elaboration of fine-scale criteria for homogeneous soil unit and climate contexts, two alternative approaches for short-term progress may be to: i) accept a range of values at the national level without specifying context or soil units, or ii) to apply criteria from soil units from other countries. In both cases, criteria should be adapted to national conditions and improved by collecting more data. With the implementation of national monitoring schemes, this type of data would become available. For indicators feasible for use by laymen (e.g. farmers, field managers, advisors), national monitoring could be complemented by citizen science projects, even despite errors introduced by ignorance. Successful examples are the EcoBioSol earthworm dataset, which integrates citizen-obtained data in French earthworm monitoring (Andrade et al., 2021) and the Earthworm Watch in the UK (Ashwood et al., 2024).

Ultimately, the sensitivity of each metric to soil health and environmental context will need to be further evaluated to be able to design robust soil health assessment criteria. For example, molecular methods, i.e. environmental DNA, are quite likely to develop as part of future national sampling schemes due to their low sampling effort, high data throughput and broad taxonomic coverage (from microbes to eukaryotes) (Orgiazzi et al., 2018). However, these metrics still need to be linked to soil functions and health (Zhang et al., 2025). Our case studies highlight some future research directions regarding metrics selection for criteria assessment. Firstly, indices-based metrics, such as nematode community SI and EI, and microarthropod indices based on biological forms, calculate ratios or weighted scores across multiple species, and might be less affected by differences in sampling protocols or seasonality when compared to total abundance or unweighted species richness. For example, the relative proportions of functional groups (e.g. nematode maturity and channel index) or trophic groups (SI) may remain stable despite fluctuations in total number of individuals due to environmental changes. The use of indices might therefore facilitate data compilation across studies. Secondly, value ranges for species abundance and richness are likely to fluctuate more with short-term environmental changes than indices incorporating functional traits (Buckland et al., 2005; Yeates and Bongers, 1999). Greater metrics sensitivity to short-terms changes (e.g. weather) will require adapted sampling design, with for example increased replication compared to more robust metrics. Thirdly, metrics based on morphological traits (e.g. QBS-ar) rather than species-level identification will likely be more easily implemented and scaled-out as they require less expertise for identification. In addition, species richness or abundance metrics as such do not account for functional differences between species, whereas metrics that incorporate species morphological traits can provide deeper ecological insights that can then be linked to soil functions and degradation (Bonfanti et al., 2025; Wright et al., 2016). For example, we found that earthworm biomass is much less reported compared to abundance and richness metrics, despite being a more robust indicator and easily linked to soil functions important to soil health, such as bioturbation and water infiltration (Torppa and Taylor, 2022). Finally, species richness metrics are likely to be particularly impacted by sampling intensity, to avoid biases due to varying sampling efforts, accumulation curves per land use and soil texture need to be assessed and sampling protocols should be standardized.

4. Conclusions

Building on expert's knowledge and national-level datasets on soil bioindicators in several European countries, we investigated the feasibility to derive assessment criteria using a distribution-based approach. We find, using our datasets, that it is not yet feasible to set assessment criteria for most soil bioindicators. Exceptions are earthworm indicators in Central Europe, e.g., France and Germany (Salako et al., 2024), where national surveys cover many soil textures, types of land use, and pedoclimatic areas. We identify data gaps, lack of harmonization of concepts (particularly for soil classification), and lack of protocol standardization as major challenges for the development of assessment criteria for soil bioindicators at the national and EU level. The implementation of the EU Soil Monitoring Law should help overcome data deficiencies by stimulating national structured sampling efforts across soil unit and climate contexts, building on existing experience in regional monitoring programmes. Ultimately, the development of robust assessment criteria for soil health will need data from adequate sampling strategies.

CRediT authorship contribution statement

L.G.A. Riggi: Writing – review & editing, Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **G. Pérès:** Writing – review & editing, Writing – original draft, Resources, Methodology. **C. Aponte:** Writing – review & editing, Resources, Methodology, Conceptualization. **A. Bispo:** Writing – review & editing, Conceptualization. **G. Bragato:** Writing – review & editing, Resources, Methodology, Conceptualization. **D. Cluzeau:** Writing – review & editing, Resources. **L. D'Avino:** Writing – review & editing, Resources, Methodology, Conceptualization. **A. Edlinger:** Writing – review & editing, Resources, Methodology, Conceptualization. **N. Gronchi:** Writing – review & editing, Resources. **G. Guegan:** Writing – review & editing, Resources. **F. Gschwend:** Writing – review & editing, Resources. **G.W. Korthals:** Writing – review & editing, Resources, Methodology, Conceptualization. **V. Kurm:** Writing – review & editing, Resources, Methodology. **M. Mittmannsgruber:** Writing – review & editing, Resources, Methodology, Conceptualization. **S. Mocali:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **F. Romero:** Writing – review & editing, Resources, Methodology, Conceptualization. **E. Tondini:** Writing – review & editing, Resources, Methodology, Conceptualization. **C. Trasar-Cepeda:** Writing – review & editing, Resources, Methodology, Conceptualization. **M. Viketoft:** Writing – review & editing, Resources, Methodology, Conceptualization. **J.G. Zaller:** Writing – review & editing, Resources, Methodology, Conceptualization. **J.H. Faber:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2025.114063.

Data availability

The data is available in the Supplementary material table A1

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