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A flexible selection tool for the inclusion of soil biology methods in the assessment of soil multifunctionality

Marie J. Zwetsloot[a], Giulia Bongiorno[a], Janna M. Barel[b], D. Paolo di Lonardo[a], Rachel E. Creamer[a,*]

[a] Soil Biology Group, Wageningen University, Wageningen, the Netherlands
[b] Laboratoire Ecologie Fonctionnelle et Environnement, Université de Toulouse, CNRS, Toulouse, France

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ABSTRACT

Soil biota contribute to the delivery of multiple soil functions. However, soil biological methods are highly underrepresented in the assessment of soil functionality in agricultural production systems. Here we present a flexible tool to support decision-making during the selection process of soil biological methods for monitoring soil functions. This tool is based on a structured and conceptual framework that connects soil biota to soil functions through their contribution to different soil processes. The methods assessed by the tool were selected as a result of a thorough literature review. Soil biology experts supported the development of the tool (i) by providing feedback on the reviewed methods through a survey and (ii) by determining the relevance of different soil biota to the soil processes related to soil multifunctionality during a workshop. The tool is freely accessible online at the Biological Soil Information System (BIOSIS) platform, where researchers or users with an understanding of research practices can interact with the tool to define the context of their assessment and preferences for technical criteria of the methods. By incorporating user input, this flexible tool can help inform a wide variety of research and assessment programs interested in applying soil biological methods to monitor soil multifunctionality at different scales.

1. Introduction

Selecting appropriate methods to assess soil multifunctionality is the first step towards improved understanding, monitoring and managing of agricultural soils. Soil functions represent bundles of soil processes (Kibblewhite et al., 2008) that are driven by the interaction between chemical, physical and biological soil attributes (Vogel et al., 2018), agricultural management practices and climate (Tóth et al., 2013; Smith et al., 2016; Debeljak et al., 2019). In comparison to soil chemical and physical attributes, soil biological attributes are less frequently measured in soil quality assessment programs (van Leeuwen et al., 2017; Bünemann et al., 2018), even though they are very sensitive to soil management (Bastida et al., 2008) and involved in at least 26 soil processes crucial to soil multifunctionality (Creamer et al., 2022). Hence, measuring the soil biota and the processes that they support could enhance assessment of the capacity of soils to deliver multiple soil functions as well as facilitate the identification of potential trade-offs.

Several challenges arise when selecting soil biological methods for the assessment of soil multifunctionality. First, determining the pertinence of different biological methods to soil multifunctionality is complicated as most scientific studies are specialized, focusing only on a single biological taxon or only consider one process at a time (De Ruiter et al., 1993; Blouin et al., 2013; de Groot et al., 2016). When bundling up these processes to the function level, it becomes extremely complex to summarize the contribution of different biota to soil functions and multifunctionality. In fact, we are only beginning to disentangle the mechanisms and the magnitude by which different soil biota impact soil functioning in agricultural production systems (Lemanceau et al., 2015; Bender et al., 2016). As a result, simplified biological indicators such as measurement of microbial biomass have been put forward that cannot always be related to “the capacity of the soil to function” (Andrews et al., 2004).

Second, there are also various technical criteria to consider when selecting soil biological indicators. These can relate to (i) more practical matters such as ease of field sampling, overall costs or possibility to store samples (Doran and Parkin, 1997; O’Sullivan et al., 2017), (ii)

* Corresponding author.
E-mail address: rachel.creamer@wur.nl (R.E. Creamer).

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sensitivity of a method to management and spatio-temporal variation, and (iii) interpretation of data generated by the method (Bünemann et al., 2018). Third, monitoring agricultural land can serve different purposes and therefore the context of the assessment needs to be understood (Powlson, 2020) and this is likely to change the relative importance of different technical criteria. Soil quality assessment programs targeted towards farmers may prioritize methods that can be performed by local soil laboratories and have the ultimate aim to provide information that supports on-farm soil management decisions. On the other hand, soil monitoring at national or regional scale can help governments assess the societal consequences of different land management practices or environmental policies that may not be easily detected at the farm scale. National and regional monitoring programs easily visit over a thousand field sites during one sampling campaign and therefore need to limit the amount of time spent and the amount of sample collected at each site. In conclusion, soil biological method selection for the assessment of soil multifunctionality is a complex decision-making process, which requires flexibility to weigh certain selection criteria over others depending on the objective and type of assessment.

The "logical sieve" framework developed by Ritz et al. (2009) provides a useful tool to support the decision-making process during the selection of biological methods. The original tool scores soil biological methods based on a range of scientific and technical selection criteria in the context of a national-scale monitoring scheme in the UK. The final output is a list of methods with scores, where the highest-ranking methods are identified as the most promising candidates to be included in UK soil monitoring. Others have applied this approach to select biological indicators for European monitoring programs (Stone et al., 2016; van Leeuwen et al., 2017). In this paper, we present a flexible biological method selection tool, called Biological Soil Information System (BIOSIS) tool. This tool is adapted from the original framework by Ritz et al. (2009), which enhances its efficacy and use in several ways.

The BIOSIS tool assesses methods in relation to four soil functions that are relevant to a wide range of temperate agricultural production systems (Millennium Ecosystem Assessment, 2005; Haygarth and Ritz, 2009; Schulte et al., 2014; Vogel et al., 2018): Carbon and Climate Regulation, Water Regulation and Purification, Nutrient Cycling and Disease and Pest Regulation. To define the list of methods, we use the cognitive soil function models in Creamer et al. (2022), which are based on decades of soil biology research and the models by Debeljak et al. (2019) to put a structure on the complexity of biotic interactions contributing to soil multifunctionality. We also use the structure of the cognitive models to evaluate the pertinence of different methods to individual soil functions and soil multifunctionality.

In the BIOSIS tool, the fixed scores of the methods mostly rely on scientific publications and technical information that could be derived from existing protocols rather than expert-opinion as was done in Ritz et al. (2009). The built-in flexibility of the tool lets the users determine which functions are relevant to their objectives and which technical criteria are most important to them, influencing the method scores and final output of the tool. This is similar to the approach of the 'Soil Management Assessment Framework' (SMAF) originally developed by Andrews et al. (2004), who also advocated for user input to be included during method selection for soil quality assessments. This allows the tool to make case-specific recommendations for which methods are most appropriate considering the context and objectives of the assessment.

2. Methodology

We built a flexible soil biological method selection tool that is freely accessible at the Biological Soil Information System (BIOSIS) platform (https://biosisplatform.eu/) and is connected to the structure of the four cognitive soil function models developed by Creamer et al. (2022). These models describe the relationship between actors and soil processes that contribute to the delivery of each soil function. We use the term actors to refer to soil biota.

The BIOSIS tool can support decision-making during the selection process of soil biological methods for a wide variety of soil assessment programs focusing on different stakeholders such as farmers, land managers and policy makers. However, the tool itself is meant for users that are involved in research and/or have a comprehensive understanding of research practices because a certain level of expertise is required to specify the context of the soil assessment program and to interpret the final output list of the tool.

2.1. Reviewing methods

To develop the list of methods that would be assessed by the BIOSIS tool, we conducted a thorough literature review of methods associated with the actors and processes from the four cognitive soil function models described by Creamer et al. (2022). Therefore, methods that were not directly related to the actors and processes in the cognitive soil function models were not included in the list. Different types of methods for actors and processes were included when possible, such as methods based on traditional microscopy or cultivation-based methods, biochemical methods (biomarkers), activity methods (incubations, enzymes, bioassays) and molecular methods. We aimed at having at least one commonly applied method for each actor and process. We did not include methods that we considered too specialized for soil monitoring purposes such as stable isotope-based methods and metabolomics. Some methods relate both to an actor and a process (e.g. functional genes). Here we evaluated on a case by case basis whether the method would be classified as an actor- or process-based method (see Tables S1 and S2).

Following the literature review, we shared the list of actor- and process-based methods with soil biology experts for validation and adjustments, which is explained in more detail in the next section. The final list contains 191 actor methods and 98 process methods (289 methods in total), which will be open for review through the BIOSIS platform. For each method, we have included a reference to the original protocol or a reference with an example of how the method can be applied (Tables S1 and S2).

2.2. Expert contributions

During the development of the BIOSIS tool, we asked for the input of soil biology experts on two different matters. First, we used expert opinion in the scoring of the relevance of an actor in contributing to a soil function. Second, we asked experts to review and comment on the list of methods included in the soil biological method selection tool.

To achieve the first objective, we organized an online workshop with 40 soil biology experts from across the globe on February 2nd, 2021. Using Mentimeter (https://www.mentimeter.com/), we asked the experts to score the importance of actors to the delivery of the processes that they are involved in following the structure of the four cognitive soil function models (1 = very low importance, 5 = very high importance). Experts could skip questions if they felt the question was too far from their expertise. We repeated these questions for all the actor-process links in the cognitive soil function models except for the obvious cases where only one actor is listed to contribute to a process (e.g. mycorrhizal fungi to mycorrhizal acquisition). In this case, the actor received a very high relevance score. The mean relevance score for each actor-process link evaluated by the experts was calculated to be included in the pertinence tier of the BIOSIS tool.

For the second objective, we sent out a survey with all the actor-based methods to 74 experts that validated and adjusted the methods in December 2020. From this survey we received 49 responses. A similar survey with the process-based methods was sent out to the 40 participants of the workshop in February 2021, for which we received 12 responses.

2.3. BIOSIS: Biological method selection tool

We used the "logical sieve" framework developed by Ritz et al. (2009) as the backbone of the BIOSIS tool dividing the method selection process into three tiers: (i) pertinence to soil functions, (ii) applicability to ecosystem under consideration and (iii) technical properties (Fig. 1). Within each tier, methods are assessed according to multiple criteria and assigned a numerical score (see formulae 1-4). When a method receives a final score of zero, the method is discarded. The tool assesses methods for each function individually, resulting in a final output of a maximum of four lists (one list for each function) with recommended methods. Users can indicate which functions should be considered during the selection process.

These selection criteria and scoring methodology are described in detail in Table 1. The pertinence tier (T_P) consists of three selection criteria, which aim to collectively assess the importance of the method to each respective soil function. This is achieved by scoring the level of functional information yielded by each method (S_{info}), the frequency of the actor or process within one soil function model that the method is measuring (S_{freq}), and the relevance of the actor to the delivery of the processes in each function model ($S_{relevance}$). The functional information scores represent the degree by which an actor or process method provides information about the capacity of a soil to deliver an individual soil function. The frequency scores are determined by the four cognitive models developed by Creamer et al. (2022). For each function, number of times that an actor or process appears in the cognitive model is counted. The relevance scores are based on the outcomes from the expert workshop described in the previous section. To obtain the pertinence score, these three scores are multiplied and divided by three to maintain a balance between the pertinence and technical tier.

$$T_P = \frac{S_{info} \times S_{freq} \times S_{relevance}}{3} \quad (1)$$

The applicability tier (T_A) only consists of one criterium, which scores the applicability of the method to the ecosystem under consideration ($S_A = 0$ or 1). In this paper, we focus on arable production systems, but the online BIOSIS tool will be expanded to grasslands and forests as well. We removed the discrimination criterium applied in the original framework of the logical sieve because Ritz et al. (2009) reported that the level of discrimination between samples of different contexts was extremely difficult to confirm in practice. Especially for novel methods, we do not have a complete dataset representing a large range of soil types and land uses to systematically evaluate the method's discrimination potential and sensitivity to spatial and temporal variation. The applicability score is multiplied by the pertinence tier.

$$T_A = T_P \times S_A \quad (2)$$

The technical tier (T_T) scores 13 criteria (S_c) relating to the logistical aspects of the assessment or monitoring program. These include among others logistics related to field sampling, sample storage, lab analysis, sample archivability, data processing and interpretation, and regional infrastructure. We derived the information needed to score the technical criteria associated with each methods from scientific publications or protocols (Tables S1 and S2). These technical scores are normalized between 0 and 1. After this, the user can choose which technical criteria should be considered and what the associated weighting factors (W_{ci}) should be. The level of the weighting factor should reflect the importance of the criteria to the context of a given assessment program. A total of 50 weighting credits are distributed among the technical criteria according to their importance. Finally, an user-filter based on the technical criteria ($F_i = 0$ or 1) can be applied by the user that excludes methods that do not meet minimum logistical user's requirements, for example whether it is possible to return to the sampling site multiple times. These filters associated with the different technical criteria are multiplied. Weights and technical scores are multiplied and subsequently added, where S_{ci} is the score for technical criteria *i*, W_{ci} is the weighting factor assigned by the user for technical criteria *i*, and *n* is the number of technical criteria.

$$T_T = \left(F_1 \times F_2 \times \ldots \times F_n\right) \times \sum_{i=1}^{n} (S_{ci} \times W_{ci}) \quad (3)$$

Finally, the aggregated score (A_S) is determined by multiplying the technical tier by the applicability tier. Note that at each tier the method score can turn into a zero, in which case the method is sieved out.

$$A_S = T_A \times T_T \quad (4)$$

Subsequently, the aggregated scores are ranked. For each soil function a list is created showing the scores and ranks of each method. This list should support the user in selecting the appropriate methods for the assessment at hand.

2.4. Interpretation of output

The final output of the BIOSIS tool is a list of methods with associated scores ranked from high to low. The individual method scores are the result of multiplication and addition algorithms using the pre-defined scores and user-selected weighting factors. As the user can apply different weighting factors and exclusion filters, the output scores of different runs should not be compared. A higher score indicates a more suitable method. We recommend that the user picks a combination of both high-ranking actor and process methods from this list. Yet, method scores should not be followed blindly because multiple methods for the same process or actor may be recommended and subtle preferences of the user may not be considered by the tool. For example, if a user is interested in Nutrient Cycling, but primarily in the cycling of nitrogen, the user could selectively look at the highest scoring nitrogen

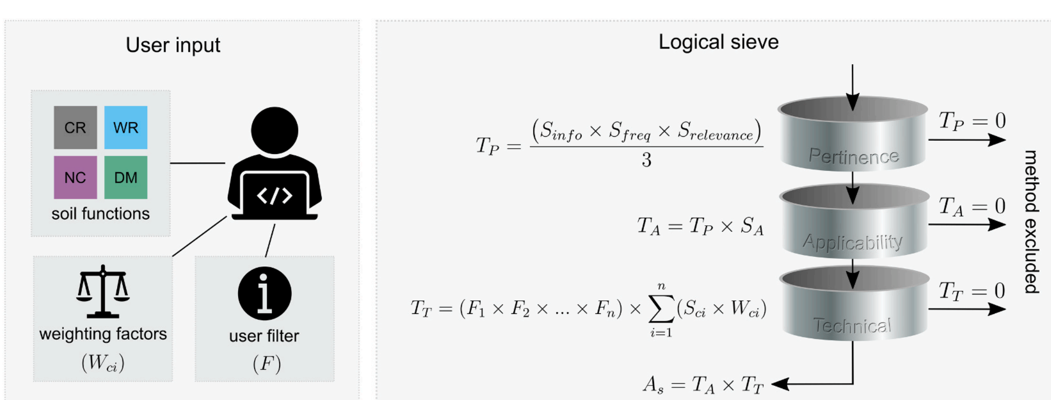

Fig. 1. BIOSIS tool: a flexible biological method selection tool adapted from the original logical sieve framework by Ritz et al. (2009).

Table 1
Description of selection criteria and scoring protocol modified from Ritz et al. (2009).

Tier	Criteria	Description	Scoring information	User-defined weighting factor	User-defined filter (F)	Compared to criteria from Ritz et al. (2009)
Pertinence	Functional information	The degree by which an actor or process method provides information about the functional capacity of a soil to deliver an individual soil function.	1 = Actor abundance (actor) 2 = Actor phylogenetic information 3 = Actor functional information (e.g., functional gene, functional group), potential process (DNA) 4 = Potential process (e.g., RNA, enzymes) 5 = Actual process (e.g., lab incubation, field measurement)	No	No	New
Pertinence	Frequency	Number of occurrences of an actor or a process within a given function model.	0 = no occurrence 1 = occurs once 2 = occurs twice 3 = occurs more than twice but not in the top 10% most frequently occurring actors 4 = part of top 10% most frequently occurring actors	No	No	New
Pertinence	Relevance	Expert-opinion score on the relevance of the actor to the delivery of a process. If actors contributed to multiple processes, scores were averaged to obtain a relevance score at the function level.	Continuous score rescaled between 0 and 4 with most relevant actor scoring 4 5 = process	No	No	New
Applicability	Applicability	Applicability of method to the ecosystem(s) under consideration	1 = applicable 0 = not applicable	No	No	Same
Technical	Throughput	The amount of samples that can be processed per week	1 = < 20 samples per week 2 = 20–50 samples per week 3 = 50–100 samples per week 4 = > 100 samples per week	Yes	No, F = 1.	Same
Technical	Storage	Allowed storage time before post-sampling measures (laboratory analysis)	0 = not possible 1 = within 1 week 2 = within 1 month 3 = within 6 months to one year	Yes	Yes, default score: F = 1. User can activate filter to exclude methods where samples cannot be stored for >1 month (F = 0).	Modified
Technical	Temporal sample collection	The number of sampling times (field visits) needed	0 = more than one sampling needed 1 = one time is enough	Yes	Yes, default score: F = 1. User can activate filter to exclude methods that require multiple field visits (F = 0).	Modified
Technical	Spatial sample collection	Number of samples needed per location or treatment	0 = more than one replicate needed 1 = one composite sample is enough	Yes	No, F = 1.	New
Technical	Archivability	Potential for long-term storage as dried or frozen sample	0 = not archivable 1 = archivable as fixated or extracted sample 2 = archivable as dried soil sample	Yes	No, F = 1.	Modified
Technical	Amount of sample	Soil mass needed for sampling and determination	1 = large mass (>1 kg or >2 L) 2 = small mass (<1 kg or < 2 L) 3 = very small mass (<100 g or < 0.5 L)	Yes	Yes, default score: F = 1. User can activate filter to exclude methods which require sample size >100 g.	Modified
Technical	Lab analysis cost per sample	Labour, hardware and consumable costs per sample	1 = high costs 2 = moderate costs 3 = low costs	Yes	Yes, default score: F = 1. User can activate filter to exclude methods with high costs (F = 0)	Modified
Technical	Ease of use in laboratory	The level of skill required to realise the method in the laboratory	1 = specialized 2 = moderate 3 = straightforward	Yes	No, F = 1.	Modified
Technical	Ease of data processing & interpretation	The level of skill required to process and interpret the data	1 = specialized 2 = moderate 3 = straightforward	Yes	No, F = 1.	New

(continued on next page)

Table 1 (*continued*)

Tier	Criteria	Description	Scoring information	User-defined weighting factor	User-defined filter (F)	Compared to criteria from Ritz et al. (2009)
Technical	Reference material	Option to include or develop reference material	0 = none 1 = potential but not often applied in practice 2 = yes, an internal reference 3 = yes, an international standard	Yes	Yes, default score: F = 1. User can activate filter to exclude methods without internal reference or international standard (F = 0).	Modified
Technical	Reproducibility (user bias)	Inherent ability of method to yield reproducible results	1 = low 2 = moderate 3 = high	Yes	Yes, default score: F = 1. User can activate filter to exclude methods with moderate or low reproducibility (F = 0).	Modified
Technical	Deployment status	Level of method development	0 = not ready, years of development needed 1 = developed for experimental use 2 = developed for routine use	Yes	No, F = 1.	Same
Technical	Regional Infrastructure	The state of regional infrastructure to realise the assessment	1 = none/few specialized labs 2 = moderate infrastructure 3 = ubiquitous infrastructure	Yes	Yes, default score: F = 1. User can activate filter to exclude methods for which specialized infrastructure is required (F = 0).	Modified

transformation methods. Therefore, it is up to the users to make the final selection of methods that they want and can apply. The selection tool attempts to optimize a complicated decision-making process, for which there may not be a perfect solution. Hence, users' judgment is needed to pick the methods from this output list that ultimately suit their context and objectives.

In the case of soil multifunctionality, the lists of the four soil functions need to simultaneously be assessed by the user. Here, we also recommend picking a combination of actor and process methods that represent the four individual soil function models and thereby soil multifunctionality.

2.5. Future developments

The transparent framework of the BIOSIS tool allows for further development, inclusion of new methods and expansion to other ecosystems of interest. We invite the scientific community to collaborate with us to achieve these goals. The R script and list of methods with the associated pertinence, applicability and technical scores are available to be used and reviewed by others through the Database 4TU.ResearchData (https://doi.org/10.4121/14431418). Moreover, the online version of the tool can be tested out at the BIOSIS platform (https://biosisplatform.eu/) and we are open for suggestions to improve the usability of the tool. Currently the BIOSIS tool requires basic scientific background of the user to define the relevant technical criteria and to be able to translate the findings of the sample results from the analyses finally performed in labs. Therefore one future direction of the tool would be the inclusion of field-based methods that can be applied by farmers where they are relevant and simplifying some of the technical criteria that require prior research knowledge. With the rapid development of new molecular, spectroscopic and isotopic methods (Bünemann et al., 2018; Fierer et al., 2021), we made sure that this tool can easily incorporate future methods. To further expand the tool, we also consider building an algorithm into the current framework that generates a list of recommended methods for soil multifunctionality taking into account the frequency of the actors and processes across the four function models and the relevance of different actors and processes to soil multi-functionality.

3. Case studies

3.1. Description

To demonstrate the flexible framework and use of the BIOSIS tool

Table 2
User-preferences considered by the soil biological method selection tool for case 1 (farm-scale soil quality assessment) and case 2 (monitoring soil multi-functionality at European scale).

	User-preferences for biological method selection	
	Case 1: Farm-scale soil quality assessment program	Case 2: Monitoring soil multi-functionality at European scale
Soil functions	All four	All four
Ecosystem of interest	Arable	Arable
User filter	No specialized infrastructure	No multiple field visits
Weighting factors		
Throughput	Very low importance (score = 1)	Very high importance (score = 5)
Sample storage	Low importance (score = 2)	Very high importance (score = 5)
Temporal sample collection	Very low importance (score = 1)	Very high importance (score = 5)
Spatial sample collection	Very low importance (score = 1)	Very high importance (score = 5)
Archivability	Not important (score = 0)	Very high importance (score = 5)
Amount of sample	Low importance (score = 2)	Very high importance (score = 5)
Lab analysis cost per sample	Very high importance (score = 5)	Medium importance (score = 3)
Ease of use in laboratory	High importance (score = 4)	Very low importance (score = 1)
Ease of data processing & interpretation	Very high importance (score = 5)	Low importance (score = 2)
Reference material	Medium importance (score = 3)	Very high importance (score = 5)
Reproducibility (user bias)	Medium importance (score = 3)	Very high importance (score = 5)
Deployment status	High importance (score = 4)	Very high importance (score = 5)
Regional Infrastructure	Very high importance (score = 5)	Very low importance (score = 1)

and how it can benefit a variety of assessment programs, we developed two case studies (Table 2). In the first case study, an applied researcher working with farmers is looking for soil biological methods to be included in a soil multifunctionality assessment program at the field scale. Here, the ease of data processing and interpretation as well as costs of sample analysis were considered to be among the most important technical criteria. Moreover, we implemented a filter to exclude methods that required highly specialized infrastructure that the researcher and farmers may not have access to in their local surroundings. The second case focuses on the development of a European soil monitoring program to assess soil multifunctionality. Technical criteria related to the sampling logistics, laboratory throughput and quality control were considered as most important, while costs and level of specialization to carry out the method or process the data were marked

Table 3
Recommended methods for case study 1 (assessment of soil multifunctionality for farmers). Columns show the top-4 scoring methods for each function from the final output of the soil biological method selection tool. Colors in front of actor or process indicate to which soil functions the method is applicable. Methods in **bold** are recommended to be included in the assessment of multifunctionality and exclude duplicate methods suggested for multiple soil functions. If multiple methods for the same actor or process were among the top-4 methods, we only selected the highest-scoring method to show here. If multiple methods for the same actor or process obtained the same score, we show both or all three methods. For the full list of method scores see Table S3.

Carbon and climate regulation	Water regulation and purification	Nutrient cycling	Disease and pest regulation
■■■ **Nematodes (actor)** Score = 440 Method: 1. Morphological ID , field sampling, extract animals, microscope, and feeding groups	■■■ **Aggregation (process)** Score = 660 Method: 1. Soil Morphology analysis in situ separating biogenic structures	■■■ Earthworms (actor) Score = 403 Method: 1. Hand-sorting and extraction of earthworms followed by quantitative identification based on morphological characteristics	■■■ **Protozoa (actor)** Score = 403 Method: 1. Molecular quantification, qPCR amplification
■■ **Microbial respiration (process)** Score = 392 Method: 1. Soil respiration with incubation and measure of CO2 evolution with alkali trap 2. Soil respiration with incubation and measure of CO2 evolution with gas analyzer	■■■ **Bioturbation (process)** Score = 507 Method: 1. Earthworms activity (burrowing and cast formation) 2. Visualization of bioturbation patterns in the field or in lab	■■ Mineralisation (process) Score = 392 Method: 1. Potentially mineralisable N anaerobic incubation (1 week) 2. Soil respiration with incubation and measure of CO2 evolution with gas analyser Soil respiration with incubation and measure of CO2 evolution with alkali trap	■■■ Nematodes (actor) Score = 400 Method: 1. Morphological ID , field sampling, extract animals, microscope, and feeding groups
■■ **Denitrification (process)** Score = 376 Method: 1. Lab incubation with acetylene inhibition	■■■ **Earthworms (actor)** Score = 413 Method: 1. Hand-sorting and extraction of earthworms followed by quantitative identification based on morphological characteristic	■■ Denitrification (process) Score = 376 Method: 2. Lab incubation with acetylene inhibition	■ **Parasitism (process)** Score = 346 Method: 1. Laboratory assays for assessing parasitism by nematodes 2. Estimate percentage fungal parasitism against nematodes with bioassays in vitro 3. Fungal egg parasitism of nematodes in vitro
■■ **Nitrification (process)** Score = 376 Method: 1. Gross nitrification by inhibition	■■■ **Microbial assimilation (process)** Score = 336 Method: 1. Bacterial growth rate with TdR and Leucin incorporation 2. Fungal growth rate with acetate	■■ Nitrification (process) Score = 376 Method: 1. Gross nitrification by inhibition	■ **Disease and pest control (subfunction)** Score = 339 Method: 1. Disease bioassays (2. Soil suppressiveness assay for nematodes 3. Soil fatigue test

as less important. In the second case, we also implemented a filter to exclude methods for which multiple field visits to the same site were required.

It is important to note that the outcomes of the case studies presented here should not be used as universal guidelines for developing a soil assessment program for farmers (case study 1) or a soil monitoring program at the regional or continental scale (case study 2). Even when assessment programs are targeted towards the same stakeholders, the objective and logistical context of assessment programs can vary. These differences can be considered by means of adjusting the weighting factors and exclusion filters of the BIOSIS tool.

3.2. Outcomes

The output of the BIOSIS tool recommends both case studies to measure similar actors and processes (Tables 3 and 4) because their pertinence to soil functions will not change depending on the logistical context of different assessment programs. Overall, we find that earthworms, nematodes, protozoa, bacteria and fungi are important actors to monitor in both case studies. These actors frequently occur in the cognitive soil function models developed by Creamer et al. (2022) and were scored as highly relevant to soil functions by soil biology experts. In addition, the output of the tool also contains high-scoring process methods, of which aggregation, bioturbation, mineralisation and parasitism are recommended to be measured in both case studies.

The differences between case studies become visible when we compare the specific methods recommended. For case study 1, commonly applied methods are recommended that are easy to interpret. In contrast, the output for case study 2 recommends more specialized and novel methods, which also maximize ease of field sampling and

Table 4
Recommended methods for case study 2 (monitoring soil multifunctionality at European scale). Columns show the top-4 scoring methods for each function from the final output of the soil biological method selection tool. Colors in front of actor or process indicate to which other soil functions the method is applicable. Methods in bold are recommended to be included in the assessment of multifunctionality and exclude duplicate methods suggested for multiple soil functions. If multiple methods for the same actor or process were among the top-4 methods, we only selected the highest-scoring method to show here. If multiple methods for the same actor or process obtained the same score, we show both or all three methods. For the full list of method scores see Table S4.

Carbon sequestration & climate regulation	Water regulation and purification	Nutrient cycling	Disease and pest regulation
■■■■ **Microbial community (actor)** Score = 474 Method: 1. Metagenomic of environmental samples	■■■ **Aggregation (process)** Score = 584 Method: 1. Near infrared spectroscopy (NIRS) that measure the different origins of aggregate classes based on their spectral signatures	■■■■ Microbial community (actor) Score = 485 Method: 1. Metagenomic of environmental samples	■■■ Protozoa (actor) Score = 451 Method: 1. Molecular quantification, qPCR amplification
■■■ **Nematodes (actor)** Score = 451 Method: 1. Quantification of selected (groups of) species, field sampling, extraction, qPCR (quantitative for targeted markers)	■■■■ Microbial community (actor) Score = 451 Method: 1. Metagenomic of environmental samples	■■■ Earthworms (actor) Score = 343 Method: 1. Qualitative identification, field sampling, environmental samples, sequencing of selected DNA (Metabarcoding)	■■■■ Microbial community (actor) Score = 411 Method: 1. Metagenomic of environmental samples
■■■ **Protozoa (actor)** Score = 348 Method: 1. Molecular quantification,qPCR amplification	■■■ **Bioturbation (process)** Score = 354 Method: 1. Earthworms activity (burrowing and cast formation) 2. Visualization of bioturbation patterns in the field or in lab	■■■ Protozoa (actor) Score = 341 Method: 1. Molecular quantification, qPCR amplification	■■■ Nematodes (actor) Score = 410 Method: 1. Quantification of selected (groups of) species, field sampling, extraction, qPCR (quantitative for targeted markers)
■ **Methanotrophy (process)** Score = 342 Method: 1. Incubation experiments measuring methane consumption (5 days)	■■■ **Earthworms (actor)** Score = 351 Method: 1. Qualitative identification, field sampling, environmental samples, sequencing of selected DNA (Metabarcoding)	■■ **Mineralisation (process)** Score = 330 Method: 1. Potentially mineralisable N anaerobic incubation (1 week) 2. Soil respiration with incubation and measure of CO2 evolution with gas analyser 3. Soil respiration with incubation and measure of CO2 evolution with alkali trap	■ **Parasitism (process)** Score = 294 Method: 1. Molecular detection of parasites with metabarcoding

throughput where possible. To give an example, both cases are recommended to measure earthworms in the assessment of Water Regulation and Purification and Nutrient Cycling. In case study 1, hand-sorting the earthworms in the field and quantifying them based on morphological characteristics to derive functional groups (Römbke et al., 2018) is the highest scoring method. In case study 2, metabarcoding of eDNA is recommended instead because this substantially simplifies field logistics. Another example is the assessment of the microbial community. Metagenomic analyses will provide case study 2 with a plethora of information including functional genes related to many of the processes that the bacterial and fungal community support in the cognitive function models (resulting in a high frequency score (S_{freq})). Moreover, metagenomic analyses allow for high throughput and only require small amounts of soil, which were two technical criteria listed as highly important in case study 2. Overall, this resulted in the high score for metagenomics in case study 2 focusing on monitoring soil functions at the European scale. Large-scale monitoring programs such as the LUCAS Soil survey are already testing the implementation of such advanced molecular methodologies in subsets of their sampling sites (Orgiazzi et al., 2018). Metagenomics is still very specialized in terms of local infrastructure, data processing and interpretation (Thomas et al., 2012; Laudadio et al., 2019). Therefore, this method was not advised to be used in assessment programs targeted towards farmers (case study 1). Instead, microbial process-based methods such as microbial respiration and mineralisation are performed by many local laboratories and are more intuitive to explain.

Another difference between the two case studies is that the BIOSIS tool recommends fewer process-based methods for monitoring soil functions at European scale than for the on-farm assessment of soil multifunctionality. This is again attributed to the user-defined technical criteria. Process-based methods are easier to interpret, yet are often more time-consuming lowering the high throughput desired for large-scale monitoring programs (Griffiths et al., 2018; Fierer et al., 2021). While process-based methods link more directly to soil multifunctionality, actors often contribute to multiple processes. Hence, both provide valuable information to the assessment of soil multifunctionality. Therefore, we would recommend measuring a combination of both actor- and process-based methods when possible. Depending on the user-defined technical criteria, the balance between actor- and process-based methods will shift to match the requirements of the assessment program.

The BIOSIS tool evaluates methods based on three tiers (pertinence, applicability and technical). While the pertinence and the applicability scores are fixed, the user defines the scoring of the technical tier by determining the weighting factors and/or implementing exclusion filters. Within the technical tier, criteria are scored, multiplied by user-determined weighting factors and subsequently added. In certain cases, users may have technical demands for which there is no perfect match. In case study 1 the applied researcher is looking for biological methods which are considered *low cost* (defined in the weighting of technical criteria) and do not require *specialized facilities* (applied in the exclusion filter as they do not have access to such facilities). Nematodes received a high pertinence score due to their high frequency in two of the four cognitive models (carbon sequestration and climate regulation and disease and pest regulation) and high relevance score given by the experts. The selection of nematode methods is then further assessed according to the technical criteria and exclusion filter. In this case the exclusion filter removes any methods that require *specialized facilities* and the remaining method is listed as morphological analysis of nematodes which is the nearest possible solution to the demands of the researcher in case study 1. If the user does not want to consider expensive methods at all, an additional exclusion filter could be implemented. Yet, it is important to note that too many exclusion filters may result in very few or no recommendations for particular actors or processes.

4. Conclusion

Soil biology is crucial to the delivery of soil multifunctionality. However, few soil biological methods are included in soil monitoring of agricultural production systems. Here we presented a flexible tool to support decision-making during the selection process of soil biological methods for the assessment of soil multifunctionality. The BIOSIS tool is based on a structured and scientific framework that connects soil biota to soil functions through their contribution to different soil processes (Creamer et al., 2022) . Users interact with the tool to define the context of their assessment and technical considerations. By incorporating user input, the BIOSIS tool can be used by a wide variety of researchers and practitioners interested in applying soil biological methods to monitor soil multifunctionality at different scales.

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

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References

Andrews, S.S., Karlen, D.L., Cambardella, C.A., 2004. The soil management assessment framework: a quantitative soil quality evaluation method. Soil Sci. Soc. Am. J. 68, 18.

Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of soil quality indices: a biological perspective. Geoderma 147, 159–171. https://doi.org/10.1016/j.geoderma.2008.08.007.

Bender, S.F., Wagg, C., van der Heijden, M.G.A., 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. Trend. Ecol. Evol. 31, 440–452. https://doi.org/10.1016/j.tree.2016.02.016.

Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L., Peres, G., Tondoh, J.E., Cluzeau, D., Brun, J.-J., 2013. A review of earthworm impact on soil function and ecosystem services. Europ. J. Soil Sci. 64, 161–182. https://doi.org/10.1111/ejss.12025.

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – a critical review. Soil Biol. Biochem. 120, 105–125. https://doi.org/10.1016/j.soilbio.2018.01.030.

Creamer, R.E., Barel, J.M., Bongiorno, G., Zwetsloot, M.J., 2022. The life of soils: integration the who and how of multifunctionality. Soil Biology and Biochemistry. https://doi.org/10.1016/j.soilbio.2022.108561. In this issue.

de Groot, G.A., Jagers op Akkerhuis, G.A.J.M., Dimmers, W.J., Charrier, X., Faber, J.H., 2016. Biomass and diversity of soil mite functional groups respond to extensification of land management, potentially affecting soil ecosystem services. Front. Environ. Sci. 4 https://doi.org/10.3389/fenvs.2016.00015.

De Ruiter, P.C., Van Veen, J.A., Moore, J.C., Brussaard, L., Hunt, H.W., 1993. Calculation of nitrogen mineralization in soil food webs. Plant and Soil 157, 263–273. https://doi.org/10.1007/BF00011055.

Debeljak, M., Trajanov, A., Kuzmanovski, V., Schröder, J., Sandén, T., Spiegel, H., Wall, D.P., Van de Broek, M., Rutgers, M., Bampa, F., Creamer, R.E., Henriksen, C.B., 2019. A field-scale decision support system for assessment and management of soil functions. Front. Environ. Sci. 7, 115. https://doi.org/10.3389/fenvs.2019.00115.

Doran, J.W., Parkin, T.B., 1997. Quantitative indicators of soil quality: a minimum data set. In: Methods for Assessing Soil Quality. John Wiley & Sons, Ltd, pp. 25–37. https://doi.org/10.2136/sssaspecpub49.c2.

Fierer, N., Wood, S.A., Bueno de Mesquita, C.P., 2021. How microbes can, and cannot, be used to assess soil health. Soil Biol. Biochem. 153, 108111 https://doi.org/10.1016/j.soilbio.2020.108111.

Griffiths, B.S., Faber, J., Bloem, J., 2018. Applying soil health indicators to encourage sustainable soil use: the transition from scientific study to practical application. Sustainability 10, 3021. https://doi.org/10.3390/su10093021.

Haygarth, P.M., Ritz, K., 2009. The future of soils and land use in the UK: soil systems for the provision of land-based ecosystem services. Land Use Policy. Land Use Futures 26, S187. https://doi.org/10.1016/j.landusepol.2009.09.016. –S197.

Kibblewhite, M. g, Ritz, K., Swift, M. j, 2008. Soil health in agricultural systems. Philosophical Transac. Royal Soc. B: Biolo. Sci. 363, 685–701. https://doi.org/10.1098/rstb.2007.2178.

Laudadio, I., Fulci, V., Stronati, L., Carissimi, C., 2019. Next-generation metagenomics: methodological challenges and opportunities. OMICS: J. Integrat. Biol. 23, 327–333. https://doi.org/10.1089/omi.2019.0073.

Lemanceau, P.P., Maron, P.-A., Mazurier, S., Mougel, C., Pivato, B., Plassart, P., Ranjard, L., Revellin, C., Tardy, V., Wipf, D., 2015. Understanding and managing soil biodiversity: a major challenge in agroecology. Agron. Sustain. Develop. 35, 67–81. https://doi.org/10.1007/s13593-014-0247-0.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-Being. Island Press, Washington, DC.

Orgiazzi, A., Ballabio, C., Panagos, P., Jones, A., Fernández-Ugalde, O., 2018. LUCAS Soil, the largest expandable soil dataset for Europe: a review. Europ. J. Soil Sci. 69, 140–153. https://doi.org/10.1111/ejss.12499.

O'Sullivan, L., Bampa, F., Knights, K., Creamer, R.E., 2017. Soil protection for a sustainable future: options for a soil monitoring network for Ireland. Soil Use Manage. 33, 346–363. https://doi.org/10.1111/sum.12351.

Powlson, D.S., 2020. Soil health—useful terminology for communication or meaningless concept? Or both? Front. Agricul. Sci. Eng. https://doi.org/10.15302/J-FASE-2020326.

Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., Wood, C., 2009. Selecting biological indicators for monitoring soils: a framework for balancing scientific and technical opinion to assist policy development. Ecological Indicators 9, 1212–1221. https://doi.org/10.1016/j.ecolind.2009.02.009.

Römbke, J., Dorow, W.H.O., Jänsch, S., 2018. Distribution and diversity of earthworms (Lumbricidae) in Hesse (Central Germany): current knowledge. Soil Organ. 90, 171–185. https://doi.org/10.25674/bmwx-qs58, 171–185.

Schulte, R.P.O., Creamer, R.E., Donnellan, T., Farrelly, N., Fealy, R., O'Donoghue, C., O'hUallachain, D., 2014. Functional land management: a framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. Environmental Science & Policy 38, 45–58. https://doi.org/10.1016/j.envsci.2013.10.002.

Smith, P., House, J.I., Bustamante, M., Sobocká, J., Harper, R., Pan, G., West, P.C., Clark, J.M., Adhya, T., Rumpel, C., Paustian, K., Kuikman, P., Cotrufo, M.F., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bondeau, A., Jain, A.K., Meersmans, J., Pugh, T.A.M., 2016. Global change pressures on soils from land use and management. Global Change Biology 22, 1008–1028. https://doi.org/10.1111/gcb.13068.

Stone, D., Blomkvist, P., Hendriksen, N.B., Bonkowski, M., Jørgensen, H.B., Carvalho, F., Dunbar, M.B., Gardi, C., Geisen, S., Griffiths, R., Hug, A.S., Jensen, J., Laudon, H., Mendes, S., Morais, P.V., Orgiazzi, A., Plassart, P., Römbke, J., Rutgers, M., Schmelz, R.M., Sousa, J.P., Steenbergen, E., Suhadolc, M., Winding, A., Zupan, M., Lemanceau, P., Creamer, R.E., 2016. A method of establishing a transect for biodiversity and ecosystem function monitoring across Europe. Applied Soil Ecology, Soil biodiversity and ecosystem functions across Europe: a transect covering variations in bio-geographical zones. Land Use Soil Properties 97, 3–11. https://doi.org/10.1016/j.apsoil.2015.06.017.

Thomas, T., Gilbert, J., Meyer, F., 2012. Metagenomics - a guide from sampling to data analysis. Microbial Inform. Exp. 2, 3. https://doi.org/10.1186/2042-5783-2-3.

Tóth, G., Gardi, C., Bódis, K., Ivits, É., Aksoy, E., Jones, A., Jeffrey, S., Petursdottir, T., Montanarella, L., 2013. Continental-scale assessment of provisioning soil functions in Europe. Ecol. Proces. 2, 32. https://doi.org/10.1186/2192-1709-2-32.

van Leeuwen, J.P., Saby, N.P.A., Jones, A., Louwagie, G., Micheli, E., Rutgers, M., Schulte, R.P.O., Spiegel, H., Toth, G., Creamer, R.E., 2017. Gap assessment in current soil monitoring networks across Europe for measuring soil functions. Environ.l Res. Lett. 12, 124007 https://doi.org/10.1088/1748-9326/aa9c5c.

Vogel, H.-J., Bartke, S., Daedlow, K., Helming, K., Kögel-Knabner, I., Lang, B., Rabot, E., Russell, D., Stößel, B., Weller, U., Wiesmeier, M., Wollschläger, U., 2018. A systemic approach for modeling soil functions, 2018 SOIL 4 (Issue 1), 83–92. https://doi.org/10.5194/soil-4-83-2018, 4, 83–92.