Report

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SQID: Prioritising biological indicators of soil quality for deployment in a national-scale soil monitoring scheme

Summary report
Defra Project No. SP0529

H.I.J. Black[1], K. Ritz[2], C.D. Campbell[3], J.A. Harris[2], C. Wood[1], P.M. Chamberlain[1], N. Parekh[1], W. Towers[3] and A. Scott[1]

[1]Centre for Ecology and Hydrology, Bailrigg, Lancaster LA1 4AP
[2]Cranfield University, Silsoe, Bedfordshire MK45 4DT
[3]Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH

Contents page

Executive summary

This project prioritised thirteen biological indicators of soil quality which showed high current potential for deployment in a national-scale soil monitoring scheme. These indicators met a range of scientific and technical criteria that related soil functions and feasibility within large-scale surveys. The priority indicators with associated methodologies are:

- Eight soil microbial groups [ammonia oxidisers, denitrifiers, fungi, bacteria, Archaea, methanogens, methanotrophs and actinomycetes] identified from TRFLP fingerprinting
- Soil microbial community structure and biomass characterised from PLFA profiles
- Multiple substrate induced respiration (MSIR) derived by GC or Microresp™
- Multi-enzyme profiling via microplate fluorometric assay
- Nematode community structure from Baermann extractions
- Microarthropod community structure from Tullgren dry extractions

The selection process was robust, repeatable and auditable. A structured framework denoted a "logical sieve" was developed to support the incorporation and analysis of a large number of assessments against a wide range of technical and scientific criteria relevant to national scale soil monitoring. This enabled a consistent synthesis of available information and the semi-objective assessment of 183 potential biological indicators identified from the literature. Stakeholder priorities for technical criteria were identified through consultation, with the UK-SIC and the expert reviewers, and incorporated into the final prioritisation phase of the logical sieve. The power of this approach is that it provides a clear audit trail on the decision-making process and would allow the inclusion of further indicators into the framework.

The process was initially reviewed by experts familiar with biological indicators and soil monitoring and then assessed at a two-day expert workshop. Comments and discussions on the relative importance and robustness of potential indicators and future research priorities proved invaluable to the final selection. As a consequence, the logical sieve was modified to prioritise biological indicators for all three soil functions rather than simply biological indicators with the highest universal scores. The final priority indicators were selected by reviewing the outputs from the logical sieve. Each priority indicator, with associated method, was assessed for relevance to ecological services, obvious surrogacy, the range of indicator indices produced and practicalities of use. Each priority indicator was reviewed and outstanding issues relating to deployment identified.

Statistical analyses of existing field survey/experimental data for PLFAs, soil invertebrates and community-level physiological profiling of the soil microbial community (BIOLOG™) highlighted generic technical, policy-related and scientific issues which were considered in the recommendations for a field evaluation of the priority indicators.

1. Background

The properties, activities of, and interactions between soil biota are critical requirements for the provision of most soil functions through their role in the provision of "ecological services", in particular food and fibre production, environmental interactions, and support of habitats and biodiversity (Table 1). Although a mechanistic understanding of relationships between soil biota and soil functions[1] remains somewhat elusive, it is reasonable to postulate that biological components of soils have considerable potential as indicators of soil quality[2] since they are a fundamental requirement for maintaining soil health (RCEP, 1996).

Table 1. The functions that soils perform. Adapted from Blum (1993), Defra (2005) and Hågvar (1998).

Soil function	Endpoint
Food and fibre production	Maintaining soil in a suitable state for plant and animal biomass production e.g. nutrient/water supply, disease control, stable rooting environment etc.
Environmental interactions (between soils, air and water)	Maintaining the capacity for soil to store, transform and regulate soil processes for environmental protection and sustainability e.g. trace gas exchanges, pollutant degradation, N/P retention, water flow regulation, etc.
Support of ecological habitats and biodiversity	Maintaining soil for the maintenance, protection and restoration of habitats and above-ground biodiversity as well as retaining the ecological, utilitarian and ethical value of soil biodiversity.
Protection of cultural heritage	Maintaining soil to protect archaeological remains and cultural landscapes.
Providing a platform	Using soil as a foundation for constructions
Providing raw materials	Using soil as a direct source of minerals and resources e.g. peat and topsoil.

National-level requirements for biological indicators were outlined in the Soil Action Plan for England (Defra, 2004) with consequent actions being addressed at a UK level by the UK-Soil Indicators Consortium (Defra, 2005). These actions parallel other country-level initiatives within Europe addressing the incorporation of biological indicators into monitoring of soil quality (Parris, 2004). Within the UK, the feasibility of using biological indicators in an extensive soil monitoring scheme was established through Countryside Survey 2000 (Black *et al*., 2003). The focus of effort is now on establishing the most appropriate biological indicators for monitoring from an immense number of potential indicators and associated methods. The Defra-funded SQID project [*scoping biological indicators of soil quality;* SP0529] operated under the umbrella of the UK-SIC consortium with the task to *objectively assess the relative performance of current potential biological indicators and produce a prioritised list of candidate biological indicators for subsequent piloting across relevant soil:land use combinations*.

The specific objectives were:

1. Complete an objective assessment, based upon currently available data, of the power of potential bio-indicators to discriminate between soil/land use combinations and their interpretability in relation to soil functional capacity;
2. Formulate a priority list of candidate bio-indicators based on such an assessment;

[1] As defined in the Soil Action Plan for England. Defra (2004)
[2] Soil quality: the capacity for a specific type of soil to carry out functions demanded of it

3. Assess the technical and scientific requirements for a field evaluation of the priority candidate bio-indicators Detail the scientific, technical and funding requirements for a field evaluation of the priority candidate bio-indicators.

This report details the approach by which a priority list of thirteen candidate biological indicators was identified and issues relating to field evaluation established. This includes the development and application of a structured assessment framework, denoted as a 'logical-sieve', the assessment of 183 potential biological indicators and analyses of existing large-scale soil biological datasets to assess methodologies, discrimination between different soil:land use combinations and the potential to establish target ranges or expected values across the UK environment.

2. Approach

In recent years, numerous reviews and reports have been published on biological indicators of soil quality with many of these having direct relevance to national-scale monitoring of soil quality (c.f. Environment Agency, 2003; Nielson and Winding, 2002) while biological indicators already been deployed in schemes throughout Europe (e.g. Breure *et al*., 2005; Mulder *et al*., 2005; Winding *et al*., 2005;) and elsewhere (Nielson and Winding, 2002; Sparling and Schipper, 2002). Although comparability between different international schemes may be desirable, from a scientific and political perspective, careful consideration is required to ensure that biological indicators chosen for deployment in a UK monitoring scheme are capable of addressing UK soil protection policy requirements, in the first instance. The sheer number and scope of published information on biological indicators of soil quality have expanded rapidly in recent years and beyond the scope of any meta-data analysis within the current project. Instead the literature was used to identify an extensive list of potential indicators for comparison within a well defined framework. The primary aim was to ensure that the prioritisation of biological indicators for national-scale monitoring within the UK was comprehensive and addressed, as far as possible, the full range of ecological services that soil biota support[3]. The robustness of this process was dependant upon clear and consistent criteria being used to assess the relative performance of the different biological indicators. The project identified a set of technical and scientific criteria with specific relevance to soil functions highly dependant upon soil biota (*viz*. food and fibre production; environmental interactions; support of habitats and biodiversity) and the suitability of each biological indicator to national-scale soil monitoring, including key practical requirements.

2.1. Linking soil biology to soil functions

The SQID approach focused on soil functions as the ultimate end-point; specifically, food and fibre production, environmental interactions and habitat/biodiversity maintenance. Research directly linking soil biology to the maintenance of soil functions, as part of a wider provision of ecosystem services, is still very much in its infancy and constrains current development of bio-indicators to some extent. However, it is widely acknowledge that soil biology is essential for a healthy soil since components of the soil community are fundamental to the delivery of individual ecological services (otherwise known as *ecological processes*). For the purposes of this project, a pragmatic approach was adopted that various combinations of ecological services are required for the maintenance of soil function and that all

[3] ecological services are the fundamental link between biological indicators and the delivery of individual soil functions.

ecological services should be maintained to some degree since, in their absence, a system's adaptability, or resilience, will otherwise be compromised in the face of environmental change. There are various definitions of ecological services and their relationships to individual soil functions and therefore a reference table was established for this project (Table 2). The soil functions under consideration were taken from Table 1. The individual ecological services maintained/regulated by soil organisms (c.f. Giller *et al*., 1997) which correspond to the maintenance of a specific soil function were then listed accordingly. This list was used to support the identification of biological indicators (organism, biochemical/genetic profile, process, index, etc) with specific relevance to individual ecological services. Indicators were then matched to the delivery of individual soil functions. As outlined below, the selection of biological indicators for specific ecological services would be possible, albeit with many more expert scorings required.

Table 2. Relationships between soil functions and ecological services

Soil Functions	Ecological services	Examples of related soil biota
Food and Fibre Production	C cycling	microbial biomass, methanogens
	Decomposition of organic matter	microarthopods, saprotrophic fungi
	N cycling	nitrifiers, denitrifiers
	P cycling	phosphatase, mycorrhiza
	S cycling	sulphur-reducing bacteria
	N fixation	rhizobia
	Primary (microbial) activity	microbial community structure and activity
	Soil food web transfers	microbial community & food web structure
	Disease & pest transmission/suppression	predators, pathogens
	Nutrient supply from symbioses	mycorrhiza, N-fixers
	Redistribution by bioturbation	earthworms, ants
	Bio-aggregation of soil	fungi, worms
Environmental Interactions	Degradation/immobilisation of pollutants	fungi, worms
	C retention/release	microbial biomass, methanogens
	N retention/release	nitrifiers, denitrifiers
	P retention/release	microbial activity, mycorrhiza
	Tolerance/Resistance (toxins)	soil community structure and activity
	S retention/release	sulphur-reducing bacteria
	Redistribution by bioturbation	earthworms, ants
	Bio-aggregation of soil	fungi, worms
Supporting ecological habitats and biodiversity	Habitat for rare soil species	wax cap fungi, Southern Wood Ant
	Germination zone for plants	plant roots, mycorrhiza
	Nutrient supply from symbioses	mycorrhiza
	Food source (aboveground)	fungi, insects
	Reservoir for soil biodiversity (taxonomic)	soil species and diversity
	Reservoir for soil biodiversity (genetic)	community DNA and RNA
	Reservoir for soil biodiversity (functional)	nitrifiers, trophic structure, worms

2.2. Biological indicators of soil quality in the literature

A comprehensive database on literature on biological indicators of soil quality was established by searching and reviewing sources available to the consortium. Full details on search terms and publications are available in the appended full report. A broad-spectrum meta-analysis was not feasible within the confines of this project (Table 3); such an objective approach may be more manageable for individual methods or specific soil functions. The literature database was therefore used to produce a relatively comprehensive list of potential indicators including specific methods, wherever possible, for a formal assessment. The final list considered was, inevitably, not totally comprehensive but it did include indicators in routine or common use plus those showing a high degree of future promise; the comprehensive coverage was subsequently confirmed in the consultation phases of the project.

Table 3. Number of published documents (n) on soil biological indicators by year. Figures pre. 1980 are likely under-estimates due to lesser representation in digital databases.

Year	n	Year	n	Year	n	Year	N	Year	n
1931	1	1964	4	1976	7	1986	27	1996	594
1932	1	1966	1	1977	9	1987	26	1997	699
1939	1	1967	2	1978	4	1988	35	1998	795
1946	1	1968	2	1979	6	1989	33	1999	867
1952	1	1970	6	1980	14	1990	73	2000	933
1958	1	1971	6	1981	17	1991	269	2001	1016
1960	1	1972	2	1982	17	1992	301	2002	1022
1961	1	1973	1	1983	13	1993	368	2003	1213
1962	1	1974	5	1984	17	1994	376	2004	1735
1963	1	1975	9	1985	22	1995	477		

3. Development of the Logical Sieve

The indicator list was transferred into an MS Excel based database which was structured as follows: each indicator was arbitrarily assigned a unique ID number for reference purposes and given a short descriptor. The complete list of biological indicators reviewed is presented in the appended full report. After consideration and exploration of different ways to compare and prioritise the indicators, it became apparent that an effective approach would be to adopt a formalised method for assessing the relative strengths, weaknesses and suitability of each indicator for national scale soil monitoring. To this end, the project developed a defined framework, and associated MS Excel based software tool - the "logical-sieve" - to support a consistent synthesis of available information and the semi-objective assessment of biological indicators against a series of scientific and technical criteria relevant to applying indicators in national scale soil monitoring. The power of this approach is that it provides a clear record and audit trail on the decision-making process and also would allow the inclusion of further indicators into the framework should this be required in the future. The process in the development and application of the logical sieve is summarised below with full details available in the appended full report. It must be stressed that the logical sieve was designed to act as a decision-support tool to assist in formulating a prioritised list of potential indicators, and not be an unequivocal and definitive list – the issues are far too complex for such rigidity to be appropriate.

3.1. The basic concept

The logical sieve is a conceptual framework designed from first principles to provide a semi-objective means of establishing the applicability of potential indicators to a wide variety of monitoring scenarios, circumstances or soil functions. It also provides a means of ranking potential indicators according to user-prescribed priorities by capturing the remarkably complex issues that surround the notion of indicators. It is deemed semi-objective due to the combination of objective information with expert opinion and incomplete knowledge of the nature and mechanistic operation of the soil biota. The framework has been designed to be sufficiently flexible so that it can be re-tuned according to the precise nature of the users' needs, and can be updated as new knowledge is accrued. The 'sieving' functions are also flexible and operate on the principle of a form of grading according to user-defined scenarios. Expert decisions have been captured in the sieve, and can be altered when new information or priorities become available. The current version of the logical sieve is operated via a

series of interlinked spreadsheets in MS Excel. The basic concept is as follows. A potential bio-indicator is assessed by expert knowledge in relation to a range of criteria categorised in three tiers (or sieves) which relate to:

- Pertinence to defined soil functions (Table 4);
- Applicability to range of ecosystems under consideration and ability to discriminate between soils that are intrinsically different in relation to the considered criteria (Table 5);
- Methodological criteria relevant to implementation in a national-scale soil monitoring scheme (Table 6).

These tiers were assigned numerical factors, denoted F_{SF}, F_{AD} and F_M respectively. The assessment takes the form of assigning numerical scores for each indicator with respect to these criteria. These scores are then aggregated according to formulae that include weighting functions defined by the user according to their requirements and priorities, to produce an aggregate score (F_A) for each indicator. Full details on the categorisations, scoring, populating, weighting and prioritisations process are provided in the appended full report. The approach was reviewed by email consultation with a group of experts in biological indicators and soil monitoring.

Table 4. Soil functions (F_{SF}) tier of the logical sieve.

SOIL FUNCTION	**SCORES**
FOOD AND FIBRE PRODUCTION (FF) Maintaining soil in a suitable state for plant and animal biomass production [supplying nutrients and water, disease control, physical condition]	0 = not pertinent 1 = pertinent 2 = highly pertinent
ENVIRONMENTAL INTERACTIONS (EI) Protecting the capacity of soils to store, transform and regulate soil processes [gas exchanges, degradation and retention of solid materials e.g. pollutants and organic matter, water flow regulation] critical to environmental sustainability	
SUPPORT OF ECOLOGICAL HABITATS AND BIODIVERSITY (HB) Maintaining the ecological, utilitarian and ethical value of soil biodiversity including maintenance of semi-natural habitats and biodiversity above-ground	

Table 5. Applicability and discrimination tier (F_{AD}) of the logical sieve.

CATEGORY	**SCORES**
APPLICABILITY: Is the property, measured by this method, intrinsically applicable in all circumstances (e.g. ecosystems) under consideration ?	0 = Not applicable, i.e. not ubiquitous 1 = Universally applicable
DISCRIMINATION: What level of discrimination would method provide between, e.g. 5/10/20 samples from variety of contexts	0 = None 1 = Some discrimination 2 = Moderately high discrimination 3 = Very high discrimination 4 = Extremely high discrimination

Table 6. Methodological tier (F_M) of the logical sieve.

CATEGORY	SCORES
THROUGHPUT: How many samples can be processed, in monitoring context, with optimised laboratory systems and dedicated staff ? Assumes soils are in ready-to-weigh into the method state (i.e. excludes post-sampling preparation time); rating is for one fully-trained operator.	1 = few per week 2 = dozens per week 3 = hundreds per week
STORAGE: Given appropriate preservation, how soon do post-sampling measures need to be applied ?	0 = not possible 1 = soon (few days) 2 = can be delayed if suitably stored
ARCHIVABILITY: What is the potential for archiving soil samples (i.e. over decades) in order to accurately re-determine these properties ?	0 = not archivable 1 = archivable by freezing, freeze-drying or pickling
SAMPLE COLLECTION: Is one-stop sampling in the field tenable ?	0 = no 1 = yes
HOW MUCH SOIL: What mass of soil is needed for sampling and determination ?	1 = large mass required (kg) 2 = core (g or less)
COST – HARDWARE: What are hardware costs to realise the method, assuming off-the-shelf technology?	1 = very expensive 2 = moderately expensive 3 = low cost
COST- LABOUR: What are the human resource costs to realise method and initial interpretation (including consideration of skill level required and associated salary) ?	1 = very expensive 2 = moderately expensive 3 = low cost
EASE OF USE: What is the amenability of the method to ready application via a standard operating procedure when presented to a competent technician; includes training element ?	1 = specialised 2 = moderate 3 = straightforward
POTENTIAL REFERENCE MATERIAL: Is the method amenable to the prescription and provision of such material ?	0 = no 1 = yes
REPRODUCIBILITY OF RESULTS: What is the inherent ability for the method to generate reproducible results, given that full quality-control protocols are available and applied, including (assumed) availability of reference material	1 = inherently poor 2 = moderate 3 = high
DEPLOYMENT STATUS: Is the method “off-the-shelf” at the moment, with SOPs or ISO accreditation?	0 = not ready, years development needed 1 = likely to be ready for deployment with some months development 2 = fully deployable, in routine use
INTERNATIONAL COMPARISONS: Is the method used in soil monitoring schemes elsewhere?	0 = no 1 = yes
UK INFRASTRUCTURE: What is the state of the UK infrastructure to realise large-scale monitoring programmes using this method ?	1 = none/few specialised labs 2 = moderate infrastructure 3 = ubiquitous infrastructure

3.2. Consolidation of logical sieve output

In the final iteration of the logical sieve was completed after an expert workshop where various issued were discussed and amendments to the approach agreed. This included weighting to zero the methodology category relating to deployment status which effectively excluded deployment status from contributing to the overall aggregated score (F_A). Deployment status was then used to consolidate the final sieve output as lists of currently deployable indicators versus those not yet deployable. To consolidate the final F_A list, i.e. to produce a set of indicators most suited to the highest-level aim of this project, those indicators with deployment status of 2 (i.e. currently deployable) and aggregated F_A score >100 were extracted and each of these indicators was then considered in turn, moving down the F_A rank. If it was unique, it was transferred to a consolidated list, but if it was repetitive, i.e. already covered by a previous (higher-ranking) indicator it was passed over, or if there were mitigating secondary reasons e.g. extreme cost implications for national-scale survey. It is notable that only one indicator was discarded for secondary reasons which supports the robustness of this approach. This procedure was repeated for all indicators with

deployment status of one and F_A>100, and again for deployment status of zero. Table 7 therefore represents the top-ranking biological indicators with respect to an aggregate score across all soil functions. The sieve was adapted and the consolidation exercise repeated to obtain aggregated scores for each soil function (Table 8) demonstrates that many indicators are in common, but others are more appropriate to the particular function under consideration. The final stage in the process was to confirm that the top-ranking indicators would be able to inform on the wide range of ecological services required to deliver each of the three soil functions (Table 9). Future potential for sieve revolves around the expansion (or contraction) of the categories both in relation to the scoring criteria and the classification of indicators. For example: Soil Functions (F_{SF}) tier be expanded to include the wider subset of ecological processes that underpin the functions; Applicability and discrimination tier (F_{AD}): Expansion of ecosystems to include individual environmental strata (e.g. broad habitats, soils, land uses). This would allow sieving to only those environmental strata under consideration; The fidelity of the matrix may be improved by wider consultation, notwithstanding that this is a significant task even with the current resolution in the tiers.

Table 7. Consolidated list of highest scoring biological indicators across all soil functions ranked according to F_A (cut off > 100) and categorised according to deployment status.

(i) Deployment status = 2

Indicator	**Indicator descriptor**	**F_A**
TRFLP - Ammonia oxidisers/denitrifiers	Genetic profile - specific group	769
PLFA profiles	Composition -total community	615
TRFLP - ITS fungal	Genetic profile - specific group	437
Multiple substrate induced respiration (MSIR) GC	Activity capability profile: total community	311
Nematode Baermann extraction procedure	Numbers, composition & size of nematode community	302
TRFLP – Bacteria	Genetic profile - specific group	295
Microarthropods Tullgren dry extraction	Numbers, composition & size of invertebrates in soil	188
On site visual recording - flora and fauna	Numbers estimate of animals	173
Microplate fluorometric multi-enzyme assay	Enzyme potential activity	172
TRFLP – Archaea	Genetic profile - specific group	146
TRFLP - Methanogens/ methanotrophs	Genetic profile - specific group	123
Invertebrates pitfall traps	Numbers, composition & size of ground-dwelling invertebrates	123
TRFLP - Actinomycetes	Genetic profile - specific group	121

(ii) Deployment status = 1

Indicator	**Indicator descriptor**	**F_A**
TRFLP - Nematodes	Genetic profile	437
Multiple substrate induced respiration (MSIR) MicroResp	Activity capability profile	313
TRFLP - Protozoa	Genetic profile	291
qPCR AM Fungi	Genetic profile	111

(iii) Deployment status = 0

Indicator	**Indicator descriptor**	**F_A**
Functional gene arrays	Genetic profile	788
Phylogenetic gene arrays	Genetic profile	511
FISH - keystone species	Genetic profile	138

Table 8. Consolidated listing of highest ranking distinct biological indicators for each soil function, ranked according to F_A score (cutoff > 100) sieved for deployment status =2.

F_{FF} F_A	**Food & Fibre Function (F_{FF})**	F_{HB} F_A	**Habitat & Biodiversity Function (F_{HB})**	F_{EI} F_A	**Environmental Interaction Function**
277	TRFLP - Ammonia oxidisers/denitrifiers	332	TRFLP - Bacteria	277	TRFLP - Ammonia oxidisers/denitrifiers
277	PLFA profiles	328	TRFLP - Archaea	277	TRFLP - Methanogens/ methanotrophs
164	TRFLP - ITS fungal	328	TRFLP - ITS fungal	233	Multiple substrate induced respiration (MSIR) GC
157	Plant seed bank - counts	302	Nematode Baermann extraction procedure	221	TRFLP - Bacteria
155	Microplate fluorometric assay - multi-enzyme	282	Microarthropods Tullgren dry extraction	221	PLFA profiles
151	Nematode Baermann extraction procedure	277	TRFLP - Ammonia oxidisers/denitrifiers	219	TRFLP - Actinomycetes
141	Microarthropods Tullgren dry extraction	277	PLFA profiles	219	TRFLP - Archaea
119	Metabolic quotient, qCO2	276	Invertebrates Pitfall traps	219	TRFLP - ITS fungal
119	N fixers direct isolation	273	TRFLP - Actinomycetes	193	Microplate fluorometric assay - multi-enzyme
116	Multiple substrate induced respiration (MSIR) GC	221	TRFLP - Methanogens/ methanotrophs	151	Nematode Baermann extraction procedure
112	N min anaerobic incubation method	209	Plant seed bank - counts	122	In situ multiple trace gas (Ecoprobe 5)
111	TRFLP - Bacteria	173	On site visual recording - flora and fauna	109	TRFLP - Coliforms
104	On site visual recording - flora and fauna	155	Multiple substrate induced respiration (MSIR) GC	109	Microbial quotient (Cmicro/Corg)
		125	%GC profiling	104	On site visual recording - flora and fauna
		110	Enchytraeids (O'Connor wet extraction)	103	Methane uptake - headspace analysis
		109	TRFLP - AM fungi		

4. Candidate biological indicators for field evaluation

The following provides a short narrative on the thirteen priority biological highlighting the information that the indicator, and the specific method chosen, would provide under a national-scale soil monitoring scheme and issues relating to deployment.

4.1 Soil microbial taxa

Many soil microorganisms cannot be cultivated[4] under laboratory conditions even when it can be demonstrated that they are metabolically active. At present there are only two approaches that overcome the problems of culturability with respect to monitoring. These are the nucleic acid technologies (e.g. TRFLP) and the use of signature lipid biomarkers (see below). Partly for these reasons, several nucleic acid methods scored highly in relation to measuring soil microbial diversity. They are also starting to have other more practical advantages e.g. soil can be stored frozen for later analysis and high throughput is possible for some methods. It was felt that although there is some loss of information in using high throughput methods, this was compensated for by ease of analysis when dealing with a high number of samples.

[4] A fundamental problem with many traditional physiological and biochemical methods is that they depend on the cultivation or growth of the microorganisms and/or the analysis of their phenotypic expression (e.g. respiration, enzymes, catabolic potential).

Table 9. Matching the top-ranking biological indicators against ecological services for each soil function.

	BASED ON FA SCORE	FOOD AND FIBRE PRODUCTION													ENVIRONMENTAL INTERACTION							SUPPORT HABITATS AND BIODIVERSITY								
		C cycling	Decomposition of organic matter	N cycling	P cycling	S cycling	N fixation	Primary (microbial) activity	Soil food web transfers	Disease & pest transmission/suppression	Nutrient supply from symbioses	Redistribution by bioturbation	Bio-aggregation of soil	Degradation/immobilisation pollutants	C retention/release	N retention/release	P retention/release	Tolerance/Resistance (toxins)	S retention/release	Redistribution by bioturbation	Bio-aggregation of soil	Habitat for rare soil species	Germination zone for plants	Nutrient supply from symbioses	Food source (aboveground)	Reservoir for soil biodiversity (taxonomic)	Reservoir for soil biodiversity (genetic)	Reservoir for soil biodiversity (functional)
Fsf	**DEPLOYMENT STATUS = 2**																												
769	TRFLP - Ammonia oxidisers/denitrifiers			x												x										x	x	x
615	PLFA profiles	x		x				x			x				x	x							x	x		x		x
437	TRFLP - ITS fungal	x	x		x					x	x		x	x	x		x	x			x	x	x	x	x	x	x	x	x
311	Multiple substrate induced respiration (MSIR) GC	x	x					x						x				x											x
302	Nematode Baermann extraction procedure							x	x	x								x					x			x	x	x	x
295	TRFLP - Bacteria	x	x	x	x	x							x	x	x	x	x	x	x		x		x			x	x	x	x
188	Microarthropods Tullgren dry extraction		x						x						x			x				x			x	x			x
173	On site visual recording - flora and fauna											x	x							x	x	x			x	x			x
172	Microplate fluorometric assay - multi-enzyme	x	x	x	x	x								x	x	x	x	x	x				x						x
146	TRFLP - Archaea														x											x	x	x	x
123	TRFLP - Methanogens/methanotrophs														x												x	x	x
123	Invertebrates pitfall traps		x						x	x								x				x			x	x			x
121	TRFLP - Actinomycetes	x	x											x	x			x								x	x	x	x
	DEPLOYMENT STATUS = 1																												
437	TRFLP - Nematodes							x	x	x								x								x	x	x	x
313	Multiple substrate induced respiration (MSIR) MicroResp	x	x					x						x	x			x											x
291	TRFLP - Protozoa							x	x	x								x								x	x	x	x
111	qPCR AM Fungi				x					x	x		x				x	x			x		x	x		x	x	x	x

Hence, as high-throughput methods, TRFLP was considered the most appropriate for the microbial taxa. TRFLP is one of several methods for DNA/RNA fingerprinting and provides profiles that are representative of the genetic structure of the community, as defined by the selected genetic primers. *Outstanding issues:*

- Despite routine use in many laboratories, Standard Operating Procedures (SOP) would be required that detail the steps used in extraction, PCR, restriction/incubation conditions and fingerprint analysis.
- Not all taxa are routinely characterised. Bacteria, fungal ITS, ammonia oxidisers and archaea methods are widely available but there has been less work on actinomycetes, methanogens, methanotrophs and denitrifiers. Work is required to identify the most suitable primers and optimise the PCR, restriction and fingerprinting steps.
- The methods, including different primers, have not been applied to a wide range of soil types in the UK with no systematic understanding of discrimination potential and sensitivity to large-scale spatial and temporal variation. Consequently it would sensible to pilot their applicability across a range of representative UK soils.

4.2. Soil microbial community structure and biomass

The use of extracted lipids, in particular phospholipid fatty acids (PLFA), as signature lipid biomarkers, has become widely used to study soil microbial communities (c.f. Zelles, 1999) because, like nucleic acid methods, it is not dependent on the growth or morphology of organisms. The total PLFA content is indicative of the total viable biomass while individual PLFAs, or small suites of PLFAs, can be related to community structure as they are found predominantly in one group e.g. fungi, bacteria, Gram negative bacteria, actinomycetes. The main advantage of PLFA is that it is a semi-quantitative method and it gives wide coverage of the soil microbial community. Total PLFA is reported to be well correlated with other methods for estimating biomass and the markers detected cover bacteria, fungi, actinomycetes and other eukaryotes all in one analysis. The large body of data on PLFAs in soil has shown they can be highly discriminatory of land use, soil type, management and pollution. There appear to be some trends across studies which show that ratios of bacteria to fungi change in predictable ways e.g. extensification of grassland and heavy metal pollution. At the expert workshop, there was clear acceptance of PLFAs as a valuable method for microorganisms with its main strengths being recognised as the breadth of soil microbial groups being covered, quantification of biomass for each group and interpretability of certain summary data e.g. total biomass:C ratio, fungal:bacterial ratios. One of the main advantages seen was its value for money since information on community structure as well as microbial biomass could be obtained from the same analysis. *Outstanding issues:*

- PLFA is potentially more time consuming than other methods and may require to be measured in more than one laboratory if there is a large number of samples. There is however considerable variation in current methods. Development of a SOP with QC reference soils should be tested in an inter-laboratory trial to be fully confident of reproducibility of results between laboratories.
- PLFA analysis has been widely used but as with most methods there has been no systematic study of the full range of soil types that might be covered by a soil monitoring exercise. Further optimisation of sample size for each soil type would be required for PLFAs fractions.
- Variable numbers of PLFA peaks can be identified depending on the rigour and time available to a lab. Hence a study of a systematic set of samples could look at the number of PLFAs required to optimise discrimination and sensitivity compared to the level of effort required.

4.3. Multiple substrate induced respiration

Carbon cycling is fundamental to soil function and the respiration of CO_2 from soils, from community-level biotic activity, is a basic indicator of intrinsic C cycling. Measurement of this property in isolation does not provide useful discrimination, and hence ranked low in the logical sieve. However, assays of C mineralisation that put the basal respiration rate into some form of wider context are considerably more powerful. The concept underlying the multiple substrate-induced respiration (MSIR) approach, also referred to as community-level catabolic profiling, is to characterise how a soil community responds to exposure to a range of carbon substrates of differing chemical status (Degens and Harris, 1997). The principle is to add a range of substrates, separately but simultaneously, to aliquots of a soil sample and measure the short-term respiratory responses that ensue. The resultant catabolic profiles reflect the ability of the extant soil microbial community to utilise the substrates as an energy source, and provide a measure of the prevailing functional diversity of the soil microbial community. In monitoring terms, changes in MSIR profiles may therefore be interpretable in indicating shifts between ecological states, or act as indicators that the metabolic capability of the soil community is changing, moreover in an interpretable manner given the nature of the compounds that are involved. The respiratory responses of soils to such substrate addition can be measured by a variety of techniques. All approaches scored highly via the logical sieve, with a small-scale version (MicroResp^{TM}; Campbell *et al*., 2003) ranking top by a small margin but falling into Deployment Status 1 since it has yet to be tested in a variety of laboratories or deployed across a range of soil: land use combinations. This method is certainly more suited to high-throughput processing of soil samples and does not require specialist equipment beyond a 96-well microplate reader. It therefore offers exceptional potential as a candidate indicator. Respiration determination by use of gas chromatograph (GC; Degens and Harris, 1997) is feasible to determine MSIR profiles but is extremely laborious, restricting potential throughput of samples, and unlikely to be feasible for large-scale soil monitoring. *Outstanding issues:*

- The immediately deployable method (MSIR by GC) is highly laborious and therefore the potential to achieve high through-put using MicroResp^{TM} system should be determined. This will require a comparative assessment of the two techniques to ensure comparable substrate responses and reproducibility between laboratories.

4.4. Multi-enzyme profiling

Biochemical reactions in soils are mediated by enzymes produced by the soil biota as part of their metabolic machinery. It follows that measuring the activity of enzymes provides information about the functional repertoire of the biota. There is a plethora of enzymes that can be profiled, that can relate to virtually any defined biochemical transformation (Burns and Dick, 2002). Many individual enzymes were considered in the logical sieve framework, but ranked lower than the multiple enzyme fluorometic approach since this assay can inform on more than one ecological process. An increasingly wide range of fluorescently-labelled substrates are available which enable sensitive measurements to be made on small samples, permitting high-throughput assay systems that can profile user-prescribed suites of enzymes (Marx *et al*., 2001). This method is suited primarily to enzymes involved in C-cycling, since the majority of fluorescently labelled substrates available target C-transforming enzymes. However, fluorescently labelled substrates that relate to phosphatase and sulphatase are also commercially available, and others may enter the market over time. Enzyme activities are expressed at rates of activity (units of substrate utilised per unit time) and are therefore transferable between studies, assuming unification of assay conditions. Multivariate profiles can be summarised using data-reduction techniques, however these affect the transferability of such data. *Outstanding issues:*

- There are apparently no published inter-laboratory trials utilising this technique, so it is unclear how reproducible the assays are under such circumstances.
- No published consideration has been given to potential reference samples to quality-control this assay. In principle, prescribed purified enzymes, or mixtures thereof, could be utilised for this purpose, for example based on stipulation of number of International Enzyme Units (IU). This concept would be appropriate to explore if the assay were to be applied in a full-scale monitoring programme.

4.5. Nematodes

Nematodes are among the most abundant multi-cellular soil organisms and their potential as biological indicators of soil quality is widely acknowledged (Mulder *et al*., 2005) with changes in nematode community structure corresponding to changes in soil nutrient cycling, plant growth and plant species composition. Nematodes can be passively extracted over a short-time period from soil samples of a known weight or volume into water, with the soil gently heated from overhead lights to encourage the nematodes to move out of the soil. The efficiency of the extraction varies with soil type and the exact methodology. The Baermann method has however been in used for many decades and proved reliable in obtaining estimates of nematode populations. It is also relatively cost-effective method to set-up and run. The principal effort comes after extraction in the enumeration and identification of the individual nematode taxa. Several indicators have been proposed from nematode taxa and community structure (Mulder *et al*., 2005). The most widely appreciated indicator is the Maturity Index (MI) which reflects the distribution of nematodes across functional groups (Bongers 1990). More amenable indicators are currently the total number of nematode taxa and abundance of individual functional groups which are proving reliable in discriminating between different management practices within the Dutch Soil Quality Network (Mulder *et al*., 2005). An important consideration for all indicators is the sampling period, since community structure alters throughout the year with respect to seasonality. At the expert workshop there was general acceptance that nematodes were a potentially useful biological indicator since changes in functional groups correspond to changes in soil C and N cycling, as well as the crop pest importance of some groups. Discussions on the methodologies identified that other methods can extract more of the nematode community but these are often more labour and equipment intensive and Baermann extraction is a simple and effective method for general assessments of the nematode community structure and for handling large numbers of soil samples.
Outstanding issues:

- Most laboratories use their own variations of the Baermann extraction technique with in-house constructed equipments. Therefore a standard operating procedure is required to establish consistency between survey periods and laboratories.
- Consideration is required to identify which metrics show the greatest discrimination and sensitivity to environmental pressures and drivers.
- Identification to functional group and species relies heavily on highly trained experts. Nucleic acid techniques have potential to help ease the reliance on a dwindling reserve of taxonomists and also offer the potential for consistent identification and rapid through-put.

4.6. Microarthropods

This group, in particular acari (*mites*) and collembola (*springtails*), are amongst the most numerous and widespread soil invertebrates in British soils where their primary functional significance is C/N release from decomposition and the soil microbial community and food sources for the soil food web and beyond, in particular for birds. Both the acari and

collembola have been proposed as reliable biological indicators and have been used in a number of soil quality monitoring projects, including CS2000 across Great Britain. With both groups, the enumeration from Tullgren dry extraction is fairly straight-forward although higher levels of identification requires expert skills and reliable keys for identification. There is currently no published key for UK soil mites however a Collembola key will be published shortly by the Field Studies Council. Quality control is mainly through checking the efficiency of individual personnel with reference specimen. Tullgren extractions support the passive extraction of invertebrates from soil samples, of known weight or volume into a preservative, through the application of heat over a set period of time, typically several days. The extraction process itself is relatively cost-effective and easy to use with much of the effort going into the identification and enumeration of the invertebrates post-extraction. Once the invertebrates are extracted into a preservative, the samples can be stored for a long period prior to further analyses and are amenable to long-term archiving. *Outstanding issues:*

- The original Tullgren extraction method has been modified over the years with many different adaptations in current use. This hampers comparisons and compatibility. Standardisation could be introduced via equipment specification, length of extraction period used and testing extraction efficiencies.
- Identification to functional group and species level relies heavily on trained staff and expert taxonomists. With a rapidly declining pool of taxonomic experts, there is pressing need to investigate the potential to use molecular techniques and/or digital recognition for consistent identification and rapid through-put.
- Some consideration is required to determine which metrics show the greatest discrimination between soil:land use combinations and sensitivity to environmental pressures and drivers.

4.7. On-site visual recording of soil fauna and flora

This method (as a combination of different potential indicators) scored highly as it is one of the few methods that could be used with relative ease to assess the presence of key groups of soil organisms that would otherwise be under-represented; namely ants, fungal fruiting bodies and earthworms via casts. Truly reliable on-site recording does however require a consistent set of methodologies (c.f. Swift & Bignell, 2001) which have not yet been developed for the UK environment. *Outstanding issues:*

- Review the requirements to develop a consistent set of methodologies that could be applied across the range of soil:land use combinations of the UK.

4.8. Ground-/soil-dwelling invertebrates

There is a substantial body of literature on the use of ground-dwelling/soil invertebrates as biological indicators and pitfall traps are a well established technique for assessing the presence and activity of ground-dwelling soil invertebrates which have been widely used for environmental surveillance. However, this method requires return visits to a sampling site (e.g. 2 weeks after deployment) which was considered impractical for a national-scale soil monitoring scheme.

5. Analyses of existing data

The development of national-level target values/ranges for biological indicators of soil quality is reliant not only upon sufficient data on across the country but also upon the availability of supporting environmental data (RCEP, 1996). These supporting data enable not only the discrimination across environmental factors to be established but also the

attribution of changes/responsiveness to specific pressures or drivers against the background of other causative agents e.g. sampling time, weather conditions etc. Approaches to establish the discriminatory power, and sensitivity, of putative indicators are necessarily multivariate, and statistical tools are well developed to analyse such data and identify key discriminators. Preliminary analyses suggest that simple ordination of primary data can be remarkably effective (e.g. Bentham *et al*. 1992). Whilst a desirable goal for indicators is simplicity, there has been relatively little consideration in the literature of the practicalities in relation to soil biological monitoring. while these issues all carry the assumption that biological indicators will meet targets or fall within specified ranges, and those outwith such ranges indicate potentially adverse changes. This project used a series of case studies to investigate the potential for setting target values and ranges based on primary data and multivariate summaries of a number of key measurements. A summary of the findings and key lessons from each case study is presented below with the full description available in the appended Full Report. Several datasets were used from collaborating institutions and UK research programmes including the SEERAD Micronet project, Countryside Survey 2000 and NERC Thematic Programmes (Soil Biodiversity, GANE-2 and EDGE Programmes). The case studies examined specific issues regarding:

- The availability of data to set target values-ranges for different soils and land uses;
- The discriminatory power of different biological indicators, and their associated methodologies, with the aid of multivariate data analyses;
- The range of soil:land-use combinations that would underwrite an effective pilot-scale study of application of the identified biological indicators, and prioritise these.

5.1. Case Study I: Combined analysis of PLFA datasets from three sources

Many individual studies have demonstrated that soil PLFA profiles discriminate between different soil:land-use combinations, and can be used to assess impacts of environmental pressures on soil microbial communities. These studies indicate that there is some consistency in PLFA profiles, and associated responses to environmental factors. However, the project team were unable to identify any published instance where PLFA data had been combined from a number of sources to test the extent to which such consistencies prevail. This is a pertinent issue in the context of broad-scale or national-level monitoring since the utility of existing datasets would be enhanced if they were able to be combined effectively. The aim of this case study was to explore the consistency of soil PLFA profiles and issues involved with attempting to combine datasets relating to soil PLFA profiles derived from a number of sources. PLFA profile datasets, covering a wide range of habitats from a variety of studies carried out within each organisation, were provided by each project partner. A total of 522 soil PLFA profiles were collated with their associated habitat and soil type. It was not possible within the confines of this study to obtain a complete set of ancillary data which could be used as covariates in an analysis, such as soil type or associated chemical properties. A total of 55 distinct PLFAs were apparent across these datasets. However, of these, only 18 were unequivocally in common in all datasets (Table 10). Other PLFAs may be in common, but the variation in nomenclature and some uncertainty in identification made cross-comparison uncertain. Only unequivocally common PLFAs were used to produce a combined dataset for further analyses. This necessary caveat would certainly have resulted in a loss of resolution in the data, since dimensionality was reduced in parallel (see Appendix 1: Full Report). The combined PLFA profiles were analysed using the data-reduction technique of principal component analysis (PCA), and resultant components ordinated graphically and further analysed by one-way analysis of variance applied to the first three principal components (PC1, PC, PC3), which accounted for 36, 20 and 19% of the variation respectively. Four PLFAs were predominantly attributable to the

separation between profiles, with four others contributing relatively highly. Principal components were highly significantly aggregated according to vegetation classes, habitat (Figure 1) and source organisation. This conclusion is entirely in agreement with the cohort of antecedent studies, and it is notable that even with reduced datasets such discrimination prevails. Four PLFAs were predominantly attributable to the separation between PLFA profiles, with another four PLFA's contributing relatively highly. There were no obvious instances where "common" soil:land-use combinations were being analysed at any two centres and hence it was not possible to test the hypothesis that PLFA profiles from the same soils/land uses would be intrinsically similar from different laboratories. There was also a very skewed distribution of number of observations between different categories, with n ranging over two orders-of-magnitude which confounds statistical analysis since information about the variance of data where n is low is compromised. *This exercise provided the following key lessons:*

- There is no published information on inter-laboratory variation in PLFA analyses, and the dataset collated did not inform this issue due to the lack of common soil samples.
- A large range of individual PLFAs are detectable under a wide range of circumstances. Different laboratories appear to identify slightly different PLFA suites which is confounded by different laboratory methodologies and a diverse nomenclature which makes cross-referencing difficult; this requires standardisation.
- A robust standard operating procedure (SOP), with quality-control standards, is needed to ensure consistency between PLFA profiles and compatibility between analyses from different laboratories.
- A standard suite of PLFAs for multivariate characterisation of soil phenotypes is a desirable goal and these analyses suggested that it may be achievable; 4 PLFAs explained 36 % of the variation in the datasets examined here. A more extensive dataset would be required to inform the prescription of such a suite.
- Analyses of combined data indicated that PLFAs can discriminate effectively between habitats and vegetation types.

5.2. Case Study II: soil invertebrate data from a single national-scale survey

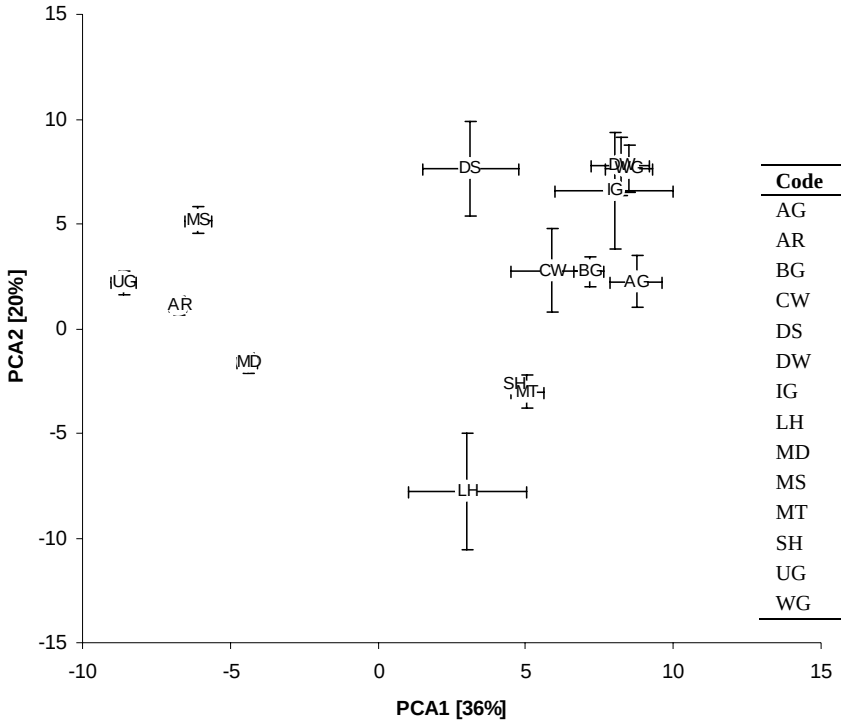
Consistent patterns and ranges for soil invertebrates can be discerned for environmental strata use combinations if sufficient information has been collected in a coherent and directly comparable manner (Black *et al*., 2003; Ruf & Beck, 2005). A large dataset is available from a national-scale assessment of soil microbial (see below) and invertebrate diversity carried out during Countryside Survey 2000 (see Haines-Young *et al*., 2000 for details on Countryside Survey). The biological methods for CS2000 were chosen for a variety of reasons, including practicalities and costs i.e. not specifically as reliable indicators. In brief, invertebrate and microbial assessments were made on soil taken ~1200 locations in 1998/99 with sampling supporting close correspondence to vegetation and other available soil information. For this case study, data on acari (soil mites) and collembola (springtails) were analysed since these two groups occurred in >80 % of all soil samples and were numerically dominant from dry extractions. Soil invertebrate numbers, including acari and collembola, are known to be highly variable both in time and space and generally require a large number of samples to detect change against the background of this inherent variability. Variability can be reduced by sub-sampling to reduce individual sample variability and/or by sampling a large number of locations. The Countryside Survey data form the latter and resulted in a significant number of samples with no records. Although this does not prevent discrimination analyses due to the large sample n (<1000), it does make interpretation of absolute populations more difficult. Consideration of the proportions of acari and collembola can help to address this issue since proportions of both

discriminate between, and within, a range of environmental strata across the British countryside.

Table 10. Individual PLFAs detected by source; those in bold are common to all three sources.

PLFA	MLI	CEH	CRA	PLFA	MLI	CEH	CRA	PLFA	MLI	CEH	CRA
C12:0	1			**C16:1w5**	1	1	1	C17:0br	1	1	
C13:0	1			**C16:1w7 c**	1	1	1	**C17:0cy**	1	1	1
C14:0	1	1	1	**C16:1w7 t**	1	1	1	**C18:2w6,9**	1	1	1
C14:0i	1			**C17:0**	1	1	1	C18:2w8,12	1		
C14:1w9	1			**C17:0i**	1	1	1	C18:3w6,8,13	1		
C14:1w9t	1			C17:1w7	1			C19:0	1		
C15:0	1	1	1	C17:1w8c	1	1		C19:0c			1
C15:0ai	1	1	1	C17:1w8t	1			**C19:0cy**	1	1	1
C15:0i	1	1	1	**C18:0**	1	1	1	C19:1w6	1	1	
C15:1			1	C18:0(10Me)	1			C19:1w8	1		
C16:0	1	1	1	C18:0br	1	1		C19:2			1
C16:0(10Me)	1			C18:1w10 or 11	1	1		C20:0	1		1
C16:0ai			1	C18:1w13	1	1		C20:1	1		
C16:0br	1			**C18:1w7**	1	1	1	C20:1w9	1		
C16:0i	1	1	1	**C18:1w9**	1	1	1	C20:4w2,6,10,14	1		
C16:1			1	C17:0(10Me)	1			C20:4w3,6,9,12	1		
C16:1i	1		1	C17:0(7me)		1		C20:4w6,9,12,15	1		
C16:1w11c	1			**C17:0ai**	1	1	1	C20:5w3	1		
C16:1w11t	1	1									

MLI = Macaulay Institute; CEH = Centre for Ecology & Hydrology; CRA = Cranfield University; SUM = number of representatives across sources



Code	Habitat group	n
AG	Acid grassland	10
AR	Arable	85
BG	Bog	29
CW	Coniferous woodland	4
DS	Desert	3
DW	Deciduous woodland	8
IG	Improved grassland	2
LH	Lowland heath	2
MD	Meadow	66
MS	Miscellaneous	36
MT	Montane	24
SH	Dwarf shrub heath	186
UG	Grassland	48
WG	Wetland grassland	12

Figure 1. First and second principal components from principal components analyses of PLFA profiles from collated datasets, aggregated according to habitat groups as denoted in the legend below. Bars show s.e. (n= as in legend).

A range of statistical analyses were carried out using mixed-model ANOVA and summary statistics produced to illustrate the potential for setting ranges/targets between and within the different strata (see Appendix 1: Full Report). The majority of summary statistics are based on medians with 25-75% quartiles plus non-outlier ranges since these reflect the entire populations sampled. Statistical results based on discrimination across vegetation types (Table 11) translate into fairly distinct ranges and give some impression of what might happen to the proportions of acari and collembola under land use change. Various soil:land use combinations were analysed (see appended full report) to illustrate the potential to establish expected values for different combinations although testing discrimination is constrained by sample size. Soil properties were also shown to be important in discrimination (Figure 2) with statistical analyses indicating soil organic matter and soil pH are major determinants in proportions of acari and collembola within JNCC Broad Habitat types.

Table 11. Results from mixed model ANOVA Type 1 Tests of Fixed Effects

Group % (arcine)	Treatment	Num d.f.	Den d.f.	F	P	Tukey post-hoc comparison to determine differences between treatment means
Acari	CVS AVC	7	778	33.18	<0.0001	Moorland grass mosaic and heath&bog means greater than means in all other habitats
Acari	NVC	112	817	3.32	<0.0001	
Acari	Broad Habitat	14	781	17.34	<0.0001	Arable+horticulture and improved grass means less than means in all other habitats
Collembola	CVS AVC	7	702	17.14	<0.0001	Infertile grass means less than means in crops& weeds, tall grass & herb, fertile grass
Collembola	NVC	112	792	2.16	<0.0001	
Collembola	Broad Habitat	14	702	6.80	<0.0001	Arable+horticulture means less than means in all other habitats

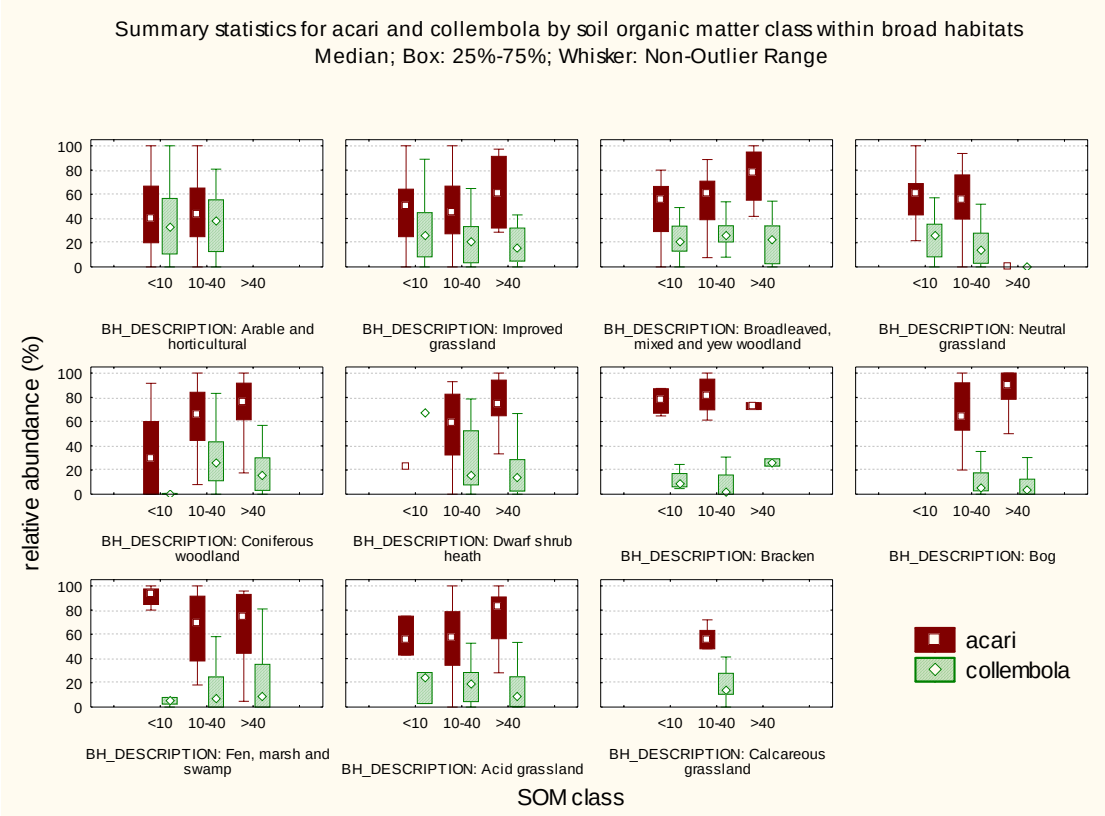


Figure 2. Ranges of acari and collembola proportions by JNCC Broad Habitats and soil organic matter classes.

This exercise provided the following key lessons:

- A large sample size will be required to establish discrimination or sensitivity against background variability; Bloem et al. (2003) recommend 20 replicates per "soil type".
- Proportions can be used to reduce issues of variability in soil invertebrates.
- Simple statistics may be used to establish expected ranges, or reference values, when adequate data are available. The most appropriate statistics, or methods of displaying indicator ranges/values, will be dependant upon a) type of indicator output e.g. numerical versus ordinal, b) knowledge of population, c) requirement for absolute values versus suitability e.g. triggers vs. normality ranges.
- Soil invertebrates discriminate better at habitat level than soil type level. The choice of habitat classification will depend upon policy requirements, sample size and the level at which vegetation information is collected.
- Measured soil properties (e.g. pH and SOM) can be important predictors of indicator values. An optimal set for co-analyses needs refining.

5.3 Community level physiological profiling in a single survey

The deployment of BIOLOG™ for community level physiological profiling (CLPP) in CS2000 provided useful insights into issues likely to be encountered in deploying a microbial indicator in soil monitoring. For example, logistical factors are important since monitoring will probably result in large numbers of samples to the analytical laboratory over extended periods of time. This issue will be exacerbated by methods that require immediate processing. The BIOLOG™ method assays the utilisation of 95 different C sources which results in a large multivariate data set. The integrated data analysis of all 95 substrates is complex and it can be difficult to summarise findings simply without overlooking the complexity of the details. In the CS2000 BIOLOG™ data, for example, there were over 900 samples analysed from across all UK major habitats. For data from CS2000 there was some discrimination between JNCC Broad Habitats but wide scatter for categories where sample N was relatively low. The resulting ordination plot is complex and is sensitive to the number of replicates per group (Figure 3); this plot indicates that discrimination between sites is virtually absent. Therefore, great care must be taken in interpreting the differences. The use of average responses (e.g. average well colour development), total number of substrates utilised (substrate richness) or simple (diversity) indices to summarise the data are also possible. Table 12 shows how the catabolic versatility (*sensu* Wenderoth and Reber, 1999) varies with broad habitat type. There are alternatives CLPP methods (e.g. Degens and Harris, 1997; Campbell *et al*., 2003) that may overcome some of the technical and scientific difficulties associated with BIOLOG™ but the same issues about temporal (seasonal) influences, sample storage, soil preparation and conditioning, data analysis requirements and QC would need to be evaluated.

This exercise provided the following key lessons:

- Careful consideration of logistical issues will be required for implementation of similar indicators requiring relatively fast turnover of samples for lab. analyses;
- Further work is require to determine which primary measurements and/or indices from CLLP-type approaches would be the most useful for national-scale soil monitoring.

Table 12. Mean % substrate use in BIOLOG™ assay by CS2000 soils, classified according to JNCC Broad Habitats. ANOVA showed no significant differences between Broad habitats.

JNCC Broad Habitats	% Substrate Use	Sample N	s.d.
Broadleaved, mixed and yew woodland	94.23	60	12.25
Coniferous woodland	94.82	66	9.68
Boundary and linear features	100	1	0
Arable and horticultural	92.87	226	8.73
Improved grassland	93.18	290	16.96
Neutral grassland	92.19	41	21.59
Calcareous grassland	97.37	6	2.56
Acid grassland	93.87	50	19.64
Bracken	90.23	18	22.93
Dwarf shrub heath	92.69	78	17.08
Fen, marsh and swamp	97.52	31	3.23
Bog	94.36	64	14.08
Inland rock	100	1	0
Built-up areas and gardens	92.42	10	18.45

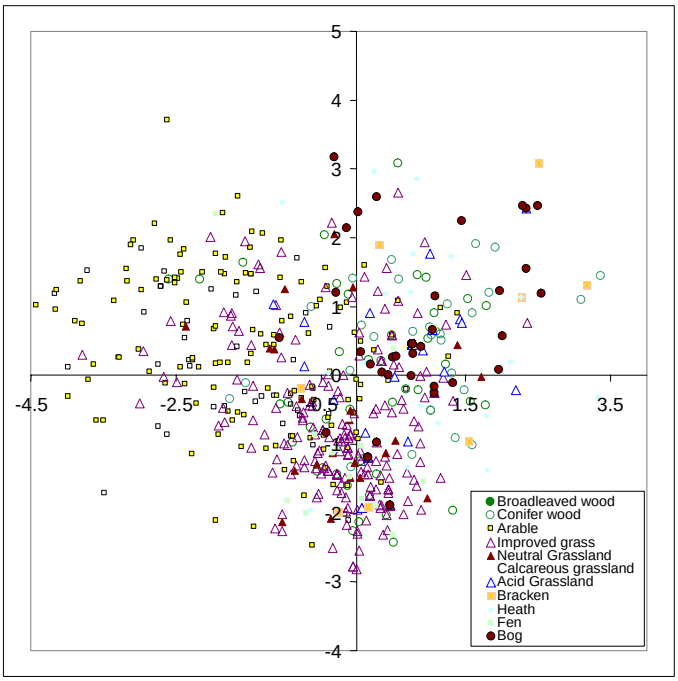


Figure 3. Ordination plot from canonical variate analysis (CVA) using substrate utilisation by CS2000 soils after 4 days incubation in Biolog GN plates, categorised by JNCC Broad Habitats. Data reduced to 16 components by PCA (covariance method) prior to CVA. Sample N<5 excluded.

6. Conclusion

This project successfully identified a priority list of 13 biological indicators that are highly relevant to monitoring soil quality at a national scale with the adopted approach robust, repeatable and auditable. The logical-sieve framework enabled a consistent synthesis of available information and the semi-objective assessment of a large number biological indicators (183) against a series of scientific and technical criteria relevant to national scale soil monitoring and stakeholder priorities. A complete report of the outputs from the logical sieve is available on request. The power of this approach is that it provided a clear record and audit trail on the decision-making process and would allow the inclusion of further indicators into the framework should this be required in the future. The basic framework of the logical sieve could be expanded according to need. Important issues regarding

deployment of biological indicators in national-scale soil monitoring were highlighted from the selection process, review of the priority indicators with recommended methods and analyses of existing data Specific objectives for field evaluation of the priority biological indicators are identified as;

1) Bring the Microresp™ method for multiple substrate-induced respiration (MSIR) to a deployable status for soil monitoring, as an alternative to MSIR by GC.
2) Establish standard operating procedures for the priority biological indicators.
3) Test the priority indicators for their comparative ability to discriminate between a range of soil:land use combinations.
4) Test the priority indicators for their sensitivity to distinct environmental pressures.
5) Determine the degree of surrogacy between these biological indicators.

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