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Selecting cost effective and policy-relevant biological indicators for European monitoring of soil biodiversity and ecosystem function

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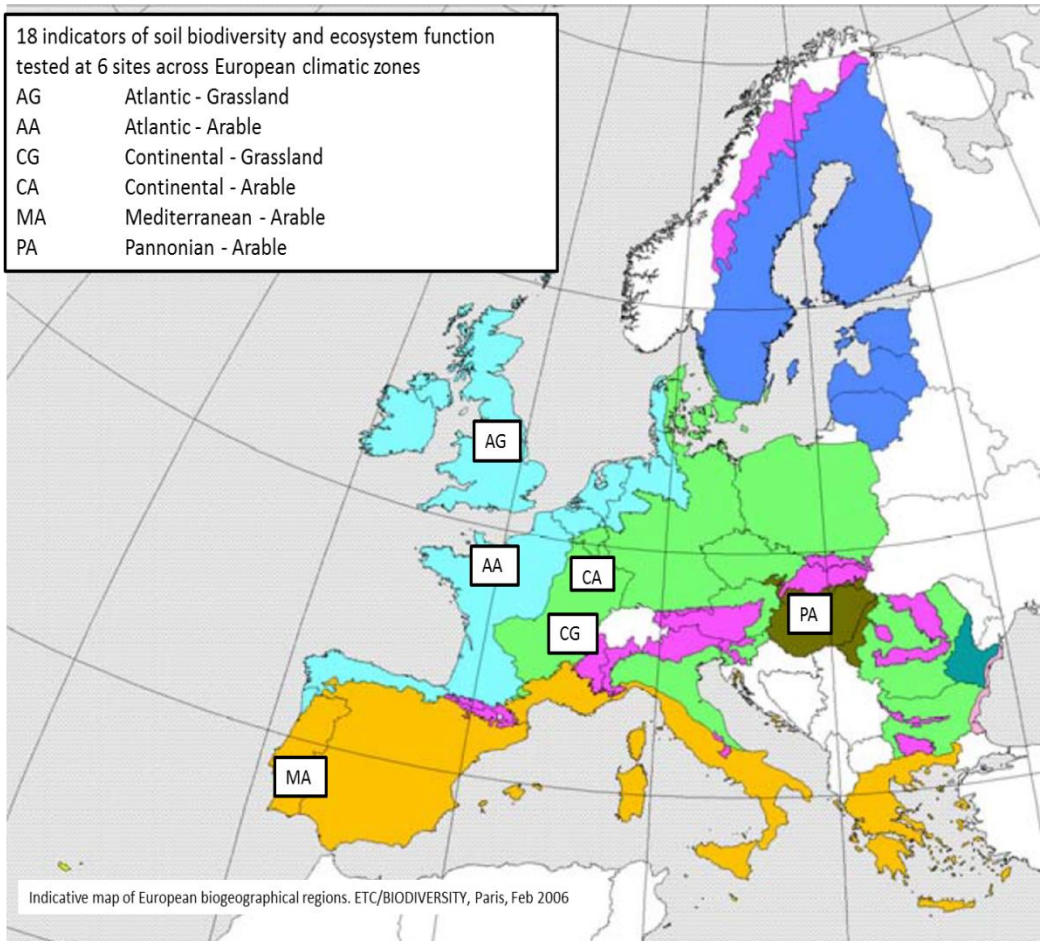
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Highlights

- Eighteen potential indicators selected using a logical sieve
- Indicators tested at six European sites across climatic zones
- No single indicator sensitive to all differences in land use intensity
- Recommended indicators for function are : earthworms; functional genes; and bait lamina
- For monitoring of biodiversity all taxonomic groups need to be addressed

Graphical Abstract



Abstract

Soils provide many ecosystem services that are ultimately dependent on the local diversity and belowground abundance of organisms. Soil biodiversity is affected negatively by many threats and there is a perceived policy requirement for the effective biological monitoring of soils at the European level. The aim of this study was to evaluate and recommend policy relevant, cost-effective soil biological indicators for biodiversity and ecosystem function across Europe. A total of 18 potential indicators were selected using a logical-sieve based approach. This paper considers the use of indicators from the 'top down' (i.e. concerned with the process of indicator selection), rather than from the 'bottom up' detail of how individual indicators perform at specific sites and with specific treatments. The indicators assessed a range of microbial, faunal and functional attributes newer nucleic acids based techniques, morphological approaches and process based measurements. They were tested at 6 European experimental sites already in operation and chosen according to land-use, climatic zone and differences in land management intensity. These were 4 arable sites, one each in Atlantic, Continental, Mediterranean and Pannonian climate zones, and 2 grassland sites, one each in Atlantic and Continental zones. At each site we sampled three replicated plots of contrasting management intensity and, while the treatments varied from site to site, their disturbance effects were quantified in terms of land use intensity. The field sampling and laboratory analysis were standardised through a combination of ISO protocols, or standard operating procedures if the former were not available. Sites were sampled twice, in autumn 2012 and spring or autumn 2013, with relative costs of the different indicators being determined each time. A breakdown of the cost effectiveness of the indicators showed the expected trade-off between effort required in the field and effort required in the laboratory. All the indicators were able to differentiate between the sites but, as no single indicator was sensitive to all the differences in land use intensity, we suggest that an indicator programme should be based upon a suite of different indicators. For monitoring under the European climatic zones and land uses of this study, indicators for ecosystem functions related to the services of water regulation, C-sequestration and nutrient provision would include a minimum

suite of: earthworms; functional genes; and bait lamina. For effective monitoring of biodiversity all taxonomic groups would need to be addressed.

1. Introduction

Human societies are highly dependent upon healthy soils for the delivery of ecosystem goods and services, including provisioning (food, fibre, timber, fuel), regulation (climate, disease, natural hazards), waste treatment, nutrient cycling and cultural services (Millennium Ecosystem Assessment, 2005). Many of the key functions supporting these ecosystem services depend to a large extent upon the diversity, abundance and activity of organisms that inhabit the soil. This diversity varies in terms of its taxonomic richness, relative abundance and distribution according to soil type, climatic conditions, vegetation and land use. Against this background, soil biodiversity is also subject to various threats associated with human activity, including soil erosion, organic matter decline, and contamination, salinization, sealing, compaction of soil and climate change; all these threats impair soil biodiversity and functioning with negative consequences on ecosystem service delivery (Hooper et al., 2005; Gardi et al., 2013; Wall et al., 2012). Increasing agricultural intensity, for example, has been shown to generally reduce soil biodiversity (e.g., Tsiafouli et al., 2015), although this response is likely to be non-linear given the variation in management practices and soil conditions across sites and regions, and differences in the sensitivity of soil organism groups to management intensity. As a result, there is a strong and increasing policy requirement for the effective monitoring of soils at local, regional and national scales (EU, 2006a, b; Ritz et al., 2009; Turbé et al., 2010; Cluzeau et al., 2012). Moreover, this need has been stimulated by the Convention on Biological Diversity (http://www.cbd.int/), which includes a requirement for indicators capable of monitoring changes in soil biodiversity (Pulleman et al., 2012).

Most soil processes are mediated by soil biota in direct relationship with the physico-chemical properties of their environment. Furthermore, soil organisms have the ability to adapt rapidly to changes in climate and soil management in an integrative way, which makes them good

53 indicators (e.g. as argued for by Ritz and Trudgill, 1999). Biodiversity is a soil attribute in itself and
54 therefore relevant to an ecosystem level approach (Doran and Zeiss, 2000; Loreau, 2000; Lemanceau
55 et al., 2015). Biological indicators, therefore, are relevant for use in supporting policy and decision
56 making to achieve sustainable soil management (Francaviglia 2008; Pulleman et al., 2012; Havlicek,
57 2012). The application of biological indicators to assess changes in the delivery of ecosystem
58 functions is accepted practice both at national and European scales (Feld et al., 2009; Pulleman et
59 al., 2012; Faber et al., 2013; Lemanceau et al., 2015). Some applications derive from an
60 ecotoxicological perspective (e.g. Van Straalen 1998; Becaert and Deschenes, 2006). However, no
61 reference set of standardised biological indicators is available yet (Pulleman et al., 2012), largely
62 because of the variation in scope, goal and duration of monitoring schemes (Turbé et al., 2010).
63 Biological indicators have long been developed and applied in specific environmental situations,
64 making the extrapolation of values and applicability under different conditions difficult.
65 Furthermore, despite recent efforts to standardise, a wide range of different methods and
66 procedures are applied, preventing meaningful comparison of conclusions. National (Gardi et al.,
67 2009; Rutgers et al., 2009) and European (e.g. ENVASSO, Bispo et al. 2009) initiatives have been
68 undertaken to recommend indicators across Europe and elsewhere (Ditzler and Tugel, 2002; Black et
69 al., 2003; Turbé et al., 2010; Pulleman et al., 2012).
70 Reviews have compared a large range of biological indicators for scientific and technical
71 relevance to assist policy-makers in land management (Ritz et al., 2009; Pulleman et al., 2012; Turbé
72 et al., 2010; Aalders et al; 2009; Bispo et al., 2009; Paz-Ferreiro and Fu, 2016), with the consensus
73 being that major efforts remain to be made in order to standardise operational procedures and to
74 validate them for different types of land use (Faber et al., 2013). The selection of potential biological
75 indicators is only a step in developing a practical monitoring scheme (Doran and Zeiss, 2000), as
76 there are operational issues to be solved such as: ease of application, robustness, sensitivity,
77 laboratory accuracy, throughput, economic value and descriptiveness. The selection criteria for
78 biological indicators are well described (e.g. Turbé et al., 2010; Ritz et al., 2009; Aalders et al., 2009)

79 but consideration also has to be given to the cost-effectiveness of the indicators and the
80 interpretation of the results from the monitoring. Different stakeholders have different information
81 needs, and different indicators have to be developed to answer their specific requirements (Turbé et
82 al., 2010).
83 The aim of this study was to evaluate and recommend policy relevant and cost-effective soil
84 biological indicators for biodiversity and ecosystem function across Europe. Indicators were selected
85 and validated by a detailed examination at European experimental sites chosen according to land-
86 use, climatic zone and differences in land management intensity. We sought to develop indicators
87 applicable across Europe with no particular management treatment in mind, using a range of
88 treatments / land-use / climatic zones to assess the generality of the indicator performance and
89 information on their practicalities and costs to determine cost-effectiveness. As we are concerned
90 with the process of indicator selection we consider the use of indicators from a 'top down' approach,
91 rather than the 'bottom-up' detail of how individual indicators perform at specific sites and with
92 specific treatments. The objectives of this study were to describe: the selection process for potential
93 indicators to field test; criteria for the selection of sites at which to evaluate the indicators;
94 standardisation of the field sampling; interpretation of the data; and recommendations for the use
95 of biological indicators for soil biodiversity and ecosystem function at the European scale.
96

97 **2. Materials and Methods**

98

99 *2.1. Selection of indicators*

100

101 There are approaching 200 biological methods that could potentially be used in a soil
102 monitoring programme (Ritz et al., 2009; Aalders et al., 2009). To reduce this to a manageable
103 number for testing an initial list of 30 potential indicators (Table 1),including developing as well as

104 established methods in soil ecology, was prepared by a panel of approximately 50 European soil
105 biology researchers. There was an even spread of experts with experience in at least one of the
106 following fields: soil functional determinations; soil fauna; microbial ecology; and the use of state-of-
107 the-art molecular analyses. This 30 was then reduced to a logistically feasible number for evaluation
108 using a logical sieve assessment, as proposed by Ritz et al. (2009), to rank potential indicators for the
109 purpose of monitoring soil biodiversity and ecosystem function across Europe. As further described
110 by Stone et al. (2016) this approach enabled a structured ordering of potential indicators by
111 applying the following steps: (1) establishment of the purpose for which the monitoring will be
112 applied; which in our case was for monitoring changes in soil biodiversity and ecosystem functions
113 across Europe; (2) listing of potential biological indicators, derived from a wide range of sources
114 including literature, past European and national-scale studies included in a meta-analysis (Faber et
115 al., 2013) and a panel of European experts; and (3) classification of indicators into three operational
116 categories, namely: microbial, faunal and functional techniques. The indicators were then ranked in
117 order of their relevance to specific criteria (further described in Faber et al. (2013), albeit with the
118 specific definitions being modified). Indicators needed to be: measurable (related to the availability
119 of the necessary laboratory equipment and technical skills); cost-effective (includes capital and
120 consumable costs as well as the labour intensiveness in the field and the laboratory); policy-relevant
121 (to provide data on biodiversity and ecosystem functions for informed decision making); sensitive to
122 likely changes such as land use and disturbance; and fit for use (meaningful, spatiotemporally
123 relevant, understandable and open to standardization). An algorithm then calculates an overall
124 ranking score from these individual criteria (See Ritz et al., 2009; Stone et al., 2016). Eighteen
125 potential indicators were thus selected for subsequent evaluation by this ranking procedure,
126 following the tenets of the logical sieve, from the 30 originally considered (Table 1).
127
128 *2.2. Selection of sites for indicator evaluation*
129

As the objective was to determine how sensitive the selected indicators are to typical disturbances in the European situation, six sites across Europe were chosen that had: a consistent agricultural management history over several years; were characteristic of recognised European climatic zones; consisted of at least three, independent, replicated plots of two contrasting treatments which varied in intensity of management. Site details are given in Table 2 and summarised here as four arable and two grassland sites: Lusignan, Atlantic arable site in France, with rotations of grass-arable (least intensive) and continuous arable (most intensive) (http://www.soere-acbb.com/index.php/fr/, Kunrath et al. 2014 and Senapati et al. 2014); Scheyern, Continental arable site in Germany with long-term plots of minimum-tillage with small fertilisation (least intensive) and conventional tillage with large fertilisation (most intensive) as described by Zeitz et al. (2004); Moskanjci, Pannonian arable site in Slovenia, with long-term plots of minimum-tillage (least intensive) and conventional tillage (most intensive) according to Kaurin et al. (2015); Castro Verde, Mediterranean arable site in Portugal, where the least intensive plots were grass-arable rotation with minimum tillage and no fertilisation and the most intensive plots were conventionally ploughed and fertilised, as described by Marta-Pedroso et al., (2007); Yorkshire Dales, Atlantic grassland site in the UK, with paired plots of extensive and intensive grassland at three locations within the Yorkshire Dales National Park, as described by de Vries et al.,(2012); Hainich, Continental grassland site in Germany, with paired plots of extensive and intensive grassland as described by Fischer et al., (2010).

The management options chosen are typical for European soils. The intensity of land use at each site was calculated using the equation of Blüthgen et al. (2012), modified to include time since tillage and tillage depth. Thus:

Land use index (LUI) = N/mean + C/mean + LU/mean + 5(T/mean) + 5(D/mean)

Where: N = kg N ha^{-1} yr^{-1}

C = grass cuts yr^{-1} x 50 (to give C an equivalent weighting to N, LU, T and D))

LU = livestock units ha^{-1} yr^{-1} (grazing intensity)

T = days since tillage, converted to a negative exponential scale ($T = 187.47e^{-0.014x}$, where x = days since tillage) to account for tillage effects being most evident immediately after tillage.

D = depth (cm) of tillage

Mean = average (N, C, LU, T or D) for all plots at all sites

(T/mean) and (D/mean) were multiplied by 5 as a weighting to reflect that the impact of tillage was greater than that of mowing, grazing or fertilisation on soil biological processes.

2.3. Sampling and standardisation

The selected indicators chosen for validation required different sampling procedures: with some having to be measured *in-situ*; some requiring intact soil cores; and the rest needing a composite bulk soil sample. Samples were then sent to the various analysing laboratories around Europe, such that each indicator was measured by only one laboratory. A coding system was developed to give each sample a unique identifier, linked to a searchable database designed for long-term data storage of the results collected. For standardisation of sampling, detailed standard operating procedures (SOPs, with step by step instructions and photographs to ensure clarity) and a video of the composite soil sampling were distributed before sampling began (http://www.youtube.com/watch?v=_k7BEInBXEc&feature=youtu.be).

Samples were taken on two occasions at each site, 2012 (autumn) and 2013 (spring or autumn), to ensure at least a minimum temporal variation, and included the *in-situ* measurements, intact cores and composite soil samples as outlined above. Sampling followed a prescribed pattern within an 8 m x 8 m area selected at a random location in each replicate plot (Figure 1). Within each sampling area there was: a composite soil sample prepared by mixing five soil samples taken by

auger from the top 15cm, this consisted of soil from a central auger and then four more augers 1 m away from the central point in North-South and East-West directions; five intact soil cores (5 cm diameter and 5 cm deep) were each collected for separate microarthropod and enchytraeid extraction (ISO 23611-2, 2006; ISO 23611-3, 2007), three soil pits of 35 cm x 35 cm (the depth varied according to the site conditions, but it was always between 10 and 20 cm) were dug for the hand-sorting and formaldehyde extraction of earthworms (ISO 23611-1, 2006), with collected earthworms being preserved in 70% ethanol in the field; bait lamina sticks were laid out in five blocks within each area (ISO 18311, 2012); water infiltration was determined using a double ring infiltrometer (DIN 19682-7, 2007). In the field, composite soil samples and intact soil cores were kept in an insulated box containing frozen 'cool blocks' until they could be stored at 4^{o}C in a laboratory. The composite soil samples were sieved through a 4mm diameter mesh and divided into aliquots appropriate for the different soil analyses. Aliquots of composite soil and intact soil cores were repackaged in insulated boxes with frozen 'cool blocks' and dispatched by 24hr courier to the analysing laboratories.

2.4. Laboratory analysis

Soil from the composite samples was analysed as follows: DNA was extracted (ISO 11063, Petric et al., 2011) and DNA yield quantified (Plassart et al., 2012). Extracted DNA was used to determine the structure of the microbial community by terminal restriction fragment length polymorphism of the archaeal, bacterial and fungal communities (TRFLP, Plassart et al., 2012), and also for quantitative PCR to determine abundances of the total bacterial community (16S rRNA) and of functional genes involved in nitrogen cycling by using the *amoA*, *nirK*, *nirS* and *nosZ1* genes as molecular markers (Bru et al., 2011). Ergosterol was quantified following alkaline extraction (de Ridder-Duine et al. 2006); Multiple substrate utilisation with MicroResp (Campbell et al., 2003, as modified by Creamer et al., 2009) used eight substrates: Water, L-Arginine, L-Malic Acid, Gamma Amino Butyric Acid, n-Acetyl Glucosamine,

D(+) Glucose, Alpha ketogluterate and Citric Acid; Extra-cellular Enzyme Activity (EEA; Johansen et al., 2005; Hendriksen et al., 2015) from the activity of β-1,4-glucosidase and cellobiohydrolase, α-1,4-glucosidase, β-*N*-acetyl-glucosaminidase, β-1,4-xylosidase, aminopeptidase, phosphatase and arylsulphatase; Nematodes, which were extracted by an Oostenbrink elutriation and Baermann funnel technique from 100 g of fresh soil for directed-T-RFLP analysis (Donn et al., 2012); Potentially Mineralisable Nitrogen (PMN, Canali et al., 2006); Hot Water extractable Carbon (HWC, Ghani et al., 2003); Microbial resilience to antibiotic (resilience) was determined in 96 well micro-titre plates as the difference in the lag-phase until growth of bacteria from a suspension of composite soil either with or without penicillin. We used six replicates of 200 µl of soil suspension (1.5 g dry weight equivalent soil 100 ml^{-1} Neff's modified amoeba saline (Page, 1988)) added to 200 µl of 1/10 strength Luria-Bertani medium either with 15 µg penicillin $well^{-1}$ (Penicillin from AppliChem, BioChemica, Penicillin G-Kaliumsalz) or without penicillin, incubated at 20°C and the optical density (450 nm) measured every 1 hr for 72 hrs with automatic agitation for 5 s prior to reading; Potential nitrification (Kandeler (1996), adapted to microplate reader as described by Sousa et al.,2004); Phospholipid Fatty Acid (PLFA) determinations were based on the guideline ISO/DTS 29843-2 (2011). The method proposed in this SOP results from a modification of the ISO guideline, including a second method for fatty acid identification (Francisco et al., 2015). Earthworms were counted, adults identified to species and juveniles to genus, and then weighed. Soil physical and chemical determinations were performed by the Soil Analysis Laboratory of INRA in Arras, France, which is accredited for soil and sludge analysis.

Soil from intact cores (5 cm diameter) was used to extract enchytraeids and microarthropods. Enchytraeids were extracted with O'Connor's hot/wet funnel method (O'Connor, 1962) (ISO 23611-3, 2006). Specimens were identified to species using the keys and techniques of Schmelz and Collado (2010, 2012), together with primary literature. Microarthropod extraction followed ISO 23611-2 (2004) using a Macfadyen high gradient extractor for 7 days, slide mounted and identified to species.

232 *2.5. Statistical analysis*

233

234 Data for individual measures were checked for homogeneity of variances and were
235 transformed to ensure a normal distribution for analysis. Abundances of enchytraeids and
236 microarthropods were converted to natural logarithms, while an angular transformation was used for
237 percent composition data. All analyses were performed using Genstat 14th edition. Univariate data
238 were analysed by two-way ANOVA while bait lamina data were analysed as a factorial ANOVA, using
239 the mean feeding activity per plot and depth and making a comparison between depth distribution
240 and treatment. Multivariate data [i.e. T-RFLP (bacteria, archaea, fungi and nematodes analysed
241 separately), PLFA, EEA, MicroResp] were analysed using principal component analysis (sums of
242 squares and products) and the resulting principal component (PC) scores treated as univariate data.
243 Shannon and inverted-Simpson were calculated as diversity indices for: microbial T-RFLP, mites,
244 enchytraeids and earthworms.
245 For the global data across all sites, the univariate data were analysed by ANOVA using site,
246 management and year as the factors. For specific site effects, because the management was specific
247 to each site, the ANOVA analysis was run comparing control and treatment at each individual site
248 using treatment and year as the factors. A global multivariate analysis was run, as above, with: hot
249 water extractable C; potentially mineralisable N; Ergosterol; molecular biomass; 16S rRNA, *amoA*
250 from bacteria (AOB) and archaea (AOA), *nirK*, *nirS* and *nosZ1* gene abundances; enchytraeid
251 abundance; enchytraeid diversity (inverse Simpson); earthworm abundance; earthworm diversity
252 (inverse Simpson); mite abundance; mite diversity (inverted Simpson); resilience; nitrification;
253 nematode PC1; nematode PC2; PLFA PC1; PLFA PC2; MicroResp PC1; MicroResp PC2; EEA PC1; EEA
254 PC2; T-RFLP PC1; T-RFLP PC2 (T-RFLP PCs calculated separately for bacteria, archaea and fungi).

255

256 *2.6. Relative costs of analysis*

The costs associated with each indicator were estimated on a relative basis for the three areas of: 1) operation in the field; 2) operation in the laboratory; and 3) equipment / instrumentation. On each sampling occasion the field teams recorded the person-hours required for each task. The laboratory analytical teams then made observations on the ease of running each assay, the throughput (samples per unit time) and whether the assay yielded multiple or single endpoints. Finally, the capital costs of the main instruments used in each assay were noted. The methods were then ranked under each category for comparison.

3. Results

3.1. Selection of indicators

The result of the selection process based on a logical sieve was a weighted score for each of the 27 potential indicators (Table 1). Most of the higher-ranking indicators were taken forward for evaluation (Table 1), but some of them were not considered because of methodological or practical limitations. For example, the molecular methods for faunal indicators were only considered advanced enough for nematodes (Vervoort et al., 2012). Litter bags and bait lamina (with a logical sieve score of 500 and 492, respectively) were statistically indistinguishable and logistical considerations favoured the bait lamina assay. Protistan morphology was considered too laborious and specialist, while the other low-scoring potential indicators were considered inappropriate or duplicated by higher scoring indicators. Some low-scoring potential indicators were evaluated, such as water infiltration because of its direct relevance to the key ecosystem service of water retention. Basal respiration was not evaluated independently but could be inferred from the MicroResp assay using the substrate 'water'. Although denitrification is an important component of the nitrogen

282 cycle, determination is complicated by the partitioning between end products (N_2O and N_2) and is
283 better represented by the functional gene assay (see for example Wessén et al., 2011). The choice
284 between pyrosequencing (or other new sequencing technologies) and T-RFLP is discussed below,
285 while chip and other 'omic' technologies are currently too expensive and technically demanding for
286 routine monitoring purposes. A rapid indicator for microbial resilience to antibiotic, estimated as
287 growth in the presence of penicillin, was developed and tested.
288
289 *3.2. Indicator performance across all sites*
290
291 The land use intensity (LUI) at each site (Table 3), and especially the difference in LUI
292 between the differently managed plots, provides a means of comparing the intensity of the
293 management options at different sites. Thus, the arable sites (Castro Verde, Lusignan, Moskanjci,
294 Scheyern) had greater average LUIs (5.4, 12.4, 18.4 and 25.4 respectively) than the grassland sites
295 (Hainich and Yorkshire Dales, 5.2 and 1.2 respectively). The arable sites also generally had the
296 greatest differences in LUI (Table 3), an exception being at Lusignan where there was no difference
297 in LUI because of the incorporation of a grass ley in the arable rotation had been imposed three
298 years earlier, and so for the last three years the two management options had received exactly the
299 same treatment.
300 Some of the indicators, namely bait-lamina and water infiltration, could not be used across
301 all sites because of logistical constraints. The bait-lamina test requires the sticks with substrate to
302 remain *in-situ* for several weeks, depending on the climatic conditions and biological activity at the
303 field site, which was not always compatible with the field operations for actively managed arable
304 sites. The method used for water infiltration needs a large volume of water (up to 200 L per
305 individual determination) and can take more than 2hrs for a single determination, so for sites
306 remote from water sources this was simply not practical.

All indicators were sensitive to site (i.e. the sites could be differentiated on the response of the indicators). A principal component analysis of all data (Figure 2) showed that the grassland sites (Yorkshire Dales and Hainich) clustered together in the PC3-PC4 plot explaining 16% of variation and separately from the arable sites which formed a separate cluster. Sites were different from one another according to the principal component analysis using all the data, but also by analysis of individual indicators. For example: indicators that separated the Yorkshire Dales from Hainich were: MicroResp; enchytraeids; AOB; 16S; nosZ1; AOB; PMN; ergosterol; soil water content; nitrification; and T-RFLP (archaeal, bacterial and fungal). Lusignan gave a significantly different response than Moskanjci to: EEA; AOB; NirS; resilience; nitrification; and T-RFLP (archaeal, bacterial and fungal) (Supplementary Tables S1 and S2). Measures of biodiversity, although showing significant differences between sites, were idiosyncratic (Supplementary Table S1). Thus, for example: using enchytraeid H' the Yorkshire Dales and Hainich sites were more diverse than Moskanjci and Scheyern; with earthworm H' Yorkshire Dales was equally as diverse as Moskanjci and Scheyern but Hainich was less diverse; with mite H' Scheyern was the least diverse site. The grassland sites, Yorkshire Dales and Hainich, had the greatest faunal diversity with Castro Verede and Scheyern having the least. Microbial biodiversity as determined from T-RFLP was also idiosyncratic, but showed that Yorkshire Dales had the greatest biodiversity of archaea while Moskanjci tended to have a large biodiversity of bacteria and fungi.

3.3. Indicator performance at individual sites

The effects of management at the individual sites could be distinguished by a range of indicators, albeit a different set of indicators at each site (Table 4). In general, as the difference in LUI increased so the number of indicators showing an effect of the management increased, with the exception of Scheyern which had the greatest difference in LUI (20), yet the conventionally managed

plots were only differentiated from the organically managed plots by four of the indicators used, namely resilience, bait lamina, earthworms and enchytraeids (Table 4). This was the same number of differentiating indicators as at the sites with the lowest LUI, i.e., Lusignan and Yorkshire Dales. At Lusignan there were no management effects detected by the functional indicators, although we were not able to use the bait lamina method there, and at Scheyern no management effects were detected by the microbial indicators (Table 4). Water infiltration was only measured at Moskanjci, Scheyern, Castro Verde and Lusignan in 2012 and rates were only significantly different at Scheyern between the organically managed (24 mm hr^{-1}) and conventionally managed (506 mm hr^{-1}, $P < 0.01$) plots. Differences due to management were not significant at the other sites in autumn 2012 but water infiltration did differ between sites. Thus infiltration rates for least intensive and most intensive plots (respectively) were at: Moskanjci 1.4 and 1.9 mm hr^{-1}; Castro Verde 0.44 and 0.39 mm hr^{-1}; and Lusignan 130 and 202 mm hr^{-1}.

The indicators of biodiversity also responded differently to the treatments at each site with: enchytraeid H' differentiating treatment and control plots at Castro Verde, Hainich and Scheyern; earthworm H' only differentiating at Lusignan; mite H' only differentiating at Moskanjci; and microbial T-RFLP differentiating at Castro Verde, Hainich and Yorkshire Dales.

3.4. Relative indicator costs

Table 5 shows the groupings of the indicators and within each column the first group scores best for that attribute. For 'ease of field work', the 'easy' group of indicators required a composite soil sample that could be readily collected within a single day, the 'moderate' group could also be collected in a single day by a single visit to each site but sampling was more involved than the composite soil, while in the 'difficult' group the bait lamina assay required revisiting the site at a variable time later (i.e. when about 50% of the substrate has been eaten) and the water infiltration

assay required that a large volume of water be readily available and took more than a single day at some sites. The 'utility of the assay' grouped those indicators that gave several endpoints (i.e. EEA and MicroResp assayed eight substrates simultaneously; faunal groups give information on biodiversity, organisms abundance/biomass and functional attributes; PLFA informs on microbial biomass, bacterial: fungal ratio and indicator peaks) and grouped those that only give a single endpoint. The 'ease of laboratory assay' had three groups, the 'basic skill level' group containing indicators requiring a simple incubation and/or extraction (nitrification for example requires a single extraction with a salt solution), a 'moderate' group requiring a more complex extraction or setup (thus DNA or ergosterol need a sequential extraction), and a 'technically demanding' group requiring the most sophisticated extractions and analysis (such as TRFLP which needs additional processing once the DNA has been extracted). The 'laboratory throughput' gave a 'high' and a 'low' group according to the rate and number of samples that can be handled at any one time. Finally the 'setup cost' ranked the indicators in three groups as the 'least expensive', which tend to be incubations whose endpoint is determined by colorimetric reaction, through the 'moderately expensive' to the 'most expensive' techniques which need gas chromatographs and nucleic acid sequencers with a relatively high associated capital and consumable cost. The table of relative costs was broken down into these different scenarios, mainly because most laboratories would be starting out with different amounts of essential equipment in place. So start-up costs would be different in each case.

4. Discussion

The objective of the study was to recommend indicators for soil biodiversity and ecosystem function. Although samples were collected from European agricultural sites, the outcomes would be relevant for non-agricultural soils, especially those of a mineral or organo-mineral texture. A breakdown of the cost effectiveness of the indicators showed the expected trade-off between the

382 intensity of work in the field and intensity in the laboratory. Thus, earthworms and water infiltration,
383 which are labour intensive in the field, require relatively little laboratory time, while DNA based
384 analyses from the easily obtained composite soil sample require the most laboratory effort.
385 An indicator programme should be based upon a suite of different indicators, as shown by
386 the fact that none of the indicators were able to detect all management effects across all sites, to
387 enhance reliability. However a balance between reliability (larger set of indicators) and costs
388 (smaller set of indicators) is always at stake, during the design of any monitoring system. The
389 ENVASSO project (Bispo et al., 2009), which was carried out to propose a set of suitable indicators
390 for monitoring the decline in soil biodiversity, selected indicators both from a literature review and
391 an inventory of national monitoring programs. ENVASSO recommended indicators in a different way
392 by having a tiered approach, with Level I being done at all times, Level II at times relevant for specific
393 issues or if resources were available and Level III was optional. ENVASSO also recommended
394 separate indicators for biodiversity (Level I = earthworm species, or enchytraeids at sites with acid
395 soils, and Collembola species; Level II = macrofauna, mites, nematodes, bacteria and fungi, Level III =
396 protists and faunal activity from litter bags or bait lamina) and function (Level I = basal respiration,
397 Level II = bacterial and fungal activity, Level III = faunal activity).
398 For monitoring under the European climatic zones and land uses we also suggest different
399 indicators of ecosystem function than for monitoring of soil biodiversity. For ecosystem functions
400 related to the services of water regulation, C-sequestration and nutrient provision (which are all
401 carried out by the general biological community), we would recommend at least three of the
402 selected indicators, one from each group (faunal, microbial and functional), which would be
403 earthworms, functional genes and bait lamina based on the results given in Table 4. In any
404 monitoring scheme there will be over-riding considerations of resources, time and expertise
405 available, so any decision to apply extra tiers, further indicators or more complete datasets then
406 becomes an internal matter that is different for each monitoring scheme. For diversity, our results
407 showed that diversity of the microbial and faunal groups responded differently to the changes in

land use intensity and that their ranking of biodiversity varied between sites (Supplementary Table S1). For example, sites such as the Yorkshire Dales had a consistent increase in diversity (Shannon) in the least intensive management for earthworms, mites, Collembola and archaea but not for enchytraeids, bacteria and fungi. Other sites had contrasting trends, such as Moskanjci which had greater earthworm diversity but less enchytraeid diversity in the least intensive management, or Lusignan which had greater earthworm and archaeal diversity but less enchytraeid and bacteria diversity in the least intensive management (Table S1). For effective monitoring of biodiversity, therefore, all taxonomic groups would need to be addressed because changes in the biodiversity of one group cannot be used to infer changes in other taxonomic groups .

These bioindicators will require standardisation and their deployment will need to be cost-effective and policy-relevant in order to be efficiently applied throughout Europe. This will allow us to identify the land uses which have the most severe impact on soil functioning and to quantify threats to soil ecosystem functions.

4.1. Indicator selection

The logical sieve (Ritz et al., 2009; Stone et al., 2016) based approach that we used is considered to be a scientifically valid and objective selection process that has been used in similar scientific studies for indicator selection in the face of a large number of methods to choose from (Aalders et al., 2009). The scores from this method can then inform judicial selection of the indicators, because this process resulted in several indicators ending up with similar scores. For example, basal respiration, bait lamina and litter bags all scored around 500, so we chose between these equivalent indicators based on the availability of equipment and expertise in the participating laboratories. In other cases, some indicators that scored relatively poorly but had high relevance to the ecosystem services of interest were included, for example water infiltration (which had a score

433 of 398) which is highly relevant for water regulation. The scores for the faunal indicators were all
434 similar with no real difference between the scores for molecular analysis and morphological analysis.
435 This is probably because the molecular methods for fauna provide information on the same targeted
436 endpoint, i.e. the composition of the specific faunal group. So they are different methods, but
437 aiming at the same result. The equivalence of scores may also reflect the fact that molecular
438 methods for faunal analysis are not yet commonplace and so the advantages in higher throughput
439 have not fully been appreciated (Thompson and Newmaster, 2014). The possibility of
440 metabarcoding, which will allow identification of all faunal groups simultaneously (Creer et al., 2010;
441 Taberlet et al., 2012) is only beginning to be explored for soil systems, so we believe that molecular
442 methods for faunal indicators will become increasingly preferred. In contrast, some of the molecular
443 methods for microbial analysis were the least favoured. This may result from several factors,
444 including the fact that the output represents new information (i.e. unlike the molecular faunal
445 methods you do not get the same information as existing methods such as T-RFLP and PLFA) and so
446 the interpretation is less straightforward. Faunal analysis has traditionally been based on a list of
447 species (i.e. Bongers, 1990) so the developing faunal molecular methods speed up and simplify the
448 process of acquiring the list of species (sequences). This approach was not widespread in the study
449 of microbial ecology, or relied on cultivation based techniques, and the equivalent associations
450 between microbial taxa and traits is an emerging science (Fierer et al., 2014). There is also the fact
451 that developments in molecular technology are moving so rapidly that standardization maybe
452 considered premature. We acknowledge that the new sequencing technologies such as the Illumina
453 or Ion torrent platforms are becoming cheaper, which would provide more information on the
454 specific indicator taxa responding to land use change. However we note they are still not as cost
455 effective as T-RFLP assays and, for detecting change at the community level at least, there are
456 currently few advantages in applying sequencing over T-RFLP (Thomson et al, 2015).
457 Most of the non-molecular methods selected for testing are already in use in monitoring schemes
458 from individual European countries (Turbe et al., 2010, Pulleman et al., 2012, Faber et al., 2013). As

459 such these methods have undergone thorough scientific validation and their usefulness as indicators
460 has been demonstrated. There was little difference between the functional indicators as those
461 receiving the lowest score (water infiltration and MicroResp) still received 80% of the score of the
462 highest scoring (nitrification). This could be a reflection that functional methods have been
463 effectively evaluated in previous monitoring schemes (Faber et al., 2013) and generally have a
464 proven track record for all aspects of the logical sieve approach. This study also tested methods not
465 currently used as indicators across Europe. We focused on functional genes for nitrogen cycling
466 because previous studies showed that they were good candidate bioindicators for soil monitoring
467 and are increasingly common in the scientific literature (Ritz et al. 2009, Wessén and Hallin, 2011;
468 Jones et al., 2014). Bait lamina (Van Gestel et al, 2003) and water infiltration (Tejedor et al., 2013)
469 have been used previously and standardized methods (ISO 18311, 2014 and DIN 19682-7:2007-07,
470 respectively) already exist, but they have not been widely used in European monitoring schemes.
471 Our experience suggested that water infiltration was too time-consuming and logistically
472 demanding, given that it required an abundant and easily available water supply to be practical for
473 monitoring. Although there were constraints to using bait lamina sticks at some of our sites
474 (cultivation occurring too soon after deployment), its ease of use, functional relevance (Römbke,
475 2014), and sensitivity led us to recommend its use. MicroResp and extracellular enzyme activity
476 (EEA) are developments of the multi-substrate assay approach and comparable to methods such as
477 BIOLOG that can also be used as biological indicators of soil quality (Rutgers et al., 2006). The
478 resilience assay (microbial resilience to antibiotic) was the only truly novel method tested in this
479 study and, although it proved to be a straightforward assay with high-throughput, results would
480 need to be tested for relevance to ecosystem functions in more detail. Here we chose to analyse the
481 lag phase of the growth curve because the initial biomass differences between samples is more
482 likely to influence the subsequent exponential growth. Also, certain fast growing microbes are likely
483 to benefit proportionally more than others which will be especially pronounced in later growth
484 phases, and in nutrient rich media total abundances eventually often merge as the closed systems

485 have limited carrying capacities. The analysis of other growth parameters could be explored. These
486 methods were included because of their relevance to soil biodiversity and ecosystem function.

487

488 *4.2. Indicator performance*

489

490 We used a variety of management treatments, typical of European practice, to ascertain the
491 capabilities of the selected indicators. The indicators selected clearly distinguished between the
492 different field sites, which gives a measure of confidence that the indicators selected are valid to
493 include in a European wide survey. The samples did not include examples of forest soil, nor organic-
494 matter rich soils. To extend the analyses to these systems or soil types would have required some of
495 the methodology to be modified, for example it would have been impractical to rely on earthworm
496 diversity and abundance as they are rare in acid or highly organic soils (Petersen and Luxton, 1982;
497 Lavelle and Spain, 2004) and DNA and other chemical extractions from humic-rich soils would
498 require different protocols (Miao et al., 2014). Although the equation used to calculate land use
499 intensity was originally developed for grassland (Blüthgen et al. 2012), our modification of this
500 calculation provided a means to compare the sites used in this study objectively as they also
501 included non-grassland. At the Lusignan site, where the transition from grass to arable took place
502 three years before sampling, the LUI equation calculated no difference in intensity between the
503 control (grass – arable) and treatment (continuous arable) plots. The treatments were obviously
504 very similar given the minor changes observed but earthworms may have been responding to the
505 extra organic matter incorporated from the grass, even after three years (van Eekeren et al., 2008).
506 The effects the grass phase of the rotation maybe equivalent to small additions of fertiliser, as at the
507 Yorkshire Dales site where earthworm biomass in the fertilised plots (67 g m^{-2}) was greater than in
508 the unfertilised plots (37 g m^{-2}) although this effect was not significant (P = 0.06). The LUI at
509 Scheyern did not match the indicator results, fewer of which responded than at Moskanjci which

510 also had differences in tillage intensity as the treatment. In fact the Scheyern site used no-till which
511 might be expected to lead to bigger differences from normally tilled plots than minimum or
512 conservation tillage (van Capelle et al., 2012).

513 Some indicators did not respond to the treatments at the test sites (no difference between
514 control and treatment) i.e. abundance of epigeic enchytraeids, diversity of endogeic earthworms,
515 number of earthworm species, potentially mineralisable nitrogen, fungal biomass measured by
516 ergosterol, and the abundance of 16S and *NirK* functional genes. This might be the consequence of
517 not sampling the complete suite of land uses, texture types, other soil characteristics, climate zones,
518 and soil management intensities across Europe. All indicators will probably demonstrate sensitivity
519 in some situations that we did not include in our sampling design. It is likely that earthworms at the
520 sites studied are represented by too few species to be a reliable indicator of biodiversity, although
521 the presence of anecic species is strongly related to water infiltration (Spurgeon et al., 2013; Fischer
522 et al., 2014). Enchytraeids did prove to be a good indicator of biodiversity, but the abundance of
523 epigeic species was more variable than that of the other enchytraeid groups. The abundance of
524 functional genes was normalized (gene copy number per ng of DNA), which could explain why some
525 were less discriminatory.

526 With the biodiversity indicators a better coverage of changes was given from examination of
527 both microbial and faunal groups. The application of metabarcoding approaches (Fierer et al., 2014)
528 has become more prominent, so that the diversity of the major faunal groups might be determinable
529 from the same sample as used for microbial groups in the near future. For instance, recent
530 developments in the measurement of environmental DNA (e-DNA, Taberlet et al., 2012; Wilcox et
531 al., 2013; Bohmann et al., 2014), would greatly increase the ease of determining faunal communities
532 using DNA-based approaches.

533

534 **Conclusion**

For undertaking a large-scale biological indicator programme this study has shown that standardisation of methods is an absolute necessity, otherwise it is not possible to properly compare results. This would include an inter-laboratory comparison for the small number of indicators finally selected (i.e. Creamer et al., 2009). It would also necessitate accurate prescription of sampling appropriate for the land uses and edaphic conditions within the monitoring area. An easily accessible database needs to be established to detect temporal changes, so that the results can show a trajectory of system improvement or decline rather than just being a point measure of status. A suite of complementary indicators is necessary, ideally linking biodiversity to soil functioning to give a more meaningful outcome. The ongoing developments in nucleic acid based analyses of biodiversity are likely to improve the throughput and resolution of biodiversity indicators, which need to cover both microbial and faunal groups. Indicators for ecosystem functions related to the services of water regulation, C-sequestration and nutrient provision would include a minimum suite of: earthworms; microbial functional genes; and bait lamina.

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564

565 **Appendix A. Supplementary data**

566

567 Supplementary data associated with this article can be found, in the online version, at

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Table 1. Weighted score from the logical sieve style assessment of potential biological indicators of soil biodiversity and ecosystem function. Indicators were grouped as faunal, microbial or functional, and addressed issues of biodiversity (BD), ecosystem function (EF) or both. Indicators selected for evaluation are in bold. DNA abundance and resilience were not assessed in this exercise. EEA - extra-cellular enzyme activity; T-RFLP - Terminal Restriction Fragment Length Polymorphism of archaea, bacteria and fungi; PLFA - phospho-lipid fatty acid analysis. Indicators evaluated in the field but not ranked in this assessment are included for completeness and scored n/a.

Potential indicator	Indicator Group	Issue Addressed	Weighted score
Nematodes: molecular	**Fauna**	**BD/EF**	**659**
Nematodes: morphological	Fauna		640
Enchytraeids: molecular	Fauna		639
Mites: molecular	Fauna		639
Collembola: molecular	Fauna		639
Earthworms: morphological	**Fauna**	**BD/EF**	**633**
Collembola : morphological	**Fauna**	**BD/EF**	**623**
Enchytraeids: morphological	**Fauna**	**BD/EF**	**623**
Mites: morphological	**Fauna**	**BD/EF**	**611**
Earthworms - molecular	Fauna		599
Fungi (ergosterol)	**Microbe**	**BD**	**549**
Protista – molecular	Microbe		539
Nitrification	**Function**	**EF**	**525**
Potentially mineralisable N	**Function**	**EF**	**525**
Hot water extractable C	**Function**	**EF**	**525**
Respiration	Function	EF	507
Bait Lamina	**Function**	**EF**	**492**
EEA	**Function**	**EF**	**474**
Microbial – T-RFLP	**Microbe**	**BD**	**473**
PLFA	**Microbe**	**BD**	**459**
Functional genes	**Function**	**BD/EF**	**448**
Protista – morphology	Microbe		446
Denitrification	Function		422
Pyrosequencing	Microbe		415
MicroResp	**Function**	**EF**	**398**
Water infiltration	**Function**	**EF**	**398**
Molecular Chip technology	Microbe		383
Other 'omic' methods	Microbe		328
DNA abundance	**Microbe**	**EF**	**n/a**
Resilience	**Microbe**	**EF**	**n/a**

Table 2. European sites where selected indicators were tested. Each site had three replicated plots of the contrasting management options. At Scheyern, fertilisation is abbreviated to fert.

Climatic Zone	Land Use	Soil texture	Management	Country	Site name
Continental	Arable	Silt Loam	till, conventional fert vs no-till, minimal fert	Germany	Scheyern
Atlantic	Arable	Silt Loam	arable vs grass/arable	Lusignan	Lusignan
Pannonian	Arable	Clay Loam	till vs no-till	Slovenia	Moskanjci
Mediterranean	Arable	Sandy Loam	cereal vs fallow	Portugal	Castro Verde
Continental	Grass	Clay / Silty Clay	intensive vs extensive	Germany	Hainich
Atlantic	Grass	Sandy Silt Loam	intensive vs extensive	UK	Yorkshire Dales

Table 3. Land use intensity (LUI) of the agricultural treatments applied at the sites where the selected biological indicators were tested and the difference in LUI within each site. The treatments are either control (C), least intensive management at that site, or treatment (T), the most intensive treatment at that site.

Site	Land Use		Treatments	Climatic Zone	Country	LUI	Difference
Scheyern	Arable	C	Organic	Continental	Germany	15.4	20.0
		T	Conventional			35.4	
Moskanjci	Arable	C	Minimum tillage	Pannonian	Slovenia	14.8	7.3
		T	Conventional tillage			22.1	
Castro Verde	Arable	C	2 year fallow after arable	Mediterranean	Portugal	0.1	10.7
		T	Conventional tillage			10.8	
Lusignan	Arable	C	Continuous arable	Atlantic	France	12.4	0.0
		T	3 years arable after pasture			12.4	
Hainich	Grass	C	Extensive grass (species rich)	Continental	Germany	3.0	4.5
		T	Intensive grass (fertilised)			7.6	
Yorkshire Dales	Grass	C	Extensive grass (species rich)	Atlantic	UK	0.4	1.6
		T	Intensive grass (fertilised)			2.0	

Table 4. Biological indicators, by group, detecting differences between treatments at each site. Sites are ranked according to the difference in land use intensity (dLUI, see table 3). Abbreviations for the indicators are: extra-cellular enzyme activity (EEA); MicroResp (Mresp); Resilience (resil); Nitrification (Nit); Bait Lamina (BL); Hot water extractable carbon (HWC); Earthworms (EW); Enchytraeids (Enc); Nematodes (Nem); Microarthropods (Mpod); terminal restriction fragment length polymorphism (T-RFLP); DNA abundance (DNA). Bait lamina (BL) tests could not be used at Lusignan and Hainich (x).

Site	dLUI		Functional							Faunal					Microbial		
Lusignan	0.0						x			EW		Nem			T-RFLP	DNA	
Yorkshire Dales	1.6				Resil		BL						Mpod		T-RFLP		FG
Hainich	4.5		EEA	Mresp		Nit	x			EW	Enc				T-RFLP		FG
Moskanjci	7.3			Mresp		Nit	BL	HWC		EW	Enc	Nem	Mpod		T-RFLP	DNA	FG
Castro Verde	10.7		EEA	Mresp	Resil	Nit	BL			EW	Enc	Nem			T-RFLP		
Scheyern	20.0				Resil		BL			EW	Enc						

Table 5. Relative cost-effectiveness of the selected indicators, grouped according to: ease of collecting soil samples from the field (three categories, 1 – easy; 2 – moderate; 3 – difficult); utility in terms of getting more than one piece of information from the test (1 – single endpoint, 2 – multiple endpoint); ease of laboratory operations or skill-level required for operation (1 – basic skill level; 2 – moderate; 3 – technically demanding); potential laboratory throughput of samples (1 – high; 2 – low); capital costs to set up analysis from new (1 – least expensive, 2 – moderately expensive, 3 – most expensive). Indicators are abbreviated as in Table 1 apart from: DNA abundance (DNA) and resilience (resil). Indicators are listed alphabetically and not ranked within categories.

Ease of Field sampling	Utility	Ease of Lab test	Lab throughput	Setup costs
1 DNA	1 BL	1 BL	1 BL	1 BL
EEA	EEA	EW	DNA	EEA
ERG	ENCH	HWC	ERG	EW
EW	EW	INFIL	FG	INFIL
FG	MA	NEM	HWC	MRESP
HWC	MRESP	NIT	INFIL	NIT
MRESP	NEM	PMN	NEM	PMN
NEM	PLFA	RESIL	NIT	RESIL
NIT	T-RFLP		PMN	
PLFA		2 DNA	RESIL	2 ENCH
PMN		EEA	EEA	HWC
RESIL	2 DNA	ENCH	EW	MA
T-RFLP	ERG	ERG	T-RFLP	DNA
	FG	MRESP	MRESP	ERG
2 ENCH	HWC		PLFA	
MA	NIT	3 FG		3 FG
	PMN	MA		NEM
3 BL	RESIL	PLFA	2 ENCH	PLFA
INFIL	INFIL	T-RFLP	MA	T-RFLP

Supplementary table 1

Mean value (n=3) of indicators sampled at control and treatment plots from the six European sites in 2012 and 2013.
Indicators are: Extracellular Enzyme Activity (EEA) in nM (MUF/AMC)/h/g dry weight with substrates EEA-1 to EEA-8 (arylsulfatase, alfa-glucosidase, beta-glucosidase, cellobiosidase, beta-xylosidase, chitinase, phosphomonoesterase, leucin aminopeptidase) and principal components PC1 and PC2; MicroResp (µg CO2-C/g/h) with substrates MR-1 to MR-8 (Water, L-Arginine, L-Malic Acid, Gamma Amino Butyric Acid, n-Acetyl Glucosamine, D(+) Glucose, Alpha ketogluterate and Citric Acid) with PC1 and PC2; Earthworms (EW) with total abundance (tot, no/m2), biomass (mass, g/m2), shannon diversity (H') and number of species (spp); Enchytraeids (EN) abundance (tot, no/m2), diversity (H') and number of species (spp); Nematodes with relative abundance of bacterial- (BF), fungal- (FF), plant- (PF) feeders, omnivores (OM), carnivores (CA) and PC1 and PC2; Mites abundance (tot, no/m2), diversity (H') and number of species (spp); Collembola (Coll) abundance (tot, no/m2), diversity (H') and number of species (spp); TRFLP (TRF) of archaea (A), bacteria (B) and fungi (F) showing diversity (H') and PC1 and PC2 from relative abundance of peaks; Functional gene abundance (genes/ ng DNA) of 16S rRNA, amoA from bacteria (AOB) and archaea (AOA), nirK, nirS and nosZ1 gene; phospho-lipid fatty acid (PLFA) analysis with total PLFA (tot, µg/g) and PC1 and PC2 from relative abundance; hot water extractable carbon (HWC,µg/g); potentially mineralisable nitrogen (PMN, µg/g); ergosterol (Ergost, µg/g) ; DNA abundance (DNA, ng/g) ; Resilience (difference in lag time, h); nitrification (Nit, ng NO2-N/g/h), infiltration rate (Infilt, mm/h) and bait lamina % feeding activity (Bait lam, angular transformation).
ND = not determined

Indicator	Site	Castro Verde				Hainich				Yorkshire Dales				Lusignan				Moskanjci				Scheyern			
	Plot	control		treatment		control		treatment		control		treatment		control		treatment		control		treatment		control		treatment	
	Year	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
EEA	EEA-1	42.9	10.0	30.7	13.6	90.8	99.2	80.3	40.0	303.4	197.3	230.3	111.1	58.0	38.1	56.2	42.4	32.4	54.2	47.1	39.9	54.4	50.0	64.9	62.7
	EEA-2	161.6	84.8	135.0	114.6	229.1	320.3	527.7	316.6	181.0	148.3	193.3	224.5	102.0	122.4	98.8	96.7	129.4	299.4	185.5	186.3	132.9	231.7	162.7	301.3
	EEA-3	1252	816	1533	1518	1753	1914	3749	1713	1429	1133	1399	1398	920	950	828	762	753	1793	1596	1069	892	1681	949	2017
	EEA-4	109.9	24.0	123.2	62.8	194.5	253.3	597.1	182.6	223.6	149.3	212.8	192.6	65.8	84.0	75.0	69.9	67.3	214.1	142.1	107.7	104.4	227.4	188.3	251.6
	EEA-5	170	106	164	144	330	410	721	317	596	518	368	280	137	152	137	130	88	253	133	144	148	214	155	251
	EEA-6	764	332	710	416	758	1188	792	366	792	561	612	572	511	490	483	338	286	541	664	338	386	487	387	532
	EEA-7	6468	1135	5432	1491	3434	4148	3680	2354	9191	7438	8182	8608	6953	7465	8850	6502	2606	4484	4238	3941	6791	7634	5553	7799
	EEA-8	2808	1325	1707	1325	4654	7100	10236	9589	3629	4434	2920	2284	2796	3756	2372	4042	2421	4999	2439	3557	1353	1915	1405	3579
	EEA PC1	-1192	1850	-1081	1658	2354	3406	5983	6332	-2705	-895	-2402	-3134	-1609	-1377	-3330	-486	1430	1781	301	1183	-2414	-2601	-1433	-1620
	EEA_PC2	-51	-4709	-1500	-4364	-568	1760	4067	2360	2386	1779	1179	977	190	1239	1094	787	-2951	364	-1726	-1186	-991	106	-1745	1503
MicroResp	MR-1	0.50	0.87	0.52	1.09	0.75	1.01	1.11	1.19	1.09	2.38	1.00	2.48	0.85	2.24	0.63	1.94	0.49	2.05	0.41	1.40	0.78	1.77	0.65	1.84
	MR-2	0.33	1.55	0.38	2.10	0.04	2.55	0.07	2.65	0.21	4.31	0.21	4.73	0.20	3.29	0.13	3.12	0.28	3.35	0.26	2.48	0.09	3.55	0.04	3.29
	MR-3	1.33	1.65	1.46	2.35	3.28	4.03	5.06	5.08	4.86	5.93	4.51	6.02	2.70	4.00	2.43	4.50	2.12	4.46	1.47	3.40	2.40	4.41	2.09	3.98
	MR-4	0.74	1.01	0.77	1.41	1.05	1.43	1.49	1.49	2.35	3.25	2.20	3.75	0.20	2.48	0.13	2.45	0.88	2.62	0.68	2.01	1.08	2.28	0.86	2.42
	MR-5	0.93	1.16	0.87	1.56	1.63	2.06	2.33	2.22	2.72	3.71	2.89	4.46	1.50	2.93	1.26	2.82	1.50	3.45	1.04	2.58	1.52	3.14	1.23	2.83
	MR-6	1.55	1.83	1.81	2.81	3.15	3.39	4.03	3.79	5.56	5.52	5.35	6.28	2.15	3.59	1.91	3.99	2.21	4.27	1.64	3.33	2.47	4.29	1.91	4.07
	MR-7	3.57	2.78	2.98	3.64	5.01	4.81	6.00	4.21	6.84	6.76	7.48	7.26	4.12	5.49	3.73	5.58	4.45	6.00	3.21	5.19	4.28	5.86	3.55	5.48
	MR-8	1.98	1.84	2.33	2.70	3.74	4.05	5.56	4.61	4.48	5.73	5.97	6.12	3.77	5.16	3.37	5.58	2.47	4.49	1.64	3.63	3.54	5.24	2.90	4.92
	MR_PC1	-4.24	-3.72	-4.16	-1.86	-1.21	0.41	1.46	1.15	2.32	5.43	2.92	6.67	-2.42	2.23	-3.11	2.66	-2.88	2.82	-4.44	0.47	-2.25	2.89	-3.35	2.20
	MR_PC2	-0.21	-1.58	-0.32	-1.35	1.43	-0.60	2.46	-0.61	2.46	-0.94	3.02	-1.01	0.83	-1.14	0.64	-0.64	0.46	-1.00	-0.32	-0.83	0.81	-0.90	0.35	-1.02
Earthworms	EW tot	14.81	0.00	13.00	0.00	5.14	7.26	1.81	1.51	85.56	82.84	167.20	110.36	538.78	496.15	203.17	330.16	22.37	46.98	16.33	32.65	71.96	5.74	41.72	26.91
	EW mass	2.63	0.00	1.55	0.00	1.41	1.31	0.27	0.13	43.10	31.53	80.63	53.76	145.21	146.71	57.08	84.27	17.41	47.56	11.68	19.56	24.68	1.68	17.42	10.47
	EW H'	0.40	0.00	0.80	0.00	1.11	0.94	0.28	0.90	1.72	1.77	1.32	1.62	1.26	1.30	1.22	0.62	1.25	1.40	1.37	1.52	1.60	0.94	1.64	1.59
	EW spp	2.33	0.00	2.67	0.00	2.67	2.33	1.00	1.67	7.33	6.67	6.67	6.33	5.67	6.00	4.67	4.67	4.00	3.67	3.67	3.33	8.33	2.67	7.33	5.67
Enchytraeids	EN tot	8353	0	0	0	74796	67896	74888	48699	76552	42065	148988	74882	43864	14846	30028	22752	169411	18171	7268	18660	59825	2656	23273	6989
	EN H'	1.10	0.00	0.00	0.00	2.17	2.35	1.12	1.79	2.35	2.33	2.40	2.14	1.52	1.64	1.58	1.49	1.10	1.42	1.03	1.27	1.65	1.00	1.13	0.85
	EN spp	4.33	0.00	0.00	0.00	17.00	16.00	5.67	8.00	22.33	19.00	21.67	18.33	6.67	7.33	6.67	6.33	6.00	6.67	3.67	5.67	8.67	4.00	6.67	3.67
Nematodes	Nem - BF	31.00	24.24	14.58	5.74	15.31	33.46	37.16	54.15	30.30	32.37	25.90	44.91	48.58	18.24	44.78	15.25	15.98	22.15	43.88	20.42	23.18	20.90	23.60	18.64
	Nem - FF	30.81	63.18	64.97	68.95	2.10	18.22	5.10	0.00	11.52	1.61	7.84	6.31	6.82	0.59	12.69	2.13	28.14	31.06	37.76	57.16	10.91	3.43	5.82	6.17
	Nem - PF	8.88	0.00	1.19	0.00	28.02	11.31	6.50	2.32	0.92	11.64	6.22	6.02	1.76	14.72	5.84	19.40	7.30	7.62	13.53	15.97	8.45	5.28	11.26	7.39
	Nem - Om	29.30	12.58	11.63	12.48	22.51	6.31	15.90	20.86	37.36	28.00	24.35	22.51	42.83	19.25	8.17	11.48	5.30	12.42	4.83	4.84	14.61	7.66	5.02	7.86
	Nem - Ca	0.00	0.00	7.62	12.83	32.06	30.69	35.34	22.68	19.89	26.37	35.70	20.25	0.00	47.20	28.52	51.75	43.28	26.75	0.00	1.62	42.86	62.73	54.30	59.94
	Nem PC1	0.413	0.733	0.561	0.545	-0.327	-0.096	-0.155	-0.180	0.045	-0.230	-0.185	-0.107	0.293	-0.459	-0.080	-0.480	-0.062	0.135	0.515	0.574	-0.220	-0.467	-0.378	-0.439
	Nem PC2	0.244	-0.114	-0.301	-0.492	0.074	0.010	0.099	0.431	0.204	0.235	0.029	0.262	0.554	0.032	0.085	-0.098	-0.337	-0.145	0.097	-0.189	-0.099	-0.178	-0.173	-0.195
Mites	Mite tot	ND	3565	ND	6763.9	ND	43048	ND	33452	ND	21596	ND	23105	ND	7288	ND	8599	ND	23962	ND	16621	ND	2412	ND	1258
	Mite H'	ND	1.57	ND	1.94	ND	2.24	ND	2.41	ND	2.22	ND	2.15	ND	1.61	ND	2.12	ND	1.82	ND	2.29	ND	1.65	ND	1.30
	Mite spp	ND	7.00	ND	11.67	ND	23.00	ND	21.67	ND	19.33	ND	22.33	ND	9.00	ND	13.67	ND	14.67	ND	19.33	ND	6.67	ND	4.33
Collembola	Coll tot	ND	ND	ND	ND	ND	ND	ND	ND	ND	11608	ND	8506	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Coll H'	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.10	ND	1.68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Coll spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	16.67	ND	9.67	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Supplementary table 1 continued																									
TRFLP	TRF-A H'	1.82	1.67	1.61	1.93	1.53	1.15	1.69	1.18	3.10	2.30	2.71	1.66	2.64	1.26	1.57	1.15	1.86	1.56	2.14	1.62	1.51	1.24	1.61	1.31
	TRF-B H'	3.11	3.95	3.42	3.98	3.58	4.20	3.60	4.37	3.56	3.95	3.54	4.06	3.65	4.13	3.66	4.20	3.66	4.17	3.69	4.25	3.75	4.12	3.73	4.20
	TRF-F H'	3.70	4.40	3.88	4.44	4.49	4.33	4.70	4.25	4.14	4.08	4.51	4.06	4.17	4.48	4.25	4.41	4.31	4.51	4.45	4.60	4.23	4.36	4.28	4.42
	TRF-A PC1	0.033	0.084	-0.231	0.213	-0.122	-0.163	0.168	-0.146	0.079	0.240	0.083	0.328	-0.024	-0.023	-0.134	-0.189	0.193	-0.014	0.178	-0.015	-0.067	-0.171	-0.169	-0.152
	TRF-A PC2	-0.034	-0.170	0.080	-0.162	-0.156	0.073	-0.141	0.110	0.294	0.103	0.130	0.158	0.073	0.165	-0.095	0.027	-0.010	-0.088	0.045	-0.103	-0.096	-0.031	-0.099	-0.026
	TRF-B PC1	-0.137	-0.070	-0.096	-0.072	0.020	-0.026	-0.008	-0.020	-0.060	-0.068	-0.033	-0.045	0.067	0.051	0.077	0.060	0.061	0.059	0.051	0.047	0.033	0.050	0.026	0.034
	TRF-B PC2	-0.050	-0.020	-0.043	-0.022	0.063	-0.017	0.066	-0.002	0.039	0.041	0.061	0.022	-0.030	0.013	-0.066	0.043	-0.048	-0.014	-0.024	-0.003	0.026	-0.026	0.008	-0.015
	TRF-F PC1	-0.214	-0.013	-0.130	-0.019	0.024	-0.004	0.028	-0.022	0.013	-0.063	0.002	-0.067	0.071	0.023	0.040	0.082	0.044	-0.009	0.061	-0.002	0.030	0.061	0.055	0.033
	TRF-F PC2	-0.002	0.059	-0.028	0.055	-0.003	0.008	0.006	0.019	0.109	-0.061	0.046	-0.062	0.064	-0.042	-0.013	-0.037	-0.047	0.018	-0.045	0.024	-0.029	0.010	-0.037	0.010
Functional	16Sbact	142696	131264	176079	139651	132648	93419	124108	118967	87547	103653	77871	118098	136795	180443	131465	181071	154033	163172	141672	156016	127001	161145	83716	173197
genes	AOA	1333	744	1180	249	252	983	3848	19411	78	159	128	437	5214	11246	2203	7710	1854	17445	1563	5847	3159	6349	3633	3773
	AOB	26	17	101	199	37	50	182	566	26	20	155	154	300	340	195	375	617	1380	726	1225	452	769	497	718
	nirK	9919	11156	12764	9576	15422	11966	13737	12030	13859	17642	10995	17053	16059	15520	14132	18912	15955	17024	14814	15352	15692	16108	9673	16607
	nirS	5394	5363	4903	4692	6737	6026	12838	21923	8414	7016	10972	8989	9772	10791	12584	14193	16094	15383	18126	17192	18455	17979	10950	21139
	nosZ1	21415	26293	26527	25848	29388	17621	19980	19203	41998	34589	24652	28729	30288	29228	29141	28825	29273	20758	30540	24621	28642	22035	9511	27661
PLFA	PLFA tot	93.5	89.3	17.8	15.2	62.2	71.9	39.1	37.7	56.5	62.6	38.6	27.4	15.4	12.1	17.2	16.3	10.8	6.3	11.9	4.9	11.4	9.6	28.4	23.6
	PLFA PC1	-17.71	-16.65	-7.29	-4.12	5.21	5.39	5.94	6.29	4.24	5.31	5.83	7.19	-1.13	-4.30	2.51	1.79	-0.54	-1.85	2.70	2.72	-6.90	-5.95	3.79	5.53
	PLFA PC2	-1.88	-0.87	-1.33	-2.45	3.96	3.84	2.84	2.27	3.50	4.11	2.17	1.06	2.64	1.82	-5.06	-4.93	2.05	1.95	0.75	-6.10	0.90	1.66	-1.57	-10.78
Single	HWC	266	406	249	306	1700	2179	2029	1999	2139	1956	2021	2460	345	417	392	389	596	568	443	475	501	522	433	639
endpoint	PMN	23.96	27.80	27.65	36.61	157.51	202.00	195.51	210.73	133.27	147.92	135.10	156.40	26.44	18.69	25.34	19.33	49.32	41.98	23.24	26.53	27.47	32.66	34.96	35.27
indicators	Ergost	0.87	0.50	0.84	0.38	2.52	2.97	3.72	2.12	0.64	0.08	0.28	0.17	0.44	0.21	0.53	0.35	0.99	0.78	1.03	0.45	0.37	0.44	0.72	0.50
	DNA	6182	25759	17000	24293	170787	134340	159888	93634	161406	100183	132036	117461	53093	22597	35522	22697	53275	39716	39005	28151	62988	34639	55921	33386
	Resilience	21.67	21.81	15.67	13.09	18.67	6.00	24.67	6.00	30.33	10.07	20.67	12.59	37.33	23.83	34.33	23.83	18.33	25.50	32.00	21.14	22.00	6.04	15.33	9.06
	Nit	23.72	35.30	12.05	21.50	180.91	804.63	2752.61	4028.07	79.94	67.40	378.52	493.30	263.00	265.97	182.04	265.33	727.46	987.30	464.08	515.13	693.74	766.87	702.16	633.13
	Infilt	0.44	ND	0.36	ND	ND	ND	ND	ND	ND	ND	ND	ND	153.4	ND	79.4	ND	96.2	ND	75.9	ND	24.00	ND	506.0	ND
	Bait lam	0.97	1.23	0.74	0.68	ND	ND	ND	ND	0.28	0.41	0.40	0.38	ND	ND	ND	ND	0.71	0.64	ND	ND	0.73	0.90	0.64	0.72

Supplementary table 2

Standard deviation (n=3) of indicators sampled at control and treatment plots from the six European sites in 2012 and 2013.

Indicators are: Extracellular Enzyme Activity (EEA) with substrates EEA-1 to EEA-8 (arylsulfatase, alfa-glucosidase, beta-glucosidase, cellobiosidase, beta-xylosidase, chitinase, phosphomonoesterase, leucin aminopeptidase) and principal components PC1 and PC2; MicroResp with substrates MR-1 to MR-8 (Water, L-Arginine, L-Malic Acid, Gamma Amino Butyric Acid, n-Acetyl Glucosamine, D(+) Glucose, Alpha ketogluterate and Citric Acid) with PC1 and PC2; Earthworms (EW) with total abundance (tot), biomass (mass, g m-2), shannon diversity (H') and number of species (spp); Enchytraeids (EN) abundance, diversity and number of species; Nematodes with relative abundance of bacterial- (BF), fungal- (FF), plant- (PF) feeders, omnivores (OM), carnivores (CA) and PC1 and PC2; Mites abundance, diversity and number of species; Collembola (Coll) abundance, diversity and number of species; TRFLP (TRF) of archaea (A), bacteria (B) and fungi (F) showing diversity and PC1 and PC2; Functional gene abundance of 16S rRNA, amoA from bacteria (AOB) and archaea (AOA), nirK, nirS and nosZ1 gene; phospho-lipid fatty acid (PLFA) analysis with total PLFA and PC1 and PC2; hot water extractable carbon (HWC; potentially mineralisable nitrogen; (PMN); ergosterol (Ergost) ; molecular biomass (Mol Biom) ; Resilience; nitrification (Nit), infiltration rate (Infilt) and bait lamina (Bait lam).

ND = not determined

Site		LCV				LHA				LLN				LLS				LMO				LSH			
Plot		control		treatment		control		treatment		control		treatment		control		treatment		control		treatment		control		treatment	
Year		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
EEA	EEA-1	15.75	1.39	5.81	0.96	12.65	45.23	12.96	5.30	97.83	25.97	100.06	50.65	11.16	9.04	10.49	5.17	0.84	13.50	11.03	6.42	12.99	9.34	15.67	22.01
	EEA-2	22.87	6.31	44.50	7.56	35.88	105.99	11.68	86.58	32.84	47.65	46.34	23.72	23.68	41.06	17.66	16.05	15.02	58.29	20.84	9.13	28.06	12.63	42.25	62.36
	EEA-3	94.64	54.69	599.36	131.48	490.65	876.46	429.96	232.92	238.13	444.87	240.18	259.15	115.59	211.29	257.36	28.19	121.87	341.99	842.08	113.23	51.65	378.34	224.63	336.75
	EEA-4	25.01	9.96	54.21	8.38	64.81	141.69	198.41	35.30	78.76	66.63	60.41	50.36	23.44	15.18	21.59	4.53	21.89	38.44	41.33	10.46	17.00	29.52	124.36	59.05
	EEA-5	29.70	9.07	62.01	9.06	101.96	177.35	230.61	92.80	281.64	207.26	51.12	103.12	28.19	18.72	25.09	11.60	14.19	65.70	13.05	24.62	17.13	35.31	24.29	44.73
	EEA-6	60.96	87.71	352.76	73.94	77.83	265.59	117.96	28.78	156.14	117.47	51.94	90.43	56.43	50.70	159.77	87.28	40.90	139.32	422.66	46.83	46.90	35.36	45.04	67.95
	EEA-7	1059	395	1873	444	1151	1416	849	527	1100	211	600	762	1896	1445	2842	790	736	1076	572	947	273	674	473	3985
	EEA-8	408	205	447	312	2073	1373	1429	2718	1295	1081	582	1330	385	273	370	629	422	722	657	120	213	684	44	1103
	EEA_PC1	874	174	1076	152	2213	737	740	1889	1320	587	717	1107	1681	1204	2092	367	636	819	802	724	214	330	385	3693
	EEA_PC2	720	410	1653	503	927	1948	1372	1968	1115	1004	384	1098	954	849	1971	933	550	1045	528	628	258	908	277	1829
MicroResp	MR-1	0.08	0.22	0.01	0.17	0.14	0.13	0.38	0.13	0.11	1.85	0.13	0.78	0.29	0.44	0.03	0.25	0.15	0.40	0.05	0.11	0.06	0.47	0.06	0.16
	MR-2	0.10	0.25	0.02	0.34	0.01	0.78	0.02	0.32	0.06	2.05	0.17	0.83	0.07	0.45	0.02	0.52	0.16	0.41	0.04	0.19	0.03	0.32	0.01	0.22
	MR-3	0.10	0.26	0.05	0.45	0.80	0.77	0.84	0.35	1.24	1.94	1.76	1.22	0.14	0.83	0.16	0.25	0.13	0.53	0.28	0.12	0.33	0.43	0.20	0.24
	MR-4	0.09	0.24	0.13	0.20	0.10	0.26	0.43	0.26	0.72	2.03	0.35	0.72	0.07	0.33	0.02	0.33	0.26	0.46	0.12	0.11	0.23	0.53	0.04	0.23
	MR-5	0.08	0.19	0.04	0.26	0.15	0.56	0.54	0.21	0.98	1.94	0.59	0.31	0.10	0.18	0.13	0.37	0.28	0.31	0.18	0.10	0.30	0.51	0.01	0.27
	MR-6	0.10	0.33	0.07	0.49	0.49	0.82	1.10	0.42	1.95	2.38	1.35	1.08	0.41	0.83	0.31	0.41	0.43	0.60	0.39	0.42	0.25	0.24	0.15	0.39
	MR-7	0.40	0.55	0.13	0.80	0.92	0.68	0.81	0.23	0.64	2.06	2.50	1.51	0.05	0.65	0.18	0.38	0.26	0.36	0.84	0.38	0.53	0.27	0.65	0.33
	MR-8	0.22	0.28	0.37	0.38	0.71	0.86	0.64	0.51	0.14	2.15	2.27	1.05	0.29	0.74	0.24	0.08	0.07	0.56	0.37	0.27	0.54	0.10	0.44	0.34
	MR_PC1	0.29	0.75	0.24	1.12	1.28	1.50	1.05	0.78	2.08	5.63	3.49	2.66	0.27	1.61	0.40	0.60	0.52	1.05	0.76	0.54	0.79	0.78	0.48	0.74
	MR_PC2	0.30	0.24	0.11	0.18	0.68	0.61	0.60	0.23	0.52	0.77	1.76	0.56	0.07	0.19	0.20	0.39	0.04	0.46	0.52	0.29	0.38	0.36	0.35	0.16
Earthworms	EW tot	1.89	0	15.61	0	2.28	4.71	1.81	1.39	60.81	47.48	65.80	21.51	104.58	193.03	83.88	112.67	1.39	6.07	8.31	5.95	11.41	2.09	18.87	10.19
	EW mass	0.91	0	2.06	0	0.76	0.98	0.30	0.14	10.53	21.05	31.02	26.63	14.16	41.61	29.38	36.33	3.57	3.69	9.26	16.26	5.18	0.97	5.41	6.22
	EW H'	0.35	0	0.14	0	0.10	0.42	0.40	0.29	0.40	0.24	0.09	0.14	0.15	0.07	0.13	0.12	0.26	0.28	0.49	0.24	0.13	0.40	0.33	0.19
	EW spp	1.15	0	0.58	0	0.58	1.53	1.00	1.53	0.58	0.58	2.08	0.58	1.53	1.73	0.58	0.58	0.00	1.15	1.15	0.58	1.53	0.58	1.53	1.15
Enchytraeids	EN Tot	5469	0	0	0	26500	29144	46925	41065	33862	9317	39062	60230	32779	11779	7678	8516	98957	6971	3140	12299	12125	2152	3583	4847
	EN H'	0.27	0	0	0	0.23	0.16	1.00	0.45	0.20	0.32	0.07	0.32	0.12	0.14	0.10	0.10	0.26	0.26	0.18	0.32	0.16	0.86	0.39	0.48
	EN spp	1.15	0	0	0	3.46	1.00	4.16	1.00	4.16	2.65	3.06	6.03	1.15	1.15	0.58	0.58	1.73	1.15	1.15	0.58	2.08	2.65	2.08	2.08
Nematodes	Nem - BF	26.02	7.84	4.69	9.94	6.96	18.91	21.47	22.81	23.88	10.26	8.17	9.27	4.14	4.86	23.03	9.98	6.10	6.01	11.36	7.99	5.33	2.87	7.03	8.33
	Nem - FF	47.75	11.04	1.53	15.00	3.64	31.56	0.57	0.00	14.86	1.44	3.26	9.69	6.29	1.03	11.03	3.68	18.18	17.51	9.35	6.03	7.12	2.53	2.56	6.24
	Nem - PF	7.71	0	2.07	0	32.81	15.81	9.20	4.01	1.60	12.62	1.77	5.51	3.05	3.41	2.50	7.65	7.77	0.98	9.74	5.84	1.79	1.95	1.21	1.90
	Nem - Om	29.92	3.66	3.15	6.42	18.78	5.47	17.39	2.62	13.78	12.97	9.80	10.89	9.94	7.42	1.81	13.92	2.97	5.30	1.74	4.09	5.42	4.98	2.11	3.68
	Nem - Ca	0	0.00	6.61	13.64	11.55	26.59	47.48	20.71	20.41	14.53	20.87	11.03	0.00	4.47	35.98	26.98	30.08	27.44	0.00	2.80	19.41	9.83	6.31	15.69
	Nem PC1	0.43	0.07	0.16	0.29	0.03	0.64	0.41	0.29	0.16	0.19	0.21	0.22	0.09	0.08	0.49	0.31	0.37	0.44	0.07	0.08	0.22	0.12	0.06	0.26
	Nem PC2	0.58	0.14	0.14	0.24	0.17	0.05	0.53	0.30	0.47	0.18	0.17	0.11	0.09	0.02	0.20	0.22	0.10	0.16	0.12	0.15	0.10	0.06	0.08	0.06
Mites	Mite tot	ND	1105	ND	4114	ND	18832	ND	17226	ND	15623	ND	10815	ND	9778	ND	4836	ND	14990	ND	4978	ND	636	ND	545
	Mite H'	ND	0.11	ND	0.38	ND	0.14	ND	0.09	ND	0.29	ND	0.39	ND	0.44	ND	0.29	ND	0.06	ND	0.17	ND	0.36	ND	0.46
	Mite spp	ND	0	ND	4.51	ND	4.58	ND	4.04	ND	3.21	ND	10.97	ND	3.61	ND	3.51	ND	2.08	ND	3.51	ND	1.53	ND	2.31
Collembola	Coll tot	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Coll H'	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Coll spp	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Supplementary table 2 continued																									
TRFLP	TRF-A H'	0.18	0.09	0.28	0.20	0.12	0.31	0.19	0.14	0.55	0.51	0.54	0.34	0.04	0.31	0.13	0.19	0.17	0.06	0.55	0.17	0.06	0.18	0.12	0.05
	TRF-B H'	0.05	0.05	0.11	0.06	0.04	0.12	0.07	0.07	0.26	0.10	0.23	0.19	0.03	0.06	0.14	0.04	0.02	0.07	0.08	0.01	0.11	0.06	0.07	0.10
	TRF-F H'	0.73	0.11	0.19	0.11	0.20	0.06	0.52	0.24	0.12	0.08	0.19	0.04	0.13	0.07	0.12	0.35	0.21	0.02	0.13	0.03	0.12	0.12	0.34	0.15
	TRF-A PC1	0.10	0.09	0.23	0.08	0.15	0.08	0.02	0.02	0.08	0.12	0.02	0.07	0.28	0.14	0.10	0.08	0.13	0.05	0.12	0.09	0.41	0.14	0.23	0.04
	TRF-A PC2	0.07	0.04	0.04	0.06	0.03	0.04	0.03	0.04	0.12	0.09	0.10	0.14	0.08	0.04	0.07	0.08	0.02	0.03	0.08	0.03	0.04	0.06	0.01	0.06
	TRF-B PC1	0.00	0.01	0.03	0.01	0.01	0.02	0.02	0.02	0.08	0.01	0.08	0.04	0.01	0.02	0.00	0.01	0.01	0.02	0.00	0.01	0.02	0.01	0.02	0.01
	TRF-B PC2	0.01	0.02	0.00	0.01	0.01	0.00	0.02	0.01	0.04	0.02	0.03	0.01	0.03	0.01	0.05	0.00	0.02	0.02	0.03	0.00	0.02	0.01	0.01	0.02
	TRF-F PC1	0.18	0.01	0.05	0.01	0.01	0.01	0.03	0.02	0.03	0.02	0.01	0.02	0.01	0.05	0.04	0.09	0.02	0.00	0.01	0.00	0.02	0.02	0.02	0.02
	TRF-F PC2	0.01	0.01	0.03	0.01	0.02	0.01	0.01	0.01	0.07	0.03	0.03	0.00	0.01	0.01	0.03	0.05	0.02	0.00	0.02	0.01	0.01	0.01	0.02	0.01
Functional	16Sbact	52849	38246	47184	43631	26478	32861	16301	24244	13833	12154	23291	7424	6737	22028	32960	41422	30185	10880	28997	31291	28856	32362	15111	10235
genes	AOA	50	682	194	25	148	1141	1358	5939	79	165	92	260	2521	6890	706	596	328	9296	532	3686	843	1577	1003	1233
	AOB	0.55	4.77	35.48	214.46	8.73	43.09	88.99	703.98	11.01	7.97	98.11	148.94	71.22	87.42	19.78	78.84	92.65	291.08	60.84	491.62	214.71	350.16	60.15	35.33
	nirK	5117	3757	6240	3760	4851	1364	2443	2126	1919	1004	1902	1461	2072	1586	3255	4074	1364	1237	3015	3733	5151	3095	1061	1608
	nirS	1671	1615	1278	1024	2153	3622	3132	8754	1746	185	5801	3230	2716	2081	4961	5541	7041	3211	7373	3628	7728	5945	2305	3798
	nosZ1	11511	7261	9031	7823	12677	4192	1963	5851	4993	1150	6093	6557	3046	4849	10360	10588	6095	3396	8005	3880	14213	3097	2884	2586
PLFA	PLFA tot	2.23	4.05	3.94	1.10	7.18	16.34	10.32	11.50	18.80	11.68	13.28	9.25	2.18	2.38	7.54	6.32	4.39	1.52	0.61	0.53	1.42	0.27	10.34	7.27
	PLFA PC1	7.45	6.44	1.85	0.30	0.52	1.68	0.72	0.68	1.47	1.27	0.94	1.57	3.22	1.53	2.00	2.74	1.32	0.78	0.55	1.08	1.37	1.41	1.18	5.46
	PLFA PC2	2.23	1.75	1.81	0.98	0.25	0.55	1.14	0.53	0.83	0.65	0.61	6.35	0.98	0.41	3.68	5.17	0.39	0.33	1.44	2.39	0.27	0.42	3.44	17.46
Single	HWC	39.75	63.19	47.77	40.30	164.16	909.72	523.72	574.54	820.67	397.55	173.89	173.68	67.39	12.27	61.81	77.70	127.94	125.01	19.72	41.18	93.69	153.02	46.80	71.70
endpoint	PMN	5.92	7.52	0.40	4.86	37.15	85.24	29.20	102.31	80.44	63.96	54.82	59.24	17.37	1.35	8.23	6.51	34.42	8.23	3.25	4.12	6.74	1.86	2.91	9.98
indicators	Ergost	0.87	0.13	0.35	0.12	0.34	2.32	1.49	0.18	0.10	0.03	0.19	0.10	0.17	0.09	0.11	0.13	0.10	0.03	0.44	0.09	0.15	0.19	0.33	0.21
	DNA	4896	4703	785	3521	28202	42862	54897	30988	25847	21086	2460	7801	11526	658	4976	1237	2510	5050	2686	1441	10392	15717	2378	1803
	Resilience	2.08	0.58	5.51	1.74	4.73	1.73	2.52	1.00	0.58	1.01	6.66	0.71	6.43	2.10	16.04	1.54	1.15	7.82	17.44	7.60	1.00	1.74	1.15	1.01
	Nit	4.53	3.30	2.56	16.12	117.16	762.78	1272.06	1869.57	45.57	34.52	501.29	669.55	54.31	28.56	39.62	103.33	102.24	115.22	59.06	71.68	105.48	127.21	147.39	23.10
	Infilt	0.30	ND	0.32	ND	ND	ND	ND	ND	ND	ND	ND	ND	85.46	ND	29.53	ND	27.14	ND	15.61	ND	9.37	ND	176.19	ND
	Bait lam	0.091	0.044	0.139	0.029	ND	ND	ND	ND	0.011	0.041	0.160	0.054	ND	ND	ND	ND	0.041	0.042	ND	ND	0.082	0.073	0.138	0.228

Figure 1. Sampling plan for each of the indicator sites. This common plan was implemented across all six sites in both sampling years.

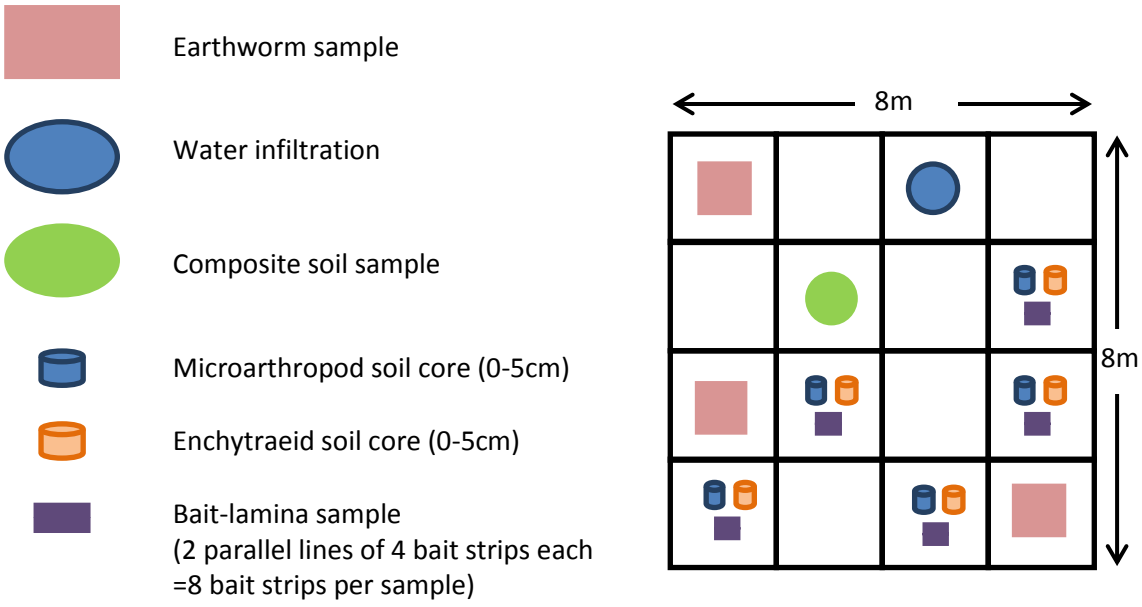


Figure 2. Mean scores, and percentage variance accounted for, of the first four principal components (PC) from analysis of all the indicator results, over both sampling occasions and treatments, for each site. Bar represents the least significant difference (lsd, P<0.05).

