Article (refereed) - postprint

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

biodiversity and ecosystem function

B.S. Griffiths[a,*], J. Römbke[b], R.M. Schmelz[b], A. Scheffczyk[b], J.H. Faber[c], J. Bloem[c], G. Pérès[d], D. Cluzeau[e], A. Chabbi[f], M. Suhadolc[g], J.P. Sousa[h], P. Martins da Silva[h], F. Carvalho[h], S. Mendes[h], P. Morais[h], R. Francisco[h], C. Pereira[h], M. Bonkowski[i], S. Geisen[i], R.D. Bardgett[j], F.T. de Vries[j], T. Bolger[k], T. Dirilgen[k], O. Schmidt[l], A. Winding[m], N.B. Hendriksen[m], A. Johansen[m], L. Philippot[n], P. Plassart[n], D. Bru[n], B. Thomson[o], R.I. Griffiths[o], M.J. Bailey[o], A. Keith[p], M. Rutgers[q], C. Mulder[q], S.E. Hannula[r], R. Creamer[s], D. Stone[s]

[a] Crop and Soil Systems Research Group, SRUC, Edinburgh, EH9 3JG, UK.
[b] ECT Oekotoxikologie GmbH, Flörsheim, Germany.
[c] Alterra, Wageningen UR , PO Box 47, 6700AA, The Netherlands.
[d] Agrocampus Ouest INRA Rennes, France.
[e] University of Rennes, CNRS OSUR-EcoBio, France.
[f] INRA Poitou Charentes, UR P3F ans Ecosys Grignon, France.
[g] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia.
[h] Department of Life Sciences, University of Coimbra, Portugal.
[i] Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Str 47b, D-50674 Cologne, Germany.
[j] Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Manchester, M13 9PT, UK.
[k] School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland.
[l] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland.
[m] University of Aarhus, Dept. Environmental Science, Roskilde, Denmark.
[n] INRA Dijon, France.
[o] Centre for Ecology & Hydrology, MacLean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, UK.
[p] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK.
[q] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
[r] NIOO-KNAW, Netherlands.
[s] Teagasc, Johnstown Castle Research Centre, Ireland.

* Corresponding author at SRUC, Edinburgh, EH9 3JG, UK. Bryan.Griffiths@sruc.ac.uk

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

biodiversity and ecosystem function

B.S. Griffiths[a,*], J. Römbke[b], R.M. Schmelz[b], A. Scheffczyk[b], J.H. Faber[c], J. Bloem[c], G. Pérès[d], D. Cluzeau[e], A. Chabbi[f], M. Suhadolc[g], J.P. Sousa[h], P. Martins da Silva[h], F. Carvalho[h], S. Mendes[h], P. Morais[h], R. Francisco[h], C. Pereira[h], M. Bonkowski[i], S. Geisen[i], R.D. Bardgett[j], F.T. de Vries[j], T. Bolger[k], T. Dirilgen[k], O. Schmidt[l], A. Winding[m], N.B. Hendriksen[m], A. Johansen[m], L. Philippot[n], P. Plassart[n], D. Bru[n], B. Thomson[o], R.I. Griffiths[o], M.J. Bailey[o], A. Keith[p], M. Rutgers[q], C. Mulder[q], S.E. Hannula[r], R. Creamer[s], D. Stone[s]

[a] Crop and Soil Systems Research Group, SRUC, Edinburgh, EH9 3JG, UK.
[b] ECT Oekotoxikologie GmbH, Flörsheim, Germany.
[c] Alterra, Wageningen UR , PO Box 47, 6700AA, The Netherlands.
[d] Agrocampus Ouest INRA Rennes, France.
[e] University of Rennes, CNRS OSUR-EcoBio, France.
[f] INRA Poitou Charentes, UR P3F ans Ecosys Grignon, France.
[g] University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia.
[h] Department of Life Sciences, University of Coimbra, Portugal.
[i] Department of Terrestrial Ecology, Institute of Zoology, University of Cologne, Zülpicher Str 47b, D-50674 Cologne, Germany.
[j] Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Manchester, M13 9PT, UK.
[k] School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland.
[l] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland.
[m] University of Aarhus, Dept. Environmental Science, Roskilde, Denmark.
[n] INRA Dijon, France.
[o] Centre for Ecology & Hydrology, MacLean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire OX10 8BB, UK.
[p] Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK.
[q] National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
[r] NIOO-KNAW, Netherlands.
[s] Teagasc, Johnstown Castle Research Centre, Ireland.

* Corresponding author at SRUC, Edinburgh, EH9 3JG, UK. Bryan.Griffiths@sruc.ac.uk

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

18 indicators of soil biodiversity and ecosystem function
tested at 6 sites across European climatic zones

AG   Atlantic - Grassland
AA   Atlantic - Arable
CG   Continental - Grassland
CA   Continental - Arable
MA   Mediterranean - Arable
PA   Pannonian - Arable

Indicative map of European biogeographical regions. ETC/BIODIVERSITY, Paris, Feb 2006
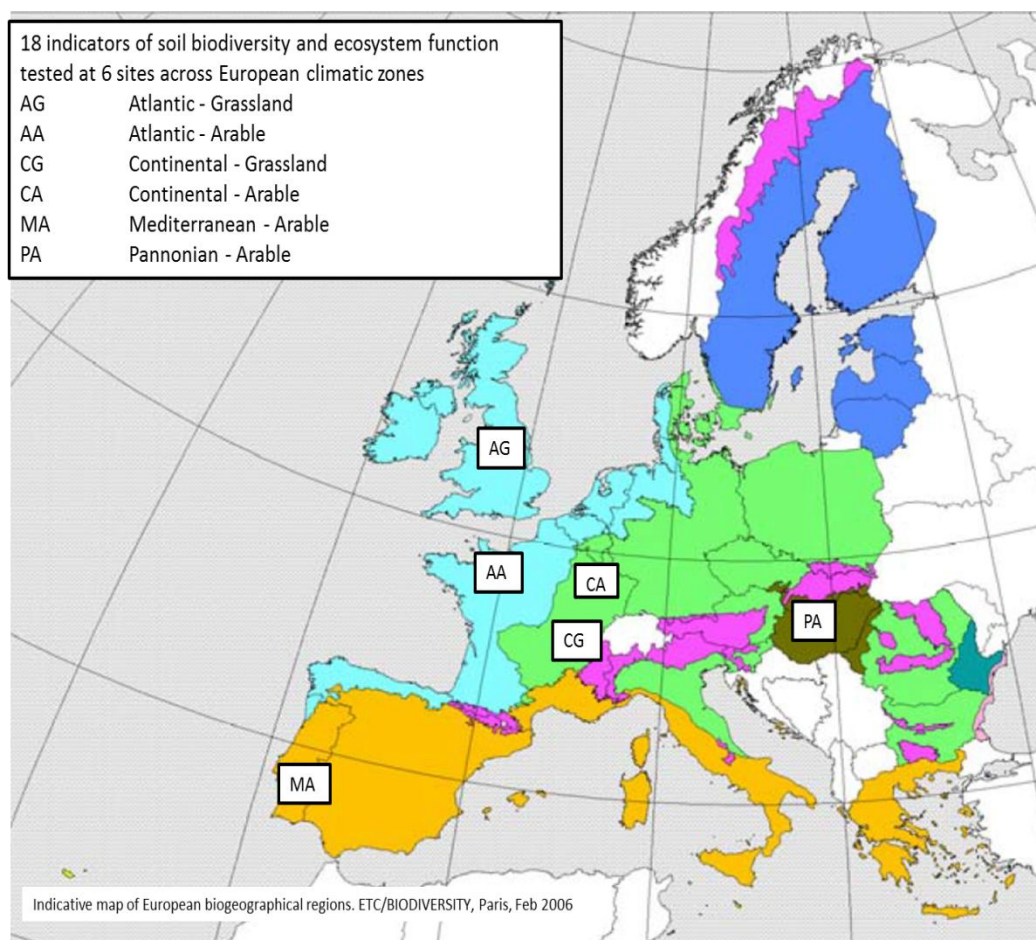
1   **Abstract**

2   Soils provide many ecosystem services that are ultimately dependent on the local diversity

3   and belowground abundance of organisms. Soil biodiversity is affected negatively by many threats

4   and there is a perceived policy requirement for the effective biological monitoring of soils at the

5   European level. The aim of this study was to evaluate and recommend policy relevant, cost-effective

6   soil biological indicators for biodiversity and ecosystem function across Europe. A total of 18

7   potential indicators were selected using a logical-sieve based approach. This paper considers the use

8   of indicators from the 'top down' (i.e. concerned with the process of indicator selection), rather than

9   from the 'bottom up' detail of how individual indicators perform at specific sites and with specific

10   treatments. The indicators assessed a range of microbial, faunal and functional attributes newer

11   nucleic acids based techniques, morphological approaches and process based measurements. They

12   were tested at 6 European experimental sites already in operation and chosen according to land-use,

13   climatic zone and differences in land management intensity. These were 4 arable sites, one each in

14   Atlantic, Continental, Mediterranean and Pannonian climate zones, and 2 grassland sites, one each

15   in Atlantic and Continental zones. At each site we sampled three replicated plots of contrasting

16   management intensity and, while the treatments varied from site to site, their disturbance effects

17   were quantified in terms of land use intensity. The field sampling and laboratory analysis were

18   standardised through a combination of ISO protocols, or standard operating procedures if the

19   former were not available. Sites were sampled twice, in autumn 2012 and spring or autumn 2013,

20   with relative costs of the different indicators being determined each time. A breakdown of the cost

21   effectiveness of the indicators showed the expected trade-off between effort required in the field

22   and effort required in the laboratory. All the indicators were able to differentiate between the sites

23   but, as no single indicator was sensitive to all the differences in land use intensity, we suggest that

24   an indicator programme should be based upon a suite of different indicators. For monitoring under

25   the European climatic zones and land uses of this study, indicators for ecosystem functions related

26   to the services of water regulation, C-sequestration and nutrient provision would include a minimum

27   suite of: earthworms; functional genes; and bait lamina. For effective monitoring of biodiversity all

28   taxonomic groups would need to be addressed.

29

30   **1. Introduction**

31   Human societies are highly dependent upon healthy soils for the delivery of ecosystem

32   goods and services, including provisioning (food, fibre, timber, fuel), regulation (climate, disease,

33   natural hazards), waste treatment, nutrient cycling and cultural services (Millennium Ecosystem

34   Assessment, 2005). Many of the key functions supporting these ecosystem services depend to a

35   large extent upon the diversity, abundance and activity of organisms that inhabit the soil. This

36   diversity varies in terms of its taxonomic richness, relative abundance and distribution according to

37   soil type, climatic conditions, vegetation and land use. Against this background, soil biodiversity is

38   also subject to various threats associated with human activity, including soil erosion, organic matter

39   decline, and contamination, salinization, sealing, compaction of soil and climate change; all these

40   threats impair soil biodiversity and functioning with negative consequences on ecosystem service

41   delivery (Hooper et al., 2005; Gardi et al., 2013; Wall et al., 2012). Increasing agricultural intensity,

42   for example, has been shown to generally reduce soil biodiversity (e.g., Tsiafouli et al., 2015),

43   although this response is likely to be non-linear given the variation in management practices and soil

44   conditions across sites and regions, and differences in the sensitivity of soil organism groups to

45   management intensity. As a result, there is a strong and increasing policy requirement for the

46   effective monitoring of soils at local, regional and national scales (EU, 2006a, b; Ritz et al., 2009;

47   Turbé et al., 2010; Cluzeau et al., 2012). Moreover, this need has been stimulated by the Convention

48   on Biological Diversity (http://www.cbd.int/), which includes a requirement for indicators capable of

49   monitoring changes in soil biodiversity (Pulleman et al., 2012).

50   Most soil processes are mediated by soil biota in direct relationship with the physico-

51   chemical properties of their environment. Furthermore, soil organisms have the ability to adapt

52   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

130   As the objective was to determine how sensitive the selected indicators are to typical

131   disturbances in the European situation, six sites across Europe were chosen that had: a consistent

132   agricultural management history over several years; were characteristic of recognised European

133   climatic zones; consisted of at least three, independent, replicated plots of two contrasting

134   treatments which varied in intensity of management. Site details are given in Table 2 and

135   summarised here as four arable and two grassland sites: Lusignan, Atlantic arable site in France,

136   with rotations of grass-arable (least intensive) and continuous arable (most intensive)

137   (http://www.soere-acbb.com/index.php/fr/, Kunrath et al. 2014 and Senapati et al. 2014);

138   Scheyern, Continental arable site in Germany with long-term plots of minimum-tillage with small

139   fertilisation (least intensive) and conventional tillage with large fertilisation (most intensive) as

140   described by Zeitz et al. (2004); Moskanjci, Pannonian arable site in Slovenia, with long-term plots of

141   minimum-tillage (least intensive) and conventional tillage (most intensive) according to Kaurin et al.

142   (2015); Castro Verde, Mediterranean arable site in Portugal, where the least intensive plots were

143   grass-arable rotation with minimum tillage and no fertilisation and the most intensive plots were

144   conventionally ploughed and fertilised, as described by Marta-Pedroso et al., (2007); Yorkshire

145   Dales, Atlantic grassland site in the UK, with paired plots of extensive and intensive grassland at

146   three locations within the Yorkshire Dales National Park, as described by de Vries et al.,(2012);

147   Hainich, Continental grassland site in Germany, with paired plots of extensive and intensive

148   grassland as described by Fischer et al., (2010).

149   The management options chosen are typical for European soils. The intensity of land use at

150   each site was calculated using the equation of Blüthgen et al. (2012), modified to include time since

151   tillage and tillage depth. Thus:

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

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

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

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

156   T = days since tillage, converted to a negative exponential scale (T = $187.47e^{-0.014x}$,

157   where x = days since tillage) to account for tillage effects being most evident

158   immediately after tillage.

159   D = depth (cm) of tillage

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

161   (T/mean) and (D/mean) were multiplied by 5 as a weighting to reflect that the

162   impact of tillage was greater than that of mowing, grazing or fertilisation on soil

163   biological processes.

164

165   *2.3. Sampling and standardisation*

166

167   The selected indicators chosen for validation required different sampling procedures: with

168   some having to be measured *in-situ*; some requiring intact soil cores; and the rest needing a

169   composite bulk soil sample. Samples were then sent to the various analysing laboratories around

170   Europe, such that each indicator was measured by only one laboratory. A coding system was

171   developed to give each sample a unique identifier, linked to a searchable database designed for

172   long-term data storage of the results collected. For standardisation of sampling, detailed standard

173   operating procedures (SOPs, with step by step instructions and photographs to ensure clarity) and a

174   video of the composite soil sampling were distributed before sampling began

175   (http://www.youtube.com/watch?v=_k7BEInBXEc&feature=youtu.be).

176   Samples were taken on two occasions at each site, 2012 (autumn) and 2013 (spring or

177   autumn), to ensure at least a minimum temporal variation, and included the *in-situ* measurements,

178   intact cores and composite soil samples as outlined above. Sampling followed a prescribed pattern

179   within an 8 m x 8 m area selected at a random location in each replicate plot (Figure 1). Within each

180   sampling area there was: a composite soil sample prepared by mixing five soil samples taken by

181   auger from the top 15cm, this consisted of soil from a central auger and then four more augers 1 m

182   away from the central point in North-South and East-West directions; five intact soil cores (5 cm

183   diameter and 5 cm deep) were each collected for separate microarthropod and enchytraeid

184   extraction (ISO 23611-2, 2006; ISO 23611-3, 2007), three soil pits of 35 cm x 35 cm (the depth varied

185   according to the site conditions, but it was always between 10 and 20 cm) were dug for the hand-

186   sorting and formaldehyde extraction of earthworms (ISO 23611-1, 2006), with collected earthworms

187   being preserved in 70% ethanol in the field; bait lamina sticks were laid out in five blocks within each

188   area (ISO 18311, 2012); water infiltration was determined using a double ring infiltrometer (DIN 19682-

189   7, 2007). In the field, composite soil samples and intact soil cores were kept in an insulated box

190   containing frozen 'cool blocks' until they could be stored at 4°C in a laboratory. The composite soil

191   samples were sieved through a 4mm diameter mesh and divided into aliquots appropriate for the

192   different soil analyses. Aliquots of composite soil and intact soil cores were repackaged in insulated

193   boxes with frozen 'cool blocks' and dispatched by 24hr courier to the analysing laboratories.

194

195   *2.4. Laboratory analysis*

196

197   Soil from the composite samples was analysed as follows: DNA was extracted (ISO 11063, Petric

198   et al., 2011) and DNA yield quantified (Plassart et al., 2012). Extracted DNA was used to determine the

199   structure of the microbial community by terminal restriction fragment length polymorphism of the

200   archaeal, bacterial and fungal communities (TRFLP, Plassart et al., 2012), and also for quantitative PCR

201   to determine abundances of the total bacterial community (16S rRNA) and of functional genes involved

202   in nitrogen cycling by using the *amoA*, *nirK*, *nirS* and *nosZ1* genes as molecular markers (Bru et al.,

203   2011). Ergosterol was quantified following alkaline extraction (de Ridder-Duine et al. 2006); Multiple

204   substrate utilisation with MicroResp (Campbell et al., 2003, as modified by Creamer et al., 2009) used

205   eight substrates: Water, L-Arginine, L-Malic Acid, Gamma Amino Butyric Acid, n-Acetyl Glucosamine,

206   D(+) Glucose, Alpha ketogluterate and Citric Acid; Extra-cellular Enzyme Activity (EEA; Johansen et al.,

207   2005; Hendriksen et al., 2015) from the activity of β-1,4-glucosidase and cellobiohydrolase, α-1,4-glucosidase,

208   β-*N*-acetyl-glucosaminidase, β-1,4-xylosidase, aminopeptidase, phosphatase and arylsulphatase; Nematodes,

209   which were extracted by an Oostenbrink elutriation and Baermann funnel technique from 100 g of

210   fresh soil for directed-T-RFLP analysis (Donn et al., 2012); Potentially Mineralisable Nitrogen (PMN,

211   Canali et al., 2006); Hot Water extractable Carbon (HWC, Ghani et al., 2003); Microbial resilience to

212   antibiotic (resilience) was determined in 96 well micro-titre plates as the difference in the lag-phase

213   until growth of bacteria from a suspension of composite soil either with or without penicillin. We

214   used six replicates of 200 µl of soil suspension (1.5 g dry weight equivalent soil 100 ml^{-1} Neff's

215   modified amoeba saline (Page, 1988)) added to 200 µl of 1/10 strength Luria-Bertani medium either

216   with 15 µg penicillin well^{-1} (Penicillin from AppliChem, BioChemica, Penicillin G-Kaliumsalz) or

217   without penicillin, incubated at 20°C and the optical density (450 nm) measured every 1 hr for 72 hrs

218   with automatic agitation for 5 s prior to reading; Potential nitrification (Kandeler (1996), adapted to

219   microplate reader as described by Sousa et al.,2004); Phospholipid Fatty Acid (PLFA) determinations

220   were based on the guideline ISO/DTS 29843-2 (2011). The method proposed in this SOP results from

221   a modification of the ISO guideline, including a second method for fatty acid identification (Francisco

222   et al., 2015). Earthworms were counted, adults identified to species and juveniles to genus, and then

223   weighed. Soil physical and chemical determinations were performed by the Soil Analysis Laboratory

224   of INRA in Arras, France, which is accredited for soil and sludge analysis.

225   Soil from intact cores (5 cm diameter) was used to extract enchytraeids and

226   microarthropods. Enchytraeids were extracted with O'Connor's hot/wet funnel method (O'Connor,

227   1962) (ISO 23611-3, 2006). Specimens were identified to species using the keys and techniques of

228   Schmelz and Collado (2010, 2012), together with primary literature. Microarthropod extraction

229   followed ISO 23611-2 (2004) using a Macfadyen high gradient extractor for 7 days, slide mounted

230   and identified to species.

231

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*

257

258   The costs associated with each indicator were estimated on a relative basis for the three

259   areas of: 1) operation in the field; 2) operation in the laboratory; and 3) equipment /

260   instrumentation. On each sampling occasion the field teams recorded the person-hours required for

261   each task. The laboratory analytical teams then made observations on the ease of running each

262   assay, the throughput (samples per unit time) and whether the assay yielded multiple or single

263   endpoints. Finally, the capital costs of the main instruments used in each assay were noted. The

264   methods were then ranked under each category for comparison.

265

266   **3. Results**

267

268   *3.1. Selection of indicators*

269

270   The result of the selection process based on a logical sieve was a weighted score for each of the 27

271   potential indicators (Table 1). Most of the higher-ranking indicators were taken forward for

272   evaluation (Table 1), but some of them were not considered because of methodological or practical

273   limitations. For example, the molecular methods for faunal indicators were only considered

274   advanced enough for nematodes (Vervoort et al., 2012). Litter bags and bait lamina (with a logical

275   sieve score of 500 and 492, respectively) were statistically indistinguishable and logistical

276   considerations favoured the bait lamina assay. Protistan morphology was considered too laborious

277   and specialist, while the other low-scoring potential indicators were considered inappropriate or

278   duplicated by higher scoring indicators. Some low-scoring potential indicators were evaluated, such

279   as water infiltration because of its direct relevance to the key ecosystem service of water retention.

280   Basal respiration was not evaluated independently but could be inferred from the MicroResp assay

281   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.

307 All indicators were sensitive to site (i.e. the sites could be differentiated on the response of

308 the indicators). A principal component analysis of all data (Figure 2) showed that the grassland sites

309 (Yorkshire Dales and Hainich) clustered together in the PC3-PC4 plot explaining 16% of variation and

310 separately from the arable sites which formed a separate cluster. Sites were different from one

311 another according to the principal component analysis using all the data, but also by analysis of

312 individual indicators. For example: indicators that separated the Yorkshire Dales from Hainich were:

313 MicroResp; enchytraeids; AOB; 16S; nosZ1; AOB; PMN; ergosterol; soil water content; nitrification;

314 and T-RFLP (archaeal, bacterial and fungal). Lusignan gave a significantly different response than

315 Moskanjci to: EEA; AOB; NirS; resilience; nitrification; and T-RFLP (archaeal, bacterial and fungal)

316 (Supplementary Tables S1 and S2). Measures of biodiversity, although showing significant

317 differences between sites, were idiosyncratic (Supplementary Table S1). Thus, for example: using

318 enchytraeid H' the Yorkshire Dales and Hainich sites were more diverse than Moskanjci and

319 Scheyern; with earthworm H' Yorkshire Dales was equally as diverse as Moskanjci and Scheyern but

320 Hainich was less diverse; with mite H' Scheyern was the least diverse site. The grassland sites,

321 Yorkshire Dales and Hainich, had the greatest faunal diversity with Castro Verede and Scheyern

322 having the least. Microbial biodiversity as determined from T-RFLP was also idiosyncratic, but

323 showed that Yorkshire Dales had the greatest biodiversity of archaea while Moskanjci tended to

324 have a large biodiversity of bacteria and fungi.

325

326 *3.3. Indicator performance at individual sites*

327

328 The effects of management at the individual sites could be distinguished by a range of

329 indicators, albeit a different set of indicators at each site (Table 4). In general, as the difference in

330 LUI increased so the number of indicators showing an effect of the management increased, with the

331 exception of Scheyern which had the greatest difference in LUI (20), yet the conventionally managed

332 plots were only differentiated from the organically managed plots by four of the indicators used,

333 namely resilience, bait lamina, earthworms and enchytraeids (Table 4). This was the same number of

334 differentiating indicators as at the sites with the lowest LUI, i.e., Lusignan and Yorkshire Dales. At

335 Lusignan there were no management effects detected by the functional indicators, although we

336 were not able to use the bait lamina method there, and at Scheyern no management effects were

337 detected by the microbial indicators (Table 4). Water infiltration was only measured at Moskanjci,

338 Scheyern, Castro Verde and Lusignan in 2012 and rates were only significantly different at Scheyern

339 between the organically managed (24 mm hr^{-1}) and conventionally managed (506 mm hr^{-1}, P < 0.01)

340 plots. Differences due to management were not significant at the other sites in autumn 2012 but

341 water infiltration did differ between sites. Thus infiltration rates for least intensive and most

342 intensive plots (respectively) were at: Moskanjci 1.4 and 1.9 mm hr^{-1}; Castro Verde 0.44 and 0.39

343 mm hr^{-1}; and Lusignan 130 and 202 mm hr^{-1}.

344   The indicators of biodiversity also responded differently to the treatments at each site with:

345 enchytraeid H' differentiating treatment and control plots at Castro Verde, Hainich and Scheyern;

346 earthworm H' only differentiating at Lusignan; mite H' only differentiating at Moskanjci; and

347 microbial T-RFLP differentiating at Castro Verde, Hainich and Yorkshire Dales.

348

349 *3.4. Relative indicator costs*

350

351   Table 5 shows the groupings of the indicators and within each column the first group scores

352 best for that attribute. For 'ease of field work', the 'easy' group of indicators required a composite

353 soil sample that could be readily collected within a single day, the 'moderate' group could also be

354 collected in a single day by a single visit to each site but sampling was more involved than the

355 composite soil, while in the 'difficult' group the bait lamina assay required revisiting the site at a

356 variable time later (i.e. when about 50% of the substrate has been eaten) and the water infiltration

357 assay required that a large volume of water be readily available and took more than a single day at

358 some sites. The 'utility of the assay' grouped those indicators that gave several endpoints (i.e. EEA

359 and MicroResp assayed eight substrates simultaneously; faunal groups give information on

360 biodiversity, organisms abundance/biomass and functional attributes; PLFA informs on microbial

361 biomass, bacterial: fungal ratio and indicator peaks) and grouped those that only give a single

362 endpoint. The 'ease of laboratory assay' had three groups, the 'basic skill level' group containing

363 indicators requiring a simple incubation and/or extraction (nitrification for example requires a single

364 extraction with a salt solution), a 'moderate' group requiring a more complex extraction or setup

365 (thus DNA or ergosterol need a sequential extraction), and a 'technically demanding' group requiring

366 the most sophisticated extractions and analysis (such as TRFLP which needs additional processing

367 once the DNA has been extracted). The 'laboratory throughput' gave a 'high' and a 'low' group

368 according to the rate and number of samples that can be handled at any one time. Finally the 'setup

369 cost' ranked the indicators in three groups as the 'least expensive', which tend to be incubations

370 whose endpoint is determined by colorimetric reaction, through the 'moderately expensive' to the

371 'most expensive' techniques which need gas chromatographs and nucleic acid sequencers with a

372 relatively high associated capital and consumable cost. The table of relative costs was broken down

373 into these different scenarios, mainly because most laboratories would be starting out with different

374 amounts of essential equipment in place. So start-up costs would be different in each case.

375

376 **4. Discussion**

377

378   The objective of the study was to recommend indicators for soil biodiversity and ecosystem

379 function. Although samples were collected from European agricultural sites, the outcomes would be

380 relevant for non-agricultural soils, especially those of a mineral or organo-mineral texture.   A

381 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

408   land use intensity and that their ranking of biodiversity varied between sites (Supplementary Table

409   S1). For example, sites such as the Yorkshire Dales had a consistent increase in diversity (Shannon)

410   in the least intensive management for earthworms, mites, Collembola and archaea but not for

411   enchytraeids, bacteria and fungi. Other sites had contrasting trends, such as Moskanjci which had

412   greater earthworm diversity but less enchytraeid diversity in the least intensive management, or

413   Lusignan which had greater earthworm and archaeal diversity but less enchytraeid and bacteria

414   diversity in the least intensive management (Table S1). For effective monitoring of biodiversity,

415   therefore, all taxonomic groups would need to be addressed because changes in the biodiversity of

416   one group cannot be used to infer changes in other taxonomic groups .

417   These bioindicators will require standardisation and their deployment will need to be cost-

418   effective and policy-relevant in order to be efficiently applied throughout Europe. This will allow us

419   to identify the land uses which have the most severe impact on soil functioning and to quantify

420   threats to soil ecosystem functions.

421

422   *4.1. Indicator selection*

423

424   The logical sieve (Ritz et al., 2009; Stone et al., 2016) based approach that we used is

425   considered to be a scientifically valid and objective selection process that has been used in similar

426   scientific studies for indicator selection in the face of a large number of methods to choose from

427   (Aalders et al., 2009). The scores from this method can then inform judicial selection of the

428   indicators, because this process resulted in several indicators ending up with similar scores. For

429   example, basal respiration, bait lamina and litter bags all scored around 500, so we chose between

430   these equivalent indicators based on the availability of equipment and expertise in the participating

431   laboratories. In other cases, some indicators that scored relatively poorly but had high relevance to

432   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**

535   For undertaking a large-scale biological indicator programme this study has shown that

536   standardisation of methods is an absolute necessity, otherwise it is not possible to properly compare

537   results. This would include an inter-laboratory comparison for the small number of indicators finally

538   selected (i.e. Creamer et al., 2009). It would also necessitate accurate prescription of sampling

539   appropriate for the land uses and edaphic conditions within the monitoring area. An easily accessible

540   database needs to be established to detect temporal changes, so that the results can show a

541   trajectory of system improvement or decline rather than just being a point measure of status. A

542   suite of complementary indicators is necessary, ideally linking biodiversity to soil functioning to give

543   a more meaningful outcome. The ongoing developments in nucleic acid based analyses of

544   biodiversity are likely to improve the throughput and resolution of biodiversity indicators, which

545   need to cover both microbial and faunal groups. Indicators for ecosystem functions related to the

546   services of water regulation, C-sequestration and nutrient provision would include a minimum suite

547   of: earthworms; microbial functional genes; and bait lamina.

548

564

565   **Appendix A. Supplementary data**

566

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

References

Aalders, I., Hough, R.L., Towers, W., Black, H.I.J., Ball, B.C., Griffiths, B.S., Hopkins, D.W., Lilly, A., McKenzie, B.M., Rees, R.M., Sinclair, A.S., Watson, C. and Campbell, C.D. (2009) Considerations for Scottish soil monitoring in the European context. Eur. J. Soil Sci. 60: 833–843.

Becaert, V. and Deschenes, L., 2006. Using soil health to assess ecotoxicological impacts of pollutants on soil microflora. Rev. Environ. Contam. Toxicol. 188:127–148.

Bispo, A., Cluzeau, D., Creamer, R., Dombos, M. Graefe, U., Krogh, P. H., Sousa, J. P., Pérès, G., Rutgers, M., Winding, A. and Römbke, J. (2009). Indicators for monitoring soil biodiversity. Integr. Environ. Assess. Manag. 5: 717–719.

Black, H.I.J., Parekh, N.R., Chaplow, J.S., Monson, F., Watkins, J., Creamer, R., Potter, E.D., Poskitt, J.M., Rowland, P., Ainsworth, G. and Hornung, M. (2003). Assessing soil biodiversity across Great Britain: national trends in the occurrence of hetero-trophic bacteria and invertebrates in soil. J. Environ. Manage. 67:255–266.

Blüthgen, N., Dormann, C.F., Prati, D., Klaus, V.H., Kleinebecker, T., Holzel, N., Alt, F., Boch, S., Gockel, S., Hemp, A., Muller, J., Nieschulze, J., Renner, S.C., Schoning, I., Schumacher, U., Socher, S.A., Wells, K., Birkhofer, K., Buscot, F., Oelmann, Y., Rothenwohrer, C., Scherber, C., Tscharntke, T., Weiner, C.N., Fischer, M., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.D. and Weisser, W.W. (2012) A quantitative index of land-use intensity in grasslands: Integrating mowing, grazing and fertilization. Basic Appl. Ecol. 13: 207-220.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W. and de Bruyn, M. (2014) Environmental DNA for wildlife biology and biodiversity monitoring. Trends Ecol. Evol. 29: 358 – 367.

Bongers, T. (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14-19.

Bru, D., Ramette, A., Saby, N. P. A., Dequiedt, S., Ranjard, L., Jolivet, C., Arrouays, D. and Philippot, L. (2011). Determinants of the distribution of nitrogen-cycling microbial communities at the landscape-scale. The ISME J. 5: 532-542.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M. (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. Appl. Environ. Microb. 69, 3593-3599.

Canali, S. and Benedetti, A. (2006). Soil nitrogen mineralization. In: Bloem J., Hopkins, D.W., Benedetti, A. (Eds), Microbiological Methods for Assessing Soil Quality, CABI, Wallingford, UK, pp. 127-135.

Cluzeau, D., Guernion, M., Chaussod, R., Martin-Laurent, F., Villenave, C., Cortet, J., Ruiz-Camacho, N., Pernin, C., Mateille, T., Philippot, L., Bellido, A., Rougé, L., Arrouays, D., Bispo, A. and Pérès, G. (2012). Integration of biodiversity in soil quality monitoring: Baselines for microbial and soil fauna parameters for different land-use types Eur. J. Soil Biol. 49: 63-72.

Creamer, R.E., Bellamy, P., Black, H.I.J., Cameron. C.M., Campbell, C.D., Chamberlain, P., Harris, J., Parekh N., Pawlett, M., Poskitt, J., Stone, D., Ritz, K. (2009) An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring Biol. Fertil. Soils 45, 623-633.

Creer, S., Fonseca, V.G., Porazinska, D.L., Giblin-Davis, R.M., Sung, W., Power, D.M., Packer, M., Carvalho, G.R., Blaxter, M.L., Lambshead, P.J.D. and Thomas, W.K. (2010) Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. Mol. Ecol. 19: 4–20.

de Ridder-Duine, A.S., Smant, W., van der Wal, A., van Veen, J.A. and de Boer, W. (2006). Evaluation of a simple, non-alkaline extraction protocol to quantify soil ergosterol. Pedobiologia 50: 293-300.

de Vries, F.T., Bloem, J., Quirk, H., Stevens, C.J., Bol, R. and Bardgett, R.D. (2012). Extensive

 management promotes plant and microbial nitrogen retention in temperate grassland. PLOS ONE

 7: e51201.

DIN 19682-7:2007-07 - Soil quality - Field tests - Part 7: Determination of infiltration rate by double

 ring infiltrometer. Beuth Verlag GmbH, Berlin, Germany.

Ditzler, C.A. and Tugel, A.J. (2002). Soil quality field tools: experiences of USDA-NRCS soil quality

 institute. Agron. J. 94:33–38.

Donn, S., Neilson, R., Griffiths, B. S. and Daniell, T. J. (2012). A novel molecular approach for rapid

 assessment of soil nematode assemblages – variation, validation and potential applications.

 Meth. Ecol. Evol. 3: 12–23.

Doran, J.W. and Zeiss, M.R. (2000). Soil health and sustainability: managing the biotic component of

 soil quality. Appl. Soil Ecol. 15:3–11.

EU (European Union) 2006a. Communication from the Commission to the Council, the European

 Parliament, the Economic and Social Committee and the Committee of the Regions. Thematic

 Strategy for Soil Protection plus Summary of the Impact Assessment. COM 231 (2006) final.

 Brussels, 12 + 8 pp.

EU (European Union) 2006b. Proposal for a Directive of the European Parliament and of the Council

 establishing a framework for the protection of soil and amending Directive 2004/35/EC. COM 232

 (2006) final. Brussels, 30 pp.

Faber, J. H., Creamer, R. E., Mulder,C., Römbke,J., Rutgers,M., Sousa, J. P., Stone, D. and Griffiths, B.

 S. (2013). The practicalities and pitfalls of establishing a policy-relevant and cost-effective soil

 biological monitoring scheme. Integr. Environ. Assess. Manag. 9: 276–284.

Feld, C.K., Martins da Silva, P., Sousa, J.P., De Bello, F., Bugter, R., Grandin, U., Hering, D., Lavorel, S.,

 Mountford, O., Pardo, I., Pärtel, M., Römbke, J., Sandin, L., Jones, B. and Harrison, P. (2009).

 Indicators of biodiversity and ecosystem services: a synthesis across ecosystems and spatial

 scales. Oikos 118:1862-1871.

Fischer, C., Roscher, C., Jensen, B., Eisenhauer, N., Baade, J., Attinger, S., Scheu, S., Weisser, W.W., Schumacher, J. and Hildebrandt, A. (2014). How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? PLOS ONE 9: e98987.

Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D. and Weisser, W.W. (2010). Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. Basic Appl. Ecol. 11: 473-485.

Fierer, N., Barberán, A. and Laughlin, D.C. (2014). Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. Frontiers Microbiol. 5: 614

Francaviglia, R. (2008). Agricultural Soil Erosion and Soil Biodiversity: Developing Indicators for Policy Analyses. OECD, Paris.

Francisco, R., Abreu, P., Creamer, R.E., Costa, M.S., Sousa, J.P. and Morais, P.V. (2015) Methodological improvements of the simple phospholipid-derived fatty acids (PLFA) extraction method applied to a comparative analysis of European soils. Applied Soil Ecology, under review.

Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Jolivet, C., Mulder, C., Ranjard, L., Römbke, J., Rutgers, M., Menta, C. 2009. Soil biodiversity monitoring in Europe: ongoing activities and challenges. Eur. J. Soil Sci. 60:807-819.

Gardi, C., Jeffery, S. and Saltelli, A. (2013). An estimate of potential threats levels to soil biodiversity in EU. Glob. Change Biol. 19: 1538-1548.

Ghani, A., Dexter, M. and Perrott, K.W. (2003). Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biol. Biochem. 35: 1231–1243.

Havileck, E. (2012). Soil biodiversity and bioindication: From complex thinking to simple acting. Eur. J. Soil Biol. 49: 80-84.

Hendriksen, N.B., Creamer, R.E., Stones, D. and Winding, A. (2015). Soil exo-enzyme activities across Europe – the influence of climate, land-use and soil properties. Appl. Soil Ecol. (in press).

Hooper, D.U., Chapin, F.S. III, Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H.,Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J.,Vandermeer, J. and Wardle, D.A. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol. Monogr. 75:3–35.

ISO 11063:2012 Soil quality -- Method to directly extract DNA from soil samples. International Organization for Standardization, Geneva, Switzerland.

ISO 23611-1:2006. Soil quality — Sampling of soil invertebrates:— Part 1: Hand-sorting and formalin extraction of earthworms. International Organization for Standardization, Geneva, Switzerland.

ISO 23611-2:2006. Soil quality -- Sampling of soil invertebrates -- Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina). International Organization for Standardization, Geneva, Switzerland.

ISO 23611-3:2007. Soil quality — Sampling of soil invertebrates: — Part 3: Sampling and soil extraction of enchytraeids. International Organization for Standardization, Geneva, Switzerland.

ISO/DIS 18311. Soil quality — Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test. International Organization for Standardization, Geneva, Switzerland.

ISO/TS 29843-2:2011 Soil quality - Determination of soil microbial diversity – Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method. International Organization for Standardization, Geneva, Switzerland.

Johansen, A., Knudsen, I.M.B., Binnerup, S.J., Winding, A., Johansen, J.E., Jensen, L.E., Andersen, K.S., Svenning, M.,M. and Bonde, T.A. (2005). Non-target effects of the microbial agents *Pseudomonas*

fluorescens DR54 and *Clonostachys rosea* IK726 in soils cropped with barley followed by sugar beet: A greenhouse assessment. Soil. Biol. Biochem. 37: 2225-2239.

Jones, C.M., Spor, A., Brennan, F.P., Breuil, M-C., Bru, D., Lemanceau, P., Griffiths, B.S., Hallin, S. and Philippot, L. (2014) Recently identified microbial guild mediates soil N_2O sink capacity. Nature Climate Change 4: 801-805.

Kandeler, E. (1996). Potential nitrification. In: Schinner, F., Öhlinger, R., Kandeler, E., Margesin, R. (Eds.), Methods in Soil Biology. Springer, Berlin, pp. 146-149.

Kaurin, A., Mihelic, R., Kastelec, D., Schloter, M., Suhadolc, M. and Grcman, H. (2015). Consequences of minimum soil tillage on abiotic soil properties and composition of microbial communities in a shallow Cambisol originated from fluvioglacial deposits. Biol. Fertil. Soils (in press).

Kunrath, T.R., de Berranger, C., Charrier, X., Gastal, F., César de Faccio Carvalho, P., Lemaire, G., Emile, J.C. and Durand, J.L. (2014). How much do sod-based rotations reduce nitrate leaching in a cereal cropping system? Agric. Water Manage. 150: 46-56.

Lavelle, P. and Spain, A.V. (2005). Soil Ecology. Springer, Dordrecht: 654 pp.

Lemanceau, P., Maron, P-A., Mazurier, S., Mougel, C., Pivato, B., Plassart, P., Ranjard, L., Revellin, C., Tardy, V. and Wipf, D. (2015). Understanding and managing soil biodiversity: a major challenge in agroecology. Agron. Sustain. Dev. 35: 67-81.

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91:3-17.

Miao, T.J., Gao, S., Jiang, S.W., Kan, G.S., Liu, P.J., Wu, X.M., An, Y.F., and Yao, S. (2014). A method suitable for DNA extraction from humus-rich soil. Biotech. Lett. 36: 2223-2228.

Marta-Pedroso, C., Domingos, T., Freitas, H. and De Groot, R.S. (2007). Cost–benefit analysis of the Zonal Program of Castro Verde (Portugal): Highlighting the trade-off between biodiversity and soil conservation. Soil Till. Res. 97: 79–90.

Millennium Ecosystem Assessment. (2005). Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

O'Connor, F.B.(1962). The extraction of Enchytraeidae from soil. In: Murphy, P.W. (Ed.), Progress in Soil Zoology. Butterworths, London, pp. 279-285.

Page F. C. (1988). A new key to freshwater and soil gymnamoebae. Freshwater Biological Association, Ambleside, UK.

Paz-Ferreiro, J. and Fu, S. (2016). Biological indices for soil quality evaluation: perspectives and limitations. Land Degrad. Develop. 27: 14–25.

Petersen, H. and Luxton, M. (1982). A comparative analysis of soil fauna populations and their role in decomposition. Oikos 39: 288-388.

Philippot, L., Čuhel, J., Saby, N.P.A., Chèneby, D., Chroňáková, A., Bru, D., Arrouays, D., Martin-Laurent, F. and Šimek, M. (2009). Mapping field-scale spatial patterns of size and activity of the denitrifier community. Environ. Microbiol. 11: 1518–1526.

Plassart, P., Terrat, S., Thomson, B., Griffiths, R., Dequiedt, S., Lelievre, M., Regnier, T., Nowak, V., Bailey, M., Lemanceau, P., Bispo, A., Chabbi, A., Maron, P-A., Mougel, C. and Ranjard, L. (2012). Evaluation of the ISO standard 11063 DNA extraction procedure for assessing soil microbial abundance and community structure. PLOS ONE 7: e44279.

Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelosi, C., Pérès, G. and Rutgers, M. (2012). Soil biodiversity, biological indicators and soil ecosystem services—an overview of European approaches. Curr. Opinion Environ. Sustain. 4: 529–538.

Ritz, K. and Trudgill, D. L. (1999). Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges. Plant Soil 212: 1–11.

Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A. and Wood, C. (2009). Selecting biological indicators for monitoring soils: a framework for balancing scientific and technical opinion to assist policy development. Ecol. Ind. 9: 1212-1221.

Römbke, J. (2014). The feeding activity of invertebrates as a functional indicator in soil. Pl. Soil 383:43–46

Rutgers, M., Breure, A.M. and Insam, H. (2006). Substrate utilization in Biolog plates for analysis of CLPP. In: Bloem J, Benedetti A, Hopkins DW, editors. Microbiological methods for assessing soil quality. Wallingford: CAB International Publishing. pp. 212–227.

Rutgers, M., Schouten, A.J., Bloem, J., van Eekeren, N., de Goede, R.G.M., Jagers op Akkerhuis, G.A.J.M., van der Wal, A., Mulder, C., Brussaard, L., and Breure, A.M. (2009). Biological measurements in a nationwide soil monitoring network. Eur. J. Soil Sci. 60:820–832.

Schmelz, R.M. and Collado, R. (2010). A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). Soil Organisms 82, 1-176

Schmelz, R.M. and Collado, R. (2012). Guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta): first supplement. VTI Agriculture and Forestry Research, Special Issue 357, 53-66.

Senapati, N., Chabbi, A., Gastal, F., Smith, P. Mascher, N., Loubet, B., Cellier, P. and Naisse, C. (2014). Net carbon storage measured in a mowed and grazed temperate sown grassland shows potential for carbon sequestration under grazed system. Carbon Manage. 5: 131-144.

Sousa, J.P., Rodrigues, J.M.L., Loureiro, S., Soares, A.M.V.M., Jones, S.E., Forster, B. and Van Gestel, C.A.M. (2004). Ring-testing and field-validation of a terrestrial model ecosystem (TME) - an instrument for testing potentially harmful substances: Effects of carbendazim on soil microbial parameters. Ecotoxicology 13: 43-60.

Spurgeon, D.J., Keith, A.M., Schmidt, O., Lammertsma, D.R. and Faber, J.H. (2013). Land-use and land-management change: relationships with earthworm and fungi communities and soil structural properties. BMC Ecol. 13: 46.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C. and Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21: 2045-2050.

Tardy, V., Spor, A., Mathieu, O., Lévèque, J., Terrat, S., Plassart, P., Reignier, T., Bardgett, R.D., van der Putten, W.H., Roggero, P.P., Bagella, S., Lemanceau, P., Ranjard, L. and Maron, P-A. (2015).

Shifts in microbial diversity through land use intensity as drivers of carbon mineralization in soil. Soil Biol. Biochem. In Press.

Tejedor, M., Neris, J. and Jimenez, C. (2013). Soil Properties Controlling Infiltration in Volcanic Soils (Tenerife, Spain). Soil Sci. Soc. AM. J. 77: 202-212.

Thompson, K.A. and Newmaster, S.G. (2014). Molecular taxonomic tools provide more accurate estimates of species richness at less cost than traditional morphology-based taxonomic practices in a vegetation survey. Biodiv. Cons. 23: 1411–1424.

Thomson, B.C., Tisserant, E., Plassart, P., Uroz, S., Griffiths, R.I., Hannula, S.E., Buee, M., Mougel, C., Ranjard, L., van Veen, J.A., Martin, F., Bailey, M.J. and Lemanceau, P. (2015). Soil conditions and land use intensification effects on soil microbial communities across a range of European field sites. Soil Biol. Biochem. 88: 403-413.Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., D' Hertefeldt, T., Hotes, S., Hol, W.H.G., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V. and Hedlund, K. (2015). Intensive agriculture reduces soil biodiversity across Europe. Glob. Change Biol., 21: 973–985.

Turbé, A., De Toni, A., Benito, P., Lavelle, P., Lavelle, P., Ruiz, N., Van der Putten, W. H., Labouze, E. and Mudgal, S. (2010). Soil biodiversity: functions, threats and tools for policy makers. Bio Intelligence Service, IRD, and NIOO, Report for European Commission (DG Environment).

Van Capelle, C. Schrader, S. and Brunotte, J. (2012). Tillage-induced changes in the functional diversity of soil biota - A review with a focus on German data. Eur. J. Soil Biol. 50: 165 – 181.

Van Eekeren, N.J.M., Bommele, L., Bloem, J., Schouten, T., Rutgers, M., De Goede, R.G.M., Brussaard, L. and Reheul, D. (2008). Soil biological quality after 36 years of ley-arable cropping, permanent grassland and permanent arable cropping. Appl. Soil Ecol. 40: 432-446.

Van Gestel, C.A.M., Kruidenie,r M. and Berg, M.P. (2003). Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. Biol. Fertil. Soils 37: 115–123.

Van Straalen, N.M. (1998). Evaluation of bioindicator systems derived from soil arthropod communities. Appl. Soil Ecol. 9:429–437.

Vervoort, M.T.W., Vonk, J.A., Mooijman, P.J.W., van den Elsen, S.J.J., van Megen, H.H.B., Veenhuizen, P., Landeweert, R., Bakker, J., Mulder, C. and Helder, J. (2012). SSU Ribosomal DNA-based monitoring of nematode assemblages reveals distinct seasonal fluctuations within evolutionary heterogeneous feeding guilds. PLOS ONE 7: e47555.

Wall, D.H., Bardgett, R.D., Behan-Pelletier, V., Herrick, J.E., Jones, T.H., Ritz, K., Six, J., Strong, D.R. and van der Putten, W.H. (eds.) (2012). Soil Ecology and Ecosystem Services. Oxford University Press, Oxford. 424 pp.

Wessén, E. and Hallin, S. (2011). Abundance of archaeal and bacterial ammonia oxidizers – possible bioindicator for soil monitoring. Ecol. Ind. 11: 1696–1698.

Wessén, E., Söderström, M., Stenberg, M., Bru, D., Hellman, M., Welsh, A., Thomsen, F., Klemedtson, L., Philippot, L. and Hallin, S. (2011). Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. ISME J 5: 1213-1225.

Wilcox, T.M., McKelvey, K.S., Young, M.K., Jane, S.F., Lowe, W.H., Whiteley, A.R. and Schwartz, M.K. (2013). Robust detection of rare species using environmental DNA: the importance of primer specificity. PLOS ONE 8: e59520.

Zeitz. J., Habeker, R. and Heckl, M. (2004). Auswirkungen verschiedener Bodenbearbeitungsverfahren auf das Vorkommen von Regenwürmern in ökologisch und integriert bewirtschafteten Flächen – Ein Versuch in Scheyern. Report, Technical University of Munich. 64 pp.

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	2 ENCH	NEM
3 BL	RESIL	PLFA	MA	PLFA
INFIL	INFIL	T-RFLP		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 / Plot / Year	Castro Verde control 2012	control 2013	treatment 2012	treatment 2013	Hainich control 2012	control 2013	treatment 2012	treatment 2013	Yorkshire Dales control 2012	control 2013	treatment 2012	treatment 2013	Lusignan control 2012	control 2013	treatment 2012	treatment 2013	Moskanjci control 2012	control 2013	treatment 2012	treatment 2013	Scheyern control 2012	control 2013	treatment 2012	treatment 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	3.98
	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	3.33	3.67	3.33	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	11.47	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 genes	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
	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 endpoint indicators	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
	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
	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	153.4	ND	79.4	ND	96.2	ND	75.9	ND	24.00	ND	506.0	ND	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	1.53	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	6.16	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	47.84	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

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 genes	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
	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 endpoint indicators	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
	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
	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.

Earthworm sample

Water infiltration

Composite soil sample

Microarthropod soil core (0-5cm)

Enchytraeid soil core (0-5cm)

Bait-lamina sample
(2 parallel lines of 4 bait strips each
=8 bait strips per sample)

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).

Figures

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

Earthworm sample

Water infiltration

Composite soil sample

Microarthropod soil core (0-5cm)

Enchytraeid soil core (0-5cm)

Bait-lamina sample
(2 parallel lines of 4 bait strips each
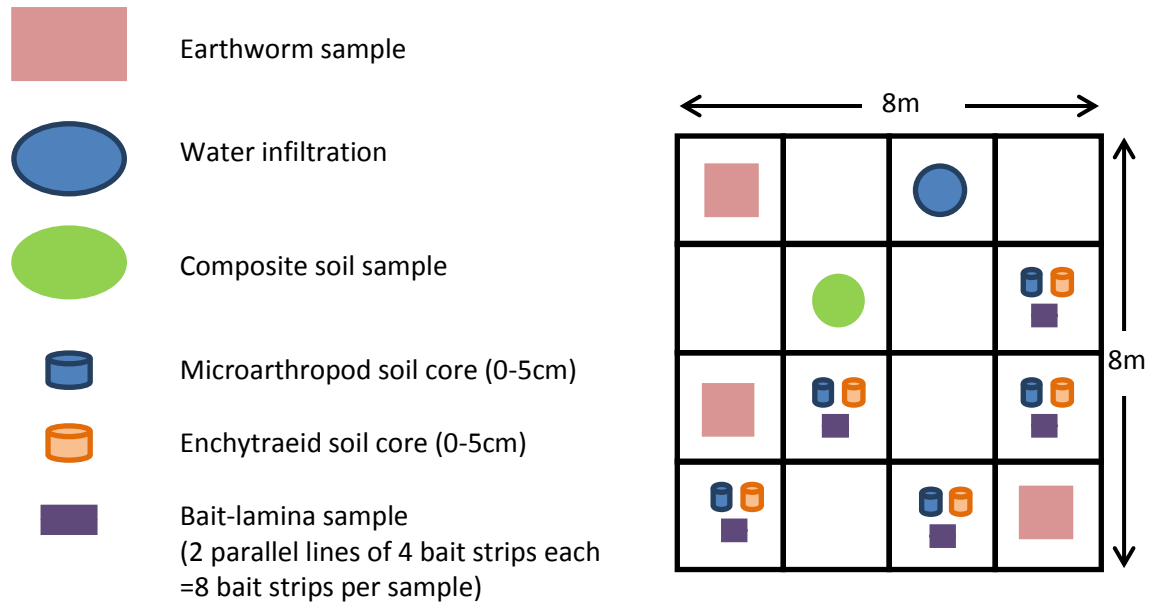=8 bait strips per sample)

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).
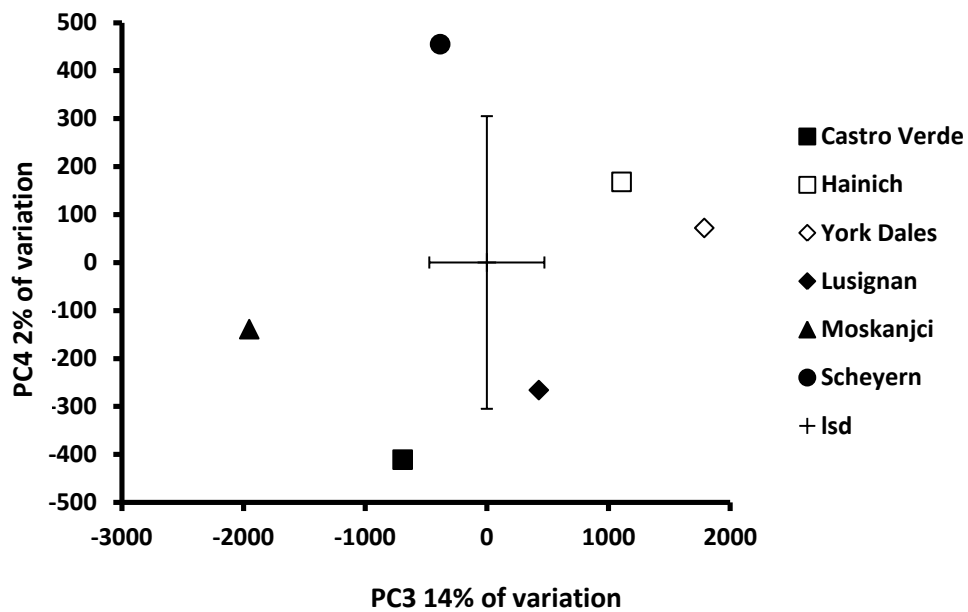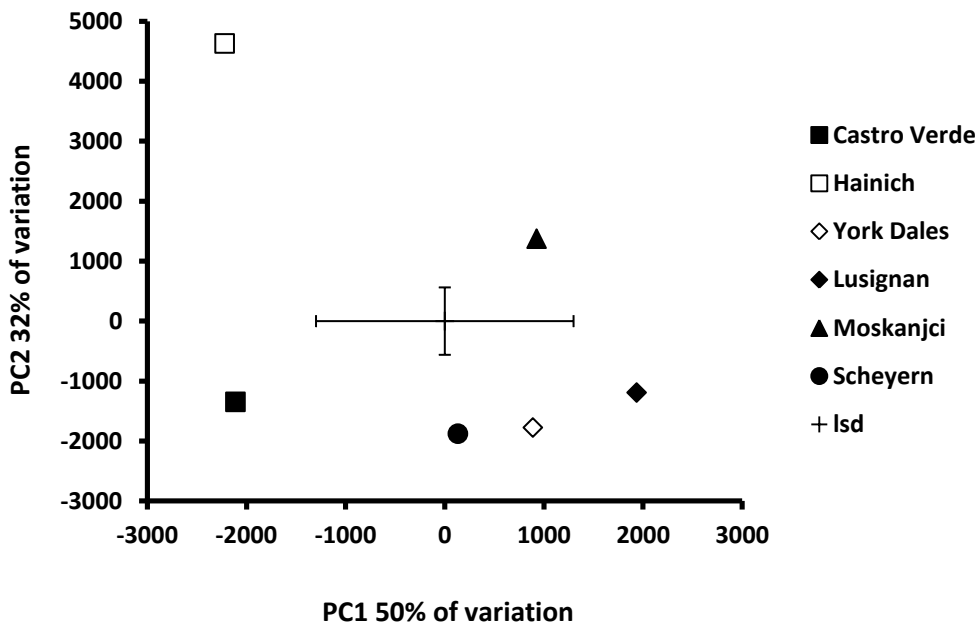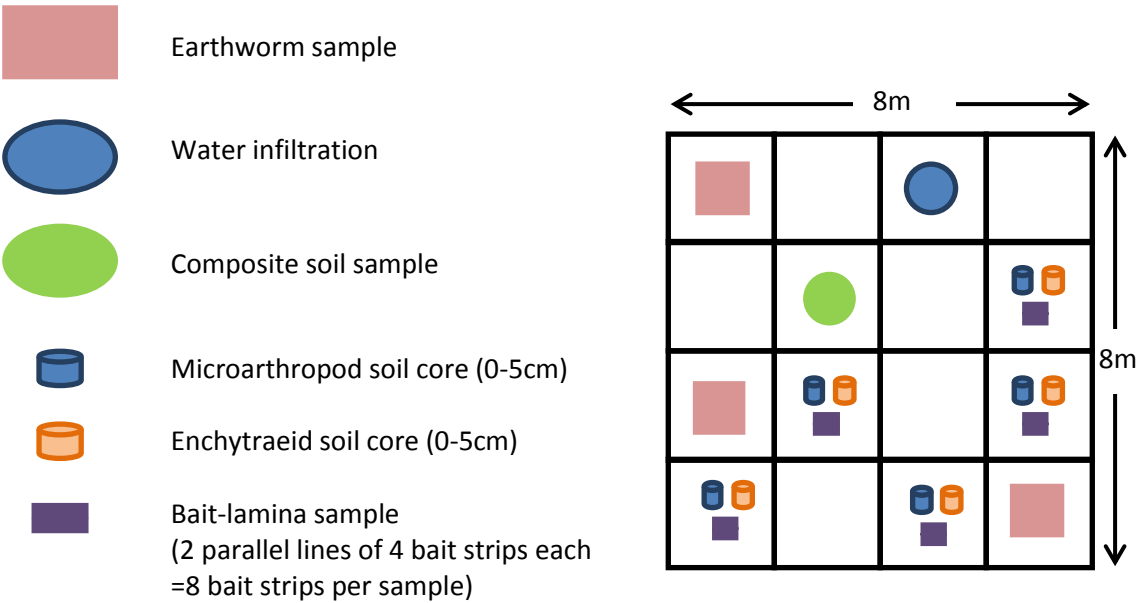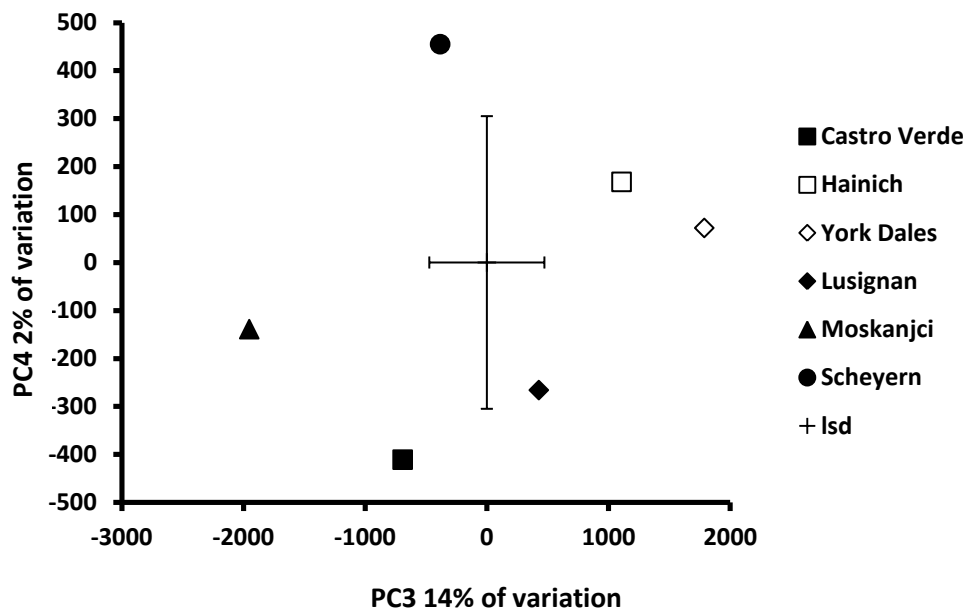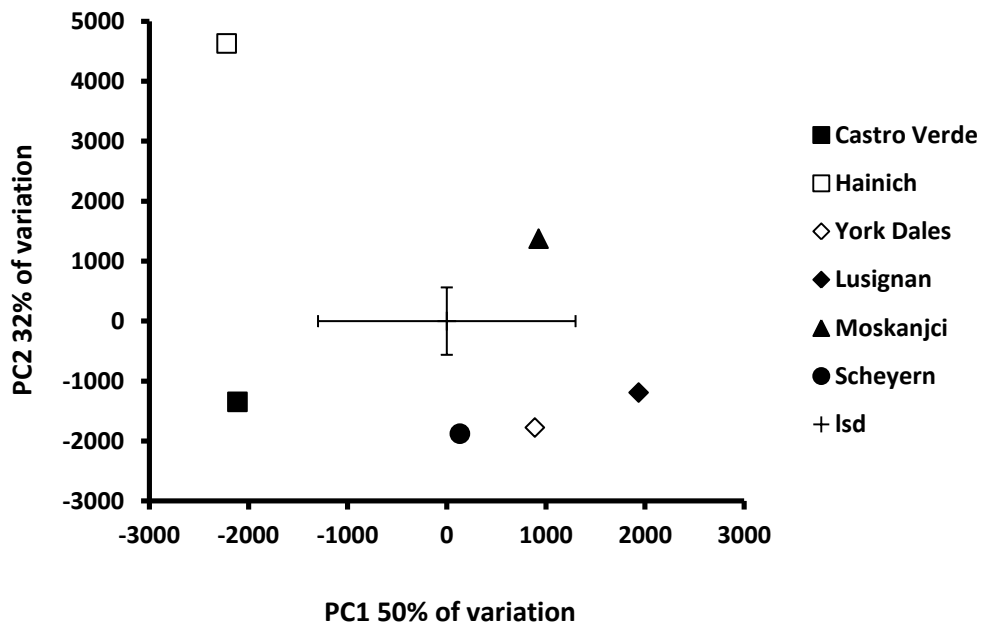
Tables

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 Material

Indicator	Site / Plot / Year	Castro Verde control 2012	control 2013	treatment 2012	treatment 2013	Hainich control 2012	control 2013	treatment 2012	treatment 2013	Yorkshire Dales control 2012	control 2013	treatment 2012	treatment 2013	Lusignan control 2012	control 2013	treatment 2012	treatment 2013	Moskanjci control 2012	control 2013	treatment 2012	treatment 2013	Scheyern control 2012	control 2013	treatment 2012	treatment 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	-0.64	-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	4.67	4.00	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	12.48	12.83	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	11608	ND	8506	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Coll H'	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	ND
	Coll spp	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	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	153.4	ND	79.4	ND	96.2	ND	75.9	ND	24.00	ND	506.0	ND	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	0.58	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	6.16	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	47.84	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 genes	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
	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 endpoint indicators	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
	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
	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	85.46	ND	29.53	ND	27.14	ND	15.61	ND	9.37	ND	176.19	ND	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