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1 **Identification of new microbial functional standards for soil quality assessment**

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3 **Sören Thiele-Bruhn [1], Michael Schloter [2], Berndt-Michael Wilke [3], Lee A. Beaudette [4], Fabrice Martin-**

4 **Laurent [5], Nathalie Cheviron [6], Christian Mougin [6], Jörg Römbke [7]**

5

6 [1] Universität Trier, Bodenkunde, Behringstr. 21, 54286 Trier, Germany

7 [2] Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Abteilung für

8 vergleichende Mikrobiomanalysen, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

9 [3] TU Berlin, FG Bodenkunde, Ernst-Reuter-Platz 1, 10587 Berlin, Germany

10 [4] Environment and Climate Change Canada, 335 River Road, Ottawa, Ontario, K1A 0H3, Canada

11 [5]AgroSup Dijon, INRA, Université Bourgogne, Université Bourgogne Franche-Comté, Agroécologie, 17 rue

12 Sully, 21065 Dijon Cédex, France

13 [6] UMR ECOSYS, Platform Biochem-Env, INRA, AgroParisTech, Université Paris-Saclay, 78026, Versailles,

14 France

15 [7] ECT Oekotoxikologie GmbH, Böttgerstr. 2-14, 65439 Flörsheim, Germany

16

17 **Correspondence:** Sören Thiele-Bruhn (thiele@uni-trier.de)

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20 **Abstract.** The activity of microorganisms in soil is important for a robust functioning soil and related
21 ecosystem service. Hence, there is a necessity to identify the indigenous soil microbial community for its
22 functional properties using soil microbiological methods in order to determine the natural properties,
23 functioning and operating range of soil microbial communities, and to assess ecotoxicological effects due
24 to anthropogenic activities. Numerous microbiological methods currently exist in the literature and new,
25 more advanced methods continue to be developed; however, only a limited number of the methods are
26 standardized. Consequently, there is a need to identify the most promising non-standardized methods for
27 assessing soil quality and develop these into standards. In alignment with the "Ecosystem Service
28 Approach", new methods should focus on soil microbial function, including nutrient cycling, pest control
29 and plant growth promotion, carbon cycling and sequestration, greenhouse gas emission, and soil
30 structure. The few existing, function-related standard methods available focus on the estimation of
31 microbial biomass, basal respiration, enzyme activities related to nutrient cycling, and organic chemical
32 biodegradation. This paper sets out to summarize and expand on recent discussions within the
33 International Organization for Standardization (ISO), Soil Quality - Biological Characterization sub-
34 committee (ISO TC 190/SC 4) where a need was identified to develop scientifically sound methods which
35 would best fulfil the practical needs of future users for assessing soil quality. Of particular note was the
36 current evolution of molecular methods in microbial ecology that uses quantitative real time PCR (qPCR)
37 to produce a large number of new endpoints and is more sensitive as compared to 'classical' methods.
38 Quantitative PCR assesses the activity of microbial genes that code for enzymes that catalyse major
39 transformation steps in nitrogen and phosphorus cycling, greenhouse gas emissions, chemical
40 transformations including pesticide degradation, and plant growth promotion pathways. In the
41 assessment of soil quality methods, it was found that fungal methods were significantly underrepresented.
42 As such, techniques to analyse fungal enzyme activities are proposed. Additionally, methods for the
43 determination of microbial growth rates and efficiencies, including the use of glomalin as a biochemical

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44 marker for soil aggregation, are discussed. Furthermore, field methods indicative of carbon turnover,
45 including the litter bag test and a modification to the tea bag test, are presented. As a final note, it is
46 suggested that endpoints should represent a potential function of soil microorganisms rather than actual
47 activity levels, as the latter can largely be dependent on short-term variable soil properties such as
48 pedoclimatic conditions, nutrient availability, and anthropogenic soil cultivation activities.

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49 **1 Introduction**

50 Soils are one of the world's hotspots for biodiversity (Parker, 2010). Biota – both micro- and macro-
51 organisms - in soil form strong networks and complex food webs, which determines the efficacy of the soil
52 ecosystem functions (e.g. nutrient cycling, C storage and turnover, water retention, and modulation of soil
53 structure) (Creamer et al., 2016). These functions support a range of ecosystem services that are
54 indispensable for soil use in agri-, horti- or silviculture (Nannipieri et al., 2017). At the same time, soil biota
55 are strongly impacted by various anthropogenic activities including ongoing global and climate change,
56 pollution, and degradation and destruction of the terrestrial environment (Gomiero, 2016; Montgomery,
57 2008; Wagg et al., 2014). Consequently, investigations of the soil biome structure and function became an
58 emerging topic in soil and environmental sciences (Griffiths and Philippot, 2013). As such, the number of
59 publications on soil ecology and ecosystem functioning has increased significantly over the past few
60 decades and has resulted in the development of new methods (e.g. Guillaume et al., 2016; Tian et al.,
61 2018). In comparison, the ecotoxicological assessment of human impacts (e.g. chemical pollution and
62 mechanical compaction) using single species tests, which are well-established methods, has remained
63 constant (Brookes, 1995; Joergensen and Emmerling, 2006).

64 Characterizing the natural state of a soil's biome is quite a challenging task. In addition to its huge
65 structural and functional diversity, the soil biome is influenced by strong temporal dynamics including
66 seasonal weather conditions and the enormous spatial heterogeneity which ranges from field scale to
67 microscale (Kuffner et al., 2012; Regan et al., 2014; Suriyavirun et al., 2019). All of these intrinsic properties
68 hinder the interpretation of data obtained from the analysis of soil biomes and the measurement of their
69 functional traits.

70 Despite the fundamental methodological advances over the past years, which allow for an in-
71 depth analysis of microbiomes and, to some extent, other soil-living organisms (e.g. Joergensen and
72 Emmerling, 2006; Paul, 2015; Yates et al., 2016), only a limited number of soil biological methods have

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73 been standardized (for details see section 3). As a result, large deviations are observed between non-
74 standardized method protocols (e.g. Strickland and Rousk, 2010). Therefore, comparability between
75 datasets generated by different laboratories using different methods or modified protocols of the same
76 method are problematic. Consequently, the development of quality indices and threshold values,
77 respectively, for assessing soil quality is nearly impossible (Bastida et al., 2008). Presumably, this is why
78 the number of meta-analyses in soil biology remains small.
79 Given that there is a lack of harmonization between existing methods and, at the same time, a
80 proliferation of new methods, there is a need to identify the most promising methods described in the
81 literature that can be standardized to produce reliable indicators for soil quality (e.g. Philippot et al., 2012).
82 At the Annual International Organization for Standardization (ISO) meeting of TC 190 (Soil Quality) in
83 Fukuoka, Japan in October 2013, a decision was made to compile a list of available methods and to identify
84 those that would be suitable for assessing soil quality. Additionally, during a subsequent meeting of ISO
85 TC 190/SC 4/WG 4 (Microbiological Methods) held in Paris, France in March 2014, further discussions
86 focused the criteria for suitable methods to be comprised of microbial functional indicators. In this paper,
87 we summarize the major outcomes of the discussions which took place over the past several years within
88 ISO TC 190/SC 4. Therefore, besides collating a list of criteria for the selection of test methods for the
89 future analysis of microbial functions in soil, the aim of this paper is to present our opinion, as members
90 of the ISO TC 190 committee, to initiate further discussion on possible methods that should be
91 standardized for future soil quality assessments.
92
93 **2 Criteria for the selection of methods**
94 Several papers addressing the task to identify suitable methods to be used as biotic indicators (usually
95 including faunal indicators) were published in the last few years, mainly in the context of EU research
96 projects (e.g. Bispo et al., 2009; Faber et al., 2013; Ritz et al., 2009; Römbke et al., 2010). Here, we propose

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97 to base the selection of soil quality methods more on the "Ecosystem Service Approach" (MEA 2005) which
98 is increasingly recognized by both environmental scientists and regulatory agencies (Breure et al., 2012;
99 Galic et al., 2012) and that soils have been raised to the rank of a natural resource to be protected. As a
100 consequence, and in addition to method development and application (including the assessment of
101 biodiversity as a prerequisite for soil function), the focus of future activities should be the determination
102 of soil microbial function as recommended endpoints (Kvas et al., 2017; Nienstedt et al., 2012; van der
103 Putten et al., 2010; TEEB, 2010). Consequently, we propose to assess both existing and new methods for
104 the selection of microbial functional tests that support various soil ecosystem services. This structures our
105 approach and simplifies the identification of ecologically relevant methods, as well as, presumably
106 increasing their acceptance by users, including the regulatory and stakeholder community. The following
107 soil **Functions** and ecosystem services have been defined and are proposed to be used as a starting point
108 for the development of future methods (MEA, 2005; Ockleford et al., 2017):
109 (1) Biodiversity, genetic resources, cultural services;
110 (2) Food web support;
111 (3) Biodegradation of pollutants;
112 (4) Nutrient cycling (for example N and P);
113 (5) Pest control and plant growth promotion;
114 (6) Carbon cycling and sequestration;
115 (7) Greenhouse gas emissions; and
116 (8) Soil structure affecting soil water, gas balance and filtration function.
117 A second major criterion for selecting methods for standardization is its usability. The method should be
118 applicable in regulations (e.g. European and National agencies registering chemicals or products) and for
119 the evaluation of soil ecology and functioning as a fundamental aspects of soil quality (e.g. by stakeholders
120 and researchers). Moreover, the routine use of methods to inform farmers and site owners on soil quality

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121 as continuous assessments of their land and land-use practises, could be an additional condition that
122 would require the choice of easy-to-use methods or possibly encourage the simplification of existing
123 methods. Frequently used methods generate more data, which in turn is of high importance for the
124 validation of threshold values.

125 To assess possible methods, a list of criteria was used based on the 'logical sieve' approach (Ritz
126 et al., 2009). The list of criteria for the identification of functional indicators and associated methodologies
127 (Table 1) was an outcome of the EU FP7 EcoFINDERS project (Faber et al., 2013). The criteria were compiled
128 after sending a questionnaire to 25 partner institutions primarily working in the field of environmental
129 science; mainly representing academia but also regulators and subcontracting laboratories. These criteria
130 are applicable for different kinds of indicators and methods, including those addressing the functions of
131 soil microbial communities. In the following sections, we assume that existing ISO standardized methods
132 already fulfil these criteria. Additionally, the most appropriate new methods, including those proposed in
133 this article, need to be evaluated using the same criteria required for the standardization of ISO methods.
134 Therefore, the aim of this whole process is to identify methods which are scientifically sound and that best
135 fulfil the practical needs of future users.

136

137 **3 Existing and new methods**

138 Current methods that have already been implemented as ISO standards are found in Table 2, whereas
139 methods that might be considered for future standardization are in Table 3. The compilation in Table 2
140 comprises methods to quantify microbial biomass (e.g. through fumigation extraction of microbial biomass
141 carbon (MBC) and DNA) (**Function 6**) as well as for (further) analysis of structural microbial diversity (e.g.
142 determination of microbial fingerprints by phospholipid fatty acids (PLFA) analysis) (**Function 1**).
143 Additionally, microbial biomass, measured as respiratory activity, has been included in Table 2, although
144 not directly linked to one of the ecosystem services, as it provides important information on the activity

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145 of the complete microbiome (i.e. microflora and microfauna). Soil basal respiration normalized to MBC
146 (ISO 14240-1 and ISO 14240-2, 1997, Table 2) yields the metabolic quotient qCO_2, which is a sensitive
147 indicator for microbial carbon use efficiency (Anderson and Domsch, 1993). Interestingly, its use as an
148 endpoint to assess anthropogenic and natural impacts on the soil microbiome has been controversially
149 discussed in literature (Wardle and Ghani, 1995).

150 The biodiversity function (**Function 1**) addresses parameters related to the structural diversity of
151 the soil microbiome. Here, respective ISO guidelines analysing PLFA, phospholipid ether lipids (PLEL)
152 (ISO/TS 29843-1, 2010; ISO/TS 29843-2, 2011) and DNA (ISO 11063, 2012; ISO 17601, 2016), have already
153 been well implemented into guidelines (Table 2). Although microbial diversity, per se, is not strongly
154 correlated with a particular functional capacity, it is clear that the loss of diversity can have an impact on
155 microbial function (Thiele-Bruhn et al., 2012); at least for relatively specific functions performed by narrow
156 microbial guilds or taxa. This applies even more, when certain taxa are closely linked to very specific
157 functions including nitrifiers, methanogens, arbuscular- and ecto-mycorrhizal fungi, and biocontrol
158 microorganisms like *Trichoderma* (e.g. Hartmann et al., 2009; Hayat et al., 2010; Lugtenberg and Kamilova,
159 2009; Peng et al., 2008; Singh et al., 2007; Xia et al., 2011). Therefore, the interpretation of the outcomes
160 from microbial community-based testing tends to be straightforward and closely linked to **Function 4** and
161 **Function 5**.

162 Food web support (**Function 2**) of higher trophic levels no doubt starts from soil microorganisms
163 and propagates through the trophic levels (e.g. earthworms) that are consumed by birds and mammals
164 (Haynes, 2014; Scheu et al., 2002; Scheu et al., 2005). However, the role of the microbiota in the soil food
165 web is not fully understood, since many eukaryotic organisms can be considered as meta-organisms, which
166 carry their "own microbiome" that itself is essential for life supporting functions. From this, it is unclear if
167 environmental microbiomes and host specific microbiomes complement one another. So far there are no
168 comprehensive methods (especially not those addressing microbial functions) or standards available to

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169 assess this problem. A future considering may employ the use of stable isotope labelling of select carbon
170 sources as a promising approach to follow food webs and degradation pathways (e.g. Coban et al., 2015;
171 Traugott et al., 2013).

172 Methods to assess the biodegradation of pollutants (**Function 3**), as described above, are already
173 implemented into ISO guidelines (Table 2) and are part of legal frameworks including pesticide directives
174 (EU Regulation1107/2009/EC; European Commission, 2009). A number of standard methods for the
175 determination of the potential of soils to degrade organic chemicals (**Function 3**) under both aerobic (ISO
176 14239, 2017) and anaerobic (ISO 15473, 2002) conditions are available. This emphasizes that in the past,
177 the development of standard methods was mainly driven by the need to assess the ecotoxicological effects
178 of anthropogenic activities, such as chemical contamination of soils, rather than to describe and
179 understand the natural properties and functions of soils. However, defining methods for the
180 determination of adverse effects of contaminants on soil biota was not only being done in ISO, but it was
181 also a major task of other organizations such as the Organization for Economic Co-Operation and
182 Development OECD). For example, there are OECD guidelines, tests No. 216 and 217, for testing the long-
183 term effects of single exposure chemicals on soil microbial nitrogen and carbon transformation,
184 respectively (OECD, 2000a; 2000b). As a result, it was decided early that the standardization of methods
185 for toxicity testing would not be the primary aim of the ISO sub-committee (ISO TC 190/SC 4).

186 Some of the existing standard methods that are listed in Table 2 focus on the estimation of enzyme
187 activities useful for soil quality assessment, which mainly contribute to **Function 4**. Here, the potential
188 dehydrogenase activity measurement is an indicator for general (potential) oxidoreductase activity in soil.
189 Since this measurement has been frequently used, there are large amounts of baseline data available on
190 the toxic effects of a range of pollutants in soil. Recently, additional potential enzyme activities related to
191 the C, N, P and S cycle have been used and are either standardized or are in the process for standardization.
192

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193 The current evolution of molecular methods in microbial ecology has resulted in a large number
194 of new endpoints. It is well known that many of the new endpoints (e.g. using quantitative real-time PCR
195 (qPCR)) are more sensitive than classical methods that had been standardized in the past (Ribbons et al.,
196 2016; Schulz et al., 2016). This new metagenomics approach will be of high importance in the future, as it
197 allows for the implementation of information on new functional traits that can be standardized into an
198 analytical pipeline. For the assessment of new methods linked to **Functions 4 to 8**, qPCR from soil DNA
199 extracts (ISO 17601, 2016) plays a very important role in determining the abundance of single marker gene
200 sequences, which are indicative of specific transformation processes or soil functions. For example, the
201 quantification of nitrogen fixing microbes, nitrifiers and denitrifiers has been successfully implemented
202 using the *nifH*, *amoA* and *nirS/nirK* genes as markers, respectively (Henry et al., 2004; Hirsch et al., 2010;
203 Ollivier et al., 2010; Sessitsch et al., 2006). Similarly, the quantification of microorganisms involved in the
204 β-ketoadipate pathway has been implemented by targeting *pcaH* (El Azhari et al., 2008) and *catA* (El Azhari
205 et al., 2010) gene sequences. Various methods for the assessment of soil microbial **Function 4** (nutrient
206 cycling), **Function 5** (pest control and plant growth promotion) and **Function 7** (greenhouse gas emissions)
207 are proposed based on the qPCR analysis of gene sequences coding for enzymes which trigger the
208 respective function (e.g. Fish et al., 2013; Ribbons et al., 2016; Smith and Osborn, 2009). Additionally, it
209 should be noted that molecular methods based on the assessment of specific marker genes for estimating
210 the degradation potential in soil have already been proposed both for PAHs (e.g. Cebron et al., 2008) and
211 individual pesticides (e.g. Martin-Laurent et al., 2004). These could be interesting for future
212 standardization; however, if a method is very compound-specific and targeted, this could limit its
213 application range. Thus, these specific approaches will not be discussed further in this article.
214 Major advantages of qPCR assays to quantify gene sequence numbers, which can be used as
215 proxies for a given microbial process, are that they are: (i) highly standardized, sensitive, selective and
216 reproducible, (ii) designed for high throughput analysis, (iii) available for a wide range of targets, and (iv)

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217 methods that are relatively cheap once the necessary analytical devices are on hand. Some training on the
218 method is required, however, once trained the assays are easy to perform. For example, numerous studies
219 have already used the microbial functional genes involved in nitrogen cycling to determine the status and
220 to assess induced changes in the soil microbial community (Levy-Booth et al., 2014; Nannipieri and Eldor,
221 2009; Wallenstein et al., 2006). Consequently, the number of functional genes that are suited for use as
222 specific indicators of soil function are continuing to grow in the literature as researcher gain experience in
223 this field and data becomes more prevalent.
224 Disadvantages, on the other hand, are that: (i) the quality of qPCR data depends on soil DNA
225 extracts (PCR inhibition), (ii) primer pairs, even degenerated ones, might not successfully amplify all
226 microbes of the functional group of interest, (iii) only genetic potential is resolved, and (iv) there is no
227 differentiation between active, dormant or dead microorganisms, when working with DNA as a template
228 for the qPCR reaction. The analysis of total RNA and of mRNA, which could help to overcome the latter
229 problem, is currently not a suitable alternative as it is highly dynamic in time and space and needs special
230 care to stabilize the RNA extracted from complex environmental matrices to avoid its degradation.
231 Another problem of DNA analysis is the biological representativeness of the results is solely based on a
232 relatively small amount of soil (from few hundred mg to ten g of soil) from which the DNA extracted. The
233 use of small soil samples (< 1 g) simplifies the sample preparation process for molecular biologists;
234 however, it provides a poor representation of the indigenous soil microbial community in the naturally
235 inhomogeneous soil. Typically, the α-biodiversity declines with sample size while that of β-biodiversity
236 increases (Nicol et al., 2003; Penton et al., 2016). Lastly, it must be noted that the high repeatability and
237 reproducibility of molecular biology methods, including qPCR assays, depends on extraction, purification
238 and amplification of DNA or RNA. This is typically performed using commercial extraction kits; however,
239 by simply changing the commercial supplier of a kit can substantially change the results (Brooks et al.,

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240 2015; Feinstein et al., 2009). This clearly challenges standardization since standard methods must not
241 hinge on a specific supplier.

242 Recently, successful examples of microbial phosphorous turnover (**Function 4)** have been
243 published (Bergkemper et al., 2016) where metagenomics data have been used for the construction of
244 primers for P mineralization, transport and uptake. As another example, the relevance of anaerobic
245 ammonium oxidation (anammox) for N cycling in soils has increased (Levy-Booth et al., 2014) along with
246 the development of analytical methods for high throughput analysis. Among the microorganisms in soil
247 that substantially govern pest control and plant growth promotion (**Function 5**), the most numerous
248 organisms are the arbuscular mycorrhizae and ectomycorrhizal fungi. These microorganisms are especially
249 abundant in the rhizosphere (Hartmann et al., 2009; Hayat et al., 2010; Lugtenberg and Kamilova, 2009).
250 Methods related to **Function 5** are listed in Table 3.

251 Several options exist for (additional) standardized methods to test **Function 6** (carbon cycling and
252 sequestration) (Table 3). For **Function 6**, there is a need to implement more fungal activity analysis as most
253 tests described this far only assess bacterial activities. Thus, the integration of more fungal enzyme
254 activities into the suite of standardized methods for soil quality assessment is essential (for example
255 determining the turnover of complex natural compounds such as lignin) (Baldrian, 2006). The ligninolytic
256 enzymes laccase and Mn-peroxidase, as well as the chitin degrading 1,4-β-*N*-acetylglucosaminidase, are
257 typical fungal enzymes of interest for ecosystem services (Jiang et al., 2014; Šnajdr et al., 2008). However,
258 since other organisms also produce these enzymes, including bacteria and plants (Bollag, 1992; de Gonzalo
259 et al., 2016), current methods do not specifically target fungal enzyme activities. As a result, the
260 implementation of molecular methods for assessing fungal communities are far less developed than those
261 for bacterial communities (Table 3).

262 The method of community level physiological profiling (CLPP) using the Biolog™ system (Biolog,
263 Hayward CA, USA) was first developed in the late 1980s to identify bacteria of clinical importance by

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264 assessing the consumption of 95 different carbon sources in a microtiter plate. The technique was then
265 extended to identify bacterial strains from environmental mixed microbial communities samples using
266 select carbon sources (Garland, 1997). Currently, the technique is frequently used to assess the effects of
267 contaminants on soil microbial activity (Bloem and Breure, 2003; Schmitt et al., 2004). As such, the CLPP
268 method has become a measure of microbial functional diversity in soil (e.g. Gomez et al., 2006) and was
269 used to distinguish the biodiversity of soil microbial communities in monitoring programs (Rutgers et al.,
270 2016). Even though the method is easy to use, it does have some drawbacks (Winding and Hendriksen,
271 2007). The technique is based on the utilisation of select carbon sources, which when consumed result in
272 reduction, and thus colour change, of a tetrazolium indicator dye (Garland and Mills, 1991). This reaction
273 is based on the dehydrogenase enzyme activity of cultivable, fast growing, aerobic, eutrophic
274 microorganisms (largely bacteria). Consequently, this technique does not reflect the full spectrum of
275 microbial species within a mixed soil community. Additionally, due to the artificial growth conditions
276 required in the test, it is argued that the method does not reflect the microbial community diversity and
277 its function of a given soil (Glimm et al., 1997). On the other hand, however, standardized conditions allows
278 for direct comparisons between microbial communities in different sites, for example, independent of the
279 abiotic conditions, thus making CLPP a popular method for toxicology testing (Preston-Mafham et al.,
280 2002).

281 Isothermal micro-calorimetry is another technique that involves the direct measurement of
282 energetics in soil and provides a functional link between energy flow and the composition of belowground
283 microbial communities at a high taxonomic level (Herrmann et al., 2014). With this method, an integrative
284 determination of the metabolic activity of soil bacteria and fungi is achieved. The integrated assessment
285 of substances' and energy turnover has high potential to elucidate the regulation of soil ecological
286 functions. However, the substantial costs for the acquisition of this very specific instrumentation is
287 considered as a major drawback. Furthermore, the measurement requires water saturation of the soil and,

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288 thus, the samples are modified. Since calorimetry has been rarely used and data and publications are few,
289 this method is considered not ready for standardization.
290 The methods targeting thymidine or leucine incorporation into microbial biomass can be used to
291 determine microbial growth rates and efficiencies (Bååth et al., 2001; Rousk, 2016). Growth rate is a
292 fundamental reference for numerous other microbial properties and functions. For example, it is required
293 to calculate microbial carbon use efficiency (CUE) as a key-parameter describing C-substrate turnover and
294 storage in soil (Liu et al., 2018; Spohn et al., 2016; Takriti et al., 2018). Furthermore, the method can be
295 used to assess the adverse effects of toxic chemicals on the microbial community (Modrzyński et al., 2016;
296 Rousk et al., 2009a). The drawbacks of these two methods are: (i) specific training is required, (ii)
297 laboratories must have a permit to manipulate radioactive isotopes, and (iii) there are higher costs for
298 proper handling and disposal of ^{3}H-labelled radioactive material. As an alternative, the incorporation of
299 the stable isotope ^{18}O from labelled water into soil microbial DNA can be used to distinguish growing and
300 non-growing microorganisms based on the gradient-separation of [^{18}O]DNA and [^{16}O]DNA (Schwartz,
301 2007). The ^{18}O stable isotope method has been improved by sequencing a marker gene from fractions
302 retrieved from ultracentrifugation to produce taxon density curves; thus enabling researchers to estimate
303 the percent isotope composition of each microbial taxon's genome (Schwartz et al., 2016). This method
304 continues to be advanced and, although not used often, could have a high potential for future
305 standardization.
306 There are simplistic methods available to determine organic matter decomposition which are
307 indicative of C cycling (**Function 6**). The tests listed in Table 3 are based on measuring the weight loss of
308 introduced organic materials of different complexity in soil over time. The tests are relatively easy to
309 perform and inexpensive, however, degradation activity is not exclusive to microorganisms but can also
310 include invertebrates. The OECD litter bag test (OECD, 2006) for site specific assessment of organic matter
311 decomposition uses wheat straw as the substrate and provides clear evidence of cellulose degradation. In

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312 general, the litter bag tests provides evidence for the degradation of naturally occurring plant material in
313 soil. Results do, however, depend on the mesh size of the litter bags (increasing exclusion of soil animals
314 with decreasing mesh size). On the other hand, plant material or litter is hard to standardise with the
315 results largely depending on the composition of the plant material. As such, artificial cellulose has been
316 successfully used for a laboratory procedure to assess organic matter decomposition (Kvas et al., 2017).
317 Another alternative to the litter bag test is the use of tea bags (Keuskamp et al., 2013). Tea bags can be
318 purchased to contain a consistent quality of material, and so this method is preferred by citizen science
319 (e.g. farmers to assess the soil quality of their land). In order to better distinguish the degrading abilities
320 of different soil microbiomes, the test could be modified to use different types of tea that contain
321 recalcitrant material to a different extent. Another test for future method development is the Bait Lamina
322 test (ISO 18311, 2016) used to assess the degradation of organic matter in field soil by grazing
323 invertebrates (Jänsch et al., 2013; Kvas et al., 2017). It is a simple test that can easily be adapted for use
324 under controlled laboratory conditions (Jänsch et al., 2017).
325 Methods for the determination and assessment of greenhouse gas emissions from soil (**Function**
326 **7**) have already been standardized or are well advanced in the standardization process (Table 2). They are
327 mostly focused on measuring concentrations of greenhouse gases, like CO_2, CH_4 and N_2O, as well as their
328 fluxes as endpoints. In addition, molecular biology methods that estimate the relative abundance of
329 functional microbial guilds or taxa gives new insight into the ecology of microorganisms involved in the
330 formation of greenhouse gases. For example, the qPCR measurement of key N_2O functional genes has
331 allowed researchers to link N_2O reduction capacity to reduced greenhouse gas emissions in soil amended
332 with organic matter (Xu et al., 2018). Additionally, the quantification of functional gene sequences related
333 to methane generation and methane oxidation, respectively, yields detailed insights into the functional
334 potential of climate change-affected permafrost soils (Yergeau et al., 2010).

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335 For **Function 8** (soil structure affecting soil water, gas balance and filtration function), there is clear
336 evidence that microbial activity and biomolecules substantially contribute to the formation and stability
337 of micro-aggregates, and thus to the structure, pore system and pre-consolidation stress of soils (Six et al.,
338 2004). While existing parameters, such as enzyme activities, are not clearly indicative in this regard (Beck
339 and Beck, 2000), glomalin can be considered as a biochemical marker of soil aggregation. This glycoprotein
340 is produced by microorganisms, especially arbuscular mycorrhiza fungi, and significantly increases
341 aggregate formation and stability (Rillig, 2004; Rillig and Mummey, 2006). The existing protocols for
342 extraction (chemical extraction combined with autoclaving) and determination of glomalin, either by using
343 the Bradford protein assay, enzyme-linked immunosorbent assay (ELISA), or LC-MS method (Bolliger et al.,
344 2008; Janos et al., 2008), open the possibility for its standardization in the near future. It should be noted,
345 however, that a well-equipped and experienced laboratory is required to perform this method.
346
347 **4 Transforming standardized methods into indicators of soil quality**
348 As recently underlined by the European Food Safety Agency (EFSA) in a scientific opinion 'addressing the
349 state of the science on risk assessment of plant protection products for in-soil organisms', there is an
350 urgent need to modernize pesticide risk assessment by implementing specific protection goals for in-soil
351 organisms which are key drivers of a wide range of functions supporting ecosystem services (Ockleford et
352 al., 2017). There currently exists a multitude of methods that can potentially be used for this task. Here,
353 we have identified in the body of this paper a number of methods that are presumably suitable for further
354 evaluation and standardization with regard to their scientific value and practical applicability. These
355 prospective standardized methods will not only be useful to identify adverse effects on the soil
356 microbiome, but also to conduct comparable studies in laboratories all over the world to define normal
357 operating ranges of microbial activity in soil and respective quality indices and threshold values.

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358 It is clear that all parameters taken together reflect the potential of a microbial community to
359 perform a certain function and not solely a specific (actual) activity. This is important to understand to
360 interpret the values of a given endpoint in relation to both energy fluxes and compound transformation
361 rates, which can largely depend on intrinsic properties such as pedoclimatic conditions, and nutrient
362 availability as well as extrinsic properties such as anthropogenic effects, and soil cultivation measures. To
363 make use of these methods as indicators for soil quality, there are several requirements that need to be
364 included. These involves the assessment of the normal operating range of soil that include natural and
365 dynamic fluctuations of a given endpoint. The methods need to be implemented into a framework, which
366 takes into account site-specific conditions including soil type, pedoclimate and land-use. Additionally,
367 there is a requirement for the assessment of resistance and resilience of a given microbial endpoint to see
368 how much it is affected by a soil disturbance and whether or not it can recover (e.g. return to its original
369 state) after the disturbance has disappeared. Also, the use of a test battery to measure a range of
370 interconnected endpoints is recommended (Ockleford et al., 2017) to integrate different biological and
371 other parameters (e.g. soil pH, organic carbon content) into multiparametric indices (Bastida et al., 2008;
372 Kvas et al., 2017). Finally, to fully understand soil microbial functioning, a task was envisioned to
373 investigate the linkage between the genetic functional potential and the available resources, termed the
374 soil metaphenome (Jansson and Hofmockel, 2018). This will require even further integration and
375 assessment of multiple parameters and test methods. Reaching that goal will surely promote soil
376 ecological research but, at the current stage, may clearly go beyond the applied aim of standardization to
377 release easy-to-use targeted methods.

378 The critical evaluation of existing and non-standardized methods is required to further select and
379 standardize new methods to assess soil quality. For methods linked to the molecular analysis of soil
380 microbiomes; there is a need to ensure that worldwide activities are synchronized to propose important
381 standards that are well accepted by the scientific community. For example, recently, the Earth Microbiome

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382 Project (www.earthmicrobiome.org) has proposed primer pairs to barcode soil bacteria in a standardized
383 manner. Furthermore, new bioinformatic pipelines have been developed that are being used more and
384 more as standard procedures. Finally, to improve the reproducibility of data it has been agreed that a
385 complex mixture of microorganisms (MOC) must be implemented as a control in every experiment. The
386 exact composition of the MOC is still under discussion, however, it is clear that if further developments of
387 microbial bar coding and/or metagenomics methods are to be implemented into ISO guidelines, an MOC
388 is required.

389 ISO standardization committees are open circles and the presented selection and valuation of
390 methods may not be complete. Environmental scientists are solicited to propose new work items enlarging
391 the current catalogue of biological methods for future standardization. Accordingly, this opinion paper
392 aims at initiating a broader discussion intended to improve the measurement of microbial functions for
393 soil quality assessment.

394

395

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397

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403

404 **References**

https://doi.org/10.5194/soil-2019-42
Preprint. Discussion started: 10 July 2019
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405 Anderson, T.H., and Domsch, K. H.: The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter
406 to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest
407 soils, Soil Biology and Biochemistry, 25, 393-395, 10.1016/0038-0717(93)90140-7, 1993.
408 Azhari, N., Devers-Lamrani, M., Chatagnier, G., Rouard, N., and Martin-Laurent F.: Molecular analysis of
409 the catechol-degrading bacterial community in a coal wasteland heavily contaminated with PAHs.
410 Journal of Hazardous Material, 177, 593-601, 2010.
411 Bååth, E.: Growth rates of bacterial communities in soils at varying pH: A comparison of the thymidine and
412 leucine incorporation techniques Microbial Ecology, 36, 316-327 doi:10.1007/s002489900118,
413 1998.
414 Bååth, E., Pettersson, M., and Söderberg, K. H.: Adaptation of a rapid and economical microcentrifugation
415 method to measure thymidine and leucine incorporation by soil bacteria, Soil Biology and
416 Biochemistry, 33, 1571-1574, 10.1016/S0038-0717(01)00073-6, 2001.
417 Bach, C. E., Warnock, D. D., Van Horn, D. J., Weintraub, M. N., Sinsabaugh, R. L., Allison, S. D., and German,
418 D. P.: Measuring phenol oxidase and peroxidase activities with pyrogallol, l-DOPA, and ABTS: Effect
419 of assay conditions and soil type, Soil Biology and Biochemistry, 67, 183-191, 2013.
420 Baldrian, P.: Fungal laccases - occurrence and properties, Fems Microbiology Reviews, 30, 215-242, 2006.
421 Bastida, F., Zsolnay, A., Hernández, T., and García, C.: Past, present and future of soil quality indices: A
422 biological perspective, Geoderma, 147, 159-171, 10.1016/j.geoderma.2008.08.007, 2008.
423 Beck, T., Beck, R.: Bodenenzyme. In: Blume, H.-P., Felix-Hennigsen, P., Frede, H.-G., Guggenberger, G.,
424 Horn, R., and Stahr, K. (eds.) Handbuch der Bodenkunde. Wiley-VCH, Weinheim, Germany, pp
425 supplement 8, 244 pg., 2000.
426 Bergkemper, F., Kublik, S., Lang, F., Krüger, J., Vestergaard, G., Schloter, M., and Schulz, S.: Novel
427 oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in
428 soil, Journal of Microbiological Methods, 125, 91-97, 10.1016/j.mimet.2016.04.011, 2016.
429 Bispo, A., Cluzeau, D., Creamer, R., Dombos, M., Graefe, U., Krogh, P. H., Sousa, J. P., Peres, G., Rutgers,
430 M., Winding, A., and Römbke, J.: Indicators for monitoring soil biodiversity, Integrated
431 Environmental Assessment and Management, 5, 717-719, 10.1897/IEAM-2009-064.1, 2009.
432 Bispo, A., Andersen, L., Angers, D. A., Bernoux, M., Brossard, M., Cécillon, L., Comans, R. N. J., Harmsen, J.,
433 Jonassen, K., Lamé, F., Lhuillery, C., Maly, S., Martin, E., Mcelnea, A. E., Sakai, H., Watabe, Y., and
434 Eglin, T. K.: Accounting for carbon stocks in soils and measuring GHGs emission fluxes from soils: Do
435 we have the necessary standards? Frontiers in Environmental Science, 5,
436 10.3389/fenvs.2017.00041, 2017.
437 Bockhorst, S., and Wardle, D.A.: Microclimate within litter bags of different mesh size: Implications for the
438 'arthropod effect' on litter decomposition. Soil Biology and Biochemistry, 58, 147-152, 2013.
439 Bollag, J.M.: Decontaminating soil with enzymes, Environmental Science and Technology, 26, 1876-1881,
440 1992.
441 Bolliger, A., Nalla, A., Magid, J., de Neergaard, A., Dole Nalla, A., and Bøg-Hansen, T. C.: Re-examining the
442 glomalin-purity of glomalin-related soil protein fractions through immunochemical, lectin-affinity
443 and soil labelling experiments, Soil Biology and Biochemistry, 40, 887-893,
444 10.1016/j.soilbio.2007.10.019, 2008.
445 Breure, A.M., De Deyn, G.B., Dominati, E., Eglin, T., Hedlund, K., Van Orshoven, J., Posthuma, L., 2012.
446 Ecosystem services: A useful concept for soil policy making! Current Opinion in Environmental
447 Sustainability 4(5), 578-585.
448 Brookes, P. C.: The use of microbial parameters in monitoring soil pollution by heavy metals, Biology and
449 Fertility of Soils, 19, 269-279, 10.1007/BF00336094, 1995.
450 Brooks, J. P., Edwards, D. J., Harwich, M. D., Jr., Rivera, M. C., Fettweis, J. M., Serrano, M. G., Reris, R. A.,
451 Sheth, N. U., Huang, B., Girerd, P., Strauss, J. F., III, Jefferson, K. K., and Buck, G. A.: The truth about

452 metagenomics: Quantifying and counteracting bias in 16S rRNA studies ecological and evolutionary
453 microbiology, BMC Microbiology, 15, 10.1186/s12866-015-0351-6, 2015.
454 Cebron, A., Norini, M.P., Beguiristain, T., and Leyval, C.: Real-time PCR quantification of PAH-ring
455 hydroxylating dioxygenase (PAH-RHD alpha) genes from Gram positive and Gram-negative Bacteria
456 in soil and sediment samples, Journal of Microbiological Methods, 73, 148-159, 2008.
457 Creamer, R. E., Hannula, S. E., Leeuwen, J., Stone, D., Rutgers, M., Schmelz, R. M., Ruiter, P., Hendriksen,
458 N., Bolger, T., Bouffaud, M. L., Buee, M., Carvalho, F., Costa, D., Dirilgen, T., Francisco, R., Griffiths,
459 B. S., Griffiths, R., Martin, F., Silva, P. D., Mendes, S., Morais, P. V., Pereira, C., Philippot, L., Plassart,
460 P., Redecker, D., Römbke, J., Sousa, J. P., Wouterse, M., and Lemanceau, P.: Ecological network
461 analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected
462 by land use across Europe, Applied Soil Ecology, 97, 112-124, 10.1016/j.apsoil.2015.08.006, 2016.
463 Coban, H., Miltner, A., Kästner, M.: Fate of fatty acids derived from biogas residues in arable soil, Soil
464 Biology and Biochemistry, 91, 58-64, 2015.
465 Dandie, C.E., Miller, M.N., Burton, D.L., Zebarth, B.J., Trevors, J.T., and Goyer, C.: Nitric oxide reductase-
466 targeted real-time PCR quantification of denitrifier populations in soil, Applied Environmental
467 Microbiology, 73, 4250–4258, 2007.
468 De Gonzalo, G., Colpa, D.I., Habib, M.H.M., Fraaije, M.W., 2016. Bacterial enzymes involved in lignin
469 degradation. Journal of Biotechnology 236, 110-119.
470 Eichlerová, I., Šnajdr, J., and Baldrian, P. (2012): Laccase activity in soils: Considerations for the
471 measurement of enzyme activity, Chemosphere, 88, 1154-1160,
472 https://doi.org/10.1016/j.chemosphere.2012.03.019, 2012.
473 El Azhari, N., Bru, D., Sarr, A., and Martin-Laurent, F.: Estimation of the density of the protocatechuate-
474 degrading bacterial community in soil by real-time PCR, European Journal of Soil Science, 59, 665-
475 673, 2008.
476 El Azhari, N., Devers-Lamrani, M., Chatagnier, G., Rouard, N., and Martin-Laurent, F.: Molecular analysis of
477 the catechol-degrading bacterial community in a coal wasteland heavily contaminated with PAHs,
478 Journal of Hazardous Materials, 177, 593-601, 10.1016/j.jhazmat.2009.12.074, 2010.
479 European Commission: EU-Regulation 1107/2009/EC of the European Parliament and the council of 21
480 October 2009 concerning the placing of plant protection products on the market and repealing
481 Council Directives 79/117/EEC and 91/414/EEC, 2009.
482 Faber, J.H., Creamer, R.E., Mulder, C., Römbke, J., Rutgers, M., Sousa, J.P., Stone, D., and Griffiths, B.S.: The
483 practicalities and pitfalls of establishing a policy-relevant and cost-effective soil biological
484 monitoring scheme, Integrated Environmental Assessment and Management, 9, 276-284, 2013.
485 Feinstein, L. M., Woo, J. S., and Blackwood, C. B.: Assessment of bias associated with incomplete extraction
486 of microbial DNA from soil, Applied and Environmental Microbiology, 75, 5428-5433,
487 10.1128/AEM.00120-09, 2009.
488 Fish, J.A., Chai, B., Wang, Q., Sun, Y., Brown, C.T., Tiedje, J.M., and Cole, J.R.: FunGene: The Functional Gene
489 Pipeline and Repository, Frontiers in Microbiology, 4, 291, 2013.
490 Gaby, J.C., and Buckley, D.H.: A comprehensive evaluation of PCR primers to amplify the *nifH* gene of
491 nitrogenase, PLoS ONE, 7, 2012.
492 Galic, N., Schmolke, A., Forbes, V., Baveco, H., van den Brink, P.J., 2012. The role of ecological models in
493 linking ecological risk assessment to ecosystem services in agroecosystems. Science of the Total
494 Environment 415, 93-100.
495 Garland, J. L., and Mills, A. L.: Classification and characterization of heterotrophic microbial communities
496 on the basis of patterns of community-level sole-carbon-source utilization, Applied and
497 Environmental Microbiology, 57, 2351-2359, 1991.

498 Garland, J. L.: Analysis and interpretation of community-level physiological profiles in microbial ecology,
499 FEMS Microbiology Ecology, 24, 289-300, 10.1016/S0168-6496(97)00061-5, 1997.
500 Glimm, E., Heuer, H., Engelen, B., Smalla, K., and Backhaus, H.: Statistical comparisons of community
501 catabolic profiles, Journal of Microbiological Methods, 30, 71-80, 10.1016/S0167-7012(97)00046-8,
502 1997.
503 Gomez, E., Ferreras, L., and Toresani, S.: Soil bacterial functional diversity as influenced by organic
504 amendment application, Bioresource Technology, 97, 1484-1489, 10.1016/j.biortech.2005.06.021,
505 2006.
506 Gomiero, T.: Soil degradation, land scarcity and food security: Reviewing a complex challenge,
507 Sustainability (Switzerland), 8, 10.3390/su8030281, 2016.
508 Griffiths, B. S., and Philippot, L.: Insights into the resistance and resilience of the soil microbial community,
509 Fems Microbiology Reviews, 37, 112-129, 2013.
510 Guillaume, T., Maranguit, D., Murtilaksono, K., and Kuzyakov, Y.: Sensitivity and resistance of soil fertility
511 indicators to land-use changes: New concept and examples from conversion of Indonesian
512 rainforest to plantations, Ecological Indicators, 67, 49-57, 10.1016/j.ecolind.2016.02.039, 2016.
513 Hartmann, A., Schmid, M., van Tuinen, D., and Berg, G.: Plant-driven selection of microbes, Plant and Soil,
514 321, 235-257, 2009.
515 Hayat, R., Ali, S., Amara, U., Khalid, R., and Ahmed, I.: Soil beneficial bacteria and their role in plant growth
516 promotion: A review, Annals of Microbiology, 60, 579-598, 10.1007/s13213-010-0117-1, 2010.
517 Haynes, R.J.: Nature of the belowground ecosystem and its development during pedogenesis, Advances in
518 Agronomy, 127, 43-109, 2014.
519 Henry, S., Baudoin, E., Lopez-Gutiérrez, J.-C., Martin-Laurent, F., Brauman, A., and Philippot, L.:
520 Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR, Journal of
521 Microbiological Methods, 59: 327-335, 2004.
522 Herrmann, A.M., Coucheney, E., and Nunan, N.: Isothermal microcalorimetry provides new insight into
523 terrestrial carbon cycling, Environmental Science and Technology, 48, 4344-4352,
524 10.1021/es403941h, 2014.
525 Hirsch, P.R., Mauchline, T.H., and Clark, I.M.: Culture-independent molecular techniques for soil microbial
526 ecology, Soil Biology and Biochemistry, 42, 878-887, 10.1016/j.soilbio.2010.02.019, 2010.
527 ISO 11063: Soil quality -- Method to directly extract DNA from soil samples, International Organization for
528 Standardization, Geneva, Switzerland, 2012. https://www.iso.org/standard/50025.html
529 ISO 11266: Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil
530 under aerobic conditions, International Organization for Standardization, Geneva, Switzerland,
531 1994. https://www.iso.org/standard/19244.html
532 ISO 14238: Soil quality -- Biological methods -- Determination of nitrogen mineralization and nitrification
533 in soils and the influence of chemicals on these processes, International Organization for
534 Standardization, Geneva, Switzerland, 2012. https://www.iso.org/standard/56033.html
535 ISO 14239: Soil quality -- Laboratory incubation systems for measuring the mineralization of organic
536 chemicals in soil under aerobic conditions, International Organization for Standardization, Geneva,
537 Switzerland, 2017. https://www.iso.org/standard/69583.html
538 ISO 14240-1: Soil quality -- Determination of soil microbial biomass -- Part 1: Substrate-induced respiration
539 method, International Organization for Standardization, Geneva, Switzerland, 1997.
540 https://www.iso.org/standard/21530.html
541 ISO 14240-2: Soil quality -- Determination of soil microbial biomass -- Part 2: Fumigation-extraction
542 method, International Organization for Standardization, Geneva, Switzerland, 1997.
543 https://www.iso.org/standard/23951.html

https://doi.org/10.5194/soil-2019-42
Preprint. Discussion started: 10 July 2019
© Author(s) 2019. CC BY 4.0 License.

544 ISO 15473: Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil
545 under anaerobic conditions, International Organization for Standardization, Geneva, Switzerland,
546 2002. https://www.iso.org/standard/27189.html
547 ISO 15685: Soil quality -- Determination of potential nitrification and inhibition of nitrification -- Rapid test
548 by ammonium oxidation, International Organization for Standardization, Geneva, Switzerland,
549 International Organization for Standardization, Geneva, Switzerland, 2012.
550 https://www.iso.org/standard/53530.html
551 ISO 16072: Soil quality -- Laboratory methods for determination of microbial soil respiration, International
552 Organization for Standardization, Geneva, Switzerland, 2002.
553 https://www.iso.org/standard/32096.html
554 ISO 17155: Soil quality -- Determination of abundance and activity of soil microflora using respiration
555 curves, International Organization for Standardization, Geneva, Switzerland, 2012.
556 https://www.iso.org/standard/53529.html
557 ISO 17601: Soil quality -- Estimation of abundance of selected microbial gene sequences by quantitative
558 PCR from DNA directly extracted from soil, International Organization for Standardization, Geneva,
559 Switzerland, 2016. https://www.iso.org/standard/60106.html
560 ISO 18311: Soil quality -- Method for testing effects of soil contaminants on the feeding activity of soil
561 dwelling organisms -- Bait-lamina test, International Organization for Standardization, Geneva,
562 Switzerland, 2016. https://www.iso.org/standard/62102.html
563 ISO 23753-1: Soil quality -- Determination of dehydrogenase activity in soils -- Part 1: Method using
564 triphenyltetrazolium chloride (TTC), International Organization for Standardization, Geneva,
565 Switzerland, 2019. https://www.iso.org/standard/70145.html
566 ISO 23753-2: Soil quality -- Determination of dehydrogenase activity in soils -- Part 2: Method using
567 iodotetrazolium chloride (INT), International Organization for Standardization, Geneva, Switzerland,
568 2019. https://www.iso.org/standard/70146.html
569 ISO/DIS 20130 EN: Soil quality - Measurement of enzyme activity patterns in soil samples using colorimetric
570 substrates in micro-well plates, International Organization for Standardization, Geneva, Switzerland,
571 2018. https://www.iso.org/standard/67074.html
572 ISO/DIS 20951: Soil Quality -- Guidance on methods for measuring greenhouse gases (CO_2, N_2O, CH_4) and
573 ammonia (NH_3) fluxes between soils and the atmosphere, International Organization for
574 Standardization, Geneva, Switzerland, under development, 2018.
575 https://www.iso.org/standard/69534.html
576 ISO/CD 23265: Soil quality -- Test for measuring organic matter decomposition in contaminated soil,
577 International Organization for Standardization, Geneva, Switzerland, under development, 2018.
578 https://www.iso.org/standard/75115.html
579 ISO/TS 20131-1: Soil quality -- Easy laboratory assessments of soil denitrification, a process source of N_2O
580 emissions -- Part 1: Soil denitrifying enzymes activities, International Organization for
581 Standardization, Geneva, Switzerland, under development, 2018.
582 https://www.iso.org/standard/67075.html
583 ISO/TS 20131-2: Soil quality -- Easy laboratory assessments of soil denitrification, a process source of N_2O
584 emissions -- Part 2: Assessment of the capacity of soils to reduce N_2O, International Organization for
585 Standardization, Geneva, Switzerland, under development, 2018.
586 https://www.iso.org/standard/67076.html
587 ISO/TS 22939: Soil quality -- Measurement of enzyme activity patterns in soil samples using fluorogenic
588 substrates in micro-well plates, International Organization for Standardization, Geneva, Switzerland,
589 2010. https://www.iso.org/standard/41226.html

https://doi.org/10.5194/soil-2019-42
Preprint. Discussion started: 10 July 2019
© Author(s) 2019. CC BY 4.0 License.

590 ISO/TS 29843-1: Soil quality -- Determination of soil microbial diversity -- Part 1: Method by phospholipid
591 fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis, International Organization
592 for Standardization, Geneva, 2010. https://www.iso.org/standard/45703.html
593 ISO/TS 29843-2: Soil quality -- Determination of soil microbial diversity -- Part 2: Method by phospholipid
594 fatty acid analysis (PLFA) using the simple PLFA extraction method, International Organization for
595 Standardization, Geneva, 2011. https://www.iso.org/standard/54070.html
596 Jänsch, S., Scheffczyk, A., and Römbke, J.: The bait lamina test – A possible screening method for
597 earthworm toxicity testing, Euro-Mediterranean Journal for Environmental Integration, 2, 5. 11pp.
598 DOI: 10-1007/s41207-017-0015z, 2017.
599 Janos, D. P., Garamszegi, S., and Beltran, B.: Glomalin extraction and measurement, Soil Biology and
600 Biochemistry, 40, 728-739, 10.1016/j.soilbio.2007.10.007, 2008.
601 Jansson, J. K., and Hofmockel, K. S.: The soil microbiome — from metagenomics to metaphenomics,
602 Current Opinion in Microbiology, 43, 162-168, 10.1016/j.mib.2018.01.013, 2018.
603 Joergensen, R. G., and Emmerling, C.: Methods for evaluating human impact on soil microorganisms based
604 on their activity, biomass, and diversity in agricultural soils, Journal of Plant Nutrition and Soil
605 Science, 169, 295-309, 10.1002/jpln.200521941, 2006.
606 Jung, J., Choi, S., Jung, H., Scow, K.M., and Park, W.: Primers for amplification of nitrous oxide reductase
607 genes associated with Firmicutes and Bacteroidetes in organic-compound-rich soils, Microbiology,
608 159, 307-315. doi: 10.1099/mic.0.060194-0, 2013.
609 Karpouzas, D.G., Tsiamis, G., Trevisan, M., Ferrari, F., Malandain, C., Sibourg, O., and Martin-Laurent, F.:
610 'LOVE TO HATE' pesticides: felicity or curse for the soil microbial community? An FP7 IAPP Marie
611 Curie project aiming to establish tools for the assessment of the mechanisms controlling the
612 interactions of pesticides with soil microorganisms, Environmental Science and Pollution Research,
613 18, 18947-18951, 2016.
614 Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, L., Sarneel, J.M., and Hefting, M.M.: Tea Bag Index: a novel
615 approach to collect uniform decomposition data across ecosystems, Methods in Ecology and
616 Evolution, 4, 1070–1075, 2013.
617 Knacker, T., Förster, B., Römbke, J., and Frampton, G. K.: Assessing the effects of plant protection products
618 on organic matter breakdown in arable fields - Litter decomposition test systems, Soil Biology and
619 Biochemistry, 35, 1269-1287, 10.1016/S0038-0717(03)00219-0, 2003.
620 Kolb, S., Knief, C., Stubner, S. and Conrad, R.: Quantitative detection of methanotrophs in soil by novel
621 pmoA-targeted real-time PCR assays, Applied Environmental Microbiology, 69, 2423–2429. doi:
622 10.1128/AEM.69.5.2423-2429.2003. PMCID: PMC154495, 2003.
623 Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R.,
624 Schindlbacher, A., and Sessitsch, A.: Effects of season and experimental warming on the bacterial
625 community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing, FEMS
626 Microbiology Ecology, 82, 551-562, 10.1111/j.1574-6941.2012.01420.x, 2012.
627 Kvas, S., Rahn, J., Engel, K., Neufeld, J.D., Villeneuve, P.J., Trevors, J.T., Lee, H., Scroggins, R.P., and
628 Beaudette, L.A.: Development of a microbial test suite and data integration method for assessing
629 microbial health of contaminated soil, Journal of Microbiological Methods, 143, 66-77, 2017.
630 Levy-Booth, D. J., Prescott, C. E., and Grayston, S. J.: Microbial functional genes involved in nitrogen
631 fixation, nitrification and denitrification in forest ecosystems, Soil Biology and Biochemistry, 75, 11-
632 25, 10.1016/j.soilbio.2014.03.021, 2014.
633 Liu, W., Qiao, C., Yang, S., Bai, W., and Liu, L.: Microbial carbon use efficiency and priming effect regulate
634 soil carbon storage under nitrogen deposition by slowing soil organic matter decomposition,
635 Geoderma, 332, 37-44, 10.1016/j.geoderma.2018.07.008, 2018.

https://doi.org/10.5194/soil-2019-42
Preprint. Discussion started: 10 July 2019
© Author(s) 2019. CC BY 4.0 License.

636 Lugtenberg, B., and Kamilova, F.: Plant-growth-promoting rhizobacteria, Annual Review of Microbiology,
637 63, 541-556, 10.1146/annurev.micro.62.081307.162918, 2009.
638 Martin-Laurent, F., Cornet, L., Ranjard, L., Lopez-Gutiérrez, J.C., Philippot, L., Schwartz, C., Chaussod, R.,
639 Catroux, G., and Soulas, G.: Estimation of atrazine-degrading genetic potential and activity in three
640 French agricultural soils, FEMS Micobiology Ecology, 48, 425-435. ,2004.
641 Martin-Laurent, F., Kandeler, E., Petric, I., Djuric, S., and Karpouzas, D.: ECOFUN-MICROBIODIV: an FP7
642 European project for developing and evaluating innovative tools for assessing the impact of
643 pesticides on soil functional microbial diversity: towards new pesticide registration regulation?
644 Environmental Science and Pollution Research, 2, 1203-1205, 2013.
645 MEA (Millennium Ecosystem Assessment): Ecosystems and Human Well-being: Synthesis, Island Press,
646 Washington, D.C., 2005.
647 Modrzyński, J. J., Christensen, J. H., Mayer, P., and Brandt, K. K.: Limited recovery of soil microbial activity
648 after transient exposure to gasoline vapors, Environmental Pollution, 216, 826-835,
649 10.1016/j.envpol.2016.06.054, 2016.
650 Montgomery, D. R.: Dirt: The Erosion of Civilizations, University of California Press, Berkeley, Calif.; London,
651 2008.
652 Nannipieri, P., and Eldor, P.: The chemical and functional characterization of soil N and its biotic
653 components, Soil Biology and Biochemistry, 41, 2357-2369, 10.1016/j.soilbio.2009.07.013, 2009.
654 Nannipieri, P., Greco, S., and Ceccanti, B.: Ecological Significance of the Biological Activity in Soil, in: Soil
655 Biochemistry: Volume 6: Volume 6, edited by: Bollag, J. M., and Stotzky, G., Routledge, New York,
656 293-355, 2017.
657 Nicol, G. W., Glover, L. A., and Prosser, J. I.: Spatial analysis of archaeal community structure in grassland
658 soil, Applied and Environmental Microbiology, 69, 7420-7429, 10.1128/AEM.69.12.7420-7429.2003,
659 2003.
660 Nienstedt, K., Brock, T.C.M., Van Wensem, J., Montforts, M., Hart, A., Aagaard, A., Alix, A., Boesten, J.,
661 Bopp, S.K., Brown, C., Capri, E., Forbes, V., Köpp, H., Liess, M., Luttik, R. Maltby, L. Sousa, J.-P.,
662 Streissl, F., and Hardy, A.R.: Development of a framework based on an ecosystem services approach
663 for deriving specific protection goals for environmental risk assessment of pesticides, Science of the
664 Total Environment, 415, 31-38, 2012.
665 Norton, J.M., Alzerreca, J.J., Suwa, Y., and Klotz, M.G.: Diversity of ammonia monooxygenase operon in
666 autotrophic ammonia-oxidizing bacteria, Archives of Microbiology, 177, 139-149, 10.1007/s00203-
667 001-0369-z, 2002.
668 Ockleford, C., Adriaanse, P., Berny, P., Brock, T., Duquesne, S., Grilli, S., Hernandez-Jerez, A. F., Bennekou,
669 S. H., Klein, M., Kuhl, T., Laskowski, R., Machera, K., Pelkonen, O., Pieper, S., Stemmer, M., Sundh, I.,
670 Teodorovic, I., Tiktak, A., Topping, C. J., Wolterink, G., Craig, P., de Jong, F., Manachini, B., Sousa, P.,
671 Swarowsky, K., Auteri, D., Arena, M., and Rob, S.: EFSA PPR Panel (EFSA Panel on Plant Protection
672 Products and their Residues) Scientific Opinion addressing the state of the science on risk
673 assessment of plant protection products for in-soil organisms, EFSA Journal, 15, 255,
674 doi:10.2903/j.efsa.2017.4690, 2017.
675 OECD: Test No. 216: Soil Microorganisms: Nitrogen Transformation Test. Paris, France, 2000a.
676 OECD: Test No. 217: Soil Microorganisms: Carbon Transformation Test. Paris, France, 2000b.
677 OECD: OECD Series on Testing and Assessment No. 56. Guidance document on the breakdown of organic
678 matter in litterbags. ENV/JM/MONO 23. Paris, France, 2006.
679 Ollivier, J., Kleineidam, K., Reichel, R., Thiele-Bruhn, S., Kotzerke, A., Kindler, R., Wilke, B.M., and Schloter,
680 M.: Effect of sulfadiazine-contaminated pig manure on the abundances of genes and transcripts
681 involved in nitrogen transformation in the root-rhizosphere complexes of maize and clover, Applied
682 and Environmental Microbiology, 76, 7903-7909, 2010.

683 Parker, S.S.: Buried treasure: Soil biodiversity and conservation, Biodiversity and Conservation, 19, 3743-
684 3756, 2010.
685 Paul, E. A.: Soil Microbiology, Ecology and Biochemistry, 4th ed., Academic Press, Burlington, MA, 2015.
686 Peng, J., Lü, Z., Rui, J., and Lu, Y.: Dynamics of the methanogenic archaeal community during plant residue
687 decomposition in an anoxic rice field soil, Applied and Environmental Microbiology, 74, 2894-2901,
688 10.1128/AEM.00070-08, 2008.
689 Penton, C. R., Gupta, V. V. S. R., Yu, J., and Tiedje, J. M.: Size matters: Assessing optimum soil sample size
690 for fungal and bacterial community structure analyses using high throughput sequencing of rRNA
691 gene amplicons, Frontiers in Microbiology, 7, 10.3389/fmicb.2016.00824, 2016.
692 Philippot, L., Ritz, K., Pandard, P., Hallin, S., and Martin-Laurent, F.: Standardisation of methods in soil
693 microbiology: Progress and challenges, FEMS Microbiology Ecology, 82, 1-10, 10.1111/j.1574-
694 6941.2012.01436.x, 2012.
695 Prado, A.G.S., Airoldi, C.: The effect of the herbicide diuron on soil microbial activity, Pest Management
696 Science, 57, 640-644 doi:10.1002/ps.321, 2001.
697 Prado, A.G.S., Airoldi, C.: A toxicity view of the pesticide picloram when immobilized onto a silica gel
698 surface, Analytical and Bioanalytical Chemistry, 376, 686-690, doi:10.1007/s00216-003-1927-9,
699 2003.
700 Preston-Mafham, J., Boddy, L., and Randerson, P. F.: Analysis of microbial community functional diversity
701 using sole-carbon-source utilisation profiles - A critique, FEMS Microbiology Ecology, 42, 1-14,
702 10.1016/S0168-6496(02)00324-0, 2002.
703 Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelose, C., Peres, G., and Rutgers, M.: Soil biodiversity,
704 biological indicators and soil ecosystem services – an overview of European approaches, Current
705 Opinion in Environ. Sustainability, 4, 529-538, 2012.
706 Regan, K. M., Nunan, N., Boeddinghaus, R. S., Baumgartner, V., Berner, D., Boch, S., Oelmann, Y.,
707 Overmann, J., Prati, D., Schloter, M., Schmitt, B., Sorkau, E., Steffens, M., Kandeler, E., and Marhan,
708 S.: Seasonal controls on grassland microbial biogeography: Are they governed by plants, abiotic
709 properties or both?, Soil Biology and Biochemistry, 71, 21-30, 10.1016/j.soilbio.2013.12.024, 2014.
710 Ribbons, R.R., Levy-Booth, D.J., Masse, J., Grayston, S.J., McDonald, M.A., Vesterdal, L., and Prescott, C.E.:
711 Linking microbial communities, functional genes and nitrogen-cycling processes in forest floors
712 under four tree species, Soil Biology and Biochemistry, 103, 181-191, 10.1016/j.soilbio.2016.07.024,
713 2016.
714 Rillig, M.C.: Arbuscular mycorrhizae, glomalin, and soil aggregation, Canadian Journal of Soil Science, 84,
715 355-363 doi:10.4141/S04-003, 2004.
716 Rillig, M.C., and Mummey, D.L.: Mycorrhizas and soil structure, New Phytologist, 171, 41-53
717 doi:10.1111/j.1469-8137.2006.01750.x, 2006.
718 Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., and Wood, C.: Selecting biological indicators for monitoring
719 soils: A framework for balancing scientific and technical opinion to assist policy development,
720 Ecological Indicators, 9, 1212-1221, 2009.
721 Römbke, J., Jänsch, S. Meier, M., Hilbeck, A., Teichmann, H., and Tappeser, B.: General recommendations
722 for soil ecotoxicological tests suitable for the environmental risk assessment (ERA) of genetically
723 modified plants (GMPs), Integrated Environmental Assessment and Management, 6, 287-300, 2010.
724 Rousk, J.: Biomass or growth? How to measure soil food webs to understand structure and function, Soil
725 Biology and Biochemistry, 102, 45-47, 10.1016/j.soilbio.2016.07.001, 2016.
726 Rousk, J., Brookes, P.C., and Bååth, E.: Contrasting soil pH effects on fungal and bacterial growth suggest
727 functional redundancy in carbon mineralization, Applied and Environmental Microbiology, 75, 1589-
728 1596 doi:10.1128/AEM.02775-08, 2009b.

https://doi.org/10.5194/soil-2019-42
Preprint. Discussion started: 10 July 2019
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729 Rousk, J., Demoling, L. A., and Bååth, E.: Contrasting short-term antibiotic effects on respiration and
730 bacterial growth compromises the validity of the selective respiratory inhibition technique to
731 distinguish fungi and bacteria, Microbial Ecology, 58, 75-85, 10.1007/s00248-008-9444-1, 2009a.
732 Rutgers, M., Wouterse, M., Drost, S. M., Breure, A. M., Mulder, C., Stone, D., Creamer, R. E., Winding, A.,
733 and Bloem, J.: Monitoring soil bacteria with community-level physiological profiles using Biolog™
734 ECO-plates in the Netherlands and Europe, Applied Soil Ecology, 97, 23-35,
735 10.1016/j.apsoil.2015.06.007, 2016.
736 Sakakibara, S.M., Jones, M.D., Gillespie, M., Hagerman, S.M., Forrest, M.E., Simard, S.W., and Durall, D.M.:
737 A comparison of ectomycorrhiza identification based on morphotyping and PCR-RFLP analysis,
738 Mycological Research, 106, 868-878, 2002.
739 Savazzini, F., Longa, C.M., Pertot, I., and Gessler, C.J.: Real-time PCR for detection and quantification of the
740 biocontrol agent Trichoderma atroviride strain SC1 in soil, Microbiological Methods, 73, 185-94. doi:
741 10.1016/j.mimet.2008.02.004, 2008.
742 Scheu, S., Schlitt, N., Tiunov, A. V., Newington, J. E., and Jones, T. H.: Effects of the presence and community
743 composition of earthworms on microbial community functioning, Oecologia, 133, 254-260,
744 10.1007/s00442-002-1023-4, 2002.
745 Scheu, S., Ruess, L., and Bonkowski, M.: Interactions between microorganisms and soil micro- and
746 mesofauna, in: Microorganisms in Soils: Roles in Genesis and Functions, edited by: Varma, A., and
747 Buscot, F., Soil Biology, 3, 253-275, 2005.
748 Schmitt, H., Van Beelen, P., Tolls, J., and Van Leeuwen, C. L.: Pollution-induced community tolerance of
749 soil microbial communities caused by the antibiotic sulfachloropyridazine, Environmental Science &
750 Technology, 38, 1148-1153, 2004.
751 Schwartz, E.: Characterization of growing microorganisms in soil by stable isotope probing with $H_2{}^{18}O$,
752 Applied and Environmental Microbiology, 73, 2541-2546, 2007.
753 Schwartz, E., Hayer, M., Hungate, B.A., Koch, B.J., McHugh, T.A., Mercurio, W., Morrissey, E.M., Soldanova,
754 K.: Stable isotope probing with ^{18}O-water to investigate microbial growth and death in
755 environmental samples, Current Opinion in Biotechnology, 41, 14-18, 2016.
756 Schulz, S., Bergkemper, F., De Vries, M., Schöler, A., and Schloter, M.: qPCR for quantitative validation of
757 metagenomic data [qPCR zur quantitativen Validierung von Metagenomdaten], BioSpektrum, 22,
758 265-269, 10.1007/s12268-016-0684-1, 2016.
759 Sessitsch, A., Hackl, E., Wenzl, P., Kilian, A., Kostic, T., Stralis-Pavese, N., Sandjong, B. T., and Bodrossy, L.:
760 Diagnostic microbial microarrays in soil ecology, New Phytologist, 171, 719-736, 10.1111/j.1469-
761 8137.2006.01824.x, 2006.
762 Singh, B. K., Tate, K. R., Kolipaka, G., Hedley, C. B., Macdonald, C. A., Millard, P., and Murrell, J. C.: Effect of
763 afforestation and reforestation of pastures on the activity and population dynamics of
764 methanotrophic bacteria, Applied and Environmental Microbiology, 73, 5153-5161,
765 10.1128/AEM.00620-07, 2007.
766 Six, J., Bossuyt, H., Degryze, S, and Denef, K.: A history of research on the link between (micro)aggregates,
767 soil biota, and soil organic matter dynamics, Soil and Tillage Research, 79, 7-31
768 doi:10.1016/j.still.2004.03.008, 2004.
769 Smith, C.J., and Osborn, A.M.: Advantages and limitations of quantitative PCR (Q-PCR)-based approaches
770 in microbial ecology, FEMS Microbiology Ecology, 67, 6-20, 10.1111/j.1574-6941.2008.00629.x,
771 2009.
772 Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability
773 of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil
774 Biology and Biochemistry 40(9), 2068-2075.

775 Spohn, M., Pötsch, E. M., Eichorst, S. A., Woebken, D., Wanek, W., and Richter, A.: Soil microbial carbon
776 use efficiency and biomass turnover in a long-term fertilization experiment in a temperate
777 grassland, Soil Biology and Biochemistry, 97, 168-175, 10.1016/j.soilbio.2016.03.008, 2016.
778 Steinberg, L.M., and Regan, J.M.: *mcrA*-targeted real-time quantitative PCR method to examine
779 methanogen communities, Applied and Environmental Microbiology, 75, 4435-4442, 2009.
780 Strickland, M. S., and Rousk, J.: Considering fungal: Bacterial dominance in soils - Methods, controls, and
781 ecosystem implications, Soil Biology and Biochemistry, 42, 1385-1395,
782 10.1016/j.soilbio.2010.05.007, 2010.
783 Suriyavirun, N., Krichels, A. H., Kent, A. D., and Yang, W. H.: Microtopographic differences in soil properties
784 and microbial community composition at the field scale, Soil Biology and Biochemistry, 131, 71-80,
785 10.1016/j.soilbio.2018.12.024, 2019.
786 Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy Alves, R. J.,
787 Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., and Richter, A.: Soil organic matter quality exerts a
788 stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal
789 transect, Soil Biology and Biochemistry, 121, 212-220, 10.1016/j.soilbio.2018.02.022, 2018.
790 TEEB: The Economics of Ecosystems and Biodiversity Ecological and Economic Foundations. Edited by
791 Pushpam Kumar. Earthscan, 2010.
792 Thiele-Bruhn, S., Bloem, J., de Vries, F. T., Kalbitz, K., and Wagg, C.: Linking soil biodiversity and agricultural
793 soil management, Current Opinion in Environmental Sustainability, 4, 523-528, 2012.
794 Tian, J., He, N., Hale, L., Niu, S., Yu, G., Liu, Y., Blagodatskaya, E., Kuzyakov, Y., Gao, Q., and Zhou, J.: Soil
795 organic matter availability and climate drive latitudinal patterns in bacterial diversity from tropical
796 to cold temperate forests, Functional Ecology, 32, 61-70, 10.1111/1365-2435.12952, 2018.
797 Traugott, M., Kamenova, S., Ruess, L., Seeber, J., Plantegenest, M.: Empirically characterising trophic
798 networks: What emerging DNA-based methods, stable isotope and fatty acid analyses can offer,
799 Advances in Ecological Research, 49, 177-224, 2013.
800 Van der Putten, W.H., Mudgal, S., Turbé, A., Toni, A.D., Lavelle, P., Benito, P., and Ruiz, N.: Soil biodiversity:
801 functions, threats and tools for policy makers, Bio Intelligence Service, Paris, France, 2010.
802 Voříšková, A., Jansa, J., Püschel, D., Krüger, M., Cajthaml, T., Vosátka, M., and Janoušková, M.: Real-time
803 PCR quantification of arbuscular mycorrhizal fungi: does the use of nuclear or mitochondrial markers
804 make a difference? Mycorrhiza, 27, 577-585. doi: 10.1007/s00572-017-0777-9, 2017.
805 Wagg, C., Bender, S. F., Widmer, F., and Van Der Heijden, M. G. A.: Soil biodiversity and soil community
806 composition determine ecosystem multifunctionality, Proceedings of the National Academy of
807 Sciences of the United States of America, 111, 5266-5270, 10.1073/pnas.1320054111, 2014.
808 Wallenstein, M. D., Myrold, D. D., Firestone, M., and Voytek, M.: Environmental controls on denitrifying
809 communities and denitrification rates: Insights from molecular methods, Ecological Applications, 16,
810 2143-2152, 10.1890/1051-0761(2006)016[2143:ECODCA]2.0.CO;2, 2006.
811 Wardle, D.A., and Ghani, A.: A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of
812 disturbance and ecosystem development, Soil Biology and Biochemistry, 27, 1601-1610,
813 10.1016/0038-0717(95)00093-T, 1995.
814 Winding, A., and Hendriksen, N. B.: Comparison of CLPP and enzyme activity assay for functional
815 characterization of bacterial soil communities, Journal of Soils and Sediments, 7, 411-417,
816 10.1065/jss2007.11.262, 2007.
817 Wright, S.F., Upadhyaya, A., and Buyer, J.S.: Comparison of N-linked oligosaccharides of glomalin from
818 arbuscular mycorrhizal fungi and soils by capillary electrophoresis, Soil Biology and Biochemistry,
819 30, 1853-1857, 10.1016/S0038-0717(98)00047-9, 1998.

https://doi.org/10.5194/soil-2019-42
Preprint. Discussion started: 10 July 2019
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SOIL Discussions

820 Xia, W., Zhang, C., Zeng, X., Feng, Y., Weng, J., Lin, X., Zhu, J., Xiong, Z., Xu, J., Cai, Z., and Jia, Z.:
821 Autotrophic growth of nitrifying community in an agricultural soil, ISME Journal, 5, 1226-1236,
822 10.1038/ismej.2011.5, 2011.
823 Xu, S., Feng, S., Sun, H., Wu, S., Zhuang, G., Deng, Y., Bai, Z., Jing, C., and Zhuang, X.: Linking N_2O
824 emissions from biofertilizer-amended soil of tea plantations to the abundance and structure of
825 N_2O-reducing microbial communities, Environmental Science and Technology, 52, 11338-11345,
826 10.1021/acs.est.8b04935, 2018.
827 Yates, M. V., Nakatsu, C. H., Miller, R. V., and Pillai, S. D.: Manual of Environmental Microbiology, Fourth
828 Edition, American Society of Microbiology, 2016.
829 Yergeau, E., Hogues, H., Whyte, L. G., and Greer, C. W.: The functional potential of high Arctic permafrost
830 revealed by metagenomic sequencing, qPCR and microarray analyses, ISME Journal, 4, 1206-1214,
831 10.1038/ismej.2010.41, 2010.

https://doi.org/10.5194/soil-2019-42
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832 **Table 1.** List of criteria for the selection of indicators for microbial functional indicators, based on Faber et
833 al. (2013) and Pulleman et al. (2012), with slight modifications by the authors.

	Criteria	Measured by	Low Score	High Score
a)	Practicability	Lab equipment	Very few labs have the equipment needed	All labs would be able to carry out the work
		Skills	Specialist skills are needed	General skills would suffice
b)	Cost efficiency	Capital start-up	More than €100 000	Less than €2000
		Cost per sample	More than €100	Less than €2
		Labour needed in the lab	High labour demand	Low labour demand
		Labour needed in the field	High labour demand	Low labour demand
c)	Policy relevance	Focus on ecosystem processes and services	Weak links with existing or planned legislation	Strong links with existing or planned legislation
d)	Sensitivity	Effect of soil properties	No response or idiosyncratic response	The indicator responds characteristically to change
		Effect of land use	No response or idiosyncratic response	The indicator responds characteristically to change
		Effect of disturbance	No response or idiosyncratic response	The indicator responds characteristically to change
e)	Selectivity		Endpoint affected by numerous variables	Endpoint only affected by parameter under investigation
f)	Reproducibi-lity		Low or largely varying reproducibility among replicates	Highly reproducible
g)	Use as an indicator	Status quo	Not in use already	In use already
h)	Handling and availability of organisms[1]		Rare and/or difficult to obtain Difficult to keep Largely varying quality/fitness Seasonal availability	Easy to obtain Easy to keep Easy to provide with constant quality/fitness Year-round availability
i)	Fit for use as an indicator	Significance / explanatory power	Weak relationship to ecological function	Strong relationship to ecological function
		Standardized	Methods are not ready for general use or standardization (i.e. low experience, no SOPs [2])	Methods are already in general use, preferably as standard (e.g. OECD)
		Spatio-temporally relevant	Spatio-temporally only relevant for a small plot at one point in time	Representative for more than one site and/or more than one point in time
		Understandable	Difficult to explain in a policy situation	Easily understood in a policy situation

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j)	Experience	Literature data	Low amount of information on performance and outcome, e.g. <10 publications	High amount of information on the performance and outcome, e.g. >10 publications, existing ring test(s)
k)	Data evaluation	Database	No or hardly any existing data available or not freely available	Freely available and sound database for data evaluation

834 [1] Only relevant for faunal species. Does not apply to soil microorganisms that are tested with their natural
835 abundance in mixed communities.
836 [2] Standard operating procedures

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837 **Table 2.** Methods already validated and published as ISO standards for determining potential microbial
838 biomass and activities for soil quality.

Microbial biomass and respiration (some relations to Functions 1 and 6)	
ISO 14240-1	Determination of soil microbial biomass – Part 1: Substrate induced respiration method
ISO 12240-2	Determination of soil microbial biomass – Part 2: Fumigation – extraction method
ISO 16072	Laboratory method for determination of microbial soil respiration
ISO 17155	Determination of the activity of the soil microflora using respiration curves
ISO 11063	Direct soil DNA extraction
ISO 17601	Quantification of the abundance of microbial groups in soil DNA extract
ISO/TS 29843-1	Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis (
ISO/TS 29843-2:	Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method
(Potential) microbial enzymatic activities: C, N and P turnover (Functions 4 and 6)	
ISO/TS 22939 [1]	Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates
ISO/DIS 20130 EN[2]	Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates
ISO/TS 23753-1	Determination of dehydrogenase activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC)
ISO/TS 23753-2	Determination of dehydrogenase activity in soils — Part 2: Method using iodotetrazolium chloride (INT)
ISO 14238	Biological methods – Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes
ISO 15685	Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation
Potential microbial activities: biodegradation of pollutants (Function 3)	
ISO 11266	Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions
ISO 14239	Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions
ISO 15473	Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions
Potential microbial activities: turnover greenhouse gases (Function 7)	
ISO/DIS 20951	Guidance on methods for measuring greenhouse gases (CO_2, N_2O, CH_4) and ammonia (NH_3) fluxes between soils and the atmosphere
ISO/TS 20131-1	Easy laboratory assessments of soil denitrification, a process source of N_2O emissions -- Part 1: Soil denitrifying enzymes activities
ISO/TS 20131-2	Easy laboratory assessments of soil denitrification, a process source of N_2O emissions -- Part 2: Assessment of the capacity of soils to reduce N_2O
Potential microbial activities: organic matter decomposition (Function 6)	
ISO/CD 23265 [3]	Test for measuring organic matter decomposition in contaminated soil

839 [1] Measured enzyme activities: Arylsulfatase E.C. 3.1.6.1; α-glucosidase E.C. 3.2.1.20; β-glucosidase E.C.
840 3.2.1.21; β-xylosidase E.C. 3.2.1.37; cellobiosidase E.C. 3.2.1.91; N-acetylglucosaminidase E.C. 3.2.1.52;

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841 phosphodiesterase E.C. 3.1.4.1; phosphomonoesterase E.C. 3.1.3.2; leucine-aminopeptidase E.C.
842 3.4.11.1; alanine-aminopeptidase E.C. 3.4.11.12.
843 [2] Measured enzyme activities: Arylamidase E.C. 3.4.11.2; arylsulfatase E.C. 3.1.6.1; α-glucosidase E.C.
844 3.2.1.20; β-glucosidase E.C. 3.2.1.21; β-galactosidase E.C. 3.2.1.22; N-acetylglucosaminidase E.C.
845 3.2.1.52; phosphatase E.C. 3.1.4.1; acid phosphatase E.C. 3.1.4.1; alkaline phosphatase E.C. 3.1.4.1;
846 urease E.C. 3.5.1.5.
847 [3] Degradation of cellulose under laboratory conditions.

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848 **Table 3.** Potential new methods for the ISO standardization process and assessment according to the
849 "logical sieve" selection criteria (described in Table 1).

Method	Source	Function addressed	a	b	c	d	e	f	g	h	i	j	k
			Assessment [1]										
Function 4. Nutrient cycling (N and P)			a	b	c	d	e	f	g	h	i	j	k
Functional genes assessed by real time qPCR													
Ammonium monoxygenase gene (*amoA*)	Levy-Booth et al., 2014	quantify the abundance of nitrifying microbes	1-2	3	5	5	5	5	5	na[2]	4	4	3
Ammonium monoxygenase gene (*amoB*)	Norton et al., 2002	quantify the abundance of nitrifying microbes	1-2	3	5	5	5	5	5	na	4	4	3
Nitrogenase gene (*nifH*)	Gaby and Buckley, 2012	quantify the abundance of N fixing microbes	1-2	3	5	5	5	5	5	na	4	4	3
Various genes driving P turnover	Bergkemper et al, 2016	quantify the abundance of microbes driving P transformation	1-2	3	5	5	5	5	5	na	4	4	3
Function 5. Pest control and plant growth promotion													
Specific mtDNA sequences assessed by real time qPCR	Voříšková et al., 2017	quantify the abundance of arbuscular mycorrhiza	1-2	3	5	5	5	5	5	na	4	3	2
Specific ITS sequences assessed by real time qPCR	Sakakibara et al., 2002	quantify the abundance of ectomycorrhizal fungi	1-2	3	5	5	5	5	5	na	4	3	2
Specific ITS sequences assessed by real time qPCR	Savazzini et al., 2008	quantify the abundance of biocontrol active Trichoderma fungi	1-2	3	5	5	5	5	5	na	4	3	2
Function 6. Carbon cycling and sequestration													
Enzyme activity of fungi	Eichlerová et al., 2012	determine activity of laccases	4	4	5	5	3	5	5	na	4	5	4
	Bach et al., 2013	determine activity of phenoloxidases	4	4	5	5	3	5	5	na	4	5	4
Community level physiological profiling (CLPP, "Biolog")	Garland and Mills, 1991	determine degradation of a set of carbon sources	3	4	3	1	1	5	3	na	1	5	3
Microcalorimetry	Prado and Airoldi, 2001; 2003	quantify microbial energy turnover	1	2	1	3	3	5	1	na	3	3	1
[^{3}H]-leucine or [^{3}H]-thymidine incorporation	Bååth, 1998; Bååth et al., 2001; Rousk et al., 2009b	quantify microbial growth rate and efficiency	1	2	5	5	4	5	5	na	4	4	2
[^{18}O] incorporation into DNA from labelled water	Schwartz, 2007; Schwartz et al., 2016	quantify microbial growth rate and efficiency	2	3	5	5	4	5	5	na	3	2	2
Organic matter decomposition	OECD, 2006; Knacker et al., 2003	assess organic matter degradation and therefore C cycling	5	5	4	4	5	4	5	na	5	5	5
Litter bag technique	Bockhorst and Wardle, 2013	assess the degradation of plant litter material											
Tea bag technique	Keuskamp et al., 2013	assess the degradation of tea leaves	5	5	4	4	5	4	5	na	5	5	5

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Table 3. Continued

Method	Source	Function addressed	Assessment [1]										
Funct. genes within C cycle assessed by real time qPCR	El Azhari et al., 2008	quantify the abundance of microbes able to degrade protocatechuate (*pcaH*) a key intermedi-ary metabolite of the β-ketoadipate pathway	1-2	3	5	5	5	5	5	na	4	4	3
	El Azhari et al., 2010	quantify the abundance of microbes able to degrade catechol (*cat A*) a key intermediary metabolite of the β-ketoadipate pathway											
Function 7. Greenhouse gas emissions													
Methyl coenzyme M reductase (*mcrA*) assessed by real time qPCR	Steinberg and Regan, 2009	quantify the abundance of methane producing microbes	1-2	3	5	5	5	5	5	na	4	4	3
N_2O reductase gene (*nosZ*) assessed by real time qPCR	Jung et al., 2013	quantify the abundance of N_20 reducing microbes	1-2	3	5	5	5	5	5	na	4	4	3
Methane reductase gene *pmoA*)	Kolb et al., 2003	quantify the abundance of methane reducing microbes	1-2	3	5	5	5	5	5	na	4	4	3
Nitric oxide reductase gene *cnorA*) assessed by real time qPCR	Dandie et al., 2007	quantify the abundance of methane reducing microbes	1-2	3	5	5	5	5	5	na	4	4	3
Function 8. Soil structure affecting soil water, gas balance and filtration capacity													
Determination of glomalin	Bolliger et al., 2008; Janos et al., 2008; Wright et al., 1998	determine the content of glomalin in soil as a proxy of soil aggregation	3	3	4	4	4	5	3	na	4	4	2

[1] Overall scoring in case of several measures for one criterion. Fulfilment of criterion described by numbering (colour code): 1 (red) very low; 2 (orange) low; 3 (yellow) medium; 4 (light green) good; 5 (dark green) very good.

[2] na = not applicable