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on behalf of the SOIL Editorial Board

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Dear Natascha Töpfer, dear editorial board members,

As the corresponding author and on behalf of all coauthors of the manuscript soil-2019-42 "*Identification of new microbial functional standards for soil quality assessment*" I herewith re-submit a revised version of the manuscript. First of all we want to express our appreciation of the very constructive and sound suggestions and criticism of both reviewers. Following these advice, we significantly revised the manuscript and added text passages. Additionally we did some further polishing in style and language.
Please find our response to the reviewers' suggestions in the following text (marked as text in italics).

I very much hope that the revised version, which we think was substantially improved, finds your and the reviewers approval.

With kind regards,

S. Thiele-Bruhn

Reviewer #1 Robert Griffith

This paper discusses new methodologies and opportunities offered by molecular methodologies to provide microbiological indicators for assessing soil quality. In essence, it reports on key issues raised during recent discussions within the International Organization for Standardization (ISO), and identifies the need to focus on soil functions of relevance to ecosystem services as recognised for example in the Millennium Ecosystem Assessment. A key focus of the paper is in highlighting and scoring the potential of qPCR approaches for quantifying functional gene abundances of relevance to providing simple metrics relevant for quantification of biogeochemical fluxes (which are difficult to quantify directly).

The paper is generally well written, interesting, and delivers on synthesising the current broad status with respect to these issues, and additionally proposes some potentially new indicator approaches which could be implemented. As such, I feel it makes a useful contribution. The paper could be improved by offering more critical analyses of the approaches; as well as authoritatively defining the new science needed to facilitate the implementation of more robust soil microbiological indicators.

We especially added further aspects and discussions on soil microbial methods, which also relate to the points raised by reviewer # 2, E. Hannula.

Three areas which could be elaborated on further I feel are highlighted below. Perhaps fully covering them in detail extends beyond the remit of this manuscript, so I leave it to the editor to decide whether they should be expanded upon in the article (alternatively I guess these publically viewable comments may constitute a contribution to the "discussion" format of the journal. . ..).

1. Indicator targets within the global soil geographic context. The paper briefly mentions this on line 365 ("methods need to be implemented into a framework, which takes into account site-specific conditions"), but offers no specific ways forward for this critical issue. Are elevated abundances of a functional indicator always "desirable", and how might indicator target values, and indeed the indicators themselves differ for different soil systems? I'm not sure if we even have a good soil classification system or framework that allows us to set regionalised targets for the simple variable of soil carbon, and I sense this is what causes pushback on soil targets from industry and policymakers. Given this, could proposing even more microbiological variables be deemed somewhat premature?

This is a very good point and emphasizes the need to gather a database of soil microbial parameters in worldwide soils (with links to soil chemical, physical and pedoclimatic data) in order to get some idea about 'normal' value ranges. We fully agree that 'higher' values do not necessarily mean 'better' and such typical value ranges are needed to identify unusual aberrations. However, this fundamental discussion would lead a bit beyond the scope of this paper, which is focused on the methods that are required to receive the results, independent from how we store and assess these results. Consequently and pointing into the direction raised by R. Griffiths we added on lines 397-399: "Undoubtedly, this requires further joint efforts in order to generate comprehensive databases from which normal operating ranges of values for a given proxy can be read. Such a task calls for standardized methods to obtain comparable data".

2. Relatedly, what is the evidence that gene abundance relates to functions of relevance to ecosystem services? It is often stated that you cannot infer anything about processes from gene abundance alone, but I feel there is little literature actually specifically addressing this with robust contrasts within an ES indicator context. For example comparative data for ammonia oxidation gene abundances does actually appear to relate to nitrification rates in certain studies, so do we need a critical meta-analyses of this now for a variety of indicators? Again, relating to the point above, do we always want high nitrification, high litter decomposition, high enzyme activity etc in all soil systems; and is there any evidence that molecular detection of elevated pathogens reliably informs on plant health...Essentially what do these measures really tell us about desirable ES outcomes, and if there is little information available, then what can be done to progress?

We share the view of the reviewer that we are only at the beginning to understand the meaning of microbial activity and functional gene abundance in terms of ecosystem services and functioning of soils. The following phrases of the added text mostly apply to this comment.
250-256 "Also, evidence is increasing that functional gene abundance and community structure are closely linked to related microbial activities and their increase or decrease, e.g. through agricultural fertilizer regime or soil contamination (Levy-Booth et al., 2014; Ouyang et al., 2018; Xue et al., 2018). However, also contrasting findings have been reported, pointing to the fact that functional gene abundance and diversity is less affected by short-term changes, e.g. due to soil moisture changes (Zhang et al., 2019). A critical meta-analysis of existing data and reports, respectively, would be timely to better identify and generalize the linkage of functional gene abundance and ecosystem services."
402-408: "Here the use of DNA based methods, which provide a measure of a microbial community's potential to perform a given process, might be more useful than using RNA. The RNA rather indicates actual activities, which may highly fluctuate in time and space, and thus are of less significance as an indicator. However, free DNA released from dead microbes is often highly resistant in soil, which might result in an over estimation of a potential function. This needs to be taken into account when interpreting the data. Recently, methods that extract DNA only from living cells have been described, but their use has not been yet introduced into recent standardization activities."

3. Standardisation: essential for policy, but bad for science? Given the paper's policy focus, it appears to heavily endorse standardisation. However molecular ecology is a rapidly growing field, and technologies change (eg sequencing platforms) which causes issues with implementing standardised protocols. Scientific developments must be free to progress in order to develop the deep and often complex understanding of processes required to implement meaningful process indicators. It would be useful to highlight this potentially conflicting issue.

We agree that standardization is a balancing act. We aim to receive largely comparable data (asking for defined methods) but want to use the latest methods for that purpose (asking for highest flexibility). So we added on lines 429-436: "Lastly, it must be noted that standardization of methods is inevitably a balancing act. On one hand, standardization provides defined methods that are essential to obtain comparable data, e.g. for integration in large, joint databases. On the other hand, it requires setting a specific method for several years. Consequently, scientific progress cannot be easily adopted, or at least with a delay, considering that standards are revised every five years, which may be a barrier to the introduction of new approaches

resulting from technological evolution, especially in the fast developing field of molecular biology methods. Hence, it is also the aim of this paper to have an open discussion to identify the best suitable methods with an assumed longer period of validity."

Reviewer #2 Emilia Hannula
The topic of relating currently available measurement techniques to the soil functions is a very timely issue. Using logical sieve approach, this article investigates the suitability of commonly used methods to evaluate the soil functions and ecosystem services, one at the time. The paper is very clearly written and presents solid, interesting research. It could benefit from section on future directions and more updated list of currently available (molecular) techniques. Will these be better or do we already have a golden standard that will tell us all we want to know?

For sure we don't have the golden standard (otherwise new method development would be obsolete). We added further text and discussion especially on soil molecular biology methods and parameters.
200-210: metagenomics for degrading activities.
218-226: Problems with data handling and bioinformatics, respectively.
250-256: Discussion on linkage between functional gene abundance and function.

It is clear that the work has started already in 2013 and in the past six years huge developments have been achieved in the toolbox available to measure soil functions and especially diversity. Authors mention the 'Earth microbiome project' and standardization of primer sets and pipelines to study bacterial diversity as an emerging technique. However, in reality, this method is the most used method to study fungi and bacteria in soils and is considered fairly standard as all labs use the same regions (V4 and ITS2) and often same primers. There is a recent article on the methodological comparison on bacteria (Ramirez et al. 2018, Detecting macroecological patterns in bacterial communities across independent studies of global soils, Nature microbiology). There is much discussion on using 'mock' (not MOC)-communities to standardize the methods and most labs use these already.

Discussion on barcoding using high throughput sequencing and in special the use of primer pairs targeting the V4 region of the 16S rRNA gene and ITS2 region for bacterial and fungal barcoding was added to the text. Also we evaluate the suitability of bioinformatics pipelines for cross comparison. (Lines 157-167).

Furthermore, the field is moving towards true (shotgun) metagenomics sequencing which yields data on all soil organisms and their functions. This approach is emerging but will definitely be worth discussing in this context. It will have no bias of PCR but the quantity of soil used and DNA extraction efficiency related issues will remain. In short, the 'newer' methods should be discussed. For the methods used to study diversity and future avenues in that field, following paper can be cited (Geisen et al. 2019, A methodological framework to embrace soil biodiversity, SBB).

Metagenomics are further discussed now on lines 218-226. We thank for the helpful literature reference that was included in the new added text.

In the abstract, it is mentioned that especially fungal functions are difficult to measure. The article presents a suite of measures traditionally used and recommends to use enzyme production to measure fungal functions. For bacteria, qPCR based methods are recommended. This discrepancy in recommendations should be discussed. Why is it not feasible in all case to look at process rates (i.e. decomposition), but details on the amount of enzymes and/or amount of organisms performing the task are needed? In which scenarios and which scales which measurement is needed? Considering this would make the article stronger and bring more to the field. In the evaluation of function 6 (carbon cycling) it is simply stated that because these enzymes are often produced by other organisms, molecular methods are less developed. This is partly true but can be related also that the enzyme measurements are pretty good and give the actual rate of enzyme measured. Furthermore, there are existing primer sets for quite some of the CAZys (for example: Edwards et al. 2011: Simulated Atmospheric N Deposition Alters Fungal Community Composition and Suppresses Ligninolytic Gene Expression in a Northern Hardwood Forest, PlosOne Gorfer et al. 2011: Community profiling and gene expression of fungal assimilatory nitrate reductases in agricultural soil, ISMEJ Chen et al. 2013: Comparative analysis of basidiomycetous laccase genes in forest soils reveals differences at the cDNA and DNA levels. Plant and Soil Hannula & van Veen (2016) Primer Sets Developed for Functional Genes Reveal Shifts in Functionality of Fungal Community in Soils. Frontiers in Microbiology These measurements from DNA have problems as different fungi have different copy numbers/ types of genes and not everything that is in the DNA is expressed. Indeed, work is needed to get these methods ISO certified but more discussion on the future directions would be welcome.

Fungi: Information on methods targeted towards fungi was added and the recommended literature included. Fct. 6. The specific problem of linking copy numbers of fungal genes to the size of a functional population was emphasized (lines 291-297).

Decomposition rates: We present and propose molecular methods (being an indirect measure of activities, rather looking on the organism side) but also on activities such as the litter bag and the tea bag method, thus targeting much more the effect point of view (see Table 3). We also added new text on the discussion in how far functional gene abundance can serve as a measure of true soil microbial functioning (lines 250-256).

Scenarios and scales: We agree that a bundle of methods is proposed with differences regarding application range and purpose (despite the function represented). To discuss this, would lead much deeper into the criteria (Table 1) d) sensitivity (with effect of soil properties, of land use and of disturbance on the test result), e) selectivity, and g) use as an indicator. We feel that this topic is surely relevant but would require too much detail information and discussion, making the manuscript too voluminous. We decided to leave the manuscript more concise and hope to find the reviewers' consent for that.

Identification of new microbial functional standards for soil quality assessment

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Abstract. The activity of microorganisms in soil is important for a robust functioning of soil and related ecosystem services. Hence, there is a necessity to identify the composition, diversity and function of the soil microbiome ~~indigenous soil microbial community for its functional properties using soil microbiological methods~~ in order to determine its ~~the~~ natural properties, functioning and operating range ~~of soil microbial communities, and~~ as well as to assess ecotoxicological effects due to anthropogenic activities. Numerous microbiological methods currently exist in the literature and new, more advanced methods continue to be developed; however, only a limited number of these methods are standardized. Consequently, there is a need to identify the most promising non-standardized methods for assessing soil quality and ~~develop~~ to transform them~~these~~ into standards. In ~~alignment~~ agreement with the "Ecosystem Service Approach", new methods should focus more on soil microbial functions, including nutrient cycling and greenhouse gas emission, pest control and plant growth promotion, carbon cycling and sequestration, ~~greenhouse gas emission, and~~as well as soil structure development and filter function. The few existing~~,~~ ~~function-related standard~~standardized methods available that focus on the function of the soil microbiome mostly include measurements, like basal respiration, enzyme activities, and biodegradation of organic matter, under well-defined conditions in the lab~~, and include~~focus on the estimation of microbial biomass, basal respiration, enzyme activities ~~related to nutrient cycling, and organic chemical biodegradation of organic chemicals~~. This paper sets out to summarize and expand on recent discussions within the International Organization for Standardization (ISO), Soil Quality - Biological Characterization sub-committee (ISO TC 190/SC 4) where a need was identified to develop scientifically sound methods, which would best fulfil the practical needs of future users for assessing soil quality, going beyond the existing test systems. Of particular note ~~was~~ is the current evolution of molecular methods in microbial ecology that use~~s~~ quantitative real time PCR (qPCR) to produce a large number of new functional endpoints ~~and~~ which are more sensitive as compared to 'classical' methods. Quantitative PCR assesses the abundance~~activity~~ of microbe~~sial genes~~ that ~~code for enzymes that~~ catalyse major transformation steps in nitrogen and phosphorus cycling, greenhouse gas emissions, chemical transformations including pesticide degradation, and plant growth promotion pathways based on the assessment of marker gene sequences that drive the related processes. In the assessment of soil quality methods, it was found that most methods focus on bacteria and related endpoints. Techniques to describe fungal communities as well as their functional traits are far less represented~~methods were significantly underrepresented~~. As such, techniques to analyse fungal enzyme activities are proposed. Additionally, methods for the determination of microbial growth rates and efficiencies, including the use of glomalin as a biochemical marker for soil aggregation, are discussed. Furthermore, field methods indicative of carbon turnover, including the litter bag test and a modification to the tea bag test, are presented.

However, it is obvious, that with increasing developments in high throughput sequencing technologies and big data analyses, including metagenomics analysis, it will be possible to implement these technologies into~~in~~ the standardisation process for assessing the functions of the soil microbiome. ~~As a final note~~, ~~Overall~~Overall, it is suggested that endpoints should represent a potential function of soil microorganisms rather than actual activity levels, as the latter can largely be dependent on short-term variable soil properties such as pedoclimatic conditions, nutrient availability, and anthropogenic soil cultivation activities.

1 Introduction

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

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

Despite the fundamental methodological advances over the past years, which allow for an in-depth analysis of microbiomes and, to some extent, other soil-living organisms (e.g. Joergensen and Emmerling, 2006; Paul, 2015; Yates et al., 2016), only a limited number of soil biological methods have been standardized (for details see section 3). As a result, large and often significant deviations are observed in the results obtained ~~between~~when non-standardized method~~s protocols~~ are used (e.g. Strickland and Rousk, 2010). This is especially true for methods that are based on high throughput sequencing approaches, where variability and bias in data can occur from the "wet-lab" steps right through the various bioinformatics pipeline analysis steps (Quince et al., 2017). Therefore, the comparability between datasets generated by different laboratories using different methods or modified protocols of the same method ~~are~~is problematic. Consequently, the development of quality indices and threshold

values, respectively, for assessing soil quality is nearly impossible (Bastida et al., 2008). Presumably, this is why the number of meta-analyses in soil biology remains small.

Given that there is a lack of harmonization between existing methods and, at the same time, a proliferation of new methods, there is a need to identify the most promising methods described in the literature that can be standardized to produce reliable indicators for soil quality (e.g. Philippot et al., 2012). At the Annual International Organization for Standardization (ISO) meeting of TC 190 (Soil Quality) in Fukuoka, Japan in October 2013, a decision was made to compile a list of available methods and to identify those that would be suitable for assessing soil quality. Additionally, during a subsequent meeting of ISO TC 190/SC 4/WG 4 (Microbiological Methods) held in Paris, France in March 2014, further discussions focused the criteria for suitable methods to be comprised of microbial functional indicators. In this paper, we summarize the major outcomes of the discussions which took place over the past several years within ISO TC 190/SC 4. Therefore, besides collating a list of criteria for the selection of test methods for the future analysis of microbial functions in soil, the aim of this paper is to present our opinion, as members of the ISO TC 190 committee, to initiate further discussion on possible methods that should be standardized for future soil quality assessments.

2 Criteria for the selection of methods

Several papers addressing the task to identify suitable methods to be used as biotic indicators (usually including faunal indicators) were published in the last few years, mainly in the context of EU research projects (e.g. Bispo et al., 2009; Faber et al., 2013; Ritz et al., 2009; Römbke et al., 2010). Here, we propose to base the selection of soil quality methods more on the "Ecosystem Service Approach" (MEA 2005) which is increasingly recognized by both environmental scientists and regulatory agencies (Breure et al., 2012; Galic et al., 2012) and which takes into account that ~~that~~ soils have been raised to the rank of a natural resource to be protected. As a consequence, and in addition to method development and application (including the assessment of biodiversity as a prerequisite for soil function), the focus of future activities should be the determination of soil microbial function as recommended endpoints (Kvas et al., 2017; Nienstedt et al., 2012; van der Putten et al., 2010; TEEB, 2010). Consequently, we propose to assess both existing and new methods for the selection of microbial functional tests that support various soil ecosystem services. This structures our approach and simplifies the identification of ecologically relevant methods, as well as, presumably increases~~ing~~ their acceptance by users, including the regulatory and stakeholder community. The following **soil functions~~Functions~~** and **ecosystem services** have been

defined and are proposed to be used as a starting point for the development of future methods (MEA, 2005; Ockleford et al., 2017):

(1) Biodiversity, genetic resources, cultural services;

(2) Food web support;

(3) Biodegradation of pollutants;

(4) Nutrient cycling (for example N and P);

(5) Pest control and plant growth promotion;

(6) Carbon cycling and sequestration;

(7) Greenhouse gas emissions; and

(8) Soil structure affecting soil water, gas balance and filtration function.

A second major criterion for selecting methods for standardization is its usability. The method should be applicable in regulations (e.g. European and National agencies registering chemicals or products) and for the evaluation of soil ecology and functioning as ~~a~~ fundamental aspects of soil quality (e.g. by stakeholders and researchers). Moreover, the routine use of methods to inform farmers and site owners on soil quality as continuous assessments of their land and land-use practises~~,~~ could be an additional condition that would require the choice of easy-to-use methods or possibly encourage the simplification of existing methods. Overall ~~F~~frequently used methods generate more data, which in turn is of high importance for the validation of threshold values. Therefore, the aim of this process is to identify methods that are scientifically sound and best fulfil the practical needs of future users. The most appropriate new methods, including those proposed in this article, need to be evaluated using the criteria required for the standardization of ISO methods.

To assess possible methods, a list of criteria was used based on the 'logical sieve' approach (Ritz et al., 2009). The list of criteria for the identification of functional indicators and associated methodologies (Table 1) was an outcome of the EU FP7 EcoFINDERS project (Faber et al., 2013). The criteria were compiled after sending a questionnaire to 25 partner institutions primarily working in the field of environmental science; mainly representing academia but also regulators and subcontracting laboratories. These criteria are applicable for different kinds of indicators and methods, including those addressing the functions of soil microbial communities. In the following sections, we assume that existing ISO standardized methods partly already fulfil these criteria, but not all relevant endpoints can be measured. ~~. Additionally, the most appropriate new methods, including those proposed in this article, need to be evaluated using the same criteria required for the standardization of ISO~~

~~methods. Therefore, the aim of this whole process is to identify methods which are scientifically sound and that best fulfil the practical needs of future users.~~

3 Existing and new methods

Current methods that have already been implemented as ISO standards are found in Table 2, whereas methods that might be considered for future standardization are in Table 3. The compilation in Table 2 comprises methods to quantify microbial biomass (e.g. through fumigation extraction of microbial biomass carbon (MBC) and DNA) (**Function 6**) as well as for (further) analysis of structural microbial diversity (e.g. determination of microbial fingerprints by phospholipid fatty acids (PLFA) analysis) (**Function 1**). Additionally, microbial biomass, measured as respiratory activity, has been included in Table 2, although not directly linked to one of the ecosystem services, as it provides important information on the activity of the complete microbiome (i.e. microflora and microfauna). Soil basal respiration normalized to MBC (ISO 14240-1 and ISO 14240-2, 1997, Table 2) yields the metabolic quotient qCO_2, which is a sensitive indicator for microbial carbon use efficiency (Anderson and Domsch, 1993). ~~Interestingly,~~However, its use as an endpoint to assess anthropogenic and natural impacts on the soil microbiome has been controversially discussed in literature (Wardle and Ghani, 1995). The microbial quotient (MBC related to organic carbon content of a soil) is an indicator revealing changes in the microbial dynamic equilibrium of soils in response to exposure to natural or anthropogenic stressors (Pankhurst et al., 2001).

The biodiversity function (**Function 1**) addresses parameters related to the structural diversity of the soil microbiome. Here, respective ISO guidelines analysing PLFA, phospholipid ether lipids (PLEL) (ISO/TS 29843-1, 2010; ISO/TS 29843-2, 2011) and DNA (ISO 11063, 2012; ISO 17601, 2016), have already been well implemented into guidelines (Table 2). In addition, high throughput sequencing of barcodes of the ribosomal operon (16S rRNA gene for bacteria and archaea and ITS [internal transcribed spacer] region for fungi) have generated a large amount of data (Schöler et al., 2017). These approaches have been also used successfully for other microbial groups like protists (using the 18S rRNA gene as a target). As it is well accepted that the use of different primer pairs introduce different biases (Ramirez et al., 2018), standards have been recommended by international initiatives. For example, the Earth Microbiome project (www.earthmicrobiome.org) recommended a primer pair targeting the V4 region of the 16S rRNA gene and ITS2 region for bacterial and fungal barcoding, respectively. Bioinformatics pipelines used for barcoding approaches are already well standardized and shared worldwide among the scientists, which makes possible the cross comparison of various datasets from different

labs. Unfortunately, this is not yet the case for the pipelines to analyse metagenomics datasets that are still under constant evolution making difficult cross-comparisons.

Although microbial diversity, per se, is not strongly correlated with a particular functional capacity, it is clear that the loss of diversity can have an impact on microbial function (Thiele-Bruhn et al., 2012); at least for relatively specific functions performed by narrow microbial guilds or taxa. This applies even more, when certain taxa are closely linked to very specific functions including nitrifiers, methanogens, arbuscular- and ecto-mycorrhizal fungi, and biocontrol microorganisms like *Trichoderma* (e.g. Hartmann et al., 2009; Hayat et al., 2010; Lugtenberg and Kamilova, 2009; Peng et al., 2008; Singh et al., 2007; Xia et al., 2011). Therefore, the interpretation of the outcomes from microbial community-based testing tends to be straightforward and closely linked to **Function 4** and **Function 5**.

Food web support (**Function 2**) of higher trophic levels no doubt starts from soil microorganisms and propagates through the trophic levels (e.g. earthworms) that are consumed by birds and mammals (Haynes, 2014; Scheu et al., 2002; Scheu et al., 2005). However, the role of the microbiota in the soil food web is not fully understood, since many eukaryotic organisms can be considered as meta-organisms, which carry their "own microbiome" that itself is essential for life supporting functions. From this, it is unclear if environmental microbiomes and host specific microbiomes complement one another. So far there are no comprehensive methods (especially not those addressing microbial functions) or standards available to ~~assess~~ address this problem. ~~A future considering may employ t~~The use of stable isotope labelling of select carbon or nutrient sources as a promising approach to follow food webs and degradation pathways might provide one possibility in the future to assess food webs in soil. This would be accomplished by combining carbon and nitrogen stable isotope fluxes determination with phylogenetic analysis of the microorganisms labelled with the stable isotopes (e.g. Coban et al., 2015; Traugott et al., 2013; Lueders et al., 2004).

Methods to assess the biodegradation of pollutants (**Function 3**), as described above, are already implemented into ISO guidelines (Table 2) and are part of legal frameworks including pesticide directives (EU Regulation1107/2009/EC; European Commission, 2009). A number of standard methods for the determination of the potential of soils to degrade organic chemicals (**Function 3**) under both aerobic (ISO 14239, 2017) and anaerobic (ISO 15473, 2002) conditions are available. ~~This emphasizes that~~However, in the past, the development of standard methods was mainly driven by the need to assess the ecotoxicological effects of anthropogenic activities, such as chemical contamination of soils, rather than to describe and understand the natural properties and functions of soils. ~~However, d~~Defining methods for the determination of adverse effects of contaminants on

soil biota was not only ~~being~~ done by~~in~~ ISO, but it was also a major task of other organizations such as the Organization for Economic Co-Operation and Development OECD). For example, there are OECD guidelines, tests No. 216 and 217, for testing the long-term effects of single exposure chemicals on soil microbial nitrogen and carbon transformation, respectively (OECD, 2000a; 2000b). As a result, it was decided early that the standardization of methods for toxicity testing sh~~w~~ould not be the primary aim of the ISO sub-committee (ISO TC 190/SC 4). Metagenomics lead to the rapid discovery of new genes, which catalyse degradation processes of xenobiotics and, consequently, offer new insight in the study of ecology of microbial degraders. The transfer of this knowledge into operational bioindicators for the estimation of soil filtration capabilities (e.g. by using qPCR or RT-qPCR assays to quantify those genes and their expression in soils, respectively) will be one of the major tasks in soil science in the upcoming years. Jeffries et al. (2018) for example were able to successfully predict the degradation rates of organophosphorus based pesticides in a contaminated soil by using metagenomics based functional profiling.

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

The current evolution of molecular methods in microbial ecology has resulted in a large number of new endpoints. It is well known that many of the new endpoints (e.g. using quantitative real-time PCR (qPCR)) are more sensitive than classical methods that had been standardized in the past (Ribbons et al., 2016; Schulz et al., 2016). This new metagenomics approach will be of high importance in the future, as it allows for the implementation of information on new functional traits that can be standardized into an analytical pipeline. Direct sequencing of soil DNA extracts, called metagenomics, is nowadays~~-~~ a method that can easily be implemented to obtain an overview of in-soil living organisms, including microorganisms. It is recognized that metagenomics is no longer limited by sequencing possibilities but more by the availabilities of large-scale computing clusters to analyse the amount of data generated. The future~~s~~ success of metagenomics in soil surveys will mainly depend~~s~~ on the possibilities to standardize bioinformatics pipelines as well as on the availability of tools for big data analysis and artificial intelligence. However, it must be mentioned that even a well~~-~~standardized and automated

workflow will generate only data on the relative abundance of nucleotide sequences and not absolute values (Geisen et al., 2019). Thus at the moment for the assessment of new methods linked to **Functions 4 to 8**, qPCR from soil DNA extracts (ISO 17601, 2016) plays a very important role in determining the abundance of single marker gene sequences, which are indicative of specific transformation processes or soil functions. For example, the quantification of nitrogen fixing microbes, nitrifiers and denitrifiers has been successfully implemented using the *nifH*, *amoA* and *nirS/nirK* genes as markers, respectively (Henry et al., 2004; Hirsch et al., 2010; Ollivier et al., 2010; Sessitsch et al., 2006). Similarly, the quantification of microorganisms involved in the β-ketoadipate pathway has been implemented by targeting *pcaH* (El Azhari et al., 2008) and *catA* (El Azhari et al., 2010) gene sequences. Various methods for the assessment of soil microbial **Function 4** (nutrient cycling), **Function 5** (pest control and plant growth promotion) and **Function 7** (greenhouse gas emissions) are proposed based on the qPCR analysis of gene sequences coding for enzymes which trigger the respective function (e.g. Fish et al., 2013; Ribbons et al., 2016; Smith and Osborn, 2009). Additionally, it should be noted that molecular methods based on the assessment of specific marker genes for estimating the degradation potential in soil have already been proposed both for PAHs (e.g. Cebron et al., 2008) and individual pesticides (e.g. Martin-Laurent et al., 2004). These could be interesting for future standardization; however, if a method is very compound-specific and targeted, this could limit its application range. Thus, these specific approaches will not be discussed further in this article.

Major advantages of qPCR assays to quantify gene sequence numbers, which can be used as proxies for a given microbial process, are that they are: (i) highly standardized, sensitive, selective and reproducible, (ii) designed for high throughput analysis, (iii) available for a wide range of targets, and (iv) methods that are relatively cheap once the necessary analytical devices are on hand. Some training on the method is required, however, once trained the assays are easy to perform. For example, numerous studies have already used the microbial functional genes involved in nitrogen cycling to determine the status and to assess induced changes in the soil microbial community (Levy-Booth et al., 2014; Nannipieri and Eldor, 2009; Wallenstein et al., 2006). Consequently, the number of functional genes that are suited for use as specific indicators of soil function are continuing to grow in the literature as researcher gain experience in this field and data becomes more prevalent. Also, evidence is increasing that functional gene abundance and community structure are closely linked to related microbial activities and their increase or decrease, e.g. through agricultural fertilizer regime or soil contamination (Levy-Booth et al., 2014; Ouyang et al., 2018; Xue et al., 2018). However, also contrasting findings have been reported, pointing to the fact that functional gene abundance and diversity is less affected by short-term changes, e.g. due to

soil moisture changes (Zhang et al., 2019). A critical meta-analysis of existing data and reports, respectively, would be timely to better identify and generalize the linkage of functional gene abundance and ecosystem services.

Disadvantages, on the other hand, are that: (i) the quality of qPCR data depends on soil DNA extracts (PCR inhibition), (ii) primer pairs, even degenerated ones, might not successfully amplify all microbes of the functional group of interest, (iii) only genetic potential is resolved, and (iv) there is no differentiation between active, dormant or dead microorganisms, when working with DNA as a template for the qPCR reaction. The analysis of total RNA and of mRNA, which could help to overcome the latter problem, is currently not a suitable alternative as it is highly dynamic in time and space and needs special care to stabilize the RNA extracted from complex environmental matrices to avoid its degradation. Another problem of DNA analysis is the biological representativeness of the results is solely based on a relatively small amount of soil (from few hundred mg to ten g of soil) from which the DNA extracted. The use of small soil samples (< 1 g) simplifies the sample preparation process for molecular biologists; however, it provides a poor representation of the indigenous soil microbial community in the naturally inhomogeneous soil. Typically, the α-biodiversity declines with sample size while that of ß-biodiversity increases (Nicol et al., 2003; Penton et al., 2016). Lastly, it must be noted that the high repeatability and reproducibility of molecular biology methods, including qPCR assays, depends on extraction, purification and amplification of DNA or RNA. This is typically performed using commercial extraction kits; however, by simply changing the commercial supplier of a kit can substantially change the results (Brooks et al., 2015; Feinstein et al., 2009). This clearly challenges standardization since standard methods must not hinge on a specific supplier.

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

Several options exist for (additional) standardized methods to test **Function 6** (carbon cycling and sequestration) (Table 3). For **Function 6**, there is a need to implement more fungal activity analysis as most tests

295 described ~~this far only~~mostly assess bacterial activities. Thus, the integration of more fungal enzyme activities into
296 the suite of standardized methods for soil quality assessment is essential (for example determining the turnover of
297 complex natural compounds such as lignin) (Baldrian, 2006). The ligninolytic enzymes laccase and Mn-
298 peroxidase, as well as the chitin degrading 1,4-α-*N*-acetylglucosaminidase, are typical fungal enzymes of interest
299 for ecosystem services (Jiang et al., 2014; Šnajdr et al., 2008). However, since other organisms also produce these
300 enzymes, including bacteria and plants (Bollag, 1992; de Gonzalo et al., 2016), current methods do not specifically
301 target fungal enzyme activities. As a result, the implementation of molecular methods for assessing fungal
302 communities are far less developed than those for bacterial communities (Table 3). First approaches to close this
303 gap have been made in recent years. For example, molecular systems to detect genes coding for laccases and other
304 ligninolytic enzymes as well as other fungal activities in carbon cycling have been published and applied (Edwards
305 et al., 2011; Chen et al., 2013; Hannula and van Veen, 2016). Also for genes involved in the fungal nitrogen
306 turnover, primer pairs have been successfully developed (Gorfer et al., 2011). However here, even more than for
307 bacteria, it is critical to link copy numbers of genes directly to the size of the respective functional population, as
308 in many cases one organism can harbour multiple operons coding for the same genes involved in a given function.
309
310 The method of community level physiological profiling (CLPP) using the Biolog™ system (Biolog,
311 Hayward CA, USA) was first developed in the late 1980s to identify bacteria of clinical importance by assessing
312 the consumption of 95 different carbon sources in a microtiter plate. The technique was then extended to identify
313 bacterial strains from environmental mixed microbial communities samples using select carbon sources (Garland,
314 1997). Currently, the technique is frequently used to assess the effects of contaminants on soil microbial activity
315 (Bloem and Breure, 2003; Schmitt et al., 2004). As such, the CLPP method has become a measure of microbial
316 functional diversity in soil (e.g. Gomez et al., 2006) and was used to distinguish the biodiversity of soil microbial
317 communities in monitoring programs (Rutgers et al., 2016). Even though the method is easy to use, it does have
318 some drawbacks (Winding and Hendriksen, 2007). The technique is based on the utilisation of select carbon
319 sources, which when consumed result in reduction, and thus colour change, of a tetrazolium indicator dye (Garland
320 and Mills, 1991). This reaction is based on the dehydrogenase enzyme activity of cultivable, fast growing, aerobic,
321 eutrophic microorganisms (largely bacteria). Consequently, this technique does not reflect the full spectrum of
322 microbial species within a mixed soil community. Additionally, due to the artificial growth conditions required in
323 the test, it is argued that the method does not reflect the microbial community diversity and its function of a given
324 soil (Glimm et al., 1997). On the other hand, however, standardized conditions allows for direct comparisons

between microbial communities in different sites, for example, independent of the abiotic conditions, thus making CLPP a popular method for toxicology testing (Preston-Mafham et al., 2002).

Isothermal micro-calorimetry is another technique that involves the direct measurement of energetics in soil and provides a functional link between energy flow and the composition of belowground microbial communities at a high taxonomic level (Herrmann et al., 2014). With this method, an integrative determination of the metabolic activity of soil bacteria and fungi is achieved. The integrated assessment of substances' and energy turnover has high potential to elucidate the regulation of soil ecological functions. However, the substantial costs for the acquisition of this very specific instrumentation is considered as a major drawback. Furthermore, the measurement requires water saturation of the soil and, thus, the samples are modified. Since calorimetry has been rarely used and data and publications are few, this method is considered not ready for standardization.

The methods targeting thymidine or leucine incorporation into microbial biomass can be used to determine microbial growth rates and efficiencies (Bååth et al., 2001; Rousk, 2016). Growth rate is a fundamental reference for numerous other microbial properties and functions. For example, it is required to calculate microbial carbon use efficiency (CUE) as a key-parameter describing C-substrate turnover and storage in soil (Liu et al., 2018; Spohn et al., 2016; Takriti et al., 2018). Furthermore, the method can be used to assess the adverse effects of toxic chemicals on the microbial community (Modrzyński et al., 2016; Rousk et al., 2009a). The drawbacks of these two methods are: (i) specific training is required, (ii) laboratories must have a permit to manipulate radioactive isotopes, and (iii) there are higher costs for proper handling and disposal of ^{3}H-labelled radioactive material. As an alternative, the incorporation of the stable isotope ^{18}O from labelled water into soil microbial DNA can be used to distinguish growing and non-growing microorganisms based on the gradient-separation of [^{18}O]DNA and [^{16}O]DNA (Schwartz, 2007). The ^{18}O stable isotope method has been improved by sequencing a marker gene from fractions retrieved from ultracentrifugation to produce taxon density curves; thus enabling researchers to estimate the percent isotope composition of each microbial taxon's genome (Schwartz et al., 2016). This method continues to be advanced and, although not used often, could have a high potential for future standardization.

There are simplistic methods available to determine organic matter decomposition, which are indicative of C cycling (**Function 6**). The tests listed in Table 3 are based on measuring the weight loss of introduced organic materials of different complexity in soil over time. The tests are relatively easy to perform and inexpensive, however, degradation activity is not exclusive to microorganisms but can also include invertebrates. The OECD litter bag test (OECD, 2006) for site specific assessment of organic matter decomposition uses wheat straw as the

substrate and provides clear evidence of cellulose degradation. In general, the litter bag tests provides evidence for the degradation of naturally occurring plant material in soil. Results do, however, depend on the mesh size of the litter bags (increasing exclusion of soil animals with decreasing mesh size). On the other hand, plant material or litter is hard to standardise with the results largely depending on the composition of the plant material. As such, artificial cellulose has been successfully used for a laboratory procedure to assess organic matter decomposition (Kvas et al., 2017). Another alternative to the litter bag test is the use of tea bags (Keuskamp et al., 2013). Tea bags can be purchased to contain a consistent quality of material, and so this method is preferred by citizen science (e.g. farmers to assess the soil quality of their land). In order to better distinguish the degrading abilities of different soil microbiomes, the test could be modified to use different types of tea that contain recalcitrant material to a different extent. Another test for future method development is the Bait Lamina test (ISO 18311, 2016) used to assess the degradation of organic matter in field soil by grazing invertebrates (Jänsch et al., 2013; Kvas et al., 2017). It is a simple test that can easily be adapted for use under controlled laboratory conditions (Jänsch et al., 2017).

Methods for the determination and assessment of greenhouse gas emissions from soil (**Function 7**) have already been standardized or are well advanced in the standardization process (Table 2). They are mostly focused on measuring concentrations of greenhouse gases, like CO_2, CH_4 and N_2O, as well as their fluxes as endpoints. In addition, molecular biology methods that estimate the relative abundance of functional microbial guilds or taxa gives new insight into the ecology of microorganisms involved in the formation of greenhouse gases. For example, the qPCR measurement of key N_2O functional genes has allowed researchers to link N_2O reduction capacity to reduced greenhouse gas emissions in soil amended with organic matter (Xu et al., 2018). Additionally, the quantification of functional gene sequences related to methane generation and methane oxidation, respectively, yields detailed insights into the functional potential of climate change-affected permafrost soils (Yergeau et al., 2010).

For **Function 8** (soil structure affecting soil water, gas balance and filtration function), there is clear evidence that microbial activity and biomolecules substantially contribute to the formation and stability of micro-aggregates, and thus to the structure, pore system and pre-consolidation stress of soils (Six et al., 2004). While existing parameters, such as enzyme activities, are not clearly indicative in this regard (Beck and Beck, 2000), glomalin can be considered as a biochemical marker of soil aggregation. This glycoprotein is produced by microorganisms, especially arbuscular mycorrhiza fungi, and significantly increases aggregate formation and stability (Rillig, 2004; Rillig and Mummey, 2006). The existing protocols for extraction (chemical extraction

385 combined with autoclaving) and determination of glomalin, either by using the Bradford protein assay, enzyme-
386 linked immunosorbent assay (ELISA), or LC-MS method (Bolliger et al., 2008; Janos et al., 2008), open the
387 possibility for its standardization in the near future. It should be noted, however, that a well-equipped and
388 experienced laboratory is required to perform this method.
389
390 **4 Transforming standardized methods into indicators of soil quality**
391 As recently underlined by the European Food Safety Agency (EFSA) in a scientific opinion 'addressing the state
392 of the science on risk assessment of plant protection products for in-soil organisms', there is an urgent need to
393 modernize pesticide risk assessment by implementing specific protection goals for in-soil organisms which are
394 key drivers of a wide range of functions supporting ecosystem services (Ockleford et al., 2017). There currently
395 exists a multitude of methods that can potentially be used for this task. Here, we have identified in the body of this
396 paper a number of methods that are presumably suitable for further evaluation and standardization with regard to
397 their scientific value and practical applicability. These prospective standardized methods will not only be useful
398 to identify adverse effects on the soil microbiome, but also to conduct comparable studies in laboratories all over
399 the world to define normal operating ranges of microbial activity in soil and respective quality indices and
400 threshold values.
401 It is clear that all parameters taken together reflect the potential of a microbial community to perform a
402 certain function and not solely a specific (actual) activity. This is important to understand in order to interpret the
403 values of a given endpoint in relation to both energy fluxes and compound transformation rates, which can largely
404 depend on intrinsic properties such as pedoclimatic conditions, and nutrient availability as well as extrinsic
405 properties such as anthropogenic effects, and soil cultivation measures. To make use of these methods as indicators
406 for soil quality, there are several requirements that need to be included. These involves the assessment of the
407 normal operating range of soil that include natural and dynamic fluctuations of a given endpoint. The methods
408 need to be implemented into a framework, which takes into account site-specific conditions including soil type,
409 pedoclimate and land-use. Undoubtedly, this requires further joint efforts in order to generate comprehensive
410 databases from which normal operating ranges of values for a given proxy can be read. Such a task calls for
411 standardized methods to obtain comparable data. Additionally, there is a requirement for the assessment of
412 resistance and resilience of a given microbial endpoint to see how much it is affected by a soil disturbance and
413 whether or not it can recover (e.g. return to its original state) after the disturbance has disappeared. Here the use
414 of DNA based methods, which provide a measure of a microbial community's potential to perform a given process,

might be more useful than using RNA. The RNA rather indicates actual activities, which may highly fluctuate in time and space, and thus are of less significance as an indicator. However, free DNA released from dead microbes is often highly resistant in soil, which might result in an over estimation of a potential function. This needs to be taken into account when interpreting the data. Recently, methods that extract DNA only from living cells have been described, but their use has not been yet introduced into recent standardization activities.

Also, the use of a test battery to measure a range of interconnected endpoints is recommended (Ockleford et al., 2017) to integrate different biological and other parameters (e.g. soil pH, organic carbon content) into multiparametric indices (Bastida et al., 2008; Kvas et al., 2017). At present, it appears to be favourable to use a suite of different methods, i.e. functional gene analysis and microbial enzyme and/or degradation activities, for soil quality testing. Finally, to fully understand soil microbial functioning, a task was envisioned to investigate the linkage between the genetic functional potential and the available resources, termed the soil metaphenome (Jansson and Hofmockel, 2018). This will require even further integration and assessment of multiple parameters and test methods. Reaching that goal will surely promote soil ecological research but, at the current stage, may clearly go beyond the applied aim of standardization to release easy-to-use targeted methods.

The critical evaluation of existing and non-standardized methods is required to further select and standardize new methods to assess soil quality. For methods linked to the molecular analysis of soil microbiomes,~~:~~ there is a need to ensure that worldwide activities are synchronized to propose important standards that are well accepted by the scientific community. ~~For example, recently, the Earth Microbiome Project (www.earthmicrobiome.org) has proposed primer pairs to barcode soil bacteria in a standardized manner. Furthermore, new bioinformatic pipelines have been developed that are being used more and more as standard procedures. Finally, t~~To improve the reproducibility of data it has been agreed that a complex mixture of microorganisms (MOCK) must be implemented as a control in every experiment, but so far no common agreement on the composition of a MOCK community has been reached. ~~The exact composition of the MOC is still under discussion, h~~However, it is clear that if further developments of microbial bar coding and/or metagenomics methods are to be implemented into ISO guidelines, an MOCK is required.

ISO standardization committees are open circles and the presented selection and valuation of methods may not be complete. Environmental scientists are solicited to propose new work items enlarging the current catalogue of biological methods for future standardization. Accordingly, this opinion paper aims at initiating a broader discussion intended to improve the measurement of microbial functions for soil quality assessment. Lastly, it must be noted that standardization of methods is inevitably a balancing act. On one hand, standardization

provides defined methods that are essential to obtain comparable data, e.g. for integration in large, joint databases. On the other hand, it requires setting a specific method for several years. Consequently, scientific progress cannot be easily adopted, or at least with a delay, considering that standards are revised every five years, which may be a barrier to the introduction of new approaches resulting from technological evolution, especially in the fast developing field of molecular biology methods. Hence, it is also the aim of this paper to have an open discussion to identify the best suitable methods with an assumed longer period of validity.

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References

Anderson, T.H., and Domsch, K. H.: The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils, Soil Biology and Biochemistry, 25, 393-395, doi 10.1016/0038-0717(93)90140-7, 1993.

Azhari, N., Devers-Lamrani, M., Chatagnier, G., Rouard, N., and Martin-Laurent F.: Molecular analysis of the catechol-degrading bacterial community in a coal wasteland heavily contaminated with PAHs. Journal of Hazardous Material, 177, 593-601, 2010.

Bååth, E.: Growth rates of bacterial communities in soils at varying pH: A comparison of the thymidine and leucine incorporation techniques Microbial Ecology, 36, 316-327 doi:10.1007/s002489900118, 1998.

Bååth, E., Pettersson, M., and Söderberg, K. H.: Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria, Soil Biology and Biochemistry, 33, 1571-1574, doi 10.1016/S0038-0717(01)00073-6, 2001.

Bach, C. E., Warnock, D. D., Van Horn, D. J., Weintraub, M. N., Sinsabaugh, R. L., Allison, S. D., and German, D. P.: Measuring phenol oxidase and peroxidase activities with pyrogallol, l-DOPA, and ABTS: Effect of assay conditions and soil type, Soil Biology and Biochemistry, 67, 183-191, 2013.

Baldrian, P.: Fungal laccases - occurrence and properties, Fems Microbiology Reviews, 30, 215-242, 2006.

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

Beck, T., Beck, R.: Bodenenzyme. In: Blume, H.-P., Felix-Hennigsen, P., Frede, H.-G., Guggenberger, G., Horn, R., and Stahr, K. (eds.) Handbuch der Bodenkunde. Wiley-VCH, Weinheim, Germany, pp supplement 8, 244 pg., 2000.

Bergkemper, F., Kublik, S., Lang, F., Krüger, J., Vestergaard, G., Schloter, M., and Schulz, S.: Novel oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil, Journal of Microbiological Methods, 125, 91-97, doi 10.1016/j.mimet.2016.04.011, 2016.

Bispo, A., Cluzeau, D., Creamer, R., Dombos, M., Graefe, U., Krogh, P. H., Sousa, J. P., Peres, G., Rutgers, M., Winding, A., and Römbke, J.: Indicators for monitoring soil biodiversity, Integrated Environmental Assessment and Management, 5, 717-719, 10. doi 10.1897/IEAM-2009-064.1, 2009.

Bispo, A., Andersen, L., Angers, D. A., Bernoux, M., Brossard, M., Cécillon, L., Comans, R. N. J., Harmsen, J., Jonassen, K., Lamé, F., Lhuillery, C., Maly, S., Martin, E., Mcelnea, A. E., Sakai, H., Watabe, Y., and Eglin, T. K.: Accounting for carbon stocks in soils and measuring GHGs emission fluxes from soils: Do we have the necessary standards? Frontiers in Environmental Science, 5, 10. doi 10.3389/fenvs.2017.00041, 2017.

Bockhorst, S., and Wardle, D.A.: Microclimate within litter bags of different mesh size: Implications for the 'arthropod effect' on litter decomposition. Soil Biology and Biochemistry, 58, 147-152, 2013.

Bollag, J.M.: Decontaminating soil with enzymes, Environmental Science and Technology, 26, 1876-1881, 1992.

Bolliger, A., Nalla, A., Magid, J., de Neergaard, A., Dole Nalla, A., and Bøg-Hansen, T. C.: Re-examining the glomalin-purity of glomalin-related soil protein fractions through immunochemical, lectin-affinity and soil labelling experiments, Soil Biology and Biochemistry, 40, 887-893, 10. doi 10.1016/j.soilbio.2007.10.019, 2008.

Breure, A.M., De Deyn, G.B., Dominati, E., Eglin, T., Hedlund, K., Van Orshoven, J., and Posthuma, L.:, 2012. Ecosystem services: A useful concept for soil policy making! Current Opinion in Environmental Sustainability 4(5), 578-585,. 2012.

Brookes, P. C.: The use of microbial parameters in monitoring soil pollution by heavy metals, Biology and Fertility of Soils, 19, 269-279, 10. doi 10.1007/BF00336094, 1995.

Brooks, J. P., Edwards, D. J., Harwich, M. D., Jr., Rivera, M. C., Fettweis, J. M., Serrano, M. G., Reris, R. A., Sheth, N. U., Huang, B., Girerd, P., Strauss, J. F., III, Jefferson, K. K., and Buck, G. A.: The truth about metagenomics: Quantifying and counteracting bias in 16S rRNA studies ecological and evolutionary microbiology, BMC Microbiology, 15, 10. doi 10.1186/s12866-015-0351-6, 2015.

Cebron, A., Norini, M.P., Beguiristain, T., and Leyval, C.: Real-time PCR quantification of PAH-ring hydroxylating dioxygenase (PAH-RHD alpha) genes from Gram positive and Gram-negative Bacteria in soil and sediment samples, Journal of Microbiological Methods, 73, 148-159, 2008.

Chen, X., Su, Y., He, X., Liang Y., and Wu J.: Comparative analysis of basidiomycetous laccase genes in forest soils reveals differences at the cDNA and DNA levels. Plant and Soil, 366, 321-331, 2013.

Creamer, R. E., Hannula, S. E., Leeuwen, J., Stone, D., Rutgers, M., Schmelz, R. M., Ruiter, P., Hendriksen, N., Bolger, T., Bouffaud, M. L., Buee, M., Carvalho, F., Costa, D., Dirilgen, T., Francisco, R., Griffiths, B. S., Griffiths, R., Martin, F., Silva, P. D., Mendes, S., Morais, P. V., Pereira, C., Philippot, L., Plassart, P., Redecker, D., Römbke, J., Sousa, J. P., Wouterse, M., and Lemanceau, P.: Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe, Applied Soil Ecology, 97, 112-124, 10. doi 10.1016/j.apsoil.2015.08.006, 2016.

Coban, H., Miltner, A., Kästner, M.: Fate of fatty acids derived from biogas residues in arable soil, Soil Biology and Biochemistry, 91, 58-64, 2015.

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Dandie, C.E., Miller, M.N., Burton, D.L., Zebarth, B.J., Trevors, J.T., and Goyer, C.: Nitric oxide reductase-targeted real-time PCR quantification of denitrifier populations in soil, Applied Environmental Microbiology, 73, 4250–4258, 2007.

De Gonzalo, G., Colpa, D.I., Habib, M.H.M., Fraaije, M.W., 2016. Bacterial enzymes involved in lignin degradation. Journal of Biotechnology 236, 110-119.

Edwards, I.P., Zak, D.R., Kellner, H., Eisenlord, S.D., and Pregitzer, K.S.: Simulated atmospheric N deposition alters fungal community composition and suppresses ligninolytic gene expression in a Northern Hardwood forest. PLoS ONE, 6, doi 10.1371/journal.pone.0020421, 2011.

Eichlerová, I., Šnajdr, J., and Baldrian, P. (2012): Laccase activity in soils: Considerations for the measurement of enzyme activity, Chemosphere, 88, 1154-1160, https://doi.org/10.1016/j.chemosphere.2012.03.019, 2012.

El Azhari, N., Bru, D., Sarr, A., and Martin-Laurent, F.: Estimation of the density of the protocatechuate-degrading bacterial community in soil by real-time PCR, European Journal of Soil Science, 59, 665-673, 2008.

El Azhari, N., Devers-Lamrani, M., Chatagnier, G., Rouard, N., and Martin-Laurent, F.: Molecular analysis of the catechol-degrading bacterial community in a coal wasteland heavily contaminated with PAHs, Journal of Hazardous Materials, 177, 593-601, ~~10.~~ doi 10.1016/j.jhazmat.2009.12.074, 2010.

European Commission: EU-Regulation 1107/2009/EC of the European Parliament and the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, 2009.

Faber, J.H., Creamer, R.E., Mulder, C., Römbke, J., Rutgers, M., Sousa, J.P., Stone, D., and Griffiths, B.S.: The practicalities and pitfalls of establishing a policy-relevant and cost-effective soil biological monitoring scheme, Integrated Environmental Assessment and Management, 9, 276-284, 2013.

Feinstein, L. M., Woo, J. S., and Blackwood, C. B.: Assessment of bias associated with incomplete extraction of microbial DNA from soil, Applied and Environmental Microbiology, 75, 5428-5433, ~~10.~~ doi 10.1128/AEM.00120-09, 2009.

Fish, J.A., Chai, B., Wang, Q., Sun, Y., Brown, C.T., Tiedje, J.M., and Cole, J.R.: FunGene: The Functional Gene Pipeline and Repository, Frontiers in Microbiology, 4, 291, 2013.

Gaby, J.C., and Buckley, D.H.: A comprehensive evaluation of PCR primers to amplify the *nifH* gene of nitrogenase, PLoS ONE, 7, 2012.

Galic, N., Schmolke, A., Forbes, V., Baveco, H., and van den Brink, P.J., 2012. The role of ecological models in linking ecological risk assessment to ecosystem services in agroecosystems. Science of the Total Environment 415, 93-100.

Garland, J. L., and Mills, A. L.: Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization, Applied and Environmental Microbiology, 57, 2351-2359, 1991.

Garland, J. L.: Analysis and interpretation of community-level physiological profiles in microbial ecology, FEMS Microbiology Ecology, 24, 289-300, ~~10.~~ doi 10.1016/S0168-6496(97)00061-5, 1997.

Geisen, S., Briones, M.J.I., Gan, H., Behan-Pelletier, V.M., Friman, V.P., de Groot, G.A., Hannula, S.E., Lindo, Z., Philippot, L., Tiunov, A.V., and Wall, D.H.: A methodological framework to embrace soil biodiversity. Soil Biology and Biochemistry, 136, doi 10.1016/j.soilbio.2019.107536, 2019.

Glimm, E., Heuer, H., Engelen, B., Smalla, K., and Backhaus, H.: Statistical comparisons of community catabolic profiles, Journal of Microbiological Methods, 30, 71-80, ~~10.~~ doi 10.1016/S0167-7012(97)00046-8, 1997.

Gomez, E., Ferreras, L., and Toresani, S.: Soil bacterial functional diversity as influenced by organic amendment application, Bioresource Technology, 97, 1484-1489, ~~10.~~ doi 10.1016/j.biortech.2005.06.021, 2006.

Gomiero, T.: Soil degradation, land scarcity and food security: Reviewing a complex challenge, Sustainability (Switzerland), 8, ~~10.~~ doi 10.3390/su8030281, 2016.

Gorfer, M., Blumhoff, M., Klaubauf, S., Urban, A., Inselsbacher, E., Bandian, D., Mitter, B., Sessitsch, A., Wanek, W., and Strauss, J.: Community profiling and gene expression of fungal assimilatory nitrate reductases in agricultural soil. ISME Journal, 5, 1771-1783, 2011.

Griffiths, B. S., and Philippot, L.: Insights into the resistance and resilience of the soil microbial community, Fems Microbiology Reviews, 37, 112-129, 2013.

Guillaume, T., Maranguit, D., Murtilaksono, K., and Kuzyakov, Y.: Sensitivity and resistance of soil fertility indicators to land-use changes: New concept and examples from conversion of Indonesian rainforest to plantations, Ecological Indicators, 67, 49-57, ~~10.~~ doi 10.1016/j.ecolind.2016.02.039, 2016.

Hannula, S.E., and van Veen, J.A.: Primer sets developed for functional genes reveal shifts in functionality of fungal community in soils. Frontiers in Microbiology, 7, 1897, doi 10.3389/fmicb.2016.01897, 2016.

Hartmann, A., Schmid, M., van Tuinen, D., and Berg, G.: Plant-driven selection of microbes, Plant and Soil, 321, 235-257, 2009.

Hayat, R., Ali, S., Amara, U., Khalid, R., and Ahmed, I.: Soil beneficial bacteria and their role in plant growth promotion: A review, Annals of Microbiology, 60, 579-598, ~~10.~~ doi 10.1007/s13213-010-0117-1, 2010.

Haynes, R.J.: Nature of the belowground ecosystem and its development during pedogenesis, Advances in Agronomy, 127, 43-109, 2014.
Henry, S., Baudoin, E., Lopez-Gutiérrez, J.-C., Martin-Laurent, F., Brauman, A., and Philippot, L.: Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR, Journal of Microbiological Methods, 59: 327-335, 2004.
Herrmann, A.M., Coucheney, E., and Nunan, N.: Isothermal microcalorimetry provides new insight into terrestrial carbon cycling, Environmental Science and Technology, 48, 4344-4352, ~~10.~~ doi 10.1021/es403941h, 2014.
Hirsch, P.R., Mauchline, T.H., and Clark, I.M.: Culture-independent molecular techniques for soil microbial ecology, Soil Biology and Biochemistry, 42, 878-887, ~~10.~~ doi 10.1016/j.soilbio.2010.02.019, 2010.
ISO 11063: Soil quality -- Method to directly extract DNA from soil samples, International Organization for Standardization, Geneva, Switzerland, 2012. https://www.iso.org/standard/50025.html
ISO 11266: Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions, International Organization for Standardization, Geneva, Switzerland, 1994. https://www.iso.org/standard/19244.html
ISO 14238: Soil quality -- Biological methods -- Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes, International Organization for Standardization, Geneva, Switzerland, 2012. https://www.iso.org/standard/56033.html
ISO 14239: Soil quality -- Laboratory incubation systems for measuring the mineralization of organic chemicals in soil under aerobic conditions, International Organization for Standardization, Geneva, Switzerland, 2017. https://www.iso.org/standard/69583.html
ISO 14240-1: Soil quality -- Determination of soil microbial biomass -- Part 1: Substrate-induced respiration method, International Organization for Standardization, Geneva, Switzerland, 1997. https://www.iso.org/standard/21530.html
ISO 14240-2: Soil quality -- Determination of soil microbial biomass -- Part 2: Fumigation-extraction method, International Organization for Standardization, Geneva, Switzerland, 1997. https://www.iso.org/standard/23951.html
ISO 15473: Soil quality -- Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions, International Organization for Standardization, Geneva, Switzerland, 2002. https://www.iso.org/standard/27189.html
ISO 15685: Soil quality -- Determination of potential nitrification and inhibition of nitrification -- Rapid test by ammonium oxidation, International Organization for Standardization, Geneva, Switzerland, International Organization for Standardization, Geneva, Switzerland, 2012. https://www.iso.org/standard/53530.html
ISO 16072: Soil quality -- Laboratory methods for determination of microbial soil respiration, International Organization for Standardization, Geneva, Switzerland, 2002. https://www.iso.org/standard/32096.html
ISO 17155: Soil quality -- Determination of abundance and activity of soil microflora using respiration curves, International Organization for Standardization, Geneva, Switzerland, 2012. https://www.iso.org/standard/53529.html
ISO 17601: Soil quality -- Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil, International Organization for Standardization, Geneva, Switzerland, 2016. https://www.iso.org/standard/60106.html
ISO 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, 2016. https://www.iso.org/standard/62102.html
ISO 23753-1: Soil quality -- Determination of dehydrogenase activity in soils -- Part 1: Method using triphenyltetrazolium chloride (TTC), International Organization for Standardization, Geneva, Switzerland, 2019. https://www.iso.org/standard/70145.html
ISO 23753-2: Soil quality -- Determination of dehydrogenase activity in soils -- Part 2: Method using iodotetrazolium chloride (INT), International Organization for Standardization, Geneva, Switzerland, 2019. https://www.iso.org/standard/70146.html
ISO ~~/DIS~~ 20130 ~~-EN~~: Soil quality - Measurement of enzyme activity patterns in soil samples using colorimetric substrates in micro-well plates, International Organization for Standardization, Geneva, Switzerland, 2018. https://www.iso.org/standard/67074.html
ISO ~~/DIS~~ 20951: Soil Quality -- Guidance on methods for measuring greenhouse gases (CO_2, N_2O, CH_4) and ammonia (NH_3) fluxes between soils and the atmosphere, International Organization for Standardization, Geneva, Switzerland, under development, 2018. https://www.iso.org/standard/69534.html
ISO/CD 23265: Soil quality -- Test for measuring organic matter decomposition in contaminated soil, International Organization for Standardization, Geneva, Switzerland, under development, 2018. https://www.iso.org/standard/75115.html

ISO/TS 20131-1: Soil quality -- Easy laboratory assessments of soil denitrification, a process source of N_2O emissions -- Part 1: Soil denitrifying enzymes activities, International Organization for Standardization, Geneva, Switzerland, under development, 2018. https://www.iso.org/standard/67075.html

ISO/TS 20131-2: Soil quality -- 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, International Organization for Standardization, Geneva, Switzerland, under development, 2018. https://www.iso.org/standard/67076.html

ISO/TS 22939: Soil quality -- Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates, International Organization for Standardization, Geneva, Switzerland, 2010. https://www.iso.org/standard/41226.html

ISO/TS 29843-1: Soil quality -- Determination of soil microbial diversity -- Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis, International Organization for Standardization, Geneva, 2010. https://www.iso.org/standard/45703.html

ISO/TS 29843-2: 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, 2011. https://www.iso.org/standard/54070.html

Jänsch, S., Scheffczyk, A., and Römbke, J.: The bait lamina test – A possible screening method for earthworm toxicity testing, Euro-Mediterranean Journal for Environmental Integration, 2, 5. 11pp. DOI: 10-1007/s41207-017-0015z, 2017.

Janos, D. P., Garamszegi, S., and Beltran, B.: Glomalin extraction and measurement, Soil Biology and Biochemistry, 40, 728-739, ~~10.~~ doi 10.1016/j.soilbio.2007.10.007, 2008.

Jansson, J. K., and Hofmockel, K. S.: The soil microbiome — from metagenomics to metaphenomics, Current Opinion in Microbiology, 43, 162-168, ~~10.~~ doi 10.1016/j.mib.2018.01.013, 2018.

Jeffries, T.C., Rayu, S., Nielsen, U.N., Lai, K., Ijaz, A., Nazaries, L., and Singh, B.K.: Metagenomic functional potential predicts degradation rates of a model organophosphorus xenobiotic in pesticide contaminated soils. Frontiers in Microbiology, 9, doi 10.3389/fmicb.2018.00147, 2018.

Joergensen, R. G., and Emmerling, C.: Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils, Journal of Plant Nutrition and Soil Science, 169, 295-309, ~~10.~~ doi 10.1002/jpln.200521941, 2006.

Jung, J., Choi, S., Jung, H., Scow, K.M., and Park, W.: Primers for amplification of nitrous oxide reductase genes associated with Firmicutes and Bacteroidetes in organic-compound-rich soils, Microbiology, 159, 307-315. doi~~:~~ 10.1099/mic.0.060194-0, 2013.

Karpouzas, D.G., Tsiamis, G., Trevisan, M., Ferrari, F., Malandain, C., Sibourg, O., and Martin-Laurent, F.: 'LOVE TO HATE' pesticides: felicity or curse for the soil microbial community? An FP7 IAPP Marie Curie project aiming to establish tools for the assessment of the mechanisms controlling the interactions of pesticides with soil microorganisms, Environmental Science and Pollution Research, 18, 18947-18951, 2016.

Keuskamp, J.A., Dingemans, B.J.J., Lehtinen, L., Sarneel, J.M., and Hefting, M.M.: Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems, Methods in Ecology and Evolution, 4, 1070–1075, 2013.

Knacker, T., Förster, B., Römbke, J., and Frampton, G. K.: Assessing the effects of plant protection products on organic matter breakdown in arable fields - Litter decomposition test systems, Soil Biology and Biochemistry, 35, 1269-1287, ~~10.~~ doi 10.1016/S0038-0717(03)00219-0, 2003.

Kolb, S., Knief, C., Stubner, S. and Conrad, R.: Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays, Applied Environmental Microbiology, 69, 2423–2429. doi~~:~~ 10.1128/AEM.69.5.2423-2429.2003. PMCID: PMC154495, 2003.

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., and Sessitsch, A.: Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing, FEMS Microbiology Ecology, 82, 551-562, ~~10.~~ doi 10.1111/j.1574-6941.2012.01420.x, 2012.

Kvas, S., Rahn, J., Engel, K., Neufeld, J.D., Villeneuve, P.J., Trevors, J.T., Lee, H., Scroggins, R.P., and Beaudette, L.A.: Development of a microbial test suite and data integration method for assessing microbial health of contaminated soil, Journal of Microbiological Methods, 143, 66-77, 2017.

Levy-Booth, D. J., Prescott, C. E., and Grayston, S. J.: Microbial functional genes involved in nitrogen fi xation, nitrification and denitrification in forest ecosystems, Soil Biology and Biochemistry, 75, 11-25, ~~10.~~ doi 10.1016/j.soilbio.2014.03.021, 2014.

Liu, W., Qiao, C., Yang, S., Bai, W., and Liu, L.: Microbial carbon use efficiency and priming effect regulate soil carbon storage under nitrogen deposition by slowing soil organic matter decomposition, Geoderma, 332, 37-44, ~~10.~~ doi 10.1016/j.geoderma.2018.07.008, 2018.

Lueders, T., Manefield, M., and Friedrich, M.W.: Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. Environmental Microbiology, 6, 73-78, 2004.

Lugtenberg, B., and Kamilova, F.: Plant-growth-promoting rhizobacteria, Annual Review of Microbiology, 63, 541-556, 10. doi 10.1146/annurev.micro.62.081307.162918, 2009.

Martin-Laurent, F., Cornet, L., Ranjard, L., Lopez-Gutiérrez, J.C., Philippot, L., Schwartz, C., Chaussod, R., Catroux, G., and Soulas, G.: Estimation of atrazine-degrading genetic potential and activity in three French agricultural soils, FEMS Micobiology Ecology, 48, 425-435. ,2004.

Martin-Laurent, F., Kandeler, E., Petric, I., Djuric, S., and Karpouzas, D.: ECOFUN-MICROBIODIV: an FP7 European project for developing and evaluating innovative tools for assessing the impact of pesticides on soil functional microbial diversity: towards new pesticide registration regulation? Environmental Science and Pollution Research, 2, 1203-1205, 2013.

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

Modrzyński, J. J., Christensen, J. H., Mayer, P., and Brandt, K. K.: Limited recovery of soil microbial activity after transient exposure to gasoline vapors, Environmental Pollution, 216, 826-835, 10. doi 10.1016/j.envpol.2016.06.054, 2016.

Montgomery, D. R.: Dirt: The Erosion of Civilizations, University of California Press, Berkeley, Calif.; London, 2008.

Nannipieri, P., and Eldor, P.: The chemical and functional characterization of soil N and its biotic components, Soil Biology and Biochemistry, 41, 2357-2369, 10. doi 10.1016/j.soilbio.2009.07.013, 2009.

Nannipieri, P., Greco, S., and Ceccanti, B.: Ecological Significance of the Biological Activity in Soil, in: Soil Biochemistry: Volume 6: Volume 6, edited by: Bollag, J. M., and Stotzky, G., Routledge, New York, 293-355, 2017.

Nicol, G. W., Glover, L. A., and Prosser, J. I.: Spatial analysis of archaeal community structure in grassland soil, Applied and Environmental Microbiology, 69, 7420-7429, 10. doi 10.1128/AEM.69.12.7420-7429.2003, 2003.

Nienstedt, K., Brock, T.C.M., Van Wensem, J., Montforts, M., Hart, A., Aagaard, A., Alix, A., Boesten, J., Bopp, S.K., Brown, C., Capri, E., Forbes, V., Köpp, H., Liess, M., Luttik, R. Maltby, L. Sousa, J.-P., Streissl, F., and Hardy, A.R.: Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides, Science of the Total Environment, 415, 31-38, 2012.

Norton, J.M., Alzerreca, J.J., Suwa, Y., and Klotz, M.G.: Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria, Archives of Microbiology, 177, 139-149, 10. doi 10.1007/s00203-001-0369-z, 2002.

Ockleford, C., Adriaanse, P., Berny, P., Brock, T., Duquesne, S., Grilli, S., Hernandez-Jerez, A. F., Bennekou, S. H., Klein, M., Kuhl, T., Laskowski, R., Machera, K., Pelkonen, O., Pieper, S., Stemmer, M., Sundh, I., Teodorovic, I., Tiktak, A., Topping, C. J., Wolterink, G., Craig, P., de Jong, F., Manachini, B., Sousa, P., Swarowsky, K., Auteri, D., Arena, M., and Rob, S.: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues) Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, EFSA Journal, 15, 255, doi:10.2903/j.efsa.2017.4690, 2017.

OECD: Test No. 216: Soil Microorganisms: Nitrogen Transformation Test. Paris, France, 2000a.

OECD: Test No. 217: Soil Microorganisms: Carbon Transformation Test. Paris, France, 2000b.

OECD: OECD Series on Testing and Assessment No. 56. Guidance document on the breakdown of organic matter in litterbags. ENV/JM/MONO 23. Paris, France, 2006.

Ollivier, J., Kleineidam, K., Reichel, R., Thiele-Bruhn, S., Kotzerke, A., Kindler, R., Wilke, B.M., and Schloter, M.: Effect of sulfadiazine-contaminated pig manure on the abundances of genes and transcripts involved in nitrogen transformation in the root-rhizosphere complexes of maize and clover, Applied and Environmental Microbiology, 76, 7903-7909, 2010.

Ouyang, Y., Reeve, J.R., and Norton, J.M.: Soil enzyme activities and abundance of microbial functional genes involved in nitrogen transformations in an organic farming system. Biology and Fertility of Soils, 54, 437-450, 2018.

Pankhurst, C.E., Yu, S., Hawke, B.G., and Harch, B.D.: Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biology and Fertility of Soils, 33, 204-217, 2001.

Parker, S.S.: Buried treasure: Soil biodiversity and conservation, Biodiversity and Conservation, 19, 3743-3756, 2010.

Paul, E. A.: Soil Microbiology, Ecology and Biochemistry, 4th ed., Academic Press, Burlington, MA, 2015.

765 Peng, J., Lü, Z., Rui, J., and Lu, Y.: Dynamics of the methanogenic archaeal community during plant residue
766 decomposition in an anoxic rice field soil, Applied and Environmental Microbiology, 74, 2894-2901, 10.
767 doi 10.1128/AEM.00070-08, 2008.
768 Penton, C. R., Gupta, V. V. S. R., Yu, J., and Tiedje, J. M.: Size matters: Assessing optimum soil sample size for
769 fungal and bacterial community structure analyses using high throughput sequencing of rRNA gene
770 amplicons, Frontiers in Microbiology, 7, 10. doi 10.3389/fmicb.2016.00824, 2016.
771 Philippot, L., Ritz, K., Pandard, P., Hallin, S., and Martin-Laurent, F.: Standardisation of methods in soil
772 microbiology: Progress and challenges, FEMS Microbiology Ecology, 82, 1-10, 10. doi 10.1111/j.1574-
773 6941.2012.01436.x, 2012.
774 Prado, A.G.S., Airoldi, C.: The effect of the herbicide diuron on soil microbial activity, Pest Management Science,
775 57, 640-644 doi:10.1002/ps.321, 2001.
776 Prado, A.G.S., Airoldi, C.: A toxicity view of the pesticide picloram when immobilized onto a silica gel surface,
777 Analytical and Bioanalytical Chemistry, 376, 686-690, doi:10.1007/s00216-003-1927-9, 2003.
778 Preston-Mafham, J., Boddy, L., and Randerson, P. F.: Analysis of microbial community functional diversity using
779 sole-carbon-source utilisation profiles - A critique, FEMS Microbiology Ecology, 42, 1-14, 10. doi
780 10.1016/S0168-6496(02)00324-0, 2002.
781 Pulleman, M., Creamer, R., Hamer, U., Helder, J., Pelose, C., Peres, G., and Rutgers, M.: Soil biodiversity,
782 biological indicators and soil ecosystem services – an overview of European approaches, Current Opinion
783 in Environ. Sustainability, 4, 529-538, 2012.
784 Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., and Segata, N.: Shotgun metagenomics, from sampling to
785 analysis. Nature Biotechnology, 35, 833-844, 2017.
786 Ramirez, K.S., Knight, C.G., De Hollander, M., Brearley, F.Q., Constantinides, B., Cotton, A., Creer, S., Crowther,
787 T.W., Davison, J., Delgado-Baquerizo, M., Dorrepaal, E., Elliott, D.R., Fox, G., Griffiths, R.I., Hale, C.,
788 Hartman, K., Houlden, A., Jones, D.L., Krab, E.J., Maestre, F.T., McGuire, K.L., Monteux, S., Orr, C.H.,
789 Van Der Putten, W.H., Roberts, I.S., Robinson, D.A., Rocca, J.D., Rowntree, J., Schlaeppi, K., Shepherd,
790 M., Singh, B.K., Straathof, A.L., Bhatnagar, J.M., Thion, C., Van Der Heijden, M.G.A., and De Vries, F.T.:
791 Detecting macroecological patterns in bacterial communities across independent studies of global soils.
792 Nature Microbiology, 3, 189-196, 2018.
793 Regan, K. M., Nunan, N., Boeddinghaus, R. S., Baumgartner, V., Berner, D., Boch, S., Oelmann, Y., Overmann,
794 J., Prati, D., Schloter, M., Schmitt, B., Sorkau, E., Steffens, M., Kandeler, E., and Marhan, S.: Seasonal
795 controls on grassland microbial biogeography: Are they governed by plants, abiotic properties or both?,
796 Soil Biology and Biochemistry, 71, 21-30, 10. doi 10.1016/j.soilbio.2013.12.024, 2014.
797 Ribbons, R.R., Levy-Booth, D.J., Masse, J., Grayston, S.J., McDonald, M.A., Vesterdal, L., and Prescott, C.E.:
798 Linking microbial communities, functional genes and nitrogen-cycling processes in forest floors under four
799 tree species, Soil Biology and Biochemistry, 103, 181-191, 10. doi 10.1016/j.soilbio.2016.07.024, 2016.
800 Rillig, M.C.: Arbuscular mycorrhizae, glomalin, and soil aggregation, Canadian Journal of Soil Science, 84, 355-
801 363 doi:10.4141/S04-003, 2004.
802 Rillig, M.C., and Mummey, D.L.: Mycorrhizas and soil structure, New Phytologist, 171, 41-53
803 doi:10.1111/j.1469-8137.2006.01750.x, 2006.
804 Ritz, K., Black, H.I.J., Campbell, C.D., Harris, J.A., and Wood, C.: Selecting biological indicators for monitoring
805 soils: A framework for balancing scientific and technical opinion to assist policy development, Ecological
806 Indicators, 9, 1212-1221, 2009.
807 Römbke, J., Jänsch, S. Meier, M., Hilbeck, A., Teichmann, H., and Tappeser, B.: General recommendations for
808 soil ecotoxicological tests suitable for the environmental risk assessment (ERA) of genetically modified
809 plants (GMPs), Integrated Environmental Assessment and Management, 6, 287-300, 2010.
810 Rousk, J.: Biomass or growth? How to measure soil food webs to understand structure and function, Soil Biology
811 and Biochemistry, 102, 45-47, 10. doi 10.1016/j.soilbio.2016.07.001, 2016.
812 Rousk, J., Brookes, P.C., and Bååth, E.: Contrasting soil pH effects on fungal and bacterial growth suggest
813 functional redundancy in carbon mineralization, Applied and Environmental Microbiology, 75, 1589-1596
814 doi:10.1128/AEM.02775-08, 2009b.
815 Rousk, J., Demoling, L. A., and Bååth, E.: Contrasting short-term antibiotic effects on respiration and bacterial
816 growth compromises the validity of the selective respiratory inhibition technique to distinguish fungi and
817 bacteria, Microbial Ecology, 58, 75-85, 10. doi 10.1007/s00248-008-9444-1, 2009a.
818 Rutgers, M., Wouterse, M., Drost, S. M., Breure, A. M., Mulder, C., Stone, D., Creamer, R. E., Winding, A., and
819 Bloem, J.: Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-
820 plates in the Netherlands and Europe, Applied Soil Ecology, 97, 23-35, 10. doi
821 10.1016/j.apsoil.2015.06.007, 2016.

Sakakibara, S.M., Jones, M.D., Gillespie, M., Hagerman, S.M., Forrest, M.E., Simard, S.W., and Durall, D.M.: A comparison of ectomycorrhiza identification based on morphotyping and PCR-RFLP analysis, Mycological Research, 106, 868-878, 2002.

Savazzini, F., Longa, C.M., Pertot, I., and Gessler, C.J.: Real-time PCR for detection and quantification of the biocontrol agent Trichoderma atroviride strain SC1 in soil, Microbiological Methods, 73, 185-94. doi: 10.1016/j.mimet.2008.02.004, 2008.

Scheu, S., Schlitt, N., Tiunov, A. V., Newington, J. E., and Jones, T. H.: Effects of the presence and community composition of earthworms on microbial community functioning, Oecologia, 133, 254-260, 10. doi 10.1007/s00442-002-1023-4, 2002.

Scheu, S., Ruess, L., and Bonkowski, M.: Interactions between microorganisms and soil micro- and mesofauna, in: Microorganisms in Soils: Roles in Genesis and Functions, edited by: Varma, A., and Buscot, F., Soil Biology, 3, 253-275, 2005.

Schmitt, H., Van Beelen, P., Tolls, J., and Van Leeuwen, C. L.: Pollution-induced community tolerance of soil microbial communities caused by the antibiotic sulfachloropyridazine, Environmental Science & Technology, 38, 1148-1153, 2004.

Schöler, A., Jacquiod, S., Vestergaard, G., Schulz, S., and Schloter, M. Analysis of soil microbial communities based on amplicon sequencing of marker genes. Biology and Fertility of Soils, 53, 485-489, 2017.

Schwartz, E.: Characterization of growing microorganisms in soil by stable isotope probing with $H_2{}^{18}O$, Applied and Environmental Microbiology, 73, 2541-2546, 2007.

Schwartz, E., Hayer, M., Hungate, B.A., Koch, B.J., McHugh, T.A., Mercurio, W., Morrissey, E.M., Soldanova, K.: Stable isotope probing with ^{18}O-water to investigate microbial growth and death in environmental samples, Current Opinion in Biotechnology, 41, 14-18, 2016.

Schulz, S., Bergkemper, F., De Vries, M., Schöler, A., and Schloter, M.: qPCR for quantitative validation of metagenomic data [qPCR zur quantitativen Validierung von Metagenomdaten], BioSpektrum, 22, 265-269, 10. doi 10.1007/s12268-016-0684-1, 2016.

Sessitsch, A., Hackl, E., Wenzl, P., Kilian, A., Kostic, T., Stralis-Pavese, N., Sandjong, B. T., and Bodrossy, L.: Diagnostic microbial microarrays in soil ecology, New Phytologist, 171, 719-736, 10. doi 10.1111/j.1469-8137.2006.01824.x, 2006.

Singh, B. K., Tate, K. R., Kolipaka, G., Hedley, C. B., Macdonald, C. A., Millard, P., and Murrell, J. C.: Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria, Applied and Environmental Microbiology, 73, 5153-5161, 10. doi 10.1128/AEM.00620-07, 2007.

Six, J., Bossuyt, H., Degryze, S, and Denef, K.: A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics, Soil and Tillage Research, 79, 7-31 doi:10.1016/j.still.2004.03.008, 2004.

Smith, C.J., and Osborn, A.M.: Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology, FEMS Microbiology Ecology, 67, 6-20, 10. doi 10.1111/j.1574-6941.2008.00629.x, 2009.

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biology and Biochemistry 40(9), 2068-2075.

Spohn, M., Pötsch, E. M., Eichorst, S. A., Woebken, D., Wanek, W., and Richter, A.: Soil microbial carbon use efficiency and biomass turnover in a long-term fertilization experiment in a temperate grassland, Soil Biology and Biochemistry, 97, 168-175, 10. doi 10.1016/j.soilbio.2016.03.008, 2016.

Steinberg, L.M., and Regan, J.M.: *mcrA*-targeted real-time quantitative PCR method to examine methanogen communities, Applied and Environmental Microbiology, 75, 4435-4442, 2009.

Strickland, M. S., and Rousk, J.: Considering fungal: Bacterial dominance in soils - Methods, controls, and ecosystem implications, Soil Biology and Biochemistry, 42, 1385-1395, 10. doi 10.1016/j.soilbio.2010.05.007, 2010.

Suriyavirun, N., Krichels, A. H., Kent, A. D., and Yang, W. H.: Microtopographic differences in soil properties and microbial community composition at the field scale, Soil Biology and Biochemistry, 131, 71-80, 10. doi 10.1016/j.soilbio.2018.12.024, 2019.

Takriti, M., Wild, B., Schnecker, J., Mooshammer, M., Knoltsch, A., Lashchinskiy, N., Eloy Alves, R. J., Gentsch, N., Gittel, A., Mikutta, R., Wanek, W., and Richter, A.: Soil organic matter quality exerts a stronger control than stoichiometry on microbial substrate use efficiency along a latitudinal transect, Soil Biology and Biochemistry, 121, 212-220, 10. doi 10.1016/j.soilbio.2018.02.022, 2018.

TEEB: The Economics of Ecosystems and Biodiversity Ecological and Economic Foundations. Edited by Pushpam Kumar. Earthscan, 2010.

Thiele-Bruhn, S., Bloem, J., de Vries, F. T., Kalbitz, K., and Wagg, C.: Linking soil biodiversity and agricultural soil management, Current Opinion in Environmental Sustainability, 4, 523-528, 2012.

Tian, J., He, N., Hale, L., Niu, S., Yu, G., Liu, Y., Blagodatskaya, E., Kuzyakov, Y., Gao, Q., and Zhou, J.: Soil organic matter availability and climate drive latitudinal patterns in bacterial diversity from tropical to cold temperate forests, Functional Ecology, 32, 61-70, ~~10.~~ doi 10.1111/1365-2435.12952, 2018.

Traugott, M., Kamenova, S., Ruess, L., Seeber, J., Plantegenest, M.: Empirically characterising trophic networks: What emerging DNA-based methods, stable isotope and fatty acid analyses can offer, Advances in Ecological Research, 49, 177-224, 2013.

Van der Putten, W.H., Mudgal, S., Turbé, A., Toni, A.D., Lavelle, P., Benito, P., and Ruiz, N.: Soil biodiversity: functions, threats and tools for policy makers, Bio Intelligence Service, Paris, France, 2010.

Voříšková, A., Jansa, J., Püschel, D., Krüger, M., Cajthaml, T., Vosátka, M., and Janoušková, M.: Real-time PCR quantification of arbuscular mycorrhizal fungi: does the use of nuclear or mitochondrial markers make a difference? Mycorrhiza, 27, 577-585. doi~~:~~ 10.1007/s00572-017-0777-9, 2017.

Wagg, C., Bender, S. F., Widmer, F., and Van Der Heijden, M. G. A.: Soil biodiversity and soil community composition determine ecosystem multifunctionality, Proceedings of the National Academy of Sciences of the United States of America, 111, 5266-5270, ~~10.~~ doi 10.1073/pnas.1320054111, 2014.

Wallenstein, M. D., Myrold, D. D., Firestone, M., and Voytek, M.: Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods, Ecological Applications, 16, 2143-2152, ~~10.~~ doi 10.1890/1051-0761(2006)016[2143:ECODCA]2.0.CO;2, 2006.

Wardle, D.A., and Ghani, A.: A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development, Soil Biology and Biochemistry, 27, 1601-1610, ~~10.~~ doi 10.1016/0038-0717(95)00093-T, 1995.

Winding, A., and Hendriksen, N. B.: Comparison of CLPP and enzyme activity assay for functional characterization of bacterial soil communities, Journal of Soils and Sediments, 7, 411-417, ~~10.~~ doi 10.1065/jss2007.11.262, 2007.

Wright, S.F., Upadhyaya, A., and Buyer, J.S.: Comparison of N-linked oligosaccharides of glomalin from arbuscular mycorrhizal fungi and soils by capillary electrophoresis, Soil Biology and Biochemistry, 30, 1853-1857, ~~10.~~ doi 10.1016/S0038-0717(98)00047-9, 1998.

Xia, W., Zhang, C., Zeng, X., Feng, Y., Weng, J., Lin, X., Zhu, J., Xiong, Z., Xu, J., Cai, Z., and Jia, Z.: Autotrophic growth of nitrifying community in an agricultural soil, ISME Journal, 5, 1226-1236, ~~10.~~ doi 10.1038/ismej.2011.5, 2011.

Xu, S., Feng, S., Sun, H., Wu, S., Zhuang, G., Deng, Y., Bai, Z., Jing, C., and Zhuang, X.: Linking N_2O emissions from biofertilizer-amended soil of tea plantations to the abundance and structure of N_2O-reducing microbial communities, Environmental Science and Technology, 52, 11338-11345, ~~10.~~ doi 10.1021/acs.est.8b04935, 2018.

Xue, K., Zhou, J., Van Nostrand, J., Mench, M., Bes, C., Giagnoni, L., and Renella, G.; Functional activity and functional gene diversity of a Cu-contaminated soil remediated by aided phytostabilization using compost, dolomitic limestone and a mixed tree stand. Environmental Pollution, 242, 229-238, 2018.

Yates, M. V., Nakatsu, C. H., Miller, R. V., and Pillai, S. D.: Manual of Environmental Microbiology, Fourth Edition, American Society of Microbiology, 2016.

Yergeau, E., Hogues, H., Whyte, L. G., and Greer, C. W.: The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses, ISME Journal, 4, 1206-1214, ~~10.~~ doi 10.1038/ismej.2010.41, 2010.

Zhang, K., Li, X., Cheng, X., Zhang, Z., and Zhang, Q.: Changes in soil properties rather than functional gene abundance control carbon and nitrogen mineralization rates during long-term natural revegetation. Plant and Soil, 443, 293-306, 2019.

926 **Table 1.** List of criteria for the selection of indicators for microbial functional indicators, based on Faber et al.
927 (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
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

928 [1] Only relevant for faunal species. Does not apply to soil microorganisms that are tested with their natural
929 abundance in mixed communities.
930 [2] Standard operating procedures

Table 2. Methods already validated and published as ISO standards for determining potential microbial 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

[1] Measured enzyme activities: Arylsulfatase E.C. 3.1.6.1; α-glucosidase E.C. 3.2.1.20; β-glucosidase E.C. 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; phosphodiesterase E.C. 3.1.4.1; phosphomonoesterase E.C. 3.1.3.2; leucine-aminopeptidase E.C. 3.4.11.1; alanine-aminopeptidase E.C. 3.4.11.12.

[2] Measured enzyme activities: Arylamidase E.C. 3.4.11.2; arylsulfatase E.C. 3.1.6.1; α-glucosidase E.C. 3.2.1.20; β-glucosidase E.C. 3.2.1.21; β-galactosidase E.C. 3.2.1.22; N-acetylglucosaminidase E.C. 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; urease E.C. 3.5.1.5.

[3] Degradation of cellulose under laboratory conditions.

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

Method	Source	Function addressed	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 Litter bag technique	OECD, 2006; Knacker et al., 2003 Bockhorst and Wardle, 2013	assess organic matter degradation and therefore C cycling assess the degradation of plant litter material	5	5	4	4	5	4	5	na	5	5	5
Tea bag technique	Keuskamp et al., 2013	assess the degradation of tea leaves	5	5	4	4	5	4	5	na	5	5	5
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											

945 **Table 3.** Continued

Method	Source	Function addressed	Assessment [1]
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

946 [1] Overall scoring in case of several measures for one criterion. Fulfilment of criterion described by numbering
947 (colour code): 1 (red) very low; 2 (orange) low; 3 (yellow) medium; 4 (light green) good; 5 (dark green) very good.
948 [2] na = not applicable
949

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