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Interactive comment on "Identification of new microbial functional standards for soil quality assessment" *by* Sören Thiele-Bruhn et al.

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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 resubmit 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). Reviewer #1 Robert Griffith This paper discusses new methodologies and op-portunities offered by molecular methodolo-gies to provide microbiological indicators for assessing soil quality. In essence, it reports on key issues raised during recent dis-

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cussions within the International Organization for Stand-ardization (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 quantifica-tion 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 contribu-tion. 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 elevat-ed 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 regional-ised 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 micro-biological variables be deemed somewhat premature?

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This is a very good point and emphasizes the need to gather a database of soil microbial pa-rameters in worldwide soils (with links to soil chemical, physical and pedoclimatic data) in or-der 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 aberra-tions. 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 am-monia oxidation gene abundances does actually appear to relate to nitrification rates in cer-tain 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 decomposi-tion, 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 availa-ble, 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 func-tioning 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 struc-ture 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 func-tional gene abundance and ecosystem services." 402-408: "Here the use of DNA based methods, which provide a measure of a microbial com-munity'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 re-cent 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 imple-menting 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 is-sue.

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 meth-ods 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, scien-tific 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 result-ing 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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