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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 #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

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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 develop-ments 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 defi-nitely 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 em-brace 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 pro-duction 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 be-cause 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 pret-ty 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 Ex-pression 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 re-veals 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 Com-munity in Soils. Frontiers in Microbiology These measurements from DNA have problems as different fungi have different copy numbers/ types of genes and

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not everything that is in the DNA is expressed. Indeed, work is needed to get these methods ISO certified but more dis-cussion on the future directions would be welcome.

Fungi: Information on methods targeted towards fungi was added and the recommended litera-ture 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 regard-ing 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.

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