

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

Emilia Hannula (Referee)

e.hannula@nioo.knaw.nl

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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?

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

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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.

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).

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

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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.

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