Recommendations for soil microbiome analyses

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As a result of the recent developments in high-throughput molecular biology methods, our understanding of the soil microbiome has increased significantly over the last decades. Using microbiome DNA- or RNA-based analyses, such as amplicon sequencing and shotgun metagenomics, a large number of new phyla could be identified and some could be assigned to important traits, which drive keystone functions for ecosystem services provided by soils. However, there is still a lack of information with respect to the major drivers that trigger microbial community composition and function on all scales, i.e., from the pedon (microhabitat, at the micrometer scale) to the region. A large number of publications that included microbiome analyses have been submitted in the last year's *Biology and Fertility of Soils*, and some have addressed such questions. However, in many cases, the provided information, despite the studies being sound in analytics, did not strongly contribute to our understanding of the impact on, as well as the function of, the soil microbiome for several reasons.

- 1) As a result of the high costs for analyses and the complex bioinformatics analysis, only one sampling time point is often chosen for analysis, although there is a large body of evidence on the high temporal dynamics of the soil microbiome, in particular when activity hotspots like the

rhizosphere, the mycosphere, or the detritosphere are considered. Changes over time can be higher in magnitude than those observed between treatments at a single sampling point, for instance due to differences in management, plant cover, and other variables (Nannipieri et al. 2019). In many cases, samples from agricultural soils are taken at crop harvest time. Thus, such studies mostly describe the response of the soil microbiome to the disturbance (and stress) caused by the harvest instead of depicting the typical response pattern and dynamics of the soil microbiome. It is deemed essential to select at least two sampling time points, with at least one incurring a minimal disturbance (mostly in spring), using this as a reference for the analysis of management-related factors like fertilization, tillage, or cropping sequence.

- 2) Besides the temporal dynamics of soil microbiomes, there is also a huge heterogeneity in space, and so intricate statistical techniques are required to grasp this variation. However, the multiple soil samples taken from individual field plots are often composited to yield just one sample covering a plot. In more elaborate studies, spatial heterogeneity should be captured in order to statistically compare variances with plot and treatment differences, using an appropriate number of replicates. Experimental designs with low number of replicates (e.g., three) reduce the statistical power of both univariate and multivariate statistics.
- 3) Linking microbial community composition or potential function to the transformation processes that occur in the soil is often performed only taking into account the concentration of the product/substrate of the target process, ignoring the fact that the levels of these compounds in the soil may also be dependent on other biotic/abiotic processes. For example, changes in the concentration of exchangeable ammonium or nitrate depend not only on the activity of autotrophic nitrification but also on the activity of other N cycling processes. Therefore, target process rates should be determined to establish such linkages and the quantification and/or presence/absence of functional guilds should be included in the discussion.

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- 4) The detection or quantification of a target gene is often directly linked to a process in which the predicted protein is presumed to be a central catalyst. However, it is important to consider and discuss that such a presumption is risky, as the predicted protein may or may not be active and be a central player within the microbial community. As a second confounding factor, using microbiome DNA as a proxy for potential function is often biased due to the presence of DNA from dead microbes.
- 5) Data generated by amplicon sequencing (coined by some authors as “barcoding” or “metabarcoding”) and shotgun metagenomics constitute “relative” data. Such data, by definition, do not provide any information on the total abundances of the identified clades. Thus, mainly in soil systems in which changes of microbial biomass occur, including for key organisms or functions, ribosomal RNA gene-based qPCR analyses are needed to define absolute abundances, which could serve as proxies for potential transformation rates.
- 6) The Earth Microbiome project (www.earthmicrobiome.org) has provided a number of important standards for the analysis of soil microbiomes, which are very helpful if the data from single projects are to be compared with studies of relevance from other researchers in a meta-

analysis. If no clear arguments exist against these standards, they should be implemented into all analytical pipelines as soon as possible.

We strongly recommend that the aforementioned six issues are implemented in all future manuscripts submitted to *Biology and Fertility of Soils*, as has been advocated and summarized in two recent papers (Schöler et al. 2017; Vestergaard et al. 2017).

References

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