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Assessment of spike-AMP and qPCR-AMP in soil microbiota quantitative research

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ABSTRACT

Relative microbiome profiling (RMP) using new sequencing approaches has limited capacity to detect shifts in microbial abundances. The growing need for absolute abundances has led to advances in absolute microbiome profiling (AMP). However, the performance and universal applicability of these various AMP methods remain unclear. Here, the two most popular AMP methods, spike-in method (spike-AMP) and quantitative PCR combined with high-throughput sequencing (qPCR-AMP), were evaluated in soil microbiota research. Our results showed that the quantitative results based on spike-AMP were inconsistent with expected trends. The spike-derived absolute abundance was indeterminate and highly dependent on the amount of spike added. Furthermore, no good correlation was found between the addition of spike copies and output of spike reads, especially at low spike levels, contradicting the theoretical assumption of the spike-in method. Spike addition consumed substantial sequencing resources, and more importantly, it altered the original microbial community structure, explaining 16.1%–36.2% of structural variation. In contrast, the more common qPCR-AMP method provided valuable insights into the understanding of soil microbial dynamics in response to straw addition. Our results showed that the straw-induced variations in some dominant phyla such as *Proteobacteria*, *Actinobacteriota* and *Ascomycota* could only be detected by absolute rather than relative microbial profiling. We inferred microbial networks based on absolute and relative data matrices, respectively, and observed that the choice of data type essentially impacted the patterns of co-occurrence networks and the recognition of module hubs. The keystones and enriched phyla only detected by absolute microbial profiling were confirmed to be involved in straw decomposition by a stable isotope probing experiment. Overall, AMP can provide valuable insights into the understanding of soil microbial dynamics in response to environmental fluctuations. Given its stability and technical feasibility, qPCR-AMP may be broadly applicable to soil microbiota quantitative research.

1. Introduction

Microorganisms are diverse forms of life and thrive in almost all environments. Their composition and function have substantial impacts on human health (Fan and Pedersen, 2021), global element cycling (Crowther et al., 2019), crop production (Charpentier and Oldroyd, 2010) and plant disease resistance (Kwak et al., 2018). Advances in high-throughput sequencing technologies have contributed to the surge of microbial sequencing data (White et al., 2016), but similar to previous fingerprinting approaches, such as denaturing gradient gel electrophoresis or terminal restriction fragment length polymorphism, relative microbiome profiling (RMP) obtained from sequencing data overlooks absolute microbial abundance. However, without absolute quantification, it is challenging to build a more comprehensive understanding of how dynamics of microbiome abundance vary across space, time, and in response to environmental fluctuations (Vandeputte et al., 2017; Zhang

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