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Use of an improved high-throughput absolute abundance quantification method to characterize soil bacterial community and dynamics

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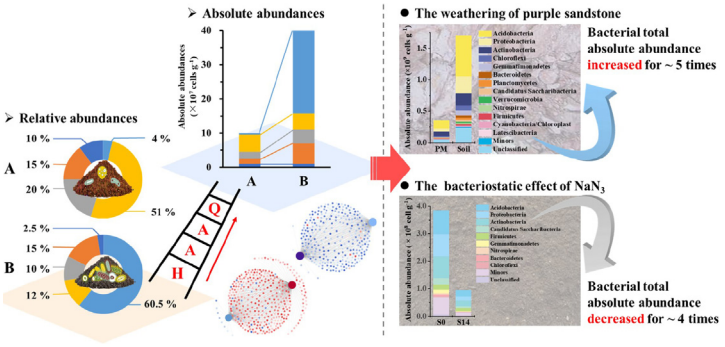
HIGHLIGHTS

- Developed an improved bacterial abundance quantification by adding ISS to samples
- The improved HAAQ method can simultaneously obtain relative and absolute abundances.
- Absolute abundances of soil bacterial genera fit well to a partial log-normal distribution.
- The improved HAAQ can better characterize soil bacterial community and dynamics.

GRAPHICAL ABSTRACT



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ABSTRACT

High-throughput sequencing has dramatically expanded our understanding of bacterial communities based on the information of the species types and their relative abundances. Recently, researchers have also become aware of a deficiency in not considering the absolute abundance in this technique. Combining two or more different methods has typically been used to achieve absolute quantification of microbial communities. However, making a combination of different methods not only is time-consuming but also involves potential uncertainty due to variations in the experimental conditions. To simplify the experimental procedure and improve the high-throughput absolute abundance quantification (HAAQ) of a soil bacterial community, we propose an HAAQ method that uses an internal standard strain (ISS) HAAQ-GFP to simultaneously obtain both the relative and absolute abundances in the soil bacterial community. The results showed that a soil bacterial community and its dynamics can be better characterized by the HAAQ method when the optimal concentrations of ISS HAAQ-GFP (10^5 to 10^7 cells g^{-1}) were used, and a 16S rRNA gene copy number adjustment was applied. Based on the HAAQ method, we first found that soil bacterial absolute abundances at the genus level fitted well to the partial log-normal distribution function, and most genera concentrations were in the range of $10^{3.5}$ to $10^{6.5}$ cells g^{-1} in the test soils. Our case studies also indicated that more comprehensive descriptions of soil bacterial communities and their dynamics can be achieved by both the relative and absolute abundances than by the relative abundance alone. The improved HAAQ method can be potentially applied to other microbial ecological studies and to stimulating the development of quantitative bacterial ecology studies.

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1. Introduction

Soil microorganisms and their community play essential and vital roles in shaping terrestrial ecosystems and in regulating biogeochemical cycles of carbon, nutrients and pollutants (van der Heijden et al., 2008). This subject has been extensively studied by using conventional culture-based methods and, more recently, by culture-independent methods such as microarray hybridization and rRNA gene sequencing.

A community, as defined in ecology, is mainly described by three basic attributes, the absolute abundance, relative abundance and types of species (Molles, 2001). The relative abundance of species can be calculated from the absolute abundance, but not vice versa. In quantitative ecology, the absolute abundance provides exact information for one specie regardless of its population is declining, growing, or stable along spatial and temporal shifts, while the relative abundance describes how different species are composed together in a single community (Molles, 2001).

With the recently developed powerful and expedient high-throughput sequencing technology, thousands of operational taxonomic units (OTUs) can be detected in a single community, which dramatically expands our insightful understanding for their types of species and relative abundances (Roesch et al., 2007). Although the relative abundance can describe the dominant species in microbial communities, it is not as efficient as the absolute abundance when comparing the changes in species across multiple communities (Smets et al., 2016; Stokell et al., 2016; Props et al., 2017; Tourlousse et al., 2017; Zhang et al., 2017). Recently, high-throughput sequencing was combined with microbial quantification techniques, such as flow cytometry (FCM), quantitative PCR (qPCR), phospholipid fatty acids (PLFAs), to obtain the absolute abundances of microbial communities in aerosols, water, and soil (Lecuyer, 2014; Prest et al., 2014; Stokell et al., 2016; Props et al., 2017; Zhang et al., 2017; Lou et al., 2018). The inconsistent trends of the relative and absolute abundances in microbial communities were reported in these studies.

It is worthwhile to note that an accurate measurement of the total microbial quantification is critical to obtaining reliable absolute abundances in the microbial community. Measurements of the same sample with several methods are time-consuming, and a combination of different methods could add uncertainty to the results and, consequently, the conclusions. For example, the average of the total bacterial biomass in the soil samples of Beijing detected by the FCM method was higher than that in the soil samples of Tibet, whereas the averages of the total bacterial biomass indicated by the MBC measurement in the samples of Beijing and Tibet were the opposite (Zhang et al., 2017). Obviously, the calculated soil absolute bacterial abundances based on the results of FCM and MBC would be inconsistent. Therefore, it is important to propose a simple and consistent method that can not only reduce the operational procedures or uncertainties but also efficiently obtain reliable high-throughput absolute abundance quantification (HAAQ) of the microbial community.

In theory, the total absolute abundance of the soil bacteria can be estimated by monitoring a marked microorganism, which serves as an internal standard, with its known absolute and relative abundances. This approach is similar to the capture-mark-recapture method in which individuals in the first capture session are marked and then released back into the population. The ratio of marked to unmarked individuals in the subsequent recapture sessions was used to estimate the population size (Petit and Valiere, 2006; Rees et al., 2011). Then, the absolute abundances of each individual can be calculated from the corresponding relative abundance multiplied by the total absolute abundance.

Despite the use of bacterial DNA (Smets et al., 2016) or synthetic 16S rRNA genes (Tourlousse et al., 2017) as the internal standard for quantifying the absolute abundances of soil bacteria, many questions still remain, e.g., the optimal additional concentration of the internal standard, or the feasibilities of bacterial cells as an internal standard strain. To overcome the existing deficiencies in soil bacterial quantification methods, the objectives of this study were to (1) propose an HAAQ method for quantitatively characterizing a soil bacterial community and its dynamics by adding an internal standard strain (ISS) HAAQ-GFP, (2) identify optimal experimental conditions for the method, and (3) demonstrate the application of this improved method to reveal the bacterial population distribution and its dynamics across samples at different development stages (soil and its parent material) and in sodium azide (NaN_3) treated soils.

2. Materials and methods

2.1. General description of the HAAQ method

The main steps of the HAAQ method are outlined in Fig. 1. Firstly, the ISS HAAQ-GFP was absolutely quantified by the microscope count method. Then, the defined amount of ISS HAAQ-GFP suspension was added to the soil and stirred thoroughly with the soil particles prior to the soil DNA extraction. After the soil DNA extraction, the DNA sample was high-throughput sequenced for the 16S rRNA gene (relative abundance). With the relative and absolute abundances of ISS HAAQ-GFP, the total absolute abundance of the soil indigenous bacteria was calculated first. Then, the absolute abundances of each taxon were obtained through the total absolute abundance multiplied by its corresponding relative abundance. The quantitative bacterial ecology in the test soil was revealed by the absolute abundances at each taxon level and other derivative indexes.

2.2. Internal standard strain and its absolute quantification

The ISS HAAQ-GFP was generated from *Escherichia coli* O157:H7 strain EDL933 (ATCC 43895) and was labeled by green fluorescent protein (GFP) with the expression of the plasmid pGFPuv. The plasmid pGFPuv contained the complete GFP coding sequence and an ampicillin resistant gene, which was introduced into the competent strain EDL933 by the calcium chloride method, as described by Joseph Sambrook et al. (1989). Transformants were selected on the Luria-Bertani (LB) (Becton Dickinson, Franklin Lakes, USA) plate, which contained ampicillin (100 $\mu g\ mL^{-1}$), and with the emission of green fluorescence under an ultraviolet lamp as well as with fluorescent microscopy.

The ISS HAAQ-GFP cells were cultured in LB medium with ampicillin (100 $\mu g\ mL^{-1}$) for inoculation following the method by Wang et al. (2014). The bacterial cells were harvested by centrifugation at 4 °C (6,000 $\times g$ for 10 min) and washed three times with sterile water. The cell pellets were resuspended in sterile water and were subjected to 2-fold serial dilutions to examine the fluorescence signals from GFP of ISS HAAQ-GFP by a two-photon excitation laser scanning confocal microscope (Zeiss LSM 710 NLO, Jena, Germany). The GFP fluorescence of ISS HAAQ-GFP was excited by 488 nm and detected at 493–649 nm. To minimize the error caused by air bubbles, 8.00×10^{-3} mL of bacterial suspension was added to the interlayer (approximate 14.00 μm) between the slide and coverslip (22 mm × 26 mm). In each visual field of the microscope (which contained a liquid volume of 1.61×10^{-6} mL), cells ranging from 10 to 500 were countable, which corresponds to concentrations from 6.21×10^6 to 3.10×10^8 cells mL^{-1}. Ten images (339.74 μm × 339.74 μm) were taken randomly from different areas to quantify the ISS HAAQ-GFP. At the same time, the plate count and qPCR methods were also performed to count the ISS HAAQ-GFP (Material and methods S1 and S2, Supporting information).

2.3. Soil samples and experiment setup

The test soil samples were collected from the surface layer (A horizon) of the vegetable field and partially weathered layer (C horizon) of the soil profile in the provinces of Fujian (26°16′ N, 117°37′ E) and Zhejiang (29°18′ N, 119°18′ E), China, respectively. Three composite soil samples of each sampling site were taken to the laboratory using