Improved analysis of fungal communities using the next-generation-sequencing analysis of rpb2 genes

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Current exploration of the ecology of soil fungal and bacterial communities and microbe-catalyzed processes in soils largely rely on community composition analysis using next-generation-sequencing of PCR amplicons (1). Typically, the relative abundance of individual members of microbial communities are derived from the analyses of 16S rRNA region of prokaryotic microorganisms and 18S rRNA or internal transcribed spacer (ITS) region of the rDNA for fungi and other microeukaryots. The analysis of fungal ITS sequences is helpful tool for molecular systematics at the species level, and even within species, but the quantitative information on the relative abundance of individual taxa is skewed due to the presence of multiple rDNA gene copies per genome, ranging from 10 to 200 (2). On the other hand, it was demonstrated that there is a group of genes like the elongation factor-1 alpha (*tef1*) or RNA polymerase II second largest subunit (*rpb2*) that are consistently present in one copy per fungal genome and exhibit sufficient variation to be used for phylogenetic analysis and taxonomic assignment (3). The use of such genes offers the possibility to directly count fungal genomes and improve the knowledge on the relative importance of individual taxa of fungi in the environmental processes. Here we demonstrate that the amount of ITS copies per nanogram DNA shows high variation among soil basidiomycetes and even closely related species largely differ in this respect. We also demonstrate that the use of the *rpb2* gene is applicable for analysis of soil fungal communities and that the data derived using this molecular marker are largely different from those based on the amplicon sequencing of the ITS. Although the phylogeneti discriminative power of the *rpb2* gene is limited, it still offers a suitable tool to infer fungal taxonomy at least on the level of families.

References

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