Response to Comment on “Global diversity and geography of soil fungi”

Leho Tedersoo,1* Mohammad Bahram,2,3 Sergei Põõme,1 Sten Anslam,2 Taavi Rii,2 Urmas Kõljalg,2 R. Henrik Nilsson,4 Falk Hildebrand,5 Kessy Abarenkov1 Schadt and Rosling (Technical Comment, 26 June 2015, p. 1438) argue that primer-template mismatches neglected the fungal class Archaeorhizomycetes in a global soil survey. Amplicon-based metabarcoding of nine barcode-primer pair combinations and polymerase chain reaction (PCR)–free shotgun metagenomics revealed that barcode and primer choice and PCR bias drive the diversity and composition of microorganisms in general, but the Archaeorhizomycetes were little affected in the global study. We urge that careful choice of DNA markers and primers is essential for ecological studies using high-throughput sequencing for identification.

S chadt and Rosling (1) argue that primer-template mismatches neglected the fungal class Archaeorhizomycetes in a global soil survey. Fungi are the dominant eukaryote kingdom in soil (2). The recently described fungal class Archaeorhizomycetes accounts for a high proportion of soil fungal communities around the world (3, 4), ranging from 0 to 20% in our recent global study (5). Schadt and Rosling argue that the relative importance of this group has been substantially underestimated in our study because our molecular identification approach may have involved a primer bias—i.e., distorted taxon distribution as a result of imperfect primer matches with some templates. We decided not to account for two unique mismatches of the ITS4ngs primer to Archaeorhizomycetes (2, 3), owing to its relatively low taxonomic diversity with two described species (0.002% of all described taxa) and a few hundred species-level taxa based on DNA sequences recovered from the soil environment (4, 6).

To gain insight into the magnitude of this primer bias, we generated a data set comprising amplicon-based sequencing of nine barcode-primer pair combinations (Fig. 1A) and polymerase chain reaction (PCR)–free shotgun metagenomics for a subset of the samples from Papua New Guinea (2, 5). Although differences in taxonomic richness and relative abundance of all fungal classes were highly significant (Fig. 1, B and C), there was no evidence for specific discrimination against Archaeorhizomycetes by one or two central mismatches in the primers SSU515ngs, ITS4ngs, or LR0Rngs (2). However, mismatches near the 3′ end of a primer reduced the perceived abundance of particular taxa (for example, the primers ITS4ngs in Sordariomycetes, ITS1Fngs in Mucoromycetes, ITS2 in Tremellomycetes and Glomeromycetes, and LF402 in Chytridiomycetes). Introns within barcodes accounted for another potential source of bias in Geoglossomycetes (the ITS1ngs-ITS2 and LR3R-LR3 primer pairs). In addition, a greater proportion of G and C nucleotides and greater barcode length reduce the chances of successful amplification and/or emulsion PCR of DNA molecules (7–9). Despite all these PCR and primer biases, the median amplicon-based abundance of fungal classes was strongly correlated with abundance estimates based on the shotgun metagenomics approach (F = 111.9; R2 = 0.867; P < 0.001). Furthermore, the metagenomics data analysis confirmed that Archaeorhizomycetes, as well as arbuscular mycorrhizal Glomeromycetes, exhibit low relative abundance in these tropical forest soils (5). The extreme dominance of Archaeorhizomycetes in some previous studies may have derived from a cloning bias favoring relatively

Fig. 1. Overview of barcode-primer pair combinations. (A) Primer map indicating variable domains, position of primers (identifier-tagged primers in red), and intron sites within barcodes (triangles). (B) Total taxonomic richness based on samples rarefied to 8609 sequences, with different letters indicating significantly different groups. (C) Proportion of sequences corresponding to the 16 most common fungal classes (median amplicon-based abundance >0.1% sequences; shaded columns) as based on the average values of amplicons (open columns) and shotgun metagenomes (red columns). Error bars, letters, asterisks, and triangles indicate standard error, significantly different groups, primer-template mismatches, and introns within barcodes, respectively. Numbers behind class names indicate variation (R2adj) explained by the choice of primers. ITS1a, primer pair ITS1Fngs-ITS2; ITS1b, ITS1ngs-ITS2; ITS2a, ITS3mixtag-ITS4ngs; ITS2b, gITS7-ITS4ngs (5).
short internal transcribed spacer (ITS) amplicons characteristic of this fungal class (2).

Schadt and Rosling also argue that the primer discrimination against Archaeorhizomycetes affects the perceived proportion of functional groups. Indeed, our results showed that the choice of primers explains 8.6 and 62.0% of variation in the proportion of ectomycorrhizal (ITS data set: \(F_{3,72} = 70.14; P < 0.001\)) and arbuscular mycorrhizal (full data set: \(F_{8,205} = 90.64; P < 0.001\)) symbionts, respectively. However, the disparity in ectomycorrhizal fungi is ascribed to the overestimation of relative abundance of Agaricomycetes, which comprise ~95% of all ectomycorrhizal fungal species (10,11), by the ITS1 barcode. Because the class-level abundance of fungi based on ITS3tagmix-ITS4ngs primer pair (4) had the strongest correlation among all nine primer pairs to the respective median values (\(F_{1,37} = 827.4; R_{Pearson} = 0.978; P < 0.001\)), we reckon that the abundance estimates of functional groups were relatively little biased in our global study. Furthermore, these primers recovered the greatest species- and class-level diversity of all primer pairs tested (Fig. 1B), which encourages their use in future studies (2).

In conclusion, thorough consideration of primers and barcodes is clearly essential for the highly sensitive metabarcoding analyses. Similarly, anticipating unaccounted biases is of utmost importance in molecular studies of plant, animal, and microbial community ecology.

REFERENCES

9 January 2015; accepted 29 July 2015
10.1126/science.aaa5594
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Science 349 (6251), 936.
DOI: 10.1126/science.aaa5594