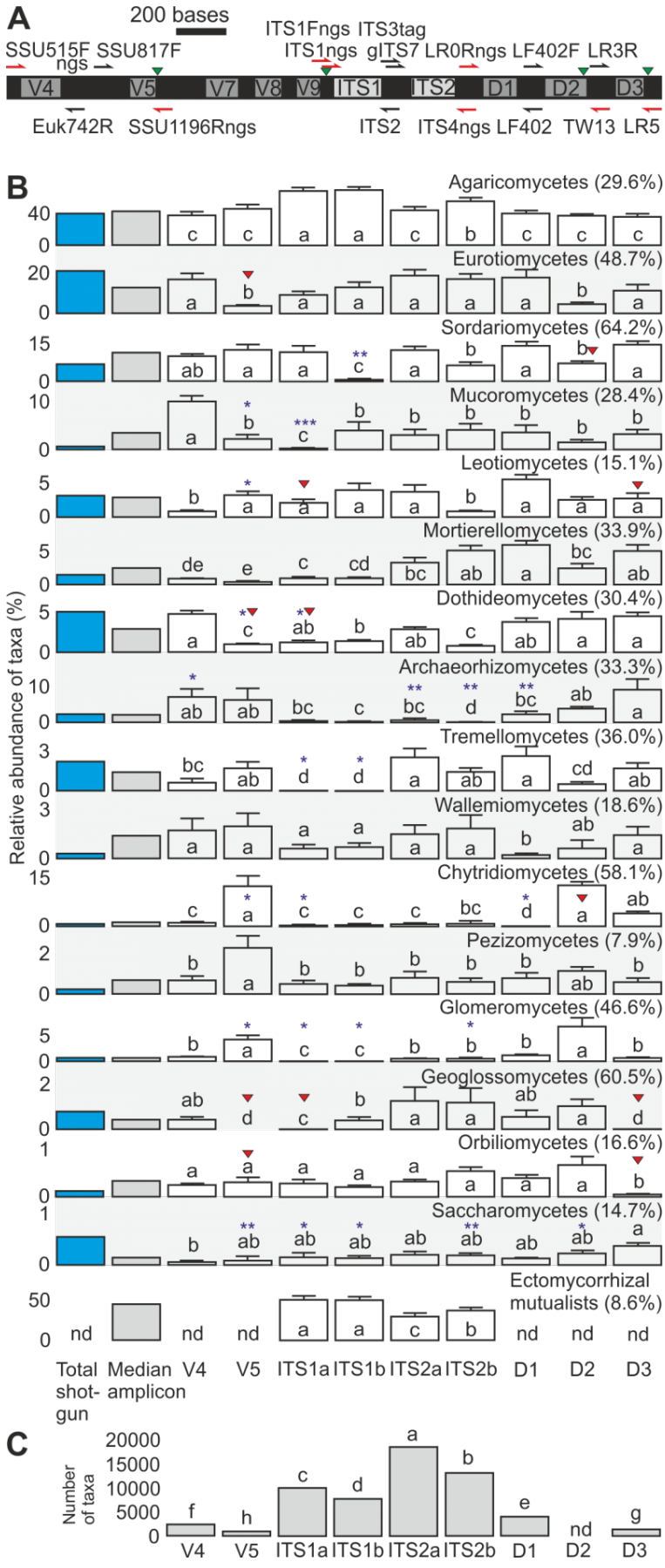
# Microbial diversity insights are often strongly biased

September 2 2015


A
200 bases
SSU515F ngs
SSU817F
ITS1Fngs
ITS3tag
ITS1ngs
gITS7
LR0Rngs
LF402F
LR3R
V4
V5
V7
V8
V9
ITS1
ITS2
D1
D2
D3
Euk742R
SSU1196Rngs
ITS2
ITS4ngs
LF402
TW13
LR5
B
Agaricomycetes (29.6%)
Eurotiomycetes (48.7%)
Sordariomycetes (64.2%)
Mucoromycetes (28.4%)
Leotiomycetes (15.1%)
Mortierellomycetes (33.9%)
Dothideomycetes (30.4%)
Archaeorhizomycetes (33.3%)
Tremellomycetes (36.0%)
Wallemiomycetes (18.6%)
Chytridiomycetes (58.1%)
Pezizomycetes (7.9%)
Glomeromycetes (46.6%)
Geoglossomycetes (60.5%)
Orbiliomycetes (16.6%)
Saccharomycetes (14.7%)
Ectomycorrhizal mutualists (8.6%)
Relative abundance of taxa (%)
Total shot-gun
Median amplicon
V4
V5
ITS1a
ITS1b
ITS2a
ITS2b
D1
D2
D3
C
Number of taxa
20000
15000
10000
5000
0

Overview of barcode-primer pair combinations: (A) primer map indicating variable domains, position of primers and intron sites within barcodes (triangles); (B) 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 (blue 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; (C) total taxonomic richness based on samples rarefied to 8609 sequences with different letters indicating significantly different groups. Credit: Dr. Leho Tedersoo

Substantial methodological biases in soil fungal diversity were demonstrated by an Estonian-German research consortium (University of Tartu and EMBL). It turns out that even sophisticated and innovative approaches such as DNA barcoding and PCR-free analyses are likely to end up with biased information when researching the taxonomic community composition of soil biota. The study is published in both open access journal *MycoKeys* and *Science* magazine.

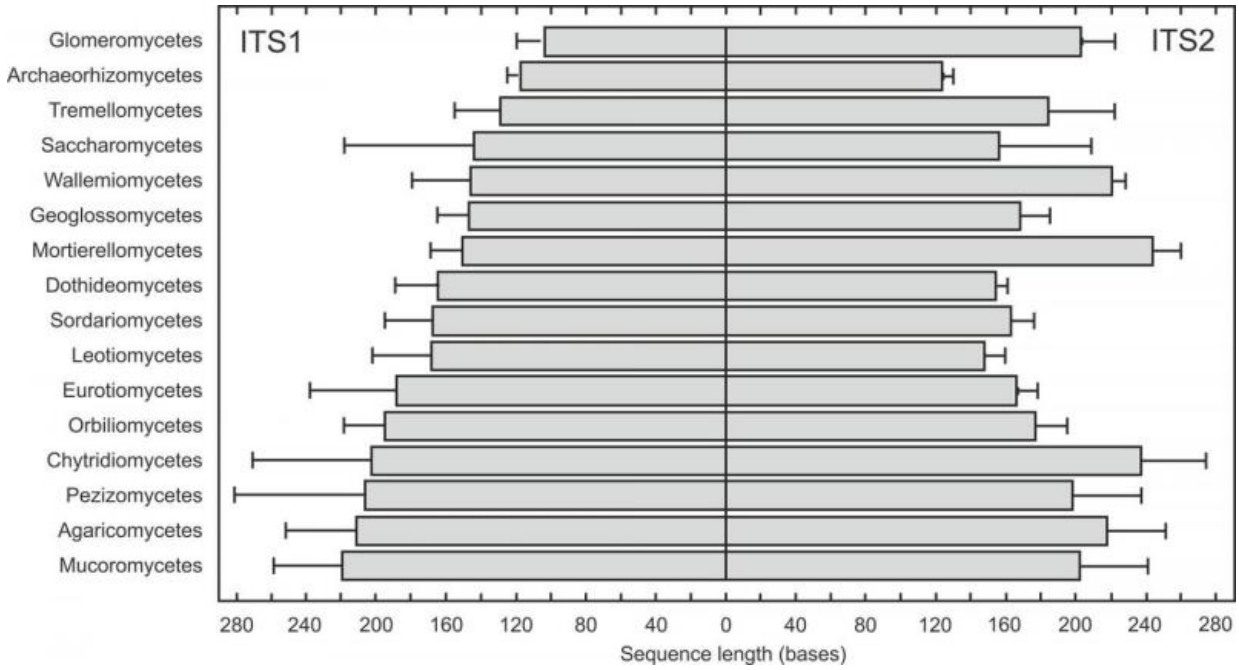
High-throughput Illumina sequencing of DNA metabarcodes and the whole soil metagenome revealed strong methodological biases in taxonomic insights into soil fungal diversity. All methods had their inherent biases and shortcomings, but reached roughly similar ecological conclusions indicating the greatest role of floristic variables on soil fungal communities in the mountainous Papua New Guinea.

"I was motivated by a criticism on our last year's *Science* article on global

fungal diversity, where the abundance of certain fungi were suspected of being underestimated", explained Dr. Leho Tedersoo, soil ecologist and mycologist from the University of Tartu, Estonia, and leading author of the present paper.

"The most intriguing result is that all these innovative, high-throughput molecular methods have their serious inherent biases. For example, amplicon-based methods depend strongly on taxonomic resolution of the barcode (DNA fragment used for identification), primer-template mismatches, the presence of introns and the overall length of the barcode; but conversely, PCR-free methods are affected strongest by the availability of taxonomic reference information, which differs enormously for fungal classes and phyla", added Leho.

In conclusion, Dr. Tedersoo pointed out that the recently developed PCR-free methods do not provide a magic wand for understanding the taxonomic community composition of soil biota, but nonetheless, these methods have a great potential in understanding the functional capacity of microorganisms.




Differences in sequence length in the ITS1 and ITS2 barcodes of 16 most abundant fungal classes as revealed based on amplicon libraries in this study. Columns, and error bars represent mean values and standard deviation, respectively. Credit: Dr. Leho Tedersoo

**More information:** Tedersoo L, Anslan S, Bahram M, Põlme S, Riit T, Liiv I, Kõljalg U, Kisand V, Nilsson RH, Bork P, Hildebrand F, Abarenkov K. 2015. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. *MycoKeys* 10: 1-43. DOI: 10.3897/mycokeys.10.4852

Tedersoo L, Bahram M, Põlme S, Anslan S, Riit T, Kõljalg U, Nilsson RH, Hildebrand F, Abvarenkov K. 2015. Response to Comment on "Global diversity and geography of soil fungi": Analytical biases in microbial diversity studies. *Science* 359: 936. DOI: 10.1126/science.aaa5594

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