

The gut mycobiome in pediatric multiple sclerosis: Establishing a bioinformatics pipeline

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Introduction

- Studies examining the role of the microbiota in multiple sclerosis (MS) often focus on the gut bacteria; few have considered a potential role of gut fungi (mycobiota).^{1, 2, 3}
- Methods for evaluating gut mycobiota are lacking and require systematic evaluation of sequencing protocols, reference databases, and bioinformatics pipelines to properly investigate possible gut mycobiome influences on MS.^{1, 4, 5}

Objectives

To evaluate the performance of different sequencing conditions and analytical approaches for characterizing the gut mycobiome in a mock fungal community for downstream assessment of unaffected controls, and individuals with monophasic acquired demyelinating syndrome (mono ADS) or pediatric-onset MS.

Methodology

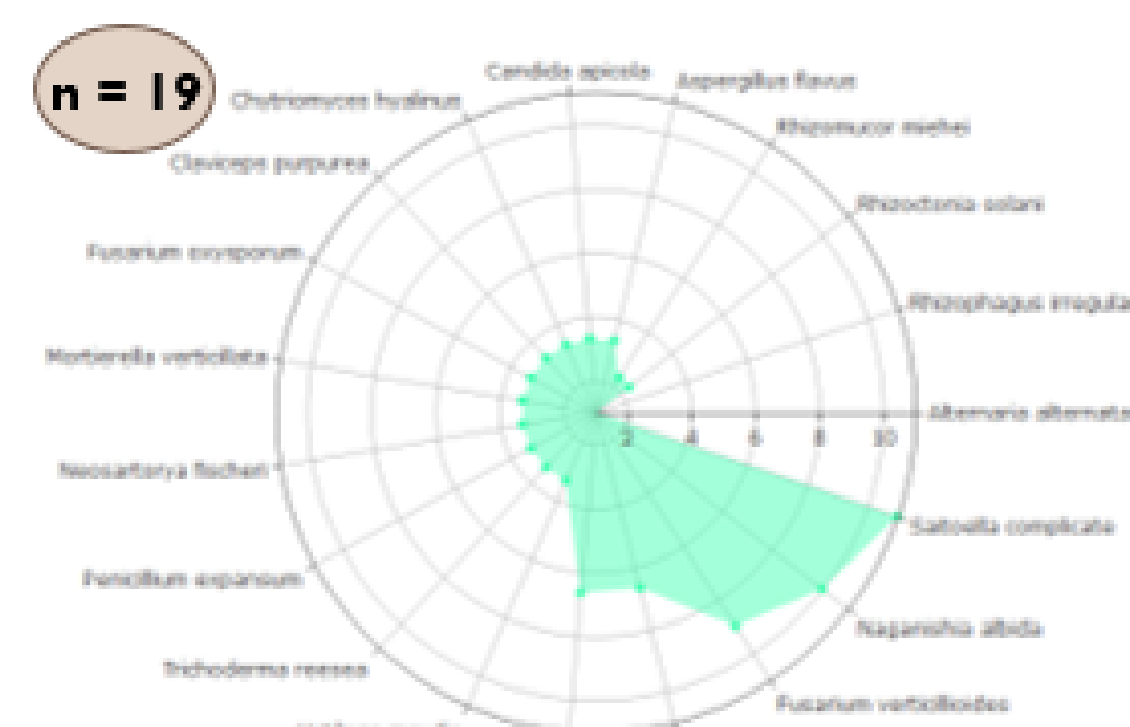


Figure 1. Log₂-transformed expected relative abundances of a 19-taxon fungal mock community⁶. Ribosomal gene copies are estimated by 18S rDNA qPCR.

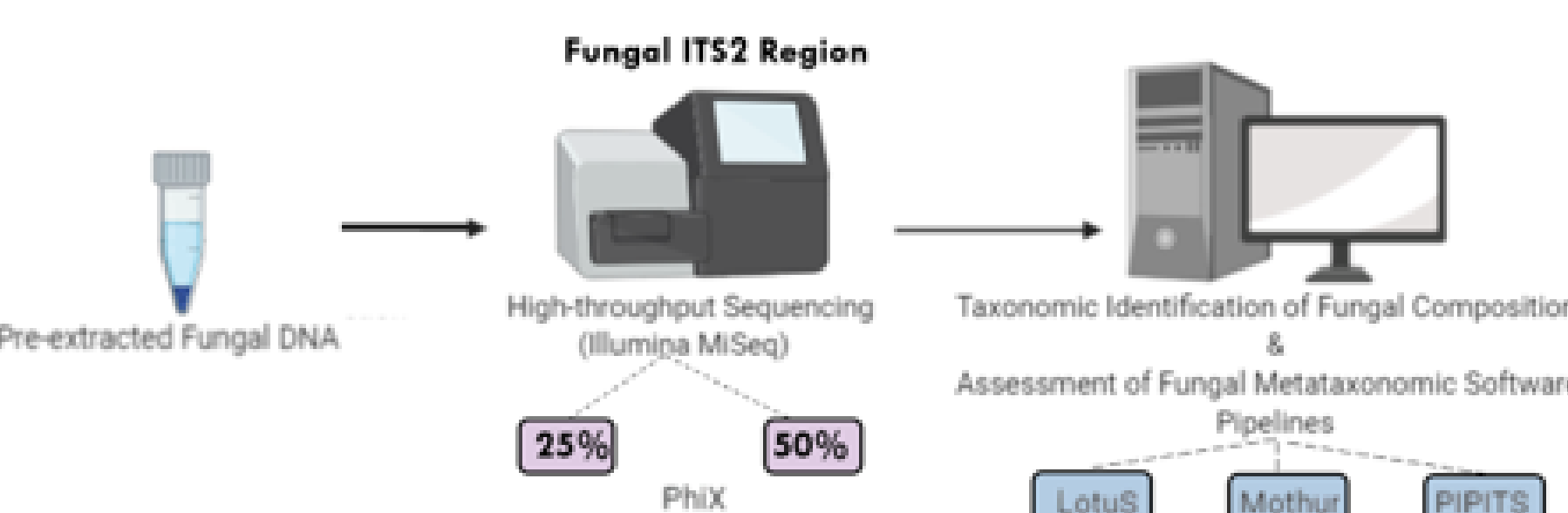


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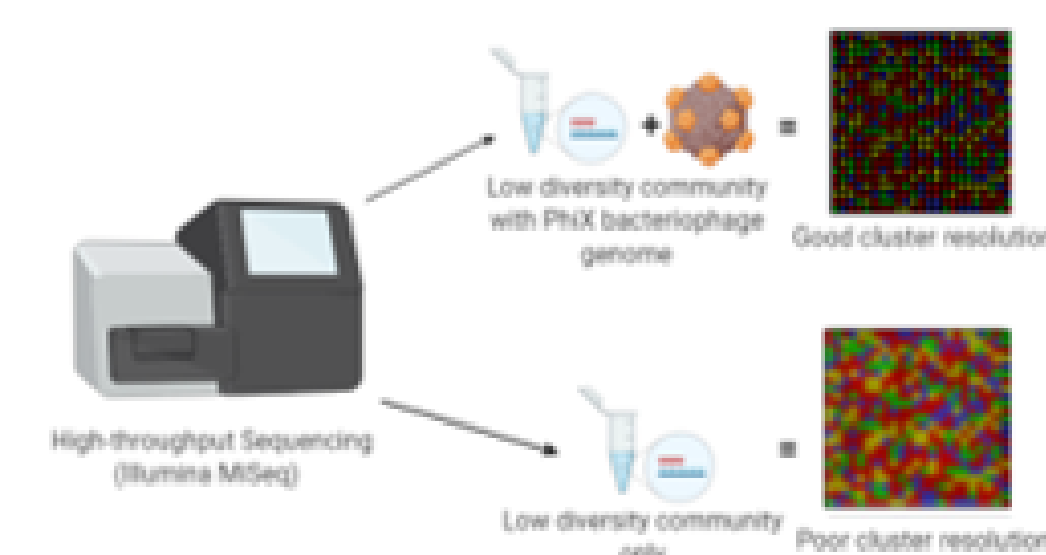


Figure 3. Addition of PhiX genome to low-diversity samples improves signal quality.

Results

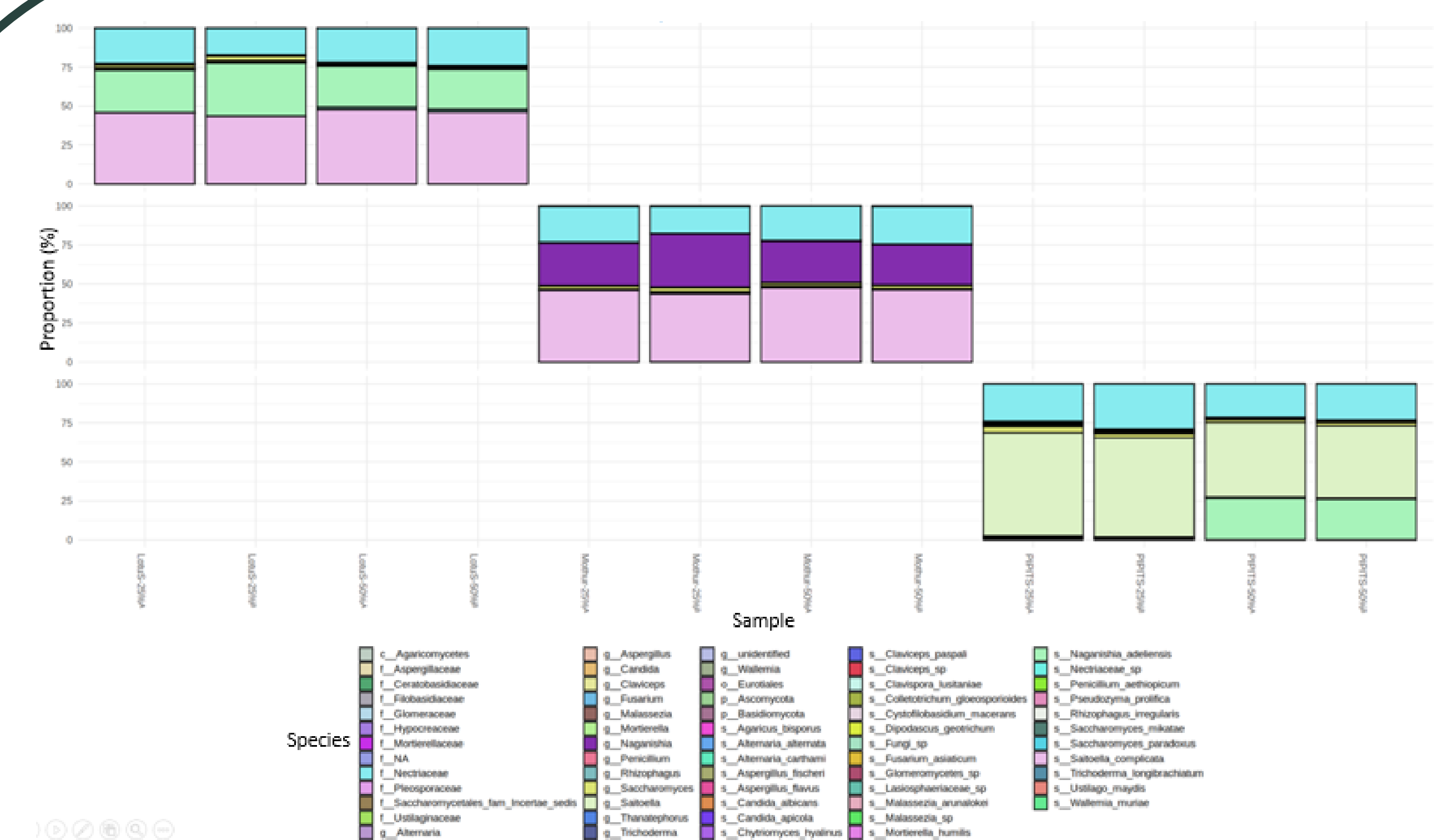


Figure 4. Stacked bar chart showing fungal compositions by comparing PhiX spike-in concentrations (25% or 50%) between technical replicates per sequencing pipeline. Abbreviations per pipeline is as follows: PIPITS stands for (pip)eline for analyses of fungal internal transcribed spacer (ITS) sequences; Lotus stands for (l)ess (OTU) (s)cripts; including mothur; all pipelines are open source software packages for fungal metataxonomic analysis.

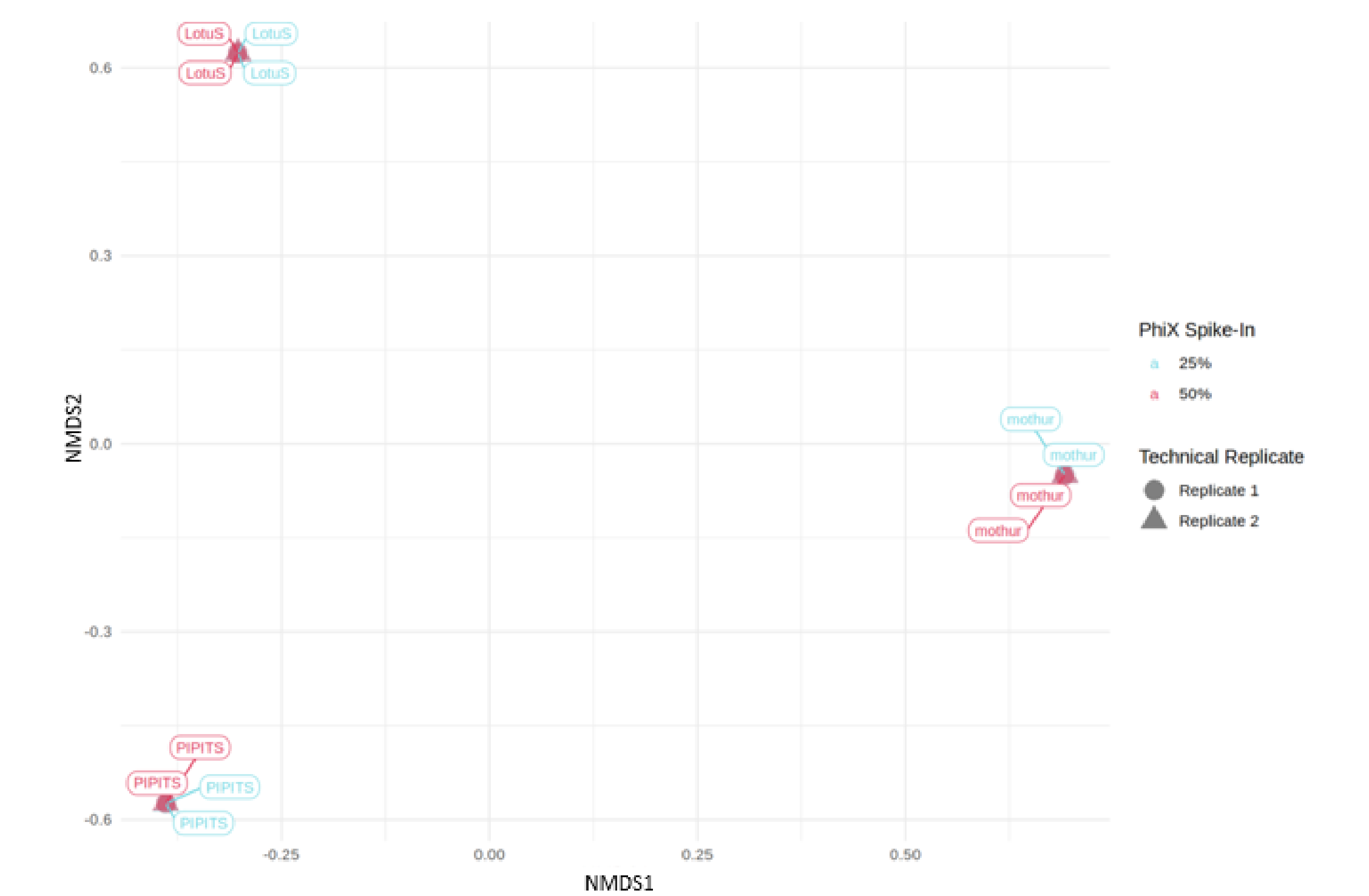


Figure 5. Beta diversity between mock sequenced samples using nonmulti-dimensional scaling. Points are labeled by the percent PhiX used during sequencing, technical replicate number, and pipeline used for analysis. The distance metric used is the Morisita-Horn index.

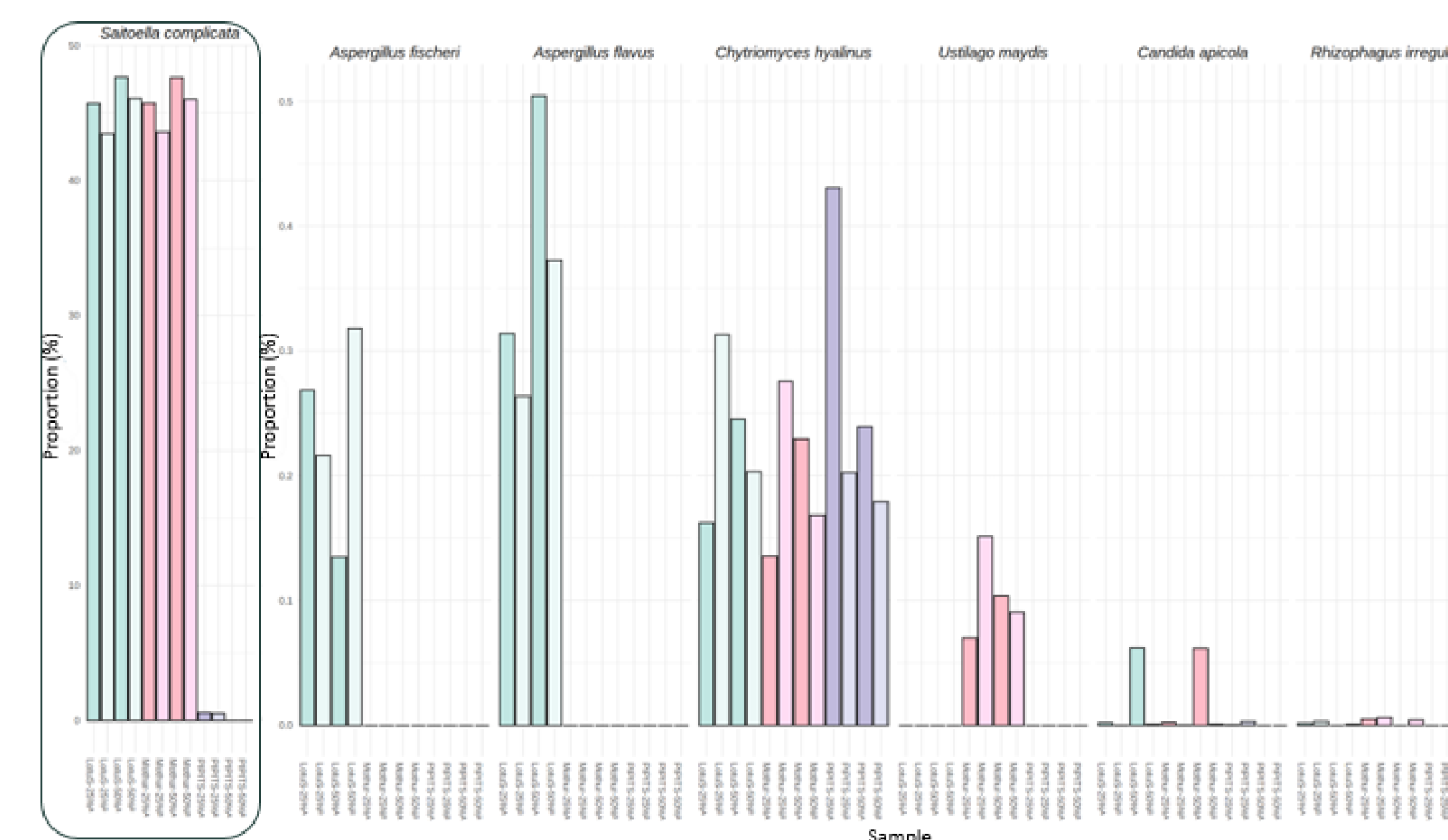


Figure 6. Filtering result for the 19 expected fungal mock community organisms. Samples are named by pipeline, followed by PhiX spike-in level and paired by technical replicates.

Conclusions

- Fungal metataxonomic pipelines tested: Lotus, mothur, and PIPITS all differ in compositional identification.
- Sequencing using 25% PhiX yields a slight identification advantage.
- The Lotus pipeline best identified fungal taxa in our mock-community, with optimal resolution to species level.
- Establishment of this validated experiment, confirmed using a mock-community with known fungal identities, will aid characterization of gut mycobiomes for our cohort of individuals with/without pediatric-onset MS.

References

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Disclosures/financial relationships with any commercial interest, past 12 months of the authors

Mr. Mok, Dr. Knox, Dr. Forbes, Dr. Graham, Ms. Hart, Dr. O'Mahony, and Mr. Zhu have no disclosures. Dr. Arnold reports personal fees for consulting from Acorda, Biogen, Celgene, F. Hoffmann-La Roche, Frequency Therapeutics, Genentech, Merck-Serono, Novartis, and Sanofi-Aventis; grants from Biogen, Immunotec and Novartis; and an equity interest in NeuroRx Research. Dr. Bar-Or serves on scientific advisory boards for Alara Biotherapeutics, Biogen Idec, Celgene/Receptos, Janssen/Actelion, Merck/EMD Serono, Novartis, Roche/Genentech and Sanofi-Genzyme, and has sponsored research agreements with Biogen Idec, Novartis, EMD-Serono and Roche/Genentech. Dr. C.N. Bernstein is supported in part by the Bingham Chair in Gastroenterology. He has been on the speaker's bureau for Abbvie Canada, Janssen Canada, Takeda Canada and Medtronic Canada. He has received unrestricted educational grants from Abbvie Canada, Janssen Canada, Pfizer Canada, and Takeda Canada and has done contract research with Abbvie, Janssen, Pfizer, Celgene, Boehringer Ingelheim, and Roche. Dr. Marrie has received research funding from CIHR, MS Society of Canada, Crohn's and Colitis Canada, NMSS, CMSC, the Arthritis Society outside of the submitted work. Dr. Yeh reports grants from CMSC, NMSS, MS Society of Canada/MS Scientific Research Foundation, Biogen, CIHR, CIRM, SCN, SickKids Foundation, CBMH, meeting support from the Guthy Jackson Foundation, Honoraria from MS At the Limits, Exomed, Alexion, Biogen. All outside of the submitted work. Dr. Van Domselaar has received research support in the last 3 years from Genome Canada and CIHR. Dr. Banwell serves as a consultant to Roche, UCB, Novartis, and Janssen. Dr. Banwell has served as a non-remunerated advisor to Biogen IDEC and Teva Neuroscience. Dr. Banwell receives grant funding from the Canadian MS Society and Research Foundation, NMSS, and NIH. Dr. Waubant has received honoraria for presentation from ANA, AAN, MS@theLimit, and for consulting from Jazz Pharma, and Emerald. She is funded by the NIH, the NMSS, the Race to Erase MS & PCORI. Dr. Tremlett is the Canada Research Chair for Neuro-epidemiology & MS and has received research support in the last 3 years from the: National MS Society, Canadian Institutes of Health Research, Canada Foundation for Innovation, MS Society of Canada, and the MS Scientific Research Foundation.

University of Manitoba



The VADA Program
Visual and Automated Disease Analytics
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Multiple Sclerosis Society of Canada



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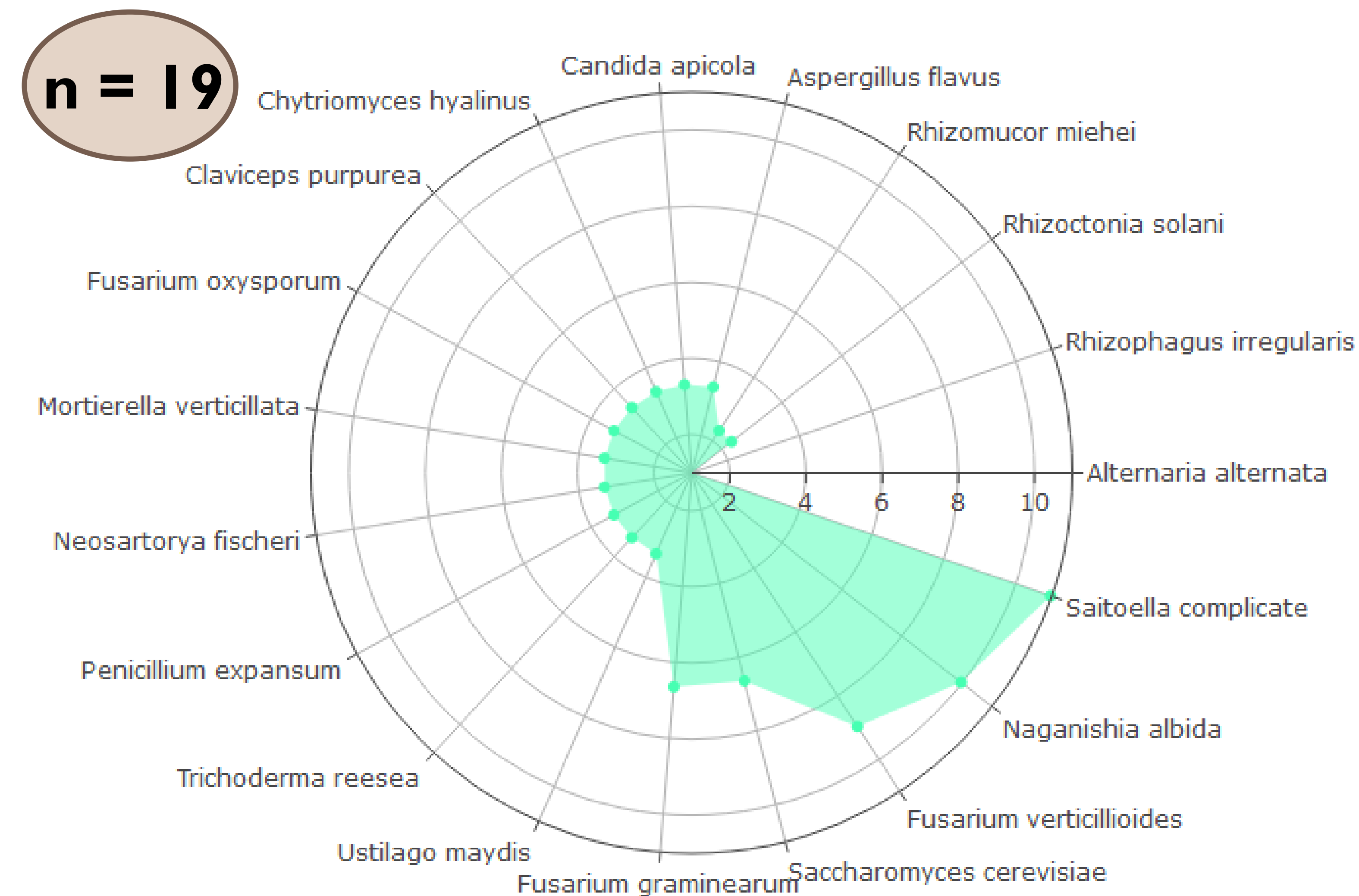


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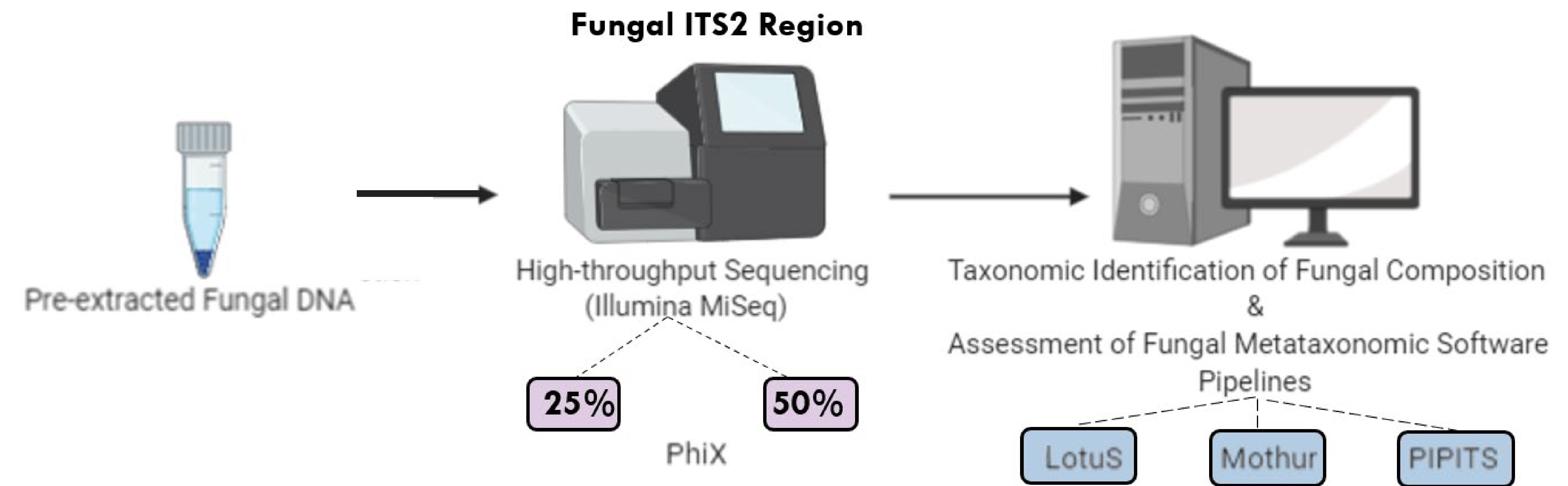


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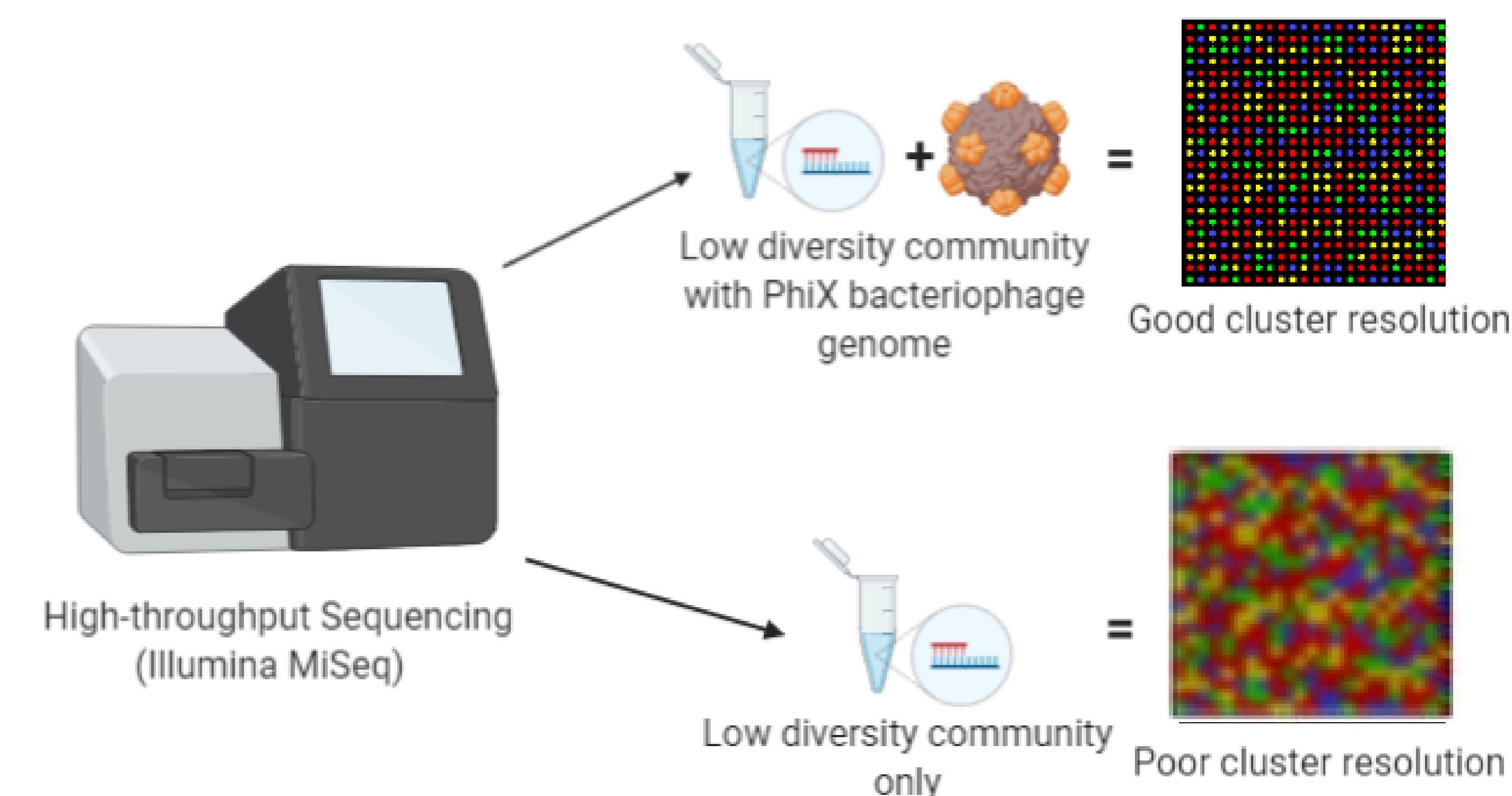


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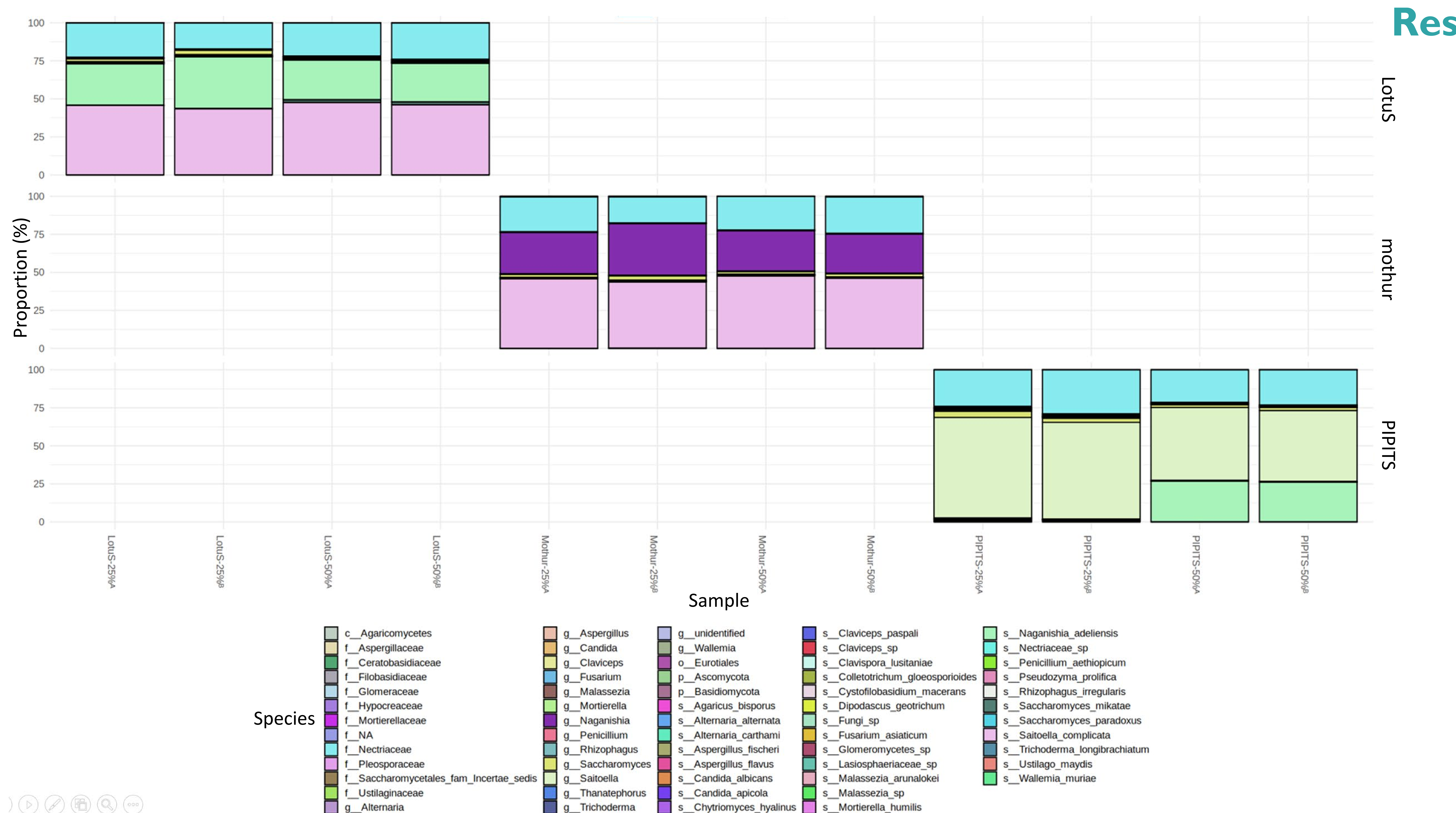


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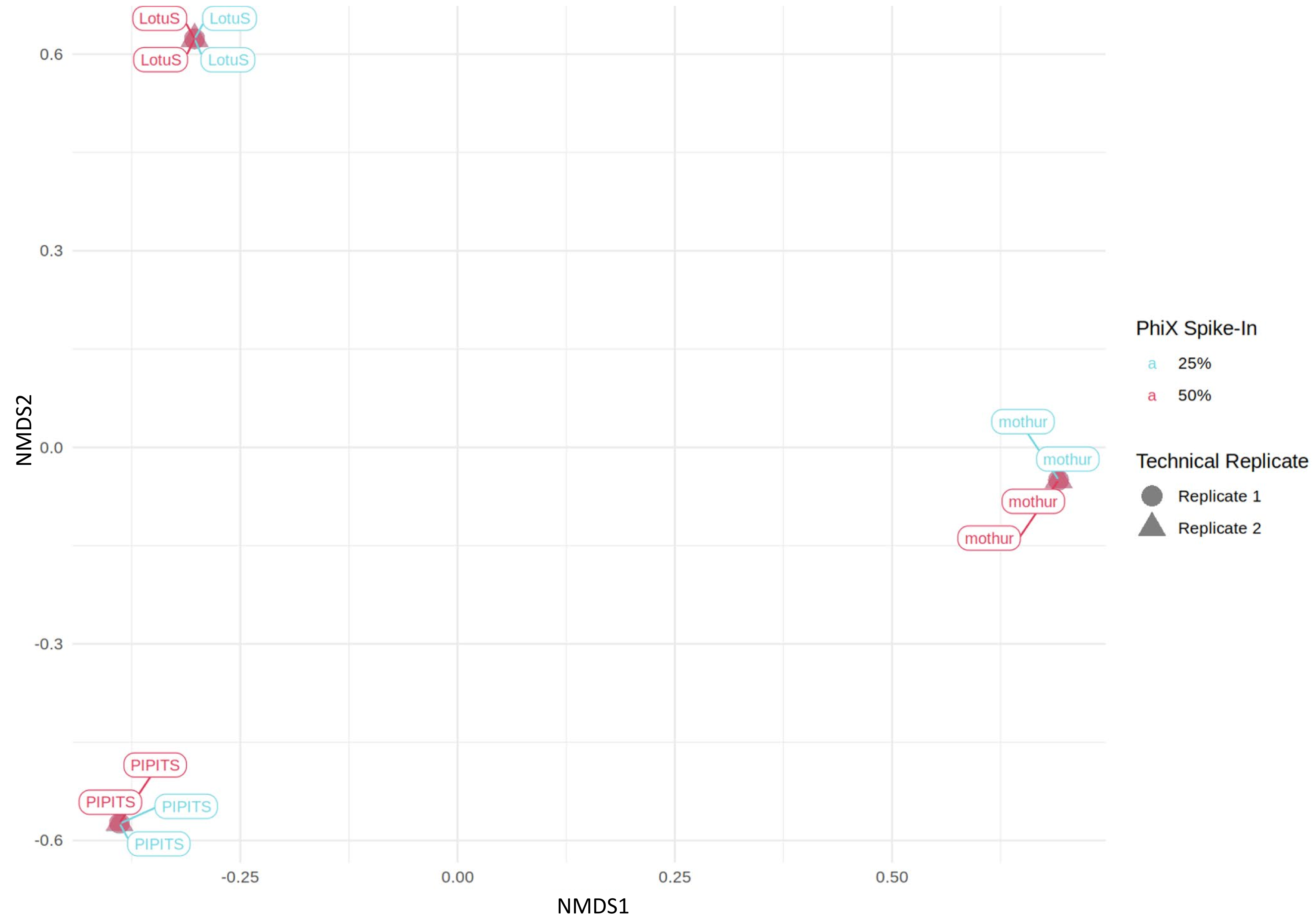


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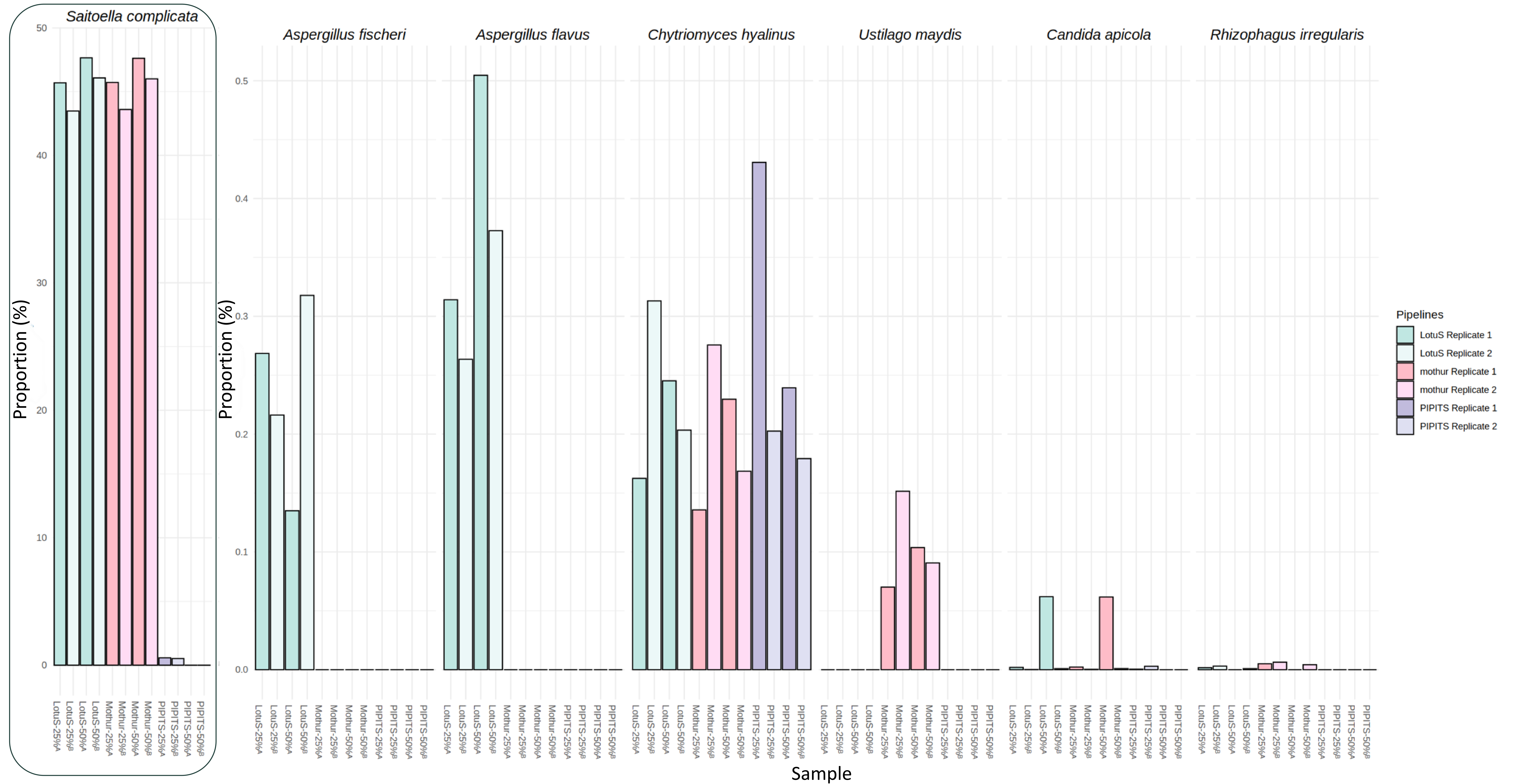


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