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Its all fun guys: a comparison of bioinformatic pipelines for metabarcoding plant and soil fungal communities

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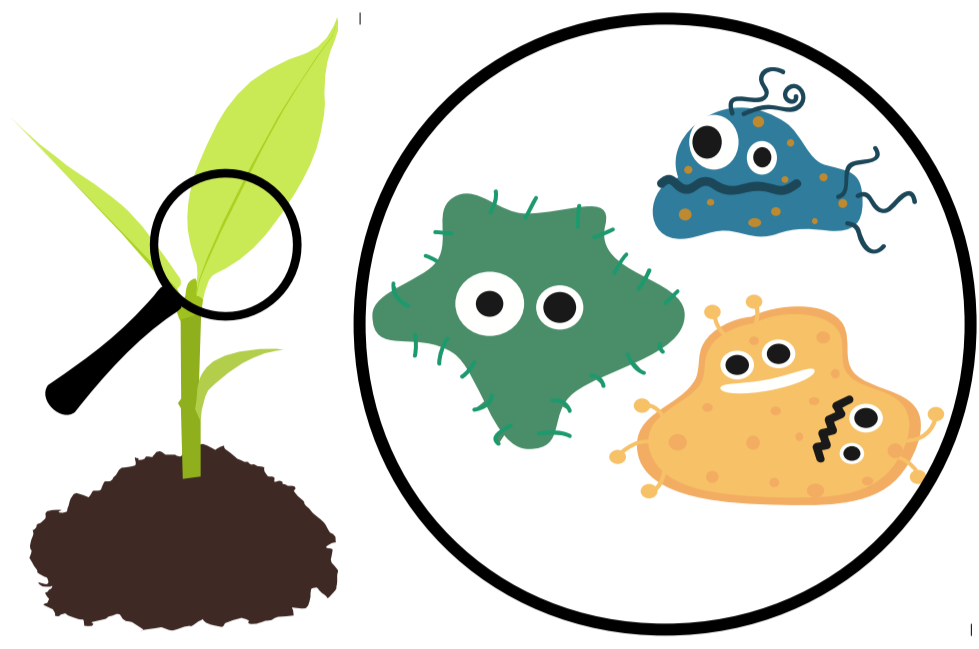


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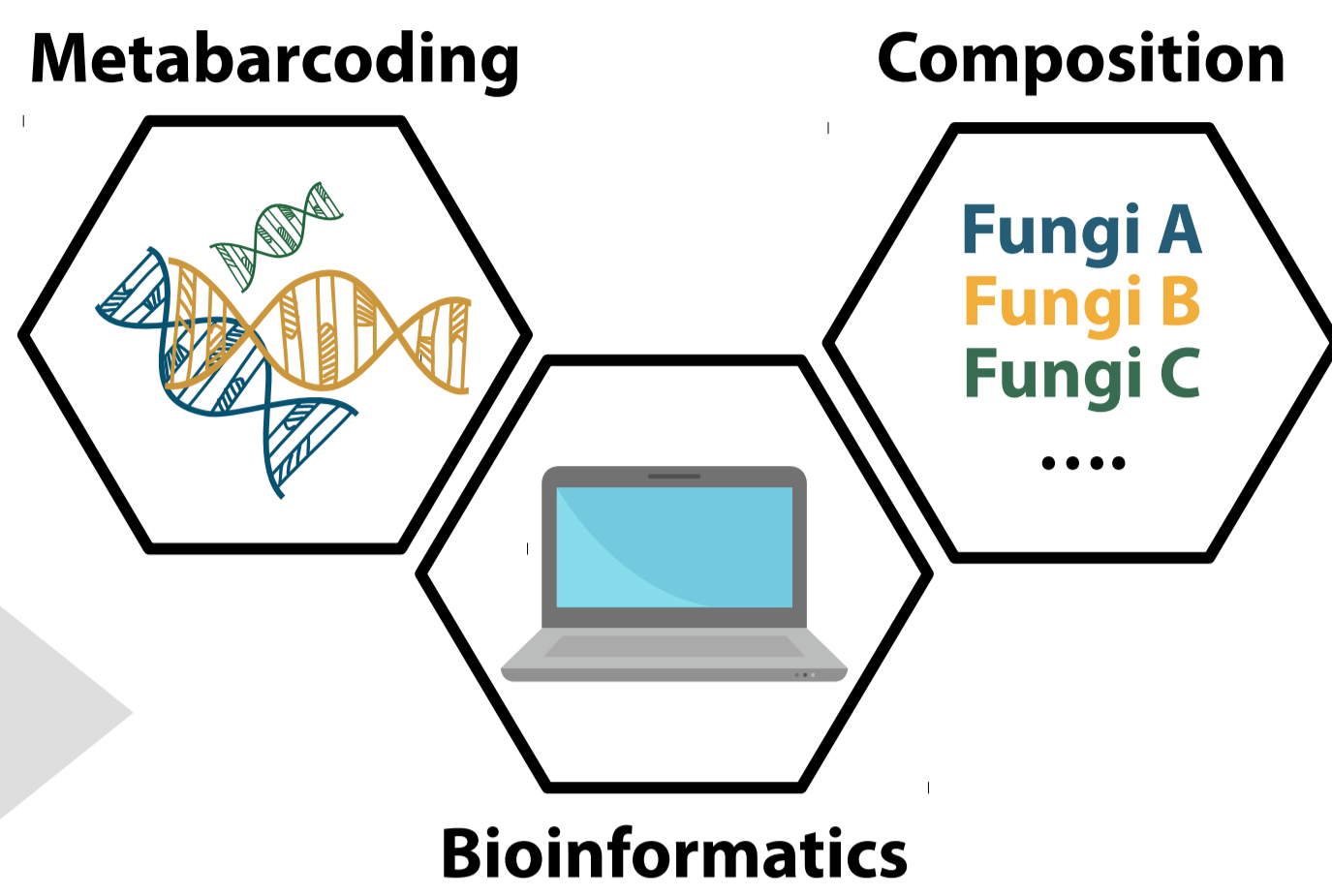
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Introduction

Plant and soil fungal communities influence plant fitness and ecosystem functioning.



These fungi are classically studied by **metabarcoding** approaches targeting the ribosomal internal transcribed spacer (ITS)



But there is **no clear consensus** concerning the most appropriate bioinformatic pipeline.

Objectives

1. Make and sequence a **mock community** of 189 Dikarya strains commonly found in agricultural and forest soils and in plant tissues.
2. Compare the ability of 288 **combinations of bioinformatic softwares and parameters** to recover the fungal strains present in this mock community, in the expected proportions.

Methods

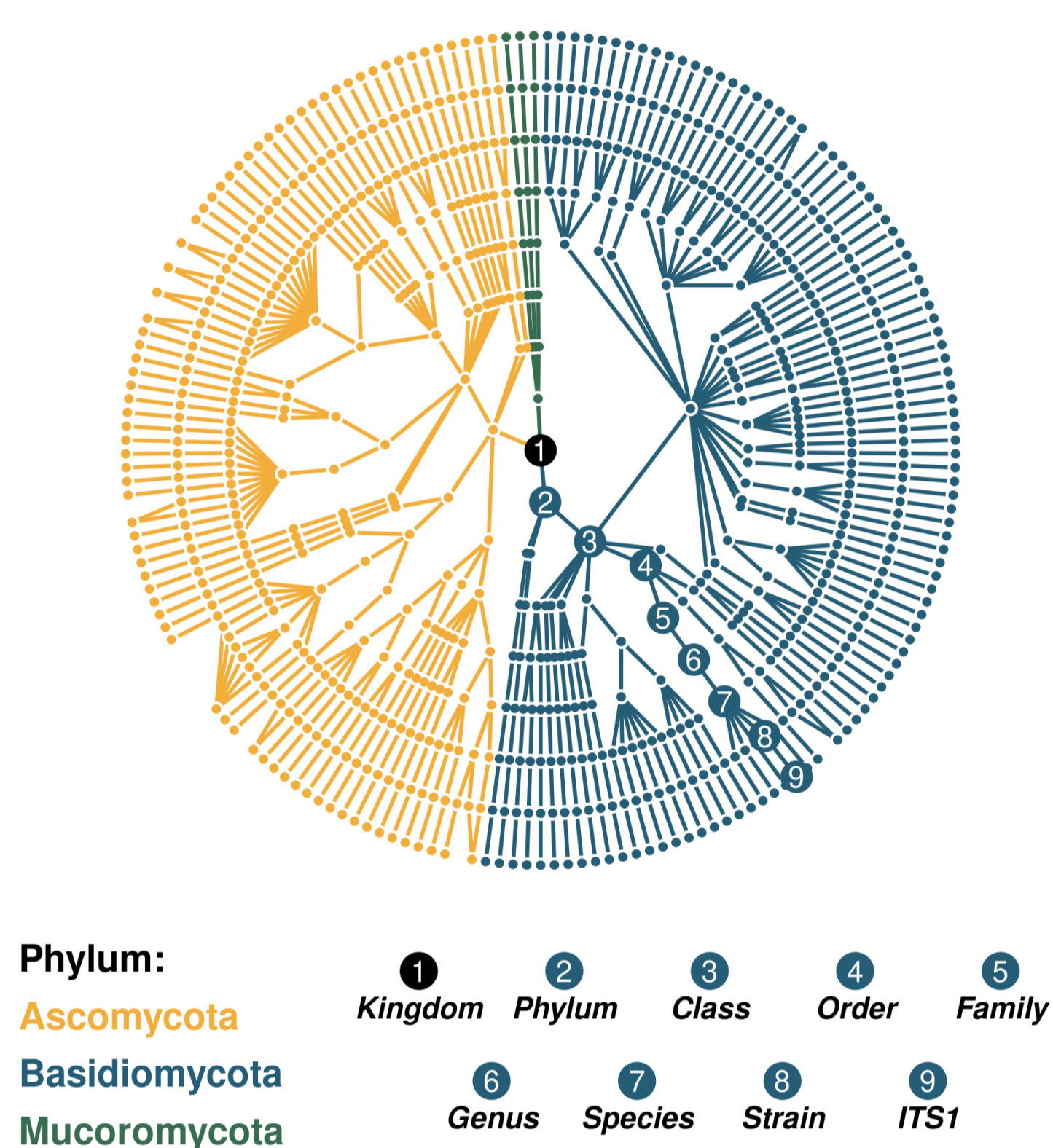


Fig 1 Taxonomic composition of the artificial fungal community. The nodes of the tree are the ITS1 sequences ($n = 175$) of the fungal strains ($n = 189$) that constitute the artificial or « mock » community.

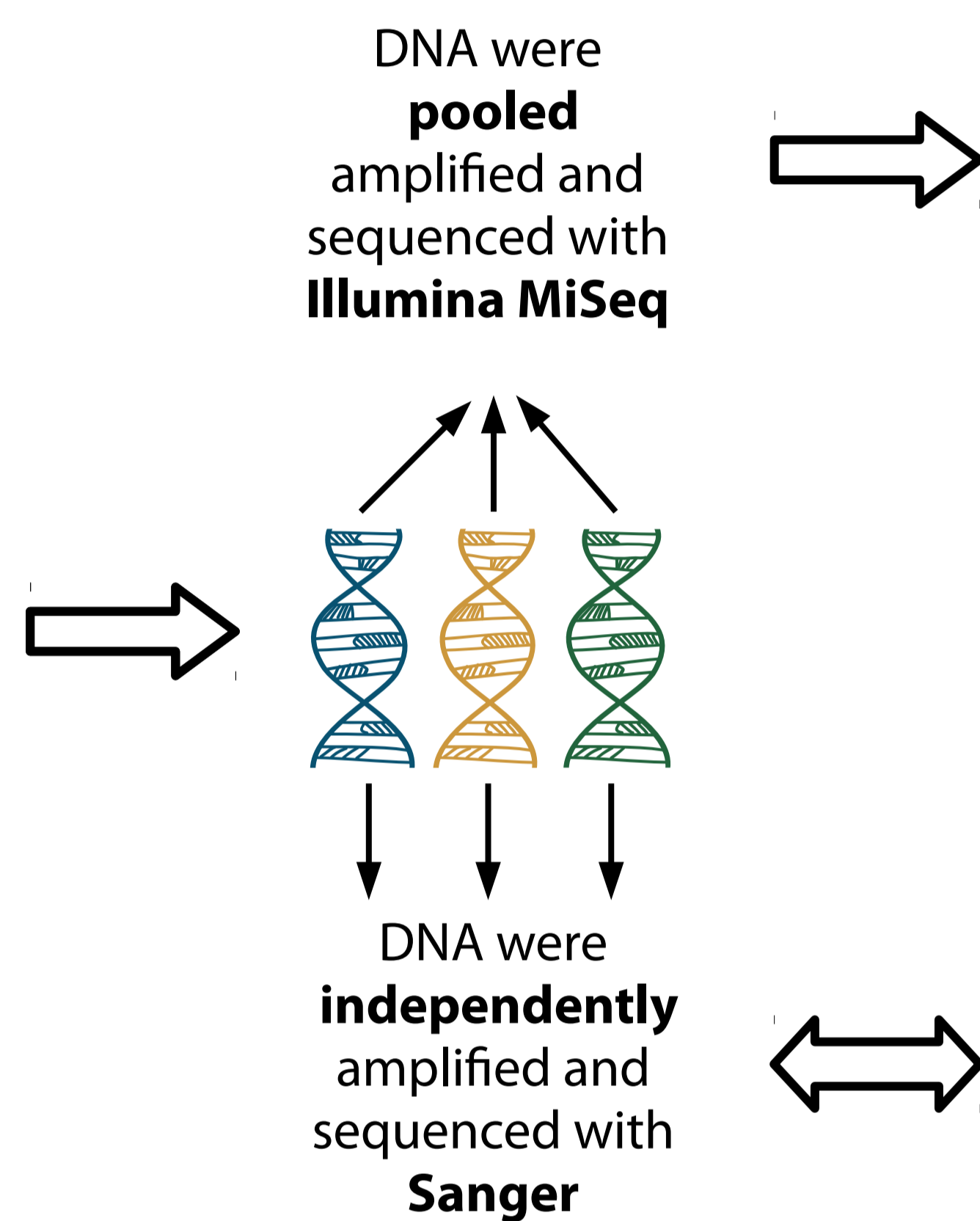
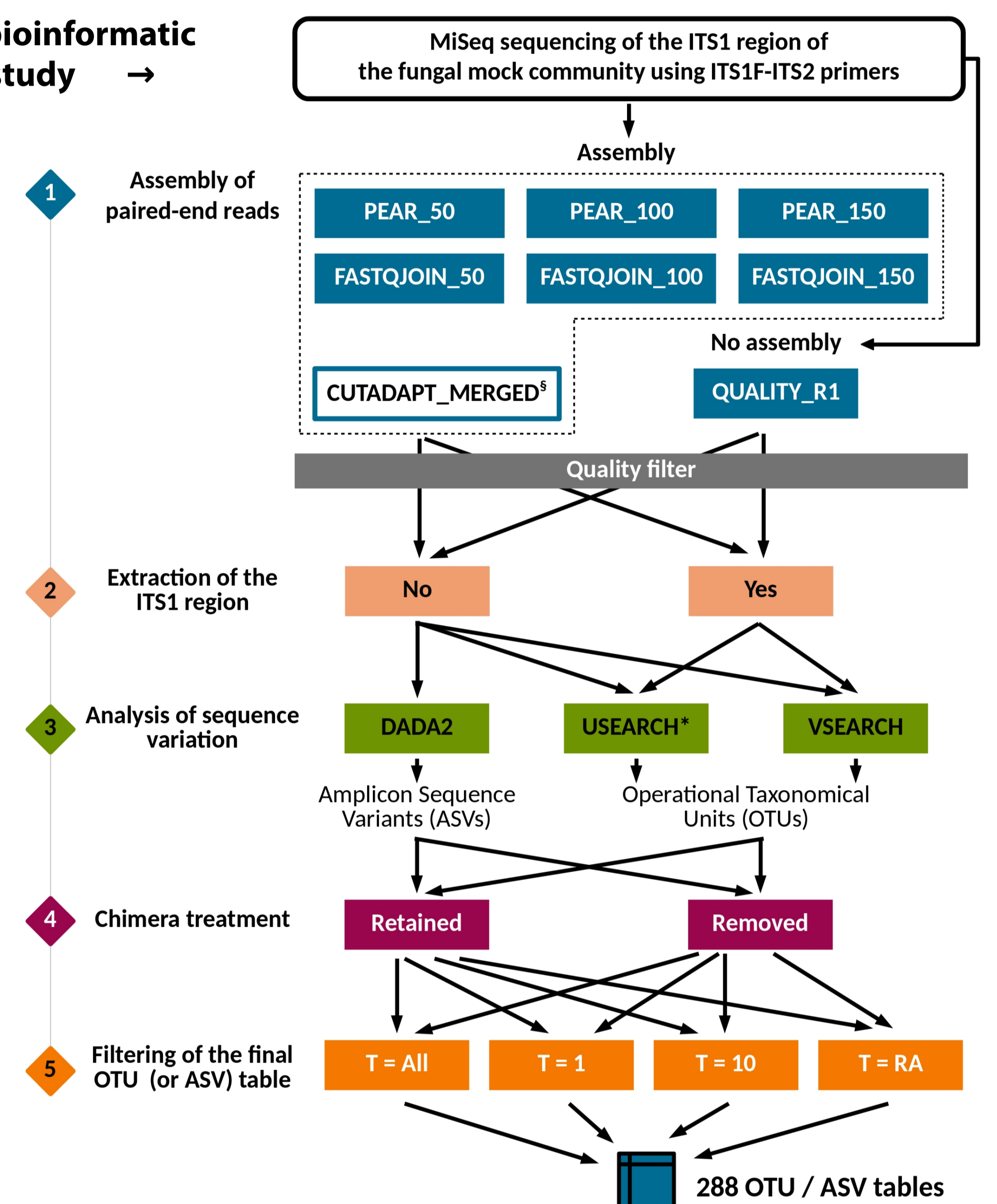
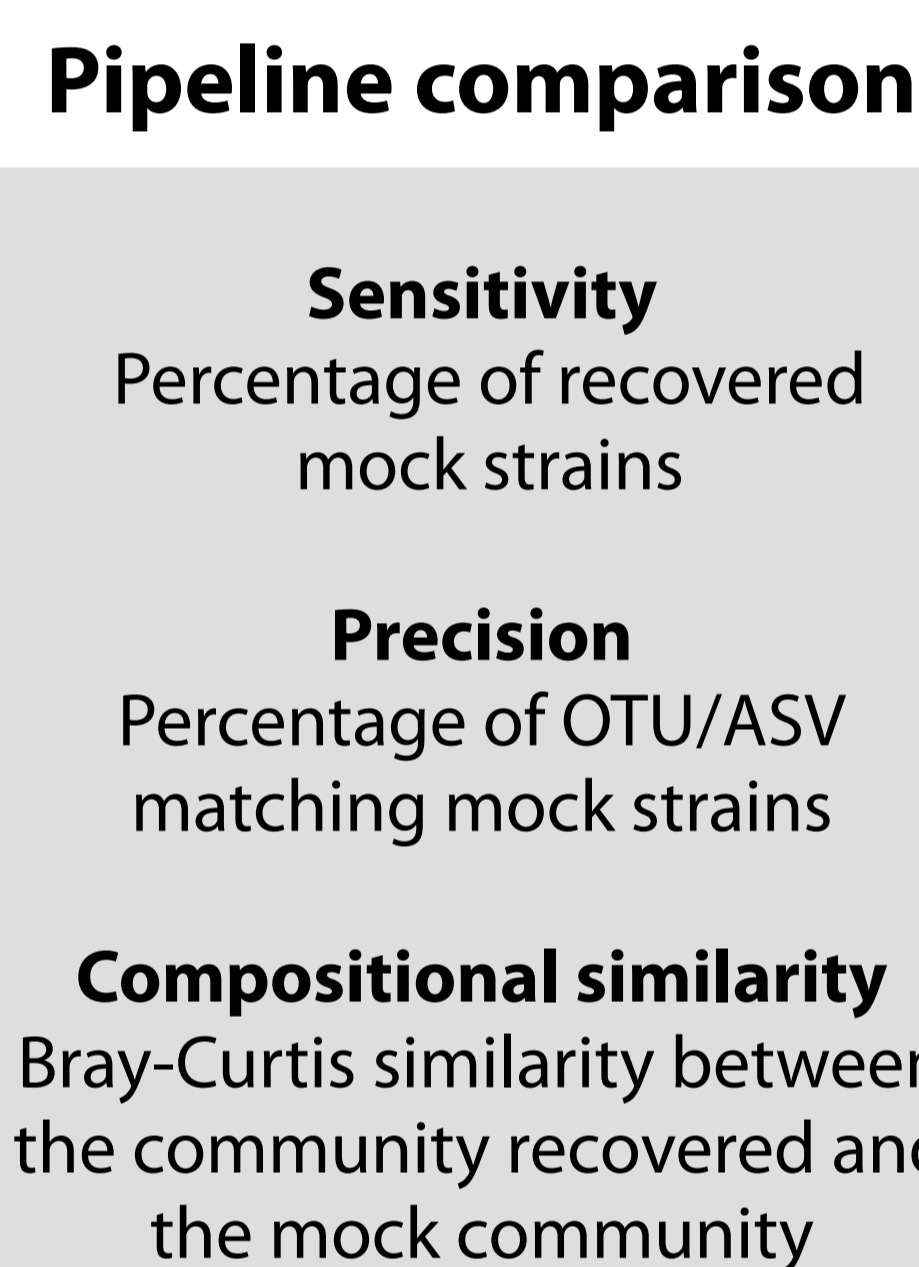


Fig 2 Overview of the bioinformatic pipelines compared in this study

The MiSeq sequences were analyzed with 288 unique combinations of bioinformatic softwares and parameters (referred to hereafter as pipelines)



Results

	Highest Sensitivity	Highest Precision	Highest Similarity	Best Trade-off
1. Assembly	QUALITY_R1	FASTQJOIN_150	QUALITY_R1	QUALITY_R1
2. Extraction	No	No	No	No
3. Variation	VSEARCH	DADA2	DADA2	DADA2
4. Chimeras	Retained	Removed	Retained	Removed
5. Filtering	All	10	All	All

Table 1 Pipelines with the best performances for each three criteria and the pipeline with the best trade-off. Pipelines are labeled below the table and the steps are numbered as in Fig 2.

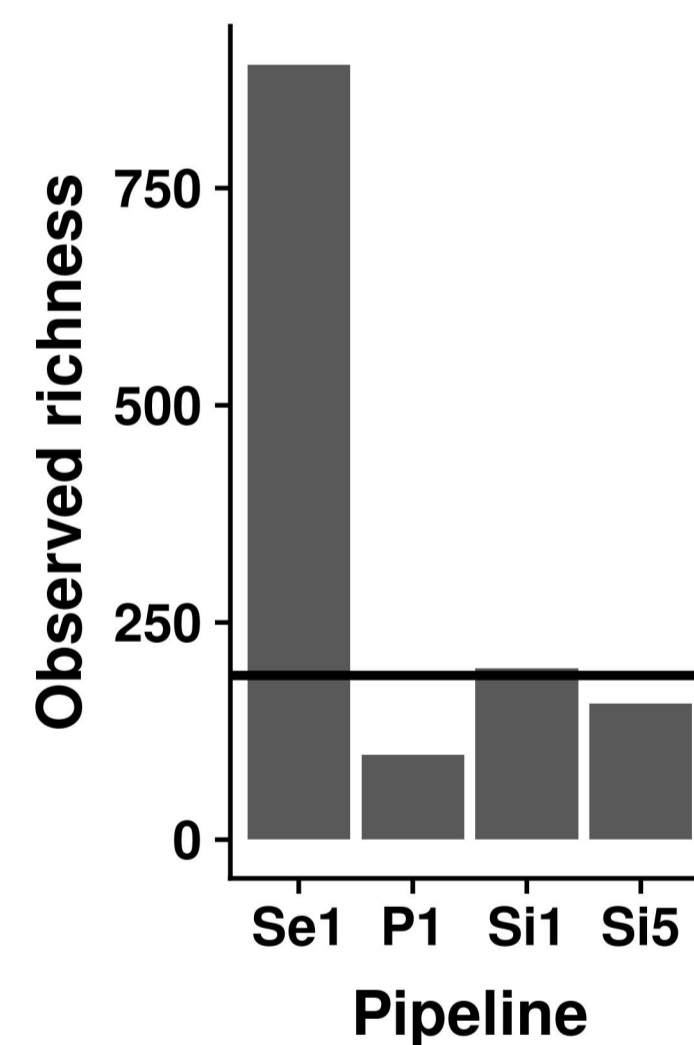


Fig 3 Total number of OTUs (or ASVs) retrieved by the top four pipelines (Table 1). The black horizontal line indicates the expected richness.

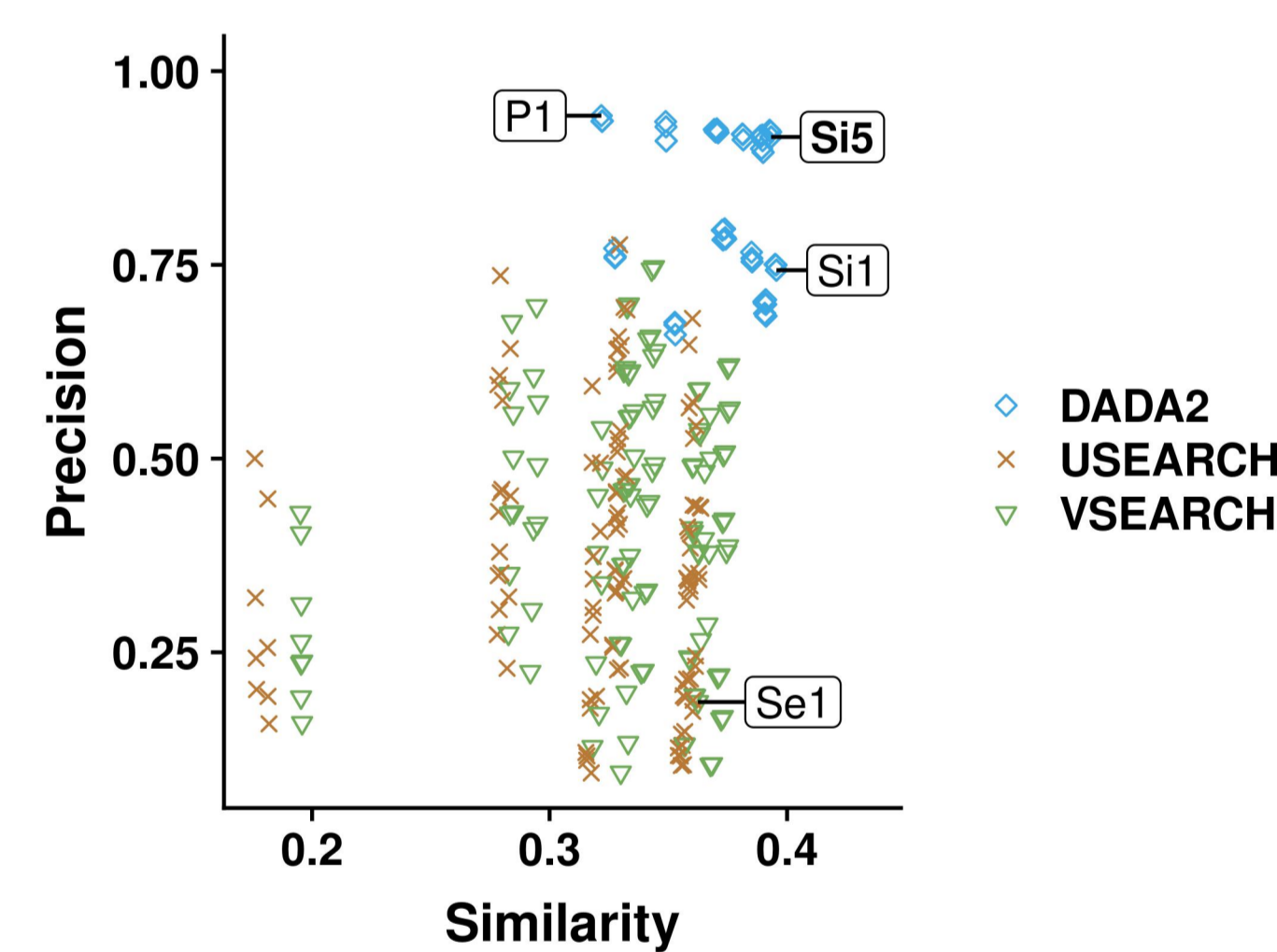


Fig 4 Values of precision and compositional similarity to the mock fungal community, for all 288 bioinformatic pipelines. Only the top four pipelines are labeled

None of the pipelines scored highest in all three criteria (Table 1).

Observed fungal richness depended strongly on the bioinformatic pipeline (Fig 3).

Community richness and composition were well recovered by DADA2 (Fig 3 & 4).

Conclusion

The **Si5 pipeline** used single forward (R1) sequences with DADA2 and no filter other than the removal of low-quality and chimeric sequences.



No overestimation of the number of fungal strains. One of the best to recover the composition of the mock community. Good performance on precision and sensitivity as well.

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