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Metagenomic assembly of complex ecosystems with highly accurate long-reads

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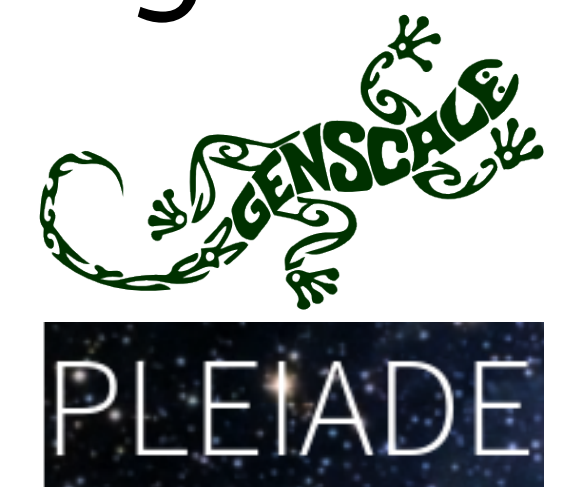
Metagenomic assembly of complex ecosystems with highly accurate long-reads



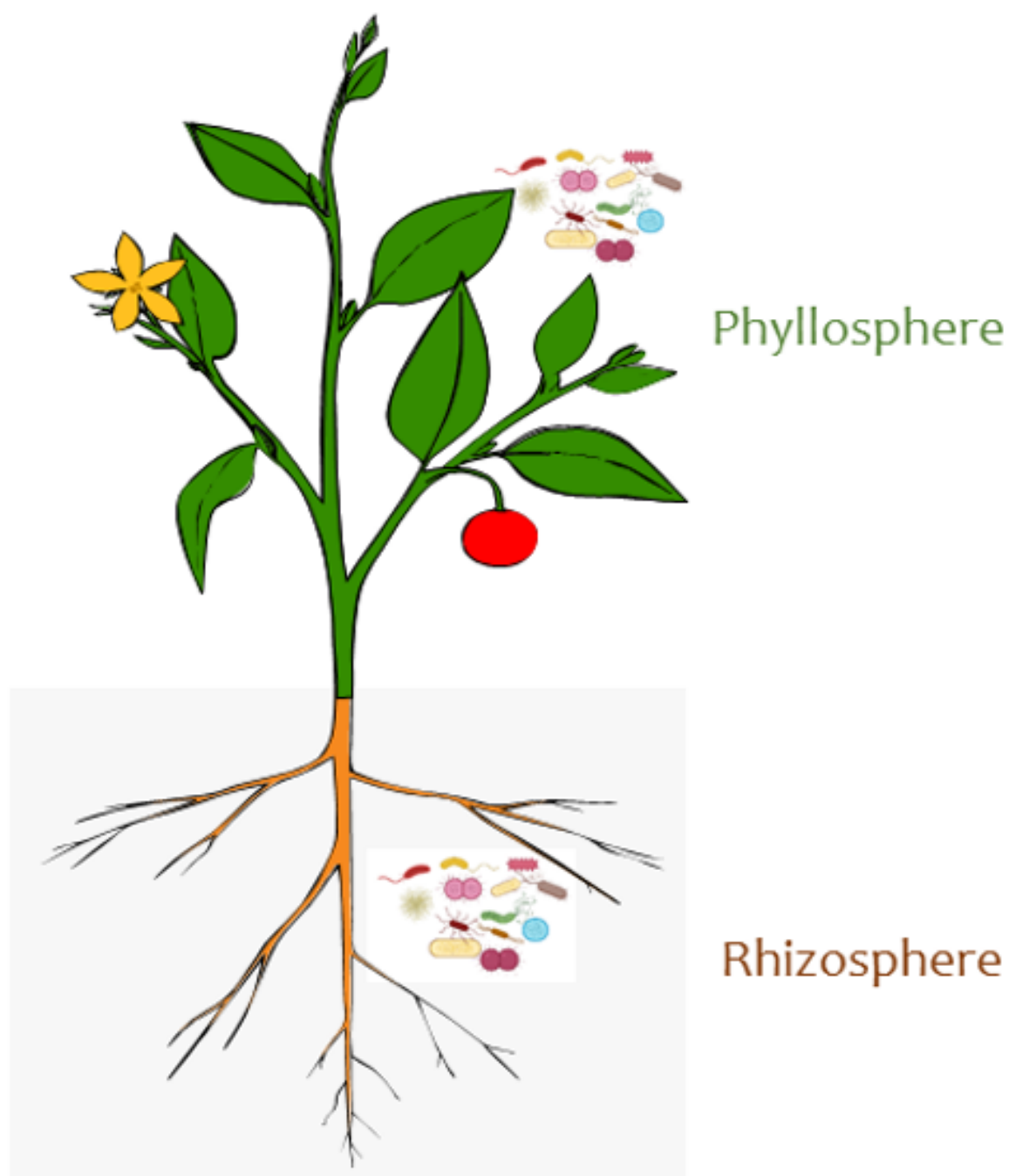
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Context



Plant microbiota

Plants can recruit micro-organisms from the millions (10^3 - 10^8 /g) found in soil, to regulate key functions such as :

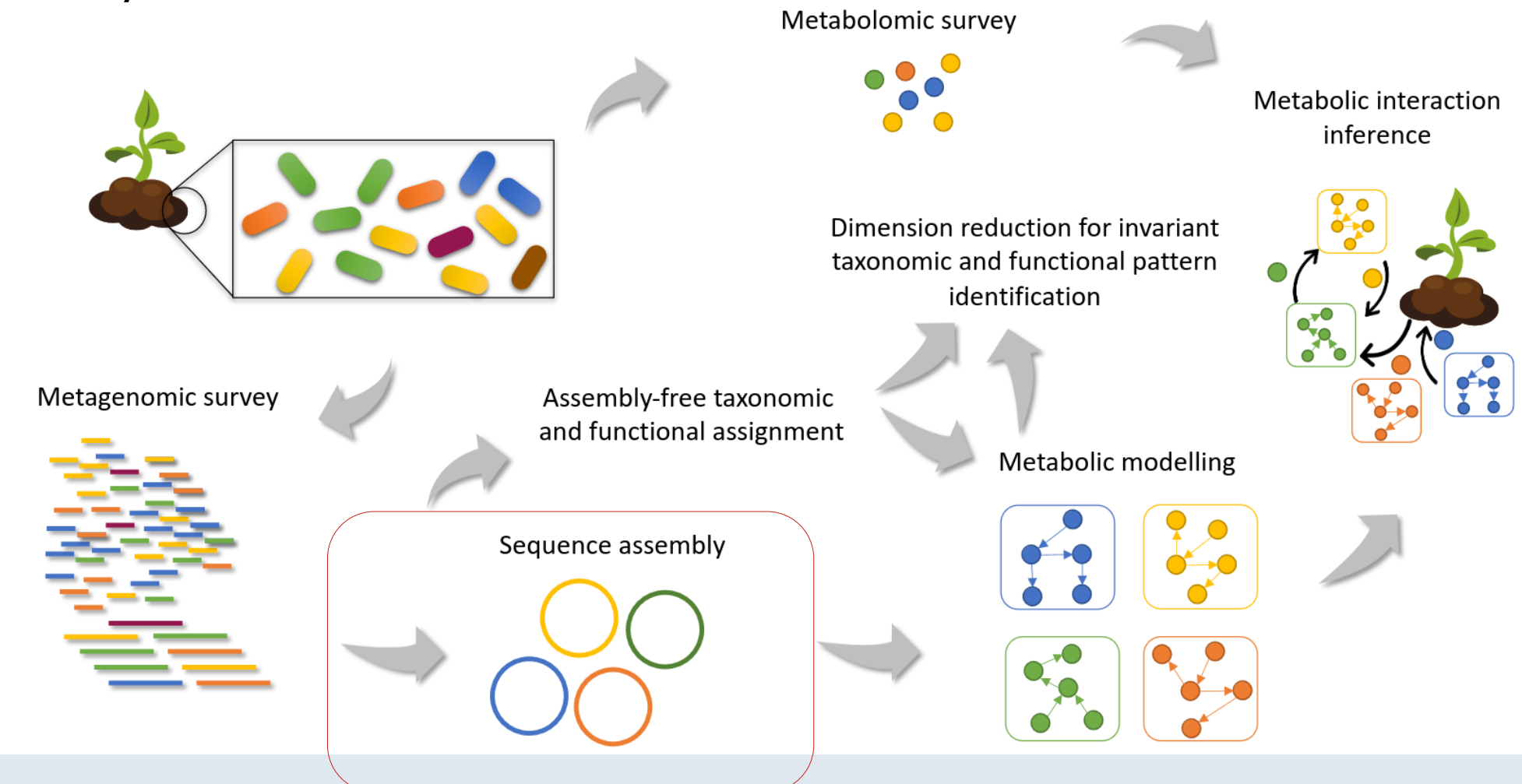
- Nutrition and growth,
- Abiotic stress,
- Biotic stress.

Still, both ecosystems are poorly characterised, as culturomics, transcriptomics and short-read metagenomics cannot recover enough complete genomes needed to modelise and characterise those communities.

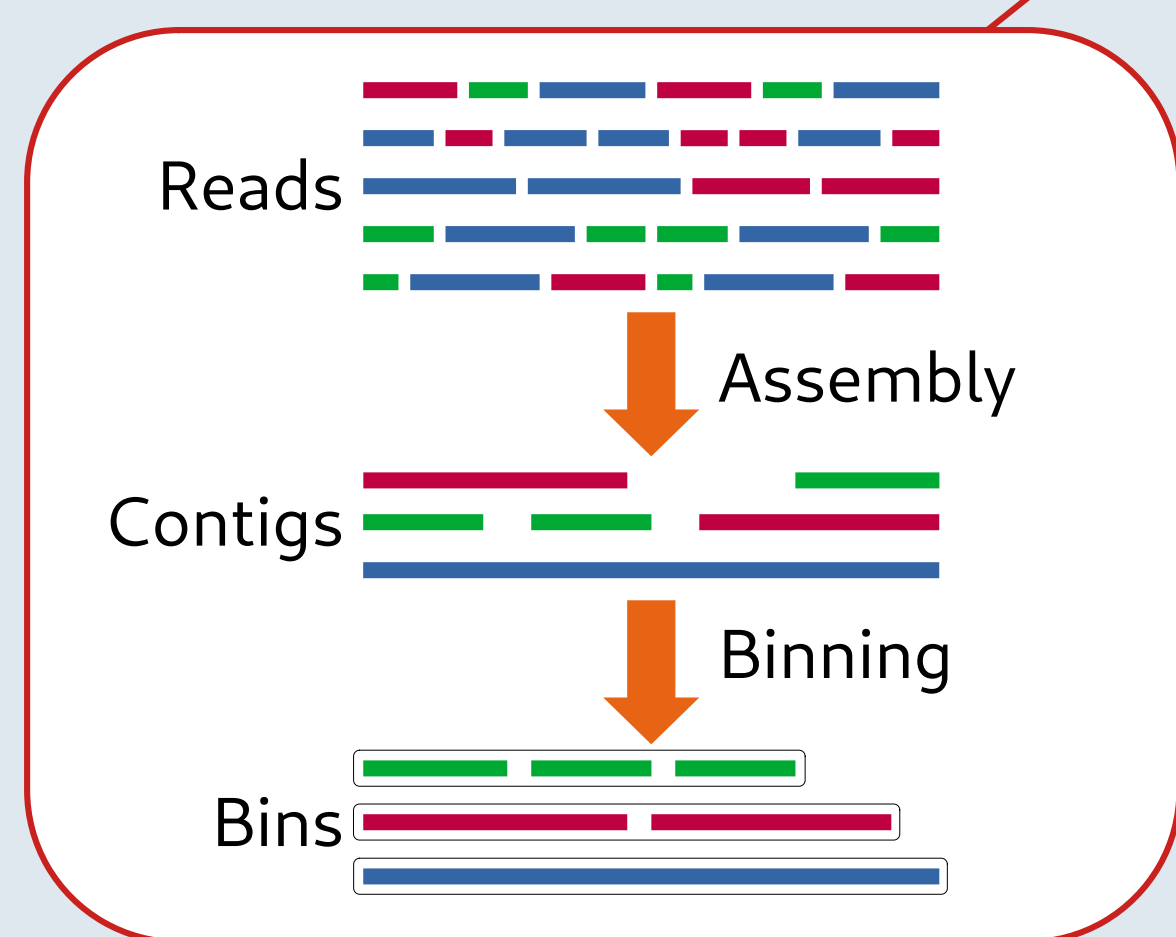
MISTIC Project : project.inria.fr/mistic

Methodological development for microbial community dynamics modelling

- WP1 : Top down approach from large-scale data on natural communities,
- WP2 : Bottom up approach, building dynamic models of increased complexity under controlled conditions.



Motivations



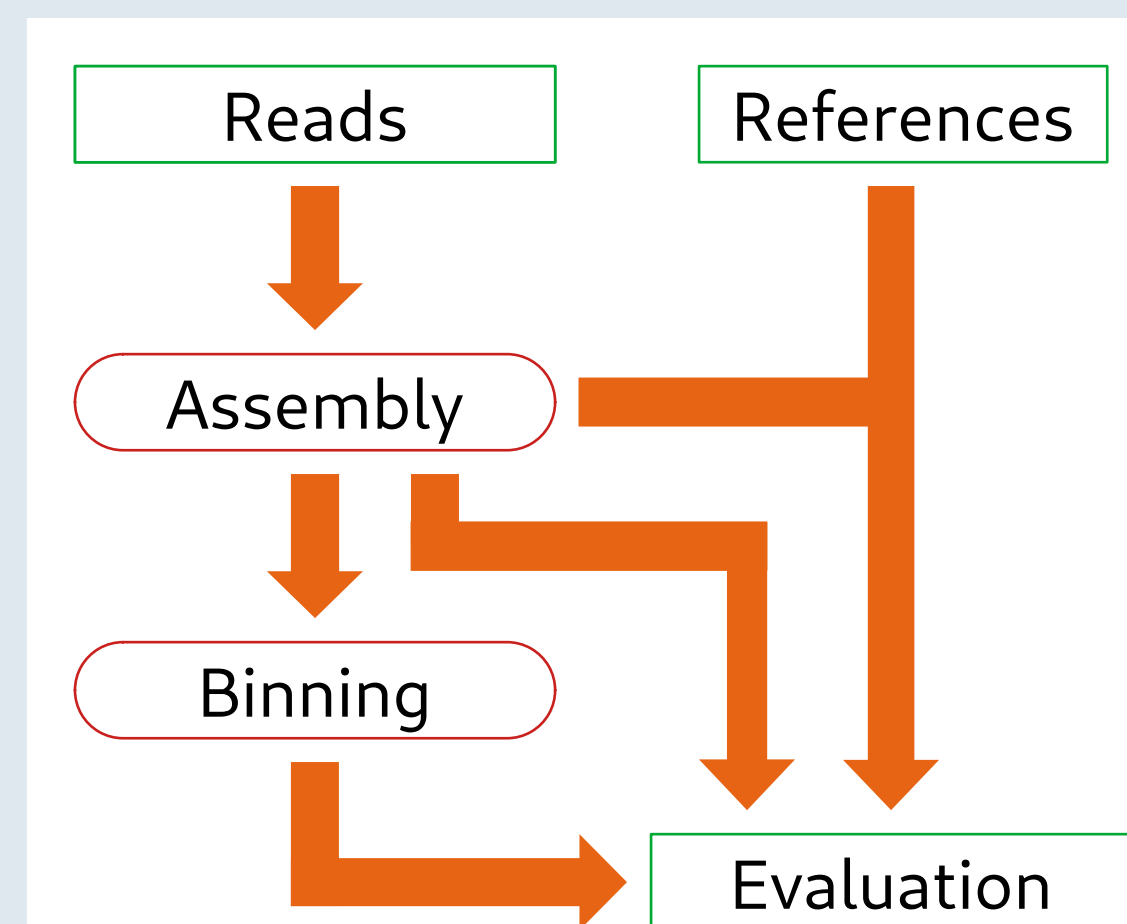
Emerging sequencing technologies

	Read type	Read length (bp)	Error rate
Illumina	Short reads	151 bp	~0.1
ONT R9.4	Long reads	~20kb	~10%
PacBio CLR		~10kb	~10%
ONT R10.4	Highly accurate	~20kb	~1%
PacBio Hi-Fi	long reads	~10kb	~1%

Higher quality reads lead to higher quality assemblies.

How to exploit those highly accurate long-reads to recover high-quality genomes from complex environments such as soil and rhizosphere ?

Mapler : gitlab.inria.fr/mistic/mapler



Pipeline

Built with Snakemake and Slurm integration.

Benchmarks three highly accurate long reads assemblers :

- Metaflye¹,
- Hifiasm-meta²,
- MetaMDBG³.

Datasets

	Total size (Gb)	Median size (Kb)	Taxonomic complexity
Zymo D6331	18.0	8.1	Mock community
Human gut	15.2	8.4	+
Rhizosphere	19.5	5.8	++
Soil	13.4	6.0	+++

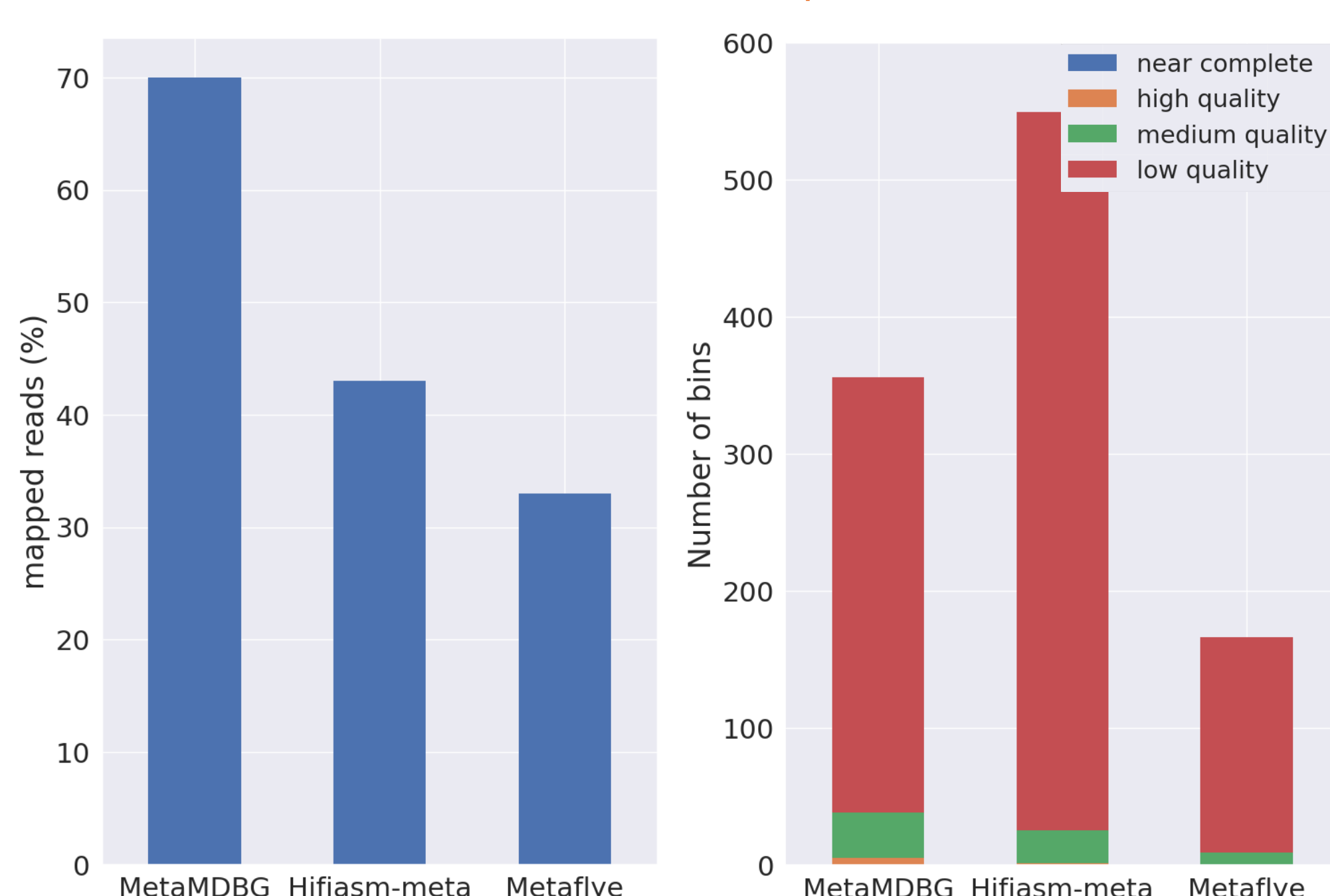
Evaluation metrics

- Aligning contigs on reference genomes, if available : NGA50 and genome fraction,
- Aligning reads on contigs : counting reads that map to the assembly,
- Evaluating the bins completeness and contamination with marker genes, then categorizing bins by level of quality.

Mapler : Results

Assembler comparison

On the soil sample

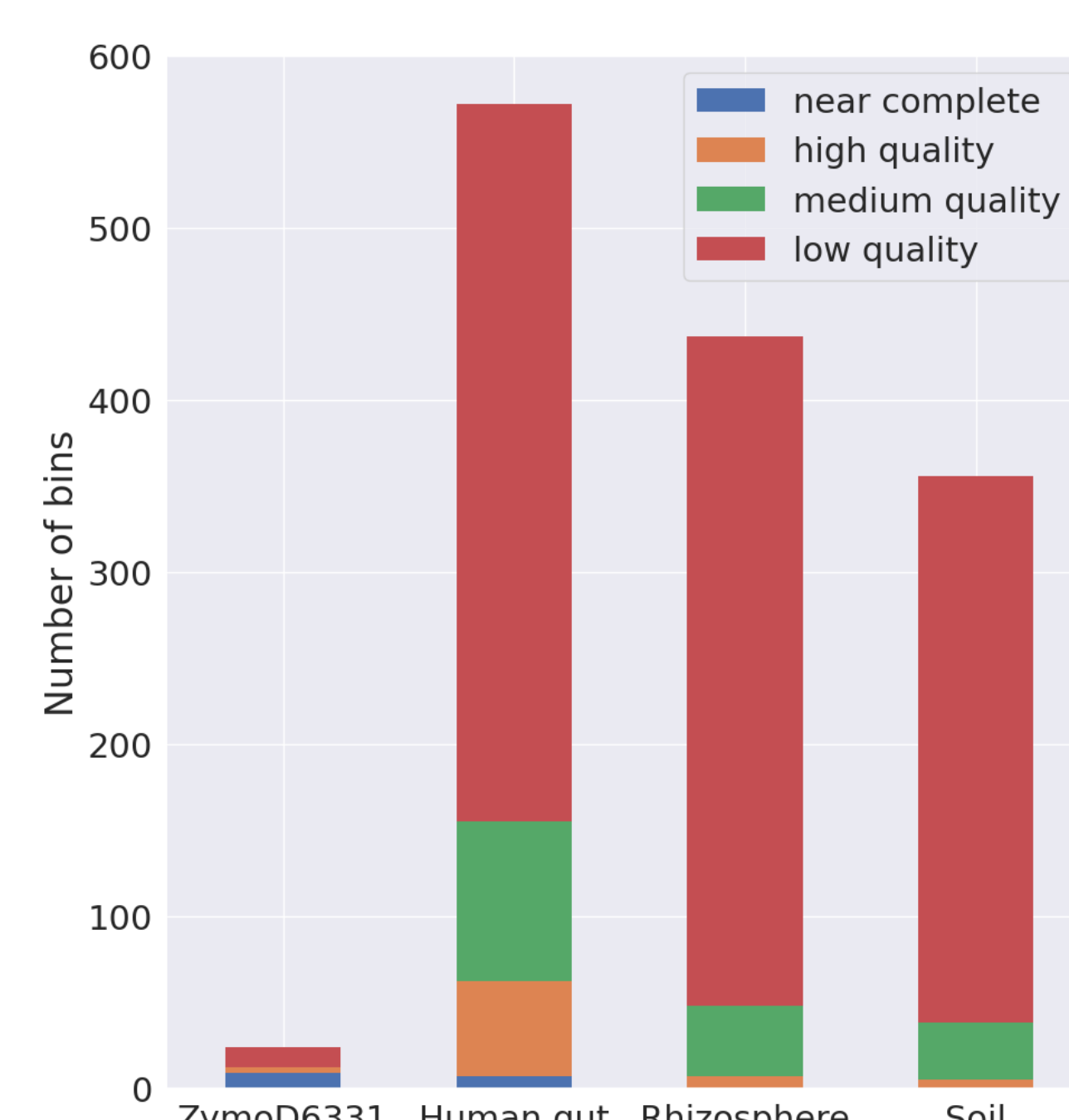


MetaMDBG captures :

- A greater proportion of reads,
- More high-quality bins,
- More medium-quality bins.

Sample comparison

Using metaMDBG

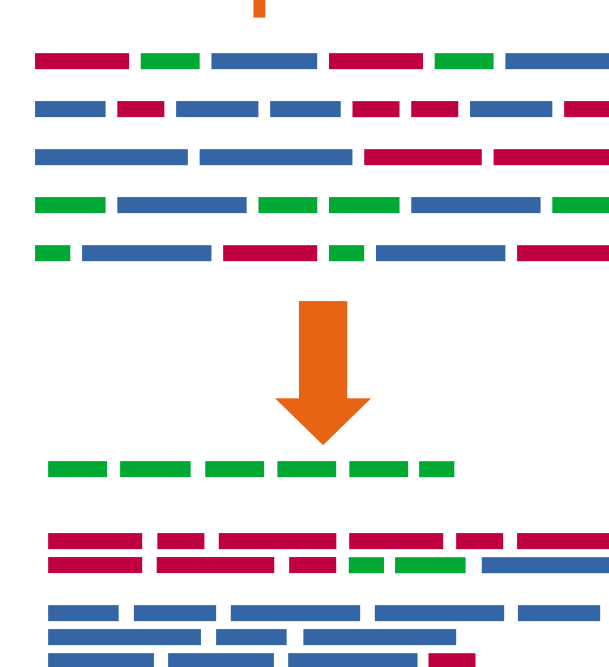


As taxonomic complexity increases :

- Quality of bins decreases,
- Proportion of captured taxa decreases.

Methodological avenues of improvement

Read partitioning

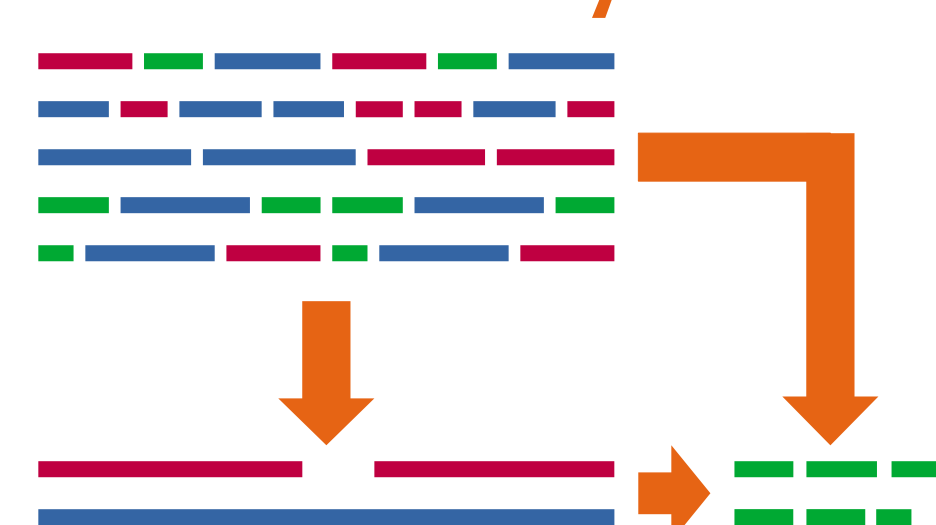


By partitioning the reads into smaller sets via :

- Taxonomic assignation,
- Composition-based clustering,
- Abundance-based clustering.

We could reduce the complexity of each subset, simplifying the assemblies.

Read recovery



By mapping the reads on contigs, we could extract unassembled reads, and assemble those separately with reduced complexity.

Hybrid assembly or binning

Highly accurate long reads could be complemented by a higher coverage of short reads, which could be used to better assess the abundance of each long-read, leading to a better assembly or binning.

[1] M.Kolmogorov et Al. metaFlye: scalable long-read metagenome assembly using repeat graphs, 2020

[2] X. Feng et Al. Metagenome assembly of high-fidelity long reads with hifiasm-meta, 2022

[3] G. Benoit et Al. High-quality metagenome assembly from long accurate reads with metaMDBG, 2024