

# **Microbiome analysis based on 16S-rRNA gene full length sequencing with PacBio and ONT**

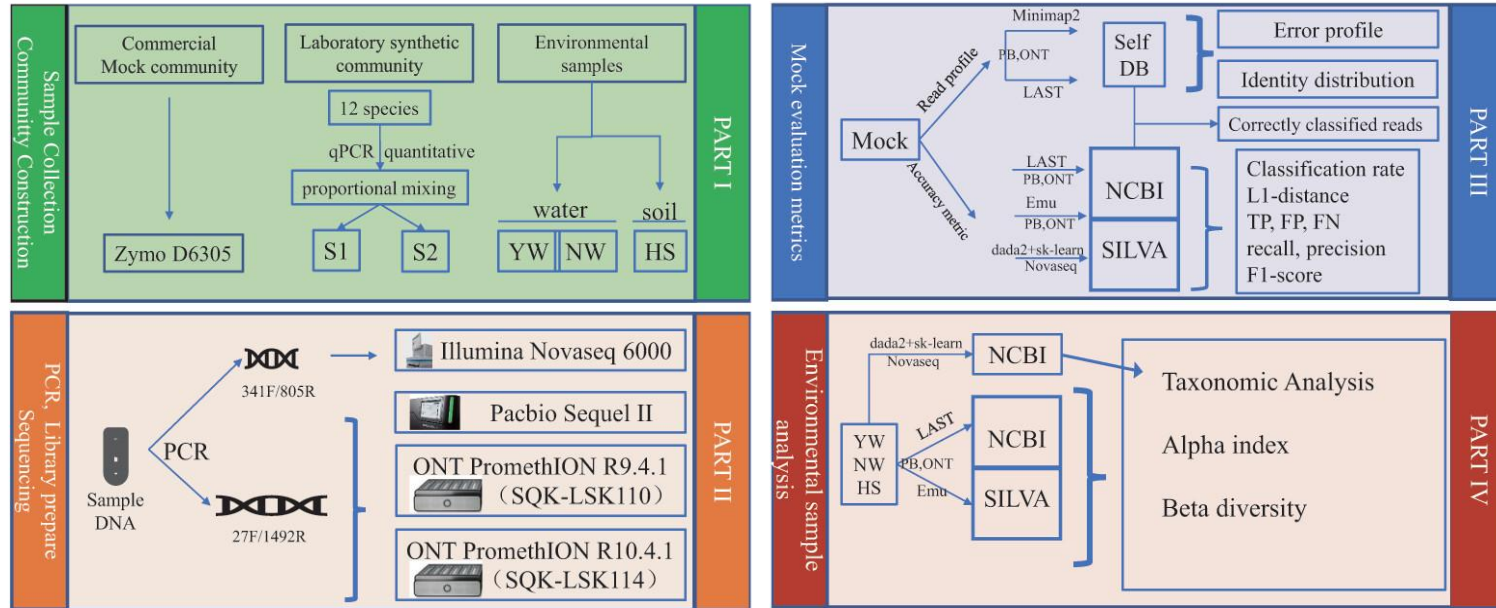
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Nguyen Quang Khai

# The newest Oxford Nanopore R10.4.1 full-length 16S rRNA sequencing enables the accurate resolution of species-level microbial community profiling

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## AFFILIATIONS



**FIG:** The current workflow for the evaluation of R10.4.1 full-length 16S sequences. (<https://doi.org/10.1128/aem.00605-23>)

To simplify the content for the course, in this will focus on the soil sample (denoted as **HS**) sequenced using PacBio and ONT (R10.4.1).

# Dataset

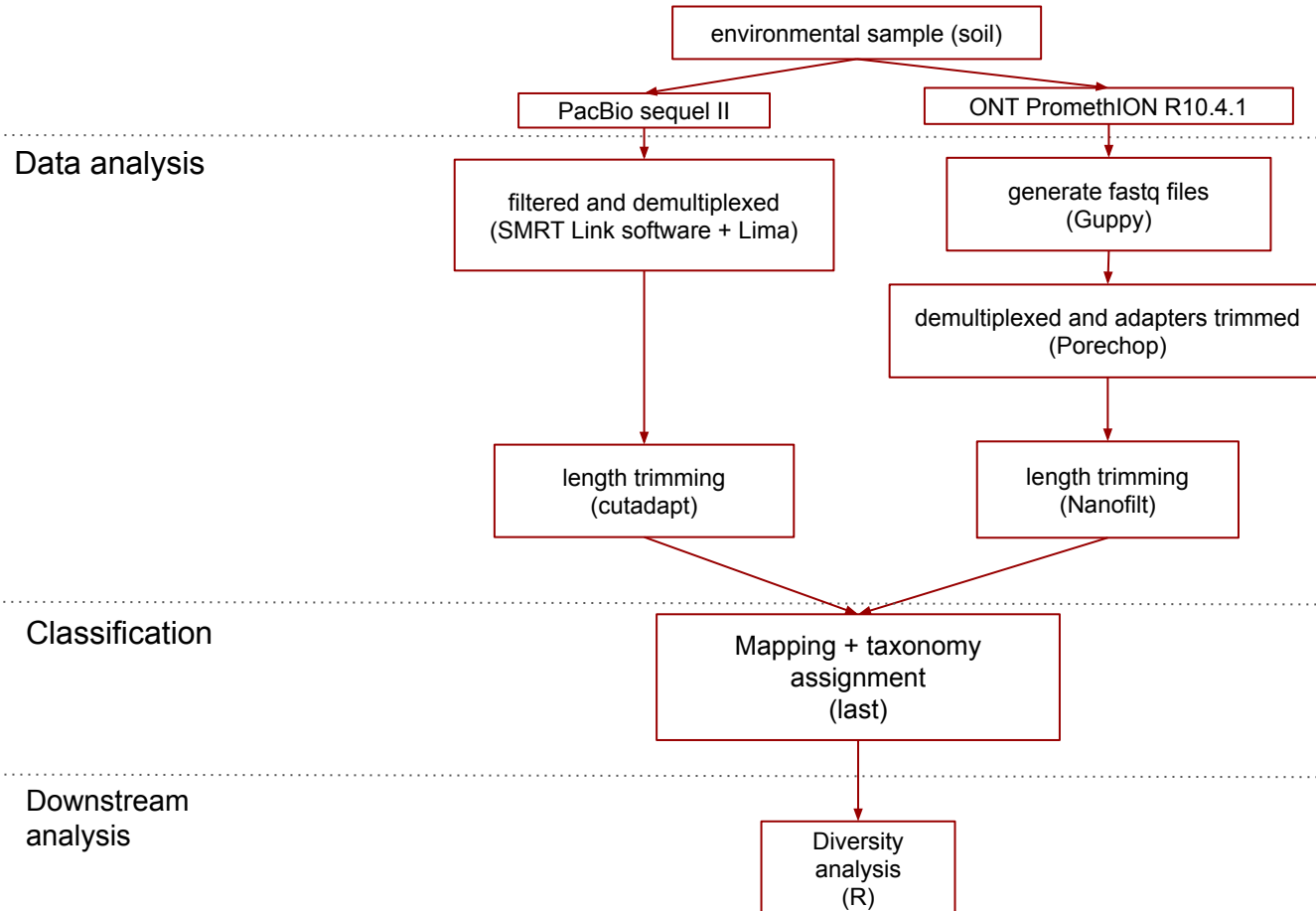
Soil samples “**HS**” were collected in three replicates from the foot of Shizi Mountain (114.37°E, 30.48°N, Huazhong Agricultural University, Wuhan, China). <https://doi.org/10.1128/aem.00605-23>

	PacBio	ONT (R10.4.1)
Biological replicate 1	SRR23176518	SRR23176498
Biological replicate 2	SRR23176517	SRR23176497
Biological replicate 3	SRR23176516	SRR23176496

More information about this BioProject (PRJNA925180): [metadata](#)

# Bioinformatics workflow

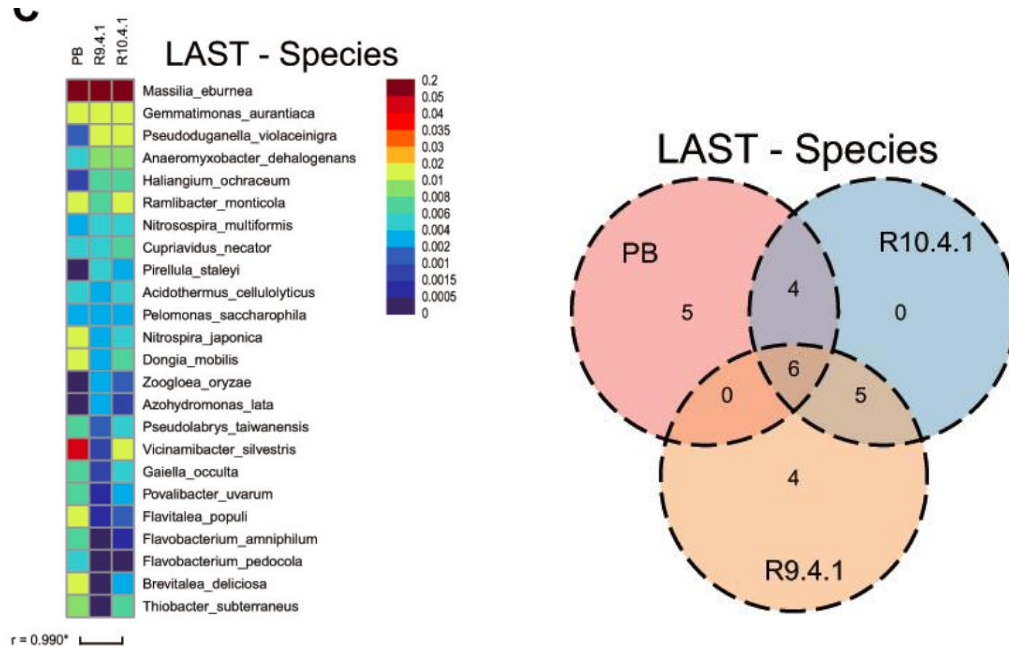
(redraw based on information from [the paper](#))



# Results

**TABLE:** Statistics of the number of taxa at different phylogenetic levels identified via different platforms

Sample	Method	Platforms	Phylum	Class	Order	Family	Genus	Species
HS	LAST	PB	20	51	116	229	549	1,088
HS	LAST	R10.4.1	19	51	122	243	702	1,436



→for HS samples, there were 10 common species detected by R10.4.1(ONT) and PB.

the average abundance of the top 15 species across different platforms

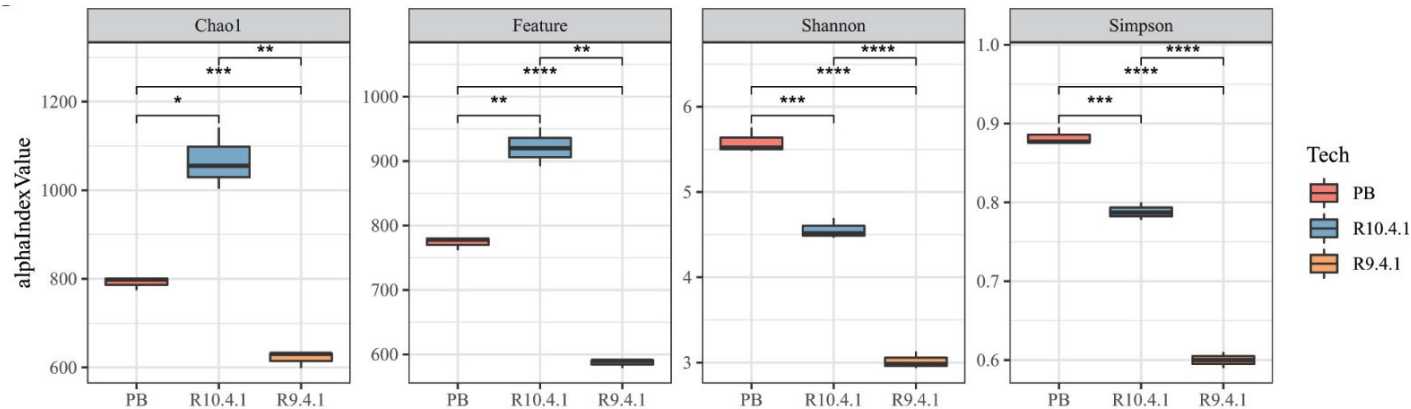
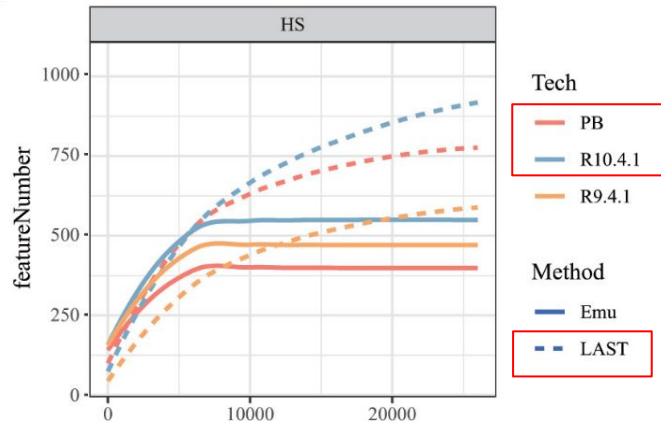


FIG: Boxplots of alpha diversity across two platforms. A *t*-test was used for calculating whether these samples are significantly different with a *P*-value of <0.05 (\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001; \*\*\*\**P* < 0.0001).



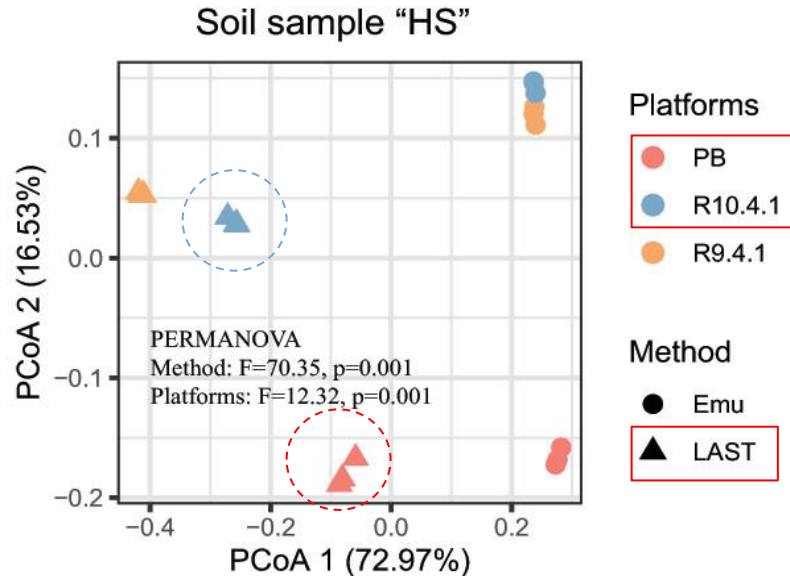
→ R10.4.1 can obtain a higher PacBio for Chao1 and feature. In addition, PB and R10.4.1 lie closer on the Shannon and Simpson indices.

All samples were rarified based on the minimal number of reads (26,000 reads) and  $\alpha$ -diversity index was calculated

Fig: Rarefaction curves based on reads obtained from R10.4.1 (blue) compared with PB (red).

# Principal Coordinate Analysis(PCoA) and permutational multivariate analysis of variance(PERMANOVA) analysis for soil samples

Comparing different platforms based on the Bray–Curtis matrix.



Current results suggest that species-level community composition was significantly diverse across different platforms (HS: F = 13.32, P = 0.001)

FIG: The PCOA and PERMANOVA analysis of soil sample



**Table showing comparison of long read technologies**

	<b>PacBio HiFi sequencing</b>	<b>ONT Nanopore sequencing</b>
Input	DNA, cDNA	DNA, RNA
Read length	500 to 20 kb	20 to >4 Mb
Read accuracy	Q33 (99.95%)	~Q20 <sup>2,3,4</sup>
Typical run time	24 hours	72 hours
Typical yield per cell	90 Gb (Revio)	50-100 Gb <sup>7</sup>
Base calling	Yes, on-instrument (\$0)	Off-instrument, often requires additional costly GPU server
Variant calling - SNV	Yes	Yes
Variant calling - Indels	Yes	No
Variant calling - SVs	Yes	Yes
Detectable DNA modifications	5mC on-instrument calling (\$0)	5mC, 5hmC, and 6mA. Off-instrument calling, often requires additional costly GPU server
Platforms	Revio, Sequel, and Sequel IIe	PromethION, MinION, GridION, and Flongle
Typical output file size (type)	55 GB (BAM)	1,300 GB (fast5/pod5)
Storage cost per month*	\$1.30 USD	\$30.00 USD
Table footnote: * AWS S3 Standard cost per month is calculated based on USD \$0.023 per GB storage pricing. Oxford Nanopore Technologies information gathered from <a href="https://nanoporetech.com/platform">https://nanoporetech.com/platform</a>		

## Some points

- For soil samples, ONT (R10.4.1) can obtain more species than PacBio.
- Among the major species, ONT (R10.4.1) and PacBio platforms reveal a similar microbiome.
- Considering the advantages of high throughput and portability of ONT sequencing, we believe that more researchers will employ ONT sequencing to explore environmental microbes.
- Consider PacBio if high accuracy is concerned.

**Thank you for watching!**