Diverse Rhizosphere Bacterial Communities Associated With Wild Edible Mushroom *Astraeus asiaticus* -Metagenomics Insights.

Vineet Vishal[1*], Sabdar Rahaman[1], Somnath Singh Munda[2], Geetanjali Singh[3], Shalini Lal[4#]
[1]Department of Botany, Bangabasi Evening College, Calcutta University, Kolkata-700009, India
[2,3,4]Department of Botany, Dr Shyama Prasad Mukherjee University, Ranchi-834008 , India

*Presenting Author- vineet.vishal73@gmail.com; #Corresponding Author- shalini.kumari@gmail.com

ABSTRACT :

The symbioses of ectomycorrhizal mushroom and seed-bearing plants have shown to host a rich diversity of microbiota that interact with their hosts. Here, we collected soil samples using standard protocols from Bandhgaon village, West Singhbhum Jharkhand, India to study diverse rhizospheric soil bacterial communities associated with ectomycorrhizal fungi *Astraeus asiaticus* compared to bacterial communities in bulk soil by using MinION oxford nanopore sequencing of 16S rRNA amplicons. For controls, adjacent bulk soil samples were taken at 20m distances from fungi. A total of 37,240 raw read were analyzed, out of which only 2,664 reads were valid read with 662 OTUs was found in plant roots and bulk soil has 22,848 raw reads, out of which 1,796 were valid reads and has 495 OTUs generated using MinION oxford nanopore sequencing and EzBioCloud 16S database version PKSSU4.0 a data analysis platform to reveal the abundance of phyla -*Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria and Gemmatimonadetes*. The present work analyzed ectomycorrhizosphere presents a diverse microbiome, which likely influence the functioning of the plant-fungus interactions.The data is available at the NCBI – BioProject ID PRJNA615789, Sequence Read Archive with accession number: SRX8009931 for EcM rhizosphere and SRX8009932 for bulk soil. This is the first report of the rhizospheric microbiome belonging to a wild edible mushroom *Astraeus asiaticus*.

Keywords: Ectomycorrhiza, Mushroom, MinION, EzBioCloud, India

INTRODUCTION:

Positive interaction between seed-bearing plants and microbes contribute substantially to the ecological integrity of the biosphere unveil rich diversity of plant microbiota on earth that interact with their host. High-throughput next-generation sequencing of these microbiota genes has shed new light, exclusively the role of bacteria in the eukaryotic system. Recent rhizospheric metagenome analysis of the mycorrhizal symbiosis between plant root tip and fungal hyphae reveals presence bacterial phyla such as *Bacteroidetes*, *Chlorobi*, *Actinobacteria*, *Cyanobacteria*, *Proteobacteria* and *Firmicute* play a quintessential role to promote the functioning of mycorrhizae, where mycorrhizae dependent on nutrients released from bacterial action and bacteria feeding on fungal hyphae, assist in the fungal-plant recognition system, receptivity of the plant root to the mycorrhizal fungus. These associations are expressively imperative in agroecosystems where the sustainability of soil fertility is of prime importance. Yet paltry of work done to explore the richness of bacterial diversity associated with ectomycorrhizal fungi due to lesser focus on fungal taxonomic coverages.

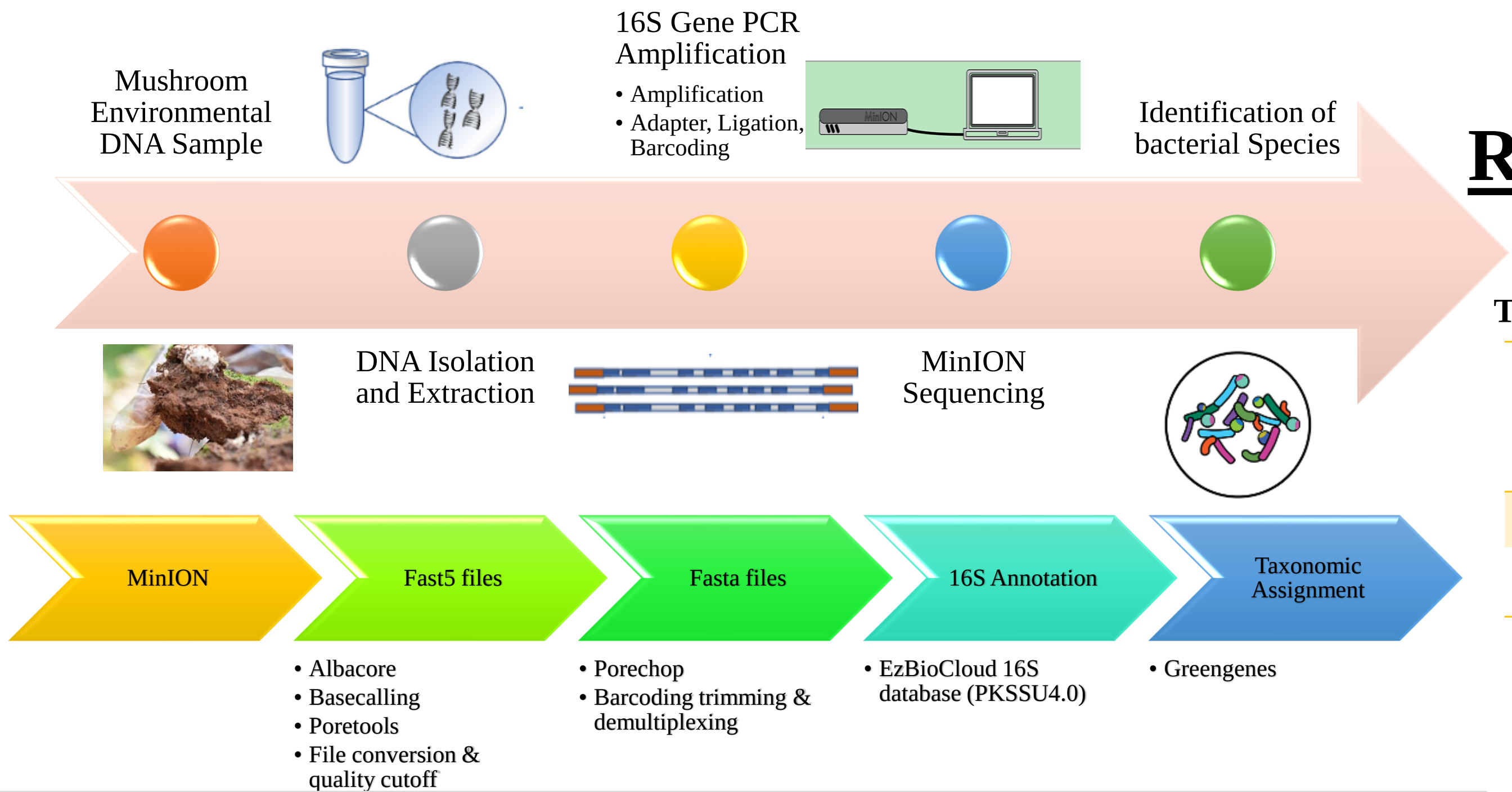
OBJECTIVES:

In order to gain better insight of the relationship between ectomycorrhizal fungi *Astraeus* and bacteria, it is necessary to determine whether the bacterial communities associated with ectomycorrhizal fungi are consistent across space and time.

Our main objectives are to-

- Explore the bacterial diversity of the EcM rhizosphere,
- Study dominant bacterial population in term of abundance and structure associated with plant root versus the bulk soil,
- Explore if the bacterial population in bulk soil and roots are spatially auto-correlated,
- Soil physicochemical analysis provided us with beneficial information regarding soil productiveness.

WORKFLOW:



Mushroom Environmental DNA Sample

DNA Isolation and Extraction

16S Gene PCR Amplification
- Amplification
- Adapter, Ligation, Barcoding

MinION Sequencing

Identification of bacterial Species

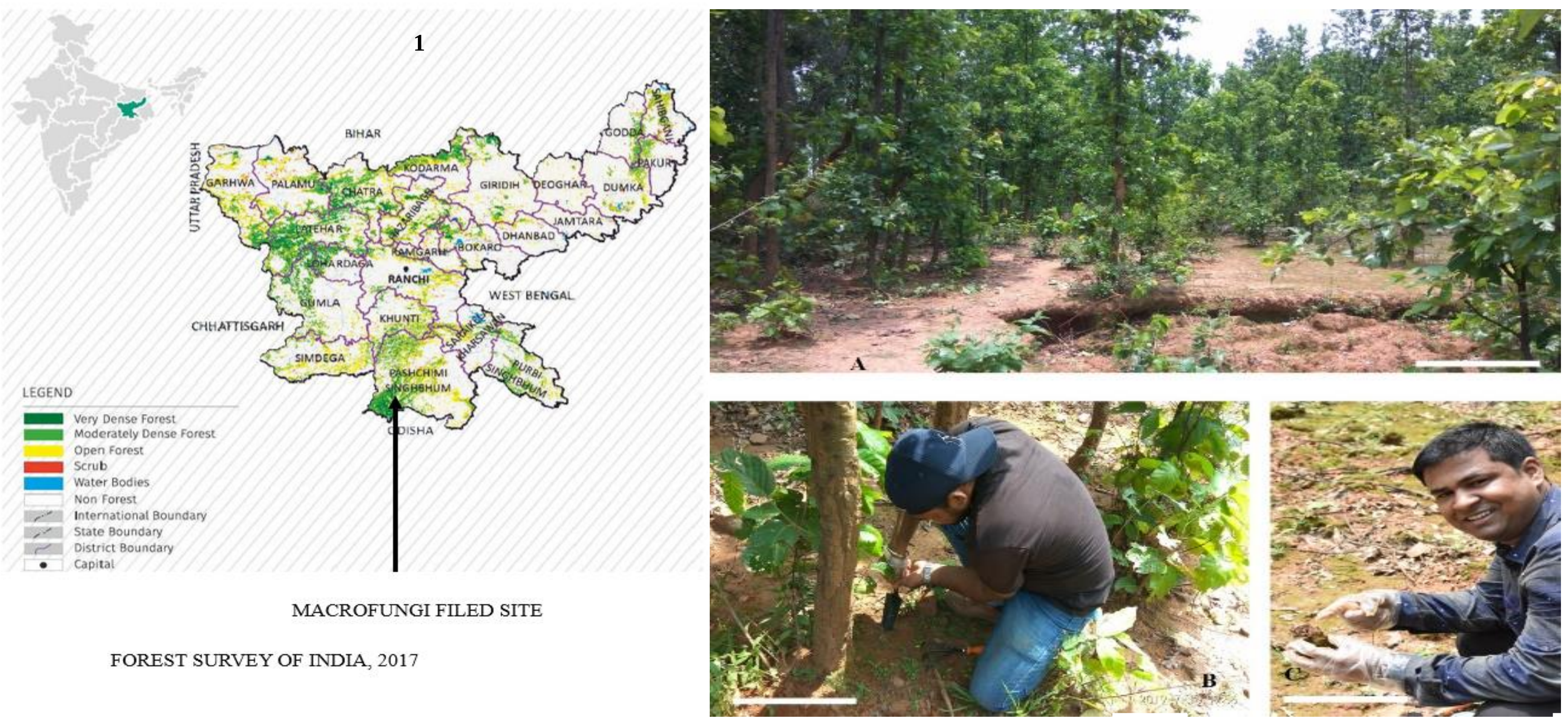
MinION → Fast5 files → Fasta files → 16S Annotation → Taxonomic Assignment

Fast5 files:
- Albacore
- Basecalling
- Poretools
- File conversion & quality cutoff

Fasta files:
- Porechop
- Barcoding trimming & demultiplexing

16S Annotation:
- EzBioCloud 16S database (PKSSU4.0)

Taxonomic Assignment:
- Greengenes



MACROFUNGI FILED SITE

FOREST SURVEY OF INDIA, 2017

Fig 1 –Mushroom Environmental Sample Study site : 1) Geographical Map; (A-C) Saranda Reserve Forest, Jharkhand, India

RESULTS:

Table 1-The physiochemical analyses of the soil sample A) EcM rhizosphere and sample B) bulk soil .

Samples	pH	EC (ds/m)	Organic Carbon(%)	N (ha/Kg)	P (ha/Kg)	K (ha/Kg)	B (mg/Kg)	Fe (mg/Kg)	Mn (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)
A	5.32	0.17	1.005	535	31.6	264.77	0.335	12.34	15.57	1.19	0.468
B	5.12	0.16	0.855	487	23.13	271.49	0.401	12.23	13.13	1.22	0.308

Table 3- Ecological diversity indices were calculated to compare species richness and evenness of samples using OTU information of sample A) EcM rhizosphere and sample B) bulk soil.

Soil Sample	Estimation of species richness			Estimation of species evenness		
	ACE	Chao1	Jackknife	Shannon	Simpson	NP Shannon
Sample A	2107.4	1452.2	1972.5	5.393	0.012	5.627
Sample B	1671.5	1275.6	1496.7	5.251	0.012	5.481

Table 2- Nanoplot statistical analysis and number of reads mapped by EzBioCloud MTP server of sample A) EcM rhizosphere and sample B) bulk soil.

Statistical Parameters	Mean read length	Mean read quality	Median read length	Median read quality	No. of reads	Read length N50	Total bases	Reads above quality cut-off > Q5	Reads above quality cut-off > Q7	Reads above quality cut-off > Q10	Reads above quality cut-off > Q12	Reads above quality cut-off > Q15	No. of reads mapped by EzBioCloud MTP	Total valid reads count	Total OTUs
Sample A	303.4	9.8	257.0	9.8	59,358.0	302.0	18,009,487.0	59358 (100.0%)	59358 (100.0%)	25319 (42.7%)	2487 (4.2%)	6 (0.0%)	37,358	2644	662
Sample B	274.0	9.9	222.0	9.9	28,239.0	262.0	7,738,587.0	28239 (100.0%)	28239 (100.0%)	13124 (46.5%)	1795 (6.4%)	12 (0.0%)	22,848	1796	495

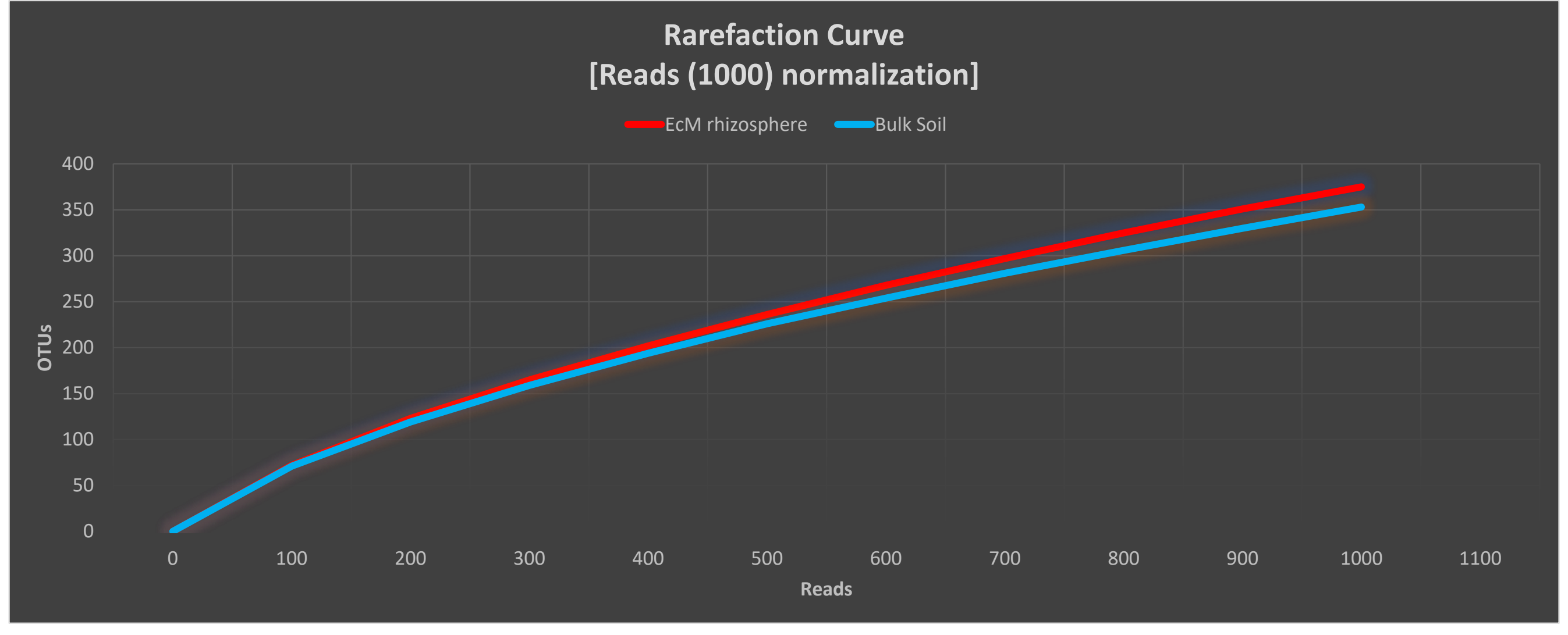


Fig 2 -Bacterial richness in EcM rhizosphere (Red) and bulk rhizospheres (Blue) estimated using rarefaction.

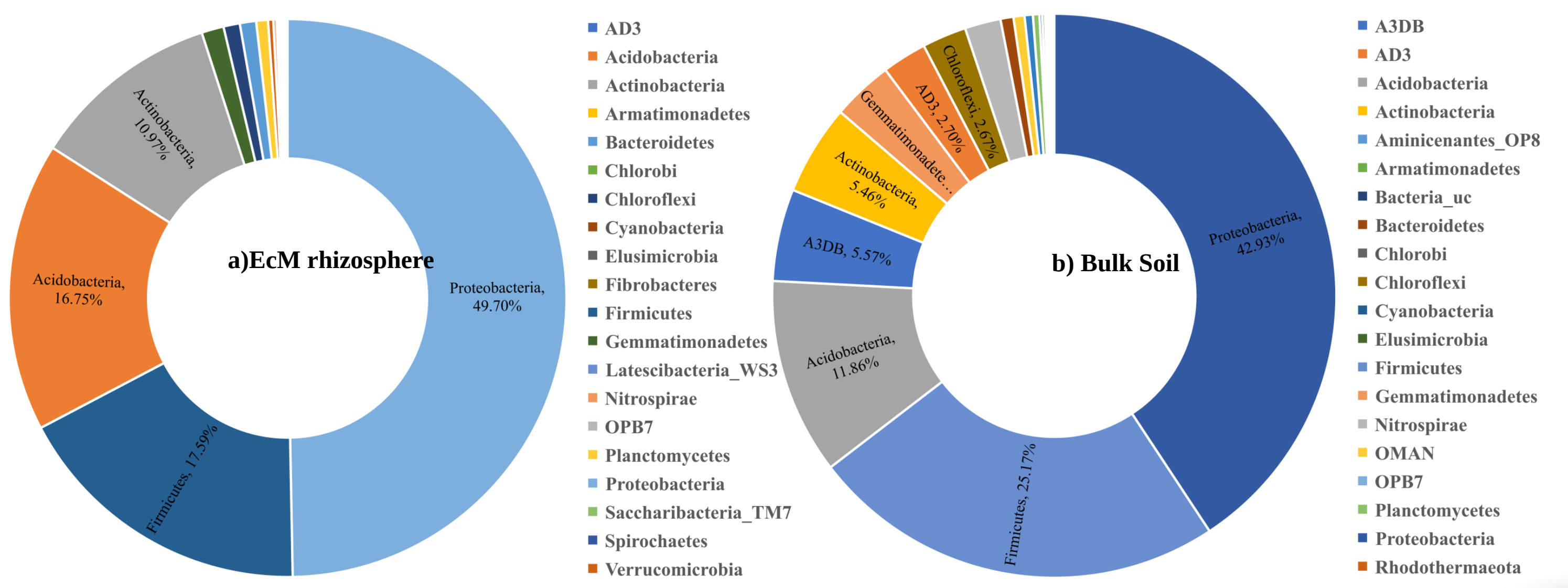


Fig 3: Distribution of various microbial phylum in the rhizospheric sample of a) EcM rhizosphere; b) Bulk Soil.

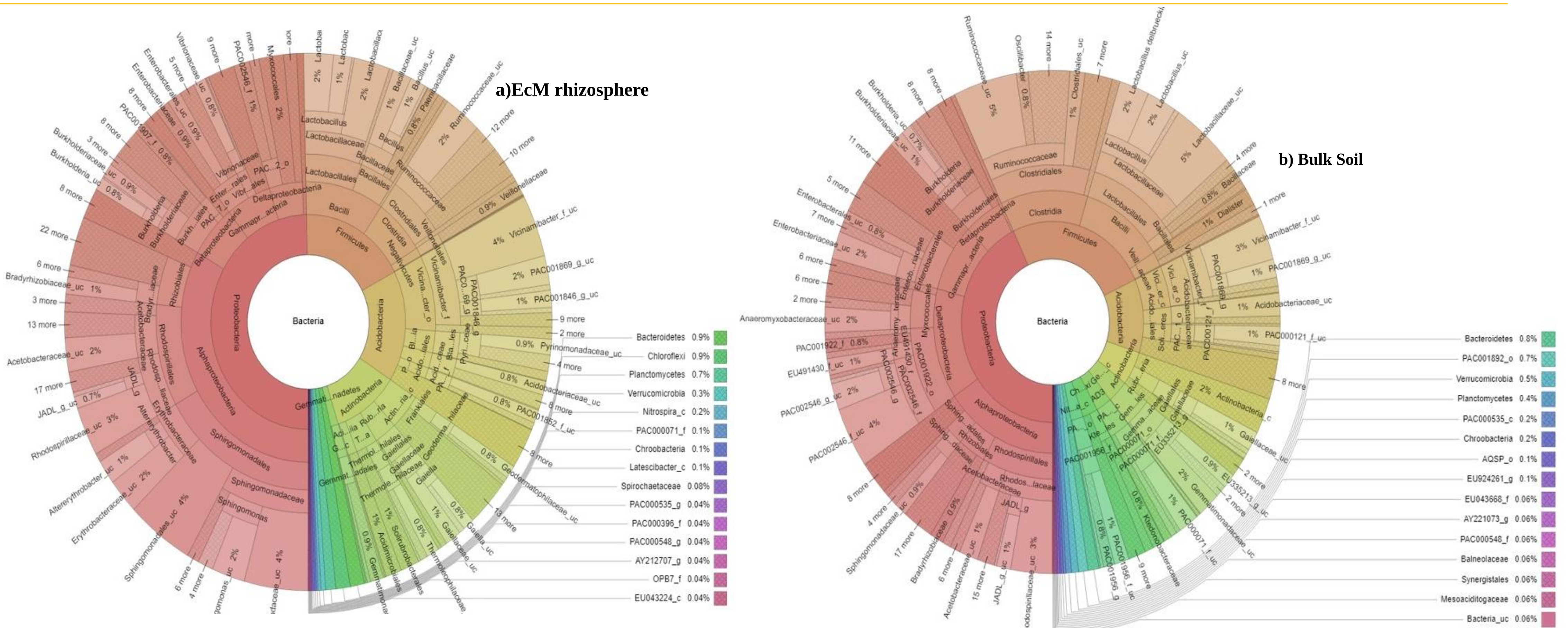


Fig 4 Krona map showing phylogenetics diversity - a) EcM rhizosphere and b) Bulk Soil

EcM rhizosphere(%)

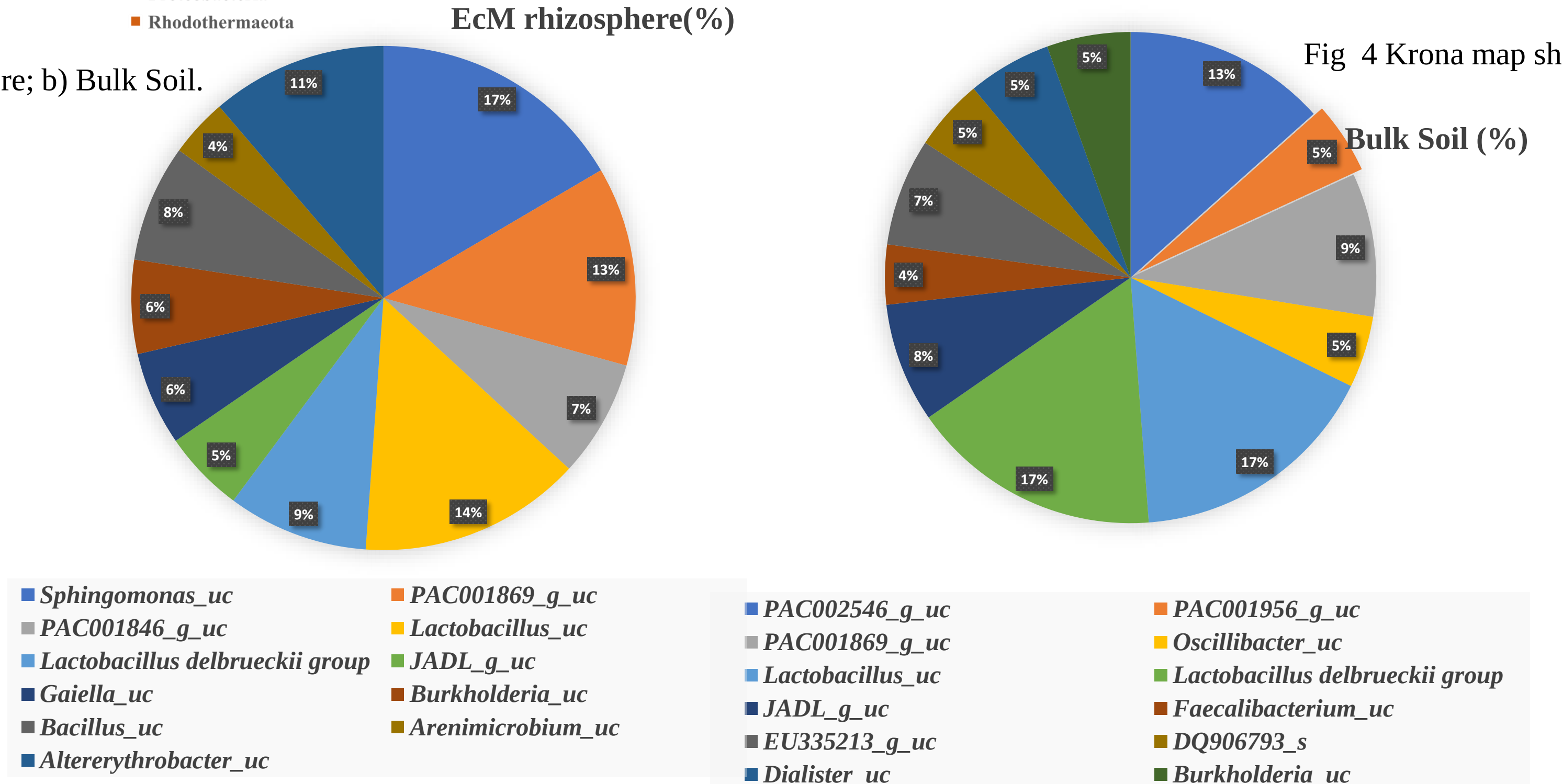


Fig 5: Shows the most abundant species identified in the a) EcM rhizosphere; b) Bulk Soil.

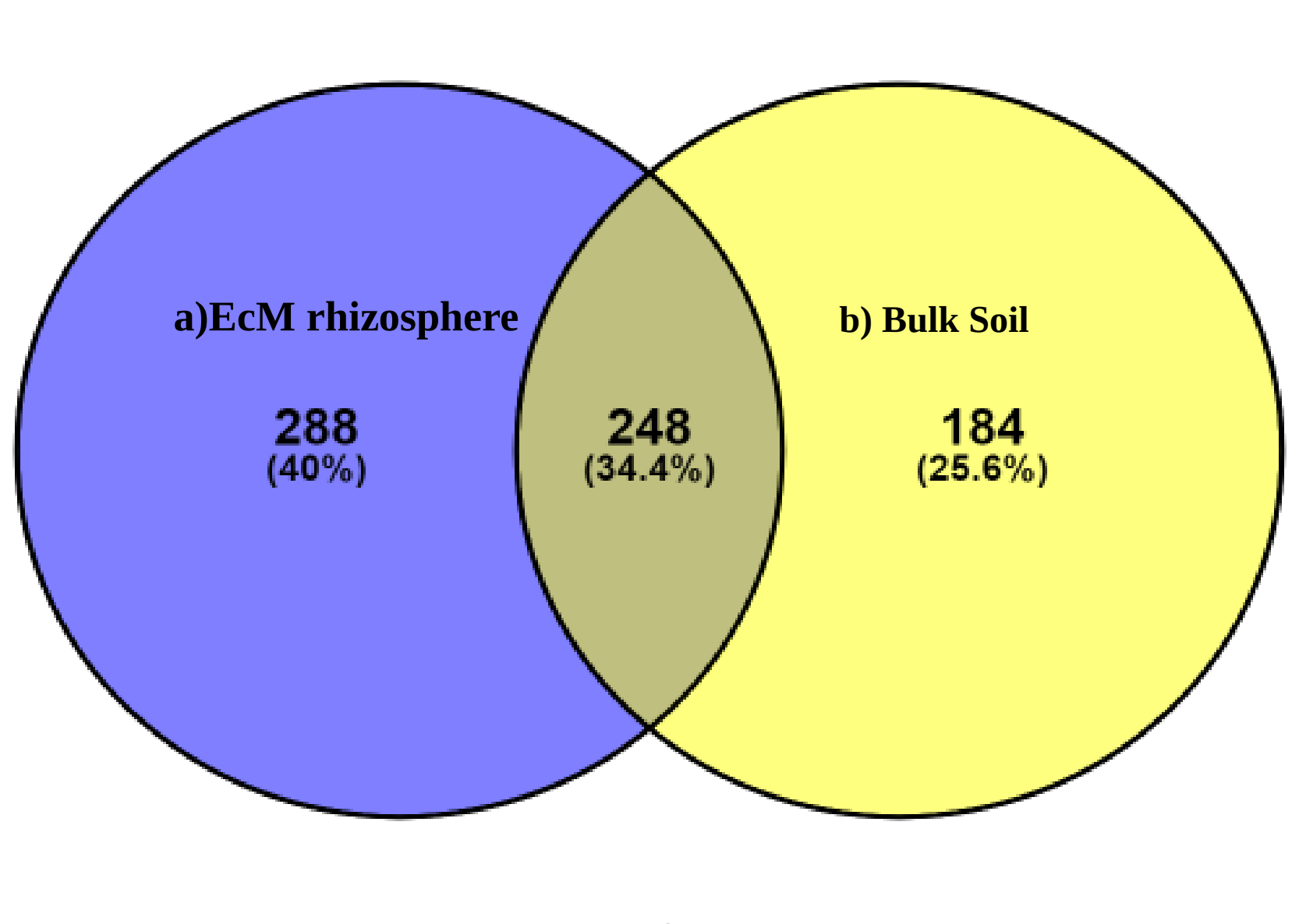


Fig 6: Venn diagram showing the number of common genera shared between phylogenetics diversity – a) EcM rhizosphere and b) Bulk Soil.

Discussion:

- The phylogenetic analysis of the metagenomic libraries classified into 20 phylum, 62 class, 136 order, 296 family, 537 genus, 662 species of EcM rhizosphere over 23 phylum, 63 class, 121 order, 262 family, 432 genus, 495 species of bulk soil.
- The rarefaction curve that expresses species diversity for both the EcM rhizosphere and adjacent bulk soil approached based on the number of OTUs, indicating, higher species richness than bulk soil.
- The abundant phyla in EcM rhizosphere were *Proteobacteria* (49.6%) followed by, *Acidobacteria* (16.7%) and *Actinobacteria* (10.9%) whereas, the abundant phyla in the bulk soil were *Firmicutes* (25.9%), *Gemmatimonadetes* (3.6%), AD3 (2.7%), *Chloroflexi* (2.6%) and *Nitospirae* (2.1%).
- The most abundant species in the EcM rhizosphere are *Sphingomonas_uc*, are ligninolytic bacteria and play significant role into the nutrient cycling whereas, in the bulk soil *Lactobacillus delbrueckii* is the dominant species.
- In this study, about 40% of the bacterial OTUs were unique to EcM rhizosphere, whereas, 25.6% of the OTUs were unique to bulk soil.
- Higher existence of unclassified bacteria within the EcM rhizosphere may be due to the fact that the bacterial diversity in this environment is poorly understood.

CONCLUSION:

Our study provides a first insight into the richness and diversity of the EcM rhizosphere bacterial communities associated with the wild edible mushroom *Astraeus asiaticus* symbiosis with *Shorea robusta* using universal 16S rRNA gene. Our analysis reveals that the EcM rhizosphere bacterial communities qualitatively resemble those of the bulk soil environments at the phylum and genus levels but have significant quantitative differences, due to the specificity of the EcM rhizosphere. However, the lack of a highly exclusive microbiome within the plant root samples may specify that the bacterial community structure plays a less significant role in the composition bacterial community structure between the soil and plant root microbiota.

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