



The impact of breastfeeding on the composition of the infant faecal microbiome



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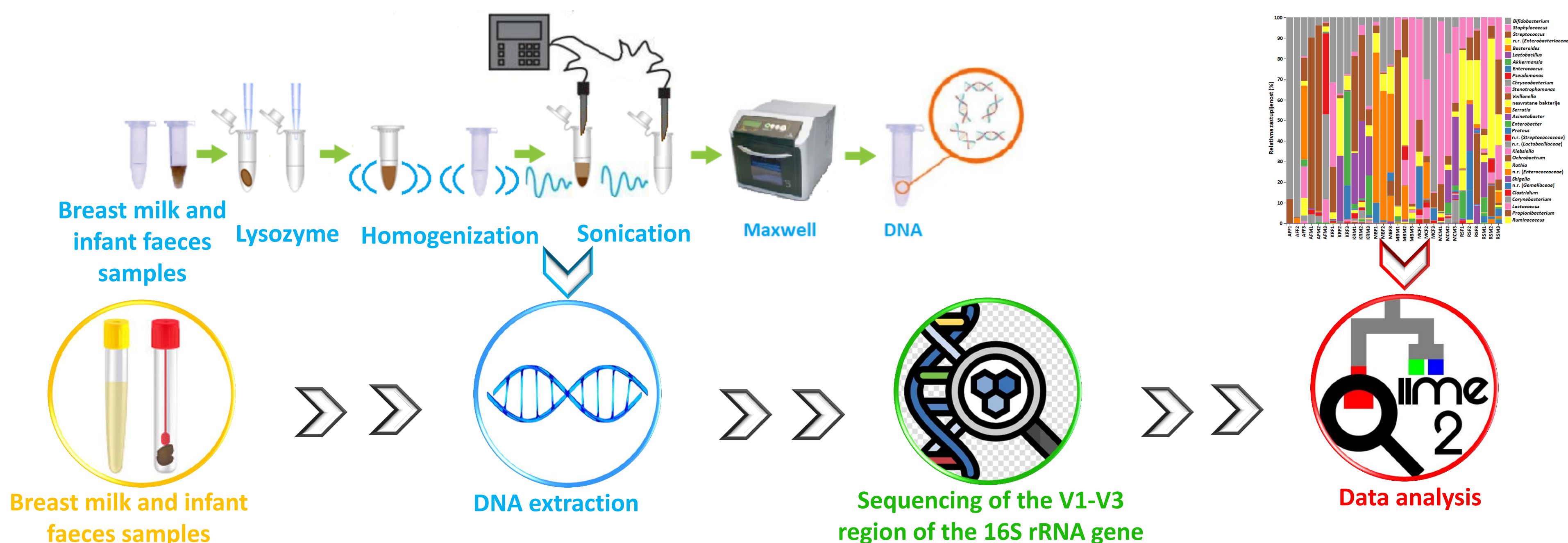
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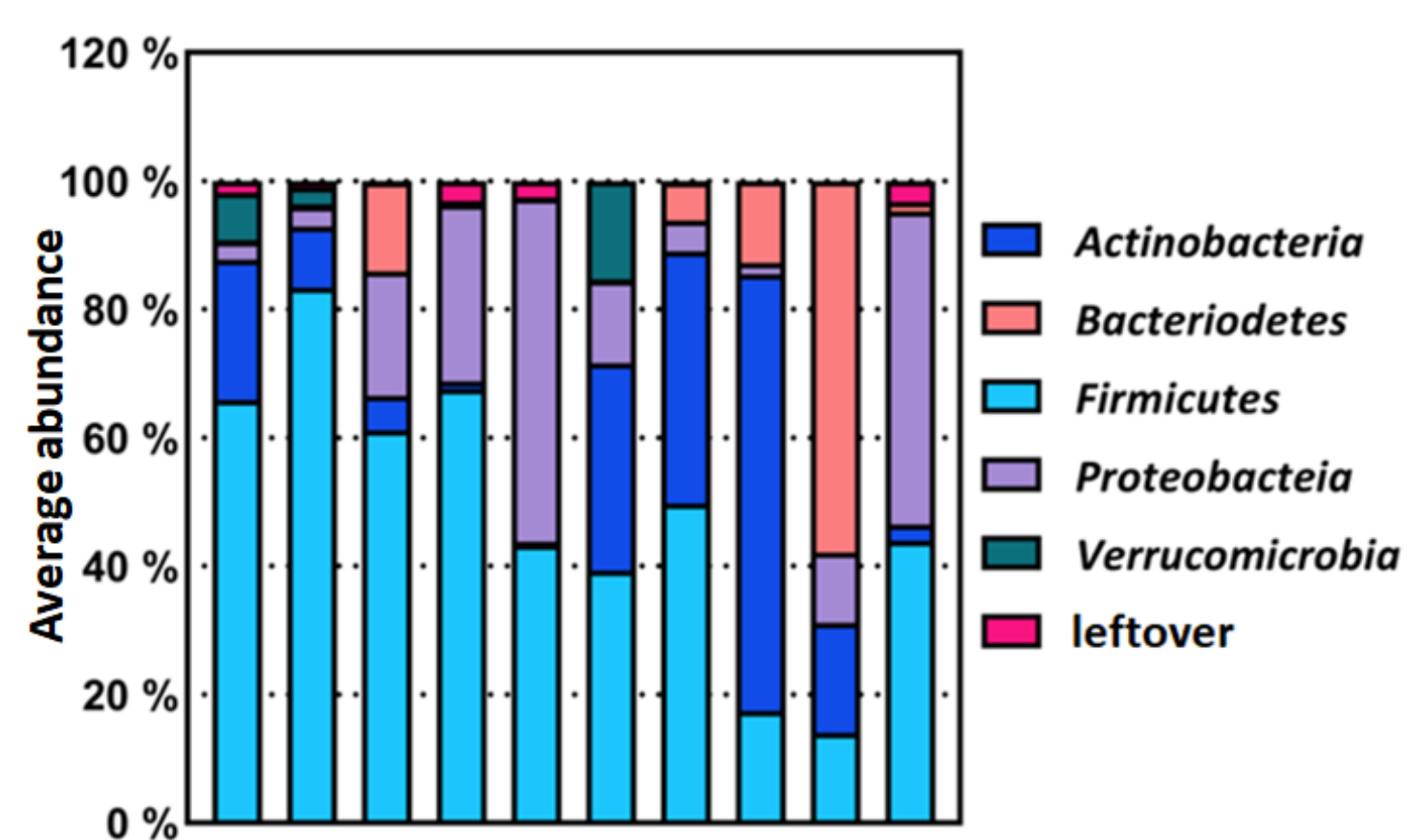
INTRODUCTION

Breast milk not only provides a perfect balance of nutrients to meet all the needs of the infant in the first months of life, but also contains a variety of bacteria that play a key role in tailoring the neonatal gut microbiome. To investigate the impact of breast milk on the composition of the infant faecal microbiome, DNA was extracted from samples of breast milk (n=15) and infant faeces (n=15) collected from 5 mother-infant pairs at 3 different time points. The V1-V3 region of the 16S rRNA gene was amplified, and amplicon sequencing was performed using the Illumina MiSeq platform. Analysis of the sequencing data using the QIIME 2 platform revealed the unique composition of each breast milk and infant faeces microbiome and confirmed the correlation of their composition in each mother-infant pair. *Firmicutes* (64.28 %) and *Proteobacteria* (21.31 %) were the most predominant phyla in breast milk microbiome, whereas *Firmicutes* (32.92 %) and *Actinobacteria* (31.86 %) dominated the faecal microbiome. The analysis confirmed that the composition of the breast milk microbiome changes during lactation, as does the faecal microbiome during infant growth and development. α -diversity analysis revealed that the breast milk microbiota is more diverse, i. e. species-rich, than the infant faecal microbiota.

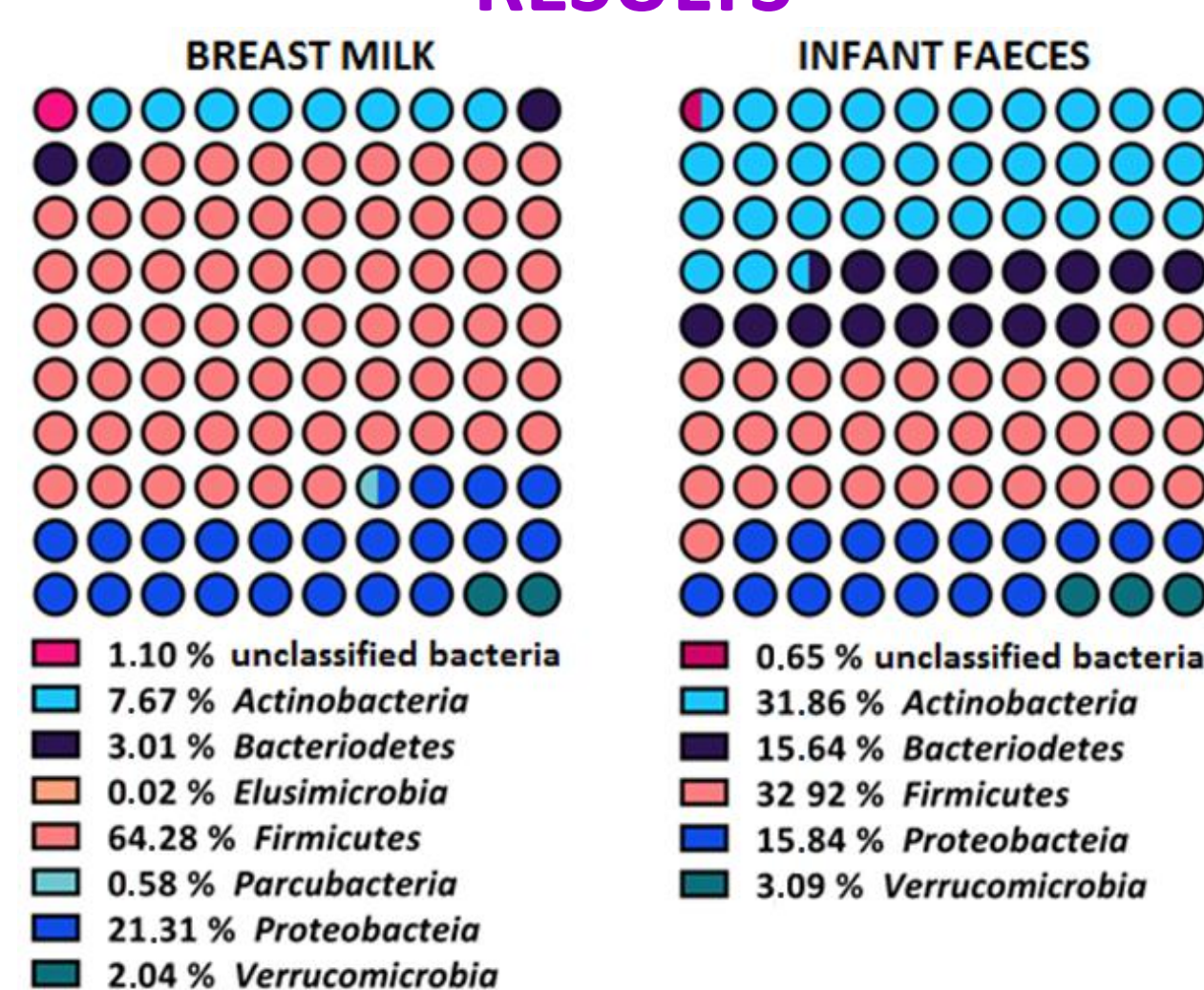
STUDY DESIGN



RESULTS

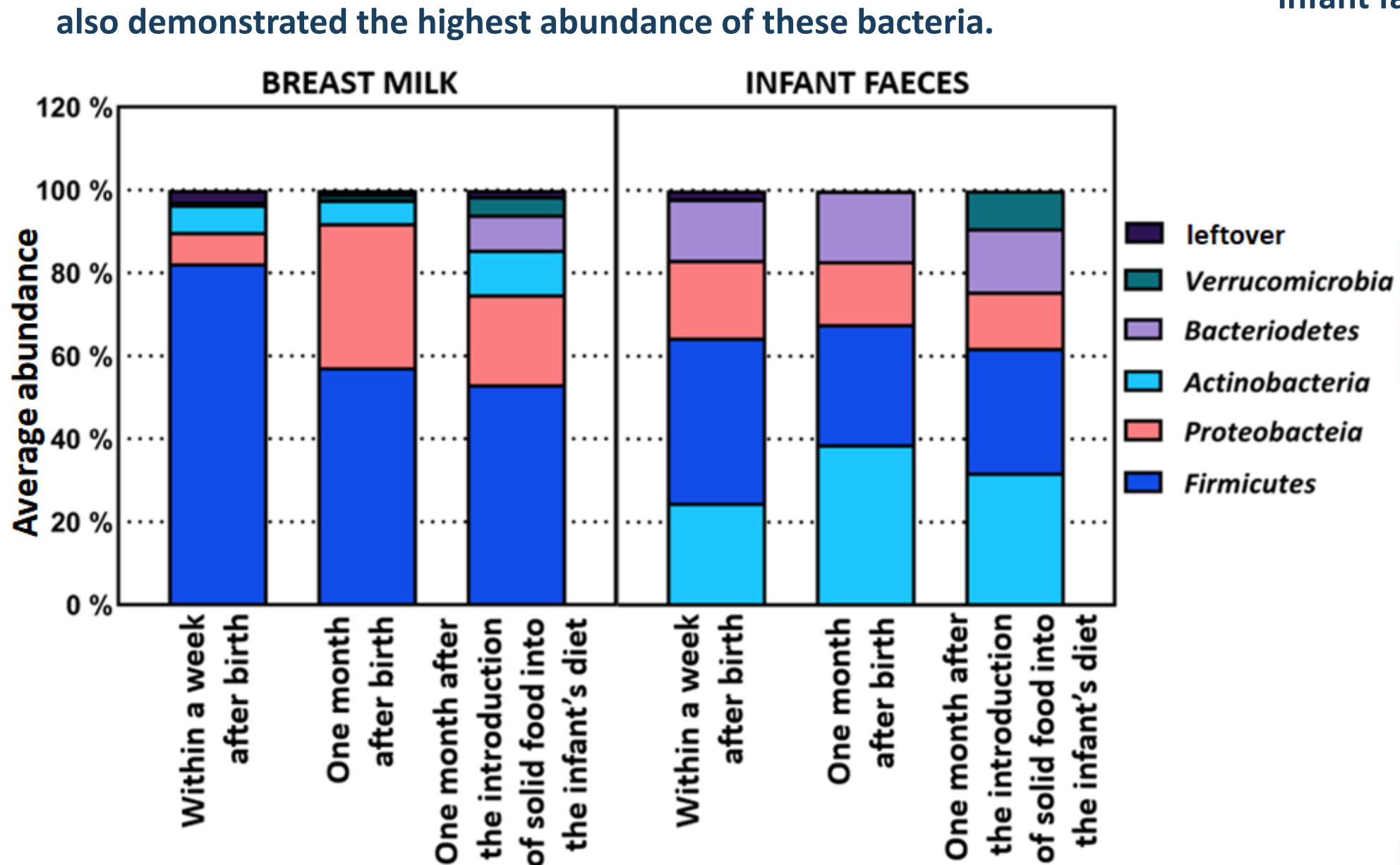


Average abundance of OTUs in milk samples from the same mother, i. e. faeces from the same child, at phylum level

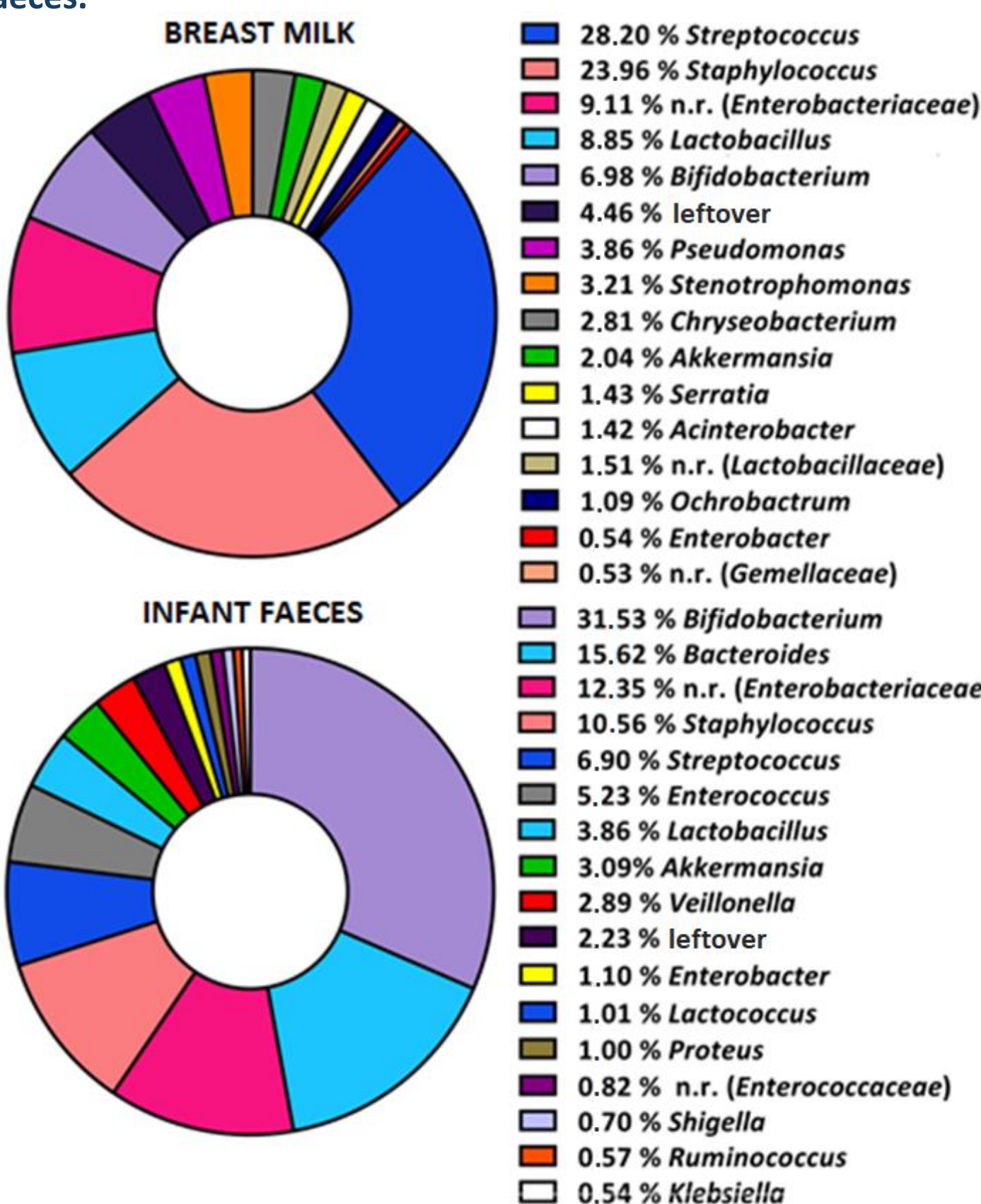


Average abundance of OTUs in breast milk and infant faeces at the phylum level

- The presence of bacteria from 7 genera (predominantly belonging to *Firmicutes* and *Proteobacteria*) was observed in breast milk, whereas the presence of bacteria from only 5 genera (predominantly *Firmicutes* and *Actinobacteria*) was observed in infant faecal samples. This suggests higher diversity of the microbiome of breast milk compared to the microbiome of infant faeces.

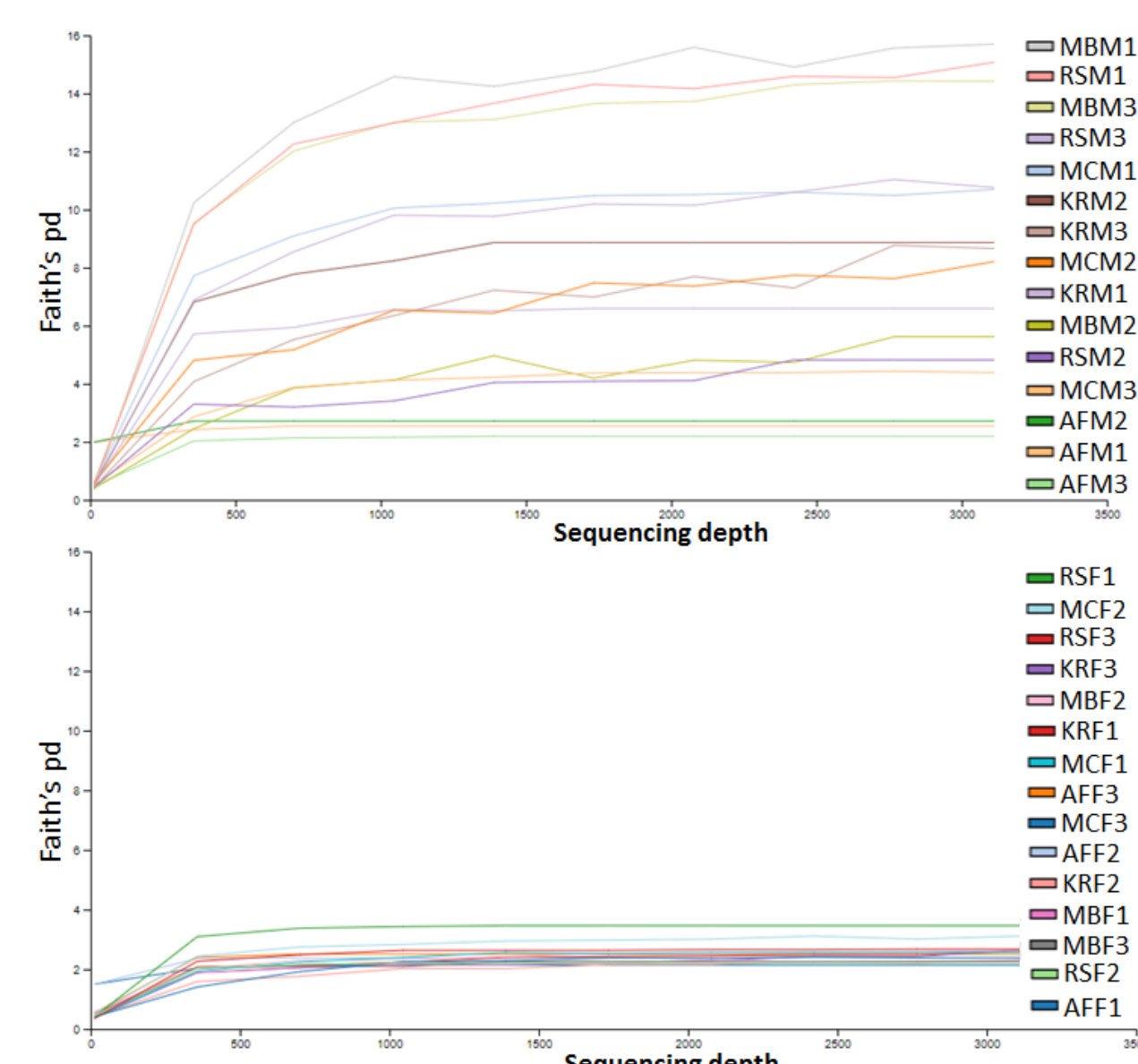


Average abundance of OTUs in samples of breast milk and infant faeces collected within one week after birth, one month after birth, and one month after the introduction of solid food into the infant's diet



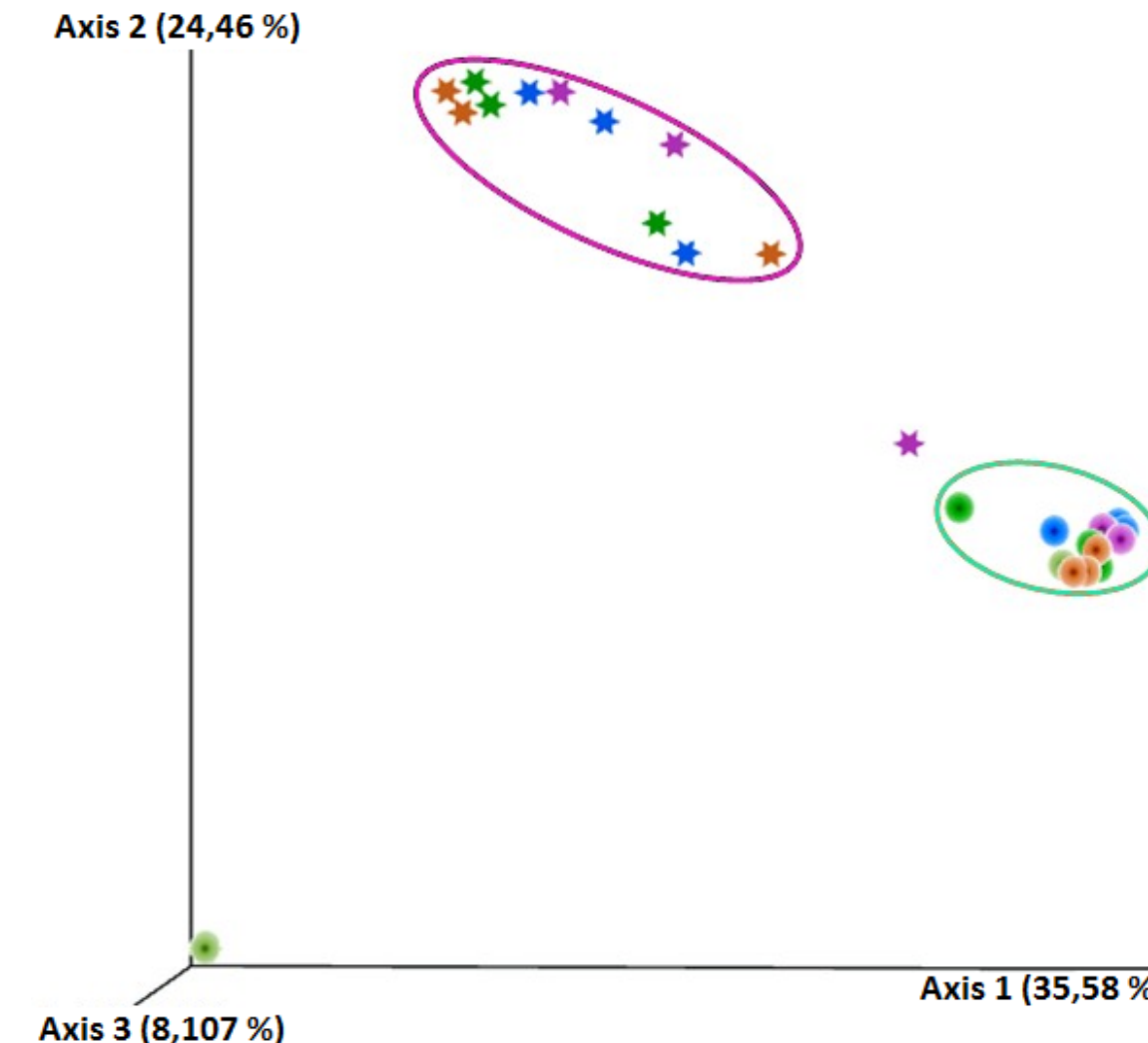
Average abundance of OTUs in all samples of breast milk and infant faeces at the genus level

- At the genus level, bacteria from the genera *Streptococcus* and *Staphylococcus* were predominantly found in breast milk. Bacteria from the genera *Bifidobacterium* and *Bacteroides* dominated the infant faecal microbiome.



α -diversity of the breast milk and infant faecal microbiome calculated by Faith's phylogenetic diversity level metric

- α -diversity of the breast milk microbiome was greater than that of the infant faeces, meaning that the breast milk microbiome is richer in species than the faecal microbiome. A correlation in the compositional biodiversity of breast milk and infant faeces was also established, as the samples of milk and faeces with the lowest α -diversity belonged to the same mother-infant pair.



PCoA analysis of β -diversity calculated by the Unweighted UniFrac metric. Breast milk samples are represented by an asterisk and faecal samples by a circle. Samples from the same mother-infant pair are marked with the same colour. The pink ellipse surrounds a cluster of breast milk samples whereas the blue ellipse surrounds a cluster of infant faecal samples

- PCoA analysis formed two clusters, one comprising the breast milk samples and the other the infant faecal samples. The smallest distance was observed between the samples labelled with the same colour, belonging to the same mother or infant, implying that the microbiome of breast milk and faeces of each individual is unique.

CONCLUSION

Analysis of the 16S rRNA sequencing data using the QIIME 2 platform revealed that the microbiome of each breast milk and each infant's faeces is unique, and showed the correlation in the composition of the microbiome of mothers' milk and their infants' faeces. The results also implied that the composition of the breast milk microbiome changes during lactation, as does the faeces microbiome. By analyzing the α -diversity of the collected samples, it was found that the breast milk microbiome is more diverse, i. e. richer in species, than the infant faecal microbiome. PCoA analysis of the β -diversity of the breast milk microbiome and the faecal microbiome of the infant, calculated with the Unweighted UniFrac metric, showed the smallest distance between milk samples belonging to the same mother and faeces belonging to the same infant, implying that each person's microbiome is unique.

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