

# Comparison between shotgun metagenome and 16S rRNA sequencing for the taxonomic characterization of the gut microbiota in patients with osteoarthritis

## Introduction

- Osteoarthritis (OA) is a chronic painful musculoskeletal condition.
- Currently no cure, only interventions to manage its symptoms.
- Typically thought of as a disease of 'wear and tear' but now known to be due to complex interactions connecting biomechanics, inflammation, and the immune system.
- A multidisciplinary approach to understanding the multifaceted aetiology is needed, for innovative new management and treatment pathways to be identified and developed.
- The gut microbiome has been proposed to be involved in osteoarthritis pathogenesis.

## Aim

- To explore the relationship between osteoarthritis and the gut microbiota by evaluating the ability of 16S rRNA and shotgun metagenomic sequencing to characterise bacterial taxonomic species profile in the faecal microbiome of patients with knee osteoarthritis.

## Methodology

- Faecal samples were collected at baseline as part of a randomised control trial evaluating the efficacy of an Internet-Based Exercise programme Aimed at Treating knee Osteoarthritis (iBEAT-OA) in the community.
- Samples from 10 randomly selected Caucasian participants were analysed.

Study ID	Age	Gender	BMI
S4	68	Female	30.7
S6	82	Female	30.9
S8	74	Female	27.0
S16	71	Unknown	22
S21	77	Female	25.1
S58	63	Male	32
S63	47	Male	31.5
S75	54	Female	33.2
S78	71	Female	27.5
S84	60	Unknown	36.3

- Both 16S rRNA and shotgun raw reads were processed, taxonomically classified using reference databases, and Relative Species Abundance (RSA) computed using Python and R scripts and Galaxy Europe.

## Results Comparing Sequencing Methodology

Principal coordinate analysis was used to examine and compare the microbiota community structures (Fig 1).

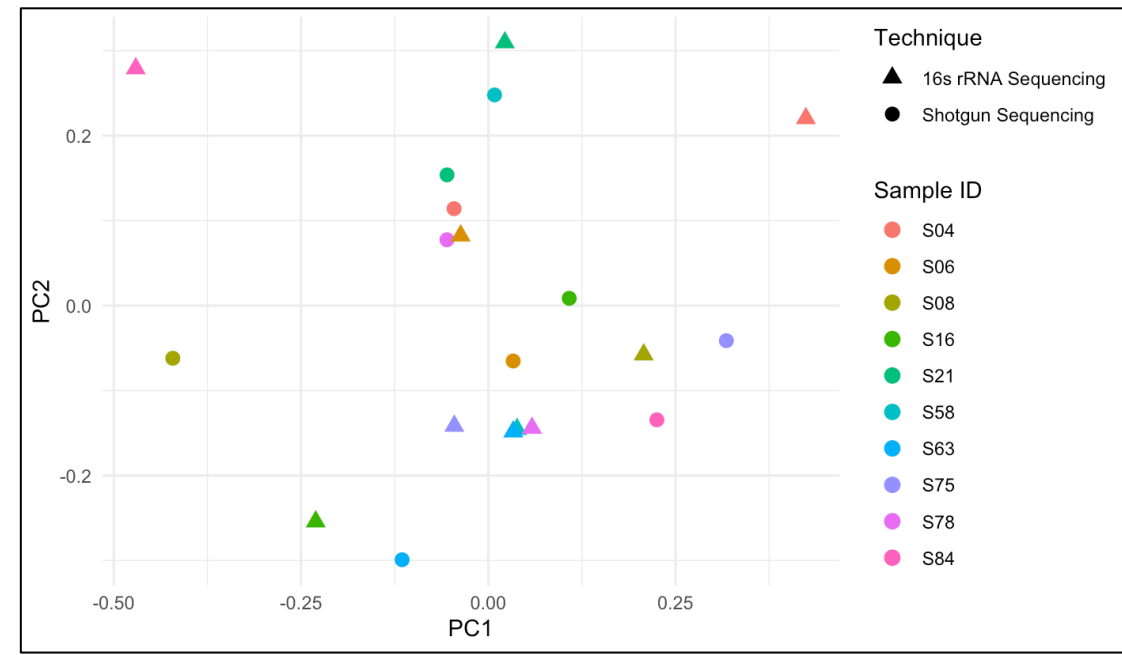


Fig 1 Principal coordinate analysis comparing sequencing techniques

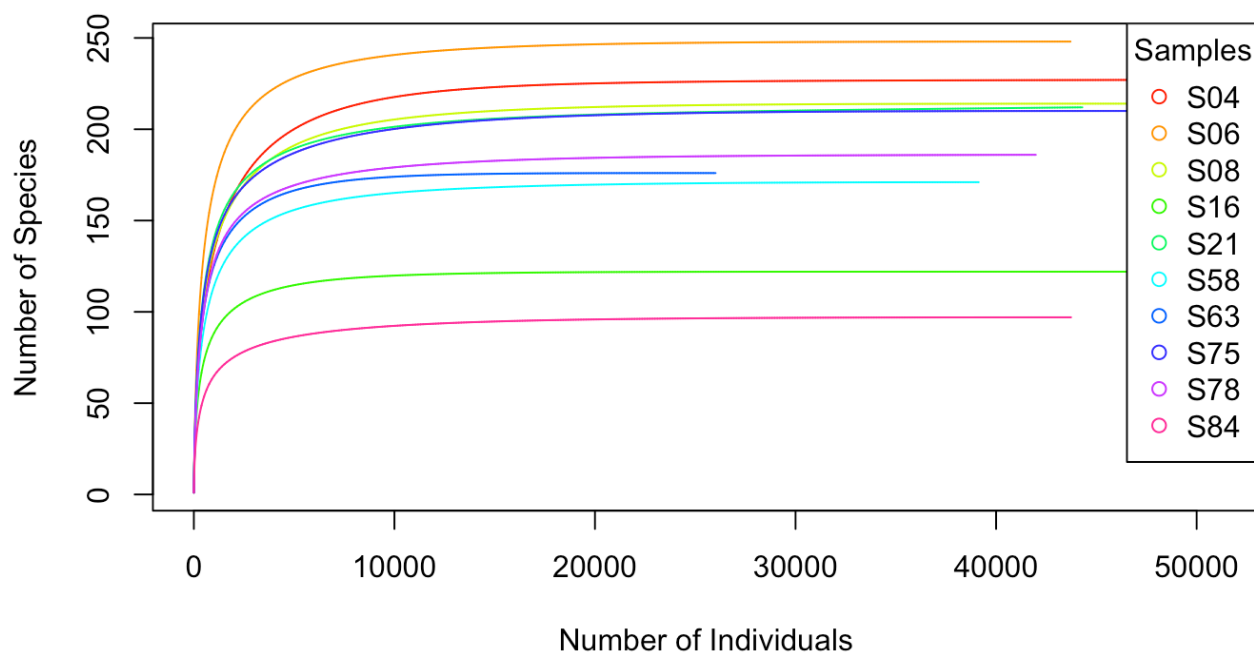


Fig 2 Rarefaction curve: 16S rRNA sequencing

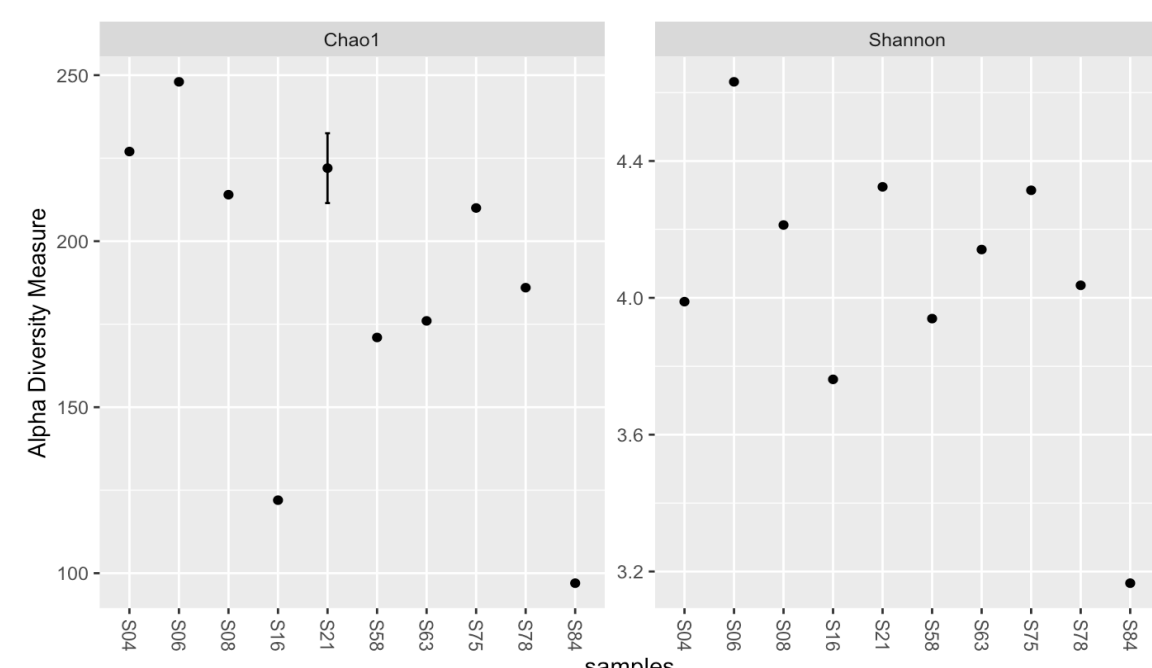


Fig 4 Alpha diversity : 16S rRNA sequencing

Richness and diversity of the gut microbiota were examined through rarefaction curves as an objective way to compare species richness (Fig 2 & 3).

To identify the total number of species in each sample and compare the differences between the two sequencing methodologies the Chao1 index was used to measure the species richness and the Shannon index was used to estimate the species diversity (Fig 4 & 5).

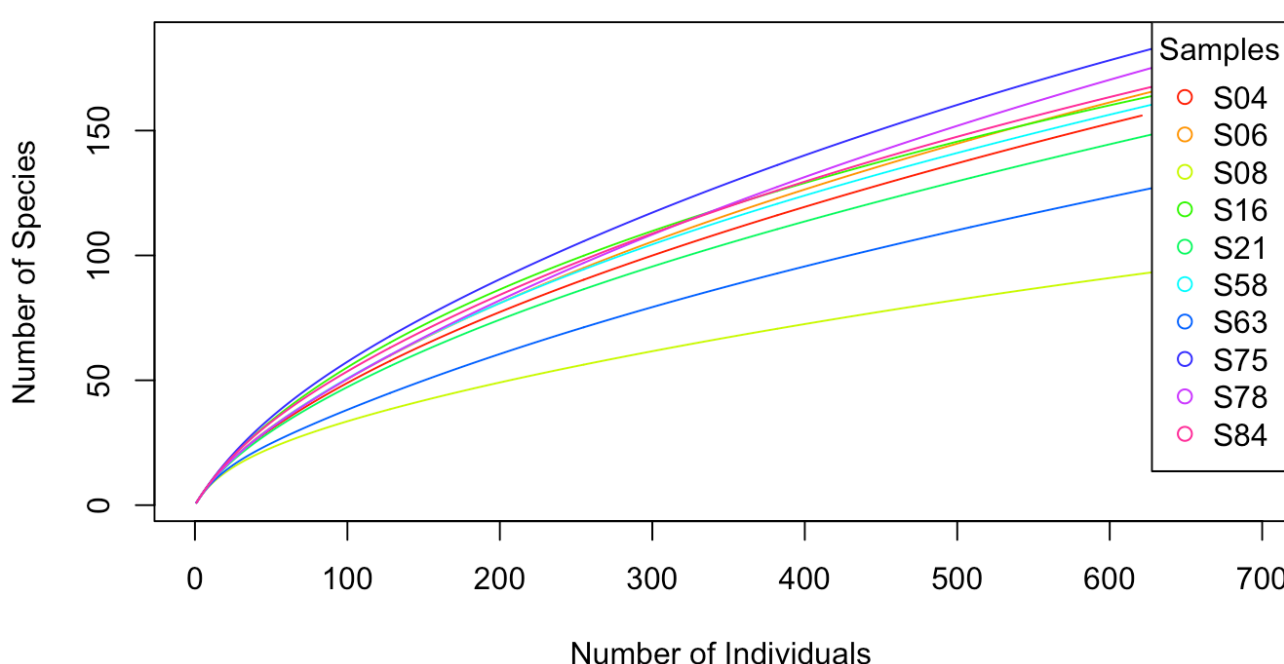


Fig 3 Rarefaction curve: Shotgun sequencing

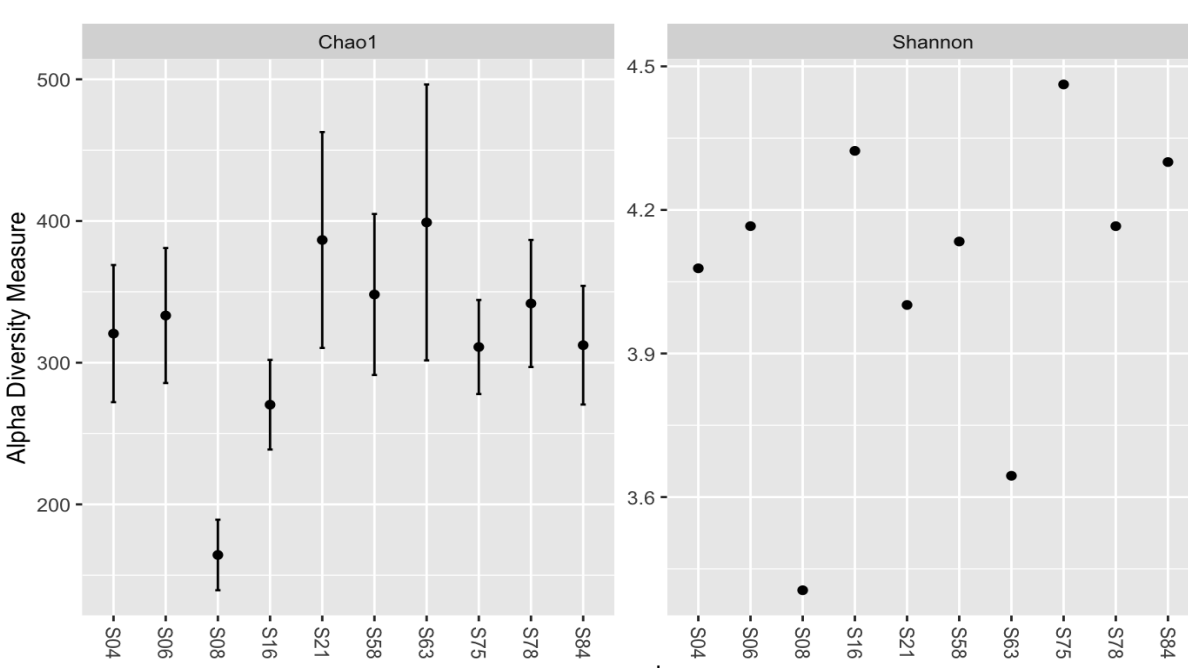


Fig 5 Alpha diversity : Shotgun sequencing

Krona charts comparing the taxonomic hierarchy, identification and relative abundance of the most abundant bacteria identified through 16S rRNA (Fig 6) and Shotgun (Fig 7) sequencing.

Fig 6 Krona Chart: 16S rRNA sequencing

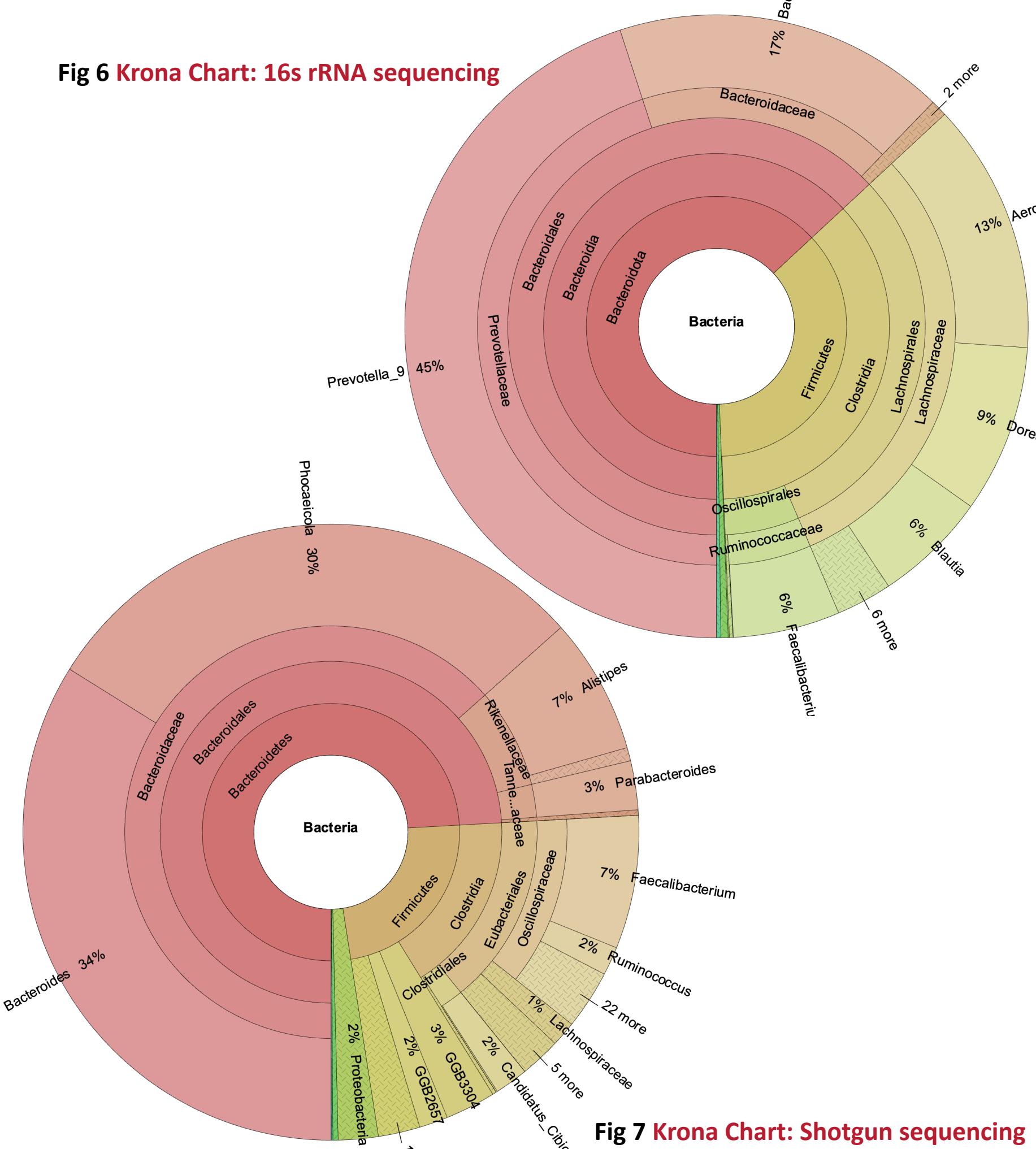


Fig 7 Krona Chart: Shotgun sequencing

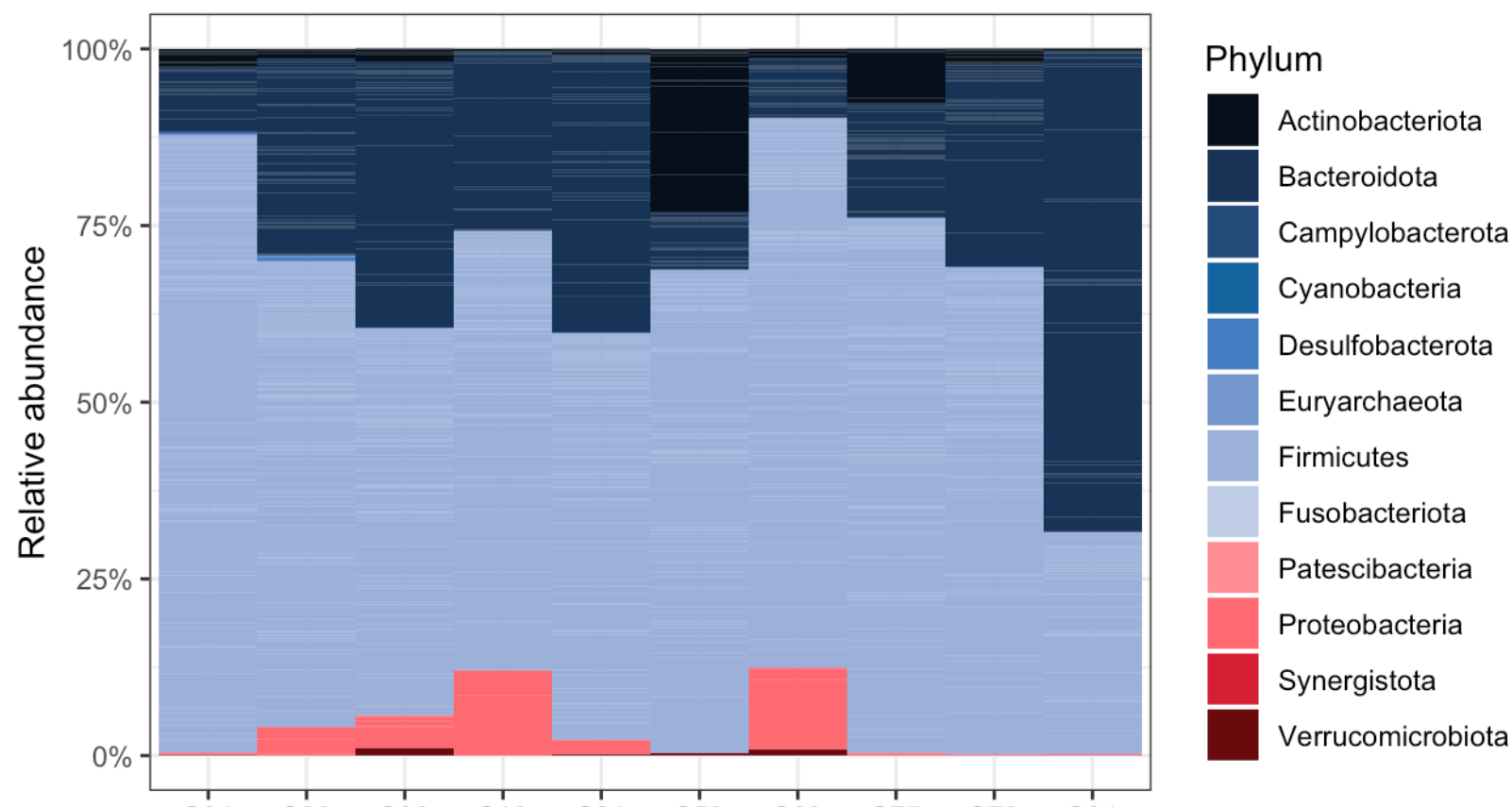


Fig 8 Relative phylum abundance: 16 s rRNA sequencing

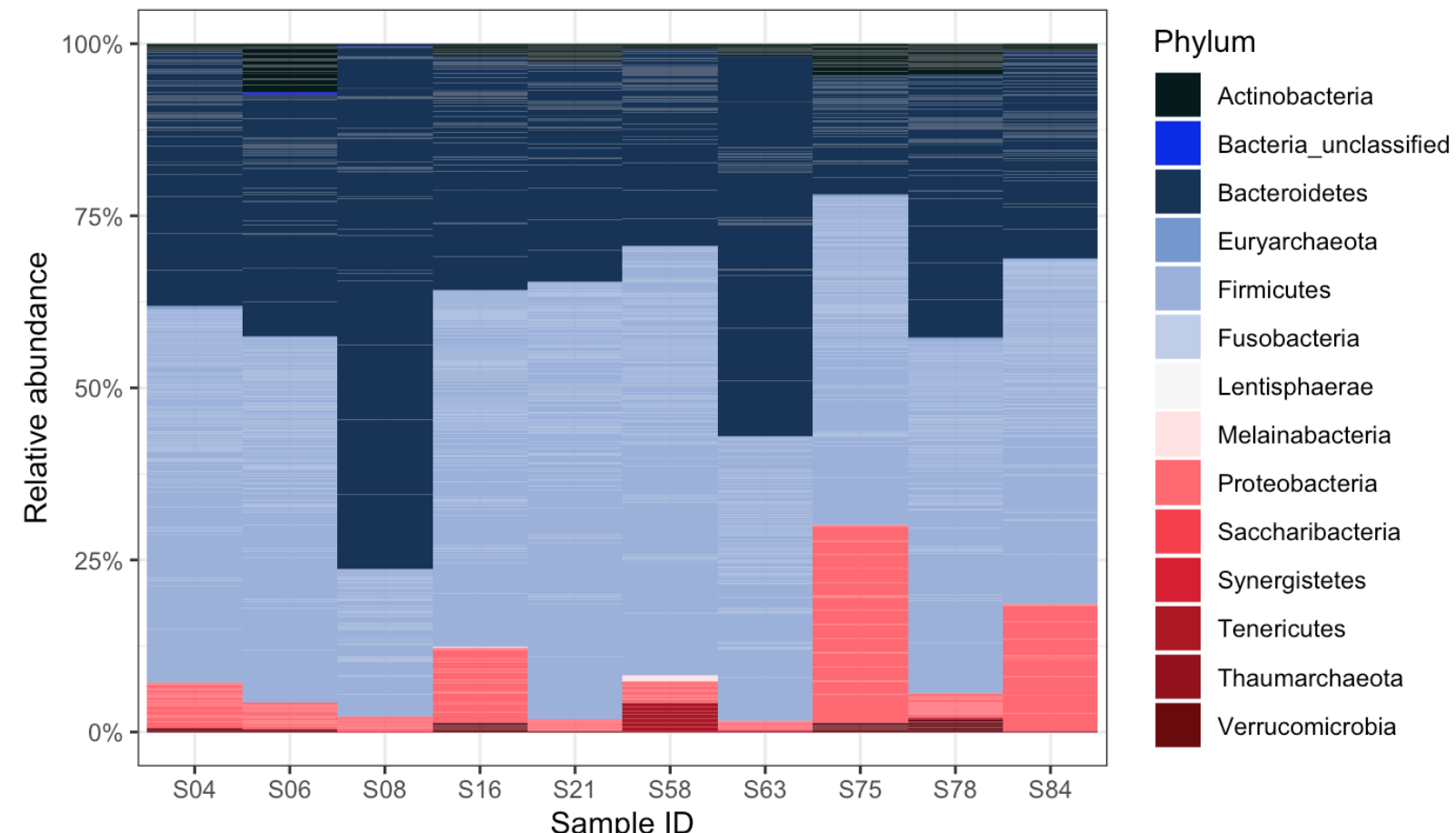


Fig 9 Relative phylum abundance: Shotgun sequencing

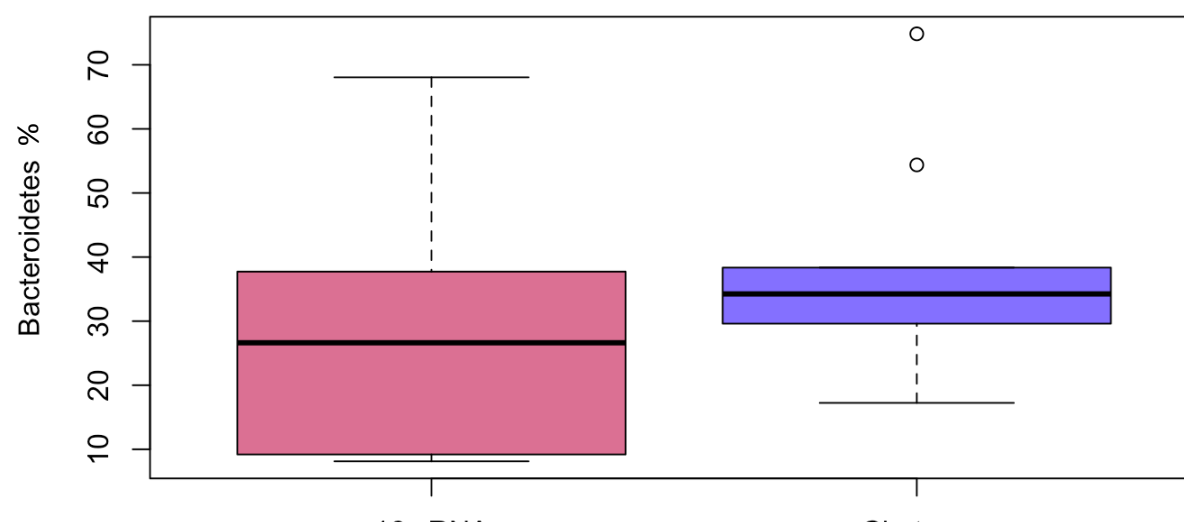


Fig 10 Bacteroidetes abundance by sequencing technique

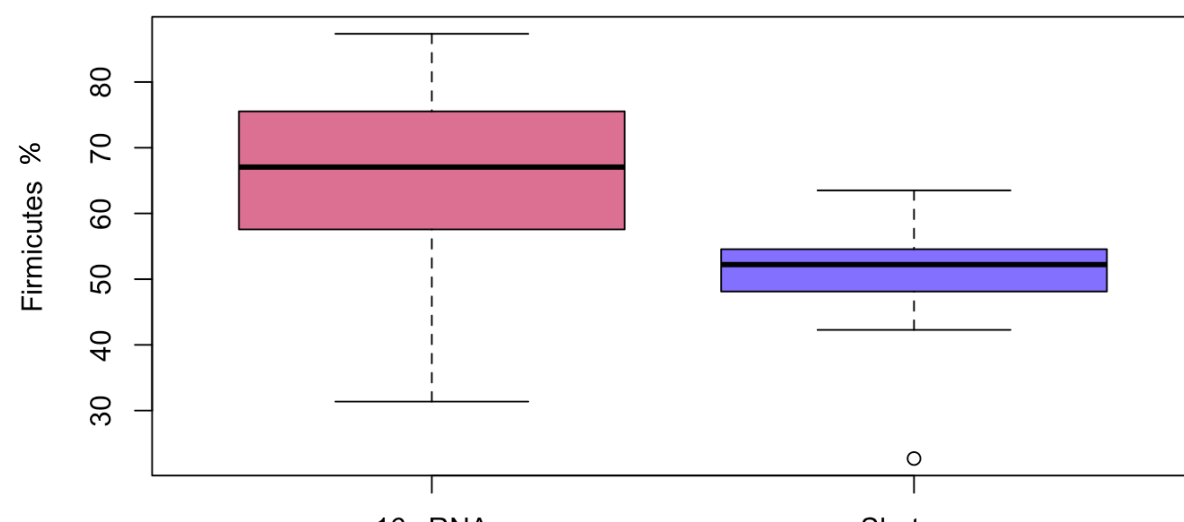


Fig 11 Firmicutes abundance by sequencing technique

## Results Relevant to OA Patients

Across all samples, **shotgun** sequencing identified **811** unique bacterial species whereas only **165** were identified using **16S rRNA** sequencing. The most relatively abundant species were:

- **Prevotella copri**: associated with musculoskeletal inflammatory diseases including ankylosing spondylitis and new-onset untreated rheumatoid arthritis; suggested to be immune-relevant in rheumatoid arthritis pathogenesis.
- **Faecalibacterium prausnitzii**: reported to alleviate inflammatory arthritis and other immune-related disorders.
- **Bacteroides uniformis**: described as the most driving species in dermatan sulfate degradation, which has been suggested to promote rheumatoid arthritis and osteoarthritis by affecting articular cartilage.
- **Anaerostipes hadrus** reported to be enriched in osteoarthritis patients compared to healthy subjects.

Numerous taxonomic differences were observed following analysis by 16S rRNA and shotgun sequencing. At the phylum level, Using 16S rRNA sequencing the overall abundance of bacteria in our study cohort at a phylum level was identified as 63% Bacteroidetes and 36% Firmicutes. However, using NGS this was 74% Bacteroidetes (range 17.24 to 74.83%) and 23% Firmicutes (range 22.7 to 63.5%). (Figures 8-11)

## Conclusion

The higher number of taxa found using shotgun sequencing showed that 16S rRNA gene sequencing detects only part of the gut microbiota community compared to shotgun sequencing, which was able to identify less abundant taxa. When compared to healthy individuals these gut bacterial signatures could help to provide biomarkers to predict osteoarthritis and help in future mechanistic and clinical intervention studies.

Joanne Stocks<sup>1,2,3</sup> Afroditi Kouraki<sup>1,2</sup> Amrita Vijay<sup>1,2</sup> Tony Kelly<sup>1,2</sup>  
Sameer Gohir<sup>1,2</sup> Ana Valdes<sup>1,2</sup>

<sup>1</sup>Academic Unit of Injury, Recovery and Inflammation Sciences, School of Medicine, University of Nottingham, Nottingham, UK <sup>2</sup>NIHR Nottingham Biomedical Research Centre, Nottingham, UK, <sup>3</sup>School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK



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Joanne.Stocks@Nottingham.ac.uk  
@DrJoanneStocks  
@JoStocks  
www.JoanneStocks.com