Understanding the impact of chlorine/chlorine-free cleaning approaches and other factors on the microbial composition of raw milk

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Study Background

- Chlorine-based detergents are highly effective and commonly used in the dairy industry
- Formation of total organic chlorine residues
  - Affect end-product quality
  - Pose potential risk to consumer health
- Shift towards the use of chlorine-free cleaning products
- Unsure of influence of different cleaning methods (chlorine & non-chlorine) on microbial composition of milk

Study aim

Aim: Understand the effect of the cleaning method (chlorine & non-chlorine) on the microbial composition of bulk tank raw milk

- Any differences?
- Any other factors that influence the microbial composition?

Study design

- **Sampling:** Raw milk from bulk milk tanks from farms across Ireland were sampled in April, August and November 2019

Farms chosen by 4 milk processors classified into **3 cleaning categories**:

- Chlorine used for machine and bulk milk tank (C)
- Chlorine-free used for machine and bulk milk tank (CF)
- Chlorine-free used for bulk milk tank only (BTCF)

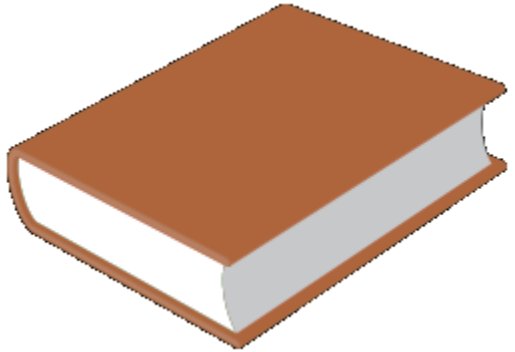
Study design

- **Microbial analysis**
  - Total bacteria counts (TBC), Thermoduric, Thermophilic and other tests
- **Chlorine residue analysis**
  - Perchlorate, Chlorate and Trichloromethane

Previous study (David & Lizandra)

- **High-throughput sequencing**
  - DNA extraction from samples
  - Shotgun metagenomic sequencing on Illumina NextSeq
  - Bioinformatics analysis

High-throughput sequencing



- DNA – letters
- Genes – sentences
- Genome – book
- Samples (metagenomes) – shelves with different books

Why develop sequencing-based approaches?

- Not all bacteria can be grown on agar
- Different bacteria require different conditions to grow
- Microbes in samples – more than just bacteria present

High-throughput sequencing

The quick brown fox jumped over the lazy dog.

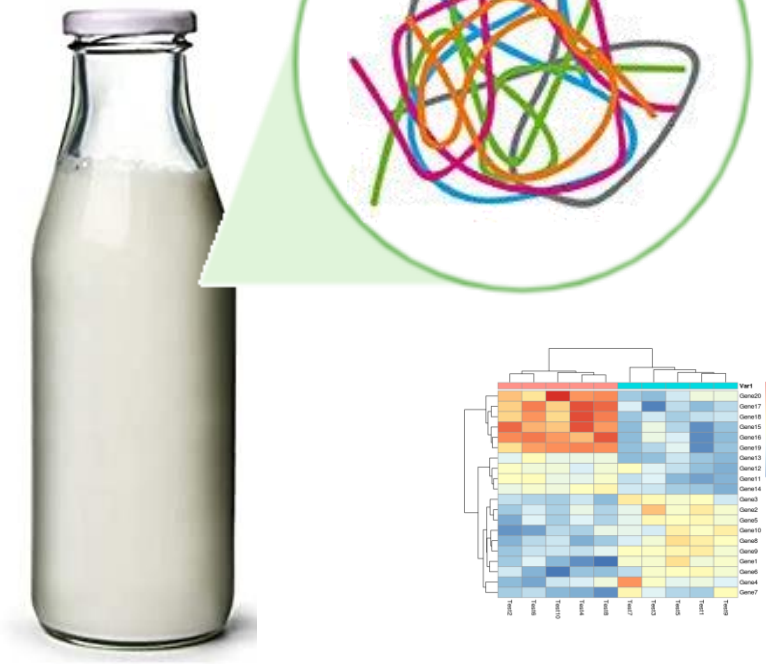
The quick brown fox

brown fox jumped

fox jumped over the

over the lazy dog.

DNA extraction

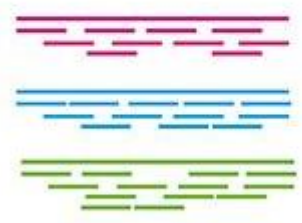


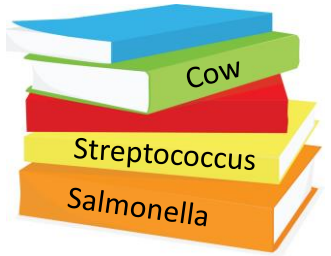
Fragmentation

DNA sequencing

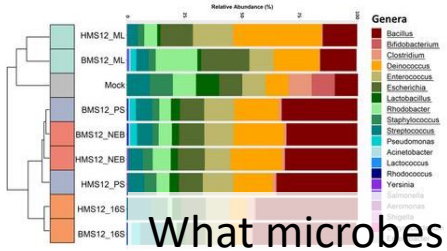
GCGCGATATGCGTATTT
GCGTTAAATGCGCTATT
CGAGTTCCCGGTATATA
AGTTAACGATTAGGCAT
CGGATAGGTTAGTATCG
GCGCGATATGCGAATTC

Read classification

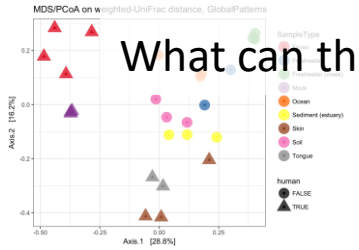




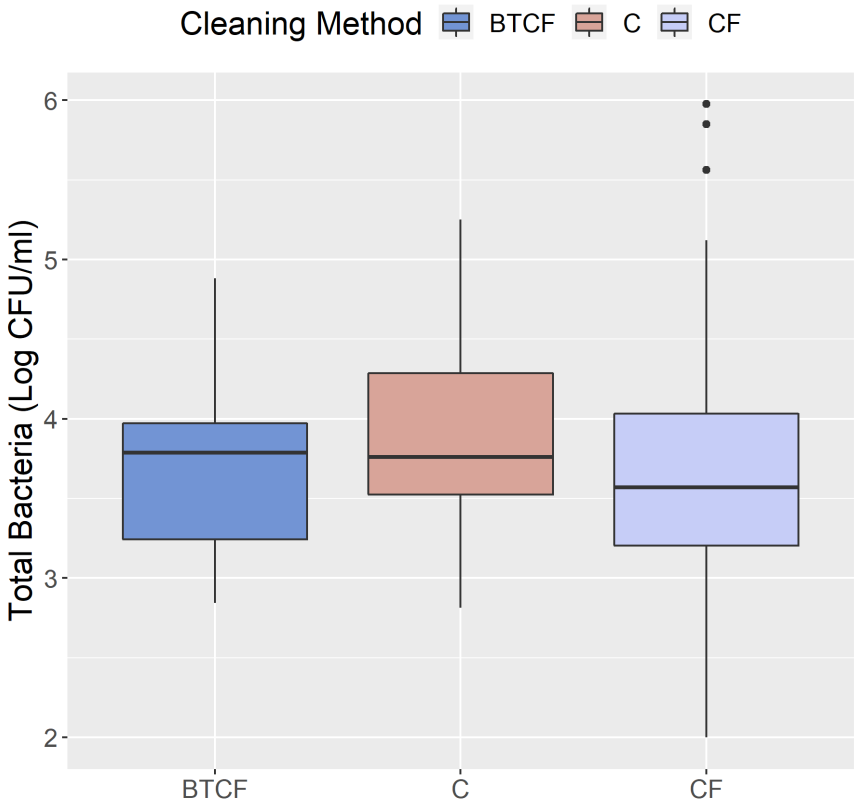
What microbes are there?

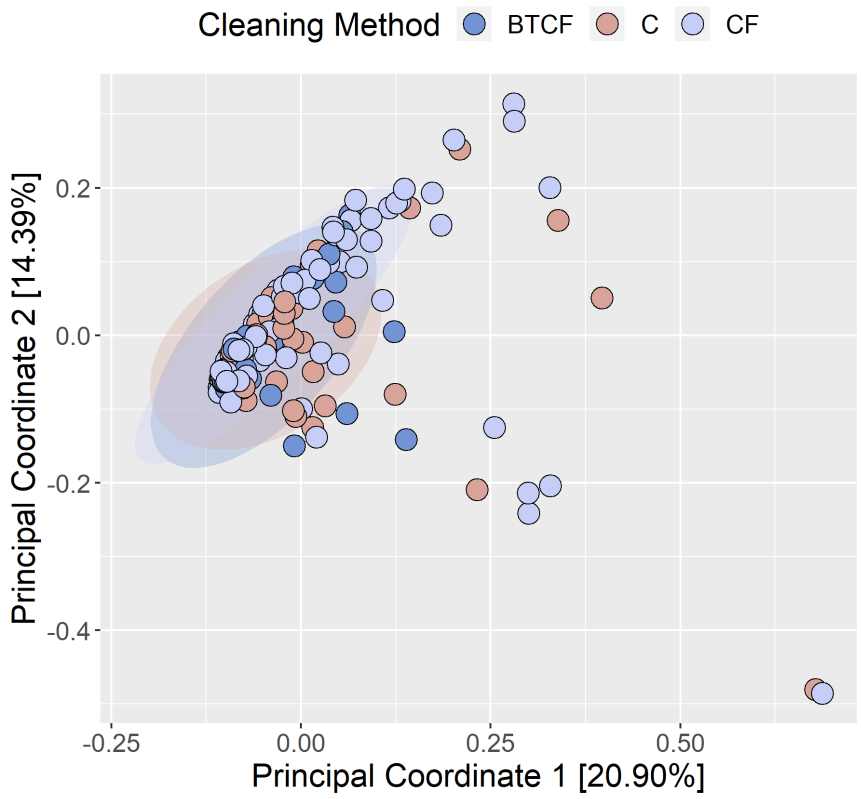


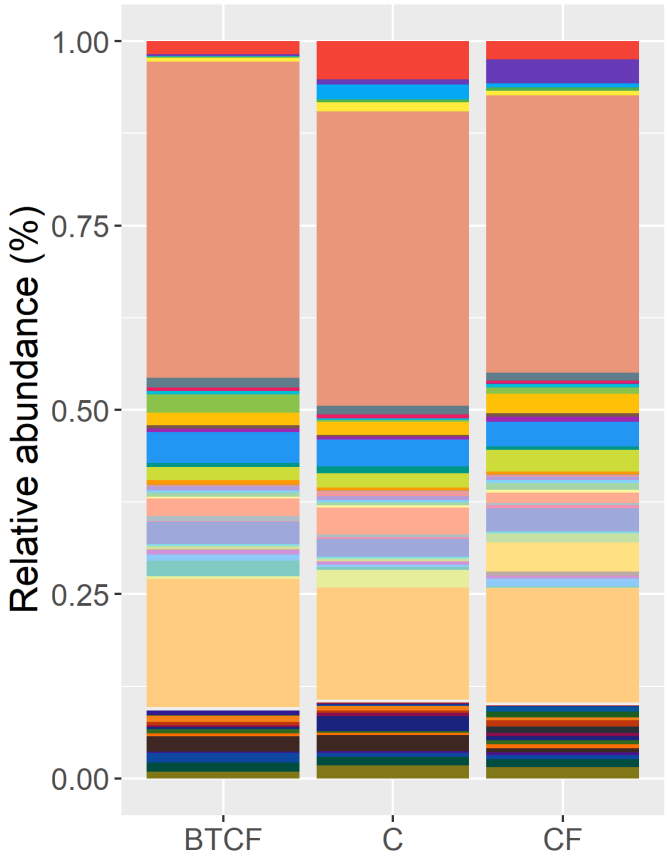
What can they do?



Cleaning method did not impact microbial composition





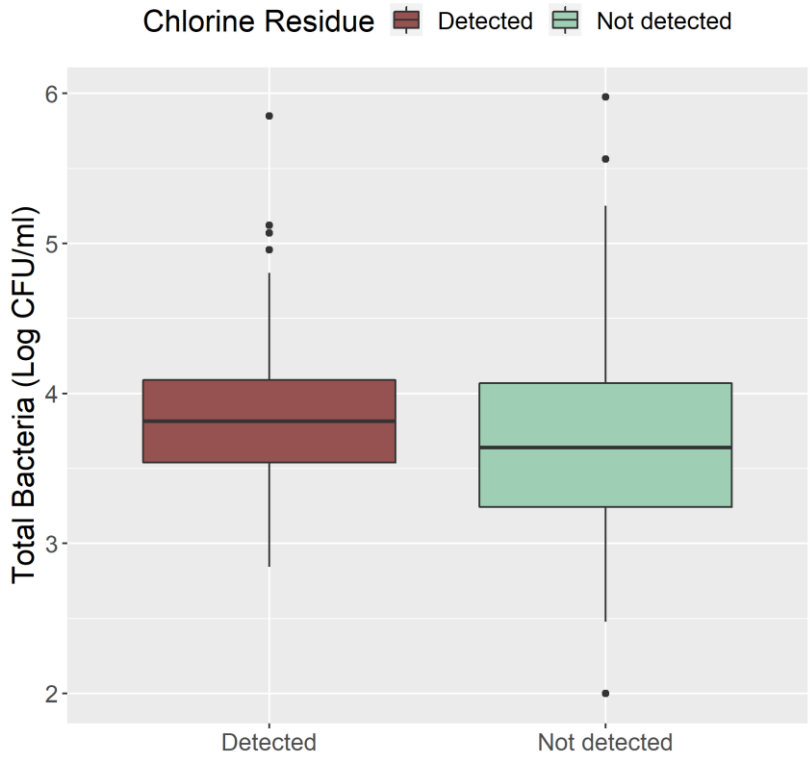


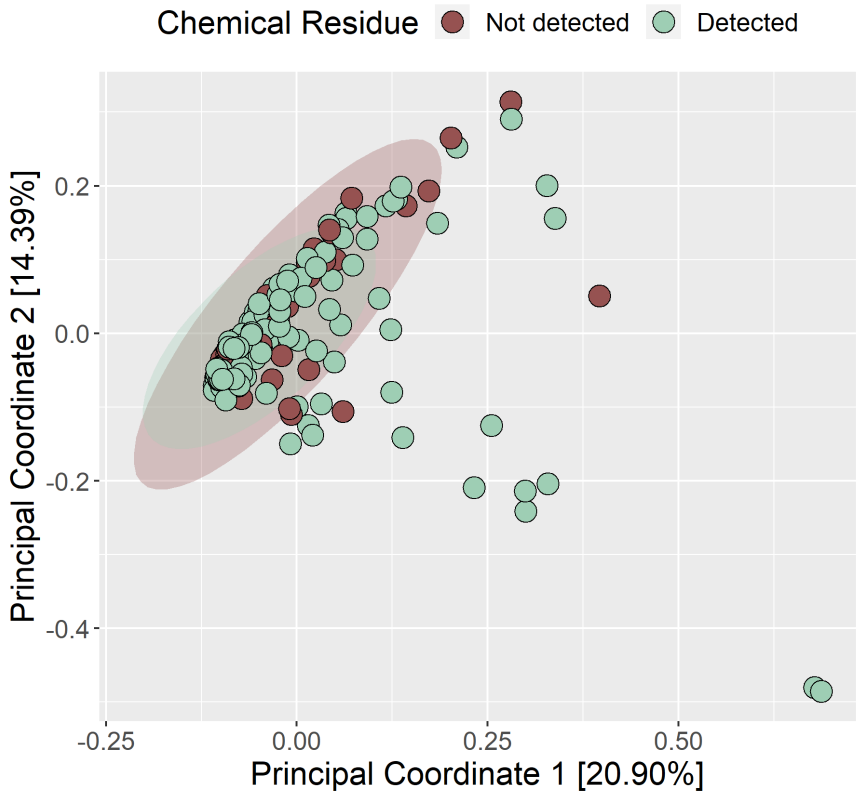
Detection of chlorine residues was not associated with a different microbial composition

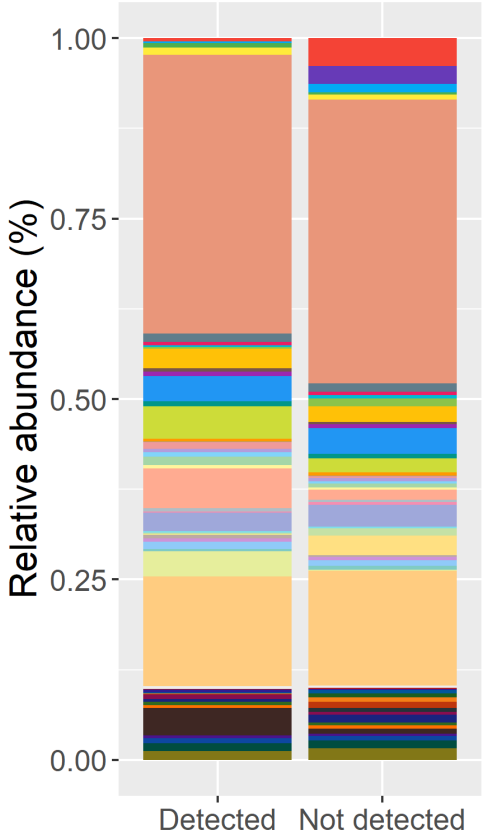
Chlorine residue detected

Greater than 0.0015 mg/kg trichloromethane

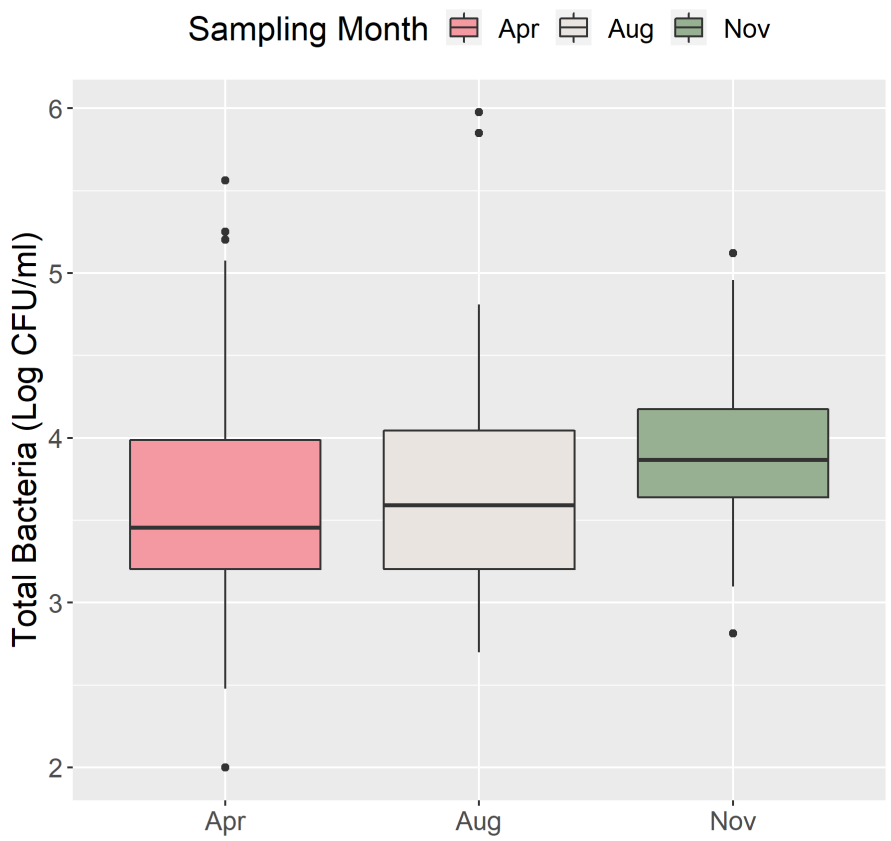
Or greater than 0.002 mg/kg chlorate detected

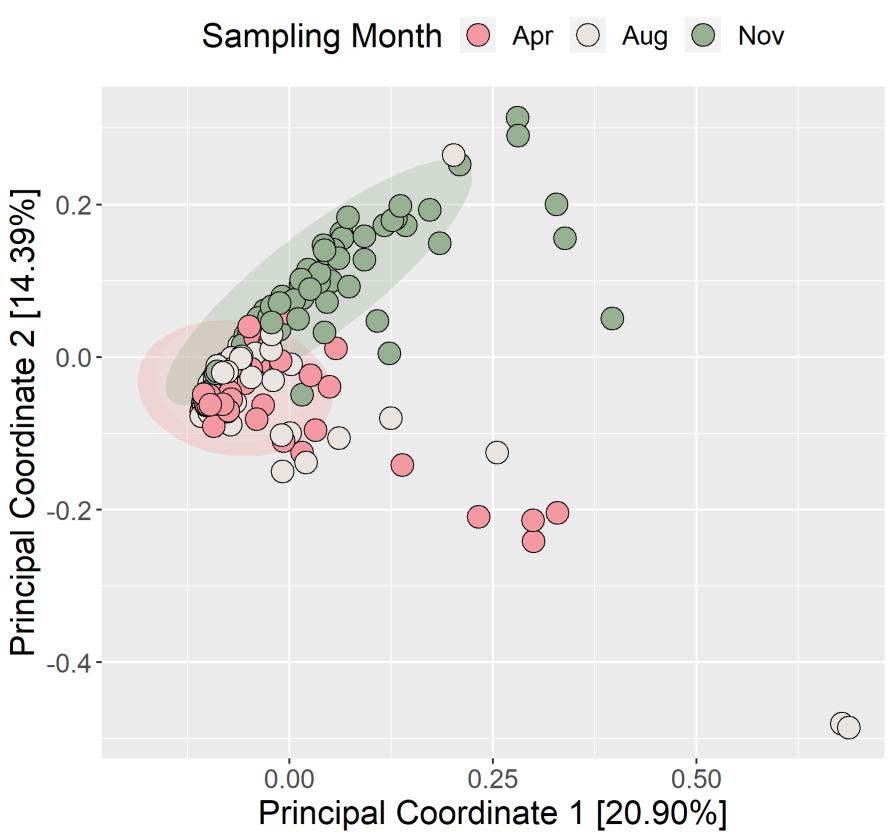


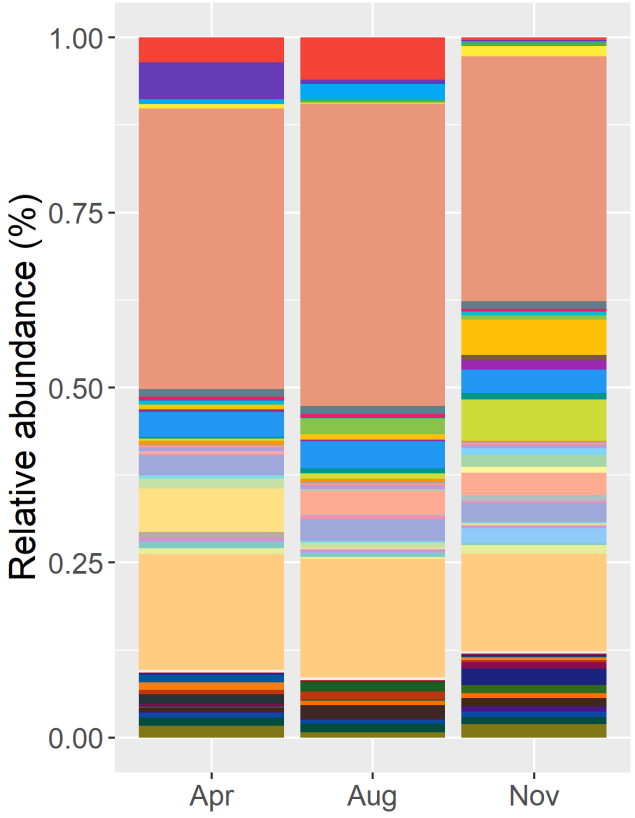




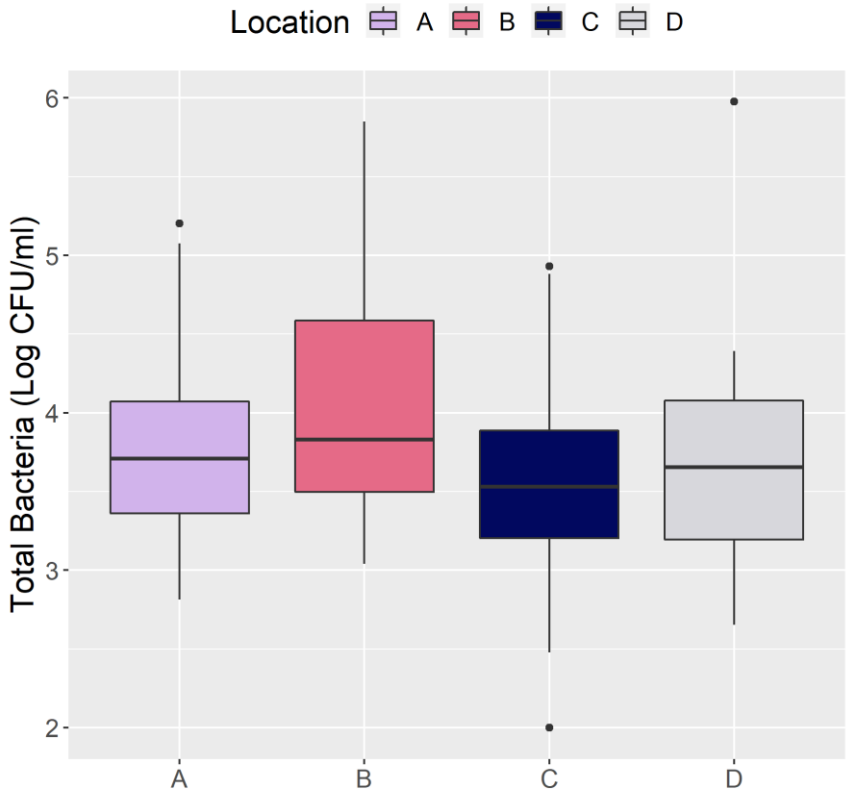
Samples taken in Nov had a different microbial composition

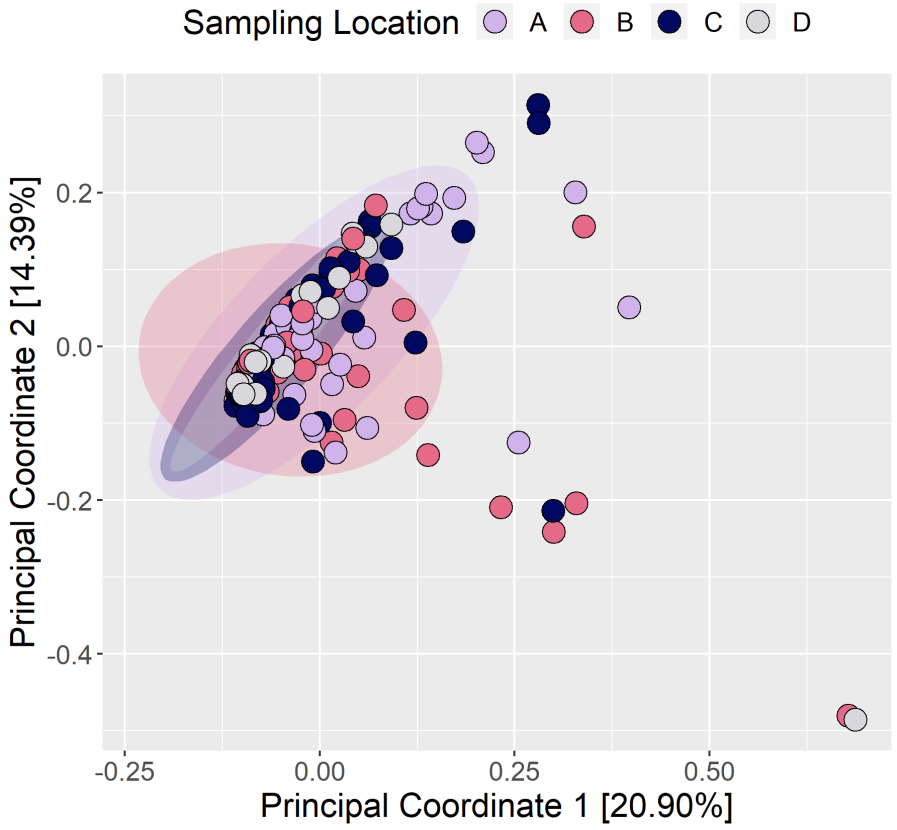


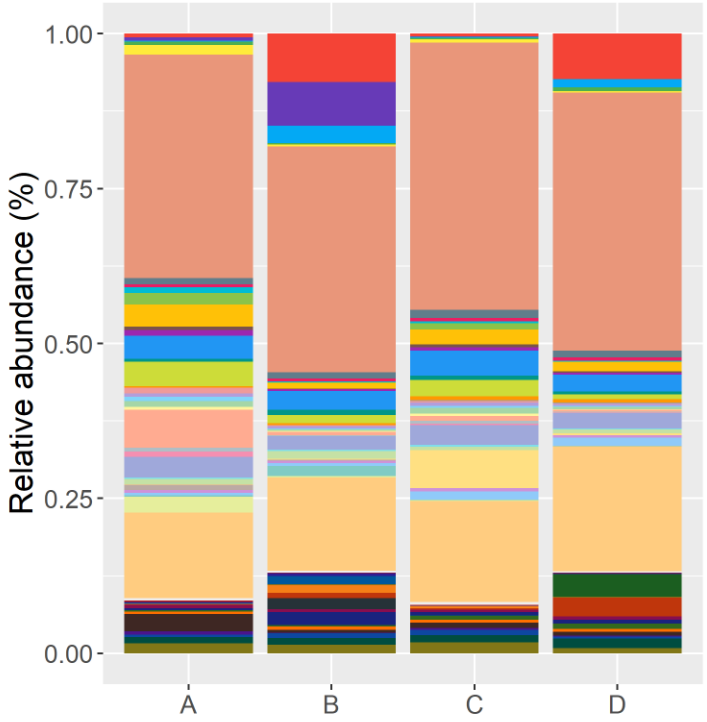




Microbial composition of raw milk differed between sampling locations







Conclusions

- Sampling month and location had greater impact on microbial composition of bulk tank raw milk

- Chlorine and chlorine-free cleaning are comparable – no significant differences between microbial composition

- Sequencing can provide more information on the microbes present in dairy samples

Acknowledgements:

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Thank you.

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