

# Can wastewater antibiotic resistant bacteria survive a healthy human gut microbiome?

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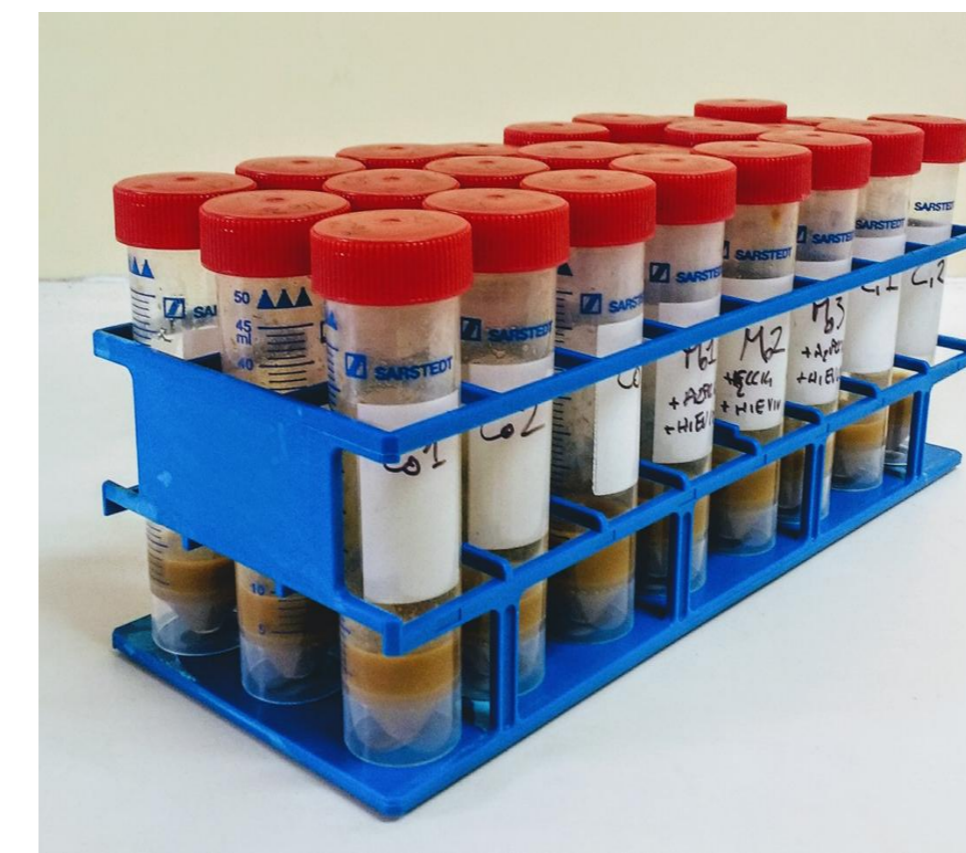
## Background and Objectives

The use of treated wastewater to irrigate crops may be a source of antibiotic resistant bacteria and antibiotic resistance genes (ARB&ARGs) capable of contaminating the human food chain, and therefore threat human health. The major question is how this contamination may threaten the human health, since entry via food chain is a likely possibility.

This study aims to assess whether the human gut microbiome may hamper the survival of known wastewater ARB.

## Methodology

Faeces-based microcosm assays (FMAs) spiked with wastewater ARB harboring known ARGs



FMAs containing faecal microbiome from healthy donor, never subjected to antibiotherapy

Antibiotic Resistant Bacteria (ARB)	Antibiotic Resistant Genes (ARG)
<i>Escherichia coli</i> A2FCC14	<i>bla</i> <sub>TEM</sub> , <i>bla</i> <sub>CTX</sub> , <i>bla</i> <sub>OXAA</sub>
<i>Enterococcus faecalis</i> H1EV10	<i>vanA</i>

Test conditions:

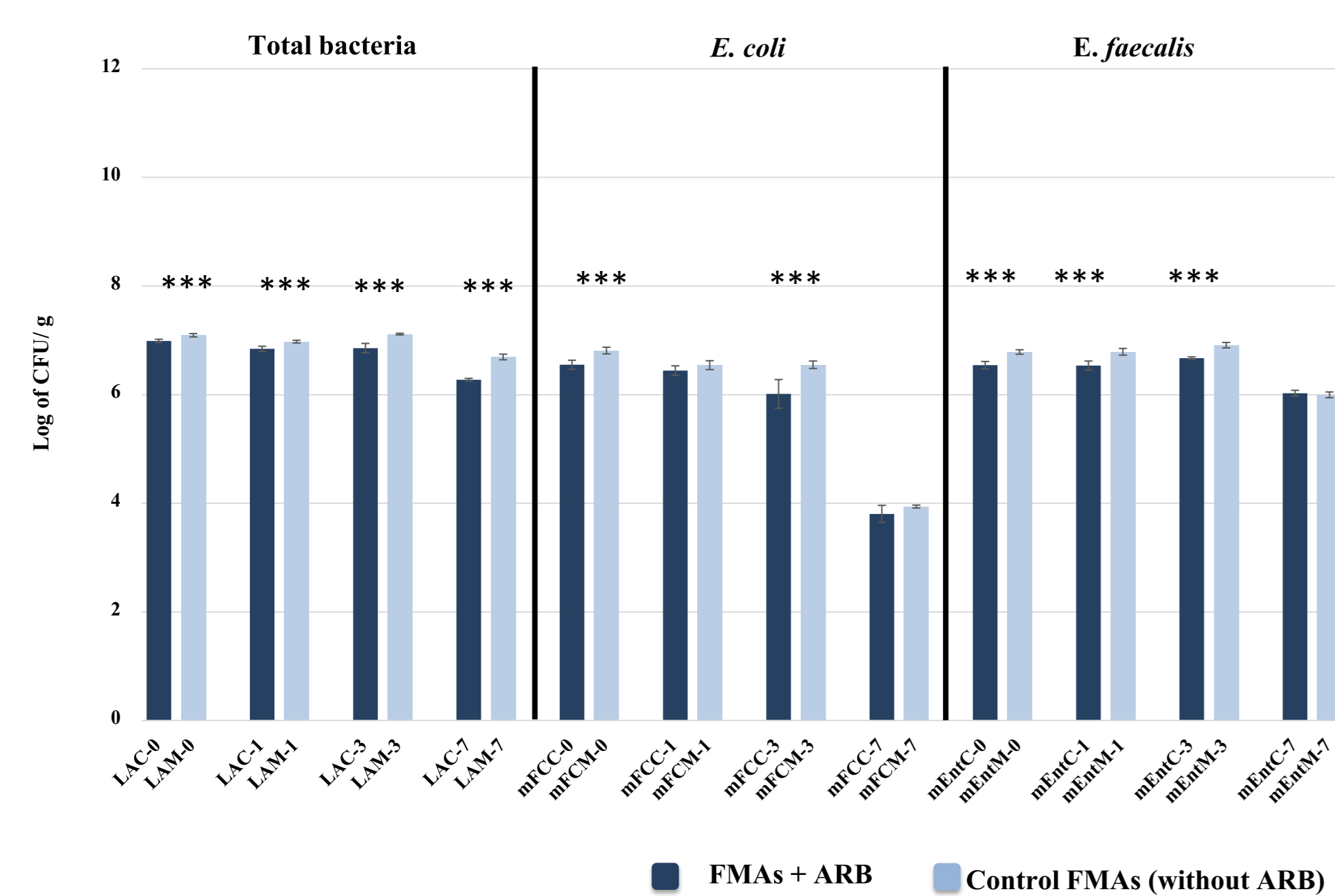
- Aerobic
- Anaerobic
- Selective pressure: cefotaxime (CTX, 4 mg/L) or vancomycin (VAN, 16 mg/L)

FMAs were monitored for 7 days based on cultivable bacteria counts, ARGs quantification by qPCR, and bacterial community analysis with Illumina Miseq.

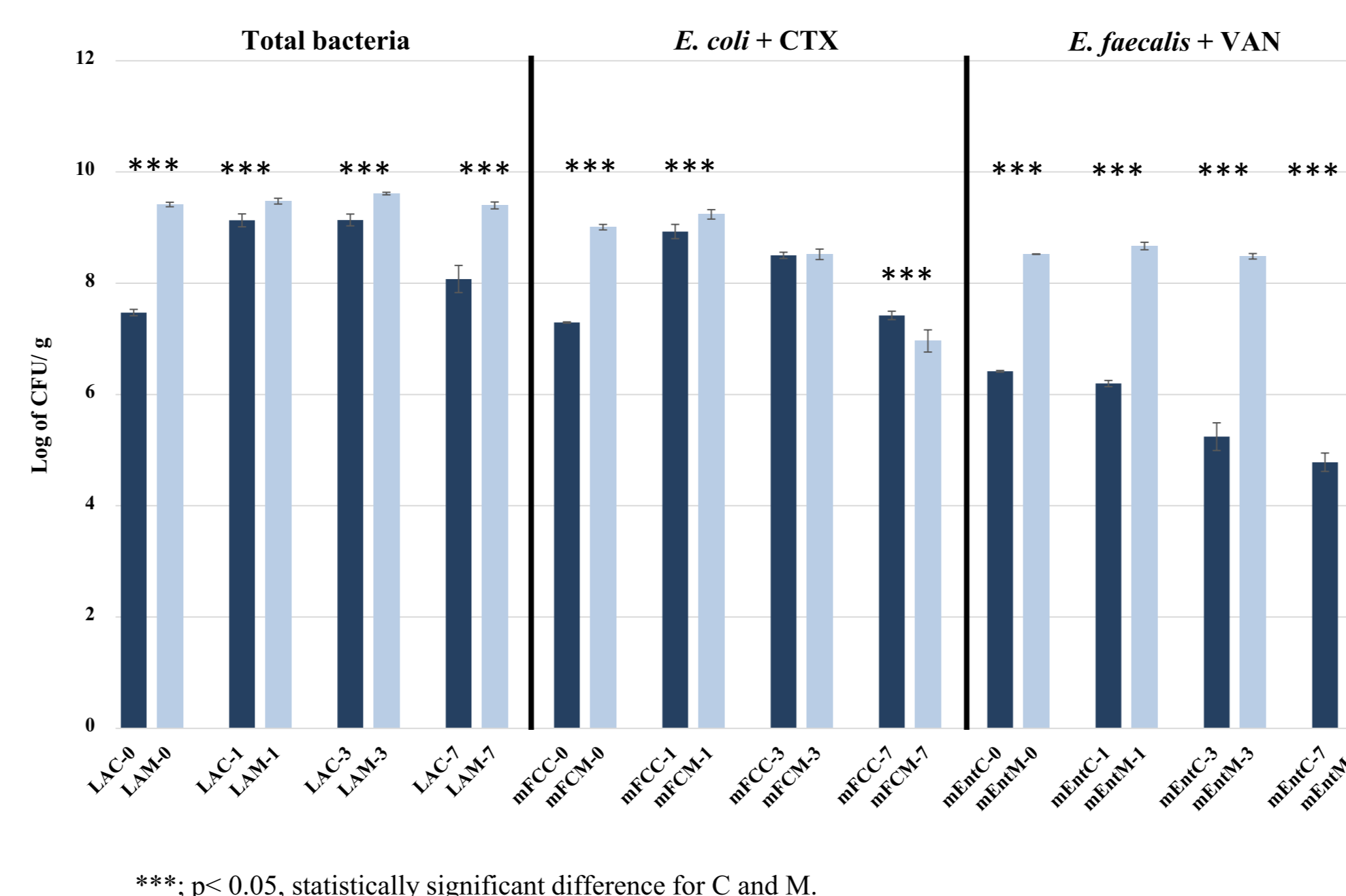
## Results

### ARB survival, ARGs abundance & microbiome analysis

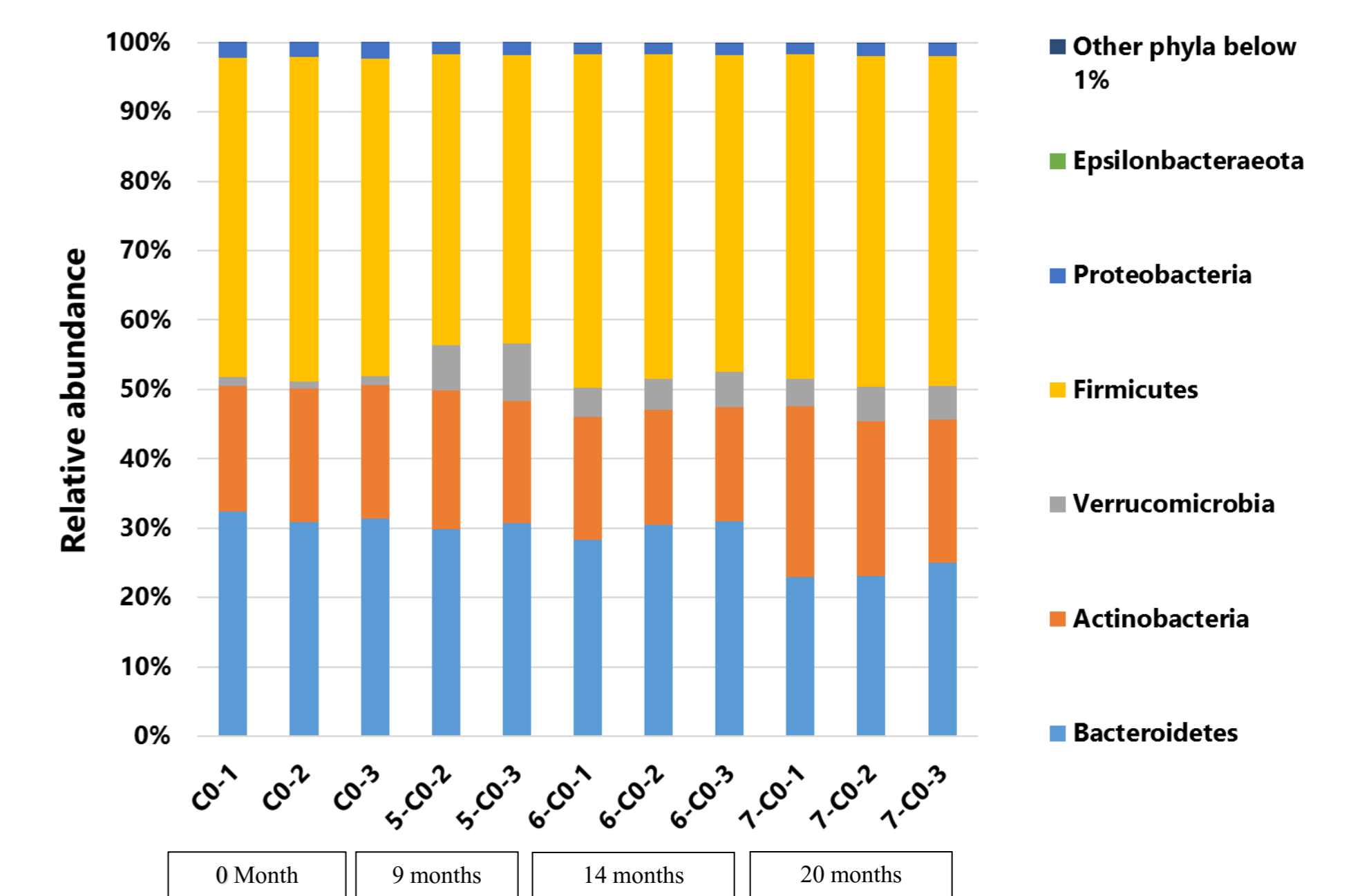
#### ARB SURVIVAL in anaerobic FMAs



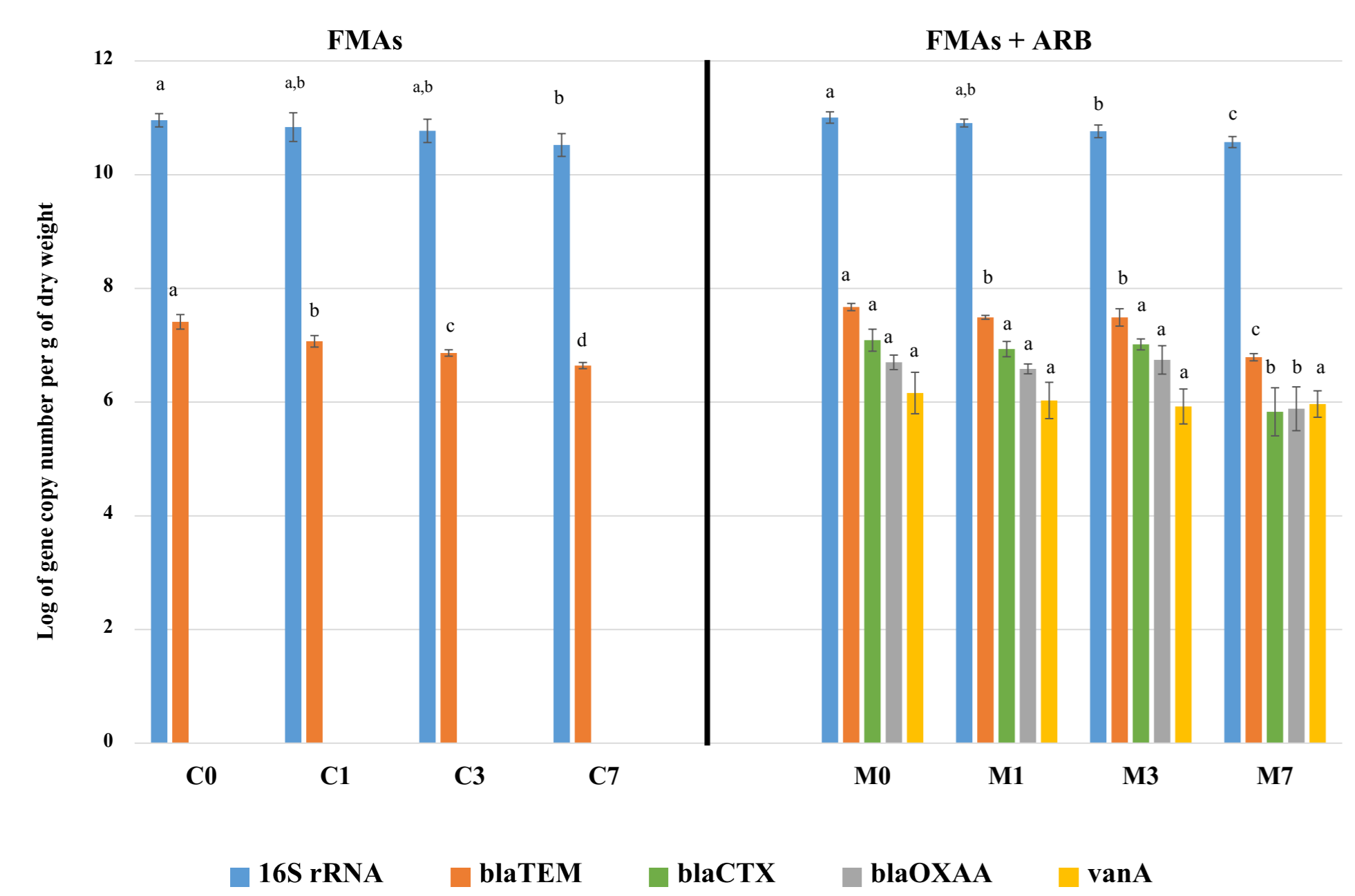
#### ARB SURVIVAL with selective pressure



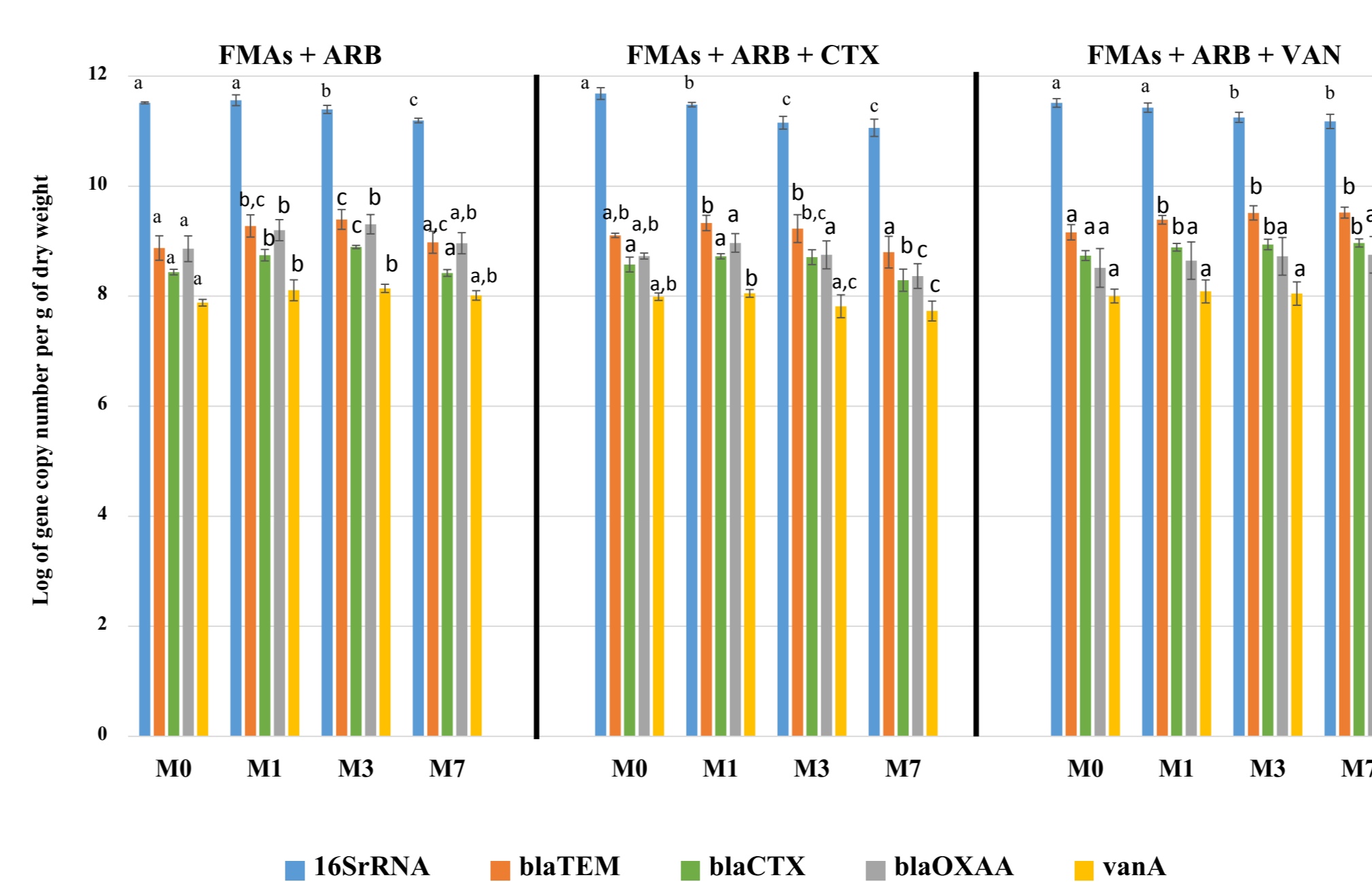
#### Microbial community evolution



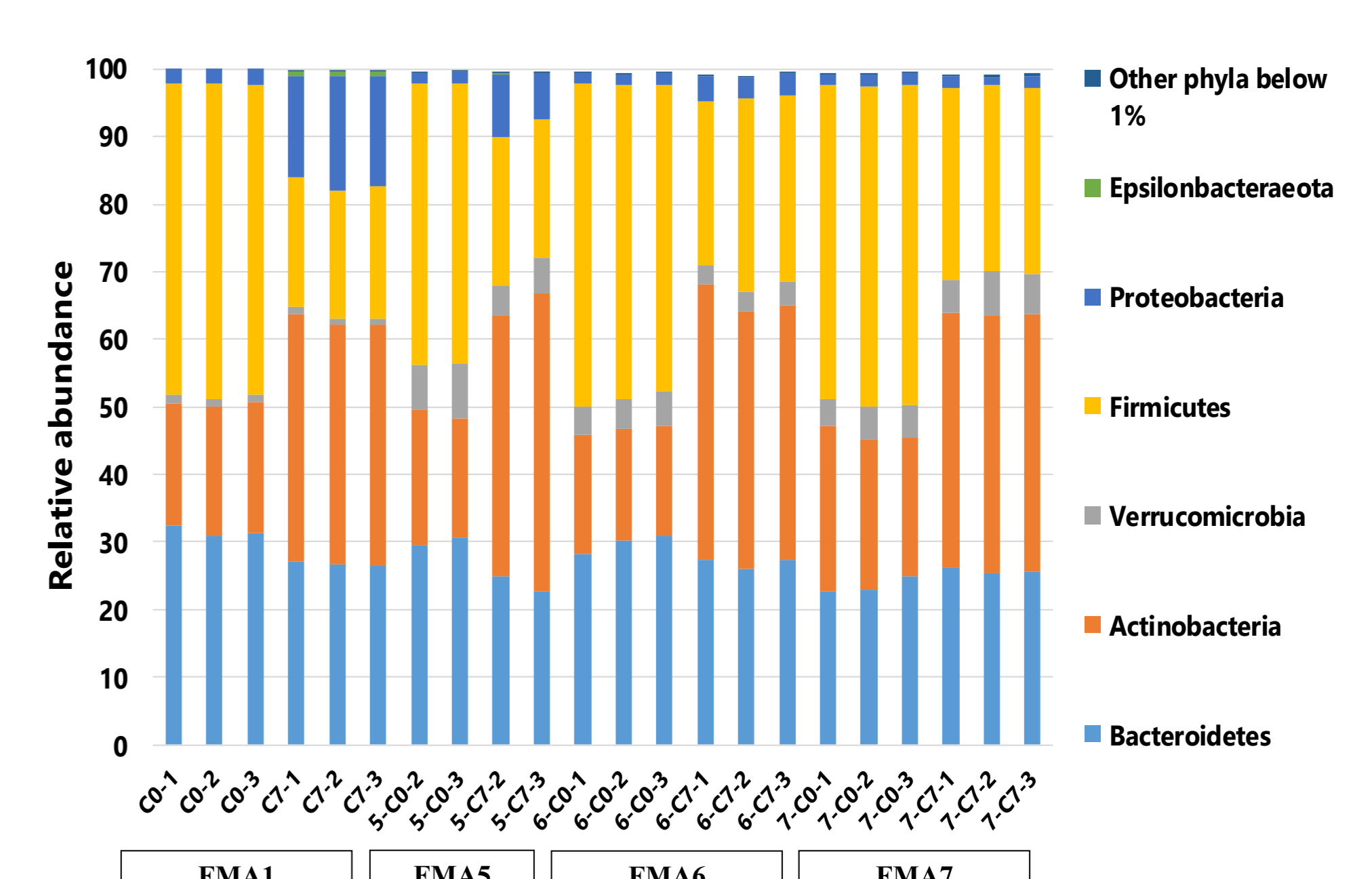
#### ARGs ABUNDANCE in anaerobic FMAs



#### ARGs ABUNDANCE in FMAs with antibiotic



#### Microcosm effect



a-c; statistically significant difference (p < 0.05) along time for each sample (Control and FMA+ARB).

## Conclusions

- In both aerobic and anaerobic conditions, the spiked ARB were able to survive with the faecal microbiota for a week and their ARGs could be quantified at least for one month. However, anaerobic conditions may have had a slight positive effect on ARB elimination.
- The presence of sub-inhibitory concentrations of antibiotics did not affect the survival of the ARB. However, possible interferences with the faecal microbiome are being analyzed.
- Preliminary results show that the microbial population is quite stable along time. The *Proteobacteria*, *Actinobacteria* and *Firmicutes* abundance seems to be influenced by the microcosm effect.

**A major conclusion of this research is that ARB with origin in wastewater are able to survive and are not outcompeted by the autochthonous faecal microbiota until a week, while their genes can persist for at least one month.**

## Acknowledgements

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