

# ➤ Conductive materials stimulate ruminal methanogenesis and induce microbial changes indicative of improved electron transfer

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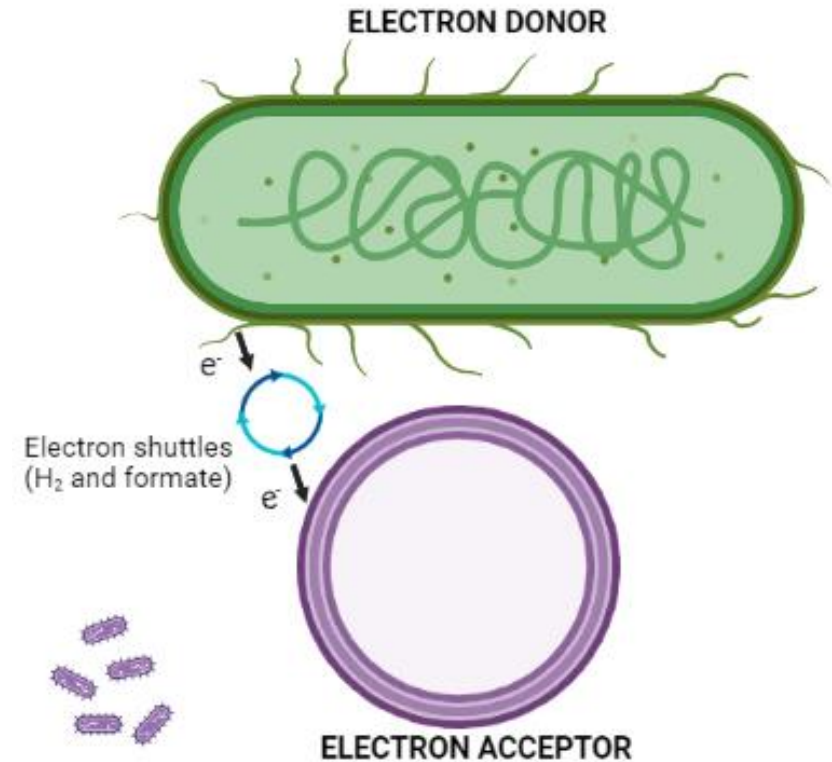
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# ➤ Methanogenesis and electron transfer in microbial ecosystems

## Interspecies Electron Transfer (IET)

- Dominant mechanism in the rumen
- Electron shuttles → H<sub>2</sub> and formate
- Electron donor: bacteria, protozoa and fungi
- Electron acceptor: methanogenic archaea

## Extracellular electron transfer (EET)



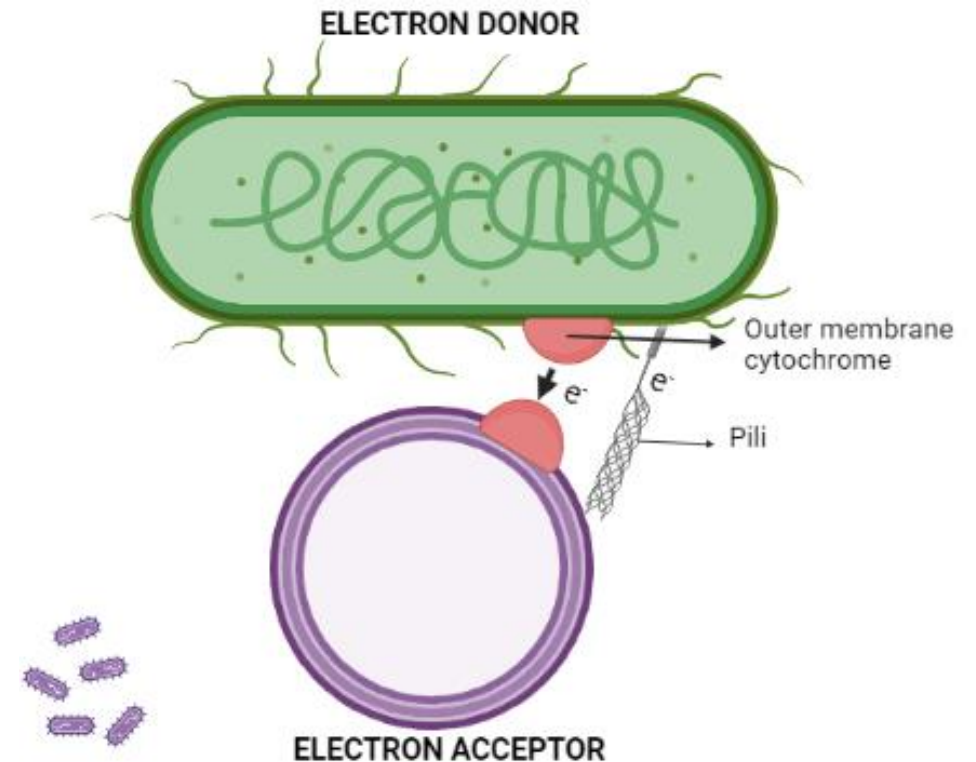
# ➤ Methanogenesis and electron transfer in microbial ecosystems

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## Extracellular electron transfer (EET)

- Exchange of intracellular electrons with an extracellular electron donor/acceptor
  - Electroactive microbes → electrogens & electrotrophs
- GIT → flavins, sulfhydryl, humic compounds, Fe(III) oxides



In the rumen, the extent and importance of EET mechanisms is not known

## ➤ Strategies to explore Extracellular Electron Transfer (EET)

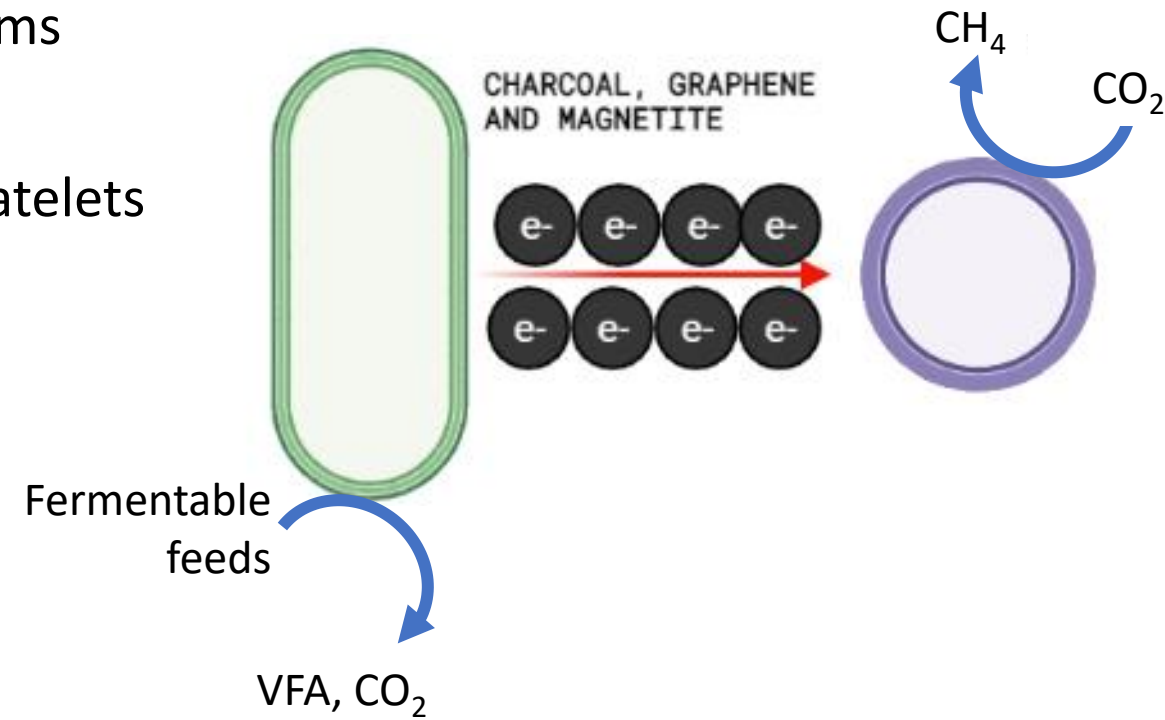
- Conductive materials (CMs) increases EET
  - Can promote biological processes
  - ↑ methane production in some ecosystems

### ➤ CARBON-BASED MATERIALS

- Activated charcoal and Graphene nanoplatelets
- Provide an electron-conducting surface

### ➤ MINERAL-BASED MATERIALS

- Magnetite [Fe<sub>3</sub>O<sub>4</sub>; Iron(II,III) oxide]
- Acts as an electron conduit



## ➤ Hypothesis

- Incubation of CMs in the rumen would increase methane production and the abundance of microbial communities associated with EET
  - Does it change fermentation parameters?
  - Which microbes could be associated with this presumed EET mechanism in the rumen?

## ➤ Objective

- To test the effect of CMs on rumen fermentation and on microbial communities

## ➤ METHODS

### A - In vitro experiment

➤ In vitro batch culture technique (48 h)

➤ Donors of ruminal fluid: Four rumen-cannulated sheep

➤ Treatments

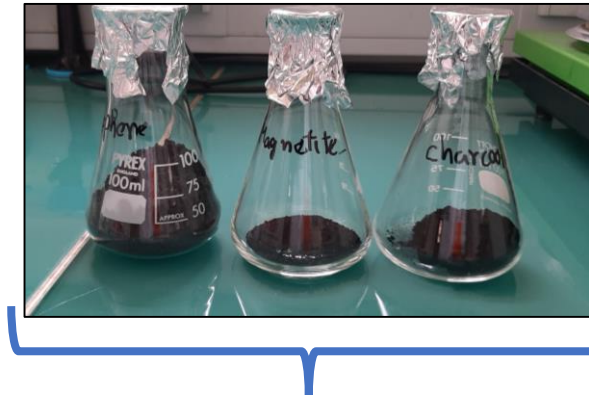
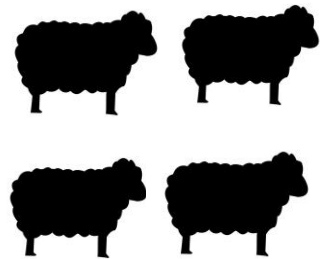
- Control

Substrate: alfalfa:wheat; 3:1 ratio

2:1 buffer-rumen fluid mixture

- Control + 5 and 10% of

- Activated Charcoal (AC)
- Graphene (GPH)
- Magnetite (MAG)



## ➤ METHODS

### B - In sacco experiment

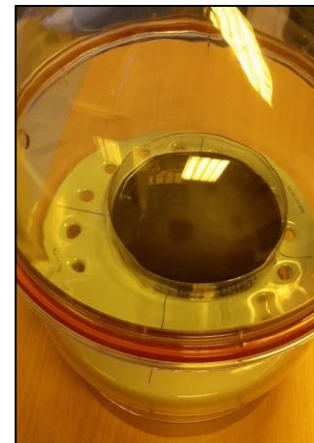
#### ➤ Conductive materials (AC, GPH, MAG)

- Membranes of the inert polymer polydimethylsiloxane containing CMs

#### ➤ Incubated in the rumen of four rumen-cannulated sheep

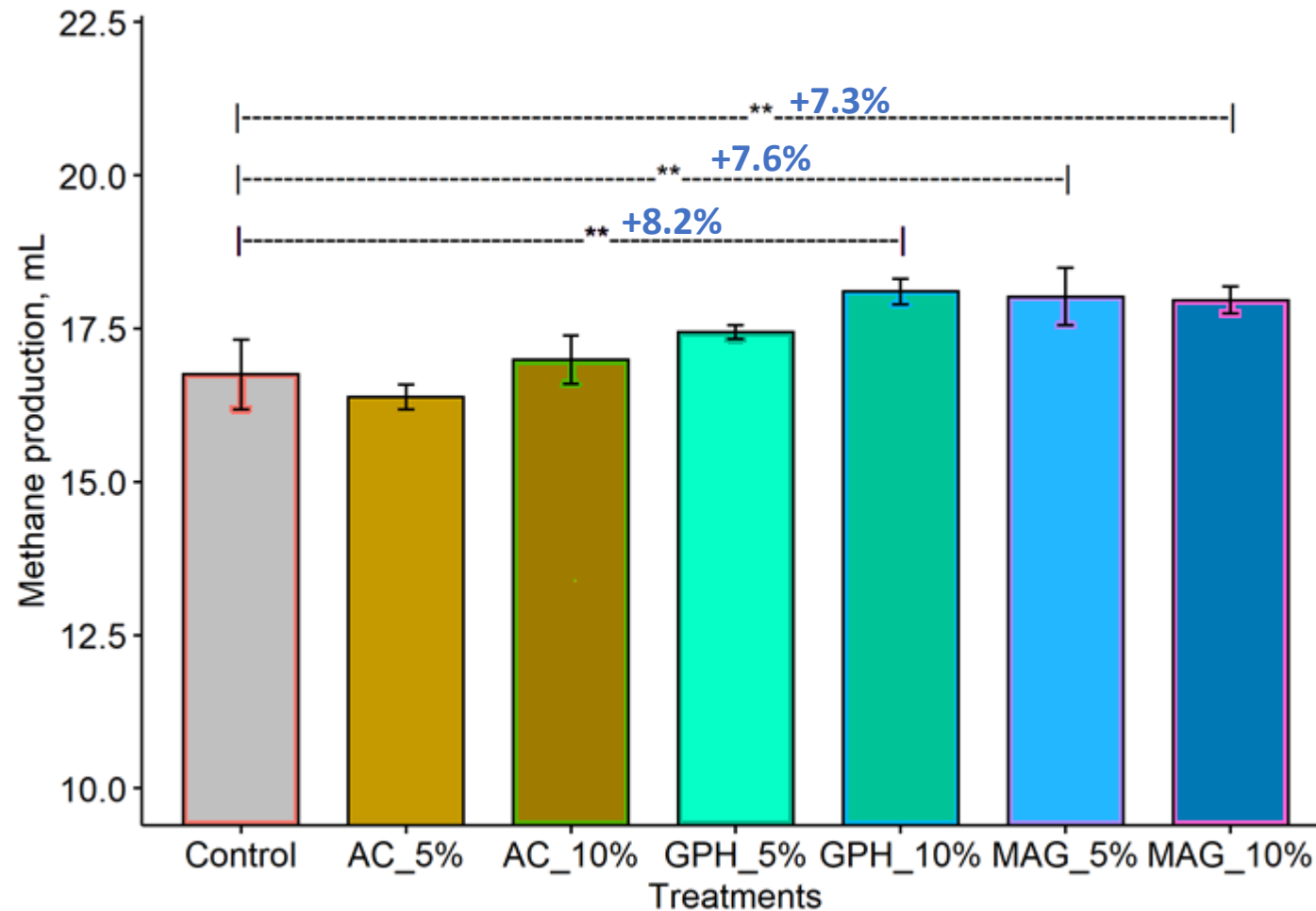
- 1 d
- 7 d
- 28 d

#### ➤ 16S rRNA sequencing



## ➤ RESULTS

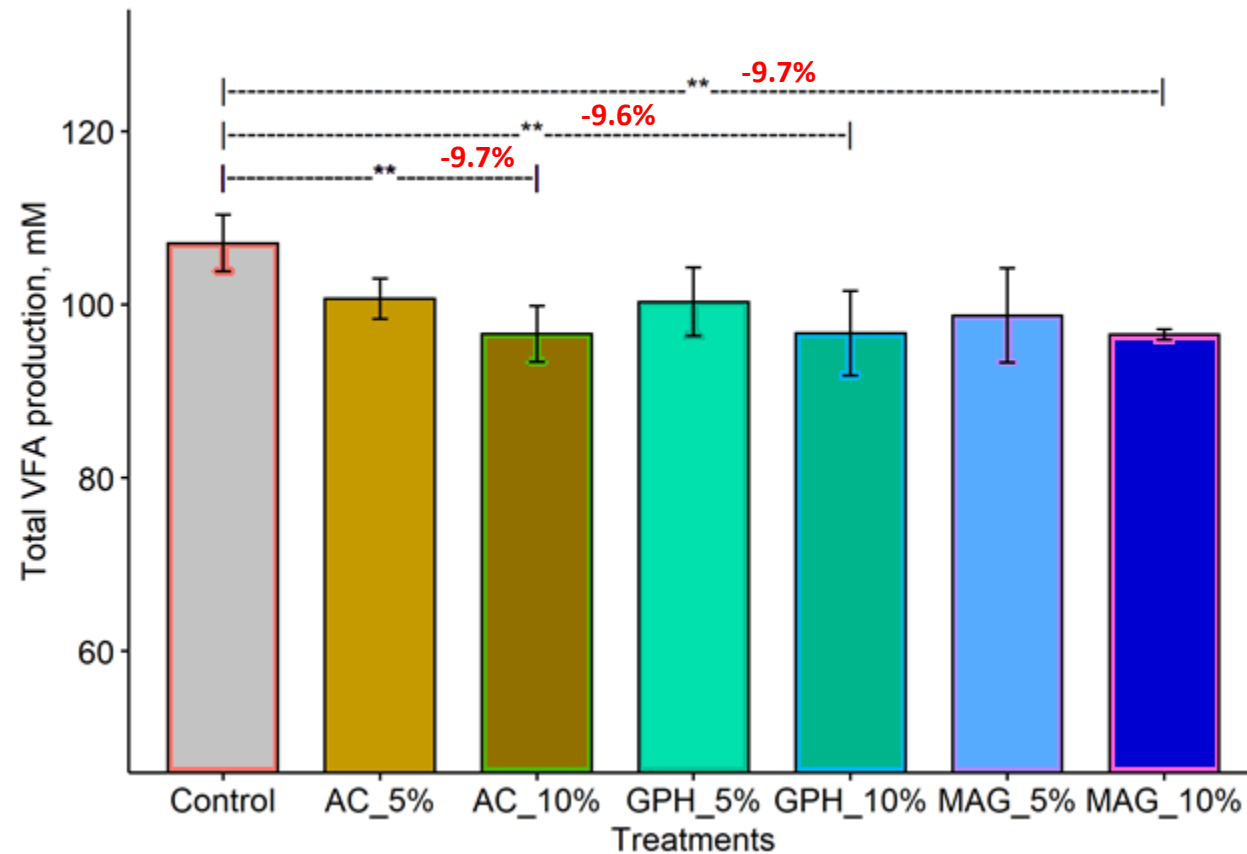
### Graphene and Magnetite increased methane production





## ➤ RESULTS

- No changes in total gas production and VFA profile
- Conductive materials at 10% reduced total VFA production



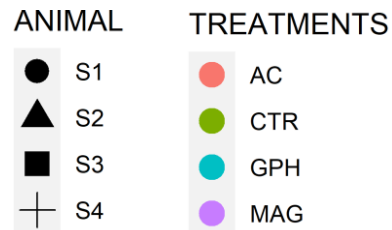
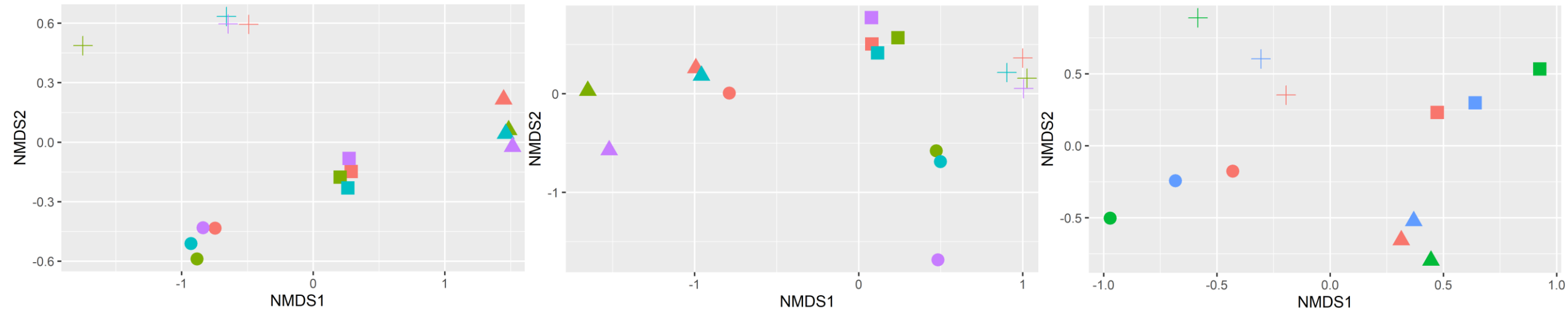
# ➤ Microbial diversity associated to conductive materials

- No differences in  $\alpha$  diversity
- Microbial communities differed between animals ( $P < 0.01$ )
- Within animals, the microbial communities differed between treatments

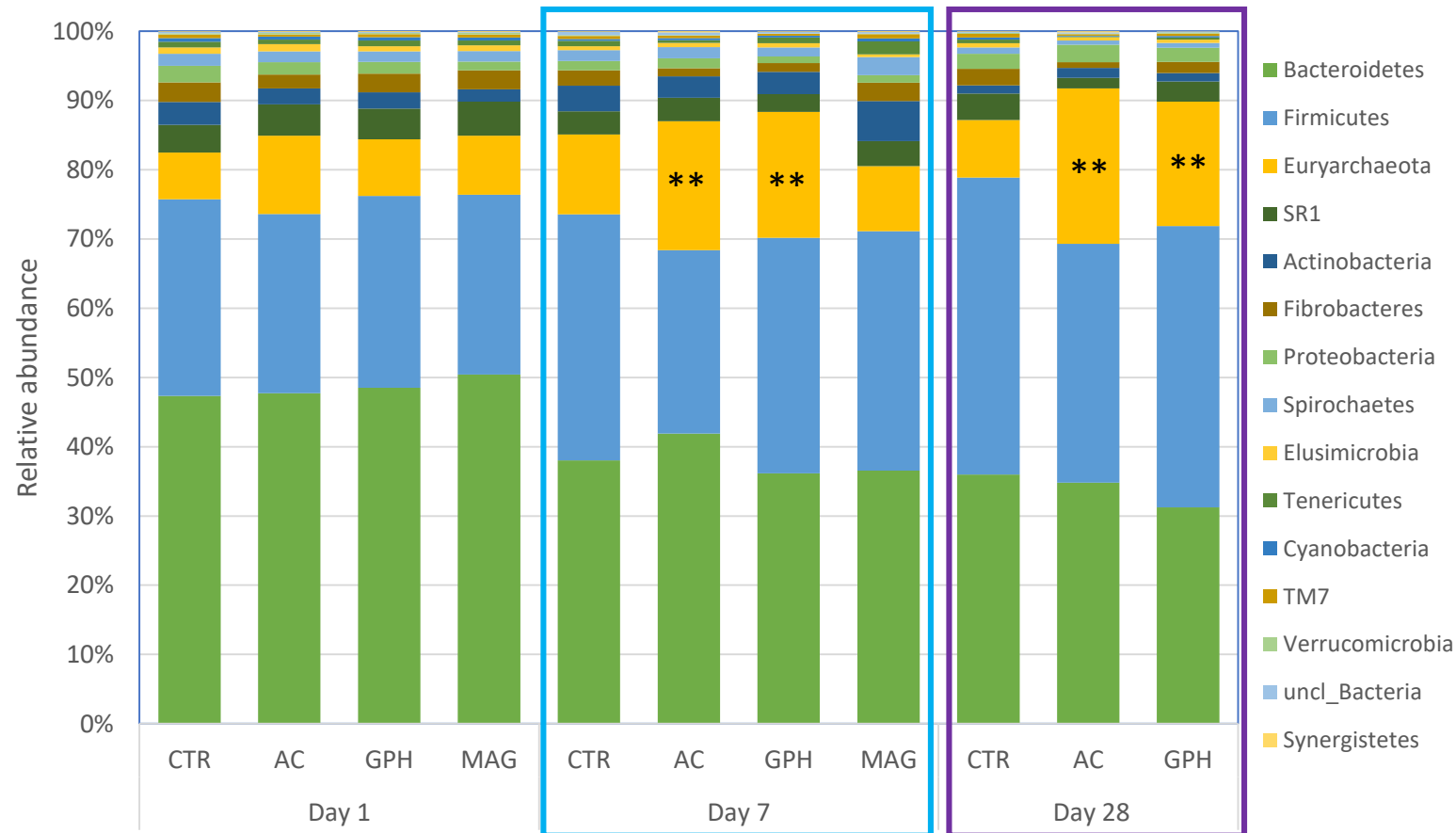
**Day 1**  
Treatment  $P < 0.001$

**Day 7**  
Treatment  $P = 0.003$

**Day 28**  
Treatment  $P = 0.005$

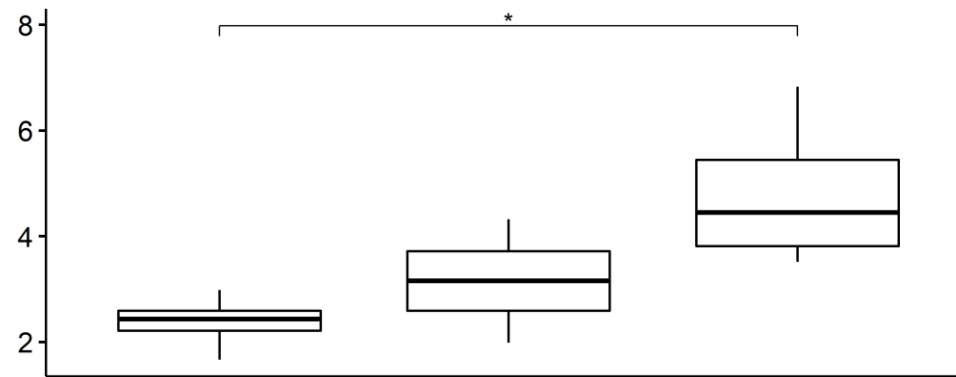


# ➤ Activated Charcoal & Graphene increased the relative abundance of Euryarchaeota



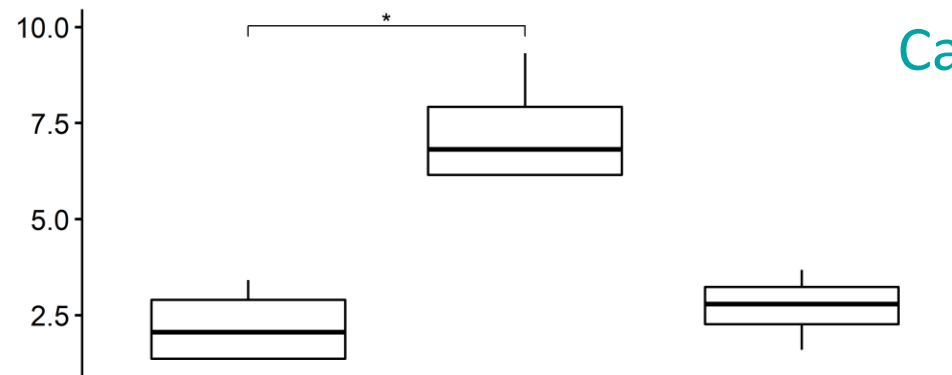
## Differences in methanogens genera at day 28

Kruskal-Wallis,  $\chi^2(2) = 6.04$ ,  $p = 0.049$ ,  $n = 12$



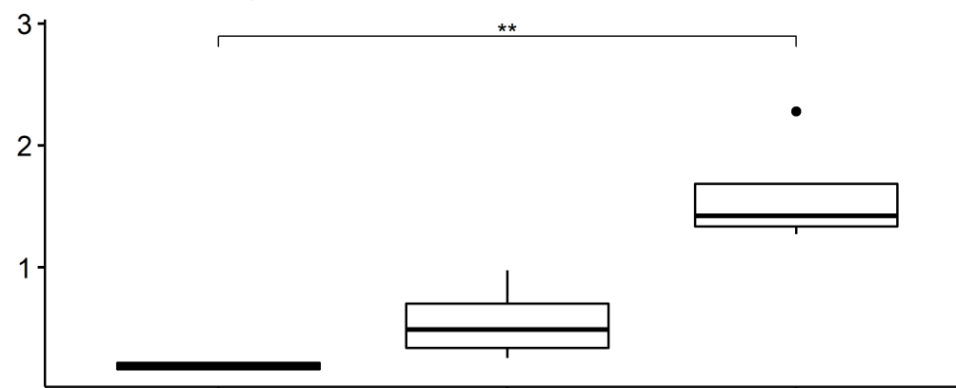
*Methanobrevibacter*

Kruskal-Wallis,  $\chi^2(2) = 7.73$ ,  $p = 0.021$ ,  $n = 12$



*Cand. Methanomethylophilus*

Kruskal-Wallis,  $\chi^2(2) = 9.85$ ,  $p = 0.0073$ ,  $n = 12$



*Methanosphaera*

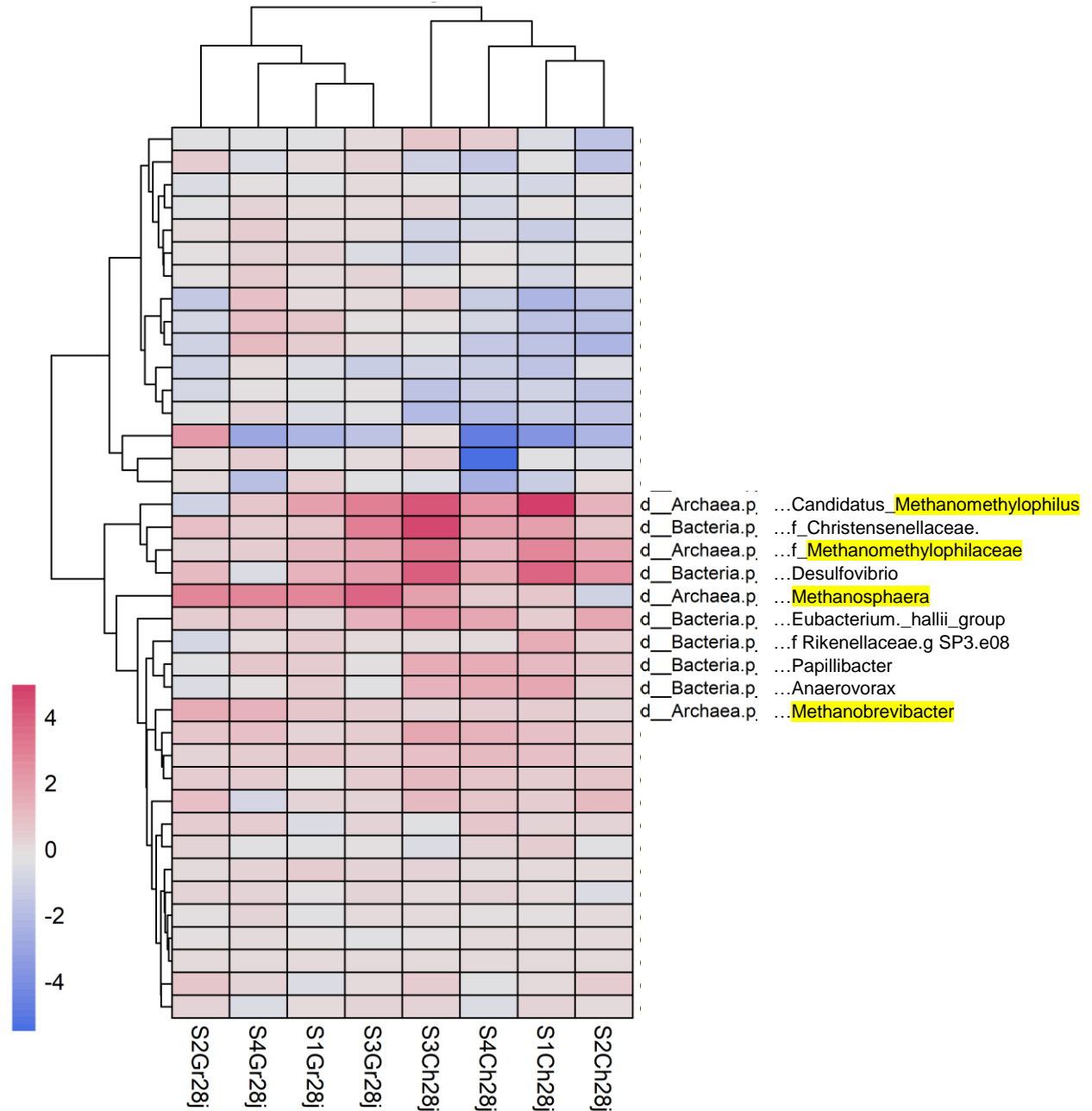
Control

Charcoal

Graphene

pwc: Dunn test; p.adjust: Bonferroni

## ➤ Changes at the genus level



## ➤ CONCLUSION

➤ Increased methane production

➤ Shift in microbes associated to conductive materials

→ suggests the presence of EET in the rumen

○ EET and mechanisms involved should be validated with additional tools

○ Its extent in the rumen should be assessed as it could be another mechanism to consider for modulating methanogenesis

## ➤ Acknowledgments



Thank you for your  
attention



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**INRAE**

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