Extracellular electron transfer in the rumen ecosystem is stimulated by conductive materials

Abimael Ortiz-Chura, Milka Popova, and Diego Morgavi

INRAE, Herbivores Research Unit, France

RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

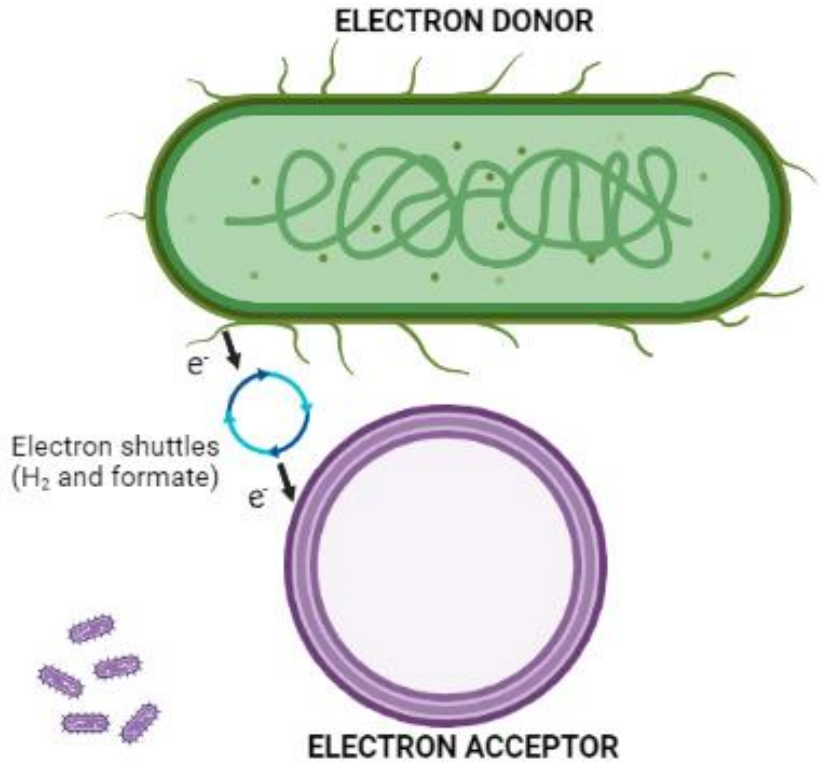
INRAE

2024 Congress on Gastrointestinal Function
Beckman Institute for Advanced Science and Technology
University of Illinois, Urbana-Champaign

Methanogenesis and electron transfer in microbial ecosystems

Interspecies Electron Transfer (IET)

Extracellular electron transfer (EET)



Methane is a biosignature gas

“Bovine flatulence — the intimate intestinal activities of cows, reindeer, elephants, and elk — is detectable over interplanetary distances, while the bulk of the activities of mankind are invisible.

We would not ordinarily consider the flatulence of cattle as a dominant manifestation of life on Earth, but there it is.”

Carl Sagan

The Cosmic Connection: An Extraterrestrial Perspective,

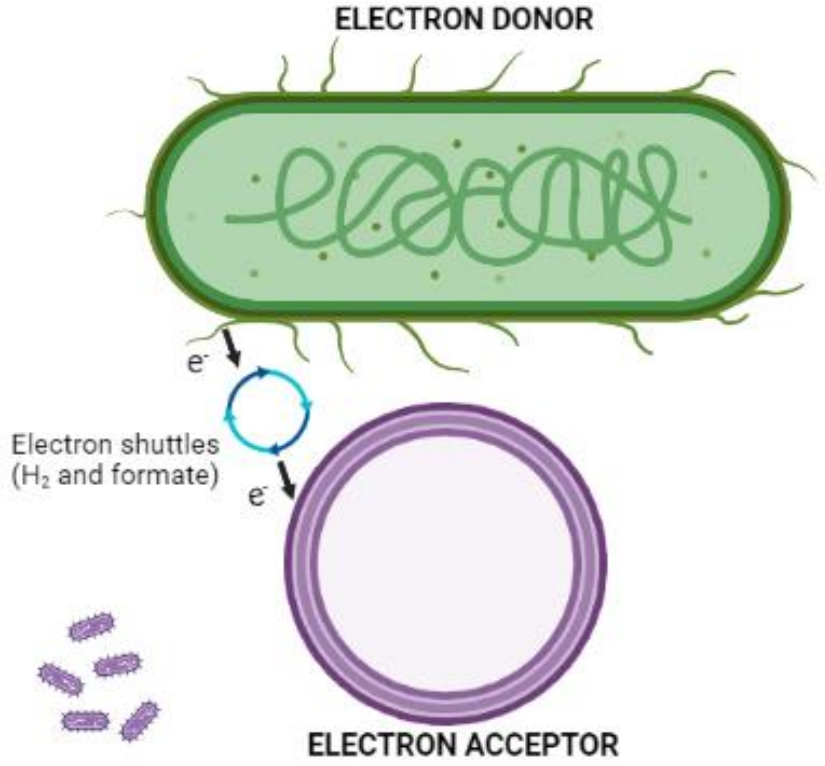
Chapter 22 (p. 150), Dell Publishing, Inc. 1975

Methanogenesis and electron transfer in microbial ecosystems

Interspecies Electron Transfer (IET)

- Dominant mechanism in the rumen
- Electron shuttles → H_2 and formate
- Electron donor: bacteria, protozoa and fungi
- Electron acceptor: methanogenic archaea

Extracellular electron transfer (EET)



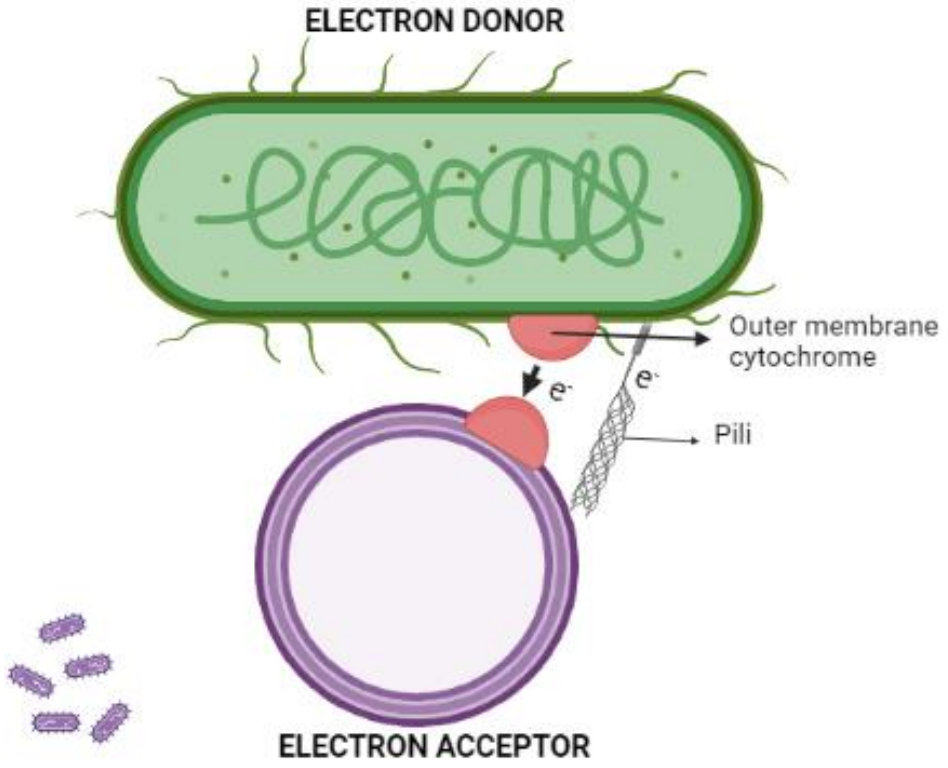
Methanogenesis and electron transfer in microbial ecosystems

Interspecies Electron Transfer (IET)

- Dominant mechanism in the rumen
- Electron shuttles → H_2 and formate
- Electron donor: bacteria, protozoa and fungi
- Electron acceptor: methanogenic archaea

Extracellular electron transfer (EET)

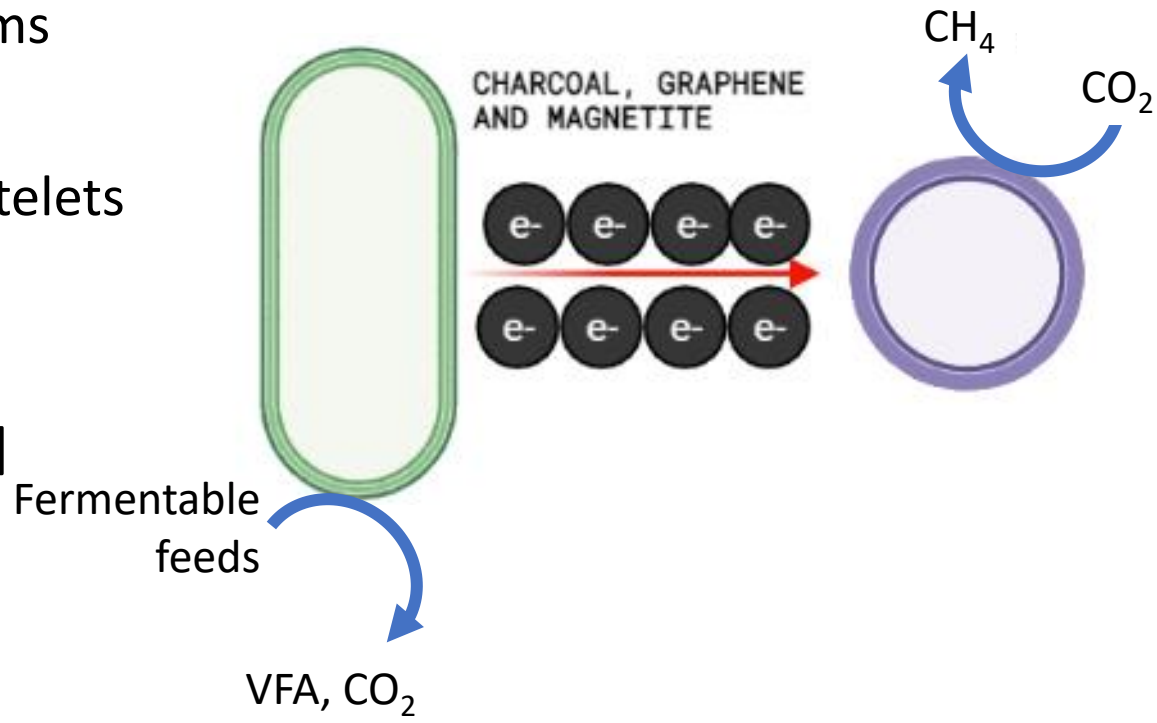
- Exchange of intracellular electrons with an extracellular electron donor/acceptor
  - Electroactive microbes → electrogens & electrotrophs
- GIT → flavins , sulfhydryl, humic compounds, Fe(III) oxides



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

Strategies to explore Extracellular Electron Transfer (EET)

- Conductive materials (CMs) increase 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 [$Fe^{2+}Fe_2^{3+}O_4$; Iron(II,III) oxide]
  - Acts as an electron conduit



Lovley, 2017. DOI 10.1016/j.coelec.2017.08.015
Lovley, 2017. DOI 10.1146/annurev-micro-030117-020420

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

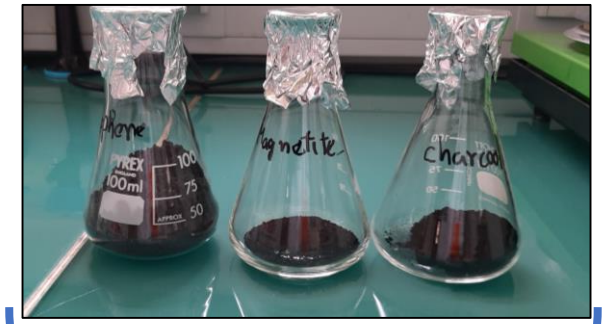
METHODS

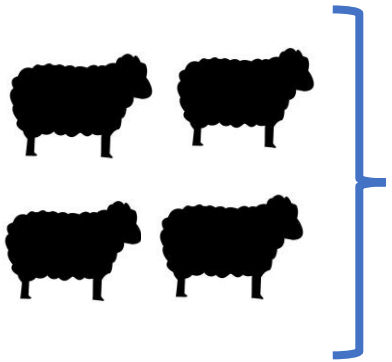
1. In vitro batch culture (48 h)
2. Consecutive batch culture (enrichment)
3. In sacco experiment

METHODS

1. In vitro batch culture (48 h)

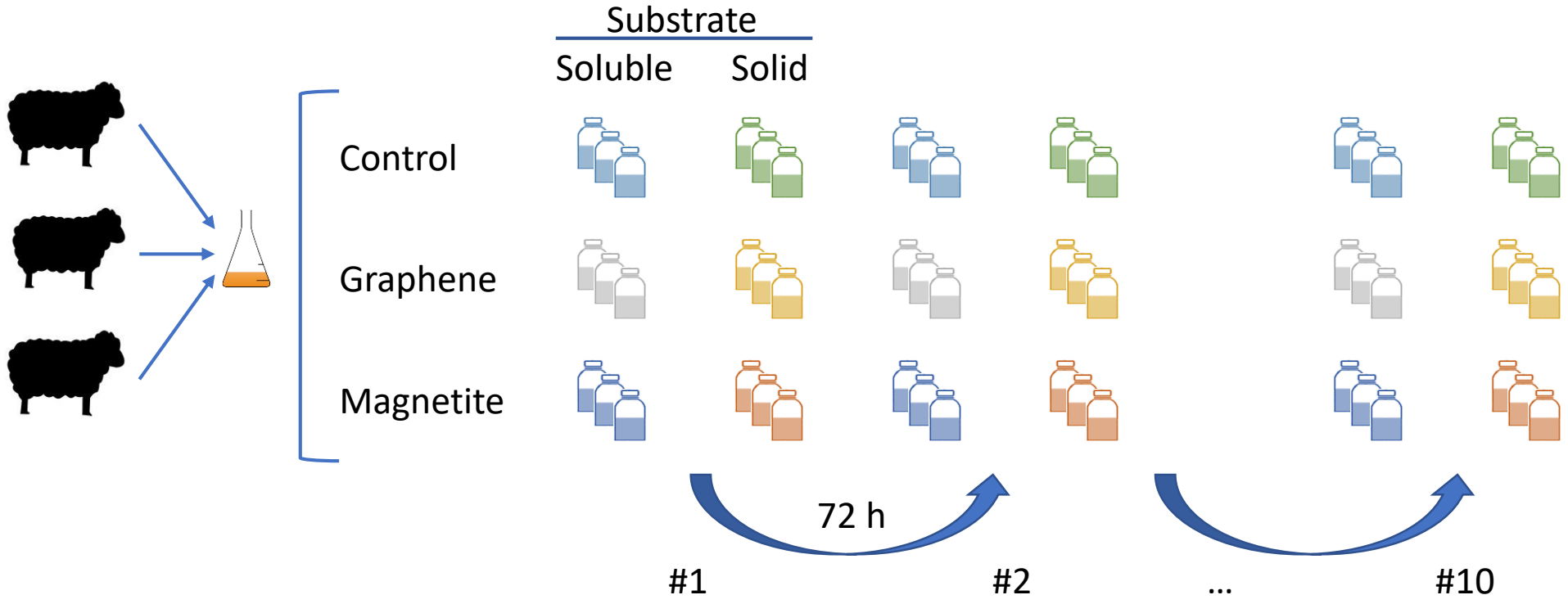
- Donors of ruminal fluid: Four sheep
- Treatments
  - Control (substrate alone)
  - Control + 5 and 10% of
    - Activated Charcoal (AC)
    - Graphene (GPH)
    - Magnetite (MGN)





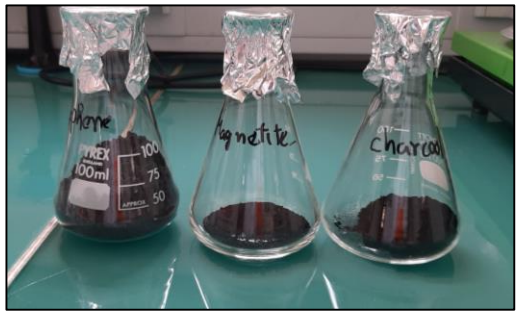
METHODS

1. In vitro batch culture (48 h)
2. Consecutive batch culture (enrichment)



METHODS

1. In vitro batch culture (48 h)
2. Consecutive batch culture (enrichment)
3. In sacco experiment
   - Conductive materials (AC, GPH, MGN)
   - 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



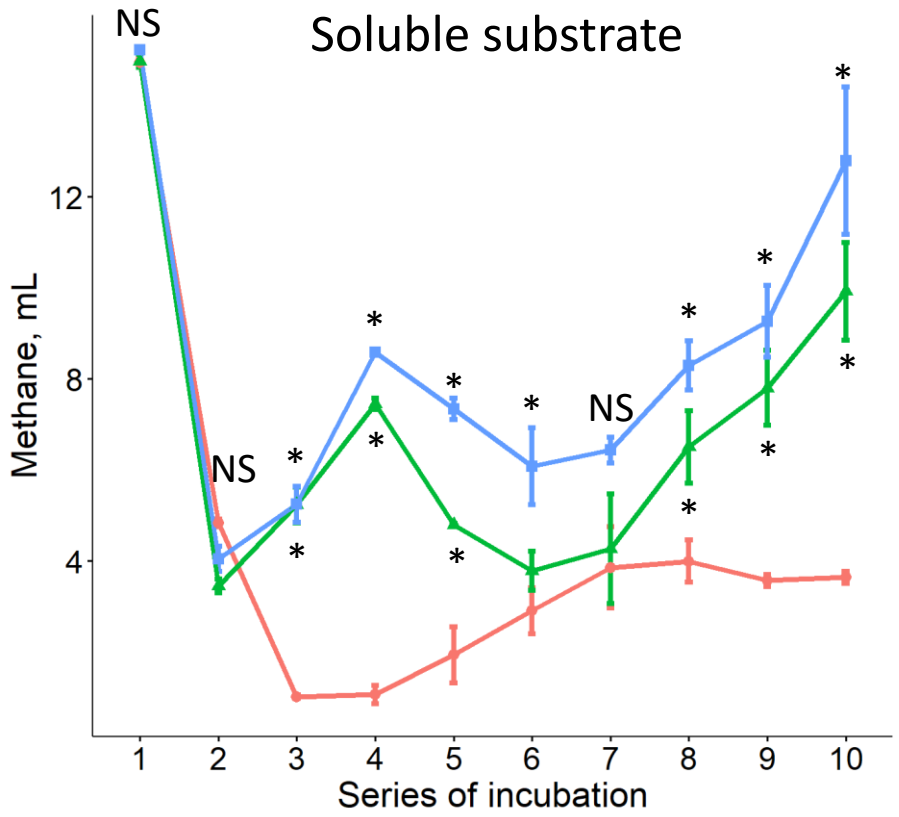
RESULTS

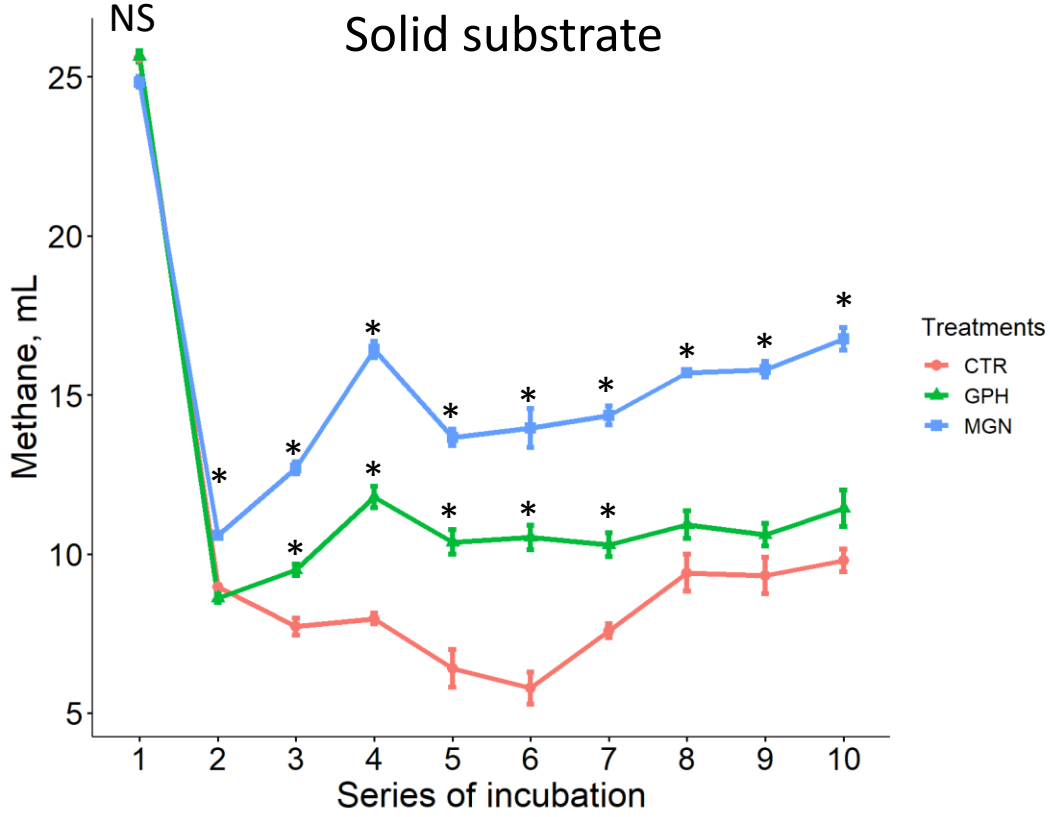
In vitro batch culture (48 h)

- Graphene and Magnetite increased methane production ~8%
  - No changes in total gas production and VFA profile
  - Conductive materials at 10% reduced total VFA production

RESULTS

Consecutive batch culture



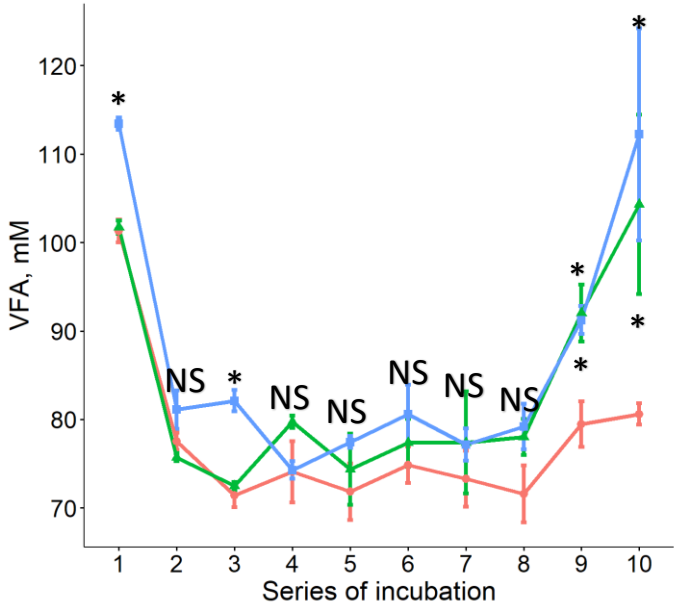


Conductive materials increased methane production

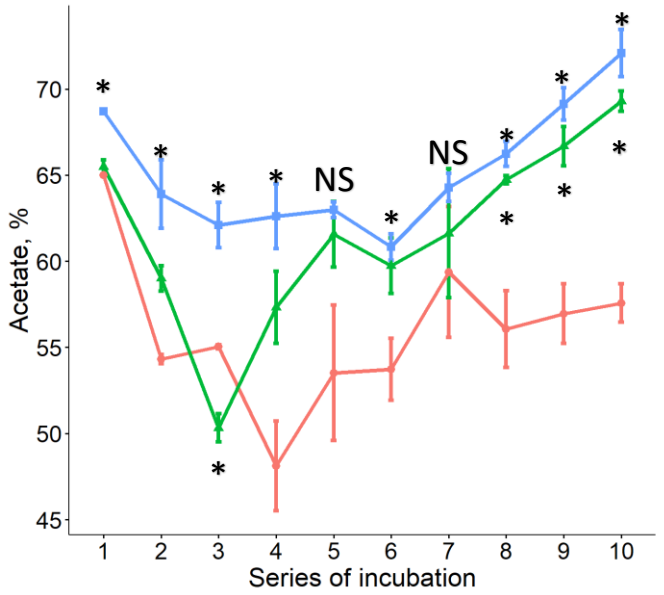
RESULTS

Consecutive batch culture - Soluble substrate

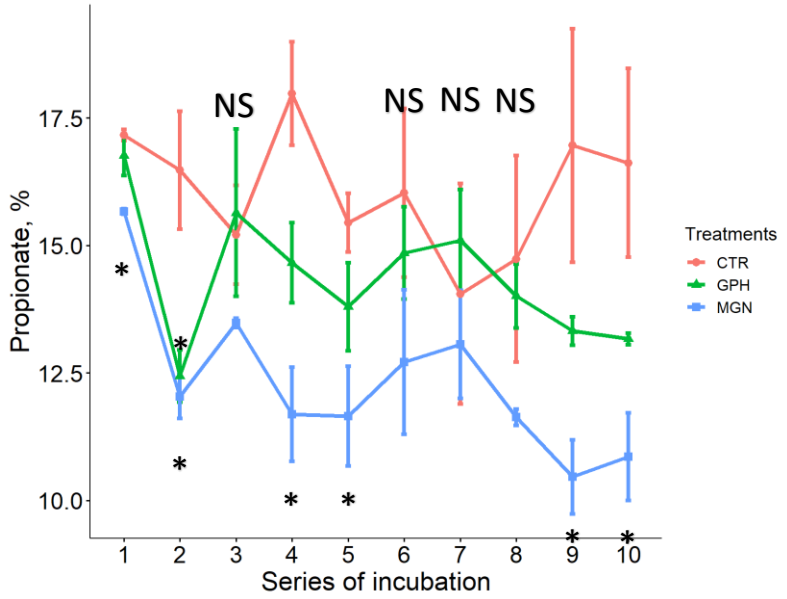
VFA



Acetate



Propionate



Conductive materials

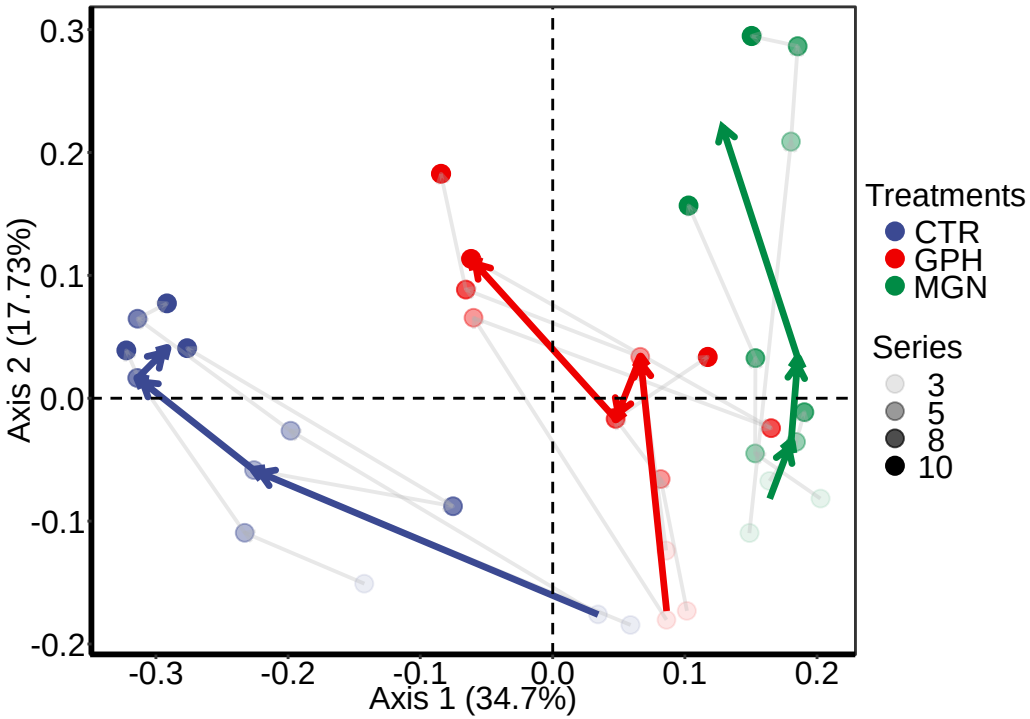
↑ VFA production

↑ Acetate

↓ Propionate

RESULTS

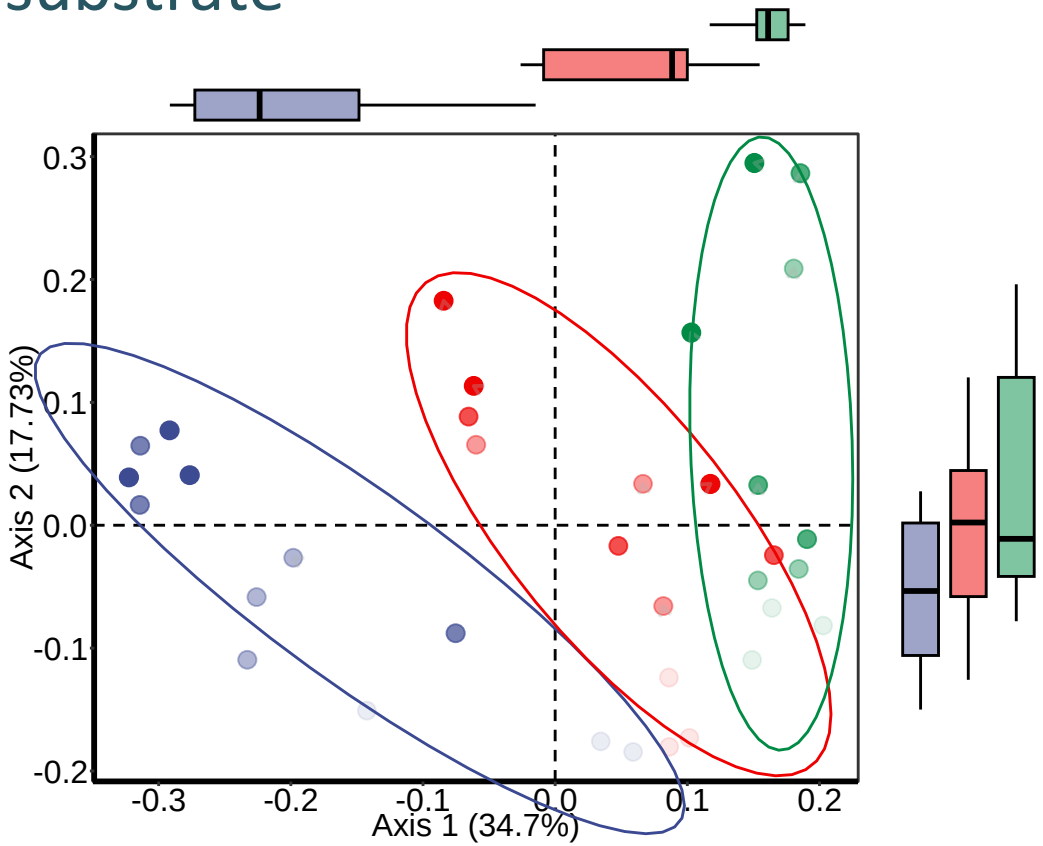
Consecutive batch culture - Soluble substrate



WUniFrac
S3 to S10

Linear mixed model

NS



PERMANOVA

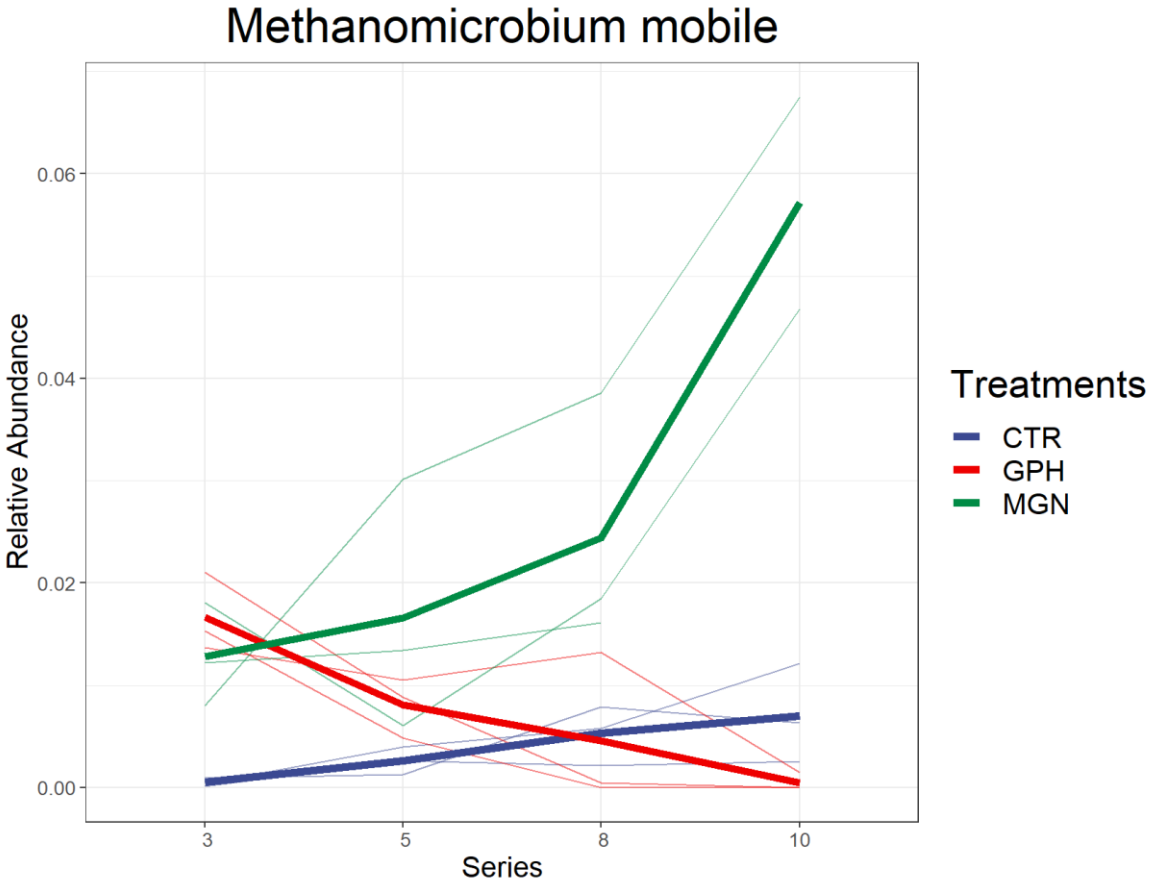
Variable	R.Squared	P.Value
Series	0.212	0.001
Treatments	0.327	0.001

RESULTS

Consecutive batch culture - Soluble substrate

MaAslin2: S3 to S10

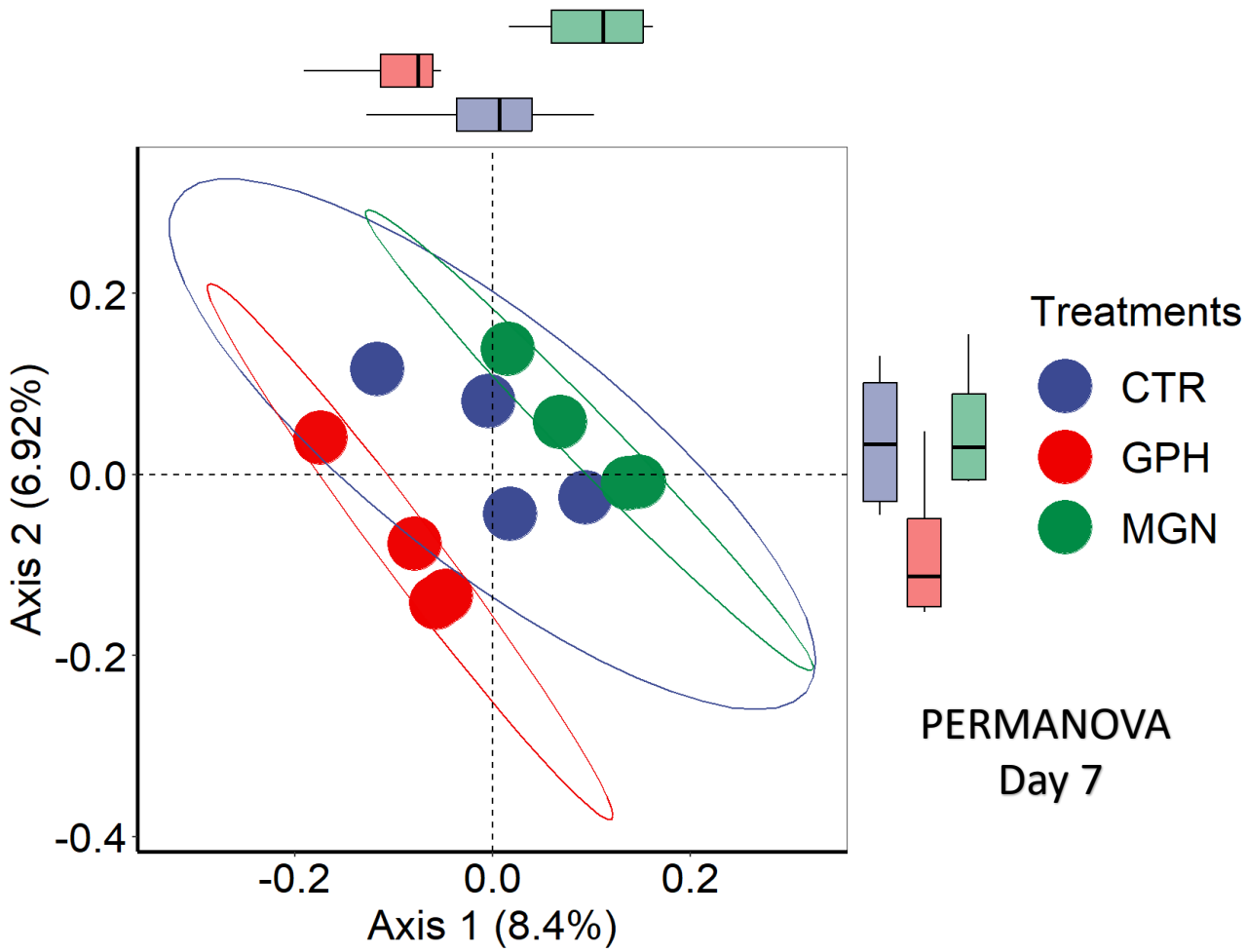
Species enriched	CTR	GPH	MGN	q-value
Treponema_D bryantii	0,77	2,39	5,32	0,000
Clostridium_G cochlearium	1,94	5,15	4,35	0,099
Methanomicrobium mobile	0,39	0,74	2,49	0,078
CAG-873 sp004553485	0,00	1,66	1,06	0,000
Prevotella heparinolytica	0,20	0,15	0,70	0,006
Succinivibrio hippei_B	0,03	0,16	0,34	0,000
JAAVLZ01 sp012034405	0,00	0,11	0,31	0,000
Synergistes jonesii	0,03	0,12	0,29	0,004
Marseille-P3106 sp900169975	0,00	0,03	0,26	0,003
UBA1217 sp902788805	0,00	0,01	0,24	0,008
SFEB01 sp004558105	0,04	0,07	0,18	0,034
Succiniclasticum ruminis	0,00	0,00	0,15	0,004
PeH17 sp900542285	0,00	0,09	0,13	0,000
Succinivibrio dextrinosolvens_B	0,00	0,00	0,11	0,038
ER4 sp000765235	0,01	0,03	0,09	0,000
Desulfovibrio_R_446353 desulfuricans_A_446137	0,08	1,32	0,06	0,039
SFLA01 sp004553575	0,02	0,05	0,06	0,050
RUG13615 sp902790905	0,01	0,00	0,05	0,000
Methanimicrococcus blatticola	0,00	0,00	0,03	0,007
Atopobium minutum	0,00	0,02	0,03	0,006
Proteiniphilum saccharofermentans	0,00	0,01	0,02	0,027
Methanoculleus_A_2118 hydrogenitrophicus	0,00	0,03	0,00	
Total	3,51	12,15	16,28	



RESULTS

In sacco experiment

- No differences in α diversity
- Microbial communities differed between animals (P<0.01)
- Within animals, the microbial communities differed between treatments
- (Some) microbial similarities with in vitro enrichment



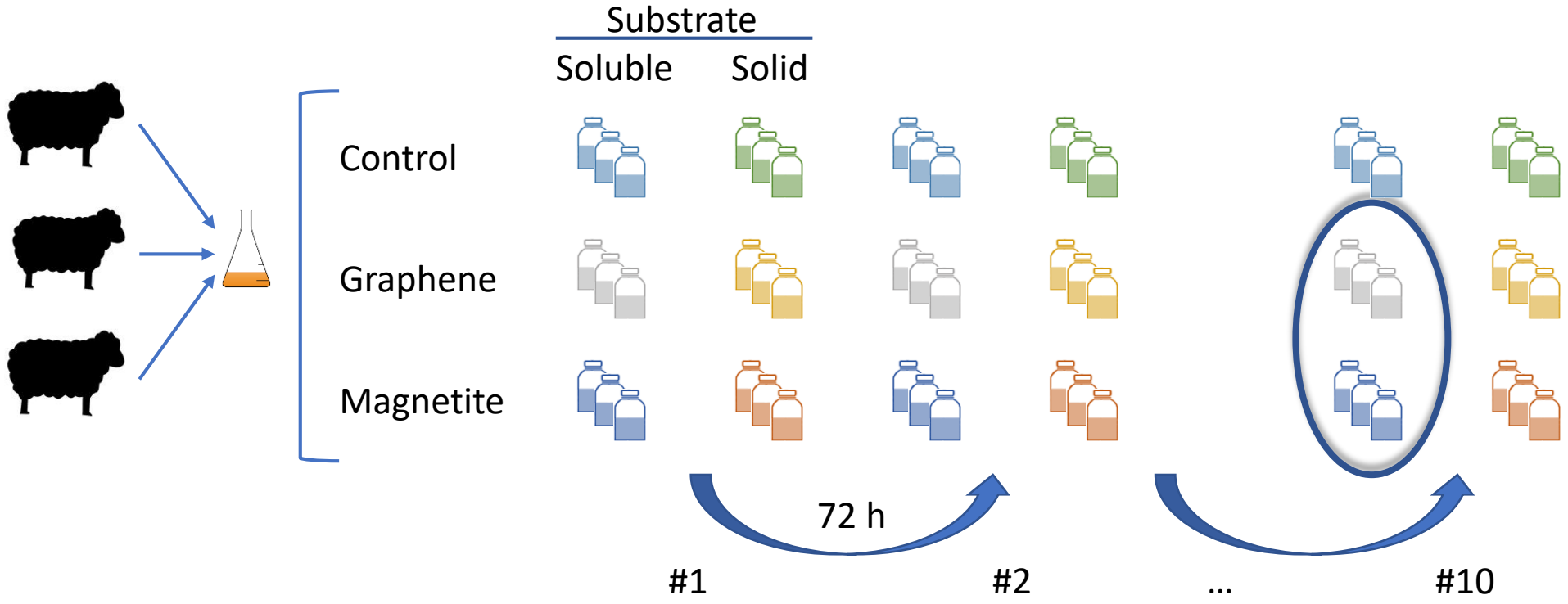
Distance	Variable	R.Squared	P.Value
WUniFrac	Animal	0.430	0.001
WUniFrac	Treatments	0.399	0.002

METHODS

1. In vitro batch culture (48 h)
2. Consecutive batch culture (enrichment)

Genome-centric metagenome analysis

Graphene: 69 MAGs (1 archaea), 51 HQ, 17 MQ
Magnetite: 120 MAGs (3 archaea), 80 HQ, 36 MQ



RESULTS

Consecutive batch culture - Soluble substrate

MaAslin2: S3 to S10

Species enriched	CTR	GPH	MGN	q-value	Cytochrome C-type	Pili	Flavin	Flagella	
Treponema_D bryantii	0,77	2,39	5,32	0,000	No	No	Yes	Yes	
Clostridium_G cochlearium	1,94	5,15	4,35	0,099	Yes	Yes	Yes	Yes	
Methanomicrobium mobile	0,39	0,74	2,49	0,078	Cytochrome b6-f	No	Yes	Archaella	Putative flagella-related protein H
CAG-873 sp004553485	0,00	1,66	1,06	0,000	No	No	No	No	
Prevotella heparinolytica	0,20	0,15	0,70	0,006	Yes	Yes	Yes	Yes	Prevotella sp
Succinivibrio hippei_B	0,03	0,16	0,34	0,000	Yes	No	Yes	Yes	Succinivibrio sp
JAAVLZ01 sp012034405	0,00	0,11	0,31	0,000	No	No	No	No	
Synergistes jonesii	0,03	0,12	0,29	0,004	Yes	Yes	Yes	Yes	
Marseille-P3106 sp900169975	0,00	0,03	0,26	0,003	No	Yes	Yes	Yes	
UBA1217 sp902788805	0,00	0,01	0,24	0,008	Yes	No	No	Yes	
SFEB01 sp004558105	0,04	0,07	0,18	0,034	No	No	No	No	
Succiniclasticum ruminis	0,00	0,00	0,15	0,004	No	Yes	No	Yes	Succiniclasticum sp
PeH17 sp900542285	0,00	0,09	0,13	0,000	No	No	No	No	
Succinivibrio dextrinosolvens_B	0,00	0,00	0,11	0,038	Yes	No	Yes	Yes	Succinivibrio sp
ER4 sp000765235	0,01	0,03	0,09	0,000	No	No	No	Yes	ER4 sp
Desulfovibrio_R_446353 desulfuricans_A_446137	0,08	1,32	0,06	0,039	Yes	Yes	Yes	Yes	Desulfovibrio sp
SFLA01 sp004553575	0,02	0,05	0,06	0,050	No	No	No	No	
RUG13615 sp902790905	0,01	0,00	0,05	0,000	No	No	No	Yes	RUG13615 sp
Methanimicrococcus blatticola	0,00	0,00	0,03	0,007	No	No	No	No	No MAG
Atopobium minutum	0,00	0,02	0,03	0,006	No	No	No	No	
Proteiniphilum saccharofermentans	0,00	0,01	0,02	0,027	Yes	No	No	Yes	Proteiniphilum acetatigenes
Methanoculleus_A_2118 hydrogenitrophicus	0,00	0,03	0,00		Cytochrome b6-f	No	Yes	Archaella	Putative flagella-related protein H
Total	3,51	12,15	16,28						

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

CLIFF – GRADS
Climate, Food and Farming Network
GRA Development Scholarships
GLOBAL RESEARCH ALLIANCE ON AGRICULTURAL GREENHOUSE GASES
CGIAR
RESEARCH PROGRAM ON Climate Change, Agriculture and Food Security
CCAFS
HoloRuminant
Understanding microbiomes of the ruminant holobiont

Thank you for your attention

www.holoruminant.eu