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Differential effects of *Ostertagia ostertagi* vaccination and infection on the rumen microbiome

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Presentation outline

- Background:
 - The rumen microbiome
 - *Ostertagia ostertagi*
 - Objectives
- Materials and methods
 - Experimental trial
 - Diversity
 - Partial least squares discriminant analysis
- Results and discussion
 - Diversity
 - Microbial genera
 - Microbial genes
- Conclusions
- Acknowledgements

Background

The rumen microbiome

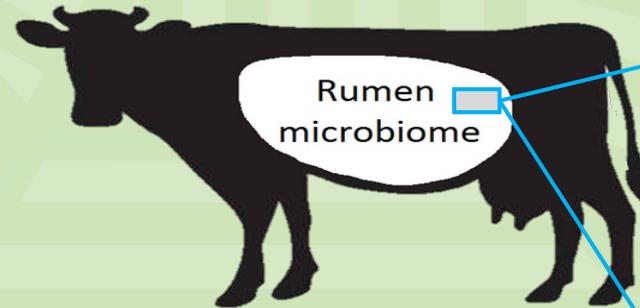
Host Genetics

↳ Breed

Environment

- ↳ Diet
- ↳ Parasite burden

Ostertagia ostertagi

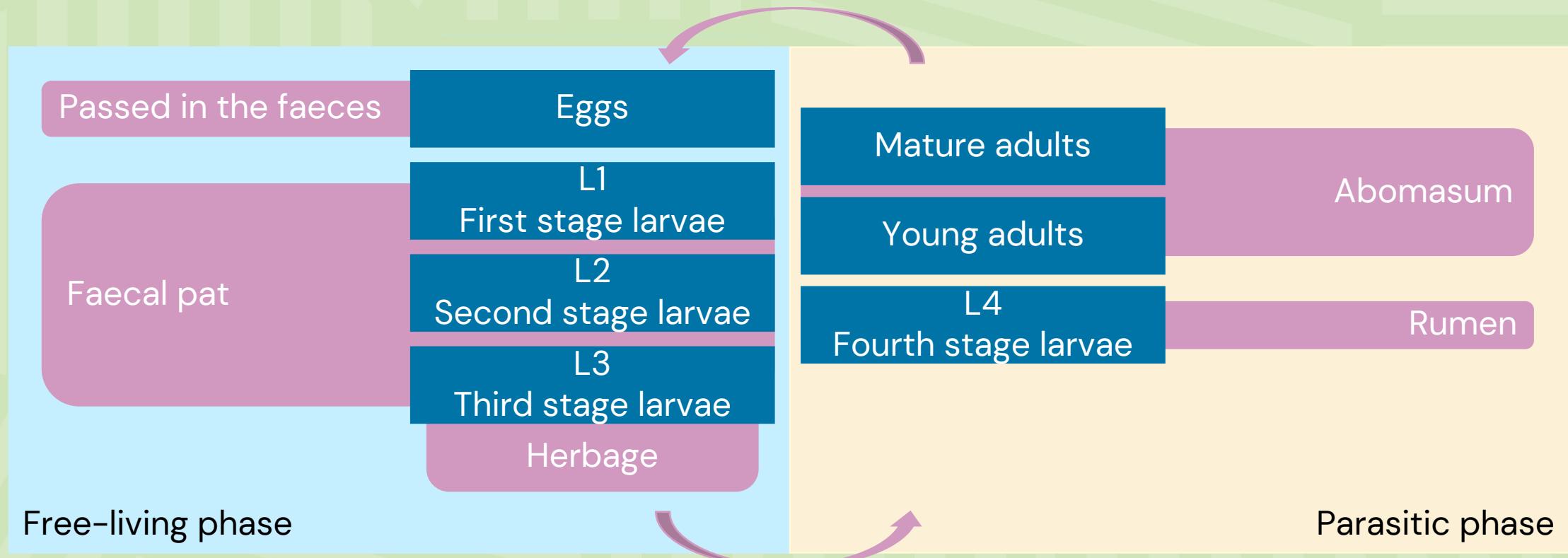


Microbial fermentation

Volatile Fatty Acids
Microbial protein
Vitamins

CO_2
 H_2 CH_4
(archaea)

Background *Ostertagia ostertagi*



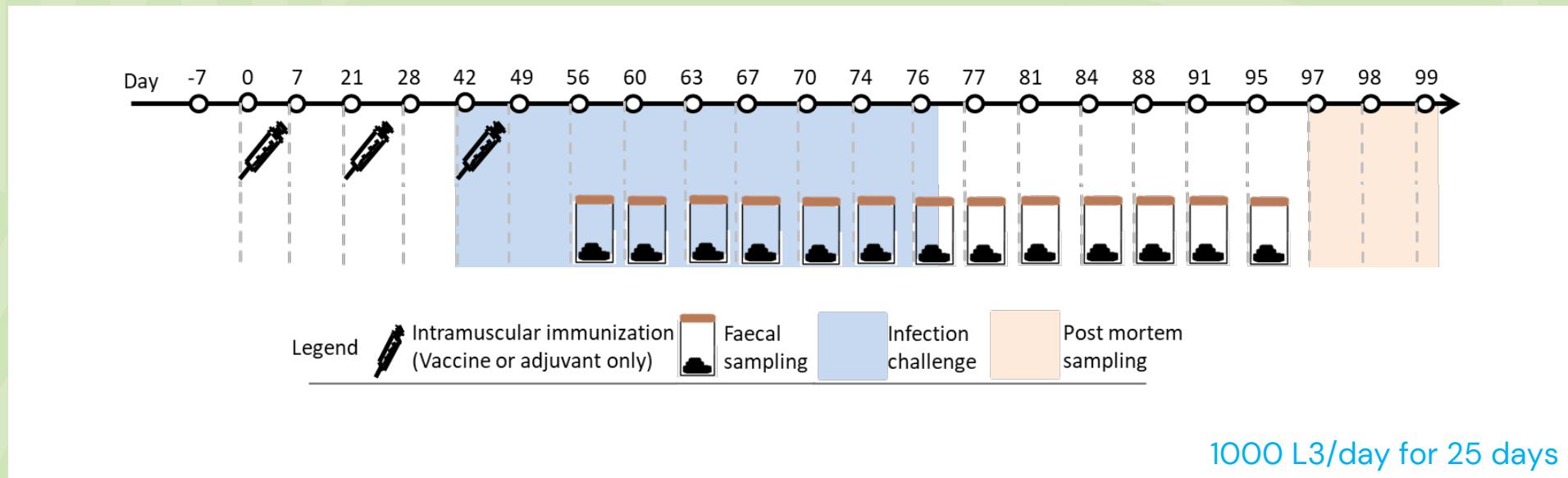
Objectives

- To investigate whether parasitism by *O. ostertagi* impacts the rumen microbiome composition, and, if so:
 - main changes at the taxonomic level
 - main changes at the functional level
- To understand whether a native vaccine against *O. ostertagi* influenced the rumen microbiome profiles.

Materials and Methods

Experimental trial

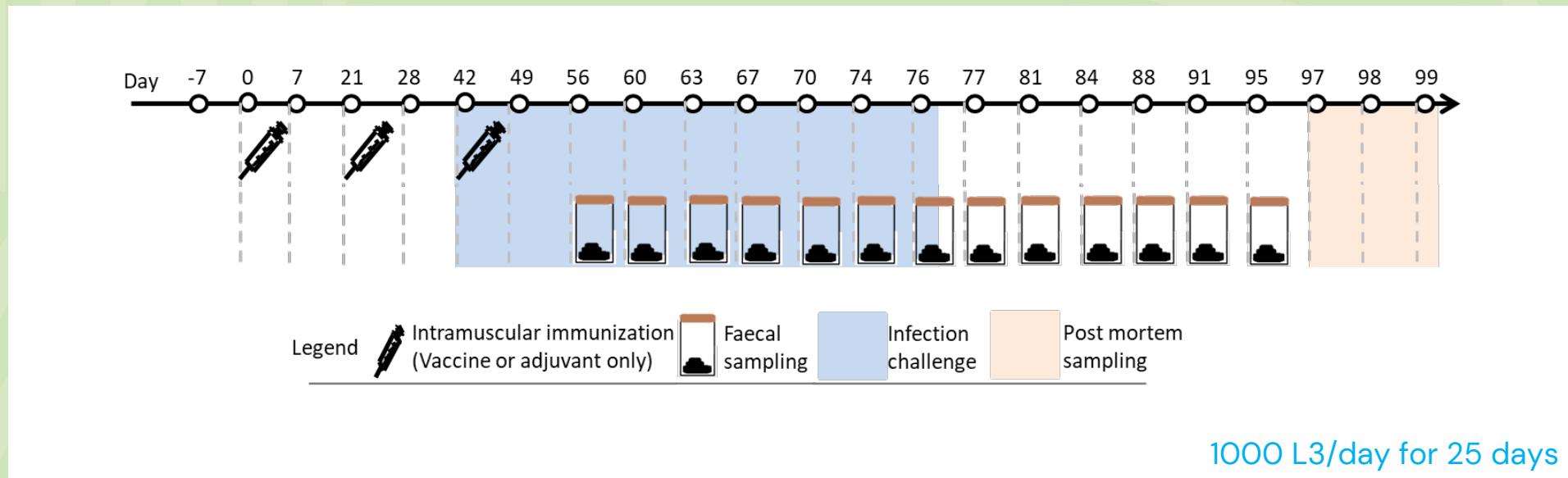
- 10 animals received vaccine
- 10 animals received adjuvant-only
- 4 animals - control



Materials and Methods

Experimental trial

- 10 animals received vaccine → 4 VAC
- 10 animals received adjuvant-only → 4 high cumulative faecal egg count - CHE
→ 4 low cumulative faecal egg count - CLE
- 4 animals - control → 4 UNF



Materials and Methods

Diversity indices

- 16 samples
 - 3 domains
 - 45 phyla
 - 461 families
 - 1200 microbial genera
 - 8393 microbial genes
- Archaea:Bacteria ratio
 - ANOVA
- Alpha diversity
 - Adjusted Shannon index
 - ANOVA
- Beta diversity
 - Bray–Curtis dissimilarity matrices
 - PERMANOVA and non-metric multidimensional scaling (NMDS)

Materials and Methods

Partial Least Squares Discriminant Analysis

- Variables with relative abundance lower than 0.001% and taxa absent from at least one animal were removed from the datasets.
- Abundances were centred-log ratio transformed to account for the compositional nature of the data.
- PLSDA with all permutations possible (40,320)

Materials and Methods

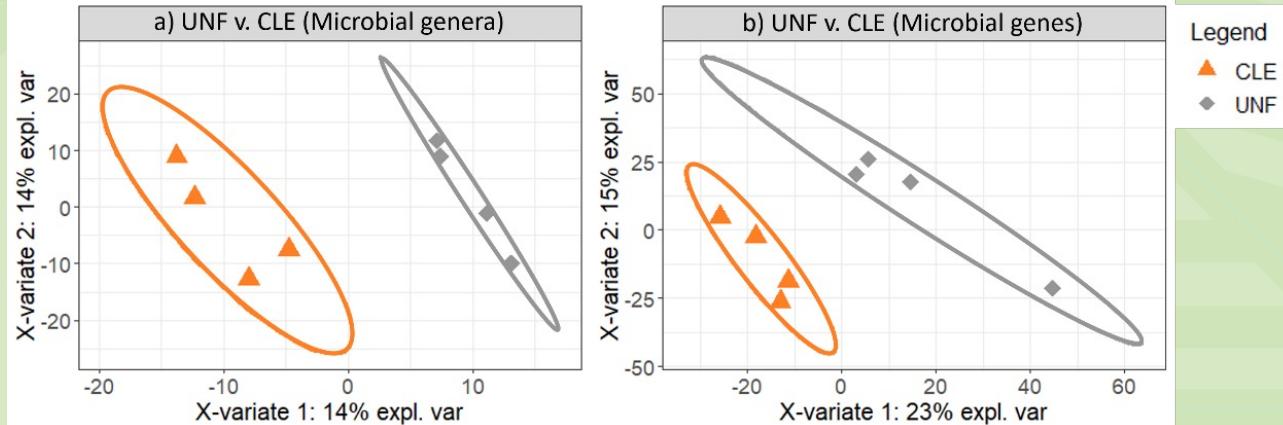
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Materials and Methods

Partial Least Squares Discriminant Analysis

(899 microbial genera and 3124 microbial genes)

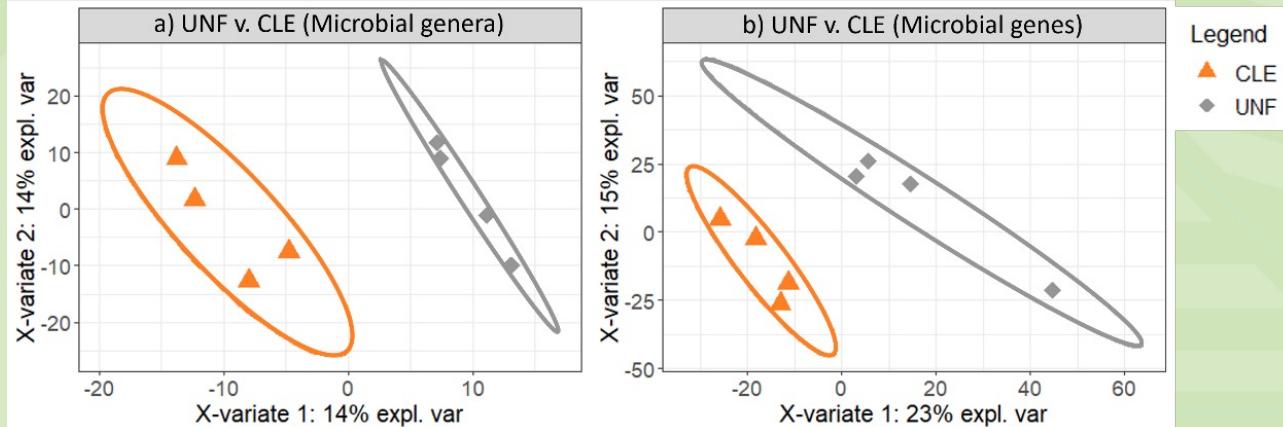


294 microbial genera and 1060 microbial genes considered important
for the discrimination ($VIP \geq 1$)

Materials and Methods

Partial Least Squares Discriminant Analysis

(899 microbial genera and 3124 microbial genes)



294 microbial genera and 1060 microbial genes considered important for the discrimination ($VIP \geq 1$)

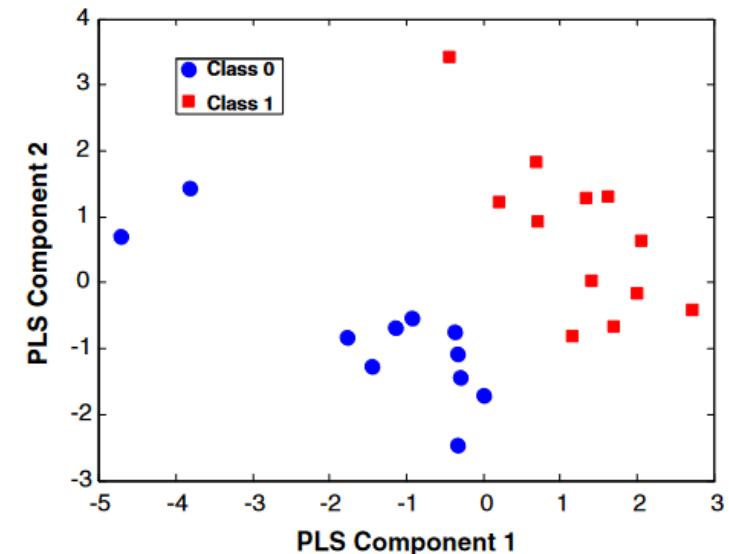


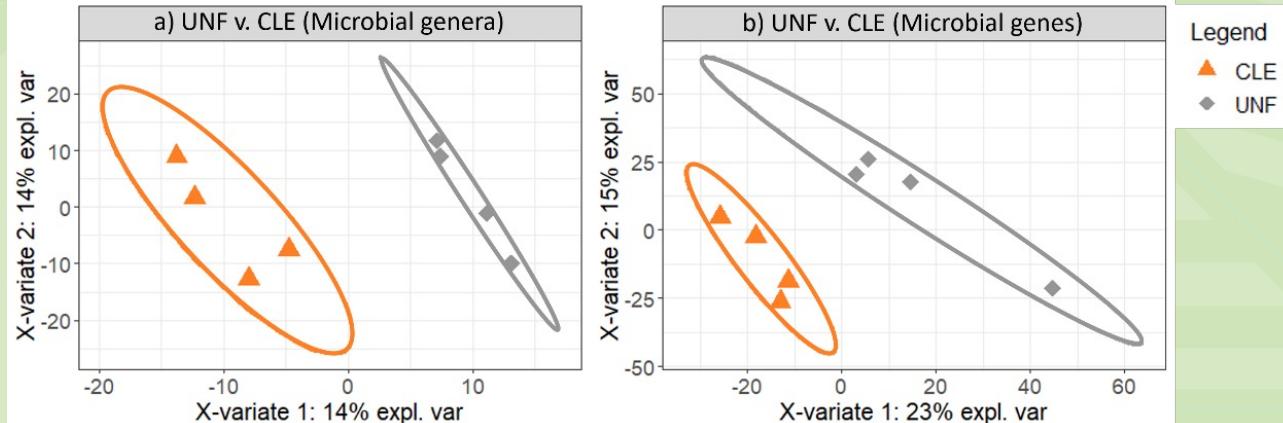
Fig. 1 PLSDA score plot of an NMR data set of healthy volunteers which were arbitrarily divided into two classes. Q^2 value of this model was -0.18 . Still a clear separation between the classes is observed in this score plot

Westerhuis, J. A., Assessment of PLSDA cross validation. Metabolomics (2008) 4:81–89 DOI 10.1007/s11306-007-0099-6

Materials and Methods

Partial Least Squares Discriminant Analysis

(899 microbial genera and 3124 microbial genes)



294 microbial genera and 1060 microbial genes considered important for the discrimination ($VIP \geq 1$)

*"The obvious conclusion from our experiments is that **it is a terrible idea to use PLS-DA blindly** with all datasets. In spite of its attractive ability to identify features that can separate the classes, **it is clear that any data set with sufficiently large number of features is separable** and that most of the separating hyperplanes are just "noise".*

Daniel, R.-P., So you think you can PLS-DA?. BMC Bioinformatics 2020, 21(Suppl 1):2
<https://doi.org/10.1186/s12859-019-3310-7>

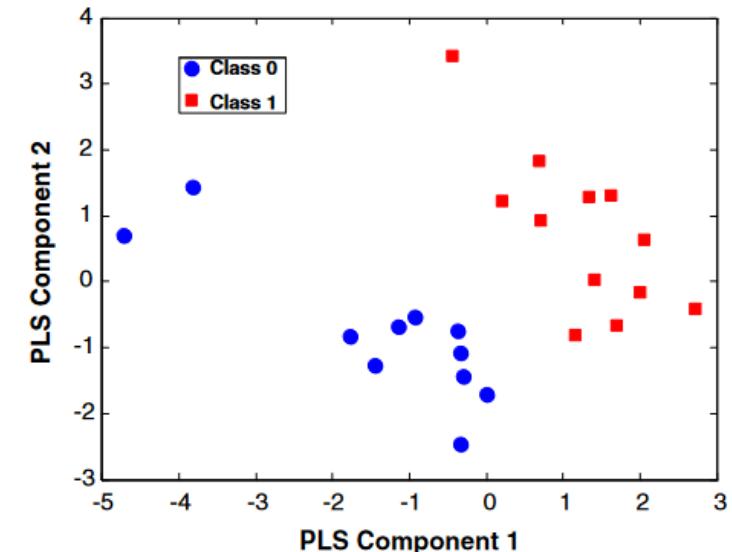


Fig. 1 PLSDA score plot of an NMR data set of healthy volunteers which were arbitrarily divided into two classes. Q^2 value of this model was -0.18 . Still a clear separation between the classes is observed in this score plot

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Materials and Methods

Partial Least Squares Discriminant Analysis

Label	Animal	Microbiome
Pink	1	1
Pink	2	2
Pink	3	3
Pink	4	4
Blue	5	5
Blue	6	6
Blue	7	7
Blue	8	8

Original labelling

Label	Animal	Microbiome
Pink	1	1
Pink	6	2
Pink	3	3
Pink	4	4
Blue	5	5
Blue	2	6
Blue	7	7
Blue	8	8

Permuted labelling (example 1)

Label	Animal	Microbiome
Pink	1	1
Pink	4	2
Pink	3	3
Pink	2	4
Blue	5	5
Blue	6	6
Blue	7	7
Blue	8	8

Permuted labelling (example 2)

- 40,320 PLSDA models
- Distribution of Variable Importance in Projection (VIP) was created.
- P-value for each variable was calculated. ($p < 0.05$, $VIP \geq 1$)

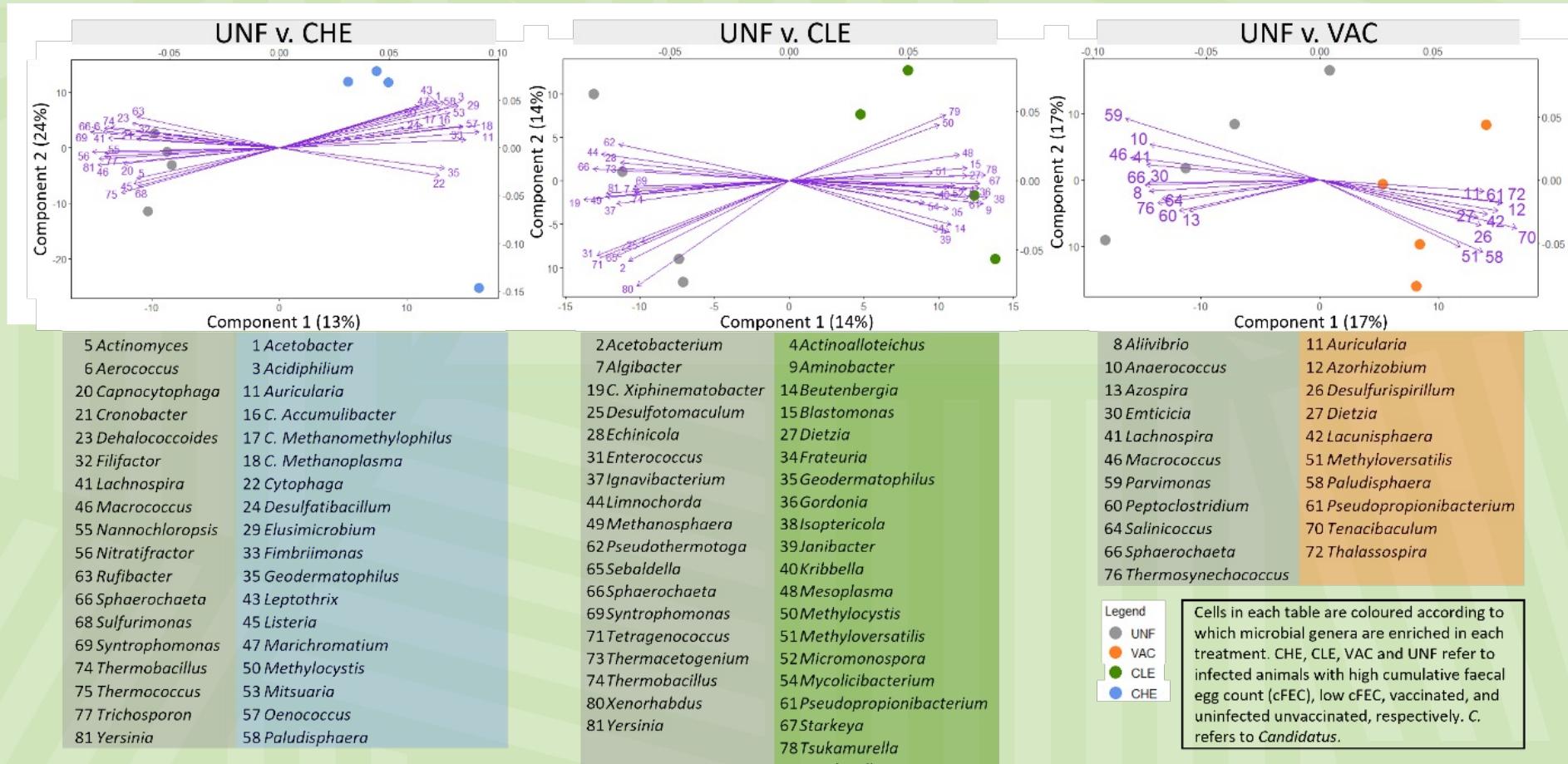
Results

Diversity indices

- Archaea:Bacteria ratio
 - No significant differences
- Alpha diversity
 - No significant differences
- Beta diversity
 - No significant differences

Results

Microbial genera discriminating between treatments



36 microbial genera

38 microbial genera

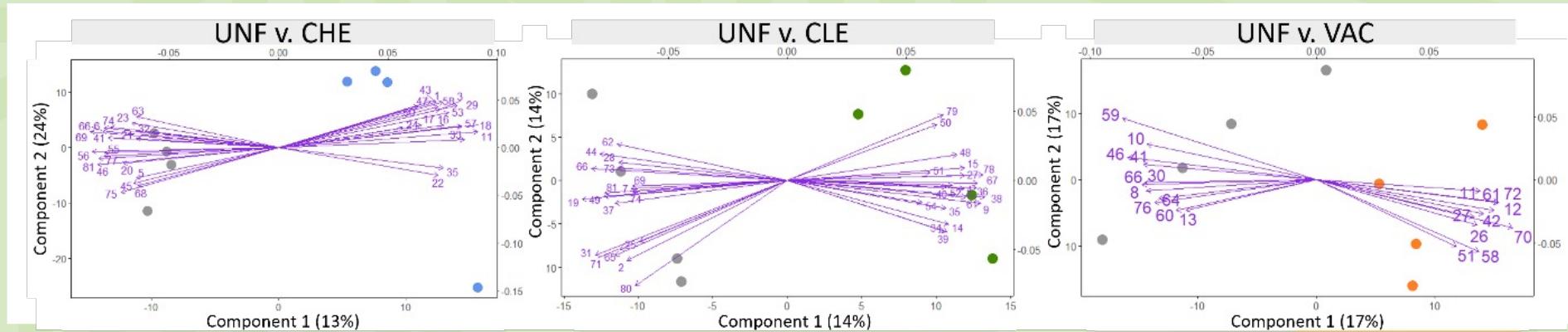
21 microbial genera

Results

Microbial genera discriminating between treatments



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UNF = uninfected
CHE = infected, high cFEC
CLE = infected, low cFEC
VAC = vaccinated

5 <i>Actinomyces</i>	1 <i>Acetobacter</i>
6 <i>Aerococcus</i>	3 <i>Acidiphilum</i>
20 <i>Capnocytophaga</i>	11 <i>Auricularia</i>
21 <i>Cronobacter</i>	16 <i>C. Accumulibacter</i>
23 <i>Dehalococcoides</i>	17 <i>C. Methanomethylphilus</i>
32 <i>Filifactor</i>	18 <i>C. Methanoplasma</i>
41 <i>Lachnospira</i>	22 <i>Cytophaga</i>
46 <i>Macrococcus</i>	24 <i>Desulfatibacillum</i>
55 <i>Nanochloropsis</i>	29 <i>Elusimicrobium</i>
56 <i>Nitratiruptor</i>	33 <i>Fimbriimonas</i>
63 <i>Rufibacter</i>	35 <i>Geodermatophilus</i>
66 <i>Sphaerochaeta</i>	43 <i>Leptothrix</i>
68 <i>Sulfurimonas</i>	45 <i>Listeria</i>
69 <i>Syntrophomonas</i>	47 <i>Marichromatulum</i>
74 <i>Thermobacillus</i>	50 <i>Methylocystis</i>
75 <i>Thermococcus</i>	53 <i>Mitsuaria</i>
77 <i>Trichosporon</i>	57 <i>Oenococcus</i>
81 <i>Yersinia</i>	58 <i>Paludisphaera</i>

36 microbial genera

2 <i>Acetobacterium</i>	4 <i>Actinoalloteichus</i>
7 <i>Algibacter</i>	9 <i>Aminobacter</i>
19C. <i>Xiphinematobacter</i>	14 <i>Beutenbergia</i>
25 <i>Desulfotomaculum</i>	15 <i>Blastomonas</i>
28 <i>Echinicola</i>	27 <i>Dietzia</i>
31 <i>Enterococcus</i>	34 <i>Frateuria</i>
37 <i>Ignavibacterium</i>	35 <i>Geodermatophilus</i>
44 <i>Limnochorda</i>	36 <i>Gordonia</i>
49 <i>Methanospaera</i>	38 <i>Isoptericola</i>
62 <i>Pseudothermotoga</i>	39 <i>Janibacter</i>
65 <i>Sebaldella</i>	40 <i>Kribbella</i>
66 <i>Sphaerochaeta</i>	48 <i>Mesoplasma</i>
69 <i>Syntrophomonas</i>	50 <i>Methylocystis</i>
71 <i>Tetragenococcus</i>	51 <i>Methyloversatilis</i>
73 <i>Thermacetogenium</i>	52 <i>Micromonospora</i>
74 <i>Thermobacillus</i>	54 <i>Mycobacterium</i>
80 <i>Xenorhabdus</i>	61 <i>Pseudopropionibacterium</i>
81 <i>Yersinia</i>	67 <i>Starkeya</i>
	78 <i>Tsukamurella</i>
	79 <i>Virgibacillus</i>

38 microbial genera

8 <i>Aliivibrio</i>	11 <i>Auricularia</i>
10 <i>Anaerococcus</i>	12 <i>Azorhizobium</i>
13 <i>Azospira</i>	26 <i>Desulfurispirillum</i>
30 <i>Emticicia</i>	27 <i>Dietzia</i>
41 <i>Lachnospira</i>	42 <i>Lacunisphaera</i>
46 <i>Macrococcus</i>	51 <i>Methyloversatilis</i>
59 <i>Parvimonas</i>	58 <i>Paludisphaera</i>
60 <i>Peptoclostridium</i>	61 <i>Pseudopropionibacterium</i>
64 <i>Salinicoccus</i>	70 <i>Tenacibaculum</i>
66 <i>Sphaerochaeta</i>	72 <i>Thalassospira</i>
76 <i>Thermosynechococcus</i>	

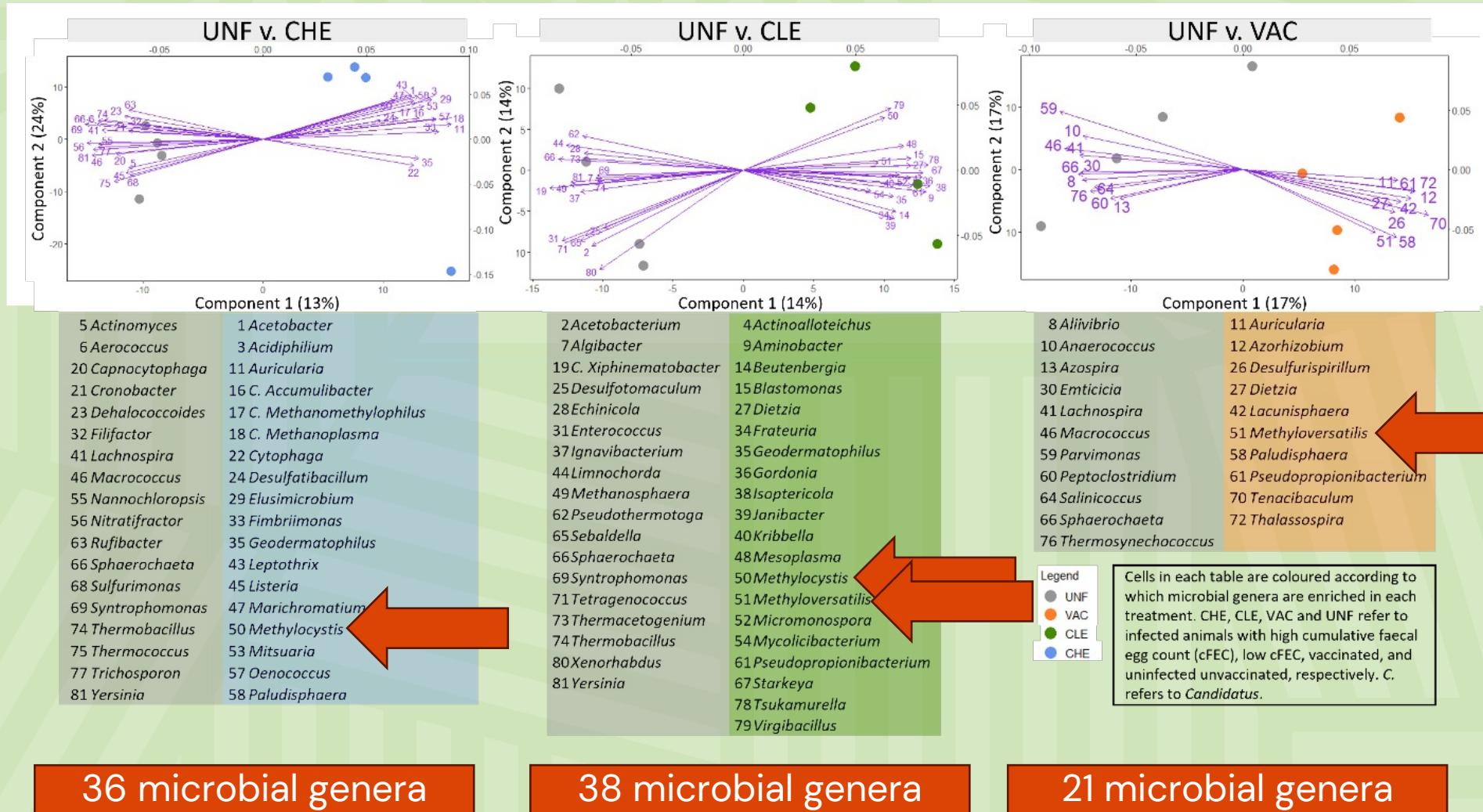
Legend
● UNF
● VAC
● CLE

Cells in each table are coloured according to which microbial genera are enriched in each treatment. CHE, CLE, VAC and UNF refer to infected animals with high cumulative faecal egg count (cFEC), low cFEC, vaccinated, and uninfected unvaccinated, respectively. C. refers to *Candidatus*.

21 microbial genera

Results

Microbial genera discriminating between treatments

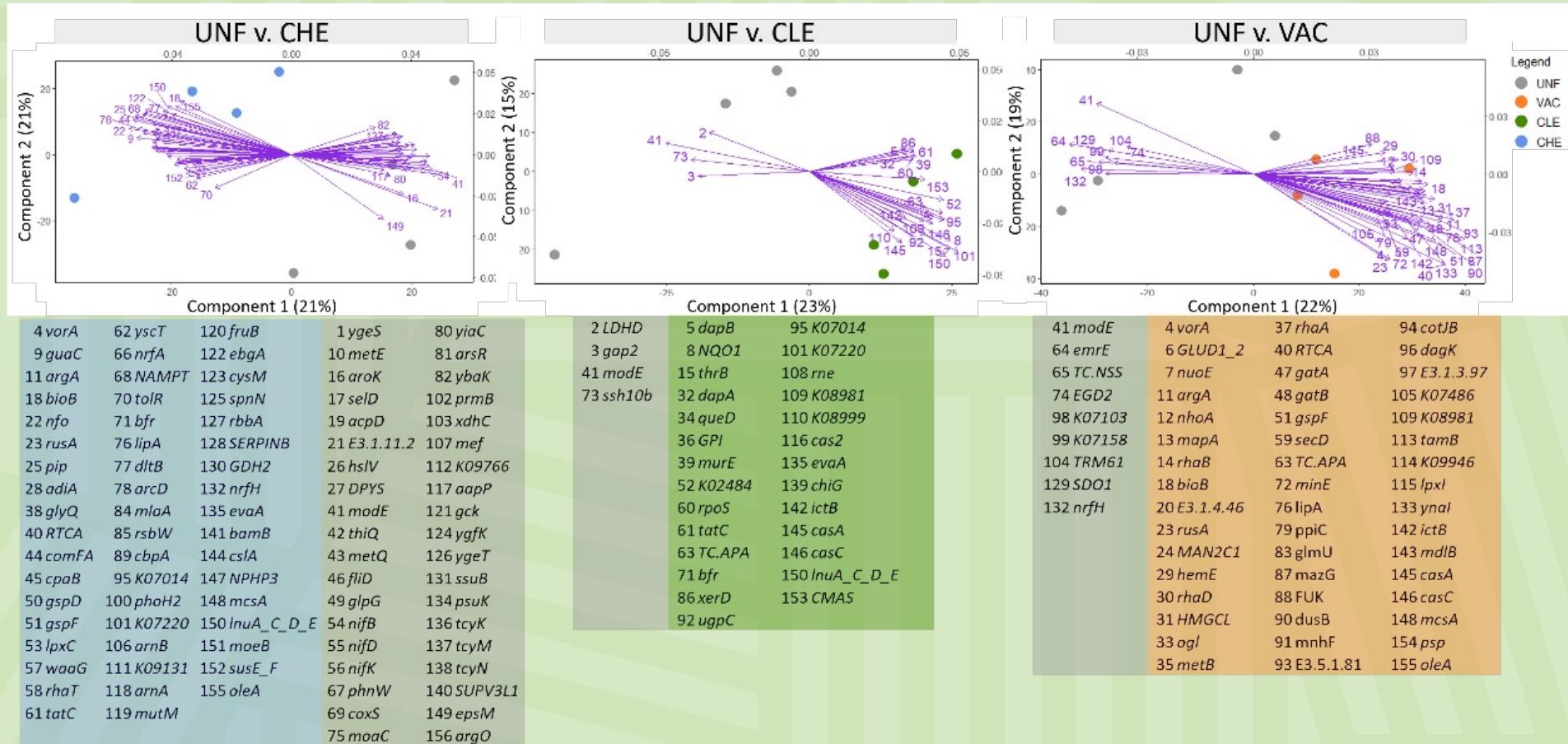


Results

Microbial genes discriminating between treatments



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91 microbial genes

31 microbial genes

57 microbial genes

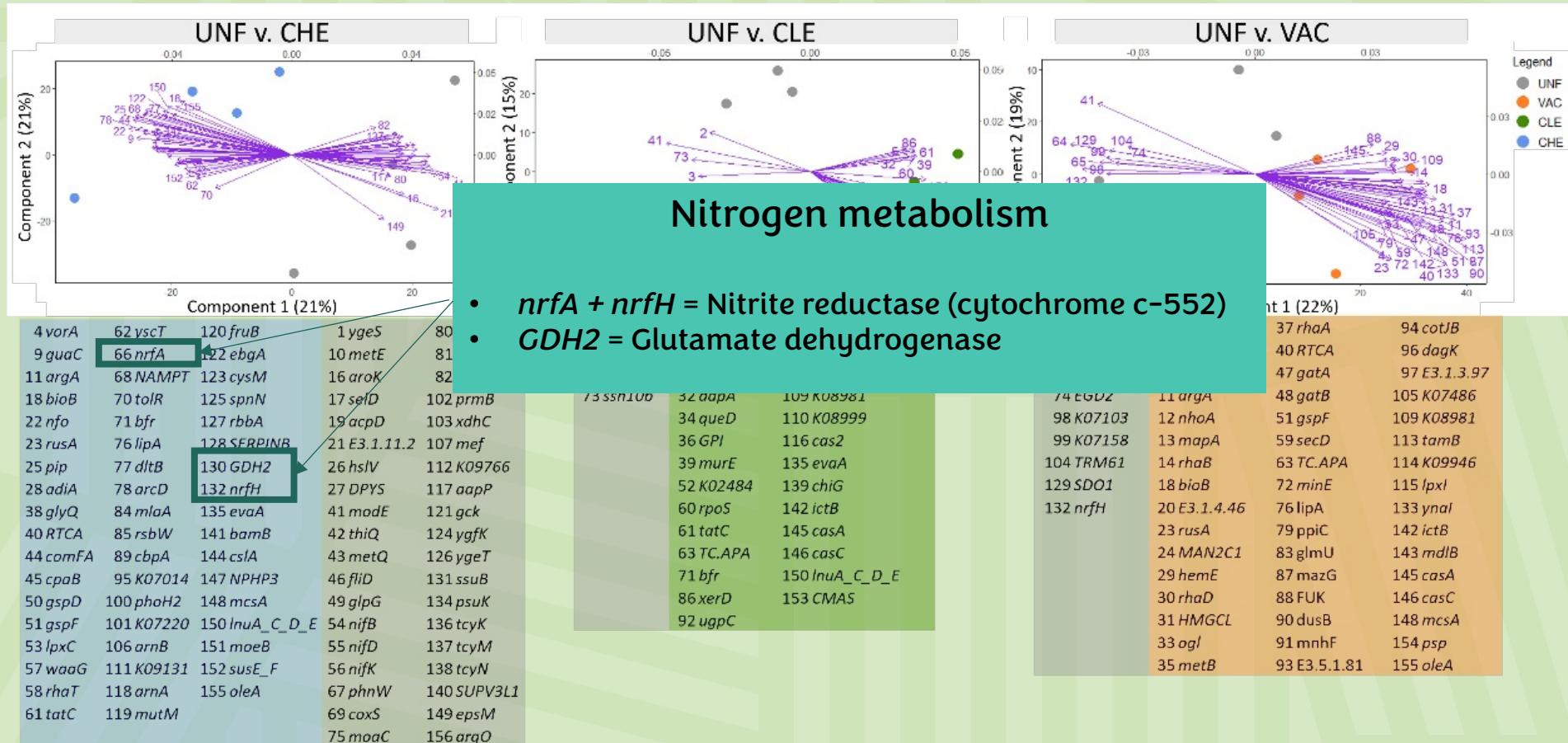
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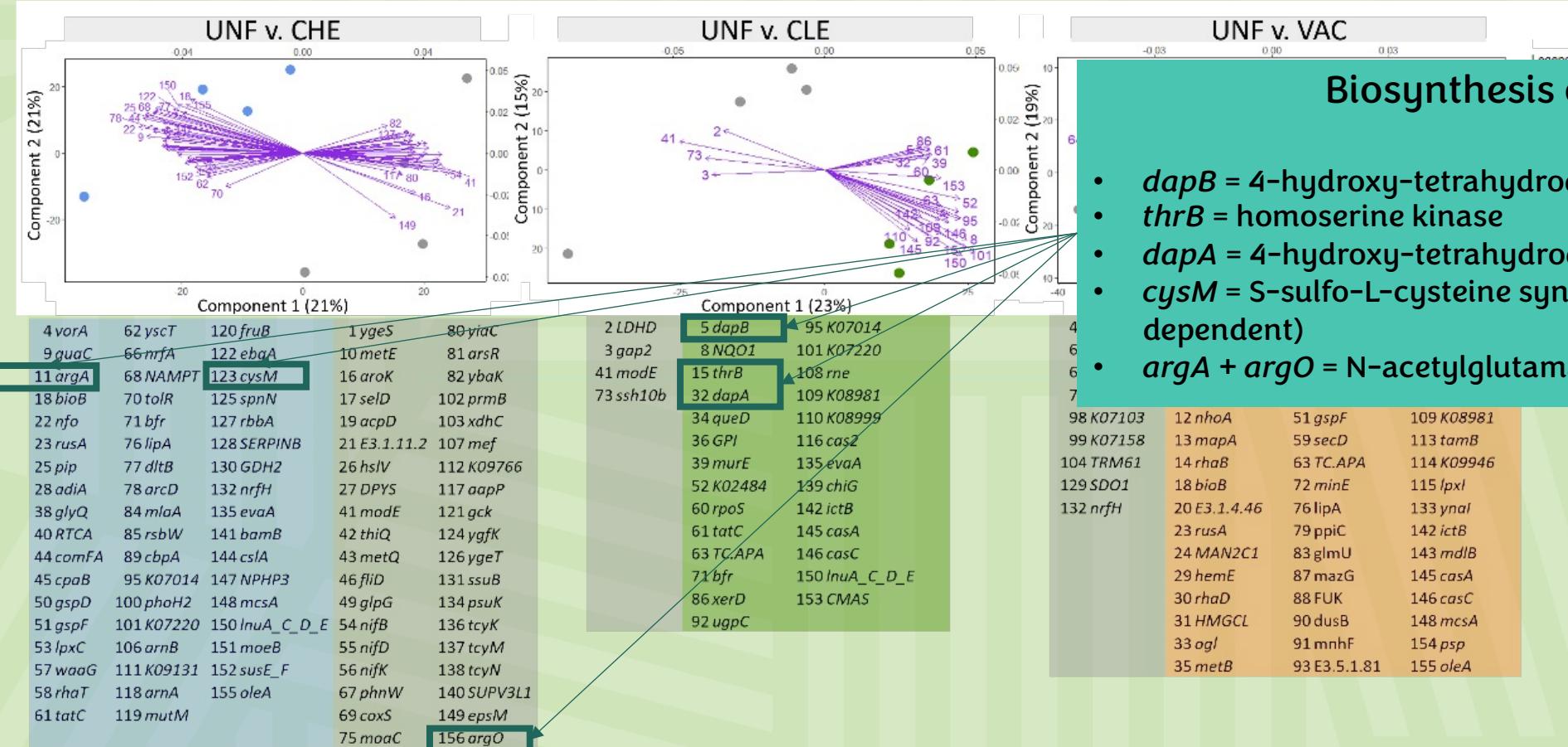
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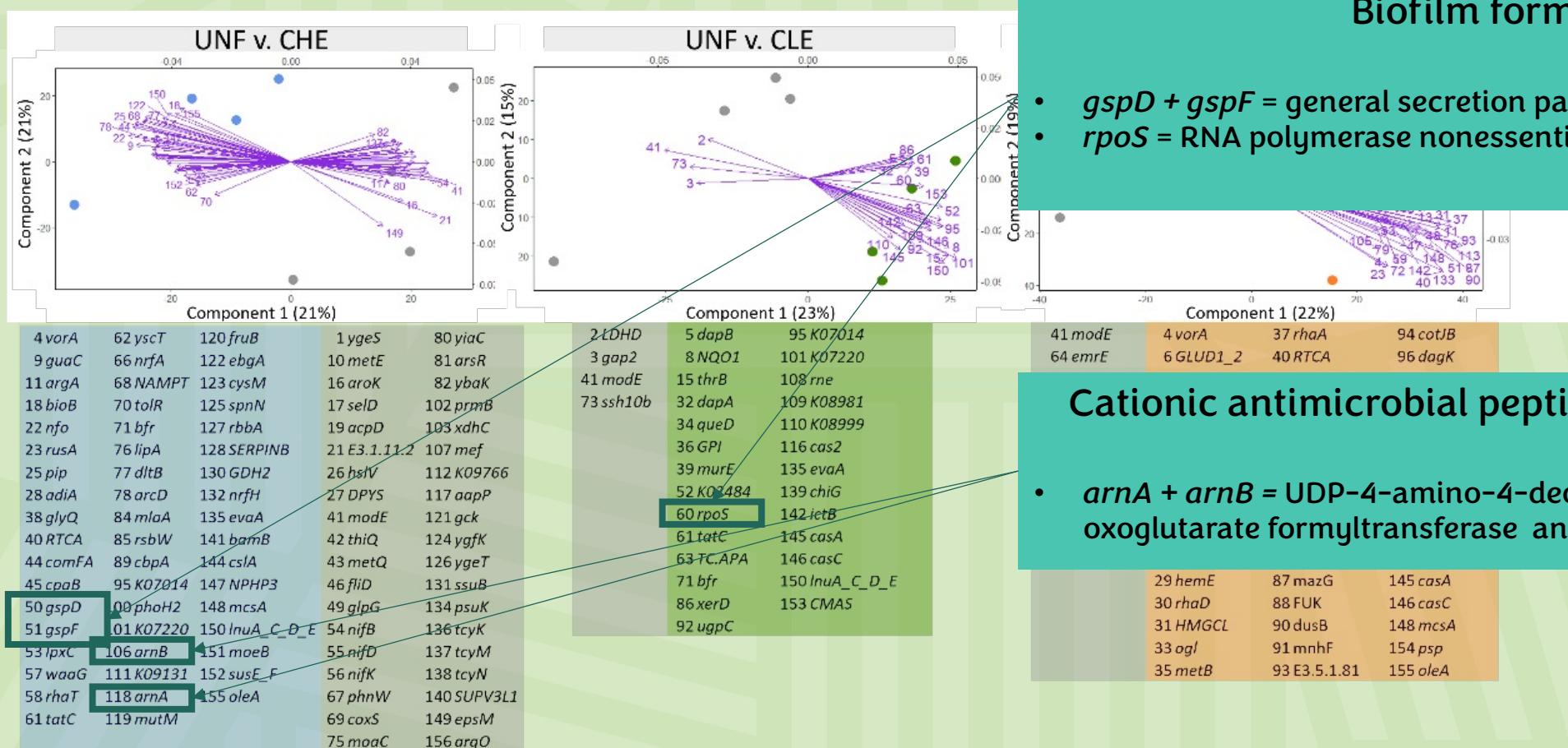
Microbial genes discriminating between treatments



- *dapB* = 4-hydroxy-tetrahydrodipicolinate reductase
- *thrB* = homoserine kinase
- *dapA* = 4-hydroxy-tetrahydrodipicolinate synthase
- *cysM* = S-sulfo-L-cysteine synthase (O-acetyl-L-serine-dependent)
- *argA + argO* = N-acetylglutamate synthase

Results

Microbial genes discriminating between treatments



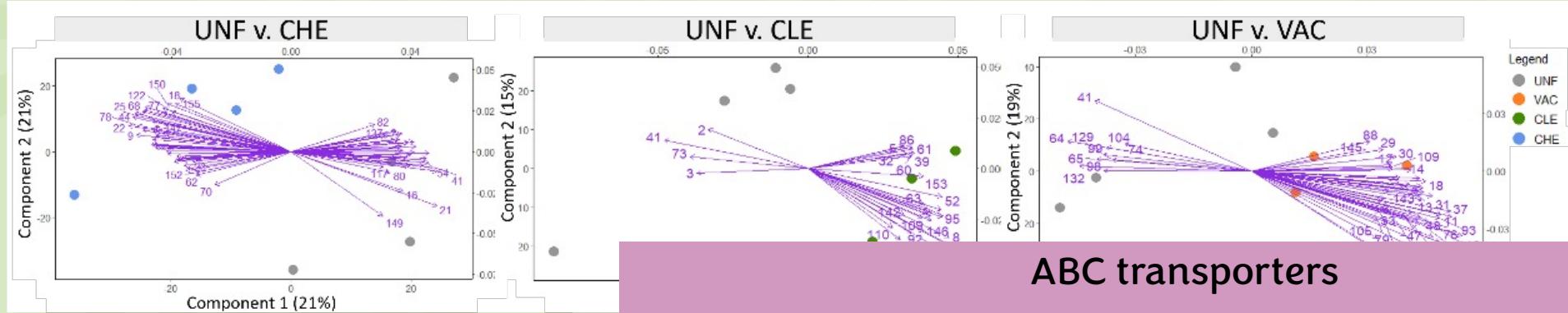
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Results

Microbial genes discriminating between treatments



4 vorA	62 yscT	120 fruB	1 ygeS	80 yiaC	2 LD
9 guaC	66 nrfA	122 ebgA	10 metE	81 arsR	3 ga
11 argA	68 NAMPT	123 cysM	16 aroK	82 ybaK	41 mc
18 bioB	70 tolR	125 spnN	17 selD	102 prmB	73 sst
22 nfo	71 bfr	127 rbbA	19 acpD	103 xdhC	
23 rusA	76 lipA	128 SERPINB	21 E3.1.11.2	107 mef	
25 pip	77 dltB	130 GDH2	26 hslV	112 K09766	
28 adiA	78 arcD	132 nrfH	27 DPYS	117 aapP	
38 glyQ	84 mlaA	135 evaA	41 madF	121 gck	
40 RTCA	85 rsbW	141 bamB	42 thiQ	124 ygfk	
44 comFA	89 cbpA	144 cslA	43 metQ	126 ygeT	
45 cpaB	95 K07014	147 NPHP3	46 fliD	131 ssuB	
50 gspD	100 phoH2	148 mcsA	49 glpG	134 psuK	
51 gspF	101 K07220	150 lnuA_C_D_E	54 nifB	136 tcyK	
53 lpxC	106 arnB	151 moeB	55 nifD	137 tcyM	
57 waaG	111 K09131	152 susE_F	56 nifK	138 tcyN	
58 rhaT	118 arnA	155 oleA	67 phnW	140 SUPV3L1	
61 tatC	119 mutM		69 coxS	149 epsM	
			75 moaC	156 argO	

- ABC transporters**
- *thiQ* = thiamine transport system ATP-binding protein
 - *metQ* = D-methionine transport system substrate-binding protein
 - *aapP* = general L-amino acid transport system ATP-binding protein
 - *ssuB* = sulfonate transport system ATP-binding protein
 - *tcyK* = L-cystine transport system substrate-binding protein
 - *tcyM* = L-cystine transport system permease protein
 - *tcyN* = L-cystine transport system ATP-binding protein

33 ogl	91 mnHF	154 psp
35 metB	93 E3.5.1.81	155 oleA

91 microbial genes

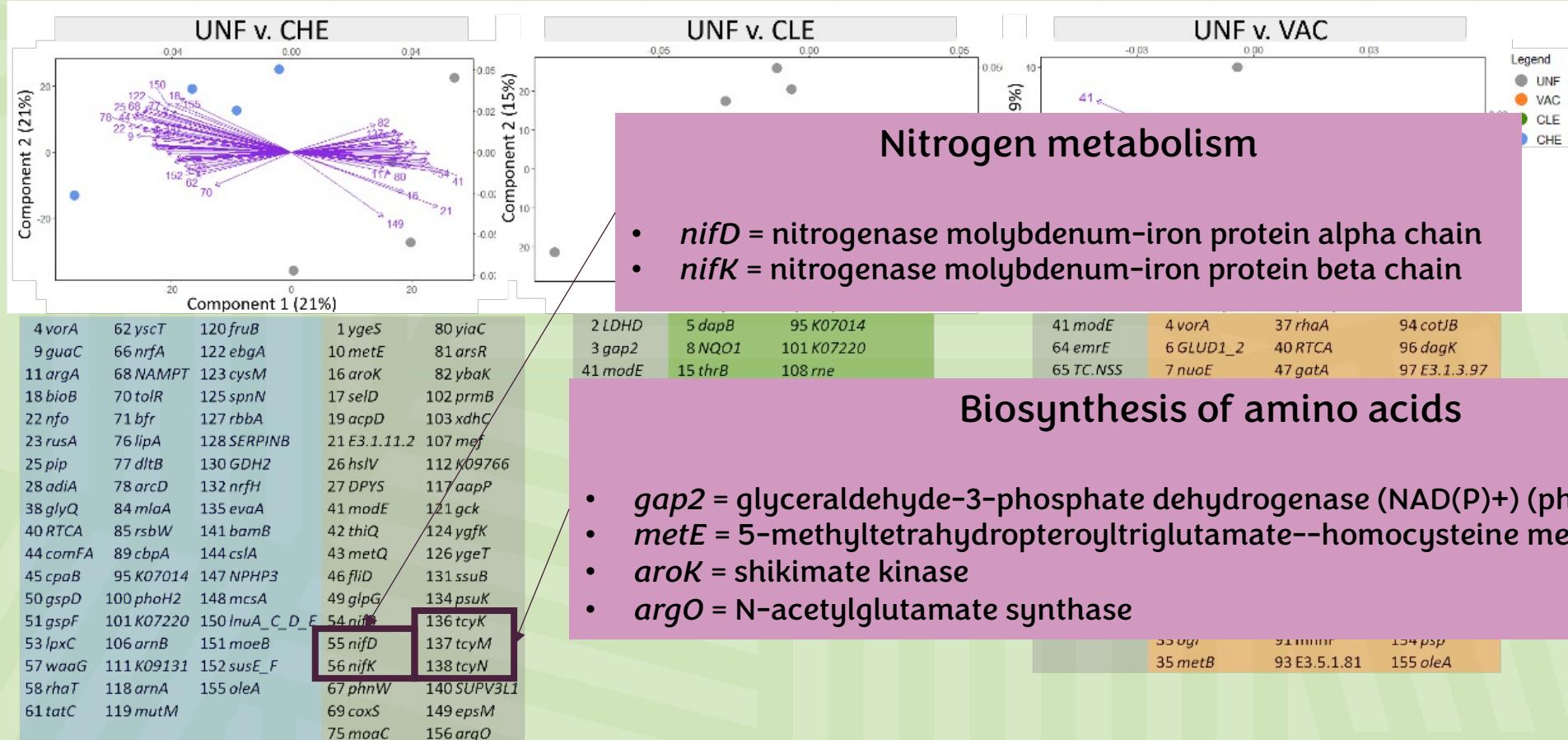
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Results

Microbial genes discriminating between treatments



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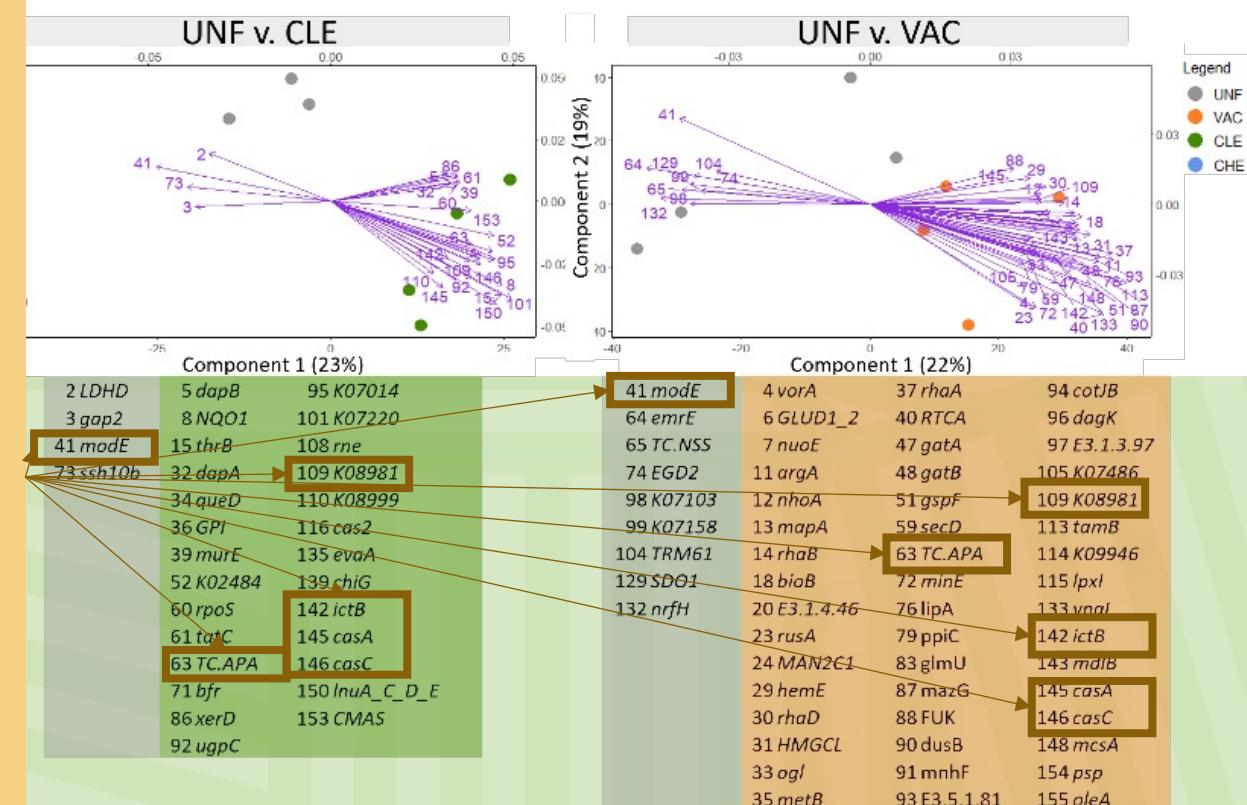
Microbial genes discriminating between treatments

Increased in CLE and VAC

- ictB* = putative inorganic carbon (*hco3(-)*) transporter
- casA* = CRISPR system Cascade subunit CasA (Prokaryotic defense system)
- casC* = CRISPR system Cascade subunit CasC (Prokaryotic defense system)
- TC.APA* = basic amino acid/polyamine antiporter, APA family
- KO8981 = putative membrane protein

Decreased in CLE and VAC

- modE* = molybdate transport system regulatory protein



31 microbial genes

57 microbial genes

Conclusions

- The abomasal nematode *Ostertagia ostertagi*, and the native vaccine against this parasite, **affects the rumen microbial communities**, with consequences on their functionality.
- Negative **consequences of parasitism** by nematodes in cattle (animal health and welfare issues, inappetence, and lower productivity), are **not exclusively due to the abomasal inflammation**, but also **due to dysbiosis** of the rumen microbiome
- **Vaccination** has a positive effect on the rumen microbiome, **potentially preventing alterations of the microbiome** associated with high levels of infection



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