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

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Presentation outline

SRUC

- 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

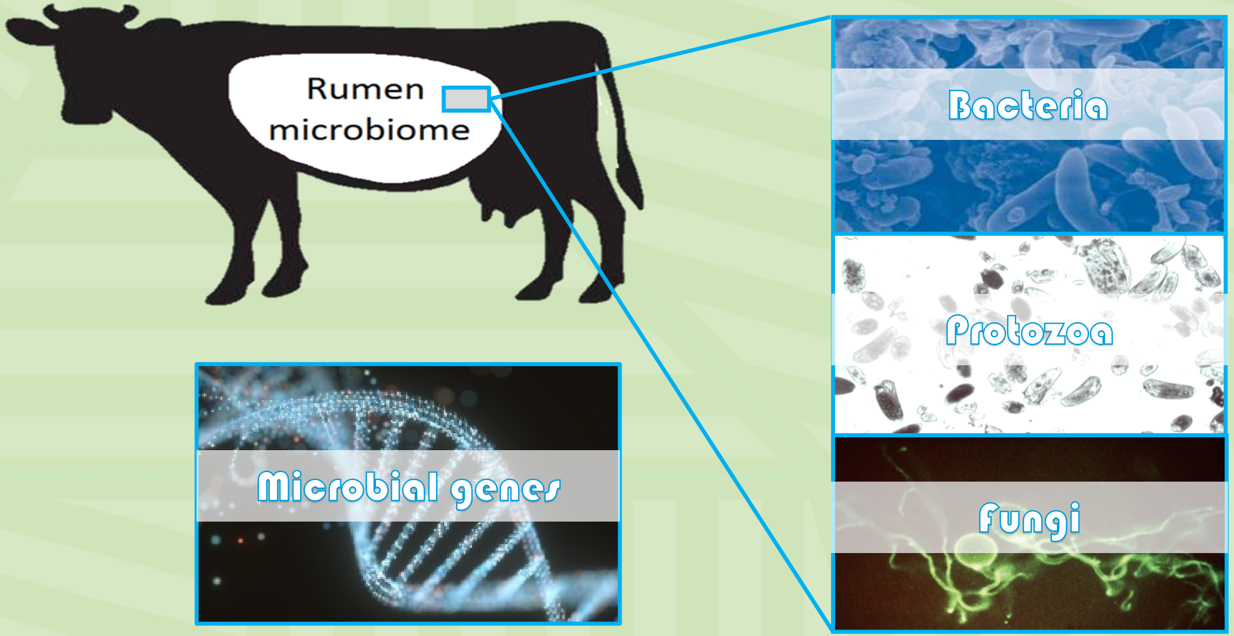
Background
The rumen microbiome

Host Genetics

- Breed

Environment

- Diet
- Parasite burden



Ostertagia ostertagi

Microbial fermentation

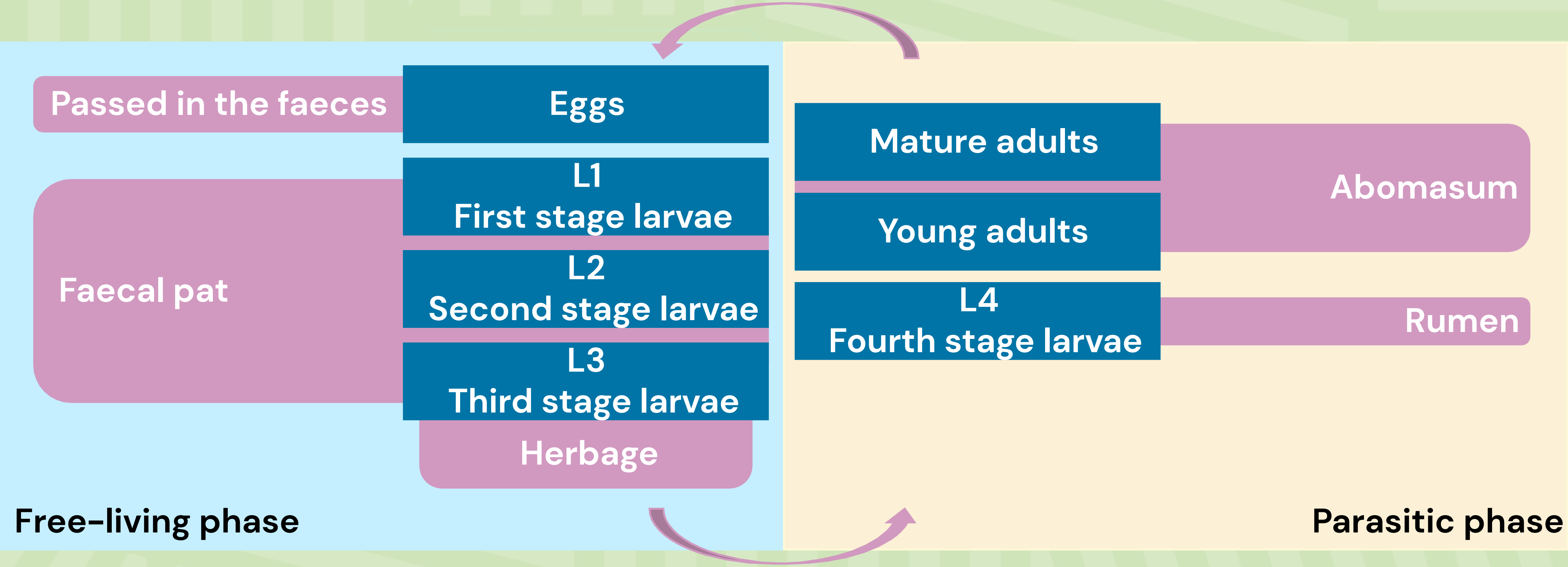
Volatile Fatty Acids
Microbial protein
Vitamins

CO_2
H_2

CH_4
(archaea)

Background
Ostertagia ostertagi

SRUC



Passed in the faeces

Eggs

L1
First stage larvae

L2
Second stage larvae

L3
Third stage larvae

Faecal pat

Herbage

Free-living phase

Mature adults

Young adults

L4
Fourth stage larvae

Abomasum

Rumen

Parasitic phase

Objectives

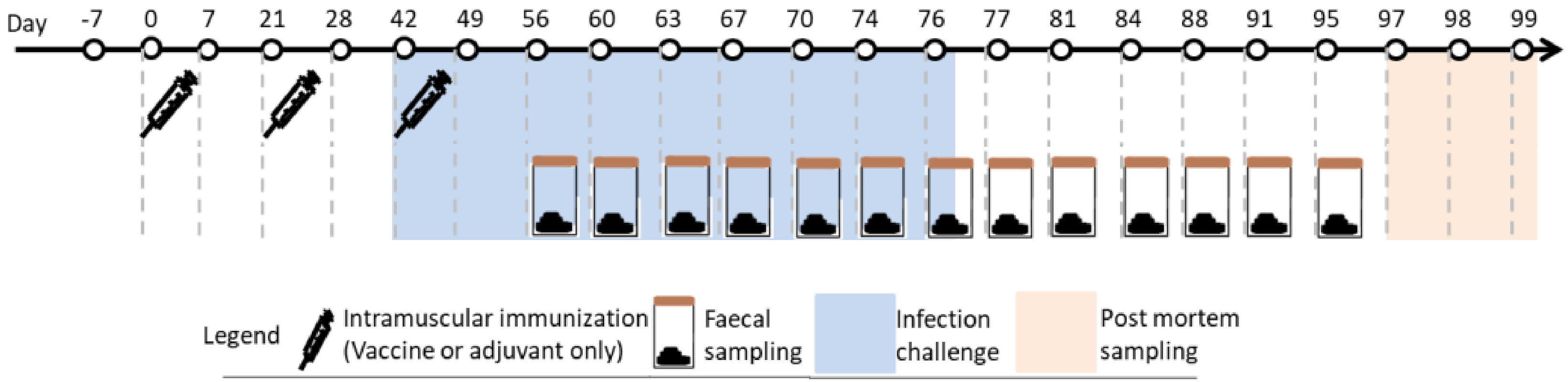
- To investigate whether parasitism by *O. ostertagi* impacts the rumen microbiome composition, and, if so:
  - main changes at the taxonomic level
  - main changes at the functional level

- To understand whether a native vaccine against *O. ostertagi* influenced the rumen microbiome profiles.

Materials and Methods

Experimental trial

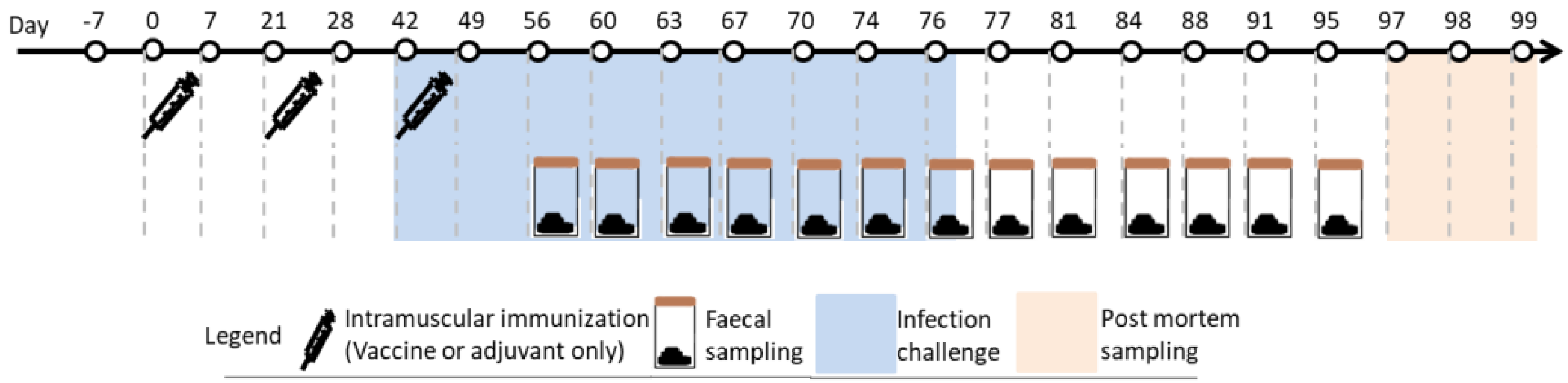
- 10 animals received vaccine
- 10 animals received adjuvant-only
- 4 animals - control



1000 L3/day for 25 days

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



1000 L3/day for 25 days

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)

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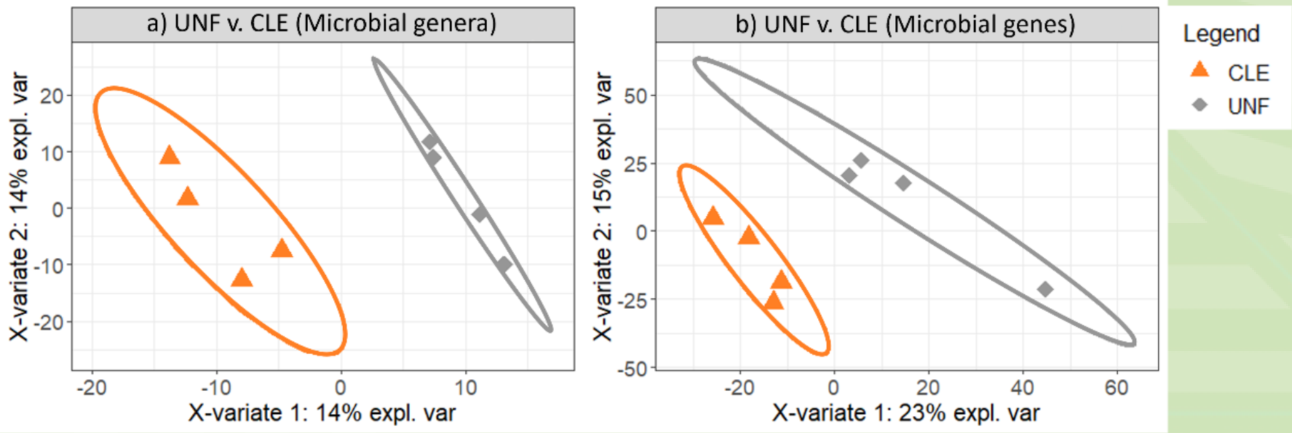
Materials and Methods

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 ≥ 1)

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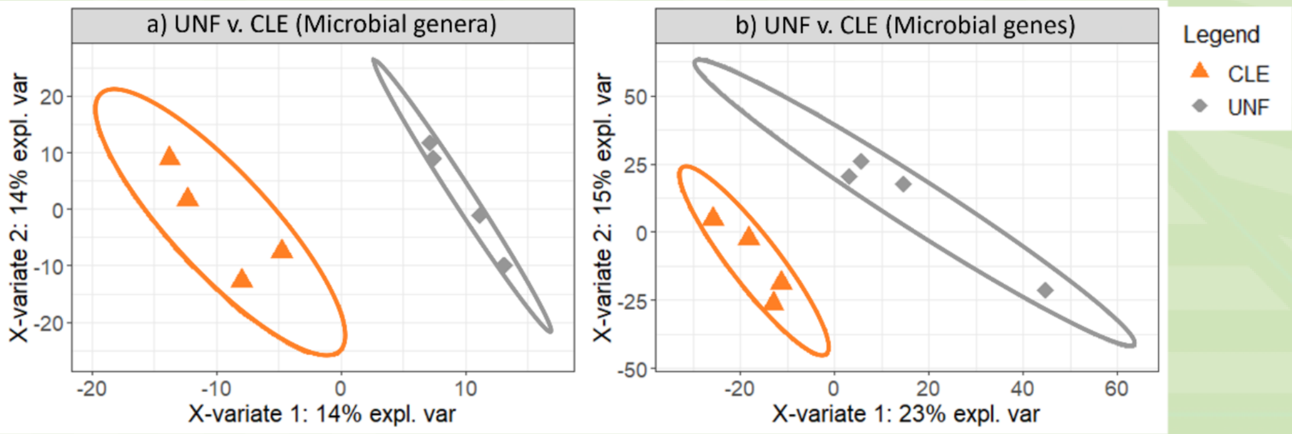

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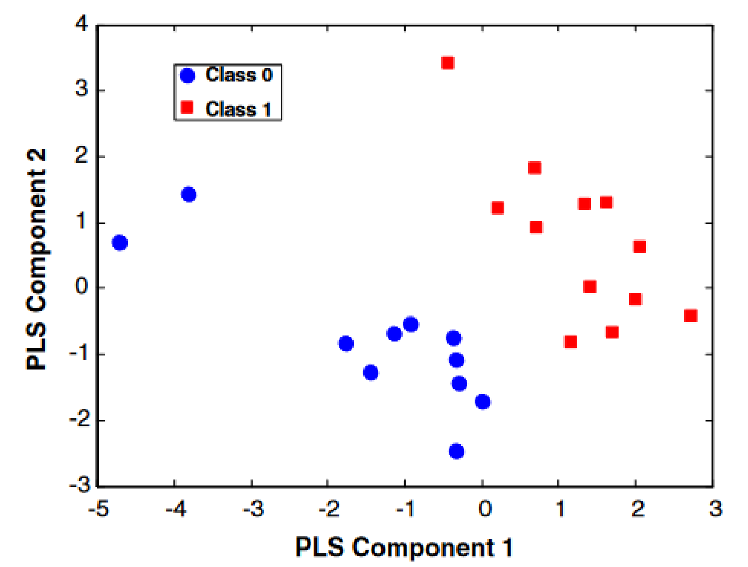

Fig. 1 PLSDA score plot of an NMR data set of healthy volunteers which were arbitrarily divided into two classes. Q^2 value of this model was −0.18. Still a clear separation between the classes is observed in this score plot

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

Materials and Methods

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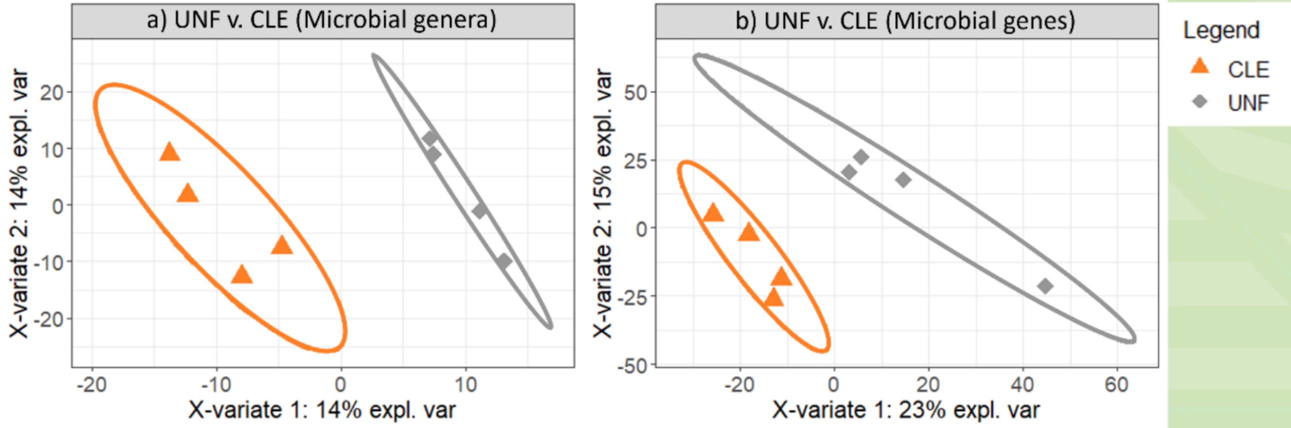

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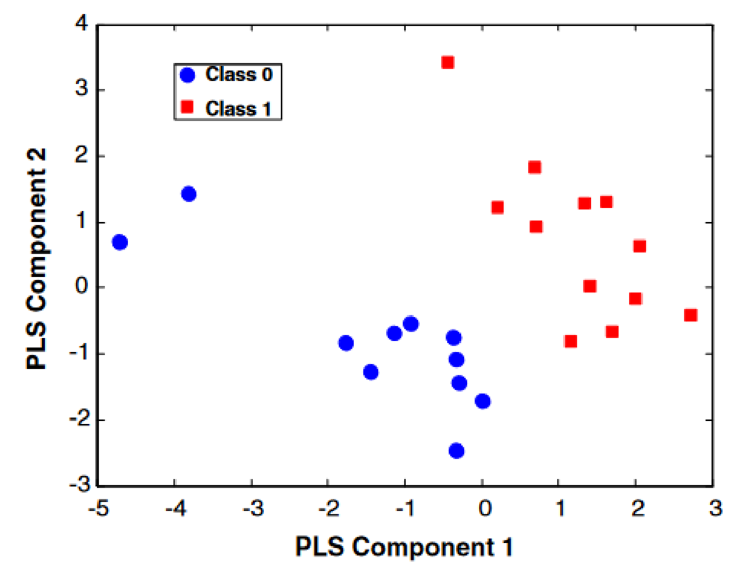

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> *“ **The obvious conclusion from our experiments is that it is a terrible idea to use PLS-DA blindly with all datasets. In spite of its attractive ability to identify features that can separate the classes, it is clear that any data set with sufficiently 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

Materials and Methods

Partial Least Squares Discriminant Analysis

SRUC

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)

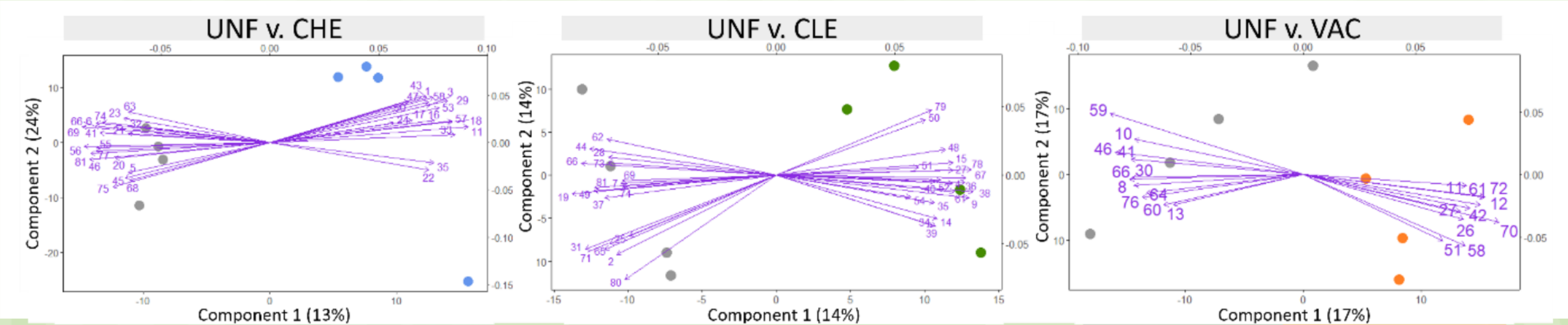
Results
Diversity indices

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

Results

Microbial genera discriminating between treatments

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



5 *Actinomyces*
6 *Aerococcus*
20 *Capnocytophaga*
21 *Cronobacter*
23 *Dehalococcoides*
32 *Filifactor*
41 *Lachnospira*
46 *Macrococcus*
55 *Nannochloropsis*
56 *Nitratifractor*
63 *Rufibacter*
66 *Sphaerochaeta*
68 *Sulfurimonas*
69 *Syntrophomonas*
74 *Thermobacillus*
75 *Thermococcus*
77 *Trichosporon*
81 *Yersinia*

1 *Acetobacter*
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37 *Ignavibacterium*
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70 *Tenacibaculum*
72 *Thalassospira*

Legend
● UNF
● VAC
● CLE
● CHE

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*.

36 microbial genera

38 microbial genera

21 microbial genera

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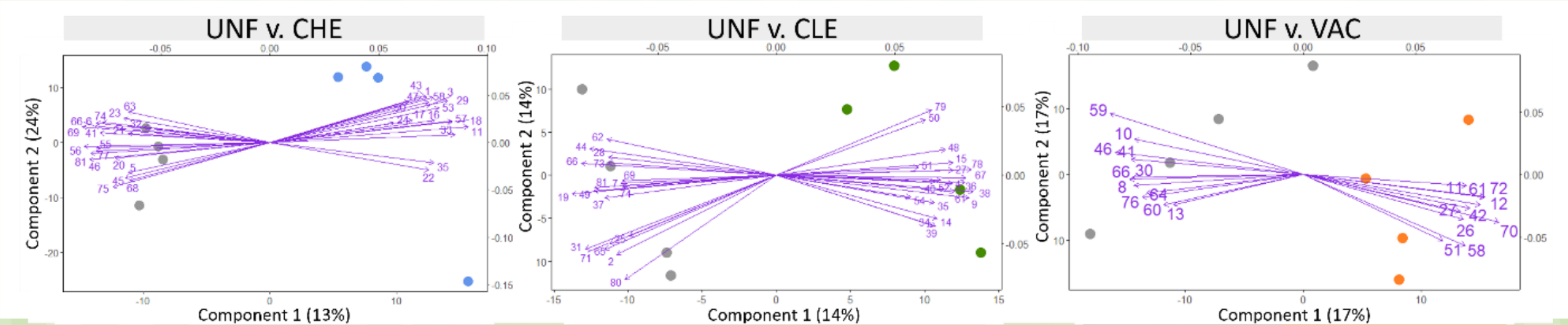

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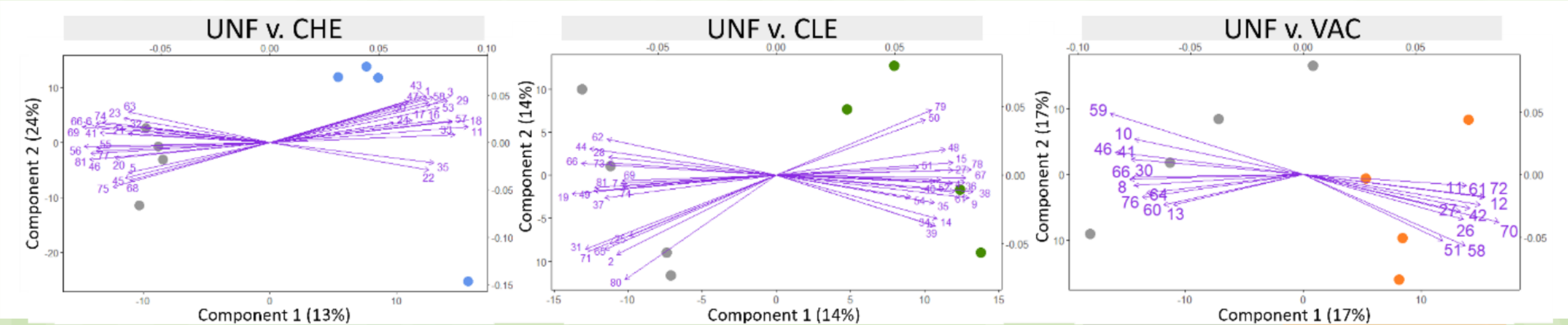

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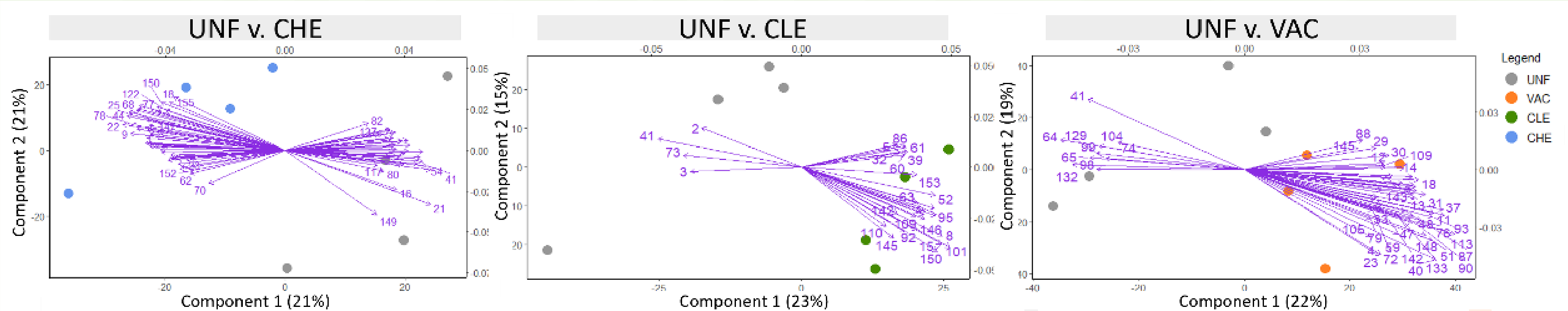
Microbial genes discriminating between treatments



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VAC = vaccinated

4 *vorA*
9 *guaC*
11 *argA*
18 *bioB*
22 *nfo*
23 *rusA*
25 *pip*
28 *adiA*
38 *glyQ*
40 *RTCA*
44 *comFA*
45 *cpaB*
50 *gspD*
51 *gspF*
53 *lpxC*
57 *waaG*
58 *rhaT*
61 *tatC*
62 *yscT*
66 *nrfA*
68 *NAMPT*
70 *tolR*
71 *bfr*
76 *lipA*
77 *dltB*
78 *arcD*
84 *mlaA*
85 *rsbW*
89 *cbpA*
95 *K07014*
100 *phoH2*
101 *K07220*
106 *arnB*
111 *K09131*
118 *arnA*
119 *mutM*
120 *fruB*
122 *ebgA*
123 *cysM*
125 *spnN*
127 *rbbA*
128 *SERPINB*
130 *GDH2*
132 *nrfH*
135 *evaA*
141 *bamB*
144 *cslA*
147 *NPHP3*
148 *mcsA*
150 *lnuA_C_D_E*
151 *moeB*
152 *susE_F*
155 *oleA*

1 *ygeS*
10 *metE*
16 *aroK*
17 *selD*
19 *acpD*
21 *E3.1.11.2*
26 *hslV*
27 *DPYS*
41 *modE*
42 *thiQ*
43 *metQ*
46 *fliD*
49 *glpG*
54 *nifB*
55 *nifD*
56 *nifK*
67 *phnW*
69 *coxS*
75 *moaC*
80 *yiaC*
81 *arsR*
82 *ybaK*
102 *prmB*
103 *xdhC*
107 *mef*
112 *K09766*
117 *aapP*
121 *gck*
124 *ygfK*
126 *ygeT*
131 *ssuB*
134 *psuK*
136 *tcyK*
137 *tcyM*
138 *tcyN*
140 *SUPV3L1*
149 *epsM*
156 *argO*

91 microbial genes

2 *LDHD*
3 *gap2*
41 *modE*
73 *ssh10b*

5 *dapB*
8 *NQO1*
15 *thrB*
32 *dapA*
34 *queD*
36 *GPI*
39 *murE*
52 *K02484*
60 *rpoS*
61 *tatC*
63 *TC.APA*
71 *bfr*
86 *xerD*
92 *ugpC*
95 *K07014*
101 *K07220*
108 *rne*
109 *K08981*
110 *K08999*
116 *cas2*
135 *evaA*
139 *chiG*
142 *ictB*
145 *casA*
146 *casC*
150 *lnuA_C_D_E*
153 *CMAS*

31 microbial genes

41 *modE*
64 *emrE*
65 *TC.NSS*
74 *EGD2*
98 *K07103*
99 *K07158*
104 *TRM61*
129 *SDO1*
132 *nrfH*

4 *vorA*
6 *GLUD1_2*
7 *nuoE*
11 *argA*
12 *nhoA*
13 *mapA*
14 *rhaB*
18 *bioB*
20 *E3.1.4.46*
23 *rusA*
24 *MAN2C1*
29 *hemE*
30 *rhaD*
31 *HMGCL*
33 *ogl*
35 *metB*
37 *rhaA*
40 *RTCA*
47 *gatA*
48 *gatB*
51 *gspF*
59 *secD*
63 *TC.APA*
72 *minE*
76 lipA
79 ppiC
83 glmU
87 mazG
88 FUK
90 dusB
91 mnhF
93 E3.5.1.81
94 *cotJB*
96 *dagK*
97 *E3.1.3.97*
105 *K07486*
109 *K08981*
113 *tamB*
114 *K09946*
115 *lpxI*
133 *ynaI*
142 *ictB*
143 *mdlB*
145 *casA*
146 *casC*
148 *mcsA*
154 *psp*
155 *oleA*

57 microbial genes

Results

Microbial genes discriminating between treatments

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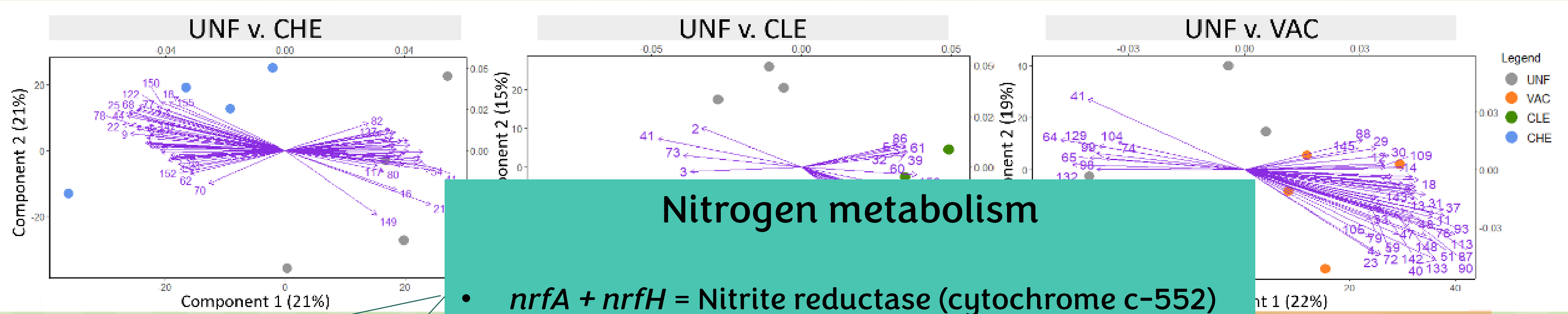
VAC = vaccinated



Nitrogen metabolism

- *nrfA + nrfH* = Nitrite reductase (cytochrome c-552)
- *GDH2* = Glutamate dehydrogenase

- 4 *vorA*
- 9 *guaC*
- 11 *argA*
- 18 *bioB*
- 22 *nfo*
- 23 *rusA*
- 25 *pip*
- 28 *adiA*
- 38 *glyQ*
- 40 *RTCA*
- 44 *comFA*
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- 70 *tolR*
- 71 *bfr*
- 76 *lipA*
- 77 *dltB*
- 78 *arcD*
- 84 *mlaA*
- 85 *rsbW*
- 89 *cbpA*
- 95 *K07014*
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- 101 *K07220*
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- 147 *NPHP3*
- 148 *mcsA*
- 150 *lnuA_C_D_E*
- 151 *moeB*
- 152 *susE_F*
- 155 *oleA*

- 1 *ygeS*
- 10 *metE*
- 16 *aroK*
- 17 *selD*
- 19 *acpD*
- 21 *E3.1.11.2*
- 26 *hslV*
- 27 *DPYS*
- 41 *modE*
- 42 *thiQ*
- 43 *metQ*
- 46 *fliD*
- 49 *glpG*
- 54 *nifB*
- 55 *nifD*
- 56 *nifK*
- 67 *phnW*
- 69 *coxS*
- 75 *moaC*
- 80
- 81
- 82
- 102 *prmB*
- 103 *xdhC*
- 107 *mef*
- 112 *K09766*
- 117 *aapP*
- 121 *gck*
- 124 *ygfK*
- 126 *ygeT*
- 131 *ssuB*
- 134 *psuK*
- 136 *tcyK*
- 137 *tcyM*
- 138 *tcyN*
- 140 *SUPV3L1*
- 149 *epsM*
- 156 *argO*

- 73 *ssh10b*

- 32 *dapA*
- 34 *queD*
- 36 *GPI*
- 39 *murE*
- 52 *K02484*
- 60 *rpoS*
- 61 *tatC*
- 63 *TC.APA*
- 71 *bfr*
- 86 *xerD*
- 92 *ugpC*
- 109 *K08981*
- 110 *K08999*
- 116 *cas2*
- 135 *evaA*
- 139 *chiG*
- 142 *ictB*
- 145 *casA*
- 146 *casC*
- 150 *lnuA_C_D_E*
- 153 *CMAS*

- 74 *EGD2*
- 98 *K07103*
- 99 *K07158*
- 104 *TRM61*
- 129 *SDO1*
- 132 *nrfH*

- 11 *argA*
- 12 *nhoA*
- 13 *mapA*
- 14 *rhaB*
- 18 *bioB*
- 20 *E3.1.4.46*
- 23 *rusA*
- 24 *MAN2C1*
- 29 *hemE*
- 30 *rhaD*
- 31 *HMGCL*
- 33 *ogl*
- 35 *metB*
- 37 *rhaA*
- 40 *RTCA*
- 47 *gatA*
- 48 *gatB*
- 51 *gspF*
- 59 *secD*
- 63 *TC.APA*
- 72 *minE*
- 76 lipA
- 79 ppiC
- 83 glmU
- 87 mazG
- 88 FUK
- 90 dusB
- 91 mnhF
- 93 E3.5.1.81
- 94 *cotJB*
- 96 *dagK*
- 97 *E3.1.3.97*
- 105 *K07486*
- 109 *K08981*
- 113 *tamB*
- 114 *K09946*
- 115 *lpxI*
- 133 *ynaI*
- 142 *ictB*
- 143 *mdlB*
- 145 *casA*
- 146 *casC*
- 148 *mcsA*
- 154 *psp*
- 155 *oleA*

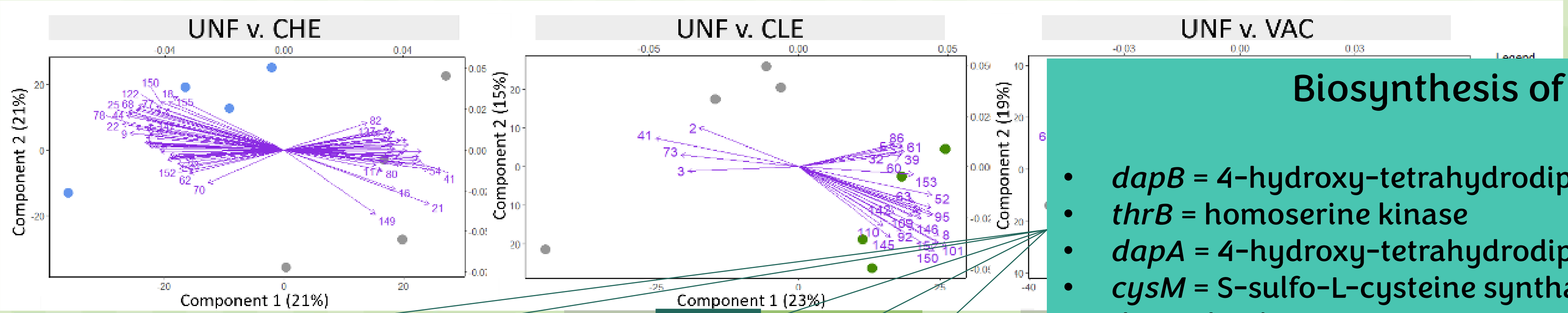
91 microbial genes

31 microbial genes

57 microbial genes

Results

Microbial genes discriminating between treatments



UNF = uninfected

Biosynthesis of amino acids

- *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

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

2 *LDHD*
3 *gap2*
41 *modE*
73 *ssh10b*
5 *dapB*
8 *NQO1*
15 *thrB*
32 *dapA*
34 *queD*
36 *GPI*
39 *murE*
52 *K02484*
60 *rpoS*
61 *tatC*
63 *TC.APA*
71 *bfr*
86 *xerD*
92 *ugpC*
95 *K07014*
101 *K07220*
108 *rne*
109 *K08981*
110 *K08999*
116 *cas2*
135 *evaA*
139 *chiG*
142 *ictB*
145 *casA*
146 *casC*
150 *lnuA_C_D_E*
153 *CMAS*

4
6
6
7
98 *K07103*
99 *K07158*
104 *TRM61*
129 *SDO1*
132 *nrfH*
12 *nhoA*
13 *mapA*
14 *rhaB*
18 *bioB*
20 *E3.1.4.46*
23 *rusA*
24 *MAN2C1*
29 *hemE*
30 *rhaD*
31 *HMGCL*
33 *ogl*
35 *metB*
51 *gspF*
59 *secD*
63 *TC.APA*
72 *minE*
76 lipA
79 ppiC
83 glmU
87 mazG
88 FUK
90 dusB
91 mnhF
93 E3.5.1.81
109 *K08981*
113 *tamB*
114 *K09946*
115 *lpxI*
133 *ynaI*
142 *ictB*
143 *mdlB*
145 *casA*
146 *casC*
148 *mcsA*
154 *psp*
155 *oleA*

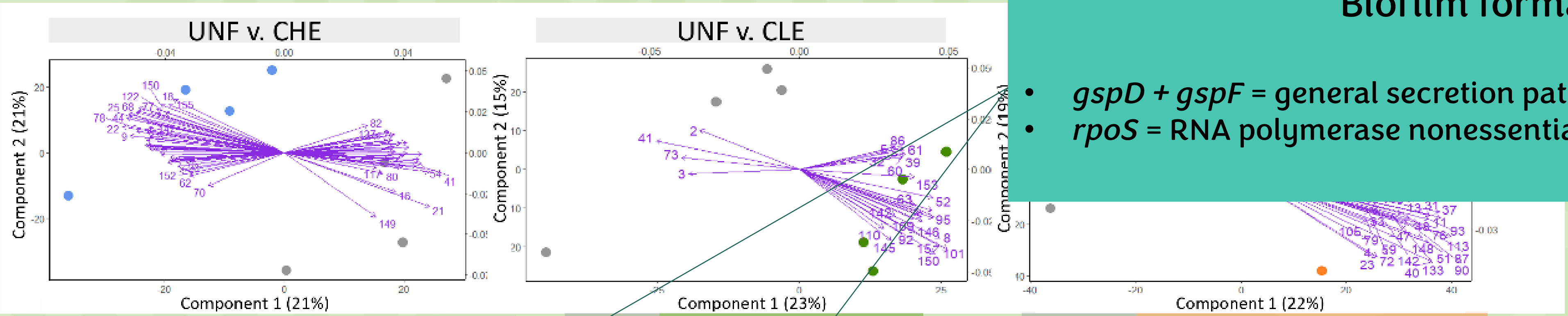
91 microbial genes

31 microbial genes

57 microbial genes

Results

Microbial genes discriminating between treatments



4 *vorA*
9 *guaC*
11 *argA*
18 *bioB*
22 *nfo*
23 *rusA*
25 *pip*
28 *adiA*
38 *glyQ*
40 *RTCA*
44 *comFA*
45 *cpaB*
50 *gspD*
51 *gspF*
53 *lpxC*
57 *waaG*
58 *rhaT*
61 *tatC*
62 *yscT*
66 *nrfA*
68 *NAMPT*
70 *tolR*
71 *bfr*
76 *lipA*
77 *dltB*
78 *arcD*
84 *mlaA*
85 *rsbW*
89 *cbpA*
95 *K07014*
100 *phoH2*
101 *K07220*
106 *arnB*
111 *K09131*
118 *arnA*
119 *mutM*
120 *fruB*
122 *ebgA*
123 *cysM*
125 *spnN*
127 *rbbA*
128 *SERPINB*
130 *GDH2*
132 *nrfH*
135 *evaA*
141 *bamB*
144 *cslA*
147 *NPHP3*
148 *mcsA*
150 *lnuA_C_D_E*
151 *moeB*
152 *susE_F*
155 *oleA*

1 *ygeS*
10 *metE*
16 *aroK*
17 *selD*
19 *acpD*
21 *E3.1.11.2*
26 *hslV*
27 *DPYS*
41 *modE*
42 *thiQ*
43 *metQ*
46 *fliD*
49 *glpG*
54 *nifB*
55 *nifD*
56 *nifK*
67 *phnW*
69 *coxS*
75 *moaC*
80 *yiaC*
81 *arsR*
82 *ybaK*
102 *prmB*
103 *xdhC*
107 *mef*
112 *K09766*
117 *aapP*
121 *gck*
124 *ygfK*
126 *ygeT*
131 *ssuB*
134 *psuK*
136 *tcyK*
137 *tcyM*
138 *tcyN*
140 *SUPV3L1*
149 *epsM*
156 *argO*

2 *LDHD*
3 *gap2*
41 *modE*
73 *ssh10b*

5 *dapB*
8 *NQO1*
15 *thrB*
32 *dapA*
34 *queD*
36 *GPI*
39 *murE*
52 *K0[illegible]484*
60 *rpoS*
61 *tatC*
63 *TC.APA*
71 *bfr*
86 *xerD*
92 *ugpC*
95 *K07014*
101 *K07220*
108 *rne*
109 *K08981*
110 *K08999*
116 *cas2*
135 *evaA*
139 *chiG*
142 *ictB*
145 *casA*
146 *casC*
150 *lnuA_C_D_E*
153 *CMAS*

41 *modE*
64 *emrE*

4 *vorA*
6 *GLUD1_2*
29 *hemE*
30 *rhaD*
31 *HMGCL*
33 *ogl*
35 *metB*
37 *rhaA*
40 *RTCA*
87 mazG
88 FUK
90 dusB
91 mnhF
93 E3.5.1.81
94 *cotJB*
96 *dagK*
145 *casA*
146 *casC*
148 *mcsA*
154 *psp*
155 *oleA*

Biofilm formation

- *gspD + gspF* = general secretion pathway protein D and F
- *rpoS* = RNA polymerase nonessential primary-like sigma factor

Cationic antimicrobial peptide (CAMP) resistance

- *arnA + arnB* = UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate formyltransferase and aminotransferase

91 microbial genes

31 microbial genes

57 microbial genes

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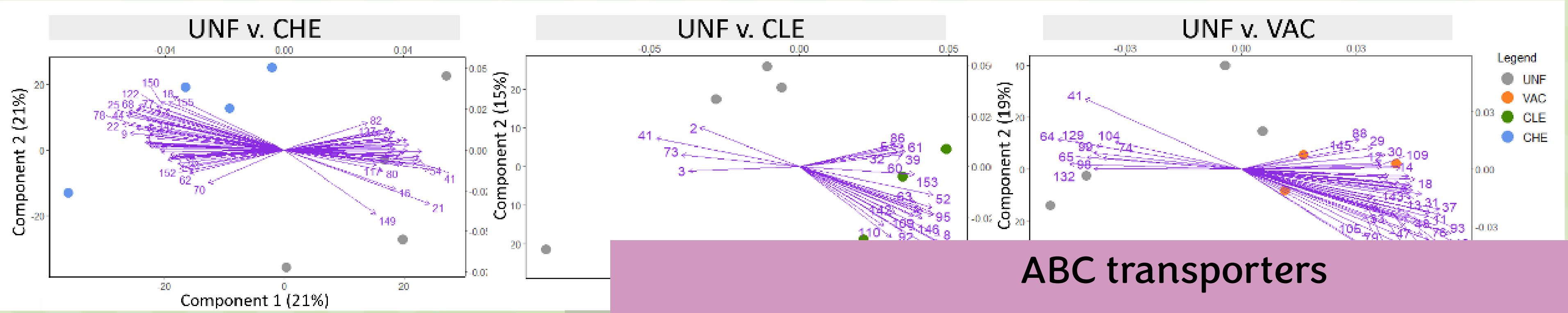
Microbial genes discriminating between treatments



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

4 *vorA*
9 *guaC*
11 *argA*
18 *bioB*
22 *nfo*
23 *rusA*
25 *pip*
28 *adiA*
38 *glyQ*
40 *RTCA*
44 *comFA*
45 *cpaB*
50 *gspD*
51 *gspF*
53 *lpxC*
57 *waaG*
58 *rhaT*
61 *tatC*

62 *yscT*
66 *nrfA*
68 *NAMPT*
70 *tolR*
71 *bfr*
76 *lipA*
77 *dltB*
78 *arcD*
84 *mlaA*
85 *rsbW*
89 *cbpA*
95 *K07014*
100 *phoH2*
101 *K07220*
106 *arnB*
111 *K09131*
118 *arnA*
119 *mutM*

120 *fruB*
122 *ebgA*
123 *cysM*
125 *spnN*
127 *rbbA*
128 *SERPINB*
130 *GDH2*
132 *nrfH*
135 *evaA*
141 *bamB*
144 *cslA*
147 *NPHP3*
148 *mcsA*
150 *lnuA_C_D_E*
151 *moeB*
152 *susE_F*
155 *oleA*

1 *ygeS*
10 *metE*
16 *aroK*
17 *selD*
19 *acpD*
21 *E3.1.11.2*
26 *hslV*
27 *DPYS*
41 *modE*
42 *thiQ*
43 *metQ*
46 *fliD*
49 *glpG*
54 *nifB*
55 *nifD*
56 *nifK*
67 *phnW*
69 *coxS*
75 *moaC*

80 *yiaC*
81 *arsR*
82 *ybaK*
102 *prmB*
103 *xdhC*
107 *mef*
112 *K09766*
117 *aapP*
121 *gck*
124 *ygfK*
126 *ygeT*
131 *ssuB*
134 *psuK*
136 *tcyK*
137 *tcyM*
138 *tcyN*
140 *SUPV3L1*
149 *epsM*
156 *argO*

2 *LD*
3 *ga*
41 *m*
73 *ssl*

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*
35 *metB*

91 mnhF
93 E3.5.1.81

154 *psp*
155 *oleA*

91 microbial genes

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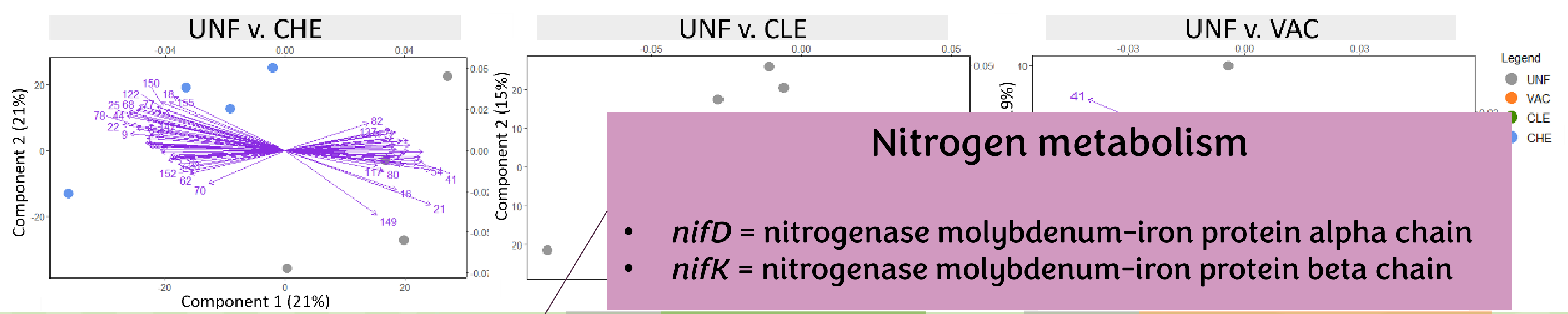
57 microbial genes

Results

Microbial genes discriminating between treatments

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



Nitrogen metabolism

- *nifD* = nitrogenase molybdenum-iron protein alpha chain
- *nifK* = nitrogenase molybdenum-iron protein beta chain

4 *vorA*	62 *yscT*	120 *fruB*	1 *ygeS*	80 *yiaC*
9 *guaC*	66 *nrfA*	122 *ebgA*	10 *metE*	81 *arsR*
11 *argA*	68 *NAMPT*	123 *cysM*	16 *aroK*	82 *ybaK*
18 *bioB*	70 *tolR*	125 *spnN*	17 *selD*	102 *prmB*
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 *modE*	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*

2 *LDHD*	5 *dapB*	95 *K07014*
3 *gap2*	8 *NQO1*	101 *K07220*
41 *modE*	15 *thrB*	108 *rne*

41 *modE*	4 *vorA*	37 *rhaA*	94 *cotJB*
64 *emrE*	6 *GLUD1_2*	40 *RTCA*	96 *dagK*
65 *TC.NSS*	7 *nuoE*	47 *gatA*	97 *E3.1.3.97*
	35 *metB*	93 E3.5.1.81	155 *oleA*

Biosynthesis of amino acids

- *gap2* = glyceraldehyde-3-phosphate dehydrogenase (NAD(P)+) (phosphorylating)
- *metE* = 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
- *aroK* = shikimate kinase
- *argO* = N-acetylglutamate synthase

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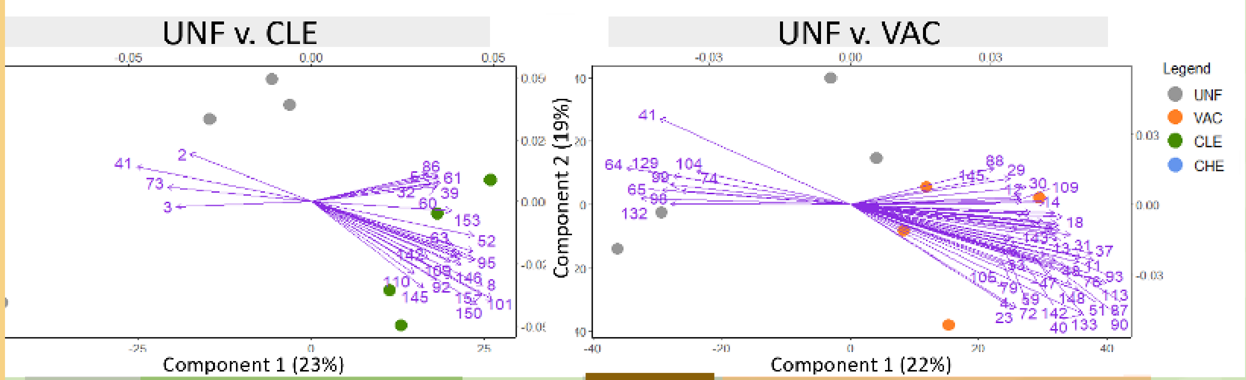
SRUC

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
- K08981 = putative membrane protein

Decreased in CLE and VAC

- *modE* = molybdate transport system regulatory protein



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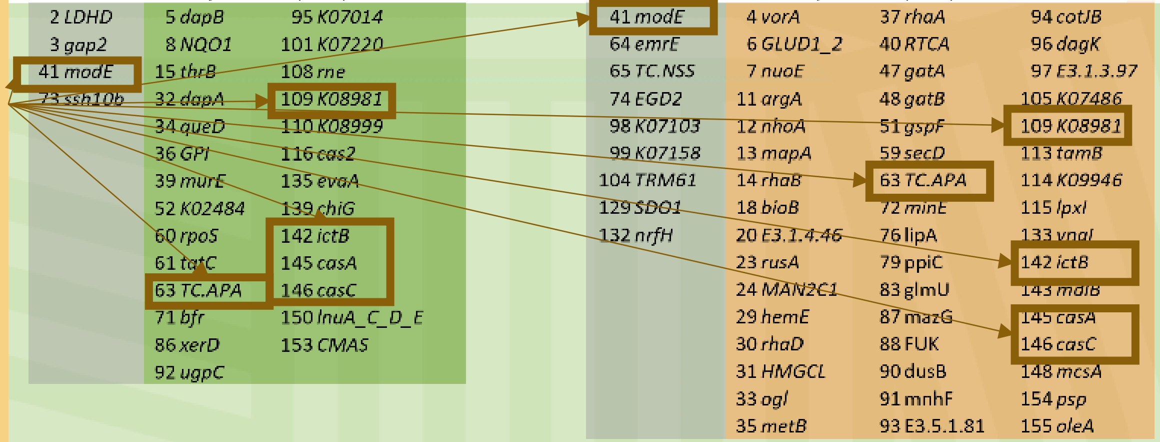
CLE = infected, low cFEC
VAC = vaccinated



2 LDHD
3 gap2
41 modE
73 ssh10b

5 dapB
8 NQO1
15 thrB
32 dapA
34 queD
36 GPI
39 murE
52 K02484
60 rpoS
61 tatC
63 TC.APA
71 bfr
86 xerD
92 ugpC

95 K07014
101 K07220
108 rne
109 K08981
110 K08999
116 cas2
135 evaA
139 chiG
142 ictB
145 casA
146 casC
150 lnuA_C_D_E
153 CMAS

41 modE
64 emrE
65 TC.NSS
74 EGD2
98 K07103
99 K07158
104 TRM61
129 SDO1
132 nrfH

4 vorA
6 GLUD1_2
7 nuoE
11 argA
12 nhoA
13 mapA
14 rhaB
18 bioB
20 E3.1.4.46
23 rusA
24 MAN2C1
29 hemE
30 rhaD
31 HMGCL
33 ogl
35 metB

37 rhaA
40 RTCA
47 gatA
48 gatB
51 gspF
59 secD
63 TC.APA
72 minE
76 lipA
79 ppiC
83 glmU
87 mazG
88 FUK
90 dusB
91 mnhF
93 E3.5.1.81

94 cotJB
96 dagK
97 E3.1.3.97
105 K07486
109 K08981
113 tamB
114 K09946
115 lpxI
133 vnaI
142 ictB
143 malB
145 casA
146 casC
148 mcsA
154 psp
155 oleA

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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

Acknowledgements

SRUC

- Moredun Research Institute
  - Tom McNeilly
  - Philip Steele

- Our team
  - Rainer Roehe
  - Marina Martinez-Alvaro
  - Tuan Nguyen

- Scottish Rural College / University of Edinburgh

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