

1 **Alterations in gut microbiota linked to provenance, sex, and chronic wasting disease in**
2 **white-tailed deer (*Odocoileus virginianus*)**

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11

12 **Abstract**

13 Chronic wasting disease (CWD) is a fatal, contagious, neurodegenerative prion disease affecting
14 both free-ranging and captive cervid species. CWD is spread via direct or indirect contact or oral
15 ingestion of prions. In the gastrointestinal tract, prions enter the body through microfold cells
16 (M-cells), and the abundance of these cells can be influenced by the gut microbiota. To explore
17 potential links between the gut microbiota and CWD, we collected fecal samples from farmed
18 and free-ranging white-tailed deer (*Odocoileus virginianus*) around the Midwest. Farmed deer
19 originated from farms that were depopulated due to CWD. Free-ranging deer were sampled
20 during annual deer harvests. All farmed deer were tested for CWD via ELISA and IHC, and we
21 used 16S rRNA gene sequencing to characterize the gut microbiota. We report significant
22 differences in gut microbiota by provenance (Farm 1, Farm 2, Free-ranging), sex, and CWD
23 status. CWD-positive deer from Farm 1 and 2 had increased abundances of *Akkermansia*,
24 *Lachnospireacea* UCG-010, and RF39 taxa. Overall, differences by provenance and sex appear
25 to be driven by diet, while differences by CWD status may be linked to CWD pathogenesis.

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28

29 **Introduction**

30 Chronic wasting disease (CWD) is a fatal, contagious, neurodegenerative prion disease affecting
31 both free-ranging and captive cervid species, including white-tailed deer (*Odocoileus*
32 *virginianus*), mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus elaphus*), and moose (*Alces*
33 *alces*). First identified in Colorado, USA in the 1960s, CWD was given the designation as a
34 transmissible spongiform encephalopathy (TSE) in 1978 ^{1,2}. Other TSEs include bovine
35 spongiform encephalopathy, transmissible mink encephalopathy, kuru, and variant and sporadic
36 Creutzfeldt-Jakob Disease (CJD) ¹. Since the 1960s, CWD has spread across North America and
37 has been identified in cervids in 26 states ³. Outside of the United States, CWD has been
38 documented in Korea, Canada ¹, and Norway ⁴. Clinical signs of CWD include progressive
39 weight loss, altered posture, head tremors, ataxia, and polydipsia and polyphagia¹.
40 Pathologically, CWD causes spongiform lesions within the central nervous system caused by an
41 abnormal, diseased isoform (PrP^{CWD}) of the normal cellular prion protein (PrP^C). PrP^C is
42 typically composed of multiple alpha-helices, but the abnormal isoform undergoes a
43 transformation into a beta-sheet conformation, making it resistant to proteases, high
44 temperatures, and standard disinfection protocols ¹. The extreme hardiness of the diseased prion,
45 as well as an incubation period ranging from 18 months to 5 years ¹, makes CWD extremely
46 challenging to control and manage.

47
48 CWD is commonly shed in the saliva, urine, feces, and skin and is spread via direct or indirect
49 contact with infectious prions and environmental fomites ⁵. There is evidence that after oral
50 ingestion and passage into the intestinal tract, prions enter the body through microfold cells (M-
51 cells) ^{6,7}. M-cells are specialized cells found in lymphoid follicles, the appendix, mucosal
52 associated lymphoid tissue (MALT), and in the follicle-associated epithelium (FAE) of Peyer's
53 patches in the gut ⁸. M-cells are considered the gatekeeper of the intestine, as they continuously
54 sample and internalize material from the lumen of the intestine via transcytosis to the underlying
55 lymphoid tissue in the Peyer's patch for initiation of mucosal and systemic immune responses <sup>7-
56 9</sup>. Studies in mice have shown that after oral entry of a TSE agent, prions initially accumulate in
57 Peyer's patches and mesenteric lymph nodes in the gut ^{7,10}. Increased M-cell abundance has
58 been linked to an increased susceptibility to orally acquired prion diseases, and the absence of
59 M-cells at the time of oral exposure to infectious prions blocks neuroinvasion and disease

60 development⁶. Importantly, M-cell abundance can be influenced by microbes in the gut as well
61 as by enteric inflammation, and M-cell induction and development has been linked to
62 inflammatory cytokine stimulation and pathogen infection^{11–13}. Further, a 2009 study¹⁴ found
63 that mice with intestinal inflammation as a result of increased levels of *Salmonella* had a
64 significantly higher risk of prion disease. Therefore, increased abundance of M-cells in the gut
65 due to a concurrent inflammation or due to increased levels of specific microbes, such as
66 *Salmonella*^{14,15}, could potentially enhance uptake of prions from the gut lumen¹².

67
68 The gut microbiota serves as a defense system against pathogens and other disease-causing
69 agents¹⁶. Furthermore, the gut microbiome plays an important role in host immune development
70¹⁷, neurogenesis¹⁸, brain development¹⁹, and microglia function in the central nervous system
71 (CNS)^{20,21}. The gut microbiome has also been linked to human neurologic conditions via the
72 “gut-brain axis²¹.” Both Parkinson’s Disease (PD) and Alzheimer’s Disease (AD) have
73 similarities to prion diseases and involve abnormal protein aggregates and protein misfolding
74 occurring in the brain, including a conversion of alpha-helical structures to beta-sheet structures
75 in PD^{22–26}. As a result of these similarities, the “prion hypothesis” suggests that PD is a prion-
76 like disease²⁷. Studies indicate a critical relationship between the gut microbiota and neurologic
77 diseases, including PD, AD, amyotrophic lateral sclerosis (ALS), and autism^{21,28–32}. In a 2016
78 study, alpha synuclein-overexpressing mice (a mouse model for PD) treated with antibiotics had
79 an altered gut microbiota and exhibited reduced brain pathology and motor deficits, identifying
80 direct links between alterations in the gut microbiota and brain pathology associated with PD.
81 Further, microbial colonization of germ-free mice with stool samples from patients with PD
82 resulted in the disease-typical protein-misfolding-mediated motor deficits³¹. Although there is
83 growing evidence for the role of gut microbes in neurologic diseases, there has been very little
84 work examining the role of gut microbiota in prion diseases and no published studies, to our
85 knowledge, on gut microbial communities and chronic wasting disease.

86
87 In this study, we used 16S rRNA gene sequencing to examine the gut microbiota of white-tailed
88 deer (*Odocoileus virginianus*) from two deer farms (breeding facilities) that were depopulated
89 due to the presence of CWD. Additionally, we characterized the gut microbiota of free-ranging
90 white-tailed deer harvested from Cleveland Metroparks in northeast Ohio as part of its deer

91 population management program. Based on previous studies that have reported differences in the
92 gut microbiota of wild and captive ruminants, including deer³³, we hypothesized that microbial
93 communities would differ between deer by provenance (Farm 1, Farm 2, and Free-ranging) with
94 the greatest differences being observed between farmed and free-ranging deer. Based on studies
95 that have reported alterations in gut microbiota associated with neurologic disease, we
96 hypothesized that we would observe differences in farmed-deer gut microbial communities by
97 CWD status (CWD-positive, CWD non-detect).

98

99 **Methods**

100 **Fecal Sample Collection**

101 Per United States Department of Agriculture (USDA) regulations, all deer on Farm 1 (n=101)
102 and Farm 2 (n=30) (Wisconsin, USA) were euthanized after a deer from each farm tested
103 positive for CWD at harvest. Farm 1 was depopulated in May 2018, and Farm 2 was depopulated
104 in May 2019. Post-euthanasia, deer were transported to the Wisconsin Veterinary Diagnostic
105 Laboratory (WVDL) for CWD enzyme-linked immunosorbent assay (ELISA) testing. The
106 WVDL is a National Animal Health Laboratory Network Level 1 laboratory and is accredited by
107 the American Association of Veterinary Laboratory Diagnosticians. Regulatory surveillance
108 samples were shipped to the National Veterinary Services Laboratory (NVSL) for CWD
109 Immunohistochemistry (IHC).

110 Fecal samples were collected digitally from the rectum of all deer and stored on dry ice until they
111 were transferred into a -80°C freezer. Fresh gloves were donned for sampling each deer. Samples
112 remained at -80°C until DNA extraction was performed. All deer carcasses were disposed of
113 after sampling via an alkaline tissue digester at the WVDL. One hundred and one deer were
114 sampled from Farm 1; thirty deer were sampled from Farm 2 (**Table 1**).

115 **Table 1: Farm 1, Farm 2, and Free-Ranging Deer Demographics**

116

Farm 1		
	CWD Positive	CWD Non-Detect
Total Number of Deer	20	81
Age at Depopulation (mean \pm SD)	2.59 \pm 1.02	4.37 \pm 3.43
Sex (n, %)		
Female	1 (1)	35 (35)
Male	19 (19)	46 (45)
Farm 2		
Total Number of Deer	6	24
Age at Depopulation (mean \pm SD)	2.76 \pm 0.39	3.22 \pm 2.71
Sex (n, %)		
Female	0 (0)	12 (40)
Male	6 (20)	12 (40)
Free-Ranging Deer		Total
Total Number of Deer		100
Age at Depopulation (mean \pm SD)		1.70 \pm 1.01
Sex (n, %)		
Female		50 (50)
Male		50 (50)

117

118 One hundred fecal samples were also obtained from free-ranging white-tailed deer harvested in
119 the Cleveland Metroparks (January – March, 2018; **Table 1**), as part of a deer population
120 management program that includes regular CWD testing. Cleveland Metroparks deer herds were
121 tested for CWD in 2008 (125 deer), 2011 (53 deer), 2012 (50 deer), 2016 (277 deer), and 2020
122 (135 deer), and none were found to have detectable CWD. Harvested deer were brought to a
123 central location within four hours of death, and a fecal sample was obtained from the rectum of
124 each deer, placed in a sterile plastic bag, and frozen at -20°C. Samples were transferred into a -
125 80°C freezer within 24 hours of collection where they remained until DNA extraction.

126 Samples from deer on Farm 1 and 2 were collected under USDA APHIS permit #136689. Post-
127 mortem collection of feces was deemed exempt by the IACUC.

128

129 **CWD Sample Collection and Testing**

130 The head was removed from all farmed deer greater than one year of age and the obex region of
131 the brainstem and medial retropharyngeal lymph nodes were collected following USDA APHIS
132 guidelines³⁴. IHC and ELISA-based testing for the abnormal prion protein were performed on
133 the dorsal motor nucleus of the vagus nerve in the obex and medial retropharyngeal lymph

134 nodes. For IHC testing, tissues were preserved in 10% neutral buffered formalin, embedded in
135 paraffin, sectioned at 5 μ m, mounted on slides, and examined using IHC with monoclonal
136 antibody (Mab) F99/97.6.1³⁵. Animals were considered CWD-positive if any one of the tissues
137 examined contained detectable PrP^{CWD}. Animals in which tissues did not contain detectable
138 PrP^{CWD} were considered CWD non-detect animals.

139 **DNA Extraction, Amplification, and Sequencing**

140 DNA extraction on all fecal samples was performed as follows: Approximately 0.25 grams of
141 stool was used for each extraction with QIAamp PowerFecal DNA Kits (Qiagen, Venlo,
142 Netherlands). Following DNA isolation, DNA concentration and purity was measured using a
143 Qubit Fluorometer 4 (Invitrogen, Carlsbad, CA, USA) and a NanoDrop 1000 Spectrophotometer
144 (Thermo Scientific, Waltham, MA, USA), respectively. Ethanol precipitation was performed on
145 all DNA samples from Farm 1 to improve DNA purity using a protocol³⁶ from MRC Holland
146 (Amsterdam, Netherlands). Briefly, 4 μ l of sodium acetate and 132 μ L of 200 proof ethanol was
147 added to 40 μ L of the DNA. This was incubated for 30 minutes at 4°C then centrifuged for 30
148 minutes at 4°C. After removing the supernatant, 250 μ L of 70% ethanol was added to the DNA
149 and centrifuged for 15 minutes. The supernatant was again removed and the DNA pellet was
150 resuspended in 40 μ L of the C6 elution buffer from the PowerFecal (Qiagen) DNA isolation kits.
151 All DNA samples were submitted for library preparation and 16S rRNA gene sequencing on an
152 Illumina MiSeq (Farm 1 and Free-ranging: The Ohio State University Molecular and Cellular
153 Imaging Center; Farm 2 samples: Argonne National Laboratory). Earth Microbiome Project
154 primers (515F and 806R) were used to amplify the V4 hypervariable region of the bacterial 16S
155 rRNA gene³⁷.

156

157 **Sequence Processing and Analysis**

158 A total of 231 samples were submitted for sequencing. Raw, paired-end reads were processed
159 and denoised in QIIME2 v. 2020.2³⁸. Taxonomy was assigned using the SILVA 132 99%
160 amplicon sequence variants (ASVs) database from the 515F/806R classifier^{39,40}, and samples
161 were filtered at a sequencing depth of 10,000 features. This resulted in the retention of 229
162 samples with the loss of 2 samples – one CWD non-detect male deer from Farm 1 and one CWD
163 non-detect female deer from the free-ranging population. After filtering, 5,803,410 reads from

164 229 samples were used for analysis (average of 25,342 reads per sample). Reads per sample
165 ranged from 10,049 to 92,179 reads. Alpha (Shannon Diversity Index) and beta diversity were
166 analyzed using QIIME 2³⁸. Beta diversity indices were compared using permutational
167 multivariate analysis of variances (PERMANOVA) between weighted and unweighted Unifrac
168 distance matrices. P-values were corrected for multiple comparisons using the Benjamini-
169 Hochberg FDR correction, and values less than 0.05 were considered significant. An analysis of
170 composition of microbes (ANCOM) was used to determine differentially abundant taxa between
171 groups after filtering out taxa that had fewer than 10 reads and taxa that occurred in fewer than
172 two deer. We performed ANCOMs at both the L7 and amplicon sequence variant (ASV) levels.
173 The L7 level is roughly equivalent to a species level while an ASV is roughly equivalent to a
174 bacterial strain and may differ from another ASV by as few as one nucleotide⁴¹. Multiple ASVs
175 may be classified as a single L7 level taxa. However, deeper genome sequencing is necessary for
176 true species and strain differentiation as this is not feasible with amplicon sequencing alone. The
177 single CWD-positive female was not included in statistical analyses comparing CWD-positive
178 and CWD non-detect animals to reduce any confounding introduced by sex. Sequencing data is
179 available at NCBI Bioproject PRJNA688284.

180

181 **Results**

182 **Microbial Composition and Diversity by Provenance and Sex**

183 When we examined the gut microbiota of all deer (n=229), we found significant differences in
184 gut microbial composition and diversity by provenance (Farm 1, Farm 2, Free-ranging), with
185 farmed deer having greater microbial diversity than free-ranging deer (Unweighted UniFrac
186 PERMANOVA $p = 0.001$, Shannon Diversity Index $q = 6.5 \times 10^{-11}$, Weighted UniFrac
187 PERMANOVA $p = 0.001$; **Fig. 1a, b, Supp. Fig. 1a**). Moreover, farmed deer from both farms
188 had more similar gut microbiota to each other than to free-ranging deer (Farm 1 to Farm 2
189 pseudo-F = 9, $q = 0.001$; Farm 1 to Free-ranging pseudo-F = 38, $q = 0.001$; Farm 2 to Free-
190 ranging pseudo F = 18, $q = 0.001$; **Fig. 1c**).

191

192 To identify microbial taxa that were differentially abundant between farmed and free-ranging
193 deer, we combined all deer from Farm 1 and 2 – excluding CWD-positive deer – and compared
194 these against the free-ranging deer. Through an ANCOM at the L7 (roughly species) level, we

195 identified 82 taxa that were differentially abundant (**Supp. Table 1**). Twenty-six of these taxa
196 were in the order Bacteroidales (phylum Bacteroidetes) and seven of these were in the family
197 *Prevotellaceae*. The vast majority of the Bacteroidales taxa (22 of 26) were significantly
198 increased in the farmed deer. On the other hand, free-ranging deer had significantly greater
199 abundances of taxa (25 of 38) in the Clostridiales order (phylum Firmicutes), all of which were
200 in the *Ruminococcaceae* and *Lachnospiraceae* families. Based on these results, we decided to
201 compare log Firmicutes:Bacteroidetes (F:B) ratios for farmed and free-ranging deer. Log F:B
202 ratios are associated with dietary energy harvest and higher ratios indicate greater energy
203 extraction⁴²⁻⁴⁴. We found significantly higher F:B ratios in the free-ranging deer as compared to
204 the farmed deer (Log F:B ratios (mean \pm SE), Free-ranging: 0.39 ± 0.03 ; Farmed: 0.08 ± 0.02 ;
205 Kruskal-Wallis $p < 0.0001$).

206
207 We also discovered significant differences in microbial composition but not diversity by sex on
208 Farm 1 and in free-ranging deer (CWD non-detect deer only; Farm 1: Unweighted UniFrac
209 PERMANOVA $p = 0.008$, Shannon Diversity Index $p = 0.34$, Weighted UniFrac PERMANOVA
210 $p = 0.003$; Free-ranging: Unweighted UniFrac PERMANOVA $p = 0.018$, Shannon Diversity
211 Index $p = 0.53$, Weighted UniFrac PERMANOVA $p = 0.066$; **Fig. 2a, b, Supp. Fig. 1b**). No
212 significant differences in microbial composition or diversity were detected by sex on Farm 2
213 (CWD non-detect deer only; Farm 2: Unweighted UniFrac PERMANOVA $p = 0.179$, Shannon
214 Diversity Index $p = 0.15$, Weighted UniFrac PERMANOVA $p = 0.115$; **Fig. 2a, b, Supp. Fig.**
215 **1b**). There were also no differentially abundant microbial taxa detected by sex on Farm 2.
216 However, on Farm 1, we identified a single taxa that was significantly increased in males. This
217 was an uncultured bacterium from the order Bacteroidales, family RF16 (ANCOM, L7 - roughly
218 species level, $W = 626$). In the free-ranging deer population, there were multiple differentially
219 abundant taxa by sex, with the two most differentially abundant including a microbe in the
220 genera *Oscillibacter* and a microbe in the family *Lachnospiraceae*, genera GCA-900066575.
221 Both of these taxa were significantly increased in males (**Supp. Table 2**).

222

223 **Microbial Composition and Diversity by CWD Status**

224 Based on the microbial composition differences observed by sex and the fact that there was only
225 one CWD-positive female in the entire data set, we opted to analyze only male deer in relation to

226 CWD status. The single CWD-positive female deer was still included in data visualizations.
227 Microbial composition differed significantly in CWD-positive deer on both farms (Males only;
228 Farm 1: Unweighted UniFrac PERMANOVA $p = 0.003$ Weighted UniFrac PERMANOVA $p =$
229 0.011 ; Farm 2: Unweighted UniFrac PERMANOVA $p = 0.003$, Weighted UniFrac
230 PERMANOVA $p = 0.002$; **Fig. 1a, Supp. Fig. 1a**). Increased microbial diversity (Shannon
231 Index), although not significant, was also observed in CWD-positive males on both farms (Farm
232 1 $p = 0.07$; Farm 2 $p = 0.26$; **Fig. 2b**).

233
234 We further discovered several differentially abundant microbes at the L7 and ASV levels
235 between CWD-positive and CWD non-detect males on both farms. (Note, multiple ASVs may be
236 classified as a single L7, roughly species level, taxa.) On Farm 1, at the L7 level, multiple taxa
237 were differentially abundant between CWD-positive and CWD non-detect males, the top four of
238 which included: an uncultured bacterium from the class Bacilli (formerly Mollicutes), order
239 RF39, increased in CWD-positive males (ANCOM $W = 80$; **Fig. 3a**); an uncultured *Paludibacter*
240 species increased in non-detect males (ANCOM $W = 54$); an uncultured bacterium in the order
241 Gastranaerophilales also increased in non-detect males (ANCOM $W = 34$); and a microbe in the
242 family *Lachnospiraceae* UCG-10 increased in CWD-positive males (ANCOM $W = 28$; **Fig. 3c**)
243 (**Supp. Table 3**). (ANCOM W values represent the number of times the null hypothesis is
244 rejected in pairwise comparisons of microbial species ratios between groups. In other words, for
245 the Bacilli RF39 L7 level taxa, the null hypothesis was rejected 80 times when comparing
246 microbial species ratios between CWD positive and non-detect animals.)

247
248 On Farm 2, at the L7 level, two microbes were found to be differentially abundant. Both were
249 increased in CWD-positive males and included a unidentified rumen bacterium from the class
250 Bacilli, order RF39 (ANCOM $W = 132$; **Fig. 3b**) and a microbe from the family
251 *Lachnospiraceae* UCG-10 (ANCOM $W = 115$; **Fig. 3c**). On Farm 2, multiple ASVs were also
252 differentially abundant (**Supp. Table 4**), the top three of which, all increased in CWD-positive
253 males, were an ASV from the class Bacilli (formerly Mollicutes), order RF39 (ANCOM $W =$
254 132 ; **Fig. 3e**), an ASV from the family *Lachnospiraceae* UCG-10 (ANCOM $W = 176$; **Fig. 3f**),
255 and an uncultured ASV from the family *Akkermansia* (ANCOM $W = 90$; **Fig. 3g**). On Farm 1, at
256 the ASV level, only one microbe was found to be differentially abundant: an ASV in the

257 *Akkermansia* family which was increased in CWD-positive males (ANCOM $W = 1958$; **Fig. 3h**).
258 *Akkermansia* taxa at the L7 level were not differentially abundant on either farm (**Fig. 3d**).

259

260 **Discussion**

261 In this study, we used 16S rRNA gene sequencing to compare the gut microbiota of farmed and
262 free-ranging white-tailed deer (*Odocoileus virginianus*). We hypothesized that deer gut
263 microbiota would differ by provenance (Farm 1, Farm 2, and Free-ranging) and disease status
264 (CWD-positive, CWD non-detect). Indeed, microbial composition and diversity did vary with
265 provenance. Moreover, composition but not diversity varied with sex (Farm 1 and Free-ranging
266 only) and with CWD status (Farm 1 and Farm 2).

267

268 *Drivers of microbial community composition by provenance*

269 Multiple factors could contribute to the gut microbial differences we observed based on
270 provenance, including diet, spatial proximity, host genetics, and biogeography. Diet is one of the
271 main factors that influences gut microbial composition and diversity^{45,46}. The free-ranging deer
272 in this study had diets that primarily consisted of browse, small plants, shrubs, grasses and
273 occasional agricultural, landscaping, and garden plants⁴⁷. The farmed deer had access to
274 pastures and were also fed a variety of commercial deer feeds, grains, hay, and supplemental
275 items, including peanuts, roasted soybeans, and dandelions. As diets differed between farmed
276 and free-ranging deer, it was not surprising that farmed and free-ranging deer had significantly
277 different microbial communities or that there were significant differences in gut microbiota
278 between the two farms with different feeding regimens. Multiple previous studies have also
279 reported gut microbial differences between wild and captive animals⁴⁸⁻⁵⁰, including
280 ruminants^{33,51}.

281

282 Spatial proximity (or social interaction) has also been shown to influence the gut microbiota in
283 other species: individuals with more contact share more similar gut microbiota⁵². Farmed deer
284 sharing the same pen are likely to have increased direct and indirect contact with each other
285 while free-ranging deer within the same herd (typically matrilineal family groups or bachelor
286 herds) will also have more contact with each other than with non-herdmates. Host genetics can
287 also play a role in shaping the gut microbiome⁵³; although, these effects are subtle compared to

288 other environmental factors⁵⁴, and one previous study on white-tailed deer, albeit small (n=66),
289 did not find any significant effects of host genetic relatedness on the gut microbiota⁴⁶. Both
290 farms in this study were breeding facilities and maintained a number of genetically related
291 animals. During breeding season, a single male was commonly penned with 10-12 females for
292 breeding. Breeders that produced high quality traits might be maintained at the farm for multiple
293 seasons, generating several years of related offspring. Biogeography – including factors like
294 habitat or soil type and water source – could also uniquely influence the gut microbiota of deer at
295 each location⁵⁵.

296

297 We hypothesized that deer gut microbial communities would differ by provenance. Specifically,
298 we predicted that based on differing diets, host genetics, and biogeography, we would observe
299 distinct microbial signatures in deer from each location (Farm 1, Farm 2, Free-ranging) (**Fig. 1a**).
300 We further predicted that within locations, farmed deer would have more similar (less distant)
301 microbiota due to more regulated diets and more limited “home ranges” as compared to free-
302 ranging deer (**Fig 1c**, Farm 1 to Farm 1, Farm 2 to Farm 2, Free-ranging to Free-ranging).
303 Finally, we hypothesized that the greatest differences in microbial communities would be
304 observed between farmed and free-ranging deer since farmed deer generally share more similar
305 diets (formulated commercial feeds, grains, hay, pasture) than free-ranging deer (**Fig. 1c**, Farm 1
306 to Farm 2, Farm 1 to Free-ranging, Farm 2 to Free-ranging). Our results supported each of these
307 predictions. Although we cannot parse the individual effects of diet, spatial proximity, host
308 genetics, and biogeography in this data set, the differentially abundant taxa identified between
309 farmed and free-ranging deer strongly support a role for diet as a key driver of the microbial
310 community differences we observed. Free-ranging deer consume a plant and fiber-rich diet full
311 of shrubs and browse, while farmed deer consume a starchier diet of grains and commercial feed
312 in addition to pasture and hay. Microbial taxa in the *Lachnospiraceae* and *Ruminococcaceae*
313 families were increased in abundance in the free-ranging deer, while Bacteroidales taxa, like
314 *Prevotellaceae*, were increased in the farmed deer (**Supp. Table 1**). *Lachnospiraceae* and
315 *Ruminococcaceae* taxa are associated with plant-rich diets, and these taxa metabolize plant
316 materials such as cellulose and hemicellulose^{50,56,57}. Bacteroidales and *Prevotellaceae* are more
317 commonly associated with starch consumption, and in ruminants, Bacteroidales, including
318 *Prevotella*, increase in animals on concentrate / grain diets^{50,58–60}.

319

320 Firmicutes:Bacteroidetes ratios also indicated diet as a driver of differing microbial compositions
321 between farmed and free-ranging deer. Free-ranging deer exhibited higher F:B ratios, which are
322 associated with increased energy extraction⁴² and fermentation efficiency. In humans, increased
323 F:B ratios are associated with obesity; in farmed ruminants, increased F:B ratios are positively
324 correlated with average daily gain^{42,43}. In foregut-fermenting primates (which have ruminant-
325 like digestion), wild primates exhibited higher F:B ratios than captive primates⁴⁴. This was
326 attributed to the need for the wild primates to maximize energy extraction from “low-quality”
327 food items such as fibrous plants, bark, and seeds, while captive primates, with “high quality”
328 diets rich in soluble carbohydrates, were less dependent on efficient energy harvest⁴⁴. Similarly,
329 free-ranging deer gut microbiota may maximize energy extraction from a fibrous browse diet,
330 while the grain-rich diets of farmed deer reduce the need for fermentation efficiency and create a
331 niche for microbial taxa capable of metabolizing soluble starches and sugars. Taken together, our
332 results suggest that diet is playing a key role in the microbial differences we observe by
333 provenance.

334

335 *Microbial community structure by sex*

336 Interestingly, we also identified microbial composition differences by sex on Farm 1 and in free-
337 ranging deer. This analysis only included CWD non-detect farmed deer. No differences in
338 microbial composition by sex were observed on Farm 2; however, Farm 2 had the smallest
339 sample size (n=18 males, 12 females) which may have limited our power to detect these
340 differences. Microbial community structure alterations associated with sex could be attributed to
341 a number of factors, including differential feeding by sex or hormonal influences on the gut
342 microbiome. We received anecdotal reports of differential feeding by sex on Farm 1 based on
343 deer breeding and growth requirements. While we did not characterize the diet of free-ranging
344 deer by sex in this study, a previous study on wild sheep reported differential feeding between
345 males and females, leading to differences in gut microbiota composition between the sexes⁶¹. A
346 separate study on white-tailed deer reported that, in winter, female deer in the Midwest
347 consumed more grass (higher quality feed) and less browse than male deer⁶². Our samples were
348 also collected from free-ranging deer in the Midwest during winter; thus, differential feeding
349 could contribute to the microbial differences we observed between sexes. Male and female deer

350 also maintain different home ranges⁶³, which can differ in vegetation – further driving potential
351 dietary differences by sex.

352

353 Besides diet, breeding hormones have been linked to gut microbial changes in wild animals,
354 including ground squirrels (*Spermophilus dauricus*)⁶⁴ and black rhino (*Diceros bicornis*)⁶⁵. It is
355 thus possible that hormones are influencing gut microbiota in male and female white-tailed deer.
356 Free-ranging deer were sampled January through March which corresponds to estrous cycling or
357 pregnancy in females and post-rut (declining testosterone levels) in males⁶⁶. Notably, our results
358 contrast with a 2017 study on white-tailed deer that observed no differences in microbial
359 composition between sexes; although, sampling season differed between our studies, as the 2017
360 study sampled deer in March and June⁴⁶.

361

362 Differentially abundant taxa between male and female deer included a microbe in the order
363 Bacteroidales, family RF16 - increased in males on Farm 1; and microbes in the genera GCA-
364 900066575 (family *Lachnospiraceae*) and *Oscillibacter* - both increased in free-ranging males.
365 Bacteroidales and *Lachnospiraceae* taxa, discussed above, have ties to diet and energy
366 extraction. *Oscillibacter* species increase in humans on diets high in resistant starch and low in
367 carbohydrates⁶⁷, which is consistent with the browse-rich winter diet of free-ranging male deer
368⁶². These differentially abundant taxa underscore the role of diet in microbial community
369 differences observed by sex.

370

371 *Chronic wasting disease and the gut microbiota*

372 On both farms, we observed significant differences in microbial composition in CWD-positive
373 deer as compared to non-detect deer. Twenty-five of the 26 total CWD-positive deer across both
374 farms were male. Previous studies in wild deer have reported that CWD prevalence is two times
375 higher in males, and that males have a threefold greater risk of CWD infection as compared to
376 females⁶⁸. These differences in infection risk and prevalence by sex are thought to be linked to
377 increased CWD transmission amongst male social groups outside of breeding season⁶⁸.

378 Alternately, models of CWD outbreaks in captive deer predict that density-dependence and
379 indirect transmission⁶⁹ play an important role in CWD spread. On at least one of the farms in
380 this study (Farm 1), male deer were penned with females during rut (fall) and then separated into

381 bachelor herds for the remainder of the year. As such, both transmission through male social
382 groups and indirect, density-dependent transmission (in bachelor pens) could have played a role
383 in the predominantly male infections observed in farmed deer. Because of this skew by sex, we
384 opted to analyze only males in relation to CWD status. This within-farm, male-only analysis
385 mitigated potential gut microbial confounders, including sex, diet, and biogeography.

386

387 Differentially abundant microbial taxa common across both farms and increased in CWD-
388 positive animals included: two different microbes in the class Bacilli, order RF39 (formerly
389 Mollicutes RF39) – one increased on Farm 1 and one increased on Farm 2; a microbe in the
390 family *Lachnospiraceae* UCG-10; and two different ASVs in the *Akkermansia* family – one
391 increased on Farm 1, and one increased on Farm 2 (**Fig. 3**). The fact that these three taxa (RF39,
392 *Lachnospiraceae* UCG-10, *Akkermansia*) emerged as CWD-associated on two independently run
393 farms over 100 miles apart is intriguing and merits further attention. In a previous study, RF39
394 was found to be increased in a mouse model of the relapse-remitting form of multiple sclerosis
395 (MS) ⁷⁰, which is a disease that shares many features with prion diseases, including CJD ⁷¹. Taxa
396 in the Bacilli (formerly Mollicutes) class have been associated with CWD in other studies ⁷².
397 Specifically, Bastian et al. reported the presence of *Spiroplasma* DNA in the brains of eight out
398 of ten sheep with scrapie, six out of seven cervids with CWD, and two humans with CJD ⁷². All
399 matched normal sheep, cervid, and human brains were negative for *Spiroplasma* DNA. However,
400 no *Spiroplasma* could be detected in a hamster model of scrapie ⁷³. Further, Bastian et al.
401 induced spongiform changes in the brains of deer, sheep, and goats inoculated intracranially with
402 *Spiroplasma* species ⁷⁴. This could not be replicated in a subsequent study on neonatal goats ⁷⁵;
403 although, differences in methodology between the studies was noted. *Spiroplasma* species are
404 not in the order RF39.

405

406 Besides RF39, we also observed an increase in *Lachnospiraceae* UCG-010 in CWD-positive
407 animals on both farms at the L7 level (**Fig. 3b, c**). *Lachnospiraceae* taxa have been reported in
408 other studies on wild and captive deer gut microbiota ^{76,77}. *Lachnospiraceae* has also been noted
409 in association with neurologic diseases. However, it is decreased, rather than increased, in
410 several studies on Parkinson's disease (PD), and this decreased abundance is associated with
411 more severe cognitive and motor impairments ^{78,79}. Decreases in *Lachnospiraceae* have also

412 been observed in Alzheimer’s Disease (AD) and amyotrophic lateral sclerosis (ALS) ^{28,80}.
413 Moreover, multiple studies highlight the ability of Lachnospiraceae species to produce butyrate
414 which helps maintain the epithelial barrier ^{81,82}. However, *Lachnospiraceae* family taxa have
415 also been associated with type 2 diabetes ⁸² and intestinal inflammation⁸³.
416
417 Like *Lachnospiraceae*, *Akkermansia* taxa are commonly associated with health⁸⁴ and even touted
418 as promising probiotics ^{84,85}; although, more recent evidence has promoted caution in defining
419 *Akkermansia* as exclusively a “good bug” ⁸⁶. In fact, the mucin-degrading *Akkermansia* is
420 reportedly increased in multiple neurologic diseases, including PD, multiple sclerosis, and AD
421 ^{32,87–90}, although it has also been shown to reduce pathological alterations (amyloid beta-protein
422 accumulation) and cognitive impairments in one mouse model of AD ⁹¹. *Akkermansia* has
423 additionally been associated with fasting or malnutrition, as it can utilize host mucin as its sole
424 energy source while other microbes require dietary substrates consumed by the host ^{50,92}. A
425 single *Akkermansia* ASV was significantly increased on Farm 1, while a different *Akkermansia*
426 ASV was increased on Farm 2 (**Fig. 3a,b,g,h**), suggesting potential species or strain differences
427 in these taxa by Farm. Future work with deeper sequencing is necessary to assess true species
428 and strain level differences between farms.
429
430 We hypothesized that we would observe differences in gut microbial communities by CWD
431 status, and our results support this hypothesis. However, how and why these three taxa (RF39,
432 *Lachnospiraceae* UCG-010, *Akkermansia*) are associated with CWD are the next important
433 questions to answer. Do these taxa contribute to a gut environment that is more permissive to
434 orally-ingested prions? *Akkermansia*, for example, can degrade mucin, thinning the protective
435 mucus barrier that lines the gut. In concert with a pro-inflammatory *Lachnospiraceae* species,
436 these microbes could create an inflammatory environment that induces colonic M-cells ^{11,14,15,93},
437 enhancing susceptibility to prion disease ^{6,94}. Gut inflammation has also been linked to the
438 progression of neurodegenerative disease including AD and PD ^{30–32}. Alternately, are these taxa
439 increased as a result of prion disease? Early clinical signs of CWD can include behavioral and
440 locomotive changes followed by eventual wasting and weight loss¹. Subtle behavioral changes
441 could conceivably alter diet and drive dietary differences in the gut microbiota between deer with
442 and without CWD. *Akkermansia* can also thrive in the face of malnutrition as it only needs host

443 mucin to survive; therefore, *Akkermansia* could increase in a host that is consuming less food.
444 Finally, could these taxa be providing protective effects in the presence of a prion disease? This
445 seems less likely from an evolutionary perspective, but *Lachnospiraceae* and *Akkermansia* are
446 associated with many health benefits, and increased relative abundances of these species are
447 associated with protection against metabolic diseases and reduced pathological changes in AD
448 ^{28,82,86,91,95}. Bacilli (e.g. RF39 - formerly in phylum Tenericutes, now in Firmicutes) have also
449 been posited to play a protective role in the gut as they are decreased in relative abundance in the
450 presence of DSS-colitis ⁹⁶. It is important to note that all three taxa were also observed in the
451 free-ranging deer at varying and often comparable levels to the levels observed in CWD-positive
452 farmed deer (**Fig. 3**). Given the significant differences in microbial composition and diversity
453 between farmed and free-ranging deer, these results are more challenging to interpret but suggest
454 that, while the differentially abundant taxa in CWD-positive animals may play a role in CWD
455 pathogenesis, these results need to be interpreted carefully and within context.

456
457 This study represents the first investigation, to our knowledge, of white-tailed deer gut
458 microbiota in relation to CWD. We acknowledge several limitations to the present study. First,
459 while our results suggest that differential diets are the major driver of microbial community
460 differences by provenance and sex, we cannot explicitly rule out the potential effects of spatial
461 proximity, host genetics, or biogeography. Second, as farmed and free-ranging deer had
462 significantly different microbial communities, we cannot be certain that microbial composition
463 differences observed in farmed deer based on CWD-status are generalizable to free-ranging deer.
464 Third, the free-ranging deer in this study were not explicitly tested for CWD but were presumed
465 CWD non-detect based on extensive CWD testing on Cleveland Metroparks deer herds in years
466 antecedent and subsequent to 2018. Further, until December 2020, CWD had never been
467 detected in any free-ranging deer in the state of Ohio. Fourth, microbial composition is not
468 representative of microbial function ⁹⁷, and future studies using shotgun metagenomics and
469 metabolomics are warranted to capture function. Fifth, while *Akkermansia*, RF39, and
470 *Lachnospiraceae* UCG-010 are associated with CWD, further work is needed to clarify if these
471 differences preceded or succeeded disease. Finally, fecal samples from Farm 1 and free-ranging
472 deer underwent library preparation and sequencing on an Illumina MiSeq at The Ohio State
473 University Molecular and Cellular Imaging Center, while fecal samples from Farm 2 underwent

474 library preparation and sequencing on an Illumina MiSeq at Argonne National Laboratory. While
475 differences between laboratories and sequencing facilities can lead to differing results in
476 microbiome studies⁹⁸, we limited these effects by using the same methodology and kits (Qiagen
477 PowerFecal) for all DNA extractions, the same region and primers for sequencing (V4 -515F and
478 806R), and all sequencing data was combined and underwent sequence processing and taxonomy
479 assignment together. Further, our results by sex and CWD status would not be affected, as these
480 results were analyzed independently for each location (Farm 1, Farm 2, Free-ranging).

481
482 In conclusion, we report differences in gut microbiota in white-tailed deer by provenance (Farm
483 1, Farm 2, Free-ranging), sex, and CWD status. Differences by provenance and sex are likely
484 driven by diet, while differences by CWD status are more challenging to interpret and include
485 increased abundances of *Akkermansia*, *Lachnospireacea* UCG-010, and RF39 taxa in CWD-
486 positive deer. Priorities for future research include determining how these taxa play a role in CWD
487 susceptibility or pathogenesis, characterizing the gut microbiota of free-ranging cervids with
488 CWD, and assessing M-cell presence and abundance in CWD-positive and CWD non-detect
489 animals to elucidate potential relationships between gut microbiota, M-cells, and chronic wasting
490 disease.

491 492 **Author Contributions**

493 DM collected the samples from the farmed deer, performed DNA extraction on all samples,
494 assisted with data analysis of sequencing results using QIIME 2, and drafted the manuscript.
495 CM assisted with DNA extraction of all samples, DNA purification of Farm 1 samples,
496 coordinated laboratory activities, and assisted with editing of the manuscript.
497 ME assisted with data analysis and processing of sequencing results using QIIME 2 and provided
498 feedback on the manuscript.
499 GB provided expertise on neuropathology in cervids and feedback on the manuscript.
500 DB and KP facilitated CWD testing and sample collection from farmed deer, provided expertise
501 on CWD in cervids, and provided feedback on the manuscript.
502 PD facilitated sample collection from free-ranging deer and provided feedback on the
503 manuscript.

504 VLH conceived the presented idea and designed and directed the study. Additionally, VLH
505 assisted with analysis of sequencing results, manuscript preparation, and figure generation.

506

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513

514 **Conflicts of Interest**

515 No authors report any conflicts of interest.

516

517 **References**

- 518 1. Haley, N. J. & Hoover, E. A. Chronic wasting disease of cervids: current knowledge and
519 future perspectives. *Annual Review of Animal Biosciences* **3**, 305–325 (2015).
- 520 2. Hannaoui, S., Schatzl, H. M. & Gilch, S. Chronic wasting disease: emerging prions and
521 their potential risk. *PLOS Pathogens* **13**, e1006619 (2017).
- 522 3. United States Geological Survey. Expanding distribution of chronic wasting disease.
523 *Expanding Distribution of Chronic Wasting Disease*
524 [https://www.usgs.gov/centers/nwhc/science/expanding-distribution-chronic-wasting-](https://www.usgs.gov/centers/nwhc/science/expanding-distribution-chronic-wasting-disease?qt-science_center_objects=0#qt-science_center_objects)
525 [disease?qt-science_center_objects=0#qt-science_center_objects](https://www.usgs.gov/centers/nwhc/science/expanding-distribution-chronic-wasting-disease?qt-science_center_objects=0#qt-science_center_objects).
- 526 4. Benestad, S. L., Mitchell, G., Simmons, M., Ytrehus, B. & Vikøren, T. First case of chronic
527 wasting disease in Europe in a Norwegian free-ranging reindeer. *Veterinary Research* **47**,
528 (2016).
- 529 5. Gough, K. C. & Maddison, B. C. Prion transmission: prion excretion and occurrence in the
530 environment. *Prion* **4**, 275–282 (2010).

- 531 6. Donaldson, D. S., Sehgal, A., Rios, D., Williams, I. R. & Mabbott, N. A. Increased
532 abundance of m cells in the gut epithelium dramatically enhances oral prion disease
533 susceptibility. *PLOS Pathogens* **12**, e1006075 (2016).
- 534 7. Press, C. M., Heggebø, R. & Espenes, A. Involvement of gut-associated lymphoid tissue of
535 ruminants in the spread of transmissible spongiform encephalopathies. *Adv. Drug Deliv.*
536 *Rev.* **56**, 885–899 (2004).
- 537 8. Corr, S. C., Gahan, C. C. G. M. & Hill, C. M-cells: origin, morphology and role in mucosal
538 immunity and microbial pathogenesis. *FEMS Immunology & Medical Microbiology* **52**, 2–
539 12 (2008).
- 540 9. Mabbott, N. A., Donaldson, D. S., Ohno, H., Williams, I. R. & Mahajan, A. Microfold (M)
541 cells: important immunosurveillance posts in the intestinal epithelium. *Mucosal*
542 *Immunology* **6**, 666–677 (2013).
- 543 10. Maignien, T., Lasmézas, C. I., Beringue, V., Dormont, D. & Deslys, J. P. Pathogenesis of
544 the oral route of infection of mice with scrapie and bovine spongiform encephalopathy
545 agents. *J. Gen. Virol.* **80** (Pt 11), 3035–3042 (1999).
- 546 11. Bennett, K. M. *et al.* Induction of colonic m cells during intestinal inflammation. *The*
547 *American Journal of Pathology* **186**, 1166–1179 (2016).
- 548 12. Donaldson, D. S. & Mabbott, N. A. The influence of the commensal and pathogenic gut
549 microbiota on prion disease pathogenesis. *Journal of General Virology* **97**, 1725–1738
550 (2016).
- 551 13. Terahara, K. *et al.* Comprehensive gene expression profiling of peyer’s patch m cells,
552 villous m-like cells, and intestinal epithelial cells. *The Journal of Immunology* **180**, 7840–
553 7846 (2008).

- 554 14. Sigurdson CJ *et al.* Bacterial colitis increases susceptibility to oral prion disease. *The*
555 *Journal of infectious diseases* **199**, 243–52 (2009).
- 556 15. Tahoun, A. *et al.* Salmonella transforms follicle-associated epithelial cells into m cells to
557 promote intestinal invasion. *Cell Host & Microbe* **12**, 645–656 (2012).
- 558 16. Kamada, N., Chen, G. Y., Inohara, N. & Núñez, G. Control of pathogens and pathobionts
559 by the gut microbiota. *Nature Immunology* **14**, 685–690 (2013).
- 560 17. Round, J. L. & Mazmanian, S. K. The gut microbiota shapes intestinal immune responses
561 during health and disease. *Nature Reviews Immunology* **9**, 313–323 (2009).
- 562 18. Ogbonnaya, E. S. *et al.* Adult hippocampal neurogenesis is regulated by the microbiome.
563 *Biol Psychiatry* **78**, e7-9 (2015).
- 564 19. Diaz Heijtz, R. *et al.* Normal gut microbiota modulates brain development and behavior.
565 *Proc Natl Acad Sci U S A* **108**, 3047–3052 (2011).
- 566 20. Erny, D. *et al.* Host microbiota constantly control maturation and function of microglia in
567 the CNS. *Nature Neuroscience* **18**, 965–977 (2015).
- 568 21. Fung, T. C., Olson, C. A. & Hsiao, E. Y. Interactions between the microbiota, immune and
569 nervous systems in health and disease. *Nat Neurosci* **20**, 145–155 (2017).
- 570 22. Chu, Y. & Kordower, J. H. The prion hypothesis of Parkinson’s disease. *Curr Neurol*
571 *Neurosci Rep* **15**, 28 (2015).
- 572 23. Goedert, M. Alzheimer’s and Parkinson’s diseases: the prion concept in relation to
573 assembled A β , tau, and α -synuclein. *Science* **349**, 1255555 (2015).
- 574 24. Herva, M. E. & Spillantini, M. G. Parkinson’s disease as a member of prion-like disorders.
575 *Virus Res.* **207**, 38–46 (2015).

- 576 25. Tan, J. M. M., Wong, E. S. P. & Lim, K.-L. Protein misfolding and aggregation in
577 Parkinson's disease. *Antioxid. Redox Signal.* **11**, 2119–2134 (2009).
- 578 26. Weickenmeier, J., Jucker, M., Goriely, A. & Kuhl, E. A physics-based model explains the
579 prion-like features of neurodegeneration in Alzheimer's disease, Parkinson's disease, and
580 amyotrophic lateral sclerosis. *Journal of the Mechanics and Physics of Solids* **124**, 264–281
581 (2019).
- 582 27. Olanow, C. W. & Brundin, P. Parkinson's disease and alpha synuclein: is Parkinson's
583 disease a prion-like disorder? *Mov. Disord.* **28**, 31–40 (2013).
- 584 28. D'Argenio, V. & Sarnataro, D. Microbiome influence in the pathogenesis of prion and
585 Alzheimer's diseases. *Int J Mol Sci* **20**, (2019).
- 586 29. Kang, D.-W. *et al.* Microbiota transfer therapy alters gut ecosystem and improves
587 gastrointestinal and autism symptoms: an open-label study. *Microbiome* **5**, 10 (2017).
- 588 30. Rowin, J., Xia, Y., Jung, B. & Sun, J. Gut inflammation and dysbiosis in human motor
589 neuron disease. *Physiol Rep* **5**, (2017).
- 590 31. Sampson, T. R. *et al.* Gut microbiota regulate motor deficits and neuroinflammation in a
591 model of Parkinson's disease. *Cell* **167**, 1469-1480.e12 (2016).
- 592 32. Vogt, N. M. *et al.* Gut microbiome alterations in Alzheimer's disease. *Sci Rep* **7**, 13537
593 (2017).
- 594 33. Guan, Y. *et al.* Comparison of the gut microbiota composition between wild and captive
595 sika deer (*Cervus nippon hortulorum*) from feces by high-throughput sequencing. *AMB*
596 *Express* **7**, 212 (2017).

- 597 34. USDA APHIS | Cervids: Chronic Wasting Disease.
598 <https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease->
599 [information/cervid/cervids-cwd/cervid-cwd](https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/cervid/cervids-cwd/cervid-cwd) (2020).
- 600 35. Keane, D. P. *et al.* Chronic wasting disease in a Wisconsin white-tailed deer farm. *Journal*
601 *of Veterinary Diagnostic Investigation* **20**, 698–703 (2008).
- 602 36. Ethanol precipitation protocol - MRC Holland Technical Support.
603 <https://support.mrcholland.com/kb/articles/ethanol-precipitation-protocol>.
- 604 37. Apprill, A. & Parada, A. E. 16S Illumina amplicon protocol: Earth microbiome project.
605 <http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/>.
- 606 38. Boylen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science
607 using QIIME 2. *Nature Biotechnology* **37**, 852–857 (2019).
- 608 39. Yilmaz, P. *et al.* The SILVA and ‘all-species Living Tree Project (LTP)’ taxonomic
609 frameworks. *Nucleic Acids Research* (2014) doi:10.1093/nar/gkt1209.
- 610 40. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: Improved data
611 processing and web-based tools. *Nucleic Acids Research* (2013) doi:10.1093/nar/gks1219.
- 612 41. Callahan, B. J. *et al.* DADA2: high resolution sample inference from Illumina amplicon
613 data. *Nat Methods* **13**, 581–583 (2016).
- 614 42. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for
615 energy harvest. *Nature* **444**, 1027–1031 (2006).
- 616 43. Min, B. R., Gurung, N., Shange, R. & Solaiman, S. Potential role of rumen microbiota in
617 altering average daily gain and feed efficiency in meat goats fed simple and mixed pastures
618 using bacterial tag-encoded FLX amplicon pyrosequencing. *J Anim Sci* **97**, 3523–3534
619 (2019).

- 620 44. Clayton, J. B. *et al.* Associations between nutrition, gut microbiome, and health in a novel
621 nonhuman primate model. *Scientific Reports* **8**, 11159 (2018).
- 622 45. David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature*
623 **505**, 559–563 (2014).
- 624 46. Delgado, M. L. *et al.* Intestinal microbial community dynamics of white-tailed deer
625 (*Odocoileus virginianus*) in an agroecosystem. *Microbial Ecology* **74**, 496–506 (2017).
- 626 47. Rogers, L. L., Mooty, J. J. & Dawson, D. *Foods of white-tailed deer in the upper Great*
627 *Lakes region: a review*. (North Central Forest Experiment Station, Forest Service, U.S.
628 Dept. of Agriculture, 1981).
- 629 48. Gibson, K. M. *et al.* Gut microbiome differences between wild and captive black rhinoceros
630 – implications for rhino health. *Scientific Reports* **9**, 7570 (2019).
- 631 49. Guo, W. *et al.* Comparative study of gut microbiota in wild and captive giant pandas
632 (*Ailuropoda melanoleuca*). *Genes (Basel)* **10**, (2019).
- 633 50. Hale, V. L. *et al.* Gut microbiota in wild and captive Guizhou snub-nosed monkeys,
634 *Rhinopithecus brelichi*. *American Journal of Primatology* **81**, e22989 (2019).
- 635 51. Prabhu, V. R., Wasimuddin, Kamalakkannan, R., Arjun, M. S. & Nagarajan, M.
636 Consequences of domestication on gut microbiome: a comparative study between wild gaur
637 and domestic mithun. *Front. Microbiol.* **11**, (2020).
- 638 52. Moeller, A. H. *et al.* Social behavior shapes the chimpanzee pan-microbiome. *Science*
639 *Advances* **2**, e1500997 (2016).
- 640 53. Goodrich, J. K. *et al.* Human genetics shape the gut microbiome. *Cell* **159**, 789–799 (2014).
- 641 54. Rothschild, D. *et al.* Environment dominates over host genetics in shaping human gut
642 microbiota. *Nature* **555**, 210–215 (2018).

- 643 55. Loo, W. T., García-Loor, J., Dudaniec, R. Y., Kleindorfer, S. & Cavanaugh, C. M. Host
644 phylogeny, diet, and habitat differentiate the gut microbiomes of Darwin's finches on Santa
645 Cruz Island. *Scientific Reports* **9**, 18781 (2019).
- 646 56. Amato, K. R. *et al.* The gut microbiota appears to compensate for seasonal diet variation in
647 the wild black howler monkey (*Alouatta pigra*). *Microb Ecol* **69**, 434–443 (2015).
- 648 57. Clayton, J. B. *et al.* The gut microbiome of nonhuman primates: lessons in ecology and
649 evolution. *Am J Primatol* **80**, e22867 (2018).
- 650 58. Khafipour, E. *et al.* Effects of grain feeding on microbiota in the digestive tract of cattle.
651 *Anim Fron* **6**, 13–19 (2016).
- 652 59. Liu, C. *et al.* Dynamic alterations in yak rumen bacteria community and metabolome
653 characteristics in response to feed type. *Front. Microbiol.* **10**, (2019).
- 654 60. Wu, G. D. *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science*
655 **334**, 105–108 (2011).
- 656 61. Zhu, Z. *et al.* Seasonal variation and sexual dimorphism of the microbiota in wild blue
657 sheep (*Pseudois nayaur*). *Frontiers in Microbiology* **11**, (2020).
- 658 62. Beier, P. Sex differences in quality of white-tailed deer diets. *Journal of Mammalogy* **68**,
659 323–329 (1987).
- 660 63. Dechen Quinn, A. C., Williams, D. M. & Porter, W. F. Landscape structure influences
661 space use by white-tailed deer. *J Mammal* **94**, 398–407 (2013).
- 662 64. Yang, X. *et al.* Seasonal breeding leads to changes for gut microbiota diversity in the wild
663 ground squirrel (*Spermophilus dauricus*). [https://www.researchsquare.com/article/rs-](https://www.researchsquare.com/article/rs-96089/v1)
664 96089/v1 (2020) doi:10.21203/rs.3.rs-96089/v1.

- 665 65. Antwis, R. E., Edwards, K. L., Unwin, B., Walker, S. L. & Shultz, S. Rare gut microbiota
666 associated with breeding success, hormone metabolites and ovarian cycle phase in the
667 critically endangered eastern black rhino. *Microbiome* **7**, 27 (2019).
- 668 66. Gordon, I. R. *Controlled Reproduction in Horses, Deer, and Camelids*. (Cab International,
669 1997).
- 670 67. Walker, A. W. *et al.* Dominant and diet-responsive groups of bacteria within the human
671 colonic microbiota. *The ISME Journal* **5**, 220–230 (2011).
- 672 68. Samuel, M. D. & Storm, D. J. Chronic wasting disease in white-tailed deer: infection,
673 mortality, and implications for heterogeneous transmission. *Ecology* **97**, 3195–3205 (2016).
- 674 69. Miller, M. W., Hobbs, N. T. & Tavener, S. J. Dynamics of prion disease transmission in
675 mule deer. *Ecological Applications* **16**, 2208–2214 (2006).
- 676 70. Gandy, K. A. O., Zhang, J., Nagarkatti, P. & Nagarkatti, M. The role of gut microbiota in
677 shaping the relapse-remitting and chronic-progressive forms of multiple sclerosis in mouse
678 models. *Scientific Reports* **9**, (2019).
- 679 71. Ebringer, A., Rashid, T., Wilson, C., Boden, R. & Thompson, E. A possible link between
680 multiple sclerosis and Creutzfeldt–Jakob disease based on clinical, genetic, pathological
681 and immunological evidence involving *Acinetobacter* bacteria. *Medical Hypotheses* **64**,
682 487–494 (2005).
- 683 72. Bastian, F. O., Dash, S. & Garry, R. F. Linking chronic wasting disease to scrapie by
684 comparison of *Spiroplasma mirum* ribosomal DNA sequences. *Experimental and*
685 *Molecular Pathology* **77**, 49–56 (2004).
- 686 73. Alexeeva, I. *et al.* Absence of *Spiroplasma* or other bacterial 16S rRNA genes in brain
687 tissue of hamsters with Scrapie. *Journal of Clinical Microbiology* **44**, 91–97 (2006).

- 688 74. Bastian, F. O. *et al.* Spiroplasma spp. from transmissible spongiform encephalopathy brains
689 or ticks induce spongiform encephalopathy in ruminants. *J Med Microbiol* **56**, 1235–1242
690 (2007).
- 691 75. French, H. M. Characterization of Spiroplasma mirum and its role in transmissible
692 spongiform encephalopathies.
- 693 76. Ricci, S. *et al.* Impact of supplemental winter feeding on ruminal microbiota of roe deer
694 Capreolus capreolus. *wbio* **2019**, 1–11 (2019).
- 695 77. Sun, C.-H., Liu, H.-Y., Liu, B., Yuan, B.-D. & Lu, C.-H. Analysis of the gut microbiome of
696 wild and captive Père David’s deer. *Front Microbiol* **10**, (2019).
- 697 78. Barichella, M. *et al.* Unraveling gut microbiota in Parkinson’s disease and atypical
698 parkinsonism. *Movement Disorders* **34**, 396–405 (2019).
- 699 79. Pietrucci, D. *et al.* Dysbiosis of gut microbiota in a selected population of Parkinson’s
700 patients. *Parkinsonism & Related Disorders* **65**, 124–130 (2019).
- 701 80. Radisavljevic, N., Cirstea, M. & Brett Finlay, B. Bottoms up: the role of gut microbiota in
702 brain health. *Environ Microbiol* (2018) doi:10.1111/1462-2920.14506.
- 703 81. Geirnaert, A. *et al.* Butyrate-producing bacteria supplemented in vitro to Crohn’s disease
704 patient microbiota increased butyrate production and enhanced intestinal epithelial barrier
705 integrity. *Scientific Reports* **7**, 11450 (2017).
- 706 82. Vacca, M. *et al.* The controversial role of human gut Lachnospiraceae. *Microorganisms* **8**,
707 (2020).
- 708 83. Zeng, H., Ishaq, S. L., Zhao, F.-Q. & Wright, A.-D. G. Colonic inflammation accompanies
709 an increase of β -catenin signaling and Lachnospiraceae/Streptococcaceae bacteria in the

- 710 hind gut of high-fat diet-fed mice. *The Journal of Nutritional Biochemistry* **35**, 30–36
711 (2016).
- 712 84. Zhang, T., Li, Q., Cheng, L., Buch, H. & Zhang, F. Akkermansia muciniphila is a
713 promising probiotic. *Microb Biotechnol* **12**, 1109–1125 (2019).
- 714 85. Zhou, K. Strategies to promote abundance of Akkermansia muciniphila, an emerging
715 probiotics in the gut, evidence from dietary intervention studies. *J Funct Foods* **33**, 194–
716 201 (2017).
- 717 86. Cirstea, M., Radisavljevic, N. & Finlay, B. B. Good bug, bad bug: breaking through
718 microbial stereotypes. *Cell Host Microbe* **23**, 10–13 (2018).
- 719 87. Berer, K. *et al.* Gut microbiota from multiple sclerosis patients enables spontaneous
720 autoimmune encephalomyelitis in mice. *PNAS* **114**, 10719–10724 (2017).
- 721 88. Cekanaviciute, E. *et al.* Gut bacteria from multiple sclerosis patients modulate human T
722 cells and exacerbate symptoms in mouse models. *PNAS* **114**, 10713–10718 (2017).
- 723 89. Heintz-Buschart, A. *et al.* The nasal and gut microbiome in Parkinson’s disease and
724 idiopathic rapid eye movement sleep behavior disorder. *Mov Disord* **33**, 88–98 (2018).
- 725 90. Hill-Burns, E. M. *et al.* Parkinson’s disease and PD medications have distinct signatures of
726 the gut microbiome. *Mov Disord* **32**, 739–749 (2017).
- 727 91. Ou, Z. *et al.* Protective effects of Akkermansia muciniphila on cognitive deficits and
728 amyloid pathology in a mouse model of Alzheimer’s disease. *Nutrition & Diabetes* **10**, 1–
729 10 (2020).
- 730 92. Belzer, C. & de Vos, W. M. Microbes inside—from diversity to function: the case of
731 Akkermansia. *The ISME Journal* **6**, 1449–1458 (2012).

- 732 93. Ganesh, B. P., Klopfleisch, R., Loh, G. & Blaut, M. Commensal *Akkermansia muciniphila*
733 exacerbates gut inflammation in *Salmonella* Typhimurium-infected gnotobiotic mice. *PLOS*
734 *ONE* **8**, e74963 (2013).
- 735 94. Donaldson, D. S. *et al.* M cell-depletion blocks oral prion disease pathogenesis. *Mucosal*
736 *Immunology* **5**, 216–225 (2012).
- 737 95. Derrien, M., Belzer, C. & de Vos, W. M. *Akkermansia muciniphila* and its role in
738 regulating host functions. *Microbial Pathogenesis* **106**, 171–181 (2017).
- 739 96. Nagalingam, N. A., Kao, J. Y. & Young, V. B. Microbial ecology of the murine gut
740 associated with the development of DSS- colitis. *Inflamm Bowel Dis* **17**, 917–926 (2011).
- 741 97. Huttenhower, C. *et al.* Structure, function and diversity of the healthy human microbiome.
742 *Nature* **486**, 207–214 (2012).
- 743 98. Sinha, R. *et al.* Collecting fecal samples for microbiome analyses in epidemiology studies.
744 *Cancer Epidemiology Biomarkers & Prevention* **25**, 407–416 (2016).
- 745

746 **Figure 1 – Microbial composition and diversity by provenance**

747 a) Microbial composition (Unweighted UniFrac) differed significantly by provenance

748 (PERMANOVA $p = 0.001$). Farm 1 deer are featured in green circles and red diamonds.

749 Farm 2 deer are featured in blue circles and yellow diamonds. Free-ranging deer are

750 featured in purple circles.

751 b) Microbial diversity as measured by the Shannon Diversity Index differed significantly by

752 provenance ($p = 6.5 \times 10^{-11}$). All pairwise comparisons $*p < 0.001$.

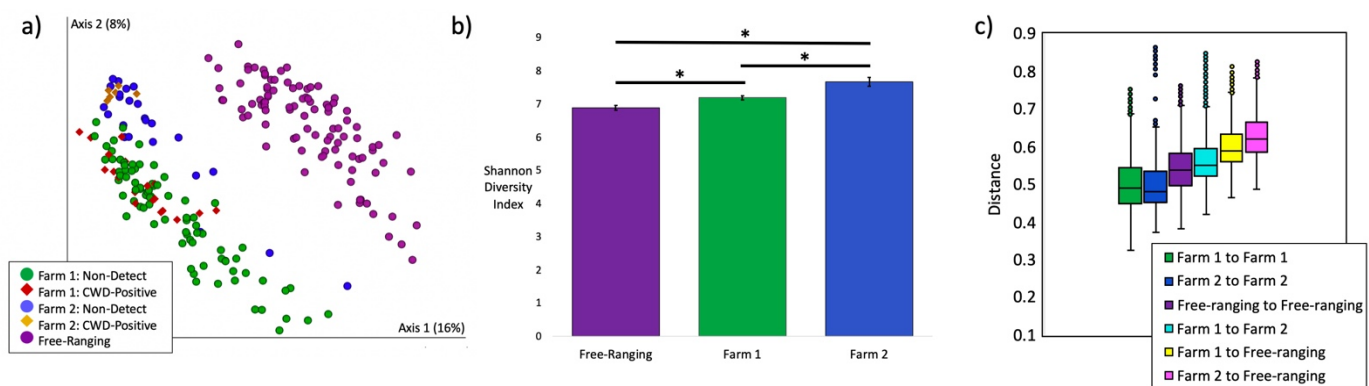
753 c) Farmed deer have more similar microbial communities to each other than to free-ranging

754 deer (Unweighted UniFrac pairwise PERMANOVA: Farm 1 to Farm 2 pseudo-F = 9, $q =$

755 0.001; Farm 1 to Free-ranging pseudo-F = 38, $q = 0.001$; Farm 2 to Free-ranging pseudo

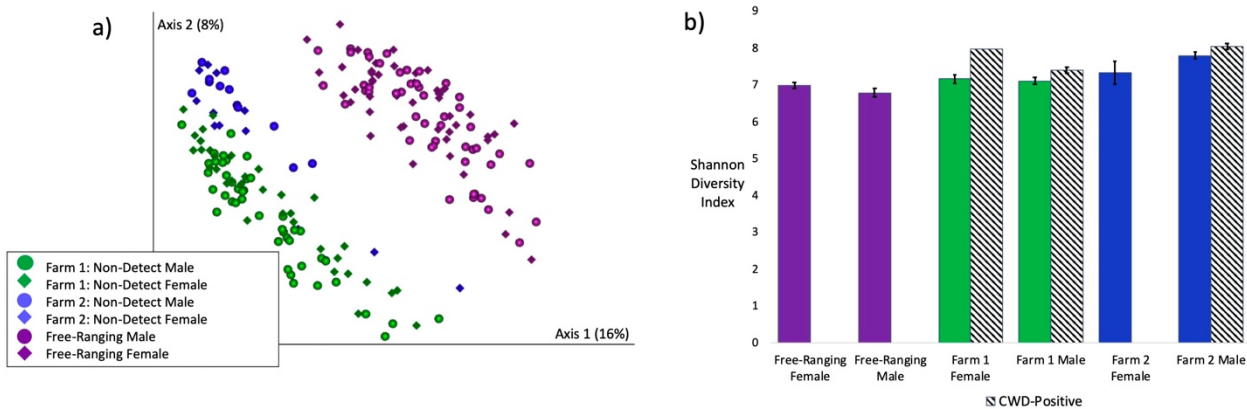
756 F = 18, $q = 0.001$);).

757



758 **Figure 2 – Microbial composition and diversity by sex**

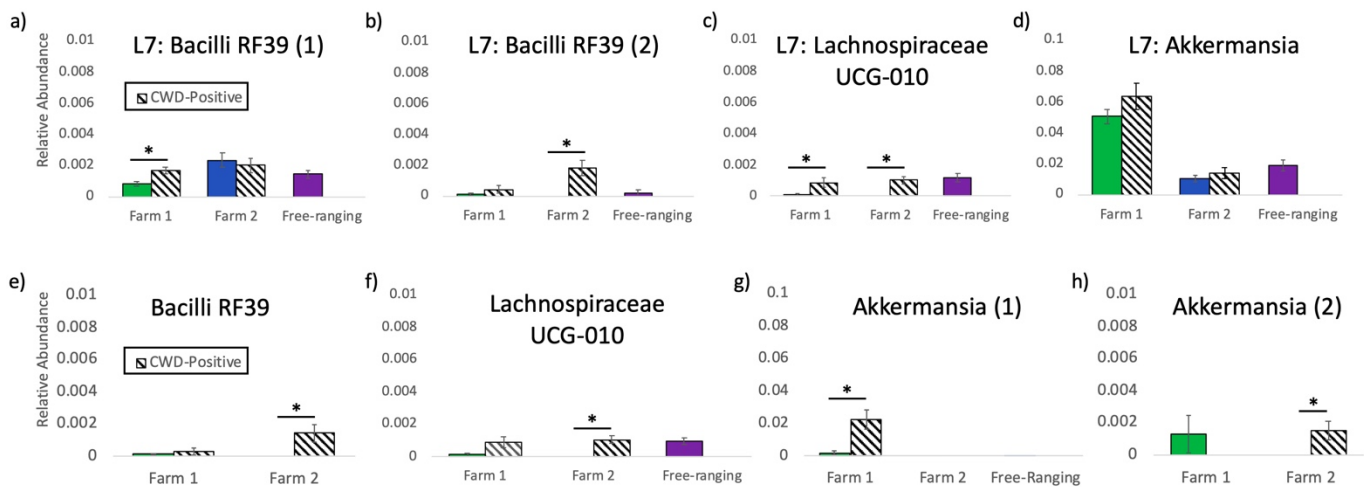
- 759 a) Gut microbial composition (unweighted UniFrac) differed significantly by sex on Farm 1
760 (PERMANOVA $p = 0.008$) and in Free-ranging deer (PERMANOVA $p = 0.018$), but not
761 on Farm 2 (PERMANOVA $p = 0.179$). Farm 1 = green. Farm 2 = blue. Free-ranging =
762 purple. Males = circles. Females = diamonds.
- 763 b) Microbial diversity as measured by the Shannon Diversity Index did not differ
764 significantly by sex (Farm 1: $p = 0.34$, Farm 2: $p = 0.15$, Free-ranging: $p = 0.53$) or CWD
765 status (Farm 1: $p = 0.07$, Farm 2: 0.26).



766

767 **Figure 3 – Differentially abundant microbial taxa by CWD Status**

768 Differentially abundant taxa by CWD Status, including a) an L7 (roughly species) level
769 taxa in the Bacilli class, order RF39, b) a second L7 level taxa in the Bacilli class, order
770 RF39, c) an L7 level taxa in the Lachnospiraceae UCG-10 family, d) an L7 level taxa in
771 the Akkermansia family, e) an ASV (roughly strain level) in the Bacilli class, order RF39
772 (formerly Mollicutes RF39), f) an ASV in the Lachnospiraceae UCG-10 family, g) an
773 ASV in the Akkermansia family, h) a second ASV also in the Akkermansia family. Free-
774 ranging deer (all male) did not contain any reads of the Bacilli RF39 or Akkermansia (2)
775 ASVs.



776 **Supplemental Figure 1 – Microbial composition by provenance and sex:**

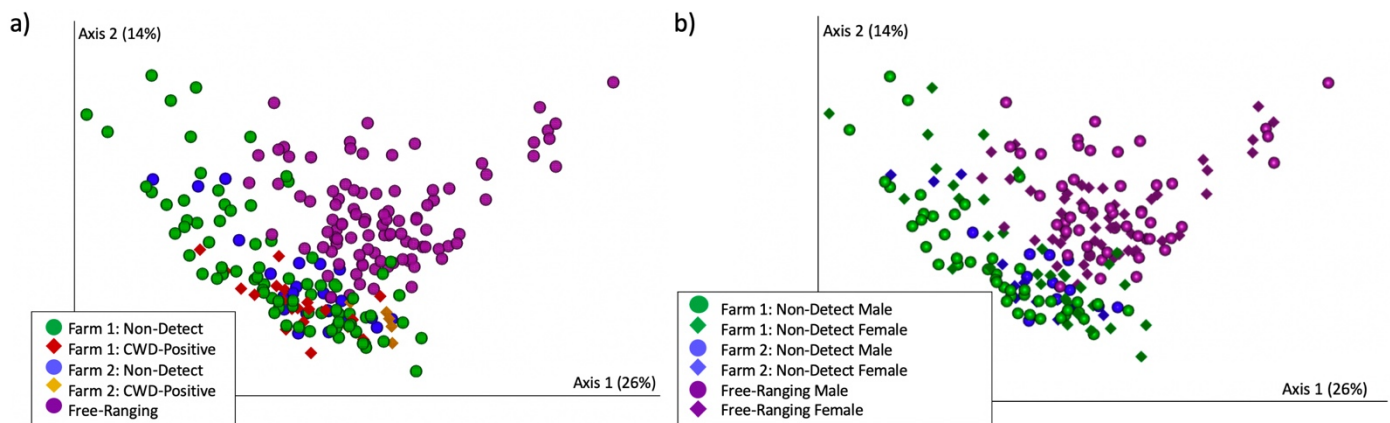
777 a) Microbial composition (weighted UniFrac) differed significantly by provenance
778 (PERMANOVA $p = 0.001$). Farm 1 deer are featured in green circles and red diamonds.

779 Farm 2 deer are featured in blue circles and yellow diamonds. Free-ranging deer are
780 featured in purple circles.

781 b) Gut microbial composition (weighted UniFrac) differed significantly by sex on Farm 1
782 (PERMANOVA $p = 0.003$), trended toward significance in free-ranging deer

783 (PERMANOVA $p = 0.066$), but did not differ significantly on Farm 2 (PERMANOVA p
784 $= 0.115$). Farm 1 = green. Farm 2 = blue. Free-ranging = purple. Males = circles. Females

785 = diamonds



786

Supplemental Table 1: Differentially abundant taxa (ANCOM) between farmed and free-ranging white-tailed deer.

Microbial Taxa	Increased in Free-Ranging	W	Reject null hypothesis
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] oxidoreducens group;D_6_uncultured bacterium	YES	606	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Roseburia;	YES	603	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales RF16 group;	NO	602	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-001;D_6_uncultured bacterium	YES	602	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-007;D_6_uncultured bacterium	YES	602	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteria;D_4_Burkholderiaceae;	YES	602	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-003;D_6_uncultured Bacteroidales bacterium	NO	599	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Acetivomaculum;D_6_uncultured bacterium	YES	594	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Acidaminococcaceae;D_5_Phascoloractobacterium;D_6_uncultured Veillonellaceae bacterium	NO	591	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005;D_6_metagenome	YES	588	TRUE
D_0_Bacteria;D_1_Spirochaetes;D_2_Spirochaetia;D_3_Spirochaetia;D_4_Spirochaetia;D_5_Treponema 2;D_6_uncultured bacterium	NO	588	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Oscillibacter;D_6_uncultured bacterium	YES	587	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae FCS20 group;D_6_uncultured bacterium	YES	586	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Candidatus Stoqueficoccus;D_6_uncultured bacterium	YES	585	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Acidaminococcaceae;D_5_Phascoloractobacterium;D_6_uncultured bacterium	YES	585	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Anaerostipes;D_6_uncultured bacterium	YES	581	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-001;D_6_uncultured Lachnospiraceae bacterium	YES	580	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Marinifilaceae;D_5_Butyricimonas;	NO	572	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales RF16 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	571	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-002;D_6_uncultured bacterium	NO	563	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Bacteroides] pectinophilus group;D_6_uncultured rumen bacterium	YES	562	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobivibrionales;D_4_Desulfobivibrionaceae;D_5_Mailhella;D_6_uncultured bacterium	NO	562	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae Ga6A1 group;D_6_uncultured bacterium	YES	560	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005;D_6_uncultured rumen bacterium	YES	560	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;	YES	559	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmatales;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	556	TRUE
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Bifidobacteriales;D_4_Bifidobacteriaceae;D_5_Aeriscardovia;D_6_uncultured bacterium	NO	555	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Barnesiellaceae;D_5_uncultured;D_6_uncultured Porphyromonadaceae bacterium	NO	555	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Negativibacillus;D_6_uncultured bacterium	NO	552	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-002;	NO	552	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Marvinbryantia;D_6_uncultured bacterium	YES	545	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Barnesiellaceae;	YES	543	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group;D_6_uncultured rumen bacterium	NO	543	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteria;D_4_Burkholderiaceae;D_5_Parasuterella;D_6_uncultured Burkholderiales bacterium	NO	543	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Dorea;D_6_uncultured Lachnospiraceae bacterium	NO	541	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Marvinbryantia;	YES	533	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae NK4A214 group;D_6_uncultured bacterium	YES	529	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Family XIII A03011 group;	NO	522	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_uncultured;	NO	521	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Mogibacterium;D_6_uncultured rumen bacterium	NO	521	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] ruminantium group;D_6_uncultured bacterium	NO	515	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4A136 group;D_6_uncultured Lachnospiraceae bacterium	YES	514	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Alloprevotella;D_6_uncultured Bacteroidales bacterium	NO	513	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005;	YES	512	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Blautia;	YES	506	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_uncultured;D_5_uncultured Bacteroidales bacterium;D_6_uncultured Bacteroidales bacterium	NO	505	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidaceae;D_5_Bacteroides;D_6_uncultured bacterium	NO	503	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Aeromonadales;D_4_Succinivibrionaceae;D_5_Succinivibrio;D_6_uncultured Succinivibrio sp.	NO	500	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Barnesiellaceae;D_5_uncultured;	YES	499	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_GCA-900066575;D_6_uncultured bacterium	YES	499	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Anaerostipes;	YES	496	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-003;	NO	495	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Alistipes;D_6_uncultured Bacteroidetes bacterium	NO	495	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Rikenellaceae RC9 gut group;D_6_uncultured Bacteroidales bacterium	NO	493	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 1;D_6_Ruminococcus flavefaciens	NO	492	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Paludibacteraceae;D_5_uncultured;D_6_uncultured Paludibacter sp.	NO	489	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_EMP-G18;D_4_uncultured Bacilli bacterium;D_5_uncultured Bacilli bacterium;D_6_uncultured Bacilli bacterium	NO	487	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Rikenellaceae RC9 gut group;	NO	485	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Agathobacter;D_6_uncultured bacterium	NO	485	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Marinifilaceae;D_5_Odoribacter;D_6_uncultured Bacteroidales bacterium	NO	484	TRUE

Supplemental Table 1 Continued: Differentially abundant taxa (ANCOM) between farmed and free-ranging white-tailed deer.

Microbial Taxa	Increased in Free-Ranging	W	Reject null hypothesis
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae NK3831 group;D_6_uncultured bacterium	NO	484	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-004;D_6_uncultured bacterium	NO	484	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Enterobacteriales;D_4_Enterobacteriaceae;D_5_Escherichia-Shigella;__	NO	484	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Candidatus Soleaferrea;__	YES	483	TRUE
D_0_Bacteria;D_1_Lentisphaerae;D_2_Lentisphaeria;D_3_Victivallales;D_4_Victivallaceae;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	482	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmatales;D_4_uncultured organism;D_5_uncultured organism;D_6_uncultured organism	YES	480	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-001;D_6_uncultured Bacteroidales bacterium	NO	478	TRUE
D_0_Archaea;D_1_Euryarchaeota;D_2_Methanobacteria;D_3_Methanobacteriales;D_4_Methanobacteriaceae;D_5_Methanobrevibacter;__	NO	476	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_F082;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	474	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_dgA-11 gut group;D_6_uncultured bacterium	NO	474	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidaceae;D_5_Bacteroides;D_6_uncultured Bacteroides sp.	NO	473	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Paludibacteriaceae;D_5_Paludibacter;__	NO	473	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_[Eubacterium] nodatum group;D_6_uncultured bacterium	NO	471	TRUE
D_0_Archaea;D_1_Euryarchaeota;D_2_Methanomicrobia;D_3_Methanomicrobiales;D_4_Methanocorpusculaceae;D_5_Methanocorpusculum;D_6_uncultured methanogenic archaeon	NO	469	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminiclostridium 1;D_6_uncultured rumen bacterium	NO	468	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-010;__	YES	467	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-010;D_6_uncultured bacterium	YES	464	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Aeromonadales;D_4_Succinivibrionaceae;D_5_Succinivibrio;__	YES	463	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Alistipes;D_6_uncultured bacterium	NO	459	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Faecalibacterium;__	YES	457	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_uncultured;__;	YES	457	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4A136 group;D_6_uncultured organism	YES	455	TRUE
Taxa in the Ruminococcaceae and Lachnospiraceae families are highlighted in green. 25 of 38 of these taxa are increased in free-ranging deer.			
Taxa in the Bacteroidales order are highlighted in yellow. 22 of 26 of these taxa are increased in the farmed deer.			
**formerly phylum Tenericutes, class Mollicutes			

Supplemental Table 2: Differentially abundant taxa (ANCOM) by sex in free-ranging white-tailed deer.

Microbial Taxa	Increased in Males?	W	Reject null hypothesis
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Oscillibacter;__	YES	49	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_GCA-900066575;D_6_uncultured bacterium	YES	47	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Aeromonadales;D_4_Succinivibrionaceae;D_5_Succinivibrio;__	YES	13	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_EMP-G18;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	11	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;__;__	YES	7	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_uncultured;D_5_gut metagenome;D_6_gut metagenome	YES	6	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Eubacteriaceae;D_5_Anaerofustis;D_6_uncultured bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Dorea;D_6_uncultured Lachnospiraceae bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-007;D_6_uncultured rumen bacterium	NO	5	TRUE
D_0_Archaea;D_1_Euryarchaeota;D_2_Thermoplasmata;D_3_Methanomassiliicoccales;D_4_Methanomethylphilaceae;__;__	YES	4	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Marinifilaceae;D_5_Sanguibacteroides;D_6_Gabonibacter massiliensis	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4B4 group;__	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured Clostridiaceae bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Acetivibrio] ethanolognignens group;__	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4A136 group;D_6_uncultured rumen bacterium	YES	3	TRUE
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;__;__;__	NO	2	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;__;__;__	YES	2	TRUE
D_0_Bacteria;D_1_Deferribacteres;D_2_Deferribacteres;D_3_Deferribacterales;D_4_Deferribacteraceae;D_5_Mucispirillum;D_6_bacterium 'Lincoln Park 3'	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_bacterium AC2043	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiaceae 1;D_5_Clostridium sensu stricto 1;D_6_human gut metagenome	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Mogibacterium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_uncultured;__	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Acetitomaculum;D_6_uncultured rumen bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coprococcus 2;D_6_uncultured rumen bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Eisenbergiella;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_GCA-900066575;__	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK3A20 group;D_6_uncultured Firmicutes bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-009;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-010;D_6_uncultured organism	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Shuttleworthia;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_uncultured;D_6_uncultured rumen bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group;D_6_uncultured Ruminococcaceae bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Candidatus Soleaferrea;__	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured rumen bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured organism	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Erysipelatoclostridium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Acidaminococcaceae;D_5_Phascolarctobacterium;__	NO	2	TRUE
D_0_Bacteria;D_1_Lentisphaerae;D_2_Lentisphaeria;D_3_Victivallales;D_4_Victivallaceae;D_5_uncultured rumen bacterium;D_6_uncultured rumen bacterium	YES	2	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmatales;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Opitales;D_4_Punicococcaceae;D_5_uncultured;D_6_uncultured bacterium	NO	2	TRUE

**formerly in phylum Tenericutes, class Mollicutes

Supplemental Table 3: Differentially abundant taxa (ANCOM) by CWD Status on Farm 1 at the L7 (roughly species) level.

Microbial Taxa	Increased in CWD-Positive	W	Reject null hypothesis
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_RF39;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	80	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Paludibacteraceae;D_5_uncultured;D_6_uncultured Paludibacter sp.	NO	54	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	34	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-010;	YES	28	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-001;D_6_uncultured Bacteroidales bacterium	NO	20	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Paludibacteraceae;D_5_Paludibacter;	NO	18	TRUE
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria	NO	16	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae;D_6_uncultured bacterium	YES	16	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales;D_5_Clostridium sensu stricto 1;	NO	14	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_RF39;D_4_uncultured Erysipelotrichaceae bacterium;D_5_uncultured Erysipelotrichaceae bacterium;D_6_uncultured Erysipelotrichaceae bacterium	YES	14	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured bacterium	YES	11	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Erysipelotrichales;D_5_Erysipelotrichaceae;D_6_Turicibacter;	YES	9	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_djA-11 gut group;D_6_uncultured bacterium	YES	8	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales;D_5_Bacteroides;D_6_uncultured bacterium	NO	7	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Candidatus Stoquefichus;D_6_uncultured bacterium	NO	7	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Alistipes;	NO	6	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-007;D_6_uncultured bacterium	YES	6	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_uncultured;D_6_uncultured organism	NO	6	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Breznakia;D_6_uncultured bacterium	NO	6	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmatales	NO	6	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_p-251-o5;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae;D_6_uncultured bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-009;D_6_uncultured bacterium	YES	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;	YES	5	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales RF16 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_hoS-07005 gut group;D_6_uncultured Bacteroidales bacterium	YES	4	TRUE
D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Elusimicrobiales;D_4_Elusimicrobiaceae;D_5_Elusimicrobium;	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Lactobacillales;D_4_Streptococcaceae;D_5_Streptococcus;	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_Clostridiales bacterium enrichment culture clone 06-1235251-67;D_6_Clostridiales bacterium enrichment culture clone 06-1235251-67	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4 Family XIII;D_5 Family XIII UCG-001;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group;D_6_uncultured bacterium	YES	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	YES	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014;D_6_uncultured Clostridiales bacterium	YES	4	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales RF16 group;D_5_uncultured Paludibacter sp.;D_6_uncultured Paludibacter sp.	NO	3	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Barnesiellaceae;D_5_Barnesiella;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophilales;D_4_uncultured rumen bacterium;D_5_uncultured rumen bacterium;D_6_uncultured rumen bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_[Eubacterium] ruminantium group;	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Butyrylvibrio;	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Erysipelotrichaceae;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Actinobacteria;D_2_Actinobacteria;D_3_Bifidobacteriales;D_4_Bifidobacteriaceae;D_5_Bifidobacterium;	NO	2	TRUE
D_0_Bacteria;D_1_Actinobacteria;D_2_Coriobacteria;D_3_Coriobacteriales;D_4_Eggerthellaceae;D_5_Slackia;	NO	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Muribaculaceae;D_5_Muribaculum;D_6_Parabacteroides sp. YL27	NO	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotella 1;D_6_unidentified rumen bacterium RFN29	NO	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae Ga6A1 group;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Tannerellaceae;D_5_Parabacteroides;D_6_Parabacteroides goldsteinii ClO2712C30	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_uncultured;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4 Family XIII;D_5_[Eubacterium] nodatum group;D_6_Eubacterium sp. AB3007	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4 Family XIII;D_5 Family XIII AD3011 group;D_6_uncultured Clostridiales bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Anaerocolumna;	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-009;	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Pseudobutyrylvibrio;	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Peptococcaceae;D_5_uncultured;	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014;D_6_gut metagenome	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014;D_6_uncultured rumen bacterium SCD-12	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 1;D_6_gut metagenome	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 1;D_6_uncultured Ruminococcus sp.	NO	2	TRUE
D_0_Bacteria;D_1_Lentisphaerae;D_2_Lentisphaeria;D_3_Victivallales;D_4_Victivallaceae;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Patescibacteria;D_2_Saccharimonadia;D_3_Saccharimonadales;D_4_Saccharimonadaceae;D_5_Candidatus Saccharimonas;D_6_uncultured bacterium	NO	2	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmatales;D_4_gut metagenome;D_5_gut metagenome;D_6_gut metagenome	NO	2	TRUE

YELLOW highlights indicate a taxa similar to a taxa identified as one of the top 5 differentially abundant taxa on Farm 2 and increased or decreased in the same direction as the taxa on Farm 2.

**formerly in phylum Tenericutes, class Mollicutes

Supplemental Table 4: Differentially abundant taxa (ANCOM) by CWD Status on Farm 2 at the ASV (similar to strain) level.

Microbial Taxa	Increased in CWD Positive	W	Reject null hypothesis
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-010	YES	176	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_RF39;D_4_unidentified rumen bacterium RF9;D_5_unidentified rumen bacterium RF9;D_6_unidentified rumen bacterium RF9	YES	132	TRUE
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Verrucomicrobiales;D_4_Akkermansia;D_5_Akkermansia;D_6_uncultured bacterium	YES	90	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae NKA4214 group;D_6_uncultured rumen bacterium	YES	76	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	YES	31	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	22	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Intestinimonas	NO	22	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	22	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_uncultured prokaryote	NO	19	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	YES	17	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	YES	17	TRUE
D_0_Bacteria;D_1_Lentisphaerae;D_2_Lentisphaeria;D_3_Victivallales;D_4_Victivallaceae;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	14	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Defluvitaleaceae;D_5_Defluvitaleaceae UCG-011;D_6_uncultured bacterium	NO	13	TRUE
D_0_Bacteria;D_1_Elusimicrobia;D_2_Elusimicrobia;D_3_Elusimicrobiales;D_4_Elusimicrobiales;D_5_Elusimicrobiales;D_6_uncultured bacterium	NO	11	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	8	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_dga-11 gut group;D_6_uncultured bacterium	YES	7	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales 1	NO	6	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group	YES	6	TRUE
D_0_Bacteria;D_1_Verrucomicrobia;D_2_Verrucomicrobiae;D_3_Ophthalae;D_4_Punicococcaceae;D_5_uncultured;D_6_metagenome	NO	6	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales 1;D_5_Clostridium sensu stricto 1	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coprococcus 2;D_6_uncultured bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group;D_6_uncultured bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	5	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Erysipelotrichaceae UCG-004;D_6_uncultured bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Gammaproteobacteria;D_3_Betaproteobacteria;D_4_Rhodocyclaceae	NO	5	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmales;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	5	TRUE
D_0_Bacteria;D_1_Actinobacteria;D_2_Coriobacteria;D_3_Coriobacteriales;D_4_Atopobacteria;D_5_Atopobium;D_6_uncultured rumen bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Marinifilaceae;D_5_Odoribacter;D_6_uncultured bacterium	YES	4	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainobacteria;D_3_Gastranaerophilales	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_bacterium YE57	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_uncultured prokaryote	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_uncultured rumen bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales 1	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Family XIII AD3011 group;D_6_uncultured Clostridiales Family XIII bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Mogibacterium;D_6_uncultured rumen bacterium 3C0d-10	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Acetivomaculum;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coprococcus 1;D_6_uncultured rumen bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospira;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-008;D_6_uncultured rumen bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Shuttleworthia;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-002	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	YES	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured Clostridiales bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 1	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 2;D_6_uncultured rumen bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Dielma;D_6_uncultured bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Negativicutes;D_3_Selenomonadales;D_4_Acidaminococcaceae;D_5_Phascococcobacterium;D_6_uncultured Veillonellaceae bacterium	NO	4	TRUE
D_0_Bacteria;D_1_Lentisphaerae;D_2_Lentisphaeria;D_3_Victivallales;D_4_Victivallaceae	NO	4	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiaceae;D_5_Aureimonas	NO	4	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Deltaproteobacteria;D_3_Desulfobrivionales;D_4_Desulfobrivionaceae;D_5_Desulfobrivio	NO	4	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotella 1;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Prevotellaceae;D_5_Prevotellaceae UCG-004;D_6_uncultured Bacteroidales bacterium	YES	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales 1	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinBB60 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	3	TRUE

Supplemental Table 4 Continued: Differentially abundant taxa (ANCOM) by CWD Status on Farm 2 at the ASV (similar to strain) level.

Microbial Taxa	Increased in CWD Positive	W	Reject null hypothesis
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured rumen bacterium;D_6_uncultured rumen bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Eubacteriaceae;D_5_Anaerofustis;D_6_uncultured bacterium	YES	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Anaerovorax;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Family XIII UCG-001;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Acetomaculum;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_[Eubacterium] coprostanoligenes group;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Oscillibacter	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-002	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae NK4A214 group;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-002	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005;D_6_uncultured Ruminococcaceae bacterium	YES	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 1	YES	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Saccharofermentans;D_6_uncultured rumen bacterium SCD4	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Brennania;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Erysipelatoclostridium;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_uncultured;D_6_uncultured bacterium	NO	3	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_Rhizobiales;D_5_Alfarhizobium-Neorhizobium-Pararhizobium-Rhizobium	NO	3	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhizobiales;D_4_uncultured;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	3	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmales	NO	3	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_RF39	NO	3	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidaceae;D_5_Bacteroides;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Bacteroidales RF16 group;D_5_uncultured Porphyromonadaceae bacterium;D_6_uncultured Porphyromonadaceae bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Barnesiellaceae;D_5_uncultured	NO	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Munibaculaceae;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Bacteroidetes;D_2_Bacteroidia;D_3_Bacteroidales;D_4_Rikenellaceae;D_5_Alistipes	YES	2	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophiales	NO	2	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophiales	YES	2	TRUE
D_0_Bacteria;D_1_Cyanobacteria;D_2_Melainabacteria;D_3_Gastranaerophiales;D_4_uncultured rumen bacterium;D_5_uncultured rumen bacterium;D_6_uncultured rumen bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_uncultured Clostridia bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Christensenellaceae;D_5_Christensenellaceae R-7 group;D_6_uncultured prokaryote	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Clostridiales vadinB860 group;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Family XIII;D_5_Mogibacterium;D_6_uncultured rumen bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Blautila	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Coprococcus 3;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae AC2044 group;D_6_uncultured rumen bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae NK4A136 group;D_6_uncultured Lachnospiraceae bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Lachnospiraceae UCG-010;D_6_uncultured rumen bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Lachnospiraceae;D_5_Tyzzerella 4;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_GCA-900066225;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Oscillospira;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Papillibacter;D_6_uncultured Clostridiales bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae;D_6_uncultured Clostridium 1	YES	2	TRUE

Supplemental Table 4 Continued: Differentially abundant taxa (ANCOM) by CWD Status on Farm 2 at the ASV (similar to strain) level.

Microbial Taxa	Increased in CWD Positive	W	Reject null hypothesis
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-005;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-010;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-013;D_6_uncultured bacterium	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcaceae UCG-014;D_6_unidentified rumen bacterium JW32	YES	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Ruminococcus 1	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_Saccharofermentans	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Clostridia;D_3_Clostridiales;D_4_Ruminococcaceae;D_5_uncultured	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae	NO	2	TRUE
D_0_Bacteria;D_1_Firmicutes;D_2_Erysipelotrichia;D_3_Erysipelotrichales;D_4_Erysipelotrichaceae;D_5_Candidatus Stoquefichus;D_6_uncultured bacterium	NO	2	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_uncultured;D_5_gut metagenome;D_6_gut metagenome	NO	2	TRUE
D_0_Bacteria;D_1_Proteobacteria;D_2_Alphaproteobacteria;D_3_Rhodospirillales;D_4_uncultured;D_5_uncultured bacterium;D_6_uncultured bacterium	NO	2	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Anaeroplasmatales;D_4_Anaeroplasmataceae;D_5_Anaeroplasmataceae;D_6_uncultured bacterium	YES	2	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_Izimaplasmatales;D_4_uncultured bacterium;D_5_uncultured bacterium;D_6_uncultured bacterium	YES	2	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_RF39	NO	2	TRUE
**D_0_Bacteria;D_1_Firmicutes;D_2_Bacilli;D_3_RF39	NO	2	TRUE
YELLOW highlights indicate a taxa similar to a taxa identified as one of the top 5 differentially abundant taxa on Farm 1 and increased or decreased in the same direction as the taxa on Farm 1.			
**formerly phylum Tenericutes, class Mollicutes			