A cross-sectional study of the nasal and fecal microbiota of sows from different health status within six commercial swine farms

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Background. Cull sows represent a unique population on swine farms, often representing poor producing or compromised animals, and even though recent studies have reported that the microbiome is associated with susceptibility to diseases, the microbiome in the cull sow population has not been explored. The main objective of this study was to investigate whether there were differences in fecal and upper respiratory tract microbiota composition for groups of sows of different health status (healthy, cull, and compromised/ clinical sows) and from different farms (1 to 6). **Methods.** Six swine farms were visited once. Thirty individual fecal samples and nasal swabs were obtained at each farm and pooled by five across health status and farm. Samples underwent 16S rRNA gene amplicon sequencing and nasal and fecal microbiota were analyzed using QIIME2 v.2021.4. **Results.** Overall, the diversity of the nasal microbiota was lower than the fecal microbiota ($p < 0.01$). No significant differences were found in fecal or nasal alpha diversity by sow's health status or by farm. There were significant differences in nasal microbial composition by farm and health status (PERMANOVA, $p < 0.05$), and in fecal microbiota by farm (PERMANOVA, $p < 0.05$), but not by health status. Lastly, at the L7 level, there was one differentially abundant taxa across farms for each nasal and fecal pooled samples. **Discussion.** This study provided baseline information for nasal and fecal microbiota of sows under field conditions, and results suggest that farm of origin can affect microbial diversity and composition. Furthermore, sow's health status may have an impact on the nasal microbiota composition.

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21 ## Abstract

23 **Background.** Cull sows represent a unique population on swine farms, often representing poor

24 producing or compromised animals, and even though recent studies have reported that the

25 microbiome is associated with susceptibility to diseases, the microbiome in the cull sow

26 population has not been explored. The main objective of this study was to investigate whether

27 there were differences in fecal and upper respiratory tract microbiota composition for groups of

28 sows of different health status (healthy, cull, and compromised/ clinical sows) and from different

29 farms (1 to 6).

30 **Methods.** Six swine farms were visited once. Thirty individual fecal samples and nasal swabs

31 were obtained at each farm and pooled by five across health status and farm. Samples underwent

32 16S rRNA gene amplicon sequencing and nasal and fecal microbiota were analyzed using

33 QIIME2 v.2021.4.

34 **Results.** Overall, the diversity of the nasal microbiota was lower than the fecal microbiota ($p <$

35 0.01). No significant differences were found in fecal or nasal alpha diversity by sow's health

36 status or by farm. There were significant differences in nasal microbial composition by farm and

37 health status (PERMANOVA, $p < 0.05$), and in fecal microbiota by farm (PERMANOVA, $p <$

38 0.05), but not by health status. Lastly, at the L7 level, there was one differentially abundant taxa

39 across farms for each nasal and fecal pooled samples.

40 **Discussion.** This study provided baseline information for nasal and fecal microbiota of sows

41 under field conditions, and results suggest that farm of origin can affect microbial diversity and

42 composition. Furthermore, sow's health status may have an impact on the nasal microbiota
43 composition.
44
Introduction
46
47 Culling refers to the process of removing animals from a breeding herd in order to optimize
48 productivity and profitability. This is an essential and common practice conducted on
49 commercial swine herds and it is typical for large systems to cull up to 50% of the herd on an
50 annual basis (D'Allaire, Stein & Leman, 1987; Stein et al., 1990; Stalder et al., 2003). Sows,
51 which refer to reproductively mature females, are normally culled for reasons that include but are
52 not limited to low production efficiency, age, poor reproductive traits, and/ or presumed
53 compromised immune status. However, the definitive underlying reason for culling is often not
54 completely elucidated or recorded. In addition, culls sows may remain on the farm, mixed with
55 the general population, for a significant time period as transportation of mature pigs is
56 logistically challenging. This results in the maintenance of a consistent subpopulation of
57 potentially compromised animals that may serve as a source of pathogens to the general
58 population of healthy sows. Even though recent work has focused on the role of cull sows on
59 disease transmission outside of the farm (i.e. auctions, sale yards etc.) (Sutherland, 2018), little to
60 no work has been devoted to assessing the role of cull sows in the maintenance and re-emergence
61 of pathogens and disease on-farm.
62
63 The microbiome is defined as the microbial community within or on a body site and is known to
64 play an important role in human and animal health (Round & Mazmanian, 2009; Young, 2017;
65 Nowland et al., 2019) and animal production (Jami, White & Mizrahi, 2014). The study of the
66 swine microbiome and its impact in respiratory and systemic diseases has been an emerging area
67 of study within the past few years (Niederwerder, 2017). More specifically, published papers
68 have focused on investigating the associations between the piglet gut or fecal microbiome and
69 intestinal immunity development (Schokker et al., 2014), and performance upon the presence of
70 pathogenic viruses and their co-infections (Niederwerder et al., 2016; Ober et al., 2017). A recent
71 paper has reviewed several publications on piglets' intestinal microbiome and its association
72 with post weaning diarrhea, with a focus on antimicrobial use (Gresse et al., 2017). A few other
73 studies have also described the associations between the piglet nasal microbiota and presence of
74 pathogenic bacteria such as *Staphylococcus sp* and *Haemophilus parasusis* (Weese et al., 2014;
75 Espinosa-Gongora et al., 2016)
76
77 Previous work has explored the impact of health status and geographic location on swine
78 microbiota (Correa-Fiz, Fraile & Aragon, 2016); in addition, recent work conducted under
79 laboratory and semi-experimental conditions has determined that the gut and nasal microbiota
80 are associated with susceptibility to respiratory conditions such as porcine respiratory and
81 reproductive syndrome (PRRS) (Niederwerder et al., 2016), porcine circovirus type 2 associated
82 disease (Niederwerder et al., 2016), and Glasser's disease (Correa-Fiz, Fraile & Aragon, 2016).

83   However, the vast majority of the studies published in the literature focused on piglet
84   populations and did not investigate the effect of health status or farm of origin on the microbiota
85   of sows.
86
87   Therefore, the objectives of this study were to:
88   1) Characterize baseline fecal and nasal microbiota of adult sows under field conditions,
89   2) Investigate the association between health status (including healthy, cull, and compromised/
90   clinical sows) and nasal and fecal microbial composition and diversity. For the purpose of this
91   study, cull sows were defined as an adult female that was identified to be removed from the herd;
92   and compromised or clinical sows were defined as sows presenting acute clinical signs of disease
93   that could be visually assessed by the attending veterinarian.
94   3) Assess the association between farm of origin and nasal and fecal microbial composition and
95   diversity.
96
97   ## Materials & Methods
98
99   *Study population and sampling protocol*
100
101   This study has been approved by The Ohio State University's Institutional Animal Care and Use
102   Committee (IACUC Protocol #2017A00000060), as well as by the Institutional Biosafety
103   Committee (IBC Protocol #2017R00000041).
104
105   A cross-sectional observational study was conducted during July of 2017. The project included
106   enrollment of sows distributed within six privately owned commercial farrow-to-wean swine
107   farms from the same production company located in the U.S. Mideast region. A commercial
108   farm was defined as a swine farm that raised pigs with the intent to commercialize them (in this
109   case of farrow-to-wean farms, the farms were producing and selling weaned piglets to
110   downstream farms that would raise them to market). All sows were fed commercial diets from
111   the same source that met the Nutrient Requirements of Swine (National Research Council,
112   2012). Basic farm descriptors are shown in Table 1. Healthy sows were defined as an adult
113   female with no evidence of clinical signs of disease at the time of data collection. Healthy sows
114   were included regardless of stage of production (i.e. pregnant, lactating or dry). Cull sows were
115   defined as adult females that was identified to be removed from the herd based on the standard
116   operating procedure implemented by the farm staff. Investigators were using sow cards (paper
117   records) in order to identify culled sows. The primary reasons for animals to be culled was if
118   they did not achieve acceptable reproductive performance. Compromised or clinical sows (the
119   two terms are used interchangeably throughout the manuscript) were defined as sows presenting
120   acute clinical signs of disease that could be visually assessed by the attending veterinarian. This
121   included the following categories: lameness, severe shoulder ulcers, active abortion, and poor
122   body condition score. Each farm contributed an equal number of sampled sows for each health

123   status examined herein (healthy, cull and compromised / clinical); with 10 sows sampled for
124   each health status in each farm.
125
126   Farms were visited once during the month of July of 2017 for collection of fecal samples and
127   nasal swabs from all sows within a farm on the same day. Fecal samples were used in this study
128   as a non-invasive way to examine the gut microbiota. A total of 30 fecal samples and 30 nasal
129   swabs were obtained per farm (ten for each type of animal) for a total of 360 samples (180 fecal
130   samples and 180 nasal swabs). A schematic of sampling design is presented in Figure 1, along
131   with relative geographical location for the sampled farms.
132
133   For nasal swab sampling, pigs were restrained using a snare and the swab (Puritan™ PurFlock™
134   Ultra Sterile Flocked Swabs) was inserted into the ventral passage of each nostril. Nasal samples
135   were collected for three seconds (per nasal passage) and placed in a dry tube. Following this, a
136   fecal sample was collected using digital palpation of the rectum to remove feces with a sterile
137   glove. Fecal samples were then placed into sterile bags.
138
139   *Sample pooling, genomic DNA extraction and sequencing*
140
141   Fecal and nasal swabs were collected and placed in tubes on dry ice immediately after collection.
142   Samples were then transported to the Food Animal Health Research Program (The Ohio State
143   University) laboratory in Wooster, Ohio, and transferred into a -80C freezer within 24 hours
144   where they remained until extraction. For nasal swabs, two milliliters of phosphate-buffered
145   saline (PBS) were added to each nasal swab tube, tubes were vortexed for 30 sec. The samples
146   were divided into 2 subsamples: one 1 ml sample was stored at -80 C for future use and the other
147   1 ml sample was centrifuged at 13,000 g for 15 min. The resulting pellet was resuspended in 250
148   μl of PBS and used for DNA extraction. Fecal and nasal swab samples were pooled across 5
149   animals of the same status by farm; so each farm contributed two pools of each health status
150   containing samples of five animals each, as shown in Figure 1. The samples selected to be part of
151   the same pool were randomly selected, and the nasal and fecal pool sets were composed of the
152   same animals. The pools consisted of 0.5 ± 0.015 g of feces per sample and 45 μl of nasal swab
153   suspension per sample. There were 72 pooled samples in total (36 fecal and 36 nasal swab
154   pooled samples) and the pooled sample was considered our unit of analysis. Genomic DNA
155   extraction of the pooled fecal (0.5 ± 0.015 g) or nasal (225 μl) swab samples was conducted
156   using the PureLink Microbiome DNA Purification Kit (Life Technologies, Invitrogen Corp.)
157   followed by RNAse treatment (10 units for 1 hour), as previously described (Deblais et al.,
158   2018). Four blank (empty) extractions were also performed as negative controls and sequenced
159   along with the rest of the samples. It is important to note that this commercial kit lyses the cells
160   using chemical (lysis buffer + enhancer [guanidine isothiocyanate]), thermic (65°C for 10
161   minutes), and mechanical (bead beating at full speed for 10 minutes) approaches. Next

162   generation sequencing library preparations and Illumina MiSeq sequencing were conducted at
163   the Bioscience Division, Los Alamos National Laboratory, NM.

164

165   Briefly, DNA samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA)
166   and DNA quality was checked on a 0.6% agarose gel using E-gel electrophoresis system
167   (ThermoFischer Scientific, Grand Island, NY). A total of 50 ng DNA was used to generate
168   amplicons of the V3-V4 hypervariable regions of the bacterial and Archaeal 16S rRNA gene
169   using primers 341F and 806R. The first round of PCR amplified the V3-V4 region using KAPA
170   HIFI HotStart Ready Mix (Kapa Biosystems, Wilmington, MA) with following PCR conditions;
171   95°C for 3 minutes, 20 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30
172   seconds and an extension of 72°C for 5 minutes. The second round of PCR added the Illumina
173   specific sequencing adapter sequences and unique indexes using the Nextera XT Index Kit v2
174   (Illumina, San Diego, CA) and KAPA HIFI HotStart Ready Mix (Kapa Biosystems, Wilmington,
175   MA) with following PCR conditions; 95°C for 3 minutes, 8 cycles of 95°C for 30 seconds, 55°C
176   for 30 seconds and 72°C for 30 seconds and an extension of 72°C for 5 minutes. The amplicons
177   were cleaned up using AMPure XP beads (Beckman Coulter, Indianapolis, IN). The
178   concentration of the amplicons pool was obtained using the Qubit dsDNA HS Assay
179   (ThermoFisher Scientific, Grand Island, NY). The average size of the library was determined by
180   the Agilent High Sensitivity DNA Kit (Agilent, Santa Clara, CA). An accurate library
181   quantification was determined using the Library Quantification Kit – Illumina/Universal Kit
182   (KAPA Biosystems, Wilmington, MA). The amplicon pool was sequenced on the Illumina
183   MiSeq generating paired end 300 bp reads. The amplicon pool was demultiplexed using
184   Illumina's bcl2fastq. Sequencing data are publicly available at NCBI SRA: PRJNA674564.

185

186   *Taxonomic microbiota analyses and statistical analyses*
187   Raw, single-end sequence reads were processed using QIIME2 v. 2021.4 (Bolyen et al., 2019).
188   The DADA2 plugin was used to truncate reads at 280 bp and to denoise the single-end reads
189   (Callahan et al., 2016). We opted for single end reads over paired end due to the poor quality of
190   the R2 reads. Taxonomy was assigned in QIIME2 using SILVA version 138.1, with a 99%
191   similarity threshold using the full length 16S rRNA gene classifier (Quast et al., 2013; Yilmaz et
192   al., 2014). All fecal samples with fewer than 5600 reads and all nasal samples with fewer than
193   500 reads were removed from analyses. To compare alpha diversity between nasal and fecal
194   samples, all samples were rarefied at 500 reads. We also rarefied at 500 reads to compare nasal
195   samples by farm and health status. To compare fecal samples by farm and health status, we
196   rarified at 5600 reads. In total, we retained all fecal samples (n=36) and 19 out of 36 nasal
197   samples for further analysis. To assess the effects of rarefaction, we also analyzed fecal and nasal
198   samples without rarefaction (excluding fecal samples with fewer than 5600 reads and nasal
199   samples with fewer than 500 reads). We also analyzed nasal samples (rarefied and non-rarefied)
200   excluding samples with fewer than 1000 reads.

201

202   Alpha diversity was analyzed using Shannon and Faith's Phylogenetic Diversity (PD) indices. A
203   Kruskal-Wallis test was used to compare alpha diversity and read numbers by health status and
204   farm. An adjusted P-value lower than 0.05 was considered significant.

205

206   Beta diversity indices were compared using permutational multivariate analysis of variances
207   (PERMANOVA) and permutational analysis of multivariate dispersions (PERMDISP) between
208   Bray Curtis distance matrices. P-values were corrected for multiple comparisons using the
209   Benjamini-Hochberg FDR correction, and values less than 0.05 were considered significant.
210   PERMANOVAs were performed on the fecal and nasal samples by farm and by health status. An
211   analysis of composition of microbes (ANCOM) was used to identify differentially abundant
212   nasal and fecal taxa by farm and health status. An ANCOM performs pairwise comparisons of
213   microbial species ratios and generates a W-value, which indicates how many times a null
214   hypothesis is rejected across comparisons. The maximum W value in any data set is limited by
215   the total number of microbial taxa within that data set. For all samples, we filtered out taxa that
216   had fewer than 10 reads and occurred in fewer than two samples prior to ANCOM analysis. No
217   additional filtering was performed on nasal samples due to the low microbial diversity in the
218   majority of samples. ANCOMs were performed at the L7 level, which is roughly equivalent to a
219   species level; although deeper genome sequencing is necessary for true species differentiation.
220
221   **Results**
222
223   One hundred and eighty animals were sampled across the six farms; comprising of a total of 60
224   healthy sows, 60 cull sows, and 60 compromised / clinical sows. Reasons for sows to be in the
225   'compromised' or 'clinical' group included low body condition score (48.3%), acute abortion
226   (23.3%) and shoulder ulcer (15.0%). For the 'cull' group, the main reason for culling was low
227   performance (which included low number of weaned pigs, high pre-weaning mortality, and low
228   number of born alive piglets; 50.0%), unknown (missing information on the sow card; 28.3%),
229   and return to estrus (10.0%). A breakdown for most common culling reasons by farm is provided
230   on Table 1. The mean parity for compromised/ clinicalanimals was 2.9 (SD= 2.0); for cull sows,
231   5.2 (1.9), and for heathy sows, 3.0 (2.1). A breakdown for parity distribution by farm is provided
232   on Table 1.
233
234   *16S rRNA Gene Amplicon Sequencing*
235   We obtained a total of 1,759,761 reads with a mean read length of 294 base pairs (Supplemental
236   Table 1). Of the four negative controls, only one yielded any reads (3 reads total), and all 3 reads
237   were identified as chloroplasts. Chloroplasts, mitochondria, and eukaryotic reads were then
238   removed bioinformatically prior to further analyses. A total of 493 taxa were identified across all
239   samples. The mean number of reads per nasal swab pool (n=36) was 2,572; the median was
240   914.5, with a range of 1-27,870 reads (Supplemental Table 2). Nasal samples with fewer than
241   500 reads (n=17) were excluded from analysis. For the 36 pooled fecal samples, we obtained a
242   total of 901,440 reads. The mean number of reads per fecal pooled sample was 25,040, and the
243   median was 22,256, with a range of 5,694-62,677 reads (Supplemental Table 3). All fecal
244   samples were included in our analyses, which were performed rareified at 5600 reads and

245 unrarified. To facilitate a direct comparison between nasal and fecal samples, we also performed
246 a separate analysis with fecal and nasal samples together rarefied at 500 reads and unrarefied.
247
248 *Alpha diversity results*
249 Overall, nasal samples harbored a significantly lower microbial diversity compared to the fecal
250 samples (Faith's PD, p < 0.001; Shannon, p < 0.001; Supplemental Figure 1a). No significant
251 differences in fecal or nasal diversity (Shannon index) were detected by health status (healthy,
252 cull and compromised / clinical) or by farm (Kruskal-Wallis, all p >0.05, Figure 2, Supplemental
253 Figure 1b, Supplemental Table 4).
254
255 *Beta diversity results*
256 There were significant differences in overall nasal microbial composition by farm (Bray-Curtils
257 PERMANOVA, pseudo-F = 1.83, p = 0.002, Figure 3a, Supplementary Figure 2a, Supplemental
258 Table 4); although, no pairwise comparisons were significant. Overall nasal microbial
259 composition by health status also differed significantly (PERMANOVA, pseudo-F = 1.64, p =
260 0.017, Figure 3b, Supplementary Figure 2b) with healthy versus cull as the only significant
261 pairwise comparison (p = 0.033). These differences were not explained by dispersion
262 (PERMDISP by farm: pseudo-F = 0.577, p = 0.795; by health status: pseudo-F = 2.11, p = 0.148)
263 or number of reads by farm or health status (Kruskal-Wallis by farm: p = 0.36; by health status: p
264 = 0.28).When we examined the pooled fecal microbiota of sows, we found significant
265 differences in overall microbial composition by farm (PERMANOVA, pseudo-F = 3.16, p =
266 0.001, Figure 4a, Supplementary Figure 3a, Supplemental Table 4) with significant pairwise
267 differences between all farms (p < 0.05) with the exception of farms 4 and 5 (p = 0.08) and farms
268 5 and 6 (p = 0.1). No significant differences in pooled fecal microbial composition were found
269 based on health status (p = 0.663, Figure 4b, Supplementary Figure 3b).
270
271 In terms of relative abundances, the most abundant phyla in the pooled nasal samples was
272 Proteobacteria, followed by Firmicutes, Actinobacteria and Bacteroidetes (Figure 5a). The most
273 abundant nasal genera across all pooled samples was *Moraxella*. At the L7 level, we identified
274 one differentially abundant nasal taxa by farm: a taxa in the Micrococcaeae family, genera
275 *Nesterenkonia* (ANCOM, W = 32), which was only present on farm 6 (Supplemental Table 5).
276 No nasal taxa were differentially abundant by health status. In terms of relative abundance in the
277 pooled fecal samples, Firmicutes was the most abundant phyla, followed by Bacteroidetes,
278 Euryarchaeotes, Spirochetes, Actinobacteria and Proteobacteria (Figure 5b). Within the
279 Firmicutes phyla, the most abundant family was Lachnospiraceae, and the most abundant genera
280 across all fecal samples were *Lactobacillus* and *Methanobrevibacter* (an Archaea). At the L7
281 (roughly species) level in fecal samples, we identified one differentially abundant taxa by farm:
282 it was a taxa in the order Bacteroidales, genera F082 (ANCOM, W = 140), which was broadly
283 present at low abundances across all samples from farms 1, 4, 5 and 6 but almost entirely absent

284   on farms 2 and 3 (Supplemental Table 6). No fecal taxa were differentially abundant by health
285   status.
286
Discussion
287
288
289   Cull sows are important from both a disease transmission and welfare perspective but are an
290   understudied subpopulation of animals within commercial swine farms. Studies looking at both
291   fecal and nasal microbiota of adult female pigs are lacking in the literature. Therefore, the main
292   objective of this study was to characterize nasal and fecal microbiota of sows that differed by
293   health status and farm of origin under field conditions.
294
295   Our study did not show statistical differences in nasal microbial alpha diversity by health status,
296   or by farm. Few studies have examined swine nasal microbial diversity and reports vary: one
297   study reports significantly decreased diversity linked to Glasser's disease (Correa-Fiz, Fraile &
298   Aragon, 2016) while another reports no change in diversity linked to methicillin-resistant
299   *Staphylococcus aureus* (MRSA) carriage (Weese et al., 2014). A few studies have also reported
300   changes in nasal microbiota upon use of antimicrobial treatments, all conducted in young
301   animals (which are different from our population of interest). A study conducted in Spain
302   reported the use of peri-natal treatment with antimicrobials (including penicillin, streptomycin
303   and tulathromycin in one farm, and ceftiofur and tulathromycin in a second farm) in 3-4 week
304   old piglets to be associated with a decrease in nasal microbiota diversity as compared to a period
305   without using these peri-natal treatments. (Correa-Fiz et al., 2019). Likewise, a study conducted
306   in the US showed that oxytetracycline administration reduced nasal microbiota diversity in 3-
307   week-old piglets (Mou et al., 2019). In this study, on-farm antimicrobial treatments were not
308   available to investigators, which did not allow for further investigations. Additionally, the only
309   disease issue reported amongst all six farms in the month prior to sampling was a recent swine
310   influenza outbreak on farm 1. Despite this, we observed no differences in microbial diversity by
311   farm or health status. All farms have reported to not have had outbreaks for the main pathogens
312   of importance in swine including porcine reproductive and respiratory syndrome, *Mycoplasma*
313   *sp.* and porcine epidemic diarrhea.
314
315   Nasal microbial composition differed both by farm and by health status; and fecal microbial
316   composition differed by farm (all except 4 and 5 and 5 and 6). The differences observed between
317   farms could also not be explained by their geographical location (Figure 1). Of note, both farm
318   size (number of sows) and parity distribution are higher for farm 5, which could have been
319   contributors for the observed trends in microbiota composition.
320
321   In terms of relative abundance in nasal swab samples, Proteobacteria was the largest represented
322   phyla, followed by Firmicutes, Actinobacteria and Bacteroidetes. These results are similar to
323   swine-related nasal microbiota studies conducted in Canada, the United Kingdom and Spain

324   (Weese et al., 2014; Slifierz, Friendship & Weese, 2015; Correa-Fiz, Fraile & Aragon, 2016)
325   which report Proteobacteria as the most abundant phyla, followed by a large abundance in
326   Bacteroidetes, and Firmicutes. In contrast, the abundance of Actinobacteria in our study is higher
327   than what was previously reported (Weese et al., 2014; Correa-Fiz, Fraile & Aragon, 2016), and
328   the abundance of Tenericutes (particularly Mycoplasmacetae family), phyla which has been
329   recently reclassified into the Firmicutes phylum (Parks et al., 2018), was more prominent in the
330   European study (Correa-Fiz, Fraile & Aragon, 2016) compared to our current study. These
331   differences could be related to animal age given previous studies were conducted with piglets or
332   slaughter animals [approximately six months of age], which are younger than our adult female
333   study population (1-3 years of age). *Nesterenkonia* was the only taxa identified as differentially
334   abundant by farm, and was only found on farm 6. The relevance of this taxa in swine is unclear;
335   although it has been identified previously in studies on the human lower repiratory tract and gut
336   microbiota (Chander et al., 2017; Li et al., 2019).
337
338   Most of the shared taxa for fecal samples in our study were in the Firmicutes and Bacteroidetes
339   families. This agrees with a meta-analysis published in 2017, which reported that those two
340   phyla accounted for nearly 85% of the 16S rRNA gene amplicon sequences among over 930
341   swine gastrointestinal samples (Holman et al., 2017). This meta-analysis also pointed out that the
342   main factors influencing swine gut microbiota were study itself, including technical factors such
343   as extraction and sequencing, gastrointestinal sample location, and animal age, which makes
344   comparisons between studies a challenge. A study by Kim et al. (Kim & Isaacson, 2015)
345   analyzed fecal samples of various aged pigs (including sows) and reported that the most common
346   microbial families in sows were Prevotellaceae, Ruminococcaceae, Lachnospiraceae, and
347   Streptococcaceae, which were also present across most of the fecal samples in our study. Those
348   authors also reported a large proportion of "unclassified" taxa, which did not occur in our case.
349
350   Even though the farms enrolled in the current study were under the same management practice
351   (i.e. herd veterinarian, health and biosecurity protocols, treatment protocols, feed source), there
352   were likely inherent differences regarding past disease challenges, herd productivity, and
353   physical characteristics of the farm (e.g. farm size, number of workers, environment, biosecurity
354   etc.) that were not fully captured or available to investigators in this study, and may have
355   influenced differences observed. For example, farm size and parity distribution were among
356   some of the differences observed between farms that were different in regards to microbial
357   composition metrics (Table 1).
358
359   An important limitation of this study includes the pooling of samples (five sows were
360   represented in each pooled sample). This resulted in only two samples per health status per farm,
361   a small sample size that may have limited our statistical power. This pooling further limited our
362   ability to evaluate the relationship between individual-level variables such as parity, age,
363   production stage and reproductive performance on nasal and fecal microbiota. A second

364   limitation includes the lack of clean/blank swabs extracted as negative controls. Low biomass
365   samples, such as some of the nasal samples in this study, are highly subject to contamination,
366   and we cannot rule out the possibility of swab-contaminants in these samples. However, we did
367   include negative controls for extraction (kits) and sequencing, and we analyzed the nasal sample
368   data with and without rarefaction and after removing samples with fewer than 500 or 1000 reads
369   in an attempt to eliminate the samples at greatest risk of contamination. The low number of reads
370   in the nasal samples likely further limited our statistical power and ability to distinguish true
371   differences between groups. Moreover, in the nasal sample analysis, rarefaction at 500 reads is a
372   low sampling depth that could have limited our ability to detect and compare the true level of
373   diversity within these samples. Despite this, when we analyzed nasal samples with and without
374   rarefaction at a minimum threshold of either 500 or 1000 reads, the results were comparable in
375   all 4 analyses (Supplemental Table 4). With all these considerations, the results presented herein
376   should be considered preliminary, and interpreted as an initial baseline for our population (s) of
377   interest.
378
379   In addition, we further acknowledge the potential for misclassification bias when selecting study
380   subjects. This stems from the fact that on swine farms, the use of "sow cards", which are paper-
381   based animal records, is a common way of keeping production records and "flagging" animals
382   that need to be culled. In many cases, the reason for culling is not always specified. As such,
383   during farm visits, investigators relied on these cards to identify animals that were to be culled,
384   which were then included in the study. Confirmation with the farm manager or farm personnel
385   was attempted whenever possible to minimize this potential source of bias. Future studies could
386   expand sample size and should focus on exploring the association between the fecal and nasal
387   microbiota and detailed demographic (e.g. age), health (e.g. antimicrobial treatment history), and
388   production parameters at the animal or herd levels.
389

Conclusions

391
392   In conclusion, this study provided baseline information for nasal and fecal microbiota of sows
393   under field conditions. Our results suggest that farm of origin can affect nasal and fecal microbial
394   diversity and composition, and health status of animals (compromised / clinical, healthy and cull)
395   can affect alpha diversity. Given the study limitations, these analyses should be expanded to a
396   larger number of animals in the future.
397

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404   28622.

405

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506   clinicians. *Bmj* 356.

507

508

509

510   Figure titles and legends:

511

512   Figure 1

513   Title: Sampling protocol with summarized sample processing steps and relative locations of the

514   six participating farms. Numbers represent farms 1 to 6. Actual map is not shown for

515   confidentiality reasons.

516

517   Figure 2

518   Title: Alpha diversity by farm and health status. Microbial diversity (Shannon index) did not

519   differ by farm or health status for pooled fecal samples and pooled nasal swabs. Median and

520   quartiles are shown in the box and whiskers plots. Fecal samples were rarefied at 5600 reads and

521   nasal samples were rarefied at 500 reads.

522

523   Figure 3

524   Title: Nasal microbial communities (Bray Curtis) by a) farm and b) health status.

525   Legend: Nasal samples were rarefied to 500 reads and differed significantly by farm

526   (PERMANOVA p = 0.002) and by health status (PERMANOVA p = 0.017). No pairwise

527   comparisons were significant by farm. Healthy versus cull was significant (p = 0.033) by health

528   status.

529

530   Figure 4

531   Title: Fecal microbial communities (Bray Curtis) by a) farm and b) health status

532   Legend: Fecal samples were rarefied to 5600 reads and differed significantly by farm

533   (PERMANOVA p = 0.001) but not by health status (PERMANOVA p = 0.663). Pairwise

534   comparisons were significant between all farms (p < 0.05) with the exception of farms 4 and 5 (p

535   = 0.08) and farms 5 and 6 (p = 0.1). No significant differences were found based on health status

536   (p = 0.663).

537

538   Figure 5

539   Title: Microbial taxa bar plots at the phyla level in a) nasal samples and b) fecal samples. Farm

540   and health status of each sample is identified. All taxa with > 1% abundance are included.

541

542   Supplemental Figure 1

543   Title: Alpha diversity analysis (Faith PD index) for pooled fecal and nasal swabs.

544   Legend: Microbial diversity by a) sample type and b) farm and health status. In A, all nasal and

545   fecal samples were rarefied at 500 reads. In B, fecal samples were rarefied at 5600 reads and

546   nasal samples were rarefied at 500 reads. Median and quartiles are shown in the box and

547   whiskers plots. N= 36 fecal and 19 nasal swabs. *p <0.001

548

549   Supplemental Figure 2

550   Title: Nasal microbial communities (Jacaard) by a) farm and b) health status.
551
552   Supplemental Figure 3
553   Title: Fecal microbial communities (Jacaard) by a) farm and b) health status.
554
555   Supplemental Table 1
556   Title: Number of reads in each sample. (Chloroplasts, mitochondria, and eukaryotic reads have
557   been removed.)
558
559   Supplemental Table 2
560   Title: Nasal samples - Average number of reads by Farm and Health Status. (Samples with fewer
561   than 500 reads were excluded.)
562
563   Supplemental Table 3
564   Title: Fecal samples - average number of reads by Farm and Health Status. (Samples with fewer
565   than 5600 reads were excluded.)
566
567   Supplemental Table 4
568   Title: Microbial community analysis of nasal samples with and without rarefaction at 500 reads
569   and 1000 reads. Microbial community analysis of fecal samples with and without rarefaction at
570   5600 reads.
571
572   Supplemental Table 5
573   Title: L7 feature table for pooled fecal samples
574
575   Supplemental Table 6
576   Title: L7 feature table for pooled nasal swab samples
577

Farm demographics and general information for the source population of the study

Low performance included notes that has any of the following parameters: low born alive, low number of weaned pigs, low milk production, and low performance.

1 **Table 1.** Farm demographics and general information for the source population of the study

Descriptor	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Date sampled	6/26/2017	7/10/2017	7/10/2017	7/12/2017	7/12/2017	7/18/2017
Number of sows	1,300	2,700	1,100	2,400	5,500	4,000
Cull sow housing type	Crates	Crates	Crates	Crates/ Pens	Crates/ Pens	Crates
Cull sow removal frequency	Every 2 weeks	Every 1-2 weeks	Every 2 weeks	Every 2 weeks	Weekly	Every 2 weeks
Mean (SD) parity	3.4 (1.6)	3.5 (2.2)	3.3 (1.8)	3.9 (2.2)	4.3 (3.0)	3.4 (1.8)
Top three listed reasons for 'compromised / clinical' sows (%)	Low body condition (50), abortion/ discharge (40), lameness (10)	Low body condition (40), abortion/ discharge (30), shoulder ulcer, abcess, other/ kissing (10 for each)	Low body condition (40), shoulder ulcer (20), other/ missing (20)	Low body condition (50), abortion/ discharge (30), lameness and shoulder ulcer (10 each)	Low body condition (70), abortion/ discharge (20), shoulder ulcer (10)	Low body condition and shoulder ulcer (40 each), abortion/ discharge (20)
Top three listed reasons for 'cull' sows (%)	Low performance (70), return to estrus (30)	Low performance (40), return to estrus (30), missing information (30)	High parity (40), low performance (30), missing information (30)	Low performance (100)	Missing information (100)	Low performance (80), missing information (20)

2 Low performance included notes that has any of the following parameters: low born alive, low
3 number of weaned pigs, low milk production, and low performance.

Figure 1

Sampling protocol with summarized sample processing steps (A) and relative locations (B) of the six participating farms.

Numbers represent farms 1 to 6. Actual map is not shown for confidentiality reasons.
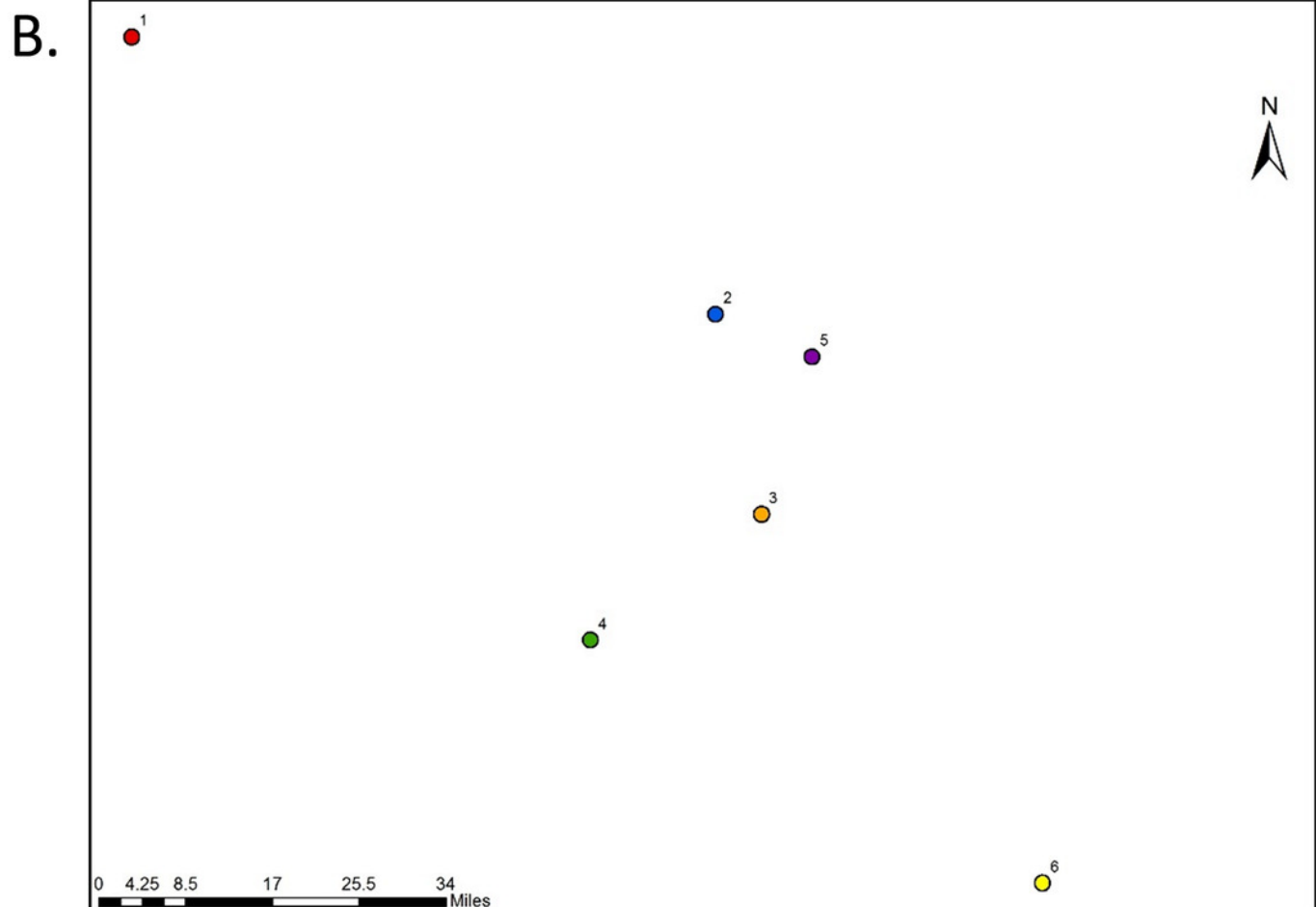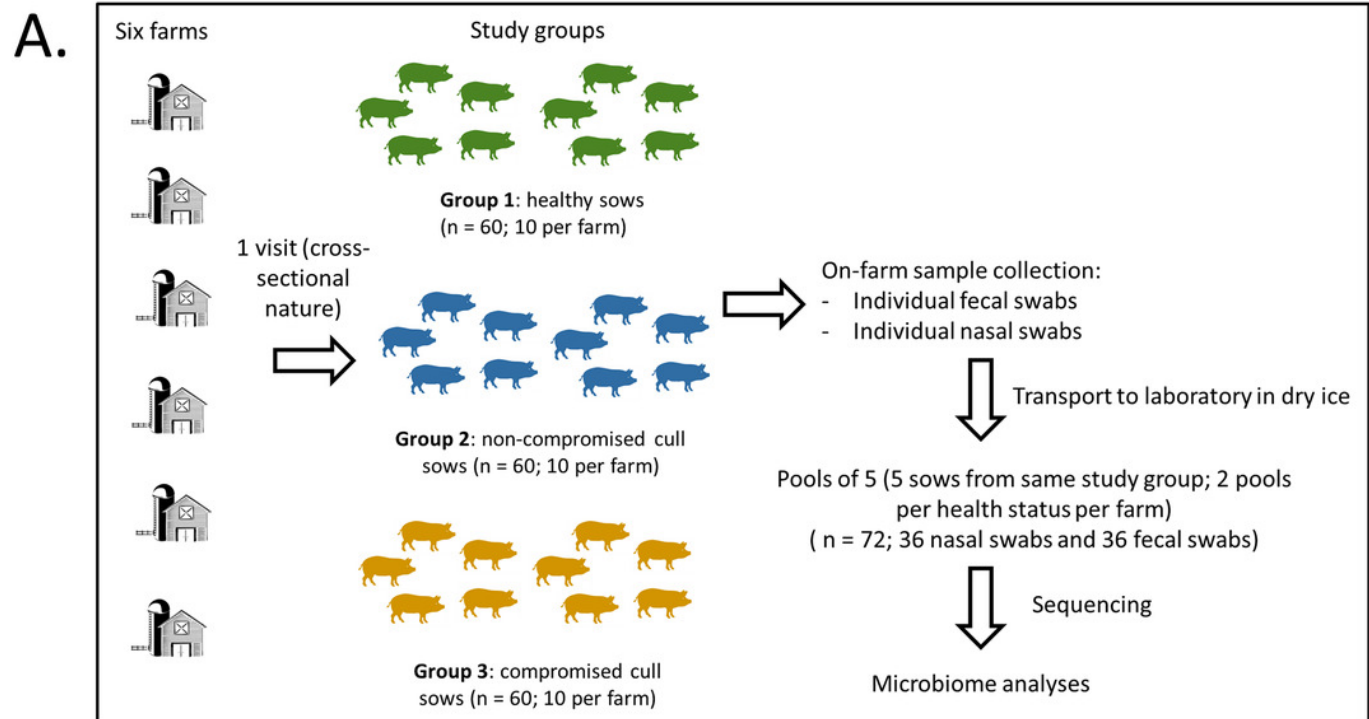
Figure 2

Alpha diversity by farm and health status.

Microbial diversity (Shannon index) did not differ by farm or health status for pooled fecal samples and pooled nasal swabs. Median and quartiles are shown in the box and whiskers plots. Fecal samples were rarefied at 5600 reads and nasal samples were rarefied at 500 reads.
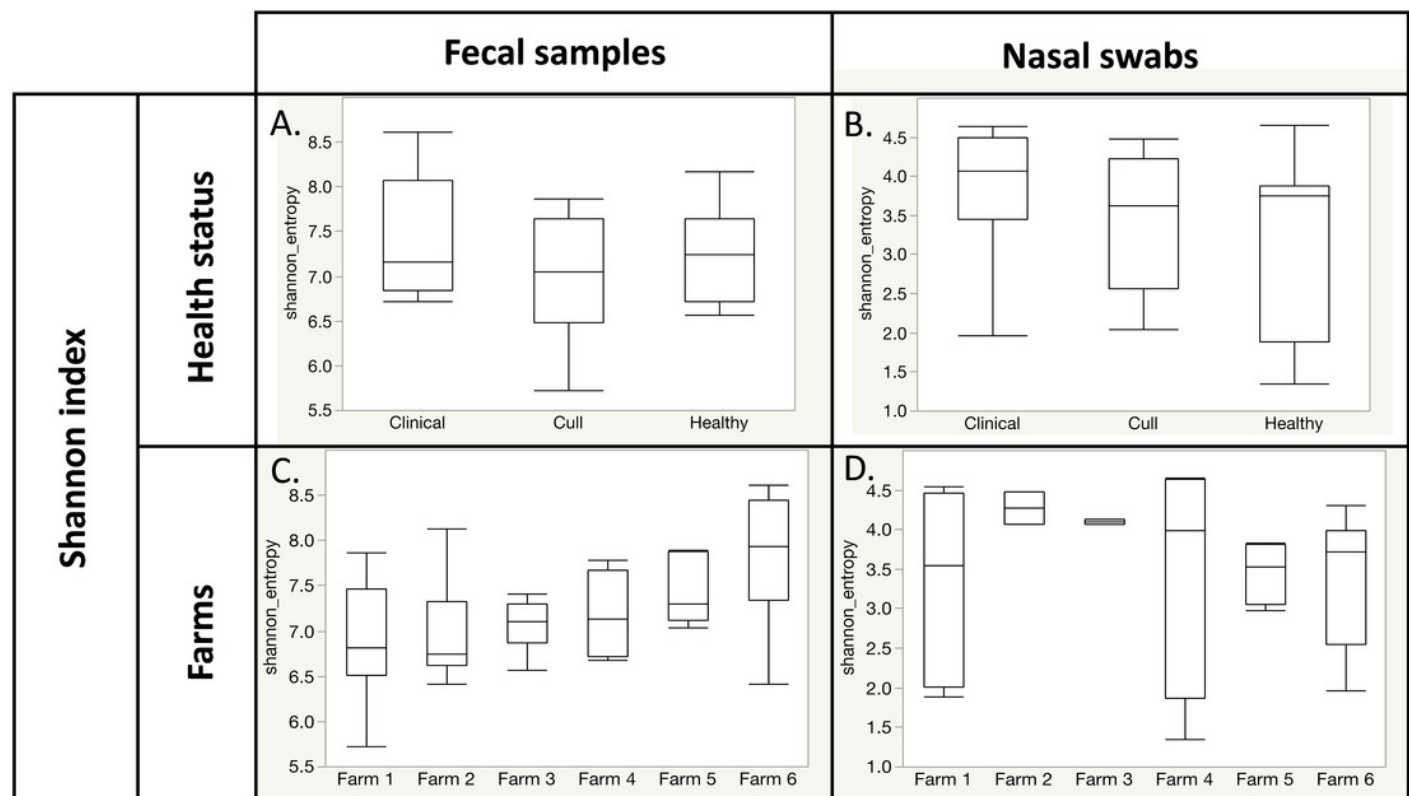
Figure 3

Nasal microbial communities (Bray Curtis) by a) farm and b) health status.

Nasal samples were rarefied to 500 reads and differed significantly by farm (PERMANOVA p = 0.002) and by health status (PERMANOVA p = 0.017). No pairwise comparisons were significant by farm. Healthy versus cull was significant (p = 0.033) by health status.
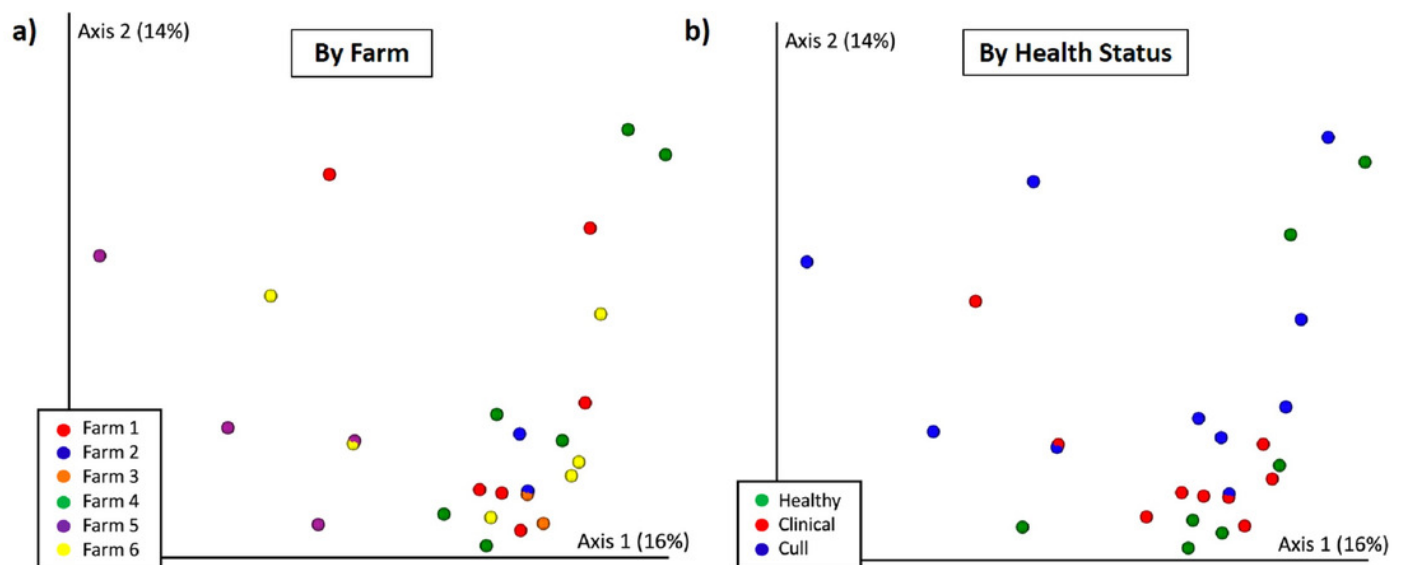
Figure 4

Fecal microbial communities (Bray Curtis) by a) farm and b) health status

Fecal samples were rarefied to 5600 reads and differed significantly by farm (PERMANOVA p = 0.001) but not by health status (PERMANOVA p = 0.663). Pairwise comparisons were significant between all farms (p < 0.05) with the exception of farms 4 and 5 (p = 0.08) and farms 5 and 6 (p = 0.1). No significant differences were found based on health status (p = 0.663).
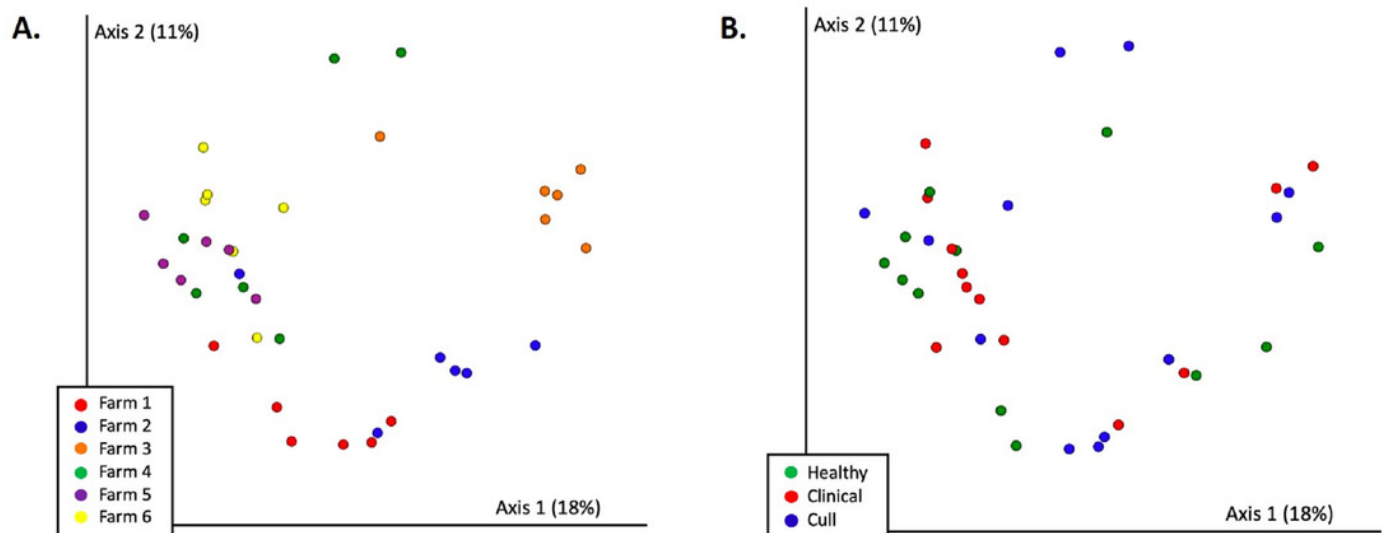
Figure 5

Microbial taxa bar plots at the phyla level in a) nasal samples and b) fecal samples.

Farm and health status of each sample is identified. All taxa with > 1% abundance are

included.