

Supplementations of *Bacillus* spp. on gut microbiota of weaned pigs under diarrheal stress

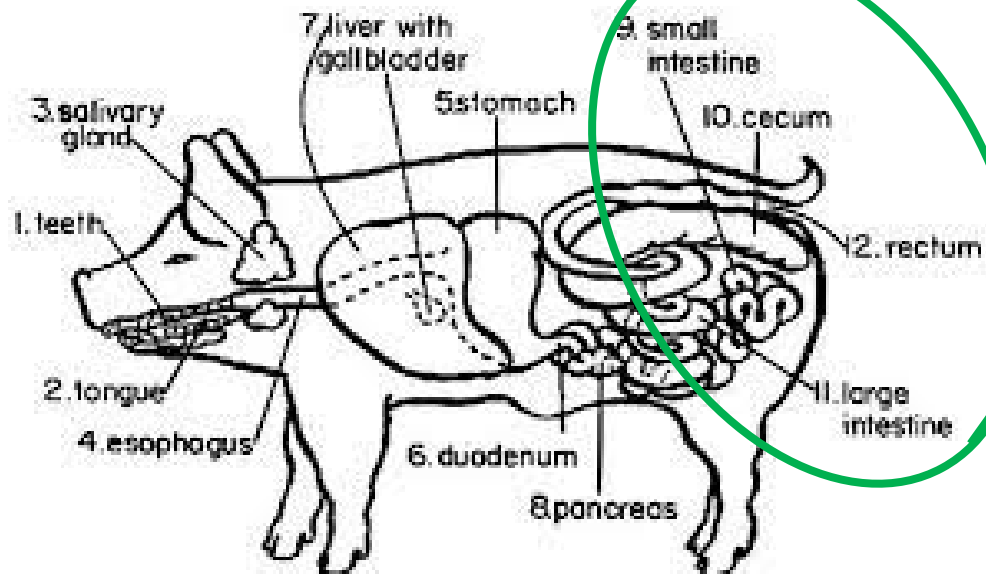
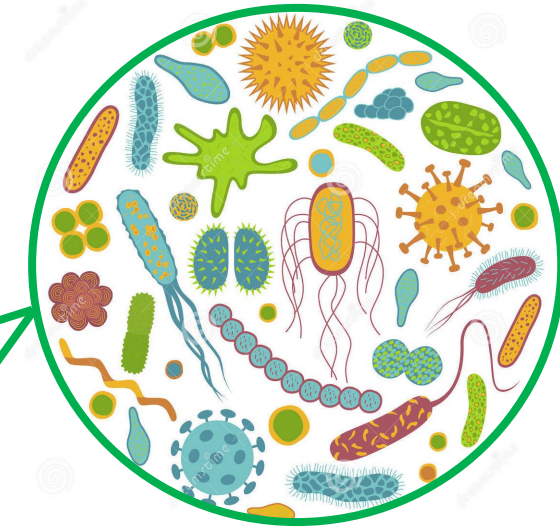
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Gut microbiota

- The digestive system (gut) is crucial for nutrient metabolism and immunity regulation in pigs

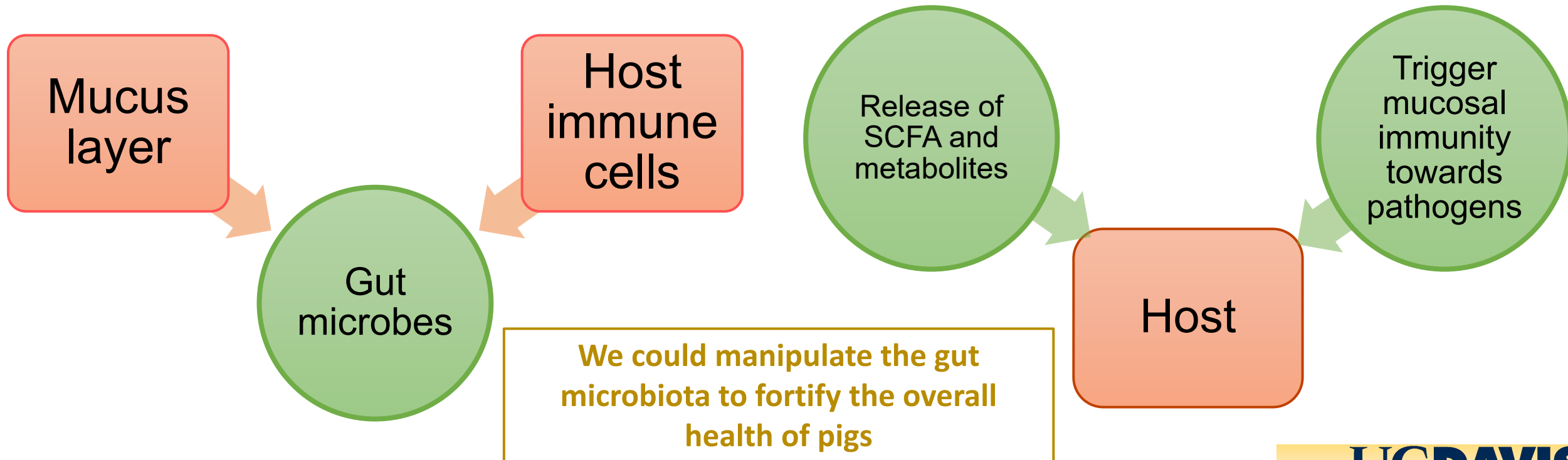


extension.purdue.edu

- Microbes are to harbor innumerably in the gut and have been overlooked to understand their benefits for the pig (host)
- The dynamic and diverse population of microbes in the gut is collectively referred to as the **gut microbiota**

Gut microbiota

- Microbes and their host holds a **symbiotic relationship**, in which the host allows microbes to habituate in the intestines while the microbes benefits the host's health



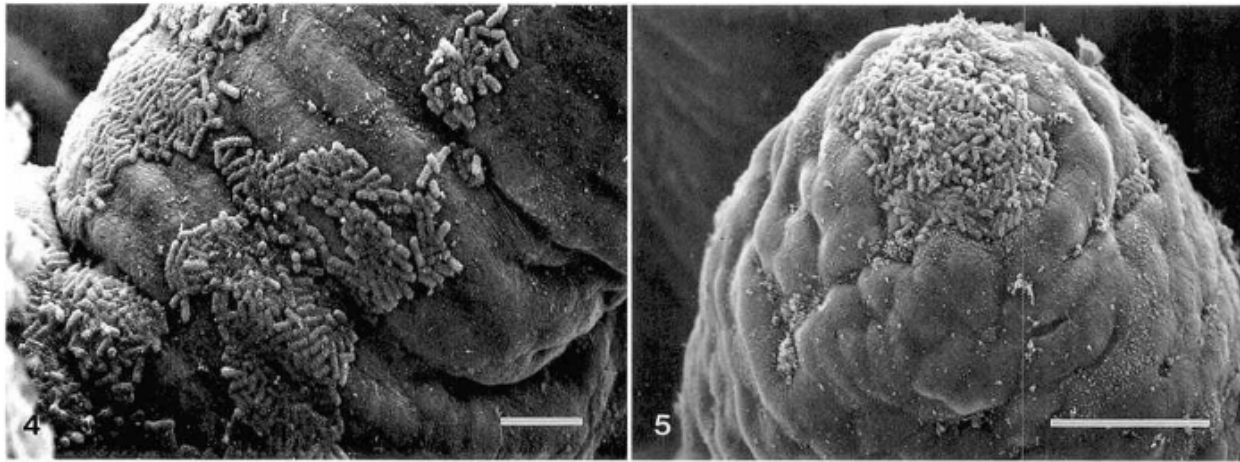
Post-weaning stress

- **Weaning** becomes one of the most stressful moments in pigs
 - Separation from sow and possibly their littermates
 - Change of environment
 - Diet change
- **Weaning stress** reduces feed intake, which can cause a cascading effect toward pig's health
 - Lower feed intake = less metabolizable energy = disturbance of intestinal development in growing pigs



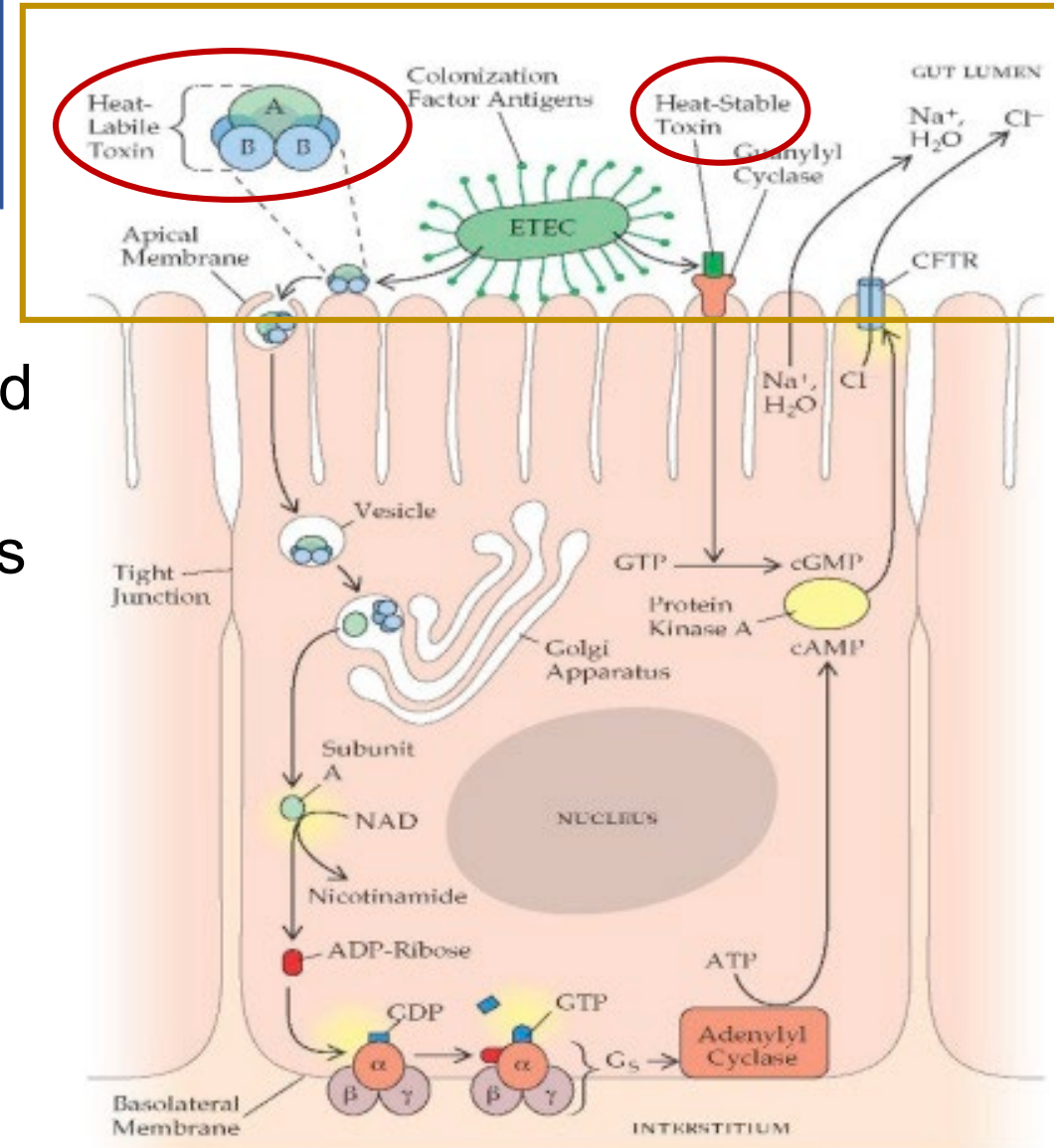
Post-weaning diarrhea

- Post-weaning stress can cause diarrhea
- Post-weaning diarrhea is commonly induced by enterotoxigenic *E. coli* (**ETEC**)
- Leads to high morbidity rate in weaning pigs along with huge economical loss in the swine industry



Vet Pathol 29:239-246 (1992)

ETEC pathogenesis

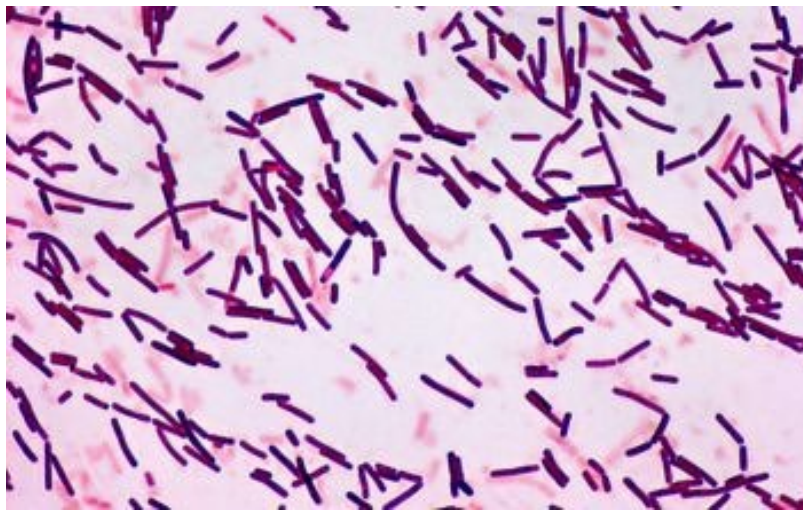


Microbial pathogenesis 117: 162-169 (2018)

Bacillus spp.: Potential feed additives

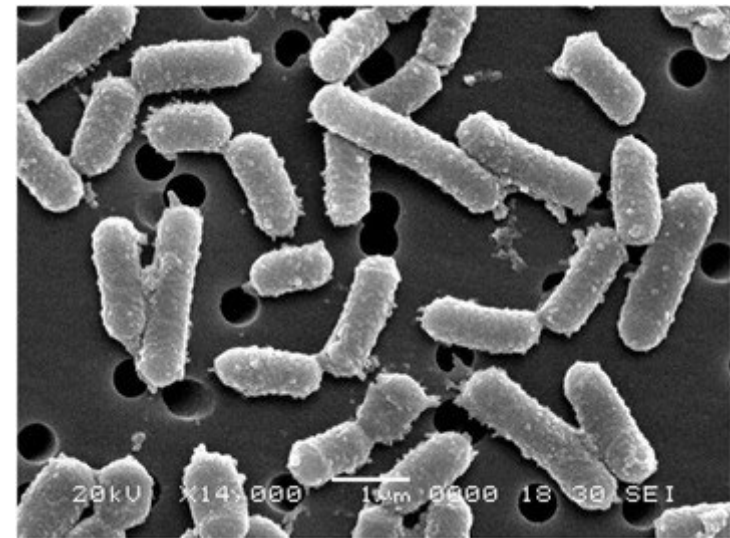
- Our previous study (He et al., 2020) observed that supplementing *B. subtilis* reduced diarrhea and enhanced growth performance of weaned pigs experimentally infected with ETEC

microbe-canvas.com



Bacillus subtilis

Annals of clinical microbiology and antimicrobials, 14(1), 1-11.



Bacillus amyloliquefaciens

Bacillus spp.: Potential feed additives

- However, impact of *Bacillus* spp. on gut microbiota of weaned pigs under post-weaning diarrhea is not yet understood

Objective


1

Supplementations of *Bacillus (B.) subtilis* on intestinal microbiota on weaned pigs challenged with ETEC

Objective

2

B. amyloliquefaciens (BAM) supplementation on performance, systemic immunity, and intestinal microbiota of weaned pigs challenged with ETEC



Supplementations of *B. subtilis* on intestinal
microbiota on weaned pigs challenged with ETEC

Objective 1

Materials & methods

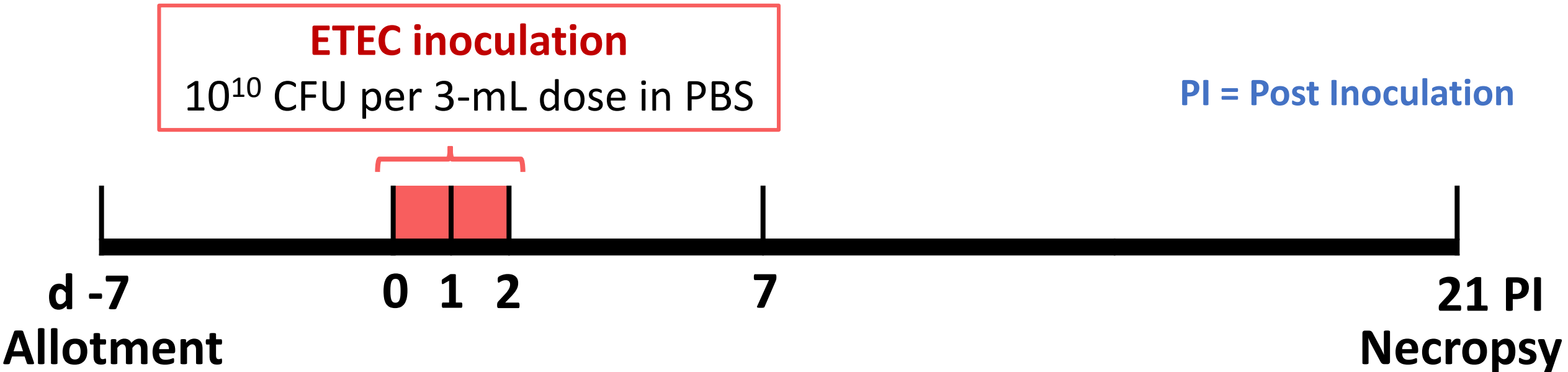
- 48 weaned pigs (6.17 ± 0.36 kg)
 - Around 21-24 day of age
- 4 treatments (12 pigs / treatment)

Sham	NC	Negative control with basal diet
ETEC	PC	Positive control with basal diet
	AGP	Basal diet with 50 mg/kg carbadox
	DFM	Basal diet with 500 mg/kg <i>B. subtilis</i>

AGP = antibiotics growth promoter (carbadox)

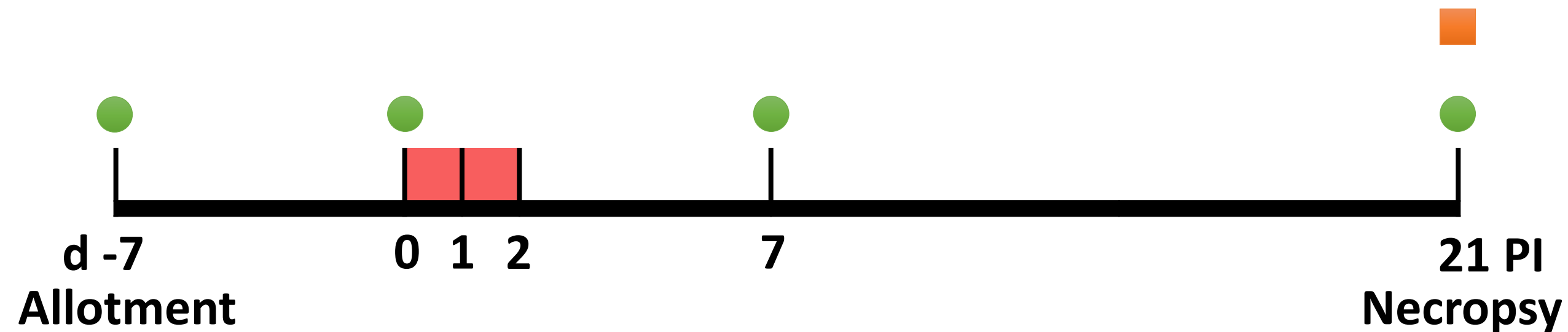
DFM = Direct fed microbials (*B. subtilis*)

Timeline



- Pigs were screened for ETEC sensitivity prior to experimentation prior to study
- *Ad libitum* water and feed provided throughout the study

Sample collection



● Fecal samples collected

■ Jejunal digesta, ileal digesta, and cecal content

16S rRNA sequencing

1

Library prep

- DNA extraction
- PCR
- Illumina sequencing



2

Downstream analysis

- sabre
- QIIME2



3

Data analysis

- R program
- Statistical analysis
- Data visualization



Gut microbiota analysis



Alpha diversity:

- Shannon index (richness & evenness)
- Chao1 index (richness only)

Beta diversity:

- Bray-Curtis dissimilarity

Taxonomic analysis:

- Relative abundance in phylum and genus levels

Statistical analysis

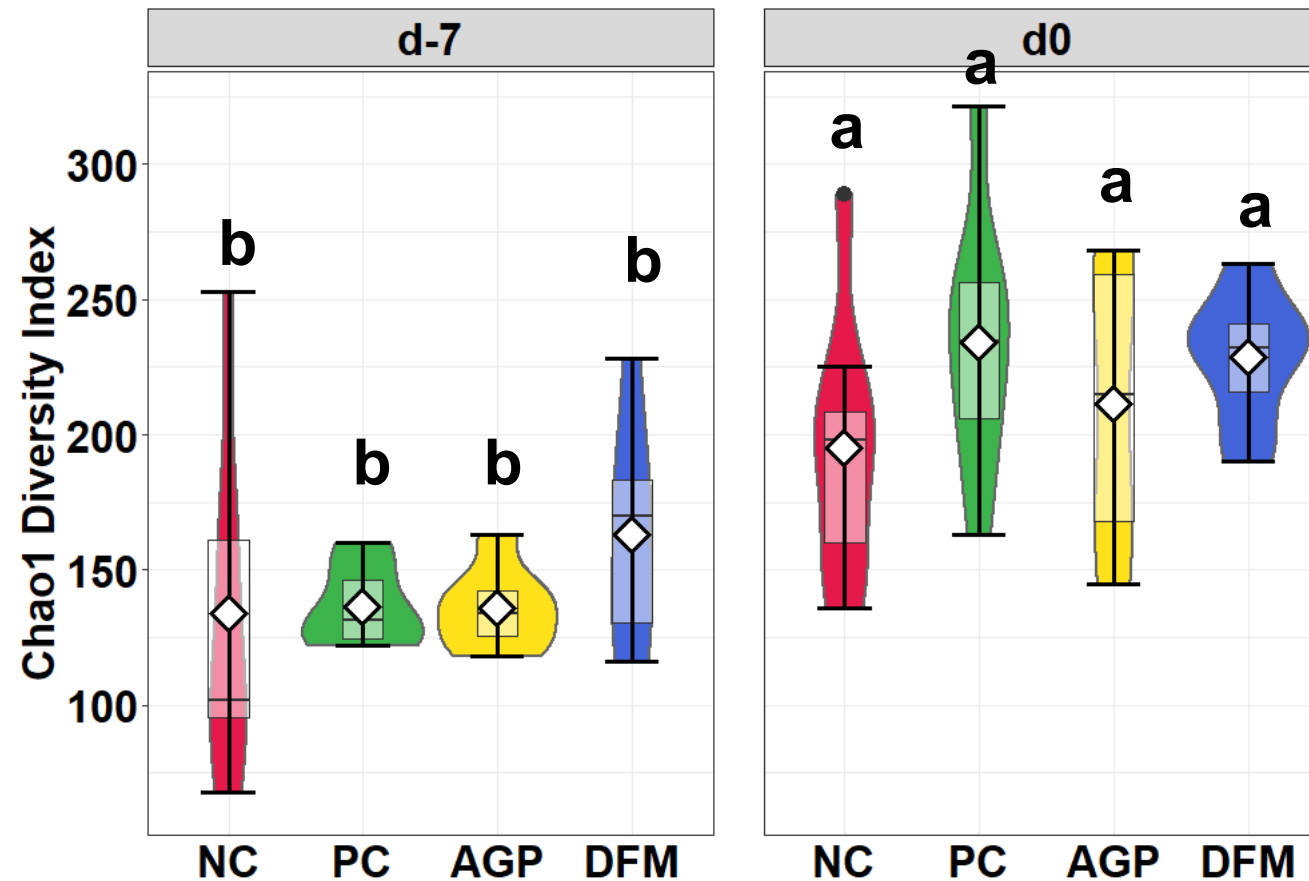
- **Alpha diversity and relative abundance**
 - Normal distribution = ANOVA followed by estimated marginal means (EMMEANS)
 - Non-normal distribution = Kruskal-Wallis followed by Conover test
- **Beta diversity**
 - Betadisper followed by adonis function using *vegan* package in R

Results: Fecal microbiota

No significant difference observed in Shannon diversity (richness & evenness)

Alpha diversity: Chao1 index (richness only)

- Chao 1 index was increased between d -7 and d 0 for all dietary treatments

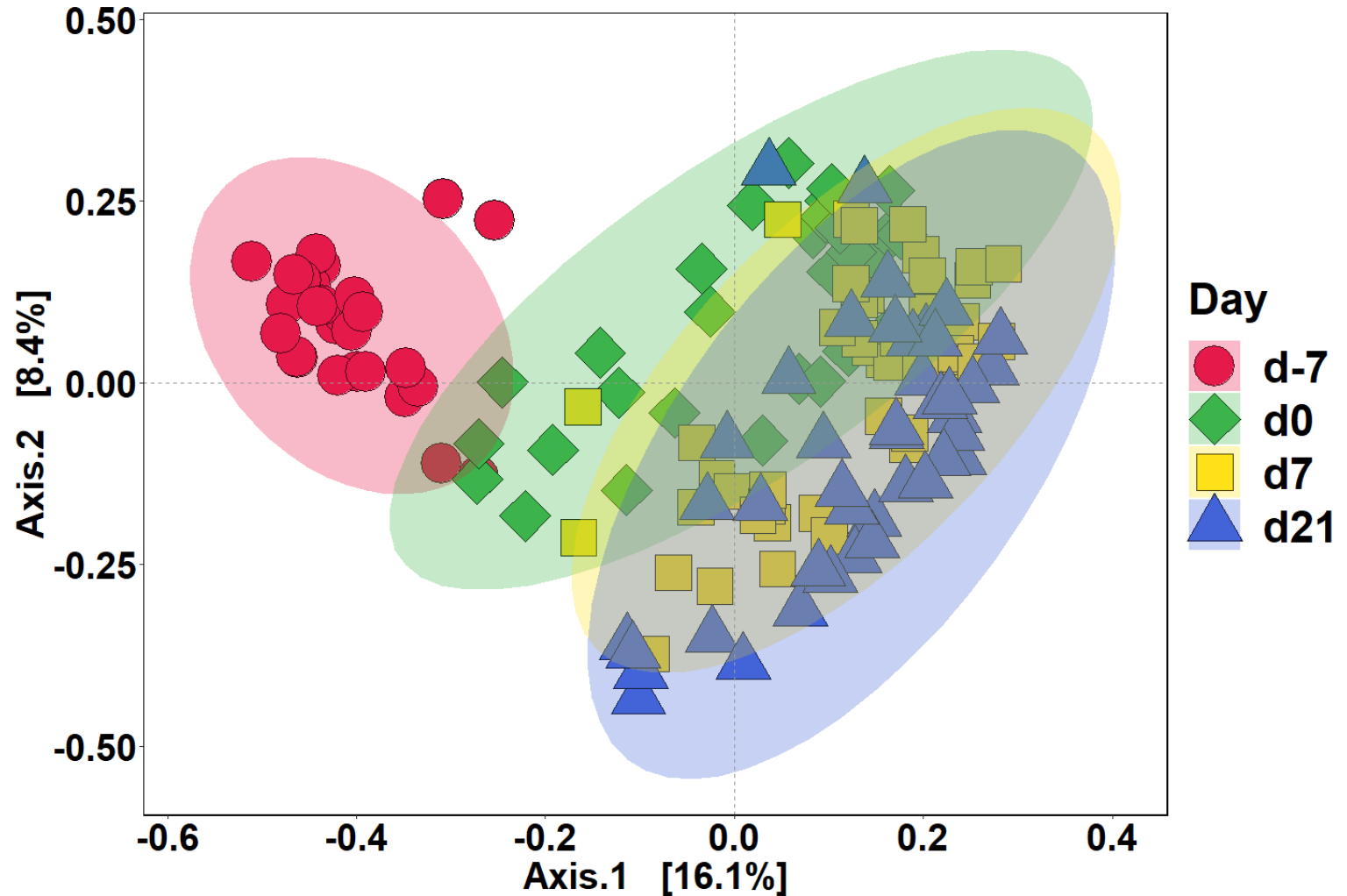


NC = no ETEC
PC = w/ ETEC
AGP = carbadox
DFM = *B. subtilis*

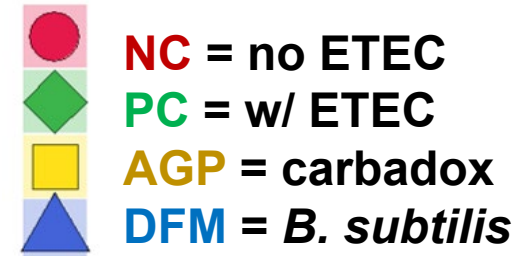
$P < 0.05$

Feces: Beta diversity

- Each point represent a sample
- Color and shape represent day sampled
- The farther the point is from another point, the more likely there is difference in microbial composition between the 2 points

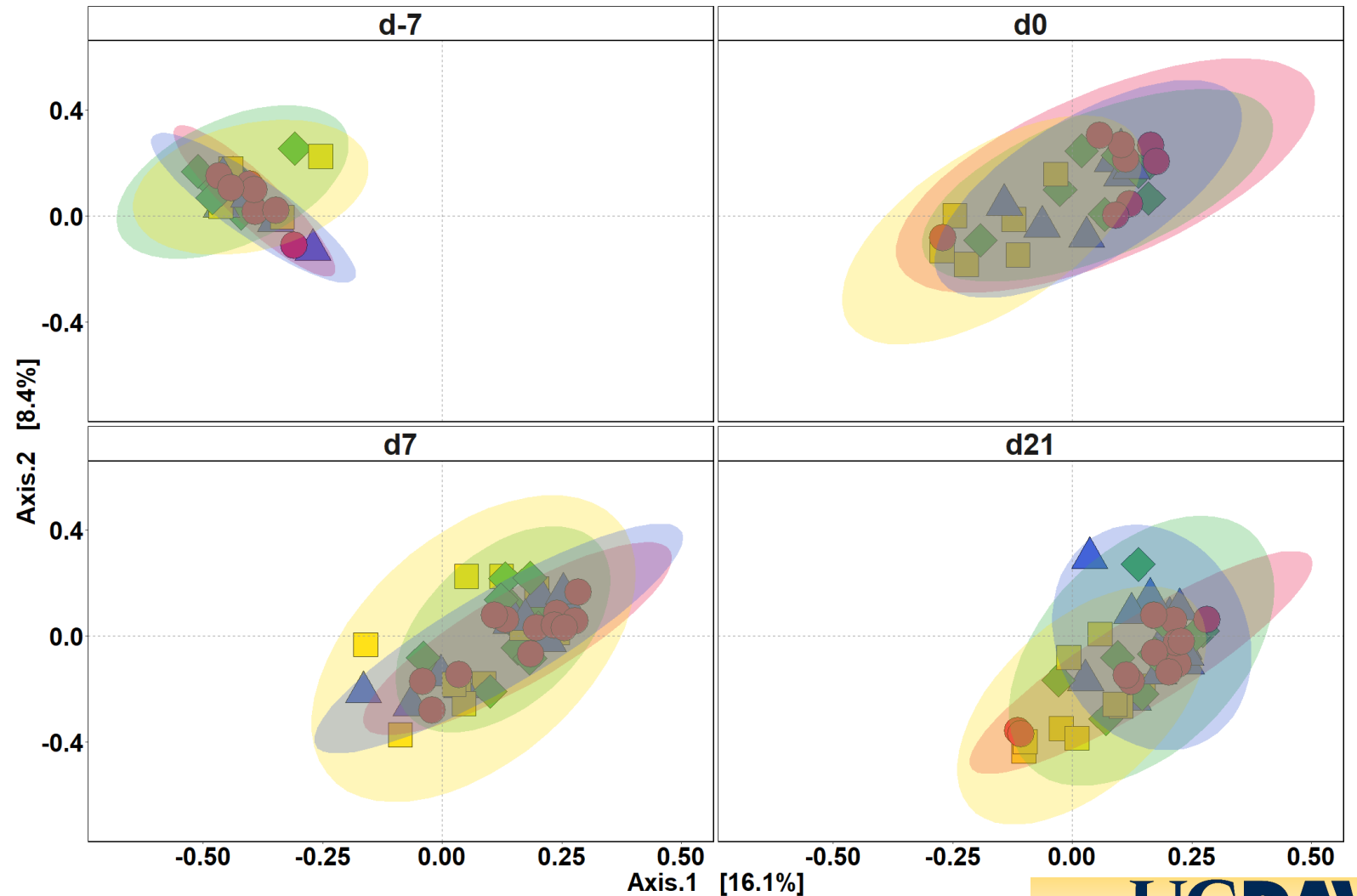


Feces: Beta diversity



Treatment*Day interaction

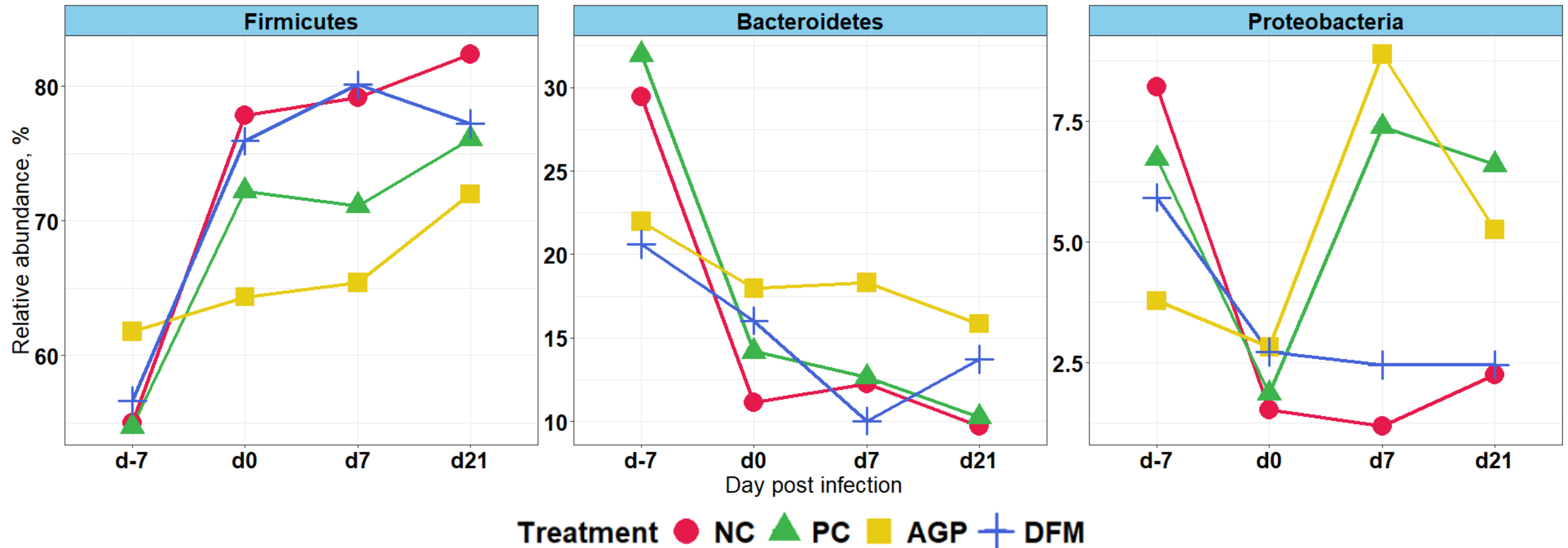
- Facetted by sampling days
- Color and shape represent treatment



Feces: Phylum

- **Firmicutes** increased over time
- **Bacteroidetes** decreased over time
- **Proteobacteria** remained the same

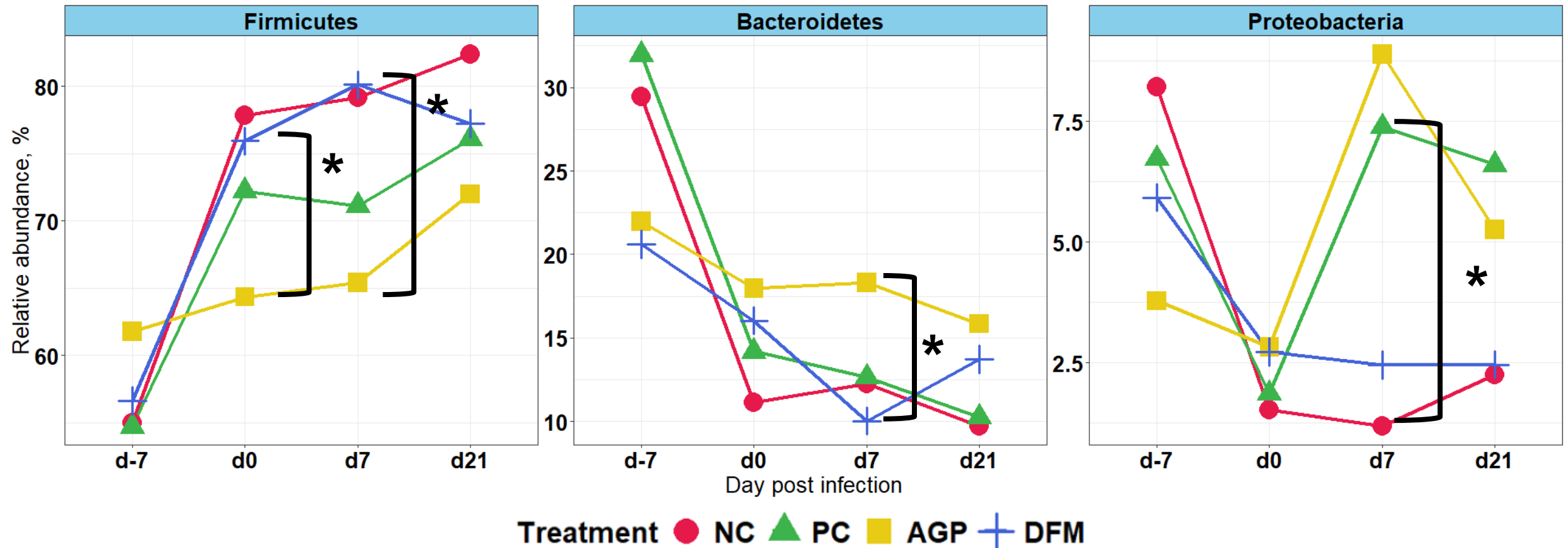
Relative abundance: Phylum level



Feces: Phylum

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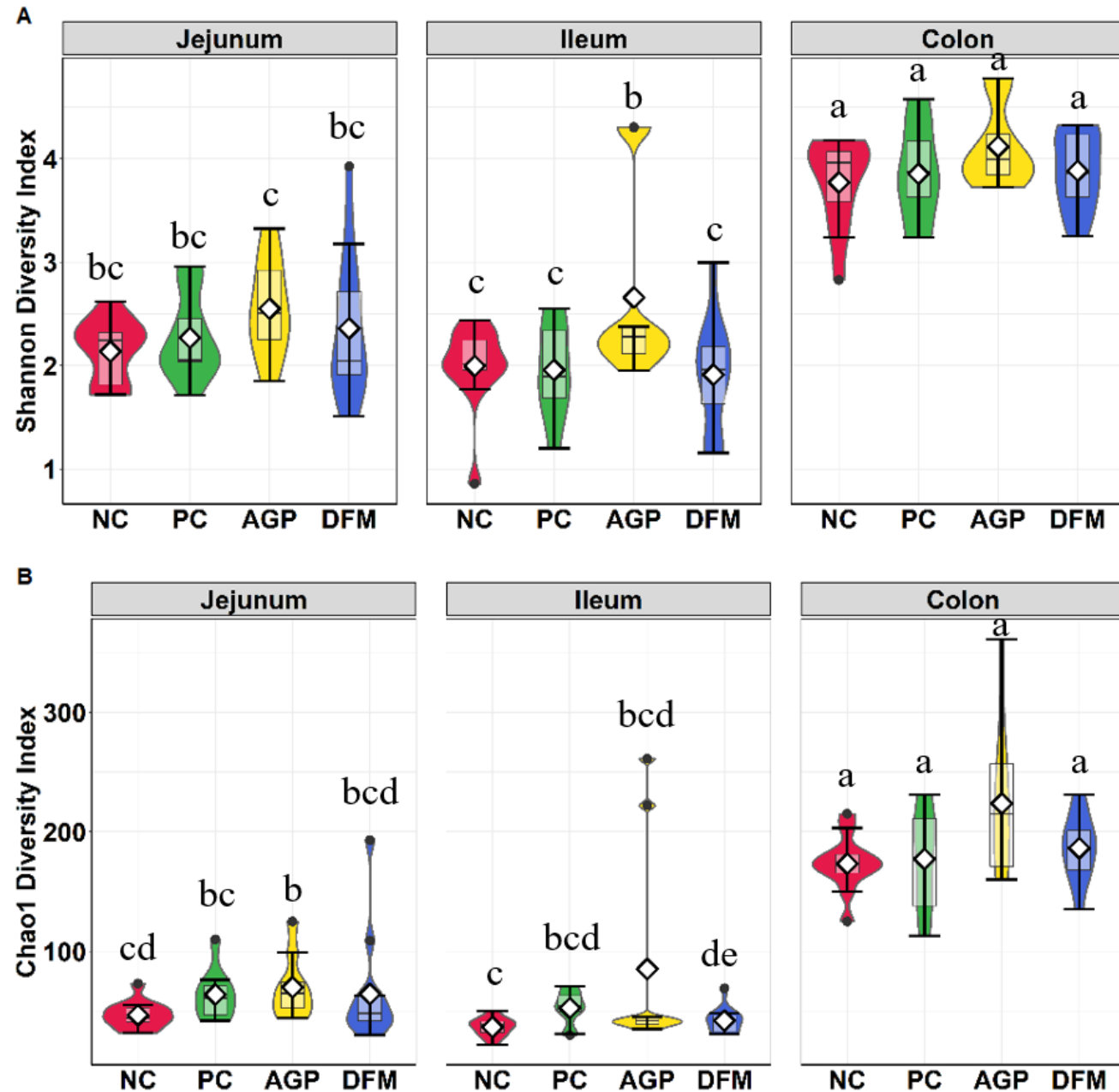
Relative abundance: Phylum level



Results: Intestines

Alpha diversity: Intestinal sites

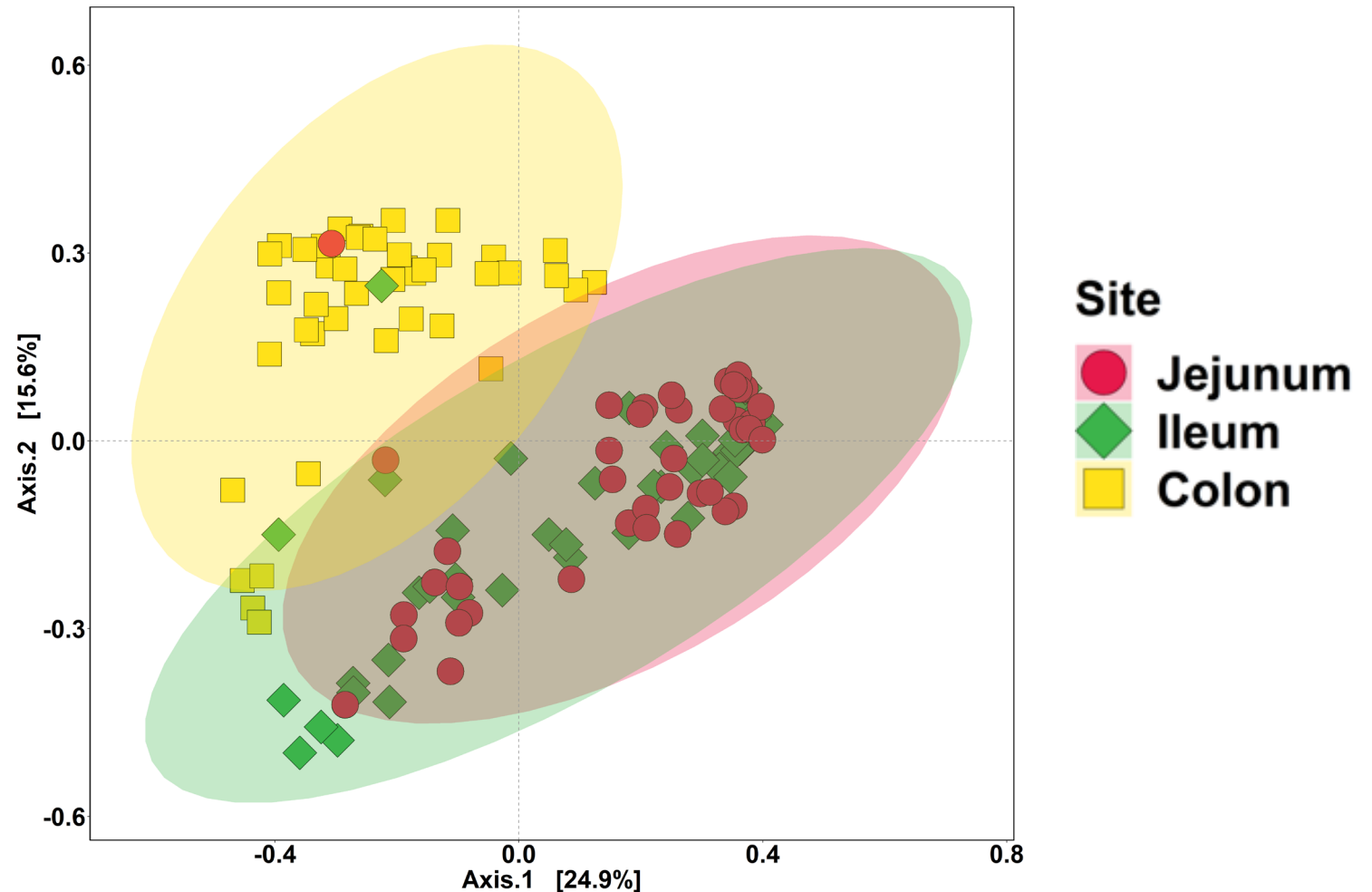
- Samples were collected on d 21 PI
- Shannon and Chao1 indices were greatest in **colon**



Intestines: Beta diversity

Beta diversity: Intestinal sites

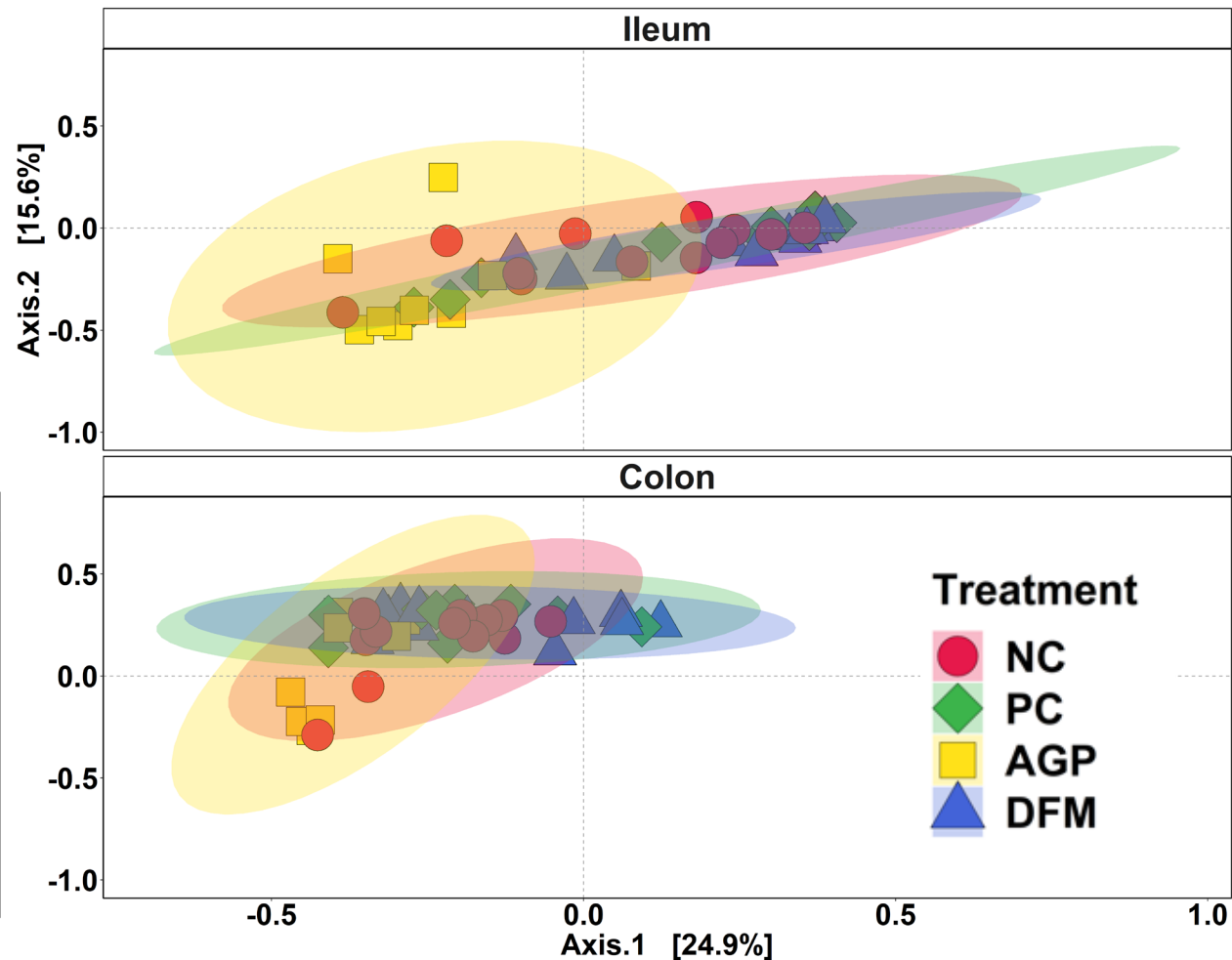
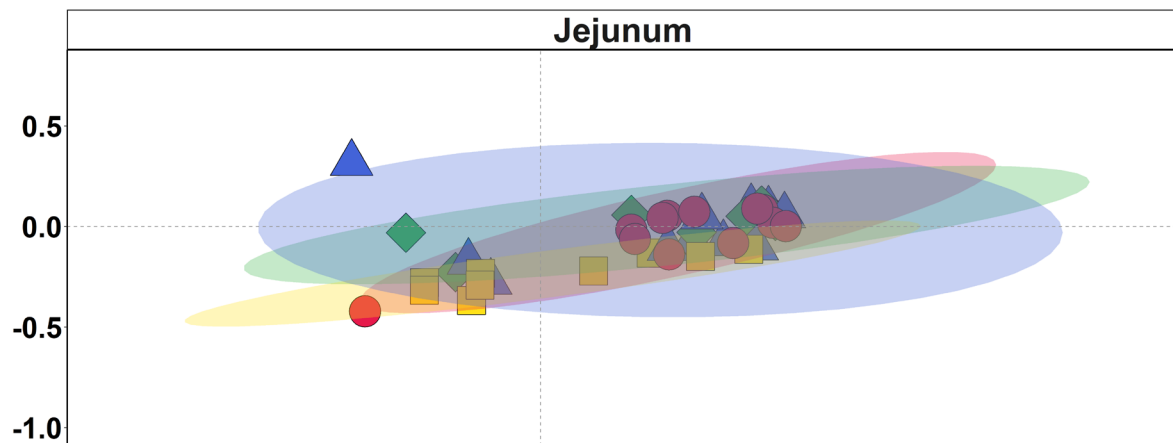
Color and shape represent intestinal site (jejunum, ileum, or colon)



Intestines: Beta diversity

Beta diversity: Site*Treatment

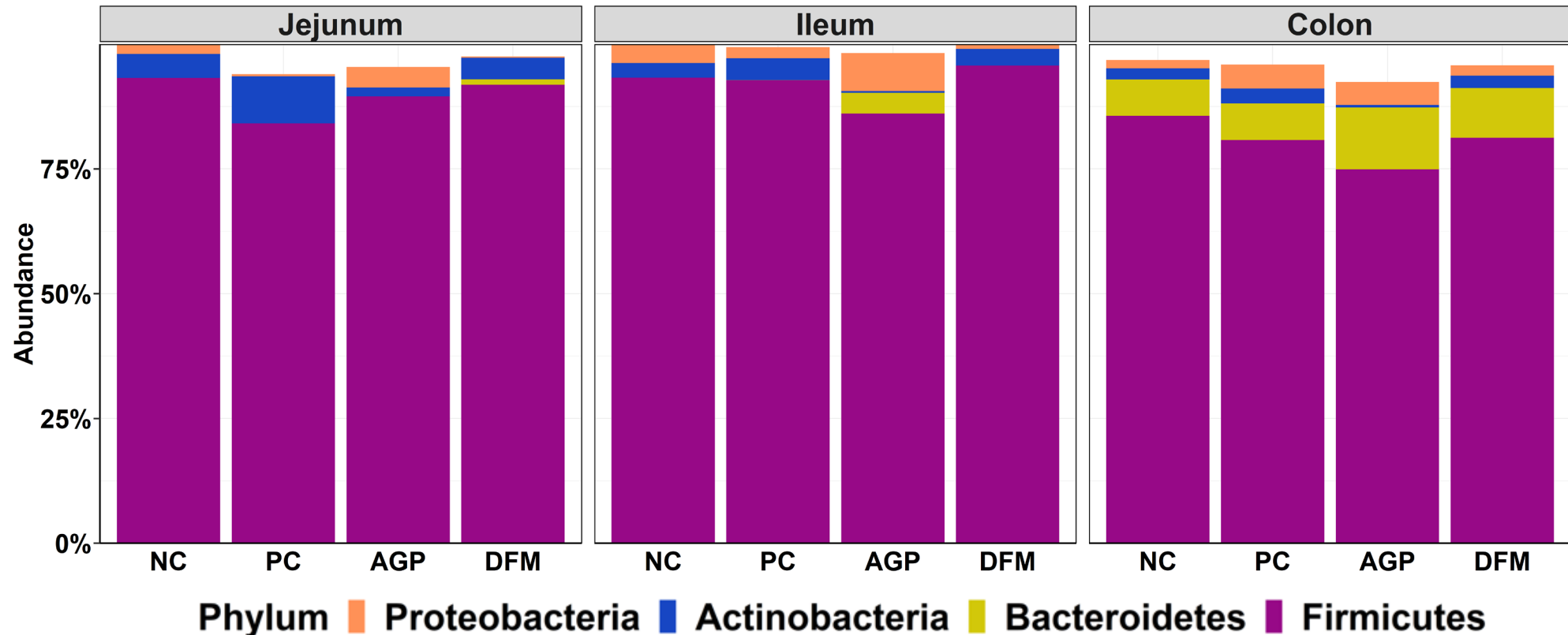
Color and shape represent treatment



Intestines: Phylum

Relative abundance: Phylum level

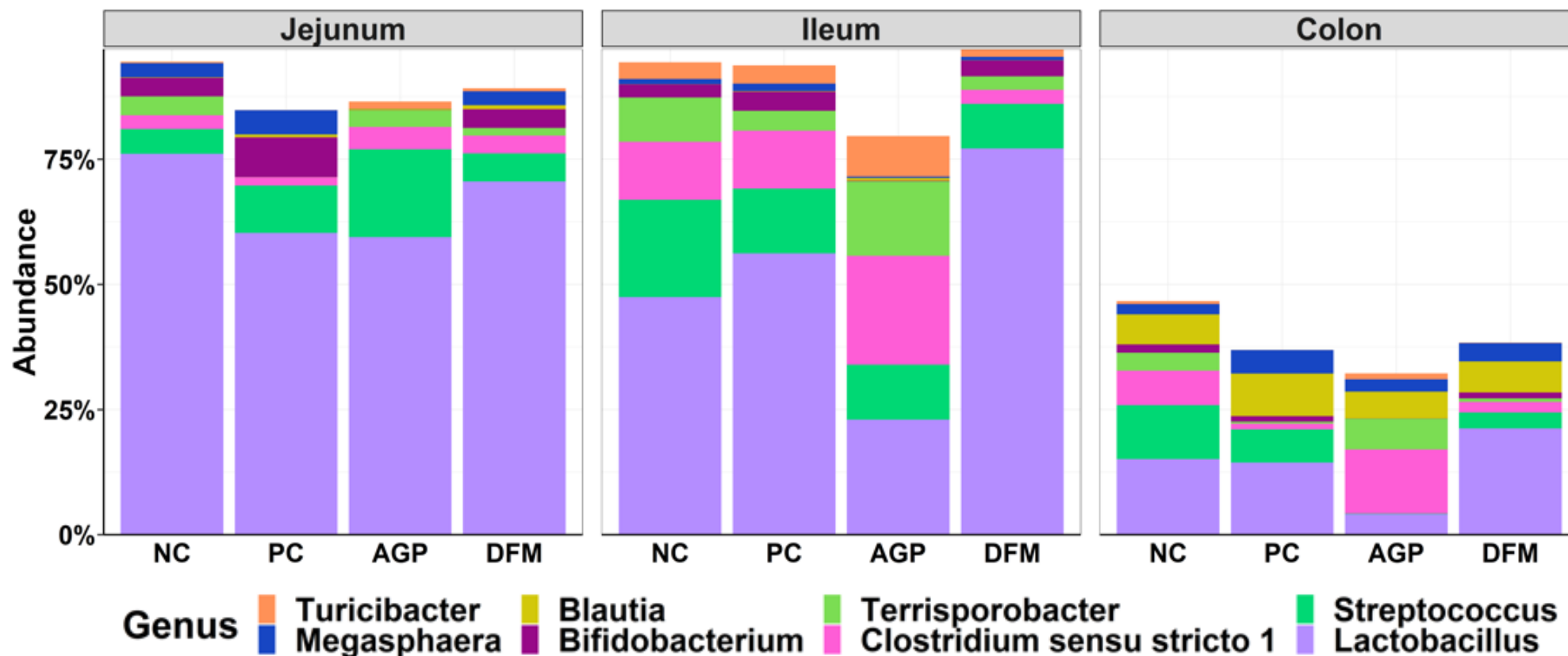
- **Actinobacteria** was greater in ileal digesta from PC than from AGP
- **Bacteroidetes** was greater in AGP than in DFM in ileal digesta
- **Proteobacteria** was greater in AGP than in DFM in jejunal and ileal digesta



Intestines: Genus

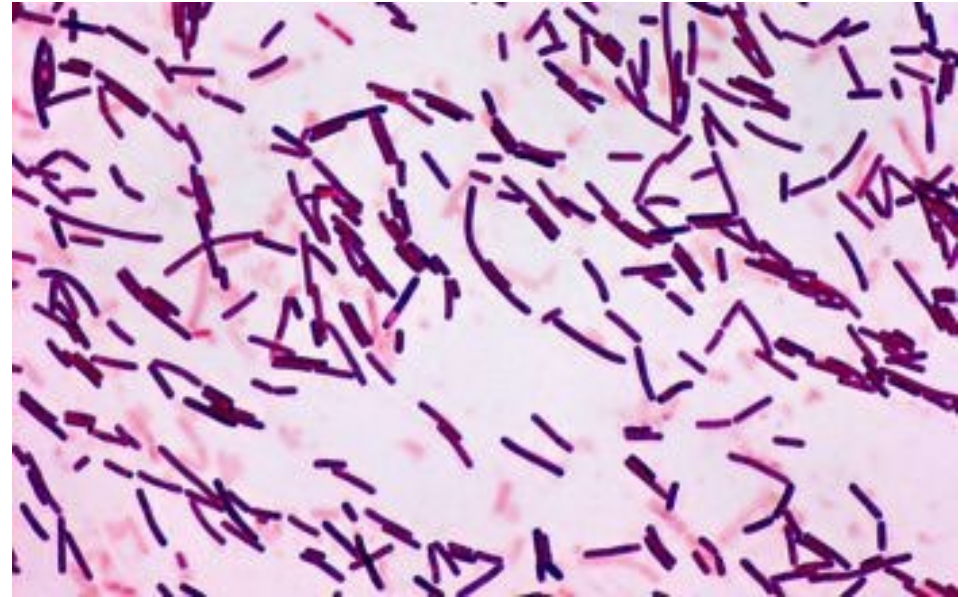
- *Lactobacillus* and *Bifidobacterium* were greater in DFM than AGP and PC in jejunum, ileum, and colon on d 21 PI

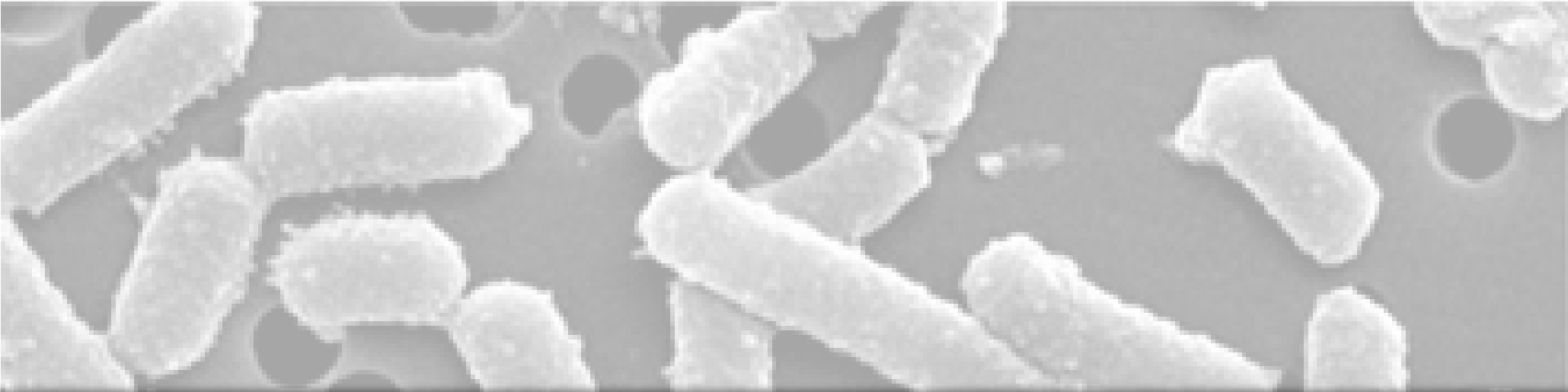
Relative abundance: Genus level



Conclusion

- Age, intestinal sites, ETEC, and diet all contributed to the modulation of gut microbiota
- **Carbadox** supplementation increased relative abundance of gram-negative bacteria including Bacteroidetes and Proteobacteria
 - Could increase risk of antibiotics resistance
- ***B. subtilis*** supplementation was associated with increase of beneficial microbes in the intestinal microbiota of weaned pigs under ETEC challenge





B. amyloliquefaciens (BAM) supplementation on performance, systemic immunity, and intestinal microbiota of weaned pigs challenged with ETEC

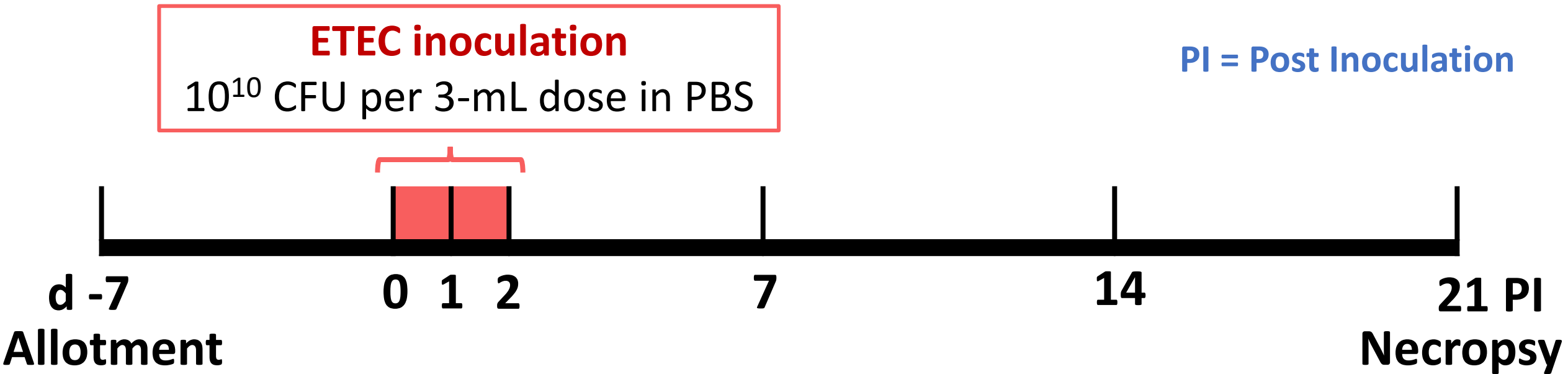
Objective 2

Materials & methods

- 50 weaned pigs (7.41 ± 1.35 kg)
 - Around 21-24 day of age
- 5 treatments (10 pigs / treatment)

Sham (-)	CON -	Control diet
	BAM -	0.10% inclusion rate with 10 ⁹ CFU/kg BAM
ETEC (+)	CON +	Control diet
	BAM +	0.10% inclusion rate with 10 ⁹ CFU/kg BAM
	AGP +	50 mg/kg of Carbadox

Timeline



- Pigs were screened for ETEC sensitivity prior to experimentation
- *Ad libitum* water and feed provided

Measurements

1. Growth performance

- Bodyweight
- Average daily gain (**ADG**)
- Average daily feed intake (**ADFI**)

2. Diarrhea frequency

- Diarrhea score (1 = normal feces, 5 = watery diarrhea)

3. Systemic immunity

- Total and differential blood cell count

4. Fecal and ileal microbiota

- Alpha and beta diversity
- Relative abundance

Statistical analysis

- **PROC MIXED of SAS**
 - Randomized complete block design
 - Pig as experimental unit
 - Diet and challenge as main effect
 - Block as random effect

Statistical analysis: Gut microbiota

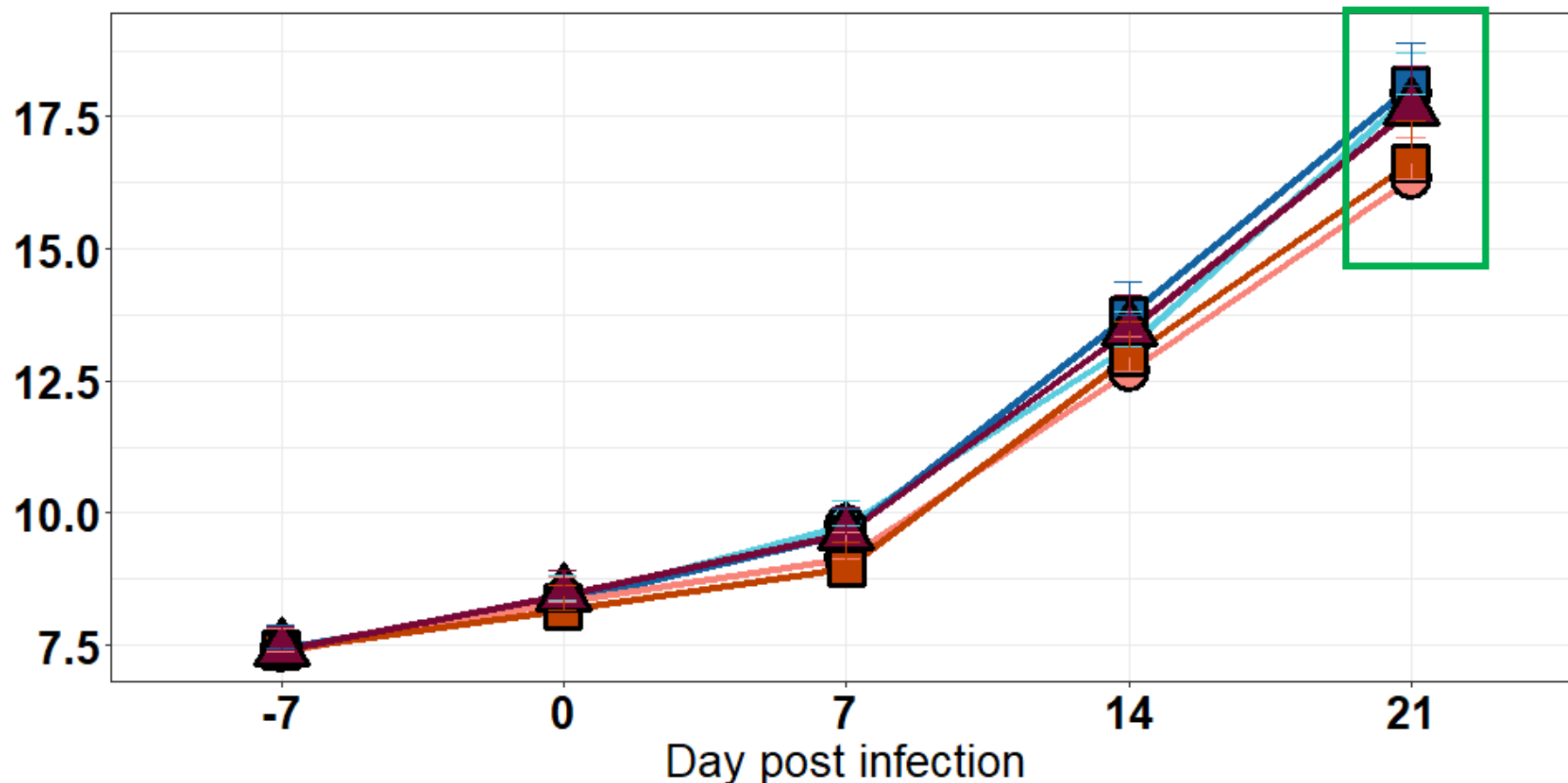
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Results

Bodyweight

	SHAM (-)		ETEC (+)		
	CON	BAM	CON	BAM	AGP
d 21 PI	17.92 ^{ab}	18.09 ^a	16.31 ^c	16.63 ^{bc}	17.65 ^{ab}

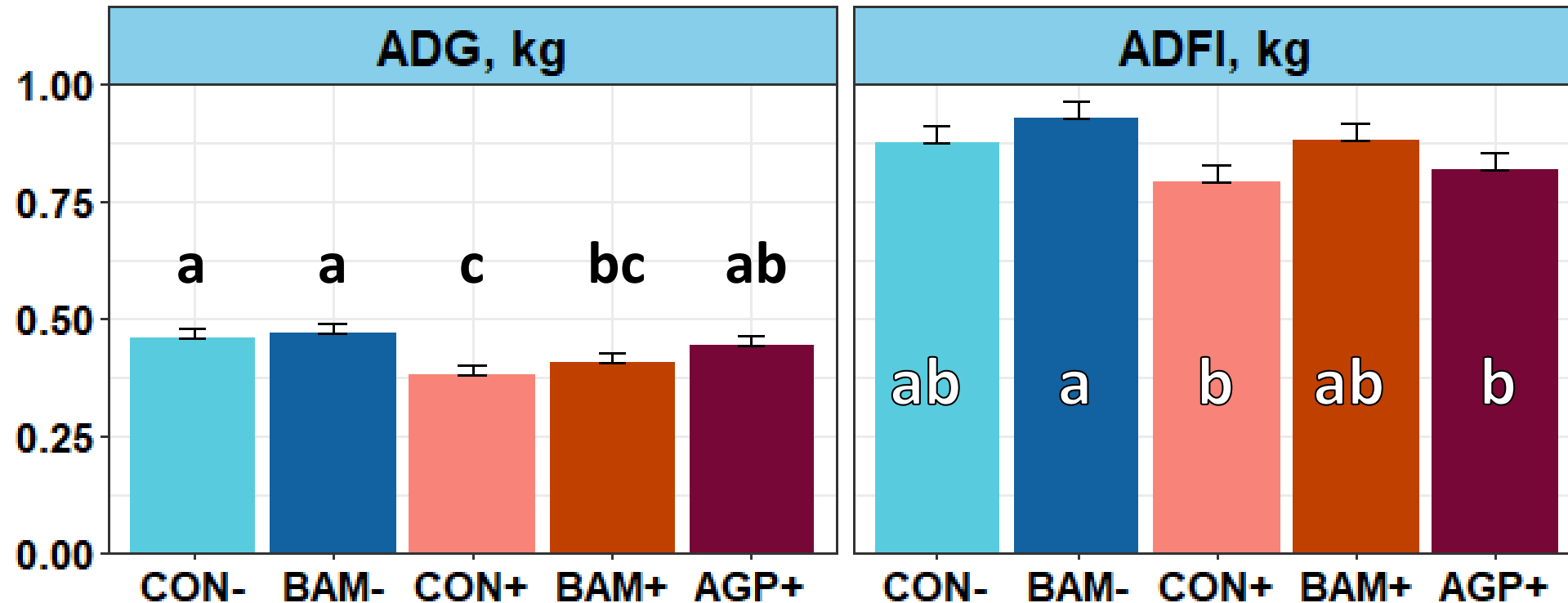
$P < 0.05$



- CON- = no ETEC
- BAM-
- CON+ = w/ ETEC
- BAM+
- ▲ AGP = carbadox

Growth performance

d0 to d21 PI



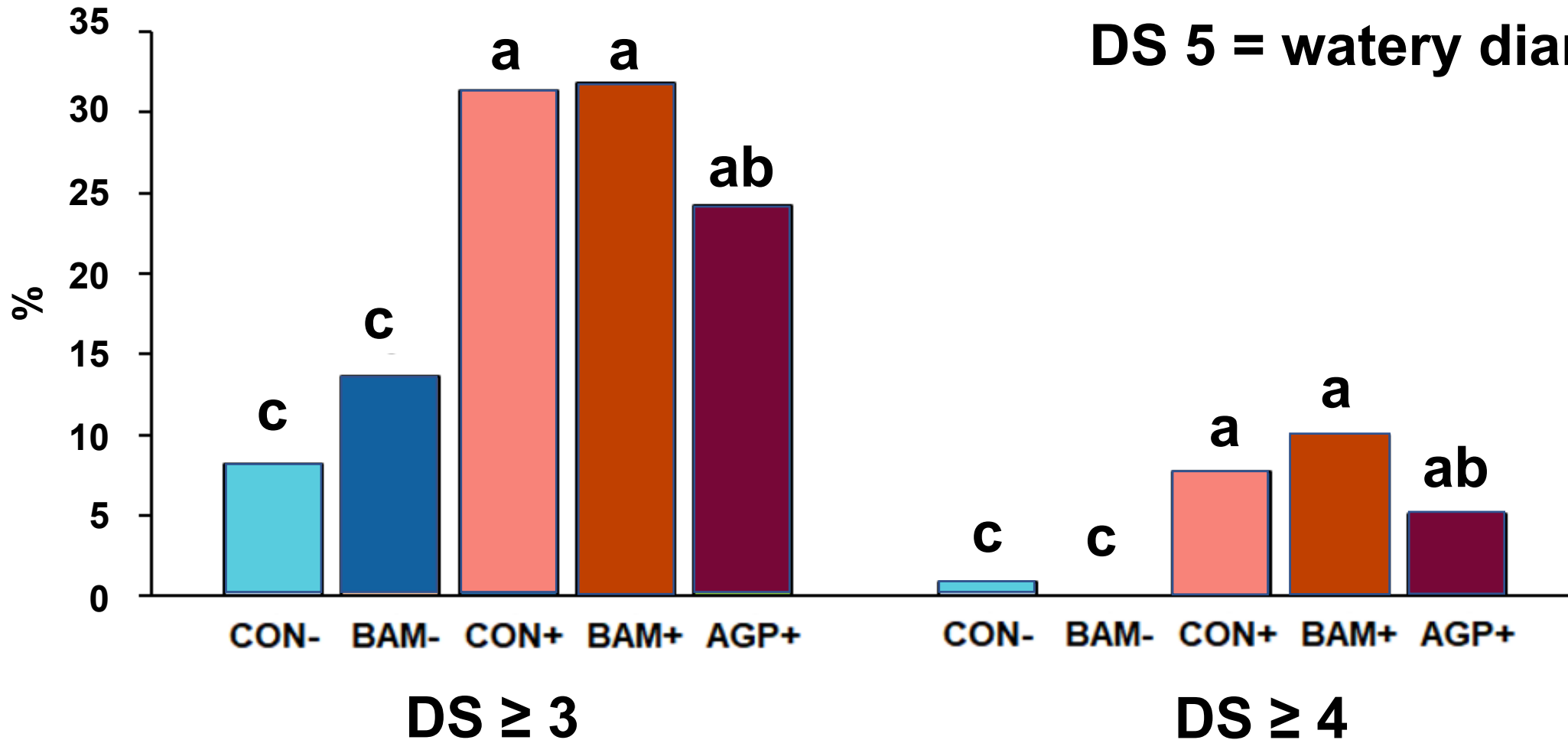
$P < 0.05$

Frequency of diarrhea

DS = Diarrhea score

DS 1 = normal feces

DS 5 = watery diarrhea



P < 0.05

WBC counts

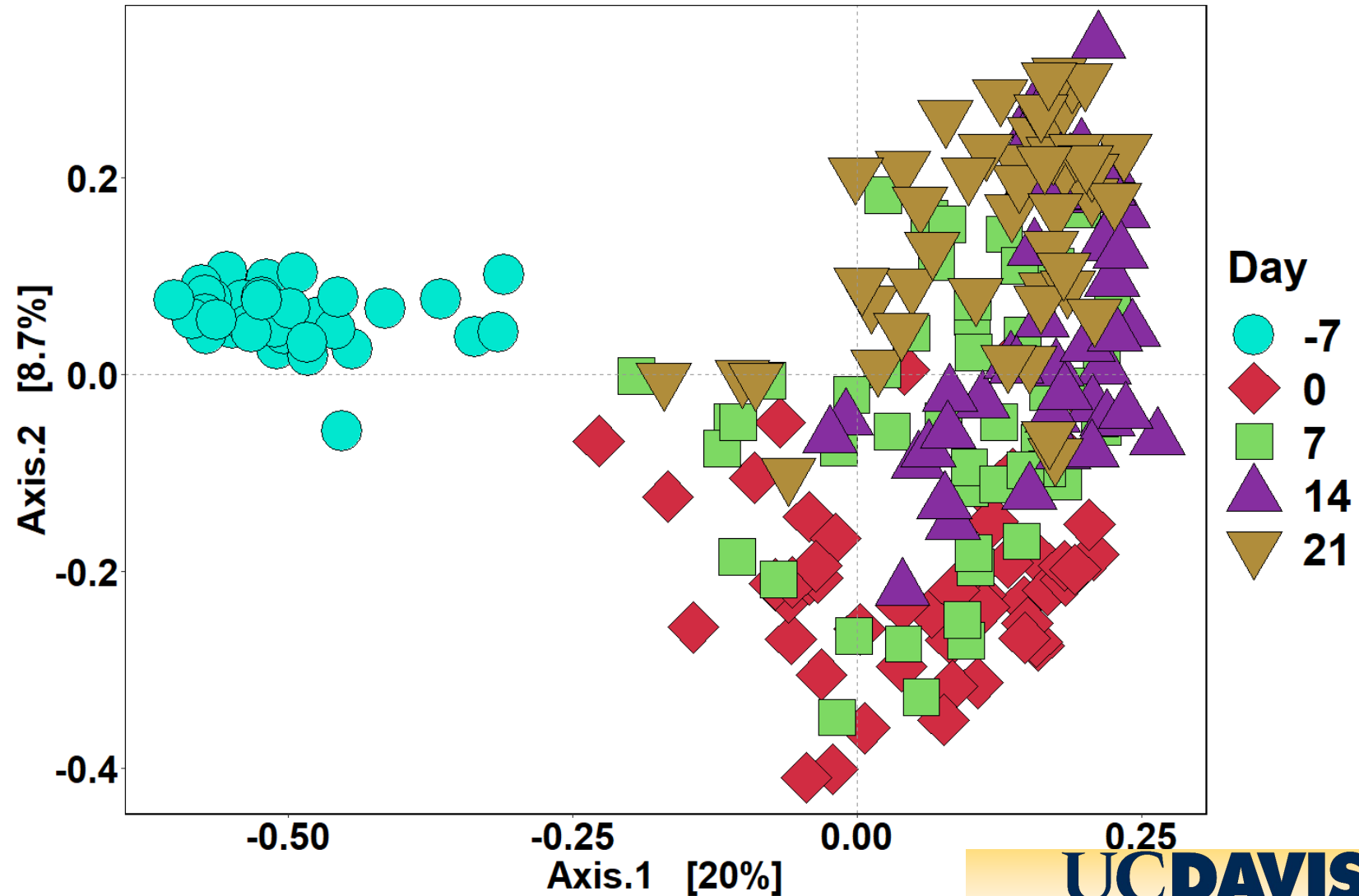
$P < 0.05$

Day PI	SHAM (-)		ETEC (+)		
	CON	BAM	CON	BAM	AGP
Lymphocyte, 10³/μL					
0	4.86 ^b	4.75 ^b	5.23 ^b	6.31 ^{ab}	7.14 ^a
7	5.28 ^b	6.21 ^{ab}	8.71 ^a	8.26 ^a	8.22 ^a
21	5.59 ^b	5.63 ^b	7.30 ^a	5.36 ^b	6.79 ^{ab}
Neutrophil, 10³/μL					
14	7.62 ^b	7.22 ^b	9.49 ^a	7.92 ^b	7.27 ^b
	ab	ab	a	bc	c

Fecal microbiota

Beta diversity

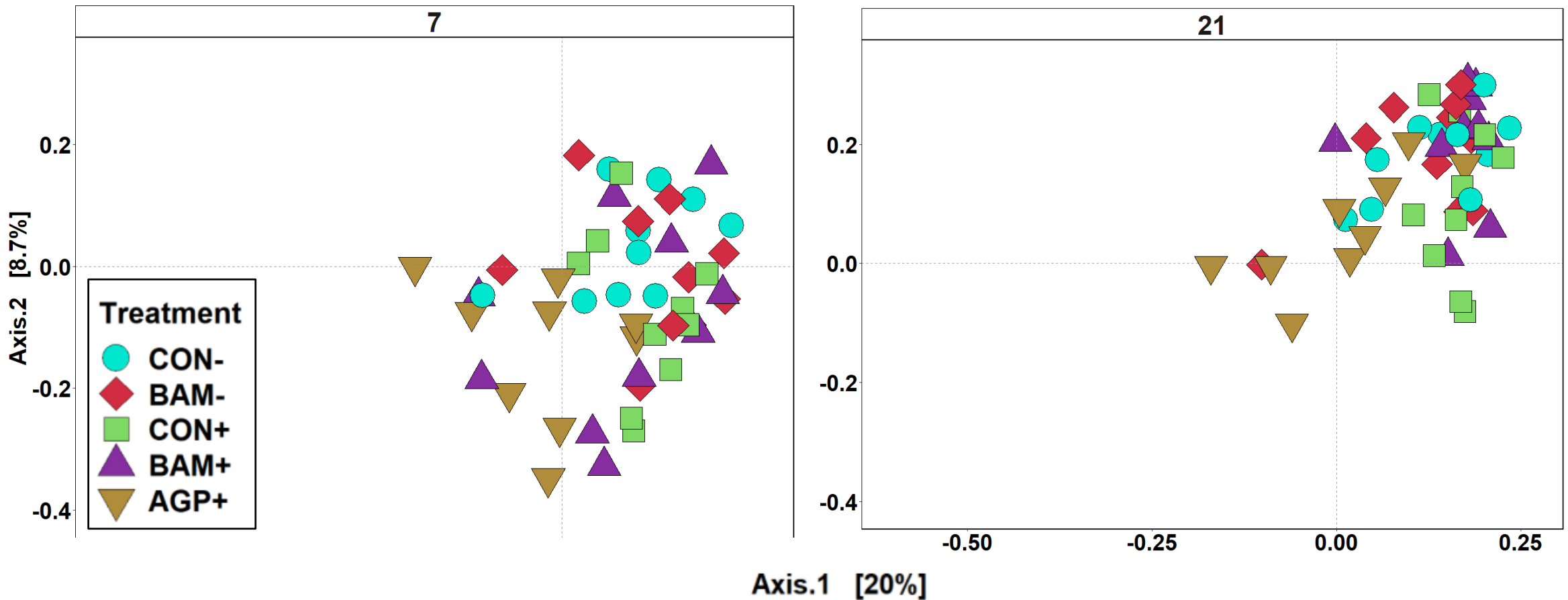
Color and shape represent sampling day



Feces: Beta diversity

Treatment*Day

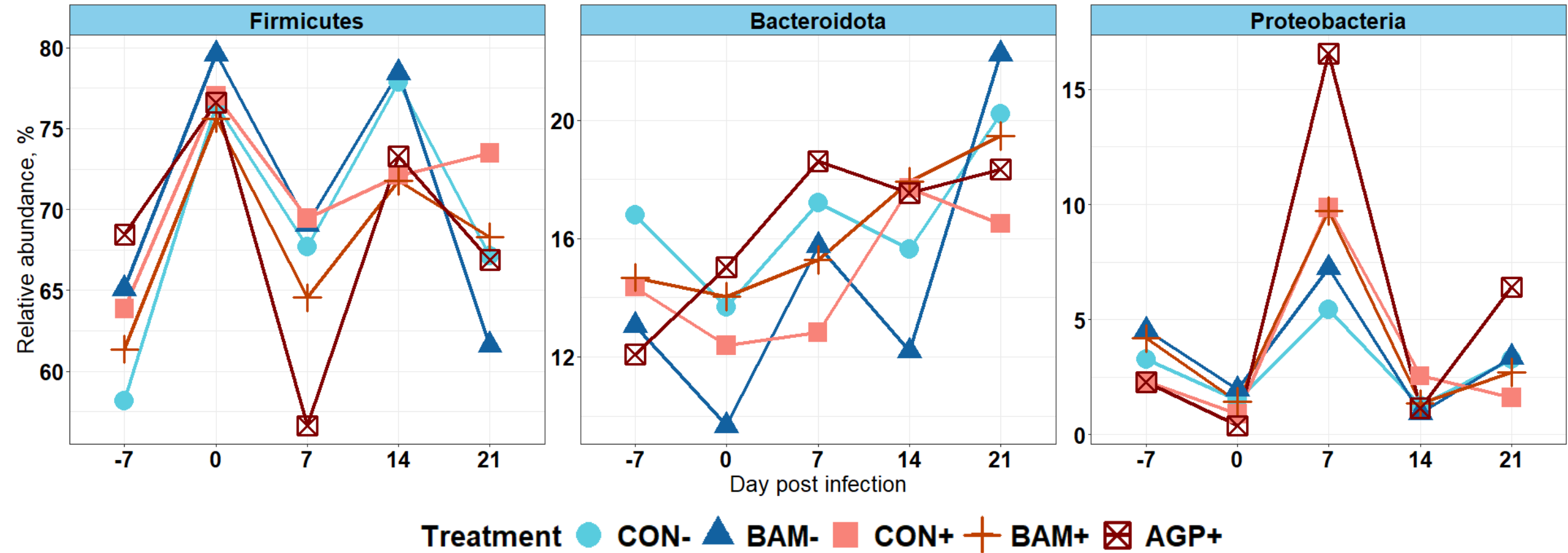
- AGP+ samples were clustered together



Feces: Phylum

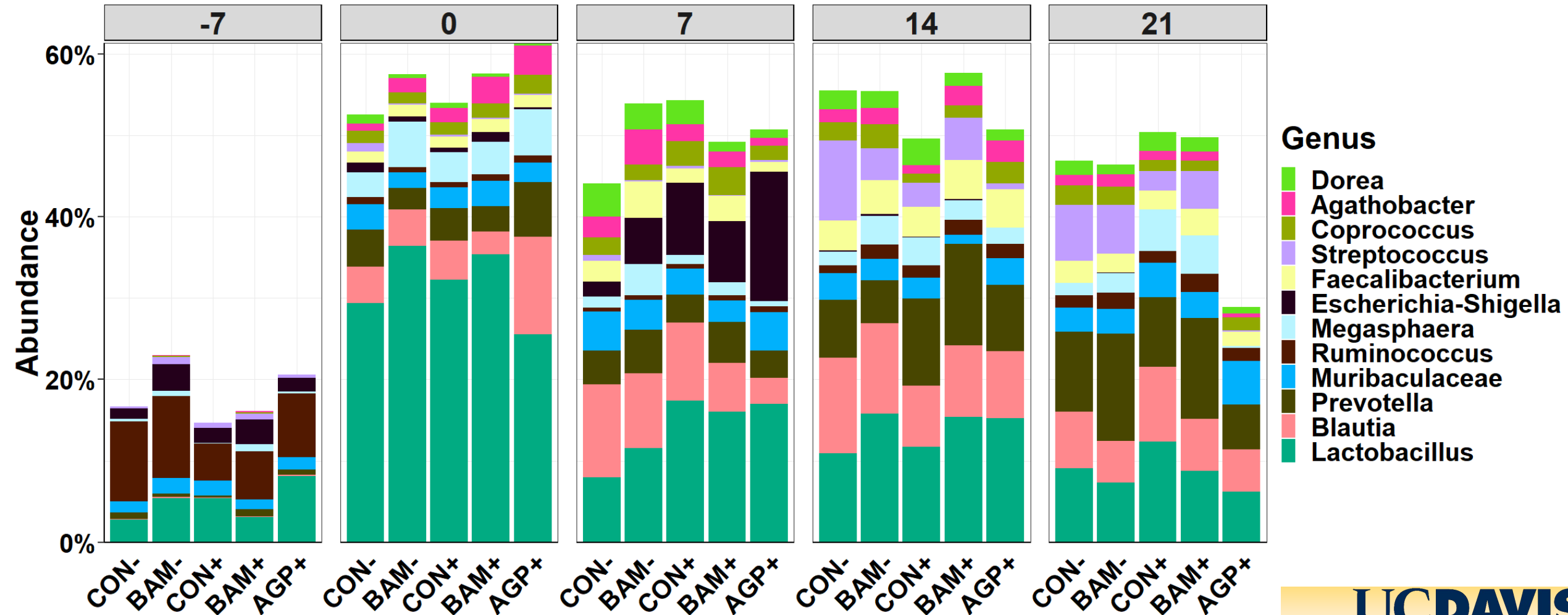
Firmicutes decreased between d 0 and 7 PI in AGP+

Proteobacteria increased between d 0 and 7 PI in CON and AGP



Feces: Genus

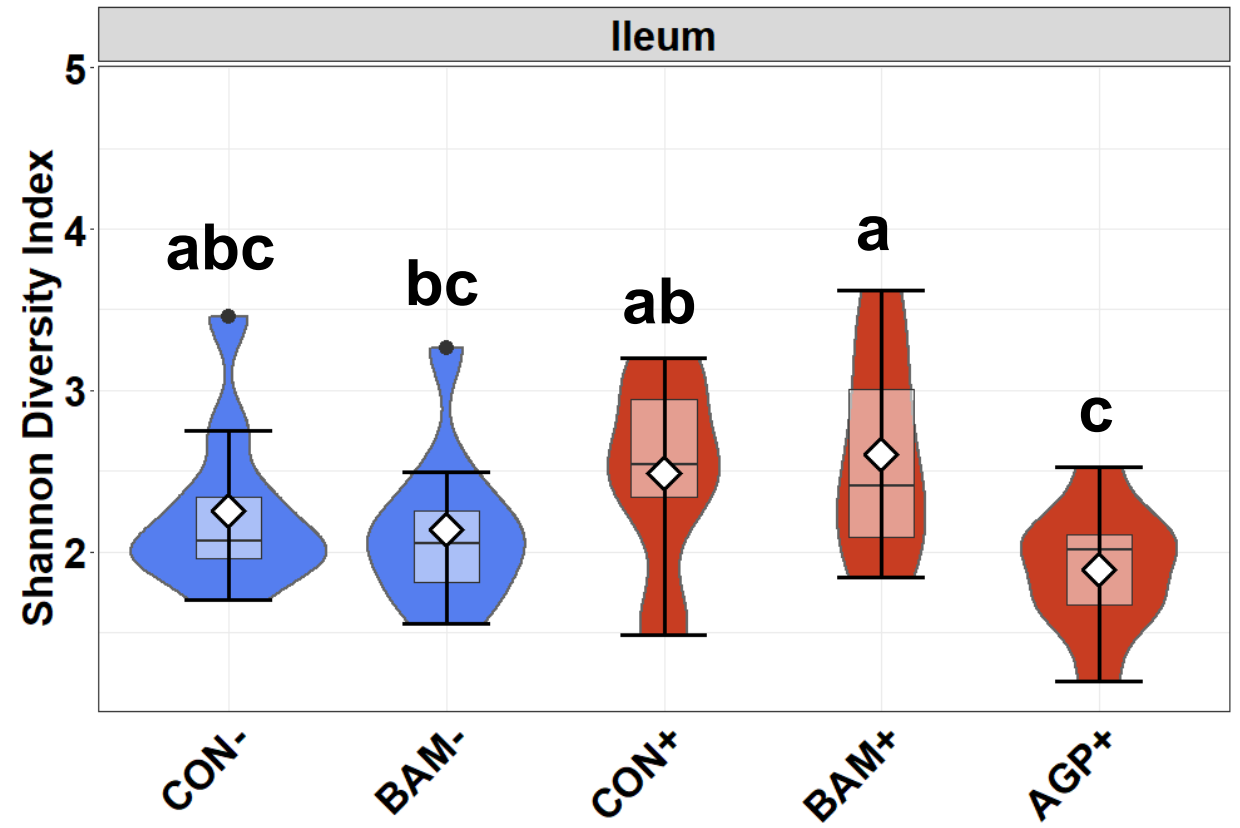
- *Lactobacillus* was greater in CON+ than CON- on d 7 PI
- *Blautia* and *Prevotella* was greater in AGP+ than in BAM+ on d 0
- *Prevotella* was greater in BAM+ than AGP+ in d 21 PI



Ileal microbiota

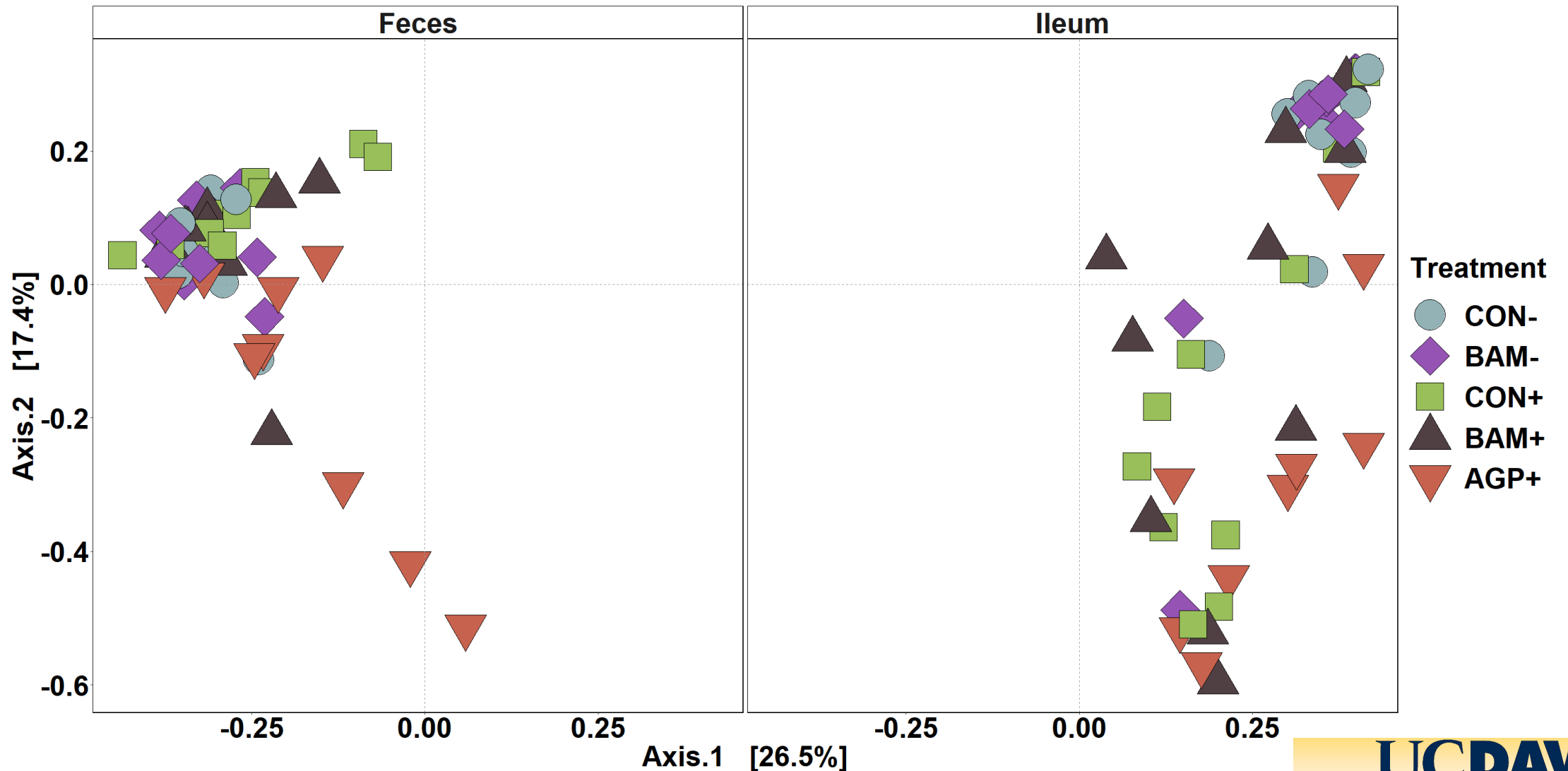
Alpha diversity (Shannon)

- Ileal digesta collected on d 21 PI
- CON+ and BAM+ had greater Shannon diversity index than AGP+ in ileum



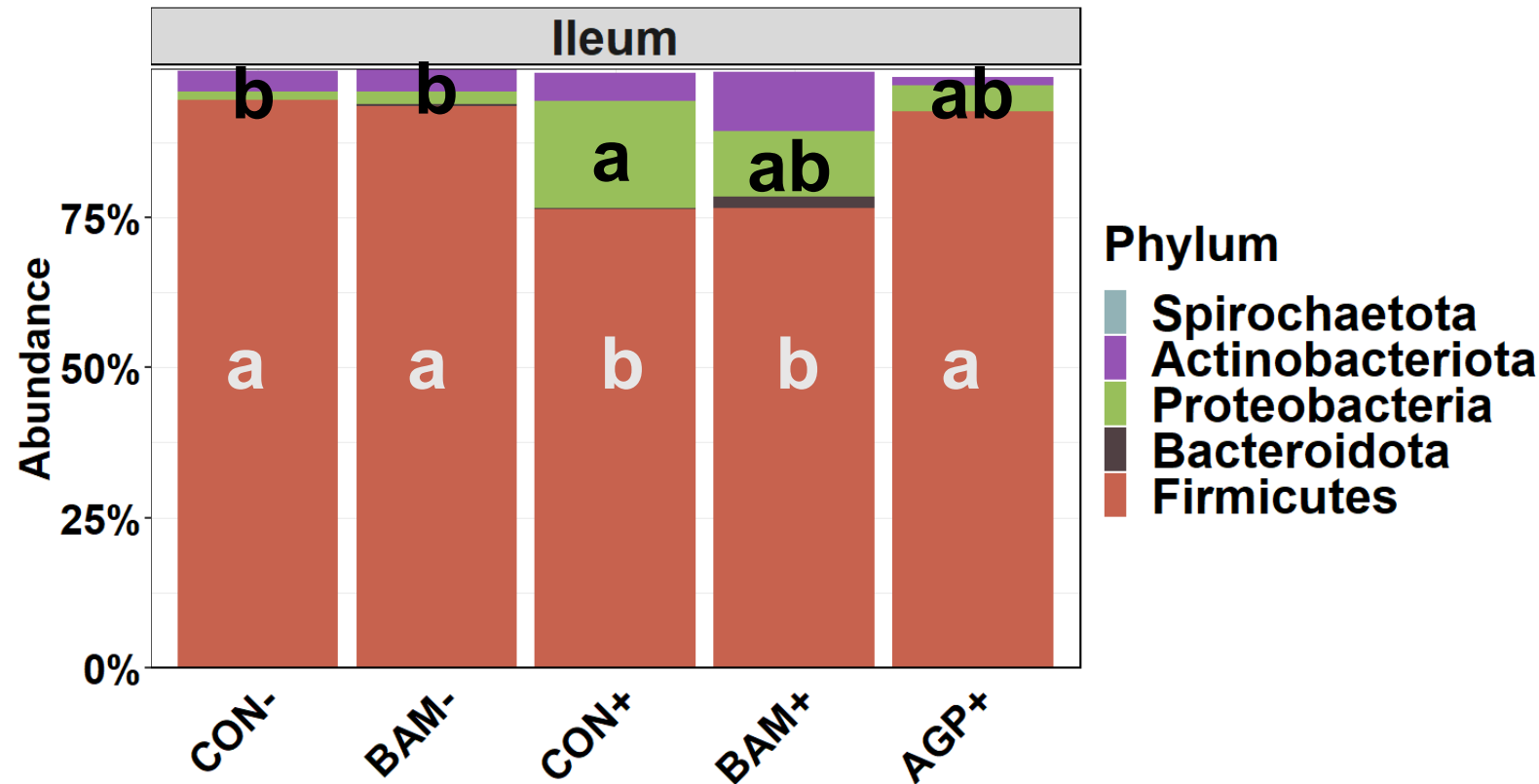
Ileum: Beta diversity

Separated clusters in ETEC infected and non-infected treatment groups in ileal digesta



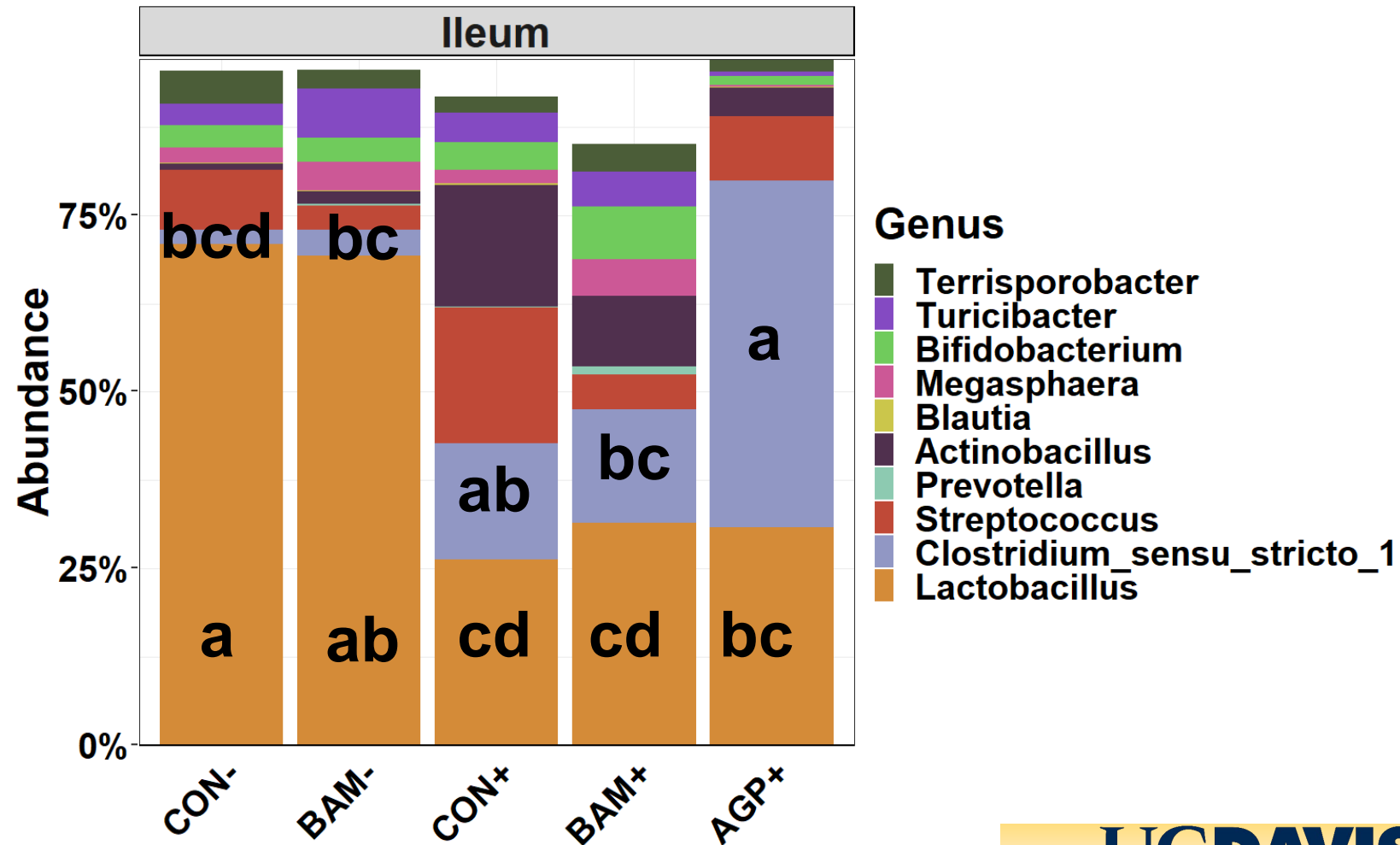
Ileum: Phylum

- **Firmicutes** was greater in AGP+ than in CON+ and BAM+ in ileal digesta
- **Proteobacteria** was greater in CON+ than in CON-



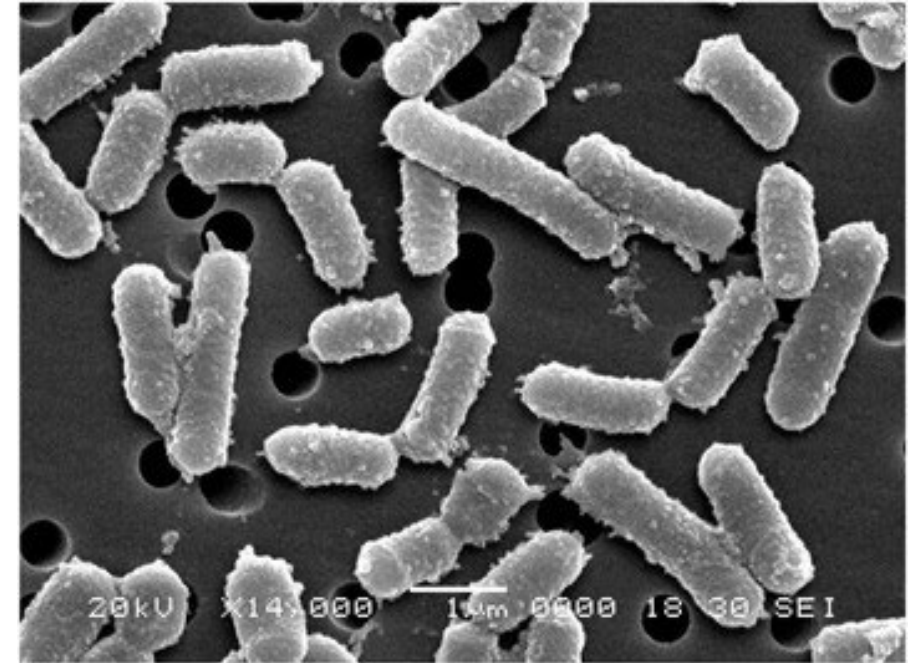
Ileum: Genus

- *Lactobacillus* was greater in CON- than in ETEC infected groups
- *Clostridium sensu stricto 1* was more abundant in AGP+ than BAM+ in ileal digesta



Conclusions

- *B. amyloliquefaciens* supplementation to weaned pigs challenged with ETEC
 - Tended to enhance growth performance
 - Had limited effects on diarrhea
 - Reduced systemic inflammation
 - Altered fecal and ileal microbiota differently from carbadox supplementation
- *B. amyloliquefaciens* solely may not provide weaned pigs with growth enhancement and acute diarrheal alleviation as similarly as carbadox



***Bacillus* spp. can vary in how they are used how they impact different animal species**

Acknowledgement

Major thanks

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 - Dr. Xiang Yang

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- Sidd

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