

# Dietary supplementation of *Bacillus subtilis* modified fecal microbiome of weaned pigs in comparison to antibiotics

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## ABSTRACT

The objective of this study was to investigate the effects of supplementing *Bacillus subtilis* on fecal microbiota of pigs experimentally infected with F-18 *Escherichia coli* (*E. coli*), in comparison to carbadox. Forty-eight weaned pigs ( $6.17 \pm 0.36$  kg BW) were individually housed and randomly allotted to one of four treatment (n =12): negative control (NC), positive control (PC), antibiotics (50 mg/kg of carbadox), and direct fed microbials (DFM, 500 mf/kg of *Bacillus subtilis*). The experiment lasted 28 days with 7 days before and 21 days after first *E. coli* inoculation (d 0). Pigs in the NC, PC, and DFM groups were orally inoculated with F18 *E. coli* for 3 consecutive days with 10 CFU/3 mL/dose. Fecal samples were collected on d -7 and 0 before *E. coli* inoculation, and d 7 and 21 post inoculation (PI). DNA were extracted from all fecal samples to perform 16S rRNA gene sequencing at the V4 hypervariable region. All data were analyzed with QIIME2 (2019.4) and R. Chao1 index was greatest ( $P < 0.05$ ) in feces collected on d 0 before *E. coli* inoculation and lowest ( $P < 0.05$ ) on d -7 feces. Pigs supplemented with DFM had lower ( $P < 0.05$ ) Chao1 index than pigs fed with antibiotics on d 21 PI. Bray-Curtis PCoA displayed separate clusters among days but overlaps among treatments. Bacteroidetes and Proteobacteria were most ( $P < 0.05$ ) abundant on d -7 and lowest ( $P < 0.05$ ) on d 21 PI. However, Actinobacteria and Firmicutes were most ( $P < 0.05$ ) abundant on d 21 PI. Pigs in the NC and DFM groups had greater ( $P < 0.05$ ) relative abundance of Firmicutes than pigs fed with antibiotics on d 0 and 7. Supplementation of antibiotics reduced ( $P < 0.05$ ) the relative abundance of *Lactobacillaceae* compared with other treatments on d 0 PI. In conclusion, both animal age and dietary treatments influenced the fecal microbiome of weaned pigs.

### Keywords

*Bacillus subtilis*, fecal microbiome, weaned pigs

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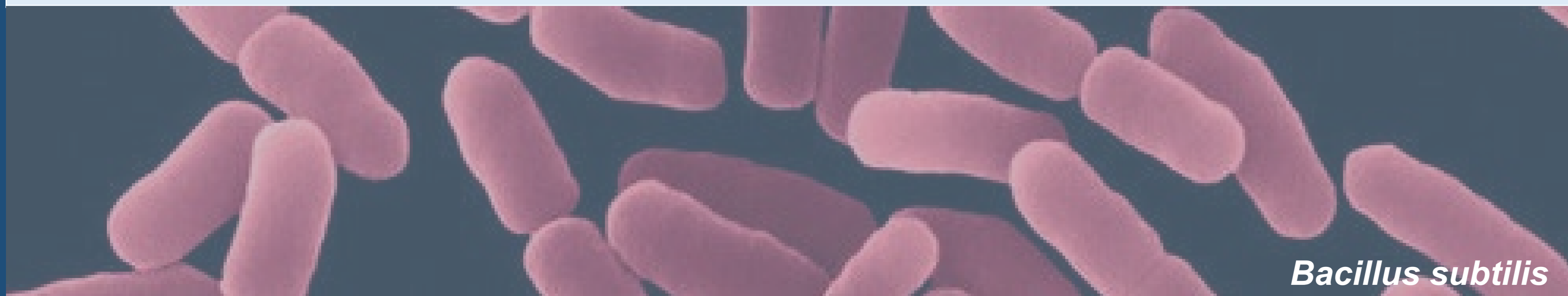
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## INTRODUCTION

- Enterotoxigenic *E. coli*
  - Common type of *E. coli* that causes post-weaning diarrhea
- Post-weaning diarrhea
  - Can be induced by stress, sudden change in diet, increased exposure to enterotoxigenic pathogens
  - Often leads to weight loss and possibly death
  - Economical losses
- Probiotics
  - Live microorganisms confer a health benefit on host when administered in adequate amounts (UNFAO/WHO 2001)
  - Potential alternatives to antibiotics
- *Bacillus subtilis*
  - Gram positive, aerobic bacteria
  - Stable in ambient temperature
  - Spores resistant to harsh environment



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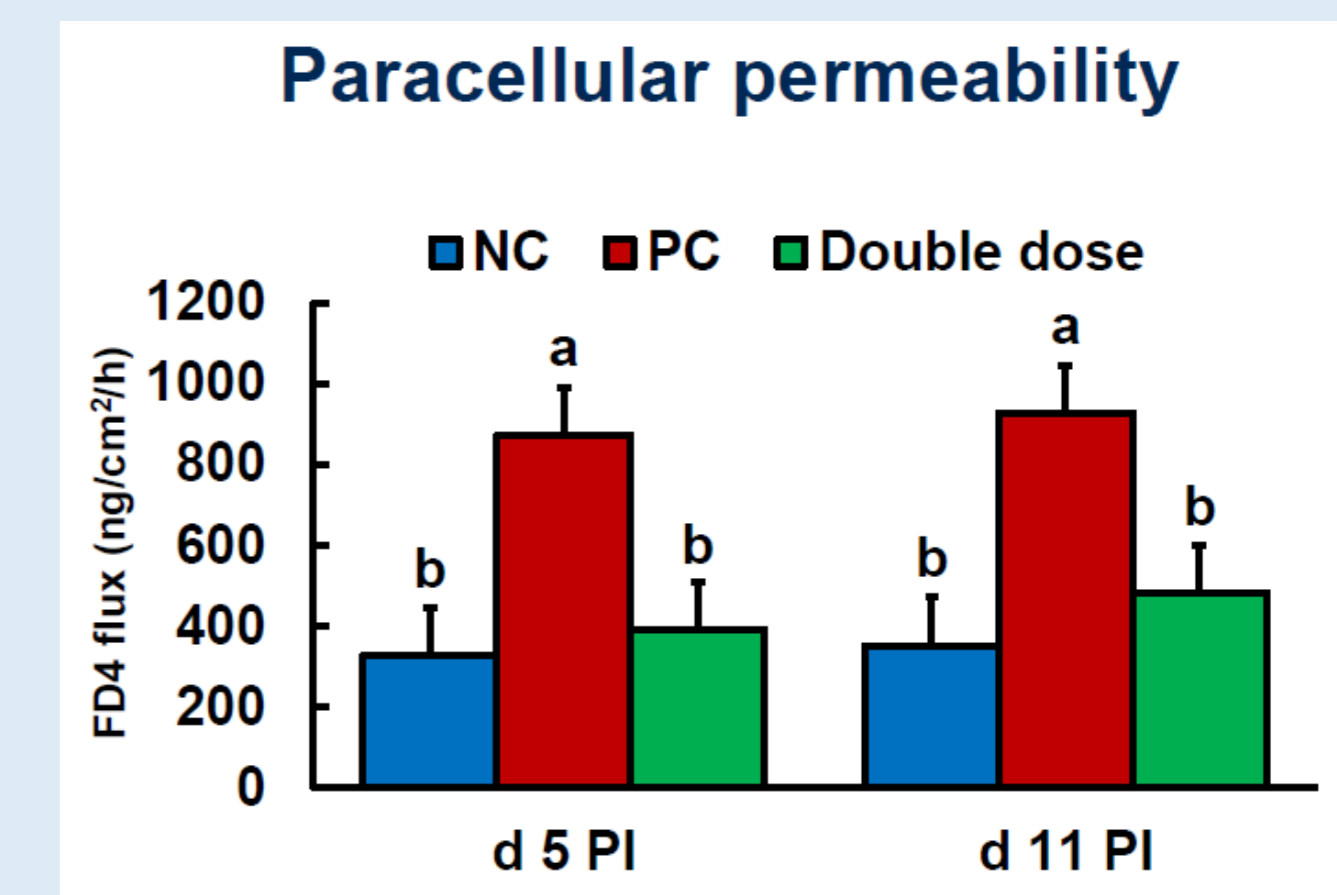
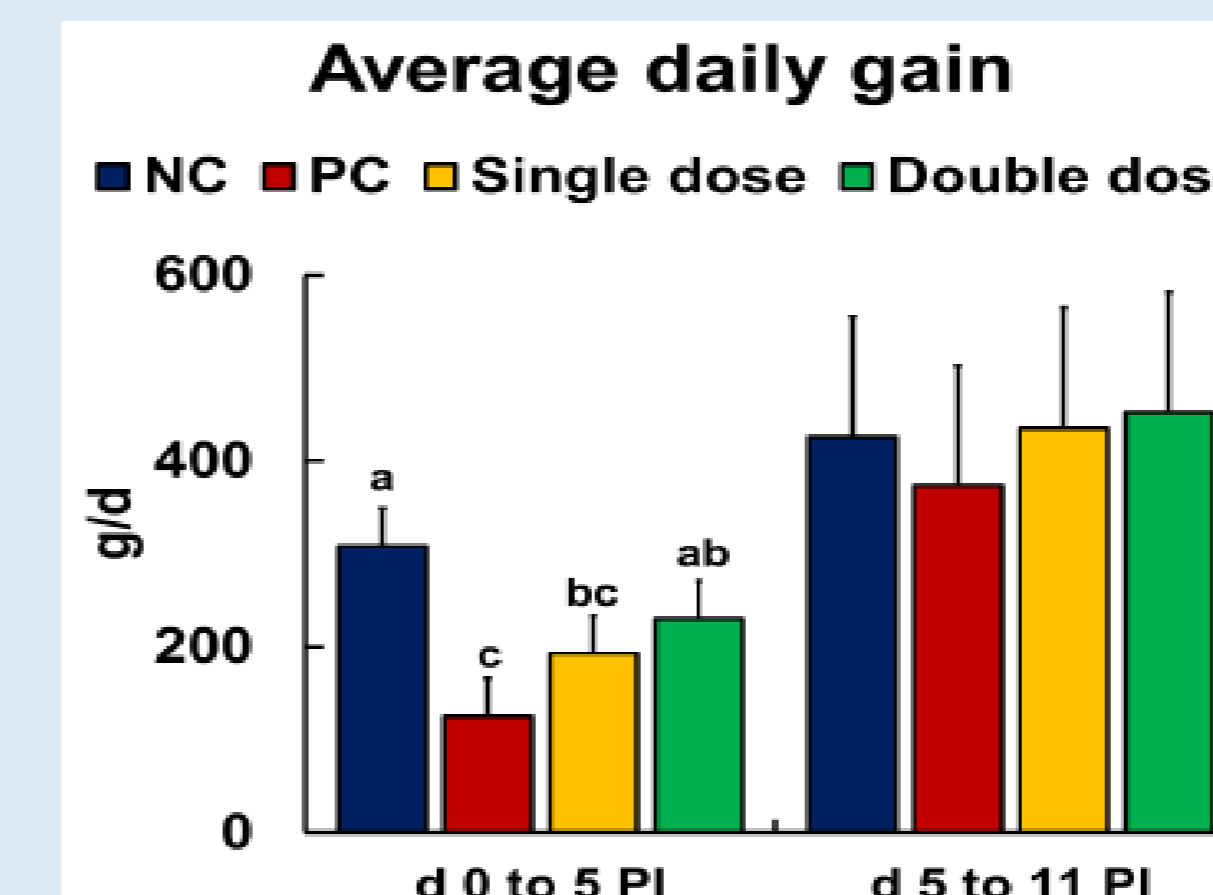
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## SUPPLEMENTATION OF *B. SUBTILIS* TO WEANED PIGS

- Dietary supplementation of *B. subtilis* affected weaned pigs challenged with F18 *E. coli* (Kim et al., 2019)
  - Improved growth rate
  - Reduced leaky gut
  - Enhanced gut barrier function



## WHY LOOK INTO MICROBIOTA?

- Gut microbiota = collection of microbe living inside the gut
- Microbes in intestines play a role in keeping the host health
  - Nutrition: produce VFAs
  - Physiology: maintain gut structure and barrier function
  - Immune system: sIgA regulation

## OBJECTIVE

To observe the effects of supplementing *Bacillus subtilis* on fecal microbiota of pigs experimentally infected with F-18 *Escherichia coli*.

## MATERIALS AND METHODS

### Step 1: Animal experiment

#### Pigs

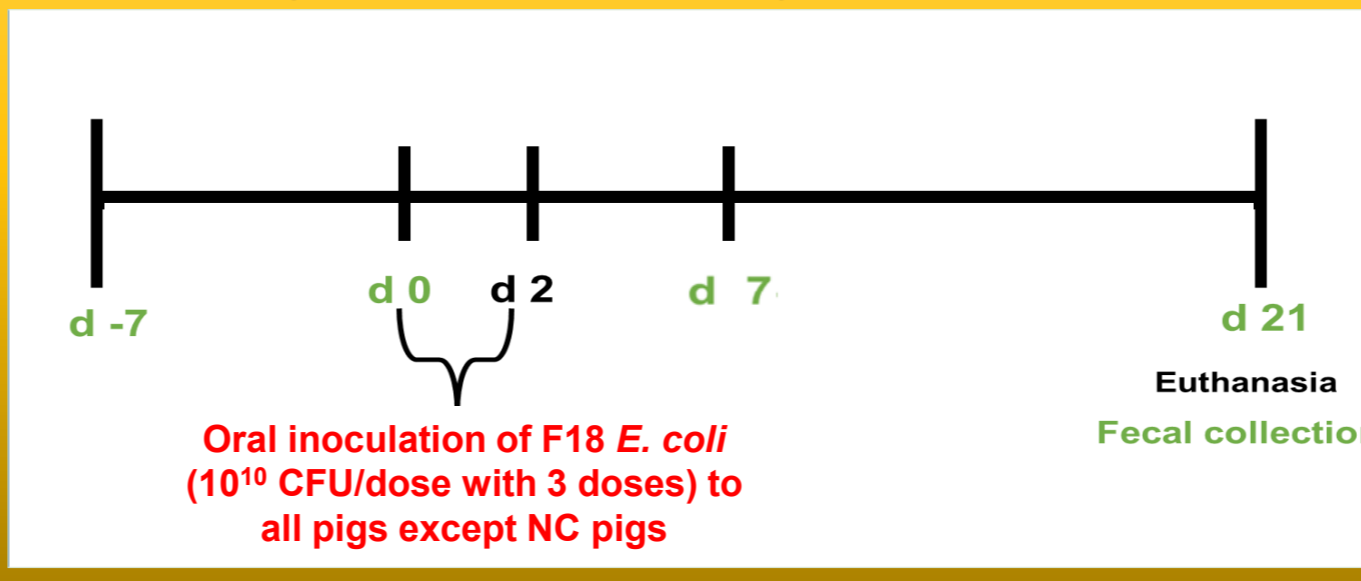
- 48 weanling pigs
- Average BW 6.17 kg
- Weaned at 21 d of age into 3 confined nursery rooms
- Pigs are individually housed and had access to feed and water *ad libitum*

#### Treatment

- 12 pigs per treatment
- **Negative control (NC):** nursery basal diet not infected with *E. coli*
- **Positive control (PC):** nursery basal diet infected with *E. coli*
- **Antibiotics (AGP):** 50 mg/kg carbadox
- **Directed fed microbials (DFM):** 500 mg/kg *Bacillus subtilis*

#### Design

- Randomized complete block design
- Blocking factors: BW x gender



#### DNA extraction

- Zymo Research miniprep kit

#### PCR

- Amplified at V4 hypervariable region (primers 806R & 515F with 8 bp barcode and Illumina adapter on the 5' end)
- Samples pooled after PCR
- Purified with Qiagen purification kit

#### Sequencing

- Illumina MiSeq platform
- 250bp paired-end sequencing
- Sequencing outputs were in Fastq files

### Step 2: 16S rRNA Sequencing: Library preparation

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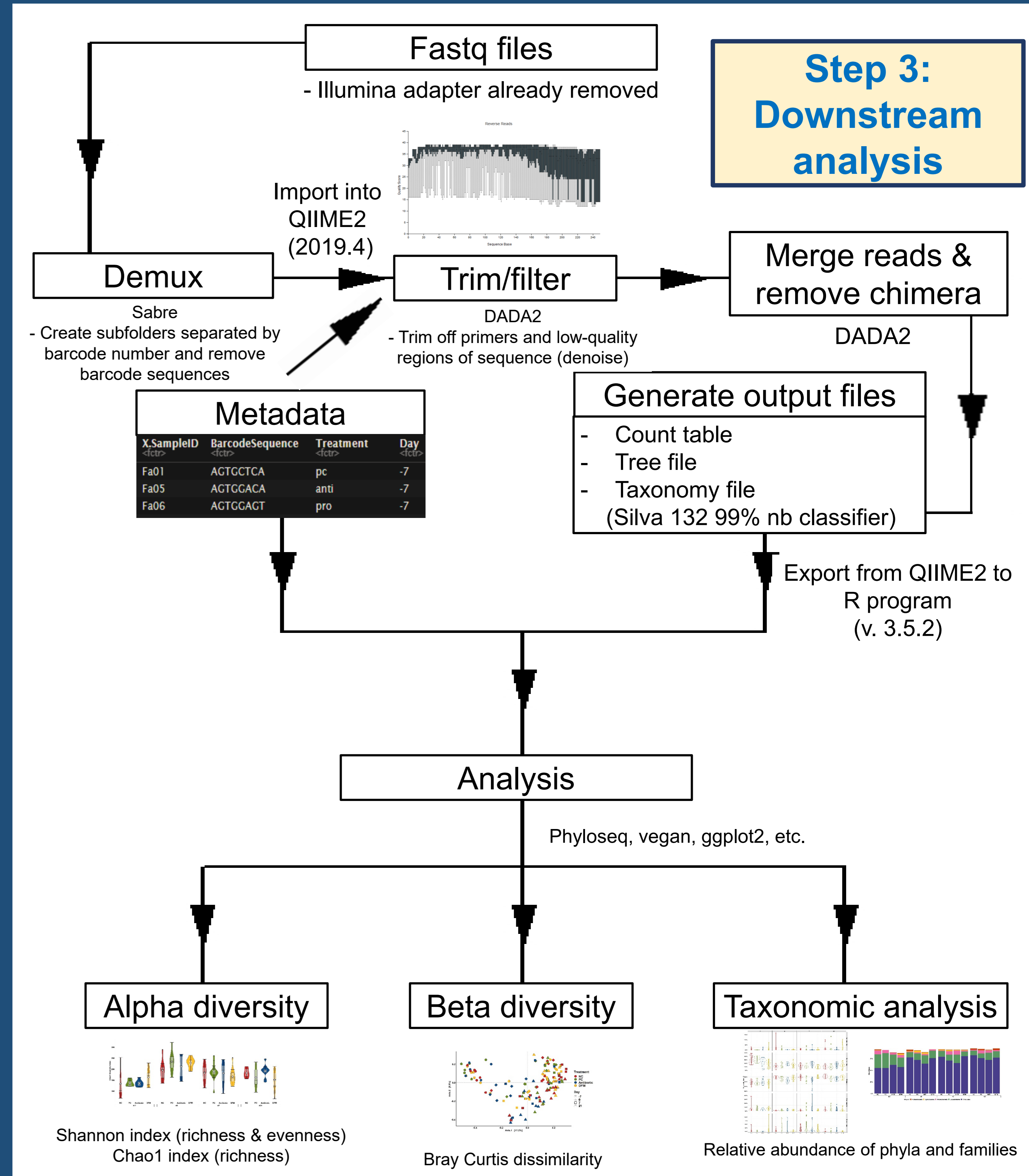
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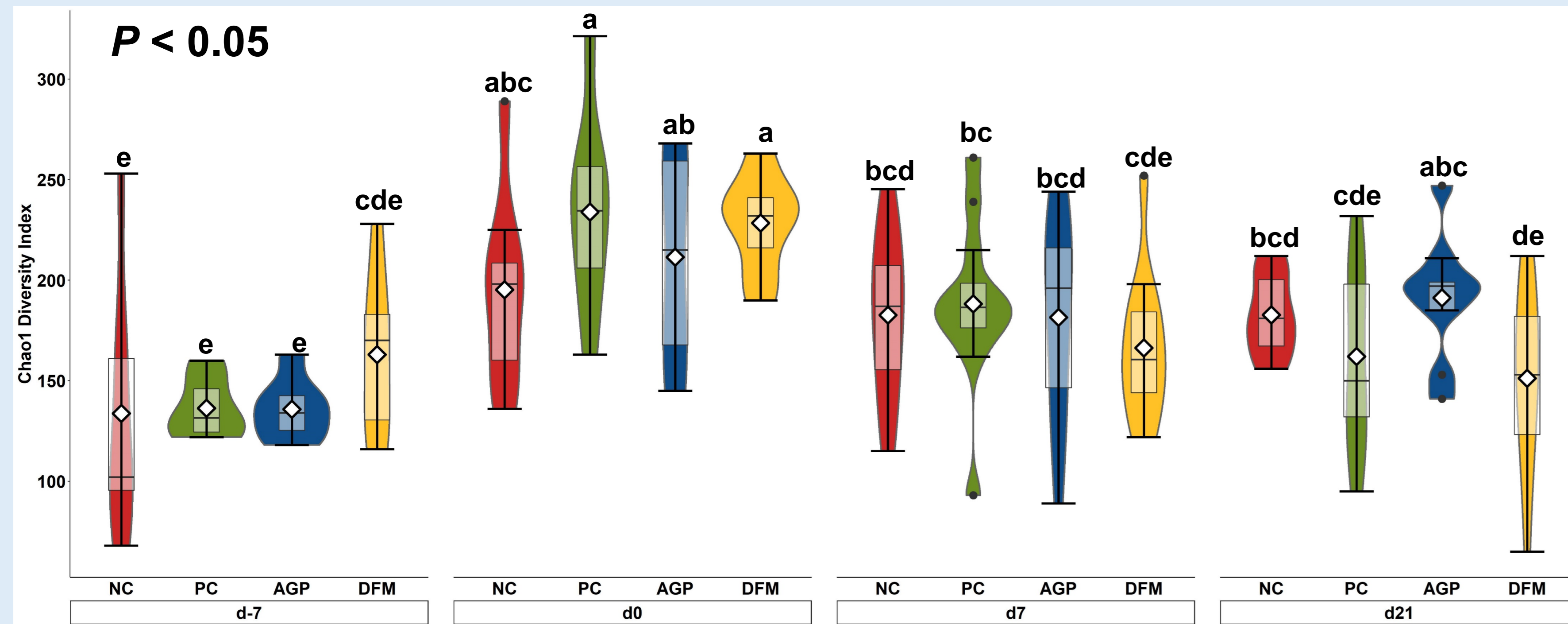
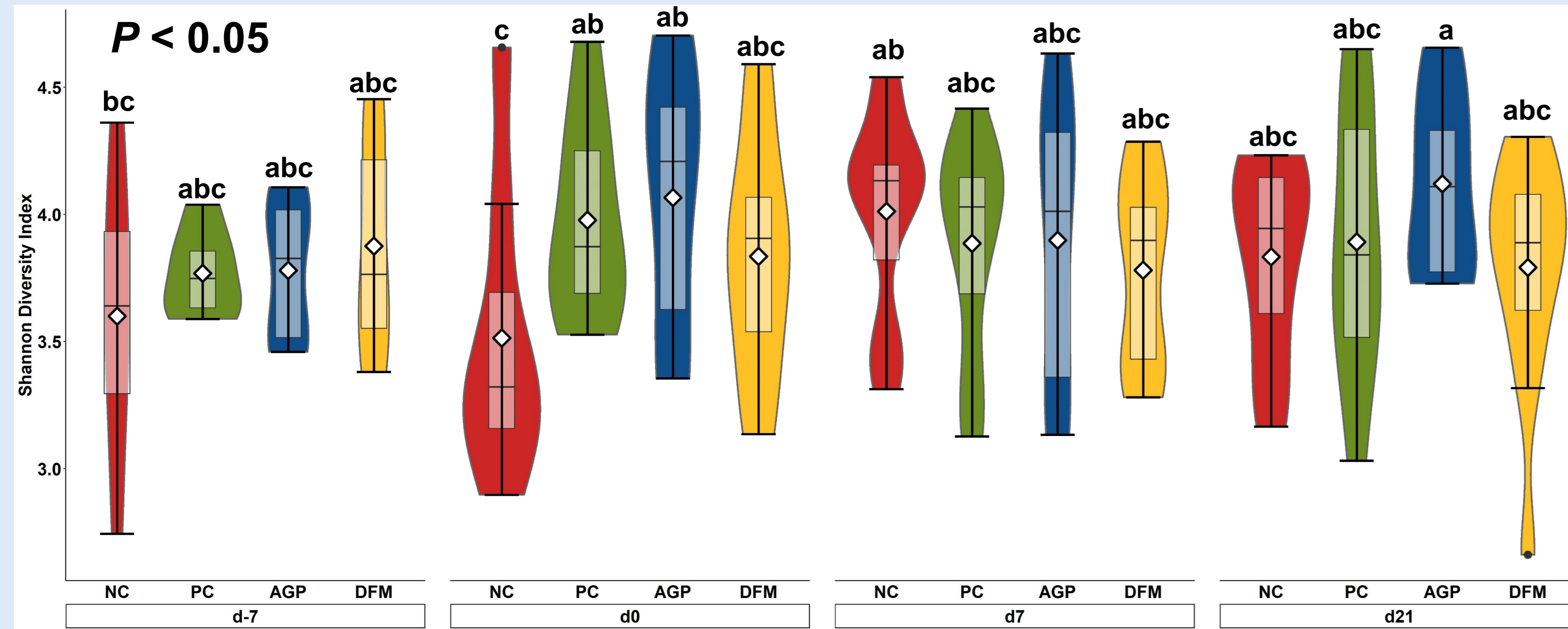


### Step 3: Downstream analysis

Statistical analysis using R:  
Chao1 index and taxonomic analysis were analyzed with Kruskal-Wallis and Conover test using agricolae package.  
Shannon index was analyzed using ANOVA and emmeans package.  
Beta diversity was analyzed with Adonis and betadisper function using vegan (v. 2.5-4) & pairwiseAdonis packages.

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## ALPHA DIVERSITY



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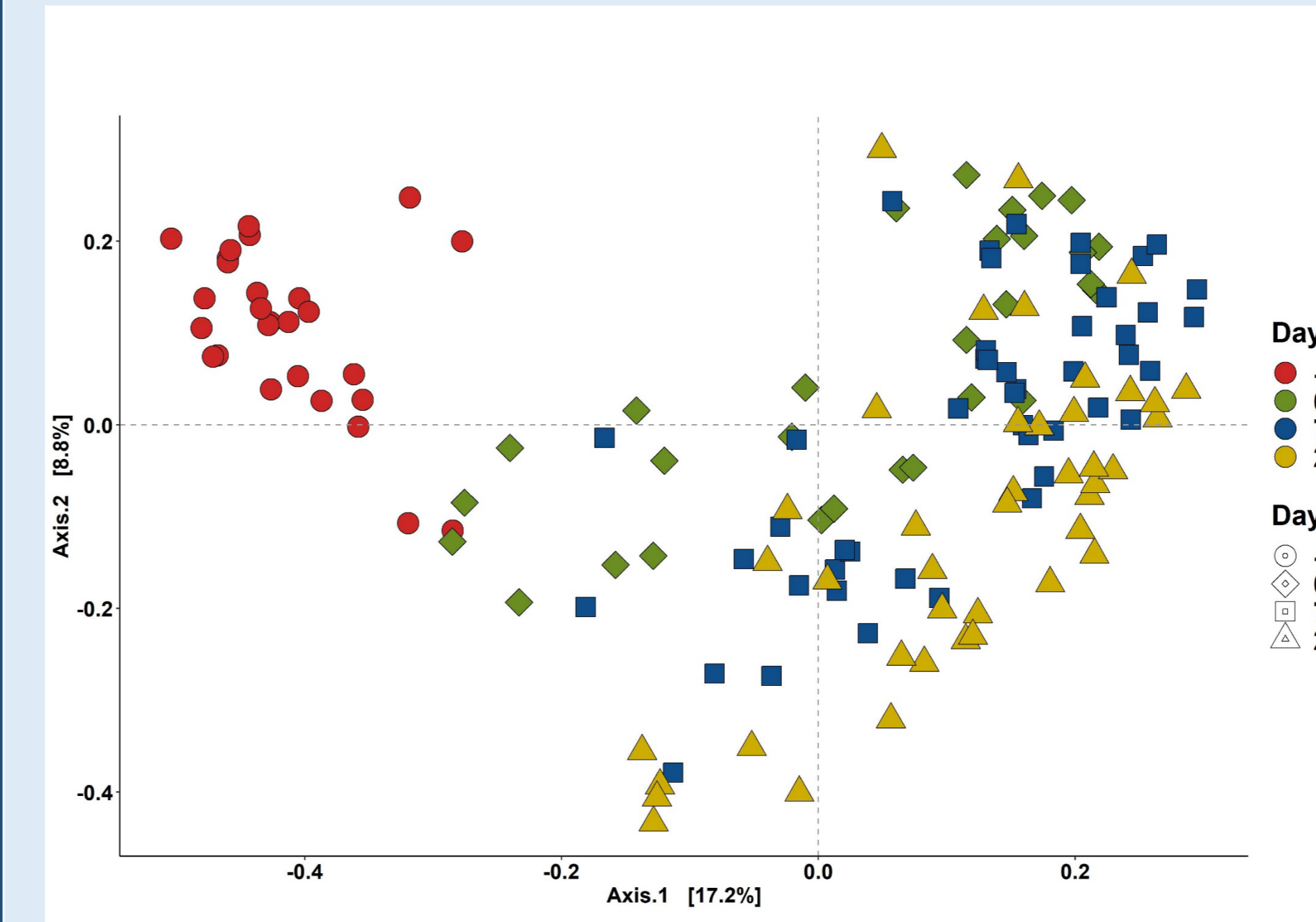
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## BETA DIVERSITY

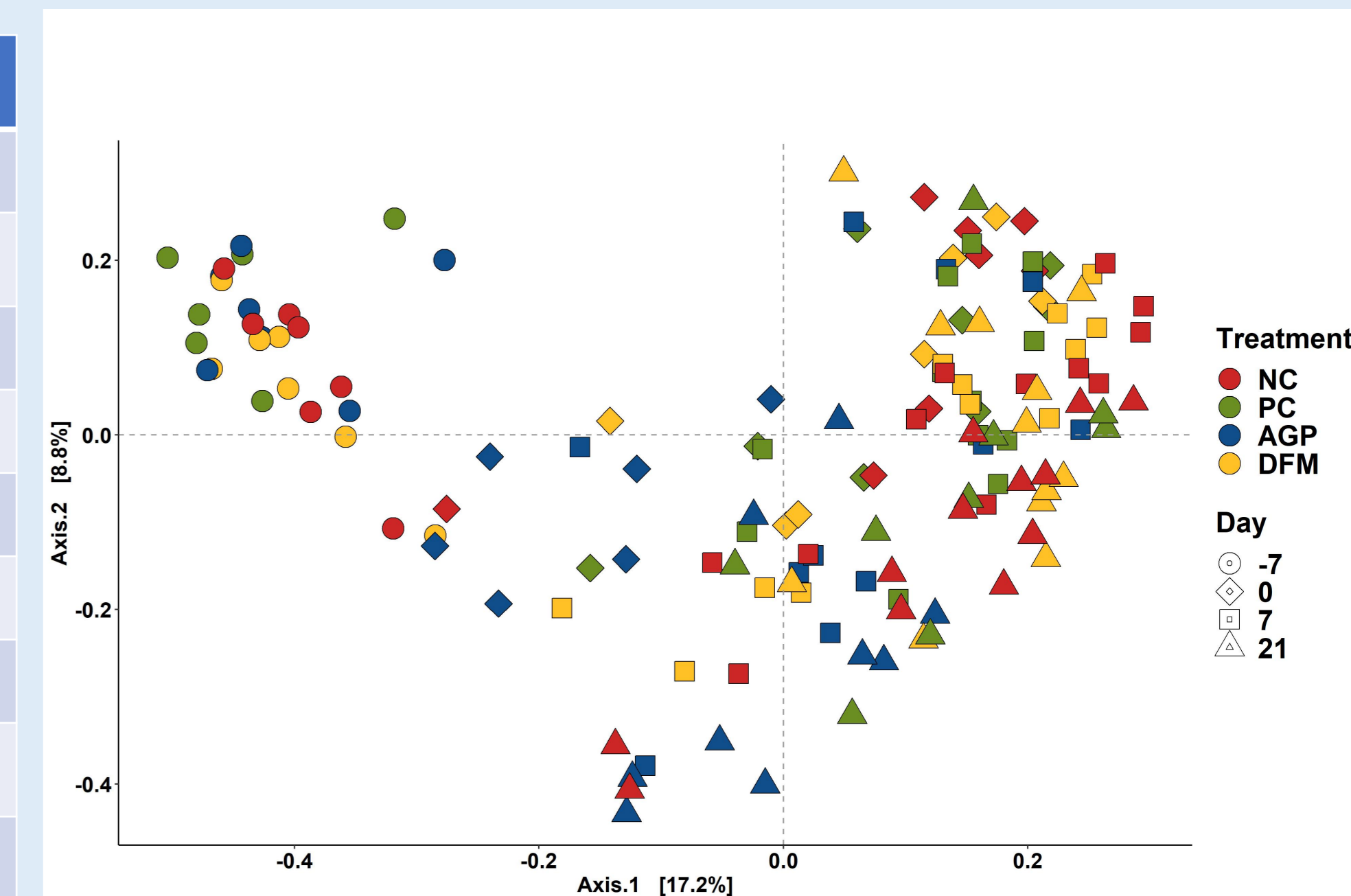


### Pairwise Adonis [Day]

pairs <fctr>	Df <dbl>	SumsOfSqs <dbl>	F.Model <dbl>	R2 <dbl>	p.value <dbl>
-7 vs 0	1	3.552898	13.021772	0.19429167	0.001
-7 vs 7	1	5.400005	21.265348	0.22800910	0.001
-7 vs 21	1	5.548391	20.283238	0.23238412	0.001
0 vs 7	1	1.000001	4.122887	0.05277438	0.001
0 vs 21	1	2.016086	7.731386	0.10075911	0.001
7 vs 21	1	1.771063	7.152440	0.07596659	0.001

### Pairwise Adonis [Day:Treatment] with $P < 0.05$

Day	Pairs	Df	SumsOfSqs	F.Model	R2	p.value
d 0	PC vs AGP	1	0.51	1.93	0.14	0.014
	AGP vs DFM	1	0.50	1.82	0.14	0.010
d 7	AGP vs NC	1	0.69	2.59	0.18	0.006
	PC vs NC	1	0.38	1.85	0.08	0.007
	AGP vs NC	1	0.47	2.00	0.09	0.017
d 21	DFM vs NC	1	0.39	1.81	0.08	0.031
	PC vs AGP	1	0.60	2.21	0.12	0.016
	AGP vs DFM	1	0.80	3.15	0.14	0.004
	AGP vs NC	1	0.69	2.72	0.13	0.013
	DFM vs NC	1	0.42	1.87	0.08	0.017



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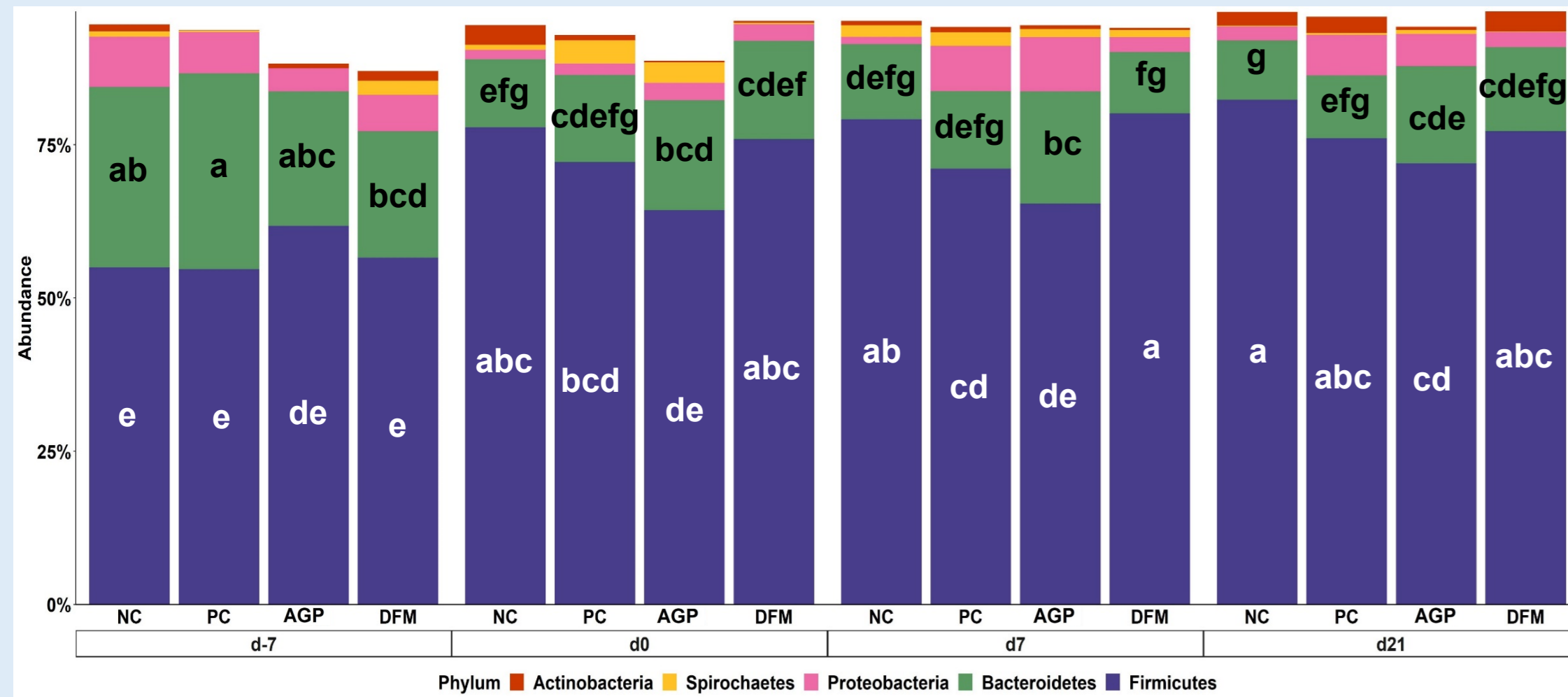
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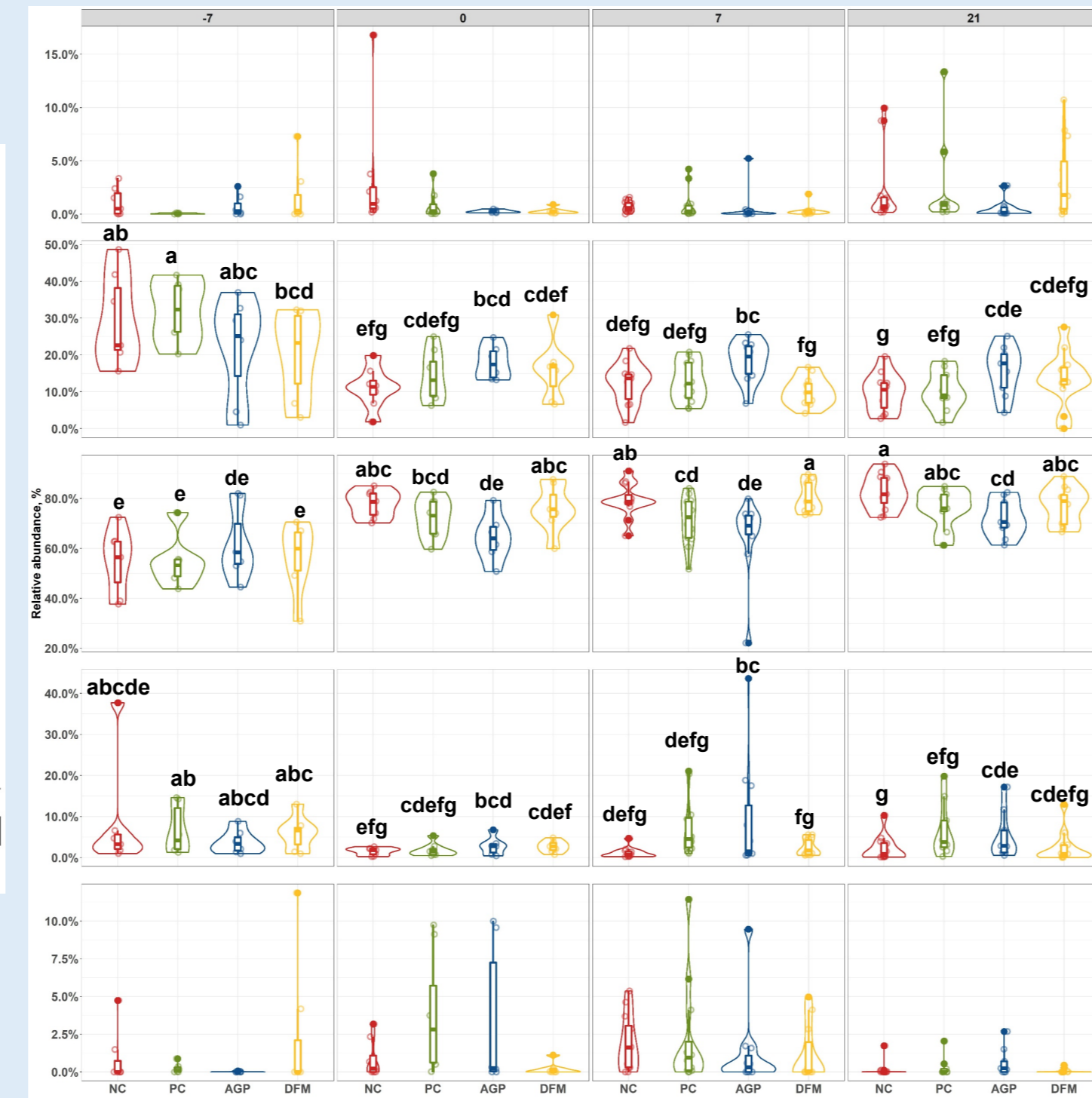
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## RELATIVE ABUNDANCE

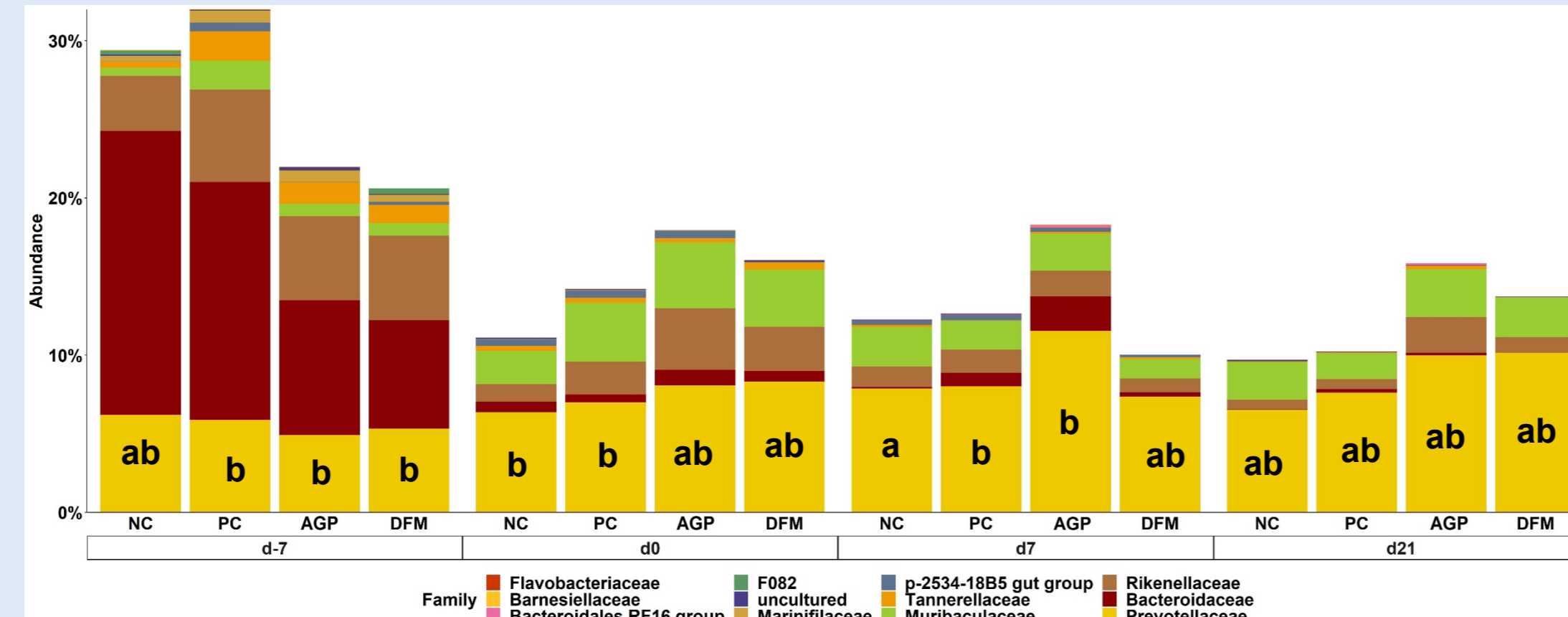
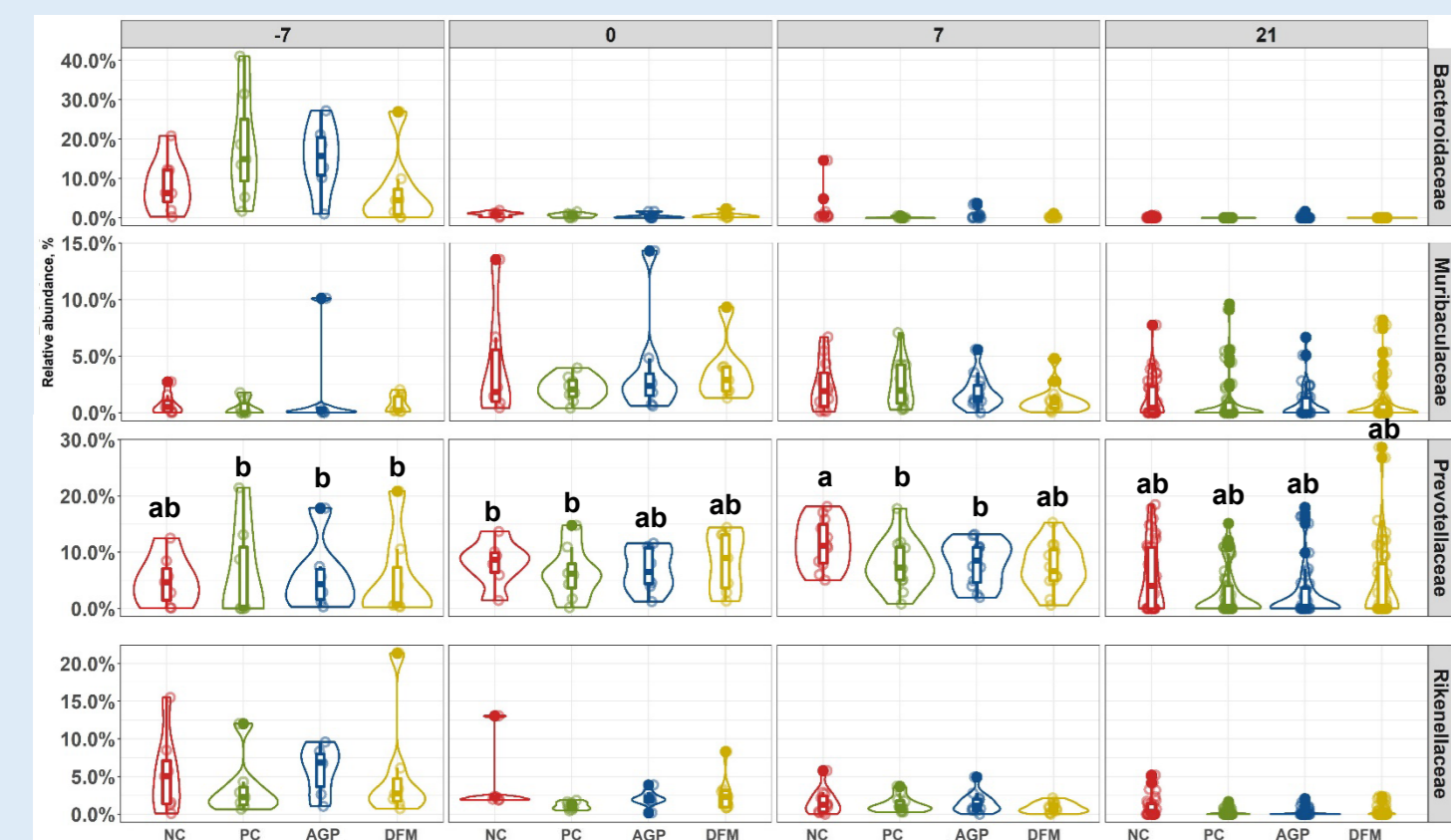
### Phylum



$P < 0.05$



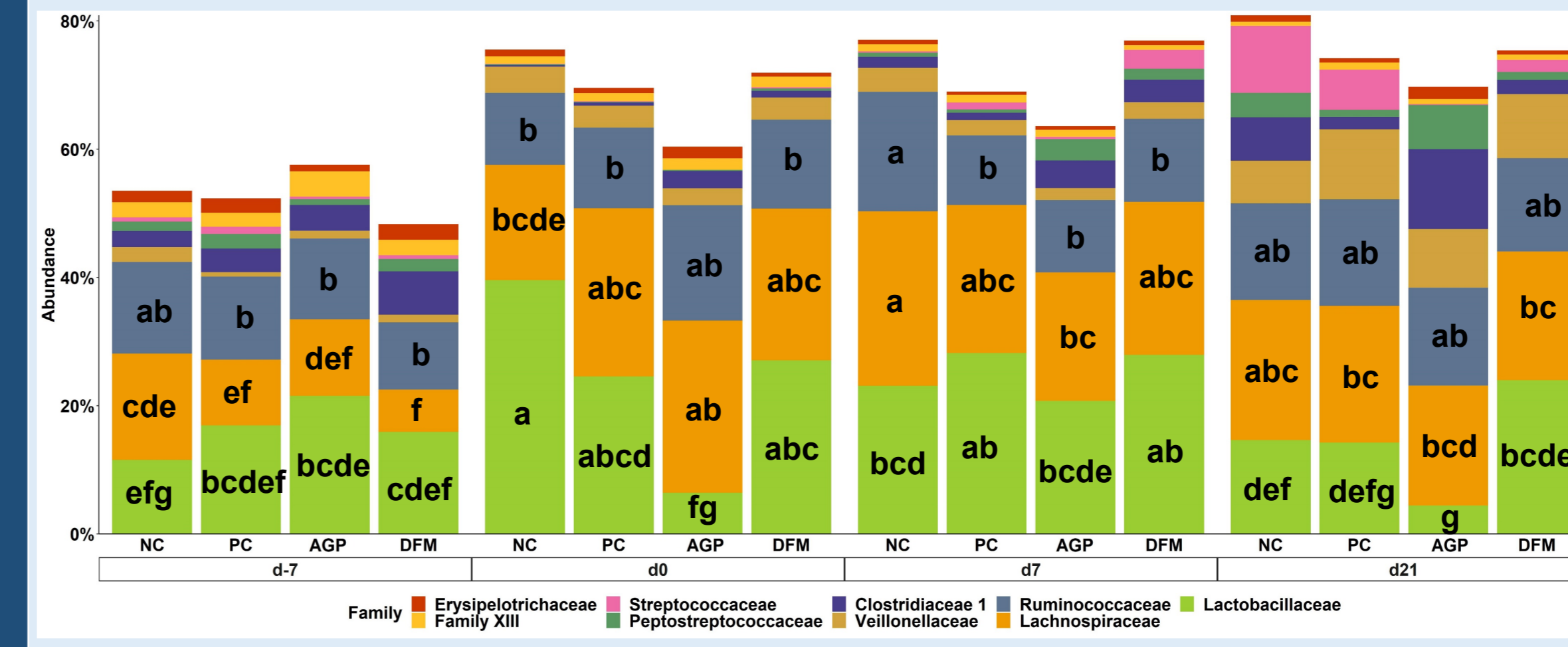
### Bacteroidetes family



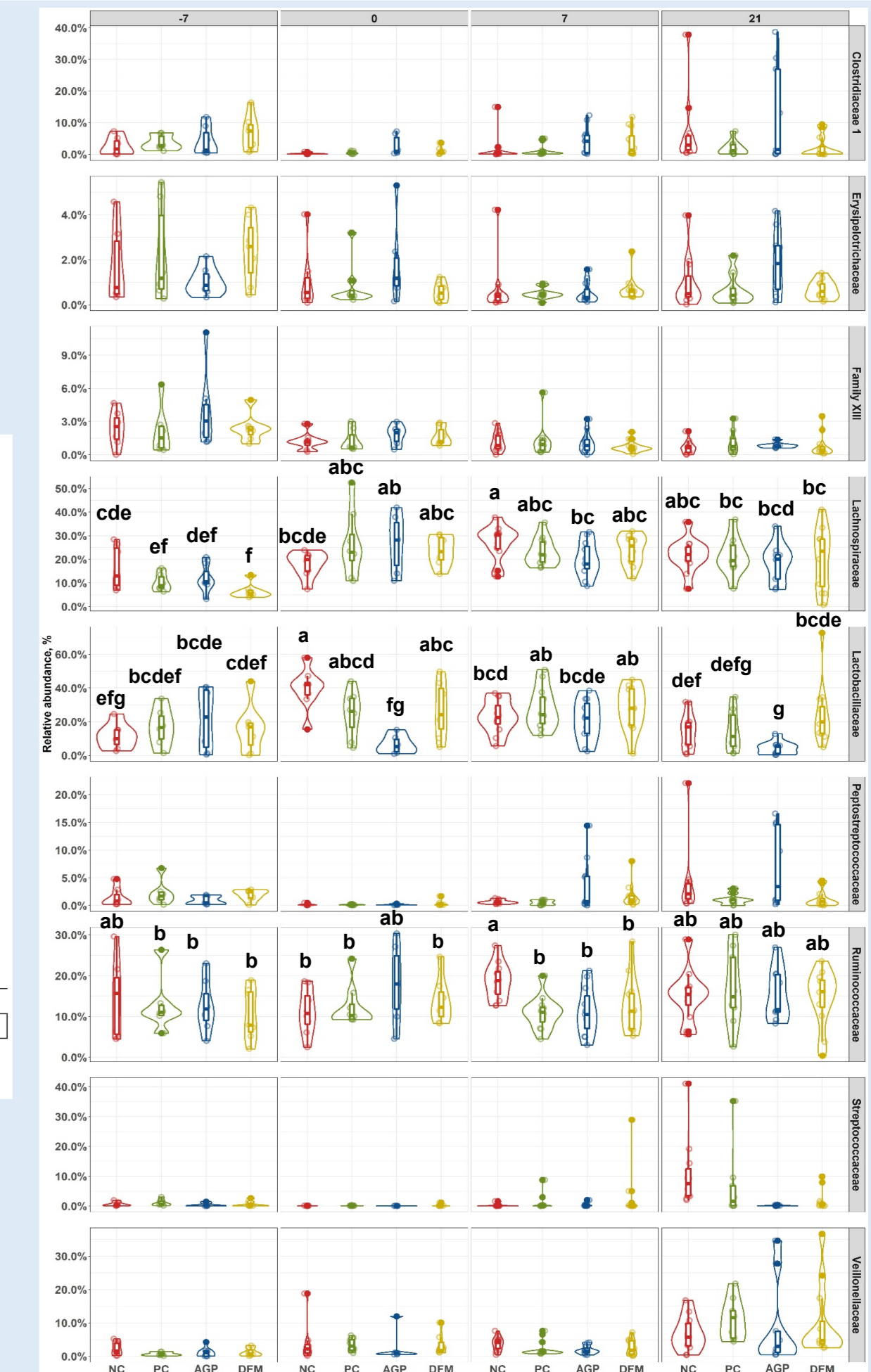
## RELATIVE ABUNDANCE

### Firmicutes family

$P < 0.05$



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## SEQUENCE SUMMARY

- Number of taxa: 3430 species
- Sample sequencing depth

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
1	10902	13369	13803	16159	30047

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## CONCLUSIONS

- Supplementation of *Bacillus subtilis* alters the fecal microbiome of weaned pigs challenged with pathogenic *E. coli*
- Fecal microbiome altered differently when supplementing *B. subtilis* and antibiotics to weaned pigs
- Further analysis will evaluate the metagenomic of the microbiota of weaned pigs supplemented with *Bacillus subtilis*

## REFERENCES

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- Kim, K., Y. He, X. Xiong, A. Ehrlich, X. Li, H. Raybould, E. R. Atwill, E. A. Maga, J. Jørgensen, and Y. Liu. 2019. Dietary supplementation of *Bacillus subtilis* influenced intestinal health of weaned pigs experimentally infected a pathogenic *E. coli*. *J. Anim. Sci. Biotechnol.* 10:52-63.

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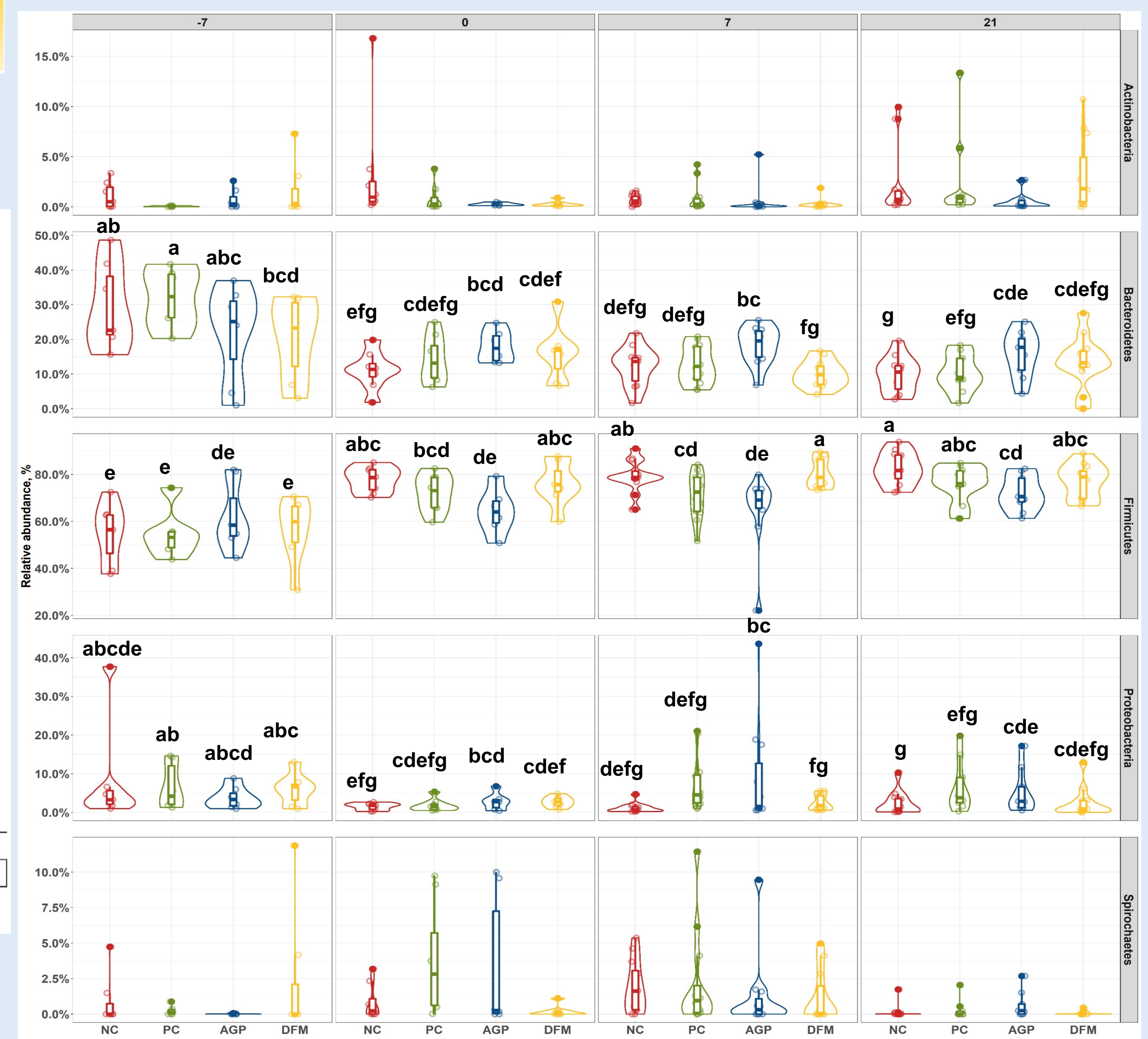
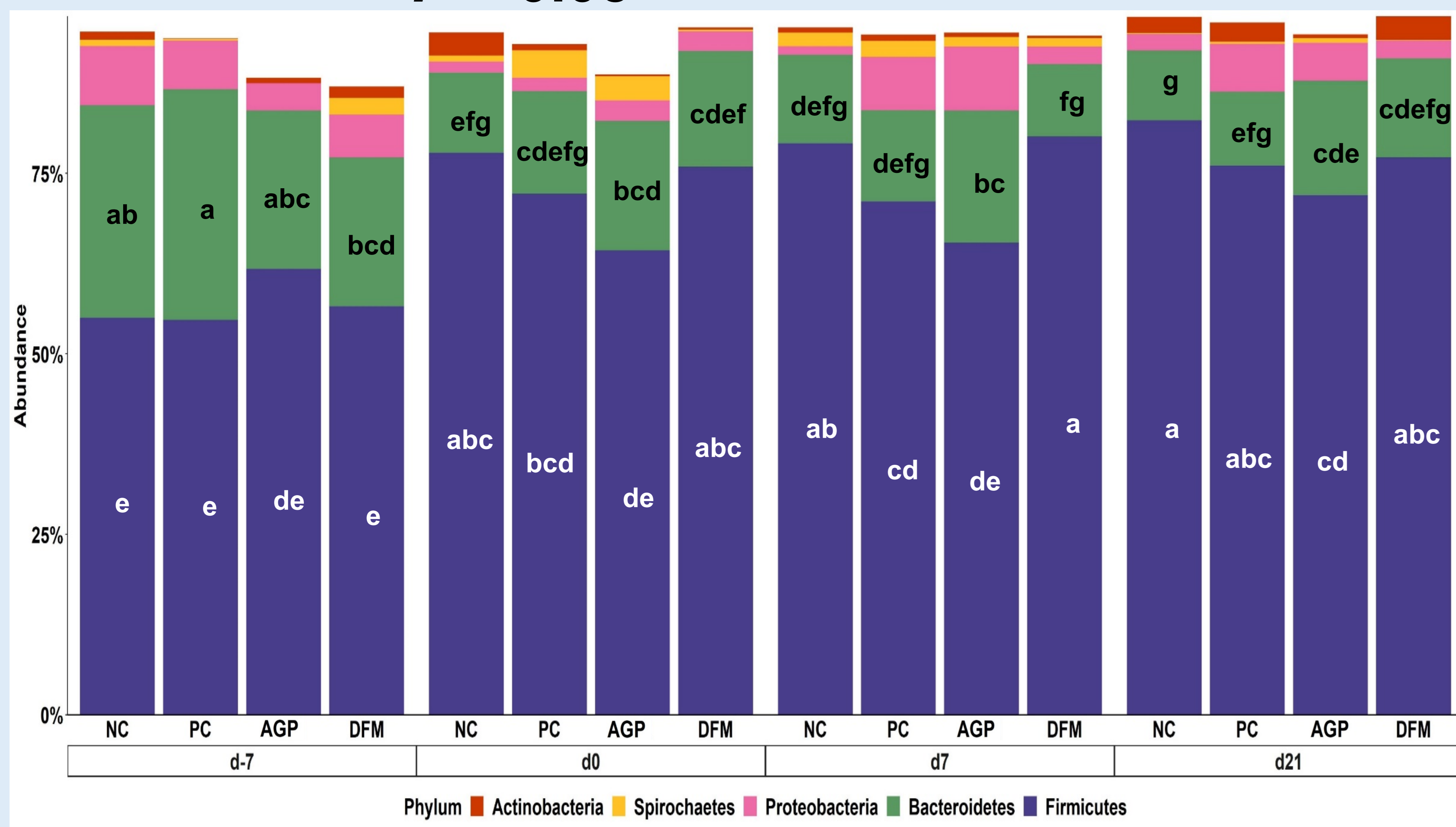
PHYLA

FIRMICUTES  
FAMILY

BACTEROIDETES  
FAMILY

## Phyla

$P < 0.05$



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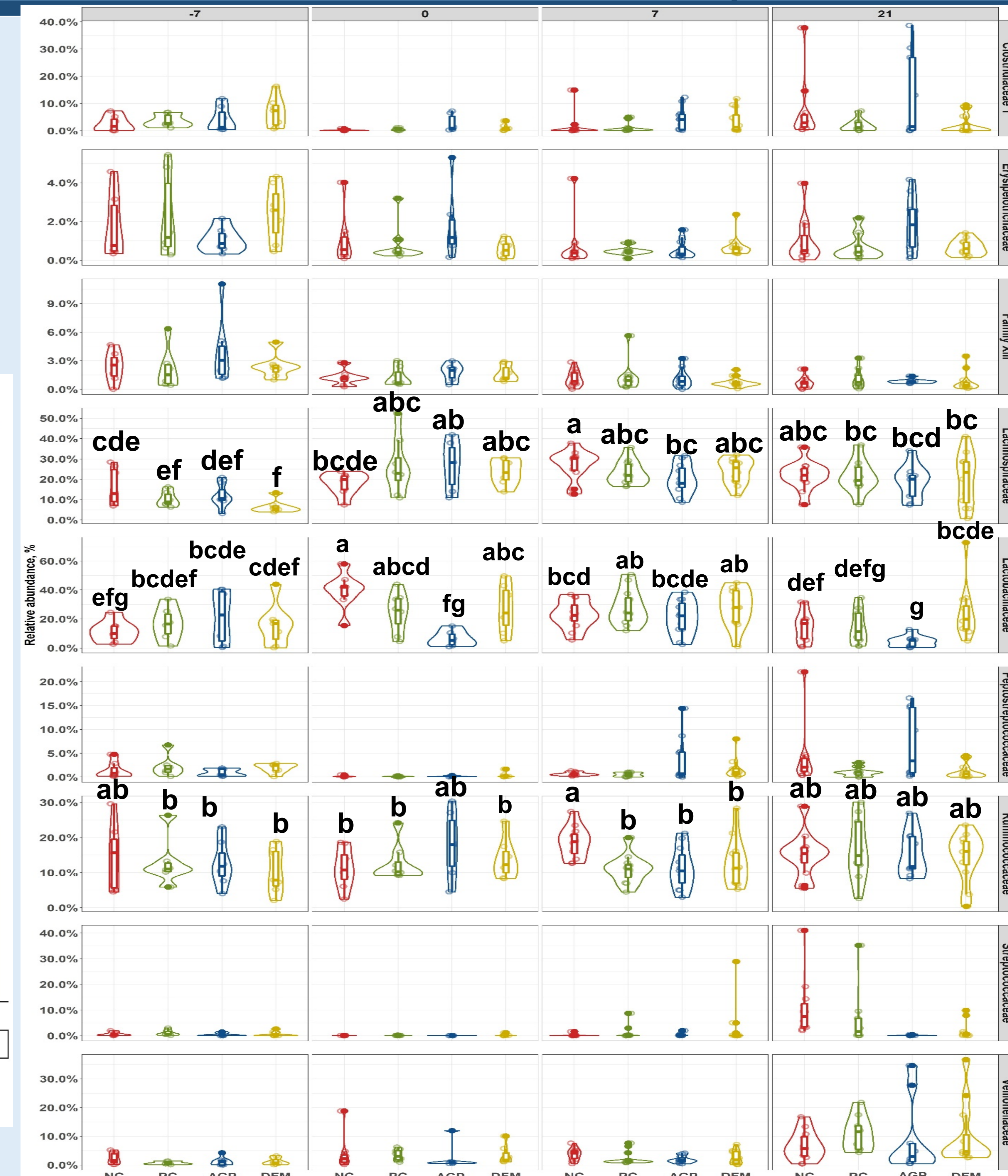
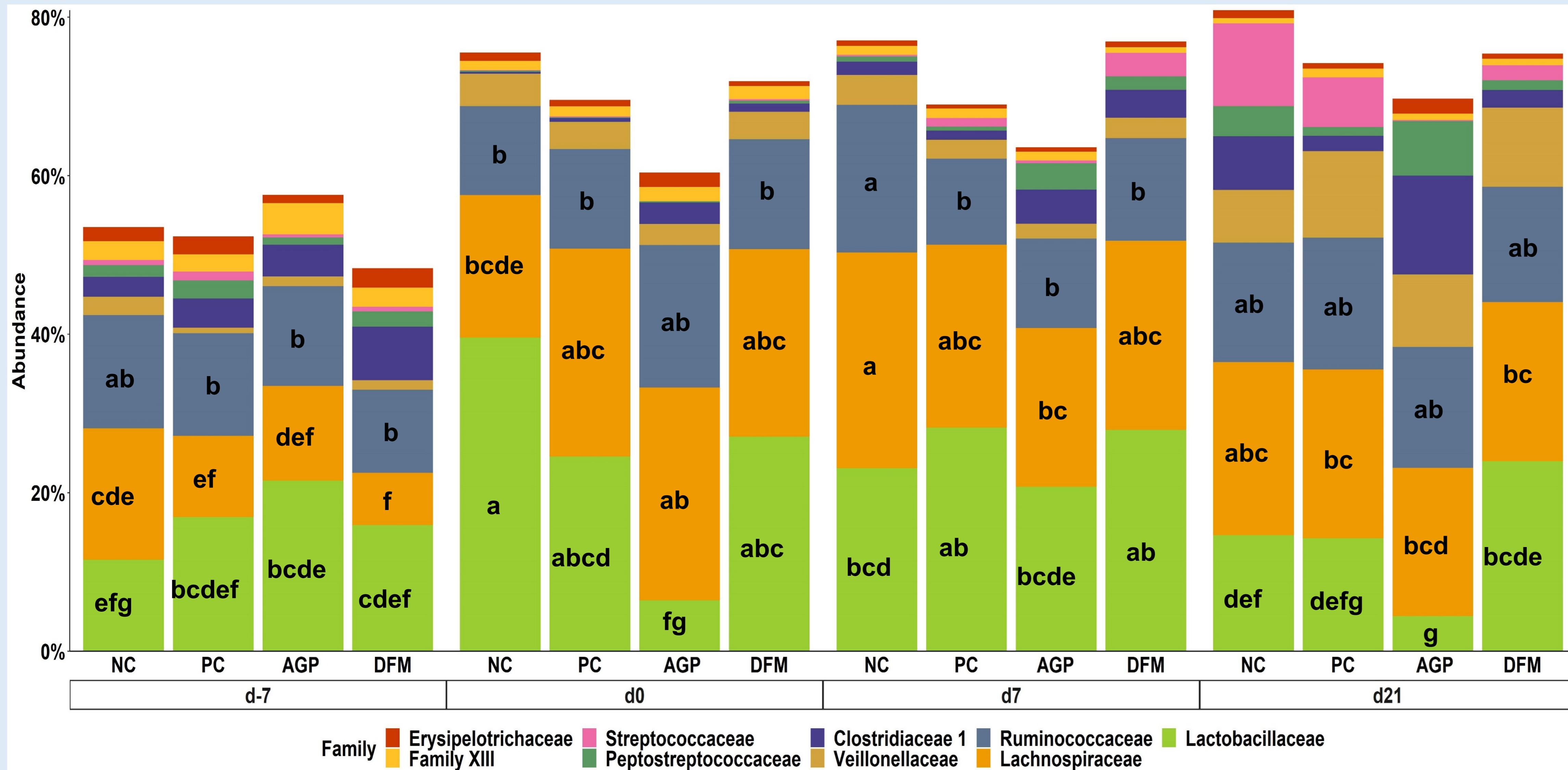
PHYLA

FIRMICUTES  
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FAMILY

## Firmicutes family

$P < 0.05$



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FAMILY

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FAMILY

## Bacteroidetes family

$P < 0.05$

