

# ➤ **Implantation of the porcine gut microbiota: dynamics and links with robustness at weaning**

**Jordi Estellé**

UMR 1313 GABI, INRAE Jouy-en-Josas

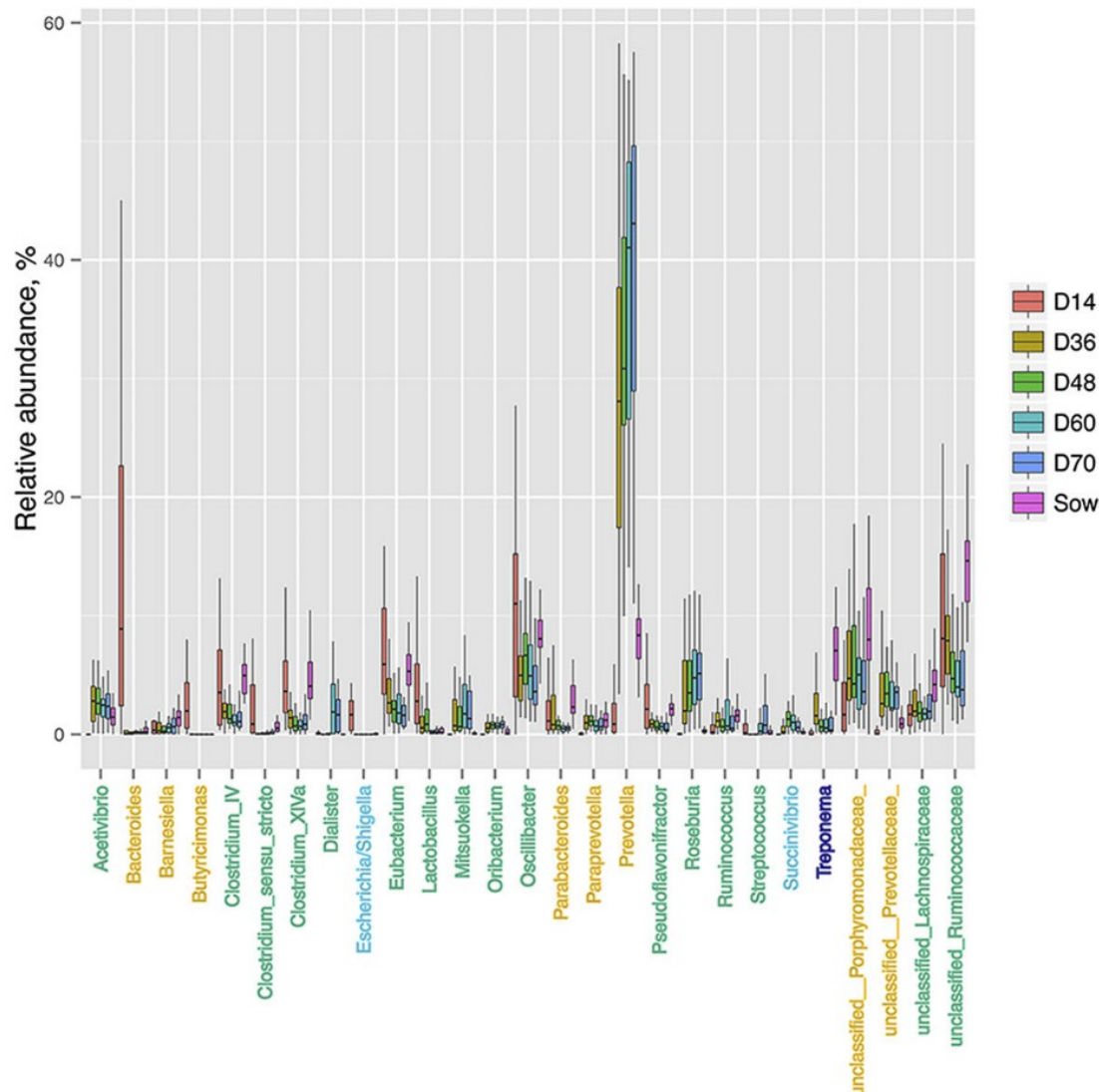
[Jordi.Estelle@inrae.fr](mailto:Jordi.Estelle@inrae.fr)

# Weaning of piglets in intensive pig production

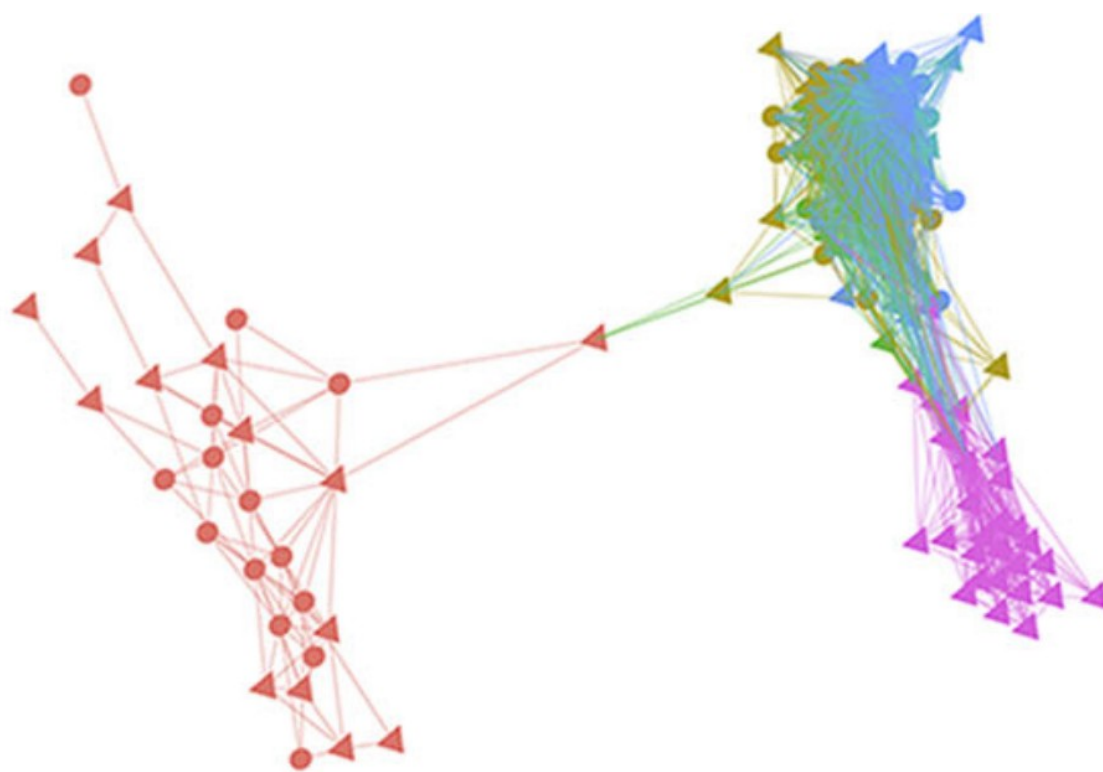
- Practiced at 3-4 weeks of age, while natural weaning occurs around 17 weeks after birth
- Sudden and stressful changes in diet, social, and environmental life conditions



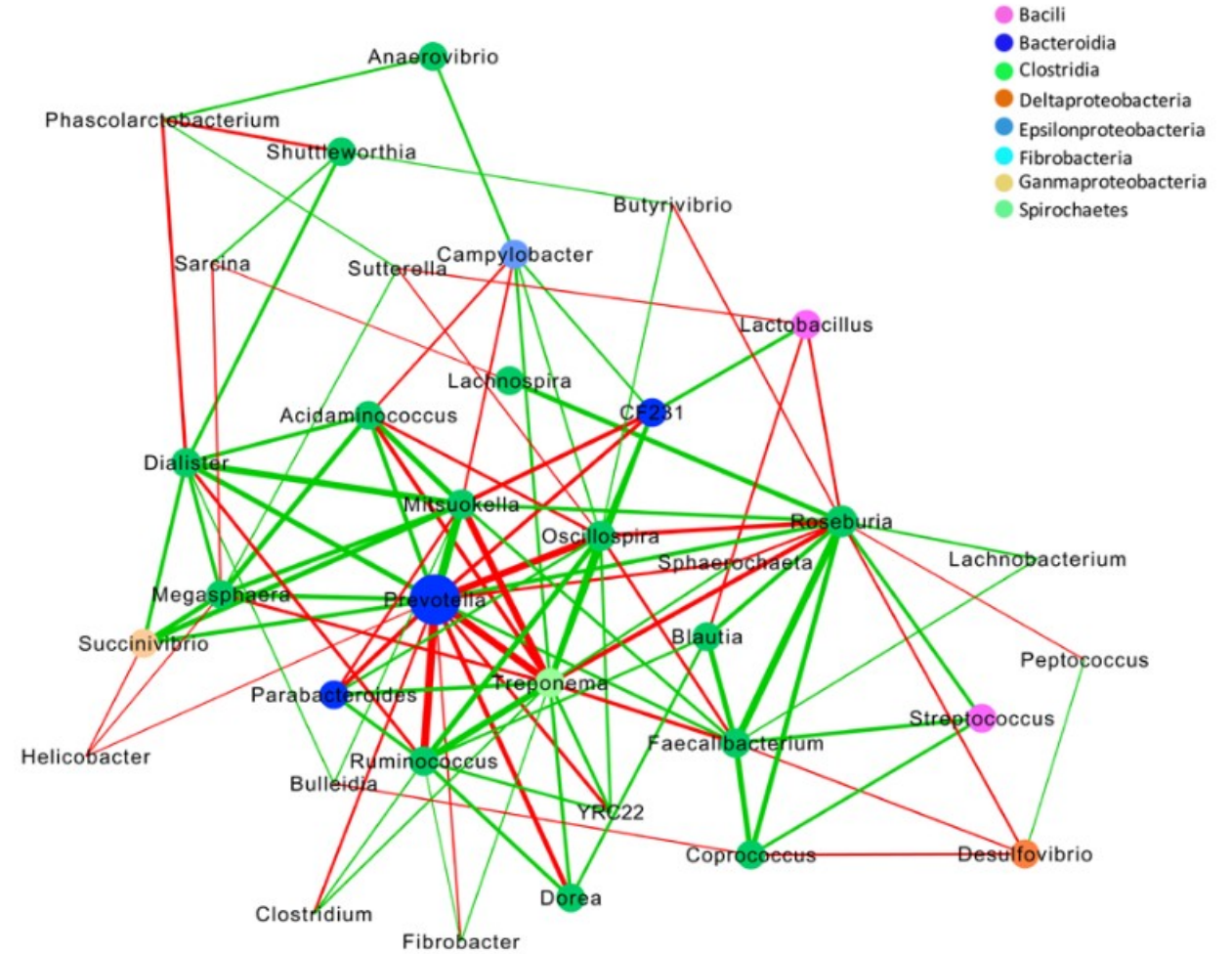
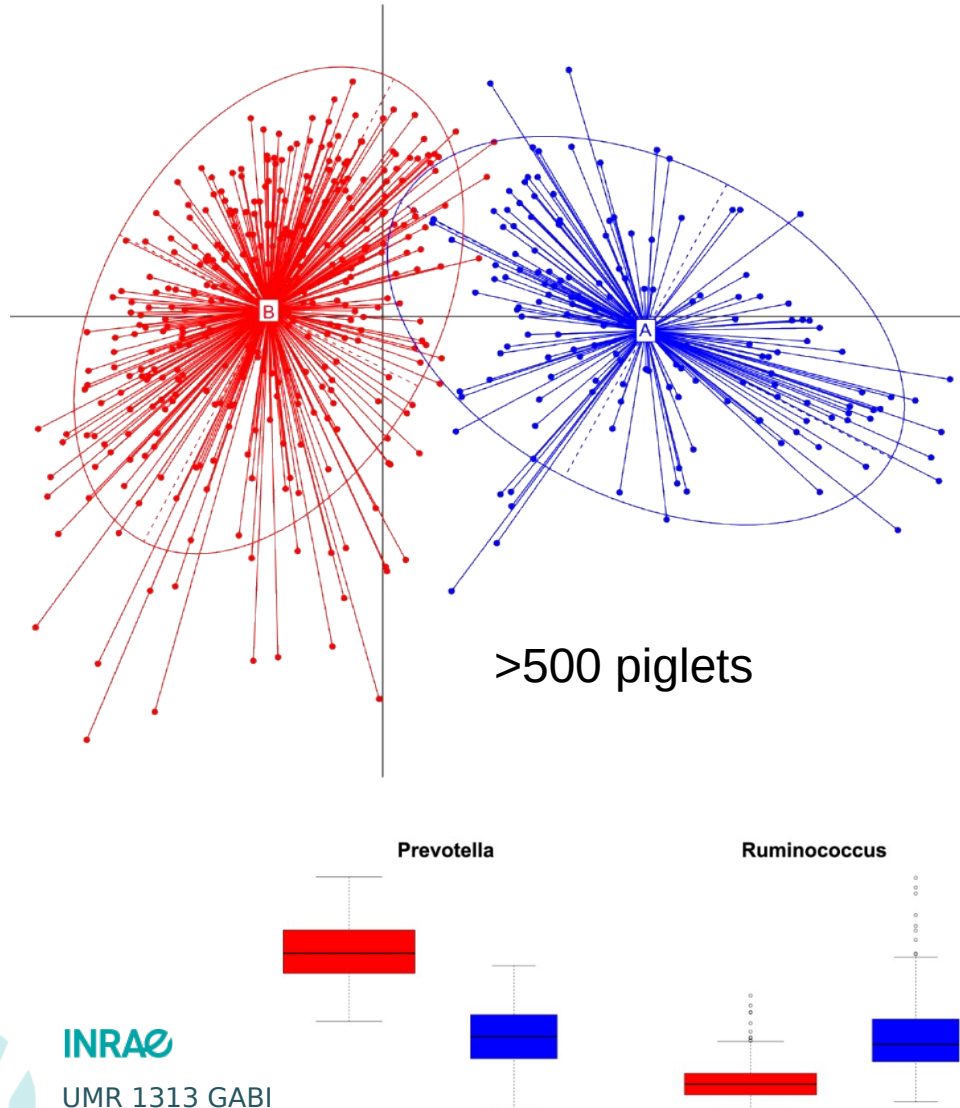
# Weaning represents a big microbiota shift for piglets



31 piglets weaned at 28 days-of-age



# Microbiota tends to get more stable at 60 days of age, but still individual variability (e.g. enterotypes)



ANR SUSFLORA; Ramayo-Caldas et al. 2016. ISME Journal

# Weaning of piglets in intensive pig production

- Practiced at 3-4 weeks of age, while natural weaning occurs around 17 weeks after birth
- Sudden and stressful changes in diet, social, and environmental life conditions
- **Undesired consequences:** anorexia, growth check, intestinal inflammation, unbalanced gut microbiota & **DIARRHEA**
- **A strong need of antimicrobial alternatives for diarrhea treatment and prevention!**



# Jambon de porcs sans antibiotiques\*\*?





# Jambon sans “antimicrobiens”?



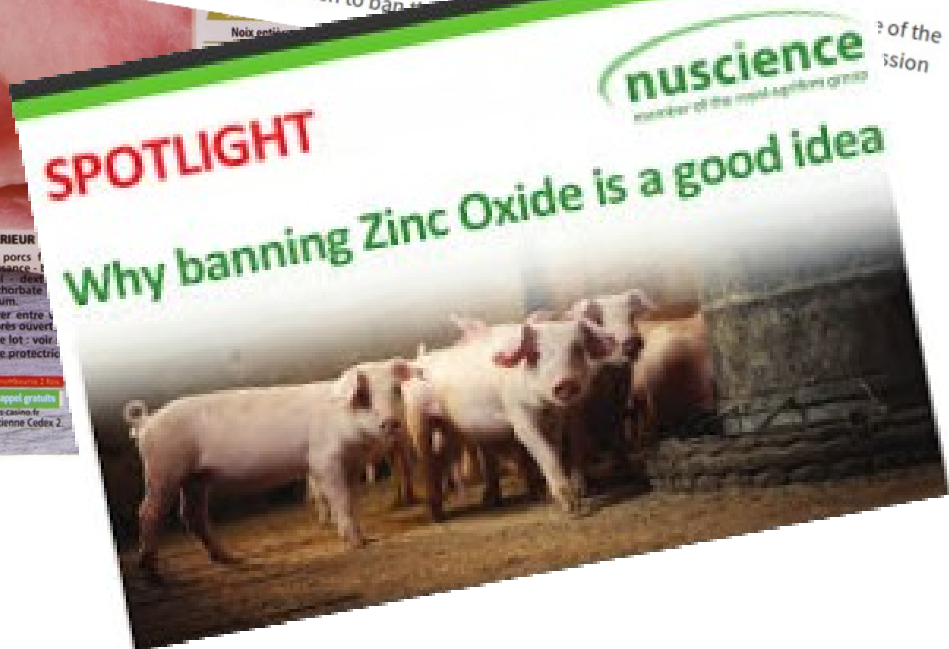
Health News | Jun 26, 2017 | 6 comments

## EU to end high-level zinc oxide use for piglets in 5 years

If they were using zinc oxide to control post-weaning diarrhoea, pig producers in the European Union will soon have to start looking for alternatives. The European Commission has voted to phase out zinc oxide as a veterinary instrument in the entire union within 5 years.

The outcome of the commission does not mean that zinc oxide had already been to ban the use of zinc oxide in the

Committee for Medicinal Products



# ANR PIGLETBIOTA Objective: find alternative to antimicrobials to promote robustness at weaning

- **Biorepository:**

- >1600 TEMPUS tubes (blood RNA)

- ~1000 EDTA blood tubes (DNA)

- > 1600 plasma tubes

- Frozen PBMCs (>1600 samples)

- ~**10 000 feces tubes** (DNA and RNA)

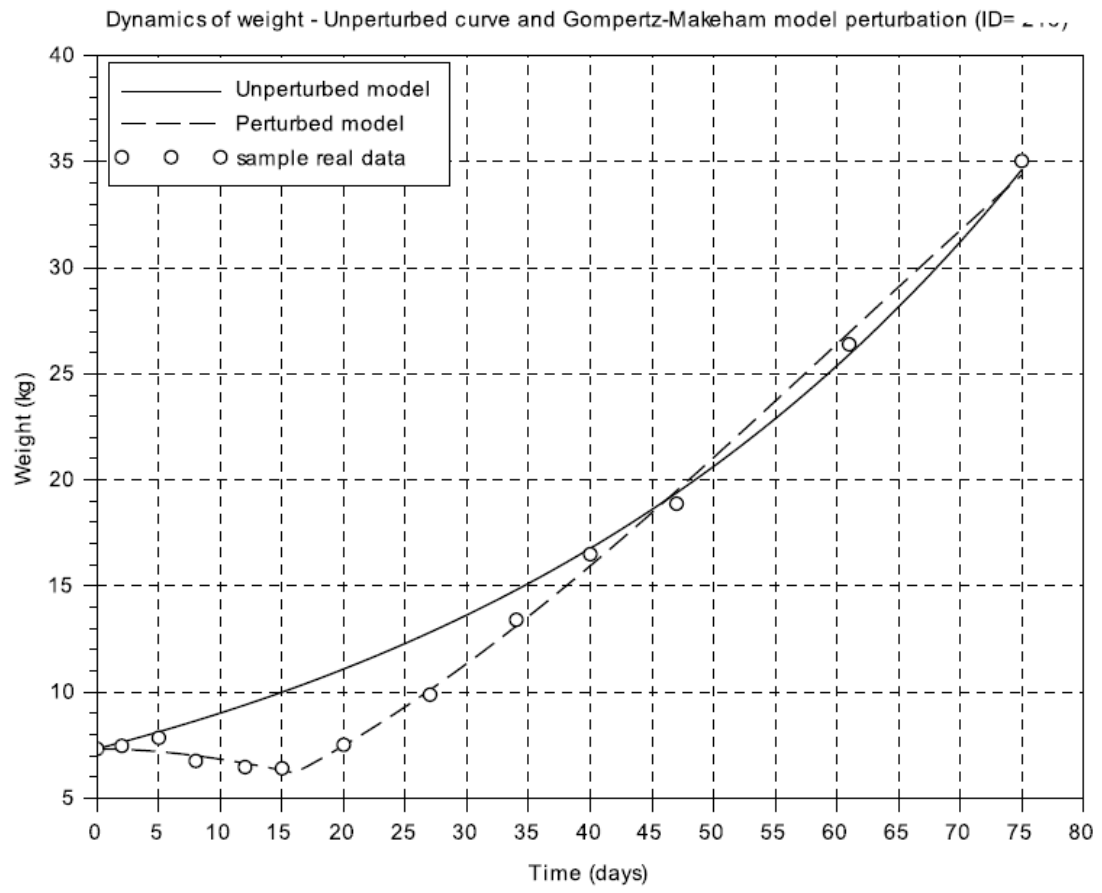
- **Growth and health phenotypes at weaning**



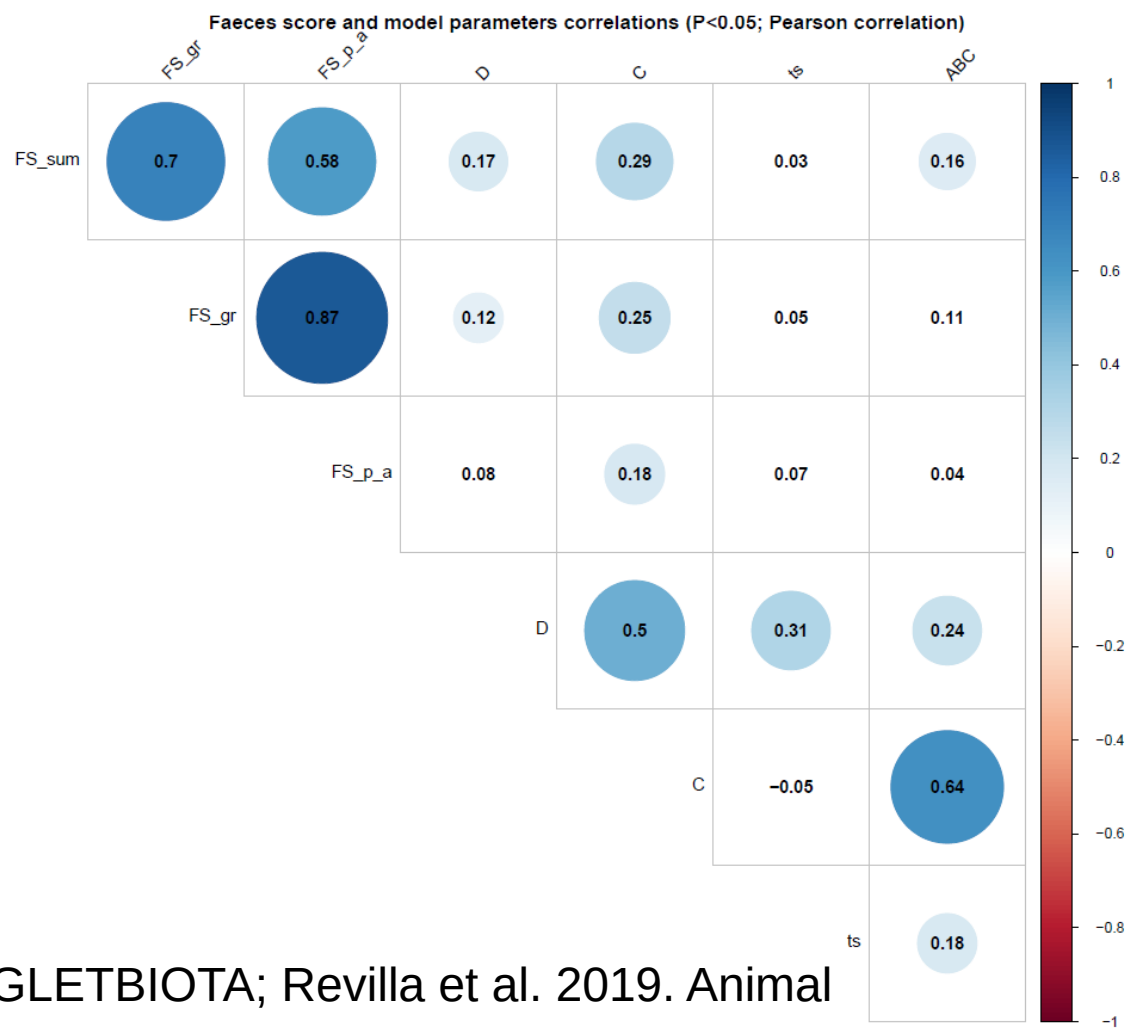


# Challenge: to model weaning robustness with growth

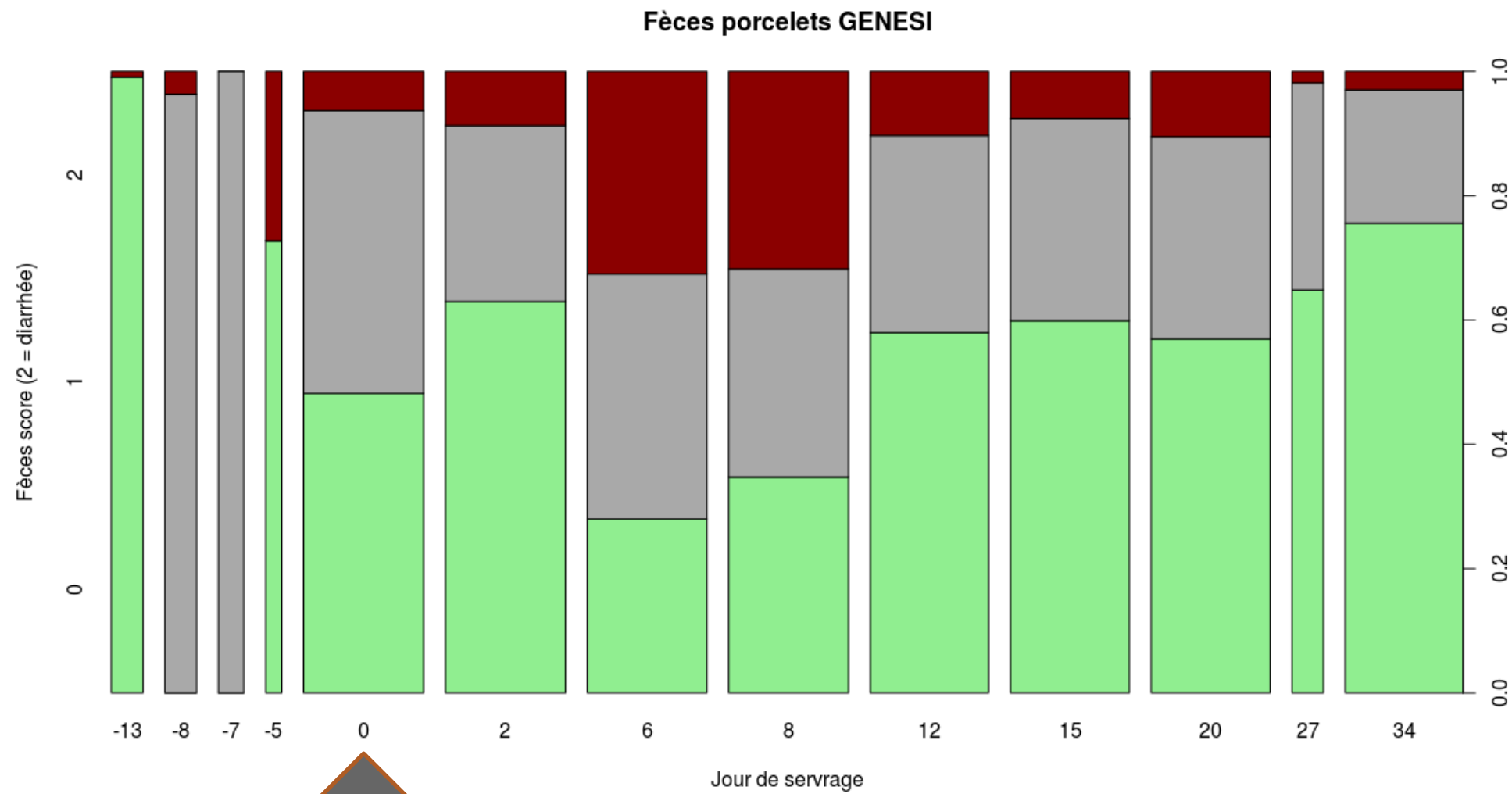
Expected vs. Observed growth: area between the curves (ABC)



ABC is significantly correlated with several health traits at weaning



# Diarrhea in piglets: ~1 week after weaning



# Challenge: adapt our structure and @BRIDGE to medium-throughput microbiota DNA extraction

- **~10 000 feces tubes** (DNA and/or RNA)
  - At sampling, anticipate the next steps by pre-alliquoting samples (~200mg per crytube) in order to save pre-processing time at the lab
  - At the lab, adapt manual protocol to semi-automated microbiota DNA extraction using @BRIDGE facilities at GABI => increase repeatability and efficiency of extractions



# Challenge: adapt our structure and @BRIDGE to medium-throughput microbiota DNA extraction

- International Human Microbiome Standards (FP7, Coord. J.Doré)
- SOP “H” adapted to @BRIDGE’s STAR nucleic acid workstation (Hamilton, Perkin Elmer)



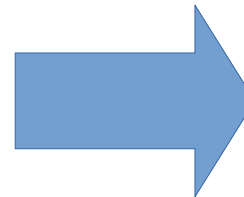
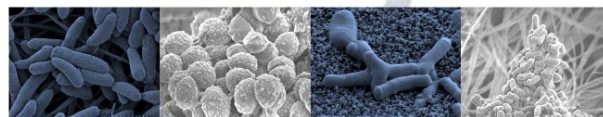
IHMS Consortium	IHMS - QUALITY PROTOCOL SOP FOR FECAL SAMPLES DNA EXTRACTION Protocol H	Code : IHMS_SOP 07 V2 Version : 2 Date : 2015-04-12 Number of pages : 11 Page n° : 1	Last Contributor : Sebastian BURZ Approved by: IHMS CONSORTIUM Date : 2015-01-31
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IHMS – QUALITY PROTOCOL  
International Human Microbiome Standards  
<http://www.microbiome-standards.org>

IHMS SOP 07 V1: STANDARD OPERATING PROTOCOL FOR FECAL SAMPLES

DNA EXTRACTION

Protocol H



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UMR 1313 GABI

@JordiEstelle

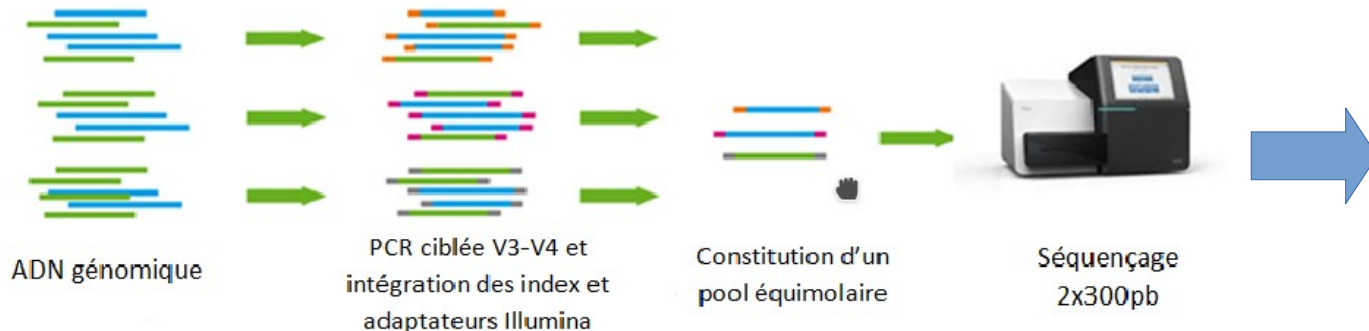
# How weaning age affects gut microbiota in piglets?

- PIGLETBIOTA ANR experiment at UE PAO (INRAE):

N. piglets	Weaned at	Birth (d0)	d14	d21	d28	d35	d42	d49	d60
12	14 days	→	Faeces (12 pigs)	Faeces (12 pigs)	-----→				Faeces (6 pigs)
12	21 days	→		Faeces (12 pigs)	Faeces (12 pigs)	-----→			Faeces (6 pigs)
12	28 days	→			Faeces (12 pigs)	Faeces (12 pigs)	-----→		Faeces (6 pigs)
12	42 days	→					Faeces (12 pigs)	Faeces (12 pigs)	Faeces (6 pigs)

# How weaning age affects gut microbiota in piglets?

- Dynamics of microbiota implantation by 16S seq.

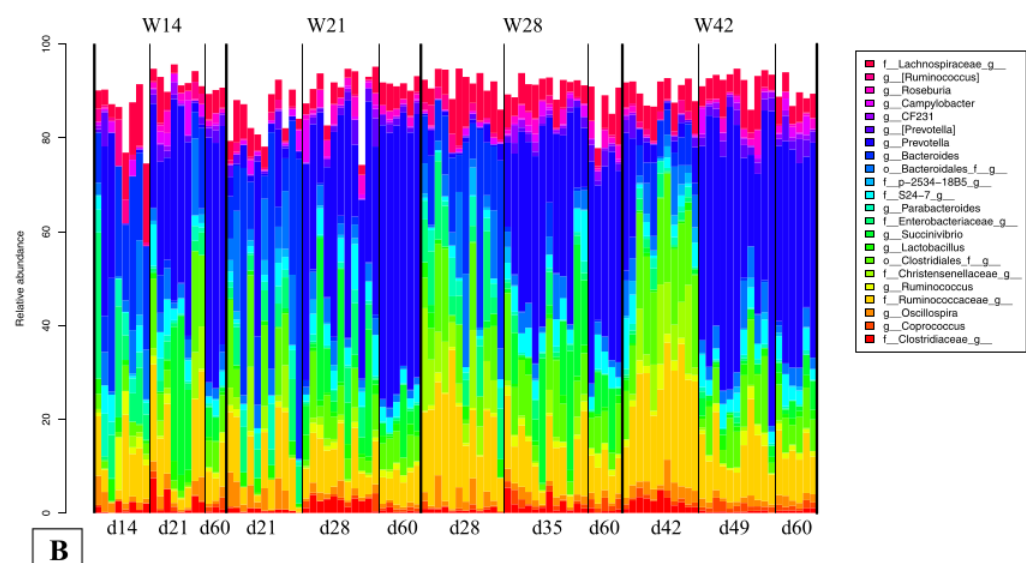
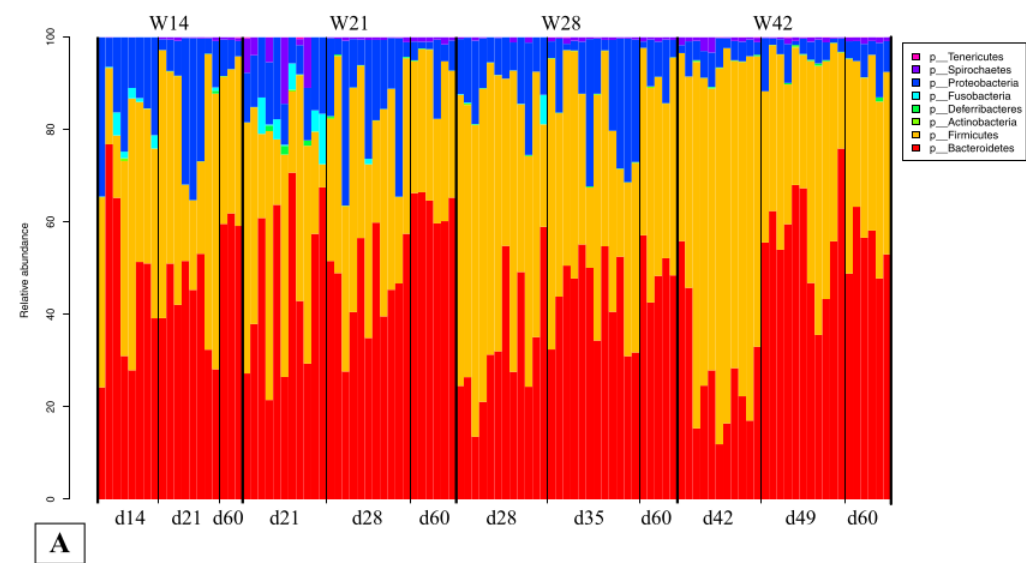
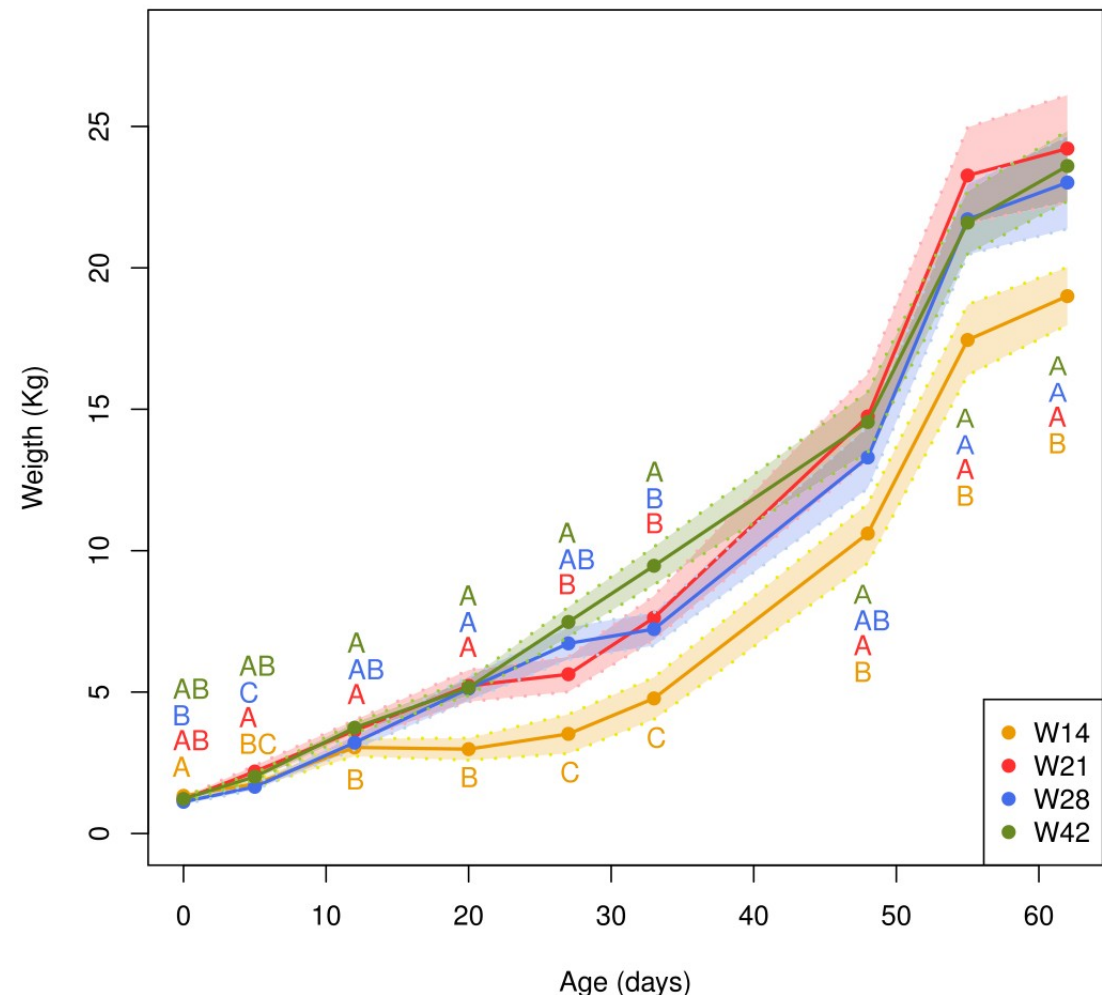


1. Inspect Illumina reads
2. Clean adapters / primers (depending on protocol)
3. Join paired end reads
4. Remove bad quality reads
5. Perform OTU calling
6. Remove low abundance OTU
7. Remove chimera OTU
8. Summary statistics

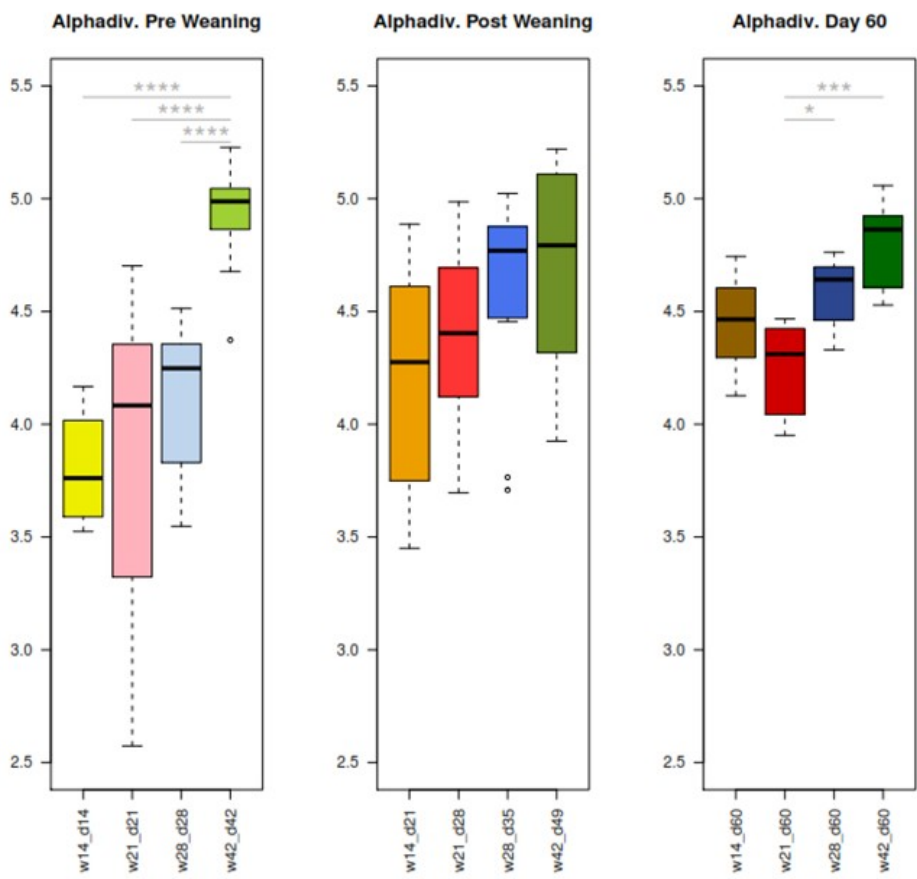




# How weaning age affects gut microbiota in piglets?

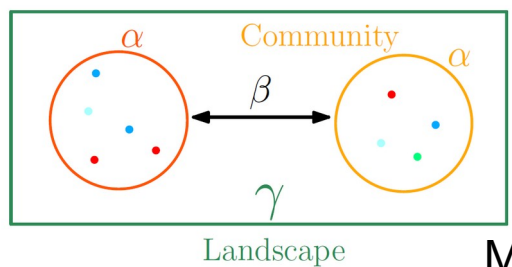
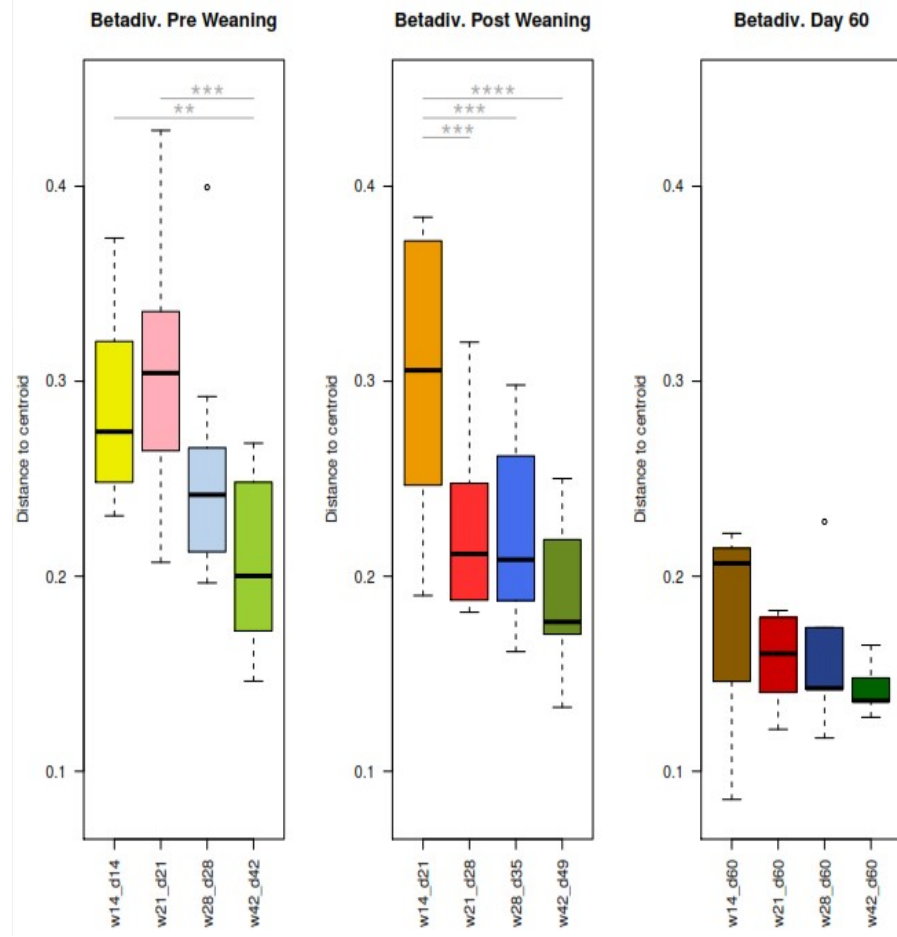


# How weaning age affects gut microbiota in piglets?



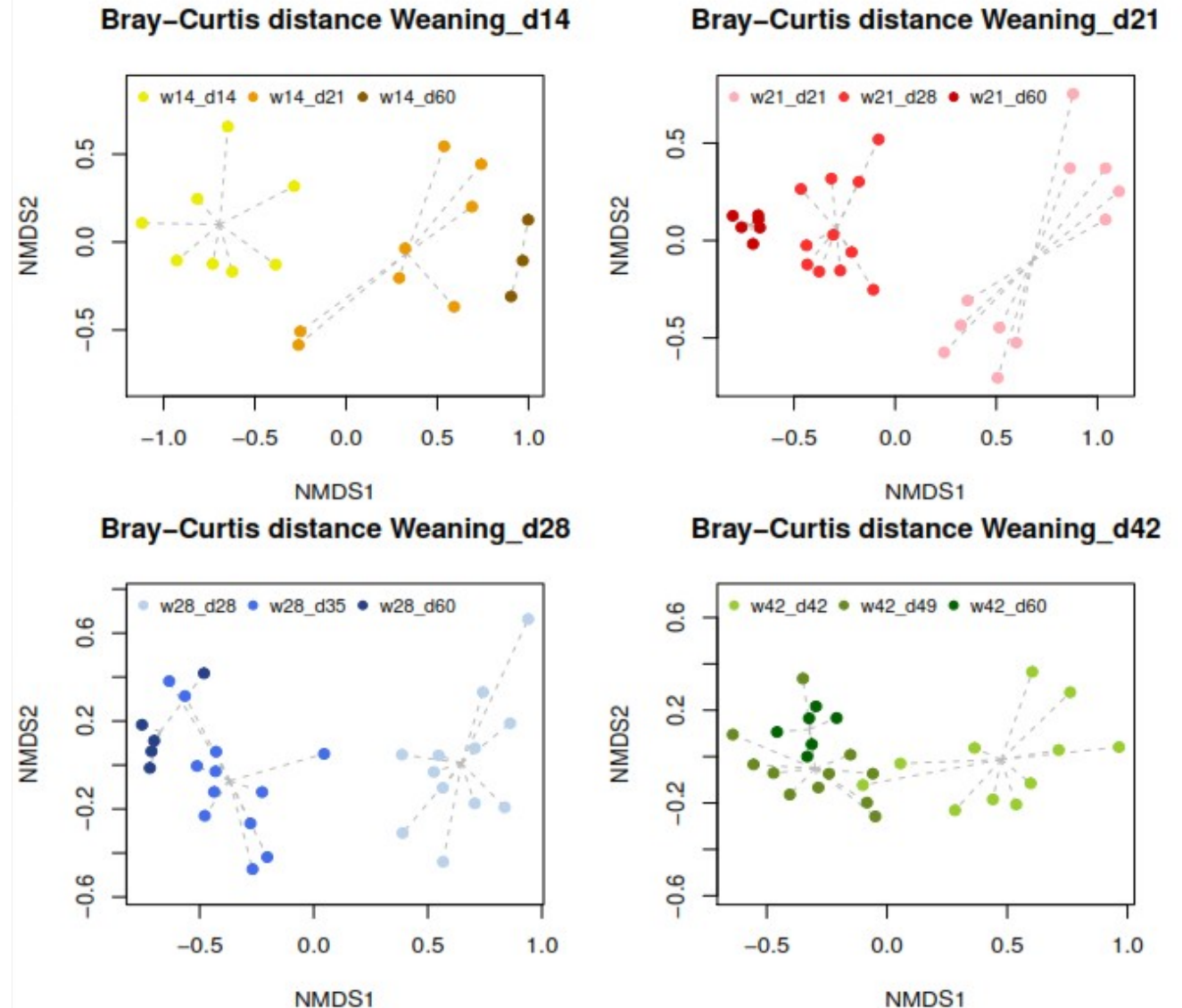
The **group weaned at 42 days old** has a **higher alpha-diversity** during the pre-weaning, post-weaning and until the day 60. It has also lower beta-diversity (the composition is more homogeneous among the animals in this group)

**=> RICHER IS BETTER!**



# How weaning age affects gut microbiota in piglets?

- Each weaned group shows drastic changes between pre- and post-weaning gut microbiota composition
- Results in accordance with literature and our own previous results (Mach et al. 2015)



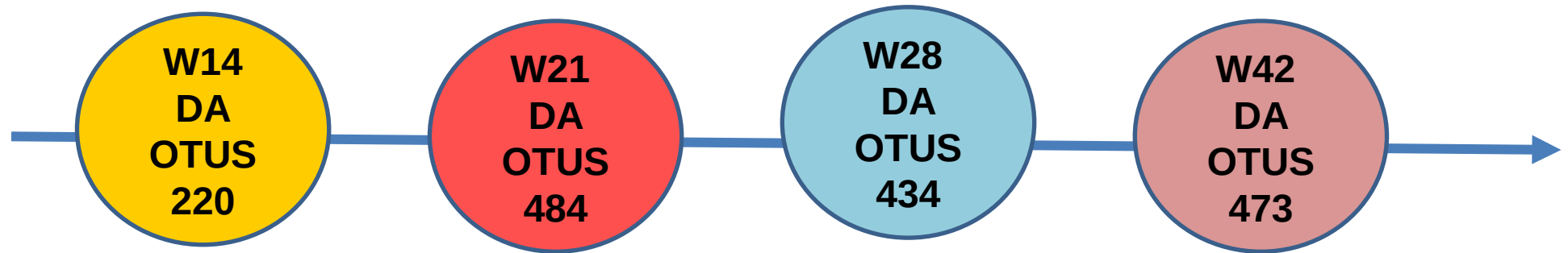


# How weaning age affects gut microbiota in piglets?

- Differential abundance analysis of OTUs:

Pre-Weaning:

More abundances of OTUs annotated as *Bacteroides* spp., *Ruminococcus* spp., *Oscillospira* spp., *Clostridium* spp., ...



Post-Weaning:

More abundance of OTUs annotated as *Succinivibrio* spp., *Prevotella* spp., *Campylobacter* spp., *Faecalibacterium prausnitzii*, ...

# How weaning age affects gut microbiota in piglets?

[Front Microbiol.](#) 2017 Jun 30;8:1226. doi: 10.3389/fmicb.2017.01226. eCollection 2017.

## Functional Characterization of Novel *Faecalibacterium prausnitzii* Strains Isolated from Healthy Volunteers: A Step Forward in the Use of *F. prausnitzii* as a Next-Generation Probiotic.

[Martín R](#)<sup>1</sup>, [Miquel S](#)<sup>1,2</sup>, [Benevides L](#)<sup>1,3</sup>, [Bridonneau C](#)<sup>1</sup>, [Robert V](#)<sup>1</sup>, [Hudault S](#)<sup>1</sup>, [Chain F](#)<sup>1</sup>, [Berteau O](#)<sup>1</sup>, [Azevedo V](#)<sup>3</sup>, [Chatel JM](#)<sup>1</sup>, [Sokol H](#)<sup>1,4,5</sup>, [Bermúdez-Humarán LG](#)<sup>1</sup>, [Thomas M](#)<sup>1</sup>, [Langella P](#)<sup>1</sup>.



## *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients

Harry Sokol<sup>††</sup>, Bénédicte Pigneur<sup>††</sup>, Laurie Watterlot\*, Omar Lakhdari\*, Luis G. Bermúdez-Humarán\*, Jean-Jacques Gratadoux\*, Sébastien Blugeon\*, Chantal Bridonneau\*, Jean-Pierre Furet\*, Gérard Corthier\*, Corinne Grangette<sup>§</sup>, Nadia Vasquez<sup>¶</sup>, Philippe Pochart<sup>¶</sup>, Germain Trugnan<sup>‡</sup>, Ginette Thomas<sup>‡</sup>, Hervé M. Blottière\*, Joël Doré\*, Philippe Marteau<sup>¶</sup>, Philippe Seksik<sup>††††</sup>, and Philippe Langella<sup>\*,\*\*\*†</sup>

[Gastroenterol Res Pract.](#) 2014;2014:872725. doi: 10.1155/2014/872725. Epub 2014 Mar 27.

## Association between *Faecalibacterium prausnitzii* Reduction and Inflammatory Bowel Disease: A Meta-Analysis and Systematic Review of the Literature.

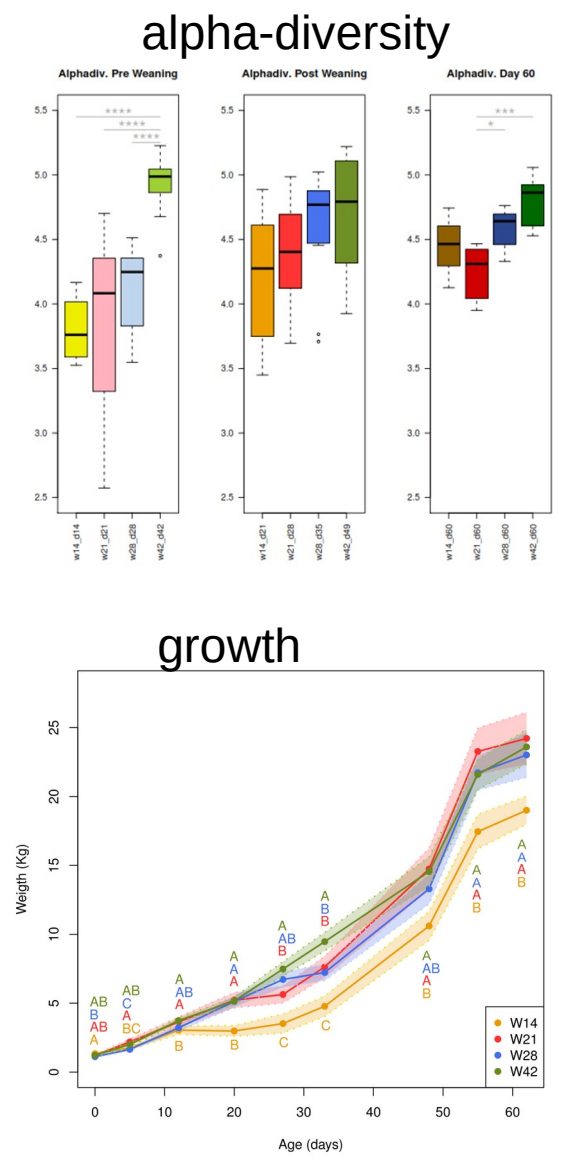
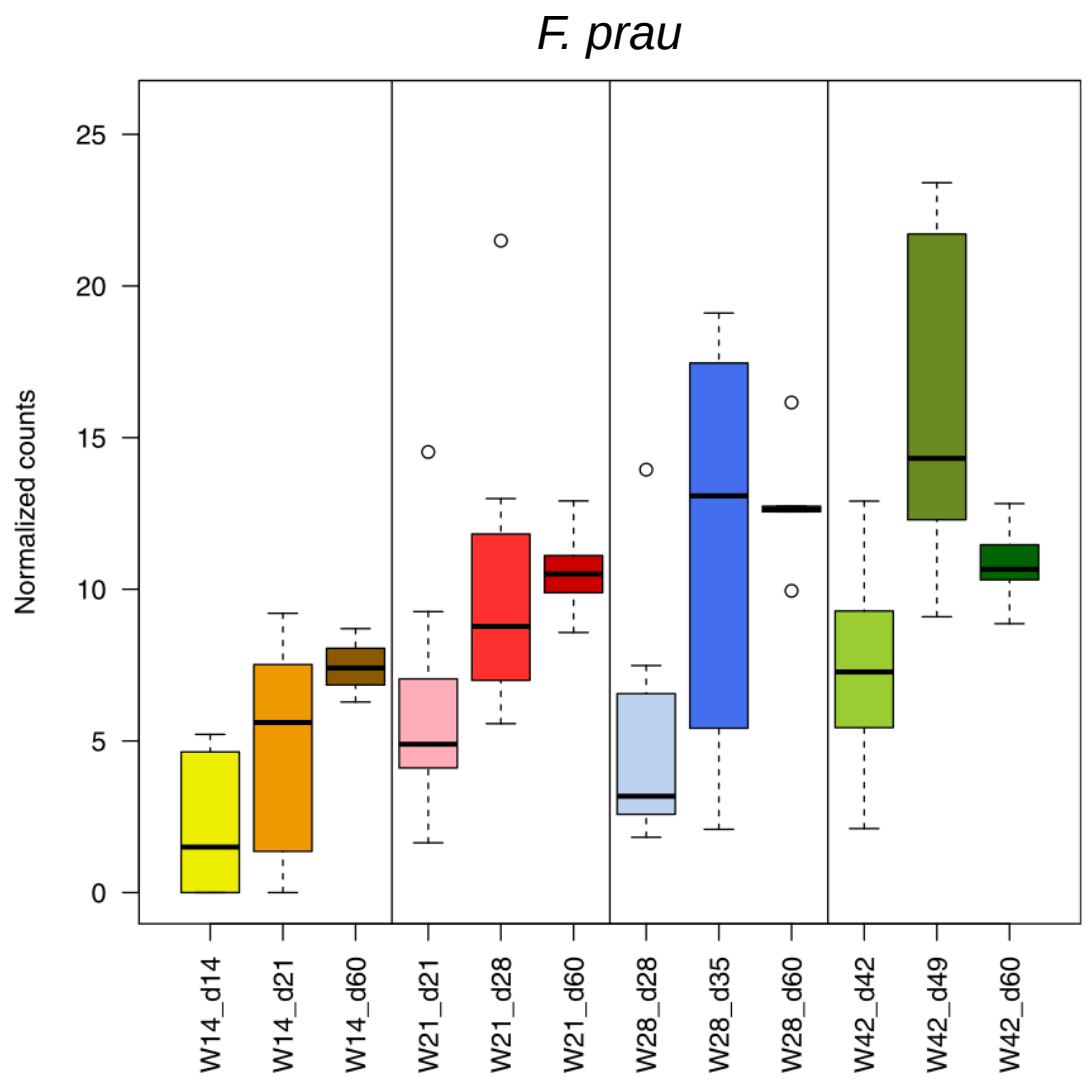
[Cao Y](#)<sup>1</sup>, [Shen J](#)<sup>1</sup>, [Ran ZH](#)<sup>1</sup>.

[Appl Environ Microbiol.](#) 2015 Nov;81(21):7582-92. doi: 10.1128/AEM.02006-15. Epub 2015 Aug 21.

## Mucosa-associated *Faecalibacterium prausnitzii* phylotype richness is reduced in patients with inflammatory bowel disease.

[Lopez-Siles M](#)<sup>1</sup>, [Martinez-Medina M](#)<sup>1</sup>, [Abellà C](#)<sup>1</sup>, [Busquets D](#)<sup>2</sup>, [Sabat-Mir M](#)<sup>3</sup>, [Duncan SH](#)<sup>4</sup>, [Aldeguer X](#)<sup>2</sup>, [Flint HJ](#)<sup>4</sup>, [Garcia-Gil LJ](#)<sup>5</sup>.

# How weaning age affects gut microbiota in piglets?





# *F.prau* as a useful pro-biotic in weanling piglets?

Massacci et al. *Animal Microbiome* (2020) 2:2  
<https://doi.org/10.1186/s42523-020-0020-4>


Animal Microbiome

## RESEARCH ARTICLE

## Open Access

### Late weaning is associated with increased microbial diversity and *Faecalibacterium prausnitzii* abundance in the fecal microbiota of piglets



Francesca Romana Massacci<sup>1,2,3\*</sup> , Mustapha Berri<sup>4</sup>, Gaetan Lemonnier<sup>1</sup>, Elodie Guettier<sup>5</sup>, Fany Blanc<sup>1</sup>, Deborah Jaret<sup>1</sup>, Marie Noelle Rossignol<sup>1</sup>, Marie-José Mercat<sup>6</sup>, Joël Doré<sup>7,8</sup>, Patricia Lepage<sup>7</sup>, Claire Rogel-Gaillard<sup>1</sup> and Jordi Estellé<sup>1\*</sup>



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@JordiEstelle

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PIGLETBIOTA

- Late weaning at 42 days-of age would be a good compromise for enhancing gut health in piglets
- Coherent with general weaning practices performed in organic farming, but the implementation of late weaning in industrial conventional production would be challenging
- Possible perspective for probiotic candidates like *F. prau* to be used in pre-weaning piglets in order to limit the dysbiosis in the gut!

# ➤ **Implantation of the porcine gut microbiota: dynamics and links with robustness at weaning**

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