Microbiomics of Monogastric Farm Animals

Elucidating the **INTERPLAY** of Microbiota with its Mammalian Host

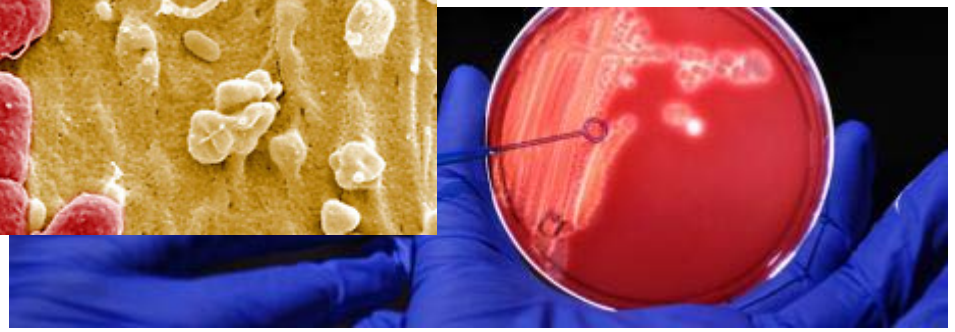
August 29th, 2013, **Hauke Smidt**

EAAP, Nantes

WAGENINGEN UNIVERSITY
WAGENINGEN UR

The world inside us – the intestinal ecosystem

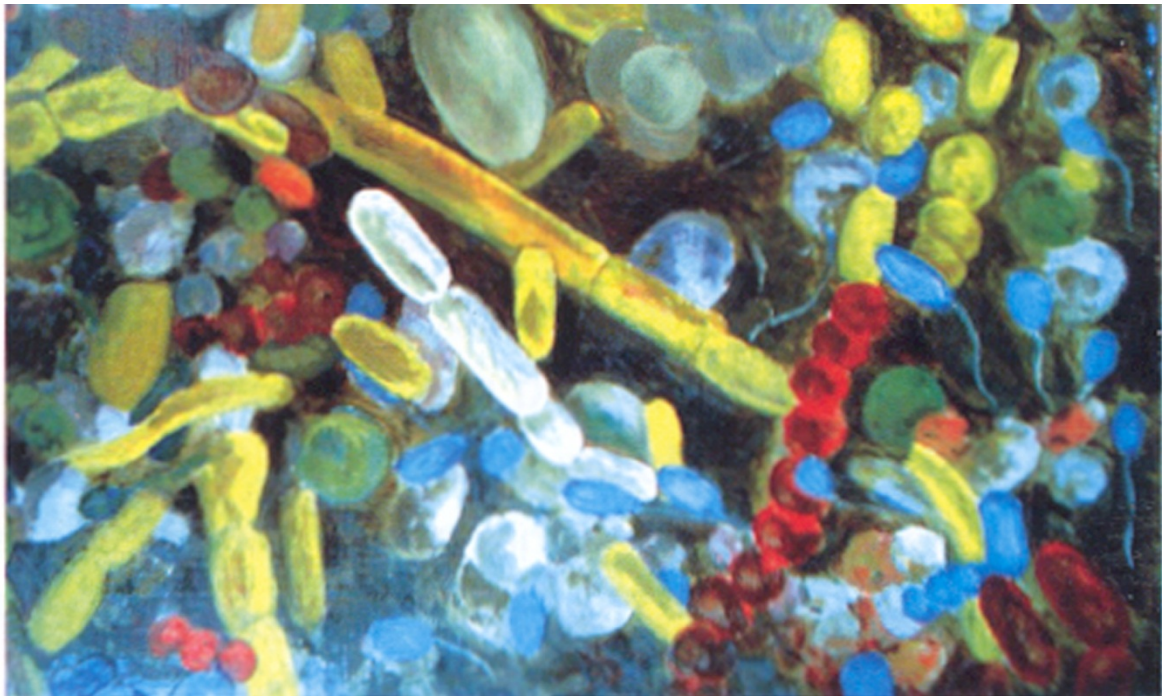
Intestinal pathogens are front-page news



Worries about increasing spread of antibiotic resistance in bacteria in humans & farm animals

The world inside us – the intestinal ecosystem

But there is so much more than just the bad guys



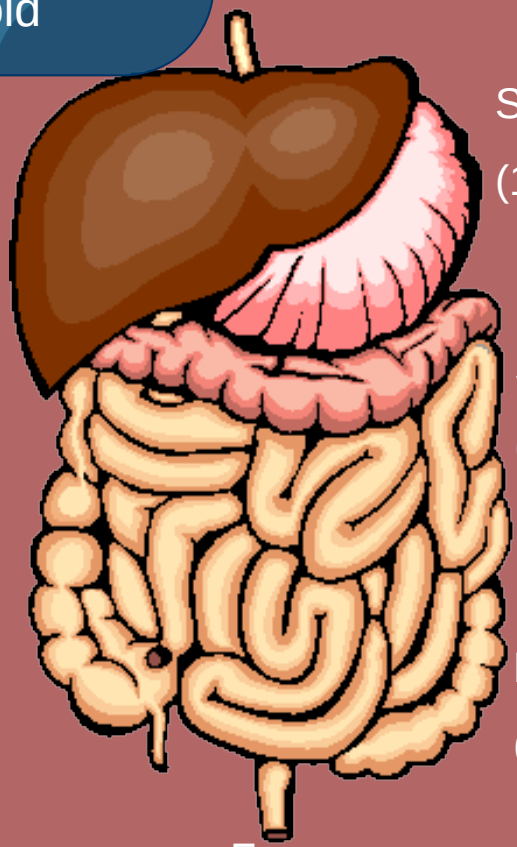
microbial world impressions of an artist & pioneer microbial ecologist

Antoon Akkermans

The world inside us – the intestinal ecosystem

Microbial cells outnumber host cells by 10-fold

More than 1000 different species of microbes in the intestine



Stomach & duodenum

(10^3-10^5 bacteria/g)

Small intestine

(10^4-10^8 bacteria/g)

Large intestine

(10^{10}-10^{12} bacteria/g)

Feces

(10^{10}-10^{12} bacteria/g)

Microbial metagenome outnumbers host genome by 100-fold

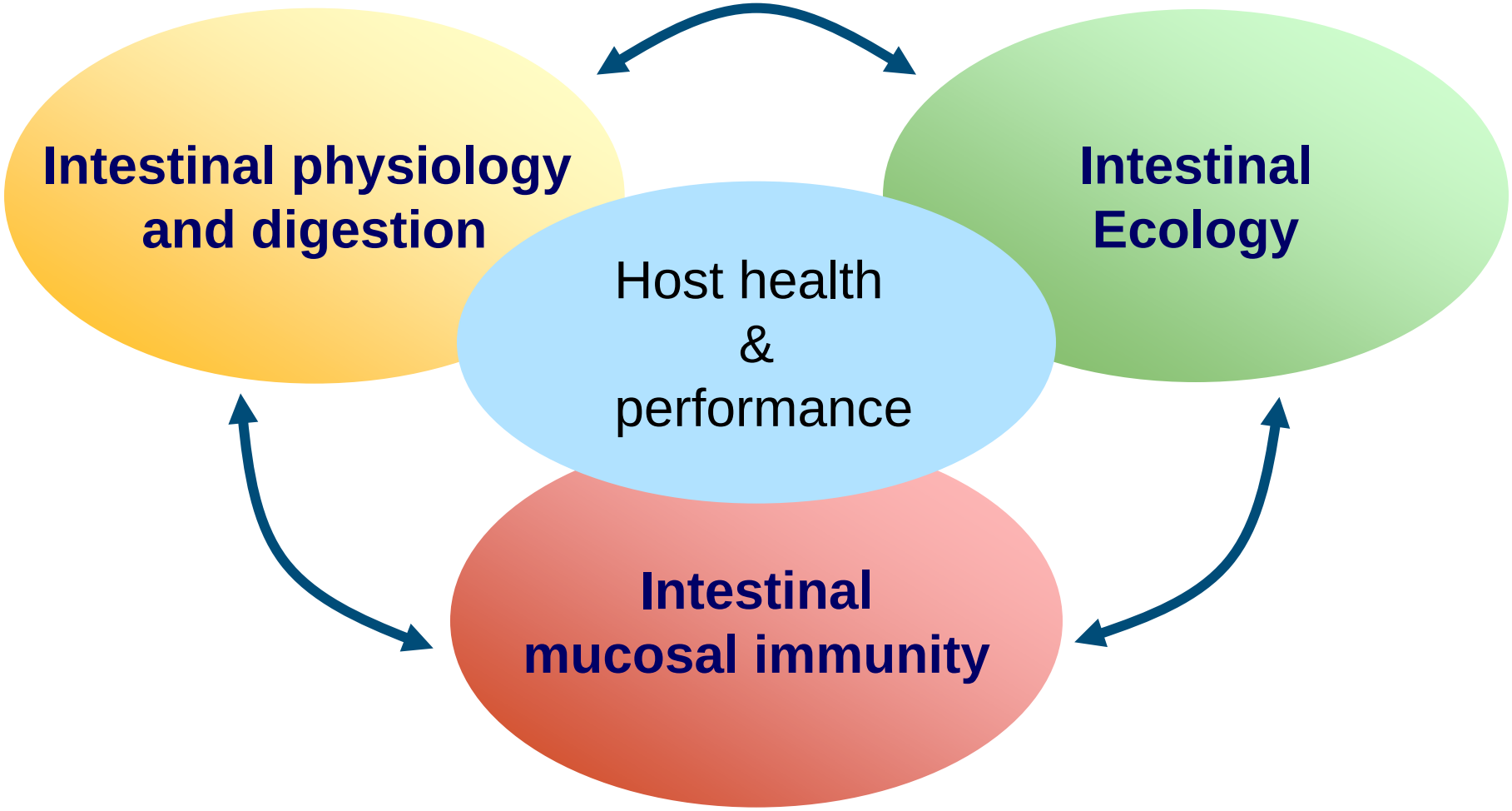
Microbiota impact on health:

IBD, allergy, obesity…

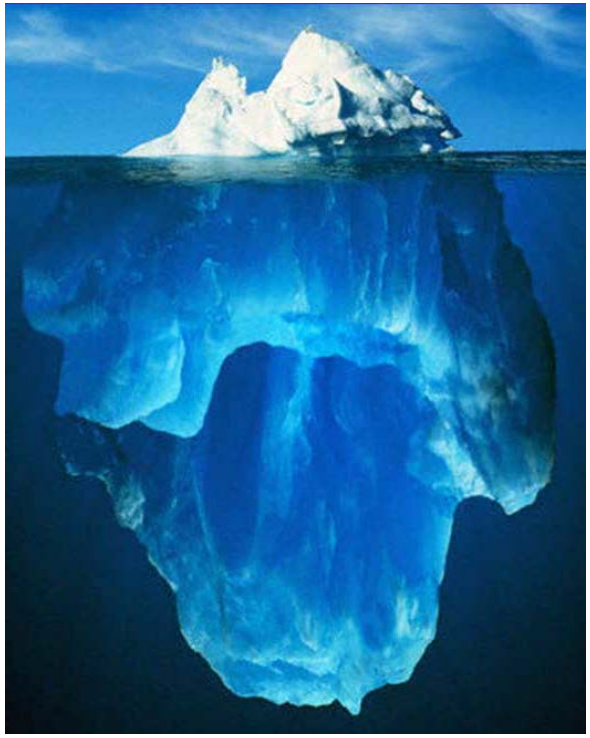
What is the microbiota doing in the intestine?

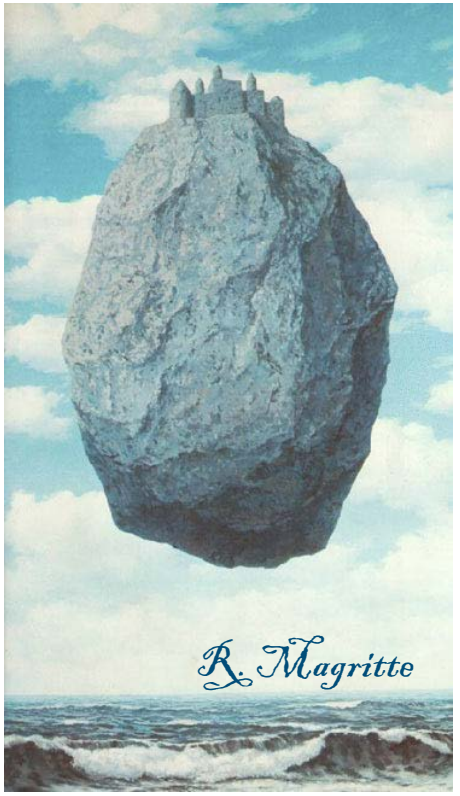
- Digest food and provide nutrients
  - Short chain fatty acids
  - Amino acids
  - Vitamins
- Alter gut morphology and physiology
- Protect against pathogens

The “Golden Triangle” of intestinal interactions



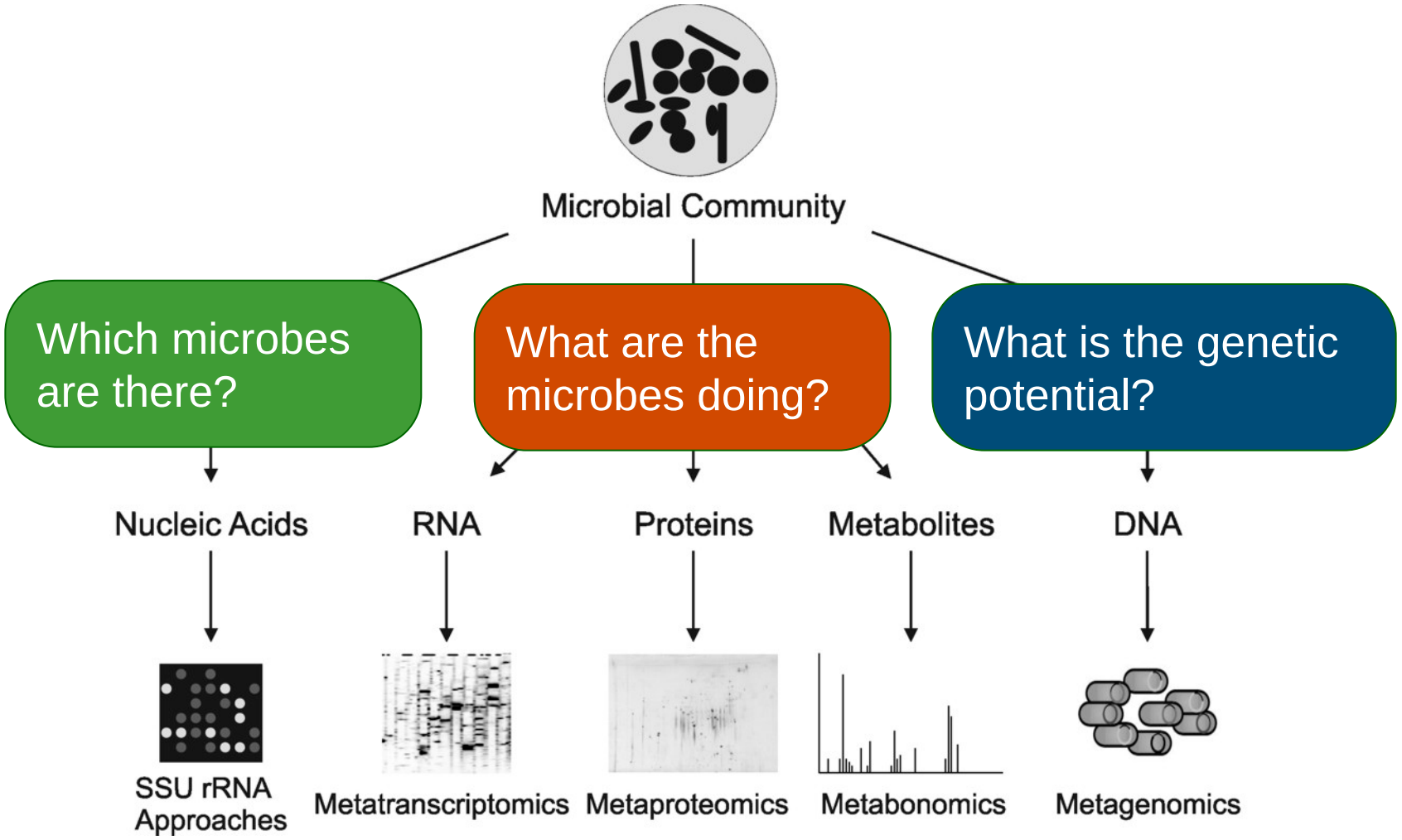
Most micro-organisms have not been cultured





- Innovative cultivation &
  Molecular genomics-based approaches needed

How to learn more about (gut) microbes



Microbial biomarkers for ecosystem functioning

Questions driving farm animal microbiomics

What is the role of microbes in the triangle with animal nutrition, physiology and immunology?

- **AWAY FROM BLACK BOX APPROACHES**

For many years, antibiotics used as growth promoters, YET:

- How do they work?
- Banned since 2006 (EU)
- Quest for alternative additives

Model systems for human research

Monogastric farm animal research

Focus on Pigs

HealthyPigut

FPH Feed for Pig Health

INTERPLAY

INTERPLAY

FP7 project

11 partners

2009 - 2013

Nanjing ★

INTERPLAY

Focus on intestinal development in pigs

- Early colonization
- Effect of diet & environment
- Effect of host genotype

Nanjing ★

Pig Microbiomics Research – Key questions

- Which are the first bacterial colonizers in the pig intestine?
- Effect of environment factors, diet and host on bacterial colonization?
- What is the impact of early microbiota on health later on in life?
- Alternatives to antibiotics: Improved dietary intervention strategies possible (pre/probiotics)?

The 4 Central Hypotheses of INTERPLAY

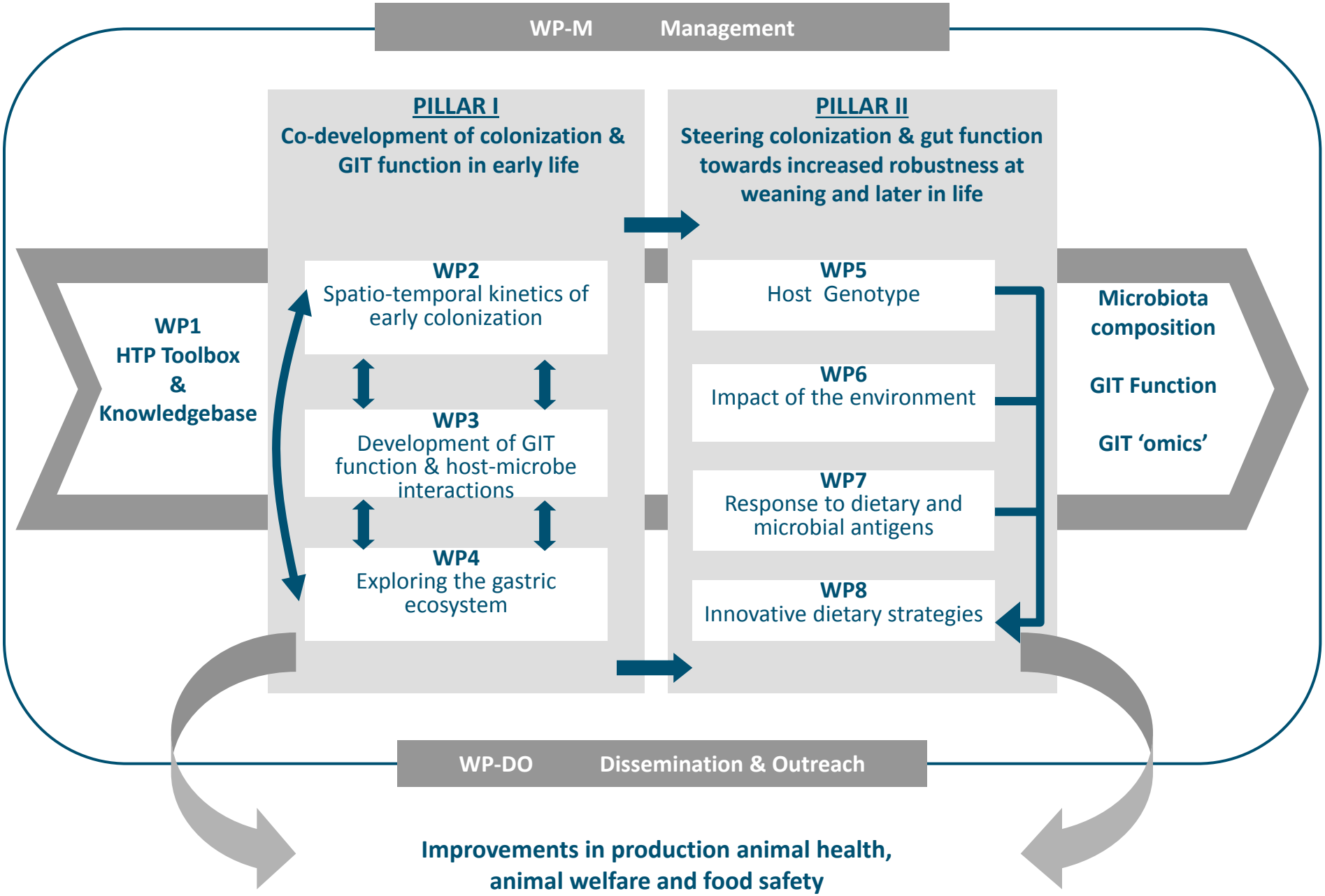
1. Kinetics of early GIT colonization drives the dynamics of microbiota composition and activity, intestinal function and host-microbe interaction after birth and later in life

2. Sows can affect the co-development of intestinal microbiota and gut function
   i. Through their genotype
   ii. Through their own microbiota

The 4 Central Hypotheses of INTERPLAY

3. Rearing environment, including postnatal antibiotic treatment, affects interplay of microbial colonisation and gut function development

4. Improved management strategies can be developed based on innovative pre- and probiotics towards sustainable pig production

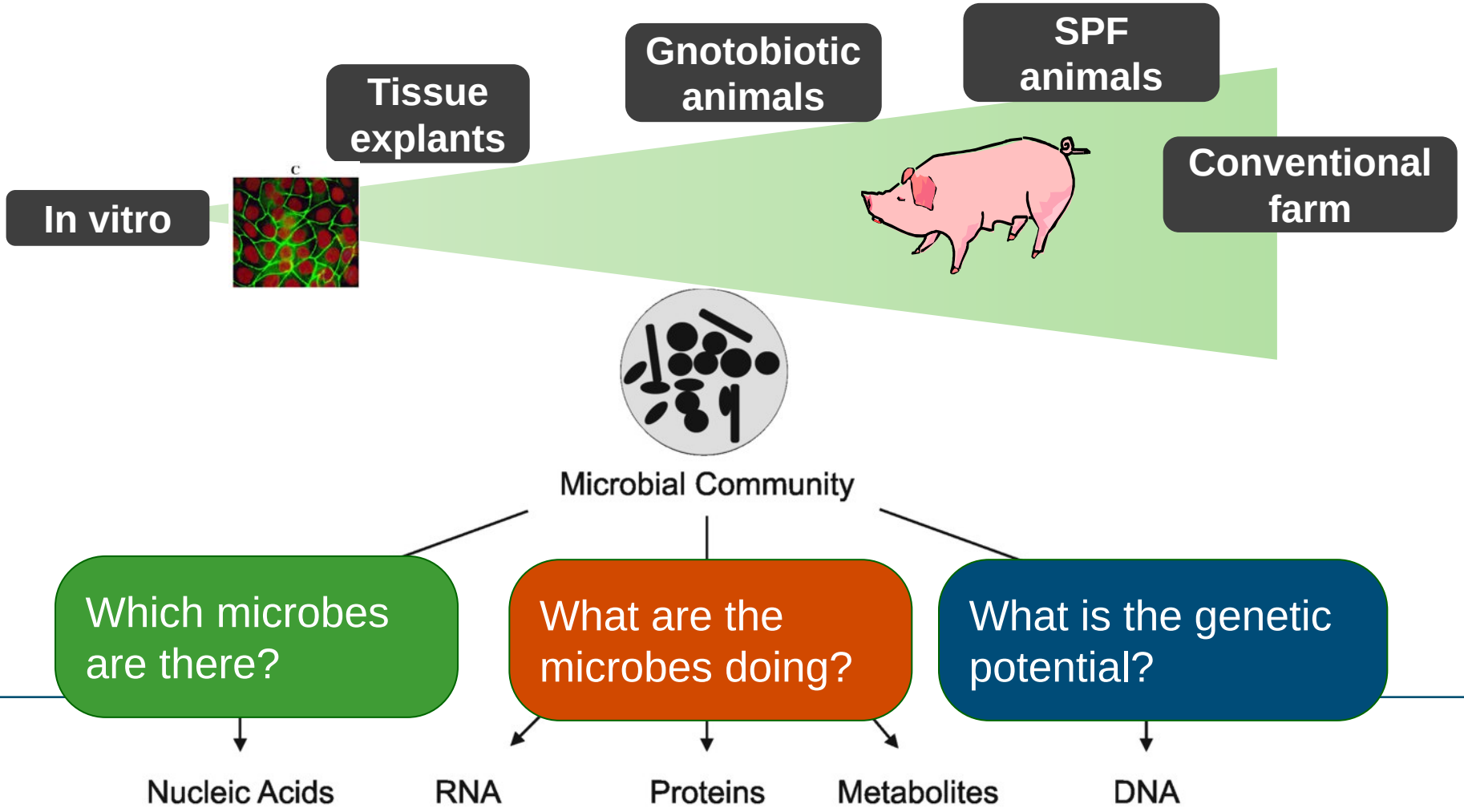

WP-M Management
PILLAR I
Co-development of colonization & GIT function in early life
PILLAR II
Steering colonization & gut function towards increased robustness at weaning and later in life
WP1
HTP Toolbox
&
Knowledgebase
WP2
Spatio-temporal kinetics of early colonization
WP3
Development of GIT function & host-microbe interactions
WP4
Exploring the gastric ecosystem
WP5
Host Genotype
WP6
Impact of the environment
WP7
Response to dietary and microbial antigens
WP8
Innovative dietary strategies
Microbiota composition
GIT Function
GIT 'omics'
WP-DO Dissemination & Outreach
Improvements in production animal health, animal welfare and food safety

Piglet models for studying microbial colonization

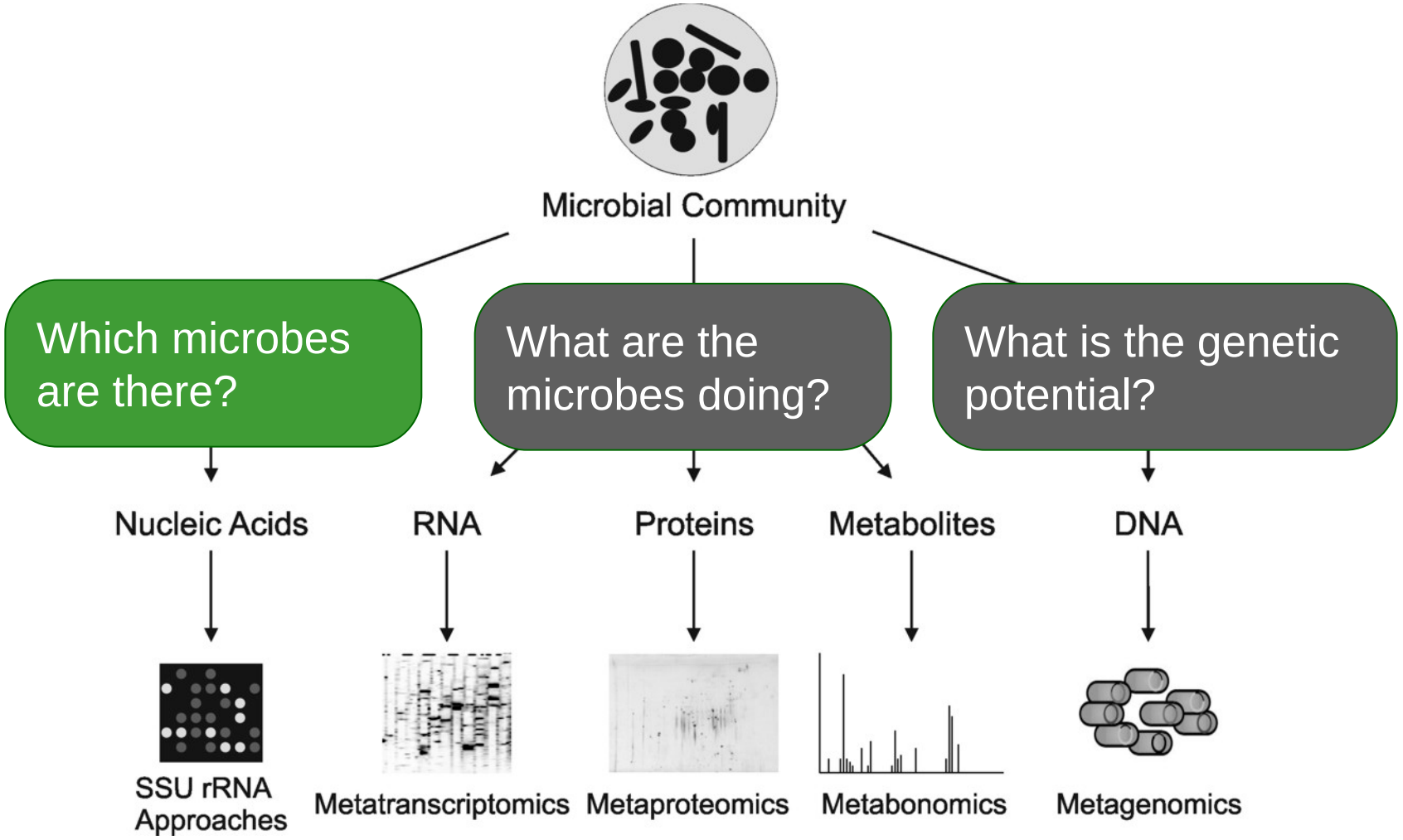
Interaction between early microbial colonization of the GIT and development of gut function

Implications for gut health and welfare throughout life

How to learn more about (gut) microbes & their interaction with the host

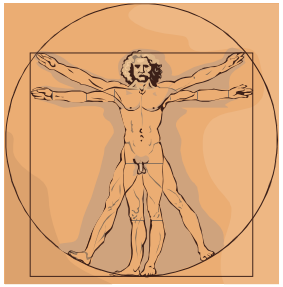


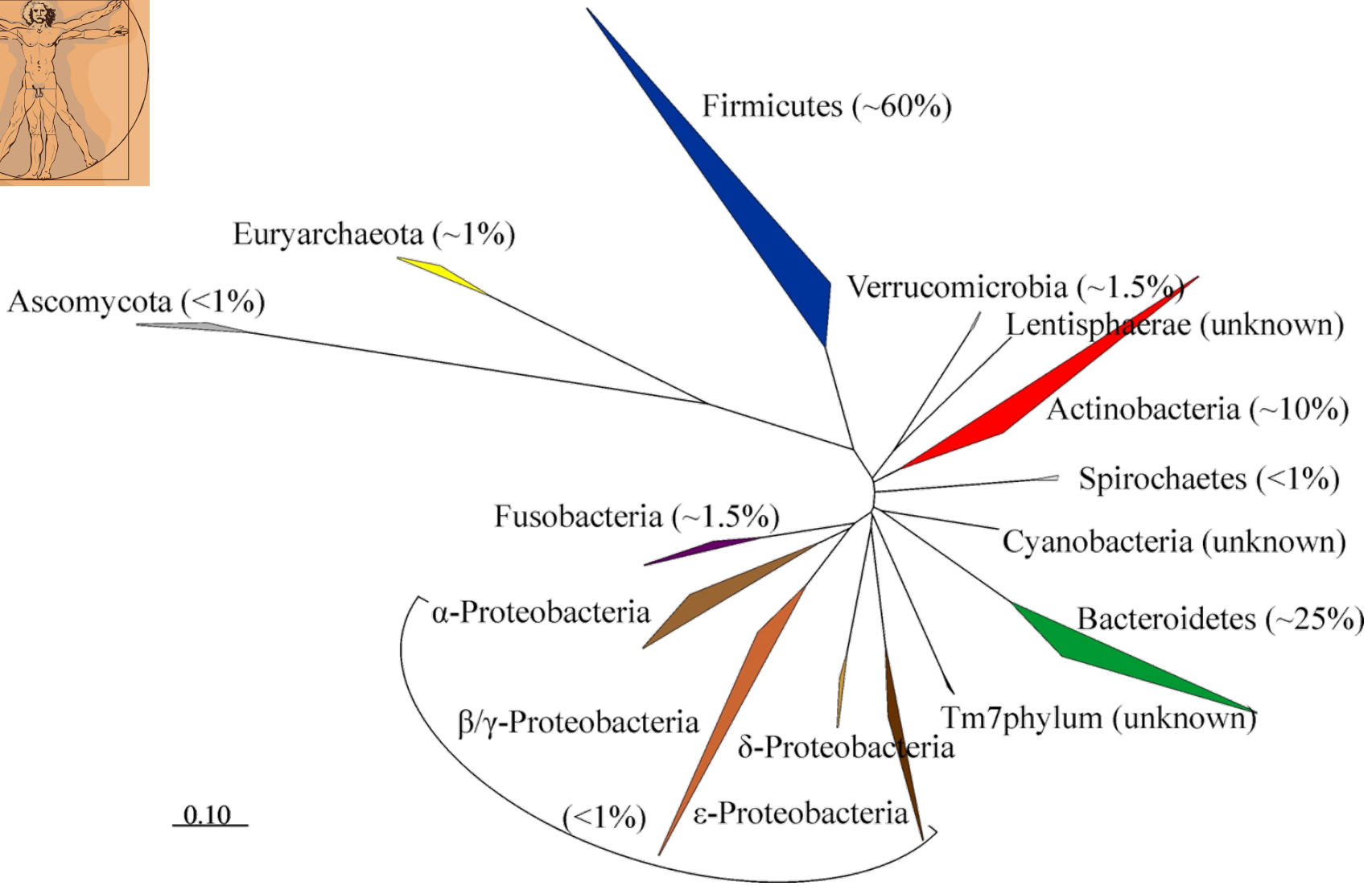
How to learn more about (gut) microbes



Ribosomal RNA as ideal universally applicable marker

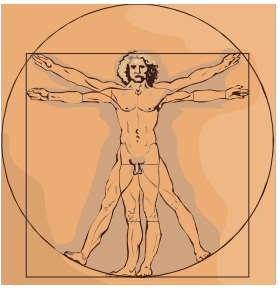
Which microbes can be found in the intestine?

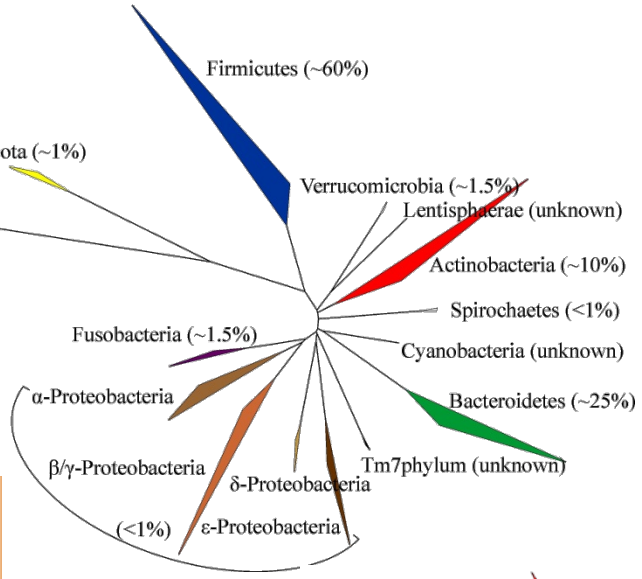


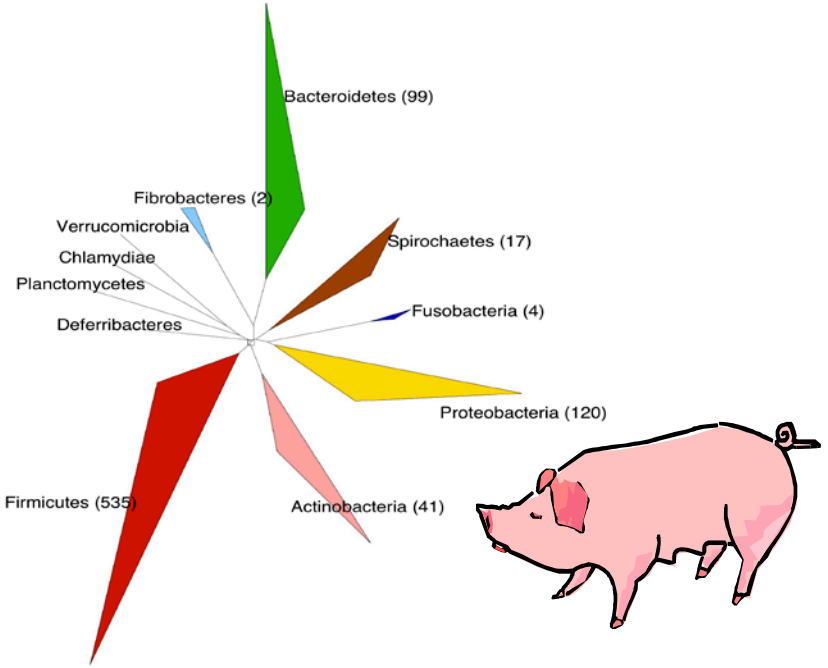


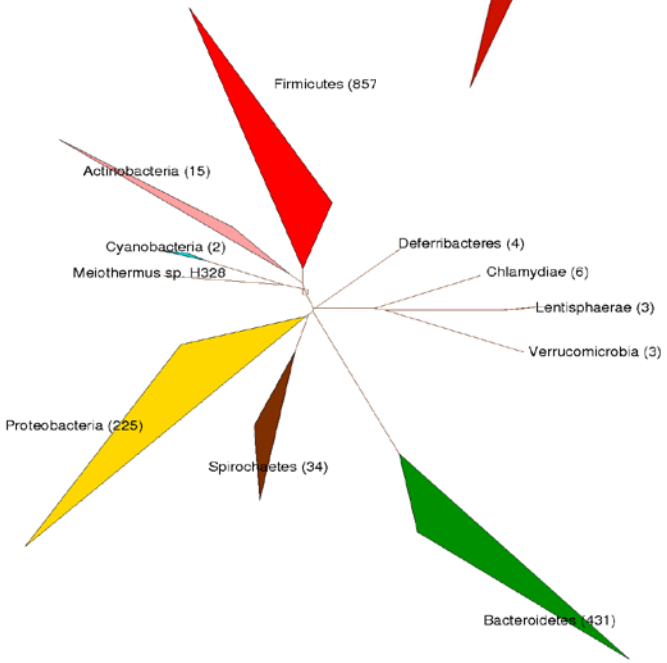
Rajilić-Stojanović et al., 2007. Env. Microbiol.

Which microbes can be found in the intestine?

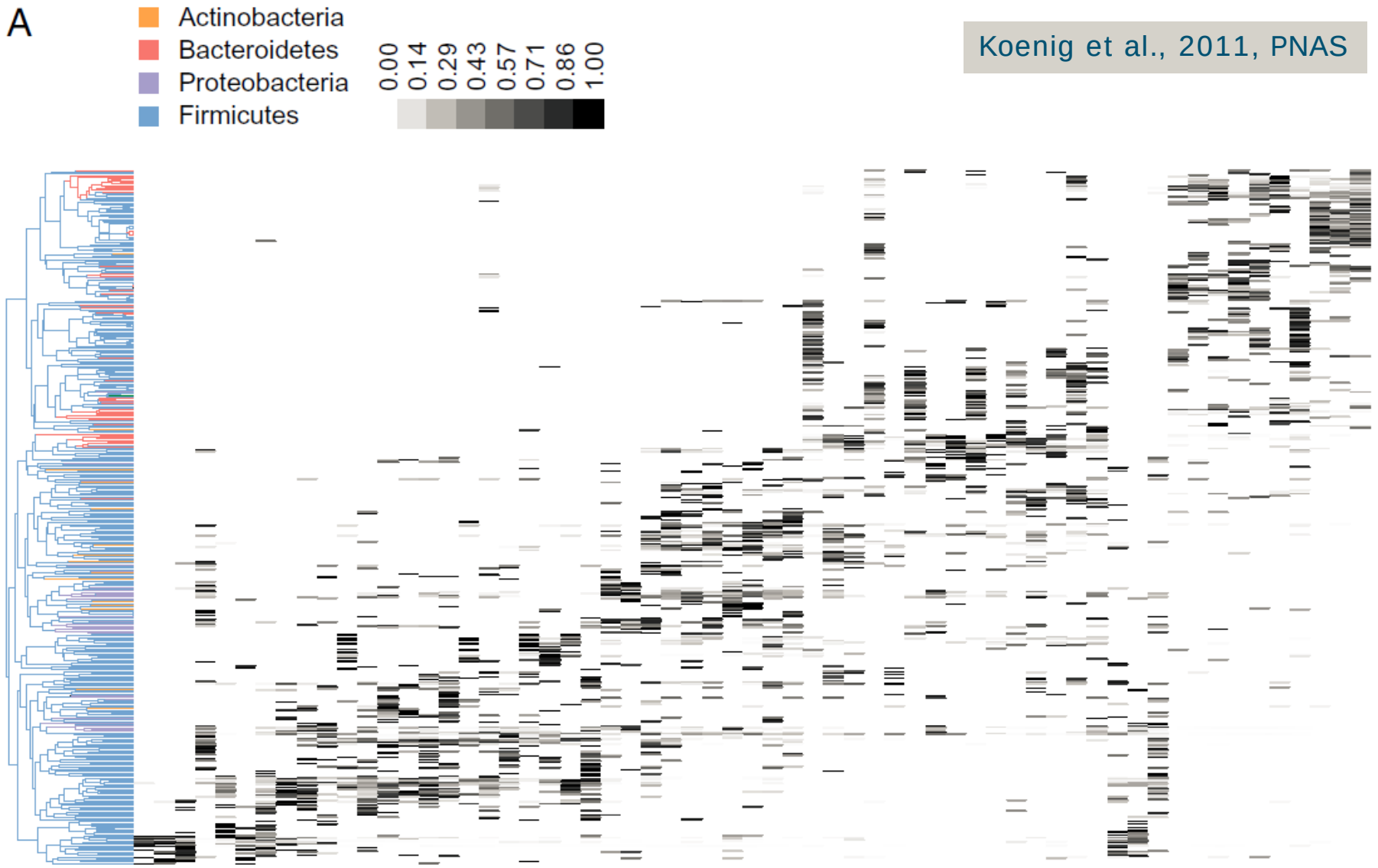








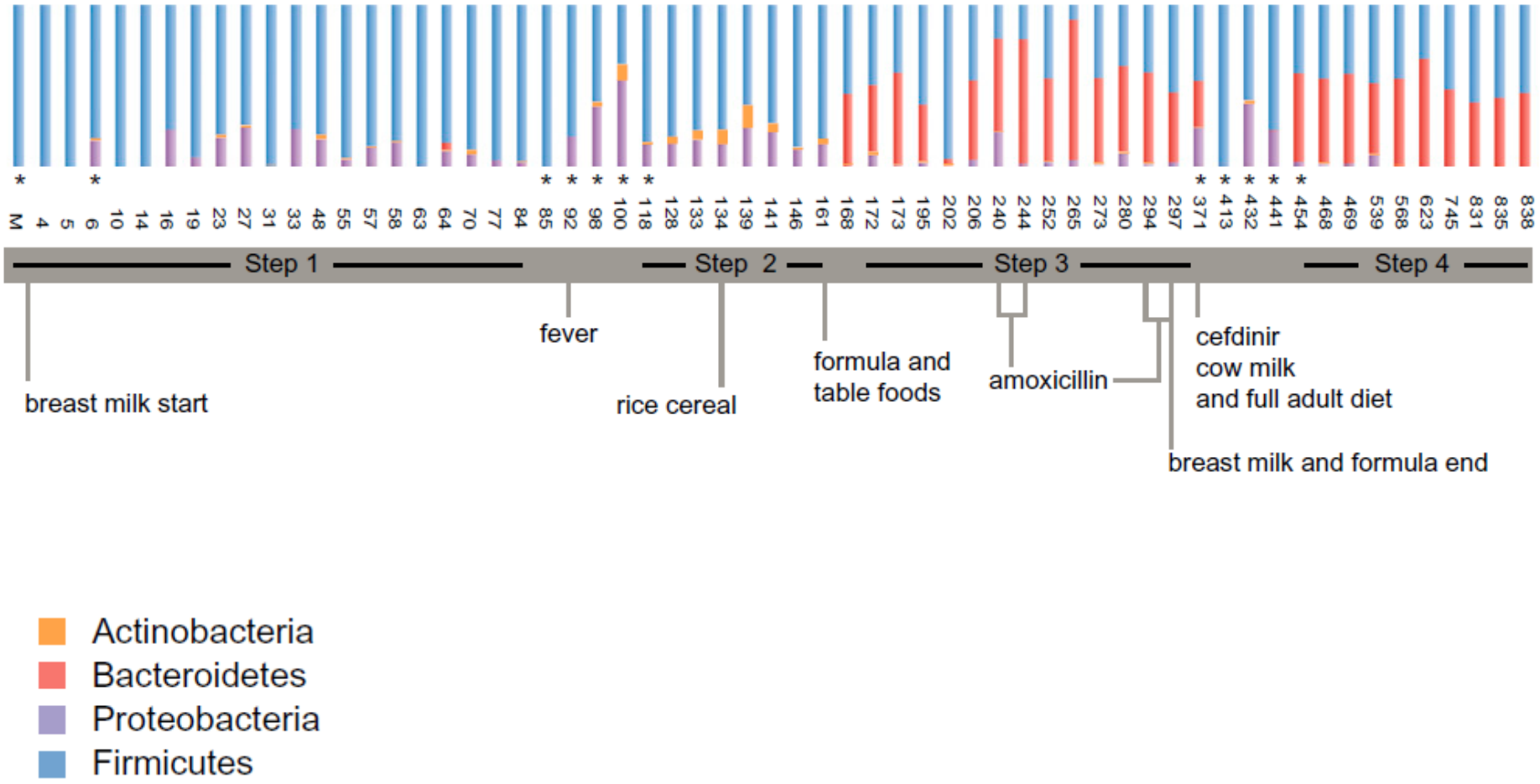
Microbial succession in newborns



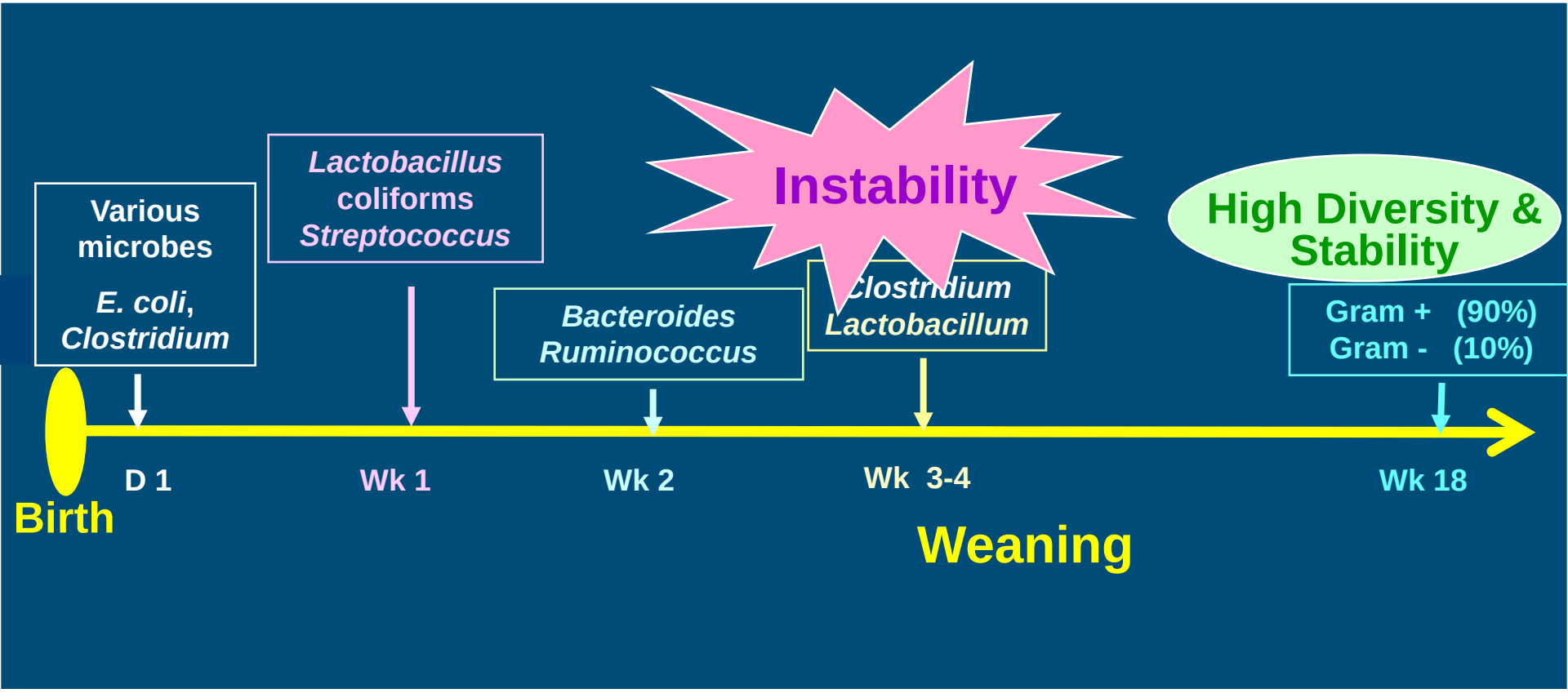
Koenig et al., 2011, PNAS

Microbial succession in newborns

Koenig et al., 2011, PNAS



Microbial succession in piglet feces



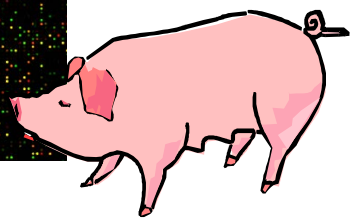
Robust high throughput tools available

The Intestinal Tract Chip Suite

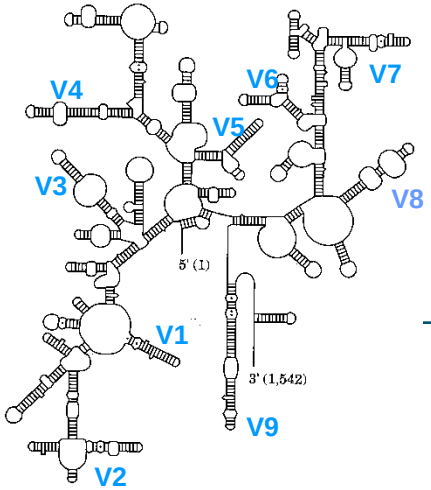
HITChip

MITChip

PITChip



CHICKChip

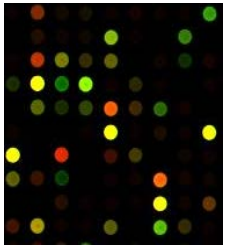


Agilent Technologies

X-Chip Combines Phylogenetic Fingerprinting and Phylotype Quantification

SAMPLES
(Microbial community)

DNA extraction
16S rRNA gene amplification
Labelling

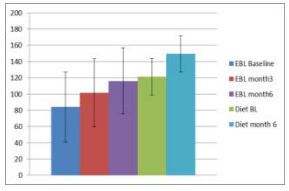


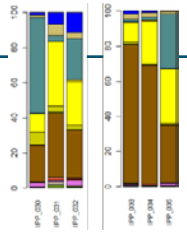
Hybridization

X-Chip: Data analysis

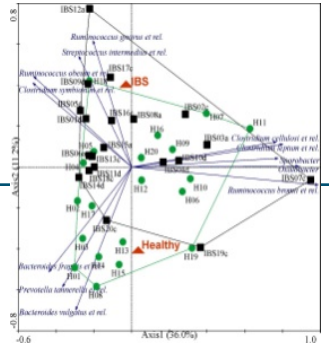
- Profiling
- Identification
- Quantification

Diversity analysis

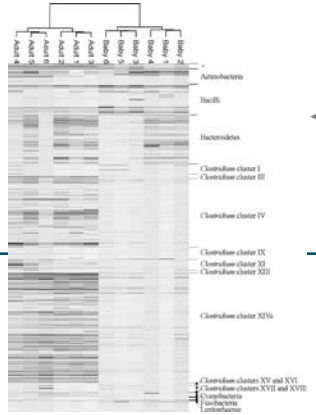




Multivariate statistics (RDA/PCA)



Phylotype clustering



Effect of antibiotics

Assess impact of AB treatment of sows on offspring colonization

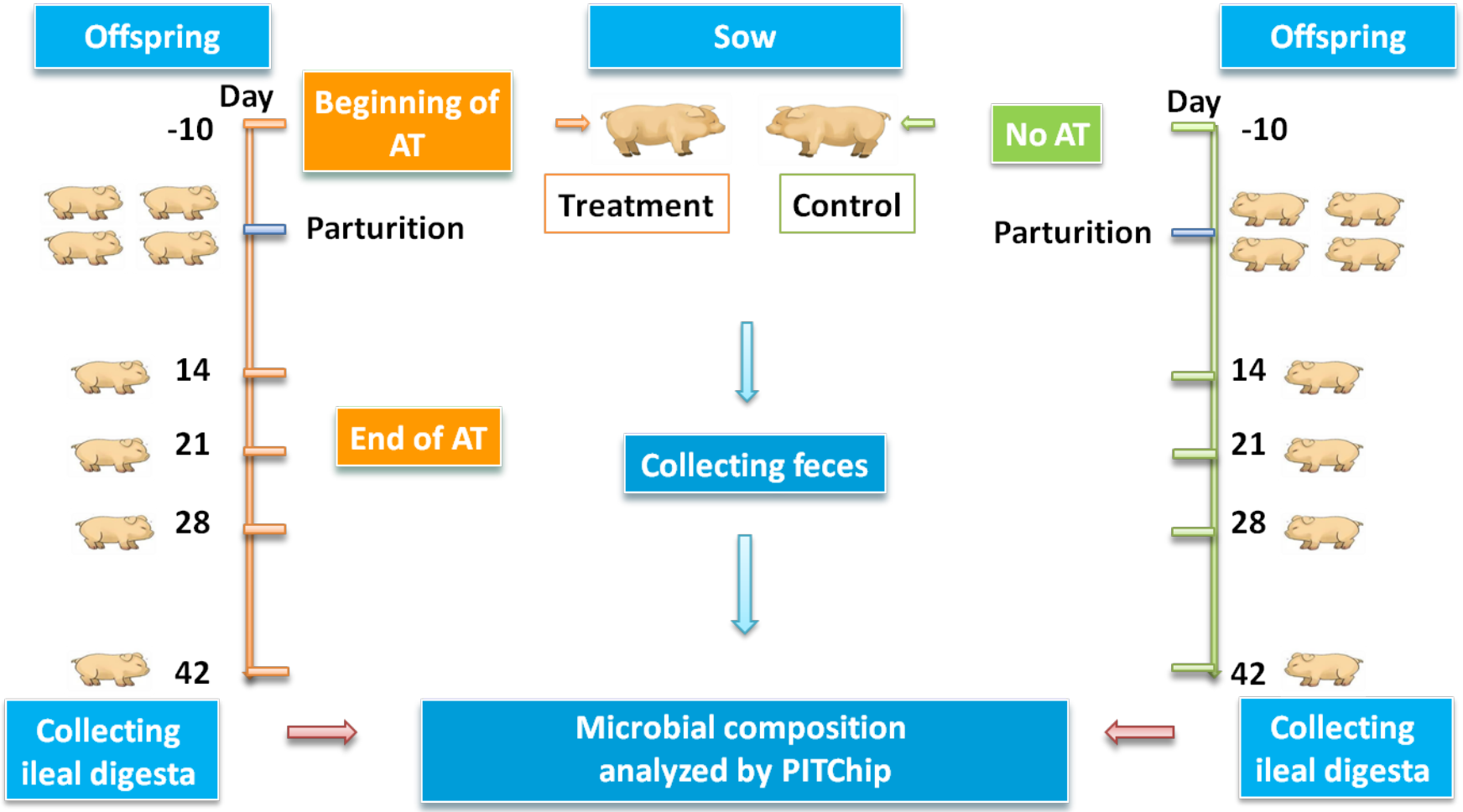
AB's used for controlling infections of genitourinary tract and mammary gland peri- or post-partum in sows

WAGENINGEN UNIVERSITY
WAGENINGEN UR

INTERPLAY

INRA

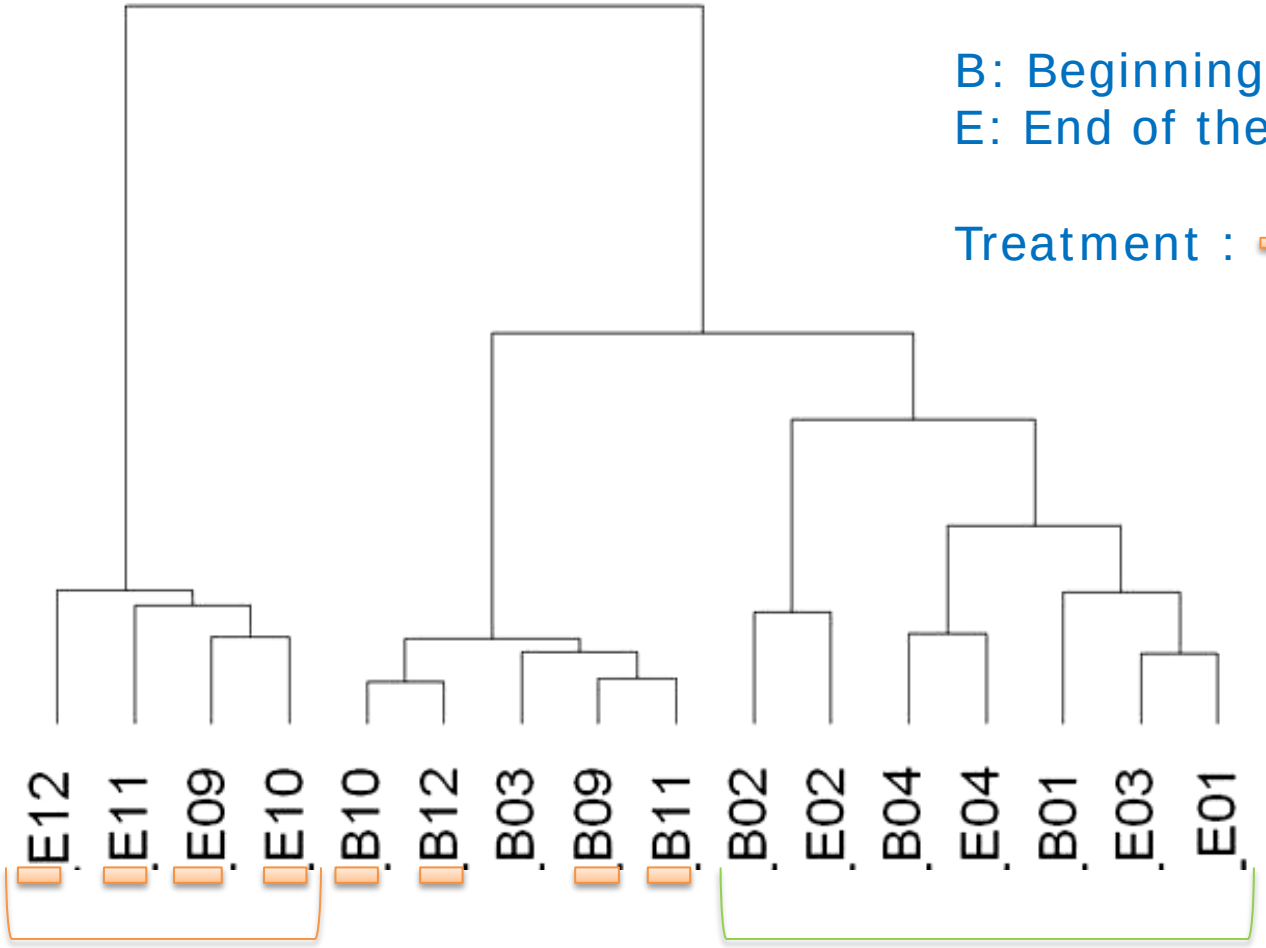
Experimental design



Amoxicillin 40 mg/kg BW/d on feed

Presentation J-P Lallès

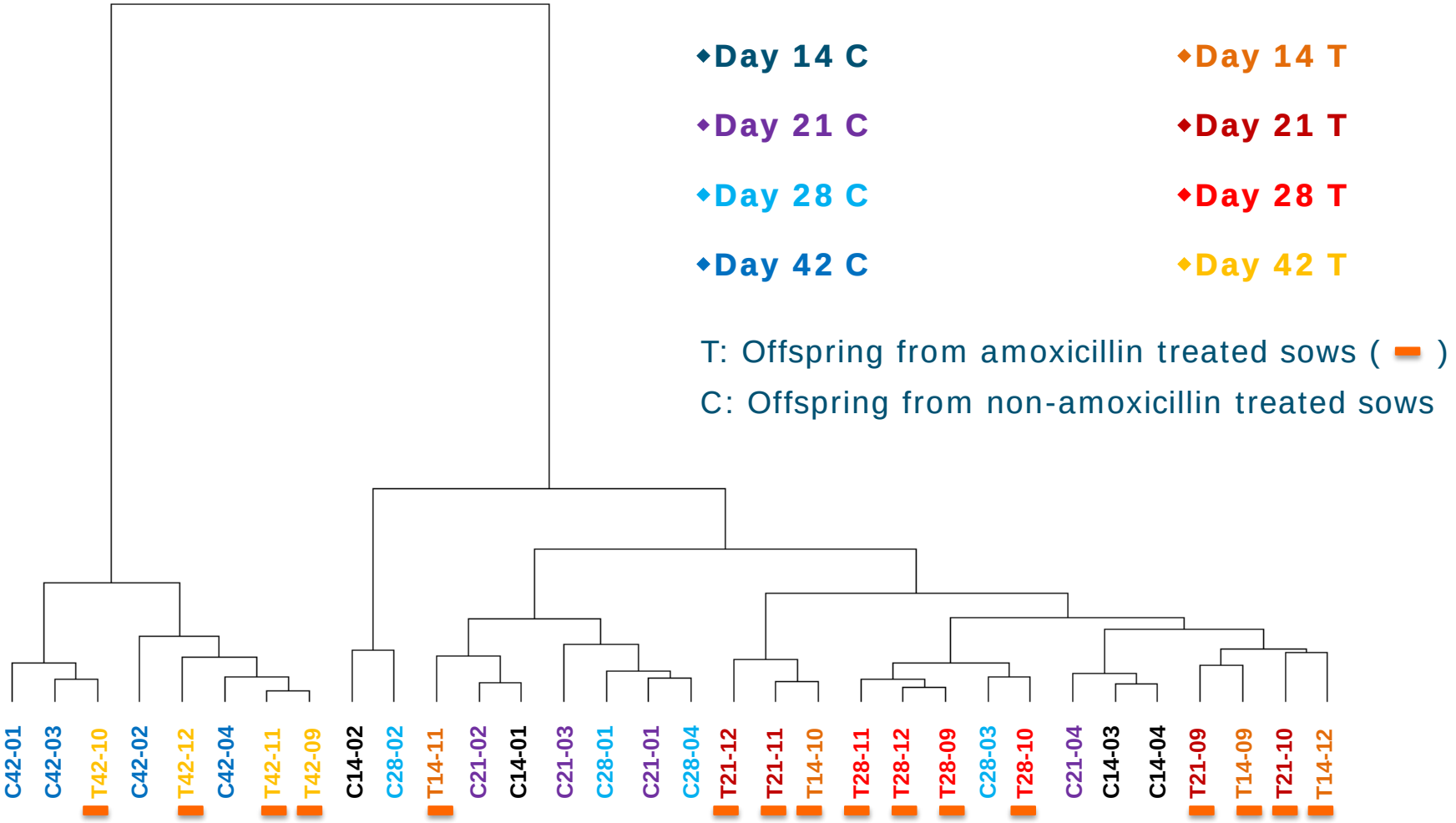
Effect of AB treatment on sows' microbiota



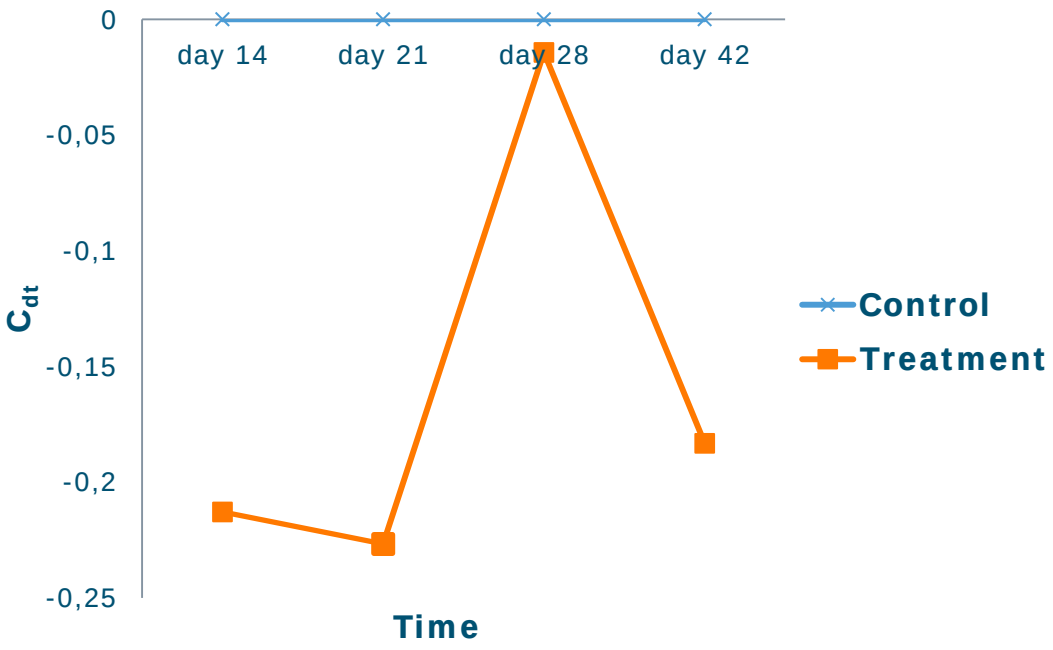
B: Beginning of the experiment
E: End of the experiment

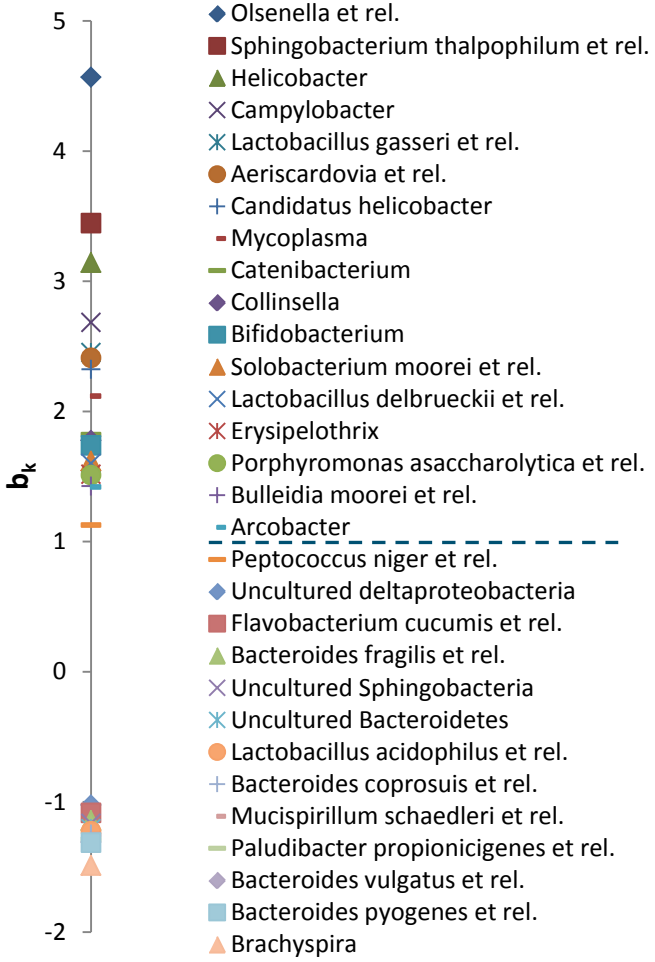
Treatment :

Effect of AB treatment on piglets' ileal microbiota



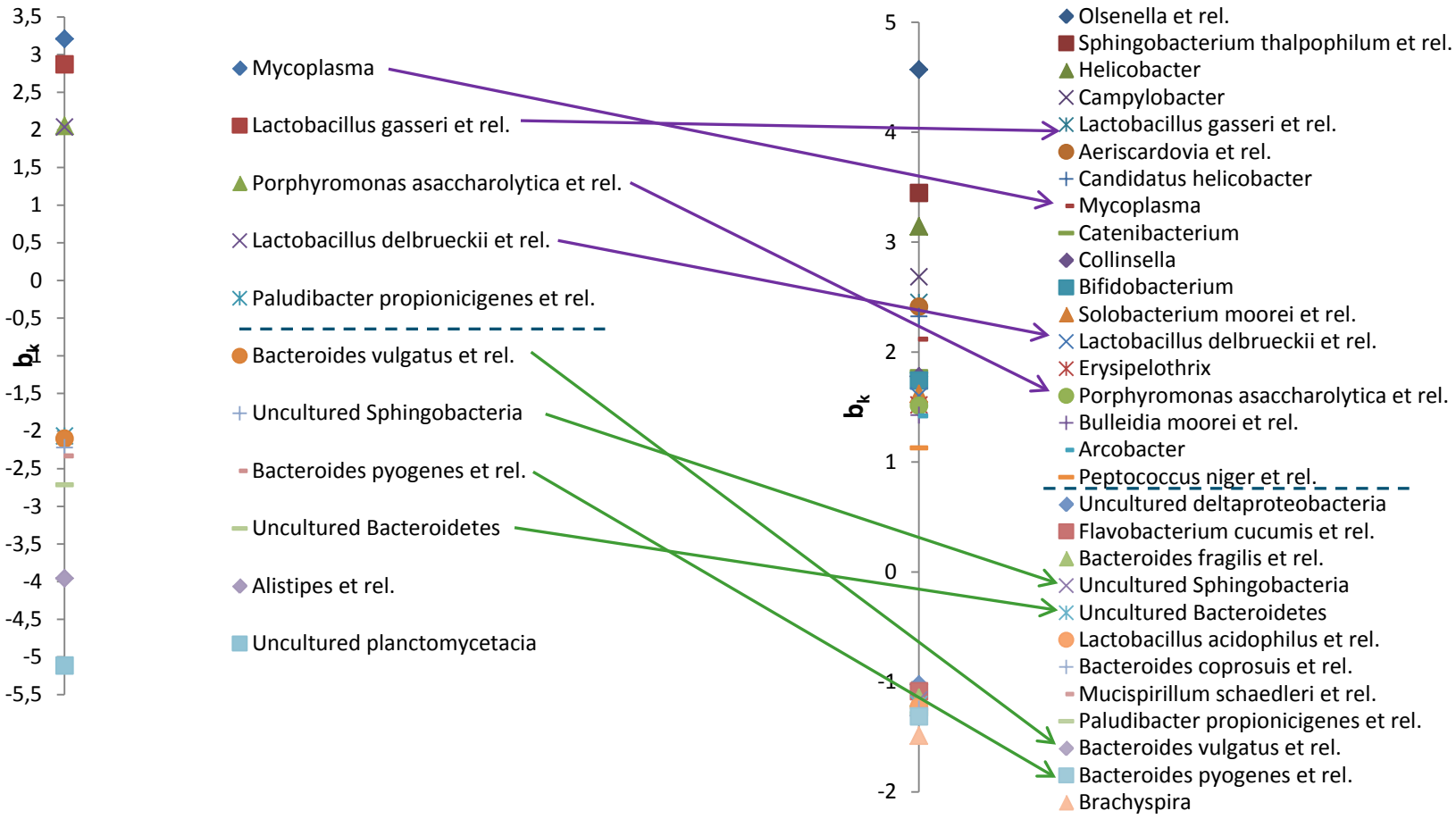
Short term effect on piglets





Difference between control & treatment increases after weaning

Similar effects in sows & piglets (d42)



Conclusions

- significant differences in microbiota composition in piglets especially before and after weaning
- effect of AB treatment in sows amplified in piglets AFTER weaning
    - Microbial groups adapted to post-weaning (adult) diet
- Next step – long term effects on microbiota composition & gut function
    - See presentation J.P. Lallès

The Team

WAGENINGEN UR
For quality of life

MICROBIOLOGY

- Odette Perez
- Jing Zhang
- Xin Gao
- Lingli Zhang

INRA

- Jean-Paul Lallès
- Gaëlle Boudry
- Isabelle Luron

Impact of host genotype

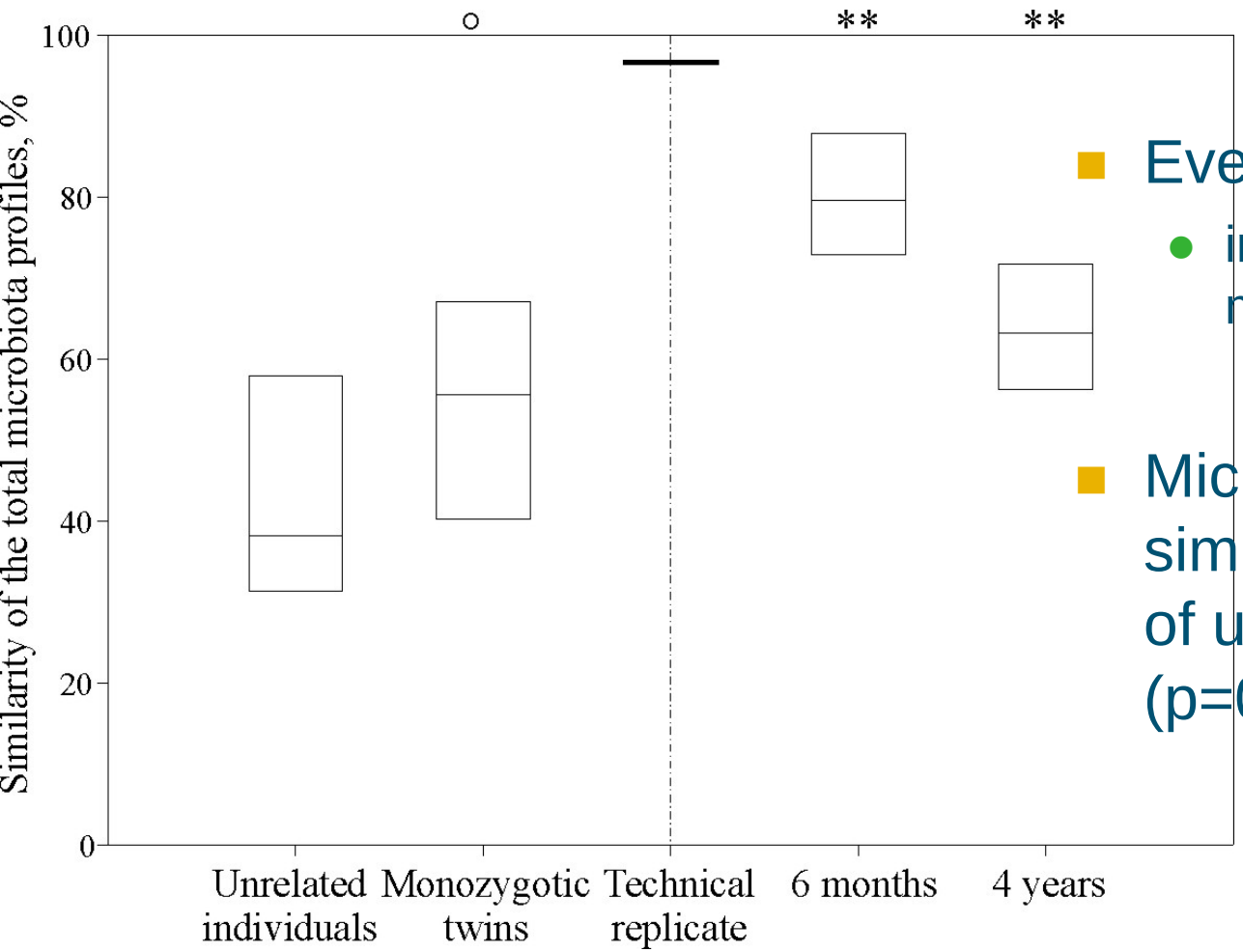
WAGENINGEN UNIVERSITY
WAGENINGEN UR

INTERPLAY

南京農業大學

Humans: Some are more equal than others...

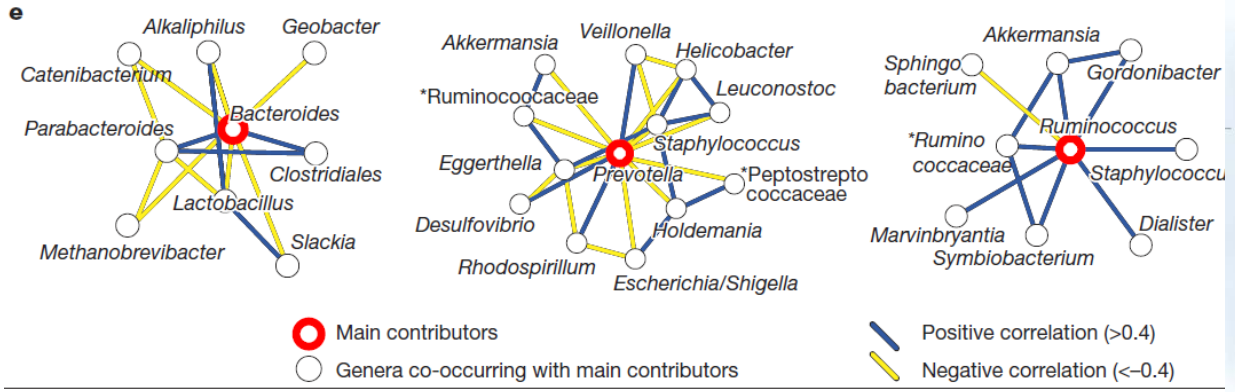
Dynamics of the microbiota in healthy adults



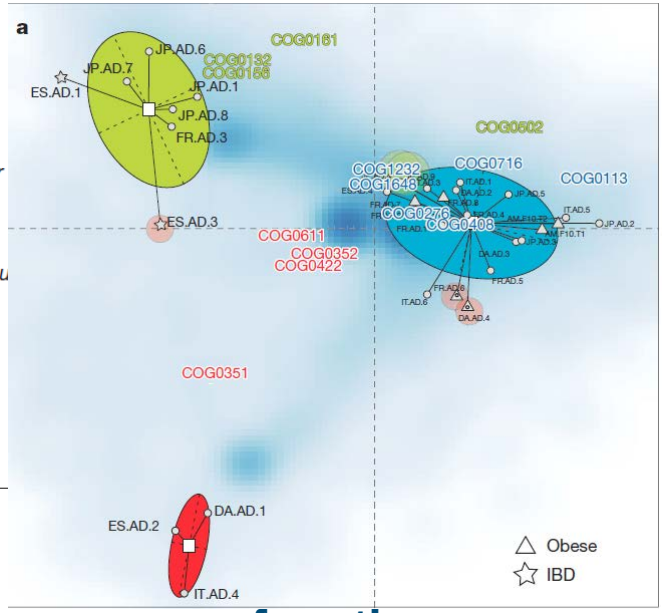
- Even after 4 years:
  - individual-specific microbiota
- Microbiota of twins more similar than microbiota of unrelated individuals (p=0.06)

Three major networks in global population

Enterotypes based on metagenomes & HITChip analyses



Composition



function

Impact on Personalized Nutrition and Pharma

Amurugam et al., 2011. Nature

Some are more equal than others...

How about farm animals?

- Host genetic influence within & across breeds has not been properly investigated in farm animals
- Can we discern genotype/phenotype-specific enterotypes?
- Potential links with
  - Efficiency of feed conversion
  - Emission of greenhouse gasses (methane)
  - Overall health & performance

Many different genotypes...

Effect of host genotype & environment

Cross-fostering experiment

Landrace

Meishan

Sow A Sow B

Oestrus synchronization

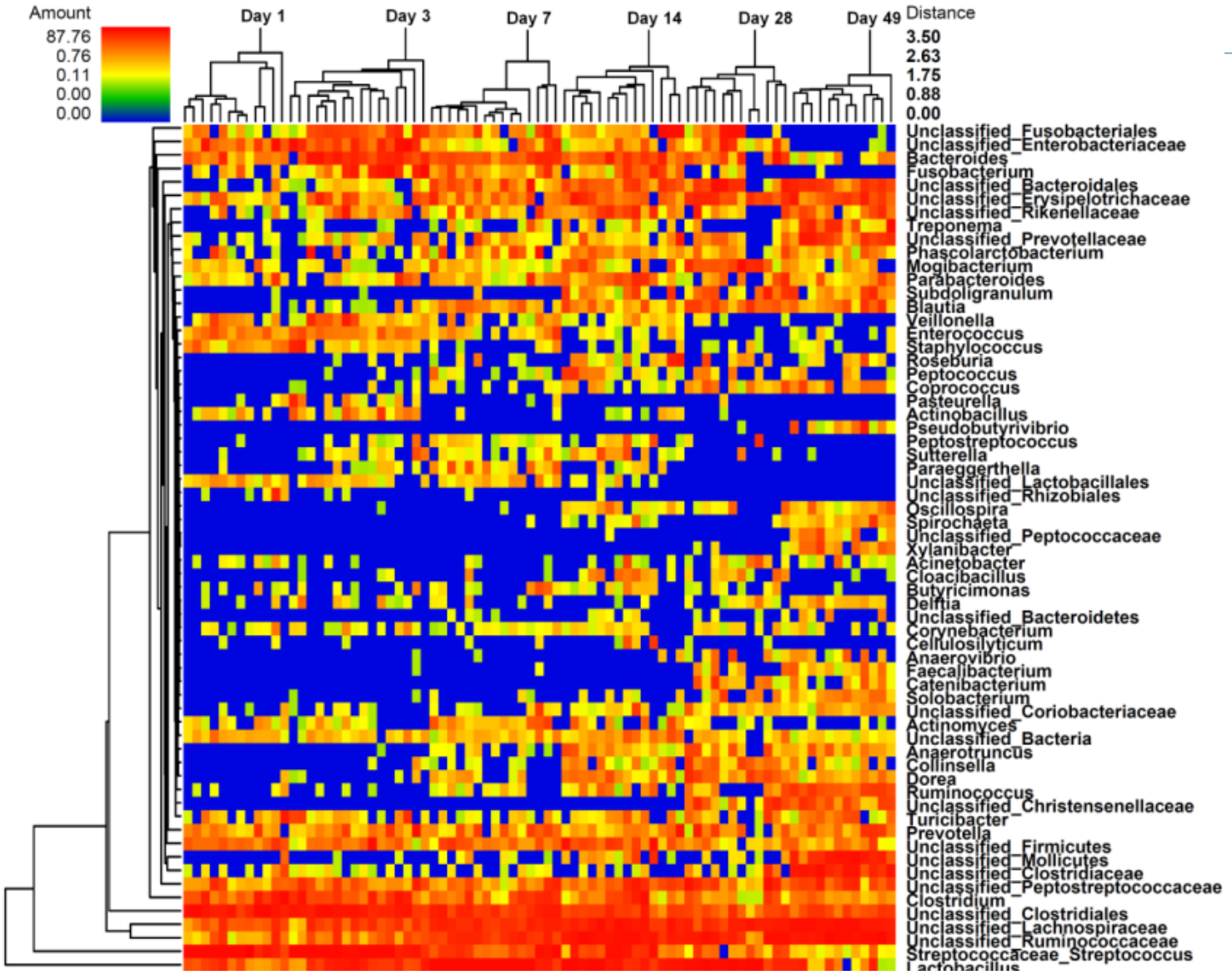
Piglets A Piglets B

Cross-fostering

Sow A Sow B

Follow piglets in time with respect to microbiota composition & function, physiology and immune system development

Microbial succession most strongly affected by age

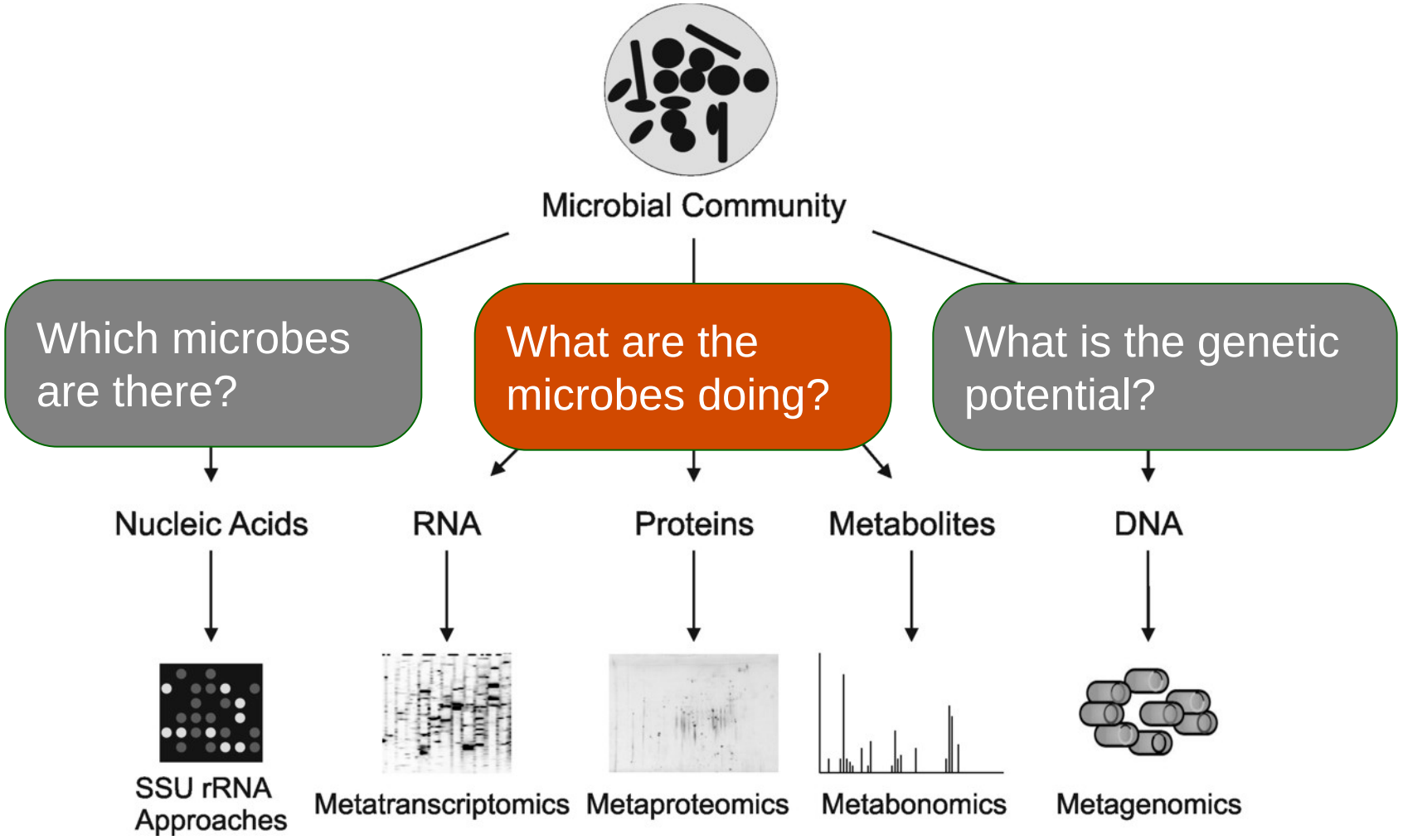


The Team

- Odette Perez
- Jing Zhang
- Lingli Zhang

- Wei-yun Zhu
- Su Yong
- The Nanjing Team

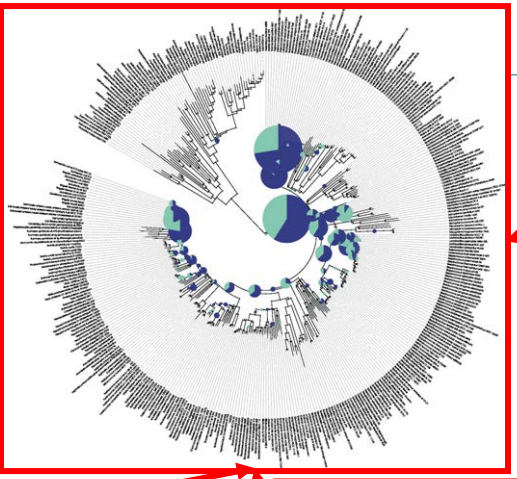
How to learn more about (gut) microbes



Microbial biomarkers for ecosystem functioning

Functional microbiomics of the human small intestine - **Metatranscriptomics**

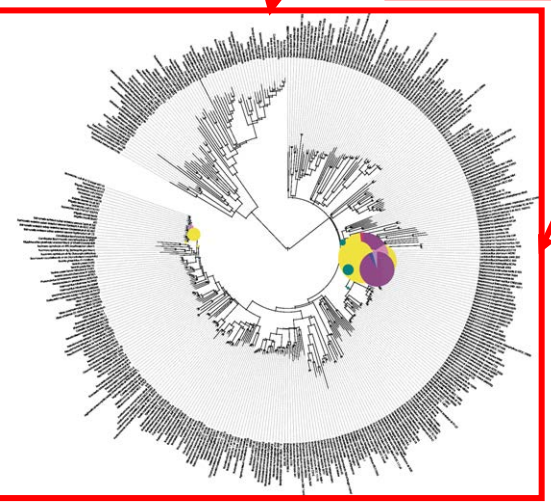
Subconsortium activity
Streptococcus
Escherichia



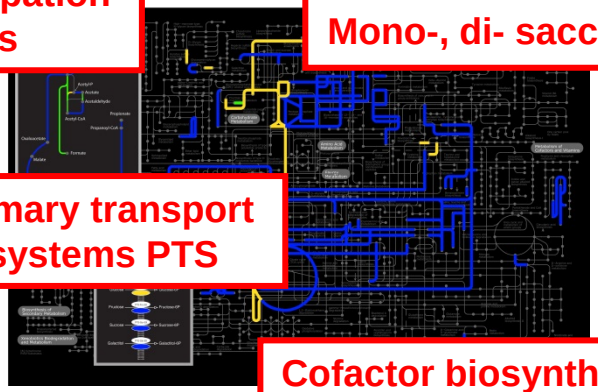
“All” members participate

Pyruvate dissipation pathways

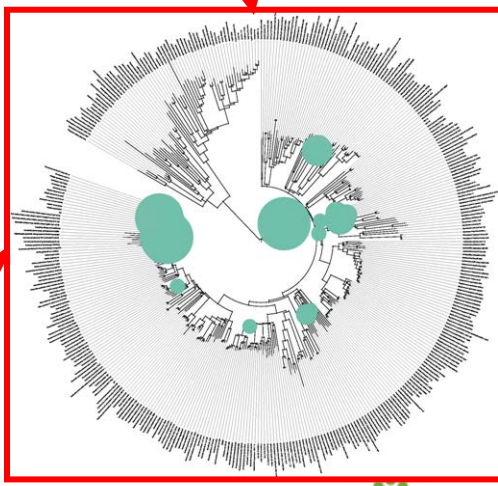
‘simple’ carbohydrate utilization
Mono-, di- saccharides



Primary transport systems PTS



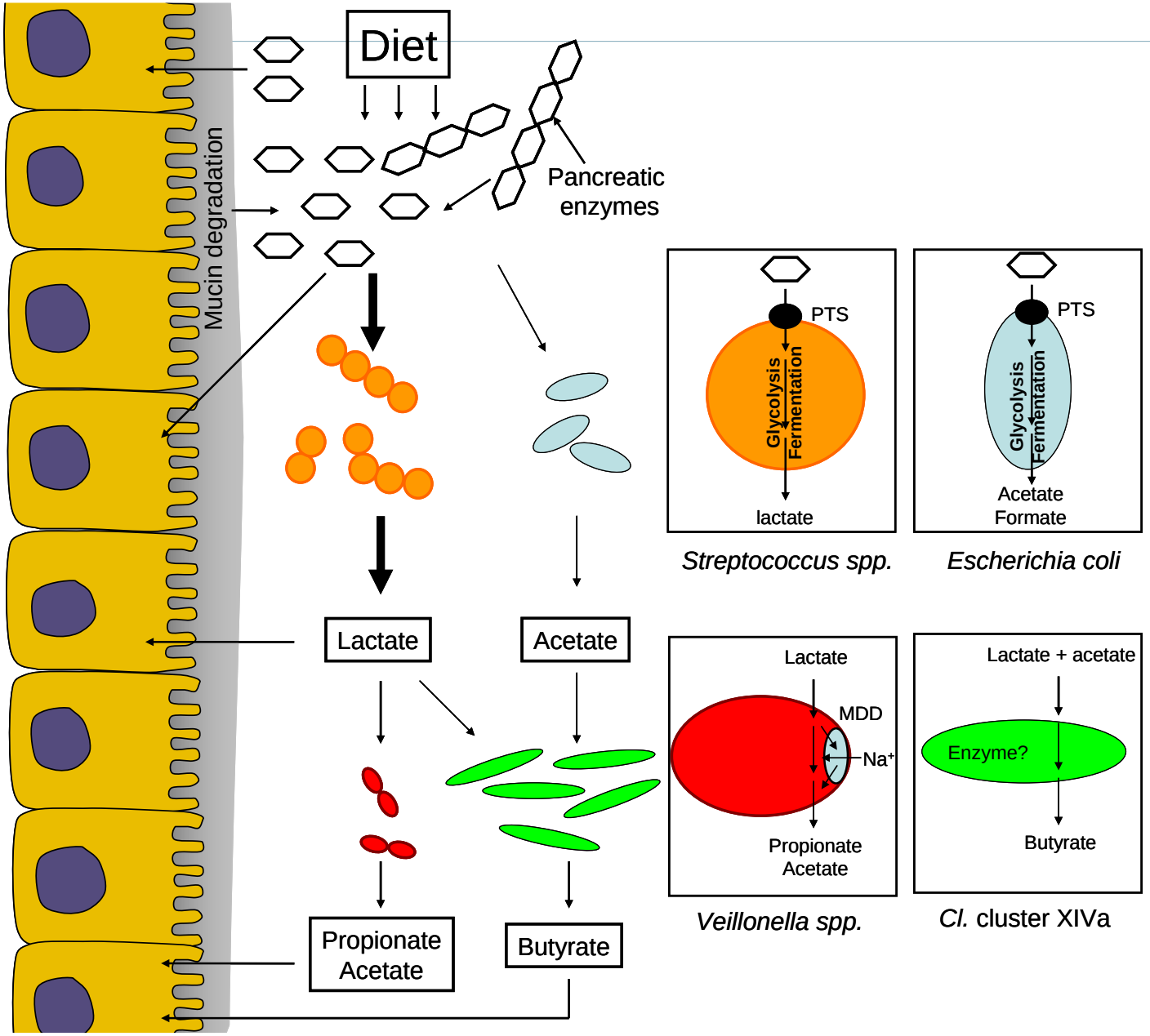
Cofactor biosynthesis biotin



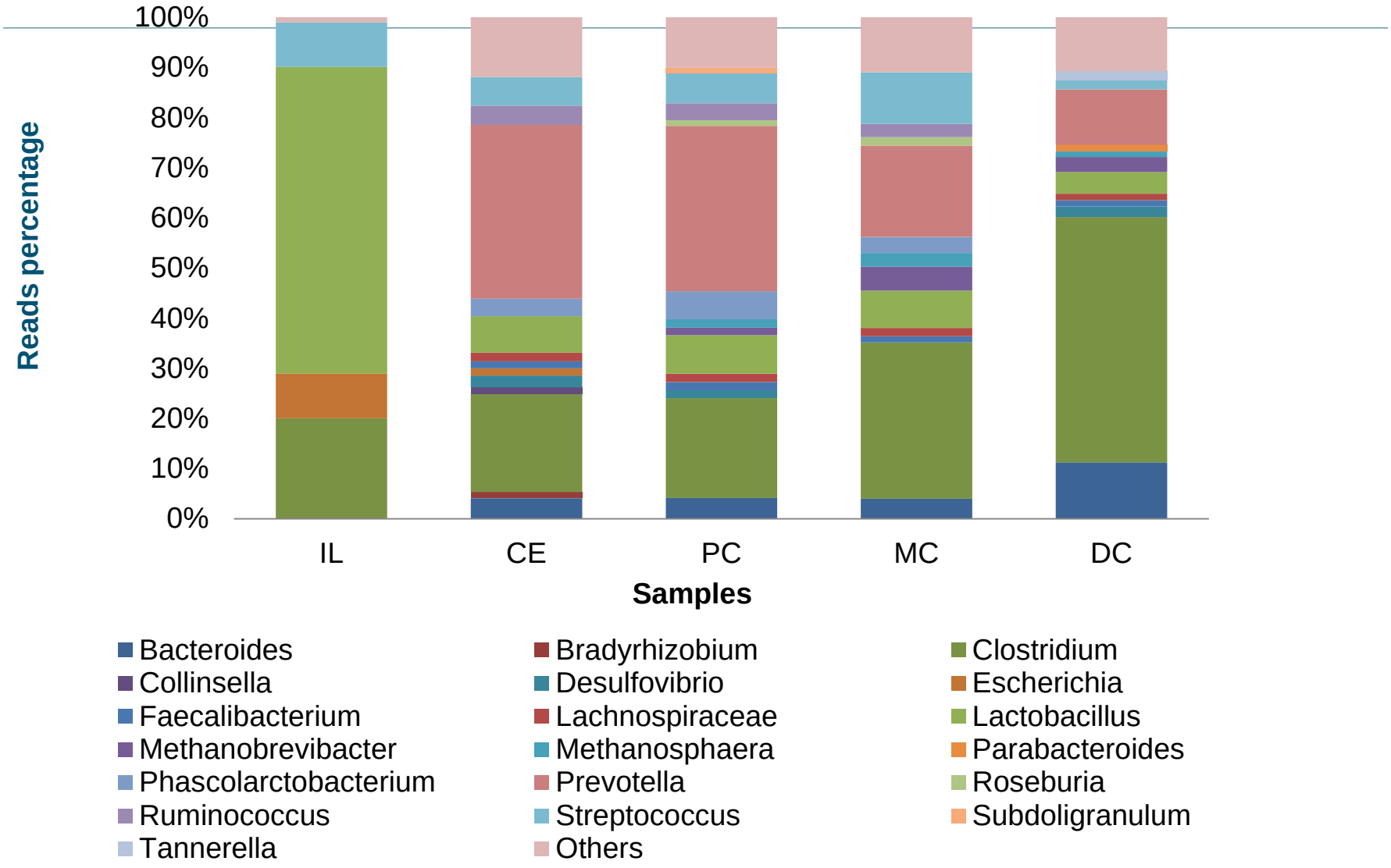
Zoetendal, Raes et al. 2012, ISME J

EMBL

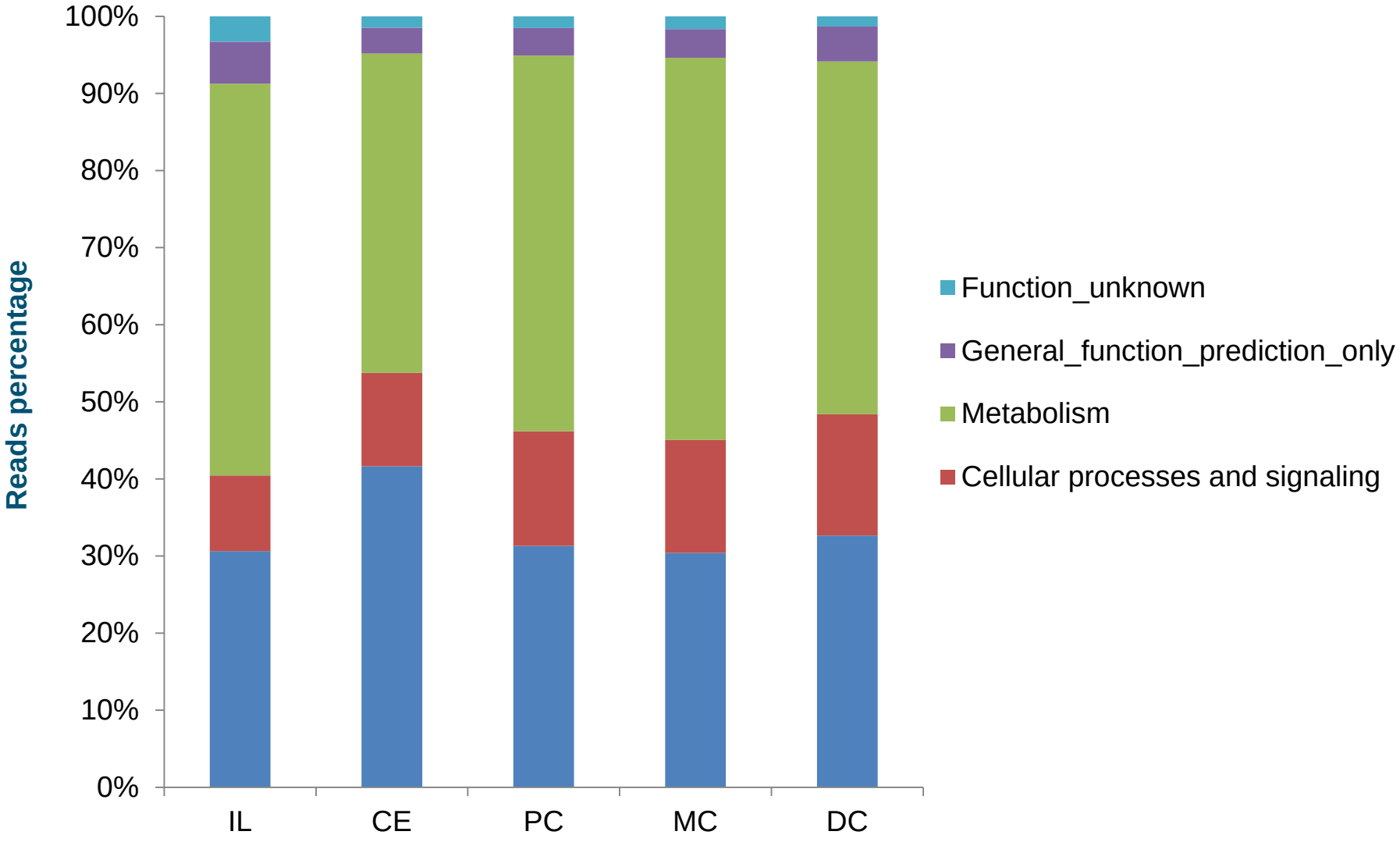
Life in the small intestine – a first glimpse



Phylogenetic distribution of activity along pig GIT



Functional distribution of activity along GIT



Take Home Messages

- High diversity intestinal microbiota; > 1000 phylotypes
- Need for functional genomics-based approaches for comprehensive characterization
- Microbiota is influenced by host (genetic background); age, environment & feed
- Period around weaning is characterized by instability → chance for potential pathogens
- Potential for pre- and probiotics; other feed additives incl. organic acidifiers and bioactive plant extracts

Together forever in a microbial world

- We need microbes
- Microbes need us
- Microbes need each other

INTERPLAY today at EAAP 2013