



Gut microbiota analyses for sustainable European local porcine breeds: a TREASURE pilot study

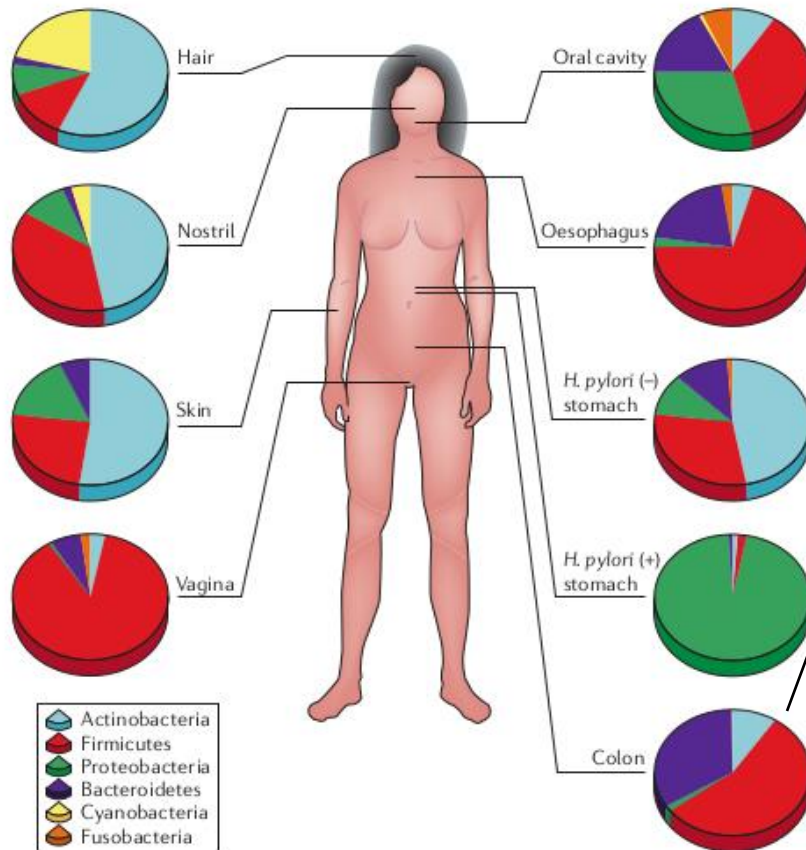
J.Estellé, M. Čandek-Potokar, M. Škrlep, Č. Radović, R. Savić, D. Karolyi, K. Salajpal, M.J. Mercat, G. Lemonnier, O. Bouchez, J. García-Casco, P. Palma-Granados, R. Nieto, A.I. Fernández, B. Lebret and C. Óvilo

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Horizon 2020
Grant agreement No 634476

Symbiotic microbiota



Microbiota

- Population of microorganisms living in symbiosis with its host
- Intestine:
 - 10^{14} microorganisms (~ 1,5 Kg)
 - Implantation at early age
 - Relative stability along host's life

Dysbiosis

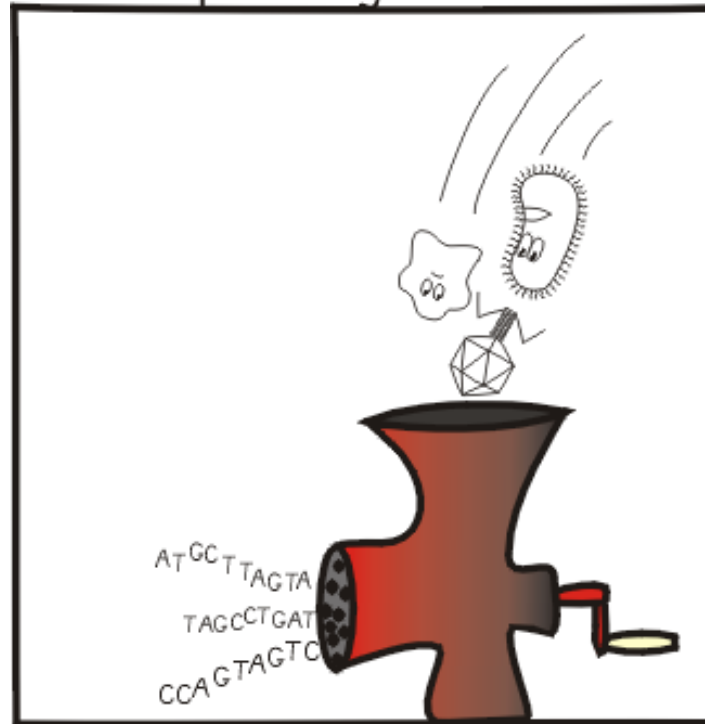
- Disequilibrium of microbiota composition that can let to pathology

How to look at microbiota?



VS.

Mass sequencing



“Shotgun”

16S

by Viktor S. Poór

Objective of microbiota pilot study

- To conduct a pilot characterization of intestinal microbiota in order to test its usefulness to characterize pig population and production systems.
 - **Production systems**
 - Breeds inside production systems
 - ...



Target populations

- Differentiate production systems
 - Krškopolje pigs under conventional and organic production systems (Slovenia)
 - Iberian pigs under “montanera” forest and standard industrial feeds (Spain)
 - Turopolje pigs under to evaluate effects of acorn supplementation in organic production (Croatia)



Target populations

Krškopolje



Turopolje



Iberian

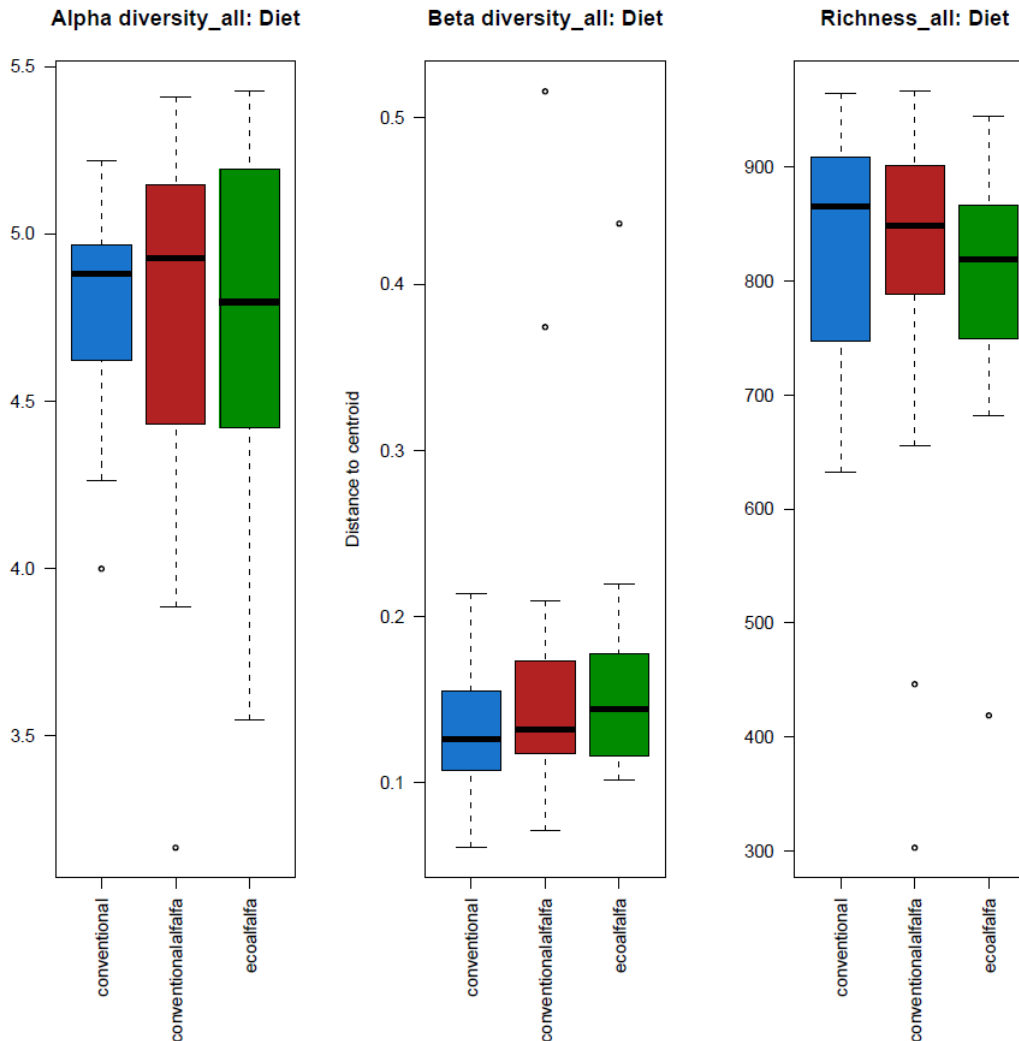


Krškopolje pigs (Slovenia)

- Pigs under conventional and organic production feed with alfalfa supplementation.
 - Design: 36 pigs distributed in 3 diets, 16S in two sample points (155d & 228d)
 - Samples (n=106) processed for DNA extraction and 16S sequencing in an Illumina MiSeq system



Diversity analysis



Conventional
 Conventional+alfalfa
 Eco-alfalfa

α DIVERSITY:
 is the within-sample diversity

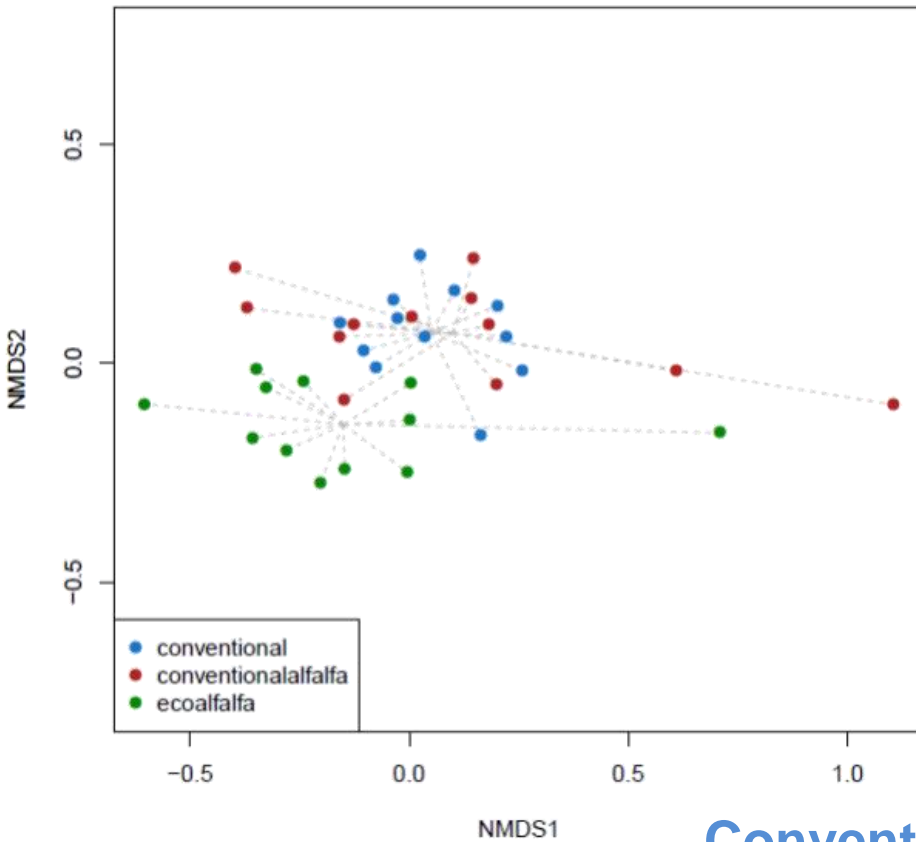
β DIVERSITY:
 is the between samples diversity

SPECIES RICHNESS:
 is the number of different species
 represented in an ecological community

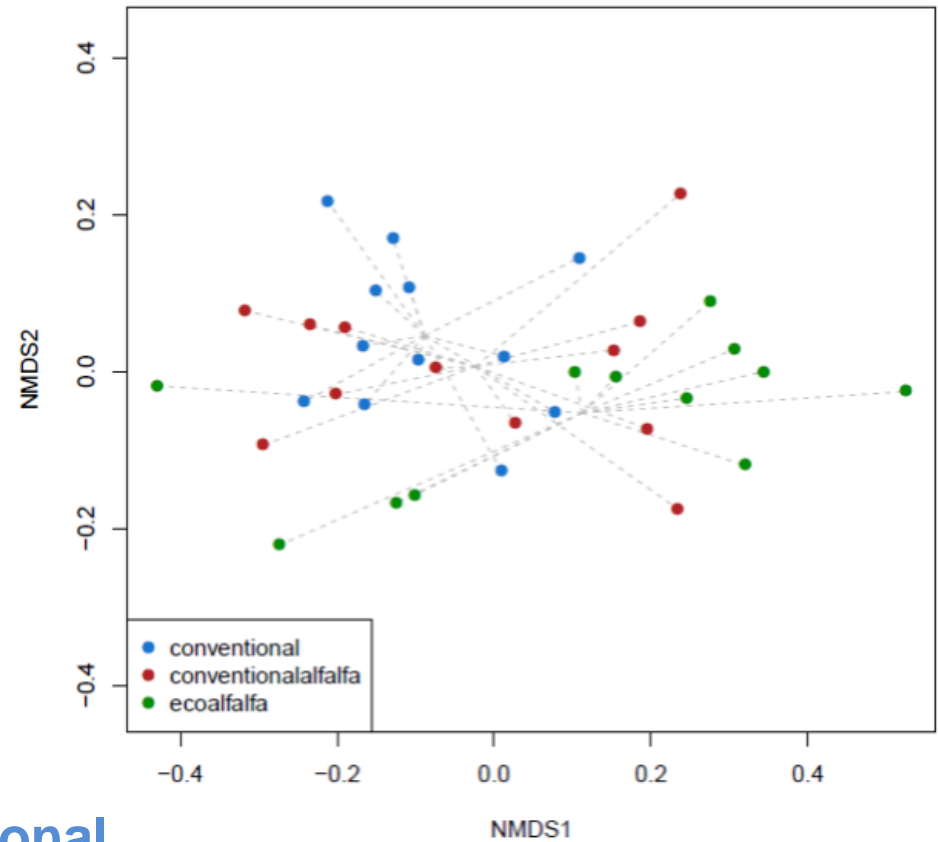


NMDS by sample time

NMDS (Bray-Curtis distance) OTU counts by Diet (T0)



NMDS (Bray-Curtis distance) OTU counts by Diet T1

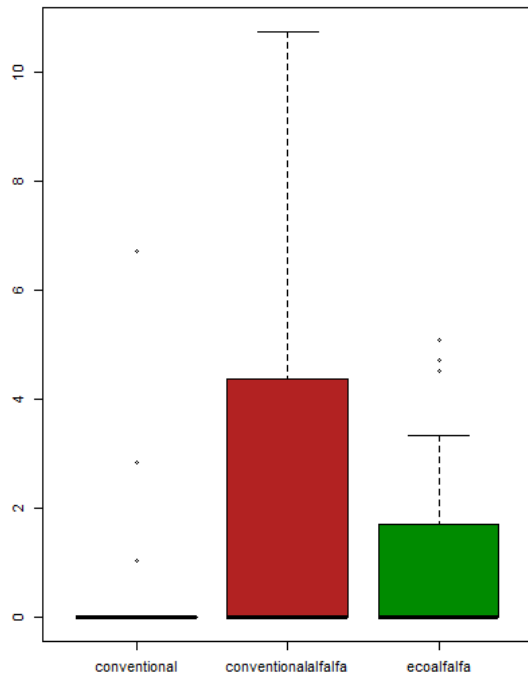


Conventional
 Conventional+alfalfa
 Eco-alfalfa



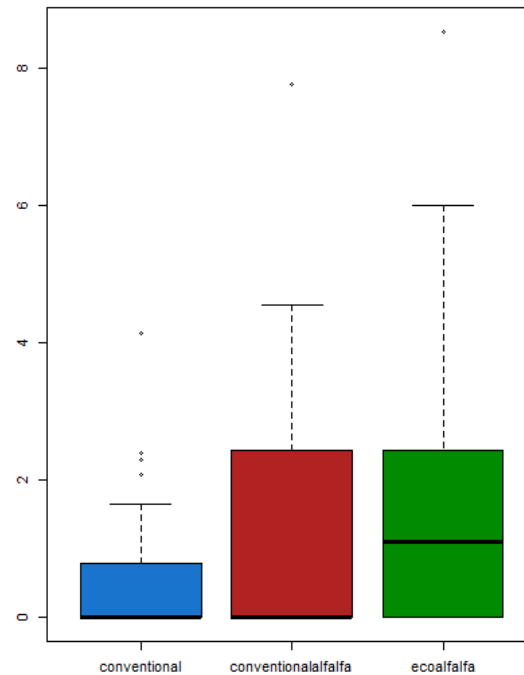
Diff. abundant Genera

Genus Bacteroides



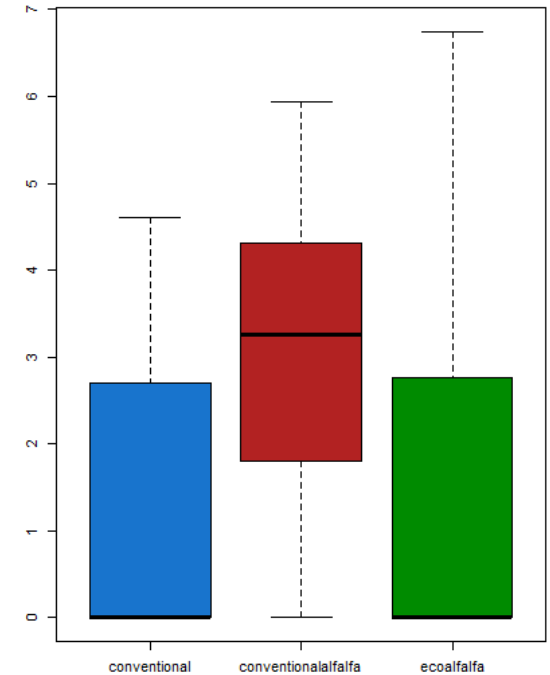
352617 OTU normalized abundance

Genus Paludibacter



New.ReferenceOTU1853 normalized abundance

New.ReferenceOTU3162 (order: Rickettsiales)



New.ReferenceOTU3162 normalized abundance

Conventional
Conventional+alfalfa
 Eco-alfalfa



49.26: Gut microbiota composition in Krškopolje pigs under conventional and organic production systems.

J. Estellé, F.R. Massacci, D. Esquerré, D. Jardet, G. Lemonnier, C. Óvilo, M. Skrlep, K. Poklukar and M. Čandek-Potokar



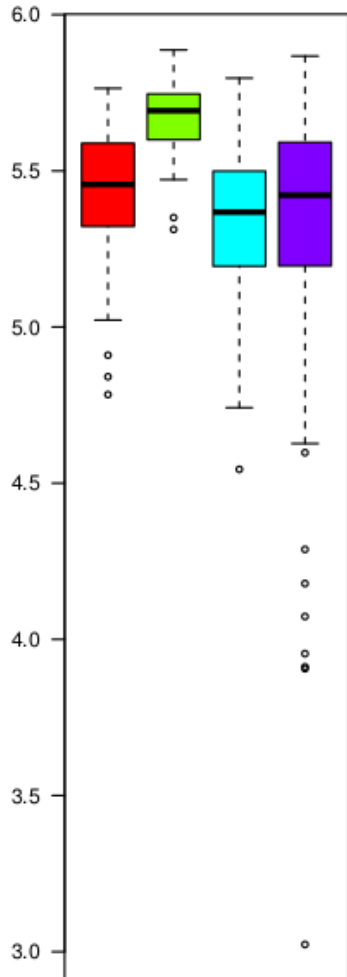
Iberian pigs (Spain)

- Pigs under “montanera” forest and standard feeding (“cebo”)
 - Sampling completed 336 samples:
 - 106 samples on “bellota” accorn diet
 - 145 samples on standard feeding (“cebo”)
 - 43 samples on enriched commercial diet
 - 42 samples on enriched diet + acorns
 - Microbiota 16S analyses in an Illumina MiSeq system

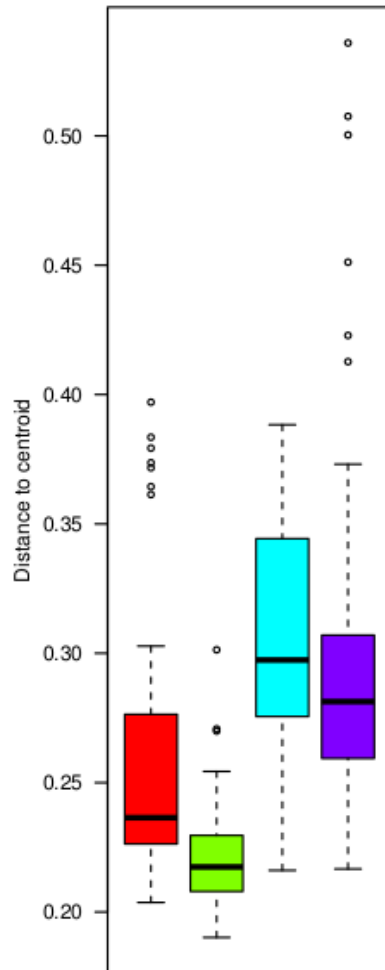


Diversity analysis

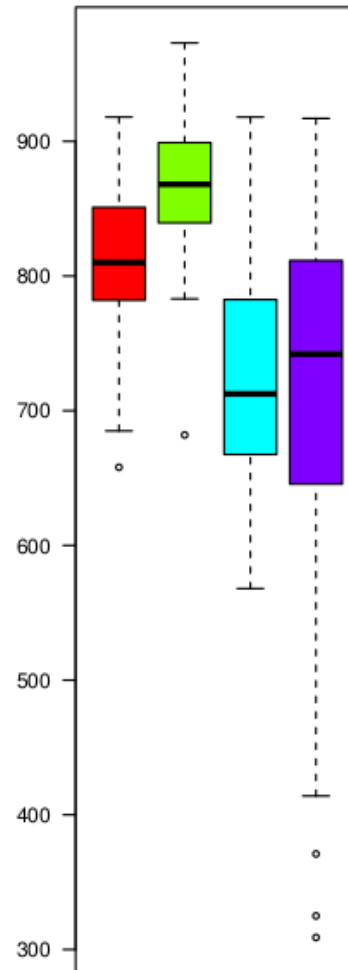
Alpha diversity_all: diet



Beta diversity_all: diet



Richness_all: diet

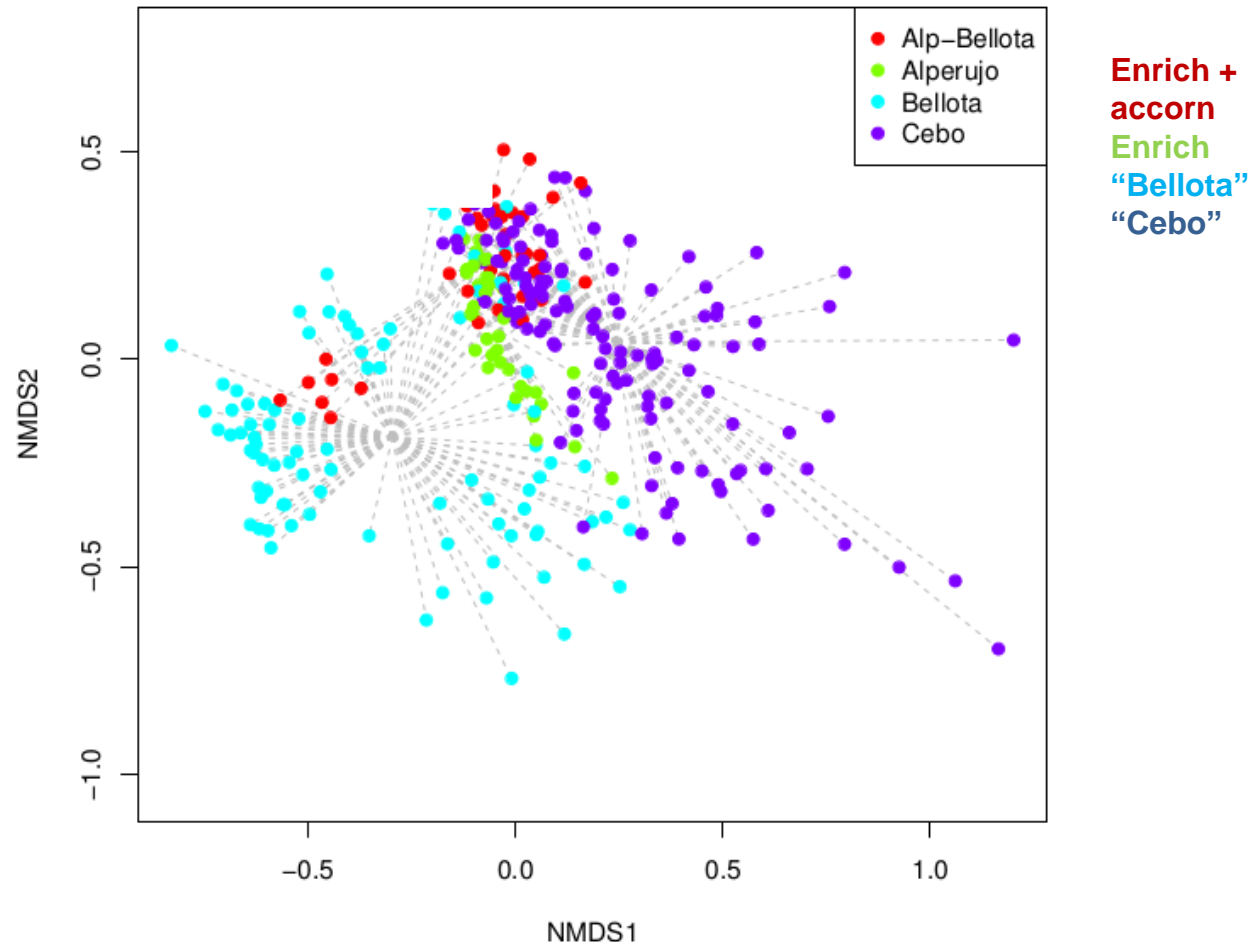


**Enrich +
accorn**
Enrich
“Bellota”
“Cebo”



NMDS analysis (all samples)

NMDS (Bray–Curtis distance) all OTU counts by diet



ADONIS PERMANOVA: Date $p < 1e-05$ ***

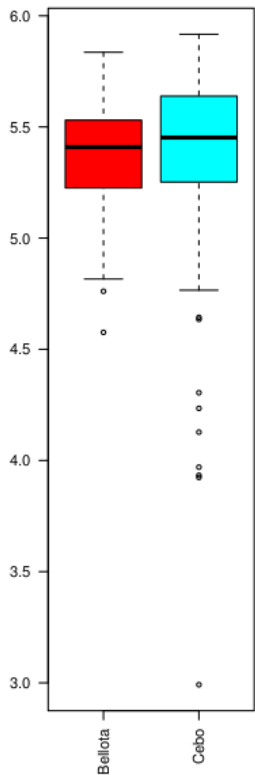
Diet $p =$



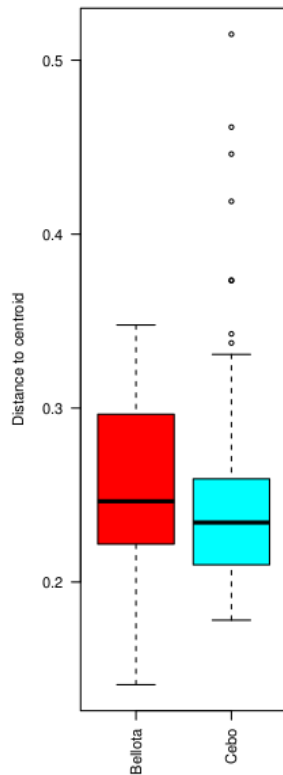
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“Montanera” vs. “Cebo”

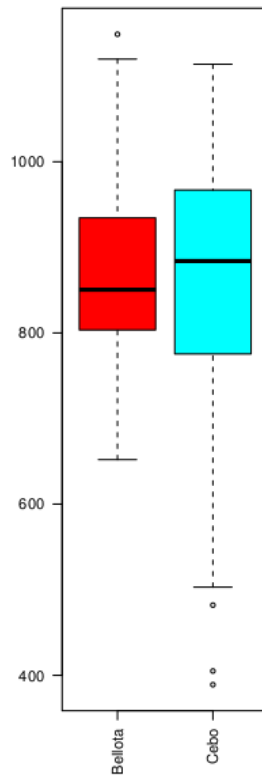
Alpha diversity_all: diet



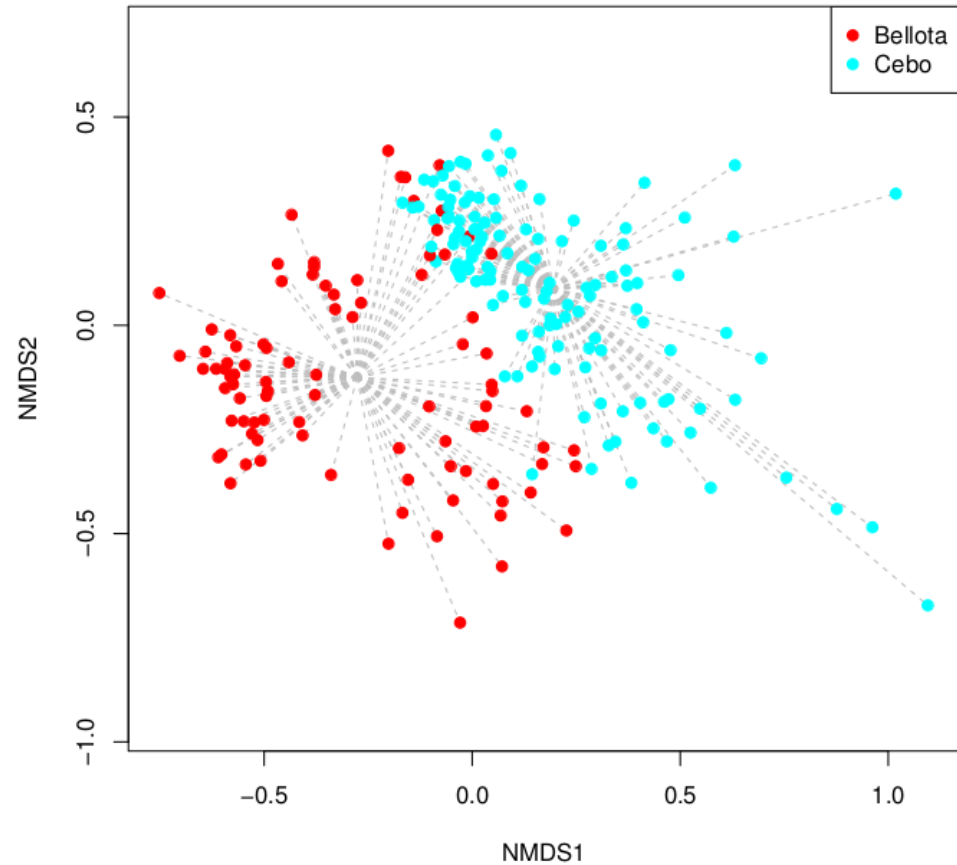
Beta diversity_all: diet



Richness_all: diet

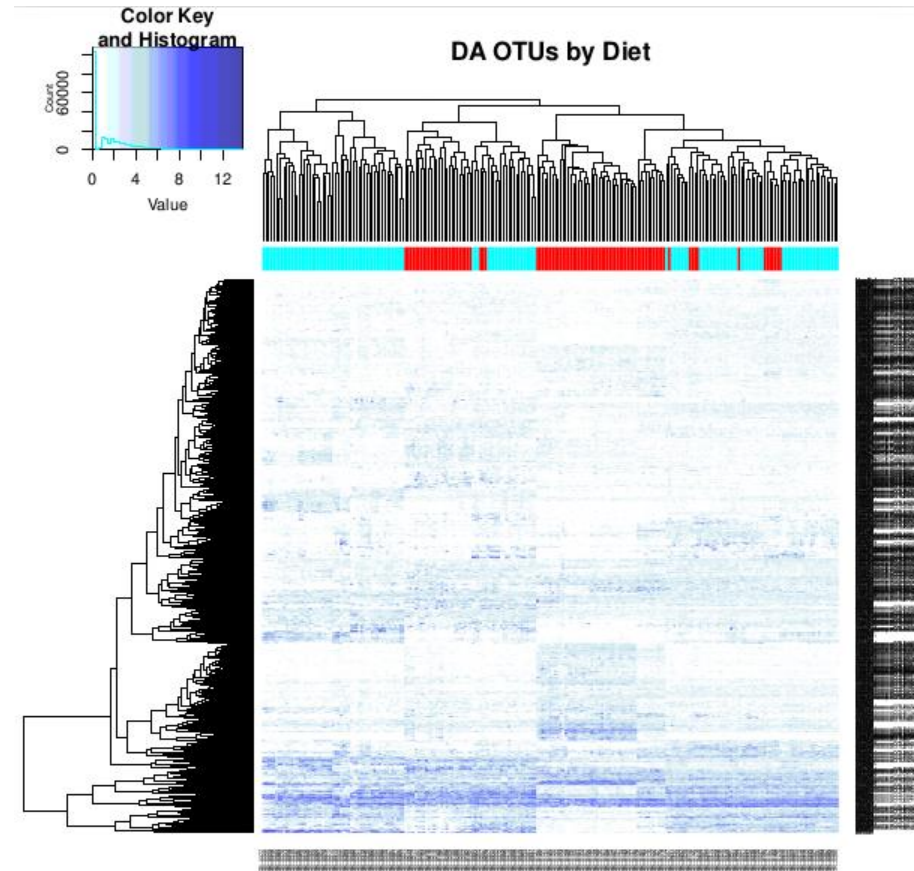
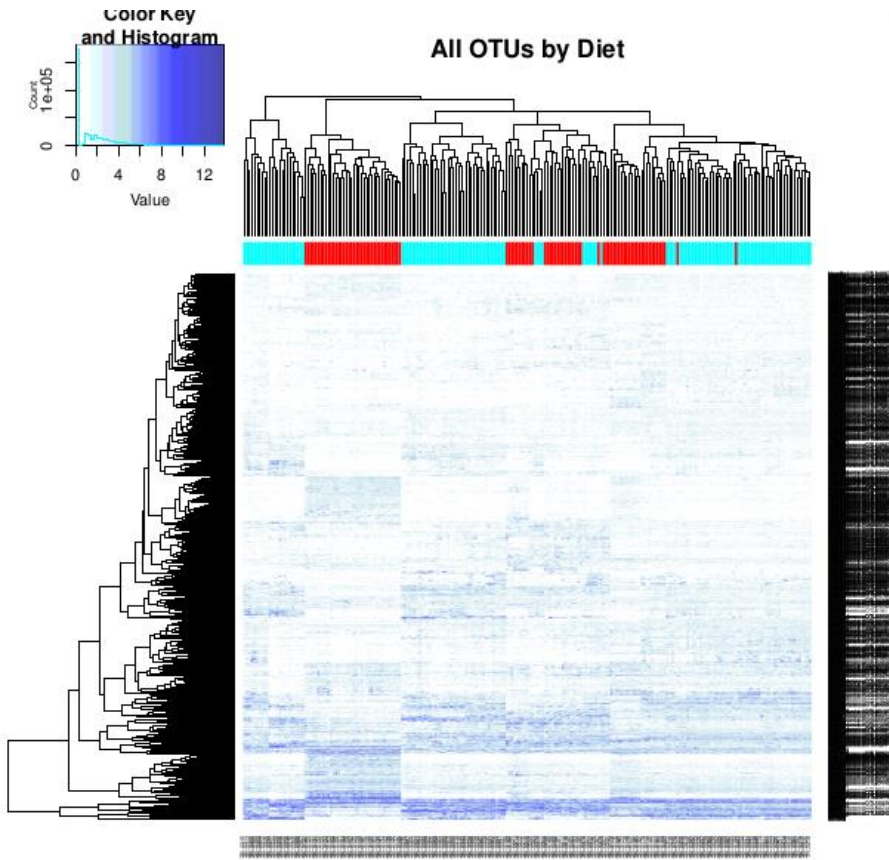


NMDS (Bray–Curtis distance) all OTU counts by diet



Diff. abundant OTUs

977 OTUs are DA!



Poster presentations

49.23: Major differences in gut microbiota composition of Iberian pigs in montanera vs commercial systems.

J.M. García-Casco, M. Muñoz, G. Lemonnier, J.M. Babilliot, O. Bouchez, A.I. Fernández, F.R. Massacci, M.A. Fernández-Barroso, A. López-García, C. Caraballo, C. Óvilo and J. Estellé

49.24: Gut microbiota composition in Iberian pigs fed with olive oil by-products during the growing period

M. Muñoz, J.M. García-Casco, G. Lemonnier, D. Jardet, O. Bouchez, M.A. Fernández-Barroso, F.R. Massacci, A.I. Fernández, A. López-García, C. Caraballo, E. González-Sánchez, C. Óvilo and J. Estellé

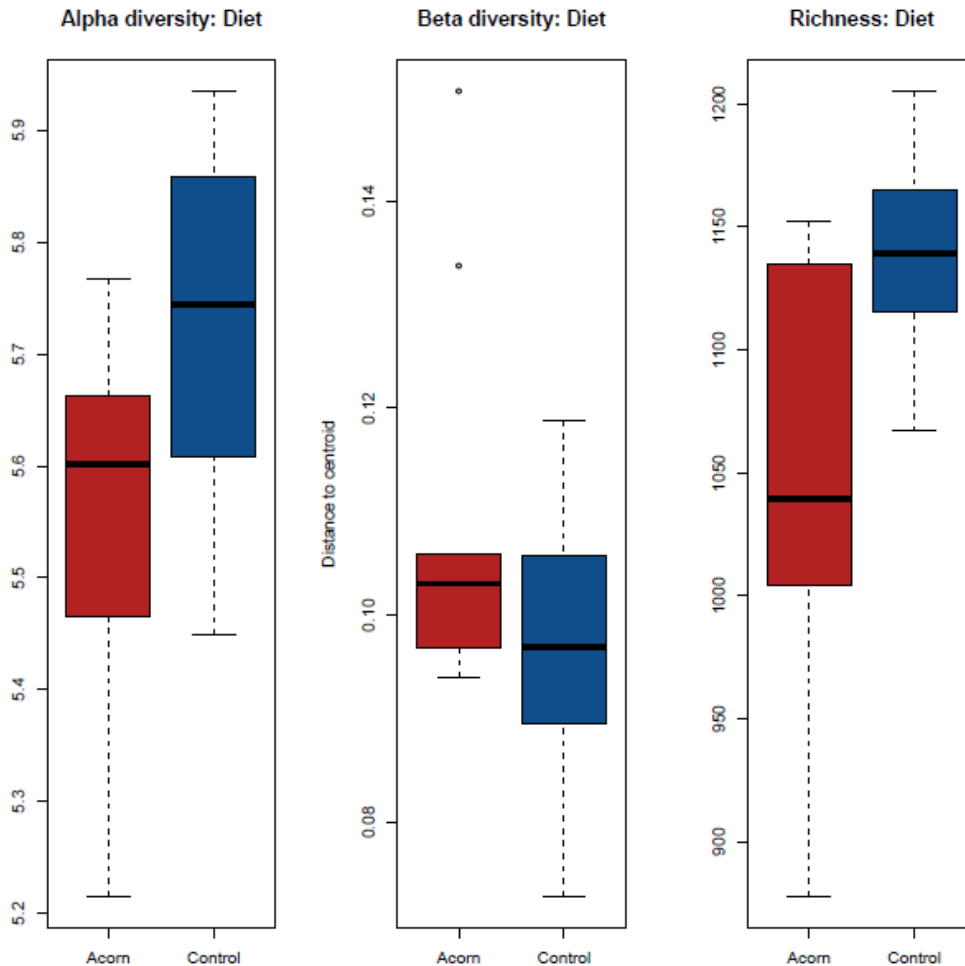


Turopolje pigs (Croatia)

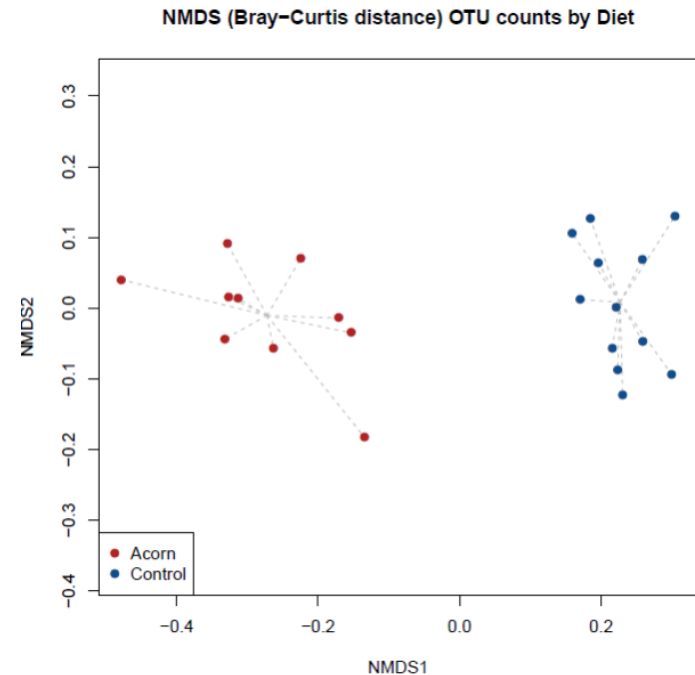
- Turopolje pigs under organic production conditions
 - Design: case-control study to evaluate the effect of acorn supplementation in open-air organic production
 - Samples: 10 acorn supplemented vs 14 controls, 16S sequencing in an Illumina MiSeq system



Diversity analysis

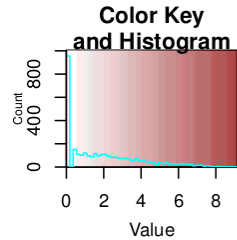


Acorn diet
Control diet

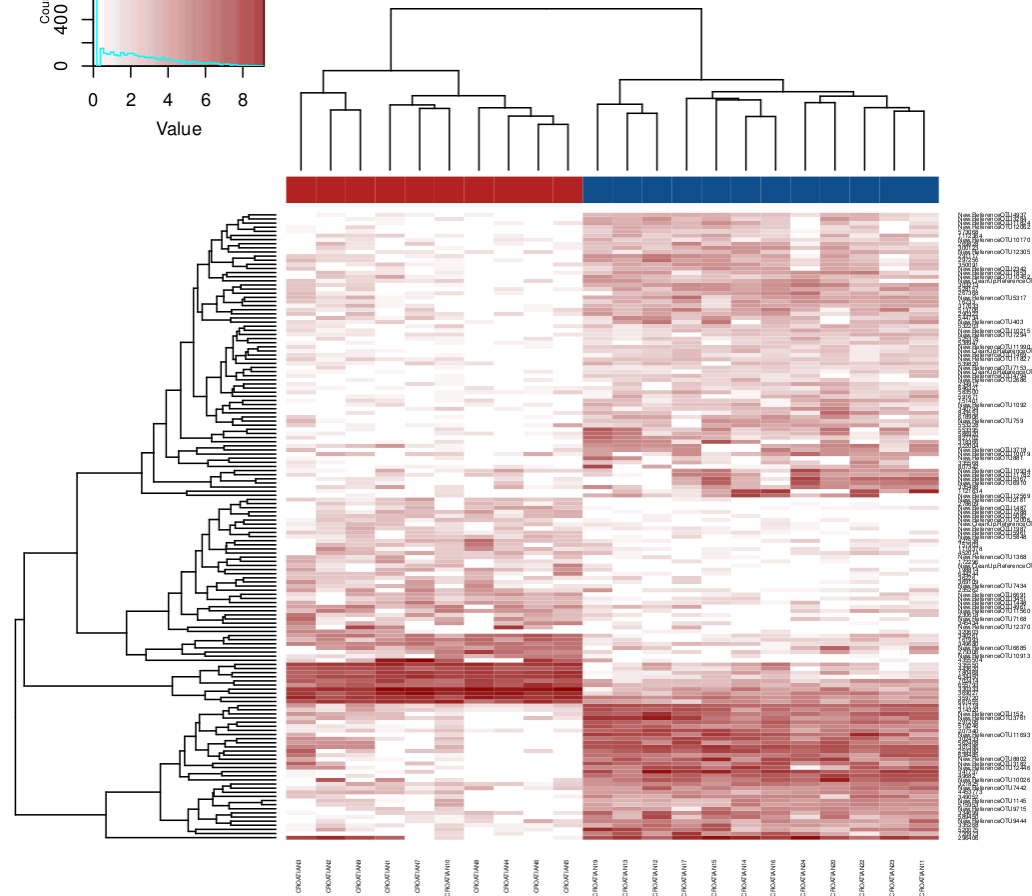


Diff. abundance analysis

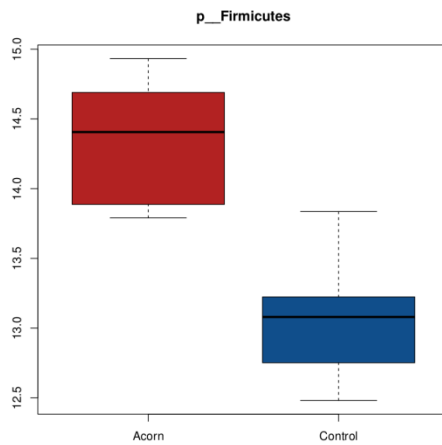
152 OTUs are DA!
(among the global 1466 OTUs)



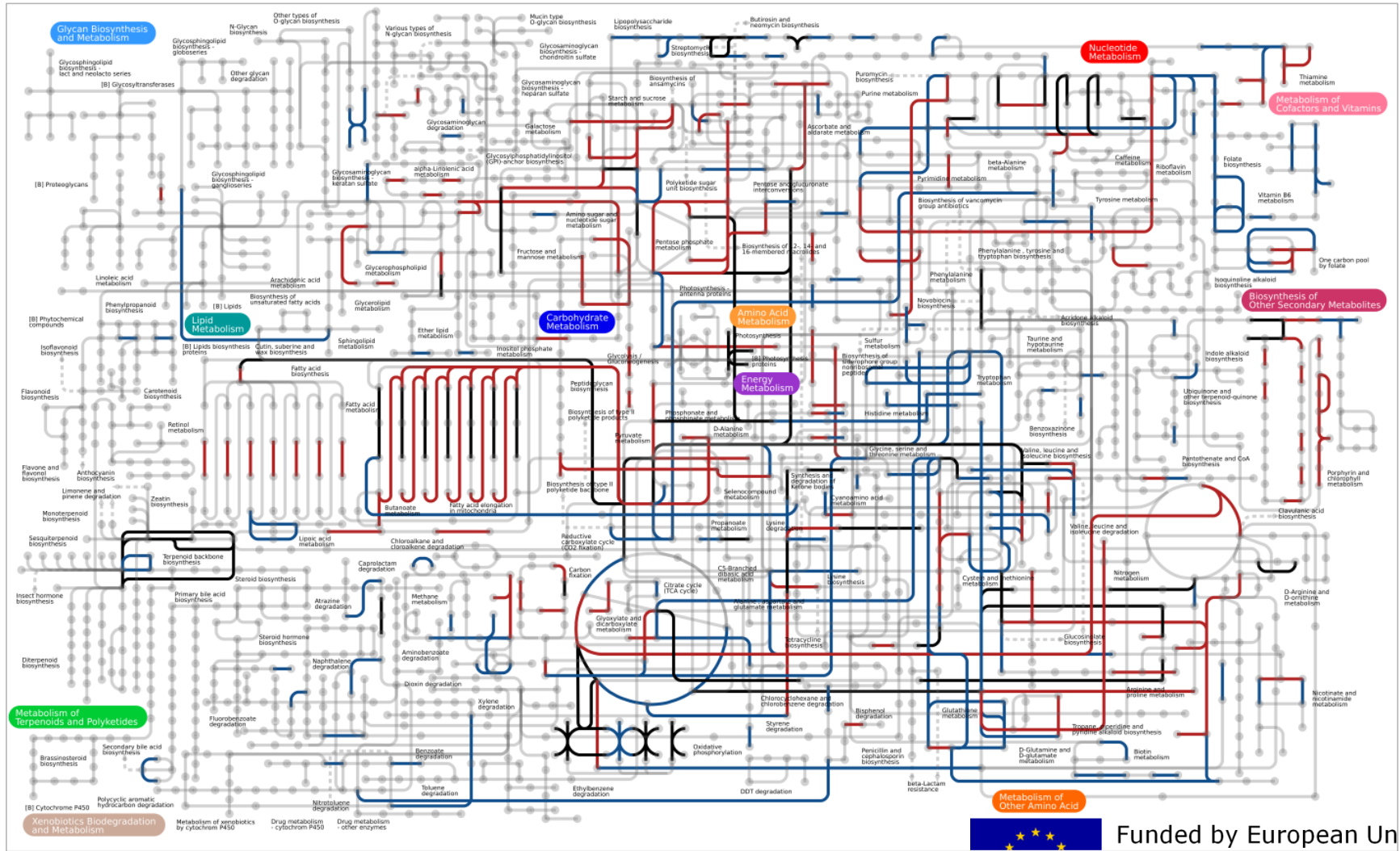
Significant OTUs by diet



Firmicutes is DA



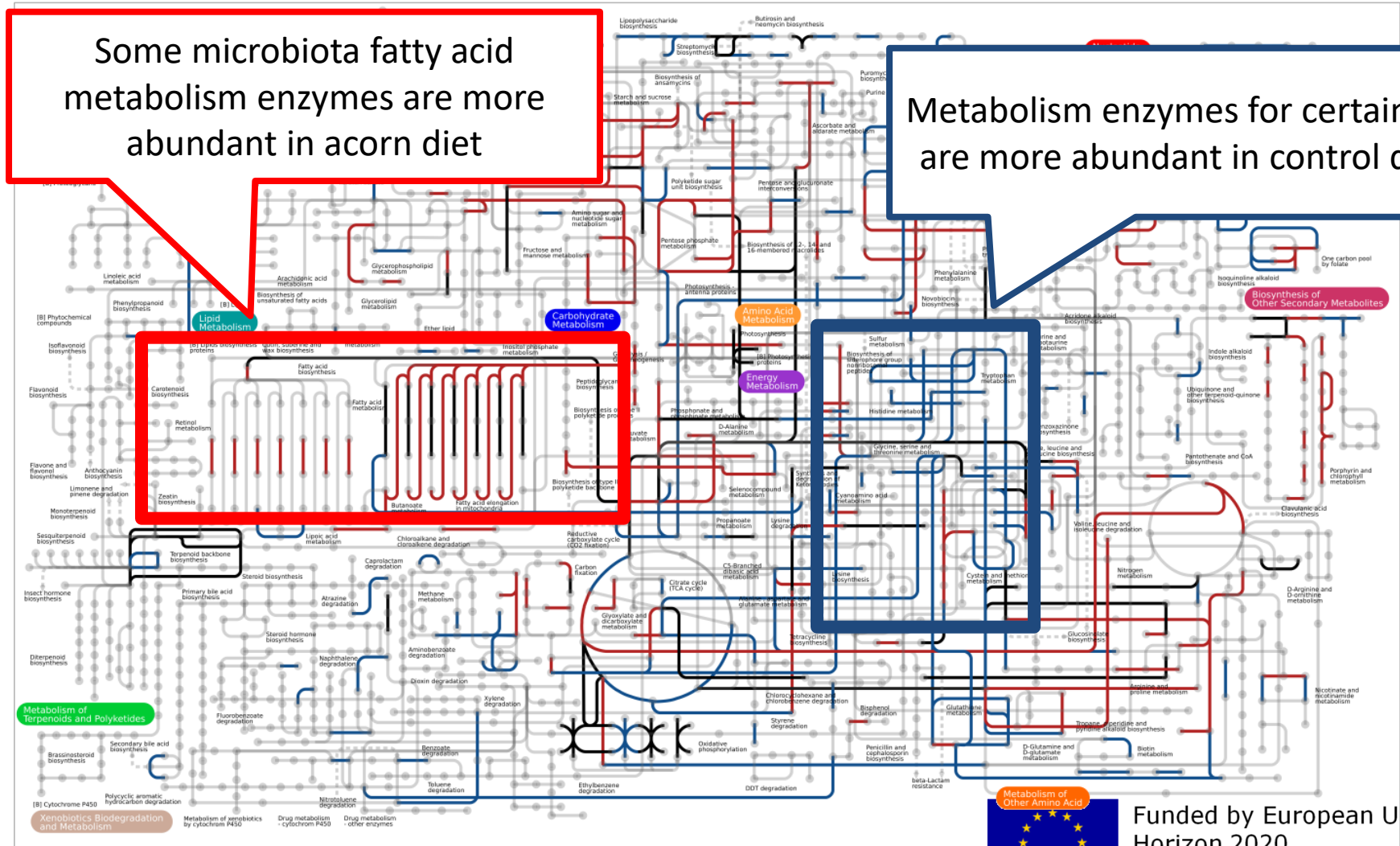
DA KOs on metabolic map:



DA KOs on metabolic map:

Some microbiota fatty acid metabolism enzymes are more abundant in acorn diet

Metabolism enzymes for certain AA are more abundant in control diet



Metabolism of Terpenoids and Polyketides

Metabolism of Other Amino Acids



49.27: Gut microbiota composition of Turopolje pigs in outdoor production and acorn supplementation.

J. Estellé, F.R. Massacci, D. Esquerré, D. Jardet, G. Lemonnier, C. Óvilo, M. Čandek-Potokar, K. Salajpal and D. Karolyi



GLOBAL CONCLUSIONS

- Gut microbiota analysis provides useful information of the study local European porcine breeds:
 - Study of the physiology and function of microbiota gives insights on particular diets effects.
 - Towards the development of next-generation **certification tools** to protect the particular production systems





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