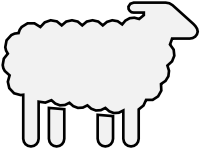


# The rumen microbiota is modified in lambs divergently selected for residual feed intake



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# Introduction



**Feed efficiency** is a key trait in animal farming and breeding

- **Residual Feed Intake** (RFI; Koch *et al.*, 1963): one feed efficiency criterion
- Heritable trait : 0.11-0.45 (Snowder & Van Vleck, 2003 ; Cammack *et al.*, 2005 ; Tortereau *et al.*, 2020)

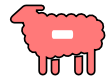


The promises of the **rumen microbiome** as a **biomarker/predictor** for the RFI

- Biomarkers for RFI of beef cattle (Clemmons *et al.*, 2019)
- Proxies of ewe lambs RFI with a forage based-diet (Ellison *et al.*, 2019)
- Biomarkers of lambs RFI with a concentrate based-diet (Zhang *et al.*, 2021)

# RFI\* divergent lines

\***RFI** = Daily Feed Intake – [  $\mu + \beta_A \times \text{Daily Weight Gain} + \beta_B \times \text{Body Weight}^{0.75} + \beta_M \times \text{Muscle} + \beta_F \times \text{Fat}$  ]



**RFI - : most efficient**

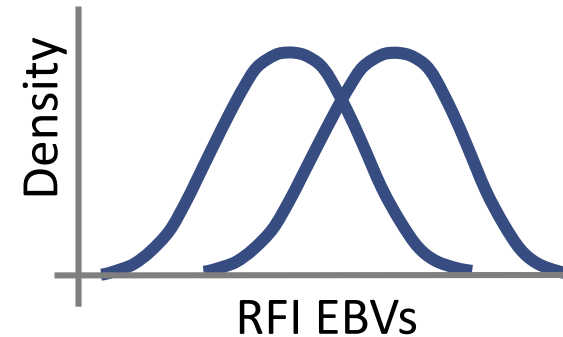
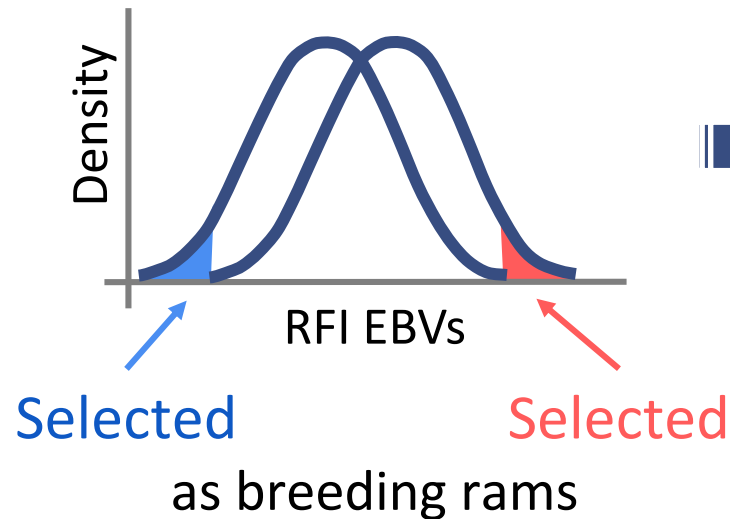


**RFI+ : least efficient**

**Generation**

**G2** 103 animals

**G3** 174 animals



**Mean RFI**

(by generation and line)

$$\mu_{RFI-} = -81.3 \text{ g/day}$$

$$\mu_{RFI+} = 49.9 \text{ g/day}$$

$$\Delta \approx 0.9 \sigma_p$$

$$\mu_{RFI-} = -69.6 \text{ g/day}$$

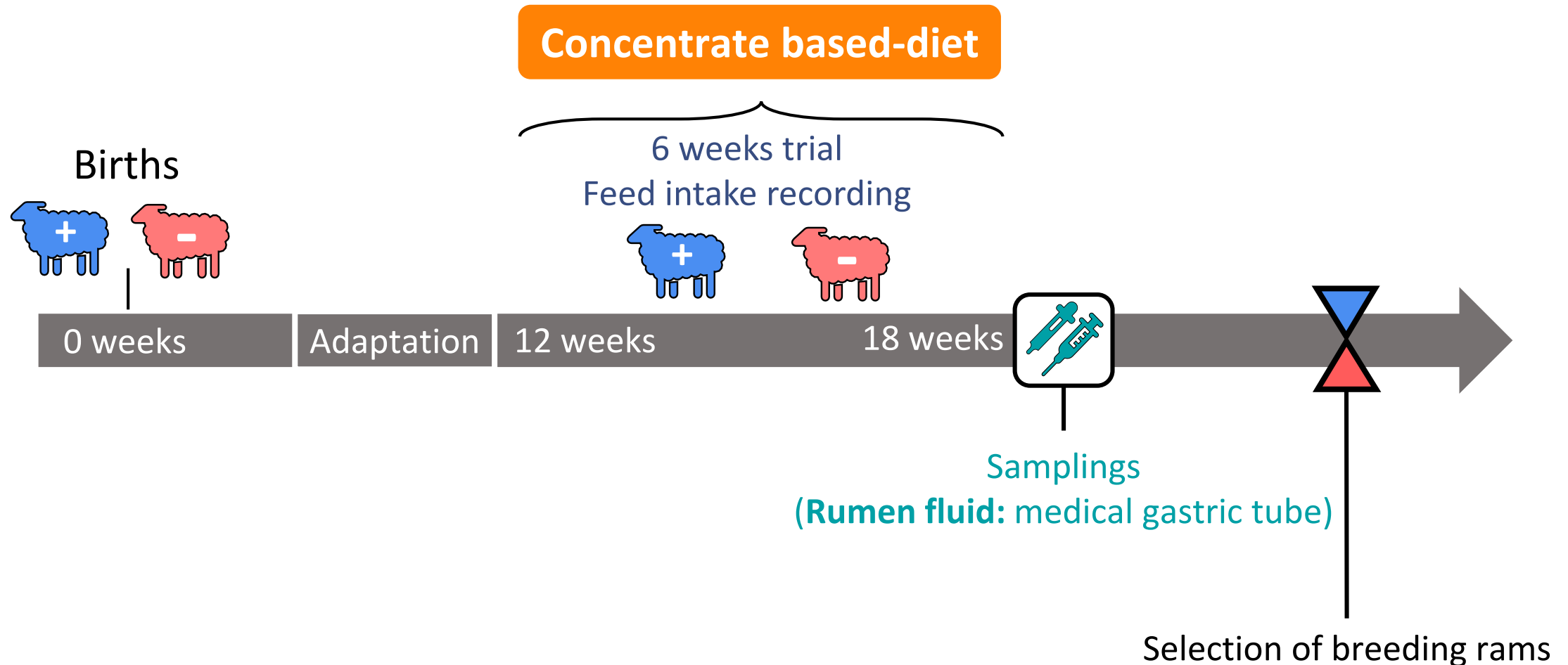
$$\mu_{RFI+} = 78.1 \text{ g/day}$$

$$\Delta \approx 1.0 \sigma_p$$



# Experimental design

277 Romane male lambs raised between 2018 and 2020 (i.e. G2 and G3)



# Operational Taxonomic Units

- 1 16S and 18S sequences processing and clustering  
(Frogs pipeline, Escudié *et al.* 2018)



Data is not rarefied

- 2 Filters :  
Relative abundances (<0.005%, Bokulich *et al.* 2013)  
Prevalence (<2 animals)

## 16S sequencing

Bacteria + Archaea

**994** retained OTUs

## 18S sequencing

Protozoa (+ Fungi)

**213** retained OTUs

# Discriminant analysis

① 16S and 18S sequences processing and clustering



② Filters



③ Zero imputation

Geometric Bayesian Multiplicative replacement (GBM)



④ Transformation

Centered LogRatio (CLR)



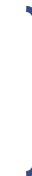
⑤ Linear models

To adjust abundances for the age and technical or environmental effects



⑥ Discriminant analysis

Sparse Partial Least Squares Discriminant Analysis (sPLS-DA)



Cross-validation strategy to tune and assess the model



Divergent lines

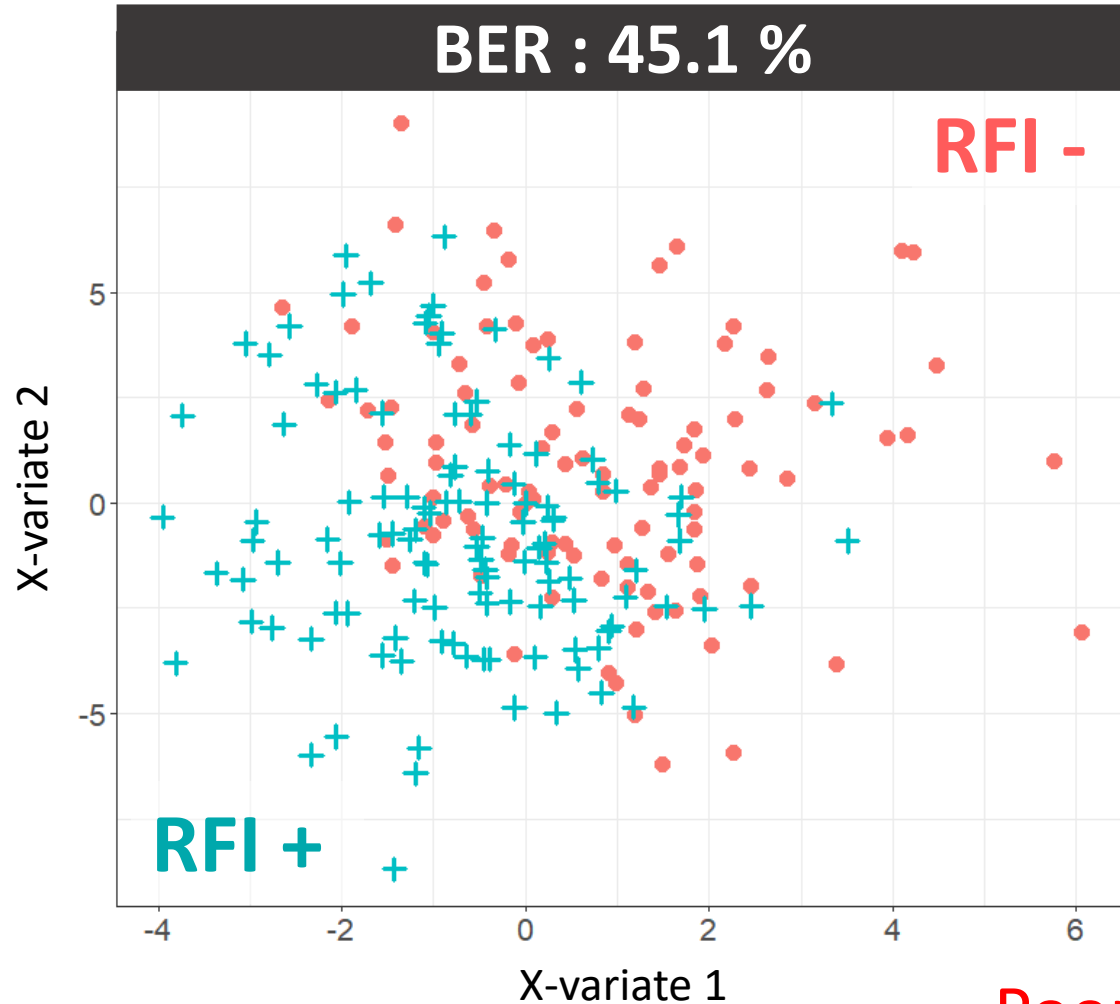
Compositional approach,  
see Martinez Boggio et al. (2021)



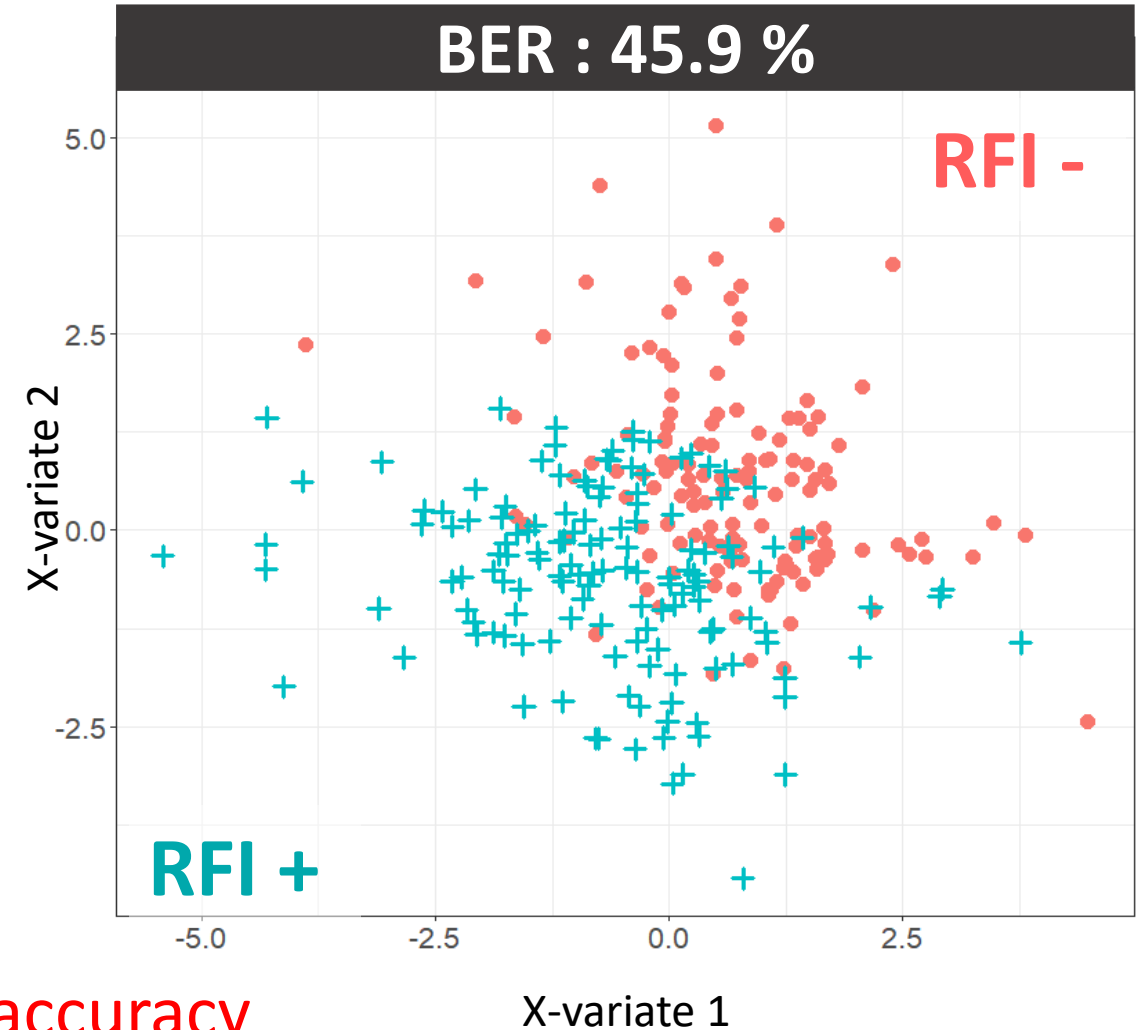
# Discriminant analysis with RFI lines

Balanced Error Rate (BER): average of the prediction errors on each class

sPLS-DA (16S data)



sPLS-DA (18S data)



Poor accuracy

# Discriminant analysis with other traits

- The RFI line status of a lamb :**
- ➡ is inherited from its sire
  - ➡ may not completely be consistent with RFI EBVs

Line	RFI EBVs*	
	EBV-	EBV+
RFI- line	129	7
RFI+ line	9	131

\*EBVs : Estimated breeding values



# Discriminant analysis with other traits

The RFI line status of a lamb : ➡ is inherited from its sire

➡ may not completely be consistent with RFI EBVs

Line	RFI EBVs*		Phenotypic <sup>1</sup> RFI		Phenotypic <sup>2</sup> ADFI	
	EBV-	EBV+	RFI-	RFI+	ADFI-	ADFI+
RFI- line	129	7	95	41	95	41
RFI+ line	9	131	43	97	43	98

\*EBVs : Estimated breeding values

➡ is even less consistent with the animal phenotypes

<sup>1</sup>Adjusted for the pen; <sup>2</sup>Adjusted for the pen, year, and age

➡ Thus, would it be more accurate to discriminate animals based on these traits ?

# Discriminant analysis with other traits (16S data)

Comparison of sPLS-DA balanced error rates

Technique	Line	RFI EBVs	Phenotypic RFI	Phenotypic ADFI
16S	45.1%	41.0%	42.1%	38.3%

Most contributing OTUs to the ADFI discriminant analysis (16S)

OTU	Phyla	Genus	Prevalence* (%)	Higher abundance
1	Firmicutes	Saccharofermentans	44.8	ADFI+
2	Firmicutes	Ruminococcaceae UCG 004	91.7	ADFI+
3	Firmicutes	Lachnospiraceae NK3A20 gr.	52.3	ADFI+
4	Bacteroidetes	(Rikenellaceae) U29 B03	25.2	ADFI+

\*Fraction of the animals for which the OTU was detected

# Discriminant analysis with other traits (18S data)

Comparison of sPLS-DA balanced error rates

Technique	Line	RFI EBVs	Phenotypic RFI	Phenotypic ADFI
18S	45.9%	48.5%	39.6%	43.2%

## Most contributing OTUs to the RFI discriminant analysis (18S)

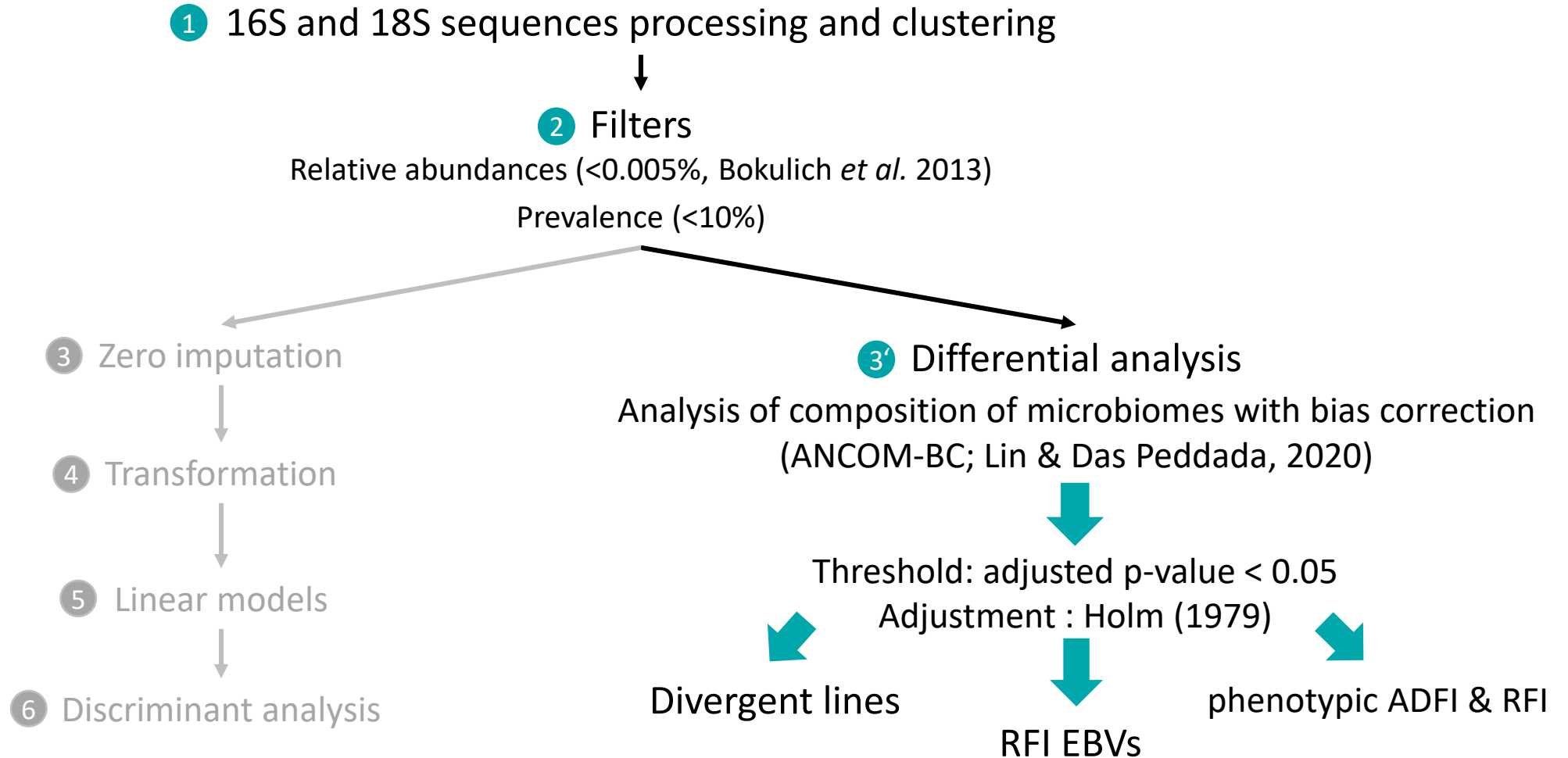
OTU	Phyla	Genus	Prevalence* (%)	Higher abundance
1	Ciliophora	Metadinium	10.0	RFI+
2	Ciliophora	Metadinium	25.3	RFI+
3	Ciliophora	Entodinium	21.2	RFI+
4	Ciliophora	Entodinium	2.7	RFI+

\*Prevalence: fraction of the animals for which the OTU was detected

# Differential analysis

**Discriminant analysis** : Identifying biomarkers characterizing the groups

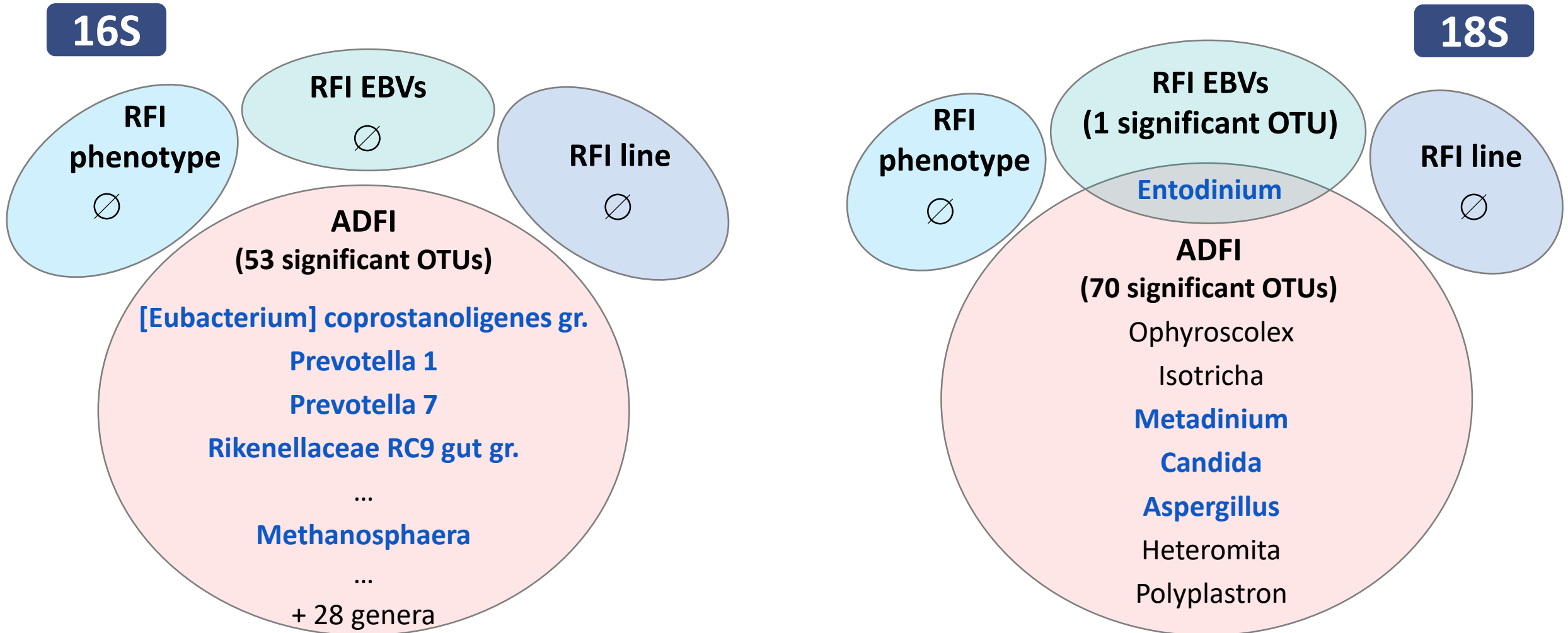
**Differential analysis** : Identifying significantly and differentially abundant OTUs



# Differential analysis

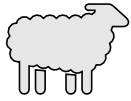
## Genera of significant OTUs (adjusted p-value < 0.05)

Silva 132 16S & Silva 138.1 18S reference databases (Yilmaz et al., 2014)



Some of the **most discriminating OTUs** are also significantly differentially abundant.

# Conclusion



Ruminal microbiota is not informative enough to discriminate lambs from RFI divergent lines

Ruminal microbiota might not be significantly modified by the selection

Strong environmental / sequencing effects



Might be difficult to find RFI proxies with the ruminal microbiota

# Perspectives



Data integration to predict the feed efficiency (microbiome, genome, metabolome, NIRS...)

Relationships between the rumen microbiome and other traits will be assessed :



Growth performances

Body composition

Greenhouse gases emissions



With the RFI divergent lines



## Questions and exchanges



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