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COMPARISON OF CLASSICAL AND MOLECULAR METHODS TO STUDY THE MICROBIOTA ALONG THE SILO FACE ON FARM CORN SILAGE

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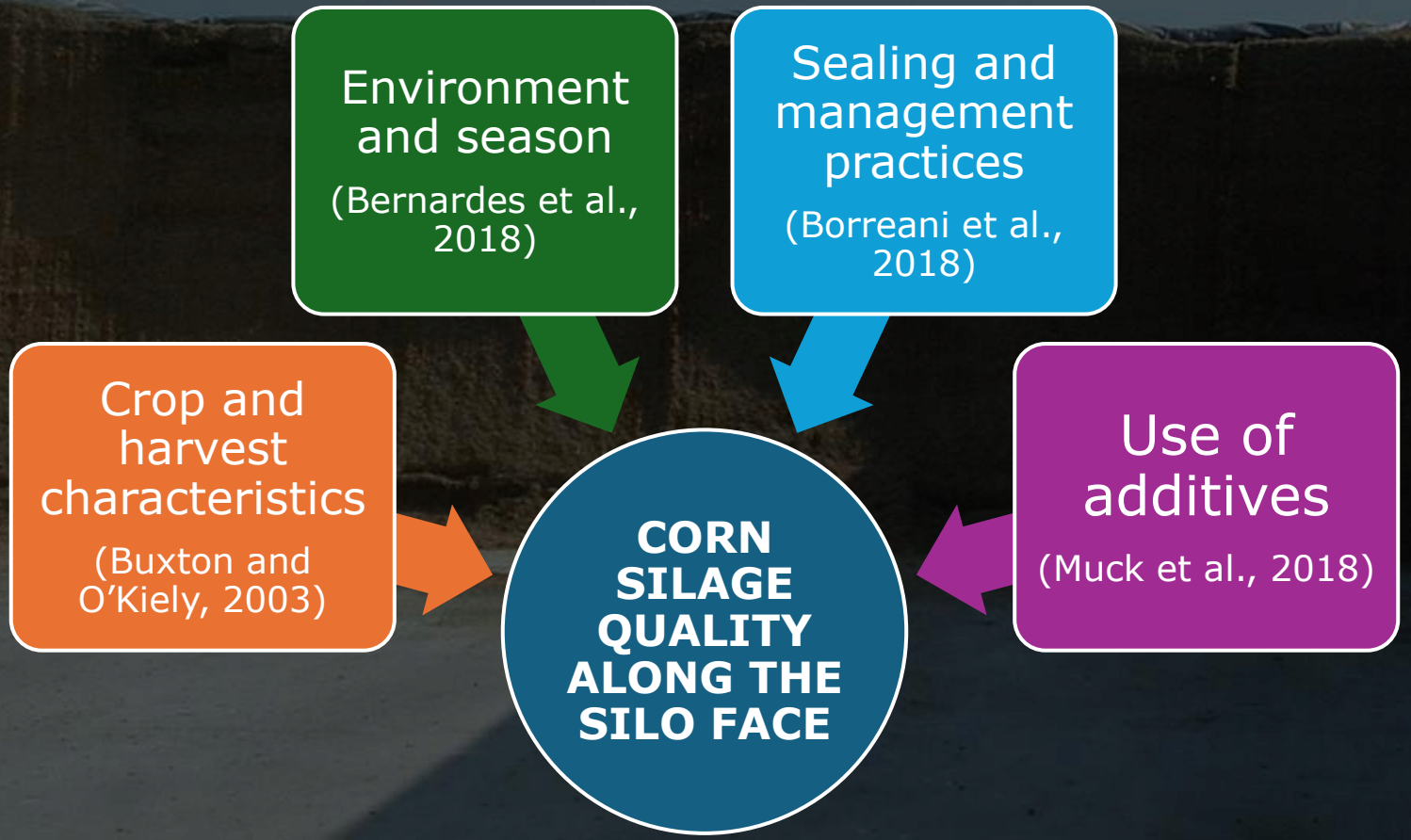
The significance of investigating bunker silo variability

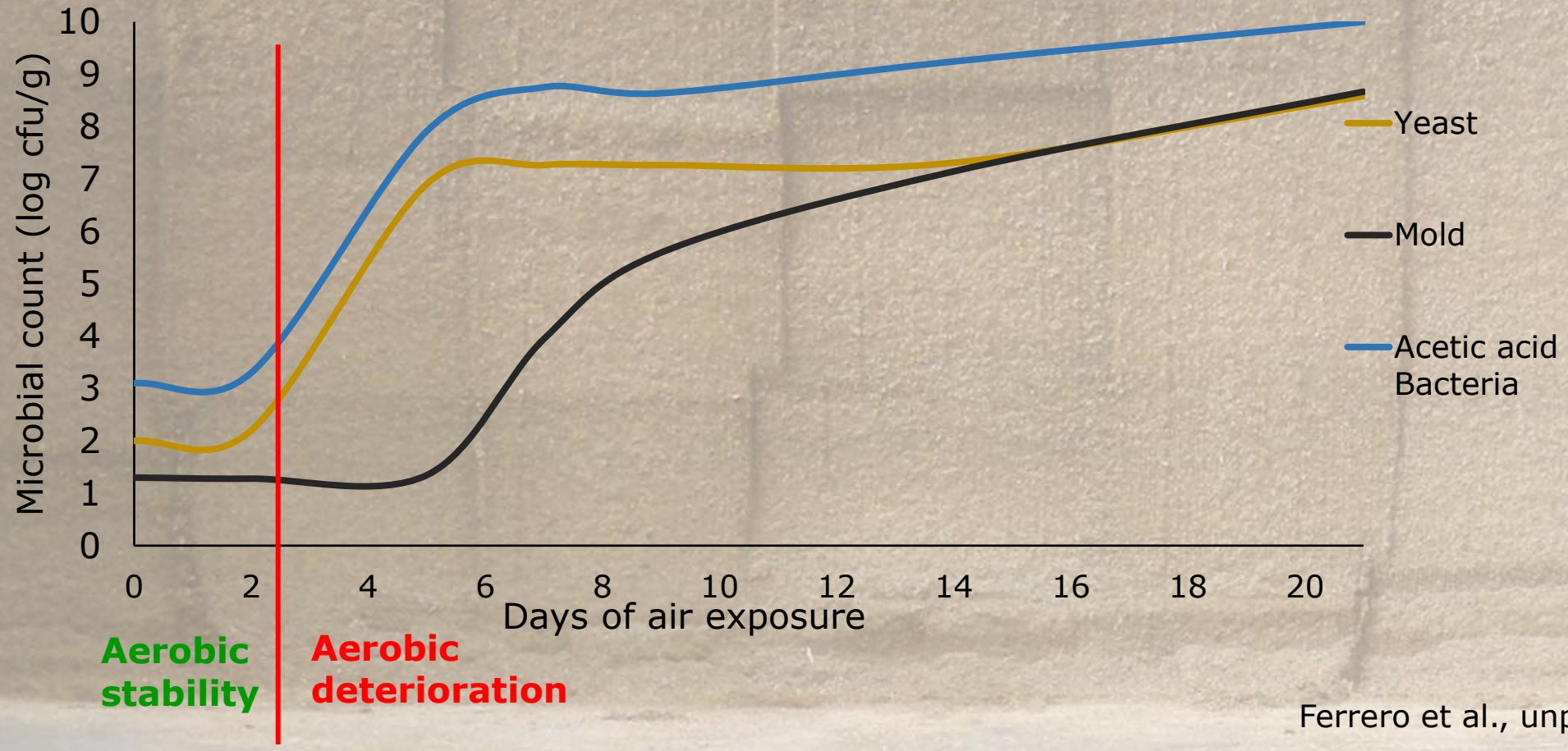


Strategic objectives for optimal bunker silo management in animal feed production

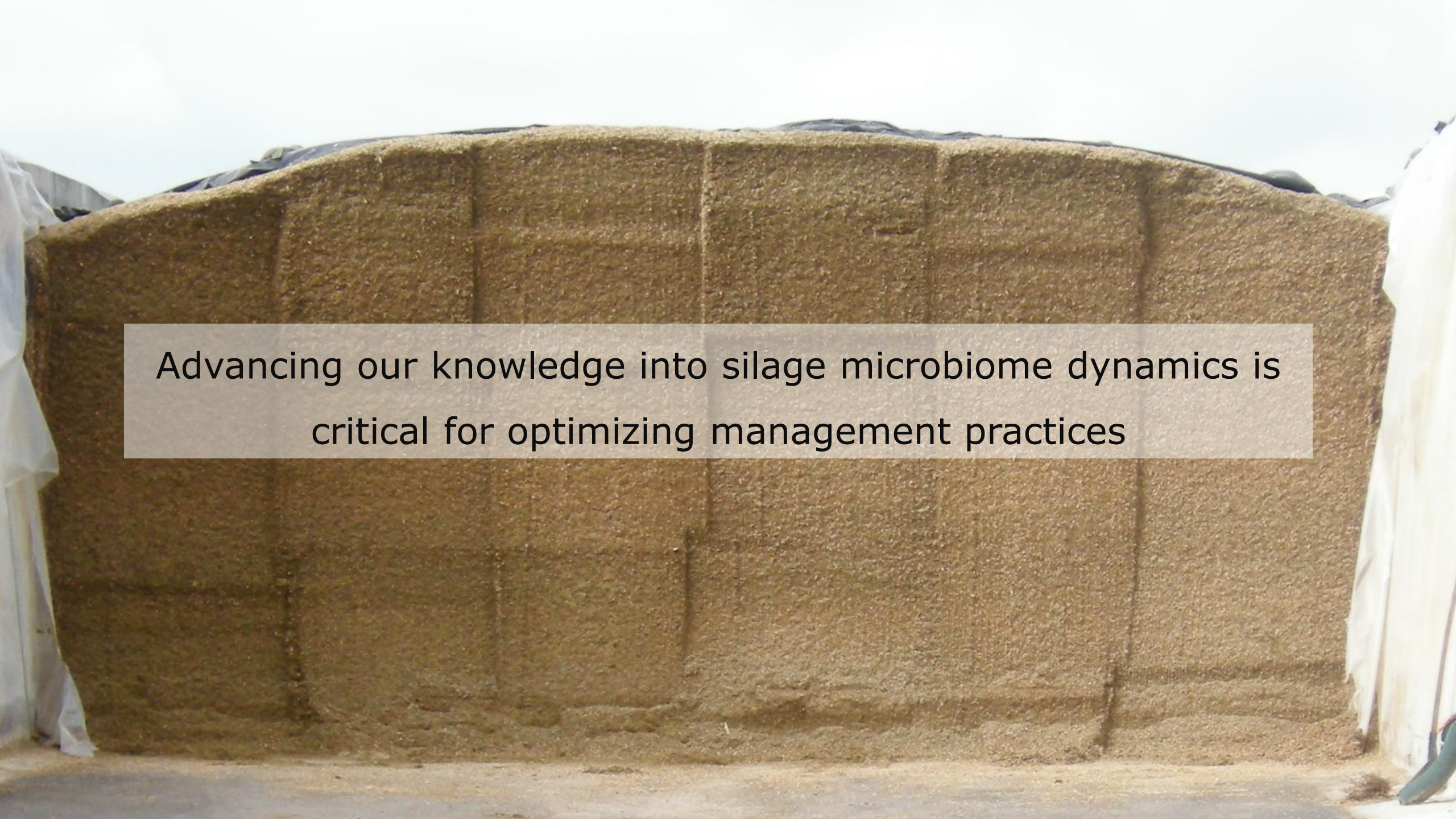
Reducing losses of aerobic deterioration

(Improve aerobic stability and healthy silage)

MAIN PROBLEM ON BUNKER SILOS OF CORN SILAGE ON FARM



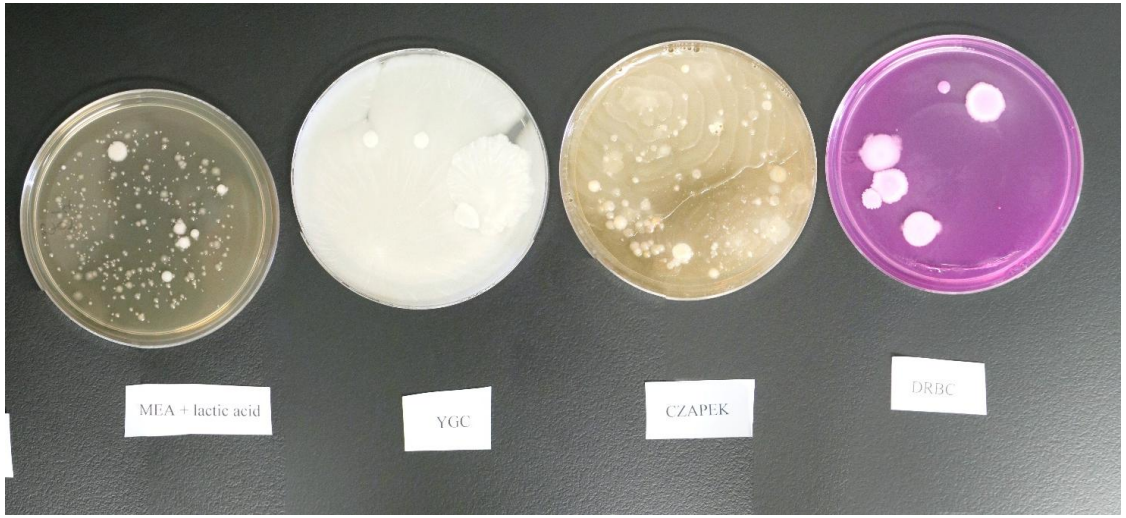
Ferrero et al., unpublished data



Advancing our knowledge into silage microbiome dynamics is critical for optimizing management practices

Currently available techniques for microbial analysis

Culture-**dependent** enumeration methods



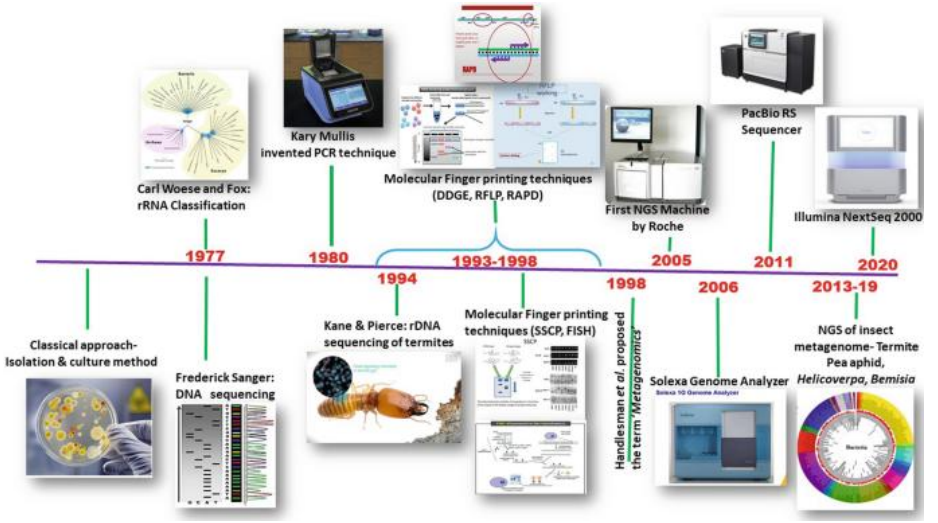
- Specific media for each group of microorganisms
- Time consuming analysis
- Actual count
- Possibility to isolate and to identify single colony

Culture-**independent** methods

J. Dairy Sci. 101:4060–4074
https://doi.org/10.3168/jds.2017-13704

Silage review: **Using molecular approaches to define the microbial ecology of silage[1,2]**

T. A. McAllister,*[3] L. Dunière,†‡ P. Drouin,§ S. Xu,* Y. Wang,* K. Munns,* and R. Zaheer*



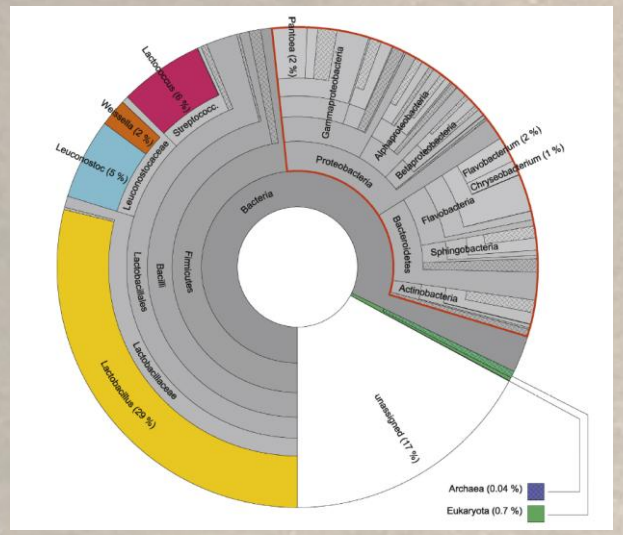
- Significant advancements over the past 2 decades
- Identification of non-culturable microorganism
- Difficulties to reach species level
- Currently the count estimate is often unreliable

McAllister et al., 2018; Drouin and Ferrero, 2020; Chellappan et al., 2021

Role of microbiota characterization in the ensiling process: BACTERIA

Culture-dependent

- Count of desirable microorganisms (lactic acid bacteria)
- Count of undesirable microorganisms (Enterobacteriaceae, Clostridia, *Bacillus*, etc.)

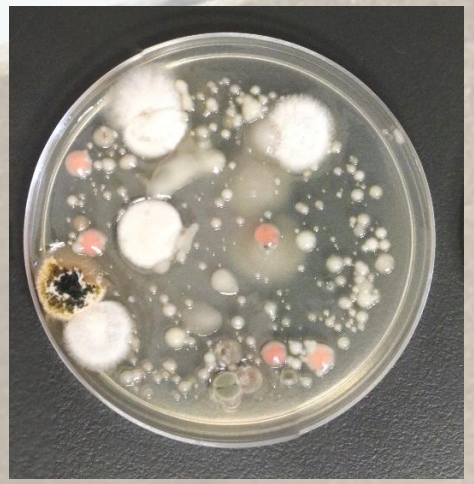


Culture-independent

- LAB identification
- Acetic acid bacteria identification
- Sporeformers identification
- Detection of pathogenic microorganisms

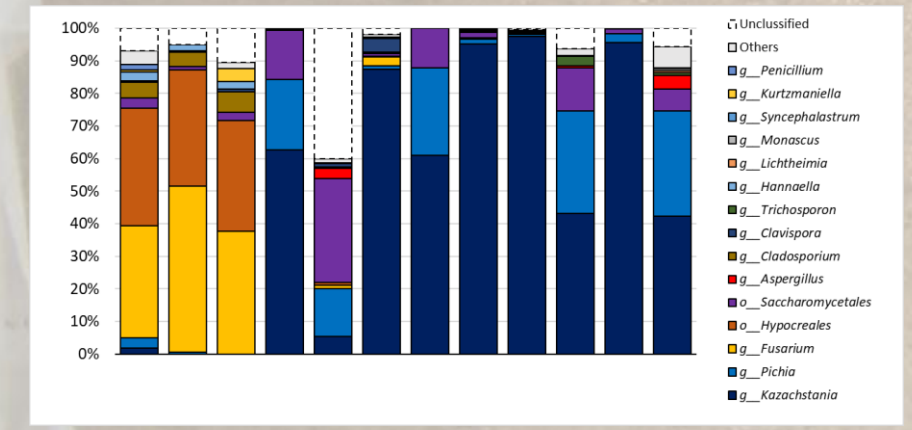
Eikmer et al., 2013; McAllister et al., 2018; Drouin and Ferrero, 2020

Role of microbiota characterization in the ensiling process: FUNGI



Culture-dependent

- Yeast count correlated to aerobic deterioration
- Detection and count of molds



Culture-independent

- Identification of yeasts and molds
- Presence of mycotoxigenic fungi

McAllister et al., 2018; Drouin and Ferrero, 2020; Ferrero et al., 2023

Aim of the study

To analyze the **uniformity of microbiota along the silo face** of corn silages on five commercial dairy farms characterized by different silo management strategies

Microbiota was analyzed using:

- culture-**dependent** enumeration methods
- culture-**independent** methods (i.e. 16S rDNA gene and ITS amplicon sequencing)

Material and Methods

5 dairy farms
North-West Italy

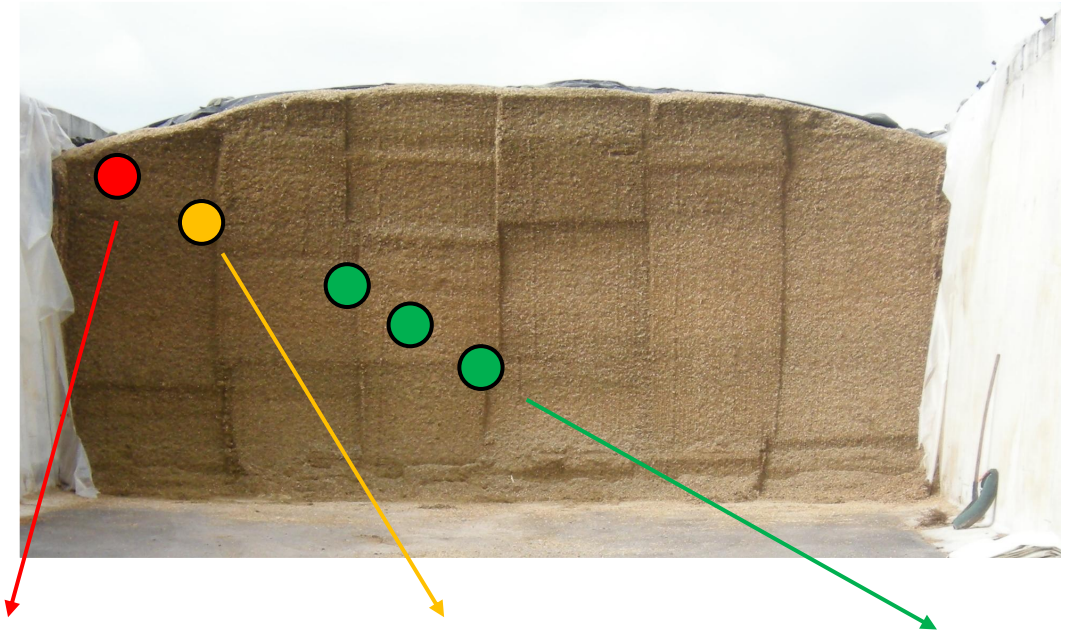
Corn silage

Sampling during **summer**

Survey of bunker silo **management practices** (such as feed out rate, sealing strategies, additives, etc.)

Quantification of **surface of visible spoiled** of the silo working face and silage **temperature**

Sampling procedure
(5 kg silage sample)



A1
Close to the sealant film

BA1
Below visible spoiled silage, not discarded by farmers

C
Core, composite sample

Material and Methods

- **Chemical** analyzes: DM content, pH, nitrate, fermentative profile by HPLC (acids and alcohols)
- **Culture-dependent** methods: microbial count using specific media for **yeast** and **mold** (YCG), **LAB** (MRS), **acetic acid bacteria** (mod. From Spoelstra et al., 1988), **enterobacteria** (VRBA)
- **Culture-independent** methods:
  - **DNA** extraction following Drouin and Ferrero (2020)
  - Microbiota studied by amplifying the V3 and V4 region of the **16S** rRNA for bacteria and the internal transcribed spacer **(ITS)-1** region for the fungi
  - **Alpha diversity** was assessed by Shannon diversity index
  - **High-throughput sequencing** and bioinformatics analysis following Drouin and Ferrero (2020)
- Statistical analysis using RStudio (v4.3.2)

Results

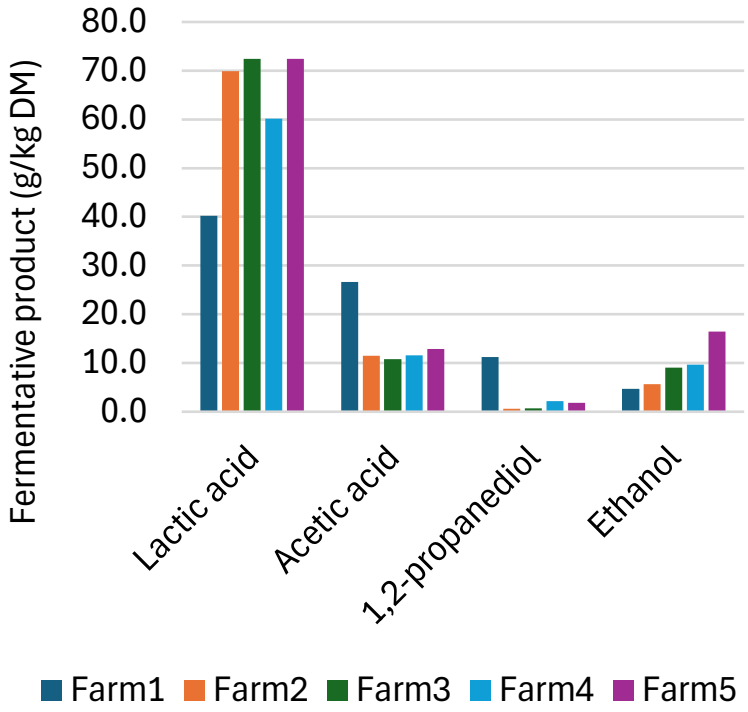
Bunker silo characteristics

		Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Days of conservation		349	293	278	288	297
Days after silo opening		137	112	216	69	73
Temperature (°C)						
Daily average temperature range				27 - 35		
	C	23.1	24.3	21.2	20.0	20.8
Temperature	**BA1**	33.9	31.7	39.1	29.0	28.2
	A1	44.7	39.8	48.9	38.0	34.6
Dry matter (%)		34.5	36.0	34.2	33.5	33.8
Surface visible moldy (%)		2.7	0.8	2.4	0.0	9.5
Feed out rate (m/d)		0.123	0.157	0.109	0.212	0.251
Plastic film on the wall		yes	no	no	yes	yes
Number of plastic film		1	1	1	2	2
Weighting		Tires	Tires	Tires	Tires	Tires + bags
Inoculum treatment		**None**	***LB***	**None**	***LP + LB***	**None**

** LB = Lentilactobacillus buchneri; LP = Lactiplantibacillus plantarum*

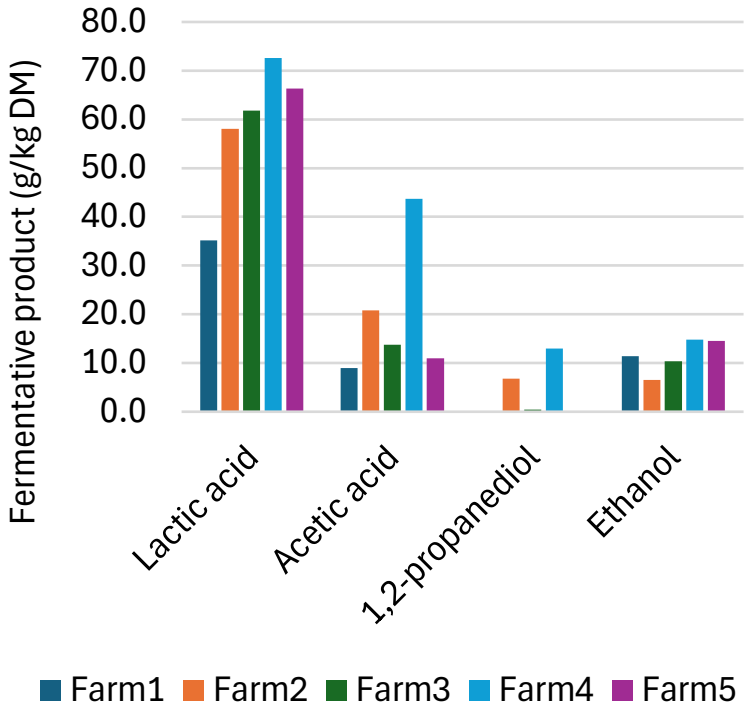
Fermentative profile of silage

CORE (C)



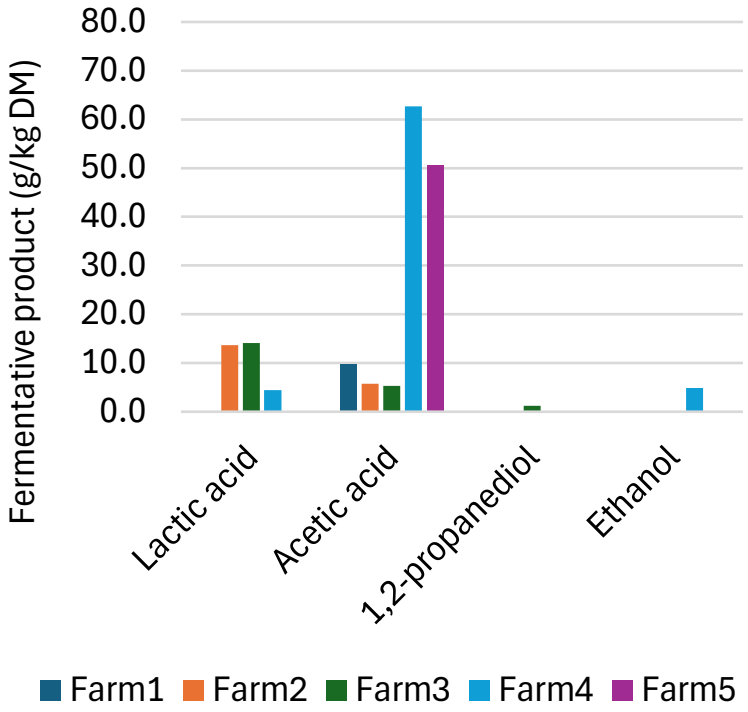
Heterolactic fermentation in Farm 1. No evidence of activity of LAB inoculum in Farm 2 and 4

Below spoiled area (BA1)



Evidence of heterolactic LAB inoculum higher in BA1 than C in Farm 2 and 4

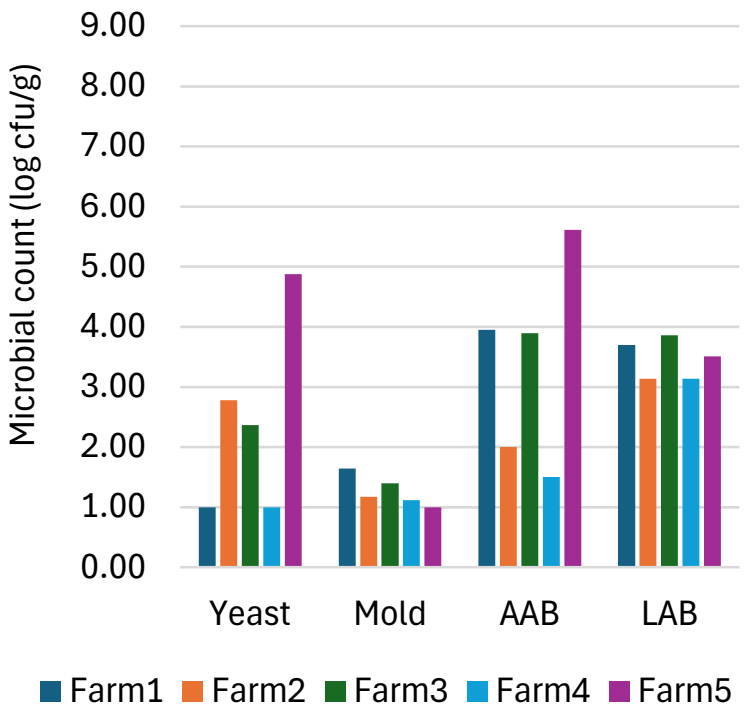
Spoiled area (A1)



Depletion of fermentative products due to aerobic deterioration

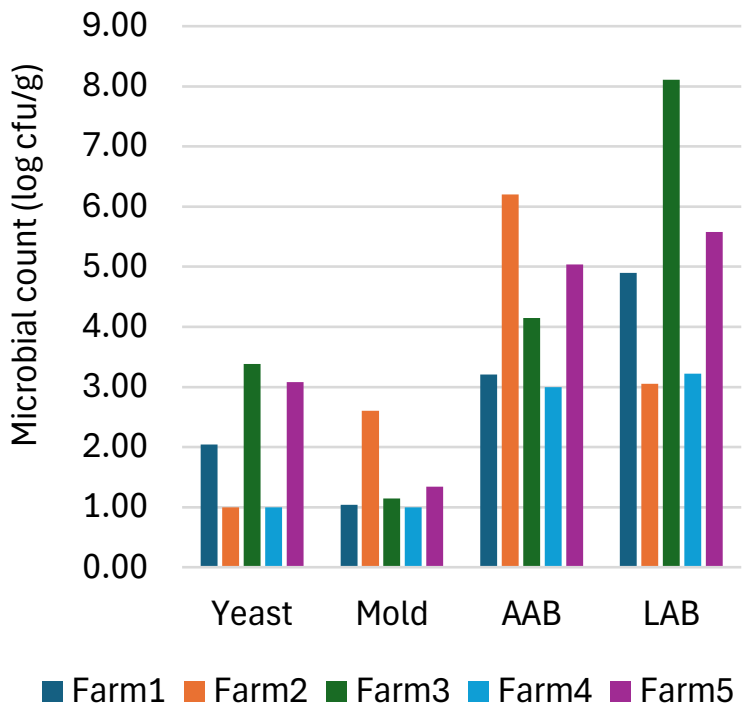
Microbial count of silages

CORE (C)



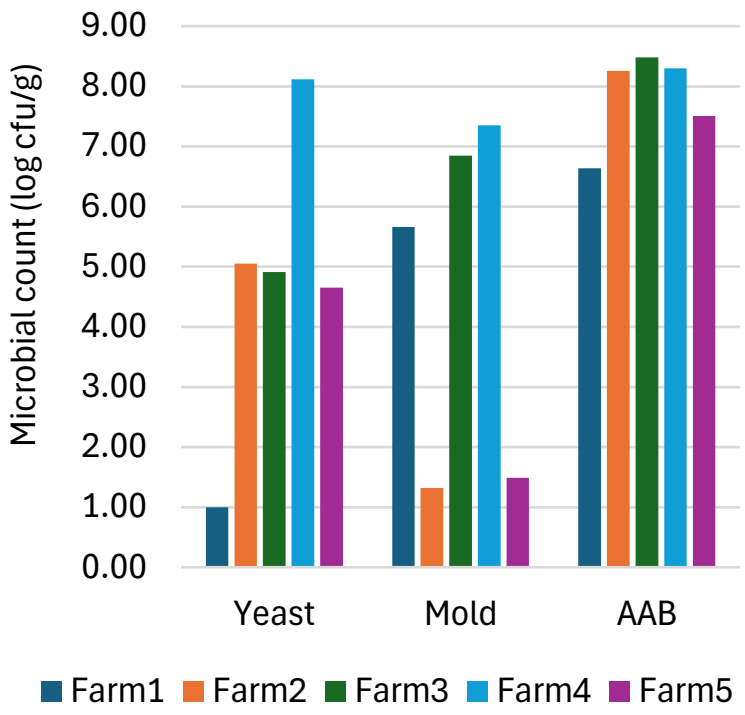
Strongly reduction of yeast count in Farm 1 and 4

Below spoiled area (BA1)



Reduction of yeast count in treated silages of Farm 2 and 4

Spoiled area (A1)

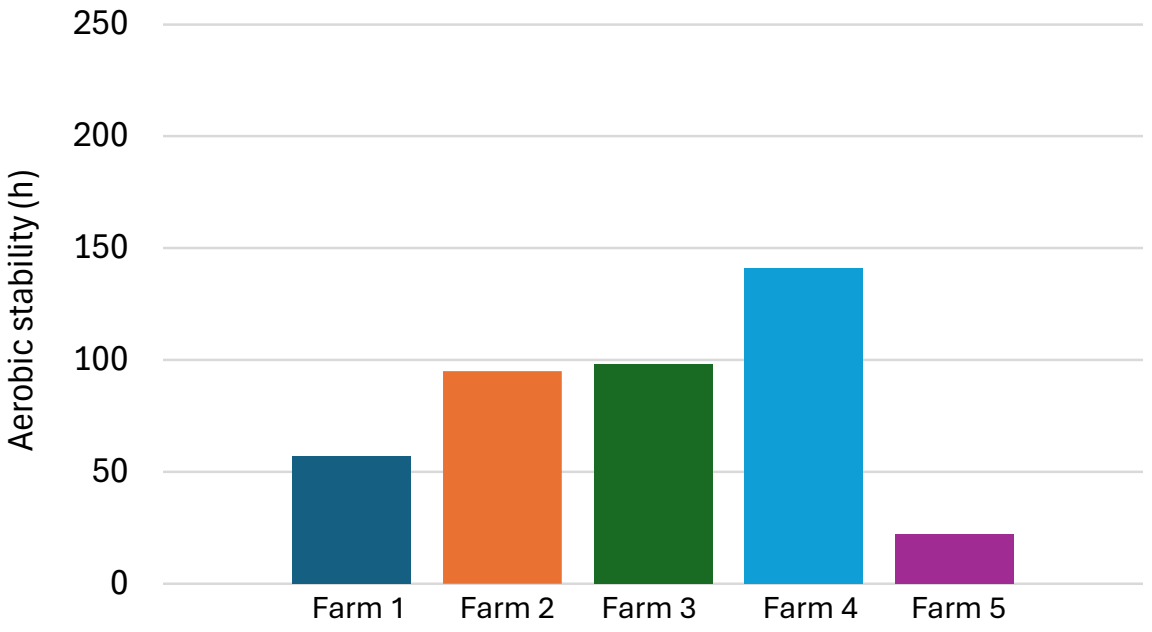


High yeast and AAB counts

AAB = acetic acid bacteria, LAB = lactic acid bacteria

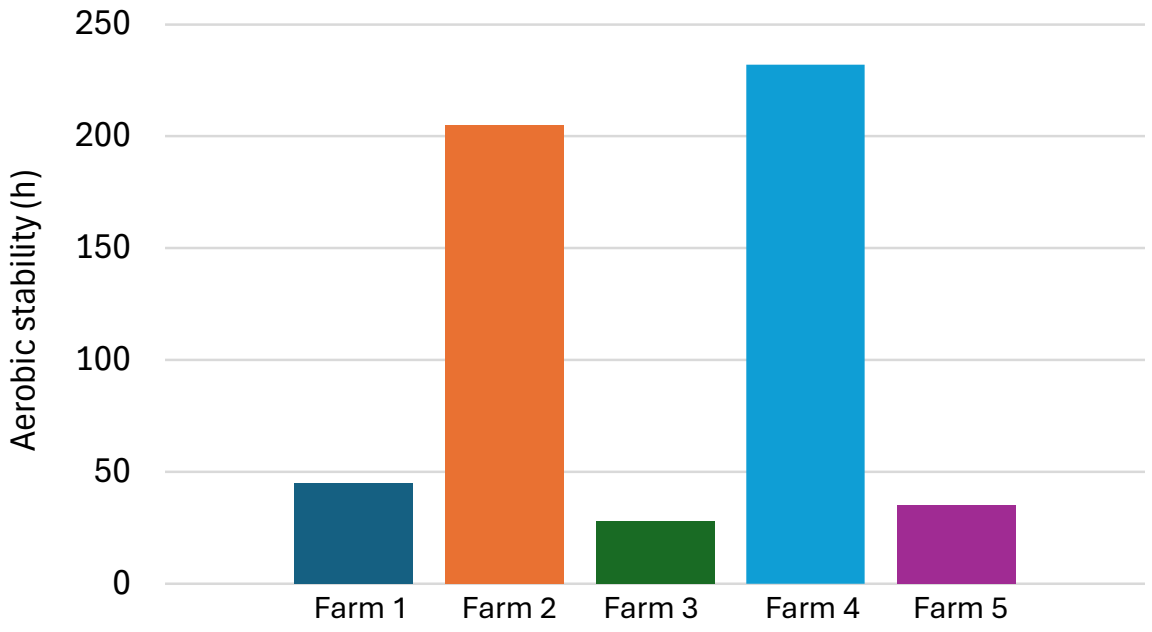
Aerobic stability of silages

CORE (C)



Lower AS in C than BA1

Below spoiled area (BA1)

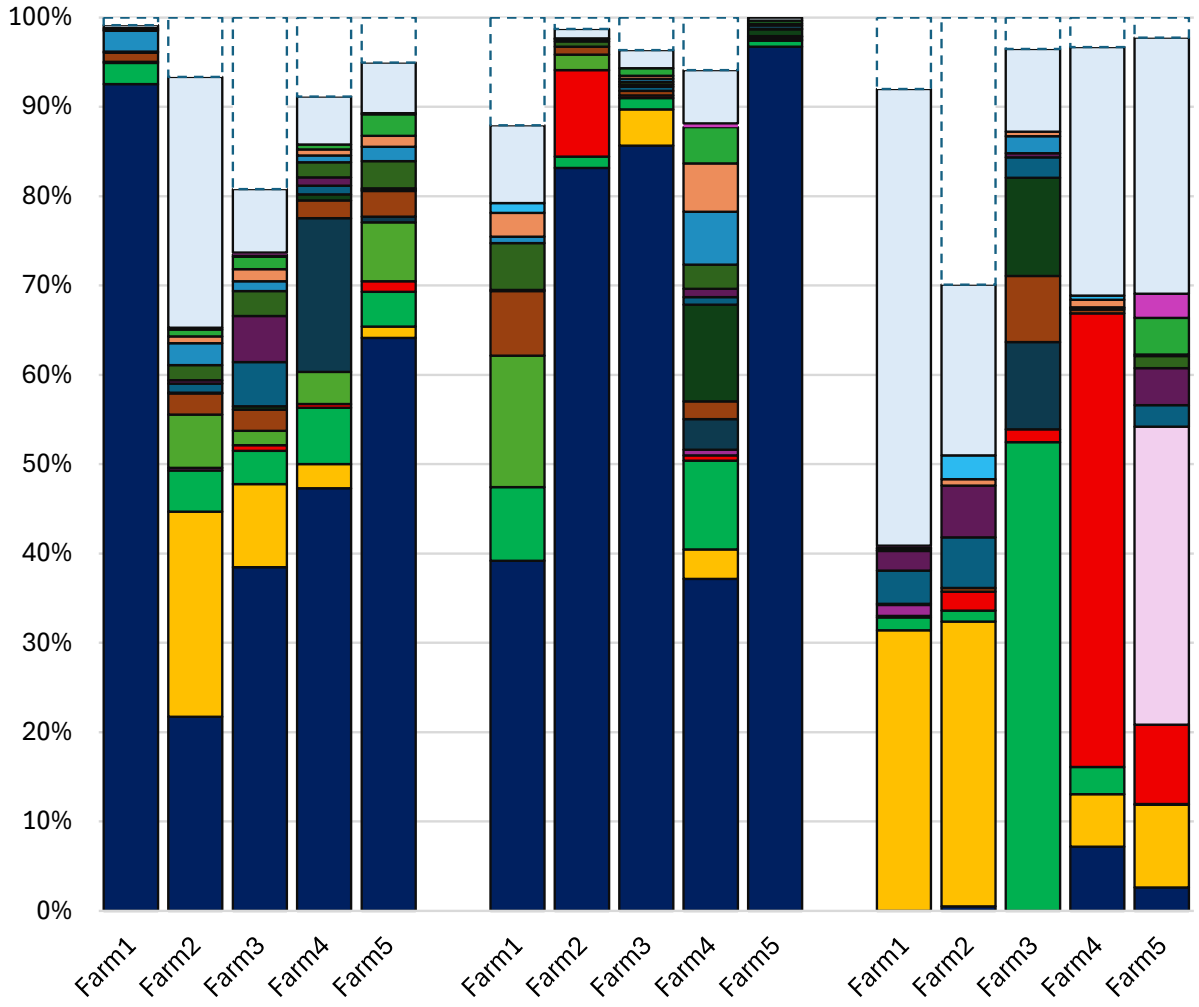


Effect of heterolactic LAB inoculum higher in BA1 than C (Farm 2 and 4)

Spoiled area (A1)

Zero aerobic stability of A1

Bacteria microbiome (16S)



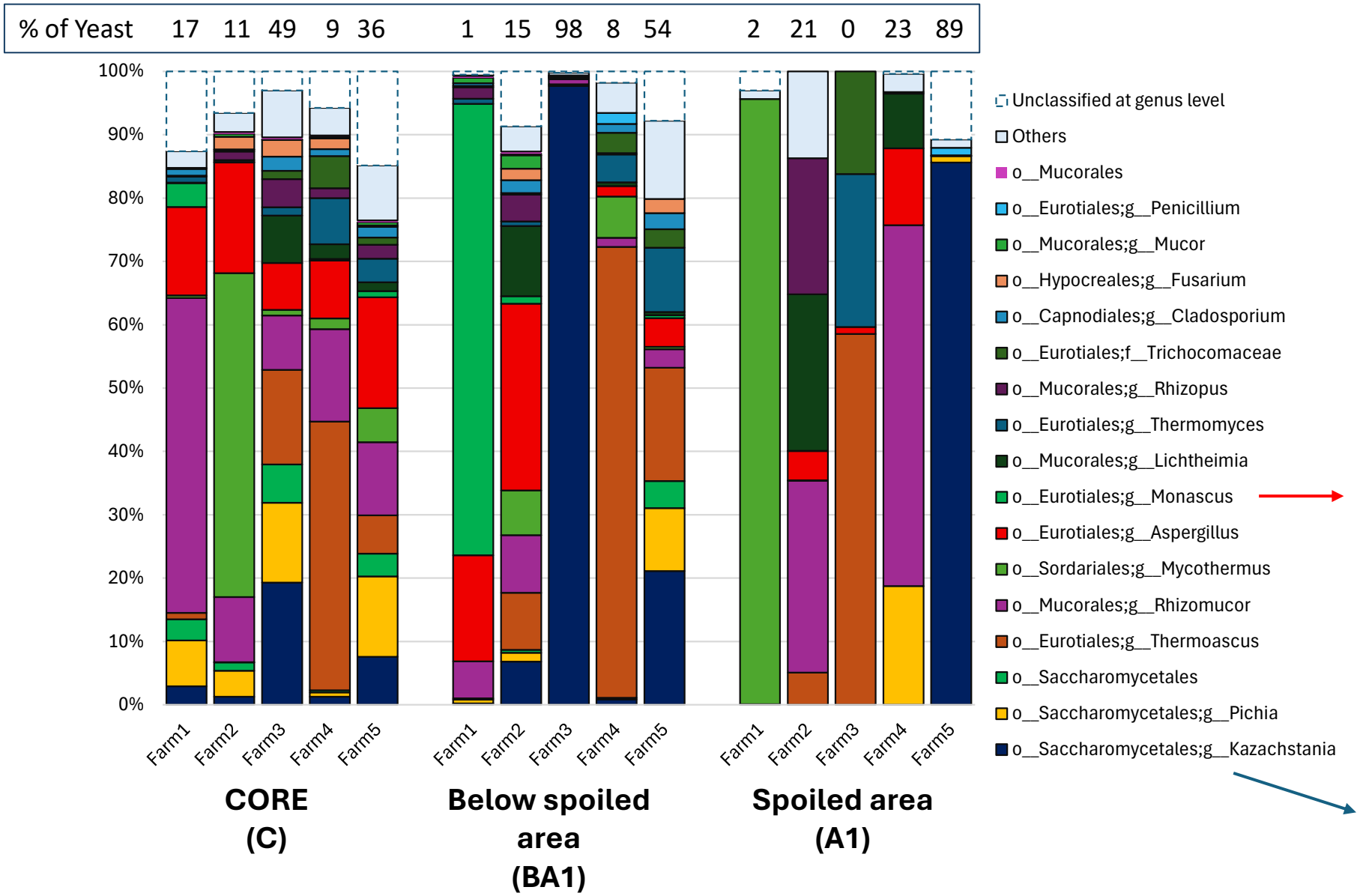
- Unclassified at genus level
- Others
- o__Clostridiales;g__Anaerocolumna
- o__Enterobacterales
- o__Clostridiales
- o__Bacillales;g__Paenibacillus
- o__Bacillales;g__Thermoactinomyces
- o__Clostridiales;g__Clostridium
- o__Burkholderiales;g__Comamonas
- o__Pseudomonadales;g__Pseudomonas
- o__Bacillales;g__Brevibacillus
- o__Bacillales;f__Bacillaceae
- o__Bacillales;g__Ureibacillus
- o__Burkholderiales;g__Massilia
- o__Burkholderiales;f__Alcaligenaceae
- o__Rhodospirillales;g__Acetobacter
- o__Bacillales;g__Bacillus
- o__Sphingobacteriales;g__Sphingobacterium
- o__Lactobacillales;g__Lactobacillus

Low presence, mainly in A1

Dominate bacteria microbiome in A1

Dominate bacteria microbiome in C and BA1, when LAB count > 4 log cfu/g

Fungal microbiome (ITS)

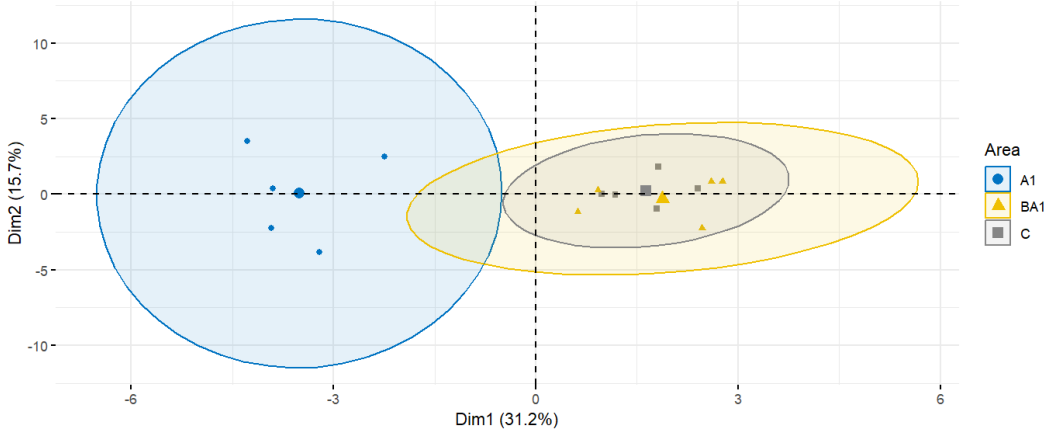


Eurotiales fungal order is the main detected, with *Aspergillus* genus dominated A1 samples

Presence of *Monascus* in microaerobic condition with high temperature

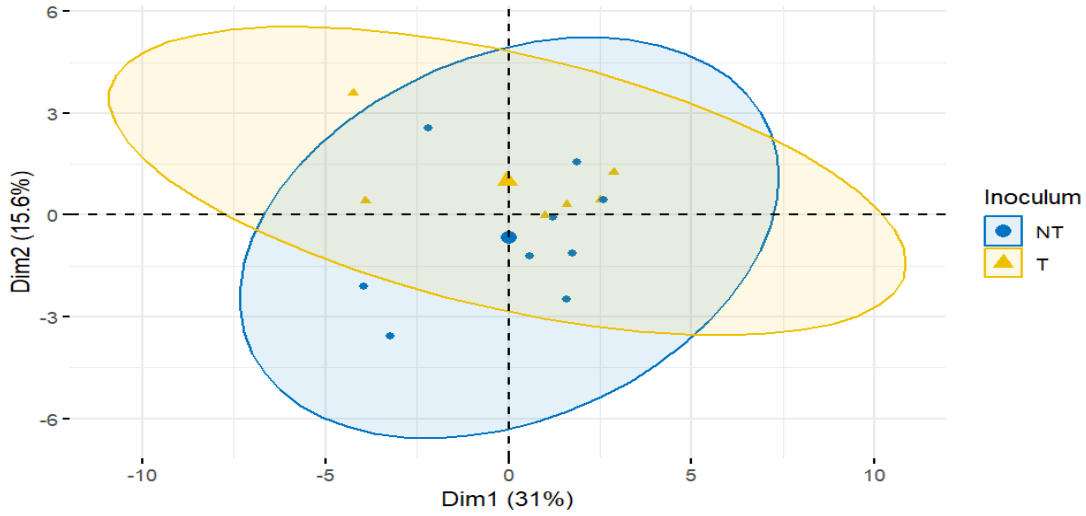
Kazachstania was the main representative yeast genera with yeast count higher than 3 log cfu/g

Principal component analysis (PCA) of samples characteristics



Sampling area of bunker and presence of aerobic spoilage determined a separation of samples

Other factor (e.g. treatments with LAB inoculum) did not show any differentiation



Conclusion

- Microbial communities of silages allows to evaluate the **qualitative microbial quality** of silages, however this technique needs to be integrated with traditionally culture-dependent methods to have a **quantitative approach**
- **Bunker management influenced the fermentative and microbial characteristics of silages**, in particular for critical areas
- Better microbial qualitative characterization will **help farmers in managing bunker and preventing aerobic deterioration**


Thank you for your attention