



Unraveling Silage Fermentation with Metagenomics and Metabolomics

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History of ensiling

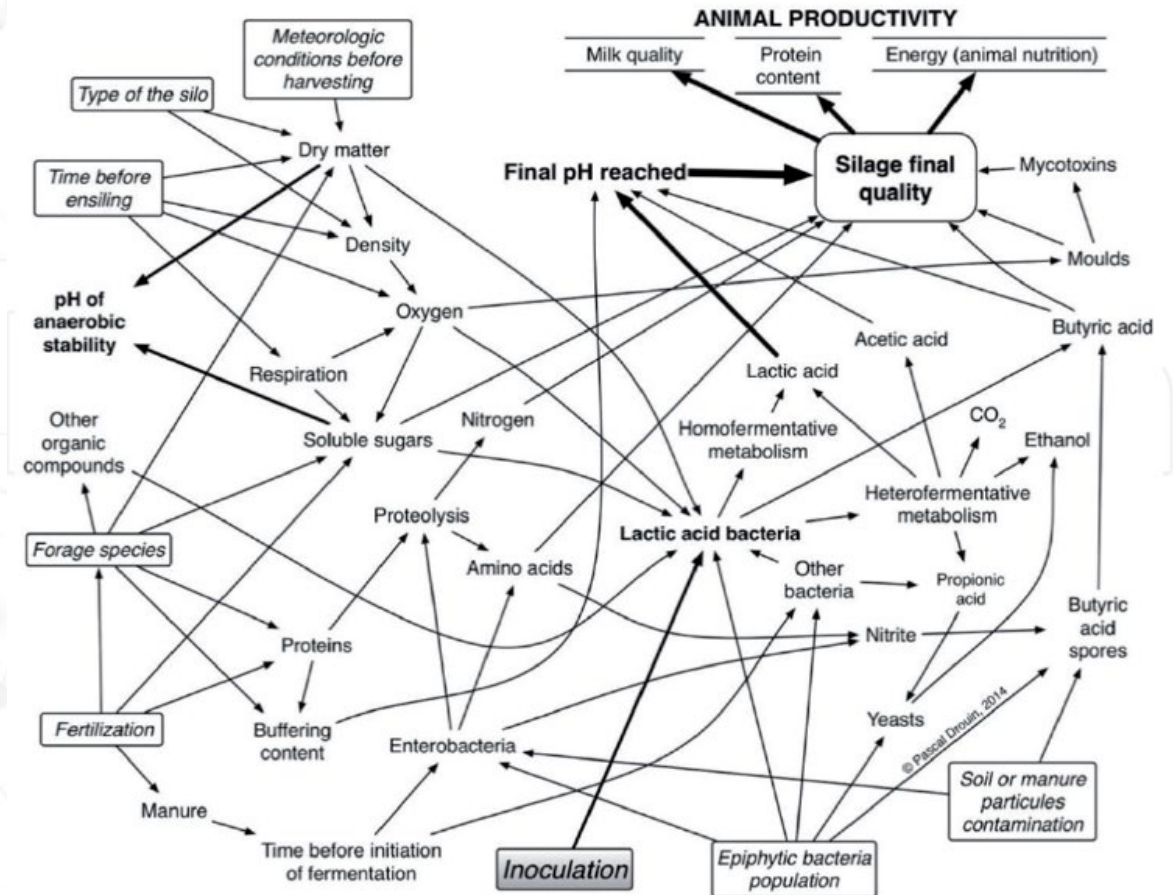
- First mention of ensiling nearly 3000 years ago
 - Ancient Egyptians ensiled cereal grains to preserve the harvest
- Used in Medieval Europe
- Still remains as the most important forage conservation method → minimal changes in the practice itself



<https://beefpoint.com.br/silagem-uma-breve-historia-65427/>

Why is ensiling important?

- Preservation of nutrients at harvest
 - Minimize dry matter losses during fermentation and feed-out
- Inhibition of undesirable microorganisms
- Improve the aerobic stability of silage during feed-out



Drouin et al., 2019
Intech Open

Silage Microbiology

First mention of silage in literature

> J Boston Soc Med Sci. 1901 Feb 19;5(7):378.

CONCERNING THE THEORIES OF SILAGE FORMATION

H L Russell, S M Babcock

PMID: 19971374 PMID: PMC2048453

First mention of silage microbiology

> Am J Vet Res. 1956 Apr;17(63):231-4.

The resistance of milk samples to the activity of Streptococcus agalactiae as affected by feeding silage

W D POUNDEN, N A FRANK, R W BROWN, R K SCHERER

PMID: 13302644

Sequencing of "silage-like flora"

Changes in microbial population during fermentation of feedlot waste with corn

G R Hrubant¹

Affiliations + expand

PMID: 16350022 PMID: PMC187127 DOI: 10.1128/am.30.1.113-119.1975

First publication of metagenomics in silage

> J Biotechnol. 2009 Jun 1;142(1):38-49. doi: 10.1016/j.biotech.2009.02.010. Epub 2009 Feb 27.
Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and metagenome sequence data obtained by 454-pyrosequencing

Magdalena Kröber¹, Thomas Bebel, Narytza N Diaz, Alexander Goemann, Sebastian Jannicke, Lutz Krause, Dieter Miller, Kai J Runte, Prisca Viehöver, Alfred Pühler, Andreas Schüler

Affiliations + expand

PMID: 19480946 DOI: 10.1016/j.biotech.2009.02.010

First mention of sequencing in silage

Maize silage: incidence of moulds during conservation

J Pelhate

PMID: 328359

1901

1950

1955

1960

1965

1970

1975

2005

2010

THE STRUCTURE OF DNA

J. D. Watson¹ and F. H. C. Crick

Cavendish Laboratory, Cambridge, England

DNA Structure (Watson & Crick)

Structure of a Ribonucleic Acid

ROBERT W. HOLLEY, JEAN APOAR, GEORGE A. EVERETT, JAMES T. MADISON, MARK MARQUSEE, SUSAN H. MERBIL, JOHN ROBERT PENSWICK, AND ADA ZAMIR [Authors](#)

[Info & Affiliations](#)

SCIENCE • 19 Mar 1965 • Vol 147, Issue 3664 • pp. 1462-1465 • DOI: 10.1126/science.147.3664.1462

First reported nucleic acid sequencing from *Saccharomyces cerevisiae*

The amino-acid sequence in the glycyl chain of insulin. 2. The investigation of peptides from enzymic hydrolysates

F Sanger^{1,2}, E O P Thompson^{1,2}

First sequencing: two chains of insulin protein



Next generation sequencing

Article | Published: 08 April 1976
Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene

W. Fiers, R. Contreras, F. Duerinck, G. Haegeman, D. Iserentant, J. Merregaert, W. Min Jou, F. Molemans, A. Raeymaekers, A. Van den Bergh, G. Volckaert & M. Ysebaert

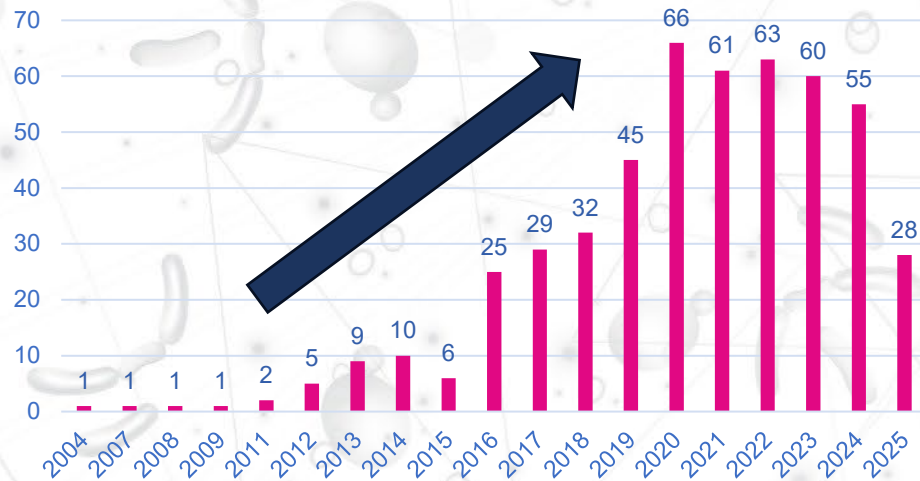
[Nature](#) 260, 500-507 (1976) | [Cite this article](#)

First organism to be completely sequenced

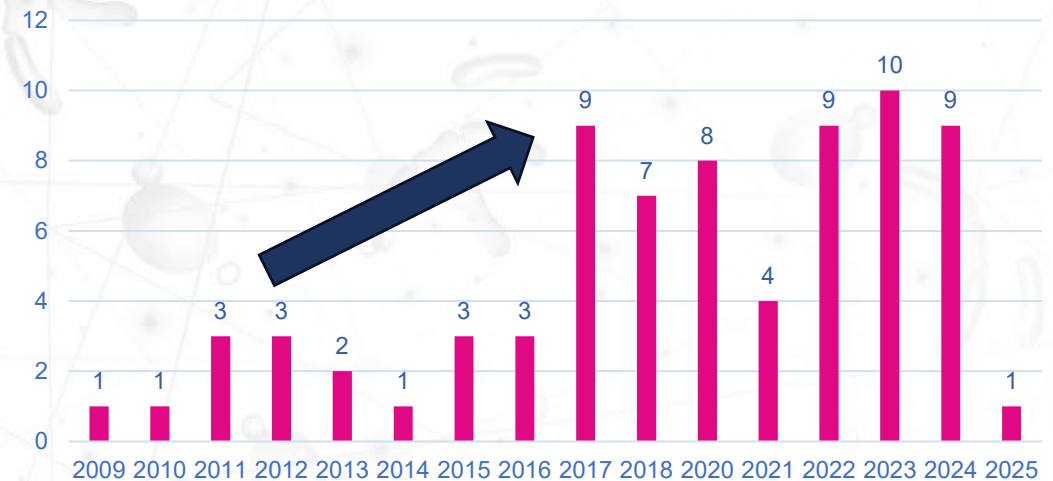
Molecular biology

The application of molecular biology in silage is relatively new

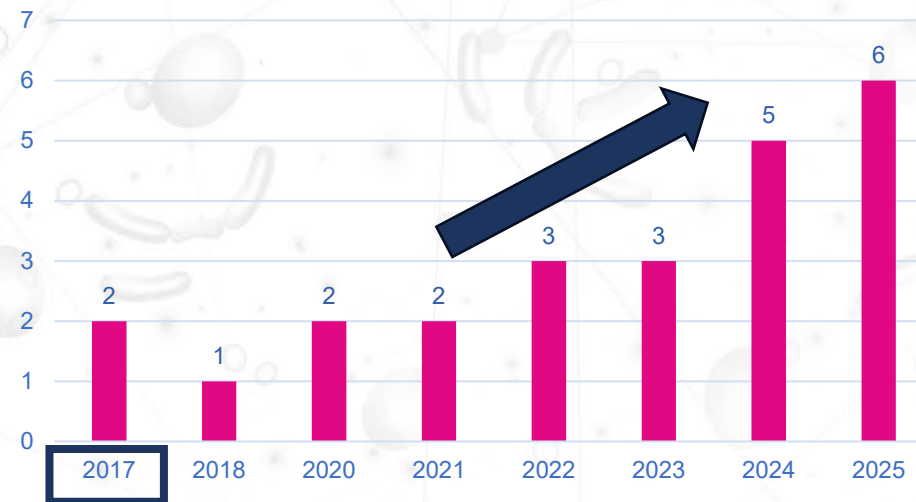
PubMed: "silage", "microbiome"



PubMed: "silage", "metagenomics"



PubMed: "silage", "OMICS"



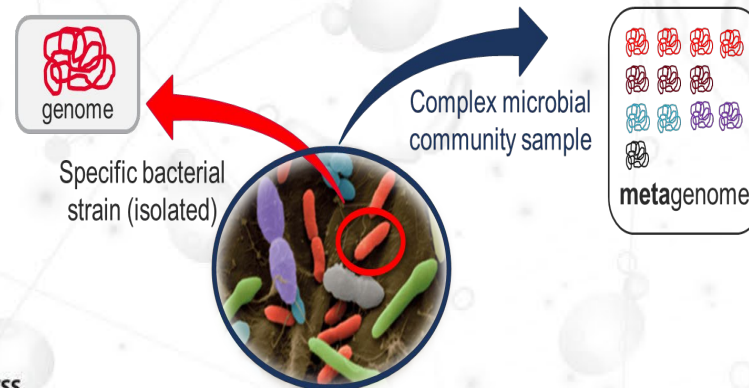
Defining OMICS and OMICS-based approaches

- Application of molecular biology to understand different levels of complexity in biological systems
- “...analyzing large amount of data representing structure and function of an entire makeup of a given biological system at a particular level.” (Dai and Shen, 2022)

~ome complete set of genes, proteins, metabolites...

~omics analysis of one ~ome

Meta~ analysis from multiple species



Metagenomics

External factors

Pre-harvest conditions
Forage type
Moisture content
Etc.



Genomics/ metagenomics

Who is there?
Community structure
Potential functions

- Genomic potential of *L. buchneri* CD034 (Heinl 2012)
- Community structure of corn silage during aerobic exposure after inoculation with different strains (Drouin et al., 2020)

Metabolomics

What happened?
Activity under tested
conditions

- Transformation of alfalfa proteins during ensiling with *L. plantarum* (Xia et al., 2025)

Transcriptomics/ metatranscriptomics

Functional potential
Activity level

- Gene expression analysis of high-moisture corn with different inoculation treatments (Drouin et al., 2023)

Proteomics/ Metaproteomics

What happened?
Activity under
tested conditions

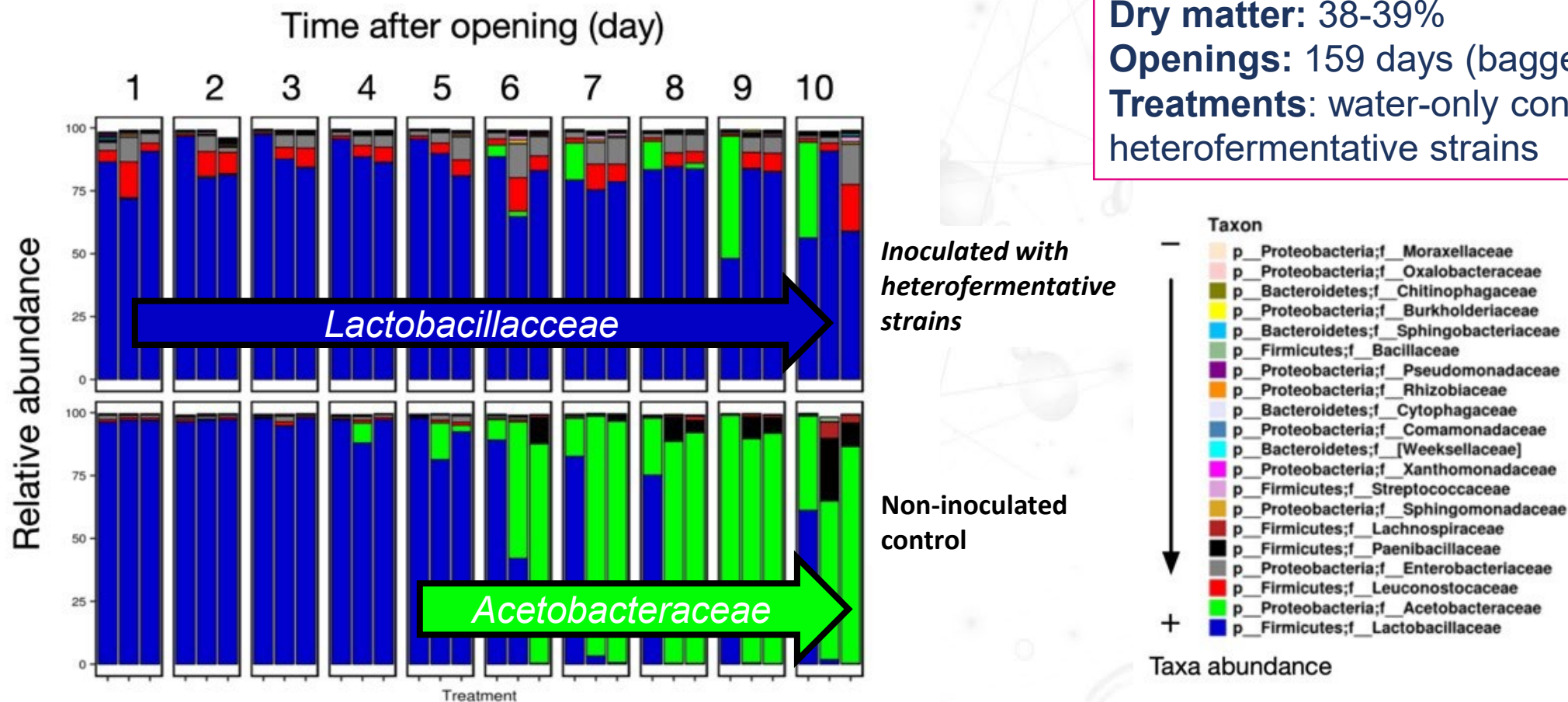
- Transformation of alfalfa proteins during ensiling with *L. plantarum* (Xia et al., 2025)

External factors
Storage conditions
Feed-out



Metagenomics example: amplicon sequencing to characterize changes during aerobic exposure

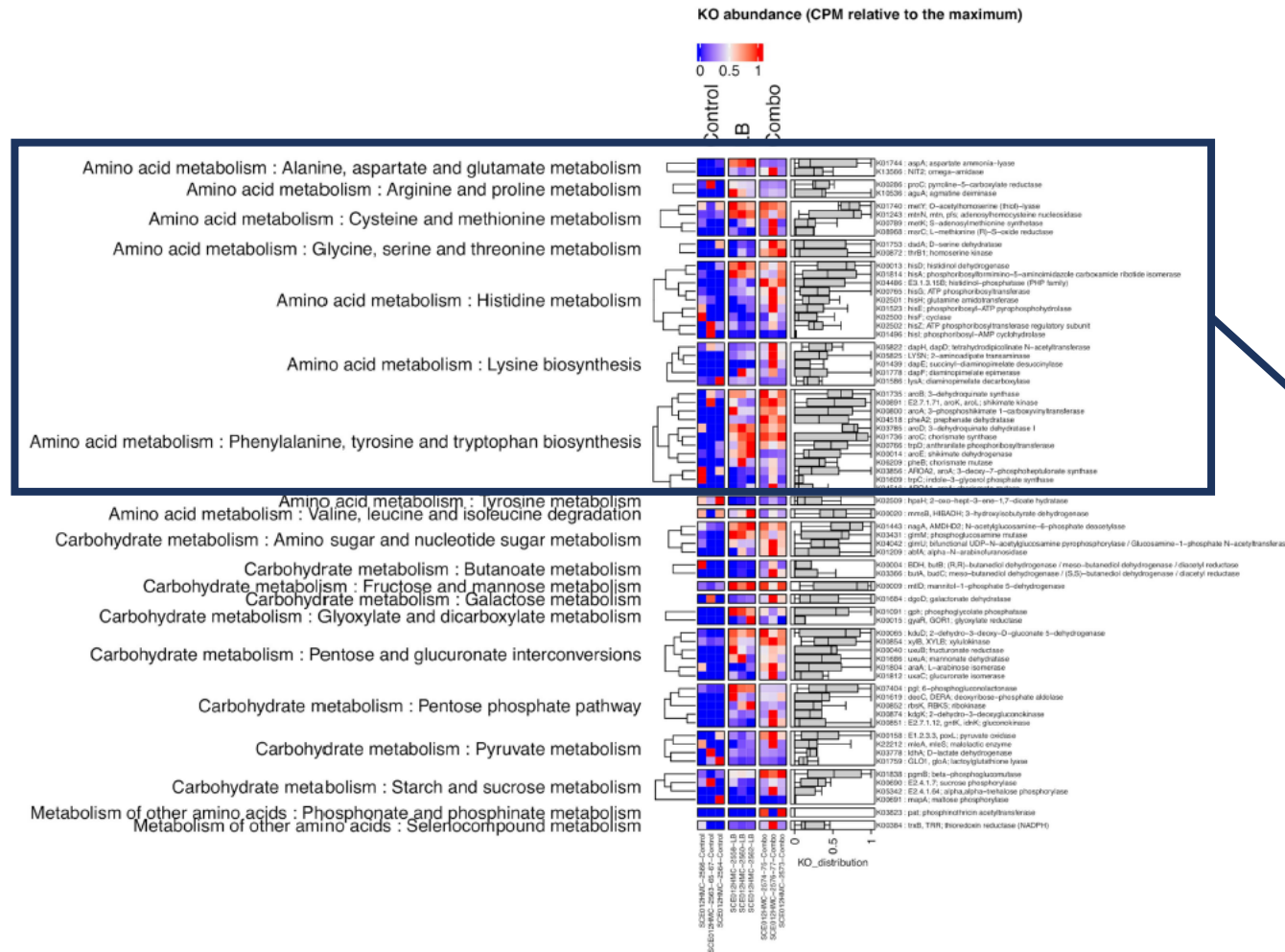
Forage type: corn silage
 Dry matter: 38-39%
 Openings: 159 days (bagged silage)
 Treatments: water-only control, two heterofermentative strains



Key message: 16S amplicon sequencing highlights changes in bacterial communities during aerobic deterioration in treated and untreated silage over 10 days.

Transcriptomics example: gene expression analysis of high-moisture corn silage

Forage type: High-moisture corn
Dry matter: 70.8%
Openings: 120 days (RNA)
Treatments: water-only control, *L. buchneri*, two heterofermentative strains



Inoculation increased gene expression associated with protein and amino acid degradation → *improved starch-D*.

Key message: Metatranscriptomics highlighted changes in gene expression across treatments.

Proteomics example: characterizing individual strains

Forage type: Alfalfa silage
Dry matter: ~ 38%
Openings: 50 days
Treatments: sterile water, two strains of *L. plantarum* applied individually.

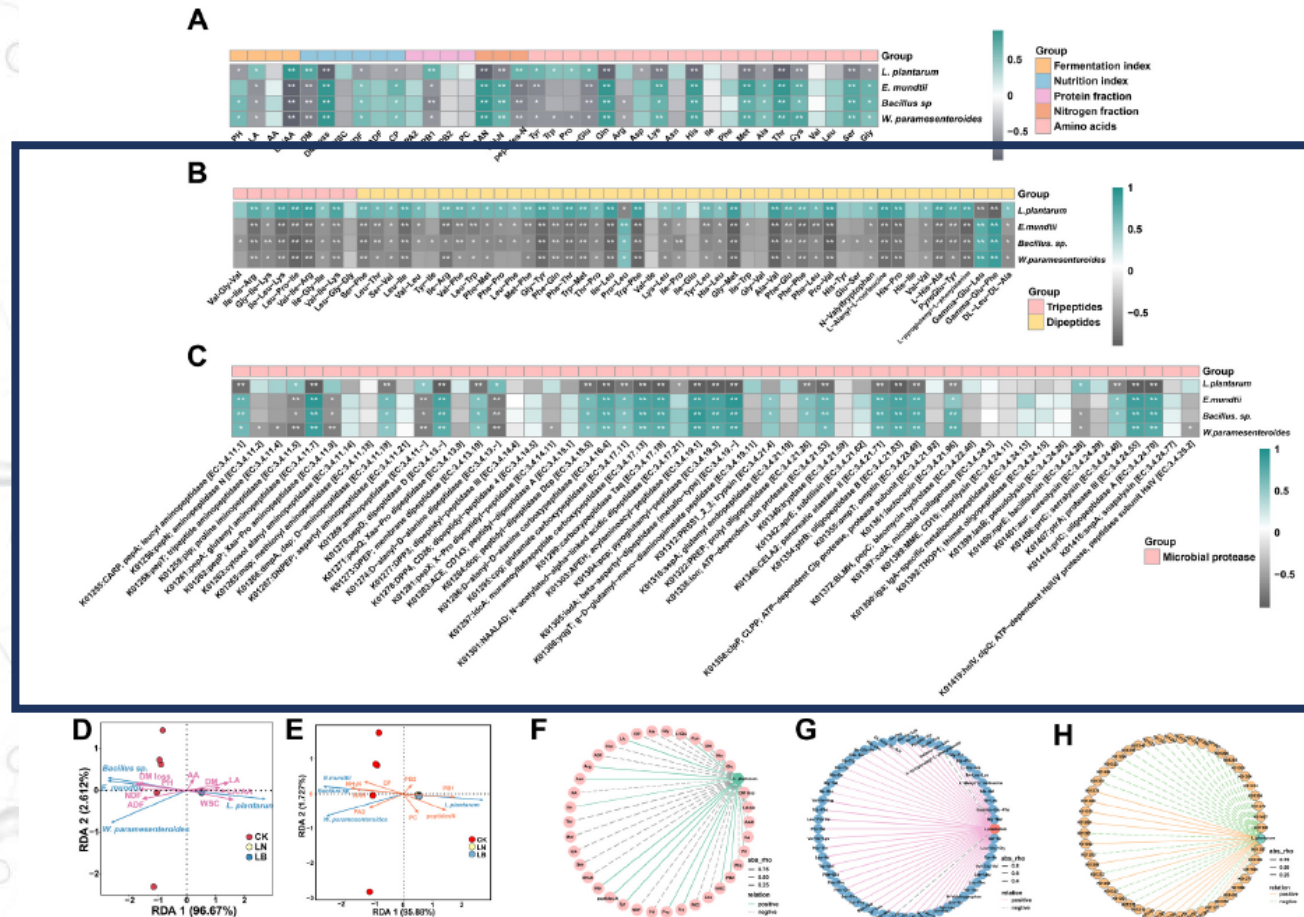


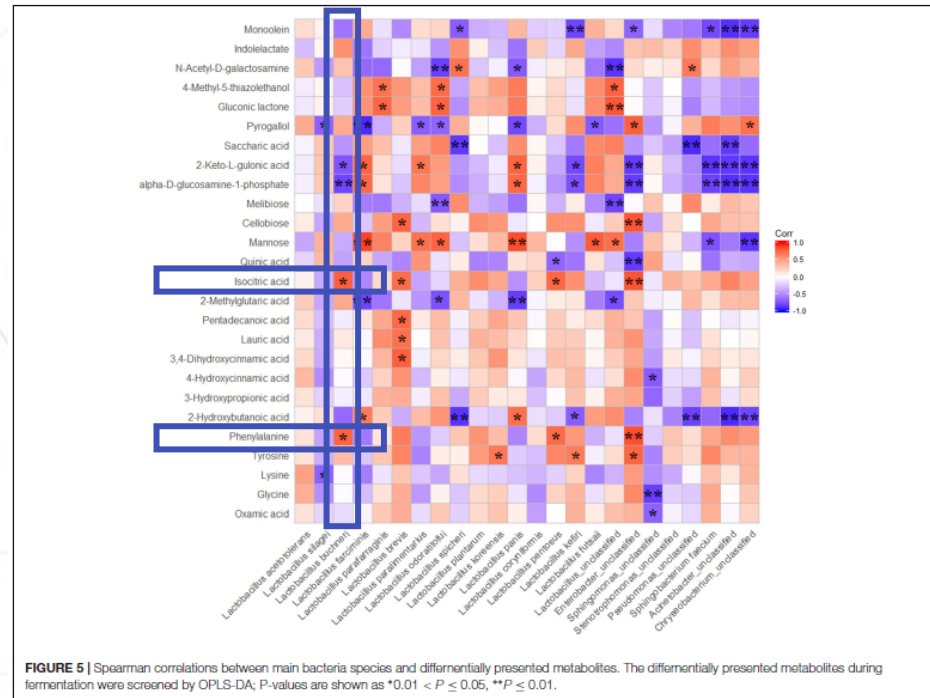
Fig. 9. The relationship between microbial and metabolite identifies the key species. A, the heatmap showing the correlation between bacteria (*L. plantarum*, *E. mundtii*, *Bacillus* sp., *W. paramesenteroides*) abundance and fermentation indexes, nutritional indexes, and amino acids. B, the heatmap showing the correlation between peptides and bacteria (*L. plantarum*, *E. mundtii*, *Bacillus* sp., *W. paramesenteroides*) abundance. C, the heatmap showing the correlation between microbial enzyme genes and bacteria (*L. plantarum*, *E. mundtii*, *Bacillus* sp., *W. paramesenteroides*) abundance. D, E, redundancy analysis between fermentation, nutrition index and *L. plantarum*, protein, nitrogen fraction and *L. plantarum*, respectively. F, G, H, circos plot showing the correlation between amino acids and *L. plantarum*, peptides and *L. plantarum*, microbial enzyme genes and *L. plantarum*. * $p < 0.05$ and ** $p < 0.01$ versus the CK.

Key message: Proteomics was applied to characterize protein metabolism of alfalfa silage → *L. plantarum* inoculation improved peptide-N availability.

Metabolomics example: mapping metabolic differences between silage treatments

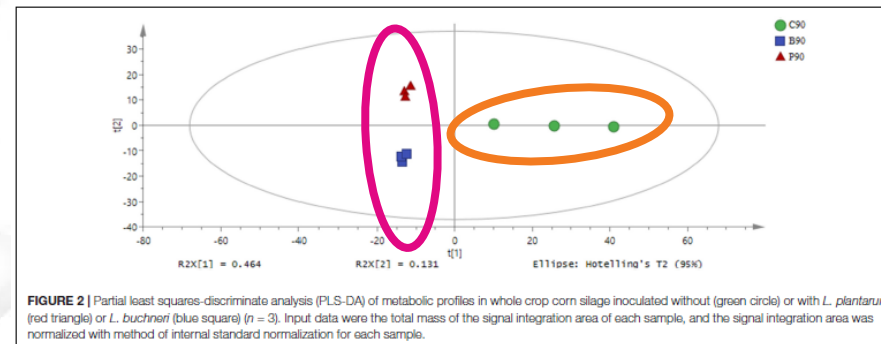
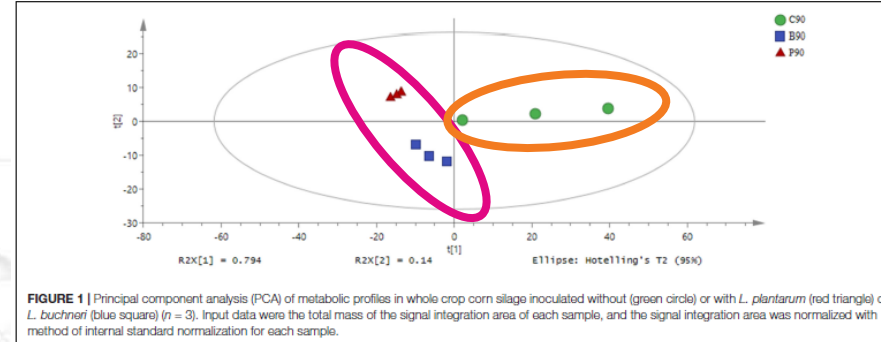
Xu et al.

Metabolome and Microbiota in Silage



Xu et al.

Metabolome and Microbiota in Silage



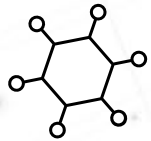
Forage type: Corn silage
Dry matter: 23.4%
Openings: 90 days
Treatments: Untreated, *L. plantarum*, *L. buchneri*

Key message: Metabolomics highlighted differences in corn silage between *L. plantarum* and *L. buchneri* treatments. Additionally, PCoA highlighted the consistency and distinction of treated from *untreated corn silage*.

Non-exhaustive list of sub-disciplines within OMICS

Applied to silage

- **Volatomics/ Volatilomics** – Sub-discipline of metabolomics studying volatile organic compounds (VOCs)



- PubMed: 5 publications
- Which volatiles are associated with specific fermentations?
- Which ensiling volatiles should we “watch” from an environmental perspective?

- **Multi-omics** – Combination of multiple OMICS disciplines to characterize a given system.



- PubMed: 4 publications**

- **Culturomics** – High-throughput cultivation and identification of previously unculturable microorganisms



- PubMed: 1 publication → [Wang et al., 2024](#)
- Identify other microorganisms in silage

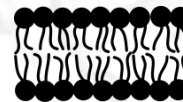
Yet to be applied to silage

- **Glycomics** – Characterization of all the carbohydrate structures produced by a defined system



- PubMed: none
- Improve our understanding of nutrient degradation during ensiling
- Better functional characterization of enzymes during ensiling

- **Lipidomics** – Characterization of lipids in a biological system.



- PubMed: none
- Improve our understanding of fatty acid changes during ensiling → *dietary implications??*

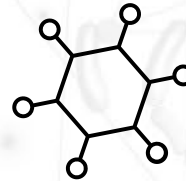
Future Perspectives

■ The importance of ensiling is increasing in a changing world

- Improve nutrient retention and nutritive value (reduce DM losses)
- Reduce feed-costs → *increased use of on-farm feeds* → relies on maximizing forage quality
- Reduce production of harmful byproducts and metabolites
- Improve sustainability on-farm through changes in current practices

■ OMICs approaches will continue to improve and expand their reach

- *Method improvements* → reduced cost → better access for researchers → feasible commercial applications
- Expansion of artificial intelligence into OMICs



■ What have we learned in ~15 years of OMICs approaches in silage?

- Microbial successions in ensiling are dynamic and evolve with time
- Characterizing different populations across forages types
- “Pay attention” to other metabolites in silage

■ What is next for OMICs in silage?

- Further characterization of microbial communities
- Expanded functional mapping:
 - “Other metabolites” → secondary metabolites, enzymes, bioactives
 - Confirming metabolic activity of specific strains
- Improving our understanding of how ensiling alters nutritional composition
- Understanding ensiling with alternative forage types and varying conditions
- Strengthen the link between silage and animal well-being/ performance

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