



European Federation of Animal Science

Effect of the use of *Lactiplantibacillus plantarum* strain 5BG and a commercial mix of polygalacturonase and glucanase on the quality of artichoke bracts silage

Greco R.¹, Ferrara M.², De Bellis P.², Giannico F.¹, Mulè G.², Maggiolino A.¹, De Palo P.¹

¹Dept. of Veterinary Medicine, University of Bari, 70010, Italy; ²Institute of Sciences of Food Production, CNR-ISPA, Bari, Italy

Consiglio Nazionale delle Ricerche



ISTITUTO DI SCIENZE DELLE
PRODUZIONI ALIMENTARI



UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO



Italy produced 378.110 tons of artichoke in 202,

The 60-85% of the artichoke fresh weight represent a by-product:

- leaves;
- external bracts;
- stem.

Artichoke bracts



No edible for human



Rich in macronutrients



Rich in fiber content

...Experimental hypothesis

Stabilize by ensiling the huge amount of
this seasonal matrix
making it a functional ingredient in the
ruminant feeding

It's useful in ruminant nutrition in a circular economy perspective...

Experimental design:

Artichoke by-products were stabilized by spontaneous fermentation or by inoculation of a selected bacterial strain (*Lactiplantibacillus plantarum* 5BG) alone or associated with enzymes (polygalacturonase and glucanase). The microbial population dynamic has been monitored by applying a microbiological approach and metabarcoding analysis.

Supplementations

Control : distilled water

5BG : 10^8 cfu/g starter

5BG low : 10^7 cfu/g starter

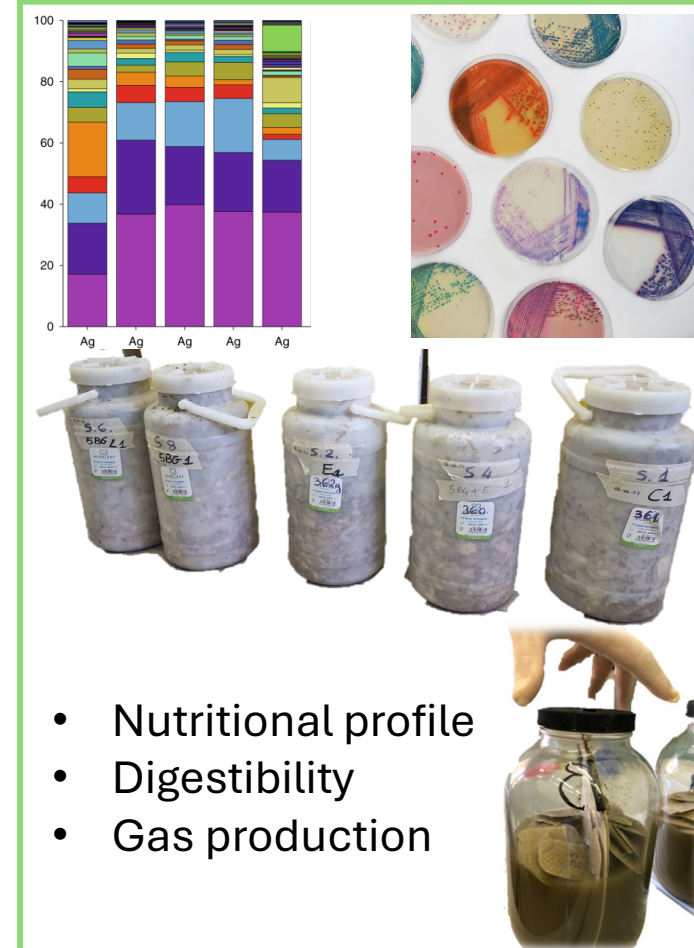
5BG+E : 10^8 cfu/g starter + 1% enzymes

E : 1% enzymes

Artichoke by-products



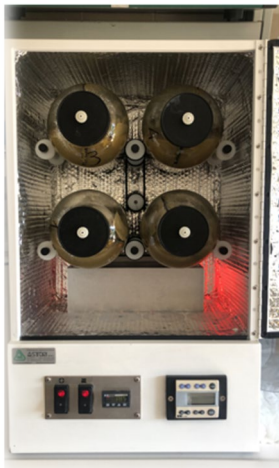
30 days of ensiling process...



Chemical analysis & *in vitro* ruminal digestion:

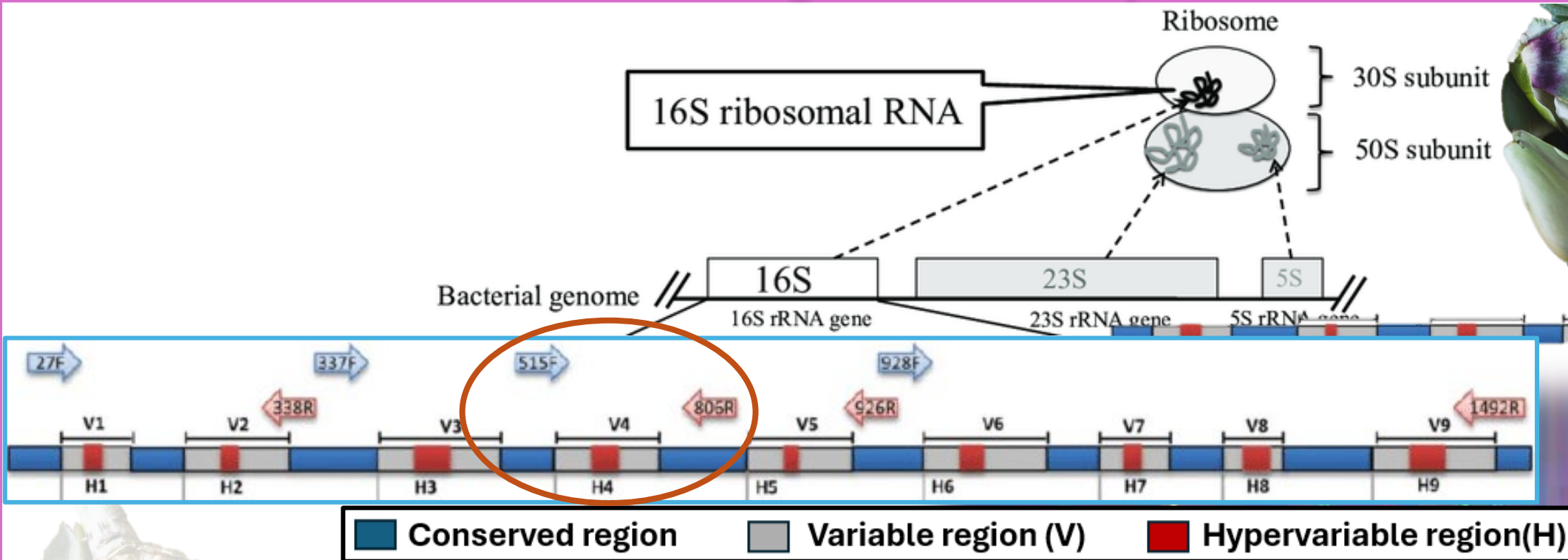
- Chemical gross composition →
- *In vitro* digestion (Daisy II, Ankom);
- *In vitro* gas production (Ankom Gas Analyzer).

- Dry matter
- Lipids
- Crude protein
- Neutral detergent fiber
- Acid detergent fiber
- Crude fiber



Each replicate of *in vitro* digestion was performed using a rumen fluid pool sampled from 4 subjects (heifers Belgian Blu x Holstein) after the slaughter procedure.

Metagenomic analysis



- Library creation
- Sequencing by IONTorrent system



Statistical analysis:

Data obtained by...

- chemical compositions;
- *In vitro* digestion ;
- gas production.

- metagenomic profiles

...were subjected to...

...The pairwise comparisons between the means of the group levels (t-test).

The Benjamini & Hochberg (1995) correction for multiple testing at a confidence level of $\alpha=0.05$.

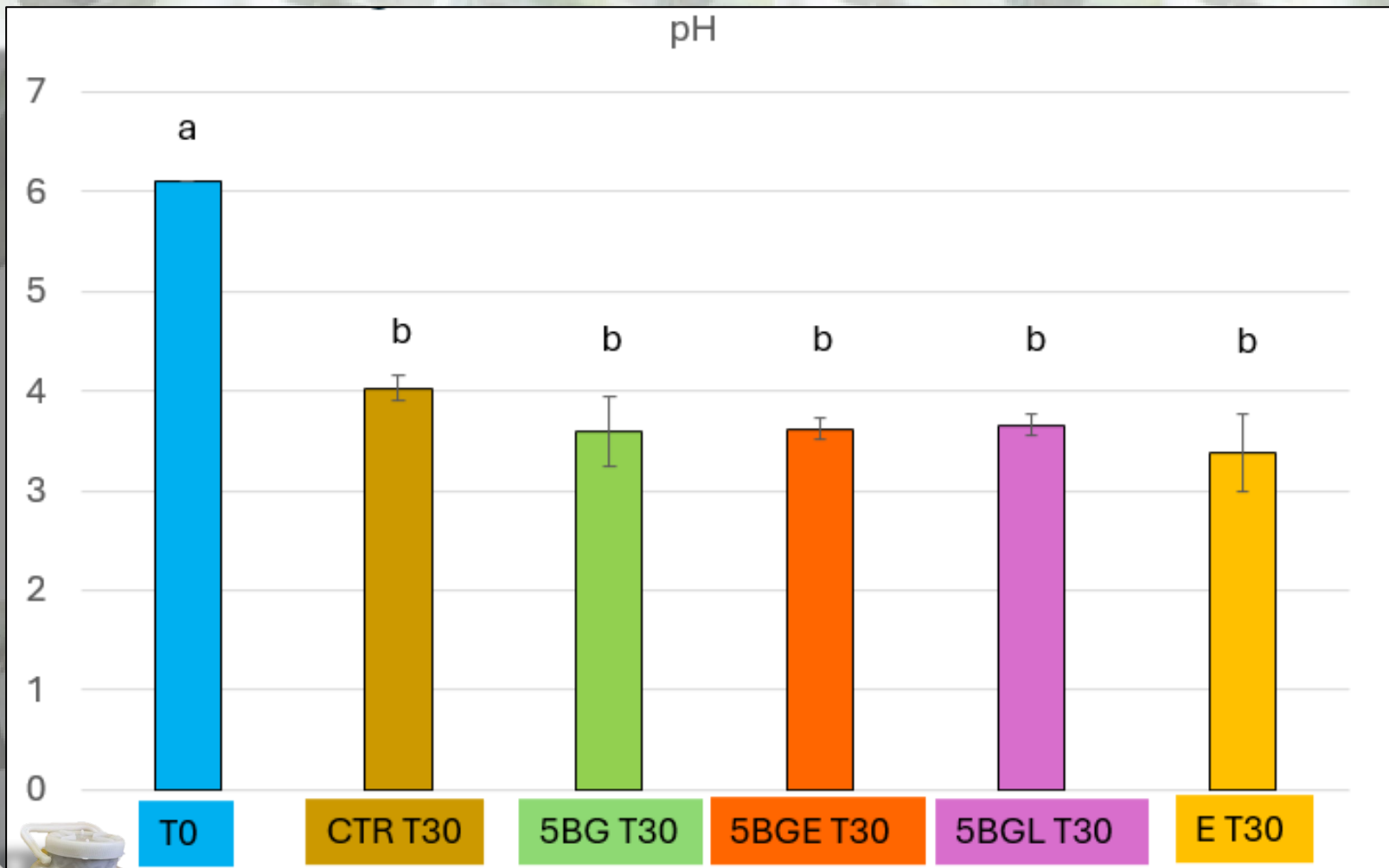
Open-source R software (R Core Team, 2023).

...Bioinformatic analysis by DADA2 vs SILVA
SSU database V138.1

Welch's test for single group comparison

STAMP software

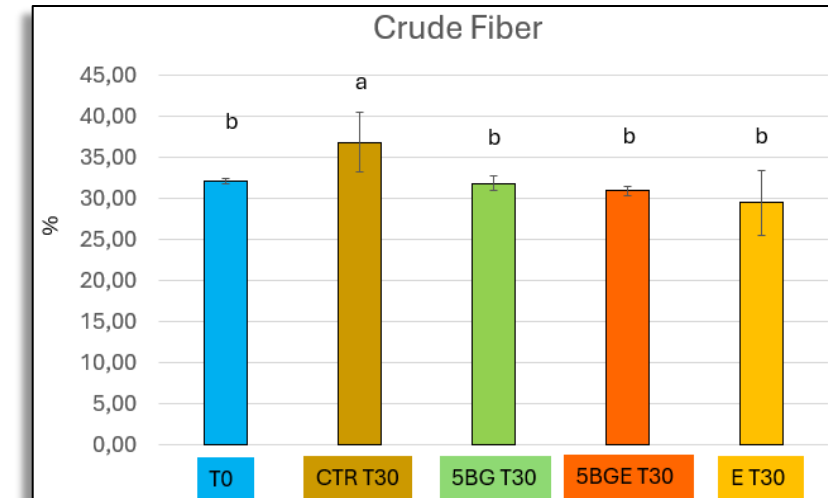
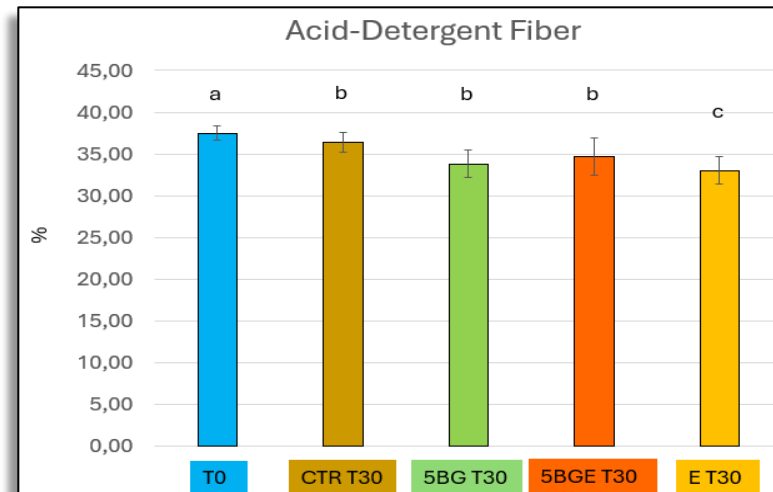
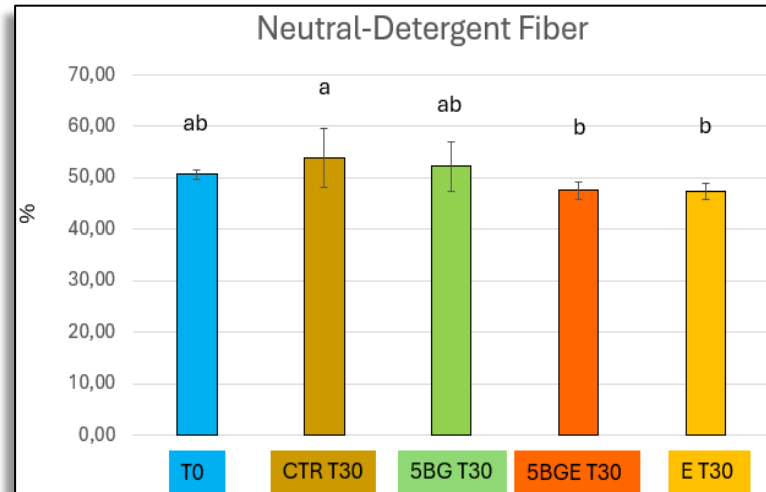
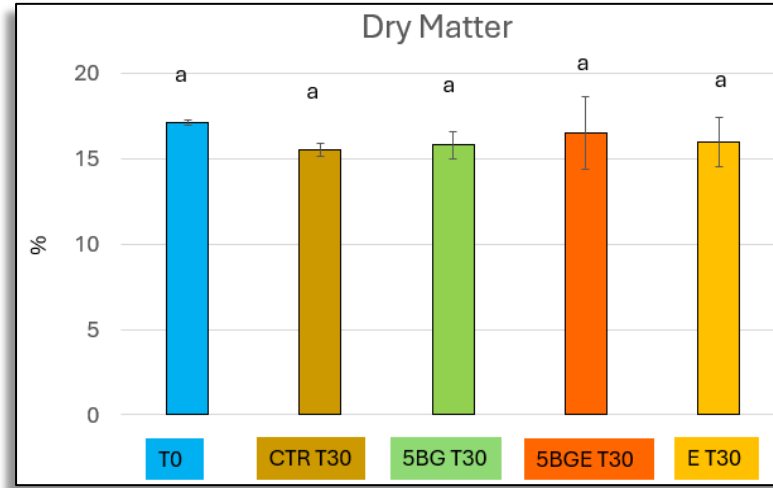
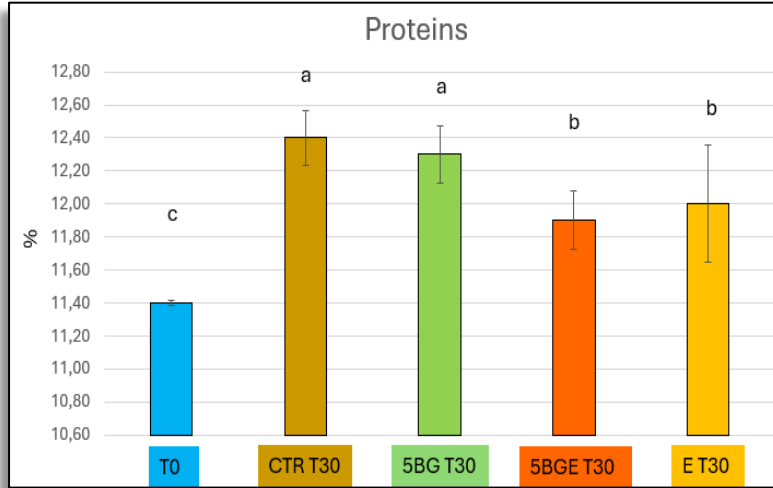
Post-silage process



Different letters indicate significant differences (a, b: $P \leq 0.05$)



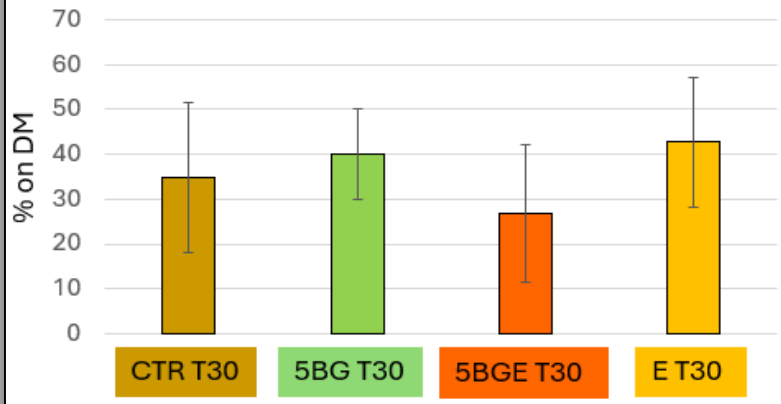
Post-silage process



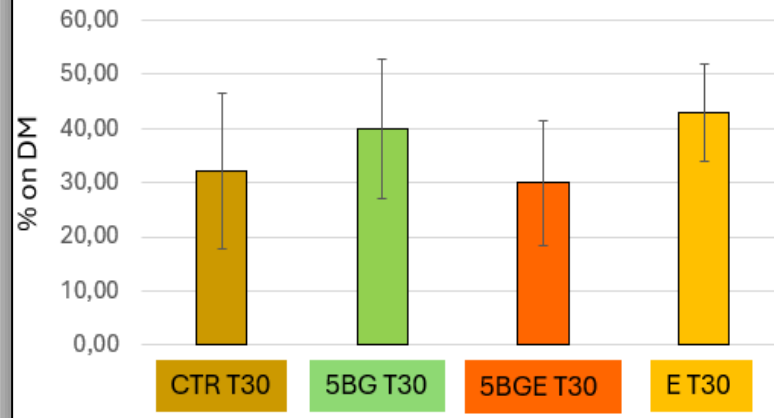
Different letters indicate significant differences (a, b, c: $P \leq 0.05$)

In vitro digestion disappearance

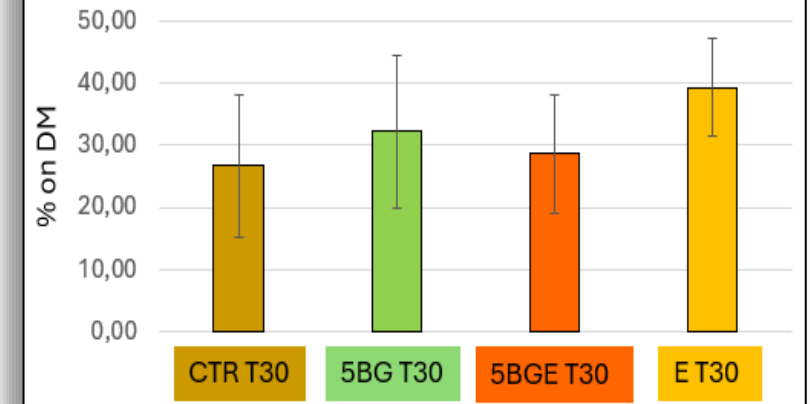
crude fiber



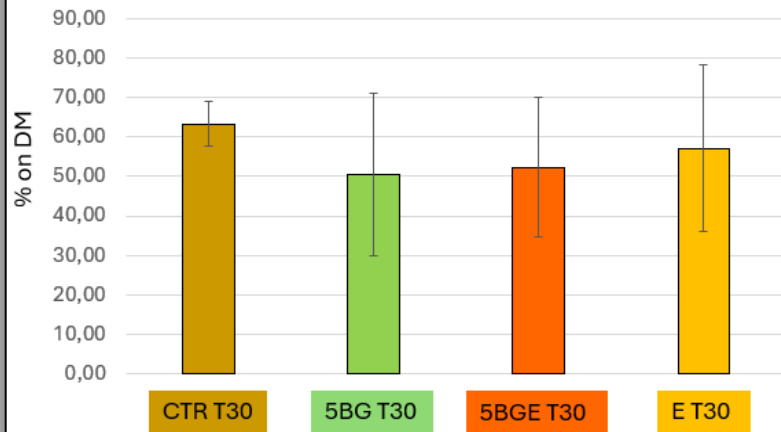
Neutral-detergent fiber



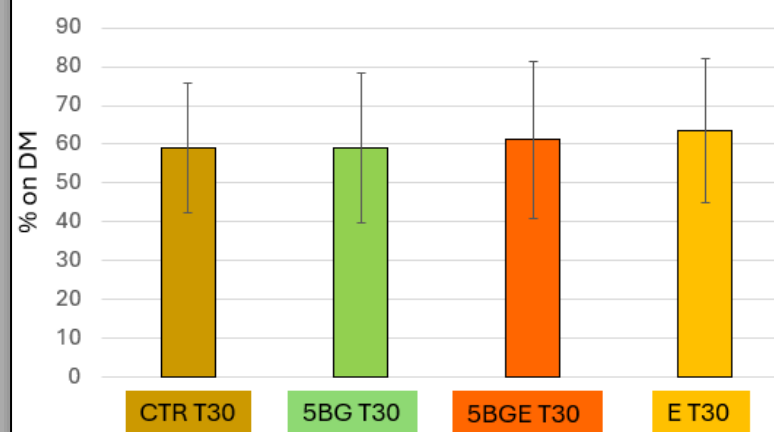
Acid-detergent fiber



Lipids

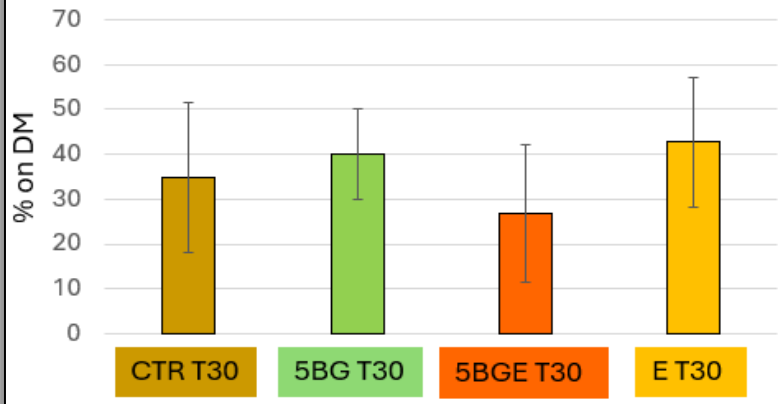


Proteins

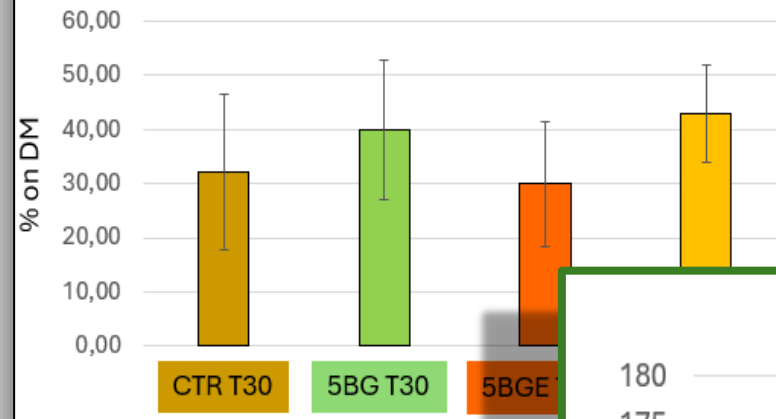


In vitro digestion disappearance

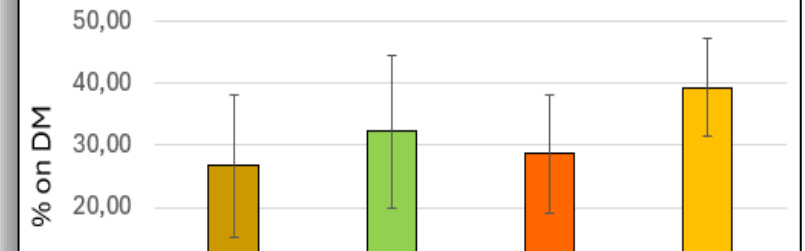
crude fiber



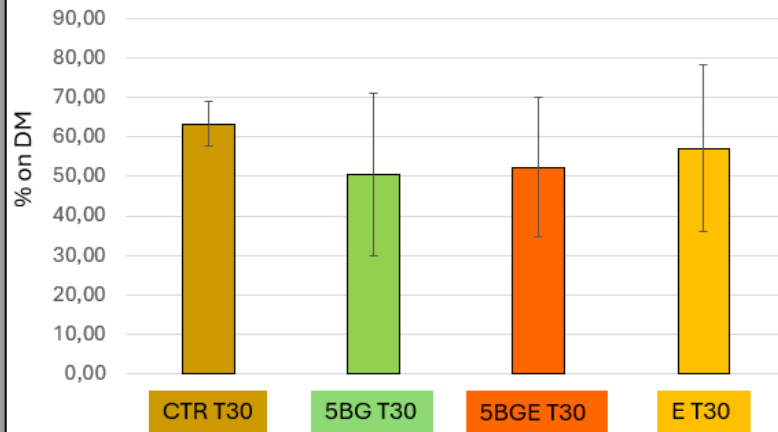
Neutral-detergent fiber



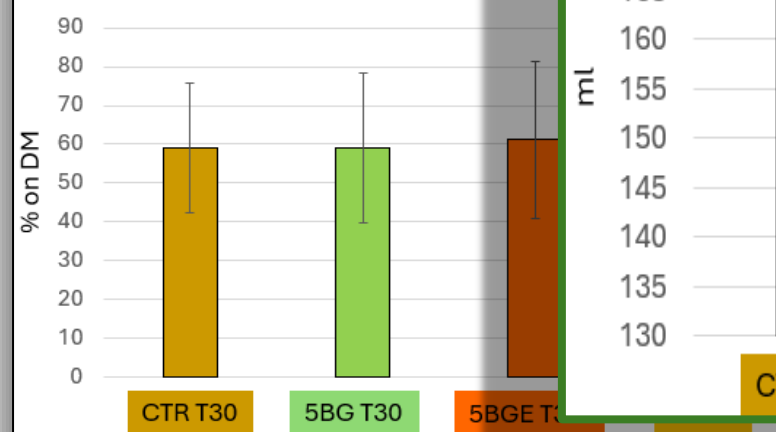
Acid-detergent fiber



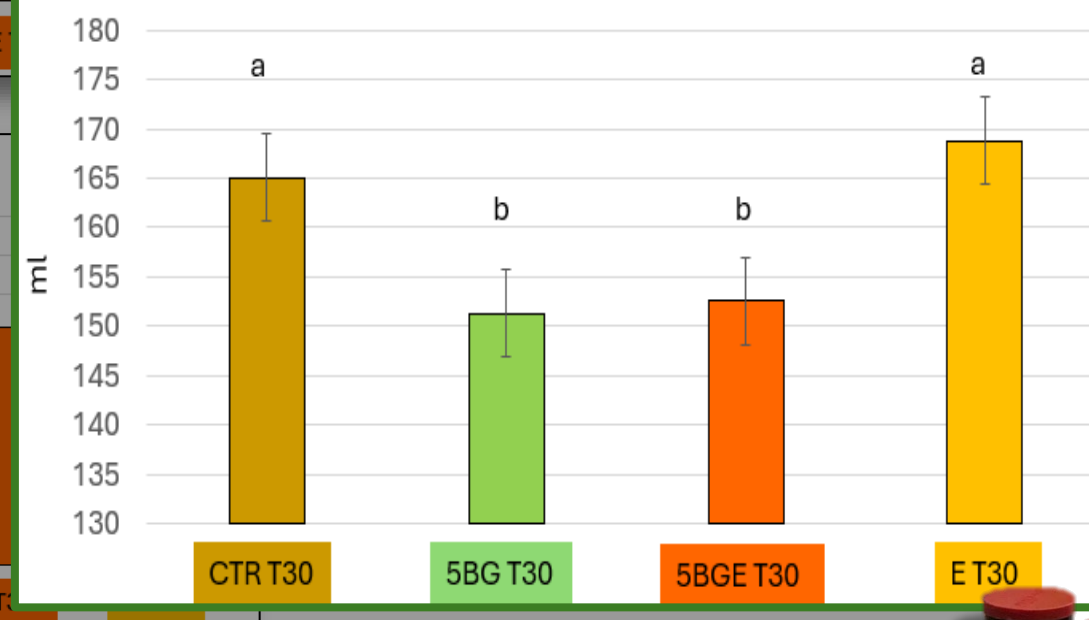
Lipids



Proteins



Gas Production



In vitro gas production

Different letters indicate significant differences (a, b: $P \leq 0.05$)

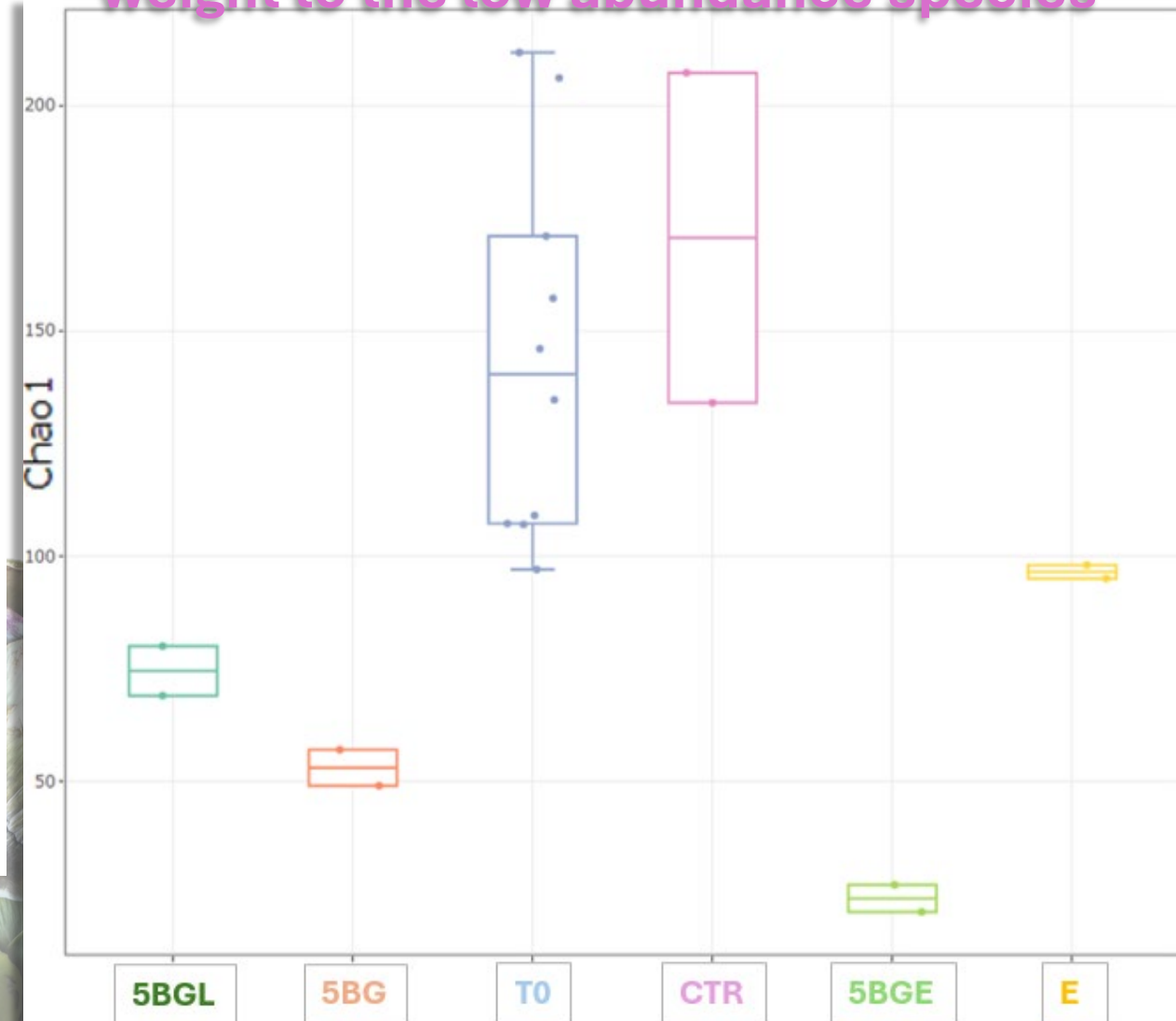


Species alpha diversity

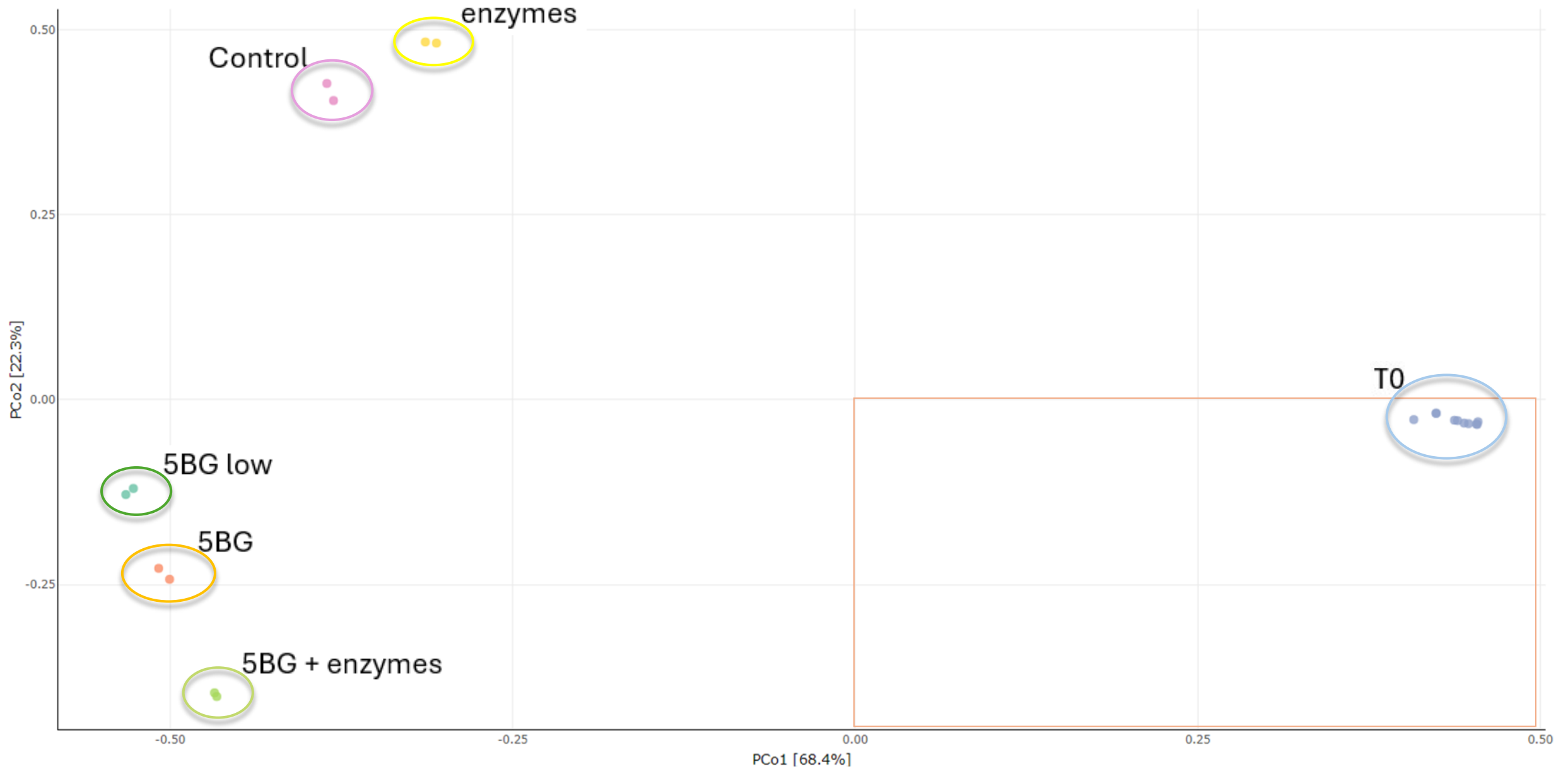


“Species Richness” is the total number of different organisms present in a sample.

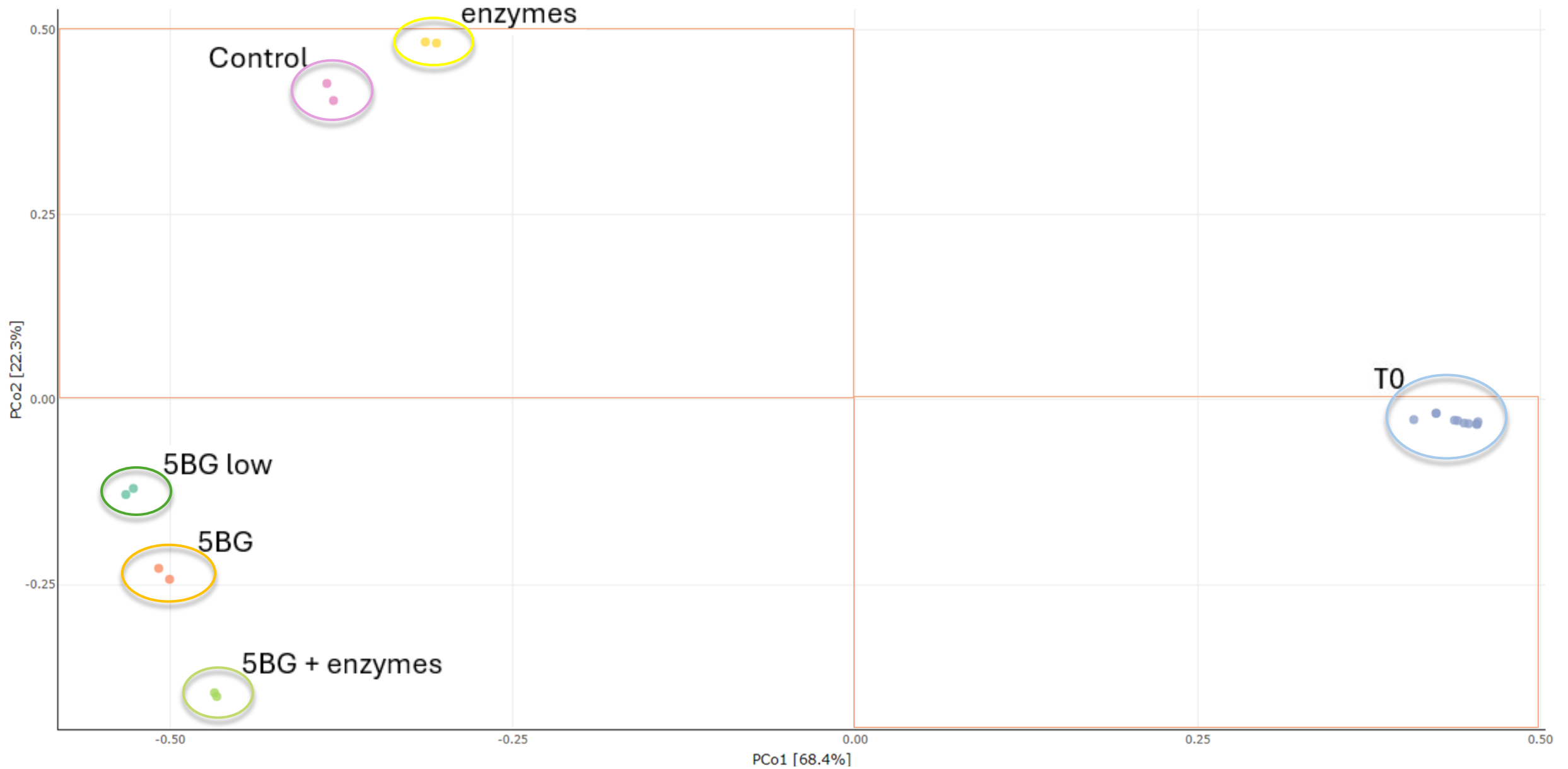
Chao1 richness estimator gives more weight to the low abundance species



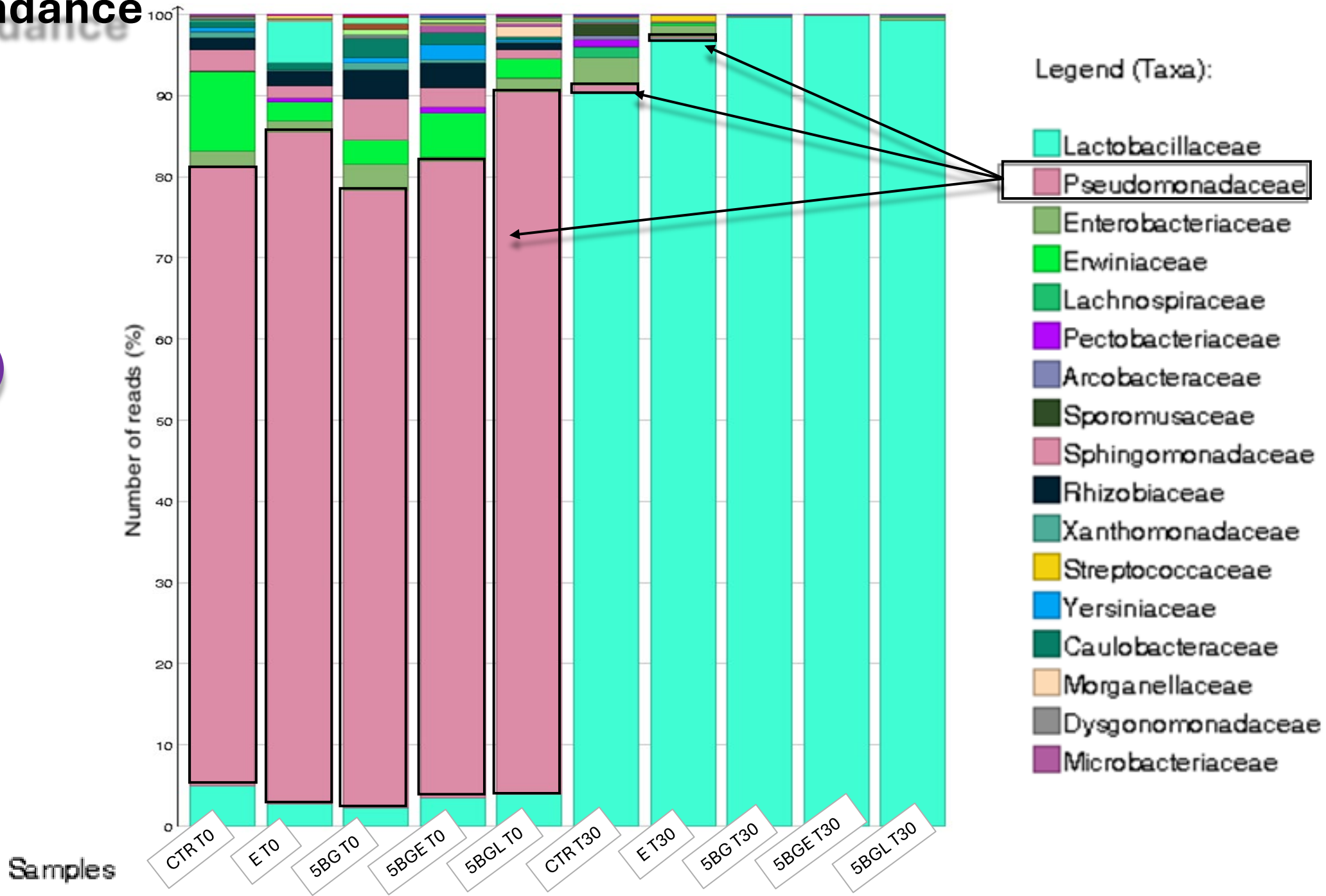
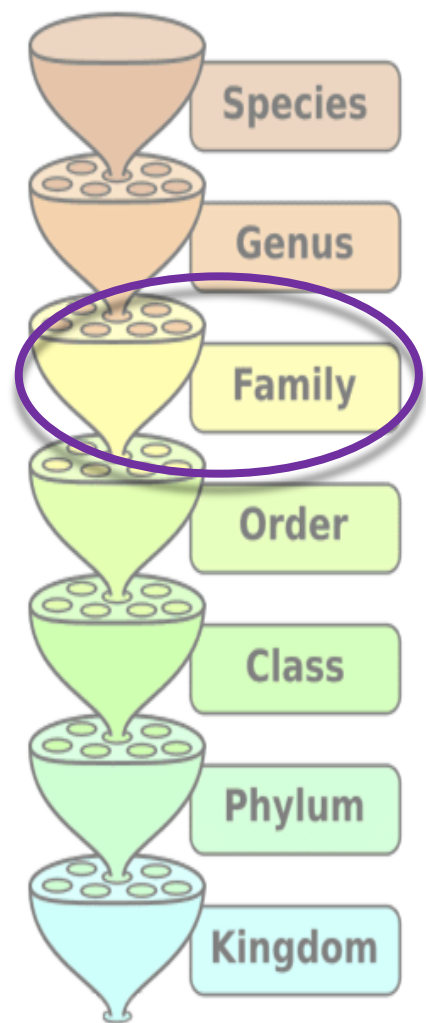
PCoA with bray distance

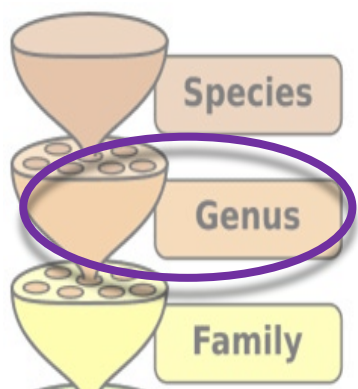


PCoA with bray distance



Relative abundance





Comparisons between pre and post-silage process

T0
CTR T30

T0
E T30

T0
5BG T30

T0
5BG+E T30

T0
5BGL T30

Pseudomonas



Weissella



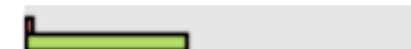
N.S.



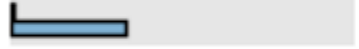
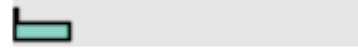
Leuconostoc



N.S.



Lactiplantibacillus

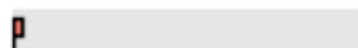


N.S.

Lactococcus



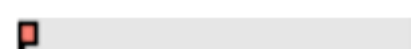
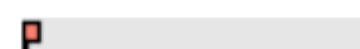
Sphingomonas



Klebsiella

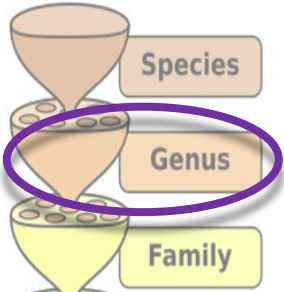


Pantoea



Welch's test for single-group comparison

N.S.= no significant differences between the groups



Comparisons between control and experimental treatments

CTR T30
5BG T30

CTR T30
E T30

CTR T30
5BGL T30

CTR T30
5BG+E T30

Leuconostoc

Weissella

Lactiplantibacillus

Klebsiella

Pseudomonas

Sphingomonas

Novosphingobium

Loigolactibacillus

Paenibacillus

N.S.

N.S.

N.S.

N.S.

0.0 59.2
Mean proportion (%)

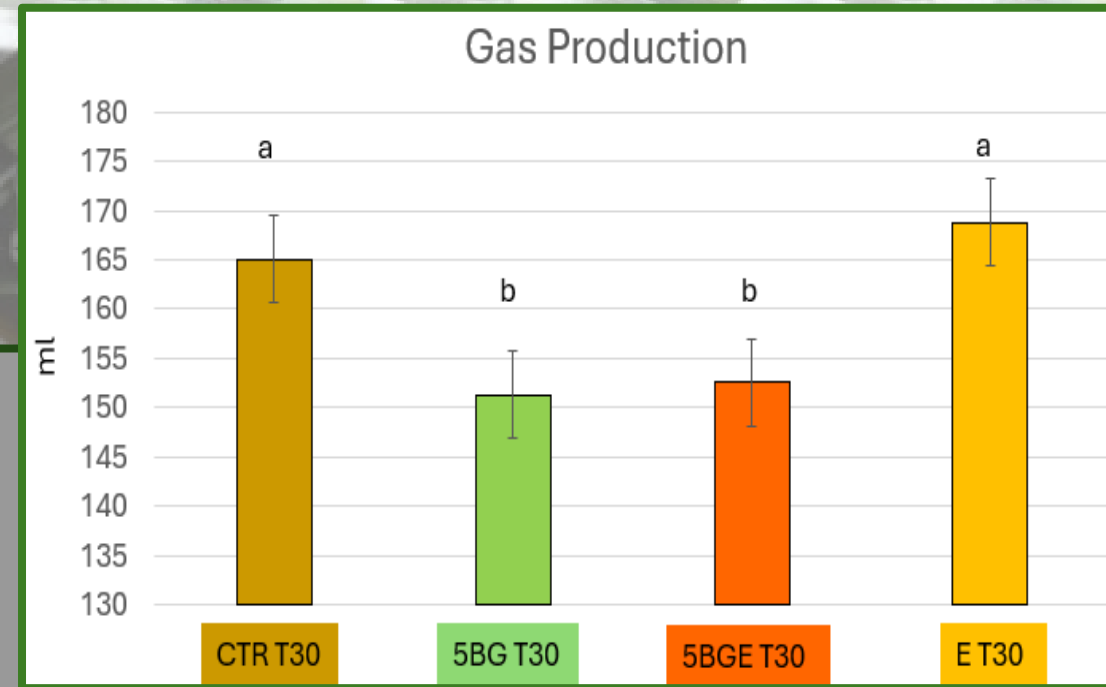
0.0 59.2
Mean proportion (%)

0.0 73.9
Mean proportion (%)

Welch's test for single-group comparison
N.S.= no significant differences between the groups

Conclusions: Chemical composition, *in vitro* disappearance, and gas production...

- pH reduction in all the silages;
- Differences in chemical composition of the silages;
- Similar performances of *in vitro* digestion;
- 5BG caused a reduction of gas production.

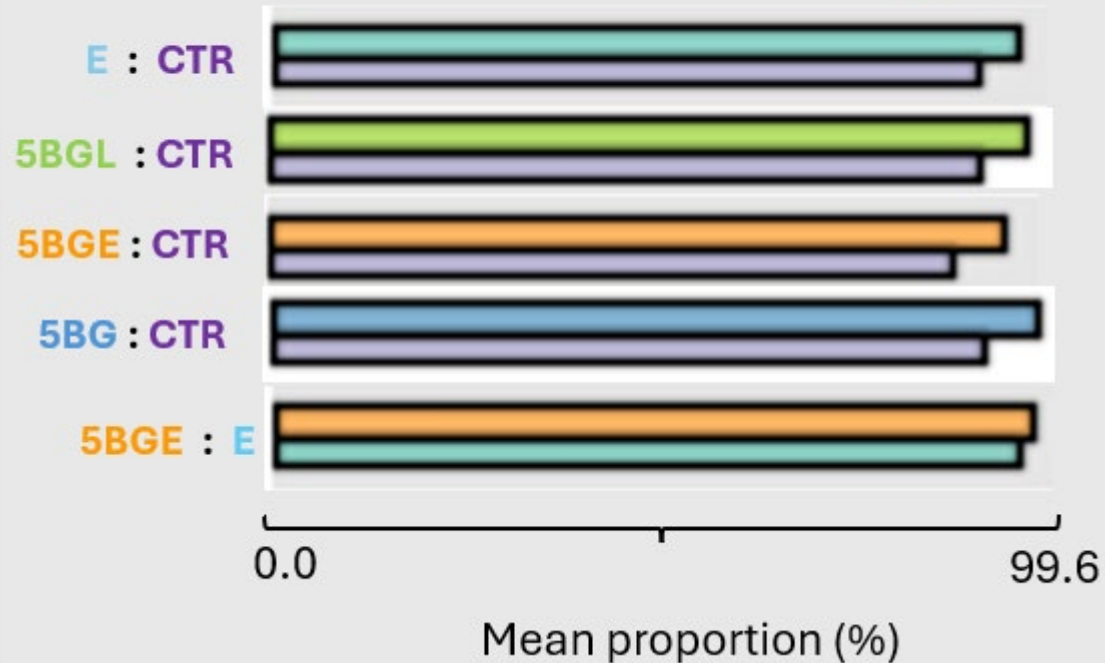


Conclusions: *Metagenomic profiles...*

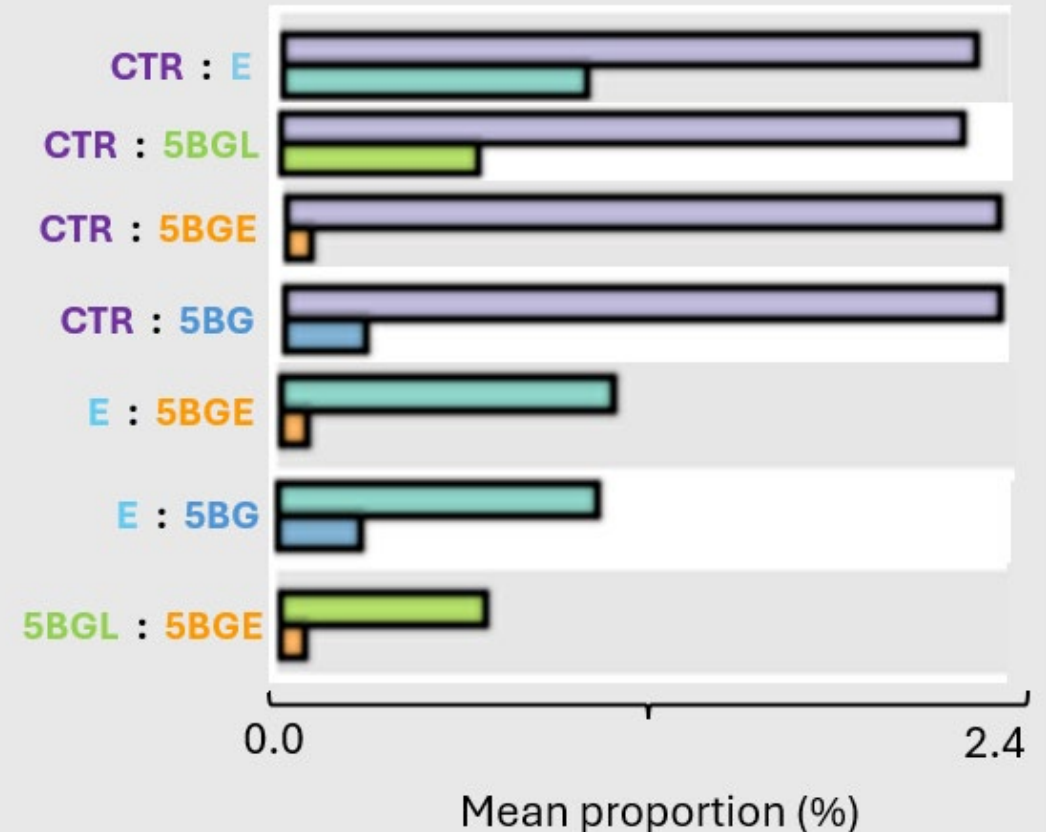
- The ensiling process was effective in the microbial stabilization of the artichoke by-products;
- Predominance of Lactobacillaceae in all the silages;
- The 5BG starter guaranteed a better control of Enterobacteriaceae up to 99%



Lactobacillaceae family



Enterobacteriaceae family



Thank you for
your attention



Acknowledgments

Consiglio Nazionale delle Ricerche



ISTITUTO DI SCIENZE DELLE
PRODUZIONI ALIMENTARI

Mulè Giuseppina;

Ferrara Massimo;

De Bellis Palmira.



UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

Greco Roberta;

De Palo Pasquale;

Maggiolino Aristide;

Giannico Francesco.