

Introduction

Salame Piemonte is a typical fermented sausage of Nord-West of Italy that is preserved by protected geographical indication (PGI). These kind of products constitute a significant part of the Mediterranean diet and have a long tradition originating from Europe. Their microbiota is specific of the region or area where they are produced and it is essential in the better management of microbial resources to protect the sensory characteristics of the product (Aquilanti *et al.*, 2007). With the aim of the selection of autochthonous starter cultures for this local fermented sausage, the ecology and microbial dynamics during the fermentation process of three different productions from the same factory were evaluated. The study of fermented sausages has been carried out by culture-dependent and independent methods in addition to volatile profile, chemicals and sensory analysis (Ferrocino *et al.*, 2018; Greppi *et al.*, 2015).

Materials and Methods

Sampling:

- 3 batch: February (Y), March (Z), May (X)
- 6 sampling times: T0, T4, T8, T15, T30, T50

Analysis:

- Culture - dependent analysis: LAB (MRS), CNC (MSA), Fungi/Yeast (AMT)
- Culture - independent analysis: V1-V4 region of the 16S rRNA (Illumina MiSeq)

Results



Fig.1: a. pH and aw measurements of the fermented sausages during maturation; b. Growth of LAB, CNC, yeast and fungi during the maturation process.

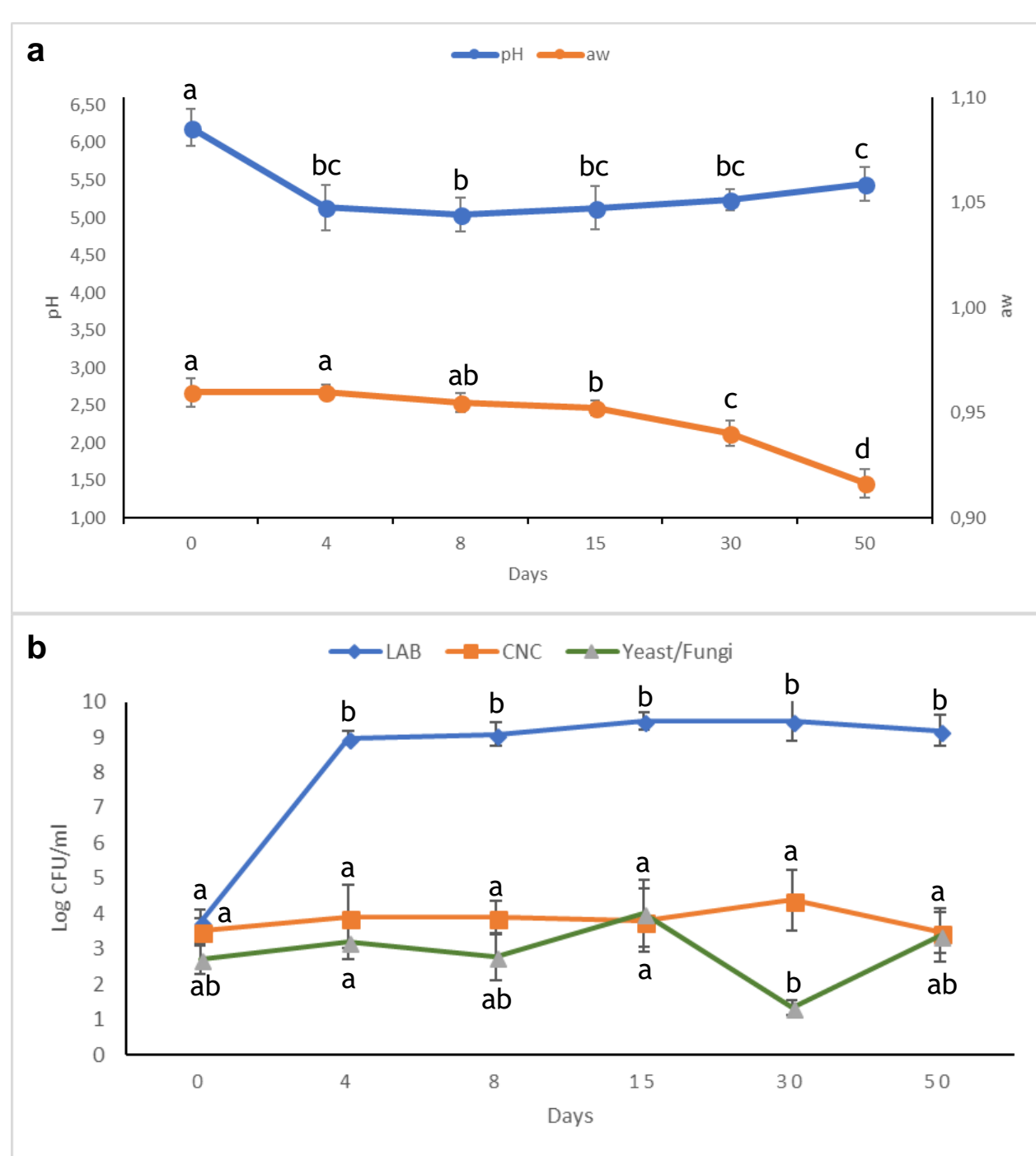


Fig.2: Relative abundance of the major taxonomic groups detected by means of 16S sequencing. Only OTUs with an incidence above 0.2% in at least two samples are shown

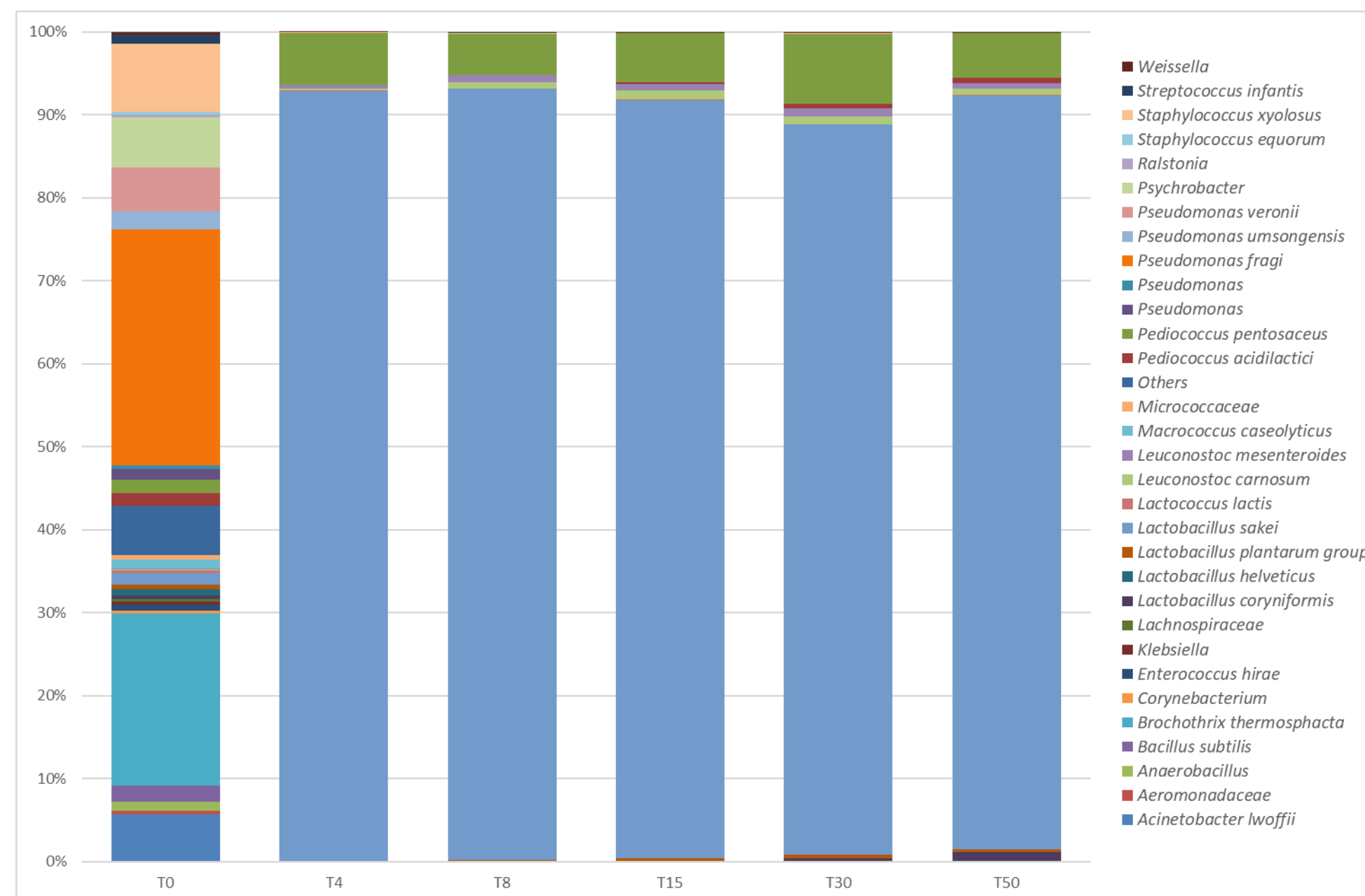


Fig.3: Principal coordinates analysis of weighted UniFrac distances for 16S rRNA gene sequence data. Samples are color-coded by batch: blue (Y; February), green (Z; March), red (X; May)

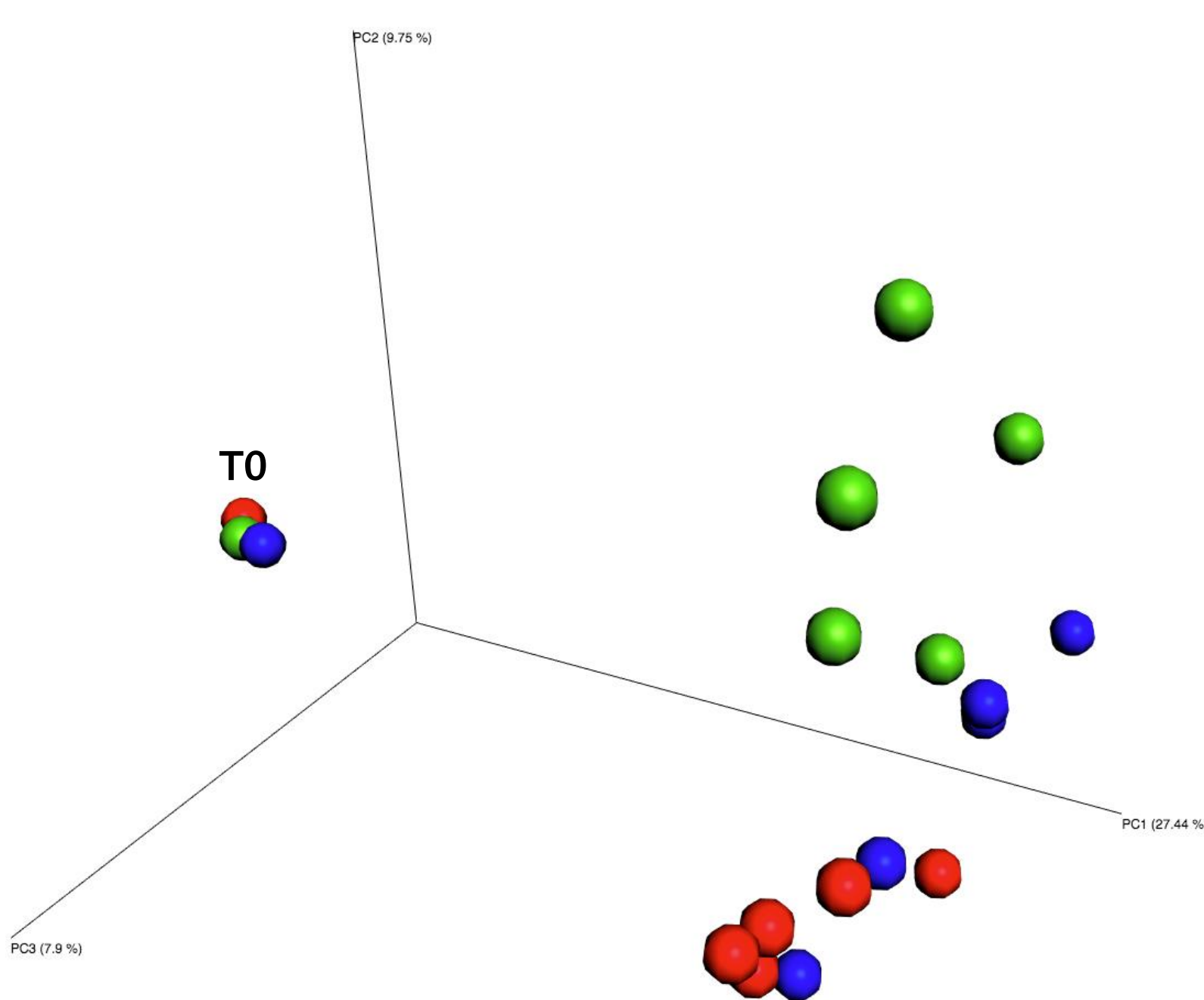
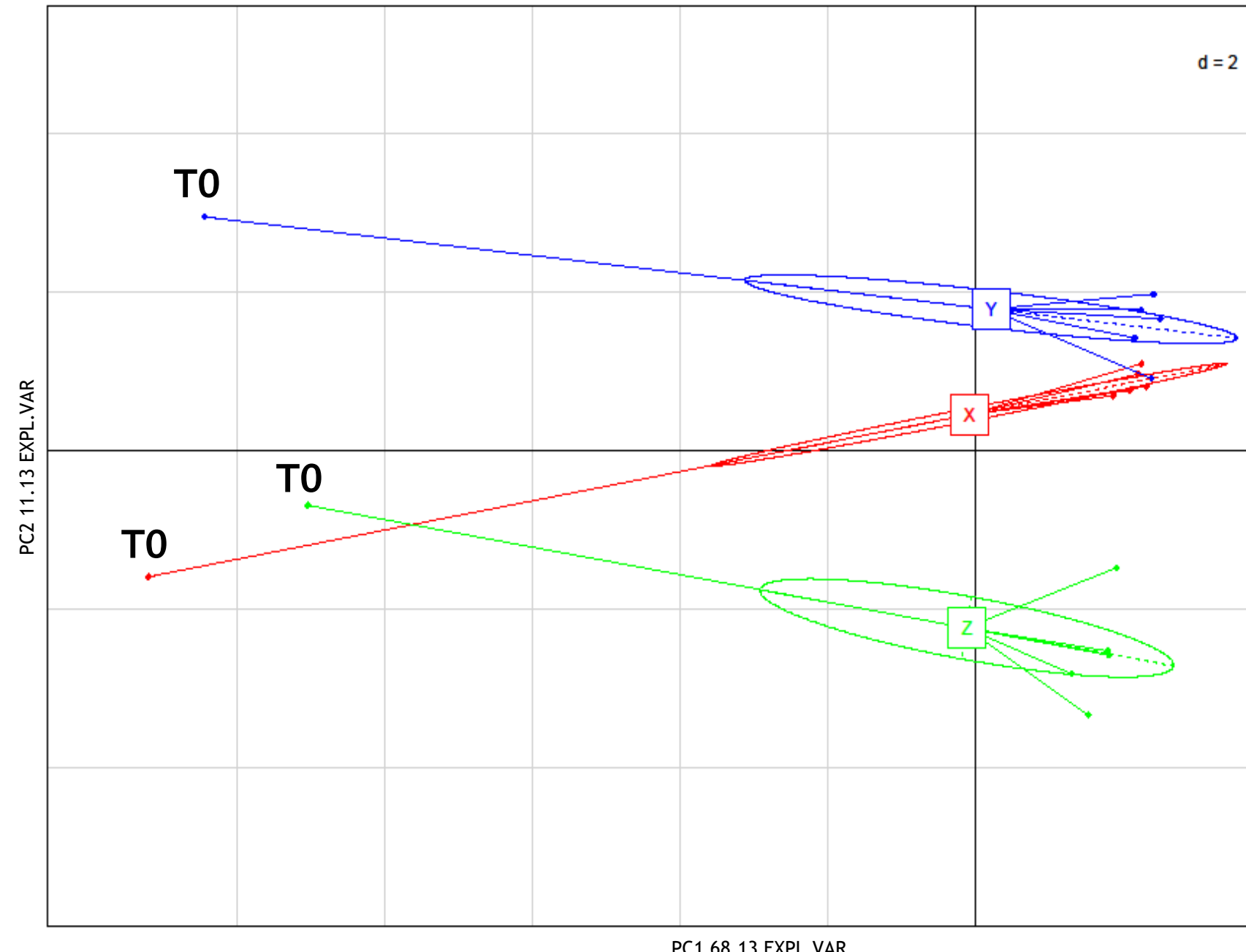


Fig.4: Principal-component analysis (PCA) based on the OTUs relative abundance of the three different batch: blue (Y; February), green (Z; March), red (X; May)



Conclusions...

- *Lb. sakei* was the most abundant OTU in all samples after 4 days until the end of fermentation, its abundance was never lower than 50%.
- Through principal coordinate analysis (PCoA) with a weighted UniFrac distance matrix, it was possible to show that meat samples at time 0 separated well all salami samples on the basis of the microbiota
- Through Principal-component analysis (PCA), it was possible to show that samples from batch Z grouped together and that they were well separated from batch X and Y on the basis of their microbiota
- Through predicted metagenomes, it was possible to show that T0 of all three batch has a similar pathways correlations

... and future steps

- With the aim of selection of new starter cultures, REP characterization for all isolated strains will be performed
- Shotgun metagenomics and volatile profile (with GC-MS analysis) will be performed to confirm the metabolic pathways and the microbial ecology

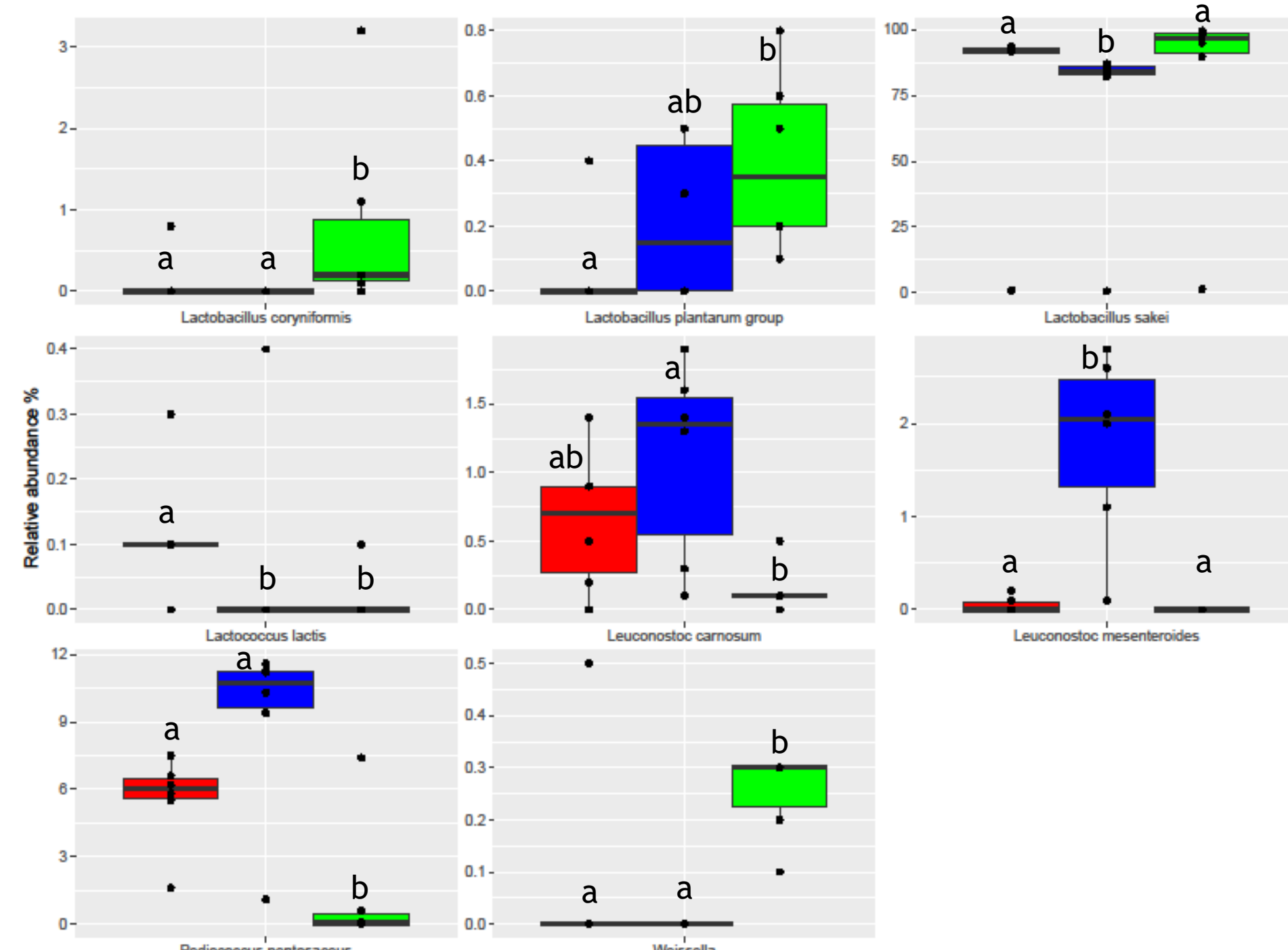
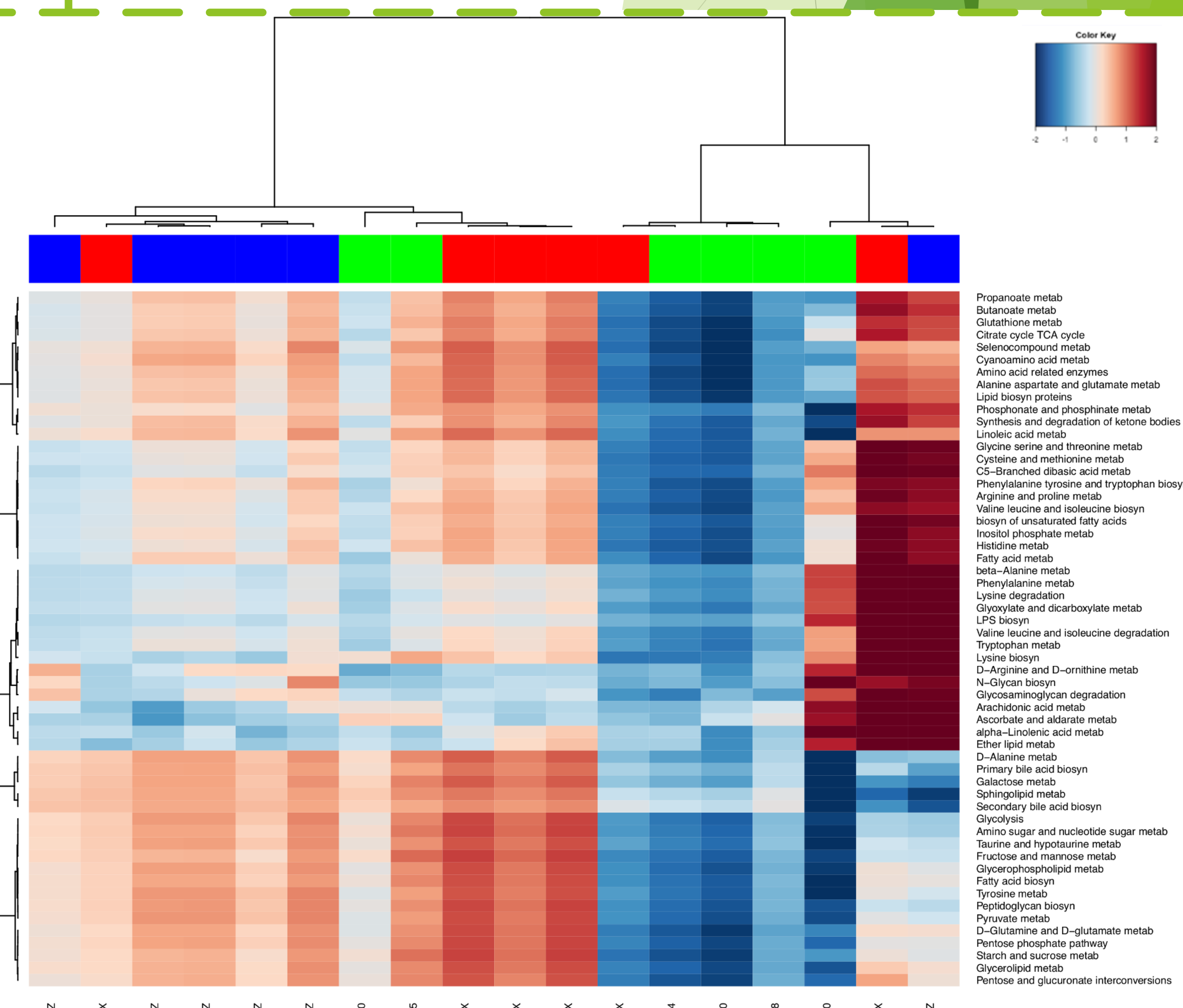


Fig. 5: Boxplots showing the relative abundance at species or genus level of the OTUs differentially abundant based on Wilcoxon matched pairs test ($p < 0.05$) in three different batch: blue (Y; February), green (Z; March), red (X; May)

Fig. 6: Heat plot showing Spearman's correlations between OTUs occurring at 0.2% in at least 2 samples and predicted metabolic pathways, filtered for KO gene sample presence >10 in at least 10 samples, related to amino acid, lipid AND carbohydrate metabolism. Rows and columns are clustered by Ward linkage hierarchical clustering. The intensity of the colors represents the degree of correlations between the OTUs and KO as measured by the Spearman's correlations.

- Blue = Z March
- Green = Y February
- Red = X May



References:

- Aquilanti L., Santarelli S., Silvestri G., Osimani A., Petruzzelli A., Clementi F. (2007). *The microbial ecology of a typical Italian salami during its natural fermentation*. Int. J. Food Microbiol. 120, 136–145.
- Ferrocino I., Bellio A., Giordano M., Macori G., Romano A., Rantsiou K., Decastelli L., Cocolin L. (2018). *Shotgun metagenomics and volatile profile of the microbiota of fermented sausages*. Appl. Environ. Microbiol. 84 (3), e02120-17.
- Greppi A., Ferrocino I., La Storia A., Rantsiou K., Ercolini D., Cocolin L. (2015). *Monitoring of the microbiota of fermented sausages by culture independent rRNA-based approaches*. Int. J. Food Microbiol. 212, 67-75.