

1. Introduction

Seafood and fishery products are very fragile commodities with a short shelf-life as the consequence of organoleptic and microbiological qualities quick deterioration. Spoilage, resulting from microbial growth and activity, is responsible for up to 25% of food losses in post-harvest and industry¹. In this context and to meet the consumer's demand for minimally processed food, developing mild preserving technologies such as biopreservation, represents a crucial challenge. In this work, we studied the impact of 6 lactic acid bacteria (LAB), selected by Wiernasz *et al.* (2017)², used as bioprotective agents on dill Gravlox microbial ecosystem, organoleptic properties and volatilome.

3. Results

3.5. Gravlox volatilome

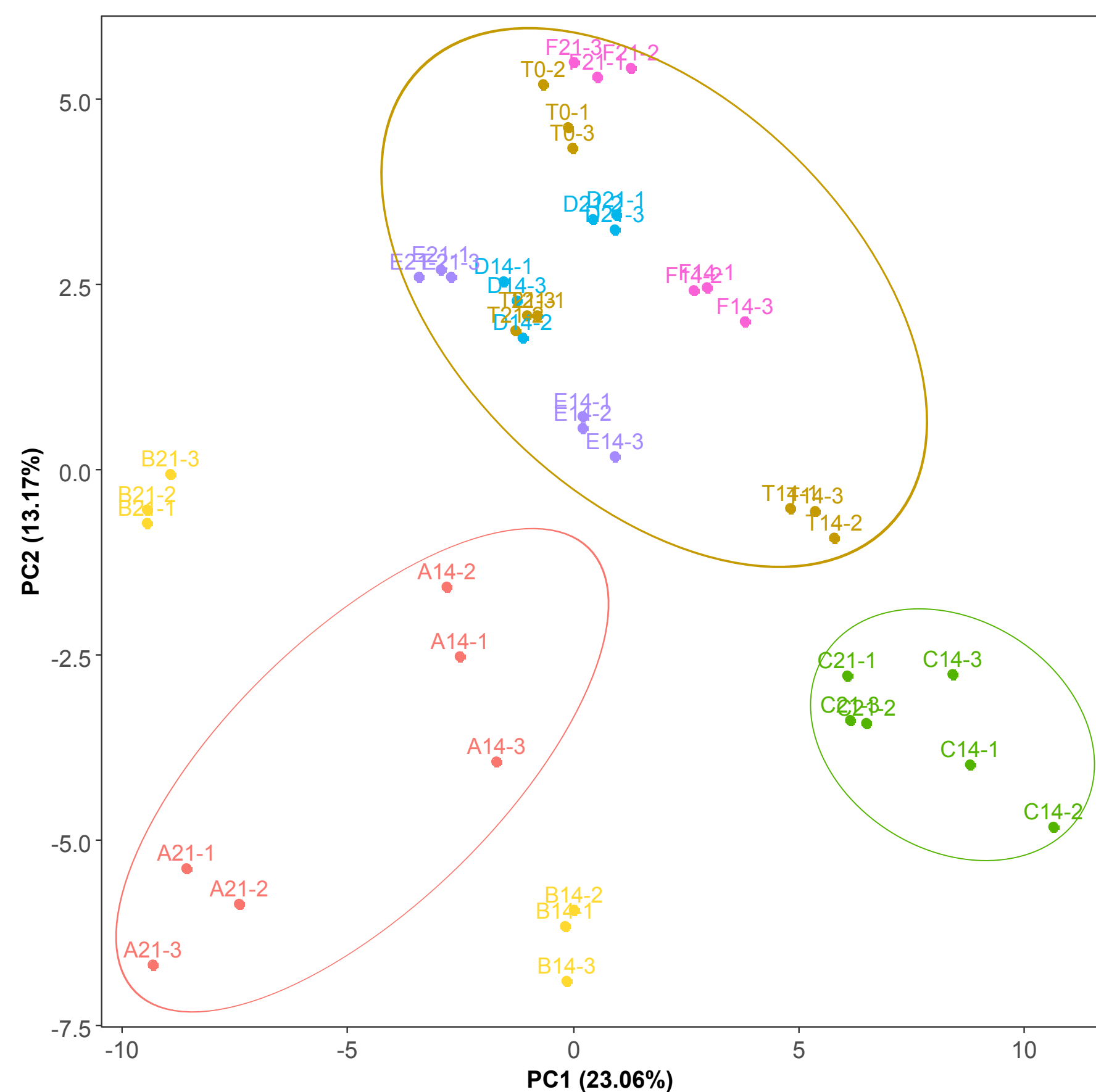


Figure 4. Principal component analysis (PCA) representation of dill Gravlox volatilome after inoculation with the 6 LAB. Volatile organic compounds (VOCs) were identified and quantified by HS-SPME/GC-MS.

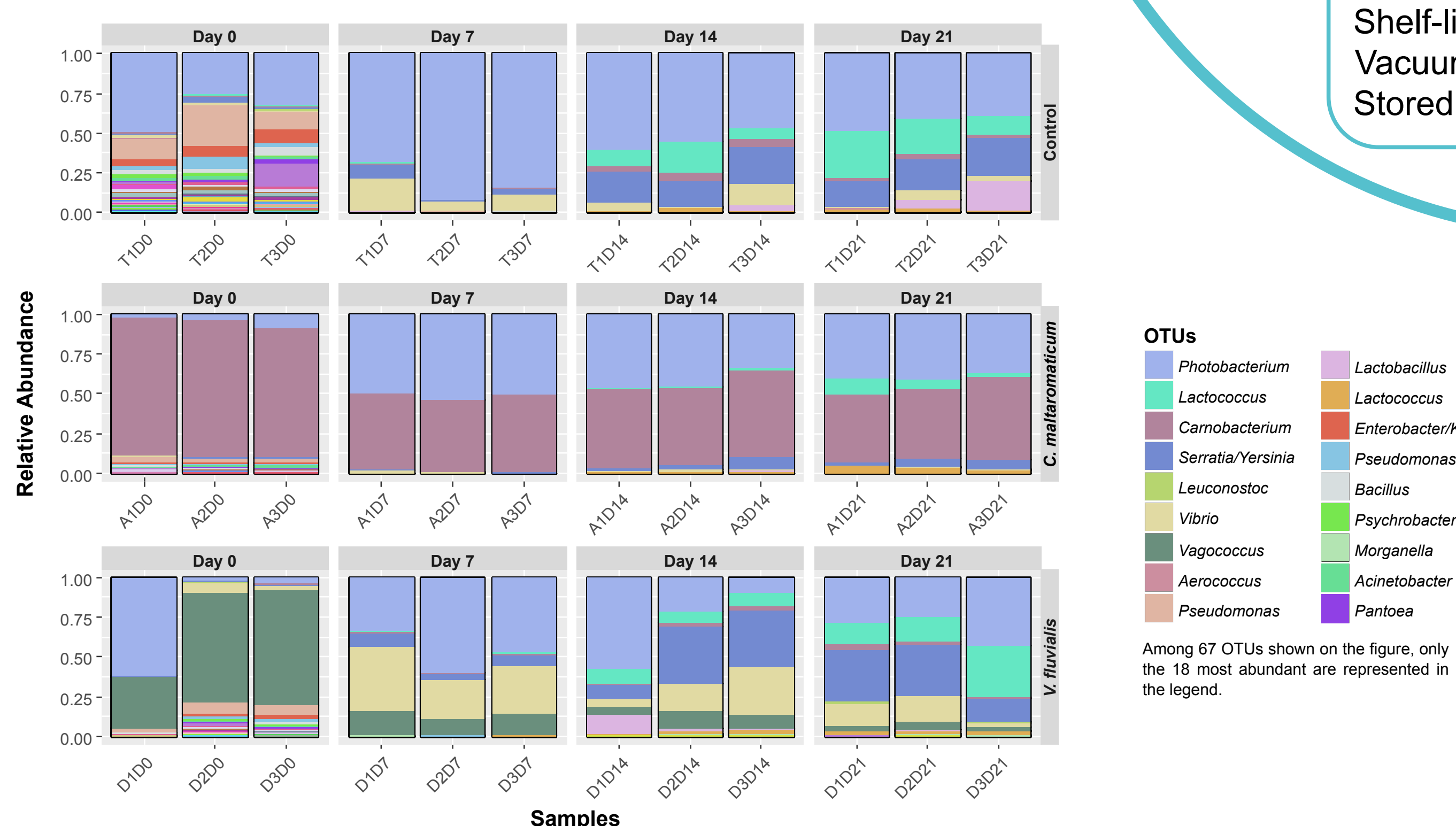
Strain
● Control
● *C. maltaromaticum*
● *L. piscium*
● *L. gelidum*
● *V. fluvialis*
● *C. inhibens*
● *A. viridans*

V. fluvialis, *C. inhibens*, *A. viridans* were closed to the control with a volatilome mainly composed by ethanol, ethyl acetate, acetic acid, hexanal, decane and dimethylamine.

- *C. maltaromaticum* was associated with VOCs such as, 3 and 2-methylbutanal (malt odor), 3-methyl-1-butanol and dimethylamine.
- In presence of *L. gelidum*, ethanol, acetic acid and many derivatives were strongly produced.
- After 14 days *L. piscium* were associated with the production of hexanal, pentanal, 1-penten-3-ol and dimethylamine; while after 21 days, volatilome was driven by high concentration in terpenes (limonene, sabinene)

3.4. Gravlox ecosystem monitoring through metabarcoding

Figure 3. Ecosystem evolution during storage. The hypervariable region V4 of the 16S rRNA gene was targeted³. Amplicons were sequenced with Illumina MiSeq technology and output data were treated with FROGS pipeline⁴. On bar plots of ecosystem composition, only figure results obtained for the control and samples inoculated with *C. maltaromaticum* and *V. fluvialis*.



Two patterns were observable:

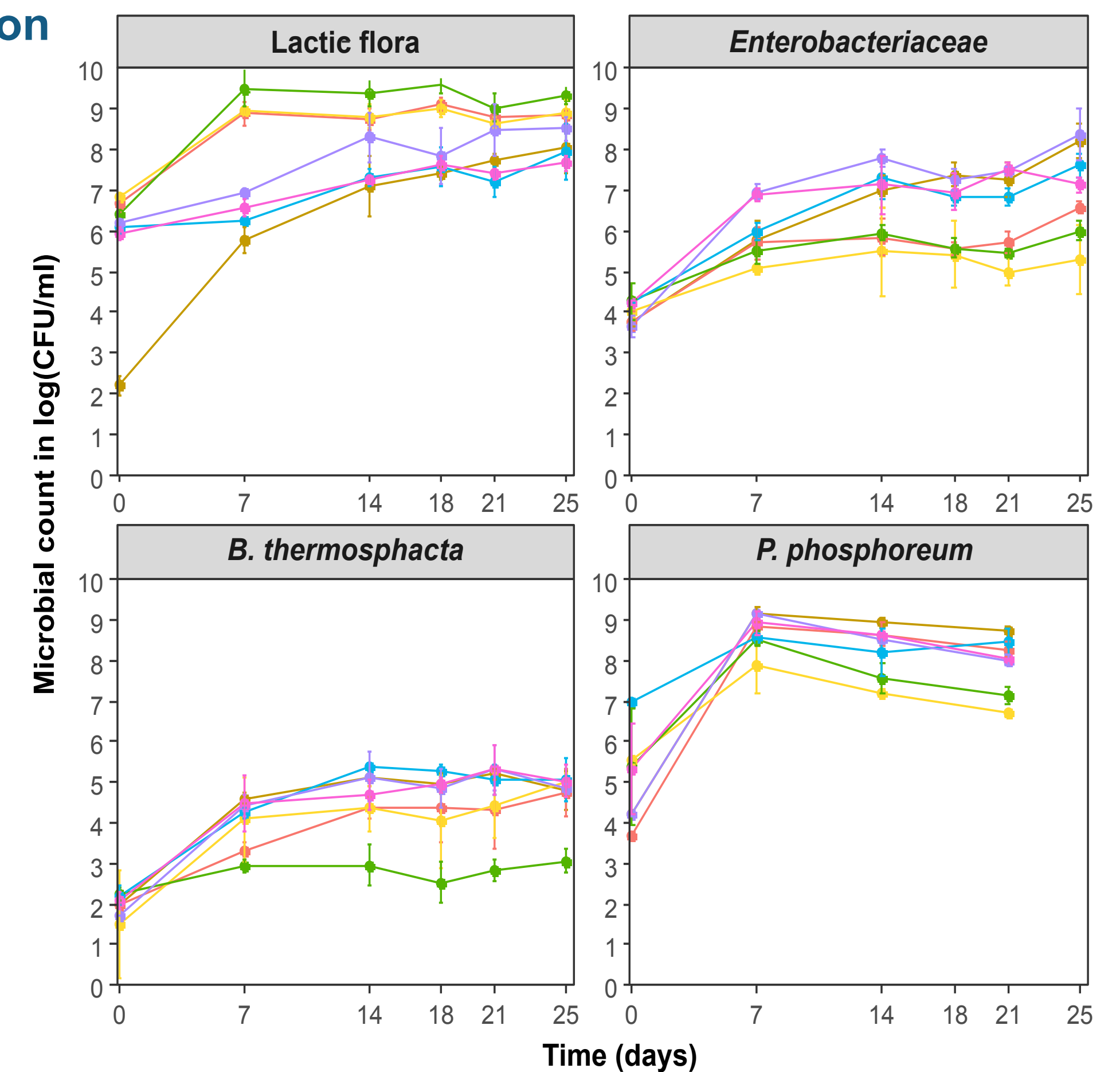
- *C. maltaromaticum*, *L. piscium*, *L. gelidum* were well implanted in the product and remain predominant during storage.
- *V. fluvialis*, *C. inhibens*, *A. viridans* were not competitive and became a minority after 1 week of storage (ecosystem closed to the control).

3.1. Microbial enumeration

Figure 1. Evolution of different microbial flora during storage, in presence of the 6 LAB. *B. thermosphacta* was enumerated on STAA, lactic flora on NAP, *Enterobacteriaceae* on VRBG and *P. phosphoreum* by q-PCR targeting the luxA gene.

Slight inhibition exerted by *C. maltaromaticum*, *L. piscium* and *L. gelidum* on main spoilage bacteria.

Strain
● Control
● *C. maltaromaticum*
● *L. piscium*
● *L. gelidum*
● *V. fluvialis*
● *C. inhibens*
● *A. viridans*



3.2. Biochemical analyses

- pH: quick acidification for *L. piscium* and *L. gelidum* (cooked aspect).
- No significant differences with the control for the 6 biogenic amines quantified (histamine, tyramine, cadaverine, putrescine, spermine, spermidine).
- Significant decrease in gravlox TVBN content in presence of *L. piscium*, *L. gelidum*, *C. inhibens* and *A. viridans*.
- No Trimethylamine production during storage.

3.3. Impacts on sensory quality

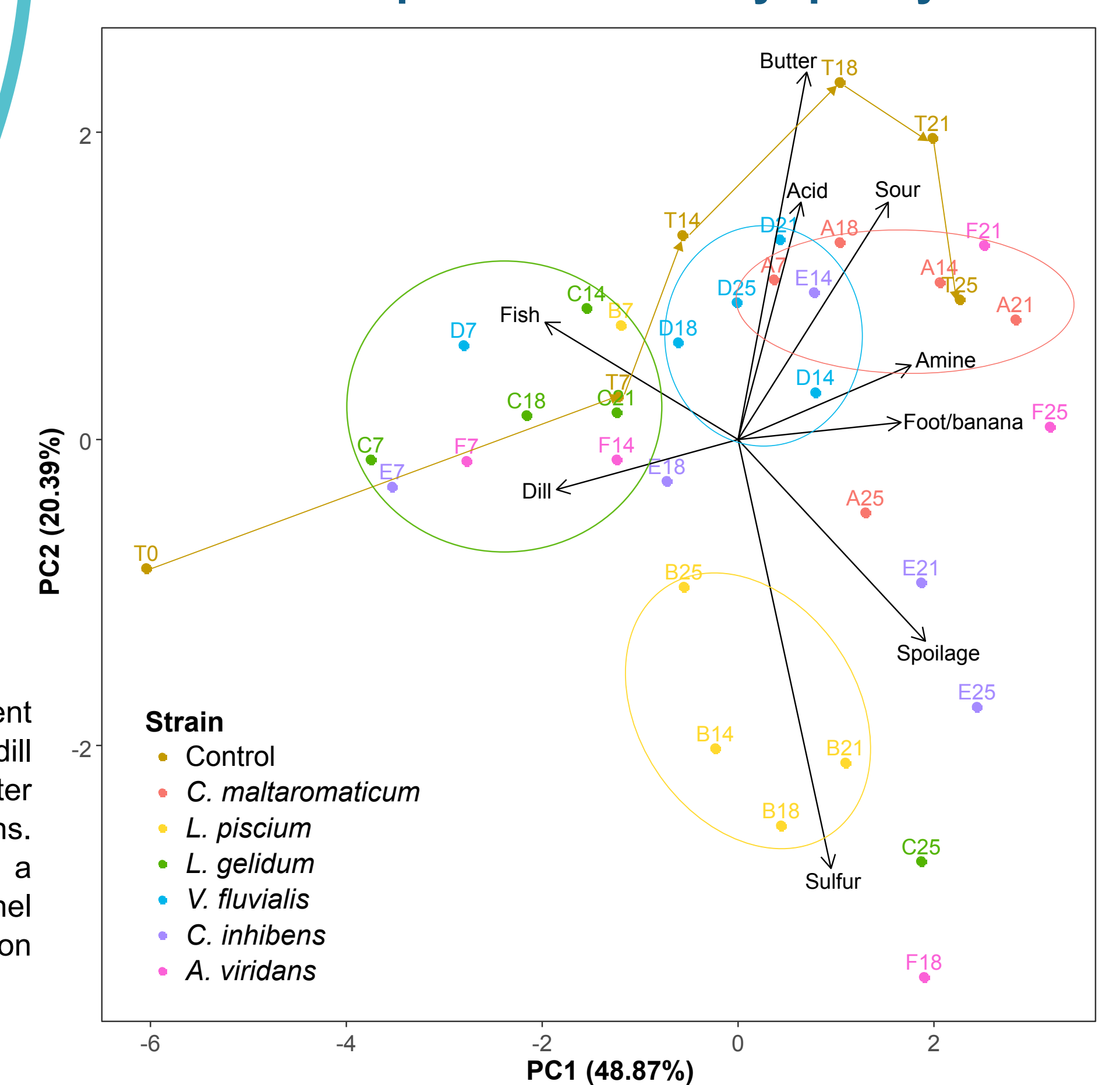


Figure 2. Principal component analysis (PCA) representation of dill Gravlox slices sensory profiles after inoculation with the 6 LAB strains. Odor descriptors were scored on a scale from 0 to 10 by a trained panel of 20 judges. PCA was performed on descriptors scores mean.

- Control was strongly spoiled after 21 days with mainly amine and acid odors.
- Although very close to the control, *V. fluvialis*, was the only strain able to improve and maintain the sensory quality to an acceptable level after 25 days.
- *C. maltaromaticum* was associated with foot/banana and sour odor.
- *L. piscium* related to a strong sulfur odor production after only 7 days.
- *L. gelidum* was a good candidate with dill and fishy odor until 21 days, but a production of gaz and slime was visible after only 14 days.

4. Conclusion

The 6 LAB effect on dill gravlox quality could be classified in two main scenarios:

- I) *C. maltaromaticum*, *L. piscium*, *L. gelidum* were competitive in the product by dominating the ecosystem till the end of the experiment, expressed antimicrobial activity against spoilage bacteria but also against *Listeria monocytogenes* (data not shown), and possessed their own sensory signature and volatilome specificity.
- II) *V. fluvialis*, *C. inhibens*, *A. viridans* were not well implanted at the gravlox surface, with a sensory and volatilome profil very closed to the control, and did not demonstrate antimicrobial activity. Nevertheless, *V. fluvialis* maintained the gravlox sensory quality below the rejection threshold after 25 days (control was rejected after 21 days).

Biopreservation of naturally contaminated products remains complex to apprehend and may further relies on metabolic interactions between microorganisms from an ecosystem more than antimicrobial activity.



5. References

1. Gram, L. & Dalgaard, P. Fish spoilage bacteria – problems and solutions. *Curr. Opin. Biotechnol.* 13, 262–266 (2002).
2. Wiernasz, N. et al. Lactic Acid Bacteria Selection for Biopreservation as a Part of Hurdle Technology Approach Applied on Seafood. *Front. Mar. Sci.* 4, (2017).
3. Caporaso, J. G. et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci.* 108, 4516–4522 (2011).
4. Escudie, F. et al. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinforma. Oxf. Engl.* 34, 1287–1294 (2018).

6. Acknowledgement

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