

Answer to reviewers: PCIMicrobiol #158

We would like to thank both reviewers for their careful review of our paper and their excellent suggestions for improvement.

We have shortened and clarified the text, which has been edited by Victoria Hawken, UK.

We have also redrawn several figures to improve their presentation and readability (Fig 2, Fig 3, Fig 4, Fig 5, Fig 7 and 8), and to better illustrate the main messages of our study.

The other changes are detailed below.

Review by Thibault Nidelet

This article is very interesting and overall well written. I recommend it for publication with minor corrections of the text and figures.

- **Title and abstract**

- Does the title clearly reflect the content of the article? Yes, No (please explain), I don't know
- Does the abstract present the main findings of the study? Yes, No (please explain), I don't know

- **Introduction**

- Are the research questions/hypotheses/predictions clearly presented? Yes, No (please explain), I don't know
- Does the introduction build on relevant research in the field? Yes, No (please explain), I don't know

- **Materials and methods**

- Are the methods and analyses sufficiently detailed to allow replication by other researchers? Yes, No (please explain), I don't know
- Are the methods and statistical analyses appropriate and well described? Yes, No (please explain), I don't know

Some minor justifications have to be justified. They consider the different salt concentration as equivalent replicates for other factors as there is no salt effect. That is not the most common practice but possible. It should be better justified.

- **Results**

- In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? Yes, No (please explain), I don't know
- Are the results described and interpreted correctly? Yes, No (please explain), I don't know

- **Discussion**

- Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? Yes, No (please explain), I don't know
- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? Yes, No (please explain), I don't know

Here is the list of my comments.

Line 45 to 47: this phrase could be reformulated : Maximal Enterobacteriaceae counts were higher in cabbage (8 vs 7 logCFU/g), while lactic acid bacteria counts were higher in carrot (9 vs 8 logCFU/g).

Answer: done; we also shortened the revised version by removing from the initial text all carrot and cabbage comparisons, according to the other reviewer's recommendation, and consistently with what we stated in our discussion: "However, the experiment was not designed to address the comparison of carrot and cabbage in itself".

85 is "legumes" the good word. For me "legumes" correspond to soybean, faba bean, lentil, broad bean, etc and not cabbage or carrots.

Answer: corrected, thank you

Line 103 to 105: this sentence "The environmental aerobic or facultatively anaerobic microorganisms first grow and are progressively replaced by a succession of heterofermentative and then LAB" need a reference

Answer: three references were added: (Pederson & Albury, 1969; Buckenhueskes, 2015; Thierry, Baty, et al., 2023).

Line 117 to 119: this sentence "In a recent study, carried out on 75 samples produced at a domestic scale, the age of samples ranged from 2 weeks to 4 years with a median value of 6 months. 84 % of analysed samples still contained alive LAB" need a reference.

Answer: one reference (Thierry, Madec, et al., 2023) added. Please note that several other references have been added in the introduction section (see the answer to reviewer 2).

Last paragraph of the introduction, you speak about a slight reduction of salt but from 1% to 0,8% it is a decrease of 20% that is not a small reduction, even if the total concentration of salt is small

Answer: You are right, thank you. We suppressed the mention "slightly different", leading to the following sentence: "We also studied two salt concentrations, a concentration of 1%, which is the minimum concentration of salt normally used, and, with a view to further reduce salt rates to follow health recommendations, a concentration of 0.8%."

Methods :

Why have vitamins not been sampled à T3 ? Figure 1 the corresponding time of the sample could be added to the figure and not only indicated in the legend

Answer: The asterisk (*) corresponding to vitamin analysis in Fig 1 was erroneously placed in the initial version. As indicated in the M&M section, vitamins were analysed at T0 and/or T3. Times corresponding to T1, T2 etc were also added. Fig 1 has been corrected in the revised version.

Line 204 "either" implicates a second option

Answer: modified, thank you

Line 284 "to visualise barplots" which barplot do you speak off ? I think Phyloseq has also been used to calculate the abundances

Answer: The line was changed to "to calculate relative abundances"

Line 369 as the different vitamins were not measure at the same time, it will be important to precise it in the Fig 1

Answer: The *corresponding to vitamin analysis in Fig 1 was erroneously placed, as indicated in the M&M vitamins were analysed at T0 and/or T3. Fig 1 has been corrected in the revised version.

Lines 388 the used packages have to be cited with a specific reference

Answer: Sorry for omission to cite R package authors. The following references were added in the material and method section: (Lenth, 2024) for emmeans package, (Lê et al., 2008) for FactoMineR package, McMurdie 2013 for phyloseq (v1.44) R package, Gu et al, 2016 for ComplexHeatmap (v2.16.0) R package, and Rohart et al, 2017 for R package mixOmics.

Line 395 why do you take out the factors that were not significant from your anova ?

Answer: We initially chose the option of omitting the non-significant interactions and factors from the ANOVA model, if relevant, for each variable studied, so as to simplify the model and increase the degrees of freedom of the residuals, in an attempt to increase the statistical power of the model.

Following your comment, we have systematically compared the p-values of factors in the complete and in the simplified ANOVA models. We observed that the p-values were very similar in both models and, consequently, that the same conclusions could be drawn concerning the effect of factors. We have therefore decided to keep the complete model for all variables.

The results of the ANOVAs have been added in a supplementary Table (S3), and are cited in the text (lines 422, 439, 517).

Accordingly, we suppressed the sentence "The factors and interactions that were not significant (p -value > 0.05) were further removed from the model" from the revised M&M section.

Line 400 it will be interesting to add the total number of variables used in the PCA, its help to analyse de percentage of variance explained by the PCA's axes

Answer: the number of variables is now indicated in the M&M section (line 402), in the result section (line 552) and in figure 5 legend.

Results

Line 420 "were significantly impacted by the vegetable studied, the fermentation stage, and, to a lesser extent, the cutting type." Add the corresponding p-values of these tests in the text of in a table

Answer: As explained above, the p-values of ANOVA have been added in the supplementary Table S3 and cited in the text in the two first result paragraphs (lines 423, 441, 519, 529).

Line 422 "Therefore, four replicates instead of two were available at each sampling point for statistical analyses." This is due to the suppression from the model of the NaCl effect and considering the different NaCl concentrations as equivalent replicates. It have to be justified or at least specified here or in material and methods

Answer: as for the ANOVA model, please see above the explanations given to the comment L 395.

In figures 2 and 4, we chose to keep the same representation in the revised version. It was explained in the material and method section (Lines 396-398) and in the legend of these figures, as follows: "Values are means of the results observed in two to four independent jars, [...], where the four values corresponded to the duplicate jars at the two salt concentrations, gathered because either no (cabbage) or limited (carrot) effects of salt concentration were observed.

Figure 2 : adding the indication of sampling numbers T1, T2, T3, etc. in the graph will be interesting as they are used in the text. In the text you explained that the sampling times were not the same in the different medium as the speed of fermentation was different. However in the graph they appear identical ? The time unity is not indicated. The order of the panels don't follow the order of the text that is enterobacteria, LAB and pH. It will be better to have the same order in both the text and the graph

Answer: Figure 2 has been modified as follows:

- The T1, T2 etc labels and X-axis legends were added, the order of panels modified to follow that used in the text.
- The times of sampling slightly differed at T2 stage only (2.7 days for carrot and 3.6 days for cabbage, as stated in Figure 1 legend). Supplemental times T2a (carrot) and T2c (cabbage) and the corresponding labels were also added in Fig 2.
- The time-course of titratable acidity was also added in Fig 2.
- Moreover, lines and columns were inverted, as suggested for Figure 4, to facilitate comparisons between rough and thin cuttings.

Line 458 : add the fact that 7 months is not represented in figure 2

Answer: done (cf Lines 422, 436).

Lines 469 : remind what is TTA

Answer: done (line 432)

Lines 496 to 500 : as for yeast there is no statistical information indicating any effect

Answer: Statistical information was added (lines 485-486)

Figure 3 : As you have two jars you can calculate averages and variances of concentrations and therefore add error bars on the graph

Answer: We chose to keep the same representation that shows the results for each duplicate jars, because it is a way of illustrating the variability from one jar to another. In addition, the high variability between replicate jars would have made figure 3 difficult to read.

The presentation of figure 3 was however improved using R instead of Excel. Moreover, the high variance between replicate jars would have hamper legibility

Line 518 to 532 no indication of statistic tests

Answer: Statistical information was added (lines 519 – 532).

Line 539 figure 4 is not in bold

Answer: done. In the first version, we used bold only for the first citation of figures and tables, but, in the revised version, we have used it systematically.

Figure 3 : in the same manner that the type of cutting the salt concentration could be add in the upper tree

Answer: done; As stated above, figure 3 was improved using R instead of excel, and vegetable, cutting type and salt concentration added above.

Figure 4 as you compare the different types of fermentation more than the time of fermentation inverting the lines and the column will help to the main comparison. Time unity is not indicated

Answer: Figure 4 has been modified in a similar way to Figure 2: The T1, T2 etc labels and X-axis legends have been added, the order of panels modified to follow that used in the text, and lines and columns have been inverted, as suggested. This change effectively facilitates comparisons between roughly- and thinly-cut samples.

You could also superpose the four graphs with different colors. That is also possible for Figure 2.

We also tried to superimpose the variables (see below). The figure is acceptable for carrot, but not readable for cabbage. Therefore we have not kept this option.

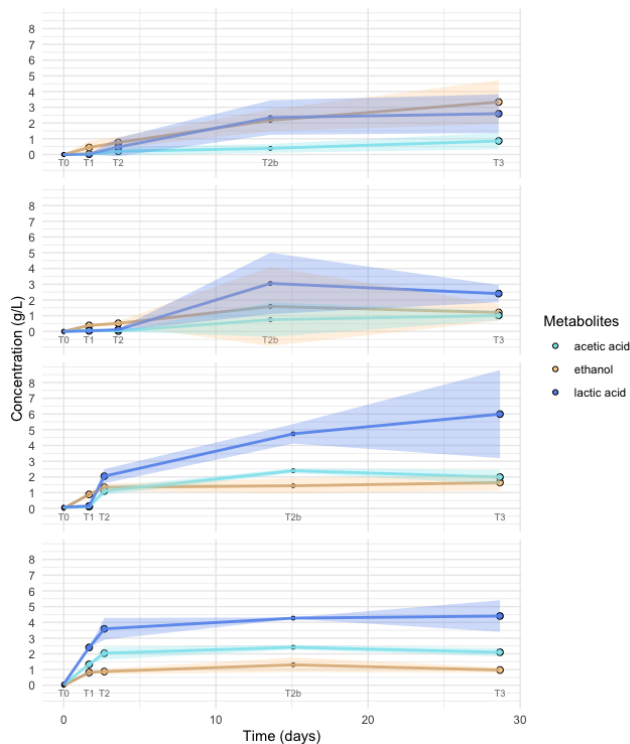


Figure 5 : is often better to do the PCA on the averages and not on the replicates, in particular for reasons of legibility. You have to add indicators for the three different panels like A) individuals graph, B) variables graph and C) confidence zones for different factors. In the last panel it will be better to have different color pallets for the type of factors. Here green been T2, S1 or cabbage thin depending on the three graphs.

Answer: Figure 5 has been redrawn to better illustrate the message of our paper, as suggested by the second reviewer. Figure's legend has been completed and the presentation of PCA individual graph modified to better illustrate the time-course of changes over fermentation of the vegetables and their cutting type. I unfortunately did not succeed in modifying colours in panels 5C and 5D

Line 575 yo 578 : it will be interesting to make the link between these separation and the statistical effects of the different factors previously shown

Answer: We have made the link between the results presented in Figures 2 and 4 and PCA results, in the rewritten paragraph that describe PCA results. Please see lines 556-563; 572-577.

Line 596 to 597 : add the fact that this minor metabolites have been not used to calculate the PCA's axes, supplementary variables is not very clear by itself

Answer: thank you. This detail has been added in the legend of Figure 5B.

Line 607 "27, 15, and 10 volatiles" : it not clear at what the numbers make reference to

Answer: this sentence effectively needed to be clarified, thank you (lines 603-607)

Figure 6 : the graph of the variable is important to interpret the PCA, if there is to much variables you can plot only the 20 most important variables for the PCA construction. You can make filter for that and the FactoMineR package. Add indication of the panel A and B for more clarity

Answer: Panels names were added in Fig 6. Graphs of variables were added in supplementary figures S1 and S2.

Line 630 : same remark than for the line 607, it could be rephrased for clarity

Answer: done (paragraph lines 622-630)

Lines 667 to 669 : add the corresponding p values

Answer: p-value has been added (line 648)

Table 1 add the standard deviation to the average in the table as well as letter* indicating the significant difference between samples

Answer: Table 1 was clarified as suggested and letters added to

Figure 7 : increase the size of the label especially for the sample of the first heatmap. I will split the two heatmaps in two separate figures. Here the A and B indicating the two panels are absent

Answer: the size of labels has been increased and (previous) Fig 7B was transferred in supplementary data (now Supplementary Figure S3)

Lines 767 yo 769 : can you give examples of the differences between duplicate jars

Answer: we added examples, as follows "Furthermore, it is worth noting that there were pronounced differences in taxonomic profiles between the duplicate jars (coded a and b). For example, the abundance of the genus *Leuconostoc* detected with the gyrB marker was much higher in carrot at T1 in replicate 421-b-T1 than in 421-a-T1; the abundance of the genus *Lactiplantibacillus* detected with the 16S marker was much higher in carrot in replicate 411-b-T3 than in 411-a-T3. In the case of cabbage, differences were particularly marked in roughly-cut cabbage. For example the abundance of the genus *Enterobacter* detected with the gyrB marker was much higher in replicate 331-a-T3 than in 331-b-T3." (lines 742-749)

Moreover, we have also highlighted the differences between duplicate jars at several other places in text, e.g. lines 488-489 for yeasts, lines 517-518 for metabolites (“There were large differences, mostly quantitative, were observed at T3 between the duplicate jars”), and in the comments of PLS-DA lines 782-785, and this point discussed (Lines 841-864)

Line 796 figure 8 is not in bold

Answer: done

Figure 8 : the labels are too small to be read. Use A and B to indicate the two panels. The legend is incomplete as you only speak of the variable in brown and not the other one.

Figure 8 was improved and the legend has been modified as follows:

“Multiblock PLS-DA results for cabbage (A, B) and carrot (C, D) samples at stage T3 (one-month fermentation). The left-hand panels (A, C) show the alignment of samples in the latent space, where each round point represents the centroid of all datasets for a given sample, and the arrow tips indicate the sample's position within each block. The blocks are colour-coded as follows: blue for 16S rRNA gene taxonomic data, red for microbial counts, green for gyrB taxonomic data, orange for metabolites, and yellow for volatiles. The right-hand panels (B, D) present correlation circle plots showing the relationships between variables as scatter plots, with variables coloured according to their respective blocks. The microbial count variables correspond to seven targeted microbial groups: lactic acid bacteria (LAB), total aerotolerant bacteria (tot_aero_bact), halotolerant bacteria, aerotolerant Gram-negative bacteria (tot_aero_Gneg), yeasts, bile-tolerant Enterobacteriaceae (enterobacteria), and enterococci.”

Line 785 to 833 it will be interesting to put more in light the new results shown by the Partial Least Squares-Discriminant Analysis (PLS-DA) compared with previous analyses. Why this additional analysis?

Answer: Thank you for your valuable feedback. We appreciate the opportunity to clarify the rationale for using PLS-DA in our study.

In the previous result sections, we have shown that the fermentation rate differs according to the type of cut, suggesting a possible effect on fermentation dynamics. However, in this study we wanted to investigate whether these differences extend to the fermentation profiles after one month of fermentation, or whether they merely reflect variations in the fermentation rate. To address this, we used multi-block PLS-DA, a powerful method for integrating different data sets - metataxonomic profiles, metabolite concentrations, viable counts and volatile compounds - and systematically identifying patterns across data blocks. This approach allowed us to investigate whether cutting type and salt concentration resulted in different final fermentation signatures, and to identify the variables driving these differences.

Our results confirmed that cutting type influenced not only the rate but also the final fermentation profile, with thinly cut cabbage characterised by higher concentrations of lactic acid, acetic acid and mannitol, as well as increased abundance of *Leuconostoc*. In contrast, salt concentration did not produce a clear discriminant signature at T3. By identifying the variables most strongly associated with these differences, PLS-DA provided important mechanistic insights into how cutting type shapes fermentation. This level of integration and detail could not have been achieved with univariate analyses alone. We hope this explanation clarifies our methodological approach.

We performed the following changes in the text:

-M&M section, lines 412-413: “This method was chosen for its ability to model heterogeneous, multi-block data, allowing for the identification of the best variables that discriminated the samples.”

-Result section, lines 758-761: “In earlier sections of this paper, it was shown that the fermentation rate varied by cutting type and, to a lesser extent, by salt concentration. Additionally, it was important to investigate whether the fermentation profiles still differed or eventually converged at one month. A multi-block Partial Least Squares-Discriminant Analysis (PLS-DA) was therefore performed {...}.

-Result section, lines 790-793: "Therefore, carrot profiles did not exhibit a strong discriminating signature by cutting type across different blocks (except for gyrB and metabolites), as convergence between samples with different cutting types occurred at one month."

Discussion

Lien 842 "slight reduction" 20% is not slight

Answer: slight compared to the variations occurring in domestic products. Changed into 20%-reduction throughout the text

Lines 856 to 858 "As a direct consequence, quadruplicate samples instead of duplicate samples were available at each stage to investigate the effect of the other factors examined, i.e. the cutting and the fermentation stage." That way of using statistic is very specific and has to be better justified

Answer: This sentence was suppressed from the discussion section. Hence, we chose to keep all the factors in the ANOVA models, as explained above (cf. comment on L395). In Figures 2 and 4, however, as specified in the legend, "Values are means of the results observed in [...] independent jars, [...] the four values corresponded to the duplicate jars at the two salt concentrations, gathered because either no (cabbage) or limited (carrot) effects of salt concentration were observed."

Line 861 the intra-jar variation is not specifically shown by any of this graph. A specific graph in supplementary information could be interesting showing the value for each sample rather than the average and confidence interval

Answer: Examples of variations between replicate jars can be seen:

-in Figure 3 for the three main metabolites: for more clarity we specified in the legend "in two replicate jars coded a and b".

-in the PCA Figure 5 in which all observations were kept

- and in the heatmaps of Figure 7 and Supplementary Figure S5 that illustrate metabarcoding results

We also clarified that point in the discussion section, by using the term "duplicate jars" instead of "replicates" (cf lines 846-849: "For example, yeasts were detected in only one of the duplicate jars in different samples (leaf cabbage at 40 h fermentation, sliced carrot at one month of fermentation).") Variability between replicate jars was also evidenced from the metabolite profiles (Figure 3) and metataxonomic profiles (Figure 7).

Line 1173 "DOI of the webpage hosting the supplementary information" has to be removed.

Answer: done

2nd reviewer Kate Howell

The authors have described extensive experiments aimed to understand the result of slicing width on the fermentation dynamics of fermented vegetables. The paper describes the rationale for the experiment which arose from a previous study from the same group which was a citizen science program to understand composition of fermented vegetables made in the home. The paper describes a single experiment that has been comprehensively studied with a range of methods. This is a drawback to the paper, as we don't know if the same conclusions would be reached with cabbage or carrots of a different origin. The paper is generally well presented but needs work on expression to improve clarity and flow of the manuscript. The methods are comprehensively described and the information presented is complete. The methods encompass traditional plate counts to identify microbes of interest as well as culture independent methods. The results are presented sequentially and have a lot of detail- I suggest too much as detailed below. Subheadings in the results could be used for great effect to highlight the main results you would like the reader to understand. The results are

long and a little repetitive- I would prefer better summaries so that your main points are highlighted. I can see a nice manuscript, but I feel that the presentation and data representations are not sophisticated and succinct. The information is interesting, but there is a tendency to display everything found, rather than a careful presentation of relevant results and their discussion. I suggest a review of language usage to clean up expression and remove erroneous words. Your paper will be more impactful and useful if this advice is followed.

[Answer: We would like to thank you for your careful review of our paper and your excellent suggestions for improvement.](#)

[We have also shortened and clarified the text, which has been edited by Victoria Hawken, UK. We have added informative subheadings in the results section to help the reader follow the main results.](#)

[We have redrawn several figures to improve their presentation and readability \(Fig 2, Fig 3, Fig 4, Fig 5, Fig 7 and 8\) and to better illustrate the main messages of our study.](#)

Major points

Title. Your expression is slightly confusing for me. Do you mean that ‘the cutting rate of vegetables influences the rate of spontaneous fermentation?’. Having fermentation/fermented twice in the heading makes it sound like the vegetables are fermented and then cut.

[Answer: Thank you for this comment and suggestion, we modified the title as follows: “The cutting type of vegetables influences the spontaneous fermentation rate”](#)

Introduction.

- There are some unreferenced claims in the introduction that should be considered. For example, line 95 ‘Fermentation is most generally spontaneous and due to an endogenous lactic acid bacteria (LAB) community’ does not have a reference but relates very closely to the outcomes of your paper given the wide variation you found in the results.

[Answer: references have added in the introduction section \(Thierry, Madec, et al., 2023\)\(Thierry, Baty, et al., 2023\) \(Buckenhueskes, 2015; Ashaolu & Reale, 2020\) \(Rezac et al., 2018\) \(Pederson & Albury, 1969;](#)

- I’m not clear on your rationale- what have your plate counts shown you that the amplicon sequencing doesn’t show? “it is crucial to combine cultural methods with culture-independent methods such as 16S metataxonomics or shotgun metagenomics, to better understand the dynamics of the microbiota of fermented vegetables.”

[Answer: To introduce the two microbial approaches used, the initial sentence of the introduction section “Given the microbial changes over fermentation time, it is crucial to combine cultural methods with culture independent methods such as 16S metataxonomics or shotgun metagenomics, to better understand the dynamics of the microbiota of fermented vegetables”](#)

[has been modified as follows:](#)

[“Culture methods and culture-independent methods such as 16S metataxonomics are complementary as each method contributes specific information and potential biases \(Parente et al., 2022\). Culture methods enable quantification of the living share of the cultivable microorganisms present, while, by contrast, culture-independent methods provide access to all the microorganisms present in the sample, whether or not they are viable at the time of analysis” \(lines 125-130\)](#)

[For example, the death of enterobacteria, which is important from a safety point of view, was observed using a culturomic approach rather than a taxonomic approach.](#)

Results

- I really counsel against showing all of your results- simply because you have them. For example, ‘Regarding the other media used to enumerate bacteria, the counts enumerated on BHI-YEnp, a medium used to enumerate Gram-negative aerotolerant bacteria, were very similar or a bit lower compared to the counts on VRBG, suggesting that the same bacteria grew on both these media. I

suggest simplifying your data representation and descriptions so that this data is not included if it is not informative. Your main results are lost when everything is included. Similarly for ‘Two other, unidentified, compounds were detected by using HPLC-UV, at retention times 26 min and 28 min, named RT26 and RT28.’

Answer: Thank you for this comment. We have done our best to remove as much detail as possible from the initial version. For example, the unidentified metabolites RT26 and RT28 were suppressed from the text and PCA data in Figure 5; the results of the culturomics were greatly simplified, and the clustering made from PCA data removed and the corresponding comments.

- I like the presentation of figures 2 and 4, but suggest that the elementary presentation of results in figure 3 needs to be improved away from a stacked column graph made in excel which is very difficult to interpret and apply statistical tests.

Answer: rebuilt using R software instead of excel. The results of the ANOVAs carried out at the T3 stage on the variables shown in this figure showed no significant effect of either the cutting type or the salt factors on the concentrations of the metabolites, except for ethanol. (specified in the text as follows (lines 526-528): “By contrast, ethanol production, was significantly affected (p-value <0.001) by the cutting type, with concentrations of 3.3 and 1.2 g/kg in leaf and shredded cabbage, respectively (Figures 3 and 4).”

- How were the yeast identified?

Answer: Instead of the reference given, we added the following sentence: “Bacteria and yeast were identified by 16S rRNA gene and D1/D2 domain of 26S rRNA gene sequencing, respectively” (lines 266-267)

- The use of PCA to represent metabolic/volatile data is fine, but as there is little separation in some cases, perhaps a better method could be made. You don’t really talk this through in your discussion- I suggest you delete this representation.

Answer: We decided to keep the PCA analyses, but modified both the graphical representations and the corresponding comments. We suppressed the clustering (and thus cluster description) in order to focus on the main objective, which was to summarise the effects of the two factors studied, ‘cutting type’ and ‘salt concentration’, making links with the previous paragraphs.

The PCA graphs now clearly illustrate the specific course of changes for each vegetable/cutting over one month of fermentation (Figure 5A) and the absence of effect of salt content on the microbial and biochemical concentrations (Figure 5D).

- Are carrot and cabbage comparisons relevant to your aims? Given your aims are salt addition and cutting type, I don’t think so. You could clean up and trim these results from your descriptions to streamline your results. Indeed, you state in your discussion ‘However, the experiment was not designed to address the comparison of carrot and cabbage in itself, since the vegetable cultivar, culture conditions, harvest and storage conditions (time, temperature) can also impact their fermentation’.

Answer: we totally agree, It was tempting to compare the two vegetables, but as we ourselves said, the experiment was not designed to explore this. We therefore removed from the abstract, the results and the discussion section any elements relating to a comparison between carrots and cabbages.

- I’m missing the rationale for combining your results in a PCA (figure 5). Sure it gives you a ‘global picture’, but how does this help answer your research questions?

Our microbial and biochemical analyses generated many variables that we chose not to represent all individually. For example, Figures 2 and 4 show the time-course of changes for 4 and 3 variables in respectively, for four of the eight cases of our experimental design (the two salt concentrations were gathered).

We chose PCA as a multivariate analysis because this unsupervised multivariate analysis that reduces data's complexity while retaining as much as possible of the data's variation. It extracts latent principal

factors that contribute to the most variation of the data. It helps to find similarities and differences between samples and to highlight the important variables (the major contributors to the first few components).

As detailed above, we modified both the graphs and the associated comments to focus on the illustration of the effects of the factors studied, 'cutting type' and 'salt concentration' and time-course of changes.

- Figure 7 is glorious and comprehensive. But do you need both a and b? Why not send one to supp data and focus on the one that helps you tell your story.

Answer: we chose to keep (previous) Fig 7A in the main text and (previous) 7B as supplementary material (now Supplementary Figure S3)

- Figure 8 is confusing for me. Why do you need to pool your data and present it in this way?

Answer: Thank you for your valuable feedback. We appreciate the opportunity to clarify the rationale for using PLS-DA in our study, as answered to the other reviewer:

In the previous result sections, we have shown that the fermentation rate differs according to the type of cut, suggesting a possible effect on fermentation dynamics. However, in this study we wanted to investigate whether these differences extend to the fermentation profiles after one month of fermentation, or whether they merely reflect variations in the fermentation rate. To address this, we used multi-block PLS-DA, a powerful method for integrating different data sets - metataxonomic profiles, metabolite concentrations, viable counts and volatile compounds - and systematically identifying patterns across data blocks. This approach allowed us to investigate whether cutting type and salt concentration resulted in different final fermentation signatures, and to identify the variables driving these differences.

Our results confirmed that cutting type influenced not only the rate but also the final fermentation profile, with thinly cut cabbage characterised by higher concentrations of lactic acid, acetic acid and mannitol, as well as increased abundance of *Leuconostoc*. In contrast, salt concentration did not produce a clear discriminant signature at T3. By identifying the variables most strongly associated with these differences, PLS-DA provided important mechanistic insights into how cutting type shapes fermentation. This level of integration and detail could not have been achieved with univariate analyses alone. We hope this explanation clarifies our methodological approach.

We performed the following changes in the text in the M&M and result sections to clarify the objectives of multi-block PLS-DA and better highlight the benefits of this analysis:

-M&M section, lines 412-413: This method was chosen for its ability to model heterogeneous, multi-block data, allowing for the identification of the best variables that discriminated the samples.

-Result section, lines 753-766: In earlier sections of this paper, it was shown that the fermentation rate varied by cutting type and, to a lesser extent, by salt concentration. Additionally, it was important to investigate whether the fermentation profiles still differed or eventually converged at one month. A multi-block Partial Least Squares-Discriminant Analysis (PLS-DA) was therefore performed [...].

-Result section, lines 788-791: [...] Therefore, carrot profiles did not exhibit a strong discriminating signature by cutting type across different blocks (except for *gyrB* and metabolites), as convergence between samples with different cutting types occurred at one month.

Discussion

In general, I find the discussion long and detailed. Keep the detail but edit thoroughly to be concise and direct about the results. I like the informative subheadings, but the paragraphs underneath them are not well formed and tend to blurt the information without structure or narrative considerations. It

is so very difficult to follow the story of your paper when these dense lines of text, without highlights and signposts are presented and hides the value of your paper.

I really like the section titled 'A thin cutting favours...etc'. this part should be preserved, and the preceding sections considerably edited and shortened. Remember that this is your main result and outcome for the paper! The section on the vitamins can be considerably shortened.

Answer: Thank you for these comments. We have done our best to clarify and organise the content of the paragraph and to shorten most of the parts in order to highlight and discuss the main messages. Anything related to the comparison between the two vegetables studied has been omitted, and the section on vitamins has been considerably shortened.