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Meta-Omics Analyses of Conventional and Regenerative Fermented Vegetables: Is There an Impact on Health-Boosting Potential?

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Abstract: Fermented vegetables contain probiotic microbes and metabolites, which are transformed from fresh vegetables, potentially providing health benefits. The kind of vegetable used to ferment and how it is grown may determine the types of health-promoting properties. To understand the possible benefits of fermented vegetables under different growing conditions, we compared the microbiomes and metabolomes of three different types of naturally fermented vegetables—carrots, peppers, and radishes—that were grown either under conventional or regenerative growing systems. We profiled bacterial and fungal communities via 16S rRNA short-read (V4 region), long-read, and ITS2 sequencing, in tandem with untargeted metabolomics (LC-MS). The results showed that the microbiomes and metabolomes of the fermented vegetables under each growing system are unique, highlighting distinctions in amino acid content and potentially probiotic microbes ($p < 0.05$). All fermented vegetables contained high amounts of gamma-aminobutyric acid (GABA), a critical neurotransmitter. However, GABA was found to be in higher abundance in the regenerative fermented vegetables, particularly in carrots ($p < 0.01$) and peppers ($p < 0.05$), and was associated with higher abundances of the typically probiotic *Lactiplantibacillus plantarum*. Our findings indicate that the growing system may impact the microbiome and metabolome of plant-based ferments, encouraging more research on the health-boosting potential of regeneratively grown vegetables.

Keywords: fermented vegetables; microbiome; metabolome; regenerative agriculture; conventional agriculture; GABA

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1. Introduction

A key strategy to feed the world's growing population under an industrialized food system has been agricultural intensification, which is the process of increasing agricultural inputs through fertilizers and pesticides for disease-free crops and increased yields [1]. Yet, multiple studies have demonstrated that wide-spread herbicide and pesticide use leads to a depletion of soil biodiversity [2–5] and can have negative health impacts [1]. Conventional farming practices, which also include intensive tillage and nitrogen fertilization, in addition to synthetic pesticide use, are thought to contribute to declining nutrient density through disrupting crop symbioses with soil life [6,7]. Furthermore, pesticide use has been associated with a loss of gut microbial diversity [8,9]. The impact of the loss of microbes in the environment, likely including those in our food system, has been described by the "old

friends hypothesis", which states that the lack of exposure to non-harmful or commensal microbes in the environment and in foods has been an important mechanism explaining an increase in the prevalence of diseases that affect the immune system [10,11].

Fermented foods have been a traditional part of the diet in all cultures [12]; however, under food system industrialization in Western countries, they have become largely absent in the human diet. Individual types of fermented foods vary among cultures and geographic regions [13,14], potentially hosting distinct populations of microorganisms and nutrients [15]. The microorganisms involved in fermentation, as well as microbial metabolites such as bioactive peptides, organic acids, amino acids, fatty acids, and vitamins in fermented foods [16], have been shown to positively influence gut microbiome composition and function [17–19], intestinal integrity [20], macronutrient digestion and absorption [21,22], and immune system function [19,23–26]. Therefore, incorporation of fermented foods back into the daily diet of Western populations may be one way to increase microbial exposures, thereby helping to boost health.

Furthermore, the microbial ecosystem of a fermented food, and particularly, fermented vegetables, provides a unique opportunity to explore the analeptic properties and bioprospecting of probiotic microorganisms and health-boosting compounds, given their important relationship and proximity to soil ecosystems.

In this regard, regenerative agriculture (RA) has gained momentum from farmers, NGOs, governments, and corporations as a solution to current wide-spread challenges including climate change, loss of biodiversity, and human health [27]. While RA refers to farming practices to rebuild soil organic matter, definitions vary depending upon culture and geography and are constantly evolving [27–29]. These farming practices may include the use of cover crops, crop rotation, no-till farming, and other practices that actively rebuild soil communities, including the integration and rotation of livestock on the land, which are thought to result in more microbial soil life, and perhaps, even more nutrient-rich food [6,29], although much more research is needed in this area.

In this study, we sought to understand the differences in the microbiomes and metabolomes of different fermented vegetables grown under conventional or RA farming practices by conducting multi-omics analyses, including short-read and long-read 16S rRNA gene amplicon sequencing to profile bacterial communities, ITS2 amplicon sequencing to profile fungal communities, and liquid chromatography-mass spectrometry (LC-MS) to profile metabolites. Considering the innumerable factors that can influence the outcome of a study like this, our goal was to determine future research questions and methodologies that may one day shed light on how RA could impact the abundance of potentially probiotic microorganisms and metabolites of importance for human health and nutrition.

2. Materials and Methods

2.1. Selection of Conventional and Regenerative Organic Produce

Our first consideration was locating an RA farm growing vegetables close to a conventionally farmed one to maintain soil type. The farms identified closest to one another (28 miles apart) are located around the Delano region of Minnesota (MN) (45.0419° N, 93.7891° W) (Delano, MN, USA) where each farmer/grower described the soil as sandy loam. Vegetables were chosen based upon the availability in common from each farmer: carrots, radishes, and peppers. However, it should be noted that the exact vegetable subspecies, or where the seeds had come from, was not thoroughly discussed with each farmer. Therefore, having different seeds/subspecies of vegetables from each farm may have impacted the results shown here. In order to help mitigate this issue, including the three different vegetables allows for the identification of any similar trends viewed across all three, which may be likely due to each farm rather than to genotype.

We purchased 1.36 kg of each vegetable and brought it to the University of Minnesota where it was washed with a vegetable brush and chlorinated water before undergoing fermentation. Consequently, harvesting and post-harvesting washing and storing may also impact study results presented here.

2.2. About the Farms

The conventional farmer had been farming for 25 years and specialized in rare greenhouse plants and flowers in addition to vegetables. Herbicide and pesticides were minimally used and included Marathon (Imidacloprid), Safari insecticide, and Neem Oil. The RA farmer had been farming the land for 29 years utilizing many RA practices including cover crops, green manure crops, compost, and mined minerals as soil inputs, with very little to no tilling. Crop rotation was also utilized to prevent weeds, diseases, and insects, as well as to feed soil microbes. The RA farmer claimed no synthetic pesticides or herbicides have been used on the farm for the last 29 years. The vegetables that come from this farm are described as "RA" vegetables.

2.3. Fermentation Preparation and Sample Collection

Vegetables were purchased and brought to a certified food lab at the University of Minnesota and prepared with basic in-home fermentation techniques, utilizing what is described as "wild" or "natural" fermentation, which relies on naturally occurring bacteria and yeast to ferment foods. Briefly, all jars, lids, and glass fermentation weights were washed and sanitized prior to use. As noted previously, vegetables were thoroughly washed in cold chlorinated water and gently scrubbed with a vegetable brush prior to fermentation. The carrots and peppers were cut into smaller pieces and radishes were cut in half. For each conventional and organic vegetable, nine (32 oz) wide-mouth ball jars were filled with ¾ of the cut vegetables and enough salt water (50 g/L, Diamond Crystal Kosher salt) to cover them. The sanitized glass fermentation weights and Masontops "pickle pipes" (for lids) were put on top of six of each the conventional and organic vegetable jars. The remaining 3 jars from each of the conventional and organic vegetables were autoclaved for 30 min as noted in Raghuvanshi et al. (2019) [30]. The autoclaved jars were used as sterile controls, to assess the extent to which the native/soil microbial communities of each vegetable contributed to their probiotic or nutritional (metabolite) content. Samples and pH readings (to ensure proper and safe fermentation) from each vegetable were taken on day 1 (24 h), day 3 (72 h), day 7 (168 h), and day 14 (336 h) along the fermentation process for a total of n = 216 samples (please see Supplementary Table S1 for pH records). All samples were moved to a −80 °C freezer immediately after sampling. On day 14, samples of the vegetables and vegetable brine were taken for metabolomic analyses. For a detailed view of the study's schematic design, please see Figure 1.

2.4. Microbiome Analyses—16S rRNA and ITS2 Short Amplicon Sequencing

DNA Extraction, Sequencing, and Data Processing

Genomic DNA was extracted using the Power Soil DNA extraction kit of MoBio (Carlsbad, CA, USA). To determine bacterial composition, the V4 variable region of the 16S rRNA gene was amplified using 16S-515F (GTGCCAGCMGCCGCGGTAA) and 16S-806R (GGACTACHVGGGTWTCTAAT) primers. To examine fungal composition, the internal transcribed spacer 2 (ITS2) by amplification of ITS3 (GCATCGATGAAGAACGCAGC) and ITS4 (TCCTCCGCTTATTGATATGC) primers was used. Sequencing of pooled libraries was carried out using the Illumina MiSeq platform at the University of Minnesota Genomics center (UMGC) to generate 2x300 bp of sequences. 16S rRNA short-read and ITS2 sequences were processed using custom-made Perl scripts and the Qiime2 pipeline (qiime2.org). Briefly, raw sequencing data were processed to remove primers and low-quality reads

(phred quality score < 30). These high-quality reads were considered for denoising, merging, and chimera removal, and to generate unique amplicon sequence variants (ASVs) using the Dada2 plugin within Qiime2 [31]. Representative sequences of each ASV were aligned using MAFFT and phylogenetic trees both rooted and unrooted were constructed with FastTree [32]. Taxonomic assignments of bacterial ASVs were carried out by trained naive Bayes classifiers on reference sequences (clustered at 99% sequence identity) from Greengenes 13_8, and fungal ASVs were carried out with the UNITE database. For both taxonomic assignments, Qiime2 plugins feature-classifier fit-classifier naive-bayes and feature-classifier classifier-sklearn were used [31]. Generated bacterial and fungal ASV counts, and frequency tables were converted to relative proportions using total reads per sample, and the ASVs that were not present in at least 5 samples (~3% of total samples) were omitted from the data set.

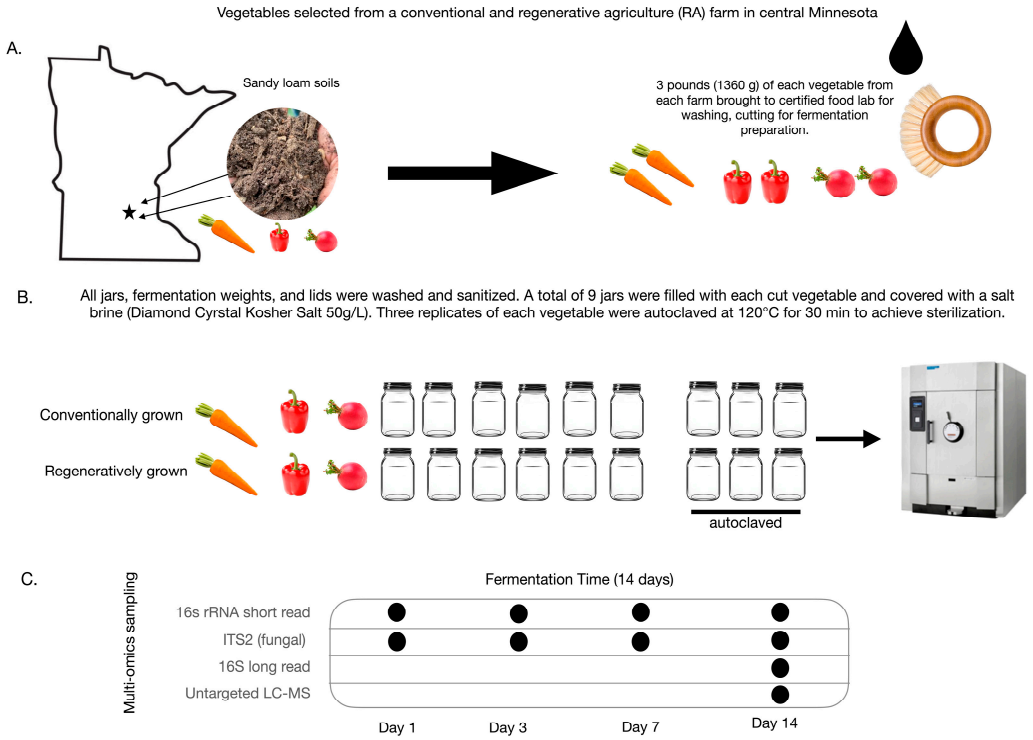

Figure 1. Study schematic design. (**A**) Carrots, peppers, and radishes were purchased from two different farms—one conventional farm and one regenerative farm in Central Minnesota. (**B**) Vegetables were washed, chopped, and covered in a salt brine (50 g/L Diamond Crystal kosher salt) for a "natural fermentation" period of 14 days. Three jars from each group were autoclaved for 30 min at 120 °C to sterilize as controls. (**C**) Samples were taken and pH recorded on days 1, 3, 7, and 14 for microbiome short-read analysis and samples were taken on day 14 for 16S long-read and metabolomic analysis (n = 216 total samples).

2.5. Microbiome Analyses—16S rRNA Long Read Sequencing

Given the probiotic content potential of fermented foods, we also profiled bacterial communities targeting the entire 16S rRNA gene to obtain high resolution of bacterial taxa down the species and strain level. However, long-read sequencing was exclusively performed on the last day of fermentation (D14). Extracted DNA was sent to Loop Genomics and processed through the Loop Genomics pipeline described in [33,34]. Briefly, as described, the LoopSeq^{TM} protocol uses unique molecular barcoding labeling of individual 16S rRNA genes. This unique molecular barcode is evenly distributed throughout the 16S rRNA gene and leads to its fragmentation. The barcoded 16S rRNA gene fragment sequences enable sequencing by short reads on an Illumina sequencing platform, with

subsequent reconstruction of the full-length 16S rRNA gene. Therefore, all hypervariable 16S rRNA regions (V1–V9) can be identified and sequenced. The libraries were read on an Illumina NovaSeq 6000 sequencer (Illumina, San Diego, CA, USA), using a paired-end 2 × 150 bp reading system. Coverage was 200–250 million paired-end reads per library of 24 samples. The short-read raw data were collected in real-time on Illumina's BaseSpace, which generates FASTQ files, and were then uploaded to the Loop Genomics unique analytic pipeline. The sequencing raw data (2 × 150 bp PE, NovaSeq, Illumina) were transferred to the Loop Genomics unique barcode identifier cloud. It is a data analysis pipeline that is used for the low-quality base trimming, unique sample barcode demultiplexing, and synthetic long-read reconstruction. The demultiplexing and synthetic long-read reconstruction is a process that enables the de novo assembly to the full-length 16S long-read data after rearranging the short reads tagged with the same unique barcode. The complete preparation and sequencing protocol has been detailed previously [33].

2.6. Metabolomic Analyses

2.6.1. Sample Preparation for LC-MS Analysis

Vegetable brine samples for metabolomic analyses were taken on the last day (D14) of fermentation. The brine samples were quenched with 5 volumes of acetonitrile containing 5 µM sulfadimethoxine and extracted by vortexing and sonication for 10 min, followed by centrifugation at 18,000× *g* for 10 min to remove the insoluble fraction. All samples were stored at −80 °C.

2.6.2. Chemical Derivatization

For detecting organic acids, which are the main products of carbohydrate fermentation, the samples were derivatized with 2-2′-dipyridyl disulfide (DPDS), triphenylphosphine (TPP), and 2-hydrazinoquinoline (HQ) prior to the LC-MS analysis [35]. Briefly, 2 µL of sample or organic acid standard was added into 100 µL of freshly prepared acetonitrile solution containing 1 mM DPDS, 1 mM TPP, 1 mM HQ, and 100 µM deuterated d_4-acetic acid as internal standard. The reaction mixture was incubated at 60 °C for 30 min, chilled on ice, and mixed with 100 µL of H_2O. This mixture was centrifuged at 18,000× *g* for 10 min, and the supernatant was transferred into an HPLC vial for LC-MS analysis.

For detecting and quantifying amino acids, the quenched brine samples were derivatized by dansyl chloride prior to LC-MS analysis. In brief, 5 µL of sample was mixed and co-incubated with 5 µL of 50 µM deuterated d_5-tryptophan (internal standard), 50 µL of 10 mM sodium carbonate, and 100 µL of DC (3 mg/mL in acetone) at 60 °C for 15 min. Then the mixture was centrifuged at 13,000× *g* for 10 min. The acquired supernatant was transferred to an HPLC vial for LC-MS analysis.

2.6.3. LC-MS Analysis

The workflow of LC-MS analysis was described previously [36]. Briefly, 5 µL aliquot of the HQ derivatized solution was injected into an Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) and separated in a BEH C18 column with a gradient of mobile phase ranging from water to 95% aqueous acetonitrile consisting of 0.05% glacial acidic acid and 2 mM ammonium acetate in a 10 min run. The DC-derivatized samples were separated in a BEH C18 column with a gradient of mobile phase ranging from water to 95% aqueous acetonitrile containing 0.1% formic acid in a 10 min run. Non-derivatized quenched brine samples were separated in a BEH Amide column with a gradient of mobile phase ranging from acetonitrile to water containing 0.1% formic acid in a 10 min run, with sulfadimethoxine as the internal standard in both positive and negative mode. The LC eluate was directly introduced into a Xevo-G2-S QTOF (Waters, Milford, MA, USA) mass spectrometer for the accurate mass measurement and

ion counting. Capillary voltage and cone voltage for electrospray ionization (ESI) was maintained at 0.2 kV and 40 V for positive-mode detection and −0.2 kV and −40 V for negative mode detection, respectively. Nitrogen was used as both cone gas (50 L/h) and desolvation gas (600 L/h) and argon as collision gas. For accurate mass measurement, the mass spectrometer was calibrated with sodium formate solution (range m/z 50–1200) and monitored by the intermittent injection of the lock mass leucine encephalin ($[M + H]^+$ = m/z 556.2771, $[M - H]^-$ = 554.2615). Mass chromatograms and mass spectral data were acquired and processed by MassLynx^{TM} software V4.2 (Waters, Milford, MA, USA) in centroided format. Additional structural information was obtained by tandem MS (MS/MS) fragmentation with collision energies ranging from 10 to 50 eV in the positive mode and −10 to −50 eV in the negative mode.

2.7. Analysis of Spectral Data

Metabolites were quantified by fitting the peak area (normalized by corresponding internal standards) with the standard curves via Quanlynx (Waters).

Chromatographic and spectral data of samples were analyzed using the MarkerLynx software V4.2 (Waters, Milford, MA, USA). A multivariate data matrix containing information on sample identity, ion identity (retention time (RT) and m/z), and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The intensity of each ion was calculated by normalizing the single ion counts (SIC) versus the total ion counts (TIC) in the whole chromatogram. The total ion intensity was set arbitrarily as 10,000. The data matrix and sample list integrity were assessed by exporting spectral data into SIMCA-P$^+$ software version 13.0 (Sartorius, Göttingen, Germany). The chemical identities of markers were determined by elemental composition analysis, isotope modeling, search in database (ECMDB, accessed on 16 January 2021, https://ecmdb.ca/; HMDB, accessed on 16 January 2021, https://ecmdb.ca/; and KEGG, accessed on 16 January 2021, https://www.genome.jp/kegg/), and comparison with authentic standards if possible.

2.8. Statistical Analyses

2.8.1. Microbial Community Analysis

Microbial community analyses were performed in the R statistical platform (version 4.2.3 R Foundation for Statistical Computing). Briefly, for alpha diversity, beta diversity, and permutational multivariate analyses of variance (PERMANOVA), multiple R packages such as vegan, ape, phyloseq were used [37–39]. Significantly discriminating bacterial and fungal taxa were identified using species indicator analysis using the labdsv package in R, which calculates the indicator value using the fidelity and relative abundance of species [40]. Wilcoxon rank-sum and Kruskal–Wallis chi squared tests within the R statistical interface were used to assess the statistical significance of univariate measures (e.g., taxonomic abundance, alpha diversity) between or across treatment groups and across fermentation days—1, 3, 7, and 14).

2.8.2. Metabolomic Analysis

Principal component analyses and heatmaps were generated using the Metaboanalyst 5.0 online platform [41]. Data were normalized by sum, log transformed, and auto scaled (mean centered divided by the square root of the standard deviation of each variable). Univariate statistical tests for metabolite abundances were performed as described above.

2.8.3. Correlation and Network Analysis

Correlation analysis was conducted with Spearman's rank correlation, and corresponding p-values corrected based on the Holm–Bonferroni method using the cor.test function

in R between the normalized metabolite data and the 16S long-read relative abundance data and plotted with ggplot2. Network analysis was conducted using significant positive correlations between GABA and bacterial taxa ($r > +0.5$, $p < 0.05$) with Cytoscape 3.10.1.

3. Results

3.1. Microbial Alpha Diversity for Ferments Under Conventional or Regenerative Farming Practices

3.1.1. Bacteriome

After 24 h of fermentation (D1), the only differences observed in bacterial alpha diversity (Shannon Index) between the conventional and regenerative ferments was seen in the radishes and carrots, with higher diversity for the conventional version at D1 and D3 (Figure 2A; Wilcoxon rank-sum test; $p < 0.005$). Yet, by the end of fermentation, D14, no bacterial diversity differences were seen between the conventional and regenerative ferments for any vegetable. Remarkably, by day 14 of the fermentation process, the autoclaved conventional samples showed greater bacterial diversity compared with their regenerative counterparts ($p < 0.05$). Bacterial richness, expressed as the number of amplicon sequence variants (ASVs), showed greater values for radishes only, at D14 (Figure S1A; $p = 0.03$).

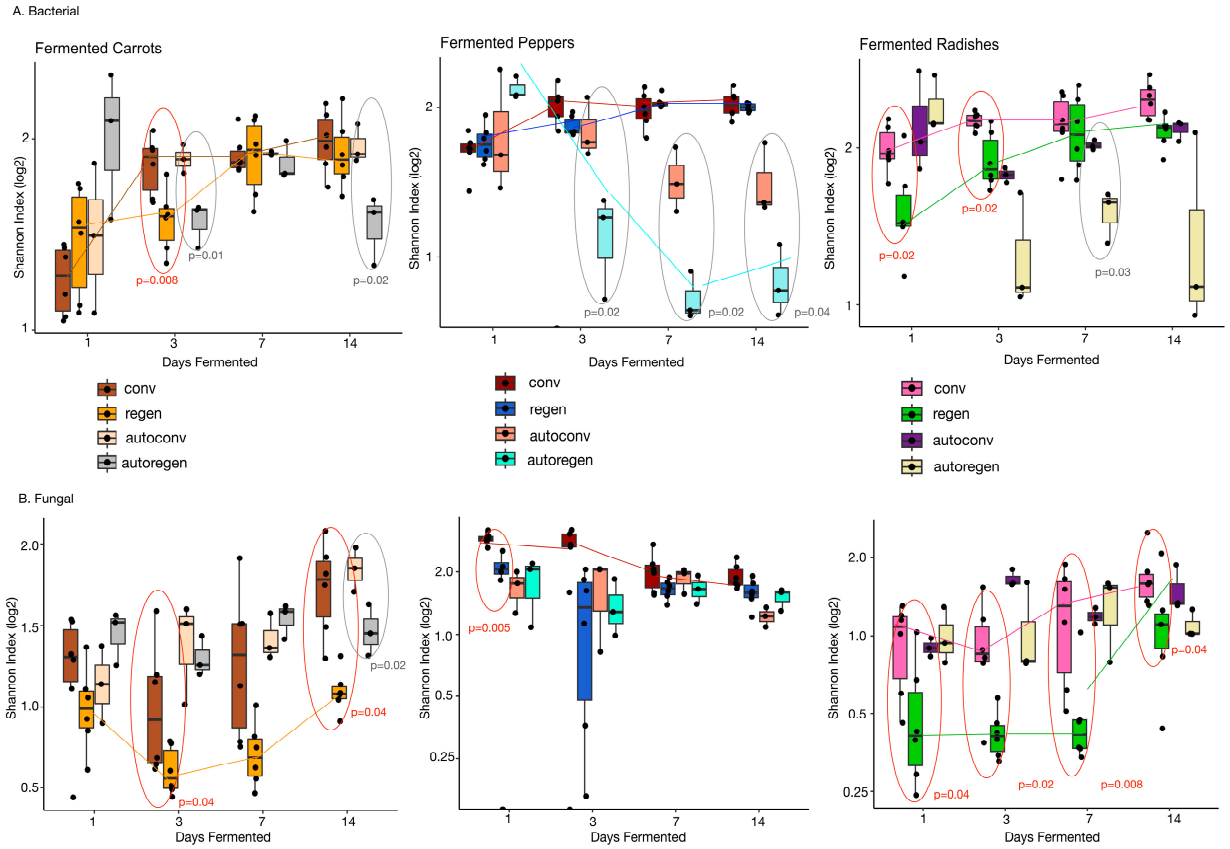

Figure 2. Bacterial and Fungal differences in alpha diversity of fermented vegetables. Alpha diversity (Shannon Index) for bacteriome (**A**) and mycobiome (**B**) of regenerative and conventional fermented carrots, peppers, and radishes during a 14-day fermentation period. Statistical significance calculated with Wilcoxon rank-sum test within a day between conventional and regenerative samples (red circles) and autoclaved conventional and autoclaved regenerative samples (gray circles). Color line is shown only if there were significant differences across fermentation days for each ferment group according to the Kruskal–Wallis test.

3.1.2. Mycobiome

Fungal Shannon diversity tended to be higher for all conventional vegetables compared with the regenerative versions across all fermentation days (Figure 2B), with significance detected for D3 and D14 for carrots, D1 for peppers, and all fermentation days for radishes ($p < 0.05$). Although fungal richness tended to be higher in regenerative carrots, especially in the last two days of fermentation (D7 and D14), no statistical significance was reached (Figure S1B). Like the patterns observed with the bacteriome, autoclaved conventional samples showed greater fungal diversity, but this was only statistically significant for carrots on day 14 of fermentation ($p < 0.05$). Fungal richness was generally higher across all days for conventional peppers, with statistical significance reached at D1, D7, and D14 ($p < 0.05$, Figure S1B).

3.2. Alpha Diversity Differed Across Fermentation Time in Conventional and Regenerative Ferments

3.2.1. Bacteriome

In the bacteriome, significant differences were observed in all the conventional and regenerative ferments compared to their autoclaved counterparts, showing the bacterial communities slightly increasing in diversity over time until D14 (Figure 2A; Kruskal–Wallis test for all ferment groups, $p < 0.01$). Notably, their autoclaved counterparts showed greater variability in Shannon diversity over time. For example, both the autoclaved organic carrots and radishes showed a decrease in bacterial diversity from D1–D3, an increase in D3–D7, and another decrease in D7–D14. This pattern was similar in the autoclaved conventional radishes. However, we only found a significant difference in Shannon diversity across fermentation days in the autoclaved regenerative peppers, showing a decreasing trend over time (Figure 2A; Kruskal–Wallis test; $p = 0.05$).

3.2.2. Mycobiome

In the mycobiome, a significant difference across fermentation days was observed with the regenerative fermented carrots (Figure 2B Kruskal–Wallis test; $p = 0.002$) and the conventional fermented peppers ($p = 0.03$), while both the conventional and regenerative radishes showed a significant difference across fermentation days ($p = 0.03$). However, these time-dependent trends in the mycobiome were inconsistent and did not follow a defined increasing or decreasing trend across days for any vegetable type.

3.3. Conventional and Regenerative Fermented Vegetables Display Unique Bacteriome and Mycobiome Composition

Principal coordinates analyses (PCoA plots) based on weighted Bray–Curtis distances of the relative abundances of ASVs showed significant stratification in both the bacteriome (Figure S2A) and mycobiome (Figure S2B) between fermented carrots, radishes, and peppers. These analyses indicated that each vegetable, regardless of the system (conventional or regenerative), or whether they are autoclaved or not, harbors a distinct microbiome composition across all fermentation days. However, these distinctions appear less pronounced for fungal communities. For example, at the beginning of fermentation (D1), the mycobiome of fermented carrots and radishes showed substantial overlap, except for the regenerative carrots, which showed more unique compositional patterns through the fermentation process. In addition, the mycobiome of autoclaved peppers, regardless of the system, seemed to cluster more closely with the conventional and autoclaved carrots, mostly at D7 and D14 (Figure S2B).

Throughout the 14-day fermentation period, conventional and regenerative ferments displayed significantly different microbiome compositions (Figure 3A,B). For the most part, although the autoclaved samples clustered close to their conventional or regenerative

counterparts, they usually showed unique compositional patterns. However, in the radishes mycobiome, all conventional samples continually showed substantially distinct fungal communities compared with the autoclaved or organic samples. Interestingly, in both the bacteriome and mycobiome of the fermented peppers, the conventional and regenerative samples remain very distinct from their autoclaved counterparts.

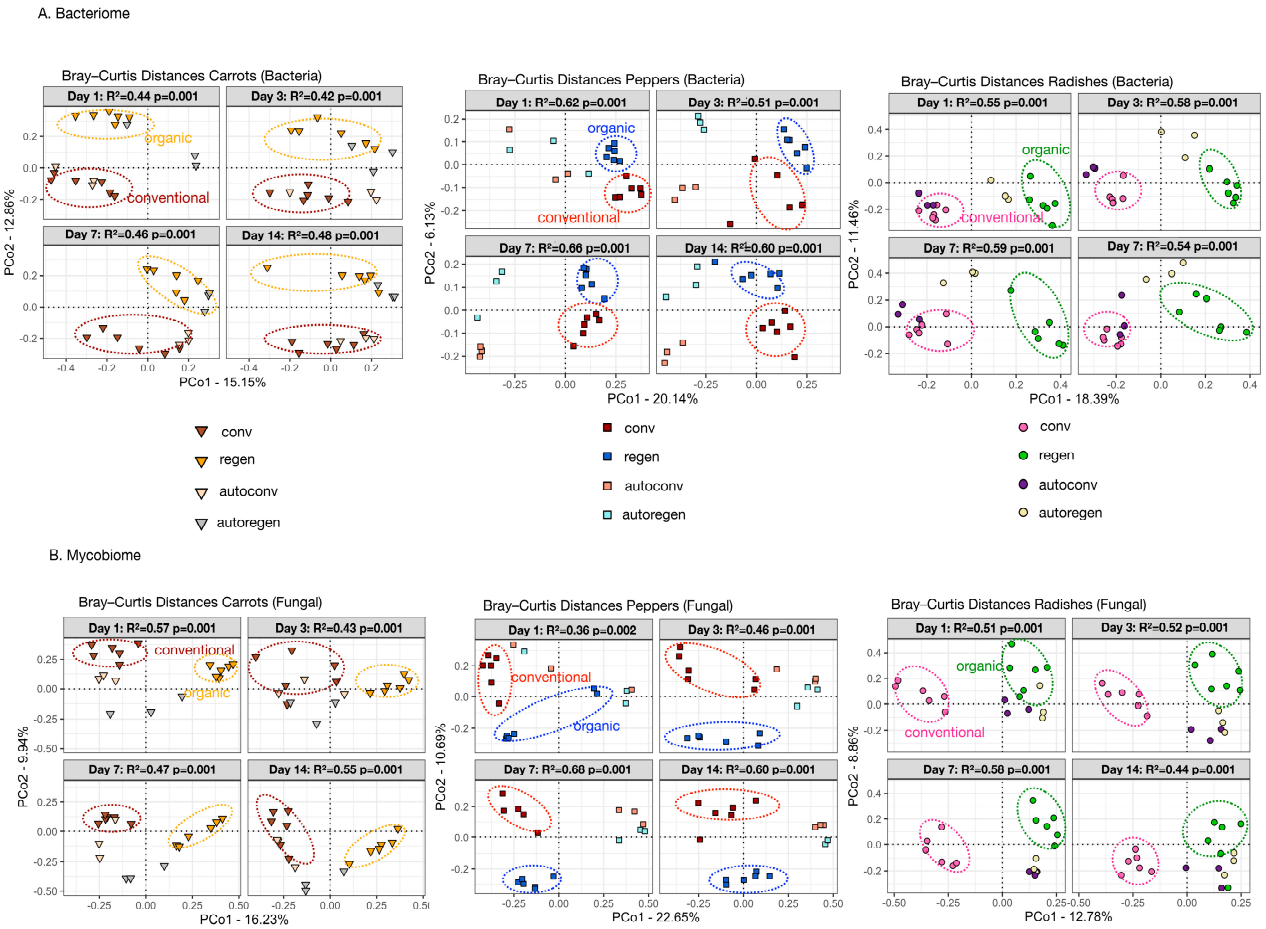

Figure 3. Beta Diversity of bacterial and fungal communities reveal unique differences in microbiome composition. Unweighted Bray–Curtis dissimilarity metric of (**A**) bacterial communities and (**B**) fungal communities in conventional, regenerative, autoclaved conventional, and autoclaved regenerative fermented carrots, radishes, and peppers across 14-day fermentation period.

3.4. Distinct Taxonomic Signatures Differentiate Conventional and Regenerative Fermented Vegetables

To characterize ASVs and strains of bacteria and fungi that contributed to differences in the microbiome composition between conventional and regenerative fermented vegetables, we conducted an indicator species analysis at D14, the last day of fermentation. Indicator taxa can be seen in Figure 4A for 16S rRNA long-read taxa, and Figure 4B for ITS2 ASVs (indicator value > 0.5, $p < 0.05$ (Wilcoxon rank-sum test with FDR). For all indicator values, see Supplementary Table S2).

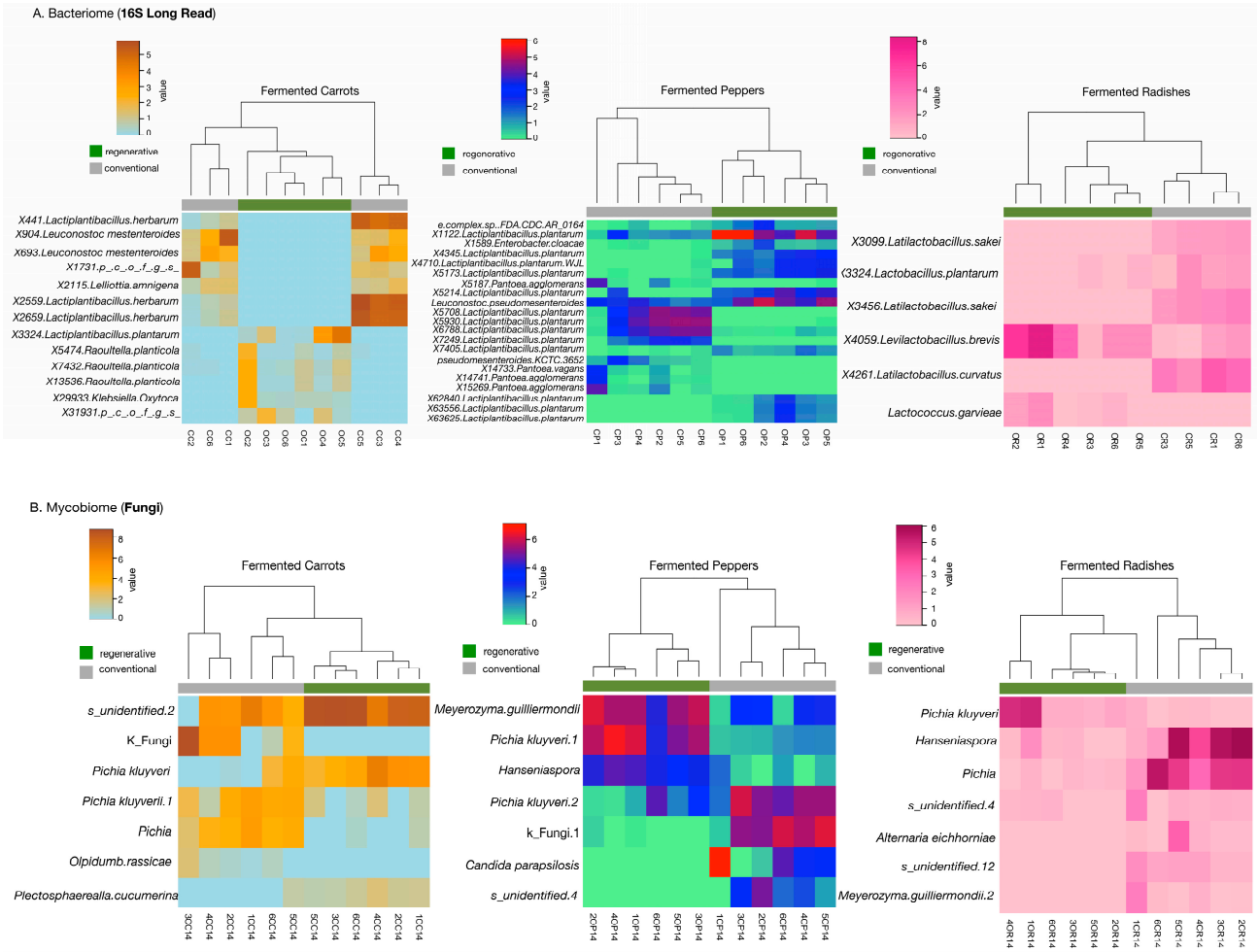

Figure 4. Heatmaps of indicator species on day 14 of fermentation. (**A**) Indicator species analysis of (**A**) 16s rRNA long-read and (**B**) ITS2-mycobiome. Indicator values > 0.5 and $p < 0.05$ (Wilcoxon rank-sum test with FDR).

The indicator species of the conventional fermented carrots were mainly LAB such as *Lactiplantibacillus herbarum* and *Leuconostoc mesenteroides*, while the indicator species of the regenerative fermented carrots were mainly strains of *Raoultella planticola* and *Klebsiella Oxytoca*, both gram-negative bacteria commonly found in soil, but also a strain of *Lactiplantibacillus plantarum* (*L. plantarum*).

The long-read indicator taxa detected for the fermented peppers were strains identified as *L. plantarum*, which characterized both the conventional and regenerative peppers, although different strains were indicative of each group. Taxa unique to the conventional peppers were *Lactiplantibacillus pentosus*, *Leuconostoc pseudomesenteroides*, and certain species belonging to the Enterobacteriaceae family, including *Pantoea agglomerans* and *Pantoea vagans*. The regenerative fermented peppers displayed many different strains of *Lactiplantibacillus plantarum*, as well as *Enterobacter cloacae* and *E. cloacae complex* (Figure 4A, middle panel).

Long-read bacterial species characteristic of the fermented radishes were all identified as LAB. Only two indicator species were found in the regenerative fermented radishes, *Levilactobacillus brevis* and a strain of *Lactococcus garvieae*, while species indicative of the conventional radishes included *Latilactobacillus sakei*, *Lactiplantibacillus plantarum*, and *Latilactobacillus curvatus* (Figure 4A, right panel).

Like in other studies [42,43], our analysis of fungal communities also showed that many of the ITS2 sequences generated could not be assigned to any taxonomic group.

Nonetheless, some indicator species were evident. Regenerative fermented carrots displayed an unidentified fungus ASV, *Pichia kluyveri* and *Plectosphaerealla cucumerina*. Conventional fermented carrots were characterized by abundances of a different ASV of *Pichia kluyveri*, the genus *Pichia* and *Olpidium brassicae* (Figure 4B, right panel). Indicator species discovered in the fermented regenerative peppers showed *Meyerozyma guilliermondii, Hanseniaspora,* and an ASV of *Pichia kluyveri*. Conventional peppers were characterized by another ASV of *Pichia kluyveri* and higher abundances of unidentified fungi and *Candida parapsilosis* (Figure 4B, middle panel). Like the fermented peppers, the fermented regenerative radishes were mainly distinguished by a higher abundance of *Pichia kluyveri*, while the conventional peppers displayed more indicator species, including another ASV of the genus *Pichia*, some unidentified fungi, *Alternaria eichhorniae*, and *Meyerozyma guilliermondi.2* (Figure 4B, right panel).

3.5. Metabolome Profiles for Conventional and Regenerative Fermented Vegetables

As seen with the microbiome, the global metabolome of each fermented vegetable showed unique profiles, regardless of the system or autoclaving (Figure S3A). However, when looking at differences within each vegetable, mainly radishes and carrots showed metabolome distinctions driven by conventional, regenerative, or autoclaving methods. In contrast, the metabolomes of the conventional and regenerative peppers and their autoclaved counterparts were not readily distinguishable (Figure S3B). When analyzing metabolome profiles by metabolite category, minor differences were found in the organic acid profiles between conventional and organic ferments, according to a PCA (Figure S4). In contrast, all vegetables demonstrate greater distinctions in amino acid profiles depending on conventional or regenerative production systems (Figure 5). Here, we focus the analyses on the organic and amino acids detected in all fermented vegetables.

3.6. No Differences Observed in the Abundance of Organic Acids in Conventional vs. Regenerative Growing Systems

As expected, lactic acid was found to be several folds higher in abundance compared to all the organic acids detected by day 14. In addition, the relative abundance of lactic-acid was significantly higher in all the fermented vegetables compared with their autoclaved counterparts (Figure S5A; carrots-Wilcoxon rank-sum test; $p = 0.002$; peppers-Wilcoxon rank-sum test; $p = 0.02$ and radishes-Wilcoxon rank-sum test; $p = 0.001$). When conducting pair-wise tests between growing systems, it was found that the regenerative carrots had significantly higher abundances of lactic acid by day 14 compared to the conventional version (Figure S5B; Wilcoxon rank-sum test; $p = 0.04$). On the other hand, for peppers and radishes, the opposite pattern was observed, that is, more lactic acid was seen in the conventional vegetables, but with statistically significant results for radishes only (Wilcoxon rank-sum test; $p = 0.015$ for radishes, and $p = 0.243$ for peppers; Figure S5B). The data on other organic acids do not point to clear distinctions between fermented vegetables and their autoclaved counterparts or common patterns between growing systems.

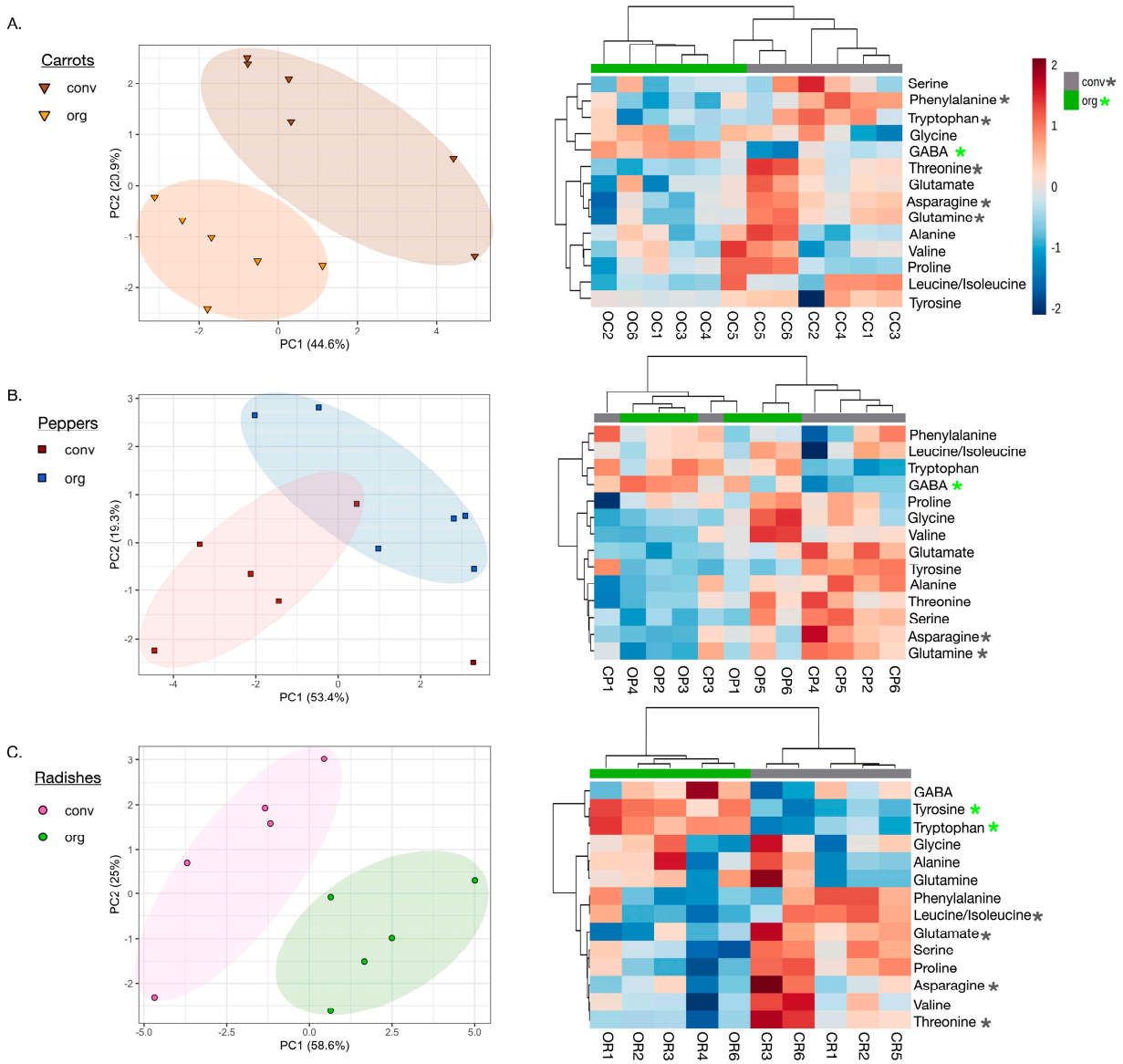

Figure 5. PCA and Heatmap of identified amino acid metabolites in fermented vegetables on day 14. (**A**) Conventional and regenerative fermented carrots, (**B**) conventional and regenerative radishes, and (**C**) conventional and regenerative peppers. PCAs based upon normalized data (normalization by sum; log transformation; autoscaling) and heatmaps based upon normalized data, Euclidean distances, and Ward clustering utilizing the average abundance of amino acids detected in each fermented vegetable. $p < 0.05$ according to Wilcoxon rank-sum test indicated by * for regenerative and * for conventional.

3.7. Higher Amounts of GABA Discovered in All Fermented Vegetables

Similar to the microbiome, the principal component analysis (PCA) of detected amino acids on day 14 of fermentation found distinctions between the regenerative and conventional growing systems (Figure 5). Overall, amino acids appeared to be higher in the conventional carrots (Figure 5A). Intriguingly, gamma-aminobutyric acid (GABA) was found to be the most abundant amino acid detected in all the fermented vegetables (Figure S11). Given the importance of GABA as the main inhibitory neurotransmitter in the central nervous system, we investigated its abundance more closely.

GABA was not only the most abundant amino acid detected (several fold) in all the regenerative and conventional vegetables compared to all other amino acids (Figure S11), but also, the abundance of GABA was significantly higher in all the fermented vegetables

compared with their autoclaved counterparts (Figure S12—carrots-Wilcoxon rank-sum test; $p = 0.0001$; peppers-Wilcoxon rank-sum test; $p = 0.0004$ and radishes-Wilcoxon rank-sum test; $p = 0.02$). The lower abundance in autoclaved vegetables demonstrates that the native microbial communities responsible for the fermentation process are the main drivers of GABA in fermented vegetables. To further investigate patterns in the abundances of GABA in the context of the production system, we conducted specific pairwise tests for the abundance of this metabolite between regenerative and conventional ferments. GABA was observed to be significantly higher in the regenerative fermented carrots and peppers (Figure 6A,B) (Wilcoxon rank-sum test; $p = 0.003$ carrots; Wilcoxon rank-sum test; $p = 0.02$ peppers; Wilcoxon rank-sum test; $p = 0.15$ radishes). Although the GABA abundance was also found to be higher in the regenerative radishes, the difference was not statistically significant (Figure 6C).

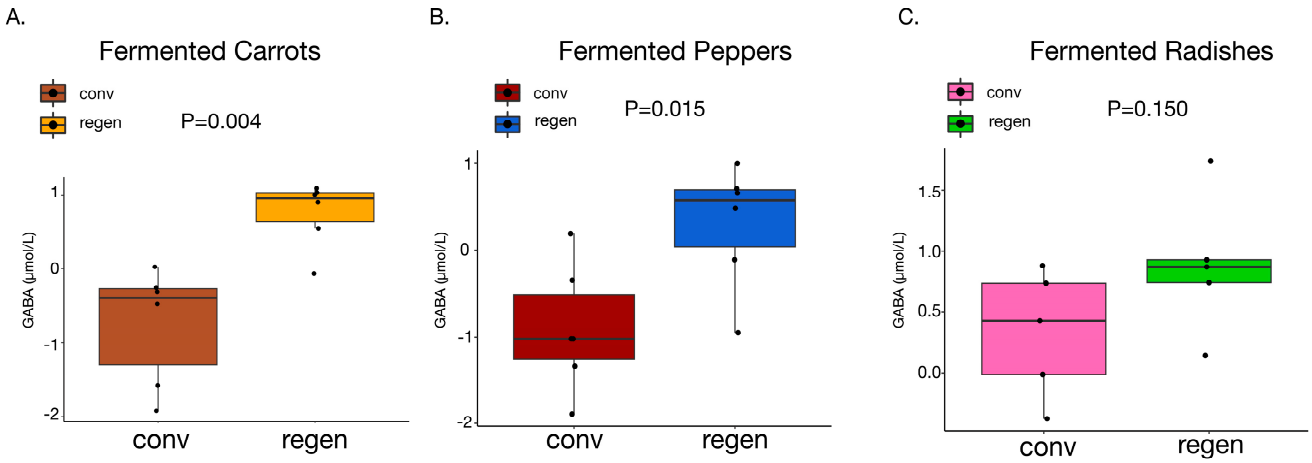

Figure 6. Amounts of GABA found in conventional and regenerative fermented vegetables. Boxplots of the amounts of GABA found between conventional and regenerative fermented vegetables (normalization by sum; log transformation; auto-scaling) for (**A**) Fermented Carrots (**B**) Fermented Peppers (**C**) Fermented Radishes. Statistical significance calculated by Student's *t*-test.

To further understand the possible drivers of increased GABA in regenerative vegetables, we performed a Spearman's rank correlation analysis based on the relative abundance of taxa detected via 16S long-read sequencing and the normalized abundance of this metabolite. This analysis showed that a specific strain of *Lactiplantibacillus plantarum* (*L. plantarum*) has the strongest association with GABA ($r = 0.73$; $p = 1.183 \times 10^{-6}$) across all the dataset, including both conventional and regenerative samples. We then separated samples according to system (only the regenerative fermented vegetables and only the conventional fermented vegetables) and repeated the analysis. There was a significant positive correlation between GABA and *L. plantarum* observed in the regenerative fermented vegetables, and specifically, in the regenerative fermented peppers and radishes, which seemed to drive this strong correlation (Figure 7; $r = 0.87$; p-value $= 4.691 \times 10^{-6}$). On the other hand, no significant correlation was detected with the conventional fermented vegetables between GABA and *L. plantarum*. These analyses are concordant, to some extent, with the differential abundance of bacterial taxa (Figure 4), in which it was shown that different strains of *L. plantarum* were the most distinctive markers of regenerative versus conventional vegetables, particularly in peppers.

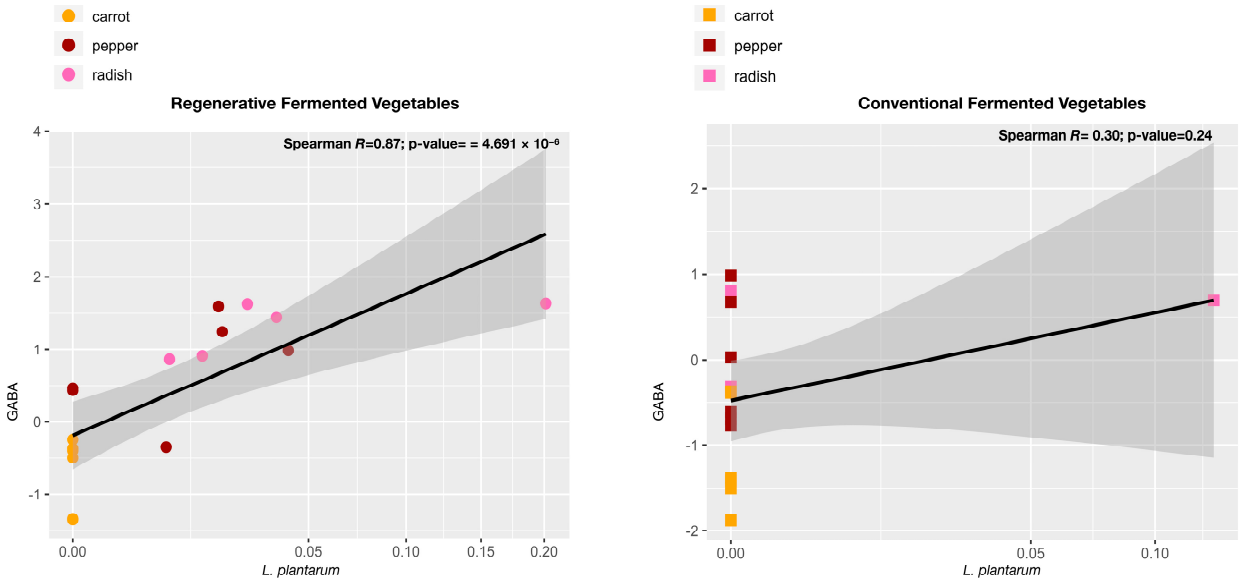

Figure 7. GABA is positively correlated with a strain of *L. plantarum* in the regenerative fermented vegetables. Scatterplots of Spearman's correlation between GABA (normalization by sum; log transformation; auto-scaling) and the relative abundance of a strain of *L. plantarum* in the organic fermented vegetables (Spearman $R = 0.87$; $p = 4.691 \times 10^{-6}$) and the conventional fermented vegetables (Spearman $R = 0.30$; $p = 0.24$). Correlation coefficients were calculated using the Spearman method, and corresponding *p*-values corrected based on the Holm–Bonferroni method.

3.8. Network Dynamics Reveal GABA Is More Strongly Correlated to Probiotic Bacterial Taxa in Fermented Vegetables from the Regenerative Growing System

Assuming more taxa could potentially drive the abundances of GABA in fermented vegetables, we also considered all significant correlations between any given taxa (as determined via long read analyses) and GABA. Analysis of only the strong positive correlations (spearman correlation; r > +0.5; adjusted *p*-value < 0.05) between GABA and all bacterial taxa from either conventionally grown fermented vegetables or regeneratively grown fermented vegetables showed that GABA has a greater number of stronger positive correlations with more bacterial taxa in regenerative system (Figure 8). Among these associations were bacteria known for their probiotic potential, including strong co-variation with different strains of *L. plantarum* and *Levilactobacillus brevis* (*L. brevis*) (Figure 8; Regenerative Network; highest correlation GABA to *L. plantarum*; r = 0.87, *p*-value= 4.69×10^{-6} as stated above, and GABA to *L. brevis*; r = 0.82; *p*-value = 4.69×10^{-6}, among other strains).

This observation was in contrast with the conventional network where there were fewer numbers of bacterial taxa found to be strongly correlated to GABA (Figure 8; conventional network; highest correlation GABA to *Klebsiella michiganensis*; r = 0.76; *p*-value= 0.0005 and GABA to *Leuconostoc pseudomesenteroides*; r = 0.73; *p*-value= 0.001). In the conventional network, GABA was also associated with some potentially probiotic bacteria such as different strains of *Leuconostoc mesenteroides*, *L. plantarum*, and *Lactococcus lactis*. However, there were fewer and less strong associations between these bacterial taxa and GABA. In addition, different strains of *Enterobacter cloacae* were found to be associated with GABA in the conventional network.

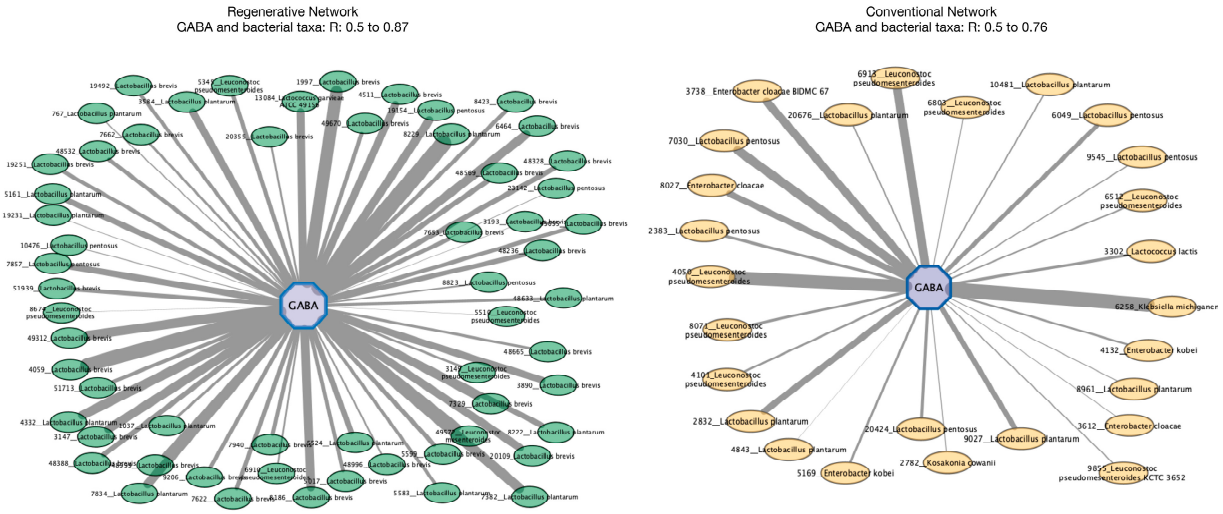

Figure 8. Networks between GABA and bacterial taxa in regenerative and conventional fermented vegetables. Analysis of the strong positive correlations between GABA and bacterial taxa ($r > +0.5$; p-value < 0.05) of all the regenerative fermented vegetables and conventional fermented vegetables shows that GABA has more significant positive correlations in the regenerative network. Node is showing the bacterial taxa, and the edge line demonstrates the strength of the correlation between GABA and any given taxa.

4. Discussion

This report presents an overview of the differences observed in the microbiomes and metabolomes of naturally fermented carrots, peppers, and radishes over a 14-day fermentation period, considering conventional or regenerative farming practices. The results demonstrate that each vegetable harbors a unique microbiome and metabolome under different growing conditions. Here, we discuss the significance of the data in the context of potential health benefits of plant ferments produced from either conventional or regenerative production systems.

4.1. Regenerative and Conventional Fermented Vegetables Display Minor Differences in Microbial Alpha Diversity but Reveal Unique Microbiome Compositions

Notable differences in alpha diversity were seen in the fungal communities of the fermented carrots and radishes, with a higher mycobiome diversity in conventional fermented carrots and radishes at day 14 (last day of fermentation), compared to the regenerative ferments (Figure 2B). The high fungal diversity in conventional vegetables is noteworthy. In the context of food safety, a higher diversity of fungal communities might result in the risk of fungi producing higher levels of mycotoxins in the fermented vegetable [44]. A number of strains of LAB are reported to produce antifungal metabolites (lactic acid, phenyl lactic acid, indole, and bioactive peptides) that can reduce both fungal growth and mycotoxin synthesis, which may result in competition for the occupied niche and nutrients needed for growth [45]. Therefore, a potential avenue of further research may not only focus on whether conventional plant ferments harbor more mycotoxins, but also, on whether LAB communities in the regenerative fermented vegetables produce more anti-fungal metabolites in the fermentation environment, likely reducing fungal diversity in comparison with the conventional ferments. This research focus can shed light on whether ferments derived from organic production systems are safer for consumption compared with conventional vegetable ferments. Evidence shows that concentrations of mycotoxins are usually similar or reduced in organically produced crops [46,47] but there is no evidence showing that regeneratively (or organically) produced fermented vegetables may be safer for consumption than conventionally produced ones.

4.2. Environmental Bacteria Found to Be More Characteristic of Fermented Carrots

The species distinctive of the regenerative fermented carrots were mostly species belonging to the Enterobacteriaceae family, which are abundant in soil microbiomes (Figure 4A). The long-read 16S rRNA analysis revealed different species of *Raoultella planticola,* which characterized the organic carrots. *R. planticola* are gram-negative bacteria ubiquitous in water and soil, previously isolated from plants such as herbs and fresh vegetables [48]. They are also known colonizers of animals and humans—mainly in the upper respiratory and gastrointestinal tracts—but can become pathogenic in immunocompromised people [49]. This species of bacteria is also a known producer of histamine, which is a common biogenic amine found in many foods and beverages, including fermented vegetables [50]. Like other biogenic amines, histamine is a concern to human health, as it is responsible for several toxicological symptoms such as pseudo-allergic reactions (histaminic intoxication), food-induced migraines, and hypertensive crisis due to the interaction between monoamines and monoamine-oxidase inhibitor drugs [50]. However, a strain of *L. plantarum,* which is known to degrade histamine [51], was also identified as characteristic of the regenerative carrots. In that sense, the biological relevance or potential health effects of consuming ferments with more abundance of soil or environmental bacteria in contrast with LAB should be further investigated, especially in ferments, and particularly, in carrots, produced in regenerative systems.

4.3. Different Strains of Lactiplantibacillus plantarum Dominate Both Regenerative and Conventional Fermented Peppers

Lactiplantibacillus plantarum (L. plantarum) was the signature species identified in both the conventional and regenerative peppers, but different strains were detected between the two kinds of vegetables (Figure 4A). Unfortunately, the 16S rRNA long-read analysis could not identify the specific strains. *L. plantarum* is a common species detected in vegetable fermentations and numerous strains are known to produce bacteriocins, which are biologically active proteins or protein complexes that display antimicrobial properties, usually toward closely related species [52]. Studies indicate some strains of *L. plantarum* may be highly beneficial for health, albeit with different modes of action. For example, the bacteriocin activity of *L. plantarum* SF9C has been shown to effectively exert antibacterial activity against *Listeria monocytogenes* and *Streptococcus aureus*, both common contaminants found in fermented foods (56). Furthermore, the probiotic potential of *L. plantarum* SF9C has been demonstrated, as far as its capacity to successfully colonize the gastrointestinal tract [53].

Interestingly, another strain of *L. plantarum* (strain K21) has been shown to positively influence lipid metabolism. Supplementation of K21 given to mice on a high-fat diet was shown to alleviate body weight gain and epididymal fat mass accumulation, reduce plasma leptin levels, decrease cholesterol and triglyceride levels, and strengthen intestinal integrity [54]. Therefore, although this taxon was found across the three vegetables analyzed, mainly in carrots, and peppers, in particular, the high prevalence of *L. plantarum* in both regenerative and conventional peppers would place this fermented vegetable as an important source of this probiotic strain, at least compared with carrots and radishes. Interestingly, *L. plantarum* has also been identified as a key bacterium driving the production of important aroma compounds in fermented peppers [55], underscoring its important role in this ferment, both from organoleptic and potentially health-conferring standpoints.

4.4. Regenerative and Conventional Radishes Display Distinct LAB

The taxonomic signatures of the conventional radishes were species such as *Latilactobacillus sakei* (*L. sakei*), *Latilactobacillus curvatus* (*L. curvatus*), and *L. plantarum. L. sakei*, first

discovered as a "contaminant" of the rice wine sake, has been well studied due to its ability to colonize many different food habitats including sourdoughs, fermented vegetables, and fermented meats [56]. Similar to *L. plantarum*, it has been shown to produce potent bacteriocins, and the strain OK67, which was isolated from a kimchi, has also been shown to exert anti-obesogenic effects by reducing inflammation and increasing the expression of colon tight junction proteins in mice on a high-fat diet [57,58]. While less research has been conducted on strains of *L. curvatus*, a study on strain GH5L showed it was a powerful antioxidant [59], and another strain, HY7601, also demonstrated anti-obesogenic effects [60]. The regenerative fermented radishes were characterized by *Levilactobacillus brevis (L. brevis),* as well as *Lactococcus garvieae.* Strains of *L. brevis*, also commonly found in fermented dairy products, have been shown to be active producers of GABA and have probiotic potential, including beneficial effects against hyperlipidemia and neurodegeneration [61,62]. Therefore, these data do not indicate that, as far as the bacteriome at least, regeneratively produced ferments exhibit more potentially probiotic bacteria than their conventional counterparts. Perhaps, the probiotic potential in terms of content is similar, but may result in different kinds of benefits. This is a contention that requires further analyses.

4.5. Typical Fungi Characteristic of Fermented Foods and Beverages Found in All Fermented Vegetables but a Unique Species, Plectosphaerealla cucumerina, Discovered in Regenerative Carrots

All the fermented vegetables, regardless of growing system, were characterized by higher amounts of *Pichia kluyveri* and *Hanseniaspora*, which have been noted to be highly present in the middle to late stages of vegetable fermentations [63]. *Pichia kluyveri* is a common yeast found in different fermented foods and beverages, notably, in the wine and beer industry, as a bioprotective agent to reduce the use of sulfur dioxide (SO2) [64]. Research has shown that the metabolism of *Pichia kluyveri* allows it to increase esters and thiols (aroma-active compounds), which increase the quality of beverages [65]. Moreover, research on other species and strains of *Pichia,* such as *Pichia kudriavzevii,* have demonstrated possible probiotic qualities, showing that *Pichia* can survive the journey through the gastrointestinal tract [66,67]. When used as a starter culture along with *Lactobacillus fermentum* in a millet cereal (fermented gruel), which is often used as supplemental nutrition in addition to breast-feeding for young children in African countries, *Pichia kudriavzevii* was noted for its increase in intracellular folate after 24 h of growth [66]. *Hanseniaspora* is also a common non-*Saccharomyces* yeast frequently found in food and beverage fermentations, and particularly, in wine, where they play an important role in the beginning of fermentation, producing enzymes and aroma compounds that result in different wine colors and flavors [68].

Plectosphaerealla cucumerina (*P. cucumerina*) was identified as indicator fungi in all the regenerative carrots. While the literature on *P. cucumerina* is somewhat limited, it is known as a filamentous fungus that can cause root and collar rot in plants [69]. Recently, it has been identified as a possible bioherbicide, showing promising results against *Cirsium arvense* (a weed also known as creeping thistle) [70]. Furthermore, a comprehensive study aimed at characterizing natural compounds in *P. cucumerina* extract showed that it inhibited virulence factors and biofilm formation, including the disruption of pre-formed biofilms of *P. aeruginosa PAO1* [71]. This is of particular interest since it has been reported that nearly 80% of human infections are induced by microorganisms that produce biofilms, many of which are resistant to antibiotics [71,72].

Thus, the potential of both regenerative and conventional fermented vegetables as probiotic vectors may be similar, despite differences in the LAB and fungal taxa detected in each type of vegetable ferment. Both the conventional and regenerative peppers contained high amounts of *L. plantarum* compared to the different LAB discovered in the fermented radishes such as *L. brevis* in the regenerative version and *L. curvatus* and *L. sakei* in the

conventional one. Furthermore, the fermented carrots displayed higher amounts of *L. mesenteroides* and *L. herbarum*, while the regenerative fermented carrots showed much more environmental bacteria such as *R. planticola*. Exactly how these different bacteria interact with the detected fungal microorganisms such as *pichia kluyveri* and *hanseniaspora* to produce important and possibly health-boosting metabolites, especially in fermented foods, is a growing area of research. For example, a recent study identified non-conventional yeasts from a wide range of environmental sources (flowers, fruits, leaves, and mixed-fermentation beers) and determined that *Pichia kluyveri* (LAR001) and *Hanseniaspora uvarum* (PIT001) showed antimicrobial activity against potential pathogenic bacteria [67].

4.6. GABA Found in High Amounts in All Fermented Vegetables but Significantly Higher in Regenerative Ones

The metabolomic data show no clear distinctions in the abundance of most potentially health-conferring organic acids (acetate, propionate, butyrate, valerate, and lactic acid) between conventional and regenerative vegetables (Figure S4). However, the regenerative peppers displayed higher levels of butyrate, which has been substantially researched in the context of health benefits [73–75]. Whether regenerative fermented peppers can offer more health benefits as better butyrate sources, at least in comparison with conventional pepper ferments, needs to be tested further.

Out of all the amino acids detected, GABA was found to have the highest concentrations in all the fermented vegetables (Figure S11); it was found to be significantly higher in the regenerative carrots and peppers (Figure 6) and higher in the radishes, albeit not statistically significant. GABA is the main central nervous system (CNS) inhibitory neurotransmitter, and it plays an important role in regulating neuronal activity and improving sleep and mood [76–78]. In addition, GABA has been shown to exert other health benefits including anti-diabetic, anti-hypertensive, and anti-inflammatory properties [76,79]. Furthermore, alterations in central GABA receptor expression are implicated in the development of anxiety and depression, which are highly comorbid with bowel disorders [80].

Many of the aforementioned LAB found in the fermented vegetables are known to be important GABA producers including *L. brevis*, *L. plantarum*, *Lactococcus*, *Leuconostoc*, and *Weissella* [81]. The regenerative radishes contained high amounts of *L. brevis*, which has been shown to contain two distinct glutamic acid decarboxylase (GAD) systems for survival in high lactic acid conditions [82,83]). GABA is the end product of the decarboxylation of glutamic acid in LAB, which makes *L. brevis* a potent synthesizer of GABA [83].

It was notable that all the ferments had higher amounts of GABA compared to the autoclaved controls (Figure S12), demonstrating the important role of the starter microorganisms natively found on vegetables and/or in soil in the production of this essential amino acid in fermented vegetables, and placing fermented plants as a key nutritional resource for healthy brain metabolism and function [79]. These observations show that naturally fermented vegetables, and particularly, those of regenerative origin, could be better sources of metabolites that promote brain health. These benefits may be more significant than those conferred by nonfermented versions (e.g., vinegar pickling) or vegetables devoid of native soil microbiomes.

In this regard, increasing evidence has shown that the gut microbiome influences behavior through the bi-directional communication between the gut and brain, via the vagus nerve, immune system, and systemic circulation [80,84–86]. Critical to this communication are the two major classes of GABA receptors, which are currently the targets for anti-depressive and anti-anxiety medications [80,87,88]. Some studies have also shown the beneficial impacts of fermented foods on mood, including decreased social anxiety and depression in humans. For example, van de Wouw and colleagues (2020) found that mice consuming two different milk kefirs had a higher prevalence of LAB in their gut micro-

biome, specifically, *L. reuteri*, which was associated with an increase in GABA synthesis and a decrease in depressive-like behaviors. In fact, several studies have demonstrated that different LAB strains have been shown to reduce depressive symptoms in mouse models [80,89–91]. The mechanism proposed is that an increase in the LAB introduced in the gut (from fermented food ingestion) stimulates gut-derived GABA synthesis; however, it remains unclear whether gut-derived GABA can cross the blood–brain barrier and how this increase can ultimately improve mood [89,92].

The reasons behind the increased abundance of GABA in regenerative plant ferments, in comparison with conventional ones in this dataset, warrant further investigation. One mechanism we propose is that the use of herbicides and pesticides, even if minimal, could affect the capacity of bacteria such as *L. plantarum* and *L. brevis* to produce GABA [81]. This idea is supported by the association analyses shown in Figure 8, which indicate that these taxa may be important contributors to the high GABA pool found in the regenerative ferments, in addition to the higher diversity of other strains associated with GABA abundance in these ferments compared with the conventional versions. To the best of our knowledge, no other study so far has shown that the microbiome of fermented vegetables produced from regenerative farming might harbor greater potential to produce GABA in comparison to ferments made from conventionally grown vegetables. However, these contentions need to be tested in a wider pool of ferments produced from both systems, across different farms, including animal models that can demonstrate the health-conferring properties of ferments with differential GABA content, for instance, including models that address disorders affecting the CNS [93].

4.7. Study Limitations

Besides the type of land management and farming practices used, there are numerous factors that determine the nutritional quality and safety of vegetables including genotype (variety); geographical location (climate); soil factors, such as pH, available nutrients, texture, organic matter content, and soil-water relationships; weather factors, including temperature, rainfall, and light intensity; and post-harvest handling and storage; to name a few [27,94]. Additionally, several factors can influence the final product of a fermented vegetable, including those aforementioned, as well as the methods used to wash the vegetables, the cutting style, the type and amount of salt applied, whether the vegetables were salted directly or submerged in a brine, the fermentation environment, the temperature, the duration, and the use of any additional herbs or spices.

5. Conclusions

The interactions between the microbiome found in the production system soil and that in fermented vegetables may potentially influence human health when ferments are consumed, yet this relationship is still poorly understood [95,96]. These data show that fermented vegetables grown under different conventional or regenerative systems are unique in their microbiome compositions and amino acid profiles. The regenerative fermented carrots, in particular, harbored more microbial taxa from the environment on day 14 of fermentation, but all conventional and regenerative vegetables contained different strains of different potentially probiotic bacteria, such as *L. plantarum*, *L. brevis*, *pichia kluyveri*, and *hanseniaspora*. In addition, all fermented vegetables contained high amounts of GABA, which makes them a potential source of metabolites that positively modulate mood and behavior. However, it is remarkable that all regenerative fermented vegetables in our model showed more abundance of GABA compared to the conventional ferments. This observation should be further investigated in an experiment that controls for all the potential confounding variables and with greater sample sizes that especially

test whether regenerative farming systems stimulate the abundance of bacteria with more capacity to produce GABA. Furthermore, these data cannot assess any health benefits of the vegetables tested, meaning that the physiological effects of consuming different regenerative or conventional fermented vegetables need to be assessed in vivo. Because many microbes existing in the environment are difficult to culture in the laboratory [97], we speculate that studying the fermentation of vegetables under regenerative farming systems may be an innovative way to reveal how diverse bacterial and fungal species in the environment, and their metabolites, can positively impact human health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation11010022/s1. Figure S1: Alpha Diversity (Observed ASVs) of the Bacteriome and Mycobiome; Figure S2: Beta Diversity (Bray–Curtis Distances) of the Bacteriome and Mycobiome; Figure S3: Principal Component Analysis of Detected Metabolites on Day 14; Figure S4: PCA and Heatmap of Organic Acids Detected on Day 14; Figure S5: Lactic Acid Detected in Fermented Vegetables on Day 14; Figure S6: Pyruvate Detected in Fermented Vegetables on Day 14; Figure S7: Acetic Acid in Fermented Vegetables Detected on Day 14; Figure S8: Butyric Acid Detected in Fermented Vegetables on Day 14; Figure S9: Propionic Acide Detected in Fermented Vegetables on Day 14; Figure S10: Valeric Acid Detected in Fermented Vegetables on Day 14; Figure S11: Relative Abundances of Amino Acid Metabolites in Fermented Vegetables on Day 14; Figure S12: Relative Abundance of GABA (D14) in Fermented and Autoclaved Vegetables.

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