Meta-Omics Analyses of Organic and Conventional Fermented Vegetables Reveal Differences in Health-Boosting Potential

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Abstract

Naturally fermented vegetables may provide specific health benefits as they contain diverse nutrients, complex polysaccharides, probiotic microbes, and metabolites, which are transformed from fresh vegetables. Therefore, the kind of vegetable used to ferment and how they are grown may determine the types of health-promoting properties. To further understand the possible benefits of diverse fermented vegetables under distinct growing conditions, we compared the microbiome and metabolome of three different types of naturally fermented vegetables – carrots, peppers, and radishes, that were grown under conventional or regenerative organic growing systems. We profiled bacterial communities via 16S rRNA short read (V4 region) and long-read sequencing and fungal communities using ITS2 sequencing, in tandem with untargeted metabolomics (LC-MS). Results showed that the microbiome and metabolome of the fermented vegetables under each growing system is unique, highlighting distinctions in amino acid metabolites and potentially probiotic microbes (P<0.05). Regardless of the growing system, 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 organic 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 different vegetables grown in similar soils under different farming practices may influence the microbiome and metabolome of a fermented vegetable, with implications for their overall health-promoting potential.

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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][3][4][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 human 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 in the "old friends hypothesis," which states that lack of exposure to non-harmful or commensal microbes in the environment and in foods has been an important mechanism behind the increase of diseases, including those that dysregulate the immune system [10][11].

Fermented foods have been a traditional part of the diet in every known culture around the world[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 present in fermented foods [16], have been shown to positively influence gut microbiome composition and function [17][18][19], gut permeability [20], macronutrient digestion and absorption [21][22], and have anti-inflammatory and immunomodulatory effects [17][23][24][25][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.

In addition, the concept of "bugs as drugs," in which microbial ecosystems are explored as a repository from which bioactive or novel drugs may be mined and translated to human health [27], is an area of ongoing research. For example, rapamycin (RAP), a lipophilic macrolide that helps suppress and regulate the immune system, was discovered from a strain of S*treptomyces hygroscopicus* isolated from a soil sample obtained from the Vai Atore region of Easter Island (Rapa Nui) [28][29]. Thus, the microbial ecosystem of a fermented food, particularly fermented vegetables, provides a unique opportunity to explore analeptic properties and bioprospecting of probiotic microorganisms and health boosting compounds, given their important relationship and proximity to soil ecosystems.

Regenerative, or "regenerative organic" agriculture refers to farming practices that go beyond organic certifications to rebuild soil organic matter and soil health. These farming practices, which include cover crops, crop rotation, no-till and other practices that actively rebuild soil communities, including the integration and rotation of livestock on the land, are thought to result in more microbial soil life, and therefore, more nutrient-rich food [6][30]. However, the way these regenerative farming practices impact the microbiome of diverse fermented vegetables as probiotic vectors or vehicles of health boosting metabolites, in contrast with vegetables produced from conventional systems (that permit the use of pesticides), has not been thoroughly explored.

In this study, we conducted multi-omics analyses, including short-read and long-read 16S rRNA gene amplicon sequencing to profile bacterial communities at increased resolution, ITS2 amplicon sequencing to profile fungal communities and Liquid Chromatography-Mass Spectrometry (LC-MS) to profile metabolites in three different vegetables: carrots, peppers, and radishes. These vegetables were grown under either conventional or regenerative farming practices at closely located farms in Minnesota and microbiome was screened along 14-days of fermentation. By investigating the microbiome composition and metabolome profiles of different fermented vegetables produced under these two production systems, we shed light on how regenerative and conventional farming practices impact the abundance of potentially probiotic microorganisms and metabolites of importance for human nutrition and health maintenance.

Materials and Methods

Selection of Conventional and Regenerative Organic Produce

Conventional and certified organic vegetable farms located around the Delano region of Minnesota (MN) (45.0419° N, 93.7891° W) were selected for their carrots (*Daucus carota subsp. sativus*), peppers (conventional = *Capsicum* and organic = *Capsicum annuum*) and radishes (*Raphanus sativus*). Farms were located 28 miles from each other. Each farmer/grower described the soil as sandy loam, which are typical soil types in this region of Central MN. Three pounds of each vegetable were purchased from the farms. In an effort to ferment all the vegetables on the day of purchase, the conventional vegetables were picked up at a local farmer's market. The certified regenerative organic vegetables were picked from the ground with the farmer and washed at the farm. All produce was brought to the University of Minnesota to undergo natural lactic acid fermentation.

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 organic farmer had been farming the land for 29 years. During this time, the land has been farmed with regenerative agricultural practices including the utilization of cover crops, green manure crops, compost, and mined minerals as soil inputs and very little to no tilling. Crop rotation was also utilized to prevent weeds, diseases, and insects, as well as to feed soil microbes. 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 "regenerative organic" or "organic" in this study.

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. Vegetables were thoroughly washed 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

(50g/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 minutes. The autoclaved jars were used as 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 hours), day 3 (72 hours), day 7 (168 hours) and day 14 (336 hours) along the fermentation process for a total of n=216 samples (please see **Supplementary Table 1** 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 the metabolomic analyses. For a detailed view of the study schematic design, please see **Figure 1**.

Figure 1 - Schematic Study Design

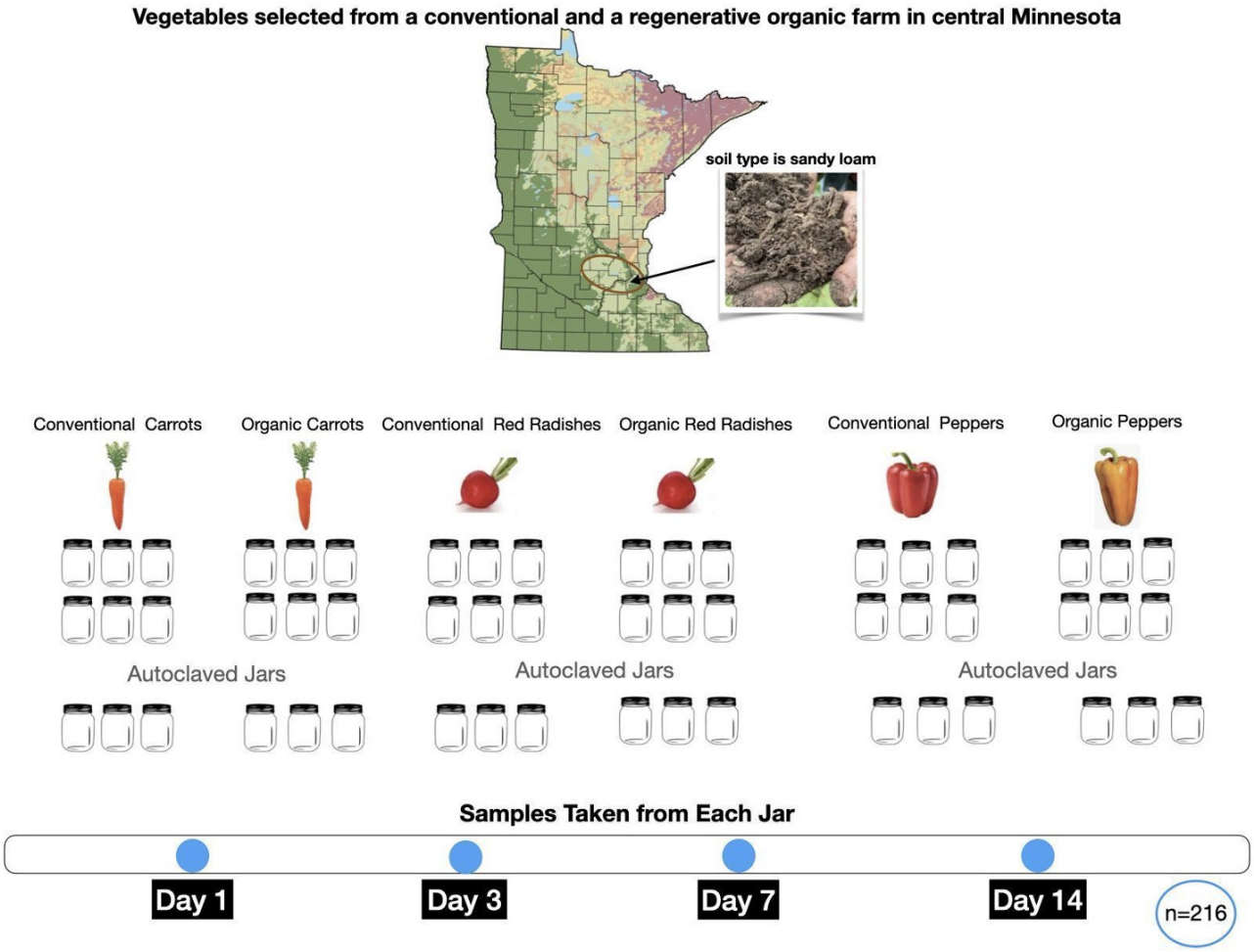

Figure 1. Study schematic design. Carrots, peppers and radishes were purchased from two different farms - one conventional farm and one regenerative organic farm in Central Minnesota. Vegetables were washed, chopped, and covered in a salt brine (50g/L Diamond Crystal kosher salt) for a “natural fermentation” period of 14 days. Three jars from each group were autoclaved for 30 minutes as controls. Samples were taken and pH recorded on days 1, 3, 7 and 14 (n=216 total samples).

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). 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 were used. Sequencing of pooled libraries was carried out using Illumina MiSeq platform at the University of Minnesota Genomics center (UMGC) to generate 2*300 bp of sequences. 16S rRNA 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 which were not present in at least 5 samples (~3% of total samples) were omitted from the data set.

Microbiome Analyses - 16S rRNA long read sequencing

Given the probiotic content of fermented foods, we also profiled bacterial communities targeting the entire 16S rRNA gene to obtain 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] and [34]. Briefly, the LoopSeq™ 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 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 then were 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, the unique sample barcode demultiplexing, and synthetic longread 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-leads tagged with the same unique barcode. The complete preparation and sequencing protocol can be found in has been detailed

previously [33].

Metabolomic analyses

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.

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 or amino acid standard 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.

LC-MS analysis. The workflow of LC-MS analysis was described previously(Ma et al. 2019). 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 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.

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 (Waters). 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.

Statistical Analyses

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, permutational multivariate analyses of variance (PERMANOVA), multiple R packages such as vegan, ape, phyloseq were used [36][37][38]. 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 [39]. 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).

Metabolomic Analysis. Principal component analyses and heatmaps were generated using the Metaboanalyst 5.0 online platform [40]. 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.

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.

Results

Microbial alpha diversity for ferments under conventional or organic farming practices

Bacteriome: After 24 hours of fermentation (D1) the only differences observed in bacterial alpha diversity (*Shannon Index*) between conventional and organic 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 conventional and organic ferments for any vegetable. Remarkably, by day 14 of the fermentation process, the autoclaved conventional samples showed greater bacterial diversity compared with their organic 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**; $P=0.03$).

Mycobiome: Fungal Shannon diversity tended to be higher for all conventional vegetables compared with the organic 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 organic carrots, especially in the last two days of fermentation (D7 and D14), no statistical significance was reached (**Figure S1B**). Like patterns observed with the bacteriome, autoclaved conventional samples showed greater fungal diversity, but 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**).

Alpha diversity differed across fermentation time

Bacteriome: In the bacteriome, significant differences were observed in all the conventional and organic 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 organic peppers, showing a decreasing trend over time **(Figure 2A**; Kruskal-Wallis test; $P=0.05$).

Mycobiome: In the mycobiome, a significant difference across fermentation days was observed with the organic fermented carrots (**Figure 2B** Kruskal-Wallis test; $P=0.002$) and the conventional fermented peppers ($P=0.03$), while both the conventional and organic radishes showed 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.

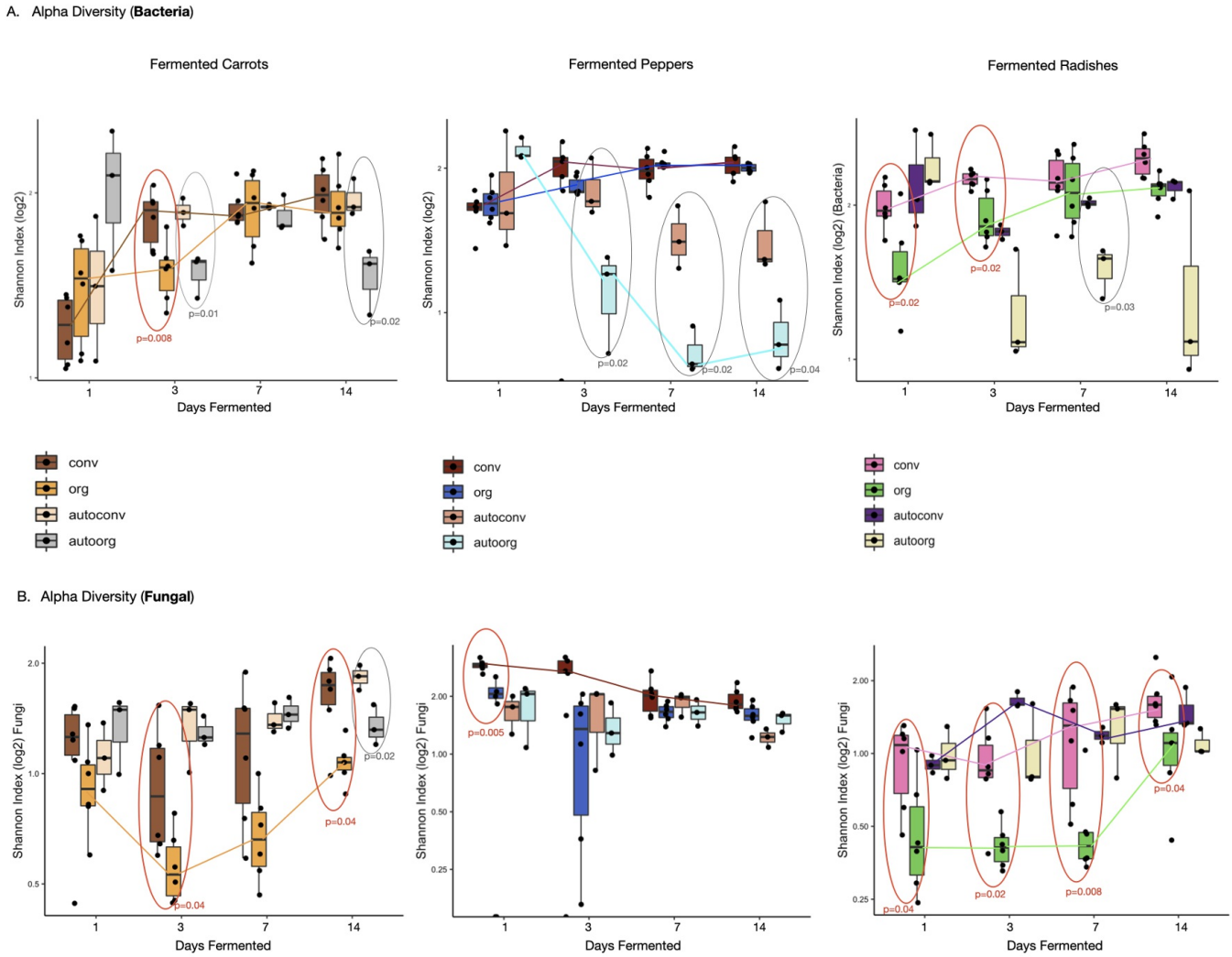

Figure 2. Bacterial and Fungal differences in alpha diversity of fermented vegetables. Alpha diversity (Shannon Index) for bacteriome (**A**) and mycobiome (**B**) of regenerative organic and conventional fermented carrots, peppers and radishes during a 14-day fermentation period. Statistical significance calculated with Wilcoxon rank-sum test within a day between conventional and organic samples (red circles) and autoclaved conventional and autoclaved organic 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.

Conventional and organic 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 system (conventional or regenerative organic), or whether they are autoclaved or not, harbors 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 organic carrots, which showed more unique compositional patterns through the fermentation process. In addition, the mycobiome of autoclaved peppers, regardless of 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 organic ferments displayed significantly different microbiome composition (**Figure 3A and 3B**). For the most part, although the autoclaved samples clustered close to their conventional or organic counterparts, they usually showed unique compositional patterns. However, in the radishes mycobiome, all conventional samples continually showed substantially distinct fungal communities compared with autoclaved or organic samples. Interestingly, in both the bacteriome and mycobiome of the fermented peppers, the conventional and organic samples remain very distinct from their autoclaved counterparts.

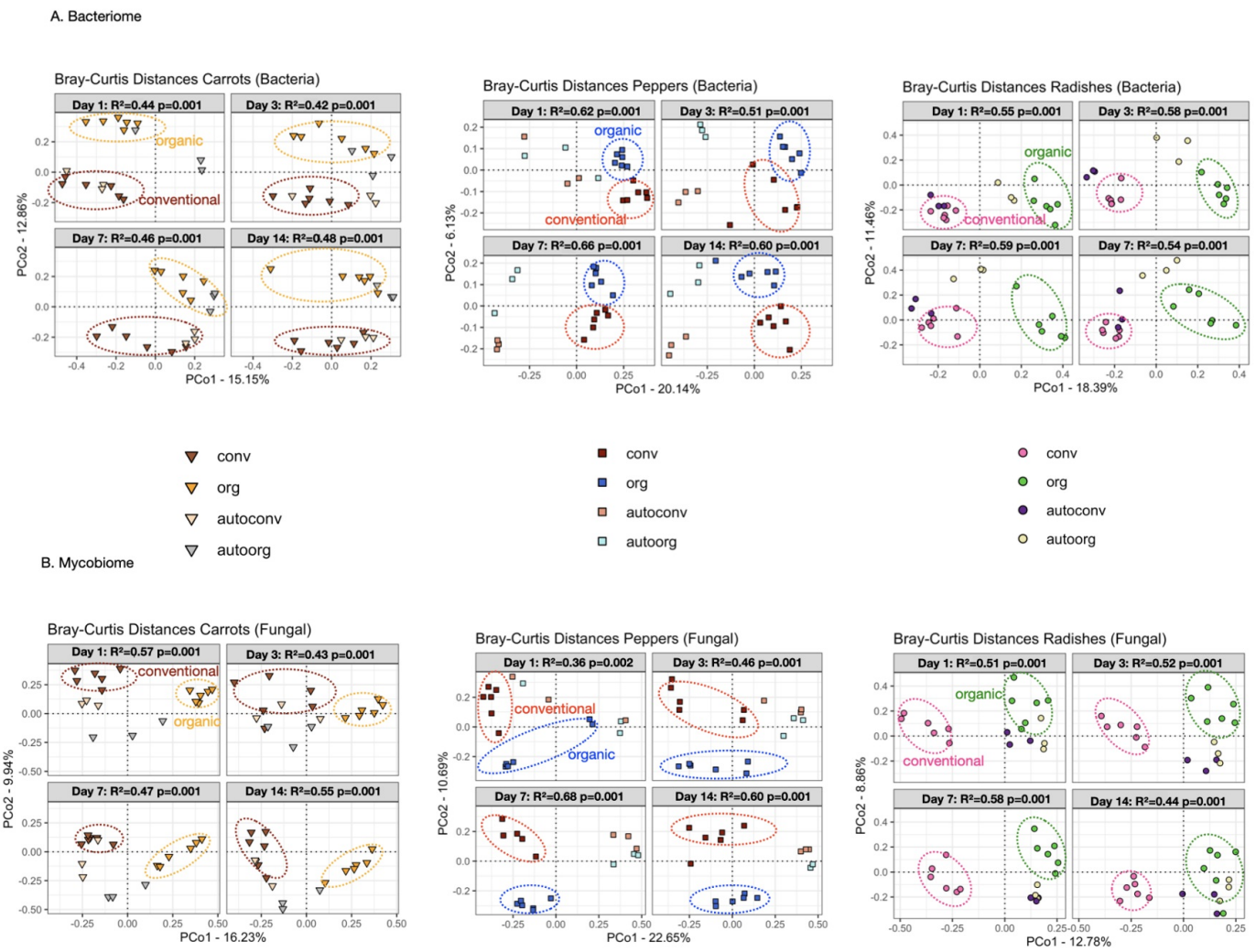

Figure 3. Beta Diversity of bacterial and fungal communities reveal unique differences in microbiome composition. UnweightedBray-Curtis dissimilarity metric of (A) bacterial communities and (B) fungal communities in conventional, organic, autoclaved conventional, and autoclaved organic fermented carrots,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

Distinct taxonomic signatures differentiate conventional and organic fermented vegetables

To characterize ASVs and strains of bacteria and fungi that contributed to differences in 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 ASVs, **Figure 4B** for 16S rRNA long-read taxa, and

Figure 4C for ITS2 ASVs. (Indicator value > 0.5, $P < 0.05$ (Wilcoxon rank-sum test with FDR) – for all indicator values, see **supplementary Table 2**).

Bacterial ASVs commonly known to be present in fermented vegetables such as*Leuconostoc mesenteroides* and other lactic-acid bacteria (LAB) in the families Leuconostocaceae and Lactobacillaceae, characterized the fermented conventional carrots. In contrast, bacteria typically found in soil environments, such as the Enterobacteriaceae family, distinguished fermented organic carrots (**Figure 4A, left panel**). In the fermented peppers, LAB such as*Levilactobacillus brevis* and Lactobacillaceae were found to be signatures of the organic fermented peppers, while the conventional fermented peppers showed higher abundance of Enterobacteriaceae but also *Leuconostoc* (**Figure 4A, middle panel)**. Organic fermented radishes were mainly characterized by higher abundance of *Lactococcus garvieae* and *Levilactobacillus brevis.* In contrast, conventional fermented radishes showed higher amounts of*Leuconostoc mesenteroides*, Lactobacillaceae and *Weissella* (**Figure 4A, right panel**).

In general, indicator species analysis based on long,16S rRNA reads displayed different discriminant taxa compared with the ASV-level analyses for all vegetables (**Figure 4B**), indicating taxonomic disparities between the two methods. For instance, indicator species of the conventional fermented carrots were mainly LAB such as *Lactipantibacillus herbarum* and Leuconostoc *mesenteroides,* while the indicator species of the organic 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 quite different compared with the analyses at the ASV level. Many strains identified as *L. plantarum* characterized both the conventional and organic 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 organic fermented peppers displayed many different strains of*Lactiplantibacillus plantarum*, as well as *Enterobacter cloacae* and *E. cloacae complex* (**Figure 4B, middle panel**).

Long-read bacterial species characteristic of the fermented radishes were all identified as LAB. Only two indicator species were found in the organic 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 4B, right panel**).

Similar to other studies[41][42] our analysis of fungal communities also showed that many of ITS2 sequences generated could not be assigned to any taxonomic group. Nonetheless, some indicator species were evident. Organic 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 4C, right panel**). Indicator species discovered in the fermented organic 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 4C, middle panel**). Like the

fermented peppers, the fermented organic radishes were mainly distinguished by 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 4C, right panel**).

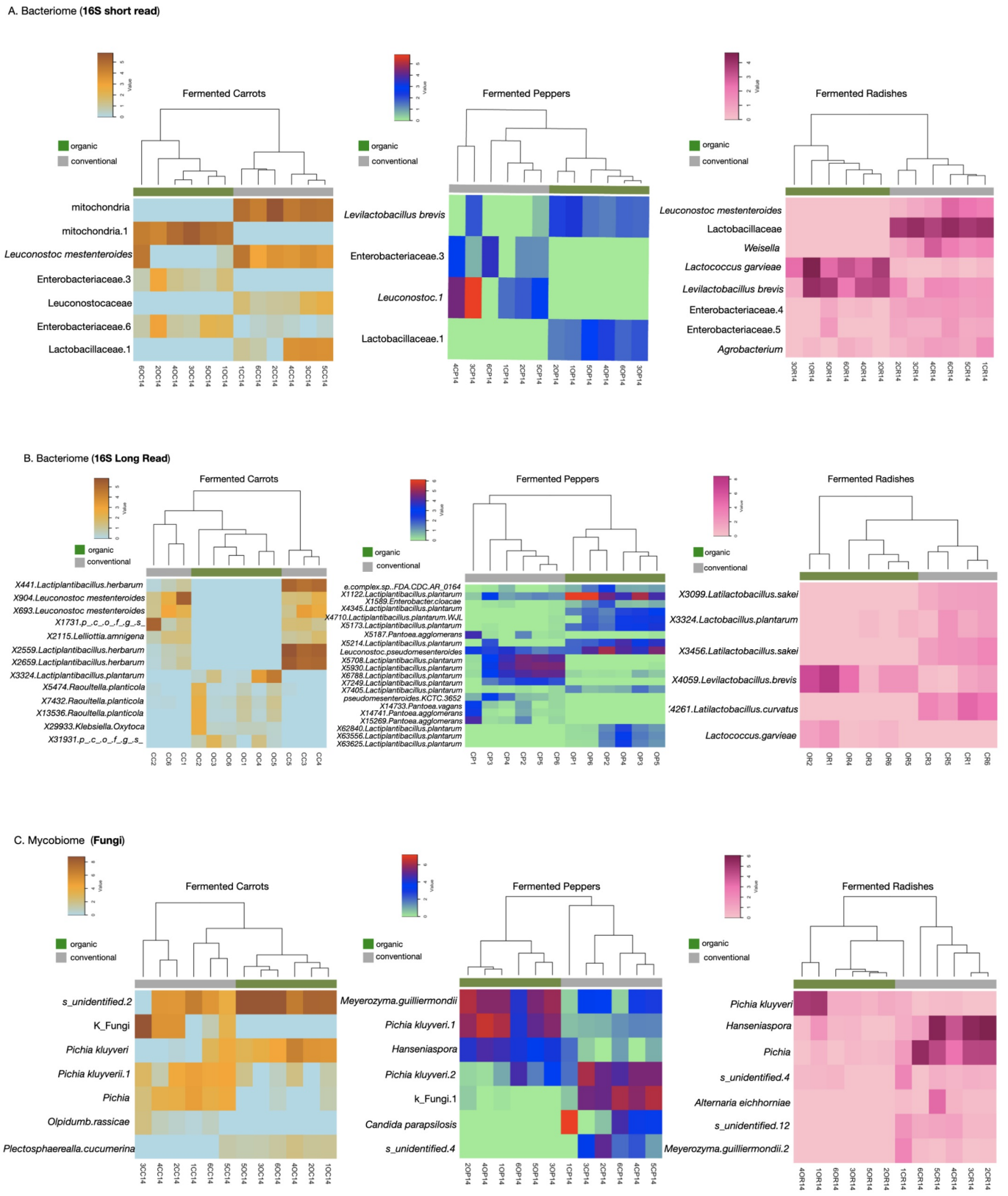

Figure 4. Heatmaps of indicator species on day 14 of fermentation (A)Indicator species analysis of (A) bacteriome (16s rRNA short-read)

(B)16s rRNA long-read and (C) ITS2-mycobiome. Indicator values > 0.5 and *P* <0.05 (Wilcoxon rank-sum test with FDR).

Metabolome profiles for conventional and organic fermented vegetables

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

No pattern observed in the abundance of organic acids in conventional vs. organic growing systems

As expected, lactic-acid was found to be several fold 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). As expected, the same trend was found with pyruvate, an intermediate for lactic acid production, with its abundance always more prevalent in fermented vegetables compared with autoclaved vegetables (P<0.05; Figure **S6A**). When conducting pair-wise tests between growing systems, it was found that the organic 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. For instance, short chain fatty acids, which are considered to be potential health-conferring metabolites in these foods, only show a differential abundance between fermented and autoclaved vegetables only in radishes and specifically for butyrate, propionate and valerate (P<0.05, **Figures S8-S10)**. However, just as with lactic acid, patterns between conventional and organic systems were inconsistent; for example, in carrots, acetate, propionate, and butyrate were always higher in conventional vegetables (P<0.05, **Figures S7B, S8B, S9B**). However, for peppers, butyrate and propionate were more abundant in organic versions of the vegetable. For radishes, except for lactic acid, no other organic acid was differentially abundant between growing systems.

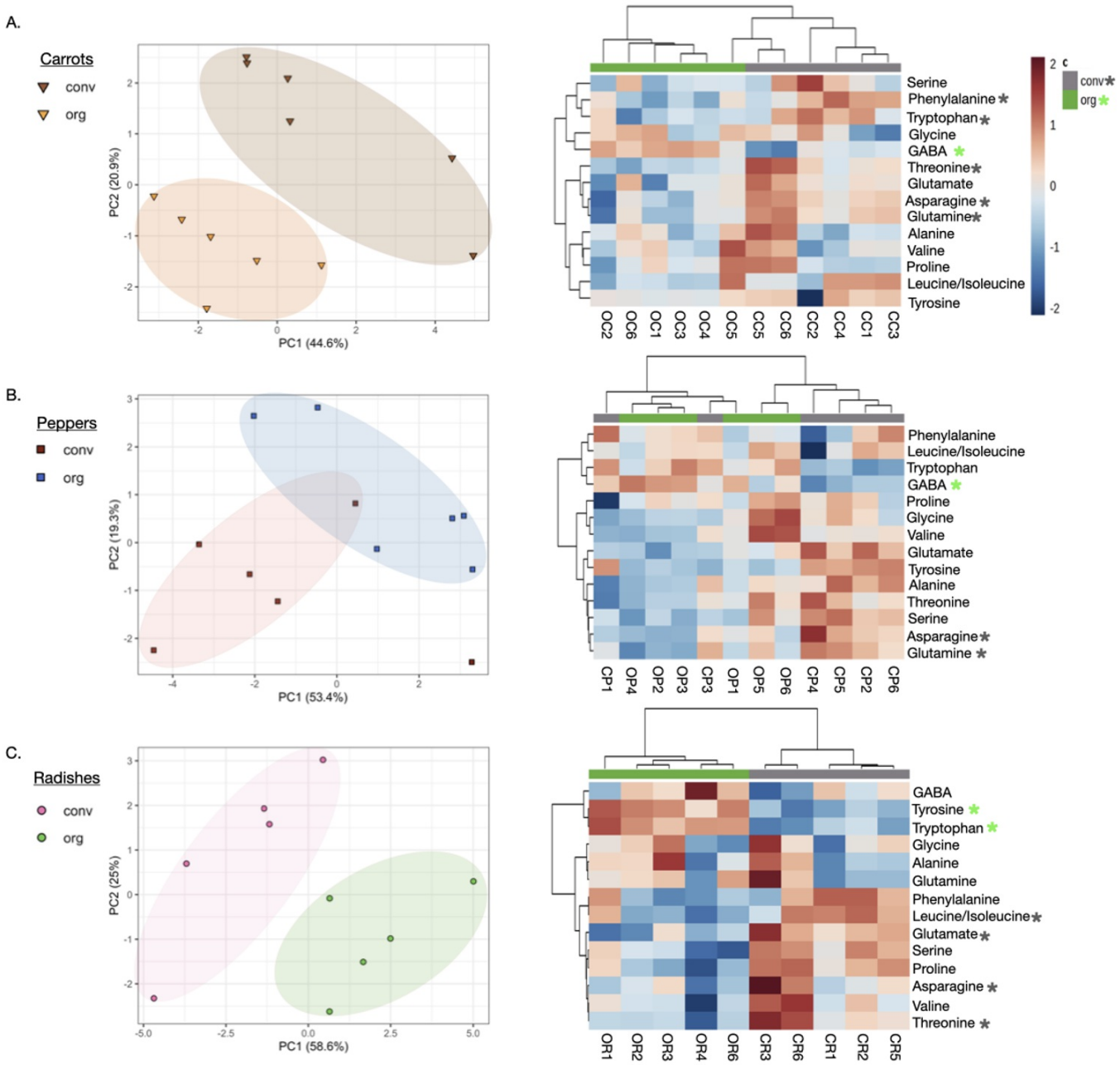

Figure 5. PCA and Heatmap of identified amino acid metabolites in fermented vegetables on day 14. (A)organic and conventional fermented carrots (B) conventional and organic radishes and (C) conventional and organic peppers. PCAs based upon normalized data (normalization by sum; log transformation; autoscaling) 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 *.

Higher amounts of GABA discovered in all fermented vegetables

Similar to the microbiome, principal component analysis (PCA) of detected amino acids on Day 14 of fermentation found distinctions between the organic 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 organic and conventional vegetables compared to the 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 organic and conventional ferments. GABA was observed to be significantly higher in the organic fermented carrots and peppers (**Figure 6, A-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 GABA abundance was also numerically higher in organic radishes, the difference was not statistically significant (**Figure 6C**).

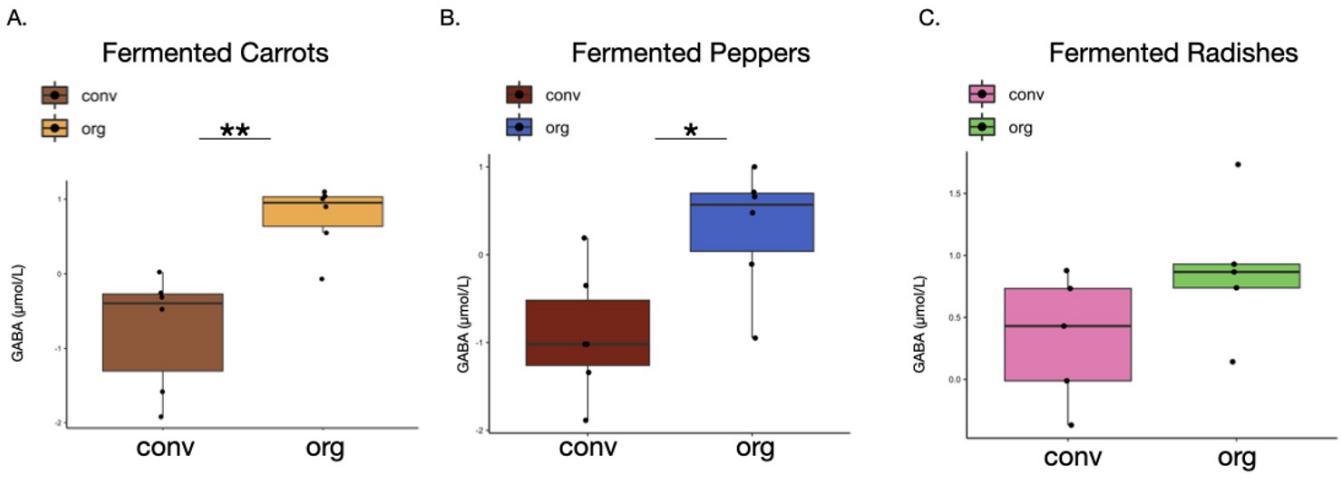

Figure 6. Amounts of GABA found in conventional and organic fermented vegetables. Boxplots of the amounts of GABA found between conventional and organic fermented vegetables (normalization by sum; log transformation; auto-scaling). Statistical significance calculated by Wilcoxon rank-sum test; * p-value ≤ 0.05; ** p-value ≤ 0.01.

To further understand the possible drivers of increased GABA in organic vegetables, we performed a Spearman's rank correlation analysis based on the relative abundance of taxa detected via 16S long read taxa 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=1.183e-06) across all the dataset, including both conventional and organic samples. We then separated samples according to system (only the organic 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 organic fermented vegetables; specifically, the organic fermented peppers and radishes, which seemed to drive this strong correlation (**Figure 7**; r=0.87; p-value=4.691e-06). On the other hand, no significant correlation was detected with the conventional fermented vegetables between GABA and *L. plantarum*, despite similar abundance patterns of GABA. 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

between organic and conventional vegetables, particularly in peppers.

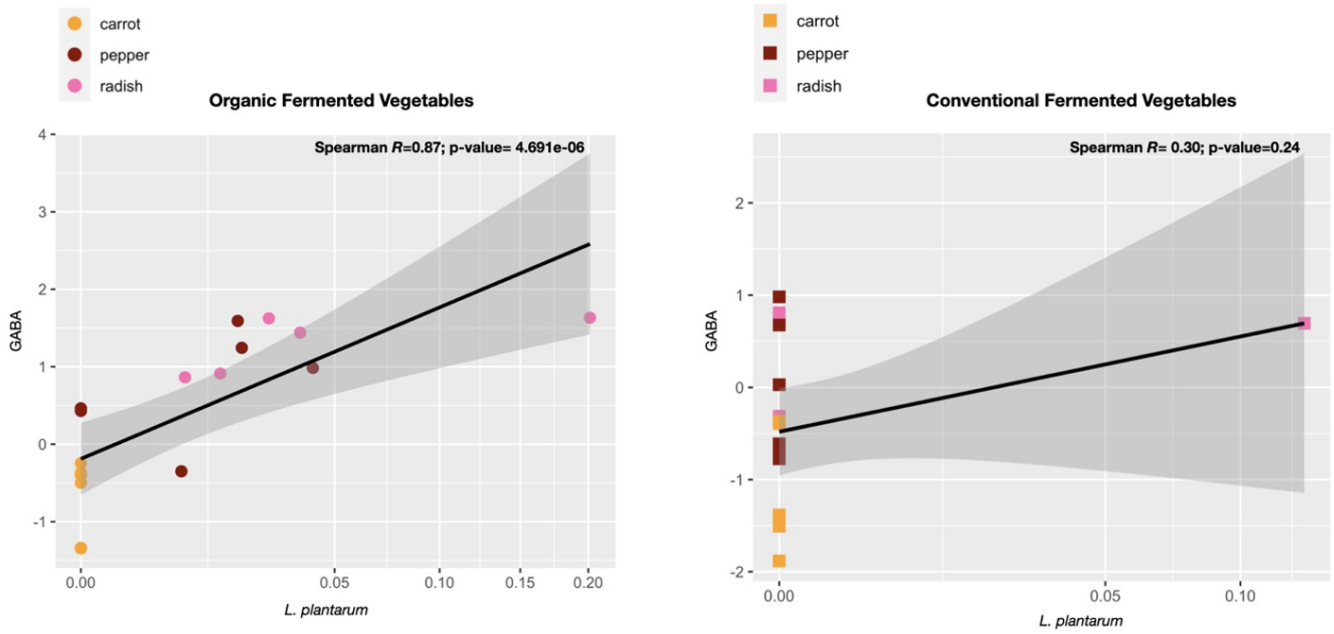

Figure 7. GABA is positively correlated with a strain of *L. plantarum* in the organic fermented vegetables. Scatterplots of Spearman's correlation between GABA (normalization by sum; log transformation; auto-scaling) and the relative abundance of a strain of *L. plantarum* in the organic fermented vegetables (Spearman *R*=0.87; *P*=4.691e-06) 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.

Network dynamics reveal GABA is more strongly correlated to probiotic bacterial taxa in fermented vegetables from the regenerative organic growing system

Assuming that 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; p-value<0.05) between GABA and all bacterial taxa from either conventionally grown fermented vegetables or regenerative/organically grown fermented vegetables, showed that GABA has a greater number of stronger positive correlations with more bacterial taxa in organic systems (**Figure 8**). Among these associations were bacteria known for their probiotic potential including strong associations with different strains of *L. plantarum* and *Levilactobacillus brevis* (*L. brevis*) (Figure 8; Organic Network; highest correlation GABA to*L. plantarum*; r=0.87, p-value= 4.69E-06 as stated above, and GABA to*L. brevis*; r=0.82; p-value=4.69E-06, 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 *L. mesenteroides*, *L. plantarum* and *Lactococcus lactis*. However, there were smaller numbers 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.

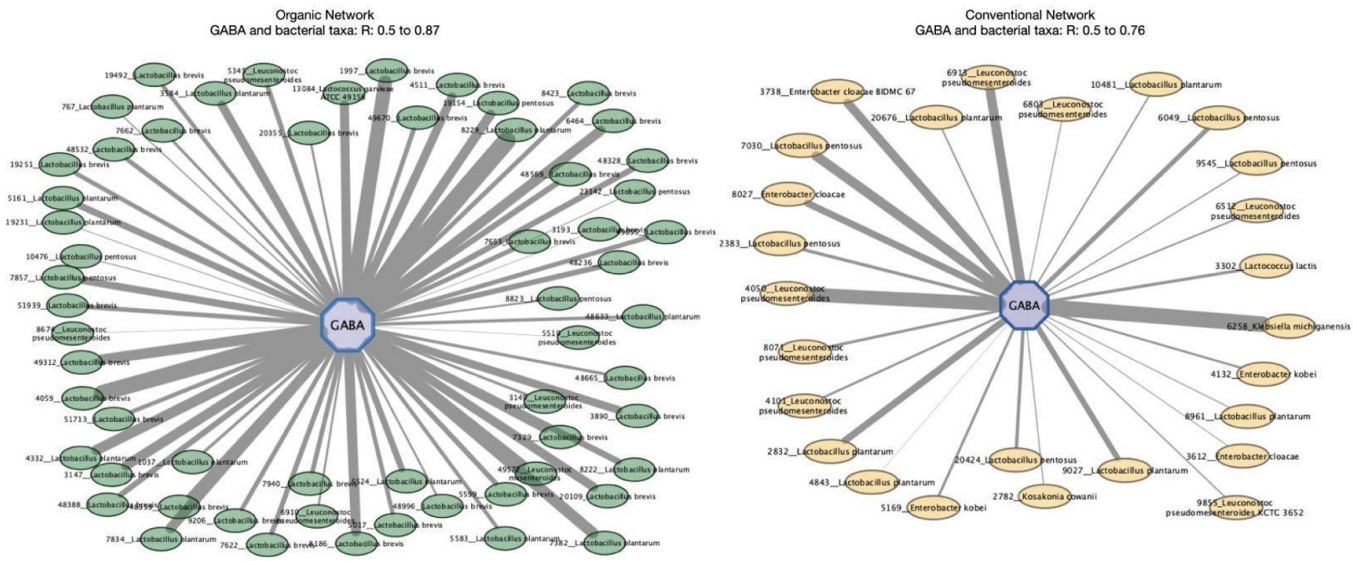

Figure 8. Networks between GABA and bacterial taxa in organic and conventional fermented vegetables. Analysis of the strong positive correlations between GABA and bacterial taxa ($r > +0.5$; p-value<0.05) of all the organic fermented vegetables and conventional fermented vegetables shows that GABA has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

Discussion

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

Regenerative organic and conventional fermented vegetables display minor differences in microbial alpha diversity but have unique microbiome compositions

We originally hypothesized that organic fermented vegetables would harbor more microbial alpha diversity compared with conventional ferments, since, in theory, regenerative organic soil practices yield higher soil microbial diversity, as shown previously [43][44][45]. However, the opposite was observed across several fermentation days. For instance, only minor differences were noted in the bacteriome, with generally greater bacterial diversity in conventional ferments mostly early in the fermentation process. However, no significant differences were detected on the last day of fermentation (D14) in this experiment. Significant differences were noted in the fungal communities of the fermented carrots and radishes, with higher mycobiome alpha diversity observed for conventional fermented carrots and radishes at day 14 (last day of fermentation), compared to the organic ferments (**Figure 2B)**.

The high fungal diversity in conventional vegetables is noteworthy. In the context of food safety, higher diversity of fungal communities may result in the risk of fungi producing higher levels of mycotoxins in the fermented vegetable [46]. A number of strains of LAB are reported to produce antifungal metabolites (lactic acid, phenyllactic acid, indole, 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 [47]. Therefore, a potential avenue of further research may focus on whether LAB communities in the organic 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 [48][49] but there is no evidence showing that organically produced fermented vegetables may be safer for consumption than a conventionally produced one.

Environmental bacteria found to be more characteristic of fermented carrots

Species distinctive of the organic regenerative fermented carrots were mostly species belonging to the Enterobacteriaceae family, which are abundant in soil microbiomes (**Figure 4B**). The long read 16S rRNA analysis, which provided better taxonomic resolution, 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 [50]. They are also known colonizers of animals and humans - mainly in the upper respiratory and gastrointestinal tracts but can become pathogenic in immunocompromised people [51]. This species of bacteria is also a known histamine producer, which is a common biogenic amine found in many foods and beverages, including fermented vegetables [52]. 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 [52]. However, a strain of *L. plantarum,* which is known to degrade histamine [53], was also identified as characteristic of the regenerative organic 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 carrots, produced in organic systems.

Different strains of *Lactiplantibacillus plantarum* dominate both regenerative organic and conventional peppers

Lactiplantibacillus plantarum (L. plantarum) was the signature species identified in both the conventional and organic peppers, but different strains were detected between the two kinds of vegetable **(Figure 4B).** 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 [54]. Studies indicate some strains of *L. plantarum* may be highly beneficial for health, albeit with different modes of action. For example, 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[55]. Furthermore, *L. plantarum* SF9C was shown to survive harsh gastrointestinal tract conditions in a rat model, making it an important probiotic strain [55]. 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, as well as strengthen intestinal integrity [56]. 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 organic and conventional peppers, would place this vegetable as an important source of this probiotic strain, at least compared with carrots and radishes. Interestingly, *L. plantarum* has also been identified as key bacteria driving the production of important aroma compounds in fermented peppers [57], underscoring its important role in this ferment, both from organoleptic, and potentially health conferring standpoints.

Regenerative organic and conventional radishes display distinct LAB

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 its ability to colonize many different food habitats including sourdoughs, fermented vegetables and fermented meats [58]. Similar to *L. plantarum*, it has been shown to produce potent bacteriocins, and 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 [59][60]. While less research has been conducted on strains of *L. curvatus*, a study on strain GH5L showed it was a powerful antioxidant[61] and another strain, HY7601, also demonstrated anti-obesogenic effects [62]. The organic 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 [63][64]. Therefore, these data do not indicate that, as far as the bacteriome at least, organically produced ferments exhibit more potentially probiotic bacteria than their conventional counterparts. Perhaps, the probiotic potential as far as content is similar, but may result in different kinds of benefits. This is a contention that requires further analyses.

Typical fungi characteristic of fermented foods and beverages found in all fermented vegetables but a unique species, *Plectosphaerealla cucumerina,* discovered in regenerative organic 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[65]. *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) [66]. 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[67].

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[68][69]. When used as a starter culture along with *Lactobacillus fermentum* in a millet cereal (fermented gruels), which are 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 hours of growth [68]. *Hanseniaspora* is also a common non-*Saccharomyces* yeast frequently found in food and beverage fermentations, 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 [70].

Plectosphaerealla cucumerina (P. cucumerina), was identified as indicator fungi in all the regenerative organic 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 [71]. Recently, it has been identified as a possible bioherbicide, showing promising results against *Cirsium arvense* (a weed also known as creeping thistle)[72]. Furthermore, in a comprehensive study to understand more about natural compounds that can disrupt biofilm formation from certain microorganisms, it was discovered that *P. cucumerina* extract both inhibited virulence factors and biofilm formation and disrupted pre-formed biofilms of*P. aeruginosa PAO1* [73]. 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 [73][74].

Thus, the potential of both regenerative organic 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 organic peppers contained high amounts of *L. plantarum* compared to the different LAB discovered in the fermented radishes such as *L. brevis* in the organic radishes and*L. curvatus* and *L. sakei* in the conventional version. Furthermore, the fermented carrots displayed higher amounts of *L. mesenteroides* and *L. herbarum*, while the organic 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 [69].

Also, no clear patterns emerged as far as the abundance of potentiality health conferring organic acids (acetate, propionate, butyrate, valerate and lactic acid) between conventional and organic vegetables (**Figure S4**). The organic peppers displayed higher levels of butyrate, which has been substantially researched in the context of health benefits [75][76][77]. Whether organic fermented peppers can offer more health benefits as better butyrate sources, at least in comparison with conventional pepper ferments, needs to be tested further.

GABA found in high amounts in all fermented vegetables but significantly higher in organics

Out of all the amino acids detected, GABA was found to have the highest concentrations in all of the fermented vegetables (Figure S11), and it was found to be significantly higher in the organic 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 [78][79][80]. In addition, GABA has been shown to exert other health benefits including anti-diabetic, anti-hypertensive, and anti-inflammatory properties [78][81]. Furthermore, alterations in central GABA receptor expression are implicated in the development of anxiety and depression, which are highly comorbid with bowel disorders [82].

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* [83]**.** The organic radishes contained high amounts of*L. brevis*, which has been shown to contain two distinct glutamic acid decarboxylase (GAD) systems for acid resistance [84][85]. GABA is the end product of the decarboxylation of glutamic acid in LAB, which makes *L. brevis* a potent synthesizer of GABA [85]*.* 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 soil in the production of this essential amino acid in fermented vegetables, placing fermented plants as a key nutritional resource for healthy brain metabolism and function [81]. These observations show that naturally fermented vegetables, and particularly those of organic 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 the gut microbiome influences behavior through the bi-directional communication between gut and brain axis via the vagus nerve [82][86][87][88]. Critical to this communication are the two major classes of GABA receptors, which are currently the targets for anti-depressive and anti-anxiety medications [82][89][90]. 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 higher prevalence of LAB in their gut microbiome, 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 [82][91][92][93]. 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 [91][94]. In addition, the reasons behind the increased abundance of GABA in organic plant ferments, in comparison with conventional versions, warrant further investigation. A clear path would center on investigating how pesticides affect the soil and phyllosphere microbiome as starter cultures and sources of this key metabolite. This pathway should also include testing whether organic plant ferments have more beneficial effects on behavior outcomes.

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]. These

data show that fermented vegetables grown under different conventional or regenerative organic systems are unique in their microbiome composition and amino acid profile. The organic fermented carrots, in particular, harbored more microbial taxa from the environment on day 14 of fermentation, but all conventional and organic 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 organic fermented vegetables showed more abundance of GABA than the conventional ferments. The reasons behind this observation need to be further investigated with greater sample sizes, especially whether organic farming systems stimulate the abundance of bacteria with more capacity to produce GABA. However, these data cannot assess any health benefits of the vegetables tested, meaning that the physiological effects of consuming different organic or conventional fermented vegetables need to be assessed in vivo. Most microbes existing in the environment are difficult to culture in the laboratory [96] and we posit that the studying the fermentation of vegetables under regenerative organic farming systems may be an innovative way to reveal novel bacterial and fungal species in the environment and their bioprospecting potential, as sources of metabolites able to positively impact human health.

Supplementary material

This material is available from the Supplementary data section and can be downloadedhere.

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