

APPENDIX Tables and Figures:

Appendix A

Table A1 Chemical characteristics of biological soil amendments collected from 6 farms*

Collection farm	Compost type	Total Nitrogen (%)	Carbon (%)	C:N (%)	Organic matter (%)	Moisture content (%)	EC (soluble salts; mmhos/cm)	pH
Poultry farm #1	Active compost	1.64±0.02	27.77±1.12	16.97±0.45	51.95±2.76	39.71±1.15	10.47±0.46	8.20±0
	Finished compost	1.85±0.13	28.525±1.31	15.41±0.36	60.15±4.88	56.67±0.16	6.18±0.25	6.45±0.07
Poultry farm #2	Active compost	3.13±0.07	38.37±0.69	12.27±0.49	73.45±0.64	25.68±0.06	19.20±2.27	8.70±0
	Finished compost	3.50±0.01	34.19±1.55	9.77±0.47	65.10±2.83	22.95±0.09	18.88±0.35	8.75±0.07
Poultry farm #3	Active compost	1.78±0.07	21.36±0.71	12.02±0.9	34.42±0.25	26.9±0.01	5.24±0.03	8.75±0.07
	Finished compost	1.59±0.1	17.55±2.13	11.01±0.65	27.19±3.05	32.54±1.51	3.62±0.09	8.40±0
Dairy farm #1	Active compost	1.52±0.06	24.43±0.91	16.08±0.04	46.55±0.92	26.74±0.06	5.12±0.33	8.5±0
	Finished compost	1.62±0.02	22.22±1.07	13.74±0.85	36.3±1.41	24.38±0.01	4.94±0.21	8.45±0.07
Dairy farm #2	Active compost	1.48±0.03	19.57±0.62	15±0.17	40.7±0.17	53.5±0.84	6.84±0.48	8.45±0.07
	Finished compost	1.72±0.03	19.57±0.62	11.34±0.17	35.95±3.18	47.56±3.53	6.94±0.48	7.85±0.07
Dairy farm #3	Active compost	2.47±0.11	72.4±4.93	29.35±0.76	86.78±0.04	83.08±1.38	7.11±0.71	8.8±0.14
	Finished compost	3.21±0.66	52.94±10.27	16.53±0.25	77.27±0.81	81.77±3.63	5.32±0.86	8.65±0.07

*Chemical characteristics were calculated by dry weight.

Table A2 Microbiological analysis of biological soil amendments collected from 6 farms*

Collection farm	Compost type	Total aerobic bacteria (log CFU/g)	Heterotrophic (log CFU/g)	Thermophiles (log CFU/g)	Enterobacteriaceae (log CFU/g)	Yeast/Mold (log CFU/g)	Actinomycetes (log CFU/g)
Poultry farm #1	Active compost	8.99±0.02	8.4±0.06	7.48±0.06	5.72±0.07	5.64±0.06	6.9±0.11
	Finished compost	7.77±0.05	7.66±0.09	7.1±0.12	5.7±0.03	6.31±0.14	7.17±0.08
Poultry farm #2	Active compost	8.33±0.06	7.07±0.02	5.37±0.01	< 2.13±0	3.27±0.11	8.3±0.08
	Finished compost	6.84±0.07	5.58±0.01	3.29±0	2.23±0.08	< 2.11±0	5.69±0.08
Poultry farm #3	Active compost	8.06±0.05	7.22±0	7.41±0.03	4.86±0.03	< 2.47±0.05	7.35±0.05
	Finished compost	7.58±0.04	7.68±0.22	6.49±0.16	4.94±0.1	4.32±0.02	7.27±0.03
Dairy farm #1	Active compost	9.72±0.06	7.43±0.06	8.31±0.08	4.25±0.01	3.12±0.06	7.49±0.08
	Finished compost	7.78±0.07	6.9±0.01	7.72±0.06	< 2.12±0	< 2.12±0	6.7±0.04
Dairy farm #2	Active compost	9.48±0.02	8.85±0.02	7.21±0.07	2.34±0.01	3.05±0.13	8.67±0.02
	Finished compost	8.7±0.01	8.61±0.05	6.58±0.01	5.12±0.03	< 2.28±0.02	7.56±0.1
Dairy farm #3	Active compost	9.18±0.03	8.29±0.01	8.55±0.03	6.14±0.03	< 2.21±0	7.72±0.09
	Finished compost	8.46±0.02	7.92±0.02	7.4±0.08	6.12±0.06	< 2.19±0	7.59±0.05

* Note: Bacterial population was calculated to log CFU/g based on dry weight of compost.

Table A3 Selected genera identified from compost samples by 16S targeted sequencing, used as biological control agents

Biological control genera	Biological control application on agricultural field	Reference
<i>Lysobacter</i>	Antagonistic activity against fungal pathogens that causes plant disease.	Qian et al. 2009
<i>Bacillus</i>	Against <i>Fusarium solani</i> and <i>oxysporum</i> on root rot of olive, and <i>Botrytis spp</i> that causes scape and umbel blights of onion.	Bouzoumita et al 2020; Abo-Elyousr et al 2020
<i>Enterobacter</i>	Against <i>Fusarium solani</i> and <i>oxysporum</i> on root rot of olive.	Bouzoumita et al 2020
<i>Pseudochrobactrum</i>	Control of soil-borne phytopathogens including <i>F. oxysporum</i> and <i>S. rolfsii</i> .	Nepomuceno et al. 2019
<i>Streptomyces</i>	Control of damping-off disease of pea caused by <i>Aphanomyces euteiches</i>	Oubaha et al. 2019
<i>Paenibacillus</i>	<i>P. polymyxa</i> promoted growth of barley, cucumber, pepper, sesame, and produces antimicrobial compounds that protect plants against pathogenic and bacteria.	Jeong et al. 2019
<i>Pseudomonas</i>	Biological agents of soilborne pathogens.	Weller et al. 2007
<i>Burkholderia-Paraburkholderia</i>	Against the causal agent of mango anthracnose, <i>Colletotrichum gloeosporioides</i> .	de Los Santos-Villalobos et al. 2018
<i>Rhizobium</i>	Against <i>Sclerotium (Athelia) rolfsii</i> on the common bean.	Volpiano et al. 2018
<i>Stenotrophomonas rhizophila</i>	Biocontrol of <i>Colletotrichum gloeosporioides</i> on mango.	Reyes-Perez JJ et al. 2019
<i>Pantoea</i>	In vivo control of Alternari alternate that infected fruits and vegetables.	Tekiner et al. 2019
<i>Lysinibacillus</i>	Protection of tomato plants from <i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i>	Kavroulakis et al. 2010
<i>Serratia</i>	Protection of tomato plants from <i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i>	Kavroulakis et al. 2010
<i>Alcaligenes</i>	Protection of tomato plants from <i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i>	Kavroulakis et al. 2010

Table A4 Kruskal-Wallis analysis for variables that affect the alpha diversities

Factors		Shannon	Inverse_Simpson	Chao_richness	Observed_species
Compost related	C:N	2.306e-08***	0.0005435**	2.668e-12**	2.637e-12***
	pH	7.177e-05***	2.844e-07***	0.2879	0.2763
	Compost sources	0.01989*	3.436e-06***	0.358	0.3640
	Composting stage	0.0005404***	0.0769	0.0218*	0.0197*
	Collection location	2.2e-16***	2.2e-16**	2.2e-16***	2.2e-16***
Experimental variables	Moisture contents	0.0017*	0.00254*	0.0103*	0.0098*
	Incubation time	0.0264*	0.01122*	0.0145*	0.0157*
	<i>Listeria</i> inoculation	0.8385	0.9149	0.5064	0.5083

P-values in bold indicate significant correlations: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table A5 Test the significance of factors using ANOSIM and PERMANOVA^a

Factors			ANOSIM		PERMANOVA	
			R	Corrected <i>P</i> -values	F	Corrected <i>P</i> -values
Compost related	Compost sources	Dairy vs Poultry	0.1594	0.000999 ***	32.158	0.000999 ***
	Composting stage	Active vs Finished	0.1837	0.000999 ***	25.242	0.000999 ***
	Collection location	Various	0.2198	0.000999 ***	37.286	0.000999 ***
Experimental variables	Moisture contents	40% vs 80%	0.005995	0.07992	2.0888	0.07093
	Incubation time	0 h vs 72 h	0.01523	0.005994**	3.0293	0.01499 *
	<i>Listeria</i> inoculation	LM vs no LM	-0.00535	0.99401	0.18877	0.997

^a R and F values in bold indicate significant difference observed between different factors; with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table A6 Canonical correspondence analysis output for each farm

Farms	Experimental factors	F	P-values ^a
Dairy farm #1	Experimental moisture	4.8637	0.001***
	Incubation time	6.2962	0.001***
	<i>L. monocytogenes</i> inoculation	0.6487	0.776
Dairy farm #2	Experimental moisture	1.1631	0.242
	Incubation time	1.8146	0.094
	<i>L. monocytogenes</i> inoculation	0.6486	0.677
Dairy farm #3	Experimental moisture	2.8826	0.011*
	Incubation time	2.9677	0.016*
	<i>L. monocytogenes</i> inoculation	0.5883	0.871
Poultry farm #1	Experimental moisture	1.2030	0.211
	Incubation time	2.3772	0.050
	<i>L. monocytogenes</i> inoculation	0.5597	0.917
Poultry farm #2	Experimental moisture	6.3641	0.001***
	Incubation time	9.1743	0.001***
	<i>L. monocytogenes</i> inoculation	0.5080	0.896
Poultry farm #3	Experimental moisture	1.5417	0.088
	Incubation time	2.7471	0.006**
	<i>L. monocytogenes</i> inoculation	0.6603	0.909

^a P-values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table A7 Selected discriminatory microbial members that increased in relative abundance after LM inoculation,

as identified from each farm

Farms	Genus	Increased after LM inoculation	Contribution to dissimilarity (%)
Dairy farm #1	<i>Sphaerobacter</i>	in both active and finished compost with 40 and 80% MC.	12.44%
	<i>Bacillus</i>	in finished compost with 80% MC, and active compost with both MCs.	7.57%
	<i>Rhodothermus</i>	in finished compost with 80% MC, and in active compost with 40% MC.	4.51%
Dairy farm #2	<i>Brumimicrobium</i>	slightly in both active and finished compost with 40% MC.	5.95%
	<i>Flavobacterium</i>	in finished compost with 40% MC.	4.38%
	<i>Luteivirga</i>	slightly in both active compost with 40 and 80% MC.	3.57%
Dairy farm #3*	<i>Flavobacterium</i>	increased after 72 h incubation.	3.22%
Poultry farm #1	<i>Pseudolabrys</i>	in both active and finished compost at all conditions.	6.2%
	<i>Rhodanobacter</i>	in both active and finished compost at all conditions.	4.5%
	<i>Steroidobacter</i>	in both active and finished compost, especially in the compost with 80% MC.	3.7%
Poultry farm #2	<i>Lentibacillus</i>	in both active and finished compost at all conditions.	12.2%
	<i>Brachybacterium</i>	in both active and finished compost, especially in the compost with 80% MC.	10.4%
	<i>Salinicoccus</i>	in active compost only.	7.4%
Poultry farm #3	<i>Steroidobacter</i>	highest increasing observed from active compost with 80% MC.	4.5%
	<i>Chryseolinea</i>	increased for all conditions.	4.3%
	<i>Bacillus</i>	increased for all conditions.	4.3%

* All other top discriminatory microbial members were decreased after LM inoculation in the compost sample collected from dairy farm #3.

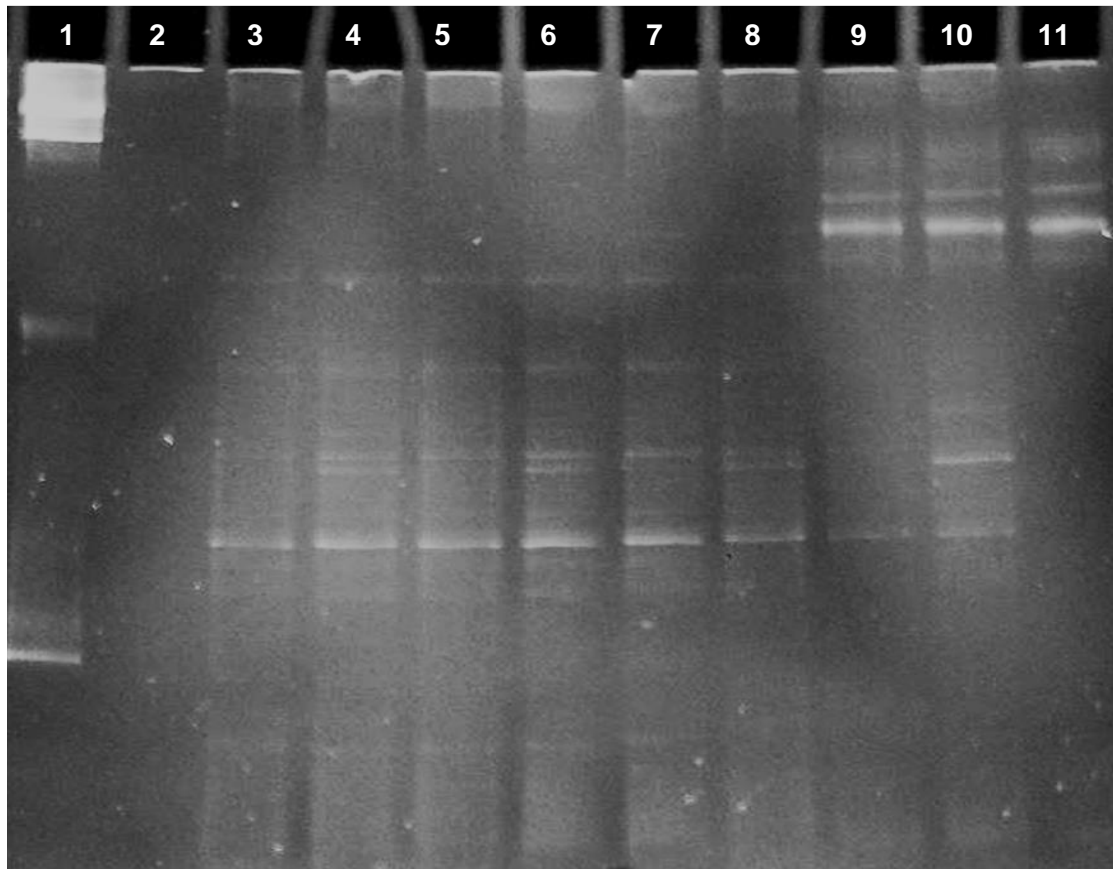


Figure A1 DGGE profiles of PCR-amplified 16S rDNA fragments from finished chicken litter compost with 80% MC.

Lane 1, 1Kb Ladder; Lane 2, space lane without sample; Lanes 3-4, compost samples w/o *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 5-6, compost samples with ca. 5 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 7-8, compost samples with ca. 7 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 9-10, compost samples with ca. 9 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lane 11, *L. monocytogenes* strain only.

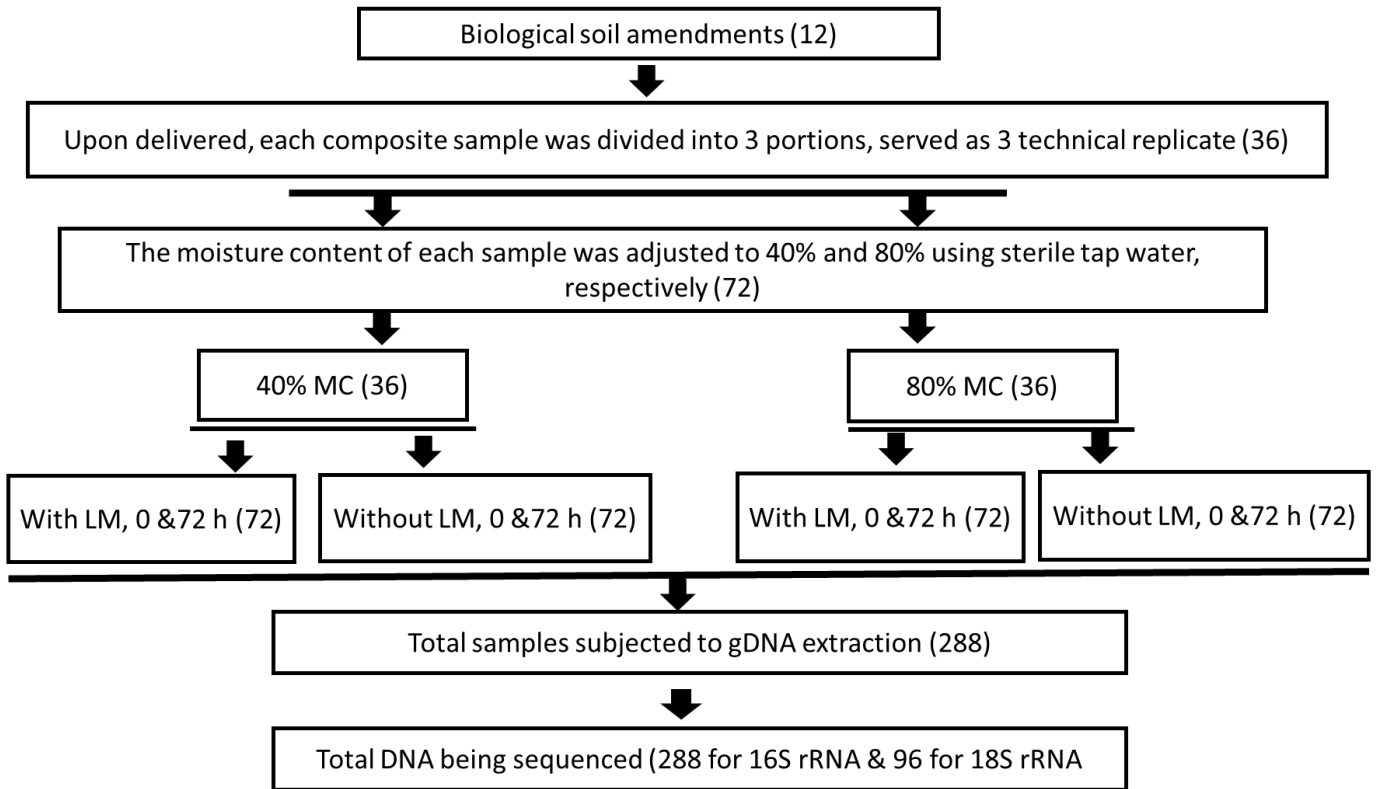


Figure A2 Flow chart for DNA extraction of compost samples.

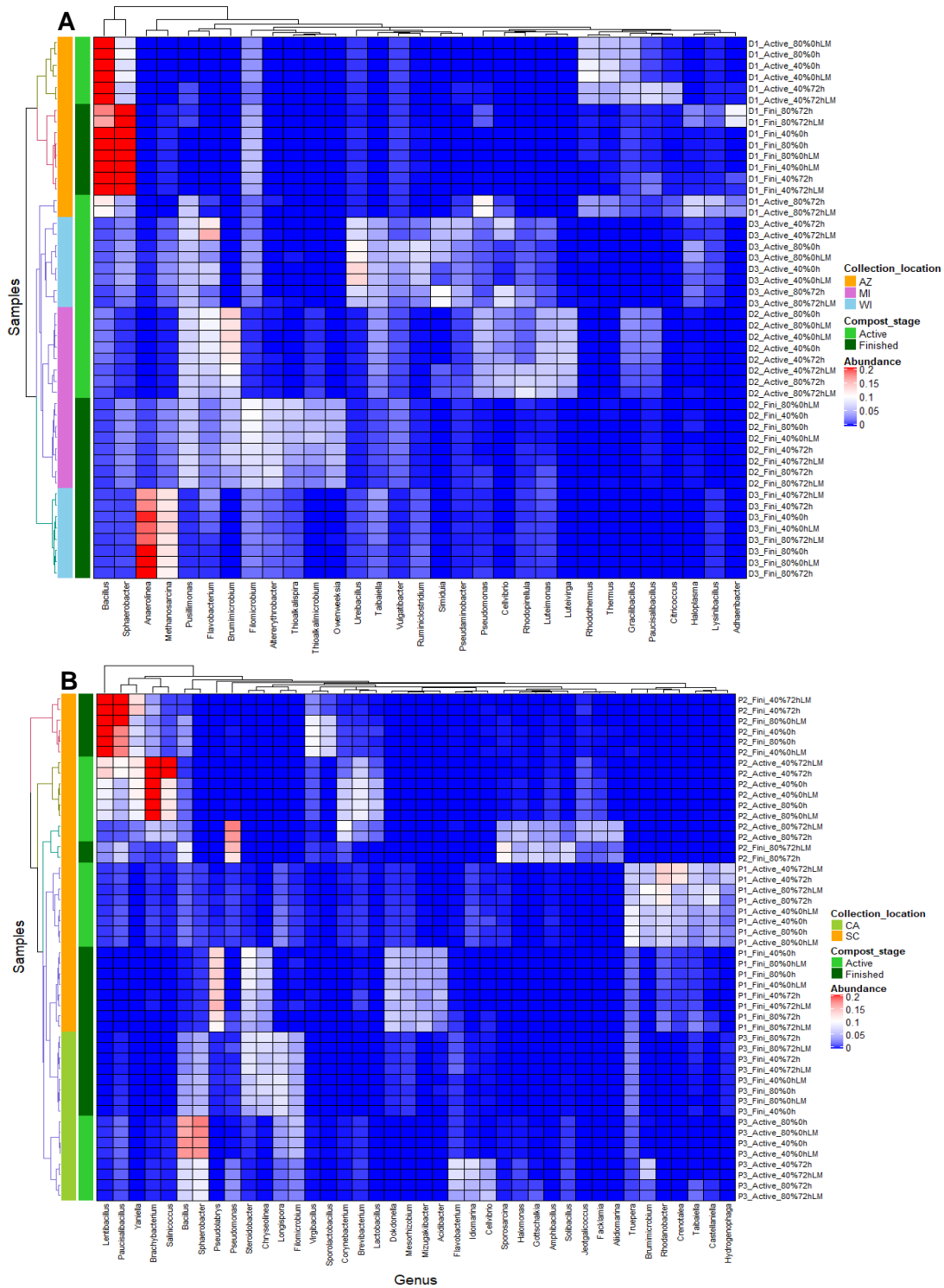


Figure A3 Clustered heatmap of bacterial distribution of key genus from dairy (A) and poultry (B) composts. Rows represent different samples, and columns stand for relative percentage of each bacterial genus. The relative abundance for each bacterial genus was depicted by color intensity, with the legend indicated on the right of the figure.

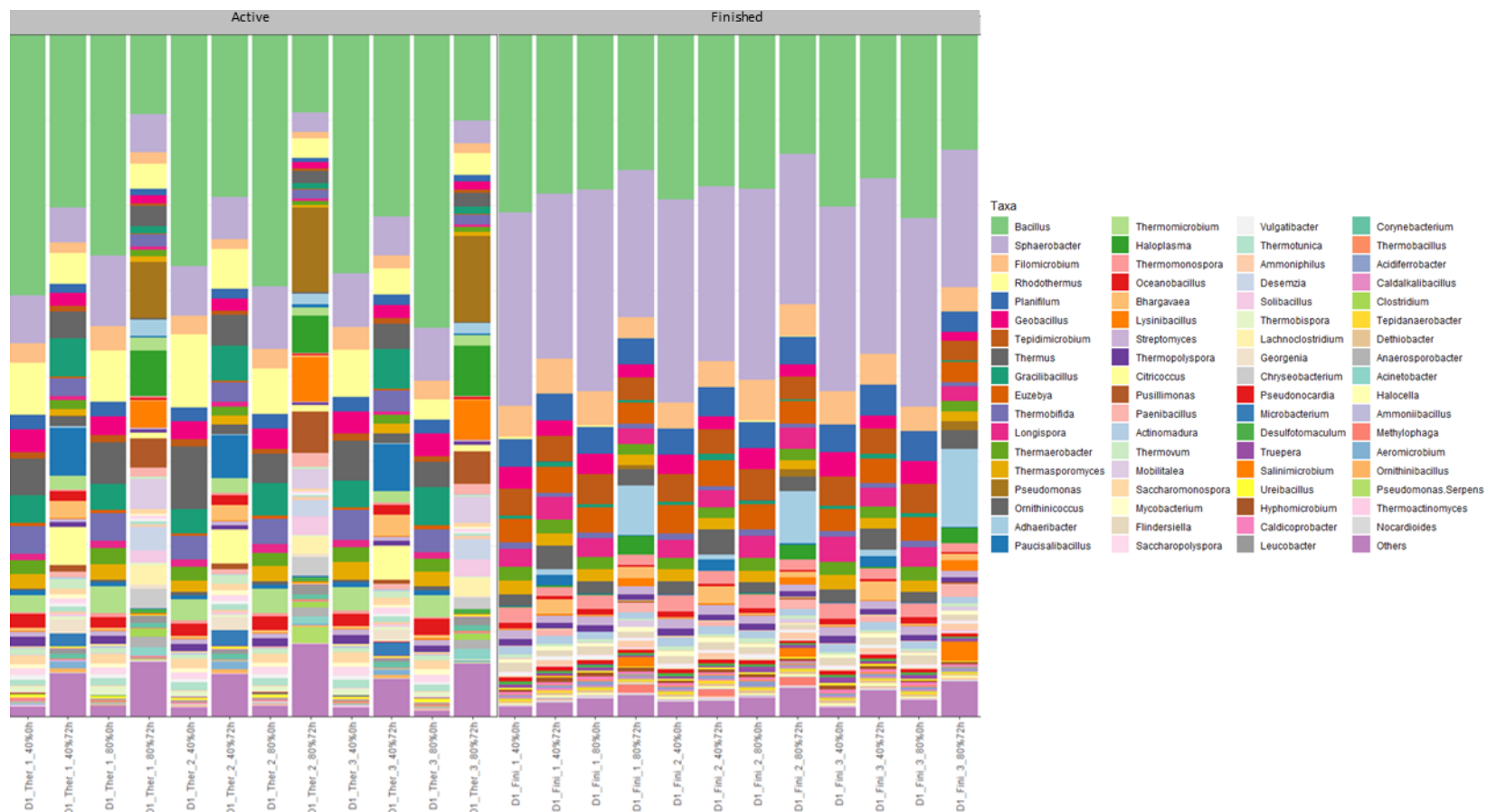


Figure A4 Bacterial community composition in dairy farm #1 composts; X-axis represents different samples, and Y-axis stands for relative percentage of each bacterial genus. The left 12 lanes are for active compost samples, and the right 12 lanes are for finished compost samples.

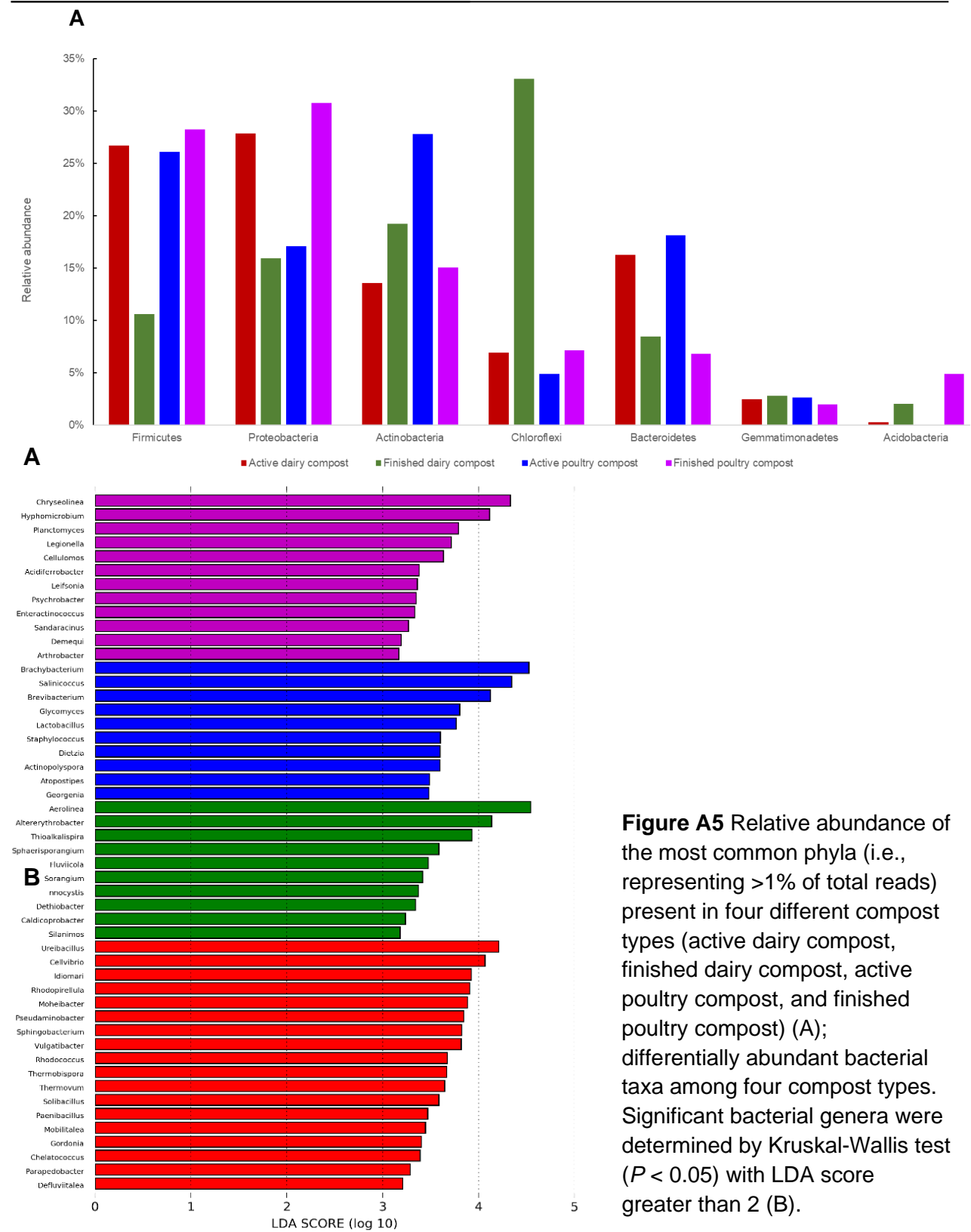


Figure A5 Relative abundance of the most common phyla (i.e., representing >1% of total reads) present in four different compost types (active dairy compost, finished dairy compost, active poultry compost, and finished poultry compost) (A); differentially abundant bacterial taxa among four compost types. Significant bacterial genera were determined by Kruskal-Wallis test ($P < 0.05$) with LDA score greater than 2 (B).

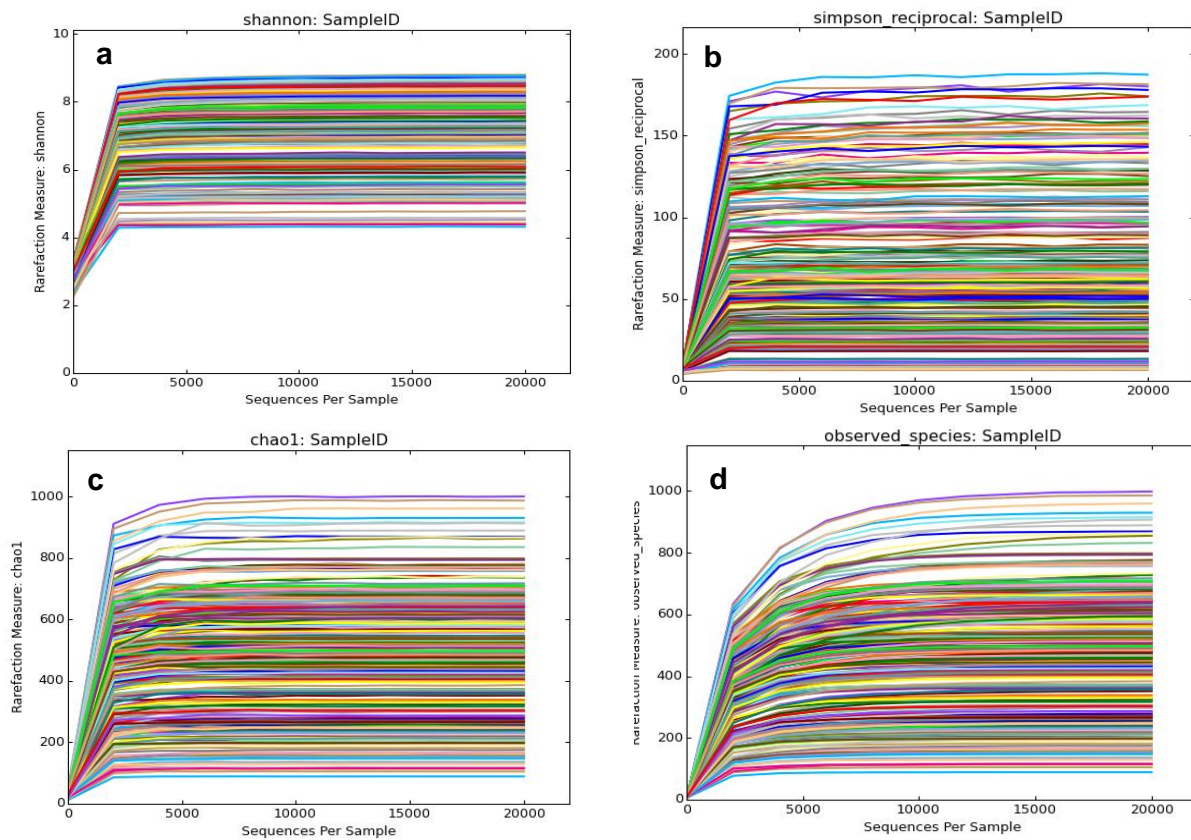


Figure A6 Rarefaction curves for each sample (a. Shannon, b. Inverse Simpson, c. Chao1, d. Observed species).

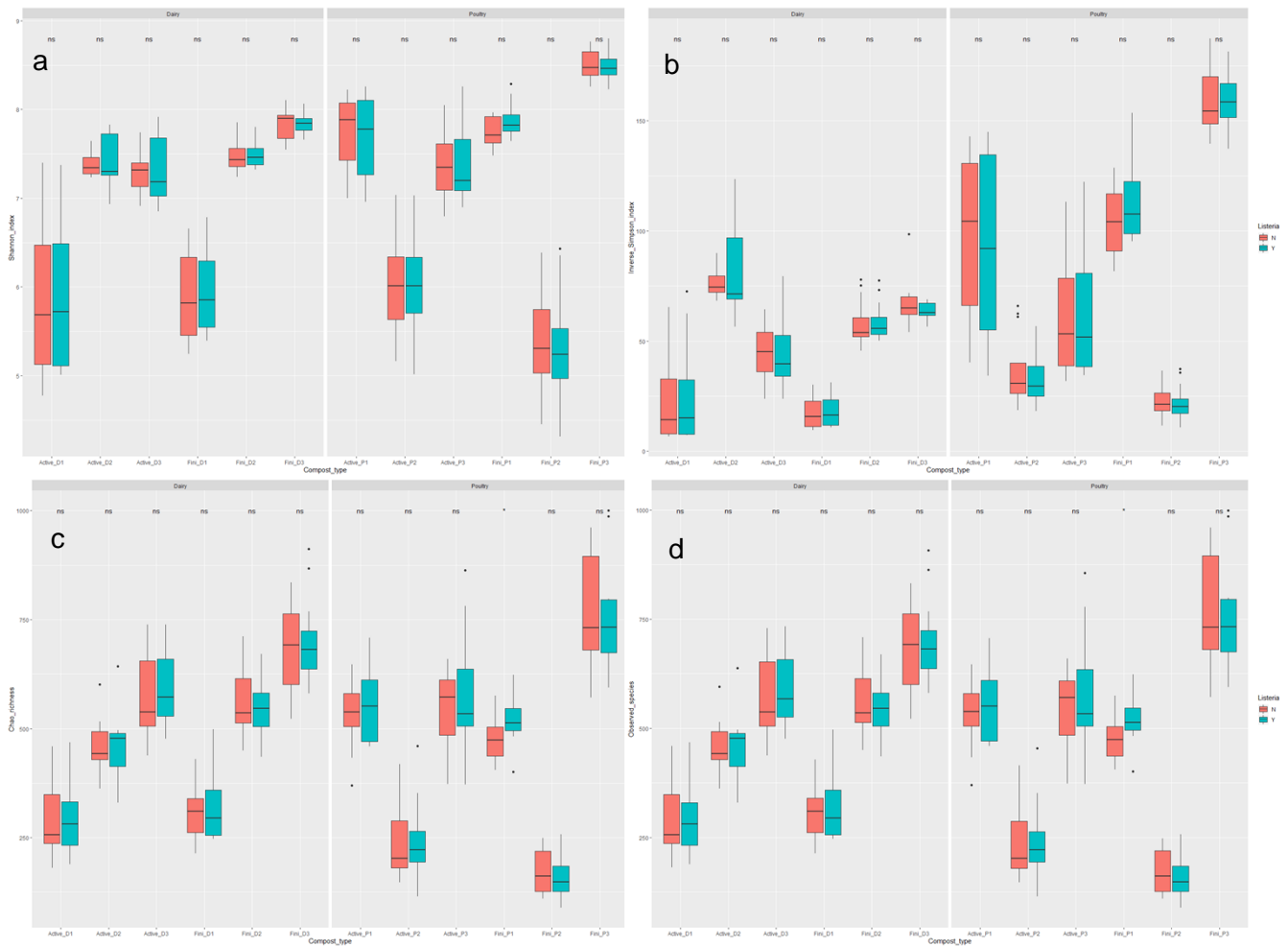


Figure A7 Alpha diversity in different compost samples, including boxplot of Shannon index (a), Inverse Simpson index (b), Chao1 richness (c), and Observed species (d). Wilcoxon method was used to compare means between LM (blue) and no-LM groups (red), and the significant levels were added on the boxplot (ns; no significant difference with P -value > 0.05).

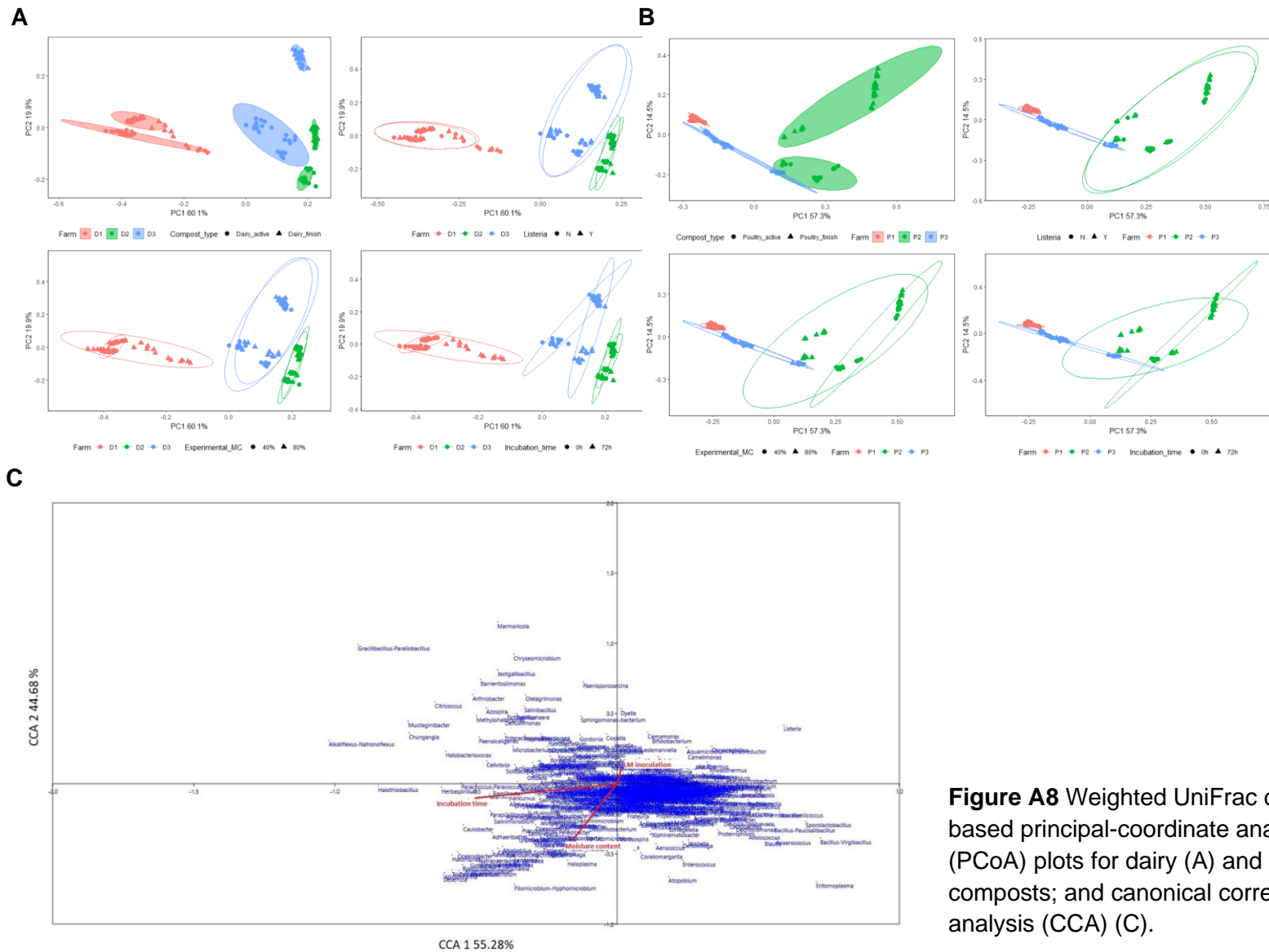


Figure A8 Weighted UniFrac distance-based principal-coordinate analysis (PCoA) plots for dairy (A) and poultry (B) composts; and canonical correspondence analysis (CCA) (C).

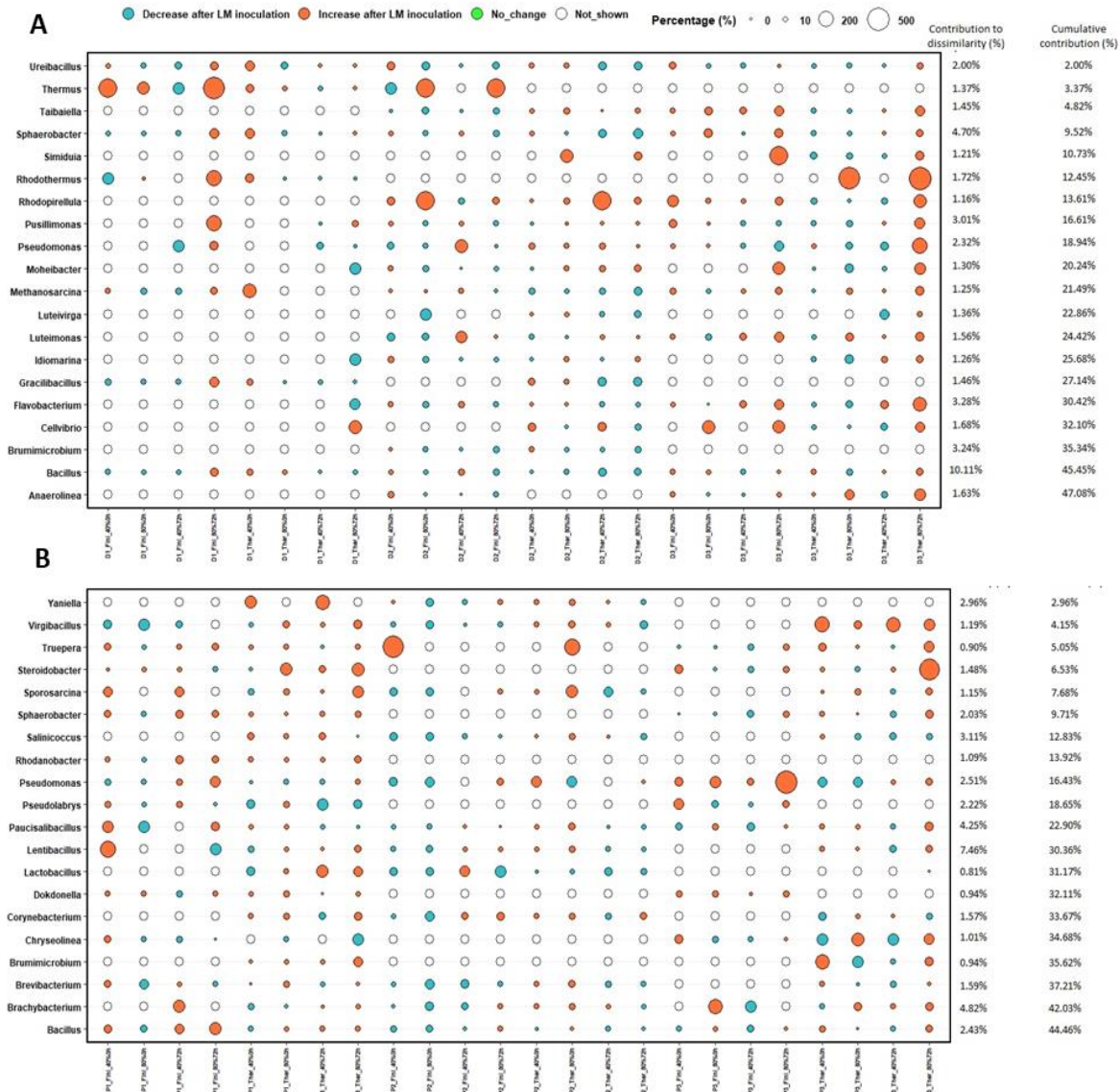


Figure A9 Similarities percentage (SIMPER) analysis (A, B).

Appendix B

Table B1 Relative proportions of functional categories found in active dairy compost samples that significantly changed among different treatments annotated by SEED subsystem level 1 and level 2, as revealed by metagenomic sequencing

Functional categories		No LM	With LM	<i>P</i> -value*		
Level 1	Level 2	0h (%)	72h (%)	0h (%)	72h (%)	
Carbohydrates	Monosaccharides	14.37±0.18	14.4±0.11	14.38±0.05	14.67±0.29	0.01
Clustering-based subsystems	One-carbon Metabolism					
	Monosaccharides - #1	0.17±0.01	0.16±0.01	0.17±0.01	0.18±0.01	0.01
	Pyruvate kinase associated cluster	0.27±0.01	0.28±0.01	0.27±0.01	0.24±0.06	0.01
	Fatty acid metabolic cluster	1.88±0.03	1.85±0.05	1.88±0.02	1.95±0.11	0.02
Phages, Prophages, Transposable elements, Plasmids	Phosphate metabolism	Low in proportions	Low in proportions	Low in proportions	Low in proportions	0.02
	Catabolism of an unknown compound	0.12±0.01	0.12±0.01	0.011±0.01	0.14±0.02	0.04
	Pathogenicity islands	22.71±0.77	22.47±0.81	22.28±0.5	19.93±3.55	0.04
RNA Metabolism	Phages, Prophages	61.1±0.83	61.22±1.3	61.62±0.63	63.58±1.78	0.03
	Unclassified	0.64±0.02	0.65±0.03	0.63±0.01	0.75±0.17	0.03
	Bacteriocins, ribosomally synthesized antibacterial peptides	1.07±0.04	1.04±0.12	1.05±0.05	1.18±0.07	0.03
Virulence, Disease and Defense	Protein processing and modification	11.87±0.06	11.84±0.11	11.83±0.07	11.99±0.15	0.04
Protein Metabolism	Social motility and nonflagellar swimming in bacteria	0.33±0.04	0.33±0.03	0.32±0.03	0.6±0.52	0.04
Motility and Chemotaxis						

* Significant at *P*-value < 0.05.

**Numbers highlighted in bold indicate a significant increase due to *L. monocytogenes* inoculation after 72 h.

Table B2 Relative proportions of selected functional categories found in active dairy compost samples that significantly changed among different treatments annotated by SEED subsystem level 1 and level 2 as revealed by metatranscriptomic sequencing

Functional categories		No LM		With LM		P-value*
Level 1	Level 2	0h (%)	72h (%)	0h (%)	72h (%)	
Amino Acids and Derivatives	Arginine; urea cycle, polyamines Gram-Positive cell wall components	8.02±0.02	9.65±0.34	7.85±0.12	11.7±0.18	< 0.001
Cell Wall and Capsule	Hypothetical Related to Low in	1.17±0.03	2.2±0.01	1.06±0.17	3.3±0.11	< 0.001
Clustering-based subsystems	Dihydroorrate Dehydrogenase proportions	0.73±0.01	0.92±0.02	0.69±0.02	0.76±0.01	< 0.001
Clustering-based subsystems	Two related proteases	62.35±0.05	61.11±0.4	62.98±0.16	56.84±0.12	< 0.001
DNA Metabolism	DNA repair	4.87±0.07	6.73±0	4.79±0.12	9.07±0.44	< 0.001
DNA Metabolism	Unclassified	17.69±2	48.57±3.2	15.11±0.18	78.81±1.21	< 0.001
Phages, Prophages, Transposable elements, Plasmids	Phages, Prophages	78.97±2.1	48.21±3.97	82.23±0.52	17.76±0.6	< 0.001
Phages, Prophages, Transposable elements, Plasmids	Pathogenicity islands Resistance to antibiotics and toxic compounds	88.2±0.13	84.09±0.35	87.89±0.24	79.55±0.94	< 0.001
Virulence, Disease and Defense						

* Significant at P -value < 0.001.**Numbers highlighted in bold indicate a significant increase due to *L. monocytogenes* inoculation after 72 h.

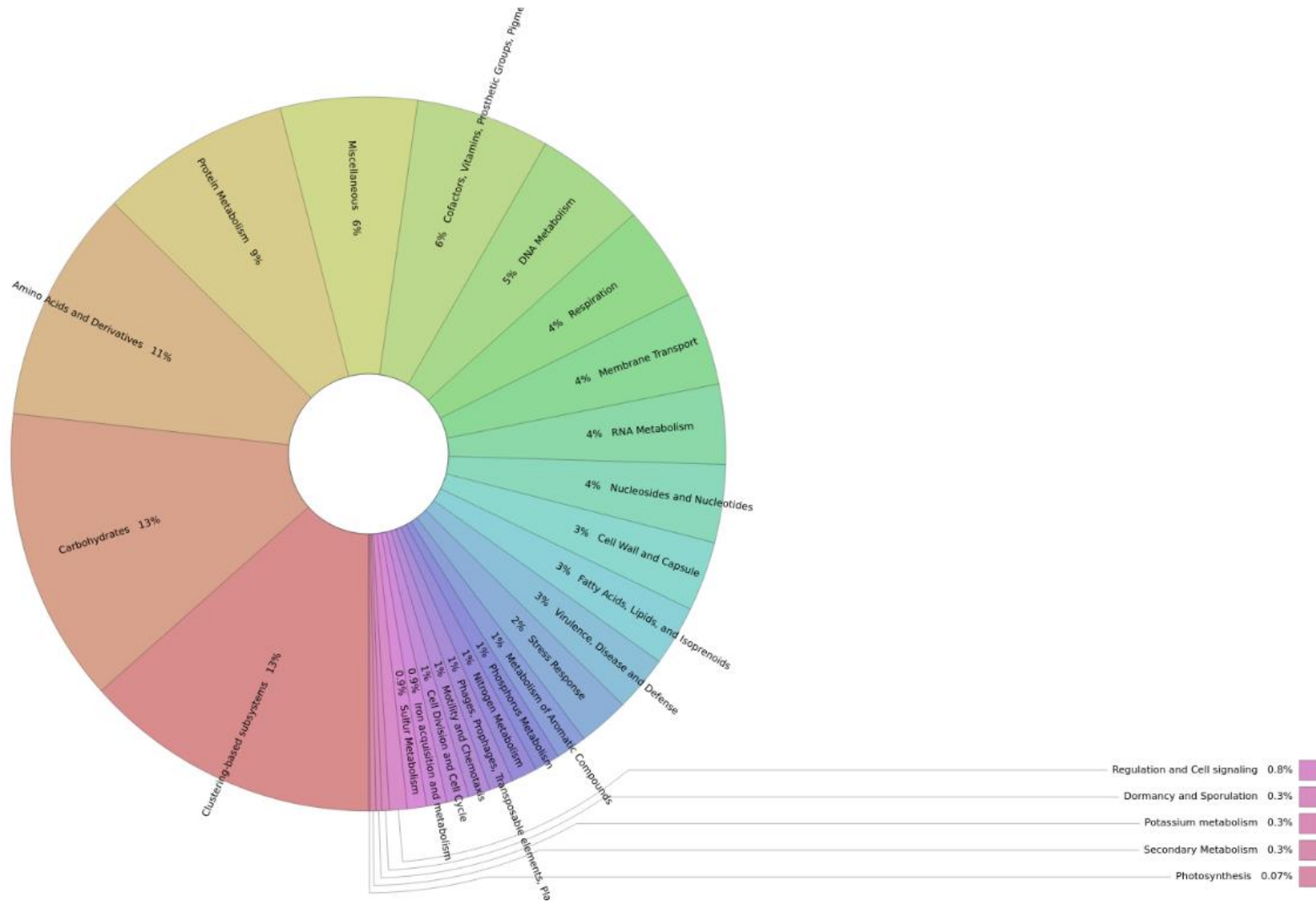


Figure B1 KRONA plot of functional profiles detected in active dairy compost microbiome annotated with SEED subsystem.

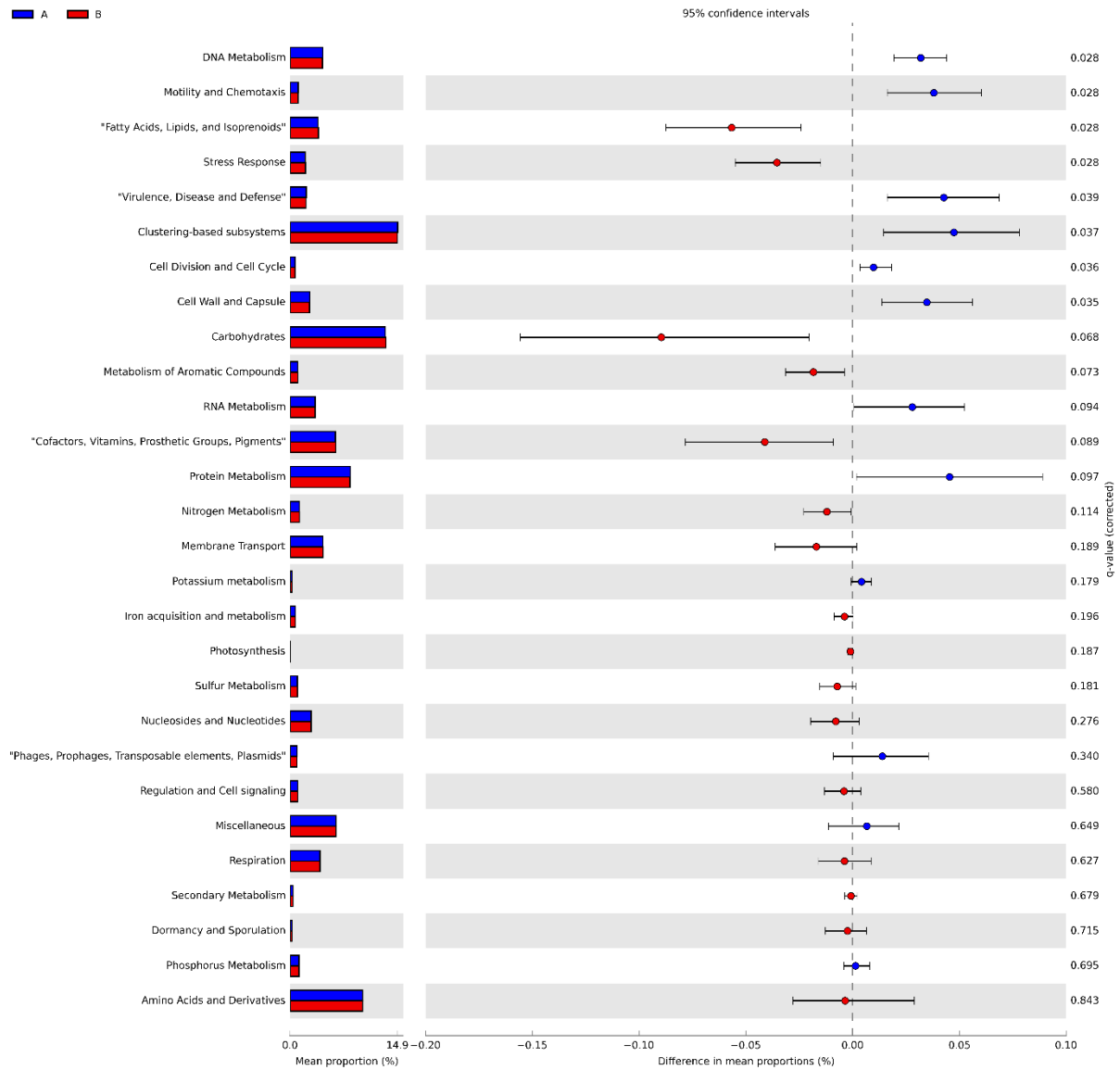


Figure B2 Functional profiles for the microbial metagenomes of active dairy compost from two separate collections (A and B). Extended error bar plot compared the functional profiles for the microbial metagenomes in active dairy compost from two separate collections based on the SEED subsystem level 1. Points and bars indicate the differences between collections A and B (blue and red, respectively), and the values at the right show the P -values were derived from a White's non-parametric t-test with Benjamini-Hochberg FDR correction.

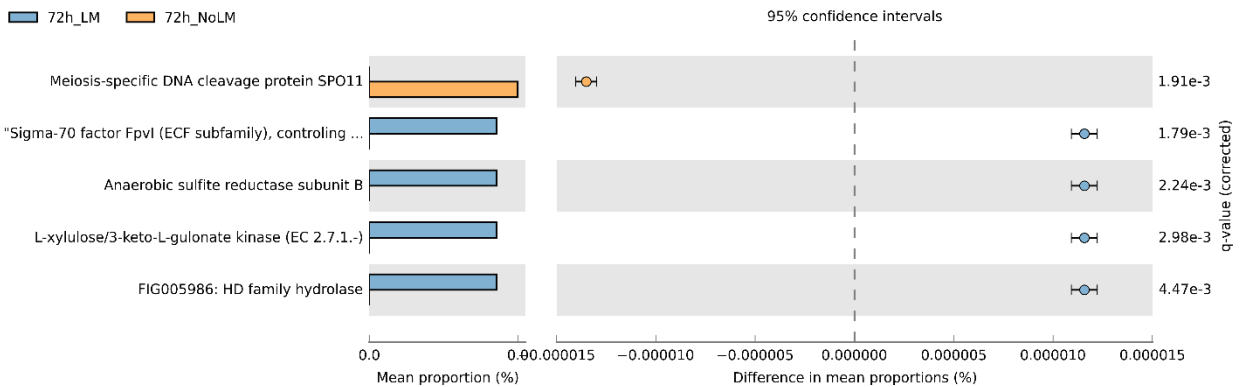


Figure B3 Extended error bar plot indicating the microbial functional potentials changed in active dairy compost from collection B due to the inoculation of *L. monocytogenes* after 72 h incubation based on the SEED subsystem functional gene entries. Points and bars indicate the differences between *L. monocytogenes*-inoculated and uninoculated compost (light blue and light orange, respectively), and the values at the right show the *P*-values were derived from a White's non-parametric t-test with Benjamini–Hochberg FDR correction.

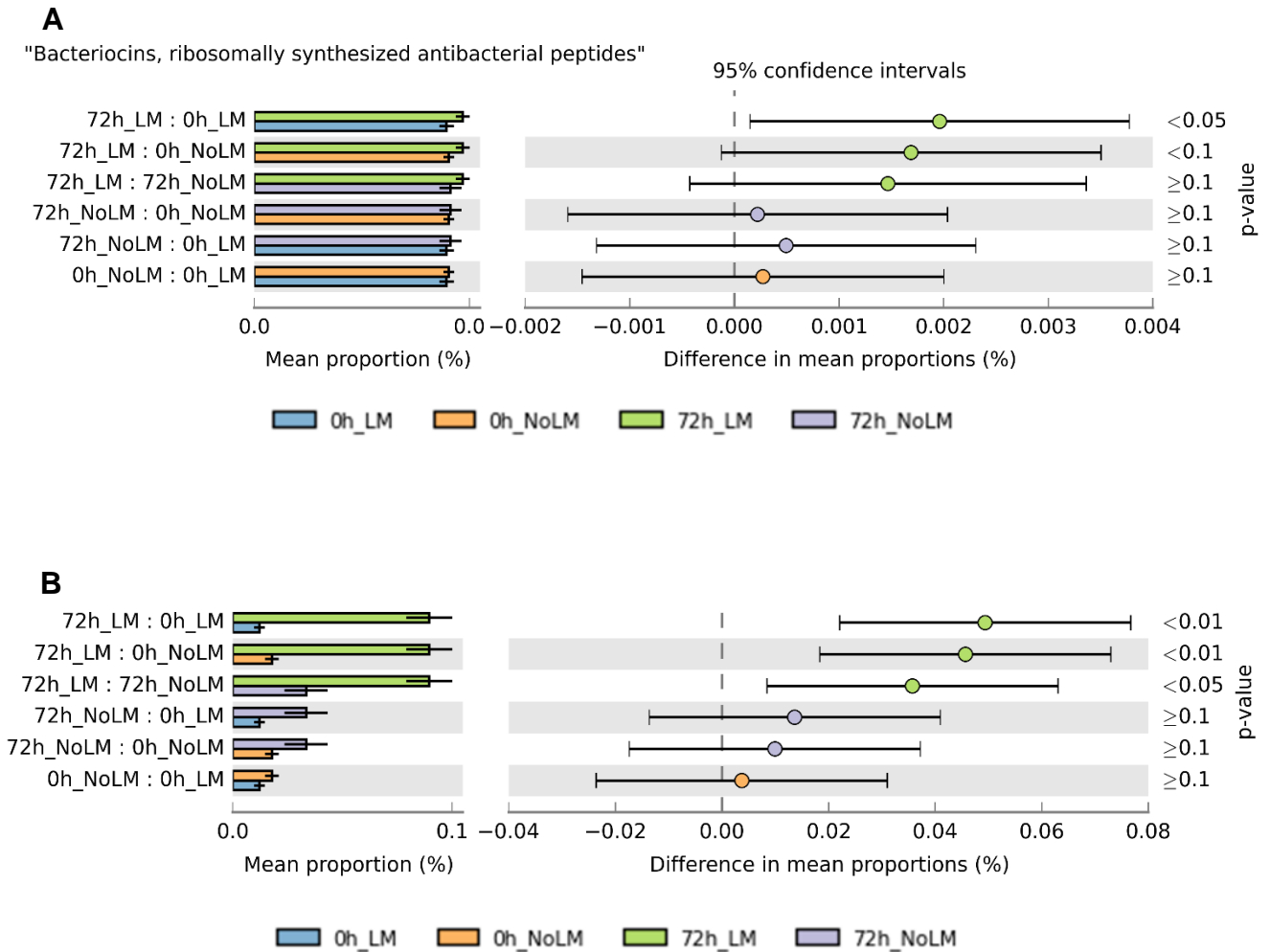


Figure B4 Post-hoc plot for bacteriocins, ribosomally synthesized antibacterial peptides, as revealed by metagenomic (A) and metatranscriptomic (B) sequencing. The light blue, orange, green, and purple bars indicate compost samples at 0h with LM, 0h without LM, 72h with LM, and 72h without LM, respectively.

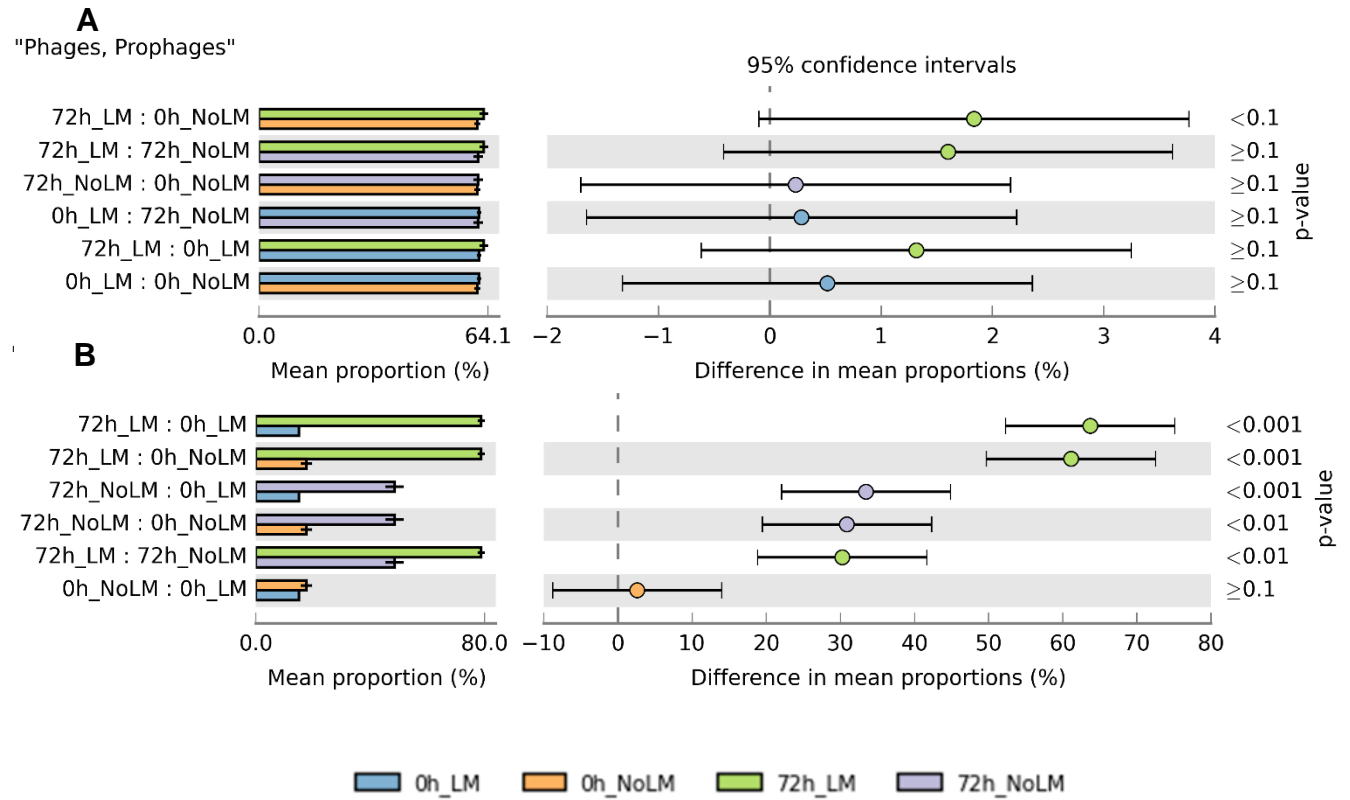


Figure B5 Post-hoc plot for phages, prophages as revealed by metagenomic (A) and metatranscriptomic (B) sequencing. The light blue, orange, green, and purple bars indicate compost samples at 0h with LM, 0h without LM, 72h with LM, and 72h without LM, respectively.

Appendix C

Table C1 New colonies that appeared on PYG agar plates from the highest countable dilution on day 14 and 21

Collection farm	Compost stage	Colonies (CFU)	
		Day 14	Day 21
Poultry farm #1	Active compost	17	10
Poultry farm #1	Finished compost	8	12
Poultry farm #2	Active compost	42	140
Poultry farm #3	Active compost	25	7
Dairy farm #1	Active compost	9	4
Dairy farm #1	Finished compost	6	5
Dairy farm #2	Active compost	16	21
Dairy farm #3	Active compost	43	57

Table C2 Competitive exclusion strains with the antagonistic activities against *Listeria* strains isolated from composts

Sanger sequencing identification	Compost sources*	Culture plates	Anti- <i>L. monocytogenes</i> strain			Biological control application	Reference
			R9-5506	LCDC 81-861	ScottA		
<i>Bacillus aerius</i>	P1	TSA	+	-	-	Control fusarium wilt in tomatoes.	Rocha et al. 2017
<i>Bacillus alkalitelluris</i>	D3	MPN + Rpf	+	+	+		
<i>Bacillus altitudinis</i>	D3/P1	TSA	-	+	+	Biocontrol agent for potato common scab.	Li et al. 2019
<i>Bacillus deserti</i>	D3	MPN + Rpf	-	+	+		
<i>Bacillus haynesii</i>	P1	TSA	-	+	-	Biological control agent for <i>Aeromonas</i> .	Mulyani et al. 2018
<i>Bacillus licheniformis</i> (I)	P1	TSA	+	-	-	Inhibit the growth of phytopathogenic fungi including <i>Phoma medicaginis</i> .	Slimene et al. 2015
<i>Bacillus licheniformis</i> (II)	P3	TSA	+	+	+	Biological control of tomato gray mold caused by <i>Botrytis cinerea</i> .	Lee et al. 2006
<i>Bacillus maritimus</i>	D1	MPN + Rpf/TSA	+	+	+		
<i>Bacillus mediterraneensis</i>	D1	TSA	+	+	+		
<i>Bacillus rhizosphaerae</i>	P1	TSA	+	-	-		
<i>Bacillus sporothermodurans</i>	D3	MPN + Rpf	+	+	+		
<i>Bacillus velezensis</i>	P3	TSA	+	-	+	Biological Control of strawberry <i>Fusarium Wilt</i> .	Nam et al. 2009

* P1-3, Poultry farm #1-3; D1-3, Dairy farm #1-3.

Table C2 cont. Competitive exclusion strains with the antagonistic activities against *Listeria* strains isolated from composts

Sanger sequencing identification	Compost sources	Culture plates	Anti- <i>L. monocytogenes</i> strain			Potential Biological control applications	Reference
			R9-5506	LCDC 81-861	ScottA		
<i>Brevibacillus halotolerans</i>	P2	TSA	+	+	+	Inhibit major soilborne pathogens of soybean.	Brunda et al. 2018
<i>Kocuria flava</i>	D3	MPN + Rpf	+	+	+		
<i>Kocuria oceani</i> *	D1	MPN + Rpf	+	+	+		
<i>Kocuria rosea</i>	D3	MPN + Rpf	-	+	+		
<i>Kocuria salina</i>	D3	MPN + Rpf	+	+	+		
<i>Paenibacillus dendritiformis</i>	P3	TSA	+	+	+	Reduce disease indices and increase yield in potato crops.	Lapidot et al. 2015
<i>Paenibacillus popilliae</i>	P3	TSA	+	+	+	<i>Paenibacillus</i> spp produces antifungal compound	Chung et al. 2000
<i>Paenibacillus woosongensis</i>	P3	TSA	+	+	+	Producer of biologically active substances with antifungal activities.	Bakaeva et al. 2017
<i>Planococcus maitriensis</i>	D1	TSA/MPN+Rpf	-	+	+		
<i>Planococcus plakortidis</i>	D3	TSA	+	+	+		

* *Kocuria* spp, such as *Kocuria rhizophila* is a biological control for fungal rice pathogens (Chaiharn et al. 2009).

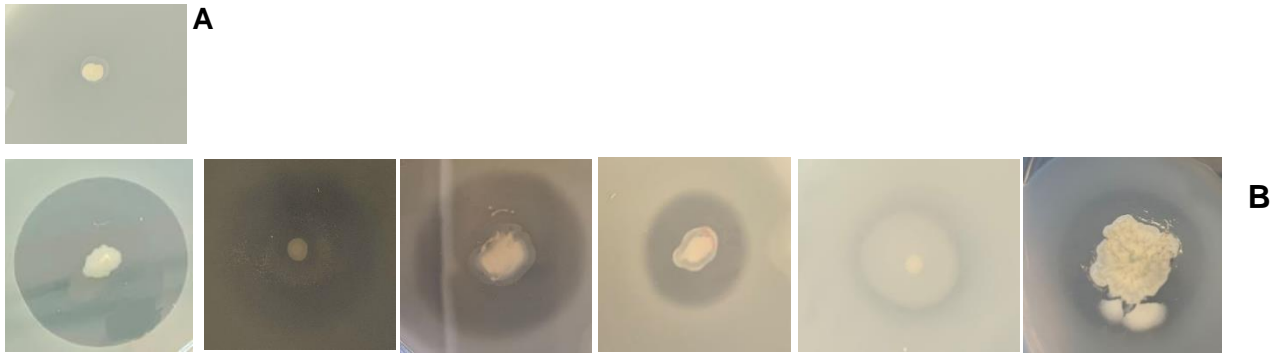


Figure C1 Selection of competitive exclusion microorganisms from compost samples against *L. monocytogenes*. The isolates showed no inhibition zone on *Listeria* lawn (A), and with various sizes of inhibition zones (B).

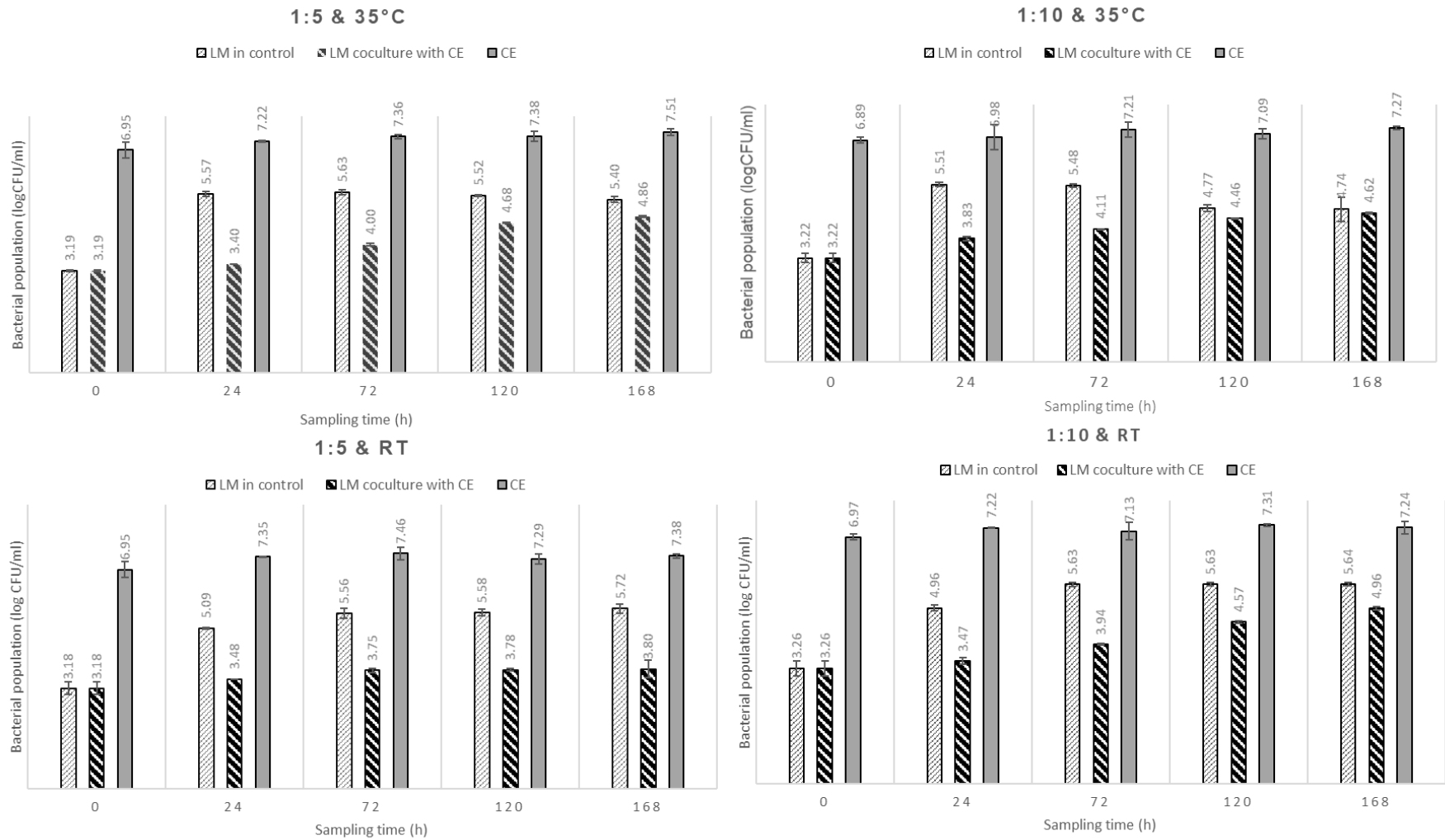


Figure C2 Inhibition of 3 strains of *L. monocytogenes* in the presence of CE in 1:5 and 1:10 dairy compost extracts at 35°C and room temperature. Data are expressed as average log CFU/ml from two separate trials.

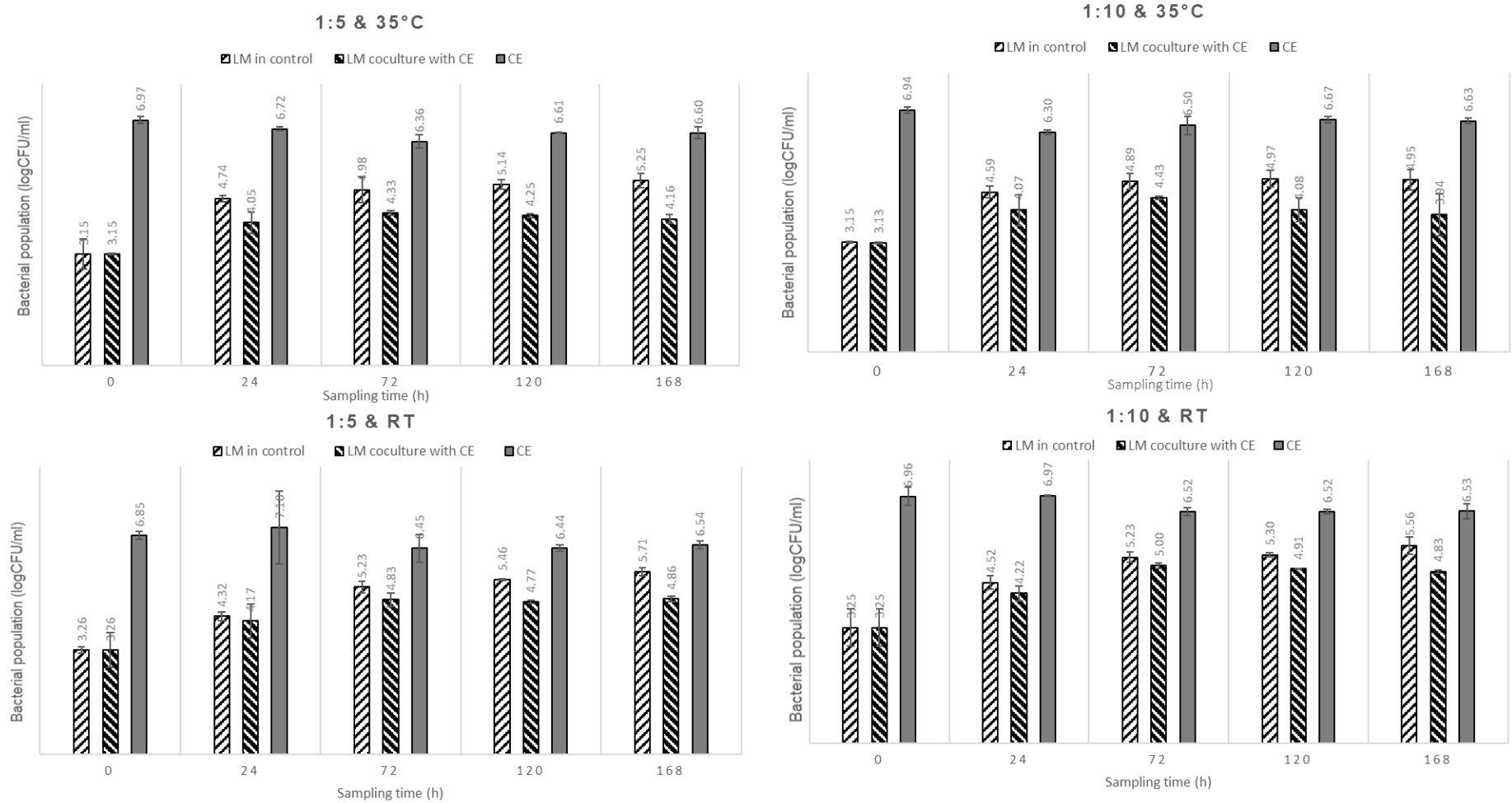


Figure C3 Inhibition of 3 strains of *L. monocytogenes* in the presence of CE in 1:5 and 1:10 poultry compost extracts at 35°C and room temperature. Data are expressed as average log CFU/ml from two separate trials.