

Supplementary Materials

Bacterial Communities of House Flies from Dairy Farms Highlight Their Role as Reservoirs, Disseminators, and Sentinels of Microbial Threats to Human and Animal Health

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Supplementary Figures

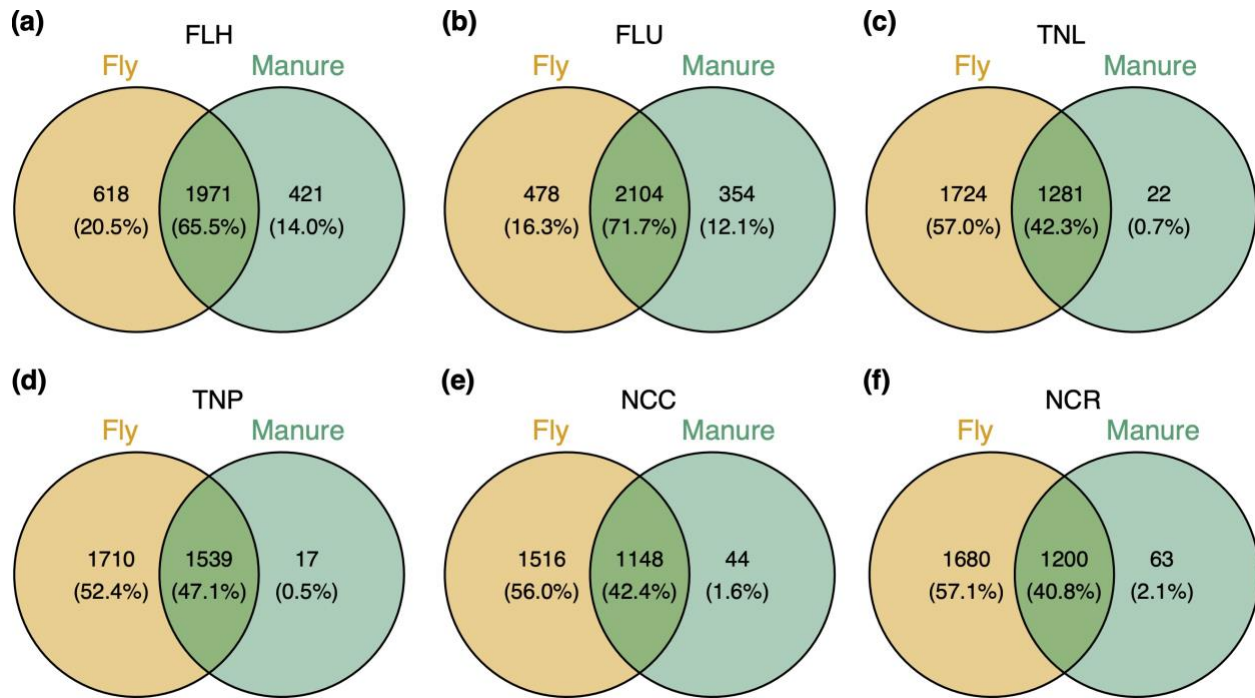


Figure S1: Bacterial communities (observed operational taxonomic units, OTUs) in house fly and manure samples within a farm. Overlapping sections represent the shared bacterial communities, observed number of OTUs, between house fly and manure samples, and non-overlapping sections represent OTUs unique to the sample types (house fly or manure) at dairy farms: (a) FLH, (b) FLU, (c) TNL (upper panel) and (d) TNP, (e) NCC, (f) NCR (lower panel).

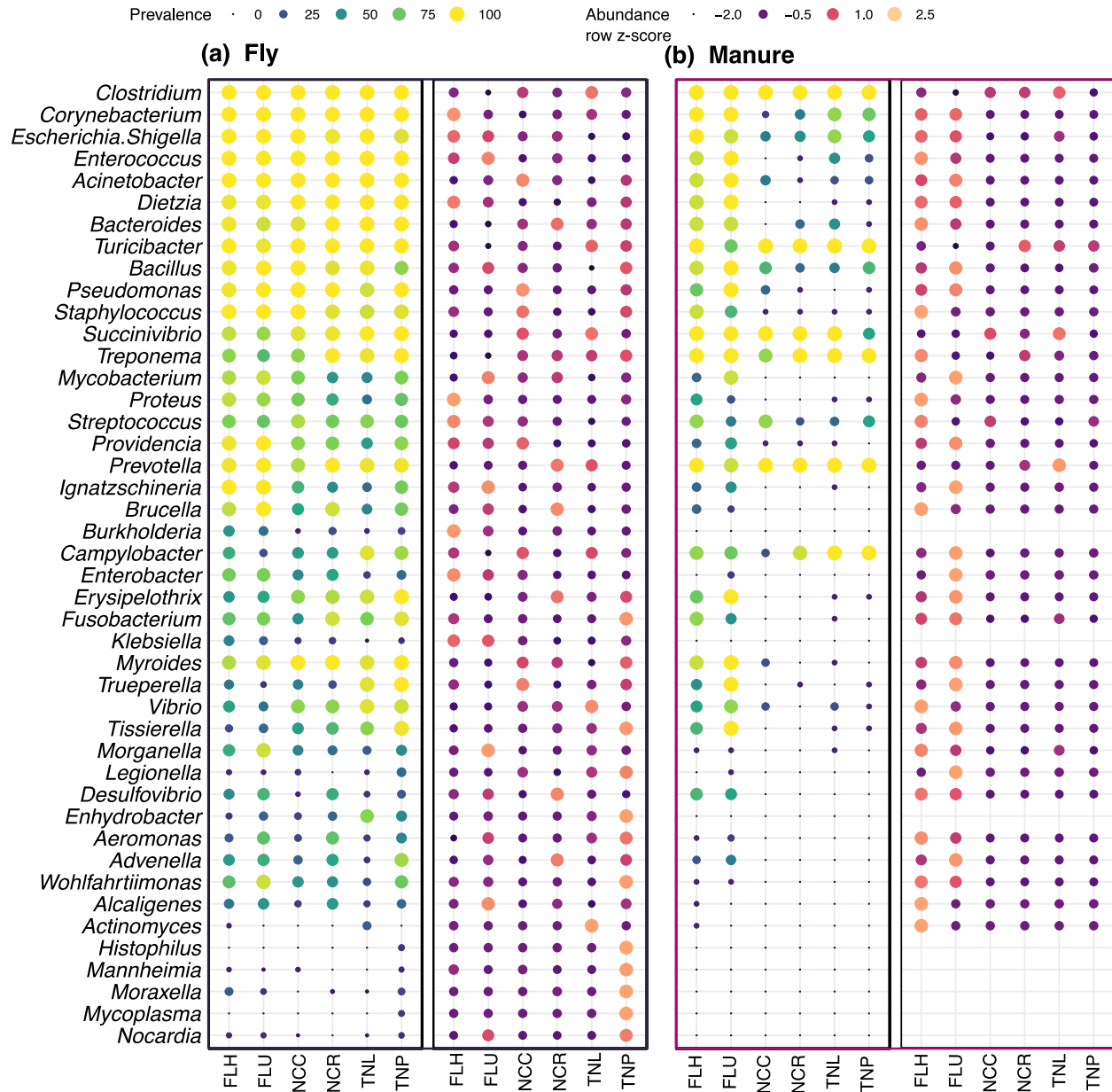


Figure S2: Prevalence and abundance profiles of potential bacterial pathogens in house fly and manure samples associated with dairy farms. (a) prevalence (%; n = 40 per farm except FLU and TNL, n = 38) and abundance (row z-score of mean relative abundance; n = 40, except FLU and TNL, n = 38) in house fly samples. (b) prevalence (%; n = 12 per farm) and abundance (row z-score of mean relative abundance; n = 12 per farm) in manure samples.

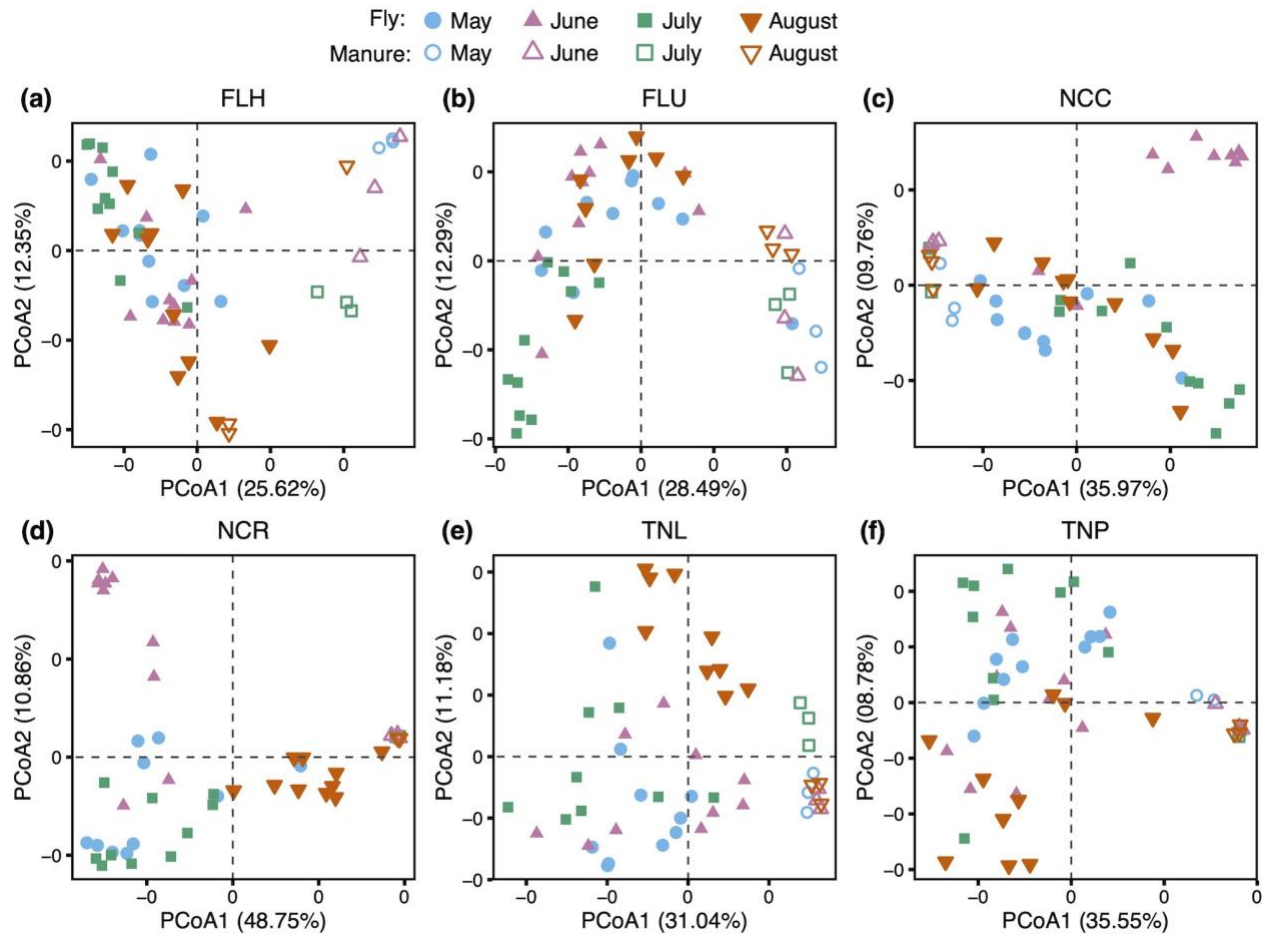


Figure S3: Bacterial community composition in house flies associated with dairy farms. The first and second Principal coordinate analysis (PCoA) axes show the bacterial community composition in individual house fly and manure samples collected in May, June, July, and August at (a) FLH, (b) FLU, (c) NCC (upper panel) and (d) NCR, (e) TNL, (f) TNP (lower panel) dairy farms.

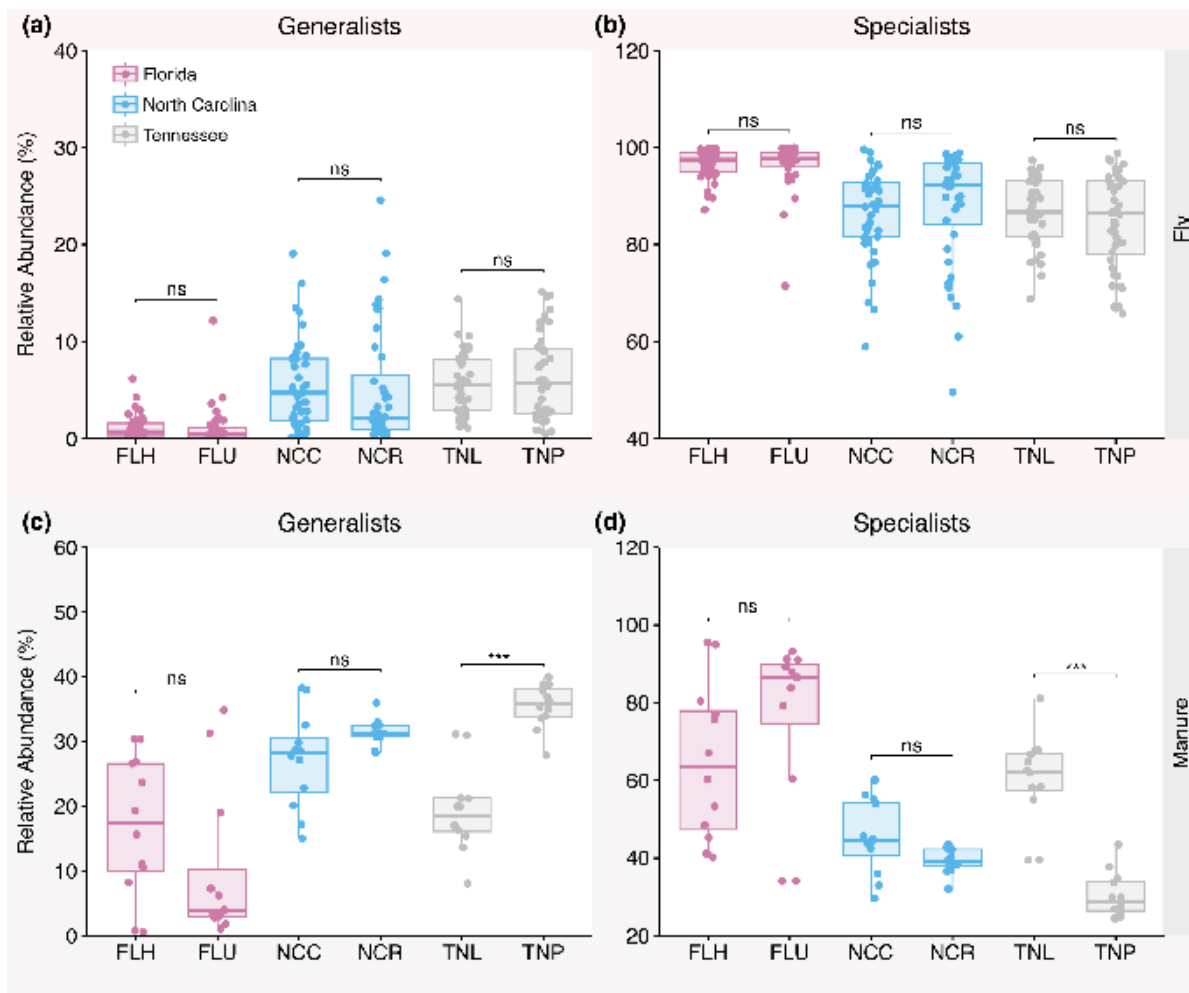


Figure S4: Generalist and specialist bacterial communities in house fly and manure samples from dairy farms. Relative abundance of bacterial generalists in (a) fly and (c) manure samples and specialists in (b) fly and (d) manure samples for each farm (see main text for descriptions). Plots depict the median and interquartile ranges (25th – 75th percentile; boxes) with whiskers representing upper and lower values, respectively with outlier points. Asterisks (***) indicate significant differences between farm within a farm's geographic location (state) at $p < 0.0001$ and ns indicates non-significant. All farms had $n = 40$ samples (except FLU and TNL $n = 38$) of house flies and $n = 12$ manure samples.

Supplementary Tables

Table S1: Metadata associated with sampling sites (farms).

State	Farm	Latitude, Longitude	Cattle breed; Age & Sex	Diet at pen	Lot capacity (head)	Manure management	Fly management	Antibiotic use	Other animals nearby	Sampling Date (year 2021)
Florida	FLH	29.61 N, 82.09 W	Holstein, Jersey and cross; mixed male and female	Total mix ration (TMR)	825	Spread, dry scrape	Yes	Yes	Yes (donkeys)	May 7, June 9, July 21, August 24
Florida	FLU	29.79 N, 82.41 W	Holstein, Jersey and cross; adult females	Grain / TMR (silage)	700/500	Piled in pen	Yes	Yes	Yes (beef cattle)	May 7, June 9, July 21, August 24
North Carolina	NCC	35.92 N 79.12 W	Jersey; mixed	Pasture, hay, roughage	60	Piled nearby, spread	Yes	Yes	Yes (pigs)	May 18, June 11, July 7, August 10
North Carolina	NCR	35.73 N 78.68 W	Holstein, Jersey, Brown Swiss; mixed	Grain, silage, roughage and pasture	380	pile nearby, pile in pen, spread, compost	Yes	Yes	Yes (beef, poultry, swine within a mile)	May 17, June 8, July 6, August 9
Tennessee	TNL	35.66 N, 84.20 W	Holstein; heifers and cows	Grain silage	1500	Spread, hailed away	Yes	Yes	No	May 20, June 24, July 20, August 26
Tennessee	TNP	35.68 N, 84.39 W	Holstein; mixed female	Grain silage	2000+	Spread	Yes	No	No	May 20, June 24, July 20, August 24

Table S2. Effects of farm and sample type on relative abundances of potential pathogens in house fly and manure samples associated with dairy farms.

Taxon	Farm		Sample		Farm:Sample	
	F _(5, 296)	p-value	F _(1, 296)	p-value	F _(5, 296)	p-value
<i>Clostridium</i>	8.79	<0.0001	86.78	<0.0001	7.88	<0.0001
<i>Corynebacterium</i>	18.01	<0.0001	39.52	<0.0001	3.02	0.0113
<i>Escherichia-Shigella</i>	2.95	0.0129	14.81	0.0001	0.87	0.5029
<i>Enterococcus</i>	10.56	<0.0001	25.71	<0.0001	2.19	0.0551
<i>Acinetobacter</i>	1.67	0.1407	15.08	0.0001	1.50	0.1895
<i>Dietzia</i>	2.43	0.0350	5.38	0.0210	0.39	0.8543
<i>Bacteroides</i>	1.35	0.2436	8.46	0.0039	2.02	0.0760
<i>Turicibacter</i>	9.10	<0.0001	24.36	<0.0001	2.73	0.0196
<i>Bacillus</i>	0.72	0.6118	4.50	0.0347	0.17	0.9732
<i>Pseudomonas</i>	6.15	<0.0001	10.27	0.0015	2.97	0.0125
<i>Staphylococcus</i>	3.19	0.0080	8.27	0.0043	0.97	0.4380
<i>Succinivibrio</i>	33.38	<0.0001	100.14	<0.0001	25.31	<0.0001
<i>Treponema</i>	3.57	0.0038	134.73	<0.0001	12.57	<0.0001
<i>Mycobacterium</i>	0.86	0.5074	2.56	0.1110	0.19	0.9647
<i>Proteus</i>	2.49	0.0313	1.54	0.2151	0.74	0.5908
<i>Streptococcus</i>	0.77	0.5726	2.62	0.1069	0.20	0.9608
<i>Providencia</i>	2.47	0.0329	4.52	0.0343	0.75	0.5892
<i>Prevotella</i>	23.25	<0.0001	22.70	<0.0001	10.63	<0.0001
<i>Ignatzschineria</i>	6.46	<0.0001	7.30	0.0073	2.00	0.0790
<i>Brucella</i>	6.03	<0.0001	12.83	0.0004	1.83	0.1063
<i>Burkholderia</i>	2.28	0.0468	2.03	0.1552	0.69	0.6333
<i>Campylobacter</i>	0.70	0.6253	7.09	0.0082	4.39	0.0007
<i>Enterobacter</i>	1.17	0.3222	2.55	0.1114	0.35	0.8799
<i>Erysipelothrix</i>	3.93	0.0018	3.66	0.0568	9.38	<0.0001
<i>Fusobacterium</i>	3.51	0.0043	4.13	0.0430	1.14	0.3405
<i>Klebsiella</i>	1.05	0.3906	5.48	0.0198	0.32	0.9005
<i>Myroides</i>	2.52	0.0298	8.52	0.0038	2.25	0.0498
<i>Trueperella</i>	2.17	0.0578	2.59	0.1089	1.56	0.1707
<i>Vibrio</i>	1.38	0.2312	1.87	0.1722	0.87	0.5037
<i>Tissierella</i>	8.18	<0.0001	18.10	<0.0001	37.33	<0.0001
<i>Morganella</i>	2.40	0.0376	4.47	0.0354	0.75	0.5897
<i>Legionella</i>	1.76	0.1204	3.50	0.0624	0.67	0.6441
<i>Desulfovibrio</i>	4.88	0.0003	1.21	0.2731	2.06	0.0708
<i>Enhydrobacter</i>	1.95	0.0857	1.25	0.2651	0.59	0.7105
<i>Aeromonas</i>	0.59	0.7099	2.94	0.0874	0.18	0.9682

<i>Advenella</i>	1.92	0.0903	9.44	0.0023	0.61	0.6937
<i>Wohlfahrtiimonas</i>	3.93	0.0018	3.86	0.0503	1.18	0.3165
<i>Alcaligenes</i>	1.39	0.2268	3.53	0.0613	0.45	0.8129
<i>Actinomyces</i>	2.14	0.0602	0.69	0.4080	0.69	0.6351
<i>Histophilus</i>	2.26	0.0483	0.68	0.4087	0.68	0.6380
<i>Mannheimia</i>	0.98	0.4329	0.66	0.4159	0.29	0.9157
<i>Moraxella</i>	1.04	0.3949	0.41	0.5221	0.31	0.9051
<i>Mycoplasma</i>	3.48	0.0045	1.05	0.3057	1.05	0.3897
<i>Nocardia</i>	0.89	0.4898	0.72	0.3966	0.27	0.9294

Table S3: Effects of farm and sampling month on relative abundances of potential pathogens in house fly associated with dairy cattle farms.

Pathogen	Farm		Month		Farm:Month	
	F _(5, 212)	p-value	F _(3, 212)	p-value	F _(15, 212)	p-value
<i>Clostridium</i>	2.12	0.0643	5.97	0.0006	1.46	0.1230
<i>Corynebacterium</i>	16.57	<0.0001	1.68	0.1731	1.71	0.0509
<i>Escherichia-Shigella</i>	2.96	0.0133	0.67	0.5728	0.97	0.4861
<i>Enterococcus</i>	10.67	<0.0001	1.66	0.1763	2.30	0.0047
<i>Acinetobacter</i>	2.17	0.0590	3.45	0.0174	2.15	0.0091
<i>Dietzia</i>	1.78	0.1191	7.17	0.0001	2.86	0.0004
<i>Bacteroides</i>	2.44	0.0356	1.53	0.2086	3.16	0.0001
<i>Turicibacter</i>	8.44	<0.0001	13.21	<0.0001	2.84	0.0004
<i>Bacillus</i>	0.71	0.6151	2.58	0.0543	1.03	0.4226
<i>Pseudomonas</i>	7.85	<0.0001	9.88	<0.0001	1.79	0.0375
<i>Staphylococcus</i>	3.10	0.0101	0.71	0.5453	0.61	0.8688
<i>Succinivibrio</i>	16.17	<0.0001	6.52	0.0003	5.49	<0.0001
<i>Treponema</i>	9.23	<0.0001	3.11	0.0272	3.18	0.0001
<i>Mycobacterium</i>	0.82	0.5338	0.24	0.8678	1.35	0.1738
<i>Proteus</i>	2.61	0.0257	1.75	0.1571	1.59	0.0785
<i>Streptococcus</i>	0.75	0.5855	0.66	0.5757	1.10	0.3555
<i>Providencia</i>	2.42	0.0369	0.70	0.5535	0.56	0.9023
<i>Prevotella</i>	11.59	<0.0001	4.38	0.0051	1.79	0.0380
<i>Ignatzschineria</i>	7.02	<0.0001	2.11	0.0997	1.64	0.0664
<i>Brucella</i>	9.89	<0.0001	9.69	<0.0001	8.62	<0.0001
<i>Burkholderia</i>	2.65	0.0240	3.88	0.0100	2.63	0.0012
<i>Campylobacter</i>	1.18	0.3222	1.78	0.1524	1.26	0.2328
<i>Enterobacter</i>	1.22	0.2988	1.29	0.2792	1.43	0.1366
<i>Erysipelothrix</i>	9.10	<0.0001	8.76	<0.0001	4.08	<0.0001
<i>Fusobacterium</i>	4.35	0.0009	1.91	0.1286	3.83	<0.0001

<i>Klebsiella</i>	1.05	0.3905	1.71	0.1651	0.81	0.6652
<i>Myroides</i>	3.90	0.0021	3.14	0.0264	3.78	<0.0001
<i>Trueperella</i>	3.34	0.0064	2.95	0.0338	4.59	<0.0001
<i>Vibrio</i>	1.74	0.1278	0.93	0.4249	1.57	0.0853
<i>Tissierella</i>	18.70	<0.0001	3.44	0.0177	2.84	0.0005
<i>Morganella</i>	2.39	0.0394	0.33	0.8048	1.05	0.4056
<i>Legionella</i>	1.99	0.0820	1.37	0.2531	1.93	0.0216
<i>Desulfovibrio</i>	5.01	0.0002	0.57	0.6338	1.22	0.2568
<i>Enhydrobacter</i>	1.94	0.0885	0.69	0.5570	0.80	0.6774
<i>Aeromonas</i>	0.61	0.6899	0.60	0.6159	1.48	0.1138
<i>Advenella</i>	2.30	0.0464	0.59	0.6196	3.66	<0.0001
<i>Wohlfahrtiimonas</i>	4.65	0.0005	3.76	0.0117	3.17	0.0001
<i>Alcaligenes</i>	1.48	0.1980	0.85	0.4666	1.41	0.1447
<i>Actinomyces</i>	2.32	0.0446	2.03	0.1113	1.86	0.0290
<i>Histophilus</i>	2.31	0.0455	1.05	0.3705	1.04	0.4138
<i>Mannheimia</i>	0.99	0.4231	0.74	0.5297	1.09	0.3698
<i>Moraxella</i>	1.05	0.3876	0.99	0.3974	0.94	0.5193
<i>Mycoplasma</i>	3.58	0.0040	1.20	0.3097	1.17	0.2935
<i>Nocardia</i>	0.88	0.4934	0.72	0.5419	0.99	0.4718

Table S4: Effects of farm and sampling month on relative abundances of potential pathogens in manure samples associated with dairy cattle farms.

Taxon	Farm		Month		Farm:Month	
	F _(5, 48)	p-value	F _(3, 48)	p-value	F _(15, 48)	p-value
<i>Clostridium</i>	19.16	<0.0001	2.44	0.0762	3.49	0.0005
<i>Corynebacterium</i>	12.09	<0.0001	4.33	0.0089	1.99	0.0368
<i>Escherichia-Shigella</i>	3.34	0.0114	1.32	0.2788	0.94	0.5301
<i>Enterococcus</i>	5.67	0.0003	0.88	0.4572	0.85	0.6161
<i>Acinetobacter</i>	4.83	0.0012	0.72	0.5445	0.76	0.7143
<i>Dietzia</i>	6.19	0.0002	5.27	0.0032	2.99	0.0020
<i>Bacteroides</i>	3.87	0.0050	1.47	0.2353	1.81	0.0616
<i>Turicibacter</i>	8.21	<0.0001	4.23	0.0099	3.67	0.0003
<i>Bacillus</i>	6.49	0.0001	0.18	0.9101	0.82	0.6523
<i>Pseudomonas</i>	6.85	0.0001	1.94	0.1356	1.46	0.1571
<i>Staphylococcus</i>	1.72	0.1476	0.92	0.4400	0.95	0.5152
<i>Succinivibrio</i>	25.53	<0.0001	1.00	0.4005	1.83	0.0575
<i>Treponema</i>	6.69	0.0001	5.25	0.0033	3.29	0.0009
<i>Mycobacterium</i>	36.16	<0.0001	4.23	0.0098	2.56	0.0070
<i>Proteus</i>	5.31	0.0006	1.44	0.2430	1.31	0.2351

<i>Streptococcus</i>	5.53	0.0004	0.36	0.7793	2.43	0.0102
<i>Providencia</i>	2.70	0.0312	1.91	0.1405	1.52	0.1344
<i>Prevotella</i>	23.38	<0.0001	3.47	0.0232	1.33	0.2216
<i>Ignatzschineria</i>	3.17	0.0150	1.54	0.2155	1.60	0.1108
