

Research article

Comparative 16S rRNA amplicon profiling of cattle manure and Lake Van sediment: selective environmental filtering of fecal-associated bacterial communities in a polyextreme soda lakeKoray Tuncay¹  and Fatih Ş. Erkuş^{2*} ¹Department of Biosystems Engineering, Institute of Natural and Applied Sciences, Van Yüzüncü Yıl University, Van 65080, Türkiye²Department of Biosystems Engineering, Faculty of Agriculture, Van Yüzüncü Yıl University, Van 65080, Türkiye*Corresponding author: Fatih Ş. Erkuş, fatiherkus@yyu.edu.tr

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Article information: Tuncay K and Erkuş FŞ, 2026. *One Health Microbiol. Infect.* Vol. 2, No. 1: 48–59. <https://doi.org/10.66585/ohmi.2026.2.0015>**Abstract**

The agricultural dissemination of livestock-derived fecal microbiota into aquatic ecosystems presents a significant One Health challenge. However, polyextreme environments, such as the highly alkaline and saline Lake Van, are hypothesized to act as natural environmental filters against non-adapted, allochthonous microorganisms. This study evaluated the selective environmental filtering potential of the Lake Van ecosystem against cattle manure inputs. We compared the microbial communities of cattle manure (CM, $n = 5$) and Lake Van coastal sediment (LS, $n = 5$) using 16S rRNA gene amplicon sequencing at the strict amplicon sequence variant (ASV) level. The analysis revealed a profound compositional separation between the two compartments (PERMANOVA pseudo- $F = 5.21$, $p = 0.011$), with only 8.6% of the 45,842 detected ASVs shared across environments. A sharp alpha diversity contrast in favor of LS was accompanied by a phylum-level inversion between *Bacillota* (LS-dominant) and *Pseudomonadota* (CM-dominant). Crucially, classic fecal and zoonotic indicators (*Escherichia-Shigella*, *Enterobacteriaceae*, *Acinetobacter*, *Burkholderiaceae*, and *Streptococcus*) exhibited a systematic 3- to 42-fold reduction in the sediment. Conversely, obligately anaerobic *Bacillota* families, including spore-forming *Clostridium*, persisted in the LS environment. The polyextreme physicochemical conditions of the soda lake impose a strong environmental filtering effect on manure-derived microbial diversity. While this filter substantially attenuates aerobically-tolerant fecal microbiota, the persistence of strict anaerobes indicates that the filtering effect is selective rather than absolute. The minimal shared-ASV fraction places a strict upper bound on strain-level fecal–sediment connectivity, underscoring the severe selective pressures of the extreme soda-lake environment.

Keywords: Cattle manure, Environmental filtering, Lake sediment, Lake Van, Soda lake microbiome**Introduction**

The One Health framework requires an integrated understanding of the dynamic interactions among environmental, animal, and human microbiomes [1]. Cattle manure is produced in large volumes and can disseminate fecal microorganisms into soil and water systems. Within this framework, manure-associated microbiota may link livestock health, environmental contamination, and human exposure [2]. In agricultural systems, cattle manure (CM) serves as a critical link in this network due to its high microbial load and concentration of potential pathogens. Research on the cattle manure microbiome has demonstrated that manure harbors diverse fermentative and hydrolytic microbial communities, making it a suitable proxy for animal-derived microbial reservoirs [3]. Cattle feces typically contain enteric bacteria of fecal origin (e.g., *Clostridia* and *Bacteroidia*) alongside various opportunistic pathogens [4]. When this material enters aquatic ecosystems through surface runoff from agricultural fields, it can serve as a significant environmental reservoir for disease transmission [5].

In freshwater systems, allochthonous fecal microorganisms can persist for extended periods, particularly in sediments, where decay rates remain consistently lower than in the water column [6, 7]. By

contrast, polyextreme soda lakes impose a markedly different selective regime. Their stable alkaline conditions (pH 9–11) and sodium-carbonate-driven salinity favor haloalkaliphilic specialists while limiting the survival of most non-adapted fecal taxa [8, 9].

Lake Van, located in eastern Türkiye, is the world's largest soda lake, characterized by a stable pH of approximately 9.7 and a salinity of roughly 22 g/L [10]. With a 2024 livestock inventory in the adjacent Van Province comprising over 150,000 cattle and 3.3 million small ruminants, livestock manure represents a realistic nonpoint-source contamination risk to the lake via surface runoff and tributary transport [11, 12, 13]. Previous studies of Lake Van have documented high bacterial diversity in both water and sediment, alongside a dominance of *Proteobacteria* (*Pseudomonadota*). In soda lakes, elevated pH and salinity act as major environmental pressures that shape microbial community composition [14]. Because these extreme conditions limit the viability of non-adapted enteric bacteria [15, 16], Lake Van sediments are expected to function as a natural environmental filter against such agricultural fecal inputs.

Despite its ecological importance, the initial persistence and adaptation of allochthonous, manure-derived microbiota under soda-lake conditions remain poorly characterized. To evaluate this natural filtering effect, we

used 16S rRNA gene amplicon sequencing to compare the bacterial communities in cattle manure (CM) and Lake Van coastal sediments (LS). We hypothesized that CM would exhibit greater enrichment of zoonotic and opportunistic taxa, whereas LS would reflect the selective filtering imposed by the soda-lake environment.

Materials and methods

Sample collection and DNA extraction

Two sample types were analyzed in five biological replicates: cattle manure (CM) and Lake Van coastal sediment (LS), all collected in September 2025. Fresh cattle manure was obtained from healthy, lactating Holstein cows at the Van Yüzüncü Yıl University Livestock Application and Research Center, maintained on a standardized total mixed ration (48% corn silage, 16% wheat straw, 19% alfalfa hay, 16% dairy concentrate, dry-matter basis). The manure (moisture content $88.51 \pm 0.72\%$) was transported to the laboratory in insulated thermoses with hot-water bags to maintain ambient temperature.

Lake Van sediment was sampled from the littoral zone, 80 m from shore ($38^{\circ}29'47.9''\text{N}$, $43^{\circ}19'10.3''\text{E}$), using an Ekman-type bottom grab sampler, which retrieves the uppermost ~0–15 cm of surface sediment. Sediment

(moisture content $57.55 \pm 0.82\%$) was transported in thermoses pre-cooled to 4°C and stored at 4°C until analysis to preserve microbial viability and chemical integrity. The sediment sampling point was located at the discharge of a surface drainage channel that conveyed runoff from a catchment hosting numerous smallholder cattle husbandry operations into Lake Van, thereby establishing a direct source-to-sink pathway between the manure-bearing catchment and the sampled sediment.

Prior to molecular analysis, both compartments were characterized physicochemically (Table 1). Total and volatile solids were determined gravimetrically (APHA 2540; drying at 105°C for 24 h, ignition at 550°C for 3 h); pH was measured with a WTW pH 3110 meter; soluble-salt content was determined from the electrical conductivity of a 1:5 (w/v) extract equilibrated at 25°C [17]; total carbon and nitrogen were measured by elemental analysis (Thermo Scientific Flash 2000). Overlying water temperature and dissolved oxygen at the lake site were recorded *in situ* with a multiparameter water-quality meter. The sediment was strongly alkaline and saline (pH 9.76; soluble salt 43.36 g/kg), consistent with regional values for Lake Van [10] and confirming polyextreme soda-lake conditions, whereas the manure was near-neutral and low in salinity (pH 7.52; 6.89 g/kg).

Table 1: Physicochemical characteristics of the sampled compartments (mean ± SD).

Parameter	Lake Van sediment (LS)	Cattle manure (CM)
pH	9.76±0.11	7.52±0.03
EC, 1:5 (dS/m)	13.55 ± 0.17	2.15 ± 0.10
Soluble salt (g/kg)	43.36 ± 0.54	6.89 ± 0.31
Total solids (%)	42.45 ± 0.92	11.49 ± 0.72
Volatile solids (% of TS)	7.67 ± 0.01	76.92 ± 0.46
Total C (%)	11.20 ± 0.25	35.20 ± 0.42
Total N (%)	1.18 ± 0.03	2.45 ± 0.08
C/N	9.50 ± 0.30	14.37 ± 0.55
Water temperature (°C, overlying water)	12.7 ± 0.1	—
Dissolved oxygen (% sat., overlying water)	165 ± 20	—

pH = power of hydrogen (acidity); EC = electrical conductivity; dS/m = decisiemens per metre; C/N = carbon-to-nitrogen ratio; SD = standard deviation; "—", not applicable.

Genomic DNA was extracted from the samples using a commercial DNA Isolation Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Briefly, cell pellets were obtained by centrifugation at $10,000 \times g$ for 10 minutes. The pellets were homogenized with a lysis buffer, followed by mechanical disruption via bead-beating. Total DNA concentration was quantified fluorometrically using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

16S rRNA amplification and sequencing

Total community DNA served as a template for amplifying the V3–V4 hypervariable region of the prokaryotic 16S rRNA gene. Amplification was performed on a SimpliAmp Thermal Cycler using the 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-

GACTACHVGGGTATCTAATCC-3') primer pair, which broadly targets both bacterial and archaeal lineages [18]. PCR conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of touchdown PCR (95°C for 30 s, $53\text{--}50^{\circ}\text{C}$ for 30 s, and 72°C for 30 s), and a final hold at 4°C .

Amplicon products were purified using the Qiaseq Beads Clean-Up Kit (Cat. No.: 180795, Qiagen, Hilden, Germany). Sequencing libraries were prepared using the Nextera XT DNA Library Prep Kit (Cat. No.: FC-131-1096, Qiagen, Hilden, Germany), and indexing was carried out with the TG Nextera XT Index Kit v2 Set A (Cat. No.: TG-131-2001, Qiagen, Hilden, Germany), following the manufacturer’s protocols. Final library concentrations were determined using the iQuant™ dsDNA HS Assay Kit (ABP Biosciences, Rockville, MD, USA). Sequencing was

performed on an Illumina iSeq 100 platform (Illumina, Inc., San Diego, CA, USA), generating 2×150 bp paired-end reads.

Per-sample raw read depth ranged from 576,597 (LS1) to 1,200,562 (CM3), with group-mean depths of $864,492 \pm 251,601$ reads for the LS group and $855,536 \pm 213,713$ reads for the CM group. There was no significant difference in sequencing depth between the two groups ($p = 0.953$, Welch's t-test), indicating high comparability for downstream microbiome profiling.

Bioinformatics processing

Raw paired-end reads were processed in QIIME 2 (version 2025.7) using the standard DADA2 workflow for denoising, primer trimming, merging, and amplicon sequence variant (ASV) inference. The resulting ASV-level feature table contained 45,842 unique sequence variants across the ten study samples. Taxonomic assignments were made against the SILVA 138.1 reference database utilizing the QIIME 2 sklearn classifier. Across the ten samples, the phylum-assigned bacterial fraction accounted for $49.19 \pm 0.68\%$ of total reads, with very low between-sample variation ($CV \approx 1.4\%$), making between-sample relative comparisons robust at this denominator. In accordance with updated prokaryotic nomenclature, the previously used phylum names *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were re-designated as *Bacillota*, *Bacteroidota*, and *Pseudomonadota*, respectively [19, 20].

Statistical analysis

The analytical pipeline followed the framework described by Ozbayram et al. [3] for comparative microbiome studies, adapted to the present sample comparison and re-implemented at a strict ASV level. Alpha-diversity statistics, including the number of observed ASVs (S_{obs}), Chao1 richness estimator, Shannon entropy (H'), Simpson index ($1 - D$), inverse Simpson ($1/D$), and Pielou's evenness (J), were calculated according to standard formulas [21]. Group differences in alpha diversity were assessed using Welch's two-sample t-test (heteroscedastic) and the non-parametric Mann-Whitney U test ($n = 5$ per group). To contextualize the per-group sample size, standardized effect sizes (Cohen's d) and the minimum detectable effect size at 80% power (two-sided $\alpha = 0.05$) were computed for the alpha-diversity contrasts; achieved post-hoc power was also estimated, recognizing that observed power is a monotone function of the p -value and of limited inferential value [22].

Beta diversity was assessed on column-normalized (relative-abundance) ASV-level profiles using the Bray-Curtis dissimilarity index. Principal coordinate analysis (PCoA) was performed on the resulting dissimilarity matrix via classical multidimensional scaling (eigendecomposition of the double-centered squared

distance matrix). PCoA was selected because rank-preserving NMDS produced a degenerate compressed solution at this sample size; furthermore, PCoA preserves true Bray-Curtis distances and yields interpretable variance-explained axes. Group separation was tested by one-way PERMANOVA on the Bray-Curtis matrix with 999 random permutations. No rarefaction was applied; alpha-diversity indices, richness estimators, and the shared-ASV (Venn) analysis were computed on the full (unfiltered) ASV table, and beta-diversity on relative-abundance profiles, because per-sample sequencing depth did not differ between groups (Welch $p = 0.953$; Table 2) and rarefying is statistically inadmissible [23]. The abundance-weighted indices emphasized here (Shannon, inverse Simpson, Pielou's J) are largely insensitive to read depth, whereas observed-ASV richness is depth-sensitive and is interpreted cautiously.

Family-level differential abundance (for taxa with a mean relative abundance $> 0.5\%$ in at least one group) was evaluated as an exploratory analysis via Welch's t-test on per-sample percentages, complemented by the non-parametric Mann-Whitney U test; these exploratory p -values are reported without multiple-test correction, and interpretation rests on the directional consistency across taxa and the agreement of the two tests rather than on any single corrected p -value.

Following Ozbayram et al. [3], shared and unique taxa between groups were visualized with a Venn diagram at the strict ASV level, using a presence-call threshold of count > 0 in any sample of the corresponding group. Statistical analyses were executed in R (v4.3) and Python (v3.12) environments, utilizing the pandas, numpy, scipy.stats, and scikit-learn (manifold.MDS) libraries, with figures rendered via matplotlib.

Results

Read recovery and taxonomic resolution

Total read counts per sample (Table 2) ranged from 576,597 to 1,200,562, with group-mean depths of $864,492 \pm 251,601$ for the LS group and $855,536 \pm 213,713$ for the CM group. There was no significant difference in sequencing depth between the two groups (Welch $p = 0.953$), excluding sequencing-depth bias as a confounder of compositional differences. After taxonomic curation, $49.19 \pm 0.68\%$ of reads were assigned to a defined bacterial phylum and were retained as the denominator for all compositional analyses. The remainder consisted of "Unassigned" reads (mean $164,782 \pm 51,712$ per sample) and reads classified to the domain Bacteria but unresolved at the phylum level (mean $271,298 \pm 71,124$ per sample). Archaeal reads were detected only in trace amounts (≤ 155 reads per sample), indicating a negligible archaeal presence in these specific environments despite the broad targeting capabilities of

the primer pair used. As reported in Table 2, neither the unassigned fraction (Welch $p = 0.392$) nor the bacterial fraction unclassified at the phylum level ($p = 0.567$) differed significantly between LS and CM, and the proportion of reads retained as phylum-assigned was near-identical between groups ($49.5 \pm 0.8\%$ vs $48.9 \pm 0.3\%$); the excluded fractions are therefore balanced across compartments and do not bias the between-group

comparison. The magnitude of the unresolved fraction is expected for V3–V4 amplicons classified against SILVA, particularly for sediment and soda-lake communities rich in uncultured lineages under-represented in reference databases. All absolute percentages are reported conditional on the phylum-assigned-bacteria denominator.

Table 2: Read statistics and read-retention summary (n=10).

Sample	Group	Total reads	Unassigned	Bact. uncl. phylum*	Phylum-assigned bacteria	% retained
LS1	LS	576,597	105,132	192,664	278,678	48.3
LS2	LS	955,279	156,408	321,041	477,551	50.0
LS3	LS	987,397	173,709	329,663	483,650	49.0
LS4	LS	1,170,132	202,864	377,659	588,249	50.3
LS5	LS	633,053	113,403	202,996	315,730	49.9
CM1	CM	725,653	158,034	210,239	357,055	49.2
CM2	CM	913,841	197,771	271,244	441,976	48.4
CM3	CM	1,200,562	266,982	350,167	582,885	48.6
CM4	CM	773,557	159,262	233,993	379,856	49.1
CM5	CM	664,068	114,259	223,317	325,794	49.1
LS mean $\pm$ SD		864,492 $\pm 251,601$	150,303 $\pm 41,290$	284,805 $\pm 80,272$	428,772 $\pm 124,916$	49.5 ± 0.8
CM mean $\pm$ SD		855,536 $\pm 213,713$	179,262 $\pm 60,168$	257,792 $\pm 60,128$	417,513 $\pm 99,830$	48.9 ± 0.3
p (Welch)		0.953	0.392	0.567	0.880	—

*Bact. uncl. Phylum refers to reads classified to the domain bacteria but unclassified at the phylum level. LS: = Lake Van sediment; CM = Cattle manure

Phylum-level composition

At the phylum level, a marked compositional contrast separated the two compartments (Figure 1, Table 3). The LS was strongly dominated by *Bacillota* (53.66% pooled, mean $53.74 \pm 5.43\%$), followed by *Bacteroidota* (21.29% pooled, mean $21.38 \pm 2.88\%$) and *Pseudomonadota* (19.21% pooled, mean $18.88 \pm 3.91\%$), with all remaining phyla collectively contributing $<5\%$. The CM compartment exhibited a substantially different signature: *Pseudomonadota* (36.16% pooled), *Bacteroidota* (30.03% pooled), and *Bacillota* (23.44% pooled), with notable contributions from *Thermodesulfobacteriota* (4.46%), *Actinomycetota* (0.86%), *Chloroflexota* (0.81%), and *Spirochaetota* (0.62%). The collapse of *Bacillota* from $\sim 54\%$ in LS to $\sim 23\%$ in CM, mirrored by an inverse rise of *Pseudomonadota* from $\sim 19\%$ to $\sim 36\%$, was the dominant compositional axis distinguishing the two groups. Within-group variability was modest in LS (*Bacillota* CV = 10.1%; *Pseudomonadota* CV = 20.7%) and somewhat higher in CM (*Bacillota* CV = 22.2%; *Pseudomonadota* CV = 22.9%), reflecting biological variability among individual cattle manure samples. *Bacteroidota* was the second most dominant phylum in both compartments but was higher in CM, consistent with this phylum encompassing both gut-associated and sediment-associated lineages.

Family-level composition and differential abundance

Of the 44 families with a mean relative abundance $\geq 0.5\%$ in at least one group, 16 (36.4%) were significantly differentially abundant between LS and CM at Welch's $p < 0.05$ (Figure 2, Figure 3, Table 4). Notably, 15 of these 16 families were also significant under the non-parametric Mann–Whitney U test ($p < 0.05$), with 11 reaching the minimum possible two-sided p -value (0.008) for two groups of $n = 5$ with completely non-overlapping ranks.

Anaerobic-fermenter families were strongly enriched in LS: *Oscillospiraceae* (13.86% vs. 4.97%, $p = 0.001$), *Lachnospiraceae* (6.87% vs. 1.88%, $p < 0.001$), *Eubacterium coprostanoligenes* group (3.91% vs. 0.36%, $p < 0.001$), *Bacteroidaceae* (4.07% vs. 1.30%, $p = 0.001$), *Ruminococcaceae* (2.18% vs. 0.28%, $p < 0.001$), *Prevotellaceae* (2.88% vs. 0.24%, $p = 0.008$), *Monoglobaceae* (1.16% vs. 0.04%, $p < 0.001$), *Bacteroidales* RF16 group (1.03% vs. 0.10%, $p < 0.001$), and *Peptococcaceae* (0.84% vs. 0.02%, $p = 0.010$). *Butyricicoccaceae* (0.65% vs. 0.13%, $p = 0.021$) and an unclassified *Bacillota* family (3.22% vs. 1.42%, $p = 0.003$) were also elevated in this compartment.

Conversely, families typical of aerobic/facultative environments were strongly enriched in CM: *Rhodocyclaceae* (15.13% vs. 0.04%, $p < 0.001$), an

unclassified *Pseudomonadota* family (3.17% vs. 0.28%, $p = 0.001$), *Desulfomicrobiaceae* (1.80% vs. 0.05%, $p = 0.021$), and an unclassified *Bacteroidota* family (2.92% vs. 0.97%, $p = 0.028$). Interestingly, *Pseudomonadaceae* showed the opposite trend, being enriched in LS (10.27% vs. 4.46%, $p = 0.046$); this is consistent with *Pseudomonas* encompassing both opportunistic-pathogen lineages and ubiquitous environmental species.

The overall pattern was internally consistent, with gut-associated obligate anaerobes showing higher abundance in LS whereas *Pseudomonadota*, which are typically associated with aerobically perturbed manure or environmental nitrogen cycling, were more abundant in CM.

Table 3: Phylum-level relative abundance (LS: Lake Van sediment; CM: Cattle manure).

Phylum	LS pooled %	LS mean $\pm$ SD %	CM pooled %	CM mean $\pm$ SD %	Δ (LS – CM, pooled)
Bacillota	53.66	53.74 $\pm$ 5.43	23.44	23.92 $\pm$ 5.32	+30.22
Bacteroidota	21.29	21.38 $\pm$ 2.88	30.03	29.72 $\pm$ 7.02	–8.74
Pseudomonadota	19.21	18.88 $\pm$ 3.91	36.16	36.44 $\pm$ 8.33	–16.95
Campylobacterota	2.12	2.30 $\pm$ 2.85	0.02	0.02 $\pm$ 0.05	+2.10
Thermodesulfobacteriota	1.56	1.54 $\pm$ 0.92	4.46	4.34 $\pm$ 1.82	–2.90
Verrucomicrobiota	0.74	0.76 $\pm$ 0.40	0.43	0.51 $\pm$ 0.58	+0.31
Patescibacteria	0.27	0.26 $\pm$ 0.06	0.33	0.34 $\pm$ 0.08	–0.06
Actinomycetota	0.27	0.25 $\pm$ 0.15	0.86	0.84 $\pm$ 0.39	–0.59
Spirochaetota	0.22	0.21 $\pm$ 0.05	0.62	0.59 $\pm$ 0.70	–0.40
Chloroflexota	0.20	0.21 $\pm$ 0.24	0.81	0.74 $\pm$ 0.93	–0.61
Cyanobacteriota	0.16	0.16 $\pm$ 0.03	0.09	0.11 $\pm$ 0.15	+0.07

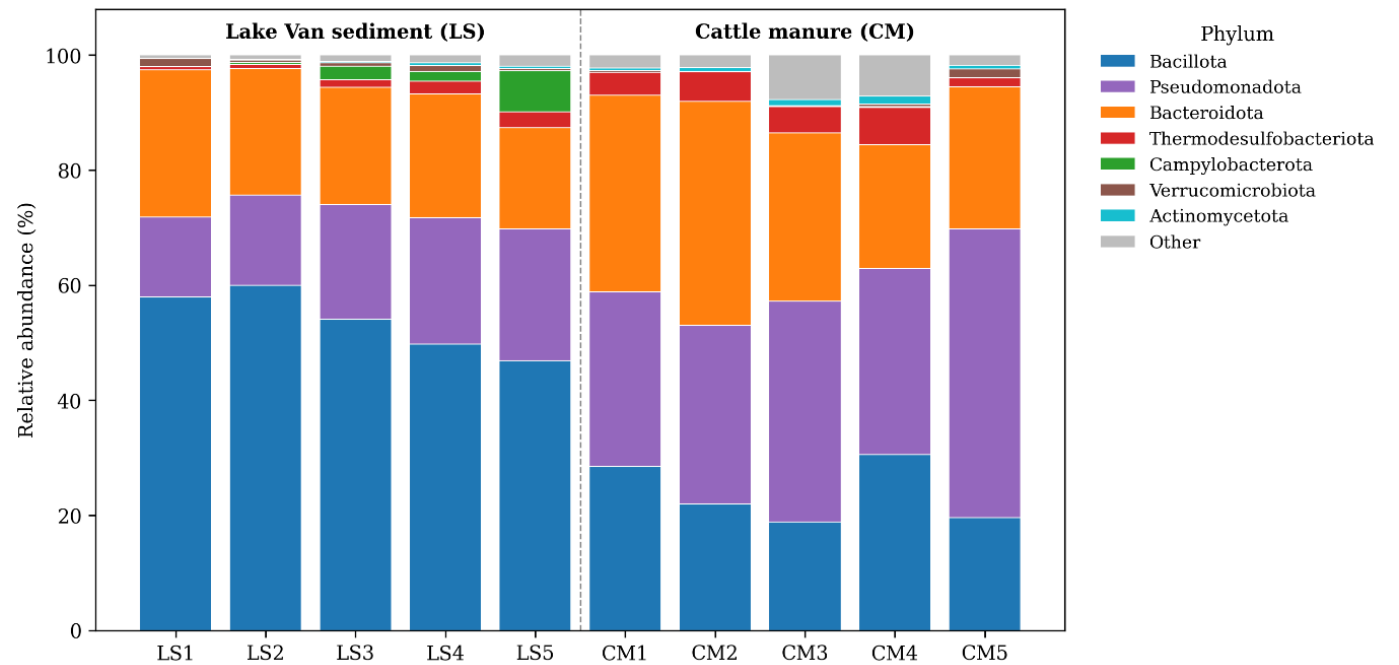


Figure 1: Phylum-level relative abundance of phylum-assigned bacterial reads in the five LS and five CM replicates. Phylum-level bacterial composition (n=5+5).

Zoonotic and opportunistic indicator taxa

To address the central hypothesis of the study, which proposes that LS reflects environmental filtering of fecal-derived microbiota, we focused on a curated set of canonical zoonotic and opportunistic indicator taxa (Table 5, Figure 4). The pattern was internally consistent: classic fecal/zoonotic markers were systematically reduced in LS relative to CM. *Escherichia–Shigella*, a primary fecal

coliform indicator, was 7.9-fold lower in LS than in CM (0.017 vs. 0.130% pooled); the broader *Enterobacteriaceae* family was 10.1-fold lower (0.017 vs. 0.168%). The opportunistic genus *Acinetobacter* (*Moraxellaceae*) was 3.2-fold lower in LS (0.97 vs. 3.07%), with substantial within-group variability (CM range 0.91–8.67% across replicates). *Streptococcus* was 2.9-fold lower in LS, *Enterococcus* 1.6-fold lower, and *Burkholderiaceae*

42.3-fold lower. Conversely, environmental aquatic and sediment-associated lineages were strongly enriched in LS: *Shewanella* (0.50 vs. 0.008%, ~64-fold higher in LS), *Aeromonas* (0.046% in LS, undetectable in CM), *Pseudomonas* (10.77 vs. 4.97%, 2.2-fold higher in LS), and *Clostridium* (sensu lato, 0.42 vs. 0.04%, ~10-fold

higher in LS). The directional consistency of the indicator pattern, across taxa spanning *Pseudomonadota* and *Bacillota*, supports the interpretation that the observed contrast reflects systematic environmental filtering rather than stochastic compositional variation.

Table 4: Differentially abundant families (Welch's t-test $p < 0.05$).

Family	Phylum	LS mean $\pm$ SD %	CM mean $\pm$ SD %	$\log_2(\text{LS/CM})$	$p(\text{Welch})$	$p(\text{M-W})$
<i>Rhodocyclaceae</i>	<i>Pseudomonadota</i>	0.04 $\pm$ 0.05	15.13 $\pm$ 1.92	-8.48	< 0.001	0.012
<i>Ruminococcaceae</i>	<i>Bacillota</i>	2.18 $\pm$ 0.35	0.28 $\pm$ 0.13	+2.94	< 0.001	0.008
<i>Bacteroidales</i> RF16 group	<i>Bacteroidota</i>	1.03 $\pm$ 0.21	0.10 $\pm$ 0.19	+3.42	< 0.001	0.008
[<i>Eubacterium</i>] <i>coprostanoligenes</i> group	<i>Bacillota</i>	3.91 $\pm$ 0.65	0.36 $\pm$ 0.24	+3.46	< 0.001	0.008
<i>Lachnospiraceae</i>	<i>Bacillota</i>	6.87 $\pm$ 1.13	1.88 $\pm$ 1.10	+1.87	< 0.001	0.008
<i>Monoglobaceae</i>	<i>Bacillota</i>	1.16 $\pm$ 0.22	0.04 $\pm$ 0.05	+4.77	< 0.001	0.008
<i>Oscillospiraceae</i>	<i>Bacillota</i>	13.86 $\pm$ 2.73	4.97 $\pm$ 1.37	+1.48	0.001	0.008
<i>Bacteroidaceae</i>	<i>Bacteroidota</i>	4.07 $\pm$ 0.25	1.30 $\pm$ 0.79	+1.65	0.001	0.008
<i>Pseudomonadota</i> Unclassified	<i>Pseudomonadota</i>	0.28 $\pm$ 0.06	3.17 $\pm$ 0.78	-3.51	0.001	0.008
<i>Bacillota</i> Unclassified	<i>Bacillota</i>	3.22 $\pm$ 0.74	1.42 $\pm$ 0.45	+1.18	0.003	0.008
<i>Prevotellaceae</i>	<i>Bacteroidota</i>	2.88 $\pm$ 1.22	0.24 $\pm$ 0.16	+3.57	0.008	0.008
<i>Peptococcaceae</i>	<i>Bacillota</i>	0.84 $\pm$ 0.40	0.02 $\pm$ 0.03	+5.26	0.010	0.008
<i>Desulfomicrobiaceae</i>	<i>Thermodesulfobact.</i>	0.05 $\pm$ 0.05	1.80 $\pm$ 1.06	-5.16	0.021	0.016
<i>Butyrificoccaceae</i>	<i>Bacillota</i>	0.65 $\pm$ 0.33	0.13 $\pm$ 0.18	+2.26	0.021	0.016
<i>Bacteroidota</i> Unclassified	<i>Bacteroidota</i>	0.97 $\pm$ 0.15	2.92 $\pm$ 1.30	-1.59	0.028	0.016
<i>Pseudomonadaceae</i>	<i>Pseudomonadota</i>	10.27 $\pm$ 3.55	4.46 $\pm$ 4.19	+1.20	0.046	0.095

All families with Welch's $p < 0.05$ and mean ≥ 0.5 % in at least one group, sorted by ascending Welch p -value. Negative $\log_2$ fold-changes denote enrichment in CM. The Mann-Whitney U test reaches its minimum two-sided p (≈ 0.008) for two groups of $n = 5$ with completely non-overlapping ranks; values above this threshold indicate at least one rank crossing between groups; LS = Lake Van sediment; CM = Cattle manure.

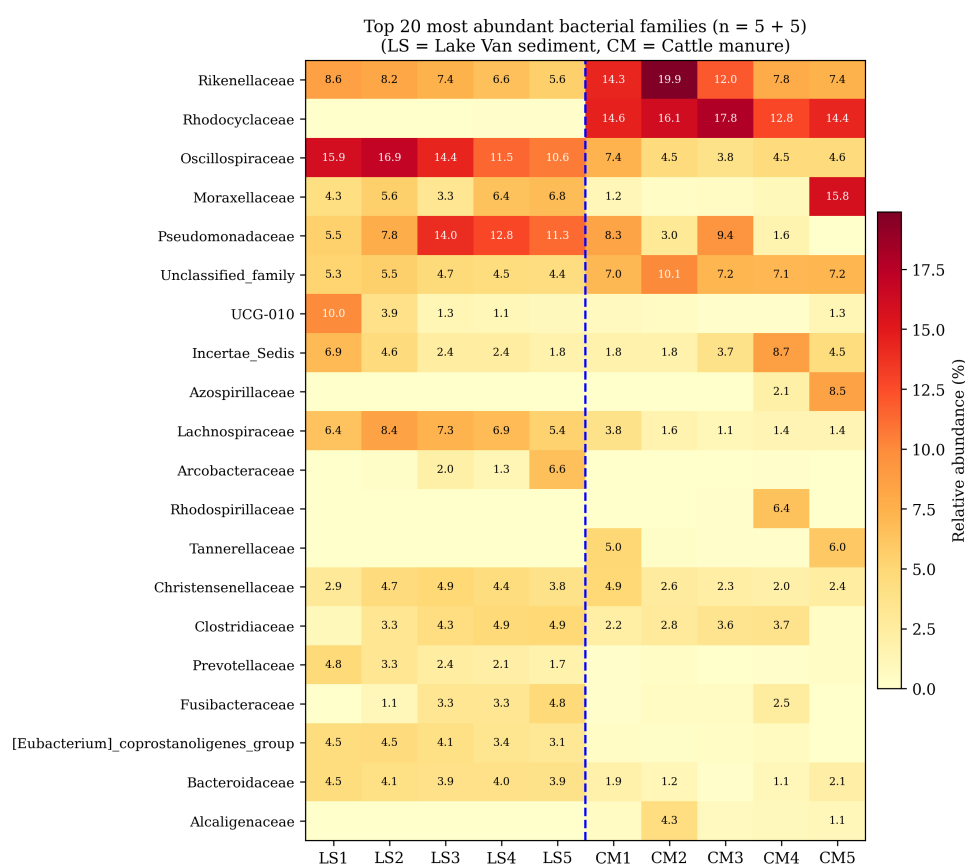


Figure 2: Heat-map of the 20 most abundant bacterial families across all ten samples. Cell labels denote per-sample relative abundance (%) where ≥ 1 %. Color scale linear from 0 to dataset maximum. Dashed blue line separates groups. LS: Lake Van sediment; CM: Cattle manure.

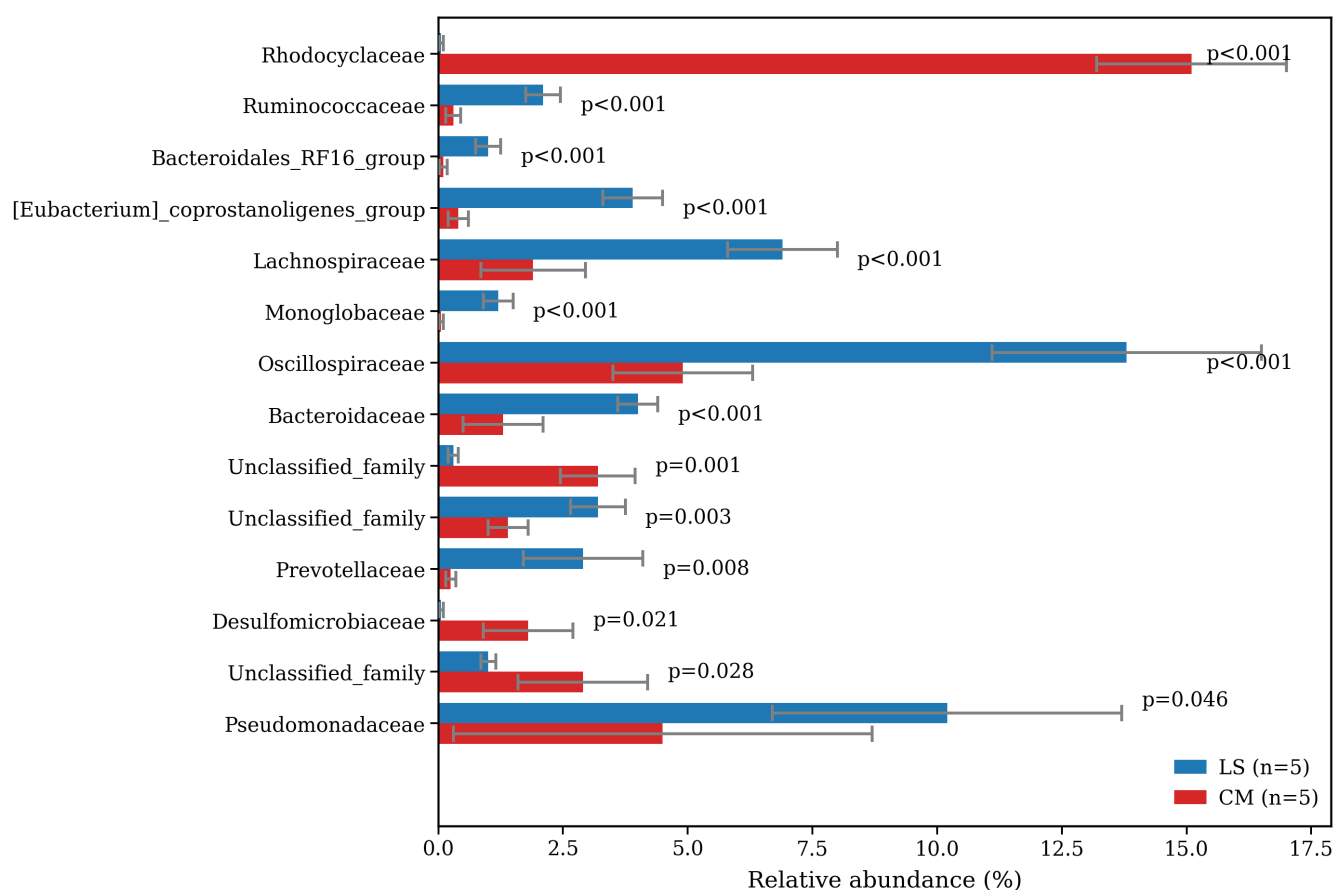


Figure 3: Family-level differential abundance: mean (bars) $\pm$ SD (whiskers) in groups LS (blue, n = 5) and CM (red, n = 5), with Welch's t-test p-values. Filtering: $p < 0.05$, max group mean $> 1\%$.

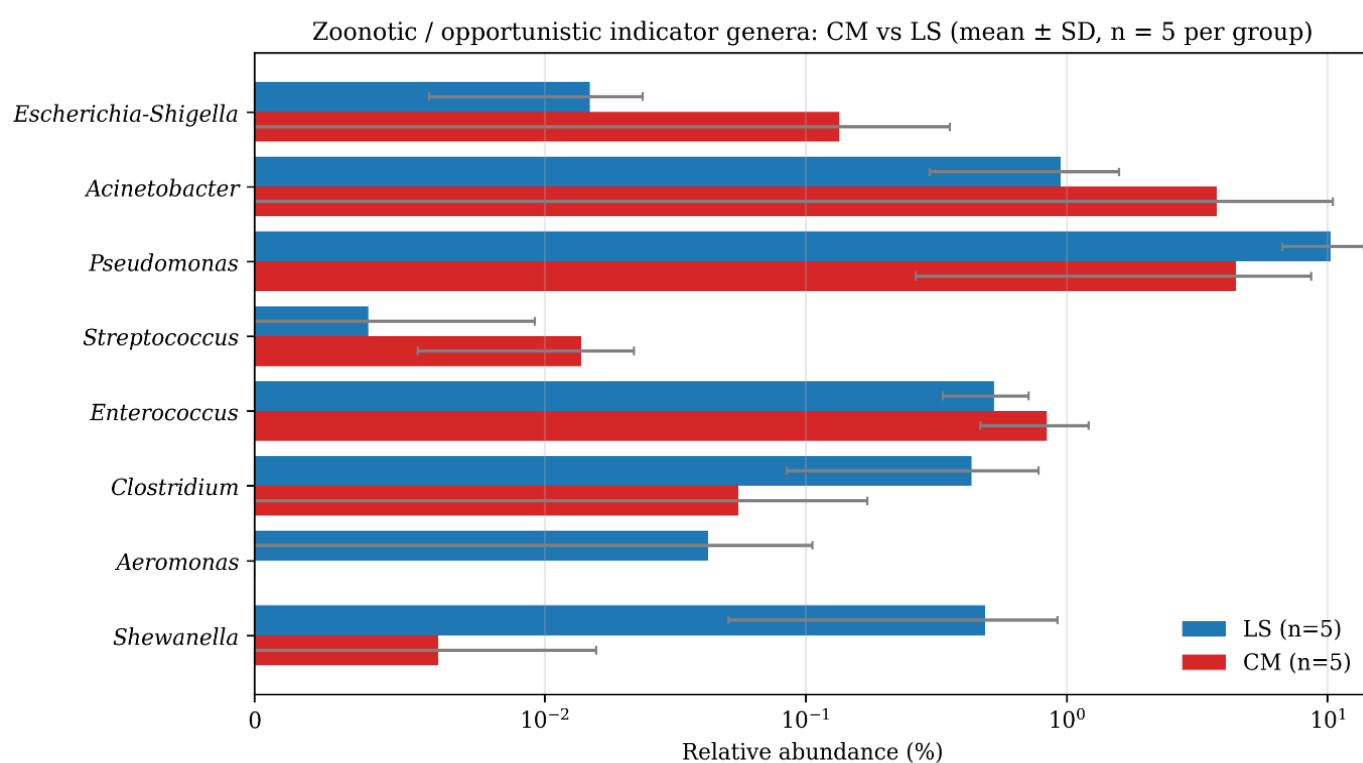


Figure 4: Zoonotic and opportunistic indicator genera in CM and LS (mean $\pm$ SD, n = 5 per group). X-axis is symmetric-log scale to span the four orders of magnitude that separate the most abundant from the rarest taxa. LS: Lake Van sediment; CM: Cattle manure.

Table 5: Zoonotic and opportunistic indicator taxa (pooled relative abundance %).

Taxon	Rank	LS pooled %	CM pooled %	CM/LS ratio	Indicator role
<i>Escherichia-Shigella</i>	Genus	0.017	0.130	7.85	Fecal coliform / opportunistic
<i>Enterobacteriaceae</i>	Family	0.017	0.168	10.12	Fecal coliform
<i>Acinetobacter</i>	Genus	0.972	3.066	3.15	Opportunistic / nosocomial
<i>Streptococcus</i>	Genus	0.005	0.015	2.86	Opportunistic
<i>Enterococcus</i>	Genus	0.543	0.859	1.58	Fecal indicator / opportunistic
<i>Burkholderiaceae</i>	Family	0.001	0.032	42.30	Opportunistic / environmental
<i>Erysipelotrichaceae</i>	Family	0.133	0.049	0.37	Gut-associated / opportunistic
<i>Pseudomonas</i>	Genus	10.773	4.971	0.46	Opportunistic / environmental
<i>Clostridium</i>	Genus	0.420	0.043	0.10	Spore-forming
<i>Aeromonas</i>	Genus	0.046	0.000	0.00	Opportunistic / aquatic
<i>Shewanella</i>	Genus	0.498	0.008	0.02	Aquatic / sediment

LS = Lake Van sediment; CM = Cattle manure. CM/LS ratios > 1 indicate enrichment in cattle manure (consistent with the fecal-reservoir hypothesis); ratios < 1 indicate enrichment in sediment (environmental lineages). All values are pooled relative abundances based on phylum-assigned bacterial reads.

Alpha and beta diversity (ASV level)

Alpha-diversity indices computed at the strict ASV level (Table 6) revealed significantly higher diversity in LS than in CM. Shannon entropy was 8.08 ± 0.18 (LS) vs. 7.20 ± 0.28 (CM), Welch $p < 0.001$ (Mann-Whitney $p = 0.008$); inverse Simpson $1,231 \pm 268$ (LS) vs. 410 ± 206 (CM), $p < 0.001$ (M-W $p = 0.008$); Simpson $(1 - D) p = 0.027$ (M-W $p = 0.012$); and Pielou's evenness 0.90 ± 0.02 (LS) vs. 0.82 ± 0.03 (CM), $p = 0.004$ (M-W $p = 0.008$). Observed ASV richness was numerically higher in LS ($8,041 \pm 1,421$ vs. $6,557 \pm 519$) but did not reach significance under Welch's test ($p = 0.079$). The ASV-level Bray-Curtis dissimilarity matrix (Table 7) showed mean within-LS dissimilarity = 0.602 ± 0.101 , mean within-CM dissimilarity = 0.738 ± 0.130 , and mean between-group dissimilarity = 0.925 ± 0.024 . The between-group dissimilarity was approximately 1.5-fold higher than the within-LS mean

and 1.25-fold higher than the within-CM mean, indicating clear compositional separation. All 25 between-group pairwise dissimilarities were ≥ 0.889 ; the smallest between-group distance (LS3 ↔ CM1 = 0.889) exceeded all 10 within-LS distances and 9 of 10 within-CM distances. PCoA ordination (Figure 5) showed clear group separation along the first principal coordinate axis (PCoA1, 40.8% variance explained), with all five LS replicates clustered tightly between PCoA1 ≈ -0.36 and -0.32 , and all five CM replicates between PCoA1 = $+0.14$ and $+0.45$. The minimum gap between the two clusters along PCoA1 was 0.45 dissimilarity units, with no overlap. PERMANOVA returned pseudo-F = 5.21, $p = 0.011$ (999 permutations), confirming the visual separation as statistically significant. This result is closely consistent with the QIIME 2 location-grouped PERMANOVA performed on the original metadata (pseudo-F = 5.19, $p = 0.007$).

Table 6: Alpha-diversity indices (ASV level, mean $\pm$ SD), LS: Lake Van sediment; CM: Cattle manure.

Index	LS (n = 5)	CM (n = 5)	p (Welch)
Observed ASVs	$8,041 \pm 1,421$	$6,557 \pm 519$	0.079
Shannon (H')	8.08 ± 0.18	7.20 ± 0.28	< 0.001
Inverse Simpson	$1,231 \pm 268$	410 ± 206	< 0.001
Pielou's Evenness (J)	0.90 ± 0.02	0.82 ± 0.03	0.004

Table 7: Bray-Curtis dissimilarity matrix (ASV level, n = 10).

	LS1	LS2	LS3	LS4	LS5	CM1	CM2	CM3	CM4	CM5
LS1	0.000	0.602	0.702	0.700	0.762	0.929	0.967	0.983	0.974	0.897
LS2	0.602	0.000	0.512	0.598	0.652	0.902	0.937	0.950	0.938	0.899
LS3	0.702	0.512	0.000	0.479	0.537	0.889	0.919	0.933	0.911	0.912
LS4	0.700	0.598	0.479	0.000	0.475	0.905	0.930	0.936	0.909	0.911
LS5	0.762	0.652	0.537	0.475	0.000	0.912	0.930	0.934	0.904	0.927
CM1	0.929	0.902	0.889	0.905	0.912	0.000	0.575	0.603	0.783	0.801
CM2	0.967	0.937	0.919	0.930	0.930	0.575	0.000	0.570	0.712	0.882
CM3	0.983	0.950	0.933	0.936	0.934	0.603	0.570	0.000	0.668	0.901
CM4	0.974	0.938	0.911	0.909	0.904	0.783	0.712	0.668	0.000	0.881
CM5	0.897	0.899	0.912	0.911	0.927	0.801	0.882	0.901	0.881	0.000

Within-LS mean = 0.602 ± 0.101 ; within-CM mean = 0.738 ± 0.130 ; between-group mean = 0.925 ± 0.024 . PERMANOVA: pseudo-F = 5.21, $p = 0.011$ (999 permutations). LS = Lake Van sediment; CM = Cattle manure.

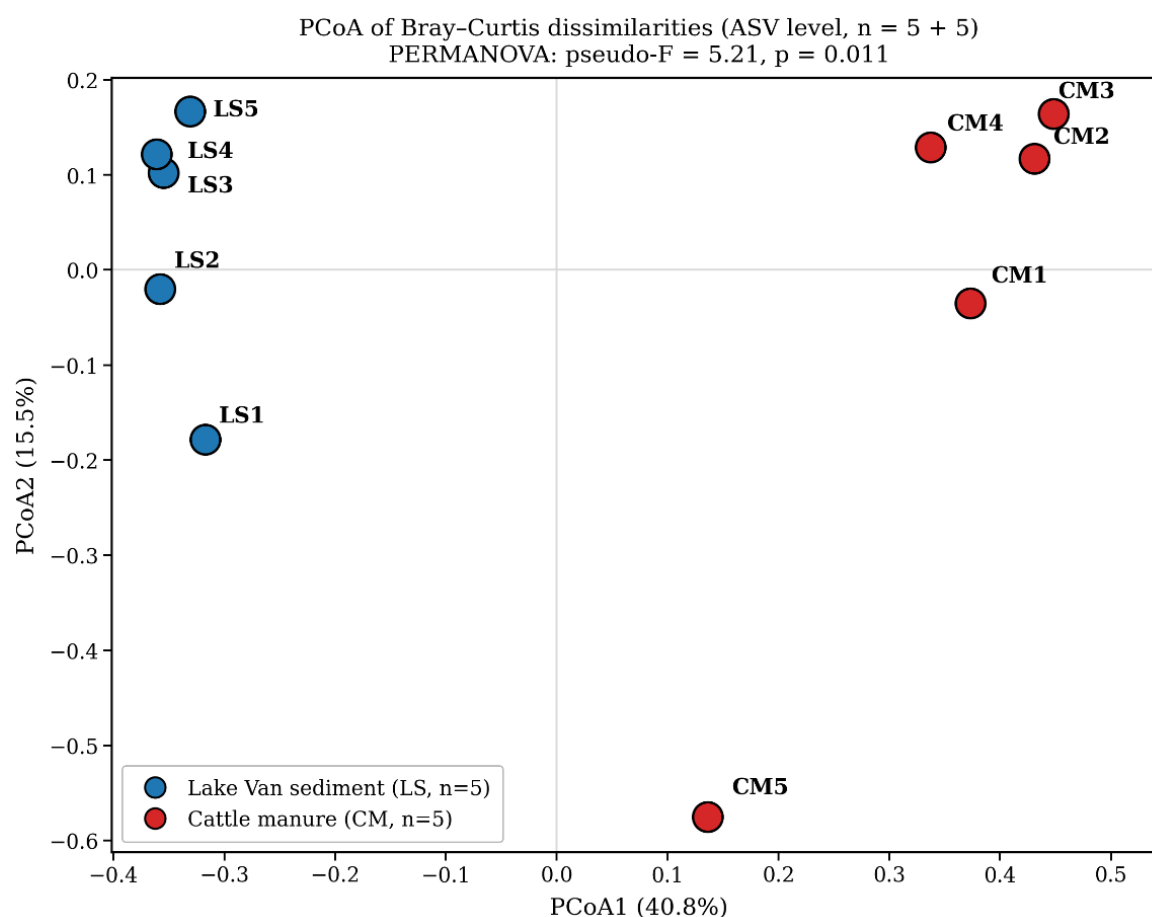


Figure 5: PCoA ordination of Bray–Curtis dissimilarities computed on column-normalized ASV-level relative abundances ($n = 5 + 5$). PCoA1 (40.8 % variance) cleanly separates LS (left, blue) from CM (right, red). PCoA2 (15.5 % variance) captures within-group heterogeneity. PERMANOVA: pseudo- $F = 5.21$, $p = 0.011$ (999 permutations).

Shared and unique ASVs

Across all ten samples, 45,842 unique ASVs were detected (Figure 6). Of these, only 3,952 (8.6%) were shared between groups, while 21,161 (46.2%) were unique to LS and 20,729 (45.2%) were unique to CM. Because single-nucleotide ASV resolution rarely recovers shared variants even between related communities, overlap was also assessed at coarser, ecologically interpretable ranks using the same presence criterion (Table 8): shared fractions rose to 31.1% of species, 43.4% of genera, and 54.1% of families. The 8.6% ASV figure therefore represents a strict strain-level upper bound rather than evidence of disjoint communities; nonetheless, substantial turnover persisted at every rank (≈ 46 –57% of genera and families were compartment-specific). This contrasts sharply with what would be expected under a model of free, unfiltered fecal–environmental coupling, in which a substantial fraction of manure-derived ASVs should be retained in the receiving sediment. Combined with the strong reduction of classic fecal/zoonotic indicators, the significant alpha-diversity contrast, and the significant PERMANOVA outcome ($p = 0.011$), the Venn pattern indicates that the soda-lake compartment imposes a strong selective filter on incoming manure-derived microbial diversity at the strain level.

Taxa called present at count > 0 in any sample of a group (the criterion used for the ASV-level Venn). Named taxa at each rank; using all collapsed bins gives near-identical values (e.g., 45.5% genera, 54.7% families). The ASV row is reproduced from Figure 6.

Shared and unique ASVs (strict ASV/OTU level)
Total: 45,842 ASVs; Shared = 8.6%

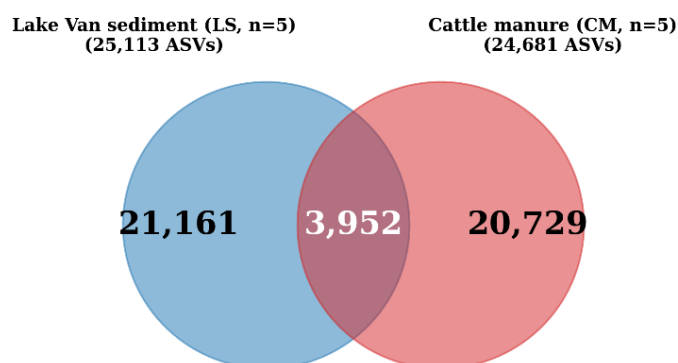


Figure 6: Venn diagram of shared and unique ASVs between LS and CM ($n = 5$ each). Of 45,842 ASVs detected, only 3,952 (8.6 %) were shared between compartments.

Table 8: Shared and unique taxa between LS and CM at increasing taxonomic resolution, LS: Lake Van sediment, CM: Cattle manure.

Taxonomic level	Total	Shared	% shared	Unique LS	Unique CM
ASV (strain)	45,842	3,952	8.6	21,161	20,729
Species	941	293	31.1	271	377
Genus	645	280	43.4	136	229
Family	340	184	54.1	48	108

Discussion

Differential persistence of fecal/zoonotic indicators in Lake Van sediment

The central hypothesis of this study, which proposes that the polyextreme physicochemistry of Lake Van (high alkalinity, high salinity) acts as an environmental filter on allochthonous, manure-derived microbiota, is strongly supported by four converging lines of evidence: (i) the systematic, 3- to 42-fold reduction of canonical fecal/zoonotic indicator taxa in LS relative to CM (Table 5, Figure 4); (ii) the highly significant alpha-diversity contrast in favor of LS (Shannon $p < 0.001$, inverse Simpson $p < 0.001$, Pielou's $J p = 0.004$; Table 6); (iii) the small shared-ASV fraction (8.6%, Figure 6); and (iv) the significant PERMANOVA outcome (pseudo- $F = 5.21$, $p = 0.011$) confirming statistical separation of the LS and CM communities at strain level. The pattern is consistent with sediment communities being more diverse and more even than cattle-gut-derived communities, in line with the established generalization that low-stress, resource-heterogeneous sediment habitats sustain higher microbial richness than the highly selected gut compartment [24]. *Escherichia-Shigella* and the broader *Enterobacteriaceae*, which are routinely used as quantitative tracers of fecal contamination in surface waters [25], were 8- to 10-fold lower in LS than in CM. *Acinetobacter*, a globally important opportunistic and frequently multidrug-resistant pathogen [26], was 3-fold lower in LS, while *Burkholderiaceae* was 42-fold lower. The directionality of the effect was internally consistent across all classic fecal/zoonotic and gut-opportunist groups examined.

Several non-mutually-exclusive mechanisms can account for this pattern: (i) physicochemical inactivation of non-adapted *Enterobacteriaceae* and *Acinetobacter* by sustained alkaline pH and elevated $\text{Na}^+/\text{CO}_3^{2-}$ concentrations, which compromise membrane integrity in non-haloalkaliphilic organisms [9]; (ii) competitive displacement by autochthonous haloalkaliphilic and aquatic lineages such as *Shewanella* (~64-fold enriched in LS), *Aeromonas* (detected only in LS), and sediment-adapted *Pseudomonadaceae*; and (iii) preferential adsorption / sedimentation kinetics that may differentially partition larger fecal particles into sediment horizons not sampled here. The complementary enrichment of *Shewanella* and *Aeromonas* in LS is consistent with both their well-documented preference for

aquatic/sediment habitats and an active microaerophilic-to-anaerobic metabolism in the lake's organic-rich sediment layers [27].

Persistence of anaerobic fermenter lineages in LS

A nuance that complicates a simple "sanitization" reading is the enrichment of strictly anaerobic, gut-associated families (*Oscillospiraceae*, *Lachnospiraceae*, *Prevotellaceae*, *Ruminococcaceae*, *Bacteroidaceae*) in LS relative to CM (Table 4, Figure 3). At least three interpretations are compatible with these observations and are not mutually exclusive. First, the CM samples in our dataset show high *Pseudomonadota* loads (mean 36%, range 30–50%), particularly of *Rhodocyclaceae* (~15% pooled), unclassified *Pseudomonadota*, and aerobic *Pseudomonadaceae*, which is typical of aged or aerobically-perturbed manure rather than fresh feces [26]. Importantly, because the manure was collected fresh, this enrichment most likely reflects the rapid aerobic succession that freshly deposited cattle feces undergoes within hours of excretion and air exposure [26], accelerated by the warm, same-day transport used to preserve anaerobic viability rather than aged or stored manure. Under that scenario, the original anaerobic-fermenter content of these CM samples may have been progressively replaced by environmental *Pseudomonadota*, artificially lowering the CM contribution of fermenter families. Second, the strict-anaerobe families enriched in LS may represent viable, deposited fecal signal that persists in the anoxic sediment compartment, a finding consistent with the long-known capacity of *Bacillota* spore-formers to survive harsh transit [28] and with the strong vertical anoxia gradient typical of soda-lake sediments. Third, fermentative anaerobes are not exclusively gut-associated: *Oscillospiraceae* in particular includes lineages adapted to a wide range of low-redox aquatic and terrestrial environments [29], and other strict-anaerobe *Bacillota* families such as *Lachnospiraceae* are well represented in anoxic sediments and soils [30, 31]. The persistence of these families in LS therefore does not contradict the environmental-filtering interpretation of the canonical-pathogen results; rather, it reflects the ecological versatility of strict-anaerobe *Bacillota* lineages.

Implications within the One Health framework

Read together, the indicator-taxon pattern (Section 4.1), the anaerobic-fermenter pattern (Section 4.2), and the significant statistical separation of the two compartments

at ASV level (Section 3.5) point to a selective, not absolute, environmental-filtering effect of the Lake Van sediment compartment. Aerobically-tolerant fecal indicators and opportunistic pathogens (*Escherichia-Shigella*, *Acinetobacter*, *Enterobacteriaceae*, *Burkholderiaceae*, *Streptococcus*) appear strongly attenuated, whereas obligately anaerobic *Bacillota* lineages, including taxa with potential pathogenic species (e.g., spore-forming *Clostridium* representatives, here detected at 10-fold higher relative abundance in LS than CM), persist or are even enriched.

From a One Health surveillance standpoint, this suggests that any single-marker approach, such as classical *E. coli* enumeration, would underestimate the residual microbiological risk in soda-lake-receiving environments, and that microbiome-based indicator panels combining aerobic *Enterobacteriaceae*, opportunistic *Acinetobacter*, and obligate-anaerobe *Clostridium*/Clostridiaceae markers provide a more complete picture of fecal-derived contamination retention. The framework outlined by Skoufos et al. [1] for cross-compartment microbiome integration is directly applicable to such evaluations at agricultural-extreme-aquatic interfaces.

The very small shared-ASV fraction (8.6%, Section 3.6) further suggests that, beyond compositional shifts, the strain-level filtering effect is severe, with most manure-derived sequence variants either inactivated below detection or outcompeted in the sediment compartment. Although *Acinetobacter* was 3.2-fold lower in LS, it nonetheless persisted at ~1% of the sediment community; as a clinically important, frequently multidrug-resistant opportunist, this residual presence in a multi-use lake is a non-trivial public-health signal that single-marker (e.g., *E. coli*) monitoring would overlook, reinforcing the case for microbiome-based multi-marker surveillance. Profiling of antimicrobial-resistance genes (e.g., by long-read metagenomics) in these compartments is a priority future direction [32]. Finally, the modest per-group sample size ($n = 5$) limited power for small-effect endpoints: whereas the dominant diversity contrasts were detected with very high power (Cohen's $d = 3.1-3.7$, power ≥ 0.99), observed-ASV richness ($d = 1.4$) fell below the minimum detectable effect ($d \approx 2.0$ at 80% power) and remained non-significant, and is therefore treated as suggestive; the conclusions rest on effect sizes and the convergence of multiple indices rather than on p -values alone.

In conclusion, The 16S rRNA gene amplicon profiling revealed a strong environmental filtering effect of the Lake Van sediment on manure-derived microbial diversity. While the extreme conditions substantially attenuate many fecal-associated and opportunistic taxa, the persistence of certain anaerobes indicates that the filtering is selective rather than absolute. These findings

underscore the importance of integrated environmental surveillance in extreme ecosystems.

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