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Loss of Aerobic Respiration and Hydrocarbon Degradation Potential in Soils Acutely Contaminated with Used Vegetable Oil

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Abstract

The improper disposal of used vegetable oil (UVO) presents a significant environmental issue as it can obstruct soil pores and rapidly induce anoxia. However, the genomic-scale disturbance that occurs immediately after a spill remains poorly understood. Here, we used comparative 16S rRNA metagenomics to investigate early-stage dysbiosis in tropical alluvial–regosol soils from North Sulawesi subjected to acute UVO contamination (<3 months). We observed a significant decline in alpha diversity and a fundamental restructuring of the soil microbiome, characterized by an expansion of opportunistic *Proteobacteria*, particularly the order *Burkholderiales*, displacing a diverse native flora. Importantly, predictive functional profiling using PICRUSt2 revealed a critical metabolic bottleneck: although genes associated with aromatic-compound degradation (e.g., toluene and catechol pathways) were selectively enriched, core aerobic respiration pathways were strongly suppressed, especially cytochrome c oxidase. This physiological decoupling suggests that, while indigenous "first responder" communities retain enzymatic potential to degrade pollutants, their catabolic activity is severely constrained by physical oxygen limitation. In contrast to this acute-state pattern of Proteobacterial proliferation coupled with respiratory impairment, our prior observations from chronically UVO-contaminated soils in Bitung indicate a *Firmicutes*-dominated endpoint consistent with a stable, fermentation-associated community under long-term oxygen limitation. Collectively, these findings highlight immediate soil aeration as a critical intervention to unlock the latent bioremediation potential of indigenous bacterial communities.

Keywords: *Metabolic bottleneck; used vegetable oil; early dysbiosis; Proteobacteria; PICRUSt2; alluvial-regosol.*

INTRODUCTION

The improper disposal of used vegetable oil into terrestrial ecosystems presents a growing environmental challenge, particularly in developing regions undergoing rapid urbanization and expanding culinary tourism sectors. In North Sulawesi cities such as Manado and Bitung, the increasing volume of used vegetable oil (UVO) generated by food processing activities often bypasses proper treatment facilities, ending up in open lands and drainage systems. Unlike petroleum-based hydrocarbons, UVO is chemically distinct, and consists mainly of triglycerides, free fatty acids, and glycerol. Although these compounds are, in principle, biodegradable, their rapid and high-load input into soils can trigger a cascade of physicochemical alterations that can severely disrupt ecosystem stability. During high-temperature cooking, vegetable oils undergo oxidation and polymerization, generating secondary byproducts such as aldehydes and ketones. These compounds are not only recalcitrant but can exhibit direct toxicity to the indigenous soil microbiota, imposing immediate selective pressure on the microbial community (Tang et al., 2012).

The ecological impact of this contamination is intensified by the physical properties of the pollutant itself. The specific soil formations of the region, typically low-altitude mixtures of alluvial and regosol types, differ significantly in their interaction with viscous fluids compared to clay-heavy soils. When viscous UVO saturates the soil matrix, it coats soil particles and drastically reduces hydraulic conductivity (Abioye et al., 2012). This physical occlusion of soil pores creates a barrier to gas exchange, rapidly depleting soil oxygen levels and preventing atmospheric replenishment. Consequently, the soil microenvironment is forced to transition from an aerobic state to a hypoxic or anoxic state. For the indigenous microbial biomass, which is typically dominated by aerobic bacteria capable of degrading naturally occurring organic matter, this shift creates severe physiological constraints. Thus, UVO contamination may create an apparently paradoxical state in which soils become carbon-rich yet metabolically constrained due to impaired respiration, thereby halting biodegradation processes before the microbial community can mount an effective adaptive response (Ren et al., 2025).

Despite the critical nature of this initial disturbance, most of the research on oil contamination has prioritized long-term adaptation or the evaluation of bioremediation strategies in chronically polluted sites. There remains a significant knowledge gap regarding the acute phase of contamination, the critical window immediately following spillage where the ecosystem equilibrium is first disrupted. It is during this early stage that the trajectory of the microbiome is determined, shifting from a diverse, healthy community structure toward a simplified, dysbiotic state. Understanding the specific loss of metabolic function during this acute phase is essential for predicting the natural attenuation capacity of the soil and for designing timely interventions that might prevent permanent ecosystem collapse (Al-Darbi et al., 2005).

Our motivation for the present study arises directly from findings obtained in our earlier investigation of permanently polluted (chronic-phase) WCO-contaminated soil in Bitung, North Sulawesi (Mantiri et al., 2023). That work demonstrated that long-term contamination is associated with profound restructuring of soil bacterial community composition, including a marked shift toward *Firmicutes* dominance and strong overrepresentation of the genus *Weissella*. In particular, *Weissella paramesenteroides* constituted approximately 46.68% of metagenomes recovered from WCO-contaminated soil, whereas *Weissella* represented only ~1.25% in the natural soil baseline. Because *Weissella* is commonly associated with fermentation-linked ecological strategies, the chronic-phase endpoint is consistent with sustained oxygen limitation and a stable, anaerobically structured microbial configuration. These chronic observations raised an unresolved mechanistic question: how does a newly contaminated soil transition toward this *Firmicutes*/*Weissella* fermentative endpoint, and what metabolic constraints emerge immediately after contamination begins?

To address this gap, we investigate bacterial community structure and predicted functional capacity in alluvial–regosol soils acutely contaminated with UVO compared to an unpolluted reference. We apply comparative 16S rRNA amplicon profiling with modern bioinformatics (QIIME 2 and DADA2) and infer community metabolic potential using PICRUSt2 (Mantiri et al., 2025; Mantiri et al., 2023; Douglas et al., 2020). We focus on pathways central to aerobic energy metabolism (notably cytochrome c-associated respiration) and pathways relevant to degradation of aromatic intermediates, which can represent breakdown routes for thermally altered oil byproducts. By resolving the early “first responder” state and identifying whether respiration capacity becomes constrained even when degradation pathways are enriched, this study provides a genomic framework to explain why UVO-contaminated soils may fail to self-remediate and how early-stage constraints could drive progression toward the chronic fermentative configuration previously observed in permanently polluted soils.

METHODS

This study employs a comparative metagenomic approach focusing on alluvial-regosol soil formations in the cities of Manado and Bitung, North Sulawesi, Indonesia. To evaluate the acute impact of contamination, two datasets representing distinct ecological conditions were integrated. The dataset for Unpolluted soil (Control) was obtained from our previous study, which characterized the baseline microbial community of a protected zone with no history of contaminant exposure (Mantiri et al., 2023). This was compared against a newly generated dataset for Early polluted soil, collected from a site subjected to acute used vegetable oil (UVO) spillage for a duration of less than three months.

For the early polluted condition, soil samples were obtained from the topsoil layer (5–20 cm) using sterile trowels. Composite samples were transferred to sterile containers, transported on ice, and stored at -80°C (Rochelle et al., 1994). Total genomic DNA was isolated from 250 mg of soil using a commercial soil DNA extraction kit, PrimeWaySoil DNA Extraction Kit (1st Base, KIT-9060-250), following the manufacturer's protocol. The hypervariable V3-V4 region of the bacterial 16S rRNA gene was amplified using universal primers 341F and 805R (Klindworth et al., 2013), and the resulting amplicons were sequenced on the Illumina platform to generate paired-end reads, ensuring methodological consistency with the reference control dataset.

Raw sequencing data from both the early polluted samples and the retrieved unpolluted control files were processed uniformly using the QIIME 2 (v. 2024.10) pipeline (Bolyen et al., 2019) to ensure comparability. Quality control revealed low quality scores in reverse reads; therefore, a single-end analysis approach was adopted. Forward reads were denoised, quality-trimmed, and resolved into Amplicon Sequence Variants (ASVs) using the DADA2 algorithm (Callahan et al., 2016). Taxonomic classification was performed using a Naive Bayes classifier trained on the SILVA 138 reference database (Quast et al., 2013).

To assess the metabolic implications of acute contamination, the functional potential of the bacterial communities was predicted using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas et al., 2020). ASVs were placed into a reference phylogenetic tree, and gene family abundances (EC numbers and KO terms) were predicted based on nearest-neighbor genomes. The analysis specifically targeted metabolic pathways related to aerobic respiration (cytochrome c oxidases) and hydrocarbon degradation to quantify the metabolic bottleneck hypothesis.

Downstream data analysis and visualization were performed using Python (v. 3.x) with Pandas and Seaborn libraries. Relative abundances of taxa and metabolic pathways were calculated and visualized using heatmaps and bar charts. Functional shifts were evaluated by comparing the normalized abundance counts of specific metabolic pathways between the archived unpolluted data and the new early polluted data.

RESULTS

Sequencing Performance and Immediate Loss of Community Diversity

Amplicon sequencing generated datasets of sufficient quality to support comparative ecological interpretation between unpolluted (control) and early polluted soils. Following DADA2 quality control (single-end processing due to low reverse-read quality), both datasets maintained high base-calling accuracy (Q30 > 86%). Read retention was comparable across conditions (Effective %: 57.66% in unpolluted vs 58.68% in early polluted; **Table 1**), suggesting that the observed ecological contrasts are unlikely to be driven by major sequencing artifacts. Rarefaction curves reached a plateau in both conditions (**Figure 1**), supporting that sequencing depth adequately captured the dominant community members and that the diversity collapse described below reflects a biological signal rather than undersampling.

Table 1. Summary of Sequencing Quality

Soil Condition	Raw Reads (#)	Clean tags (#)	Effective tags (#)	Base (nt)	AvgLen (nt)	Q20	Q30	GC (%)	Effective %
Unpolluted	176419	156085	114218	27412320	240	95,76	89,81	57,66	0,65
Early polluted	100000	80345	61726	14814240	240	94,06	86,23	58,68	0,62

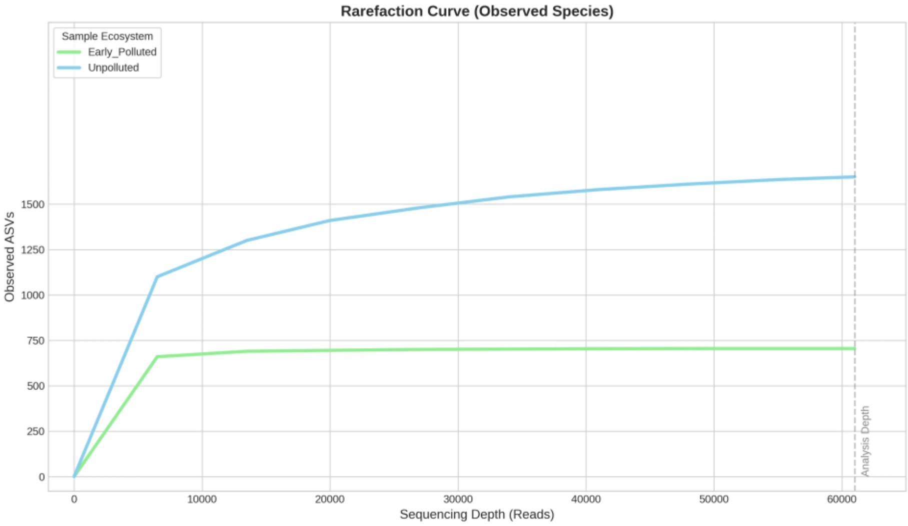

Figure 1. Rarefaction curves of observed species, showing saturation of sequencing depth for both soil conditions.

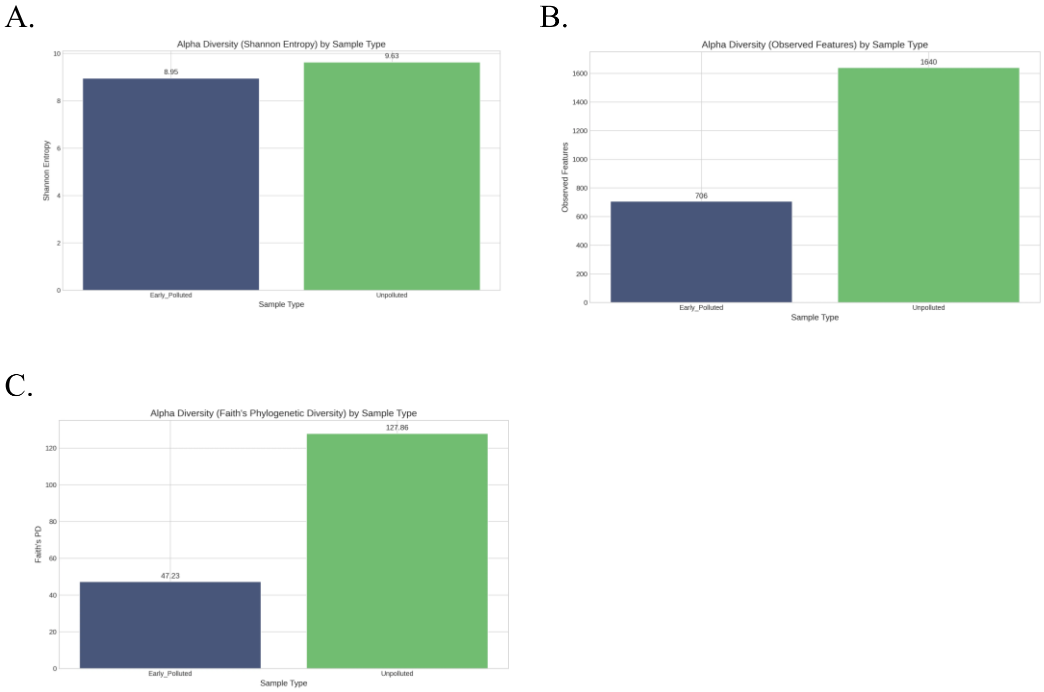

Figure 2. Comparison of Alpha Diversity indices (Shannon Entropy and Faith's Phylogenetic Diversity) between Unpolluted and Early Polluted soils.

The most pronounced immediate effect of UVO contamination was evident in the alpha diversity metrics. While the unpolluted soil exhibited a rich and phylogenetically diverse community (Shannon Index = 9.63; Faith's PD = 127.86), the early polluted soil showed a sharp collapse in diversity (Shannon Index = 8.95; Faith's PD = 47.23) (**Figure 2**). The drastic reduction in Faith's Phylogenetic Diversity (a drop of ~63%) indicates that acute oil exposure acts as a severe environmental filter, eliminating entire evolutionary lineages of bacteria that are sensitive to toxicity or physical pore-clogging. This interpretation is consistent with Huang et al. (2021), who reported that hydrocarbon contamination can rapidly restructure microbial communities by favoring stress-tolerant taxa adapted for oxidative stress and hydrophobic conditions.

Beta diversity analysis using Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity confirmed that the Early Polluted community is structurally distinct, forming a separate cluster from the Unpolluted baseline (PC1 axis separation) (**Figure 3**). This indicates that within less than three months of exposure, the soil microbiome had already undergone a fundamental reorganization, shifting away from the stable natural configuration toward a dysbiotic state (Shade et al., 2012).

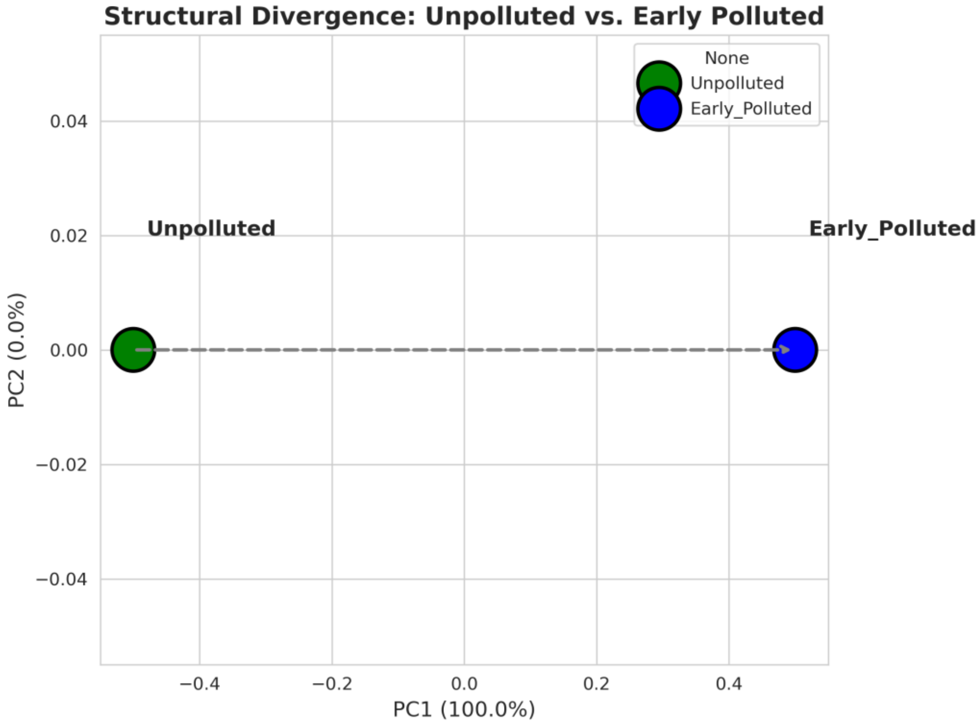

Figure 3. Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity showing the structural divergence of the Early Polluted community from the Unpolluted control.

Taxonomic Restructuring in the Acute Phase: Proteobacterial “First Responders” Precede the Chronic Endpoint

Acute UVO exposure resulted in a pronounced taxonomic shift toward *Proteobacteria* dominance. In the unpolluted soil, the community composition was relatively balanced, with *Proteobacteria* (17.81%) and *Actinobacteriota* (17.44%) as major contributors. In contrast, early polluted soil showed a substantial increase in *Proteobacteria* to 28.56% (**Figure 4A**), with expansion driven by *Gammaproteobacteria* (15.87%) and *Alphaproteobacteria* (12.68%) (**Figure 4B**). At the order level, *Burkholderiales* increased to 8.29% compared with 5.55% in the control (**Figure 4C**). Members of the *Burkholderiales* are well-documented "r-strategists" or rapid growers that possess versatile catabolic genes for degrading organic pollutants (Siles & Margesin, 2018). This pattern is consistent with an early “first-responder bloom,” in which fast-growing and metabolically flexible lineages rapidly exploit newly available carbon while tolerating the initial disturbance regime (Halbrook et al., 2024).

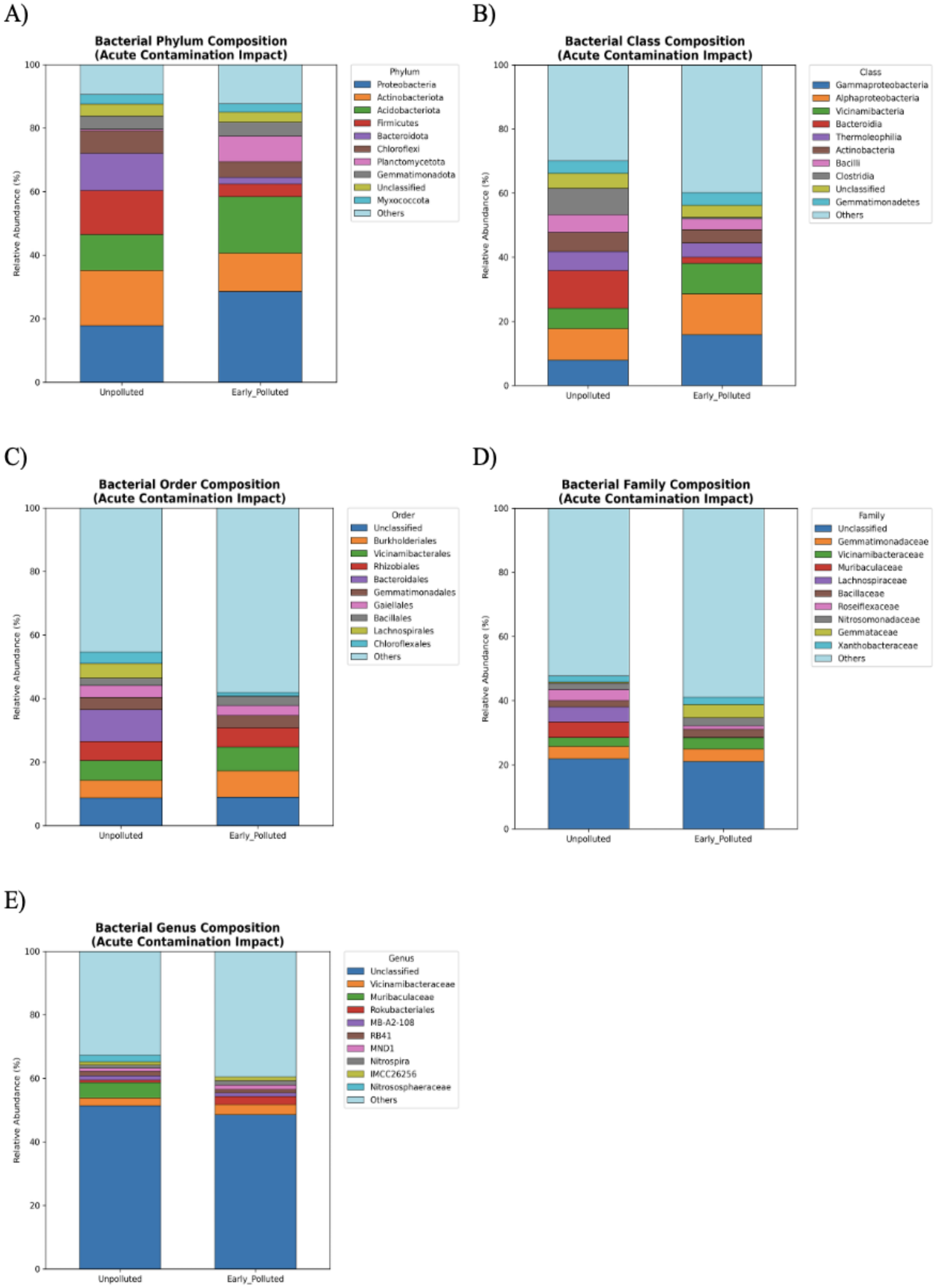

Figure 4. Relative abundance of bacterial Phyla (A), Classes (B), Orders (C), Families (D), and Genera (E).

Importantly, the acute-phase configuration observed here should be contextualized with our earlier findings from permanently polluted (chronic-phase) WCO-impacted soils in Bitung, North Sulawesi (Mantiri et al., 2023). In that chronic system, the community shifted toward *Firmicutes* dominance, and *Weissella* became highly overrepresented, with *Weissella paramesenteroides* constituting ~46.68% of the metagenomes in WCO-contaminated soil. In contrast, only ~1.25% *Weissella* was represented in the natural baseline. Because *Weissella* is frequently associated with fermentation-linked ecological strategies, this chronic enrichment is consistent with a late-stage community configuration shaped by prolonged oxygen limitation and sustained habitat restructuring under persistent oil loading (Potts et al., 2022).

When viewed together, the contrast between the present acute dataset (low *Firmicutes*, ~4%) and the prior chronic dataset (*Firmicutes*/*Weissella* dominance) supports a successional transition model, in which early contamination favors *Proteobacterial* opportunists while oxygen remains partially available, whereas persistent contamination progressively pushes the system toward stronger anoxia and a fermentative *Firmicutes* endpoint. This is not just a descriptive difference between two sites. It further frames the central mechanistic question motivating this study: how does the microbiome transition from an early *Proteobacteria*-driven responder community into the chronic *Firmicutes*/*Weissella* configuration, and what functional constraints appear immediately after contamination begins?

Metabolic Bottlenecks: Early Functional Constraints That May Set the Trajectory Toward the Chronic Fermentative Endpoint

To address the mechanistic question raised by the chronic-phase *Weissella* enrichment, we examined whether acute contamination immediately constrains core energy metabolism in a way that could plausibly drive the longer-term shift toward fermentation-associated taxa. PICRUSt2-based functional profiling revealed broad suppression of central metabolic and energy-generation pathways in early polluted soil relative to the unpolluted baseline (**Figure 5**). Most notably, predicted abundance for Aerobic Respiration I (cytochrome c) decreased strongly, with concurrent reductions in TCA cycle I (prokaryotic) and glycolysis-associated functions. This pattern provides community-level genomic support for the hypothesis that acute UVO inputs rapidly induce oxygen stress, likely through physical pore occlusion that limits oxygen diffusion and reduces the feasibility of high-yield aerobic respiration.

This acute suppression of aerobic respiration is conceptually important because it provides a plausible bridge mechanism from the acute state to the chronic state. If oxygen limitation emerges quickly and persists, it can progressively favor taxa that (i) tolerate low oxygen, (ii) rely more on fermentative or microaerophilic strategies, and/or (iii) exploit niches created by reduced aerobic competition. In other words, the early decrease in aerobic respiration capacity may be one of the earliest functional “selection pressures” that ultimately helps explain why chronic soils become dominated by fermentation-associated taxa such as *Weissella* in our previous work.

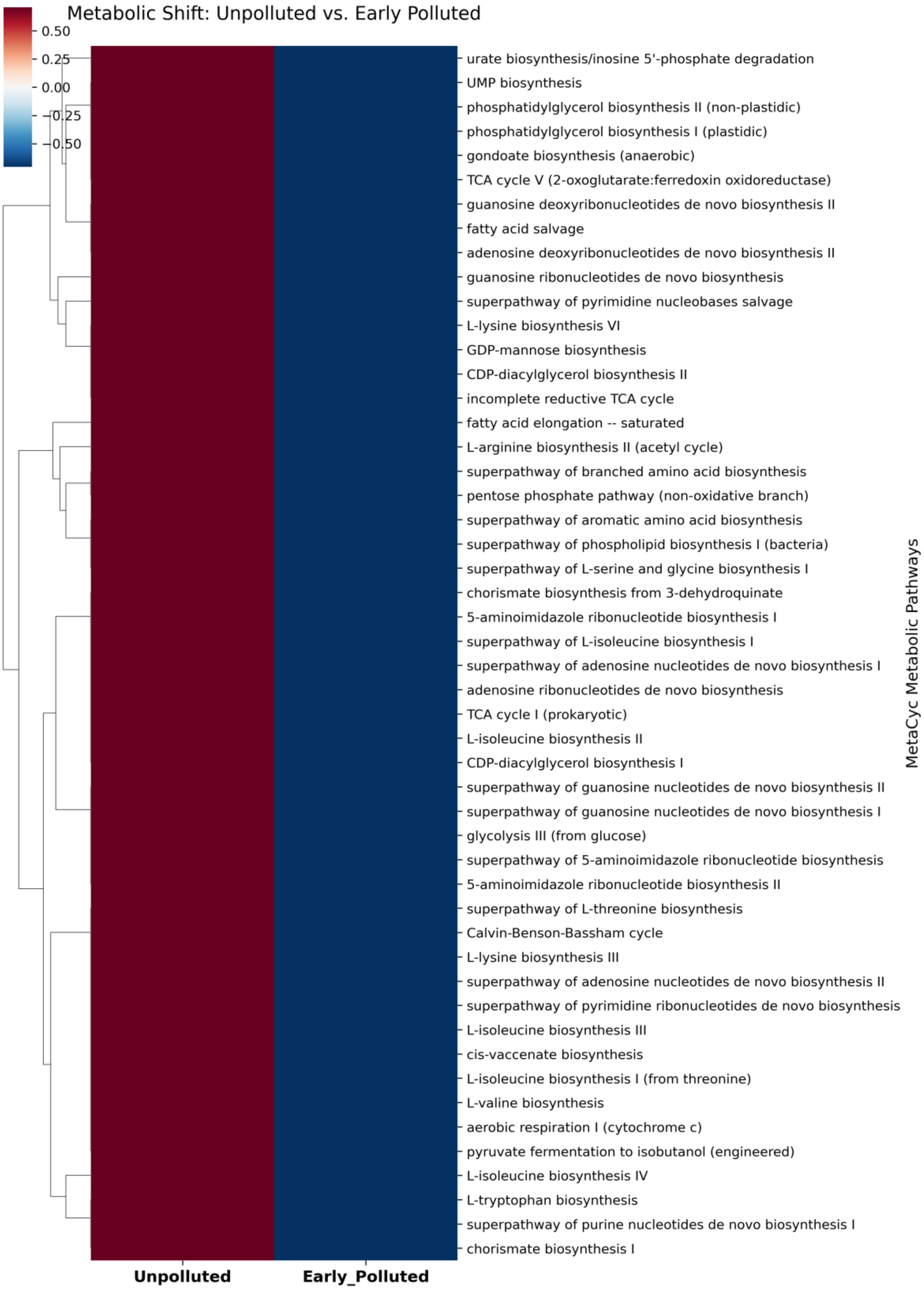

Figure 5. Heatmap of the Top 50 predicted metabolic pathways (MetaCyc) showing the downregulation of central metabolism genes in Early Polluted soil.

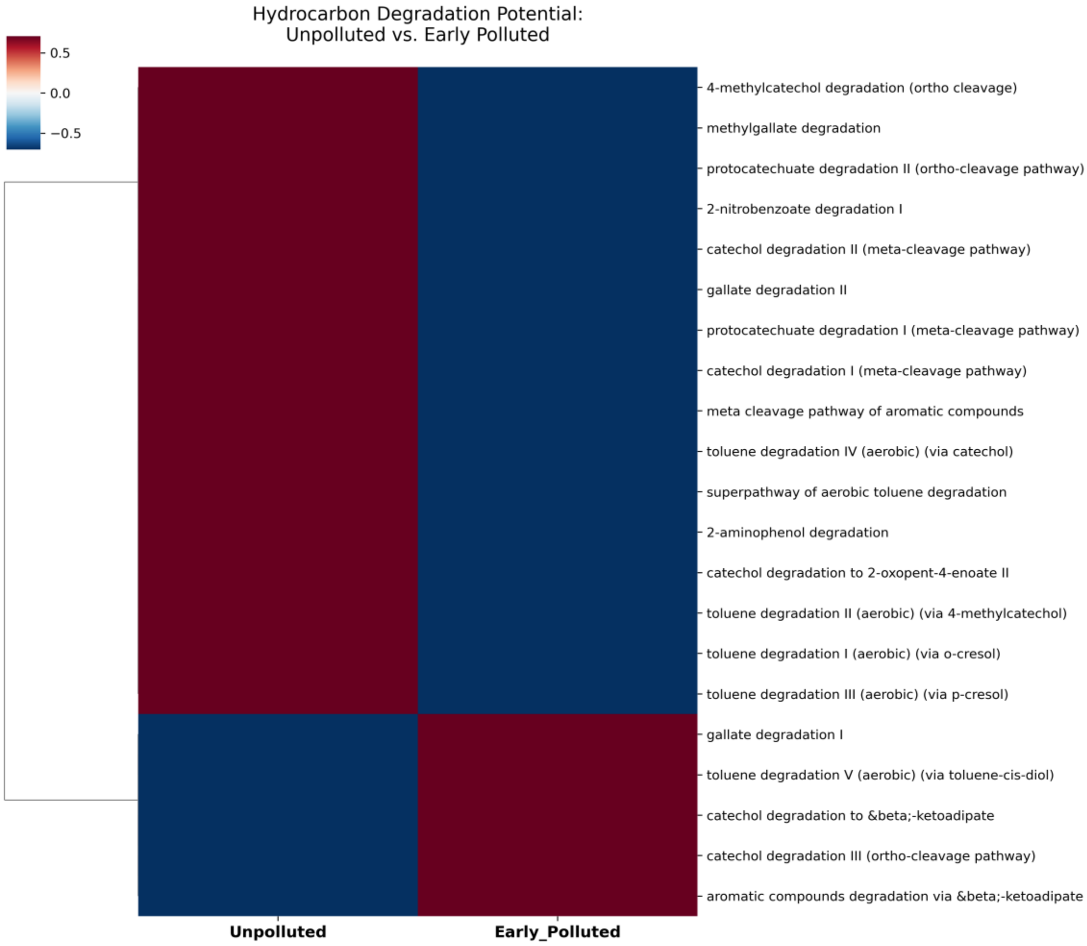

Figure 6. Targeted heatmap of hydrocarbon and aromatic compound degradation pathways.

Despite the predicted suppression of respiratory capacity, the early polluted soil showed selective enrichment of predicted pathways involved in aromatic compound degradation, including toluene degradation V (via toluene-cis-diol) and catechol degradation (β-ketoadipate route) (**Figure 6**). The β-ketoadipate route is a key funnel for aromatic intermediates toward central metabolism, suggesting that the community retains (or even enriches) genetic potential to process complex oxidized organics consistent with thermally altered cooking oil byproducts (Harwood & Parales, 1996). However, these catabolic potentials appear partially "uncoupled" from the energy-generating machinery needed to drive complete oxidation efficiently, consistent with a metabolic bottleneck model: degradative potential exists, but respiration is constrained by oxygen limitation.

Integrated synthesis: The Acute "Bottleneck" As an Early Driver of The Chronic Firmicutes/Weissella Endpoint

The integration of taxonomic and functional data suggests that acute UVO contamination creates a unique, transient ecological state. The soil is not yet anaerobic enough to support fermentation specialists, but it is too toxic and oxygen-limited to support the broad metabolic versatility of the original flora. The high abundance of degradation genes (catechol/toluene pathways) paired with the reduced abundance of respiration genes (cytochrome c) points a metabolic bottleneck, in which the community possesses the enzymatic tools to break down the pollutant but lacks the bioenergetic capacity (respiration) to do so efficiently due to physical oxygen limitation. This functional constraint provides a coherent mechanistic explanation for why a chronically contaminated soil could later stabilize into a fermentation-associated community dominated by *Firmicutes*/*Weissella*, as observed previously. The data indicate that sustained oxygen limitation can gradually shift competitive advantage away from aerobic degraders and toward taxa adapted to low-oxygen or fermentative niches.

From an applied standpoint, these results imply that early bioremediation should prioritize interventions that restore oxygen transfer and pore connectivity (e.g., aeration or physical soil loosening), because the acute limitation appears to be bioenergetic (limited respiratory capacity) respiratory rather than a shortage of catabolic potential. Timely relief of the respiratory bottleneck (Ren et al., 2023) may help prevent the system from crossing a threshold into the stable chronic fermentative state exemplified by *Weissella paramesenteroides* dominance in permanently polluted soils (Mantiri et al., 2023).

CONCLUSION

This study shows that the acute phase of used vegetable oil (UVO) contamination triggers an immediate and profound dysbiosis in alluvial-regosol soils, characterized by a rapid collapse in microbial diversity and a fundamental uncoupling of metabolic potential. While the taxonomic community shifts toward opportunistic *Proteobacteria* capable of hydrocarbon degradation, the functional profile reveals a critical "metabolic bottleneck" driven by the suppression of aerobic respiration pathways. The significant reduction in cytochrome c abundance, despite the enrichment of genes for aromatic compound degradation (e.g., catechol and toluene pathways), suggests that physical oxygen limitation acts as the primary constraint on natural attenuation during the initial weeks of spillage. Consequently, bioremediation strategies targeting this acute phase must prioritize soil aeration to relieve respiratory inhibition, thereby unlocking the latent catabolic potential of the indigenous "first responder" community before the ecosystem transitions into a permanently anoxic fermentative state.

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