International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 4 issue 3 Sept, 2025 Research Article

Metagenomic Assessment of Bacterial Community Structure on Carbon Steel Surfaces Treated with shoot Extract of *Ocimum gratissimum* for the Control of Microbiologically Induced Corrosion

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ABSTRACT

This study investigated the inhibitory impact of leaf/stem extract of *Ocimum gratissimum* on microbial communities responsible for microbiologically influenced corrosion (MIC) of steel. Steel coupons preconditioned with the plant extract as corrosion inhibition treatment were buried in simulated soil and incubated anaerobically for 28 days. Metagenomic DNA was extracted from the soil samples, sequenced and analyzed to characterize shifts in microbial community structure after 28 days compared to untreated baseline and control samples. Results showed that *Proteobacteria* was the most dominant phylum, increasing in relative abundance from 53.27% at baseline at day 0 (E0) to 96.58% in the untreated control sample at day 28 (E28), while sample treated with *O. gratissimum* (B) resulted in a slightly lower relative abundance of 89.91%. The family *Pseudomonadaceae* followed a similar trend, increasing from 5.72% (E0) to 24.76% in E28 as compared to a lower abundance of 11.50% in B. At the genus level, *Pseudomonas* rose in abundance from 5.72% (E0) to 24.76% (E28) with a marked variation of 5.18% in treated sample (B). Likewise, *Sphingomonas* increased in abundance to 3.11% in E28 from 0.20% at baseline, while lower (0.42%) in treated sample B. Notably, *Pseudomonas balearica,* a bacterial specie known to form biofilms on metal surfaces and cause corrosion, varied between 1.91% in E28 to 0.22% in B, indicating microbial inhibition by the extract. These findings indicate that *O. gratissimum* leaf/stem extract effectively inhibited several key corrosion-associated bacteria, highlighting their potential as eco-friendly alternatives for managing MIC on buried steel infrastructures.

Keywords: Microbiologically Induced Corrosion (MIC), *O. gratissimum*, Biocorrosion Control, Bacterial community structure.

Introduction

Microbiologically induced corrosion (MIC) remains a persistent and costly challenge in the maintenance and longevity of buried pipeline systems worldwide. MIC is primarily driven by complex interactions between metal surfaces and diverse microbial communities, which accelerate corrosion processes through the formation of biofilms, production of corrosive metabolites such as sulfides and organic acids, and direct electron transfer (Beech & Sunner, 2004; Jia *et al*., 2019). Sulfate-reducing bacteria (SRB), iron-oxidizing bacteria (IOB), and acid-producing bacteria (APB) are frequently implicated in these processes, collectively accounting for significant infrastructure degradation and economic losses (Enning & Garrelfs, 2014; Little and Lee, 2014).

Traditional control of MIC has predominantly relied on the use of synthetic biocides and corrosion inhibitors, which, although effective, pose substantial environmental and health concerns due to their toxicity and persistence (Videla & Herrera, 2005; Férnandez-Segovia *et al.,* 2020). Consequently, there is growing interest in exploring eco-friendly alternatives, including the application of plant-derived extracts with bioactive phytochemicals known for their antimicrobial and anti-corrosive properties (Verma & Quraishi, 2015; Umoren *et al.,* 2019). *Ocimum gratissimum* (African basil) is one of the plants that have attracted considerable attention due to its rich profiles of phenolics, flavonoids, and terpenoids, which exhibit potent antibacterial and antioxidant activities (Venuprasad *et al.,* 2014; Adetunji *et al.,* 2020; Adegoke *et al.,* 2023).

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Recent studies have demonstrated that extracts from *O. gratissimum* can effectively inhibit corrosion processes and reduce bacterial colonization on metal surfaces (Immanuel et al., 2016; Adindu *et al.,* 2017; Adetunji *et al.,* 2020; Ajayi *et al*., 2020). However, the precise impact of these treatments on the structure and dynamics of microbial communities associated with buried pipeline systems remains inadequately characterized. Understanding these microbial shifts is crucial, as changes in community composition can influence the resilience and recurrence of MIC under field conditions (Gu *et al.,* 2019).

Advancements in high-throughput sequencing and metagenomic tools have revolutionized our capacity to investigate microbial communities in complex environments without the biases of traditional culture-dependent techniques (Handelsman, 2004; Thomas *et al.*, 2012). Metagenomic approaches, such as 16S rRNA gene amplicon sequencing and shotgun metagenomics, enable comprehensive profiling of microbial diversity, functional potential, and shifts in community structure in response to various interventions (Franzosa *et al.,* 2015; Kim *et al.,* 2021a). This is particularly valuable for studying MIC systems, where uncultivable and previously unrecognized taxa may play critical roles in corrosion processes (Li *et al*., 2019).

Applying metagenomic tools to evaluate bacterial community changes following the treatment of buried pipeline metals with extract of *O. gratissimum* can provide vital insights into the microbial mechanisms underlying MIC control. Such studies can identify reductions in the relative abundances of key MIC-associated taxa, reveal suppression of functional genes involved in sulfate reduction or acid production, and detect enrichment of potentially protective microbial consortia (Wang *et al.,* 2020; Zuo, 2019). This knowledge not only supports the development of sustainable MIC mitigation strategies but also informs the long-term monitoring and management of microbial communities in pipeline infrastructures.

Given the substantial environmental and economic implications of MIC, and the urgent need for green alternatives to conventional corrosion inhibitors, this study employs metagenomic analyses to elucidate the bacterial community dynamics associated with MIC control on buried pipeline metals treated with shoot extract of *Occimum gratissimum* comprising of the leaf and soft stem parts. By integrating phytochemical-based interventions with modern molecular microbiology, this research aims to advance our understanding of microbial induced corrosion management in buried pipeline systems.

Materials and Methods

Sample Collection

Flat Carbon steel coupons (0.1% C, 0.4% Mn, 0.03% S, 0.06% P, and 99.41% Fe) with a diameter of 6mm and dimensions of 4cm x 3cm x 1.7cm were fabricated at the University of Port Harcourt Science and Engineering workshop, meticulously polished with abrasive paper, and subsequently rinsed in 20% HCl, followed by cleaning with gauze saturated in absolute ethanol to eliminate rust particles from the coupon surfaces (NACE International, 2018).

Extract Formulation and Biocorrosion Simulation

400g/L concentration of *O. gratissimum* leaf/stem aqueous extract was formulated by submerging (for 24 hours) 100g of grinded leaf and stem parts of *O. gratissimum* in 125ml of distilled water to produce *O. gratissimum* leaf/stem aqueous extract (OGAE). After the 24hours, the test biocides were extracted by percolation through filter paper (Whatmann No.1) at room temperature (Umar *et al.,* 2016; Amise *et al*., 2016).

Produced water from an oil well in Seplat Energy PLC was used to enrich clayey-loam soil in sterile plastic containers labelled appropriately (B for treatment setup with the inhibitor and E for control setup without the inhibitor) and was used to simulate a biocorrosion system for the corrosion inhibition study. Pre-weighed and labelled carbon steel coupons were aseptically conditioned with the formulated *O. gratissimum* leaf/stem aqueous extract (OGAE) by submerging the steels in the extract for 24 hours, to serve as an inhibitor for microbiologically influenced corrosion of the carbon steels (Adindu *et al*., 2017; Briggs *et al.,* 2019). These carbon steels conditioned with the OGAE (inhibitor) were buried in the soil setup labelled B (treated setup), while carbon steels buried without the inhibitor in the soil setup labelled E served as control. The setups were incubated at room temperature for 28 days under anaerobic conditions.

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Metagenomic Sample Collection and Preservation

Three soil samples were collected from the soil surrounding the buried metal coupons and experimentally set up to assess the bacterial genome of the MIC ecosystem in this study. The experimental samples consisted of the baseline soil collected on day 0 before any treatment (E0), a control soil collected from the setup without any inhibitor application on day 28 (E28) and soil collected from the setup containing metal coupons treated with aqueous leaf/stem extract of *Ocimum gratissimum* on day 28 (B). Each soil sample was carefully collected under aseptic conditions using sterile spatulas and immediately transferred into sterile 5mL sample bottles pre-filled with a nucleic acid preservation buffer.

This buffer served to stabilize and protect microbial DNA, thereby preserving the original microbial community structure present at the time of sampling. The bottles were securely closed and gently inverted to ensure uniform mixing of the buffer with the soil matrix. Thereafter, all samples were placed in insulated containers with ice packs to maintain a low temperature and transported under strict cold-chain conditions to Laragen Inc. in the United States for metagenomic analyses.

DNA Extraction

Bacterial DNA was extracted from approximately 250mg of each soil sample using the Zymo Research Quick-DNA™ Fungal/Bacterial Miniprep Kit and following the manufacturer's protocol. This kit was selected for its efficiency in lysing a broad range of microbial cell walls, including those of Gram-positive and Gram-negative bacteria, as well as potential fungal contaminants. (Zymo Research, 2023).

The extraction process combined mechanical lysis via bead beating with chemical lysis to ensure thorough disruption of microbial cells. DNA was eluted in a low-salt buffer and stored at -20°C until further processing (Li et al., 2020). The concentration and purity of each DNA extract were measured using a NanoDrop spectrophotometer, with absorbance ratios at 260/280nm and 260/230nm used to confirm the absence of protein and solvent contaminants that might inhibit downstream enzymatic reactions (Desjardins and Conklin, 2010).

Amplification of 16S rRNA Genes and Illumina MiSeq Sequencing

The extracted DNA samples were used as templates for amplifying the bacterial 16S ribosomal RNA gene, targeting the hypervariable V3–V4 regions which are commonly employed for microbial community profiling due to their high taxonomic resolution (Klindworth *et al.,* 2013; Kim *et al.,* 2021b). Amplifications were carried out using PCR with primers compatible with Illumina sequencing workflows. The PCR thermal cycling was optimized to achieve high specificity and yield. Amplification success was verified by running the PCR products on agarose gels, which consistently produced single, sharp bands of approximately 460 base pairs (Illumina, 2023). These amplicons were then purified, quantified, and prepared into sequencing libraries following Illumina's standard library preparation protocols. The libraries were sequenced on the Illumina MiSeq platform in paired-end mode (2 × 300 bp), which provided overlapping reads that improved assembly accuracy and reliability of subsequent taxonomic classifications (Kozich *et al.*, 2013; Callahan *et al.,* 2016).

Bioinformatic Processing and OTU Picking

Following the procedure of Bolyen *et al.,* 2019, the raw sequence reads obtained from the MiSeq platform were analyzed using the QIIME2 version 2021.8 bioinformatics pipeline (https://qiime2.org/). Initially, the reads were demultiplexed to assign sequences to their respective samples. Quality control steps included trimming low-quality regions, filtering out ambiguous bases, and removing chimeric sequences using the DADA2 algorithm within QIIME2 (Callahan *et al.,* 2016). Paired-end reads were merged to reconstruct full-length V3–V4 amplicon sequences. Operational Taxonomic Units (OTUs) were then clustered using a de novo approach at a 97% sequence similarity threshold, allowing distinct bacterial taxa to be identified independently of reference-based constraints (Edgar, 2010).

Representative sequences from each OTU were aligned and compared against established reference databases such as SILVA or Greengenes to assign taxonomic identities from the phylum down to the genus level (DeSantis *et al*., 2006; Quast *et al*., 2013).

Diversity Analysis and Visualization

Alpha diversity metrics, including observed OTU richness and the Shannon diversity index, were calculated to evaluate species richness and evenness within each sample (Hill *et al*., 2003). These indices provided insights into the internal diversity of the bacterial communities present under different treatment conditions. Beta diversity was assessed using Bray-Curtis dissimilarity matrices to examine compositional differences between samples (Anderson et al., 2006). These matrices were visualized through principal coordinates analysis (PCoA) plots, which illustrated the extent to which bacterial communities clustered or diverged as a result of the treatments (Lozupone & Knight, 2005). Additionally, taxonomic bar plots were generated to show the relative abundances of dominant bacterial groups across samples, offering a clear visualization of how the treatment with *O. gratissimum* extract influenced key microbial taxa associated with MIC (Caporaso et al., 2012; Bokulich *et al.,* 2018). This comprehensive methodology enabled a detailed investigation of the bacterial community dynamics in soils exposed to plant-based corrosion control strategies on buried pipeline systems.

Results

The sequencing yielded a sum of 200 paired end amplicons of 250 nucleotide quality sequences. The frequency of occurrence of each sequence varied across the three samples. The classified (identified) sequences were 1485, 40380 and 41529 operational taxonomic units (OTUs) for baseline (E0), control without inhibitor (E28) and treatment with OGAE (B) samples, repectively. Unclassified (unidentified) sequences in the ecosystems were 74 (E0), 27(E28) and 56(B). The metagenomes from the three samples (E0, E28 and B) analyzed showed microbial communities with taxonomically large diversity but with variations in their abundance. Percentage relative abundance of the metagenomes (both classified and unclassified) were calculated for the various taxa groups and the resultant data presented in Figures 1- 6.

The charts (Figure 1 – 6) vividly demonstrate how *O. gratissimum* (B) treatment reshaped the bacterial community structure compared to the control (E28) and baseline (E0).

Amplicon sequencing revealed that all microbial communities belonged to the Kingdom Bacteria, with classification into 10 phyla, 19 classes, 31 orders, 59 families, and 65 genera.

Starting at the phylum level (Figure 1), Proteobacteria, Firmicutes, Actinobacteria, Bacteriodetes, Acidobacteria, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, Crenarchaeota and Chloroflexi were identified. The relative abundance of Proteobacteria was highest in the control (E28) at 96.58%, while slightly decreasing at 89.91% under *O. gratissimum* treatment (B). The baseline (E0) had the lowest proportion at 53.27%, indicating that corrosion conditions without any plant extract allowed this phylum to flourish massively. Meanwhile, Firmicutes rose from 5.18% (combined minor phyla including Firmicutes, since individual values were very low in E0) to 7.81% under B, highlighting some shifts among less dominant phyla. Actinobacteria and Acidobacteria under E0 was 25.19% and 6.20%, but declined to 1.92% and 0.08% under E28, respectively, indicating that the corrosive changes that took place in the systems were not favourable to most groups under these phyla. The same decline was observed in B, with relative abundance of 2.02% and 0.16%, for Actinobacteria and Acidobacteria respectively.

For the Bacteriodetes, even though the abundance at the beginning of the study (E0 - 2.0%) decreased by day 28 (E28 - 0.77%, B - 0.08%) in both the control and treatment samples, treated sample still showed lower abundance. Unclassified phyla decreased drastically from 4.98% in E0 to 0.13% in B, reflecting better taxonomic resolution or a community becoming dominated by well-identified groups under stress.

At the class level (Figure 2), 19 classes were identified from the three samples. Among the classes were, Gamma-proteobaceria, Beta-proteobaceria, Bacilli, Alpha-proteobacteria, Actinobacteria, Gemma-timonadetes, Bacteroidia, Saprospirae, Cytophagia and Planctomycetia. Gammaproteobacteria and Betaproteobacteria were the most dominant in the community. Gammaproteobacteria was dominant in all samples, increasing from 22.29% in E0 to 49.47% in the control (E28), then slightly higher still in B at 52.71%. The same trend was observed for Betaprotobacteria, from 22.09% in E0 to 28.96% in E28 and 21.59% in B.

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This suggests that *O. gratissimum* allowed certain gammaproteobacterial and betaproteobacterial groups to persist or even proliferate slightly. Bacilli (4.31% in E0, 0.32% in E28 and 7.31% in B) on the other hand declined significantly as the days progressed in the control sample, but increased in the treatment sample, suggesting that *O. gratissimum* enhanced their proliferation in the environment. The class Saprospirae showed a decrease from 1.145% in E0 to 0.005% in B compared to the observed increase in E28 at 0.728%, indicating these were sensitive to the treatment. Unclassified class sequences also dramatically fell under the treatment to 0.152% in B down from 7.205% in E0.

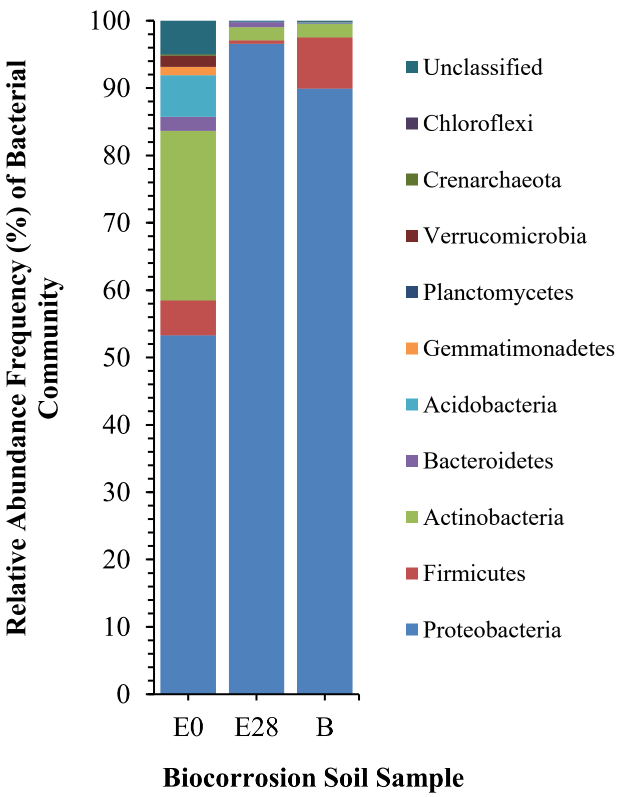

Figure 1: Relative Abundance of Bacterial Community Phylum Taxa

Key: E0 – Baseline soil sample from biocorrosion setup collected on Day 0;
E28 – Soil from setup without inhibitor (extract) collected on Day 28 (Control);
B – Soil collected from Setup treated with *O. gratissimum* leaf/stem aqueous extract (Treatment).

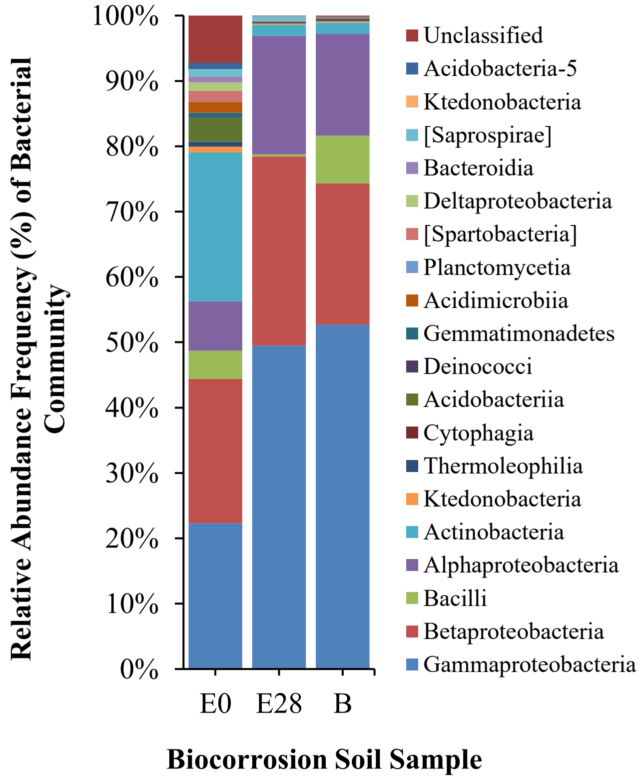

Figure 2: Percentage Relative Abundance of Bacterial Community Class Taxa

Key: E0 – Baseline soil sample from biocorrosion setup collected on Day 0;
E28 – Soil from setup without inhibitor (extract) collected on Day 28 (Control);
B – Soil collected from Setup treated with *O. gratissimum* leaf/stem aqueous extract (Treatment).

Looking at the order level (Figure 3), some of the order assigned from the analysis were: Xanthomonadales, Burkholderiales, Clostridiales, Pseudomonadales, Rhodospirillales, Desulfuromonadales, Actinomycetales, Myxococcales, Lactobacillales and Caulobacterales. Burkholderiales was the most dominant with abundance of 22.088% in E0 (baseline), 28.952% in E28 (control) and 21.522% in B (treatment) samples, the treatment showing little or no effect on these groups of bacteria. Pseudomonadales, strongly tied to corrosion, showed a decrease in treated sample (B) at 12.952% compared to the untreated control (E28) at 24.762%, clearly illustrating suppression by the extract.

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Another group which showed significant decrease in the treatment sample compared to the control without the inhibitor was the Sphingomonadales (0.202% in E0, 3.108% in E28 and 0.424% in B), also indicating suppression by the extract. Additionally, Desulfuromonadales effectively vanished, dropping from 0.135% in E0 to almost zero in the treatment (just 0.017% in B and 0% in E28).

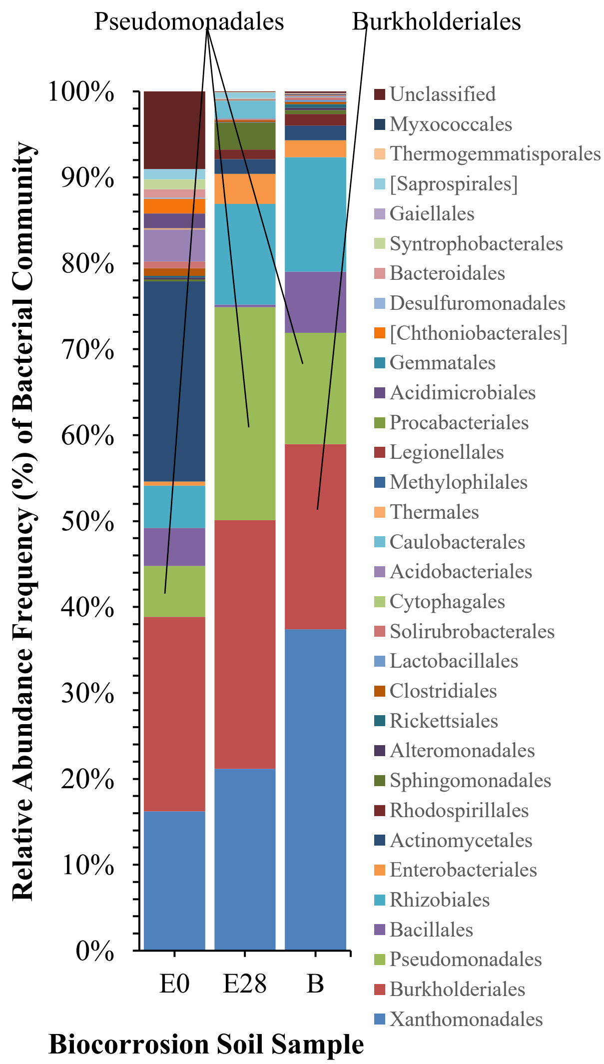

Figure 3: Percentage Relative Abundance of Bacterial Community Order Taxa

Key: E0 – Baseline soil sample from biocorrosion setup collected on Day 0;
E28 – Soil from setup without inhibitor (extract) collected on Day 28 (Control);
B – Soil collected from Setup treated with *O. gratissimum* leaf/stem aqueous extract (Treatment).

The family level is presented in Figure 4.

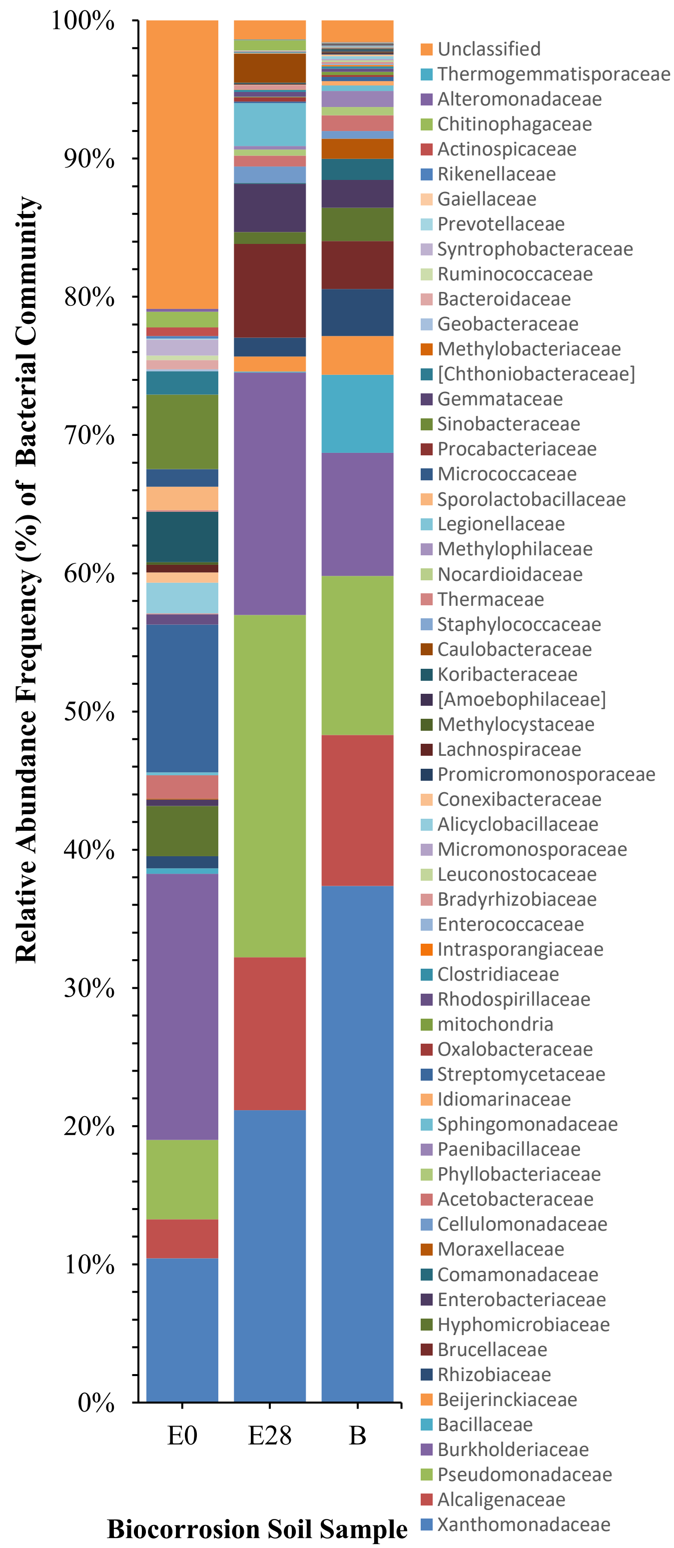

Figure 4: Percentage Relative Abundance of Bacterial Community Family Taxa

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The family level which included, Xanthomonadaceae, Pseudomonadaceae, Sphingomonadaceae, Clostridiaceae, Sinobacteriaceae, Chitinophagaceae, Bacteroidaceae, Actinospicaceae and others, reinforces this. Xanthomonadaceae increased markedly under *O. gratissimum* to 37.374%, compared to 21.149% in the control, suggesting that when Pseudomonadaceae (corrosion-promoters) dropped significantly in treatment at 11.503% (B) while control at 24.762% (E28) increased, this family expanded to occupy the ecological space. Meanwhile, Sphingomonadaceae dropped in abundance in the treatment to 0.424% (B) compared to control which increased at 3.108% (E28) from baseline (E0) at 0.202%, also indicating sensitivity to these treatments. Unassigned sequences in this taxon were E0- 20.875%, E28- 1.394%, B- 1.568% and D- 1.805% in abundance.

From the genus-level chart (Figure 5), *Stenotrophomonas* (E0- 0%, E28- 6.565%, B- 31.571%), *Pseudomonas* (E0- 5.724%, E28- 24.762%, B- 5.184%) and *Burkholderia* (E0- 15.152%, E28- 9.946%, B- 5.541%) were the most predominant. *Stenotrophomonas* dramatically increased to 31.571% under *O. gratissimum* (B) and decreased to 6.565% in E28. *Pseudomonas* increased from 5.72% in E0 to 24.762% in E28, while decreasing significantly to 5.184% in B. This suggests *Stenotrophomonas* opportunistically filled niches opened by the inhibition of *Pseudomonas*. Similarly, *Burkholderia* dropped to 5.541% under B in comparism with 9.95% seen in E28, highlighting the antimicrobial ability of the *O. gratissimum* extract. *Geobacter*, a genus in the order Desulfuromonadales was present in the samples, with an abundance of 0.135%, 0% and 0.017% in E0, E28 and B, indicating their scarce presence in the community across all three samples. *Pseudomonas, Burkholderia, Sediminibacterium Salinispora, Sphingomonas, Sphinopyxis, Pseudoxanthomonas, Mycoplana, Phenylobacterium, Chitinophaa, Enterobacter, Sphinobium, Dokdonella, Clostridium, Rhodanobacter, Azospirillum, Marinobacter, Bosea Ochrobactrum,* and *Cupriavidus* were the genera that showed significant decrease in abundance in the treatment sample (B), an indication of the effectiveness of the *O. gratissimum* stem/leaf aqueous extract in inhibiting the growth and activities in a soil environment. The relative abundance of unassigned sequences in the genus taxa were: E0- 52.391%, E28- 13.747% and B- 27.318%.

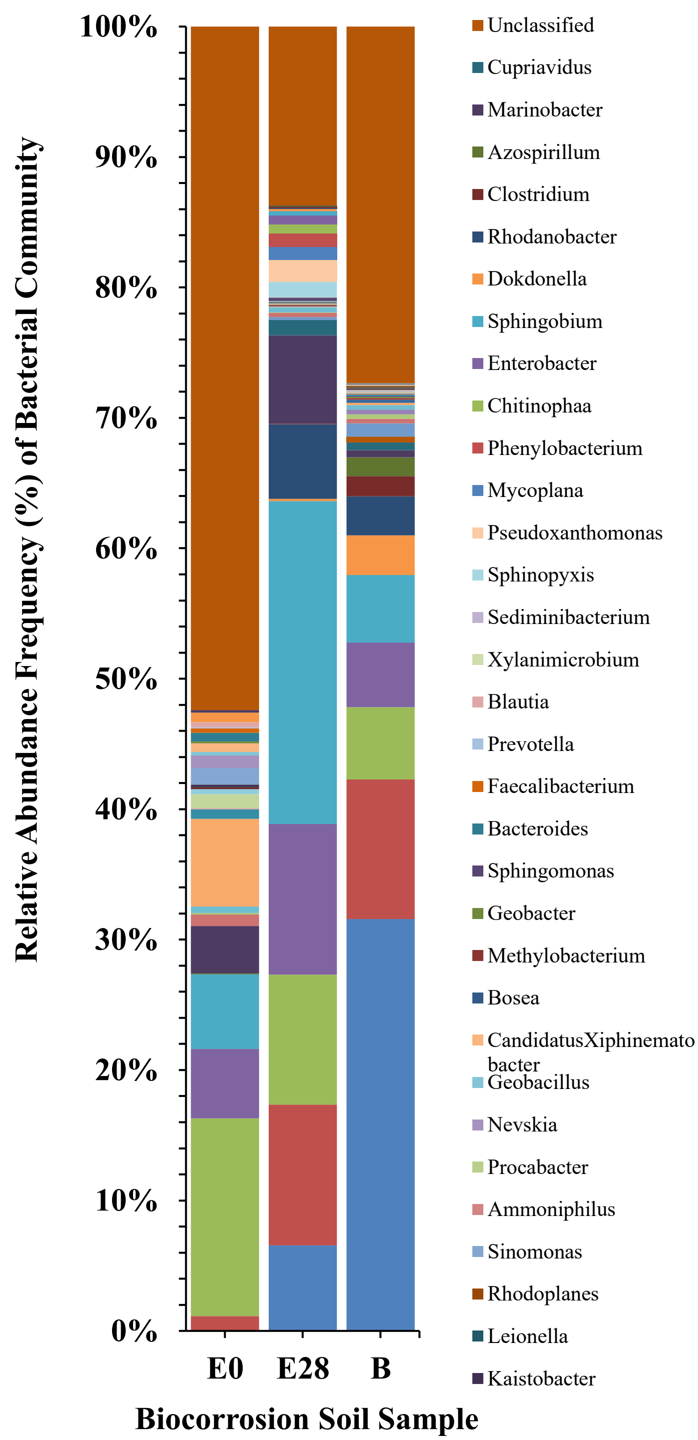

Figure 5: Percentage Relative Abundance of Bacterial Community in Genus Taxa

Key: E0 – Baseline soil sample from biocorrosion setup collected on Day 0; E28 – Soil from setup without inhibitor (extract) collected on Day 28 (Control); B – Soil collected from Setup treated with *O. gratissimum* leaf/stem aqueous extract (Treatment).

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At the species level (Figure 6), *Pseudomonas balearica* was a clear target of inhibition: from 0.34% abundance in baseline (E0) to 1.907% in untreated sample after 28 days (E28), but decreased in the treatment (B) at 0.222%. In contrast, *Ochrobactrum intermedium* remained roughly constant at 2.234% (E28) and 2.232% (B), indicating resistance to the extract. Meanwhile, *Stenotrophomonas acidaminiphila* surged under *O. gratissimum* to 23.124%, showing that certain bacteria thrived when competitors were suppressed. The charts quantitatively demonstrate that the extract significantly reduced corrosion-associated taxa such as *Pseudomonas* and *Desulfuromonadales*, while allowing genera like *Stenotrophomonas* to flourish. The relative abundance shifts, backed by these numerical values, highlight the selective modulation of the microbial community structure by this plant-based treatment.

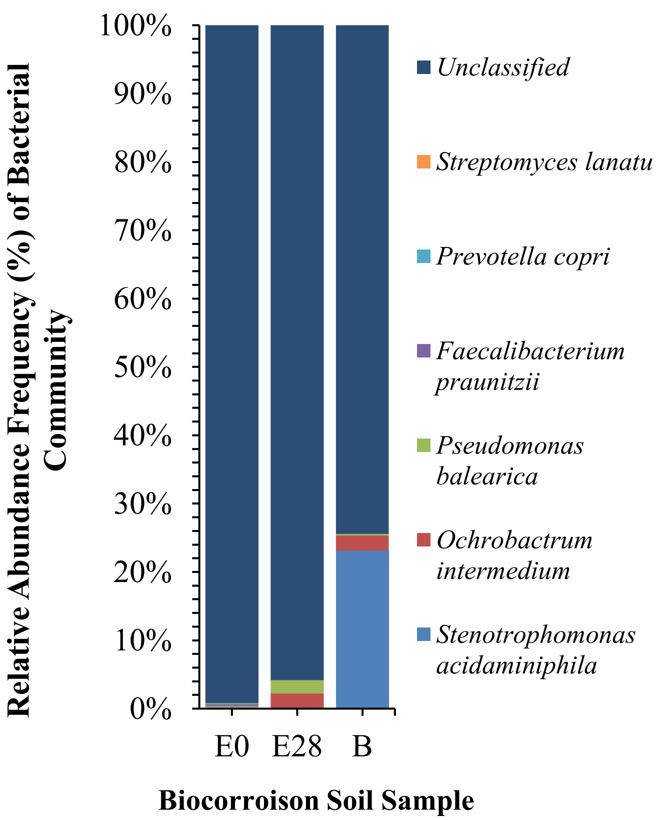

Figure 6: Percentage Relative Abundance of Microbial Community in Species Taxa

Key: E0 – Baseline soil sample from biocorrosion setup collected on Day 0; E28 – Soil from setup without inhibitor (extract) collected on Day 28 (Control);
B – Soil collected from Setup treated with *O. gratissimum* leaf/stem aqueous extract (Treatment).

Discussion

Metagenomes from the three study samples (E0, E28 and B) that were analyzed for their community structure and diversity revealed diverse bacterial groups present in the communities across the three samples. The analysis of microbial community structure, diversity, and abundance in the three samples (E0, E28 and B) demonstrated notable variations influenced by the application of the plant extract in treatment setup. The baseline sample (E0) provided a reference for initial microbial diversity and abundance at Day 0, while the control sample (E28) represented untreated conditions after 28 days. Treated sample B, which was exposed to *O. gratissimum* leaf/stem aqueous extract, exhibited distinct shifts in microbial composition compared to the control (E28).

The present study elucidated the impact of *Ocimum gratissimum* leaf/stem aqueous extract on the bacteria community structure associated with microbiologically induced corrosion (MIC) on carbon steel, using high-throughput amplicon sequencing to characterize the taxonomic shifts. The results demonstrate substantial alterations in the bacterial community composition, particularly among key taxa implicated in corrosion processes.

16S rRNA gene analysis of the metagenomes from the four samples revealed the presence of bacteria belonging to Proteobacteria, Firmicutes, Bacteriodetes, Actinobacteria and Acidobacteria as the dominant taxonomic groups in the simulated soil studied. Similar taxonomic groupings have been detected by other researchers using metagenomics in oilfield samples and biocorrosion ecosystems (Hassan *et al.,* 2018; Nasser, 2021). At the phylum level, Proteobacteria dominated across the treatment and controls, with relative abundances increasing from 53.27% in the baseline (E0) to 96.58% in the 28-day untreated control (E28), while slightly lower at 89.91% under *O. gratissimum* (B) treatment. The prevalence of Proteobacteria in corrosion biofilms has been extensively documented, given their metabolic versatility and roles in iron and sulfur cycling (Beese-Vasbender *et al.,* 2015; Tay, 2018; Li *et al.,* 2022). The marginal decrease in the treated setup suggests that the *O. gratissimum* leaf/stem extract exerted partial inhibitory effects on this phylum's proliferation, thereby potentially disrupting electron transfer processes critical for corrosion.

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At a finer taxonomic resolution, the class Gammaproteobacteria emerged as the most dominant across samples, with relative abundance reaching 52.71% under *O. gratissimum*. This class encompasses genera such as *Pseudomonas* and *Stenotrophomonas*, which are frequently linked to biofilm formation on steel surfaces (Zhang *et al.,* 2021a). Notably, while *Pseudomonas* was substantially inhibited by the extract—increasing from 5.72% in E0 to 24.76% in E28 while decreasing to 5.18% under *O. gratissimum* treatment (B)—*Stenotrophomonas*, a genus often associated with biofilm formation and biocorrosion, conversely proliferated, rising from 0% in E0 to 6.56% in E28 and 31.57% in B. Such shifts are indicative of competitive dynamics within biofilm communities, where suppression of one group may open ecological niches for others (Kumar & Anand, 1998).

At the order level, Burkholderiales was the most dominant group across all three samples, with abundances of 22.088% (E0), 28.952% (E28) and 21.522% (B). This group which includes the *Burkholderia* and *Achromobacter* have been implicated in MIC of buried oil pipeline metals (Nasser, 2019; Mand & Enning, 2020), and its robust presence suggests its adaptability to both treated and untreated conditions, a trend that has also been observed in previous researches (Liew *et al*., 2017; Nasser, 2019). Meanwhile, Pseudomonadales, encompassing the *Pseudomonas* genus, demonstrated a marked decrease in abundance (B - 12.95%) under the plant extract treatment compared to the control (E0 - 24.76%), aligning with previous studies that documented the inhibitory effects of plant-derived compounds on *Pseudomonas* species (Chen *et al*., 2019). This is of particular interest as *Pseudomonas* genus which has diverse properties, have been principally isolated from oil polluted environments around the world (Ahmadi *et al*., 2017; Salva-Serra *et al*., 2023) and are well-known agents of extracellular electron transfer that facilitate steel dissolution (Javaherdashti, 2008). Their suppression underscores the potential of these phytochemicals as eco-friendly alternatives to synthetic biocides. Furthermore, the near-complete disappearance of *Desulfuromonadales* under treatment (from 0.135% in E0 to 0.017% in B) is significant, as members of this order are linked to sulfate and sulfur compound reduction, processes that exacerbate localized corrosion (Enning & Garrelfs, 2014; Immanuel *et al*., 2016).

Family-level analyses revealed a dynamic interplay. For instance, the family *Pseudomonadaceae* increased in abundance from 5.72% in E0 to 24.76% in E28 in contrast to 11.50% relative abundance in B, affirming the genus-level suppression. Meanwhile, *Xanthomonadaceae* surged under *O. gratissimum* to 37.37%, suggesting compensatory growth by other heterotrophic groups possibly less directly involved in corrosion but capable of exploiting available nutrients (Costerton *et al.,* 1995). Similarly, the family *Sphingomonadaceae* decreased significantly in the treatment, consistent with its sensitivity to various antimicrobial plant metabolites (Dey *et al*., 2020).

The bacterial community in the baseline Day 0 (E0) sample had 26 classified genera. The most dominant groups among them were *Burkholderia, Pseudomononas, Salinospora, Streptacidiphilus, Sinomonas,* and *Dokdonella*. The control E28 (no inhibitor) sample, had a community comprised of 51 microbial genera present. Among them, the seven afore mentioned genera along with *Stenotrophomonas, Achromobacter, Dyella, Ochrobactrum, Cellulomonas, Sediminibacterium, Phenylobacterium, Pseudoxanthomonas* and *Mycoplana* were the most dominant in that community. The treatment with *O. gratissimum* extract (B) sample had a total of 53 identified genera in their community. The most dominant of them were *Stenotrophomonas, Achromobacter, Burkholderia, Dyella, Pseudomonas, Agrobacterium, Ochrobactrum, Comamonas, Acinetobacter and Paenibacillus.* Several other genera implicated in biocorrosion on pipelines, showed remarkable decrease in abundance in the treatment sample, indicating the effectiveness of the *O. gratissimum* leaf/stem extract in inhibiting biocorrosion caused by microbial presence on buried carbon steel. The genera *Burkholderia* (E0-15.15%, E28- 9.95%, B- 5.54%), *Salinospora* (E0- 3.64%, E28- 6.75%, B- 0.56%), *Sphingomonas* (E0- 0%, E28- 0.20%, B- 0.01%), *Pseudoxanthomonas* (E0- 0%, E28- 1.65%, B- 0%) and *Phenylobacterium* (E0- 0%, I.0%, B- 0%) have been reported for their presence in oil polluted environments and their involvement in microbiologically induced corrosion (Revathy *et al*., 2015; Mand & Enning, 2020; Krohn *et al.,* 2021).

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At the species-level, the identified species were: *Stenotrophomonas acidaminiphila*, *Ochrobactrum intermedium*, *Pseudomonas balearica*, *Faecalibacterium prausnitzii*, *Prevotella copri*, and *Streptomyces lanatus*. Among these, *Ochrobactrum intermedium* emerged as the most dominant species amongst the four samples, corroborating findings by Zhang *et al.* (2021b) on its adaptability in metal-enriched environments. Species-level patterns provided more direct insights into corrosion mitigation. *Pseudomonas balearica*, a species associated with denitrification and biofilm resilience (Martínez-Checa *et al.,* 2019), decreased significantly in treated sample under *O. gratissimum* (0.22%) as compared to the control sample with sharp increase in abundance (1.91%) after the 28 days. This suggests potent suppression by the extracts, likely linked to their rich content of phenolic and flavonoid compounds which can disrupt cell membranes and interfere with quorum sensing (Cowan, 1999; Cushnie & Lamb, 2011). *P. balearica* is known to thrive in diverse environments, including soil, water, and within different ecosystems. The *Pseudomonas* genus has been reported numerous times to be involved in corrosion processes and are lead colonizers in biofilm formation (Abdolahi *et al.,* 2014; Jia *et al.,* 2017; Xu *et al.,* 2017). Conversely, *Ochrobactrum intermedium* which can thrive in crude oil environments and degrade crude oil and is known for its resistance to many conventional antibiotics including betalactams (Chai *et al.,* 2015), showed stable abundances (~2.23%) across the treatment and control samples, indicating possible resistance or lesser involvement in processes directly targeted by the plant bioactives. The selective inhibition observed may relate to the unique phytochemical profiles of *O. gratissimum*. Studies have highlighted that this plant contain eugenol, thymol, and other phenolic derivatives capable of exerting broad-spectrum antibacterial effects by destabilizing membrane integrity and disrupting metabolic pathways (Prakash *et al.,* 2010; Okwu & Omodamiro, 2005). This aligns with our observation that despite overall shifts in community structure, specific corrosion-associated taxa such as *Pseudomonas* were disproportionately impacted. Moreover, the substantial reduction in unclassified sequences under the treatment sample falling from 13.75% in E28 to 27.31% in B at the genus level may reflect reduced microbial diversity due to the selective pressure imposed by the extract. Reduced diversity in biofilms has been linked to decreased metabolic redundancy, which may impair the complex cooperative interactions necessary for MIC (Dang & Lovell, 2016). In sum, the data compellingly indicate that *O. gratissimum* extract selectively suppress key MIC-associated microbial groups, particularly *Pseudomonas* spp. and sulfur-reducing orders, thereby potentially mitigating corrosion processes. These findings advance current understanding of biocorrosion control by introducing plant-derived alternatives to traditional chemical biocides, which are often associated with environmental persistence and toxicity (Videla & Herrera, 2005). Future studies should aim to elucidate the precise molecular mechanisms underlying these inhibitory effects and evaluate the long-term performance of these extracts in complex industrial systems.

Conclusion

This study demonstrated that the aqueous leaf/stem extract of *Ocimum gratissimum* has significant inhibitory effects on the microbial communities associated with microbiologically influenced corrosion on buried steel surfaces. The leaf/stem extracts selectively suppressed key bacterial taxa known to drive corrosion processes, notably reducing the relative abundances of genera such as *Pseudomonas*, *Sphingomonas*, *Burkholderia*, *Salinospora* and *Phenylobacterium* which are often implicated in biofilm formation and extracellular electron transfer critical for corrosion progression. Notably, treatment with this extract resulted in substantial shifts in community composition across multiple taxonomic levels. The reduction in the abundance of orders like *Pseudomonadales* and families such as *Pseudomonadaceae* in treated samples highlights the potential of this phytochemical to disrupt microbial processes that facilitate metal deterioration. The consistent dominance of *Proteobacteria* despite treatment suggests a selective, rather than broad-spectrum, antimicrobial effect, which may help maintain certain ecological functions while targeting corrosion-associated microbes. The observed decline in species such as *Pseudomonas balearica* under the plant extract treatment further underscores the capacity of this botanical to mitigate specific microbial drivers of corrosion. By contrast, taxa like *Ochrobactrum intermedium* remained relatively unaffected, suggesting differential susceptibilities among community members.

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Recommendations

Based on these findings, it is recommended that:

1. The active phytochemical constituents responsible for microbial inhibition be isolated and characterized, paving the way for development of standardized green corrosion inhibitors.
2. Future studies integrate metatranscriptomic or metaproteomic analyses to unravel functional gene expression changes in microbial communities exposed to these plant extracts.
3. Policies promoting the adoption of eco-friendly corrosion management strategies include provisions for using locally sourced plant extracts, thereby reducing reliance on synthetic biocides and minimizing environmental impact.

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