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Catabolic gene indices of hydrocarbon diminution in Ultisol treated with cropped *Bacillus altitudinis*-amendments

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ABSTRACT

This study evaluated catabolic gene expression as an index of hydrocarbon breakdown in ultisol treated with *Bacillus altitudinis*-Cropped Biofertilizers derived from Biosolid and Brewer Spent Grain (BSG) using phenotypic and molecular methods. We observed significant reductions in Total Petroleum Hydrocarbons (TPH) concentrations with both amendments, albeit the biosolid based amendment was markedly more effective. Concurrently, there was a substantial increase in hydrocarbon-degrading bacteria. Notably, the bulk of Operational Taxonomic Units (OTUs) in biosolid (95.77 %) and BSG (93.00 %) amended Ultisol were Proteobacteria, Acidobacteria, Actinobacteria, and Planctomycetes, which are key players in hydrocarbon bioremediation. We observed the presence of eleven hydrocarbon-degrading genes through M5nr analysis. These genes encompass essential catalysts for aliphatic hydrocarbon degradation and hydrocarbon desulfurization. Notably, these enzymes/genes include Bacterial Flavin-bound Monooxygenase (AlmA), Alkane Monooxygenase (AlkM), Alpha Ketoglutarate Dependent Dioxygenase (*alkB*), Propane Monooxygenase (PrM), Cytochrome P450 (cP450), Methane Monooxygenase/Ammonia Monooxygenase (MMO/AMO) subunits A, B, and C, Alkane Sulfonate Monooxygenase (*ssuD*), Alkane Sulfonates Transport System Permease Protein (*ssuC*), Dibenzothiophene Monooxygenase (*dszC*), Dibenzothiophene-Sulfone Monooxygenase (*dszA*), and Dibenzothiophene-5,5-Dioxide Monooxygenase (*dszB*). These genes play pivotal roles in the degradation of aliphatic hydrocarbons and hydrocarbon desulfurization. Interestingly, unique gene expression patterns were observed for each amendment, with Actinomycetales and Bulkhoderiales orders expressing the majority of identified genes. These findings have revealed the amendment-specific microbial and genetic alterations and, the diversity and potential of the annotated genes induced by the *Bacillus altitudinis*-Cropped Biofertilizers (biosolid and BSG amendments) for effective remediation of hydrocarbon-contaminated soils.

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Introduction

The relentless pursuit of energy resources has led to an escalation in anthropogenic hydrocarbon contamination, posing severe threats to both human health and environmental sustainability. A considerable fraction of these pollutants infiltrates the soil, predominantly Ultisols in Southern Nigeria, disrupting their natural equilibrium and degrading the intrinsic soil microbial community. Crude oil spills and their consequent contamination of surrounding environments represent enduring threats to the ecological, societal, and economic well-being of affected regions [1–5]. In response, considerable efforts have been dedicated to devising strategies for the prevention and management of oil spills. In recent times, there has been a noteworthy shift toward the remediation of contaminated soil through approaches that prioritize non-destructive, minimally disruptive, and cost-effective technologies.

One such method gaining prominence is Remediation by Enhanced Natural Attenuation (RENA), which offers a comprehensive bioremediation approach for addressing soil, sediment, and sludge contamination. Despite the merits of bioremediation, its efficacy is often hampered primarily by the limited bioavailability of hydrocarbons to microorganisms. This challenge arises from the inherent low solubility of hydrocarbons and their strong, often irreversible, sorption to the soil matrix [1,2,6].

The capacity of microorganisms to degrade hydrocarbons and utilize them as energy sources is well-documented. However, in polluted soils, the native microbial communities frequently prove insufficient to efficiently manage the degradation process. Consequently, the implementation of bioaugmentation techniques becomes imperative to bolster microbial degradation capabilities. Among the various methods devised to enhance the bioavailability of hydrocarbons, Remediation by Enhanced Natural Attenuation (RENA) stands out, with a particular emphasis on biological sources.

The majority of studies in this domain have concentrated on enhancing remediation through biostimulation and bioaugmentation (BB) protocols [1,2,7]. In tandem with bio-augmentation, organic amendments have gained significant traction as a strategy to stimulate the existing microbial communities. As a by-product of beer production, Brewer Spent Grain (BSG) is an abundant organic waste product rich in lignin, cellulose, and hemicellulose, making it a promising candidate for organic amendment. Biosolids, rich in organic matter and nutrients, also serve as an excellent source of bio-augmentation. Their application to contaminated soils provides an influx of microorganisms capable of metabolizing hydrocarbons, thus fostering enhanced remediation [8].

Exploring the abundance and diversity of microbial communities within polluted areas and understanding the influence of environmental conditions on their degradative capacities is crucial for regulating remediation rates in affected regions. Despite numerous studies evaluating microbial abundance, diversity, and gene expression during hydrocarbon degradation in contaminated sites, a substantial knowledge gap exists regarding the metabolic potentials of these microbes [9–11]. Continuation of metagenomic studies is essential to delve into this ecological process further. Gene expression profiles of specific catabolic genes like CYP153, *alkB*, and xylE etc. serve as pivotal indicators, offering insights into the activities and interactions within microbial communities that drive hydrocarbon degradation. The presence and expression levels of these genes elucidate crucial pathways and enzymatic processes involved in breaking down hydrocarbons [12]. Understanding these genetic expressions aims to provide a comprehensive understanding of the potential for enhanced remediation in contaminated soils, leveraging insights from these specific catabolic genes. Ultimately, this research significantly contributes towards the development of sustainable, biotechnologically-driven solutions for managing environmental pollution."

Materials and methods

The amendments used in this study were derived from biosolid and BSG. A vivid description of derivation of the amendments via

A1 0.5% Hydrocarbon (12.05mg/kg) + 10 kg bacterized biosolid	A2 1.0% Hydrocarbon (15.15mg/kg) + 10 kg bacterized biosolid	A3 1.5% Hydrocarbon (21.25mg/kg) + 10kg bacterized biosolid	AB4 (control-1) 1.5% Hydrocarbon (21.25mg/kg) +no amendment
B1 0.5% Hydrocarbon (12.05mg/kg) + 10 kg bacterized BSG	B2 1.0 % Hydrocarbon (15.15mg/kg) + 10 kg bacterized BSG	B3 1.5% Hydrocarbon (21.25mg/kg) + 10kg bacterized BSG	

Fig. 1. Experimental design.

composting and characterization of same have been described elsewhere [7,8]. The amendments were bacterized with a strain of *Bacillus altitudinis* (GenBank accession number KY569499.1) as described by Maheshwari et al. [13] and stored for field trial. The field trial was conducted at the Experimental Farm of the Department of Botany and Ecological Studies, located between Latitude N05°02′25.3″ and Longitude 07°58′42.9″, University of Uyo Main Campus, Uyo, Nigeria.

Treatments were completely randomized. In the research station, three 2 m x 2 m plots were prepared as crop beds and contaminated with 0.5 %, 1.0 % and 1.5 % levels ($^{V}/_{W}$)) of hydrocarbon source. Thereafter the hydrocarbon simulated soil beds (A1-A3) for biosolids, (B1-B3) for BSG were amended with 10 kg of bacterized BSG and biosolid as basal dressings [14]. A control experiment comprising hydrocarbon contaminated but un-amended soil (AB4) was also set up (Fig. 1).

The determination of Total Petroleum Hydrocarbon (TPH) content in both control and treated soils involved an extraction process wherein 10 g of soil sample was subjected to dichloromethane extraction using a Soxhlet apparatus over a 16-hour period. Subsequently, the extracted solution was concentrated to 1 ml using a rotary evaporator, and the residual TPH was quantified through Gas Chromatography-Mass Spectrometry (GC–MS) analysis. This experimental procedure was carried out in triplicate, with TPH quantification being performed on a mixture of the three subsamples, adhering to established protocols as previously documented by Offiong et al. [15].

Following preliminary experimentation, a metagenomic approach was employed to analyze the treatments that exhibited the highest efficiency in TPH removal (A3 & B3), alongside control experiment (AB4). The extraction of DNA from soil samples was executed using the ZYMO Soil DNA Extraction Kit (Model D 6001, Zymo Research, USA), in strict accordance with the manufacturer's guidelines. The filtered DNA was subsequently amplified via Polymerase Chain Reaction (PCR). The resulting PCR products underwent sequencing and alignment using Vecton NTI Suite 9 (Infor Max, Inc.).

Bioinformatic analysis was conducted employing NCBI-BLAST-2.2.24 and CLC bio-Genomics Workbench v7.5.1. Raw data obtained from the PacBio platform underwent preprocessing to eliminate redundant or non-informative reads, followed by analysis using the Metagenome Rapid Annotation using Subsystem Technology (MG-RAST). Genes that yielded the best-hit classifications were subjected to M5nr annotation, with the application of specific parameters such as a minimum percentage identity, E-value, and alignment length cut-off set at 70 %, $1e^{-5}$, and 15 bp, respectively.

Functional categorization and hierarchical classification of the genes were extrapolated by referencing the KO (KEGG orthologs), NOG (non-supervised orthologous groups), and COG (cluster of orthologous groups) subsystems.

Statistical data analysis was conducted utilizing XLSTAT PREMIUM Software (Evaluation 2023.1.5.1409 Version). The significance of differences among various treatment groups was assessed using Welch's analysis of variance (ANOVA). Principal Component Analysis (PCA) and correlation coefficients were calculated to elucidate the interrelationships among variables and determine which treatment types accounted for the majority of sample variability.

Results and discussion

The microbiological and chemical properties of the amendments is presented in Table 1. The spent grain (BSG) is rich in carbon source but acidic and very poor in resource elements, viz organic matter along with nitrogen, phosphorus and potassium (NPK) while the sewage sludge contains relevant characteristics including richer levels of nutritive salts and resource elements such as organic matter with remarkable levels of NPK. Levels of nitrogen (10.05 %) and sulphate (4.33 mg/kg), were significantly ($p < 0.05$) higher in biosolid compared to BSG while same trend was observed for levels of phosphorus (2.87 %) and ammonium (11.2 mg/kg) in BSG compared to biosolid.

The TPH levels in the amended and control soils are presented in Table 2-4 while the levels for other categories of treatment are presented in Figure S1 (Supplementary Information). Post-contamination analysis showed little variability within each treatment (beds) for the initial TPH levels (12.05 to 21.25 mg kg^{-1}) before the addition of amendments indicating a homogeneous initial distribution of the hydrocarbon contaminates in soil. Result further revealed that Biosolid was significantly ($p < 0.05$) more effective at driving TPH reduction in amended beds than BSG, although both treatments showed promising results when compared to the naturally

Table 1
Microbiological and chemical properties of amendment used.

Properties	Biostimulant Type	
	Biosolid	BSG
HET (logCFU/g)	9.20	8.30
HDB(logCFU/g)	2.10	–
Fungi(logCFU/g)	3.6	2.80
pH	7.34	6.67
Organic Matter (%)	22.16	10.4
Nitrogen (%)	10.05	1.91
Phosphorus (%)	0.76	0.81
Potassium (mg/kg)	2.89	2.80
Phosphate (mg/kg)	1.13	2.87
Ammonium ion (mg/kg)	0.84	11.2
Sulphate (mg/kg)	4.33	1.29
Chlorides (mg/kg)	1.07	0.002

Table 2
Levels of TPH and residual weight percentages in cropped biosolid amended ultisols.

Compound	A (mg/kg)	B (mg/kg)	C (mg/kg)	% Degradation
C12	1.04	0.04	1.00	96.15
C13	1.75	0.08	1.67	95.43
C14	2.00	0.06	1.94	97.00
C15	1.98	0.03	1.95	98.48
C16	1.60	0.06	1.54	96.25
C17	0.43	0.05	0.38	88.37
C18	1.25	0.04	1.21	96.80
C19	1.33	0.14	1.19	89.47
C20	1.05	0.01	1.05	99.52
C21	1.41	0.07	1.34	95.04
C22	1.46	0.01	1.46	99.66
C23	1.06	0.09	0.97	91.51
C24	1.34	0.03	1.31	97.76
C25	0.36	0.05	0.31	86.11
C26	0.43	0.05	0.38	88.37
C27	0.09	0.08	0.01	11.11
C28	0.01	0.00	0.01	100.00
C29	1.30	0.14	1.16	89.23
C34	0.34	0.13	0.21	61.76
C38	1.02	0.04	0.98	96.08
Total	**21.25**	**1.19**	**20.06**	**94.40**

A: TPH load in Contaminated Bed; B: TPH load in Amended Bed; C: Amount of TPH Degraded.

attenuated soil (AB4). The recorded levels for residual TPH loads at expiration of study were 1.19, 2.30 and 8.86 mg/kg, for A3, B3 and AB4 respectively. More specifically, the residual weight percentages of the C-12 to C-32 carbon chains have shown that aliphatic components of the hydrocarbon were remarkably degraded in soil amended with cropped biosolid biofertilizer (Table 2). Though found to be present in very minimal concentrations in contaminated ultilsols, C-17 (44.19 %) and C-34 (17.65 %) were poorly degraded in soil treated with cropped BSG biofertilizer while 100 % degradation was recorded for C-27 and C-28 chain hydrocarbons in soil amended with same amendment (Table 3).

Bioremediation encounters formidable challenges imposed by a spectrum of environmental, physical, and chemical factors. A prominent impediment in Total Petroleum Hydrocarbon (TPH) bioremediation revolves around the limitation of nutrients within the soil matrix [16]. Addressing this issue, biostimulation through nutrient supplementation has emerged as an effective strategy to augment the biodegradation of TPH in soil [17–19]. Empirical evidence underscores the necessity of employing mixed consortia of microorganisms adept at crude oil utilization, possessing broad enzymatic capabilities, to effectively tackle the degradation of intricate hydrocarbon mixtures in diverse environmental settings, encompassing soil, freshwater, and marine environments [16,19].

The activities of oil-degrading microorganisms are markedly influenced by the availability of essential nutrients, particularly nitrogen and phosphorus, in the soil. Augmented levels of these nutrients, facilitated through soil amendments, have the potential to

Table 3
Levels of TPH and residual weight percentages in cropped BSG amended ultisols.

Compound	A (mg/kg)	B (mg/kg)	C (mg/kg)	% Degradation
C12	1.04	0.08	0.96	92.31
C13	1.75	0.30	1.45	82.86
C14	2.00	0.33	1.67	83.50
C15	1.98	0.24	1.74	87.88
C16	1.60	0.03	1.57	98.13
C17	0.43	0.24	0.19	44.19
C18	1.25	0.04	1.21	96.80
C19	1.33	0.16	1.17	87.97
C20	1.05	0.02	1.03	98.10
C21	1.41	0.04	1.37	97.16
C22	1.46	0.01	1.45	99.32
C23	1.06	0.06	1.00	94.34
C24	1.34	0.03	1.31	97.76
C25	0.36	0.007	0.35	98.06
C26	0.43	0.03	0.40	93.02
C27	0.09	0.09	0.00	100
C28	0.01	0.01	0.00	100
C29	1.30	0.09	1.21	93.08
C34	0.34	0.28	0.06	17.65
C38	1.02	0.22	0.80	78.43
Total	**21.25**	**2.30**	**18.94**	**89.16**

A: TPH load in Contaminated Bed; B: TPH load in Amended Bed; C: Amount of TPH Degraded.

Table 4
Levels of TPH and residual weight percentages in naturally attenuated ultisol (AB4).

Compound	A (mg/kg)	B_1 (mg/kg)	C (mg/kg)	% Degradation
C12	1.04	0.02	1.02	98.08
C13	1.75	0.09	1.66	94.86
C14	2.00	0.08	1.92	96.00
C15	1.98	0.10	1.88	94.95
C16	1.60	0.05	1.55	96.88
C17	0.43	0.03	0.40	93.02
C18	1.25	0.01	1.24	99.20
C19	1.33	1.28	0.05	3.76
C20	1.05	0.05	1.00	95.24
C21	1.41	1.03	0.38	26.95
C22	1.46	1.03	0.43	29.45
C23	1.06	1.06	0.00	0.00
C24	1.34	1.03	0.31	23.13
C25	0.36	0.26	0.10	27.78
C26	0.43	0.08	0.35	81.40
C27	0.09	0.09	0.00	0.00
C28	0.01	0.01	0.00	0.00
C29	1.30	1.21	0.09	6.92
C34	0.34	0.30	0.00	0.00
C38	1.02	1.01	0.01	0.98
Total	**21.25**	**8.86**	**12.39**	**58.49**

A: TPH load in Contaminated Bed; B_1: TPH load in attenuated bed; C: Amount of TPH Degraded.

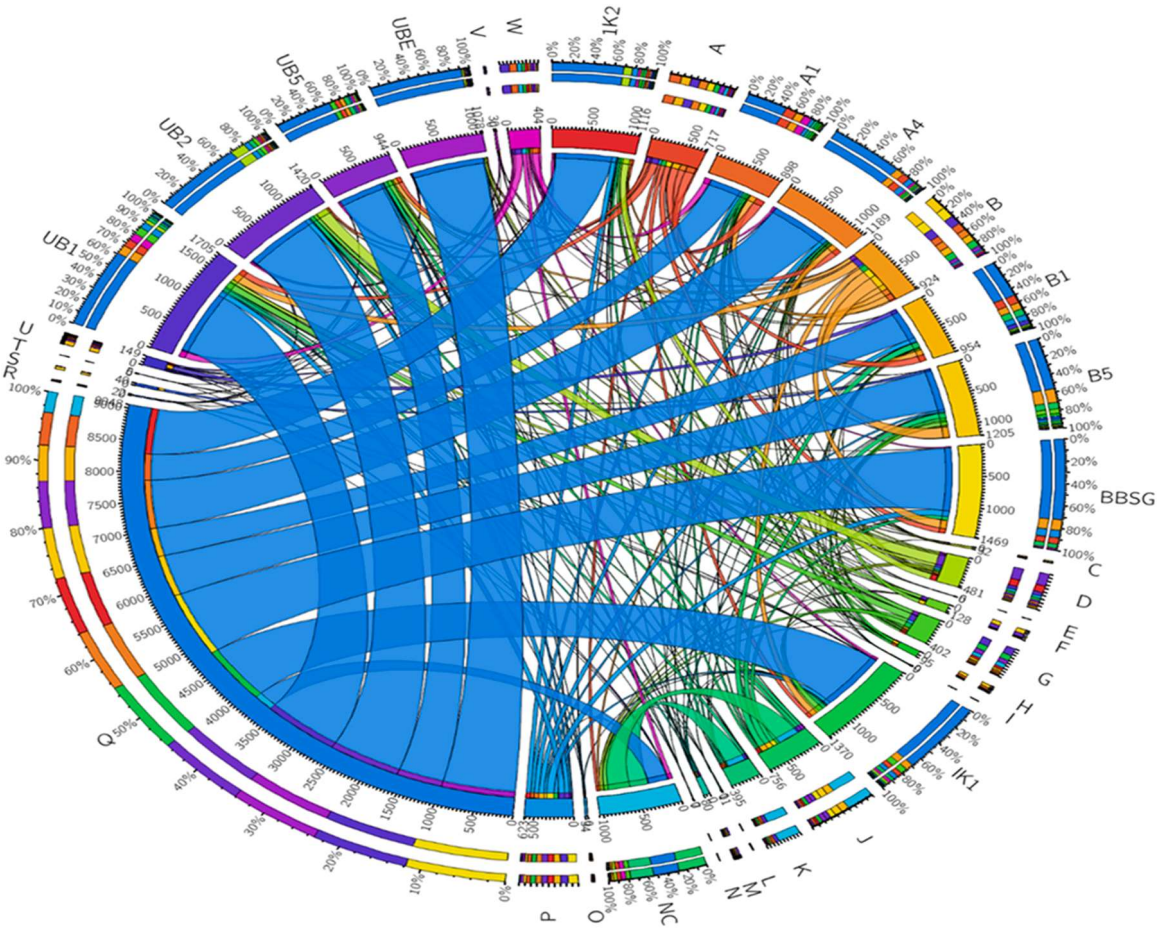

Fig. 2. Genomic circus showing the total metagenome of the research area.

bolster the proficiency of oil-degrading microorganisms [16]. In conjunction with a readily utilizable source of carbon, microorganisms necessitate nutrients like nitrogen, phosphorus, and potassium (NPK) to fuel cellular metabolism and facilitate robust growth [20]. In contaminated sites where organic carbon content typically remains elevated, the available nutrient pool can be rapidly depleted during microbial metabolism [21]. Consequently, it becomes imperative to supplement contaminated soil with essential nutrients to complement the carbon utilization by microorganisms effectively.

When comparing the limited degradation observed in C17 and C34 to that of other compounds, it appears that C17 and C34 consist of slowly digestible recalcitrant materials that are resistant to enzymatic breakdown by the microbiota [22]. In biosolid-amended utisol, C17 and C34 experienced more pronounced degradation compared to BSG amended. This heightened degradation can be attributed to the superior organic support and mass provided by biosolids. Biosolids, which are derived from human waste and composted alongside organic materials like woodchips, have gained widespread use in rangeland soil management. Various studies have demonstrated their effectiveness in addressing soil health concerns and combating land degradation. One such study conducted by Ploughe et al. in 2021 highlighted the utility of biosolids in soil management practices [23]. Additionally, a comprehensive meta-analysis by Gravuer et al. in 2019 investigated the ecosystem benefits and consequences of employing organic amendments in rangeland environments. This analysis revealed that biosolids have the potential to exert positive impacts by increasing plant biomass, enhancing soil organic matter (SOM) and soil organic carbon (SOC) levels, and neutralizing soil pH [24].

The total metagenome of the research area is presented in Fig. 2. It was observed that although 3.23 % of organisms were unclassified, the majority of the OTUs (95.77 %) identified from soil amended with biosolid (A3) belonged to Proteobacteria (51.44 %), Acidobacteria (14.42 %), Actinobacteria (10.38 %) (Fig. 3). At the order level, Rhizobiales (10.0 %), Acidobacteriales (7.7 %), Actinomycetales (6.5 %), Rhodospirillales (6.3 %), Burkholderiales (5.9 %), Xanthomonadales (4.6 %) and Bacillales (4.2 %) were the dominant OTUs (Fig. 4). While beds amended with BSG (B3) had 4.70 % of unclassified organisms, the majority of the OTUs (93 %) identified belonged Proteobacteria (48.61 %), Actinobacteria (12.37 %) and Planctomycetes (7.57 %) (Fig. 3). At the order level, Rhizobiales (10.8 %), Burkholderiales (7.7 %), Actinomycetales (5.9 %), Xanthomonadales (5.9 %), Rhodospirillales (3.6 %), Solirubrobacterales (3.5 %) and Bacillales (3.3 %) were the dominant OTUs (Fig. 4). For the soil left to natural attenuation (AB4), only 2.44 % of organisms were unclassified, while the majority of the OTUs (96.5 %) identified belonged to Proteobacteria (67.99 %), Actinobacteria (7.39 %) and Acidobacteria (7.23 %). At the order level, Burkholderiales (9.3 %), Chromatiales (8.1 %), Rhizobiales (7.7 %), Myxococcales (5.8 %), Rhodospirillales (5.4 %) and Xanthomonadales (5.0 %) were the dominant OTUs. While the pristine garden soil (AB5) had majority of the OTUs comprising mostly of Proteobacteria (42.2 %), Actinobacteria (16.97 %) and Acidobacteria (10.66 %). At the order level, Burkholderiales (9.3 %), Rhizobiales (7.0 %), Actinomycetales (6.4 %) and Solirubrobacterales (5.0 %) were the dominant OTUs (Fig. 4).

Studies on the genome sequence dataset of *B. altitudinis* have shown that the bacterium has hydrocarbon degrading capabilities which can potentially be useful in the remediation of hydrocarbon contaminated sites [12]. Six enzymes (including three alcohol dehydrogenase, one 4-carboxy mucono-lactone decarboxylase, one 4-oxocrotomate tautomerase, and one catechol 2,3-doxigenase) responsible for aromatic hydrocarbons degradation are predictably associated with the bacterium [12]. The implication is that the bio-augmenting agent could successively drive crude oil catabolism in soil. The M5nr analysis of the sequences revealed the elaboration of eleven (11) genes known to be involved in hydrocarbon degradation. Six genes/enzymes including AlmA, AlkM, *alkB*, PrM, cP450, MMO/AMO subunits A, B and C were involved in degradation of aliphatic hydrocarbon, while five genes namely; *ssuD, ssuC, dszC, dszB* were involved in the desulfurization of hydrocarbon. More microbial orders (17) expressed genes/enzymes in soil left to natural attenuation (AB4) compared to 15 in soils amended with both biosolid (A3) and BSG (B3) (Table 5). In biosolid amended soils, *ssuD* and *ssuC* (10) were the most expressed genes by microorganisms, while *ssuD* (9), AlkM and *ssuC* (8) were the most expressed in BSG amended soils. Same trend was observed for soil (AB4) left to natural attenuation, although the expression of other genes/enzyme such as AlmA and Mt/AmM were more pronounced compared to other treatment types. It was also observed that expression of AlkM

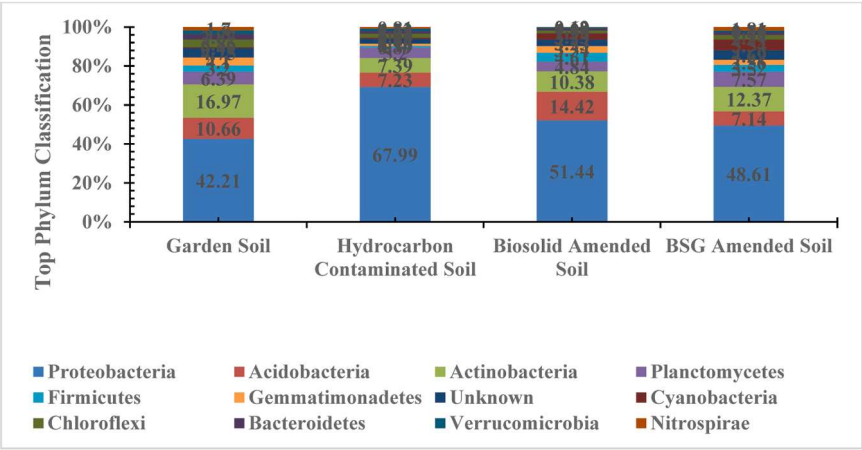

Fig. 3. Microbial diversity top phylum classification in the remediated soil.

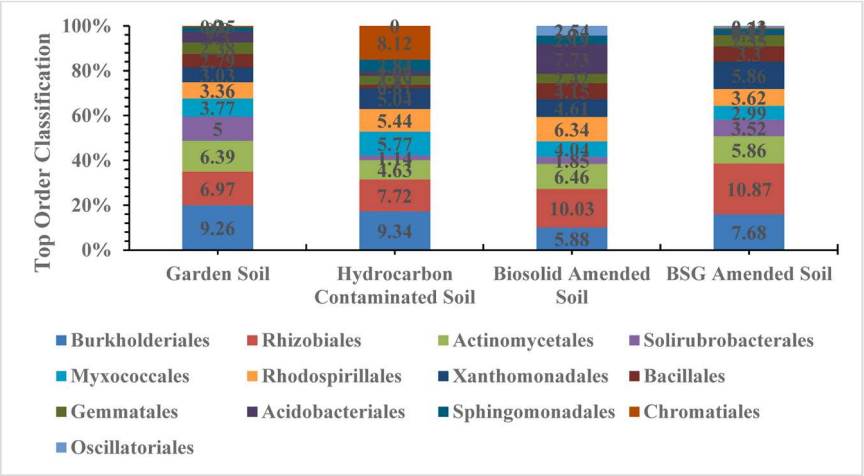

Fig. 4. Microbial diversity top order classification in the remediated soil.

Table 5
Distribution and occurrence of gene expression by microbial group encountered in research area.

S/N	Microbial Group	AB4	A3	B3	No. of Genes Expressed
1	Burkholderiales	+	+	+	9
2	Rhizobiales	+	+	+	6
3	Actinomycetales	+	+	+	10
4	Rhodospirillales	+	+	+	6
5	Sphingomonadales	+	–	+	2
6	Bacillales	+	+	+	4
7	Pseudomonadales	+	+	–	6
8	Oscillatoriales	+	+	+	2
9	Caulobacterales	+	+	+	2
10	Myxococcales	+	+	+	2
11	Solirubrobacterales	+	+	+	3
12	Rhodobacteriales	+	+	+	6
13	Clostridiales	+	+	–	1
14	Alteromonadales	+	–	–	2
15	Oceanospirillales	+	–	–	2
16	Sphingobacterales	+	+	–	6
17	Chromotiales	–	–	+	1
18	Nostocales	–	+	+	2
19	Bifidobacterales	–	+	–	1
20	Nitrosomonadales	–	–	+	1
21	Enterobacterales	–	+	–	3
22	Legionellales	–	–	+	1
23	Flavobacterales	+	–	–	1
	Total	**17**	**16**	**15**	

and AlmA were more pronounced in soil left to natural attenuation while the expression of *dszB* was suppressed compared to amended soils where levels were higher. Microorganisms in the order Actinomycetales (11), Burkholderiales (9) and Rhizobiales (7) expressed most of the genes/enzymes encountered in all treatment types (Table 5).

The Principal Component Analysis (PCA) presented in Fig. 8 provides a graphical representation of the relationships between different treatments and their associated gene expressions. In this case, two principal components of gene expression explained 100 % of the total variation (71.3 % and 28.7 % respectively), illustrating that the genes expressed can fully account for the observed differences in the treatment responses. As per the analysis, genes highly correlated with the first Principal Component (PC1) were AlmA, PrM, Cp450, *ssuD, DszC, DszB*, and *DszA*, implying these genes were the primary contributors to the variance in the treatment responses in PC1. On the other hand, *ssuD* and *ssuC* were major contributors to the second Principal Component (PC2). The PCA plot visualizes a clear separation between the different treatments, A3 (Biosolid amended beds), B3 (BSG-amended beds), and AB4 (soil left to naturally attenuate). This separation indicates distinct gene expression profiles associated with each treatment. Biosolid amended beds (A3) were characterized by the expression of PrM and Cp450, genes involved in aliphatic hydrocarbon degradation, and *ssuD*. BSG-amended beds (B3), in contrast, were marked by the expression of *DszA*-C, which are involved in hydrocarbon desulfurization, along with AlmA and AlkM. Lastly, the naturally attenuated soil (AB4) was chiefly characterized by the expression of Mt/AmM, alkane hydroxylating enzymes, and *ssuC*. These findings imply that the type of treatment applied to contaminated soil can significantly influence the types of

genes expressed by the microbial community, ultimately affecting the soil's capacity to degrade and remediate hydrocarbons. Understanding these relationships can be instrumental in optimizing soil bioremediation strategies.

The utilization of the Kyoto Encyclopedia of Genes and Genomes (KEGG) for data analysis unveiled the extensive metagenomic capacity within both hydrocarbon-contaminated and amended soils. This metagenomic repertoire appeared sufficiently expansive to accommodate the majority of well-known metabolic pathways essential for microbial growth and activity. Many of these genes and metabolic pathways primarily contribute to routine microbial functions, including the metabolism of macromolecules and other essential cellular processes.

Genes play a pivotal role in the adaptation, survival, and proliferation of microorganisms, particularly in environments characterized by elevated concentrations of petroleum hydrocarbons. These genetic processes not only play a critical role in the subsequent breakdown of hydrocarbon compounds but are also directly linked to the efficiency and duration of their complete degradation. As elucidated by Abbasian et al. [12], soluble CYP153 cytochrome P450 monooxygenases assume responsibility for the mono-oxidation of alkanes possessing carbon chains ranging from C5 to C12, while flavin-binding monooxygenase (AlmA) is accountable for the oxidation of long-chain hydrocarbons spanning from C20 to C36. These genes are expressed by a diverse array of phylogenetic groups, including Burkholderiales, Myxococcales, Rhodospirillales, Actinomycetales, Pseudomonadales, and Rhizobiales.

It is however important to mention that Oceanospirillales, Flavobacterales and Alteromonadales were only found in soil left to natural attenuation (AB4). These organisms are often referred to as the primary aliphatic hydrocarbon degraders and they are very important in the initial degradation of petroleum hydrocarbon [25,26]. While Legionellales and Nitrosomonadales could only thrive in beds amended with BSG. These organisms are known to be very fastidious. Legionellales in particular, requires large input of protein (L-cysteine) to thrive, while Nitrosomonadales are often found in areas with high levels of nitrogenous compounds where they use electron obtained from ammonia oxidation to produce energy. They are thus considered to very useful in bioremediation. Species of Nitrosomonas has been identified as being able to degrade a variety of halogenated compounds including benzene and trichloroethylene [27]. The DBT (dibenzothiophene) degrading enzyme system typically assumes an operon configuration comprising three structural genes, namely *dszC, dszA*, and *dszB*, where *dszC* codes for 2,2-hydroxybiphenyl benzensulfunate desulfinase. This enzyme system is instrumental in the conversion of poly-aromatic sulfur heterocyclic compounds into 2-hydroxybiphenyl (2-HBP) [12,28]. Notably, this study revealed a notable abundance of these genes (as depicted in Figs. 5, 6, 7 and 8), consistent with prior research findings documented by Offiong et al. [4], Yergeau et al. [29], and Liang et al. [30].

In our study, we identified a set of genes/enzymes within the treated soil that are known to be associated with various stages of hydrocarbon degradation in microorganisms. While our investigation did not directly elucidate the specific mechanism of hydrocarbon degradation in this context, the presence of these genes suggests the potential for hydrocarbon degradation by the microbial community inhabiting the soil investigated.

Among the identified genes, we observed the presence of enzymes responsible for key stages of hydrocarbon degradation (Fig. 9). For instance, the presence of Bacterial Flavin-bounded monooxygenase (AlmA) indicates a possible role in the initial activation of alkanes for uptake and metabolism. Similarly, the identification of Alkane monooxygenase (AlkM) and Alpha-ketoglutarate dependent dioxygenase (*alkB*) suggests the potential for initial hydrocarbon breakdown and partial oxidation [31–33].

Furthermore, the presence of Propane monooxygenase (PrM) implies that the microbial community may be capable of complete mineralization of hydrocarbons, including propane. The detection of Cytochrome P450 (cP450) enzymes hints at the possibility of aromatic compound degradation within the environment.

To meet their energy requirements, microorganisms often utilize enzymes like Methane monooxygenase/ammonia monooxygenase (MMO/AMO) subunits A, B, and C for the oxidation of methane and ammonia. The presence of these subunits suggests that the microbial community may harness hydrocarbon degradation as an energy source.

Additionally, we observed genes associated with the degradation of sulfur-containing compounds, *ssuD, ssuC, dszC, dszA*, and *dszB* (Fig. 9). This finding implies the potential for the microbial community to degrade sulfur-containing hydrocarbons [32,34].

Conclusion

In the quest to remediate hydrocarbon-contaminated soils, this study sheds light on the substantial potential of amendments, specifically biosolid and BSG. These amendments, with their unique compositions of nitrogen, sulphate, phosphorus, and ammonium, significantly contributed to the reduction of Total Petroleum Hydrocarbons (TPH) with biosolid proving to be more efficacious than BSG. However, both treatments performed commendably when juxtaposed with the naturally attenuated soil. Through metagenomic analysis, we unveiled a vibrant microbial community with a prevalence of Operational Taxonomic Units (OTUs) such as Rhizobiales, Acidobacteriales, Actinomycetales among others. These findings suggest a strong correlation between microbial diversity and their effectiveness in bioremediation. Although a wider array of hydrocarbon degradation genes was expressed in the naturally attenuated soil, it did not lead to superior TPH reduction. This observation underscores the complexity of microbial interactions and their role in soil remediation. Furthermore, the study recorded distinctive gene expression patterns across different treatment types, a phenomenon that begs further investigation. Actinomycetales, Burkholderiales, and Rhizobiales emerged as central figures in the expression of these pivotal genes across all treatments. The characterization of enzyme-induced diminution of hydrocarbon has provided insight and indices for the elucidation of the molecular processes that plausibly may occur in microbes-mediated catabolism of hydrocarbon in tropical soils. These genes also show promise as bio-indicators of hydrocarbon availability in soil. Summarily, this study demonstrates that amendments like biosolid and BSG can enhance the bioremediation process of hydrocarbon-contaminated soils. However, it's the intricate interplay of microbial community and gene expressions that could hold the key to optimizing this process.

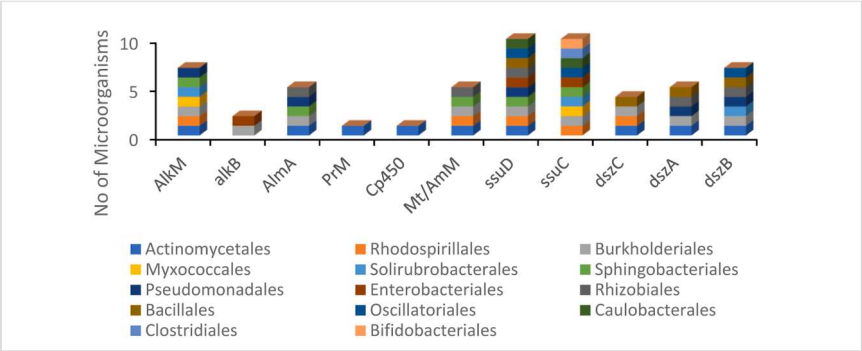

Fig. 5. Genes and Microorganisms associated with hydrocarbon degradation in an ultisol contaminated with hydrocarbon and remediated with Biosolid.

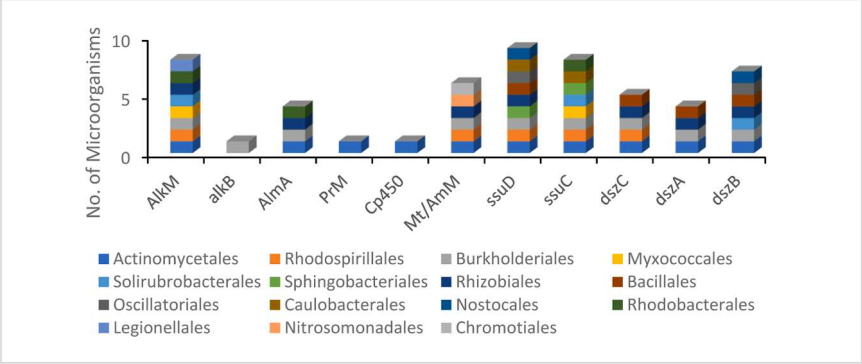

Fig. 6. Genes and Microorganisms associated with hydrocarbon degradation in an ultisol contaminated with hydrocarbon and remediated with BSG.

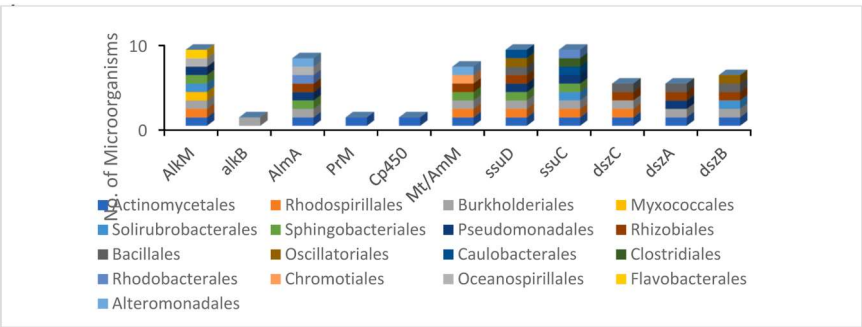

Fig. 7. Genes and Microorganisms associated with hydrocarbon degradation in an ultisol contaminated with hydrocarbon and left to be naturally attenuated.

CRediT authorship contribution statement

Opeyemi K. Fatunla: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Data curation, Funding acquisition, Writing – original draft, Writing – review & editing. **Anthony A. Adegoke:** Investigation, Validation, Formal analysis, Data curation, Funding acquisition, Writing – review & editing. **Nnanake-Abasi O. Offiong:** Methodology, Writing – review & editing. **Utibe A. Ofon:** Writing – review & editing. **Solomon E. Shaibu:** Methodology, Writing – review & editing. **Edu J. Inam:** Validation, Writing – review & editing. **P. Reddy:** . **Joseph P. Essien:** Supervision, Conceptualization, Validation, Writing – review & editing.

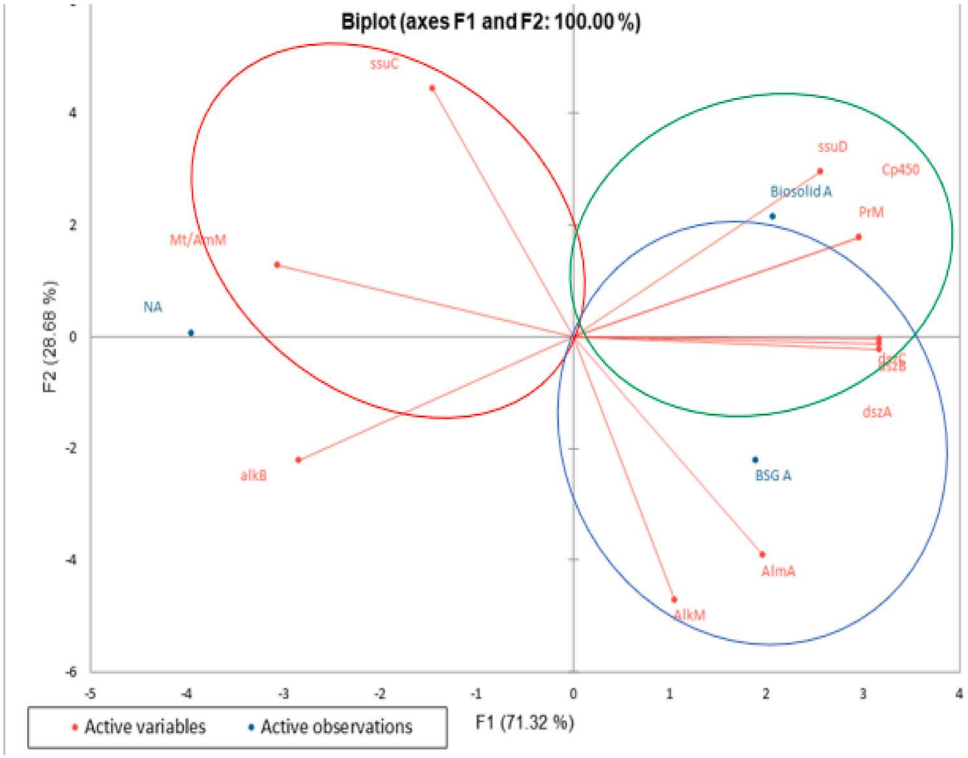

Fig. 8. PCA between the treatment types (A3, B3 and AB4).

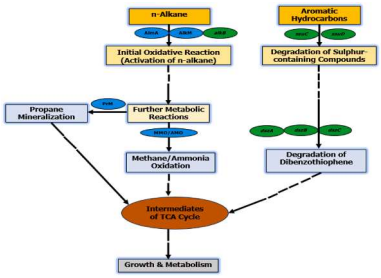

Fig. 9. Schematic illustration of the probable hydrocarbon degradation pathway(s) in the amended soil depicting the involvement of genes/enzymes.
Key: The enzymes; Bacterial Flavin-bound Monooxygenase (AlmA), Alkane Monooxygenase (AlkM),), Propane Monooxygenase (PrM), Methane Monooxygenase/Ammonia Monooxygenase (MMO/AMO) subunits A, B, and C are depicted in blue, while the genes; Alpha Ketoglutarate Dependent Dioxygenase (alkB) Alkane Sulfonate Monooxygenase (ssuD), Alkane Sulfonates Transport System Permease Protein (ssuC), Dibenzothiophene Monooxygenase (dszC), Dibenzothiophene-Sulfone Monooxygenase (dszA), and Dibenzothiophene-5,5-Dioxide Monooxygenase (dszB) are depicted in green.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analysed during this study are included in this published article.

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