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RESEARCH ARTICLE

PHYLOGENETIC AND TAXONOMIC ANALYSIS OF BACTERIAL COMMUNITIES IDENTIFIED FROM THE METHANE EMISSION SITES USING 16S rRNA METAGENOMIC SEQUENCING

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Abstract

Since methane is a much more potent greenhouse gas than CO_2, scientists are worried about methane emissions from various sources. The major fields for methane production are fuel production sites, crop production fields, and waste disposal sites due to the anaerobic decomposition of animal and vegetation wastes at these sites by bacterial communities. Considering this, we studied bacterial communities in the Oil-Natural Gas Field and Paddy Field, two major sites for methane emission. In this study, the Illumina MiSeq platform was used to identify the methanotrophs and other bacterial communities involved in methane and natural gas production, explicitly using their 16S amplicon (V3-V4) hypervariable regions. This study observed several methane-oxidizing and producing bacterial families at the Oil-Natural Gas Field and Paddy Field.

Introduction:-

The Oil-Natural Gas Field (ONGF)and a Paddy Field (PF) are actively utilised to fulfil human needs but are methane emitters. The soil physiology, microelements composition, and microbial diversity of the ONGF and PF may greatly vary due to geographical conditions and environmental stress. The ONGF sites are usually enriched with aromatic hydrocarbons and heavy metals where hydrocarbon-degrading, surfactant producers, and sulfate/nitrite reducers are usually found. The anaerobic and facultative microorganisms are also found due to low redox potential at the oil reservoir (Hidalgo et al. 2021). On the other hand, PF is flooded chiefly, which is required for rice cultivation, exposed to pesticides and fertilisers, and provides favourable breeding conditions for mosquitoes. These conditions result in the temporary aquatic microbial community, pesticide-degrading microorganisms, and human pathogens transmitted by a mosquito (Fernando 1993).

Several previous studies have reported the presence of such bacterial communities in ONGF and PF. The methanogenic bacteria convert crude oil into methane at an ONGF (Berdugo-Clavijo and Gieg 2014) and degrade organic matter into methane at a PF(Min et al. 1997). The rice field is the major source of atmospheric methane, with 15 to 20% (Singh et al., 2018),whereas ~15% of the methane is emitted from the activity of oil and gas fields (Christophe McGlade, K.C. Michaels, and Tim Gould 2020). The methylotrophic bacteria grow by utilising methane or methanol as the carbon source. This study has compiled the functions of bacterial species identified in ONGF and PF soil samples. The first sampling site (ONGF) was Borholla Gas Gathering Station (GGS), an Oil and Natural Gas Corporation Limited (ONGC). This place is an active site for producing crude oil located along the Brahmaputra valley in the Jorhat district of Assam, a North-Eastern state of India. Another sampling site is a private PF located in

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a small village (Bara) in the Banka district of Bihar, another North-Eastern state located approximately 1000 km from the first ONGF.

Metagenomic studies aided with Next-Generation Sequencing (NGS) made the exploration of microbiota from different biomass, hosts, and ecosystems in a culture-independent method. The metagenomic approach involves directly isolating DNA from complex environmental samples, such as soil, water, sewage, ocean, and salt lakes, to identify the actual microbial composition of that environment (Bevivino et al. 2014; Caporaso et al. 2010). The metagenomic NGS is based on amplifying specific DNA regions, such as the 16S amplicon (Carrino-Kyker et al., 2013). This study used the V3 and V4 hypervariable regions of the 16S rRNA gene to identify the bacterial communities at ONGF and PF. The amplified V3 and V4 hypervariable regions of the 16S rRNA gene sequences were clustered into Operational Taxonomic Units (OTUs).

The OTU richness and the community composition were observed to be much higher in PF compared to the ONGF. Here, we analysed the taxonomic composition of bacterial communities from the samples obtained from ONGF and PF gradually from phylum to species level taxa. The phylum *Proteobacteria* were dominant in both samples, whereas the second dominant phylum differed (*Bacteroidetes* in ONGF and *Firmicutes*in PF). Similarly, the bacterial classes, orders, families, genera, and species were analysed for both samples. The top five bacterial populations at each level of taxa from phylum to species have been listed (**Table 1**) and represented the hierarchical data of both samples at various levels, including bacterial phylum to bacterial family distribution, using the Krona plot. The 64 classified species in ONGF and 104 in PF were also discussed with their functions and roles in different habitats. The species richness was also studied for both samples using the Rarefaction curve and Shannon index, which was found to be higher in the PF sample.

At last, a comparative analysis was done to observe the relative abundance of bacterial communities in both samples. Conclusively, this study has identified the bacterial species located explicitly in ONGF and PF. There were many bacterial species found familiar in both locations, which indicates the adaptability of those species to different habitats irrespective of the changes in physiological and environmental conditions.

Materials And Methods:-

Soil sampling

The soil samples were collected fromONGF sites of Jorhat, Assam (26°45’25.5” N, 94°14’41.2” E)and PF soil samples of Bara, Bihar (24°57’46.4868” N, 86°37’34.4238” E). Both soil samples were collected in sterile blue-capped bottles and immediately stored at -20°C in the laboratory. Permits from INBIGS & ONGC were required to carry out the sample collection from ONGF sites, whereas no permit was required in the case of PF soil collection.

Extraction and sequencing of metagenomic DNA

The total metagenomic DNA was extracted from both soil samples. One gram of soil was taken in a spin tube provided in FastDNA SpinKit for soil DNA isolation (MP Biomedicals, USA), following the manufacturer’s instructions. DNA was extracted from the soil samples and quantified and qualified by determining the A260/280 ratio using NanoDrop (NanoDrop Spectrophotometer 2000, Thermo Fisher Scientific, USA).

Preparation of 16S rRNA library

Nextera XT Index Kit (Illumina inc.) was used to prepare the amplicon libraries following the 16S Metagenomic Sequencing Library preparation protocol (Part #15044223 Rev. B). The primers were designed and synthesized at Eurofins Genomics Lab to amplify the bacterial-specific region. The bacterial 16S region was amplified using 3 µl of PCR product and resolved on 1.2% agarose gel for ~60 min at 120V. The primers for amplifying the V3-V4 hypervariable region of the bacteria 16S rRNA gene were designed and synthesized. The Forward and Reverse sequences consisted of 5’-GCCTACGGGNGGCWGCAG-3’ and 5’-ACTACHVGGGTATCTAATCC-3’, respectively. As per the standard Illumina protocol, the QC-passed amplicons were amplified using i5 and i7 primers containing multiplexing index sequences and the respective standard adaptors (P5 and P7). A Qubit Fluorometer was used to quantify the amplicon libraries purified with AMPureXP beads.

Bioinformatics analysis of taxonomic and functional diversity in ONGF and PF

Next-generation sequencing is increasingly being used to profile complex microbial communities. Using the MiSeq system, researchers can go from sample to analysed data in as little as eight hours. The 16S (V4) region in most microbial species is 254 bp long, but some species vary by a few base pairs from this length. As a result, the 16S

(V3-V4) regions are the most studied regions in sequencing analysis (High-Speed, Multiplexed 16S Microbial Sequencing on the MiSeq® System n.d.).The metagenomic sequencing was performed on an Illumina MiSeq platform in a 2 X 300 paired-end mode using FLASH. The Quantitative Insights into Microbial Ecology (QIIME) pipeline, which combines multiple standard tools for community analysis, was used in this study to analyse microbial communities post-sequencing. Chimeras were filtered from the stitched data using FLASH (v1.2.11). The OTUs were selected based on sequence similarity within the reads, and the 16S sequences from each OTU were compared with the Greengenes database (version 13_8). By using a reference database, OTU was assigned to a taxonomic identity. Using UCLUST's algorithm, this sequence clustering is based on 97% sequence similarity.

Results And Discussion:-

Taxonomic composition analysis

The 16S rRNA-based NGS analysis of bacterial metagenomics obtained from the ONGF revealed 29 classifieds (1 unclassified) bacterial and two classified Archaeal phyla, which further classified into 77 classified and four unclassified classes, 114 classified and 30 unclassified orders, 136 classified and 91 unclassified families, 176 classified and 179 unclassified genera, 64 classified and 332 unclassified species.However, this study has focussed only on the role of classified species listed in **Table 2**. Similar to this, the analysis of the bacterial metagenomics data from the PF revealed 36 classifieds (1 unclassified) and three classified Archaeal phyla, which were further classified into 103 classified and 15 unclassified classes, 156 classified and 61 unclassified orders, 203 classified and 160 unclassified families, 253 classified and 309 unclassified genera, and 104 classified and 525 unclassified species. The classified species observed at PF and their environmental role are listed in **Table 3**. The top five taxa for ONGF and PF at each classification level are listed in **Table 1**. The sample obtained from the ONGF was observed with fewer phyla than from the PF. Additionally, the lower level of sub-clustering was observed even at the lower taxonomic levels in the ONGF sample than in the PF sample. It may be due to the restrictions for people other than the ONGC employees at the ONGF, leading to less anthropogenic activities than the PF, which is surrounded by and exposed to various human activities such as farming, littering, and faecal and urine excretion. Therefore, the PF sample has higher bacterial diversity and population than the PF.

Table 1:- Taxonomic hierarchy and relative abundance in different geographical locations.

Taxonomic abundance at the Phylum level (Top five against 31 classified in ONGF and 39 classifieds in PF)					
ONGF	*Proteobacteria* (50.45%)	*Bacteroidetes* (28.84%)	*Chloroflexi* (9.45%)	*Actinobacteria* (2.81%)	*Firmicutes* (2.03%)
PF	*Proteobacteria* (35.86%)	*Firmicutes* (28.54%)	*Bacteroidetes* (9.36%)	*Actinobacteria* (8.13%)	*Acidobacteria* (4.40%)
Taxonomic abundance at Class level (Top five against 77 classifieds in ONGF and 103 classifieds in PF)					
ONGF	*Gammaproteobacteria* (35.61%)	*Bacteroidia* (25.94%)	*Anaerolineae* (9.27%)	*Alphaproteobacteria* (7.03%)	*Betaproteobacteria* (6.83%)
PF	*Clostridia* (17.49%)	*Betaproteobacteria* (17.20%)	*Bacilli* (10.89%)	*Alphaproteobacteria* (8.00%)	*Gammaproteobacteria* (7.43%)
Taxonomic abundance at the Order level (Top five against 114 classifieds in ONGF and 156 classifieds in PF)					
ONGF	*Bacteroidales* (25.94%)	*Chromatiales* (17.39%)	*Xanthomonadales* (11.14%)	*Anaerolineales* (7.91%)	PYR10d3 (5.51%)
PF	*Clostridiales* (17.45%)	*Burkholderiales* (16.42%)	*Bacillales* (10.29%)	*Bacteroidales* (6.05%)	*Pseudomonadales* (5.41%)
Taxonomic abundance at the Family level (Top five against 136 classifieds in ONGF and 203 classifieds in PF)					
ONGF	*Porphyromonadaceae* (22.54%)	*Ectothiorhodospiraceae* (17.31%)	*Xanthomonadaceae* (10.61%)	*Anaerolinaceae* (7.91%)	*Oxalobacteraceae* (4.41%)
PF	*Oxalobacteraceae* (15.18%)	*Clostridiaceae* (11.33%)	*Bacillaceae* (8.22%)	*Moraxellaceae* (5.16%)	*Prevotellaceae* (4.06%)
Taxonomic abundance at Genus level (Top five against 176 classifieds in ONGF and 253 classifieds in PF)					
ONGF	*Pseudoxanthomonas* (6.65%)	SHD-14 (5.01%)	*Prevotella* (2.52%)	T78 (2.07%)	*Flavobacterium* (1.17%)
PF	*Bacillus* (8.18%)	*Clostridium* (7.02%)	*Acinetobacter* (5.09%)	*Prevotella* (4.06%)	*Tepidibacter* (2.49%)
Taxonomic abundance at Species-level (Top five against 64 classifieds in ONGF and 104 classifieds in PF)					
ONGF	*copri* (2.24%)	*succinicans* (0.90%)	*aminovorans*	*lividum* (0.40%)	*nitroreducens*

			(0.42%)		(0.35%)
PF	*copri* (3.67%)	*selenatarsenatis* (0.56%)	*lividum* (0.37%)	*prausnitzii* (0.36%)	*stercorea* (0.29%)

On the phylum level,the dominant phyla observed in both samples were *Proteobacteria*, accounting for 50.45% in the ONGF and 35.86% in the PF (**Supplementary file**, **Fig. S1** and **S2**). Followed by the *Proteobacteria*, other bacterial phyla observed in ONGF were *Bacteroidetes* (28.84%)(**Supplementary file,Fig. S3**), *Chloroflexi*(9.45%), *Actinobacteria* (2.81%), and *Firmicutes* (2.03%) (**Fig. 1a**). In contrast, the bacterial phyla followed by the *Proteobacteria* observed in the PF were *Firmicutes* (35.86%) (**Supplementary file, Fig. S4**), *Bacteroidetes*(9.36%), *Actinobacteria*(8.13%), and *Acidobacteria* (4.40%) (**Fig. 1b**). *Proteobacteria* is the dominant microbial phyla found on Earth (Bradley and Pollard 2017a; Spain, Krumholz, and Elshahed 2009) and has been found in diverse ecosystems such as terrestrial, marine, and deep ocean (Delmont et al. 2018; Hauptmann et al. 2014; Huber et al. 2007; Zehr, Carpenter, and Villareal 2000). It can also be found in the animal microbiome (Moon et al. 2018) and human microbiome (Bradley and Pollard 2017b; Flaugnatti et al. 2021). Therefore, the dominancy of *Proteobacteria* in both the sampling sites (ONGF and PF) is not astonishing. Similar to*Proteobacteria*, *Bacteroidetes* are also found in different biomes of Earth, such as soil, ocean, and freshwater, as well as in dairy products, animal microbiota and diseased animals (Thomas et al., 2011).As per the previous studies, *Firmicutes* are one of the dominant chitins-degrading bacteria in soil (Gooday 1990b, 1990a)and are also found in the rhizosphere and human microbiota (Browne et al. 2021; Mariat et al. 2009). The *Firmicutes* were found in a high proportion (35.86%) in the PF due to active farming of paddy crops, the presence of rhizosphere, constant exposure to humans and animals and their usual activities such as planting, pruning, spraying, and faecal excretion. Along with the bacterial kingdom, the ~2% *Archaebacteria*of total microbial compositionwere also found in the samples obtained from the ONGF (**Fig. 2a**). The Archaebacterial composition of the ONGF is further classified into two phyla (*Euryarchaeota* and *Crenarchaeota*), five classes (*Methanobacteria*, *Methanomicrobia*, *Thaumarchaeota*, MCG, and *Thermoplasmata*), nine orders (*Methanobacteriales*, *Methanosarcinales*,*Nitrososphaerales*, NRA6, *Methanosarcinales*, pGrfC26, *Methanomicrobiales*, E2, and *Methanocellales*), nine classified families (*Methanobacteriaceae*, *Methanosarcinaceae*, *Nitrososphaeraceae*, *Methanosaetaceae*, *Methanomassiliicoccaceae*, *Methanocellaceae*, and *Methanoregulaceae*), eight genera (*Methanobacterium*, *Methanosarcina*, *CandidatusNitrososphaera*, *Methanosaeta*, *Methanocella*, *Methanobrevibacter*, *Methanomassiliicoccus*, *CandidatusMethanoregula*), and four classified species (*beijingense*, *mazei*, SCA1145, and SCA1170).Likewise, in the ONGF, 2% Archaebacterial of total microbial composition was also found in the PF. However, one additional Archaebacterial phylum, i.e., *Parvarchaeota*, was also observed along with *Euryarchaeota* and *Crenarchaeota* (**Fig. 2b**). It was further classified into seven classes (*Methanobacteria*, *Thaumarchaeota*, MCG, *Methanomicrobia*, *Parvarchaea*, *Thermoplasmata*, and *Halobacteria*), eleven orders (*Methanobacteriales*, *Methanosarcinales*, *Nitrososphaerales*, pGrfC26, *Methanocellales*, *Methanomicrobiales*, *Nitrososphaerales*, YC-E6, YLA114, E2, *Halobacteriales*), ten families (*Methanobacteriaceae*, *Methanosarcinaceae*, *Nitrososphaeraceae*, *Methanosaetaceae*, *Methanocellaceae*, *Methanoregulaceae*, *Methanospirillaceae*, *Methanomassiliicoccaceae*, *Methanocorpusculaceae*, and *Halobacteriaceae*), thirteen genera (*Methanobacterium*, *Methanosarcina*, *CandidatusNitrososphaera*, *Methanosaeta*, *Methanocella*, *CandidatusMethanoregula*, *Methanospirillum*, *Methanobrevibacter*, *Methanomassiliicoccus*, *Methanocorpusculum*, *Methanolobus*, *Natronomonas*, *Halogranum*), and four classified species (*beijingense*, *bryantii*, *mazei*, and SCA1170).

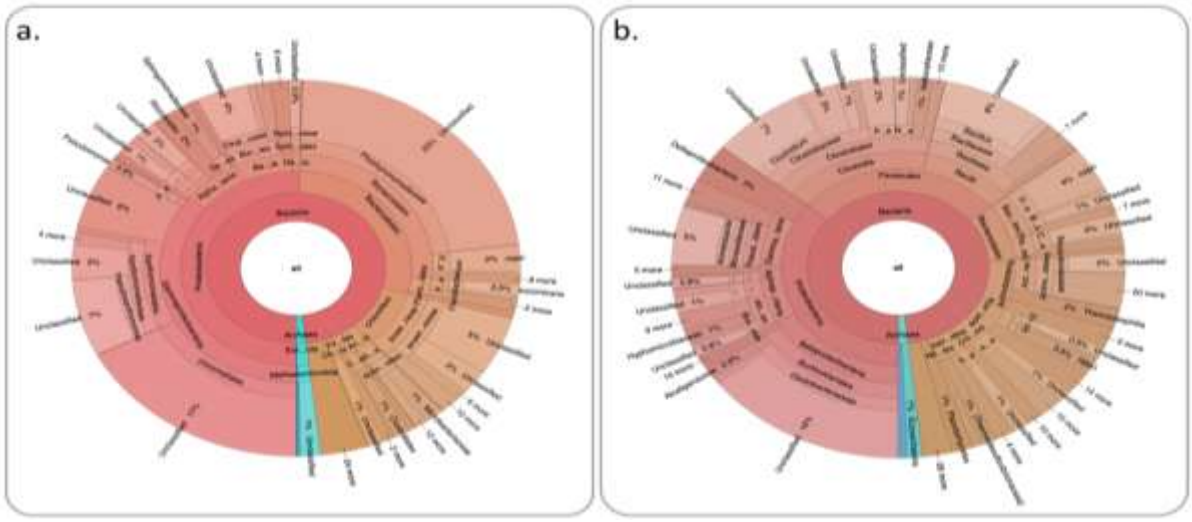

Figure 1:-The bacterial community in (a) ONGF and (b) PF samples was detected through the Metagenome MiSeq Illumina Next-Generation Sequencing platform displayed on the Krona website.

In this study, the Archaebacterial classes, *Methanobacteria*, *Methanomicrobia*, *Thaumarchaeota*, MCG, and *Thermoplasmata*, were common in both ONGF and PF. *Methanobacteria* and *Methanomicrobia* are the dominant methanogenic communities classified under Archaebacteria, known for methane production (Z. Yu et al. 2018). These methanogens are widespread and commonly found in soil, mud, sewage, sludge, and animal rumen (Shukla, Khan, and Rao 2021). *Thaumarchaeota* is a dominant archaebacterial class in most soil environments capable of ammonia (NH_3) oxidation. In low-NH_3 environments such as oligotrophic open ocean waters, acidic forest soils, geothermal habitats, and nutrient-poor soils, *Thaumarchaeota* are dominant among ammonia-oxidizing organisms due to their high affinity for the substrate (NH_3) (Holmes, Dang, and Smith 2019). Miscellaneous Crenarchaeotal Group (MCG) is an uncultivated and predominant archaeal group mainly found in anoxic environments. It has not yet been characterised for its specific functions but may have significant roles in biogeochemical cycles (Meng et al. 2014). *Thermoplasmata* an extensive and ecologically essential archaebacterial class, comes under the phylum *Euryarchaeota*(Hu et al. 2021) and have widespread habitats such as soil, marine habitats, and the intestinal tract of mammals and termites (Paul et al. 2012). Previous studies have reported that the *Thermoplasmata*have been found in healthy patients' oral cavities (Radaic and Kapila 2021) and bovine rumen as methylamine-degrading microorganisms(Poulsen et al. 2013).

In PF, two additional Archaebacterial classes, i.e., *Halobacteria* and *Parvarchaea*, were found. *Halobacteria* are facultative aerobic halophilic archaebacteria growing in salt-rich environments(Chang 2011). *Halobacteria* possess pigments such as halorhodopsin and bacteriorhodopsin in their membrane (Raven 2001)and have photosynthetic properties(Lake et al. 1985). Previous studies reported that *Parvarchaea* had been detected earlier from acid mine drainage and hot springs. Its putative role has been suggested in iron cycling and is known to interact physically with the phyla *Thermoplasmata*(Chen et al. 2018).

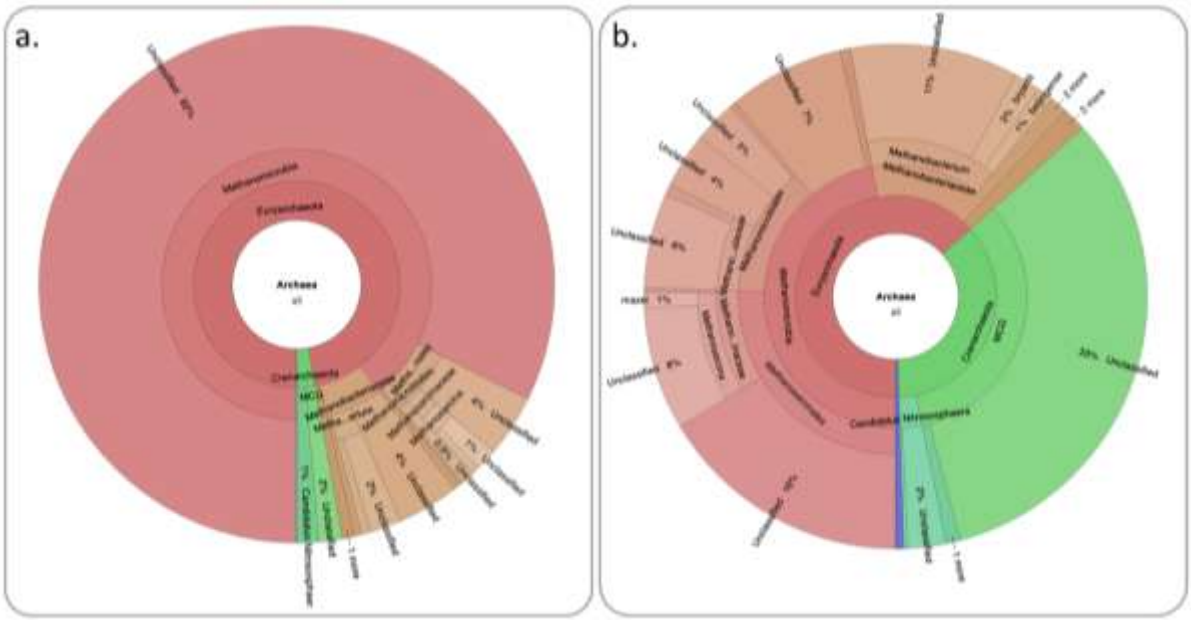

Figure 2:-Archaebacterial community present in (a) ONGF and (b) PF soil samples detected through Metagenome MiSeq Illumina platform of Next-Generation Sequencing displayed through Krona website.

Among the top five bacterial classes, *Gammaproteobacteria* were the most dominant, with an abundance of 35.61% of total bacterial composition in ONGFs (**Supplementary file**, **Fig. S5**), followed by the *Bacteroidia* (25.94%), *Anaerolineae* (9.27%), *Alphaproteobacteria* (7.03%), and *Betaproteobacteria* (6.83%). On the other hand, *Clostridia* were the most dominant class with an abundance of 17.49% of total bacterial composition observed in the ONGF (**Supplementary file**, **Fig. S6**), followed by the *Betaproteobacteria*(17.20%), *Bacilli*(10.89%), *Alphaproteobacteria*(8.00%), and *Gammaproteobacteria*(7.43%). The bacterial population categorised under *Proteobacteria* and *Firmicutes* have been reported to be involved in the metabolism of methane, nitrate, and sulphate (Haldar and Nazareth 2018). In this study, most bacterial classes obtained from the OF belonged to *Proteobacteria*, whereas from PF belonged to *Proteobacteria* and *Firmicutes*.

Among the top five bacterial orders, *Bacteroidales*were observed as the most dominant, with an abundance of 25.94% of total bacterial composition in the ONGF (**Supplementary file**, **Fig. S7**), followed by the *Chromatiales*(17.03%), *Xanthomonadales*(11.14%), *Anaerolineales*(7.91%), and PYR10d3 (5.51%).*Bacteroidales*are gram-negative bacteria primarily found in the human intestine and have also been reported to prevent the intestinal inflammatory disease induced by the *Helicobacter hepaticus*(H. Tan et al. 2018a). Sulfide oxidation to intermediate sulfur is rare but observed during the oil/water separation at crude oil processing plants, which may cause corrosion (Basafa and Hawboldt 2019). *Chromatiales* belong to the phylum *Gammaproteobacteria* which oxidises the sulfide and carbon and is phylogenetically close to the *Halothibacillus*, which is a carbon-fixing, non-photosynthetic and sulfide-oxidizing bacterium (Lavy et al. 2018). *Xanthomonadalesare* diverse in their physiological characteristics and therefore found in habitats ranging from adverse conditions such as hot springs to contaminated soil as non-pathogenic species. They may also have pathogenic nature against plants and humans (Bayer-Santos et al. 2019). *Anaerolineales* have been reported to possess the CO_2 fixing metabolic potential via the Wood-Ljungdahl pathway (X. Shi et al. 2021). PYR10d3, yet not got genus or species tag, was previously found in a bacterial community originating from oil-contaminated coastal sediment (Païssé et al. 2010)

In PF, *Clostridiales* were observed as the most dominant order with an abundance of 17.45% of total bacterial composition (**Supplementary file,Fig. S8**), followed by the *Burkholderiales* (16.42%),*Bacillales*(10.29%), *Bacteroidales* (6.05%),and *Pseudomonadales*(5.41%). *Clostridiales* include a broad range of gram-positive and obligate anaerobic bacteria found ubiquitously in decaying organic matter, soil, and water. Some bacterial species of *Clostridiales* also produce neurotoxins and cause neurological disorders in animals and humans, such as botulism and tetanus (Zeiller et al. 2015). The *Burkholderiales* have been reported to be found in the mycorrhizal and non-mycorrhizal roots of the plant Medicago truncatula(Offre et al. 2008)and can infect patients having chronic lung disease and admitted in intensive care units (Voronina et al. 2015a). The bacterial families such as *Bacillaceae*,

Planococcaceae, *Paenibacillaceae*, *Staphylococcaceae*, and *Thermoactinomycetaceae* were found in this study from the PF under order *Bacillalesare* diverse and found in different habitats such as saline soil, hot springs, hydrothermal vents, salt lakes (Mandic-Mulec, Stefanic, and van Elsas 2015a), marine sediments, compost, sputa (Carrillo and Benítez-Ahrendts 2014), and some are hardiest non-spore-forming bacteria which can survive non-physiologic conditions (Toltzis 2018). *Bacteriodales* are found mainly in the human microbiota, such as the mouth, upper respiratory and gastrointestinal tract, female gentile tract, and intestine. They have also been reported to form syntropic interactions and carry unique and strong carbohydrate-utilizing abilities (H. Tan et al. 2018b), (Zitomersky et al. 2013), (Kumari and Kokkiligadda 2021). The order *Pseudomonadales* are found in plant microbiota, human infections, and associated with the remediation of contaminated soil (Liao et al. 2020).

Among the top five bacterial families, *Porphyromonadaceae* were found to be the most dominant, with an abundance of 22.54% of total bacterial composition in the ONGF (**Supplementary file, Fig. S9**), followed by the *Ectothiorhodospiraceae* (17.31%), *Xanthomonadaceae* (10.61%), *Anaerolinaceae* (7.91%), *Oxalobacteraceae* (4.41%). Most species under the family *Porphyromonadaceae* are found in the human and animal microbiota, especially in the gastrointestinal tract and oral cavity. Some are reported to cause infections in humans and animals (Sakamoto 2014). *Ectothiorhodospiraceae* includes the alkaliphilic and halophilic purple sulfur bacteria closely related to *Chromatiaceae* involved in carbon fixation by utilising sulfide as an electron source (Canniffe and Hitchcock 2021). *Xanthomonadaceae* includes pathogenic and non-pathogenic species of *Gammaproteobacteria* that infects plants and humans(Assis et al. 2017). The hydrocarbons are found in oil reservoirs in high proportion, and methanogenesis is required to degrade the hydrocarbon into methane (Jones et al. 2008). This study found that the abundance of *Anaerolinaceae* was 7.91% of the total microbial composition in the sample obtained from the ONGF. *Anaerolinaceae* forms syntrophic cooperation with *Methanosaeta* in an acetoclastic methanogenesis pathway, where the acetate released by the *Anaerolinaceae* is utilised by *Methanosaeta*(B. Liang et al., 2015, McIlroy et al., 2017). *Anaerolinaceae* also have aromatic hydrocarbon-degrading properties (Owusu-Agyeman et al. 2019). In PF, *Oxalobacteraceae* were found to be the most dominant family with an abundance of 15.18% (**Supplementary file, Fig. S10**), followed by the *Clostridiaceae* (11.33%), *Bacillaceae* (8.22%), *Moraxellaceae* (5.16%), and *Prevotellaceae* (4.06%). *Oxalobacteraceae* have been reported to be a major rhizosphere and root-colonizing bacterial family for many plants species (Ofek, Hadar, and Minz 2012), as well as also promote plant growth and improve nitrogen acquisition(P. Yu et al. 2021). Recently a novel cellulose-degrading bacterium belonging to the family *Oxalobacteraceae*has been isolated from the rice (paddy) rhizosphere (Du et al. 2021). *Clostridiaceae* have been reported with ubiquitous distribution in diverse environments such as soil, water, and the gastrointestinal tract of humans and mammals (Bauer and Kuijper 2017). *Bacillaceae*, due to its resistant endospore-forming properties, is the most robust bacteria on the Earth, involved in the cycling of organic matter, stimulating plant growth and health by suppressing plant pathogens (Mandic-Mulec, Stefanic, and van Elsas 2015b). *Moraxellaceae* is composed of a heterogeneous group of bacteria in different environments, such as soil and water, food, and human and animal skin. Few of them have been reported to have pathogenic nature (Teixeira and Merquior 2014).

Among the top five bacterial genera, *Pseudoxanthomonas* were found to be the most dominant, with an abundance of 6.65% of the total bacterial composition, followed by the SHD-14 (5.01%), *Prevotella* (2.52%), T78 (2.07%), and *Flavobacterium* (1.17%). The primary source of hydrocarbons is crude oil, but it is also found in coal, natural gas and petroleum (Sparkman, Penton, and Kitson 2011). In this study, *Pseudoxanthomonas*, a dominant bacterial genus found in the sample obtained from ONGF, have been reported to have hydrocarbon-degrading and nitrite and nitrate reduction capabilities (Mohapatra et al. 2018). In PF, *Bacillus* was the most dominant genus with an abundance of 8.18% of the total bacterial composition, followed by *Clostridium* (7.02%), *Acinetobacter* (5.09%), *Prevotella* (4.06%), *Tepidibacter* (2.49%). It has been reported that bacteria from the *Bacillus* genera can be used in place of chemical fertilizers and pesticides, promote plant growth, and prevent against pathogens by secreting several metabolites. *Bacillus* can withstand unfavourable environmental conditions (Radhakrishnan, Hashem, and Abd_Allah 2017). This study found *Bacillus* to be the dominant genera in samples obtained from the PF. Another dominant bacterial genus found in the PF was *Clostridium*. *Clostridium* can be pathogenic and non-pathogenic, form spores, survive under adverse conditions for long periods, and be found in habitats such as soil and animal and human intestines(Walker 1990). *Acinetobacter* is a ubiquitous bacterium and can be found in various sources such as soil, sewage, water, and food (Poduch and Kotra 2007). The presence of cattle around the crop field is ubiquitous, and the bacteria belonging to *Prevotella* in this study were found in the PF sample. They were earlier reported to be found in the rumen of cattle and sheep (Flint and Stewart 1999).

On the species level, 64 classified and 332 unclassified species were obtained from the ONGF, whereas 104 classified and 525 unclassified species were obtained from the PF. Among all 64 classified species obtained from the ONGF, 29 (most) belonged to the phylum *Proteobacteria*, 15 belonged to phylum *Firmicutes*, eight belonged to *Bacteroidetes*, seven belonged to *Actinobacteria*, two belonged to *Crenarchaeota*, two belonged to *Euryarchaeota*, and only one (least) belonged to the *Fusobacteria*. Most bacterial species in the ONGF were reported to possess regulatory roles in the biogeochemical cycles related to sulfur, nitrate, carbon, andhydrocarbons. Few bacterial species were reported to possess pathogenic nature against humans and animals, and some have other roles, such as antimicrobial testing, food preparation, and bioremediation. The features/roles of the bacterial species obtained from the ONGF have been listed in **Table 2**.

Table2:-List of classified bacterial species identified from the ONGF.

Phylum	Bacterial species	Role/Function	Reference
Actinobacteria	*Kocuriarhizophila*	• Found in the rhizosphere of narrow-leaf cattail (a herbaceous plant) • To develop the colour and flavour of fermented meats • Used as a control in antimicrobial susceptibility testing	(Takarada et al. 2008) (Q. Shi et al. 2021)
Bacteroidetes	*Flavobacterium succinicans*	• A commensal species with the potential to act as an opportunistic pathogen responsible for bacterial gill disease in fishes	(Good et al. 2015)
	Sphingobacterium faecium	• Opportunistic human pathogen • It consists of a high concentration of Sphingophospholipids	(Lambiase et al. 2009)
Firmicutes	*Doreaformicigenerans*	• Found in the human intestine • Positively associated with Obesity, waist circumference, and body mass index	(Companys et al. 2021)
Proteobacteria	*Acinetobacter lwoffii*	• Found in the human oropharynx, skin, perineum, and urinary tract mucosa • Related to comprised catheter-related bloodstream infections • Cause bacteraemia in immunocompromised patients	(Ku et al. 2000)
	Albidovuluminexpectatum	• A non-photosynthetic and Slightly Thermophilic Bacterium from a Marine Hot Spring • Closely related *Rhodovulum*, a photosynthetic genus	(Albuquerque et al. 2002)
	Brevundimonas diminuta	• Found in clinical specimens, including blood and urine • An etiological agent of nosocomial infections • Used as a test organism to validate reverse osmosis filtration devices for drinking water purification • Used to test the porosity of pharmaceutical-grade filters (0.2 mm) due to their tiny size • Potential bioremediatory strains of marine oil pollution, including diesel, n-alkanes, and polycyclic aromatic	(Ryan and Pembroke 2018a) (Lupande-Mwenebitu et al. 2021; Ryan and Pembroke 2018b)

		hydrocarbons	
	Desulfoglaeba alkanexedens ALDC	• Found in an oil field • Oxidize n-alkanes • A sulfate-reducing and alkane-degrading bacterial strain	(Davidova et al. 2006) (Davidova et al. 2021)
	Methylococcus capsulatus	• Found in soil, water, sewage, mud, and lake sediments • An obligate methanotroph used for single-cell protein production	(Indrelid et al. 2017) (Lieven et al. 2018)
	Petrobactersuccinatimandens	• Found in oil well • Moderately thermophilic • Nitrate-reducing bacterium	(Salinas et al. 2004)
	Photobacterium damselae	• Found in tropical and semitropical aquatic environments • Causing wound infections and haemorrhagicsepticaemia in marine animals • Opportunistic human pathogen	(Chart 2012) (Rivas et al. 2011)
	Prosthecomicrobium pneumaticum	• Ability to use methanol as a sole carbon source	(Yee et al. 2010)
	Pseudomonas nitroreducens	• Found in petroleum-contaminated soil • Synthesise polyhydroxy butyrate homopolymer from medium chain length fatty acids • Denitrifying bacteria • Apropensity for petroleum hydrocarbons and crude oil	(Onwosi and Odibo 2012) (Iyer, Iken, and Damania 2017; J. Yao et al. 1999)
	Pseudomonas fragi	• Found in spoiled meat • Psychrotrophic species responsible for meat spoilage stored aerobically at refrigeration temperatures	(Ercolini et al. 2007) (G. Wang et al. 2017)
	Pseudoxanthomonas kalamensis	• Found usually in soil contaminated with polycyclic aromatic hydrocarbons and polychlorinated biphenyls • Form yellow-pigmented colonies on heterotrophic media. • Reduces nitrite to nitrous oxide	(Harada, Campbell, and Li 2006)
	Stenotrophomonas maltophilia	• Found in aqueous habitats, including plant rhizospheres, animals, foods, and water sources • The organism is commonly found in respiratory tract infections • Causes nosocomial infections in clinical environments • People who are immunosuppressed, immunocompromised, or who have medical implants are prone to infection and death	(Brooke 2012) (Adegoke, Stenström, and Okoh 2017)
	Xanthobacter autotrophicus	• Found in several environmental samples • Stimulate growth and yields of rice,	(Manuel Sánchez-Yañez 2022)

		tomato, and lettuce at a reduced dose of nitrogen or phosphate fertilizer • Bioremediation of environmental pollution by chemicals	

Among all 104 classified species obtained from the PF, 39 (most) belonged to the phylum *Proteobacteria*, 32 belonged to phylum *Firmicutes*, 18 belonged to *Actinobacteria*, nine belonged to *Bacteroidetes*, three belonged to *Euryarchaeota*, and only one (least) belonged to the *Crenarchaeota*, *Chlamydiae*, and *Fusobacteria*, each. A variety of Bacterial species were obtained from the PF, as few were related to plant growth and diseases, few were related to human and animal infections, and few were related to methane/methanol oxidation.

Several other bacterial species were also identified with unique features. *Rhodococcusequi*, a model organism to study estrogen degradation from wastewater treatment plants. *Bacteroides*uniformis improves mice's lipid profile, metabolic disorders, and immunological dysfunctions. Clostridium *butyricum* is used as a probiotic for treating diarrhoea in humans, and *Clostridiumacetobutylicum* is used for biofuel production. Along with the above-mentioned bacterial species, several other species and their roles/features have been discussed in **Table 3**. Several other bacterial species were commonly observed in both the samples obtained from ONGF and PF (**Table 4**).

Table 3:-List of classified bacterial species and their biological/environmental roles found in the sample obtained from PF.

Phylum	Bacterial species	Biological/Environmental role	Reference
Actinobacteria	*Actinomaduravinacea*	• Soil-borne organisms • Associated with the decomposition of organic material	(Wells et al. 2018)
	Arthrobacter nitroguajacolicus	• Found in soils, the aerial surface of plants, and wastewater sediment • It is capable of degrading 4-nitroguaiacol (4-NG)	(Gobbetti and Rizzello 2014) (Kotoučková et al. 2004)
	Propionibacterium granulosum	• Found in the skin, gut, lymph nodes and lung tissues of healthy individuals • Cause infections in patients compromised by recent surgery, trauma, or implanted devices (e.g., prosthetic heart valves) and cerebrospinal fluid shunts) but are isolated more commonly in the clinical laboratory as culture contaminants	(SHARMA 2009a) (Buckingham 2009; SHARMA 2009b)
	Rhodococcus equi	• A model organism to study oestrogen degradation • Causes cavitary pneumonia and lung abscess, especially in immunocompromised hosts • A soil organism that is carried in the gut of many herbivores and widespread in animal dung, manures, soils of grazing fields, and	(Harthern-Flint et al. 2021) https://www.ncbi.nlm.nih.gov/books/NBK441978/

		other related farm environments	
	Rhodococcus fascians	• Found in water, soil, and marine habitats, including harsh ecological regions such as the Arctic, deserts, and heavily polluted areas • Disrupt hormone balances, and cause disease in plants • It produces neoplastic orhyperplastic diseases in plants	(Park et al. 2021) (Srivastava 2002; Vereecke et al. 2000)
	Streptomyces mirabilis	• Found in uranium mining sites, featuring high ambient concentrations of metal ions in the soils • Degrade cellulose, hemicelluloses or lignocellulose found in wood • It produces miramycin and nitroreductase	(Brangsch et al. 2022) (Bontemps et al. 2013; J. Yang et al. 2012)
	Streptomyces lanatus	• A respiratory allergen in humans • It causes streptomyces lanatus-mediated pneumonia in humans	(Sharma, Gautam, and Saxena 2014)
	Bacteroides uniformis	• Found in human faeces • Improve lipid profile, leptin levels, and TNF-α production in high-fat–fed mice on oral administration of *B. uniformis* • Ameliorate immunological dysfunctions and metabolic disorders related to intestinal dysbiosis in obese mice	(Renouf and Hendrich 2011) (Dahiya et al. 2019)
	Flavobacterium columnare	• Found in freshwater fish • The causative agent of columnaris disease • It helps pathogens resist antibiotic and disinfectant treatments by forming a biofilm	(Waśkiewicz and Irzykowska 2014) (Cai, de La Fuente, and Arias 2019)
	Estrella lausannensis	• Forms peculiar star-shaped elementary bodies (EBs) • It has a pathogenic role towards human or animal	(Kebbi-Beghdadi and Greub 2014; Lienard et al. 2011)

Bacteroidetes	*Methanobacterium bryantii*	• Found in sewage sludges • Obligate anaerobes methanogens that produce methane • Classified as acetate fermenters, obligate methylotrophs, and autotrophic hydrogen oxidizers	(Borja 2011) (Karadagli and Rittmann 2005)
	Ammoniphilusoxalaticus	• Found in the rhizosphere of sorrel (herbaceous plant) and decaying wood • Use oxalate as the sole organic source of carbon and energy for growth	(Zaitsev et al. 1998)
	Bacillus selenatarsenatis	• Involved in the bioremediation of environments contaminated with selenium and arsenic • It reduces selenate to selenite through anaerobic respiration and subsequently into elemental selenium	(Yamamura et al., 2007) (Kuroda et al. 2015)
Chlamydiae	*Bacillus firmus*	• Promote host plants growth, such as tomato, cotton, and Bermudagrass • It has nematicidal properties against a wide range of nematodes • *B. firmus* increases plant height, plant biomass, fruit number, and fruit weight	(Huang et al. 2021)
Euryarchaeota	*Bacillus foraminis*	• Found in non-saline-alkaline groundwater • Have relatively good resistance against potentially toxic elements (PTEs), which is necessary for efficient bioleaching	(Golzar-Ahmadi and Mousavi 2021; Tiago et al. 2006)
Firmicutes	*Brevibacillusreuszeri*	• Found in diverse environments, including rocks, dust, aquatic environments, and guts of various insects and animals • Gram-positive, spore-forming, and strictly aerobic bacterium • The only functional gene from *B. reuszeri* is L-methionine-Ncarbamoylase, which will be a potential biocatalyst for the production of L--	(Panda et al. 2014) (J. Wang et al. 2015)

		amino acids	
	Clostridium butyricum	• Found in environments including soil, cultured milk products, and vegetables • Used as a probiotic to treat and prevent diarrhoea and intestinal microflora disorder in human beings and to enhance the humoral immune response	(Stoeva et al. 2021) (Fu et al. 2021)
	Clostridium intestinale	• Found in the faeces of a cattle • Ferment sorbitol may help distinguish this organism from other aerotolerant clostridia • It can cause bacteremia	(Elsayed and Zhang 2005)
	Clostridium hungatei	• An obligate anaerobic and spore-forming bacterium, found in soil. • It ferments carbohydrates, such as cellulose or D-glucose • Nitrogen-fixing bacteria	(Poehlein et al. 2017)
	Clostridium neonatale	• Found in stools of preterm neonates • It has a potential role in the pathogenesis of necrotizingenterocolitis	(Cassir et al. 2021) (Hosny et al. 2019)
	Clostridium cellulovorans	• It has the potential to ferment all main plant polysaccharides, namely cellulose, hemicellulose and pectin's • Ability to produce n-butanol from lignocellulosic wastes, a process that would significantly reduce the cost of bio-butanol	(Costa et al. 2021)
	Clostridium acetobutylicum	• Used for industrial-scale production of the organic solvents acetone, n-butanol, and ethanol (ABE) through a process known as ABE fermentation • An attractive candidate for biofuel production	(Fierobe, Mingardon, and Chanal 2012) (Herman et al. 2017)

	Clostridium perfringens	• Found infresh meat and poultry products • It uses its arsenal of >16 toxins to cause histotoxic and intestinal infections in humans and animals • Cause gas gangrene in humans and several animal species • cause necrotizing enteritis and enterotoxaemia mainly in neonatal individuals of several animal species	(Labbé 2003) (Uzal et al. 2014)
	Desulfosporosinusmeridiei	• Found in gasoline-contaminated groundwater • Capable of reducing sulfate in the oxidized mine waste materials • Survive in a fluctuating environment such as a shallow, sandy aquifer due to the formation of endospores • Serves as a 'hydrogen scavenger' for fermenters or acetogens	(Mardanov et al. 2016) (Robertson et al. 2001)
	Doreaformicigenerans	• Found in human faeces • DfFAE belongs to type-A FAE and has a higher preference for hydrolyzing short-chain ester substrate (p-nitrophenyl acetate, pNPA)	(Taras et al. 2002) (Shen et al. 2022)
	Enterococcus haemoperoxidus	• Found in seawater • The strain can decompose hydrogen peroxide into oxygen and water when cultivated on blood-agar media	(Svec et al. 2001)
	Lysinibacillusboronitolerans	• A novel bacterial species closely related to the Bacillus fusiformis with 97.2% sequence similarity and *Bacillus sphaericus* with 96.9% sequence similarity.	(Ahmed et al. 2007)
	Paenibacilluschondroitinus	• Found in soil, fresh and salt water, sewage, sediments, caves, humus, compost, rhizosphere, food, plants, insect larvae and clinical samples • Closely related to the organism that shares the ability to hydrolyze various	(Sáez-Nieto et al. 2017) (SHIDA et al. 1997)

		β-glucans, curdlan, and alginate	
	Veillonelladispar	• Found in the microbiota of the mouth, gastrointestinal tract, and urogenital area • A gram-negative anaerobic bacteria cause disease in human	(Cobo et al. 2020)
	Acinetobacter johnsonii	• Found in environmental samples and animals • It can occasionally colonize human skin • It can cause clinical infections such as catheter-related bloodstream infections or peritonitis associated with the infections in patients with chronic lung disease and admitted to intensive care units.	(Montaña et al. 2016) (Voronina et al. 2015b)
	Azospirillum brasilense	• Found in the rhizosphere of various grass species • It can increase plant growth by fixing atmospheric Nitrogen non-symbiotically and producing plant growth substances such as plant hormones (auxins).	(Tien, Gaskins, and Hubbell 1979) (Miransari 2016)
	Cupriavidusgilardii	• Found in respiratory secretions of cystic fibrosis patients • An emerging pathogen in immunocompromised patients due to its innate antimicrobial resistance and its ability to acquire new resistances	(Kobayashi et al., 2016) (Karafin et al., 2010)
	Desulfovibrio mexicanus	• Found in sludge wastewater • An amino acid-degrading, sulfate-reducing bacterium	(Hernandez-Eugenio et al. 2000)
	Desulfovibrioputealis	• Found in the deep subsurface of water • Strictly anaerobic • Able to use sulfate, sulfite, and thiosulfate, with the production of sulfide	(Basso, Caumette, and Magot 2005)
Proteobacteria	*Ensiferadhaerens*	• Gram-negative soil bacteria that attach endwise to various living gram-positive and gram-negative bacteria	(Germida and Casida 1983)

		• It is a participant in a predatory chain involving other bacteria	
	Enterobacter cloacae	• Found in water, sewage, soil, and food • It can degrade or inactivate antibiotics to prevent by expressing detoxifying enzymes	(Davin-Regli and PagÃ¨s 2015)
	Erwinia dispersa	• Its virulence provides essential insights into the functions of this sRNA in biofilm control and systemic infection.	(Peng, Schachterle, and Sundin 2021)
	Massiliaaerilata	• Aerobic, Gram-negative, and rod-shaped bacteria • It degrades casein, hypoxanthine, tyrosine, and Tween 80.	(Weon et al. 2008)
	Methylomicrobium agile	• Found in sediment samples from wetlands • It shares 99.16% sequence similarity with the *Methylomicrobiumalbum* BG8, which is an obligate, gram-negative, *gammaproteobacterial* methanotroph • It uses methane or methanol as its sole carbon and energy source.	(Hamilton et al. 2015) (Villada et al. 2022)
	Methylosarcina lacus	• Obligate methanotrophic bacterial strains grow on methane • It possesses particulate methane monooxygenase (MMO) and assimilates formaldehyde via the ribulose monophosphate (RuMP) pathway	(Kalyuzhnaya et al. 2005)
	Methylosarcina quisquiliarum	• Found in a landfill site • Obligate methane-oxidizing bacteria • It utilizes only methane and methanol as carbon sources	(Wise, McArthur, and Shimkets 2001)
	Methyloteneramobilis	• Found in lake sediment • Obligate methylamine utilizing bacteria • It oxidizes the methylamine via methylamine dehydrogenase and assimilates formaldehyde via the RuMP pathway	(Kalyuzhnaya et al. 2006)
	Nannocystis exedens	It plays a significant role in the	(TAYLOR and DRAUGHON 2001)

		control of the population of many plant disease bacteria and fungi in aerated soils	
	Nevskiaramosa	• It is widely distributed epineustonic bacterium, which can specifically be deleted by its flat and hydrophobic rosettes on ammonia-free media	(Stürmeyer et al. 1998) (Pladdies, Babenzien, and Cypionka 2004)
	Paucimonaslemoignei	• It is unique among PHA-degrading bacteria because it can synthesize at least six different extracellular PHA depolymerases	(Handrick et al. 2001)
	Phaselicystis flava	• A novel arachidonic acid-containing soil myxobacterium.	(Garcia et al. 2009)
	Rubrivivax gelatinosus	• Found in freshwater ponds, sewage ditches, activated sludge, and food processing wastewater • It carries out anoxygenic photosynthesis using electrons derived from organic acids and energy from sunlight • It performs aerobic respiration using organic acids • Fix nitrogen into ammonium to support cell growth.	(Nagashima et al. 2012) (Wawrousek et al. 2014)
	Sulfuricurvumkujiense	• It is a facultatively anaerobic, chemolithoautotrophic sulfur-oxidizing epsilon proteobacterium • It can grow anaerobically using thiosulfate or sulfide as the electron donor and nitrate as the electron acceptor	(Kodama and Watanabe 2004) (Cron et al. 2019)
	Variovorax paradoxus	• Found in the human oral cavity • It plays an essential role in the natural cycling of biogenic chemicals • It is able also able to degrade a variety of contaminants, including pesticides and crude oil-associated S-metabolites	(Jamieson et al. 2009) (Han et al. 2011)

Table 4:- List of classified common bacterial species and their biological/environmental roles found in the samples obtained from ONGF and PF.

Phylum	Bacterialspecies	Biological/Environmentalrole	Reference
Actinobacteria	*Propionibacterium acnes*	• Found in the skin, oral cavity, large intestine, conjunctiva, and the external ear canal • It is an opportunistic pathogen • It plays a role in acne • It causes a range of postoperative and device-related infections • It includes infections of the bones and joints, mouth, eyes, and brain	(Perry and Lambert 2011)
	Bifidobacterium longum	• Found in the human intestine • It inhibits inflammation by regulating the balance of the immune system, improving the intestinal barrier function, and increasing acetate production	(Quigley 2017) (S. Yao et al. 2021)
	Bifidobacterium adolescentis	• Found in the healthy human and animal intestinal tract • It displays distinct anti-inflammatory effects • It is a producer of folate in the colon	(Bifidobacterium adolescentis n.d.) (Pompei et al. 2007)
	Collinsellaaerofaciens	• Found in the gastrointestinal tract of healthy humans • It can ferment a range of plant and animal-origin carbohydrates and for producing H_2, ethanol, short-chain fatty acids, and lactate in the human colon • It is the major utilizer of lactose in the human colon	(Bag, Ghosh, and Das 2017)
	Nocardioidesfurvisabuli	• Isolated from black sand from Samyang Beach on Jeju Island	(S. D. Lee 2007)
Bacteroidetes	*Bacteroides fragilis*	• Found in the oral cavity, intestinal tract, and female reproductive tract • It causes endogenous infection and can invade the human bloodstream and cause bacteremia or septicemia • It plays a role in alleviating disease conditions • Restoring systemic immune defects	(Y. Yang et al. 2021) (C. Wang et al. 2021)
	Bacteroides ovatus	• Found in the human colon • It is a symbiont with anti-inflammatory properties such as relieving LPS-induced inflammation, promoting intestinal homeostasis, and protecting DSS-induced chronic colitis in mice	(Garrett & Onderdonk, 2015) (C. Wang et al., 2021)

	Bacteroides caccae	• Found in normal microbial flora in the human gastrointestinal tract • It is also a conditional pathogen that invades the blood and causes bloodstream infection when the intestinal mucosa of the host is damaged • Causes inflammatory bowel disease in humans • It can invade the mucosa of the intestine and cause various abdominal suppurative infections	(Y. Yang et al., 2021) (Cheng et al., 2019)
	Parabacteroides distasonis	• Found in the gastrointestinal tract of numerous species • Alleviates obesity and obesity-related dysfunctions in mice • It generates succinate and secondary bile acids in the gut in mice • It also activates intestinal gluconeogenesis (IGN) and farnesoid X receptor (FXR) pathways in the gut in mice	(Ezeji et al., 2021) (K. Wang et al., 2019)
	Prevotellacopri	• Found in the human gut • Associated with high-fibre non-Western diets as they possess extensive repertoires of carbohydrate-active enzymes that allow this species to metabolise complex polysaccharides	(Yeoh et al. 2022)
	Prevotellastercorea	• Found in the human gut • Its genomes possess several carbohydrate esterases that may be involved in releasing ester modifications from carbohydrates to facilitate their degradation	(Yeoh et al. 2022)
	Eubacterium biforme	• It is widely used in the production of butyrate	(Mukherje e et al. 2020)
Crenarchaeota	*Candidatus Nitrososphaera SCA1170*	• Found in the open ocean, soils, arctic, hot springs, and marine sponges • Ammonia-oxidizing Archaea bacteria	(Zhalnina et al. 2014)
Euryarchaeota	*Methanobacterium beijingense*	• Found in anaerobic digesters • It is an anaerobic degradation of organic compounds	(Ma et al., 2005) (Kabaivanova et al., 2022)
	Methanosarcina mazei	• Found in semi-aquatic environments such as sewage receptacles and anoxygenic, moist soils (i.e. riverbeds and ponds) • Digest organic waste in a semiaquatic environment • It could be used in a waste treatment process • It is a methanogen; it is possible to harness that metabolic endpoint to produce alternative fuels	https://microbewiki.kenyon.edu/index.php/Methanosarcina_mazei#Practical_Application
Firmicutes	*Bacillus selenatarsenatis*	• Found in the effluent drain of a glass-manufacturing plant • The bacterium is a facultative anaerobe that respires oxygen, selenate, arsenate, and nitrate as terminal electron acceptors	(Yamamura et al. 2007b)

	Bacillus flexus	• Found in milk, cheese, and fermented beans • Stimulate the proliferation of human peripheral blood lymphocytes in vitro • This strain exhibited nitrogen fixation • Produced siderophore, ammonia • Enhance the host plant growth under salt stress conditions	(Gayathri and Krubha 2021) (T.-T. Wang et al. 2017)
	Clostridium difficile	• It is the leading cause of nosocomial antibiotic-associated diarrhoea and pseudomembranous colitis worldwide	(Chandrasekaran and Lacy 2017)
	Clostridium pasteurianum	• Found in fermentative and non-photosynthetic green anaerobic bacteria • Used to produce chemicals and fuels such as n-butanol and 1,3 propanediol	(Doelle 1975) (Sabra et al. 2016)
	Enterococcus asini	• It plays a role in flavour development and fermentation • Used as a starter culture in the production of fermented salami and several types of ripened cheese	(Ghosh and Zurek 2015)
	Faecalibacterium prausnitzii	• Found in animals and human • It is a potentially active component of probiotic formulations and appears to be a promising therapeutic strategy for inflammatory bowel diseases and colorectal cancer	(Parsaei et al. 2021) (Ferreira-Halder, Faria, and Andrade 2017)
	Lactobacillus pontis	• It is used in the starter for making sourdough bread. • The bacteria begin fermentation by breaking down the sugars, forming lactic acid in the process	(Thiele et al., 2002)
	Lactobacillus ruminis	• Found in the intestinal tract of humans and animals • It has potential immunomodulatory properties • A possible role in suppressing antibiotic-resistant pathogens	(S. Wang et al. 2020) (O' Donnell et al., 2015)
	Peptostreptococcus anaerobius	• It is an anaerobic bacterium selectively enriched in the faecal and mucosal microbiota of patients with colorectal cancer (CRC)	(Long et al., 2019)
	Ruminococcus torques	• Found in the human gut microbiome • It is generally more abundant after circadian rhythm disruption when compared to the baseline composition and the normal LD group of microbiomes	(P. L. Tan and Kim 2021) (Deaver et al., 2018)
	Ruminococcus gnavus	• Found in the human gut microbiome • It synthesizes and secretes a complex glucorhamnan polysaccharide with a rhamnose backbone and glucose sidechains	(Henke et al., 2019)
	Ruminococcus bromii	• It has a pivotal role in the fermentation of RS3 in the human large intestine	(Ze et al., 2012)

	Streptococcus luteciae	• Found in aerobic environments of the skin surface • Streptococcus luteciae is increased during colorectal carcinogenesis.	(X. Liang et al., 2015)
	Sebaldella termitidis	• Found in Mediterranean termites • It is the only species in the genus Sebaldella within the fusobacterial family '*Leptotrichiaceae*' • The sole and type strain of the species was first isolated about 50 years ago from the intestinal content of Mediterranean termites	(Harmon-Smith et al., 2010)
Fusobacteria	*Acinetobacter guillouiae*	• Found in gasworks effluent • An amikacin-susceptible environmental species	(Nemec et al. 2010) (Yoon et al., 2014)
Proteobacteria	*Bdellovibrio bacteriovorus*	• Found in the human gut • Associated with natural biofilms	(Iebba et al. 2014) (Harini et al., 2013)
	Boseagenosp	• Found in • It is a diazotrophic, solubilized inorganic phosphorus and is involved in biocontrol	(Estendorfer et al., 2020)
	Hyphomicrobiumzavarzinii	• Found in soils, freshwater environments, and activated sludge • Mainly for the biotechnological potential of the exceptional formaldehyde dehydrogenase	(Martineau et al., 2015)
	Janthinobacteriumlividum	• It is involved in the spoilage of pasteurised milk • Able to cause opportunistic infections, including fatal septicaemia • It produces a metallo-β-lactamase conferring resistance to several β-lactam antibiotics	(Pantanella et al., 2006)
	Massiliaaerolata	• They are commonly found on the hands, mobile phones, plant-related substances, or plant roots	(S. Lee et al., 2021)
	Methylobacteriumorganophilum	• Ability to form biofilms • It exhibits tolerance to cleaning and disinfecting agents and high temperatures • Predominantly found in the hospital environment, particularly in tap water and endoscope channels	(Kovaleva et al., 2014)
	Paracoccusaminovorans	• N, N-dimethylformamide (DMF)-utilizing bacteria have been reported to date	(Urakami et al., 1990)
	Paracoccusmarcusii	• Found in marine and terrestrial habitats, including associations with insects, corals, and bryozoans • It has the potential to produce bioactive secondary metabolites	(Leinberger et al., 2021)
	Pseudomonas alcaligenes	• Found in soil and water • It causes nosocomial bloodstream infection, a very uncommon neonatal pathogen, in a preterm neonate	(Suzuki et al. 2013) (Flores-Carrero et al., 2016)

	Pseudoxanthomonas mexicana	• Found in human urine, riverside urban soil, and anaerobic digester • Members of this species can be distinguished from the other mesophilic species by their inability to use D-galactose, D-glucosamine, lactulose, and D-xylose • Susceptible to most classes of antibiotics except aminoglycosides	(Thierry et al., 2004)
	Sphingomonaswittichii	• Isolated from Elbe River in Germany • It is a potent degrader of toxic dioxin pollutants • It entirely mineralizes the organic backbone of the dibenzo-p-dioxin structure	(Chai et al. 2016) (Miller et al., 2010)
	Sphingomonaschangbaiensis	• Found in soil from the Changbai Mountains • Strictly aerobic, gram-negative, heterotrophic, oxidase- and catalase-positive • Cells are rods of 0.3–0.4 mm in diameter and 1.5–2.5 mm in length and are motile employing peritrichous flagella	(Zhang et al., 2010)
	Xylophilusampelinus	• Bacterial blight of grapevine in its only known host, *Vitis vinifera*	(Komatsu and Kondo 2015)

The number of species found in a particular area is known as species richness, and the average species diversity in a specific habitat is called alpha diversity (Alpha, Beta, and Gamma Diversity 2022). In the case of microbial ecology or diversity assessment, alpha diversity is a measure of microbial diversity found in a single sample (Willis 2019). It also provides insight into an ecological community structure with respect to species richness. Here, a rarefaction curve, a statistical method used to evaluate the species richness, was used to estimate the microbial richness of the samples obtained from the ONGF and PF. The rarefaction curve analysed the operational taxonomic units derived from the clustering of 16S rRNA gene sequences obtained as a result of bacterial metagenomic sequencing in the case of each ONGF and PF sample. The rarefaction curve can also determine the identity of a specific sample and infer whether a sample group is from the same community (Boussarie et al. 2022). In **Fig. 3**, the X-axis (horizontal axis) represents the number of sequences per sample, whereas the Y-axis (vertical axis) represents the community's diversity. The initial steep slope on the left in the case of both the samples (blue colour represents the ONGF and the red colour represents the PF) indicates the existence of many undiscovered species. The graph depicts the observed OTUs of ONGFs and PF. The ONGF shows 1,064 OTUs, whereas the PF shows 2,654 OTUs among 40000 sequences (**Table 5**). Observing the rarefaction curve, it can be concluded that the sample obtained from the PF has higher species richness than the ONGF. As per the previous studies, an ecosystem with high species richness is considered a diverse ecosystem which is more productive and capable of combating environmental stress and natural catastrophes.

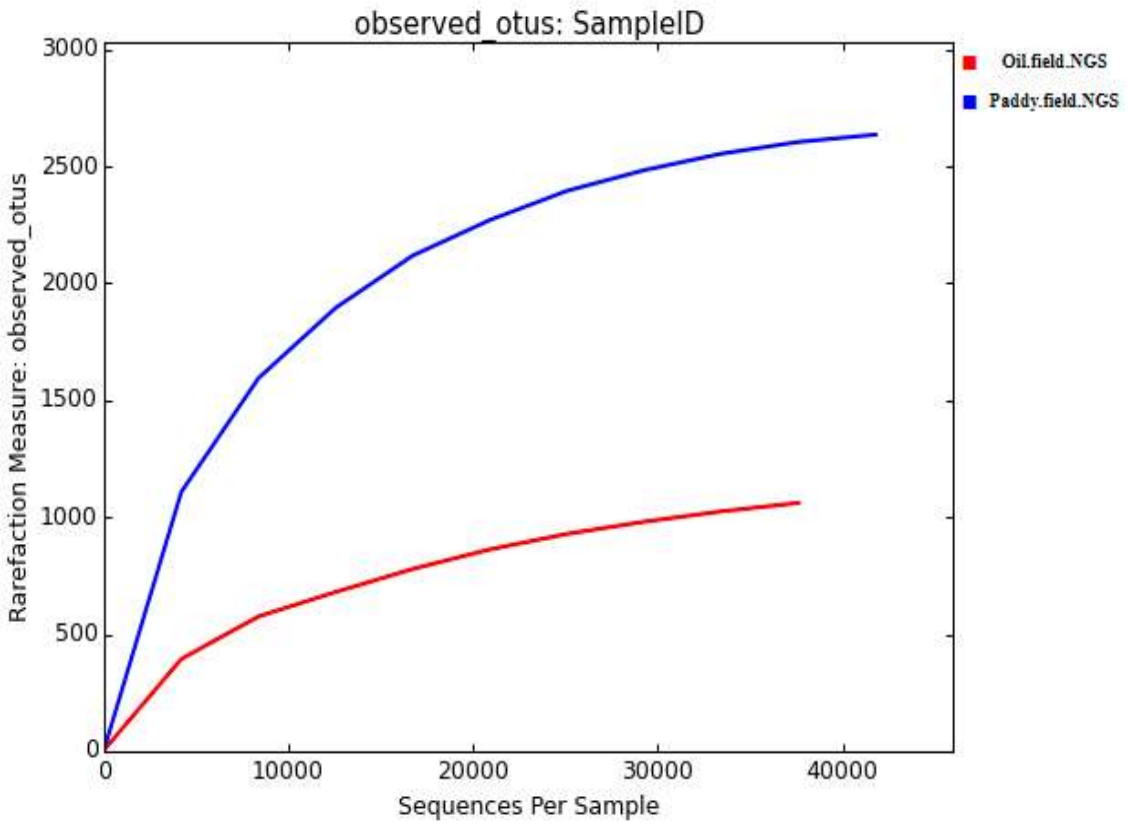

Figure 3:- Rarefaction plots for ONGF and PF samples representing observed OTUs (in the y-axis) and the number of sequences per sample (in the x-axis). The red and blue lines represent samples from the oil field and paddy field, respectively.

The Shannon index can also be used to estimate species richness. The Shannon index says that "The more species you observe, and the more even their abundances are, the higher the entropy or, the higher the uncertainty of predicting which species you would see next if you were to look at another read from this sample" (Denise Lynch n.d.). The observed Shannon diversity index for the ONGF is 5.43, and for the PF is 8.86 (**Table 5**).

Table 5:- Alpha diversity metrics of ONGF and PF samples.

S.no.	Sample name	Observed OTU	Shannon alpha diversity	Observed species	
				Classified	**Unclassified**
1.	ONGF	1,064	5.43	64	332
2.	PF	2,654	8.86	104	525

Comparative analysis of bacterial community

The comparative analysis of the bacterial diversity obtained from the ONGF, and PF revealed *proteobacteria* as the dominant phyla despite different geographical regions and environmental conditions (**Supplementary file, Fig. S11**).

To compare the bacterial phyla, the heatmap was generated, where each row represents an OTU (bacterial phyla), and each column corresponds to a sample (ONGF and PF). Heatmaps with higher relative abundances of OTUs show more intense colours at the corresponding positions. In the sample, red contributes a low number of OTUs. In contrast, purple contributes a high number (**Fig. 4**). The interpretation of this heatmap is that a high rate of OTUs is observed in the PF compared to the ONGF, which concludes that the PF sample has much more taxonomic diversity than the ONGF sample.

Among all the microbial communities, few bacterial phyla such as *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *VerrumicrobiaChloroflexi*, and *Actinobacteria* showed contrasting colour patterns in their OTUs, representing the difference in their relative abundance. The remaining bacterial phyla in both samples showed red, representing the lower abundance. *Proteobacteria* are considered the most diverse microbial phyla and are successfully found in most of the biomes on Earth (Zhou et al. 2020). Following the previously reported studies, *Proteobacteria* was the most dominant phyla in samples obtained from both locations. In the heat map (**Fig. 4**) the purple colour and the value near 0.48 for the sample obtained from the ONGF indicate a high abundance of *Proteobacterial* phyla.

In contrast, the light green colour with a relative abundance value near 0.36 indicates a lower abundance of Proteobacterial phyla in the PF. The orange colour with a relative abundance value near 0.10 indicates the lower abundance of *Bacteroidetes* phyla in the PF compared to the ONGF, where the resulting colour is yellow with a relative abundance value near 0.24. Unlike the distribution of *Bacteroidetes*, the *Firmicutes* abundance was high in the PF (yellow colour and relative abundance value of ~0.24) and lower in the ONGF (red colour with relative abundance value of ~0.04). Similarly, by observing the OTUs colour pattern, the *Chloroflexi* abundance was higher in the ONGF than the PF. In contrast, the Actinobacterial abundance was higher in the PF compared to the ONGF.

As observed by the comparative analysis of bacterial communities via heat map, the two dominant bacterial phyla, i.e., *Proteobacteria* and *Bacteroidetes*, were found in the ONGF, whereas *Proteobacteria* and *Firmicutes* were the dominant phyla found in the PF. Other than the dominant bacterial phyla in the ONGF and PF, the phyla, such as *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Verrucomicrobia*, were also found to have significant distributions. Further analysis of these bacterial phyla at the class level showed that both the sampling sites (ONGF and PF) had varying distributions of bacterial classes of each bacterial phylum, as discussed above.

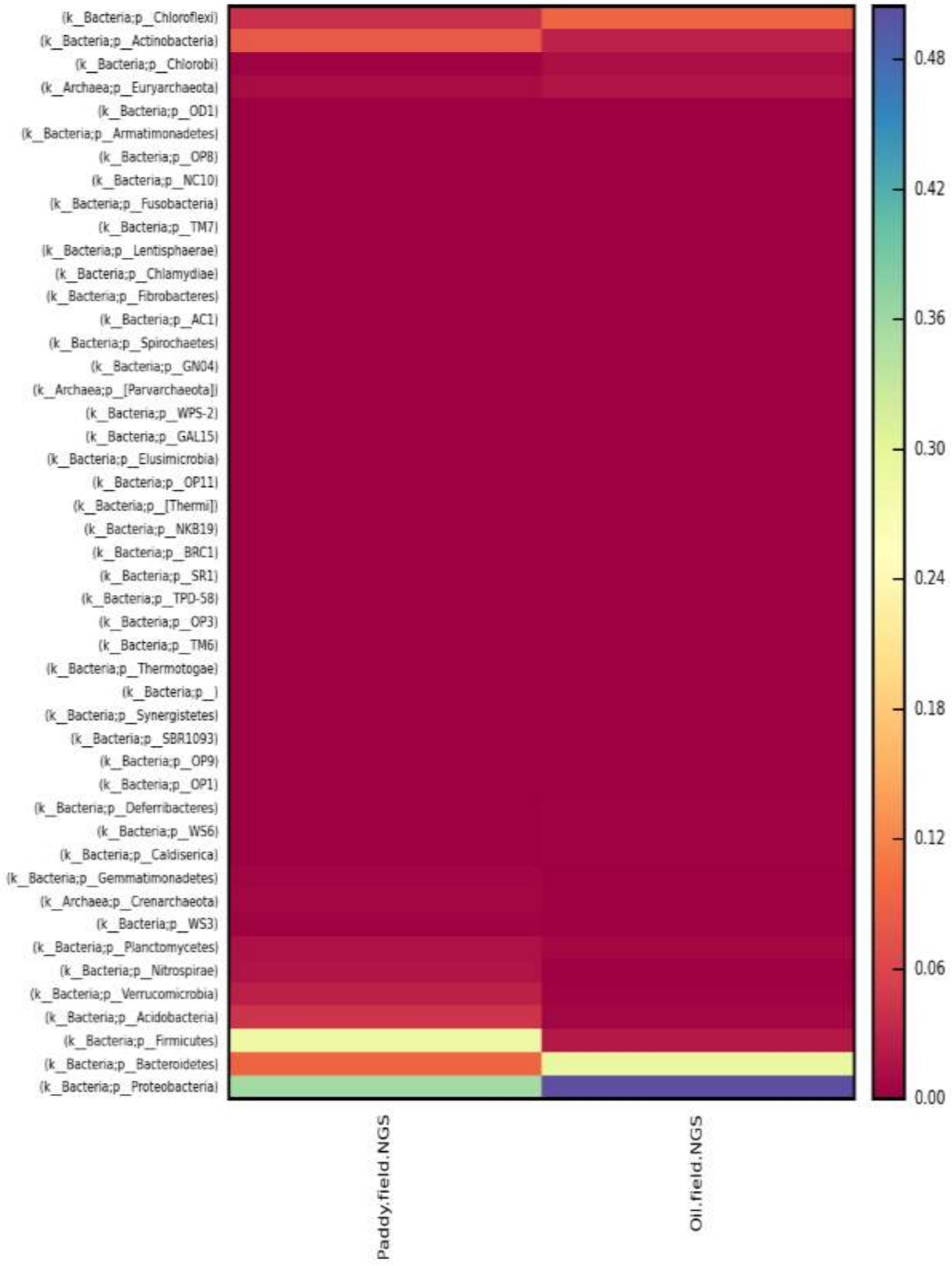

Figure 4:- Heatmap to visualize the Operational Taxonomic Unit (OTU) at the Phyla level. The transverse axis of the heat map represents "ONGF" and "PF", and the longitudinal axis represents different taxonomic levels. Red contributes a low percentage of OTUs to the sample, while purple contributes a high percentage of OTUs.

The different classes of *Proteobacteria*, such as *Alphaproteobacteria*, showed an abundance of 35% in ONGF's & 8% in PF, *Beta-proteobacteria* showed an abundance of 6.8% in ONGF's & 17.2% in PF, *Gammaproteobacteria* showed an abundance of 7% in the ONGF and 7.4% in PF, and *Delta-proteobacteria* showed an abundance of 0.96% in ONGF and 3.17% in PF (**Fig. 5a**). The different classes of *Bacteroidetes*, such as *Sphingobacteriia*, showed an abundance of 0.34% in ONGFs and 0.22% in PF, *Flavobacteriia* showed an abundance of 2.26% in the ONGF and 0.12% in PF, *Bacteroidia* showed an abundance of 25.94% in the ONGF and 6.05% in PF, and *Saprospirae* showed an abundance of 0.17% in the ONGF and 2.7% in PF (**Fig. 5b**). The different classes of *Firmicutes*,such as *Erysipelotrichi*, showed an abundance of 0.08% in the ONGF and 0.155 in the PF; *Clostridias*showed an abundance of 1.3% in the ONGF and 17.49% in the PF, and *Bacilli* showed an abundance of 0.6% in the ONGF and 10.89% in PF (**Fig. 5c**). The major classes of phylum *Acidobacteria*, such as *Solibacteres*, showed an abundance of 0.44% in ONGF and PF both, *Acidobacteria*-6 showed an abundance of 0.18% in ONGFs and 1.3% in PF, and *Chloracidobacteria* showed an abundance of 0.07% in the ONGF and 1.2% in PF (**Supplementary file, Fig. S12**). The major classes of phylum *Actinobacteria*, such as *Thermoleophilia*, showed an abundance of 0.05% in the ONGF and 1.6% in the PF, OPB41 showed an abundance of 0.7% in the ONGF and 0.30% in PF, *Actinobacteria* showed an abundance of 2.32% in the ONGF and 4.76% in PF, and *Acidimicrobiia* showed an abundance of 0.30% in the ONGF and 0.78% in PF (**Supplementary file, Fig. S13**). The major classes of phylum *Chloroflexi*, such as *Thermomicrobia*, showed an abundance of 0.14% in the ONGF and 0.13% in the PF, Ellin6529 showed an abundance of 0.01% in the ONGF and 0.58% in the PF, *Anaerolineae*showed an abundance of 9.27% in the ONGF and 2.82% in PF (**Supplementary file, Fig. S14**). The classes of phylum *Verrucomicrobia*, such as *Verrucomicrobiae*showed an abundance of 0.04% in the ONGF and 1.3% in the PF, *Opitutae* showed an abundance of 0.18% in the ONGF and 0.04% in PF, *Spartobacteria* showed an abundance of 0.05% in the ONGF and 0.30 in PF, and *Pedospaerae* showed an abundance of 0.09% in the ONGF and 0.82% in PF (**Supplementary file, Fig. S15**).

Table 6:- Details of accessions of the submitted samples.

SRA Accession	Study	BioProject_Accession	Biosample_accession	Submission ID	Library_ID	Taxonomy ID
SRR21460292	SRP395956	PRJNA876823	SAMN30678034	SUB12014851	ONGF	410658
SRR21460291	SRP395956	PRJNA876823	SAMN30678035	SUB12014851	PF	410658

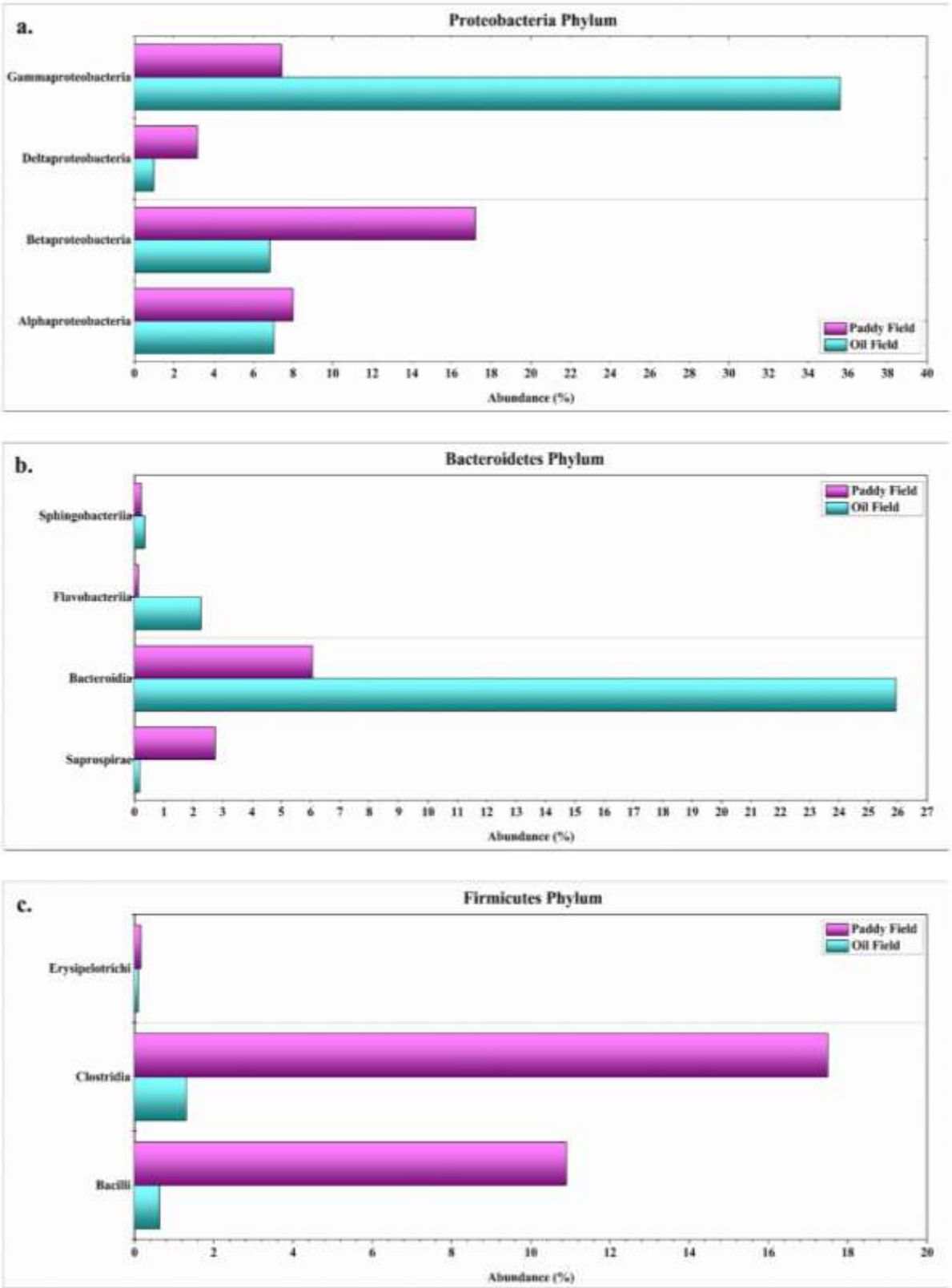

Figure 5:- Bar chart showing the taxonomic composition of microbial communities at the level of Phyla (*Proteobacteria*, *Bacteroidetes* and *Firmicutes*). The figure depicts the abundance of bacterial classes within the respective phyla in the oil and paddy fields.

Conclusion:-

Soil has a large diversity of microbial flora and fauna. It can be determined by various factors such as soil depth, pH, porosity, and the concentration of carbon dioxide, oxygen, and organic compounds. This metagenomic study examined the bacterial and archaebacterial diversity from two geographically isolated regions with varying ecological conditions and soil composition. Higher abundance of ecologically important *Proteobacteria* and *Bacteroidetes* at ONGF, whereas *Proteobacteria* and *Firmicutes* at PF, indicate that both regions are ecologically rich habitats. This study hypothesised that the bacterial composition at ONGF and PF will be primarily different and have specific environmental roles. As per the initial hypothesis, the bacterial species found at the ONGF, such as *CandidatusNitrososphaera*associated withammonia oxidation, *Brevundomonasdiminuta*withbioremediation of oil pollution, *Desulfoglaebaalkanexedens*withn-alkane oxidation, *Prosthecomicrobiumpneumaticum* with methane oxidation, and*Pseudoxanthomonaskalamensis* with nitrite reduction. The bacterial species found at the PF such as *Rhodococcusfascians* was associated with plant disease, *Streptomycesmirabilis* with cellulose degradation, *Bacillusfirmus* with plant growth, *Clostridiumcellulovorans* with plant polysaccharide fermentation, *Azospirillumbrasilense* with nitrogen fixing bacteria, *Nannocystisexedens* with preventing plant disease, and *Rubrivivaxgelatinosus* with anoxygeic photosynthesis and nitrogen fixation. The archaebacterial species such as *Methanobacteriumbeijingense*,considered an anaerobic digester of organic compounds involved in methane production, and *Methanosarcinamazei*, which can digest organic waste, used in waste treatment, and methane production,were common at ONGF and PF. The PF has high species richness and many classified species compared to the ONGF. Despite the non-specific bacterial communities, several opportunistic pathogenic bacterial strains related to human infections were also found at ONGF and PF. Therefore, maintaining good hygiene is essential for people working at ONGF and PF, including periodic disinfection and bioremediation. However, the 16S rDNA amplicon sequencing has limitations when it comes to elucidating the extent of variation in the bacterial communities found at ONGF and PF, whether it is caused by environmental conditions, soil composition, or anthropogenic pressures. Although, these results can be used in the future as an example to strengthen microbiome research findings in oil-and-gas fields and rice fields. In addition, this metagenomic research also suggests that sites like ONGFs and PFs may be used to isolate the bacterial species identified in this study.

Acknowledgement:-

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Statements And Declarations

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Competing Interests

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 article.

Author Contributions

AV is involved in Conceptualization, Methodology, Data Curation and analysis, Writing-original draft, and Editing; SK is involved in Data analysis, Writing-original draft, and Editing; SSM is involved in the Supervision,Conceptualisation, Validation, Writing, reviewing& editing. All authors made important contributions to the manuscript and approved publication.

Data Availability

The datasets generated for this study can be found in NCBI accession numbers shown in **Table 6**, SAMN30678034, SAMN30678035, SRR21460292, SRR21460291, SRX17464001, SRX17464002 (https://www.ncbi.nlm.nih.gov/bioproject/876823).

Supplementary data

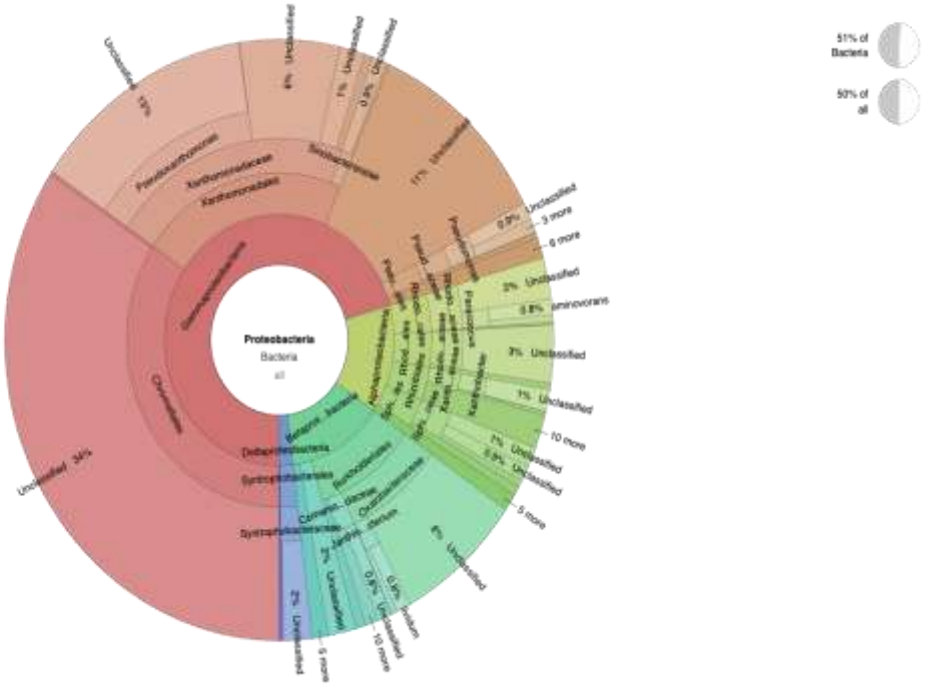


Figure S1:- Krona graph showing taxonomic assignment for the phylum *Proteobacteria* (50.45%) at ONGF.

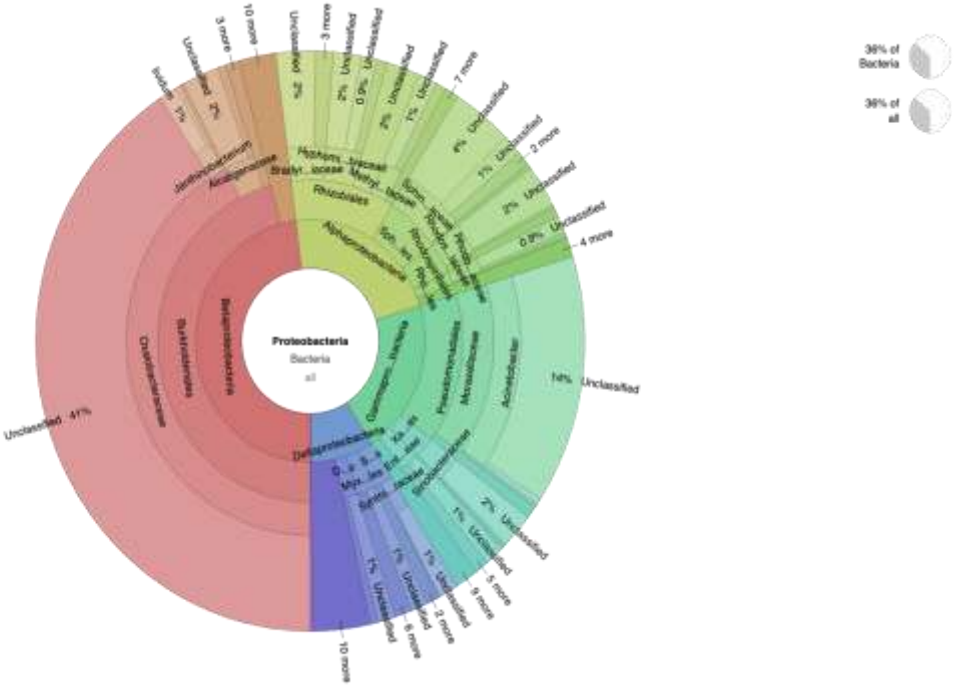


Figure S2:- Krona graph showing taxonomic assignment for the phylum *Proteobacteria* (35.86%) at PF.

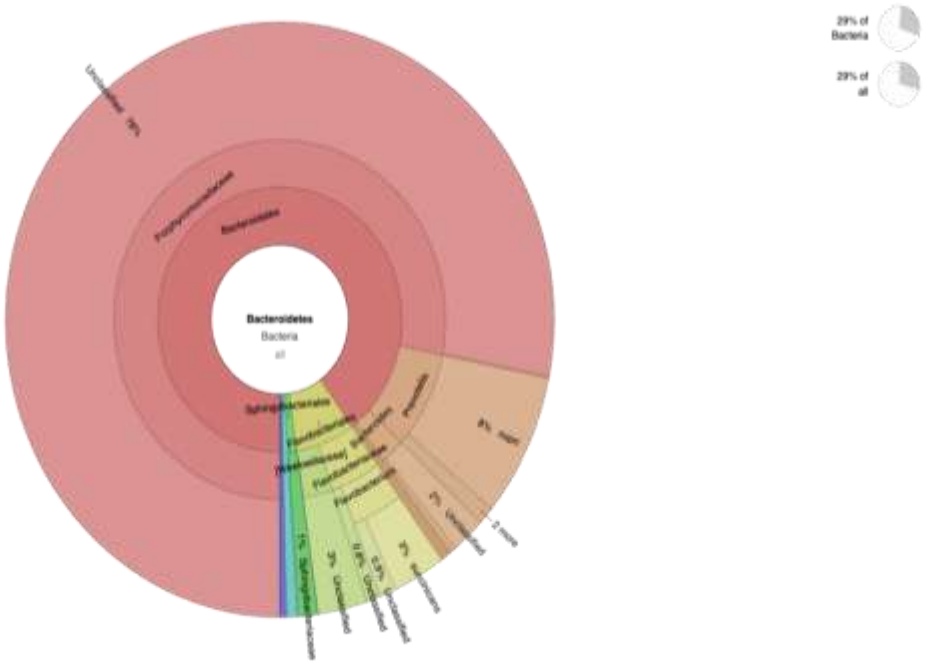


Figure S3:- Krona graph showing taxonomic assignment for the phylum *Bacteroidetes* (28.84%) at ONGF.

Figure S4:- Krona graph showing taxonomic assignment for the phylum *Firmicutes* (28.54%) at PF.

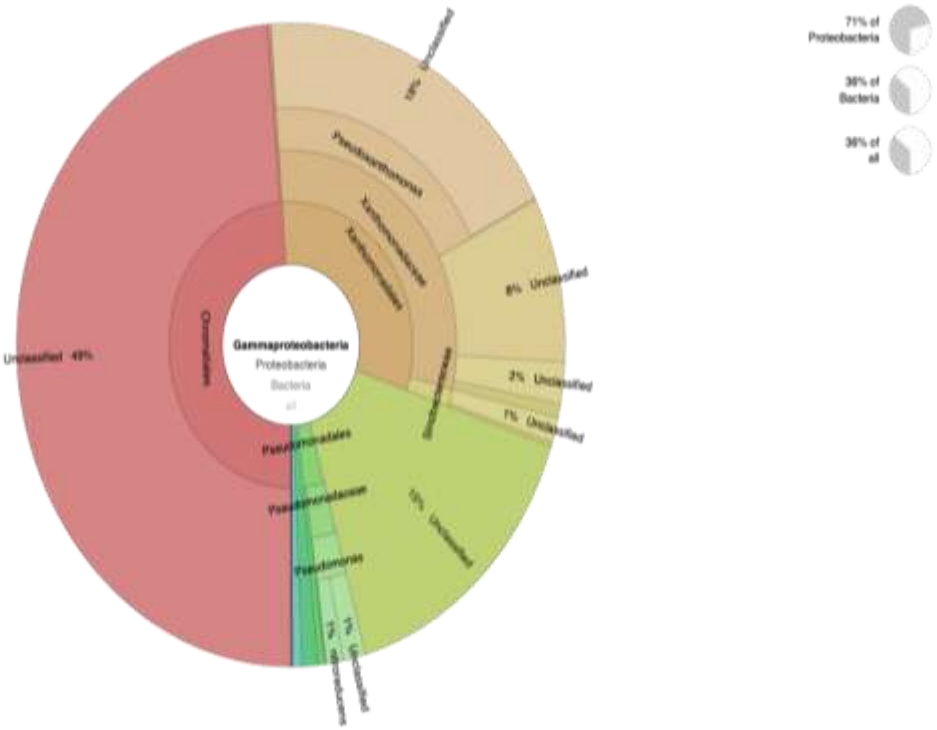
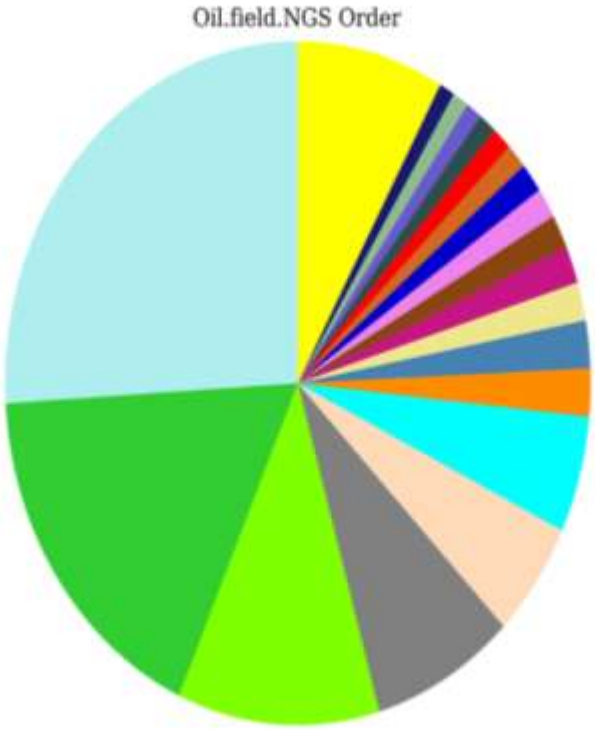


Figure S5:- Krona graph showing taxonomic assignment for the class *Gamma-Proteobacteria* (35.61%) at ONGF.

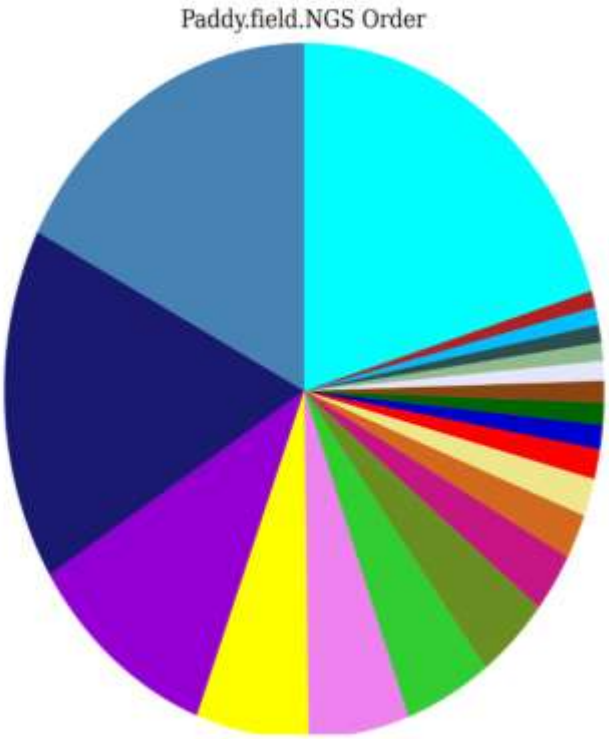
Figure S6:- Krona graph showing taxonomic assignment for the class *Clostridia* (17.49%) at PF.



Oil.field.NGS Order legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	25.94%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Chromatiales	17.39%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales	11.15%
	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__Anaerolineales	7.91%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__PYR10d3	5.51%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	5.51%
	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales	2.27%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	2.26%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales	1.81%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales	1.75%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	1.61%
	k__Archaea;p__Euryarchaeota;c__Methanomicrobia;o__NRA6	1.44%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales	1.41%
	k__Bacteria;p__Chlorobi;c__Ignavibacteria;o__Ignavibacteriales	1.18%
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	1.11%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales	1.0%
	k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Syntrophobacterales	0.9%
	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SBR1031	0.89%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MOB121	0.85%
	Others	8.09%

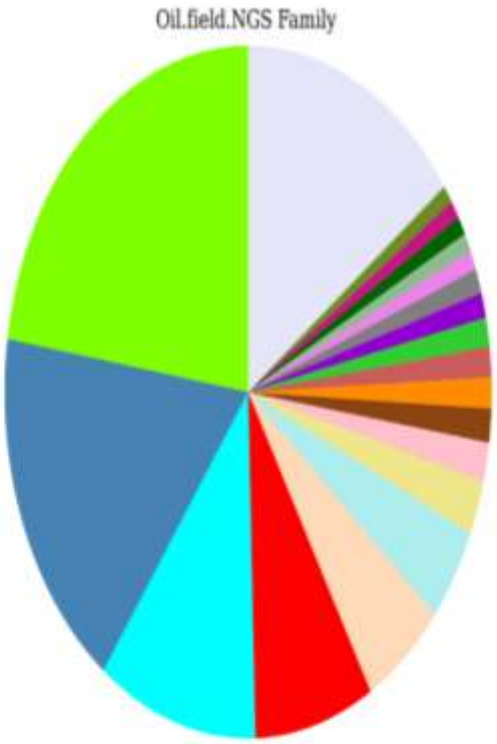
Figure S7:- Pie chart showing the absolute abundance of each order within each bacterial community at ONGF. The most abundant bacterial order is *Bacteroidales* (25.94%).



Paddy.field.NGS Order legend

Legends	Taxonomy	Abundance
	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	17.45%
	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	16.43%
	k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales	10.29%
	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	6.06%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales	5.42%
	k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	4.7%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales	3.96%
	k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales]	2.75%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales	2.09%
	k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales	1.76%
	k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales	1.39%
	k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales	1.12%
	k__Bacteria;p__Acidobacteria;c__Acidobacteria-6;o__iii1-15	1.03%
	k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__GCA004	1.01%
	k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41	0.92%
	k__Bacteria;p__Actinobacteria;c__Thermoleophilia;o__Gaiellales	0.85%
	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales	0.82%
	k__Bacteria;p__Verrucomicrobia;c__[Pedosphaerae];o__[Pedosphaerales]	0.82%
	k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales	0.78%
	Others	20.35%

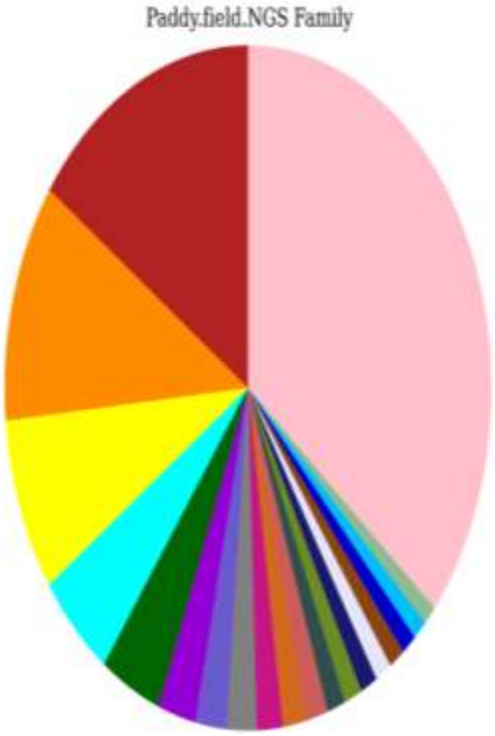
Figure S8:- Pie chart showing the absolute abundance of each order within each bacterial community at PF. The most abundant bacterial order is *Clostridiales* (17.45%).



Oil.field.NGS Family legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyromonadaceae	22.55%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Chromatiales;f_Ectothiorhodospiraceae	17.31%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Xanthomonadales;f_Xanthomonadaceae	10.62%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_Anaerolineales;f_Anaerolinaceae	7.91%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_PYR10d3;f_Unclassified	5.51%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Oxalobacteraceae	4.42%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae	2.53%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhodobacterales;f_Rhodobacteraceae	1.81%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhodospirillales;f_Rhodospirillaceae	1.58%
	k_Archaea;p_Euryarchaeota;c_Methanomicrobia;o_NRA6;f_Unclassified	1.44%
	k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales;f_Flavobacteriaceae	1.42%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Microbacteriaceae	1.35%
	k_Bacteria;p_Chlorobi;c_Ignavibacteria;o_Ignavibacteriales;f_Ignavibacteriaceae	1.18%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae	1.15%
	k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Syntrophobacterales;f_Syntrophobacteraceae	0.9%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_SBR1031;f_SHA-31	0.87%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae	0.86%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_MOB121;f_Unclassified	0.85%
	k_Bacteria;p_Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales;f_[Weeksellaceae]	0.84%
	Others	14.9%

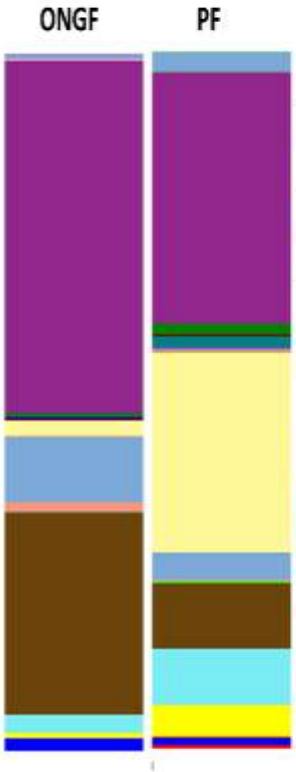
Figure S9:- Pie chart showing the absolute abundance of each family within each bacterial community at ONGF. The most abundant bacterial family is *Porphyromonadaceae* (22.55%).



Paddy.field.NGS Family legend

Legends	Taxonomy	Abundance
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Oxalobacteraceae	15.19%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae	11.34%
	k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Bacillaceae	8.23%
	k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae	5.17%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae	4.07%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptostreptococcaceae	2.52%
	k_Bacteria;p_Bacteroidetes;c_[Saprospirae];o_[Saprospirales];f_Chitinophagaceae	2.07%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sphingomonadales;f_Sphingomonadaceae	1.99%
	k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Actinomycetales;f_Nocardioidaceae	1.72%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae	1.63%
	k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae	1.39%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Bradyrhizobiaceae	1.21%
	k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae	1.19%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhizobiales;f_Hyphomicrobiaceae	1.14%
	k_Bacteria;p_Nitrospirae;c_Nitrospira;o_Nitrospirales;f_[Thermodesulfovibrionaceae]	1.1%
	k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae	1.06%
	k_Bacteria;p_Chloroflexi;c_Anaerolineae;o_GCA004;f_Unclassified	1.01%
	k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae	0.95%
	k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rhodospirillales;f_Rhodospirillaceae	0.92%
	Others	36.11%

Figure S10:- Pie chart showing the absolute abundance of each family within each bacterial community at PF. The most abundant bacterial family is *Oxalobacteraceae* (15.19%).



Legend	Taxonomy	Oil.field.NGS	Paddy.field.NGS
	k__Bacteria;p__Proteobacteria	50.50%	35.90%
	k__Bacteria;p__Bacteroidetes	28.80%	9.40%
	k__Bacteria;p__Firmicutes	2.00%	28.50%
	k__Bacteria;p__Chloroflexi	9.50%	4.00%
	k__Bacteria;p__Actinobacteria	2.80%	8.10%
	k__Bacteria;p__Acidobacteria	0.70%	4.40%
	k__Bacteria;p__Verrucomicrobia	0.40%	2.60%
	k__Archaea;p__Euryarchaeota	1.70%	1.10%
	k__Bacteria;p__Planctomycetes	0.60%	1.50%
	k__Bacteria;p__Nitrospirae	0.10%	1.80%
	k__Bacteria;p__Chlorobi	1.20%	0.30%
	k__Archaea;p__Crenarchaeota	0.10%	0.60%
	k__Bacteria;p__Gemmatimonadetes	0.00%	0.50%
	k__Bacteria;p__Caldiserica	0.30%	0.00%

Figure S11:- Stacked bar chart showing the relative abundance of each phylum within each sample.

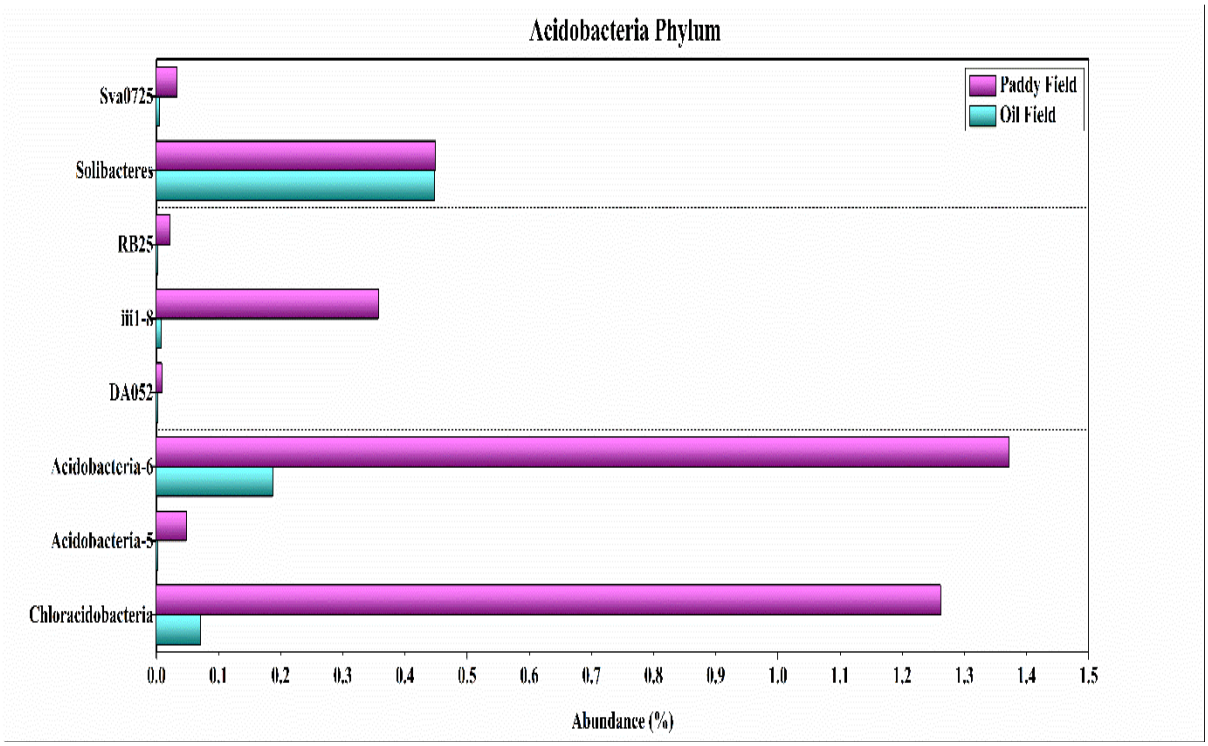


Figure S12:- Bar chart showing the taxonomic composition of microbial communities of Phyla *Acidobacteria*. The Figure depicts the abundance of bacterial classes within the *Acidobacteria*present in the Oil and Paddy fields.

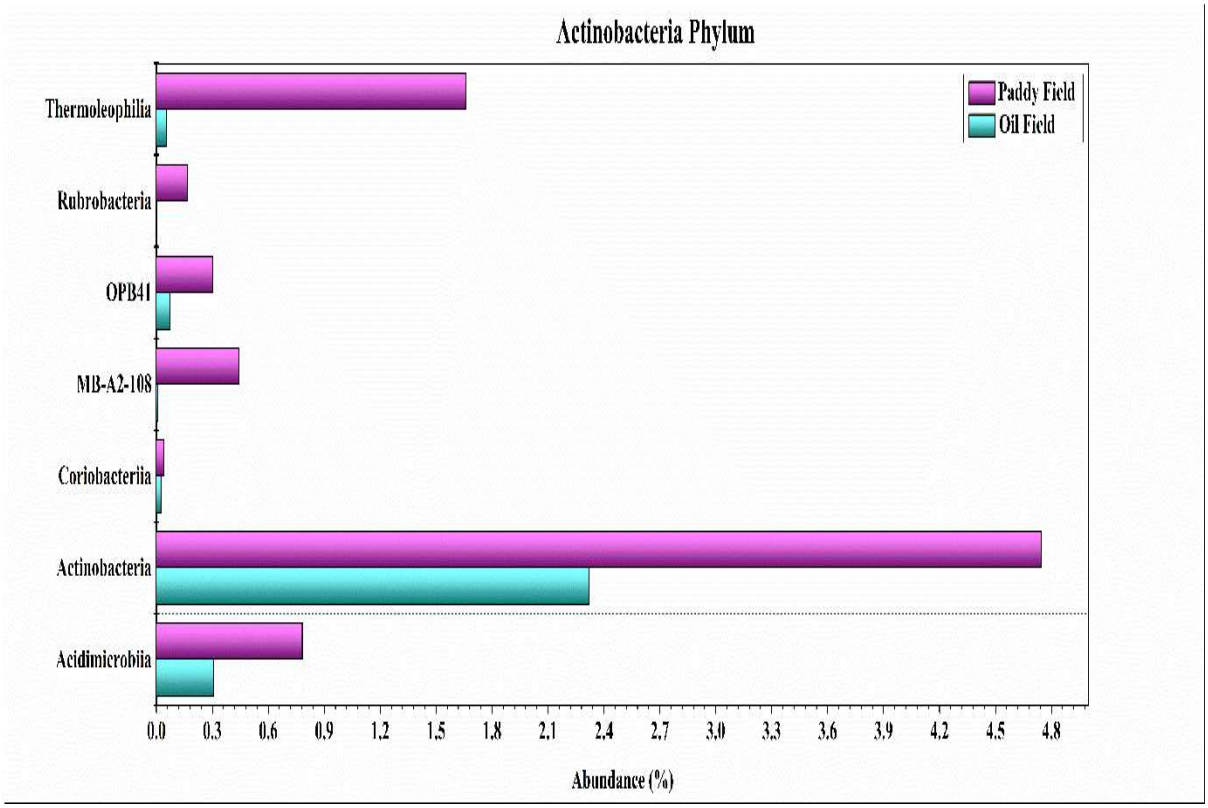


Figure S13:- Bar chart showing the taxonomic composition of microbial communities of Phyla *Actinobacteria*. The Figure depicts the abundance of bacterial classes within the *Actinobacteria*in the Oil and Paddy fields.

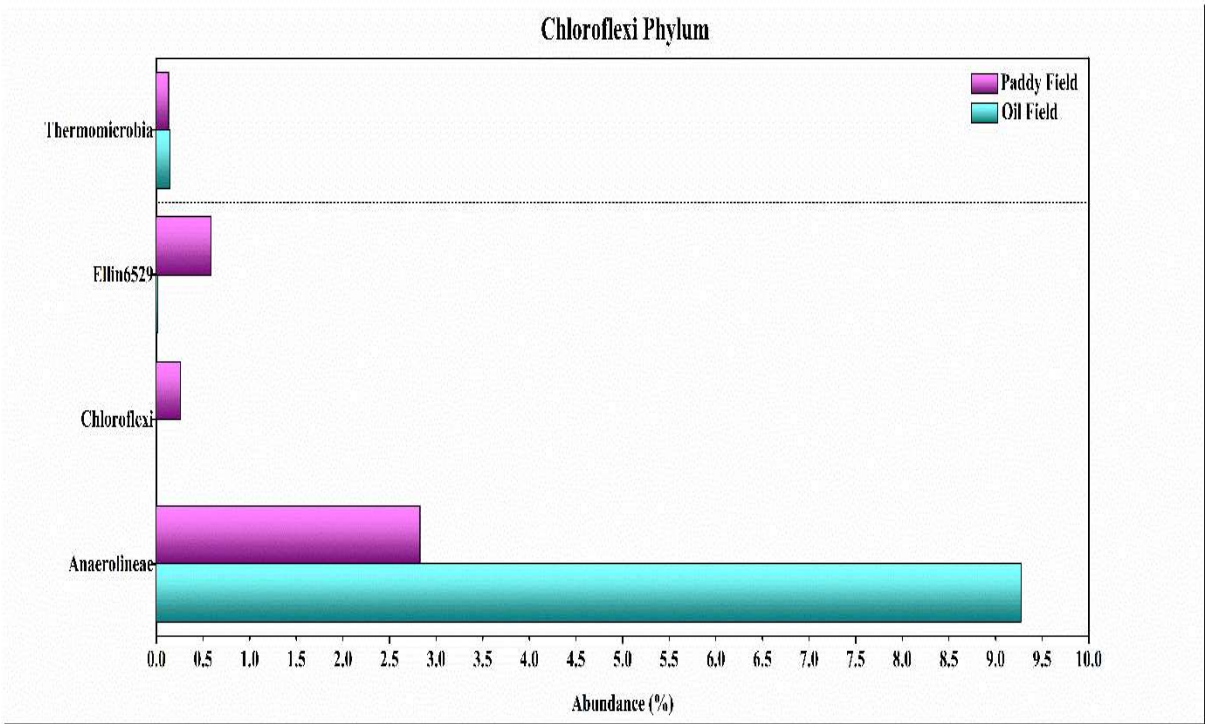


Figure S14:- Bar chart showing the taxonomic composition of microbial communities of Phyla *Chloroflexi*. The Figure depicts the abundance of bacterial classes within the *Chloroflexi*present in the Oil and Paddy fields.

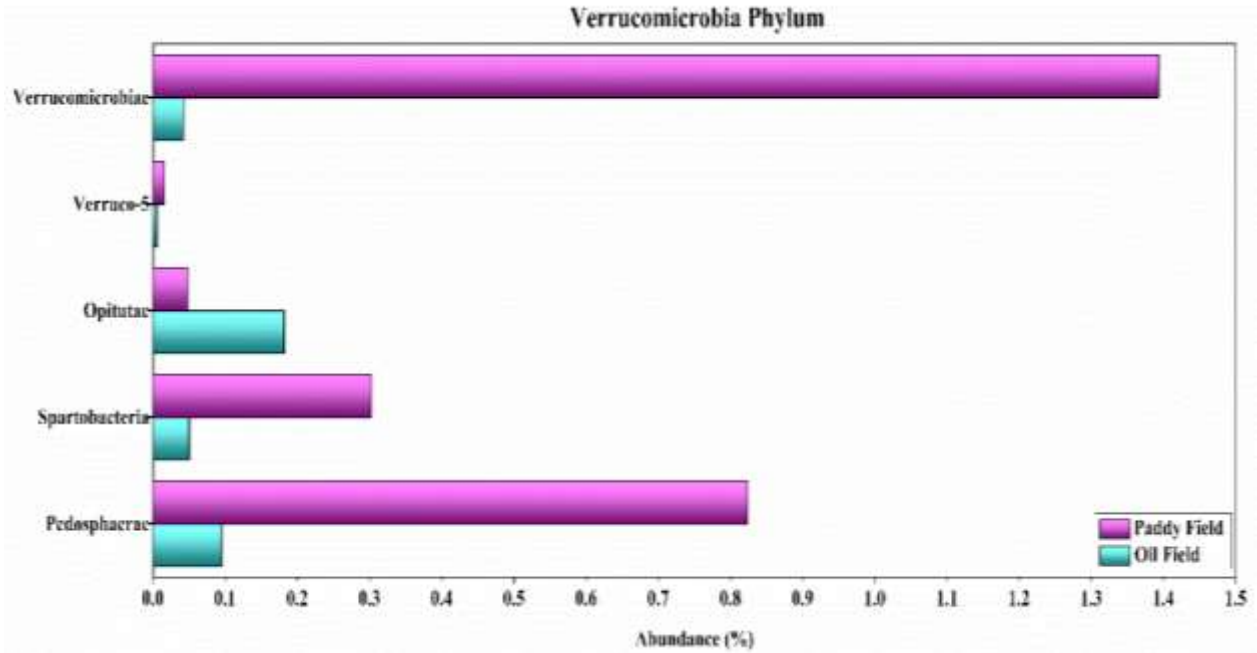


Figure S15:- Bar chart showing the taxonomic composition of microbial communities of Phyla *Verrucomicrobia*. The Figure depicts the abundance of bacterial classes within the *Verrucomicrobia* in the Oil and Paddy fields.

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