Chapter 1

Review of literature

"Natural science does not simply describe and explain nature; it is part of the interplay between nature and ourselves."

-Werner Heisenberg

1. Introduction

Geomicrobiology is active and rapidly advancing research field at the intersection of geology and microbiology, it focuses on the interconnection of diversity, distribution, and activities of microbes with their (geological) surroundings. Microbes are ubiquitous in nature and janitor of the earth as microbial activities play a vital role in maintaining biogeochemical cycling, bioremediation, metal and radionuclide transformations, mineral formation, mineral degradation (includes bioweathering and bioleaching), soil and sediment formation, chemical and isotopic fractionation (Jiao et al. 2021; Gadd 2010; Bianchi 2007). Microbial biomass, microbial physiological, and taxonomic characteristics can serve as indicators of soil quality and soil health (Anderson 2003; Schloter et al. 2003). Deep subsurface microbiology is an arising field in geomicrobiology since the subsurface biosphere is physiologically and phylogenetically highly complex and performs diverse biogeochemical processes (Amend and Teske 2005). Prokaryotes (i.e., bacteria and archaea) are dominant in subsurface environments as deep continental subsurfaces comprise ~2-6×10^{29} prokaryotic cells (Magnabosco et al. 2018). Soil and shallow terrestrial microbial biomass harbors ~0.5–5 ×10^{15} g of C (i.e., 0.5–5 Pg C) before vegetation occurred at ca. 0.5 billion years ago (McMahon and Parnell 2018) and in recent times the earth's continental subsurface harbor ~23 to 31 Pg of C as prokaryotic biomass (Magnabosco et al. 2018). Moreover, subsurface microbial activities play a crucial role in maintaining subsurface ecosystem functioning and groundwater quality (Bell et al. 2020; Madsen 1995). These suggest that named "stewards of the biosphere" given by Jansson and Fredrickson (2010) for microbes is more fitting for earth's surface and subsurface biosphere than anywhere else on the planet. Additionally, for a more holistic understanding of subsurface ecosystem functioning knowledge about subsurface microbial characteristics together with knowledge about physical and chemical conditions that control the microbial parameters within subsurface environments have pronounced importance. However, earlier microbial studies of subsurface environments were restricted to shallow depth but nowadays due to advancement in drilling techniques it has become possible to collect subsurface samples from various sedimentary and rock environments up to greater depths for microbiological analysis (Kieft 2010).

1.1 Characteristics of subsurface environments

Deep subsurface soils and sediments possess distinct physical and chemical characteristics than the surface (Holden and Fierer 2005). Earth's subsurface environment is a low nutritive oligotrophic mineralogical environment that is isolated from phototrophic energy sources and often limited in electron donors or electron acceptors. The deep biosphere includes different subsurface habitats, such as marine sediments and the basaltic ocean crust, unsaturated (vadose) zones and terrestrial deep aquifer systems, mines, river floodplain sediments, and fluvial terraces (represent a former level of the floodplains), deeply buried hydrocarbon reservoirs. Subsurface ecosystems comprise distinct stratigraphic sequences (lithofacies/geological formations) depending on the distribution of different particles (i.e. silt, clay, sand). Stratigraphic sequences of subsurface sedimentary environments deposited during different geological periods and derived from various depositional sources such as fluvial, marine, aeolian, estuarine, lacustrine (lake). Tectonic uplifts, sea-level changes, and paleoclimatic variations drive sedimentation and influence deposition of different lithofacies therefore shapes assortment in subsurface sedimentary environments (Berryman et al. 2000; Hong et al. 2007). Stratigraphic sequences of sedimentary ecosystem comprise complex and distinct mineralogical and geochemical composition depending on the source of parent material (i.e. felsic, intermediate, mafic, and ultramafic igneous rocks) [mafic and ultramafic igneous rocks have higher contents of Ni, Cr, Co, Cu than felsic and intermediate igneous rocks (Brügmann et al. 1987)], depositional source [marine sediment is rich in salinity and nitrogen content compare to fluvial sediment], paleoclimatic conditions [e.g. paleosol horizons observed in river sedimentary ecosystem indicates a period of alluviation followed by prolonged periods of pedogenesis due to climatically dry period (Juyal et al. 2000)], anthropogenic activities that took place at source area of sediments from where sediment is eroded (Karkanas 2010; Waters et al. 2014) and also depending on anthropogenic activities observed at depositional location (Swennen and Sluys 2002).

Availability of oxygen also varies considerably in different subsurface ecosystems. D'Hondt et al. (2015) demonstrated the presence of oxygen in the south Pacific Gyre sediment even 75 meters below seafloor while Lin et al. (2012) reported oxic subsurface sediments up to depth ~18 m (below that anoxic condition was observed) at the Hanford Site and Wood and Petraitis (1984) reported 16 to 19% reduction in oxygen concentration at 20 m below surface in

the unsaturated zone of the southern high plains of Texas. Low variability in pH, temperature, and moisture is observed in deep subsurface as compared to surface and near-surface soils and sediments (Fierer et al. 2003; Holden and Fierer 2005). However, groundwater recharge rate (or water percolation rate) could able to cause spatial and temporal variation in physicochemical characteristics of subsurface ecosystems including variation in pH, moisture content, and nutrient availability as percolation of water is responsible for migration of dissolved organic matter, minerals, and various contaminants from land surface to subsurface horizons (McNabb and Dunlap 1975; Bleam 2016). In arid and semiarid regions, the groundwater recharge rate is generally low as a result subsurface environment is relatively stable in arid and semiarid regions as compared to high water recharge rate regions.

Additionally, at the microscale, aggregates present in soils or sediments provide spatial heterogeneity and different microbial niches (i.e. microhabitats) depending on their size, their physical and chemical properties as well as their pore water chemistry and serve as hotspots for microbial diversity and activities (Kuzyakov and Blagodatskaya 2015; Bach et al. 2018). Subsurface soil or sediment ecosystems also comprise microhabitats like aggregates and simultaneously support both aerobic and anaerobic microbial processes (Hansel et al. 2008). On the whole, complexity and heterogeneity in physicochemical characteristics of subsurface sedimentary environments are also observed at a local scale hence subsurface sedimentary ecosystems provide a diverse microbial niche and ultimately diverse microbial characteristics.

1.2 Abundance, distribution, and taxonomic diversity of prokaryotes in different subsurface ecosystems

Microbial abundance is generally measured by culture dependent and culture independent methods (unculturable methods). Culture dependent methods include most probable number (MPN) and colony forming unit (CFU) enumeration (Balkwill 1989) and culture independent methods include direct microscopic counts, nucleic acid probing, quantitative real-time PCR (qPCR). Microbial diversity and their distribution can be determined using various techniques includes traditional culturable (plate count) and microbial physiology-based methods namely sole carbon source utilization patterns/community level physiological profiling (CLPP), phospholipid fatty acids (PLFA) analysis, diglyceride fatty acids (DGFAs) analysis (Kieft et al. 1998); molecular-based techniques namely amplified ribosomal DNA restriction analysis

(ARDRA) or restriction fragment length polymorphism (RFLP), terminal restriction fragment length polymorphism (T-RFLP), denaturing/ temperature gradient gel electrophoresis (DGGE/TGGE), repetitive element palindromic (REP) PCR, automated ribosomal intergenic spacer analysis (ARISA) (Kirk et al. 2004) and next-generation sequencing approaches (metagenomics). Metagenomics is the study of genetic material retrieved directly from environmental samples regardless of the nature of the sample and abundance of microbial entities (Oulas et al. 2015). Next-generation sequencing (NGS) technology such as ion torrent sequencing, pyrosequencing, Illumina sequencing is widely used for metagenomics analysis. A typical metagenomic analysis involves the isolation of metagenomic DNA from the samples, followed by single-gene amplicon sequencing (i.e., metabarcoding) e.g. 16S rRNA gene sequencing from the environmental sample or random shotgun studies of all environmental genes, analysis of sequencing reads, and finally the determination of phylogenetic and functional classification, interspecies interaction within the microbial community of different environmental samples (Fig. 1.1).

Despite the challenging environment of the subsurface (section 1.1) microbial life has been detected up to several kilometers below the surface as e.g. Balkwill (1989) reported terrestrial igneous rock-hosted deep biosphere, Onstott et al. (1998) revealed presence of microbial life up to depth ~2.8 km in the Taylorsville basin Triassic sediments of early to middle Carnian [230 million years ago (Ma)] age and Purkamo et al. (2020) revealed presence of microbial life up to depth 4.4 km within crystalline bedrock. Table 1.1 represents microbial abundance reported in different subsurface ecosystems together with methods by which microbial abundance was determined. Culturable counts determined by CFU and MPN enumeration in different subsurface environments revealed that depth up to which detectable culturable microbial population was observed is varied in different subsurface environments e.g. Balkwill (1989) reported the presence of culturable microbial communities up to depth 265 m at Savannah River Plant (SRP) facility near Aiken, South Carolina while Kieft et al. (1998) reported no detection of the culturable microbial population at greater depth in 14.9 m and 38 m deep core profiles (Table 1.1). Several studies of culture independent methods indicate that as depth increase microbial abundance decrease (Kieft et al. 1998; Taylor et al. 2002) while other studies reported that microbial abundance did not influence by depth (Fredrickson et al. 1989; Buongiorno et al. 2017) or decrease up to certain depth after that depth no further reduction in microbial

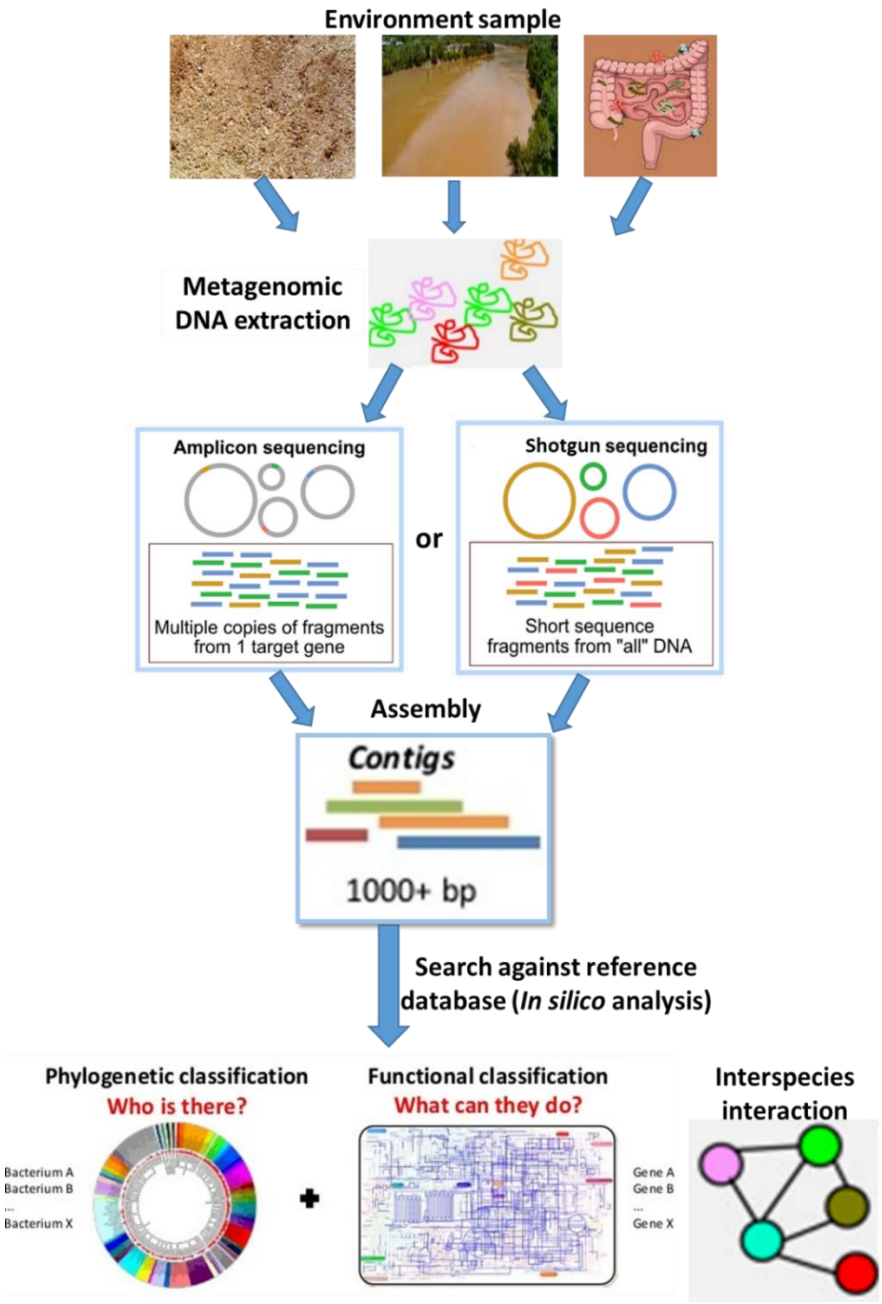

Figure 1.1: Schematic presentation of metagenomic analyses of environmental samples (modified from https://www.arraygen.com/metagenomics.php, https://astrobiomike.github.io/, and Dash and Das 2018).

abundance was observed at greater depth (Breuker et al. 2011; Schippers et al. 2012) (Table 1.1). Microbial abundance determined by culturable methods is usually lower than abundance determined by culture independent methods (Kieft et al. 1998; Taylor et al. 2002) (Table 1.1).

This phenomenon is consistent with the great plate count anomaly concept which indicates that only 0.1 to 1% of the total bacteria can be enumerated by the culturable method (Staley and Konopka 1985). However, culturable methods are still essential to understand microbial adaptations to various environmental conditions because this method provides insight into the physiological capacities of microbes and assists in understanding the ecological functioning of bacterial species in the environment (Fry 2000).

Assessment of microbial diversity in several subsurface environments revealed that microbial diversity decrease with depth, as determined by PLFA (Blume et al. 2002; Fierer et al. 2003), DNA fingerprinting techniques (LaMontagne et al. 2003; Agnelli et al. 2004; Goberna et al. 2005) or 16S rRNA gene sequences (Eilers et al. 2012) (Table 1.2). In contrast, Balkwill (1989), Zlatkin et al. 1996; Wilms et al. (2006), Rastelli et al. (2019) demonstrate that microbial diversity did not decrease with depth (Table 1.2). However, microbial community composition changes with soil depth (Balkwill 1989; Fierer et al. 2003; Eilers et al. 2012; Hong et al. 2019). Several studies reported that bacteria is dominating at subsurface as compare to archaea (Purkamo et al. 2020; Hong et al. 2019; Lin et al. 2012) while other studies revealed a similar abundance of both domains (Schippers et al. 2012; Lloyd et al. 2013) or higher abundance of archaea (Biddle et al. 2006) in subsurface environments. Among bacterial communities, earlier studies often show that compared to the relative abundance of gram-negative bacteria, a relative abundance of gram-positive bacteria is high at greater depth (Blume et al. 2002; Fierer et al. 2003). On the contrary, other studies reported dominance of gram-negative bacteria and gram-negative bacterial phyla Proteobacteria at deep subsurface (Balkwill 1989; Biddle et al. 2011; Lin et al. 2012). Various studies demonstrate oxygen-limited condition or anoxic condition in subsurface and dominance of anaerobes and anaerobic microbial processes in subsurface ecosystems (Jones et al. 1989; Lovley and Chapelle 1995). In contrast, Balkwill (1989) reported the presence of aerobic, chemoheterotrophic bacteria up to depth 265 m at Savannah River Plant (SRP), South Carolina; Ridley and Voordouw (2018) reported the presence of aerobic microbial taxa within 220 and 320 m deep oil sand cores at Northern Alberta, Canada and D'Hondt et al. (2015) revealed the presence of aerobic microbial communities up to 75 meters below seafloor (mbsf) at the south Pacific Gyre sediment together with also demonstrate that 9–37% of the global seafloor possess oxygen and aerobic microbial communities throughout the entire sediment sequence.

Table 1.1: Microbial abundance reported in different subsurface environments

	Methods	Major findings	Depth up to sample collected	Type of subsurface environments	References
Culturable methods	Colony forming unit (CFU) counts	~10^7 g^{-1} dry wt of soil	4.2 m	Soil samples comprising different texture collected from agricultural field in Iowa and Michigan, USA	Taylor et al. 2002
		Top sediments have 10^6-10^7 CFU/g which declined up to 1.35% at the shallowest sample, further declined to 0% in the at Washtucna and declined up to 0.0003% at Winona site	14.9 m and 38 m core	Core samples of Washtucna (14.9 m deep) and Winona (38 m deep), USA (unsaturated, buried loess sediments of >1 million years to <10,000 years older)	Kieft et al. 1998
		Average 5×10^5 g^{-1} sediment	179 m	Sediment cores located on the San Juan basin at Cerro Negro, New Mexico	Zlatkin et al. 1996
		➔ >10^6 heterotrophic bacteria g^{-1} dry wt (weight) sediment was observed within 60% of the sediments samples ➔Depth do not influence on bacterial counts and certain subsurface sediment samples have as high or higher bacterial counts than 0.1 m deep surface sample ➔Heterotrophic bacterial counts were lower in sediments of high clay content and low pH	260 m	Sediment samples were collected from three boreholes drilled at the U.S. Department of Energy's Savannah River Plant (SRP) in South Carolina	Fredrickson et al. 1989
		10^5-10^8 g^{-1} sediment	265 m	Sediment samples collected from Savannah River Plant (SRP) facility near Aiken, South Carolina [complete core profile comprise vadose (unsaturated) zone samples, several transmissive, aquifer zones samples, and samples from clay confining layers between the aquifers]	Balkwill 1989
	Most probable number (MPN) enumeration	Presence of 210 to 0.4 denitrifiers g^{-1} soil up to depth 3 m	3 m	Four different subsurface soil core profiles used for corn and soybean production in north- central Iowa	Yeomans et al. 1992
		7.91 to 3.24 oligotrophic denitrifiers g^{-1} soil up to depth 4 m	4 m	Soil core samples collected from the national agricultural research center at Kyushu Okinawa region in the southern part of Japan	Hashimoto et al. 2006
		2.1×10^3 fermenters, 2.4×10^2 iron reducers at Washtucna from 0.3 m depth; >2.4×10^5 fermenters, 4.6×10^2 iron reducers at Winona from 0.5 m depth; fermenters reduce up to <10 and iron reducers reduce up to <3 at higher depth in both sites (per gram sediment)	14.9 m and 38 m	Core samples of Washtucna (14.9 m deep) and Winona (38 m deep), USA (unsaturated, buried loess sediments of >1 million years to <10,000 years older)	Kieft et al. 1998

		➔Nitrifying bacteria were repeatedly detected within one borehole only and lower in numbers (≤ 10 g^{-1} dry wt sediment) ➔Sulphur oxidizing bacteria were detected up to 30 g^{-1} dry wt sediment within 60% of the sediment samples ➔Highest number of nitrifiers were observed in the surface samples while sulfur-oxidizing bacteria were not detected or very less in numbers within surface samples	260 m	Sediment samples collected from three boreholes drilled at the U.S. Department of Energy's Savannah River Plant (SRP) in South Carolina	Fredrickson et al. 1989
		10^1 to 10^8 aerobic chemoheterotrophic bacterial cells g^{-1} dry wt sediment (except few sediment samples in which no MPN counts was detected) present in different sediment samples	265 m	Sediment samples collected from Savannah River Plant (SRP) facility near Aiken, South Carolina [complete core profile comprise vadose (unsaturated) zone samples, several transmissive, aquifer zones samples, and samples from clay confining layers between the aquifers]	Balkwill 1989
		Fe(III)-reducing and sulfate-reducing bacteria were observed within the range of 1 to 10^4 cells g^{-1}	2800 m	Sediment samples collected up to depth 2800 m from Taylorsville basin Triassic sediments of early to middle Carnian [230 million years ago (Ma)] age	Onstott et al. (1998)
Un-culturable methods	Direct microscopic counts	~10^8 to 10^{10} bacterial cells g^{-1} dry wt soil determined by DAPI – as depth increases, bacterial cells decrease.	4.2 m	Soil samples comprising different texture collected up to 4.2 m depth from agricultural field in Iowa and Michigan, USA	Taylor et al. 2002
		10^8 to 10^9 cells g^{-1} sediment within top sediment samples decline to 10^6-10^7 cells g^{-1} sediment in both core profiles as determined by 4'6- diamidoino-2-phenylidole (DAPI)- as depth increase number of cells decrease	14.9 m and 38 m deep	Core samples of Washtucna (14.9 m deep) and Winona (38 m deep), USA (unsaturated, buried loess sediments of >1 million years to <10,000 years older)	Kieft et al. 1998
		10^9 to 10^{10} cells/ml at surface sediment which decrease to 10^7 to 10^8 cells/mL below 1 mbsf and further no remarkable reduction observed at higher depth (up to 10 mbsf) as determined by SYBR Green (II) direct counts (SGDC) using the protocol without detaching cells	10 mbsf	Organic rich subsurface marine core sediments up to a depth of 10 mbsf at six stations from the anoxic Black Sea and Benguela upwelling system off Namibia	Schippers et al. 2012
		~10^7 to 10^9 /cm^3 microbial cells determine by acridine orange (AO), SYBR Gold, and SYBR Green I direct count	87 mbsf	Samples were collected during IODP Expedition 347 from four different Baltic Sea sites up to depth ~87 mbsf	Buongiorno et al. 2017
	Nucleic acid probing	1×10^7 to 4×10^7 active sulfate-reducing bacteria cm^{-3} present in 5 m deep core profile as determine by catalysed reporter	5 m	5 m sediment core samples collected from North-eastern margin of the Janssand tidal flat located in the back-	Gittel et al. 2008

		deposition -fluorescence *in situ* hybridization (CARD-FISH)		barrier area of the island of Spiekeroog, North Sea, Germany	
		Equal dominance of living bacteria and archaea was observed by CARD-FISH in subsurface sediment samples	10 mbsf	Organic rich subsurface marine core sediments collected from six stations from the anoxic Black Sea and Benguela upwelling system off Namibia	Schippers et al. 2012
	qPCR	16S rRNA gene abundance decrease as depth increase; 10^4 to 10^8 bacterial 16S rRNA gene copies g^{-1} sediment while archaeal 16 rRNA genes undetectable in several samples, and comprised less than 8% of total 16S rRNA gene copies	9–52 m	Vertical stratified sediments located at the Hanford site 300 area near Richland, Washington State, USA	Lin et al. 2012
		Between 10^9 to 10^{10} cells g^{-1} observed at the surface sample; reduce to 10^6 cells g^{-1} at 20 m depth; between 20 to 140 m number of cells were highly variable and not correlated with depth	140 m	Fifty sediment samples were taken up to 140 m depth at the Chesapeake Bay impact structure (CBIS), Virginia, USA	Breuker et al. 2011
		➔Bacterial 16S rRNA gene copies observed between 10^1 to 10^2 g^{-1} rock samples and bacteria were more dominant than archaea within deep crystalline bedrock	2569 m to 4275 m	2569 m to 4275 m depth crushed rock materials collected from geothermal drilling wells located at the municipal area of Espoo, Finland	Purkamo et al. 2020
		1×10^8 to 6×10^8 archaeal 16S rRNA gene copies g^{-1} sediment i.e. 10 fold lower than bacterial 16S rRNA gene abundance	10 mbsf	Subsurface sediment samples collected from the Sonora Margin (Guaymas Basin)	Vigneron et al. 2014

Few studies described in Table 1.2 revealed the presence of Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, Spirochaetes, and Fusobacteria phyla in different subsurface ecosystems by cultivation method. Ribosomal Database Project (RDP) database of 16S rRNA gene inventory (https://rdp.cme.msu.edu/hierarchy/hb_intro.jsp) with a search keyword of "subsurface" retrieves 1637 sequences from various bacterial isolates. The majority of these bacterial isolates are taxonomically affiliated to four phyla includes Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes [Fig. 1.2; Table A-I (Appendix)]. Based on the RDP database lower percentage of bacterial isolates belonging to phylum Aquificae, Thermotogae, Chloroflexi, Deinococcus-Thermus, Fusobacteria, Tenericutes, Verrucomicrobia together with unclassified culturable bacteria were also observed in different subsurface ecosystems [Fig. 1.2; Table A-I (Appendix)].

Likewise, RDP database of 16S rRNA gene inventory (https://rdp.cme.msu.edu/hierarchy/hb_intro.jsp) with a search keyword of "subsurface"

retrieves 34119 sequences of uncultured microorganisms belonging to various subsurface environments. Among uncultured microbes and within the bacterial domain majority of hits belonging to phylum Bacteroidetes, Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes were observed [Fig. 1.2; Table A-II (Appendix)]. Further, based on extracted RDP database total 33 different bacterial phyla were observed with less than 1% abundance in different subsurface ecosystems when the data was retrieved for uncultured bacteria and these phyla were not observed when the data was retrieved for culturable bacterial isolates. These phyla include Ignavibacteriae, Nitrospirae, Spirochaetes, Gemmatimonadetes, Aminicenantes, Candidatus Saccharibacteria (Candidate Division TM7), Cyanobacteria, Armatimonadetes, Elusimicrobia, Parcubacteria, Synergistetes, Latescibacteria, Caldiserica, SR1, Lentisphaerae, Deferribacteres, Microgenomates, Chlorobi, BRC1, Marinimicrobia, Fibrobacteres, Candidate division ZB3, Thermodesulfobacteria, Omnitrophica, Candidate division WPS-1, Acetothermia, Cloacimonetes, Chlamydiae, Hydrogenedentes, Nitrospinae, Chrysiogenetes, Atribacteria, and Candidate division WPS-2 (represented as others in Fig. 1.2) (Table A-II, Appendix).

Table 1.2: Microbial diversity and distribution in different subsurface sedimentary ecosystems

	Methods	**Major finding**	**Depth up to sample collected**	**Type of subsurface environments**	**References**
Culturable methods	Isolation and identification of isolates by FAME, partial 16S rDNA sequences, and by rep-PCR genome fingerprint	➔Total 158 aerobic bacterial strains procured from depth up to 179 m and identification of isolates revealed that 67% of isolates were gram-positive bacteria mainly with high %G +C DNA, 19% isolates were α-*Proteobacteria* and 14% isolates were *Flavobacteria*	179 m	Sediment cores located on the San Juan basin at Cerro Negro, New Mexico	Zlatkin et al. 1996
	Plating and observation of bacterial colony and cell morphology	➔11 to 62 morphologically distinct colonies were observed from most aquifer sediments ➔Analysis of colony morphology indicate that diversity did not decrease with depth, but bacterial community composition varied extensively depending on the geological formation ➔ More than 80% of isolates were gram-negative rods and almost 95% of colonies contained non-streptomycete bacteria	265 m	Sediment samples collected from Savannah River Plant (SRP) facility near Aiken, South Carolina [complete core profile comprise vadose (unsaturated) zone samples, several transmissive, aquifer zones samples, and samples from clay confining layers between the aquifers]	Balkwill 1989

Un-culturable methods	PLFA	➔ The changes in the fatty acid profiles, linked with sample depth ➔Relative abundance of gram-positive bacteria increase with depth	1.7 m	Two soil core profiles; a Tracy sandy loam and a Lauramie silt loam collected from Purdue University's Piney and O'Neil Agricultural Research Centers, respectively	Blume et al. 2002
		➔Microbial communities change significantly with soil depth and also decline with depth ➔Two soil depth profiles have different microbial community composition ➔Relative abundance of gram-positive bacteria and *Actinomycetes* increases with depth while relative abundances of gram-negative bacteria observed lower in the subsurface	2 m	Samples collected from two unsaturated Mollisol profiles located near Santa Barbara, CA, USA. One profile is on Santa Ynez Valley and another profile is on a Quaternary terrace site	Fierer et al. 2003
	Clone library preparation	➔Operational taxonomic units (OTUs) affiliated with *Proteobacteria, Acidobacteria, Nitrospirae, Chloroflexi, Actinobacteria, Planctomycetes, Gemmatimonadetes, Bacteroidetes, Firmicutes, Candidate Division* GAL 15, NC10 and SPAM was observed ➔Stratigraphic sequences, redox differences and small-scale physical and chemical heterogeneities govern microbial community composition as microbial communities of oxic samples and anoxic samples clusters distinctly together with that depth-dependent clustering was also observed ➔ Deeper samples were dominated by Proteobacterial OTUs such as *Pseudomonas*, *Stenotrophomonas*, *Bradyrhizobium* and *Enhydrobacter*	9–52 m	Subsurface vertical stratified sediments at the Hanford site 300 area near Richland, Washington State, USA	Lin et al. 2012
	T-RFLP	➔Lowest Shannon diversity index value was observed in the deepest sample, lower diversity observed at higher depth ➔Depth dependent variation observed in bacterial communities ➔ OTUs related to *Pseudomonas*, *Variovorax Actinobacteria* and *Firmicutes* were observed	4 m	Soils samples collected from two vertical Mediterranean grassland transects [one is a depositional site (valley) and another is an adjoining plateau (terrace)] located in the University of California Sedgwick Natural Reserve (Santa Ynez, CA) in California	LaMontagne et al. 2003
	ARISA	➔α-diversity (i.e. richness of bacterial OTUs or number of peaks observed in ARISA) and their equitability (Pielou evenness index	1.5 m	Vertical sediment core profile retrieved from Izu-Bonin Trench	Rastelli et al. 2019

		value) high throughout the vertical sediment profile ➔β-diversity i.e. turnover diversity observed within ranged from 31 to 90% dissimilarity of the bacterial community composition			
	CLPP and DGGE	➔Microbial diversity differ in different soil profiles and at deeper depth low diversity was observed	~ 0.7 m	Disturbed and undisturbed semiarid Mediterranean forest (southeast Spain) soils	Goberna et al. 2005
	DGGE	➔High diversity observed in upper A horizons (A1 and A2) as a large number of bands were observed in DGGE profiles while less diverse microbial communities observed in the deeper B horizons (Bw1, Bw2, Bcb1, Bcb2)	~ 1.5 m	Soil samples collected from forest soil profile comprising different horizons namely A1 (0-0.1 m), A2 (0.1-0.23 m), Bw1 (0.23-0.42 m), Bw2 (0.42-0.71 m), Bcb1(0.71-1.09 m) and Bcb2 (1.09-1.5 m) located at the Vallombrosa National Reserve, on the west flanks of the Apennine Mountains (Italy)	Agnelli et al. 2004
		➔Phylogenetic diversity of bacteria in surface and deeper layers (1.6 to 1.8 m) were differ ➔*Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Spirochaetes*, and *Chloroflexi* are dominant phyla ➔DGGE banding pattern revealed that microbial diversity did not decrease with depth and deeper layers appeared to be more diverse ➔γ-*proteobacteria* observed only at upper sand-dominated horizons whereas *Firmicutes*, *Bacteroidetes*, and *Chloroflexi* were observed at 2.2 m layer and below that.	3.6 m	Stratified sediment cores collected from a backbarrie tidal-flat area close to the island of Spiekeroog, Germany	Wilms et al. 2006
	Metagenomics	**Shotgun sequencing** ➔ *Proteobacteria*, *Firmicutes*, *Euryarchaeota*, and *Chloroflexi* observed frequently within all samples	8 mbsf ; 1,16,32, 50 mbsf	Metagenomics of sediment sample of 8 mbsf retrieved from IODP Expedition 308 site 1320 located in the Brazos-Trinity Basin IV in the Gulf of Mexico and its comparison with Peru margin metagenomics sequences of 1,16,32, 50 mbsf samples	Biddle et al. 2011
		16S rRNA gene (amplicon) sequencing or metabarcoding ➔Archaeal abundance gradually increased with depth. However, a relative abundance of bacteria was higher throughout the depth ➔ Microbial community	1 m	A sediment core sample collected from the mouth of the York River Estuary at Gloucester Point VA, within the Chesapeake Bay	Hong et al. 2019

		composition differs significantly from the surface to a depth of 1 m ➔γ-*proteobacteria*, β-*proteobacteria*, *Acidibacteria*, *Acidimicrobiia*, *Chloroplast*, *Flavobacteriia*, Mb-NB09, *Nitrospira* dominant in surface to 0.7 m sediment whereas δ-*proteobacteria*, *Dehalococciodetes*, and *Phycisphaerae* were dominant in the 0.8–0.9 m depth			
		16S rRNA gene (amplicon) sequencing or metabarcoding ➔ Diversity is highest in the top 0.1 m depth which decreases 20-40% at deepest horizons sampled ➔ Microbial community in all nine pits most variable at the surface while relatively similar at deeper soil depths ➔Microbial community distribution significantly affected by depth – a relative abundance of *Bacteroidetes* was decreased with depth and relative abundance of *Verrucomicrobia* was observed high at 0.1 and 0.5 m depth	0.2 to 1.8 m	Samples were collected from the nine soil pits located in a forested montane watershed in Colorado, USA	Eilers et al. 2012
		16S rRNA gene (amplicon) sequencing or metabarcoding ➔Bacterial class γ-*proteobacteria*, α-*proteobacteria*, β-*proteobacteria*, *Actinobacteria*, *Cytophagia*, *Bacilli*, *Bacteroidia*, *Deinococci*, *Sphingobacteriia*, and *Flavobacteriia* observed in both core profiles with >0.1% average abundance ➔Additionally, microbial community analysis indicate that aerobic taxa including *Pseudomonas* and *Acinetobacter* were dominated while anaerobic taxa were observed occasionally in subsurface oil sand samples	220 to 320 m	Subsurface oil sand core samples retrieved from two adjacent sites comprising McMurray formation located within the Athabasca Sedimentary Basin, Northern Alberta, Canada	Ridley and Voordouw 2018
		16S rRNA gene (amplicon) sequencing or metabarcoding ➔ γ-*proteobacteria* and *Actinobacteria were* dominant in rock samples ➔Greater relative abundance of *Firmicutes*, *Bacteroidetes*, β-*proteobacteria* was also observed in some rock samples ➔Majority of γ-proteobacterial OTUs affiliated with *Pseudomonas*, *Acinetobacter*, *Enhydrobacter*, and unclassified *Enterobacteriaceae*	2569 m to 4275 m	2569 m to 4275 m depth crushed rock materials collected from geothermal drilling wells located at the municipal area of Espoo, Finland	Purkamo et al. 2020
		16S rRNA gene (amplicon) sequencing/metabarcoding and shotgun sequencing ➔ Microbial cells have been concentrated using	~15 mbsf	Collected samples from ultramafic and mafic rocks and sediments of the	Goordial et al. 2021

		fluorescence-activated cell sorting (FACS) to overcome the limitations of bulk DNA extraction from low biomass samples ➔Acidobacteria, Candidatus Dadabacteria, Dehalococcoidia/Chloroflexi, were most abundant ➔Genes associated with heterotrophy and aerobic carbon monoxide and formate cycling metabolisms were present within the shallow subsurface biosphere of the Atlantis Massif and genes related to autotrophy were rare in these samples.		southern wall of the Atlantis Massif. These samples comprise low microbial biomass.	

Fierer et al. (2009) established global patterns of belowground communities in which they reported dominance of phylum Acidobacteria, Actinobacteria, Proteobacteria, and Bacteroidetes (revealed using barcoded pyrosequencing approach) by surveying 88 different subsurface soil samples collected from different geographic location includes desert, tundra, temperate grassland, boreal forest, temperate deciduous forest, temperate coniferous forest, and tropical forest. However, in this study, they did not include different subsurface sediment samples. Several studies reported in Table 1.2 revealed the presence of Proteobacteria, Acidobacteria, Nitrospirae, Chloroflexi, Actinobacteria, Planctomycetes, Gemmatimonadetes, Bacteroidetes, Firmicutes, Candidate Divisions, Deinococcus-Thermus, Spirochaetes, Cyanobacteria and Verrucomicrobia phyla in different subsurface ecosystems by unculturable methods suggesting that different subsurface ecosystems are highly diverse in their microbial community composition.

Largely, microbial community composition, their abundance and depth up to which different microbial communities has been detected are different in different subsurface ecosystems reflected due to difference in availability of nutrients as well as electron acceptors and donors in various subsurface environments (Lovley and Chapelle 1995; Rastelli et al. 2019), distinctive physicochemical characteristics (Shao et al. 2020; Fierer et al. 2009), distinctive paleoclimatic and depositional conditions (Delgado-Baquerizo et al. 2017; Thomas et al. 2015) and difference in geological succession observed within diverse subsurface ecosystems (Lv et al. 2016).

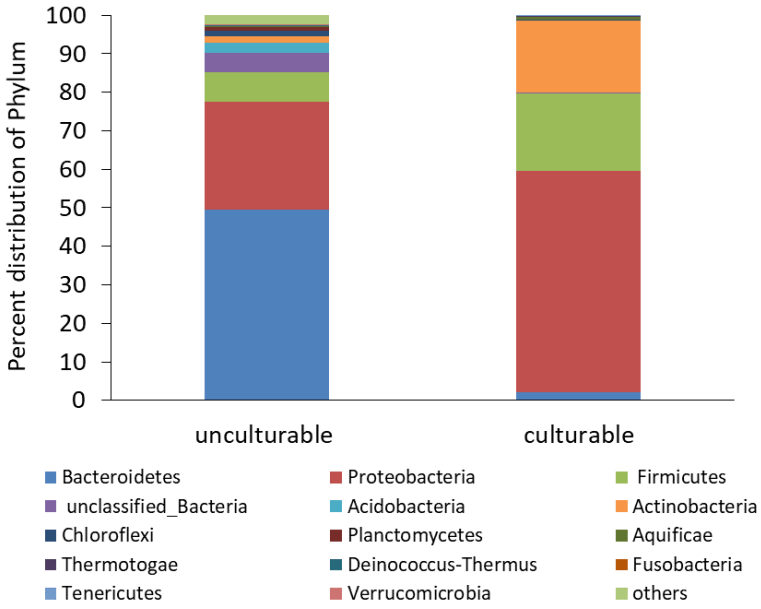

Figure 1.2: Phylum level distribution of culturable (cultivable isolates) and unculturable bacterial communities obtained from various subsurface ecosystems. Data Retrieved from the Ribosomal Database Project (RDP) database of 16S rRNA gene inventory (https://rdp.cme.msu.edu/hierarchy/hb_intro.jsp) with a search keyword of "subsurface". Detailed information about this data has been shown in Table A-1 and A-II (Appendix). Phyla comprising <1% and not detected via the culturable method are represented as others.

1.3 Subsurface sampling for microbiological analysis by core drilling

Drilling technologies have continued to advance and have enabled sampling from different subsurface environments. Methods of core drilling include auger drilling, cable tool drilling, and rotary drilling (Kieft 2010; Fig. 1.3). The auger drilling process is the simplest method, can perceive the soil types present at the site but cannot provide the specific depths of the samples and is limited to unconsolidated sediments (Kieft 2010; Rix et al. 2019). Cable tool rigs are readily available, but slow as allowed, drilling of 10–20 m depth per day and is also limited to unconsolidated sediments (Kieft 2010). The rotary drilling method allows drilling of >300 m depth and sample collection from a variety of lithologies including consolidated sediments or hard rock formations (Kieft 2010; Rix et al. 2019). In the rotary drilling method, a drill bit is rotated at the bottom end of sections of hollow steel drill pipe that is connected by threaded joints. The core collection is achieved by dropping a core barrel through the center of

the drill string using a wireline system (Fig. 1.3). Core barrels used in the rotary drilling process are of three types: single tube, double tube (include rigid and swivel double tube core barrels), and triple tube (Fig. 1.4). The basic operating principle of all core barrels is pumping drilling fluid through the drill rods and core barrel as the core bit cuts the rock (Rix et al. 2019). The single-tube core barrel consists of a case-hardened, hollow steel tube with a drilling bit attached at the bottom while the double-tube core barrel is an outer tube barrel connected to the bit with an inner liner that holds the cut core. The double-tube core barrel is offered with either a rigid or swivel inner liner. In the rigid double-tube core barrel, the inner liner is fixed to the outer core barrel so that it rotates with the outer tube while in the swivel type double-tube core barrel is supported on a ball bearing carrier that allows the inner tube to remain immobile during rotation of the outer barrel (Fig. 1.4). The triple-tube core barrel consists of another separate, nonrotating transparent plastic solid tube or a split thin metal liner to the double-tube core barrel which is used to retain the sample (Fig. 1.4).

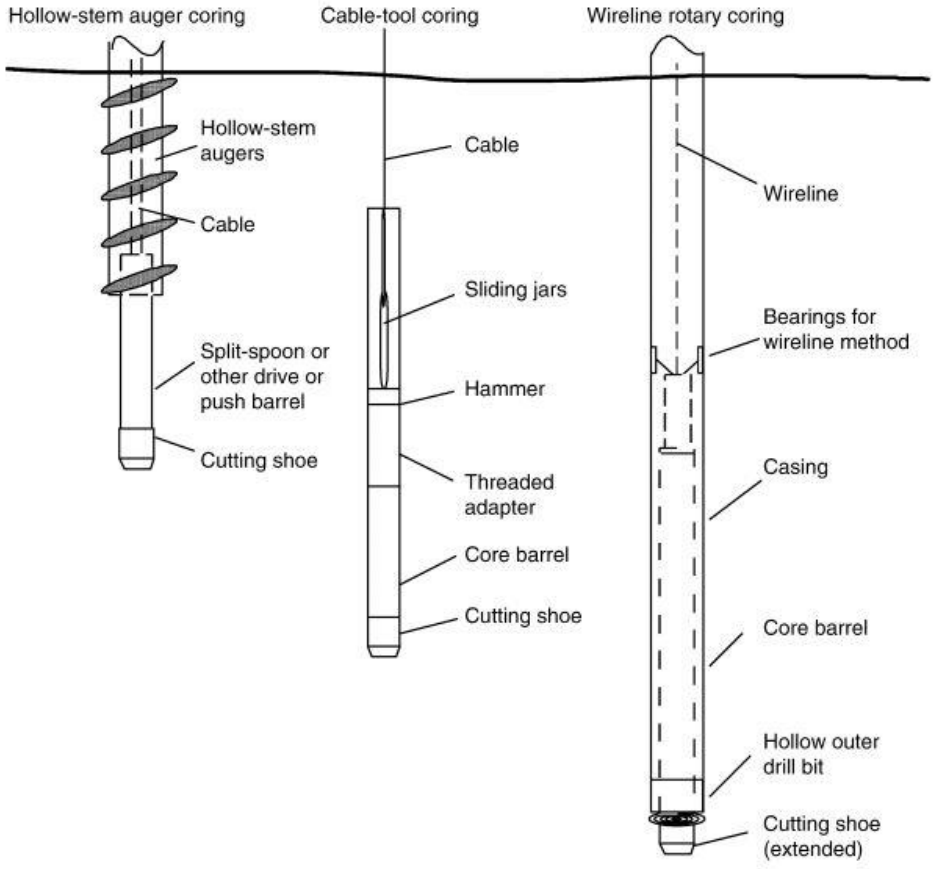

Figure 1.3: Auger, cable-tool, and rotary drilling process of core sample collection (adapted from Kieft 2010).

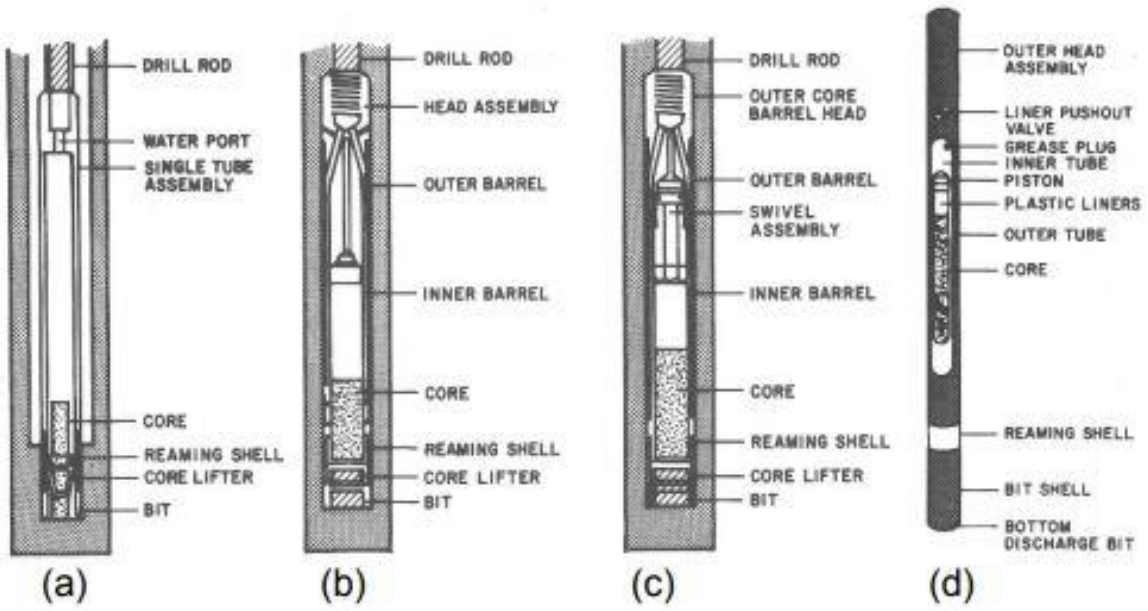

Figure 1.4: Diagram showing types of rock core barrels (a) single tube (b) rigid double tube (c) swivel double tube (d) triple tube (adapted from Rix et al. 2019).

There are many sources of contamination including the circulating drilling fluid, equipment used in drilling, personal and air-born transport during subsurface sample collection (Griffin et al. 1997). To avoid contamination caused due to drilling equipment, disinfection of drilling tools, core liners with steam or 10% bleach or 5 mg/1 chlorine solution followed by cleaning of drilling tools with sterile water is required (Kieft 2010; Griffin et al. 1997). Since complete elimination of contamination is not possible, it's essential to quantify or trace it to assuring sample quality. For that, two primary components in a field sampling program are required (1) the implementation of a tracer which provides information on the magnitude and type of contamination that may have been introduced during sample collection and processing and (2) the use of controls, blanks, and replicate samples provide information about contamination that has been introduced during sample handling (Griffin et al. 1997). Particulate tracers such as fluorescent microspheres, serendipitous native bacteria in drilling fluids and solute tracers such as rhodamine, bromide, perfluorocarbons, potassium as Cl or Br salt (for tracing chemical contamination), pigmented strains, fluorescent strains, genetically modified strains (for tracing microbial contamination) can be used (Kieft 2010; Griffin et al. 1997). Tracers can be placed into a plastic bag within the shoe (at the bottom of the corer), or can be

added to the drilling fluid or can be introduced into the borehole immediately prior to coring. In each of these cases, the outer portion of the core is exposed to the tracers along with actual sources of contamination such as drilling fluids, sloughed material from the borehole. After retrieving the core, it is dissected, the tracer-detected portion of the core being removed and the remaining core can be used for microbiological and geological investigation (Kieft 2010). Samples spiked with known bacteria or chemical contaminants are used as controls to examine the detection sensitivity and limits of analytical techniques, as well as any potential physical influences on samples during processing and shipment (Griffin et al. 1997). Blank samples are sterile samples that are ideally similar to those collected in the field. In the field, blanks are processed in the same way as real samples to see if contamination was introduced during processing or during shipping. Replicates, or sample "splits," are collected on a regular basis for independent laboratory examinations to ensure that analytical results are repeatable. Analytical results for controls, blanks, and replicates can be compared to results from actual samples to see if artifacts were introduced during core processing or subsequently by laboratory methods (Griffin et al. 1997).

1.4 Microbial activity and functional diversity

In terrestrial ecosystems, soils and sediments provide the main platform for microbial activities such as biogeochemical cycling, element biotransformations, metal and mineral transformations, decomposition, and bioweathering (Fig. 1.5) (Gadd 2004, 2010; Long et al. 2016; Khurana et al. 2021). Metals and elements such as C, N, P, and S are major components of soils and sediments where their presence in the mineral fraction, organic fractions, provide many essential nutrients for microbes, and important solid components that can have a fundamental effect on biogeochemical processes (Huang et al. 2004). Elements and metals present in organic fractions of soils and sediments, recycled as a result of organic matter decomposition (Fig. 1.5). Further, the mechanisms such as leaching, chelation by microbial metabolites and siderophores, redox transformations, biosorption, by which microorganisms effect changes in metal speciation and mobility are fundamental components of biogeochemical cycles for metals as well as all other elements, including C, N, P and S (Fig. 1.5) (Gadd 2004, 2010). There are various methods used to determine soil microbial activity such as enzyme

activities, respiration activity, $^{32}PO_4$ incorporation, etc. Some important methods used to measure the microbial activity of soil or sediment samples are described in Table 1.3.

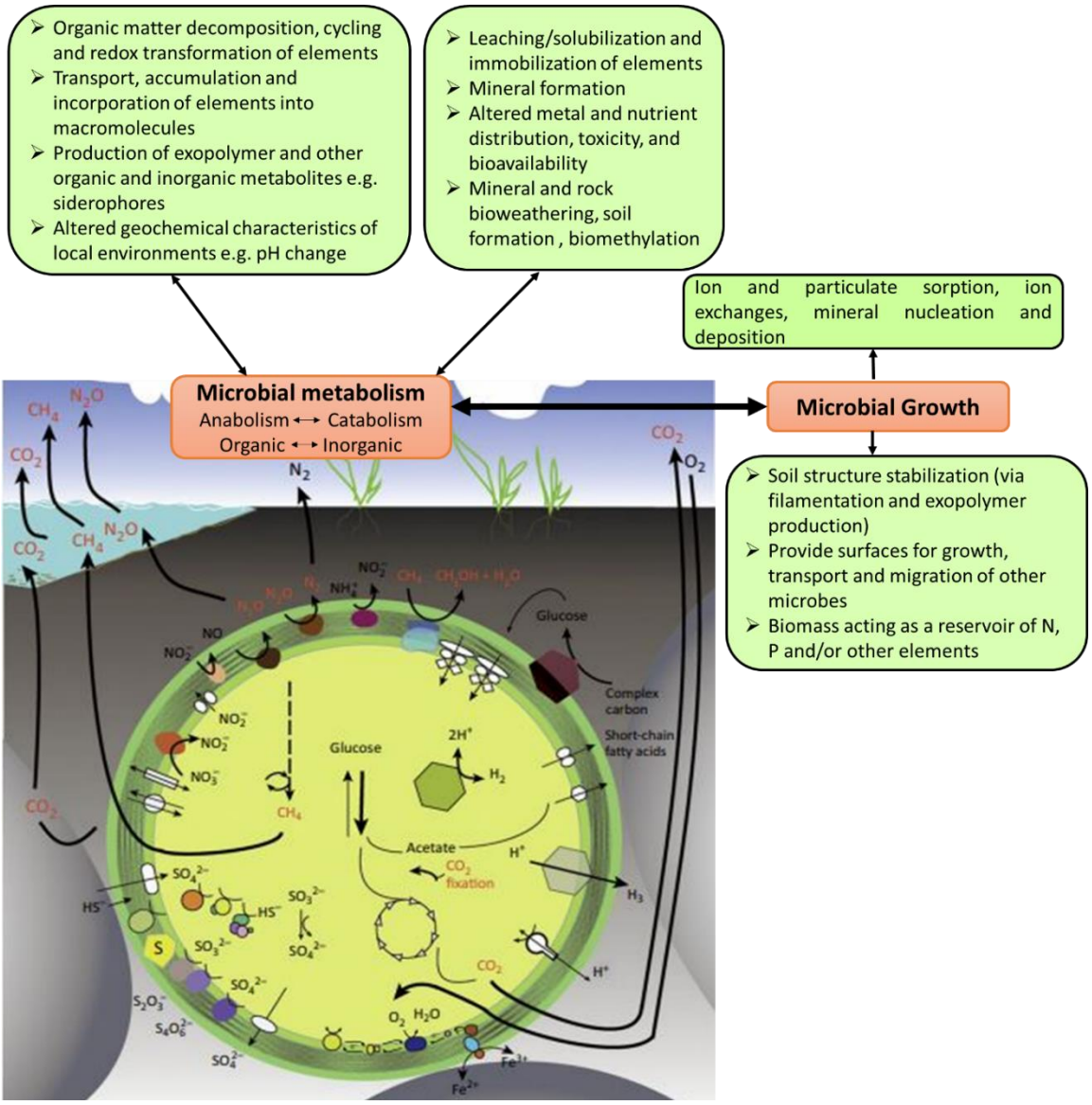

Figure 1.5: Microbial activities that occur in the terrestrial ecosystem and some of the important roles of microbes in biogeochemical processes. Microbial activities take place in natural ecosystems such as terrestrial ecosystems dependent on the microbial community composition and physicochemical parameters of sediments. Most of these parameters are interlinked with each other, directly or indirectly depending on the mode of microbial growth (such as unicellular, filamentous, colonial, biofilm) and mode of metabolism, in turn, dependent on the availability of nutrient and energy source(s) (modified from Gadd 2010 and Long et al. 2016).

Table 1.3: Methods used for the measuring microbial activity of soil or sediment samples

Name	Method description	References
Soil respiration	Soil respiration often measures in the absence (i.e., basal respiration) or in the presence of specific organic substrates or organic residues (i.e., substrate-induced respiration) in which the CO_2 emission rates have been measured from incubated soil samples.	Nannipieri et al. 1990; Alef 1995
Soil enzyme activities	The most widely measured enzyme activities include dehydrogenase, alkaline and acidic phosphatases, β-D-glucosidase, arylsulphatase, protease, etc. Chromogenic e.g. p-nitrophenol (pNP) and fluorogenic e.g. methylumbelliferone (MUB or MUF) based substrates have facilitated measurement of these enzyme activities.	Sinsabaugh et al. 2008, Taylor et al. 2002
FDA hydrolysis	Fluorescein diacetate (FDA), a colorless compound hydrolyzed due to microbial activity into fluorescein which is colored.	Adam and Duncan 2001
Use of labeled isotopes of precursors	Labeled isotopes of precursors such as $^{32}PO4$, ^{3}H-acetate, ^{14}C-acetate, ^{3}H-thymidine, ^{14}C-glucose have been used widely. In the laboratory, to measure anaerobic activity, N_2:CO_2 (90:10%) gas passed from sampling tubes. To measure aerobic activity, the ambient condition has been used. Isotopes incorporation measured over time during incubation and rate of incorporation or mineralization will be observed.	Phelps et al. 1989, 1994
Ammonification of arginine/amino acids or N mineralization	A method used to determine microbial potentials of N mineralization (i.e., amount of inorganic N released from the soil during a defined period) based on ammonification of arginine or other amino acids.	Alef and Kleiner 1985

Determination of soil enzyme activities is a simple, rapid, and inexpensive method (Alkorta et al. 2003; Verchot and Borelli 2005). The enzymes activities have been proximate agents of organic matter decomposition and related to rates of microbial metabolism, biogeochemical cycling (Sinsabaugh et al. 2008; Dilly and Nannipier 1998), microbial community structure (Waldrop et al. 2000; Schnecker et al. 2015), soil physicochemical characters (Turner et al. 2014; Błońska et al. 2017), natural and anthropogenic disturbance (García and Hernández 1997; Mora et al. 2005), and succession (Tscherko et al. 2003).

The detailed description of several enzyme activities used to determine soil biogeochemical cycling processes such as C, N, P, and S are listed below.

1.4.1 Microbial enzyme activities and fluorescein diacetate hydrolysis activity

1.4.1.1 Dehydrogenase activity (DHA)

Dehydrogenase enzyme is an intracellular enzyme and the activity of this enzyme encompasses oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors which are part of the respiration pathways of microorganisms. Therefore, dehydrogenase enzyme activity in the soil gives indications of the metabolic efficiency of viable microorganisms and potential of the soil to support organic matter decomposition which are essential for maintaining biogeochemical processes, mainly C cycling (Das and Varma 2011). Dehydrogenase activity was found to be a sensitive assay for determining the effect of soil pH, soil redox status, natural and anthropogenic disturbances on metabolically active soil microbial biomass (Kumar et al. 2013).

1.4.1.2 β-glucosidase activity (β-GA)

A β-glucosidase enzyme is involved in the hydrolysis of various β-glucosides which are commonly supplied to soil from plant residues. β-glucosidase is characteristically useful as a soil quality indicator and correlated well with soil microbial biomass, soil organic matter (Stott et al. 2010). Therefore, β-glucosidase activity serves as an indicator of changes in soil organic matter and importance in C cycling.

1.4.1.3 Phosphatases activity (PA)

Phosphatases describe a broad group of enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Laskowski 1961). In soil and sediment ecosystems, phosphatase plays a crucial role in the phosphorus (P) cycle (Speir and Ross 1978). Among phosphatases, acid phosphatase (acid PA), alkaline phosphatase (alkaline PA), and phosphodiesterases are considered the predominant phosphatases in most types of soil (Tabatabai 1994). The activities of these phosphatases are influenced by various soil properties such as soil pH, soil weathering, organic phosphorus, nutrient availability, and soil microbial biomass (Margalef et al. 2017).

1.4.1.4 Protease activity (PTA)

Protease enzyme activity is an important process concerning N cycling in many ecosystems, as proteolysis is considered to be a rate-limiting step during N mineralization in soils (Vranova et al. 2013). Protease activity, like any other enzyme activity, is influenced by the soil's physical properties such as texture, porosity, and bulk density (Das and Varma 2011).

1.4.1.5 Arylsulphatase activity (ASA)

Arylsulphatase catalyzes the hydrolysis of ester sulfates ($R\text{-}O\text{-}SO3^-$) into phenols (R-OH) and sulfate or sulfate sulfur ($SO4^{-2}$ or SO4–S) in the soil (Tabatabai 1994) and are secreted by bacteria into the external environment as a response to sulfur limitation (McGill and Colle 1981). Arylsulphatase activity is correlated with the rate of S immobilization, microbial biomass, and availability of organic matter (Whalen and Warman 1996).

1.4.1.6 Fluorescein diacetate hydrolysis activity (FDA HA)

The hydrolysis of fluorescein diacetate (FDA, 3' 6'- diacetyl-fluorescein) is a measurement of the contribution of several enzymes such as esterases, proteases, and lipases, all of which are involved in the decomposition of organic matter in the soil (Adam and Duncan 2001). FDA hydrolysis is dependent on the contribution of both extracellular and intracellular enzyme activities. FDA hydrolysis activity is thought to reflect overall soil microbiological activity which contributed to C, N, and P cycling (Schnurer and Rosswall 1982).

1.4.2 Microbial activity in subsurface sedimentary environment

In a deep subsurface environment, microbial activities depend on the availability of water, org. C, nutrients, energy, terminal electron acceptors, and other environmental factors such as pH and temperature (Holden and Fierer 2005). Because light is not available at deep subsurface, microbes obtained their energy from energy sources that have been buried in the sediment or entered as dissolved components in the recharge water. Further, the availability of energy sources is dependent on the porosity and permeability of the subsurface material (which in turn depends on sediment texture and pore size), and the redox potential of the sedimentary environment (Holden and Fierer 2005). These energy sources are organic matter or reduced compounds such as Mn(II), Fe(II), ammonia, and sulfide (Lovley and Chapelle 1995). Figure

1.6 illustrated the primary ways in which microorganisms acquire energy from the oxidation of organic matter. These processes involve electron transfer reactions from an electron donor to a terminal electron acceptor, referred to as the terminal electron-accepting process (TEAP). Energy yield from terminal electron-accepting processes depended on reduction potential difference between two redox couples. If the reduction potential difference between two redox couples is greater, the reaction will be more exergonic, thus providing more energy (Fig. 1.6). Therefore, terminal electron acceptors are used sequentially and the most exergonic reactions are favored until the most reactive electron acceptor is depleted. As result, the sequence of respiration metabolisms in the sedimentary environment is perceived as shown in Fig. 1.6. This sequence is O_2 reduction (aerobic respiration), NO_3^- reduction (denitrification and dissimilatory nitrate reduction), Mn(IV) and Fe(III) reduction, sulfate reduction, as well as carbon dioxide reduction (methanogenesis) (Lovley and Chapelle 1995; Gobat et al. 2004). In the sedimentary environment, buried organic matter present in a complex mixture of polymeric organic molecules. The microorganism secrets extracellular enzymes that hydrolyze complex organic matter into simple organic compounds such as sugars, amino acids, aromatic compounds, and long-chain fatty acids. All of these compounds are defined as primary substrates (Lovley and Chapelle 1995; Gobat et al. 2004). Further, microorganisms completely degrade these primary substrates into CO_2 using oxygen and nitrate as terminal electron acceptors (Fig. 1.6).

Under depleted oxygen conditions, anaerobically respiring microbes reduced Mn(IV), Fe(III), and sulfate and oxidized simple organic compounds (primary substrates). For example, long-chain fatty acids (LCFA) are oxidized by LCFA oxidizers, aromatic compounds oxidized by aromatic oxidizers, sugars, and amino acids fermented into organic acids (such as acetate, propionate, butyrate, lactate, or succinate), alcohols (Fig. 1.6). All of these compounds are defined as secondary substrates (Lovley and Chapelle 1995; Gobat et al. 2004). When conditions become more reduced so that Mn(IV), Fe(III), and sulfate are also depleted, then some microorganisms can still degrade the rest of organic matter to CO_2 and CH_4 by methanogenesis. Further, organic acids (larger than two carbon) and alcohols degrade into acetic acid or formic acid by proton-reducing acetogenesis that oxidizes organic compounds by reducing protons (H^+) to hydrogen. But this reaction provides very low energy to sustain life, as a result, it can only be carried out by microorganisms if the H_2 concentration is retained very low in the surrounding environment, which is executed by methanogenic partners (syntrophy)

(Gobat et al. 2004). The overall microbial processes involved in the decomposition of organic matter in sedimentary environments are illustrated in Fig. 1.6.

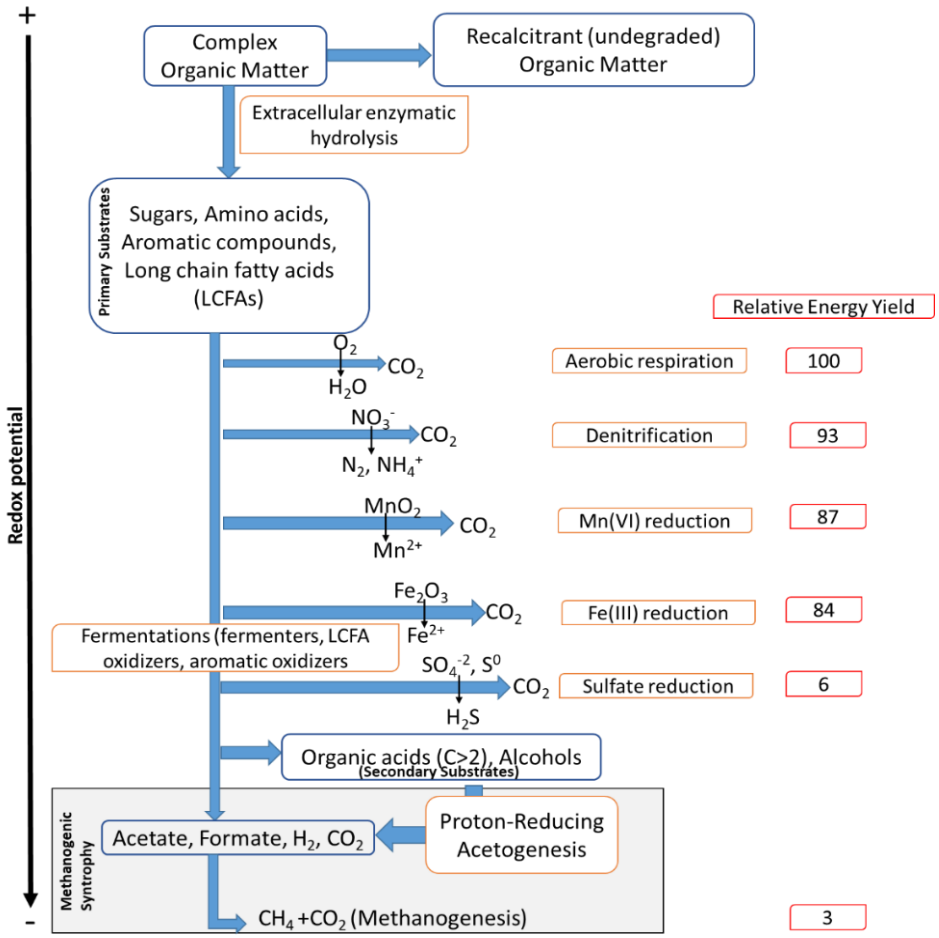

Figure 1.6: Overview of the predominant terminal electron-accepting processes (TEAPs) take place in sedimentary environments (modified from Gobat et al. 2004, the value of relative energy yield obtained from Lovley and Chapelle 1995).

Several studies reported that rates of microbial activities per mass of soil decrease with depth (Stone et al. 2014; Goberna et al. 2006; Fierer et al. 2003). The activities of enzymes involved in C, N, P, and S cycling show similar patterns with depth (Taylor et al. 2002; Stone et al. 2014). For intense, dehydrogenase activity (Taylor et al. 2002), β-galactosidase activity (Kieft et al. 1998), phosphatase (Stone et al. 2014), FDA hydrolysis (Federle et al. 1986) decrease with depth within the unsaturated zone. The decrease in the rates of microbial activity is primarily a result of the parallel decrease in nutrient availability, quality of degradable

organic matter as depth increases (Goberna et al. 2006; Fierer et al. 2003; Agnelli et al. 2004). However, few other studies reported notable microbial activities even in subsurface sediment and revealed that subsurface microbial activity depends on soil or sediment physicochemical characteristics such as soil/sediment texture, moisture content, organic C content (Phelps et al. 1989; Albrechtsen and Winding 1992).

1.4.3 Microbial functional diversity in subsurface sedimentary environment

Functional diversity has been widely recognized as the essential link between biodiversity patterns and ecosystem functioning (Escalas et al. 2019). Many studies applied the quantification and diversity of a different functional group of organisms such as denitrifying bacteria, nitrifying bacteria, alkaline phosphomonoesterase producing bacteria, sulfate-reducing organisms as a functional diversity measure (Smith et al. 2007; Miletto et al. 2007; Sánchez-Andrea et al. 2012; Vigneron et al. 2014; Liu et al. 2021). Escalas et al. (2019) reported a list of more than 400 microbial genes responsible for various functions related to biogeochemical cycles including C, N, P, and S. There are several methods listed in Table 1.4 used to determine functional gene abundance and diversity within soil/sediment samples.

Table 1.4: Phenotypic and genotypic methods used for functional microbial diversity assessment.

Method	**References**
Determination of functional gene abundance by qPCR	Kandeler et al. 2006; Smith et al. 2007; Kondo et al. 2008; Liu et al. 2021
Functional gene diversity using TGGE/DGGE or cloning	Geets et al. 2006; Smith et al. 2007; Miletto et al. 2007
community-level physiological profiles (CLPP) using BIOLOG^{R}- systems	Garland et al. 1997
Fluorescence in situ hybridization (FISH) combined with microautoradiography	Amann et al. 1995; Lee et al. 1999
High throughput technology such as GeoChip microarray; metagenomics, metaproteomics	Vigneron et al. 2014; Long et al. 2016; Bell et al. 2020

In the subsurface sedimentary environment, depending on physicochemical characteristics and redox status of sediment (as mentioned in section 1.4.2) microbial functional gene abundance and diversity has been detected (Lovley and Chapelle 1995; Vigneron et al. 2014; Long et al. 2016). Therefore, the determination of functional gene abundance and diversity in subsurface sedimentary environments provides a fundamental understanding of ecosystem functioning.

1.4.4 Role of microbes in heavy metal bioremediation

Microbial bioremediation is a process in which native or nonnative microbes (introduced to polluted sites, sometimes, ones that have been genetically engineered) are being used to reduce pollution levels (Hou et al. 2020). Though many physical and chemical remediation techniques are available, the use of microorganisms for remediation has many advantages like cost-effectiveness, environment-friendly as it does not employ any hazardous chemicals, less labor-intensive and after bioremediation treatment soil can be reused (reusability).

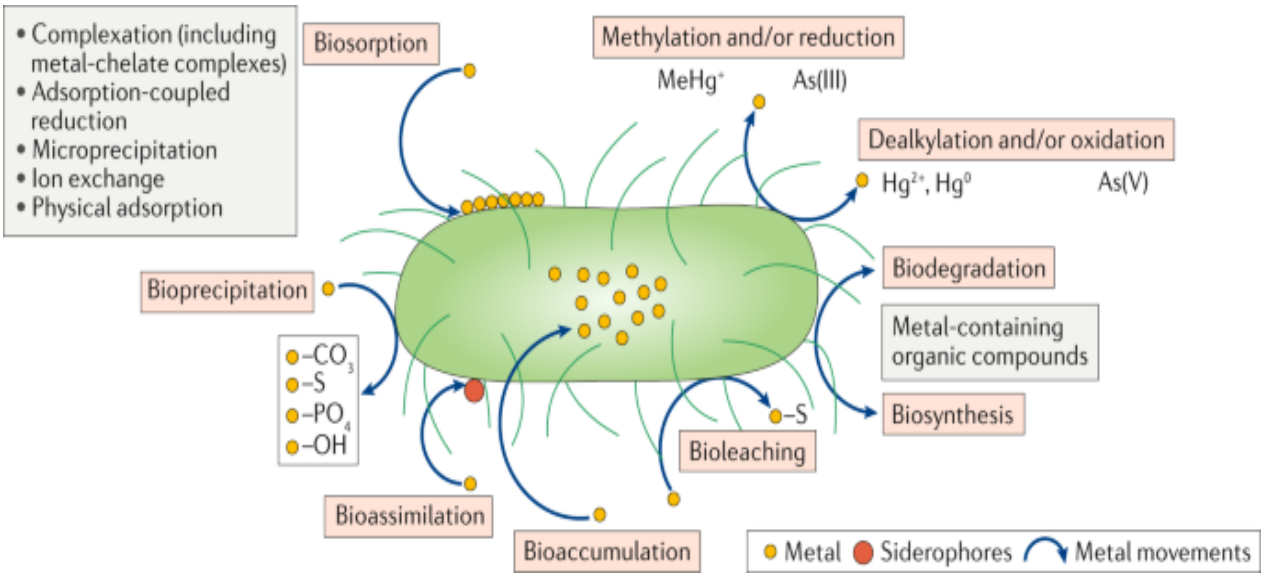

Figure 1.7: Microbial mediated bioremediation of heavy metals/metalloids (adapted from Hou et al. 2020).

Microbes can remove or reduced metal contamination from environments by various mechanisms such as by accumulating metals on the cell surface (biosorption), accumulating metals in the intracellular space by using proteins in their cellular processes (bioaccumulation), assimilating metals via iron-assimilation pathways using siderophores (bioassimilation), reducing or oxidizing metal species, synthesizing or degrading metal-containing organic

compounds via various metabolic activities (biosynthesis or biodegradation), releasing acids and dissolving metal-containing compounds for leaching of metals (bioleaching), precipitating metals (bioprecipitation) e.g. sulfur-oxidizing bacteria produced sulfuric acid which causes leaching of metals, sulfate-reducing bacteria can precipitates metal by the formation of low-mobility sulfides (Fig. 1.7; Hou et al. 2020). Removal of Cd^{+2} and Zn^{+2} up to 78% via biosorption carried out by *P. aeruginosa* B237 and *Tsukamurella paurometabola* A155 (Limcharoensuk et al. 2015), 98% reduction of Cr^{+6} by *Bacillus* sp. CSB-4 (Dhal et al. 2010) in a batch culture experiment has been demonstrated. White et al. (1998) reported >90% metal removal from artificially contaminated soil with multiple heavy metals include Cd^{+2}, Cr^{+6}, Cu^{+2}, Co^{+2}, Ni^{+2}, Mn^{+2}, and Zn^{+2} through bioleaching followed by bioprecipitation approach carried out by sulfur reducing bacteria and sulfur oxidizing bacteria respectively. Several other reports on microbial mediated removal of different heavy metals from contaminated soils or sediments in the laboratory conditions (i.e., microcosm study) have been listed in Table 1.5.

Table 1.5: Heavy metals bioremediation studies in the laboratory (i.e., microcosm study)

Bioremediation (microcosm) study	References
Stabilization of **copper** in the subsurface by biopolymers using **laboratory drainage flow studies**	Etermadi et al. 2003
Stimulated with acetate and bioaugmented with *Geobacter sp.* For **uranium** bioremediation in Northwestern Colorado-**Laboratory column experiment**	Williams et al. 2009
Nonlinear reactive- transport model bioaugmented with *Geobacter sp.* **column packed with the sediment from old Rifle** for **uranium** bioremediation	Zhao et al. 2011
Bioleaching using *Acidithiobacillus thiooxidans DMC and P. aeruginosa* ZH in the **laboratory column filled with Xiangjiang River alkaline sediment** contaminated by **zinc**, **manganese**, **copper**, **cadmium** heavy metals	Zhu et al. 2014
Removal of the **cadmium** in subsurface **vertical flow constructed wetlands** planted with *Iris sibirica* in the low temperature season	Ma et al. 2017

1.5 Linkage between microbial and geochemical parameters

The spatial distribution of microorganisms on our planet is often articulated according to Baas Becking's hypothesis i.e., "everything is everywhere but the environment selects" (Baas Becking 1934). Fondi et al. (2016) proved that environmental factors (ecological niche) shape microbial assemblages via studying global geolocalization of microbial genes reported through

metagenomics sequencing of different environmental samples and further extended the Bass Becking hypothesis. There are many soil/sediment characteristics such as nature of parent materials, texture (lithology) of soil or sediment, topography, soil water content, soil organic matter quality and quantity, pH, salinity, mineralogy and geochemistry, nutrient availability affects the distribution of microbes, microbial activities, and composition of microbial communities in different terrestrial ecosystems (Chen et al. 2016; Freixa et al. 2016; Stegen et al. 2016; Curd et al. 2018; Shao et al. 2020).

Lovley and Chapelle (1995) exemplified a model which demonstrated the influence of lithology (sediment texture) on microbial characteristics (Fig. 1.8). In brief, the clayey bed is enriched in organic C content and the microorganisms present their carried out fermentation of this organic matter into fatty acids or other simpler organic compounds. These simpler organic molecules can diffuse from clayey beds to sandy sediments, where these organic molecules (acids) consumed by microorganisms present there and oxidized into CO_2. In this way, sediment lithology supports the gradient of organic matter and distinctive microbial activity as well as diversity (Fig. 1.8).

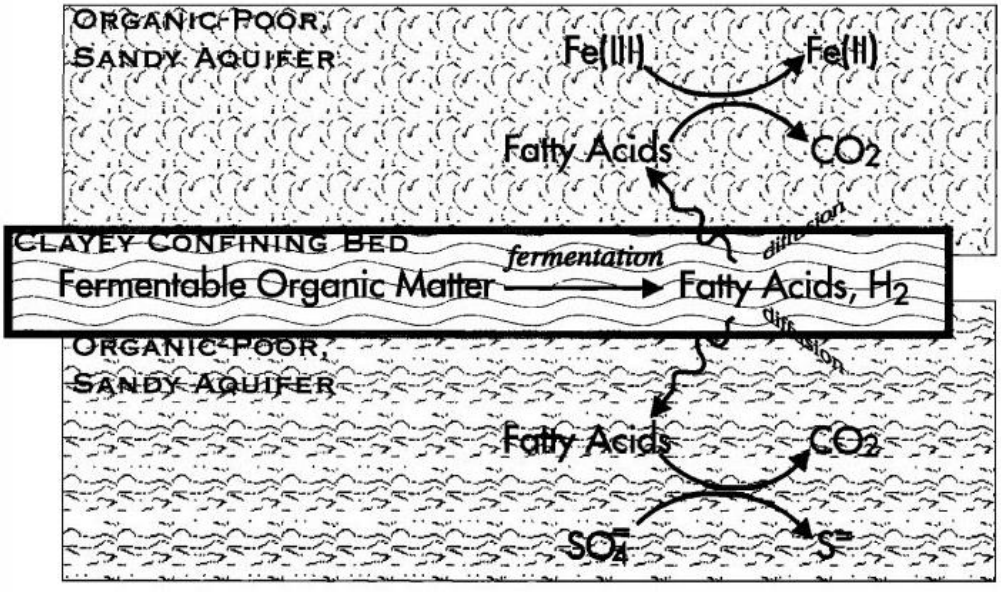

Figure 1.8: Model demonstrating the influence of lithology on organic matter metabolism in deep aquifers. In this, organic matter is fermented within the clayey beds and the fermentation acids diffuse into the sandy sediments, where they are consumed by respiratory processes such as Fe(III) reduction and sulfate reduction (from Lovley and Chapelle 1995).

Albrechtsen and Winding (1992) reported that sediment texture was correlated with microbiological properties especially clay content was correlated with higher numbers of total cells in Quaternary glacio-fluvial sediments in Denmark. Albrechtsen (1994) revealed that viable cell counts and the aerobic microbial activity in terms of C-14 substrate mineralization were highest in the silt-sized particle fraction. Contradictory to that, several studies also reported that clay content was negatively correlated and sand content positively correlated with culturable aerobic bacteria (Balkwill 1989; Fredrickson et al. 1989; Phelps et al. 1994) and anaerobic bacteria (Phelps et al. 1989, 1994), total cell numbers (Sinclair and Ghiorse 1989), aerobic and anaerobic C-14 substrate mineralization activity (Hicks and Fredrickson 1989; Phelps et al. 1989, 1994). The main influences of sediment texture on microbial parameters appear to be due to textural direct relationship with basic sediment physicochemical characteristics including porosity, permeability, organic matter (substrate) availability, water-holding capability, and particle mineralogy (Schjønning et al. 1999; Anawar et al. 2010; Lininger et al. 2018).

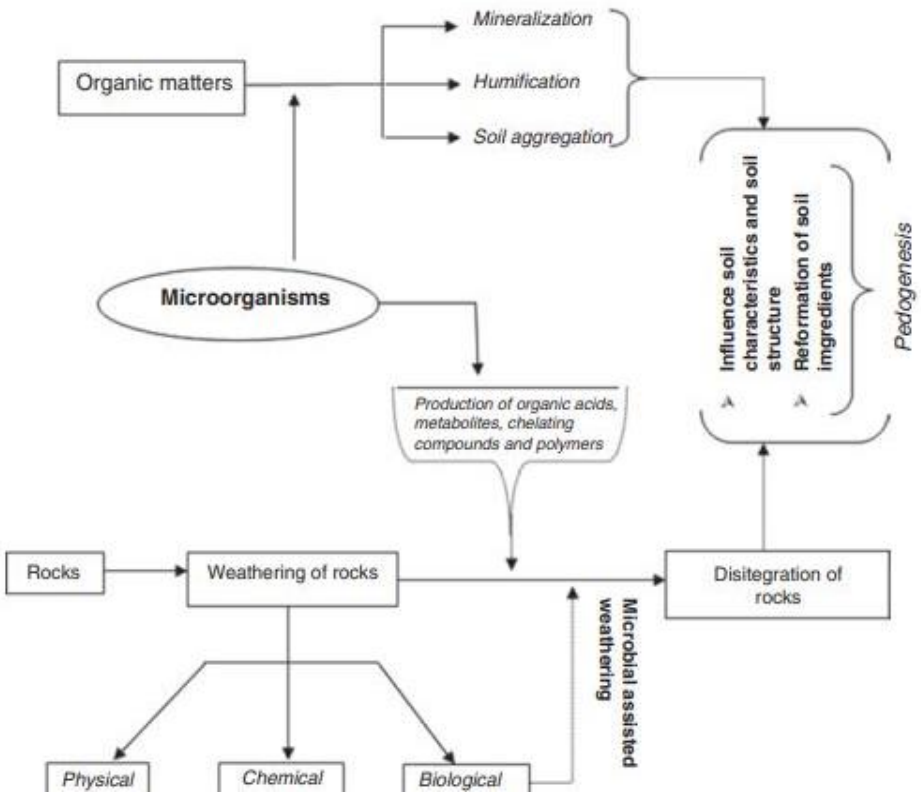

Figure 1.9: Microbial mediated process involved in the decomposition of organic matter and weathering of rocks for pedogenesis (from Kaviya et al. 2019).

The other way around, microbial activities also have remarkable influence on soil/sediment physicochemical properties as microbial activities have an important role in soil formation, pedogenesis, and ecosystem developmental process (Buscot and Varma 2005; Kaviya et al. 2019; Jiao et al. 2021). Figure 1.9 illustrates the role of microorganisms in the pedogenesis process via playing key roles in mineralization, humification process, and the formation of soil aggregates. By the mineralization process, microorganisms recycle nutrients and elements, and by the humification process microbes convert organic matter into humus. Another important function carried out by microbes is 'soil aggregation' where soil particles reside together in the form of stable clumps. Several studies demonstrated that microorganisms produce various extracellular polymeric substances (EPS) that can interact with soil particles (organic material, clay material, and polyvalent metal) and leads to the development of soil aggregates (Davinic et al. 2012; Costa et al. 2018). All these processes influence soil characteristics such as fertility, water-holding capability, nutrients availability, pore size, and pH of the soil (Buscot and Varma 2005; Kaviya et al. 2019). On the whole, microbial and physicochemical parameters have strong relationships with each other and all together determine soil health and quality (Fig. 1.10).

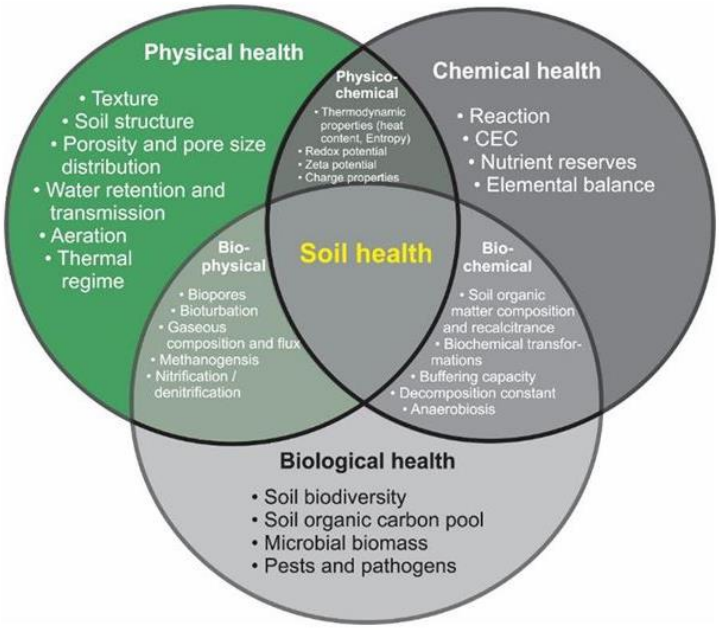

Figure 1.10: Indicators of soil health source: http://www.fertilizer.co.za/knowledge-centre/sustainability/263-soil-health-looking-beyond-soil-fertility). CEC: Cation Exchange Capacity.

1.6 Heterogeneity of river floodplain ecosystems

Natural freshwater ecosystems (include rivers, streams, lakes, ponds, wetlands as well as groundwater) represent the terrestrial phases of the global hydrological cycle and constitute approximately one-tenth of the global land surface area (Reid et al. 2019). The freshwater ecosystems are often subdivided into being "lotic" (flowing) or "lentic" (still) systems. Among which the lotic ecosystem is more imperative as this system serves as an open system that transfers matter, energy, and solutes, including pollutants, from one place to a neighboring place and is connected via a four-dimensional hierarchy (Reid et al. 2019). River ecosystems can be viewed as lotic ecosystems as they comprise highly spatio-temporal variations which manifested due to the dynamic and four-dimensional hierarchical nature of lotic ecosystems. This four-dimensional hierarchy includes horizontal, vertical, and lateral hierarchical arrangements and their integration with hydrologic and geomorphic processes occurring within a temporal hierarchy (Ward 1989). Lateral interactions (that connect aquatic and terrestrial environments) are developed due to the dynamics of floodplain sediments which are considered an integral part of the river ecosystem (Ward 1989).

Moreover, river floodplain sediments mobilized from upstream catchments and harbor a mixture of particles of different sizes, river-born nutrients, and organic matter. Floodplain soils or sediments also provide an important link between the land-ocean aquatic continuums. River terraces represent former levels of the floodplains and have been recognized as sinks and sources of older sediments deposited as stratigraphic sequences over the course of geologic time. Characteristics of river terrace stratigraphic sequences depended on past flood dynamics or periods of inundation, tectonic uplift, changes in climatic conditions, and sea-level fluctuations (Gibling et al. 2011). The dynamics of source and origin, erosion, delivery, and transport of sediments regulate physical and chemical properties of floodplain sediments such as sediment texture, elemental composition, quantity, and quality of organic matters (Nesbitt and Young 1982; Taylor and McLennan 1985; Koiter et al. 2015). Therefore, at a larger scale, river floodplain ecosystems are highly heterogeneous in their physicochemical characteristics nevertheless at a smaller scale each stratum and each location comprise relatively constant physical and chemical properties (Ghiorse and Wilson 1988). Further, around the world, river ecosystems and their floodplains have great economic importance as river landscapes can be

used for various man-made activities such as agriculture, urbanization, and industrial developments.

1.7 Geomorphic setting of the Mahi River Basin

The Mahi River is the third largest west flowing river of India after the Narmada and Tapi Rivers. The Mahi River emerges from the Arravalli Mountains near the Sardarpur district of Madhya Pradesh and meets the Arabian Sea (Gulf of Cambay/Khambhat) near the Vadodara district of Gujarat (Fig. 1.11). Alluvial plains of the Mahi River comprise a total 34,842 km^2 area (which is nearly 1.1% of the total geographical area of the country) from which Gujarat has 11694 km^2 area with semiarid climatic condition (Sharma and Kumar 2018).

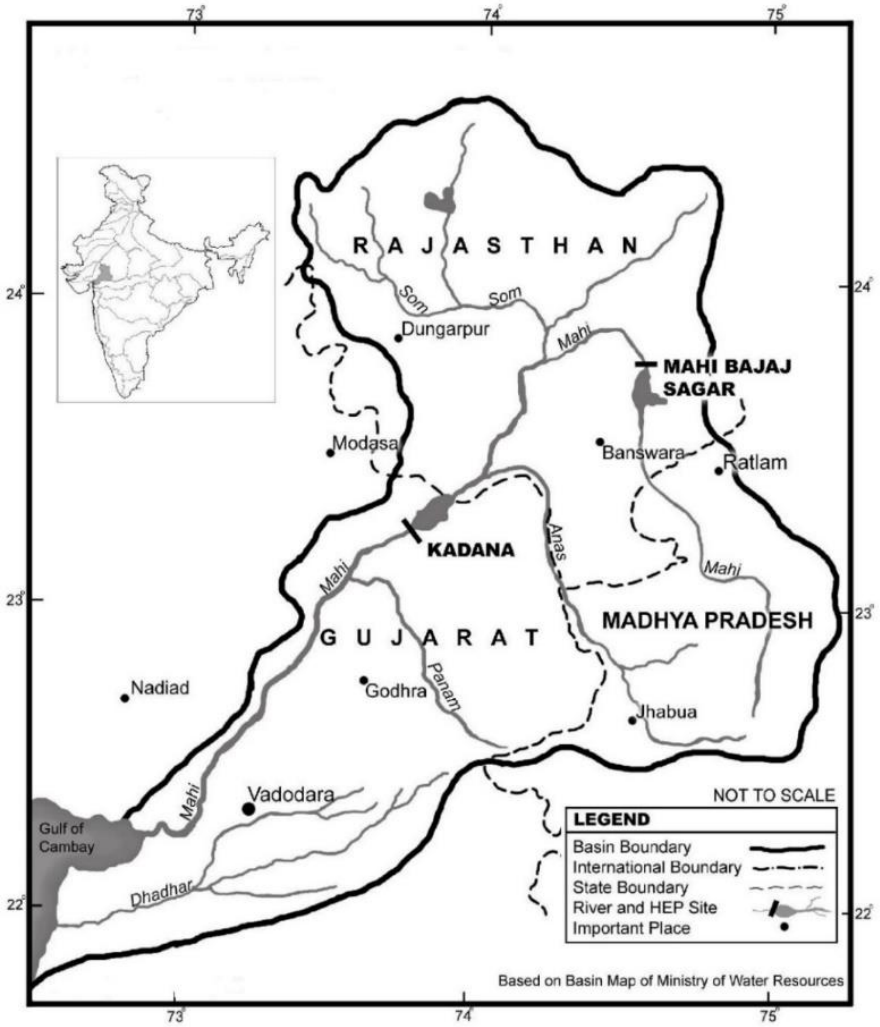

Figure 1.11: Map of the alluvial plain of the Mahi River (Source: https://sandrp.in/themes/basin-maps/).

The geomorphic setting of the lower Mahi river basin is shown in Fig. 1.12. The lower Mahi river basin possesses older and younger two different terraces. The older terrace comprises aeolian, fluvial, and marine sediment deposits (15-35 m cliff) which dated back to ~125 ka (Juyal et al. 2000, 2006). The younger terrace comprises tidal-flat estuarine deposits of the Middle Holocene (5-7 m cliffs) into the pre-existing Late Pleistocene fluvial valley (Maurya et al. 1997a, 2000; Kusumgar et al. 1998).

As shown in Fig. 1.12 Rayka and Rampura (near Nandesari) located at the older terrace surface of the left bank of a river. Dabka, Majpur, and Chokari are located at the younger terrace surface of the left bank of a river while Kothiakhad and Dahevan are located at the younger terrace surface of the right bank of the river (Fig. 1.12). Depositional source of stratigraphic sequences observed at older and younger terraces of the Mahi River basin with their chronological age have been well documented in the earlier studies (Maurya et al. 1997a, 1997b, 2000; Kusumgar et al. 1998; Raj et al. 1999; Juyal et al. 2000, 2006).

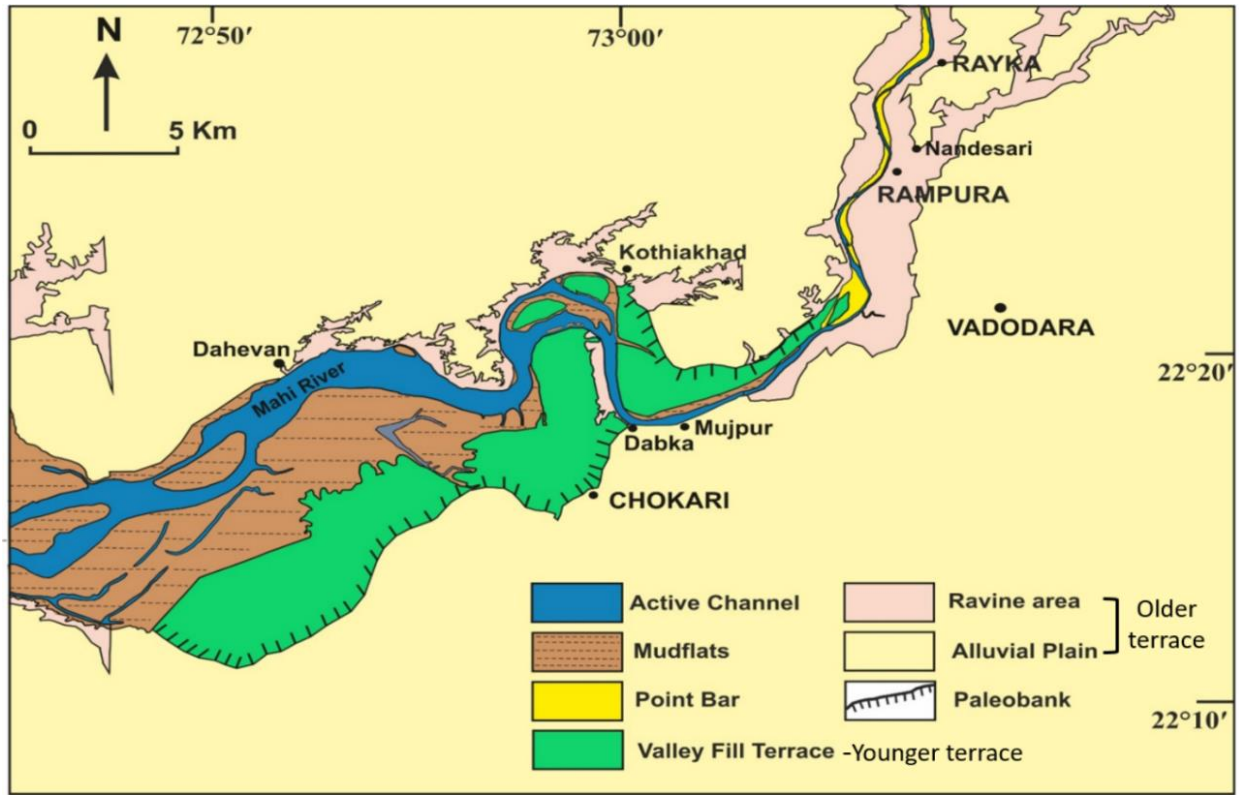

Figure 1.12: Geomorphic map of the lower Mahi River basin. An older terrace comprises a ravine area and alluvial plain while a younger terrace comprises a Holocene valley fill terrace (adapted from Maurya et al. 1997a).

1.8 Aims and scope of the thesis

Microbial parameters and their correlation with physicochemical characteristics provide vital information regarding soil health. Further, a comprehensive understanding of the crucial abiotic parameters that control microbial characteristics in river floodplain may have important implications for improving river floodplain ecosystem functioning, sustainable development, and environmental management (Doran and Zeiss 2000; Baritz et al. 2018). Microbial dynamics and microbial ecology of alluvial and estuarine surface sediments has been widely studied in the different countries of the world and within the Indian subcontinent (Mai et al. 2020; Das et al. 2020; Samson et al. 2019; Verma and Pandey 2019; Lv et al. 2016; Hill et al. 2010; Sinsabaugh and Findlay 1995). In the different countries of the world, microbial characteristics and their correlation with sediment physicochemical properties in the buried subsurface soils/sediments of river floodplains has also been documented (Stegen et al. 2016; Lin et al. 2012; Takeuchi et al. 2009; Kieft et al. 1998). However, understanding microbial characteristics and their abiotic interlinkages within subsurface river floodplain deposits is not yet explored well in the Indian subcontinent. As per our knowledge only a single recent study reported microbial diversity and their relationship with bulk sediment geochemistry in relatively few subsurface sediment samples (spanning 435 to 440 m depth) collected from Krishna Godavari (KG) river basin (Nema et al. 2019). Therefore, in the present study, we investigated the microbial characteristics and their correlation with sediment physicochemical parameters within subsurface sediment deposits of the Mahi River (also known as Mahisagar River) basin for which a reasonably well constrained geological model exists (section 1.7).

Microbiological characteristics of alluvial and estuarine sediments of the Mahi river basin have been studied previously (Subrahmanyam et al. 2011a; 2011b; 2014; 2016; 2021; Subrahmanyam 2013). However, these studies were restricted to surface and exposed cliff sediments. A major finding of these studies revealed that estuarine sediment samples had high microbial activity as compared to alluvial soil/sediment samples (Subrahmanyam et al. 2011a); organic carbon, moisture content, palynofacies components (fungal spores, brown C, structured carbon) Al_2O_3, FeO, MgO, MnO, and Zr are the critical determinants of microbial activity within the exposed cliff sediment samples of Rayka spanning 29 m depth (Subrahmanyam et al. 2021); chronologically older palaeosol (45 ka) was showed high microbial activity and diversity as compared to younger palaeosol (30 ka) (Subrahmanyam et al. 2011b). It is interesting to

study further how stratigraphic sequences of different chronological ages, their mineralogical and textural composition of unexposed (buried) subsurface floodplain sediments influence microbial parameters as buried subsurface sediments have distinct physicochemical characteristics then surface and exposed cliff sediments (refer to section 1.1 for detail). Therefore, in the present study, an attempt is made to understand the relationship of geochemical characteristics with microbial parameters in three sediment cores of the Mahi River basin (Gujarat, India) spanning 28 m in depth. Among these three cores, two cores (located at Rayka and Rampura, denoted as RYD and RMD respectively) lie within the older terrace while one core (located at Chokari, denoted as CRD) lies within the younger terrace. Analysis of laterally deposited cores provides insight into discrepancy of geological and microbial characteristics in both horizontal and vertical directions together with the linkage between microbial and geological parameters provided perception into the microbial ecology of Late Quaternary river floodplain ecosystem.

1.9 Objectives

- To understand the correlation of physicochemical characteristics of the sedimentary sequences and microbial activities in subsurface sediment cores
- Study of microbial diversity in the stratigraphic sequence of sediment cores by culture dependent and culture independent methods
- Determination of relative abundance of genes for selected enzymes from different biogeochemical cycles and establishing functional diversity in the stratigraphic sequence of sediment cores

To fulfill the above objectives the thesis is divided into **three chapters** (Chapters 3 to 5) as follows

1) Microbial characteristics and their association with sediment geochemical properties in 17 m deep estuarine core at Chokari, Mahi River Basin
2) Linkage of sediment indigenous properties with microbial parameters in two laterally deposited Late Quaternary sediment cores of the Mahi River basin: A case study from Rayka and Rampura
3) Evaluation of bacterial strains isolated from subsurface sediment samples at Rayka and Rampura for heavy metal tolerance and Cr(VI) removal ability