Chapter 2
Microbial Diversity, Life Strategies, and Adaptation to Life in Extreme Soils

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2.1 Introduction: What Is an Extreme Environment?

There is no general consensus on how to define an extreme environment. From an anthropocentric point of view, physicochemical conditions supporting mammalian life appear as normal, and conditions deviating from these are considered as extreme. However, what is extreme and what is normal for microbes remains debatable, and the concept "extreme" as we use it may not necessarily be appropriate for micro-organisms (Gorbushina and Krumbein 1999). Micro-organisms dwell in virtually all types of soil habitats. These range from extremely dry and cold deserts in the Antarctic and deep into permafrost soils to geothermal and humid soils in volcanic areas, from extremely acid mines with sulfuric acid to high alkaline areas. Microbial life can also exist in salt crystals, under extremely low water activity, and low nutrient concentrations. As a group, micro-organisms have the highest ability of all life forms to adapt to extreme and stressful environments. This includes new types of habitats created by anthropogenic activities, such as those polluted with heavy metals, radionuclides, and high concentrations of toxic xenobiotic compounds (e.g., polychlorinated biphenyls, hydrocarbons, and pesticides).

Environments which we consider extreme can be inhabited by well-adapted microbiota, and if the environment is stable the resident micro-organisms may not experience any stress but metabolize and grow successfully under strictly limiting conditions, which appear normal to them. During the approximately 3.8 billion years that micro-organisms inhabited Earth, dramatic changes in physicochemical conditions of Earth's surface have periodically occurred. Thus, conditions that can be considered as "normal" for life have also changed.

An alternative view is that any stable environment can be regarded as "normal," and that an extreme environment is one with highly fluctuating conditions where the organisms experience episodic or periodic dramatic environmental changes. In unstable and extreme environments, the metabolic costs to survive stress may be

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high, and most organisms will probably die. However, some micro-organisms have very high physiological and ecological plasticity, which makes them well adapted to survive even in environments where the conditions may change suddenly and dramatically. Organisms that can tolerate considerable environmental stress caused by fluctuating conditions have been termed poikilotrophic or poikilophilic (*poikilo* = various; Gorbushina and Krumbein 1999). Poikilo-environments have prevailing hostile conditions for life (extremely low water potential, extreme temperatures, low nutrients, high levels of toxic substances), but the conditions may occasionally and sporadically change and become suitable for microbial activity and growth. The best examples of poikilotrophic organisms are rock-dwelling prokaryotes and fungi. Organisms living in deserts or arid fell-field soils with extremely low nutrients, precipitation, and highly variable temperatures can also be regarded as poikilotrophic.

A more objective view of extreme environments is one based on the fact that there are specific physical and chemical limitations to cellular processes. These limitations are related to the characteristics of biomolecules and biochemical reactions and set the boundaries for cellular life. Extreme conditions can, according to this view, be defined as those near the limits for cell functioning, that is, limiting for enzyme activities or damaging to biomolecules (Rothschild and Mancinelli 2001; Marion et al. 2003). The best example of a physical limit to life is the presence of liquid water. Life is not possible without water, because it is the solvent necessary for all biochemical reactions. Other constraints are extreme conditions typical at the end of gradients, such as low and high temperature, low and high pH and E_h, high salinity, high radiation doses, high concentrations of toxic compounds, and extremely low nutrient concentrations. Despite the physicochemical limits to biochemical processes and stability of biomolecules, the evolving micro-organisms have extended the boundaries for their life processes.

Organisms living under extreme conditions are divided into different categories according to the nature of their adaptation (Table 2.1). They are classified as thermo- (high temperature), psychro- (low temperature), halo- (high salt), acido- or alkali- (extreme low or high pH), and xero- (low water activity). The suffix -phile is used for those that require the extreme condition for growth, and -troph or -tolerant for those that tolerate the extreme condition. These designations are not exclusive, because two or more factors can be extreme in the same environment, as independent or interrelated conditions. The organisms may therefore belong to multiple categories, and, for example, be considered as both psychro- and xerophile.

The extremophiles are adapted to and limited by very narrow sets of environmental conditions, and they thrive in or require the extreme conditions. Extremotrophic or extremotolerant organisms can survive and proliferate under a wider set of environmental conditions. They tolerate extreme environments but normally grow better at moderate conditions.

In ecology, the predominant growth strategy of organisms is often described as r- and K-strategy (Panikov 1999; van Elsas et al. 2006). Organisms with a predominant K-strategy can live near the carrying capacity of the environment. They have relatively low growth rates, but compensate this by competitive advantages such as

Table 2.1 Micro-organisms in extreme soil

Environmental Conditions	Types of Extreme Micro-organisms	Definitions	Adaptation Mechanisms	Examples
Temperature (low)	Psychrophile	Growth ≤0–20°C; T_{opt}<20°C	Production of unsaturated fatty acids to counteract decreased membrane fluidity. Reduced cell size. Increased fraction of ordered cellular water	*Psychrobacter*
	Psychrotroph	Growth ≤5–30°C; T_{opt}>20°C		
Temperature (high)	Thermophile	Growth 60–80°C	Heat-stable biomolecules (proteins, nucleic acids, lipids). Production of long-chained, saturated, branched fatty acids and cyclic lipids	*Picrophilus sp.*; *Thermobaculum terrenum*; *Pyrolobus fumarii*
	Hyperthermophile	Growth >80°C		
Radiation	Radiation resistant/ tolerant	Tolerate high levels of ionizing radiation (e.g., UV, γ-radiation)	Strong pigmentation (melanoids, carotenoids, etc). Efficient DNA repair mechanisms	*Deinococcus radiodurans*; *Rubrobacter sp.*
Desiccation	Xerophile/xero-tolerant	Water potential MPa: −4 to −10 (bacteria) −10 to −70 (actinomycetes and fungi)	Accumulation of inorganic or organic osmolytes; Production of extracellular polysaccharides; Differentiation into desiccation resistant cells (spores)	Actinobacteria: "group-1 Rubrobacteria" and members of Actinomycetales; Cyanobacteria, fungi
Salinity	Halophile/halotolerant Osmophile	2–5 M NaCl; water potential −1.5 to −40 Mpa	Accumulating osmolytes. Salt-tolerant (and salt-dependent) enzymes	*Haloarcula japonica*; Cyanobacteria
pH (low)	Acidophile	pH_{opt} ≤2–3	Proton exclusion, efficient efflux system. Acid stable membrane. Proton-driven secondary transporters	*Acidithiobacillus; Picrophilus sp.*; *Ferroplasma sp*
pH (high)	Alcaliphile	pH_{opt} ≥9	Membrane impermeability for OH-ions. Efficient proton uptake mediated by membrane antiporters. Negatively charged cell wall polymers	*Natronobacterium; Bacillus firmus OF4*
Heavy metals	Metalo-resistant/ tolerant	Tolerate high metal concentrations	Efflux pump. Sequestering and/or detoxification of metals (reduction, alkylation)	*Ferroplasma acidarmanus*
Toxins and xenobiotics	Toxin-resistant/tolerant; Xenobiotic decomposers	Tolerate high concentrations of toxic compounds	Efficient efflux pump. Detoxification or decomposition of toxins and xenobiotics	*Ralstonia sp.*; *Pseudomonas putida*

high affinity for substrates, low maintenance energy, the ability to uncouple growth from transport, and accumulation of storage polymers. In contrast, r-selected organisms have the potential of rapid proliferation and fast response to abundant and readily available substrates. A specific life strategy, designated L-strategy, is used to characterise organisms that are selected under unfavorable conditions and are highly tolerant or resistant to stress (Panikov 1999). This also includes micro-organisms with specific adaptation to manmade stress conditions. Thus, soils may be characterized as extreme which support the growth of micro-organisms that can tolerate anthropogenic disturbance and adverse conditions caused by high concentrations of pollutants and toxic compounds.

2.2 Physicochemical Factors Limiting to Life

2.2.1 Water

Soil water is either adsorbed onto surfaces or present as free water in pores or films between soil particles. The soil water status is described by the water potential, which is a measure of the energy and forces that hold and move water in the soil. It relates to water activity (a_w) and is the difference in free energy (in pascals or Pa; energy per unit mass) between pure water and soil water. The main components of soil water potential are the matric potential (the energy with which soil water binds to solid surfaces or is retained in pores) and the osmotic potential (a function of dissolved salt concentrations; Vetterlein and Jahn 2004). Because these components reduce the free energy of water, the water potential is negative. This indicates the energy that organisms must exert to withdraw water from soil, and as the water potential decreases, water becomes less available and the stress level of organisms increases.

Extreme soil water stress occurs periodically in most soils, even in climates with ample precipitation, where the water availability depends on soil composition, rainfall drainage, and plant cover. Soil prokaryotes live in water films surrounding particles or inside water-filled pores, and are therefore very susceptible to water depletion. Soil fungi are normally more tolerant to water stress than prokaryotes. Furthermore, they can tolerate drought due to hyphal growth, which allows them to cross dried pores and obtain water from smaller pores where the water remains for longer periods (Killham 1994). Prokaryotic cells have a turgor pressure, with a concentration of solutes inside the cell being slightly higher than that outside. Changes in water activity in the environment are rapidly followed by a water flux across the semipermeable cell membrane from high to low water potential. This can cause swelling and lysis of the cells under hypotonic conditions or dehydration under hypertonic conditions (Kempf and Bremer 1998). Therefore the cell must maintain an intracellular water potential similar to that existing outside the cells.

To maintain cell integrity at nonextreme temperatures (10–40°C) usually requires soil water potential above −4 MPa (0.95 a_w) for most bacteria, and above −22 MPa (0.86 a_w) for actinomycetes and most fungi. It is generally considered that the lower limit of water potential for life is −70 MPa (0.60 a_w; Zvyagintsev et al. 2005), but recently it has been demonstrated that spore germination and elongation of some actinomycetes can occur at water potential of −96 MPa (0.50 a_w; Doroshenko et al. 2005; Zvyagintsev et al. 2005). Some organisms, for example lichens, can even survive on water vapor rather than liquid water. Inasmuch as cell damage cannot be repaired during desiccation, these organisms must exhibit an efficient repair system upon rehydration.

Xerotolerant or xerophile micro-organisms are able to withstand water and salt stress because they can counterbalance a low water potential in the environment by accumulating highly soluble small molecules in the cytoplasm (Kempf and Bremer 1998). The solutes can be inorganic salts or organic molecules (amino acids, polyols, carbohydrates, quaternary ammonium compounds). The accumulation of solutes results in decreased internal water potential. These molecules can influence and modulate specific enzyme activities, but do not inhibit the overall metabolism of the cells, and are therefore termed compatible solutes or osmoprotectants (see Section 2.2.2). Some compatible solutes are constitutively produced, whereas others are induced. Osmoregulation is an energy-requiring process, but seems to be a general mechanism enabling soil micro-organisms to preserve the activity of intracellular enzymes under long-term and severe water stress.

Other strategies that protect prokaryotes from desiccation are the production of extracellular polysaccharides which retain water (Wright et al. 2005). Formation of microaggregates of cells where elevated water activities are retained may further protect micro-organisms from desiccation. Actinomycetes are particularly well osmoregulated, as their cell membranes have restricted permeability and keep salt ions out and organic solutes inside the cells. Like fungi, they can differentiate into dormant cells that are resistant to drying (Dose et al. 2001).

2.2.2 Salinity

Salt or osmotic stress is closely related to water stress because solutes strongly affect the water activity. In contrast to water stress which occurs frequently in most terrestrial habitats, high salinity typically occurs in restricted habitats. Soils with high salinity are often characterized by highly uneven temporal and spatial water distribution (Brown 1976). Such fluctuations cause special stress for the microbes and reduce their ability to survive, because they need to respond rapidly to desiccation and adapt to high salt concentrations (see Chapter 5).

Saline terrestrial habitats are typical for naturally arid regions with high evaporation rates. They may also be the result of pollution from mining activities or chemical and metallurgic industries. Most of the micro-organisms that inhabit saline soils are salt tolerant (halotolerant), but also halophilic micro-organisms

that require salt for maintaining their membrane integrity and enzyme stability and activity are present. Extremely halophilic prokaryotes can tolerate very low water potential, and grow well at −40 Mpa (0.75a_w, the value of saturated NaCl solution). This limit is determined by the solubility of salt rather than by the physiology of the cells (Brown 1976). As with the xerophile and xerotolerant organisms, the halophiles and halotolerant organisms accumulate a variety of small molecules in the cytoplasm (osmolytes or compatible solutes; see Section 2.2.2) to counteract the external osmotic pressure (Kempf and Bremer 1998; Roberts 2005). Generally, bacteria produce and accumulate compatible organic solutes that are zwitterions (e.g., proline, glycine betaine, ectoine, methylamines, and derivatives) or nonionic (such as polyols, carbohydrates, neutral peptides, and amino acids and derivatives).

In archaea the osmolytes are often inorganic cations that are taken up by passive diffusion or by selective ion transport across the membrane. Many archaea have evolved negatively charged acid polypeptides that require cation counterions such as K^+ for proper protein folding and activity. Organic osmolytes that accumulate in archaea belong to the same types as for bacteria, but the majority of the solutes in archaea are anionic (due to negatively charged groups such as carboxyl, phosphate, and sulfate groups; Martin et al. 1999). In addition to their functions as osmotically active substances, the compatible solutes may function as chemical chaperones that protect proteins from denaturation and increase their activity.

2.2.3 Temperature

The temperature limits of life are related to the boiling and freezing points of water. However, many micro-organisms have developed mechanisms to extend the temperature ranges beyond the values for pure water and atmospheric pressure. At present, the temperature limitations for microbial activity are regarded as ranging from approximately −40°C to +130°C (Kashefi and Lovley 2003; Price and Sowers 2004). Arctic micro-organisms are well adapted to an extremely cold climate and several authors have reported microbial activities at temperatures as low as −10 to −20°C (Panikov and Dedysh 2000; Bakermans et al. 2003; Jakosky et al. 2003; Callaghan et al. 2004; Gilichinsky et al. 2005; see Chapter 7). At subzero temperatures there can still be liquid water present in soils, as adsorbed water forms a thin liquid film on the surface of soil particles (hygroscopic water; Rivkina et al. 2000; Steven et al. 2006).

Growth at low temperatures requires significant membrane alterations in order to maintain the fluidity necessary for nutrient transport across the membrane. The low temperature modifications involve less saturated and less branched membrane fatty acids. Below the minimum growth temperature the membrane becomes solid and transmembrane transportation stops. Life at subzero temperatures is also facilitated by accumulation of antifreeze compounds (high concentrations of salts, hydrocarbons, or amino acids) in the cytoplasm. Archaea have

many of the same mechanisms for adaptations to low temperatures as bacteria; these involve altered membrane composition (cold-adapted lipids) as well as cold-active proteins involved in fundamental cell functions (e.g., protein synthesis; Cavicchioli et al. 2000).

In psychrophiles, the specific cold adaptation implies such drastic changes in the chemical composition of the cell that life outside of cold environments becomes impossible. For example, micro-organisms adapted to low temperatures have enzymes and ribosomes becoming unstable at temperatures 1–2°C above their optimum temperatures. Accordingly, the psychrophiles have optimum temperatures at or below 15°C and maximum temperatures below 20°C. The psychrotrophic organisms can also grow at temperatures close to or even below 0°C, but their optimum temperature is above 15°C, and their maximum temperature can be as high as 30 to 40°C. Price and Sowers (2004) studied the temperature dependence of metabolic rates in different environments including permafrost. These authors distinguished three categories of metabolic rates: first, rates sufficiently high to allow growth; second, intermediate rates sufficient for maintenance of functions, but too low for growth; and third, basal rates sufficient for survival of cells and repair of damaged macromolecules, but otherwise permitting only cell dormancy. They did not observe any minimum temperature for metabolism, but at low temperatures the metabolic rate was extremely low. At elevated temperatures, micro-organisms from permafrost showed metabolic rates similar to those found in temperate soils.

At the other extreme of the temperature range for life are the thermophiles and the thermotrophs. Thermophilic organisms cannot grow at temperatures below 50°C, whereas the thermotrophs have a lower temperature limit (20–30°C). At their upper temperature limit, cells undergo instability and irreversible denaturation of their proteins and nucleic acids, and therefore the ability of these molecules to perform their functions is lost. Thermal soils, with temperatures above 50°C, can be natural or manmade (see Chapters 8 and 14). Thermophilic micro-organisms have been isolated from natural thermal soils such as decomposing litter, volcanic, geothermal and tropical desert soils, and from manmade thermal soils such as compost piles and coal refuse piles (Botero et al. 2004).

Micro-organisms adapted to high temperatures have mechanisms for protecting their proteins and nucleic acids from irreversible denaturation. Biomolecules from such organisms are thermostable and remain active at temperatures that generally inactivate proteins, lipids, and nucleic acids in mesophilic organisms (Rothschild and Mancinelli 2001). In some proteins, the stabilization is caused by changes in amino acid residues that make the proteins more hydrophobic and increase the stability of subunit interactions (Singleton and Amelunxen 1973). The nucleic acids are also thermostabilized, for example as the result of interactions with histonelike proteins. At high temperatures, the membrane fatty acids acquire longer chains, and they become more saturated and more branched. Such changes in the membrane structure and composition lead to decreased membrane fluidity and consequently better thermostability (Pakchung et al. 2006).

2.2.4 pH

Taken as a group, prokaryotes can live in environments with pH values ranging from below 0 to 13 (Marion et al. 2003), although most prokaryotes grow at relatively narrow pH ranges close to neutrality. Extreme acidophiles grow in the pH range 0 to 3. It seems that a general adaptation to extreme pH is to regulate the intracellular pH and keep it close to neutral. The bacterium *Acidithiobacillus ferrooxidans* (previously *Thiobacillus ferrooxidans*) lives in acidic environments with a pH of about 1 (see Chapter 10). However, its intracellular pH is around 5.5, which indicates an active mechanism for excluding protons.

Whereas all known cytoplasmic enzymes have pH optima from pH 5 to pH 8, some enzymes found in the bacterial outer membrane tend to have low pH optima. Among archaea there are acidophiles that can grow at pH 0. Members of the cell wall-lacking archaeon *Ferroplasma* have been isolated from highly acidic environments associated with sulfide ores, solfatara fields, and the like (Golyshina and Timmis 2005). They can mobilize metals from sulfide ores and tolerate high concentrations of heavy metals (Edwards et al. 2000; Baker-Austin et al. 2005). The extremely thermoacidophilic archaea *Picrophilus torridus* and *P. oshimae* were first isolated from solfataric Japanese soils. They have optimal growth rates at pH 0.7 and 60°C (Schleper et al. 1995). In contrast to most other acidophilic micro-organisms, the intracellular pH is very low (pH 4.6), and *P. torridus* cannot grow above pH 4.0. A common feature of acidophile and acidotolerant micro-organisms is the presence of acidophilic lipids with cyclic rings and alkyl side chains in their fatty acids. Adaptation to acid environments is also enhanced by unusual tetraether lipids in the cell membrane (in *Ferroplasma* and *Picrophilus*) or lipopolysaccharides with a low content of fatty acids (in *Acidithiobacillus*). The tetraether lipids render the membrane of acidophilic archaea impermeable to protons, and a proton pump expels protons from the cytoplasm. High ratios of genes for proton-driven secondary transporters over ATP-consuming primary transporters indicate that the cells utilize the proton gradient for solute transport into the cell (Futterer et al. 2004).

Extreme alkaliphilic micro-organisms have pH optimum between 9 and 11, and do not grow near neutral pH. However, the cytoplasmic pH is at least two units lower than the external pH (Krulwich et al. 1998), which indicates that the cell membrane presents an efficient barrier to fluxes of OH^- ions and that there is an efficient inward proton translocation system (e.g., Na^+/H^+ or K^+/H^+ antiporters, proton-translocating ATP synthase). pH in bulk soil is generally between 4.0 and 8.5 (Lynch 1979). Decomposition of plant litter tends to reduce the pH, and soil with accumulated organic matter is normally acid. Redox reactions also influence the soil pH. For example, oxidation of NH_4^+, S, and FeS_2 results in production of mineral acids such as HNO_3 and H_2SO_4, and thereby decreases the pH. Thus, it is apparent that prokaryotes exert a profound influence on their own environment. As a result of metabolic activity, the pH in microbial microhabitats can be at least 3–4 pH units lower than in the bulk soil. High pH is common in soils containing alkaline minerals, or occurs temporally in restricted zones, for instance due to the presence of animal excretions.

Anaerobic reactions, such as the reduction of nitrate to N_2 and of sulfate to sulfide, also increase the pH. Recently alkaliphilic psychrotolerant bacteria were isolated from permafrost soil in the Qinghai-Tibet Plateau (Zhang et al. 2007). The colony-forming units of alkaliphilic bacteria in these soils varied between 10^2 and 10^5 cells g^{-1} of dry soil. The isolates could grow at pH 6.5–10.5 with optimum pH of 9.0–9.5, and optimum growth temperatures of 10–15°C.

2.2.5 Radiation

High doses of ionizing radiation and ultraviolet (UV) light are lethal to most microbes, although some of them can tolerate surprisingly high radiation doses (see Chapter 16). Normally high correlations are observed between tolerance towards radiation, desiccation, and DNA damaging chemicals (Shukla et al. 2007). *Deinococcus radiodurans* is the most radiation-resistant organism known and can survive doses of 1,000 J/m^2 UV-light, and more than 20 kGy of γ-radiation (1 Gy = 100 Rad), which is approximately 4,000 times the dose that will kill a human (Battista 1997; Marion et al. 2003; Rainey et al. 2005). This micro-organism is remarkably well adapted to extreme conditions as it can survive drought and lack of nutrients, in addition to extremely high radiation dosages (Battista 1997). This red spherical bacterium was discovered in 1957, in a can of ground meat that was spoiled despite having been sterilized by radiation. The bacterium is widely distributed and has been found in a variety of soil environments as well as in granite in Antarctic dry valleys.

Dehydration and radiation cause very similar types of DNA damage. Resistance is conferred on *Deinococcus* by a particularly efficient system for DNA repair. Desiccation or high doses of radiation lead to massive double-strand breaks in DNA. *Deinococcus* has 4–10 copies of its chromosome (Battista 1997) and repairs the fragments by intrachromosomal recombination that reconstitute an intact chromosome in just a few hours (Minton and Daly 1995; Sale 2007). Prokaryotes and algae on surfaces of barren polar soil have adaptive strategies that allow them to avoid, or at least minimize UV injury. In this case, substances such as pigments and amino acids (e.g., melanoids, carotenoids, scytenomin, mycosporine-like amino acids) protect against the excessive light radiations and oxidative damage (Ehling-Schulz et al. 1997; Bowker et al. 2002; Wright et al. 2005).

2.2.6 Low Nutrients

Environments characterized by extreme physical or chemical conditions, such as desert and permafrost soils, are often poor in organic and inorganic nutrients. Most of the indigenous micro-organisms in such environments are probably oligotrophs, which means they are adapted to low nutrient supply rates. However, high numbers of oligotrophic prokaryotes may also be found in bogs and other soils with high

amounts of organic matter. In such soils the major organic compounds are humic matter that is recalcitrant and not readily decomposable (Koch 2001; Fierer et al. 2007). Bacteria in the phylum Acidobacteria are generally regarded as oligotrophic. They are especially abundant in soils with low resource availability and their abundance decreases after amendment with a readily available carbon source (Fierer et al. 2007). Prokaryotes that are adapted to grow in oligotrophic environments are normally K-selected (Bernard et al. 2007). They have low growth rates, but very efficient uptake systems with low half-saturation constants (down to nM levels) for uptake of organic substrates. This adaptation often results in their inability to grow under high nutrient levels.

Other mechanisms for adaptation to low nutrient levels are the ability to use many different substrates simultaneously (Eichorst et al. 2007). In environments where nutrient supplies fluctuate, prokaryotes can store nutrients as intracellular polymers (e.g., polysaccharides, poly-β-hydroxybutyrate, polyphosphate). However, in constant oligotrophic environments, especially cold environments, the nutrient supply probably is too low to support any intracellular storage. It has been suggested that the organisms' affinity for substrates decreases at low temperature due to loss of membrane fluidity that impedes active transport, and that the minimum substrate concentration needed for growth therefore increases near the organisms' lower temperature limits (Wiebe et al. 1992). If liquid water is present, growth limitation by decreased temperature may be the result of reduced active uptake of nutrients, which eventually becomes so low that the cell's minimum maintenance requirements is no longer met (Nedwell 1999).

2.2.7 Pollution

Acid deposition has affected soil microbial communities and activities for some decades. This pollution is caused by acid precipitation, the result of nitrogen oxide (NO_x) and sulfur dioxide (SO_2) emitted into the atmosphere and oxidised to SO_4^{2-} and NO_3^-. Despite the effort to reduce the primary sources of acid input, the effect is still apparent in many regions. The effect of acid deposition on soil ecosystems depends on the concentration of SO_4^{2-} and NO_3^-, the amount of precipitation, and the buffering capacity of the soils (the cation exchange capacity through bases). Nitrogen and sulfur provided by acid rain may stimulate growth of some soil micro-organisms. On the other hand, even low-level but prolonged acid rain will result in soil acidification that may have adverse effects on soil bacteria, whereas the effect on fungi seems to be minor (Pennanen et al. 1998a; Bååth and Anderson 2003).

The effect of acid deposition can be direct or indirect. The lower pH and reduced concentrations of divalent cations (Ca^{2+}, Mg^{2+}) can lead to mobilization and increased bioavailability of heavy metals and other toxic compounds (Francis 1986). Acidification of soils may also reduce the solubility of organic matter and thereby reduce the substrate availability for microbes. Increased soil acidity does not seem to affect prokaryotic biomass to any extent, but rather to reduce prokaryotic

growth rates and activity (Francis 1986; Pennanen et al. 1998b). Reduced activity of a number of soil enzymes, such as dehydrogenases, ureases, and phosphatases have been observed at significant pH reductions (Killham et al. 1983). The reduced microbial growth observed with increased acidity may indicate that more metabolic energy is used for maintenance rather than for biosynthesis of cell materials. It has been suggested that increased metabolic quotient (qCO_2, the ratio of basal respiration to microbial biomass) indicates a shift in energy use from growth to maintenance, and that increased energy demand is a sensitive indicator of physiological adaptation to environmental stress (Post and Beeby 1996; Liao and Xie 2007).

Soil can have naturally high concentrations of heavy metals as the result of weathering of parental material with high amounts of heavy metal minerals (e.g., mineral sulfides). Other sources are contaminations associated with mines and metal smelters, which have led to increased soil concentrations of heavy metals such as zinc, cadmium, copper, and lead. Sewage sludge may also contain heavy metals, and it has been demonstrated that long-term application of heavy metal containing sewage sludge to agricultural soils can have profound effects on the microbial diversity and community composition (Sandaa et al. 1999; Gans et al. 2005). The effect of heavy metal toxicity depends on soil abiotic factors such as organic matter and clay content, divalent cation concentrations (cation exchange capacity), and pH (Giller et al. 1998). These factors influence complex formation and immobilization of heavy metals.

Irrespectively of soil types, however, the relative toxicity of different metals seems to be the same, namely Cd > Cu > Zn > Pb (Bååth 1989). In soil contaminated for 40 years with high concentrations of Cr and Pb, the microbial biomass and activity was reduced and soil organic carbon accumulated (Shi et al. 2002). These results indicated that Pb presented greater stress to soil microbes than Cr. Soil micro-organisms vary widely in their tolerance to heavy metal contamination, and the proportion of culturable resistant micro-organisms can range from 10% to nearly 100%. The activity of enzymes in soil may serve as indicators for heavy metal contamination as there are generally high correlations between reduced enzyme activities (of, e.g., dehydrogenases, acid phosphatases, and ureases) and increased heavy metal contamination (Bååth 1989). It has been reported that heavy metal contamination has a different effect on soil bacteria and fungi (Rajapaksha et al. 2004). Metal addition decreased bacterial activity whereas it increased fungal activity, and the increased fungal activity was found to persist in contaminated as compared to control soil after 35 days. The different effect of heavy metals was also demonstrated by an increase in the relative fungal/bacterial ratio (estimated using phospholipids fatty acid analysis) with increased metal concentrations.

Mechanisms for metal resistance include stable complex binding (chelation) with organic ligands (extracellular or intracellular sequestering), transportation out of the cells, and biotransformation of the ions to less bioavailable or less toxic metal species. Genes for metal resistance (e.g., mercury resistance) are often harbored on plasmids and can easily be disseminated through a population or a community in response to selection pressure associated with toxic metal exposure (Drønen et al. 1998).

Hydrocarbon contamination of soils caused by human activities increasingly occurs in all parts of the world. Petroleum is a rich carbon source and most of the hydrocarbon components are biodegradable by micro-organisms. The rate of degradation is normally rather low, because crude oil has low concentrations of phosphorus and nitrogen, which do not allow extensive growth of indigenous hydrocarbon-degrading micro-organisms in petroleum-contaminated soils. However, growth can be stimulated by addition of phosphorus and nitrogen fertilizers. In many extreme environments, hydrocarbon-polluted areas are found (Margesin and Schinner 2001). The success of bioremediation in such environments depends on the presence of biodegrading microbes that are adapted to the prevailing environmental conditions.

Pesticides are classified according to their primary target organisms, that is, herbicides, fungicides, and insecticides (Johnsen et al. 2001). Normally the pesticides are very specific and restricted to a narrow range of target organisms. However, they can be modified in the environment and become toxic also to nontarget organisms. For instance, triazines, which normally target photosynthetic enzymes in C3 plants, may be chlorinated in the triazine ring and thus become toxic to a wide range of organisms. The effect of pesticides on soil microbes depends on their bioavailability, which in turn is influenced by the crop being grown, as well as soil properties affecting the sorption and leaching of pesticides. The micro-organisms can develop resistance to the pesticides through their ability to decompose or transform them to less toxic compounds.

2.3 Soil as Habitat for Micro-Organisms

Soil has been defined as the upper weathered layer of the earth's crust, with a complex mixture of particulate materials derived from abiotic parent minerals, living biota, and particulate organic detritus and humic substances (Odum 1971). Formation of soil is the result of climate (temperature and moisture), parental material, time, topography, and organisms (Jenny 1994), and involves complex interactions of physical, chemical, and biological processes. Soil texture (the relative proportion of particles with different sizes) and mineral constituents depend on the parent material (rocks), and transportation by water, ice, and wind. Soil structure is the distribution of pores of various sizes that occur between soil particles. The pore sizes depend on the level of aggregation of soil particulate material, and the pores contain gases and water.

The vegetation and soil biota affect soil development by weathering and controlling organic matter accumulation and mineralization. The recognition of close interactions between soils and vegetation is reflected in the division of soils into major types, which are associated with climatic vegetation zones. Micro-organisms are able to modify and shape their physical and chemical environment. They dissolve and alter minerals derived from the parental material, contribute to and mineralize soil organic matter, and recycle nutrients. Microbes produce biopolymers

(polysaccharides) as cell envelopes. Such polymers facilitate formation and stabilization of soil aggregates, and thereby improve the soil water-holding capacity. Together with colloid clay particles and humus, the polymers create complex structures with extensive surfaces, which adsorb minerals and organic molecules. Adsorption of proteins and nucleic acids to surfaces protects them from biodegradation and denaturation. Adsorbed DNA remains available for horizontal gene transfer by transformation of competent cells (Lorenz and Wackernagel 1994). The activity of extracellular enzymes is maintained or even increased by adsorption on minerals, whereas adsorption to humic substances can either maintain or decrease their activity (Nannipieri et al. 1990; Allison 2006). The adsorption to soil colloids may strongly reduce the availability of organic molecules as nutrients for micro-organisms, and contribute to soils being oligotrophic environments.

Surfaces of soil minerals, especially clay colloids, can serve as catalyst for abiotic chemical reactions. Clay particles are coated with metal oxides and hydroxides and have net electronegative charges. They can mediate electron transfer reactions and catalyse oxidation of phenols and polyphenols. They also contribute to humus formation by catalysing reactions such as deamination, polymerization, and condensation of organic molecules. It has been suggested that microbial processes such as decomposition and mineralization of organic substances prevail under moderate conditions, whereas abiotic reactions are more dominant under harsh conditions where microbial activities are hampered (Huang 1990; Ruggiero et al. 1996).

Soils are among the habitats that have been shown to support the highest abundance and diversity of micro-organisms. Soil habitats are distinguished from aquatic habitats by being much more complex and spatially heterogeneous. A characteristic feature is the wide range of steep physicochemical gradients (e.g., of substrate concentrations, redox potential, pH, available water) which may occur across short distances approaching the size of a soil aggregate. Thus, even an aggregate of a few mm can offer many different microenvironments that would collectively be colonized by different types of micro-organisms (Standing and Killham 2006). The size scale of microhabitats is typically a few μm for unicellular prokaryotes, but may be much larger for filamentous actinomycetes and fungi. Microhabitats for prokaryotes exist either within or between aggregates. Intra-aggregate habitats have typically small pores that are often water-filled and anaerobic, whereas inter-aggregate habitats are more frequently aerobic. However, the living conditions in these habitats can undergo considerable changes both in space and time, therefore soils are highly dynamic systems.

The distribution, activity, and interactions (e.g., predation) of soil biota depend on soil properties such as texture, structure, and available nutrients and water. The growth conditions are normally most favorable on surfaces, and most (80–90%) of the soil micro-organisms are attached to surfaces (Hattori et al. 1997), often aided by extracellular biopolymers which stick to particles. However, surfaces also expose micro-organisms to the highest risks of desiccation and predation. Specific soil habitats such as organic litter aggregates, biofilms, rhizosphere, and animal droppings, are rich in readily available organic nutrients and can support very high microbial activities. The bulk soil on the other hand often contains low levels of

easily decomposable substrates, and most of the organic matter is refractory. Thus the distribution of biomass and activities of soil microbes is generally very patchy, and the space that is occupied by micro-organisms may be less than 5% of the overall space in soil (Nannipieri et al. 2003).

Moderate soils with no stress factors are characterized by a high microbial abundance (10^9-10^{10} prokaryotes g^{-1} soil dry weight) and high genetic, phylogenetic, and functional diversity of microbial communities (Giller et al. 1997; Torsvik and Øvreås 2006). Micro-organisms are by far the most active and functionally diverse component of the soil biota. It has been estimated that 80–90% of the soil processes are mediated by the microbiota, including prokaryotes and fungi. Generally, about one third of the organic carbon added to temperate soils is transformed to humus and microbial biomass, whereas about two thirds of the carbon is respired to CO_2 by micro-organisms (Stotzky 1997).

Interestingly, the deep subsurface terrestrial environments, which can extend for several hundred meters below the soil surface, have been proven to sustain ample microbial biomasses. Although the cell numbers are much lower than in the surface soil, a variety of micro-organisms, primarily prokaryotes, is present in deep subsurface soils. For example, in samples collected aseptically from bore holes drilled down to 300 m, a diverse array of micro-organisms has been found. These organisms most likely have access to organic nutrients present in the groundwater percolating down the subsurface material and flowing through their habitat. Studies on the microbial ecology of deep basalt aquifers have shown that both chemoorganotrophic and chemolithotrophic prokaryotes are present, but that the chemolithotrophs are dominating in these environments (Stevens and McKinley 1995).

2.4 Extreme Soils

Extreme soil microbiology deals with micro-organisms adapted to extreme or stressful soil conditions. Soil properties are determined by the parental material (geological properties), climate, and biota, and are influenced by anthropogenic activities. Odum (1971) divided soils in two categories, those which are mainly controlled by climate and vegetation types, and those which are mainly controlled by parent materials or other pedological or environmental factors (topography, drainage, pollution, etc.). These controlling factors can strengthen each other. In regions with extreme climate, minor differences in edaphic factors may create large differences in the structure and activity of soil microbiota.

In many temperate climate zones, soil water and/or temperature stress occurs periodically. Also nutrient stress occurs periodically in many soils, and this will influence the soil microbiota so that organisms adapted to periodic stress become dominant. In some areas, wide fluctuations in environmental conditions occur. It has been reported that in Antarctic desert soil the temperature could change from −15°C to nearly 30°C in three hours (Cowan and Tow 2004). For the micro-organisms to survive freeze–thaw cycles and sudden differences of more than 40°C, very

specialized adaptations are required. Thus, in soil where a stress situation is maintained over an extended time period, the microbiota will develop specialized adaptations and life strategies that differentiate them from microbiota in nonextreme environments. However, such specialization may correspond to a tradeoff between life under adverse and harsh situations and loss of adaptability. Indeed, extremophiles are often not able to adapt to less extreme conditions, and do not compete effectively under moderate conditions.

Soil microbial communities under nonextreme and relatively stable environmental conditions are characterized as functionally redundant. Moderate environmental stress and perturbations seem to have little impact on overall soil processes such as respiration and mineralisation, although the microbial community structure can be profoundly changed. This is explained by the insurance hypothesis (Yachi and Loreau 1999), which states that in an ecosystem there are many different populations which can perform the same function, so that when some micro-organisms disappear others proliferate and take over the function (Giller et al. 1997). Microbial communities in extreme environments, especially those with fluctuating conditions, often comprise some numerically dominant species. In such environments, ecological processes may be more sensitive to changes in diversity imposed by additional stress factors. Lack of functional redundancy in extreme soils is illustrated by the observation that, in these environments, microbiota plays an increasing role at all trophic levels. For example, cyanobacteria and microalgae contribute significantly to primary production when the conditions become so harsh that higher plants can no longer grow.

Two types of extreme soils are dominant on Earth, namely desert and tundra soils. Typical of these biomes is that vegetation is sparse and consists mainly of low vegetation, with no trees being present.

2.4.1 Desert Soils

Water is an overall limiting factor in terrestrial ecosystems, and within a specific climate zone the annual net primary production correlates well with annual precipitation. Deserts occur in regions having less than 250 mm of rainfall per year (Odum 1971). Arid areas, that can be either extremely hot or cold, cover more than 30% of Earth's terrestrial surface (Rainey et al. 2005). Dry soil ecosystems are characterized by spatial patterns and high spatial heterogeneity. In temperate deserts, spatial variability is strongly influenced by vegetation, whereas in polar deserts, which often lack vascular plants, physical processes control the spatial variation in soil properties. An example is the formation of frost fissure patterns.

The micro-organisms living in such environments have to deal with unfavorable life conditions such as absence of water, high or low temperatures, and lack of nutrients. The most extreme deserts are found in Antarctica (Ross Desert, Dry Valleys), in northern Chile (Atacama Desert ; see Chapter 6), and in central Sahara where there is virtually no rainfall. The low precipitation is caused by high

subtropical atmospheric pressure (Sahara), position in rain shadow areas (Chile), high altitude (Tibet and Gobi), or latitude (Antarctic Dry Valley). In hot deserts, most of the matric water evaporates during the day and micro-organisms obtain moisture by absorbing dew water during cool nights. The cold deserts in Antarctica suffer from extreme temperature in addition to extreme water stress, although in some areas and during restricted periods water can come from melting snow. The air temperature is −10°C on average during the summer and down to −55°C during winter, and there are often strong winds, which are responsible for high sublimation rates.

In the past, doubts were raised if any organism could proliferate under such climatic conditions, but micro-organisms have been isolated from even the harshest desert environments. In deserts, the microbiota often inhabit pores in sandstones or they tend to form biological soil crusts. In the Ross Desert, an Antarctic cold desert, cryptoendolithic micro-organisms grow in the near-surface layer of porous sandstone rocks, where the microclimate is less hostile. They transform and mobilize iron compounds, and depend on the unsteady interactions between biological and environmental factors for survival. If the balance between these factors changes and becomes unfavorable, they will die but leave behind trace fossils and a characteristic iron-leaching pattern caused by their activity (Friedmann and Weed 1987). In the most extreme cold deserts, conditions suitable for microbial metabolism may occur only 2–10 days per year.

The crust communities are composed of prokaryotes, fungi, microalgae, and lichens. They are extremely important in desert ecosystems as they form stable soil aggregates with increased water retention responsible for functions such as primary production, nitrogen fixation, and nutrient cycling. Lichens are especially well adapted to extreme conditions, as they can withstand desiccation for long periods. Under cold conditions, the lichen algae keep the water in their cytoplasm in liquid form by producing and accumulating polyol intracellular solutes. The desiccation tolerance characteristic for desert micro-organisms is often correlated with salinity tolerance, extreme oligotrophy, and radiotolerance. Chanal et al. (2006) analysed microbial diversity in the Tataouine sand dunes in south Tunisia. The climate at this site is arid with a high seasonal variation in precipitation. The mean annual rainfall is 115 mm, and there is almost no precipitation in summer. Despite these unfavorable conditions, an unexpectedly high diversity of micro-organisms was revealed. The community contained a broad spectrum of micro-organisms, with 16S rRNA sequences affiliated with 11 bacterial divisions and some archaeal lineages. After irradiation of this soil with 15 kGy, radiotolerant organisms affiliated with *Bacillus, Deinococcus/Thermus*, and the Alphaproteobacteria could be isolated. In fact, many of the environments from which radioresistant organisms have been isolated are extremely dry, and many of these isolates are also desiccation-resistant (Rainey et al. 2005; Chanal et al. 2006).

In deserts with the most extreme dry habitats, micro-organisms can be totally dried out, and in arctic deserts they may actually be freeze-dried. The best strategy for micro-organisms to survive in such extreme environments may be to completely

abolish their metabolism during the most unfavorable time period, and switch into a dormant state until the conditions improve. Therefore many of them have resting stages or spores (Barak et al. 2005).

2.4.2 Tundra Soils

Climate has an overriding effect on species diversity on a global scale and biodiversity generally decreases with increased latitudes and altitudes. For eukaryotes, this trend is seen in polar regions both in terms of number of species and growth forms. However, on smaller spatial scales, biodiversity may not be any lower in Arctic tundra than in temperate soils. Permafrost represents approximately 26% of terrestrial soil ecosystems and can extend hundreds or even thousands of meters down into the subsurface (Steven et al. 2006). The permafrost environment is considered extreme because indigenous micro-organisms must survive long-term exposure to subzero temperatures and withstand background radiation. Low temperature and a short growing season (approximately 60 days) characterize extreme tundra and high altitude fell-field soils. Here such soils are considered and described together as extreme environments.

On a global scale, most of the tundra consists of Arctic wetlands covered by vegetation. However, the most extreme high Arctic tundra offers bare soil without vegetation except for sparse areas of lichens, sedges, and grasses. In the high Arctic we find permafrost soils, where the ground is permanently frozen except for a few dm of active layer during the growth season (see Chapters 7 and 12). Characteristic in permafrost soils are the ice-wedge polygon structures. These are topographic features formed by a network of ice-wedges, with either a depressed central area caused by thawing of the ice-rich permafrost in the centre, or a relatively elevated central area due to melting of the surrounding ice-wedges (see Chapter 7). Alpine fell-field tundra occurs in high mountains in temperate zones, and such tundra soils do not have permafrost.

In some areas such as the Antarctic deserts, several harsh environmental factors interact, such as low temperature, low annual precipitation, and strong desiccating winds. The Antarctic Dry Valleys are regarded as the coldest and driest place on Earth. The precipitation is only a few millimetres a year and occurs mainly as snow. As most of this snow is blown away, the potential evaporation exceeds precipitation. These regions are further characterised by a long period of winter darkness and low temperatures, followed by a very short summer with 24 h daily light for a few weeks, and even then the temperature rarely exceeds 0°C. Organisms in these environments must therefore tolerate long periods of desiccation and dormancy, and a common opinion has been that the microbial biomass is very low in these soils (Horowitz et al. 1972; Virginia and Wall 1999; Smith et al. 2006). Recent investigations suggest that the biomass is several orders of magnitude higher than previously recognized (Cowan et al. 2002).

In polar tundra areas there may also be profound microclimate differences. One factor which exerts a major influence on soil temperature is the snow depth. In Siberia and Alaska, it has been observed that, whereas exposed areas with low snow cover had soil temperatures down to −30 to −40°C, soils under the snow cover had temperatures around −5 to −10°C. During the active summer season the diurnal temperature fluctuations in the upper soil layers can vary considerably over short time periods. The amplitude of such fluctuations is influenced by the soil water content and vegetation cover, but in dry barren mineral soils temperatures can vary by more than 20°C, sometimes by nearly 40°C, with minima below 0°C. As a result of such abrupt temperature changes, freeze–thaw cycles occur that can be lethal to soil organisms. The organisms have therefore developed mechanisms that allow them to survive repeated freeze–thaw cycles.

Survival of adapted microbes depends on their hydration state, their compatible solute content, and their ability to switch metabolism to cryoprotectant synthesis. In some arid mineral soils, the micro-organisms are also subjected to osmotic stress due to accumulated salts. However, the presence of salt may result in water remaining liquid in cold environments, and active microbes can exist in thin films of liquid water present in permafrost or in permafrost brine lenses, called cryopegs, at below freezing temperatures (Gilichinsky et al. 2003). Cryopegs are layers of unfrozen ground that are perennially cryotic (forming part of the permafrost), but in which freezing is prevented by freezing-point depression due to high concentrations of dissolved substances in the pore water. An unfrozen cryopeg is entirely surrounded by frozen ground (Gilichinsky et al. 2005). Such habitats allow for microbial growth at −10°C and metabolic activity at −20°C and even lower (Bakermans et al. 2003).

2.5 Microbial Diversity and Community Structure in Extreme Soils

The term microbial diversity describes different aspects of complexity and variability within microbial populations and communities. This comprises genetic variability within taxons (species), variability in community composition, complexity of interactions, trophic levels, and number of guilds, this latter parameter defining the functional diversity. Diversity is expressed in different ways: as inventories of taxonomic groups or as single numbers (diversity indices), which are based on the number of taxons or OTUs (operational taxonomic units). Diversity may also be represented as phylogenetic trees, or appreciated from the number of functional guilds.

In moderate and stable environments, soil microbial communities will normally develop into complex systems with high phylogenetic and functional diversities. Therefore, such communities are among the most difficult to characterize phenotypically and genetically. In addition, huge and coherent discrepancies between the total and cultivable cell numbers in natural environments has led to the introduction of “the great plate count anomaly” concept (Staley and Konopka 1985). This means that diversity measurements based on cultured micro-organisms are restricted to a

subset of 1% or less of the community members (Torsvik et al. 1990; Ward et al. 1990) and applying culture-dependent methods will only reveal information about the very small fraction of micro-organisms able to grow under the given conditions (Sørheim et al. 1989).

Molecular methods and direct in situ studies circumvent the selective and biased culturing step and allow both cultured and noncultured members of a community to be surveyed (Pace et al. 1986; Torsvik et al. 1998). Some molecular methods allow for an in situ detection of prokaryotes in more or less intact soil samples whereas other methods require effective separation of cells from soil particles. Analysis of total DNA extracted directly from a community generates information derived from all the community members, and provides estimates of the microbial diversity and a comprehensive picture of soil microbial community composition (Fig. 2.1). The information contained in nucleic acids can be used to address diversity at different levels from the entire microbial community and populations to within species levels. A schematic overview of various methods used to obtain such information is given in Fig. 2.2 and in Øvreås (2000).

The total genetic diversity can be estimated by measuring the reassociation rate of community DNA (Britten and Kohne 1968; Torsvik et al. 1990), which is a low-resolution method that allows analyses of broad-scale differences in microbial communities (Torsvik and Øvreås 2006).

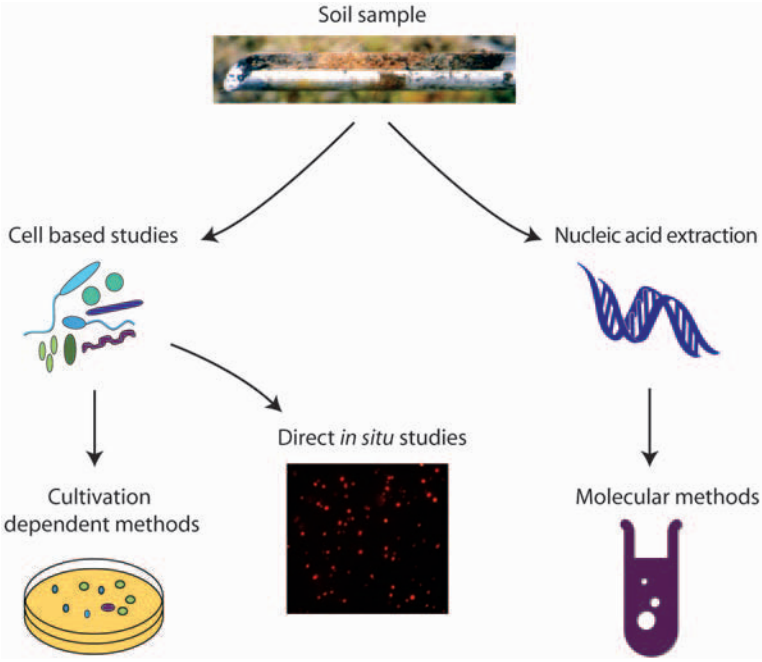

Fig. 2.1 Schematic drawing showing the overall approaches available for measuring bacterial diversity in soil

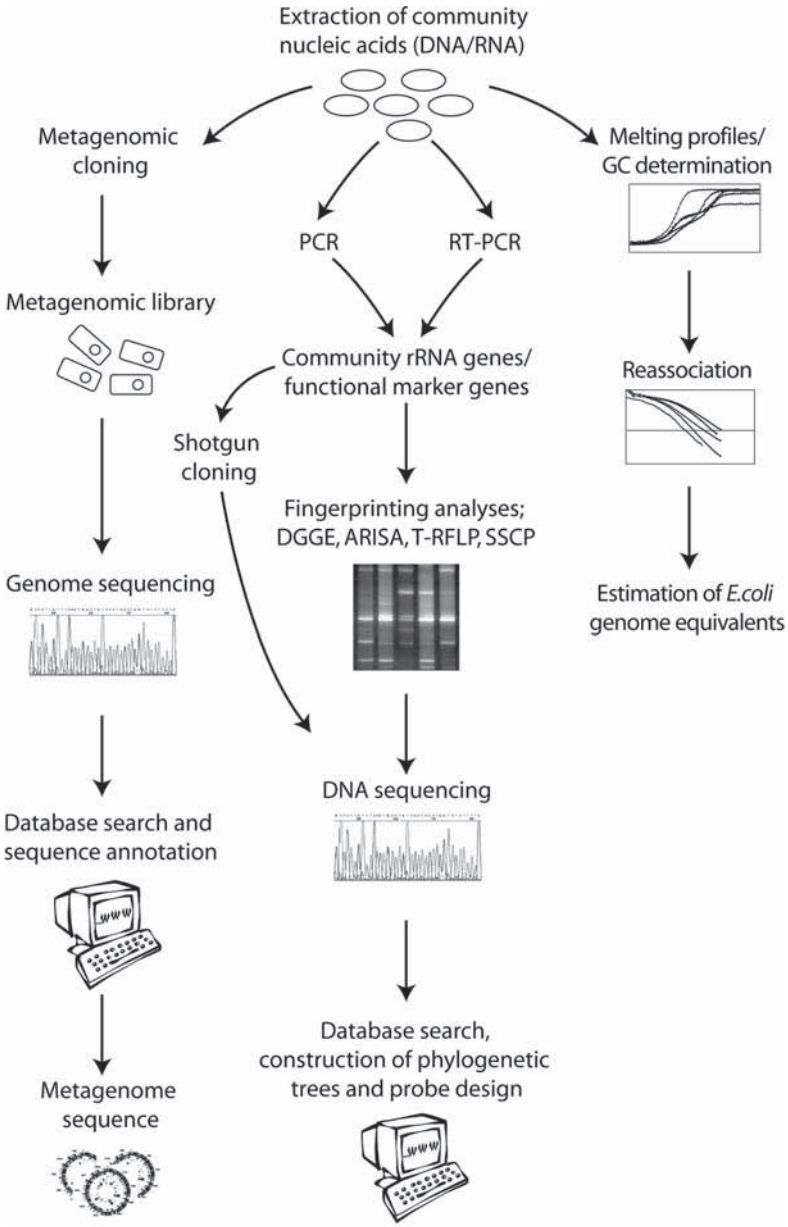

Fig. 2.2 Schematic drawing of the basic principles of some molecular methods

The metagenome can be regarded as the sum of all the microbial genomes in a given sample. Therefore, the metagenome approach represents a different whole community DNA-based analysis. It circumvents the cultivation anomaly as well as the PCR biases by cloning and sequencing genes directly from the environmental

DNA. This method involves construction of complex community libraries by direct cloning of large genomic DNA fragments (40–80 kb) from environmental samples into fosmid or BAC (bacterial artificial chromosome) vectors. The challenge of this application lies in the ability to extract DNA of high molecular weight and high purity. The metagenome approach can be used to generate information on the potential functioning of individual microbial species in soil environments in order to study the broader role of micro-organisms in the ecosystem (Rondon et al. 2000; Tringe et al. 2005).

The most common approach for assessing microbial diversity is to use polymerase chain reaction (PCR) to amplify 16S rRNA genes (rDNA) from the community (Pace et al. 1986). The amplified genes can then be cloned, and clones can be identified by DNA sequencing, which are then amenable to comparative analyses. Cloning and sequencing approaches are time-consuming and labor-intensive for routine analysis of large sample sets. To screen for changes in time and space, community fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), automated ribosomal intergenic spacer region analysis (ARISA), and single strand conformational polymorphism analysis (SSCP) of PCR amplified 16S rRNA genes are frequently used (Muyzer et al. 1993; Macnaughton et al. 1999a,b; Kozdroj and van Elsas 2001; Kuske et al. 2002; Hoj et al. 2005, 2006; Nakatsu et al. 2005; Neufeld and Mohn 2005; Becker et al. 2006; Joynt et al. 2006; Loisel et al. 2006; Hewson et al. 2007).

All these methods provide information about the numerically dominant community members, and the motivation for choosing one particular method instead of another lies in the expertise and equipment available in various laboratories. Furthermore, the phylogenetic affiliation of the numerically dominant organisms can be assessed by subsequent sequence analysis of, for instance, DGGE-separated PCR products. Such rDNA-based fingerprinting and cloning approaches offer higher resolution than DNA reassociation analysis, and have led to the discovery of several new prokaryotic taxa, even some entirely new divisions (Hugenholtz et al. 1998, 2001). Many studies have showed that most of the 16S rRNA sequences obtained from a given soil sample are unique (Hugenholtz et al. 2001; Smith et al. 2006). Due to the complexity of soil communities and the efforts required for the cloning and sequencing-based approach, only a limited number of soil environments have been surveyed using such methods, and our understanding of the extent of microbial diversity in soils is still very limited. More detailed descriptions of molecular methods for analysing microbial communities are provided in recent review papers (Johnsen et al. 2001; Prosser 2002; Lynch et al. 2004; van Elsas et al. 2006).

Regardless of their limitations, the culture-independent molecular methods have greatly expanded our view of extreme soils as habitats for micro-organisms. Several investigations have addressed the effect of extreme conditions created by human activities and pollution on microbial communities. Microbial communities in soils treated for many years with heavy metal-contaminated sewage sludge were investigated with respect to diversity and composition (Sandaa et al. 1999).

The control soil was amended with "uncontaminated" sewage sludge, whereas the contaminated soils received sewage sludge with two different levels of heavy metal concentrations (resulting in low and high levels of metal contamination). The total genetic diversity in microbial communities in unpolluted soil was high. In this case, the complexity of the community genome corresponded to approximately 9,800 different bacterial genomes with an average *E. coli* genome size. The diversity of the metal-polluted soils was reduced and depended on the level of pollution. The complexity of DNA isolated from the soils with low and high levels of metal pollution corresponded to a diversity of approximately 4,600 and 1,500 *E. coli* genomes, respectively. Thus, it seems that the genetic diversity can be an indicator of environmental stress caused by pollution.

It was further observed that environmental stress induces profound changes in the community structure. Pollution and perturbation lead both to reduced species richness and evenness, as some species become numerically dominant. It can be concluded that, under extreme stress conditions or strong pollution, microbial diversity may be reduced, and microbial community structure changed to the extent that functioning of this community is altered (Giller et al. 1997; Griffiths et al. 2004).

Both DNA reassociation and clone library analysis suggest that the overall prokaryotic diversity in pristine Arctic tundra soils can be very high (see Chapter 7), even higher than in soils from temperate regions (Øvreås et al. 2004; Neufeld and Mohn 2005). The prokaryotic communities in tundra soils have representatives of the same phylogenetic divisions as found in soils at lower latitudes (Cowan et al. 2002; Smith et al. 2006). They also carry out the same microbial processes as in temperate soils although at slower rates.

The increased human activities in polar regions depend on petroleum hydrocarbon for power generation and transportation. As a consequence of this and the exploitation of oil field reservoirs in the Arctic, increased oil pollution has become a significant problem in these cold environments (Aislabie et al. 2006; see Chapter 12). Several studies of hydrocarbon-contaminated polar soils indicate that hydrocarbon degraders are widely distributed in polar soils and that oil spill will result in marked enrichment of these micro-organisms (Atlas 1986; Aislabie et al. 2004, 2006; Sunde 2005). In comparative studies of pristine and oil-polluted Arctic and Antarctic tundra, significant shifts in the microbial diversity and community composition have been observed as a result of oil contamination. Disturbed tundra soil had lower microbial diversity than pristine soils, and in the polluted soils some populations were very predominant (Neufeld and Mohn 2005; Saul et al. 2005; Smith et al. 2006).

An investigation from Arctic tundra at Svalbard, Norway, showed that the proportion of clones with sequence similarities to cultured bacteria was much higher in polluted (36%) than in pristine soil (6%). Even then, the phylogenetic groups that were most abundant in pristine tundra soil have so far only been represented in the databases by a limited number of sequences from cultured organisms (Yndestad 2004). These, as well as clone library data from Antarctic pristine tundra soils, indicate that most of the sequences are derived from unknown and uncultured

micro-organisms, and may represent new and undescribed taxa. Enrichment cultures with three different oil types incubated at 4°C demonstrated that different oils promoted the establishment of different communities. In these experiments, the efficient oil-degrading organisms showed phylogenetic affiliation to well-known hydrocarbon-degrading organisms within the Proteobacteria and the Gram positive bacteria (Sunde 2005). In oil-contaminated soil, biodegradation of petroleum hydrocarbons by indigenous cold-adapted microbial populations at low temperatures has been observed (Whyte et al. 1999, 2001; Rike et al. 2003; Sunde 2005), but the in situ rates of degradation were low. Therefore, the activity of the indigenous hydrocarbon-degrading microbes is limited in cold soil, most likely by a combination of unfavorable conditions including low temperature and moisture, nutrient limitation, alkalinity, and potentially inhibitory hydrocarbons.

2.6 Conclusions: The Significance of Studying Extremophiles in Soil

Extreme soils have highly selective physicochemical properties and many of them have low microbial diversity relative to nonextreme soils. Therefore, they can serve as model systems for exploring fundamental ecological principles such as the relationships between diversity and activity of micro-organisms and soil environmental conditions (Smith et al. 2006). Furthermore, studies of microbial community composition and functions in extreme soils may be of great value for applications in environmental cleanup, pollution prevention, or energy production.

Improved knowledge about extreme ecosystems will lead to important advances in the understanding of microbial adaptation mechanisms, and facilitate the design of biotechnological applications for enzymes and other compounds adapted to function under extreme physicochemical conditions. In addition, extreme prokaryotes may be interesting for bioprospecting, as they can be expected to contain a number of bioactive compounds potentially useful in medicine as well as in the pharmaceutical and environmental industries.

Defining the limiting conditions for life on our planet can aid us in speculation on comparable limits in the universe. Extreme environments on Earth may resemble those that exist on other planets and moons. Thus, investigation of the most challenging environments on Earth, can give us some clues as to under which conditions we can expect to find life on other planets (see Chapters 6, 7, and 10). It can also provide some hints of what to search for when looking for signs of extraterrestrial life (see Chapter 11). The study of microbes in extreme soils is therefore highly relevant for astrobiology. It will advance our understanding, at the molecular and physiological levels, of specializations and adaptations required for the maintenance and proliferation of remote and as yet unrecognized forms of life.

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References

Aislabie J, Saul DJ, Foght JM (2006) Bioremediation of hydrocarbon-contaminated polar soils. *Extremophiles* 10:171–179

Aislabie JM, Balks MR, Foght JM, Waterhouse EJ (2004) Hydrocarbon spills on Antarctic soils: Effects and management. *Environ Sci Technol* 38:1265–1274

Allison SD (2006) Soil minerals and humic acids alter enzyme stability: Implications for ecosystem processes. *Biogeochem* 81:361–373

Atlas RM (1986) Fate of petroleum pollutants in Arctic ecosystems. *Water Sci Tech* 18:59–67

Baker-Austin C, Dopson M, Wexler M, Sawers RG, Bond PL (2005) Molecular insight into extreme copper resistance in the extremophilic archaeon '*Ferroplasma acidarmanus*' Fer1. *Microbiol* 151:2637–2646

Bakermans C, Tsapin AI, Souza-Egipsy V, Gilichinsky DA, Nealson KH (2003) Reproduction and metabolism at −10 degrees C of bacteria isolated from Siberian permafrost. *Environ Microbiol* 5:321–326

Barak I, Ricca E, Cutting SM (2005) From fundamental studies of sporulation to applied spore research. *Mol Microbiol* 55:330–338

Battista JR (1997) Against all odds: The survival strategies of *Deinococcus radiodurans*. *Ann Rev Microbiol* 51:203–224

Becker JM, Parkin T, Nakatsu CH, Wilbur JD, Konopka A (2006) Bacterial activity, community structure, and centimeter-scale spatial heterogeneity in contaminated soil. *Microb Ecol* 51:220–231

Bernard L, Mougel C, Maron P-A, Nowak V, Leveque J, Henault C, Haichar FeZ, Berge O, Marol C, Balesdent J, Gibiat F, Lemanceau P, Ranjard L (2007) Dynamics and identification of soil microbial populations actively assimilating carbon from ^{13}C-labelled wheat residue as estimated by DNA- and RNA-SIP techniques. *Environ Microbiol* 9:752–764

Botero LM, Brown KB, Brumefield S, Burr M, Castenholz RW, Young M, McDermott TR (2004) *Thermobaculum terrenum* gen. nov., sp. nov.: A non-phototrophic gram-positive thermophile representing an environmental clone group related to the Chloroflexi (green non-sulfur bacteria) and Thermomicrobia. *Arch Microbiol* 181:269–277

Bowker MA, Reed SC, Belnap J, Phillips SL (2002) Temporal variation in community composition, pigmentation, and fv/fm of desert Cyanobacterial soil crusts. *Microb Ecol* 43:13–25

Britten RJ, Kohne DE (1968) Repeated sequences in DNA. *Science* 161:529–540

Brown AD (1976) Microbial water stress. *Bact Rev* 40:803–846

Bååth E (1989) Effects of heavy metals in soil on microbial processes and populations (a review). *Water Air Soil Poll* 47:335–379

Bååth E, Anderson TH (2003) Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol Biochem* 35:955–963

Callaghan TV, Björn LO, Chernov Y, Chapin T, Christensen TR, Huntley B, Ims RA, Johansson M, Jolly D, Jonasson S, Matveyeva N, Panikov N, Oechel W, Shaver G, Elster J, Henttonen H, Laine K, Taulavuori K, Taulavuori E, Zöckler C (2004) Climate change and UV-B impacts on arctic tundra and polar desert ecosystems; Biodiversity, distributions and adaptations of arctic species in the context of environmental change. *Ambio* 33:404–417

Cavicchioli R, Thomas T, Curmi PMG (2000) Cold stress response in Archaea. *Extremophiles* 4:321–331

Chanal A, Chapon V, Benzerara K, Barakat M, Christen R, Achouak W, Barras F, Heulin T (2006) The desert of Tataouine: An extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. *Environ Microbiol* 8:514–525

Cowan D, Russell N, Mamais A, Sheppard D (2002) Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. *Extremophiles* 6:431–436

Cowan DA, Tow LA (2004) Endangered Antarctic environments. *Ann Rev Microbiol* 58:649–690

Doroshenko EA, Zenova GM, Zvyagintsev DG, Sudnitsyn II (2005) Spore germination and mycelial growth of streptomycetes at different humidity levels. *Microbiol* 74:690–694

Dose K, Bieger-Dose A, Ernst B, Feister U, Gomez-Silva B, Klein A, Risi S, Stridde C (2001) Survival of microorganisms under the extreme conditions of the Atacama desert. *Ori Life Evol Biosph* 31:287–303

Drønen AK, Torsvik V, Goksøyr J, Top EM (1998) Effect of mercury addition on plasmid incidence and gene mobilizing capacity in bulk soil. *FEMS Microbiol Ecol* 27:381–394

Edwards KJ, Bond PL, Gihring TM, Banfield JF (2000) An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. *Science* 287:1796–1799

Ehling-Schulz M, Bilger W, Scherer S (1997) UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune. J Bacteriol* 179:1940–1945

Eichorst SA, Breznak JA, Schmidt TM (2007) Isolation and characterization of bacteria from soil that define *Terriglobus gen. nov*., in the phylum *Acidobacteria. Appl Environ Microbiol* 73:2708–2717

Fierer N, Bradford M, Jackson R (2007) Towards an ecological classification of soil bacteria. *Ecology* 88:1354–1364

Francis AJ (1986) Acid rain effects on soil and aquatic microbial processes. *Cell Molec Life Sci* (*CMLS*) 42:455–465

Friedmann EI, Weed R (1987) Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic cold desert. *Science* 236:703–705

Futterer O, Angelov A, Liesegang H, Gottschalk G, Schleper C, Schepers B, Dock C, Antranikian G, Liebl W (2004) Genome sequence of *Picrophilus torridus* and its implications for life around pH 0. *Proc Natl Acad Sci USA* 101:9091–9096

Gans J, Wolinsky M, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science* 309:1387–1390

Gilichinsky D, Rivkina E, Bakermans C, Shcherbakova V, Petrovskaya L, Ozerskaya S, Ivanushkina N, Kochkina G, Laurinavichuis K, Pecheritsina S (2005) Biodiversity of cryopegs in permafrost. *FEMS Microbiol Ecol* 53:117–128

Gilichinsky D, Rivkina E, Shcherbakova V, Laurinavichuis K, Tiedje J (2003) Supercooled water brines within permafrost - An unknown ecological niche for microorganisms: A model for astrobiology. *Astrobiology* 3:331–341

Giller KE, Beare MH, Lavelle P, Izac AMN, Swift MJ (1997) Agricultural intensification, soil biodiversity and agroecosystem function. *Appl Soil Ecol* 6:3–16

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. *Soil Biol Biochem* 30:1389–1414

Golyshina OV, Timmis KN (2005) *Ferroplasma* and relatives, recently discovered cell wall-lacking archaea making a living in extremely acid, heavy metal-rich environments. *Environ Microbiol* 7:1277–1288

Gorbushina AA, Krumbein WE (1999) The poikilotrophic micro-organism and its environment. In: Seckbach J (ed) *Enigmatic Microorganisms and Life in Extreme Environments*. Kluwer Academic, Dordrecht, pp 177–185

Griffiths BS, Kuan HL, Ritz K, Glover LA, McCaig AE, Fenwick C (2004) The relationship between microbial community structure and functional stability, tested experimentally in an upland pasture soil. *Microb Ecol* 47:104–113

Hattori T, Mitsui H, Haga H, Wakao N, Shikano S, Gorlach K, Kasahara Y, El BA, Hattori R (1997) Advances in soil microbial ecology and the biodiversity. *Ant v Leeuw* 72:21–28

Hewson I, Jacobson-Meyers ME, Fuhrman JA (2007) Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. *Environ Microbiol* 9:923–933

Hoj L, Olsen RA, Torsvik VL (2005) Archaeal communities in High Arctic wetlands at Spitsbergen, Norway (78[deg]N) as characterized by 16S rRNA gene fingerprinting. *FEMS Microbiol Ecol* 53:89–101

Hoj L, Rusten M, Haugen LE, Olsen RA, Torsvik VL (2006) Effects of water regime on archaeal community composition in Arctic soils. *Environ Microbiol* 8:984–996

Horowitz NH, Cameron RE, Hubbard JS (1972) Microbiology of the Dry Valleys of Antarctica. *Science* 176:242–245

Huang PM (1990) Role of soil minerals in transformation of natural organics and xenobiotics in soil. In: Bollag J-M, Stotzky G (eds) *Soil Biochemistry*. Marcel Dekker, New York, pp 29–115

Hugenholtz P, Goebel BM, Pace NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *J Bacteriol* 180:4765–4774

Hugenholtz P, Tyson GW, Webb RI, Wagner AM, Blackall LL (2001) Investigation of candidate division TM7, a recently recognized major lineage of the domain bacteria with no known pure-culture representatives. *Appl Environ Microbiol* 67:411–419

Jakosky BM, Nealson KH, Bakermans C, Ley RE, Mellon MT (2003) Subfreezing activity of microorganisms and the potential habitability of Mars' polar regions. *Astrobiology* 3:343–350

Jenny H (1994) *Factors of Soil Formation. A System of Quantitative Pedology*. Dover Press, New York. (Reprint, with Foreword by R. Amundson, of the 1941 McGraw-Hill publication). Reprint McGraw-Hill, New York

Johnsen K, Jacobsen C, Torsvik V, Sørensen J (2001) Pesticide effects on bacterial diversity in agricultural soils – A review. *Biol Fertil Soils* 33:443–453

Joynt J, Bischoff M, Turco R, Konopka A, Nakatsu CH (2006) Microbial community analysis of soils contaminated with lead, chromium and petroleum hydrocarbons. *Microb Ecol* 51:209–219

Kashefi K, Lovley DR (2003) Extending the upper temperature limit for life. *Science* 301:934

Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch Microbiol* 170:319–330

Killham K (1994) *Soil Ecology*. Cambridge University Press, Cambridge

Killham K, Firestone MK, JGM (1983) Acid rain and soil microbial activity: Effects and their mechanisms. *J Environ Qual* 12:133–137

Koch AL (2001) Oligotrophs versus copiotrophs. *BioEssays* 23:657–661

Kozdroj J, van Elsas JD (2001) Structural diversity of microbial communities in arable soils of a heavily industrialised area determined by PCR-DGGE fingerprinting and FAME profiling. *Appl Soil Ecol* 17:31–42

Krulwich TA, Ito M, Hicks DB, Gilmour R, Guffanti AA (1998) pH homeostasis and ATP synthesis: studies of two processes that necessitate inward proton translocation in extremely alkaliphilic *Bacillus* species. *Extremophiles* 2:217–222

Kuske CR, Ticknor LO, Miller ME, Dunbar JM, Davis JA, Barns SM, Belnap J (2002) Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Appl Environ Microbiol* 68:1854–1863

Liao M, Xie XM (2007) Effect of heavy metals on substrate utilization pattern, biomass, and activity of microbial communities in a reclaimed mining wasteland of red soil area. *Ecotox Environ Safety* 66:217–223

Loisel P, Harmand J, Zemb O, Latrille E, Lobry C, Delgenes JP, Godon JJ (2006) Denaturing gradient electrophoresis (DGE) and single-strand conformation polymorphism (SSCP) molecular fingerprintings revisited by simulation and used as a tool to measure microbial diversity. *Environ Microbiol* 8:720–731

Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol Mol Biol Rev* 58:563–602

Lynch JM (1979) The terrestrial environment. In: Lynch JM, Poole NJ (eds) *Microbial Ecology: A Conceptual Approach*. Blackwell Scientific, Oxford, pp 67–91

Lynch JM, Benedetti A, Insam H, Nuti MP, Smalla K, Torsvik V, Nannipieri P (2004) Microbial diversity in soil: Ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. *Biol Fertil Soils* 40: 363–385

Macnaughton S, Stephen JR, Chang YJ, Peacock A, Flemming CA, Leung K, White DC (1999a) Characterization of metal-resistant soil eubacteria by polymerase chain reaction - Denaturing gradient gel electrophoresis with isolation of resistant strains. *Can J Microbiol* 45:116–124

Macnaughton SJ, Stephen JR, Venosa AD, Davis GA, Chang YJ, White DC (1999b) Microbial population changes during bioremediation of an experimental oil spill. *Appl Environ Microbiol* 65:3566–3574

Margesin R, Schinner F (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl Microbiol Biotechnol* V56:650–663

Marion GM, Fritsen CH, Eicken H, Payne MC (2003) The search for life on Europa: Limiting environmental factors, potential habitats, and Earth analogues. *Astrobiology* 3:785–811

Martin DD, Ciulla RA, Roberts MF (1999) Osmoadaptation in *Archaea. Appl Environ Microbiol* 65:1815–1825

Minton KW, Daly MJ (1995) A model for repair of radiation-induced DNA double-strand breaks in the extreme radiophile *Deinococcus radiodurans. BioEssays* 17:457–464

Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S ribosomal-RNA. *Appl Environ Microbiol* 59:695–700

Nakatsu CH, Carmosini N, Baldwin B, Beasley F, Kourtev P, Konopka A (2005) Soil microbial community responses to additions of organic carbon substrates and heavy metals (Pb and Cr). *Appl Biochem Microbiol* 71:7679–7689

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. *Eur J Soil Sci* 54:655–670

Nannipieri P, Grego S, Ceccanti B (1990) Ecological significance of the biological activity in soil. In: Bollag J-M, Stotzky G (eds) *Soil Biochemistry*. Marcel Dekker, New York, pp 293–355

Nedwell DB (1999) Effect of low temperature on microbial growth: Lowered affinity for substrates limits growth at low temperature. *FEMS Microbiol Ecol* 30:101–111

Neufeld JD, Mohn WW (2005) Unexpectedly high bacterial diversity in Arctic tundra relative to boreal forest soils, revealed by serial analysis of ribosomal sequence tags. *Appl Environ Microbiol* 71:5710–5718

Odum EP (1971) *Fundamentals of Ecology*, 3rd edn. W.B. Saunders, Philadelphia

Øvreås L (2000) Population and community level approaches for analysing microbial diversity in natural environments. *Ecol Lett* 3:236–251

Øvreås L, Daae FL, Yndestad S, Jørgensen SL, Torsvik V, Brandvik PJ (2004) Microbial community analysis in pristine and polluted environments from Arctic. In: *10th International Symposium for Microbial Ecology (ISME10)*, Cancún, Mexico

Pace NR, Stahl DA, Lane DJ, Olsen GJ (1986) The analysis of natural microbial populations by ribosomal-RNA sequences. *Adv Microb Ecol* 9:1–55

Pakchung AAH, Simpson PJL, Codd R (2006) Life on earth. Extremophiles continue to move the goal posts. *Environ Chem* 3:77–93

Panikov NS (1999) Understanding and prediction of soil microbial community dynamics under global change. *Appl Soil Ecol* 11:161–176

Panikov NS, Dedysh SN (2000) Cold season CH_4 and CO_2 emission from boreal peat bogs (West Siberia): Winter fluxes and thaw activation dynamics. *Global Biogeochem Cycles* 14:1071–1080

Pennanen T, Fritze H, Vanhala P, Kiikkila O, Neuvonen S, Baath E (1998a) Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. *Appl Environ Microbiol* 64:2173–2180

Pennanen T, Perkiomaki J, Kiikkila O, Vanhala P, Neuvonen S, Fritze H (1998b) Prolonged, simulated acid rain and heavy metal deposition: Separated and combined effects on forest soil microbial community structure. *FEMS Microbiol Ecol* 27:291–300

Post RD, Beeby AN (1996) Activity of the microbial decomposer community in metal-contaminated roadside soils. *J Appl Ecol* 33:703–709

Price PB, Sowers T (2004) Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc Natl Acad Sci USA* 101:4631–4636

Prosser JI (2002) Molecular and functional diversity in soil micro-organisms. *Plant Soil* 244:9–17

Rainey FA, Ray K, Ferreira M, Gatz BZ, Nobre MF, Bagaley D, Rash BA, Park MJ, Earl AM, Shank NC, Small AM, Henk MC, Battista JR, Kampfer P, da Costa MS (2005) Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus *Deinococcus* obtained from a single soil sample. *Appl Environ Microbiol* 71:5225–5235

Rajapaksha RMCP, Tobor-Kaplon MA, Bååth E (2004) Metal toxicity affects fungal and bacterial activities in soil differently. *Appl Environ Microbiol* 70:2966–2973

Rike AG, Haugen KB, Borresen M, Engene B, Kolstad P (2003) In situ biodegradation of petroleum hydrocarbons in frozen arctic soils. *Cold Reg Sci Tech* 37:97–120

Rivkina EM, Friedmann EI, McKay CP, Gilichinsky DA (2000) Metabolic activity of permafrost bacteria below the freezing point. *Appl Environ Microbiol* 66:3230–3233

Roberts MF (2005) Organic compatible salutes of halotolerant and halophilic microorganisms. *Sal Syst* 1:5–30

Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, Tiong CL, Gilman M, Osburne MS, Clardy J, Handelsman J, Goodman RM (2000) Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Appl Environ Microbiol* 66:2541–2547

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. *Nature* 409:1092–1101

Ruggiero P, Dec J, Bollag J-M (1996) Soil as a catalytic system. In: Bollag J-M, Stotzky G (eds) *Soil Biochemistry*. Marcel Dekker, New York, pp 79–122

Sale JE (2007) Radiation resistance: Resurrection by recombination. *Curr Biol* 17:R12–R14

Sandaa R-A, Torsvik V, Enger O, Daae FL, Castberg T, Hahn D (1999) Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. *FEMS Microbiol Ecol* 30:237–251

Saul DJ, Aislabie JM, Brown CE, Harris L, Foght JM (2005) Hydrocarbon contamination changes the bacterial diversity of soil from around Scott Base, Antarctica. *FEMS Microbiol Ecol* 53:141–155

Schleper C, Puehler G, Holz I, Gambacorta A, Janekovic D, Santarius U, Klenk HP, Zillig W (1995) *Picrophilus gen. nov., fam. nov.*: A novel aerobic, heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. *J Bacteriol* 177:7050–7059

Shi W, Bischoff M, Turco R, Konopka A (2002) Long-term effects of chromium and lead upon the activity of soil microbial communities. *Appl Soil Ecol* 21:169–177

Shukla M, Chaturvedi R, Tamhane D, Vyas P, Archana G, Apte S, Bandekar J, Desai A (2007) Multiple-stress tolerance of ionizing radiation-resistant bacterial isolates obtained from various habitats: correlation between stresses. *Curr Microbiol* 54:142–148

Singleton JR, Amelunxen RE (1973) Proteins from thermophilic microorganisms. *Bacteriol Rev* 37:320–342

Smith JJ, Tow LA, Stafford W, Cary C, Cowan DA (2006) Bacterial diversity in three different Antarctic cold desert mineral soils. *Microb Ecol* 51:413–421

Staley JT, Konopka A (1985) Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Ann Rev Microbiol* 39:321–346

Standing D, Killham K (2006) The soil environment. In: van Elsas JD, Jansson JK, Trevors JT (eds) *Modern Soil Microbiology*, 2nd edn. CRC Press, Taylor and Francis, Boca Raton, FL, pp 1–22

Steven B, Léveillé R, Pollard WH, Whyte LG (2006) Microbial ecology and biodiversity in permafrost. *Extremophiles* 10:259–267

Stevens TO, McKinley JP (1995) Lithoautotrophic microbial ecosystems in deep basalt aquifers. *Science* 270:450–454

Stotzky G (1997) Soil as an environment for microbial life. In: van Elsas JD, Trevors JT, Wellington EMH (eds) *Modern Soil Microbiology*, 1st edn. Marcel Dekker, New York, pp 1–20

Sunde IR (2005) Enrichment, isolation and phylogenetic characterisation of hydrocarbon-degrading bacteria from oil-contaminated tundra. In: *Department of Biology*. University of Bergen, Bergen, p 103

Sørheim R, Torsvik VL, Goksoyr J (1989) Phenotypical divergences between populations of soil bacteria isolated on different media. *Microb Ecol* 17:181–192

Torsvik V, Daae FL, Sandaa RA, Øvreas L (1998) Novel techniques for analysing microbial diversity in natural and perturbed environments. *J Biotechnol* 64:53–62

Torsvik V, Goksoyr J, Daae FL (1990) High diversity in DNA of soil bacteria. *Appl Environ Microbiol* 56:782–787

Torsvik V, Øvreås L (2006) Microbial phylogeny and diversity in soil. In: van Elsas JD, Jansson JK, Trevors JT (eds) *Modern Soil Microbiology*, 2nd edn. CRC Press, Taylor and Francis Group, Boca Raton, FL, pp 23–54

Tringe SG, von Mering C, Kobayashi A, Salamov AA, Chen K, Chang HW, Podar M, Short JM, Mathur EJ, Detter JC, Bork P, Hugenholtz P, Rubin EM (2005) Comparative metagenomics of microbial communities. *Science* 308:554–557

van Elsas JD, Torsvik V, Hartmann A, Øvreås L, Jansson JK (2006) The bacteria and archaea in soil. In: van Elsas JD, Jansson JK, Trevors JT (eds) *Modern Soil Microbiology*, 2nd edn. CRC Press, Taylor and Francis, Boca Raton, FL pp 83–105

Vetterlein D, Jahn R (2004) Combination of micro suction cups and time-domain reflectometry to measure osmotic potential gradients between bulk soil and rhizosphere at high resolution in time and space. *Eur J Soil Sci* 55:497–504

Virginia RA, Wall DH (1999) How soils structure communities in the Antarctic Dry Valleys *BioScience* 49:974–983

Ward DM, Weller R, Bateson MM (1990) 16S ribosomal-RNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* 345:63–65

Whyte LG, Slagman SJ, Pietrantonio F, Bourbonniere L, Koval SF, Lawrence JR, Inniss WE, Greer CW (1999) Physiological adaptations involved in alkane assimilation at a low temperature by *Rhodococcus sp* strain Q15. *Appl Environ Microbiol* 65:2961–2968

Whyte LG, Goalen B, Hawari J, Labbe D, Greer CW, Nahir M (2001) Bioremediation treatability assessment of hydrocarbon-contaminated soils from Eureka, Nunayut. *Cold Reg Sci Tech* 32:121–132

Wiebe WJ, Sheldon WM, Jr., Pomeroy LR (1992) Bacterial growth in the cold: Evidence for an enhanced substrate requirement. *Appl Environ Microbiol* 58:359–364

Wright DJ, Smith SC, Joardar V, Scherer S, Jervis J, Warren A, Helm RF, Potts M (2005) UV irradiation and desiccation modulate the three-dimensional extracellular matrix of *Nostoc commune* (Cyanobacteria). *J Biol Chem* 280:40271–40281

Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proc Natl Acad Sci USA* 96:1463–1468

Yndestad S (2004) Microbial diversity in oil polluted and pristine tundra on Svalbard (Norwegian). In: *Department of Biology*. University of Bergen, Norway, Bergen

Zhang G, Ma X, Niu F, Dong M, Feng H, An L, Cheng G (2007) Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai-Tibet Plateau permafrost region. *Extremophiles* 11:415–424

Zvyagintsev DG, Zenova GM, Sudnizin II, Doroshenko EA (2005) The ability of soil *Actinomycetes* to develop at an extremely low humidity. *Doklady Biol Sci* 405:461–463