# 9 Microbial Life in Terrestrial Permafrost: Methanogenesis and Nitrification in Gelisols as Potentials for Exobiological Processes

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The comparability of environmental and climatic conditions of the early Mars and Earth is of special interest for the actual research in astrobiology. Martian surface and terrestrial permafrost areas show similar morphological structures, which suggests that their development is based on comparable processes. Soil microbial investigations of adaptation strategies of microorganisms from terrestrial permafrost in combination with environmental, geochemical and physical analyses give insights into early stages of life on Earth. The extreme conditions in terrestrial permafrost soils can help to understand the evolution of life on early Mars and help searching for possible niches of life on present Mars or in other extraterrestrial permafrost habitats [1, 2].

## 9.1 Permafrost Soils and Active Layer

In polar regions huge layers of frozen ground are formed - termed permafrost - which are defined as the thermal condition, in which soils and sediments remain at or below 0 °C for two or more years in succession. Terrestrial permafrost, which underlay more than 20% of the world‘s land area, is above all controlled by climatic factors and characterized by extreme terrain condition and landforms. On Earth the permafrost thickness can reach several hundreds of meters, e.g., in East Siberia (Central Yakutia) about 600-800 m. During the relatively short period of arctic/antarctic summer only the surface zone of permafrost sediments thaws. This uppermost part of the permafrost (active layer) includes the so called Gelisols [3], which contains permafrost in the upper 100 cm soil depth. Gelisols are characterized by gelic material that have the evidence of cryoturbation and ice segregation. Permafrost soils may be cemented by ice which is typical for the Arctic regions, or, in the case of insufficient interstitial water, may be dry like the Antarctic polar deserts.

Permafrost can be divided into three temperature regimes (Fig. 9.1), which characterize the extreme living conditions: (i) The surface near upper active layer (0.2-2.0 m thickness) is subjected to seasonal freezing and thawing with an extreme temperature regime from about +15 °C to −35 °C, (ii) the correlated upper, perennially frozen permafrost sediments (10-20 m thickness) with smaller seasonal temperature variation of about 0 °C to −15 °C above the zero annual amplitude and (iii) the deeper permafrost sediments which are characterized by a stable temperature regime of about −5 °C to −10 °C [4].

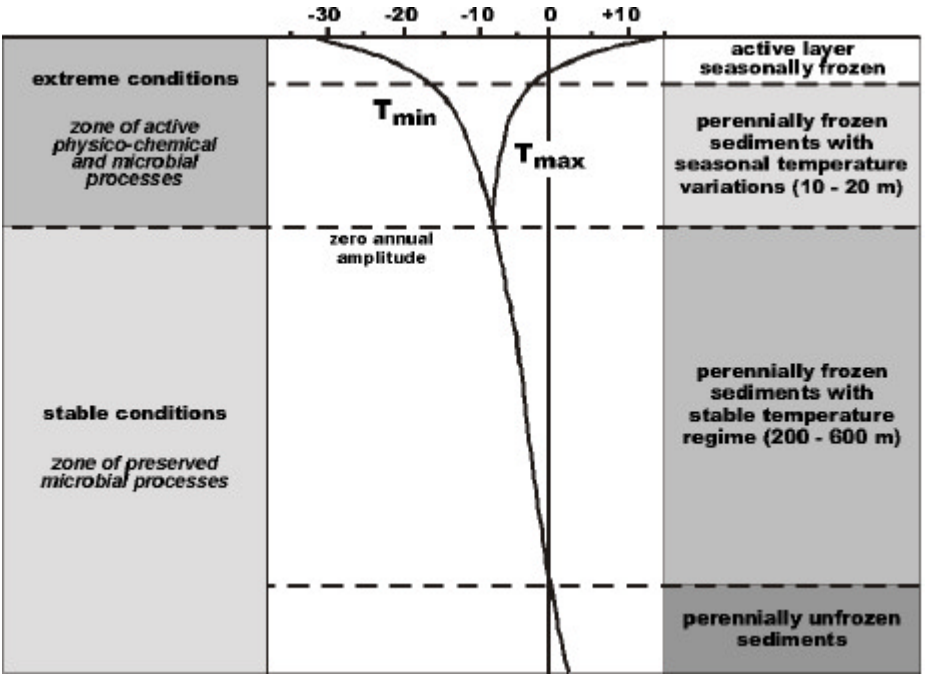


**Fig. 9.1** Scheme of temperature amplitude in permafrost sediments (according to French [4], modified).

The main Gelisol-forming processes in permafrost landscapes are cryopedogenesis, which include freezing and thawing, frost stirring, mounding, fissuring and solifluction. The repeating cycles of freezing and thawing leads to cryoturbation features (frost churning) that includes irregular, broken or involuted horizons and an enrichment of organic matter and other inorganic compounds, especially along the top of the permafrost table. As a result of cryopedogenesis many Gelisols are influenced by a strong micro-relief (patterned ground, Fig. 9.2). The type of patterned ground has effects on soil formation and soil properties.

Ice wedge polygons for example of the Siberian lowlands (Fig. 9.3-A) which are typical for high arctic, are characterized by two different soil conditions: The Gelisols of the polygon center (*Historthels*) are water saturated and have a large amount of organic matter due to the accumulation under anaerobic conditions (Fig. 9.3-B). The Gelisols of the polygon border (*Aquiturbels*) show evidence of cryoturbation in more or less all horizons of the active layer (Fig. 9.3-C). These soils drain into the polygon center, which leads to dryer conditions in the upper layer of the border.

These examples demonstrate that the Gelisols of the active layer and upper permafrost sediments are the zone with active physico-chemical processes under extreme conditions. Therefore, microbial life in permafrost soils and sediments is influenced by extreme gradients of temperature, moisture and chemical properties. However, deeper permafrost layers characterize living conditions, which have been stable for long periods of time and microbial life is preserved (see Chap. 8, Gilichinsky).

## 9.2 Microbial Life under Extreme Conditions

Over 80% of the Earth's biosphere - including the polar regions - is permanently cold. Most natural environments have a temperature regime colder than 5 °C. Temperature is one of the most important parameters regulating the activity of microorganisms because it controls all metabolic activity of living cells [5]. The temperature in the upper zone of the cryolithosphere (active layer and upper permafrost sediments) ranged between –50 °C to +30 °C [6]. Especially permafrost soils are characterized by extreme variation in temperature. Previously the potential of growth as well as the molecular, physiological and ecological aspects of microbial life at low temperatures were investigated [7, 8]. Many microorganisms are able to survive in cold permafrost sediments, but this adaptation can be a tolerance or a preference. According to Morita [9] bacteria can be described by their temperature range of growth: psychrophiles ($T_{min}$ <0 °C, $T_{opt}$ ≤15 °C, $T_{max}$ ≤20 °C), psychrotrophs ($T_{min}$ ≤0 °C, $T_{opt}$ ≥15 °C, $T_{max}$ ≤35 °C) and mesophiles ($T_{opt}$ 25-40 °C). The minimum temperature for growth of bacteria was recently reported with –20 °C [10], whereas the minimum temperature for enzyme activity was –25 °C [11].

The seasonal variation of soil temperature influences also the availability of pore water. The presence of unfrozen water is an essential biophysical requirement for the survival and activity of microorganisms in permafrost. Temperature below zero stands for an increasing loss of water. At the same time freezing of water leads to an increase of salt content in the remaining pore solution. However, in clayey permafrost soils liquid water was analyzed at temperatures up to –60 °C [12]. The most important feature of this water is the possible transfer of ions and nutrients [13]. Furthermore, McGrath et al. [14] showed that the intercellular water in fossil bacteria from permafrost soils was not crystallized as ice even at an extreme temperature of –150 °C.

For studying microbial life under extreme conditions it is also necessary to consider whether and where these conditions are changing or stable in a permafrost profile. The seasonal variation in soil temperature, particularly freeze-thaw cycles in the active layer, results in drastic changes of other environmental conditions like salinity, soil pressure, changing oxygen conditions (anoxic, microaerophilic, oxic) and nutrient availability. Therefore, besides the physico-chemical conditions of permafrost, the physiological properties of microorganisms are relevant for the adaptation to extreme conditions. On account of this potential, they developed strategies to resist salt stress, physical damage by ice crystals and background radiation [15]. Survival could be also possible by anabiosis (dormant stage of life) or by reduced metabolic activity in unfrozen waterfilms (see Chap. 8, Gilichinsky).

## 9.3 Microbial Key Processes

Terrestrial permafrost is colonized by high numbers of chemoorganotrophic bacteria as well as microbes like methanogenic archaea and nitrifying bacteria [16-18], which are highly specialized organisms. They are characterized by litho-autotrophic growth

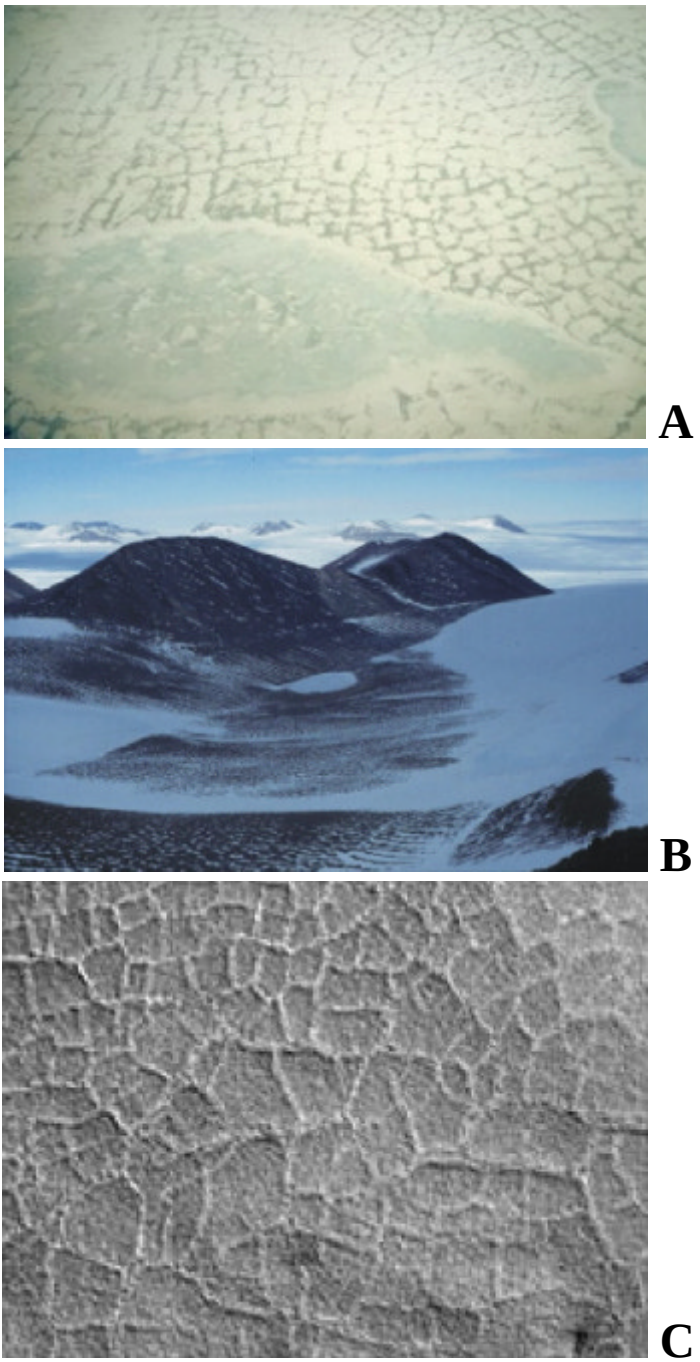


**Fig. 9.2** Permafrost structures. **A:** Lena Delta, Russian Arctic (April 1999, photo W.Schneider, AWI); **B:** Haskard Highlands, Antarctica (December 1994, photo W.-D. Hermichen, AWI); **C:** Mars, Northern Hemisphere (May 1999, photo NASA).

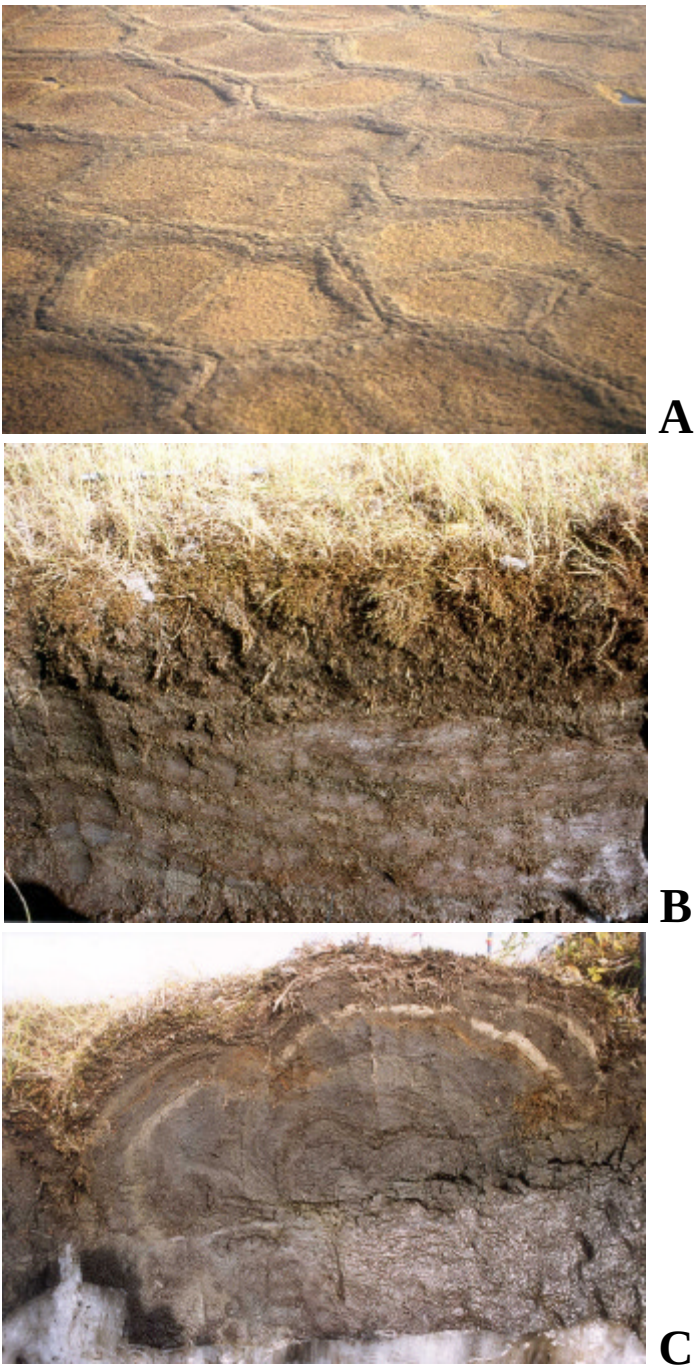


**Fig. 9.3** Landscape and soils of polygon tundra, Lena Delta/Siberia. **A:** Low-centered polygons; **B:** *Typic Historthel* of the polygon center; **C:** *Glacic Aquiturbel* of the polygon border (photos L. Kutzbach, AWI).

gaining energy by the oxidation of inorganic substances. Carbon dioxide can be used as the only carbon source. Lithoautotrophic growth is an important presumption for long-term survival [19] of microbes in extreme environments like terrestrial permafrost or maybe on other planets of our Solar System.

### 9.3.1 Methanogenesis

Responsible for the biogenic methane production (methanogenesis) is a small group of microorganisms called methanogenic archaea [20]. Methanogenesis represents the terminal step in carbon flow in many anaerobic habitats, including permafrost soils, marshes and swamps, marine and freshwater sediments, flooded rice paddies and geothermal habitats. Although methanogens are widely spread in nature they show an extremely specialized metabolism. They are able to converse only a limited number of substrates (e.g., hydrogen, acetate, formate, methanol, methyl- amines) to methane. In permafrost soils two main pathways of energy-metabolism dominate: (i) the reduction of $CO_2$ to $CH_4$ using $H_2$ as a reductant and (ii) the fermentation of acetate to $CH_4$ and $CO_2$. In the case of $CO_2$-reduction organic carbon is not necessary for growth of methanogenic archaea [21].

At present, 68 species of methanogenic archaea are known including common genera like *Methanosarcina*, *Methanobacterium* and *Methanococcus.* Phylogenetically, they are classified as ARCHAEA [22], a group of microbes that are distinguished from BACTERIA by some specific characteristics (e.g., cell wall composition, coenzymes). They show a high adaptability at extreme environmental conditions like temperature, salinity and oxygen. Besides the mesophilic species, also thermophilic methanogens are known (see Chap. 11, Stetter). In newer times, more attention has been paid on the search for psychrophilic strains since many of methanogenic habitats belong to cold climates [23]. A lot of methanogens (e.g., *Methanogenium cariaci*, *Methanosarcina thermophila*) are able to adapt to high salinity by the accumulation of compatible solutes to equalize the external and internal osmolarity [24]. Although, they are regarded as strictly anaerobic organisms without the ability to form spores or other resting stages, they are found in millions of years old permafrost sediments [25] as well as in other extreme habitats like aerobic desert soils [26] and hot springs [27].

Because of the specific adaptations of methanogenic archaea to conditions like on early Earth (e.g., no oxygen, no or less organic compounds), they are considered to be one of the initial organisms from the beginning of life on Earth.

### 9.3.2 Nitrification

Nitrifying bacteria play a main role in the global nitrogen cycle by the transformation of reduced nitrogen compounds. Two groups of distinct organisms - the ammonia and nitrite oxidizers - are responsible for the oxidation of ammonia to nitrite and further to nitrate [28]. The genera of ammonia oxidizers have the prefix nitroso- whereas the nitrite oxidizers start with nitro-. The best known nitrifiers are *Nitrosomonas* and *Nitrobacter*. Up to now, 5 genera of ammonia oxidizers (with 16 species) and 4 genera of nitrite oxidizers (with 8 species) have been described [29, 30]. Phylogenetically,

ammonia and nitrite oxidizers are affiliated to different subclasses of the *Proteobacteria* with the exception of *Nitrospira* (and maybe *Nitrospina*), which belong to a separate phylum [31, 32]. Although *Nitrosomonas* and *Nitrobacter* are usually the most isolated nitrifiers, they are not obligatelly the most abundant ones in a given habitat. For example, organisms of the genus *Nitrospira* seem to have a higher ecological importance than previously assumed since they were recognized as dominant nitrite oxidizers in several aquatic habitats (reviewed by Spieck and Bock [33]). These bacteria are the phylogenetic most ancient nitrifiers since they belong to a deep branching phylum. Here, a high diversity of new species was detected recently.

Nitrifiers exist in most aerobic environments where organic matter is mineralized (soils, compost, fresh- and seawater, waste water). In general, cell growth is slow with regard to the poor energy sources but can be adapted to changing environmental conditions. Especially for *Nitrobacter*, mixotrophic and heterotrophic growth with organic compounds is an alternative to the oxidation of nitrite. Nitrifiers are also active in low oxygen and anaerobic environments like sewage disposal systems and marine sediments where they are able to act as denitrifiers [34]. Although they form no endospores, they can survive long periods of starvation and dryness. Therefore, nitrifying bacteria were also detected in e.g., antarctic soils [35], natural stones [36], heating systems [32] as well as in subsurface sediments in a depth of 260 m [37]. Especially ammonia oxidizers form dense cell clusters, where cells are embedded in a dense layer of EPS (extracellular polymeric substances). These microcolonies may protect the cells against stress factors like dryness. Another protecting mechanism is the production or accumulation of compatible solutes (e.g., trehalose, glycine betaine or sucrose, see Chap. 12, Kunte et al.). Due to salt stress and dryness an increasing amount of compatible solutes was found in cells of *Nitrobacter* [38].

## 9.4 Methods for Analogue Studies of Microbial Processes in Terrestrial and Extraterrestrial Habitats

Increased attention has been paid on the processes of methanogenesis and nitrification in the last decades, because the involved bacteria [39] influence the global climate by the generation and transfer of climate-relevant trace gases like methane and gaseous nitrogenous oxides (NO, $N_2O$, $NO_2$). Recently, these microorganisms are also important in the area of astrobiology research because of their adaptation ability to extreme location-conditions and consequently for the search of extraterrestrial lives of particular interest.

In order to understand the described microbial key-processes in permafrost soils, it is necessary to know the microbial community which is involved in methanogenesis and nitrification. The most important members of this community are those archaea/bacteria, which are metabolic active under such extreme conditions in the soils. To learn about these microbes and their adaptation strategies, they must be isolated and characterized. For quantitative aspects, bacterial cell numbers and microbial biomass have to be determined using classical microbiological and modern molecular biological techniques.

### 9.4.1 Methanogenic and Nitrifying Populations

A polyphasic approach is needed to reveal the diversity, population dynamics and ecological significance of bacteria in permafrost soils and sediments. Enrichment and isolation of microorganisms is necessary for taxonomical and ecophysiological characterization of microbial populations in order to understand their adaptation strategies and potential to extreme environmental conditions.

Traditionally, cell numbers of methanogenic archaea and nitrifying bacteria were quantified by the most probable number (MPN) technique in selective chemolithoautotrophic media [28, 40]. The highest dilution serves as initial inoculum for cultivation studies like identification and characterization of the relevant bacteria. Viable methanogens and nitrifiers were detected in the Kolyma-Indigirka Lowland in northeast Siberia by Russian and German scientists [41]. The bacteria occurred in high cell numbers in the upper layers and in decreasing numbers in more ancient deposits. MPN counts of methanogenic archaea varied between $2.0 \times 10^2$ and $2.5 \times 10^7$ cells $g^{-1}$. Soina et al. [42] detected mesophilic nitrifying bacteria with $2.5 \times 10^2$ cells $g^{-1}$ soil in a depth of 28 m. Lebedeva and Soina [17] found nitrifying bacteria in geological horizons up to 3 millions of years in a depth of 60 m. With increasing age of the sediments, psychrotrophic nitrifiers were found to be replaced by psychrophilic ones**,** although the permafrost communities are dominated by psychrotrophs [43]. Nevertheless, psychrophilic bacteria have a significant part in the microbial community in cold environments like permafrost soils [44]. The investigations of the methanogenic community on Taimyr Peninsula [45] and in the Lena Delta [46] gave hints for the adaptation to the low *in situ* temperatures. However, isolation of psychrophilic methanogens and nitrifiers from permafrost soils seems to be more complicated than in other physiological defined groups like acetogenic [47] and methane-oxidizing bacteria [48] as well as clostridia [49]. Cultivation at 5 °C of the slow growing microbes is hindered by prolonged lag-periods, which amounted to 2 - 14 months in the case of nitrite oxidizers [50]. Therefore, the organisms had to be incubated at higher temperatures of e.g., 17 °C. So far, there was only one methanogenic archaea isolated from Ace Lake/Antarctica, which showed psychrophilic growth characteristics [51].

In order to obtain pure cultures for physiological characterization (e.g., determination of the temperature optima) isolation of typical bacteria is required. This can be done by serial dilution in liquid growth media or deep-agar tubes (Agar-shakes), plating on agar plates under aerobic or anaerobic conditions and is also possible by percoll density gradient centrifugation [52]. However, separation of aggregated cells is problematically and requires further treatment. Identification of isolates and enriched organisms was performed by traditional light and electron microscopy with genus-specific morphology and ultrastructure as criteria. Classified by their spiral cell shape, the ammonia oxidizers isolated from soil samples taken during the expedition “Beringia“ in 1991 and 1992 were identified as members of the genus *Nitrosospira* (or *Nitrosovibrio*). Among nitrite oxidizers, *Nitrobacter* was identified by its pleomorphic morphology and a polar cap of intra-cytoplasmic membranes [50]. In surface samples which were taken during the expedition “Lena 1999“ [46], the coexistence of *Nitrobacter* and *Nitrospira* in enrichment cultures was demonstrated by their typical morphology (Fig 9.4) of pleomorphic short rods (with a diameter of 0.8 µm) respectively spiral rods (with a diameter of 0.2 µm).

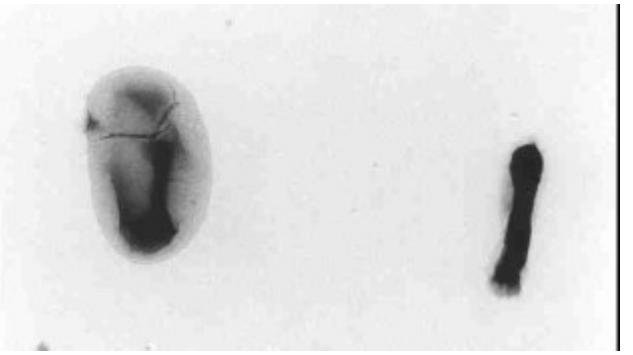

**Fig. 9.4** Enriched bacteria in nitrite oxidizing medium from the active layer of a permafrost soil (Samoylov/Lena Delta) with a morphology similar to *Nitrospira* respectively *Nitrobacter*. Negative staining was performed with uranyl acetate. Magnification 20 300×.

Since the isolated organisms may not be the ecologically relevant ones, the development of new detection strategies was necessary to monitor the enrichment procedure. Modern microscopic techniques like CLSM (confocal laser scanning microscopy) in combination with fluorescent dyes enable specific or unspecific labeling of viable cells. In Fig. 9.5 an unspecific labeling of bacteria probably belonging to the genus *Nitrospira* is presented. Here, the organisms were affiliated by the formation of characteristic cell clusters. Like many ammonia oxidizers the *Nitrospira*-like bacteria were aggregated to micro-colonies.

New molecular techniques were developed for the detection of ecological relevant bacteria without cultivation [53, 54]. Fluorescence *in situ* hybridization (FISH) using population-specific gene probes targeting 16S rRNA enables direct microscopic enumeration of single cells (Fig. 9.6). Demanding low amounts of cell material, such methods are well suited for methanogenic archaea and nitrifying bacteria. Molecular 16S rDNA sequence analysis is required for phylogentic affiliation of new isolates.

An immunological approach for the identification of nitrite as well as ammonia oxidizers was developed by Bartosch et al. [55] and Pinck et al. [56]. They used monoclonal and polyclonal antibodies, respectively, recognizing the key-enzymes of these functional groups of bacteria as phylogenetic marker. Nitrite oxidizers enriched from permafrost sediments were identified immunologically as members of the genus *Nitrobacter* [55]. These nitrifiers originated from sediments with an age of 40 000 years. Further on, in the active layer of a permafrost soil from Samoylov Island/Lena Delta nitrite oxidizers of the genus *Nitrospira* were detected by Hartwig [57]. Depending on the substrate concentration, *Nitrospira* together with *Nitrobacte* (0.2 g $NaNO_2$ $l^{-1}$) or *Nitrobacter* alone (2 g $NaNO_2$ $l^{-1}$) could be enriched. Both genera of nitrite oxidizers could be distinguished in Western blot analysis by different molecular masses of the β-subunit of their nitrite oxidizing systems (Fig. 9.7). This protein of *Nitrobacter* has a molecular mass of 65 kDa, whereas in *Nitrospira* 46 kDa were determined [55].

Phospholipid analysis in microbial ecology is a further method to study the biomass, population structure, metabolic status and activity of natural communities [59]. Specific groups of microorganisms (like the nitrite oxidizers) contain characteristic phospholipid ester-linked fatty acids (PLFA), whereas methanogenic archaea are characterized by ether-linked glycerolipids [60]. Lipid biomarkers are important for the detec-

tion of single taxons. Such a characteristic new fatty acid (11-methyl-palmitate) was recently found in *Nitrospira moscoviensis* [61].

### 9.4.2 *In situ* Activity

The activity of microorganisms depends not only on their own physiological capability but is influenced also by habitat-qualities like the grain size or the availability of nutrients. That is why, besides the characterization of the microflora, their activity in

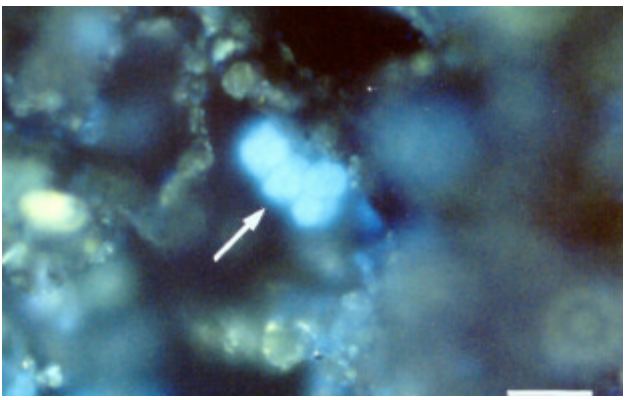

**Fig. 9.5** With DAPI (4,6-diamidino-2-phenylindol) stained micro-colony of *Nitrospira*-like bacteria (arrow), enriched from the active layer of a permafrost soil (Samoylov/Lena Delta). Bar = 25 µm. (photo C. Hartwig, University of Hamburg).

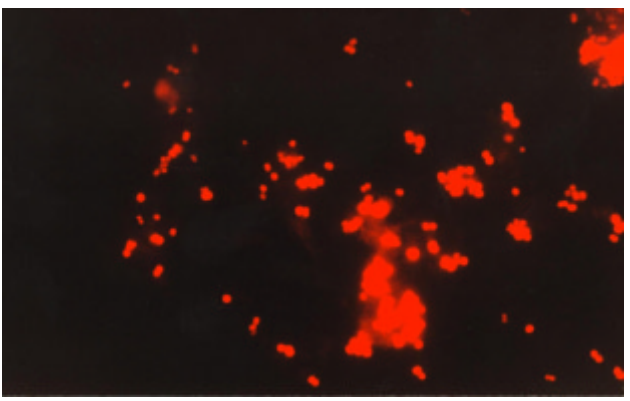

**Fig. 9.6** Confocal microscopy of archaea from the family *Methanomicrobiales* enriched from permafrost soils (Lena Delta/Siberia). The culture was grown with $H_2/CO_2$ (80:20, v:v) at 10 °C. The hybridization was carried out with the oligonucleotide probe MG1200 [54] (photo S. Kobabe, AWI).

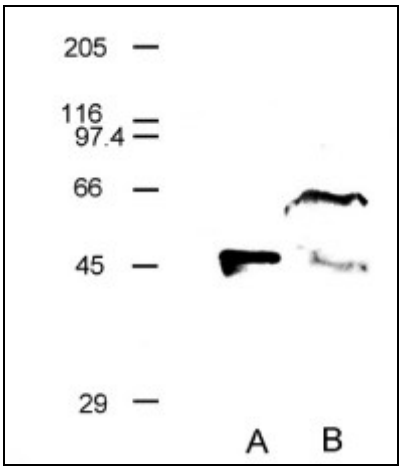


**Fig. 9.7** Immunoblot of an enrichment culture derived from permafrost soil using monoclonal antibodies recognizing the β-subunit of the nitrite-oxidizing system. The values on the left are molecular masses in kDa. **A:** pure culture of *Nitrospira moscoviensis*, **B:** enrichment culture using 0.2 g $NaNO_2$ $l^{-1}$ (modified from Bartosch et al. [58]).

the natural habitat is of importance for the understanding of life under extreme conditions. There are different methods for analyzing *in situ* activities, i.e. determination of concentration gradients [62], flux measurements [63] and assay of activity in soil samples [64]. A new technique for the determination of nitrification rates *in situ* is the introduction of microelectrodes (e.g., for ammonia and nitrate). These sensors make it possible to monitor metabolic reactions in the nitrogen cycle [65].

The activity of methanogenic archaea can be followed by the measurement of the metabolic end product $CH_4$ over a period of time. Methane generation takes place only under anaerobic conditions in the permafrost soils and sediments, for example in the water saturated soils of the polygon center. *In situ* rates of methanogenesis can only be obtained if the anaerobic food chain is not affected by the experimental procedure because methanogenesis depends on the substrates produced by other microorganisms. The *in situ* methane production can be investigated by incubation of soil samples from permafrost sites. Figure 9.8 shows the *in situ* methane production in dependence from the natural temperature gradient of a permafrost soil. For this investigation, fresh soil material was used. The prepared soil samples were re-installed in the same layers of the soil profile from which the samples had been taken [46].

The influence of soil texture on the activity of microorganisms can be examined by incubation experiments with model soils of a different grain size [66]. Changing temperature and pressure conditions as well as the impact of different substrates on microbial activity can be studied in special simulation experiments with undisturbed soil samples (see 9.4.4).

To estimate the nitrifying activity in permafrost sediments, the potential activity of soil bacteria was determined under optimal laboratory conditions. For that purpose, soil samples were taken from drill cores and transferred to the laboratory under sterile

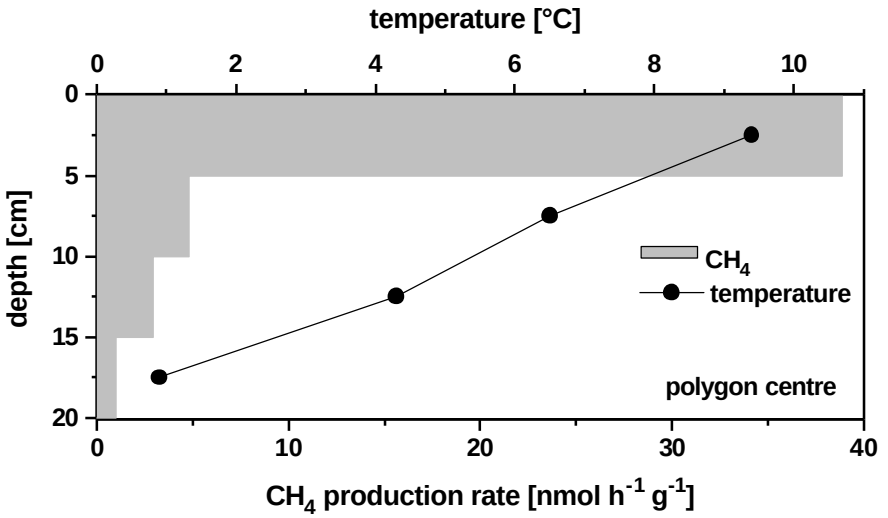


**Fig. 9.8** Vertical profile of *in situ* methane production and soil temperature for a permafrost soil of the polygon center.

conditions. In the active layer and in sediments with an age of 40 000 years the nitrifying activity was higher at 28 °C in comparison to 17 °C, whereas in more ancient deposits (0.6-1.8 million of years and 2.5-3 million of years) the bacteria preferred lower temperatures of 17 °C (Lebedeva, pers. comm.).

Further investigations about nitrifying bacteria in permafrost sediments included measurements of ammonia, nitrite and nitrate as substrates respectively products of nitrification. Janssen [50] determined the concentrations of these nitrogen compounds in the soil samples by high-performance-liquid chromatography. The profiles showed that nitrite and nitrate were always found in the ppm range in sediments up to 150 000 years and occasionally in deeper layers. Ammonia concentrations amounted up to 100 ppm with increasing amounts in sediments with an age of 1-5 millions years. Nitrite and nitrate correlated with the presence of nitrifying bacteria although nitrifiers were also detected in samples without these nitrogen salts. The detection of the chemical unstable metabolic intermediate nitrite in correlation with the presence of viable ammonia oxidizers gave first evidence of modern microbial activity in permanently frozen sediments.

### 9.4.3 Isotopic Analysis: Carbon Fractionation via Microbial Processes in Permafrost

It is well known that microbial processes tend to fractionate the C-isotopes of organic matter in soils and sediments by favoring the lighter $^{12}$C-carbon over the heavier $^{13}$C-compounds. Methanogenesis for example leads to the strongest C-discrimination in nature with the result that soil organic matter will be enriched with $^{13}$C-carbon (e.g.,

$\delta^{13}$C-values of about –16‰ to –22‰) while the product of anaerobic decay - methane - will be depleted with $^{13}$C (values of about –60‰ [67]). In anaerobic zones of permafrost soils with methane production the soil organic matter showed an absolute enrichment of $^{13}$C-carbon of about 3.7‰ to 8.3‰ [45, 68]. Therefore, isotope-related analysis in combination with the microbial studies may be a powerful tool to search for traces of microbial life in extraterrestrial habitats, even if the applicability for extraterrestrial environments could not be examined until now sufficiently [69].

### 9.4.4 Simulation Experiments

The influence of environmental conditions on the activity and survival of microorganisms could be investigated by simulation experiments with bacterial cultures and with undisturbed soil material.

Thawing and freezing processes influence not only the soil temperature regime but also the availability of liquid water, the pressure conditions and the salinity of pore water. They produce also granular, platy and vesicular soil structure in the surface near horizons and a massive structure in the subsurface zones. Undisturbed soil samples (soil cores of different size) save the structure, pore system and stratification of the natural soil, which influence the interaction between microbes and soil matrix. Simulation of freeze-thaw cycles can help to understand: how will the microbial population be influenced by the natural permafrost system and by the interaction of the combined parameters?

The viability of the permafrost microflora under the environmental conditions of Martian atmosphere can be investigated by simulation experiments in special ice laboratories (Alfred Wegener Institute for Polar and Marine Research, AWI) and in a special Martian simulator (German Aerospace Center, DLR). Natural soil material and pure cultures of bacteria isolated from terrestrial permafrost habitats can be exposed to extreme cold temperatures (–60 °C), lower pressure (560 Pa), higher background radiation (UV 200 nm), drier soil moisture conditions and varying ice contents in comparison with well known terrestrial permafrost.

## 9.5 Conclusion and Future Perspectives

Microbial life in permafrost soils depends on available water (see Chap. 5, Brack). If inorganic compounds like hydrogen as well as ammonia or nitrite are present as substrates, conditions are favorable for the growth of methanogenic archaea and nitrifying bacteria. Since cell synthesis is carried out by the assimilation of carbon dioxide there is no further need for organic material. This mineralic basis resembles the situation on mars (e.g., C, H, O, N, P, K, Ca, Mg and S, reviewed by Horneck [70]). Lithoautotrophic bacteria are well investigated and ubiquitous distributed organisms on Earth. They survived even in terrestrial permafrost for several millions of years [41]. Here, they demonstrate the residue of the autochthon population within the paleosoils which was enclosed during deposition of fresh sediments. The frozen microorganisms in the deeper permafrost sediments are thought to have not evolved significantly during the

past several million years because it was not necessary to adapt to their environment [16]. In contrast, the microbes living in the active layer and the transition permafrost sediments are influenced by extreme changes of live-decisive environmental conditions. Preserving their viability in such an extreme environment they had to develop different strategies to resist desiccation, freezing processes and nutrient-lack. The isolation and characterization of methanogenic archaea and nitrfying bacteria from permafrost soils should clarify the possible growth characteristic (psychrophile and psychrotroph) and ecological significance of these microbes.

The data obtained from future research on living conditions and adaptation strategies of microorganisms in terrestrial permafrost soils should be compared with the postulated environmental conditions on early Mars [1, 71]. They were characterized by liquid water, a moderate climate and a postulated biosphere which had been dominated by anaerobic processes and diversification of anaerobic organisms. Furthermore, the comparative system studies will serve for understanding the modern Mars cryosphere and other extraterrestrial permafrost habitats. This knowledge represents an essential basis for the understanding of the origin of life and the environmental development on extreme habitats.

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