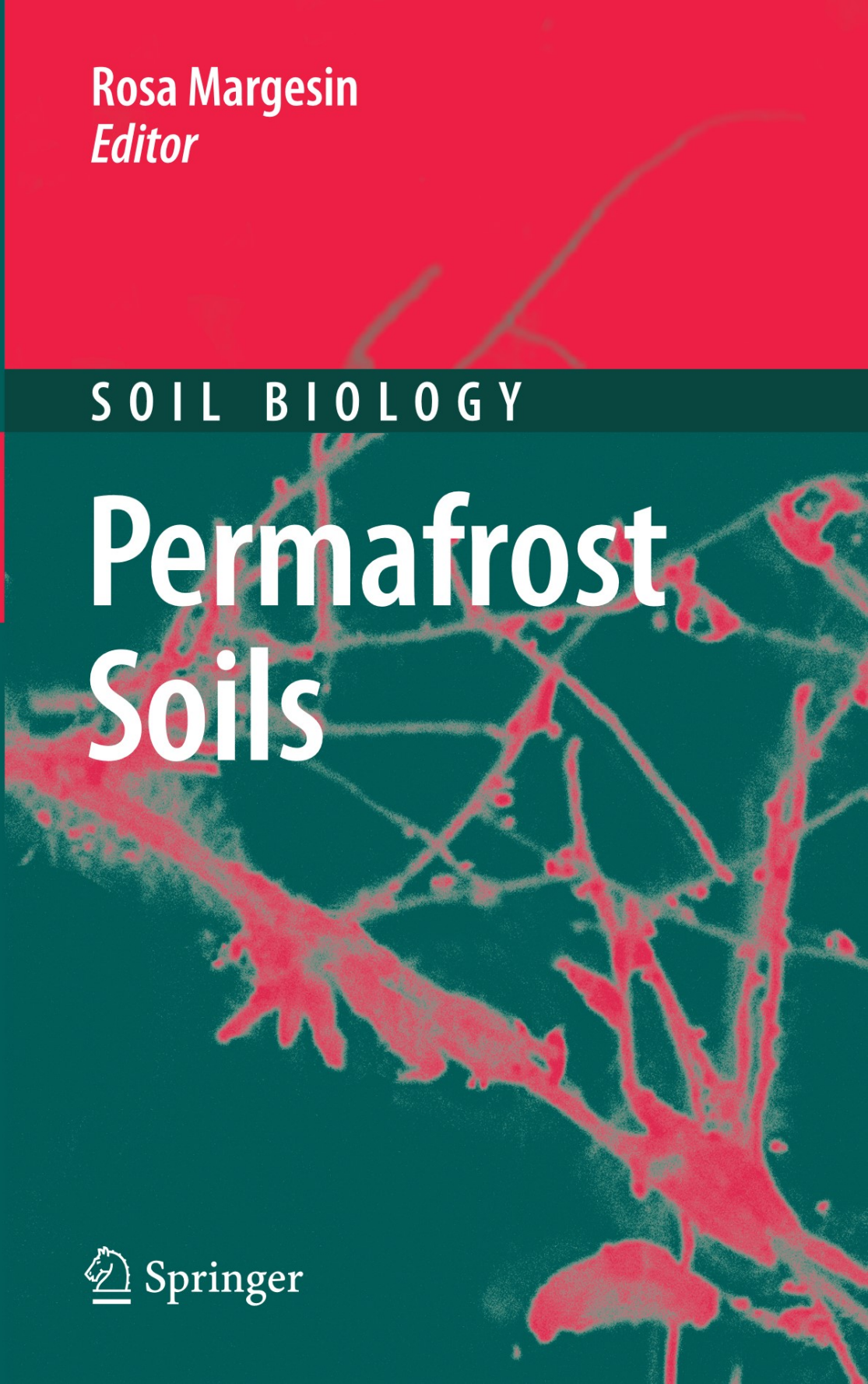
Rosa Margesin
Editor

SOIL BIOLOGY

Permafrost Soils



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Preface

Most of the Earth's biosphere is characterized by low temperatures. Vast areas (>20%) of the soil ecosystem are permanently frozen or are unfrozen for only a few weeks in summer. Permafrost regions occur at high latitudes and also at high elevations; a significant part of the global permafrost area is represented by mountains.

Permafrost soils are of global interest, since a significant increase in temperature is predicted for polar regions. Global warming will have a great impact on these soils, especially in northern regions, since they contain large amounts of organic carbon and act as carbon sinks, and a temperature increase will result in a release of carbon into the atmosphere. Additionally, the intensified release of the climate-relevant tracer gas methane represents a potential environmental harzard.

Significant numbers of viable microorganisms, including bacteria, archaea, phototrophic cyanobacteria and green algae, fungi and protozoa, are present in permafrost, and the characteristics of these microorganisms reflect the unique and extreme conditions of the permafrost environment. Remarkably, these microorganisms have been reported to be metabolically active at subzero temperatures, even down to −20°C.

This book summarizes recent knowledge on various aspects of permafrost and permafrost-affected soils, including typical properties of these soils, distribution and biodiversity of permafrost microorganisms, examples for microbial activity in frozen soils, and genomic and proteomic insights into cold adaptation of permafrost bacteria. The impact of global warming on microbial communities, carbon dynamics, geomorphology, and frozen-ground engineering are further discussed. Other chapters describe the feasibility and limitations of methods for removing contaminants in frozen ground. Finally, terrestrial permafrost is considered as a model for extraterrestrial habitats.

I wish to thank all authors, who are authorities in their field, for their excellent contributions. I also thank Dr. Franz Schinner for many interesting discussions and Dr. Jutta Lindenborn and Dr. Dieter Czeschlik, Springer Life Sciences, for continuous support during the preparation of this volume.

Innsbruck, April 2008 Rosa Margesin

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Part I
Geological, Chemical and Physical Properties of Permafrost

Chapter 1
Arctic Permafrost Soils

Charles Tarnocai

1.1 Introduction

The Arctic region, the portion of the Northern Hemisphere lying north of the arctic tree line, covers a land area of approximately 7.2×10^6 km^2. Approximately equal extents of most of this area (66%) occur in Canada and Russia, with lesser extents occurring in the United States (Alaska), Greenland and Scandinavia. Glaciers cover approximately 1.9×10^6 km^2 (26%) of this land area, with most of the glaciers (92%) occurring in Greenland.

At the beginning of the twentieth century, German and Russian soil scientists carried out soil studies in the Eurasian Arctic region (Kvashnin-Samarin 1911; Sukachev 1911; Meinardus 1912; Blanck 1919). These scientists used primarily a geological approach to study Arctic soils. In the North American Arctic, Everett (1968), Leahey (1947), Tedrow and Douglas (1964) and Tedrow et al. (1968) carried out the early pedological studies in the Arctic. Although these North American scientists applied a pedological approach to their studies, they viewed these soils as merely frozen versions of temperate soils — formed by much weaker, but basically similar, processes to those taking place in unfrozen soils.

During the early 1970s, Canadian soil scientists carried out extensive pedological work in northern Canada. When they realized that the development of these soils was dominated by cryogenic processes, they developed the Cryosolic Order for the Canadian System of Soil Classification (Canada Soil Survey Committee 1978). This new approach was very quickly embraced by American soil scientists, and eventually led to the creation of the Gelisol soil order in the US Soil Taxonomy (Soil Survey Staff 1998). This concept enjoyed wide acceptance in Western Europe, and resulted in the establishment of the new Cryosolic major soil group for permafrost-affected soils in the World Reference Base for Soil Resources (Spaargaren 1994). The current state of knowledge about permafrost-affected soils was summarized by international experts in 37 papers in the book "Cryosols: Permafrost-Affected Soils" (Kimble 2004).

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In this chapter the geological, physical and chemical properties of permafrost-affected soils in the Arctic region will be discussed, along with the cryogenic processes that produce their unique characteristics. In addition, data for selected Arctic soils are presented in Tables 1.1 and 1.2.

Table 1.1 Location and source of data for selected pedons

Pedon no.	Field no.	Area	Latitude (N)	Longitude	Source of data
1	12–89–22	Ellesmere Is., Canada	81° 23′ 35″	76° 44′ 30″ W	Tarnocai (unpubl)
2	Isachsen 3	Ellef Ringnes Is., Canada	78° 47.098′	103° 33.125′.W	Ping (unpubl)
3	12–81–26	Prince Patrick Is., Canada	76° 14′	119° 20′ W	Tarnocai 2004
4	DB-4	Bathurst Is., Canada	75° 40′	97° 41′ W	Tarnocai 2004
5	SO1AK185006	Howe Is., USA	70° 18.986′	147° 59.647′ W	Ping (unpubl)
6	94FN825009	Chersky, Siberia, Russia	69° 27′ 49″	161° 45′ 57″ E	USDA Lab[a]
7	N5b	Tuktoyaktuk, Canada	69° 26′	133° 01′ W	Pettapiece et al. 1978
8	Y66	Yukon, Canada	68° 55′	137° 50′ W	Tarnocai (unpubl)

[a] US Department of Agriculture Soil Laboratory, Lincoln, NE, USA

Table 1.2 Site parameters for selected pedons

Pedon no.	1	2	3	4	5	6	7	8
Landform[a]	CB	DG	I	R	L	U	L	U
Drainage[b]	W	P	MW	W	I	MW	P	I
Parent material[c]	C	GM	C	C	A	L	P	C
Depth to permafrost (cm)	60	34	40	58	55	62	30	24
Patterned ground[d]	NC	TH	NC	SP	NC	IWP	IWP	EH
Vegetation[e]	D	ML	MS	ML	LT	GST	ST	ST
Soil class. (Canada)[f]	RTC	CTC	OETC	OETC	OETC	GTC	MOC	GTC
Soil class. (US)[g]	TP	GAT	TUT	TUT	MT	TAT	TH	TAT

[a]Landform: *CB* colluvial blanket; *DG* dissected; *I* inclined; *L* level; *R* rolling; *U* undulating

[b]Drainage: *W* well; *MW* moderately well; *I* imperfect; *P* poor

[c]Parent material: *A* alluvium; *C* colluvium; *GM* glaciomarine; *L* loess; *P* peat

[d]Patterned ground: *EH* earth hummocks; *IWP* ice-wedge polygons; *NC* nonsorted circles; *SP* small (15–40 cm diam) polygons; *TH* turf hummocks

[e]Vegetation: *D* dryas–sedge tundra; *GST* grass–shrub tundra; *LT* lichen–shrub tundra; *ML* moss–lichen–saxifrage tundra; *MS* moss–sedge–lichen–willow tundra; *ST* shrub tundra

[f]Soil classification (Canada: Soil Classification Working Group 1998): *CTC* Glacic Turbic Cryosol; *GTC* Gleysolic Turbic Cryosol; *MOC* Mesic Organic Cryosol; *OETC* Orthic Eutric Turbic Cryosol; *RTC* Regosolic Turbic Cryosol

[g]Soil classification (US: Soil Survey Staff 1998): *GAT* Glacic Aquaturbel; *MT* Molliturbel; *TAT* Typic Aquaturbel; *TH* Typic Hemistel; *TP* Typic Psammoturbel; *TUT* Typic Umbriturbel

1.2 Arctic Environment

The Arctic climate is characterized by short, cold summers and long, extremely cold winters. It has 24 h of daylight during much of the summer, and darkness during much of the winter. Mean daily temperatures above 0°C occur only during the warmest part of the summer. The range of mean July temperatures is 7–10°C in the southern part of the Arctic and 3–5°C in the northern part. The coldest month is February, with temperatures of −20 to −40°C. Total annual precipitation is generally low (60–160 mm) and occurs mostly as snow.

The Arctic vegetation is a nearly continuous cover of shrub-tundra in the south, grading to a sparse cover of dwarf shrubs, herbs, mosses and lichens in the north. Permafrost is continuous, and reaches a thickness of 100–500 m in North America and >500 m in Siberia. The active layer (the surface layer which freezes and thaws annually) is about 30–60 cm thick. The soil surface is generally associated with patterned ground, which refers to a land surface that displays an ordered and repeated, more-or-less symmetrical, morphological pattern. A number of patterned ground classification systems occur in the literature, but the one most commonly used was developed for mineral terrain by Washburn (1980). This classification uses descriptive terminology based on geometric forms and the presence or absence of sorting of stones (coarse) and finer materials. The patterned ground forms for mineral terrain are circles, nets, steps, stripes, and polygons.

1.3 Geological Setting

The bedrock geology of the Arctic is dominated by large areas of sedimentary, igneous and metamorphic rocks. Repeated glaciations and erosional processes reshaped the landscapes and deposited various thicknesses of surficial materials. During the glacial periods, large parts of the Canadian and Scandinavian Arctic were covered by glacial ice, which deposited variable thicknesses of glacial materials. Remnants of this ice still remain in Greenland, and as ice caps in the northeastern part of the Canadian Arctic. Coastal areas usually are associated with marine deposits, because of sea-level changes and glacial rebound.

A large part of the Arctic in Eurasia and northwestern North America (Alaska and part of Yukon) was unglaciated, and is covered with thick surficial materials of eolian (loess), colluvial and lacustrine origin. Most of the Siberian Arctic is associated with deep yedoma sediments derived from windblown, reworked colluvial materials.

Peat deposits are common surficial deposits, especially in the southern part of the Arctic. These deposits, which are usually about 2–3 m thick, result from peat deposition during the last 5,000–8,000 years. They usually occur in lowlands, and are associated with ice-wedge polygons. These peat deposits play an important role in the carbon budget of the area.

1.4 Soil-Forming Processes

All soils are formed by the interaction of soil-forming factors, but because of the cold climate in the Arctic region, cryogenic processes, which lead to the formation of permafrost-affected soils, dominate the soil genesis. The presence and mobility of unfrozen soil water, as it migrates towards the frozen front along the thermal gradient in the frozen system, drives this process. The cryogenic processes that affect the genesis of Arctic soils are freeze–thaw, cryoturbation (frost churning), frost heave, cryogenic sorting, thermal cracking, and ice build-up. Other soil-forming processes that can leave an imprint on these soils include the gleying process, brunification, eluviation and salinization.

1.5 Properties of Arctic Soils

The presence of ice in permafrost-affected soils causes complex physico-chemical processes. Formation of ice in these soils creates stresses and pressures that result in deformation and rearrangement of the soil horizons, and translocation of materials and solutes. This leads to unique macromorphologies and micromorphologies, thermal characteristics, and physical and chemical properties.

1.5.1 Macromorphology

The morphologies of both the surface and subsurface of Arctic soils are shaped by cryogenic processes (Figs. 1.1 and 1.2). The soil surface is associated with various types of patterned ground caused by frost heave and sorting, while the subsurface is dominated by cryoturbation that results in irregular or broken soil horizons, involutions, organic intrusions, and organic matter accumulation, usually along the top of the permafrost table. Oriented rock fragments (Fig. 1.1), silt-enriched layers and silt caps are also common (Bockheim and Tarnocai 1998). The freeze–thaw process produces granular, platy and blocky structures (Table 1.3). The subsurface soil horizons often have massive structures and are associated with higher bulk densities, especially in fine-textured soils. This massive structure results from cryostatic desiccation (cryodesiccation), which develops when the two freezing fronts (one from the surface, the other from the permafrost) merge during freeze-back. Although these macromorphological properties occur primarily in the active layer, they also can be found in the near-surface permafrost because of the dynamic nature of the permafrost (Bockheim and Tarnocai 1998).

Arctic soils generally have high moisture content, especially near the permafrost table, which acts as a moisture barrier. As a result, gleying associated with grayish colours and redoximorphic features is a common occurrence, especially in loamy and fine-textured soils.

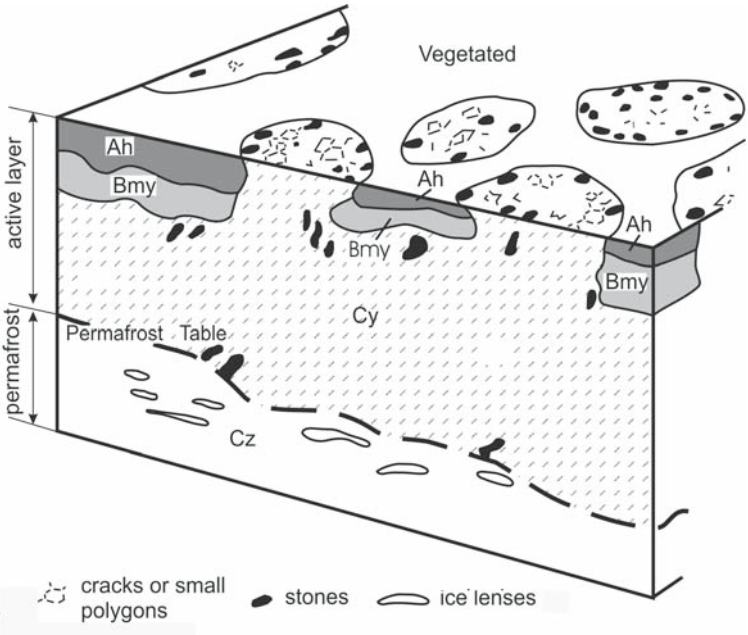

Fig. 1.1 Schematic diagram showing a nonsorted circle type of patterned ground with discontinuous and broken cryoturbated soil horizons (*y*) and oriented stones in the active layer, and ice lenses in the permafrost layer

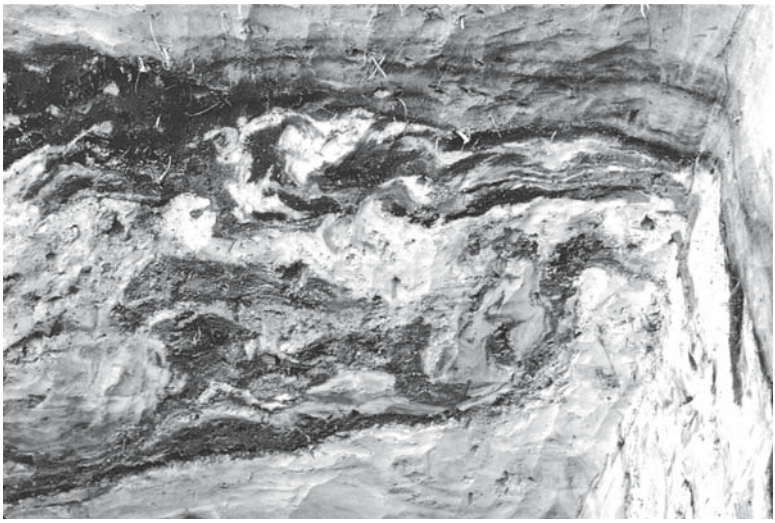

Fig. 1.2 Strongly cryoturbated soil with contorted and broken soil horizons

Table 1.3 Morphological characteristics of selected pedons

Pedon no.	Horizon	Depth (cm)	Colour	Texture[a]	Structure[b]	Ice content	Special features
1	Ck	0–15	10YR 4/1.5m	fSL	sbk	–	10% gravel
	Cky	15–60	10YR 3/2m	SiL	sbk	–	–
	Ckyz	60–100	10YR 3/2.5f	SiL	sbk	medium	ice crystals
2	Ajj1	0–10	10YR 3/2m	C	fgr	–	–
	Ajj2	10–18	10YR 4/3m	C	fgr	–	–
	Bw	18–34	10YR 4/3m	C	lenticular	–	–
	Wf/Bgf	34–40	10YR 4/2f	C	lenticular	high	–
	Wf	40–42	–	–	–	ice	pure ice
	Wf/Cf	42–57	10YR 4/2f	C	lenticular	high	–
	Wf	57–110	–	–	–	ice	pure ice
3	Ah	0–8[c]	10YR 2/1m	–	gr	–	–
	Bmy1	0–14	10YR 4/3m	SL	gr	–	–
	Bmy2	14–55	10YR 4/2m	SL	gr	–	–
	Cy	55–100	10YR 4/2m	SL	sg	–	oi[d]
	Ahyz	40–45[c]	10YR 2/1f	–	sl	high	–
	Cyz	45–80[c]	10YR 4/2f	SL	sl	high	oi[d]
4	Bmky	2–44[c]	10YR 3.5/2m	SL	gr	–	vesicular
	BCky	10–32[c]	10YR 3/3m	SL	gr	–	vesicular
	Ahky	1–15[c]	10YR 2/1m	SL	sg	–	vein ice
	Ckz	46–100	10YR 4/1m	SL	sl	–	ice crystals
5	A1	0–5[c]	10YR 3/2m	fSL	platy	–	–
	A2	5–40[c]	10YR 3/2m	fSL	platy	–	–
	Ajj	62–70[c]	7.5YR 3/2m	fSL	fgr	–	oi[d]
	C1	0–5[c]	2.2Y 4/2m	fSL	reticular	–	–
	C2	5–25[c]	10YR 3/2m	fSL	reticular	–	–
	Bwjj1	20–60[c]	10YR 4/2m	fSL	lenticular	–	–
	Bwjj2	20–65[c]	10YR 4/2m	fSL	reticular	–	–
	Bwjj3	55–68[c]	2.5Y 5/1f	fSL	massive	–	–
	Wfm/Cf	68–110[c]	7.5YR 4/1f	fSL	ataxitic	high	70% ice
	Cf	80–110[c]	2.5Y 4/1f	fSL	platy	–	–
6	Oi	0–11[c]	5YR 3/2m	Peat	–	–	–
	A	0–8[c]	2.5Y 3/2m	SiL	gr	–	–
	Bw	0–42[c]	2.5Y 3/2m	SiL	sbk	–	–
	Bwj	0–62[c]	2.5Y 5/3m	SiL	sbk	–	–
	Bgfm	12–15[c]	2.5Y 3/2f	SiL	sbk	–	–
	Ajfm	0–10[c]	10YR 2/1f	SiL	platy	–	–
	Oajfm	0–9[c]	10YR 2/2f	Organic	massive	–	–
	BCgfm	0–10[c]	2.5Y 3/2f	SiL	massive	–	–
7	Oh	0–30	2.5YR2.5/2m	Peat	–	–	–
	Ohz	30–40	5YR 2.5/2m	Peat	–	–	–
	Omz1	40–150	7.5YR 3/2f	Peat	–	medium	–
	Omz2	150–215	7.5YR 3/2f	Peat	–	medium	–
	Wz	215–268	–	–	–	ice	pure ice
	Cz	268–288	–	Si	–	high	–

(continued)

Table 1.3 (continued)

Pedon no.	Horizon	Depth (cm)	Colour	Texture[a]	Structure[b]	Ice content	Special features
8	L,H	0–6[c]	10YR 3/2m	–	litter	–	–
	Bmgy1	0–12	10YR 5/3m	SiL	gr	–	oi[d]
	BCgy1	12–24	10YR 4/4m	SiL	sbk	–	oi[d]
	BCgyz1	24–47	10YR 4/4m	SiL	sbk	–	oi[d]
	Cz1	47–60	5Y 3/1m	SiL	massive	–	–
	Cz2	60–100	5Y 3/1m	SiL	massive	–	–
	Bmgy2	0–24	10YR 4/2m	SiL	gr	–	oi[d]
	BCgy2	24–34	5Y 3/1m	SiL	sbk	–	oi[d]
	BCgyz2	34–56	5Y 3/1m	SiL	sbk	–	oi[d]

[a]Texture: *SiL* silt loam; *SL* sandy loam; *fSL* fine sandy loam; *Si* silt; *C* clay
[b]Structure: *gr* granular; *fgr* fine granular; *sg* single grain; *sl* structureless; *sbk* subangular blocky
[c]Range of thicknesses-given for discontinuous, cryoturbated horizons
[d] *oi* organic intrusions

Thin eluvial or leached layers resulting from the brunification process occur primarily in sandy soils in the southern part of the Arctic. Salt crusts on the soil surface are also characteristic. These salt crusts develop during dry periods in the summer because of higher evapotranspiration from the soil surface.

Thixotropy, which results in an unstable soil surface, is frequently present in the thawed portion of permafrost-affected soils, and is often associated with soils having high silt content. When a thixotropic soil dries out, a characteristic vesicular structure develops.

1.5.2 Micromorphology

The fabric of Arctic soils varies from granular-like (granic and granoidic) in the surface horizons to mainly porphyroskelic in subsurface horizons (Fox 1985). The terminology for microfabrics associated with permafrost-affected soils, which was developed and described by Fox and Protz (1981), is summarized as follows. Orbicular fabric, which is common in cryoturbated soils, has skeletal grains organized into circular patterns, probably as a result of sorting. Suscitic fabric has skeleton grains oriented in a vertical fashion, often with an underlying accumulation of finer matrix material (Fig. 1.3). Conglomeric fabric has individual structural units enclosed by finer matrix. Ice lensing and vein ice lead to the development of lenticular or platy structure (Fig. 1.4). Cryodesiccation and cryoturbation can lead to granic (granular) or blocky fabrics.

1.5.3 Thermal Characteristics

Probably the most striking thermal characteristics of Arctic soils are the low soil temperatures, the steep vertical temperature gradient, and the perennially frozen nature of a portion of the subsoil. Although soil temperatures are directly related to

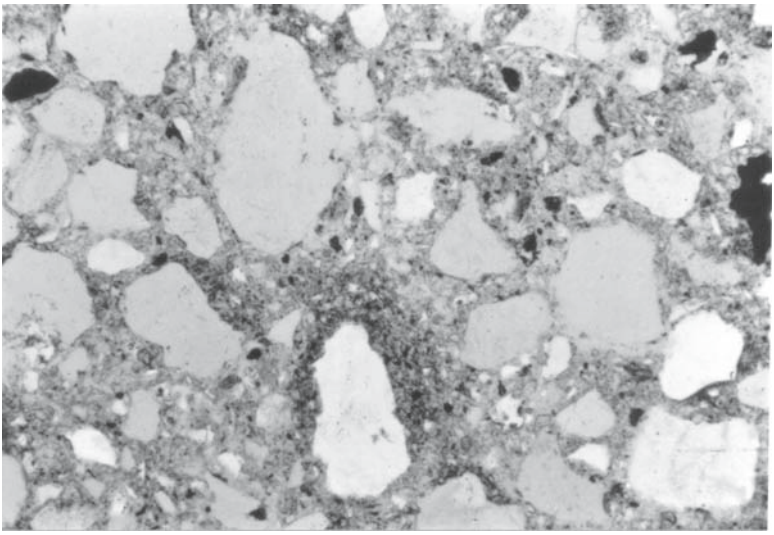

Fig. 1.3 Cryoturbated microfabric showing oriented sand grains

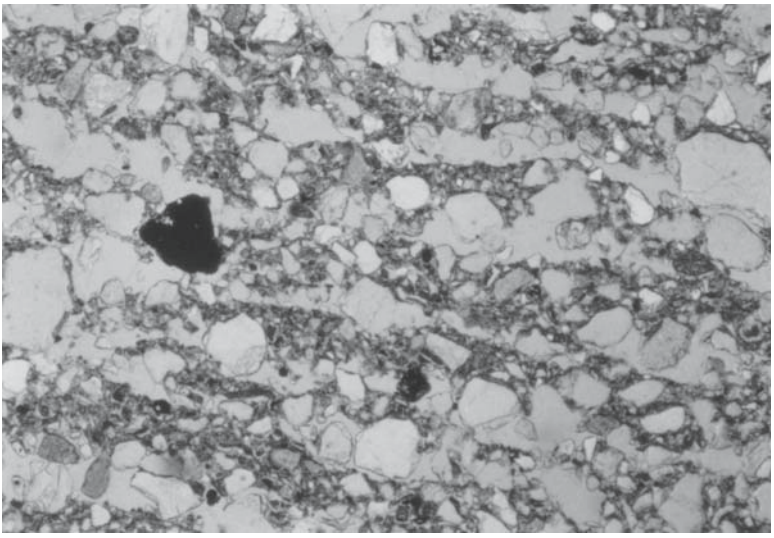

Fig. 1.4 Cryoturbated microfabric showing lenticular or platy structure

air temperature (Fig. 1.5), factors such as vegetation cover, soil moisture, thickness of snow cover, and underlying permafrost have a modifying effect. Since the active layer has very little buffering capacity, however, soil temperatures rapidly reflect fluctuating air temperatures, especially when they are cooling (Tarnocai 1980).

Relationships between air temperature and soil temperatures at depths of 50 and 100 cm at two latitudes are shown in the graphs in Fig. 1.5. The Overlord site (Fig. 1.5a)

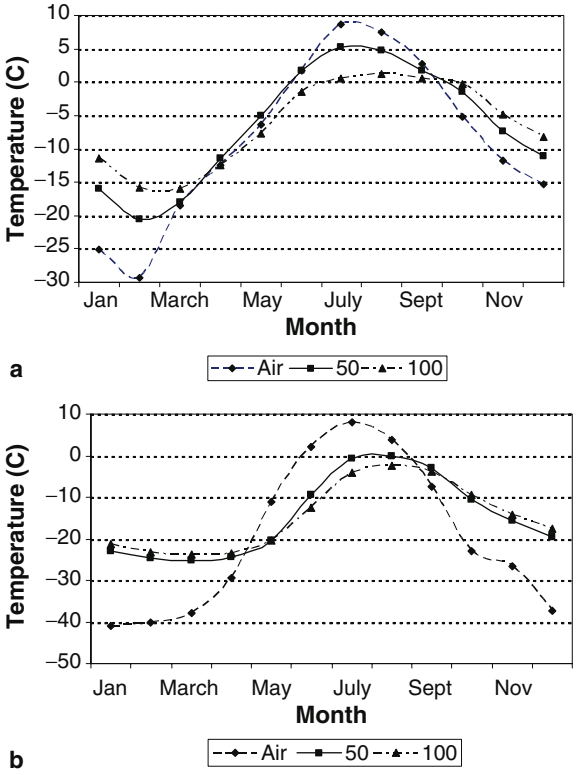

Fig. 1.5 Mean monthly soil (50 and 100 cm depths) and air temperatures measured in 1999 on southern Baffin Island **(a)** and northern Ellesmere Island **(b)** in the Canadian Arctic

on Baffin Island in the southern part of the Arctic (Lat. 66° 23′ 30″ N; Long. 65° 29′ 20″ W) has temperatures above zero at the 0–100 cm depth during the summer months. At the Lake Hazen site (Fig. 1.5b) on Ellesmere Island in the High Arctic (Lat. 81° 49′ 15″ N; Long. 71° 33′ 17″ W), however, only the surface 0–45 cm of the soil thaws during the summer months; below this depth, the soil remains frozen throughout the year.

As a result of the very thin and compacted snow cover in much of the Arctic region, the subsoil cools rapidly as the air temperatures drops, leading to a very small, or negligible, thermal gradient in the soil, especially in the High Arctic (Tarnocai 1980).

1.5.4 Physical Properties

Arctic soils have a wide range of textures, including clay, silty clay, loam, sandy loam and coarse gravelly sand (Table 1.3), with the texture depending mainly on the mode of deposition of the parent material.

Fig. 1.6 Vein ice formation in the subsoil, resulting in a lenticular or platy structure

The structure of the soil, as has already been mentioned in the macromorphology section (see Sect. 1.5.1), is the result of cryogenic processes. The granular structure (Table 1.3) is the result of freeze–thaw processes, which induce desiccation and rolling by frost action. The common platy structure (Table 1.3) is the result of vein ice formation, as is shown in Fig. 1.6. The massive structure is the result of cryo-desiccation during freeze-back.

One of the unique features of Arctic soils is that not all of the water in the permafrost layer is in the form of ice throughout the year. The ice in the subsoil is very dynamic, and increases in thickness and volume over time because of the migration of this liquid water along the thermal gradient from warm to cold.

1.5.5 Chemical Properties

The pH of Arctic soils varies greatly (Table 1.4), and depends on the chemistry of the parent materials. The similarity of the pH to that of the parent material results, in part, because of cryoturbation, which not only mixes and translocates fresh parent material to the near surface, but also mixes soil material among the soil horizons.

The nitrogen, potassium and phosphorus contents of Arctic soils are generally low (Table 1.4), since most of these nutrients are locked into the surface organic matter (Broll et al. 1999). The movement of moisture along the thermal gradient from warm to cold results in the transfer of nutrients carried by solutes, enriching the perennially frozen layer of the soils. The movement of nutrients by this process occurs in both organic and mineral soils (Tarnocai 1972; Kokelj and Burn 2005).

Table 1.4 Selected chemical and physical characteristics of selected pedons

Pedon no.	Horizons	pH	$CaCO_3$ equiv. (%)	C (%)	N (%)	CEC (meq)	Total sand (%)	Silt (%)	Clay (%)
1	Ck	7.3	10.2	2.3	0.10	–	61.0	36.8	2.2
	Cky	7.4	13.3	3.1	0.24	–	18.7	58.7	2.6
	Ckyz	7.1	7.4	2.8	0.20	–	40.2	54.7	5.1
2	Ajj1	5.0	–	3.2	0.2	21.4	18.0	36.8	45.2
	Ajj2	4.9	–	2.7	0.3	21.3	16.0	36.8	47.2
	Bw	5.0	–	2.7	0.2	20.0	14.0	38.8	47.2
	Wf/Bgf	4.9	–	2.7	0.2	26.4	16.0	38.8	45.2
	Wf/Cf	4.9	–	2.8	0.2	23.3	20.0	36.8	43.2
3	Ah	6.2	–	10.3	0.9	37.0	–	–	–
	Bmy1	7.2	1.85	1.4	0.1	11.3	62.8	23.3	13.8
	Bmy2	7.3	1.76	1.1	0.1	10.3	63.2	23.3	14.5
	Cy	7.0	1.10	2.2	0.2	16.0	64.4	23.9	11.8
	Ahyz	6.6	–	13.4	0.8	51.8	–	–	–
	Cyz	6.9	–	2.4	0.2	19.8	59.0	29.3	11.7
4	Bmky	7.4	7.5	1.7	0.1	–	72.3	16.0	11.7
	BCky	7.2	4.6	0.2	0.1	–	75.3	14.0	10.7
	Ahky	7.4	<1	5.5	0.3	–	76.9	14.2	8.9
	Ckz	7.5	13.8	0.4	<1	–	82.5	11.7	5.8
5	A1	7.9	27	2.2	0.2	11.9	54.9	38.5	6.6
	A2	7.9	25	2.4	0.2	9.7	49.2	42.2	8.6
	Ajj	8.0	–	4.2	0.2	16.0	–	–	–
	C1	8.6	22	1.5	0.1	7.9	33.2	43.4	23.4
	C2	8.3	22	0.8	0.1	7.5	36.2	42.9	20.5
	Bwjj1	8.1	23	0.8	0.1	7.2	38.1	43.3	18.6
	Bwjj2	8.0	23	4.4	0.1	7.2	38.9	44.9	16.2
	Bwjj3	8.0	33	1.8	0.1	9.1	31.3	59.4	9.3
	Wfm/Cf	7.9	22	2.8	0.1	8.5	41.0	43.6	15.5
	Cf	7.4	36	2.8	0.1	6.4	54.2	35.6	10.2
6	Oi	4.1	–	17.2	0.6	2.2	20	60	20
	A	4.1	–	2.4	0.2	0.9	20	62	18
	Bw	4.6	–	1.4	0.1	0.7	18	62	20
	Bwj	4.7	–	1.1	0.1	0.7	19	62	20
	Bgfm	4.7	–	1.6	0.1	0.7	15	66	19
	Ajfm	5.2	–	3.0	0.2	0.9	18	63	20
	Oajfm	5.3	–	14.1	0.8	1.5	14	48	38
	BCgfm	6.3	–	3.3	0.2	1.2	19	64	18
7	Oh	3.4	–	36.9	1.4	–	–	–	–
	Ohz	3.5	–	47.3	1.5	–	–	–	–
	Omz1	3.9	–	37.8	1.7	–	–	–	–
	Omz2	4.0	–	45.1	1.8	–	–	–	–
	Wz	7.0	–	–	–	–	–	–	–
8	L,H	4.2	–	43.3	1.1	70.7	–	–	–
	Bmgy1	3.9	–	1.7	0.1	11.8	22.3	54.7	23.0
	BCgyz1	3.9	–	1.5	0.1	11.5	22.8	56.0	21.2
	Cz1	4.0	–	2.3	0.1	11.7	20.4	56.2	23.4
	Cz2	4.3	–			17.0	22.3	52.6	25.1
	Bmgy2	4.1	–	3.8	0.1	15.3	18.3	52.3	29.4
	BCgyz2	4.0	–	4.3	0.2	14.0	19.9	53.2	26.9

The electrical conductivity of arctic soils is generally low, except for those soils developed on marine clays or marine shale. For example, soils developed on marine clay in the Tanquary Fiord area of Ellesmere Island have an electrical conductivity of 1.64–2.73 mmhos cm^{-1}, while soils developed on marine shale on Ellef Ringnes Island have a conductivity of 0.350–0.500 mmhos cm^{-1}. Salt crusts usually develop on the surfaces of both of these types of soils during dry periods in the summer.

One of the most striking features of Arctic soils is the large amount of organic carbon in both the active layer and the perennially frozen portion of the soils (Table 1.4). Although permafrost-affected ecosystems produce much less biomass than do temperate ecosystems, permafrost-affected soils that are subject to cryoturbation have the unique ability to sequester a portion of this organic matter and store it for thousands of years.

Organic, or peatland, soils, which occur mainly in southern areas of the Arctic, contain large amounts of organic carbon that have accumulated as a result of the gradual build-up process. Although this process may be interrupted periodically by wildfires or other environmental changes, the build-up process has continued for thousands of years. The organic carbon content of these organic soils ranges from 43 to 144 kg m^{-2} (Tarnocai et al. 2007). The organic carbon content of cryoturbated, permafrost-affected, mineral soils, which occur throughout the Arctic, is also large, ranging from 49 to 61 kg m^{-2} (Tarnocai et al. 2007).

1.6 Conclusion

The development of Arctic soils is dominated by cryogenic processes, which are driven by the formation of ice in the soils. A number of models have been developed to explain the mechanisms involved in cryoturbation, which is one of the most common cryogenic processes in these soils. The most recent model involves the process of differential frost heave (heave–subsidence), which produces downward and lateral movement of materials (Walker et al. 2002; Peterson and Krantz 2003). Other processes, such as brunification and, especially, podzolization, are not common, probably because of the lack of leaching resulting from the shallowness of the active layer. Gleyic processes are common, and can occur in soils developed on various parent materials.

Soil properties such as soil texture, pH, salinity and the presence of carbonates depend on the parent materials. The nitrogen content of Arctic soils is generally very low, and has been regarded as a more limiting factor for plant growth than phosphorus and potassium contents (Broll et al. 1999). Other limiting factors for plant growth are low soil temperatures, high stone content and, in some cases, high carbonate content and the occurrence of salts (Bölter et al. 2006).

The high amounts of organic carbon stored in Arctic soils, and the relatively rapid warming of this region as a result of climate change, are probably the main reasons so much attention has been focused on these soils in recent times. These

soils (both mineral and organic) have operated as carbon sinks for thousands of years. In general, small amounts of organic matter are produced annually by the vegetation. This organic matter is then deposited as litter on the soil surface, with some decomposing as a result of biological activity. A large portion of this litter, however, builds up on the soil surface, forming an organic soil horizon. Cryoturbation causes some of this organic material to move down into the deeper soil layers (Bockheim and Tarnocai 1998). In addition, roots contribute organic carbon that is also translocated by cryoturbation. Soluble organic materials move downward because of the effect of gravity and the movement of water along the thermal gradient toward the freezing front (Kokelj and Burn 2005). Once the organic material has moved down to the cold (0 to –15°C), deeper soil layers, where very little or no biological decomposition takes place, it may be preserved for many thousands of years. As a result, the average carbon content of cryoturbated, permafrost-affected mineral soils is approximately 49–61 kg m^{-2}, while that of organic (or peatland) soils is 43–144 kg m^{-2} (Tarnocai et al. 2007).

Little is known about soils in much of the Arctic, because the harsh climatic conditions and the relative inaccessibility of most of this vast region have made such studies very difficult. We know even less about how the climate-warming that is already affecting this region will transform these northern soils and their properties.

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Chapter 2
Antarctic Permafrost Soils

Iain B. Campbell (✉) and Graeme G.C. Claridge

2.1 Introduction

Antarctica, with an area of 14 million km^2, is the worlds largest continent, yet exposed ground on which permafrost soils occur covers a mere 49,000 km^2, or about 0.35% of the entire continent (Fox and Cooper 1994). The continent is roughly circular in outline, and its topography is dominated by two massive ice sheets (Fig. 2.1); the East Antarctic Ice Sheet with an average elevation of around 3,000 m, and the West Antarctic Ice Sheet with an average elevation of around 1,500 m. A major physiographic feature is the Transantarctic Mountains, which extend over 3,500 km and separate the two ice sheets. Bare ground areas are found scattered around the margin of the continent where the ice sheets have thinned or receded, in the Antarctic Peninsula and along the Transantarctic Mountains (Fig. 2.1). The largest ice-free area is in the Transantarctic Mountains (23,000 km^2 estimate), which includes approximately 7,000 km^2 in the Dry Valley region, the largest contiguous area of bare ground.

The climate for formation of soils and permafrost throughout Antarctica is severe. With very low mean annual temperatures, negligible effective precipitation and rare occurrences of mosses and lichens, except for the Antarctic Peninsula where plant life including some grasses are more abundant, the soils have aptly been described as Cold Desert Soils (Tedrow and Ugolini 1966; Campbell and Claridge 1969). The exposed landscapes are dominated by glacial valleys with land surfaces and deposits that show the influence of glacial activity, which has extended from the Late Pleistocene to earlier than Miocene times (Denton et al. 1993; Marchant et al. 1993). Notwithstanding the tiny proportion of the continent that is ice-free and exposed to weathering processes, a large degree of diversity is found in both the soils and permafrost, owing to the wide variations in the environmental and geomorphic forces.

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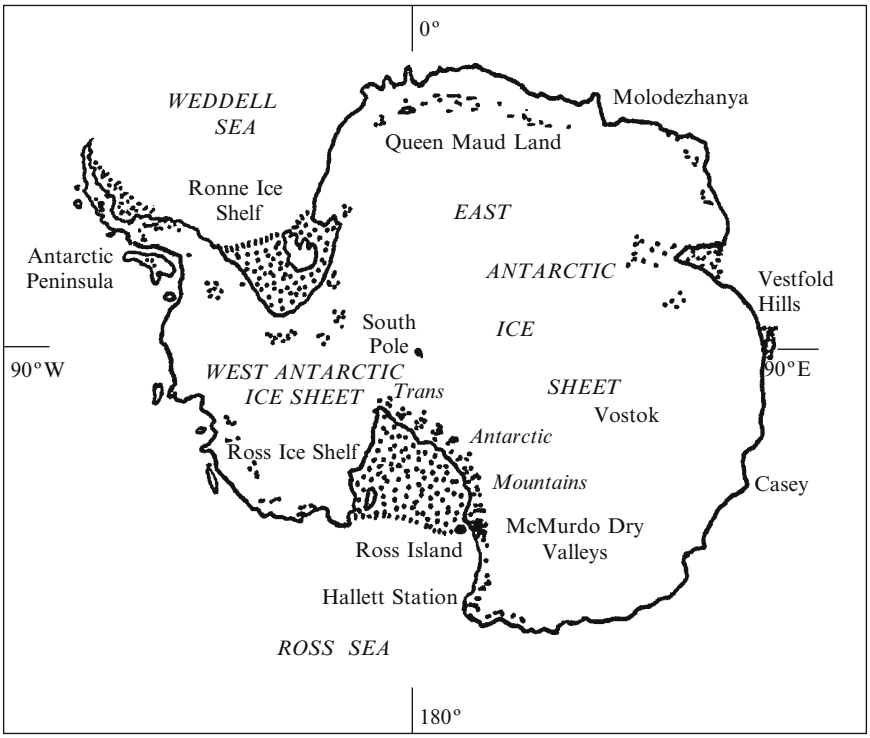

Fig. 2.1 Location map with areas of ice-free ground (not exact or to scale)

2.2 The Climatic Environment

The climate of Antarctica embraces the most extreme cold conditions found on Earth. Antarctica is cold because the solar radiation is only 16% of that at equatorial regions, and also because of the high average surface elevation of the ice sheet, which in places exceeds 4,000 m. Temperatures as low as −89°C have been recorded at Vostok (Fig. 2.1), and −49°C at the South Pole. However, mean annual air temperatures increase nearer the coast where land is exposed, and in the northernmost areas (−25°C at Mt. Fleming at the head of Wright Dry Valley near the edge of the Polar Plateau, −20°C at Vanda Station in the Dry Valleys, −18°C at McMurdo Station on Ross Island, −15°C at Hallett Station). Further north, in coastal areas of East Antarctica, warmer climates are found (MacNamara 1973; Burton and Campbell 1980). At Davis Station in the Vestfold Hills, mean annual temperature is −10.2°C, while at Molodezhnaya and Casey (Fig. 2.1) similar temperatures to those at Davis Station are experienced.

Air temperatures directly influence permafrost properties, with the active layer thickness decreasing from around 80 to 100 cm in the warmer coastal and northern regions to 2 cm or less in the cold inland high-elevation sites (Fig. 2.2) following the

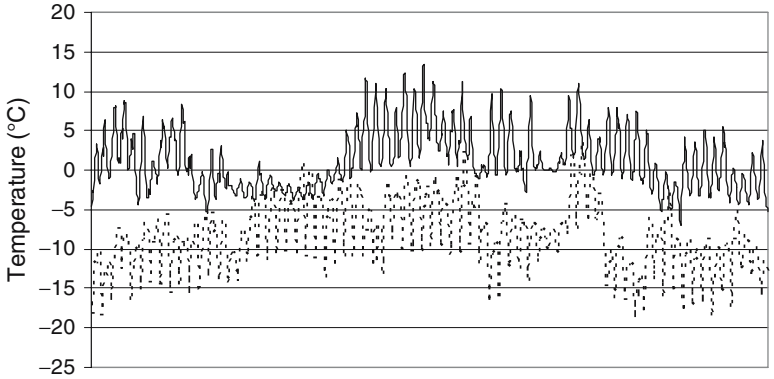

Fig. 2.2 Hourly temperature records from Marble Point (*solid line*; 70 m above sea level (asl), measurement at 7.5 cm) and Mount Fleming (*dashed line*; 2,000 m asl, measurement at 2 cm) from December 4 2002 to February 12 2003. The records illustrate the large difference that site climate has on soil thermal properties

adiabatic lapse rate (Campbell and Claridge 2006). Other soil thermal properties related to geographic differences in climate include the length of the thaw period, the number of thaw days during summer, the number of freeze/thaw cycles that occur and the length of time that the soil may be continuously above freezing. At Marble Point, for example [approximately 70 m above sea level (asl) and permafrost table at 60 cm], the thaw period (measured at 7.5 cm depth) extended over 70 days, there were 34 freeze–thaw cycles and 16 days when the soil temperature was continuously above 0°C (Fig. 2.2). By contrast, at Mt. Fleming (2,000 m asl, permafrost table approximately 2 cm) the thaw period, measured at 2 cm depth, extended over 31 days, but with only 6 days in which soil temperature was briefly above 0°C.

The mean annual precipitation over Antarctica averages around 50 mm per year, with least falling inland and most in coastal locations. In the McMurdo Dry Valleys, one of the driest areas of Antarctica, precipitation averaged 13 mm per year on the valley floor near Lake Vanda and 100 mm per year in nearby upland mountains. Around the periphery of East Antarctica, precipitation is much higher, with 650 mm per year at Molodezhnaya in Enderby Land (MacNamara 1973). The precipitation normally falls as snow, and little is available for direct soil moistening because of ablation and evaporation. Despite the minimal amounts of soil moistening, distinct soil climate zones, based on moisture availability, have been recognized (ultraxerous, xerous, xerous to subxerous, oceanic subxerous and moist zones; Campbell and Claridge 1969). Soils of the ultraxerous zone are found in arid inland areas, rarely if ever have liquid water present, and have ground temperatures that are seldom above freezing point. At the other extreme, moist soils in coastal environments may be moistened at the soil surface, and ground temperatures remain above freezing point for periods throughout the year.

In Antarctica, the soil climate and permafrost properties are strongly influenced by the surface radiation balance, since the soil thermal regime is consequent upon

the gains and losses of radiation from the soil surface. Surface radiation balance investigations for soils at several sites were reported by Balks et al. (1995), MacCulloch (1996) and Campbell et al. (1997), who found that soils with dark-coloured surfaces had low albedo values (approximately 5% at Scott Base) while soils with light-coloured surfaces had much higher albedo values (26% at Northwind Valley). Differences such as these, when coupled with available soil moisture, translate into appreciable differences in the diurnal soil thermal regime and permafrost characteristics. At Bull Pass in Wright Valley, for example, a soil surface with approximately 50% dark-coloured clasts had summer soil temperatures (measured at 2 cm) up to 5°C higher (max 17°C) than in adjacent soil with a light-coloured surface, while the mean annual soil temperature at that depth was 0.25°C greater than for the light coloured soil.

2.3 The Geologic Environment

The Antarctic plate, like other parts of Gondwanaland, is formed mainly from Precambrian to Lower Paleozoic basement rocks, intruded by granites and peneplained by weathering and glacial erosion with overlying sediments of sandstones, siltstones, coal measures and tillites. Jurassic basic igneous rocks were intruded to form widespread sills. Other more recent volcanics occur along major orogenic zones. The present glacial environment is believed to have established after the separation of Antarctica from South America which allowed the formation of a circumpolar circulation pattern.

Antarctic soils and permafrost occur in a geological setting where the time scale for landform development and weathering processes extends back to the Miocene or earlier, and in which the glacial events responsible for till deposition are related to several distinct sources. They include glaciations related to the East Antarctic Ice Sheet, the West Antarctic Ice Sheet and to Alpine glaciers (Figs. 2.3a and 2.3b).

2.3.1 The Glaciological Setting

The East Antarctic Ice Sheet is believed to have been stable since Miocene times (Denton et al. 1993; Marchant et al. 1993; Sugden et al. 1993). Evidence from dated $^{40}Ar/^{39}Ar$ in situ volcanic ashes occurring in association with soils from unconsolidated tills in the Dry Valleys, from basaltic flows interbedded with widespread tills and from reworked clasts in moraine sequences, indicate that there has been no significant expansion of this ice sheet or landscape evolution at least since mid-Miocene times. The West Antarctic Ice Sheet has a different history. It rests on bedrock mostly below sea level, and is dramatically affected by sea-level changes. There is clear evidence that during low sea levels, the associated ice shelves grounded and expanded, causing ice to flow backwards into valleys along the

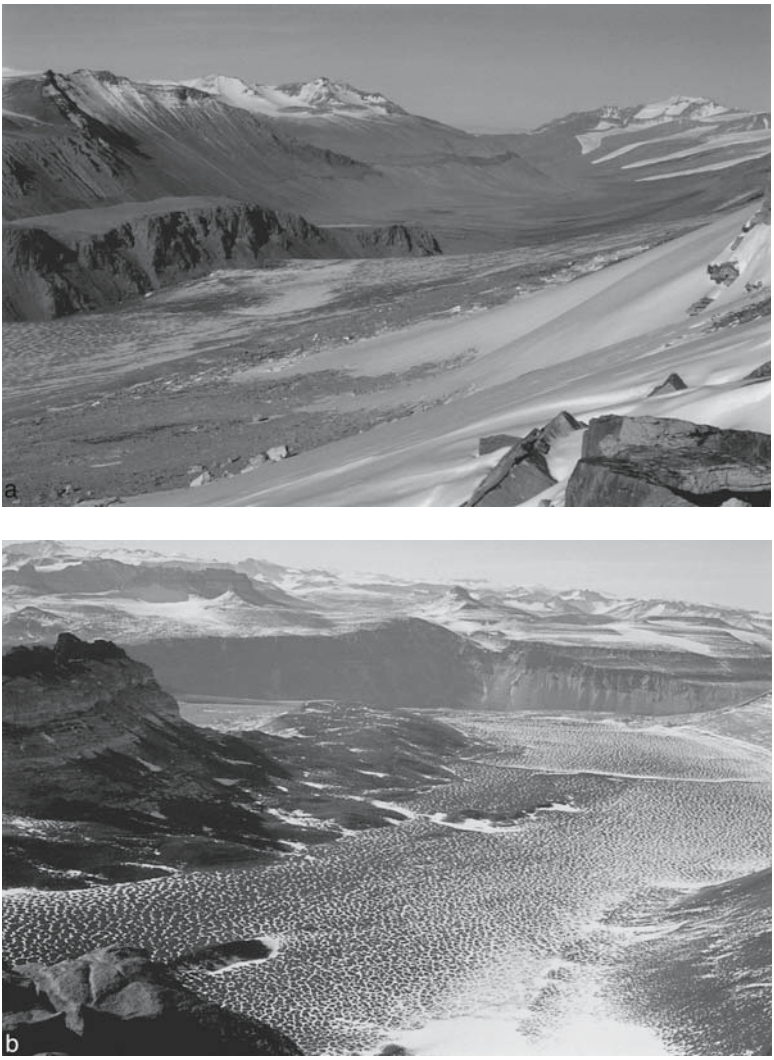

Fig. 2.3 **a** View looking east towards the coast along Wright Valley. Expansions of the Ross Ice Shelf deposited moraines in the valley mouth with earlier incursions extending far up the valley. The four alpine glaciers on the far right have moraine sequences dating to >2.1 million years. Foreground surfaces have old Miocene aged tills and soils. Wright Valley, formerly a fjord, was probably carved by a through-flowing glacier in the Oligocene. **b** View looking northwards along the Transantarctic Mountains and across Wright Valley. Tills with patterned ground are alpine moraines with weathering stage 2 soils. An older landscape and soils occur on the rounded patterned ground-free terrain in the middle

Transantarctic Mountains (Denton and Hughes 1981). The last expansion in the Late Last Glacial period (Ross Glaciation; Denton et al. 1971) resulted in widespread deposition of tills to more than 1,000 m elevation in valleys and coastal surfaces. Alpine glaciers are small and independent of the ice sheets, and comprise ice from snow accumulations in local névés, etc. These glaciers respond to changes in local conditions and, like the East Antarctic Ice Sheet, have moraine sequences which indicate that changes in their masses since the late Pliocene have been relatively small (Everett 1971).

Tills that are associated with the three ice sources, and in which the soils and permafrost occur, have broadly similar characteristics, usually diamictons which are predominantly bouldery sands or silty sands. Tills on older inland surfaces are mainly unconsolidated, often deeply weathered and sometimes include several layers separated by paleosols, which are indicative of multiple ice advances. Younger tills, especially those of the Ross Glaciation, are typically unweathered and firmly ice-cemented, while some tills are underlain by massive ice that is believed to be several million years old (Campbell and Claridge 1987; Sugden et al. 1999). Tills cover most of the exposed landscapes throughout Antarctica, but steep slopes, upland plateau and benched surfaces commonly have bedrock outcrops and felsenmeer that are estimated to make up 10–15% of all bare ground surfaces. Aeolian deposits and fluvial deposits are rarely found.

2.4 The Biological Environment

The Antarctic soil biological environment is known from many studies including those of Gressitt (1967), Cameron (1971), Holdgate (1977), Friedmann (1982), Broady (1996), Powers et al. (1995), Vishniac (1996) and Green et al. (1999); see also Chaps. 9–12 in this book. The terrestrial biota have a sporadic occurrence, being found only in very small areas where there is sufficient light, water, warmth and shelter from wind. Biodiversity is extremely low, and diminishes with increasing severity of climatic conditions. Primary producers are bryophytes, lichens, cyanobacteria and algae, and terrestrial fauna include collembola, mites and groups of microscopic organisms. In the warmer Antarctic Peninsula and other maritime areas, lichen, moss and vascular plants form communities that may give rise to peat formation, with soils that are modified by incorporation of organic matter (Blume et al. 1997). Elsewhere, and also apart from penguin nesting areas, there is no organic influence on the soils.

2.5 Physical Properties of Antarctic Soils and Permafrost

The physical properties of Antarctic soils and permafrost are known from numerous studies since the 1960s, but principally from those of Ugolini (1964), Claridge (1965), Campbell and Claridge (1975, 1987, 2006), Claridge and Campbell (1977),

Bockheim (1979), Blume et al. (1997) and Campbell et al. (1998). The two main pedological processes that operate in Antarctic soils are oxidation and salinization. Coarse particle reduction takes place mainly at the soil surface, with surface clast size becoming smaller through granular disintegration and abrasion. Within the soil, coarse particles are nearly always angular and unstained, indicating low cryoturbic activity. The organic regime is everywhere insignificant, owing to the paucity of biological communities.

2.5.1 Principal Soil Weathering Processes

Oxidation, or reddening of the soil, derives from the very slow oxidation of iron-bearing minerals in rock particles, and usually results in a thin coating of iron oxides on mineral grains. The youngest soils have colours resembling those of rock, but as soil age increases the intensity of oxidation and reddening and the depth of oxidation both increase, with alteration extending to beyond 1 m in depth. Salinization, or the accumulation of salts, is widespread, and is a consequence of high evaporation rates, which typically exceed precipitation. The salts in the soils may form distinct horizons, and are predominantly derived from atmospheric transport. Clear geographic and climate-related differences in soil salt content, as well as age-related differences in salt abundance, are found (Claridge and Campbell 1977; Campbell and Claridge 1987). Salt accumulation is essentially linear with time (Bockheim 1979), and chemical weathering is insignificant by comparison.

2.5.2 Soil Morphological Properties

Antarctic soils are coarse-textured, with coarse particles >2 mm typically exceeding 50% (Table 2.1). Horizon development is weak, and mostly restricted to colour changes that diminish in intensity with increasing depth, to lithologically related textural changes, or to the presence of salt accumulations (Fig. 2.4). The soil surface is usually a stone pavement including loose material derived from fragmentation of surface clasts. On younger surfaces, clasts are mainly angular, coarse and unweathered, while on older surfaces, clast rounding, rock pitting, ventifaction, oxidation and disaggregation may be prominent. Weakly developed vesicular structure may be present in the surface horizon as a result of freezing when the soil is moist. Where there is an increased proportion of fine material, a thin surface crust may be present. Below the surface, the soil is usually structureless and pulverulent, except where salt concentrations occur, when the soil material may be firmly cohesive. In older soils, the disaggregation of coarse-grained clasts by salt weathering results in rock ghosts that indicate a highly stable soil environment.

Table 2.1 Coarse fraction (weight %) for a typical soil from Marble Point, McMurdo Dry Valleys area

	Weight (%) of coarse fractions				
Soil depth (cm)	2–5 mm	5–20 mm	20–75 mm	0.1–75 mm	>2 mm (whole soil)
0–3	7	55	4	90	66
3–15	6	11	41	88	58
15–32	7	11	39	87	57
32–45	8	12	53	92	73
45–69	11	22	41	91	74
69–100	10	20	79	79	49

Fig. 2.4 Profile of weathering stage 3 soils from dolerite and sandstone till from the Asgard Range in Wright Valley. The surface pavement is well-developed, with moderate reduction, rounding and staining of surface boulders. A weakly developed salt horizon is present, with a concentration of salts to the right of the tape beneath a boulder that was removed. Ice-cemented permafrost is at 35 cm

2.5.3 Soil Distribution Patterns

With increasing time, soil oxidation intensity and oxidation depth, as well as the soil salt content, increase. Campbell and Claridge (1975) found that soil weathering indicated by these parameters could be expressed in terms of six soil weathering stages

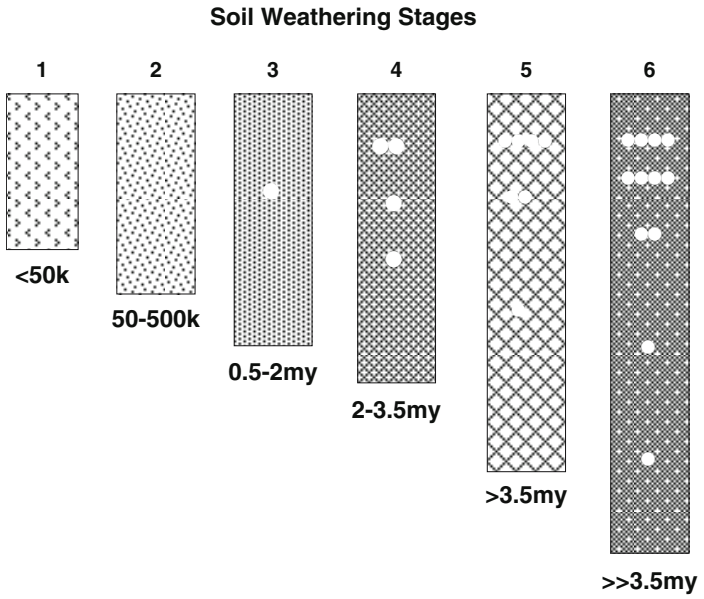

Fig. 2.5 Weathering stages identified in Antarctic soils by Campbell and Claridge (1975) are marked by increasing intensity and depth of oxidation and increasing soluble salt content. Weathering stage 5 soils may be Miocene or older judged by subsequent dating of volcanic ashes. *k* = 1,000 years; *my* = million years

covering the time between late Last Glaciation and the Miocene (Fig. 2.5). These weathering differences are intimately associated with landform differences, most commonly moraine sequences of differing ages. Coupled with the soil age differences are soil differences resulting from climate. Soils in the oceanic subxerous and moist zones, for example, have comparatively high water contents, grading from around 0.5% in surface horizons to 12% near the permafrost boundary, while soils in the arid ultraxerous zones may have a moisture content of <0.5% through the whole profile. The soil salt content likewise shows a marked geographic distribution pattern, the coastal soils having salts dominated by sodium chloride, and the arid inland soils by nitrate salts.

2.5.4 Antarctic Permafrost Properties

Antarctic soils are everywhere underlain by permafrost, which can be divided into a number of distinct types (Campbell and Claridge 2006). Ice-cemented or ice-bonded permafrost (Fig. 2.6) is easily recognized, and has an active layer that immediately overlies hard ice-bonded permafrost. The active layer depth varies according to mean annual temperature, moisture supply and the thermal radiation balance, but is usually deepest (up to 1 m) in warmer northern locations, and shallow (< 2 cm) in the coldest areas. A similar form is permafrost with massive ice

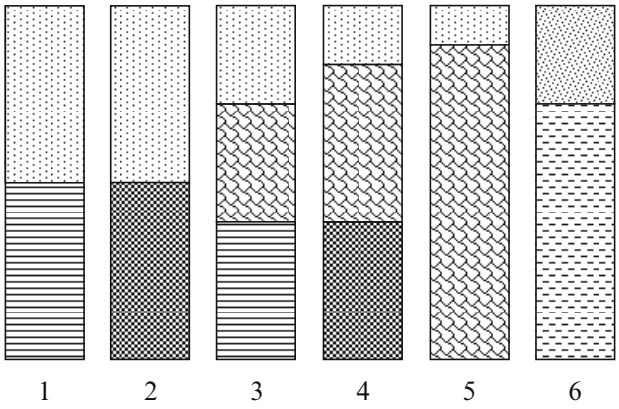

Fig. 2.6 Permafrost types in the Transantarctic Mountains region. The active layer thickness diminishes with increasing coldness and with increasing age and aridity, the permafrost changes from ice bonded to dry permafrost. *1*: active layer over ice-bonded permafrost, *2*: active layer over buried or massive ice, *3*: active layer over dry permafrost over ice-bonded permafrost, *4*: active layer over dry permafrost over buried or massive ice, *5*: active layer over dry permafrost, *6*: saline permafrost

immediately below or at some depth below the active layer. This ice is typically stagnant or old residual glacial ice (Claridge and Campbell 1968; Sugden et al. 1993), commonly associated with patterned ground surfaces (Fig. 2.4) and younger land surfaces with thermokarst terrain (Campbell and Claridge 2003).

Ice-free or dry permafrost (Bockheim 1995) is distinguished by very low water content in both the active layer and the permafrost, which is loose and non-cohesive. Ice crystals, where present, may behave like sand grains. Our measurements indicate that a gravimetric water content of around 6–7% is required for ice bonding to occur in these sandy gravel materials. In ice-bonded permafrost, weathering is restricted to the active layer but in dry permafrost, weathering occurs into the permafrost, sometimes to a depth of several meters. Intermediate forms between ice-bonded and dry permafrost are also found with dry and weathered perennially frozen permafrost overlying at variable depth ice-bonded permafrost or ancient massive ice (Claridge and Campbell 1968; Sugden et al. 1999).

Saline permafrost is found in small depressions and salty hollows, and associated soils are highly saline. In summer months, the active layer frequently contains brine, usually at a temperature several degrees below 0°C, while the soil is characterized by abundant efflorescences of soluble salts.

2.5.5 Permafrost Distribution Patterns

The distribution of the differing permafrost types, based on more than 900 observations from northern Victoria Land and through the Transantarctic Mountains, was summarized by Campbell and Claridge (2006). The permafrost table is at greatest

depth in the warmer northern regions of Antarctica, and diminishes in depth with increasing latitude and altitude, with some soils possibly being perennially frozen. There is much site variation, however, due to local differences in the heating from radiation owing to topographic shading, aspect, snow cover, surface colour and surface roughness.

Ice-bonded permafrost is most commonly found in coastal regions, on younger-aged surfaces nearest to a glacier and in areas where the precipitation or drainage regime results in moist soils. At higher elevations and greater distances inland, on the older land surfaces and areas of greatest aridity such as parts of the Dry Valleys, dry permafrost, including the intermediate form, predominates. When the transition from one form to another occurs over a short distance, it is commonly related to surface age or moisture availability differences. The ice content of ice-bonded permafrost is usually greatest in coastal regions and least in colder regions. Permafrost is also present in exposed bedrock surfaces, where it may be either ice-bonded or dry.

2.6 Chemical Properties of Antarctic Permafrost Soils

Chemical weathering is of very low intensity in these soils, because of the low temperatures and extreme aridity, but soils vary in their chemistry because of environmental variations. The soils contain very small amounts of fine particle size material and even less of clay-sized material, which is the most chemically reactive fraction of the soil. Most of the fine particle size material is produced by physical disintegration of the Beacon Supergroup sandstones, so that the fine fraction of the soil is dominated by rounded quartz grains of fine sand grade, together with smaller amounts of material produced by glacial grinding.

Clay-sized material largely originates from the matrix bonding the sandstones together, and consists of micas and vermiculites of little chemical reactivity. In some instances, these have been altered by soil weathering processes to more hydrous clay minerals, illites, hydrated vermiculites and (in rare instances) smectites. In some old soils, especially those of higher weathering stages, authigenic clay minerals may be formed. The nature of these minerals is dependent on factors such as soil pH and the chemistry of the salts,

Because the climate is extremely arid, salts, mainly derived from precipitation, accumulate in the soils and strongly influence the soil chemistry. Near the coast, where winds from the sea may carry ocean-derived salts some distance inland, the soil salts are largely chlorides and sulphates of sodium, and the soils are alkaline — up to pH 9 in some cases. This may cause the transformation by hydration of some of the micas into illites and more hydrous clays, even forming some smectites (Claridge 1965). Because the buffering capacity of the soil is very low, only small amounts of salts are needed to raise the pH of the soil to high levels. Soils close to the coast are also generally very young, and contain comparatively low amounts of salts.

Further inland, soils are older, and salts have accumulated to a much greater extent than in coastal regions, often forming thick salt horizons. The salts in these soils are considered to have been derived from the oxidation of protein material caught up

from the ocean surface and transported through the upper atmosphere, where they become completely oxidised to nitric acid and sulphuric acid (Claridge and Campbell 1977). Other mechanisms are also proposed, such as auroral fixation of nitrogen. Soils of inland regions therefore have low pH values; as low as 6.0 in ultraxerous soils of weathering stage 5 on the inland edge of the Transantarctic Mountains. The pH of the soil can be directly related to distance from the open sea.

Some breakdown of primary minerals takes place in the acid environment of these soils. The ferromagnesians in particular release iron, which causes the reddish staining on grain surfaces as the iron is oxidised in older soils. Cations such as calcium and magnesium are released, so that the soluble salts, which are such a dominant feature of the older soils of inland regions, are nitrates and sulphates of calcium and magnesium. Almost all crystalline phases that can be formed by combinations of calcium, magnesium, sodium, nitrate and sulphate can be identified in the soils (Claridge and Campbell 1977).

Because the salts in solution lower the freezing point, liquid water can be present at very low temperatures, generally as thin films on grain surfaces, and chemical processes can take place at temperatures as low as –50°C. In most of the old, weathering stage 5 soils of the inland edge of the Transantarctic Mountains, the clay-sized fraction of soils formed on till is dominated by clays derived from Beacon Supergroup rocks. However, in some soils formed directly on physically fragmented dolerite, these clays are absent, and it is possible to demonstrate the formation of authigenic clays, such as nontronitic montmorillonite, a consequence of clay mineral formation in an environment rich in iron and magnesium (Claridge and Campbell 1984). In these cases, though, most of the clay-sized material is physically disintegrated fragments of the glassy matrix of the parent rock of the soil, Ferrar Dolerite. In some situations, especially the very old soils of the inland regions, zeolites such as chabazite (Dickinson and Grapes 1997) may form.

Thus, the chemistry of the soil depends on geographic location, which determines the nature of the salts, the weathering processes operating and the secondary mineral that may be formed.

2.7 Sensitivity to Change

Because weathering processes in Antarctica are infinitely slow, terrestrial ecosystems in this harsh environment are extremely fragile. A wide-ranging review of the impacts of human activities and the susceptibility of the land systems to disturbance was carried out for the Ross Sea region (Campbell 2001), and showed that disturbances from human activities are long-lasting. Physical disturbances to the soils may persist for many hundreds of years, or in the most arid zones where recovery processes are negligible, be permanent. Chemical contaminations may also persist in the absence of significant leaching. Permafrost is likewise dramatically and rapidly

altered when physical disturbance takes place. Less clear, however, are the future impacts of global climate change. Over recent decades, a distinct warming trend has been noted in the Antarctic Peninsula region, while recent data suggests that there may be a cooling trend in the East Antarctic region.

2.8 Conclusion

The soils of Antarctica are for the most part formed in the absence of biological processes and, as a consequence of the prevailing low temperatures, are everywhere underlain by permafrost, with the active layer varying in thickness from about one metre in northern areas to a few centimeters or less in the soils of the inland edge of the Transantarctic Mountains. The permafrost is generally ice-cemented, but in older and drier soils may be loose. Because of the extreme aridity, the soils accumulate salts derived from precipitation and weathering, the composition and amount of the salts being a function of soil age, composition of the parent material and distance from the coast. Chemical weathering processes are assisted by the salts, which allow unfrozen saline solutions to be present on grain surfaces and cracks in rock particles, even at very low temperatures. Weathering comprises the breakdown of ferreomagnesian minerals, releasing iron and cations to the soil solution. The iron oxidises and is precipitated on grain surfaces, giving rise to the red colouring of older soils. The cations, especially calcium and magnesium, combine with nitric and sulphuric acids arriving in precipitation, to make up part of the thick salt horizons which are found in older soils. The concentrated salt solutions react with silica, also released by weathering, to form secondary clay minerals and in some cases, zeolites.

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Chapter 3
Mountain Permafrost

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3.1 Introduction

This chapter provides an introduction to mountain permafrost and a review of recent scientific progress. In it, we use rather few references to the scientific literature in order to make the text more easily readable. For further reading, we recommend, Haeberli et al. (2006), and Gruber and Haeberli (2007), two recent reviews in which the current state of the art is discussed in depth and in which extensive references can be found.

Permafrost is lithosphere material that permanently remains at or below 0ºC. In this context, "permanence" is often defined to be two or more consecutive years, in order to establish a minimum value for avoiding the effect of only one cold and long winter being considered permafrost. By this definition, permafrost can – but does not need to – contain water or ice. Based on this purely thermal definition, every substrate is permafrost when subject to certain temperature conditions. By definition, glaciers are not permafrost. Most permafrost areas experience seasonal thaw, during which surface temperatures rise above the melting point and a certain volume of material directly beneath the surface is thawed. The material that is subject to seasonal temperature changes crossing 0ºC is termed the "active layer", and has a typical thickness of 0.5–8 m.

Mountain permafrost is simply permafrost in mountain areas. It can be situated at low or at high latitudes and in the Arctic or Antarctic – we define mountain permafrost based on the influence that mountain topography has on its properties. Many other terms that are commonly used to classify certain types of permafrost, such as Arctic, Antarctic, polar, or plateau, can be applicable at the same time. These qualifying terms are useful to describe properties, but not to sharply dissect geographic or scientific space. The dominating characteristic of mountain areas and mountain permafrost is their extreme spatial variability with respect to nearly all surface and near-surface characteristics and properties. Examples of this are:

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(a) Elevation itself, as well as other geometric measures such as slope, aspect, curvature, or roughness
(b) Surface micro-climatology, which is dominated by differences in elevation (strongly affecting long-wave radiation and turbulent fluxes) and in short-wave solar irradiance due to shading and variable angles of insolation
(c) Subsurface material thickness and composition, which is dominated by diverse processes of erosion, grain-size fractionation, and deposition
(d) Water availability, which is affected by contributing area, surface shape, and subsurface material
(e) Snow cover, which is influenced by surface micro-climatology, precipitation patterns, wind drift and avalanches.

All these properties affect ground temperature and, as a consequence, permafrost occurrence and characteristics. Water in mountain permafrost areas drains quickly, and the water content of mountain permafrost soils is usually small when compared to the often-waterlogged substrates found in Arctic lowland areas. Data on mountain permafrost are often sparse and biased to areas with existing infrastructure, because access and measurements on most mountain slopes are difficult and expensive. This is especially true for mountain areas outside Europe, where access infrastructure is sparse.

Permafrost is invisible because it is a thermal phenomenon. It is difficult to assess at the ground surface, because it usually lies beneath an active layer. Furthermore, its reliable detection requires temperature measurements spanning at least 2 years in order to understand the seasonal temperature evolution or, alternatively, measurements at greater depths. The depth of zero annual amplitude (ZAA), where the seasonal temperature fluctuation is damped to less than 0.1°C, is usually about 10–15 m below the surface. Below this depth, single measurements can establish the presence or absence of permafrost. However, great care has to be taken to minimize the thermal disturbance caused by drilling or measuring. The difficulty in detecting permafrost, together with expensive access and extreme lateral variability, makes permafrost research in mountain areas a difficult endeavor. Understanding and predicting spatial patterns of permafrost occurrence and characteristics needs to be based on a combination of measurements and models, because the systematic variability caused by topography dominates spatial patterns already over short distances.

The scientific and practical relevance of mountain permafrost has many facets. Permafrost is an important element of landscape evolution because of the characteristic landforms such as rock glaciers, push-moraines, ice faces and hanging glaciers, which are connected to its existence, and because it affects long-term sediment transfer mechanisms. This alteration of sediment transfer systems (Fig. 3.1) leads to changing regimes of natural hazards, such as rock avalanches and debris flows. Here, permafrost warming and thaw has the potential to alter frequency and magnitude of events, and to affect geographic areas that have previously been considered safe based on historical evidence. The safe construction and maintenance of infrastructure in mountain permafrost requires special techniques for the handling of thermal perturbations and ground movement. Furthermore, in some areas, land cover and land use are connected to the presence of water tables perched on permafrost.

Fig. 3.1 North-exposed steep bedrock containing permafrost beneath the top station of the Corvatsch cable car, Switzerland. The debris on the small glacier is almost exclusively due to strong rock fall activity during 2003–2006. Most likely this is caused by permafrost degradation

3.2 Spatial Distribution

The processes that govern the existence and evolution of mountain permafrost can be categorized into the scales and process domains of climate, topography and ground conditions (Fig. 3.2). The climate scale governs the global distribution of cold climates in mountains. It refers to the influence that latitude and global circulation have on the general climatic characteristics of an area. These climatic conditions are then further modified by topography, which affects ground temperatures because of its strong influence on surface micro-climatology. This influence is due to differences in ambient air temperature caused by elevation, differences in solar radiation caused by terrain shape, or snow transport by wind and avalanches. Locally, the influence of topographically altered climate conditions on ground temperatures are modified further by ground properties and their influence on heat transfer. Here, coarse block layers result in relative ground cooling when compared to bedrock or fine-grained substrate, and a high ice content can significantly retard warming and permafrost degradation at depth.

The distinction between these three scales and process domains is not sharply defined. The effect that topography has on regional precipitation patterns, for instance, spans the scales of climate and topography, and the effect of snow redistribution on ground temperatures spans the scales of topography and ground conditions.

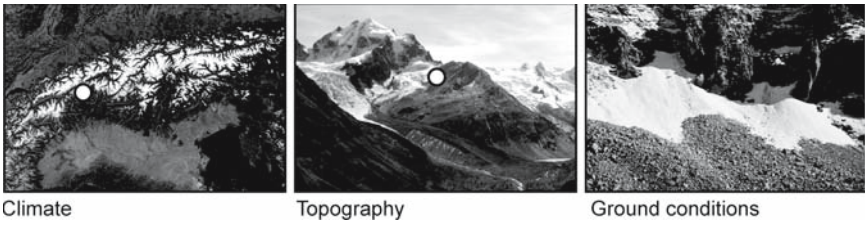

Fig. 3.2 Conceptual hierarchy of scales and process domains that influence ground temperature and permafrost conditions in mountain areas. The *white disk* in the two leftmost images refers to a location that is then depicted in the image to the right — and has its conditions further overprinted by the respective conditions of that scale

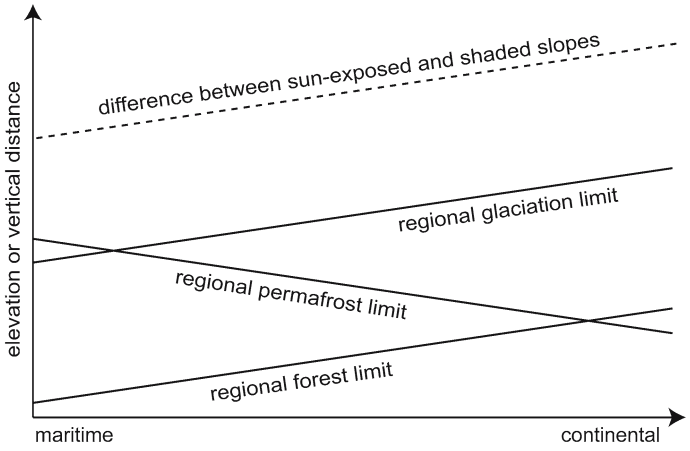

Fig. 3.3 Schematic of mountain permafrost distribution (changed from King et al. 1992). Lines indicate a general trend, but the shape (*straight line*) is a strong generalization (glaciation limits, for instance, rather rise exponentially with increasing continentality)

Nevertheless, this concept of scales is useful for understanding the diverse influences on mountain permafrost characteristics. The overall magnitude of the effect of topography and ground conditions can be as high as 15°C within a horizontal distance of 1 km — a similar difference in ground temperature in polar lowland areas would normally occur over a latitudinal distance of roughly 1,000 km.

In the European Alps, a mean annual air temperature below −3°C can be used for first-order classification of altitudinal belts that have significant amounts of permafrost. However, this rule is subject to many exceptions, and may not hold for other mountain areas. Figure 3.3 illustrates the influence that continentality has on mountain permafrost distribution. We speak of continental climates where total precipitation and cloudiness are low and total solar radiation as well as annual and diurnal temperature amplitudes are high. Maritime areas have high precipitation, often overcast skies, and rather small temperature amplitudes and solar radiation sums. The upper limit of closed forests rises along with summer air temperatures,

which are higher in continental climates. The glaciation limit rises with decreasing precipitation towards continental areas, whereas the permafrost limit rises towards maritime areas because thick snow cover provides insulation during winter and results in warmer ground temperatures. However, this only holds true for gently inclined slopes that accumulate a thick snow cover. The regional boundary for permafrost in steep bedrock is probably much less affected by continentality. The relative difference between sun exposed and shaded slopes is usually greater in steep than in moderately inclined terrain, because of the dampening effect of snow cover, and it is higher in continental areas because of the increased solar radiation. As a consequence of these patterns, permafrost can exist in forested mountain areas in continental climate, whereas in the European Alps even alpine meadows usually are a reliable sign of the absence of permafrost. In maritime climates, the glaciation limit is lower than the regional limit of permafrost. As a consequence, perennially frozen talus and rock glaciers are often absent, because their potential locations are covered by glaciers, and permafrost only exists in steep bedrock.

Permafrost in mountain areas occurs in a wide range of materials and surface cover types, which decisively influence ground temperatures. One of the most prominent surface covers are coarse block layers. They exert a cooling influence on ground temperatures and thus affect permafrost distribution patterns. For this reason, coarse rock has also received considerable attention from the engineering community as a construction material (Goering and Kumar 1996). The cooling influence of blocky layers is mainly based on three processes:

(a) Temperature-driven convection of air
(b) A reduced warming effect of the winter snow
(c) The advection of latent heat by snow that enters deep into the voids of the active layer.

During winter, ground temperature is higher than near-surface air temperature and, in deposits with sufficient permeability, free convection of air can thermally couple the atmosphere and the sub-surface effectively. Because a closed snow cover reduces or inhibits convection, the effectiveness of this cooling mechanism is greatest in areas or during times with little snow. The warming effect of the winter snow cover is based on a contrast in thermal resistance between cold and warm periods. This contrast reduces the influence that cold winter temperatures have on ground temperatures at depth. Because block layers have a very low thermal conductivity, they reduce the contrast between summer and winter by increasing the overall thermal resistance. In this way, block covers can result in significant ground cooling by reducing the warming effect of the winter snow (Gruber and Hoelzle 2008). The magnitude of this relative cooling is greatest in areas with thick snow cover. In very coarse deposits, snow can penetrate deeply into the voids of the active layer. Especially in areas with high wind speed, this process can advect significant latent heat into the ground, which is only slowly removed by heat conduction from the warming surface during summer.

Permafrost and ground temperatures in steep bedrock are discussed in depth by Gruber and Haeberli (2007). Unfortunately, little quantitative understanding exists with respect to the many intermediate conditions in the spectrum between steep

bedrock and moderately inclined coarse blocks that make up a large proportion of mountain permafrost areas. For example, the influence of water flow and summer–winter contrasts of thermal conductivity in fine-grained soil, or the influence of snow on temperatures in moderately steep rock walls, are hardly known at present.

Active talus slopes as well as active volcanic areas (Kellerer-Pirklbauer et al. 2007) often accumulate permafrost deposits consisting of debris or scoria mixed and inter-layered with snow deposits. Very ice-rich talus often begins to creep and ultimately forms rock glaciers. Figure 3.4 shows a buried perennial snow patch in aggrading permafrost, and illustrates the influence of topography and strong winds on the spatial pattern of such mixed deposits.

Unusual forms of permafrost can sometimes be found in areas that have a mean annual air temperature several degrees above freezing. Ice caves, for instance, preserve ice (and thus permafrost conditions) over several years (see Luetscher et al. 2005). The main process responsible for this effect is strong density-driven exchange of air through the cave system during winter, which terminates during summer when the cold air is stratified stably in the cave. Additionally, winter snow sometimes falls through the cave opening (bringing with it significant latent heat) and does not melt during summer because almost no solar radiation arrives inside the cave, and air exchange with the warm surface is minimal. Steeply inclined slopes of coarse blocks often have permafrost conditions at the foot of the slope, which are caused by a seasonal sub-surface ventilation pattern ("chimney effect") which can reduce the mean temperature in the lower parts of steep and blocky slopes locally by several degrees (Delaloye and Lambiel 2005).

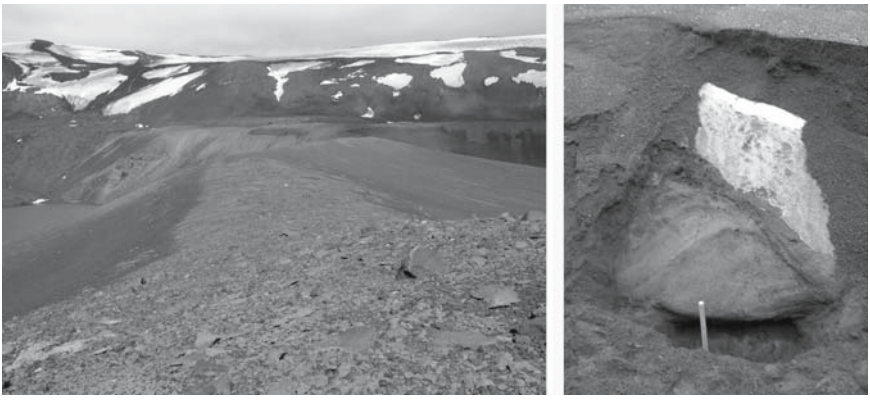

Fig. 3.4 The interplay of strong winds and topography governs the spatial distribution of permafrost characteristics and small glaciers on Deception Island, Maritime Antarctic. The contrast of light-colored substrate on the ridge in the foreground of the *left panel* and the darker lower slopes is due to wind transport of fine scoria from convex to concave areas. Similarly, snow is transported and deposited. On the *right panel*, a cross section through aggrading permafrost is shown. The sequence from top to bottom is: active layer in fine scoria; permafrost in fine scoria (above buried snow patch); buried snow patch consisting of dense ice in the lower and compact snow in the upper part; permafrost in fine sediments; and unfrozen sediments where the permafrost has been undercut by a stream

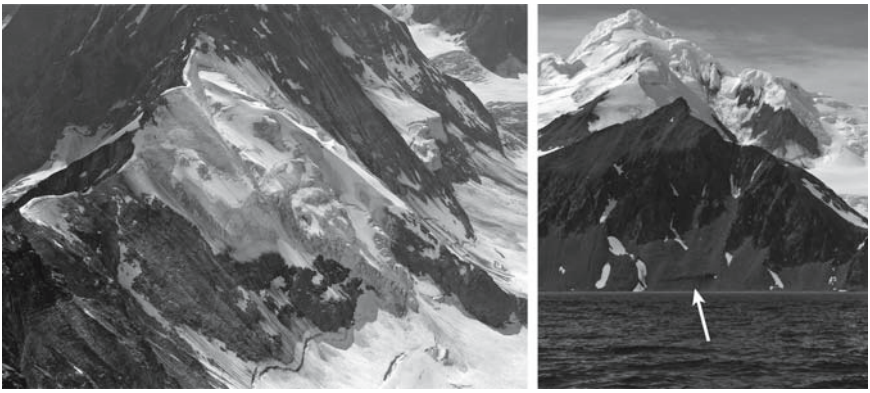

Fig. 3.5 *Left*: hanging glaciers and ice faces on the northern side of the ridge extending between the Matterhorn and the Dent d'Herens along the border between Switzerland and Italy. *Right:* an incipient rock glacier (*arrow*) at sea level as well as ice faces and hanging glaciers only a few hundred meters higher on Livingston Island, Maritime Antarctic

Two types of phenomena often visually indicate the presence of permafrost in mountain areas (Fig. 3.5). Rock glaciers and other creep phenomena form distinct landforms caused by the slow deformation of cohesive, ice-rich sediments (Haeberli et al. 2006). When thawed, relict forms can be used to infer past permafrost conditions. Ice faces and hanging glaciers, on the other hand, only indicate current permafrost conditions, because they leave no long-lived remnants after degradation. Ice faces, hanging glaciers and active rock glaciers are reliable indicators of permafrost. Their absence, however, does not indicate the absence of permafrost.

3.3 Temperature, Ice Content, and Age

A number of borehole temperature measurements exist in mountain permafrost. Some are part of monitoring networks or research projects, others have been drilled and measured during construction or mineral prospecting, and data are seldom available for the scientific community. The most prominent scientific monitoring networks include the PACE transect of boreholes from the European Alps to the Arctic island of Spitzbergen and the PERMOS permafrost monitoring network in Switzerland (Vonder Mühll et al. 2007), which have contributed significantly to the understanding of mountain permafrost temperatures. Both networks contribute data to global monitoring organizations. The thermal response of permafrost to climate change is presented in more detail in Chap. 14.

In mountain areas, temperature does not simply increase with depth (Fig. 3.6). The subsurface temperature field is usually rather complex and governed by lateral heat fluxes, which are caused by topography and variable surface conditions (Fig. 3.7). These complex patterns can result in permafrost being induced, for instance, under a

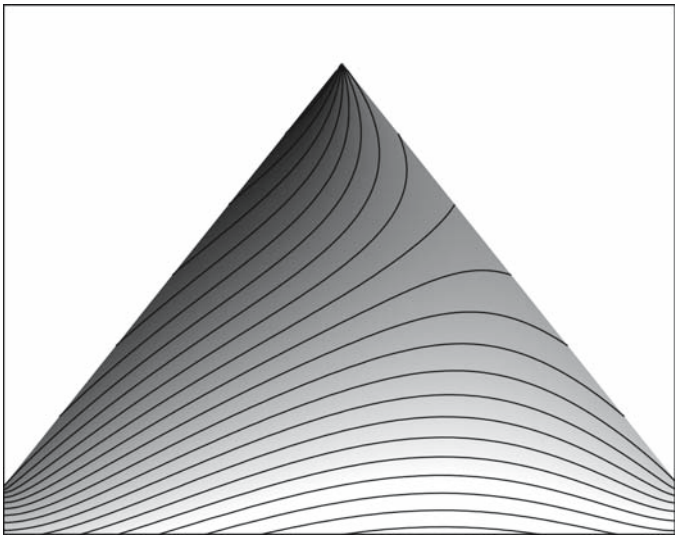

Fig. 3.6 Schematic cross-section through the steady-state thermal field of a ridge or summit. Isotherms are shown by *black lines*; *darker shading* refers to colder temperatures. In the upper part of the section, heat flow and thermal gradient are predominantly lateral

seemingly warm sun-exposed slope from the nearby cold and shaded slope (Noetzli et al. 2007). Furthermore, recent warming has already penetrated tens of meters into the ground and can thus lead to inverted temperature profiles. As a consequence, great care must be taken in the interpretation of temperature profiles, and heat fluxes at a depth of several decameters can be positive or negative, depending on location and time (Gruber et al. 2004). The thermal profiles observed in mountain permafrost are usually either cold (i.e., colder than about 0.5°C with insignificant amounts of liquid water) or temperate. Temperature profiles in temperate permafrost have large sections (sometimes tens of meters thick) of near-isothermal conditions due to phase transition of ice contained in unconsolidated material or highly fractured rock. Areas of temperate mountain permafrost will likely increase under current atmospheric warming trends.

Glaciers and permafrost interact in many ways. Permafrost exists below the interface of cold ice and rock or sediments, and the melt of parts of a temperate glacier tongue can be followed by permafrost formation in the newly exposed material. Cold glacier tongues advancing into perennially frozen sediments can deform them into so-called push-moraines, which are landforms indicative of permafrost. Many intermediate forms of creep phenomena exist between very small debris-covered glaciers, ice-cored moraines and rock glaciers (Fig. 3.8). Ice in rock glaciers (Haeberli et al. 2006) exists in many forms, ranging from massive ice with dispersed debris to relatively homogeneous ice/rock mixtures. The origin of ice in rock glaciers is difficult to trace to either glacial or non-glacial formation, because of many shared characteristics between both ice types. Especially in the rooting zone of rock glaciers, a complex and temporally variable combination of processes such as

Fig. 3.7 Variability dominates: within short distance there is bedrock, talus slopes, several intermediate forms of fractured, thinly debris covered rock, and a rock glacier at the foot of slope. The cast shadow illustrates the variable illumination conditions

metamorphosis of debris-laden avalanche snow, ice segregation, and freezing of shallow ground water occurs. Talus slopes in permafrost areas can be cemented by interstitial ice (Fig. 3.9, left) and, as a consequence, aggrade significant amounts of material protected from erosion – but possibly released in enhanced debris flow activity if thawed during climate change.

Ice in fissures and fractures is common in bedrock permafrost (Fig. 3.9, right) and has been observed both at construction sites and in the fresh detachment scars of rock fall. The percolation of water in previously ice-filled joints can lead to fast and linear thaw of permafrost and, possibly the fast destabilization of large masses of rock. The origin of ice in fractures is unclear. Both the percolation and freezing of meteoric water and ice segregation are possible, and, at present no clear evidence pointing at one or the other process exists.

Permafrost in debris slopes and the landforms associated with it are usually of Holocene age, because their locations are subject to glacier cover and removal of unconsolidated sediments during glacial cycles. By contrast, permafrost in steep

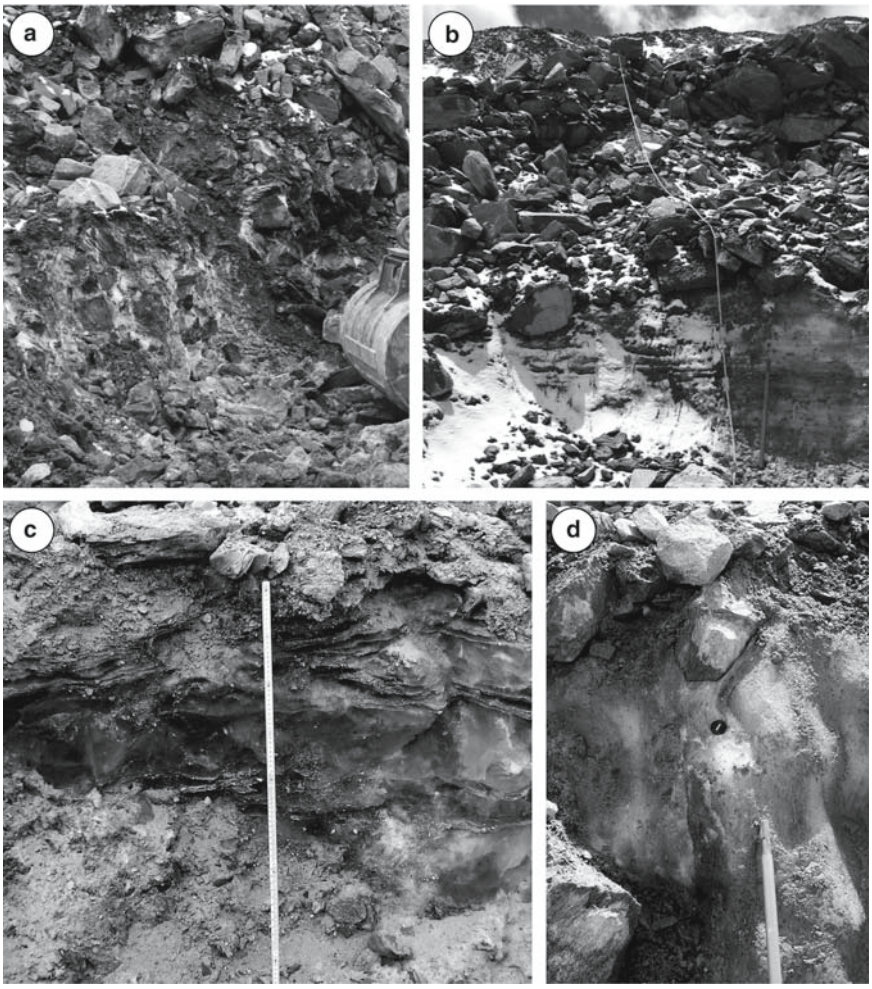

Fig. 3.8 Different forms of ground ice that have been encountered within few hundred meters distance from each other at an elevation of about 3,000 m asl. The exposures were made by excavator during the construction of a ski run north of Gornergrat, Switzerland. **a** Ice-cemented coarse blocks about 4 m below the ground surface in a perennially frozen and creeping moraine. **b** Massive ice with visible layering that is most likely a remnant of a small glacier or perennial snow patch that has formed this moraine. **c** Massive ice that is partly clear and partly cloudy. The ice contains individual large clasts and parallel but undulating layers rich in fine material. **d** Massive ice exposed just one meter below the surface of a rock glacier. The ice contains individual large clasts, as well as areas rich in pebble-size rock. Photographs by I. Roer and O. Wild

and high bedrock peaks is likely to be very old. Rock temperatures of −10°C or lower are not uncommon and, therefore, permafrost and ice in cracks and crevices of high peaks may have endured over several glacial and interglacial cycles. The age of this cold bedrock permafrost is more probably controlled by uplift and erosion than by past climate fluctuations.

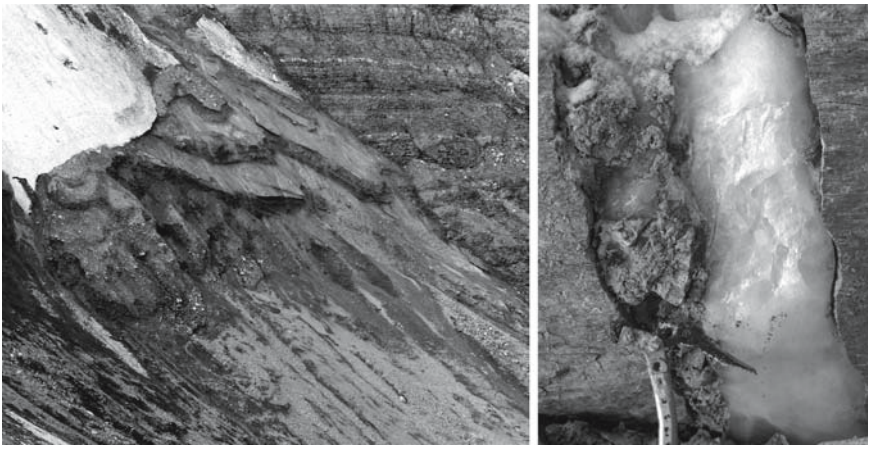

Fig. 3.9 *Left*: Eroded debris slope and exposed ice-cemented permafrost at 2,400 m asl below the 2005 Dents Blanches rock fall (photograph by B. Rey-Bellet). *Right*: Ice-filled fissure in bedrock that has been exposed during construction activities just below a cable car station at Stockhorn, 3,400 m asl, Switzerland. The fine material fill of the joint is entirely on the left side and separate from the pure ice on the right that is about 20 cm thick

3.4 Conclusion

Mountain permafrost is a fascinating phenomenon: It is invisible, extremely variable and heterogeneous, difficult to measure, difficult to model, and it currently undergoes rapid changes. These changes can affect landscape dynamics as well as human infrastructure and safety. Despite this importance, most systematic investigations of mountain permafrost at present are local in nature, because the strong heterogeneity of the system and the limited amount of available data often preclude continental-scale evaluation and modeling. In the future, however, an increased resolution of global and regional climate (or earth system) models, or the improved representation of mountain topography at the sub-grid scale, will likely allow the explicit consideration of mountain permafrost in continental-scale assessments.

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Part II
Biodiversity in Permafrost

Chapter 4
Very Old DNA

Martin B. Hebsgaard(✉) and Eske Willerslev

4.1 Introduction

The DNA molecule degrades over time, just like other cellular components if not repaired. Often the degradation is relatively fast, as fossil remains that are only a few hundred years old contain little or no amplifiable endogenous DNA. One basic question in research on ancient DNA is "how long can DNA and cells survive?" This question is not easily answered because it depends on numerous interacting factors. A maximum DNA survival of 50,000–1 million years has been suggested from theoretical considerations and empirical studies. It is clear that temperature is an important factor, because low temperatures and dry conditions slow the rate of chemical processes that degrade DNA. Given that rates of reaction generally drop an order of magnitude for every 10°C drop in temperature, colder environments are naturally better environments for long-term storage of DNA (Smith et al. 2001). Other natural processes that accelerate the degradation of the DNA molecule are endogenous and exogenous nucleases, as well as hydrolysis (Lindahl 1993; Handt et al. 1994; Hofreiter et al. 2001a). Despite the predicted maximum age of DNA, several studies have claimed to be able to extract DNA many million of years old, yet others fail to amplify DNA with a very young origin. How do we explain this discrepancy? On the one hand, we know that DNA degrades over time and that fossil remains can contain very little or no DNA. This is a problematic situation, which makes the studies very prone to contamination, giving false-positive results.

4.2 Theory

In metabolically active cells, genomic damage is effectively repaired through complex enzymatic pathways; in dead or dormant cells such as bacterial endospores, damage will accumulate over time (Nicholson et al. 2000). Most fossil remains of one hundred to a

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few thousand years old do not contain amplifiable endogenous DNA. This indicates that DNA degradation must occur at a rapid tempo (Hofreiter et al. 2001a). It also indicates that the DNA molecule is relatively unstable compared to other cellular components (Lindahl 1993). The initial degradation process begins with cells being dissolved by cellular enzymes; subsequently, rupture of the cell releases nutrients, which support the growth of environmental microorganisms that contribute further to the degradation process (Nicholson et al. 2000). Rapid desiccation, freezing, and high salt concentrations can in special cases significantly reduce this enzymatic and microbial degradation. In cases like these, slower continuous processes such as hydrolysis, oxidation, and cross-linking will modify the DNA and finally render it irretrievable (Hofreiter et al. 2001b; Willerslev et al. 2004b; Pääbo et al. 2004; Willerslev and Cooper 2005) (Fig. 4.1).

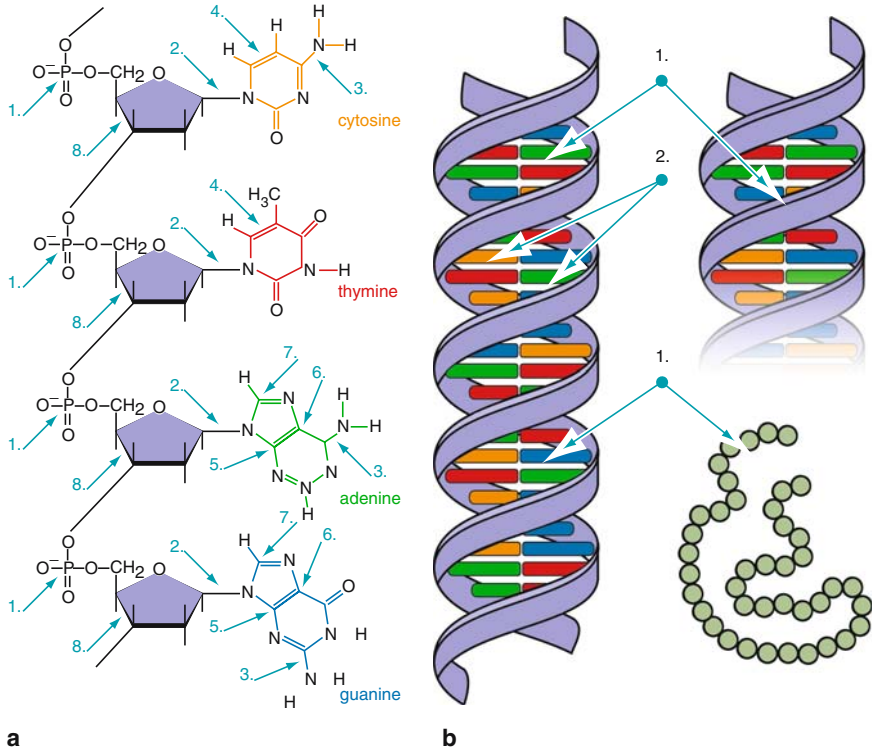

Fig. 4.1 **a** The DNA molecule is highly prone to spontaneous degradation processes such as hydrolysis and oxidation. Hydrolytic damage is responsible for breaks of the sugar backbone (*1*), for base loss (especially the purines, adenine and guanine = depurination) (*2*), and for the deamination of bases (cytosine, adenine, and guanine) (*3*). Oxidative damage perturbs the integrity of the DNA molecule by attacking the shared double bond of carbons C5 and C6 of pyrimidines (cytosine and thymine) (*4*) or the C4 (*5*), C5 (*6*) and C8 (*7*) carbons of purines. The sugar backbone can also be attacked (*8*). Hydrolytic and oxidative damage causes nicks, and blocking- or miscoding lesions. **b** A largely unrecognized DNA modification is crosslinking which includes intermolecular crosslinks such those of DNA and proteins (*1*) and interstrand crosslinks, i.e. between two DNA strands (*2*). Crosslinks prevent amplification, but might also stabilize the DNA molecule over time, so reducing fragmentation

The key question is whether we can predict the long-term survival of DNA, and what environmental conditions and genomic protection mechanisms allow the DNA to survive longest on the Earth's biosphere. Several attempts have been made to predict long-term DNA survival, such as amino acid racemization (Poinar et al. 1996), thermal age (Smith et al. 2001), and extrapolations from DNA in solution (Pääbo and Wilson 1988; Willerslev et al. 2004a). These models are in general too simple; for example, they assume that hydrolytic depurination is the only significant type of DNA damage, even though other modifications such as crosslinking have been shown to be more important for the retrieval of DNA under certain conditions (Rivkina et al. 2000; Willerslev et al. 2004a; Hansen et al. 2006) (Fig. 4.1).

Further, some bacterial cells have continuous metabolic activity, allowing genomic repair over time which extends the long-term survival of metabolically active cells compared to cells under dormancy (Johnson et al. 2007). Thus, predicting DNA survival remains complicated, among other things because the rates of DNA degradation under various environmental conditions are only poorly understood.

Even though it is difficult to predict the long-term survival of DNA, empirical claims of geologically ancient DNA in the order of 1,000-fold older than theoretical predictions for maximal DNA survival are of considerable concern. In general, models for long-term DNA preservation predict a maximum survival time of about 100,000 years for short pieces of amplifiable DNA (~100 bp) (Pääbo and Wilson 1988; Poinar et al. 1996; Smith et al. 2001). Together with the huge problems with contamination, it is very important that we evaluate and authenticate the claims of very old DNA (Hebsgaard et al. 2005).

4.3 Empirical Evidence

A series of publications claim that ancient DNA from plants, animals, and microbes — even viable bacterial cells — can survive in amber, halite, soft tissue, and sediments for up to several hundred million years (Goldenberg et al. 1990; Soltis et al. 1992; Cano et al. 1992a, b, 1993; DeSalle et al. 1992, 1993; Poinar et al. 1993; DeSalle 1994; Kennedy et al. 1994; Woodward et al. 1994; Cano and Borucki 1995; Morita 2000; Vreeland et al. 2000; Lambert et al. 2001; Vreeland and Rosenzweig 2002; Fish et al. 2002; Kim et al. 2004). These publications suggest that nucleic acids can persist over geological timescales (i.e., DNA sequences >1 million years old). Departing from the theoretical evidence, these claims bear a heavy burden of proof. Another interesting study showed that Antarctic ice samples up to 8 million years old not only contain amplifiable DNA but also living bacteria (Bidle et al. 2007). This is a very interesting result as the 8 million-year-old sample is the oldest ice sample ever studied, but also because both the bacteria DNA and the viable cells isolated from the ice are much older that expected. The result is also far reaching compared to the record of long-term DNA survival from Greenland. In a recent study, 450,000- and 800,000-year-old DNA have been extracted from the silty ice of the Dye 3 Ice Core, but not from the much older ice in the GRIP (Greenland Ice Core Project) core (Willerslev et al. 2007).

Recent studies of frozen sediments performed under very strict conditions show that DNA from extinct animals and plants can reproducibly be recovered by independent laboratories from samples dated 300,000–400,000 years old, but not from sediments dated to be 1.5–2 million years old (Willerslev et al. 2003). Another study showed that bacteria DNA can be amplified from 400,000 to 600,000 years old permafrost samples from Siberia, but not from 8.1 million-year-old samples from Antarctica (Willerslev et al. 2004a). These results from permafrost show that DNA from bacteria, extinct animals and plants can reproducibly be recovered from very old samples up to 600,000 years old. Even though these findings could potentially result from leaching of free DNA, they are within what many groups currently accept as maximum ages for DNA survival (Hofreiter et al. 2001a; Smith et al. 2001; Willerslev et al. 2004a, b; Pääbo et al. 2004; Willerslev and Cooper 2005).

The long-term survival of bacteria sealed in permafrozen sediments for up to 1 million years have also recently been investigated (Johnson et al. 2007). The study showed evidence of bacteria surviving in samples up to 500,000 years old which make this the oldest independently authenticated DNA to date obtained from viable cells. It is further shown that this long-term survival is closely tied to cellular metabolic activity and DNA repair.

4.4 Contamination

At best, most ancient samples contain no or only small amounts of amplifiable endogenous DNA. This, combined with a complex and poorly understood contamination risk in ancient DNA studies, involves a high risk of false-positive results (Cooper and Poinar 2001; Hofreiter et al. 2001b; Marota and Rollo 2002; Willerslev et al. 2004b; Pääbo et al. 2004; Willerslev and Cooper 2005). Traditional contamination is separated into laboratory and sample contamination.

To avoid laboratory contamination, all pre-PCR work should be carried out in dedicated isolated ancient DNA facilities with separate ventilation systems, nightly UV irradiation, and positive air pressure. The work should be carried out following strict protocols with bodysuits, facemasks, and gamma-sterilized gloves (Hebsgaard et al. 2005; Willerslev and Cooper 2005). Blank-extraction and PCR-amplification controls should be incorporated. Blank controls cannot by themselves guarantee detection of laboratory contamination, due to the sporadic nature of contamination and carrier effects (Cooper and Poinar 2001; Marota and Rollo 2002; Cooper 1993; Handt et al. 1994; Pääbo et al. 2004; Willerslev and Cooper 2005).

Another risk of contamination is carryover of PCR products, which can lead to high levels of amplicons rapidly spreading through laboratories, making it easy to obtain false-positive amplification products (Willerslev and Cooper 2005).

It is impossible to discount minor amounts of laboratory-based contamination, even for the most comprehensive laboratory setup. This holds especially true in human and microbial studies due to the universal distrinution of these organisms in laboratory settings (Rollo and Marota 1999; Willerslev et al. 2004b; Pääbo et al. 2004).

However, high contamination risk can also be applied to studies of rare organisms (even extinct species) if close modern relatives are processed in the same laboratory or large amounts of amplicons are produced, such as in large-scale genetic population studies (Shapiro et al. 2004). Fortunately, laboratory contamination, although a serious concern, can be detected by the following simple authentication criteria (Pääbo 1989; Cooper and Poinar 2001; Hebsgaard et al. 2005). The independent replication of results by another laboratory is the strongest argument against laboratory contamination, because it is unlikely that the same contaminant sequence will be independently sequenced in another laboratory.

Much more challenging is sample contamination, because it is much more difficult to exclude. In most human and microbial studies there is currently no way to clearly distinguish an endogenous DNA sequence or culture from that of a contaminant (Rollo and Marota 1999). The problem is especially pronounced where the samples have been handled by several individuals during excavation. In the same way, microbes can easily contaminate samples just by passive or active movement. Even microbes known to be associated with a particular specimen might have unknown relatives or even identical ecotypes in the surrounding environment (Gilbert et al. 2005a). Sample contamination can only be excluded for sequences obtained from morphologically identifiable specimens, with restricted extant distributions and well-known diversity (e.g., many vertebrates and some higher plants), though recent sequencing of DNA directly from sediments or ice (Willerslev et al. 2003, 2007; Johnson et al. 2007) complicates authentication for these groups.

4.5 Verification of Results

Since we cannot rule out that samples get contaminated on the basis of experimental setup, it is important to assess the authenticity using empirical tests. An independent line of evidence for authenticity of ancient DNA results is the application of relative rate analyses. One such approach — the evolutionary rate test — is an empirical test that exploits the temporal difference between related modern sequences and the very old DNA claims. The method infers the timing of the divergence between the ancient sequence and the modern sequences, by assuming a molecular clock and applying a published substitution rate for the particular gene. This approach can fail if the published rate of evolution is not correct for the taxa in question or the sequence in question. Furthermore, very old divergences may also be obtained if the ancient sequence is from a previously unknown modern contaminant (Hebsgaard et al. 2005).

A more solid approach is the relative rates test. Essentially, it examines if the relative distance between an outgroup and the ancient sequence is significantly different from the distance between the same outgroup and a modern sequence that is closely related to that of the ancient sequence (Fig. 4.2). A more vigorous approach is the relative rate analysis. This method estimates a likelihood function of the substitution per site, using database sequences of the most closely related

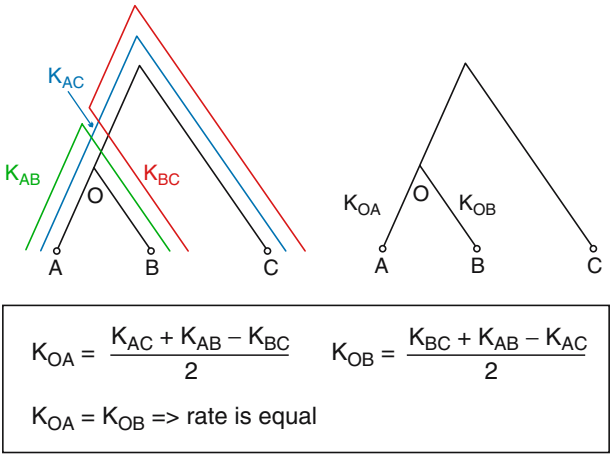

Fig. 4.2 The relative rates test uses an outgroup sequence, *C*, which is known to branch off before either sequence *A* or *B*. *O* is the common ancestor sequence of *A* and *B*. *KOA* is the relative substitution rate between *O* and *A*, and *KOB* is the relative substitution rate between *O* and *B*. Whether the genetic distance between *O* and *A* is significantly different from the distance between *O* and *B* can be evaluated by comparing KOA and KOB, which are calculated using the equations enclosed in the box

sequences. This is then translated using published substitution rates to an estimate of age using a molecular clock (Willerslev et al. 2007).

Compared to the evolutionary rate test, the relative rate test is independent of an accurate calibration date and substitution rate. But the above molecular dating and relative rates tests all assume that substitutions accumulate in a clock-like manner. More important is the rate at which the DNA evolves; as mentioned before, these rates are different for different individuals but they are also different for different genes. This means that the rate can be very different for slow-evolving genes and fast-evolving genes. For example, for 14 published insect COI genes the published evolutionary rates vary between 0.3×10^{-8} and 9×10^{-8} substitutions per year (Morgan-Richards et al. 2001).

4.6 Can We Trust Very Old Claims?

There are many examples where scientists have trusted their results at first but later the results have turned out to be wrong. Historically, ancient DNA studies have suffered much criticism since they began about 20 years ago. Unfortunatel,y the field is still recovering from the effects of early spectacular and erroneous claims, such as that of DNA being preserved in plant fossils, dinosaur bones, and amber for many millions of years (Hebsgaard et al. 2005; Willerslev and Cooper 2005). Unfortunately, unreplicated results of surprising age continue to be published,

including those from old human remains (Adcock et al. 2001), microorganisms (Cano and Borucki 1995; Vreeland et al. 2000; Fish et al. 2002), and plant fossils (Kim et al. 2004). These studies have routinely underestimated the extent to which ancient DNA research is confounded by contamination with modern DNA, and are widely thought to result from such contamination (Willerslev et al. 2004a; Hebsgaard et al. 2005; Willerslev and Hebsgaard 2005).

In recent years, a greater understanding of postmortem damage and contamination has provided a more robust foundation for the field, although the authentication of studies of human remains and microbes is still highly problematic (Willerslev et al. 2004b; Gilbert et al. 2005b; Hebsgaard et al. 2005; Willerslev and Cooper 2005).

The first report of putative Neanderthal (*Homo neanderthalsensis*) mitochondrial DNA (mtDNA) was a rare example of a remarkable ancient DNA (aDNA) result obtained using very strict criteria for authenticity, including the independent replication of results and tests of biochemical preservation (Krings et al. 1997; Cooper and Poinar 2001; Hofreiter et al. 2001a; Pääbo et al. 2004; Willerslev and Cooper 2005; Hebsgaard et al. 2007). The result is convincing, as the Neanderthal sequence differs from any known modern human (*Homo sapiens*) and chimpanzee (*Pan troglodytes*) sequences but is clearly human-like. Furthermore, subsequent independent retrieval of similar, but not identical, mtDNA from other Neanderthal specimens strongly supports the sequence's authenticity (Krings et al. 1999, 2000; Ovchinnikov et al. 2000; Schmitz et al. 2002; Serre et al. 2004; Lalueza-Fox et al. 2005; Hebsgaard et al. 2007). Although the result is convincing it has been shown that the first published Neanderthal sequence may include errors due to postmortem damage in the template molecules for PCR (Hebsgaard et al. 2007). In contrast, inadequate experimental design and a high percentage of chimeric sequences misled Pusch and Bachmann (2004) to suggest the Neanderthal sequences were products of PCR artefacts, a conclusion that later turned out to be wrong (Hebsgaard et al. 2007).

Two recent ice core studies have investigated the long-term survival of DNA in ice from Greenland (Willerslev et al. 2007) and Antarctica (Bidle et al. 2007). The first study showed that DNA can be extracted from ice core samples dated 450,000–800,000 years old from the centre of Greenland (Willerslev et al. 2007). Following strict criteria (Willerslev et al. 2004b; Hebsgaard et al. 2005; Willerslev and Cooper 2005), PCR techniques yielded short sequences (less than 120 bp) of plant and insect DNA, which were independently replicated in three different laboratories (Willerslev et al. 2007).

In the study by Bidle and colleagues (2007), ancient DNA and viable cells were isolated from up to 8 million-year-old samples from Antarctica. This is indeed remarkable, and if authentic this study is the first to amplify DNA and viable cells from ice cores as old as 8 million years. Unfortunately, as with many other results of geological ancient DNA, the study did not follow the strict criteria for ancient DNA studies and therefore suffers from inadequate experimental setup and insufficient authentication. Hebsgaard et al. (2007) showed how important it is to follow the strict criteria when working with very old DNA or geological ancient DNA. The results are also interesting compared to results from an 8 million-year-old permafrost sample from Antarctica where not even small fragments of DNA could

be amplified (Willerslev et al. 2007). In contrast to Bidle et al. (2007), this study applied strict criteria for ancient DNA work and used dedicated facilities. The results are also interesting because both the DNA and the viable cells isolated from the ice are much older that expected, and are also far reaching compared to the record of long-term DNA survival from permafrost sediment.

Studies of old permafrost samples have been in progress since Willerslev et al. (2003) showed under strict conditions that DNA from extinct animals and plants can be recovered by independent laboratories using strict criteria from samples dated to be 300,000–400,000 years old. Additionally, the study showed that it is not possible to extract DNA from sediment samples dated to be 1.5–2 million years old (Willerslev et al. 2003). A more recent study showed that bacterial DNA can be amplified from 400,000- to 600,000-year-old permafrost samples from Siberia, but not from 8.1 million-year-old samples from Antarctica (Willerslev et al. 2004a). Both of these studies used strict criteria including replication in an independent laboratory, which excluded the possibility that the results were due to laboratory contaminations. A problem with these studies is the risk associated with vertical migration of DNA across different strata. However, it has been shown that for organisms that do not produce copious amounts of liquid urine, the DNA is stratigraphically localized in the sediments, which is true for bacteria, plants and most animals (Haile et al. 2007). Additionally, the results are within the range of what many groups currently accept as maximum ages for DNA survival (Hofreiter et al. 2001b; Smith et al. 2001; Willerslev et al. 2004a, b; Pääbo et al. 2004; Willerslev and Cooper 2005).

Since the isolation of 250 million-year-old bacteria from salt crystals (Vreeland et al. 2000), the long-term survival of bacteria has been questioned in several publications (Graur and Pupko 2001; Nickle et al. 2002) and has been found very problematic (Hebsgaard et al. 2005). A recent study, however, investigated the long-term survival of bacteria in sealed permafrost samples up to 1 million years old (Johnson et al. 2007). The study followed strict criteria and showed that bacteria can survive in permafrost up to 500,000 years, which make this the first independently authenticated evidence for viable cells surviving that long.

4.7 Conclusion

The race to continue to extract DNA from older and older samples will persist, but it is important that we keep up methodologically with the race to authenticate our results. Currently, no authentication criteria can completely exclude all paths of contamination in studies of very old DNA. This holds especially true for studies on ancient human and microbial remains. However, following strict criteria for authentication such as those outlined in Hebsgaard et al. (2005) will minimize false-positive results. It is concerning that many claims of very old DNA are still published without even following the most fundamental of these authentication criteria, which

unfortunately renders these studies unreliable. It is our hope that, in order to interest a broader scientific community, the priorities change, so that age is not the most important factor but the focus is on reproducibility and authentication of results. Also, the centre of ancient DNA research should focus on what questions can be answered and not just how old the DNA is.

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Chapter 5
Bacterial and Archaeal Diversity in Permafrost

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5.1 Introduction

Microorganisms in permafrost survive in an extreme environment characterized by constant subzero temperatures, low water and nutrient availability, and prolonged exposure to background radiation. Despite the harsh conditions, considerable abundance and diversity of microorganisms inhabit permafrost. Pioneering studies focusing on permafrost microbiology simply attempted to determine if permafrost harbored viable microorganisms. For example, microorganisms cultured from Canadian (James and Sutherland 1942), Alaskan (Boyd and Boyd 1964) and Antarctic (Cameron and Morelli 1974) permafrost samples were generally poorly characterized, and the studies were hampered by an inability to demonstrate that drilling and sample handling were performed aseptically. Recent developments using fluid-less drilling (Gilichinsky et al. 1989; Khlebnikova et al. 1990; Juck et al. 2005), tracer microorganisms (Christner et al. 2005; Juck et al. 2005), nucleic acid stains (Christner et al. 2005) and fluorescent microspheres as microbial surrogates (Juck et al. 2005) have greatly improved our ability to recover intact permafrost samples and to monitor exogenous microbiological contamination of pristine permafrost samples.

Permafrost also contains various other geomorphological structures including massive ground ice, cryopegs, and ice wedges (Steven et al. 2006) that harbor microbial populations. The description of the abundance, diversity, activity and distribution of microorganisms in permafrost and associated environments will be fundamental to our understanding of how microorganisms survive in permafrost, and how they will respond to future climatic warming and permafrost thawing. Lastly, permafrost microorganisms and microbial ecosystems are considered significant terrestrial analogs for similar organisms that may inhabit permafrost environments that exist beyond the Earth, especially in light of the recent evidence of massive amounts of shallow ground ice near the surface of Mars (Gilichinsky 2002a; Gilichinsky et al. 2007).

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5.2 Viable *Bacteria* and *Archaea* in Permafrost

5.2.1 Microbial Abundance in Permafrost Environments

Substantial numbers (up to 10^9 cells g^{-1}) of microbial cells are detected in permafrost but vary over a large range among different permafrost environments (Table 5.1). In general, only a small proportion of the microbial community is represented by cultured isolates. In Arctic permafrost ca. 0.1–10% of the microbial community is recovered by standard culturing, while in Antarctic permafrost viable cell recovery is only 0.001–0.01% (Vorobyova et al. 1997). Microscopic investigations of permafrost microorganisms in situ have revealed the presence of partially degraded cells (i.e., ruptured cell walls and membranes) and empty "ghost cells" (Dmitriev et al. 2000; Soina et al. 2004); due to the constant subzero temperatures in permafrost, dead or compromised microbial cells may remain well preserved and contribute to total microbial counts. For example, Hansen et al. (2007) observed that 74% of the microbial community in Spitsbergen Island permafrost had compromised cell walls, based on differential staining and microscopy, and were considered non-viable. Intact microbial cells in permafrost are characterized by altered ultrastructures such as thickened cell walls and a non-homogenous cytoplasm that contains numerous aggregates (Soina et al. 1995, 2004). Perhaps most characteristically, Siberian permafrost appears to be dominated by populations of cells ≤1 μm in size (Dmitriev et al. 2000; Soina et al. 2004) with ultramicroforms of cells ≤0.4 μm in diameter making up as much as 80% of Siberian permafrost microbial populations (Vorobyova et al. 2001). Dwarfed cells are characteristic of the viable but non-culturable state (reviewed in Oliver 2005) and, therefore, many cells in permafrost may be in a physiological state that is recalcitrant to laboratory cultivation, partially explaining the low viable cell recovery.

The ability to recover viable cells from permafrost seems to be independent of permafrost temperature or depth, but depends on the age of the permafrost. With increasing age, both the number and diversity of bacterial isolates decrease, with an increase in the number of sterile samples (Gilichinsky et al. 1989, 1992; Khlebnikova et al. 1990). Nevertheless, viable microbial cells were recovered from Siberian permafrost as old as 3 million years (Gilichinsky 2002a). The amount of ice in permafrost also has a large effect on cell recovery, as increasing ice content often greatly reduces viable cell counts. Viable bacteria are rarely recovered from nearly pure ice systems in permafrost such as ice wedges (Gilichinsky et al. 1995; Gilichinsky 2002b) or massive ground ice formations (Steven et al. 2008a), although viable bacterial numbers of up to 10^6 CFU ml^{-1} were recovered from an Alaskan ice wedge sample (Katayama et al. 2007). Therefore, the origin, age and physiochemical characteristics of the ice presumably determine the presence and abundance of a viable microbial community.

Table 5.1 Microbial abundance in various permafrost environments

Location	Cell type	Viable cell counts[a]	Direct microscopic counts[b]	References
Antarctic Dry Valley	Aerobic heterotrophs	$0–10^5$	$10^5–10^{6c}$	Horowitz et al. (1972)
	Methanogens	$0–10^3$		Cowan et al. (2002)
	Sulfate reducers	$0–10^3$		Gilichinsky et al. (2007)
	Denitrifying bacteria	$0–10^1$		
Siberian permafrost	Aerobic heterotrophs	$0–10^8$	$10^3–10^8$	Rivkina et al. (1998)
	Methanogens	$0–10^7$		Gilichinsky (2002a)
	Sulfate reducers	$0–10^3$		
Canadian high Arctic permafrost	Aerobic heterotrophs	$10^1–10^4$	$10^7–10^8$	Steven et al. (2007a)
				Steven et al. (2007c)
Spitsbergen Island	Aerobic heterotrophs	10^5	10^9	Hansen et al. (2007)
	Anaerobic heterotrophs	10^5		
Tianshan Mountains, China (alpine permafrost)	Aerobic heterotrophs	10^5	NA[d]	Bai et al. (2006)
Qinghai-Tibet Plateau (high altitude permafrost)	Alkaliphilic and psychrotolerant bacteria	$10^2–10^5$	NA	Zhang et al. (2007)
Siberian Cryopeg	Aerobic heterotrophs	$10^2–10^5$	10^7	Bakermans et al. (2003)
	Anaerobic heterotrophs	$10^1–10^2$		Gilichinsky et al. (2003)
	Sulfate reducers	10^6		Gilichinsky et al. (2005)
	methanogens	10^2		
Alaskan ice wedge	Aerobic heterotrophs	$10^5–10^6$	NA	Katayama et al. (2007)
Canadian high Arctic ground ice	Aerobic heterotrophs	0	10^4	Steven et al. (2007c)
Greenland glacier ice/permafrost	Aerobic heterotrophs	10^2	10^7	Miteva et al. (2004)

[a]CFU g^{-1} (only the order of magnitude of the counts are presented)
[b]Cells g^{-1} (only the order of magnitude of the counts are presented)
[c]Estimated from ATP content/cell
[d]Data not available

5.2.2 Diversity of Viable Bacteria and Archaea

The catalog of viable *Bacteria* recovered from permafrost and associated environments, currently includes at least 70 genera (Table 5.2). Cultured isolates recovered from permafrost are capable of a wide range of metabolic processes including aerobic and anaerobic heterotrophy, chemolithoautotrophy, sulfate-reduction, methanotrophy, methanogenesis (Gilichinsky et al. 1995; Steven et al. 2006) and even phototrophy (Chap. 6). Both Gram-positive and Gram-negative cells are represented, and spore-forming *Bacteria* are also commonly isolated, although the abundance of spore-forming *Bacteria* varies widely between geographically separated permafrost samples. For example, spore-forming genera dominated the culturable community from 2 to 9 m (69% and 100% of isolates, respectively) Canadian high Arctic permafrost samples (Steven et al. 2007a, 2008a), whereas spore-forming genera only composed 30, 5 and 1% of Siberian (Shi et al. 1997), Spitsbergen Island (Hansen et al. 2007) and Chinese alpine (Bai et al. 2006) permafrost isolates, respectively. *Firmicutes* and *Actinobacteria* generally represent a high proportion of the permafrost microbial community, accounting for up to 100% of Canadian high Arctic isolates (Steven et al. 2008a), 60% of Chinese alpine permafrost isolates (Bai et al. 2006) and 45% of Siberian permafrost isolates (Shi et al. 1997). To date, the phylogenetic groups that account for the anaerobic *Bacteria* community in permafrost remain poorly characterized.

Cryopegs are lenses of supercooled, saline liquid water within the permafrost (Bakermans et al. 2003) that can harbor substantial numbers of viable microbial cells (Table 5.1). These include a variety of anaerobic and aerobic, spore-less and spore-forming bacteria (Table 5.2), with a *Psychrobacter*-related isolate accounting for 53% of all isolates, suggesting this organism was a dominant community member (Bakermans et al. 2003).

A single report of the microbial community in an Alaskan permafrost ice wedge indicated relatively high numbers of viable microbial cells (Table 5.1), although the diversity of the recovered isolates was low (Katayama et al. 2007). The phylogenetic groups of the isolates were similar to those identified in permafrost soils (Table 5.2).

The description of viable *Archaea* in permafrost remains limited. Methanogenic *Archaea,* generally occur in low numbers (10^2–10^3 g^{-1}) and not in all samples (Rivkina et al. 1998, 2002). Recovered isolates related to the genera *Methanosarcina* and *Methanobacterium* (Rivkina et al. 2007) and methanogenic activity detected in Siberian permafrost samples suggests that methanogenesis occurs at in situ permafrost temperatures (Rivkina et al. 2000, 2002). We recently detected halophilic *Archaea* in saline enrichment cultures from Canadian high Arctic permafrost, indicating that these organisms are members of a viable permafrost microbial community (unpublished data).

5.2.3 Increasing Representation of Cultured Isolates

Methods to increase the representation of cultured microbial isolates from permafrost have recently been applied. For example, Vishnivetskaya et al. (2000) used natural permafrost sediment (NPS) enrichment to recover microbial isolates. NPS, consisting

Table 5.2 Phylogenetic groups of *Bacteria* cultured from permafrost[a]

Phylogenetic group	Canadian high Arctic permafrost[b]	Siberian permafrost[c]	Siberian cryopeg[d]	Spitsbergen Island permafrost[e]	Antarctic permafrost[f]	Chinese alpine permafrost[g]	Alaskan ice wedge[h]
Actinobacteria							
Arthrobacter	+	+	+	+	+	+	+
Brachybacterium	+			+			+
Cellulomonas		+		+	+		
Cryobacterium				+			+
Frigoribacterium			+			+	
Kocuria	+			+			
Leifsonia				+		+	
Microbacterium		+	+			+	+
Micrococcus	+	+		+	+		
Nocardia				+		+	
Promicromonospora		+			+		
Rhodococcus	+	+	+	+	+	+	+
Streptomyces		+		+	+		
unique genera	–	1	1	10	–	5	–
CFB							
Flavobacterium	+	+				+	
Pedobacter	+			+		+	
unique genera	–	2	1	1	–	1	–
Firmicutes							
Bacillus	+	+	+	+	+	+	
Exiguobacterium		+				+	
Paenibacillus	+	+	+	+			
Planococcus	+	+				+	+
Planomicrobium		+				+	
Sporosarcina	+	+				+	
unique genera	3	–	–	–	–	2	1
Proteobacteria							
Aeromonas		+			+		
Myxococcus		+			+		
Psychrobacter		+	+			+	
Pseudomonas	+	+		+	+	+	+
unique genera	1	9	1	5	1	7	1

[a]Genera represented in at least two permafrost environments are indicated (+). The number of genera that were unique to the distinct permafrost environments are also indicated. *Bacteria* phyla are shown in **bold**

[b]Steven et al. (2007a, 2007b);

[c]Shi et al. (1997), Vorobyova et al. (1997) and Vishnivetskaya et al. (2006)

[d]Bakermans et al. (2003) and Gilichinsky et al. (2005)

[e]Hansen et al. (2007)

[f]Vorobyova et al. (1997) and Gilichinsky et al. (2007)

[g]Bai et al. (2006) and Zhang et al. (2007)

[h]Katayama et al. (2007)

of thawing permafrost at 4°C and incubating the permafrost samples for up to 12 weeks before direct plating, increased the recovery of both the numbers and diversity of viable cells from most permafrost samples. Similarly, preliminary incubation in anaerobic and aerobic liquid media prior to plating greatly increased the recovery and diversity of recovered organisms from deep Greenland ice core samples and a Spitsbergen Island permafrost sample (Miteva et al. 2004; Hansen et al. 2007). Preliminary incubations may permit damaged, stressed, or dormant cells to repair damage induced by long-term exposure to thermal, osmotic, and nutritional stresses imposed by permafrost environments. Ideally, osmoprotectants such as salts, alcohols, and/or sugars could be incorporated in culture media, not only to enhance cellular survival and recovery, but to lower the freezing point of culture media to ambient permafrost temperatures. The ability to isolate and culture permafrost microorganisms at in situ temperatures will be crucial in determining the cellular mechanisms and physiological adaptations required for indigenous microbes to survive in permafrost.

5.3 Phenotypic Characteristics of Permafrost Isolates

The recovery of viable cells from Arctic and Antarctic permafrost samples is generally facilitated by using nutrient-poor media (Gilichinsky et al. 1989; Bai et al. 2006; Steven et al. 2007a), suggesting that permafrost communities are primarily oligotrophic; although organic carbon is more abundant in Arctic permafrost (Vishnivetskaya et al. 2000; Gilichinsky 2002a; Steven et al. 2006). Microbial abundance and activity in subsurface soils is affected by soil porosity, as subsurface pores are required for the movement of liquid water, with larger pore sizes associated with an increased availability of organic compounds (Kaiser and Bollag 1990). The sequestering of liquid water as ice in permafrost reduces porosity and may therefore act to limit the availability of organic carbon, selecting for oligotrophic microbial populations.

Permafrost microorganisms also tend to be more halotolerant than organisms from the overlying active layer soil (Gilichinsky 2002a; Steven et al. 2008a). Microbial survival in extremely cold environments is under the influence of ice formation and, consequently, little biologically available liquid water is present. Therefore, water activity is probably an important factor influencing microbial survival in permafrost (Gunde-Cimerman et al. 2003). In addition, during freezing and the binding of water in ice crystals, ions are expelled and concentrate in the remaining liquid phase (Price 2007). Thus, there may be a connection between halotolerance and microbial survival at extremely low temperatures.

Permafrost microorganisms are primarily cold-adapted, with very few mesophilic or thermophilic isolates identified (Gilichinsky 2002a; Steven et al. 2006) Most isolates described are psychrotolerant (growth optimum ≥20°C) rather than psychrophilic, although both psychrotolerant and psychrophilic microorganisms capable of growth at subzero temperatures are isolated from permafrost (Ponder et al. 2005; Bai et al. 2006; Steven et al. 2007a, 2008a), suggesting the potential for growth and metabolism at the ambient subzero temperatures in permafrost.

Many of the microorganisms isolated from permafrost represent potentially novel microbial species or genera (Bakermans et al. 2003; Bai et al. 2006; Ponder et al. 2005; Rivkina et al. 2007; Steven et al. 2007a, 2008a, b). Recent genomic (see Chap. 11) and proteomic (Qiu et al. 2006; Bakermans et al. 2007; see Chap. 12) investigations of species from the genera *Exiguobacterium* and *Psychrobacter* will help define the physiological and genetic adaptations that have allowed these organisms to survive in permafrost. Presumably, these and future studies will lead to a better understanding of long-term survival at subzero temperatures and the low temperature limits for microbial growth and metabolism.

5.4 Culture-Independent Bacterial and Archaeal Diversity in Permafrost

Culture-independent methodologies have recently been applied to the study of microbial diversity in permafrost. These studies, which use molecular-based tools to analyze DNA extracted directly from permafrost (Spiegelman et al. 2005 and references therein), bypass the need for culturing and have increased the number of phylogenetic groups of *Bacteria* and *Archaea* associated with permafrost (Table 5.3). For example, the culturable microbial community in a Canadian high Arctic permafrost sample was dominated by *Firmicutes*-related isolates, whereas *Actinobacteria*- and *Proteobacteria*-related sequences were predominant in a culture-independent analysis, with the phyla *Gemmatimonadetes,* CFB and *Planctomyces* identified in the culture-independent survey but not among the isolates (Steven et al. 2007a). A diverse *Bacteria* community, comprised of 13 *Bacteria* phyla (Table 5.3), including three candidate phyla (phyla that have no cultured representatives), was detected in Spitsbergen Island permafrost 16S rRNA gene clone libraries (Hansen et al. 2007), while only four phyla (*Actinobacteria,* CFB, *Firmicutes* and *Proteobacteria*) were represented by cultured isolates (Hansen et al. 2007). 16S rRNA gene clone libraries constructed from Siberian permafrost DNA (Table 5.3) were dominated by sequences related to the *Proteobacteria*, *Actinobacteria* and *Firmicutes,* with *Arthrobacter* being abundant in both the culture-dependent and culture-independent surveys of microbial diversity (Vishnivetskaya et al. 2006). The proportion of 16S rRNA sequences related to the high G + C Gram-positive *Bacteria* was also found to increase with increasing age of Siberian permafrost (Willerslev et al. 2004a). Antarctic Dry Valley permafrost 16S rRNA gene clone libraries were composed of the phylogenetic groups *Proteobacteria* and *Actinobacteria*, with *Arthrobacter, Bacillus,* and *Pseudomonas* detected in all of the Antarctic permafrost clone libraries (Gilichinsky et al. 2007).

To date, very few studies have described the *Archaea* communities in permafrost using culture-independent methodologies. Other than a report of the detection of 16S rRNA genes related to the *Crenarchaeota* (affiliated to environmental group 1.1.b) in Chinese alpine permafrost (Ochsenreiter et al. 2003), all of the culture-independent characterizations of *Archaea* diversity in permafrost are from the Canadian high

Table 5.3 Phylogenetic groups of *Bacteria* and *Archaea* detected by culture-independent methods in various permafrost environments

Phylogenetic group	Canadian high Arctic permafrost[a,b]	Kolyma lowlands Siberia[c]	Spitsbergen Island[d]	Dry Valleys Antarctica[e]	Alaskan ice wedge[f]	Canadian high Arctic massive ground ice[b]
Bacteria						
Acidobacteria	+		+	+		
Actinobacteria	+	+	+	+	+	+
CFB	+		+	+		+
Firmicutes	+	+	+	+	+	+
Gemmatimonadetes	+					
Planctomyces	+		+			
Proteobacteria	+	+	+	+	+	+
Spirochaetes			+			
Thermomicrobia			+			
Verrucomicrobiae			+			
OD1[g]			+			
OP10[g]			+			
TM7[g]			+			
Unclassified	+		+			
Archaea						
Environmental *Crenarchaeota*	+					+
Environmental *Euryarchaeota*	+					+
Halophilic *Archaea*	+					+
Methanogenic *Archaea*						+

[a]Steven et al. (2007a)
[b]Steven et al. (2007b)
[c]Vishnivetskaya et al. (2006)
[d]Hansen et al. (2007)
[e]Gilichinsky et al. (2007)
[f]Katayama et al. (2007)
[g]Candidate divisions for which there are no cultured representatives

Arctic. Our studies have revealed that both of the major *Archaea* phyla (*Euryarchaeota* and *Crenarchaeota*) are present in Canadian permafrost, with sequences belonging to the *Euryarchaeota* being numerically dominant (Steven et al. 2007a, 2008a). Although methanogens have been isolated from Antarctic and Siberian permafrost (Rivkina et al. 1998; Gilichinsky et al. 2007), 16S rRNA gene sequences related to methanogenic *Archaea* were not detected in Canadian high Arctic permafrost, with the exception of a single sequence detected in a massive ground ice deposit (Steven et al. 2008a). An interesting result of the culture-independent characterization of *Archaea* communities in Canadian high Arctic permafrost was the detection of a significant number of sequences related to the halophilic *Archaea*, although the salinity

in the permafrost was only moderate (Steven et al. 2007a, 2008a). The detection of halophilic organisms in only moderately saline permafrost provides circumstantial evidence that the primary microbial habitat in permafrost exists as thin saline liquid water veins surrounding soil particles (Price 2007).

It should be noted that the detection of a DNA sequence is not conclusive evidence that the phylogenetically related organism is active or even viable in permafrost, as the constant subzero temperatures are ideal for DNA preservation (Willerslev et al. 2003, 2004a; see Chap. 4). Thus, developing novel methods will be essential to determine if microorganisms identified in culture-independent surveys exist as viable cells or are the microbial equivalent of mammoths, frozen in time in the permafrost environment.

5.5 Biogeography of Permafrost Microorganisms

One of the longstanding theories of microbial biogeography is the paradigm that "everything is everywhere, but the environment selects" (Baas-Becking 1934, cited in O'Malley 2007). However, various studies have started to challenge this traditional theory with research showing divergence of microbial types due to geographical constraints on microbial migration, and environmental factors driving spatial and temporal distributions (Hughes Martiny et al. 2006). Comprehensive descriptions of permafrost environments encompassing both molecular and culture-based approaches are only starting to emerge in the literature (Vishnivetskaya et al. 2006; Gilichinsky et al. 2007; Hansen et al. 2007; Steven et al. 2007a, 2008a); therefore, it may be premature to put these into a biogeography context. Nevertheless, trends are beginning to appear including the dominance of high G + C Gram-positive organisms within permafrost as revealed by culture-dependent and culture-independent methods (Tables 5.2 and 5.3). The high similarity between 16S rRNA gene sequences and isolates recovered from permafrost samples (Gilichinsky et al. 2007; Hansen et al. 2007; Steven et al. 2007a, 2008a) and those from other similar cryoenvironments (e.g., glacial ice, sea ice, and Lake Vostok accretion ice) also suggests that cosmopolitan groups of microorganisms adapted to life at subzero temperatures exist. Conversely, several *Bacteria* genera detected in each of the above mentioned studies also seem to be unique to the specific location under investigation (Tables 5.2 and 5.3). Taken together, these results indicate both cosmopolitan and endemic populations of microbes residing in geographically separated permafrost. However, one cannot conclusively prove an organism is not present in any given environment, due to the limitations of current technologies used in microbial ecology (Ramette and Tiedje 2007). It is also important to note that studies of the microbiology in permafrost are from a relatively small number of sites, and do not reflect a comprehensive survey of permafrost environments.

Work undertaken by Steven et al. (2008a) has also demonstrated the importance that comparisons between microbial communities in geographically separated permafrost should be made from similar horizons, as the composition of microbial

communities varies with permafrost depth. For example, 55% of the *Bacteria* 16S rRNA gene sequences from a 1-m depth permafrost sample (Steven et al. 2008a) were most closely related to 16S rRNA gene sequences recovered from a ca. 1-m deep Spitsbergen Island permafrost sample (Hansen et al. 2007), compared to 15% of clones from a 2-m permafrost sample, while none of the clone sequences from a 9-m sample (Steven et al. 2007a) had closest relatives identified in the Spitsbergen Island permafrost sample.

The application of new techniques in biogeography theory, taxonomic level resolution and exhaustive sampling methods, and novel molecular approaches such as microarray and metagenomic technologies (Ramette and Tiedje 2007; Xu 2006) will lead to a greater understanding of microbial biogeography and the environmental factors in permafrost that control the abundance, distribution and diversity of the microbial populations.

5.6 Permafrost Microorganisms: Ancient Survivors or an Active Ecosystem?

Without a fossil record or detectable events of when a group of specific microorganisms appeared for the first time, we have little knowledge concerning the timeline or age of microbial species, or how to calibrate their evolutionary divergence (Vreeland and Rosenzweig 2002). Therefore, the age of supposed ancient organisms, including permafrost isolates (Willerslev et al. 2004b), is assumed from the age of their surrounding environment (Drancourt and Raoult 2005). A molecular clock has been postulated estimating that there is a characteristic rate of evolution in small subunit rRNA genes (Ochmann et al. 1999; Vreeland and Rosenzweig 2002). However, there is doubt regarding the validity of assuming a universal molecular clock of sequence evolution, as rates differ between bacterial taxa, and it may be unrealistic in regard to native species of environments such as permafrost that are subjected to low nutrient levels, extremely low temperatures, and long microbial doubling times (Vreeland and Rosenzweig 2002). In addition, recent studies demonstrating microbial activity in permafrost samples at ambient subzero temperatures (Steven et al. 2006; see Chap. 9) further complicate the determination of the age of microorganisms isolated from permafrost. These findings suggest that at least a subpopulation of the permafrost microbial community may constitute an active modern microbial ecosystem rather than “ancient” frozen microbial survivors.

5.7 Conclusion

Both culture-dependent and culture-independent methods have revealed that permafrost harbors diverse and novel microbial communities. The future challenge for the study of permafrost microbiology is to begin to address the ecology of these

unique microbial ecosystems. The knowledge gained from culture-independent surveys of microbial diversity can be used to design targeted culturing strategies in order to determine if phylogenetic groups detected by molecular strategies are part of the viable microbial community. Moreover, the characterization of the microbial component of permafrost will provide important insights into how these environments will respond to climate change in regard to the increased metabolic rates associated with higher temperatures and nutrient availability due to the melting of permafrost. The application of technologies such as stable isotope probing (Dumont et al. 2006) and FISH-microautoradiography (Lee et al. 1999) could identify active microorganisms, and better define the functioning and maintenance of permafrost microbial ecosystems at ambient subzero temperatures. As microbial activities in situ are expected to be extremely slow and minute, new methods and technologies specific to the permafrost environment will be required. For example, we have recently described a method to measure microbial respiration at subzero temperatures that was effective at detecting low amounts of microbial respiration occurring at temperatures as low as −15°C from a variety of Arctic environments (Steven et al. 2007b). Developing methods to detect and characterize the active *Bacteria* and *Archaea* in permafrost will allow for the differentiation of the active microbial populations presumed to exist in permafrost from cryopreserved microbial fossils that may have remained frozen for geological time scales.

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Chapter 6
Viable Cyanobacteria and Green Algae from the Permafrost Darkness

Tatiana A. Vishnivetskaya

6.1 Introduction

Photosynthetic organisms, i.e., plants, algae, cyanobacteria and photosynthetic bacteria, have developed efficient systems to harvest the light of the sun and to use the light energy to drive their metabolic reactions, such as the reduction of carbon dioxide to sugar. It is through photosynthesis that Earth's biosphere derives its energy from sunlight. On the other hands, cyanobacteria are the most ancient oxygen-releasing photosynthetic organisms on the Earth. The stromatolite fossils and carbon isotope ratios confirm that autotrophs fixing carbon via the Calvin cycle must have existed for 3.5 billion years (Schopf and Packer 1987). The characteristic fossil structures formed by cyanobacteria were discovered on the Precambrian rocks and, probably, on meteorites (Zhmur et al. 1999; Boyd 2001). There is also an opinion that green algae were originated from symbiosis of cyanobacteria and a non-photosynthetic eukaryotic ancestor (Margulis 1993; Douglas 1998), the origin of photosynthetic eukaryotes that gave rise to the first alga having occurred 1.5 billion years (Yoon et al. 2004). Early algae probably gave rise to multicellular plants (Graham 1996).

Photoautotrophic microorganisms live mostly in aquatic environments, but some unicellular and filamentous algae and cyanobacteria dwell in moist soils; others join with fungi to form lichens. A number of microscopic algae and cyanobacteria inhabit different extreme environments, such as cold waters and ice, hot springs and geysers, acid ponds or salt waters, dry hot and cold deserts. A description of diverse communities of microalgae and cyanobacteria in cold habitats such as the Arctic and Antarctic lakes, rivers, seas, sea ice, glaciers, cold soils may be found elsewhere (Malone et al. 1973; Friedmann and Ocampo 1977; Sinclair and Ghiorse 1989; Getsen 1990; El-Sayed and Fryxell 1993; Nienow and Friedmann 1993; Palmisano and Garrison 1993; Vincent et al. 1993a, b; Abyzov et al. 1998; Priscu et al. 1998; Willerslev et al. 1999; Comte et al. 2007).

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6.2 Cyanobacteria and Green Algae from Permafrost Environments

6.2.1 Permafrost

Permafrost is defined as a subsurface frozen layer, primarily soil or rock, which remains frozen for more than 2 years. The age of permafrost ranges from a few thousand years up to 2–3 million years and even older in Antarctica. Permafrost makes up more than 20% of the land surface of the Earth, including 82% of Alaska, 50% of Russia and Canada, 20% of China, and most of the surface of Antarctica (Harris 1986; Williams and Smith 1989). Permafrost underlies the glaciers and soils of polar and alpine regions. Permafrost soils contain about 20–70% of ice and 1–7% of unfrozen water in the form of salt solutions with low water activity (a_w = 0.85) (Gilichinsky et al. 1993). Since life depends upon liquid water, permafrost is one of the most extreme environments on the Earth. In addition, permafrost is characterised by constant negative temperature, inaccessibility of nutrient supplies, and complete darkness. It is surprising to discover photoautotrophic microorganisms which need to use light energy to drive their metabolic reactions within permafrost sediments. Because of the difficulty of studying permafrost in an undisturbed form, interactions among the organisms that live in it are not yet well understood.

6.2.1.1 Arctic Permafrost

The study sites have been located on Kolyma lowland, Northeast Russia (67–70°N, 152–162°E). The Arctic permafrost represents an anaerobic oligotrophic environment with a mean annual temperature of −10°C, redox potential Eh = +40 to −250), and an organic carbon content in the range 0.05–7% (Gilichinsky 2002). Nitrogen in form of NH^{4+}, NO^{2-}, or NO^{3-} was determined (Janssen and Bock 1994). A total of 293 permafrost samples differentiated in lithology, genesis, and physico-chemical properties were screened for the presence of photosynthetic microorganisms. The distances between boreholes ranged from 50 to 300 km. The deepest sample was from a depth of 61 m, and the oldest sample was 3 million years old. The permafrost samples were hydrocarbonate-calcium fresh composition with a low salinity and neutral pH, and of marine origin with significantly higher salinity and dominance of ions Na^+ and Cl^-.

6.2.1.2 Antarctic Permafrost

The study areas have been located in the McMurdo Dry Valleys of Southern Victoria Land, Antarctica (77–78°S, 160–163°E). The temperature of the Antarctic permafrost varies from −18.5°C (Taylor Valley) through −24°C (Beacon Valley) to −27°C (Mt. Feather) (Gilichinsky et al. 2007b). The Antarctic permafrost is of

fresh-water genesis, with the alkaline pH, low clay content and organic matter often close to zero (0.05–0.25%) (Wilson et al. 1996). Since the Antarctic permafrost has a low buffering capacity, the soil pH is sensitive to the total accumulation of soil salts (Campbell and Claridge 1987). A total of 56 permafrost samples were analysed for presence of cyanobacteria and green algae. The Antarctic samples were not so anaerobic (redox potential Eh = + 260 to + 480), and the gaseous phase contained oxygen, nitrogen, methane, carbon dioxide, etc. (Rivkina and Gilichinsky 1996; Wilson et al. 1996).

6.2.2 Permafrost Sample Collection

The permafrost samples were obtained by slow rotary drilling without the use of any drilling solutions between 1991 and 1999. Evaluation of the aseptic sampling methods and contamination controls was done (Khlebnikova et al. 1990; Juck et al. 2005). The surface of extracted frozen core was trimmed away with a sterile knife, then immediately divided into sections of 5 cm long, placed in presterilized aluminum tins, sealed, and placed in frozen storage. All samples remained frozen throughout this process and during transport. In the laboratory, frozen samples were fractured in a class II positive-flow hood with a sterile knife, and only sections internal to the core were taken for microbiological analysis using sterile forceps (Shi et al. 1997; Rivkina et al. 1998).

6.2.3 Isolation and Identification

For isolation of photoautotrophic microorganisms, prolonged enrichments (8–18 weeks) of thawed but otherwise undisturbed permafrost samples under continuous illumination (1,000 lx) were applied. The enrichment cultures in BG11 (Rippka 1988), Bristol (Gollerbakh and Shtina 1969), BBM (Brown and Bold 1964) media were incubated at 4 and 20°C. Enrichments were re-examined weekly to document biodiversity (Table 6.1).

Isolates were initially examined by measuring of the fluorescence excitation spectra at 686 nm (Vishnivetskaya et al. 2001). Identification of algae and cyanobacteria was based on morphological (Komarenko and Vasil'eva 1978; Rippka et al. 1979; Andreeva 1998) and phylogenetic (Nubel et al. 1997; Krienitz et al. 2003) criteria. DNA was extracted from cyanobacteria (Smoker and Barnum 1988) and green algae (Fawley and Fawley 2004). Bacteria-specific (8F and 1492R) (Weisburg et al. 1991) and cyanobacteria-specific (CYA106F and CYA781R) (Nubel et al. 1997) primers were used to amplify 16S rRNA gene from cyanobacteria. The 18S rRNA gene from the green algae was amplified with primers NS1 and 18L (Gilichinsky et al. 2007b).

Table 6.1 The observation frequency of viable bacteria, cyanobacteria and green algae within Siberian permafrost

Sediment	Age (years)	Observation frequency[a] (%)		
		Bacteria	Cyanobacteria	Green algae
Lake-swamp loam	Holocene (1,000–10,000)	91	17	50
Alluvium sandy loam	Late Pleistocene (20,000–30,000)	80	9	18
Channel-fill sands	Late Pleistocene (20,000–30,000)	40	0	0
Marine (littoral) sands	Middle Pleistocene (100,000–200,000)	40	0	0
Lake-alluvium loam and sandy loam	Middle Pleistocene (200,000–600,000)	90	8	39
Lake-alluvium loam and sandy loam	Late Pliocene-early Pleistocene (0.6–1.8 millions)	38	6	15
Lake-alluvium loam and sandy loam	Late Pliocene-early Pleistocene (2–3 millions)	44	13	9

[a]Two hundred and ninety three Siberian permafrost samples were studied; the observation frequency is expressed as a percentage of samples with viable microorganisms

6.2.4 Cyanobacteria

Thirty viable non-axenic cyanobacterial strains were isolated from 28 Siberian permafrost cores. Filamentous heterocystous (Nostocales) and non-heterocystous (Oscillatoriales) cyanobacteria were recovered (Vishnivetskaya et al. 2001). The 16S rRNA genes from representative strains of each order were sequenced. Seven out of eight strains of the order Oscillatoriales were close to each other and to *Leptolyngbya* with identity 80–95.8%, and one strain was closely related to *Microcoleus* with identity 96.8% (Fig. 6.1). The phylogenetic analyses were confirmed by studying the morphological features of the isolates. Cyanobacteria of the Oscillatoria-Leptolyngbya group, with narrow straight uniseriate trichomes, were often isolated from both young and old permafrost sediments. The *Microcoleus*-like strain 195A20 grew at both 27°C and 4°C, with a doubling time of 20 h at 24°C. The strain 195A20 showed morphological plasticity with respect to growth temperature, trichomes usually being shorter and wider at 27°C than at low temperature (Vishnivetskaya et al. 2003). According to the 16S rRNA analysis, three cyanobacterial strains had close relatives within the order Nostocales (Fig. 6.1). Viable strains of the *Nostoc* and *Anabaena* formed heterocysts in the absence of a combined nitrogen source, and were characterized by different phycoerythrin/phycocyanin ratios depending on nitrogen source and light wavelength (Erokhina et al. 1999, 2000; Vishnivetskaya et al. 2001). Viable cyanobacteria were dominated by

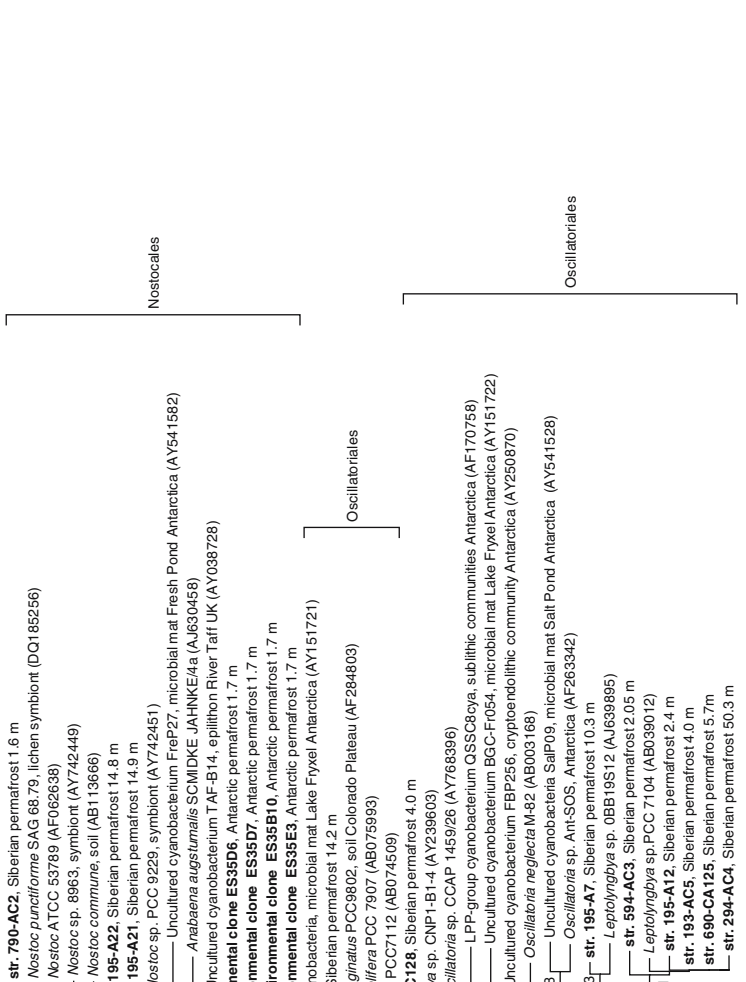

Fig. 6.1 Phylogenetic relationship of cyanobacterial isolates and environmental clones derived from Siberian and Antarctic permafrost (the phylogenetic tree was adopted from Gilichinsky et al. 2007a). Tree was produced by the neighbor-joining method (Saitou and Nei 1987). Bootstrap values, expressed as percentages of 100 replications, higher than 40% are shown. Sequences were deposited in GenBank

non-heterocystous filamentous cyanobacteria of the order Oscillatoriales. While no viable cyanobacteria were detected in any of 56 Antarctic permafrost samples, a few 16S rRNA cyanobacterial environmental clones were obtained from the total community genomic DNA extracted from Antarctic permafrost of depth 1.7 m (Gilichinsky et al. 2007b). The phylogenetic analyses of the environmental clones and isolates obtained from the permafrost samples of both Polar Regions did not show any matches. Nine environmental clones were affiliated with the genus *Anabaena*, and they were closely related to an uncultured cyanobacterium found in river epilithon (O'Sullivan et al. 2002). We have found that viable permafrost cyanobacteria were closely related to strains and more often to uncultured cyanobacterial clones derived from a microbial mat or cryptoendolithic communities in Antarctica (Gilichinsky et al. 2007b).

6.2.5 Green Algae

Viable green algae were widely distributed in Siberian permafrost and were detected in 76 out of 293 permafrost cores. A total of 106 strains of green algae were isolated, and half of them, small non-motile globular cells, were identified as *Chlorella* spp. (Vishnivetskaya et al. 2001, 2005). Along with *Chlorella* spp., the species *Chlorella vulgaris* and *Chlorella sacchorophilla* and the genera *Mychonastes* sp., *Pseudococcomyxa* sp., *Chodatia* sp. (*Chodatia tetrallontoidea), Stichococcus* sp., *Chlorococcum* sp., *Scotiellopsis* sp. were identified using morphological criteria (Komarenko and Vasil'eva 1978; Andreeva 1998). Only three strains of green algae, classified as *Chlorella* sp., *Mychonastes* sp., *Chlorococcum* sp., were found in borehole 1/99 located in Beacon Valley, Antarctica (Gilichinsky et al. 2007b). These green algae were isolated from a permafrost layer sandwiched between buried ice horizons at depths of 14.1–14.8 m.

The 18S rRNA gene sequences of the viable green algae from Siberian (six strains) and Antarctic (three strains) permafrost were analyzed (Fig. 6.2). Among unicellular green algae were representatives of the genera *Nannochloris, Chlorella* (both in the order Chlorellales), *Stichococcus* (order Microthamniales), and *Paradoxia* (uncertain position) within Trebouxiophyceae. We found that two isolates from Siberian permafrost and three isolates from Antarctic permafrost were closely related to each other and to *Nannochloris* sp. JL4–6 (99%) and *Chlorella protothecoides* (97.8%).

Thus, the algae isolated from subsurface permafrost sediments had previously characterized relatives from cold environments, mostly from Antarctica. Members of the Chlorellaceae family, which consists of unicellular coccoid algae with simple morphology and small size, are widespread in Antarctic cold freshwater environments and cryptoendolitic communities (Friedmann and Ocampo-Friedmann 1976; Friedmann 1982; Wynn-Williams 1990; Vincent et al. 1993b; Vishniac 1993).

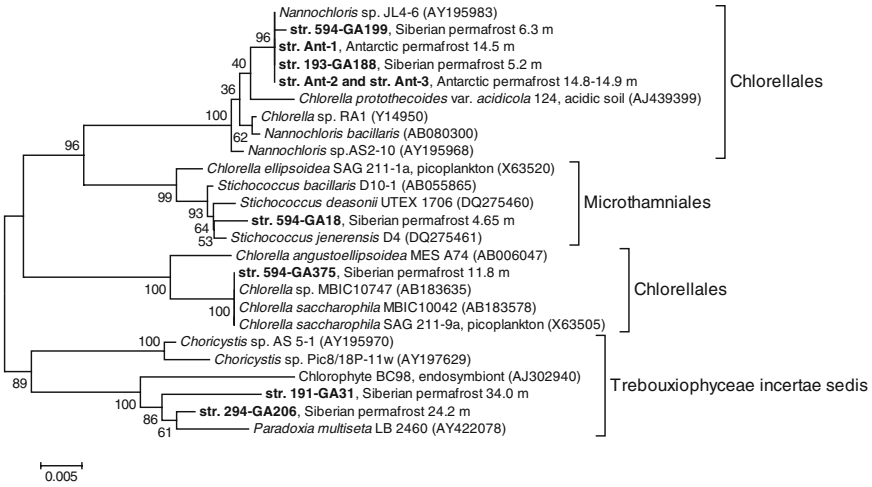

Fig. 6.2 Phylogenetic relationship of green algae isolated from Siberian and Antarctic permafrost. Tree was created as described in Fig. 6.1. Sequences were deposited in GenBank

6.3 Life in Dark and Cold Ecosystems

While the mechanisms which protect bacteria against the adverse conditions that include oxidation, cooling, high osmolarity/dehydration and starvation are well studied, our knowledge about adaptive and survival mechanisms of photoautotrophic microorganisms in cold and dark ecosystems such as permafrost remains limited. Obviously the upper soil and permafrost layers prevent photosynthetic activity of any chlorophyll-containing organisms. However, green algae and cyanobacteria do survive in the permafrost (Table 6.2). We have suggested that the permafrost algae survive in the deep dark permafrost sediments below freezing point for thousands and up to millions of years in the dormant or resting state (Vishnivetskaya et al. 2001). Permafrost photoautotrophic microorganisms endure the long-term impact of cold and darkness but they are readily reversible to proliferation and they do not lose the capability for photosynthesis (Vishnivetskaya et al. 2003). We have shown that isolates of the genus *Chlorella* grew on solid nutrient media at the dark (Vishnivetskaya et al. 2005). Recent studies have shown that contemporary unicellular algae possess the ability for heterotrophic growth as a mechanism for survival. For example, *Chlamydomonas* exhibited a remarkable resistance to starvation in the dark (Tittel et al. 2005); the marine dinoflagellate *Fragilidium subglobosum* was capable of phototrophic growth as well as of heterotrophic (phagotrophic) growth in the dark (Skovgaard 1996); unicellular green algae (*Oocystis* sp.) and cyanobacteria (*Xenococcus* sp.) were isolated from drinking water systems, and they demonstrated the ability to grow in the dark as a consequence of their heterotrophic metabolism (Codony et al. 2003).

Table 6.2 List of the viable cyanobacteria and green algae discovered in the permafrost

Arctic (Kolyma lowland, Northeast Russia)	**Antarctica** (Dry Valleys)
Green algae	
Chlorella sp. *Chlore vulgaris* *Chlorella sacchorophilla* *Chlorococcum* sp. *Chodatia* sp. *Chodatia tetrallontoidea* *Mychonastes* sp. *Nannochloris* sp. *Paradoxia* sp. *Pseudococcomyxa* sp. *Scotiellopsis* sp. *Stichococcus* sp.	*Chlorella* sp. *Chlorococcum* sp. *Mychonastes* sp.
Cyanobacteria	
Anabaena sp. *Leptolyngbya* sp. *Microcoleus* sp. *Nostoc* sp. *Oscillatoria* sp. *Phormidium* sp.	No

Our observations have shown that the appearance, morphology and growth rate of ancient permafrost algae did not differ significantly from the findings on contemporary algae from cold regions. The viable permafrost green algae grew at 27, 20 and 4°C, but cyanobacteria had good growth at room temperature only (Vishnivetskaya et al. 2003). Algae had a low growth rate, with a doubling time of 10–14 days. Rise in nitrogen, phosphorus or CO_2 concentrations did not affect the growth rate. On the other hand, the growth of the *Nostoc* sp. was completely inhibited by ammonium chloride or ferric ammonium citrate (Erokhina et al. 1999). The sources of organic ammonium such as Na-glutamine, asparagine or glycine led to the reduction of heterocysts and the development of akinetes (resistant resting cells) (Vishnivetskaya et al. 2003).

The content and composition of photosynthetic pigments in the cells of the ancient cyanobacteria and green algae based on their absorption spectra, the second-derivative absorption spectra, were studied (Erokhina et al. 1998, 2004). Comparative analysis of the absorption spectra of the Siberian permafrost cyanobacteria *Oscillatoria* sp., *Phormidium* sp., *Nostoc* sp., and *Anabaena* sp. revealed the presence of chlorophyll a, phycobiliproteins, and carotenoids in their cells (Erokhina et al. 1998). Spectral analyses of the Antarctic permafrost green algae *Chlorococcum* sp. and *Chlorella* sp. showed the presence of a low content of chlorophyll *a*, a high relative content of chlorophyll *b*, and complex composition of carotenoids (Erokhina et al. 2004; Gilichinsky et al. 2007b). The ability of *Nostoc* sp., and *Anabaena* sp. to form numerous heterocysts when grown on nitrogen-free medium, and the presence of C-phycoerythrin,

suggested that they were capable of nitrogen fixation (Erokhina et al. 1999; Vishnivetskaya et al. 2001). The permafrost nitrogen-fixing cyanobacteria were capable of complementary chromatic adaptation, which involves the regulation of the synthesis of the photosynthetic pigments, C-phycoerythrin and phycocyanin, by red or green light (Erokhina et al. 2000; Vishnivetskaya et al. 2005).

In nature, algae inhabiting surface layers of cold regions show high resistance to the temperature fluctuations which are caused by repetitive phase transitions of water through the freezing point. Deep freezing (−40°C, −100°C, −196°C) and desiccation, laboratory-tested on cyanobacterial and algal strains from maritime and continental Antarctica, caused little harm to cyanobacteria, but was fatal for more than 50% of the population of algae (Sabacka and Elster 2006). But how would permafrost microalgae conduct themselves in such a situation? The fact that algae have been recovered from permanently frozen sediments may suggest the resistance of algae to both primary and long-term freezing. The most critical steps where cells may receive injuries are the primary freezing and the thawing. The permafrost samples with relatively high algal biomass and numerous cultivable green algae units were exposed to repeated freeze–thaw cycles. During the experiments, it was shown that permafrost algae themselves could survive the stresses associated with transition through the freezing point. It appears that freezing induces the formation of protective envelopes and resting cells, and as a result the permafrost algae withstand dehydration and long-term inactivity (Vishnivetskaya et al. 2003).

6.4 Conclusion

The discovery of photoautotrophic microorganisms in permafrost is surprising, not only because of the constant subzero temperature and complete darkness of the sediments, but also because of the length of time the sediments have been frozen. These organisms may well be the only living photoautotrophs that have survived for a geologically significant period of time. These cyanobacteria and green algae inhabiting such an absolutely extreme environment exist “on the edge”, near the absolute limits of their physiological potential. Therefore, permafrost cyanobacteria and green algae represent unique material for research on evolution and low-temperature adaptation, and they defiantly possess unique mechanisms that allow them to maintain viability for very long periods of time.

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Chapter 7
Fungi in Permafrost

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7.1 Introduction

In this review, we analyze data on the occurrence of fungi in Arctic permafrost of different ages. Antarctic habitats of fungi are beyond the scope of this chapter, because a database of non-lichenized fungi from Antarctica has been created in the United Kingdom (http://www.antarctica.ac.uk/bas_research/data/access/fungi/Speciespublic2.html#Use; version 2.1.4; February 2007), and lists of fungal species identified in this region have been published (Vishniac 1993; Azmi and Seppelt 1998; Tosi et al. 2002; Onofri et al. 2005; Selbmann et al. 2005; Ruisi et al. 2007), including novel species (McRae et al. 1999; Sonjak 2007), whereas data on fungi in subsurface Antarctic horizons are very rare (Kochkina et al. 2001; Gilichinsky et al. 2007).

Arctic fungi have been the subject of meticulous studies for a long time. Mycologists focus on assembling an inventory, which would cover the taxonomic diversity of fungi inhabiting eternal ice (Gunde-Cimmerman et al. 2003; Sonjak et al. 2006), superficial horizons of Arctic landscapes of various locations (Zabawski 1982; Bab'eva and Sizova 1983; Bergero et al. 1999; Kirtsidely 1999a, b, 2001, 2002; Chernov 2002; Etienne 2002; Callaghan 2005; Kurek et al. 2007), and plant substrates (Karatygin et al. 1999). The mycobiota of Arctic permafrost have been studied over the last decade (Kochkina et al. 2001; Ozerskaya et al. 2004; Gilichinsky et al. 2005; Panikov and Sizova 2007).

Permafrost fungi are studied by culture-dependent and culture-independent methods. The limitations of microbiological techniques are due to the fact that many microorganisms actively developing in nature cannot be cultured in artificial culture media under laboratory conditions. In this context, it remains largely unknown whether the picture derived from experimental studies of the structure of a microbial community is complete, if at all. Nevertheless, the use of microbiological methods makes it possible to successfully characterize permafrost samples and their culturable microbial communities.

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In addition, such studies may result in the assembly of collections of unique microorganisms, which in turn allows the performance of various screening tests, pertaining to diverse problems and requests of biotechnology. At present, many fungal strains isolated from low-temperature habitats are kept in a number of mycological collections (e.g., CBS, ATCC, etc.). There are also specialized collections of such fungi. CCFEE (Culture Collection of Fungi from Extreme Environments) is a specialized mycological collection preserving the biodiversity of Antarctic fungi (Onofri et al. 2005). All the collections mentioned above maintain fungal strains isolated preferentially from Antarctic samples. A specialized collection of fungi isolated largely from Arctic permafrost (600 strains belonging to 112 species of 44 genera) has been assembled for the first time as part of the All-Russian Collection of Microorganisms (VKM). This specialized collection also includes about 80 strains of sterile mycelium, which cannot be identified using cultural and morphological methods; research on their molecular–biological identification is on the way.

The available data on permafrost fungi cover several aspects:

- Number of colony-forming units (CFUs)
- Taxonomic diversity
- Morphological, physiological, and biochemical characteristics of the cultures isolated, which enable the fungi to retain viability or capacity for development under the conditions of permafrost.

7.2 Amount of Fungi

Much attention has been given to questions of determining the size of the fungal population in permafrost samples. There are data on the fungi of North-West Territories (Canada), Alaska, and Russia. The age, depth, and chemical and textural composition of the samples varied considerably, due to the fact that samples were taken by different expeditions, each having its own set of goals and objectives. In general, however, the data on the fungal amount pertain to superficial Arctic horizons.

Recent studies (Kochkina et al. 2001; Ozerskaya et al. 2008) made it possible to derive a generalized picture of the amount of fungi in samples of differing age. There is almost no relation between the amount of fungi and the depth and age of permafrost; in samples of modern soil profiles, the existence of such a relation is judged from changes in the number of CFUs (Widden and Parkinson 1973; Soderstrom 1975; Mirchink 1988). Populations of fungi in permafrost samples are microfocal, in that increased numbers of CFUs may be detected in any portion of the sample, regardless of the depth or age of the sediments. This is the reason why the fungal amount varies over the range of four orders of magnitude, from less than 10 to almost 100,000 CFUs g^{-1} material. It is important to note that the peaks of numbers in individual foci are not paralleled by increased diversity of fungal species. Conversely, the ratio of the number of species to the total amount of fungal colonies (index of abundance; Odum 1971) in such cases dramatically decreases and tends to zero.

7.3 Taxonomic Diversity

Species diversity in eukaryotes, including fungi, inhabiting permafrost horizons, collected in the Arctic, has been the subject of intense research over the last decade (Dmitriev et al. 1997; Kochkina et al. 2001; Vishnivetskaya et al. 2003). However, the complete list of the fungi detected has not been reported. The published results are summed up in Table 7.1, which lists mycelial fungi detected in permafrost horizons of the Northern hemisphere. To make the picture complete, we also indicate reports on soils of Arctic tundra, in which the same fungal species can be found as in deep horizons of Arctic habitats. Table 7.1 shows that the fungi of Arctic permafrost exhibit considerable taxonomic diversity. Analysis of the occurrence frequencies of fungal species demonstrated that *Geomyces pannorum, Cladosporium* spp., and *Aspergillus* spp. were the most common. The genus *Penicillium* was represented by the greatest number of species. Interestingly, species occurring most frequently in deep horizons were also isolated from modern Arctic soils in the course of long-term studies.

Table 7.1 Fungal biodiversity in Arctic permafrost[a]

Species	References
Acremonium pteridii W. Gams et Frankland	Ozerskaya et al. (2008)
A. salmoneum W.Gams et Lodha	Ozerskaya et al. (2008)
Alternaria alternata (Fries) Keissler	Kirtsidely (1999a), Ivanushkina et al. (2005), Kurek et al. (2007)
Arthrinium sphaerospermum Fuckel	Ivanushkina et al. (2005)
Aspergillus fumigatus Fresen.	Ivanushkina et al. (2005)
A. niger van Tiegh.	Kirtsidely (1999a), Etienne (2002), Ivanushkina et al. (2005, 2007)
A. oryzae (Ahlb.) E.Cohn	Ivanushkina et al. (2005)
A. sclerotiorum G.A. Huber	Stakhov et al. (2008)
A. sydowii (Bainier et Sattory) Thom et Church	Ivanushkina et al. (2005, 2007)
A. versicolor (Vuill.) Tirab.	Zabawski (1982), Ivanushkina et al. (2005, 2007), Kurek et al. (2007)
Aureobasidium pullulans (de Bary) G. Arnaud var. *pullulans*	Ivanushkina et al. (2005), Kirtsidely (1999a)
A. pullulans (de Bary) G. Arnaud var. *melanogenum* Herm.-Nijh.	Ozerskaya et al. (2008)
Bispora antennata (Pers.) E.W. Mason	Ivanushkina et al. (2005)
Botrytis cinerea Pers.	Ivanushkina et al. (2005), Kurek et al. (2007)
Chaetomium globosum Kunze	Zabawski (1982), Ivanushkina et al. (2005)
C.indicum Corda	Ivanushkina et al. (2005)
Chaetophoma sp.	Ivanushkina et al. (2005)
Chrysosporium merdarium (Ehrenb.) J.W. Carmich	Ozerskaya et al. (2008)
Cladosporium cladosporioides (Fres.) G.A. de Vries	Cooke and Fournelle (1960), Zabawski (1982), Kirtsidely (1999a), Ivanushkina et al. (2005), Kurek et al. (2007)

(continued)

Table 7.1 (continued)

Species	References
C. herbarum (Pers.) Link	Zabawski (1982), Ivanushkina et al. (2005), Kurek et al. (2007)
C. macrocarpum Preuss	Ivanushkina et al. (2005)
C. sphaerospermum Penz.	Ivanushkina et al. (2005), Stakhov et al. (2008)
Engyodontium album (Limber) de Hoog	Ivanushkina et al. (2005)
Eurotium amstelodami L. Mangin	Ozerskaya et al. (2008)
E. herbariorum (F.H. Wigg.) Link	Ozerskaya et al. (2008)
E. rubrum W. Bremer	Ivanushkina et al. (2005)
Exophiala jeanselmei (Langeron) var. *heteromorpha* (Nannf.) de Hoog	Ivanushkina et al. (2007) (Langeron) McGinnis et A.A. Padhge
Fusarium oxysporum Schltdl.	Cooke and Fournelle (1960), Kirtsidely (1999a), Ivanushkina et al. (2005)
F. solani (Mart.) Sacc.	Ozerskaya et al. (2008)
Geotrichum candidum Link	Ivanushkina et al. (2005)
Geomyces pannorum (Link) Sigler et J.W. Carmichael	Kirtsidely (1999a), Etienne (2002), Ivanushkina et al. (2005), Kurek et al. (2007), Stakhov et al. (2008)
G.vinaceus Dal Vesco	Ozerskaya et al. (2008)
Gliocladium sp.	Ozerskaya et al. (2008)
Lecythophora mutabilis (J.F.H. Beyma) W.Gams et McGinnis	Ozerskaya et al. (2008)
Malbranchea pulchella Sacc. et Penz.	Ozerskaya et al. (2008)
Monodictys glauca (Cooke et Harkn.) S. Hughes	Ivanushkina et al. (2005)
Mucor plumbeus Bonord.	Ivanushkina et al. (2005)
Paecilomyces variotii Bainier	Ivanushkina et al. (2005)
Penicillium aurantiogriseum Dierckx	Zabawski (1982), Kirtsidely (1999a, b), Ivanushkina et al. (2005, 2007), Kurek et al. (2007), Stakhov et al. (2008)
P. brevicompactum Dierckx	Zabawski (1982), Kirtsidely (1999a), Etienne (2002), Ivanushkina et al. (2005)
P. chrysogenum Thom	Zabawski (1982), Kirtsidely (1999a), Ivanushkina et al. (2005, 2007), Kurek et al. (2007)
P. citrinum Thom	Ivanushkina et al. (2005)
P. crustosum Thom	Ivanushkina et al. (2005)
P. decumbens Thom	Ivanushkina et al. (2005)
P. glabrum (Wehmer) Westling	Zabawski (1982), Kirtsidely (1999a, b, 2001), Ivanushkina et al. (2005)
P. granulatum Bainier	Zabawski (1982), Kirtsidely (2001), Ozerskaya et al. (2008), Stakhov et al. (2008)
P. griseofulvum Dierckx	Ozerskaya et al. (2008)
P. melinii Thom	Ivanushkina et al. (2007)
P. miczynskii K.M. Zalessky	Kirtsidely (1999a, b), Ivanushkina et al. (2007)
P. minioluteum Dierckx	Ivanushkina et al. (2005, 2007)
P. puberulum Bainier	Ivanushkina et al. (2005)
P. purpurogenum Stoll	Kirtsidely (1999a), Ivanushkina et al. (2005)

(continued)

Table 7.1 (continued)

Species	References
P. restrictum J.C. Gilman et E.V. Abbott	Ivanushkina et al. (2005)
P. rugulosum Thom	Ivanushkina et al. (2005)
P. simplicissimum (Oudem.) Thom	Zabawski (1982), Kirtsidely (1999a), Ozerskaya et al. (2008)
P. variabile Sopp	Ivanushkina et al. (2005)
P. verrucosum Dierckx	Ivanushkina et al. (2005, 2007)
P. viridicatum Westling	Ivanushkina et al. (2007)
Papulaspora sp.	Ivanushkina et al. (2005)
Phialophora fastigiata (Lagerb. et Melin) Conant	Zabawski (1982), Kurek et al. (2007), Stakhov et al. (2008)
P. melinii (Nannf.) Conant	Ozerskaya et al. (2008)
Phoma crystallifera Gruyter, Noordel. et Boerema	Stakhov et al. (2008)
Ph. destructive Plowr.	Ozerskaya et al. (2008)
Ph. jolyana Piroz. et Morgan-Jones var. *jolyana*	Ozerskaya et al. (2008)
Ph. herbarum Westend.	Stakhov et al. (2008)
Ph. nebulosa (Pers.) Berk.	Ozerskaya et al. (2008), Stakhov et al. (2008)
Rhinocladiella atrovirens Nannf.	Ivanushkina et al. (2005)
Scopulariopsis candida (Guég.) Vuill.	Ivanushkina et al. (2007)
Stachybotrys chartarum (Ehrenb.) S.Hughes	Ivanushkina et al. (2005)
Sphaeronaemella mougeotii (Fr.) Sacc.	Ivanushkina et al. (2007)
Sporotrichum pruinosum J.C. Gilman et E.V. Abbott	Ivanushkina et al. (2005)
Thysanophora penicillioides (Roum.) W.B. Kendr.	Ozerskaya et al. (2008)
Trichoderma longibrachiatum Rifai	Ivanushkina et al. (2005)
Ulocladium atrum Preuss	Ozerskaya et al. (2008)
U. botrytis Preuss	Ivanushkina et al. (2005)
Valsa sordida Nitschke	Ozerskaya et al. (2008)
Verticillium sp.	Ivanushkina et al. (2005)
Xylohypha nigrescens (Pers.) E.W. Mason	Ozerskaya et al. (2008)
Mycelia sterile (white; dark; dark with sclerotia)	Zabawski (1982), Kirtsidely (1999a, b); Kurek et al. (2007), Ozerskaya et al. (2008), Stakhov et al. (2008)

[a]Fungi from superficial horizons of Arctic landscapes of various locations were studied in the following articles: Cooke and Fournelle (1960), Zabawski (1982), Kirtsidely (1999a), Kirtsidely (1999b), Etienne (2002), Kirtsidely (2001), Kurek et al. (2007)

We attempted to compare parameters characterizing the diversity of mycelial fungi from Arctic permafrost, which were determined by (1) the conventional technique of inoculating solid nutritive media with aqueous suspensions of the specimens, and (2) the method of analyzing DNA isolated directly from the same specimens (DNA identification was performed using the database of nucleic acid sequences GenBank; Lydolph et al. 2005). In comparing the two approaches, we used samples (comprising permafrost sediments of Holocene and late Pleistocene) collected in the eastern Arctic. Only at the level of families (or higher taxa) was it possible to compare the

taxonomic diversity parameters of the fungi, because the method of direct DNA isolation did not favor precise species assignment (Table 7.2).

The results of our comparative analysis made it possible to assess the concordance in species composition between the isolated complexes of mycelial fungi; for this, we used the Sorensen index of similarity, the values of which were at the level of 40%. The number of higher taxa isolated by plating was slightly lower, which

Table 7.2 Two methods of study of fungal biodiversity (*DNA* culture-independent method; *Plate* culture-dependent method)

Class	Subclass	Order	Family	Method	
Ascomycetes	Dothideomycetidae	Dothideales	Dothioraceae	**DNA**	**Plate**
			Incertae sedis	–	Plate
		Incertae sedis	Pseudoperisporiaceae	DNA	–
		Mycosphaerellales	Mycosphaerellaceae	**DNA**	**Plate**
		Pleosporales	Incertae sedis	–	Plate
			Leptosphaeriaceae	DNA	–
			Pleosporaceae	**DNA**	**Plate**
			Sporormiaceae	DNA	–
	Erysiphomycetidae	Erysiphales	Erysiphaceae	DNA	–
		Eurotiales	Trichocomaceae	**DNA**	**Plate**
	Incertae sedis	Incertae sedis	Incertae sedis	**DNA**	**Plate**
			Myxotrichaceae	–	Plate
			Pseudeurotiaceae	–	Plate
	Leotiomycetidae	Helotiales	Dermateaceae	–	Plate
			Helotiaceae	DNA	–
			Hyaloscyphaceae	DNA	–
			Sclerotiniaceae	**DNA**	**Plate**
		Rhytismatales	Rhytismataceae	DNA	–
	Sordariomycetidae	Hypocreales	Hypocreaceae	–	Plate
			Incertae sedis	–	Plate
			Nectriaceae	–	Plate
		Sordariales	Chaetomiaceae	DNA	–
			Sordariaceae	DNA	–
Basidiomycetes	Agaricomycetidae	Polyporales	Corticiaceae	DNA	–
			Cyphellaceae	DNA	–
			Fomitopsidaceae	DNA	–
			Phanerochaetaceae	DNA	–
Coelomycetes	Incertae sedis	Incertae sedis	Incertae sedis	–	Plate
Saccharomycetes	Saccharomycetidae	Saccharomycetales	Incertae sedis	**DNA**	**Plate**
Urediniomycetes	Incertae sedis	Uredinales	Melampsoraceae	DNA	–
Zygomycetes	Incertae sedis	Basidiobolales	Basidiobolaceae	DNA	–
		Mortierellales	Mortierellaceae	**DNA**	**Plate**
		Mucorales	Mucoraceae	–	Plate

may be due to problems of identification of sterile mycelium. Its identification by molecular–biological techniques may well show that higher fungi are much more abundant in permafrost than is considered to be case today.

7.4 Morphological, Physiological, and Biochemical Characteristics of Permafrost Fungi

Table 7.1 shows that the overwhelming majority of fungi isolated from permafrost strata form small unicellular conidia (e.g., *Aspergillus* spp., *Chrysosporium* spp., *Penicillium* spp., *Phialophora* spp.). Experience of successful cryopreservation of collection cultures demonstrates that fungi with small spores are better adapted to long-time preservation than fungi with other types of spores. Representatives of genera of which a characteristic is the ability to form large multicellular spores (*Alternaria, Bispora, Monodictys, Ulocladium*, etc.) contain melanin within cell wall components; this compound is widely known as a protectant against the impact of extreme temperatures (contrary to prior belief that it attenuates adverse effects of exposure to UV radiation) (Sterflinger 1998; Robinson 2001; Rosas and Casadevall 2001). Note that more than 60% melanin-containing strains were isolated at 4°C (Ivanushkina et al. 2007).

Of considerable importance for the preservation of fungi in permafrost are both the presence of natural cryoprotectants in these ecotopes and the ability of the fungi to make use of their inherent mechanisms of protection. For example, species belonging to the genera *Arthrinium, Aureobasidium, Botrytis, Fusarium, Geotrichum,* and *Oidiodendron*, as well as many others, are usually isolated from plant material and/or appear as phytopathogens. It is conceivable that plant substrates or derivatives thereof are natural cryoprotectants, which enables them to provide advantageous conditions to microorganisms when the sediments freeze. Stakhov et al. (2008) demonstrated that ancient seeds of higher plants constitute a specific habitat for microorganisms in frozen ground, which favors their preservation for millennia. The presence of such natural protectants made it possible to preserve certain microbial species specific for these plants, e.g., representatives of the genus *Phoma*.

Fungi with a broad adaptive potential, such as species of the genera *Penicillium, Aspergillus, Cladosporium,* and *Geomyces*, occur in permafrost most frequently. Lowering the ambient temperature may trigger protector mechanisms inherent in fungal cells. These mechanisms include elevation of intracellular trehalose, polyols, and unsaturated fatty acids, as well as the synthesis of enzymes operating at low temperatures (Robinson 2001). In particular, there is evidence that the temperature of cultivation affects both the content and the composition of intracellular carbohydrates and lipids in mycelial fungi. The changes increase the amount of compounds with cryoprotectant properties (e.g., unsaturated fatty acids are elevated, and the sterol to phospholipid ratio becomes lower) (Weinstein et al. 2000; Turk et al. 2004). Fungi exposed to osmotic stressors are capable of synthesizing glycerol for

maintaining their intracellular water potential at low levels (Förster et al. 1998; Teixido et al. 1998), and glycerol is known to protect cells under conditions of extreme temperatures.

The features indicated above not only facilitate survival of fungi exposed to stressors, they also favor the development of individual strains of certain species in extreme habitats. The observation that representatives of certain species, isolated from permafrost, are characterized by growth optima shifted towards lower temperatures provides indirect evidence of the ability of microbial strains to develop at extremely low temperatures. Such species are, according to our data and reports of researchers working with Antarctic strains, representatives of the genera *Penicillium, Cladosporium* and *Geomyces*, most frequently occurring in permafrost (Tosi et al. 2002). Moreover, a strain of *Geomyces pannorum*, isolated from liverwort in Antarctica, was reported to grow at a rate of 0.05 mm per day when the temperature of the environment was −2°C (Hughes et al. 2003). The cultures of this species are capable of switching cellular metabolism in response to temperature decreases (Finotti et al. 1996). The ability of these fungi to grow at subzero temperatures is in accordance with the results of studies of Arctic strains (Kochkina et al. 2007). The experiments demonstrated that the optimum growth temperature of *G. pannorum* strains, isolated from overcooled water brines (cryopegs) and frozen marine deposits, is lower than those of representatives of this species isolated from other habitats. In addition, these strains exhibited active growth at subzero temperatures (−2°C), surpassing control cultures from the temperate zone by two orders of magnitude in the growth rate.

7.5 Conclusion

The reported data demonstrate that viable fungi can be isolated from permafrost habitats. These microorganisms are exposed to diverse stressors, such as low temperatures, low water activity and hypoxia. The amount of the fungal community is generally small, which is in contrast with their pronounced species diversity. Fungal organisms in these ecotopes are likely to be in the state of survival due to conditions that may favor natural cryopreservation. Detailed studies of permafrost differing in age, performed in replicates of adequate numbers of samplings, provide evidence of the existence of extremotolerant fungi capable of retaining viability and developing under conditions of permafrost, thus exhibiting a high adaptive potential.

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Chapter 8
Ancient Protozoa Isolated from Permafrost

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8.1 Introduction

Protista is a group of eukaryotic auto- and heterotrophic organisms, rich in the number of species and their diversity. At present, this group amounts, by different estimates, to 120,000–200,000 species; these species are only a minor part of those really existing in nature (Poljansky et al. 2000).

Protozoa is a polyphyletic group of protists, which includes heterotrophic (free-living and parasitic), presumably unicellular organisms. Free-living protozoa are distributed worldwide, and inhabit almost all suitable-for-life environments. In all geographical zones, they are an obligatory component of soil biocenoses, comparable in number and diversity only to bacteria (Poljansky et al. 2000; Auer and Arndt 2001).

Protozoa are able to live over a wide temperature range and to adapt to both extremely high and low temperatures. In cold habitats, the temperature optimum for growth and reproduction of protozoa are lowered (Sukhanova 1968; Lozina-Lozinsky 1972). Owing to their highly developed adaptive strategies, these eukaryotic organisms are widespread in various biotopes of polar regions: in the cold sea and fresh waters, as a component of plankton and benthos (Tong et al. 1997; Robinson 2001; Mylnikov et al. 2002; Petz et al. 2005; De Jonckheere 2006; Petz 2007; Tikhonenkov and Mazei 2007), in the melt water and ice (Ikävalko et al. 1996; Ikävalko 1998), and in the terrestrial ecosystems of the Antarctic and high-latitude Arctic (Smith 1978; Foissner 1996; Petz 1997; Bobrov et al. 2003).

The ability to switch to cryptobiotic stages, i.e., to form resting cysts which are well-developed in soil protozoa, allows them to survive under unfavorable environmental conditions and to spread over sizeable territories (Hausmann and Hulsmann 1996; Clegg 2001). It is known that, in the state of cryptobiosis, they can sustain

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temperatures from −1 to −60°C in natural environments and from −1 to −269°C under experimental conditions (Poljansky et al. 2000). Cases of protozoa cysts have been described that kept viability after long-term (several tens of years) conservation in the ices of Greenland and the Baltic Sea (Ikävalko et al. 1996, Ikävalko and Grandinger 1997; Ikävalko 1998) and in dry soil samples (Goodney 1914; Lozina-Lozinsky 1972; Moon-van der Staay et al. 2006). Cysts of the infusorium *Colpoda steinii* and amoeba *Vahlkampfia* sp., which preserved the capability of excystation after several centuries of cryoconservation (Marquardt et al. 1966), were isolated from the Greenland ice.

There are no data on viable protozoa specimens found in permafrost sediments. In the 1930s, Kapterev reported on finding viable amoebas and ciliates in the Transbaikalian permafrost (Kapterev 1936, 1938). These organisms were, however, probably found at the bottom of the seasonal-thawing layer.

Our investigations have shown that protozoa cysts, conserved in permafrost (at stably low temperatures, in the dark, without water and oxygen), can remain viable for several hundred thousands of years (Shatilovich et al. 2005, 2007; Shatilovich and Petrovskaya 2007; Shmakova et al. 2007; Gilichinsky et al. 2007).

8.2 Ancient Protozoa from Permafrost

8.2.1 Study Area

Studies were carried out in the East Arctic sector, from the Lena delta to the lower reaches of the Kolyma in the continuous permafrost zone (Fig. 8.1). The territory is characterized by cold Arctic climate, with a mean annual air temperature of −13.5°C in the west (Tiksi settlement) and −13.4°C in the east (Chersky settlement). The permafrost samples of various age and origin, as well as soils buried in those sediments and burrows of fossil rodents, were selected for protozoological analysis. For comparative analysis, samples of modern tundra soils were taken.

The samples of buried soils and burrows were taken on the northern edge of the taiga zone at the eastern border of the Kolyma lowland from the late Pleistocene Ice complex (outcrops Stanchikovsky and Duvanny Yars). The mean annual ground temperature in this region ranges from −5 to −6°C, and the maximal depth of seasonal thawing, which was observed in the summer of 2007 (the warmest summer over the last 25 years), reached 70 cm. The buried soils were represented by peat and a profile of humus–peat gley soil (Gubin 1994); the material of fossil burrows was represented by remnants of herbaceous vegetation in the storage chambers, and included seeds of higher plants, rodent excrement, hairs of large animals, and a mixture of dusty loam. The buried soils and rodent burrows lie at a depth of ~30 m below the surface; their age, according to radiocarbon dating, is 28,000–32,000 years (Gubin et al. 2003a).

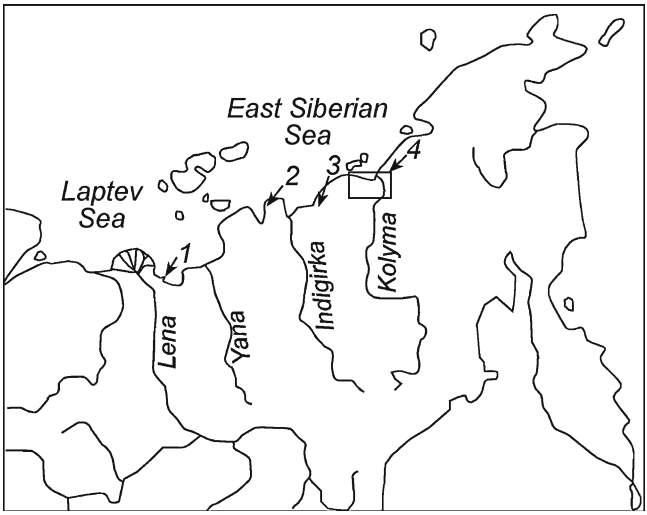

Fig. 8.1 Schematic map of study area with sampling sites. *1*, The coast of Laptev Sea, Bykovsky peninsula; *2*, Yana-Indigirka lowland, mouth of Chroma bay; *3*, Indigirka-Kolyma lowland, Khomus-Yuryakh river; *4*, Kolyma lowland

Frozen samples were taken from the cores of boreholes drilled in the tundra zone of the Laptev Sea coast (Cape Bykovsky), coast of the East Siberian Sea (outfall of the Khroma river, Cape Chukochy) and inner regions of the Kolyma lowland (valleys of the Khomus-Yuryakh, Kuropatochya and Chukochya rivers). In these regions, the mean annual ground temperature varies from −9°C in depressions to −12°C on watersheds, and the maximal depth of seasonal thawing of loamy soils does not exceed 50 cm. A protozoological study was made of samples from the main horizons of the Pleistocene cross section (Fig. 8.2). Among them, epicryogenic sediments of Holocene alases (shallow cryogenic depressions) (boreholes 1/01, 7/03, 2/04 and 2/96) and syngenetically frozen deposits of the late Pleistocene Ice complex (borehole 4/05) turned out be populated with protozoa. The most ancient single findings (borehole 4/05) were attributed to the syncryogenic mid-Pleistocene Ice complex.

8.2.2 Sample Collection

Permafrost samples were taken with a core drilling tool 12/25 (V.V. Vorovsky's Machinery Plant, Ekaterinburg, Russia), without flushing or using any chemicals. Sampling under sterile conditions was performed by a proven method, which is described in a number of papers (Shi et al. 1997; Juck et al. 2005; Gilichinsky et al. 2007). The central part of an intact frozen core (50–100 mm in diameter) was taken

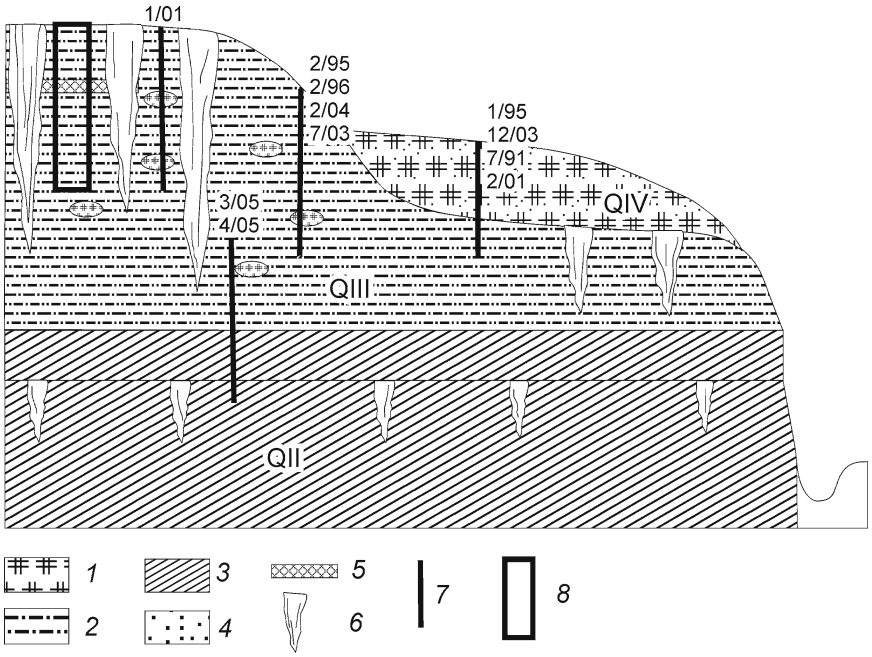

Fig. 8.2 Holocene–Pleistocene geological cross-section of sediments of North-Eastern Siberia. *1*, peat; *2*, sandy loam; *3*, loam; *4*, sand; *5*, buried soil; *6*, ice wedge; *7*, boreholes; *8*, outcrops

with all aseptic precautions: the core was handled in a field microbiological box, its surface was trimmed with a sterile scalpel and treated with 95% ethanol, and the sample was placed in a sterile aluminium container or plastic bag and sealed. The sealed samples were stored in a "fridge bore" at a temperature of −10°C. At the end of the season, they were placed in cryothermostates and delivered, in the frozen state, to the laboratory.

The samples of buried soils and the material from the burrows of fossil rodents were taken from the frozen outcrop walls. With melting material cleaned out from the wall surface and the unthawed layer exposed, the frozen rock was excavated to make a hollow of 30–40 cm depth, and a sample was taken from this hollow. After treating with 95% ethanol, the sample was placed in a sterile plastic bag and stored frozen.

In the laboratory, the samples were stored in freezers at −20°C.

8.2.3 Isolation of Protozoa

Viable protozoa were isolated from the permafrost samples by the method of enrichment cultivation. The cultures were cultivated on mineral media at two temperature regimes (+8°C and +20°C) for 4 months (Page 1988; Foissner 1992). Several media were tested, and the PJ-medium (Prescott's and James's solution)

(Page 1988) turned out to be the most suitable medium. The cultures were observed in closed, parafilm-sealed Petri dishes using an inverted light microscope. If protozoans were found, the culture was reinoculated.

The monocultures and clonal cultures of protozoa were obtained from the enrichment culture by standard techniques; the isolated protozoa were cultivated on liquid and agar media supplemented with various nutrients, such as with bacterial cells of *Escherichia coli* and *Klebsiella aerogenes*, or with rice grains (Page 1988; Zhukov 1993). A morphological study of the isolated protozoa was made on either vital or fixed preparations (Pussard and Pons 1977; Page 1988; Foissner 1991a, 1992).

The fine structure of protozoa cells in resting (cryptobiotic) stages was examined with a transmission electron microscope.

8.2.4 Taxonomical Research

We examined about 200 samples of Pleisocene and Holocene deposits, which were collected from 29 boreholes at a depth of 0.5–47 m, as well as from buried soils and the material of cryopedolith-located fossil rodent burrows. In the ice-complex sediments, viable protozoa were found in 25 of 125 samples (20% of total samples examined). Occurrence of viable protozoa was considerably higher in the buried soils (80%; 14 samples) and fossil burrows (100%; 12 samples) (Table 8.1).

The protozoological analysis of the samples revealed specimens of major protozoa macrotaxons: naked amoebas, heterotrophic flagellates, ciliates and heliozoa (Table 8.2, Fig. 8.3).

Twelve cultures of cyst-forming species of ancient ciliates were obtained from the samples of permafrost sediments, buried soils and burrows. They were represented, in their major part, by specimens of polyzonal species. Apart from ten strains of specimens of the taxonomical group Colpodea — *Colpoda steinii, C. inflata*, *C.* aff. *aspera*, *C.* aff. *augustini*, *Colpoda* sp., *Platyophrya* aff. *vorax* — the ciliates *Vorticella* sp. (Oligohimenophorea) and *Oxytricha* sp. (Spirotrichea) were isolated.

It turned out that the occurrence of viable amoeboid organisms (49%) in the permafrost sediments was higher than that of ciliates (9%). Naked amoebas were found both in the samples of permafrost sediments and in buried soils. We identified specimens of lobose (Leptomyxida, Acanthamoebidae) and heterolobose (Vahlkampfiidae) amoebas. Pure cultures of ancient naked amoebas, two Leptomyxida and eight Acanthamoebidae strains, were obtained in laboratory settings. Acanthamoebas, like colpodean ciliates, are distributed worldwide (Page 1988; Foissner 1993).

In the samples from buried burrows, we found 27 species and forms of heterotrophic flagellates from ten taxonomical groups and flagellates *incertae sedis* (Shatilovich et al. 2008). The taxonomical analysis of the ancient flagellate fauna revealed that amoeboid flagellates (Cercomonadida, Apusomonadidae) and stramenopiles (Chrysophyceae) were the most numerous and diverse groups (Fig. 8.4).

Table 8.1 Sites, age and genesis of the permafrost samples

Site	Location	Well no.	Depth (m)	Age	Genesis	Lithology
1	The coast of Laptev Sea, Bykovsky peninsula		0.40–0.56	Modern soil		
			1	QIII	Late Pleistocene sediments of ice complex	Peat
			1.95–2.05			Sandy loam with peat
			2.16			
			2.8			
			7			
		1/01	19			Sandy loam
		2/01	1.0–1.1	QIV	Sediments of Holocene alases	Sandy loam with peat
			2			
			2.25–2.33			
			2.4			
			2.5			
		7/03	4	QIII	Late Pleistocene sediments of ice complex	Peat
			4.95–5.05			Sandy loam with peat
			6.9			Peat
		12/03	3.5	QIV	Sediments of Holocene alases	Sand with inclusions of peat
			4			
2	Yana-Indigirka lowland, mouth of Chroma bay	2/04	0.71	Modern soil		
			1.15	QIII	Late Pleistocene sediments of ice complex	Loam
3	Indigirka-Kolyma lowland, Khomus-Yuryakh river	3/05	0.7	Modern soil		
			4.2	QII	Middle Pleistocene sediments of ice complex	Sandy loam
			6.5			Sandy loam
		4/05	9.3			Loam
4	Kolyma lowland, Chukochi cape	7/91	1	QIV	Sediments of Holocene alases	Loam with peat
	Kolyma lowland, Oler ltake	1/95	1.25–1.3			Loam
		2/95	0.3–0.35	Modern soil		
	Kolyma lowland, Kuropatochia river	2/96	10,6–10,7	QIII	Late Pleistocene sediments of ice complex	Loam
	Kolyma lowland, Kolyma river, Anuy river (Stanchikovsky and Duvanny yars)	Outcrop 1			Buried soils and burrows in the late Pleistocene sediments of ice complex	
		Outcrop 2				

Table 8.2 Biodiversity of ancient protozoa isolated from Siberian permafrost (Adl et al. 2005)

Taxonomic groups			Species and forms
CHROMALVEOLATA Adl et al. 2005	Alveolata Cavalier-Smith 1991	Ciliophora Doflein 1901 [Ciliata: Perty 1852, Infusoria: Butschli 1887]	*Oxytrichia* sp.
			Colpoda steinii Maupas 1883
			Colpoda inflata Kahl 1931 (Stokes 1884)
			Colpoda aff.*augustini* Foissner 1987
			Colpoda aff.*aspera* Kahl 1926
			Colpoda sp
			Platyophrya aff. *vorax* Kahl 1926
			Vorticella sp.
	Cryptophyceae Pascher 1913, emend. Schoenichen 1925	Goniomonadales Novarino and Lucas 1993	*Goniomonas truncata* (Fresenius) Stein 1878
	Stramenopiles Patterson 1989, emend. Adl et. al. (2005)	Chrysophyceae Pascher 1914	*Spumella elongata* (Stokes) Belcher and Swale 1976
			Spumella sp.
	Incertae sedis Alveolata		*Colponema edaficum* Mylnikov et Tikhonenkov 2007
EXCAVATA Cavalier-Smith 2002, emend. Simpson 2003 (P?)	Heterolobosea Page and Blanton 1985	Vahlkampfiidae Jollos 1917	*Vahlkampfia* sp.
	Fornicata Simpson 2003	Histionidae Flavin and Nerad 1993	*Reclinomonas* aff. *americana* Flavin and Nerad 1993
	Euglenozoa Cavalier-Smith 1981, emend. Simpson 1997	Euglenida Bütschli, 1884, emend. Simpson 1997	*Anisonema ovale Klebs* 1893
		Kinetoplastea Honigberg 1963	*Bodo curvifilis* Griessmann 1914
			Bodo designis Skuja 1948
			B. repens Klebs 1893
			B. minimus Klebs 1893
AMOEBOZOA Luhe 1913, emend. Cavalier-Smith 1998	Tubulinea Smirnov in Adl et al. 2005	Leptomyxida Pussard and Pons 1976, emend. Page 1987	*Leptomyxa* sp.
	Acanthamoebidae Sawyer Pons 1976, emend. Page 1987		*Acanthamoeba* sp.
	Eumycetozoa Zopf, 1884, emend. Olive 1975	Incertae sedis Eumycetozoa	*Hyperamoeba flagellata*: Alexeieff 1923

(continued)

Table 8.2 (continued)

Taxonomic groups			Species and forms
	Incertae sedis AMOEBOZOA Spongomonadida: (Hibberd 1983) emend. Karpov 1990	Spongomonadidae Karpov 1990	*Phalansterium solitarium* Sandon 1924
			Spongomonas uvella Stein 1878
OPISTOCONTA Cavalier-Smith 1987, emend. Cavalier-Smith and Chao 1995, emend. Adl et al. 2005	Choanomonada Kent 1880	Monosigidae Zhukov and Karpov 1985	*Codonosiga botrytis* Kent 1880
			Desmarella moniliformis Kent 1880
			Monosiga ovata Kent 1880
		Salpingoecidae Kent 1880	*Salpingoeca globulosa* Zhukov 1978
RHIZARIA Cavalier-Smith 2002	Cercozoa Cavalier-Smith 1998, emend. Adl et al. 2005	Cercomonadida (Poche 1913), emend. Vickerman 1983, emend. Mylnikov 1986	*Cercomonas angustus*: (Skuja 1948) Mylnikov and Karpov 2004
			Cercomonas crassicauda Dujardin 1841
			Cercomonas granulifera (Hollande 1942) Mylnikov and Karpov 2004
			Cercomonas sp.
			Heteromita minima (Hollande 1942) Mylnikov and Karpov 2004
			Heteromita aff. *globosa* (Stein) Kent 1880
		Incertae sedis Heteromitidae	*Allantion tachyploon* Sandon 1924
			Protaspis aff. *gemmifera* Larsen and Patterson 1990
			Protaspis simplex Vørs 1992
Incertae sedis EUKARYOTA	Apusomonadida Karpov and Mylnikov 1989	Apusomonadidae Karpov and Mylnikov 1989	*Apusomonas proboscidea* Alexeieff 1924
	Centrohelida: Kühn 1926	Acanthocystidae Claus 1874	*Choanocystis perpusilla* Siemensma 1991

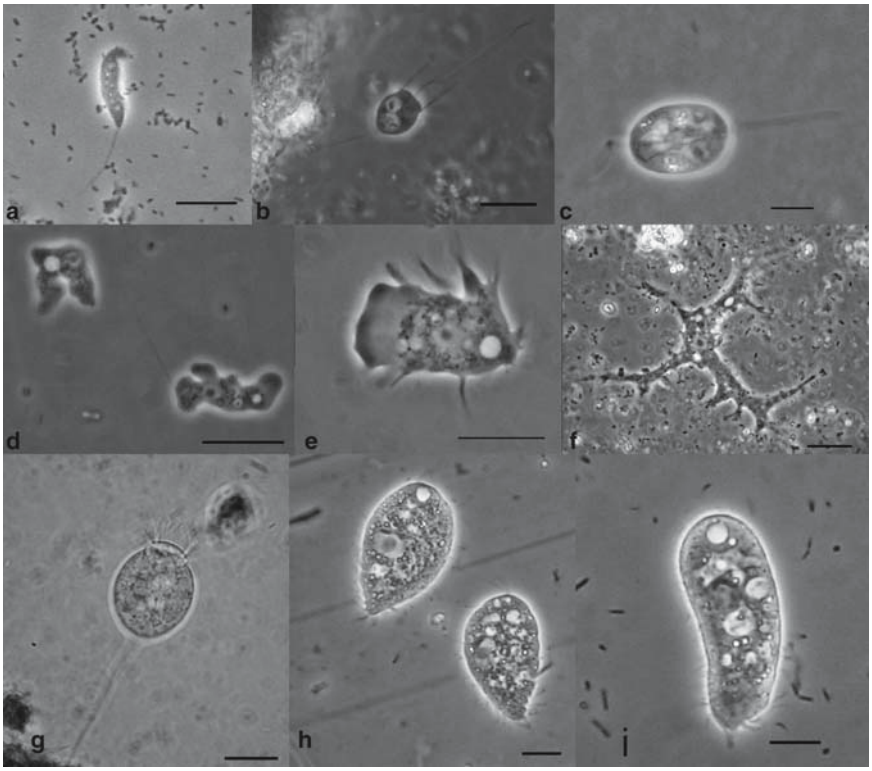

Fig. 8.3 Light micrographs of protozoans isolated from permafrost: **a**, **b**, **c** heterotrophic flagellates; **d**, **e**, **f** naked amoebas; **g**, **h**, **i** ciliates. Bars = 10 μm

Most of the species were bacteriotrophs, and four forms (*Goniomonas truncata*, *Allantion tachyploon* *Colponema edaphicum*, *Choanocystis perpusilla*) were predators. In one of the samples from buried burrows, we found a centrohelid heliozoan, *Choanocystis perpusilla*. For many species of ancient protozoa, we obtained monocultures and clonal cultures, which grew well at 20°C.

The permafrost samples that were collected up to 3 m below the surface (boreholes 1/95, 2/95, 7/91, 2/01, 1/01, 1/03, 2/04, 3/05 and 1/95) appeared to be more abundant in protozoa, since they were found in 60% of those samples. The maximal depth at which we managed to isolate viable protozoa was 19 m (borehole 1/01). The organisms found at the upper permafrost boundary are not older than a few hundred years, with single, the most ancient, findings dated to the middle Pleistocene, 200,000–300,000 years (borehole 4/05; 9.3 m deep).

There was a tendency for the number and diversity of viable protozoa species in the buried soils and burrows to be larger than those observed in the ice-complex sediments. This, probably, is explained by more favorable conditions of cryoconservation and a relatively rich initial fauna in buried soils and burrows. In addition, the collection

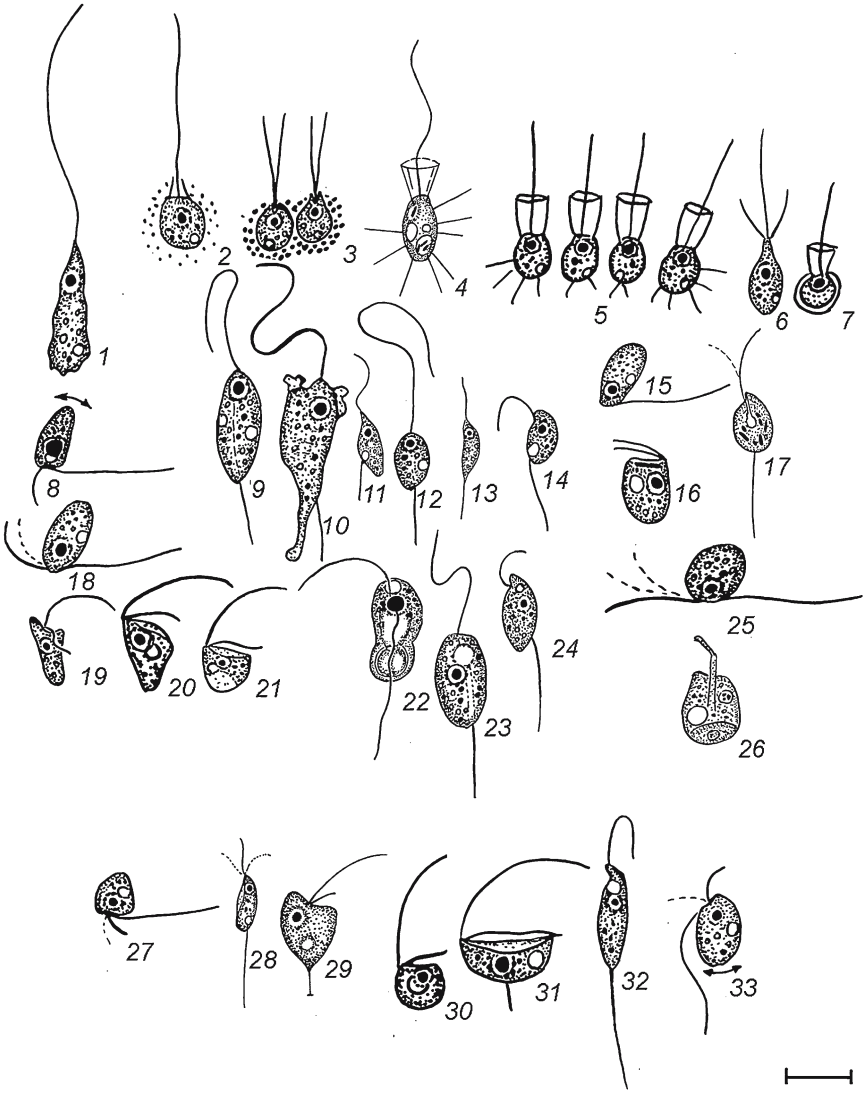

Fig. 8.4 Morphology of ancient heterotrophic flagellates: *1*, Hyperamoeba flagellat; *2*, Phalansterium solitarium; *3*, Spongomonas uvella; *4*, Codonosiga botrytis; *5*, Desmarella moniliformis; *6*, Monosiga ovata; *7*, Salpingoeca globulosa; *8*, Cercomonas angustus; *9*, Cercomonas crassicauda; *10*, Cercomonas granulifera; *11*, Cercomonas sp. 1; *12*, Cercomonas sp. 2; *13*, Cercomonas sp. 4; *14*, Heteromita aff. globosa; *15*, Allantion tachyploon; *16*, Goniomonas truncata; *17*, Protaspis aff. gemmifera; *18*, Protaspis simplex; *19*, *20*, Spumella elongat; *21*, Spumella sp. 1; *22*, Colponema edaficum; *23*, Anisonema ovale; *24*, Bodo designis; *25*, B. repens; *26*, Apusomonas proboscidea; *27*, Heteromita minima; *28*, Cercomonas sp. 3; *29*, Spumella sp. 2; *30*, Spumella sp. 3; *31*, Reclinomonas aff. americana; *32*, Bodo curvifilis; *33*, B. minimus. The bar is equal to 5 (1–27) or 10 (28–34) μm

of samples from outcrops allowed us to choose samples abundant in organics, more structured and, therefore, more suitable for protozoological examination.

No correlation was revealed between the occurrence of viable protozoa in the sediment samples and the physical–chemical properties of these sediments (moisture, grading, pH and temperature).

8.3 Survival Strategies of Protozoa in Permafrost

8.3.1 In Situ Detection of Protozoa: are Protozoa Only a Contamination?

Our studies showed that soil protozoa remain viable for tens and hundreds of thousands of years under conditions of subzero temperatures, oxygen deficiency, and lack of available water and food.

Protozoa are most often found in Holocene sediments (the first 2.5 m below the surface). The maximal depth of thawing in the regions explored can reach 0.8 m in extraordinarily warm years. Therefore, the age of protozoa at the upper permafrost boundary does not exceed a few hundred years. However, below the active layer, in the ice-cemented strata, the influence of environmental factors is largely restricted, and there is no aquifer or infiltration. Thermodiffusion and migration of protozoa through the films of non-frozen water are impossible too, since the size of protozoa is incommensurably larger than the thickness of these films, which is about 10^{-3} μm. The presence of thick icy veins directly indicates that the ice-containing sediments have never been unfrozen, i.e., the biota found could not penetrate into these layers. The biota also could not been introduced from the outside in the process of drilling: the technique of sterile core sampling has been proved many times in the microbiological studies of frozen strata. In view of the aforesaid, we can conclude that the viable protozoa species revealed in the permafrost strata were found in situ.

8.3.2 Ecological Implications

The major contribution to biodiversity of permafrost protozoa is made by species of ecological relevance, which can be found in modern polytypic aqueous and soil ecosystems.

All the extracted protozoa were characterized by relatively small sizes (5–60 μm) and the ability to use various nutrition strategies. Their life cycle includes a cryptobiotic stage, which comes under unfavorable conditions (food deficiency, water shortage, low oxygen content, low temperatures) and is often accompanied by formation of a cyst (Keilin 1959). Such adaptive properties are typical of organisms that utilize the advantages of a so-called “r-strategy”, which enables them to survive

under unstable or persistently extreme environmental conditions (Odum 1986). The r-strategy is a strategy of evolutionary development of a species, which implies intensive reproduction and short life duration, high degree of conformity to environmental changes and increased viability. This results, in particular, in a wide adaptive reaction of the organisms and in the successful colonization of polar ecotopes (MacArthur 1972; Lüftenegger et al. 1985).

It is known that at high latitudes of the Arctic and Antarctic, under extremely low conditions of temperature, organisms that apply passive–tolerant adaptive strategies (r-strategies) have an advantage. Correspondingly, more progressive taxons that realize strategies of the resistant–active type (K-strategies), and provide the basis for biodiversity of the global biota, are rare in the ecosystems of polar regions (Chernov 1984; Chernov and Matveeva 2002).

The modern soil and freshwater protistofauna of the east Arctic is practically unexplored; this makes it difficult to perform a comparative faunistical analysis of the regional ecosystems. However, the results of similar investigations in other polar regions confirm the observations described above. For example, the communities of Arctic and Antarctic soil protists were reported to be dominated by colpodean ciliates, especially by *Colpoda steinii* and *C. inflata* (Colpodea), which are typical r-strategists (Foissner 1996; Petz 1997).

Studies on the diversity of protozoan species isolated from permafrost have shown that the fauna of ancient ciliates is represented mainly by colpodean specimens. The species of the *Acanthamoebida* genus, which prevail among permafrost amoebas, are also evident r-strategists, and are distributed worldwide (Page 1988).

The fauna of heterotrophic flagellates isolated from permafrost consists mainly of eurybiontic species, and is highly similar to the typical fauna of freshwater polar ecosystems. Some species (*Allantion tachyploon, Bodo curvifilus, B.designis, Monosiga ovata, Apusomonas proboscidea, Cercomonas* sp., *Heteromita globosa, Spumella* sp.) were described earlier as inhabitants of freshwater biotopes of the Arctic and Antarctic (Mylnikov and Zhgarev 1984; Tong et al. 1997; Butler 1999; Mylnikov 2002; Tikhonenkov and Mazei 2007). Other species (*Allantion tachyploon*, *Bodo curvifilus, B.designis, Heteromita globosa, Heteromita minima, Monosiga ovata, Spumella* sp., *Goniomonas truncata*) are euryhaline, and can be found in high-latitude sea ecosystems (Patterson et al. 1993; Vørs 1993; Tong et al. 1997; Mazei and Tikhonenkov 2006). Most species of the ancient heterotrophic flagellates were described earlier in the freshwater and soil ecosystems of temperate latitudes (Zhukov 1993; Foissner 1991b; Ekelund and Patterson 1997; Auer and Arndt 2001).

It can be supposed that the adaptive mechanisms that help certain taxons to thrive in extreme ecotopes also allow them to sustain successfully an ultra-long anabiosis under permafrost conditions.

8.3.3 Adaptation Mechanisms

As revealed in numerous studies, protozoa are highly resistant to many external factors, including low temperatures (Sukhanova 1968; Lozina-Lozinsky 1972). Under natural conditions, low temperatures have a considerable influence on the

character of metabolism and the related morpho-functional processes in the protozoa cells. Affected by near-zero temperatures and the concomitant dehydration and altered chemism of the environment, protists use different survival strategies (Bradbury 1987; Gutierrez et al. 2001):

(i) In one strategy, organisms do not undergo cell differentiation, keep the general morphology of vegetative stage unchanged, and at the same time maintain metabolism at a sufficient level, until the action of the adverse factor ends. Lowering temperature below the optimum triggers protective mechanisms inside the cell, such as the increase in the content of trehalose, unsaturated fatty acids and polyols, and the synthesis of cold-resistant enzymes (Poljansky 1963; Lozina-Lozinsky 1972; Mazur 1984; Robinson 2001; Clegg 2001; Podlipaeva et al. 2006).

(ii) Alternatively, protozoa turn to the mechanisms based on cell differentiation, and pass into a more stable state, which essentially differs from the vegetative state. Accordingly, survival will be achieved by almost complete suspension of metabolic activity; that is why the strategy of this second type is often called cryptobiosis (from Greek "hidden life", according to the term given by Keilin in 1959). In many organisms, transition to the state of physiological resting is accompanied by the formation of specific morphological structures (Goldovskij 1986; Ushatinskaja 1990); in protozoa, these are resting cysts (Gutierrez et al. 1990; Hausmann et al. 2003).

Encystation of protozoan cells is accompanied by the processes of differentiation, which are characterized by alterations such as considerable dehydration of the cytoplasm, autophagic activity, deposition of storage substances, formation of a protective envelope, and changes in the organization of the nuclear apparatus (Lozina-Lozinsky 1972; Corliss and Esser 1974; Walker et al. 1980; Ushatinskaya 1990; Gutierrez et al. 1990; Guppy and Withers 1999). The resting cysts of ciliates (e.g., Colpodidae) accumulate a large amount of disaccharides, trehalose and/or sucrose (Potts 1994). In the process of dehydration, it is suggested that these polyhydroxylic compounds substitute for the aqueous hydration envelope around macromolecules and intracellular organelles, thus protecting them from damage (Clegg 1986).

The resting cysts of protozoa are protected from adverse environmental effects by a multilayer water- and gas-tight envelope (Ushatinskaya 1990; Gutierrez et al. 2001). Encysted acanthamoebas, for example, are resistant to biocides, chlorination and antibiotics (De Jonckheere and Van de Voorde 1976; Khunkitti et al. 1998; Turner et al. 2000; Lloyd et al. 2001). Little is known about the macromolecular composition of different envelope layers; their major components are proteins, glycoproteins and carbohydrates (Tomlinson and Jones 1962; Neff and Neff 1969; Gutierrez et al. 2003; Matsusaka and Hongo 1984; Benitez et al. 1991; Izquierdo et al. 1999).

The cultivation of protozoa isolated from permafrost showed that all ancient amoebas, ciliates and a part of heterotrophic flagellates formed resting cysts — as, according to the literature data, do their modern counterparts of analogous species and genera. However, there are some species in the fauna of ancient heterotrophic flagellates

(*Goniomonas truncata, Spumella elongata, Colponema edaficum, Bodo curvifilis, B. designis, B. repens, B. minimus, Phalansterium solitarium, Spongomonas uvella, Salpingoeca globulosa, Cercomonas angustus, Heteromita minima, Protaspis simplex, Apusomonas proboscidea*), which have never been reported to have resting cysts in their life cycle (Zhukov 1993; Mylnikov, personal communication).

Electron microscopy study of ancient ciliates (Colpodea) and heterolobose amoebas (Acanthamoeba) has revealed that, in different species, the number of structurally distinct layers in the envelope of their cysts varies from two to four, which was also observed in the envelope of modern specimens of those species and genera (Frenkel 1987; Díaz et al. 2000; Gutierrez et al. 2003; Chavez-Munguia et al. 2005). Acanthamoebas form a two-layer envelope, which consists of the outer ectocyst and the inner endocyst (Fig. 8.5). There may be pores on the cyst surface, so-called ostioles. The pores are covered with a protective cap, operculum, which is made of the same material. The cyst envelope of the ciliate *Colpoda inflata* (Colpodea) consists of an ectocyst, mesocyst (intermediate layer between ecto- and endocyst), endocyst and granular layer or metacyst (Fig. 8.5).

8.3.4 Conditions of Cryoconservation

We guess that the conditions under which protozoa cysts were buried and passed into the frozen state, as well as the conservation regime, had a considerable impact on the formation of the fauna of "alive fossil" protozoa. The cysts of protozoa may

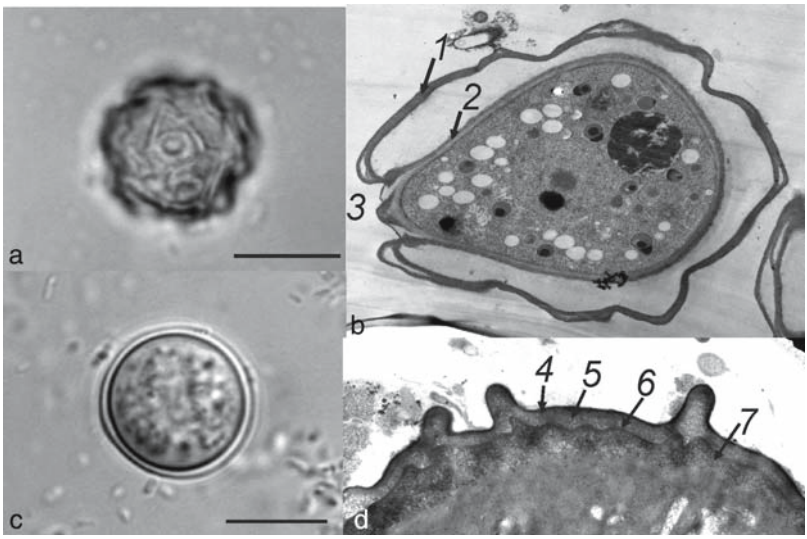

Fig. 8.5 Resting cysts of ancient protozoans. **a**, **c** Interference-contrasted images. **b**, **d** TEM-micrographs **a**, **b** *Acanthamoeba* sp. **c**, **d** *Colpoda inflata. 1*,*4* ectocyst; *2*,*6* endocyst; *3* ostiole with operculum; *5* mesocyst; *7* granular layer. Bars = 10 μm

have been buried in the course of gradual deposit formation. In this case, cells should have experienced a dramatic long-lasting stress of freezing-thawing until they finally got frozen. This factor could have been crucial for the formation of a "survivor's community". Another variant of burial implies filtration with the flow of soil moisture from the active layer to the upper permafrost boundary, as described for bacteria (Spirina and Fedorov-Davydov 1998). In this case, the transition of cells to the frozen state should have taken much less time than it would have taken in the case of a gradual burial. The conditions in the permafrost strata are relatively stable, and the duration of cryoconservation and the protective mechanisms that protozoa possess play a key role in the selection of the most resistant organisms. Taxons highly tolerant to the extreme conditions of tundra ecotopes, i.e., r-strategist, would have been favored in both cases. Our observations confirm this conclusion: the fauna of soil protozoa isolated from the sediments of icy complex and the soils buried there is characterized by low species diversity, and consists of pioneer species adapted to the extreme environmental conditions.

In the permafrost sediments of the icy complex, we found fossil burrows that should be considered as special paleoecological objects. These are suslik (ground squirrel) burrows, which belong to a species of the subgenus *Urocitellus* (Gubin et al. 2003a, b; Zanina 2005). These rodents collected seeds and plant fruits from various biotopes, and stored them in the food chambers located at the upper boundary of permafrost sediments. Brought from the surface together with the plant material, protozoa cysts were kept in dry, well-aerated chambers that were protected from abrupt temperature drops, in which they froze in a little while. As a result, we see a large increase in the diversity of viable protozoa species in fossil burrows in comparison with the diversity found in the sediments of the icy complex.

8.4 Conclusion

For the first time, we showed the ability of protozoa of different macrotaxons to survive in the state of cryptobiosis for a long time, under conditions of subzero temperatures, hypoxia, and lack of accessible water. Studying communities of viable paleoorganisms gives us a unique chance to make progress in understanding the mechanisms of psychrophily, cryoanabiosis and cryptobiosis in general, and to examine the phenomenon of survival in the cryosphere for a geologically significant period.

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Part III
Biological Activity in Permafrost

Chapter 9
Microbial Activity in Frozen Soils

Nicolai S. Panikov

9.1 Introduction

Seasonally frozen soils and permafrost are widespread on Earth, accounting for more than 50% of the Earth's land surface (Toll et al. 1999). Frozen soils have strong implications on freshwater hydrology, terrestrial ecology and the climatic system. For example, frozen soils are largely impervious to water, and during the release of water during spring, thaw may significantly increase runoff, contributing to severe flooding. In the last two decades, tundra soils and permafrost attracted serious attention from the entire global change community when it became clear that global warming is much more pronounced in the polar area than elsewhere. The climate projections suggest a continuation of the warming trend, with an increase in mean annual temperatures of 4–5°C by 2080 (Elberling and Brandt 2003; Callaghan et al. 2004). The thawing of permafrost, combined with melting of sea ice, is predicted to cause disastrous events including flooding, karst and erosion, accompanied by accelerated degradation of terrestrial carbon.

Microbiology of permafrost and frozen soils is at its infancy. Until recently, permafrost has been addressed by microbiologists primarily as a natural *depository* of ancient forms of life. However, the recent finding of measurable winter gas emission to the atmosphere (see below) demonstrated that subzero microbial activity is an important driver of the observed global changes. This activity may significantly accelerate permafrost degradation under global warming; and this acceleration should be detected well before the visible signs of permafrost thawing appear.

This review focuses on microbial activity in permafrost and frozen soils. The starting point will be methodology; how to measure subzero metabolic activity, and how to distinguish reliable data from experimental artifacts. This is followed by a survey of available data on spatial variation and magnitude of microbial activity in frozen soils, mainly in the North Slope of Alaska. Finally questions crucial for mechanistic understanding of subzero activity will be (tentatively) answered:

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- Is there mass-transfer between cells and frozen microenvironment?
- What kind of substrates are available to support subzero growth?
- What is the physiological state of active microorganisms: is it a partial dormancy, maintenance of viability without growth, or a regular metabolism resulting in cellular growth and division?
- What particular microbial species/phylotypes are responsible for subzero soil activity, what are their biological features and how can they be isolated from natural habitats?

9.1.1 Importance of Subzero Activity

Even very low microbial activity within permanently frozen ground could have a tremendous impact on geochemistry and geophysics of cryolithozone on a geological time scale of 10^3–10^4 years. In seasonally frozen winter soils, microbial activity could be an important agent of biochemical transformations, leading to restoration of soil fertility and resuscitation of stressed and metabolically injured cells (one of the reasons for the frequently observed spring burst of soil activity). There is increasing concern from conservational microbiologists that global warming might damage the most vulnerable psychrophilic members of the soil community. System analysts involved in the construction of a holistic view of the Earth under anticipated global changes can no longer ignore subzero microbial activity, which should be incorporated into comprehensive simulation models of terrestrial and marine ecosystems for the better understanding and realistic prediction of climatic changes.

Microbial subzero activity is also important for *astrobiological studies* and *biotechnological developments*. Evidence for microbial growth and activity at −20°C and lower promotes search for microbial life on cryogenic planets, moons and comets (Friedmann and Ocampo-Friedmann 1984; Finegold 1996; Cavicchioli 2002; Jakosky et al. 2003; Marion et al. 2003; Head et al. 2005). These studies could help to develop detection tools with required sensitivity, and identify the most appropriate targets, including organisms able to function in the oxygen-free extraterrestrial environment, e.g., methanogens (Rivkina et al. 2004). Isolation and growth optimization of microorganisms able to subzero growth could unlock access to new types of biocatalysts efficient at low temperature and low water content for various applications in industry, agriculture and medicine (Feller et al. 1996; Lonhienne et al. 2001; Cavicchioli et al. 2002; Georlette et al. 2004; Marx et al. 2004).

9.1.2 Indirect Evidence for Subzero Microbial Activity

Although reported in occasional publications starting in the 1960s (see below), subzero activity remains a matter of serious doubt, and is not unconditionally accepted as a significant factor in ecosystem dynamics of boreal and polar regions. The majority of texts assume that subzero temperatures reduce the intensity of

biological processes to a negligible level. The definition of psychrophiles is based on their upper temperature limit of 20°C (Morita 1975; Helmke and Weyland 2004), while the low-temperature boundary is left undefined or is assumed to be around zero. Skepticism with regard to subzero activity by the majority of biologists is based on the deeply rooted postulate that life functions are to be supported by the running of the key metabolic processes above a certain threshold level; if cooling slows metabolic reactions below this level, then cells die. Another important restriction factor is claimed to be a lack or severe deficiency in the amount of liquid water in frozen habitats. Without liquid water, the majority of cellular biocatalysts, such as DNA, RNA, enzymes, semi-fluidic membranes etc., remain functionally disabled (Kushner 1981).

In spite of these persuasive a priori arguments, there are several areas of indirect evidence which support the existence of subzero metabolic activity:

(1) Most biological processes are chemical reactions, so chemical kinetics at different temperatures, including ultra-low temperatures, may be instructive for explaining subzero metabolic activity. Generally, rates of abiotic chemical reactions decrease with cooling, but do not stop completely below the freezing point, having a global minimum in the vicinity of 0 K. It is remarkable that some chemical reactions, e.g., neutral free radicals reactions of O(3P) with hydrocarbon (Sabbah et al. 2007), have been shown to remain rapid down to temperatures as low as 20 K, and the rate coefficients increase as the temperature is lowered (Fig. 9.1). These data clearly demonstrate that temperature per se could not be the only restrictive factor; some chemical and maybe biochemical reactions may be accelerated below the freezing point (0°C, 273 K).
(2) It was shown many years ago (Michener and Elliott 1964; Gill and Lowry 1982; Geiges 1996) that frozen food is slowly degraded by bacteria and fungi. Although temperatures in industrial freezers are not maintained perfectly constant, it was concluded that frozen meat can support subzero growth down to −12°C. A psychrophilic community developing on a slaughtered cow in a freezer is probably initiated by opportunistic pathogens or by accidental saprotrophic contamination of freezers. Time for adaptation is measured at no longer than several years, which is nothing, compared with, say, Yedoma subsoils developed under permanent freezing during 30,000 years (Zimov et al. 2006). Therefore, we may safely assume that the lowest permissible temperature for a microbial community in frozen soils or subsoils may be essentially lower than −12°C.
(3) Similar observations were made recently on microbial contamination of embryos and semen cryopreserved in sealed plastic straws and stored for 6–35 years in liquid nitrogen (Bielanski et al. 2003). After such multiyear storage, plating and DNA retrieval permitted the identification of 32 bacterial and 1 fungal species which represented commensal or environmental microorganisms. *Stenotrophomonas maltophilia* was the most common organism. No doubt, significant parts of detected microbes were just survival forms, but some of them could preserve activity and slowly multiply. Indirect evidence comes from the fact that the spectrum of detected species in cryopreserved material was not identical before and after long-term storage.

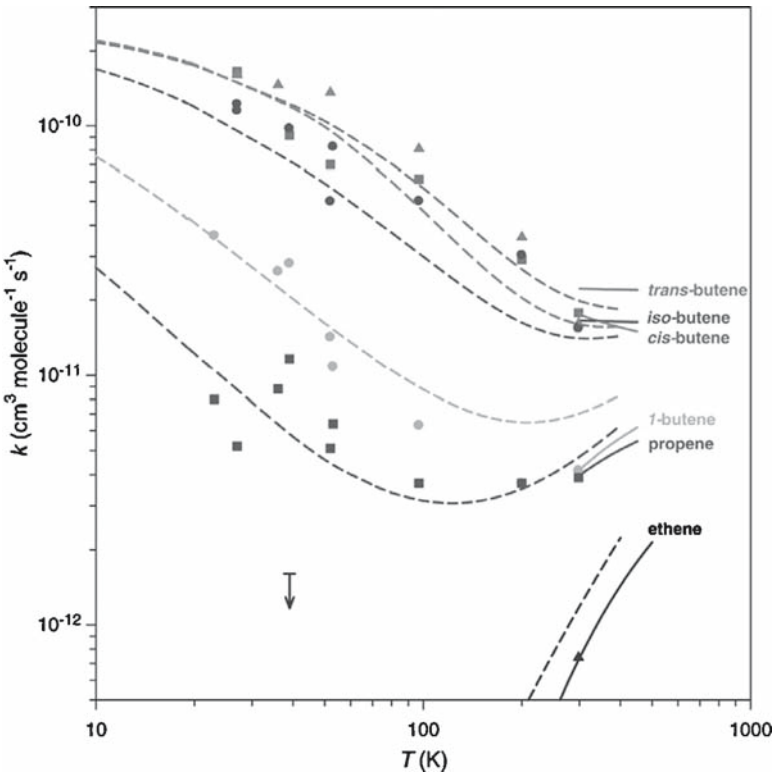

Fig. 9.1 Experimental data on the rates of reactions between O(3P) atoms and various alkenes at different subzero temperatures. The *dashed lines* show the results of calculations based on the modified microcanonical transition state theory (Sabbah et al. 2007). Note that cooling just below the water freezing point (273 K) decreases reaction rates but further cooling leads to acceleration of these radical reactions with approaching the maximum close to 10 K (with permission of Science magazine)

(4) Finally, the most impressive indirect evidence of subzero activity was recently demonstrated by the fact that winter tundra and boreal soils emit various gases: CO_2, CH_4, N_2O. The cumulative cold-season C-fluxes can account for 2–20% of the annual methane emission and up to 60% of the net CO_2 efflux from soil to atmosphere (Whalen and Reeburgh 1988; Dise 1992; Zimov et al. 1993; Melloh and Crill 1996; Brooks et al. 1997; Oechel et al. 1997; Fahnestock et al. 1998, 1999; Grogan and Chapin 1999; Panikov and Dedysh 2000). The mechanism behind the winter emission was a matter of hot discussion. Coyne and Kelley (1971) interpreted it as physical gas ejection from the soil by progressing freezing front; Zimov et al. (1993) hypothesized that soil microorganisms warm themselves up by biogenic heat production; Oechel et al. (1997) and Panikov and Dedysh (2000) suggested that cold-season C-emission was due to instant winter activity of yet unknown organisms.

All these factors are really indirect. Paradoxically, winter-season gas fluxes turned out not to be valid evidence for instant subzero activity of soil microorganisms. To explain this, the next section will focus on critical analysis of available techniques.

9.2 Techniques to Assess Microbial Activity in Frozen Ground

9.2.1 Permafrost Sampling and Sample Processing

It is usual practice to ensure that permafrost cores remain uncontaminated with chemicals or alien microorganisms (Shi et al. 1997; Rivkina et al. 2004). To achieve this goal, all mechanical parts of cutting equipment (auger, chisel, disk saw) are cleaned (e.g., with ethanol or other antiseptics) before collecting the next sample. This condition is relaxed when we analyze the surface frozen soil, which is normally subjected to intensive colonization by allochtonous forms (atmospheric deposition, run-off, borrowing animals, addition of manure, etc.) The extracted cores are immediately sealed in plastic bags and kept frozen during transportation and storage; at each step the temperature is monitored by microloggers to exclude accidental warming of samples. Microbiological analysis such as plating or DNA extraction is preceded by surface shaving of the cores with a sterile scalpel.

Several additional requirements come forward when the task is to measure subzero microbial activity:

(a) How to split frozen cores into small-size aggregates to be filled into test tubes for subsequent activity detection
(b) How to add soluble (glucose, succinate, etc.), or insoluble substrates (starch, cellulose) with minimal disturbance of frozen soil and its community
(c) How to avoid oxygen stress on strict anaerobes
(d) How to prevent sample desiccation during long-term incubations, etc.

The developed procedure (Fig. 9.2) satisfies the majority of required conditions. The homogenization is achieved by splitting the original 30–50 cm core into 2–3 cm sections following crushing into 3–8 mm aggregates inside a polypropylene sleeve under continuous cooling and N_2 flow. The easiest way to amend substrate is to use gases and volatile compounds (CO_2, ethanol, methane and other hydrocarbons, volatile fatty acids, etc.) and add them to the headspace over crushed soil. Soluble substrates require preliminary short-term permafrost melting. We tried to add soluble and insoluble substrates (cellulose) as frozen powder, but never detected their transformation below −10°C. Horizontal chest freezers are advantageus over vertical freezers, and a circulating thermostat with appropriate bath fluid (ethanol, polydimethylsiloxane) is obviously to be preferred over a dry freezer due to higher temperature stability (±0.1°C) even under frequent access. Alcohol bath (at constant temperature) and aluminum block (temperature gradient) provide a unique opportunity for headspace gas sampling: the vial with incubated permafrost is kept at a given

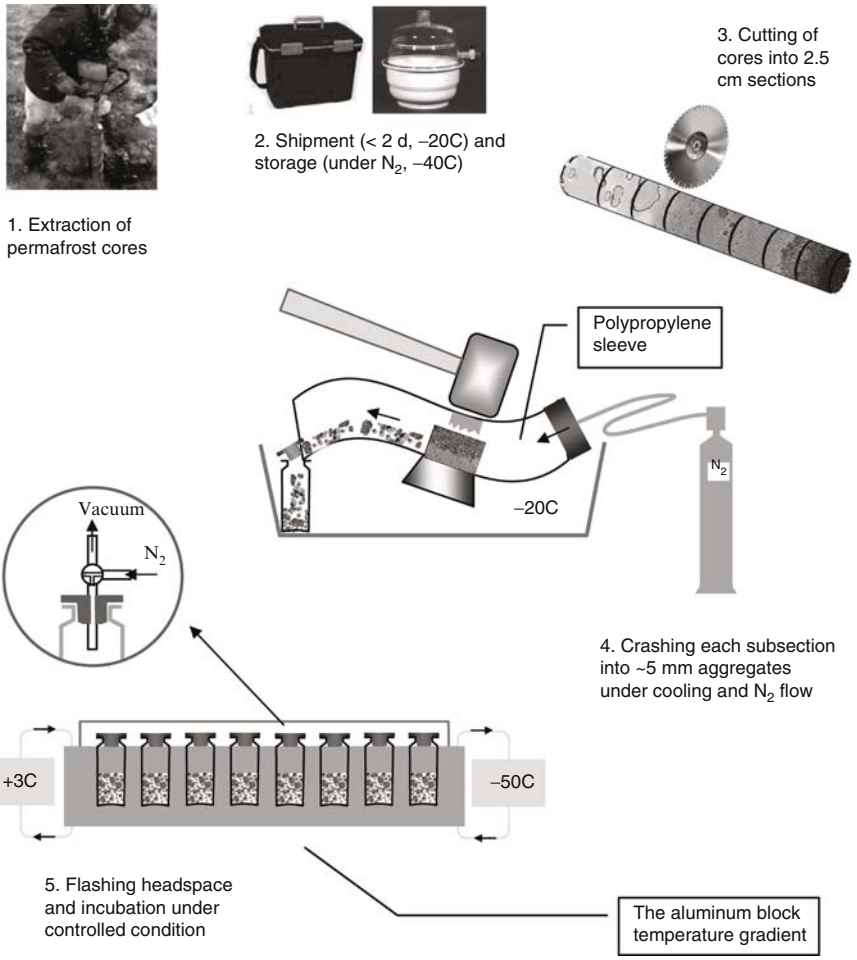

Fig. 9.2 Sampling and processing of permafrost samples for the measurement of metabolic activity of indigenous permafrost microorganisms including strictly anaerobes

below-zero temperature while the rubber septum stays outside the cooling range, remaining soft and resilient to multiple needle piercing and accessible to analysis.

9.2.2 Advantages and Disadvantages of Various Methods for Detecting Microbial Activity

The list of available techniques is shown in Table 9.1. The rate of incorporation of labeled DNA and protein precursors (thymidine and leucine, respectively) is the most popular method for testing homogeneous frozen objects, such as sea and glacier ice,

Table 9.1 Techniques used to measure microbial activity in permafrost and other naturally frozen habitats

Technique		Habitat	Temp (°C)	References	Advantages of technique	Methodological limitations
Incorporation of labeled precursors	DNA (^{3}H-thymidine) and proteins (^{14}C-Leucine)	Glacial ice bacteria	−15	Christner (2002)	• High sensitivity • Clear physiological and biochemical interpretation of data • Can be combined with subsequent analysis of labeled constituents	• Disturbance of natural community by substrate addition and thaw-refreezing • Technique is destructive (cannot be used repeatedly on the same sample)
		South Pole Snow	−17 to −12	Carpenter et al. (2000)		
	Proteins (various ^{3}H and ^{14}C-amino acids)	Arctic sea ice	−20 to + 1	Ritzrau (1997) and Junge et al. (2006)		
	Lipids (^{14}C-acetate)	Siberian permafrost	−20 to 0	Rivkina et al. (2000)		
Gas evolution	CO_2	Barrow, Alaska	−40 to 0	Panikov et al. (2006)	• High precision and sensitivity, availability of respective analytical instruments • Relevance to high-priority green-house gases research	• Overestimation of activity resulted from release of gases accumulated in sample before measurements • Underestimation because of time delay between formation and release of gases
		Tussock tundra, Alaska	−12 to 0	Mikan et al. (2002)		
	CH_4	Siberian permafrost	−16.5 to 0	Rivkina et al. (2002)		
	N_2O	Alpine tundra, Colorado	−5 to 0	Brooks et al. (1997)		
Gradient of gases		Mountain glacier, Bolivia	−40 to 0	Campen et al. (2003)		

(continued)

Table 9.1 (continued)

Technique		Habitat	Temp (°C)	References	Advantages of technique	Methodological limitations
Gas uptake	O_2	Antarctic peat	−1 to + 1	Wynn-Williams (1982)		• Low sensitivity
	Light $^{14}CO_2$ uptake	Alpine, Tibet	−10 to + 20	Kato et al. (2005)	• Highly sensitive and simple technique	• Technique is destructive because of requirement to extract the labeled cell constituents
		Endolithic lichen, Antarctica	−24 to + 5	Kappen and Friedmann (1983) and Kappen (1993)	• Does not require substrate addition and thaw of frozen sample	
	Dark $^{14}CO_2$ uptake	Permafrost and tundra, North Slope of Alaska	−80 to 0	Panikov and Sizova (2007)	• Results are not affected by gases accumulation prior to measurements	
Organic matter decomposition	Net N mineralization and nitrification	Taiga and tundra soils, Alaska	−5 to + 5	Clein and Schimel (1995)	• Assessment of the in situ processes • Provides data for entire outdoor ecosystem	• Poor temporal resolution
						• Low sensitivity
		Tundra, Alaska	−30 to + 5	Schimel et al. (2004)		• Difficulties in data interpretation stemming from stochastic and seasonal variations of temperature and other environmental factors
	Plant litter weight loss	Tussock tundra, Alaska	−30 to + 5	Hobbie and Chapin (1996)		
	Loss of K, Mg, P, phenolics and carbohydrates	Subarctic woodland, Canada	ND	Moore (1983)		
	Oxidation of ^{14}C-labeled compounds added to frozen sample	Barrow tundra, Alaska	−40 to 0	Panikov et al. (2006)	• High sensitivity • High specificity • No effect of gases present before analysis	• Disturbance of microbial community by thaw-refreezing and addition of substrate
UV Microscopy	Low-temperature cells staining and microscopy in the walk-in cold room	Arctic sea ice	−20 to −2	Junge et al. (2004)	• High spatial resolution at micro-scale	• Technique is not quantitative • Possible changes in microenvironment by staining and microscopy

polar snow, supercooled cloud droplets, etc., but sometimes it is also used for soils. This technique is sensitive, and characterizes two basic intracellular processes, DNA and protein synthesis. The major disadvantage is that the procedure is destructive, and requires preliminary ice or soil thaw which could be sources of artifacts. In addition, there are some general uncertainties (Karl 1980), e.g., strong dependence of results on the amount of added nucleoside or amino acid: if it is too small, then endogenous synthesis is not suppressed and the incorporation rate is underestimated; if the amount is too large, then the trophic status of the sample is changed.

Microscopy in combination with oligonucleotide probes and stains visualizing active cells (like 5-cyano-2,3-ditoyl tetrazolium chloride, CTC) is a potentially powerful tool; however, so far it provides only qualitative information on the state of cells in frozen samples, rather than on activity or growth rates.

The microbial activity in heterogeneous habitats (frozen soils, permafrost) is estimated most often either through exchange rates of gases (CO_2, O_2, CH_4, N_2O), or by recording decomposition processes, e.g., plant litter weight loss or N net mineralization. The second approach characterizes the in situ process, which is a great advantage but is destructive and not sensitive. The major reason for low sensitivity is that decomposition dynamics provides a time-averaged *integral curve* rather than an *instant rate* of a particular microbial activity related to the current temperature or other environmental factors. Besides, recorded data are usually difficult to interpret because the observed dynamics are a sum of several simpler processes having often opposite signs, e.g., production–consumption, decay–synthesis, immobilization–mobilization. Decomposition of labeled individual compounds (e.g., ^{14}C-glucose) is much less complex, but should be classified as potential substrate-induced microbial activity.

9.2.3 Why Might Soil Respiration Produce Misleading Results?

The gas exchange rates are measured instantly and with high precision. Care should be taken however about possible artifacts associated with the "sticky" nature of some gases, such as CO_2. Recently, Panikov et al. (2006) measured CO_2 evolution from frozen tundra samples and found abnormal response of sterile controls (autoclaved and refrozen sample): instead of declining, the rate of CO_2 evolution from sterile controls increased (Fig. 9.3, insert). The most reasonable explanation was that measured "soil respiration" is severely compromised by abiotic release of CO_2 + HCO_3^- accumulated in soil before the laboratory test. But why did autoclaving stimulate this release? We repeated measurements of CO_2 evolution from several soils under conditions preventing biological activity, e.g., with soils incubated under pure N_2, poisoned with $HgCl_2$ and benzoic acid, or desiccated (see Fig. 9.3). Abiotic flux of CO_2 was always rather intensive from humic soil layers even in non-calcareous soils, and autoclaving always stimulated CO_2 release. The dynamics of abiotic CO_2 evolution was approximated by the double exponential equation (Fig. 9.3, insert) indicating the existence of at least two pools of CO_2.We speculate

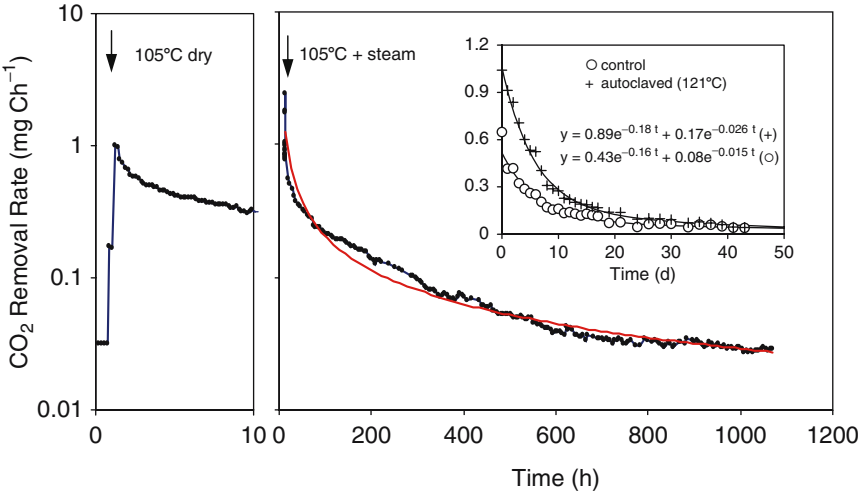

Fig. 9.3 Demonstration of abiotic CO_2 production compromising value of "soil respiration" as a measure of subzero biological activity. *Main panel:* Long-term dynamics of CO_2 release from the soil column during its flushing with N_2 and dry heating (*first arrow*) followed by treatment with overheated steam (*second arrow*). The soil is from the upper soil layer of the NJ forest soil. After 1,000 h (42 days), the cumulative amount of released CO_2 was 20.11 mg C g^{-1} soil or 38% of the total soil C. *Insert:* CO_2 evolution from the same soil at 25°C after autoclaving (30 min, 121°C) as compared with the untreated control. Note that autoclaving increases CO_2 release. Continuous curves were fitted to double exponential regression equation displayed on the graph

that the first pool is formed by free or loosely bound CO_2 (probably surface binding to soil particles and gas dissolved in soil water) which stays in equilibrium with the gas partial pressure in soil air (Henry law); therefore, it is easily removed by soil flushing. The second larger pool is represented by tightly bound CO_2 molecules. It should be a non-covalent interaction between CO_2 and soil phase and probably involves gas molecules entrapment within a soil inter-aggregate space, such as half-closed microscopic cavities formed by organo-mineral complexes. Heating and especially steam-flushing (as during autoclaving) eventually remove firmly bound CO_2, probably via competitive replacement of physically or H-bounded CO_2 with water molecules.

To release 95% of the total CO_2 we had to spent 43 days (!) of continuous soil flushing with overheated steam at 105–110°C (Fig. 9.3). It is remarkable that the size of this pool is equivalent to ca. 40% of the total soil C measured by soil ignition! It was proven that released CO_2 was not a pyrolysis artifact because heating to the same temperature without gas flow produced a negligible amount of CO_2.

The main conclusion derived from this methodology work is that cold-season in situ CO_2 emissions or laboratory-measured CO_2 evolution from frozen soils have two components: the bigger one is abiotic release of accumulated CO_2, and the smaller one could be instant respiratory activity of psychrophilic soil biota. The

total flux of unlabeled CO_2 from frozen soils should significantly overestimate an actual respiration of microbial community. Overestimation of methane and N_2O generation is probably smaller due to higher mobility of these gases, but it should be tested in future. Oxygen uptake is impractical because of low precision (too high ambient content of O_2 in atmosphere) and possible abiotic oxidation reactions.

9.2.4 Methods Based on $^{14}CO_2$ Uptake

These methods are free from limitations inherent in CO_2 evolution, because the addition of labeled CO_2 to gas phase over soil does not affect the soil trophic status, and sticky soil CO_2 does not interfere with the detection of added $^{14}CO_2$. An additional significant advantage is that exposure to $^{14}CO_2$ does not require preliminary melting of frozen soil. A minor disadvantage inherent to the majority of techniques based on radioactive indicators is that analysis is destructive: we have to sacrifice the incubated sample, and we can't set up a continuous monitoring of $^{14}CO_2$ uptake with the same sample or laboratory microcosm.

At least three groups of soil organisms are responsible for $^{14}CO_2$ uptake: (i) photoautotrophic, (ii) chemolithotrophic (chemosynthetic), and (iii) chemoorganotrophic (heterotrophic) organisms.

The contribution of the first group is quantified by using artificial illumination and calculating the difference between ^{14}C-uptake under light and the dark control. Historically, photosynthetic CO_2 uptake by Antarctic lichens was probably the very first reliable measurement of below-zero microbial activity in situ (Lange and Metzner 1965; Lange and Kappen 1972).

Dark CO_2 fixation (DF) refers tothe activity of the second (chemosynthetic) and third (heterotrophic organisms, the most abundant in soils) microbial groups. They could be differentiated by using specific autotrophic inhibitors (acetylene, allylthiourea, etc.) or by observing DF stimulation by the addition of oxidizeable inorganic substrates, such as H_2, NH_4^+, S^{2-}, S°, Fe^{2+}, etc.

Heterotrophic microorganisms always use CO_2 in biosynthetic reactions (so-called heterotrophic fixation, HF), although this process is hidden by simultaneously occurring respiratory release of CO_2. Therefore, we have to add an isotope indicator to measure HF. Specifically, CO_2 can be fixed in several fermentation pathways and in the anaplerotic reactions of the tricarboxylic acid (TCA) cycle via carboxylations of pyruvate or phosphoenol pyruvate (PEP) at the expense of ATP or by running a reverse TCA cycle.

Computational modeling of metabolic flux indicates that the net contribution of HF to total cellular synthesis is about 40% (Marx et al. 1996), but the empirically found stoichiometric ratio varies from 1% to 10%, with an average close to 6% (Johnson and Romanenko 1984; Santruchkova et al. 2005).

In the rest of this review, this technique will be referred to as DF because a differentiation between chemosynthetic and heterotrophic organisms has not been done. The brief protocol for testing DF is as follows: 2.0 g of frozen soil crumbled

in 3–10 replicated 30 ml vials with rubber septum are incubated with $^{14}CO_2$ in the headspace (at least 1,000 DPM ml^{-1}); after a certain period (usually after 1 week of incubation at −5 to −25°C) one of the replicated vials is sacrificed. The headspace is sampled for the total CO_2 (LiCor 800) and $^{14}CO_2$ (1 N NaOH trap following counts with Beckman 5800 L scintillation counter) to determine isotope dilution. Then the soil is flushed with N_2 and dried for 3 h at 95°C to remove non-reacted $^{14}CO_2$. Finally, the fixed ^{14}C is released and counted as $^{14}CO_2$ after soil ignition at 900°C (Solid Sample Module, TOC-VE, Shimadzu) (Panikov and Sizova 2007). Linear dynamics in ^{14}C uptake at least during the first month of incubation at −11°C has been demonstrated; the sterile (autoclaved and refrozen) control demonstrated zero retention of $^{14}CO_2$.

9.3 Below-Zero Microbial Activity in Alaskan Tundra and Permafrost: Variation and Underlying Mechanisms

In this section, our own data (Panikov 1999a, b; Panikov and Dedysh 2000; Panikov et al. 2006; Panikov and Sizova 2007) on the spatial variation of below-zero microbial activity in Alaskan tundra are summarized, followed by the analysis of the major environmental factors restricting subzero microbial activity. Among these factors, there will be a focus on (i) the porosity of frozen soils, which affects mass-transfer (mainly diffusion) rates within frozen soils, (ii) deficiency of available water, (iii) low temperature per se (low kinetic energy of reactants), and (iv) long-term damage caused by gamma radiation.

9.3.1 Vertical Profiles of DF

There are data on four Alaskan sites: Barrow (N 71°18′, W 156 °47′), Franklin Bluffs (N 69°40′, W 148 °41′), Sagwon (N 69°25′, W 148 °41′), Fairbanks (N 64° 52′, W 147 °52′). All four sites were represented by wet tundra with some peat accumulation; three sites are acidic (pH 4.5–5), and Franklin Bluffs is neutral carbonaceous soil. Figure 9.4 shows vertical variation of DF along soil profiles measured at −11°C. It was highest in the top soil layers, and declined with soil depth. The closest correlation was found between DF and the content of organic matter, as well as between DF and the total amount of microbial biomass estimated as phospholipids fatty acids (PLFA) (Fig. 9.5). This correlation was particularly clearly expressed in the Fairbanks profile, where two peaks of soil C were observed: the top modern humus layer at 0–25 cm and the old buried humus layer at 50–60 cm.

The coldest sites (Franklin Bluffs and Sagwon) had higher DF activity than the warmer Fairbanks site, although their activity above the freezing point of water was approximately the same (data not shown). Earlier, we have shown that also tundra and permafrost have higher below-zero activity than boreal soils, and in the Barrow

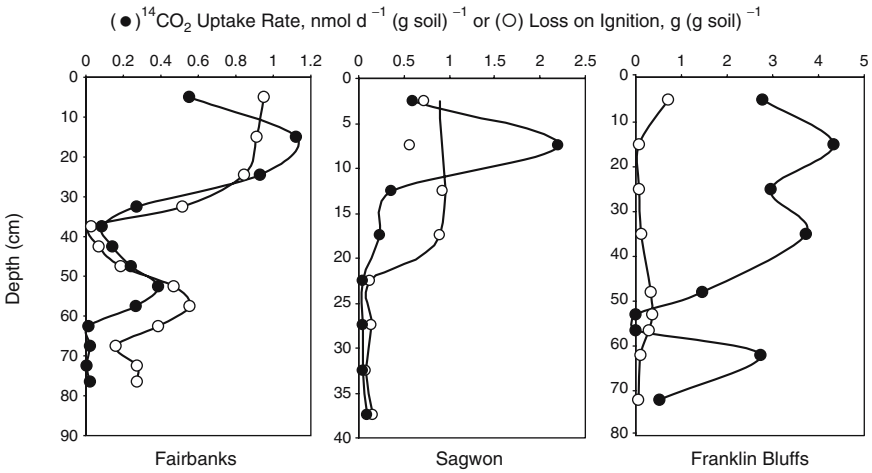

Fig. 9.4 Vertical distribution of below-zero microbial activity in three Alaskan sites: dark $^{14}CO_2$ uptake (•) and loss on ignition (○) as a measure of the organic matter content. Note the difference in scales

site soil respiration at −20°C was consistently higher in deeper permafrost layers than in the top seasonally frozen soil, while the above-zero respiration displayed the reverse trend (Panikov et al. 2006). This observation supports the view on the adaptive nature of below-zero activity, acquired by specialized microorganisms under pressure of natural selection in polar regions. It is in agreement with the emergent ecological theory on acclimation and adaptation of Arctic organisms to cold conditions. This theory has been firmly established for plants and animals (Mooney and Billings 1961; Tjoelker et al. 1999) and now can be confirmed for microorganisms.

9.3.2 Slow Molecular Diffusion in Frozen Soil as Possible Restriction Factor

Pure ice does not allow gas diffusion; that is why air entrapped in the Greenland and Antarctic ice has been used for chronological reconstruction of the Earth atmosphere (Brook et al. 1996). There are ice lenses in polar soils and subsoils which serve as barriers to gas diffusion. However, the bulk of permafrost and seasonally frozen soils represented by mosaic of frozen water, solid organo-mineral particles and fine network of gas-filled pores and channels should be conductive for gases and probably to soluble compounds. To find out the rate of gas diffusion, we used the following experimental approach. First, we obtained intact permafrost aggregates by gentle crashing the core avoiding its melting. One single intact aggregate (ca. 15 mm in diameter) was placed into a vial precooled to −20°C, $^{14}CO_2$ was

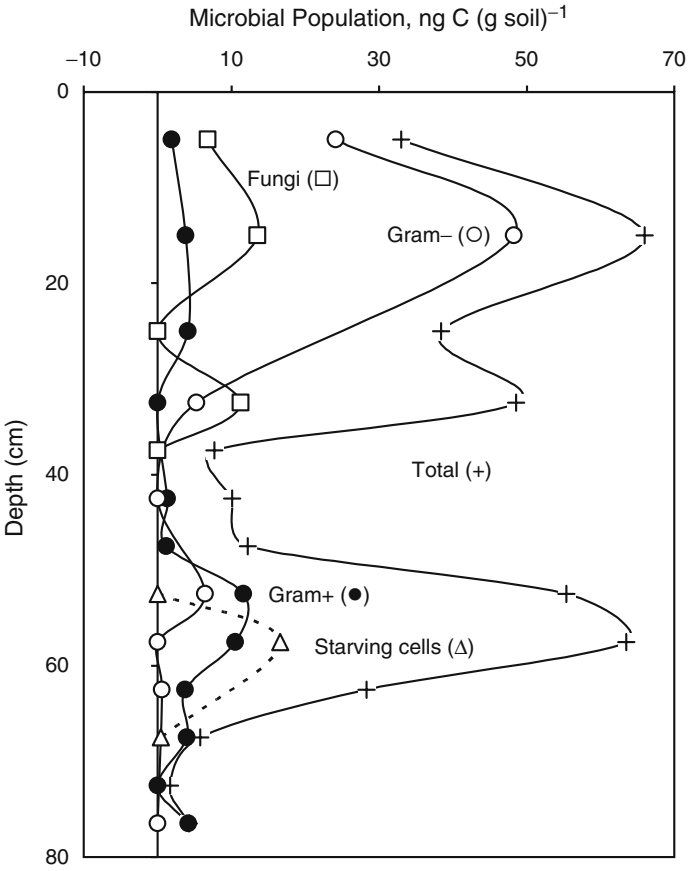

Fig. 9.5 Vertical distribution of microbial groups as determined by membrane fatty acid analysis. Site: Fairbanks, forest soil with buried organic layer

injected into headspace, and after an exposure over 0.5–4 days, the label penetration was quantified by serial washing of the aggregate with cooled (~0°C) 0.5 N NaON. The alkaline solution was used to remove layer-by-layer the surface material containing label, leaving the aggregate core frozen. The accompanying reduction in the aggregate size was recorded with a TV camera and converted to volumes by image analysis, and the leached label was counted by scintillation.

Results are presented in Fig. 9.6 for the Fairbanks soil sample taken from the second buried organic layer. Contrary to pure ice, this permafrost is highly conductive to gases. The apparent diffusion coefficient for CO_2 as estimated from its spatial gradient was found to be 6.9×10^{-9} cm^2 s^{-1}. For comparison, the diffusion coefficient for CO_2 in air at the same temperature of −20°C is 0.119 cm s^{-1}, or 10^7 times higher. Another Fairbanks sample taken from the lower mineral layer (70–80 cm) displayed 15 times slower $^{14}CO_2$ diffusion. The most probable mechanism of gas

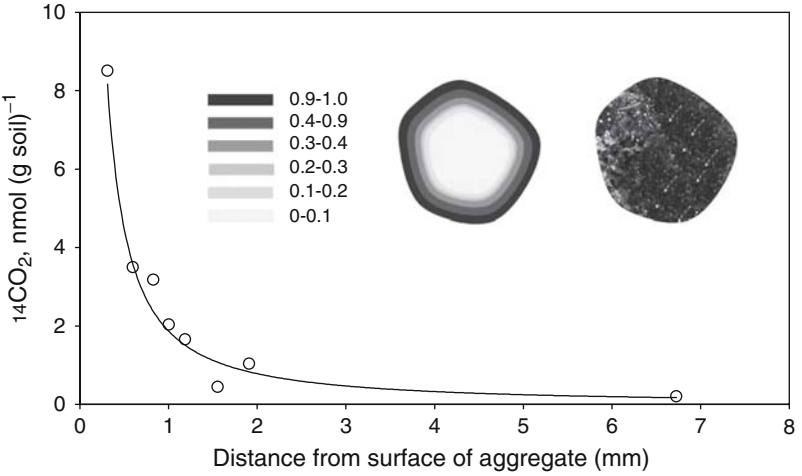

Fig. 9.6 Diffusion of $^{14}CO_2$ to inner space of the frozen aggregate. The *insert* shows $^{14}CO_2$ concentration distribution inside of 14.5 mm permafrost aggregates from Fairbanks, layer 50–60 cm, after 48 h of exposure to labeled gas at −20°C

penetration into permafrost is molecular diffusion via tiny aeration pores. Judging from gas penetration dynamics, the partial contribution of aeration pores to bulk volume of this permanently frozen organic soil layer (about 50% of organic matter) was as low as 5.8×10^{-8} (compared with the typical value of 0.2–0.4 for top soils at a moisture content of 50% of the maximum water-holding capacity). Obviously these frozen mineral soils are even less conductive. In any case, the tested soils have enough air-filled micropores to support slow aerobic growth. Apart from CO_2 and O_2, frozen soils should allow also the delivery of volatile organic substrates (alcohols, hydrocarbons, fatty acids) as a carbon and energy source for heterotrophic microorganisms.

We have not measured the mobility of non-gaseous compounds, and respective rates are expected to be slower by a factor of 10^5, the difference in diffusivity of gases in gas and liquid phases. Even such low mobility could be sufficient to deliver compounds soluble in unfrozen water films around cells. Indirect confirmation of this possibility comes from our data on oxidation of ^{14}C-glucose added to permafrost from Barrow in the temperature range from 0 to −35°C (Panikov et al. 2006).

9.3.3 Water Deficiency

All reviews on effects of freezing on microbial cells (Mazur 1980; Kushner 1981; Vorobyova et al. 1997) put the main emphasis on the state of water inside and immediately outside the cells. The formation of intracellular ice crystals is considered a critical

factor affecting survival of frozen cells, and cold-resistance is normally attributed to intracellular antifreeze compounds preventing the formation of crystals.

Some liquid water exists in soils at temperatures below freezing (Ershov 1998). The thickness of such quasi-liquid water film was calculated to be ca. 50 nm (Anderson 1967). Such thin water film could cover only a fraction of cells or form an external unfrozen water shell around the bacterial cell, but cannot provide continuous water channels to move around the icy space. Wolfe et al. (2002) used several reasonable assumptions about the geometry of surface and thermodynamic variables to derive the following simple equation relating unfrozen water content (UW) to freezing temperature (ΔT):

$$\mathrm{UW} = 3\times10^{18}\ln\frac{10^3}{\Delta T}\,\mathrm{molecules\,m^{-2}} = 5\times10^{-6}\ln\frac{10^3}{\Delta T}\,\mathrm{moles\,m^{-2}}. \qquad (9.1)$$

This equation agreed well with experimental data (Romanovsky and Osterkamp 2000) on unfrozen water content in Sagwon site (Fig. 9.7). We plotted on the same figure the temperature-dependent content of water vapor over ice, assuming that some permafrost microorganisms could acquire water from the gas phase through aquaporins; these specialized water-transporting channels in membrane play an important role in microbial freeze-resistance (Tanghe et al. 2006). The content of unfrozen water declines abruptly just below the freezing point and then decreases slowly with further cooling, while humidity (water vapor content) displays uniform decline within the entire temperature range above and below the freezing point.

9.3.4 The Effect of Temperature per se

The temperature per se is related to kinetic energy of reactants. The cooling should progressively slow-down metabolic reactions but cannot stop them completely, due to the exponential nature of energy distribution (i.e., it approaches zero when temperature is approaching 0 K). In the real processes, we can't vary incubation temperature without affecting soil water content, but we can do it mathematically by using multiple regression. Figure 9.8 shows temperature-dependent changes in metabolic activity (respiration and DF) and available water (UW and air humidity). These data were fitted to multiple non-linear regression of metabolic activity (v) on two factors, temperature, Celsius (T) and available water content (W). The best results were obtained with double exponential regression:

$$v = A\times e^{\lambda T}\times e^{kW} = A\times e^{\lambda T + kW}, \qquad (9.2)$$

where A, λ and k are kinetic constants.

The empirical exponential term $\exp(\lambda T)$ could be replaced with the more meaningful Arrhenius term containing the temperature in Kelvin (K) and the parameter E_a (energy of activation):

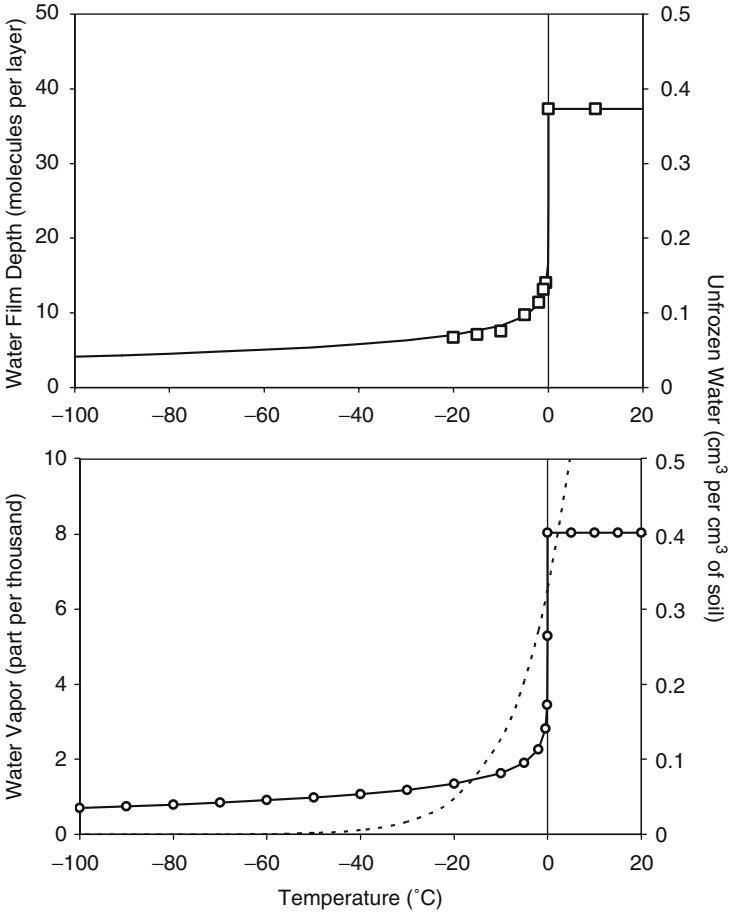

Fig. 9.7 Dependence of unfrozen water content on ambient temperature in permafrost. *Top:* The calculated unfrozen water content [*continuous line*, (9.1)] plotted vs experimental data points (Romanovsky and Osterkamp 2000) for Sagwon site, AL (□). *Bottom:* The relationship between unfrozen water content (○) and relative humidity of air over frozen soil (*dotted line*)

$$v = A \times \exp(kW) \times \exp\left(\frac{-E_a}{RK}\right) = A \times \exp\left(kW - \frac{E_a}{RK}\right). \tag{9.3}$$

The agreement between (9.2) or (9.3) and experimental points was good enough to carry out a separate account of factors T and W. We prefer to use the Celsius temperature (equation 9.2) rather than K, since this is more common in biological literature. The influence of T alone was expressed by the parameter λ which is related to the traditional parameter Q_{10}, which explains how many times the reaction rate is accelerated per every 10 degrees of temperature shift-up: Q_{10}= exp(10λ).

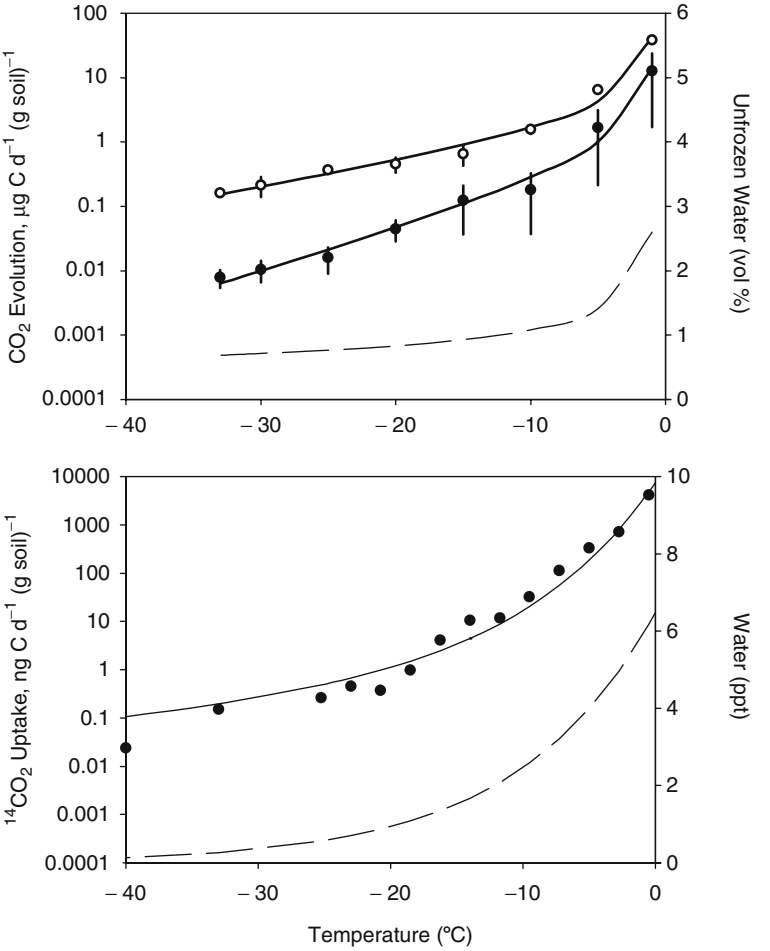

Fig. 9.8 The effect of below-freezing temperature on available water and microbial activity. *Top* (Panikov et al. 2006): the rate of $^{14}CO_2$ production from the ^{14}C-glucose (•) added to Barrow (AL) soil, the total rate of CO_2 evolution (○) and unfrozen water content (*dotted line*) determined in the field by Romanovsky and Osterkamp (2000). *Bottom:* the rate of $^{14}CO_2$ uptake (•) and water vapor concentration (*dotted line*) during Sagwon soil laboratory incubation (Panikov and Sizova 2007). *Continuous solid lines* were calculated from equation 9.2 with the following parameters: $\lambda = 0.078$, $k = 1.65$ (CO_2 evolution); $\lambda = 0.135$, $k = 1.77$ (^{14}C-glucose oxidation); $\lambda = 0.063$, $k = 1.36$ (DF)

A cooling by 10 degrees caused a 1.9-fold decrease in DF and a 2.1–3.8-fold decrease in respiration, which is very close to the "typical" Q_{10} value of 2–3 observed in a majority of above-zero biological processes. Therefore, the effect of temperature alone remains the same above and below the freezing point of water, and the experimentally observed steep decline in metabolic activity below the freezing point should be caused by the abrupt decrease in availability of water, not by temperature.

Surprisingly two metabolic processes, ^{14}C-glucose oxidation and respiration in Barrow (Fig. 9.8, top) and DF in Sagwon soil (Fig. 9.8, bottom), correlated with different forms of available water: the first was more closely related to UW, while the second one correlated better with air humidity. Further studies are needed to clarify this discrepancy and decide whether the source of the samples or the type of metabolic processes is more important.

What is the lower temperature limit for microbial growth and activity? We still do not have a clear answer, and there is a wide range of opinions from extreme skepticism denying metabolic activity at −10°C (Warren and Hudson 2003) to the overoptimistic statement that "there is no evidence of a minimum temperature for metabolism" (Price and Sowers 2004). We have found that even at the lowest tested temperature of −40°C the rate of $^{14}CO_2$ incorporation exceeded the background level of the killed control. Moreover, the entire "activity-temperature" plot was smooth and continuous, indicating a progressive decline with cooling below the freezing point rather than some threshold. Therefore, we are inclined to support the opinion expressed by Price and Sowers (2004) with the truistic reminder that any processes should stop completely before approaching 0 K, metabolic reactions being no exception. In future, it would be important to find out whether at temperatures approaching 0K (–273°C) its effect on microbial activity would deviate from equations 9.2 or 9.3. Such deviation would indicate an existence of the minimal temperature or implication of factors other than temperature and available water content. Note that testing of temperature effects below −40°C would require a rather expensive experimental setup, longer incubation times and highly sensitive analytical instruments.

9.3.5 The Effects of Addition of Nutrient Substrates

The addition of complex substrates (yeast extract, proteins, and broth) to frozen soil had mostly negative effect on DF, while volatile compounds and gases (ethanol, methanol, CH_4 but not H_2) stimulated DF as compared with unamended controls with added deionized water. The reason for inhibition by yeast extract and proteins is obscure. The regulatory repression of anaplerotic enzymes by complex substrates seems unlikely, as indicated by experiments on $^{14}CO_2$ fixation by pure bacterial cultures grown on various substrates (Hesselsoe et al. 2005). On the other hand, stimulation of DF by gases and volatile compounds is in full agreement with our finding that frozen soils allow diffusion of these compounds into internal space. Probably, microbial species able to utilize the mobile C-compounds have a selective advantage in permafrost and seasonally frozen soils.

9.3.6 Physiological State of Microorganisms in Frozen Soil

No doubt, many microorganisms found in frozen soil should be in a dormant stage (endo- and exospores, cysts, non-spore anabiotic cells, etc.). This review does not touch dormancy, as we are looking only at metabolically active cells. They may be

represented by normally growing organisms and those who maintain their viability and convert some substrates, but do not grow or multiply (so-called state of maintenance). The second state (maintenance) has been postulated for microorganisms active below freezing point (Bakermans and Nealson 2004; Price and Sowers 2004), but this hypothesis has not been tested until recently.

The state of maintenance is defined as zero growth rate with non-zero consumption rate of energy source (Panikov 1995); therefore yield at this physiological state should be zero. To measure the subzero growth yield of soil community, we incubated soil with ^{14}C-ethanol and measured label partitioning between CO_2 (oxidation of ethanol equivalent to respiration rate), cells (label incorporation equivalent to cell growth) and unused substrate + exometabolites (Panikov and Sizova 2007). It was found that the cooling of frozen soil from 0 to −16°C resulted in a dramatic decline of the respiration and incorporation rates, but their ratio remained almost constant; the growth yield remained practically constant (0.54 ± 0.09 g C-cell per g C-ethanol) and close to values reported for pure microbial cultures grown on unfrozen laboratory media.

In another experiment (Panikov and Sizova 2007), the eukaryotic consortium *Leucosporidium-Geomyces* was grown at −8°C and then subjected to a temperature shift-down by moving individual tubes with culture to various temperatures between −8 and −25°C. As shown in Fig. 9.9, the rates of respiration and DF after the temperature shift-down was biphasic: for the first 2–3 weeks the consortium remained

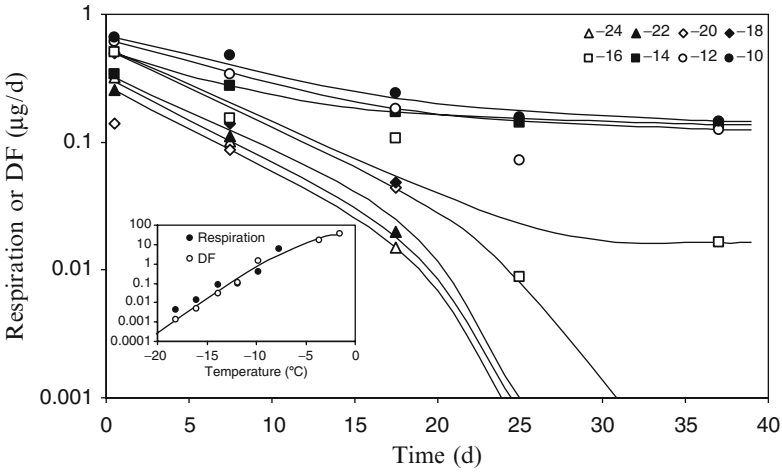

Fig. 9.9 Demonstration of subzero activity of microbial consortia isolated from permafrost during growth on microcrystalline cellulose with ethanol as a sole carbon and energy source (Panikov and Sizova 2007). The enrichment containing basidiomycetous fungi and leucosporidial yeasts was isolated from Fairbanks site on solid ethanol-mineral medium with cellulose powder at −8°C without antifreeze. At time zero, incubation temperature was shifted from −8°C to lower temperatures as indicated on legend. *Main panel*: CO_2 evolution rate (microbial respiration) as dependent on incubation temperature *Insert:* plot of microbial respiration rate (•) and DF (○) vs temperature

active even at the lowest temperature of −25°C, then growth stopped in the temperature interval −25°C to −18°C, but continued at temperatures from −16°C to −8°C. The insert panel in Fig. 9.9 plots the respiration and DF vs temperature for the second growth phases. Again, we can clearly see that respiration (energy-generating process) and DF (anaplerotic process related to cellular biosynthesis and growth) do change synchronously with cooling. Both processes stopped between −16°C and −20°C, and we never observed even transiently that cessation of growth (zero DF) was associated with non-zero respiratory activity, as should be expected at the state of maintenance. Based on the described experiments, it can be concluded that the attractive hypothesis on maintenance state (Price and Sowers 2004) has not been confirmed experimentally, and can be safely rejected as inappropriate.

How can biphasic respiration dynamics be interpreted? Most probably, the first phase was endogenous respiration of reserves accumulated at −8°C. It could be specialized reserved compounds like poly-β-hydroxyalkanes or glycogen, or non-specific endogenous substrates, such as cellular proteins, nucleic acids and cell-wall components (Panikov 1995). In chemostat, an endogenous self-digestion is used as an energy source to drive cell entry into the stationary phase, to express gene *rpoS* and synthesize hundreds of new enzymes required for survival under starvation conditions (Reeve et al. 1984; Zgurskaya et al. 1997). In the case of temperature shift-down, endogenous respiration could play a similar role of cellular reconstruction to adjust the intracellular machinery to function under colder conditions.

9.4 Microorganisms Responsible for Activity Below the Freezing Point

9.4.1 Microbial Diversity in Permafrost

Viable bacteria in permafrost were first documented as a part of investigations of mammoths in Siberia (Becker and Volkmann 1961; Cameron and Morelli 1974). High numbers of viable microorganisms (up to 10^5–10^7 CFU g^{-1}) were reported by plating, and main efforts were directed to application of molecular tools through sequencing of 16S rDNA (Shi et al. 1997; Zhou et al. 1997). The detected phylotypes formed 11 established lines of descent of bacteria and one entirely new sequence not assigned to any of the known groups. Most of the clones belonged to the alpha (20.9%) and delta (25.6%) subdivisions of the *Proteobacteria*, with lesser proportions in the beta (9.3%) and gamma (4.7%) subdivisions, groups typically isolated from soil by culture methods. The majority of permafrost-derived clones (77%) had sequences similarities less than 95–80% with those in the database, indicating the predominance of new genera or families.

In the last 5–10 years, the highest number of new microbial species have come from aquatic cold habitats: sea ice, polar lakes and snow crust. Surprisingly, sea ice presented the unique particular case of a high degree of culturability of the natural

community (up to 65% from direct microscopic count) (Junge et al. 2002). Culture-independent analysis based on 16S rRNA and conventional isolation revealed rather limited diversity of psychrophilic organisms, all of them belonging to either *Proteobacteria* or *Cytophaga-Flexibacter-Bacteroides* (Gosink and Staley 1995; Irgens et al. 1996; Gosink et al. 1998; Junge et al. 1998, 2002; Staley and Gosink 1999). Microbial communities of the continental icy habitats, including Lake Vostok accretion ice (Christner et al. 2001; Brinkmeyer et al. 2003), Tibetan plateau ancient glacier (Christner et al. 2003a, b) and cold deep Atlantic sediments (Xu et al. 2003), seem to be more diverse.

9.4.2 Development of Isolation Technique

The majority of known techniques for microbial isolation below the freezing point are based on using liquid media with glycerol or other antifreeze compounds (Breezee et al. 2004). The lowest temperature limit for isolates obtained by this approach was −10 to −12°C. The disadvantage of using supercooled liquids is obvious. First, it is technically unreliable at temperatures below −7°C, some flasks turn frozen for seemingly unknown reasons. Secondly, we cannot proceed to the lower and extremely challenging temperatures which are expected to dominate in the polar desert or outside the Earth. Thirdly, homogeneous liquid media are fine for aquatic bacteria, but often are inappropriate for terrestrial habitats such as soils and permafrost. We developed a solid-state cultivation system (Panikov and Sizova 2007) which can be used at any below-zero temperature, and more closely imitates natural growth conditions in permafrost. Solid-state cultures are grown as thin frozen film between plastic sheets or in powder of microcrystalline cellulose with ethanol or other appropriate C-sources, volatile or soluble. We never detected significant subzero degradation of cellulose or other polysaccharides. Probably, the degradation of polymeric compounds requiring the synthesis of extracellular hydrolytic enzymes is completely arrested in frozen media.

Conventional liquid and new solid-state enrichments led to the isolation of different organisms: liquid media resulted in the isolation of bacteria similar to those described in studies of polar aquatic habitats, while solid frozen media allowed the isolation of yeasts and mycelial fungi (Table 9.2). Apart from ethanol, aerobic growth in frozen media was supported by H_2 and succinate. The isolated bacteria belong to new species, but are closely related (95–99% of similarity in 16S or 26S rDNA genes) to known psychrophilic bacteria and fungi recently isolated from sea ice and Antarctic habitats (*Polaromonas, Arthrobacter, Mrakia, Cryobacterium*). The most interesting bacteria *Polaromonas hydrogenovorans* is able to grow autotrophically on the mixture of H_2 and CO_2 or heterotrophically on succinate, pyruvate, and citrate.

Surprisingly, the most active growth in frozen media was displayed by eukaryotic microorganisms, dimorphic yeasts of the genus *Leucosporidium* and ascomycetous fungi of the genus *Geomyces*. The last organisms grew exponentially at −8°C,

Table 9.2 Psychrophilic and psychrotolerant microorganisms isolated from Alaskan permafrost and top soil

Organism, strain	Isolation source	Enrichment conditions	Growth temperature (°C) Min	Max	Closely related phylotypes (BLAST)	Genbank accession number
Pseudomonas sp. 3–2005	Forest soil, Fairbanks, 10–20 cm, frozen 9 months/year	Liquid ethanol – mineral medium, 0°C	−15	25	Antarctic bacterium R-9113 isolated from lake mat (96%)	DQ 094182
Arthrobacter sp. 9–2	Permafrost, Fairbanks, 50–55 cm	Liquid ethanol– mineral medium, 0°C	−15	25	*Arthrobacter sp.* An16 isolated from deep sea sediment (98%)	DQ 094184
Polaromonas hydrogenovorans	Forest soil, Fairbanks, 10–20 cm, frozen 9 months/year	Liquid mineral medium, H_2:CO_2 in headspace, 0°C	−1	25	Polaromonas naphtalenevorans (99%)	DQ 094183
Leucosporidiales spp. MS-1, MS-3	Forest soil, Fairbanks, 10–20 cm, frozen 9 months/year	Ethanol-MCC[a] solid media frozen to −5°C and −8°C	−18	20	*Cryptococcus sp.* Ytty94 Y24 (99%), *Leucosporidium scottii* isolate (97%)	DQ 295018
Mrakia sp. MS-2	Forest soil, Fairbanks, 10–20 cm, frozen 9 months/year	Ethanol-MCC solid media frozen to −5°C	−16	18	*Mrakia sp.* and *M. frigida*, isolated from various Antarctic habitats (100%)	DQ 295019
Geomyces spp. FMCC-1, FMCC-2, FMCC-3, FMCC-4	The same	The same, – 8°C	−35	18	*Geomyces pannorum* from cryopegs (98%); *Aleurodiscus farlowii* Burt, wooddecomposing fungi (100%)	DQ499471 – 74 (ITS region) DQ520619–22 (LSU rRNA)

[a]*MCC* microcrystalline cellulose

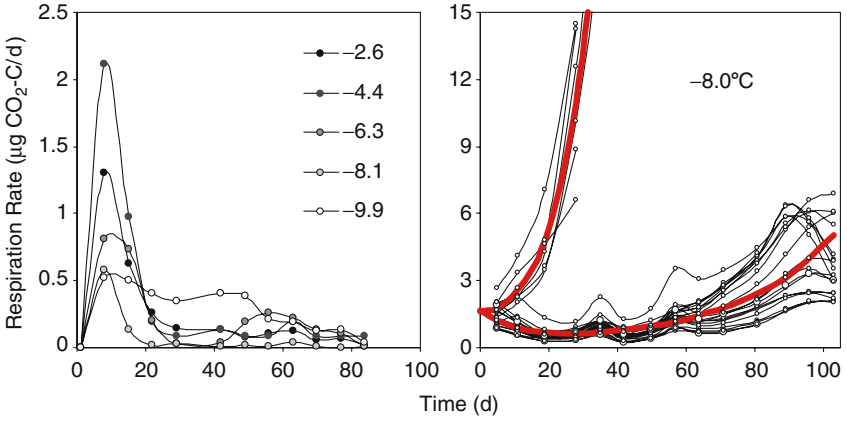

Fig. 9.10 Demonstration of competitive advantage of fungi over bacteria while growing on solid-state frozen media (after (Panikov and Sizova 2007)). *Left:* Growth dynamics of Arthrobacter sp. 9–2. 50 µl of bacterial suspension were frozen in plastic bags 2 × 6" (=inches) with ethanol-mineral medium (EMM), rolled into a tube and placed in a Hungate tube. Growth was followed from the rate of CO_2 production. The legend indicates the growth temperature. *Right:* Growth dynamics of an eukaryotic consortium in frozen ethanol-MCC powder. The eukaryotic consortium (*Geomyces* spp. – *Leucosporidium* spp.) was used as inoculum of 29 tubes containing EMM with cellulose powder. Growth was followed at −8°C as CO_2 production rate. Note that six out of 29 tubes displayed higher growth rates than other slow growers. *Heavy solid curves* are the best-fit exponential equation which ignores auto-oscillations

with a generation time of about 1 week; under further cooling the growth rate and respiratory activity progressively declined, but were still detectable at the lowest tested temperature of −24°C. For comparison, prokaryotic organisms (*Pseudomonas sp* and *Arthrobacter* sp.) grew in solid media only in a progressively declining fashion (not exponentially), indicating the presence of some unknown restriction factor (Fig. 9.10).

9.5 Conclusion

In this review, experimental data on microbial activity in permafrost and other frozen media are summarized. By a deeply rooted and fair tradition, all manifestations of life are intimately associated with the presence of free water. The search for extraterrestrial life is ultimately associated with spotting of large aquatic reservoirs on other planets, "rivers" or "oceans" being the most probable loci accommodating life. In terrestrial studies of permafrost and other cold habitats a similar trend absolutely dominates, with the primary objective of detecting any form of liquid water: brine solutions, vein water in ice, unfrozen water in permafrost. Psychrophilic microorganisms are grown in supercooled liquid media containing high concentration of antifreezes.

The main message of the author is that macroscopically discerned liquid water is not an absolute prerequisite for microbial metabolic activity below the freezing point. Cultivation of psychrophilic microorganisms can be successfully done by using solid frozen media like frozen powder or thin films which allow gas exchange and provide solid support for slowly growing cells. The cryogenic planets in the Solar system could also have spots of biological activity outside extensive bodies of liquid water. More important seem to be continuous-delivery energy sources, such as flux of volatile compounds combined with the presence of adequate electron acceptors.

Permafrost and frozen tundra soils can no longer be considered as a depository of dormant organisms. Adequate conditions for life functions are provided by non-zero gas permeability, the presence of unfrozen water and a supply of mobile oxidizeable compounds. Contrary to sea ice, which has a relatively simple and "young" microbial community with easily domesticated members, the permafrost community is more complex, containing active and dormant populations, culturable and unculturable species with unknown growth requirements. Probably, fungi including mycelial organisms and dimorphic yeasts are more resistant to hostile permafrost environment and display more vigorous growth in frozen habitats than bacteria.

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Chapter 10
Anaerobic Ammonium Oxidation (Anammox)

C. Ryan Penton

10.1 The History of Anammox

The anammox reaction is anaerobic oxidation of ammonium coupled with nitrite reduction under anoxic conditions. This alternative nitrogen removal pathway was first proposed by Richards (1965), following observations of ammonium deficits in anoxic marine basins. Throughout most of the 20th century, ammonium was believed to be inert under anoxic conditions. Canonical denitrification liberates ammonium from organic matter during respiration, resulting in net accumulation in the sediment/soil profile. The proposed 'anammox' pathway allows for the removal of ammonium under purely anoxic conditions. Early evidence for the presence of this reaction was provided by marine sediment porewater profiles where the simultaneous disappearance of nitrite and ammonium was observed (Codispoti and Richards 1976; Cline and Richards 1972). Broda (1977) soon proposed a new type of bacteria responsible for these observations, a "chemosynthetic bacteria that oxidizes ammonia to nitrogen with O_2 or nitrate as an oxidant", which was coined one of two "lithotrophs missing in nature". It was not until 1995 that the anammox process was confirmed in a fluidized bed reactor treating wastewater effluent (Mulder et al. 1995). The anammox reaction is a chemolithotrophic process in which 1 mol of ammonium is oxidized by 1 mol of nitrite to produce N_2 gas in the absence of oxygen (Strous et al. 1999a, b):

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O.$$

Compared to denitrification, this process produces twice the amount of N_2 per mol of nitrite consumed and increases N_2 production in sediments where nitrification is limited. The bacteria responsible for this process were later identified as a deep-branching planctomycete with a peculiar morphology (Strous et al. 1999a, b).

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Despite the early suggestion that many microbes cannot be isolated in pure culture (Winogradsky 1949), the presence of a microbially mediated reaction that disputed the notion that ammonium was inert under anoxic conditions was initially regarded with skepticism. Since then, numerous studies have identified anammox as a key process in the global nitrogen cycle.

10.2 Anammox Physiology and Metabolism

All currently known bacteria capable of anaerobic ammonium oxidization belong to a deep-branching lineage of the order *Planctomycetales* with high genus level diversity (Freitag and Prosser 2003; Schmid et al. 2003). The evolutionary distance among the anammox genera is large (<85% 16S rRNA gene nucleotide identity), though they share the same basic anammox metabolism and cell structure. There are currently four *Candidatus* genera whose grouping is largely based on 16S rRNA sequences: the "freshwater" Kuenenia (K. stuttgartiensis; Schmid et al. 2000) and Brocadia (B. anammoxidans (5) and B. fulgida (22)), and the "marine" anammox Scalindua (S. sorokinii, S. brodae, and S. wagneri; Schmid et al. 2003). The fourth *Candidatus* genus has one member, Anammoxoglobus propionicus (Kartal et al. 2007b), which exhibits an alternative metabolism. Anammox bacteria are characterized by a membrane-bound organelle called the anammoxosome that comprises more than 30% of the cell volume. This intracytoplasmic compartment is surrounded by unique lipids, called ladderanes (Sinninghe Damsté et al. 2002) that are unique to the anammox bacteria. Ether and ester linkages tie the lipids to a glycerol backbone in the membrane which has historically only been found in members of the domain Archaea and may reflect an early divergence of anammox in the bacterial lineage (Brochier and Philippe 2002). Due to a very dense arrangement of carbon atoms, the ladderane lipids serve as a diffusion barrier (Sinninghe Damsté et al. 2002). This may serve to protect the bacteria from the toxic anammox reaction intermediates hydroxylamine and hydrazine (Jetten et al. 2003). Due to their unique characteristics, ladderane lipids have also been used as a biomarker for the presence of anammox bacteria (Kuypers et al. 2003).

Evidence from the genome of *Candidatus* K. stuttgartiensis (Strous et al. 2006) indicates that the anammox reaction proceeds via the following steps:

$$NO_2^- \rightarrow NO,$$
$$NO + NH_4^+ \rightarrow N_2H_4 \rightarrow N_2.$$

The anammox hydroxylamine oxidoreductase (HAO) enzyme is responsible for the oxidation of hydrazine to N_2 gas and is located exclusively within the anammoxosome (Lindsay et al. 2001), a possible target for future molecular studies. The highly reactive hydrazine intermediate is stored inside the anammoxosome (Sinninghe Damsté et al. 2002), which is especially important considering the slow enzymatic turnover, resulting in a doubling time of 9 days in optimal conditions for

the "freshwater" anammox (Strous et al. 1999a, b). Anammox are reversibly inhibited by O_2, and reaction rates are the same after as before aeration (Jetten et al. 1999).

Anammox bacteria have been found to be metabolically flexible, exhibiting alternative metabolic pathways. For instance, anammox can subsequently reduce nitrate to nitrite to ammonium, followed by the conversion of ammonium and nitrite to N_2 through the anammox pathway, allowing anammox bacteria to overcome ammonium limitation. Anammox bacteria are also a potential source of N_2O production by nitric oxide detoxification (Kartal et al. 2007a). Currently the other known processes that produce N_2O are nitrification and denitrification (Fig. 10.1. As such, classical denitrification measures that depend exclusively on N_2O measures may overstate the role of denitrification in the system. Another alternative pathway is carried out by *Candidatus* Anammoxoglobus propionicus, which has been shown to co-oxidize propionate and ammonium, and out-compete denitrifiers and other anammox bacteria in the process (Kartal et al. 2007b). This supports the niche differentiation of anammox in which different "ecotypes" dominate specific habitats, and may be the reason why two different anammox species are not commonly found in the same sample. Lastly, iron and manganese oxides have also been found to be respired with formate as an electron donor (Strous et al. 2006), further expanding the metabolic diversity of the anammox bacteria.

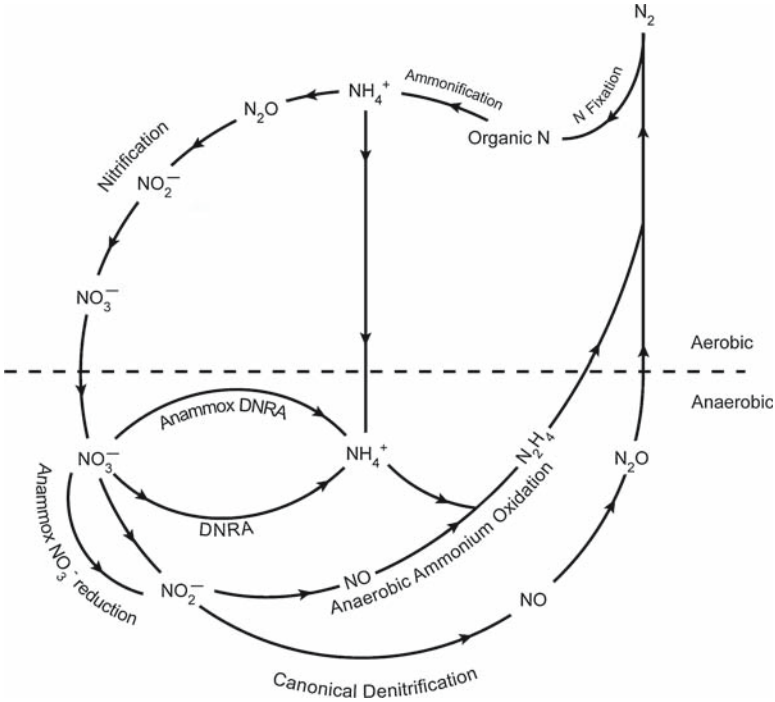

Fig. 10.1 Anaerobic ammonium oxidation pathway of nitrogen removal in context of the current nitrogen cycle

10.3 Detection of Anammox Bacteria and Activity

The isotope pairing technique (IPT) has been used as the standard measure of anammox activity, most commonly using homogenized sediments (Thamdrup and Dalsgaard 2002). Concentrations of NH_4^+, NO_3^-, and NO_2^- are first determined, the sediments are placed in airtight containers with septums, such as Exetainer tubes, and the headspace is flushed with He for a minimum of 5 minutes to replace ambient O_2. Concentrations of residual NO_x species are monitored over time until all available NO_x is removed from the incubations. Three parallel incubations are then performed: (1) $^{15}NH_4^+$ alone, (2) the combination of $^{15}NH_4^+$ and $^{14}NO_2^-$, and (3) $^{15}NO_2^-$ alone. Reactions are stopped by the addition of $ZnCl_2$.The first incubation is used as a control to detect any oxidation of ammonium without the addition of nitrite. The lack of $^{29}N_2/^{30}N_2$ is indicative of the lack of oxidants at the end of the pre-incubations. The second treatment is used to determine if anammox activity is possible. The production of $^{29}N_2$ indicates anammox activity through the oxidation of ammonium with nitrite. The combination of the first two incubations is used to establish anammox activity. Finally, the third incubation is used to estimate anammox and denitrification rates (Fig. 10.2). Anammox produces $^{29}N_2$ through the oxidation of the resident NH_4^+ pool with the added $^{15}NO_2^-$, while denitrification is measured by the production of $^{30}N_2$. However, evidence that anammox can also

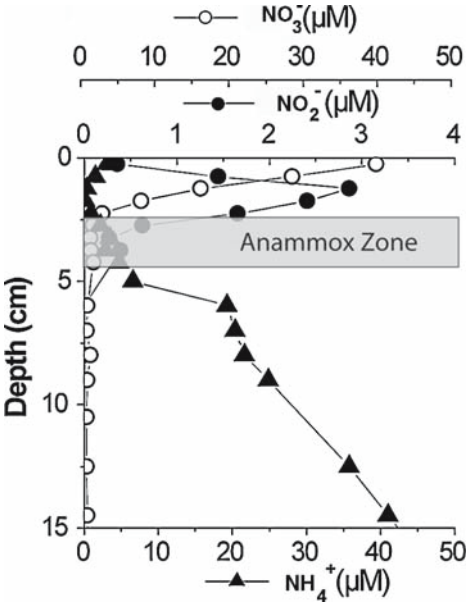

Fig. 10.2 Typical porewater nitrogen profile from a deep ocean sediment, indicating a possible zone of anaerobic ammonium oxidation

reduce $^{15}NO_3^-$ to $^{15}NO_2^-$ to $^{15}NH_4^+$ (Kartal et al. 2007a) results in the possibility that the anammox reaction can pair $^{15}NO_2^-$ with $^{15}NH_4^+$, and thus some proportion of measured denitrification may be partitioned to the anammox reaction. Several modifications to this protocol are promising, notably the addition of N_2O measures to more accurately quantify N_2 production, and the use of intact sediment cores (Trimmer et al. 2006).

Molecular methods have been extensively utilized to identify the presence of anammox bacteria in environmental and wastewater samples. Fluorescence in situ hybridization (FISH) targeting the 16S rRNA gene has been used extensively, and is described in detail by Schmid et al. (2005). Anammox bacteria have also been identified using PCR, using a variety of primers, often based on FISH probes, targeting the group as a whole or specific members (Schmid et al. 2005; Penton and Tiedje 2006). The unique ladderane lipids that constitute the anammoxosome have also been used as biomarkers for relative quantification (Kuypers et al. 2003), while distinctive hopanoid lipids may be useful in assessing relative anammox abundance in the sedimentary record (Sinninghe Damsté et al. 2004). Quantitative PCR (q-PCR) has been used for direct quantification of all known anammox-like bacteria in water columns (Hamersley et al. 2007), in wastewater enrichment cultures (Tsushima et al. 2007), and for the specific enumeration of *Candidatus* Scalindua "marine" anammox in sediments.

10.4 Anammox in the Environment

The linkage of anammox activity with the removal of fixed inorganic nitrogen in natural systems was first confirmed in the Black Sea suboxic water column (Kuypers et al. 2003). Since then, anammox has been shown to be a significant contributor to nitrogen losses in a variety of environments, responsible for 19–35% of the nitrogen loss in an anoxic coastal bay (Dalsgaard et al. 2003) and the majority of N removal in one of the most productive regions of the world's oceans, the Benguela upwelling oxygen minimal zone (Kuypers et al. 2005). These sites exhibit characteristics of oxygen minimum zones, which are thought to be responsible for 30–50% of global N removal (Brandes and Devol 2002). Evidence for the anammox reaction in sediments or soils is generally first determined by the porewater N profile. Anoxic zones where there is a concomitant reduction in both nitrite/nitrate and ammonium represent the initial conditions necessary for anammox activity (Fig. 10.3). The maximum reported contribution of anammox is 67–79%, occurring in sediments at a depth of 700 m (Engström et al. 2005), which led to the hypothesis that relative anammox contributions increase with depth. However, current evidence suggests that anammox accounts for between 13 and 51% of total N_2 production in deep ocean sediments (ca. 3,000 m).

Ammonium is typically abundant in anoxic systems, provided by organic matter oxidation. Nitrate reducing or aerobic ammonium oxidizing bacteria provide the nitrite necessary for the anammox reaction. As such, organic matter availability is

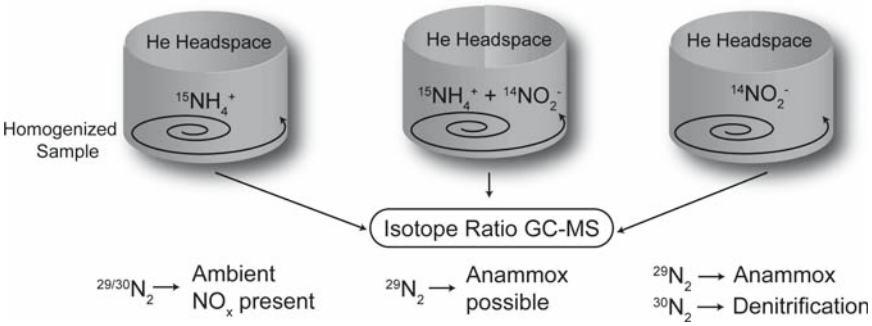

Fig. 10.3 Experimental layout of the isotope pairing technique used for estimating anammox and denitrification activities

thought to be a major factor influencing the relative significance of anammox to total N_2 production. Greater organic matter availability creates a higher demand by denitrifiers for NO_2^- and NO_3^-, and less NO_2^- is liberated for anammox consumption. As such, anammox contributed 0–9% of total N_2 production in subtropical mangrove sediments (Meyer et al. 2005), 8% in estuarine sediments (Trimmer et al. 2003), and less than 2% in eutrophic shallow coastal bay sediments (Thamdrup and Dalsgaard 2002). Although sediment reactivity has been negatively correlated with anammox contribution to total N_2 production (Trimmer et al. 2003), absolute anammox rates appear to peak at sites with intermediate reactivity (Engström et al. 2005). As such, a strict relationship between anammox activity and organic matter availability is not firmly established. Additionally, due to the slow growth of anammox and their inhibition by low concentrations of O_2 (if the "marine" anammox respond the same as the "freshwater" species), environmental stability may be an important controlling factor of anammox activity.

16S rRNA sequences identical or closely related to the marine anammox have been found widely distributed in marine systems, freshwater lakes, and subtropical wetlands (Penton and Tiedje 2006). However, relatively few studies have investigated anammox activity in natural freshwater systems, although the "freshwater" anammox bacteria are the most intensively studied due to their implementation in wastewater treatment bioreactors. Schubert et al. (2006) reported an anammox contribution of 13% in the largest freshwater anoxic lake in the world, Lake Tanganyika. Anammox 16S rRNA gene sequences with > 96% sequence identity to *Candidatus* Scalindua brodae were identified in the anoxic water column, and anammox cells were enumerated using FISH. Molecular analysis was used to assess the diversity of the anammox population in the Xinyi River (China) (Zhang et al. 2007). Sequences, obtained by targeted PCR, exhibited 16S nucleotide identities of 95% to *Candidatus* Brocadia anammoxidans and 95% to the *Candidatus* Scalindua species, including the sequence obtained from the Lake Tanganyika

study. These findings suggest that more diverse anammox communities may exist in freshwater habitats, compared to the multitude of marine studies that indicate a single, dominant anammox ecotype.

10.5 Anammox in Permafrost

Although the anammox process has not been investigated in permafrost soils, "marine" anammox 16S rRNA sequences have been identified in Siberian frozen alluvial sandy loam, deposited in the Middle Pleistocene Epoch 300,000–400,000 years ago in the Cape Svyatoi Nos tundra zone on the Laptev Sea coast (Penton and Tiedje 2006). Rysgaard and Glud (2004) found that anammox was responsible for up to 19% of total N_2 production in a Greenland Sea ice floe, but was not detectable in annual sea ice, perhaps due to increased stability. Both aerobic and anaerobic processes in microzones were found to occur simultaneously in brine pockets. This raises the possibility that anammox contributes to N_2 removal in permafrost soils.

Due to the use of N_2O as a common measure of denitrification and nitrification in permafrost soils, the anammox contribution to nitrogen losses remains an enigma. Ma and colleagues (2007) have reported that a reduction in ammonia concentrations may not be linked to nitrous oxide production in Canadian permafrost soils. Uptake by plants was listed as a possible cause, though they noted that a concomitant nitrate reduction was not observed. The anammox pathway is another possibility that would describe the uptake of ammonia that was not recorded in the N_2O emissions. Other evidence for an active N microbial consortium comes from the reported presence of "unstable" ammonia-oxidizing bacteria in Arctic permafrost (Vorobyova et al. 1997). The presence of active nitrifiers at low but finite O_2 concentrations in Vostok ice was inferred by Sowers (2001), and a novel cold-adapted nitrite oxidizing bacterium was isolated from a Siberian permafrost sample (Alawi et al. 2007). Low oxygen concentrations, anaerobic microsites and slow water transport, coupled with low organic matter availability, are ideal conditions for anammox bacteria to outcompete denitrifiers for available nitrified NO_2^- in permafrost. If anammox do indeed contribute to N losses in permafrost brine channels, system stability is a key issue that may affect activity on an annual or over an extended warming trend. However, the use of 15N isotopic measures, such as variations of the isotope pairing technique, and molecular methods are necessary to assess the viability and response of the anammox and nitrogen cycling community as a whole to ecosystem changes.

Permafrost melting increases water activity and mixing, resulting first in increased O_2 availability, which should theoretically negatively impact the anaerobic anammox community. The "explosive microbial growth" following permafrost thawing (Vorobyova et al. 1997) with high available SOM and no mineralization constraints (Uhlírová et al. 2007) would result in competition for available NO_2^- by denitrifiers. However, the use of ^{15}N isotopic measures, such as variations of the

isotope pairing technique, and molecular methods are necessary to assess the viability and response of the anammox and nitrogen cycling community as a whole to ecosystem changes.

10.6 Conclusion

Over 40 years have passed since the anaerobic oxidation of ammonium with nitrite reduction was first proposed. Currently known to be a globally important marine N sink, anammox bacteria are found distributed among a diverse variety of soils and sediments. However, the use of techniques which enable the detection of anammox as well as other pathways are necessary to quantify the full extent of N removal in a system. Detection of anammox activity in sea ice suggests that this may be an active process in permafrost, where anammox bacteria have also been identified. In the context of current warming trends, a thorough characterization of the nitrogen cycle in permafrost soils is needed in order to quantify effects on organic matter mineralization and ultimately, carbon dioxide release as a positive feedback mechanism to global warming.

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Chapter 11
Genomic Insights into Cold Adaptation of Permafrost Bacteria

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11.1 Introduction

The sequencing and analysis of whole genomes (genomics) is a powerful tool that is being applied to many microorganisms in order to identify the distinguishing molecular features and gene content of those microorganisms. Genomic analyses allow the detection of trends that may only be apparent at the genome level rather than at the level of individual genes, due to differences resulting from genetic drift. For example, biases in amino acid abundance of the genomes of hyperthermophiles have been reported, and reflect adaptations to living at high temperatures (Singer and Hickey 2003). In addition, examination of gene content has been used to better understand the metabolic capabilities of the smallest microorganisms such as *Mycoplasma genitalium* and *Chlamydia* (Fraser et al. 1995; Read et al. 2000). Similarly, genomics can be used to investigate cold adaptation of psychrophiles at the molecular level by analyzing amino acid composition, codon usage, and nucleotide content, and at the level of genes by examining gene content and other unique features.

To date, only ten cold-adapted microorganisms have been completely sequenced (see Table 11.1), accounting for a mere 2.5% of all microbial genomes sequenced (10 of 398). All of these cold-adapted organisms have been isolated from polar regions and have provided valuable information about cold adaptation. Comparative studies of cold adaptations in these organisms should reveal which adaptations are common to all psychrophiles and which are specific to the particular environment each psychrophile inhabits, or to the particular family of organisms they represent. The majority of these cold-adapted microorganisms have been isolated from low-temperature marine environments (water, ice, or sediment) which are distinctly different from low-temperature terrestrial environments such as permafrost. Marine environments have high solute concentrations, while terrestrial environments do not. Hence, when sea water freezes, fairly large channels of brine can be found

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Table 11.1 Psychrophilic microorganisms whose genomes have been sequenced

Microorganism	Environmental source	References
Colwellia psychrerythraea	Arctic sea ice	Methe et al. (2005)
Desulfotalea psychrophila	Arctic marine sediment	Rabus et al. (2004)
Methanococcoides burtonii, Methanogenium frigidum	Ace Lake, Antarctica (salinity close to sea water)	Saunders et al. (2003)
Polaribacter filamentus	Arctic surface sea water	Gosink et al. (1998)
Polaribacter irgensii	Antarctic sea water	Gosink et al. (1998)
Pseudoalteromonas haloplanktis TAC125	Antarctic sea water	Medigue et al. (2005)
Psychrobacter arcticus 273-4, *Psychrobacter cryohalolentis* K5	Siberian permafrost	Bakermans et al. (2006)
Psychromonas ingrahamii	Arctic sea ice	Auman et al. (2006)

within the ice, while liquid water in permafrost localizes to very thin films, creating a highly constrained physical environment (Rivkina et al. 2000; Bock and Eicken 2005). In addition, Siberian permafrost is a sedimentary system uniquely characterized by passage through an active layer which freezes and thaws on a seasonal basis, and subsequent burial in permanently frozen sediments that experience more stable temperatures. Consequently, genomic analysis of microorganisms isolated from permafrost may reveal unique mechanisms of cold adaptation.

Genomic analysis does not have to stop at the sequence level; complex metabolic changes at the system level can also be elucidated using postgenomic technologies. For example, microarrays can be used to study the transcriptome (all the genes expressed during specific culture conditions). Cold shock has been examined extensively using microarrays (Beckering et al. 2002; Phadtare and Inouye 2004; Gao et al. 2006); however, there are very few studies of the transcriptome during growth at low temperatures, especially at temperatures below 0°C which are essential to permafrost (Budde et al. 2006). Examination of the transcriptome enables the investigation of the underlying gene expression that results in cold adaptation, and ultimately permits the successful colonization of low-temperature environments by cold-adapted microorganisms. Here we review and summarize what has been learned about cold adaptation and active growth at temperatures below 0°C from genome sequence analysis and gene expression experiments in *Psychrobacter arcticus* 273-4, a model organism isolated from 20,000–30,000-year-old permafrost.

11.2 Model Organism – *Psychrobacter*

Among the microorganisms that have been recovered and isolated from Siberian permafrost samples, *Psychrobacter* species have remarkable capabilities at subzero temperatures which identify them as potential model organisms for the study of

low-temperature adaptations relevant to inhabiting permafrost (Vishnivetskaya et al. 2000; Bakermans et al. 2003). These *Psychrobacter* species grow quickly at low temperatures, actively reproduce at −10°C, easily survive freeze–thaw cycles, and are tolerant to 12% NaCl (Bakermans and Nealson 2004; Ponder et al. 2005; Bakermans et al. 2006, 2007). *Psychrobacter* species are commonly isolated from a variety of low-temperature environments, including: Antarctic sea ice, ornithogenic soil, and sediments; the stomach contents of the Antarctic krill *Euphausia*; sea water (NW Pacific Ocean, 300 m depth); the deep sea; and the internal tissues of a marine ascidian (Bowman et al. 1997; Maruyama et al. 2000; Romanenko et al. 2002; Yumoto et al. 2003). In addition, quantitative PCR analyses have revealed that *Psychrobacter* species are widespread in polar regions and have been found throughout Antarctica and Siberia at 16S ribosomal RNA gene copy numbers ranging from 10^3 to 10^7 per μg of total community DNA (Rodrigues 2007).

To date, genomic (and post-genomic) studies have focused on two species that were isolated from the Kolyma Lowland region of Siberia where the permafrost is continuous, approximately 800 m thick, and remains stable at −9 to −11°C (Gilichinsky et al. 1992; Shi et al. 1997). *Psychrobacter arcticus* 273-4 was recovered from a depth of 12.5 m within a 20,000–30,000-year-old sandy loam that froze as it was deposited and has remained frozen to modern times (Sher et al. 1977; Vishnivetskaya et al. 2000). *Psychrobacter cryohalolentis* K5 was recovered from a cryopeg (a highly saline, 13%, lens of water) at a depth of 11 m, within a marine layer that was deposited beneath shallow lagoons at temperatures slightly above 0°C and froze sub-aerially as the polar ocean regressed some 110,000–112,000 years ago (Bakermans et al. 2003; Gilichinsky et al. 2003, 2005). The complete genomes for both of these organisms have been sequenced in collaboration with the Joint Genome Institute, and are available at http://genome.jgi-psf.org/mic_home.html (comparative genomic studies are ongoing and will not be discussed here).

11.3 Low-Temperature Adaptations

From analysis of both the genome and transcriptome, significant advances have been made in our understanding of cold-adaptation in the permafrost organism *P. arcticus* 273-4 (Ayala-del-Río et al.; Bergholz et al.; personal communications). The adaptations observed fall into three broad categories: control of molecular motion, resource efficiency, and temperature-specific alleles.

11.3.1 Control of Molecular Motion

Low temperatures decrease the energy of motion of molecules, leading to increased stability and rigidity. For example, as temperature decreases proteins become less flexible, membrane lipids become less fluid, and secondary structures of DNA and

RNA become more stable. As a general mechanism, cold-adapted microorganisms increase the disorder within macromolecules to maintain fluidity or flexibility, and hence function at low temperatures (Feller 2007). In *P. arcticus* 273-4, a variety of adaptations are believed to facilitate the motion of biomolecules and cellular structures at low temperatures, and include amino acid composition, specific chaperone proteins, membrane components, and cell-wall structure.

Low temperatures reduce the activity of enzymes through decreased flexibility of protein structure. Cold adaptation of enzymes is commonly achieved in psychrophiles by reducing weak stabilizing interactions (ion pairs, hydrogen bonds, hydrophobic and intersubunit interactions), increasing solvent interactions with apolar or interior residues, reducing proline and arginine content, and/or clustering of glycine residues (Feller et al. 1996; Russell 2000). Consistent with these themes in amino acid alteration, the genes of *P. arcticus* 273-4 contain fewer hydrophobic and acidic residues, fewer proline residues, and more lysine and fewer arginine residues when compared to their homologs in the Swiss-Prot Database (Ayala-del-Río et al., personal communication). Having fewer acidic or proline residues was the most common modification observed in *Psychrobacter* genes. Overall, 56% of the genes of *P. arcticus* 273-4 can be classified as "cold-adapted" by at least one of these measures (less hydrophobic; fewer proline residues; less aliphatic; fewer acidic residues; or fewer arginine and more lysine residues) and, on average, each of these cold adapted genes contain three of the five types of adaptations described above.

Protein chaperones have been repeatedly identified as important components of low-temperature growth in mesophilic and psychrophilic bacteria (Phadtare and Inouye 2004). Peptide chaperones such as GroEL/ES and peptidyl-prolyl *cis-trans* isomerases (PPIase) are thought to be important for promoting correct protein folding at low temperature (Strocchi et al. 2006). Of the protein chaperones present in the genome of *P. arcticus* 273-4, only *clpB* (a protein disaggregating chaperone) was up-regulated at low temperature, suggesting that aggregation of denatured peptides at low temperature may be a hurdle at subzero temperatures. Other heat shock proteins and PPIases (except oxidative stress chaperones) were up-regulated only during growth at warm temperatures in *P. arcticus* 273-4. The amino acid changes that result in cold-adapted genes (as observed from genomic analyses) may have left *Psychrobacter* sp. dependent on the function of heat shock proteins during growth at the relatively mild temperatures of 22°C and 17°C near the upper end of their growth temperature range.

Low temperatures also stabilize the secondary structures of nucleic acids, leading to the inhibition of the processes of transcription, translation, and DNA replication. Cold-adapted microorganisms alleviate stress on these processes via RNA chaperones and specialized helicases (Jiang et al. 1997; Chamot and Owttrim 2000; Phadtare et al. 2002). RNA chaperones, such as cold-shock proteins (*csp*), are thought to prevent secondary structure formation in RNA, thereby ensuring successful translation of transcripts in conjunction with other cold-shock proteins such as DEAD box helicases (Whyte and Inniss 1992; Goldenberg et al. 1997; Lim et al. 2000; Iost and Dreyfus 2006). Likewise, increased expression of specific ribosomal

proteins may contribute to low-temperature function of the ribosome, with a trade-off in increased thermolability of that translational apparatus (Bayles et al. 2000). In *P. arcticus* 273-4, several cold-shock genes associated with molecular motion were upregulated during growth at low temperatures, including *csdA* (a DEAD-box helicase) and *rbfA* (a ribosome binding factor); however, *cspA* was constitutively expressed at all temperatures. Constitutive expression of the major cold-shock protein transcript may be the result of exposure to continuous cold temperatures in the permafrost. Interestingly, up-regulation of *cspA* was observed in the proteome (Bakermans et al. 2007); thus, regulation of CspA in *Psychrobacter* may involve posttranscriptional control of protein synthesis or degradation.

The fluidity of cell membranes can be maintained at low temperatures by increasing unsaturated lipids, decreasing acyl chain length and branch-chained lipids, or altering polar head groups and by producing compatible solutes (Russell 1990). For example, psychrophilic bacteria commonly increase the proportion of $C_{18:1}$ and/or C_{16} fatty acids at low temperatures (Russell 1990, 1997). To ensure that membrane fluidity is maintained at low temperatures, *Psychrobacter* species contain two separate mechanisms for creating unsaturated fatty acids in membrane lipids: de novo synthesis and fatty acid desaturases. Indeed, increased expression of membrane fatty acid desaturases was observed during growth of *P. arcticus* 273-4 at low temperature. A previously unreported response to low temperatures was also observed in genes responsible for the dynamic growth and elasticity of the cell wall (Yao et al. 1999). Lytic transglycosylases and D-alanyl-D-alanine carboxypeptidases were up-regulated during growth of *P. arcticus* 273-4 at low temperature. Regulation of cell-wall elasticity could play a major role in growth rate control at low temperatures. Because elastic materials stiffen in the cold, losing their resilience and resistance to stretching, *Psychrobacter* sp. may actively regulate the elasticity of the peptidoglycan wall to maintain the turgor pressure required for growth in the frozen conditions of the permafrost.

11.3.2 Efficient Use of Resources

Efficiency of resource utilization may be key to the survival of heterotrophic microbes in frozen environments over thousands to millions of years. While little is known about how low temperatures affect resource efficiency in psychrophiles, study of the proteome of *Methanococcoides burtonii* suggested that efficient carbon utilization occurs during growth at low temperatures (Goodchild et al. 2004). Genome sequence analysis reveals that *P. arcticus* 273-4 can conserve resources via the glyoxylate shunt, a bypass of the TCA cycle which allows cells to conserve carbon when growing on 2-carbon compounds, as both isocitrate lyase and malate dehydrogenase are present. In addition, the transcriptome of *P. arcticus* 273-4 indicates that resource conservation occurs during growth at low temperatures even under nutrient-replete conditions (e.g., 20 mM acetate, 5 mM ammonium, and 1 mM phosphate). *P. arcticus* 273-4 increases the expression of 12 peptidases and

five ribonucleases that probably enhance the recycling of nucleotides and amino acids over long generation times during growth at low temperatures. The ability to recycle the basic building blocks of cellular machinery at subzero temperatures is likely essential to long-term viability in permafrost.

Decreased energy metabolism at low temperatures was also a major aspect of gene expression during growth at low temperatures. The most pronounced decreases in *P. arcticus* 273-4 transcript abundance at subzero temperatures were in energy metabolism genes. These genes included ATP synthase, NADH dehydrogenase and TCA cycle genes. While it is generally thought that energy cost per generation is much higher at low temperatures than at optimal growth temperatures, instantaneous resource demands should be much lower at subzero temperatures. The energy needs of *P. arcticus* 273-4 at subzero temperatures appear to be met by low levels of expression of energy metabolism genes, suggesting that *P. arcticus* 273-4 is well adapted for heterotrophic metabolism in the permafrost.

11.3.3 Temperature-Specific Alleles

Organisms can employ temperature-specific alleles, or isozymes, as an adaptation to low temperatures by possessing two alleles of the same enzyme that have different temperature optima. While animals commonly use isozymes as an adaptation to temperature changes, few examples have been documented in bacteria (Ishii et al. 1987; He et al. 2001). Bacterial isozymes were first documented for isocitrate dehydrogenase of the psychrophile *Colwellia maris* (Ochiai et al. 1979, 1984; Ishii et al. 1987). While it is difficult to assess the extent to which isozymes occur throughout the genome of *P. arcticus* 273-4, putative isozymes have been identified in transcriptome experiments where the expression of one isozyme is increased at high temperatures and the expression of the second isozyme is increased at low temperatures (Bergholz et al., personal communication). For example, there are two genes for RNA helicase (1082 and 943). The transcript for 943 was upregulated during growth at warm temperatures, while 1082 was upregulated at low temperatures. Similar patterns of expression were documented for two dihydrolipoamide dehydrogenases, two D-alanyl-D-alanyl carboxypeptidases, and two 16S rRNA pseudouridine synthases. Certainly, these enzymes are important to cell function at any temperature; hence, the presence of isozymes would ensure that these functions are maintained regardless of growth temperature. Additional data from analysis of the proteome and the phenotype of deletion mutants suggest that isozymes (for the substrate-binding subunits of ferric-citrate and dicarboxylic acid transporters) may be involved in ensuring that key nutrients are transported across the membrane at different temperatures (Bakermans et al. 2007). The use of isozymes may be particularly useful to microorganisms that live in permafrost given that their initial habitat, prior to burial, is within the active layer of permafrost where temperatures fluctuate on a seasonal — and sometimes daily — basis around the freezing point of water.

11.4 Conclusion

Genomic analysis of the permafrost isolate *Psychrobacter arcticus* 273-4 has revealed that a variety of adaptations are employed by *P. arcticus* 273-4 to enable active growth at low temperatures. Many of these low-temperature adaptations are largely similar to adaptations found in other psychrophilic microorganisms isolated from other low-temperature environments. These similarities include: changes in amino acid abundance that favor protein mobility; RNA and protein chaperones; and desaturation of membrane lipids. Unlike other psychrophiles, *P. arcticus* 273-4 constitutively expressed the major cold-shock protein (*cspA*, an RNA chaperone) at all growth temperatures to maintain the molecular motion of RNA. The constitutive expression of *cspA* may be an advantage in permafrost, where cold temperatures reign. In addition, cell-wall elasticity may be affected by low temperatures in *P. arcticus* 273-4, and could play a major role in growth rate control or maintenance of turgor pressure in the frozen conditions of the permafrost. Low-temperature effects on the cell wall have not been reported in other psychrophiles, and could suggest a unique adaptation to the permafrost environment. Clearly, maintaining molecular motion, and hence function of those molecules, through changes in the basic structures of biomolecules (proteins, lipids, cell wall) and with the assistance of chaperones is important to actively living at low temperatures.

Isozymes can also be used to maintain molecular motion and allow key enzymatic functions to be maintained regardless of growth temperature. Isozymes may be particularly useful to microorganisms that live in the active layer of permafrost, where temperatures fluctuate on a seasonal — and sometimes daily — basis around the freezing point of water. While *P. arcticus* 273-4 was recovered from deep permafrost where the temperature has been stable at −10°C for 20,000–30,000 years (Vishnivetskaya et al. 2000), its initial habitat was at the surface within the active layer of permafrost. The presence and use of isozymes within *P. arcticus* 273-4 (and the constitutive expression of *cspA*) may reflect this ecological history.

Analysis of the transcriptome demonstrated that efficient use of resources was another strategy employed by *P. arcticus* 273-4 for living at low temperatures. Efficiency of resource utilization may be key to the survival of heterotrophic microbes over thousands to millions of years in permafrost, given that permafrost, due to the frozen state, is an environment characterized by a high degree of spatial isolation and low rates of solute transport. Hence, the introduction of new substrate is likely to be a rare event in the permafrost. Efficient use of resources has only been suggested in psychrophilic methanogens, and has not been noted in studies of other psychrophiles. Therefore, this particular strategy (efficient use of resources) may be an adaptation of these psychrophiles to physically or energetically constrained environments and not an adaptation to low temperatures per se.

Long-term survival strategies in permafrost are thought to fall into two main categories: (i) microbes maintain viability by entering a dormant state in which they can resist damage to cellular insults, or (ii) microbes maintain viability by metabolizing and repairing damage at rates sufficient to equal or exceed the rate of death

due to environmentally induced damage. *Psychrobacter* sp. clearly fall into the latter category, as the observed changes in the genome and in gene expression are primarily directed toward maintenance of molecular motion and resource efficiency for continued growth in frozen conditions. These low-temperature adaptations are consistent with an organism adapted for life under long-term freezing conditions and may be crucial to survival, considering that a recent study of ancient DNA from permafrost concluded that "long-term survival is closely tied to cellular metabolic activity and DNA repair that over time proves to be superior to dormancy as a mechanism in sustaining bacteria viability" (Johnson et al. 2007).

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Chapter 12
Proteomic Insights: Cryoadaptation of Permafrost Bacteria

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12.1 Introduction

Permafrost, which is defined as a subsurface frozen layer that remains frozen for more than 2 years, makes up more than 20% of the land surface of the earth, including 82% of Alaska, 50% of Russia and Canada, 20% of China, and most of the surface of Antarctica (Harris 1986; Williams and Smith 1989; Storad 1990). Permafrost poses unique challenges to its resident biota because of the permanently cold temperature of the soils, averaging −10 to −12°C, and the length of time over which the soils were frozen, which may be from a few thousand to even 2–3 million years.

To survive at subfreezing temperatures in permafrost, microbes have apparently developed various adaptive mechanisms. Electron microscopic examination of bacterial cells in a chip of permafrost core revealed that bacterial cells may survive due to reduction of cell size and formation of "dwarf" curved forms similar to nanoforms. The in situ permafrost bacteria, further characterized by thickened cell walls, altered structure of cytoplasm, compact nucleoid, showed similarities to cyst-like resting forms of non-spore-forming bacteria (Soina et al. 2004). The survival mechanisms may include reduction of the polar polysaccharide capsular layer, decrease of the fractional volume of cellular water, increase of the fraction of ordered cellular water, or extraction of energy by catalyzing redox reactions of ions in thin aqueous films in permafrost (McGrath and Gilichinsky 1994; Ostroumov and Siegert 1996; Mindock et al. 2001; Gilichinsky 2002). Among such adaptive processes, not only the bacteria themselves might be affected by environmental low temperature and induced cold-adapted features, but also the production of cold-induced organic molecules within them, such as polysaccharides, proteins and enzymes that sustain their metabolism at low temperatures.

Progress on low-temperature adaptation research has been achieved mainly through genomic or physiological studies. Proteomic analysis provides the dynamic information of cells which reflects the actual live status of cells. Protein patterns demonstrated that growth temperature substantially reprogrammed the proteome.

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Identification of all the proteins, including those differentially expressed under different conditions, will facilitate the understanding of the adaptation process. Comparative proteomic studies of various microorganisms during growth at different temperatures could be found (Sinchaikul et al. 2002; Goodchild et al. 2005; Kawamoto et al. 2007). Some of these differentially produced proteins displayed temperature trends: some proteins accumulated to high levels at low temperatures, while other protein expressions are elevated at high temperatures. Here we review the proteomic studies of cryoadaptation of permafrost bacteria.

12.2 Proteomic Studies of Low-Temperature Adaptations in Permafrost Bacteria

In the discussion of bacterial low-temperature adaptation, specific sets of cold-induced proteins (CIPs) have been considered to facilitate and allow cell growth at low temperature. CIPs are defined as proteins that are preferentially or uniquely present at low temperatures, and are thought to contribute specially to the ability of organisms to function at low temperatures (Fukunaga et al. 1999). CIPs could be further classified into cold-shock proteins (CSPs) and cold-acclimation proteins (CAPs).The term "CSPs" is used here for proteins that are transiently over-expressed after an abrupt shift to a low temperature, and the term "CAPs" is used for the proteins synthesized at a greater level during continuous growth at low temperatures as compared with high temperatures. CSPs and CAPs have been considered to facilitate and allow cell growth at low temperatures, and both sets of proteins may share functionality at both the molecular and cellular level (Whyte and Inniss 1992; Bayles et al. 1996; Berger et al. 1996; Panoff et al. 1997). Similarities between the CSPs and CAPs may suggest that these proteins are of significance to both shock recovery as well as constant growth in a new environment. The synthesis of CIPs in response to continuous growth at low temperatures in comparison to optimal growth temperature has been studied in two strains of the genus *Exiguobacterium* and two strains of the genus *Psychrobacter* isolated from Siberian permafrost and water brine samples (Table 12.1).

12.2.1 Cold-Inducible Proteins (CIPS)

The detection and identification of CIPs present during growth at 16°C, 4°C, and −4°C (salinity remained constant at 5%) by two-dimensional electrophoresis has been reported in *Psychrobacter cryohalolentis* K5 (Bakermans et al. 2007). Changes in the growth temperature regime differentially induce the synthesis of a large set of specialized proteins needed to maintain growth and reproduction at different temperatures. Twenty-eight of the CIPs were identified in *P. cryohalolentis* K5.

Table 12.1 List of permafrost strains studied by proteomics approaches

Strain	Origin (age)	Location, collection date	Environmental conditions	References
E. sibiricum 7-3 (VKM B 2374)	Alluvium loam and sandy loam (30,000 years)	Khomus-Yuryakh river; 68°19′N, 154°58′E; August 1989	8 m, −10°C, pH 7	Chong et al. (2000)
E. sibiricum 255-15 (DSM 17290)	Lake-alluvium loam and sandy loam (3 million years)	Bol'shaya Chykochya river; 69°10′N, 158° 4′E; July 1994	43.6 m, −10°C, pH 7.3	Qiu et al. (2006)
P. arcticus 273–4 (DSM 17307)	Alluvium sandy loam (30,000 years)	Malay Kon'kovaya river; 69°N, 158°30′E; August 1997	12.5 m; −10°C, pH 6.9	Zheng et al. (2007)
P. cryohalolentis K5 (DSM 17306)	Brine water lens within alluvial icy complex (43,000 years)	Lake Yakutskoe; 69°50′N, 159°30′E; August 1999	24 m, −11°C, pH 7.4, salinity 150 g°l^{-1}	Bakermans et al. (2007)

Sample description was adopted from Gilichinsky et al. (2005) and Vishnivetskaya et al. (2000, 2006)

Among them, 15 proteins synthesized at 16°C were overexpressed at low temperatures, eight CIPs were detected during growth at both 4°C and −4°C, and five CIPs were specifically detected during growth at −4°C. These negative temperature-inducible proteins included:
The B subunit of F1/F0 ATP synthase, AtpF
The outer membrane efflux system protein, TolC
The elongation factor Ts, EF-Ts
A hypothetical protein with a bacterial Ig-like domain, Pcryo_1988, and
The outer membrane receptor for ferric citrate transport, FecA.
The drastic increase in relative abundance of these proteins at −4°C, relative to 4°C and 16°C, suggest specific stress on energy production, protein synthesis, and transport during growth at subzero temperatures. The efflux transporter TolC (as AcrAB-TolC) has a broad substrate range, and transports antibiotics, detergents, etc. suggesting an increased need to export potentially harmful molecules at −4°C.

12.2.2 Cold-Shock Proteins (CSPS)

CSPs comprise a family of small proteins that are structurally highly conserved, bind to single-stranded nucleic acids and are involved in a variety of cellular processes, such as transcription (Ermolenko and Makhatadze 2002). Bacterial

CSPs are rich in aromatic and basic amino acids, and their expression peak occurs shortly after a rapid temperature downshift to regulate the adaptation to cold stress, but they are also present under normal conditions to regulate other biological functions (Barbaro et al. 2002; Guo and Gong 2002). The cold-shock phenomenon was originally found in *Escherichia coli* at a temperature downshift from 37°C to 10°C (Jones and Inouye 1994), and was later found to be a cold-shock response common to many bacterial species (Kim et al. 1998b; Lottering and Streips 1995; Obata et al. 1998) some eukaryotes (Somer et al. 2002), and archaea (Cavicchioli et al. 2000). The major cold-shock protein CspA of *E. coli* has high sequence similarity with eukaryotic Y-box DNA-binding proteins that are known to be involved in regulation of several transcription and translation processes (Lee et al. 1994). A homolog of CspA was found to be upregulated following cold shock in psychrotrophic bacterium *Arthrobacter globiformus* SI55, but unlike its mesophilic counterparts, it was still expressed during prolonged growth at 4°C. The synthesis of this CspA-like protein was regulated at the translational level, and it was shown that growth resumption following a temperature downshift correlated with CspA expression (Berger et al. 1997). Similarly, psychroactive bacteria from permafrost showed overexpression of the CSPs during continuous low-temperature growth.

The presence of homologous cold-shock protein C (CspC, 7.255 kDa) in *Exiguobacterium sibiricum* 7-3 and three Csps (with Mr 7.150, 7.414 and 7.444 kDa) in *E. sibiricum* 255-15 was detected by high-performance liquid chromatography (HPLC) associated with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) (Chong et al. 2000; Qiu et al. 2006). Along with CspC, the overexpression of two other CSPs (CSP CSI4B 1,924.3 kDa, CSP CSI5 1,359.7 kDa) was observed in *E. sibiricum* 7-3 during low temperature growth (Chong et al. 2000). Three major CSPs from *E. sibiricum* 255-15 were homologous with 65.15%, 66.67%, and 59.09% sequence overlap to CspA in *E. coli*, and over 74% when compared to CspB, CspC, and CspD in *Bacillus subtilis* (Qiu et al. 2006). What is interesting is that unlike in *E. coli, B. subtilis*, and *E. sibiricum* 7-3 family of CSPs, those in *E. sibiricum* 255-15 were found similarly expressed at 25°C and 4°C, and represent about 10% of the total soluble proteins in cells grown at both temperatures. This result suggests that the genes for these proteins are turned on continuously to produce "shock" proteins to protect the cells from damage during abrupt changes in environmental conditions. Such behavior has been observed in other psychroactive bacterium such as *Psychrobacter arcticus* 273-4, where it has been shown that certain proteins (e.g. ribosomal proteins, ATP-dependent helicase, Elongation factor Ts) are always synthesized (Zheng et al. 2007). Apparently these organisms, which survive for long periods of time under extreme conditions, have adapted such a continuous expression as a means of survival. However, a putative CSP (8,111 Da/4.9 pI) was detected in *P. arcticus* 273–4 at 4°C and at both 22°C and 4°C when grown in medium with 5% NaCl, but was not detected at 22°C in ½ Tryptic Soy Broth (TSB) (Zheng et al. 2007). Another strain, *P. cryohalolentis* K5, showed the presence of CSP (CspA, 7.45 kDa) only at temperatures of 4°C and −4°C (Bakermans et al. 2007).

12.2.3 Cold-Acclimation Proteins (CAPs)

A set of proteins which are distinct from CSPs, and are specifically synthesized during continuous growth at low temperatures, are termed CAPs (Roberts and Inniss 1992; Whyte and Inniss 1992; Berger et al. 1996; Colucci and Inniss 1996). Recently, CAPs distinct from CSPs have been identified in the mesophilic bacteria *Enteroccoccus faecalis* during continuous growth at 8°C and *Listeria monocytogenes* at 10°C (Panoff et al. 1997; Liu et al. 2002).

From peptide analysis of the whole-cell lysates of *E. sibiricum* 255-15, 39 proteins with Mr ranging from 7 to 95 kDa were identified to be present at an increased level at the lower temperature and were considered to be CAPs, 16 of which were not detected at 25°C (Qiu et al. 2006). Some of these CAPs, such as trigger factor (TF) and pyruvate dehydrogenase, were characterized as CSPs in *E. coli* (Kandror and Goldberg 1997; Jones et al. 2006). TF in *E. coli* is a molecular chaperone with prolyl-isomerase activity, and associates with nascent polypeptides on ribosomes, binds to GroEL, enhances GroEL's affinity for unfolded proteins, and promotes degradation of certain polypeptides (Kandror and Goldberg 1997). TF levels increased progressively as growth temperature decreased and even rose in cells stored at 4°C. *E. coli* cells with reduced TF content die faster, while cells overexpressing TF showed greater viability. Thus, TF represents an example of an *E. coli* protein which protects cells against low temperatures. Unlike the TF, the role of pyruvate dehydrogenase has not yet been well-understood. Presumably, it is involved in the intensification of glycolysis and the suppression of the tricarboxylic acid cycle, i.e., in the processes that are observed upon the retardation of cell growth, and the adaptation of cells to stresses (Graumann and Marahiel 1996; Qiu et al. 2006).

The overexpression of heat-shock protein 70 (Hsp70) molecular chaperones was observed in *E. sibiricum* 255-15 during the cold-adaptation process. The heat-shock proteins may function as molecular chaperones that play an important role in protein folding, and — like DnaK — have functions in refolding of misfolded proteins that are essential under stress. Thus, these so-called "heat-shock proteins" are not simply heat-shock-specific proteins. They should more appropriately be called "temperature-stress proteins" (Qiu et al. 2006). While Hsp70 of *P. articus* 273–4 was overexpressed only in response to low temperature, chaperonin Hsp60 was found to be induced by low temperature or salt, where it was down-regulated if both of these extremes were present (Zheng et al. 2007).

Chaperone proteins DnaK and GroEL were found to be actively synthesized in response to heat, cold, and chemical stress (Salotra et al. 1995; Phan-Thanh and Gormon 1997). The phage-shock protein A (PspA) of *E. sibiricum* 255-15 was the highest overexpressed protein at low growth temperatures, whose expression ratio was over 70 (Qiu et al. 2006). Presently, the exact function of PspA remains elusive. High-level synthesis of PspA occurs only under extreme stress conditions including heat shock, cold shock, osmotic shock, and exposure to ethanol (Brissette et al. 1990; Kleerebezem and Tommassen 1993; Model et al. 1997). These stress

conditions might all lead to the dissipation of the proton-motive force, and expression of the PspA may help the cells to maintain the proton-motive force under such stress conditions (Kleerebezem et al. 1996).

The penicillin tolerance protein of *E. sibiricum* 255-15 was also found greatly overexpressed at 4°C. In *P. articus* 273–4, 18 proteins were up-regulated at 4°C in ½ TSB and only four proteins were up-regulated at 4°C in ½ TSB supplemented with 5% NaCl (Zheng et al. 2007). These facts suggest that a single stress could induce other stress-induced proteins that are organized in a complex and highly sophisticated adaptation network.

12.2.4 Cold-Adapted Enzymes

Enzymes which exhibit high catalytic efficiency at low temperatures are called cold-adapted enzymes. Indeed, cold-adapted enzymes have been isolated from cold-adapted organisms including psychrotrophic and psychrophilic bacteria. While cold-active enzymes are characterized by a high catalytic efficiency at a low temperature, they may behave differently at moderate temperatures: some of them exhibit a high catalytic efficiency at moderate temperatures but are rather thermolabile, others inactivate rapidly at a moderate temperature (Feller et al. 1996). However, not all enzymes found in psychroactive organisms are cold-adapted. Many enzymes of psychrophiles show comparable thermostability and catalytic efficiency to the counterparts of mesophilic organisms (Brenchley 1996). In general, rates of biochemical reactions are reduced under low-temperature conditions. However, since levels of the growth rates of psychrophilic bacteria are comparable to those of homologous organisms living at a moderate temperature, relatively similar metabolic rates must be maintained in psychrophilic bacterial cells. For achieving metabolic rate compensation, two enzymatic mechanisms have been proposed: (1) alterations in the concentration of enzymes present in the cells, and (2) changes in the catalytic efficiencies of enzymes (Hochachka and Somero 1984). For instance, an increase of enzyme concentration and activity in the *Lactococcus lactis* has been reported during cold adaptation (Wouters et al. 2000). Overexpression of polynucleotide phosphorylase has been detected in *E. coli* at low temperatures (Mathy et al. 2001).

E. sibiricum 255-15 is able to grow efficiently at temperatures down to −6°C (Vishnivetskaya et al. 2007); therefore, clearly, this organism has found mechanisms of temperature compensation in order to cope with the reduction of chemical reaction rates induced by low temperatures. A proteomics study of cold-adapted cells of *E. sibiricum* 255-15 showed that 28 out of 39 identified CAPs were enzymes. The higher levels of triosephosphate isomerase, acetolactate decarboxylase and cyclohydrolase have been detected in cells of *E. sibiricum* 255-15 grown at low temperature (Qiu et al. 2006). Cold-adapted enzymes in psychrophilic organisms may catalyze rate-limiting steps in metabolism, and play essential roles in survival at a low temperature. Another mechanism for survival is to express

enzymes with temperature-independent reaction rates. This is the case of perfectly evolved enzymes, where such enzymes are relatively rare: typical examples are carbonic anhydrase, acetylcholinesterase, and triosephosphate isomerase. Perfectly evolved enzymes, apparently, do not need to be adapted to low temperatures from a kinetic point of view, therefore they could be extremely useful to probe the various hypotheses related to enzyme adaptation. It may be suggested that the possible role of these enzymes involves maintenance of the bacterial metabolism enabling the cells to adapt to cold temperatures.

12.2.5 Housekeeping Protein

Every microorganism contains a set of proteins involved in the basic functioning of a cell. These proteins are called the housekeeping proteins. The synthesis rate of these "common" proteins does not vary significantly with growth temperature. From a 2D-map of *P. cryohalolentis* K5, a total of 311 (51%) of the spots did not vary with growth temperature (−4°C, 4°C and 16°C) and accounted for 73% (v/v) of the amount of protein detected at each temperature (Bakermans et al. 2007). The proteome of *E. sibiricum* 255-15 showed that most of the proteins were similarly expressed at the two temperatures, 4°C and 25°C (Qiu et al. 2006). While housekeeping proteins are required for basic cell functions at any temperature, they may be essential for the proper function of the bacterial cells during the cold-adaptation process.

12.3 Putative Roles of Cold-Inducible Proteins in Low-Temperature Growth

The temperature regulates the growth rate, the level of biosynthesis, metabolism, and survival (Price and Sowers 2004). Comparison of the proteomic profiles of different psychroactive bacteria grown at low temperatures involves the up-regulation of the similar proteins.

Protein profiles of strains *P. cryohalolentis* K5 and *E. sibiricum* 255-15 following cold adaptation showed overexpression of translation elongation factor Ts involved in gene expression, and F1/F0-type ATP-synthase B subunit important for energy production (Qiu et al. 2006; Bakermans et al. 2007). The overexpression of translation elongation factor Tu was observed in two *Psychrobacter* strains studied (Bakermans et al. 2007; Zheng et al. 2007). The proteins involved in gene expression, e.g., CSPs, transcriptional regulators, ribosomal proteins, RNA chaperones and elongation factors, are known to be induced in the response to low temperature in order to decrease stress on transcription, translation initiation and elongation (Mihoub et al. 2003). Low-temperature-induced synthesis and accumulation of CIPs in the cells allows bacteria to maintain energy and constructive metabolism under unfavorable environmental conditions.

Bacteria of the genus *Exiguobacterium* are non-spore-forming bacteria; however, the elevated level of the sporulation control protein was observed in both *Exiguobacterium* strains studied, suggesting that cold-stressed bacteria may enter cyst-like resting states that enhance their survivability (Chong et al. 2000; Qiu et al. 2006; Soina et al. 2004). Growth at low temperatures has been shown to require more energy and be less efficient (Bakermans et al. 2003; Bakermans and Nealson 2004). Both *Exiguobacterium* strains showed low-temperature overexpression of triosephosphate isomerase that involved glycolysis which might be maximally induced under cold growth (Wouters et al. 2000). Some bacteria use different pathways at different growth temperatures; for example, psychrotrophic *Rhizobium* strains switched from respiration to lactate glycolysis in order to generate energy effectively at low temperatures (Sardesai and Babu 2000). Temperature-specific carbon source utilization has also been observed in *E. sibiricum* 255-15 and *P. arcticus* 273–4 (Ponder et al. 2005). Various carbon sources may differentially influence the protein production, suggesting that cells grown with one carbon source may be stressed by low temperatures to a greater extent than cells grown with another (Barbaro et al. 2002). The suggested induction of the glycolysis at low temperature has been further supported by observation of up-regulation the enzymes of the glycolytic pathway, e.g. malate/lactate dehydrogenases, in *P. cryohalolentis* K5 (Bakermans et al. 2007).

The affinity to substrate decreases at low temperatures; therefore the changes in transport systems are required to counteract lower rates of diffusion and solute transport across the membrane (Nedwell 1999). Bacteria of the genera *Exiguobacterium* and *Psychrobacter* were shown to be able to grow at temperatures below 0°C, therefore the processes of substrate sequestration from the environment and excretion of spent solutes from cells turn out to be very important for growth at the low temperatures. A number of transport-related proteins and membrane-associated proteins were up-regulated by cold in these strains (Chong et al. 2000; Qiu et al. 2006; Bakermans et al. 2007; Zheng et al. 2007). The drop of a temperature below 0°C leads to ice formation within the cell which might lead to cell lysis, and leads to the increase of salinity outside the cell followed by the consequent increase of an osmotic gradient across the cell membrane. The cold-shock induced ice nucleation activity in different psychroactive bacteria including *E. sibiricum* 7-3 (Ponder et al. 2005), and induced synthesis of the ice nucleation proteins which can act as a template for ice formation (Kawahara 2002). Another stress that bacteria encounter at low temperatures is oxidative stress, because oxygen radicals accumulate to higher concentrations, given that oxygen is more soluble and reduced respiration rates consume oxygen more slowly. The CIPs of diverse functions including chemotaxis, hydroperoxide detoxification, and surface proteins may maintain cell integrity and functioning during this stress (Bakermans et al. 2007).

The psychrotrophic bacteria harbored antibiotic multiresistant traits, and this feature increased with cold (Munsch-Alatossava and Alatossava 2007). While *E. sibiricum* 255-15 showed a decrease in resistance to chloramphenicol and tetracyclin at 4°C (penicillin was not tested) (Ponder et al. 2005), the high overexpression level of penicillin tolerance protein was detected in this bacterium at 4°C (Qiu et al. 2006).

During the growth at low temperatures, cells cope with amino acid starvation, oxidative stress, aberrant protein synthesis, cell-surface remodeling, alterations in degradative metabolism, and induction of global regulatory responses. A life in less than ideal environmental conditions leads to changes in the physiological state and the biochemical activity of bacterial cells, and these changes bind directly to protein synthesis.

12.4 Putative Roles of Differentially Induced Proteins in Cryotolerance

There has been growing interest in the survival mechanisms of psychroactive bacteria at repeated freeze–thaw cycles largely because successive freezing and thawing are common processes in nature. In addition, there is a considerable interest in the cryotolerance mechanisms of both bacteria related to food-spoilage and food-borne pathogens. It appears that overexpression of CSPs significantly improves cryotolerance, and helps to retard freezing or lessen the damage incurred upon freezing and thawing of the bacteria, yeasts, and plants (Kim et al. 1998a; Thomashow 1998; Broadbent and Lin 1999; Wouters et al. 1999; Thammavongs et al. 2000; Wouters et al. 2001; Minami et al. 2005).

In order to characterize freeze–thaw resistance, the single-cell isolates of the genus *Exiguobacterium* were subjected to repetitive freeze–thaw cycles (Vishnivetskaya et al. 2007). This study showed that bacteria grown in complex, structured (agar) medium had improved tolerance to the freeze–thaw challenge compared to bacteria grown in mass-action (liquid) medium, regardless of growth temperature. However, growth temperature was a determining factor of a cryotolerance in mass-action (liquid) habitat. Bacteria grown at 4°C in liquid medium tolerate freezing/thawing much better than when grown at 25°C. A subsequent study compared proteomic profiles of *E. sibiricum* 255-15 grown in liquid broth or an agar surface at both 4°C and 25°C to determine proteins important for cryotolerance (Qiu et al., unpublished). The bacteria with improved cryotolerance have revealed a general down-regulation of enzymes involved in major metabolic processes (glycolysis, anaerobic respiration, ATP synthesis, fermentation, electron transport, and sugar metabolism) as well as in the metabolism of lipids, amino acids, nucleotides and nucleic acids, while eight proteins (2′–5′ RNA ligase, hypoxanthine phosphoribosyl transferase, FeS assembly ATPase SufC, thioredoxin reductase and four hypothetical proteins) were up-regulated (Qiu et al., unpublished). It has been shown that the repression of RNA species and over-expression of enzymes involved in amino acid biosynthesis during nutritional deprivation led to improved bacterial survivability (Jain et al. 2006). The overproduction of the CSPs in the mesophilic bacterium *Lactobacillus plantarum* transiently alleviated the reduction in growth rate, and led to an enhanced capacity to survive freezing (Derzelle et al. 2003). In *E. sibiricum* 255-15, only 15% of the total cellular proteins were overexpressed more than two-fold under different growth conditions. The induction of these proteins might have a potential role in freeze–thaw resistance.

The suppression of some enzymes in the cells grown on agar or at low temperatures indicated the reduction of biochemical reaction rates at these conditions. Therefore, it is reasonable to assume that bacterial cells with slowed metabolism and an enhanced system of replication, recombination, and repair easily tolerate severe environmental factors, e.g., repetitive freeze–thaw cycles.

12.5 Conclusion

The studies described in this chapter indicate that the adaptive nature of permafrost bacteria at near-freezing temperatures is regulated by cellular physiological processes through the regulation of certain cellular proteins. Although cold adaptation is still far from being properly understood, it is possible that proteins synthesized at low temperatures may support temperature homeostasis, protect other proteins from denaturation and damage, and enable the cells to adapt to near or below-freezing temperatures.

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Part IV
Impact of Global Warming On Permafrost Properties

Chapter 13
Global Warming and Thermokarst

Julian B. Murton

13.1 Introduction

Thermokarst denotes the processes, landforms and sediments associated with ablation – usually by thawing – of excess ice in permafrost. Thaw has two important geomorphic consequences: (1) a reduction in soil strength due to the change to an unfrozen state, and (2) a reduction in soil volume (consolidation) due to the loss of excess ice. Both factors promote geomorphic and sedimentary processes that can transform the morphology of the land surface and the physical properties of the substrate. Because thermokarst activity is usually initiated by disturbances to the energy balance at or near the ground surface, thermokarst phenomena are sensitive indicators of environmental change. This chapter reviews the processes, development, activity and phenomena associated with thermokarst in permafrost soils, before considering the relationship between thermokarst and global warming. Thermokarst activity in frost-susceptible bedrock is discussed by Murton et al. (2006).

13.2 Thermokarst Processes

13.2.1 Thermokarst Subsidence

Thermokarst subsidence denotes a lowering of the ground surface following ablation of excess ice in permafrost. Ablation typically occurs by melting caused by heat conduction as the active layer deepens or surface water ponds. In permeable soils, however, it also results from heat convection by percolating rain or groundwater. The subsequent loss of excess water by drainage or evaporation allows the soil to consolidate and ground surface to subside. Subsidence is clearest where it is localised, for example above intersecting ice wedges (Fig. 13.1) or where collapse

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Fig. 13.1 High-centred ice-wedge polygons near Johnson Bay, Tuktoyaktuk Peninsula, western Arctic Canada. The pond has developed by thermokarst subsidence above intersecting ice wedges. The fissures marking the polygon margins have developed by thermal erosion. *Person* for scale. Photo courtesy of Mark Bateman

pits on the floors of drained lakes develop by melting of an ice-rich layer at the top of permafrost (Mackay 1999). Under very dry soil conditions, however, ice loss can occur directly by sublimation. In the hyperarid cold desert of southern Victoria Land, Antarctica, where soil temperatures may remain < 0°C all year, sublimation of ground ice through a porous, gravelly overburden has caused localised ground subsidence, forming prominent troughs around high-centred ice-wedge polygons (Marchant et al. 2002).

The degree of subsidence depends on the amount and distribution of excess ice prior to thaw and on the thickness of permafrost thawed (Mackay 1970). Subsidence can be predicted if the excess ice content is known, although prediction may be complicated by mass movement (Lawson 1982). The amount of subsidence varies from millimetres to many metres. Subsidence of 20 m or more can result from thaw of thick, ice-rich Pleistocene silts (Yedoma or ice-complex) which underlie more than 1 million km^2 of northern Siberia and central Alaska (Kachurin 1962; Zimov et al. 2006).

13.2.2 Thermal Erosion

Thermal erosion occurs where flowing water melts ground ice by the combined effects of heat conduction and convection, and then mechanically erodes newly released sediment. It often occurs on hillslopes during periods of snowmelt or

Fig. 13.2 Frozen badlands formed by intense thermal erosion of massive icy sediments exposed within a retrogressive thaw slump, Summer Island, Tuktoyaktuk Coastlands, Canada. Relief is ~1 m between interfluves and gullies

heavy rainfall, and can lead to rapid gully development and persistent slope instability, particularly along ice wedges (Fig. 13.1; Mackay 1974, 1988; Seppälä 1997). In thaw slumps (see Sect. 13.4.3), intense thermal erosion during hot summer days can lead to intense dissection of massive icy sediments, producing a remarkable frozen badlands topography (Fig. 13.2).

13.2.3 Surface Ablation

Excess ice that erosion exposes at the surface ablates by melting and sublimation. Ablation is fastest in summer, when melting occurs by radiation or sensible heat transfer (Lewkowicz 1986, 1988), and releases sediment that falls, slides or flows to the base of the ice exposure. Sublimation is favoured by dry winter conditions, and is apparent where pebbles or soil aggregates protrude from the ablating ice surface.

13.2.4 Combinations

Thermokarst processes often occur in close proximity or succession. For example, thermokarst ponds whose floors subside over intersecting ice wedges may deepen sufficiently to initiate flow of water along adjacent ice-wedge troughs. Likewise,

ground ice exposed in bluffs may ablate by thermal erosion and surface melt in summer, and by sublimation in winter.

13.3 Thermokarst Development

13.3.1 Initiation

Thermokarst usually commences when a disturbance to the surface energy balance raises the ground temperature sufficiently to thaw excess ice in the underlying permafrost. Many disturbances of local to regional extent initiate thermokarst (Table 13.1). For example, removal of vegetation or peat cover raises the ground surface

Table 13.1 Factors that initiate, retard or counteract thermokarst activity

Scale	Factors	Initiating disturbances	Retarding or counteracting factors
Local	Vegetation and surface organic mat	Damage or removal	Regrowth of vegetation
		Compaction of peat or organic soil	Accumulation of peat or organic soil
	Water	Ponding on ground surface or underground	Drainage of ponds, lakes or cavities
		Flowing surface or groundwater	Refreezing of underground pools
			Improved drainage or diversion of drainage
		Wetting of dry peat in summer	Drying of peat in summer
	Snow cover	Thicker snow cover	Thinner snow cover
		Reduced snow density	Increased snow density
		Early snowmelt in summer	Late snowmelt in summer
	Overburden thickness	Soil erosion exposes ice-rich ground	Deposition of sediment
		Artificial removal of soil	Burial of ice-rich ground by spoil
	Artificial substrate	Laying of gravel pad too thin to contain seasonal freezing and thawing depth	Thicker gravel pad
			Insulation placed beneath gravel
	Artificial heat source	e.g., heated buildings, pipelines, utilidors	Dissipate heat (e.g. allow cold air circulation or use thermosyphons)
Regional	Mean annual air temperature	Climate warming	Climate cooling
	Regional snowfall	Thickening snow cover	Thinning snow cover
		Early accumulation of snow in winter	Later accumulation of snow in winter
	Summer weather	Unusually warm or wet weather	Cool and dry weather
	Continentality	Increased continentality	Decreased continentality
	Large forest fires	Damage vegetation or surface organic mat	Regrowth of forest

temperature in summer and often initiates thermokarst activity, as resulted from camp construction and drilling activities during the 1940s and 1950s on the tundra of the National Petroleum Reserve, northern Alaska (Lawson 1986). Further south, in the boreal forest near Fairbanks, central Alaska, removal of spruce trees, moss and underlying peat for land development resulted in thermokarst ponds forming within 5 years of site clearance (Nicholas and Hinkel 1996). Because of its low albedo (<10%), water ponding on the ground surface warms rapidly in summer, especially if the water is shallow and darkened by dissolved organic material. With its high heat capacity, water acts as a heat source and promotes thaw of underlying ground ice (Fig. 13.1).

Disturbances that initiate thermokarst are often compound and interactive. For example, fires in the boreal forest and forest-tundra may initiate active-layer deepening and thermokarst subsidence by (a) destroying the shady vegetation canopy, (b) reducing heat loss from evapotranspiration, and (c) lowering the surface albedo due to burning of the organic cover (Mackay 1995; Burn 1998). Both climate warming and regionally extensive fires may raise ground temperatures, and so increase the susceptibility of ice-rich permafrost to thaw by local disturbances (Burn 1992). Recent thermokarst activity in central Alaska reflects increases in both in mean annual air temperature (MAAT) and winter snow cover during the twentieth century (Jorgenson et al. 2001). Furthermore, a link between rising air temperatures and increasing frequency and magnitude of forest fires may accelerate permafrost degradation.

13.3.2 Stabilization

Thermokarst activity ceases when a balance is restored between surface energy inputs and the depth of seasonal thawing. In areas of thin permafrost, this may involve the disappearance of permafrost. But where the permafrost is thick or cold, thermokarst activity often ceases and the ground surface stabilizes before all of the excess ice has ablated. For example, thermal erosion may cease where deposition of sediment buries exposed ground ice, where differential thaw along gully axes eliminates drainage gradients (Lawson 1982), or where water trapped in underground channels and pools freezes to form pool ice (Mackay 1988).

13.3.3 Recovery

Thermokarst terrain may recover from the effects of thermokarst activity if permafrost re-aggrades and incorporates excess ice or peat. Permafrost aggradation results from reduced surface energy inputs due to factors such as vegetation growth, peat accumulation, improved drainage or climate cooling (Table 13.1). Regrowth of vegetation after a forest fire may lead to active-layer thinning and incorporation

into permafrost of ice lenses formed at the bottom of the active layer (Mackay 1995), thus heaving the ground surface. Terrain recovery is also manifest in areas where lake basins have drained at different times. On the coastal plain of northern Alaska, permafrost re-aggrades beneath the floors of drained basins, allowing excess ice to build an ice-rich layer in near-surface permafrost; hence the oldest drained basins tend to be the most ice-rich and therefore show the greatest recovery (Sellmann et al. 1975). Recovery of the ground surface from thermokarst subsidence or thermal erosion in ice-wedge terrain also occurs where peat preferentially accumulates in wet ice-wedge troughs or in the centres of low-centred polygons.

13.3.4 Complexities

Thermokarst development can be complex, varying locally according to factors such as microtopography, ice content, erosion and the geotechnical properties of thawed sediment (Lawson 1986). A second cause of complexity is where permafrost soils experience alternating episodes of ice loss and gain. These are common where part of the ice-rich layer in near-surface permafrost episodically thaws due to changes in active-layer depth or lake levels and then re-forms (Sellmann et al. 1975; Shur et al. 2005), or where ice wedges experience alternate thermal erosion and pool-ice formation (Mackay 1988).

13.4 Thermokarst Activity and Phenomena

Thermokarst activity is expressed geologically by lowering of the ground surface, retreat of slopes or hollowing out of the substrate. These expressions, occurring singly or in different combinations, allow us to distinguish here six fundamental modes of thermokarst activity in permafrost soils: (1) active-layer deepening, (2) ice-wedge melting, (3) thaw slumping, (4) groundwater flow, (5) shoreline thermokarst, and (6) basin thermokarst. A more complex classification of modes of permafrost degradation specific to the boreal forest is given by Jorgenson and Osterkamp (2005).

13.4.1 Active-Layer Deepening

Active-layer deepening is inevitable in areas of ice-rich permafrost — because of interannual or longer term variations in thaw depth — and leads to thermokarst subsidence. But evidence for subsidence is usually clear only where deepening has been substantial, where remnants of the unaffected ground surface remain, or where subsidence has altered the surface hydrology and vegetation. In the cryostratigra-

phy, evidence for former active-layer deepening occurs where a thaw unconformity truncates excess ice below the base of the modern active layer (Fig. 13.3). Pullman et al. (2007), in a study of potential thaw settlement following severe disturbance to vegetation on the tundra of the Alaskan Arctic Coastal Plain, determined values between 0 cm (in sandy soils) and 103 cm (in silty soils). Mackay (1995) estimated that, following a forest-tundra fire near Inuvik, Canada, active-layer deepening of 10–78 cm produced ground subsidence of 5–39 cm during the succeeding 5–20 years. Osterkamp et al. (2000) reported subsidence commonly of 2 m for discontinuous permafrost in the boreal forest of the Mentasta Pass area, southeast Alaska, where active-layer deepening attributed to climate warming has led to the replacement of spruce stands by wet sedge meadows whose surface is typically 1–3 m below that of the original spruce forest.

As the active layer deepens, thaw consolidation produces melt-out horizons and may trigger soft-sediment deformation. Mineral particles released from thawing ice assume a tighter packing than sediment dispersed in the ice, and so form distinctive melt-out horizons that record thaw events (Fig. 13.3). Soft-sediment deformation is most likely to occur during rapid thaw of ice-rich clayey sediments, when the soil is reduced to a fluid-like consistency. Under such conditions, processes associated with water-escape, buoyancy and subsidence form thermokarst involutions (Murton and French 1993; Harris et al. 2000). Other consequences of active-layer deepening

Fig. 13.3 Thaw unconformity marking the base of the early Holocene active layer truncates massive ice and icy sediments (basal Laurentide ice), Summer Island, Tuktoyaktuk Coastlands, Canada. Melt-out till containing thermokarst involutions in a relict active layer overlies the thaw unconformity. *Person* for scale

through ice-rich permafrost may include the enlargement of mud hummocks and, on hillslopes, enhanced gelifluction and triggering of active-layer detachment slides.

13.4.2 Ice-Wedge Melting

Melting of ice wedges often produces high-centred polygons (Fig. 13.1) or, where thermokarst activity is pronounced, thermokarst mounds (French 1975; Lawson 1986). Mounds 3–15 m in diameter and 0.3–2.5 m high started to form — mainly by thermokarst subsidence — within 2–3 years of vegetation clearance in cultivated fields near Fairbanks as surface water ponded in small disconnected depressions, accelerating thaw of the underlying ice wedges (Péwé 1954, 1982). On eastern Banks Island, Canada, thermal erosion of ice wedges by streams has produced conical thermokarst mounds that may exceed 8 m in height and 2–3 m in summit diameter (French 1974).

Thermal erosion of ice wedges beneath hillslopes often forms gullies and tunnels. In the Tuktoyaktuk Peninsula area, Canada, gullies are initiated by (1) collapse of tunnels formed by water flowing through interconnected ice-wedge cracks during the snowmelt period, (2) surface flow through ice-wedge troughs, (3) overtopping of snow dams followed by rapid erosion at lake outlets, and (4) diversion of lake outlets through ice-wedge systems (Mackay 1974, 1988). The gullies can develop rapidly to depths of several metres when lakes drain catastrophically. In central Yakutia, Siberia, sinkholes and underlying tunnels form where thermokarst mounds collapse into adjacent trenches and disintegrate through thermal erosion (Czudek and Demek 1970).

Thermokarst subsidence can also create or accentuate low-centred polygons. Water seeping into and moistening dry, unfrozen peat in polygon centres increases the thermal conductivity of the peat and thus the depth of thaw. Where the upper layer of permafrost is ice-rich, the resulting thermokarst subsidence may lead to ponding of surface water in the centres of low-centred polygons (Dredge and Nixon 1979). Where the soil is exceptionally ice-rich, thermokarst subsidence beneath the centres and troughs of low-centred polygons can transform them into walled or fortress polygons following a rapid lowering of the water table in ice-wedge troughs (Mackay 2000).

Thermokarst subsidence and thermal erosion sometimes coexist in gently sloping depressions, forming beaded streams: a series of pools linked by short, narrow channels (Higgins et al. 1990). The pools are 0.5–3 m deep, ≤30 m in diameter and form by melting of ground ice, usually at ice-wedge intersections. The channels tend to form by thaw of individual ice wedges, and therefore have short, straight sections, often with abrupt changes in direction at ice-wedge intersections.

Ice-wedge melting produces voids that often fill with sediment. The process of infilling (ice-wedge casting) and the resulting structures (ice-wedge pseudmorphs, involutions and tunnel fills) are strongly influenced by thaw-consolidation processes (Harris et al. 2005; Murton 2006).

13.4.3 Retrogressive Thaw Slumping

Retrogressive thaw slumping is a slope failure characterized by thaw of exposed ground ice and slumping of thawed soil. Slumping usually starts where ice-rich permafrost is exposed by erosion, mass movement, forest fires, construction or mining (Burn and Lewkowicz 1990). Where the exposure reveals massive ice, large ice wedges or dense concentrations of segregated ice, slumping may quickly enlarge it to produce a steep or vertical headwall (1 m to > 15 m high) that overlooks a low-gradient floor covered by slumped soil.

Headwall ablation occurs mainly by radiation and sensible heat transfer, and often leads to rapid slope retreat. Net radiation is dominant in some High Arctic slumps, but sensible heat transfer is more important in warmer permafrost regions (Lewkowicz 1988). Retreat rates depend on atmospheric conditions and ground-ice concentration. Rapid ablation is favoured by clear, warm and windy conditions, when radiative inputs and turbulent transfer of heat to the ice are high, and during rainstorms, which wash thawed soil from the thaw face. Rates of headwall retreat often reach several metres per year, with rates as high as 16 m per year and 23 mm h^{-1} measured in central Yukon (Burn and Lewkowicz 1990).

Permafrost degradation beneath slump floors occurs by heat conduction or convection. In the boreal forest near Mayo, central Yukon, Burn (2000) measured increases in ground temperature with time, and increased depths to permafrost with distance from a slump headwall. Permafrost degradation between 1949 and 1995 resulted from surface disturbance by slumping, which raised mean annual ground temperature (MAGT) by ~3–4°C at 1 m depth beneath the slump floor. As permafrost degraded — primarily by conductive heat flow in fine-grained soil — the active layer thickened to > 4.8 m. Where permafrost had degraded longest and reached a depth of 7 m or more, a residual thaw (unfrozen) layer developed above the permafrost and beneath the depth of seasonal frost penetration. Where slump-floor sediments are sandy and permeable, as on Summer Island, NWT (Murton 2001), convective heat flow from percolating groundwater probably contributes to degradation.

Thaw slumps eventually stabilize. Stabilization results when all of the excess ice has melted, where slumped soil insulates the headwall, or where the slope gradient above the headwall is less than that of the slump-floor deposits, which therefore bury the excess ice. The duration of thaw slumping varies from a single summer to several decades or more (e.g., Lewkowicz 1987; Burn 2000). After slumps stabilize, permafrost may re-aggrade beneath the slump floor and vegetation re-establish. Near Mayo, re-establishment of a birch/white spruce sere similar to that of the original boreal forest takes ~35–50 years after slumping (Burn and Friele 1989). Cycles of slumping and stability may occur where erosion episodically removes slumped debris.

Soil and organic material fall, slide or flow from ablating headwalls onto the slump floor, where they are often reworked by debris flows or meltwater. On eastern Banks Island, debris-flow morphology, size and activity are largely determined by the liquid limit, permeability and water content of the thawed soil (French

1974). The resulting debris-flow deposits usually comprise a mixture of soil, peat and vegetation (Murton 2001).

13.4.4 Groundwater Flow

Groundwater flowing through discontinuous bodies of ice-rich permafrost thermally erodes tunnels, cavities, caverns and pits. Near Fairbanks, surface water entering small cracks and tunnels, augmented by meltwater from ground ice, percolates downward through ice-rich silts towards a water table 5–30 m beneath the surface. The percolating water often enlarges depressions initiated by thermokarst subsidence, forming steep-walled, sinkhole-like features (1.5–6 m deep and 1–10 m across) known as thermokarst pits (Péwé 1954, 1982; Higgins et al. 1990). The pits develop within 3–30 years after vegetation clearance. Tunnels extend from the base of some pits, and current marks and waterlain silt on some tunnel floors indicate intermittent underground streamflow. In some pits, the floor of the boreal forest — held together by roots — is suspended over cavities 0.5 m deep formed by thermokarst subsidence beneath the root layer (Osterkamp et al. 2000). Caverns and pits sometimes fill with sediment to form casts that can be identified in stream banks and mining cuts in Alaska.

13.4.5 Shoreline Thermokarst

Thermokarst activity along the shorelines of rivers, lakes and seas involves thermal erosion and thermokarst subsidence. Thermal erosion at shorelines that dissect ice-rich unconsolidated sediments causes undercutting and rapid bank retreat. Undercutting by waves and currents excavates a horizontal cleft (thermo-erosional niche) that may extend 10 m or more laterally into the bank, at about water level (Fig. 13.4). Above the niche, the undermined permafrost episodically collapses in large blocks, often along ice wedges. Such erosion is common in the very ice-rich permafrost fringing the Arctic Ocean, particularly around the Laptev Sea, where mean retreat rates due to thermal erosion of the ice complex are 2–6 m per year (Are 1983).

Retreat rates show high spatial and interannual variability. For example, on the Lena River, northern Siberia, retreat exceptionally reaches 19–24 m per year, or even 40 m per year (Are 1983). On the Colville River, northern Alaska, the long-term retreat rates rarely exceed 3 m per year, although block collapse can generate an almost instantaneous retreat as much as 12 m, protecting the bank from further retreat for periods of up to a few years (Walker et al. 1987). Numerical analysis and experimental simulation of fluvial thermal erosion suggest that exceptionally high retreat rates reflect a combination of high water temperatures and river discharge, in association with some particular channel geometry (Costard et al. 2003). Along coasts exposing ice-rich permafrost, exceptionally

Fig. 13.4 Thermo-erosional niche developed in massive ice beneath the floor of a retrogressive thaw slump along the Beaufort Sea coast at North Head, Richards Island, Tuktoyaktuk Coastlands, Canada. *Spade* for scale

high retreat rates result from storm events. For example, a maximum rate of 19 m per year estimated during a stormy year contrasts with a long-term rate of 1.9 m per year for the same coastal segment of the Beaufort Sea coast, NWT (Dallimore et al. 1996).

Thermokarst subsidence occurs along coastal margins where excess ice in subsea permafrost thaws beneath the seabed. For example, where the warm waters of the Mackenzie River enter the Beaufort Sea, sea-bottom temperatures of ~2°C exist year-round in water depths shallower than ~10 m and deeper than the zone where sea ice freezes to the seabed (Rachold et al. 2000). Thus, ice-bonded permafrost can degrade continuously in a narrow coastal band. Rates of seabed subsidence of 5–7 mm per year are estimated along parts of the Canadian Beaufort Sea Shelf.

13.4.6 Basin Thermokarst

Thermokarst basins are closed depressions formed by degradation of ice-rich permafrost. They are generally 0.5–20 m deep and 0.01–5 km in diameter, and many contain standing water (thermokarst ponds and lakes). The basins are initiated by factors such as water ponding or vegetation degradation. Thermokarst ponds or

lakes sometimes develop at sites where thaw occurs beneath standing water, notably at ice-wedge intersections or in low-centred polygons, as well as under small streams (Dredge and Nixon 1979). The likelihood of such site-specific disturbances may be increased by regional disturbances such as climate warming (Burn and Smith 1990).

Basins grow by deepening and widening. Deepening is promoted by ponding of water on the basin floor, especially if water depth exceeds the maximum thickness of winter lake ice (~2 m); when this occurs, the bottom-water temperature exceeds 0°C all year, resulting in continuous thaw of underlying excess ice and subsidence of the lake floor. Ponds and lakes also thaw permafrost around their margins, causing bank subsidence, slumping of lake shorelines and submergence or tilting of vegetation (Burn 1992). In lakes with sufficient fetch, wave-induced currents and lake-ice scour erode shores, remove newly thawed sediment or initiate thaw slumping (Rampton 1974). In central Yakutia, large thermokarst basins with steep sides and a flat, grass-covered floor (alases) develop by slumping of thermokarst mounds on basin margins and then by thermokarst subsidence beneath a thermokarst lake (Czudek and Demek 1970; Soloviev 1973). Some alases are several thousand years old, whereas others have formed during the span of a human generation. Alases may eventually coalesce, forming thermokarst valleys.

Limited data are available on rates of lake-basin enlargement. Wallace (1948) estimated bank retreat of ~0.06–0.18 m per year at two thermokarst lakes in eastern Alaska, and Burn and Smith (1990) employed comparison of aerial photographs taken in 1949 and 1984 to derive a mean growth rate of 0.7 m per year for 12 thermokarst lakes in boreal forest near Mayo. Radial expansion rates of 1.5–5.0 m per year (but accelerating through time) were determined for a high-mountain thermokarst lake on the Gruben rock glacier in the Swiss Alps (Kääb and Haeberli 2001).

Basin size and shape are controlled largely by the distribution and volume of pre-existing excess ice, the time since thaw commenced, and by erosion and sedimentation. Shallow lake basins, usually no more than a few metres deep, form by thaw of the ice-rich layer in near-surface permafrost (Sellmann et al. 1975), whereas basins 10–40 m deep represent thaw of much thicker ice-rich permafrost (Czudek and Demek 1970; Carter 1988; Romanovskii et al. 2000).

Basin growth may cease by lake drainage, infilling with sediments and peat, or exhaustion of ground ice (Burn 1992). Lake drainage is sometimes rapid. On the Tuktoyaktuk Peninsula, on average two lakes drain catastrophically each year (Mackay 1988). Drainage results mainly from diversion of water through interconnecting ice-wedge systems, causing rapid thermal erosion. Lake drainage is often incomplete, leaving shallower lakes or residual ponds. Basins infill by lacustrine or colluvial sedimentation, hydroseral encroachment by plants such as sedges and Sphagnum, peat accumulation and eventually growth of ice wedges. The sediments within them represent the most widespread type of thermokarst sediments, with a high preservation potential and often a distinctive stratigraphy (Hopkins and Kidd 1988; Murton 1996).

13.5 Thermokarst and Global Warming

Global warming is one of many factors that initiate thermokarst activity (Table 13.1). But it is undoubtedly a key factor, based on the abundant evidence in the geological record for pan-Arctic thermokarst activity during the Last Glacial-to-Interglacial Transition (LGIT) and the signs of intensifying thermokarst activity in recent decades.

13.5.1 Glacial-To-Interglacial Transitions

Global warming during the LGIT initiated very widespread and intense thermokarst activity in the icy permafrost lowlands of the Arctic and sub-Arctic. The warming occurred in two short bursts, the first at ~14,500 calibrated year BP — the start of the Bølling-Allerød warm period (Greenland Interstadial 1e); and the second at ~11,500 cal year BP — the start of the Holocene (Björck et al. 1998). Evidence for LGIT thermokarst activity is preserved in thermokarst basin fills, relict active layers and ice-wedge pseudomorphs in Alaska, Canada and Siberia (McCulloch and Hopkins 1966; Rampton 1974, 1988; Tomirdiaro 1982; Burn et al. 1986; Burn 1997; Romanovskii et al. 2000; Walter et al. 2007). Interestingly, evidence for more muted thermokarst activity in northwest Europe — horizons of ice-wedge pseudomorphs, thick involuted layers and deformed brecciated bedrock (Vandenberghe and Van Den Broek 1982; Vandenberghe and Pissart 1993; Murton et al. 2003) — suggests that regional thermokarst commenced before the LGIT, probably when climate warmed in Greenland Interstadial 2 (21,800–21,200 cal year BP).

The penultimate glacial-to-interglacial transition at ~130,000 year BP also triggered regional thermokarst activity. Climate warming at that time initiated deep and rapid thaw of ice-rich permafrost in treeless terrain in the Yukon–Tanana upland, east-central Alaska. Meltwater from thawing ground ice thermally eroded gulleys, triggering block slumping and producing an irregular thermokarst terrain. Subsequently a protective cover of boreal forest developed above the thermokarst features during the Last Interglaciation (LIG), remains of which are preserved as the Eva Interglaciation Forest Bed (Péwé et al. 1997). During the LIG, the summer climate of the Arctic was markedly warmer than during the twentieth century or the late Holocene, providing a potential analogue for future global warming (Otto-Bliesner et al. 2006).

13.5.2 Last 100–150 Years

Although there is clear evidence that thermokarst activity during the last 100–150 years has spread and intensified, not all of it can be attributed to global warming. One of the clearest examples where climate warming has exacerbated thermokarst

is an abrupt increase in ice-wedge melting since 1982 in continuous permafrost of northern Alaska (Jorgenson et al. 2006). The melting probably resulted from record high summer temperatures between 1989 and 1998, and was initiated by extreme hot and wet summer weather in 1989, leading to unusually deep thaw of the active layer. This thermokarst activity coincided with a 2–5°C increase in mean annual ground temperature, partially melting ice wedges that had previously been stable for thousands of years.

Further south, in warm discontinuous permafrost of sub-Arctic regions, increased thermokarst activity since the Little Ice Age is well-established (see review in Jorgenson and Osterkamp 2005), but the causes are complex. For example, thermokarst activity in the Tanana Flats, central Alaska, has transformed large areas of birch forest into fens and bogs (Jorgenson et al. 2001). Thermokarst here probably began in the mid-1700s, associated with climate warming. But thermokarst activity during the succeeding ~250 years has been enhanced in part by (1) convective heat transfer by movement of relatively warm (2–4°C year-round) groundwater through the fens and underlying outwash gravel, (2) fires, and (3) increased snow depths. Isolating the influence of fire, snow and climate warming is difficult, because an increase in fire frequency may correlate with an increase in summer temperatures (Jorgenson and Osterkamp 2005), and because warmer winter temperatures may correlate with increased snowfall and therefore warmer MAGTs (cf. Osterkamp 2007).

In western Siberia, climate warming since the early 1970s is thought to have driven thermokarst activity in two different ways (Smith et al. 2005). In the continuous permafrost zone, the number of lakes has increased substantially, whereas in discontinuous, isolated and sporadic permafrost it has decreased. This disparity supports a conceptual model in which initial warming of cold, continuous permafrost favours thermokarst activity and lake expansion, followed by lake drainage as permafrost degrades further. In central Siberia, climate warming since the 1980s has led to increased water temperature in the Lena River and its tributaries, in turn leading to increased rates of fluvial thermal erosion along their banks (Costard et al. 2007); significantly, this recent climate warming followed a period of cooling in the mid twentieth century, when thermal erosion rates along the coast of the Laptev sea tended to decrease (Are 1983). Finally, increases in mean annual air temperature and summer air temperatures between 1992 and 2001 at Yakutsk have coincided with (1) thermokarst subsidence beneath stable inter-alas meadows and (2) flooding of young thermokarst basins, enhancing thermokarst activity at a nearby permafrost monitoring site (Fedorov and Konstantinov 2003).

13.5.3 Next 100 Years

With climate warming predicted to continue during the next century, amplified in Arctic and sub-Arctic regions (ACIA 2005), thermokarst activity will generally spread and intensify still more. Thawing of permafrost is projected to be concen-

trated in the current discontinuous permafrost zone during the next 100 years (Deslile 2007). This is of particular concern in sub-Arctic Alaska, where ~40% of the area may be susceptible to thermokarst (Jorgensen et al. 2007). Thus, global warming at high latitudes is putting large areas of ice-rich permafrost at risk of thermokarst subsidence and related disturbances (Nelson et al. 2001).

Although projected climate warming in the twenty-first century will lead to deeper ground thaw in many permafrost regions, its impacts will be modulated by site-specific conditions. For example, peat and vegetation cover may buffer permafrost from severe degradation, whereas local disturbance of ground cover or fires in the boreal forest or tundra may accelerate permafrost thaw (Yi et al. 2007). Thus, caution is needed in generalizing between projected changes in atmospheric climate and geocryological responses.

13.6 Conclusion

Thermokarst activity in ice-rich permafrost soils is significant for a number of reasons: (1) it represents a major process of landscape evolution and sedimentary disturbance; (2) it provides an important geoindicator of global warming; and (3) it poses significant geotechnical problems. Although the causes, development and processes of thermokarst activity are manifold and often complex, it is clear both from the geological record and from observations during the last 100–150 years that projected global warming will generally cause thermokarst to intensify and spread. Such issues will become increasingly important to scientists, engineers and inhabitants of permafrost regions.

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Chapter 14
Global Warming and Mountain Permafrost

Wilfried Haeberli(✉) and Stephan Gruber

14.1 Introduction

The phenomenon of permafrost or perennially frozen ground is a specific ground thermal condition (see Chap. 3). Atmospheric warming is therefore likely to have strong if not dramatic impacts on permafrost, making cold areas at high latitudes and high altitudes especially vulnerable. The phenomenon of permafrost in cold mountains, however, has long been neglected in scientific research. As a consequence, the effects of global warming on perennially frozen mountain slopes have been studied for little more than a decade only. First overviews were given by Cheng and Dramis (1992) and Haeberli et al. (1993a). They were soon followed by first thoughts about goals and possibilities of long-term monitoring (Haeberli et al. 1993b).

In the meantime, concentrated efforts were undertaken to build up a corresponding knowledge base (Haeberli et al. 1998) and to establish baselines for long-term monitoring within the framework of the Global Climate Observing System/Global Terrestrial Observing System (GCOS/GTOS). The most systematic efforts were undertaken in European mountains, which form an important longitudinal transect from Svalbard through Scandinavia and the Alps to the Sierra Nevada in Spain (Harris et al. 2001). Corresponding information (Harris et al. 2003; Isaksen et al. 2007), together with results from similar observations elsewhere — especially from high mountains in Asia (Jin et al. 2000; Marchenko et al. 2007) — is now more and more entering international climate change assessments (IPCC 2007a, b; UNEP 2007).

An intense learning process has started, for which long-term observations are key elements enabling improved process understanding. The present review can therefore only represent a brief and rather preliminary halt on a widening avenue of fascinating progress and rapid knowledge development concerning a still too little-known aspect of the global environment. With a primary focus on experience from the densely populated European Alps with their rugged topography, the review starts with a short explanation of basic principles, and continues with some outlines of available

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methodologies in order to summarize first results of observations and to compare them with numerical model studies with regard to possible consequences for human habitats in some of the most climate-sensitive regions on Earth.

14.2 Basic Principles

Extreme spatial variability with respect to microclimatic conditions, abundance of well-drained coarse sediments and bare rock on steep slopes, snow redistribution by wind and avalanches, and the reduced influence of vegetation cause permafrost in alpine topography (Fig. 14.1) to be strikingly different from permafrost in high-latitude lowlands, and to react in a specific way to climate change and global warming. Complications already start on the highest peaks with very steep to vertical and largely snow-free rock walls, where subsurface heat diffusion can be assumed to play a predominant role: permafrost inside such mountain peaks can be effectively decoupled from geothermal heat because of pronounced lateral fluxes caused by its often strongly asymmetrical three-dimensional (3D) geometry and thermal structure (Gruber et al. 2004b; Noetzli et al. 2007). Furthermore, warming trends can penetrate from two or more sides to greater depths below the surface.

Fig. 14.1 The village of Täsch and the Mischabel Group in the Matter Valley, Valais Alps, Switzerland, with numerically simulated permafrost distribution (*blue*; *red* = uncertainty zone and probably warm/degrading/already thawed permafrost). Note dams for rock fall protection on left and debris flow on right slope above village. In 2001, a debris flow from a moraine lake in marginal permafrost of the lateral valley (*centre of image*) caused heavy damage to the village (satellite imagery: © ESA/Eurimage, CNES/Spotimage, swisstopo/NPOC; permafrost simulation/visualization: S. Gruber, S. Biegger, University of Zurich)

On less inclined slopes, a spatially and temporally most variable snow cover acts as a complex interface between the warming atmosphere and the ground surface. It thereby greatly affects the radiation balance via the albedo — especially in spring and early summer — as well as the exchange of sensible heat through thermal insulation (Lütschg et al. 2004). Even greater complexities exist on the widespread slopes covered by coarse-grained, well-drained debris, because openwork active layers enable lateral and vertical heat advection through movements of air and water to play an important role (Bernhard et al. 1998; Delaloye et al. 2003; Vonder Mühll et al. 2003; Hanson and Hoelzle 2004). Additionally, the low thermal conductivity of such deposits causes a relative ground cooling when compared to other materials, because of a lower contrast between the thermal conditions during winter (snow and ground) and summer (only ground, Gruber and Hoelzle 2008).

Ice contents far in excess of the pore volume are common in perennially frozen sands and silts. They not only cause perennially frozen debris to creep at considerable rates and to form striking landforms of cohesive flow (rock glaciers) in otherwise non-cohesive material (talus, moraines), but also retard permafrost thaw through latent heat exchange. Finally, permafrost in high mountain areas often interacts with various forms of perennial surface ice such as persisting avalanche cones, perennial snow banks and glacierets as well as with polythermal to cold mountain, cirque and hanging glaciers (Haeberli 2005; Gruber and Haeberli 2007). In these cases, the warming-induced evolution of subsurface ice is intimately coupled with the vanishing of surface ice.

14.3 Methodologies

The most direct information about the reaction of mountain permafrost to climate change derives from borehole temperature measurements. Following the equipment for long-term monitoring of the first borehole drilled through an active rock glacier (Haeberli et al. 1988; Vonder Mühll and Haeberli 1990; Vonder Mühll et al. 1998), attempts were made to collect borehole measurements from mountains on several continents (Haeberli et al. 1998). The standardized bedrock boreholes to 100 m depth established by the EU-funded project Permafrost and Climate in Europe (PACE; Fig. 14.2) for climate-related monitoring of mountain permafrost through the European mountains thereby constitute a major contribution to the Global Terrestrial Network for Permafrost (GTN-P) within GTOS/GCOS (Harris et al. 2001).

A real revolution in the systematic observation of thermal conditions at surfaces of remote slopes and rock walls with difficult access (especially in wintertime) was the introduction and installation of a rapidly increasing number of miniature temperature loggers, which provide fundamentally important high-resolution information on surface temperatures and snow-cover effects (Hoelzle et al. 1999, 2003; Gruber et al. 2003).

Modern strategies of long-term permafrost monitoring at high mountain sites now combine measurements of borehole temperature with miniature temperature

Fig. 14.2 Drilling into permafrost at Juvasshøe near Jotunheimen, Norway for long-term monitoring of borehole temperatures. Permafrost temperature at 20 m depth is about −3°C, and permafrost thickness clearly exceeds 100 m (photo: K. Isaksen 2000)

logging at nearby surfaces, geophysical soundings or even time-lapse geophysics (resistivity and seismic tomography) at fixed profiles (Fig. 14.3; Krautblatter and Hauck 2007) and numerical modelling of time-dependent 3D-temperature evolution (Noetzli et al. 2007). Together with observations of temperature evolution through time (Isaksen et al. 2007), the latter is especially important to disentangle topographic and climatic effects on temperature profiles with depth, and to reconstruct past permafrost temperature histories from heat-flow anomalies (Gruber et al. 2004b). Photogrammetry and more recently also differential GPS and INSAR technologies are used to document flow patterns and their changes in time of creeping permafrost within numerous rock glaciers (Haeberli et al. 2006; Strozzi et al. 2004; Delaloye et al. 2008).

14.4 Observations

The longest, high-resolution time series of borehole temperatures is available from ice-rich, slowly creeping permafrost with an active layer consisting of coarse blocks in the active Murtèl rock glacier (Fig. 14.4). The overall trend observed since 1987 is permafrost warming by about 0.4°C per decade at 10 m depth, and roughly twice as much for the summer temperatures in the active layer. Winter temperatures strongly depend on winter snow conditions rather than on atmospheric temperatures alone. As a consequence, permafrost temperatures remained stable or

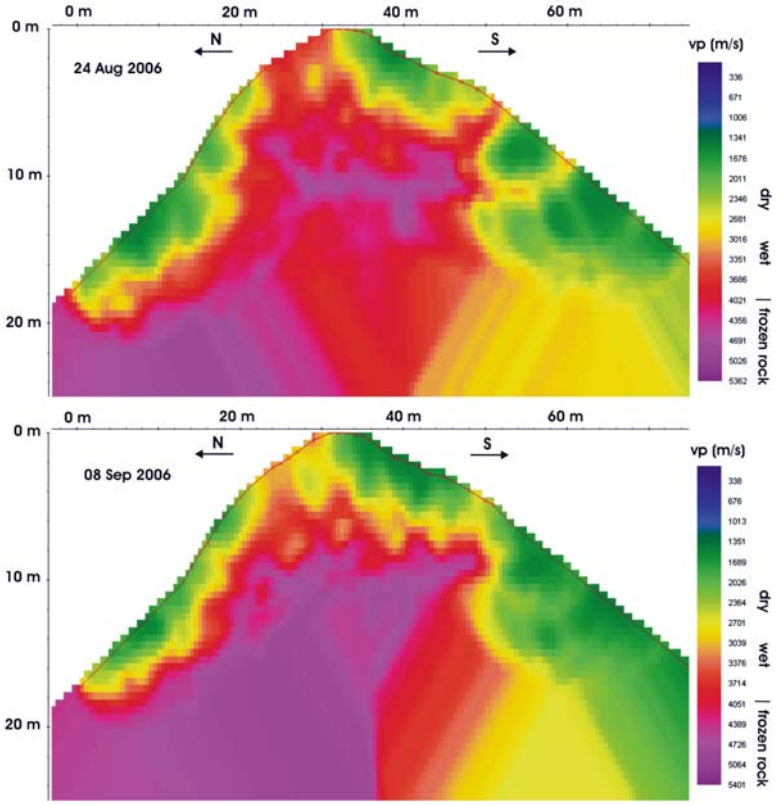

Fig. 14.3 Tomographies derived from repeated (24 August and 8 September 2006) P-wave refraction seismics on the E–W trending rock crest "Steintälli" (3,150 m above sea level) between Matter- and Turtmann Valleys, Switzerland. *Dark red* colours correspond to partly frozen rock sections, *purple* mostly to the deeply frozen permafrost core without residual water in pores. It appears that in delayed response to cool August temperatures, the frozen rock core develops towards the north face and cools inside. Simultaneously, the snow cornice on top (20–30 m) melts and gives way due to thermal heat conduction from the surface (source: M. Krautblatter 2007)

even decreased during the past decade with above-average high winter air temperatures but relatively thin snow cover. This example clearly illustrates the complexity of the atmosphere/permafrost coupling under such — quite characteristic — high-mountain conditions: snow as a "nervous", hardly predictable interface will continue to cause large uncertainties about future developments in such cases (Lütschg et al. 2003; Lütschg and Haeberli 2005), making continued monitoring indispensable with regard to improved future knowledge.

The PACE borehole temperatures exhibit clear indications of a century-long warming of permafrost within bedrock; however, conclusions can only be drawn on the basis of 4D modelling. Nevertheless, preliminary interpretation of the documented thermal anomalies with respect to an assumed steady-state profile in homogenous

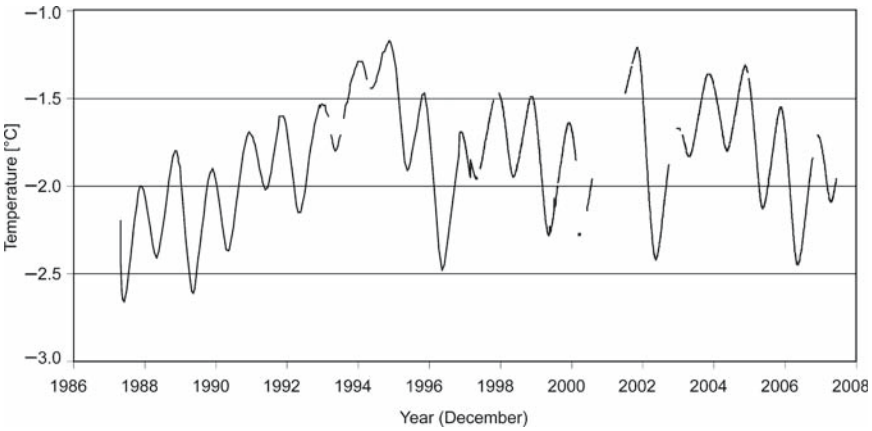

Fig. 14.4 Borehole temperatures at a depth of 11.6 m in the ice-rich permafrost of the active rock glacier Murtèl/Corvatsch, Grisons Alps, Switzerland. The overall trend is ground warming by about 0.4°C per decade, but inter-annual variations are large and snow-cover effects important

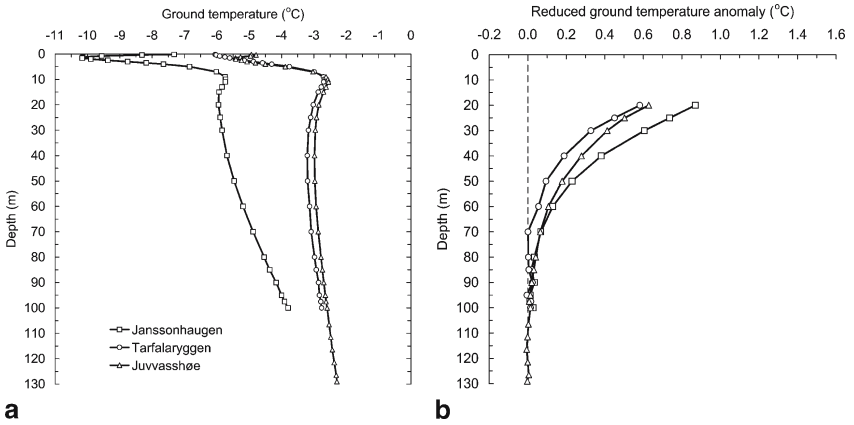

Fig. 14.5 Borehole temperatures and recent warming. **a** Ground temperature profiles in permafrost at Janssonhaugen (Svalbard, 102 m deep, 78°10'N, 16°28'E, 270 m above sea level), Tarfalaryggen (Sweden, 100 m deep, 67°55'N, 18°38'E, 1550 m a.s.l.), and Juvvasshøe (Norway, 129 m deep, 61°40'N, 08°22'E, 1894 m a.s.l.), recorded on 22 April 2005. **b** Profiles of reduced temperature anomalies; data are obtained by subtracting temperatures for assumed steady state conditions from measured temperatures for depths at which annual fluctuations are negligible. Steady-state temperatures were estimated by extrapolating the thermal gradient measured in the lowermost part of the borehole, which is assumed to be unaffected by recent warming trends. Reproduced from Isaksen et al. (2007)

bedrock of simplified half-space geometry (Harris and Haeberli 2003, Harris et al. 2003) together with first detailed analyses of time-dependent temperature changes at depth leave little doubt that temperature rise in mountain permafrost over the past century has taken place at a continental scale and at a rate which is comparable to atmospheric warming ca. (0.5 to 1.5°C per century), creating a marked thermal anomaly

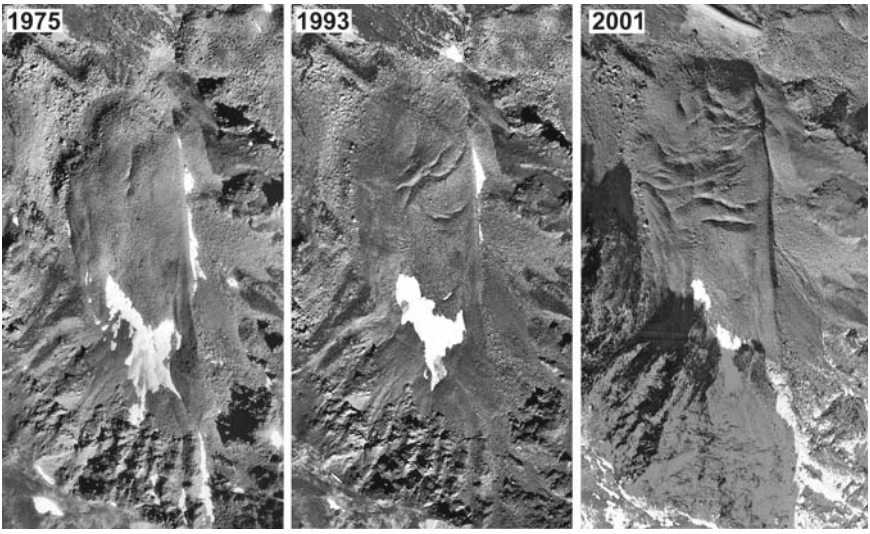

Fig. 14.6 Development in surface geometry and crevasse patterns due to strongly accelerated flow of an active rock glacier in the Turtmann Valley, Swiss Alps. Orthoimages of 20 August1975, 20 August 1993 (aerial photographs taken by Swisstopo) and 28 September 2001 (HRSC-A survey). From Roer (2007)

down to depths of 50–70m (Figure 15.6; Isaksen et al. 2007) which will continue to penetrate to greater depths. With the record warm winter 2006/2007, the outer parts of steep walls mantling mountain peaks in the European Alps may, in fact, have heated up to levels without precedent during the past millennia since at least the Upper Holocene.

Continued observation is also necessary to better understand the striking large-scale phenomenon of recently accelerated permafrost creep (Kääb et al. 2006; Delaloye et al. 2008), with in places the formation of deep crevasses indicating destabilization of large volumes of ice-rich debris (Fig. 14.6; Roer 2007).

14.5 Model Calculations

Early estimates (Haeberli 1985) already clearly indicated that latent heat effects would cause complete melting of perennially frozen rock glacier debris rich in ice to require many centuries, even with instantaneous atmospheric warming by several °C. Increasing temperatures over time and complicated effects from snow cover (Lütschg et al. 2003, 2004; Lütschg and Haeberli 2005) could easily extend such time scales beyond the millennium. In contrast to small and medium-size mountain glaciers, mountain permafrost will, therefore, continue to exist for long time periods into the future, though in a state of growing disequilibrium with respect to thermal conditions at the surface and with extreme heat-flow anomalies (reversal) down to depths of several tens of meters or more.

The same is true for permafrost in rock summits with steep slopes and walls. Time-dependent spatial heat diffusion modelling of idealized topographies provides fundamentally important insights (Fig. 14.7; Noetzli et al. 2007). After 100 years already, i.e., after twenty-first century warming, permafrost conditions may no longer exist at the surfaces of sun-exposed slopes, but frozen rocks may still be present at some depth below, as influenced by colder temperatures from both earlier centuries as well as colder slopes facing away from the sun. Roughly the 500 top meters of sharp mountain peaks are effectively decoupled from geothermal heat, and undergo changes influenced by multilateral warming as well as by strongly asymmetrical and often sub-horizontal heat flow through the mountain from warm to cold sides.

Inhomogeneities such as the occurrence of ice-filled cracks and fissures certainly cause more complex developments in reality. Penetration of surface water into such linear features, with almost stepwise increasing hydraulic permeability when thawing, is likely to lead not only to strong acceleration of deep warming but also to highly irregular structures of the thermal field inside mountain peaks. Together with modelling snow-cover effects, realistic simulation of the influence from ice-containing heterogeneities constitutes one of the primary challenges in climate-change-related research about mountain permafrost.

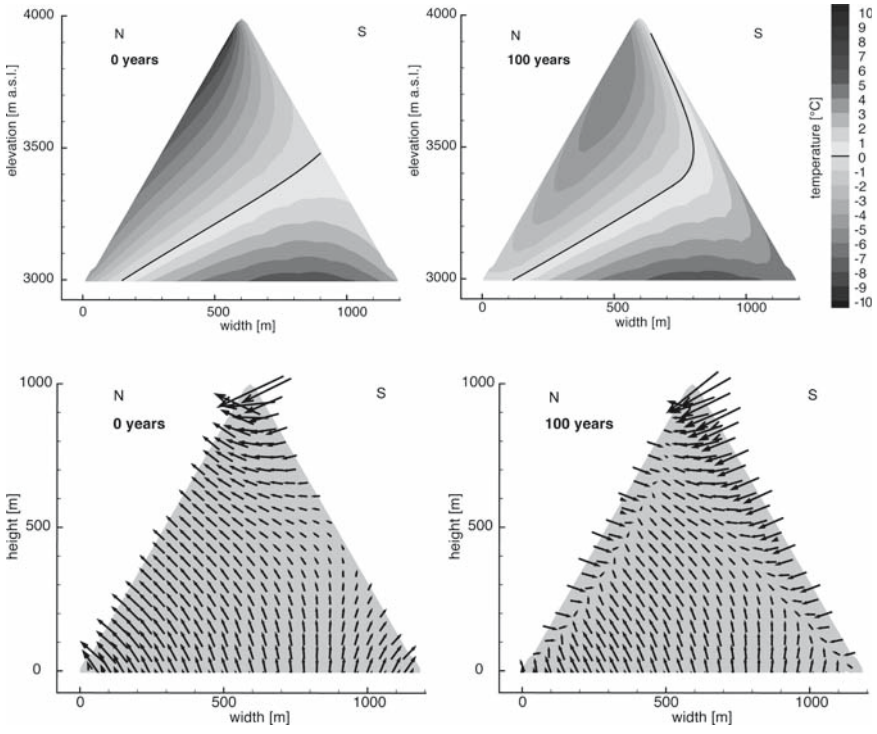

Fig. 14.7 2D-temperature field (*top*) and heat flow (*bottom*) for an idealized high mountain peak with a cold and a warm side and with a linear warming of 3°C/100 years. From Noetzli et al. (2007)

14.6 Consequences

The inertia related to diffusion of subsurface heat and the retarding effects from latent heat exchange cause global warming-induced permafrost changes to last for very long time periods (Haeberli and Burn 2002; Noetzli et al. 2007). On moderately inclined slopes with abundant fine material of mountains, with a continental climate and widespread permafrost occurrence, increasing active layer depth in degrading permafrost is likely to reduce near-surface soil humidity and, hence, change the living conditions of plants and animals (Etzelmüller et al. 2001). In rugged topography with coarser sediment cover and bedrock, reduction of slope stability is now seen to be the main problem. Steep outer slopes of thick morainic deposits from small glaciers below large rock walls and the fronts of active rock glaciers may be especially sensitive to changes in permafrost conditions (Fig. 14.8). The involved phenomena are, however, rather complex (Zimmermann and Haeberli 1992). The most delicate situation may indeed develop during the transition from conditions with to without permafrost, when the increasing active-layer thickness in the steep slope allows for deeper erosion but permafrost still forms a roughly surface-parallel hydraulic barrier at depth, which inhibits percolation, concentrates precipitation water in a near-surface layer of limited thickness and delivers the so-enhanced subsurface flow directly to the upper parts of the steep slopes.

Concerning large rock falls in steep rock slopes (see. case descriptions by Dramis et al. 1995; Deline 2001), a combination of the factors (i) slope inclination, (ii) geological structure, (iii) permafrost condition, and (iv) topographic history

Fig. 14.8 Debris flow starting zone in a Little Ice Age moraine with marginal permafrost and vanishing avalanche-fed cirque glacier. The resulting debris flow had a volume of about 500,000 m^3 and caused heavy damage in the village of Guttannen, Bernese Alps, Switzerland (photo: Flotron AG 2005)

must be considered in each individual case. Among these four primary factors, the ice-related permafrost conditions and topographic history (glacier vanishing with corresponding stress redistribution) are those now subject to the strongest and fastest change (Fig. 14.9; Fischer et al. 2006). In detail, things are again much more complicated than sometimes assumed (Gruber et al. 2004a; Gruber and Haeberli 2007). Lowest stability of bedrock with ice-filled cracks, for instance, does not occur with complete thaw but in "warm" permafrost at temperatures slightly below melting (Davies et al. 2001). With continued permafrost warming, layers at critical temperatures — allowing for ice-rock-water coexistence — will not only extend over larger vertical distances but also to greater depths below surface. The probability of large rock falls must therefore be assumed to slowly but steadily increase.

During the past 20 years in the Alps, periglacial rock falls with volumes exceeding one million m^3 and often reaching far below the timberline have occurred at time intervals of a few years. Current research strategies relating to such growing hazards from permafrost areas of cold mountain areas focus on GIS-based spatial definitions of critical factor combinations with rock walls above the timberline, and numerical modelling of flow paths resulting from potential instabilities (Fig. 14.9; Fischer et al. 2006; Noetzli et al. 2006). The goal is to recognize the most critical threats, and to enable early detection, warning and protection (Fig. 14.10) through adequate observation and monitoring. In particular, rock falls into existing lakes, or into lakes which newly form in connection with accelerated glacier shrinkage, have

Fig. 14.9 East face of Monte Rosa and Ghiacciaio del Belvedere, Valle Anzasca, Regione Piemonte, Italy. Most intense rock fall activity in this rock face correlates with warm or marginal permafrost and recently deglaciated surfaces. The detachment zone of an ice avalanche (2005) is marked with a *black circle*, the one of a rock avalanche (2007) in *white* (photo: L. Fischer 2004)

Fig. 14.10 Avalanche protection above Pontresina, Grisons Alps, Switzerland. The retention dam at the bottom of the slope is to protect against snow avalanches and debris flows from marginal permafrost (photo: W. Haeberli 2007)

the potential to produce large flood waves. Such flood waves may trigger devastating and far-reaching debris flows, constituting a serious and still inadequately recognized hazard to people and infrastructure in cold mountain regions.

14.7 Conclusion and Perspectives

The growing interest in perennially frozen ground of cold mountain ranges is justified and the rapidly developing progress in this young research field must be considered timely and most welcome. Continued if not accelerating atmospheric temperature rise indeed has the potential to cause serious and long-lasting disequilibria on the slopes as well as inside many mountain peaks on earth. Concerning impacts of climate change on mountain permafrost, system reactions deserve special attention: the effects of permafrost thaw on soil humidity and growth conditions on gentle slopes of mountain ranges with a continental-type climate, or large rock falls into already existing or newly forming lakes in areas of fast glacier retreat, constitute major threats. International programs of long-term monitoring within the framework of global climate-related observations must continue at a level of higher intensity, and the exchange of experience and scientific-technological know-how for assessing possible ecosystem changes and natural hazard conditions without historical precedence are strongly recommended.

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Chapter 15
Global Warming and Carbon Dynamics in Permafrost Soils: Methane Production and Oxidation

Dirk Wagner(✉) and Susanne Liebner

15.1 Introduction

A better understanding of the global terrestrial carbon cycle has become a policy imperative, both nationally and worldwide. The Kyoto Protocol recognizes the role of terrestrial systems as carbon sinks and sources. Terrestrial and sub-marine permafrost is identified as one of the most vulnerable carbon pools of the Earth system (Osterkamp 2001; Zimov et al. 2006). About one third of the global soil carbon is preserved in northern latitudes (Gorham 1991), mainly in huge layers of frozen ground, which underlay around 24% of the exposed land area of the northern hemisphere (Zhang et al. 1999). This carbon reservoir is of global climatic importance, in particular due to the currently observed climate changes in the Arctic (IPCC 2007; see Chap. 1 and Sect. 15.4).

Thawing of permafrost could release large quantities of greenhouse gases into the atmosphere, thus further increasing global warming and transforming the Arctic tundra ecosystems from a carbon sink to a carbon source (Oechel et al. 1993). Trace gas fluxes from permafrost ecosystems are influenced by a number of biotic and abiotic parameters (Fig. 15.1). The decomposition of soil organic matter and the generation of greenhouse gases result from microbial activity, which is affected by habitat characteristics (soil parameters) and by climate-related properties (forcing parameters). The method of gas transport determines the ratio between methane and carbon dioxide emission to the atmosphere. However, the processes of carbon release, their spatial distribution and their climate dependency are not yet adequately quantified and understood.

The world-wide wetland area has a size of about 5.5×10^6 km^2 (Aselmann and Crutzen 1989). About half of it is located in high latitudes of the northern hemisphere (> 50°N). The atmospheric input of methane from tundra soils of this region has been estimated to vary between 17 and 42 Tg CH_4 yr^{-1} (Whalen and Reeburgh 1992; Cao et al 1996; Joabsson and Christensen 2001), corresponding to about 25% of the methane emission from natural sources (Fung et al. 1991).

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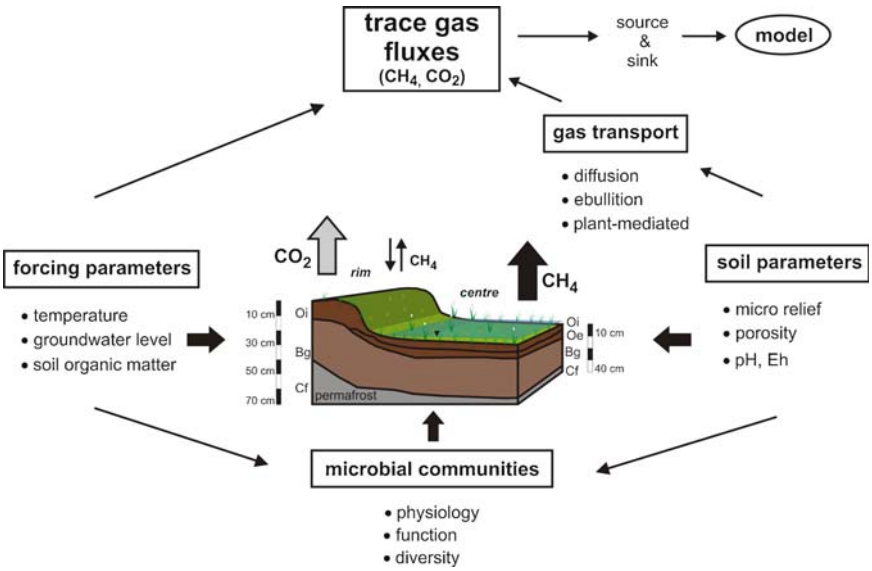

Fig. 15.1 Schematic view of the process variables influencing the formation, transport, and release of climate-relevant trace gases in permafrost soils

In the last decades, numerous studies on methane fluxes have been focused on tundra environments in Northern America and Scandinavia (Svensson and Rosswall 1984; Whalen and Reeburgh 1988; Bartlett et al. 1992; Liblik et al. 1997; Reeburgh et al. 1998; Christensen et al. 2000). Since the political changes in the former Soviet Union in the early 1990s, the large permafrost areas of Russia have been integrated into the circum-Arctic flux studies (Christensen et al. 1995; Samarkin et al. 1999; Panikov and Dedysh 2000; Tsuyuzaki et al. 2001; Wagner et al. 2003; Corradi et al. 2005; Kutzbach et al. 2007; Wille et al. 2008). All these studies revealed temporal and spatial variability of methane fluxes, ranging between −1.9 and 360 mg CH_4 m^{-2} per day. To understand these dramatic fluctuations, some studies focused on the environmental conditions and soil characteristics, comprising the water table position, soil moisture and temperature, type of substrate and vegetation as well as availability of organic carbon (Torn and Chapin 1993; Vourlitis et al. 1993; Bubier et al. 1995; Oberbauer et al. 1998; Joabsson et al. 1999; Yavitt et al. 2000). These factors influence the methane dynamics of tundra environments. Although 80–90% of total methane emissions originate from microbial activity (Ehhalt and Schmidt 1978), only a few investigations dealt with methane production and methane oxidation caused by microbiological processes in the course of carbon dynamics (Slobodkin et al. 1992; Vecherskaya et al. 1993; Samarkin et al. 1994; Schimel and Gulledge 1998; Segers 1998; Frenzel and Karofeld 2000; Høj et al. 2005; Wagner et al. 2005; Liebner and Wagner 2007; Metje and Frenzel 2007).

This review first examines the processes of the methane cycle in permafrost soils. It then describes the methane-cycling microorganisms, including possible impacts of global warming on their structure and function.

15.2 Methane Cycle in Permafrost Soils

The carbon pool estimates for permafrost soils vary between 4 and 110 kg C m^{-2} (Schell and Ziemann 1983; Tarnocai and Smith 1992; Michaelson et al. 1996). These large variations can be attributed to different soil types (from mineral to peaty soils) and varying depths of measurement (from the upper few cm to 1 m depth). Permafrost soils can function as both a source and a sink for carbon dioxide and methane (Fig. 15.2). Under anaerobic conditions, caused by flooding of the permafrost soils and the effect of backwater above the permafrost table, the mineralization of organic matter can only be realized stepwise by specialized microorganisms of the so-called anaerobic food chain (Schink and Stams 2006). Important intermediates of the organic matter decomposition are hydrogen, carbon dioxide and acetate, which can be further reduced to methane (methanogenesis) by methanogenic archaea (see Sect. 15.3.1). The fermentation of carbon by microorganisms takes place much more slowly than oxidative respiration. As a result of the prolonged anaerobic conditions and low in situ temperatures of permafrost soils organic matter accumulates (peat formation) in these environments.

Nervertheless, the quantity of organic matter provides no information on its quality. This, however, determines the availability of organic compounds as energy

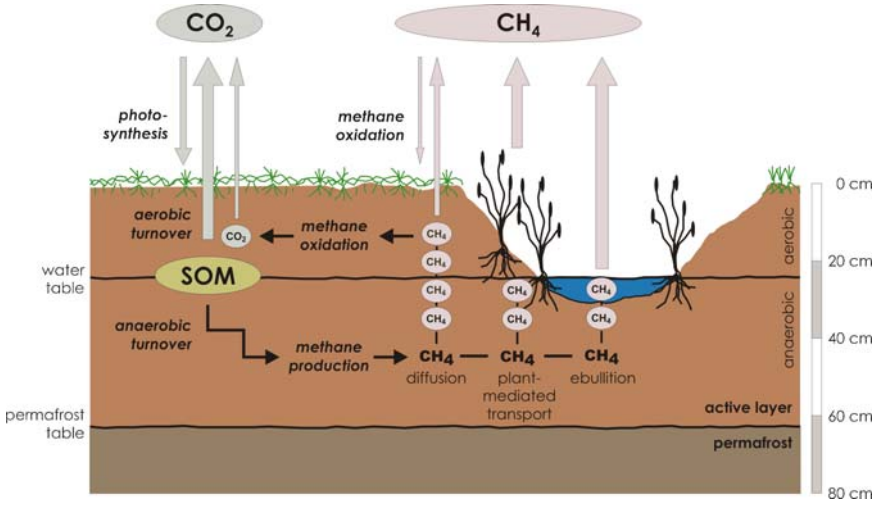

Fig. 15.2 The carbon cycle in permafrost soils. Permafrost soils can be both a source and a sink for CO_2 and CH_4. Under aerobic conditions soil organic matter (SOM) is respired to CO_2, whereas under anaerobic conditions SOM is decomposed via a sequence of microbial processes to CH_4. Methane fluxes from anaerobic soil horizons to the atmosphere result from diffusion (slow), ebullition (fast), and through plant-mediated transport (bypassing the oxic soil layer). Therefore, the method of transport determines the amount of methane that is re-oxidized by microorganisms in aerobic soil horizons. Photosynthesis provides an important sink for CO_2 in permafrost environments. Thereby, biomass is produced. In contrast, the consumption of atmospheric methane (negative methane flux) in the upper surface layer of the soils plays only a minor role for the methane budget. The thickness of the *arrows* reflects the importance of the above processes

and carbon sources for microorganisms (Hogg 1993; Bergman et al. 2000; see also Chap. 16). For this purpose, the humification index (HIX, dimensionless), for instance, is a criterion for organic matter quality and can, therefore, give suitable information with regard to microbial metabolism (Zsolnay 2003). It has been demonstrated that the availability of organic carbon in permafrost soils decreased with increasing HIX (Wagner et al. 2005). It has further been shown that the HIX increased continuously with depth in Holocene permafrost sediments (Wagner et al. 2007). This indicates that the organic carbon is less available for microorganisms with increasing depth because of the higher degree of humification. Therefore, in addition to the quantity, the quality of soil organic matter should also be taken into account with regard to permafrost environments as a huge carbon reservoir.

Wherever oxygen is present in permafrost habitats (upper oxic soil horizons, rhizosphere), methane can be oxidized to carbon dioxide by aerobic methane-oxidizing bacteria (see Sect. 15.3.2). Between 76% and up to more than 90% of the methane produced in wetlands is oxidized by these specialists before reaching the atmosphere (Roslev and King 1996; Le Mer and Roger 2001). Hence, the biological oxidation of methane represents the major sink for methane in Arctic permafrost environments.

Vegetation is another important factor occupying a central position for microbial processes and the transport of methane. Plants can have both enhancing and attenuating effects on methane emission. Through the aerenchyma of vascular plants, oxygen is transported from the atmosphere to the rhizosphere, thus stimulating methane oxidation in otherwise anoxic soil horizons (Van der Nat and Middelburg 1998; Popp et al. 2000). In the opposite direction, the aerenchyma is a major pathway for methane transport from the anoxic horizons to the atmosphere, bypassing the oxic/anoxic interface in the soil, where methane oxidation is most prominent. It has been shown that up to 68% of the total methane release from wet permafrost soils is transported through sedges like *Carex aquatilis* (Kutzbach et al. 2004). Furthermore, the vegetation provides the substrates for methanogenesis such as decaying plant material and fresh root exudates (Whiting and Chanton 1992; Joabsson et al. 1999).

15.3 Microbial Communities Involved in the Methane Cycle

The biological formation and consumption of methane are carried out by very specialized microorganisms, methanogens and methanotrophs. Thereby, methane production results solely from the activity of members of the kingdom *Euryarchaeota*, the so-called methanogenic archaea (methanogens). The group of microorganisms capable of consuming methane (methanotrophs), however, is more complex, comprising obligate aerobic members of the phyla *Proteobacteria* (Bowman 1999), and *Verrucomicrobiaea* (Dunfield et al. 2007; Pol et al. 2007), as well as anaerobically methane-oxidizing archaea in marine habitats (e.g., Boetius et al. 2000), and bacteria of a yet unknown phylum carrying out methane oxidation

in the presence of very high nitrate and methane concentration in freshwater habitats (Raghoebarsing et al. 2006). The dominant methane-consuming microorganisms in permafrost soils are those of the *Proteobacteria* phylum. Because of the pronounced distribution of methanogenic archaea and methanotrophic *Proteobacteria* in Arctic permafrost soils (reviewed by Wagner 2008, Fig. 15.3) and their significance for the global methane budget, these two groups are of particular attention in this review.

15.3.1 Methanogenic Archaea

Methanogenic archaea represent a small group of strictly anaerobic microorganisms (Hedderich and Whitman 2006). They can be found either in temperate habitats like paddy fields (Grosskopf et al. 1998), lakes (Jurgens et al. 2000; Keough et al. 2003), freshwater sediments (Chan et al. 2005), in the gastrointestinal tract of animals (Lin et al. 1997), or in extreme habitats such as hydrothermal vents (Jeanthon et al. 1999), hypersaline habitats (Mathrani and Boone 1985) or permafrost soils and sediments (Rivkina et al. 1998; Kobabe et al. 2004). In cold environments, 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 (Conrad 2005). However, only a few psychrophilic (cold-adapted) strains of methanogenic archaea have been described so far (Simankova et al. 2003; Cavicchioli 2006).

Although permafrost environments are characterized by extreme climate conditions, it was recently shown that the abundance and composition of the methanogenic population is similar to that of communities of comparable temperate soil ecosystems (Wagner et al. 2005). The highest cell counts of methanogenic archaea were detected in the active layer of permafrost, with numbers of up to 3×10^8 cells g^{-1} soil (Kobabe et al. 2004). Methanogenic archaea represented between 0.5 and 22.4% of the total cell counts. Phylogenetic analyses revealed a great diversity of methanogens in the active layer, with species belonging to the families *Methanobacteriaceae*, *Methanomicrobiaceae*, *Methanosarcinaceae*, and *Methanosaetaceae* (Høj et al. 2005; Metje and Frenzel 2007; Ganzert et al. 2007; Fig. 15.3). Other sequences detected were affiliated to the euryarchaeotal Rice Clusters II and V (Hales et al. 1996; Grosskopf et al. 1998; Ramakrishnan et al. 2001) as well as to the Group I.3b of the uncultured Crenarchaeota (non-methanogenic archaea; Ochsenreiter et al. 2003). Environmental sequences from the Laptev Sea coast form four specific permafrost clusters (Ganzert et al. 2007). Permafrost Cluster I was recovered mainly from cold horizons (with temperatures of less than 4°C) of the active layer, and was related to *Methanosarcinacea*. Permafrost Clusters II and III were related to *Methanomicrobiales*, and Permafrost Cluster IV was related to Rice Cluster II. It was hypothesized that these clusters comprise methanogenic archaea with a specific physiological potential to survive under harsh environmental conditions. The phylogenetic affiliation of the sequences recovered in this study indicated that both hydrogenotrophic and acetoclastic methanogenesis exist in

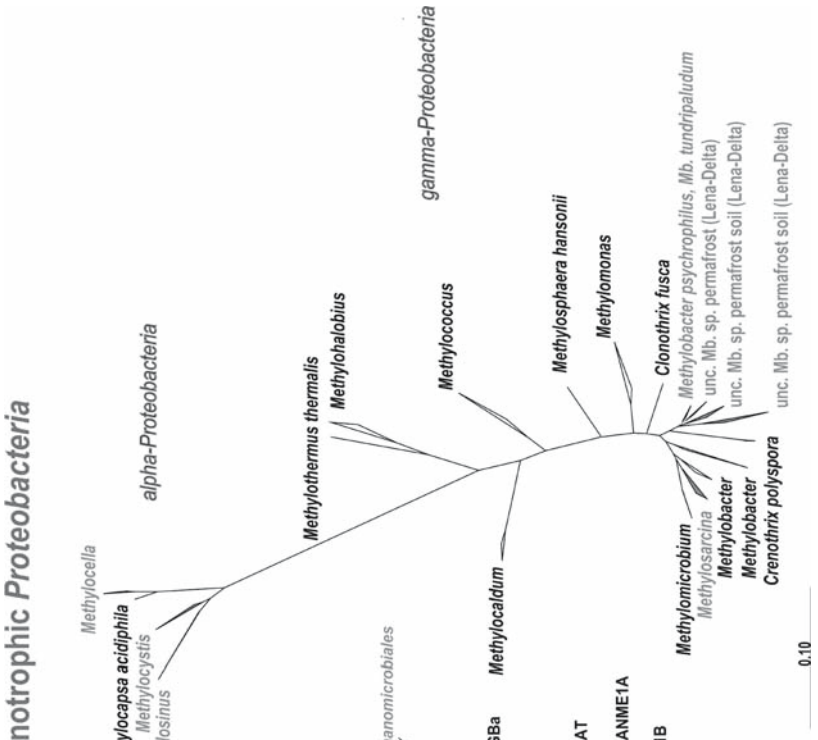

Fig. 15.3 Phylogenetic relation (based on 16S rRNA gene sequences) of methanogenic archaea and aerobic methanotrophic bacteria. *Grey squares* illustrate groups including sequences from Arctic tundra environments. *Trees* represent maximum likelihood trees using the PhyML algorithm (Guindon and Gascuel, 2003) and the ARB software package

permafrost soils. Recent studies on perennially frozen permafrost deposits from the Lena Delta (Siberia) revealed significant amounts of methane which could be attributed to in situ activity of methanogenic archaea (Wagner et al. 2007). Another study on frozen ground on Ellesmere Island reported an archaeal community composed of 61% Euryarchaeota (methane-producing archaea) and 39% Crenarchaeota, suggesting the presence of a diverse archaeal population also in the perennially frozen sediments (Steven et al. 2007; see also Chap. 5).

Methanosarcina sp. SMA-21, which is closely related to *Methanosarcina mazei*, was recently isolated from a Siberian permafrost soil in the Lena Delta. The organism grows well at 28°C and slowly at low temperatures (4°C and 10°C) with H_2/CO_2 (80:20, v/v, pressurised at 150 kPa) as substrate. The cells grow as cocci, with a diameter of 1–2 µm. Cell aggregates were regularly observed (Fig. 15.4a). *Methanosarcina* SMA-21 is characterized by an extreme tolerance to very low temperatures (−78.5°C), high salinity (up to 6 M NaCl), starvation, desiccation and oxygen exposure (Morozova and Wagner 2007). Furthermore, this archaeon survived for 3 weeks under simulated thermo-physical Martian conditions (Morozova et al. 2007; see also Chap. 21).

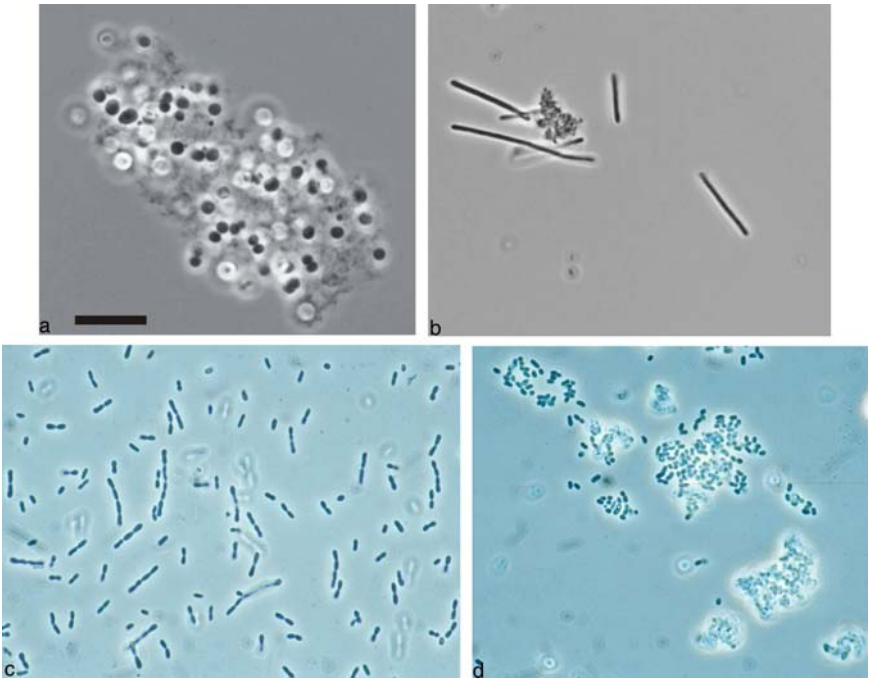

Fig. 15.4 Methane-cycling microorganisms isolated from permafrost environments. **a** *Methanosarcina* sp. SMA-21 (D. Wagner and D. Morozova, AWI; bar: 10 µm). **b** permafrost strain SMA-23 (D. Wagner and D. Morozova, AWI). **c** *Methylobacter tundripaludum* (Wartiainen et al. 2006a). **d** *Methylocystis rosea* (Wartiainen et al. 2006b)

Methanogenic activity has been observed at low in situ temperatures, with rates of up to 39 nmol CH_4 h^{-1} g^{-1} soil in the active layer of permafrost (Wagner et al. 2003; Høj et al. 2005; Metje and Frenzel 2007). The highest activities were thereby measured in the coldest zones of the profiles. Furthermore, it could be shown that methane production is limited rather by the quality of soil organic carbon than by the in situ temperature (Wagner et al. 2005; Ganzert et al. 2007). Another important factor affecting methanogenic communities in permafrost soils is the water regime. Along a natural soil moisture gradient, changes in archaeal community composition were observed, which suggest that the differences in these communities were responsible for the large-scale variations in methane emissions observed with changes in soil hydrology (Høj et al. 2006).

15.3.2 Methane-Oxidizing Proteobacteria

Based on their function as the major sink for methane in Arctic permafrost affected wetlands and tundra, methane-oxidizing *Proteobacteria* are also of importance for the greenhouse gas budget of these environments.

Methane-oxidizing *Proteobacteria* represent a subset of methylotrophic bacteria. Through the activity of their specific enzyme, methane monooxygenase, they are specialized to utilize methane as their single carbon and energy source (Hanson and Hanson 1996). The group of methane-oxidizing *Proteobacteria* comprises the three families *Methylococcaceae, Methylocystaceae, and Beijerinckiaceae* (Bowman 1999; Dedysh et al. 2000, 2001, 2002, 2004). The only exception is *Crenothrix polyspora*, a filamentous, sheathed microorganism recently discovered to be methanotrophic (Stoecker et al. 2006). *Methylococcaceae* include the genera *Methylobacter*, *Methylomonas*, *Methylomicrobium*, *Methylosarcina*, *Methylosphaera*, *Methylohalobius*, *Methylosoma*, *Methylothermus*, *Methylococcus*, and *Methylocaldum* (Hanson and Hanson 1996; Bowman et al. 1997; Wise et al. 2001; Heyer et al. 2005; Tsubota et al. 2005; Rahalkar et al. 2007). They belong to the gamma subdivision of the *Proteobacteria* phylum and are termed type I methanotrophs, except for the last two, which are also known as type X methanotrophs. The families *Methylocystaceae*, and *Beijerinckiaceae* include the genera *Methylosinus*, *Methylocystis*, *Methylocella*, and *Methylocapsa* (Hanson and Hanson 1996; Bowman 1999; Dedysh et al. 2000, 2001, 2002, 2004). Members of the *Methylocystaceae* and *Beijerinckiaceae* are termed type II methanotrophs, and belong to the alpha subdivision of the *Proteobacteria* phylum. Except for their phylogeny, type I and type II methanotrophs can also be distinguished by their carbon assimilation pathway, the structure of their intracytoplasmic membranes, their resting stages, G + C-content, the constitution of their methane monooxygenase, and by their major phospholipid fatty acids (PLFAs).

Several studies have revealed that methanotrophs are abundant and active also under very harsh environmental conditions of cold environments (review by Trotsenko and Khmelenina 2005). Viable methane oxidizers have even been detected

in deep Siberian permafrost sediments with ages of 1,000–100,000 years (Khmelenina et al. 2001). Numerous psychrophilic and psychrotrophic methanotrophs, primarily affiliated to the type I group, are known, such as *Methylobacter psychrophilus*, isolated from Siberian tundra (Omelchenko et al. 1996), *Methylobacter tundripaludum*, isolated from Arctic wetland soils (Wartiainen et al. 2006a; Fig. 15.4), *Methylosphaera hansonii*, isolated from Antarctic, marine salinity, meromictic lakes (Bowman et al. 1997), and *Methylomonas scandinavica*, isolated from deep igneous rock ground water (Kaluzhnaya et al. 1999). Type I methanotrophs have also been discovered to dominate in Arctic permafrost-affected soils (Wartiainen et al. 2003; Wagner et al. 2005; Liebner and Wagner 2007). Within the type II group, *Methylocystis rosea*, isolated from an Arctic wetland soil (Wartiainen et al. 2006b; Fig. 15.4), and representatives of the acidophilic genera *Methylocella* and *Methylocapsa* were reported to be psychrotrophs (Dedysh et al. 2002, 2004).

Methane-oxidizing *Proteobacteria* have been shown to be highly abundant in permafrost soils of the Lena Delta, Siberia, with cell numbers ranging between 3×10^6 and 1×10^8 cells g^{-1} soil and contributing up to 10% to the total number of microbial cells (Liebner and Wagner 2007). In the same area, specific clusters of methane-oxidizing *Proteobacteria* closely related to *Methylobacter psychrophilus* and to *Methylobacter tundripaludum* were detected, indicating a micro-diverse community on the species level (Liebner et al. 2008). Also, highly divergent functional gene sequences of these methanotrophs were found in soils of the high Canadian Arctic (Pacheco-Oliver et al. 2002). In contrast, the diversity of methane-oxidizing *Proteobacteria* in an Arctic wetland on the island of Svalbard was observed to be restricted to only two genera (Wartiainen et al. 2003), whereas most methanotrophic *Proteobacteria* were detected in a Russian sub-Arctic tundra (Kaluzhnaya et al. 2002).

Still, diversity and composition of methane-oxidizing bacteria in permafrost soils are only poorly explored. Also, it remains unknown whether psychrophilic or cold-adapted mesophilic methantrophs are responsible for methane oxidation at low and subzero temperatures in permafrost sediments (Trotsenko and Khmelenina 2005). A recent study, though, observed a shift between a mesophilic methanotrophic community near the surface and a psychrophilic methanotrophic community near the permafrost table of Siberian permafrost soils (Liebner and Wagner 2007). This indicates that depending on the environmental conditions both mesophilic as well as psychrophilic methanotrophs are active in Siberian permafrost soils.

15.4 Methane-Cycling Communities Under Global Climate Change

Arctic surface temperatures have increased on average to a greater extent than those of the rest of the earth (IPCC 2001), causing a particular susceptibility of Arctic permafrost to degradation. Global warming could degrade 25% of the total permafrost area by 2100 (Anisimov et al. 1999). Also, Nelson et al. (2001) predicted a

high potential for large areas of Siberian permafrost to be degraded, which would primarily lead to a thickening of the seasonally thawed layer (active layer). In the period 1956–1990, the active layer in Russian permafrost already increased by on average 20 cm (IPCC 2007). By the end of the twenty-first century, an increase of mean annual ground temperature by up to 6°C and of active-layer depth by up to 2 m is expected for East Siberia (Stendel et al. 2007). Although the estimated size of the carbon pool in Arctic permafrost-affected tundra varies between 190 Gt and, in more recent studies, approximately 900 Gt, it accounts for at least 13–15% of the global carbon pool in soils (Post et al. 1982; Zimov et al. 2006). Thawing of 10% of the total Siberian permafrost carbon reservoir was suggested to initially release about 1 Pg carbon, followed by respiration of about 40 Pg carbon to the atmosphere over a period of four decades (Dutta et al. 2006). Model calculations suggest that methane currently emitted from Arctic permafrost environments may enhance the greenhouse effect with a portion of approx. 20% (Wuebbles and Hayhoe 2002). Palaeoclimate reconstruction combined with biogeochemical biomarker analysis, for example, revealed an increase in production and release of methane from the terrestrial biosphere during the Palaeocene–Eocene thermal maximum, a period of intense global warming 55 million years ago (Pancost et al. 2007). It has also been shown that an increase of the permafrost temperature in Holocene permafrost deposits of northern Siberia would lead to a substantial rise in microbiologically produced methane (Wagner et al. 2007). Serious concerns are thus associated with the potential impact that thawing permafrost may have on the global climate system through release of greenhouse gases (Friborg et al. 2003; Christensen et al. 2004; Wagner et al. 2007). Methane flux models do indeed predict increasing methane emissions in latitudes above 60°N by 19–25% (Cao et al. 1998; Walter et al. 2001; Zhuang et al. 2004). These estimates are challenged, though, by other studies suggesting that increasing methane fluxes from Russian permafrost regions will change atmospheric methane concentrations by only 0.04 ppm (2.3%), leading to 0.012°C temperature rise globally (Anisimov 2007).

Models of modern methane emissions from Arctic wetlands determine methane production and methane oxidation rates primarily as functions of substrate availability, substrate concentration, and temperature, as well as indirectly of water table and thaw depth (Walter et al. 2001; Zhuang et al. 2004; Anisimov 2007). Changes of these parameters will consequently lead to short-term alterations of methane production and methane oxidation rates. Whether, however, the currently observed global climate change will effectively alter modern methane fluxes from Arctic permafrost-affected wetlands will particularly depend on its long-term impact on the methane-cycling communities and their ability to adapt to the new environmental conditions. This ability is very likely dependant on the level of specialisation and diversity of the indigenous microbial communities. It has been observed that an increase of temperature and precipitation altered the community structure and relative abundance of methane oxidizers in rice, forest and grassland soils (Horz et al. 2005; Mohanty et al. 2007). Also, the overall relative abundance and diversity of methanogenic archaea in a high Arctic peat from Spitsbergen increased with increasing temperature, in conjunction with a strong stimulation of methane production rates (Høj et al. 2008).

In contrast, the population structure of methanogenic archaea in permafrost-affected peat in Siberia remained constant over a wide temperature range (Metje and Frenzel 2007). Also, a psychrophilic and little diverse methanotrophic community as detected near the permafrost table of Siberian polygonal tundra soils (Liebner and Wagner 2007; Liebner et al. 2008) will likely require more time for resilience than the diverse mesophilic-psychrotolerant methanogenic community detected in permafrost soils of the same region (Ganzert et al. 2007).

There is, however, a lack of experimental research investigating the long-term effect of simulated climate change on the methane-cycling communities in permafrost soils, which would be essential to prove or disprove the previously mentioned assumptions. Also, an account of the entire plant–microbe–animal syste, and the interactions between metabolic networks which are important for methanogenesis, is missing in modern methane flux models (Panikov 1999). Due to this poor knowledge, it is worthwhile considering microbial communities in the context of global climate change in general. Simulating the effects of warming on the competition between psychrophilic and mesophilic sub-populations of *Pseudomonas*, for example, displayed a high degree of stability of this artificial community (Panikov 1999). Psychrophiles dominated the bacterial community under cold conditions, and an increase in temperature by 5°C did not affect their domination. Further warming of another 5°C resulted in a rapid 50% substitution of psychrophiles by mesophiles over 2 years, finally reaching a stable coexistence between the two sub-populations. In the same model, the main effect of rising temperatures on the carbon balance of the ecosystem was a considerable activation of organic matter decomposition due to higher production of hydrolytic enzymes. Experimental setups revealed a rather low direct impact of rising temperatures on the decomposition of soil organic matter, but rather attributed increased decomposition rates most strongly to be due to changes in local substrate characteristics and vegetation type (Zhang et al. 2005; Bokhorst et al. 2007). Still, a shift in the microbial community structure induced by warming was again observed, at least in the first study.

To summarize, there is an urgent need for modelling the response of methane-cycling communities in permafrost regions to global climate change on the one hand, and to validate these models by empirical data on the other hand. This is not only due to the importance of these communities for the atmospheric methane budget and thus for the global climate. It is also inevitable, given the close connection between physiology and function of these communities in permafrost soils that allows for a general understanding of how important the stability of microbial communities is for the greenhouse gases budget of Arctic permafrost-affected wetlands.

15.5 Conclusion and Future Perspectives

Permafrost soils and sediments are unique systems in the context of biogeochemical cycling of carbon, particularly due to the enormous amount of organic carbon stored in these environments. Recent studies demonstrate the close relationship

between apparent methane fluxes and the modes and intensities of microbiological processes of methane production and oxidation in permafrost ecosystems. Methane-producing and -consuming microorganisms are widespread, highly active and abundant in permafrost soils, despite the harsh environmental conditions they are exposed to. The permafrost environment forces an adaptation of the methane-cycling communities to low-temperature conditions, often yielding species which have not been detected in temperate ecosystems so far. In addition to soil characteristics and climate conditions, the activity and physiology of these well-adapted microbial communities dictate trace gas fluxes in permafrost soils. The future development of permafrost environments as a source of methane, therefore, primarily depends on the response of the methanogenic and methanotrophic microorganisms to a changing environment.

Anticipating this response, however, is difficult, as the sensitivity of microbial communities to permafrost degradation is completely unknown. Firstly, there is lack of experimental and theoretic studies on what determines microbial stability in general and in particular in permafrost environments. Secondly, the consequences of thawing permafrost on hydrology and morphology that indirectly influence microbial communities and their activities are very difficult to predict.

International projects such as ACD (Arctic Coastal Dynamics) and CALM (Circumpolar Active Layer Monitoring), which examine the impact of global warming on permafrost environments, should thus be linked more closely to microbiological process studies and biodiversity research. Microbial parameters important for the assessment of carbon turnover (e.g., viable cell numbers, activities, biodiversity and stability of microbial communities) should be analysed at observation areas in the Arctic, where long-term monitoring programs are undertaken. The evaluation of microbial ecology and its correlation to climatic and geochemical data represent the basis for an understanding of the role of permafrost soils in the global system, in particular in terms of feedback mechanisms related to fluxes of material and greenhouse gases in the scope of a warming Earth.

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Chapter 16
Global Warming and Dissolved Organic Carbon Release from Permafrost Soils

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16.1 Introduction

Global riverine transport of organic carbon (OC) is estimated to be 0.4–0.9 Pg annually (Meybeck 1982; Hope et al. 1994; Aitkenhead-Peterson et al. 2005). Therefore, the riverine export of OC from drainage basins to the ocean represents a major component of the global carbon cycle (Spitzy and Leenheer 1991; Hedges et al. 1997). Recent evidence from Northern Europe about increased dissolved organic carbon (DOC) concentrations in surface waters draining upland areas and wetlands (Freeman et al. 2001; Frey and Smith 2005), highlights the importance of understanding the transfer of C between soil and freshwater systems. Although the magnitude of the fluxes involved in land–atmosphere C exchange is significantly larger than that associated with surface waters, rates of DOC transport in streams draining subarctic catchments rich in organic soils are comparable to rates of C sequestration in the soil–plant system of high latitudes (Hope et al. 1994; Billet et al. 2006).

The Arctic drainage basin (~24×10^6 km^2) processes about 11% of both global runoff and DOC (Lobbes 2000; Lammers et al. 2001). Heavily influenced by permafrost, arctic river basins demonstrate the highest susceptibility to climate change. With 23–48% of the world's soil organic carbon (SOC) stored in the high-latitude region, the arctic/subarctic river basins have an enormous potential to mobilize and transport terrestrial OC to the Arctic Ocean (Guo and Macdonald 2006).

The response of permafrost soils to warming is crucial for understanding potential change in terrestrial C export to rivers. High hydraulic conductivity, low mineral content, and low DOC sorption capacity of the shallow soil active layer overlying impermeable permafrost together lead to quick DOC transport to streams and rivers, with limited microbial transformation, especially during snowmelt. As the depth, temperature and seasonal duration of the active layer increase with climate warming, new inputs of DOC may derive from thawed permafrost and/or vegetation changes (Sturm et al. 2001; Neff et al. 2006). However, significant differences in geomorphology, hydrology, permafrost distribution, soil types and

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vegetation among basins of Siberian rivers exert uncertainty in overall response of riverine DOC export to global warming. Moreover, climate change itself has both negative and positive feedbacks, and triggers complex interactions in atmosphere–vegetation–soil–river system (Serreze et al. 2000). This chapter summarizes available data on current DOC export from permafrost terrain, and attempts to assess its future projections.

16.2 DOC Production and Transport in Permafrost Soils

16.2.1 Control of DOC Production and Release

In northern boreal ecosystems, due to impeded microbial activity, organic carbon is mainly stored in the upper soil as peat or other plant debris of different decomposition stages. This highly labile organic C may greatly exceed the biomass of vegetation and is most vulnerable to climate change. Special attention has to be paid to SOC buried into permafrost and becoming bioavailable to decomposition as permafrost retreats. These carbon pools stored in high-latitude soils and peats represent the major ecosystem source of DOC (Aitkenhead-Peterson et al. 2005). On the basis of water-soluble organic matter extracted under laboratory conditions, DOC constitutes about 1% of total OC in organic soil layers (Fig. 16.1). Therefore, there is a significant

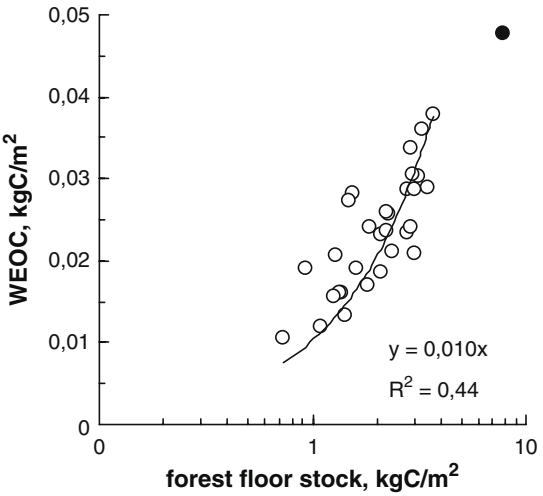

Fig. 16.1 Relationship between water extractable organic carbon (WEOC) and total organic carbon in forest floor of feather-moss dominated larch ecosystems in Central Siberia. *Black dot* represents the mean value for 50 cm deep peat of *Sphagnum fuscum*. All study sites are underlain by continuous permafrost

pool of potentially mobile OM in topsoils of permafrost terrains, which is also supposed to be renewable along with SOC decomposition (Neff and Hooper 2002).

Permafrost degradation, nevertheless, may also increase the size and frequency of fires that are important controls of carbon storage in the taiga biome of Siberia (Conard et al. 2002). Combustion of organic layers greatly reduces the amount of mobile C fraction and export of DOC to the subsoil (Shibata et al. 2003). However, deeper soil thawing activates subsoil C-cycling after a fire event.

It has been reported that about 10–40 g DOC m^{-2} are translocated annually from the organic surface layer into the mineral soil horizons in temperate forests (summarized in Michalzik et al. 2001), with only slightly lower amounts (4–17 g DOC m^{-2}) in the continuous permafrost zone of Siberia (Prokushkin et al. 2005). This means that about 10–25% of annual C input to the forest floor with litter is leached from the organic surface layers. Mobilization of organic matter in the dissolved state, driven by biotic and abiotic mechanisms of SOM degradation, is the major prerequisite for mineralization of SOM to CO_2.

Increased production of DOC has been demonstrated abiotically in freeze/thaw and drying/rewetting cycles (Kalbitz et al. 2000; Billett et al. 2006), both of which are of high importance in high latitudes. Nevertheless, there is little or contradictory information about these effects on DOC mobilization in permafrost soils in situ. Our observations in Central Siberia demonstrated lowest concentrations of DOC in organic soil leachates after earlier spring rainfalls, and highest DOC concentrations in subsoil. This may be caused by precipitation of DOC when concentrated by freezing, but such a process has not been investigated so far.

Temperature has a more profound effect through an increasing decomposition of SOM and thus DOC production by enhancing microbial activity (Christ and David 1996). The temperature regime in permafrost terrains drives the depth and timing of permafrost thawing (Fig. 16.2) and controls soil microbial activity. There is strong evidence that DOC production and CO_2 evolution in soils are coupled, and increases with rising temperatures (Neff and Hooper 2002). Increased content of DOC (Kawahigashi et al. 2004) and doubled DOC flux (Prokushkin et al. 2005) from organic soils of warm and deeper frost in south-facing slopes as compared to north-facing slopes and water-logged valleys in Central Siberia corroborates these findings. During the frost-free period, however, our previous data showed that DOC concentrations in forest floor leachates in areas with deeper frost declined with increasing litter layer temperature in the range of 7–13°C (Prokushkin et al. 2005, 2008). In contrast, DOC production in the forest floor of cooler north-facing slopes positively correlated with increasing temperatures. In addition, decomposition/oxidation of upper soil organic carbon, leading to the production of DOC in ecosystems limited by lower temperatures and soil moisture, would be enhanced in the drier and warmer climate. In particular, in Western Siberia, which stores at least 70.2 Pg C, an increase of the mean annual air temperature to values above −2°C is expected to produce a large increase of DOC export for watersheds containing 100% peat cover (Frey and Smith 2005).

Midsummer droughts may impede microbial activity in the upper soil of deeper frost areas, thereby reducing DOC export. In particular, the decline of the native

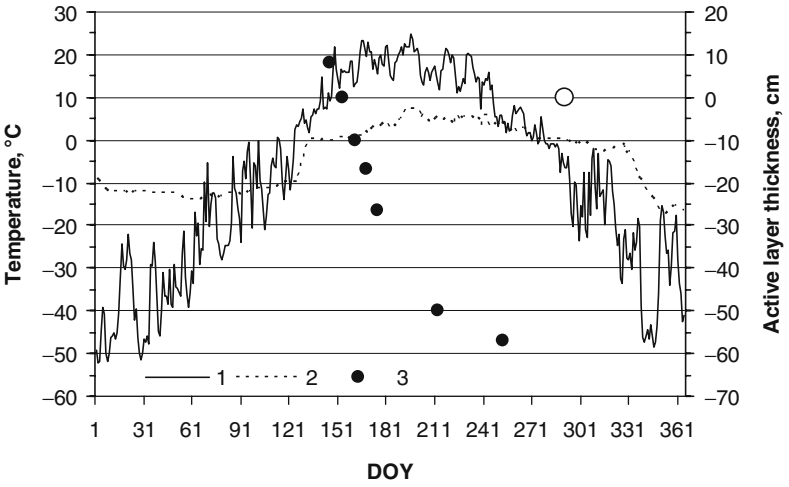

Fig. 16.2 Dynamics of the (*1*) air temperature and (*2*) humification horizon at the north-facing slope in 2002. *3* Dynamics of active layer thickness is shown during June–September. The *white points* denote the moment when the soil surface transforms into a frozen state (Prokushkin and Guggenberger 2007). *DOY* day of year

microbial communities within the permafrost zone of central Siberia has already been demonstrated at temperatures above 5°C (Šantručková et al. 2003). Thus, complex interactions between soil temperature, hydrology, and microbial activity will result in specific local responses of DOC flux in permafrost soils to changes in climate.

Precipitation constrains the yearly amount of DOC transported from the organic layers to mineral soil. Despite the decrease of DOC concentrations in solutions percolated through organic layers at higher precipitation, overall DOC flux demonstrates significant positive correlations with the amount of seepage water (Fig. 16.3). This suggests that DOC export is mainly water-limited, not C-limited. Therefore, under wetter climate conditions more DOC can be translocated into subsoil, and the retention of DOC in mineral horizons is of great importance for the fate of DOC leached from upper organic soils.

16.2.2 Retention of DOC in Soil

Sorption of DOC on mineral phases is the key geochemical process for carbon preservation in soils (McDowell and Likens 1988). In the broad range of ecosystems, most DOC leached from organic horizons is sorbed and retained in the subsoils (Kaiser and Guggenberger 2000; Kalbitz et al. 2000, 2005). The sorption depends much on the contents of sesquioxides and amount of carbon previously accumulated in soils (Kaiser et al. 2000; Kawahigashi et al. 2006). In general, immobilization

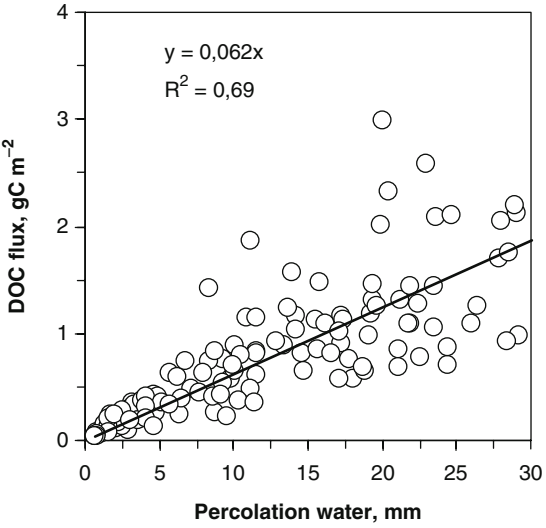

Fig. 16.3 DOC flux from forest floor in dependence from the amount of seepage water (Prokushkin et al. 2008)

of DOC has been considered an important process in the formation of stabile OC, due to its protection against microbial attack.

Sorption and mineralization of DOC in soil is not uniform, because of the heterogeneity and the complex mixture of organic molecules with different chemical characteristics, including a polymeric structure of major constituents (Schulten and Gleixner 1999). Along with the decrease of DOC concentrations on its passage through mineral soil (Fig. 16.4), there are major biochemical alterations of DOC composition. Hydrophobic compounds of high molecular weight and rich in acidic functional groups and aromatic moieties sorb most strongly (Kaiser et al. 2000; Kawahigashi et al. 2006). However, there is an introduction of "new" substances to soil solution in subsoil due to desorption of humified material and the release of hydrophilic microbial products (Kawahigashi et al. 2004; Prokushkin et al. 2007).

16.2.3 Implications for Global Change

Thus, warming in high latitudes may lead to an increased release of currently sequestered carbon through

(i) Enhancement of temperature-controlled DOC production processes
(ii) Raised precipitation, thereby increasing DOC mobilization from its large pool in the upper organic layer
(iii) Introduction of a new source of DOC from older and deeper layers, caused by permafrost degradation.

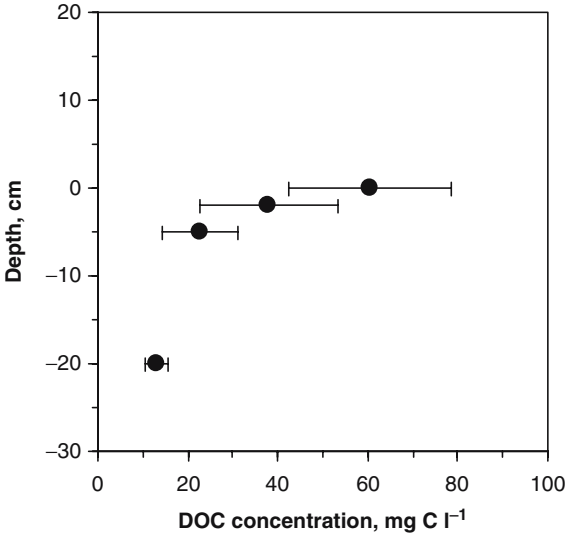

Fig. 16.4 DOC concentrations in a soil profile (forest floor leachate; 2-, 5- and 20- cm depths of mineral soil) during June–September

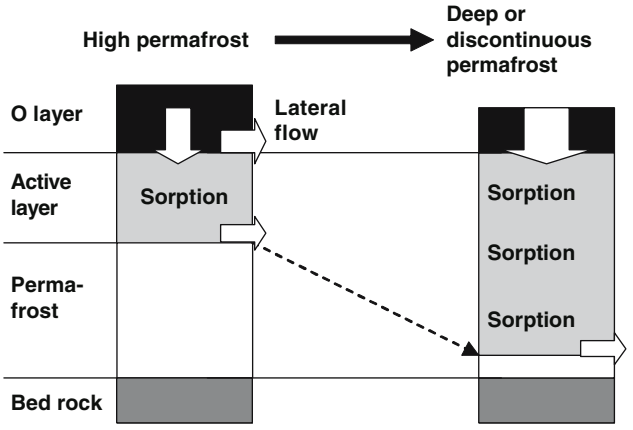

Fig. 16.5 Schematic illustration of flux of dissolved organic matter depending on the depth of the active layer (adapted from Kawahigashi et al. 2004)

The fate of DOC in soils is largely determined by the hydraulic residence of soil DOC and mineralization. As a result, although the production and release of DOC from the forest floor is greater in warmer soils, the deeper active layer increases the contact with mineral soils and thus the likelihood of DOC adsorption, allowing for C stabilization in soil and/or microbial mineralization to CO_2. The likely behavior of DOC in subsoils as affected by the permafrost degradation is illustrated in Fig. 16.5. However, on a regional basis there is large uncertainty as to how effectively

diverse soil types (highly varying in pH, content of clay, C etc.) distributed throughout the subarctic area may retain DOC. In particular, the basins of Ob' and Lena rivers, mantled with fluvial–glacial sandy soils, have likely comparatively less capacity to adsorb DOC than the basins of eastern tributaries of Yenisey river-draining clayey soils developed on basalts.

16.3 Release and Chemical Composition of Riverine DOC

16.3.1 Seasonality of Riverine DOC Export

Based on seasonal patterns of discharge and the chemical characteristics of DOC in subarctic rivers, there is a common division of annual hydrographs into spring flood, summer through autumn, and winter flow periods (Fig. 16.6). Although the start and duration of these periods may vary greatly among basins and annually, such separation is motivated by distinct changes of sources and flowpaths of water and DOC in riverine systems.

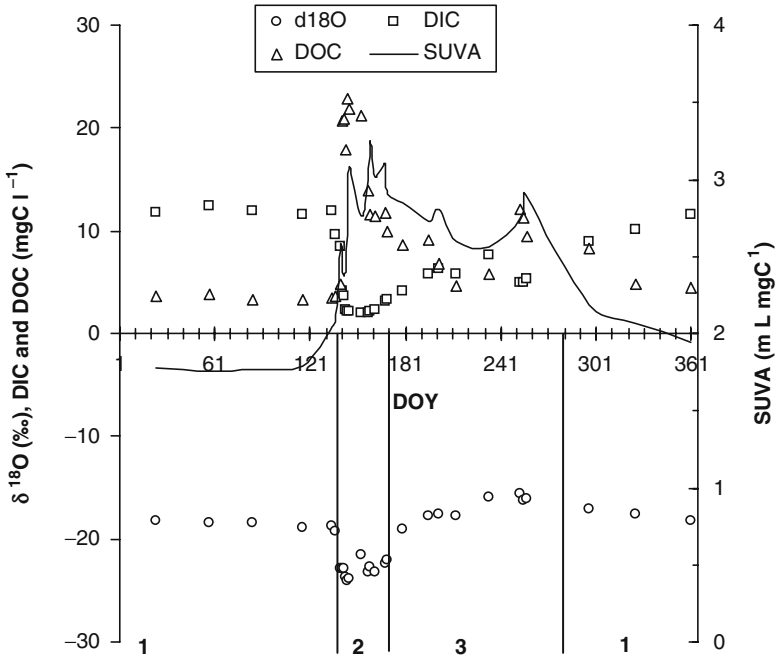

Fig. 16.6 Dynamics of $\delta^{18}O$ in water, concentration of DIC, DOC and specific ultraviolet absorbance (SUVA, 280 nm) in Kochechumo river (Central Siberia) in 2006. *1* winter; *2* spring flood; *3* summer and fall flow periods. *DOY* = day of year

A general concept observed across the subarctic area is that there are two major controls on runoff and DOC export: (1) permafrost distribution defines basin-contributing areas, as lateral flow is confined to permafrost-underlain terrains due to their ability to restrict deep percolation, and (2) surface organic soils play a key role in rapidly conveying water to the stream (Quinton et al. 2000). During the melt period, meltwater percolating from the snowpack in terrains with shallow permafrost soils infiltrates through organic soil, since deeper infiltration is restricted by the impermeable permafrost table. In areas with deeper frost (e.g. south-facing slopes) or in the absence of frost (discontinuous or sporadic permafrost regions), percolation is uninhibited unless there are ice-rich layers at depth. The isotopic signature of river water at this time becomes strongly depleted with respect to $\delta^{18}O$, suggesting large meltwater recharge (Fig. 16.6).

Chemically, DOC removed from organic soils in the meltwater solution and flushed during this runoff pulse demonstrates an enrichment in aromatic structures, originating from lignocellulose decomposition products (Kawahigashi et al. 2004; Prokushkin et al. 2007) and demonstrating contemporary ages (Neff et al. 2006). These are all attributed to relatively fresh organic matter entering the riverine systems. Such findings prove that organic solutes do not infiltrate to mineral soil, and bypass the interaction with mineral soil that remains frozen in spring. As a result, more DOC reaches rivers; therefore, subarctic river waters contain generally higher concentrations of DOC than rivers in permafrost-free areas. Furthermore, a peak in DOC concentrations is measured during spring breakup, when 40–80% of arctic river discharge occurs (Gordeev et al. 1996). Both streams and rivers of high latitudes release more than half of the annual DOC export during the 2- to 4-week-long snow melt period.

As the active layer deepens in the course of the frost-free period, deeper infiltration of organic solutes and higher retention time in soil cause a decrease of DOC concentration in subarctic rivers (Fig. 16.6) and streams (Fig. 16.7a), and an alteration of its chemical composition (Neff et al. 2006; Prokushkin et al. 2007). In particular, chemical and isotopic fingerprints of summer–autumn DOC suggest a higher input of microbially transformed and/or derived material. Therefore, the release of terrestrial DOC from permafrost-affected watersheds is controlled by the seasonal cycle of the active layer over permafrost, as shown by increasing $\delta^{18}O$ values (Carey and Quinton 2004) and $DO^{13}C$ in river waters and on the other hand, decreasing aromaticity and older ^{14}C signature of dissolved organic matter (Neff et al. 2006).

Reduced DOC export during summer through autumn in subarctic rivers contradicts suggestions that rising temperature in northern latitudes will result in a significant increase of DOC flux to the marine system. Comparative analysis of watersheds with different extent of permafrost distribution in Alaska supports the reduction scenario of DOC export in a warmer climate (MacLean et al. 1999). Recent data of Kawahigashi et al. (2004) provide further evidence of decreased riverine DOC export in Siberia, due to a significant drop of DOC concentrations in small streams along a gradient from continuous to discontinuous permafrost in the lower Yenisey River basin. Simultaneous major alteration of biochemical composition (i.e.,

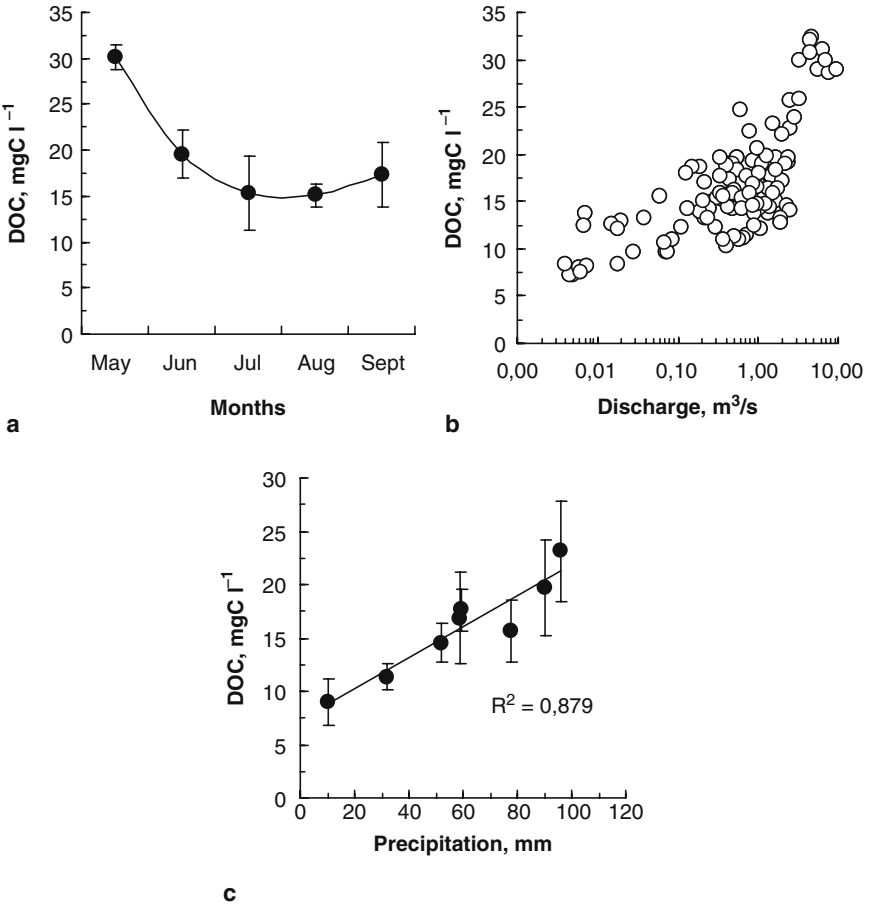

Fig. 16.7 Changes in mean concentrations of DOC in the Kulingdakan stream from May to September in 2001–2005. Relationships between stream DOC concentration (**a**) and discharge (**b**), and monthly mean DOC concentration and precipitation amount (**c**) for July 1998–2005

decrease of lignocellulose complex, increase of hydrophilic fraction) confirms the significant influence of a thickness of the active layer and distribution of permafrost on flux, composition and biodegradability of DOC in Siberian soils.

The connection between river DOC and old (aged) OC stored in permafrost remains unclear. While there is evidence that permafrost in Arctic regions is undergoing rapid change (Serreze et al. 2000), the recent (younger) DOC observed for arctic rivers shows that the release of old DOC from permafrost into the hydrological cycle is not substantial (Benner 2004; Guo et al. 2006). Extended sampling during the growing season clearly demonstrated increasing age of DOC in upland streams and the Kolyma River in Eastern Siberia (Neff et al. 2006). These findings, however, are indicative also for an increased input of deep groundwater from "taliks" (liquid water reservoirs within frozen ground) located beneath river beds.

Winter base flow in permafrost-dominated basins is largely deep beneath permafrost groundwater, having low DOC concentrations and DOC chemistry consistent with high water residence and DOC withdrawal (Striegl et al. 2005). A number of recent studies have pointed to recent trends toward increased winter discharge from the major Siberian rivers (Peterson et al. 2002). Changes in active layer depth over permafrost directly affect potential groundwater storage and river discharge throughout the winter season. The thicker active layer has more groundwater storage capacity, due to the melting of ground ice and an increased precipitation input. This increased groundwater storage in turn results in a greater contribution of subsurface water to the river systems and, hence, increases the winter season stream flow. Thus, permafrost degradation forces an elongation of the period of hydrologically active soil and an increase of the soil–water storage capacity, which in turn contribute to higher concentrations of DOC to rivers. Therefore, changes associated with the deepening of the active layer induce a reduction of DOC export from watershed in frost-free periods, and in contrast may enhance winter DOC flux.

16.3.2 Effects of Warming

There are two major scenarios of climate change in high latitudes (wet and dry) having, nevertheless, opposite effects on DOC export from terrestrial ecosystems.

Increased precipitation under "wet warming" exerts a significant control on the generation of runoff and DOC export from permafrost terrains. Streams draining permafrost-dominated watersheds have a more "flashy" hydrology than those draining permafrost-free watersheds (Woo and Winter 1993). A "flashy" hydrologic regime is characterized by low baseflows but high stormflows, with a rapid onset following rainfalls (MacLean et al. 1999). Stormflows demonstrate an increase in DOC concentrations (Fig. 16.7b) in high latitude streams, which is indicative for near-surface pathways of runoff generation within catchments. The biochemical composition of stormflow DOC [e.g., aromaticity (specific ultraviolet absorption; SUVA), lignin breakdown products etc.] clearly reflects signatures of forest floor OC, though the magnitude of rainstorms affects the contribution from various soil horizons (Prokushkin et al. 2007). Correspondingly, in wetter climates, more of the runoff is generated from the soil organic layer, resulting in higher concentrations of DOC within streams (Fig. 16.7b,c) and increased overall export of DOC from watersheds.

Wildfires, assumed to be the main disturbance factor in the boreal biome, tend to increase in frequency and severity under drier climatic conditions. In general, fires exert significant control on biogeochemical cycling within watersheds in permafrost terrains. The examination of forested watersheds in Central Siberia has demonstrated that presumably all basins of the region were affected by wildfires in the past. Analysis of DOC fluxes in streams draining basins that were largely affected by fire (>90% of area) revealed a significant decrease of DOC concentrations in streams with recent fire-effects as compared to basins covered with more

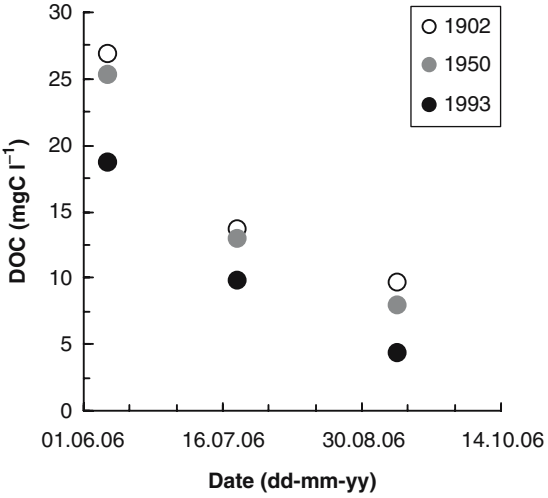

Fig. 16.8 Dynamics of DOC concentrations in small streams draining watersheds, which were totally burned in 1902, 1950 and 1993

aged forest ecosystems (Fig. 16.8). In terms of flux, DOC output from recently burned watershed in a dry year (2006) was only one fifth of that from watershed burned 100 years ago. Decreased discharge and respectively reduced DOC export may be caused by the larger water-holding capacity of the deepening active soil layer, which occurred after the fire event. Thus, under drier climatic conditions, fires imply two limitations of DOC release from watersheds: (1) decreasing mobile C-source (combustion of organic layer) and (2) free water (increased water-holding capacity of soil). Comparable concentrations of DOC in streams draining watersheds burned 50 and 100 years ago corroborate earlier estimates of a recovery time of 50 years for ecosystem structures (species composition, soil temperature etc.) (Abaimov 2005).

16.4 Conclusion

High-latitude river basins export disproportionately large amounts of terrigenous DOC to the Arctic Ocean when compared to other major river basins. As climate warms, the amount and chemical composition of DOC exported from these basins are expected to change. Clearly, the leaching of plant litter/upper soil horizons and the leaching of deeper soil horizons produce different biogeochemical fingerprints, which can then be sought in the concentration and chemical composition of organic C species in subarctic rivers, and be used as a proxy for hydrological and permafrost dynamics in these basins.

Production and fate of DOC in warmed terrestrial compartments of permafrost terrains is largely influenced by the interaction between increased microbial activity, vegetation changes, and degradation of permafrost barrier to deep infiltration of solutes. Increased DOC concentrations in Arctic rivers might be supported by enhanced terrestrial primary production, a shift from tundra to forest, increased production in organic topsoil, and release from melting permafrost.

Nevertheless, though DOC fluxes are supposed to increase, driven by warmer air temperatures, through temperature-related processes of DOC production, the increasing retention time of DOC in deep mineral soil will most likely lead to the net decrease of DOC export to rivers due to its stabilization in soils. Under this scenario, warming in high latitudes may result in the increased accumulation of C resistant to biodegradation in deep subsoil. Thus, the adsorptive properties of thawing soils distributed across the subarctic area exert the major control on this process. Another temperature-related factor influencing DOC export is wildfires, likely increased with warming. By reducing the C pool in upper organic layers and increasing the active layer thickness, fires greatly decrease the DOC output from watersheds underlain by permafrost.

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Chapter 17
Climate Change and Foundations of Buildings in Permafrost Regions

Yuri Shur(✉) and Douglas J. Goering

17.1 Design Approaches for Permafrost Regions

The impact of climate change on the integrity of structures built on permafrost has been widely discussed (US Arctic Research Commission 2003; ACIA 2005). The problem is twofold. Firstly, it is a prediction of behavior of existing buildings, and secondly, it concerns approaches to design for future conditions. Both are very difficult for design engineers to solve, because of uncertainties involved in existing climatic models and the wide range of results predicted by different climate change models. To predict climate-change impact on existing buildings, it is necessary to assess the thermal regime of the permafrost beneath the buildings, the factor of safety implemented in the designs, and change in the bearing capacity of foundations during the service life of the buildings.

Design engineers do not operate with definitions like "possible, very likely, likely to" and so on. It would be easier for engineers if the result of climate-change discussion could produce a quantitative method which could be used for design. The discussion of climate-change impact on structures in permafrost regions requires a thorough analysis of existing design approaches and of existing methods of maintenance of conditions expected in design. It also requires an analysis of current causes of existing damage to infrastructure, and understanding of their relevance or irrelevance to climate change. The most extensive engineering studies of permafrost as a base for buildings and structures were accomplished in Russia and Northern America between the 1950s and the 1970s. They led to development of design approaches and supporting engineering means (Zhukov 1958; Saltykov 1959; SN 91–60 1963; Dokuchaev 1963; Long 1966; Tsytovich 1975; Velly et al. 1977; Johnston 1981; Technical Manual 1983). Numerous studies have been performed to understand the causes of building failures on permafrost (Bondarev 1957; Shamshura 1959; Lukin 1966; Voytkovsky 1968; Goncharov et al. 1980; Kronik 2001; ACIA 2005; Alekseeva et al. 2007).

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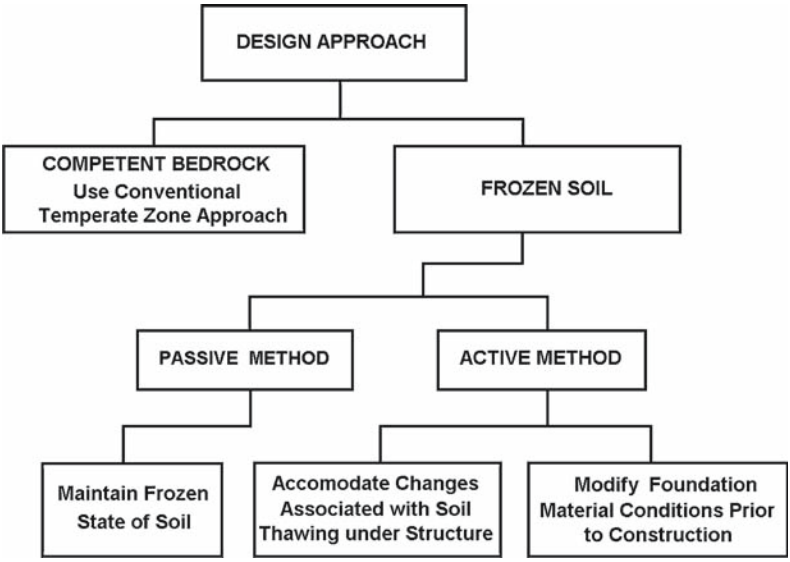

Fig. 17.1 Main design approaches for permafrost (based on Johnston 1981, Technical Manual 1983)

Although engineering means to control permafrost are constantly improving, the main approaches to design for permafrost conditions remain the same. These approaches are shown in Fig. 17.1. The two main approaches, "passive" and "active", bear names given in Russia in the 1930s and were brought to Western knowledge by Muller (1945). In Russia they are known as Principle I (use of soil in the base of structures in its permanently frozen state) and Principle II (use of soil in thawing or thawed state). The Technical Manual (1983) calls them design alternatives. They are not. An alternative implies another choice. Unfortunately, in most cases accommodation of changes associated with soil thawing under structures can not be implemented as an alternative to maintenance of the frozen state of soil.

17.1.1 Passive Method – Maintain Frozen State of Soil

This method is the main one used in the permafrost regions, but it was not fully appreciated or widely used until the 1950s after a long period of unsuccessful attempts to accommodate changes associated with permafrost thawing under structures. Numerous buildings on permafrost experienced substantial deformations because of thawing of permafrost and thaw subsidence of foundation bases. This has happened throughout the entire Russian permafrost region when methods based on accommodation of changes related to thawing permafrost were mainly applied. Even with the relatively low ice content of the silty clays in Vorkuta, many buildings have been destroyed (Zhukov 1958). Engineering means used for preservation of permafrost under buildings greatly reduced the percentage of deformed buildings.

This method is the only one which can protect structures from excessive deformations associated with thawing of ice-rich fine soils. Foundations built according to this method bear heavy load, have minimal settlement, and can be easily protected from frost heave.

The method is generally recommended for areas with a permafrost temperature of −3°C and below. "As a rough guide, the situation should be critically evaluated when the mean ground temperature is warmer than about −3°C, if the ground is to be maintained in a frozen condition following construction" (Johnston 1981, p 251). The first Russian building code for survey, design, and construction of railroads and their infrastructure in the permafrost regions recommended the active method as technically sound and economical in areas with warm permafrost (temperature above −3°C). Permafrost in the town of Skovorodino was the first example of a place recommended for application of this method. Bykov and Kapterev (1940) showed that such an approach was erroneous.

Successful applications of the passive method in areas with warm permafrost in Russia, such as Chita, Vorkuta, Igarka, and Skovorodino, and many others, showed that the passive method can be used in regions with warm permafrost.

There are several engineering means for maintaining frozen soil beneath buildings, and ventilated air space (crawl space) beneath elevated buildings is the most widely used. In Alaska and Canada this space is usually completely open, in Russia it is ventilated through relatively small openings (vents) in a foundation wall or a wall beam. The total area of openings is evaluated by using the so-called modulus of ventilation (MV), which is the ratio of the total area of openings to the footprint of a building. For buildings with the open crawl space, the MV is equal to the height of a crawl space multiplied by its perimeter. The Russian building code and some other sources provide methods evaluating MV. Saltykov (1959) presented a table which can be used for preliminary evaluation of the MV (Table 17.1). Similar MV values are recommended by the Handbook on Construction on Permafrost (Velly et al. 1977) and by Tsytovich (1975).

Design of ventilated crawl space in Russia has traditionally aimed at two goals. The first is to keep the soil beneath the buildings in the frozen state, and the second is to provide a comfortable temperature at the floor above the ventilated crawl space with minimal thermal insulation to reduce its cost. The MV approach reflects both these goals.

Table 17.1 Recommended modulus of ventilation (based on Saltykov 1959)

Thermal resistance of structure above crawl space (m^2 h°C $kkai^{-1}$)	Indoor air temperature (°C)	Modulus of ventilation for permafrost zones		
		Northern	Central	Southern
1	15	0.0025–0.005	0.005–0.02	0.02–0.03
	30	0.0075–0.015	0.015–0.05	0.05–0.08
2	15	0.0015–0.003	0.003–0.01	0.01–0.015
	30	0.0035–0.007	0.007–0.02	0.02–0.03
3	15	0.0008–0.002	0.002–0.006	0.006–0.009
	30	0.002–0.0035	0.0035–0.01	0.01–0.015

In Norilsk (northern permafrost zone), ventilation of the crawl space is designed with an MV ranging from 0.00225 to 0.004 (Shamshura 1959; Maksimov et al. 1978). For example, for a building 50 m by 20 m in Norilsk, if thermal resistance over the crawl space is equal to 3 m^2 h°C $kkai^{-1}$ and air temperature in rooms on the first floor is equal to 15°C, the total area of opening for ventilation of the crawl space can be between 0.8 m^2 and 2 m^2 (for an open crawl space with a height of 1 m, the area open for ventilation is equal to 140 m^2). Velly et al. (1977) presented an example of the evaluation of the total area of vents in the wall beam of the crawl space for a building 60 m long and 20 m wide at Dikson, a seaport in the Russian Arctic. It is expected that, during the lifetime of the building, soil temperature will increase from −6 to −3.6°C and the total area of vents should be equal to 0.66 m^2 with MV = 0.00055 (Velly et al. 1977). The ventilated area of the open crawl space of 1 m height would be equal to 160 m^2, or 240 times greater. Mean annual soil temperature under the open crawl space would decrease to about −10°C.

Thermal resistance of insulation above ventilated crawl spaces in Russia is 3–5 times smaller than required in Alaska. As a result, mean air temperature in the crawl space is intentionally kept warmer than it could be in an open crawl space, and resources in chilling permafrost remain unused when MV depends on thermal resistance of the floor above the crawl space. The example for the building in Dikson (see above) shows that the opportunity to keep the permafrost at a lower temperature was greatly reduced in an attempt to satisfy both conditions. Such an approach in reaching two competing goals has been implemented in Russian building codes for permafrost regions. This approach is at least questionable and some Russian arctic engineers do not support it. According to Dokuchaev (1963, p 121): "Preference should be given to an open crawl space (especially in regions where mean annual permafrost temperature is above −3°C) because open crawl space guarantees low permafrost temperatures. Money saved on wall beams around the crawl space could be spent on increased thermal insulation." This advice of one of the best Russian permafrost engineers has not been followed. For example in Chita, where mean annual permafrost temperature is about −0.3°C to −0.5°C, MV is equal to 0.015–0.03 for a building with continuous foundations, which is 10–15 times less that could be provided by the open crawl space.

There is one more disadvantage of the ventilated crawl space with small vents. It is not easy to observe and, thus, does not allow for easy inspection. Leaks in water or heating lines, which are usually attached to the ceiling of the crawl space, can remain undetected for a long time and badly damage frozen foundation soils before detection.

According to Shamshura (1959), permafrost temperature under an open crawl space becomes almost equal to mean annual air temperature, and permafrost temperature under a crawl space ventilated through vents is several degrees warmer. Maksimov et al. (1978) also found that mean annual soil surface temperature in an open crawl space is the practically equal to the local mean annual air temperature there. They reported that the winter temperature greatly depends on the type of crawl space. In an open crawl space, it is very close to outside temperature. In a poorly ventilated crawl space, the winter mean air temperature can be more than

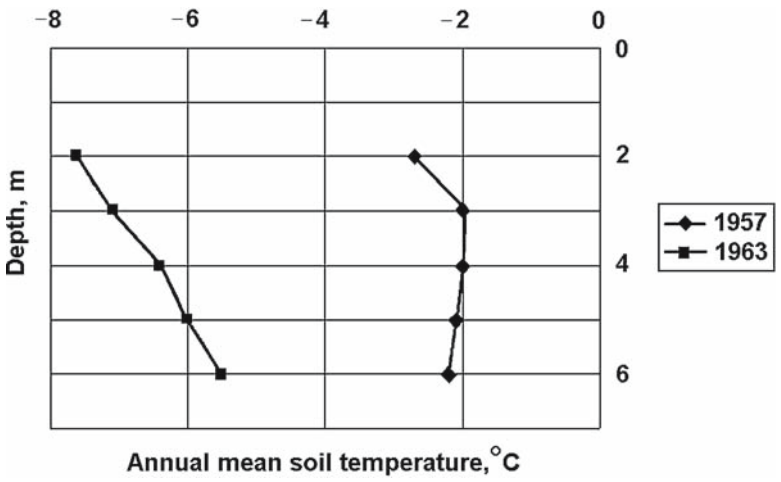

Fig. 17.2 Change in soil temperature under shoe factory in Yakutsk (based on Voytkovsky 1968)

12°C higher than the outside air temperature. Summer mean air temperature in the crawl space is about 1–2°C colder than outside air temperature (Maksimov et al. 1978).

Figure 17.2 shows a decrease in permafrost temperature in an effectively ventilated crawl space over a 6-year period in Yakutsk, Russia. The gradient in annual mean soil temperature in 1963 showed that the decrease in soil temperature continued.

An effectively ventilated crawl space reduces permafrost temperature by several degrees. It occurs during the years after construction, and can not be taken into account by design if preliminary cooling of soil prior to construction has not been applied. Design relies on permafrost temperatures during construction. Decrease of permafrost temperature under a crawl space during the service life of a building increases the Factor of Safety for the bearing capacity of foundations.

Cooling of permafrost beneath a crawl space takes years, and consequently bearing capacity of soils and foundations increases with time. The Russian Building Code (SNiP 1991) requires a decrease of soil temperature of plastic frozen soils to about −2 to −3°C. To take the advantage of such cooling into account, soil temperature should be reduced prior to construction or during construction of foundations. The simplest way is to plow snow from a site for several years prior to construction and thermally insulate the soil surface in summer. Soil can be also chilled through pipes used as piles or through holes used for the installation of piles (Maksimov et al. 1978).

A ventilated open crawl space provides a continuous decrease in permafrost temperatures and increases design bearing capacity of permafrost up to two-fold (Table 17.2). As a result, an increase permafrost temperature by several degrees due to climate change or other factors can take place without any impact on the structure

Table 17.2 Increase in bearing capacity of piles during service life (based on Lukin 1966)

	Building 1		Building 2		Building 3	
Design characteristics	1950	1963	1959	1963	1958	1963
Embedding of piles in permafrost (m)	4	5	4	5	4	5
Average design temperature along a pile (°C)	−2.8	−3.4	−1.2	−2.1	−1.5	−2.2
Design temperature at the tip of pile (°C)	−3.6	−5	−1.6	−3.1	−2.1	−3.2
Pile bearing capacity (T)	100	140	60	100	55	106
Increase in bearing capacity during service life (%)		40		67		92

integrity, and a potential climate change impact would affect buildings with open crawl spaces much later than buildings with crawl spaces with openings designed according to MV.

Permafrost temperature under outer walls determines the properties of soils used in structural design, and it is a function of permafrost temperatures beneath and outside of the building. This temperature can be decreased by several methods, such as the use of thermal piles, thermal insulation of soil outside a building, snow-plowing around a building, and a combination of these methods. A combination of thermal insulation with thermal piles resulted in greatly reduced permafrost temperature at some sites along the Trans Alaska Pipeline. A combination of thermal piles and open crawl space has been used effectively in Alaska and Russia (Vialov et al. 1993). A combination of open crawl space with heat pipes associated with piles and summer seasonal thermal insulation can keep soil in a frozen state even when the mean annual air temperature is a few degrees above 0°C.

Porkhaev (1959), whose contribution to development of methods for evaluating thermal interaction of buildings with frozen and thawing soil has so far been the most significant, found that permafrost under structures can be protected in practically the entire permafrost area. He also found that the lower permafrost temperature and the greater its thickness, the easier it is to protect permafrost. The air temperature is the defining factor, because permafrost temperature can be reduced by cooling systems and eventually becomes close to mean annual air temperature. "The thermal impact of engineering cooling systems such as ventilated crawl space, ventilated ducts and others is several times greater than the impact of natural factors" (Porkhaev 1959, p 19). Contemporary methods of frozen ground engineering have powerful means to protect the frozen state of permafrost in a wide range of climatic conditions (Khrustalev 2005).

Although general approaches to design for permafrost conditions are identical, their applications are different in Russia and North America (Table 17.3). For comparison, a building with ventilated crawl space and pile foundations is considered. The differences are important when evaluating the potential climate-change impact on permafrost as a foundation for buildings. Comparison shows that a building designed with American standards can withstand greater climatic changes.

Table 17.3 Comparison of North American and Russian approaches to designing foundations with ventilated crawl space

Characteristics	North America	Russia
Safety factor	2.5–3	1.05–1.56 (Khrustalev 2001)
Tip bearing capacity of piles	Usually not taken into account	Taken into account
Type of air space beneath a building	Open	Often closed with openings, whose area is calculated from modulus of ventilation (MV)
Central heating line in crawl space	Usually not installed	Often installed
Pile material	Steel	Concrete
Building construction material	Light	Heavy

17.1.2 Active Method – Accommodate Changes Associated with Permafrost Thawing Under Structure

At first glance, this method looks attractive in cases of degrading permafrost, but in fact it has very few successful applications. Permafrost thawing is accompanied by thaw settlement of soil and foundations if frozen soil is thaw-unstable. Thaw susceptibility of soil is determined by thaw strain — the ratio of thaw settlement to thickness of the soil layer prior to thawing. It is important to define the borderline value of thaw strain below which soil can be considered as thaw-stable. One of the old Russian Building Codes (SN 91–60 1963) defined this value as 0.03 if thaw settlement was evaluated for a load of 100 kPa. Soil with thaw strain greater than 0.03 and smaller than 0.1 is considered thaw-unstable, and soil with thaw strain greater than 0.1 as highly thaw-unstable. According to Velly et al. (1977), even soils with thaw strain equal to 0.02 require special attention. Thaw strain equal to 0.02 and less is typical of gravelly and sandy soils with dry densities greater than 1,900 kg m^{-3} and water content less than 12%. To be thaw-stable, clayey soils should be well-consolidated, should not have visible ice, and should have dry densities more than 1,800 kg m^{-3} and water content not exceeding the plastic limit of soil. Most permafrost soils are highly thaw-unstable and have thaw strain exceeding 0.1.

Thaw settlement beneath a building and differential thaw settlement should be less than the tolerable limits for such a building. Most buildings can hardly tolerate thaw settlement greater than 10 cm, and even structurally enhanced buildings can not tolerate thaw settlement greater than 30 cm. This means, for example, that for soils with thaw strain equal to 0.1, thaw depth beneath foundations can not be greater than 3 m. It is costly and difficult to design buildings which can tolerate thaw settlement, and there are numerous examples of unsuccessful applications of this method. High thaw-susceptibility of most permafrost soils, and low tolerance of buildings to settlement, limit application of the method to especially favorable conditions.

17.1.3 Active Method – Modify Foundation Material Conditions Prior to Construction

A thin layer of thaw-unstable permafrost over bedrock or over thaw-stable soil can be replaced with thaw-stable soil. More often such replacement is not feasible or not economically justified. The other method of permafrost modification prior to construction is its preliminary thawing to a specific depth. Steam and water points and electrical heating have been applied for thawing. This method has been infrequently used in Alaska and Russia.

Preliminary thawing of foundation soils is most effective in the case of coarse-grained soils where settlement is practically complete during thawing. Ice-rich clayey soils reach 60–80% of their total settlement upon thawing, and their settlement continues during and after construction. Their water content upon thawing is greater than their liquid limit and shear strength is insufficient. Thus, preliminary thawing cannot by effectively applied to such soils.

There are successful and unsuccessful examples of application of the method. This method is the first to consider in areas of degrading coarse-grained perennially frozen soils.

17.2 Building Failures in Permafrost Regions

Deformations of buildings in permafrost regions are inexcusably numerous, especially in Russia. "The percentage of dangerous buildings in large villages and cities in 1992 ranged from 22% in the town of Tiksi to 80% in the city of Vorkuta, including 55% in Magadan, 60% in Chita, 35% in Dudinka, 10% in Norilsk, 50% in Pevek, 50% in Amderma, and 35% in Dikson" (Kronik 2001; ACIA 2005). Hundreds of buildings were demolished or went through serious reconstruction (Ilichev et al. 2003).

There have been many attempts to understand the causes of such numerous failures. Bondarev (1957) was possibly the first who classified these causes as poor assessment of soil conditions at the site, mistakes in choosing foundation design approach, mistakes in design, poor construction quality, and poor maintenance.

Many failures were caused by infiltration of hot water from broken heating pipes, which resulted in the formation of deep thaw zones and severe differential settlement (Kuriachiy and Illarionov 1959; Ilichev et al., 2003; Alekseeva et al. 2007). Poor drainage and ponding of water in crawl space also cause damage (Goncharov et al. 1980; Johnston 1981). Existing building codes on foundation design in permafrost regions are focused on a separate building, and do not consider changes in permafrost conditions associated with the development of the entire area with streets, utilidors, and storm canalization (Ilichev et al. 2003).

Documented failures of building foundations constructed according to the passive method, which are attributed to changes in permafrost, very often do not

directly relate to air and permafrost temperature. Such foundations failures are not caused by permafrost warming but by climatic effects on foundations material in the active layer and in a crawl space, unaccounted for thermal stresses, and low freeze–thaw resistance of concrete in piles. Concrete piles are the most widely used foundations in the Russian permafrost region. As was found in Norilsk, Yakutsk and some other places, the upper parts of piles and their connections with concrete grillage deteriorate, and cracks in walls are often caused by crushing of the upper parts of piles. Such processes can not be directly attributed to changes in permafrost, although wetting and drying of soil of the active layer are factors contributing to fast weathering of concrete (Goncharov et al. 1980).

Thus, there is no direct correlation between failures of structures and their location. Numerous failures have occurred both in continuous and discontinuous permafrost zones, and at sites with different soil conditions and permafrost temperatures. Some deformed buildings were constructed in accordance with the passive method, while others were built to accommodate thaw settlement.

17.3 Conclusion

Reassessment of existing approaches to building construction in permafrost regions has been triggered recently by concerns associated with the potential impact of climate change on permafrost. At sites with ice-rich soils, preservation of permafrost beneath buildings remains the main approach. Most permafrost soils are highly thaw-unstable, and their thaw settlement can not practically be accommodated. Preliminary thawing of permafrost prior to construction has not found wide application so far. As long as the mean annual temperature remains below 0°C, means of permafrost protection without artificial refrigeration could be applied. Numerous building failures in permafrost regions are related to changes in permafrost due to poor design, and to poor maintenance of buildings, which are more powerful factors than the natural change in permafrost temperature.

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Part V
Contaminants in Frozen Ground

Chapter 18
Migration of Petroleum in Permafrost-Affected Regions

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18.1 Introduction

An extensive amount of effort has been undertaken by many over the last three or so decades to better understand the movement of crude oil and petroleum products through terrestrial environments. This effort is based on a desire to better characterize and remediate environments that have been impacted by releases of these substances. The presence of ice in Arctic and Antarctic soils, the influence seasonal freeze and thaw cycling has on fluid movement, and the typically shallow active layers found in these environments all impact the movement of fluids in these soils in a manner not found in temperate soils (soils that do not experience deep freezing). How the unique Arctic and Antarctic conditions affect the movement of petroleum-related substances in these environments will be discussed in this chapter.

Understanding the mobility of contaminants in these environments becomes relevant when one considers the high cost of conducting site investigations and cleanup activities at locations in the Arctic and Antarctic that are often remote. In addition, uncertainty as to how cleanup activities may possibly enhance mobility of contaminants and degradation of the ecosystem by disturbing the fragile thermal balance is of concern in any cleanup activity in the Arctic and Antarctic. At the extreme, Snape et al. (2001) discussed the directives of the Antarctic Madrid Protocol (International Council of Scientific Unions 1993) to clean up past and present waste disposal sites. At many of these contaminated sites contaminated material that cannot be treated onsite will have to be removed from the continent, an expensive process. Onsite treatment will require the shipment of treatment equipment and materials to the research stations, again an expensive process. Thus, it becomes evident why understanding the mobility of contaminants becomes important, as even a small reduction in the material to be treated or shipped will result in economic benefit.

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18.2 Background

Human activities in the Arctic and Antarctic have resulted in releases of a suite of compounds that are harmful to human and environmental health including crude oil and petroleum products. In this chapter the terms *crude oil* and *petroleum products* refer to the actual liquids, and the term *petroleum hydrocarbons* refers to compounds such as benzene that make up the liquids. Most releases that occur in the Arctic and Antarctic are insignificant in volume; however, several larger terrestrial releases have taken place. Arguably, the largest release to have occurred in the Arctic took place north of the city of Usinsk, Russia (65°N), in the Kolva River Basin (Vilchek and Tishkov 1997; AMAP 1998). By one estimate 103,000–126,000 tonnes of crude oil (other experts estimate the release to be as high as 318,000 tonnes) was released over a 2–3 month period from multiple leaks in a pipeline system that continued to pump oil even though the pipeline was leaking (Vilchek and Tishkov 1997).

Recent relatively large releases of crude oil have occurred on the Trans Alaska pipeline. In 2001, a hole was shot in the pipeline near the village of Livengood, Alaska (65°N), resulting in the release of approximately 265,000 l (Spiess 2001). Corrosion of a crude-oil transit line at Prudhoe Bay, Alaska, caused between 761,000 l and 1,010,000 l of crude oil to be released to the tundra (JIC 2006). Relatively smaller releases associated with fuel storage and transportation for industrial activities, predominately mining, and for communities in the Arctic occur with more frequency then the larger more notable releases. Such releases are a result of vehicle accidents, such as a fuel tanker truck roll-over at a large zinc and lead mine in northwest Alaska (68°N) resulting in the release of approximately 10,000 l to tundra (ADEC 2004a), or mishaps related to fuel storage, such as an approximate 9,500 l release that occurred in the village of Point Hope, Alaska (68°N), due to overfilling of a storage tank (ADEC 2004b). Rike et al. (2003) described the presence of petroleum hydrocarbons in soil at a site near the village of Longyearbyen, on Spitzbergen Island in the Svalbard archipelago (78°N), most likely resulting from small releases of petroleum over time at a fire extinction training site.

Relatively smaller releases of petroleum products have occurred in the Antarctic as well. A majority of these releases are due to poor waste management practices at research stations. In the hope that burial in frozen ground would contain waste, most waste from research stations were disposed of in dumps with little to no engineered containment systems (Snape et al. 2002). In addition, minimal attention was given to petroleum spills, owing to the belief that the frozen environment would contain the compounds (Snape et al. 2002). Investigations illustrated that containment of contaminants in this manner is not feasible. Contaminants are mobile in this environment during thawing and thawed periods much in the same manner as in more temperate environments. In addition, cryoturbation and erosion uncovers buried contaminants, exposing them to transport processes both in ground water (suprapermafrost) and surface water (Snape et al. 2001).

To understand movement of petroleum and petroleum hydrocarbons through freezing and frozen soils in the Arctic and Antarctic, an understanding of the

fundamental principles of immiscible fluid (in this case petroleum) movement through unfrozen soil is required. Several authors have presented thorough descriptions of the movement of immiscible fluid, commonly known as non-aqueous phase liquids (NAPL), through unsaturated soils (Mercer and Cohen 1990; Wilson et al. 1990; Poulsen and Kueper 1992). Petroleum is considered a light non-aqueous phase liquid (LNAPL), as the specific gravity of the fluid is less than unity. The remainder of the discussion will focus on petroleum.

Released at or near the ground-surface, petroleum will move downward through unsaturated soil toward the water table. Due to the immiscibility, the fluid migrates as a distinct liquid, separate from the air and water present in the unsaturated soil. Water and petroleum are held in the pore space of partially saturated soils by capillary forces. As petroleum migrates downward, air and possibly some water are displaced from the pore space. Once in soil pore space, individual petroleum compounds will dissolve into soil water according to the specific solubility of each compound and its mole fraction. Solubility of these compounds is low, since most petroleum hydrocarbons are non-polar. Sorption of petroleum hydrocarbons onto natural organic matter in the soil results from the non-polar nature of these compounds. The high volatility of relatively low molecular weight petroleum hydrocarbons dissolved in soil water results in partitioning of a fraction of these compounds into the gas phase. The mixture of gaseous petroleum hydrocarbons and air becomes soil gas in the pore space.

Infiltrating petroleum follows a path through unsaturated soil that is dictated by the properties of the soil encountered; primarily, permeability and pore structure. Results from field studies performed by Poulsen and Kueper (1992) illustrated how small variations in permeability result in extreme heterogeneous distribution of NAPL and some lateral migration, which is also a result of capillary forces.

Capillary forces immobilize a fraction of petroleum in the pore space as the main body of the liquid moves downward through porous medium. Results from a visualization study conducted by Wilson et al. (1990) showed that immobilized NAPL was mostly contained in pore throats and in thin films between soil water and soil gas. Soil water was also contained in pore throats that were bypassed by infiltrating NAPL, and soil gas filled the larger pore bodies.

Infiltrating petroleum that reaches the capillary fringe, sometimes referred to as the nearly saturated zone, will spread laterally as a result of the relatively high water saturations in this zone. For spill volumes that generate sufficient head to displace the water in the capillary fringe water, petroleum that migrates further downward to the water table may displace water from saturated pores and cause depression of the water table. As the water table rises and falls seasonally some petroleum is immobilized or entrapped in the capillary fringe and possibly below the water table during high water level conditions. This immobilized petroleum consists of small pockets (or ganglia) of liquid disconnected from the main body of organic liquid (Wilson et al. 1990). A dissolved phase plume results in the saturated zone below the water table, from petroleum contained above and below the water surface.

18.3 Migration of Petroleum in the Active Layer

The migration of petroleum through soil that comprises the active layer (the zone above permafrost zone that experiences season freezing and thawing) is a function of the season in which the petroleum is released. Migration of released petroleum during periods when the active layer is unfrozen or thawing will be influenced by high soil-water contents in poorly drained soils, and by the shallow nature of the active layer in many permafrost regions. During periods when the active layer is frozen, migration of released petroleum will be greatly influenced by the presence of ice in the soil. Freezing and thawing cycles will impact the distribution of petroleum in the subsurface, independent of the season the petroleum was released.

18.3.1 Petroleum Releases to Unfrozen Active Layers

In permafrost-affected regions the thickness of the active layer will be minimal — centimeters to a few meters, depending upon local conditions. The active layer begins to thaw during the spring snowmelt and continues to thicken until reaching maximum thickness in late August or September (Hinzman et al. 2005). As the active layer thaws a layer of water-saturated soil develops, which may be as thick as the entire thawed thickness. Thus, the downward flow of petroleum will be impeded due to low relative permeability to petroleum as a consequence of high soil-water saturation. With downward flow impeded, an increased flow takes place through the near surface layer of partially decayed vegetation that is typically present in many arctic ecosystems or bare ground where vegetation is not predominant. Results from field studies conducted by Mackay et al. (1974a, b, 1975) as well as Johnson et al. (1980), in which petroleum was released to unfrozen soil underlain by permafrost, illustrate how high water contents in poorly drained soils impede downward migration of released petroleum. This flow pattern leads to relatively large aerial distributions of petroleum, tempered by entrapment of the petroleum onto organic matter present in the uppermost layer of soil. However, even under these conditions petroleum does move downward through underlying mineral soil. In areas of large accumulations of petroleum, soil water will be displaced and petroleum will progress into lower mineral soils. Furthermore, over time, the petroleum may migrate deeper into the soil horizon as the active layer freezes and thaws.

In contrast, a study conducted by Mackay et al. (1975) where petroleum was released to unfrozen unsaturated (relatively low soil water contents) soils in a tundra environment resulted in infiltration of petroleum to the top of the frost line or to the water table where present. The petroleum then flowed downgradient (down slope) through a relatively thin horizontal layer of very permeable soils directly above the frost line. Using fundamental principles, the theoretical distribution of petroleum in active layer soils can be investigated.

The thin nature of the active layer and the saturated soil contained within will influence the distribution of petroleum throughout the active layer, and may allow for petroleum to be distributed as a free-phase liquid throughout the entire saturated zone. Recognizing the complex nature of characterizing the water-saturated zone contained in the active layer, due in part to the constant change taking place as thawing and refreezing occurs, the fundamental characteristics of how petroleum may distribute following a release can still be examined. Farr et al. (1990) described the distribution of free-phase LNAPL, such as petroleum, in porous media under hydrostatics considering a deep ground-water aquifer, and developed the mathematical relationships for LNAPL saturation as a function of depth from ground surface. These relationships can be re-derived to take into account the thin saturated zone typically found in a thawed or thawing active layer. As in Farr et al. (1990), total liquid saturation (water and petroleum; S_T) as a function of capillary pressure between air and petroleum (P_c^{ao}) is as follows:

$$S_T = S_w + S_o = (1 - S_r)\left(\frac{P_c^{ao}}{P_d^{ao}}\right)^{-\lambda} + S_r, \tag{1}$$

where S_w is water saturation, S_o is the petroleum saturation, S_r is residual saturation (assumed to be the same for both liquids), λ is the pore size distribution coefficient, and P_d^{ao} is the displacement pressure between air and petroleum. Similarly water saturation (S_w) as a function of capillary pressure between petroleum and water (P_c^{ow}) can be described as follows:

$$S_w = (1 - S_r)\left(\frac{P_c^{ow}}{P_d^{ow}}\right)^{-\lambda} + S_r, \tag{2}$$

where P_d^{ow} is displacement pressure between petroleum and water. Capillary pressures as a function of elevation from the frozen soil layer (z) between each fluid are as follows:

$$P_c^{ao} = \rho_o g (z - T_o), \tag{3}$$

$$P_c^{ow} = \rho_w g (z - b) + \rho_o g (T_o - z). \tag{4}$$

In (3) and (4) ρ_o is density of the released petroleum, ρ_w is water density, g is the gravitational constant, b is the thickness of the saturated zone prior to the petroleum release, and T_o is the thickness of petroleum that would be found in a monitoring screened through the entire saturated thickness. For these calculations, an assumption is made that the thickness of the water-saturated zone stays constant.

To investigate the influence the water-saturated thickness has on petroleum saturation, consider the fluid properties and soil properties for a sandy loam shown in

Table 18.1. Assume that the thickness of petroleum that would be measured in a monitoring well installed in the impacted area is 0.7 m for this example. Again acknowledging our assumptions of hydrostatic conditions and no hysteresis, (1)–(4) can be used to estimate petroleum saturation as a function of depth for different values of saturated zone thickness prior to the petroleum release. Results from these calculations are shown in Fig. 18.1.

Table 18.1 Soil and fluid properties for the example provided in the text (soil properties are from Rawls et al. 1982)

Soil property	Value
• Porosity (Φ)	0.437
• Residual saturation (S_r)	0.08
• Pore size distribution (λ)	0.553
• Air-petroleum displacement pressure (P_d^{ao})	758 kg m^{-1} s^2
• Petroleum-water displacement pressure (P_d^{ow})	330 kg m^{-1} s^2
Fluid property	
• Water density (ρ_w)	1,000 kg m^{-3}
• Petroleum density (ρ_o)	740 kg m^{-3}

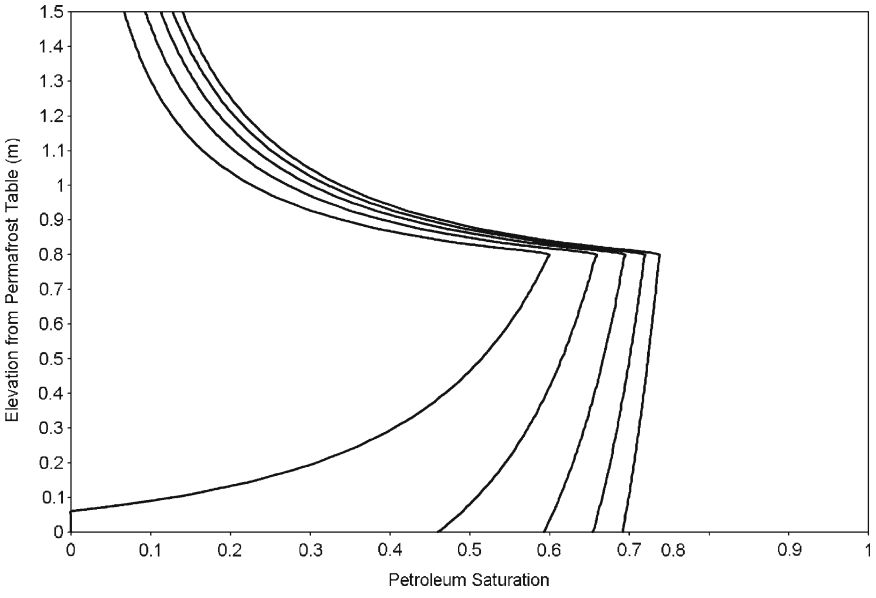

Fig. 18.1 Petroleum saturation with elevation from the top of permafrost. The petroleum saturation curve on the far left corresponds to a thick water-saturated zone where the top of the frozen layer does not interfere with the migration of the petroleum. Curves to the right of the bounding curve on the far left correspond to water saturated zone thickness prior to release of the petroleum to the active layer of 0.4, 0.3, 0.2, and 0.1 m respectively

The first notable result in Fig. 18.1 is the increase in the maximum value for petroleum saturation as the thickness of the saturated zone prior to the release of petroleum decreases. As shown in Fig. 18.1, the petroleum saturations between the top of the frozen soil layer and the elevation at which the maximum value of saturation is reached also increase, as the thickness of the saturated zone prior to release decreases. In addition, at the relatively thinner water-saturated thickness, the saturation of petroleum near the surface of the soil is greater in comparison to saturation values calculated for relatively deeper water saturation zone thicknesses.

While fluctuating water surface elevations, freeze and thaw cycling, and soil heterogeneity will most likely greatly affect the distribution of petroleum in the soil, these results indicate that petroleum release in active layers with shallow saturated zone thicknesses results in comparably greater initial mobility and, thus, a potentially wider lateral distribution of petroleum. This conclusion can be drawn due to the direct correlation between a fluid saturation and the relative permeability of porous media to that fluid. Petroleum as free product will also be distributed throughout the saturated soil thickness, leading to a widespread dissolved phase plume emanating from the source and subsequently little dilution of the dissolved phase plume. In addition, the volume of petroleum contained in a subsurface with a shallow saturated zone will most likely be greater than what would be predicted from models developed by Farr et al. (1990), Lenhard and Parker (1990), and Charbeneau et al. (1999).

18.3.2 Petroleum Releases to Frozen Active Layers

Migration of petroleum resulting from releases to frozen soils is significantly impacted by ice contained in the soil. At the minimum, ice present as pore ice will act as a solid, changing the pore geometry and thus the capillarity and permeability of the soil. In the extreme, the ground surface will be nearly impermeable, and downward migration will be minimal for the most part. Under these conditions surface flow will dominate, resulting in rapid and extensive spread of contamination upon release, though the higher viscosity at cold temperatures will inhibit lateral movement. In contrast to a release of petroleum to an unfrozen active layer, the increased exposure of the petroleum to the surface elements leads to greater losses of petroleum hydrocarbons by physical weathering (evaporation and photochemical oxidation).

Mackay et al. (1975) and Johnson et al. (1980) both conducted releases of crude oil to frozen ground in mature black spruce forests containing permafrost. Soils at both study sites were predominantly fine grain (silt). Results from sampling events shortly after each release in both studies indicated that overall there was minimal infiltration of the crude oil past the surface moss layer. Mackay et al. (1975) did document that infiltration of the crude oil did occur at spring thaw.

A laboratory study conducted by Barnes and Wolfe (2008) illustrates how pore ice in coarse soil impacts the movement of petroleum as the fluid infiltrates frozen

soil. Coarse soils are used extensively in the Arctic for foundations supporting infrastructure necessary for oil production as well as other activities, and are naturally present in Arctic and Antarctic terrain. In this study, petroleum was released to partially water-saturated sand that was frozen to −5°C. Two-dimensional petroleum flow through the frozen sand was approximated by packing moist sand between two vertical sheets of clear Plexiglas secured to a rigid frame and then freezing the entire unit. Once frozen, a volume of colored refined petroleum (JP 2) at a temperature of −5°C was introduced into the column and the progression of the petroleum was documented with time-lapse photography. Results from this study indicate that ice content far less than saturation can greatly affect the movement of petroleum, due to dead-end-pores created by ice forming in relatively smaller pore spaces, thus blocking flow paths. In addition, the formation of preferential flow paths results in deeper penetration of petroleum and unpredictable migration patterns. At the extreme, petroleum infiltration may be limited to the near surface soils due to high ice contents, as others have shown in field tests (Mackay et al. 1975; Johnson et al. 1980; Chuvilin 2001a).

Investigation of petroleum migration in frozen coarse soils in soil flumes can be taken one step further by investigating the infiltration of petroleum into a frozen heterogeneous coarse grain soil (Barnes and Adhikari, unpublished data). For this investigation, a layered soil was created in a soil flume with a layer of fine grain sand (1.3 cm thick) interbedded between coarse grain sand layers. The soil was then thoroughly wetted by introducing water to the top of the flume at timed intervals and allowing the water to drain through the sand layers. The flume was covered (to reduce evaporation) and allowed to drain for a sufficiently long enough time for gravity drainage to end. At this point, water in the pore space is held in the pore space by capillary forces at some residual level. The flume was then insulated on the sides and the bottom and placed in a cold room at −5°C to induce top-down freezing. Once frozen, colored JP2 chilled to −5°C was introduced to the top of the soil layer, and migration of the petroleum through the soil was tracked using time-lapse photography. The test was repeated in layered soil that was prepared in exactly the same manner but left unfrozen. Results from these tests are shown in Fig. 18.2.

The impact the fine grain sand layer has on the movement of petroleum through the frozen soil in comparison to the unfrozen soil is clearly evident in the images shown in Fig. 18.2. The fine sand layer in the frozen soil acts as a barrier to further downward petroleum migration. This result is due to the development of a capillary break between the fine grain sand and the underlying coarse grain sand. As water infiltrates and drains through a layered unsaturated soil, capillary breaks develop at the interface between relatively fine grain soil and underlying coarser grain soil, due to the comparably low relative permeability to water in the coarse grain soil in relation to the overlying fine grain soil. Low relative permeability in these cases is brought about by the comparably lower soil water content in this soil, owing to the larger pore dimensions and thus lower capillary forces in this layer. Once a capillary break develops, the low relative permeability in the underlying coarse soil restricts drainage of water out of the overlying fine grain soil, resulting in high water saturation in the fine grain soil. If a sufficient water saturation exists in the

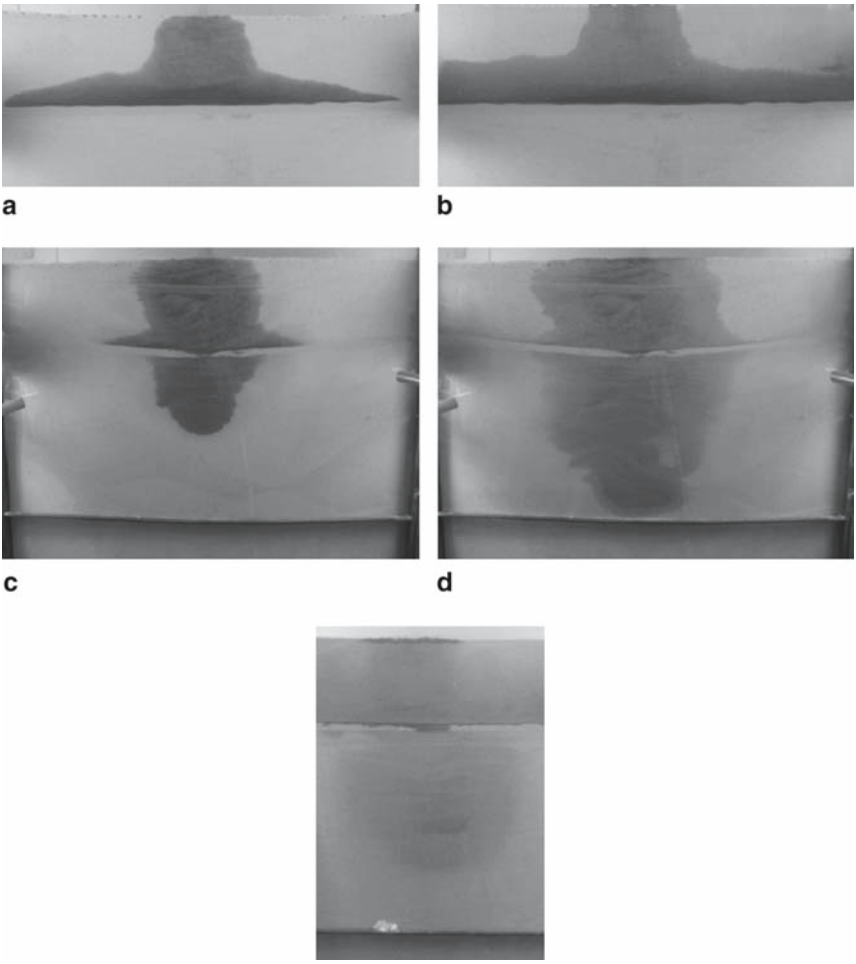

Fig. 18.2 Migration of petroleum through frozen (images **a** and **b**) and unfrozen (images **c** and **d**) layered soil. Images **a** and **c** were taken 1 h after releasing the petroleum to the soil. Images **b** and **d** were taken 1 day after releasing the petroleum to the soil. Image **e** was taken after the frozen soil from images **a** and **b** was thawed from the top down

fine grain soil prior to freezing (at least 91.7%), the pore space will be filled with ice once frozen, creating a barrier to infiltration of any liquids such as inadvertently spilled petroleum. During top-down thawing of the frozen sand in this investigation, the petroleum drained and redistributed as the thawing front advanced downward (Fig. 18.2). Some interference with the sides of the flumes was encountered, so the image has been trimmed to show the central portion of the redistributed plume. One will also note the extensive distribution of petroleum throughout the entire thickness of coarse sand above the thin layer of fine sand, which most likely developed through capillary movement of the petroleum.

In layered soil, the development of capillary breaks in frozen soil (ice-rich capillary breaks) as shown in Fig. 18.2 results in substantial increase in lateral petroleum movement upon the release of petroleum, in comparison to unfrozen soils and in comparison to frozen non-layered (homogeneous) soil (Barnes and Wolfe 2008). Others have noted the preferential lateral movement of petroleum in frozen soil Mackay et al. (1975). Damian Gore personal communications) the development of ice-rich capillary breaks may in part be the reason for these occurrences.

Ice has a substantial impact on petroleum distribution in frozen soils. Present in soil pores, ice impacts flow paths taken by infiltrating petroleum, resulting in extensive lateral distribution and possibly deeper penetration into the subsurface as petroleum seeks preferential preferential paths with relatively low ice contents. In layered frozen soils, complex distributions of petroleum will develop as infiltrating petroleum encounters soil layers saturated with pore ice. Segregated ice (ice lenses) formed in fine grain soils will also impact the flow paths taken by infiltrating petroleum by creating impermeable barriers to flow. During thawing, petroleum released to a frozen soil will redistribute as the properties of the porous media change and water is added through thawing ice contained in the soil and from infiltration from thawing snow and ice on the ground surface. In fine soils containing segregated ice, petroleum movement may be enhanced as the ice melts and petroleum flows through the relatively higher permeable soils where the segregated ice existed.

18.3.3 Influence of Freezing and Thawing Cycles on Petroleum Distribution

As is known, the freezing–thawing processes are attended by structure-forming processes which result in changes in soil properties, which in turns influence petroleum redistribution in the soil and its transformation, fractionating and formation of organic-mineral composition. Results from the experimental investigations of Chuvilin et al. (2001a, b) showed cryogenic expulsion of petroleum from freezing to thawing zone in several different freezing soils (Fig. 18.3). Barnes et al. (2004) showed with a mass balance that the primary mode of downward petroleum migration in a freezing soil is through ice formation in the pore space, resulting in displacement of petroleum out of the pore as the void is filled with ice. The resulting crystallization pressure is usually enough for petroleum displacement, due to nonpolar nature of the liquid leading to only slight connectivity with mineral particles.

Petroleum distribution in the pore space, composition of the petroleum, initial content in soils, and freezing speed all influence the efficiency of cryogenic expulsion; for example, in sandy soils the amount of petroleum expulsion into underlying unfrozen soil is more than in clay soils. A coefficient of oil expulsion can be used to quantify the efficiency of cryogenic expulsion. This coefficient is equal to the ratio of displaced petroleum to the initial petroleum content. The experimental developed relation of the coefficient of oil expulsion from freezing rate is shown in Fig. 18.4.

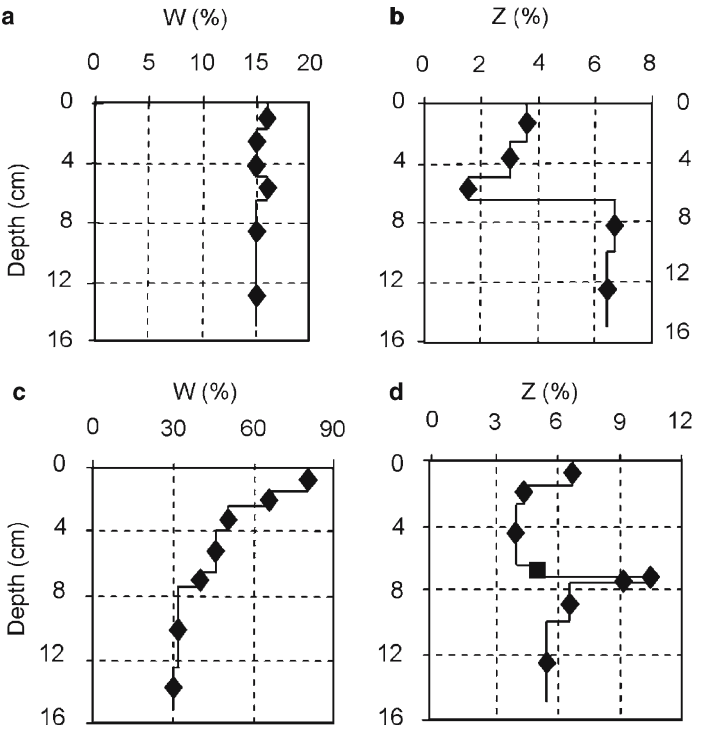

Fig. 18.3 Pattern of the water content (w) and petroleum content (Z) with height of soil freezing at −7°C. **a, b** Sand (initial water and petroleum content 16% and 5% respectively). **c, d** Clay (initial water and petroleum content 43% and 5.4% respectively)

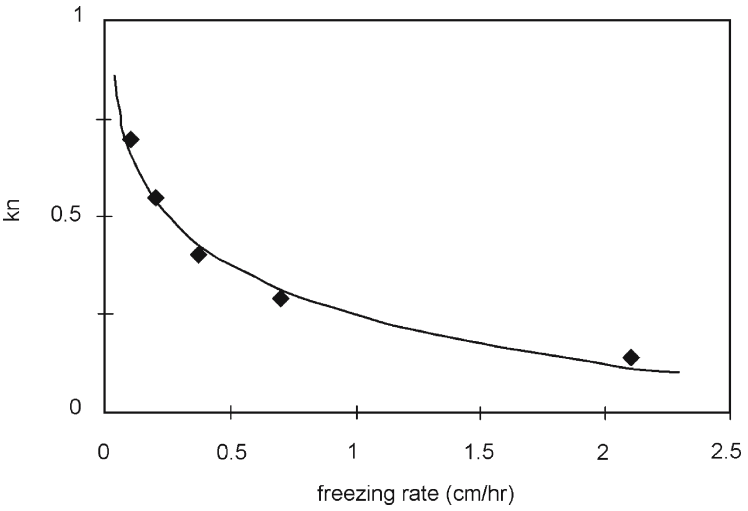

Fig. 18.4 Influence of the freezing rate on the coefficient of oil expulsion (Kn) in sand samples (initial water and oil content 16% and 5%, respectively)

The displacement of petroleum from the frozen soil to the unfrozen soil in the freezing soil sample shown in Fig. 18.4 was determined to be 70% from initial petroleum content under the favorable conditions of the test. In part, we can assume that the cryogenic expulsion is related to the petroleum "cryogenic metamorphization" — the separation of the more mobile petroleum hydrocarbon components from the petroleum. These hydrocarbons then migrate ahead of the freezing front. This process is poorly studied. One can suppose that naphthenes will be more mobile. Naphthenes are saturated hydrocarbons which don't display the associative properties under temperature reduction. In nature, the cryogenic expulsion may be the significant factor contributing to the petroleum's mobile formations and further dissipation. This process could have predominant influence in the active layer drained soils, where petroleum hydrocarbons partition into infiltrating water and migrate downward further into the soil horizon.

Laboratory studies of microstructure of freezing oil polluted sediments by White and Willams (1999) and White and Coutard (1999) have shown that their microstructure in frozen soil containing petroleum differs from frozen soil without petroleum under the same conditions. Soil structure change with addition of petroleum depends on the petroleum concentration in the soil. Relatively small concentrations of petroleum (below 200 ppm) promote the aggregation of particles and an increase in sediment porosity, resulting in an increase in hydraulic conductivity. Relatively high content of petroleum, on the contrary, prevents soil particle adhesion, resulting in sediment consolidation and an associated decrease in porosity and hydraulic conductivity. A four-fold increase in hydraulic conductivity (2.9×10^{-4}–9.8×10^{-4} cm s^{-1}) relative to uncontaminated material was observed where petroleum hydrocarbon concentrations were 50 and 200 ppm TPH (total petroleum hydrocarbons), in a silt subjected to four freeze–thaw cycles. When TPH values approached 1,000 ppm, hydraulic conductivity decreased from 2.9×10^{-4} cm s^{-1} (uncontaminated silt) to between 5.3×10^{-5} and 8.5×10^{-5} cm s^{-1}.

Grechischev et al. (2001a, b) investigated the influence of petroleum on the formation of segregated ice in fine grain soils. These researchers found that formation of ice lenses depends on composition and properties of the petroleum (crude oil in these studies) contained in the soil. Crude oil with relatively high hardening temperature (above 0°C) was found to reduce the ice segregation and cryogenic heaving of sediments. The influence of low-temperature crude oils is the opposite. Samples containing crude oil were characterized by the magnitude of the resulting cryogenic heaving. For crude oil with low hardening temperature (about −20°C), the value of ice segregation and cryogenic heaving was measured to be almost two times larger than for soils containing no crude oil (Grechischev et al. 2001a, b).

Recently, Haghighi and Ghoshai (2007) have used X-ray computed tomography (CT) to image petroleum (gasoline in this study) in freezing and thawing soils. The use of non-invasive imaging techniques allowed visualization and quantification of petroleum mobilization and displacement, and changes in petroleum blob morphology (volume, specific surface area and fractal dimension) in soil during freezing and thawing conditions. These researchers observed significant mobilization of petroleum from middle sections of the column towards the column end during

freezing. Petroleum volumes changed by up to 150% in certain regions of the column. Porosity distribution in the column changed with freezing, but porosity changes were reversible on thawing. The mean volume of the petroleum blobs increased significantly after freeze–thaw at the two column ends where petroleum migrated, and the blobs over the entire column became more spherical in shape with freeze–thaw. This research confirms redistribution of petroleum and its complicated transformation at freezing and thawing.

18.4 Migration of Petroleum into Permafrost

Petroleum hydrocarbons have been measured at depths of meters in permafrost (Biggar et al. 1998; McCarthy et al. 2004) even though petroleum migration into permafrost should typically be minimal, due to high pore-ice saturations in the upper few meters of these frozen soils. Presence of petroleum hydrocarbons in both these cases was attributed to free-phase petroleum movement through interconnected air voids in the frozen soil. These air voids may result from unsaturated compacted soil, fissures resulting from thermal contraction, or naturally occurring air voids in granular material (such as beach deposits) due to natural processes.

Frozen fine soils can contain unfrozen water at the soil surface boundary. Lacking pathways for petroleum to flow advectively into ice-rich permafrost, a possible transport mechanism is diffusion of petroleum hydrocarbons through the unfrozen water content. Aqueous phase diffusion is a relatively slow transport process in comparison to advection. The contribution this transport mechanism makes to moving contaminants into permafrost soils is most likely minimal. The role of diffusion in the movement of petroleum hydrocarbons into permafrost can be shown with a simple example. Consider the following solution to Fick's Second Law, with a constant concentration of a dissolved petroleum hydrocarbon at the top of a deep layer of permafrost that does not contain the petroleum hydrocarbon initially.

$$C_{\mathrm{w}}(z,t) = C_{\mathrm{w,o}}\, erfc\left(\frac{z}{\sqrt{4\alpha t}}\right). \tag{5}$$

In (5), $C_{\mathrm{w}}(z,t)$ is the dissolved phase concentration of the petroleum hydrocarbon in the unfrozen soil-water as a function of time and space, $C_{\mathrm{w,o}}$ is the dissolved phase petroleum hydrocarbon concentration in the soil-water at the top of the permafrost, z is the depth into the permafrost from the top of permafrost, α is the effective diffusion coefficient divided by the retardation coefficient, and *erfc* is the complementary error function.

For this example, assume that a release of petroleum has occurred in a permafrost region and that the water-saturated zone in the active layer above the permafrost contains benzene at a concentration that is equivalent to the product of the

compounds solubility and its mass fraction in the released petroleum. The retardation coefficient for benzene in this scenario is 8.55. The temperature of the permafrost is −3°C and the soil is comprised of Fairbanks Silt. From Tice et al. (1976) the unfrozen volumetric water content can be estimated to be 0.062. Assuming the soil to be ice-saturated in the region just below the top of permafrost, the porosity available for diffusion is equivalent to the volumetric unfrozen water content. The resulting effective diffusion coefficient is 2.6×10^{-11} m^2 s^{-1}. With these reasonable assumptions and considering diffusion as the only transport mechanism, after 10 years the concentration of benzene at a depth of 0.1 m into the permafrost from the top of the permafrost is only 2.2% of the initial concentration. Hence, under the conditions of this example, which are reasonable, movement of petroleum hydrocarbons into permafrost by diffusion is much too slow to be of concern.

18.5 Conclusion

Through laboratory and field studies we are beginning to gain a better understanding of how petroleum migrates through Arctic and Antarctic terrestrial environments. The presence of ice in soils found in these environments greatly influences petroleum migration at the time of release and during subsequent freezing and thawing cycles. Possibly the most predominant effect ice contained in the pore space has on the migration of released petroleum is the formation of preferential pathways, resulting in wider lateral petroleum distributions than would be expected in soils not impacted by extreme cold temperatures. Moreover, freeze and thaw cycles tend to increase the downward migration of petroleum and influence the distribution of disconnected petroleum blobs.

In addition to ice influencing petroleum migration and distribution, the typically shallow nature of the active layer and the resulting thin layer of suprapermafrost ground water impacts the vertical distribution of petroleum in the subsurface. In temperate climates with thick saturated zones, petroleum (as a free phase liquid) does not penetrate past the top few tens of centimeters of saturated soil. Given the thin nature of the saturated zone above permafrost, petroleum will distribute throughout the entire suprapermafrost saturated zone, resulting in dissolved phase plumes distributed throughout the entire depth of the saturated zone, and minimal dilution of the dissolved phase plume by uncontaminated ground water.

An understanding of these processes is necessary as petroleum-impacted areas of the Arctic and Antarctic are cleaned up over the next several decades. More study is needed, however. One of the main topics that require further attention is the validation of what is being measured in laboratory studies and described in theoretical studies against what is occurring in the field. Mackay et al. (1974a, b, 1975) as well as Johnson et al. (1980) provide well-described results from controlled field studies; however, these studies took place over 30 years ago. Laboratory and theoretical studies have focused our attention on influences that these past researchers may have not been aware of and thus not looked for during their studies. Additional

field studies will greatly improve our understanding of petroleum migration in these environments and improve our response methods.

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Chapter 19
Remediation of Frozen Ground Contaminated with Petroleum Hydrocarbons: Feasibility and Limits

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19.1 Introduction

Petroleum pollution is a significant problem in cold regions. We define cold regions as Arctic and sub-Arctic, Antarctic and sub-Antarctic, and alpine regions that exhibit permafrost or seasonally frozen ground (Filler et al. 2008b). Encountered in gravel pads, roads, and abandoned waste dumps, at remote air strips, research stations, and legacy military and mine sites, with fuel storage and dispensing facilities, and as leaked or spilled product along transport corridors (i.e., pipeline and roads), petroleum is persistent in and difficult to remove from frozen ground. Economic limitations on cleanup are associated with remoteness, access (where regulated), scant local resources, and complex logistics. Physical changes to ground brought on by sub-freezing air temperatures reduce microbial activity and alter physicochemical properties of petroleum (e.g., partial pressures — aqueous/vapor phase partitioning — and volatility). We are beginning to understand freeze–thaw effects and cryoturbation in cold contaminated soils (Biggar and Neufeld 1996; Chuvilin et al. 2001; Barnes et al. 2004; Bigger et al. 2006; Barnes and Wolfe 2008; Barnes and Biggar 2008).

Cleanup decision making is usually dictated by financial circumstances, regulatory pressure, perceived risks, and liability associated with lease responsibility or transfer of land ownership (Snape et al. 2008a). Ideally, a practical remediation strategy is chosen based on a feasibility study of alternatives, with consideration for site-specific conditions, and acceptable trade-off between cost and treatment duration. From the responsible party perspective, the cost–time relationship (Fig. 19.1) is often the single most important aspect of decision making in environmental cleanup. The regulatory perspective also considers human and ecosystem health to be of paramount importance. Irrespective of stakeholder perspective, the development of cost-effective and timely remediation strategies benefits all. Figure 19.1 illustrates cost–time relationships for developed soil treatments that have been used in cold regions.

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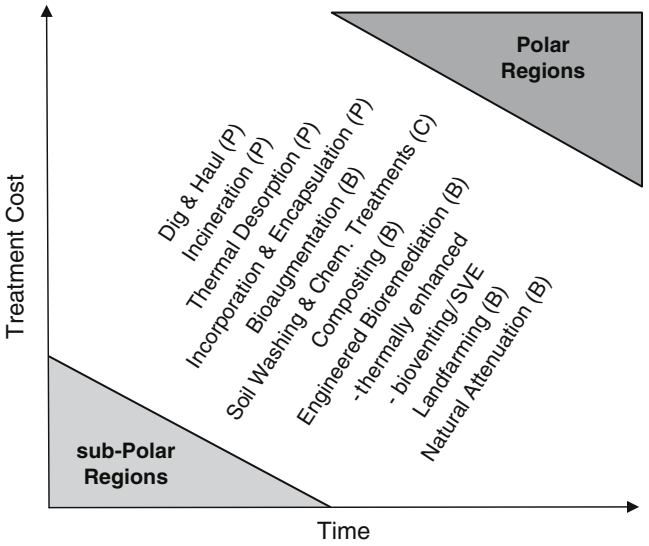

Fig. 19.1 Cost–time relationships for soil treatment methods used in cold regions. Note that cost and time for a treatment are greater in polar regions (after Snape et al. 2008a). The methods are classified as physical (*P*), chemical (*C*), and biological (*B*)

The methods identified in Fig. 19.1 are generally classified as physical (P), chemical (C), and biological (B). In cold regions, remediation of an equal volume of petroleum-contaminated soil is more expensive with physical than chemical treatments, and biological treatments are least expensive but require more time to meet cleanup standards. The exception is bioaugmentation, which can be as expensive as physical treatment because of the high costs of bioproducts and their repeated applications in order to achieve cleanup levels.

Groundwater treatment methods are less developed for cold region applications. Conventional pump and treat methods, air sparging, usually in conjunction with soil vapor extraction or bioventing, and use of oxygen release compounds have been used for sub-Arctic groundwater remediation. In the Arctic, in situ soil remediation has resulted in the degradation of petroleum in water. Emerging technologies that offer potential use in polar regions include permeable reactive barriers, two-phase partitioning bioreactors, and controlled release nutrients (i.e., bioremediation). Natural attenuation as a water treatment method is little understood, and is rarely considered for polar applications. Consequently, there is insufficient comparative information to infer cost–time trends for cold-climate groundwater treatment methods. A general distinction between the regions is that a subsurface water table is often encountered in sub-polar regions, that is more amenable to conventional treatment than is runoff or suprapermafrost water encountered above permafrost of contaminated sites in polar regions. For this reason, some methods identified in Tables 19.1 and 19.2 that are suitable for treating contaminated groundwater in the

Table 19.1 Ex situ soil remediation techniques with limitations to consider for application in cold regions

Technique	Description	Limitations
Ex situ soil treatment		
Physical processes		
Dig & haul	Excavation and transportation of contaminated soil to off-sight location for treatment	Warm season application; practical where roads and infrastructure exist; requires additional treatment; used in the Arctic and Antarctica; expensive — high transportation cost
Incineration	High-temperature thermal destruction of organic contaminants in soil	Practical Apr–Oct in the Arctic; few fixed-based incinerators available; treated soil is sterile; expensive — high energy and O&M costs; cost-prohibitive for Antarctic use
Thermal desorption	Low-temperature (600–900°C) thermal destruction of oxidizable hydrocarbons with low boiling points	Practical May–Sept. in the Arctic; mobile and fixed-base units available; treated soil is sterile; expensive — high energy and O&M costs; cost-prohibitive for Antarctic use
Incorporation & encapsulation		
	Excavation and use of contaminated soil at off-sight road or airport location. Contaminated soil is incorporated or encapsulated in roadbed, runway, or tarmac	Selective use during Arctic warm season where roads and airports exist; requires special permitting and work plans, long-term monitoring, and may incur long-term liability; can be expensive — high transportation cost; not yet considered for use in Antarctica
Chemical processes		
Soil washing	Reactor-based soil treatment whereby organic contaminants are desorbed from soil and treated via multi-stage processing.[a] Mobile washers use hot water, flotation and/or flocculation, and surfactants to remove contaminants	Practical in on-site mobile units May–Sept in the Arctic; separated contaminant residual requires additional treatment as potential hazardous waste; requires on-site power; cost-prohibitive and environmental risks high for Antarctic use
Chemical treatments	Contaminated soil is excavated and constructed as a lined heap or parceled into a liquid/solid contactor, for infusion with an oxidizer (e.g., peroxide, hydrogen peroxide, or ozone) or submersion in an alkaline/surfactant solution to liberate organic contaminants	Practical in on-site mobile units May–Sept in the Arctic; separated contaminant residual requires additional treatment and may be considered a hazardous waste; some contactors require on-site power; may have potential for Antarctic use

(continued)

Table 19.1 (continued)

Technique	Description	Limitations
Biological processes		
Bioaugmentation	Use of allochthonous microorganisms (naturally occurring, designer, or genetically engineered) to achieve bioremediation	Practical for enclosed soil treatment under controlled and optimized conditions; unregulated or semi-regulated for Arctic warm season use; comparable but much more expensive than commercially available fertilizers; science of consequence not yet established; prohibited in Antarctica
Composting	Prepared-bed treatment using a bulking agent, aeration, and heat generated from biological decomposition of organic contaminants under controlled (moisture, nutrients, and pH) conditions	Practical yet not well-developed for on-site treatment from May to Sept in the Arctic, and Dec to Feb in coastal Antarctica; beds must be enclosed and insulated for practical use in polar regions
Landfarming	Prepared-bed treatment using periodic tilling to degrade organic contaminants in soil.[b] Nutrient-enhanced landfarming induced volatilization and biodegradation to reduce hydrocarbon concentrations in soil[c]	Practical for on-site treatment from May to Sept in the Arctic, and Dec to Feb in coastal Antarctica; used in Arctic and sub-Arctic regions; highly dependent on environmental conditions
Thermally enhanced bioremediation	Biopiles engineered with mechanical systems (e.g., bioventing, nutrient infusion, and soil heating) and optimized to achieve bioremediation	Practical for on-site treatment of constructed biopiles from April to Nov in the sub-Arctic and Arctic; requires energy for mechanical systems; remote applications require alternative energy (e.g., solar, diesel-electric, fuel cell, hybrid) source; treatment regime can be manipulated independent of climatic conditions; not yet trialed in Antarctica

O&M operation and maintenance
[a]Lyman et al. (1990)
[b]Vidali (2001)
[c]Walworth et al. (2008)

sub-Arctic with vertical wells are not amenable to treatment of near-surface waters in the Arctic and Antarctica.

In this chapter, we discuss the feasibility and limitations of practical remediation of petroleum hydrocarbons in cold regions. We rely on lessons learned from cold-climate experiences in both hemispheres, and latest developments in contaminant

Table 19.2 In situ soil remediation techniques with limitations to consider for application in cold regions (see Table 19.1)

Technique	Description	Limitations
In situ soil treatment		
Chemical processes		
Soil washing	See Table 19.1 description	Not recommended for in situ use in cold regions without controlled containment and perimeter monitoring. Perceived to have negative impacts on soil ecology and permafrost. Cost-prohibitive for Antarctic use
Biological processes		
Bioaugmentation	See Table 19.1 description	Not recommended until science of consequence is established. Unregulated or semi-regulated for summer Arctic use. Comparable to but much more expensive than commercially available fertilizers. Highly susceptible to climatic conditions and temperature. Prohibited in Antarctica
Phytoremediation	The destruction, removal, or immobilization of soil contaminants brought about by plants and associated organisms	Potentially useful but not yet developed for use in cold regions. Highly susceptible to climatic conditions and temperature. Not practical for use in Antarctica
Soil vapor extraction with air sparging (SVE/AS)	Combination of vacuum enhanced recovery of volatilized hydrocarbons from the vadose zone, and use of air-injection wells to aerate and liberate hydrocarbons from groundwater	Amenable to granular soils (not fine silts and clays); used extensively from May to Oct in the sub-Arctic; not practical for use in the Arctic or coastal Antarctica with shallow contaminant zones; requires on-site energy; offers low O&M and monitoring costs; treatment durations highly variable and difficult to predict. Could be used with biopiles as ex situ engineered bioremediation in polar regions
Bioventing	The process of supplying (warmed) air to soil to stimulate aerobic biodegradation of contaminants[a]	Amenable to granular soils (not fine silts and clays); used from May to Oct in the sub-Arctic; used with thermally enhanced bioremediation in the Arctic; not practical for use in the Arctic or coastal Antarctica with shallow contaminant zones; requires on-site energy; offers low O&M and monitoring costs; treatment durations somewhat variable but more predictable than SVE/AS
Thermally enhanced		
Bioremediation	See Table 19.1 description	Biopiles can be constructed as in situ/ex situ structures; annual treatment from April to Nov in the sub-Arctic and Arctic; same energy requirements/limitations as with ex situ treatment; treatment regime can be manipulated independent of climatic conditions; maintaining permafrost integrity essential; not yet trialed in Antarctica

O&M, operation and maintenance
[a]Norris et al. (1994)

transport in freezing and frozen ground (see Chap. 18). Groundwater treatment is discussed as a consequence of soil treatment, with consideration for the relatively few documented field trials, and for emerging technologies.

19.2 Soil Remediation

The natural annual period of effective treatment at a contaminated site in the Arctic is 2–3 months, and 1–2 months in coastal Antarctica. In sub-polar regions, the treatment season varies up to 6 months. Generally, cold weather and freezing and frozen ground conditions dictate the treatment season and efficacy. However, engineered remediation can enhance conditions within the contaminant zone and extend the treatment season by a couple of months. Location may limit treatment options as a function of cost and manpower needs; restricted site access limits treatment options.

It is important to understand that no one treatment method is necessarily applicable to all cold regions. For example, soil vapor extraction coupled with air sparging is a viable in situ treatment strategy for a petroleum-contaminated site in the sub-Arctic, but it is not practical in the Arctic. Therefore, essential considerations for cold-climate environmental remediation are:

- A good understanding of (cold) temperature effects on physical and biological processes that occur in petroleum-contaminated soil
- A feasibility study of treatment alternatives
- Local (preferably site-specific) weather conditions
- Logistical requirements
- Thorough site characterization, and
- A treatability study (field trial is preferable).

The first two considerations aid decision makers with evaluating cost, (treatment) time, and risk. The middle two relate to treatment limitations, and influence site monitoring and treatment duration. The last two considerations aid the environmental practitioner with remediation design. A feasibility study evaluates treatment methods (Tables 19.1–19.4) for practicality, whereas a treatability study is then used to assess the efficacy of the chosen method under site-specific conditions. When biological treatment is relied upon, we opine that a treatability study is essential to effective treatment. Snape et al. (2008b) provide detailed discussions about the various treatability studies that are performed for bioremediation and landfarming projects.

The list of twelve soil remediation technologies identified in Fig. 19.1 represent those treatments that are routinely used in cold regions with success, or occasionally used with favorable results. These methods are identified and discussed in the following sections. Tables 19.1 and 19.2 summarize the methods as broadly divided between ex situ and in situ remediation techniques. Other remediation methods not discussed herein are either intuitively not applicable, have not been used, or may have been attempted but were unsuccessful in meeting cleanup standards.

19.2.1 Physical Treatment Methods

Dig and haul is not a treatment method per se, but rather the practice of excavating contaminated soil and hauling it to an off-site location for incorporation with other contaminated soil or treatment. The practice can be performed year-round (excavator with frost bucket in winter), is routinely used in the Arctic and Antarctica where roads and infrastructure exist, and is expensive. Permitting may limit the practice in sensitive environments (e.g., tundra, tundra lakes or marshes, and Arctic river and stream drainages) or when seed material for site reclamation is in short supply.

Incineration is a high-temperature treatment process that affords complete destruction of petroleum hydrocarbons in soil. Rotary kiln, multiple-hearth, or fluidized bed, fixed-base or mobile incinerators can treat up to 200 tons per day of petroleum-contaminated sand or gravel. Incineration is not amenable to cohesive soils, and can produce incomplete combustion products and residual ash that may have to be treated as hazardous waste. Thermal incineration is expensive as operating costs are high, and since few fixed-base facilities operate in the Arctic; mobile units incur high mobilization/demobilization and permitting costs. The Arctic operating season is from April to mid-October. Incineration is very expensive, and the environmental risks are considered too high for use in Antarctica.

Thermal desorption, or hot-air vapor extraction, is a thermal process that removes oxidizable hydrocarbons with low boiling points. The typical thermal treatment plant comprises mechanical pretreatment of the excavated soil, followed by thermal treatment in a rotary kiln, and with auxiliary treatment of exhaust gas. Operating temperatures for petroleum hydrocarbons range from 600°C to 900°C, with increased desorption rates realized at higher temperatures. The process is amenable to granular soils and non-cohesive silts. Removal efficiency is a function of temperature, residence-time volatility, and purge-gas velocity (Riser-Roberts 1998). Thermal desorption is cost-prohibitive for Antarctic use; the Arctic operating season is from May to early October. Mobile units are available that can be disassembled and reassembled at remote sites. However, expect mobilization/demobilization, permitting, energy, and labor costs to be high.

Incorporation and encapsulation refers to use of petroleum-contaminated soil in roads and airport runways and tarmacs. The contaminated soil is first screened to remove unsuitable fractions (i.e., aggregate greater than 5 cm across). Soil gradations are then performed on useable material to assess suitability for asphalt or tarmac incorporation, or highway or runway encapsulation. Highway/runway design specifications, potential long-term liability, wetlands issues, and required work plans limit use of this method in North America. Plan submittals include a runway or pavement structure design study and a leachate assessment or migration model. Modeling must demonstrate that contamination will not migrate off-site. The method is usually expensive because of high hauling costs between contaminated and use sites, and the additional expenses associated with plan submittals and long-term monitoring. Incorporation and encapsulation is more appealing when contaminated and use sites are located near each other.

19.2.2 Chemical Treatment Methods

Chemical treatment of petroleum-contaminated soil in cold regions is generally regulated as an ex situ process. Contaminated soil is excavated and constructed as a lined heap, or is stockpiled for parceling in a liquid/solid contactor. In heaps, oxidative degradation of contaminants occurs with infusion of peroxide (O_2^{2-}), hydrogen peroxide (H_2O_2), and/or ozone (O_3). In a contactor, petroleum is liberated by electro-oxidation with use of an oxidizing agent that has a high redox potential, or from soil that is submerged in an alkaline solution amended with a surfactant. In such applications, efficiency of petroleum reduction is a function of slurry temperature and oxidizing agent/surfactant concentration (Riser-Roberts 1998). Although chemical treatment of petroleum-contaminated soil is slow, removal efficiencies can exceed 90% with sands and gravels (Suthersan 1997). A disadvantage of chemical treatment of petroleum-contaminated soil is that residual waste may require additional handling as a hazardous material.

In situ soil washing is not recommended for use at petroleum-contaminated sites in polar regions where subsurface controls on migration are not in place. Furthermore, this method is cost-prohibitive (Antarctica) or may be tightly restricted for arctic use (Canada, United States, and Norway). However, although seldom used, ex situ soil washing is a useful method for petroleum-contaminated sites in cold regions. Ex situ soil washing is a chemical treatment method that is performed in a reactor. Modern reactors are two-stage (hot-water surfactant washing and flotation processes) or three-stage (with addition of biological treatment of leachate). An advantage of compartmentalized reactors is that each stage can be optimized independently. However, treatment efficiency is highly dependent on surfactant concentration; concentrations exceeding 2% can reduce slurry hydraulic conductivity and significantly increase the amount of residual waste (Riser-Roberts 1998). Mobile reactors are available for remote use, mixing could be added to the initial washing stage to accommodate peaty and some clayey soils, and a diesel–electric generator or solar collection system (Livingstone 2007) could power physical and chemical processes through the May to September arctic operating season. Furthermore, petroleum-contaminated water from the site could be used as the base washing liquid.

19.2.3 Biological Treatment Methods

It appears that petroleum-degrading microorganisms are encountered wherever petroleum is found in freezing and frozen soils (Aislabie 1997; Braddock et al. 1997; Margesin and Schinner 1998; Mohn and Stewart 2000; Whyte et al. 2002; Margesin et al. 2003). However, low temperatures (and other factors) limit microbial activity and therefore bioremediation potential (Delille et al. 2007; Rike et al. 2008). Environmental practitioners working in cold regions have developed enhanced remediation techniques to overcome cold-climate limitations in the treatment of petroleum-contaminated soils. Examples of enhanced remediation schemes

include soil vapor extraction combined with air sparging to simultaneously treat the vadose zone and underlying groundwater, and bioventing at low flow rates to treat unsaturated petroleum-contaminated soils at sub-Arctic sites. Most recently, *microbioventing* with small air-injection rods embedded in saturated peaty soil was trialed on sub-Antarctic Macquerie Island (Rayner et al. 2007). Yielding petroleum-hydrocarbon biodegradation rates of ~10–20 mg kg^{-1} per day, this method may be amenable to wet contaminated tundra sites.

Landfarming and composting, which are similar treatment methods, provide enhanced bioremediation without the use of mechanized systems. They are prepared-bed type treatments that require proper management of aeration, soil moisture and pH, nutrients, and temperature to affect biodegradation of organic contaminants in soil. Landfarming is an open-air process whereby petroleum-contaminated soil is amended with nutrients and then tilled in a lined *biocell*. A compost pile(s) can be constructed as a closed and insulated soil pile that is amended with a bulking agent (e.g., wood chips or sawdust) to enhance mixing and oxygenation, forced-air aeration, and nutrients over a smaller *footprint*. One treatability study for composting uses two or three small test piles of the soil to be treated, each amended with raw organic waste material (Savage et al. 1985). Once viable microbial populations are established in the *seed* piles, seed material is then blended with the target soil as compost piles to stimulate biodegradation. Where landfarming's biological processes are highly dependent on environmental conditions, composting offers greater control of important environmental conditions (Riser-Roberts 1998), and a closed and insulated compost pile generates heat that can potentially extend the period of annual treatment. Landfarming is now well-developed for cold regions and offers low-cost treatment of petroleum-contaminated soil in sizable biocells (Walworth et al. 2008). Ironically, composting is little used and has not yet been fully developed for use with petroleum-contaminated soils in polar regions.

Engineered bioremediation implies use of mechanized systems (e.g., forced aeration with pipe networks, heating and insulation systems, irrigation for nutrient delivery) coupled to increase biodegradation rates and improve overall bioremediation efficiency. An advantage of engineered bioremediation with a heating component for Arctic or Antarctic use is a longer annual treatment season. Environmental engineers have demonstrated that with engineered bioremediation, large volumes of petroleum-contaminated soils can be remediated to cleanup standards within two to three treatment seasons in Alaska (Filler et al. 2008a). Nevertheless, a treatability study should precede any cold-region bioremediation project. Engineered bioremediation efficiency is dependent on optimization of mechanized systems and biodegradation parameters in soil. The nominal Arctic bioremediation season is June–September, but can be enhanced by 3 months (May–November) with thermally enhanced bioremediation. Engineered bioremediation for use at remote Arctic sites is being considered, and will likely require an innovative energy scheme (e.g., hydrogen fuel cell, solar, or co-generated power) for implementation. A hybrid engineered bioremediation scheme is planned for use at Casey Station, Antarctica (Filler et al. 2006).

19.2.4 Bioaugmentation and Natural Attenuation

An interesting paradigm exists with bioaugmentation and natural attenuation (or intrinsic bioremediation) as soil treatment methods for cold regions. Bioaugmentation, while controversial and expensive, is being used because proponents report achieving cleanup in short order. On the other hand, there is a strong desire to use less expensive natural attenuation, despite knowing little about its viability in cold regions. It appears that irrespective of long-term treatment and liability, responsible parties regard low-cost remediation as highly desirable.

19.2.4.1 Bioaugmentation

Allochthonous, designer, or genetically modified or engineered microorganisms amended with an emulsion or fertilizer and an enzyme catalyst are sold commercially as bioproducts. Bioproducts have been used to remediate petroleum-contaminated sites in Alaska, Canada, Greenland, and Norway. Usually applied with repetitive tilling, practitioners claim dramatic results within a single treatment season. However, laboratory trials of various bioproducts, comparison trials of bioproducts with garden variety and arctic-blend fertilizers, and field studies, all without tilling, found that bioproducts underperformed or fared no better than the fertilizers (Venosa et al. 1992; Margesin and Schinner 1997; Whyte et al. 1999; Braddock et al. 2000; Thomassin-Lacroix et al. 2002). Bioproducts are considerably more expensive than commercially available fertilizers. With climate change, ecologists are now documenting competition and proliferation of advancing flora in boreal forests and taiga and tundra ecosystems. It stands to reason that soil ecology might also be susceptible to potentially invasive microorganisms; a better understanding of the consequences of using bioproducts in the environment must be established before their further use.

19.2.4.2 Natural Attenuation

At the edge of the cost–time relationship (Fig. 19.1) is natural attenuation, a passive remediation strategy that relies largely on intrinsic biodegradation processes by indigenous microflora. In contrast to bioaugmentation, natural attenuation is based on the principle of ubiquity proposed by Baas Becking (1934) that "everything is everywhere, but the environment selects". Degradative potential clearly exists for petroleum in cold regions, as evidenced by laboratory studies demonstrating petroleum degradation at temperatures as low as 0–7°C by bacterial isolates and soil consortia from Arctic, Antarctic and alpine locations (Whyte et al. 1997; Mohn and Stewart 2000; Yu et al. 2000; Eriksson et al. 2001; Stallwood et al. 2005; Margesin 2007). Furthermore, in Antarctic soils impacted by a 36,000 L fuel spill resulting in soil concentrations of 10,000–20,000 mg kg^{-1} soil, fuel

degradation from 40 L to 400 L per year were observed under ambient conditions (Snape et al. 2006). However, the majority of net natural attenuation was attributable to abiotic evaporative and dispersal processes (Snape et al. 2006). Other microcosm (Mohn and Stewart 2000) and field studies (Rayner et al. 2007) indicate that biodegradation rates in cold regions may be limited by oxygen and/or nutrient concentrations. By excluding environmental manipulations that biostimulate degradation, natural attenuation is a long-term strategy, yet is appealing for several reasons. Low cost is appealing to responsible parties. From a regulatory perspective, natural attenuation coupled with long-term monitoring (i.e., monitored natural attenuation) is attractive when disturbances from assessment and remediation pose greater risk to sensitive ecosystems than the contamination. In Alaska, natural attenuation is considered for long-term treatment at experimental contaminated sites with site-specific cleanup criteria and institutional controls. The science of monitored natural attenuation is not yet fully developed, and more long-term field studies are needed to evaluate the methods' effectiveness and duration for petroleum-contaminated soils in cold regions.

19.3 Groundwater Treatment

Various methods have been used, tested or proposed for the remediation of petroleum-contaminated groundwater in cold regions. These methods can be broadly divided into ex situ and in situ remediation approaches (Tables 19.3 and 19.4). The ex situ remediation methods that have been applied in cold regions are essentially variations of pump and treat, where the treatment component may include physical processes (e.g., oil–water separation, air-stripping), chemical processes (e.g., sorption to granular activated carbon), or biological processes (e.g., the use of bioreactors). In situ techniques for remediation of hydrocarbon-contaminated groundwater can also use physical techniques (e.g., construction of a barrier, air sparging), chemical treatment (e.g., permanganate addition), biological processes (e.g., bio-stimulation with nutrients), or a combination thereof. For example, air sparging can promote the physical removal of hydrocarbons from groundwater via volatilization, but it also can stimulate bioremediation by introducing oxygen to the water (i.e., biosparging). The use of natural attenuation to remediate a plume of dissolved hydrocarbons in groundwater relies on a combination of physical (e.g., dispersion), chemical (e.g., sorption to particle surfaces) and biological (e.g., degradation by microorganisms) processes to control the extent and impact of the plume.

One of the main advantages of conventional ex situ methods is that they have generally been demonstrated to be applicable under cold climate conditions. Some of the main disadvantages of the ex situ treatment methods are that:

- They tend to be relatively expensive
- They require a source of power to maintain pumping, which may be challenging at remote sites

- They require an on-site worker for ongoing operation, monitoring and maintenance activities, and
- In cold regions, they are seasonal because the extraction of groundwater for ex situ treatment is limited by freezing conditions that persist through much of the year.

There has been growing interest in the use of in situ treatment methods for treatment of hydrocarbon-contaminated groundwater. These methods offer potential cost-savings, largely because they may require little or no on-site power generation, they typically require limited operation and maintenance activities, and they can potentially be applied year-round. The main disadvantages of these in situ techniques are that:

- Some of these methods are in the developmental stage as emerging technologies, and
- Their applicability for cold regions is often not yet well established.

It is useful to consider some general statistics and trends in groundwater remediation, for all contaminant types and all climate regions, as reported by the United States Environmental Protection Agency (2007) for more than a thousand National Priority Sites:

- From 1982 through 2005, more than 90% of groundwater treatments used pump and treat methods.
- In situ groundwater treatment applications have been increasing, from none in 1982 through 1986 to a high of 31% in 2005.
- Applications of pump and treat alone decreased from about 80% before 1992 to around 20% after 2000.
- The use of monitored natural attenuation has been increasing, comprising almost half of all selections made in 2005. Monitored natural attenuation is the "reliance on natural attenuation processes… to achieve site-specific remediation objectives within a time frame that is reasonable compared to that offered by other more active methods…These in situ processes include biodegradation; dispersion; dilution; sorption; volatilization; radioactive decay; and chemical or biological stabilization, transformation, or destruction of contaminants" (United States Environmental Protection Agency 1999).
- The most common in situ technologies include air sparging, bioremediation, chemical treatment, permeable reactive barriers, and multi-phase extraction.
- Applications of in situ bioremediation and chemical treatment have increased significantly in recent years.
- In situ groundwater remediation applications generally have shorter operating periods than pump and treat remedies.

Though the above trends are not specific to cold-climate sites, they suggest that interest will continue and grow in testing and advancing in situ techniques for remediation of hydrocarbon-contaminated groundwater in cold regions.

Table 19.3 Ex situ groundwater remediation approaches with limitations to consider for application in cold regions

Technique	Description	Limitations
Ex situ groundwater treatment		
Physical/chemical processes		
Air stripping	Volatile organics are partitioned from extracted ground water by increasing the surface area of water exposed to air. Aeration methods include packed towers, diffused aeration, tray aeration, and spray aeration[a]	Requires on-site power and site crew for ongoing O&M; limited to warm season application
Carbon adsorption	Removal of hydrophobic organic contaminants from the aqueous phase to carbon (e.g., granular activated forms) by physical and chemical forces[b]	Requires on-site power and site crew for ongoing O&M; limited to warm season application
Phase filtration/ separation	Use of filter membranes and/or conventional oil–water separator to remove non-aqueous phase emulsions of hydrocarbons from water	Requires on-site power and site crew for ongoing O&M; limited to warm season application
Biological processes		
Constructed wetlands	Use of natural geochemical and biological processes inherent in an artificial wetland ecosystem to accumulate and remove contaminants from influent waters[a]	Cost of construction of artificial wetlands may be high; restricted to warm season operation; requires assessment and monitoring of impact to aquatic ecosystem
Bioreactors	A contained vessel in which biological treatment takes place[c]	Requires on-site power and site crew for ongoing O&M; limited to warm season application

O&M, operation and maintenance
[a]Van Deuren et al. (2002)
[b]Riser-Roberts (1998)
[c]Hazen (1997)

19.3.1 Ex Situ Treatment of Groundwater

Most methods for ex situ treatment of hydrocarbon-contaminated groundwater at cold-climate sites are conventional methods of pump and treat that are commonly used by engineering firms at warmer sub-Arctic sites. For general information about these conventional pump and treat methods, the reader is referred to overviews provided by Nyer (1992), Eastern Research Group, Inc. (1996), and Cohen et al. (1997). Documentation of applications of pump and treat in cold regions has typically been in the form of unpublished, proprietary reports for clients.

With some applications of pump and treat, a combination of physical, chemical and/or biological processes may be employed. For example, Mitchell and

Table 19.4 In situ groundwater remediation approaches with limitations to consider for application in cold regions (see Table 19.3)

Technique	Description	Limitations
In situ groundwater treatment		
Physical/chemical processes		
Air sparging	The injection of air below the water table in order to induce volatilization of contaminants into the unsaturated zone, which can be removed by soil vapor extraction[a]	Requires on-site power and ongoing maintenance; limited to warm season (with respect to subsurface temperature) for Arctic applications if contamination occurs in seasonally frozen active layer
Steam sparging/ flushing	Steam is forced into aquifer through injection wells to vaporize volatile and semivolatile contaminants, which are vacuum extracted from the unsaturated zone for treatment[b]	Requires on-site power and site crew for ongoing O&M; limited to warm season application
Chemical oxidation	Brings chemical oxidants (e.g., permanganate, H_2O_2) into contact with subsurface contaminants to remediate the contamination[c]	Limited by reactive capacity of added oxidant; may be compromised by unintended oxidation of non-target substances (e.g., sulfide minerals; natural organic carbon)
Hydrofracturing enhancement	Injection of pressurized water through wells to crack low permeability and over-consolidated sediments; cracks are filled with porous media that serve as substrates for bioremediation or to improve pumping efficiency[d]	Site has to be accessible by heavy equipment — generally applied as a short-term (one-event) technique in warm season; requires follow-up with another method
Multi-phase extraction	Simultaneous extraction of vapor phase, dissolved phase and separate liquid phase contaminants from vadose zone, capillary fringe, and saturated zone[e]	Requires on-site power and site crew for ongoing operation & maintenance; limited to warm season application
Treatment walls/ permeable reactive barriers	Barriers allow the passage of water while causing the degradation or removal of contaminants[d]	Limited by sorptive capacity of wall/barrier
Vertical contaminant barriers	Construction of vertical barriers such as slurry walls, grout curtains or sheet pile walls in subsurface to contain plumes of contaminated groundwater[f]	Barrier may be overtopped by groundwater flow if the annual average recharge rate exceeds the rate of evapotranspiration
Biological processes		
Intrinsic bioremediation	Unmanipulated, unstimulated, non-enhanced biological remediation of an environment; i.e., natural attenuation[g]	May be too slow for effective site remediation.

(continued)

Table 19.4 (continued)

Technique	Description	Limitations
Biosparging	The injection of air or specific gases below the water table to enhance bacterial activity for remediation[b]	Requires on-site power and ongoing maintenance; limited to warm season (with respect to subsurface temperature) for Arctic applications if contamination occurs in seasonally frozen active layer
Phytoremediation	The use of natural plants to remove contaminants through bioaccumulation or through enhancing biodegradation[a]	May be too slow for effective site remediation; limited to warm season; limited to applications for shallow water-saturated zones that are readily accessible to plant roots; limited to regions where plants can grow effectively; some jurisdictions may restrict use of non-native plant species
Bioslurping	Combines vacuum removal of petroleum hydrocarbon free product with in situ bioventing. Designed for removal of free-floating LNAPL on the water table as well as residual product in the vadose zone[a]	Requires on-site power and site crew for ongoing O&M; limited to warm season application
Biofiltering	Refers to treatment of groundwater via passage through a biologically active area in the subsurface[g]	Custom engineering design and installation may be expensive; requires ongoing subsurface monitoring; likely not practical for some settings (e.g., plumes in fractured bedrock)

O&M, operation and maintenance
[a] Riser-Roberts (1998)
[b] USEPA (2004b)
[c] USEPA (2004a)
[d] Van Deuren et al. (2002)
[e] US Army Corps of Engineers (1999)
[f] USEPA (1998)
[g] Hazen (1997)

Friedrich (2001) reported the use of a bioreactor, in combination with oil/water separation, air sparging, filtration and sorption by activated carbon. The site was Komakuk Beach in Yukon Territory, Canada, along the Arctic Ocean coast, where the mean annual air temperature is −11.4°C. Pump and treat was applied over two summers. Vacuum pumps were employed to extract fuel-contaminated groundwater along with free phase hydrocarbons via a multiphase extraction system. Following oil/water separation, the groundwater was treated in a series of two bioreactors to promote biodegradation of the hydrocarbons by indigenous bacteria. The first in the series, a fixed-film bioreactor contained polypropylene balls

as a growth medium, where groundwater was circulated, amended with urea and monopotassium phosphate as nutrients, and sparged with air. Next in series was a suspended growth bioreactor, where the groundwater was again aerated and circulated. After flow through a sedimentation tank, final treatment included bag filtration and adsorption via organically modified clay and activated carbon. Monitoring of the effluent from the first bioreactor indicated removal of 53–97% of benzene, toluene, ethylbenzene, and xylenes (BTEX) and 44–89% of the total petroleum hydrocarbons (TPH).

19.3.2 In Situ Chemical and Physical Treatment of Groundwater

Various in situ physical or chemical methods have been proposed or tested to manage or remediate hydrocarbon-contaminated groundwater in cold regions, such as:

- The construction of vertical barriers to restrict the flow of contaminated groundwater,
- The use of multiphase extraction and vacuum-enhanced recovery to remove both contaminated groundwater and free product, and
- The installation of permeable reactive barriers to sorb hydrocarbons dissolved in groundwater.

It appears that there is very limited published information that documents the success of such in situ physical/chemical applications in cold regions. Therefore, some of these methods should be viewed as emerging technologies, or as being in a research and development phase. For example, Hornig et al. (2008) reported the laboratory testing of three sorbent materials [MYCELX coated sand, granular activated carbon (GAC) and surfactant-modified zeolite (SMZ)], for capture of sparingly soluble hydrocarbons in water. The purpose was to assess these materials for their potential use in permeable reactive barriers in cold regions. Methods included batch sorption tests and various surface characterization techniques. On a mass basis, GAC was found to be the best sorbent at both 20°C and 4°C; on a surface area basis, SMZ was a better sorbent than GAC. Both sorbents had reduced adsorption efficiency at 4°C compared to 20°C.

19.3.3 In Situ Biological Treatment of Groundwater

In situ bioremediation of petroleum plumes in groundwater may involve “active” techniques to enhance the biodegradation of hydrocarbons, as outlined in Table 19.4 and below. In contrast, intrinsic bioremediation (i.e., natural attenuation) is a

"passive" approach that takes advantage of the unassisted natural biological processes that attenuate contaminants in groundwater, such as microbial degradation of petroleum hydrocarbons (Weidemeier et al. 1999).

19.3.3.1 Active in situ Bioremediation Techniques

Some applications of active in situ bioremediation at cold climate sites have been reported. Examples include:

- In situ biosparging with biostimulation (Soloway et al. 2001)
- In situ aeration with bacterial inoculation and addition of unspecified "biogenic" substances (Pawełczyk et al. 2003)
- Bioventing to treat both soil and groundwater (Barnette et al. 2005).

To date, most such reports have limited information regarding the final outcome (success or failure) and limitations of the remediation techniques employed. Also, some of the relevant publications (Carss et al. 1994; Shields et al. 1997; Pawełczyk et al. 2003) lack details regarding specific technologies or materials that were used. Consequently, there is a need to provide detailed case studies that conclusively demonstrate the applicability of in situ active bioremediation techniques for hydrocarbon-contaminated groundwater in cold regions.

Positive results were reported for some in situ bioremediation approaches, measured as disappearance or reduction of BTEX concentrations (Carss et al. 1994; Barnette et al. 2005), decline in TPH/oil concentrations (Carss et al. 1994; Pawełczyk et al. 2003), oxygen loss (Carss et al. 1994), and/or shrinkage of the plume (Shields et al. 1997). Some interpretations of field results (Curtis and Lammey 1998) or laboratory test results (Billowits et al. 1999; Cross et al. 2003) have suggested that in situ biostimulation with nutrients might enhance the bioremediation of the hydrocarbon-contaminated groundwater at cold climate sites. Following field investigations that included sulfate injection tests, Van Stempvoort et al. (2007a, b) suggested that it might be helpful to add sulfate as an electron acceptor to enhance in situ biodegradation of gas condensate plumes in groundwater in Western Canada, where groundwater temperatures were reported to range from 5 to 9°C.

19.3.3.2 Passive in situ Bioremediation

In a current review, Van Stempvoort and Biggar (2008) reported evidence that intrinsic bioremediation of petroleum hydrocarbons is a near-ubiquitous process in petroleum-contaminated groundwater in cold regions. This review noted that positive indicators for intrinsic bioremediation had been found at 16 sites in North America and 10 sites in Scandinavia and the adjacent Baltic region of Europe. Overall the annual air temperatures at these sites ranged from −12°C to 8°C. The contaminated subsurface media at these sites included sand or sand/gravel aquifers, fractured rock, gravel fill over peat, and silt/clay deposits. In these studies, the only

reported complete lack of intrinsic biodegradation of hydrocarbons in groundwater was a study of a plume of a complex mixture of contaminants at Fairbanks, Alaska (Richmond et al. 2001). However, other studies at Fairbanks (Westervelt et al. 1997; Braddock et al. 2001) have indicated evidence for significant intrinsic bioremediation of hydrocarbon plumes.

Perhaps the coldest site with published evidence for intrinsic biodegradation of a hydrocarbon plume in groundwater is one located near Barrow, Alaska (Braddock and McCarthy 1996). Here the air temperature averages −12°C annually, and rises above freezing for about 90 days each year. Soil and groundwater in sand and gravel deposits at this site had been contaminated by gasoline and jet fuel spills in the 1970s. Braddock and McCarthy (1996) reported that there were localized shallow groundwater flow systems in a thin unfrozen layer above permafrost, with groundwater temperatures ranging from 1.2 to 7.4°C. They found that 20 years after fuel was spilled, concentrations of BTEX remained elevated in the groundwater near the spill locations. Compared to groundwater outside of the plume, inside the plume the concentrations of oxygen and nitrate were lower and ferrous iron, sulfide and microbial populations were higher. These results suggested that aerobic and anaerobic microorganisms were associated with hydrocarbon degradation in the plume, utilizing oxygen, nitrate, sulfate and ferric iron as electron acceptors. Microcosm tests at 10°C indicated greater benzene mineralization potential in groundwater sampled from the plume than in groundwater sampled outside the plume. In other laboratory tests, hydrocarbon mineralization rates were stimulated by nutrient additions. Braddock and McCarthy (1996) reported that the strategy to manage the plume would incorporate intrinsic bioremediation, along with construction of a barrier to contain the plume by inducing permafrost mounding.

19.4 Conclusion

A number of soil treatment methods are available for cleanup of petroleum contamination in cold regions; few are permissible or practical for Antarctic use. In general, ex situ and in situ methods are limited to the warm season; longer annual treatment is possible inside warmed remediation enclosures. Considerable research and experience has shown that bioremediation offers the most acceptable balance between treatment cost and duration. Landfarming and thermally enhanced bioremediation are sufficiently developed technologies for seasonal use in the Arctic and Antarctica. With engineered bioremediation, it is possible to manipulate the treatment regime and lengthen the annual period of effective treatment at reasonable cost.

Further work is needed to establish the merits of monitored natural attenuation for soil treatment in cold regions. While this method offers a potential low-cost strategy for long-term remediation of sub-Arctic, sub-Antarctica and alpine petroleum-contaminated sites, its limitations and practicality are unknown. Effectiveness of this method is highly dependent on environmental conditions.

Definitive research is needed to establish the consequences of bioaugmentation use in the environment. We simply do not know anything about the potential impacts on soil ecology and the vulnerability of tundra and taiga to potentially invasive microorganisms. Bioaugmentation with non-indigenous or genetically modified/engineered microorganisms is banned in Antarctica, Norway, Iceland, and Sweden.

Various methods have been employed for ex situ treatment of hydrocarbon-contaminated groundwater (i.e., pump and treat) in cold regions. Because of their relatively high costs associated with continuous operation and maintenance, interest has grown in testing in situ treatment alternatives. However, in situ alternatives are either in the process of development as emerging technologies, or their applicability for cold regions is not yet well-established.

Methods that have been used for in situ chemical and physical treatment of hydrocarbon-contaminated groundwater in cold regions include the construction of vertical barriers to restrict the flow of contaminated water, and the use of multi-phase extraction and vacuum-enhanced recovery. The use of permeable reactive barriers to sorb hydrocarbons dissolved in groundwater has been proposed and laboratory- and field-tested.

Methods of in situ biological treatment that have been investigated for potential application in cold regions include biosparging, bioventing (to simultaneously treat soil and groundwater), and intrinsic bioremediation. The later method uses natural, unassisted biodegradation of hydrocarbons by microorganisms in groundwater. Evidence is growing that intrinsic biodegradation of petroleum hydrocarbons in groundwater may be viable for cold regions.

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Chapter 20
Application of Reactive Barriers Operated in Frozen Ground

Damian B. Gore

20.1 Introduction

Permeable reactive barriers (PRB) are a valuable weapon in the armoury of methods able to remediate contaminated ground. Developed first in temperate areas, reactive barriers are increasingly being installed in areas of freezing ground in both northern and southern hemispheres (Poland et al. 2001; Snape et al. 2001a, 2002). These environments create special challenges for the installation and operation of any remediation technology, and PRB are no exception. Particular challenges include ice formation in the barrier media, leading to temporary or permanent changes in barrier hydraulics, inefficient or ineffective reaction kinetics and exchange capacities at low temperatures, slow rates of biodegradation, and quarantine constraints on the choice of microbial agents able to be used in the degradation of organic contaminants. Despite these constraints, PRB offer particular advantages to the remediation of areas of freezing ground. Although expensive and requiring a good deal of site characterisation prior to installation, PRB are inexpensive to operate, and properly designed they can work for decades with only routine monitoring. As PRB work passively using the hydraulic gradient of the aquifer, they have low energy requirements. Barriers can be customised to suit the particular characteristics of the site, in terms of topography and the type of treatment required. These advantages are particularly important, given that many contaminated sites in areas of freezing ground are visited infrequently or seasonally.

The use of PRB is in its infancy, with few barriers having been installed earlier than 1994. Because of this youth, PRB require a greater monitoring effort to prove their success than other remediation methods. However, it is likely that, given the ongoing exploration and use of areas of freezing ground, more barriers will be installed in both hemispheres over the next decade. Whereas at present few barriers deal with petroleum hydrocarbons, it is also likely that dealing with this type of contaminant will increase in the future as hydrocarbon pollution in the Arctic increases

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(Poland et al. 2003). In Antarctica, the Protocol on Environmental Protection to the Antarctic Treaty stipulates that contaminated sites must be remediated unless doing so would result in a greater adverse environmental impact than leaving the sites untouched (Snape et al. 2001b). This favours the passive technology of permeable reactive barriers rather than more invasive remediation operations.

This chapter overviews the design of PRB, discusses the various types of reactive media used to treat a wide range of contaminants, and details the special considerations that should be given when installing, operating and decommissioning such barriers in areas of permafrost soils.

20.2 Introduction to PRB

20.2.1 PRB Function and Design

PRB can remove and retain contaminants (such as dissolved metals) travelling in groundwater, degrade some contaminant compounds (such as chlorinated hydrocarbons) directly, or facilitate their degradation (for example through the retention and biodegradation of petroleum hydrocarbons). Barriers can be used to both arrest the migration of contaminant plumes as well as remediate contaminated sites. Barriers should be optimised for specific site settings and aquifer and contaminant chemistries in order to achieve these functions. Critically though, the barrier must at all times maintain a greater permeability than the aquifer material. As a consequence, there is a broad range of designs; however, PRB are typically installed in one of two configurations.

The simplest is a continuous wall (also known as a reactive wall) extending across the width of the contaminant plume. This design is either keyed into an aquitard or impermeable substrate such as permafrost, bedrock or clay (Fig. 20.1a), or forms a “hanging wall” where the aquifer flows freely beneath the barrier (Fig. 20.1b). These wall barrier designs would be employed where the reactive media is inexpensive, or where construction of a funnel and gate system is not possible.

The alternative configuration is a “funnel and gate” design (Fig. 20.1c) consisting of impermeable walls such as sheet piling, plastic sheeting or a cement/soil–bentonite clay (+/− geofabric) slurry mixture, which direct groundwater through a permeable gate which is filled with reactive media (Starr and Cherry 1994; Gavaskar 1999). Multiple gate designs are possible, with up to four gates in a barrier in Colorado (Wilkin et al. 2002). Where soil water is focussed through a gate, the design of barrier permeability and residence times can assume great importance for the success of the remediation. Arrays of wells and injected media systems (particularly using nano-particle media) are also used (Naftz et al. 2002; Meggyes 2005). A range of PRB designs and case histories has been reviewed by Roehl et al. (2005a).

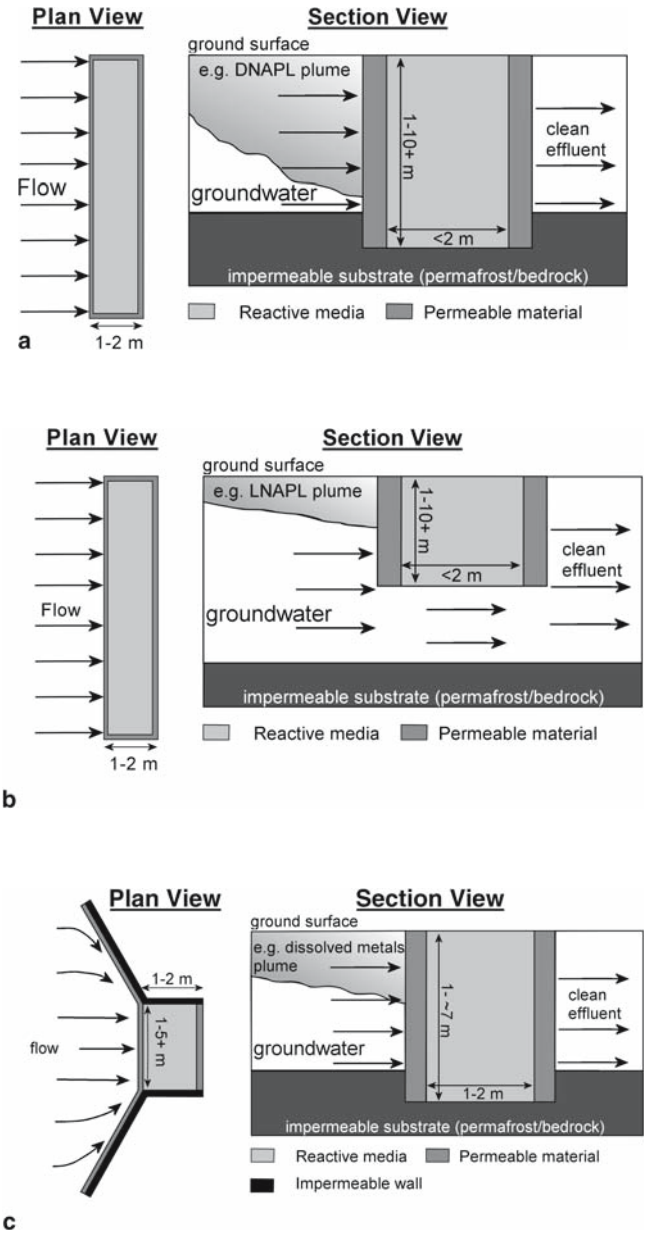

Fig. 20.1 Reactive barriers can consist of a continuous ("reactive") wall extending across the width of the contaminant plume. The wall is either keyed into an impermeable substrate (**a**) or forms a "hanging wall" where the aquifer flows freely beneath the barrier (**b**). Alternatively, a "funnel and gate" design (**c**) consisting of impermeable walls, directs groundwater through a permeable gate which is filled with reactive media

20.2.2 Types of Media

20.2.2.1 Adsorption and Ion Exchange

The calcium phosphate minerals *apatite* and *hydroxyapatite* have been demonstrated to immobilise metals including Zn, Cd, Pb and U, commonly via microbially mediated SO_4 reduction and precipitation (Fuller et al. 2002; Conca and Wright 2006; Martin et al. 2008). *Organic carbon* media function either via microbially mediated sulfate reduction and the precipitation of sparingly soluble metal sulfides (Benner et al. 1999), or with exchange of both metal ions and organic compounds (Shukla et al. 2002; Bulut and Tez 2007). *Granular activated carbon* is a particularly effective form of organic carbon material for absorption of both organics and metal ions (Ferro-García et al. 1988). Activated carbon can be made from a range of materials (Johns et al. 1998), but wood, coconut shell and coal precursors are amongst the more common forms. *Activated alumina* (Tripathi et al. 2004) and crushed rock, particularly *limestone* (Baker et al. 1998; Cravotta and Watzlaf 2002; Komnitsas et al. 2004; Turner et al. 2008), have also shown promise for the removal of inorganics including a range of metals, the former medium by adsorption and the latter by adsorption and the manipulation of aquifer pH, with resultant precipitation of metal salts. Perhaps the most widely used natural material used for the adsorbtion and ion exchange of contaminants is the *zeolite* family of minerals, particularly clinoptilolite (e.g. Ouki and Kavannagh 1997; Park et al. 2002). Zeolites may be modified, for example with coatings of surfactants such as hexadecyltrimethylammonium (Bowman 2003), to help target organic compounds such as those found in petroleum hydrocarbons, and anions such as arsenate and chromate (Haggerty and Bowman 1994; Ranck et al. 2005). The use of other promising types of sorbents has been reviewed by Bailey et al. (1999).

20.2.2.2 Oxidation

Organic contaminants can be degraded by oxidation. There is a range of potential reactive media that can be used in PRB, but most work by releasing oxygen which then mineralizes petroleum hydrocarbon contaminants. A PRB using this strategy has been installed in the Arctic (Lindsay and Coulter 2003).

20.2.2.3 Reduction

Reduction of dissolved species typically decreases their solubility, enhancing the possibility of saturated water chemistry and thus precipitation of sparingly soluble minerals and amorphous compounds containing the target contaminants. Reduction is also able to degrade some organic compounds including chlorinated solvents

(Tratnyek et al. 1997). The most common PRB media to achieve reduction is granular zero valent iron, which has been used for over a decade in North America to dissociate organic compounds (Johnson et al. 1996; Cheng and Wu 2000; Mu et al. 2004) and induce precipitation of metals (Puls et al. 1999). Organic materials have also been used to promote sulfate reduction and metal precipitation, both within PRB (Benner et al. 1997, 1999) and using naturally occurring carbon in aquifer plumes (Rectanus et al. 2007).

20.2.2.4 Microbial Degradation

Microbial activity is important for the removal of some inorganic and organic contaminants. Bacterially mediated reactions can assist in the removal of metals from groundwater via processes including sorption/ion exchange, precipitation onto live cells, attachment to dead microbial biomass and enzymatically driven redox or other chemical reactions (White et al. 1997; Benner et al. 1999; England 2006). The rate of biodegradation of organic compounds depends on their form, with aliphatic compounds generally being more readily degraded than aromatics. The addition of nutrients may be necessary to stimulate microbial growth (Walworth et al. 1997, 2007).

20.2.3 Mixed and Sequenced Multibarriers

Reactive barriers can be formed with a single type of filling, a mixture of media, or down-flow sequences of media. Barriers filled with a single type of media work effectively where all contaminants present are treated in the same manner. However, for some contaminants a mixture of media works more effectively, delivering optimal treatment while balancing hydraulic characteristics and cost. For example, mixtures of quartz sand, crushed limestone and iron and aluminum oxides were trialled to optimise the removal of phosphorus from wastewater (Baker et al. 1998). Variable mixtures within the barrier have also been proposed to reduce precipitation plugging at the entry face of the barrier (Mackenzie et al. 1999). Sequential barrier media can also form a treatment train approach, whereby different contaminants can be treated in turn. For example, it is possible to deliver nutrients in the upstream part of a barrier to encourage petroleum hydrocarbon degradation, a middle compartment to retain hydrocarbons for treatment, with an adsorbant at the back of the barrier to remove surplus nutrients from the treated effluent water. Further examples might be reductive degradation of an organic compound at the front of the barrier, with an adsorbant to remove dissolved metals at the rear of the barrier, or the treatment of a complex assemblage of organic compounds (Devlin et al. 2004; Kalin 2004; Bastiaens et al. 2005; Finkel and Bayer 2005; Ferguson et al. 2007). Conca et al. (2002) used a four-component barrier to remediate groundwater contaminated with radionuclides, other metals and nitrates.

20.3 Soil Materials, PRB Media and Barrier Operation Under Freezing Conditions

20.3.1 Grain Size

The movement of fine grained material within the barrier, either through washing of existing grains or the generation of new fine-grained particles via freeze–thaw shattering, can lead to zones of enhanced flow (leading to premature contaminant breakthrough) or clogging and reduced flow (leading to groundwater bypassing the barrier). A 1-h shake test of 24 zeolites in water showed a 1–18% loss of mass from the 65 to 40 mesh (212–420 μm) size fraction. Similarly, a 21-pore volume wash-through test using clinoptilolite zeolite revealed a 2.7–4.3% loss of mass, although these values were reduced by pre-washing to remove fines or calcining to increase grain strength (Zamzow and Murphy 1992). Low ionic strength solutions enhanced the loss of fines due to electrostatic repulsion, and shear by the slow-flowing (0.609 m per day) rinse water played only a minor role in particle movement (Abadzic and Ryan 2001).

The addition of freeze–thaw activity does not seem to create a very large additional loss of zeolite material. The <0.15 mm fraction of a 85% sand:15% ZeoponiX clinoptilolite amendment mixture, subjected to 20 freeze–thaw cycles, increased only 1.3% (Li et al. 2001) to 1.5% (Li et al. 2002) by mass, mainly at the expense of the >250 μm fraction. Similarly, clinoptilolite zeolite grains subjected to 60 freeze–thaw cycles under both drained and saturated moisture conditions, led to the <250 μm fraction increasing from 1 to 3% by mass (Gore et al. 2006). The significance of the creation of fines due to freeze–thaw activity and their redistribution or removal by flow can only be fully understood by the assessment of their three-dimensional arrangement and the resultant hydraulic characteristics of the media.

20.3.2 The Arrangement of Grains

When soils are frozen, water migrates toward the freezing front, and in doing so creates concentrations, or "segregations" of water ice and the entrained soluble components (Ostroumov et al. 2001). In doing so, ice lenses can form, depending on the grain size of the material, moisture content and rate and direction of freezing. The 9% expansion of water on freezing can displace soil particles, creating cryoturbation, frost heave, and the development of fissures and joints, particularly in silty clay soils (Eigenbrod 1996). On melting, ice lenses can transform into cavities, reducing the bulk density of the media and possibly creating macropores. The porosity of silty soil subjected to freeze–thaw cycling may increase with hydrocarbon content (White and Coutard 1999), but the void ratio of fine grained soils may also reduce under freeze–thaw (Chamberlain and Gow 1979). Small vertical cracks link together to form vertical polygons in clays (Chamberlain and Gow 1979), and

it may be that these cracks increase permeability in the vertical direction. However, an additional mechanism has been proposed to account for permeability increases in soil materials which do not exhibit cracking, and that is rearrangement of the clay particles within the voids defined by the sand and silt grain boundaries. Prior to freezing, loose clays lie in the voids, but as a consequence of the effective stress imposed by freezing, the clay particles rearrange into a denser packing and possibly also align themselves to create a greater permeability (Chamberlain and Gow 1979). Fine-grained materials can exhibit an increase in permeability of several orders of magnitude following freeze–thaw cycling (Eigenbrod 1996).

It remains unclear whether or not these effects also occur in coarser PRB media. Initial tests in the laboratory (Gore, unpublished data) show that the water-saturated bulk density of two types of clinoptilolite zeolite does not change with up to 50 freeze–thaw cycles, but the bulk density of a granular activated carbon (PicaCarb™, Pica Inc.) tends to reduce with repeated reorganisation of the grains by flotation. No evidence of long-lived cracking or macropore development was observed in the sand-sized PRB media. However, it is possible that rearrangement of finer particles in the voids may occur following freezing, increasing permeability in some places but decreasing permeability in other places where finer particles enhance the formation of pore ice (Fourie et al. 2007). Alternatively, freeze–thaw shattering may increase the total amount of fine particles, leading to a reduced permeability. X-ray computed tomography, whereby hundreds of X-ray images are used to construct a three-dimensional rendering of the grains and pore spaces with micron-scale resolution, holds great promise for the understanding of the interaction of contaminants and fluid flow, grain behaviour and the development of cracking and segregation ice in permafrost areas (de Argandoña et al. 1999; Torrance et al. 2008). Further studies of the hydraulics of contaminated soils and PRB materials are crucial to understanding the behaviour of PRB in areas of freezing ground.

20.3.3 Hydraulics of the Aquifer and Barrier Media

The permeability of the soil to both dissolved (e.g., metal ion) and free-phase (e.g., hydrocarbon) contaminants depends on the frozen and unfrozen moisture content of the media (Wiggert et al. 1997; Wolfe et al. 2003). Importantly for contaminant migration, the moisture content in both the soil and the PRB varies with depth from the surface, according to the history of wetting and the direction and characteristics of freezing. In particular, in areas (such as the high Arctic and Antarctic) where the soil is underlain by permafrost, the soil freezes both from the top down and bottom up, leading to greater water content and potentially ice saturation and the development of impermeable layers, at least seasonally, in the upper and lower parts of the active layer (Wolfe et al. 2003). There is an inverse relationship between permeability and ice content (Wiggert et al. 1997; McCauley et al. 2002). Frozen, water-saturated materials approach impermeability, a characteristic which has been

exploited for the creation of frozen soil barriers to contain hazardous materials (Andersland et al. 1996). However, Chuvilin et al. (2001) found some migration of oil, even into saturated, frozen soil, possibly as a result of movement along cracks that develop in the soil during freezing (Chamberlain and Gow 1979; Biggar et al. 1998). Cracking enhances the infiltration of water (Benson and Othman 1993) and other fluids, and so can act as an important control on the direction and flux of contaminants in soil and PRB media. Lateral patterns of contaminant movement can also be controlled by capillary suction (Barnes and Filler 2003).

The redistribution of fine-grained material by washing can create significant changes to the hydraulics of barrier media, depending on the ratio of the size of the immobile:mobile grains. If the ratio is <10, where immobile grains are <10 times larger than the mobile grains, an impermeable layer can develop in the barrier media or the aquifer dowstream. If the ratio is 10–20, hydraulic conductivity can be impaired, and if the ratio is >20 only slight changes to hydraulic conductivity (K) might occur (Abadzic and Ryan 2001). These ratios may change depending on the abundance of the mobile fraction, and the size and shape of the media.

20.3.4 Microbial Activity and Fertiliser Management

Biodegradation of petroleum hydrocarbons occurs in cold areas (e.g. Margesin and Schinner 2001), even at temperatures below freezing (Rike et al. 2003), although the rate of microbially enhanced remediation of contaminants is constrained in polar regions compared with temperate regions. In Antarctica, the Antarctic Treaty (1961; and the Agreed Measures for the Conservation of Antarctic Fauna and Flora 1964; for a reference see SCAR 2008) prohibits the importation of non-indigenous species, which includes microorganisms. The discovery and encouragement of indigenous degrading microbial populations has assumed great importance for the biodegradation of fuel spills (Braddock et al. 1997; Aislabie et al. 2000; Ferguson et al. 2003). In the Arctic, it is possible to innoculate hydrocarbon-contaminated soils to encourage biodegradation (Mohn and Stewart 2000). Of broader significance is the correct fertilisation levels, particuarly of nitrogen compounds but in some cases also phosphorus (Mohn and Stewart 2000; Walworth et al. 2001), to stimulate biodegradation rates. It is now well-established that over-fertilisation suppresses microbial activity and inhibits biodegradation of petroleum hydrocarbons. The mechanism is microbial stress due to osmotic soil water potential depression (Braddock et al. 1997; Walworth et al. 1997, 2007), which occurs as the soil dries and the ionic concentration of nutrients in the soil water increases. This effect is enhanced by the desiccation that occurs during every freeze–thaw cycle in areas of freezing ground. Thus while the addition of nutrients is important to enhance biodegradation, it is crucial that the correct applications are used, otherwise efforts at remediation will be hindered. Controlled-release nutrient sources such as nutrient-loaded zeolites and encapsulated fertilizers are able to release nutrients slowly, helping to prevent over-fertilization and allowing nutrient release during periods

when the contaminated site is unattended, which is important for reducing the operational costs of barriers in remote areas. However, encapsulated fertilizers may shatter due to the effects of freeze–thaw, particularly when moistened (Gore and Snape 2008), and this should be assessed prior to the use of controlled release nutrient sources in the field.

20.3.5 Thermal Considerations

The arrival of thaw in areas of frozen ground is a time when the PRB must be ready to accept surface and subsurface flow. In order to do so, the barrier media must be unfrozen and permeable in advance of the contaminated catchment. The barrier should be designed so that this thaw occurs. For example, if any metal used in the barrier is in contact with permafrost, then heat could be conducted downwards, chilling the barrier and delaying thaw of the media. Alternatively, thaw might be enhanced via passive solar heating of the barrier surface. Snow-lie is an important aspect of thermal management of the barrier. Modelling of the thermal characteristics of the barrier and reactive media should be part of barrier design prior to installation, and if required, heat trace and insulation of the barrier base may need to be installed to ensure barrier thaw prior to runoff generation from the aquifer. Basal insulation also helps keep the barrier keyed into the permafrost. Ideally, at the end of the melt season, the PRB should drain freely or be pumped dry, to help prevent segregation ice formation during the winter.

Temperature is an additional variable in the efficiency of contaminant capture by PRB media. Zeolites exhibit reduced exchange kinetics and capacities for metal ions at 2°C compared with 20°C (Woinarski et al. 2003, 2006), and similarly, activated carbon and surfactant modified zeolite were found to have reduced adsorption efficiency at 4°C compared with 20°C (Hornig et al. 2008). The difference in sorption behaviour with cold temperatures depends on the hydrocarbon (Hornig et al. 2008). These constraints imposed by low temperature need to be integrated into barrier design in order to treat contaminant plumes effectively. In particular, the distance of the barrier in a down-hydraulic gradient direction is critical for the duration of interaction of the contaminant plume with the media.

20.4 Long-Term Behaviour of PRB

20.4.1 Degradation of PRB Performance

The effective life of PRB depends on a complex interplay of degradation of the reactive media, either by exhaustion or coating, changes to hydraulic characteristics due to clogging, biofouling, channel formation or the production of gases. The coating of reactive surfaces with precipitates is a limiting factor on the long-term

performance of some PRB (Kamolpornwijit et al. 2003). For example, the ability of granular zero valent iron to reductively degrade trichloroethene is compromised in the presence of permanganate, which induces insoluble precipitates and oxide coatings (Okwi et al. 2005). Clogging of pores by precipitates, with the reduction of hydraulic conductivity in barrier media, was noted by Mackenzie et al. (1999), Kamolpornwijit et al. (2003) and Simon and Biermann (2005), but not by Okwi et al. (2005). Gavaskar (1999) reported no significant degradation of performance following 5 years' operation of a zero valent iron barrier in Canada. Wilkin et al. (2002) and Lai et al. (2006) noted a loss of porosity but not of hydraulic performance, indicating that the onset of clogging is specific to the size of the granular materials and the chemistry of the aquifer being treated, and as a consequence feasibility tests of the long-term performance of barrier systems should be conducted using site groundwater supplemented by numerical modelling (Blowes et al. 2000; Jeen et al. 2007). The types of minerals precipitating can show zonation within the barrier; Li et al. (2006) found that carbonates dominated on the upstream side of a zero valent iron barrier, and that ferrous hydroxide dominated on the downstream side. These spatial patterns will control the pattern of clogging and ultimately the nature and location of barrier failure.

The accumulation of gas in barrier media as a consequence of microbial activity or carbonate dissolution can reduce hydraulic conductivity over time (Oberdorfer and Peterson 1985; Soares et al. 1991; Schipper et al. 2004; Williams et al. 2007), although there is a possibility of hydraulic recovery as bubbles migrate (Fryar and Schwartz 1998). Barriers that support naturally occurring microbial activity, or in the case of petroleum hydrocarbons, are supplied with nutrients in order to encourage microbial growth and activity, can be prone to biofouling or bioclogging. The growth of biomass can lead to changes in the hydraulic conductivity of the media, causing reduced residence time in the contaminated aquifer as well as parts of the barrier (Scherer et al. 2000; Thullner et al. 2004; Seki et al. 2006). Because bioclogging might only occur in parts of the barrier, the average hydraulic performance may not be affected, and as a consequence the reduction in treatment efficiency may not be detected (Seki et al. 2006). The period to the onset of biofouling will depend on the particular site water quality, particularly in terms of dissolved nutrients, contaminants and type of barrier media. A zero valent iron barrier exhibited no sign of biofouling following 6 months operation with a trichlororethene plume (Vogan et al. 1999), although few long-term studies exist to determine over what time frame biofouling is likely (Kalin 2004). Roehl et al. (2005b) contains a range of case studies of the long-term performance of PRB.

20.4.1.1 Extrapolation from Modeling Experiments

Permeable reactive barriers are a relatively young technology, and long-term studies of their behaviour are few. As a consequence, modelling can offer valuable insights into barrier performance over longer times. Model simulations of

barriers with different hydraulic conductivity and thickness indicate that flow will be greatest (and thus residence time the least) around the edges of the gate of a barrier, and flow will be least in the centre of the gate (Benner et al. 2001; Painter 2004). Increasing barrier thickness exacerbates this effect by enhancing flow convergence. Increasing the hydraulic conductivity of the barrier increases convegent flow and water flux, but does not change the edge flow enhancement (Benner et al. 2001). Heterogeneities in aquifer hydraulic conductivity are transmitted through homogeneous barrier material, but this effect is moderated with increasing barrier thickness. Further model simulations indicate that a few localized high hydraulic conductivity layers are more effective at introducing heterogeneous flow in the barrier than smaller, better-distributed high hydraulic conductivity layers (Benner et al. 2001). These simulations have implications for the performance of barriers operated in areas of freezing ground, particularly where ice lenses develop within the barrier or the aquifer upstream or downstream. Spatial heterogeneity in hydraulic conductivity within both the barrier and aquifer control the pattern of water flow (Gupta and Fox 1999; Benner et al. 2001). The hydraulics of the aquifer control the flux of water and contaminants within thinner barriers, whereas thicker barriers are more sensitive to inhomogeneities within the barrier itself. In either case, though, contaminant breakthrough will most likely occur at localized zones of high-velocity flow, which includes the edges of homogeneous barriers. Elongating the funnel in the down-flow direction (termed "velocity equalization walls"; Christodoulatos et al. 1996) reduces this enhanced edge flow. An alternative modification to the velocity equalization walls is non-uniform barrier thickness in the down-flow direction, which places thicker barrier media in areas of enhanced throughflow, in order to achieve the desired residence time throughout the barrier (Painter 2004). A further design enhancement is the downwards extension of the funnel wall, which reduces vertical capture of groundwater flow (Painter 2004).

20.4.2 Additional Challenges in Areas of Freezing Ground

Additional challenges exist for the long-term use of PRB in areas of freezing ground. The catchment, plume and the barrier may melt out at different rates in different locations, leading to local, temporary mounds in soil water tables (see Daniel and Staricka 2000) which create changes to aquifer permeability or the direction of the hydraulic gradient or permeability, potentially bypassing barriers in areas of low gradient.

The hydraulic conductivity (K) of the barrier and upstream area is critical for remediation success, for if the hydraulic conductivity of the aquifer is greater than the barrier, then water will pond against or flow around the barrier (Benner et al. 2001) leading to contaminants bypassing the treatment. To avoid this, barriers are designed to have, at installation, a greater hydraulic conductivity than the contaminated zone upstream. The maintenance of this relative hydraulic conductivity is essential, and contaminant retention or treatment may be adversely affected

by any process which increases *K* of the contaminated upstream zone, decreases barrier *K* or causes barrier *K* to become heterogeneous, with the development of preferential flowpaths.

Disturbance by fauna is an additional hazard. In the Arctic, three barriers installed at Resolution Island, Nunavut, to treat PCB contamination have culverts placed over them to guard against disturbance by polar bears (*Ursus maritimus*; Poland et al. 2001). Interference by fauna in Antarctica is less likely, but at coastal Davis Station for example, Southern elephant seals (*Mirounga leonina*) occasionally crush piping and service lines, and any barriers installed there or at similar locations will need to be protected from disturbance.

20.4.3 Monitoring and Decommissioning

Reactive barriers are installed to prevent the spread of groundwater contamination. During operation, effluent from an operating barrier should be sampled routinely to ensure that barrier failure and contaminant breakthrough has not occurred. Barriers can fail for a range of reasons, including the development of preferential flowpaths with resultant reduced residence time, freezing damage to the media (either grain shattering or the development of macropores), media saturation, exhaustion, gas clogging and biofouling. For compounds which are reductively degraded by zero valent iron (e.g., trichloroethene), degradation products such as vinyl chloride should also be monitored. Routine water chemistry (pH, Eh, dissolved oxygen, water temperature) provides indications of barrier operation, and may provide early warnings of improper barrier operation. Datalogging is particularly important for barriers in remote areas where visits are infrequent or seasonal. Tracer tests are useful to assess the flux of water and contaminants through the PRB, as well as to measure the residence time of water within the reactive zone.

Decommissioning barriers may be necessary once they are no longer needed, or when the media needs replacement. Some barrier types can be left in the ground, where there is no environmental harm in doing so. For example, adsorbents used to trap petroleum hydrocarbons and allow biodegradation may be left intact unless the barrier has accumulated recalcitrant compounds which have resisted biodegradation. Alternatively, barriers which trap and accumulate contaminants such as metals need to be removed, to prevent later desorption and remobilisation of the contaminant. Where there is reason to remove a barrier from areas of permafrost soils, there must be a mechanism for separating the base of the barrier from the frozen ground. This may take the form of the reactive media being held within a rigid cage with lifting hooks allowing extraction, a cage placed on a sacrificial layer, or a heat trace to melt the base of the barrier out. Alternatively, the barrier media may need to be removed using an excavator with a frost claw. Consideration of the decommissioning of a PRB in permafrost soils needs to be incorporated into the design stage.

20.5 Conclusion

PRB are a passive remediation technology for groundwater contaminated with dissolved nutrients and metals, petroleum hydrocarbons and chlorinated organic compounds. Since PRB have low energy use and relatively low cost of installation and operation, they are particularly well-suited for use in remote areas. The number of contaminated sites in areas of frozen ground will continue to increase with ongoing human occupation, and the need for low-cost remediation technologies such as PRB will increase over time. Freeze–thaw cycling creates a significant challenge for the long-term operation of PRB, particularly with respect to changes in grain size and hydraulic performance of the reactive media. PRB presently installed in polar regions are being monitored for long-term performance, and promising new materials and barrier designs are being assessed for their application to a wide range of contaminants in areas of frozen ground.

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Part VI
Permafrost on Earth –A Model for Extraterrestrial Habitats

Chapter 21
Terrestrial Permafrost Models and Analogues of Martian Habitats and Inhabitants

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21.1 Introduction

Hard data indicate that biota survive over geological periods at subzero temperatures within the terrestrial cryosphere — ice sheets and permafrost. In such environments, dehydration leads to a considerable decrease of biochemical and metabolical activities. This allows the survival of ancient microbial communities that can physiologically and biochemically adapt much better in the cryosphere than in any other known habitat. The long-term subzero temperature regime of the cryosphere is not a limiting but a stabilizing factor. Organisms adapted to such balanced conditions represent a significant part of the biosphere, the cryobiosphere. Their ability to survive on a geological scale forces us to redefine the spatiotemporal limits of terrestrial and extraterrestrial biospheres.

Most planets of the Solar system, as well as their moons, asteroids, and comets, are of cryogenic nature, and the cryosphere is a common phenomenon in the cosmos. This is why the cells, their metabolic byproducts and bio-signatures (biominerals, bio-molecules and bio-gases) found in the Earth's cryosphere provide a range of analogues that could be used in the search for possible ecosystems and potential inhabitants on extraterrestrial cryogenic bodies. If life ever existed on other planets during the early stages of their development, then its traces may consist of primitive cell forms. Similar to life on Earth, they might have been preserved and could be found at depths within the ice or permafrost.

Most intriguing are the traces of past or existing life on Mars; these traces are of interest due to upcoming missions. Mars is the fourth and outermost Earth-like planet from the Sun, with an orbit between the Earth and the belt of asteroids. The orbits of both Earth and Mars are located in an intermediate position between Mercury and Venus, which are close to the Sun and therefore dehydrated, and the planets of the Jupiter group, mostly composed of volatile hydrogen, methane, and

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water. Due to such an astronomical location, Mars is known to be the only solid planet that, similar to the Earth, contains abundant water supplies that form the hydrosphere (Kasting 2003; Baker et al. 2005). The existence, quantity and phase of water on Mars during geological history and at present play a leading role in the life-searching theory based on comparison of terrestrial and Martian conditions. The exploration of Mars by spacecrafts started in 1962 and has included seven Soviet, one European, and 13 US missions. The information collected by these vehicles formed the general view of the Martian hydro- and cryospheres, and the "Mars Odyssey" observations of neutron fluxes that found water in the subsurface layer (Boynton et al. 2002) indicated Mars a "water reach" planet, where surface water mostly exists in the form of ice due to subzero temperatures. Similar to the Earth, these spheres unite into one at subzero temperatures, and the underground water represents only hydrosphere at depths below the zero isotherm.

Because of unfavorable factors, such as high irradiation intensity (~300–500 μGy per day), absence of water etc., life is unlikely to exist on the surface, and no terrestrial habitats duplicate Martian conditions. Anderson et al. (1972) and Cameron and Morelli (1974) first advanced the idea of using terrestrial permafrost analogues, and this chapter considers these analogues as a bridge to possible Martian life forms and shallow subsurface habitats where the probability of finding life is highest. Since there is a place for water, the requisite condition for life, the analogous models are more or less realistic.

21.2 Martian Hydrosphere and Cryosphere

21.2.1 History of Water on Mars

Different relief forms originating probably from liquid water activity have been observed on the Martian surface: treelike systems of fluvial valleys morphologically similar to terrestrial river systems, great mega outflow channels without inflows, which have no terrestrial analogues, and thin gullies on crater walls. Dating of these forms based on crater density shows that valley systems cut through only the oldest, most cratered surfaces of the Noachian epoch, 4.6–3.9 billion years old. It was first proposed by Sagan et al. (1973) that treelike valleys were generated by erosion of superficial run-off, and hence indicate the existence of dense atmosphere and precipitation. Thus, in the Noachian epoch Martian conditions were most similar to the Earth: precipitation fall-out and superficial run-off were formed, and constituted numerous river systems.

The mega outflow channels cut through the less cratered, i.e., younger rocks of Hesperian age, 3.9–1.5 billion years. From the beginning of the Hesperian epoch, the heating of Mars by meteorite bombing began to reduce. As a result, ground freezing started and permafrost formation took place. From this time on, Martian conditions favored a permanently frozen envelope. Geological premises for the appearance of liquid water on the surface arise only in places where permafrost was

melted through by magma as a result of tectonic and volcanic activity, in places with high concentrations of water-soluble salts in rocks, or due to hydro-explosions; in this way, huge canyons were formed (Carr 1979).

In the Amazonian epoch (1.5 billion years ago) Mars lost tectonic activity, and liquid water hypothetically was only able to get to the surface in places of groundwater (thermal or overcooled brines) seepage, and formed thin gullies on the crater walls (Malin and Edgett 2003). According to other hypotheses, these gullies were formed without water. It is important to note that some of them are probably present-day geological formations; the multiple image of one of the crater slopes in 2005 detected a new scour, filled up with light material, which was not visible on the image from the year 1999 (http://www.nasa.gov/mission_pages/mars/images/).

The recent terrestrial cryosphere is a result of the last (Cenozoic) era history. But there is evidence that this sphere periodically occupied the Earth's surface for tens and hundreds of millions of years during its early history: for example, in the early and late Proterozoic (2.4–2.1 and 1.0–0.6 billion years ago) and in the early and late Paleozoic (460–420 and 330–230 million years ago). This fact indicates that Earth and Mars underwent similar stages of development in the earliest parts of their history, which is important for the life searching theory.

21.2.2 Present-Day Situation

Permanent and seasonal polar caps occupy vast territories, and are the obvious evidence of the Martian cryosphere (Hvidberg 2005). Seasonal caps represent the up to 2 m thick CO_2 condensate, which drops out until approximately 60° latitude during the winter polar night in the corresponding hemisphere, and sublimates in spring and summer. In summertime at the poles, permanent caps remain consisting of water; but because of the ellipticity of the Martian orbit, the southern summer is shorter, and on the surface of the south cap a condensate of carbon dioxide partly remains (Mitrofanov 2005). Both caps together have a mass that is equivalent to a water layer of about 22–33 m spread over the planet's surface (Smith et al. 1999).

At present, spatiotemporal regularities of water distribution on the Martian surface and near subsurface horizons are being studied. According to the Inverse Square Law which is used to calculate the decrease in radiation intensity due to an increase in distance from the radiation source, Mars is located at 1.524 astronomic units and receives 2.32 times less solar radiation than the Earth. This fact determines the existence of a global frozen envelope, the cryosphere. Mean annual temperature on the Martian surface varies from −100°C at the poles to −50°C at the equator. The absence of atmosphere predetermines high temperature oscillations; for example, on Mars Pathfinder landing site temperature reached 2°C at noon, and fell to −80°C at night (Read and Lewis 2004).

The existence of permafrost appears in different relief forms, mainly in polygonal frost-cracking forms that are widespread in high latitudes (>45°N, >55°S) and cover the plains of different origin, flat hills and crater walls (Kuzmin 2005).

Morphological comparison of Martian and terrestrial polygons shows their similarities, but Martian polygons might be larger in size (up to 300 m).

The fluxes of neutrons and gamma rays affected by regolith have been measured on the Mars Odyssey mission since 2002 by two independent physical methods using the Gamma Subsystem, High Energy Neutron Detector and Neutron Spectrometer. Both methods, gamma-ray and neutron spectroscopy, indicate the presence of near-subsurface water-ice abundances on Mars (Boynton et al. 2002). According to these data, the 1-m thick surface ground really contains water-ice on any latitudes where thermodynamic parameters favor its existence

Martian average annual surface temperature is everywhere below the water triple point temperature. But due to the extremely low water vapor pressure in the atmosphere, the frost-point temperature is about −70°C. This means that within a latitude band of 40° ground ice may exist only at great depths (much below the accessibility depth for instruments currently searching for life and water on Mars) in unstable conditions.

Pole-ward from 40°, in both hemispheres the average annual surface temperatures are lower than the frost-point temperature, and stable ground ice exists under the thin dry regolith layer. The permafrost table occurs at depths from a few centimeters to 1 m. This dry layer protects the water ice either by reducing the sublimation rate of molecules (impeding the diffusion) and/or by attenuating the amplitudes of daily temperature oscillations above the permafrost table. The thickness of this armor regolith layer is determined by latitude, exposition, albedo, and thermal inertia of the dry layer, and corresponds to the depth where an equilibrium between steam pressure in the atmosphere and steam pressure above ice exists at the specific temperature. Such layering structure and surface distribution of water ice permafrost on Mars was predicted theoretically (Schorghofer and Aharonson 2006), and is empirically proven according to combined analysis of HEND/Odyssey and MOLA/MGS data (Mitrofanov et al. 2007).

The depth of the permafrost bottom is still not known. Estimations based on the solution of the equation on thermal conductivity with known boundary data (temperature on permafrost table) and an unknown value of heat flow from below (geological activity of Mars is lower than that of the Earth, so the value of Martian heat flow is assumed about 1/2.5 of terrestrial heat flow), indicate a thickness of permafrost of ~2 km on the equator and of 6 km on the poles (Clifford 1993). These estimations have a high degree of uncertainty due to the unknown value of heat flow from below. Mellon and Phillips (2001) calculated that the Martian subsurface temperature reaches 0°C at a depth between 150 m and 8 km, depending on soil thermal conductivity. According to empiric data from the MARSIS instrument aboard the ESA spacecraft Mars Express, the frozen sediments surrounding polar caps stretch to depths of at least 1.8 km in the north and 3.7 km in the south (Picardi et al. 2005).

From terrestrial experience, permafrost is underlain by groundwater, as a rule under pressure. This water lifts to the surface along the borehole to an elevation depending on the pressure value. The thermal groundwater decrements to the surface take place along the old faults, even in tectonically stable Arctic lowlands. For example, an outcrop of 20°C water was observed on Cape Chukochii (eastern Arctic lowlands) across the continuous permafrost (mean annual temperature −11°C) throughout the area, to depths of 600–800 m.

21.3 Icy World

Biota of the Greenland ice sheet (120,000 years old) and Antarctic ice sheet (~400,000 years old) have been widely studied to depths of more than 3 km (Abyzov 1993; Kapitsa et al. 1996; Priscu et al. 1998; Karl et al 1999; Petit et al. 1999; Skidmore et al. 2000; Deming 2002; Miteva et al. 2004; Miteva and Brenchley 2005). The age of the oldest glacial ice, as well as immured bacteria, is still under discussion: >500,000 years old at Guliya ice cap on the Tibetan Plateau (Thompson et al. 1997; Christner et al. 2003), ~2 million years at the bottom of the Vostok ice core (Salamatin et al. 2004) or even ~8.1 million years (Sugden et al. 1995; Bidle et al. 2007) in Beacon Valley, Antarctica. The data from Vostok cores showed that the upper young (<12,000 years old) layers are the most abundant ones, in spite of extremely low temperatures of −50°C (Abyzov 1993).

The number of mostly air-born microorganisms isolated from snow and seasonal ice covers are not high (10^2 cells ml^{-1}) and are of the same order as viable cells within the cores of ancient ice sheets. This fact could be interpreted as the absence of reduction of the microbial population once immured in ice during thousands of years, and could be explained by the near-zero background radiation in the ice (~2–4 mGy per year, 0.23 mGy h^{-1}).

Ice sheets are considered to be the Earths most representative analogues of icy habitats like Jupiter's ice-covered moon Europa, the icy moon in Saturn's system Enceladus, and firstly, ice caps on Martian poles. Correspondingly, microorganisms isolated from the ice cores of both hemispheres, and traces of life, such as genomic DNA well-preserved in ice cores (Willerslev et al. 1999; Christner et al. 2001), have been interpreted to be most representative analogues of inhabitants, and their fingerprints exist within these extraterrestrial icy habitats. The age of permanent Martian water-ice polar caps could be established on the basis of the amount of impact craters. On the north cap, large craters were not found, indicating the young geological age of the cap surface (not more than 100,000 years). On the south polar cap, 15 craters with a diameter >800 m were found, indicating the geological age of the cap surface to be about 7–17 million years (Hvidberg 2005). Because ice thaws under geostatic pressure, even at subzero temperatures, the existence of very old microorganisms is unlikely on the above-mentioned moons and caps. However, they and probably immured microorganisms are of the same order of age as on the Earth's ice sheets.

21.4 Soil Cover

Water ice within the top metres of the high-latitude regolith, as well as visual similarities on the terrestrial and Martian surfaces (polygons formed by frost cracking), lead to the consideration of frost-affected, seasonally thawed soil cover with a mean annual temperature below 0°C underlain by permafrost as an extraterrestrial analogue. The leading factor in differentiation of these soils, named cryosol, is temperature crossing through 0°C, resulting in freezing–thawing processes and ice–water phase exchange. Temperature oscillations crossing through the freezing point are also

observed on the Martian surface. With respect to Mars it is important to note that cryosol microbial communities, formed under the impact of multi-time freezing–thawing stress, did not change under such stress. Their maximal number and biodiversity correlate with the horizon A, decrease with depth from the surface beneath the seasonal thaw layer, and have an accumulative sharp peak on the permafrost table. In spite of the tundra, the day surface is under the influence of solar radiation, the snow and vegetation covers decrease and minimize this impact, as well as temperature oscillations. Thus, Arctic cryosol has distant similarities with the Martian surface.

The surface conditions in the Antarctic desert (the intensive level of solar radiation, the absence of snow and vegetation covers, and the ultra-low subzero temperatures, which can be as low as −60°C) and on Mars are closer. At elevations above 1,500 m, there are no summer air temperatures above freezing. However, the surface temperatures of soil or rock can be 15°C warmer than the air temperature due to solar heating, may exceed 0°C for several hours (McKay et al. 1998), and for short periods even reach 10°C (Campbell and Claridge 1987). In addition to sharp temperature oscillations and high insolation, the main similarity between Antarctic Dry Valleys and Mars is the vertical structure of their "active layers". In the Dry Valleys, the upper 10–25 cm-thick sandy layer does not form a stable soil cover on the ice-cemented permafrost table. It is dry (water content ~2%) and lacks ice-cement due to sublimation. This frosty ground throughout the upper 100 cm (including the active layer) covers 61% of Dry Valley's area (Bockheim et al. 2007). The overcooled ground, with no water and thus no ice, is often mobilized by storm winds similar to the instability of Martian dunes. Such double-layering structure and distribution of water ice within the first surface metre on Mars (dry top layer and ice-rich bottom layer) is proposed according to HEND/Odyssey and MOLA/MGS data (Mitrofanov et al. 2007), and consistent with present knowledge of environments on Mars. This is why Dry Valley's active layer which overlies permafrost could be considered as an analogue of the dry regolith layer on Mars.

The upper ~2 cm layer of the Dry Valley surface often contains a low number of viable cells compared with the underlying horizons (Horowitz et al. 1972). In some cases, these microorganisms cannot be isolated on agar plates, and correlate with a poor diversity of bacterial phylotypes, a low number of mycelia fungi strains, and a minimum of chlorophyll content. The occurrence and biodiversity of microorganisms is higher at depth than in the top of the active layer, and suggests that a search for life on Mars should not sample the surface but the bottom of the "active layer". In particular because the upper horizons contain low cell counts, Antarctic frosty soils are useful for testing equipment for searching for life on Mars (Gilichinsky et al. 2007a).

21.5 Permafrost

The most inhabited and ancient part of the cryosphere, permafrost, is defined as permanently frozen ground and underlies about a quarter of the Earth's land surface. This considerable frozen mass, up to several hundreds of meters deep, where

microorganisms are adsorbed on organic or mineral particles, harbors a high level (up to dozen millions of cells per gram) of various morphological and ecological viable microbial groups that have survived under permafrost conditions since the time of its formation. They have been isolated from frozen cores with permanently constant ground temperatures of −1 to −2°C near the south border of permafrost in Siberia, from lowest temperatures in the Arctic (−17°C on the most northern latitude: 80°N in NWT, Canada; Steven et al. 2007) and Antarctica (−27°C on the most southern latitude: 78°S in Dry Valleys; Gilichinsky et al. 2007a), down to 400 m depth in Mackenzie Delta (Gilichinsky 2002), and up to 4,700 m elevation in Qinghai–Tibet Plateau (Zhang et al. 2007). The age of the isolates corresponds to the longevity of the permanently frozen state of the sediments, and dates back from a few thousand to 2–3 million years in northeastern Arctic, and to 5–8 million years and probably older in Antarctica (Gilichinsky et al. 2007a). This great mass of the only known living communities preserved over a geologically significant time is peculiar to permafrost only, and represents a wide range of possible cryogenic ecosystems for planets without obvious surface ice.

Unfrozen water films play the leading role in the preservation of microorganisms. These films coat the soil particles and protect the viable cells adhered onto their surface from mechanical destruction by growing crystals of intrusive ice, and make possible the mass transfer of microbial metabolic by-products in permafrost, thus preventing the cells from biochemical death (Gilichinsky et al. 1993). Therefore, the unfrozen water might be considered as a main ecological niche where the microorganisms might survive. In fine dispersed Arctic permanently frozen sediments at temperatures of −3 to −12°C, the amount of unfrozen water can be estimated as 3–8% of total water mass.

Because of temperatures below −20°C in the coarse Antarctic Valley's sands, the unfrozen water amounts are so small that the instrumental methods fail to record them. The unfrozen water must therefore only be firmly bound to "liquid" water with binding molecules, and indicates a "biologically dry" environment. Based on experiments, Jakosky et al. (2003) calculated that liquid water can exist as ice grain–dust grain, and ice grain–ice grain contacts above ca. −20°C. Below this temperature, water would not be present in soils in sufficient thickness and amount to physically allow the presence of microorganisms, i.e., this temperature is the lowest at which life can function. Both conclusions are not fully clear at this moment, and not quite correct. Firstly, because for Victoria Valley it was determined that the amount of unfrozen water is 2% at −20°C and 1.5% at −30°C due to the salt content. The same amount of unfrozen water is expected in Beacon Valley, where the soil has a higher salt content (Gilichinsky et al. 2007a). Secondly, numerous studies have shown that microorganisms metabolize at extremely low temperatures in ice and permafrost, i.e., between −10°C and −20°C (Rivkina et al. 2000, 2004; Carpenter et al. 2000; Bakermans et al. 2003; Junge et al. 2004), and down to −28°C and −35°C (Rivkina et al. 2005; Panikov and Sizova 2007).

Annual maximum surface temperatures in Martian permafrost regions may rise above this level and above 0°C (for hours) up to 75° latitude in the south and up to 50° latitude in the north (Tokano 2003). But temperature at the depth of ground ice

burial never exceeds −20°C. At the same time, the Gamma Ray Spectrometer onboard the Mars Odyssey spacecraft observed some areas with rather high concentrations of Cl (Keller et al. 2006) in the upper 10–20 cm of ground, which promises the existence of enough unfrozen water at some salt-rich geologic locations to protect viable cells. This makes the near surface past and present permafrost layers potentially favorable sites to search for evidence of life similar to cryptoendolithic microbial communities within Antarctic sandstone (Friedmann 1982). Probably, in such ecological niches, thin brine films might be formed within Martian permafrost, as proposed by Dickinson and Rosen (2003) in their studies of minerals and accumulation of ground ice on Table Mountain, Sirius Group sediments.

From the astrobiological point of view, it is important that permafrost (where 92–98% of water is in a solid state) and subzero temperatures slack off the cumulative effects of background terrestrial gamma radiation on cells for thousands and millions of years. The lower the water content and the rate of metabolic processes, the less are the radio lesions of biological objects. This is why the irradiation sensitivity of soil microorganisms at temperatures above 0°C differs from the sensitivity of microorganisms preserved in permafrost. The response of permafrost microorganisms to irradiation in non-frozen and frozen state is different. At an irradiation dose of 1 kGy, there is one magnitude difference in the number of viable cells between non-frozen and frozen samples (Gilichinsky et al. 2007b), and the cell survival rate was estimated to be 1% and 10% of the initial cell number in non-frozen and frozen samples, respectively. In the model gamma-irradiation, a dose of 5 kGy was lethal for the microbial community in non-frozen samples.

Direct in situ measurements in boreholes on the Eurasian northeast showed that the dose received by the immured bacteria in frozen sands and loams is about 2 mGy per year. Taking into account the oldest (~3 million years) late Pliocene age of permafrost and bacteria, the total dose received by cells would be 5–6 kGy. Under these conditions, most of the cells survived. This fact shows that freezing increased the cells' resistance to radiation, and demonstrates the uniqueness of permafrost as an environment where microorganisms display a high resistance to radiation. From these data, the dose from radionuclides diffused through permafrost is not fatal, but should be large enough to destroy the DNA of ancient viable cells. Their viability and growth implies the capacity for DNA repair, probably in the frozen environment, i.e., at the stable rate of damage accumulation, a comparable rate of repair also exists (Rivkina et al. 2004). This is why the "biologically dry" (at temperatures below −20°C) Antarctic permafrost, with extremely low and inaccessible organic matter, is nevertheless inhabited by up to 10^3–10^5 viable cells g^{-1}, providing an analogue for Martian ecosystems.

Antarctic ice-free areas are the best terrestrial analogues of Martian permafrost for several reasons. The first one is that the temperature conditions in Antarctica are closest to conditions on Mars. For example, the annual surface temperatures on 40° latitude south, which is the warmest place with permafrost on Mars, includes a maximal temperature of 15°C, a mean temperature of −65°C, and a minimal temperature of −130°C (Tokano 2003). In Antarctic Dry Valleys (Beacon Valley), the maximal surface temperature is the same, mean and minimal temperatures are

−23°C and −45°C respectively. Due to extremely low temperatures in Antarctica, phase transfer of H_2O occurs without melting, so that sublimation is the main factor controlling the stability of ground ice exactly as on Mars. The second similarity is double-layered ground with a dry layer at the top and an ice-rich layer at the bottom, which together with the absence of vegetation and soils make the Antarctic landscape Mars-like. The third similarity is permafrost age. The development of global ice-rich permafrost is attributed to post-Noachian time (nearly 4 billion years ago). Permafrost in some areas related to Hesperian mega outflow and Amazonian volcano–ice–water geomorphic features may be substantially younger (up to 1 billion years). Mars is known to be a still geologically active planet. Aeolian transport of dust, together with the presence of a global water cycle between atmosphere and ground ice, lead to the permanent development of syncryogenic permafrost. But, as there is no addition of any new possible life-containing material from volcano eruptions or underground water (excluding local spots of possible groundwater seepage in gullies), the material of this modern permafrost still comes only from old Noachian–Hesperian–late Amazonian rocks.

Antarctic desert deposits beneath the frosty active layer are unexpectedly icy, i.e., of the same order as the more humid Arctic area. This means that ground ice instability due to the processes of sublimation at ultra-low humidity and air temperature is in a very thin surface layer only, and revises the earlier thesis of dry Antarctic permafrost. This is why we can also expect the existence of high icy subsurface layers on Mars.

Permafrost on Earth and Mars vary in age, from a few million years found in the north hemisphere on Earth (Sher 1974) to a few billion years on Mars (Carr 2000; Baker 2004; Tokano 2005); such a difference in time scale would have a significant impact on the possibility of preserving life on Mars, because the number and biodiversity of microorganisms decrease with increasing permafrost age. This is why the longevity of life forms preserved within the Arctic permafrost can only work as an approximate model for Mars. The suggested age of Antarctic permafrost (~30 million years) is somewhat closer to that of Mars. A number of studies indicate that the Antarctic cryosphere began to develop soon after the final break-up of Gondwana and the isolation of the Antarctic continent. It is believed to have been started on the Eocene–Oligocene boundary (Barrett 1996; Wilson et al. 1996; DeConto and Pollard 2003). The discussion of Neogene stability has focused mainly on the state of the ice sheet, which is the most variable part of the cryosphere. Permafrost is the more stable end-member of the cryosphere, and the conditions needed for ice degradation, even if they existed in a climatic optimum, are not sufficient to thaw the permafrost. Permafrost degradation is only possible when mean annual ground temperatures, −28°C now, rise above freezing, i.e., a significant warming to 25°C or above is required to degrade the permafrost once formed. There is no evidence to date of such significant temperature variation, which indicates that the Antarctic climatic and geological history was favorable to the formation and persistence of pre-Pliocene permafrost. For example, early Oligocene sediments (38 million years) obtained by the Cape Rogers drilling project contain cold tundra pollen spectra. Antarctic permafrost may, therefore, be more than 30 million years old

(Gilichinsky et al. 2007a) and date from Antarctic ice sheets predicted in early Oligocene times (Zachos et al. 2001).

Viable microorganisms were isolated from the cores taken in Beacon Valley from beneath an 8.1 million years volcanic ash layer (Gilichinsky et al. 2007a) that has been interpreted as a direct air-fall deposit (Sugden et al. 1995), and this age is supported by several studies (Schaefer et al. 2000). The age of isolated communities remains controversial, because recent investigation has questioned this age relationship, and calculations indicate that sublimation rates would be too high for the ice to persist for 8.1 million years (Ng et al. 2005). However, Bidle et al. (2007) isolated microorganisms from the ice beneath this ash; these authors again affirm an age of 8.1 million years. From an age perspective, the Glacigene Sirius Group sediments on Mount Feather may be even older. They were estimated to be at least 2 million years in age (Webb and Harwood 1991) and possibly as old as 15 million years (Marchant et al. 1996). The age for the superficial deposits where bacteria were sampled in the permafrost is 5 million years (Wilson et al. 2002). If this age is correct, these are, to date, the oldest confirmed viable microorganisms discovered in permafrost and the oldest viable communities reported on Earth (Gilichinsky et al. 2007a).

It would be advantageous to locate relics of the oldest Antarctic permafrost. These are possibly to be found at the high hypsometric levels of ice-free areas such as the Dry Valleys, along the Polar Plato and Trans-Antarctic Mountains, and on Northern Victoria Land. It is desirable to date the layers within them and to test for the presence of viable cells. The limiting age, if one exists, within the most ancient Antarctic permafrost cores, where the viable organisms were no longer present, could be established as the age limit for life preservation within permafrost at subzero temperatures. Any positive results obtained from Antarctic microbial data will extend the geological scale and increase the known temporal limits of cryobiosphere, i.e., duration of life preservation.

21.5.1 Volcanoes

One way to have liquid water on Mars at shallow depths would be through subglacial volcanism. Such volcano–ice interactions could be going on beneath the polar caps of Mars today, or even within the adjacent permafrost around the margins of the ice caps. Basalt lava fields are common on the Martian surface, and some cinder cones have been found near the polar caps. The rover traces on terrestrial ash fields and the Martian surface, as well as the chemical composition of basalts on Earth and Mars, are similar (Arvidson et al. 2004; Squyres et al. 2006). This is why research of terrestrial volcanoes, including the permafrost study, is expected to be a valuable step in understanding extraterrestrial volcanoes as one of the Earth's analogues, close to the extraterrestrial environment, represented by active volcanoes in permafrost areas. The key question concerning this volcanic permafrost model is the age of Martian volcanoes.

On Earth, most volcanoes are located in areas of collision of oceanic and continental plates. Despite active volcanism, permafrost often exists on slopes of high-elevation or high-latitude volcanoes (Kellerer-Pirklbauer 2007) in places such as Hawaii (Woodcock 1974), Iceland (Etzelmüller et al. 2007), Mexico (Palacios et al. 2007), Peru, North America, and Antarctica. On Mars, plate tectonics is not observed; nevertheless, more than 50% of Mars surface is known to be covered by rocks of volcanic origin, and displays of volcanism are observed everywhere (Carr 1996). The largest volcanoes are in three broad provinces: Tharsis, Elysium and Hellas. The regional elevations of Tarsus and Elysium are one of the youngest formations of Mars. But it must be mentioned that while we have no lava samples, the ages of volcanoes on Mars can only be roughly estimated by the number of impact craters, with newer regions having fewer craters (Fig. 21.1).

Tharsis is possessed of the biggest volcano in the Solar System – Olympus, which covers an area of 600 km in diameter and is 27 km high. The huge sizes of Martian volcanoes are the consequence of the stopped plate tectonic, when eruptions take place at the same point. Some volcanoes of Tarsus province undoubtedly were active in the last billion years, in that the least-cratered surfaces of lava flows of Olympus volcano were dated by Carr (1996) as a few hundred million years old or even less as ~30 million years.

The main question is: do such ecological niches as volcanoes and associated environments contain microbial communities? The task is to find thermophilic

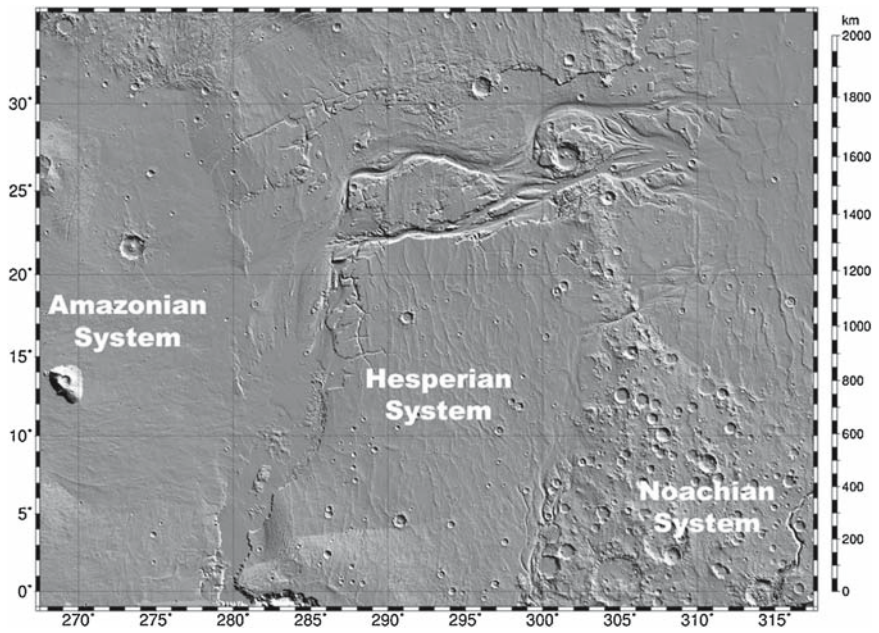

Fig. 21.1 Mars orbiter laser altimeter (MOLA) relief map, demonstrating surfaces of different age

microorganisms associated with volcanoes that have been deposited with products of eruption, and that have then survived in permafrost after the freezing of scoria and ash. Our study was carried out on the Kluchevskaya volcano group (Kamchatka Peninsula) which was formed starting from the late Pleistocene (Braitseva et al. 1995). The volcano group consists of Klyuchevsky, Bezymianny, Ushkovsky and Plosky Tolbachik, which are active volcanoes, and others that are not active today. Most of these volcanoes are higher than 3,000 m above sea level. At these points, the permafrost thickness is estimated to be 1,000 m. The mean annual ground temperature decreases from −1°C on the lower boundary of permafrost (~900 m) to −2.6°C at 1,300 m and −7°C at 2,500 m (Abramov and Gilichinsky 2008).

During the eruptions of these volcanoes in the last 2,000–3,000 years, thick (12–16 m) layers of volcanic ash, sand and scoria were accumulated on the elevations occupied by permafrost, and at that time became frozen. The last eruption was in 1975–1976, and ~500 km^2 were covered by scoria and ash; three new cinder cones and lava fields were formed (Fedotov and Markhinim 1983). The cores extracted from the borehole crossing these young volcano deposits contained biogenic CH_4 (up to 1,900 µl kg^{-1}) and viable bacteria, including thermophilic anaerobes (10^3 cells g^{-1}), and among them, methanogens growing on $CO_2 + H_2$. Because thermophiles have not previously been found before in permafrost, the only way for these bacteria to appear within frozen volcanic horizons is through the eruption of a volcano or its surrounding associated strata. The important conclusion is that thermophiles might survive in permafrost and even produce biogenic gases. For future space missions, the permafrost volcano areas are promising test sites, and provide opportunities to study analogues of possible Martian ecosystems. Their original microbial communities represent an analogue for communities that probably might be found around Martian volcanoes. The methanogenic archaea found at such sites can likely adapt to temperatures <0°C, as has been found with other studied groups of anaerobes.

21.5.2 Cryopegs

Results of the 2001 mapping of the Martian surface for the presence of chlorine by GRS spectrometer aboard the Mars Odyssey spacecraft showed significant variations of chlorine content from 0% to 1% (Keller et al. 2006). The lowest temperature at which salt-rich Martian ground water may still be in a liquid state is about −60°C (Zent and Fenale 1986). Taking into account this statement, brines may be found on Mars at inaccessible depths or at low latitudes. Areas with mean annual temperatures equal to or higher than −60°C are only found at the 30° latitudinal belt. At these latitudes Malin and Edgett (2003) found so-called gullies, freshly incised channels a few metres across. These indicate that a fluid had eroded the soil. Water is the most likely candidate responsible for the origin of these very young gullies (their age is estimated to be <1 million years), but the source of liquid water on a frozen planet is a mystery. It is quite possible that the source of liquid water is underground brine. Obviously gullies are high-priority targets for the search of life

on Mars. Unfortunately, it is impossible to land a rover on gullies because of engineering constraints. But nearby plains, which contain material accumulated from gullies, may still contain cryopeg microorganisms in a frozen state.

Terrestrial cryopegs were exposed by boreholes along the Polar Ocean coastal zone, with mean annual ground temperatures varying between −2 and −12°C on Cape Barrow (Alaska), the Barents Sea coast, the Yamal Peninsula (surrounded by the Kara Sea) and the Kolyma lowland (East Siberian Sea). At the last site, cryopegs are confined to a 20 m-thick marine horizon, sandwiched between non-saline terrigenous layers at depths of 40–50 m below the tundra surface (the mean annual ground temperature varying from −9°C to −11°C). Finely dispersed sand and sandy loams were deposited in shallow lagoons at temperatures slightly above 0°C. After regression of the Polar Ocean, the water-bottom sediments were exposed sub-aerially and froze. Because of the pressure caused by freezing, water was released as the freezing front penetrated downward. This was accompanied by a freezing out of salts in the water, to form lenses of overcooled sodium chloride brines with salinities of 170–300 g l^{-1}. Later, the marine horizon was buried by a 15–20 m thick unit of lacustrine-alluvial late Pleistocene icy complex that was built up under harsh climate conditions, was syngenetically frozen and has never thawed. Within the marine horizon, the lenses occur at different depths, their thickness varying from 0.5 m to 1.5 m and their width from 3 m to 5 m. Some of them represent non-artesian water, and some exist under low pressure with a hydrostatic head. Different salinities of the brines confirm their lenticular nature and isolated bedding.

Bacteria isolated from cryopegs were not only adapted to subzero temperatures but also tolerant to the high salt concentrations. In addition, the detected microorganisms were both halophilic and psychrophilic, and such organisms have never been isolated from natural habitats. In the cold saline conditions of cryopegs, special communities were formed. Active adaptation to low temperatures of already studied bacteria gives hope that fully active and reproducing bacteria can be discovered in saline habitats at subzero temperatures. Biotic survival in the aquatic environment on a geological time scale indicates unknown bacterial adaptations. The microbial activity detected in cryopegs at temperatures as low as −15°C documents the fact that subzero temperatures themselves do not exclude biochemical reactions, and provides reason to conclude that in overcooled water the metabolic strategy of microbial survival operates, and that this strategy does not accept that cells can multiply in situ (Gilichinsky et al. 2005).

The unfrozen water films in terrestrial permafrost, high in salts, represent the same micro-brines, even in ultra-fresh sediments, and most investigators indicate that at least part of the permafrost community (20%, according Steven et al. 2006) grows at temperatures between −2 and −10°C (Shcherbakova et al. 2004, 2005; Ponder et al. 2005; Rodrigues et al. 2006; Bakermans et al. 2006).

Biotic survival in the late Cenozoic overcooled high-salt aquatic environments for 100,000 years and in Permian–Triassic saliniferous sediments 250 million years old (Dombrowskii 1963; Vreeland et al. 2000; Stan-Lotter et al. 2002, etc.) indicate unknown bacterial adaptations. What is more, in the cold saline conditions of overcooled brines, special communities were formed, and some of them were novel

species. Because the Opportunity rover detected rocks with high S concentrations (Rieder et al. 2004), it is interesting that sulfate reducers detected in cryopegs are halophilic and psychrophilic organisms at once that have never been isolated from natural habitats. The salt tolerance may be associated with cold tolerance on a geological scale. Experimental data showed that in the presence of 25% NaCl, halophiles survive better than non-halophiles under low (−20 to −80°C) temperatures, and extreme halophiles require NaCl concentrations above 15.6% (w/v) for growth (Rothschild and Mancinelli 2001; Mancinelli et al. 2004).

Basalt is not the only rock component of the Martian surface. Stratified sediments, presumably of marine origin, were discovered on Mars, which makes it different from the Moon. It is suggested that in the Noachian epoch the northern lowlands were occupied by ocean, and a range of cratered depressions represent seas, where marine sedimentation took place (Baker et al. 1991). These bottom sediments with high solute content might represent the opportunity for free water existing as brine lenses within permafrost, formed when Mars became cold. Mars is a cryogenic planet where free water only has the opportunity to exist in the presence of high solute content, probably as brine lenses within permafrost. These brines, like their terrestrial analogues, may contain microorganisms adapted to subzero temperature and high salinity. This is why the unique halo/psychrophilic community preserved hundreds of thousands of years in mineral-enriched Arctic cryopegs and in hundreds of million of years old salt deposits, provide the plausible prototype for Martian microbial life (Gilichinsky et al. 2003) either as an “oasis” for an extant, or the last refuge of an extinct biota (Mancinelli et al. 2004).

21.6 Conclusion

The future mission priorities for the search for life on Mars must be based on studies of environments in which life might be found most likely, and the maximum period of time over which such life could be preserved. The terrestrial subsurface frozen layers represent analogues of extraterrestrial cryobiosphere, where the probability of finding life is the highest.

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Subject Index