



## Characteristics of soil filamentous fungi communities isolated from various micro-relief forms in the high Arctic tundra (Bellsund region, Spitsbergen)

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**Abstract:** Saprotrophic filamentous microfungi were isolated by means of the soil dilution method from soil samples collected from four locations in the Bellsund region of Spitsbergen (77°33'N, 14°31'E) representing the following forms of surface micro-relief: an old stormbank, a sorted circle, a frost fissure between tundra polygons, and the central part of a tundra polygon. The fungal isolates were identified and screened for their ability to grow at low temperatures. The oligotrophy of psychophilic and psychrotrophic strains was then determined as the ability of growth on silica gel without a C source added. Differences in some physico-chemical properties were found between the soils sampled from the four sites. A total of 89 taxa from 17 genera were isolated. Most of the isolates were species of *Mortierella*, *Penicillium*, *Chrysosporium* and *Phialophora*, and half of them were psychrophiles. Fungal communities isolated from a frost fissure between tundra polygons (site 3) and from the central part of a tundra polygon (site 4) were dominated by psychrophiles but those isolated from an old stormbank (site 1) and a sorted circle (site 2) were predominantly psychrotrophic. Oligopsychrophilic taxa accounted for 27% and oligopsychrotrophic for 20% of all the isolated taxa but only from 0.7% to 11.7% and from 1.2% to 6.3% of the total number of cfu (colony forming unit) isolated from an individual site, respectively. The results of the present study suggest that the abundance of fungi in Arctic soil is mostly affected by the content of organic matter in the A horizon and the plant cover, but other factors, such as the stage of soil development and the micro-relief of the surface, are more important for species richness of fungal communities.

Key words: Arctic, tundra, filamentous fungi, species richness, ecology.

## Introduction

High Arctic ecosystems may contain as much as 25–33% of the world's soil carbon (Oechel and Vourlitis 1995). However, our knowledge about the community structure of saprotrophic fungi, important decomposers of organic matter in extremely cold regions is relatively scarce. Some fundamental information was obtained when the International Biological Program (IBP) was realized. This program was drawn up by the Committee on the Productivity of Terrestrial Communities (IBP-PT) in 1966 as a major research project on different types of ecosystems, Tundra Biome being one of them (Cragg 1981). The sites included in the IBP research represented Alaska, Siberian and Scandinavian Arctic tundra, Antarctic and subantarctic tundra, subarctic tundra in Scandinavia, and alpine tundra in Europe and North America (Jonsson 1981). One of the 7 major international objectives of the studies was to estimate the number of soil microorganisms, define their main physiological activities, and determine the relationships between the numbers, biomass and activity of these organisms and soil conditions, climate, primary production, and decomposition (Heal 1981).

About 100 genera of fungi in 33 tundra areas studied within the IBP were recorded. The species most frequently isolated from soil were those of *Chyso-sporium*, *Cladosporium*, *Mortierella* and *Penicillium* genera and sterile mycelia. Fungi belonging to the genera *Trichoderma*, *Aspergillus*, *Fusarium*, *Botrytis* and *Rhizopus* were rare or absent from the IBP tundra sites (Holding 1981). It was found that microorganisms associated with decay of organic matter in tundra belong to taxonomic groups which are common in other biomes, but the number of taxa and the biomass are generally lower than in other biomes (Heal *et al.* 1981). More recent studies conducted at the beginning of the 21st century indicate that during the winter season the microbial community of Arctic soil is dominated by saprotrophic fungi (Schimel and Mikan 2005), but fungal activity during the growing season cannot be neglected because of its important role in supplying Arctic plants with mineral nutrients (Schmidt and Bölder 2002). In polar environments, fungi not bacteria are considered to be the most important group involved in cellulose decomposition. Also between 50–60% of carbon in litter can be converted to CO<sub>2</sub> by fungal respiration (Holding 1981). Under laboratory conditions 20% of the tested strains isolated from tundra sites showed an optimum of cellulose decomposition rate at approximately 6°C. Pectin decomposition was shown to be initiated at about 1°C and reached an optimum rate at 18°C or more (Holding 1981).

In cold environments such as the Arctic tundra low temperatures create other extreme conditions, including low water activity and low organic nutrient content connected with low primary production by the scanty and discontinuous plant cover. Soil microorganisms under such conditions experience not only physical stress but also starvation (Bergero *et al.* 1999); however, it has been shown re-

cently that fungi are not only able to survive but also to propagate in various environmental extremes (Gunde-Cimerman *et al.* 2003).

The aim of the present study was to estimate the structure of filamentous fungi communities in the A horizon of soils collected from four different micro-relief forms in tundra in the Bellsund region during the 1999 summer season. The measurements included determination of the total colony forming units (cfu) number, species diversity, growth temperature tests and screening for oligotrophy among the isolated psychrotrophic and psychrophilic taxa.

## Study area, material and methods

Investigated area is located in the southern part of Bellsund on the western coast of Spitsbergen in the Svalbard Archipelago (77°33'N, 14°31'E) on the lower coastal terrace in the Calypsostranda region. Mean air temperatures in summer range from 4°C to 7.6°C and the yearly mean temperature is –5°C (Klimowicz and Uziak 1996). During the summer season of 1999, from July 14 to August 12, mean air temperature was 5.5°C with max 9.6°C and min 4.0°C. The atmospheric precipitations on Spitsbergen are high compared to other Arctic regions but they are characterized by large year-to-year variability. Yearly total average precipitation is about 300 mm. In 1999, in the period from July 14 to August 12, the mean value of precipitation in the area under study was 12.9 mm (Klimowicz and Uziak 1996, Kejna *et al.* 2000).

The ground surface of the high Arctic tundra in the Bellsund region (Spitsbergen) is differentiated with respect to its micro-relief forms. This pattern has an influence on the morphological, granulometric and chemical properties of the soil developed within an individual relief form as well as its plant cover (Klimowicz and Uziak 1996).

### Sample collection

Soil samples were collected from 4 sites 6 times in the summer period of 1999 between July 13 and August 17.

**Site 1.** — An old stormbank with the surface layer almost completely covered with *Dryas octopetala*. In some places also *Silene acaulis*, *Saxifraga oppositifolia*, *Salix polaris* and black lichens were found.

**Site 2.** — A sorted circle with sparse (20%) plant cover. The plants were mainly algae, lichens and mosses with some *Silene acaulis*, *Saxifraga oppositifolia*, *Equisetum arvense* and *Salix polaris*.

**Site 3.** — A frost fissure between tundra polygons, 15 cm wide and 40 cm deep. The plant cover of the surface layer was in 100% overgrown with lichens, mosses and *Salix vesicularis*, *S. polaris*, *Silene acaulis*, *Saxifraga oppositifolia*, *S. svalbardensis*, *S. hirculus*, *S. cespitosa* and *Equisetum arvense*.

**Site 4.** — The central part of a tundra polygon. Nearly 70% of the surface was covered with the following species: *Cetraria sp.*, *Cladonia sp.*, *Silene acaulis*, *Saxifraga oppositifolia* L., *S. platysepala*, *S. hieracifolia*, *S. rivularis*, *Draba alpina*, *Cerastium arcticum*, *Sagina nivalis*, *Papaver dahlianum*, *Polygonum viviparum* and *Salix polaris*.

The altitudes of sites 1, 3 and 4 were in the range from 18 to 30 m a.s.l. and site 2 was located in a depression. The distance between the sites was less than 500 m.

The sample taken from each site for microbiological analysis was placed separately in a clean, sterile plastic bag and stored at 4°C. An additional portion (10g) of soil from each point was taken for physical and chemical analysis. Before use in the study, soil samples were sorted carefully by hand using sterilized fine forceps in order to remove any stones or plant material.

### Soil analyses

The following basic properties of soil were determined: particle size distribution by the Bouyoucos method modified by Casagrande and Prószyński (sand separated on sieves) (Lityński *et al.* 1976); calcium carbonate content using the Scheibler method (Lityński *et al.* 1976); pH by joint electrode; organic matter by loss on ignition; organic carbon content using the Tiurin method; total nitrogen by Kjeldahl (Lityński *et al.* 1976); DOC (dissolved organic carbon) in 0.5M potassium sulfate by the Tiurin method (Lityński *et al.* 1976); ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) in 2M KCl according to Kandler (Schinner *et al.* 1996); total phosphorus (P<sub>t</sub>), inorganic phosphorus (P<sub>in</sub>) and organic phosphorus (P<sub>org</sub>) – by the Kuo method (1995).

The soil dilution technique was used to determine the number of fungal cfus in soil samples and to isolate pure cultures. Plates with Martin (Martin 1950) medium inoculated with soil dilutions were incubated at 15°C for 7 days and then the number of colonies was counted.

Each type of colony originating from each soil sample was recorded, counted and isolated on a slope of Martin agar medium for species identification. For each sampling, the isolation frequency of each taxon per soil type and time of sampling was calculated as the percent of the total cfu number isolated from the same sample.

Pure cultures of isolated fungi were identified on the basis of their micro- and macromorphology according to Domsch *et al.* (1980).

### Growth temperature tests

The colony of each isolate grown on Martin medium was suspended in water, and this suspension was poured out on the surface of Martin agar medium in a petri dish. The plate was incubated at 15°C for 14 days and then a 4-mm-diameter plug was taken out and placed at the center of a petri dish containing 20 ml of Martin or

Malt Extract agar (MEA) (Azmi and Seppelt 1997) media. Three replicates of each fungal isolate were incubated at 4, 15 and 28°C for 21 days. The diameter of the colony of each replicate in each temperature and medium was measured every day. Colony diameters were measured in two dimensions at 90° to each other. Values were averaged to give the diameter of each colony.

### Screening for psychrooligotrophic fungi

The ability of strains growing at 5°C to grow oligotrophically was tested on silica gel, containing N but no added C. Fungi had been previously transferred to Czapek Dox agar (Azmi and Seppelt 1997) with no added C; after incubation at 15°C for 15 days, a 5 mm diameter plug was transferred to fresh silica gel prepared according to Funk and Krulwich (1964). Distilled water purified with a “Mili Q” filtration system was used to prepare silica gel and to wash glassware. Two replicate plates were inoculated for each strain. Plates were incubated at 5°C in order to subject fungi to concomitant low temperature and nutritional stresses. Colony diameters were measured after 21 days.

### Statistical data analysis

Values of correlation coefficients were calculated according to Armitage and Berry (1987).

## Results

Data presented in Table 1 show that there were big differences in basic physicochemical properties between soil samples taken from the four sites in the high Arctic tundra in Spitsbergen. Differences were found in total numbers of fungal cfu isolated from the A horizon (Fig. 1). The highest numbers of fungal propagules, in the range from  $9 \times 10^4$  to  $2.2 \times 10^5$ , were isolated from the frost fissure between tundra polygons (site 3) and the lowest, in the range from  $3.5 \times 10^3$  to  $1.8 \times 10^4$ , were isolated from the sorted circle (site 2). The total numbers of fungal cfu isolated from the A horizon of the four sites studied were highly positively correlated with the total content of organic C (0.94) and DOC (0.97) as well as with the total N (0.98) and P (0.96) content. Statistical analysis also showed that soil N-NH<sub>4</sub> was not a favorable N source for the isolated fungi. The present studies demonstrate that the abundance of saprotrophic filamentous fungi in soils of the high Arctic tundra reflects their heterogeneity.

The growth temperature tests showed that all the isolated (89) taxa were able to grow at 4°C. Among them, 37 were taxa which did not form a colony when incubated at 28°C and should therefore be considered as psychrophiles, while 52 were taxa of psychrotrophs growing also at 28°C (Morita 1975). Most of the psychrotrophs (44 taxa) formed biggest colonies when incubated at 15°C (Table 2, Fig. 2).

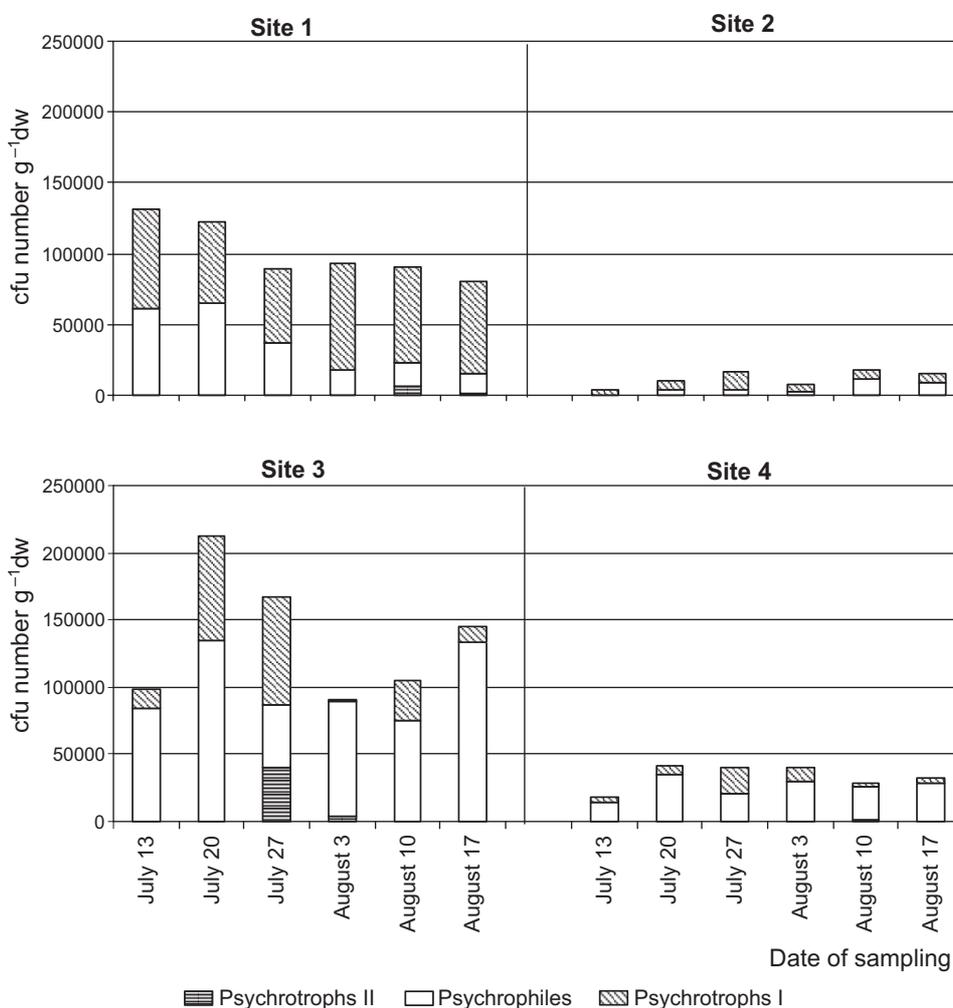


Fig. 1. Changes in the total number of fungal cfu isolated from 1 g of soil sampled from various forms of micro-relief in the high Arctic tundra during the 1999 summer season.

An average ratio of colony diameter formed at 28°C to that formed at 15°C was 0.47 (psychrotrophs I); however, for 8 taxa, the value of this ratio was 1.43 (psychrotrophs II). Psychrotrophs I isolated from the high Arctic tundra on Spitsbergen had also lower growth rates at 28°C than at 4°C, as after 21-day incubation the average 28°C/4°C ratio of colony diameter was 0.75 for rich Malt Extract agar medium.

Growth temperature tests also revealed that fungal communities isolated from soils at sites 3 and 4 were dominated by psychrophiles and those originating from sites 1 and 2 were dominated by mesophiles adapted to growth at low temperatures

Table 1  
 Some physicochemical properties of soil A (humus) horizon

Physicochemical property		Site 1	Site 2	Site 3	Site 4
Depth of A horizon [cm]		0–6	0–4	3–14	1–5
Granulometric composition [%]	>1 mm	14.3	41.7	16.9	18.8
	1–0.1 mm	48	49.8	53.2	62.8
	0.1–0.05 mm	19	6.2	13.8	11.2
	0.05–0.02 mm	24	14	23	13
	0.02–0.005 mm	8	17	5	8
	0.005–0.002 mm	0	5	1	1
	< 0.002 mm	1	8	4	4
pH <sub>KCl</sub> [1M KCl]		6.85	7.91	6.68	7.36
CaCO <sub>3</sub> [%]		1.55	20.19	0.45	3.08
C-total [%]		5.4	0.66	5.52	2.49
DOC [%]		0.042	0.019	0.044	0.02
N-total [%]		0.42	0.078	0.609	0.249
NO <sub>3</sub> [mg/100g]		1.03	0.41	<0.01	2.29
NH <sub>4</sub> [mg/100g]		12.56	15.41	10.03	>15
C:N		12.86	8.46	9.06	10
P <sub>t</sub> [μg/g]		322.35	214.925	405.85	285.706
P <sub>org</sub> [μg/g]		227.863	52.45	251.56	154.543
P <sub>in</sub> [μg/g]		94.482	162.475	154.128	131.162

(psychrotrophs I) (Fig. 3). Mesophiles able to grow at 4°C but with an optimum growth temperature at 28°C (psychrotrophs II) isolated from the four sites accounted, on average, for only 0.6 to 4% of the total cfu number and from 3.6 to 11% of the taxa isolated from individual sites.

A total of 89 taxa from 17 genera were isolated from the soil taken from the four sites (Table 2). Most (58%) of the isolates were species of *Mortierella*, *Penicillium*, and *Chrysosporium* and half of them were psychrophiles. Big differences were found in species richness between the four tested sites (Fig. 4). The soils originating from sites 1 and 2 were inhabited by fungal communities with low species richness and consisted of 10 and 12 taxa, belonging to 6 and 7 genera, respectively; however, the species of *Mortierella* and of *Penicillium* accounted for 86% of the total cfu number isolated from site 1. Much higher species richness was found while testing fungal communities isolated from sites 3 and 4. They consisted of 20 and 22 taxa belonging to 11 and 13 genera, respectively.

Fungi isolated from the four sites of the high Arctic tundra on Spitsbergen were different not only in respect of their growth temperature range but also in re-

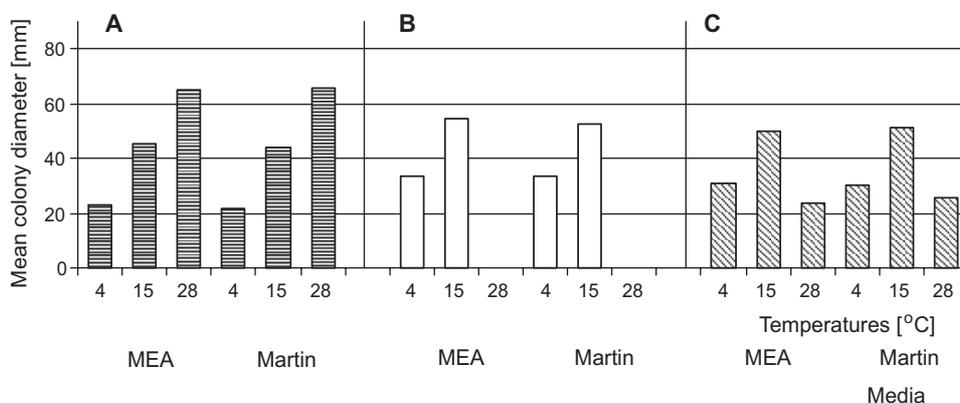


Fig 2. Effect of growth temperature on colony diameters formed on rich media by soil fungi isolated from the high Arctic tundra: A – psychrotrophs II, B – psychrophiles, C – psychrotrophs I.

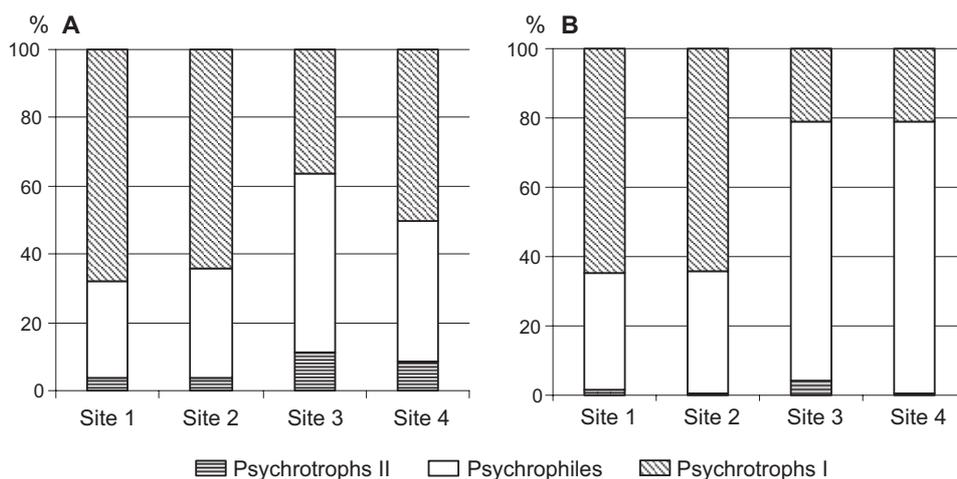


Fig. 3. Structure of fungal communities isolated from soil collected from various forms of micro-relief in the high Arctic tundra in respect of the preferred range of growth temperature: A – percentage of isolated taxa, B – percentage of the total number of isolated cfu.

spect of their nutrient requirements. The results of the oligotrophy tests indicated that taxa able to grow on silica gel without an added C source accounted for 27% of the isolated psychrophilic and 20% of the psychrotrophic species (Table 3). The ratio of mean colony diameter formed by oligopsychrophiles on silica gel to those formed on rich media such as MEA or Martin agar after 21-day incubation at 5°C was 0.73 and 0.71, respectively. The ratio values for oligopsychrotrophs were 0.59 and 0.58, respectively. Various cfu numbers of fungi with such drastically limited nutrient requirements were periodically isolated from sites 1, 2 and 3 but always from site 4 (Fig. 5).





Table 2 – continued

Genus species	Site 1			Site 2			Site 3			Site 4		
	A	B	C	A	B	C	A	B	C	A	B	C
no 4									+			+
no 5											+	
no 6											+	
<b>Dark Mycelium sterile</b>											+	
<b>Alternaria</b>												
<i>alternata</i> (Fr.) Keissler				+								
<b>Sclerotium</b>												
sp.					+			+				
<b>Cylindrocarpon</b>												
<i>magnusianum</i> (Sacc.) Wollenw. no 1									+			
<i>magnusianum</i> no 2						+						
<i>magnusianum</i> no 3									+			+
sp. 1								+				
sp. 2												+
<b>Aspergillus</b>												
<i>versicolor</i> (Vuill.) Tiraboschi								+				
<b>Arthrobotrys</b>												
<i>oligospora</i> Fres.									+			+
<b>Trichosporella</b>												
<i>cerebriformis</i> (de Vries & Kleine – Natrop) W.Gams									+			
<b>Epicoccum</b>												
<i>purpurascens</i> Ehrenb. ex Schlecht.												+
<b>Phoma</b>												
<i>exigua</i> var <i>exigua</i> Desm.												+
<b>Oidodendron</b>												
<i>cerealis</i> (Thüm.) Barron												+
<b>Botrytis</b>												
<i>cinera</i> Pers. ex Nocca & Balb											+	

## Discussion

There are reports that in tundra soils fungal biomass and abundance are lower than in the soils of other biomes (Schmidt and Bölder 2002). Meanwhile, the total numbers of isolated fungal cfu from the soil originating from the frost fissure between tundra polygons (site 3), with the deepest A horizon and the highest content of C, N and P, were in the range of those found in arable soils in the regions of the temperate climate (Maier *et al.* 2000). This finding supports earlier suggestions that the high content of soil organic matter is a more important factor directly controlling the abundance of fungi in cold environments than low temperatures (Robinson *et al.* 1996, Robinson *et al.* 2004).

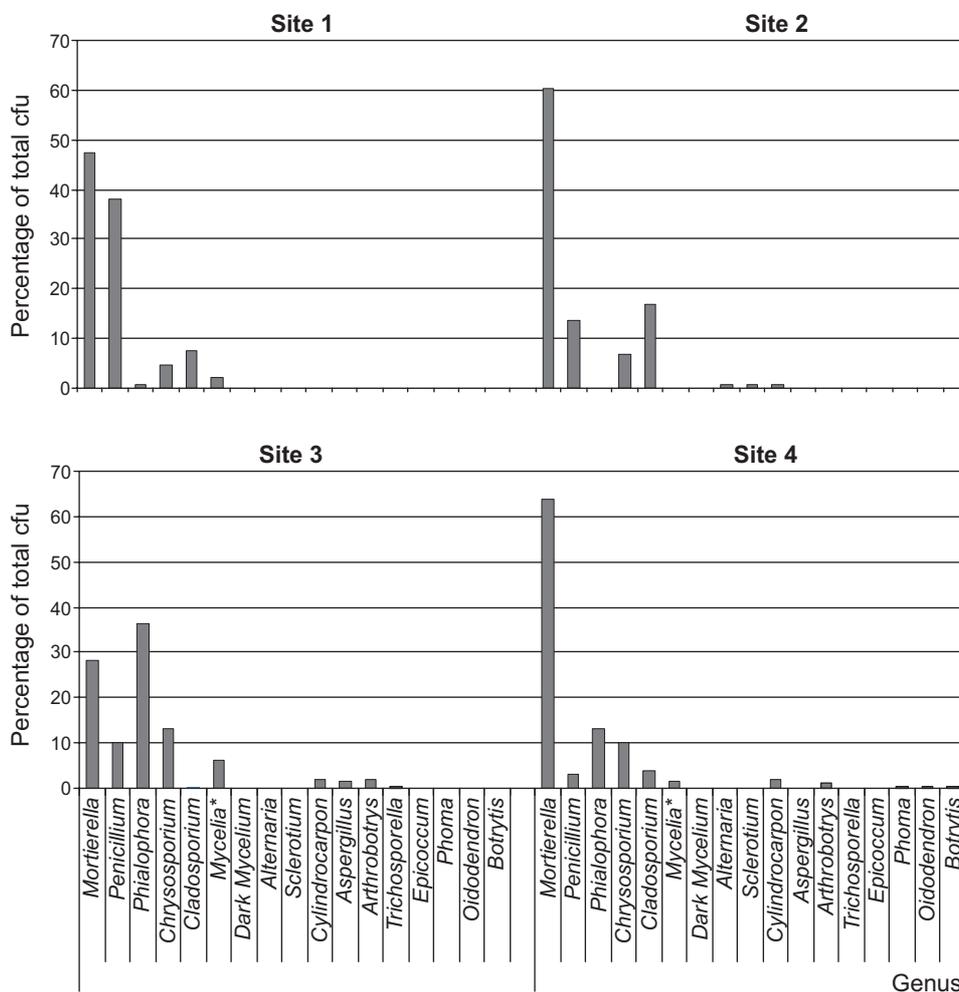


Fig. 4. Species richness of fungal communities isolated from soil collected from various forms of micro-relief in the high Arctic tundra (\*Hyaline sterile).

Psychrotrophs isolated from the high Arctic tundra on Spitsbergen had lower growth rates at 28°C than at 4°C as after 21-day incubation the average 28°C/4°C ratio of colony diameters was 0.75 for rich Malt Extract agar medium. The ratio of colony diameter formed at 26°C and 4°C by psychrotrophs isolated from cryopegs in permafrost near the East Siberian Sea coast by Gilichinsky *et al.* (2005) was 0.77 after 1-month incubation on rich medium.

The presented results are in agreement with those reported by other authors showing that fungal communities inhabiting Arctic and Antarctic soils are mainly composed of psychrotrophic species adapted to growth within a wide range of temperatures changing even during the summer seasons (Bergero *et al.* 1999; Bölter *et al.* 2002).

Table 3  
 Oligopsychrophilic and oligopsychrotrophic taxa isolated from soil collected from various forms of micro-relief in the high Arctic tundra (B – Oligopsychrophiles, C – Oligopsychrotrophs I according to the preferred range of growth temperature).

Genus species	Site 1		Site 2		Site 3		Site 4	
	B	C	B	C	B	C	B	C
<b>Penicillium</b>								
<i>lanosum</i> no 4	+							
<i>lanosum</i> no 6	+		+					
<i>expansum</i> no 1		+		+				
<i>verrucosum</i>						+		+
<b>Phialophora</b>								
<i>fastigiata</i> no 6							+	
<b>Chrysosporium</b>								
<i>pannorum</i> no 1		+						
<i>pannorum</i> no 4		+						
<i>pannorum</i> no 5	+							
<i>pannorum</i> no 6					+		+	
<i>pannorum</i> no 9			+					
<i>pannorum</i> no 11					+			
<i>pannorum</i> no 13					+			
<i>pannorum</i> no 14						+		+
<b>Hyaline Mycelium sterile</b> no 4						+		+
<b>Sclerotium</b>								
sp.			+		+			
<b>Cylindrocarpon</b>								
<i>magnusianum</i> no 1						+		
<i>magnusianum</i> no 2				+				
<i>magnusianum</i> no 3						+		+
<b>Phoma</b>								
<i>exigua</i>							+	

However, fluctuations in the total number of isolated fungal cfu from individual sites as well as in proportions between organisms with different growth temperature characteristics were observed during the tested period of the summer season 1999. This phenomenon could be connected with the reactions of microbes and the plant cover to the considerable variability in air temperature and precipitation in the 1999 summer season in the area under study (Kejna *et al.* 2000). Each of the tested sites was different in respect of their soil properties (amount and quality of organic matter), micro-relief of the surface, and the plant cover. Interactions between these factors probably affected the development of fungal communities during the vegetative seasons.

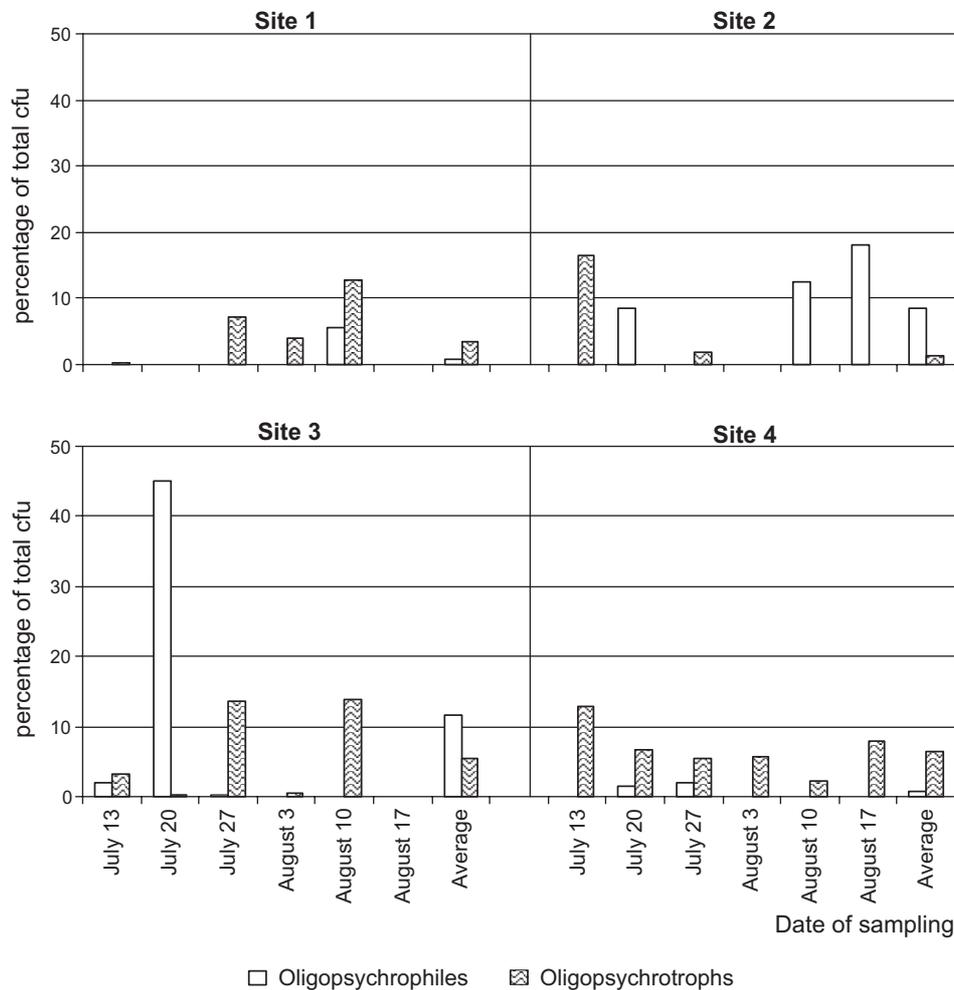


Fig. 5. Occurrence frequency of oligopsychrophiles and oligopsychrotrophs isolated from soil collected from various forms of micro-relief in the high Arctic tundra during the 1999 summer season.

The species spectrum of fungi isolated in these studies includes species isolated earlier from Arctic as well as Antarctic soils, cryopegs in permafrost and Arctic ice as well as some species entrapped in ancient glacial ice (Azmi and Seppelt 1997; Bergero *et al.* 1999; Bölter *et al.* 2002; Gilichinsky *et al.* 2005; Gundecimerman *et al.* 2003; Holding 1981; McRae *et al.* 1999; Onofri *et al.* 2004).

Large differences in species richness were found between the four tested sites. Soil originating from sites 1 and 2 was inhabited by fungal communities with low species richness consisting of 11 and 12 taxa, respectively, belonging to 7 genera. In the 2001 summer season, Robinson *et al.* (2004) found fungal communities with lower species richness and diversity in high Arctic semidesert within the *Dryas octopetala* zone of Svalbard, 3 km west of Ny-Ålesund (78°56'N, 11°50'E) in the

outer fjord zone, at an altitude of 22 m a.s.l. Low species richness was also characteristic of a fungal community isolated by Gilichinsky *et al.* (2005) from cryopegs in permafrost in Siberia and by Bergero *et al.* (1999) from a soil originating from Kane Island of Franz Josef Land. Much higher species richness was found while testing fungal communities isolated from sites 3 and 4. They consisted of 20 and 22 taxa belonging to 11 and 13 genera, respectively. This was in the same range as had been found in communities inhabiting soils originating from Cape Tegethoff and Cape Flora in Franz Josef Land (Bergero *et al.* 1999). Other authors' reports on biodiversity of fungal communities isolated from polar environments offer no information on soil characteristics. The results of the present study suggest, however, that site characteristics, including the content and quality of organic matter and micro-relief of the surface can influence the abundance and biodiversity of the isolated fungal communities.

The percentage of oligopsychrophilic and psychrotrophic fungal taxa isolated from soils of Franz Josef Land by Bergero *et al.* (1999) as well as their growth rate at 5°C were in the same range as those found for fungal taxa isolated by our team from Spitsbergen.

The presented data clearly indicate that the characteristics of fungal communities isolated from sites 3 and 4 are similar in respect of species richness and domination of psychrophiles, but fungi abundance was similar in soils collected from sites 1 and 3. Each studied site represents a different form of surface micro-relief. Among them, the tundra polygon (site 4) and the frost fissure between polygons (site 3) have been least affected by cryoturbation processes (Klimowicz and Uziak 1996). This is probably the reason why soil development processes at these sites are more advanced than at sites 1 and 2. However, the content of total organic C as well as DOC in the A (humus) horizon of the soil originating from the frost fissure between tundra polygons was twice as large as in the soil collected from the tundra polygon and in the same range as in the soil from site 1. Schmidt and Bölter (2002), studying 3 sites on Taimyr Peninsula, Central Siberia, differently affected by cryogenic processes, found a maximal value for fungal biomass in the Oe horizon (containing organic material of intermediate decomposition stage) of a tundra polygon. Other tested sites were non-sorted by steps and stone stripes. According to the authors, polygon tundra soils represent the most stable and oldest stage of soil development. These findings suggest that the abundance of fungi in Arctic soil is mostly affected by the content of organic matter in the A horizon and the plant cover but other factors such as the stage of soil development and micro-relief of the surface are more important for species richness of fungal communities.

The present study as well as other reports (Bergero *et al.* 1999, Gunde-Cimerman *et al.* 2003) also suggest that high Arctic soils, characterized by extreme conditions with regard to several ecological factors, are inhabited by physiologically flexible fungal species able to survive and grow in such an inhospitable environment.

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