CORRELATES OF VIRAL AND BACTERIAL ABUNDANCES IN SIBERIAN AND ANTARCTIC PERMAFROST

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

Despite the widespread occurrence and high abundance of bacteria and viruses in many environments, little work has been published to date on the abundances of viruses and bacteria in permafrost. Therefore, factors that affect viral and bacterial abundances in permafrost are still largely unknown. Permafrost samples from the Kolyma-Indigirka Lowland region of Siberia (70°N, 158°E), and the Beacon Dry Valley in Antarctica (77°S, 160°E) were examined for viruses. Soil type ranged from sands containing little carbon, nitrogen, and ice, to loams containing large quantities of carbon, nitrogen, and ice.

Methodology for optimal virus recovery included testing of various eluants and filter testing. Sodium pyrophosphate was the best eluant (1.34×10^8 viruses g^{-1} dry weight) over phosphate-buffered saline (8.09×10^7 viruses g^{-1} dry weight) and virus-free water (2.46×10^6 viruses g^{-1} dry weight). A glass fiber filter and 0.45 µm polyvinylidene fluoride filter was the best combination (3.32×10^8 viruses g^{-1} dry weight) over no filtering at all (too much detritus to count viruses), a 0.40 µm polycarbonate filter (2.30×10^7 viruses g^{-1} dry weight), or a 0.45 µm nitrocellulose filter (3.10×10^7 viruses g^{-1} dry weight). Sonication did not aid in virus recovery from permafrost (2.59×10^8 viruses g^{-1} dry weight, no sonication; vs. 2.49×10^8 - 2.70×10^8 viruses g^{-1} dry weight, 10 - 300 seconds, 25 Watts sonication).

Viral and bacterial presence was determined by epifluorescence microscopy; viral abundances ranged from 2.83×10^4 to 3.32×10^8 viruses g^{-1} (dry weight); bacterial abundances ranged from 1.02×10^3 to 4.09×10^8 bacteria g^{-1} (dry weight). Physical and chemical characteristics of permafrost were compared with viral and

bacterial abundances. Permafrost type was associated with viral abundance, with loamy soils giving the highest abundances (~10^8 viruses g^{-1} dry weight) and sands giving the lowest abundances (ranging from below the detection limit of $2.83x10^4$ to ~10^6 viruses g^{-1} dry weight). Viral abundance was also associated with higher carbon, nitrogen, and ice contents, and a lower pH. This is consistent with other research into bacterial abundances in permafrost, which have shown that loamy soils have higher abundances, and sands have much less abundances, usually due to the scarcity of water and organic matter available.

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INTRODUCTION

Temperature is one of the most important parameters regulating biological processes in the natural environment. Therefore it cannot be ignored when considering the impact microbes have on our ecosystem. Indeed, more than 80% of the earth's biosphere is permanently cold (below 2°C) (Gilichinsky and Wagener, 1995). Microbes have adapted to exist in all environments, including the very cold polar regions. Because of their ability to adapt to cold temperatures and live at the extreme limits of life, they can be considered the most successful colonizers on the earth.

Permafrost

Permafrost is rock or soil remaining at or below 0°C for two or more years. It is not determined by soil moisture content, overlying snow cover, or location; in fact, permafrost can contain over 30% ice, or practically no ice at all. Permafrost underlies about 20% of the Earth's land surface. It occurs in more than 50% of Russia and Canada, 85% of Alaska, 20% of China, and probably all of Antarctica (Rivkina *et al.*, 2000; Pewe, 1998).

Climate is the main factor determining the existence of permafrost. However, the spatial distribution, thickness, and temperature of permafrost are highly dependent on the temperature at the ground surface. This temperature, although strongly related to climate, is influenced by several other environmental factors such as vegetation type and density, snow cover, drainage, and soil type (Heginbottom, 2000).

Permafrost can exist for all soil types, with sands and loams at the extremes of a continuum. Loamy permafrost in Northeastern Siberia is characterized by a relatively high concentration of organic carbon (0.7-1.5% total mass) and a water pH of 5.0-7.0. Sandy permafrost generally has a lower organic content (0.1-1.0% total mass) and a lower water pH of 3.5-5.0 (Spirina and Fedorov-Davydov, 1998). Antarctic permafrost generally has little organic content, plus the very low humidity of the continent makes for very little breakdown of what organic material there is (Negoita *et al.,* 2001).

Permafrost age

In Siberia, the oldest continuously frozen permafrost in the Northern Hemisphere dates back about 3 million years (Gilichinsky, 1997). In the Southern Hemisphere, Antarctic conditions leading to continuous permafrost started 35-40 million years ago (Kennett, 1977). Permafrost sediments can be dated by several methods: paleomagnetism (dating the sediment by its magnetic field polarity) (Thompson, 1986), tephrochronology (dating volcanic products) (Perrson, 1971), luminescence dating (at deposition the sediments are 'zeroed' by their electrons becoming trapped in a crystal lattice; exposure to light or heat will cause electron luminescence, the intensity is related to how long the sediment has been buried) (Prescott and Robertson, 1997), and $^{230}Th/^{238}U$ dating (Schirrmeister *et al.,* 2002) are among the most common used. Biological material in permafrost can be additionally dated using oxygen isotope chronostratigraphy (Prell *et al.,* 1986), carbon-14 dating (Taylor, 1987), or amino acid geochronology (aminostratigraphy) (Brinton *et al.,* 1999).

Aminostratigraphy has been used to date organisms in Antarctic and Siberian permafrost cores (Brinton *et al.*, 1999). This technique measures the degree of racemization that has taken place since the death of an organism. Organisms possess mainly the L (levo-isomer) form of amino acids, and in living organisms this results in a low D/L ratio, which is maintained through repair mechanisms. After death, however, the ratio changes, and the new D/L ratio can be used to determine age of the organism. As an example (Brinton *et al.,* 1999), a Siberian permafrost sample from 4.9 m depth was determined to have an aspartic acid D/L ratio of 0.24, which gives a racemization age of 2.4 million years, which was consistent with the age determined by other stratigraphic methods such as event dating and luminescence.

Survival of viable organisms and DNA over long time periods

Originally, it was thought that permafrost would not be a likely candidate for finding viable microbes, due to its permanently frozen condition and the small quantity of liquid water present. Consequent studies have shown this is not the case, with large numbers of viable microbes recovered from various permafrost sediments. It is thought that the microbes could survive for so long because cellular DNA has a higher chance of survival if kept at a cold and dry environment, and permafrost delivers these prerequisites.

Generally, nucleic acids, if not under constant repair, will eventually degrade due to various environmental influences (such as oxidative damage or DNA-degrading enzymes). However, the particulate constituents of soils and sediments (such as clay minerals), as well as organic compounds (such as humic acids) can

bind and form complexes with DNA, allowing it to escape degradation. Some experimental observations have indicated that the half-life of DNA may be very long in sediments, especially if the DNA is inside dead cells (Marota and Rollo, 2002).

Microbes in permafrost: previous work

Viable microbes in permafrost were first discovered in the late 19th and early 20th centuries by Russian scientists studying Siberian mammoth deposits (Omelyansky, 1911, cited in Gilichinsky *et al.,* 1995). Further research in northern Canada and Alaska (Kjoller and Odum, 1971) revealed viable microbes at depths of up to 18 m and 70,000 years old. Unfortunately, these studies may have suffered from biological contamination by the drilling fluid used to extract the permafrost cores; it was not until drilling-fluid use was discontinued and rigorous non-contamination protocols were introduced that reports of viable microbes in permafrost could be trusted.

Because of permafrost's permanently frozen state and small quantity of liquid water, it was thought to be unfavorable for maintaining viable microbes. However, work by various Russian scientists (Zvyagintsev *et al.*, 1985, 1990; Gilichinsky *et al.*, 1988, 1992; Khlebnikova *et al.*, 1990), has shown that permafrost contains large numbers of viable microorganisms, which when thawed, resume physiological activity.

In the first reviews of these studies, Gilichinsky (1993) and Zvyagintsev (1993) reported the presence of viable microbial cells in permafrost samples from cold regions of both the Northern and Southern hemispheres. The deepest were recovered from depths of more than 300 m in the MacKenzie Delta. As well, a

diversity of microbes was isolated including bacteria, fungi, and actinomycetes. Aerobic and anaerobic, as well as spore-forming and sporeless microbes were isolated. Also, microbes with thick cell walls and capsules were common. Most of the microbial isolates found were psychrotrophic or mesophylic, but some thermophiles were also recovered. Generally, the microbes recovered were oligotrophs, and most were resistant to the stress of freezing and thawing. They found that the diversity and number of viable microbes was related to the age of the permafrost, the older the permafrost the lower the abundance of viable cells.

There is the suggestion that the microbes are not in a state of cryoanabiosis, but are metabolically active, with unfrozen water serving as a cryoprotector. For example, the ability of immobilized enzymes in permafrost to become instantly activated (Vorobyova and Soina, 1994), the simultaneous presence of nitrifying bacteria and nitrites (Jansen and Bock, 1994), or methangenic archea and methane (Rivkina and Gilichinsky, 1995) all suggest metabolic activity. Also, the numbers of viable microbes was related to the sediment texture, the finer the texture of sediment the higher the abundance of viable cells.

The temperature of permafrost is very stable; Siberian permafrost is -10°C for the entire year, irrespective of the air temperature. This stable environment is conducive to the continued presence of microbes, as the environmental parameters remain basically unchanged for long periods of time. Moreover, up to 7% of permafrost water remains as liquid and forms a thin film around the sediment particles. It is kept in liquid form by surface tension and pressure forces. The amount of liquid water in permafrost varies from near 0% in sandy permafrost, to 5-

7% in loamy permafrost. This water functions as a cryoprotector against ice crystal formation in cells, as a nutrient medium, and as a means of escape for metabolic waste products (Gilichinsky and Wagener, 1995).

In natural environments, the number of microbial cells varies. Epifluorescence microscopy counts in permafrost are comparable to those found in sediments and soils:

Environment	**Total Counts (cells g^{-1} dry weight)**
Soils	10^8-10^{10}
Sediments	$<10^6$-10^{10}
Permafrost sediments	10^7-10^9
Permafrost buried soils	10^5-10^9
Lake sediments	10^8-10^9
Ocean sediments	10^7-10^8
Groundwater	10^3-10^6 (cells mL^{-1})
Antarctic glaciers	~0 (cells mL^{-1})

(Adapted from Vorobyova *et al.,* 1997.)

In the Siberian permafrost, scientists have found large numbers (10^8 cells g^{-1}) of living bacteria under conditions of low temperatures, low nutrient concentrations, and a closed and generally reducing ecosystem. Generally, the number of viable microbes and overall microbial diversity depends on the permafrost age, with lower abundance and diversity in older samples (Shi *et al.*, 1997; Gilichinsky and Wagener, 1994; Gilichinsky *et al.*, 1992; Khlebnikova *et al.*, 1990; Zvyagintsev *et al.*, 1990; Gilichinsky and Wagener, 1995).

In the Antarctic less work has been done, but viable microflora have been found in permafrost older than the late Pleistoscene (1-2 million years old) (Gilichinsky *et al.*, 1995; Cameron and Morelli, 1974).

Molecular studies of permafrost microbes

To date, there has been little molecular research into permafrost microbes. One study (Shi et al., 1997) used 16S rDNA sequencing of eubacterial isolates from Siberian permafrost, in order to determine their identity and phylogenetic relationships to each other and to known microbes. Partial 16S rDNA sequences for 29 isolates from Siberian permafrost were obtained, and the relationships among these and known eubacterial species were analyzed by maximum parsimony. In this study the sequences were obtained from isolates rather than by direct extraction of nucleic acids from the environment. The maximum parsimony trees placed the Siberian isolates in four major groups, high-GC Gram-positive bacteria, β-proteobacteria, γ-proteobacteria, and low-GC Gram-positive bacteria, similar to modern-day environmental isolates from soils.

Another study (Marsic *et al.*, 1999) examined viable moss isolated from Siberian permafrost at a depth of 100 m and estimated to be about 40,000 years old. Comparative sequence analysis with other 18S RNA showed very high sequence identity to an existing moss species.

Viruses in soils and sediments

There are numerous factors that can affect persistence of viral particles and DNA in soil. Persistence of viral particles may be affected the presence or absence of proteolytic microbes, soil pH, soil temperature, predation, soil components, and water transport (Jaques and Huston, 1969; Thomas *et al.*, 1973; Lopson and Stotzky, 1983).

Viruses have a strong tendency to bind to soil and mineral particles (Hurst, 1997; Hurst *et al.,* 1980; Lefler and Kott, 1974; Moore *et al.*, 1981; Taylor *et al.*, 1980), so most viruses in soil samples will likely be adsorbed to particle surfaces. This reversible adsorption occurs by charged-colloidal-particle charged-surface interactions (Moore *et al.,* 1982.). The electrostatic interactions are pH-dependent, being stronger at lower pH levels (Taylor *et al.*, 1981); therefore the eluants used to remove viruses from soils normally range from neutral to alkaline pH. However, successful elution of the viruses not only depends on the physico-chemical characteristics of the soil, but also on the morphological characteristics of the viruses (Ostle and Holt, 1979).

One of the most common groups of viruses found in terrestrial soils are enteric viruses. Research has been done on several topics relating to soil viruses, including the extent of viral adsorption to soil particles (Moore *et al.*, 1982), the ability of viruses to persist in soil (Bitton *et al.*, 1979), and the migration of viruses through soil in association with groundwater (Hurst *et al.*, 1980).

Another major family of soil viruses are the baculoviruses, which are pathogens of insects, crustaceans, and arachnids. Many of the *Baculoviridae* are contained within a polyhedron, a proteinaceous body which seems to act as a barrier between the virion and the environment, allowing the virion to remain viable for long periods of time (Rohrmann, 1992; England *et al.*, 1998).

Viruses are also abundant in marine sediments (Danovaro *et al.,* 2001, 2002; Bird *et al.,* 2001). Marine sediments are characterized by high concentrations of

organic matter and high bacterial abundances (10^8-10^9 cells mL^{-1} in sediments, compared to 10^5-10^6 cells mL^{-1} in the overlying water column).

Viral presence in subsurface sediments has not been extensively researched to date. One study (Bird *et al.,* 2001) reported the presence of viruses in subsurface sediment layers greater than 14,000 years old. In this study, the ratio of viruses to bacteria was about 3. However, several studies (Danovaro *et al.*, 2001, 2002) have shown that in deep-sea sediments virus abundance is less than bacterial abundance, with very low virus-to-bacterium ratios (most less than 0.20). However, in these studies the samples were fixed with formalin or glutaraldehyde, which gave significant reductions of virus counts after 24 hours of sediment storage.

Viruses in polar ice

Viral genetic material has been observed in polar sea ice (Maranger and Bird, 1994) and glacial ice (Castello *et al.,* 1999). However, ancient ice is not conducive to long-term survival of biological systems. The sterility of ancient ice can be explained by several factors (Gilichinsky *et al.*, 1995), including damage to cells (and viruses) by ice crystals formed both inside and outside, cell (and virus) exclusion from the ice during freezing, and cell (and virus) degradation due to metabolic waste build-up ("biochemical death"). However, the nature of permafrost, with its thin film of liquid water around the sediment particles, means these events don't take place, thus permafrost is much more hospitable to long-term survival of microbes, including viruses.

Correlates of viruses with soil characteristics

There has been little work on the correlation of viral abundance with physical factors in soils and sediments. However, several studies (eg. Campos *et al.,* 2000; Schijven *et al.*, 2002; Quanrud *et al.*, 2003) have been done examining the retention of viruses in soils as a method for filtering out pathogens from wastewater. Moisture content, pH, organic matter content, and bacterial presence have all been implicated in affecting virus retention, with soil type and amount of water saturation having the greatest effects. Coarse-grained soils such as sands retain viruses the least, while finer-grained soils such as loams are much better at retaining viruses. As well, there is a high positive correlation between virus retention in soil and soil organic carbon content (Schijven *et al.*, 2002). Virus retention increased with increased soil moisture content (up to the saturation point). As well, it decreased with an increase in soil pH (the iso-electric pH of many viruses is less than 5.0, which is below the pH range of most soils. Therefore both the viruses and soils have a net charge, so viruses are not bound to soils by ion-exchange reactions, except in highly acidic soils.) (Campos *et al.,* 2000).

Purpose of this research

Viruses are abundant in aquatic and terrestrial systems (Fuhrman, 1999; Ashelford *et al.*, 2003), and may have an important effect on microbial loop dynamics and biogeochemical cycling of organic matter (Wilhelm and Suttle, 1999). Despite the widespread occurrence and high abundance of bacteria and viruses in many environments, little work has been published to date on the abundances of viruses and bacteria in permafrost. Therefore, factors that affect viral and bacterial

abundances in permafrost are still largely unknown. The specific questions this research will address are as follows:

1. Is there evidence of viruses in Siberian and Antarctic permafrost? Viruses have been found in many different habitats, but to date permafrost has not been examined for their presence.
2. Are there differences in virus abundance between Siberian and Antarctic permafrost? If so, what is this difference related to? Even though both geographic areas contain permafrost, the environmental conditions are not the same and so may affect the abundances of viruses.

MATERIALS AND METHODOLOGY

Permafrost sampling locations and characteristics

Permafrost samples were provided by Dr. James Tiedje from the Centre for Microbial Ecology, Michigan State University, East Lansing, USA. Samples were originally obtained from Dr. David Gilichinsky, Russian Academy of Sciences, Pushchino.

The Siberian permafrost samples came from Northeast Siberia, in the Kolyma-Indigirka Lowland region (70°N, 158°E, Figure 1). This area has an arctic climate, a mean annual temperature of -13.4°C, and annual precipitation of 229 mm (Shi *et al.*, 1997). The permafrost in this area can reach thicknesses of up to 500 m, and freezing conditions have persisted for the last 2-4 million years (Gilichinsky *et al.*, 1995). The Kolyma region permafrost comprises of three depth-horizons (Shi *et al.*, 1997): The "Alas" horizon comprises the upper layer from the surface to 3 m depth and is up to 5000-8000 years old. The "Yedoma" horizon comprises depths from 3-8 m and is 10,000-50,000 years old. The "Olyor" horizon comprises the deepest depths from 8-50 m and is 0.3-3 million years old.

The Antarctic permafrost samples came from Beacon Valley, a dry valley situated in the most westerly part of the Kukri Mountains (77°S, 160°E, Figure 2). These dry valleys form the largest ice-free area on the Antarctic continent, and are subject to low temperatures, limited precipitation and salt accumulation.

Permafrost sample collection and treatment

The cores were obtained with a drill coring device that operates without a drilling fluid, which would contaminate biological samples (see Shi *et al.,* 1997).

After removal from the corer, the cores were split widthwise into sections (5-7 cm in diameter), placed into sterile aluminum canisters, sealed, and kept frozen during storage and transport (Figure 3). Contamination with organisms nonindigenous to the frozen core sample can be excluded on the basis of previous method validation (Shi *et al.,* 1997). Briefly, 2 h before drilling, the core drilling barrel was seeded with a pure culture of *Serratia marcescens*. Also, frozen core segments were seeded with a pure culture of S. *marcescens* for several hours to several months at -10°C. In both tests, S. *marcescens* was found only on the surface of the frozen sample, never inside the frozen cores.

Upon arrival at the University of British Columbia, the cores were kept at -20°C. See Table 1 for permafrost sample information. For experimental work, the cores were fractured with a sterile knife (Figure 4). The outer surface material of the core was used for the development of methodology; the inner center of the core only was used for work requiring sterility of the samples.

Physico-chemical characteristics of permafrost

Several permafrost characteristics were examined: water (ice) content, pH, total carbon content, organic carbon content, and nitrogen content.

Ice content was determined by comparing wet vs. dry masses of permafrost samples. Samples were weighed wet, dried at 60°C for 72 h, and then weighed again dry. Meltwater from the samples was collected and the pH determined.

Organic carbon content of the samples was determined by the ash-free-dry-weight method using a muffle furnace. Approximately 1-2 g of each sample was

dried at 60°C for 72 h, weighed, and then dried at 800°C for 4 h. Samples were then cooled in a dessicator for 2 h and re-weighed.

Total carbon and nitrogen content of the samples was determined using a Carlo Erba NA-1500 Analyzer. In this procedure, samples are introduced into a combustion column reactor at 1050°C, where they melt in a temporarily enriched atmosphere of oxygen. The eluted combustion products (a mixture of CO_2, NO_x, and water) are swept into a second column (the reduction reactor). Here the excess oxygen is removed and the NO_x is reduced to N_2. The N_2, CO_2, and water are then swept through a water-absorbing filter containing magnesium perchlorate. Gas separation is on a chromatographic column maintained at 54°C, then detected by thermal conductivity. Samples were prepared as described (Verardo *et al.,* 1990). Approximately 30-35 mg dry weight of each sample was used for this procedure. After the sample run, linear regressions (least-squares method) of carbon and nitrogen vs. area counts were completed (including standards). The following algorithms were used to compute the carbon content of the samples (Verardo *et al.,* 1990):

$$A_{COR} = A_{TOT} - A_{BL}$$

$$M_C = (A_{COR}) \times (S)$$

$$\%C = (M_C/M_S) / 10$$

Where A_{COR} is the corrected area counts of the sample, A_{TOT} is the area counts of the sample, A_{BL} is the area counts of the blank standard, M_C is the mass of carbon in µg, S is the slope of the standard calibration curve, M_S is the mass of the sample in mg, and %C is in weight percent.

Statistical analysis was made on pH, ice content, total and organic carbon contents, and nitrogen contents, using the Student T-test.

Recovery of virus-like particles from permafrost

Viruses are difficult to extract from sediments due to adsorption of the viruses to particles. There are many different methods for separating viruses from sediment particles: sonication of the sediments (eg. Danovaro *et al.,* 2001, 2002), the use of eluting agents (eg. Bird *et al.,* 2001; Maranger and Bird, 1996; Hurst, 1997), physical shaking (eg. Danovaro *et al.,* 2001), and the use of filters (eg. Hurst, 1997) are some of the most common methods. In an effort to maximize virus-like particle extraction, all methods were tested.

Eluant testing

Two eluants that are commonly used for extracting viruses from sediments were tested, 10 mM phosphate-buffered saline (PBS) and 10 mM sodium pyrophosphate (NaPyr) (Bird *et al.,* 2001; Velji and Albright, 1986; Maranger and Bird, 1996; Danovaro *et al.,* 2002). Virus-free water was used as a control. One gram of permafrost sample was added to 4 mL of each eluant (in triplicate). The sample was then vortexed to dislodge particles from the sediment, and then spun using an Eppendorf Centrifuge 5403, 16A4-44 rotor at 2600 x *g* for 30 min at 4°C to pellet the sediment and collect the bacteria-and-virus-rich supernatant.

Filtering

Various filters were tested to determine which filter combination resulted in the best removal of detritus, while maximizing virus-like particles counts. An

unfiltered sample was tested for comparison. A negative control consisted of a sample of virus-free water.

Sonication

The effects of sonication were tested to determine if virus extraction could be maximized using this method. 0.3 g of permafrost sample (in triplicate) was added to 5 mL of 10 mM sodium pyrophosphate. Samples were sonicated using the Vibra-cell Ultrasonic Processor model VC250, pulsed 70% duty cycle, with a micro tip limit of 4 and 25 watts output. After the sonication treatments, the samples were incubated at 37°C for 1 h, vortexed briefly, then spun down at 800 x *g* for 15 min at 20°C. Analysis of variance was performed to determine the best sonication regime for optimal virus recovery.

Enumeration of bacteria and virus-like particles

Virus-like particles (VLPs) were enumerated by epifluorescence microscopy. Slides were made in triplicate (true replicates) of filtered permafrost samples. After eluting with NaPyr, spinning out the particulate matter, and filtering with a GF/F and 0.45 µm HV filter, 1.60 mL of each sample was gently filtered (15 kPa vacuum) onto a 0.02 µm pore-sized Anodisc 25 filter (Whatman) using a 10 place filtration unit (Hoefer Scientific Instruments). The filters were placed on 20 µl drops of SYBR-1-Green stain (18 µl virus-free water, 2 µl SYBR stain), incubated in the dark for 15 min, and rinsed once with 1 mL of virus-free water. The filters were then placed on glass slides, covered with 15 µl of SYBR mountant (50% glycerol, 50% PBS, 0.1% p-phenylenediamine) and a cover slip, and examined immediately. Statistical analysis of virus counts to soils types was by analysis of variance. The Student T-

test was used to analyze virus counts between samples from the same core at different depths.

Counts by epifluorescence microscopy were made following the protocol of Noble and Fuhrman (1998). Briefly, the protocol was as follows: For each filter, 10-20 fields were selected randomly and at least 200 VLPs and bacteria were counted using an Olympus AX70 epifluorescence microscope under 1000 x magnification under blue excitation. Virus-like particles were distinguished as bright green fluorescing points of light; bacteria were identified by the same fluorescence but much larger size. Detritus was easily identifiable by its yellow fluorescence and irregular shape. Slides were stored in the dark at -20°C until counted. Bacteria and virus-like particle counts were determined for all permafrost samples (in triplicate).

Location	Sample no.	Depth (m)	Soil Type	Notes
Siberia	3/90 (#1)	2.5	Muddy loam	Same well
	3/90 (#2)	10.0	Mud	
	6/91 (#2)[1]	1.3	Mud	Same well; sea sediment
	6/91 (#1)[1]	8.9	Mud	
	4/91[2]	21.9	Muddy silt	Sea sediment
	3/93	32.3	Muddy silt	
	N2951 (#1)	1.85-1.9	Muddy loam	Same well
	1/95 (#3)	10.1-10.15	Muddy loam	
	N5121 (#2)	15.10-15.15	Muddy loam	
	1/98 VAJ	53.0-53.5	Sand	
	2/95 VAJ	5.0	Mud	
	211-99	1.9-1.95	Loam	Buried soil
	204-99	0.03-0.05	Loam	Modern soil
	14/99	24.6-24.65	Sand	
Antarctica	2/95 HOE	2.31-2.35	Sandy mud	
	4/95[3]	0.75-0.79	Coarse sand	Same well
	4/95 DAV[3]	4.05-4.10	Sandy mud	
	X1/99 (#1)	3.6-3.9	Fine sandy mud	Same well
	X1/99 (#2)	5.4	Sandy silt	
	3/99	Surface	Fine silt	Modern soil
	11/99	0.35-0.47	Sand	
	7/99	Surface	Sandy silt	Modern soil

Table 1: Permafrost cores
Numbers in parentheses distinguish between samples collected from the same well.
[1-3] These samples were cross-referenced (Kochkina *et al.*, 2001) to determine their approximate age:
[1] 5-10,000 years old
[2] $(1.2 - 2.0)\times10^4$ years old
[3] $(1.8 - 2.1)\times10^5$ years old

Figure 1: Location of where Siberian permafrost cores were gathered. Arrow represents approximate location of Kolyma-Indigirka Lowland region.

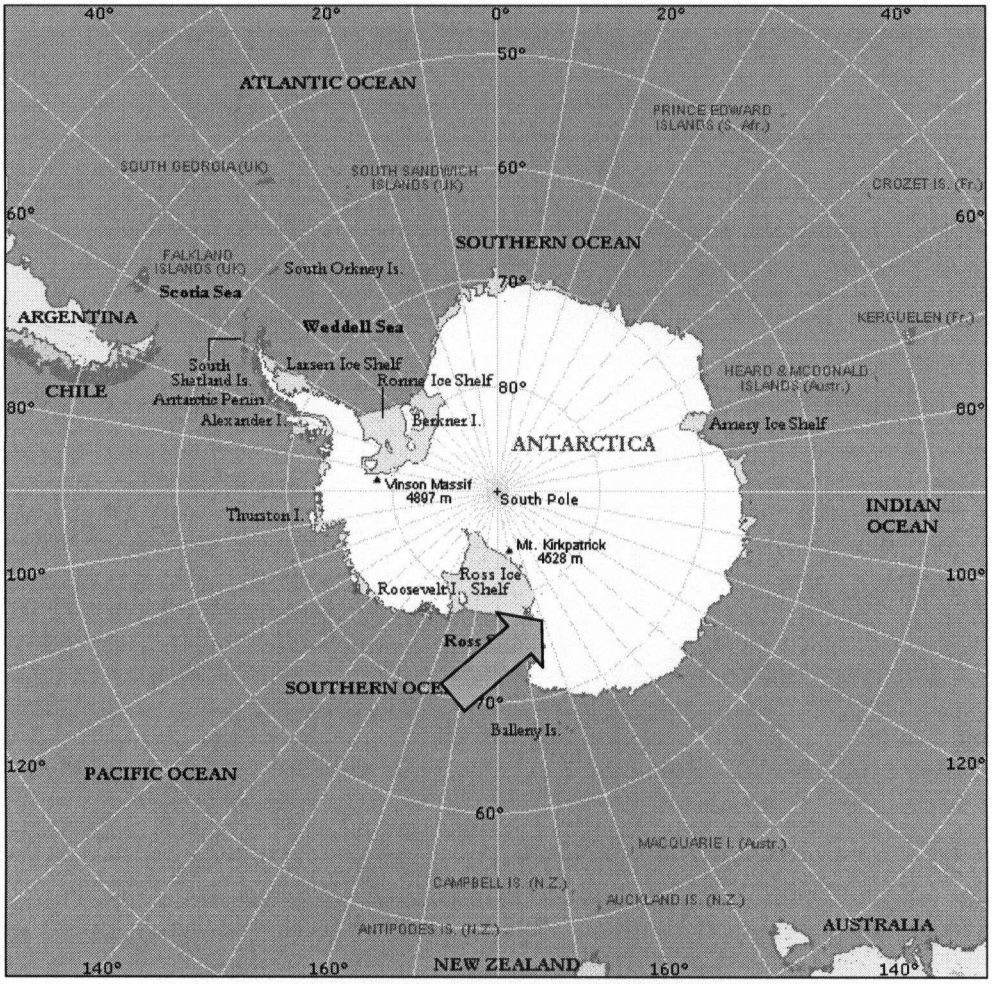

Figure 2: Location of where Antarctic permafrost cores were gathered. Arrow represents approximate location of Beacon Dry Valley.

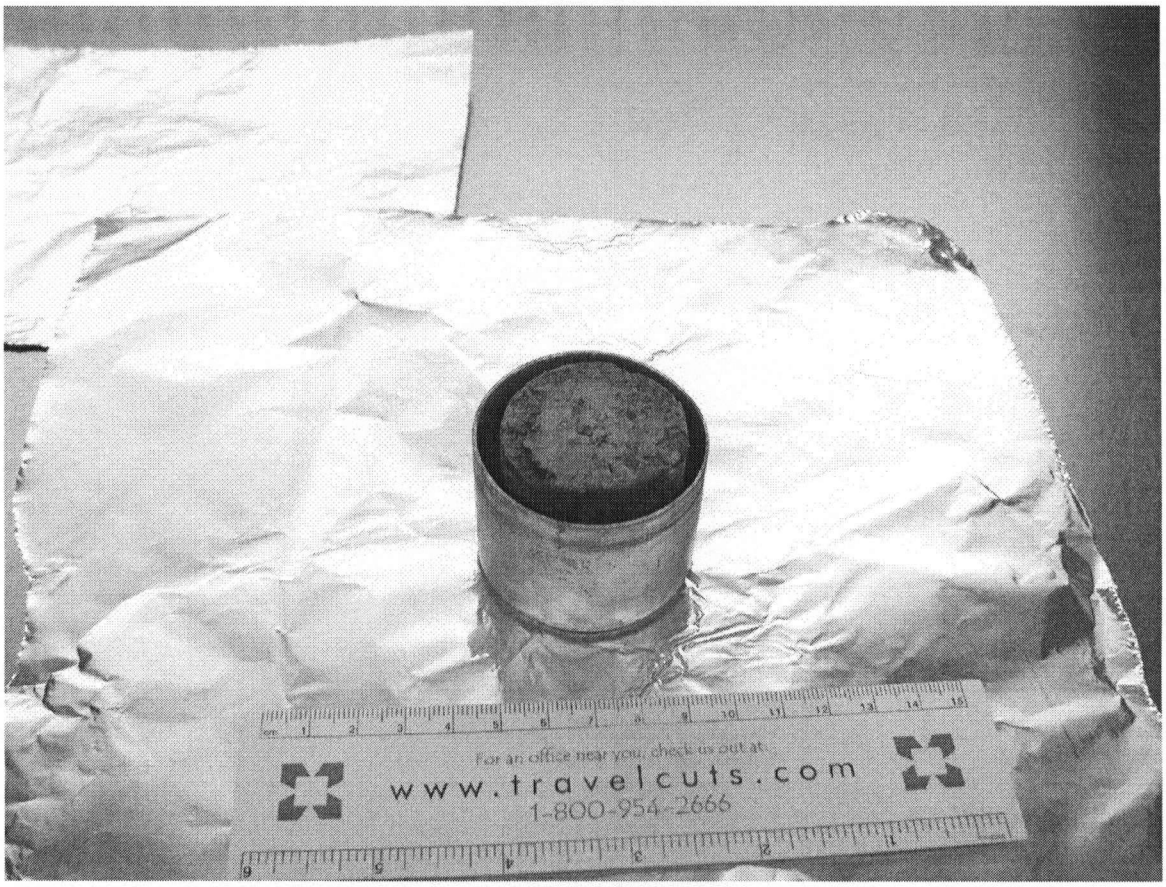

Figure 3: Siberian permafrost core 204-99 in aluminum transport canister.

The samples were sealed in these sterile canisters and kept frozen during storage and transport.

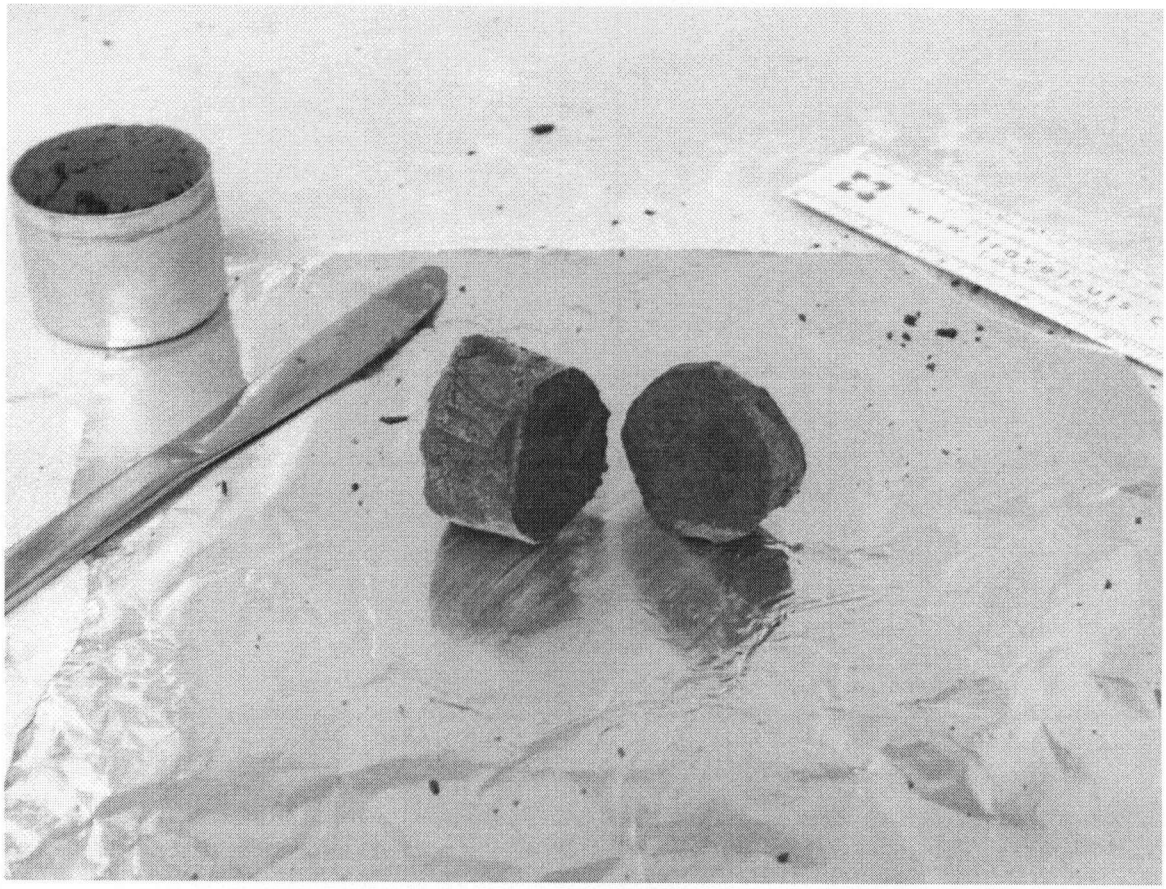

Figure 4: Siberian permafrost core 204-99, after being split to access the sterile material inside.

RESULTS AND DISCUSSION

Thesis contributions

This thesis makes several important contributions to our understanding of polar microbial communities. I was able to develop a protocol to effectively extract viruses and bacteria from permafrost communities. I present the first data on the abundances of viruses in permafrost communities. I was also able to make comparisons among viral and bacterial abundances from Arctic and Antarctic permafrost communities, and show that the characteristics of the permafrost are very important determinants of viral and bacterial abundances.

Permafrost sites and cores

Fourteen permafrost cores were obtained from Siberia, and eight cores from Antarctica. Most of the cores obtained were geographically distinct from each other, with the exception of two Antarctic samples (4/95 and 4/95 DAV; X1/99 #1 and X1/99 #2) and three Siberian samples (3/90 #1 and 3/90 #2; 6/91 #1 and 6/91 #2; N2951, 1/95, and N5121); these came from the same well but at different depths.

Soil type ranged on a continuum from sand and loam at the extremes, and with various combinations of sand, silt, mud, and loam (Table 1). Typically, the Antarctic samples were primarily sand-based, while the Siberian samples had more diverse soil types. Depth of the samples ranged from surface soils to deep subterranean sediments up to 53 m deep. Generally, the Antarctic samples were recovered from more shallow depths than the Siberian samples.

Physico-chemical characteristics of permafrost

The physical and chemical properties of the samples were examined. Ice content, pH, total carbon content, organic carbon content, and total nitrogen content were determined (Table 2).

Ice content ranged from very dry (approaching 0% of the total mass of the sample) to very wet (approaching 40% of total wet mass). Generally, the Antarctic samples were very dry and sandy, with ice content not exceeding 14%, while the Siberian samples exhibited loamy soils and more varied ice contents ranging from just over 7% to almost 40%. Ice content of the Siberian samples was significantly different ($p<0.05$) from the Antarctic samples. This corresponds with a review by Gilichinsky and Wagener (1995); they mentioned that sand contains very little liquid water (~0%), while loams typically contain 5-7% liquid water.

The pH of the Antarctic samples was generally higher than those of the Siberian samples (Table 2). The pH of the samples ranged from moderately acidic (5.32) to moderately alkaline (9.77). Siberian samples averaged a pH of 7.17; whereas Antarctic samples averaged a pH of 8.51. There was a significant difference ($p<0.05$) between the pH of Siberian permafrost and Antarctic permafrost. Soils tend to become acidic when rainwater leaches away basic ions (calcium, magnesium, potassium and sodium), carbon dioxide from decomposing organic matter and root respiration dissolve in soil water to form a weak organic acid, and strong organic and inorganic acids form (eg. nitric and sulfuric acid) from decaying organic matter and oxidation of ammonium and sulfur fertilizers (Bickelhaupt, 2003). The Antarctic environment is generally considered to be very

dry (as evidenced by the low ice content), so ions were not being lost via leaching. Also, the organic content of the Antarctic samples is very low, indicating little decomposition and acid formation to lower the pH of the permafrost. Conversely, the lower pH of the Siberian samples can perhaps be attributed to the same reasons as above. Generally for the Siberian samples, the higher the ice content, the lower the pH (Table 2).

Total and organic carbon contents were both determined for all the permafrost samples (Figure 5). Siberian samples had significantly higher total carbon content by weight than Antarctic samples (4.79% $\pm$ 3.11, n=14; and 1.23% $\pm$ 0.91, n=8 respectively; p=0.005). Siberian samples had significantly higher organic carbon contents than Antarctic samples (2.10% $\pm$ 1.98 and 0.12% $\pm$ 0.14 respectively; p=0.01), due to some Siberian samples being very organic-rich, while all Antarctic samples were organic-poor.

Total nitrogen content of all the permafrost samples was determined (Figure 5). Again, the Siberian samples had significantly higher nitrogen contents than the Antarctic samples (0.16% $\pm$ 0.10 and 0.03% $\pm$ 0.02 respectively; p=0.02), due to some samples being organic-rich.

Generally, the Siberian permafrost samples contained more organic material than the Antarctic samples. This relates to the lower pH of the Siberian samples; as well this relates with the paucity of life in Antarctica (microbial or otherwise) compared with Siberia. In Antarctica, it is difficult for organic-containing permafrost to form as there is not a lot of organic material present and very low humidity, so there is little opportunity for organic breakdown (Negoita *et al.*, 2001). Negoita *et al.*

(2001) showed that Antarctic modern soil contained low amounts of organic carbon (0.01 - 4.11% of total carbon), and nitrogen (0.014 - 1.393%). Higher abundances of organic material mean more microbes will be in evidence, and we see this with the higher bacterial and viral abundances in the Siberian samples than in the Antarctic samples.

Eluant testing

Two eluants were tested on permafrost sample 204-99: 10 mM phosphate buffered saline (PBS) and 10mM sodium pyrophosphate (NaPyr). Both PBS and NaPyr are common eluants used for extracting viruses from soil and sediment matrices. Permafrost extracted in virus-free water comprised the negative control.

Average VLP number was significantly higher ($p<0.05$) in both the PBS and NaPyr treatments compared to the water control (Figure 6). Studies (eg. Bird *et al.*, 2001; Lukasik *et al.*, 2001) have shown that both NaPyr and PBS are effective eluants for extracting viruses. Comparing PBS and NaPyr as eluants for extracting from permafrost, NaPyr was better ($p<0.05$) at extracting viruses (average VLP was 1.34×10^8 $g^{-1} \pm 3.01 \times 10^7$) over PBS (average VLP was 8.09×10^7 $g^{-1} \pm 1.88 \times 10^7$).

Filtering of permafrost samples

Due to the high sediment content of the eluted samples, filtering out the debris was necessary in order to facilitate virus counting. Several filters were tested on permafrost sample N2951 to determine the optimal combination for filtering out particulate matter while retaining viruses (Figure 7). An unfiltered sample was tested for comparison; virus-free water comprised the negative control.

With both the unfiltered sample and the sample filtered with just a GF/F filter, there was too much detritus to accurately count virus-like particles. When another filtering step was added to the GF/F treatment, detritus amounts decreased and it became possible to count virus-like particles. However, when the filter pore size was decreased from 0.45 µm to 0.20 µm, the number of recoverable virus-like particles decreased. The best results (highest counts with least detritus) came from using a GF/F filter and a 0.45 µm filter. The filter which worked best ($p<0.05$) for removing detritus but leaving the virus particles was a 0.45 µm pore size HV (polyvinylidene fluoride) filter, with 3.32×10^8 g^{-1} VLP $\pm$ 1.53×10^7 (triplicate sampling) VLPs being recovered, vs. the polycarbonate filters (2.30×10^7 g^{-1} $\pm$ 4.80×10^6, triplicate sampling) or the nitrocellulose (3.10×10^7 g^{-1} $\pm$ 8.80×10^6, triplicate sampling) filters. The polyvinylidene fluoride filter is a low protein-binding (vs. nitrocellulose), hydrophilic membrane which is ideal for filtering protein-containing eluants. Similar low protein-binding filters have been used to isolate phages (Lawrence *et al.*, 2002; Moce-Llivina *et al.*, 2003); they have been found to be very effective as viruses will not adsorb to these membranes, thus increasing virus recovery (viruses will adsorb to other non-treated filters due to electrostatic forces).

Epifluorescence microscopy showed some remaining detritus, indicating that the filtering process was not entirely successful at eliminating all soil particles. However, it was determined that further filtering would not be useful as it would only eliminate viruses with little benefit to eliminating more detritus particles. Other studies (eg. Lawrence *et al.*, 2002) have detected viruses from sediments using

0.45 µm pore size filters with no detritus masking problems under microscopic examination.

Sonication

Sonication is widely used to extract viruses from sediments and soils. Permafrost sample 204-99 was used to determine the best sonication regime. Sonication times ranged from 0 seconds (unsonicated sample) to 300 seconds (Figure 8). No significant difference ($p>0.05$) was found in VLP counts between the unsonicated sample ($2.59 \times 10^8 g^{-1} \pm 1.77 \times 10^7$) and the sonicated samples ($2.49 \times 10^8 g^{-1}$ - $2.70 \times 10^8 g^{-1}$). It is unclear why this method was not a useful tool for extraction from permafrost, considering it is widely used (eg. Danovaro *et al.*, 2001, 2002; Monpoeho *et al.,* 2001; Mignotte *et al.,* 1999; Latorre *et al.,* 1996). However, in these other studies the wattage used was considerably higher (60-100 W) than what was used in this experiment (25 W), so perhaps using a sonicator with higher wattage output would increase virus dislodgement.

Enumeration of bacteria and virus-like particles

Bacteria and virus abundances were determined for all samples (Figure 9). Generally, the Antarctic samples had less variable virus and bacteria abundances than the Siberian samples, which overall had a wider range and also contained some counts at the respective detection limits. Also, in general, the bacterial counts were an order of magnitude less than the corresponding virus counts, although in some samples (eg. 3/99, 4/91) there were higher bacterial counts than virus counts.

Virus counts were associated with permafrost soil types to determine any relationships. Samples were categorized into four main soil types: sands, silts,

muds, and loams. When virus counts were compared amongst all the soil types, there were significant differences when virus counts of the loamy samples were compared against all the other sample types (p=0.001-0.018).

Virus and bacteria counts were compared (Figure 10). Several samples contained bacteria and VLP counts at the detection limits of 28,300 VLP g^{-1} and 1020 bacteria g^{-1}. There was a moderate correlation between bacterial and virus counts (R_2=0.5508), and there was some clustering of the data. Cluster *L* contained samples from Siberian with loam soil type, and high contents of ice, carbon, and nitrogen. Both clusters *BDL* contained samples with sand or mud soil types, generally had low water contents, and had counts at the detection limits. From this data, it seems that loamy soils (Cluster *L*) are more conducive to higher viral and bacterial abundances than sandy soils.

Total carbon content, organic carbon content, and nitrogen content were compared to virus counts to determine correlations. There were no strong correlations between these and virus counts (R_2=0.0915 - 0.3398), but there was clustering of the data (Figures 11-13). Cluster *L* (Figure 11) contains samples from Siberian with loam soil types, and high nitrogen, organic carbon, and ice contents. Cluster *A* (Figure 11) contains mostly Antarctic samples; all of which have sand or mud soil types. Cluster *BDL* (Figure 11) contained samples with sand or mud soil types, and had counts below the detection limits. Cluster *L* (Figure 12) contains samples from Siberia with loam soil type, and high contents of nitrogen and ice. Cluster BDL (Figure 12) contained samples with sand or mud soil types, and counts at the detection limits. Cluster *L* (Figure 13) contains samples from Siberia with

loam soil type, and high contents of ice and carbon. Cluster *S* (Figure 13) contains mostly Antarctic samples; all of which have sand soil types and low water content. Cluster *BDL* (Figure 13) contained samples with mud or sand soil types, and had counts below the detection limits. Once again, loamy Siberian permafrost (Clusters *L*) seemed to have higher abundances of viruses over sandy (Cluster *S*) Antarctic (Cluster *A*) permafrost. These data draw a parallel with the above data comparing virus and bacterial counts, which also led to the discovery that loamy soils are more conducive to virus abundances over sandy soils.

Ice content of all samples was determined (Figure 14). Generally, Siberian permafrost contained more ice than Antarctic permafrost (average content 15.94% $\pm$ 10.45, and 6.44% $\pm$ 4.98 of total mass of the sample, respectively). This compares with the information that Antarctic permafrost is generally sandy (a poor retainer of ice) while Siberian permafrost has more diverse soil types (some types such as loams contain much ice).

Ice content of the samples was compared to virus counts to determine a correlation (Figure 15). Although no strong correlations between virus abundances and ice content were found (R_2=0.3639), there was clustering of data. Cluster *L* contains Siberian samples with loam soil type and high carbon and nitrogen contents. Cluster *S* contains mostly Antarctic samples with sand soil type; and cluster *M* contains Siberian samples with mud soil types. Cluster *BDL* had counts below the detection limits.

Since there seemed to be a difference between the loams and the other sample types, virus counts of the loamy samples were compared with all the other

sample types (sands, muds, and silts). There was a significant difference ($p<0.018$), with sands being the most different from loams in terms of virus counts ($p=0.001$). This is consistent with these results and other research, which have shown that loamy permafrost samples were correlated with high carbon, nitrogen, and water contents, and higher bacteria counts (this research, Spirina and Fedorov-Davydov, 1998).

In all cases, there were no strong correlations between the permafrost physical and chemical characteristics and virus counts, but there was consistent clustering of data. Typically, the highest virus abundances were associated with loamy permafrost with high carbon, nitrogen, and ice contents, and the lowest virus abundances were associated with sandy or muddy permafrost with low carbon and nitrogen contents. This is consistent with other research into permafrost bacterial abundances. Gilichinsky *et al.* (1998) and Tiedje *et al.* (1994), found that the largest numbers of viable bacteria (10^7-10^8 cells g^{-1} using DAPI and Acridine Orange staining) are found in relatively young layers 7-10,000 years old, and in permafrost with higher concentrations of organic matter containing carbon and nitrogen. Also, textural composition of the permafrost appears to be important. Gilichinsky *et al.* (1995) and Gilichinsky and Wagener (1995) found that under similar conditions, the number and diversity of microbes was determined by textural composition, with the finer-textured loams containing 2-3 orders more viable cells than coarser-textured sands. This is corroborated by Spirina and Fedorov-Davydov (1998), who determined that sandy soils had 10^3-10^7 bacteria g^{-1} while loamy soils had 10^5-10^8 bacteria g^{-1} (although there is considerable overlap).

In general there were fewer viruses to be found in Antarctic permafrost than in Siberian permafrost. However, since the Antarctic samples were sand-based, and sandy soils hold fewer viruses, it cannot be said that viruses are less abundant in Antarctic permafrost unless some non-sandy permafrost from Antarctica is examined. Siberia is generally considered a more hospitable region than Antarctica due to its greater potential for moisture; this can be seen in the greater virus and bacteria abundances in the permafrost samples and also the greater ice contents of the samples.

Depth of the samples was examined to determine if it had any effect on virus counts. For the samples that originated from the same core, there was no significant difference ($p>0.05$) in virus counts as depth increased (Figure 16). It was hoped that there would be a significant difference, since depth can be used as an indication of age, with the deeper samples being older than the more shallow samples, and the older samples containing fewer viruses. However, since there were only 5 samples from the same core to be examined, a larger sample size would be necessary to determine if a significant difference in virus abundances over depth exists. Other studies examining viruses in sediments (eg. Lawrence *et al.*, 2002, Bird *et al.,* 2001) have shown that virus abundance decreases with depth. Generally, diversity and numbers of bacteria are determined by the permafrost age, with older permafrost leading to lower numbers of bacteria and less diversity (Gilichinsky *et al.,* 1995; Gilichinsky and Wagener, 1995). However, given the scant number of samples from the same cores (5), more samples would have to be examined and compared to determine if this holds true for virus abundances.

Epifluorescence micrographs were taken of virus-like particles and bacteria present in the permafrost samples after the appropriate filter treatment (Figure 17). After staining with SYBR 1 Green and under observation with blue excitation, bacteria are visible as large, green-fluorescing objects, while virus-like particles are much smaller, pinprick-like, green-fluorescing objects. Most detritus has been removed by the filtering treatment as evidenced by the lack of yellow-fluorescing, irregularly-shaped objects.

Presence of viruses in permafrost

The question arises, how did the viruses get in the permafrost samples? To partially answer this: Are the host bacteria metabolically active or in a state of anabiosis? Although a definitive answer based on experimental evidence is still lacking, observations from Antarctica (Kappen *et al.,* 1996; Schroeter *et al.*, 1994) suggest that metabolic activity is taking place (in the form of CO_2 exchange) at temperatures of -10°C or below. In one study (Kappen *et al.,* 1996), lichens (which are highly freeze-tolerant) exhibited photosynthesis down to -10°C, and respiration down to -17°C. Bacteria can theoretically metabolize down to -20°C since this is the approximate temperature at which water can still exist in a liquid film around sediment particles (Bortman, 2002). Active metabolism would allow the bacteria to perform basic functions necessary for survival, including DNA repair and membrane integrity (Friedmann, 1994). If the bacteria are metabolically active, then any associated viruses in the cells could be replicating as well. Psychrophilic bacteria-virus relationships have been observed before; for example *Pseudoalteromonas* sp. has an infective virus (Middleboe *et al.,* 2002). It is unlikely the viruses traveled into

the permafrost after it formed, as the permafrost in these areas has been frozen for several million years (eg. 2-4 million years in the Kolyma-Indigirka Lowland region of Northeast Siberia), with no melting of the permafrost occurring. Therefore, it seems likely the viruses were present and replicating in the host cells as the permafrost formed, and perhaps continued this replication while the host cell was metabolically active, traveling to other cells via the liquid film of water present around the soil particles.

Age of viruses in permafrost

After examining other literature into Siberian permafrost (Kochkina *et al.*, 2001; Shi *et al.*, 1997; Dmitriev *et al.,* 1997; and Gilichinsky *et al.*, 1998), I was able to determine the possible ages of several of the samples by cross-referencing their bore-hole numbers. Ages range from recent to 3 million years old. Can viruses persist for that long? It has been proven that bacteria (Shi *et al.,* 1997), fungi (Dmitriev *et al.*, 1997), and bryophytes (Marsic *et al.*, 1999) have been isolated from very old permafrost. If bacteria and eukaryotes can survive in permafrost, then it is logical that viruses can also survive. However, they would be more likely to survive if they are residing inside host cells, and not naked in the environment, since the cryogenic properties that allow the host cells to survive would also allow any viruses inside to also survive. Therefore it seems that the viruses found in the permafrost were inside host cells during deposition, and either some of the cells were destroyed in the extraction process, allowing the viruses to be seen, or the viruses lysed the metabolically active cells while residing in the permafrost, allowing them to escape and infect other nearby cells. To establish which scenario is more likely, the

host cells in the permafrost would need to be found and their metabolic status determined.

Location	Sample	Water (% TMS[1])	pH	Total Carbon (% TMS)	Organic Carbon (% TMS)	Nitrogen (% TMS)
Siberia	3/90 (#1)	20.24	7.44	4.301	0.888	0.085
	3/90 (#2)	15.13	8.16	1.804	1.648	0.143
	6/91 (#2)	15.8	7.57	1.930	1.485	0.147
	6/91 (#1)	15.59	7.33	4.193	1.156	0.118
	4/91	13.5	6.16	4.419	0.722	0.078
	3/93	16.81	7.59	4.462	1.057	0.128
	N2951 (#1)	20.14	6.41	5.693	3.984	0.264
	1/95 (#3)	30.2	7.18	12.071	4.714	0.289
	N5121 (#2)	16.43	7.59	3.705	0.772	0.097
	1/98 VAJ	13.74	7.97	2.650	0.436	0.052
	2/95 VAJ	17.36	6.80	5.294	1.183	0.125
	211-99	38.08	6.58	10.672	6.442	0.312
	204-99	26.45	5.32	4.768	4.672	0.332
	14/99	7.53	8.32	1.084	0.192	0.035
Antarctica	2/95 HOE	10.01	8.12	2.571	0.227	0.016
	4/95 DAV	5.94	9.77	1.906	0.018	0.018
	4/95	13.22	8.10	1.490	0.421	0.018
	X1/99 (#1)	12.69	9.02	1.144	0.041	0.047
	X1/99 (#2)	0.68	7.89	0.274	0.109	0.029
	3/99	2.07	8.48	1.991	0.072	0.083
	11/99	5.13	8.08	0.086	0.043	0.018
	7/99	1.79	8.60	0.394	0.015	0.015

Table 2: Permafrost physical and chemical characteristics
[1]TMS=total mass of sample

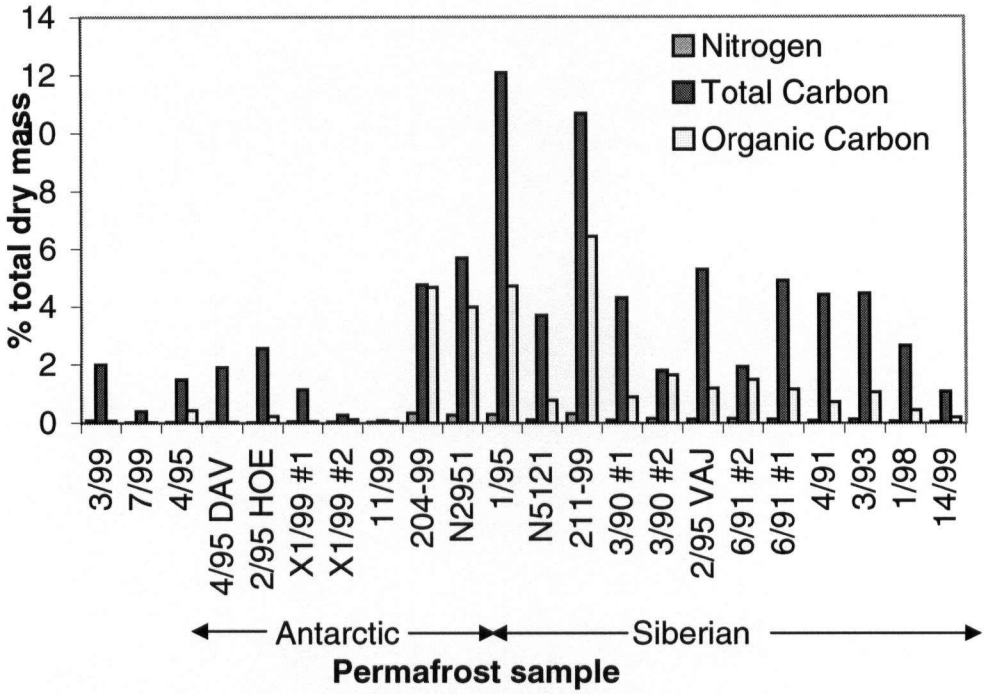

Figure 5: Carbon and nitrogen content of Antarctic and Siberian permafrost. Values are percentages of the total dry mass of the sample.

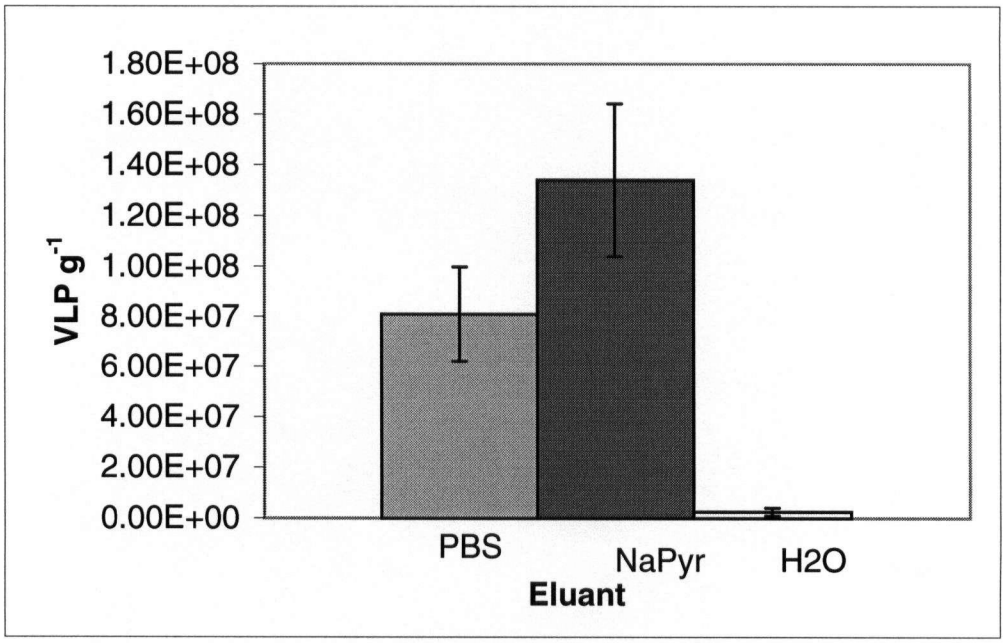

Figure 6: Effects of various eluants on VLP recovery from permafrost sample 204-99. VLP counts are values per gram dry mass of sample. Error bars represent standard deviation of triplicate samples.

PBS=phosphate-buffered saline; NaPyr=Sodium pyrophosphate H_2O=virus-free water; VLP=virus-like particle

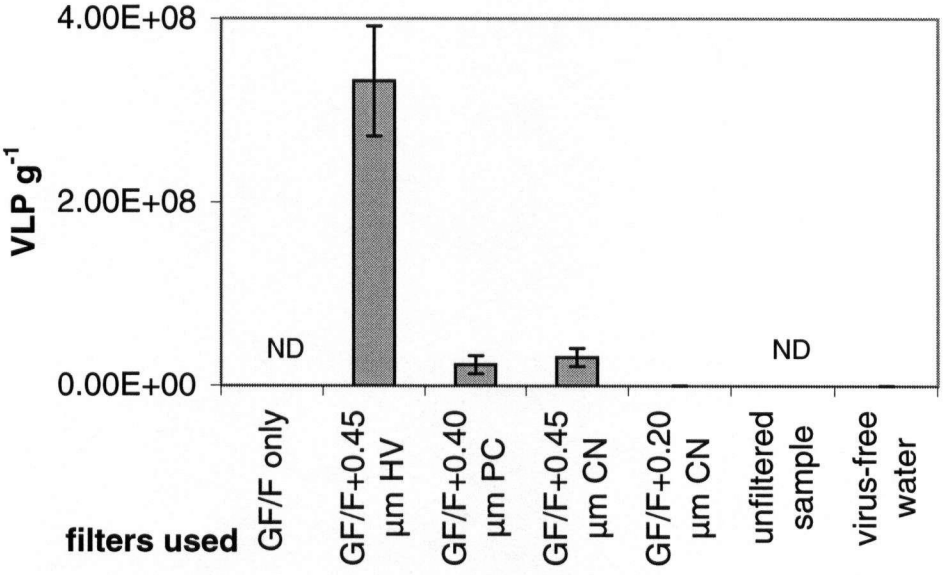

Figure 7: Effects of various filters on VLP recovery from permafrost sample N2951. Error bars represent standard deviation of triplicate samples.

ND=not determined. Both the unfiltered sample and the GF/F-only treated sample had too much detritus to allow for accurate counting.

VLP=virus-like particles; GF/F=glass fiber filter; HV=polyvinylidene fluoride filter; PC=polycarbonate filter; CN=cellulose nitrate filter.

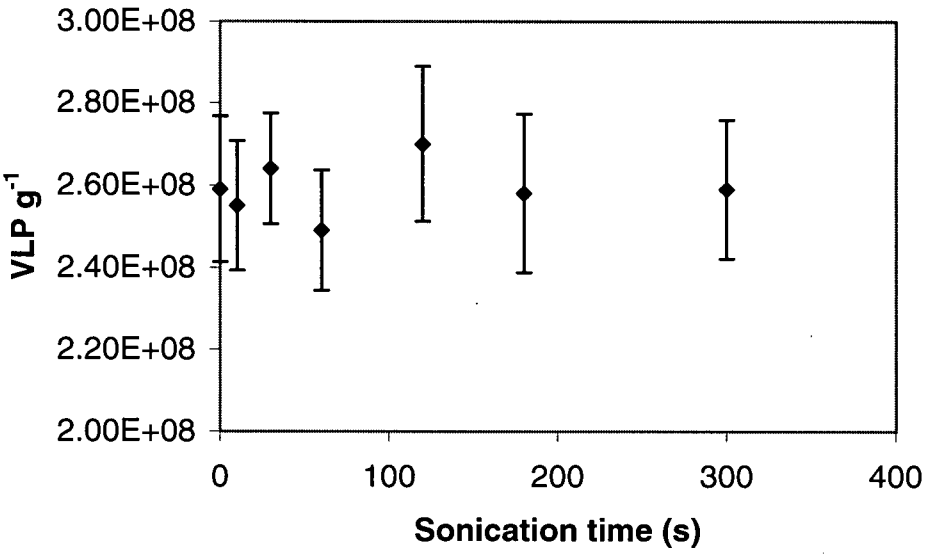

Figure 8: Ultrasonication of permafrost sample 204-99. Error bars represent standard deviation of triplicate samples. VLP=virus-like particles

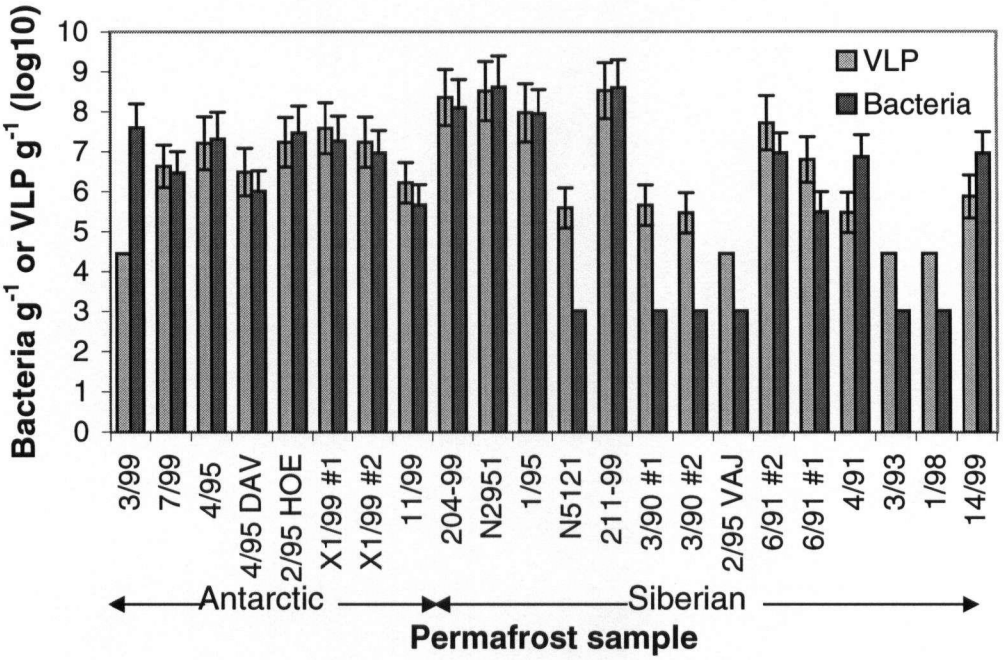

Figure 9: Bacteria and virus counts for Antarctic and Siberian permafrost. Error bars represent standard deviation of triplicate samples. Samples without error bars represent values below the detection limit. Detection limits: virus counts=28300 VLP g^{-1}; bacteria=1020 bacteria g^{-1}.

VLP=virus-like particle

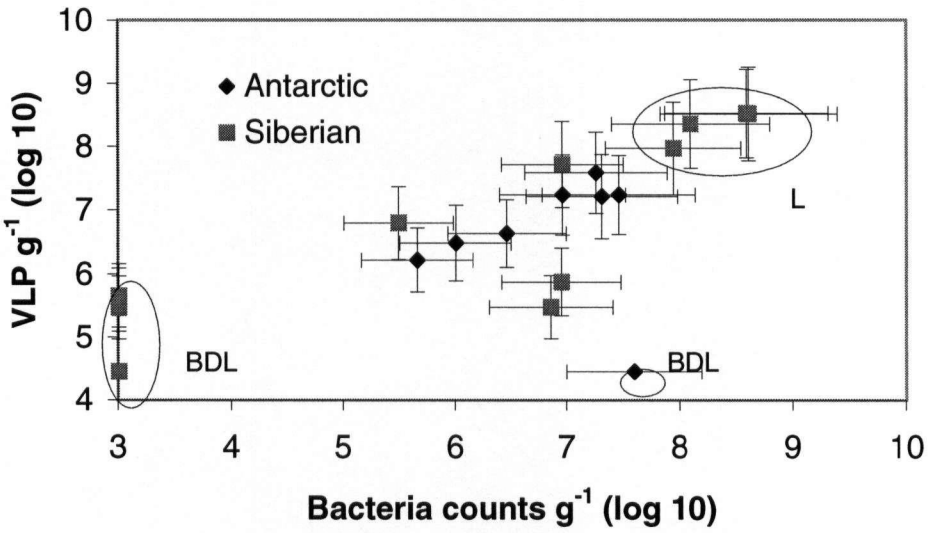

Figure 10: bacteria vs. virus counts for permafrost samples. Boxes represent Siberian samples, diamonds represent Antarctic samples. Bacteria and VLP counts are values per gram dry mass of sample. Error bars represent standard deviations of triplicate samples. Samples without error bars represent values below the detection limit. Detection limits: virus counts=28300 VLP g^{-1}; bacteria=1020 bacteria g^{-1}.

VLP=virus-like particle; BDL=below detection limit
L=loamy soil

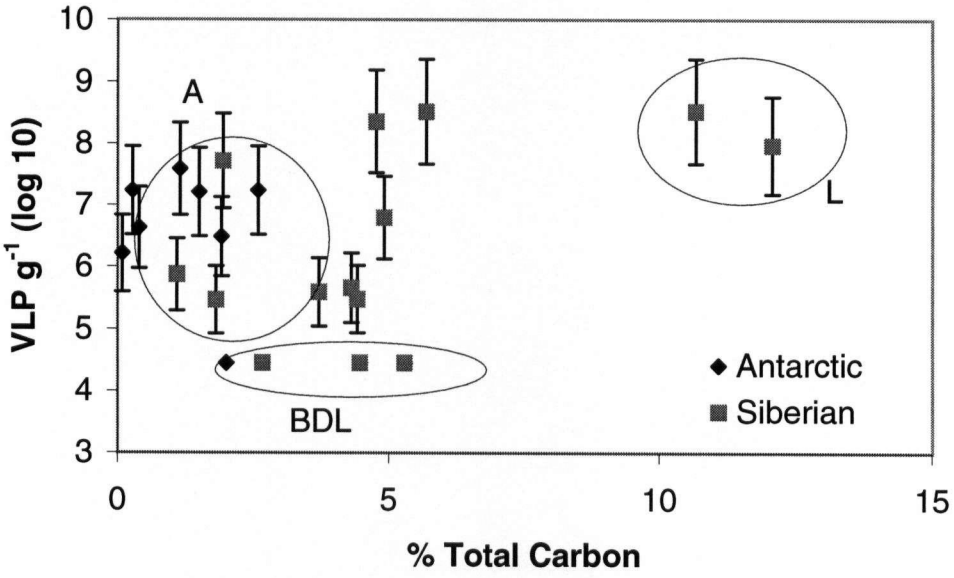

Figure 11: Virus counts vs. total carbon content for permafrost samples. Values measured as percentage of total dry mass of sample. Boxes represent Siberian samples; diamonds represent Antarctic samples. VLP counts are values per gram dry mass of sample. Error bars represent standard deviations of triplicate samples. Samples without error bars represent values below the detection limit. Detection limits: virus counts=28300 VLP g^{-1}.

BDL=below detection limit; VLP=virus-like particle
L=loamy soil; A=mostly Antarctic samples.

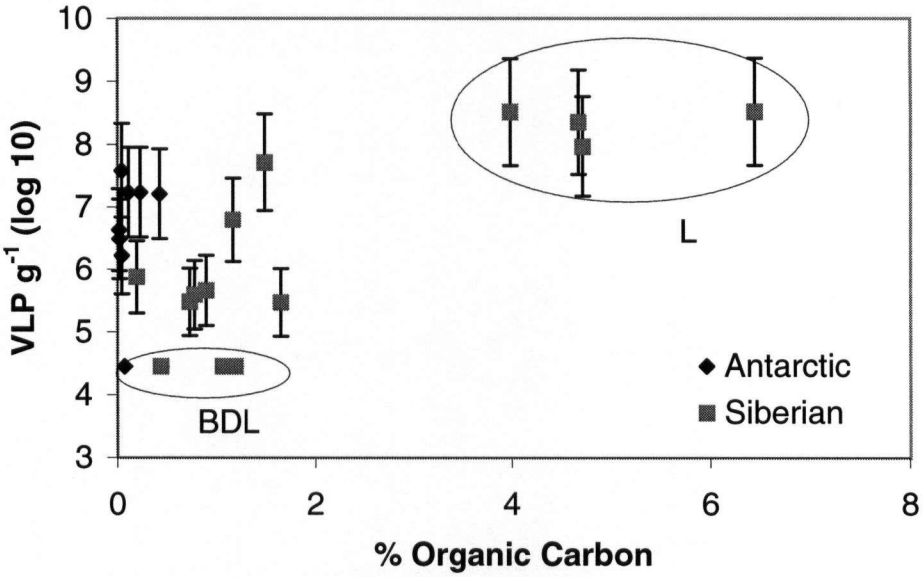

Figure 12: Virus counts vs. organic carbon content for permafrost samples. Values measured as percentage of total dry mass of sample. Boxes represent Siberian samples; diamonds represent Antarctic samples. VLP counts are values per gram dry mass of sample. Error bars represent standard deviations of triplicate samples. Samples without error bars represent values below the detection limit. Detection limits: virus counts=28300 VLP g^{-1}.

BDL=below detection limit. VLP=virus-like particle
L=loamy soil

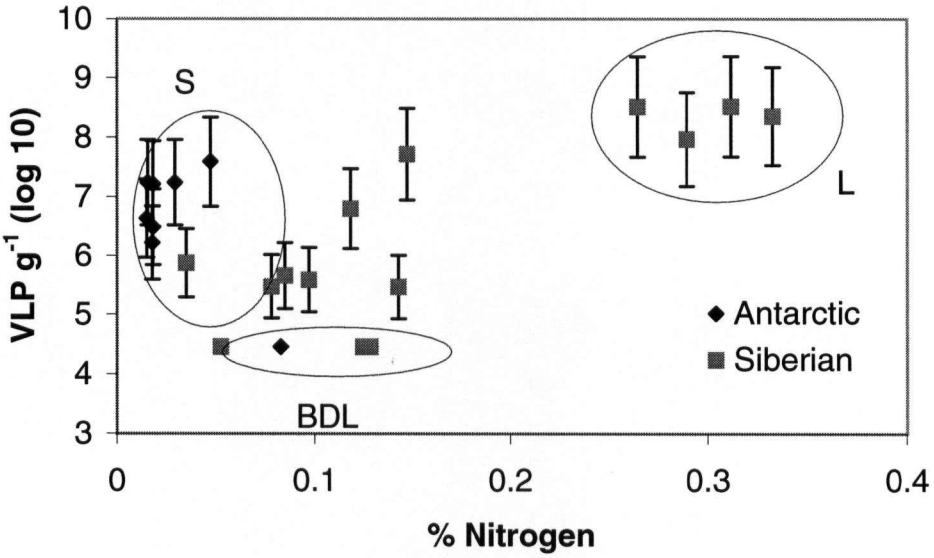

Figure 13: Virus counts vs. nitrogen content for permafrost samples. Values measured as percentage of total dry mass of sample. Boxes represent Siberian samples; diamonds represent Antarctic samples. VLP counts are values per gram dry mass of sample. Error bars represent standard deviations of triplicate samples. Samples without error bars represent values below the detection limit. Detection limits: virus counts=28300 VLP g^{-1}.

BDL=below detection limit. VLP=virus-like particle
L=loamy soil; S=sandy soil

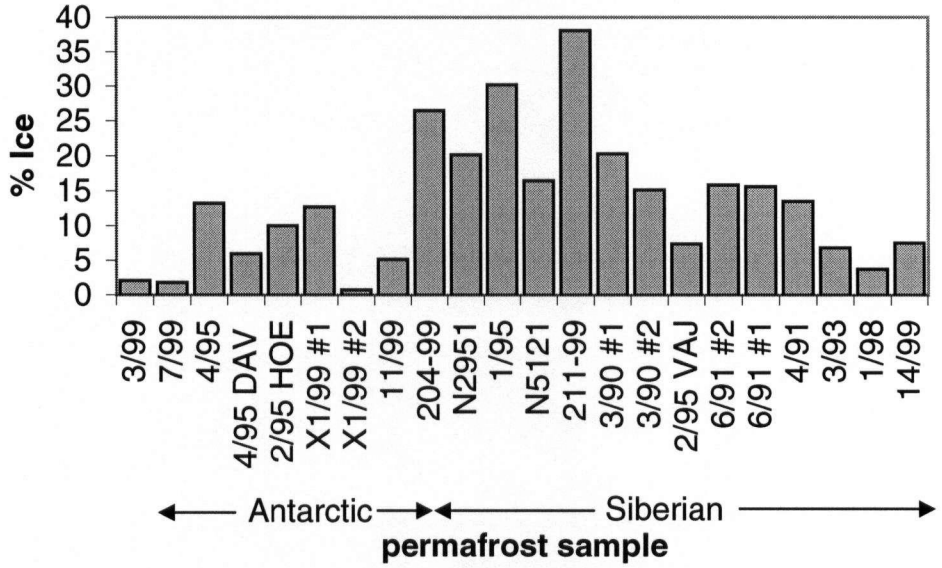

Figure 14: Ice content of Antarctic and Siberian permafrost. Values are percentage of total mass of the sample.

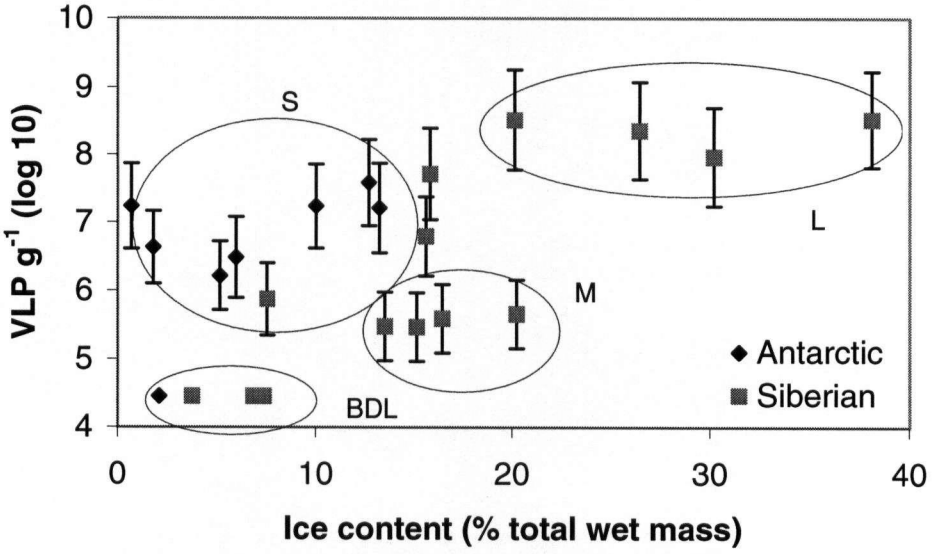

Figure 15: Virus counts vs. ice content for permafrost samples. Boxes represent Siberian samples; diamonds represent Antarctic samples. VLP counts are values per gram dry mass of sample. Error bars represent standard deviations of triplicate samples. Samples without error bars represent values below the detection limit. Detection limits: virus counts=28300 VLP g^{-1}.

BDL=below detection limit; VLP=virus-like particle
M=muddy soil; S=sandy soil; L=loamy soil

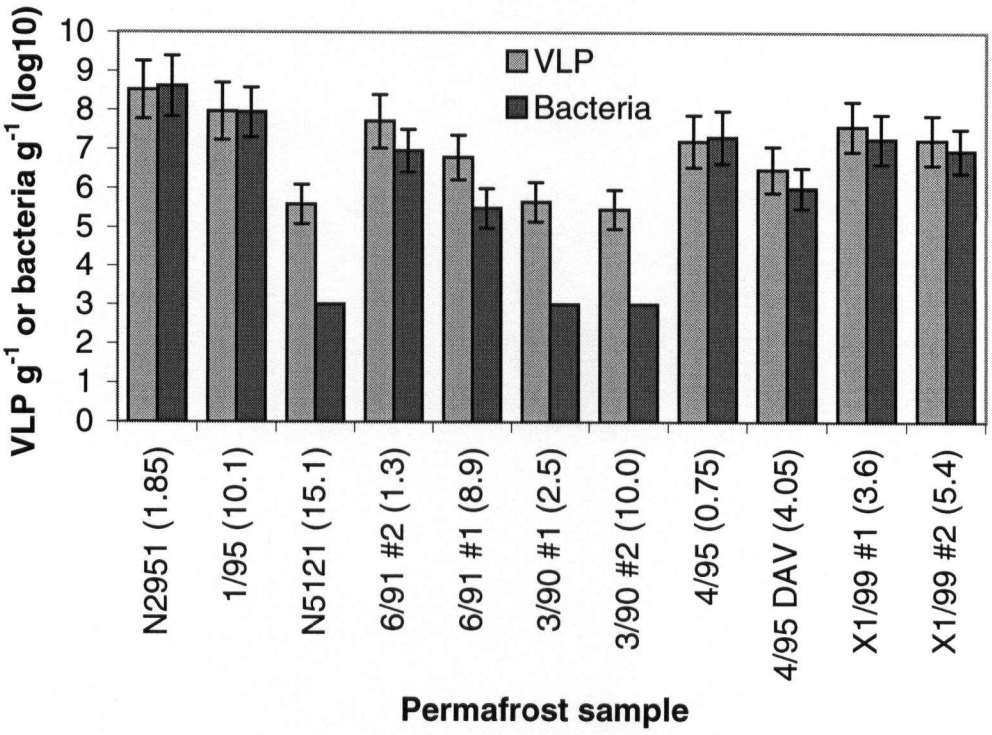

Figure 16: Virus and bacteria counts for permafrost at varying depths. Error bars represent standard deviation of triplicate samples. Samples without error bars represent values below the detection limits. Detection limits: virus counts=28300 VLP g^{-1}; bacteria=1020 bacteria g^{-1}.

VLP=virus-like particle

Permafrost samples from the same well:
N2951, 1/95, N5121;
6/91 #1, 6/91 #2;
3/90 #1, 3/90 #2;
4/95, 4/95 DAV;
X1/99 #1, X1/99 #2
Numbers in parentheses are depths (in meters) at which the core was taken.

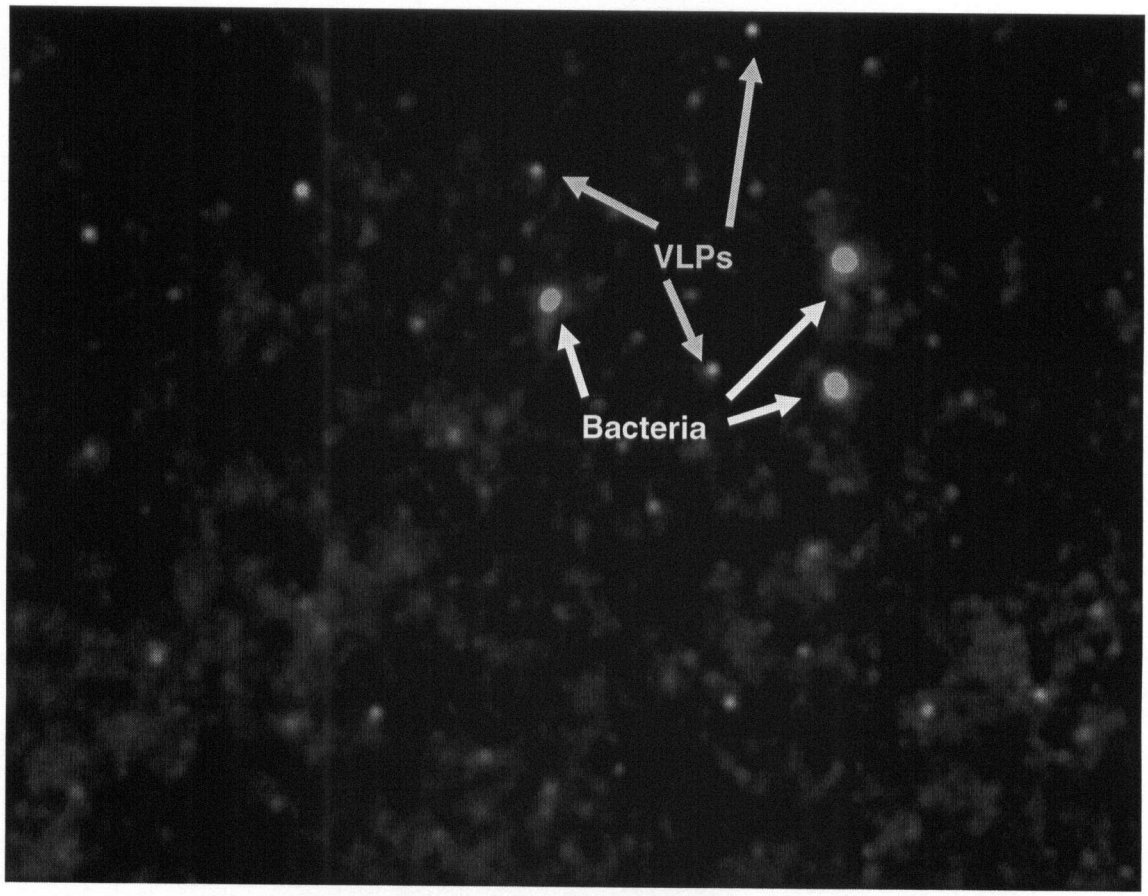

Figure 17: Epifluorescent micrograph of permafrost sample 211-99 at 1000x (oil immersion), stained with SYBR 1 Green and observed under blue excitation.

This sample has been treated with a GF/F filter plus a 0.45 µm HV filter to eliminate the masking effect of detritus. The three large objects are bacteria; the three small objects are virus-like particles (VLPs).

CONCLUSIONS

Evidence of viruses in Siberian and Antarctic permafrost

Permafrost has not previously been examined for viruses; this research has shown the presence of viruses in Siberian and Antarctic permafrost. It is possible that they are phages, and are visible as their host cell has been disrupted by the extraction method, or lysed by phage action while the cells were metabolically active in the permafrost. They could also be plant, fungal, or insect viruses, which have become adsorbed to soil particles through electrostatic interactions. Further work such as molecular studies would likely have to be done to determine exactly what type of viruses they are, and whether they are unique to permafrost habitats or are typical soil viruses (eg. baculoviruses, enteric phages, etc.).

Permafrost characteristics affect viral and bacterial abundances

Observations have shown that virus and bacterial abundances are related to the permafrost type, with loams being the most conducive to viral and bacterial presence. High contents of organic matter (carbon and nitrogen), lower pHs, and water (all characteristics of loams) are also indicators of high virus and bacteria abundances. At the other extreme, sandy permafrost was generally not a good environment for viruses and bacteria (compared to the other soil types) as it contains little water (which is essential for organisms to exist) and thus scarce amounts of organic matter for a micro-ecosystem to thrive. However, to determine if Antarctica is actually more virus-poor than Siberia, non-sandy samples from Antarctica, and additional sandy samples from Siberian would have to be examined.

Further investigations

After examination of additional permafrost samples for viruses, the next logical step would be to attempt to determine what kind of viruses are present, and what their hosts are (bacterial, fungal, plant, or otherwise). Community analysis of both bacterial and viral diversity would be helpful in further determining any differences between Siberian and Antarctic permafrost as a habitat for microbes. Molecular analyses such as pulsed-field gel electrophoresis and PCR would better elucidate the overall community involvement of viruses in the permafrost microbial habitat, and perhaps determine the role and how important viruses are in this type of environment.

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