University of Alberta

Active heterotrophic microbial communities from polar desert soils of the Canadian High Arctic

by

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Abstract

Polar warming will lead to increased labile organic carbon in Arctic soils, both from the release of organic carbon stored in permafrost and increased plant production. The impact of increasing organic carbon on Arctic soil microbial community composition and activity is of great interest because microbial decomposition of Arctic soil organic carbon is a potential major source of CO_2 to the atmosphere. I determined the activity and composition of the active heterotrophic bacteria in soil cores of the Canadian High Arctic by stable isotope probing (SIP) with a labile organic carbon analogue, ^{13}C-labeled algal lysate. The activity, measured as CO_2 production rate, was significantly higher with substrate added compared to the controls. There was a significant decrease in diversity and a shift in community composition following addition of labile carbon. These results suggest only a few groups within the community are active and responsive to increased complex organic carbon.

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1. Literature review

1.1. Introduction

Permafrost soil contains an enormous amount of total organic carbon: twice as much as the amount of carbon that is extant in the atmospheric carbon pool (Zimov et al. 2006; Schuur et al. 2008). This carbon is estimated to be 50% of the world's soil organic carbon storage (Tarnocai et al. 2009). In the northern permafrost area, the highest mean soil organic carbon contents (32.2–69.6 kg m^2) are found in organic soils (peatlands) and permafrost-affected mineral soils (Tarnocai et al. 2009). Warming in the Arctic is occurring two to three times faster than the global mean surface temperature increase (IPCC 2007). As a result, the previously inaccessible frozen organic carbon in permafrost will be subject to microbial decomposition following thaw, leading to increased carbon release to the atmosphere (Ping et al. 2008). Microbial decomposition is the main pathway for carbon turnover and its return to the atmosphere. As climate warms, this will lead to positive climate feedbacks from terrestrial ecosystems to the atmosphere (Zimov et al. 2006; Schuur et al. 2008). Most of the studies regarding these feedbacks have focused on peatlands or generally address tundra biome; however, permafrost-affected mineral soils that account for 5,000,000 square kilometers of land surface (Hogan 2011) have received little attention. Hence, it is not clear how a changing environment will affect these ecosystems or what role they may play in climate change.

The microbial organic carbon decomposition rate depends on several factors such as organic carbon quality (decomposability), environmental factors, and genetic and metabolic potential for degradation in the microbial community. Organic matter within permafrost and active layer soil is labile and decomposable (Coolen et al. 2011); thus, interactions between microorganisms and their environments are the primary regulators of soil organic matter degradation in the Arctic. Studying these ecosystems will lead to a better understanding of the functionality of microbial communities in permafrost soils and how they respond to ecosystem changes. Our knowledge of the bacterial populations present in these ecosystems is primarily via characterization of total

DNA, which may include both active and inactive cells. Thus, to specifically identify and characterize the active microbial communities that are metabolically active and responsible for nutrient cycling, other approaches must be applied.

In this thesis, microbial populations of the High Arctic (polar desert mineral soils) from different sites in dry tundra biome are investigated. The main focus is to find the links between bacterial community structure, activity, and carbon turnover in Arctic soils. This will lead to a better understanding of the role of bacterial assemblages in climate change as having a positive or negative feedback.

1.2. Global climate change and the Arctic

1.2.1. Significant changes in the Arctic terrestrial ecosystems due to global warming

1.2.1.1. Temperature

According to the International Panel on Climate Change (IPCC 2007), global mean surface temperatures have increased by 0.74°C ± 0.18°C over the last 100 years (1906–2005). Additionally, the last 50-year average atmospheric warming rate is twice the rate for the last 100-year. Temperatures are increasing more rapidly in the Arctic (especially northwestern North America and central Siberia) as compared to a general rise in global temperatures over the last 50 years (Turner et al. 2007) (Fig. 1). This exaggeration of temperature changes in high latitudes compared to lower latitudes is called polar amplification and has been shown in model projections of future climate (Holland and Bitz 2003; ACIA 2005). Polar amplification of climate warming has also been found in proxy-records of deep past warm periods (Zachos et al. 2001) as well as the more recent cold glacials (Masson-Delmotte et al. 2006). Most climate models predict an increase of 7 to 8 ^{0}C for the late 21st century in the Arctic (IPCC 2007). Since 1980s, up to 3 ^{0}C increase has been observed over the permafrost layer (IPCC 2007).

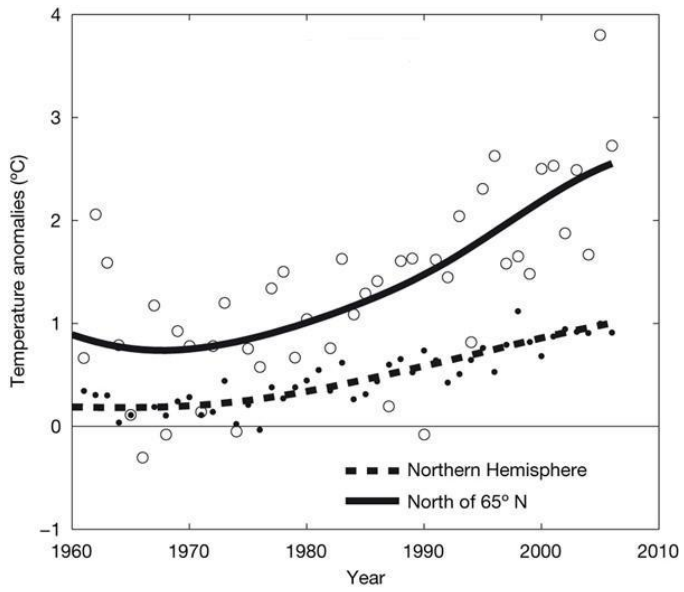

Fig 1. Amplification of Arctic temperature trend compared to Northern Hemisphere temperature trend. Temperature anomalies were measured in dark months (November-February). Solid lines and open circles represent measurements north of 65^0 N while the dashed lines and dots are for the entire Northern Hemisphere (Picture modified from Graversen et al (2008).

1.2.1.2. Increased precipitation

Another change in the Arctic that is associated with temperature elevation is increased precipitation. Findings based on six global land-area precipitation data sets, including the Global Historical Climate Network (GHCN) database (ACIA 2005) and the Fourth Assessment Report (AR4) (IPCC 2007) led to the conclusion that precipitation has risen over land north of 30 ^{0}C from 1900 to 2005. This is a general trend of 6-8 % increase for northern parts of the globe and is consistent with 1.4% per decade increase from 1900 to 2005 for the Arctic (IPCC 2005). A synthesis of new simulations for use in IPCC 2007 by Kattsov et al (2007) showed that percentage increases in the Arctic (33%) are much larger than the global mean precipitation (4.5%).

1.2.1.3. Increase in CO_2 concentration

CO_2 is the second most important greenhouse gas after H_2O in the atmosphere and its contribution to climate change through human activity has been reported to be a large factor in the context of global warming (IPCC 2007). Due to being

radiatively active and adsorbing radiant energy from the sun, the increase in its concentrations in the atmosphere will affect temperature and climate on a global scale. These effects are more tangible in northern latitudes because there will potentially be more changes in atmospheric and surface properties in those environments (Hinzman and Kane 1992). Levels of atmospheric CO_2 at present is about 390 parts per million (ppm) and may increase to concentrations between 500 ppm and 900 ppm by the end of this century (IPCC 2007). Surprisingly, CO_2 levels over the past 650,000 years never exceeded ~280–300p.p.m. by volume (Petit et al. 1999). It was during the industrial era that CO_2 abundance rose roughly exponentially to 379 ppm in 2005 (IPCC 2007).

1.2.1.4. Effects of increased temperature, precipitation, CO_2 on Arctic soils

Based on Arctic Climate Impact Assessment (ACIA 2007), permafrost has experienced warming over the past several decades in Alaska, Canada, Russia, and northern Europe as a consequence of Arctic warming. This warming strongly affects the permafrost and leads to the deepening of the active layer overlying the permafrost (Vincent et al. 2009). All the near-surface permafrost will disappear by the end of 21st century (Lawrence et al. 2008). Permafrost occupies 22% of the exposed land area of the Northern Hemisphere (Zhang et al. 2008) and 16% of the global soil area (Tarnocai et al. 2009). In colder regions with thick permafrost, climate warming turns massive ground ice and frozen water to new water bodies called thermokarst (Romanovsky et al. 2007). Thermokarst lakes are sources of methane emissions as organic matter released into the lake bottom upon permafrost thaw gets degraded anaerobically (Walter et al. 2006). Permafrost is a large carbon reservoir and once was underestimated in its size of stock organic carbon (Post et al. 1982). Recent estimates reports 1672 Pg (1 Pg = 1 billion metric tons) of organic C is stored in permafrost (Schuur et al. 2008).

The increase in precipitation will have several impacts including acceleration of permafrost degradation (Jorgenson et al. 2006), rise in soil moisture and increased plant growth and biomass (Hill and Henry 2011), all of which exert ecological modifications (Castro et al. 2010). Precipitation increase also leads to

increased nutrient deposition which leads to elevated nutrient availability. It may shift plant community composition and further quality and quantity of carbon substrates originating from litter inputs (Castro et al. 2010).

Furthermore, climate change has indirect effects on these ecosystems through aforementioned changes such as increase in nutrient availability. Higher temperatures lead to higher decomposition rates accompanied by higher availability of nutrients (Chapin III and Shaver 1996). This is specifically important regarding the fate of soil organic carbon due to climate change which raises many concerns as warming and more precipitation will lead to release of carbon to the atmosphere due to likely enhanced microbial decomposition. It is assumed that in warmer environments, microbial decomposition of soil organic matter will be triggered through increase in soil moisture, nutrient availability, and better biological conditions for enzymatic reactions (Davidson and Janssens 2006). However, the knowledge about the rate and form of the carbon release due to microbial decomposition is in its infancy (Schuur et al. 2008).

Organic matter decomposition, driven mainly by microorganisms, leads to the efflux of carbon dioxide (CO_2) with minor amounts of methane (CH_4) and hydrologic leaching of dissolved and particulate carbon compounds (Davidson and Janssens 2006). Though many studies have investigated the warming-induced acceleration of organic matter decomposition, the temperature sensitivity of soil carbon decomposition remains controversial. Several models have been proposed to predict how microbes will use SOM released by permafrost thawing; these models segregate SOM into conceptual pools with different mean residence times (MRT), such as labile and recalcitrant (Smith et al. 1997). For example, RothC model which is amongst the most wildly applied SOM simulation models, divide SOM into decomposable plant material (DPM), microbial biomass (BIO), resistant plant material (RPM), humified organic matter (HUM) and inert organic matter (IOM) (Jenkinson and Rayner 1977; Paustian et al. 1992; Brady and Weil 2002). HUM and IOM have the highest mean residence time (500-5000 years) and contain lignin, long chain n-alkanes,

humic substances and biochar, which make this pool recalcitrant (Dungait et al. 2012). RPM with 15-100 years MRT is composed of lignified tissues, waxes and polyphenols. BIO that has a shorter MRT (1-2 year(s)) contains polysaccharides from plant litter. It is less recalcitrant since many fungi and bacteria degrade plant polysaccharides. DPM (0.1-0.50 year MRT) is composed of highly labile compounds such as simple sugars and organic acids which quickly decompose in soils. The response of different pools of soil organic matter to temperature increase and a changing climate has not yet been determined due to lack of accurate forecast for global change-driven alterations in soil carbon stocks (Conant et al. 2011). Conant et al (2011) presented a conceptual model with three component processes. Changes in these processes rate could affect temperature response. These processes include depolymerization of biochemically complex compounds; production and conformation of microbial enzyme production; and processes that limit the availability of soil organic matter. Altogether, many models predict that microbial decomposition of soil organic matter will be stimulated (Lloyd and Taylor 1994; Friedlingstein et al. 2006); however field experiments showed a later decrease in CO2 release after an initial increase in the loss of CO_2 from the soils (Oechel et al. 2000; Luo et al. 2001). It has been proposed that efficiency of soil microbes in using carbon should also be considered in the soil-carbon response to climate warming and constructing models. Since many factors, including protein structure and conformation, altered protein expression, shifts in microbial community structure have never been incorporated into models (Waldrop et al. 2010), there are many uncertainties regarding the decomposition rate of organic matter in a warmer future environment.

Elevated atmospheric CO_2 has direct and indirect effects on Arctic ecosystems (Billings et al. 1983). The direct effect might be seen as enhanced carbon uptake by plants through a direct stimulation of photosynthesis. The indirect effect of enhanced CO_2 concentrations is through increase in temperature. This increase in temperature affects the photosynthesis. The indirect effect is more pronounced than the direct effect. Field experiments in some

Arctic sites (Tissue and Oechel 1987; Gwynn-Jones et al. 1997; Korner et al. 1997) revealed that responses of plant growth and biomass to increased CO_2 concentrations are normally small or nonexistent (direct effect). However, these responses might vary among different species (Dormann and Woodin 2002). Indeed, a rise in net ecosystem productivity through increase in net photosynthesis rate resulting from elevated CO_2 has been observed in many studies (Oechel and Vourlitis 1994; Norby et al. 2005; Grant et al. 2011); however, over the long-term, a simultaneous increase in temperature is needed to allow the increased carbon accumulation to persist (Oechel and Vourlitis 1994; Oechel et al. 1994). This might be due to the increase of nutrient availability in the ecosystem in the light of temperature increase (Oechel et al. 1994). It is unlikely that without an increase in nutrient availability CO_2 alone can trigger plant growth in long term.

The effect of climate change on global soil carbon stocks might exert positive or negative feedback on ecosystems. Soil respiration of plant roots and microorganisms counteracts increased net primary productivity. The carbon released from belowground to atmosphere (both in the form of CO_2 and CH_4) upon permafrost thaw will generate a positive feedback to climate change (i.e. the Arctic would act as a carbon source). Conversely, if net primary productivity exceeds soil respiration the feedback would be negative (i.e. the Arctic would act as a carbon sink). This concept is fundamental regarding Arctic terrestrial carbon fluxes as to whether Arctic terrestrial ecosystems are acting as a source or sink for GHGs (greenhouse gas) emissions.

Recent studies show the weakening of land-based CO_2 sink of the northern latitudes and a change to being a source (Grulke et al. 1990; Oechel et al. 1993; Oechel and Vourlitis 1994; Hayes et al. 2011; Lamb et al. 2011). Nonetheless, this might change due to other factors such as water content as was shown by Zona et al (2012) that CO_2 loss increased as a result of flooding. Some models reveal that Arctic is still small sink for carbon (IPCC 2007; Sitch et al. 2007). Shrubs expansion over the past 40 years in the Alaskan Arctic and more greening

and productivity (Stow et al. 2004) might contribute to negative feedback while other findings based on enhanced availability of organic material claim a positive feedback and contribution to a warmer climate. Nevertheless, uncertainty regarding the models and measurements findings remains. A more complete understanding of the feedbacks might be achieved through improving the models and field-lab experiments by incorporating information from detailed analysis of SOM substrate availability and microbial community profiles.

1.2.2. Arctic soils

The Arctic has a unique range of soils that are known to have special features. These soils are defined as being in cryosolic order in the Canadian soil classification system (Agriculture Canada 1998). Cryosols (Gelisols) cover more than 8% of the earth's ice-free land surface (Soil Survey Staff 1999). These soils can be found in a broad range of temperature, moisture and physiographic conditions. They are defined as permafrost affected soils and have two portions that are thermally distinct (Smith 2004): the lower portion that is frozen for at least two consecutive years is called permafrost (ACIA 2005) and the upper portion that thaws seasonally in summer and freezes in winter is called the active layer.

Permafrost distribution varies through the Arctic and can be continuous (90-100 % of land), discontinuous (50-90 %), sporadic (10-50 %) or isolated (<10%), primarily dependent on the latitude (Brown et al., 1998, revised Feb. 2001). Permafrost thickness differs from a few decimeters at the most southern regions to 740 m in Alaska and 1500 m in Siberia (U.S. Arctic Research Commission Permafrost Task Force 2003; Anisimov and Reneva 2006). In Canada, permafrost thickness varies from a few decimeters at the southern limit of the permafrost to over 700 meters in the High Arctic islands (Heginbottom et al. 1995).

The active layer ranges from decimeters deep in the High Arctic and extend to more than 2 m at lower latitudes (Steven et al. 2006). Permafrost thickness depends on several factors including ground temperature, soil moisture content, snow cover (French 2007), peat coverage (Kujala et al. 2008), and other factors.

The boundary separating the permafrost and active layer, known as the permafrost table, acts as a biogeochemical barrier that blocks the flow of surface water and penetration of external environmental factors into the permafrost (Gilichinsky 2002) and also accumulates water, thereby having different biogeochemistry than either the drier active layer soils or the frozen permafrost.

Arctic soil formation is dominated by cryogenic processes. These processes involve the migration of unfrozen soil water from the active layer towards the frozen front along the thermal gradient in the permafrost system (cryoturbation). This movement disturbs the ground and incorporates a large amount of soil organic matter into lower soil layers. This phenomenon leads to the formation of many special features of the soils of the Arctic; irregular or broken soil horizons, involution and organic matter accumulation in the subsoil oriented rock fragments (Bockheim and Tarnocai 1998) and can also lead to deep mixing of surficial organic carbon to great depths (in some cases, >3 m) in the soils.

The Arctic includes three main climatic subzones: polar desert, subpolar desert and the tundra (Tedrow 1977), which are equivalent to High Arctic, Middle Arctic and Sub/Low Arctic, respectively, in the Canadian classification system (Ecoregions Working Group 1989). Each of these subzones has unique features including the soil texture, vegetation cover and precipitation. The most extreme conditions are found in the High Arctic, followed by Middle Arctic, and finally Low Arctic with the mildest conditions.

Low and Middle Arctic Soils

The southernmost part of the Arctic- Low Arctic- has the winter temperatures the same as Mid-Arctic but is at least 5 ^{0}C warmer later in summer. Low Arctic receives more precipitation and has many different types of soil cover including Cryosols, Gleysols- soils permanently waterlogged- and Histosols-soils with organic matter as the primary parent material- while in the Middle Arctic, one can find Cryosols and Histosols due to less precipitation. Shrubs, dwarf shrubs, herbs and mosses are the main plant cover in both regions (Goryachkin et al. 2004).

High Arctic barren

The northern most zone of the Arctic was previously termed as polar desert by Tedrow (1977), however, “High Arctic tundra” or “High Arctic Barren” was proposed by Goryachkin et al (2004) to convey a better landscape image of this zone. These areas are different from cold deserts and are moist during early summer thaw; they receive occasional summer rainfall and some moisture from condensation (Tedrow 1991) so the term “polar desert” is not an appropriate naming for this area (Goryachkin et al. 2004). This area has lower temperatures in both summer and winter relative to the other subzones. The vegetation is limited to lichens, mosses and herbs (Goryachkin et al. 2004). Most of these soils are calcareous, with a pH above 6.0. A salt crust can often be seen on the soil surface. This phenomenon happens under polar desert conditions where there is less precipitation and the snow does not remain for a long time. Therefore, salts and carbonates do not leach and produce a thin salty crust in surface soil. Salinization and calcification do not happen in all High Arctic soils; however, there is a general tendency toward an increased salt accumulation (Tedrow 1977). It is mistakenly assumed that because of sparse vegetation, the organic carbon content of High Arctic soils is low; however, these soils undergo heavy cryoturbation and have higher soil organic matter than was assumed (Tarnocai et al. 2009).

1.3. Arctic soils biogeochemistry

Biogeochemical cycles of carbon and other elements in the Arctic terrestrial ecosystems operate at a slow rate compared to other ecosystems due to the extreme environmental conditions found there. However, the vulnerability of huge carbon stocks in the Arctic to climate change makes the carbon cycle, the most attention and research demanding of all geochemical cycles in the Arctic. Much of the organic carbon in High Arctic soils is relict, deriving from ancient sources and preserved in permafrost. Modern fixation is dominated by land plants and the response of Arctic plants to climate change is a major area of study (Callaghan et al. 2004b; Jónsdóttir 2011; Elmendorf et al. 2012).

However, since my focus in this thesis is on the role of microbial communities in soil organic carbon turnover and heterotrophic respiration and its association with CO_2 is a critical part of this research, I will focus here on heterotrophic respiration in High Arctic soils.

1.3.1. Carbon and nutrient dynamics in the terrestrial northern high latitudes

The terrestrial ecosystems of the Arctic cover approximately 5.05 x 10^6 km^2 (Walker et al. 2005). The stock of fixed C per unit area in the arctic averages approximately 550 g m^{-2} (David McGuire et al. 1997). As this number is small compared to a global average of 7150 g m^{-2} for vegetated ecosystems, Arctic ecosystems are recognized as having low primary productivity, low element input, and slow biogeochemical cycling. These features could be mainly due to the lack of trees as a result of N and P deprived soils (Callaghan et al. 2004a). Nonetheless, slow rates of decomposition and mineralization processes lead to accumulation of carbon and other elements (Jonasson et al. 2001) equivalent to that observed in other soil ecosystems.

The ratio of vegetation C assimilation to ecosystem C release is known as net primary productivity (NPP) of the ecosystem. NPP usually decreases with increasing latitude, but local factors such as topography, depth of snow cover, active layer depth, temperature, and soil moisture (Billings, 1973; Shaver et al, 1996) are also significant in influencing productivity. NPP in the High Arctic is usually limited by available nitrogen. Sources of N include atmospheric deposition and microbially-mediated N fixation (Callaghan et al. 2004a). Therefore, carbon fixation and productivity in the sparsely vegetated Canadian High Arctic is restricted to wet areas where there is more N fixation (Gold and Bliss, 1995). The cold atmosphere in the Arctic is not capable of holding high concentrations of volatile fixed N species; therefore, N deposition rates are slow. The other form of "new" nitrogen, N fixation, may account for more than 10% of plant requirement; the remainder is obtained from "old" N, provided by recycling from the soils (Lennihan et al. 1994). N loss from the system occurs through prokaryotic nitrification–denitrification and fungal denitrification; some of this is

lost as the greenhouse gas N_2O (Ma et al. 2007). Based on early field studies of N_2O fluxes from the Arctic (Christensen et al. 1999), very small releases of N_2O were expected; however, recently Lamb et al (2011) showed that at least one High Arctic terrestrial environment is a substantial emitter of N_2O. In fact, the ammonia oxidizer and denitrifier populations were as abundant as in temperate soils (Henry et al. 2006; Leininger et al. 2006; He et al. 2007; Nicol et al. 2008).

Soil Organic Matter (SOM)

There is a considerable range (1400-2400 Pg) in the quantity of global soil organic carbon reported by several studies (Jobbágy and Jackson 2000; Post et al. 1982).

The differences in estimates result from the use of different soil depths to determine the estimated average integrated soil organic carbon content per unit area (Tarnocai et al. 2009). In cryosols, cryoturbation mixes organic carbon deeply into the soils, leading to high reserves of carbon in deep soil layers (Schirrmeister et al. 2002; Zimov et al. 2006). For example, Batjes′ (1996) estimates showed that the amount of carbon in the upper-2m-layer in global terrestrial ecosystems is approximately equivalent to twice as much as in the upper-1m-layer. However, many studies focus exclusively on the surface soils, assuming that most of the organic carbon is derived from surface plants and does not migrate to a significant depth. Thus, Post et al (1982) indicates that all soils (both peatland and non-peatland soils) in the tundra zone contain about 1395 Pg based on samples of 100 cm surface soil. Meanwhile, Schuur et al (2008) finds that just non-peatland-vegetation-type northern high latitude regions hold 750 Pg carbon—almost four times as much as Post et al (1982) estimated for the entire Arctic environment—based on the upper 3 m of soil.

Peatlands are characterized as a wetland containing more than 40 cm thickness of peat (NWWG 1988). Peatlands contain 20-60 % of the total pool of organic C in permafrost, 1-20% is stored at mineral soils and the remainder in thaw lakes/thin peat areas (Bockheim 2006; Schuur et al. 2008). New estimations of the carbon pools in soils of the northern permafrost regions by Tarnocai et al (2009), revealed that organic soils (peatlands) and cryoturbated permafrost-affected

mineral soils contain the highest mean organic carbon pools (69.6 and 32.2 kg m^{-2}, respectively) compared to other soils types. This model could still be an underestimation of total C pools in mineral soils because of data scarcity (Schuur et al. 2008). For example, the most recent estimates of soil organic carbon in the Arctic, based on deep sampling, estimates the value at 1672 Pg C which is twice as much as the amount of carbon extant in the atmosphere and double that of previous estimates (Gorham 1991; Jobbágy and Jackson 2000).

Our knowledge about the quality (decomposability) of soil organic matter is limited to a few studies (Dutta et al. 2006; Ström and Christensen 2007; Coolen et al. 2011; Lee et al. 2012) and most of them are limited to few Arctic locations. The general assumption is that the quality depends on the soil genesis process; therefore, organic soils harboring peat are likely to decompose at slower rates compared to organic upland soils or mineral soils (Schuur et al. 2008). Although it was thought that permafrost C from both peatland and mineral soil have slow decomposition rate upon permafrost thaw (Schuur et al. 2008), recent research reveals much higher decomposition rates than previously expected. For example, Siberian loess permafrost, which contains a large carbon pool (450 Pg), quickly decomposes upon thaw and could release about 1 Pg C immediately followed by another 40 Pg C over the subsequent 40 years with a temperature increase to 5^0C if only 10% of total permafrost pool thaws (Dutta et al. 2006). Moist acidic tundra from Alaska has also been shown to have labile carbon that is easily decomposed (Coolen et al. 2011). Although Lee et al (2012) used a range of soils from organic and minerals soils and investigated the decomposability of carbon in aerobic and anaerobic conditions; still there remains a huge amount of work on different soils from different ecosystems of the Arctic. Many factors, including parent material of the soil, vegetation cover, soil physicochemical characteristics and climate influence the soil composition, which will affect the rate of decomposition and lability of the soil organic matter.

1.3.2. Carbon fluxes in the Arctic with focus on CO_2

Methane

Methane is both produced and consumed in soils of terrestrial ecosystems. It is produced from anaerobic decomposition of organic matter in water-saturated, anaerobic soil. Wet Arctic tundra is known as a significant contributor to methane emissions (Bartlett and Harriss 1993). One-quarter to one-third of the world's soils total methane emissions are emitted from the wet soils of high latitudes (Walter et al. 2001). The microbial process of methanogenesis is responsible for methane production in anaerobic soil zones. Conversely, in aerobic parts of the soil column, methane is consumed by microbial methane oxidation. Most of the methane consumption happens in the dry and well-drained soils (Ridgwell et al. 1999). Methanotrophy is responsible for the removal of approximately half of the produced methane. Hence, it is a very important process in regulating methane net emissions from soils. High Arctic well-drained and dry soils are not a source of methane; however, they might have a significant role in oxidation of atmospheric methane. Recent findings (Lamb et al. 2011) confirm this model as it was shown that several High Arctic dry soils act as a slight sink for methane. Based on atmospheric analyses, the Arctic is an overall net source of methane (Denman et al. 2007); therefore, these soils might play a crucial role in regulating feedbacks to a future warmer climate.

Carbon dioxide

Net CO_2 exchange from soils is an outcome of several factors: CO_2 uptake for photosynthesis minus CO_2 respiration by plants, the microbial decomposition of organic matter and CO_2 produced by fires that burn vegetation and organic matter. There is high temporal and spatial variation in C fluxes of different sites in the Arctic that makes it difficult to determine if this ecosystem as a whole will act as a source or sink of CO_2 (Aurela et al. 2001; Hobbie et al. 2002). For example, C accumulation in plant biomass is limited to vegetation-growing season (Blodau 2002; Rennermalm et al. 2005) and in summer time; many ecosystems are more

efficient in terms of ecosystem productivity via photosynthesis (Rennermalm et al. 2005). Moisture conditions also influence the uptake of CO_2 and increase in water table due to increased precipitation, proliferates net ecosystem production (Rennermalm et al. 2005). In northern wetlands of the permafrost zone, increase in temperature and moisture helps the ecosystem to act as a sink for CO_2 and produce methane in anoxic conditions (Smith et al. 2007).

Most of the models during 1990s indicate that northern high latitudes were a net sink for CO_2 (Baker et al. 2006; Gurney et al. 2004; Rödenbeck et al. 2003) Coupled climate-carbon models indicate a continuing Arctic CO_2 sink throughout the 21st century (Friedlingstein et al. 2006; Sitch et al. 2008; Qian et al. 2010). This is mainly due to a trend of increasing productivity and vegetation in response to elevated CO_2 concentrations and temperature (Norby et al. 2005; Oechel et al. 2000). On the other hand, microbial decomposition of carbon is a key determinant of changes in fluxes that might reverse these predictions by turning these soils into net sources of CO_2. In addition, episodic process of wild fire and disturbance of soil also converts soil organic carbon and adds to the release of carbon to the atmosphere and the fire response of the landscape to climate change is poorly understood (Schuur et al. 2008). But both of these processes depend enormously on the ecosystem type and climate conditions (Flannigan et al. 2005; Davidson and Janssens 2006).

When permafrost thaws, carbon that enters the soil can face two different conditions; oxic or anoxic soil conditions. In upland environments, lack of water saturation creates a desirable environment for aerobic microbial respiration. In water-saturated lowland regions, an anoxic soil condition is observed where development of thermokarst lakes results in the introduction of carbon to aquatic environments (Walter et al. 2006). Due to lack of oxygen, other elements such as nitrate, sulfate, iron and CO_2 will be used as electron acceptors for respiration. This leads to slower decomposition rates and the release of methane to the atmosphere. This process raises concerns as methane has 25 times more warming potential compared to CO_2 on a per molecule basis (IPCC 2007). To investigate the carbon balance, Lee et al (2012) examined the rate of permafrost CO_2 and

CH_4 release under aerobic and anaerobic conditions. Over a 500-day period, permafrost soils from Alaska and Siberia from both organic and mineral soil types containing 40% and 1-16% carbon, respectively, were incubated to measure the production rate of CH_4 and CO_2. As expected, higher decomposition rates were observed in carbon-rich soils on a per gram soil basis. However, when carbon release rate was expressed based on a per gram carbon basis, a measure that reflects only differences in C quality, mineral soils showed similar or greater C release to the organic soils, indicating that mineral soils may play similar roles in climate feedbacks as organic-rich peat soils. Based on these results, an aerobic upland ecosystem will probably be more critical in having larger effects on global climate change than anaerobic wetland environments in spite of methane release from wetlands. However, permafrost soils from various regions in the Arctic vary in their response and a broader study with more samples with different soil topography is needed to determine whether these findings are universal.

Laboratory studies alongside precise measurements of greenhouse gas productions and the models that take into account many key factors such as rate and extent of permafrost thaw, the hydrological and plant reaction to permafrost thaw, the decomposition timescale of organic matter and regional heterogeneities in soil properties (Von Deimling et al. 2012) will help to reach an accurate prediction of the magnitude and effect of thawing permafrost on global climate change.

1.4. Microbiology of the Arctic soils

Microorganisms in Arctic soils live in extreme environments where constant subzero temperatures, low water and nutrient availability can be seen all year round. Despite the harsh conditions of the cold environments, microorganisms manage to thrive in these extreme ecosystems. Early studies delving into the microbiology of polar regions tried to answer the question if these microbes are only surviving or actively growing (James and Sutherland 1942; Boyd and Boyd 1964; Horowitz et al. 1972; Cameron and Morelli 1974). However, they all needed to prove that there was no contamination from equipment and handling

the process of sampling from the environment. It was very likely that microbes get introduced to the ecosystem through sampling. Recently, with the development of new techniques and methods such as the utilization of fluorescent microspheres and green fluorescent protein-marked strains, there have been improvements in obtaining intact soil and permafrost samples (Shi et al. 1997; Juck et al. 2005). We now know that the frozen ground harbors ancient viable cells. It is difficult to find the timeline or age of a microbial species without a fossil record or evident events of the origin time of a microorganisms` appearance (Vreeland and Rosenzweig 2002). Thus, the age of the environment surrounding microorganisms determines their age. That is why microorganisms found in permafrost are believed to be ancient and as old as the permafrost they are isolated from. These ancient viable cells represent an important portion of the biosphere; the cryobiosphere regarding their response to future climatic warming and their role in biogeochemical cycling (Gilichinsky et al. 2008).

Precise knowledge about the abundance, diversity, viability, activity and distribution of microbial assemblages in these ecosystems is essential to understanding the ecological roles they have and their future potential response to global warming. Predicting future carbon fluxes is dependent on microbial activity in these ecosystems. Frozen conditions put limitations to the ecosystem such as low availability of water, reduced protein flexibility and enzyme activity. Therefore, these environments are very sensitive to even small changes in temperature which could exert many changes in soil composition, hydrology and thermal regimes as well as influencing microbial assemblages and their ecological roles (Doran et al. 2002).

Biodiversity and composition; importance and role in ecosystem functioning

All forms of life including, plants, animals and microbial species control ecosystem processes such as the physical formation of habitats (ecosystem engineering (Jones et al. 1994), fluxes of elements in biogeochemical cycles (for example, ecological stoichiometry (Sterner and Elser 2002), and the productivity of ecosystems (for example, via trophic cascades and keystone species (Power et

al. 1996). Biological communities' diversity has been shown to regulate these ecological processes (Hooper and Vitousek 1997; McGrady-Steed et al. 1997; Naeem et al. 1994; Symstad et al. 1998; Tilman 1997; Tilman et al. 1997). Therefore, biodiversity loss and its ecological consequences has recently been the focus of many researches (Chapin III et al. 2000; Loreau 2000; McCann 2000; Schwartz et al. 2000; Cottingham et al. 2001). Any substantial loss of biodiversity could influence these critical processes and therefore plant productivity, soil fertility, water quality, atmospheric chemistry and many other environmental and global conditions that are associated with human welfare (Naeem et al. 1999).

Microorganisms including, bacteria and their diversity is part of the aforementioned maintenance of ecosystem and human being welfare (Hunter-Cevera 1998). They are almost found and adapted to every environment on earth (Øvreås 2000).

In order to describe bacterial diversity, several culture dependent and independent methods have been used. Cultivation selects as low as 1% of the bacteria present in the environment (Perry et al. 1989)

1.4.1. Microbial diversity and ecology of permafrost and active layer

Permafrost and active layers of soils are both exposed to extreme physical and chemical conditions. Scientists have tried to unravel the complexities associated with microbial communities in both sections of cryosols. Here I review the microbial diversity and abundance of each environment as well as discussing similarities and critical dissimilarities between them.

1.4.1.1 Microbial assemblages in active layer

Bacterial abundance

Boyd and Boyd (1964) carried out the first investigation on the abundance of microorganisms in arctic soils and reported 55,000 bacteria per gram of dry soil growing at +22 °C. Later, total microbial counts of the active layer using

fluorescence in situ hybridization (FISH) showed 1.2-23 x 10^8 cells g-1 soil (Kobabe et al. 2004). These results indicate that microbial abundance in Arctic soils is comparable to peat (Dedysh et al. 2003) and soils of other regions (Atlas and Bartha1993; Richter and Markewitz 1995). Based on Kobabe et al (2004), the highest total bacterial cell count (23 x 10^8 cells g^{-1} soil) was detected in uppermost 5 cm layer of the soil and decreased with depth. However, the abundance of microorganisms rises again close to the permafrost table. The process responsible for such increase at the boundary of active layer-permafrost is likely the infiltration of melted water which carries microorganisms from the surface down to permafrost table (Spirina and Fedorov-Davydov 1998).

Bacterial community composition

In cryosolic soils of the Arctic, temperatures fluctuates between -35 and +15°C, producing a distinct temperature gradient in the surface soils that leads to the formation of a hostile environment for living cells (Wagner 2008). However, microorganisms have overcome these extreme conditions and adapted to the cold temperatures, freeze-thaw cycles, and low water and nutrient availability found in Arctic soils (Rodrigues and Tiedje 2008). In order to characterize the microorganisms thriving in this extreme environment, various culture-dependent and independent methods since the 1940s and late 1990s, respectively, have been used. Early studies using culturing showed the existence of many types of bacteria including members of: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Firmicutes, Actinobacteria, Cyanobacteria and members of Bacteroidetes - previously known as CFB group (*Cytophaga\Flexibacter\Bacteriodes*) (Nelson and Parkinson 1978; Dobrovol'skaya et al. 1996). Culture-dependent studies revealed dominance of Gram-negative bacteria such as *Burkholderia sp.*, *Collimonas sp.*, *Pedobacter sp.*, *Janthinobacter sp.*, *Duganella sp.*, *Dyella sp.*, *Achromobacter sp.*, *Pseudomonas sp.* and *Sphingomonas sp.*, over Gram-positive strains (Belova et al. 2006; Männistö and Häggblom 2006).

With advances in molecular methods and their usage in microbial ecology,

evaluating the microbial diversity and abundance has developed at an unprecedented rate. Because of this, much of the information about environmental microbial diversity is based on nucleic acid composition of microbial communities.

Neufeld and Mohn (2005) found greater bacterial diversity in active layer Arctic tundra soils than in temperate boreal forest soils. Surprisingly, the highest diversity was related to a sample from the High Arctic. The observed higher diversity at higher latitudes stands in stark contrast to the ecological patterns for plants and animals in which there is an observed decrease in species richness and diversity with the increase in latitude (Willig et al. 2003).

Zhou et al (1997) showed the presence of members of Proteobacteria (60.5%), especially from Delta (25.6%), Alpha (20.9%), Beta (9.3%) and Gamma (4.7%) subdivisions as well as Fibrobacter (16%), Gram-positive bacteria (11.6%) and members of the Bacteroidetes (2.3%) in 16S rRNA gene clone libraries from Siberian tundra soils. However, because the number of environmental clones was very small (43), these results likely do not reflect the total diversity of the soils. More extensive analysis (Neufeld and Mohn, 2005) found Proteobacteria and substantial proportions of Actinobacteria, Acidobacteria, *Firmicutes*, Bacteroidetes, Verrucomicrobia, and Cyanobacteria as the dominant groups of bacteria.

Arguably, one of the important findings of culture-independent methods is in demonstrating the presence of about 10-30 % of the total population in unclassified bacterial group- sequences that cannot be assigned to any previously described phylogenetic group- in Arctic soils. For example, Gilichinsky et al (2008) investigated the microbial soil diversity in a non-enriched soil sample in addition to aerobic and anaerobic enrichments; a high proportion of the phylotypes were related to Proteobacteria and unclassified bacteria. In general, in all their samples they had 15 phyla and about 29% of the phylotypes belonged to unclassified bacteria. In contrast, Liebner et al (2008) in a study addressing the bacterial diversity of polygonal surface tundra soils of Lena Delta, Siberia found

the majority of the community members related to Bacteroidetes, in particular Sphingobacteriaceae. This is surprising as the previous research on Arctic soils showed only a small proportion of community share of Bacteroidetes (Sørheim et al. 1989; Zhou et al. 1997; Gilichinsky et al. 2008). Yet another study on the active layer soils of the Canadian High Arctic revealed the presence of Actinobacteria (54% of clones) and Proteobacteria (42%), predominantly related to the Betaproteobacteria (25%), as well as Bacteroidetes (4%) (Steven et al. 2008). Thus, there is a significant variability in overall community composition at multiple phylogenetic levels when examining Arctic soils.

There are many factors such as organic matter content, soil physicochemical characteristics that might contribute to the minor and major differences in bacterial community composition between different sites. Unfortunately, there are few studies focusing on the differences and patterns controlling the ecological niche for microorganisms. An investigation of diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai–Tibet Plateau permafrost region showed a few differences with other permafrost environments (Shi et al. 1997) in that high content G + C Gram positive bacteria dominated the total community, although the results were solely dependent on culturable isolates (Zhang et al. 2007). Since, culture conditions can determine the type of strains isolated (Shi et al. 1997; Vishnivetskaya et al. 2000), the methodology adds to the difficulty of interpreting data. In addition, differences in physico-chemical characteristics of the soils might be another controlling factor (Zhang et al. 2007). Distinctive vegetation types has also been shown to exert changes in the community profile as shrub soils harbor different bacterial and fungal communities than tundra tussock soils (Wallenstein et al. 2007). However, the bacterial community structure in Arctic soils is not fundamentally different from the bacterial communities in lower latitudes (Chu et al. 2010). The bacterial community compositions and diversity of the Canadian, Alaskan and European Arctic soils are as variable as 85 soils from a wide range of lower latitude sites and soil pH better accounts for soil community composition than latitude or other related factors such as mean annual temperature. However, this study did not

include Arctic tundra barren sites and was only limited to the total community structure based on total DNA analysis. It is not known if the same findings are true for the active components in the community that contribute to the functions of the ecosystem.

1.4.1.2 Microbial assemblages in permafrost: composition and abundance

Bacterial abundance

Investigation of bacterial cell count in permafrost has shown quite large numbers such as 10^5-10^6 cells g^{-1} in the Antarctic (Horowitz et al. 1972; Cowan 2002), 10^3-10^8 cells g^{-1} in Siberia (Rivkina et al. 1998), and 10^7 cells g^{-1} in the Canadian High Arctic (Steven et al. 2004). Due to the extreme conditions of Arctic, a large proportion of these cells are non-viable (Giller et al. 1997; Torsvik et al. 1998; Trevors 1998). All these finding are based on plate counts of bacteria cultured and might not reflect an accurate estimation of viable cell numbers due to restrictions originating from culturing. However, the use of LIVE/DEAD stain showed that only 26% of bacteria were viable (Kirk et al. 2004), therefore, the inhospitable conditions has put cells into a recalcitrant state making the inferences for viable community limited.

Bacterial community composition

Over 70 bacterial genera belonging to aerobic heterotrophs, anaerobic heterotrophs, iron reducers, sulfate reducers, nitrifying and nitrogen fixing bacteria have been isolated from the Arctic permafrost (Gilichinsky et al. 1995; Shi et al. 1997; Vorobyova et al. 1997; Steven et al. 2009). It is technically very challenging to culture and isolate microbes including cold environment inhabiting microorganisms since the culture media itself can only favor the growth and enrichment of specific groups of bacteria and be very selective (Steven et al. 2006). As a result, cultivation can only identify a small proportion of the microbial community such that only 0.1–1% of the total cells in arctic permafrost are represented by cultured isolates (Vorobyova et al. 2001).

Nevertheless, the organisms that were successfully cultivated have the optimal growth at room temperature and therefore are psychrotolerant rather than psychrophilic- psychrophiles have an optimal growth temperature at 15 °C or below (Morita 1975); however, both psychrotolerant and psychrophilic microorganisms have been isolated (Bai et al. 2006; Steven et al. 2007; Steven et al. 2008). This finding led to the proposition of ‘‘community of survivors’’ for the permafrost microbes (Friedmann 1994), meaning they do not prefer their environment but rather are adapted and resistant to the hostile conditions of cold environments.

Both Gram-negative and Gram-positive cells, including spore-forming bacteria, are commonly isolated. However, the spore-forming genera vary in abundance in geographically distant areas of permafrost (Steven et al. 2009). For instance, Canadian High Arctic permafrost harbors between 69% and 100% of spore-forming bacteria in the total isolates from 2 and 9 m samples, respectively while Siberian (Shi et al. 1997), Spitsbergen Island (Hansen et al. 2007) and Chinese alpine (Bai et al. 2006) permafrost only contain 30%, 5% and 1%, respectively, of the total viable bacteria from spore-forming genera. Firmicutes and Actinobacteria are usually the major component of the permafrost microbial community with a 100, 60 and 45% representation in Canadian High Arctic isolates (Steven et al. 2008), Chinese alpine permafrost isolates (Bai et al. 2006) and Siberian permafrost isolates, respectively. A study on a permafrost sample from Northeast Siberian seacoast showed the dominance of phyla Firmicutes, Proteobacteria, and Actinobacteria. 85% of these isolates belong to the genera *Arthrobacter* and *Planococcus* (Hinsa-Leasure et al. 2010), both of which have been shown to be present in other permafrost samples (Steven et al. 2007; Zhang et al. 2007; Steven et al. 2009). Another study on permafrost samples from a tundra ecosystem in Northeast of Eurasia (Vishnivetskaya et al. 2006), isolated members of Actinomycetales (*Arthrobacter* and *Microbacteriaceae*), Firmicutes (*Exiguobacterium* and *Planomicrobium*), Gammaproteobacteria (*Psychrobacter*) and Alphaproteobacteria (*Sphingomonas*). They also isolated Bacteroidetes which was lacking from Northeast Siberian seacoast permafrost. The differences

observed in the occurrence of the isolates might be due the various culture conditions and culture media that have been used in different studies. Therefore, culturing techniques and strategies have been developed to improve the isolation of microorganisms from permafrost samples such as improving the culture media through using natural permafrost sediment (NPS) enrichment (Vishnivetskaya et al. 2000) and liquid media incubation prior to plating (Miteva et al. 2004; Hansen et al. 2007). This indeed has helped to retrieve more viable cells through culturing experiment (Nocker et al. 2007).

The microorganisms recovered from permafrost have adapted to the low nutrient availability of these environments. Hence, their chance of recovery from soil gets improved in nutrient-poor media (Gilichinsky et al. 1989; Bai et al. 2006; Steven et al. 2007). Generally, these isolates have distinctive characteristics that enable them to survive in these environments. One of these features is halotolerancy, which is an important factor contributing to low water tolerance of the microorganisms (Steven et al. 2009). In extreme cold conditions of permafrost, there is little biologically available liquid water and during freezing the water, ions get expelled into the remaining liquid phase (Su et al. 2012) . This generated salt stress favors halotolerant bacteria. This might explain the connection between halotolerancy and microbial survival in cold temperatures (Nowrousian 2010).

The application of molecular-based tools has added to the number of phylogenetic groups of bacteria found in permafrost. For instance, the phyla *Gemmatimonadetes*, and *Planctomyces* have been only found using culture-independent survey of a Canadian High Arctic permafrost sample (Steven et al. 2007). The phyla abundance was also found to alter using the molecular-based methods as Actinobacteria and Proteobacteria-related sequences became predominant with the decrease in Firmicutes-related sequences. In addition, phyla that have no cultured representatives such as candidate division TM7, OP10 and OD1 have been shown to be present in Spitsbergen Island of High Arctic permafrost samples (Hansen et al. 2007). Bacterial classes such as

Thermomicrobia, alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epsilonproteobacteria, Clostridia, Mollicutes, Actinobacteria, Planctomycetacia, Spirochaetes, Acidobacteria, Bacteroidetes, Flavobacteria, Sphingobacteria, and Verrucomicrobiae were present though Actinobacteria was the dominating class. *Acidobacteria* were detected in Northeast Siberian seacoast permafrost through constructing a clone library, while they could not be isolated from plating (Hinsa-Leasure et al. 2010). Another study by Yang et al (2008) investigated the community structure and diversity of bacteria in permafrost sediments in the Tianshan Mountains, China and found a rich diversity of bacteria. Their analysis revealed the presence of Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Firmicutes, and Chloroflexi with the dominance of Proteobacteria. Quantitative PCR of several bacterial Phyla in High Arctic permafrost sample from Eureka, revealed the dominancy of Actinobacteria with a codominance of Betaproteobacteria. Bacteroidetes and Alphaproteobacteria were also found in high abundance while Acidobacteria and Firmicutes were the least abundant groups measured (Melamede 1985; Hyman 1988).

Nonetheless, few reports on the microbial diversity of permafrost affected soils have been published using culture-independent methods. Because of that we find most of the knowledge about permafrost microbiology so far coming from culturing experiments. There are still many uncertainties on microbial diversity based on the detection of the DNA since much of our knowledge about microbial diversity comes from the extraction and analysis of DNA. It is unclear if they are viable and not culturable or they are just naked DNA preserved in permafrost. Although it is unlikely that dead cells will be a large proportion of community DNA due to having a reduced residence time in soil (Ronaghi 2001), constant subzero temperatures can preserve DNA for years (Simon and Daniel 2011); therefore naked DNA could be easily isolated and used as representative of microbial community.

1.4.1.3 Permafrost and the active layer similarity; a microbial community comparison

While both permafrost and active layer of the cryosols are hostile environments, there are differences in the types of challenges faced by microorganisms in these different environments. For instance, in the active layer, freeze-thaw cycles and temperature fluctuations are the main sources of stress, while in permafrost constantly below zero temperatures exert enduring cold stress (Rodrigues and Tiedje 2008).

These differences led microbial ecologists to question if there are any alterations or similarities in the microbial assemblages between the two environments. Steven et al (2008) in a study that compared the microbial diversity and composition of active layer and permafrost from Eureka in Canadian High Arctic found that diversity was similar in the two horizons. However, the composition was different as Actinobacteria (54%) and Proteobacteria (42%) were predominant in the active layer while Preteobacteria-related sequences dominated the permafrost clone library. Considering the results from culturing, the number and diversity of the bacterial isolates were higher in the active layer. In permafrost, they could only recover isolates belonging to Firmicutes. In addition, the viable cell counts and diversity of the isolates from the permafrost was lower than in the active layer. Another study on a wetland permafrost sample from Axel Heiberg Island, Canada showed that a greater diversity of isolates was recovered from permafrost than active layer, though viable cell counts were opposite (Wilhelm et al. 2011). The bacterial community composition was also different based on clone library results. Acidobacteria were dominant in the active layer while Actinobacteria were dominant in Permafrost clones. A complete list of the common occurring phyla in Canadian, Alaskan and European Arctic and a comparison of active layer and permafrost can be found in table 1.

It is likely that permafrost microbial community could be a subset of active layer as these communities formed in the surface ecosystems are trapped and

buried during sediment accumulation and freezing (Hoffmann et al. 2007). However, the differences reported between upper soil layers and underlain permafrost is possibly due to the complex vertical structure of soil/sediments and the physical/chemical differences between the horizons (Kircher and Kelso 2010). In spite of these physicochemical differences, Gilichinsky et al (2008) found similarities between active layer and permafrost of an Arctic soil. Gram-positive bacteria with high and low G+C content, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Bacterioedetes were present in both soil and sediments; however, the genera diversity was higher in permafrost. Also Gammaproteobacteria was predominant in permafrost while Alphaproteobacteria, Betaproteobacteria and Delta proteobacteria dominated the active layer.

Yergeau et al. (2010) investigated the phylogenetic and functional community composition in an active layer sample and a 2 m deep permafrost sample from the same site in Canadian High Arctic, and found *Actinobacteria* as the dominant phylum in both the active layer soil and permafrost, even though *Betaproteobacteria* was codominant with Actinobacteria in permafrost. The authors indicated that these results, which were in agreement with previous studies in finding Actinobacteria as the dominant group (Kobabe et al. 2004; Steven et al. 2007; Liebner et al. 2008; Steven et al. 2008; Wagner et al. 2009), confirms how well-adapted Actinobacteria are to cold environments.. They suggested that permafrost microbial community could be a subset of the active layer microbial community and originated from overlying active layer.

A recent metagenome sequencing study on Alaskan active layer and permafrost soils investigated the microbial phylogenetic and functional genes at the frozen state and after thaw on two replicate cores (MacKelprang et al. 2011). Active layer samples from both cores were more closely related to each other than to their underlying permafrost. The same result was observed for 2-day and 7-day incubations. Despite a higher level of similarity between active layer cores, active layer and permafrost still shared similar community composition. Actinobacteria,

Proteobacteria, Chloroflexi, Verrucomicrobia, Acidobacteria, Bacteroidetes, Firmicutes were present in both. Actinobacteria and Proteobacteria were abundant in active layer while Bacteroidetes and Firmicutes were less than 10% abundant. In comparison, Bacteroidetes and Firmicutes were dominant as more than 30% of the total community in permafrost.

1.4.2 Heterotrophs in the Arctic

Understanding heterotrophic soil respiration and its sensitivity to global warming will help fill many gaps in the knowledge about arctic climate feedbacks. Recent findings indicate that changes in the structure and diversity of terrestrial microbial communities will have important effects on CO_2 production. Allison et al (2010) indicated that microbial adaptation or shifts in microbial communities will accelerate carbon loss from the ecosystem (Allison et al. 2010). Moreover, higher concentrations of CO_2 interfere and alter soil microbial composition and activity, leading to carbon loss from ecosystems (Carney et al. 2007). Heterotrophic bacteria that oxidize organic C to inorganic forms to extract energy for growth is then considered to have the greatest role in determining the amount and rate of C transfer to the atmosphere especially in areas that that are sparsely vegetated.

Microorganisms are restricted to low nutrient availability due to the frozen state of the environment in Arctic. Actinobacteria are commonly found at high abundance in the bacterial community of Arctic soils (Vishnivetskaya et al. 2006; Steven et al. 2007; Yergeau et al. 2010). They are believed to maintain metabolic activity and DNA repair mechanisms in cold environments (Johnson et al. 2007). Furthermore, they have the capacity to degrade complex organic carbon compounds which makes them an important group in organic matter degradation in these extreme ecosystems (Yergeau et al. 2010). Based on most of the studies on Arctic soils and cold environments which was reviewed in 1.4.1, only very few studies have found *Acidobacteria* (Yang et al. 2008; Hinsa-Leasure et al. 2010) and they used culture-independent methods. A recent study also by Chu et al (2010), showed the presence of *Acidobacteria* among the dominant phyla;

however, they used a high resolution bar-coded pyrosequencing technique. It was not until very recently that researches have tried to fill the gap observed in the background literature in linking microbial communities to ecosystem function. While it has only been very recently that our knowledge has improved about the microbial diversity of permafrost-affected soils due to the application of genomic methods, we still do not know much which bacteria among the detected bacteria are growing in low temperatures in Arctic or what their role and physiological abilities are in the total microbial community. This will be the focus of the next section of my literature review.

Table 1. Phylogenitic groups of Bacteria (phylum level) in various permafrost environments of the Arctic

		Permafrost affected soils			
		Canadian Arctic	Alaskan Arctic		European Arctic	
	Phylogenetic classification	Eureka, Ellesmere Island, Nunavut Canada[a]	Hess Creek[b]	Toolik LTER,moist acidic tundra[c]	SamoylovIsland, LenaDelta, northeastern Siberia[d]	Adventdalen valley, Spitsbergen NorthernNorway[e]
active layer	proteobacteria	+	+		+	NS
	Alpha-proteobacteria			+	+	NS
	Beta-proteobacteria	+		+	+	NS
	Gamma-proteobacteria		+	+		NS
	Delta-proteobacteria			+	+	NS
	Bacteroidetes		+	+	+	NS
	Actinobacteria	+	+	+	+	NS
	Acidobacteria			+	+	NS
	Verrucomicrobia		+	+	+	NS
	Gemmatimonadetes		+	+		NS
	Chloroflexi		+		+	NS
	Planctomycetes				+	NS
	Thermomicrobia				+	NS
	Firmicutes					NS
permafrost	proteobacteria	+	+	NS	NS	
	Alpha-proteobacteria	+		NS	NS	
	Beta-proteobacteria	+		NS	NS	+
	Gamma-proteobacteria	+	+	NS	NS	+
	Delta-proteobacteria			NS	NS	+
	Bacteroidetes		+	NS	NS	+
	Actinobacteria	+	+	NS	NS	+
	Acidobacteria	+		NS	NS	
	Verrucomicrobia		+	NS	NS	
	Gemmatimonadetes	+	+	NS	NS	
	Chloroflexi		+	NS	NS	
	Firmicutes			NS	NS	

[a]Steven et al. (2008), [b]Mackelprang et al. (2011), [c]Wallenstein et al. (2007), , [d]Wagner et al. (2009), [e]Hansen et al. (2007). Ns: not studied

1.4.3 Microbial activity: identification of active microbes in cold environments

With the advances in molecular methods and the recent progress in microbial diversity analysis of permafrost-affected soils, there remains many unanswered questions regarding the biologically active microorganisms. If they are actively growing, what is the level of activity? How can the activity be linked to a function and therefore help us understand the physiological and functional changes in the community in different conditions? These questions recently have driven many microbial ecologists' attentions toward the investigation of the activity at subzero and cold temperatures of cold ecosystems. The ability of microorganisms to grow at temperatures as low as -12 °C has been shown (Breezee et al. 2004). Several recent studies have explored microbial activity in the range of -20 °C to 0 °C using various methods, including incorporation of labeled precursors such as ^{3}H-thymidine for DNA synthesis ^{14}C-Leucine for proteins synthesis (Carpenter et al. 2000; Rivkina et al. 2000; Junge et al. 2004), gas evolution (Mikan et al. 2002; Rivkina et al. 2002; Panikov et al. 2006), gas uptake (Kato et al. 2005; Panikov and Sizova 2007), organic matter decomposition (Schimel et al. 2004; Panikov et al. 2006) and UV microscopy (Junge et al. 2004). Incorporation of labeled DNA and protein precursors are normally used for homogeneous frozen objects and less frequently for soils. In the case of soils as heterogeneous habitats, gas exchange rate (CO_2, O_2, CH_4 and N2O) and decomposition processes are often used (Simon and Daniel 2011). For example, tundra soil incubations at 0 to -39^{0}C had positive and measurable CO_2 production rate during the experiment. It was shown that permafrost had higher activity than overlying active layer. Microscopy is also a powerful tool to visualize active cells; however, it does not provide activity or growth rate. All these methods have advantages and limitations of their own. For example, decomposition processes are not usually satisfactorily sensitive as it does not

generate an instant rate of a particular microbial activity; therefore, it is not indicative of current temperature conditions and only gives a time averaged integral curve (Panikov 2008). Although gas exchange rates are used as a precise and sensitive method, there is the possibility that abiotic release of gases interferes with gas release originating from instant respiratory activity of soil biota (Panikov et al. 2006).

Overall, microbial activity and growth at subzero temperatures in various cold environments have been observed but the rate is rather different from other biomes. In a study addressing temperature dependence of metabolic rates of microorganisms for growth, maintenance, and survival, Price and Sowers (2004) suggested that metabolic rates can be seen in three different situations where 1) there is active growth; 2) there is metabolism providing insufficient for growth, but capable of supporting maintenance of the organism; or 3) survival of the microorganism such that is able to repair macromolecular damage.

Measuring activity through the aforementioned methods and techniques does not provide much insight toward the key players of microbial community. A useful method that could provide access to the whole DNA pool of a microbial community that is related to a specific function is needed. Phylogeny per se does not provide much information about the function of the microorganism in the environment (Quince et al. 2009; Quince et al. 2011; Schloss et al. 2011). rRNA gene sequencing has revealed a vast microbial diversity; however, the interactions and metabolic capabilities of the bacteria related to the sequences cannot be inferred from these data. In order to link the identity of microorganisms to metabolic analyses, a favorable experimental method for microbial ecologists is to track substrate usage by a specific group of microorganisms and a few methods such as fluorescence in situ hybridization (FISH)–microautoradiography (Nocker et al. 2007) have been developed and used. In this method environmental samples are incubated with specific radio labeled substrates and then thin sections of samples are fixated and analyzed by

FISH and inverse confocal laser scanning microscopy. Bacteria that are biologically active in taking up a specific radio labeled substrate will be detected using specific 16S rRNA-targeted FISH probes. This method has been used to track nitrifiers and denitrifiers in complex microbial communities (Kindaichi et al. 2004). Another technique that helps to study identity in relation to function and activity is isotope array. This method also involves the ^{14}C-labelled substrate incubation; however, RNA is extracted and labelled with a fluorophore and analyzed with an oligonucleotide array that targets 16S rRNA of the bacteria of interest. The array will be further scanned to find the community members that have actively incorporated the radiolabeled substrate (Steven et al. 2009). Both these methods deal with radioactive material handling and there might be a high background fluorescence and non-specific binding probe that interferes with sensitivity and accuracy (Hansen et al. 2007).

Another approach that filters active populations from the total microbial DNA consuming a given substrate is called stable isotope probing (SIP).

Use of stable isotope probing in the identification of active microbial communities

An isotopically-labeled substrate of interest is provided to a microbial community and the incorporation of isotopic label in biomarker molecules is investigated. Parts of the community that assimilate the substrate are actively growing on the specific substrate. If the biomarker is DNA, the method is called DNA-SIP (Radajewski et al. 2000). Following feeding with the substrate, DNA is isolated and the labeled DNA is separated from the unlabeled DNA by CsCl density gradient ultracentrifugation. Genetic markers such as 16S rRNA genes can then be used to identify which components of the microbial assemblage are utilizing that substrate. A great example of SIP application has been the study of active methanotrophs through the use of ^{13}C-methane. In a study investigating the activity and diversity of methanotroph populations in active layer soils of Ellesmere Island in the Canadian high Arctic using SIP, active methanotrophs

revealed to be related to type I methanotrophs mostly Methylobacter tundripaludum (Steven et al. 2006). DNA-SIP has been extensively used in finding the active microbial communities responsible for biodegradation of organic pollutants. For example, Jeon et al (2003) studied the flow of carbon from pollutants to microbial community using ^{13}C-naphthalene as the stable isotope and found that a cluster of Betaproteobacteria was responsible in the metabolism of 13C-naphthalene. A combination of traditional enrichment and isolation methods with DNA-SIP led to the isolation of a strain that had been only characterized in environmental DNA sample. Overall, SIP-metagenomics has enabled microbial ecologists to dissect many biological processes in the environment including, methanotrophy (Price 2007) nitrification (Steven et al. 2009; Yergeau et al. 2010) rhizosphere–microorganism interactions and bioremediation (Turpin et al. 1993). Application of metagenomics and high-throughput sequencing alongside the DNA-SIP has enabled researchers to look for active microbial groups carrying out a specific task and find their identity.

1.5. Research objectives and hypothesis

My research objectives were divided into three main branches; 1) activity measurements of the heterotrophic bacterial communities; 2) characterization of the bacterial community and the diversity in four polar sites of the High Arctic barren; and 3) identification of the active members of the heterotrophic bacterial assemblages.

In this project, I added ^{13}C-labeled complex, labile organic carbon in the form of algal lysate to active layer high Arctic barren soils for incubation experiments at 4°C. The algal lysate was chosen to approximate the complex organic carbon present in these soils to investigate how the microbial communities will respond to increased labile organic carbon.

The soil respiration rates were measured by CO_2 efflux measurements as a measure of overall microbial activity. The goal was to examine the soil

respiration capacity in Arctic soils and their response to increased labile organic carbon. I predicted that the microbial activity (carbon consumption rate: μmols CO_2 g^{-1} soil day^{-1}) would significantly increase in the incubated samples with the addition of labile carbon compared to the control samples and that all sites would respond similarly.

DNA-SIP was used to isolate DNA of active bacterial community. I hypothesized that the addition of labile carbon to the polar desert soils would lead to a decrease in the diversity of heterotrophic bacterial community. If the total community is considered based on total DNA analysis, I expected that only a few taxa would dominate the community. I was interested to find out whether these numerically dominant taxa which are detected in the total community are also numerically functional meaning that I will observe them in enriched heavy band as well.

Following incubation with ^{13}C-labeled algal lysate, isolated DNA was separated by CsCl gradient fractionation into heavy DNA belonging to the active members and light DNA of inactive community. Real-time PCR was used to determine the 16S rRNA gene copy numbers in different fractions. High-throughput sequencing of 16S rRNA gene PCR products from SIP fractions was used to examine the microbial diversity of the samples. The last prediction for the proposed hypothesis was that there would be a directional shift toward a specific group of microbes in the incubated samples compared to non-incubated sub cores, i.e. that all sites would show higher levels of similarity in their active components than the overall assemblage. Furthermore, this directional shift will lead to a decrease in the diversity.

My second hypothesis was that there is a core Arctic desert soil microbial community found at all sites and not found in non-Arctic soils. I predicted that the microbial community composition (variability, richness and phylogenetic diversity) is similar between sites but different from other biomes i.e. temperate soils, tundra and boreal forest soils.

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2. Active heterotrophic microbial communities from polar desert soils of the Canadian High Arctic

2.1 Introduction

Permafrost soil contains an enormous amount of total organic carbon: twice as much as the amount of carbon that is extant in the atmosphere (Schuur et al. 2008; Zimov et al. 2006). Warming in the Arctic is occurring two to three times faster than the global mean surface temperature increase (IPCC 2007). As a result, the previously inaccessible frozen organic carbon in permafrost will be subject to microbial decomposition following thaw, leading to increased carbon release to the atmosphere (Ping et al. 2008). Additionally, increased productivity and extent of vegetation in a warmer climate (Norby et al. 2005; Oechel et al. 2000) will also increase labile organic carbon input in soils. Microbial decomposition is the main pathway for carbon turnover and its return to the atmosphere. As the climate warms, this will lead to positive climate feedbacks from terrestrial ecosystems to the atmosphere (Zimov et al. 2006; Schuur et al. 2008). Most studies regarding these feedbacks have focused on peatlands or generally address the tundra biome; however, few have focused on permafrost-affected mineral soils that account for 5,000,000 square kilometers of land surface (Hogan 2011). Hence, it is not clear how a changing environment will affect these ecosystems or what role they may play in climate change.

Polar desert ecosystems of the High Arctic, termed high Arctic tundra or high Arctic barren (Goryachkin et al. 2004), are permafrost affected mineral soils that undergo heavy cryoturbation and have been shown to have higher soil organic matter than was assumed (Tarnocai et al. 2009). Based on the observation that decomposition rates are similar on a per g C basis between mineral and organic Arctic soils (Lee et al. 2012), mineral soils may be a significant source of CO_2 to the atmosphere. Arctic mineral soils may also be a slight sink for atmospheric methane and substantial sources of N_2O (Lamb et al. 2011); thus, their potential impact on feedbacks to the climate system is complex and difficult to predict.

Since microbial communities play a major role in soil organic carbon turnover and heterotrophic respiration and carbon balance, therefore, more research should be focused on these environments, microbial activity and community composition and their response to future climate changes.

The microbial organic carbon decomposition rate depends on several factors such as organic carbon quality (decomposability), environmental factors, and genetic and metabolic potential for degradation in the microbial community. Organic matter within permafrost and active layer soil is labile and decomposable (Coolen et al. 2011); thus, interactions between microorganisms and their environments are the primary regulators of soil organic matter degradation in the Arctic. Changes in the structure and diversity of terrestrial microbial communities will accelerate carbon loss from the ecosystem (Allison et al. 2010). Moreover, higher concentrations of CO_2 alter soil microbial composition and activity, leading to carbon loss from ecosystems (Carney et al. 2007). Heterotrophic bacteria that oxidize organic C to inorganic forms to extract energy for growth are considered to have the greatest role in determining the amount and rate of C transfer to the atmosphere especially in areas that are sparsely vegetated.

The bacterial community structure in a variety of Arctic soils is not fundamentally different from the bacterial communities in lower latitudes (Chu et al. 2010); however, this study did not include high Arctic barrens soils. Furthermore, it is not clear whether all components of that community are active and potentially responsive to increased levels of complex organic carbon. Therefore, this study had three objectives 1) to measure the activity of heterotrophic bacterial communities 2) to characterize the bacterial community and diversity in four polar sites of the High Arctic barren and 3) to identify the active members of the heterotrophic bacterial assemblages using stable isotope probing (SIP) of DNA and high throughput barcoded-pyrosequencing. With this work, we identify for the first time the active members of the heterotrophic

bacterial assemblages of High Arctic Barren in response to labile carbon addition to active layer of permafrost-affected mineral soils.

2.2 Material and methods

2.2.1 Sites description

Soil samples were collected from three High Arctic barren sites on Ellesmere Island in the Canadian High Arctic Archipelago: Okse Bay (77 8' 88" N, 87 39' 10" W; hereafter called “Pit”), Alexandra Fjord Dome (78 51' 31" N, 75 55' 37" W, hereafter called “DP”), and Patterson River (82 35' 47" N, 63 45' 32" W, hereafter called “Pat”), as well as Resolute Bay (74° 43' 23" N, 94° 56' 10" W, hereafter called “Res”) on Cornwallis Island (Fig A1) by our colleagues at the University of Saskatchewan in summer 2010. These sites correspond to the B1 designation of the Circumpolar Arctic Vegetation Map (Walker et al. 2002). The Pit site is a small bay on the southwest peninsula of Ellesmere Island. The surrounding land is a series of raised beach crests from which the samples were taken. The top of the hills has polar desert terrain characteristics and the lower area in between is moist tundra. Soil structure is very coarse, containing mostly sand with some silt, large gravel and stones. Alexandra Fjord Dome is a valley on the east coast of Ellesmere Island. Although the lowland is a polar oasis, the DP site is in the mountainous area at the border of the valley-mountain (the dome), which is a polar desert with several glaciers covering the area. There are two different soil types at DP; dolomitic parent material to the east with a high pH (~7.6) and granitic parent material to the west with a moderately acidic pH (~5.2) (Brummell et al. 2012a). In the present study, only the dolomitic soil was used for sampling. The soil is very coarse textured, containing mostly sand with considerable amount of silt. At the Pat site on the northern edge of Ellesmere Island, the Patterson River flows from a large ice field and associated glaciers. The riverine valley is floored with polar desert terrain on glaciofluvial deposits. Soil texture is extremely coarse, at some areas sand is absent and only gravel and stones can be seen. Resolute Bay is composed of both polar desert and wetlands

on Cornwallis Island. At the Res site (polar desert-type), soil texture is similar to Pit and DP, with coarse textured soil containing mostly sand and some silt. All the soils belong to the High Arctic barren category (Goryachkin et al. 2004) with widely separated patches of plants including *Dryas integrefolia*, *Cassiope tetragona*, *Papaver radicatum*, and a few species of sedges in the genus *Carex.*

2.2.2 Soil characteristics

Soil pH, NO_3^-, NH_4^+ and organic carbon were measured at two different depths above permafrost layer for Pit and one depth for Pat and DP as previously described (Brummell et al. 2012a). *In situ* CO_2 concentrations and dark CO_2 flux were measured as was previously described (Brummell et al. 2012 a and b). *In situ* CO_2 concentrations were measured in soil using a probe and dark CO_2 flux was calculated from the accumulation or depletion of gas inside a closed, vented, non-steady-state chamber over a known time. No soil characteristic data were obtained for the Res site.

2.2.3 Sample collection and handling

In order to prevent soil disturbance and keep the structure of the soil cores intact, core collectors were used for sampling. Core collectors were manufactured by Rice engineering (AB, Canada): 1" internal diameter, seven-inch-long PVC pipes were slotted at every 0.5" on pipe surface with the slot size of 0.04", with no slots for the upper 1” at each end; one end was beveled. These 16 slots on the core collector allowed for free gas exchange. The core collectors were hammered into soil (beveled end down to provide a cutting surface) to collect the upper 10 cm of surface soil active layer. Each core contained approximately 50-60 g soil. Three replicate subsites 70 m apart were sampled at each site. This distance was chosen based on previously reported calculations that show no autocorrelation in measurements of soil parameters in High Arctic soils at >45 m distance (Banerjee et al. 2011). The cores were capped with PVC caps, sealed with plastic wrap to prevent loss of soil, and kept frozen at -20 ^{0}C during transport and storage until

further analysis. About six gram of soil was removed from the cores to use for total community analysis without any incubation as the original soil sample.

2.2.4 SIP microcosms and CO_2 production potential

Intact soil cores in the core collectors were unwrapped and placed inside a 2" diameter PVC pipe that was designed to hold the core and provide headspace (500 ml) for CO_2 production measurements (Fig A2). The outer pipes were capped with PVC caps, one of which (the top) had a butyl rubber stopper that was incorporated into the cap. PVC caps were sealed with a thin layer of vacuum grease. Core holders with no cores included were incubated as leakage controls: no significant leakage was observed.

In order to normalize the water content of the soils, field capacity was measured using cores for each site. A simple approximate method was developed to measure the field capacity (Veihmeyer and Hendrickson 1931). A sample core for each site was used to determine the amont of water to saturate soils without a rapid drainage and that amount of water was added to all cores for incubations.

Preliminary experiments designed to determine the minimal concentrations of ISOGRO (0.05%, 0.1%, 0.5% and 1% (w/w) that allowed detection of ^{13}C-label in community DNA were performed for samples from the Res site (data not shown). ISOGRO (Sigma) is an algal lysate-derived complex organic carbon mixture that heterotrophs can use as their carbon source (Bott and Kaplan 1985). All samples for preliminary experiments were incubated at 4 ^{0}C for increasing periods of time. Following incubation, we checked the existence of heavy DNA in cesium chloride gradients as previously described (Neufeld et al. 2007). ^{13}C-labelled DNA was detectable only in the 1% ISOGRO incubated samples and the minimal incubation period was approximately 49 days.

For each sub-site, six replicate cores were incubated at 4°C: three had no substrate added and acted as controls for incubation effects (“control”) while three others were incubated with 1% (w/w) ^{13}C-labeled algal lysate (ISOGRO; Sigma);

in total, there were 24 microcosms for incubations. 10-cm perforated needles (Cadence Science, Staunton, VA) were used to inject 10 mL of dH_2O containing sufficient ISOGRO for a final concentration of 1% w/w into the soil core. Soil mass was previously determined through cores used in the lab for other purposes but from the same sites. To distribute the solution evenly throughout the core, the solution was injected by squeezing the syringe very slowly in four places in the core from bottom to the top.

During the incubation, gas headspace samples (250 µl) were periodically withdrawn from the headspace of containers and analyzed for CO_2 production using a Shimadzu GC8Agas chromatograph (Fisher Scientific) equipped with thermal conductivity detector (TCD) on column HayeSep Q 80/100 (TCD temperature 120^{o}C, column temperature 35^{o}C, helium as carrier gas). A standard curve for CO_2 was prepared by injecting measured volumes from a commercially prepared CO_2 (100%). The CO_2 production rate was calculated from the slope of the linear portion of a graph of CO_2 concentration vs. time (data not shown). Incubations were continued until it was clear that CO_2 concentrations had reached their maxima. After the completion of the incubation course (49 days), soil from each core was homogenized by sieving and stored at -20°C for later DNA analysis. Statistical analysis was performed in SAS 9.3 (SAS, Toronto, Canada). The Shapiro-Wilk (Shapiro and Wilk 1965) test was used to test whether the CO_2 production rate data followed a normal distribution. Levene´s test (Levene 1960) was performed for homogeneity of variance. Since data followed the normal distribution and satisfied the homogeneity of variance, Tukey HSD (Zolman 1993) was carried out as the multiple comparison method.

2.2.5. SIP DNA extraction and ultracentrifugation in CsCl gradients

DNA was extracted from 10 g soil as previously described (Zhou et al.1996). Genomic DNA was visualized and quantified using a modification of the JGI Genomic DNA QC Using Standard Gel Electrophoresis (available at http://my.jgi.doe.gov/general/protocols.html). DNA was loaded on 1% agarose

gel along with 1 Kb Plus DNA Ladder (Invitrogen, CA). The band intensity was proportionaal to DNA concentration. Therefore, Ladder band intensity with known concentration was used as a reference in Adobe® Photoshop to calculate DNA concentrations. CsCl gradient fractionation, DNA precipitation, and DNA quantitations were carried out as described previously (Lueders et al. 2004). Briefly, ^{13}C-DNA separation was performed by cesium chloride density gradient centrifugation. CsCl solutions containing not less than 1 µg DNA were spun for 65 h at 41,000 rpm in a Vti 65.2 rotor in 13-by-51-mm polyallomer Quick-Seal centrifuge tubes (Beckman Coulter, CA). The resulting gradient was collected in 380-400 µl fractions from the bottom of each tube. The buoyant density (BD) of each fraction was determined with a Reichert AR200 refractometer (Ametek, Berwyn, PA). Each fraction was precipitated by addition of 2 vol 30% (w/v) polyethylene glycol (PEG 8000) and 20 µg glycogen (Fermentas, Burlington, Ontario, Canada). The pellet was dissolved in by placing 25 µl RNase-free water (Qiagen, Valencia, CA). Quantitative PCR (qPCR) with bacterial 16S rRNA gene primers 341F and 518R (Muyzer et al. 1993) was used to measure the abundance of bacterial DNA in gradient fractions with BD from 1.677 to 1.730 g ml^{-1}. qPCR was performed in triplicate 10 µl reactions containing 5 µl Rotor-Gene SYBR green PCR kit (Qiagen, Valencia, CA), 1 µM concentration of primers, 2µl template and 1µl Qiagen RNase-Free water. A Rotor-Gene Q thermal cycler (Qiagen, Valencia, CA) was programmed for 40 cycles at 95°C for 10 s and 60°C for 15 s. Gene copy number was calculated relative to a standard curve constructed with *Escherichia coli* genomic DNA. Denser bands, containing ^{13}C-labeled DNA, and lighter bands, containing unlabeled DNA, were separately pooled for each sample for downstream molecular analyses.

Denaturing gradient gel electrophoresis (DGGE) of bacterial PCR products was performed using a BioRad D-CODE system (BioRad, Hercules, CA) as previously described (Kulp et al. 2006). GelCompar II (Applied Maths, Austin, TX) was used to analyze DGGE banding patterns and generate a dendrogram

using a 1% band position tolerance based on Dice correlation coefficients (Kulp et al. 2006).

2.2.6 Amplicon PCR and Pyrosequencing

For each site, the V1-V3 regions of the bacterial 16S rRNA gene were amplified using the domain Bacteria-specific primer bar-coded 27F (5´-AGRGTTTGATCMTGGCTCAG-3´) (Lane, 1991) and 519R (5´-GTNTTACNGCGGCKGCTG-3´) (Turner et al., 1999). Genomic DNA was normalized to 100 ng/µl and a 100 ng (1 µl) aliquot of each samples DNA was used for a 50 µl PCR reaction (Dowd et al. 2008). Amplification was performed using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) under the following conditions: 94^{o}C for 3 minutes, followed by 28 cycles of 94^{o}C for 30 seconds; 53^{o}C for 40 seconds and 72^{o}C for 1 minute with a final elongation step at 72 ^{o}C for 5 minutes. Gene-specific PCR primer sequences were tagged with the sequencing adapters for GS FLX Titanium chemistry, 8 base barcode (only on the forward primer) and a linker sequence. All PCR reactions were performed in triplicate which were mixed in equal concentrations and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). FLX-Titanium amplicon pyrosequencing was carried out at MRDNA lab (Shallowater, TX, USA) using Genome Sequencer FLX System (Roche, Branford, CT).

2.2.7 Sequence processing and classification

To simplify sample labeling, the name of the site alone was used for the unincubated sample, the name plus C was used to represent the control sample that was incubated without the addition of substrate, the site name plus H represents the heavy fraction containing the active community, and the site name plus L represents light fractions.

The sequences obtained from pyrosequencing reads were analyzed using MOTHUR v. 1.25.0 (Schloss et al. 2009) for quality filtering, noise removal, chimera removal, identification of operational taxonomic unit (OTU), taxonomic

assignment, community comparison, and statistical analysis. Sequences were first culled based on flowgram data to set the minimum flows to 360 and maximum flows to 720, as previously recommended (Quince et al. 2011). Sequences shorter than 200 bp and those containing primer/barcode mismatches or homopolymers longer than 8 bp were excluded. Noise removal was performed using MOTHUR implementation of the PyroNoise algorithm, which corrects PCR- and pyrosequencing generated errors (Quince et al. 2011). The trimmed and quality filtered sequences were aligned to the Silva reference alignment (Silva SEED). OTUs were assigned using the average neighbor clustering algorithm for an OTU definition of 97% similarity. The assigned OTUs then were classified to phylum, class, and/or genus level using the ribosomal database project (RDP; http://rdp.cme.msu.edu/) classifier with a 51% confidence threshold. Phylotype command was used to assign sequences to OTUs based on their taxonomy and phylotypes were used to show the related phylogenetic information in each group. Based on this classification, sequences of chloroplast and mitochondria origin were identified and subsequently removed from the data set. The sequences were subsampled to the smallest library size (n=1648) for identical sequencing depth before further alpha and beta diversity analyses.

2.2.8 Community comparison analysis

To obtain an estimate of alpha-diversity (richness) of the samples, Chao1, abundance-based coverage (ACE) estimators were calculated using MOTHUR. Good's coverage (Good 1953) was used to estimate the sample coverage. Simpson's reciprocal diversity index (INVSIMPSON) (Simpson 1949) was calculated to estimate the diversity including both richness and evenness. A rarefaction curve (Gotteli and Colwel 2001) was also generated to represent the OTUs diversity and sampling effort of all samples and treatments. For Beta-diversity measurements, Venn diagrams were generated using MOTHUR to show the overlap between the communities. Unweighted and weighted UniFrac and ∫-LIBSHUFF (Schloss 2008) were employed to evaluate differences between

communities. For pair-wise comparisons among all libraries, $p < 0.0001$ was considered significant. Multivariate analysis of all samples using non-metric multidimensional scaling (NMDS) (Shepard 1966) was performed based on Yue & Clayton theta measure of dissimilarity (Yue and Clayton 2005) between the structures of two communities. A clustering analysis was also performed using the relaxed neighbor joining (RNJ) method through the Clearcut program (Sheneman et al. 2006) implemented in MOTHUR using unweighted UniFrac scores.

2.3 Results

2.3.1 Soil characteristics, CO_2 production rate, and *in situ* CO_2 flux

The three High Arctic barren sites for which we have measured soil characteristics demonstrate a wide range of soil parameters in this bioclimatic subzone (Table 2.1). These data show that these soils were all alkaline (with pHs between 7.7 and 8.6). Pit was mostly composed of sand/silt and litter. Water content (17%) was in the middle range compared to other surface soils of the High Arctic (10-20%) (Martineau et al. 2010; Steven et al. 2008; Steven et al. 2007) is a low organic content mineral soil (3.2%) based on water extractable organic carbon (WEOC) measurements and has high concentrations of inorganic nitrogen. In contrast, the soil texture of Pat was dominated by gravel. Its water content was also in the middle range (14%). Pat also falls into the category of low organic carbon mineral soils (3.3%) with high inorganic nitrogen. Similar to Pat, DP is mostly composed of gravel. DP is also a low organic carbon content soil (6.5%); however, it is considerably higher than the other two sites. The water content (10%) and nitrate and ammonia concentrations were lower compared to the Pat and Pit sites.

In situ CO_2 concentrations were in the range of 16-20 (μmoles L^{-1}; Table 2.1). Dark CO_2 flux, which is a measure of heterotrophic respiration in soils, was lowest at DP and Pat and highest at Pit (Table 2.1). In the lab, the dark CO_2 production rate (a measure of soil respiration) was measured in soil cores. There

was no significant difference for no-substrate controls in CO_2 production rate between sites ($P>0.05$; Fig. 2.1). The addition of 1% algal lysate significantly increased CO_2 production rate compared to controls at all sites except Pat ($P<0.05$). The substrate-added soil cores were not significantly different between sites excluding Pat that was significantly different from Pit. The substrate-induced CO_2 flux followed a similar trend to the *in situ* dark CO_2 flux, with Pit showing the highest mean rate, followed by DP, then Res.

Table 2.1 Soil parameters of the three High Arctic barren sites on Ellesmere Island.

Site	pH		WEOC[a] (SE) (%)		NO_3^- (SE) mg kg^{-1}		NH_4^+ (SE) mg kg^{-1}		Water content (%, v/v)	Soil Temp (^0C)	In situ CO_2 concentration (µmoles L^{-1})	Dark Flux CO_2 (SE) nmol m^{-2} s^{-1}	Soil texture
	40 APL[b]	30 APL[b]	40 APL[b]	30 APL[b]	40 APL[b]	30 APL[b]	40 APL[b]	30 APL[b]					
Okse Bay (Pit)	8.1 (0.20)	7.8 (0.15)	3.2 (0.5)	3.2 (0.3)	1.57 (0.37)	2.16 (0.19)	2.12 (0.31)	2.29 (0.22)	17 (0.04)	10	17.5 (0.8)	312 (52)	Sand/silt, litter, bare rock, bones, dung
Alexandra Fjord Dome (DP)	8.5		3.1		bdl[c]		2.47		14 (0.020)	11.2	20 (1.2)	53 (37)	Bare soil and gravel
Patterson River (Pat)	8.6 (0.09)		6.5 (1.3)		0.54 (0.36)		0.49 (0.33)		10 (0.05)	3.3	16 (0.7)	154 (29)	Gravel, sand/silt, litter

Values in parentheses are ±1 Standard Error.[a] WEOC = water-extractable organic carbon, [b] APL = above permafrost layer, [c] bdl = below detection limit

Data from Brummell et al. (submitted 2012).

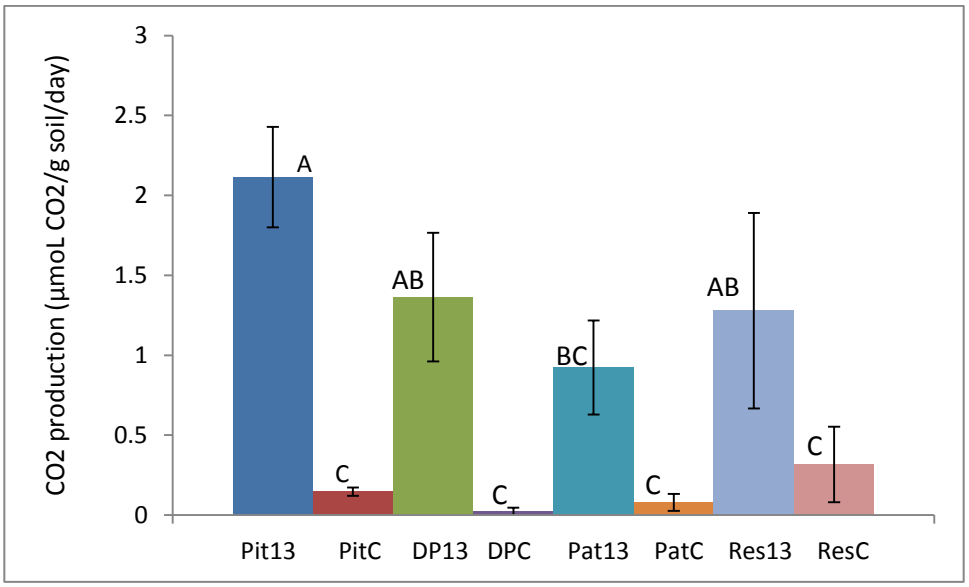

Fig 2.1 CO_2 production rates of soil samples. The 13 suffix indicates samples incubated with ^{13}C-labeled substrate while the C suffix indicates incubated without the addition of substrate. Values are means of triplicates. Error bars represent one standard deviation. Different letters on bars refer to significantly ($P<0.05$) different averages based upon the Tukey–HSD test.

2.3.2 Estimation of bacterial community diversity

We obtained a high coverage (>95%) for labeled DNA from substrate added incubations. There was moderate coverage (68-82%) for the rest of the samples (Table 2.2). Rarefaction analysis (Fig 2.2) indicated that the samples were well sampled, but not comprehensively, with the curve leveling off but not reaching an asymptote. The exception was labeled DNA of substrate-added incubations ("H" samples), which were comprehensively sampled. The rarefaction analysis is in good agreement with the coverage estimator.

The estimated OTU richness, based on the nonparametric Chao1 and ACE estimators, was high in unincubated and control samples; however, it decreased enormously upon the addition of substrate for H samples all sites. Unlabeled

DNA from Pat had the highest number of observed OTUs (1031) and ResH the lowest (88) (Table 2.2). The Simpson reciprocal index of diversity showed a high diversity for unincubated and control samples while incubation with substrate hugely reduced the diversity. The incubations alone also decreased the diversity at DP and Pit but increased diversity at Pat, with almost no change at Res. Thus, incubation alone had variable effects on assemblage diversity, while incubation with 1% algal lysate led to a strong decrease in overall diversity and especially in the active component of the community.

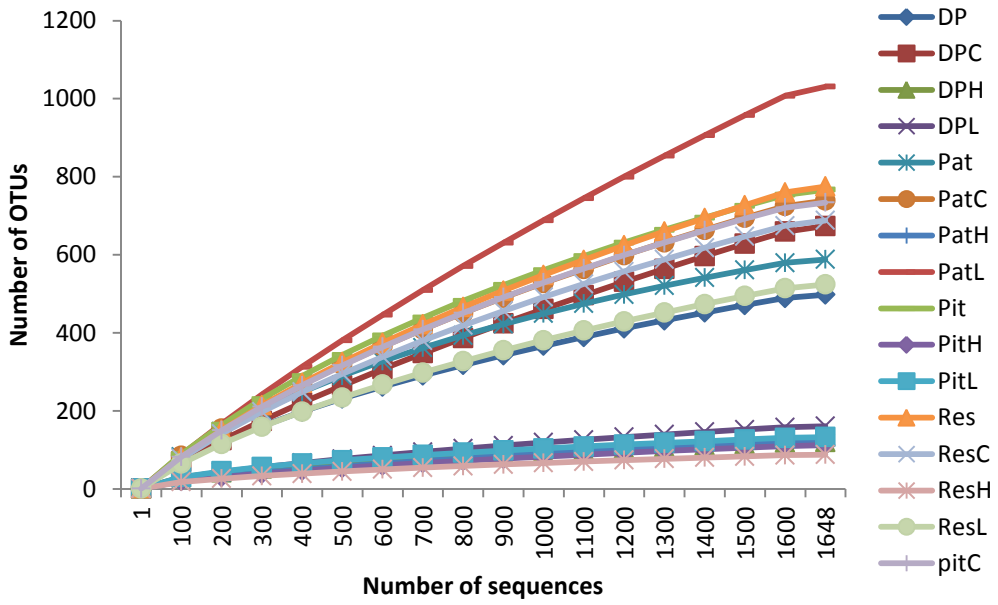

Fig 2.2 Rarefaction curve of observed 97% OTU richness with increasing sequencing depth

Table 2.2 Estimated OTU richness, diversity, and sample coverage for 16S rRNA gene tags for all samples

Sample	Number of OTUs (observed)[a]	Good's coverage(%)	Chao1	ACE	Invsimpson index
DP	498	82.16	1011 (870; 1205)	1530 (1378; 1707)	78 (70; 90)
DPC	674	69.72	1892 (1608; 2263)	3978 (3578; 4432)	49 (42; 58)
DPH	120	96.54	209 (164; 298)	317 (261; 394)	16 (15; 17)
DPL	161	94.30	312 (248; 423)	551 (456; 676)	5 (4; 5)
Pat	588	82.52	899 (817; 1010)	1212 (1114; 1327)	189 (181; 258)
PatC	739	71.00	1630 (1428; 1890)	2797 (2559; 3065)	213 (181; 257)
PatH	126	95.15	284 (211; 419)	455 (358; 591)	4 (3; 4)
PatL	1031	50.97	3343 (2890; 3906)	6283 (5674; 6970)	131 (103; 178)
Pit	767	72.39	1474 (1315; 1679)	2162 (1989; 2359)	335 (284; 407)
pitC	734	72.0	1318 (1188; 1484)	2060 (1875; 2274)	116 (96; 147)
PitH	112	96.00	270 (190; 427)	419 (338; 436)	5 (5; 6)
PitL	133	68.63	259 (197; 380)	356 (296; 436)	13 (13; 15)
Res	775	72.94	1817 (1586; 2114)	2913 (2664; 3193)	64 (52; 83)
ResC	688	97.03	1558 (1354;1825)	2637 (2405; 2899)	62 (52; 78)
ResH	88	80.58	219 (145; 383)	168 (129; 242)	6 (5-6)
ResL	524	72.94	1073 (927; 1270)	1673 (1510; 1862)	28 (24; 33)

[a] Number of OTUs calculated with MOTHUR using RDP database at 3% distance level
Values in parentheses are 95% confidence intervals

2.3.3 Community composition of active heterotrophs

SIP was performed to study labile carbon assimilating populations. Isotopic enrichment was confirmed by measuring DNA concentrations in all the fractions collected. When a peak in DNA concentration was observed in fractions with higher density than in control samples, it was considered as isotopic enriched DNA. DNA concentration peaks in fractions with similar densities to those with DNA in controls were taken as unlabeled DNA bands. DGGE fingerprinting results (Fig A3) showed smaller differences between replicates from the same site compared to between sites; therefore, DNA from replicates were pooled and later used for high throughput sequencing

For the 16 samples (before incubation, incubation without substrate, and incubation with substrate (light and heavy fraction from each of 4 sites), a total of 68878 sequences were obtained. Following quality filtering, noise removal, and removal of chimeric sequences, 54,580 sequences representing 13,880 unique sequences remained. To allow for sample comparison, all data sets were subsampled to the sample with the lowest number of samples (1648 sequences per sample).

The taxonomic information derived from phylotype classification using the RDP classifier was used along with phylotype abundance to identify the bacterial groups and their abundance. Figure 2.3-2.6 shows the relative abundance of different phylum level groups in the different samples. A broad spectrum of bacterial phyla was observed in non-incubated and no-substrate-incubated samples for all sites.

Unincubated soil samples revealed to have an overall similar phyla composition including, Proteobacteria (Alpha-Beta-Gamma), Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, however; they varied in abundance between different sites. A large fraction (17-38 %) of the phylotypes could not be classified; indeed,

the most abundant phylotype for all sites was unclassified. Planctomycetes, Verrucomicrobia, Gemmatimonadetes appeared as 1-3 % of the total community. For the classifiable phylotypes in unincubated Res samples, phylotypes related to Acidobacteria were the most abundant (27%), while in the unincubated Pit samples, Bacteroidetes (22%), Alphaproteobacteria (21%) and Acidobacteria (16%) constituted the major phyla. The dominant phyla in the DP unincubated samples were Betaproteobacteria (27%) with abundant Bacteroidetes (11%) and Actinobacteria (11%). For the unincubated Pat site, Alphaproteobacteria was the most abundant group (17%), with Bacteroidetes (12%) and Acidobacteria (15%) in high abundance as well. Thus, Bacteroidetes, Proteobacteria, Acidobacteria, and Actinobacteria are abundant at all sites without incubation, although their relative abundance changes from site to site.

Phylotypes belonging to a broad spectrum of phyla similar to unincubated samples were observed following incubation without substrate. The most abundant phylotypes in the control-incubated samples could not be classified (Tables 2.3-2.6).

Some changes in phylotype abundance were observed; for example, in Res Gammaproteobacteria increased in abundance and Bacteroidetes disappeared from the population following incubation without substrate. In Pit samples, there were also some minor changes in abundance, with the most noticeable the near disappearance of Bacteroidetes. In fact, incubation alone led to drastic decreases in Bacteroidetes in all sites. Aside from the loss of the Bacteriodetes, incubation alone had a complex influence on community; howerver, the total community was similar.

For most sites, only a few phyla had phylotypes that actively consumed the added algal lysate. For Res, Actinobacteria (73%) and Firmicutes (18%) actively consumed the added algal lysate while the other phyla were underrepresented in the labeled DNA relative to the control incubated (C) or unlabeled (L) samples (Fig 2.3). Unclassified bacteria were decreased in the labeled DNA (3%)

compared to the unincubated soil (35%) sample. Gamma- and Alpha-proteobacteria were present in the labeled DNA, but decreased to 4 and 2% of sequences, respectively. The most abundant phylotype (72%) was affiliated with the genus *Arthrobacter*. At the Pit site, unclassified bacteria totally disappeared. Actinobacteria and Gammaproteobacteria were the dominant phyla in the labeled DNA. The most abundant phylotype (46%) was affiliated with the genus *Arthrobacter*. At DP, Actinobacteria (34%), Gammaproteobacteria (26%), Alphaproteobacteria (20%) and Betaproteobacteria (11%) were predominant in the labeled DNA. Again unclassified bacteria disappeared from the population. The most abundant phylotype (34%) was affiliated with the genus *Arthrobacter*. This phylotype was the same abundant phylotype as Res and Pit.

Some of the phylotypes at phylum level are present in low abundance (1-5%) in labeled DNA fraction. In such cases, where phylotypes are more abundant in the light than the heavy band, our interpretation is that the heavy band component results from "bleed-through" from the light band; thus, we cannot confidently state that these groups are responsive to the substrate. If, on the other hand, the groups are more abundant in the heavy than the light band, our interpretation is that they directly respond to the substrate.

Res, Pit, and DP had all similar trends in community shift in response to labile carbon addition; however, at Pat, the response was quite different. Firmicutes-related phylotypes dominated (76%) the community that consumed the labeled algal lysate. Actinobacteria were present in the labeled DNA, but in less abundance (14%) compared to other sites. The most abundant phylotypes (81%) were affiliated with the family Planococcaceae and genus *Bacillus* (26%). *Arthrobacter* relatives were the third most abundant phylotype.

The most abundant phylotypes in the labeled DNA fraction are usually not found in the unlabeled fraction. We found that all sites shared similar bacterial groups without incubation. With incubation alone, they also

shared the same groups. With the addition of substrate; however, Res, Pit, and DP responded similarly, while Pat responded differently.

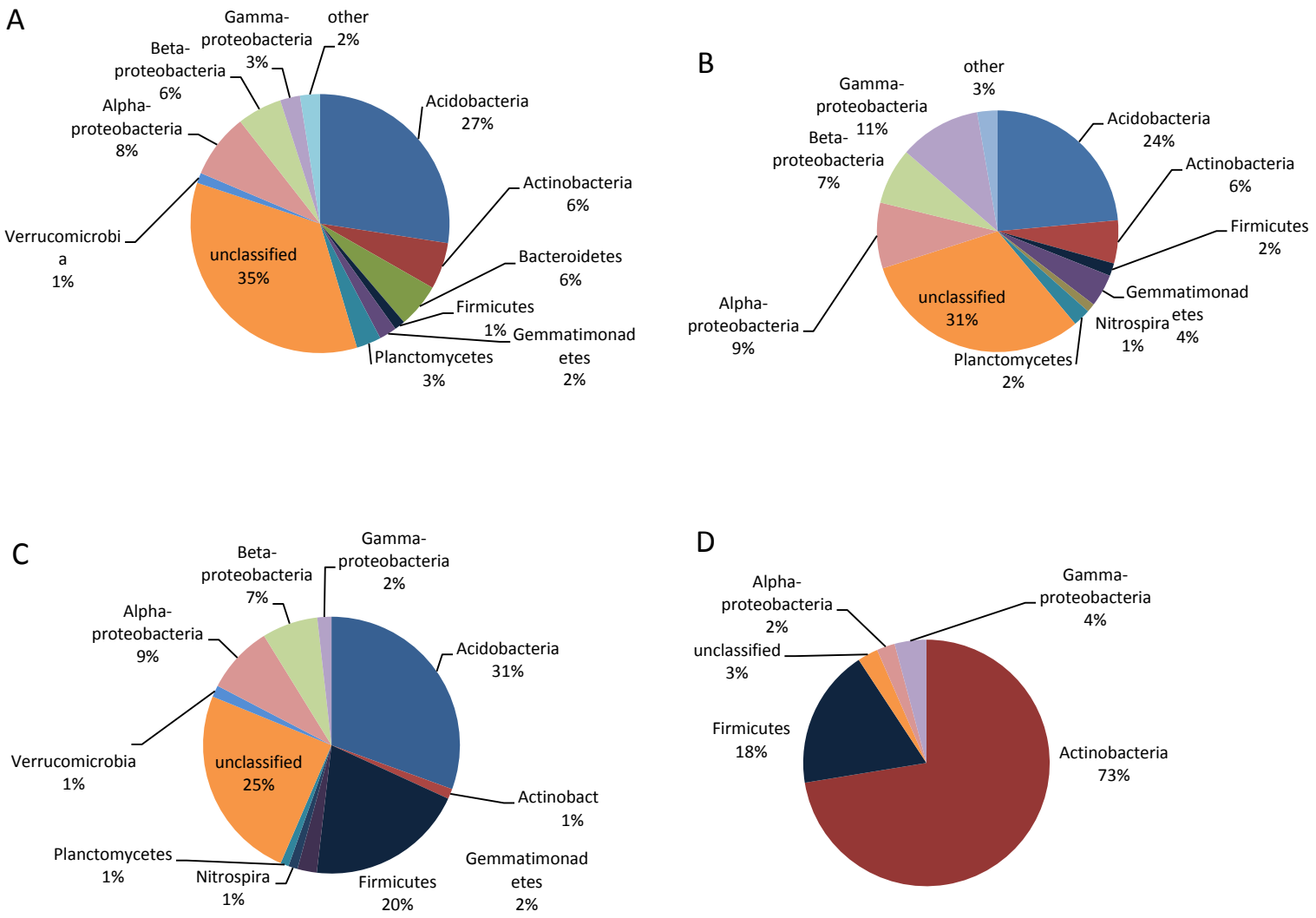

Fig 2.3 Pie charts for bacterial 16S rRNA gene (V13) phylum level assemblage composition at the Res site of A) unincubated (Res), B) no-substrate incubation (ResC), C) unlabeled DNA from incubation with substrate (ResL), and D) ^{13}C - labeled DNA from incubation with substrate (ResH; heavy fraction)

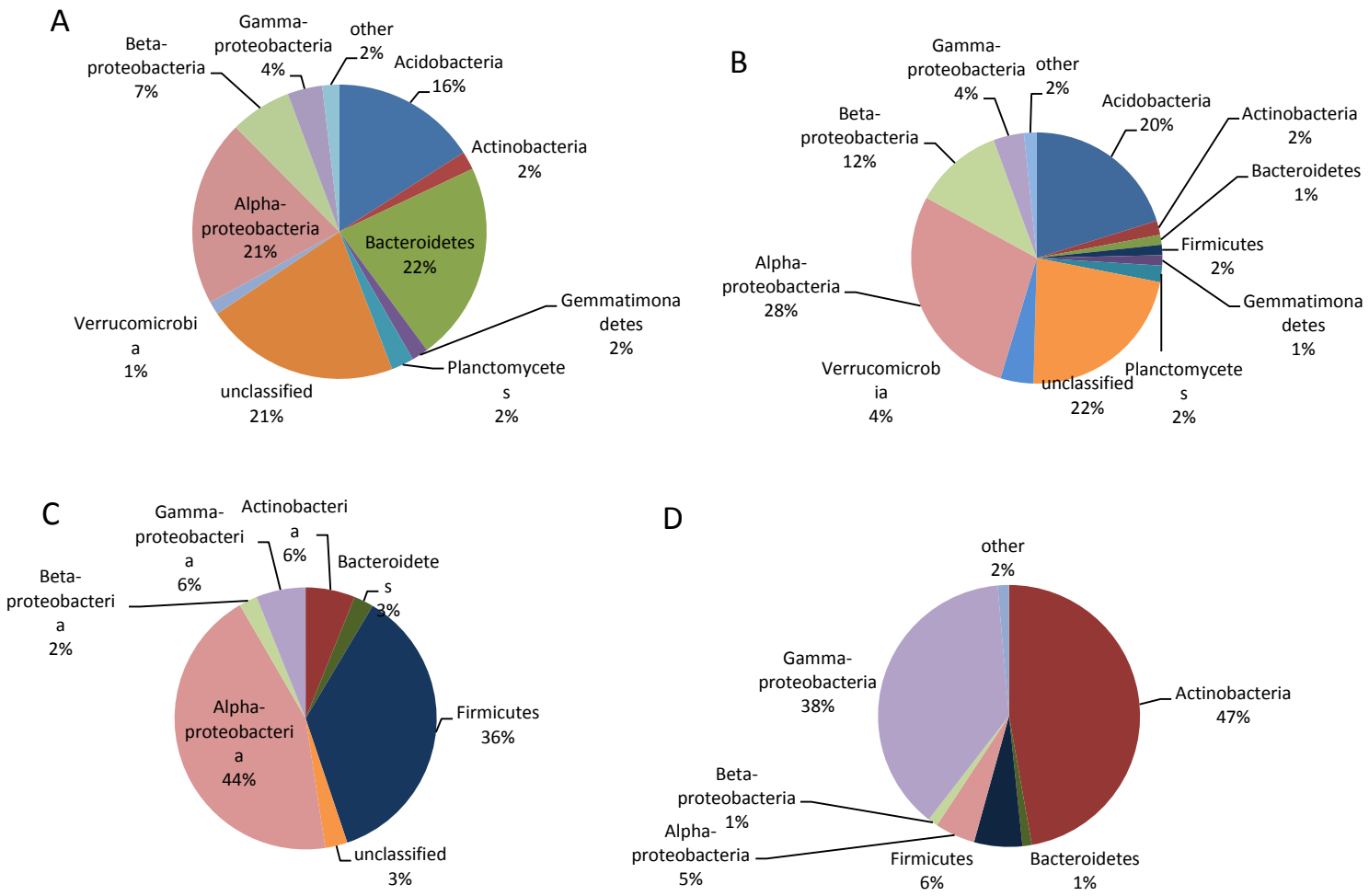

Fig 2.4 Pie charts for bacterial 16SrRNA (V13) phylum level assemblage composition at the Pit site of A) unincubated (Pit), B) no-substrate incubation (PitC), C) unlabeled DNA from incubation with substrate (PitL), and D) ^{13}C -labeled DNA from incubation with substrate (PitH; heavy fraction)

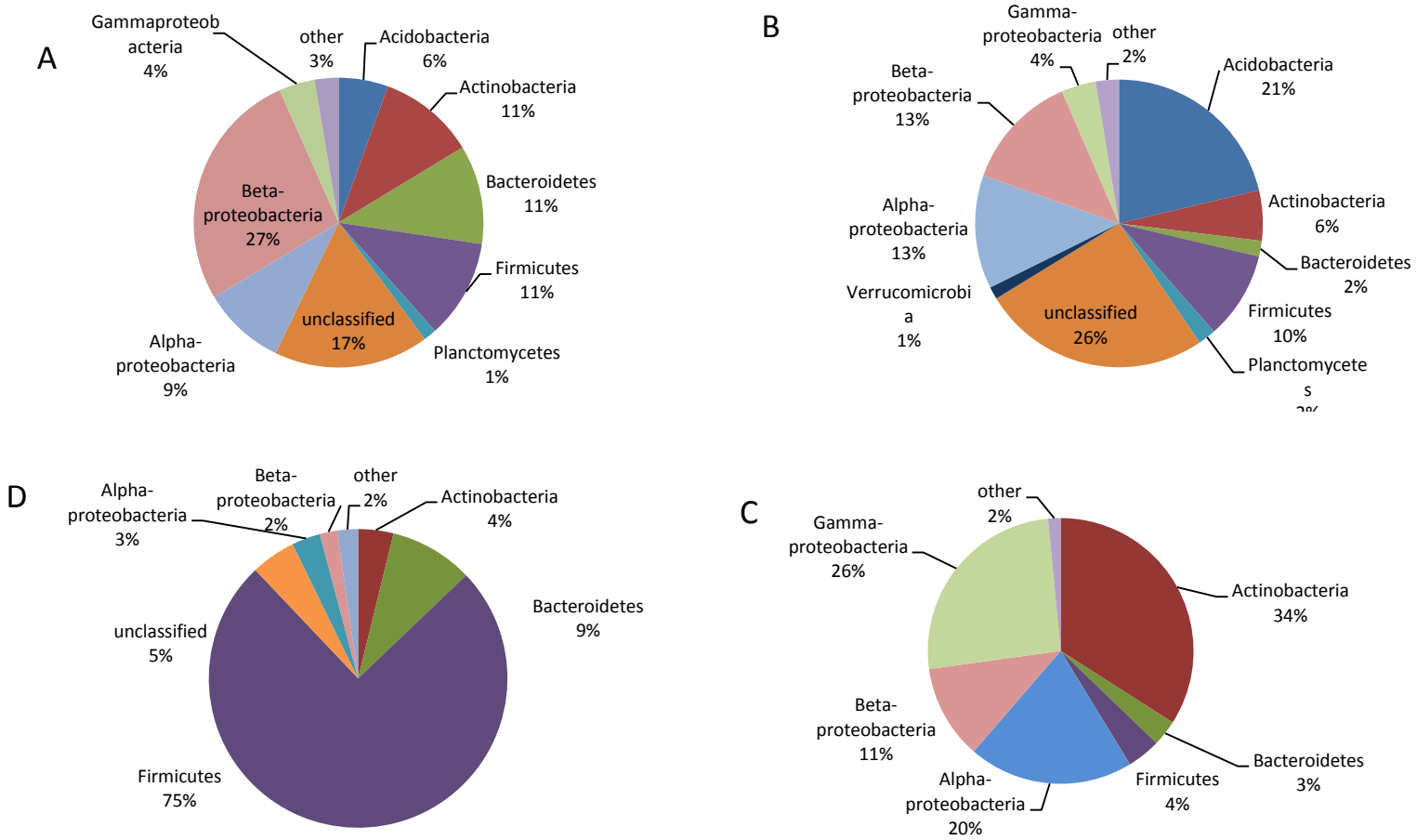

Fig 2.5 Pie charts for bacterial 16SrRNA (V13) phylum level assemblage composition at the DP site of A) unincubated (DP), B) no-substrate incubation (DPC), C) unlabeled DNA from incubation with substrate (DPL), and D) ^{13}C- labeled DNA from incubation with substrate (DPH; heavy fraction)

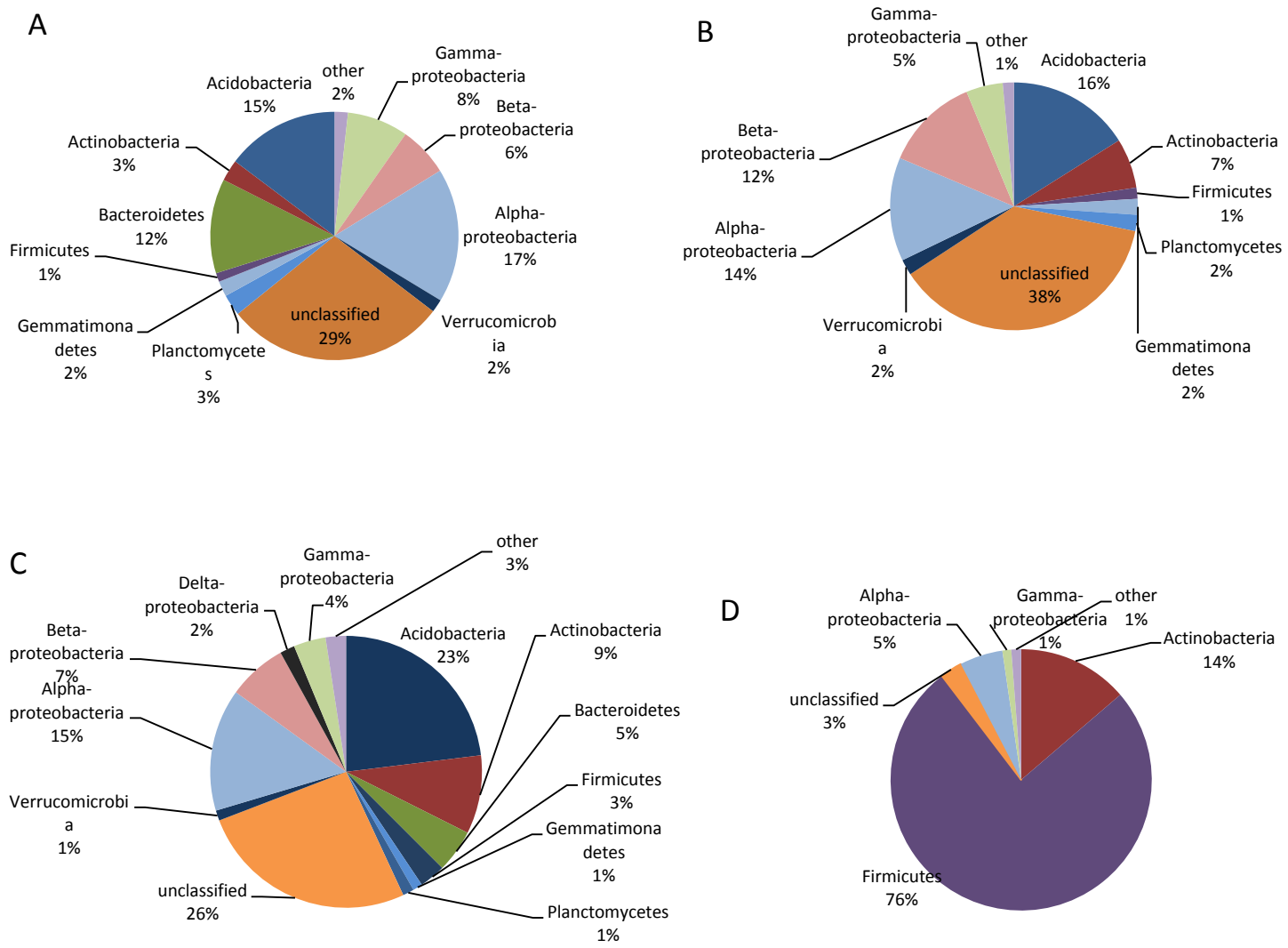

Fig 2.6 Pie charts for bacterial 16SrRNA (V13) phylum level assemblage composition at the Pat site of A) unincubated (Pat), B) no-substrate incubation (PatC), C) unlabeled DNA from incubation with substrate (PatL), and D) ^{13}C-labeled DNA from incubation with substrate (PatH; heavy fraction).

Table 2.3 Ten most abundant phylotypes from Res

Res	%[a]	ResC	%	ResL	%	ResH	%
unclassified	32.5	unclassified	29.5	unclassified	23.3	Arthrobacter	69.5
Gp4	19.9	Gp4	16.2	Gp4	19.6	Paenisporosarcina	8.2
Betaproteobacteria	3.5	Pseudomonas	4.4	Planococcaceae	7.3	Planococcaceae _incertae_sedis	5.2
Gp6	3.3	Gemmatimonas	4.4	Gp6	6.9	Pseudomonas	3.7
Chitinophagaceae	3.0	Betaproteobacteria	4.2	Paenisporosarcina	4.9	Planococcaceae	2.7
Rhizobiales	2.6	Gp6	3.8	Betaproteobacteria	4.2	Rhizobiales	1.9
Gemmatimonas	2.2	Rhizobiales	3.7	Sphingomonadaceae	3.8	unclassified	1.5
Solirubrobacterales	2.1	Xanthomonadaceae	3.0	Gp7	3.2	Bacillus	1.2
Sphingomonadaceae	2.0	Sphingomonadaceae	2.3	Planococcaceae _incertae_sedis	3.1	Proteobacteria	1.2
Planctomycetaceae	2.0	Gp7	1.9	Gemmatimonas	2.4	Microbacteriaceae	0.5

Taxonomic info is given at genus level or at higher level if the phylotype could not be classified to genus level. [a] the percentage abundance of phylotypes in each sample

Table 2.4 Ten most abundant phylotypes from Pit

Pit	%[a]	PitC	%	PitL	%	PitH	%
unclassified	18.6	unclassified	20.8	Alphaproteobacteria	15.6	Arthrobacter	44.0
Gp4	12.2	Rhizobiales	13.8	Rhizobiales	12.5	Pseudomonas	37.6
Chitinophagaceae	5.9	Gp4	13.5	Lactobacillales	12.2	Devosia	3.0
Rhizobiales	5.7	Sphingomonadaceae	5.4	Bradyrhizobiaceae	11.8	Planococcaceae	2.5
Flavobacterium	5.1	Betaproteobacteria	4.9	Planococcaceae _incertae_sedis	6.7	Microbacteriaceae	2.4
Sphingomonadaceae	4.5	Gp6	4.1	Sporosarcina	5.9	Rhizobiales	1.5
Bacteroidetes	4.0	Alphaproteobacteria	2.4	Xanthomonadaceae	4.2	Sporosarcina	0.9
Alphaproteobacteria	2.9	Spartobacteria _genera_incertae_sedis	2.4	Paenisporosarcina	3.7	unclassified	0.8
Gp6	2.8	Burkholderiales	2.2	Planococcaceae	3.2	Pedobacter	0.8
Proteobacteria	2.8	Xanthomonadaceae	1.8	unclassified	2.7	Planococcaceae _incertae_sedis	0.8

Taxonomic info is given at genus level or at higher level if the phylotype could not be classified to genus level. [a] the percentage abundance of phylotypes in each sample

Table 2.5 Ten most abundant phylotypes from DP

DP	%[a]	DPC	%	DPL	%	DPH	%
unclassified	14.9	unclassified	23.9	Planococcaceae	42.0	Arthrobacter	30.3
Polaromonas	9.3	Gp4	17.0	Paenisporosarcina	11.2	Pseudomonas	25.4
Planococcaceae	5.8	Planococcaceae	6.7	Planococcaceae _incertae_sedis	7.2	Massilia	8.6
Flavobacterium	5.5	Rhizobiales	5.7	Pedobacter	4.5	Brevundimonas	5.8
Arthrobacter	5.3	Arthrobacter	4.6	Flavobacterium	4.4	Rhizobiales	4.1
Rhizobiales	4.4	Oxalobacteraceae	4.1	Bacillus	4.3	Devosia	3.8
Albidiferax	4.1	Albidiferax	3.2	unclassified	4.2	Sphingomonas	3.6
Gp4	3.3	Sphingomonadaceae	3.1	Bacillales	3.8	Planococcaceae	2.1
Betaproteobacteria	2.8	Betaproteobacteria	2.2	Sporosarcina	3.3	Microbacteriaceae	1.9
Burkholderiales	2.5	Gp6	2.0	Arthrobacter	3.2	Flavobacterium	1.8

Taxonomic info is given at genus level or at higher level if the phylotype could not be classified to genus level. [a] the percentage abundance of phylotypes in each sample

Table 2.6 Ten most abundant phylotypes from Pat

Pat	%[a]	PatC	%	PatL	%	PatH	%
unclassified	27.7	unclassified	35.6	unclassified	23.1	Planococcaceae	42.5
Gp4	9.3	Gp4	6.4	Gp4	13.4	Bacillus	13.8
Chitinophagaceae	7.0	Betaproteobacteria	5.5	Rhizobiales	5.3	Arthrobacter	13.1
Sphingomonadaceae	6.6	Gp6	4.5	Gp6	5.0	Planococcaceae_incertae_sedis	9.2
Rhizobiales	3.8	Rhizobiales	4.4	Betaproteobacteria	3.0	Sporosarcina	5.1
Xanthomonadaceae	3.5	Arthrobacter	4.0	Proteobacteria	2.7	Devosia	2.6
Betaproteobacteria	3.4	Betaproteobacteria	2.7	Bradyrhizobium	1.7	unclassified	2.4
Gp6	2.9	Sphingomonadaceae	2.6	Actinomycetales	1.6	Paenisporosarcina	2.1
Brevundimonas	2.5	Gp17	2.4	Alphaproteobacteria	1.6	Bacillales	1.6
Lysobacter	2.2	Comamonadaceae	2.4	Bacteroidetes	1.5	Rhizobiales	1.4

Taxonomic info is given at genus level or at higher level if the phylotype could not be classified to genus level. [a] the percentage abundance of phylotypes in each sample

2.3.4 Community similarity

To determine whether the addition of substrate leads to the formation of similar bacterial communities, pairwise differences in community composition and structure were measured for all samples using ∫-LIBSHUFF statistics and both unweighted and weighted UniFrac (Table 2.7). Based on ∫-LIBSHUFF and unweighted UniFrac statistics, bacterial community composition was similar for Res-ResC (p=0.0062), DPL-DPH (p=0.0002), PitL-PitH (p=0.063) and Pat-PatC (p=0.001). Although the Pit-PitC comparison showed two different communities, the score was very low (0.0057). Large numbers indicate bigger differences between communities for Unifrac scores. Weighted UniFrac, which takes into account the OTU abundance information, indicated that no communities were significantly similar. Therefore, the assemblages at different sites and following different treatments are quite different.

Although at the detailed level of OTU similarity the communities are different, multivariate analysis of all samples using non metric multidimensional scaling (NMDS) revealed a clear clustering of bacterial communities from enriched communities (ResH, PitH, DPH and Pat H) and a high similarity between ResH, PitH and DPH (Fig 2.7). Also, control soils from different sites grouped together (PitC, PatC, ResC and DPC). Pit and Pat showed a higher similarity to one another compared to DP and Res, which tended to group together. There was more scatter observed regarding the light fraction of enriched samples from different sites, which is perhaps unsurprising as the members of the community that do not consume the algal lysate are presumably more diverse than those that do respond. A cluster analysis of samples also showed similar grouping between heavy fractions of enriched soils (Fig 2.8), supporting the findings from the NMDS analysis.

Table 2.7 Results from ∫-Libshuff and UniFrac (weighted and unweighted) analysis of bacterial 16S rRNA gene sequences

Comparisons	∫-Libshuff		Unweighted UniFrac		Weighted UniFrac	
	Score	P-value	Score	P-value	Score	P-value
Res-ResH	0.084	<0.0001	0.97	<0.0010	0.94	<0.0001
ResC-ResH	0.079	<0.0001	0.96	<0.0010	0.91	<0.0001
ResL-ResH	0.068	<0.0001	0.94	<0.0010	0.83	<0.0001
Res-ResC	**0.000**	0.0062	**0.78**	0.005	0.42	<0.0001
Pit-PitH	0.054	<0.0001	0.95	<0.0001	0.87	<0.0001
pitC-PitH	0.038	<0.0001	0.94	<0.0001	0.89	<0.0001
PitL-PitH	0.012	<0.0001	**0.83**	0.063	0.83	<0.0001
Pit-PitC	0.0057	<0.0001	0.83	<0.0001	0.53	<0.0001
Pat-PatH	0.0674	<0.0001	0.94	<0.0010	0.94	<0.0010
PatC-PatH	0.0500	<0.0001	0.94	<0.0010	0.90	<0.0010
PatL-PatH	0.0535	<0.0001	0.95	<0.0010	0.89	<0.0010
Pat-PatC	0.0016	<0.0001	**0.79**	0.001	0.50	<0.0010
DP-DPH	0.0305	<0.0001	0.66	<0.0001	0.66	<0.0001
DPC-DPH	0.0374	<0.0001	0.71	<0.0001	0.71	<0.0001
DPL-DPH	**0.0036**	0.0002	**0.81**	0.205	0.81	<0.0001
DP-DPC	0.0024	<0.0001	0.56	<0.0001	0.56	<0.0001

Numbers in bold indicate similar communities.

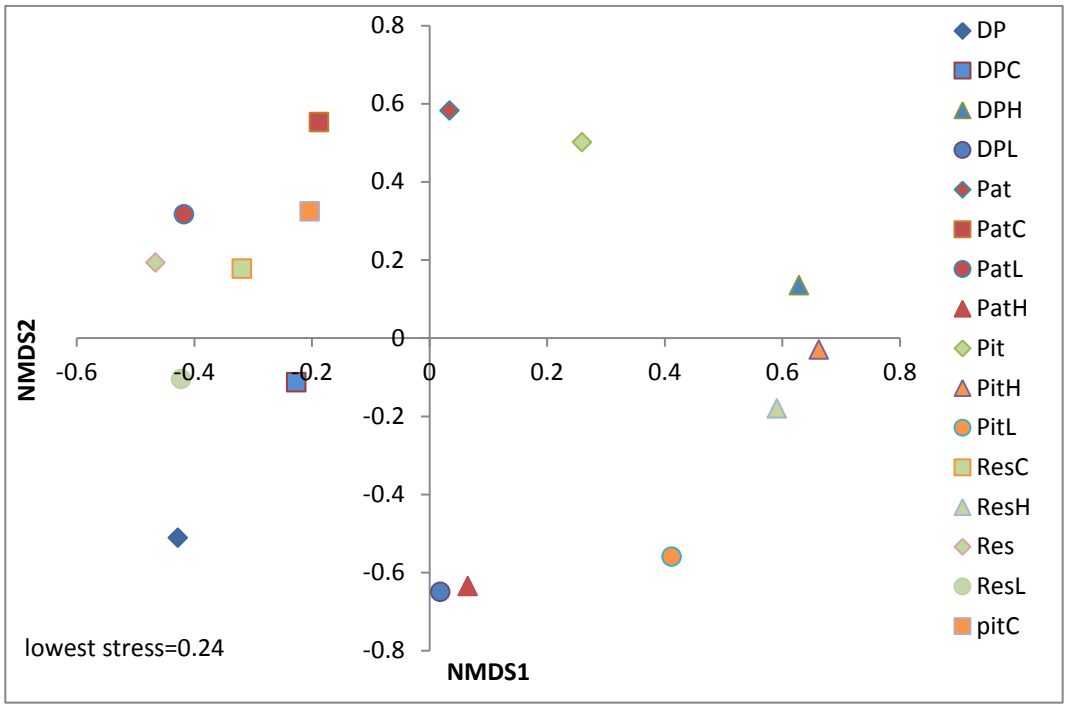

Fig 2.7 Non-metric multidimensional scaling ordination (based on Yue & Clayton theta distance matrix) showing the degree of similarity between samples. Samples within a site have been illustrated using the same color; treatments are represented by shapes.

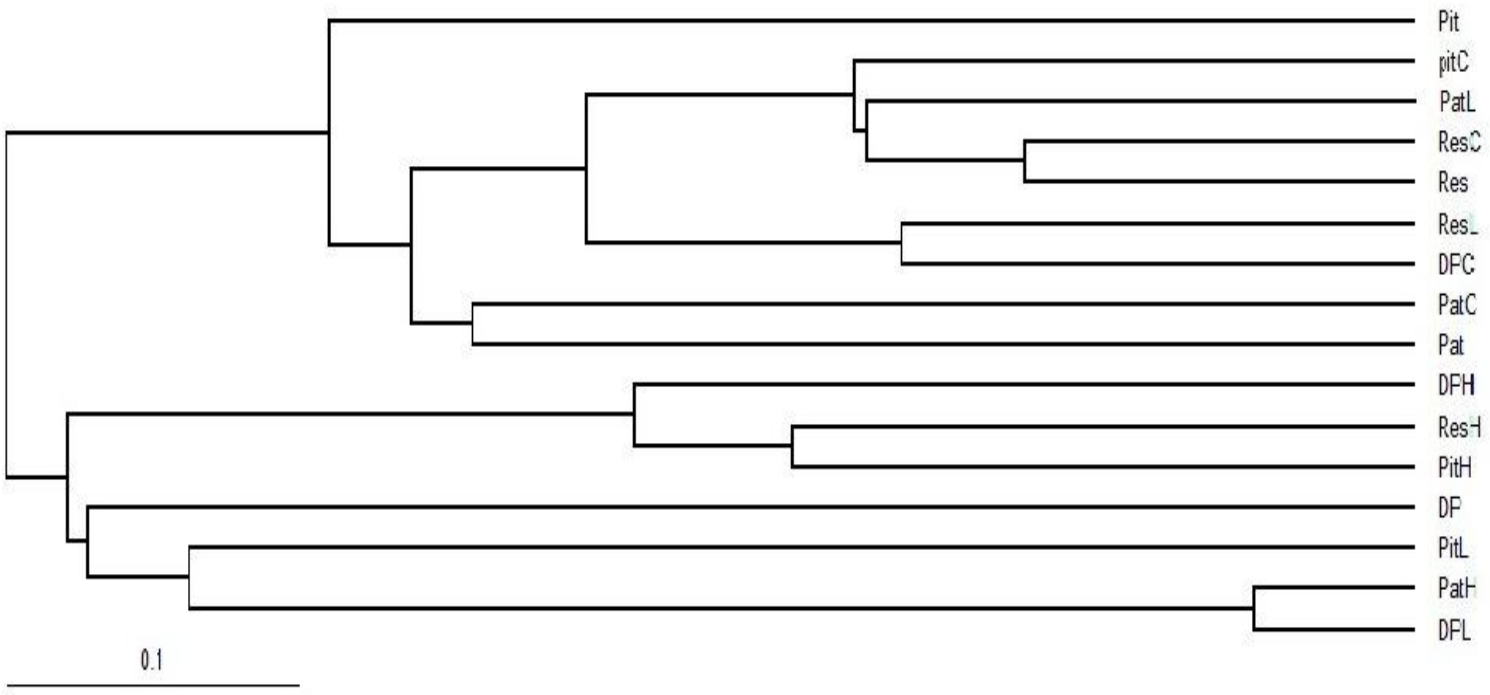

Fig 2.8 A relaxed neighbor joining dendrogram showing the clustering of the samples based on unweighted UniFrac scores. The scale bar shows the distance between groups in UniFrac units: a distance of 0 means 100% similarity and 1 means 100% dissimilarity.

2.5 Discussion

Climate warming leads to a variety of changes in local environments. As a result of the polar amplification of climate warming and the importance of ice in regulating various system traits (IPCC 2007), Arctic soils are particularly susceptible to climate effects. One such effect is increased labile carbon, both from stored organic carbon from melting permafrost (Ping et al. 2008) and increased plant productivity (Hill and Henry 2011).

Soil organic matter is a heterogeneous mixture of plant, microbial, and animal residues at various stages of biogeochemical oxidation and modification (Baldock and Skjemstad 2000). Several methodological approaches, including stable isotope probing (Radajewski et al. 2000), MICRO-FISH ((Lee et al. 1999) , FISH-SIMS (Orphan et al. 2001), and bromodeoxyuridine labeling (McMahon et al. 2011), have been used to determine the taxonomic identity of microbial assemblage members consuming specific components of organic carbon in a variety of environments; however, these methods have not been used to examine incorporation of complex organic substrates. We used ^{13}C-labeled algal lysate additions to soil cores to simulate the predicted organic carbon increases due to warming. Our goal was to directly determine which members of the microbial assemblage are active heterotrophs that would respond to the disturbance of increased labile organic carbon. To our knowledge, this study is the first attempt to characterize the entire active heterotrophic community in an intact soil core. To determine the primary consumers of the added organic carbon, it is critical to demonstrate direct consumption and incorporation of the substrate as the increased organic carbon and its consumption can have secondary effects (e.g. changes in pH, increased CO_2 concentrations in soil gas, production of secondary metabolites, etc.). We contend that utilizing ^{13}C-labeled algal lysate as a substrate for stable isotope probing directly addresses this goal and can provide insight into which components of microbial assemblages directly respond to the addition of labile organic carbon because it is a complex material that can be easily taken up

by heterotrophic bacterial communities (Bott and Kaplan 1985) and better represents the complexity of the organic carbon from natural sources. Our results suggest that the addition of complex labile carbon to active layer soils of High Arctic barren leads to increased microbial activity, decreased bacterial diversity, and shifts in bacterial community composition.

We determined the optimal concentration of algal lysate to maximize labeling while minimizing disturbance of the system. In order to run an ideal SIP experiment, the amount of substrate addition should be in the same range as *in situ* concentrations (Neufeld et al. 2007).These soils contained 3-6% total organic carbon; we added 1% algal lysate to simulate the increased organic carbon content in the soil.

As expected, the addition of algal lysate led to increased CO_2 production for all the investigated sites (Figure 2.1). The CO_2 production rate was not significantly different between sites except for Pit and Pat. These soils have distinct soil textures (Table 2.1); therefore the differences might originate from those variations. However, other factors related to site characteristics might have contributed to these variations as all these sites have various soil compositions. It has been shown that soil texture is a feature of soil physical environment that provides microenvironment for community growth and survival and influences the biological activity and microbial community composition (Chau et al. 2011). The overall activity increase due to increased carbon indicates significant implications for positive feedbacks to climate change in these ecosystems. With this information we know that these soils might contribute to global warming; however, more investigation is needed as to find the availability of soil organic matter for decomposition. Microbial efficiency of soil organic matter decomposition is one factor but other variables such as the chemical and physical composition of soils should be thoroughly studied alongside microbial community composition and physiology.

Previous studies have characterized microbial diversity in Arctic soils; however, our study focused on High Arctic barrens soils, which have not been previously studied. These ecosystems are significant to study because recent estimates of organic carbon contents of mineral soils in the northern circumpolar region, reveals higher soil organic matter than was assumed (Tarnocai et al. 2009). 581 Pg (57%) of the total estimated carbon is stored in Turbic Cryosols-mineral soils that have gone through cryoturbation. In addition, recent findings indicate a highlighted role in greenhouse gas productions of these environments and thus on climate feedbacks (Lee et al. 2012). Under aerobic conditions, regardless of soil type, the rate of total C release was shown to be six times greater than anaerobic conditions. Therefore, carbon mineralization and release to the atmosphere from these ecosystems will have significant consequences to global warming. Neufeld and Mohn (2005) found greater bacterial diversity in active layer Arctic tundra soils than in temperate boreal forest soils. Another study indicated that the bacterial community structure in Arctic soils is not fundamentally different from the bacterial communities in lower latitudes (Chu et al. 2010). Our results showed high diversity (Table 2.2 and Fig 2.2) in the soils of High Arctic barren as well. Most previous studies used methods such as clone libraries that are weak in sampling effort. The high throughput sequencing method we used was able to account for a larger fraction of the diversity (Table 2.2, Figure 2.2); however, we still did not completely sample the diversity of these soils.

We identified a diverse group of phyla including, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, and unclassified bacteria. Although all these phyla were present in all four sites, their abundance differed between sites. For example, in Res after unclassified bacteria, Acidobacteria was the dominant phyla. In DP, Betaproteobacteria dominated the assemblage. In Pit, unclassified bacteria, Alphaproteobacteria and Acidobacteria dominated the assemblage. In the furthest north site, Pat, unclassified bacteria,

Alphaproteobacteria and Acidobacteria were the most abundant. These differences are not surprising as they have been observed before; there is a significant variability in overall community composition at multiple phylogenetic levels when examining Arctic soils (Gilichinsky et al. 2008; Liebner et al. 2008; Steven et al. 2008; Zhou et al. 1997). The most abundant phylotypesfound in Res, Pit, and Pat were similar and affiliated with unclassified, GP4, Chitinophagaceae and Rhizobiales. These phylotypes have been previously found in different abundance in several Arctic environments (Zhou et al. 1997; Neufeld and Mohn, 2005). DP had the most abundant phylotypes affiliated with unclassified, Polaromonas, Planococcaceae and Flavobacterium. These results indicate that there is a similar level of variability between sites at phylum level as well as finer taxonomic levels.

Incubation of soil cores at 4 ^{0}C without the addition of algal lysate did not change the diversity (table 2.2 and Fig 2.3-2.6) and composition of phyla present; however, the abundance varied for some phyla in some sites. The most abundant phylotypes were similar in unincubated and no substrate added incubations. However, incubation alone leads to a large decrease in the abundance of Bacteroidetes in all sites. Most previous studies on Arctic soils showed only a small proportion of community related to the Bacteroidetes (Zhou et al. 1997; Gilichinsky et al. 2008). However, Liebner et al (2008) found the majority of the community members in polygonal surface tundra soils of Lena Delta, Siberia, were related to Bacteroidetes. Bacteroidetes have been shown to predominate the upper permafrost layer of moist Arctic tundra upon permafrost thaw due to availability of easily degradable carbon (Coolen et al. 2011). It appears that Bacteroidetes are particularly susceptible to disturbance in our incubations. The data here may not capture all components of the community that could consume labile organic carbon. However, the other phyla and major phylotypes don't change extensively without substrate added, so we may be picking up most of the groups.

The SIP experiments showed that Actinobacteria (*Arthrobacter*) consumed the added labile carbon at all sites (Figures 2.3-2.6). Previous studies that characterized microbial diversity in Arctic soils based on total DNA have found Actinobacteria amongst the dominant phyla. For example, Yergeau et al. (2010) found Actinobacteria as the dominant phylum in both the active layer soil and permafrost. The authors indicated that these results, which were in agreement with previous studies in finding Actinobacteria as the dominant group (Kobabe et al. 2004; Steven et al. 2007; Liebner et al. 2008; Steven et al. 2008; Wagner et al. 2009), confirms how well-adapted Actinobacteria are to cold environments. Actinobacteria are commonly found at high abundance in the bacterial community of Arctic soils (Vishnivetskaya et al. 2006; Steven et al. 2007; Yergeau et al. 2010). They are believed to maintain metabolic activity and DNA repair mechanisms in cold environments (Johnson et al. 2007). Furthermore, they have the capacity to degrade complex organic carbon compounds which makes them an important group in organic matter degradation in these extreme ecosystems (Yergeau et al. 2010). Based on our results, these bacteria are an important component of microbial community in polar desert soils of High Arctic Barren.

Arthrobacter was found to be the most abundant phylotype in the labeled fraction of Res, Pit, and DP. *Arthrobacter* can survive long periods under stressful conditions through changing to a small coccoid like state which is described as the most stable form (Mongodin et al. 2006). Finding *Arthrobacter* as the most abundant phylotype contributes to the notion that having environmentally resistant resting states favors their presence and activity in cold environments. These soils have high concentrations of ammonia. It has been shown that Actinobacteria (*Arthrobacter*) can survive high concentrations of ammonia (Kelly et al. 2012). Pat was different, as Firmicutes affiliated with Planococcaceae and unclassified at genus level was the most abundant phylotype. The finding that the same groups consume added complex organic substrate at three of the four sites might indicate that Arthrobacter is likely to be the predominant consumer of increased organic carbon resulting from warming across High Arctic Barrens. However, the finding

that Planococcaceae and not Arthrobacter is the primary consumer at one of the sites might indicate that local conditions can influence which microbes are the primary consumers of added complex organic carbon. These findings have significant implications for our understanding of climate feedbacks as different microbes have different environmental responses and capacity for organic carbon degradation. These results can be clarified with more extensive studies at other sites.

Unclassified bacteria were not enriched in the ^{13}C DNA; instead, these phylotypes were unlabeled. This is noticeable as they dominate the population in unincubated samples. Finding them in such abundance raises the question whether these sequences are representative of an ecologically significant group. As they have been mostly found through molecular based methods the question becomes if they truly have any function in the ecosystem or are the product of advanced methods biases. Given the fact that they are not present in the active heterotrophic community might indicate a lack of functionality. Alternatively, they may occupy specialized niches, which are disrupted by increased organic carbon. If the latter explanation is correct, these unclassified groups may become less prevalent in warmer, more organic-rich soils.

Addition of complex labile carbon exerts significant changes in community composition (Table 2.3). Heavy fractions from all sites were significantly different from the control and pre-incubation soil samples. In terms of phylotypes present, incubation alone did not change the communities significantly; however, their relative abundance was significantly different after incubation without substrate (Figure 2.3-2.6). In spite of the differences in the abundance of the present groups in samples between sites, all the enriched samples were the most similar and grouped together (Figure 2.7). The addition of labile organic carbon led to a convergence of the microbial communities. Given the differences in physicochemical conditions at the four study sites, and reaching this convergence

in the overall bacterial assemblage due to increased carbon, it can be concluded that our findings might be true across the Arctic for other ecosystems.

High CO_2 production rate and soil activity upon addition of labile organic carbon in polar desert soils of the High Arctic strongly indicates the possible role of these ecosystems in a future warmer climate. Our community analysis revealed a convergence in the overall community composition and presence of a few groups of active bacteria. These groups of bacteria might be of more interest, as they will be more likely to react to future climate change and contribute to global warming. Our analysis of heterotrophic bacteria using this method might be very useful for linking heterotrophy to diversity in other ecosystems and on a global basis.

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3. Conclusion

Polar warming will lead to increased labile organic carbon in Arctic soils, both from the release of organic carbon stored in permafrost and increased plant production. The impact of increasing organic carbon on Arctic soil microbial community composition and activity was the focus of this research because microbial decomposition of Arctic soil organic carbon is a potential major source of CO_2 to the atmosphere. Mainly we were interested in finding the links between bacterial community structure, activity, and carbon turnover in Arctic soils. We specifically chose permafrost-affected mineral soils in the High Arctic as these ecosystems account for 5,000,000 square kilometers of land surface (Hogan 2011) and it is not clear how a changing environment will affect these ecosystems or what role they may play in climate change. In addition, most studies regarding climate feedbacks have focused on peatlands or generally address the tundra biome. Therefore, we addressed the bacterial diversity and community composition and their response to increased carbon. We expected this study to lead us to a better understanding of the role of bacterial assemblages in climate change as having a positive or negative feedback.

This research was the first extensive study of bacterial diversity and community composition of polar desert soils in Arctic. I found as high bacterial diversity in these environments as other biomes in Arctic soils. A broad spectrum of bacterial phylogenetic groups dominated by Proteobacteria (Alpha-Beta-Gamma), Acidobacteria, Actinobacteria, Bacteroidetes and Firmicutes were found to be present in these soils. These groups have also been shown in Middle Arctic, Low Arctic and other cold ecosystems including alpine and Antarctic soils. The fact that these bacterial groups are similar in all sites studied and also similar to other biomes indicates that the total community structure of polar desert soils based on total DNA analysis is not unique to that environment and shares many phylogenetic groups at the phylum level with other environments; however there was a highly localized pattern at the OTU level. In addition, the extreme

environment of these polar desert soils does not bring strong differences in the bacterial communities. This is worth mentioning our study only addressed microbial ecology of surface soils (active layer). Another question to address would be if we could generalize these findings to permafrost layer and find similar responses for it.

With all our knowledge about microbial diversity in these ecosystems, we face the limitation of identifying total community structure only based on total DNA analysis which does not give us insight toward active members of the community. Use of SIP enabled us to 1) characterize the active heterotrophic bacterial communities 2) determine the effect of release of labile organic carbon from permafrost into Arctic soils on climate change and CO_2 production in a warmer climate through changing bacterial community composition. The results highlighted the presence of a few active and responsive groups in the community in all sites and our hypothesis that the addition of labile carbon to the polar desert soils would lead to a decrease in the diversity of heterotrophic bacterial community was supported. The presence of Actinobacteria in all sites and as the most active and responsive in three of the four sites indicates its importance and role in handling the release of organic carbon to the soil. This finding highlights their function in these ecosystems as they have been shown to exist in high abundance in cold environments and are believed to maintain metabolic activity and DNA repair mechanisms in cold environments (Johnson et al. 2007). Our results suggest their ecological role in metabolizing highly complex organic carbon in extreme cold ecosystems. Finding *Arthrobacter* as the most abundant active phylotype might contribute to the notion that having environmentally resistant resting states favors their presence and activity in cold environments. In one of the sites studied (Pat) we found Firmicutes as the phylum that most actively consumes the added algal lysate. We still do not know the reason behind their higher activity in this site or the lack of *Arthrobacter*. Pat was the most northerly site and might have some climatic or physicochemical features that possibly regulate microbial community interactions. A more extensive

longitudinal and latitudinal survey of the active bacteria across the High Arctic barrens will help to determine whether our findings are universal or more local.

Based on the taxonomic information obtained, microbial community in all sites were similar in unincubated samples considering the phylotypes at Phylum and class level and then converging to a low diversity community with the addition of substrate. Our new method in linking heterotrophic activity to diversity and identity might be helpful in identifying heterotrophic bacteria in other ecosystems. Since the overall community was similar in polar desert soils to other cold environments, our findings can be useful for those environments as well. However, we do not know certainly how other ecosystems will respond regarding labile carbon addition.

The CO_2 production capacity of these soils in response to labile carbon addition was high and similar in different sites. This finding suggests Arctic soil microbes might have an important role in CO_2 production upon the release of carbon from permafrost thaw in warmer future conditions. ^{13}C-labeled substrate we used was highly labile and an analogue to soil organic matter. However, the question becomes to what degree these results can be generalized for in situ interactions. Bioavailability of soil organic matter has been considered as a factor influencing organic carbon degradation which might vary in different permafrost environments across the Arctic (Coolen et al. 2011). Therefore, reaching a global picture on the extent of microbial contribution to climate feedbacks will be dependent on incorporating similar data to our study into climate change models to better predict soil organic matter turnover in the Arctic.

Future directions

This study focused on the bacterial communities from active layer soils. Future investigations should be directed toward determining the response of microbial communities in permafrost layer to the release of organic carbon in these ecosystems. It will be interesting to look for the active communities and find

whether they are different from what we found in active layers. Yergeau et al. (2010) suggested that since there was a highly similar functional and phylogenetic community composition between active layer and permafrost, permafrost microbial community could be a subset of the active layer microbial community and originated from overlying active layer. Further research is needed to confirm if the same bacterial communities are active in permafrost.

A systematic approach should be taken to find the environmental factors that control the active communities. In this study, we had only samples from four sites and site data only for three of them. More samples from different locations are needed to be able to statistically analyze the correlations between soil physico-chemical characteristics and bacterial taxa.

Our approach utilizing an analogue of soil organic carbon gives insight toward specific bacterial groups that might be of great importance as having ecological roles critical in climate feedbacks. In order to provide more *in situ* conditions, plant extracts could be labeled instead of algal lysate and used for SIP. Also this knowledge can narrow our focus toward studying the community at single cell level. With the advances in methods in linking environmental functions to specific microbial taxa which combines SIP with highly sensitive methods such as NanoSIMS, single cell level activity should be investigated for these soils to confirm the data and obtain a better understanding of the function of the bacteria found in our research.

3.1 Reference list

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Appendix

Fig A 1. Soil sampling sites. A-Patterson River (Pat), B-Dome Pit (DP), C-Okse Bay (Pit), D-Resolute Bay (Res). The image was obtained from Google Maps. A, B and C are located in Ellesmere Island in the Canadian High Arctic Archipelago: Okse Bay (77 8' 88" N, 87 39' 10" W), Alexandra Fjord Dome (78 51' 31" N, 75 55' 37" W and Patterson River (82 35' 47" N, 63 45' 32" W). D is located on Cornwallis Island: Resolute Bay (74° 43' 23" N, 94° 56' 10" W). These sites correspond to the B1 designation of the Circumpolar Arctic Vegetation Map (Walker et al. 2002). The Pit site is a small bay on the southwest peninsula of Ellesmere Island. The surrounding land is a series of raised beach crests from which the samples were taken. The top of the hills has polar desert terrain characteristics and the lower area in between is moist tundra. Soil structure is very coarse, containing mostly sand with some silt, large gravel and stones. Alexandra Fjord Dome is a valley on the east coast of Ellesmere Island. Although the lowland is a polar oasis, the DP site is in the mountainous area at the border of the valley-mountain (the dome), which is a polar desert with several glaciers covering the area. See text p 65.

Fig A 2. a) Incubation experiment; PVC pipes containing soil cores b) slotted PVC pipe for soil sampling and the core container.

Soil samples were collected using the slotted cores shown in Fig 2.b above. The slots on the core collector allowed for free gas exchange. The core collectors were hammered into soil (beveled end down to provide a cutting surface) to collect the upper 10 cm of surface soil active layer. The cores were then capped and secured with plastic wraps and tape, kept frozen and delivered to the lab. For the incubations, the cap was removed and the core was placed inside a larger PVC pipe shown above in Fig 2.a. The large pipes were capped and sealed applying vaccum grease. The caps had a rubber stopper built in it to allow gas sampling. For more details see text p 67.

DGGE analysis

In order to justify homogenizing triplicate samples for each site for high throughput sequencing, it was necessary to demonstrate that within site replicate variability was lower than between site variability. To test that hypothesis, DGGE profiles of PCR-amplified 16S rRNA gene fragments from triplicate samples at each site were compared, both within and between sites. Replicate samples showed higher similarity within sites than between sites for all treatments/fractions (Figure A3). Thus, the hypothesis was supported and DNA from triplicate cores was homogenized for each site prior to sending samples for high throughput 16S rRNA gene sequencing.

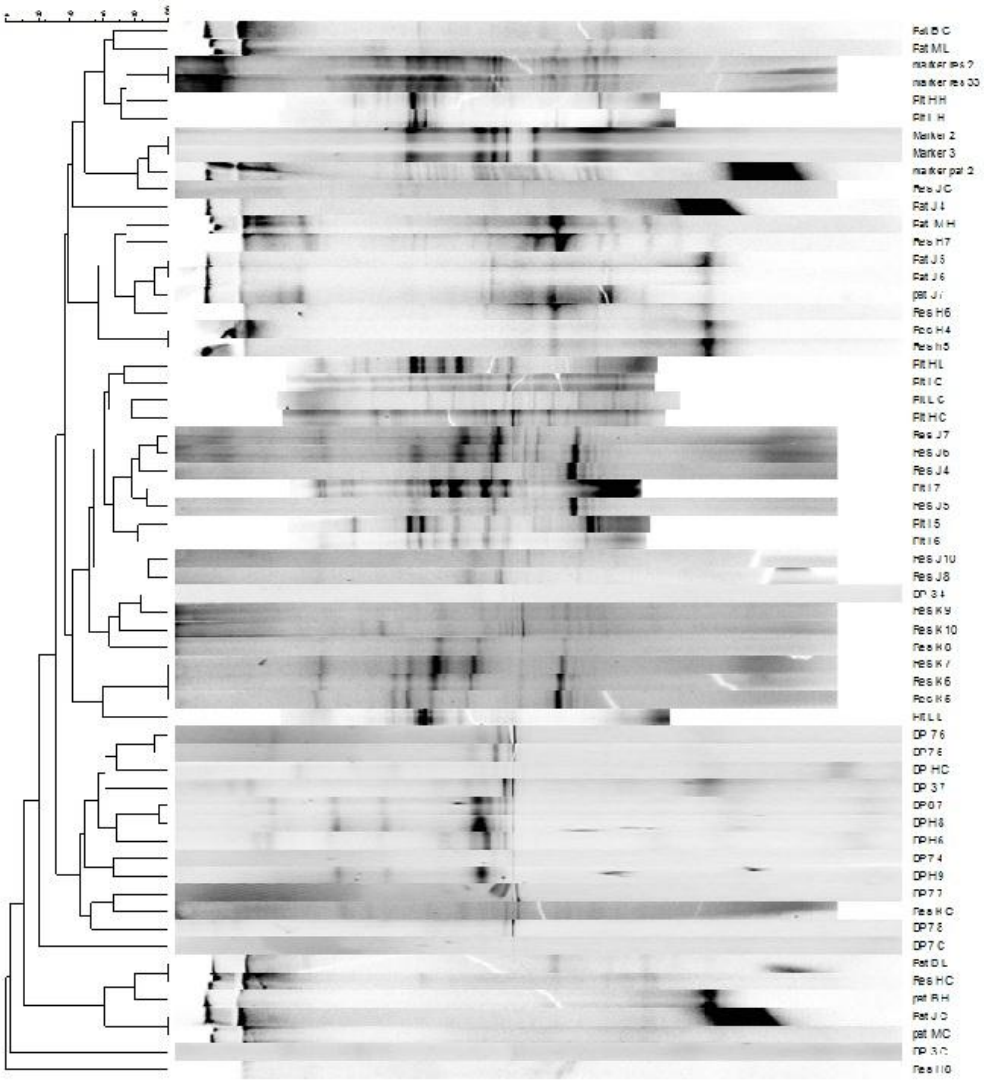

Fig A 3. DGGE and cluster analysis of PCR-amplified 16S rRNA gene fragments from DNA fractions retrieved from CsCl density gradients from three replicates of each site. For each sample, 2 or 3 heavy DNA fractions and 2 or 3 light DNA fractions were selected for DGGE analysis. Replicates include: DP 7, DPH and DP 3; Pat J, Pat M, PatH; PitH, PitI, PitL and ResJ, ResK, ResH. C refers to controls.

In order to illustrate the distribution of unique OTUs in different groups and shared OTUs between groups, Venn diagrams were generated using MOTHUR (Figure A4 a,b,c and d). In each site, different treatments were compared. In all sites, there are few shared OTUs between the different treatments. The non-incubated soils shared the highest number of OTUs with the soils incubated without the addition of substrate. Numbers of OTUs shared with heavy and light bands are much lower. The total numbers of OTUs are as follows: Res 775, Res C 688, Res H 88, Res L 524, Pit 767, Pit C 734, Pit H 112, Pit L 133, DP 496, DPC 674, DPH 120, Pit L 161, Pat 588, PatC 739, PatH 126, Pat L 1031.

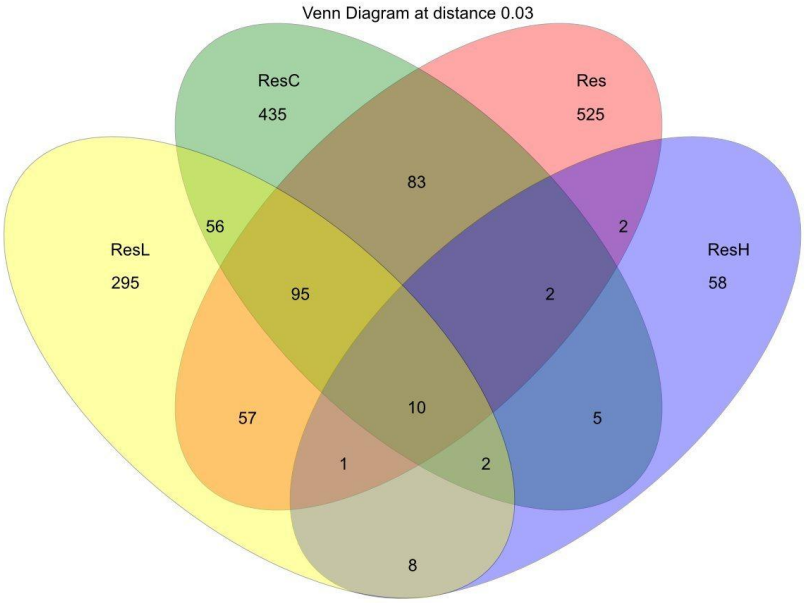

Fig A 4.A. Venn diagram for bacterial $OTUs_{0.03}$ found in site Res; Res, non-incubated soil; ResC, incubated soil without the addition of substrate; ResH, heavy fraction from incubated soil with ^{13}C-labeled algal lysate; ResL, light fraction from incubated soil with ^{13}C-labeled algal lysate. OTU 0.03 is the cut off level for generating OTUs which was used for Venn diarams as well.

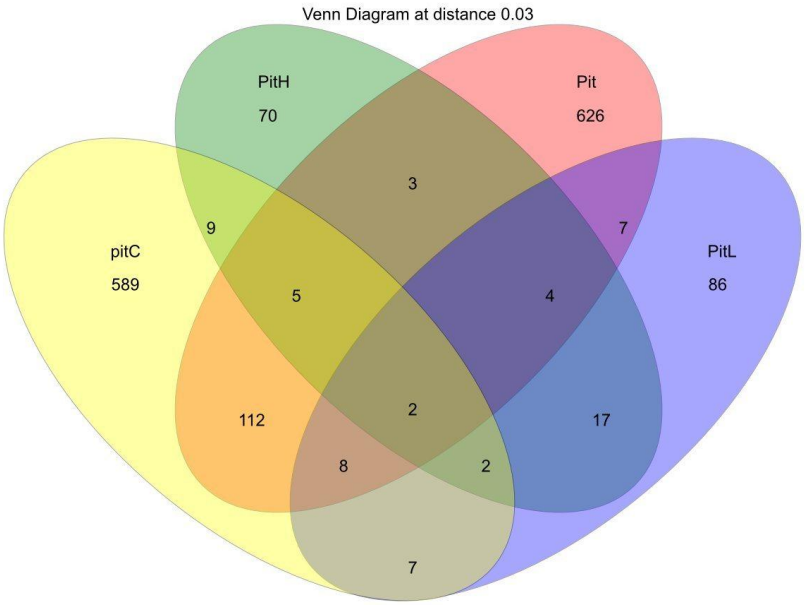

Fig A 4.b. Venn diagram for bacterial $OTUs_{0.03}$ found in site Pit; Pit, non-incubated soil; pitC, incubated soil without the addition of substrate; PitH, heavy fraction from incubated soil with ^{13}C-labeled algal lysate; PitL, light fraction from incubated soil with ^{13}C-labeled algal lysate.

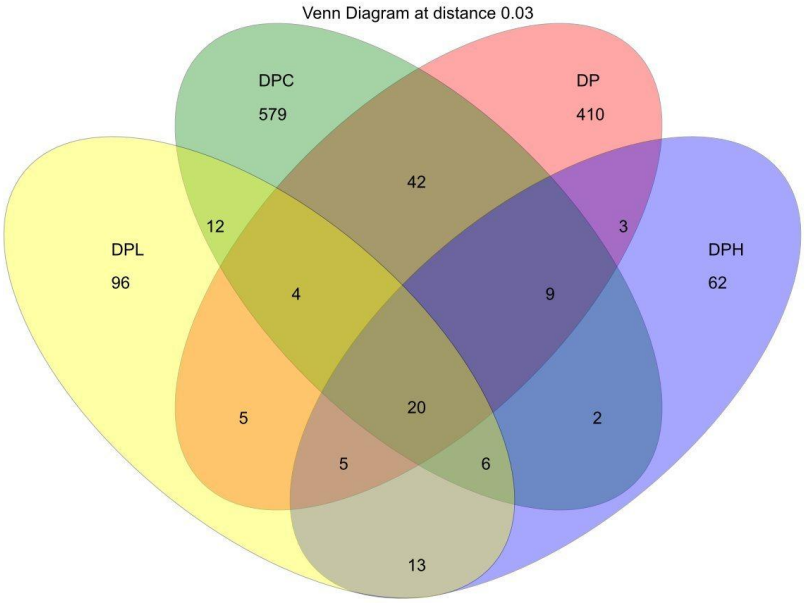

Fig A 4.c. Venn diagram for bacterial $OTUs_{0.03}$ found in site DP; DP, non-incubated soil; DPC, incubated soil without the addition of substrate; DPH, heavy fraction from incubated soil with ^{13}C-labeled algal lysate; DPL, light fraction from incubated soil with ^{13}C-labeled algal lysate.

S

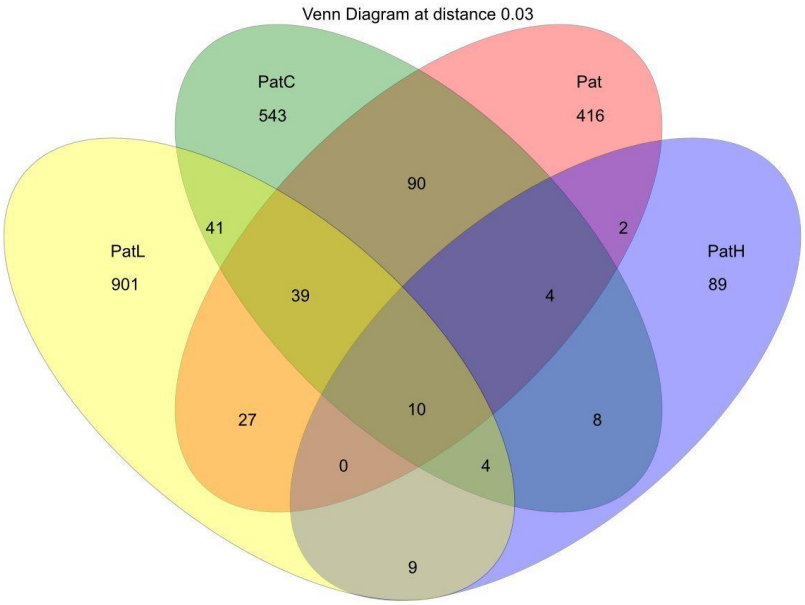

Fig A 4.d. Venn diagram for bacterial $OTUs_{0.03}$ found in site Pat; Pat, non-incubated soil; PatC, incubated soil without the addition of substrate; PatH, heavy fraction from incubated soil with ^{13}C-labeled algal lysate; PatL, light fraction from incubated soil with ^{13}C-labeled algal lysate.