ECOLOGICAL SIGNIFICANCE OF NITRIFIER AND DENITRIFIER

SPATIAL PATTERNS IN THREE ARCTIC ECOSYSTEMS

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ABSTRACT

Owing to spatial variability of soil properties, microbial communities and their functional role in biogeochemical processes may also vary across multiple spatial scales. Soil and microbial spatial heterogeneity has been studied in various tropical and temperate ecosystems yet no information is available from Arctic permafrost ecosystems. These ecosystems represent a significant proportion of global land mass and contain about one fourth of total soil carbon pool. Soil microbial N transformations such as nitrification and denitrification have significant implications for N availability and N loss in nutrient-limited Arctic ecosystems. This study aims to elucidate 1) the spatial variability of soil attributes and the overall microbial communities 2) the spatial structure of ammonia oxidizer and denitrifier abundance and their activities, and 3) relationships among microbial communities, functional processes, and soil attributes in three Arctic Cryosolic ecosystems. The results show that despite challenging climatic conditions and the regular occurrence of cryopedogenic processes, soil properties and microbial abundance are highly spatially dependent and their spatial autocorrelation is consistent within and between the ecohabitats. Despite similar abundances, the zone of spatial autocorrelation is substantially smaller than other ecosystems. The correlations between moisture content and other soil attributes in Arctic are considerably higher than temperate agricultural and tropical grassland soils, suggesting the critical role of moisture in Arctic soil ecosystems. Ammonia-oxidizing archaeal and bacterial communities and aerobic ammonia oxidation were spatially dependent. Functional groups were spatially structured within 4 m whereas biochemical processes were structured within 40 m. Ammonia oxidation was driven at small scales (<1 m) by moisture and total organic carbon content whereas gene abundance and other edaphic factors drove ammonia oxidation at medium (1-10 m) and large (10-100 m) scales. Denitrifier functional groups and

potential denitrification were spatially autocorrelated within a scale of 5 m. Soil moisture, organic carbon and nitrogen content were the predominant driving factors with *nirK* abundance also correlated to potential denitrification. This is the first study to report high spatial dependence of soil properties, overall microbial, ammonia oxidizing, and denitrifying communities, and functional processes in Canadian Arctic. It disentangles the associations among the aforementioned parameters to identify the key controls on nitrification and denitrification in Cryosolic ecosystems.

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LIST OF ABBREVIATIONS

AOA Ammonia oxidizing archaea

AOB Ammonia oxidizing bacteria

AMO Ammonia monooxygenase

amoA Gene encoding subunit A of ammonia monooxygenase in bacteria

ANOVA Analysis of variance

AOP Ammonia oxidation potential

Archaeal *amoA* Gene encoding subunit A of ammonia monooxygenase in archaea

C Carbon

DEA Denitrification enzyme activity

DNA Deoxyribose nucleic acid

DOC Dissolved organic carbon

DON Dissolved organic nitrogen

ITS Internal transcribed spacer in fungi

N Nitrogen

N_2O Nitrous oxide

N_2Of Rate of net N_2O formation

NAR Nitrate reductase

NIR Nitrite reductase

nirK Gene encoding the copper containing subunit of nitrite reductase

nirS Gene encoding the cytochrome cd1-containing subunit of nitrite reductase

NMS Non metric multidimensional scaling

NOB Nitrite oxidizing bacteria

NOR Nitrix oxide reductase

NOS Nitrous oxide reductase

nosZ Gene encoding a structural subunit of nitrous oxide reductase

HAOP Heterotrophic ammonia oxidation potential

qPCR Real-time quantitative Ploymerase Chain Reaction

r correlation coefficient

r^2 coefficient of determination

RNA Ribonucleic acid

SOM Soil organic matter

SPD Spatial dependency

θ_g Soil moisture content

TN Total nitrogen

TOC Total organic carbon

1.0   INTRODUCTION

Approximately 26% of the world's and 40% of Canada's total land area is underlain by permafrost (Bockheim and Tarnocai, 1998; Zhang et al., 2000). Global climate models show these ecosystems will be affected most severely by pervasive global climate change with an increase of 3-4°C in mean annual air temperature by 2020 (Tarnocai, 2006). Although strongly nutrient limited, these soils are the "sink" of 61% of North America's total soil organic matter pool. There is a growing concern on how climate change will impact nitrogen and other biogeochemical cycling in these ecosystems. Accumulating evidence shows a strong potential that these soils may transform into a "source" and emit enormous amounts of greenhouse gases including nitrous oxide (N_2O). Approximately 70% of the global annual N_2O emissions are contributed by soil microbial processes such as nitrification and denitrification (Mosier et al., 1998). N_2O emissions are primarily regulated by soil properties and nitrifier and denitrifier communities and the survival and distribution of the microbial communities are controlled by various soil physical and chemical properties (Bremner, 1997).

Soil resources are intrinsically variable as they fluctuate even within a short distance and generally this spatial variation is non-random and declines with distance. Furthermore, soil houses one of the most diverse and dynamic microbial assemblages in the entire biosphere (Curtis et al., 2002). Soil particles and aggregates of various sizes and shapes in numerous combinations constitute an extremely multifaceted physical environment with heterogeneity demonstrated even at finer scales (Kang, 2005). Owing to the diversity of soil physical characteristics, soil can accommodate a wide variety of microbes in close vicinity especially because of their infinitesimal size. Spatial models of microbial communities and their activities can play an important role in recognizing the processes and factors that shape their spatial distribution and services to ecosystems (Green and Bohannan, 2006). Soil resources along with

microbial abundance and community composition are the principal factors shaping most biogeochemical processes (Conrad, 1996; Schimel and Gulledge, 1998), such that microbial spatial distribution patterns have several functional implications. The relationships among microbial functional guilds, biogeochemical processes, and environmental factors can be assessed by focusing on model functional communities such as nitrifier and denitrifiers, especially because nitrification and denitrification are the traits found in many taxonomic and physiological groups of bacteria (Philippot and Hallin, 2005).

Spatial scaling patterns have been well-investigated for soil properties, plant, animal, and even microbial communities in various agricultural and pristine soils of many tropical and temperate ecosystems, resulting in a myriad of spatial ecological studies (Nunan et al., 2002; Grundmann and Delbouzie, 2000; Nunan et al., 2002; Morris, 1999; Ritz et al., 2004; Saetre and Baath, 2000). Reports are also available recently on nitrifier (Keil et al., 2011) and denitrifier (Enwall et al., 2010; Philippot et al., 2009) spatial autocorrelation patterns in various ecosystems. However, to date no study has assessed spatial heterogeneity and scale dependence of overall microbial, nitrifier and denitrifier communities in Arctic soil ecosystems. Arctic spatial ecological patterns are particularly important because these landscapes are relatively heterogeneous comprising multifaceted topographical patterns and a series of wetland soils, riparian zones, well-drained lands, ridge-top stripes, and polar desert soils (Lev and King, 1991; Pare, 2011; Walker, 2000). It would also be intriguing to examine how microbial spatial variation and scale dependence are linked to soil resources and functional processes. Moreover, ammonia oxidation and denitrification processes have not been assessed comprehensively in different types of Cryosols in the Canadian Arctic. One critical area of Arctic N cycling that remains largely unexplored is heterotrophic ammonia oxidation as studies on this process mostly

have focussed on forest ecosystems. Heterotrophic nitrification may be substantial in Cryosols with high organic matter and moisture content, although this needs to be substantiated. In general, there is a significant dearth of knowledge about Arctic N cycling processes and spatial ecology. N-cycling functional communities play a significant role in completing biogeochemical cycling of N and ecosystem functioning.

Global climate change is a matter of great concern particularly for northern ecosystems, which has let researchers surmise that "the future of Arctic is today" (Pare, 2011). Hence, the first step towards understanding the effect of global climate change on Arctic nutrient cycling and functioning is prudent assessment of the current status of these processes. A multi-level
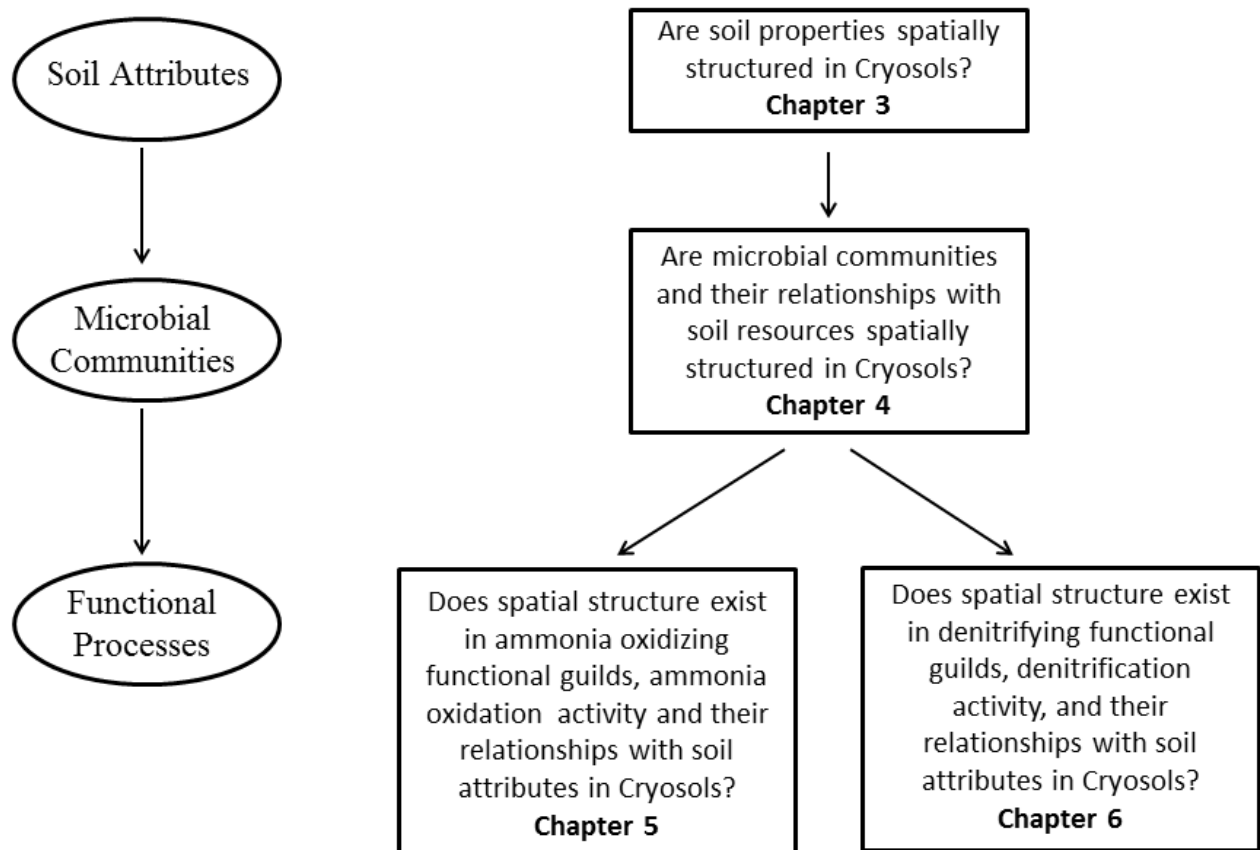
Figure 1.1 Outline of four chapters addressing individual objectives of this study

approach entailing multiple ecosystems, biogeochemical processes, functional groups, environmental factors at multiple spatial scales can be extremely valuable and this was the baseline of the current study. Specifically, the aims of this study were: 1) to assess the rate of ammonia oxidation (autotrophic and heterotrophic) and denitrification, and abundance of overall microbial, nitrifier, and denitrifier communities; 2) to characterize the spatial dependency of soil properties and the aforementioned microbial communities; and 3) to disentangle the relationships among soil resources, microbial communities, and microbial functional processes in three high Arctic ecosystems.

The following four chapters will categorically address the above-stated objectives (Fig. 1.1). Chapter 3 presents measurement data of thirteen soil physicochemical properties in 279 soil samples collected from three Canadian Arctic ecosystems. It delves into the spatial dependency of each soil attribute and elucidates their correlations with soil moisture content. This chapter was published in Soil Science Society of America Journal (Banerjee et al., 2011a). Chapter 4 estimates the abundance of archaeal, bacterial and fungal ribosomal gene copies. It examines spatial structure of three microbial communities and demonstrates how the range of spatial autocorrelation and the relationships between microbial communities and soil resources vary between different types of Cryosolic eco-habitats. This chapter was also published in Soil Science Society of America Journal (Banerjee et al., 2011b). Chapter 5 and 6 further extends the notion of microbial spatial dependency to examine how it persists in ammonia oxidizing and denitrifying communities and their functional processes. Chapter 5 measures the rate of autotrophic and heterotrophic ammonia oxidation and estimates the abundance of ammonia oxidizing archaeal and bacterial functional groups. It unravels the scale dependent associations among ammonia oxidation, ammonia oxidizing communities, and soil attributes to identify

which factors exert control on ammonia oxidation activity at fine, medium, and large scales. This chapter was accepted and is in the process of printing in Applied and Environmental Microbiology (Banerjee and Siciliano, 2012). Finally, Chapter 6 quantifies three functional groups of denitrifying communities and measures the rate of potential N_2O production and consumption in Cryosols. This chapter also characterizes spatial structure of denitrifier abundance and activities using geostatistical analyses. Furthermore, it recognizes the predominant controlling factors on N_2O production and consumption in Cryosols. This chapter is under revision in Environmental Microbiology.

2.0 LITERATURE REVIEW

2.1 Permafrost Soil Ecosystems

Arctic permafrost is located in the northern hemisphere, encircling the North Pole and extending south to the coniferous forests of the taiga. Permafrost can be defined as ground that consists of ice, soil, rock, sediment, and organic matter and temperatures persists below 0ºC permanently or for at least two consecutive years (Wagner, 2008). Approximately 26% of world's land area is underlain by permafrost (Zhang et al., 2000); however, in Canada permafrost affected soils comprise 3.7 million km^2 or about 40% of the land area (Bockheim and Tarnocai, 1998). Arctic ecosystems are completely different from other ecosystems due to their long, bitterly cold winters, short unfrozen conditions, low mean annual temperature, and less vegetation (Wagner, 2008). During the short and extreme growing season, productivity is mainly challenged by limited nutrient availability (Scott, 1995). These regions have less than four months with air temperature above 0°C and less than one month above 10°C. Average precipitation per year is usually less than 200 mm and the summer is fairly moist because of low evapotranspiration. Short summer is also accompanied by the thinner atmosphere and longer days. Permafrost regions have been predicted to have an increase of 3-4°C in mean annual air temperature by 2020 and 5-10°C by 2050 (Tarnocai, 2006).

Arctic soils are predominantly in the Cryosolic Order (CSSC, 1998), soils having one or more *cryic* horizons (from Greek *kryos*, cold, ice; a perennially frozen soil horizon) within 1 m from the soil surface for 2 or more years in succession. Presence and depth of permafrost is a key feature that distinguishes the Cryosols from other soil orders. In the majority of Northern Canadian ecosystems, permafrost table is present near the surface of both mineral and organic deposits. Based on the percentage of the land mass covered by permafrost, permafrost soil
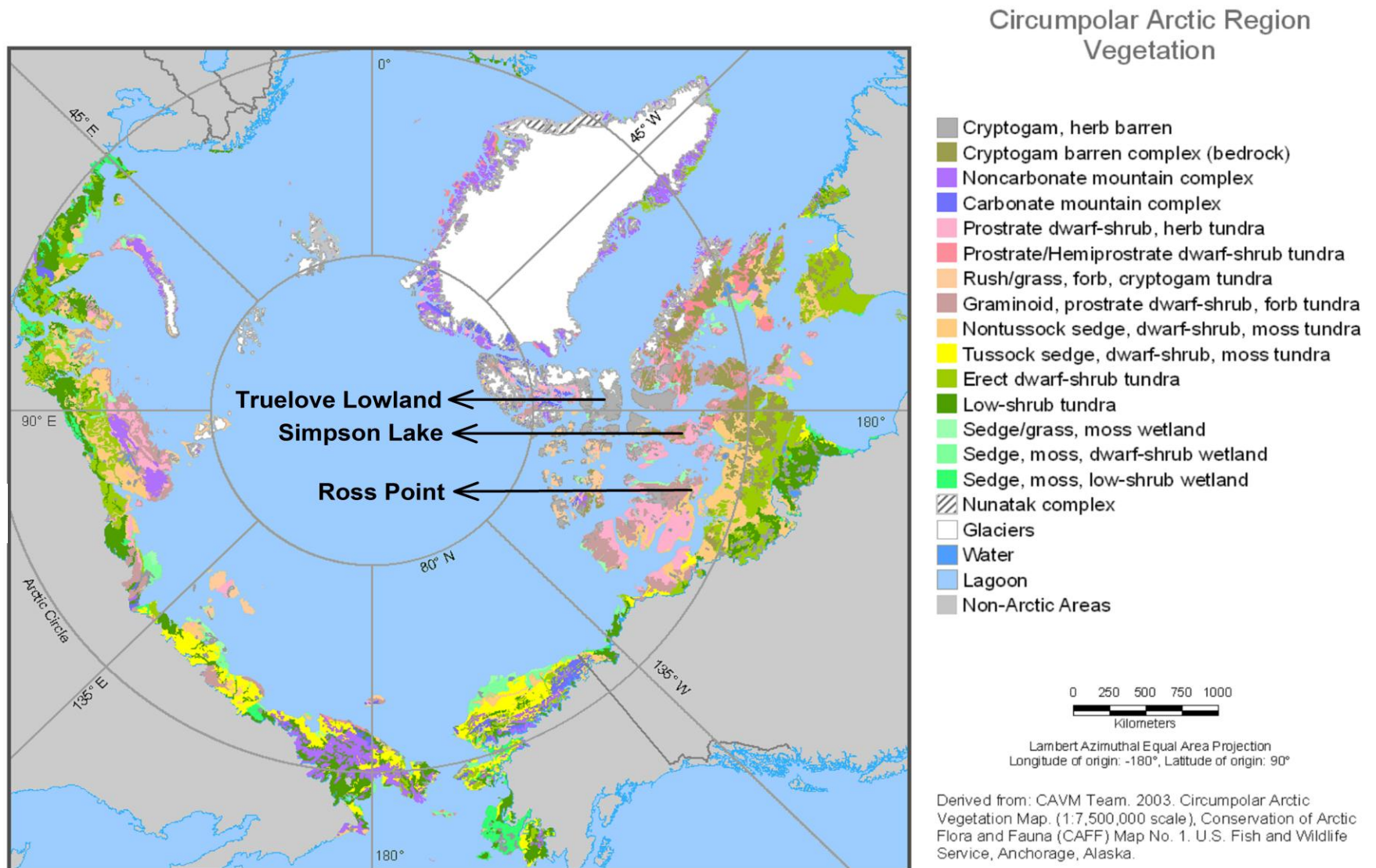
Figure 2.1. Geographic location of three experimental sites in circumpolar Arctic region: Truelove Lowland (75°40′ N 84°35′ W), Simpson Lake (68°35′ N, 91°57′ W), and Ross Point (68°31′ N, 111°10′ W). Adapted from the Toolik-Arctic Geobotanical Atlas (www.Arcticatlas.org; Alaska Geobotany Center).

regions can be subdivided into four zones: Continuous Permafrost Zone (91% to 100%), Discontinuous Permafrost Zone (51% to 90%), Sporadic Permafrost Zone (10% to 50%), and the Isolated Patches Permafrost Zone (0 to 10%). Based on the temperature gradients, Cryosols can be divided into three distinct layers: the deeper layer with less temperature variation (−5 to −10°C); the permanently frozen layer where temperature only ranges between 0 to -15°C; and the active layer, a relatively thin (<1 m) surface layer where the temperature ranges between -35°C to +15°C and only thaws briefly during summer (French 1996; Wagner, 2008). The boundary between the permanently frozen zone and the active layer is known as the permafrost table (Wagner, 2008). Cryoturbation is a predominant pedological process occurring in permafrost ecosystems, and indicates various soil movements as a consequence of frost action (Bockheim and Tarnocai, 1998). Although Cryoturbation can be found in areas of seasonal frost, it is typically associated with permafrost soils and frequently accompanied by gelifluction, fissuring, frost stirring, mounding, patterned ground such as polygons, earth hummocks, nets, stripes, steps, sorted and nonsorted circles (Wagner, 2008). Regular freeze-thaw cycles, silty parent materials, and most importantly inadequate drainage promote cryoturbation (Bockheim and Tarnocai, 1998). According to existence of cryoturbation and soil organic/mineral nature Cryosols can be divided into three Great Groups: Static Cryosols, Turbic Cryosols, and Organic Cryosols. While both Static and Turbic Cryosols have developed on mineral deposits, Turbic Cryosols are predominantly affected by cryoturbation. Turbic Cryosols exhibit distinct cryopedogenic features and consist of permafrost within 2 m of soil surface. Mixed or disturbed horizons and displaced soil materials are the diagnostic features of this great group. Moreover, the presence of organic-rich materials (Ahy) and organic horizons (Oy) close to the permafrost table and assemblage of ice on top of the permafrost layer can also found (Tarnocai and

Bockheim, 2011). Static Cryosols have formed mainly in coarse-textured parent materials with permafrost layer within 1 m of the soil surface. Existence of patterned ground and small cryopedogenic structures such as banded fabrics and silt caps are common in these soils albeit cryoturbation is typically absent. Sometimes an organic surface horizon (< 40 cm) is also present (Tarnocai and Bockheim, 2011). Organic Cryosols have formed on organic parent materials with more than 17% organic matter content by weight and the organic horizon is greater than 40 cm thick (Soil Classification Working Group 1998). These soils comprise humic (well-decomposed), mesic (moderately decomposed), and fibric (undecomposed) peat materials (Tarnocai and Bockheim, 2011). Organic Cryosols can also be moderately associated with cryogenic processes.

Soils in permafrost regions are of vital importance. Firstly, these soils have large amounts of segregated ice that upon melting may contribute to thermokarst, and these are highly susceptible to rising anthropogenic activities such as disturbance from increased interest in fossil fuel and gas hydride extraction, mining, agriculture and hydroelectric power generation. Secondly, they occur in areas of high climate warming. Arctic permafrost is the sink of 12% of the world's total soil organic matter (SOM) (Tarnocai et al., 2003). Oxidation of this huge mass of SOM could release enormous amounts of greenhouse gases including N_2O to the atmosphere (Bockheim et al., 2006).

2.2 Microbial ecology in Cryosols

Although Cryosolic ecosystems are considered to be harsh and inhospitable for microbial life cycle, a plethora of studies have shown the existence of microorganisms in permafrost environments (Steven et al., 2006; refs therein). One quarter of global soil ecosystems are underlain by layers of permafrost that range up to 1 km depth (Wagner, 2008; Williams and Smith, 1989). Although microbial communities in Cryosols are confronted with freeze-thaw

cycles, bitterly cold temperature, starvation, and desiccation, recent reports suggests these microbial groups are metabolically and functionally active (Steven et al., 2006; Wagner, 2008). Permafrost in soil ecosystems is highly heterogeneous; micro-relief and microclimatic conditions contribute to considerable small-scale spatial heterogeneity (Bardgett et al., 2007; Stewart et al., 2011). This heterogeneity becomes further compounded by frequent cryoturbation, irregular horizons, and accumulation of organic and inorganic matter on permafrost table (Wagner, 2008) and as such Cryosolic ecosystems are probably the most spatially variable habitats. As a result of this tremendous variability, microbial distribution patterns and their functioning are strongly influenced. Given that soil C and N dynamics are largely governed by microbes, permafrost microbial communities are the key drivers of Cryosolic biogeochemical and other ecosystem processes. Evidence of microbial existence in Cryosols was first provided by Omelyanski (1911) which was later followed by Kris (1940), James and Sutherland (1942), and Boyd (1958) (reviewed by Wagner, 2008). Recent reports are also available from various Arctic and sub-Arctic ecosystem. Although a large number of studies are available from tropical, temperate, and alpine soil ecosystems, relatively few reports have comprehensively assessed the microbial abundance and distribution patterns in different Cryosolic ecosystems of Canadian Arctic. Elucidation of microbial abundance and diversity in soil ecosystems under sub-zero temperature is extremely challenging and thus studies on permafrost microbial communities could also lead to exploration of novel molecular techniques. Permafrost microbial abundance and functions are highly intriguing as it could reveal their role and adaptability in a changing global climate.

2.3 Nutrient cycling in Cryosols

Microbial communities predominantly regulate soil biogeochemical cycles and as such soil nutrient cycling (Stark, 2007). These biogeochemical processes nonetheless are controlled

by environmental factors. Owing to short growing seasons, long frozen conditions, extremely low temperatures, presence of permanently frozen layer, considerable seasonal fluctuations biogeochemical processes and nutrient cycling in the Arctic are fairly slow. The recalcitrant nature of plant residues in soils also exacerbates the rate of Arctic nutrient cycling (Stark, 2007). Decomposition of SOM is historically slower than primary production in Arctic ecosystems, causing them to be a "net sink" of organic matter. Because of this imbalance Arctic soil ecosystems contain 25% of the global SOM pool (Tarnocai et al., 2008). While tropical ecosystems store the majority of organic matter in living biomass, tundra ecoregions contain a substantial biomass in the form of non-living belowground material. For instance, total carbon in an ecosystem can be divided into 1.5-3% in microbial biomass, 10-17% in the vegetation and the remaining 81-88% in the non-living biomass (Pare, 2011; Schmidt et al., 2002). Moreover, 90-95% of total N is reserved in the SOM pool (Jonasson et al., 1999). Thus SOM acts as an important nutrient reserve by storing major soil nutrients, such as C, N, and P (Schmidt et al., 2002). Arctic ecosystems are expected to experience a 4-7.5°C and 2.5-14°C temperature increase in summer and winter respectively (Wu et al, 2006). The increasing temperature in northern regions may change the rate and balance of SOM sequestration and decomposition, or even result in enhanced SOM deposition, converting these ecosystems to "net sources" and releasing enormous amount of greenhouse gases.

In the Arctic, a substantial proportion of the soil nutrient pool is immobilised by microbial communities due to low plant biomass and high SOM accumulation and often the rate of immobilisation is even higher than gross mineralisation which results in negative net mineralisation (Jonasson et al. 1999; Stark, 2007). Plants are confronted with serious competition with soil microbial communities for nutrients in Arctic ecosystems.

2.4 Nitrogen cycle processes as a contributor to nitrous oxide emissions

Nitrous oxide (N_2O) has a long atmospheric life and on a molar basis, N_2O has 298 times more global warming potential than carbon dioxide on a 100-year time frame (IPCC, 2007). Presently atmospheric N_2O concentrations are approximately 317 ppbv which has increased from 275 ppbv in 1900 (CMDL, 2001). If atmospheric N_2O concentrations were to increase at this rate, it would result in 10% decrease in the stratospheric ozone layer which again would increase the ultraviolet radiation reaching the earth by 20%. Soils play a key role in N_2O dynamics being the most significant source of N_2O and contributing up to 90% of the world's total N_2O emissions (Ma et al., 2007). Approximately 70% of global annual N_2O emissions originated from soil microbial transformations of nitrogenous compounds such as nitrification and denitrification (Mosier et al., 1998).

2.4.1 Nitrification

Nitrification is the biological oxidation of ammonium (NH_4) to nitrate (NO_3) via nitrite (NO_2^-) by autotrophic and heterotrophic microorganisms. The reactions are driven by two groups of microorganisms: the first part up to NO_2^- is conducted by the ammonia oxidizing bacteria (AOB) and archaea (AOA) or primary nitrifiers, while the second step is carried out by nitrite oxidizing bacteria or secondary nitrifiers (Ward et al., 2011) (Fig. 2.2). The first step of nitrification is production of hydroxylamine (NH_2OH) by the oxidation of ammonia catalyzed by ammonia monooxygenase (AMO). Two electrons are required for the reduction of one of the atoms of O_2 to water and are derived from the oxidation of NH_2OH to NO_2^-, which is the second step of nitrification catalyzed by the enzyme hydroxylamine oxidoreductase. The nitrite

oxidizing bacteria then uses the produced NO_2^- to form NO_3^- with the help of the enzyme nitrite oxidoreductase (Ward et al., 2011).
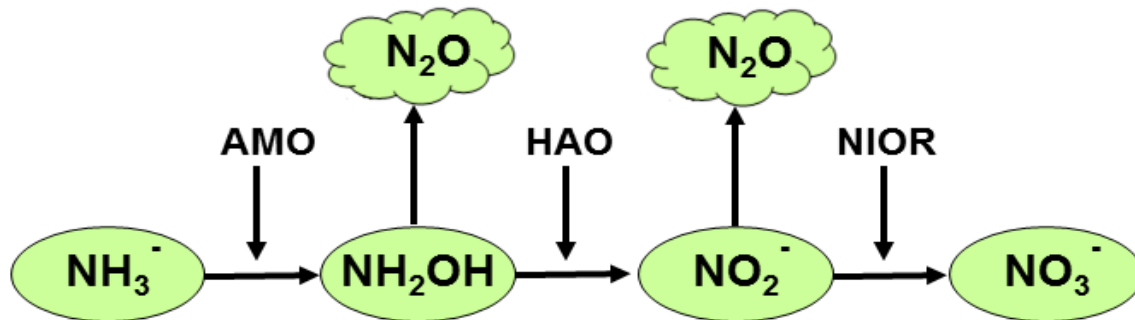
Figure 2.2. Biological nitrification pathway showing the steps of N_2O production. The soluble forms are shown in ovals and gaseous forms in clouds. AMO- ammonia monooxygenase; HAO-hydroxylamine oxidoreductase; NIOR- nitrite oxidoreductase

2.4.1.1 Autotrophic ammonia oxidation

Ammonia oxidation is one of the most critical steps of the global N cycle. AMO, the enzyme responsible for ammonia oxidation, is a membrane-bound, multisubunit enzyme. McTavish (1993) first reported the complete gene sequence (designated *amoA*) of the membrane-associated active-site polypeptide of the AMO from *Nitrosomonas europaea*. Ammonia oxidizing microorganisms, mostly chemolithoautotrophic bacteria (AOB) and archaea (AOA), are obligate aerobes. However, some species are highly tolerant of low oxygen or anoxic environments and are capable of conducting anaerobic ammonia oxidation (anammox) (Sayavedra-Soto and Arp, 2011). Anaerobic ammonia oxidizers are able to gain energy and reducing power from NH_4^+ or NH_3 and fix carbon from CO_2 in the atmosphere via Calvin cycle. However, ammonia oxidation is a thermodynamically low energy ($\Delta G^{0'} = -271$ kJ mol^{-1}) yielding process and thus the complete dependence of ammonia oxidizing microorganisms on ammonia is still a mystery (Sayavedra-Soto and Arp, 2011). The enzyme AMO is comprised of three

membrane-bound polypeptides, AmoA, AmoB, and AmoC. Autotrophic AOB mostly encompass genera *Nitrosomonas*, *Nitrosococcus*, and *Nitrosospira* whereas the AOA have been recently been placed in a separate phylum, "Thaumarchaeota", consisting of four species to date, *Nitrosopumilus maritimus*, *Cenarchaeum symbiosum*, *Nitrososphaera vienensis*, and *Nitrososphaera gargensis* (Perster et al., 2011). The AOA and AOB have been found in most aerobic environments where ammonia is available via the mineralization of SOM or anthropogenic N sources. Occurrence of ammonia oxidizing bacteria in Arctic ecosystems has also been reported recently (Siciliano et al., 2009; Wagner, 2008).

2.4.1.2 Heterotrophic ammonia oxidation

Heterotrophic ammonia oxidation in soil has also been reported in few studies (Jetten et al., 1997; Moir et al., 1996; Wehrfritz et al., 1996). Microorganisms involved in heterotrophic nitrification utilize soil organic carbon as their source of carbon and energy. Many heterotrophic bacteria also have AMO and hydroxylamine oxidoreductase enzymes. Despite their similar substrates, these enzymes differ in their structure and conformation (Moir et al., 1996; Wehrfritz et al., 1996; Robertson et al., 1988). For example, heterotrophic ammonia monooxygenase enzyme is not inhibited by acetylene. Interestingly, some heterotrophic nitrifiers are able to denitrify as well (Robertson et al., 1989). Heterotrophic ammonia oxidation can be significant, particularly in soils with low pH and high SOM content.

2.4.2 Denitrification

Denitrification, the anaerobic reduction of nitrate, nitrite and nitric oxide to N_2O or N_2, is the foremost biological mechanism through which fixed N returns to the atmosphere from soil or water, thereby closing the N-cycle (Philippot and Hallin, 2005). Denitrification is considered the dissimilatory reduction of nitrate or nitrite to gaseous nitric oxide, nitrous oxide and N, which is

coupled to electron transport phosphorylation (Fig. 2.4). The N_2O is an obligatory intermediate in denitrification pathway whereas nitric oxide may be a by-product and not intermediate (Wallenstein et al., 2006). Denitrification is carried out by both bacteria and fungi. Denitrifying bacteria may be phototrophs, lithotrophs, and organotrophs. Organotrophs and species of *Pseudomonas* predominate within this group, presumably because of their versatility and ability to compete for C substrates (Knowles, 1982). There are four reduction steps in denitrification pathway: nitrate reduction, nitrite reduction, nitric oxide reduction, and finally nitrous oxide reduction. Nitrate reduction is carried out by two homologous enzymes, membrane-bound (Nars) and periplasmic-bound (Nap) nitrate reductases (NAR). The enzymes are encoded by *narG* and *napA* respectively. The enzyme NAR has also been found in non-denitrifying bacteria therefore, *narG* and *napA* genes have not been widely
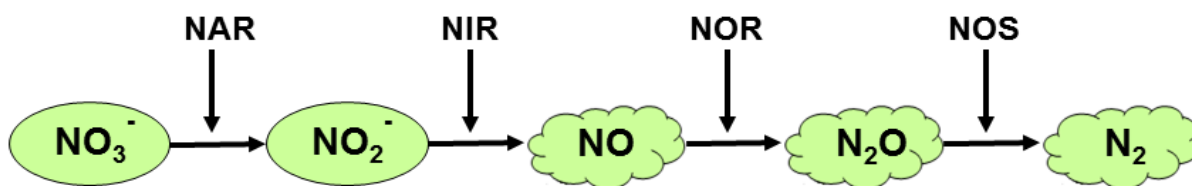
Fig. 2.3. Biological denitrification pathway adapted from Philippot and Hallin (2005). The soluble forms are shown in ovals and gaseous forms in clouds. NAR- nitrate reductase; NIR- nitrite reductase; NOR- nitric oxide reductase; NOS- nitrous oxide reductase

used to characterize the diversity of denitrifying bacteria. Nitrite reduction is catalyzed by evolutionary-unrelated enzymes that are dissimilar in their structure and metal: a copper- and a cytochrome cd_1-nitrite reductase (NIR). The cd_1 and copper nitrite reductase are encoded by *nirS* gene and the *nirK* gene, respectively. These genes were the first to be used for the assessment of denitrifier diversity and have been regularly used as molecular marker for denitrifier community studies (Cuhel et al., 2010; Enwall et al., 2010; Philippot et al., 2009). Nitric oxide reduction, regulated by nitric oxide reductase (NOR), is an important step in denitrification pathway as it

exclusively controls N_2O emissions. The enzyme is encoded by *norB* gene. The last step of denitrification pathway is nitrous oxide reduction, catalyzed by nitrous oxide reductase (NOS). The *nosZ* gene is responsible for encoding the enzyme and thus it has been of interest of researchers.

2.5 Soil properties as a driver of nitrifier and denitrifier communities and functions

In all ecosystems, soil properties play a central role in regulating the abundance of nitrifier and denitrifier functional groups. The major edaphic factors that drive nitrifier and denitrifier communities are temperature, pH, soil moisture content, total C, total N, oxygen availability, soil texture etc. (Cuhel et al., 2010; Erguder et al., 2009; Firestone et al., 1980; He et al., 2007; Knowles, 1982; Shen et al., 2008). When NO_3^- concentration is high in soils, the rate of N_2O production is preferred over N_2O consumption. When the oxygen concentration is high the enzyme nitrous oxide reductase is inhibited by O_2 (Chapuis-Lardy et al., 2007). The optimum pH for denitrification in soils ranges between 7.0 and 8.0 (Knowles, 1982) whereas the optimum temperature is between 10°C and 35°C. Soil temperature, pH, and moisture content directly affect nitrifier and denitrifier abundance and community composition by regulating their survival, growth, and functions. The enzymes produced by these microorganisms are highly sensitive and function only within a particular range of the above factors. Nitrifier and denitrifier growth and N transformations can be influenced greatly as a consequence of high amount of dissolved cations and anions in soils (Smith and Doran, 1996). On the other hand, most nitrifiers and denitrifiers except autotrophic AOB, are directly governed by soil organic matter (SOM) content. Moreover, AOB may also be affected as the differences in the level of SOM inputs may influence the rate of soil N mineralization, the central process for generating soil ammonium (Carney et al., 2004). The effects of these factors have mostly been investigated in tropical or

temperate ecosystems and little is known for Arctic ecosystems. However, the influence of these factors on microbial populations may differ considerably according to the environmental conditions and between ecosystems (Avrahami et al., 2003). Due to the unique and diverse nature of Arctic ecosystems the extent and pattern of these effects on nitrifier and denitrifier populations may vary significantly.

2.6 Functional genes of nitrifier and denitrifier communities

Traditionally, studies aiming for bacterial identification have only focused on culture-based techniques. Viable plate count or most-probable-number techniques only select for certain organisms and a substantial portion of bacterial populations are known as non-culturable (Amann et al., 1995). Therefore, these culture dependent techniques may not always reveal the true diversity in nature; these methods are inadequate to address this problem. Furthermore, some microorganisms such as AOB can be extremely slow growing thus very difficult to culture (Stephen et al., 1996). Researchers have recognized the necessity for culture-independent analyses of bacterial populations, and these kinds of analyses have been facilitated by recent advances in the molecular biology and molecular phylogeny.

In culture-independent molecular techniques, phylogenetic genes such as 16S ribosomal RNA (rRNA) gene have been used for a number of years. Due to its ubiquitous occurrence, invariable function, and considerably large size, the 16S rRNA gene has been an important phylogenetic tool and a useful target for bacterial identification, which led researchers to call rRNA the "ultimate molecular chronometer" (Woese, 1987). Many researchers have used 16S rDNA genes to characterize natural AOB (Purkhold et al., 2000) and denitrifier (Braker et al., 2001) populations in soils and to analyze their taxonomic and phylogenetic features. However, 16S rDNA and 16S rRNA based approaches have certain drawbacks. Firstly, they fail to

distinguish between close relatives, such as species within a given group or genus (Rosello-Mora and Amann, 2001). PCR primers for 16S rRNA genes may cross-react with other phylogenetic groups, particularly when they are used against environmental samples with complex microbial gene pools (Rotthauwe et al., 1997). Moreover, substantial heterogeneity can be found among organisms with almost identical 16S rDNA genes (Calvo et al., 2005). The 16S rDNA may be useful to distinguish between nitrosococci and nitrosomonads (Bruns et al., 1999) but it can be unsuccessful if examining a single genus, such as *Nitrosospira*.

Hence, during the last decade, protein encoding functional genes, such as *amoA*, *nirK*, *norB* and *nosZ*, encoding the essential enzymes in nitrification and denitrification pathways, have been used by taxonomists and molecular ecologists for diversity studies (Calvo et al., 2005; Philippot, 2005). In contrast with Ludwig and Schleifer (1999) who stated that 16S rDNA gene is the best marker to infer phylogenetic relationships, several research groups have found that functional genes are more useful at a finer scale than 16S rDNA (Rotthauwe et al., 1997; Casciotti and Ward, 2005). Moreover, the functional genes possess a greater level of sequence heterogeneity than 16S rDNA, providing greater resolution of closely-related strains and functional genes can also be amplified simultaneously (Kowalchuk and Stephen, 2001).

2.7 Measuring gene abundance: Real time quantitative polymerase chain reaction

Abundance of nitrifiers and denitrifiers can be best studied by testing their functional gene copy numbers (Siciliano et al., 2007). Several molecular techniques are employed to study the abundance and composition of microbial communities in soil and other environmental samples. This study employed polymerase chain reaction (PCR) and real-time quantitative PCR (qPCR) to assess microbial abundance and distribution. QPCR is reproducible, highly precise and accurate, has high throughput and provides additional information on amplification

efficiency (Sharma et al., 2007). Quantifying amount of amplicons (PCR amplified products) through end-point PCR has several major drawbacks such as low sensitivity, poor precision, non-automated, low resolution, inefficiency of stains, low resolution etc.Real time qPCR is one of the most widely acknowledged and sensitive techniques in microbiology (Smith and Osborn, 2009). It is a variation of the standard PCR technique to determine DNA or messenger RNA. It offers a simple yet accurate and reproducible method for quantitating abundance and expression patterns of taxonomic and functional gene markers present in environmental samples. Quantitative data measured by qPCR is used to link gene/transcript prevalence to relative difference in environmental processes and abiotic/biotic factors (Smith and Osborn, 2009). Thus, qPCR closes a long-standing gap between functional groups and ecosystem processes.

Typically PCR amplification consists of three distinct phases: exponential, linear, and plateau (Fig. 2.4). At the first phase the accumulation of PCR products is exactly double after each cycle. As the enzyme and other reagents are not limited, the amount of amplicons increases
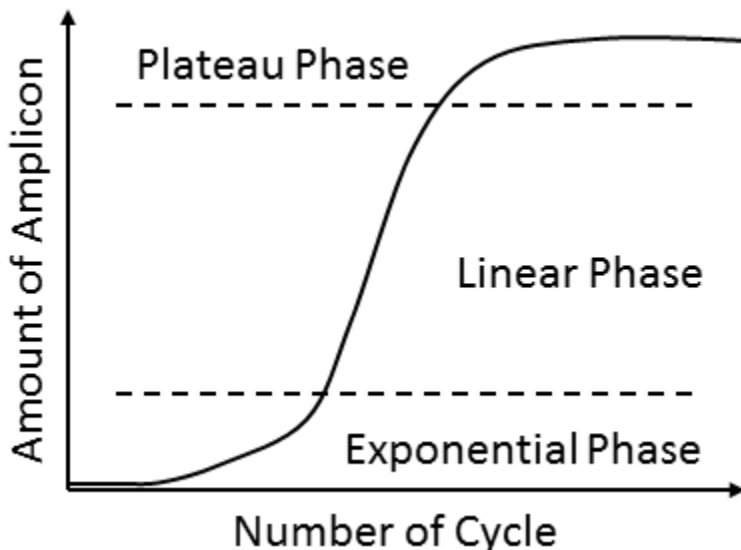
Figure 2.4. Illustration of the phases of PCR shown by a theoretical plot of PCR cycle number vs. accumulation of amplicons.

exponentially and thus this phase is called exponential phase. During the second phase the amplification gradually slows down due to depletion of reagents and the amount of PCR products increases linearly. Finally the PCR reaches a plateau i.e. reaction ceases and no more amplicons are generated and amplicons will degrade if PCR continues. Real-time quantitative PCR is fundamentally similar to conventional PCR except that the progress of the PCR is observed by fluorescence in "real-time". QPCR is based on the principle that ideally the amount of amplicons in exponential phase is proportional to the amount of initial template (Heid et al., 1996; Yuan et al., 2006). Under optimal PCR conditions such as optimized primer concentration, amplicons length, annealing temperature, and template purity, amplification efficiency can be 100% (i.e. PCR product generated in exponential phase will be twice after each cycle). QPCR can use both genomic DNA and reverse-transcribed cDNA as its templates. Monitoring the progress of PCR amplification is usually performed by DNA binding dyes like SYBR green or DNA hybridization probes such as m=Molecular Beacons or Taqman probes (Yuan et al., 2006). TaqMan dual fluorogenic probe system uses 5' nuclease activity to cut off the quencher and emit fluorescence.Hhowever, TaqMan requires extensive optimization. SYBR green anneals to the minor groove of double-stranded DNA through base pair intercalation and upon binding it emits a fluorescent signal (Pfaffl, 2001; Smith and Osborn, 2009). With the increase in number of amplified products, level of fluorescence also increases proportionately. Direct positive relationship between fluorescent dyes and the quantity of amplicons is the foundation of qPCR. As fluorescent signal is directly proportionate to the amplicon, a plot of transformed fluorescence signal against cycle number will produce a linear range. It is necessary to design highly specific primer pairs to circumvent the formation of non-specific amplicons and overestimation of amplicons as SYBR green anneals with any double-stranded DNA (Nogva and Rudi, 2004).

Furthermore, to reduce primer-dimer production, forward and reverse primers with self-complementarity should be avoided. Thus careful optimization of primer sequence and concentration is a key step of successful and precise qPCR (Smith and Osborn, 2009).

The different kinds of amplicons generated during first few cycles of qPCR include undefined long products, non-specific products, and original target amplicons (Nogva and Rudi, 2004; Ma, 2009). There are two different types of quantification in real-time PCR: relative and absolute. Relative quantification is achieved by comparing the expression of a target gene with a well-known reference gene. Relative quantification is required for pathological and physiological studies to examine the differential patterns of gene expression. On the other hand, absolute quantification is necessary in studies where exact copy number of gene/transcripts is required. Absolute quantification uses a standard curve to calculate the copy number in a sample (Yuan et al., 2006). The standard curve is obtained by plotting threshold cycle against the concentration of target DNA. Threshold and baseline is adjusted for quantitative analysis. A threshold cycle (Ct) is the particular cycle at which the fluorescence level is first detected. Ct number is primary statistical component of interest in most qPCR experiments (Yuan et al., 2006). A slope and an intercept are determined from the standard curve by linear regression. Amplification efficiency of qPCR is calculated by the following equation (Nogva and Rudi, 2004).

$$\text{Amplification Efficiency} = 10^{-\text{slope}} - 1 \qquad \text{[Equation 2.1]}$$

An amplification efficiency of 100% would result in doubling of amplicons from original concentration. Efficiency between 80% and 100% is accepted in microbial ecology studies. However, amplification efficiency is not the only determining factor. A melting curve or dissociation temperature analysis is performed after each real-time PCR run. For melting curve analysis, the double-stranded amplified product is heated in a temperature gradient and the level

of fluorescence is monitored. The temperature at which 50% double stranded DNA is denatured and the fluorescence level declines upon SYBR green dissociation is detected (Smith and Osborn, 2009). Dissociation temperature of the amplicons depends on the guanine and cytosine (G-C) content and thus, precise amplification of specific target gene results in sharp dissociation peak. This analysis is carried out to confirm that the detected fluorescence is obtained only from target genes and not from non-specific products.

Sequence-specific primers are employed to examine the relative number of copies of a sequence. Since the report by Heid et al. (1996), qPCR has been used in plethora of studies of environmental microbiology, pathogen quantification, cancer quantification, transgenic copy number determination, microarray verification, and drug therapy studies (Yuan et al., 2006). By combining the conventional 'end-point' detection PCR and fluorescent detection technologies, qPCR estimates the number of amplicons in 'real time' during each cycle of the PCR amplification (Smith and Osborn, 2009). As accumulation of amplicons is recorded during the early exponential phase of the PCR, this enables the quantification of gene (or transcript) numbers when these are proportional to the starting template concentration. Dominant amplicons generated in end-point PCR may not always reflect actual prevalence of functional groups in environmental samples whereas fluorescence based detection in qPCR is highly precise and sensitive to minute change in gene abundance (Nogva and Rudi, 2003; Smith and Osborn, 2009).

2.8 Soil spatial variability

Soil properties are characteristically heterogeneous as they vary from location to location and a thorough understanding of this spatial variability is essential to disentangle the complex correlations among the soil processes (Si et al., 2003; Zeleke and Si, 2005). In most of the cases, spatial heterogeneity is non-random and tends to decline as distance decreases between points in

space (Goovaerts, 1998). Measurements done at a number of locations at a particular time or within a small time frame are called a space series. Soil properties measured close together in space are likely more similar than measurements done far from each other. For example, soil organic N content of two soil samples tends to be highly correlated if the samples are closely spaced, and this tendency decreases as the distance between the two samples increases. Therefore, spatial dependence can be defined as the correlation of values of a variable at one point related to its value at nearby points. In a dataset, if the values at various sample points cannot be considered independent-the attribute can be spatially dependent. When the spatial structure is the same in all directions, it is known as isotropic, whereas if it is not the same, it is called anisotropic.

Scale implies a distinctive length/ area (spatial) or time (temporal) of a particular process, observation, or model (Pennock, 2004). Variability of soil properties is affected by various factors (e.g. geomorphology, predominant vegetation, land patterns) and these factors operate at multiple scales, from microscale to continental scale (Corstanje et al., 2007). Associations among the variables may not be the same across landscape. Soil N mineralization may be regulated by land use at regional scale, SOM content at the field scale and microtopography and microsites characteristics at a very fine scale. Soil spatial dependence has been extensively studied for physical (Zeleke and Si, 2005), chemical (Boyer et al., 1996; Kravchenko et al., 2006; Liu et al., 2008) and biological properties (Robertson et al., 1997; Goovaerts, 2006) in tropical and temperate soils. However, studies examining spatial heterogeneity in Cryosols are extremely limited. Arctic Cryosolic ecosystems are known for their considerable variability as they can encompass a range of landscape patterns such as well-drained lands, wetland soils, riparian zones, ridge-top stripes, and polar desert soils (Lev and King, 1991; Pare, 2011; Walker, 2000).

Thus understanding spatial heterogeneity and autocorrelation of Arctic landscapes is important to obtain information on various processes in permafrost.

2.9   Microbial spatial variability

The abundance, structure, and functioning of soil microbial communities are largely governed by soil resources. Owing to the characteristic heterogeneity of soil attributes, soil microbial communities also display spatial patterns and strong spatial dependence (Ettema and Wardle, 2002). Even in an apparently homogenous soil ecosystem, microbial biomass can be spatially heterogeneous. Spatial dependence of soil physical and chemical properties implies that microbial abundance and their functions may also show dependence. Spatial heterogeneity in soil resources results in microhabitat diversity, which can influence the distribution of microbial population (Green et al., 2006). Studies investigating soil spatial variability largely focus on physical (Iqbal et al., 2005; Zeleke and Si, 2005) and chemical (Cerri et al., 2004; Mzuku et al., 2005) attributes, and fewer studies assessed soil microbial spatial variability. Spatial autocorrelation was long considered as 'noise' in data set and scientific investigations often neglected spatial patterns until its recognition in pedology, and plant and animal community ecology. Microbial spatial variability remained largely unexplored until the end of the 1980s (Parkin 1987; Robertson, 1988). As a result of the intrinsic heterogeneity of soil resources, microbial abundance also varies spatially across multiple scales (Parkin, 1993). Microbial spatial dependency has recently been elucidated in agricultural (Bru et al., 2010), grassland (Nicol et al., 2003), and forest (Saetre and Baath, 2000) soils. However, despite the growing awareness of spatial heterogeneity of microbial diversity (Ettema and Wardle, 2002), information on microbial spatial variability in Arctic soil ecosystems is extremely limited.

2.10   Geostatistics: A tool for measuring spatial variability

Spatial autocorrelation is an inherent component of any ecological study assessing spatially heterogeneous organism distribution or environmental variables, however, most traditional statistical techniques largely ignore it and assume that the data collected are independent from each other (Ross et al., 1992). Field data collected from a research site is often spatially dependent and violates the independence assumption. Spatial autocorrelation structure can provide insight into spatial patterns such as patchiness or flatness due to a particular factor and thus facilitates data interpretation (Jung et al., 2006). There are numerous methods to elucidate spatial patterns and the technique that focuses on the statistical modeling of spatial dependence and is called "geostatistics." Geostatistics is essentially a branch of applied statistics that concentrates on the detection, modeling, and estimation of spatial patterns (Ross et al., 1992). Oliver and Webster (1991) defined geostatistics as "a technology for estimating the values at unsampled places of properties that vary in space, whether in one, two or three dimensions, from more or less sparse sample data". The earliest report conducting geostatistical study was Mercer and Hall (1911) who assessed spatial variation of crop yield at many small plots (Oliver and Webster, 1991). While geostatistics were first used by miners, it is now regularly used in soil science, ecology and microbiology. Semivariograms are central to geostatistical spatial analysis that characterize the spatial continuity or roughness of a data set by illustrating how spatial relationships change with separation distance between sampling points. A semivariogram is computed by averaging one half the difference squared of the z-values over all pairs of observations with the particular separation distance and direction (Equation 2.2).

$$\gamma(h) = \frac{1}{2N(h)} \sum_{\alpha=1}^{N(h)} [z(x_k) - z(x_k + h)]^2$$

[Equation 2.2]

27

where $z(X_k)$ is the particular soil property, z, at a location, x_k, and (X_k+h) is property z at h lag distance and N(h) is the number of data pairs for a given distance (Goovaerts, 1998; Si et al., 2003). A plot of semivariance, $\gamma(h)$, as a function of lag distance is called semivariogram. A typical semivariogram increases from a minimum value at the smallest lag distance (lag = 1; no dissimilarity) to a plateau where semivariance reaches constant with increasing lag (maximum dissimilarity). Three important attributes of a semivariogram are nugget variance, sill, and range. The nugget variance is the random variation caused by experimental error whereas sill is the
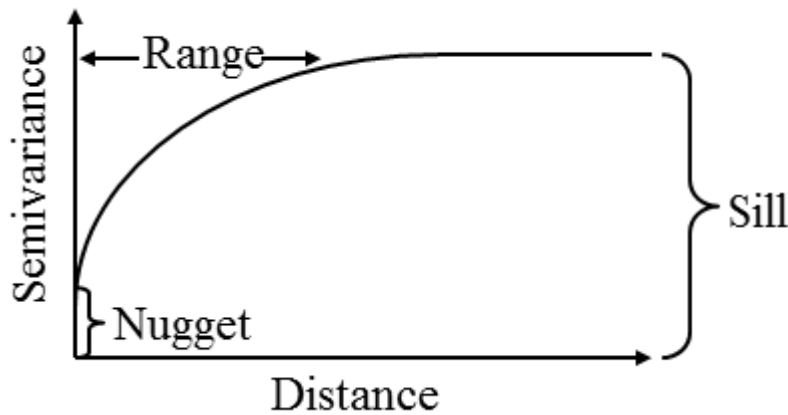
Figure 2.5. An experimental semivariogram showing key features

maximum variability reached by a property. Range is the lag distance at which the semivariance value becomes highest and as such the range of a semivariogram demonstrates the zone of spatial dependency (SPD). Therefore, samples situated closer than the range are spatially dependent while samples separated by a distance greater than the range are not spatially dependent. The spatial relationship between two variables can be determined by calculating cross-semivariance, $\gamma_{yz}(h)$ (Equation 2.3).

$$\gamma_{yz}(h) = \frac{1}{2N(h)} \sum_{k=1}^{N(h)} [y(x_k) - y(x_k + h)].[z(x_k) - z(x_k + h)] \qquad \text{[Equation 2.3]}$$

where *y* and *z* are two variables, $z(x_k+h)$ is the value at h lag distance and *N(h)* is the number of data pairs for a given distance. Cross-semivariogram is the plot of cross-semivariance, $\gamma_{yz}(h)$, as a function of *h* or lag. In a semivariogram, the shape and points near the origin are extremely important as these closest points are given highest weights in spatial interpolation and are therefore significant determinants (Si et al., 2003). Since the semivariogram is a crucial component of geostatistics, it must be computed and modeled reliably with enough data points at a pertinent scale (Oliver and Webster, 1991). Typically the larger the sample from which a semivariogram is calculated, the more accurately will it be determined. Geostatistical studies in many cases have used less than 50 samples or even as few as 24; however, only semivariograms computed with near 100 samples are satisfactory. A sample number of 150 is desirable but may not be realistic (Webster and Oliver, 1992). Geostatistical analysis can provide information regarding the minimum sampling distance to secure spatial independence, or to identify the spatial scale at which major determining processes operate in an ecosystem (Si et al., 2003). Thus, semivariance analysis can be used to examine spatial structure of a variable in a landscape and design sampling strategies such as size, shape, or direction (Si et al., 2003). Moreover, computing semivariance is the first step towards spatial interpolation or uncertainty analysis.

2.11   Conclusions

After reviewing the existing literature, it is clear that a significant dearth of knowledge remains in Arctic N dynamics and soil spatial ecology. The key research questions that can be asked here are: 1) What is the degree of correlations among soil moisture and other soil properties in various types of Cryosols? 2) Are soil physicochemical attributes spatially autocorrelated in Cryosolic ecosystems and if so how does this autocorrelation differ between

ecohabitats? 3) What are the relative abundance of overall microbial and ammonia oxidizing communities and the potential of ammonia oxidation in Canadian Arctic? 4) How are potential ammonia oxidation and ammonia oxidizing functional groups correlated with soil resources at different spatial scales? 5) What is the rate of N_2O production and consumption in different types of Cryosolic ecosystems and how does it vary from other tropical and temperate soil ecosystems? 6) Are denitrifying microbial communities spatially structured and what scale do they operate at? 7) What are the predominant driving factors of denitrifier abundance and denitrification potential in Canadian Arctic? The following four chapters will assess soil resources, N cycling communities, and functional processes in a comprehensive study conducted at three Canadian High Arctic ecosystems.

3.0   SOIL SPATIAL DEPENDENCE IN THREE ARCTIC ECOSYSTEMS[§]

Preface

Soil resources are intrinsically heterogeneous, and this heterogeneity is not random and demonstrates strong spatial structure. Thus spatial patterns and scale of soil properties can reveal the underlying controls in a soil ecosystem. Spatial variability of soil physicochemical attributes has been examined in agricultural and pristine soils of tropical and temperate ecosystems. However, little information is available for soil spatial dependency in Arctic ecosystems. This chapter presents a comprehensive report of soil spatial patterns in 279 samples collected from three Arctic ecosystems.

[§]A modified version of this chapter with the same name was published in Banerjee, S., A. Bedard-Haughn, B.C. Si, and S.D. Siciliano. 2011. Soil Science Society of America Journal 75:591-594.

3.1 Abstract

Spatial variability was examined for 13 soil physicochemical attributes and their correlation with soil gravimetric moisture content (θ_g) in 279 soil samples collected from three high Arctic ecosystems. The observed correlations between θ_g and pH, ammonium, nitrate, total organic carbon (TOC), dissolved organic carbon (DOC), and dissolved organic N (DON) contents in Arctic are considerably higher than temperate agricultural and tropical grassland soils, which suggest that θ_g plays a critical role in Arctic soil ecosystems. It was found that despite the climatic extremities Arctic soil attributes are spatially well structured and their spatial dependency is consistent within and between the studied ecosystems. However, the range of spatial dependency is considerably smaller which can be ascribed to the environmental extremities and other periglacial features. Based on the results, it is recommended that for obtaining independent samples the minimum distance between samples is 10 m in Static or Turbic Cryosols and 45 m in Organic Cryosols.

3.2 Introduction

Soil attributes typically vary from location to location making regional scale inferences about soil processes difficult. However, in most cases, spatial heterogeneity is nonstochastic and tends to decrease with the distance between spatial positions, i.e. spatial dependency (Goovaerts, 1998; Cerri et al., 2004). Spatial dependency is the correlation of values of a variable at one point related to its value at nearby points as a function of the distance separating these two points. Spatial variation of soil properties primarily results from pedogenic factors such as topography, parent material, and climate (Mzuku et al., 2005). Thus, one expects and does indeed see differences in spatial dependencies in ecosystems subjected to different geomorphology, vegetation, meteorology, or management regimes. Spatial dependencies can be thought of as

indicative of soil processes operating in the field. Understanding spatial dependencies allows one to make inferences about the long-term, ecological and physical processes that are defining an ecosystem. Spatial dependency has been extensively studied for physical (Iqbal et al., 2005), chemical (Cerri et al., 2004; Mzuku et al., 2005), and biological properties (Robertson et al., 1997; Saetre and Baath, 2000; Bengston et al., 2007) of tropical and temperate soils. However, studies examining spatial heterogeneity in permafrost soils are limited.

Permafrost soil ecosystems dominate about one-fifth of the world's and the majority (40%) of the Canadian landscape (Beer, 2008; Bockheim and Tarnocai, 1998). These ecosystems have long, cold winters and short growing seasons (50 to 60 days), frequent cryoturbation and gelifluction. North American Arctic permafrost contains 213 Gt of organic carbon, which represents about 25% of the world's total soil organic carbon and 61% of North America's total soil carbon (Tarnocai et al., 2003). Permafrost regions are likely to undergo the greatest degree of climate change and this sequestered SOM may be released to the atmosphere in the form of greenhouse gases. Efforts are underway to map the SOM present in permafrost soils as are efforts to understand the SOM dynamics and its spatial variability in these soils (Beer, 2008). Characterizing the spatial dependency of these soils will help us extrapolate from single point estimates to a larger spatial scale.

Soil moisture content is well known for its considerable spatial variability (Petrone et al., 2004). Information on spatial heterogeneity of θ_g plays a critical role in soil survey, land surface hydrological modelling and also in assessing the ecophysiographical patterns (Choi et al., 2007). Soil physicochemical properties are known to be strongly influenced by moisture content with θ_g spatially correlated to the SOM content in tropical forest (Wang et al., 2002) and upland Chernozem (Yanai et al., 2005) soils. However, the spatial relationships between θ_g and other

physicochemical attributes including SOM have not been quantified in Arctic soils. This study examined: (1) the minimum sampling distance for obtaining independent samples in Static and Organic Cryosol (2) the spatial relationships between θ_g and other physicochemical attributes in Arctic ecosystems, (3) if Arctic spatial dependency was similar to that seen in temperate and tropical ecosystems, and (4) whether this dependency was constant across three sites in the Canadian Arctic.

3.3 Materials and methods

Three high Arctic ecosystems were selected for this study: Truelove Lowland, Simpson Lake, and Ross Point. Truelove Lowland (75°40′ N, 84°35′ W) has been the subject of extensive scientific expeditions (Bliss, 1977; Chapin, 1996; Lev and King, 1999); the soils at the study-site are predominantly Static or Turbic Cryosols. Simpson Lake (68°35′ N, 91°57′ W) is situated in the middle of the Boothia Peninsula and is dominated by Static Cryosols. Ross Point (68°31′ N, 111°10′ W) is situated in the south port of Victoria Island and the soils were dominated by
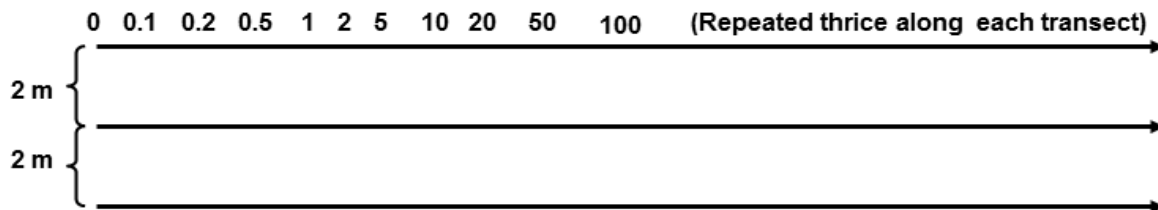
Figure 3.1. A diagram showing the soil sampling at three Arctic ecosystems

Organic Cryosols. At all three sites, a slope with a southern aspect was selected and three parallel transects (2 m lateral distance) were laid out along a 300 m section (Fig. 3.1). Soil samples were collected during 3rd and 4th week of July in 2006. Samples were collected at 31 points (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300 m) along each transect.

Soil gravimetric water content (θ_g) was calculated by measuring the weight loss of 5 g soil samples after they were dried for 24 hours at 105°C and the water content expressed as percentage of dry soil mass (Gardner, 1986). Soil pH was measured using 5 g soil in a 1:1 soil:water (deionized) mixture with an Accumet pH meter (Accumet 925, Fischer Scientific, Hanover Park, IL). The DOC and DON were measured using TOC-VCPN analyzer (Shimadzu Scientific Instruments, Columbia, MD). Various concentrations of potassium hydrogen phthalate (0-200 mg L^{-1}) and potassium nitrate (0-20 mg L^{-1}) were used as standards for total dissolved organic carbon and total dissolved N analyses respectively. A 12 ml aliquot of the diluted (1:10) soil extracts was analyzed. The amount of DON in soil was determined by subtracting the mineral N content (sum of extractable NH_4^+ and NO_3^- content) from dissolved total N content. Total organic carbon (TOC) content was determined by combustion (at 840°C) using the Leco CR-12 Carbon Analyzer (LECO Corporation, St. Joseph, MI) (Wang and Anderson, 1998). Soil total N (% mass) was determined by dry combustion using a Leco CNS-2000 elemental analyzer (Wright and Bailey, 2001). For Truelove Lowland and Simpson Lake samples, a soil standard of known concentration (5.17% C, 0.441% N) was used to calibrate the instrument. However, a plant standard of known concentration (42.9% C, 2.59% N) was used for Ross Point soils because of high soil organic matter content. For each soil sample, 0.2 g of air-dried, ball-ground sub-sample was analyzed. Soil particle size characterization was performed using the Horiba model LA-950 Laser scattering particle size distribution analyzer (Horiba Instrument, Irvine, California, USA). Analyses were performed on 0.3 g of air-dried, ball-ground, sieved (<2 mm) soils.

Prior to one-way ANOVA and geostatistical analyses, normality and homoscedasticity of the variables were checked using Anderson-Darling and Levene's test in Minitab 11 (Minitab

Inc. Pennsylvania, USA). The degree of spatial heterogeneity was assessed by semivariance analysis (Goovaerts, 1998) in GS+ version 9.0 (Gamma Design Software, Plainwell, Michigan, USA).

3.4 Results and discussion

Arctic soils are historically known to be N-limited (Chapin et al., 1993). Therefore, it is not surprising that the extractable NH_4^+ and NO_3^- content found at Truelove Lowland and Simpson Lake are significantly lower than agricultural (Robertson et al., 1997) and grassland (Jackson and Caldwell, 1993) soils (Table 3.1). However, these two sites were dominated by mineral soils, whereas Ross Point had a much higher organic matter content (Organic Cryosols), which explains the difference in DOC, DON, and TOC contents. In Arctic ecosystems, DOC and DON pools have been found to play a significant role in soil ecological processes (Bardgett et al., 2007; Buckeridge et al., 2010). Soil DOC and DON pools and fluxes offer substrates for microbial population and serve as a link between terrestrial and aquatic ecosystems (Wickland et al., 2007). There have been few studies examining spatial variability of DOC and DON content. Rover and Kaiser (1999) found that DOC content in agricultural soils has moderate spatial dependency (16-44%) and similarly Rodriguez et al. (2009) reported moderate spatial dependency (46-62%) for DON in forest soils. However, our study demonstrates that DOC and DON content in Arctic soils are highly spatially dependent (61-99%) (Table 3.2). The spatial range of Arctic soil physicochemical attributes was considerably smaller than other ecosystems (Cerri et al., 2004; Ruth and Lennartz, 2008). Moreover, the spatial range of soil properties was comparatively larger in Organic Cryosols than Static or Turbic ones (Fig. 3.2). It should be noted that Organic Cryosols are more homogeneous with similar vegetation type and moisture

Table 3.1. Mean values[§] of soil physicochemical attributes at three sites and Pearson correlation between soil moisture content (θ_g) and other soil attributes

Soil attributes	Truelove Lowland	Simpson Lake	Ross Point
θ_g	24.7 (1.66)[#]	20.4 (1.36)	60.6 (5.62)
pH	7.54 (0.013)	5.54 (0.029)	7.57 (0.039)
NO_3^- ($\mu g\ g^{-1}$ dry soil)	0.889 (0.048)	1.38 (0.126)	4.49 (0.667)
NH_4^+ ($\mu g\ g^{-1}$ dry soil)	0.744 (0.046)	0.348 (0.024)	5.28 (0.452)
DOC ($\mu g\ g^{-1}$ dry soil)	7.45 (0.242)	381 (41.6)	1887 (159)
DON ($\mu g\ g^{-1}$ dry soil)	1.61 (0.137)	53.7 (4.51)	187.1 (18.3)
TOC (% weight)	3.32 (0.270)	1.25 (0.157)	15.8 (1.12)
TIC (% weight)	2.15 (0.121)	0.221 (0.014)	1.29 (0.142)
TN (% weight)	0.370 (0.023)	0.099 (0.012)	1.068 (0.068)
C:N	15.4 (0.430)	14.6 (0.669)	16.1 (0.867)
Sand	90.8 (0.525)	51.2 (1.42)	86.0 (1.05)
Silt	7.95 (0.397)	37.2 (0.895)	13.2 (0.883)
Clay	1.20 (0.140)	11.5 (0688)	0.666 (0.229)

Correlation between θ_g and other soil attributes.

pH	-0.247*	0.013	-0.753**
NO_3^- ($\mu g\ g^{-1}$ dry soil)	0.713**	0.207*	0.225*
NH_4^+ ($\mu g\ g^{-1}$ dry soil)	0.765**	0.583**	0.790**
DOC ($\mu g\ g^{-1}$ dry soil)	0.819**	0.777**	0.906**
DON ($\mu g\ g^{-1}$ dry soil)	0.402**	0.791**	0.930**
TOC (% weight)	0.843**	0.755**	0.923**
TIC (% weight)	0.053	-0.042	-0.191
TN (% weight)	0.920**	0.554**	0.806**
C:N	-0.414**	-0.277**	-0.240*
Sand	-0.251*	0.064	0.435**
Silt	-0.276**	-0.061	-0.448**
Clay	0.163	-0.049	-0.248*

[§]Mean values (n=93) of different variables for three sites.
[#]Standard errors are shown in parentheses.
** and * indicate the relationships are significant at P<0.01 and P<0.05 respectively

content, and fewer hummocks than Static or Turbic Cryosols. Therefore, Organic Cryosols are spatially more consistent and their spatial nature and patterns extend over larger distance. For researchers interested in obtaining independent samples, the recommended minimum distance between samples is 45 m in Organic Cryosols and 10 m in Static or Turbic Cryosols.

Table 3.2. Spatial parameters of various soil properties

	Truelove Lowland				**Simpson Lake**				**Ross Point**			
	Range (m)[§]	SPD[#]	Model*	r^2	Range (m)	SPD	Model	r^2	Range (m)	SPD	Model	r^2
θ_g	1.68	0.902	Sph	0.600	0.77	0.595	Sph	0.135	1.57	0.821	Gau	0.958
pH	0.42	0.819	Exp	0.438	0.848	0.885	Exp	0.520	15.00	0.903	Exp	0.906
NO_3^-	7.96	0.732	Gau	0.728	0.75	0.999	Sph	0.490	NS**	NS	NS	NS
NH_4^+	6.58	0.829	Gau	0.689	0.77	0.698	Sph	0.197	27.60	0.500	Exp	0.706
DOC	7.44	0.712	Gau	0.813	0.625	0.999	Gau	0.339	42.60	0.684	Sph	0.728
DON	8.48	0.611	Gau	0.466	0.78	0.999	Sph	0.312	42.00	0.711	Sph	0.783
TOC	7.96	0.758	Gau	0.817	NS	NS	NS	NS	41.2	0.587	Sph	0.727
TIC	NS	NS	NS	NS	1.52	0.999	Gau	0.155	NS	NS	NS	NS
TN	8.31	0.730	Gau	0.798	9.03	0.870	Sph	0.788	6.70	0.756	Gau	0.956
C:N	10.56	0.818	Gau	0.972	0.76	0.998	Gau	0.797	70.66	0.967	Gau	0.977
Sand	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Silt	NS	NS	NS	NS	0.77	0.616	Sph	0.208	8.00	0.872	Sph	0.961
Clay	9.00	0.500	Sph	0.467	NS	NS	NS	NS	26.7	0.999	Sph	0.993
Spatial relationships with θ_g												
x pH	ND[¶]	ND	ND	ND	ND	ND	ND	ND	3.16	0.901	Gau	0.982
x NO_3^-	1.63	0.890	SPh	0.284	ND	ND	ND	ND	ND	ND	ND	ND
x NH_4^+	1.58	0.989	Sph	0.570	ND	ND	ND	ND	0.917	0.998	Gau	0.323
x DOC	1.65	0.997	Sph	0.497	0.78	0.999	Sph	0.199	1.35	0.841	Gau	0.975
x DON	ND	ND	ND	ND	0.72	0.999	Sph	0.278	2.06	0.965	Sph	0.977
x TOC	1.60	0.999	Sph	0.432	0.62	0.936	Sph	0.205	1.43	0.784	Gau	0.988
x TN	1.61	0.947	Sph	0.416	ND	ND	ND	ND	3.55	0.699	Gau	0.814

[§]Range indicates the zone of spatial dependence.
[#]SPD- Spatial dependency, calculated as $C/(C+C_0)$
*Model: Exp- Exponential; Gau- Gaussian; Sph- Spherical
**NS- Not significant ($r^2 <0.1$)
[¶]ND- Not determined
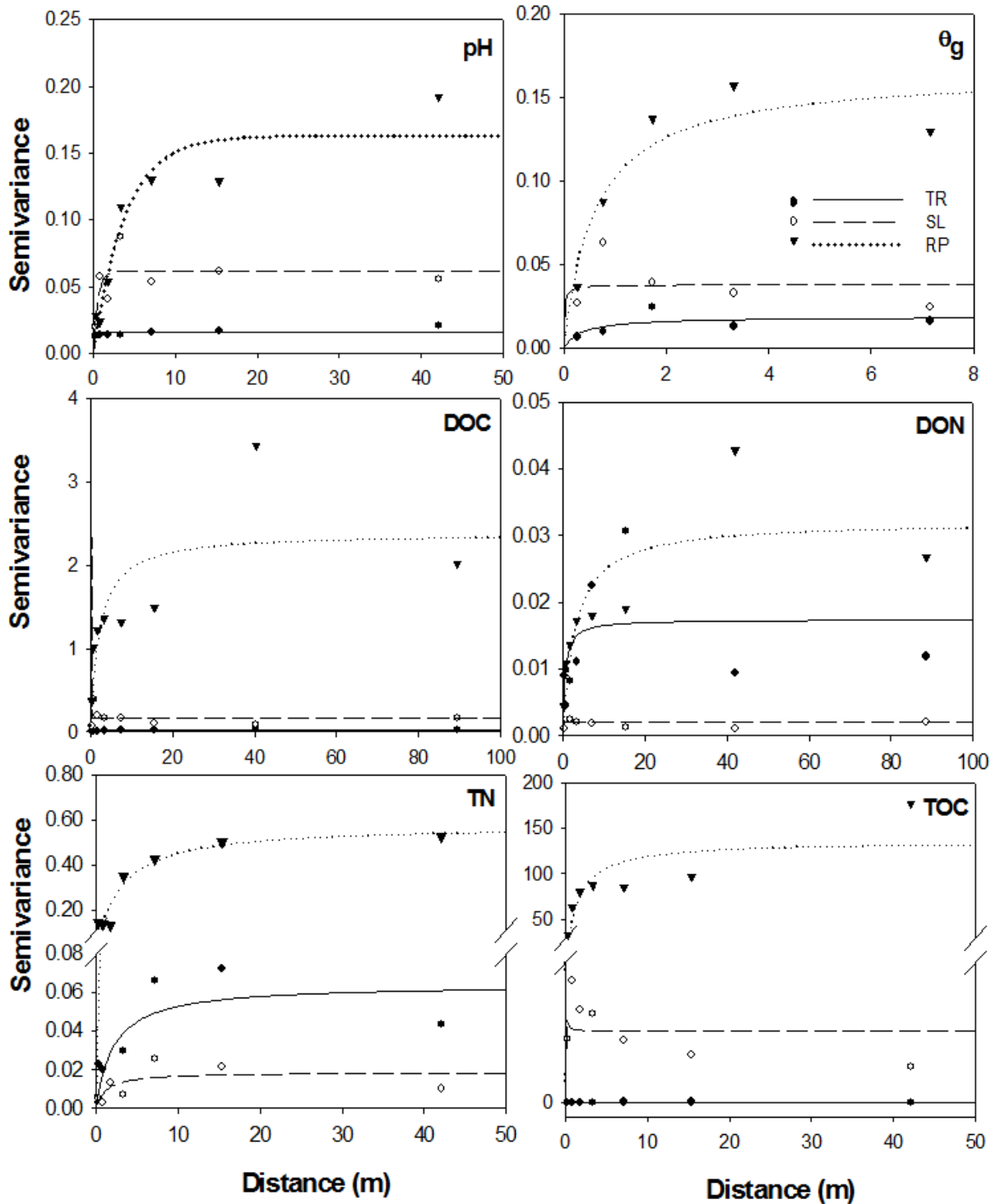
Figure 3.2. Experimental semivariograms of selected soil pH, gravimetric moisture content (θ_g), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), total nitrogen (TN), and total organic carbon (TOC) at three Arctic ecosystems. Filled circle (●) with solid line represents Truelove Lowland (TR), blank circle (○) with long dashed line represents Simpson Lake (SL), and inverted triangle (▼) with dotted line represents Ross Point (RP) site.

Soil moisture content plays a central role in regulating soluble nutrient pools, and structure and composition of microbial and plant communities (Bardgett et al., 2007; Chen et al., 2006). Soil moisture content is known to be highly variable over short temporal scale in most ecosystems, including the high Arctic (Bardgett et al., 2007); however, the impact of θ_g on microbial communities and nutrient pool is functionally stable (Wagner, 2008). Thus, strong correlations between θ_g and soil properties can be seen even when the θ_g is temporally variable. The spatial variability of θ_g affects and is affected by vegetation and physiography (Petrone et al., 2004). The role of θ_g in Arctic regions is particularly significant because of the short-term active layer dynamics and long-term permafrost dynamics and this role is further emphasized by the uptake of dissolved N by high Arctic plants (Chapin et al., 1993). It was found θ_g was correlated to other soil attributes at all three sites (Table 3.1). The correlation between θ_g and TOC found in this study is similar to tropical forest soil (r =0.86; Wang et al., 2002) but higher than that of temperate agricultural soil (r =0.44; Yanai et al., 2005), temperate grassland soil (r =0.73; Zhao et al., 2007), or tropical grassland soil (r =0.32; Jackson and Caldwell, 1993). Similarly, the correlation between θ_g and pH, DOC, DON, extractable NO_3^- and NH_4^+ contents in Cryosols is considerably higher than temperate agricultural (Rover and Kaiser, 1999) and tropical grassland soils which reconfirms the critical role of soil moisture in high Arctic ecosystems (Bardgett et al., 2007).

In summary, this study found that despite the climatic uniqueness of Arctic ecosystems, soil properties displayed strong spatial dependency. In general, the observed range of spatial dependency was smaller than non-Arctic ecosystems, which may be attributed to various periglacial features of Arctic ecosystems such as cryoturbation, gelifluction, and thermokarst.

Due to increasing concern of the impact of climate change on Arctic ecosystems, Arctic research is going through a phase of rapid development and it will most likely continue. The spatial variability information found in this study would be helpful to Arctic soil researchers when designing sampling schemes for Arctic soils.

4.0   EVIDENCE OF HIGH MICROBIAL ABUNDANCE AND SPATIAL DEPENDENCY IN THREE ARCTIC SOIL ECOSYSTEMS[§]

Preface

Chapter 3 presented a comprehensive account of soil spatial dependency and strong correlations among moisture content and other soil resources in Canadian Arctic. However, soil properties are the predominant factors shaping the distribution patterns of soil microbial communities such as archaea, bacteria, and fungi. Due to spatial variability of soil resources microbial communities may also exhibit spatial patterns. Although microbial dependency has been reported for tropical, temperate and alpine ecosystems, no information is available from Arctic ecosystems. This chapter assesses the abundance and spatial dependency of archaeal, bacterial, and fungal ribosomal gene copies in three Arctic ecosystems and identifies the soil properties that drive their spatial patterns.

4.1 Abstract

Microbial spatial heterogeneity has significant implications for ecological processes. Although microbial spatial patterns have been investigated agricultural and pristine soil ecosystems, little information is available on microbial spatial scaling in Arctic soils and how it is correlated with soil resources. This comprehensive study assessed microbial abundance in 279 soil samples collected from three Canadian Arctic ecosystems and elucidated microbial spatial heterogeneity from fine (10 cm) to large (300 m) scales. Our results demonstrate that the abundance of archaeal 16S rRNA, bacterial 16S rRNA, and fungal 18S rRNA ITS gene copies (10^8-10^{11}, 10^8-10^{10}, and 10^8-10^{10} per gram of dry soil respectively) in Arctic soils is similar to agricultural and pristine soils. Microbial spatial distribution is well structured in Arctic soils and shows high spatial dependency (0.50-0.99) at the scale of measurement of the experiment. The spatial range of microbial distribution is regularly less than 3 m, which may be attributed to the periglacial processes such as thermokarst, cryoturbation, and gelifluction. Microbial spatial distribution is significantly ($P<0.01$) correlated to soil moisture, pH, dissolved organic carbon, dissolved organic N, total organic carbon, and total N content; however, the correlation is more consistent in Organic Cryosols than Static or Turbic Cryosols.

4.2 Introduction

Permafrost-affected soils encompass about 26% of world's and 40% of Canada's total land area (Bockheim and Tarnocai, 1998). Extremely low temperature, long winter, short growing season, and regular cryoturbation typify the uniqueness of these ecosystems. Similar to other ecosystems (Heijden et al., 2008), soil microbial communities play a key role in Arctic soils. Soils in Canadian Arctic ecosystems are predominantly Static, Turbic, and Organic Cryosols. Microbial communities and their associations with soil properties may vary in different

types of Cryosolic ecosystems. Spatial variability is an integral quality of soil properties and in many cases this variability is non-random (Goovaerts, 1997). Soil spatial heterogeneity results from variations in pedologic and geologic soil forming factors, soil physicochemical and ecological processes (Ettema and Wardle, 2002). Spatial dependency of soil properties diminishes with increasing distance between points in space. Owing to the inherent variability of soil physicochemical properties soil microbial abundance may also vary across multiple spatial scales. The awareness of microbial spatial variability has increased considerably in the last 10 years. Microbial spatial patterns have been studied in agricultural (Franklin and Mills, 2003; Grundmann and Delbouzie, 2000; Nunan et al., 2002), forest (Morris, 1999; Saetre and Baath, 2000), and grassland (Nicol et al., 2003; Ritz et al., 2004) soils. However, to our knowledge, no study has elucidated microbial spatial variability in high Arctic ecosystems. Microbial spatial structure has significant functional implications for ecosystem processes. Microbially driven ecosystem functions are exhibited at multiple spatial scales and understanding of microbial spatial distribution/structure is critical to substantiate how combinations of several communities or microhabitats function together at field scale or larger scale pertinent to researchers (Franklin and Mills, 2007). Our objective in this study was to examine the overall abundance and spatial dependency of archaeal, bacterial, and fungal populations in various types of Cryosols in Canadian Arctic ecosystems.

4.3 Materials and methods

Three Arctic sites were selected for this study: Truelove Lowland, Simpson Lake, and Ross Point. Covering an area of 43 km^2, the Truelove Lowland (75°40′ N, 84°35′ W) is located on the north-eastern coast of Devon Island; it has been the subject of extensive historical scientific expeditions and the topography and soil types are well documented (Lev and King,

1999; Pare and Bedard-Haughn, 2011; Siciliano et al., 2009). Simpson Lake (68°35′ N, 91°57′ W) is situated in the middle of the Boothia Peninsula; the upperslope positions are likely comprised of Static Cryosols whereas lowerslope positions are likely Turbic Cryosols. Ross Point (68°31′ N, 111°10′ W) site is situated in the south port of Victoria Island, the second largest island in the Canadian Arctic Archipelago. The soils at this research site are predominantly Organic Cryosols. The three research sites selected in this study encompass 7° in latitude and 27° in longitude of Canadian Arctic. Furthermore, the sites consist of a polar oasis (Truelove Lowland), an Organic Cryosolic ecosystem (Ross Point) and a Mid-Arctic Static/Turbic Cryosolic ecosystem (Simpson Lake), and thus represent the diversity of Canadian permafrost ecosystem. At each site, soil samples were collected along three parallel transects (300 m each; 2 m lateral distance) at 31 points (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300 m). For spatial comparison, it was aimed to use the same design at three research sites. A 300 m x 300 m grid design was not possible to establish at Truelove Lowland. Moreover, fine scale patterns may not be captured with a grid design due to its even lag distance (minimum 2 m or 4 m). Variable (irregular) sampling design, adjacent steps separated by a repeated sequence, is a particularly useful design for substantiating multiscale patterns in an ecosystem (Fortin et al., 1989). Therefore, this study specifically employed the aforementioned variable-lag-distance transect approach to simultaneously capture the fine (0-1 m), medium (1-10 m), and large (10-300 m) scale spatial patterns of microbial communities in three Cryosolic ecosystems. In total 93 soil samples were collected from each site and 279 soil samples were collected from the three sites. At each sampling point, approximately 250 g of soil sample was collected.

Soil gravimetric water content (θ_g), pH, total organic carbon (TOC), dissolved organic carbon (DOC), extractable NH_4^+ and NO_3^-, dissolved organic nitrogen (DON), and total N (TN) contents were measured as described in Chapter 3.

Extraction of DNA from soils was performed according to the method described by Griffiths et al. (2000) with the modification that DNA samples were precipitated in polyethylene glycol overnight and RNase was not added after extraction. However, no RNA contamination was found when DNA samples were examined using agarose gel electrophoresis and RNase treatment. The variability in DNA extraction efficiency was also measured as it may vary with soil organic matter content. DNA extraction variability was not considerable. The number of bacterial 16S rRNA, archaeal 16S rRNA, and fungal 18S rRNA gene copies present in the soil DNA extracts were determined by quantitative real-time PCR using the QuantiTect™ SYBR® Green PCR Master Mix, an ABI 7500 real-time polymerase chain reaction machine (Applied Biosystems, Foster City, CA), and bacterial (Ovreas et al., 1997), archaeal (Coolen et al., 2004), and fungal (Gardes and Bruns, 1993) specific primers. Each 20 µl reaction contained 10 µl of master mix, 10 pmol of the primers, 6 µl sterilized mili-Q water, and 2 µl (20-50 ng) template DNA (1:10 diluted). Standard curves ($r^2 > 0.99$) were generated by preparing standards from purified PCR product from one of the soil DNA extracts. The amplification efficiency of the genes was between 80% and 100%. The specificity of the amplified products was examined by melting curve analysis. Amplification inhibition effects were evaluated by measuring gene abundance on three different dilutions of representative samples and selecting the dilution that minimized the inhibition (Dumonceaux et al., 2006).

The Anderson-Darling test and Levene's test were performed using Minitab 11 (Minitab Inc. State College, PA) to check for normal distribution and homoscedasticity. Non-normally

distributed variables were log or square-root transformed. Spearman rank correlations were calculated using SPSS 16.0 software (SPSS Inc. Chicago, IL). The degree of spatial heterogeneity was assessed by semivariance analysis. Semivariance (Equation 4.1), $\gamma(h)$, can be defined as half of the average squared difference between the components of a data pair:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{\alpha=1}^{N(h)} [z(x_k) - z(x_k + h)]^2 \qquad (4.1)$$

where $z(x_k)$ is the property, $z(x_k+h)$ is the value at 'h' lag distance and $N(h)$ is the number of data pairs for a given distance (Goovaerts, 1997). The key features of a semivariogram are nugget variance, sill, and range. Nugget variance (the intercept of the semivariance at $h=0$) indicates the stochastic variation resulted from experimental error or variation at scales smaller than the minimum sampling lag distance. Sill is the maximum variability attained by a variable whereas range indicates the zone of SPD (i.e. the lag distance at which the semivariance value reaches maximum). The SPD indicates what proportion of the total variability is spatially dependent and it was calculated by: $C/(C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C+C_0$ is the sill. The value of SPD is unit-less and ranges between 0 indicating no spatial dependency and 1 indicating highest spatial dependency with no nugget variance. Each lag class comprised of a minimum of 30 pairs of comparisons as recommended by Journel and Hujbregts (1978). Various models such as spherical, exponential, Gaussian and linear were fitted to the raw semivariograms and the best-fitted model was selected on the basis of lowest residual sum of squares and highest coefficient of determination (Goovaerts, 1997). Geostatistical analyses were performed using GS+ version 9.0 (Gamma Design Software, Plainwell, MI).

4.4 Results and discussion

Microbial communities in permafrost ecosystems are confronted with extreme environmental conditions such as prolonged sub-zero temperature, freeze-thaw cycles,

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desiccation and starvation (Wagner, 2008). Despite these conditions, the overall size of bacterial, archaeal, and fungal populations in these Arctic soil ecosystems (Table 4.1) resembles that of grassland (10^9-10^{11}; Jenkins et al., 2009), agricultural (10^9-10^{10}; He et al., 2007), and forest (10^8-10^9; Kemnitz et al., 2007) soils. The results of this study are also in line with Stress et al. (2010) who noted high (10^8) prevalence of bacterial 16S rRNA gene copies in alpine soils. In the active layer of Canadian High Arctic permafrost ecosystems, however, Yergeau et al. (2010) and Wilhem et al. (2011) reported relatively low abundance of fungal (8.60×10^4) and archaeal (1.49×10^4 and 3.68×10^4 respectively) ribosomal gene copies. Although, the reported bacterial 16S rRNA gene abundance (3.05×10^7 and 3.81×10^7 respectively) is somewhat similar to this study,

Table 4.1. Overall microbial abundance (copy number g^{-1} dry soil) and soil attributes at three Arctic ecosystems: Truelove Lowland, Simpson Lake, and Ross Point.

Soil and microbial attributes	Truelove Lowland[§]	Simpson Lake	Ross Point
Bacterial 16S rRNA	$2.9 \times 10^{10} (2.8 \times 10^9)$	$4.2 \times 10^8 (7.1 \times 10^7)$	$1.5 \times 10^9 (2.5 \times 10^8)$
Archaeal 16S rRNA	$2.7 \times 10^{11} (1.8 \times 10^{10})$	$5.5 \times 10^8 (1.0 \times 10^8)$	$7.9 \times 10^8 (1.4 \times 10^8)$
Fungal 18S rRNA ITS	$5.9 \times 10^{10} (8.5 \times 10^8)$	$2.2 \times 10^8 (4.6 \times 10^7)$	$7.7 \times 10^8 (1.5 \times 10^8)$
θ_g	25 (1.7)	20 (1.4)	61 (5.6)
pH	7.5 (0.013)	5.5 (0.029)	7.6 (0.040)
DOC (µg g^{-1} dry soil)	7.4 (0.24)	380 (42)	1900 (160)
DON (µg g^{-1} dry soil)	1.6 (0.14)	54 (4.5)	190 (18)
TOC (% weight)	3.3 (0.27)	1.2 (0.16)	16 (1.1)
TN (% weight)	0.37 (0.023)	0.099 (0.012)	1.1 (0.068)

[§]Mean values (n=93) of different variables for three sites. Standard errors are shown in parentheses.

the overall microbial abundance is considerably higher at the three Arctic ecosystems studied here. In particular, Truelove Lowland had considerably larger microbial populations than the other two sites. It should be noted that Truelove Lowland is a polar oasis in the middle of a vast polar desert. It is an animal-hotspot thus grazing, accumulation of fecal matter, and dead animal

body parts may favor microbial activity making this site ecologically dynamic and unique. Important factors such as snow cover, temperature, wind speed, and most importantly water and nutrient availability vary along the mesotopographic gradients in Arctic ecosystems (Billings, 1973; Giblin et al., 1991). At Truelove Lowland, transects for soil sampling were laid out on backslope (area behind raised beach crest) and snow accumulation is considerably higher in backslope area due to wind protection (Giblin et al., 1991). Presence of snowbanks in this area may have caused higher microbial abundance. This is in agreement with Zinger et al. (2009) who found high microbial diversity owing to variation in topography and differential snow cover.

The value of SPD and range indicates the degree and distance of spatial autocorrelation of an attribute respectively. With the exception of bacterial 16S abundance at Simpson Lake, microbial abundances were spatially autocorrelated and demonstrated strong spatial structures (Fig. 4.1). The value of SPD between 0.5 and 1 indicates high spatial dependency and autocorrelation. The high SPD (0.50-0.99) of microbial communities in Cryosols is comparable to grassland (0.33-0.85; Ritz et al., 2004), agricultural (0.42-1.0; Philippot et al., 2009), and forest (0.41-0.93; Saetre and Baath, 2000) soils. The r^2 value of a semivariogram reflects how clearly the experimental model incorporates the spatial variability and demarcates it from the stochastic nugget variability to show the spatial structure. The r^2 values of the semivariograms ranged between 0.49-0.99, indicating high fit. The Gaussian model was the best to the semivariograms in this study. The semivariance in Gaussian models reaches the sill asymptotically (Goovaerts, 1997) which indicates a comparatively smoother transition or gradient of the spatial patterns. Ecologically, this implies that the niches of Cryosolic microbial communities smoothly merge into one another as the soil properties change across the landscape. Range of a semivariogram indicates the distance up to which spatial autocorrelation of a particular attribute persists. Thus,
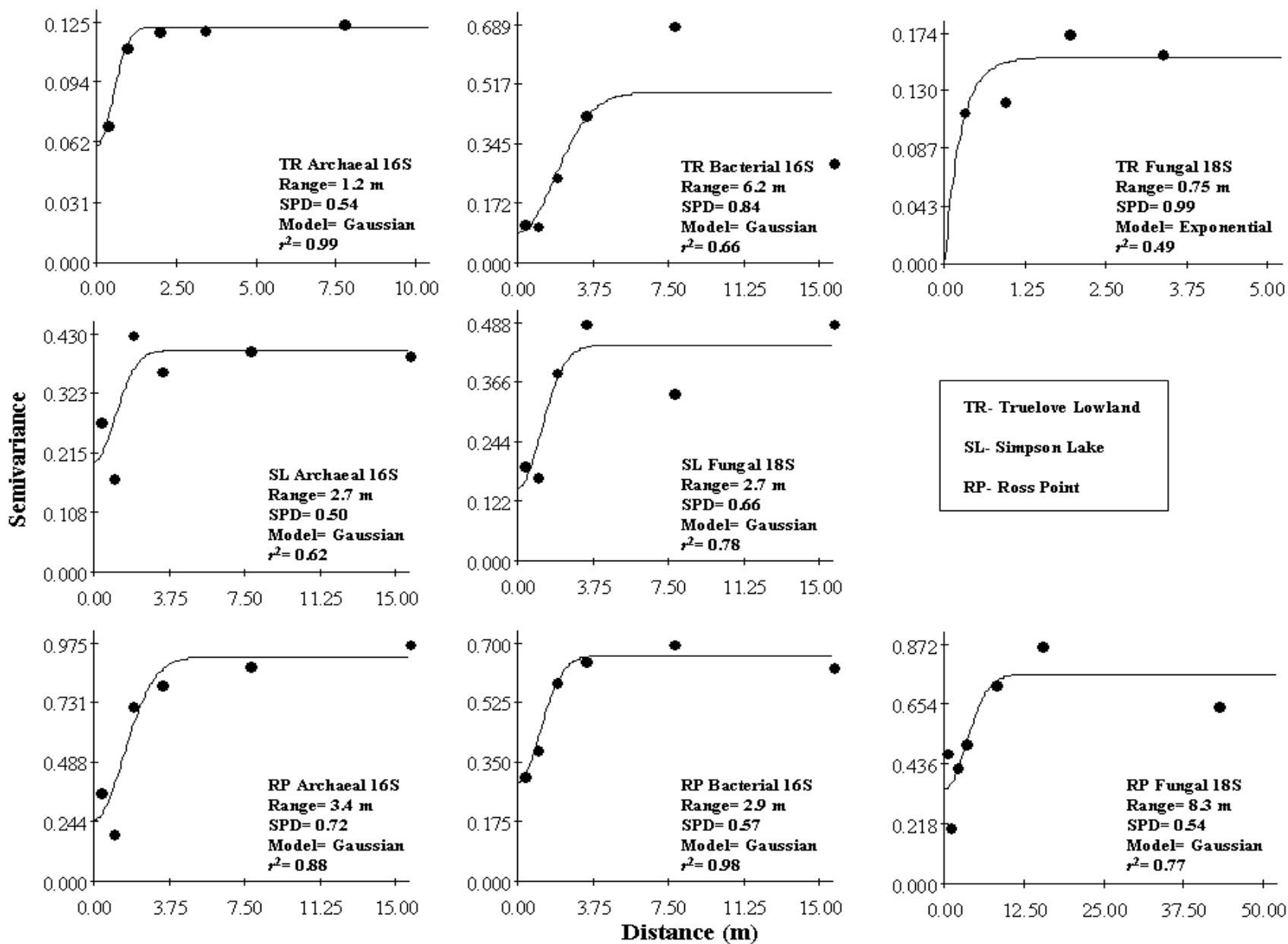
Figure 4.1. Semivariograms showing spatial variability of archaeal 16S rRNA, bacterial 16S rRNA, and fungal 18S rRNA ITS gene abundance at three Arctic ecosystems: Truelove Lowland (TR), Simpson Lake (SL), and Ross Point (RP). Range indicates the zone of spatial dependency. Spatial dependency (SPD) was calculated by: $C / (C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C+C_0$ is the sill. Various models (Gaussian, exponential etc.) were fitted (solid line) to the experimental semivariograms. Spatial dependency was considered from fine (10 cm) to large scale (300 m) but no dependency was found beyond 10 m distance. The semivariograms are shown up to specific lag distance for clarity of the spatial patterns near origin. The abundance of bacterial 16S rRNA genes was spatially independent at Simpson Lake and thus, it was not shown in the figure.

longer range reflects strong spatial autocorrelation and homogeneity in soil resources. The degree and range of microbial spatial dependency are intrinsically associated with soil physicochemical attributes and soil types (Bragato and Primavera, 1998). Banerjee et al. (2011) showed that soil physicochemical properties at Truelove Lowland and Simpson Lake are spatially well structured but the spatial range of soil physical properties is considerably smaller than Organic Cryosols of Ross Point. While the spatial range at Truelove Lowland is approximately 10 m, spatial autocorrelation diminishes beyond 1 m for most soil attributes at Simpson Lake. On the other hand, the zone of spatial dependence of soil attributes is approximately 40 m in Organic Cryosols of Ross Point. In contrast to Truelove and Simpson Lake, the Organic Cryosols at Ross Point were more homogeneous (i.e. devoid of periglacial processes such as cryoturbation, gelifluction, frost stirring, and mounding). Since these periglacial processes create irregular soil horizon, movement of soil mass, and redistribution of organic matter (Bockheim and Tarnocai, 1998), they change the spatial nature of soil properties creating high spatial heterogeneity. Thus the range of spatial autocorrelation at Ross Point is considerably larger than the other two sites.

In a low Arctic tundra landscape, Stewart et al. (2011a; 2011b) noted the importance of soil moisture, nutrient availability, and microclimate conditions in structuring the small-scale spatial variation of N fixation by cyanobacteria. Using semivariogram analysis in an alpine ecosystem, King et al. (2010) found high spatial autocorrelation of bacterial abundance and community composition. Similar results were also observed by Zinger et al. (2009) who reported high spatial variation of bacterial and fungal communities in alpine ecosystems and highlighted the importance of snow cover, which is also congruent with the present study. In a series of alpine landscapes, Zinger et al. (2011) recently reported that non-random spatial patterns of

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crenarchaeal, bacterial, and fungal communities are strongly associated with soil variables and plant community composition. In this study, consistently high correlations ($P<0.01$) were observed between the tested soil attributes and microbial abundance at all three sites with Spearman rank correlations as high as 0.93 (Table 4.2 a and b). The importance of soil pH in influencing microbial communities and ecological processes is well recognized in non-Arctic soils (Cuhel et al., 2010). Chu et al. (2010) noted that soil pH is a predominant factor structuring bacterial abundance and community composition in Arctic soil ecosystems. Similarly, in alpine ecosystems, soil pH is one of the key factors shaping bacterial communities (King et al., 2010;

Table 4.2. Associations between soil resources and the abundance of archaeal 16S rRNA, bacterial 16S rRNA and fungal 18S rRNA ITS at three Arctic ecosystems: Truelove Lowland (TR), Simpson Lake (SL), and Ross Point (RP).

a)

Abundance	θ_g			pH			DOC		
	TR	SL	RP	TR	SL	RP	TR	SL	RP
Archaea	NS[§]	NS	0.85**	-0.38**	NS	-0.77**	NS	NS	0.81**
Bacteria	NS	0.52**	0.34**	NS	NS	-0.26**	0.28**	0.60**	0.25*
Fungi	NS	0.38**	0.48**	-0.27*	NS	-0.44**	NS	0.31**	0.42**

θ_g - Soil gravimetric water content; DOC - Dissolved organic carbon
[§]NS-non-significant; * and ** indicate correlations significant at $P<0.05$ and $P<0.01$ respectively

b)

Abundance	DON			TOC			TN		
	TR	SL	RP	TR	SL	RP	TR	SL	RP
Archaea	0.22*	NS[§]	0.84**	NS	NS	0.93**	NS	NS	0.71**
Bacteria	NS	0.63**	0.30**	NS	0.48**	0.29**	NS	0.43**	NS
Fungi	NS	0.36**	0.44**	NS	0.25*	0.49**	NS	0.24*	0.35**

DON - dissolved organic nitrogen; TOC - total organic carbon; TN- total nitrogen
[§]NS-non-significant; * and ** indicate correlations significant at $P<0.05$ and $P<0.01$ respectively

Zinger et al., 2011). The present study is in agreement with this findings and it further extends the notion to report not only bacterial but archaeal and fungal abundance are also significantly correlated to soil pH. Nonetheless, Chu et al. (2010) did not find any significant correlations between bacterial communities and soil moisture, DOC, DON, and soil carbon content. Typically soil moisture is highly correlated with other soil attributes and microbial communities in Arctic ecosystems (Banerjee et al., 2011; Bardgett et al., 2007). Similarly in this study, consistently high correlations were found between soil moisture and microbial abundance at Simpson Lake and Ross Point. Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) pools in soil act as a conduit between aquatic and terrestrial ecosystems and play a critical role by offering substrates for microbial populations (Wickland et al., 2007). The importance of DOC and DON for soil ecological processes of Arctic ecosystems is well recognized with reports showing significant correlations between dissolved organic matter and microbial abundance and processes (Buckeridge et al., 2010; Chu and Grogan, 2010). This study also found strong significant correlations (r=0.25-0.84; P<0.01) between microbial prevalence and DOC and DON content. Significant association between microbial abundance and carbon content observed in this study is in line with Stres et al. (2010) who found that soil carbon alone can explain more than 70% variability in microbial abundance patterns in an alpine ecosystem.

The correlation pattern, i.e., the strength of associations between microbial parameters and soil resources differed considerably between three habitats. Correlations observed for the homogenous Organic Cryosols at Ross Point were comparatively higher than Simpson Lake and Truelove. For example, soil archaeal populations at Simpson Lake showed no correlation with the tested soil properties; on the other hand, at Ross Point, the relationships between archaeal population and soil properties were comparatively stronger (>0.7) than bacterial populations. The

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aforementioned patterns can be attributed to different regulating factors and differential niche selection of bacteria and archaea in soil (Schleper, 2010). Nonetheless, relatively few significant correlations were found at Truelove Lowland. As soil spatial dependency operates at small scales at Truelove Lowland, it is possible that microbial communities and soil properties may be correlated at finer scale, i.e. <0.2 m, that our transect design was unable to capture. Owing to the periglacial processes, permafrost soils experience pronounced small-scale variation (change in organic and inorganic compounds, microtopography, and microclimate), which thereby influences the abundance, diversity and distribution of microbial communities (Wagner, 2008). For example, in sites with large scale physical processes, the relationships between bacterial and archaeal abundance and tested soil attributes were highly consistent (i.e. Ross Point) compared to sites with finer scale physical processes (Truelove Lowland and Simpson Lake) so the spatial dependency of these microbial communities was linked to the scale of physical processes occurring at each site.

Overall this study showed consistently high abundance of archaeal, bacterial, and fungal gene copies across 7° in latitude and 27° in longitude of the Canadian Arctic. Microbial communities are spatially well structured and the zone of spatial dependency varies between the types of Cryosols with the Organic Cryosols of Ross Point having a much larger spatial dependency compared to the two Static/Turbic Cryosolic ecosystems. However, in spite of the high microbial abundance found in the present study microbial diversity may be low, which may underestimate the spatial heterogeneity. Future studies will assess spatial patterns of microbial diversity and will determine if this difference in spatial dependency holds across multiple sites.

5.0   FACTORS DRIVING POTENTIAL AMMONIA OXIDATION IN CANADIAN ARCTIC ECOSYSTEMS: DOES SPATIAL SCALE MATTER?[§]

Preface

Soil ammonia oxidation plays a pivotal role in Arctic environments confronted with paucity of nutrients, particularly N. Chapter 4 reported high spatial dependency of soil microbial populations and their strong correlations with edaphic factors. Thus, ammonia oxidation and ammonia oxidizing functional groups may also be spatially structured and driven by soil resources at multiple spatial scales. The aforementioned parameters are largely unexplored in Arctic soils and to our knowledge no study has assessed heterotrophic nitrification in Arctic soil ecosystems. This chapter examines heterotrophic and autotrophic ammonia oxidation potential and ammonia oxidizing communities in 279 soil samples and substantiates their spatial linkage with soil attributes at different spatial scales.

5.1 Abstract

Ammonia oxidation is a major process in N cycling and it plays a key role in N limited soil-ecosystems such as those in the Arctic. While mm-scale spatial dependency of ammonia oxidizers has been investigated, little is known about the field-scale spatial dependency of aerobic ammonia oxidation processes and ammonia-oxidizing archaeal and bacterial communities, particularly in Arctic soils. The purpose of this study was to explore the drivers of ammonia oxidation at the field scale in Cryosols (soils with permafrost within 1 m of the surface). This study measured aerobic ammonia oxidation potential (both autotrophic and heterotrophic) and functional gene abundance (bacterial *amoA* and archaeal *amoA*) in 279 soil samples collected from three Arctic ecosystems. The variability associated with quantifying genes was substantially less than spatial variability observed in these soils suggesting that molecular methods can be used reliably evaluate spatial dependency in Arctic ecosystems. Ammonia-oxidizing archaeal and bacterial communities and aerobic ammonia oxidation were spatially autocorrelated. Gene abundances were spatially structured within 4 m whereas biochemical processes were structured within 40 m. Ammonia oxidation was driven at small scales (<1m) by moisture and total organic C whereas gene abundance and other edaphic factors drove ammonia oxidation at medium (1-10 m) and large (10-100 m) scales. In these Arctic soils heterotrophs contributed between 29 and 47% of total ammonia oxidation potential. The spatial scale for aerobic ammonia oxidation genes differed from potential ammonia oxidation, suggesting that in Arctic ecosystems edaphic, rather than genetic, factors are an important control on ammonia oxidation.

5.2 Introduction

Approximately 13% of world's total land area is underlain by permafrost and in Canada permafrost-affected soils comprise 3.7 million km^2 or about 40% of the land mass (67). These soil ecosystems differ from others due to their low annual mean temperatures, long winters, short growing seasons, frequent cryoturbation (soil movement because of frost action) and gelifluction (slow downslope movement of waterlogged soil over permafrost layer and formation of lobe shaped features). A key feature of these Arctic ecosystems compared to temperate ecosystems is that substantial ammonia (as much as 14 mg m^{-2} NH_4^+-N) is produced over the winter period and the fate of this ammonia is crucial to the productivity of the above ground ecosystems (Buckeridge et al., 2010). Climate change will impact snow cover and mean annual temperatures and this is going to significantly increase the over-winter N mineralization (Buckeridge et al., 2010), yet we know very little about field scale ammonia oxidation processes in Arctic ecosystems. The oxidation of ammonia to nitrite is the first and rate-limiting step of nitrification. In soil, ammonia oxidation is regulated by a combination of the ammonia-oxidizing communities (Schimel and Gulledge, 1998) and soil physicochemical properties (Bremner, 1997). The purpose of this investigation was to characterize how the biology of aerobic ammonia oxidation (functional gene abundance and biochemistry) interacts with soil chemistry and pedology of Arctic ecosystems to influence field-scale ammonia oxidation processes. Understanding how molecular scales (genes) are linked to field scales will enable researchers to develop a holistic understanding of how climate change will drive evolutionary and ecological processes in Arctic ecosystems.

One approach to linking molecular to field-scale processes is through the analysis of spatial dependency of these processes. Spatial dependency arises because soil properties are

inherently heterogeneous and this spatial heterogeneity is predominantly 'non-random' (Goovaerts, 1998). In other words, as distance between two points in space declines, samples become more similar. This similarity is driven because as inter-sample distance declines, the variation of soil properties due to differences in topography, climate, soil physicochemical and biological processes and vegetation declines (Banerjee et al., 2011a; Cerri et al., 2004; Corstanje et al., 2007). Thus, the spatial dependency of a process reflects the underlying mass and energy flows of an ecosystem at a field scale. As such, a comparison of how molecular parameters for example gene abundance, are spatially dependent compared to the spatial dependency of larger processes, such as pedological characteristics, allows one to infer ecosystem-level linkages between these parameters. Microbial communities are spatially dependent (Ettema and Wardle, 2002) with previous studies focussing on grassland (Nicol et al., 2003; Rits et al., 2004), agricultural (Franklin and Mills, 2003; Grundmann and Debouzie, 2000), forest (Saetre and Baath, 2000), and Arctic (Banerjee et al., 2011b) soils. This study aims to build on this foundation by linking enzymatic processes (ammonia oxidation) to gene abundance and explore how these links are driven by pedological features of Arctic ecosystems.

Aerobic ammonia oxidation in soil is governed by chemolithoautotrophic and heterotrophic microbial communities. Unlike autotrophic ammonia oxidizers, ammonia oxidation in heterotrophic bacteria is not related to their cellular growth (DeBoer and Kowalchuk, 2001). The importance of heterotrophic ammonia oxidation in soil has long been recognized (Schimel, 1984), but no information is available on this pathway in Cryosols. This lack of knowledge regarding the autotrophic and heterotrophic ammonia oxidation processes may be due to the fact that Cryosols are typically N limited and a considerable proportion of Cryosols are acidic in nature, which reduces ammonia bioavailability for ammonia oxidation. The abundance and

functions of ammonia-oxidizing bacteria and archaea in soil are influenced by various soil factors including moisture, pH, ammonium, organic carbon content, and temperature (Erguder et al., 2009; Leininger et al., 2006; Ma et al., 2008; Nicol et al., 2008; Shen et al., 2008) that operate at multiple spatial scales. This study investigated spatial patterns of aerobic ammonia-oxidizing functional groups in three Arctic ecosystems to answer the following questions: 1) what is the relative abundance of aerobic ammonia-oxidizing bacteria and archaea in Arctic soils? 2) what is the autotrophic and heterotrophic ammonia oxidation potential in various types of Cryosols? 3) what are the driving factors of ammonia oxidation at different spatial scales in Arctic soils?

5.3 Materials and Methods

5.3.1 Site description

This study was conducted at three high Arctic sites: Truelove Lowland, Simpson Lake, and Ross Point. The Truelove Lowland site (75°40′ N, 84°35′ W) has already been extensively described (Bliss, 1977; Lev and King, 1999; Siciliano et al., 2009).The average July soil temperature in the upper 5 cm in 2008 was 13^0C with a maximum value of 24^0 and a minimum of 6^0 (Environment Canada, 2011). This coastal lowland is situated on the north-eastern coast of Devon Island and it covers an area of 43 km^2 of Devon Island's 55,000 km^2 area. The topography of Truelove Lowland is distinguished by a series of raised beach crest ridge, lower foresope, and wet sedge meadow with Regosolic Static Cryosols, Brunisolic Eutric Turbic Cryosols, and Gleysolic Turbic Cryosols, respectively (Siciliano et al., 2009). The mean annual air temperature is approximately -16°C with the highest recorded daily temperature of 21°C in July and -45°C as the lowest monthly temperatures (Bliss, 1977; Lev and King, 1999). The total annual precipitation is about 185 mm with 36 mm as rain. Simpson Lake (68°35′ N, 91°57′ W) is

located in the middle of the Boothia Peninsula, approximately 80 kilometres west of Kugaaruk and 120 kilometres east of Gjoa Haven. Historical data from Kugaaruk suggests the temperature in winter (December-January) varies between -23° to -37°C and in summer (July-August) between 4° to 24°C (Environment Canada, 2011). The total annual precipitation is about 260 mm with 110 mm as rain. The upperslope positions are comprised of Static Cryosols whereas lowerslope positions are Turbic Cryosol. Ross Point (68°31′ N, 111°10′ W) is situated in the south port of Victoria Island, the second largest island in the Canadian Arctic Archipelago. The experimental site is dominated by lichen and dry heath vegetation. Ross Point (68°31′ N, 111°10′ W) is situated in the southern part of Victoria Island. This site is about 150 km west of the town of Cambridge Bay. Historical weather data from the last 30 years in Cambridge Bay suggests the temperature in summer (July-August) ranges between 4° to 23°C and in winter (December-January) between -25° to -36°C (Environment Canada, 2011). The mean annual precipitation is about 138 mm with 69 mm as rain. Soils at Ross Point are predominantly Organic Cryosols.

5.3.2 Soil sampling design

Three parallel transects (300 m each; 2 m distance between each of the transects) were established at each research site. Soil samples were collected at 31 points (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300 m) along each transect. A GPS unit (Trimble™ GPS Systems, Sunnyvale, CA) was used to identify spacing (+/- 8 cm) between samples. Approximately 250 g of soil samples were collected at 1 to 10 cm depth using a hand trowel and sieved with a 4.75 mm sieve. Between samples, sieves and hand trowels were sterilized with 95% ethanol and dried before use. As facilities for performing chemical and molecular analyses were not

available on site, the soil samples were frozen at –20°C and were shipped to the laboratory at University of Saskatchewan.

For spatial comparison, this study used the same design at three research sites. In our experience a single long transect in the Polar Regions is often compromised because of erratic boulders, frost wedges, melt ponds and unstable slopes. Three transects provide a reasonable approximation of a typical strip of land in the Arctic regions whereas a single transect line often will not. For example, a 300 m x 300 m grid design was not possible to establish at Truelove Lowland because of erratic landscape patterns. Moreover, fine scale patterns may not be captured with a grid design due to its even lag distance (minimum 2 m or 4 m). Variable (irregular) sampling design, adjacent steps separated by a repeated sequence, is a particularly useful design for substantiating multi-scale patterns in an ecosystem (Fortin et al., 1989; Franklin et al., 2002; Mayor et al., 2009). Therefore, this study specifically used a variable-lag-distance transect approach to simultaneously capture the fine (0-1 m), medium (1-10 m), and large (10-300 m) scale spatial patterns of microbial communities in three Cryosolic ecosystems.

5.3.3 Soil analyses

Soil gravimetric water content was determined by measuring the weight loss of 5 g soil samples after they were dried for 24 hours at 105 °C and expressed as percent (Gardner, 1986). Soil pH was measured using 5 g soil in a 1:1 soil:water (deionized) mixture with an Accumet pH meter (Accumet 925, Fischer Scientific, Hanover Park, IL). For extractable ammonium NH_4^+) and nitrate (NO_3^-) content, soil subsamples were shaken with 0.5M K_2SO_4 (1:10 soil: K_2SO_4) for one hour and filtered using Whatman 90 μm filter papers (Maidstone, Kent, England). A 3 ml aliquot of extract was analyzed using a SmartChem™200 discrete chemistry analyzer (Westco Scientific Instruments Inc, Brookfield, CT). Total organic carbon (TOC) content was determined

by using the Leco CR-12 Carbon Analyzer (LECO Corporation, St. Joseph, MI). The determined quantity is expressed as a percentage of soil mass.

5.3.4 Autotrophic ammonia oxidation potential (AOP)

Potential ammonia oxidation is an estimation of the production of nitrite in soil (Torstensson, 1993). In brief, a test medium was prepared with 4 mM ammonium sulphate, 15 mM sodium chlorate and 1 mM monopotassium phosphate buffer (pH 7.2). In this test medium, ammonium sulphate is an energy source, sodium chlorate inhibits the oxidation of nitrite to nitrate (Berg and Rosswall., 1989; Gong et al., 1999) and monopotassium phosphate buffers the medium. Ammonium sulphate is added as a one-time supplement to the soil to achieve the maximum ammonia oxidation potential of a soil. The concentration of ammonium sulphate was selected from previous studies on polar soils (Schaefer et al., 2007; Schaefer et al., 2009); where it was found that average ammonia oxidation potential using this method was strongly correlated (r=0.66) with ^{15}N ammonia oxidation in polar soils (Harvey, 2011). Soil (5 g of fresh weight) was added to 20 ml of test solution and the mixture was shaken (100 rpm) at room temperature (20 ℃) for 28 hours (the time point was selected based on a time course assay) and an aliquot of 2 ml was collected. Immediately after collection, 2 ml of 4 M KCl was added to stop the ammonia oxidation and the mixture was centrifuged at 13,000 rpm for 3 minute and filtered through 0.45 µm syringe filter to remove particulate matter. Then 3 ml of filtrate was taken for nitrite analysis according to Griess Illosvay technique (Gong et al., 1999; Torstensson, 1993) using a spectrophotometer (Beckman Du-650, Beckman Coulter, CA). The concentration of nitrite was expressed as ng NO_2^--N g^{-1} dry soil h^{-1}. Typically, potential ammonia oxidation is measured at 20℃ (Gong et al., 1999; Torstensson, 1993). It should be noted that the soil samples were collected in summer (4th week of July) and these research sites regularly experience

temperature as high as 24ºC during midday in summer (Environment Canada, 2011). Soil biological assays in previous studies conducted on Truelove Lowland soils also used 20 ºC as the incubation temperature (Bliss, 1977). Thus, the incubation temperature selected for the AOP assay in this study can be representative of the midday temperature during growing season. A single incubation temperature (20ºC) was selected for consistent measurement of soil samples collected from all three sites.

5.3.5 Heterotrophic ammonia oxidation potential (HAOP)

Heterotrophic ammonia oxidation potential assay was measured using the same method as AOP assay except that 400 μg of nitrapyrin was mixed with 5 g of fresh soil and incubated for six hours before adding the soil to the test medium. Nitrapyrin is the longest known and best inhibitor of chemoautotrophic ammonia oxidizers (Chancy and Kamprath, 1987; Nielsen et al., 2007) and differentiates between heterotrophic and autotrophic ammonia oxidation (DeBoer and Kolwalchuk, 2001). The concentration of nitrapyrin was selected from a previous study (Islam et al., 2007), which found 80 mg g^{-1} soil is the most effective concentration that completely inhibited autotrophic ammonia oxidation. However, the effect nitrapyrin on ammonia-oxidizing archaeal population is not well-known, thus, heterotrophic ammonia oxidation measured in this study actually pertains to nitrapyrin-inhibited ammonia oxidation potential.

5.3.6 DNA extraction and quantification of ammonia-oxidizer abundance

DNA extraction from soils was done according to the method described by (Griffiths et al., 2000) with the modification that DNA samples were precipitated in polyethylene glycol overnight. The concentration of purified DNA was determined by spectrophotometer (Ultrospec 2000 ultraviolet (UV)/visible spectrophotometer, Pharmacia Biotech, Cambridge, UK). The number of bacterial *amoA* and archaeal *amoA* genes present in soil DNA extracts was determined

by conducting quantitative real-time PCR (qPCR) using the QuantiTect™ SYBR® Green PCR Master Mix real-time PCR kit and an ABI 7500 real-time PCR machine (Applied Biosystems, Foster City, CA). The primer sets amoA-1F/amoA-2R (Rotthauwe et al., 1997) and Arch-amoAF/Arch-amoAR (Park et al., 2006) were used for the bacterial *amoA* and archaeal *amoA* assay respectively. Each 20 µl reaction contained 10 µl of master mix, 10 pmol of the appropriate forward and reverse primers, 6 µl sterilized mili-Q water, and 2 µl template DNA (1:10 diluted). The thermal cycling program for the genes was as follows: 97 °C for 15 min, 45 × (94 °C, 20 s; 54 °C, 40 s; 72 °C, 40 s), and 77 °C for 45 s followed by a melt-curve from 50 to 95 °C. Standards were prepared by amplifying community DNA and purifying the amplified products for use in a qPCR assay. QPCR standards were also prepared using genomic DNA extracts of *Nitrosomonas europea* to compare to these community DNA standards. The number of gene copies present in the product was estimated by determining the concentration of DNA in the product (Ma et al., 2007). Only the standard curves linear over five orders of magnitude were selected and standards were run on each individual qPCR plate. For both genes, the efficiency of the reaction was between 86% and 100% (based on the slope of the standard curves). The r^2 value for the standard curves was 0.99 for all assays except bacterial *amoA* at Truelove Lowland and Simpson Lake, which was 0.98. The specificity of the amplified products was assessed by melting curve analysis. Amplification inhibition effects were assessed by assessing gene abundance on three different dilutions of representative samples and selecting the dilution that minimizes the inhibition (Dumonceaux et al., 2006).

5.3.7 Geostatistical analyses

The recorded data were tested for homogeneity of variances (Bartlett's and Levene's tests) and normality (Anderson–Darling) using Minitab software (Minitab 14, Minitab Inc. State

College, PA). This study estimated the background variability associated with molecular methods applied to soil samples. To determine the DNA extraction variability, DNA was extracted using 0.5 g of soil from 10 replicates of one soil sample, which comprises 250 g of soil and assessed for bacterial *amoA* and archaeal *amoA* abundance. Variability associated with soil subsampling was calculated as the variance of 10 independent trials of the same soil sample. The qPCR variability was estimated by averaging the variance of between 5-10 qPCR estimations of six different samples for bacterial *amoA* and seven samples for archaeal *amoA*. Variances were divided by 2 to compare with experimental semivariance of spatial analyses.

The spatial heterogeneity and spatial relationships among the variables were estimated using geostatistical analyses. In geostatistics, the degree of spatial continuity of a variable is assessed by analyzing the dissimilarity between two observations as a function of the separation distance or lag distance. One way to assess this dissimilarity is through computing semivariance, $\gamma(h)$, which is the half of the average squared difference between the components of a data pair (Equation 5.1).

$$\gamma(h) = \frac{1}{2N(h)} \sum_{\alpha=1}^{N(h)} [z(x_k) - z(x_k + h)]^2 \qquad \text{(Equation 5.1)}$$

Here $z(x_k)$ is the property, $z(x_k+h)$ is the value at h lag distance and $N(h)$ is the number of data pairs for a given distance (Goovaerts, 1998). Semivariogram is a plot of semivariance, $\gamma(h)$, as a function of *h* or lag distance. Three important attributes of a semivariogram are nugget variance, sill, and range. The nugget variance is the stochastic variation, which is contributed by measurement or experimental error. Sill is the maximum variability attained by the variable and range is the lag distance at which the semivariance value becomes highest. The range of a semivariogram indicates the zone of spatial dependency. In other words, samples spaced closer than the range are spatially dependent whereas samples separated by a distance greater than the

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range are not spatially dependent. The spatial dependence of the soil properties and gene abundance was calculated by semivariogram analysis. Spatial dependence (SPD)=$C / (C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C+C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (strong spatial dependence). The spatial association between two variables can be determined by calculating cross-semivariance, $\gamma_{yz}(h)$ (Equation 5.2).

$$\gamma_{yz}(h) = \frac{1}{2N(h)} \sum_{k=1}^{N(h)} [y(x_k) - y(x_k + h)].[z(x_k) - z(x_k + h)]$$ (Equation 5.2)

Here *y* and *z* are two variables, $z(x_k+h)$ is the value at h lag distance and $N(h)$ is the number of data pairs for a given distance. A plot of cross-semivariance, $\gamma_{yz}(h)$, as a function of *h* or lag is called cross-semivariogram. All semivariograms and cross-semivariograms were calculated with a minimum of 30 sample pairs per lag class (Journel and Juijbregts, 1978). Various models such as Gaussian, spherical, exponential were fitted to the semivariograms and cross-semivariograms using least-squares method. Three specific scales were selected for estimating scale dependent associations among the variables: fine (0-1 m), medium (1-10 m), and large (10-100 m). All geostatistical analyses were performed using GS+ version 9 (Gamma Design Software, Plainwell, MI).

5.4 Results

5.4.1 Functional gene abundance, ammonia oxidation potential, and soil attributes

This study found high abundance of bacterial *amoA* (10^5-10^7 copies g^{-1} dry soil) and archaeal *amoA* (10^6-10^8 copies g^{-1} dry soil) genes in Arctic soils (Fig. 5.1). The three Cryosolic ecosystems selected in this study showed considerably high AOP. The contribution of heterotrophs at Truelove was 35% and Simpson Lake it was 29% of total AOP, respectively, despite the moderate acidity of Simpson Lake soils. At Ross Point heterotrophs contributed up
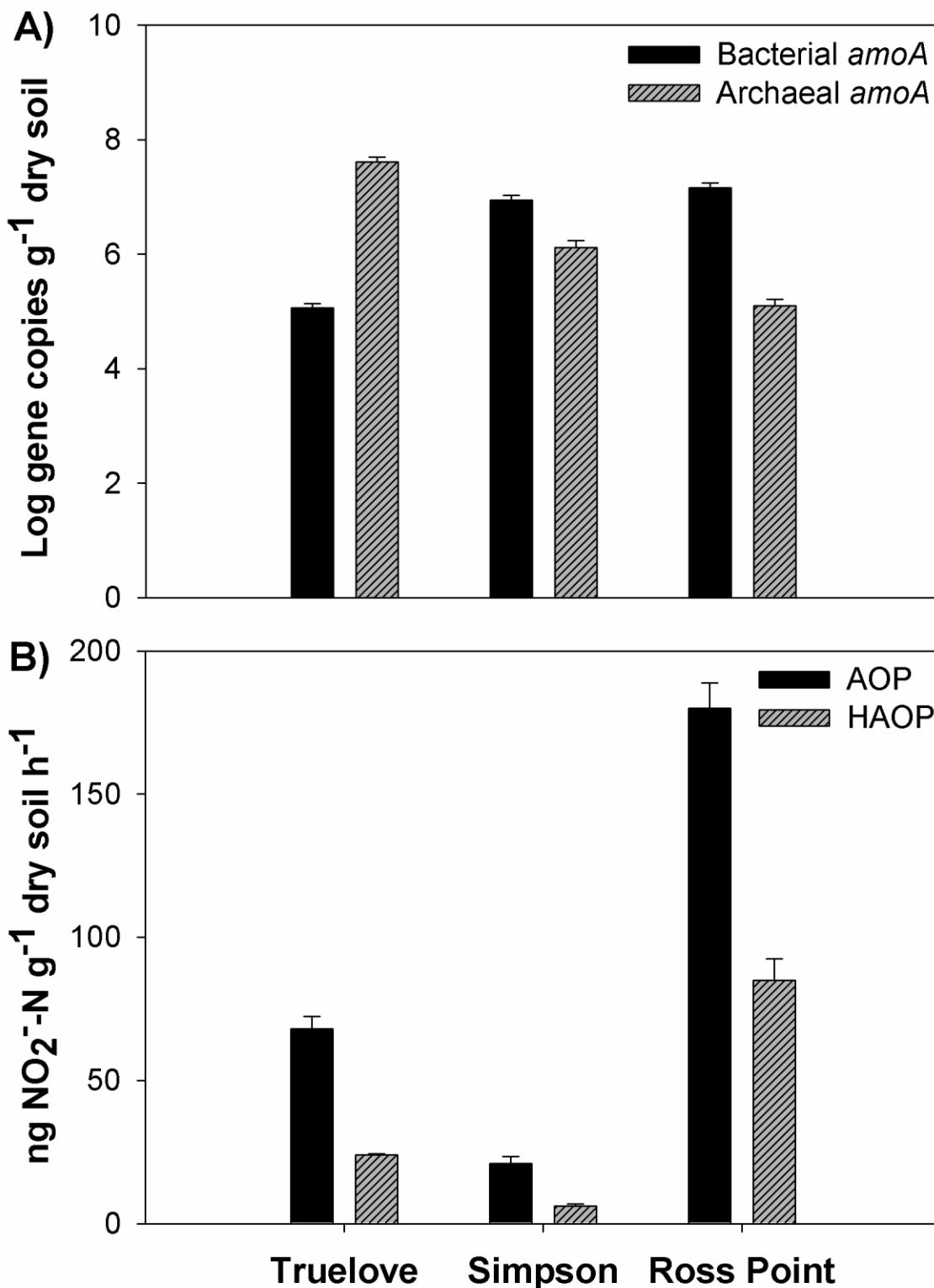
Figure 5.1 A) Log gene copy numbers of ammonia oxidizing functional groups (archaeal *amoA* and bacterial *amoA*) and B) overall ammonia oxidation potential (AOP) and heterotrophic ammonia oxidation potential (HAOP) at three Arctic sites: Truelove Lowland, Simpson Lake and Ross Point.
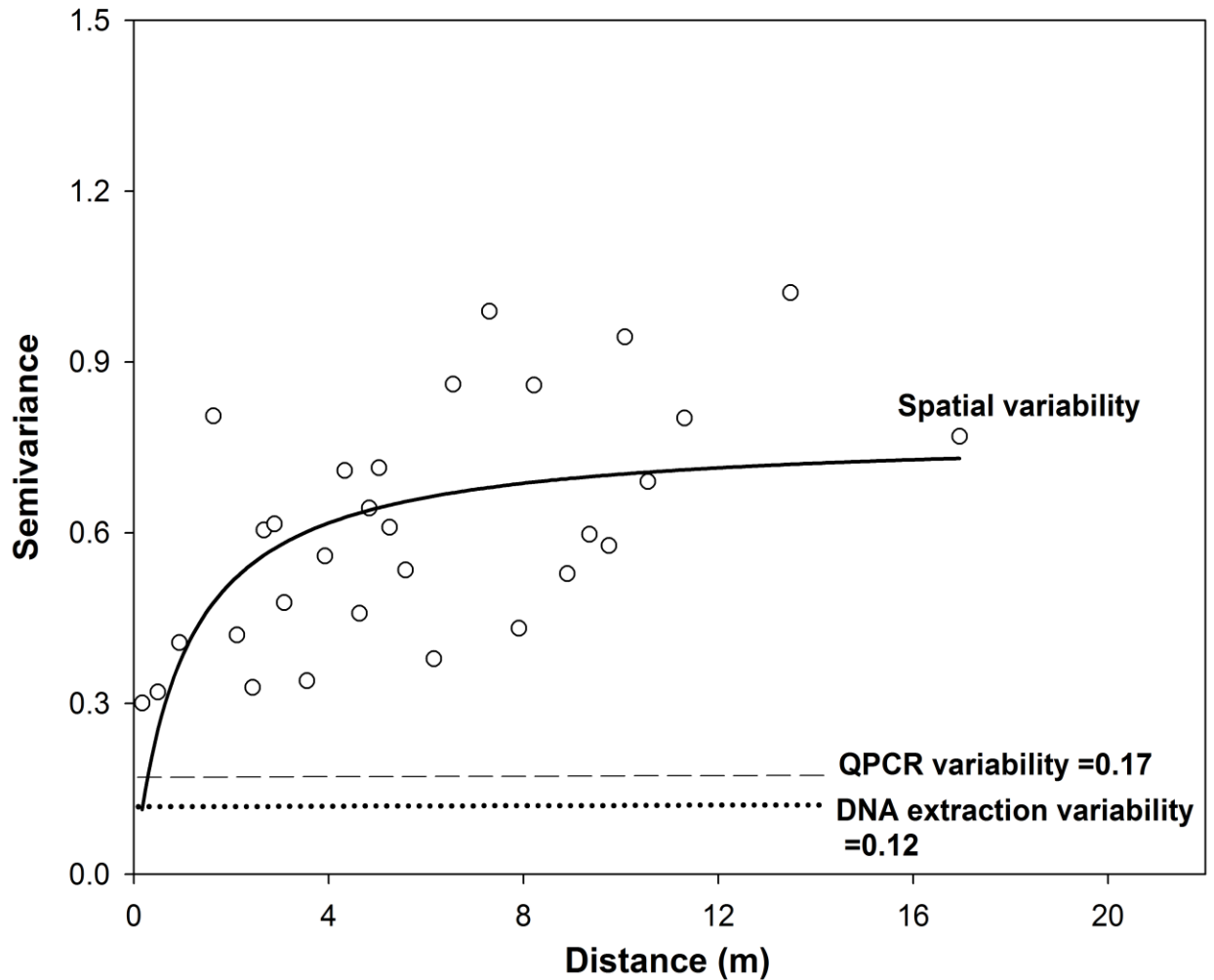
Figure 5.2. Semivariogram of bacterial *amoA* showing spatial variability at Truelove Lowland compared to experimental error associated with extracting and quantifying genes in soil. The qPCR variability (semivariance=0.17; n=6 independent DNA extracts, with 10 qPCR replicates each) and DNA extraction variability (semivariance=0.12; n = 10 independent extractions (0.5 g fresh weight) of the same 300 g soil sample, which were then analyzed by qPCR in triplicate) are indicated as long dash and dotted line, respectively.

to 47%, which may be linked to the higher TOC and moisture content compared to the other two

sites.

5.4.2 Analysis of spatial dependency

Spatial variability of the bacterial *amoA* gene (sill variance =1.02) is substantially higher

than the variance associated with DNA extraction (0.12) and quantifying genes by qPCR (0.17)

(Fig. 5.2). The bacterial *amoA* gene at Truelove is spatially autocorrelated with the spherical model providing a fit of $r^2 = 0.31$, *P*<0.01 and a spatial range of 2.6 m. Spatial dependence explains the majority of experimental variance associated with ammonia oxidizer abundance and ammonia oxidation potential (Fig. 5.3). However, the spatial dependency of archaeal *amoA* at Truelove Lowland, AOP at Simpson Lake, and HAOP at Ross Point could not be captured with the transect design, which suggests that either (a) there is no significant spatial dependency or (b) the dependency operates at less than 0.2 m distance (double our smallest lag distance). Bacterial *amoA*, archaeal *amoA*, and AOP differ from one another in their spatial structures. Bacterial *amoA* abundance is spatially structured at all three sites with a consistent spatial range (2.2 to 2.6 m). In contrast, archaeal *amoA* abundance is not consistently spatially dependent at all sites with a range between 2.2 and 4.4 m. In spite of these differences, bacterial *amoA* and archaeal *amoA* are spatially correlated (*P*<0.05) at all three sites with cross- semivariance ranges between 3 and 4.5 m and SPD between 0.38 and 0.85 (Fig 5.4). Ammonia oxidation potential has the largest spatial range (22-41 m) and considerably high spatial dependence. The spatial range of the relationships between AOP and soil properties varies greatly between sites. For example, the cross-semivariance between AOP and soil properties (*P*<0.05) has a range of ~20 m at Ross Point but only 0.3 m at Simpson Lake (Fig. 5.5). There are weak correlations between the AOP and HAOP potential at Truelove (Spearman Rank (ρ)= 0.39, *P*<0.01), none at Simpson Lake (ρ= -0.15, p=0.16) and weak links again at Ross Point (ρ= -0.28, *P*<0.01). This suggests that heterotrophic contribution to total ammonia oxidation potential, estimated as (HAOP/AOP), is variable across the site. The HAOP is spatially dependent at Truelove Lowland and HAOP/AOP are spatially dependent at two of the three sites with a range between 8.3 m and 14 m and a SPD between 0.55 and 0.65 Lake (Fig 5.6).

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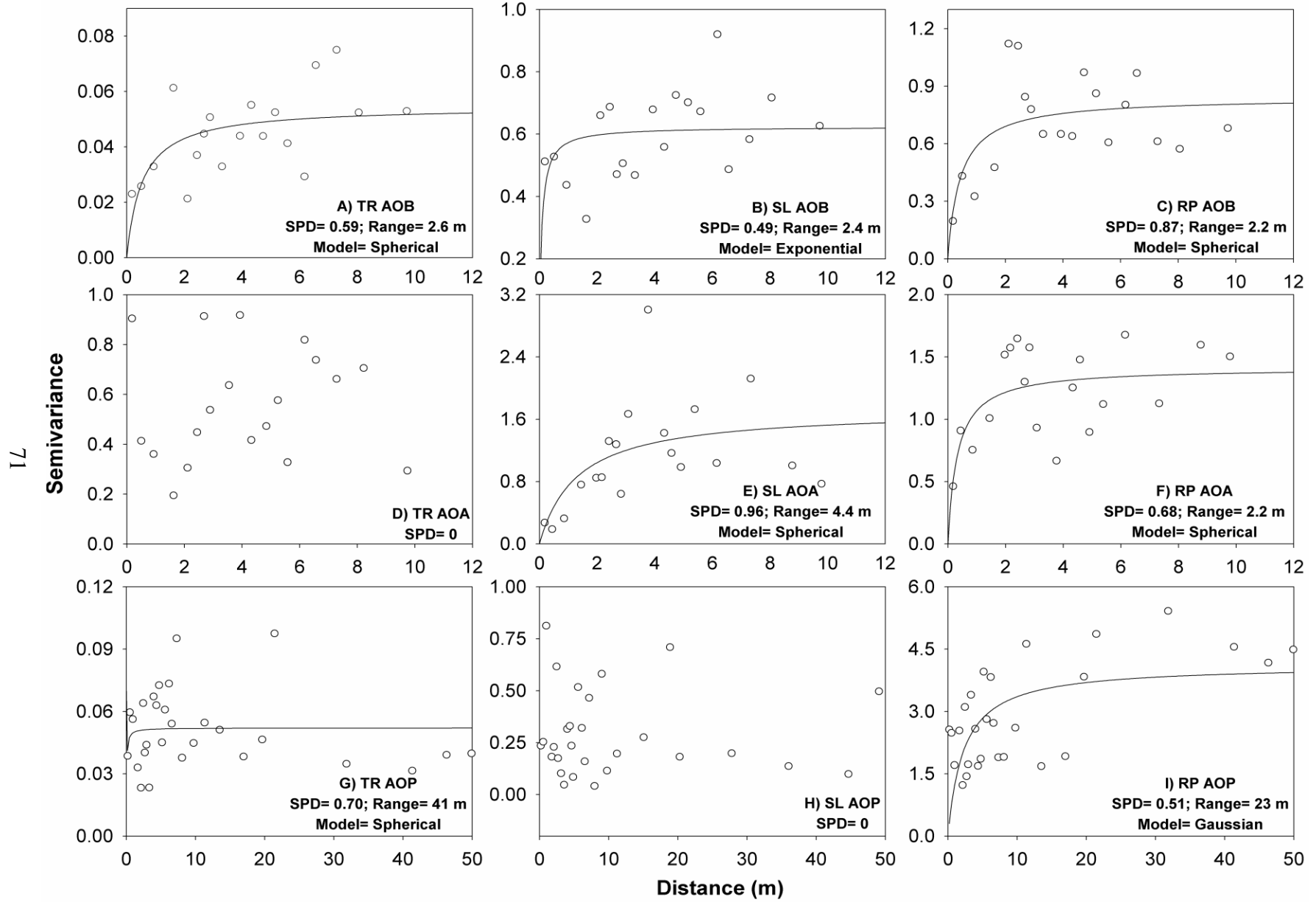
Figure 5.3. Semivariograms showing differential spatial structure of bacterial *amoA* abundance (AOB), archaeal *amoA* abundance (AOA), and potential ammonia oxidation (AOP) at three Arctic sites: Truelove Lowland (TR), Simpson Lake (SL), and Ross Point (RP). Spatial dependency (SPD) was considered from fine (10 cm) to large scale (300 m); values of SPD vary from 0 (no spatial dependence) to 1 (strong spatial dependence). Range indicates the zone of spatial dependency. Different models (Gaussian, spherical, and exponential) were fitted (solid line) to each semivariogram. Semivariograms are shown up to specific lag distance for clarity of the spatial patterns near origin.
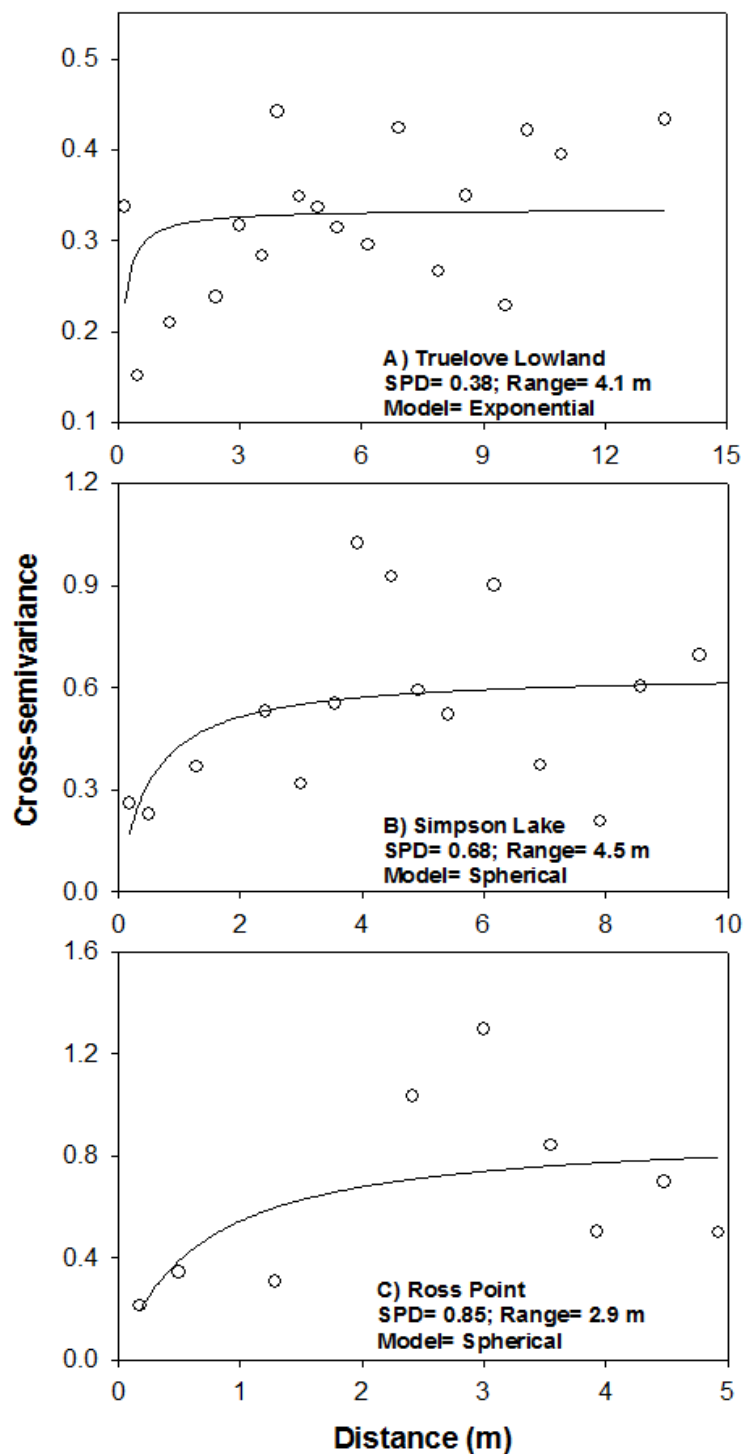
Figure 5.4. Cross-semivariograms showing the spatial relationships between archaeal *amoA* and bacterial *amoA* gene abundance at three Arctic sites. Values of SPD vary from 0 (no spatial dependence) to 1 (strong spatial dependence). Range indicates the zone of spatial dependency. Different models (Gaussian, spherical, and exponential) were fitted (solid line) to each semivariogram. Semivariograms are shown up to specific lag distance for clarity of the spatial patterns near origin.
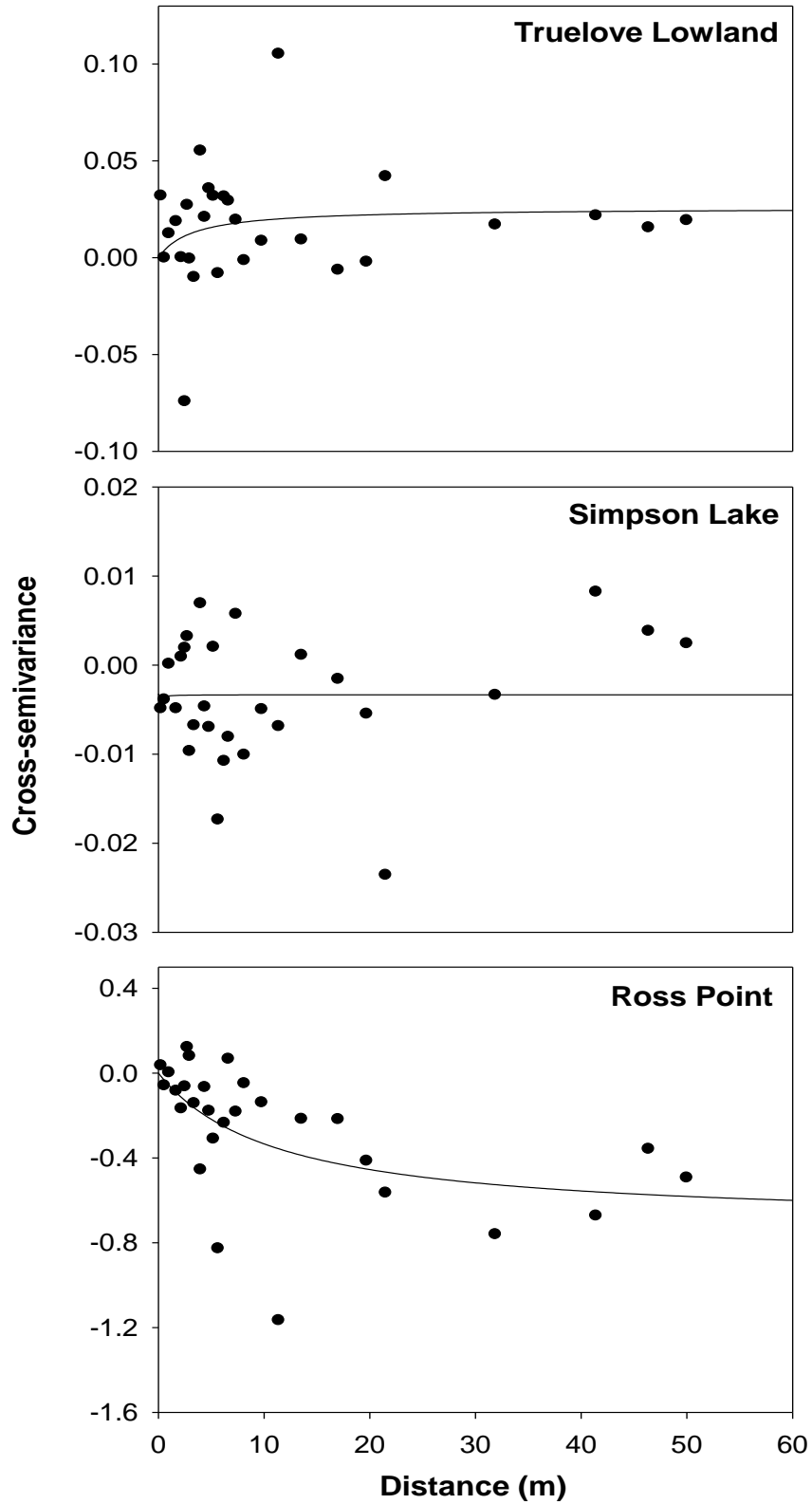
Figure 5.5 Cross-semivariograms showing the spatial range of the relationships between AOP and soil properties at three sites.
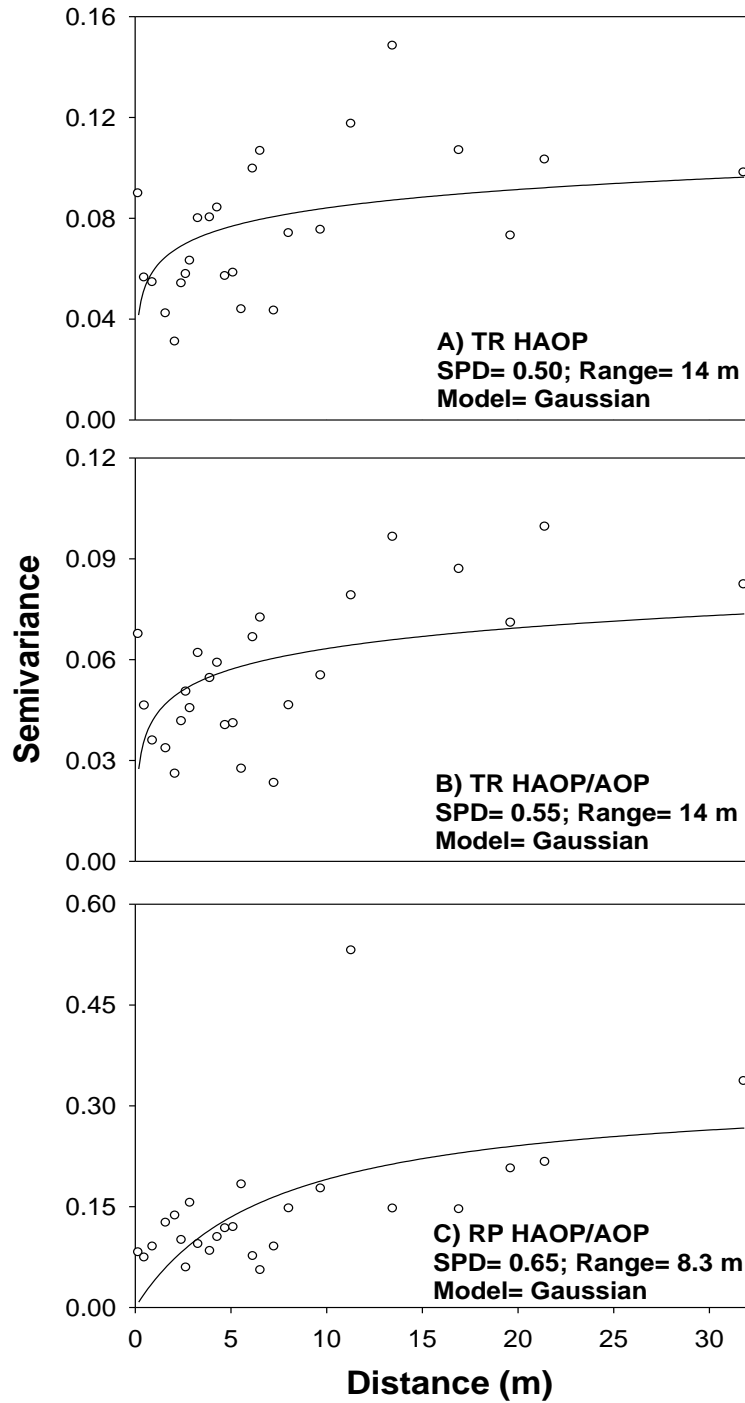
Figure 5.6 Semivariograms showing spatial patterns of heterotrophic ammonia oxidation potential and heterotrophic contribution to total potential ammonia oxidation activity, estimated as (HAOP/AOP). Values of SPD vary from 0 (no spatial dependence) to 1 (strong spatial dependence). Range indicates the zone of spatial dependency. Different models (Gaussian, spherical, and exponential) were fitted (solid line) to each semivariogram. Semivariograms are shown up to specific lag distance for clarity of the spatial patterns near origin.

Table 5.1 Spatial scale-dependent correlations between autotrophic ammonia oxidation potential (AOP), functional groups and environmental variables

	Truelove Lowland			Simpson Lake			Ross Point		
	Fine[#]	Medium	Large	Fine	Medium	Large	Fine	Medium	Large
AOP x Bacterial *amoA*	NS[§]	NS	NS	0.29*	NS	NS	NS	0.30*	NS
AOP x Archaeal *amoA*	NS	0.33*	0.35*	NS	0.42**	NS	NS	0.37*	NS
AOP x Moisture	0.27*	0.35*	0.27*	0.71**	0.64**	0.60**	0.45**	0.25*	0.35*
AOP x pH	NS	NS	NS	NS	NS	-0.36*	NS	-0.36*	-0.32*
AOP x NH_4^+	NS	0.31*	0.26*	NS	NS	0.29*	NS	NS	0.36*
AOP x NO_3^-	NS	NS	0.25*	NS	0.62**	NS	NS	NS	0.25*
AOP x TOC	NS	0.37*	0.30*	0.48**	0.42**	NS	0.38**	0.27*	0.43**

[#]Scales: fine (0-1 m), medium (1-10 m) and large (10-100 m)
[§]NS- Not significant
*   Significant at $P<0.05$ level
** Significant at $P<0.01$ level

Spatial relationships between AOP and ammonia oxidizer abundance mostly operate at medium scale (Table 5.1). The tested soil attributes that regulate the AOP at fine scale, <1 m, are moisture content and TOC. Soil moisture is strongly correlated to AOP at all scales for all three sites, similarly TOC content is also significantly correlated in most cases. Other soil attributes such as pH, ammonium and nitrate content interact with AOP at medium or large scale.

5.5 Discussion

The bacterial and archaeal *amoA* gene abundance is similar to what previously seen in Arctic (10^5-10^7 and 10^6-10^8 copies g^{-1} dry soil respectively; Siciliano et al., 2009), agricultural (10^6 and 10^7 g^{-1} dry soil respectively; He et al., 2007; Shen et al., 2008) and pristine (10^7 and 10^8 copies g^{-1} dry soil respectively; Jenkins et al., 2009; Kemnitz et al., 2007) soils. The high abundance of bacterial and archaeal ammonia-oxidizing archaea and bacteria may indicate the existence of microhabitat partitioning and distinct ecological niches in Arctic soils (Schleper, 2010). The co-spatial dependency between bacterial and archaeal *amoA* suggest that the abundance of ammonia oxidizers is being modulated by similar soil parameters that are occurring at <5 m scale. However, the qPCR primers used in this study to assess the ammonia oxidizer abundance have reduced/lower coverage and it is possible that the primers may have missed a portion of the AOA and AOB abundance. For instance, the AOA primers used in this study do not cover several archaeal genera such as *Nitrosocaldus yellowstonii*, *Nitrosopumilus maritimus*, and *Crenarcheum symbiosum*, and similarly the AOB primers also do not cover all of the bacterial ammonia oxidizers (Schleper and Nicol, 2010; Stephen et al., 1999). Therefore, these primers may have underestimated the ammonia oxidizing populations and the actual abundance in these Cryosolic ecosystems may even be larger than what reported here.

The link between DNA contents and activity in the field is not conclusive and thus, increased gene copies do not conclusively mean that activity will be increased. In addition, the archaeal ammonia monooxygenase belongs to the family of copper-containing membrane-bound monooxgenases that have a wide substrate range including methane, ammonia, and short-chained alkanes. Further, only five out of 10,000 deposited archaeal amoA sequences are actually linked to established AOA (Hanson and Hanson, 1996; Pester et al., 2011; Sayavedra-Soto et al., 2011; Schleper, 2010). Thus, the genes assessed here may also be coding for enzymes not directly linked to ammonia oxidation. Another limitation of DNA based qPCR results is that *in situ* extracellular DNA may interfere with the interpretation of our results. Although no reports are available from Arctic soil environments, microbial extracellular DNA molecules may persist anytime between 30 minutes to 70 days in soil (Nicol et al., 2008). Thus, it is also possible that a part of ammonia-oxidizer DNA molecules quantified in this study does not actually belong to active functional ammonia oxidizer population and are not essentially contributing to ammonia oxidation. Moreover, it has been found that the length of bead beating time involved in DNA extraction may also influence ammonia oxidizer abundance as AOB copy number may increase and AOA copy number decrease with the time of bead beating (Leininger et al., 2006). Therefore, the results reported in this study only intend to present a comparative account of the relative changes in the two groups of ammonia oxidizer populations. Nonetheless, the AOP at Truelove Lowland (68 ng NO_2^- N g^{-1} dry soil h^{-1}) is three times higher than that Simpson Lake (21 ng NO_2^- N g^{-1} dry soil h^{-1}) and about half of Ross Point (178 ng NO_2^- N g^{-1} dry soil h^{-1}). It should be noted that Truelove Lowland is a polar oasis whereas Simpson Lake is a typical Arctic ecosystem, which does not benefit from wind protection and increased soil moisture and temperature typical of a polar oasis such as Truelove Lowland. On the other hand, the Organic

Cryosols of Ross Point have higher moisture and ammonia content, and optimum pH, conducive for ammonia oxidation. The overall AOP at Ross Point is in the similar range as agricultural soils $(0.1\text{-}1.5 \ \mu g \ NO_2{}^- \ N \ g^{-1}$ dry soil $h^{-1})$ (He et al., 2007; Shen et al., 2008). Nonetheless, it should be noted that sodium chlorate might not be the most effective inhibitor of nitrite oxidation by *Nitrospira*, which may influence the AOP assay (Hynes and Knowles, 1983).

Heterotrophic ammonia oxidation comprises a considerable proportion of overall ammonia oxidation potential. In Arctic soils with moderate moisture and ammonium content (Truelove Lowland and Simpson Lake), heterotrophs contribute as much as one third of total ammonia oxidation. However, in a soil with high moisture, ammonium, and organic matter content (Ross Point), heterotrophs contribute up to 47% of overall soil ammonia oxidation potential. The contribution of heterotrophs to soil ammonia oxidation potential is consistent with a previous report (McLain and Martens, 2006) that heterotrophic ammonia oxidation N-cycling processes may dominate *in situ* N transformations in some soils with high organic matter. Nonetheless, the high contribution of heterotrophs at Ross Point may also have resulted from reduced effectiveness of nitrapyrin in organic soils (Chancy and Kamprath, 1987; Neufeld and Knowles, 1999). Autotrophic ammonia oxidation may not have been inhibited completely due to the sorption of nitrapyrin in organic soils. Moreover, the efficacy of nitrapyrin to inhibit ammonia-oxidizing archaeal population has not yet been tested. Aerobic ammonia oxidation by heterotrophs is presently not understood at the molecular level and the ammonia-oxidizing gene sequences in heterotrophs are not well-characterized. The present study could not link heterotrophic ammonia oxidation to functional gene abundance. The discovery and elucidation of heterotrophic ammonia-oxidizing gene targets may identify a fine scale process linking observed potential in soils to gene abundance and expression.

Spatial dependency and range may be influenced by sampling design (Fortin et al., 1989). This study employed a variable-distance sampling design to capture microbial spatial patterns at multiple spatial scales across Arctic landscapes. The high spatial dependency suggests that this study was successful to capture the majority of the spatial variability in these locations. In agriculture soils, previous research (Franklin and Mills, 2003) showed nested scaling patterns of microbial spatial heterogeneity although other ecosystems are also known to operate at multiple scales (Dale et al., 2007), and this spatial dependency will bias random sampling schemes. (Grundmann and Debouzie, 2000). The spatial range of microbial spatial autocorrelation between 1 and 7 m found in this study reflects that seen by others who have observed ranges of 0.39, 1.00, and 7 m with a minimum sampling distance of 0.1, and 2 m, respectively (Ettema and Wardle, 2002). Based on the results presented here, investigators using a statistical design that assumes sample independence, e.g. ANOVA based design, should use a spatially explicit sampling regime to estimate microbial abundance and functions in Arctic soils with a minimum distance for gene abundance studies of 5 m and for biochemical processes an intersample distance of 40 m.

Strong spatial autocorrelation of microbial communities exists in grassland (Nicol et al., 2004; Philippot et al., 2009; Ritz et al., 2004), agricultural (Franklin and Mills, 2003; Grundmann and Debouzie, 2000; Grundmann et al., 2001), forest (Pester et al., 2011), and contaminated (Becker et al., 2006) soils. Banerjee et al. (2011b) showed that in spite of the harsh environmental conditions and inherent cryopedogenic processes the overall archaeal, bacterial, and fungal populations in Arctic soils are also spatially autocorrelated. This study further extends the notion of Cryosolic spatial dependency to demonstrate that the ammonia-oxidizing populations and ammonia oxidation processes are also spatially well-structured in different types

of Cryosols. The spatial dependency of Arctic soils is similar to other ecosystems and supports the conclusion drawn by Chu et al. (2010) that broad genera-level microbial diversity does not differ across ecotypes as it appears that similar soil factors drive ecosystems across ecotypes. Nonetheless, the relationships among AOP, soil properties, and ammonia-oxidizing population are nested within one another. Although it is often thought that the scale of biological processes that lead to spatial dependency occur at medium scales, (i.e. <10 m), this study and a previous study (Bengston et al., 2007) suggest that large scale (10-100 m) biological processes can also shape spatial structure of biological properties across a study area in which soil type remains largely similar. At the large scale (10-100 m), all the studied soil properties are correlated to AOP. The high correlations between soil moisture content and AOP at all scales suggest that moisture along with soil organic carbon is a key regulator of the spatial patterns of ammonia oxidation in Arctic soils. In addition to moisture and soil organic carbon, the population size of ammonia-oxidizing bacteria and archaea was also linked to AOP at fine and medium scales (0.1-10 m), however, these correlations were weaker than soil attributes. Thus it can be surmised that soil attributes rather than functional genes are the principal determinants of potential ammonia oxidation in Arctic soils. This is the first study assessing autotrophic and heterotrophic ammonia oxidation potential and their driving factors in Arctic soils; it is not clear from this study how climate change will cascade through the soil factors to the functional genes and ammonia oxidation in Arctic soils.

6.0 SPATIALLY TRIPARTITE INTERACTIONS OF DENITRIFIERS IN ARCTIC ECOSYSTEMS: ACTIVITIES, FUNCTIONAL GROUPS, AND SOIL RESOURCES

Preface

Denitrification is a major process of N biogeochemical cycling and one of the most significant contributors of N_2O to the atmosphere. Chapter 5 showed the scale dependent spatial correlations among ammonia oxidizing prokaryotes, potential ammonia oxidation, and edaphic factors. Thus, it would also be intriguing to see if spatial relationships also persist among soil denitrifying communities and their functions. Owing to the effect of pervasive global warming, Arctic permafrost ecosystems have been predicted to experience greatest degree of climate change and knowledge of denitrifier spatial patterns may reveal the fundamental controls on N_2O dynamics in Arctic soils. In this study, the abundance and spatial autocorrelation of denitrifier functional groups were assessed in 279 soil samples collected from three research sites encompassing 7° in latitude and 27° in longitude of the Canadian Arctic.

6.1 Abstract

Soil denitrification is one of the most significant contributors to global nitrous oxide (N_2O) emissions, and spatial patterns of denitrifying communities and their functions may reveal the factors that drive denitrifier activities and functional consortia. Although denitrifier spatial patterns have been studied extensively in most soil ecosystems, little is known about these processes in Arctic soils. This study aimed to unravel the spatial relationships among denitrifier abundance, denitrification rate and soil resources in 279 soil samples collected from three Canadian Arctic ecosystems encompassing 7° in latitude and 27° in longitude. The abundance of *nirS* (10^6-10^8 copies g^{-1} dry soil), *nirK* (10^3-10^7 copies g^{-1} dry soil), and *nosZ* (10^6-10^7 copies g^{-1} dry soil) genes in these soils is in the similar range as non-Arctic soil ecosystems. The rate of potential denitrification in Organic Cryosols (1034 ng N_2O-N g^{-1} soil h^{-1}) was 5-11 times higher than Static/Turbic Cryosols and the overall denitrification rate in Cryosols was also comparable to other ecosystems. It was found denitrifier functional groups and potential denitrification were highly spatially dependent within a scale of 5 m. Functional groups and soil resources were significantly ($P<0.01$) correlated to denitrifier activities and the correlations were stronger in Organic Cryosols. Soil moisture, organic carbon and N content were the predominant controls with *nirK* abundance also linked to potential denitrification. This study suggests that the dominant control on Arctic ecosystem-level denitrifier activity is moisture and organic carbon. Further, microbial abundance controls on ecosystem level activity while undoubtedly present, are masked in the nutrient poor Arctic environment by soil resource control on denitrifier ecosystem level activity.

6.2 Introduction

Cryosols, soils with permafrost within 1m depth, can be divided into three great groups: Static Cryosols, Turbic Cryosols and Organic Cryosols, with Static and Turbic referring to the degree of cryoturbation (Bockheim and Tarnocai, 1998). Cryosolic ecosystems comprise about 13% of the Earth's and 40% of Canada's total land area; and these ecosystems contain 25% of the world's total soil organic matter pool (Bockheim and Tarnocai, 1998; Tarnocai et al., 2006) and could release enormous amounts of greenhouse gases to the atmosphere. N_2O is a potent greenhouse gas with a global warming potential 298 times higher than CO_2 (IPCC, 2007). Soils play a key role in N_2O dynamics by contributing up to 90% of the world's total N_2O emissions and in soil nearly 70% of N_2O emissions originate from microbial transformations such as nitrification and denitrification (Mosier, 1998). Although limited information is available about N_2O fluxes in Arctic ecosystems, a recent review suggests that Arctic soils contribute 0.49 kg ha^{-1} annually to world N_2O release, which is similar to emissions from temperate grassland, boreal forest, or desert ecosystems (Dalal and Allen, 2008).

Denitrification is a key regulator of soil inorganic N concentration and N losses from ecosystems via nitrate (NO_3^-) leaching and N_2O emissions. Denitrifier communities regulate N_2O production and consumption and as such, the abundance of denitrifier functional groups can serve as an essential basis for understanding N_2O dynamics in soils (Morales et al., 2010). Denitrification has been well-recognised for more than a century (Voorhees, 1902) and the denitrifier abundance and potential denitrification have been measured in various soil ecosystems including agricultural (Enwall et al., 2010; Miller et al., 2008; Morales et al., 2010; Philippot et al., 2009), grassland (Keil et al., 2011; Miller et al., 2009), riparian (Dandie et al., 2011; Rich and Myrold, 2004), and forest (Bohlen et al., 2001; Levy-Booth and Winder, 2010) soils.

However, we still know little about denitrifier abundance and activities across Cryosols types and how they are linked to denitrifier activities and soil attributes in Canadian Arctic ecosystems. The key determinants of denitrification in soil, known as proximal controls, are pH, O_2, moisture, nitrate (NO_3^-), and organic carbon availability (Knowles, 1982; Wallenstein et al., 2006). These proximal factors influence soil denitrification primarily by controlling the denitrifier physiology but also by shaping abundance and composition of denitrifiers. Therefore, unravelling the relationships among denitrifier abundance, activities and soil attributes is central to our understanding of soil denitrification and N_2O dynamics.

Microbial spatial variability remained largely unexplored until the end of the 1980s (Parkin 1987; Robertson, 1988). As a result of the intrinsic heterogeneity of soil resources, microbial abundance also varies spatially across multiple scales (Parkin, 1993). Microbial spatial dependency, in which similarity between samples declines with increasing inter-sample distance, occurs in agricultural (Bru et al., 2010), grassland (Nicol et al., 2003), and forest (Saetre and Baath, 2000) soils. Spatial heterogeneity in denitrifier communities and activities has also been examined in managed ecosystems (Enwall et al., 2010; Parkin 1987; Philippot et al., 2009; Robertson, 1988). Arctic soils are highly heterogeneous (Banerjee et al., 2011a) and recent reports demonstrated high spatial autocorrelation and scale dependence of microbial communities in permafrost soil ecosystems (Banerjee and Siciliano, 2012; Banerjee et al., 2011b). However, no study has elucidated spatial patterns of denitrifier abundance and activity in Arctic soils. As described by Enwall et al. (2010), the spatial link between ecosystem properties and the microbial communities allows us to interpret how field-scale processes influence microbial communities. Thus, for Arctic ecosystems, undergoing climate change and with the potential to release large quantities of greenhouse gases, the link between microbial spatial

structure and soil resources will allow us to downscale climate change models to soil microbial systems. This study aimed to characterize the spatial associations among denitrifier functional groups, potential denitrification and soil properties in three Canadian high Arctic ecosystems. Specifically the following hypotheses were tested: 1) potential denitrification activity is higher in Organic Cryosols than Static or Turbic Cryosols; 2) denitrifier abundance and activities are spatially dependent within a scale of 10 m in Cryosolic ecosystems; 3) the relationships between denitrifier abundance, activities, and soil attributes are more consistent in Organic Cryosols.

6.3 Materials and methods

6.3.1 Research sites

Three Arctic sites were selected for this study: Truelove Lowland, Simpson Lake and Ross Point. Truelove Lowland (75°40′ N, 84°35′ W) has been described by Lev and King (1991), Ma et al. (2007) and Siciliano et al. (2009). It is a coastal lowland covering an area of 43 km^2 on the north-eastern coast of Devon Island. The site is surrounded to the west and north by Jones Sound and two long cliffs to the east and south. The mean annual air temperature is about -16°C with the highest recorded daily temperature of 21°C in July and -45°C as the lowest daily temperatures (Bliss, 1977; King, 1969; Lev and King, 1991). The total annual precipitation is about 185 mm with 36 mm as rain. The topography of Truelove Lowland is distinguished by a series of raised beach crest ridge, lower foreslope, and wet sedge meadow with Regosolic Static Cryosols, Brunisolic Eutric Turbic Cryosols, and Gleysolic Turbic Cryosols, respectively. The lower foreslope is dominated by lichen (*Alectoria* sp.) and upland sedges (*Eriophorum* sp.); as the name indicates, the wet sedge meadow is dominated by sedges (*Carex membranacea* and *Carex stans*) and mosses. Simpson Lake (68°35′ N, 91°57′ W) is situated in the middle of the Boothia Peninsula, approximately 80 kilometres west of Kugaaruk, Nunavut. Historical data

from Kugaaruk suggests the temperature in winter (December-January) varies between -23° to -37°C and in summer (July-August) between 4° to 24°C (Environment Canada, 2011). The total annual precipitation is about 260 mm with 110 mm as rain. The experimental site is dominated by lichen and dry heath vegetation. The upperslope positions are comprised of Static Cryosols whereas lowerslope positions are Turbic Cryosol. Ross Point (68°31′ N, 111°10′ W) is situated in the southern part of Victoria Island. This site is about 150 km west of the town of Cambridge Bay. Historical weather data from the last 30 years in Cambridge Bay suggests the temperature in summer (July-August) ranges between 4° to 23°C and in winter (December-January) between -25° to -36°C (Environment Canada, 2011). The mean annual precipitation is about 138 mm with 69 mm as rain. The soil at this research site was predominantly Organic Cryosols with a relatively high degree of base saturation (indicated by pH) and thick (>15 cm) organic (peaty) horizons. The three research sites selected in this study encompass 7° in latitude and 27° in longitude of the Canadian Arctic. The sites comprise a polar oasis (Truelove Lowland), an Organic Cryosolic ecosystem (Ross Point) and a Static/Turbic Cryosolic ecosystem (Simpson Lake), and hence represent a large portion of the diversity of Canadian permafrost ecosystems.

6.3.2 Transect design

At each site, soil samples were collected along three parallel transects (300 m each) at 31 points (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300 m). The transects were separated from each other by 2 m. The variable-lag-distance transect approach was employed to simultaneously capture the fine, medium, and large scale spatial patterns of denitrifier communities in Cryosolic ecosystems (Banerjee et al., 2011). Soil samples of approximately 250 g were collected at 1 to 10 cm depth using a hand trowel and sieved with a 4.75 mm sieve.

Between samples, sieves and hand trowels were sterilized with 95% ethanol and dried before use. As facilities for performing biochemical and molecular analyses were not available on site, the soil samples were frozen at −20°C and were shipped to the laboratory at the University of Saskatchewan.

6.3.3 Soil analyses

Soil gravimetric water content (θ_g) was estimated by measuring the weight loss of 5 g soil samples after they were dried for 24 hours at 105°C and the water content expressed as percentage of dry soil mass (Gardner, 1986). Soil pH was determined using 5 g soil in a 1:1 soil:water (deionized) mixture with an Accumet pH meter (Accumet 925, Fischer Scientific, Massachusetts, USA). Total organic carbon (TOC) was determined by combustion at 840°C using the Leco CR-12 Carbon Analyzer (LECO Corporation, St. Joseph, Michigan, USA) (Wang and Anderson, 1998). The determined quantity was expressed as a percentage of soil mass. Soil total N (% mass; TN) was determined by dry combustion using a Leco CNS-2000 elemental analyzer (Wright and Bailey, 2001). For extractable nitrate (NO_3^-) content, soil subsamples were shaken with 0.5M K_2SO_4 (1:10 soil: K_2SO_4) for one hour and filtered using Whatman 90 μm filter papers (Maidstone, Kent, England). A 3 ml aliquot of extract was analyzed using a SmartChem™ 200 discrete chemistry analyzer using the manufacturer-provided methods (Westco Scientific Instruments Inc, Brookfield, CT). Samples for chemical analyses were stored at −20°C and microbial cells may have potentially thawed or lysed during sample processing. The extra NO_3^- released from microbial cells may have contributed to slightly higher NO_3^- content.

6.3.4 Denitrification enzyme activity (DEA) and nitrous oxide reductase (N$_2$Of) assay

The assay is designed to measure the overall denitrification potential (DEA) and net N$_2$O formation (N$_2$Of) in soil by alleviating environmental constraints (Smith and Tiedje, 1979; Cavigelli and Robertson, 2000). The DEA and N$_2$Of were measured according to Ma *et al.* (2011). Briefly, soil slurries were prepared in a 70 ml crimp-sealed serum bottle by mixing 10 g soil and 10 ml of a test solution containing 10 mM glucose, and 5 mM KNO$_3$. The serum-bottles were flushed three times with high purity (99.995%) helium (Praxair Inc., Danbury, CT). For DEA, 10% (V/V) acetylene was added into the slurries. Slurries were shaken (100 rpm) at room temperature (~20 ºC) for 90 minutes. The ratio of N$_2$Of to DEA (denoted as *r*N$_2$O) was calculated. A 15-ml gas sample was collected from the headspace of the slurry using a 20 cc disposable syringe equipped with a 25-gauge needle and injected into a pre-evacuated Exetainer® vial (Labco Ltd., UK). Concentrations of N$_2$O in the headspace gas were estimated by a gas chromatograph equipped with an electron capture detector (Yates et al., 2006).

Soil samples were collected during 4th week of July when these research sites regularly experience temperature as high as 24 ºC. Furthermore, soil biological assays in previous studies conducted on these soils also used 20 ºC as the incubation temperature (Banerjee and Siciliano, 2012; Bliss, 1977). Therefore, the incubation temperature selected for the DEA assay in this study can fairly represent the midday temperature during growing season. A single incubation temperature (20 ºC) was selected for consistent measurement of soil samples collected from all three sites. Soil pH in these sites varied considerably and thus the pH of the soil slurry was not adjusted. Overall, the incubation temperature and pH selected for this assay can be representative of the field conditions in these Arctic sites.

6.3.5 DNA extraction and quantification of denitrifier abundance

DNA extraction from soils was done according to the method described by Griffith et al. (2000) with the modifications that DNA samples were precipitated in polyethylene glycol overnight and RNase was not added after extraction. To assess RNA contamination, DNA samples were examined using agarose gel electrophoresis and RNase treatment. No RNA contamination was found in samples. The concentration of purified DNA was determined by spectrophotometer (Ultrospec 2000 ultraviolet (UV)/visible spectrophotometer, Pharmacia Biotech, Cambridge, UK). The number of bacterial *nirK*, *nirS*, and *nosZ* gene copies present in the soil DNA extracts was determined by performing quantitative real-time PCR (qPCR) using the Qiagen QuantiTect™ SYBR® Green PCR Master Mix real-time PCR kit (Qiagen Inc., Ontario, Canada) and an ABI 7500 real-time PCR machine (Applied Biosystems, Foster City, CA). Each 20 µl reaction contained 10 µl of master mix, 10 pmol of the appropriate forward and reverse primers (Table 6.1), 6 µl sterilized milli-Q water, and 2 µl template DNA (1:10 diluted). Two no-template controls (NTCs) were run for each qPCR assay. As concentration of DNA varied between soil samples, the number of gene copies present in each was calculated by determining the concentration of DNA in the product (Ma et al., 2007). Using the same primers, standard curves for *nirS* and *nosZ* were produced using purified PCR products obtained from the reference strain *Pseudomonas stutzeri* (ATTC 14405) and the *nirK* standard curve was prepared from purified PCR products obtained from the reference strain *Achromobacter cycloclastes* ATCC 21921. Only the standard curves linear over five orders of magnitude and r^2 value of 0.99 or higher were selected and standards were run on each individual qPCR plate. For all genes, the efficiency of the reaction was between 80% and 100% (based on the slopes of the standard

Table 6.1 Primers and thermal cycling conditions used for the quantification of denitrifier functional genes

Genes	Primer	Sequence (5'-3')	Fragment length (bp)	Annealing temp (ºC)	Reference
nirK	FlaCu R3Cu	ATC ATG GTS CTG CCG CG GCC TCG ATC AGR TTG TGG TT	473	94°C, 3 min, 1 cycle 94°C for 30 s, 57°C for 1 min, 72°C for 30 s, 80°C for 15 s, 40 cycles 95°C for 15 s, 60 to 95°C, 1 cycle	Hallin and Lindgren, 1999
nirS	nirS 1F nirS 3R	CCT A (C/T) TGGCCGCC (A/G) CA (A/G) T GCCGCCGTC(A/G)TG(A/C/G)AGGAA	256	95°C, 15 min, 1 cycle 94°C for 15 s, 55°C for 1 min, 72°C for 30 s, 80°C for 15 s, 40 cycles 95°C for 15 s, 60 to 95°C, 1 cycle	Braker et al., 1998
nosZ	nosZ F nosZ R	CGCTGTTCITCGACAGYCAG ATGTGCAKIGCRTGGCAGAA	700	94°C, 3 min, 1 cycle 95°C for 15 s, 56°C for 1 min, 72°C for 30 s, 80°C for 15 s, 40 cycles 95°C for 15 s, 60 to 95°C, 1 cycle	Kloos et al., 2001

curves). Consistency of Y-intercept values was checked between different qPCR runs and the specificity of the amplified products was assessed by melting curve analysis. Amplification inhibition effects were assessed by assessing gene abundance on three different dilutions of representative samples and selecting the dilution that minimizes the inhibition (Dumonceaux et al., 2006).

6.3.6 Statistical analysis

To assess the relationships among soil moisture, TOC and denitrifier functions multiple linear regression analysis was performed using SigmaPlot 10.0 software (Systat Software Inc, San Jose, CA). The spatial heterogeneity and spatial relationships were estimated using geostatistical analyses. The degree of spatial continuity was assessed by analyzing the dissimilarity between two observations as a function of the separation distance or lag distance. This dissimilarity is measured through calculating semivariance, $\gamma(h)$, half of the average squared difference between the components of a data pair (Equation 6.1).

$$\gamma(h) = \frac{1}{2N(h)} \sum_{\alpha=1}^{N(h)} [z(x_k) - z(x_k + h)]^2$$
(Equation 6.1)

Here $z(x_k)$ is the property, $z(x_k+h)$ is the value at h lag distance and $N(h)$ is the number of data pairs for a given distance (Goovaerts, 1998). The key features of a semivariogram (a plot of semivariance and lag distance) are range, sill, and nugget. The range of a semivariogram indicates the zone of spatial dependency i.e. the lag distance at which the semivariance value becomes highest whereas sill is the maximum variability attained by the variable. Nugget variance is the random variability due to experimental error. The spatial dependence (SPD) was calculated as: SPD=$C / (C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C+C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (high spatial dependence). All semivariograms were computed with a minimum of 30 sample pairs per lag class (Journel and

Huijbregts, 1978). Gaussian, spherical, exponential models were fitted to the semivariograms using least-squares method in GS+ version 9 (Gamma Design Software, Plainwell, MI, USA).

6.4 Results

6.4.1 Denitrifier activities and functional guilds in Arctic soils

The rate of denitrifier activities varied significantly ($P<0.05$) among three Cryosolic ecosystems with greatest denitrifier activities at Ross Point, the Organic Cryosols (Fig 6.1A). Ross Point soils contain higher moisture, organic matter and N content than the other two sites. The lowest NO_3^- content was observed in Truelove Lowland soils (0.89 μg g^{-1} dry soil) whereas Simpson Lake had the lowest pH among the study sites. The overall denitrification potential (DEA) in Ross Point soils (1034 ng N_2O-N g^{-1} dry soil h^{-1}) was 5-11 times higher than Simpson Lake (230 ng N_2O-N g^{-1} dry soil h^{-1}) and Truelove Lowland (92 ng N_2O-N g^{-1} dry soil h^{-1}) soils, which directly supports the first hypothesis that Organic Cryosols will have higher denitrifier activities. However, net N_2O formation (N_2Of) did not differ between Ross Point (206 ng N_2O-N g^{-1} dry soil h^{-1}) and Simpson Lake (177 ng N_2O-N g^{-1} dry soil h^{-1}), which had 5 times higher rates than Truelove Lowland soils (44 ng N_2O-N g^{-1} dry soil h^{-1}). The value of rN_2O at these three Arctic sites ranged between 0.35 and 0.86 with the lowest rN_2O recorded in Ross Point soils (Table 6.2). Similar to potential denitrification, functional gene abundance also varied significantly (Duncan Test; $P<0.05$) among all three sites (Fig 6.1B). Highest abundance of *nirS* and *nosZ* was found in Truelove Lowland whereas Simpson Lake had the highest abundance of *nirK*. Abundance of *nirK* functional groups in Truelove Lowland soils was substantially lower than other denitrifier groups at all three sites.
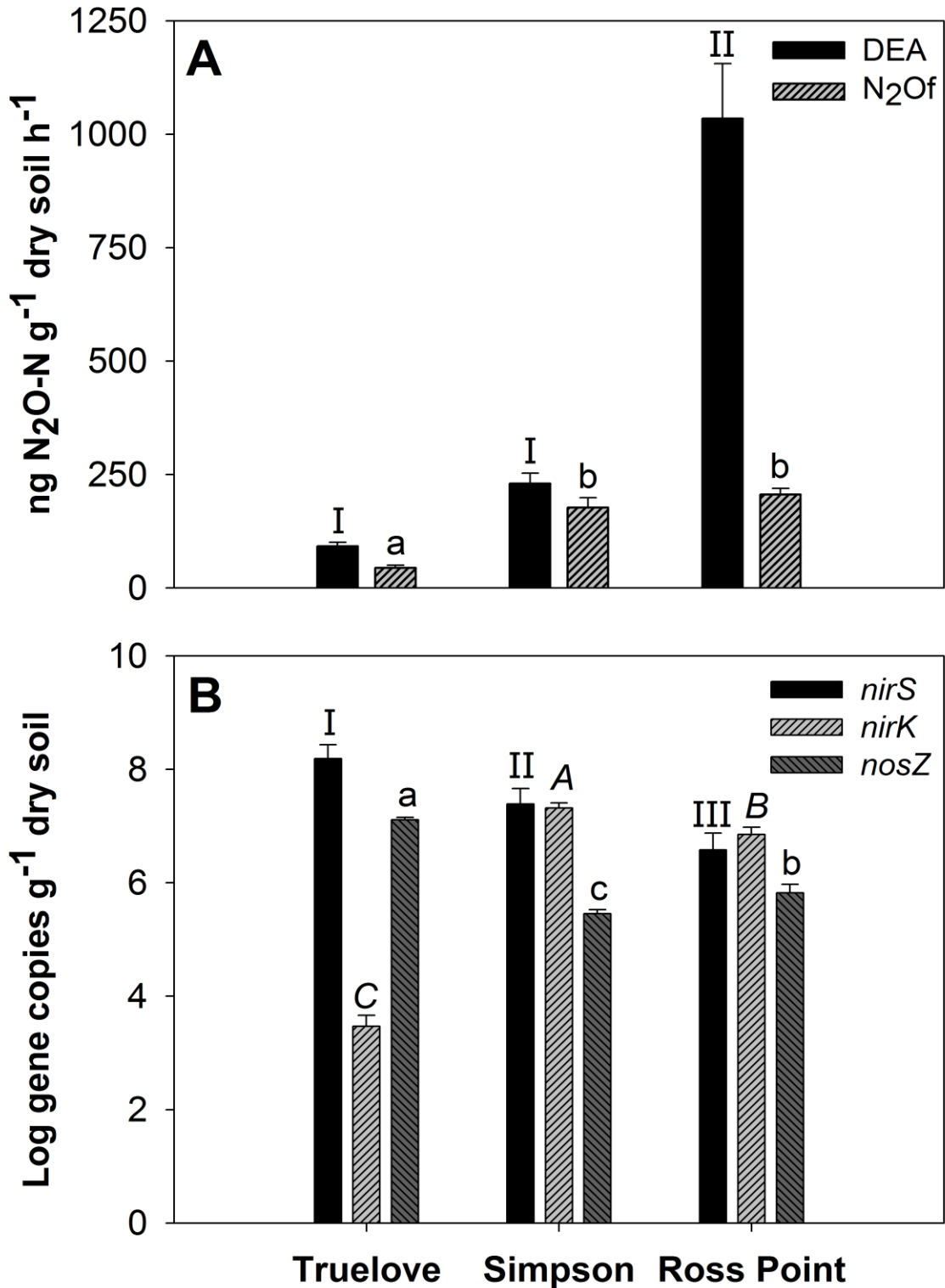
Figure 6.1 A) Denitrifying enzyme (DEA) and N_2O reductase (N_2Of) activity in three contrasting Arctic soil ecosystems: Truelove Lowland, Simpson Lake and Ross Point. B) Abundance of denitrifying functional genes (*nirS*, *nirK* and *nosZ*) in three Arctic ecosystems. Different letters/symbols indicate statistical different at $P \leq 0.05$.

Table 6.2. The rate of N$_2$O consumption (rN$_2$O) and Spearman Rank correlations between rN$_2$O and other variables at three Arctic ecosystems. The rN$_2$O was calculated as the ratio of N$_2$Of to DEA (i.e. rN$_2$O = N$_2$Of/DEA) (Ma et al., 2008; Rich and Myrold, 2004).

	Truelove Lowland	**Simpson Lake**	**Ross Point**
rN$_2$O	0.509 (0.052) [§]	0.857 (0.069)	0.349 (0.037)

Pearson correlation coefficients (r) between rN$_2$O and other variables

nosZ	-0.209	0.070	-0.150
nirS	0.016	-0.186	-0.203
nirK	-0.008	-0.020	-0.180
Moisture	-0.112	0.097	-0.425**
pH	0.151	0.098	0.391**
NO$_3^-$	-0.047	0.091	-0.051
TOC	-0.161	-0.061	-0.619**
TN	-0.096	-0.054	-0.531**

[§]Mean values (n=93) of different variables for three sites.

* and ** indicate relationships significant at *P<0.05* and *P<0.01* respectively

6.4.2 Spatial patterns of denitrifier activities and functional guilds

Spatial analysis revealed that denitrifier functional gene abundance in various Cryosols is spatially autocorrelated within a spatial scale of 5 m, (Figure 2). The value of spatial dependence (SPD) ranges between 0.5 and 0.999, which demonstrates high spatial variance and low nugget (unexplained and stochastic variance) effects. In Truelove Lowland, denitrifiers exhibited different spatial behaviour with *nirS* gene being spatially independent, *nosZ* moderately dependent, and nirK highly dependent. Overall, gene abundance in Ross Point was highly spatially structured within a range between 1.8 m and 3.4 m. Similar to functional groups, denitrifier activities also operated within 5 m spatial scale at all three study sites (Figure 6.3). Although the scale of spatial autocorrelation was small in Simpson Lake (~0.5 m), dependence was extremely high (SPD> 0.95). Ross Point had the largest scale (~4.6 m) of autocorrelation.

6.4.3 Factors driving denitrifier activities in Arctic soils

Denitrifier activities, functional guilds, and soil resources were significantly correlated ($P<0.05$) with each other across the Canadian Arctic but association among soil resources and denitrifier activities were considerably stronger than denitrifier abundance (Table 6.3). Overall, the highest and most consistent correlations were observed in the Organic Cryosols of Ross Point, supporting the third hypothesis of this study. Since soil moisture and total organic carbon content were identified as the key driving factors, their relationships with denitrifier functions were further elucidated by regression analysis. Although significant, the correlations between denitrifier activities and gene abundances were comparatively weaker than soil resources and as such they were not included in the regression analysis. Associations between TOC, moisture and denitrifier functions are highly significant ($P<0.0001$) across the Cryosolic ecosystems (Figure 6.4). In general, the rate of denitrifier activities is high in soils with both high TOC and moisture
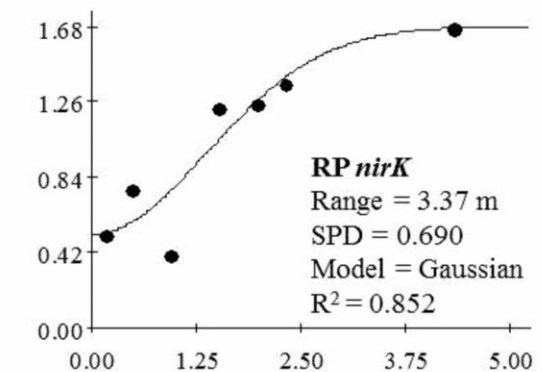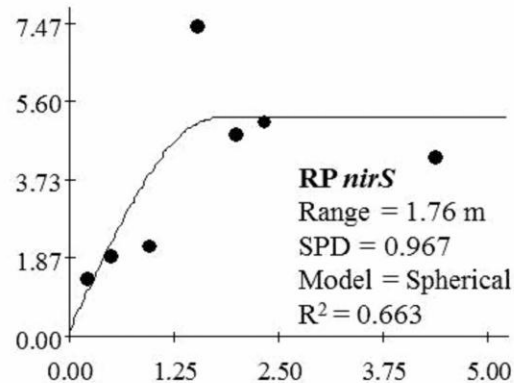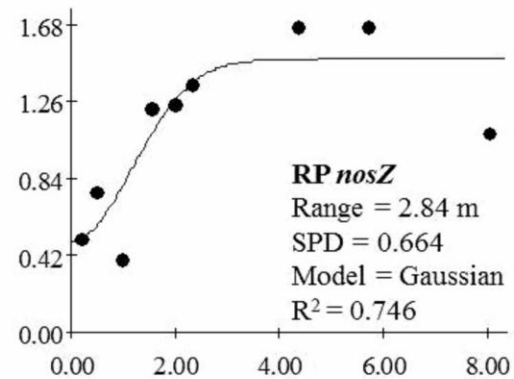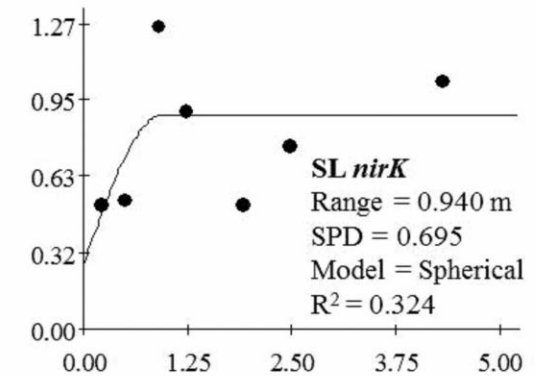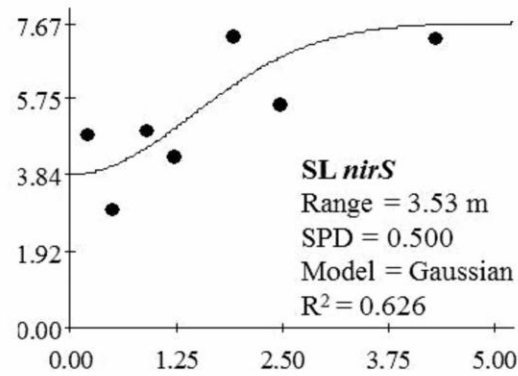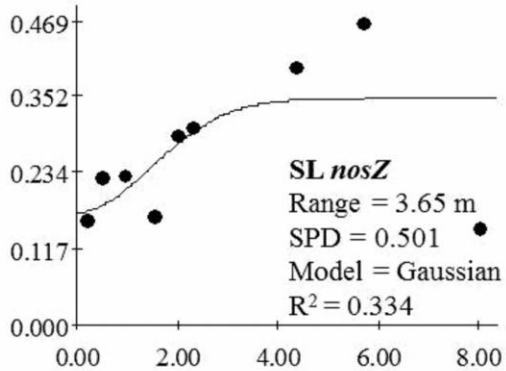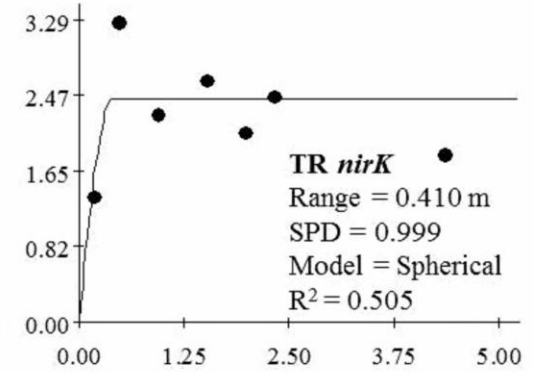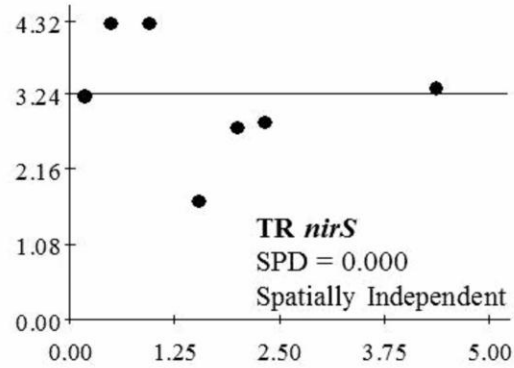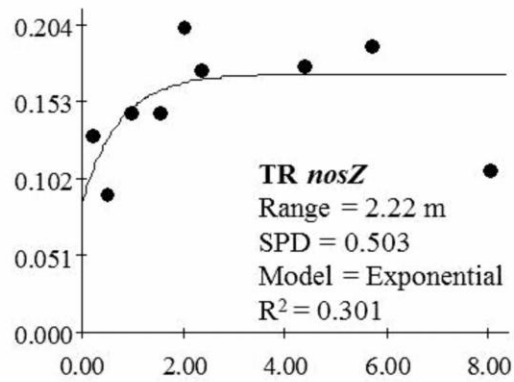
Figure 6.2. Semivariograms showing spatial structure of denitrifier functional genes (*nosZ*, *nirS* and *nirK*) in three Arctic soil ecosystems: Truelove Lowland (TR), Simpson Lake (SL), and Ross Point (RP). Range indicates the area of spatial dependence (SPD). Spatial dependence (SPD) was calculated as: SPD=$C / (C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C+C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (high spatial dependence). Various models (Gaussian, spherical, and exponential) were fitted (solid line) to each semivariogram. Spatial dependency was considered from fine (10 cm) to large scale (300 m); the semivariograms are shown up to specific lag distance for clarity of spatial patterns near origin. Each point in the semivariogram represents the mean semivariance (dissimilarity) for a single lag class, which is a group of pairs separated by a specific lag distance.

Figure 6.3. Semivariograms exhibiting spatial structure of denitrifier activity (DEA, N_2Of, and the ration of N_2Of to DEA i.e. rN_2O) in three Arctic soil ecosystems: Truelove Lowland (TR), Simpson Lake (SL) and Ross Point (RP). Range indicates the area of spatial dependence (SPD). Spatial dependence (SPD) was calculated as: $SPD=C / (C + C_0)$, where C is the structural variance, C_0 is the nugget, and $C+C_0$ is the sill. Values of SPD vary from 0 (no spatial dependence) to 1 (high spatial dependence). Various models (Gaussian, spherical, and exponential) were fitted (solid line) to each semivariogram. Spatial dependency was considered from fine (10 cm) to large scale (300 m); the semivariograms are shown up to specific lag distance for clarity of spatial patterns near origin. Each point in the semivariogram represents the mean semivariance (dissimilarity) for a single lag class, which is a group of pairs separated by a specific lag distance.

Table 6.3. Spearman Rank correlation coefficients among denitrifier abundance, denitrifier activities, and soil properties at three Arctic soil ecosystems: Truelove Lowland (a), Simpson Lake (b), and Ross Point (c).

a)

Truelove Lowland					
	nosZ	*nirK*	*nirS*	DEA	N$_2$Of
nosZ		0.140	0.351**	0.291*	0.201
nirK			0.316**	0.047	0.105
nirS				0.114	0.183
DEA					0.679**
Moisture	0.349**	0.048	0.181*	0.687**	0.677**
pH	-0.025	-0.253*	-0.107	-0.300*	-0.257*
NO$_3^-$	0.354**	0.002	0.182	0.631**	0.727**
TOC	0.362**	-0.003	0.066	0.579**	0.529**
TN	0.373**	-0.020	0.097	0.688**	0.684**

b)

Simpson Lake					
	nosZ	*nirK*	*nirS*	DEA	N$_2$Of
nosZ		0.611**	0.497**	0.338**	0.338**
nirK			0.460**	0.285**	0.288**
nirS				0.438**	0.324**
DEA					0.804**
Moisture	0.367*	0.300*	0.306*	0.518**	0.500**
pH	0.026	0.196	-0.120	0.131	0.176
NO$_3^-$	-0.089	0.215*	0.163	-0.065	0.038
TOC	0.205	0.304**	0.413	0.508**	0.431**
TN	0.284**	0.208	0.367*	0.495**	0.375**

c)

Ross Point					
	nosZ	*nirK*	*nirS*	DEA	N$_2$Of
nosZ		0.733**	0.676**	0.254*	0.280**
nirK			0.865**	0.257*	0.259*
nirS				0.246*	0.241*
DEA					0.705**
Moisture	0.264**	0.343**	0.293**	0.705**	0.746**
pH	-0.199	-0.314**	-0.252**	-0.578**	-0.555**
NO$_3^-$	0.034	0.169	0.051	0.159**	0.247**
TOC	0.286**	0.338**	0.303**	0.812**	0.704**
TN	0.319**	0.334**	0.287**	0.756**	0.682**

* and ** indicate significance at *P*<0.05 and *P*<0.001 respectively
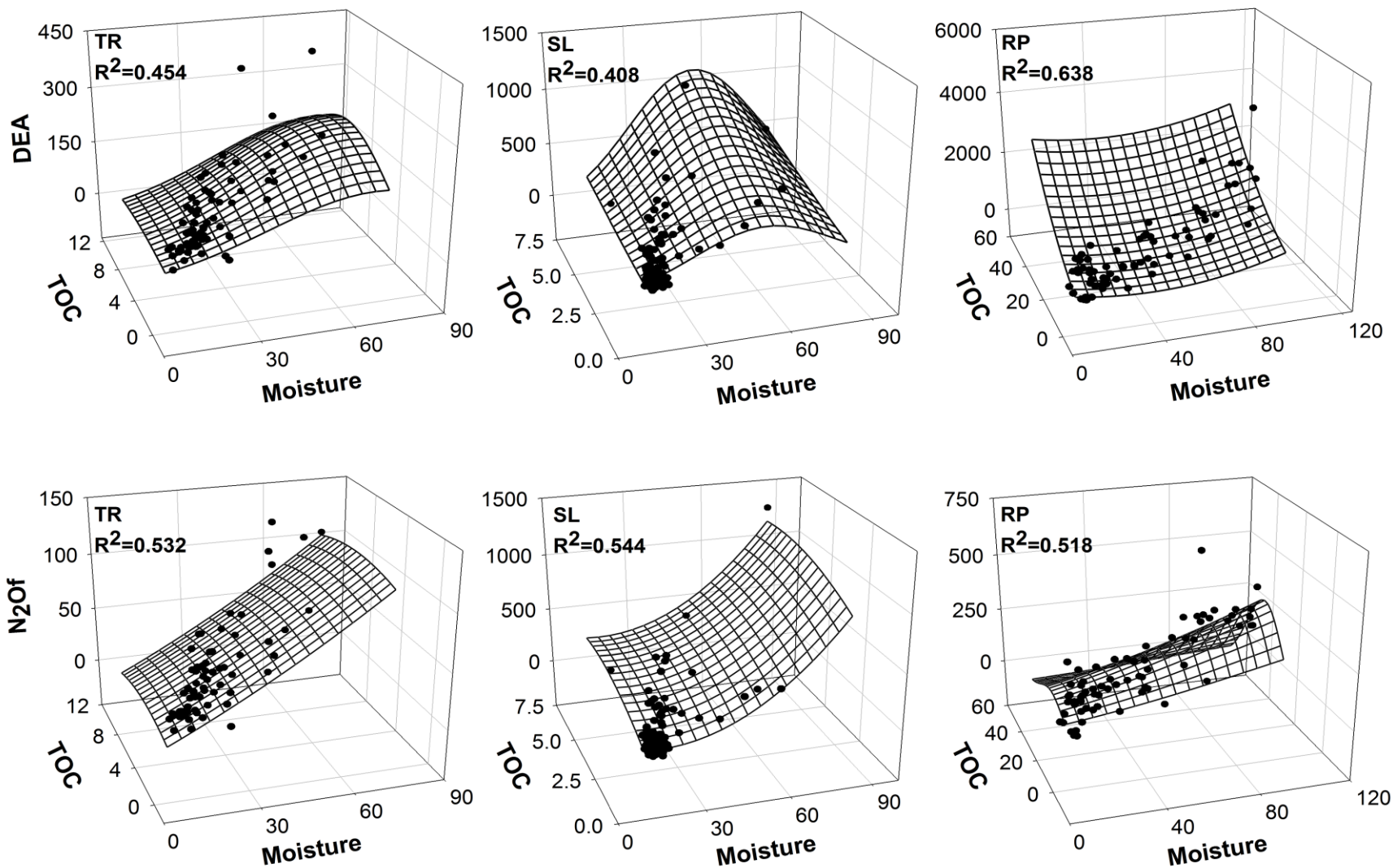
Figure 6.4. Relationships among denitrifier activities, soil moisture and total organic carbon (TOC) content at three Arctic ecosystems: Truelove Lowland (TR), Simpson Lake (SL) and Ross Point (RP). Multiple regression analysis was performed between soil resources and denitrification potential (DEA) and net N_2O formation (N₂Of). The pattern and strength of the associations are shown by response surface i.e. grid lines and R^2 respectively.

content. However, the nature (demonstrated by the response surface) of these associations varies considerably among three sites. Although the strength of correlation did not differ markedly between DEA and N_2Of and among the research sites, correlation was slightly stronger for DEA at Ross Point.

6.5 Discussion

Arctic soil ecosystems are often assumed as 'functionally inert' due to their slow turnover rate and thus very few studies have measured DEA rate in Arctic soils (Bjork et al., 2007; Chapin, 1996). The results suggest that these ecosystems possess strong denitrification potential and this is particularly true for Organic Cryosols. The denitrifier activities in Organic Cryosols are in the same range as non-Arctic soil ecosystems such as agricultural (Cavigelli and Robertson, 2000; Enwall et al., 2010), grassland (Cuhel et al., 2010; Miller et al., 2009; Philippot et al., 2009), and riparian and creek (Rich and Myrold, 2004). In general, the rates of DEA and N_2Of in Truelove Lowland and Simpson Lake were lower than agricultural or pristine soil ecosystems. The soils with high DEA activity did not necessarily have high N_2Of activity, which is consistent with the results of Philippot et al. (2011). The rate of DEA in Ross Point (Organic Cryosols) is in the similar range as a heath snowbed whereas DEA in Truelove Lowland and Simpson Lake was comparable to dry heath soils measured in Northern Sweden (Bjork et al., 2007). In Truelove Lowland soils, Chapin (1996) found that denitrification activity is primarily driven by moisture and nitrate content, however, denitrification is comparatively higher in drier hummocks, which differs from the results presented in this study. Denitrification activity is typically higher in soils with high moisture and nitrate content (Knowles, 1982; Wallenstein et al., 2006) and as such high DEA rate in Organic Cryosols is expected. Ross Point soils have higher moisture, C, and N content and thus these nutrient-rich soils can be extremely dynamic. This is consistent with the

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findings of Banerjee and Siciliano (2012) who demonstrated significantly higher nitrification potential in Organic Cryosols of Ross Point than the other two sites and this functional potential was the foundation of the first hypothesis.

The abundance of denitrifying bacteria is usually assessed by quantifying functional genes i.e., genes encoding enzymes responsible for different steps of the denitrification pathway. The functional genes involved in denitrification are *narG*, *nirS*, *nirK*, *norB*, and *nosZ* (Philippot et al., 2002). The *narG* gene can be found in both denitrifying and non-denitrifying organisms, and thus is not a suitable marker. Moreover, primers for *norB* are mainly designed for a single organism. Many *nirS* and *nirK* primers will also amplify sequences from non-denitrifying microorganisms and further, are not necessarily linked to N_2O emissions. However, given the difficulties associated with *norB* primers, *nirS* and *nirK* genes remain popular choices and have regularly been used for quantification of denitrifier abundance. In spite of the cryopedogenic processes in Arctic, the overall abundance is also similar to other ecosystems such as grassland (Keil et al., 2011; Miller et al., 2009), agricultural (Miller et al., 2008; Enwall et al., 2010), and forest (Levy-Booth and Winder, 2010). The abundance of denitrifier functional genes in this study is similar to previous reports from Arctic soils (Lamb et al., 2011; Siciliano et al., 2009). However, previous studies investigating denitrifier abundance in Arctic soils focused on a single research site and did not comprehensively elucidate abundance patterns in different types of Cryosols. Results from this study demonstrate that distribution of denitrifier functional groups varies significantly among the sites; this difference was also observed when gene abundances were calculated per ng DNA (data not shown). In general, Truelove Lowland had high denitrifier abundance which is in agreement with the findings of Banerjee et al. (2011b) who found that overall bacterial biomass at Truelove Lowland (2.9×10^{10} copies g^{-1} dry soil) was substantially

higher than at Simpson Lake (4.2×10^8 copies g^{-1} dry soil) or Ross Point (1.5×10^9 copies g^{-1} dry soil). Interestingly, Truelove soils had highest abundance of *nirS* and lowest abundance of *nirK* functional groups whereas Ross Point had similar abundance of all denitrifier groups. It should be noted that Truelove Lowland is strongly N limited with lowest nitrate content whereas Ross Point soils had highest nitrate content. It may indicate a possible niche-differentiation of nitrite reducing denitrifier groups in Static/Turbic Cryosols due to N limitation whereas these groups are all equally present in nutrient rich Organic Cryosols. However, the patterns of abundance of nitrite reducing (*nirK* and *nirS*) and nitrous oxide reducing (*nosZ*) functional groups differed between at the three study sites. Others have also found that these groups differ in their distribution pattern in Cryosols, although these other reports were in glacier forefield soils which are considerably harsher environments (due to large temperature shifts, UV radiation, and large humidity fluctuations) than those studied here (Kandeler et al., 2006; Brankatschk et al., 2010; Zumsteg et al., 2011). The *nosZ* primers selected in this study have been successfully used to estimate *nosZ* abundance in many soil microbiological studies (Geets et al., 2007; Liu et al., 2010; Ma et al., 2011; Rich et al., 2003; Rösch et al., 2002; Vlaeminck et al., 2007). However, the *nosZ* amplicons generated by these primers are longer than 500 bp and may not be ideal for qPCR work. Therefore, future studies may wish to corroborate *nosZ* abundance using primers such as those designed by Henry et al. (2006) that yield comparatively short amplicons.

The rN_2O (the ratio of N_2Of to DEA) indicates the rate of N_2O accumulation; it can be used to explain the balance between N_2O formation and reduction. Low rN_2O indicates that high N_2O consumption by N_2O reductase whereas higher values indicate that relatively little N_2O reduction is occurring. Similar to Rich and Myrold (2004), this study found rN_2O varied among the eco-habitats. Lower rN_2O is linked to greater soil carbon content while higher rN_2O is linked

to lower pH (Firestone et al., 1980; Rich and Myrold, 2004). Because rN_2O is influenced by soil properties, it explains the lack of the strict congruence between gene abundance and activity. Ross Point, with the highest organic carbon had a much lower rN_2O compared to Truelove Lowland despite similar *nosZ* abundance; Truelove soils have one-fifth less organic carbon content than Ross Point. Similarly, the high rN_2O in Simpson Lake is linked to the differences in pH among the sites.

Scale of spatial patterns has significant implications for pedological and ecological sampling strategies and in this aspect geostatistics may be highly useful. Geostatistics is a powerful statistical tool for elucidating microbial spatial variability. By focusing on the dissimilarity between data pairs as a function of distance (semivariance) and modelling spatial dependency, geostatistics estimates uncertainty and interpolate values at unsampled areas (Franklin and Mills, 2007). Overall, this study found that denitrifier functional groups and potential denitrification in Cryosolic eco-habitats are highly spatially structured and their spatial dependence is in the same range as agricultural (Enwall et al., 2010) and grassland (Keil et al., 2011; Philippot et al., 2009) soils. The range of spatial autocorrelation of denitrifier functional guilds and activities were substantially smaller than what has been previously reported in agricultural (> 200 m; Enwall et al., 2010) and grassland (~60 m; Keil et al., 2011) ecosystems. Cryoturbation, soil movement as a consequence of frost action, is a predominant cryopedogenic process occurring in permafrost ecosystems (Bockheim and Tarnocai, 1998). These cryopedogenic processes in the Arctic operate at small scales and form distinct patches. Inherent cryopedogenic processes in Arctic ecosystems, particularly cryoturbation, result in ice-segregation, movement of soil mass, irregular soil horizon, and accumulation/redistribution of organic matter (Bockheim and Tarnocai, 1998). Together, these mechanisms largely alter the

spatial nature of soil properties, which is likely why the spatial scale in these Arctic ecosystems is so much lower than that seen in temperate ecosystems. Supporting this idea that cryoturbation leads to lower spatial scales, the largest range of spatial dependence was found in Ross Point, an Organic Cryosols. Organic Cryosols are generally more homogenous and more consistent than Static or Turbic Cryosols and thus spatial similarity extends over a longer distance. A distinct similarity in spatial structure of denitrifier functional guilds and potential denitrification was detected in these soils and this indicates that DNA based estimations of gene abundances may reflect the functional potential of Arctic denitrifier communities as has been seen in agricultural soils (Morales et al., 2010).

Denitrifier functional genes have been frequently linked to denitrifier activities, however, relatively weak and sometimes no correlations have been previously reported among soil resources and denitrifier abundance (Cuhel et al., 2010; Enwall et al., 2010). This study found that denitrifier abundance was significantly correlated to the all tested soil attributes, particularly in the Organic Cryosols at Ross Point. Consistently higher correlations in Organic Cryosols may have been caused by its relatively greater spatial homogeneity, which was also noted previously (Banerjee et al., 2011b). Overall, the strong associations among denitrifier abundance, potential denitrification, and soil resources found in present study may indicate that Arctic ecosystems are confronted with severe nutrient-limitation and thus soil resources have a more readily detectable impact on microbial distribution and activities. The nature of this soil resource control on microbial activity differed between the sites, likely because the TOC and moisture contents differed significantly across the sites. For example, the relationship patterns of DEA and N_2Of, differ between Simpson Lake and Ross Point, suggesting niche-partitioning of

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denitrifier and N_2O reducing communities as reported previously in non-Arctic ecosystems (Cuhel et al., 2010; Enwall et al., 2010).

A recent report found denitrifier functional gene abundance as a key factor determining denitrification-mediated N_2O emissions from agricultural soils (Morales et al., 2010). In contrast, this current study demonstrated that soil moisture and TOC content are the major factors shaping N_2O production/consumption in Arctic soils although other attributes such as pH, TN, NO_3^-, and functional gene abundance are also correlated to denitrification potential but at weaker levels. Although strong nutrient limitation in the Arctic may be a possible reason for this direct impact, the importance of soil resources as the primary drivers of denitrifier activities is also true for other ecosystems (Attard et al., 2011). The reason for this difference is likely linked to the identity of the denitrifier community at each site. That is, environmental factors exert their effects on denitrification through the denitrifying community (Wallenstein et al., 2006) and potential denitrification is often only associated with one of the functional guilds such as *nirS* (Cuhel et al., 2010), *nirK* (Attard et al., 2011), or *nosZ* (Philippot et al., 2009). Thus, the nature of the community at the site will modulate how the soil resource $\rightarrow$ gene abundance $\rightarrow$ ecosystem activity path is expressed. This study postulates that because soil resources are typically limited in Arctic ecosystems, their influence on ecosystem activity masked gene abundance effects. In contrast, gene abundance links to ecosystem activity in agricultural ecosystems with their eutrophic environment would likely mask soil resource modulations. Previous studies reported strong association between denitrifier abundance and organic carbon pool (Henry et al., 2004; Kandeler et al., 2006). Thus, this study suggests that the dominant control on ecosystem level denitrifier activity is moisture and organic carbon. Further, microbial

abundance controls on ecosystem level activity while undoubtedly present, are over-ridden in the nutrient poor Arctic environment.

This study evaluated 279 soil samples collected from three research sites encompassing $7°$ in latitude and $27°$ in longitude of the Canadian Arctic. Arctic Cryosolic ecosystems are highly vulnerable to global warming and have been predicted to undergo greatest degree of changes. An increase of 3-4°C in mean annual air temperature by 2020 and 5-10°C by 2050, and as a consequence change in the balance between gains and losses of N have also been projected (Christensen et al., 1999; IPCC, 2007; Tarnocai, 2006). This study demonstrated that Cryosolic ecosystems, particularly Organic Cryosols, have high denitrification potential and denitrifier abundance, similar to tropical and temperate ecosystems. Soil resources and the abundance of the denitrifier functional gene, *nirK*, were significantly correlated to N_2O production/consumption rate. Soil moisture, organic carbon, and N contents are the key factors that drive denitrification in Cryosols, Denitrifier abundance and activities are spatially well-structured and the spatial dependence of these parameters comprised within 5 m scale due to cryopedogenic processes. Thus, experimental designs on denitrification in Arctic regions should likely have field replicates greater than 5 m apart to insure independence. Future work will evaluate if the soil and gene parameters identified here can predict in situ, i.e. field, denitrification activity. Overall, the high N_2O emission potential of Arctic soil ecosystems highlights that these soils could emit significant amount of N_2O gas upon favourable conditions, and this could have important implications in the light of the pervasive global climate change.

7.0 CONCLUSIONS AND FUTURE WORK

7.1 Major findings

This study examined soil and microbial spatial variability in 279 soil samples collected from Truelove Lowland, Simpson Lake, and Ross Point sites separated by 7° in latitude and 27° in longitude of Canadian Arctic. There are several aspects that this study investigated for the first time in Arctic ecosystems. The following section renders a detail account of the principal findings from this study.

7.1.1 High functional potential of Cryosols

The results showed extremely high potential for both ammonia oxidation and N_2O production/consumption. In general AOP in Organic Cryosols of Ross Point is 3-9 times higher than what was measured at Static or Turbic Cryosols of Truelove Lowland and Simpson Lake. Likewise, N_2O emission potential at Ross Point was 5-11 times higher than Simpson Lake and Truelove Lowland soils. Overall, Organic Cryosols of Canadian Arctic has strong potential for high N cycling processes resulting in N_2O emissions. This reinforces recent evidence that both nitrification and denitrification can occur simultaneously in same soil ecosystem and microbial communities. Soil organic matter preserves moisture and consequently, Organic Cryosols at Ross Point have highest moisture and total organic matter content. However, extractable ammonium and nitrate content were also high in Ross Point soils, which combined with optimum pH resulted in high functional activity. The overall N_2O emission potential in Cryosols, particularly Organic Cryosols, is in the range or even higher than what has been measured for agricultural or grassland soils.

The data presented in this study has important implications for studies examining N_2O and other greenhouse gas dynamics in Arctic soils, particularly in light of global climate change. Arctic ecosystems will experience an increase of 3-4°C in mean annual air temperature by 2020

and 5-10°C by 2050. Consequently, the slow turnover rate of these soils may soon change and the balance between gains and losses of N may disrupt. The high functional potential of Arctic soil ecosystems reveals the probability of substantial N_2O emissions upon availability of favorable conditions. The results highlight the significance of Organic Cryosols in N_2O dynamics and accordingly, greenhouse gas emission and climate change models should incorporate the significant role of Organic Cryosols in Arctic.

7.1.2 Heterotrophic ammonia oxidation potential (HAOP)

Heterotrophic bacteria and fungi may oxidize organic N or ammonium but also use organic carbon and aerobic respiration to produce energy. The heterotrophic ammonia oxidizing microorganisms occupy a unique niche in complex N cycling. Although the existence of heterotrophic ammonia oxidation has been known since as early as 1933, relatively few studies have investigated these microbes and their activities. This dearth of knowledge on heterotrophic nitrification is reinforced by the fact most studies examining this process were done on forest or grassland soils and this is first study to present the evidence in Arctic soil ecosystems. The findings show that heterotrophic ammonia oxidation may represent a substantial stake in overall ammonia oxidation potential in Arctic soils. In Static/Turbic Cryosolic ecosystems with adequate moisture and ammonium content, heterotrophs can contribute up to one third of total ammonia oxidation. Although heterotrophic nitrification is particularly important in soils with high organic matter content, such high contribution of heterotrophs in Arctic was completely unknown. Future studies should measure HAOP in other Cryosolic ecosystems and should also utilize methods such as isotope pool dilution to assess this process. Furthermore, little is known about the genes associated with heterotrophic ammonia oxidation so it was not possible to establish the

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connection between process and functional genes. Future study should examine the molecular level evidence of heterotrophic ammonia oxidation.

7.1.3 High abundance of overall microbial and N cycling functional communities in Cryosols

Soil microbial communities experience extremely harsh environmental conditions such as regular occurrence of cryopedogenic processes, prolong freeze-thaw cycles, short unfrozen condition, desiccation and starvation. Thus, one may expect low microbial abundance in Cryosolic ecosystems. This study found a high prevalence of archaeal, bacterial, and fungal communities in different types of Cryosols, which is higher than what has been reported in Arctic and alpine soil ecosystems previously and even in the same range as agricultural soils. Truelove Lowland has significantly larger microbial populations than Simpson Lake and Ross Point, which can be ascribed to its ecological uniqueness being an oasis and animal-hotspot in the middle of a vast polar desert. High abundance of microbial communities in Truelove soils is also reflected in ammonia oxidizing and denitrifying functional groups. The copy number of these ecofunctional genes at Truelove was also similar to what is reported in non-Arctic soil ecosystems.

7.1.4 Spatial structure of soil properties and microbial communities in Arctic

Spatial heterogeneity of soil resources has important ecological implications for the microbial communities harboring the multifaceted environment of soil. The structural, physical, chemical and biological complexities of the soil microhabitats exert strong effects on microbial abundance, spatial distribution, and most importantly their microbial functional processes. Since its recognition in ecology and soil science, spatial dependency has been extensively studied for most soil physical and chemical properties in agriculture, forest, grassland, contaminated ecosystems of tropical and temperate regions ensuing plethora of studies (Cerri et al., 2004; Iqbal

et al., 2005; Mzuku et al., 2005). Soil biological properties and microbial spatial structure have also been examined in such ecosystems (Becker et al., 2004; Bengston et al., 2007; Robertson et al., 1997; Saetre and Baath, 2000). Nonetheless, this is the first study examining soil and microbial spatial dependency in Arctic soils. Results comprehensively show that soil attributes in Arctic ecosystems are spatially well structured and their spatial dependency is in the line with other non-Arctic ecosystems.

Organic matter content is known for moisture holding capacity and consequently high moisture content was found in Organic Cryosols at Ross Point. These soils have fewer occurrences of typical cryopedogenic processes and comprise relatively homogenous moisture gradient and vegetation, and fewer hummocks. As a result, the zone of spatial autocorrelation operates up to 10 m in Static/Turbic Cryosols and 40 m in Organic Cryosols, which is also reflected in microbial spatial distribution and in the associations between microbial communities and soil properties. Microbial correlations with soil resources are more consistent in Organic Cryosols than Static or Turbic Cryosols. Spatial ecological parameters can provide important information whilst designing sampling scheme such as size, shape, or direction. Based on this study, it is recommended that for collecting independent soil samples the minimum distance between samples is 10 m in Static or Turbic Cryosols and 45 m in Organic Cryosols, although for microbial abundances and functional processes the recommended minimum intersample distance is 5 m.

7.1.5 Predominant controls on ammonia oxidation and denitrification activity

Ammonia oxidation is the rate limiting and a crucial step in N cycling. It constitutes the first step of nitrification and thereby offers the substrate for denitrification. On the other hand, denitrification is a major microbial respiratory process and an important component of ecosystem

N cycling. Together, ammonia oxidation and denitrification help regulate the availability of N compounds. Thus, for strongly N limited ecosystems such as the Arctic, these processes play an extremely important role. Furthermore, these processes contribute to 70% of global N_2O emissions. Findings of this study demonstrate that the factors driving ammonia oxidation and denitrification operate at multiple spatial scales. However, in general, soil attributes rather than functional gene abundance are the predominant controls on these processes. Soil moisture, TOC and TN content are the principal determinants across all studied spatial scales. Although similar factors also drive these processes in other tropical and temperate ecosystems, their associations are considerably stronger in Cryosols, highlighting their importance in Arctic ecosystems.

7.1.6 Ecological significance of nitrifier and denitrifier spatial patterns in Arctic soils

Spatial variability of soil properties is known for last four decades (Nielsen et al., 1973) and it has been investigated in various agricultural and pristine soil ecosystems of tropical and temperate regions, resulting in a myriad of journal articles. Moreover, microbial spatial variability is also known for last three decades (Parkin, 1987) and it has been elucidated in most ecosystems. Importance of scale is now widely-accepted, and such that 'scale' has become the new ecological buzzword (Wiens, 1989). Spatial scaling of soil and microbial ecosystems has attracted particular interest among ecologists. A central goal in ecology is recognizing the spatial scale at which microbial patterns and functions can be explained by underlying processes (Ramette and Tiedje, 2007). However, despite the considerable interests and research in spatial variability, little is known about the functional implications of spatial patterns. Therefore, linking spatial patterns and scaling of ecofunctional communities and ecological processes would open a new direction and would enrich spatial ecology. Since nitrification denitrification traits are widely distributed in microbial communities, nitrifier and denitrifiers can serve as model

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ecofunctional communities to study the ecological ad functional significance of microbial spatial patterns.

To further extend the notion of microbial spatial variability and how it affects the scaling of microbial/ecological processes, this study assessed the scale-dependent associations among nitrifier and denitrifier communities, potential ammonia oxidation and denitrification, and soil resources. Microbial spatial scaling was indeed reflected in their functions in Cryosolic ecosystems. This study demonstrated that ammonia oxidizing and denitrifying communities, and their functional potential were spatial structured within a similar distance. Spatial patterns of microbial communities and functional potential are highly similar to that of soil resources and more importantly, this spatial scale can also be attributed to soil spatial scaling and inherent cryopedogenic processes. Thus, microbial spatial patterns and functions can indeed be explained by underlying processes and vice versa. Furthermore, co-spatial dependency of different groups of ammonia oxidizing and denitrifying communities indicates that these functional groups are modulated by similar edaphic factors. On the other hand, considerably high abundance of individual functional groups suggests the existence of microhabitat partitioning and distinct ecological niches in Arctic soils. Overall, spatial patterns of soil factors are reflected in microbial communities, and then the spatial relationships of soil resources and functional communities are strongly reflected in ecological processes such as nitrification and denitrification. Thus, this study demonstrates the ecological significance of spatial patterns of nitrifying and denitrifying communities in Arctic soil ecosystems.

7.2 Future Directions

There is a strict congruence between knowledge and research: the more one attains the more there is to explore. This PhD project constitutes the first spatial ecology study in Arctic. It addressed some significant, yet novel, aspects of Arctic N cycling communities such that it fills an important niche in Arctic microbial ecology. Nonetheless, there are several areas and components that this project could not incorporate and following section will illustrate those areas and highlight the possibilities and technologies that future studies may find essential. Firstly, this study only assessed the ammonia oxidation potential and denitrification potential using a lab based method. Although, evidence was presented to show that these potential methods strictly reflect field-based measurements, the actual N_2O flux and rate of nitrification and denitrification may be different. Future studies should measure *in situ* N_2O flux using new methodologies such as Multicomponent Fourier Transform Infrared Gas Analyzer. Furthermore, researchers may also wish to delve into N cycling functional processes using methods based on ^{15}N such as isotope fractionation, isotope dilution, ^{15}N mass balances, and direct measurement of ^{15}N labeled gases. Secondly, this study could not incorporate spatial interpolation to construct spatial map showing distribution patterns of soil properties and microbial communities across landscapes. These spatial maps are highly useful in linking microbial and environmental factors to mesotopographical parameters or plant community distribution. Ecosystem processes at field scale is governed by a combinations of several microbial communities. It is intriguing how distribution pattern of microbial communities at medium or large scales is reflected in their functions and as such ecosystem functioning, and spatial maps can provide this invaluable information. Simple interpolation techniques such as Kriging can construct such maps, which can be finally included in larger spatial modelling maps. Soil is an extremely diverse

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Spatial interpolation and kriging

Distance → Soil attributes

Metagenomics

Microbial communities

Metatranscriptomics, microarray with qPCR

Microbial activities

In situ nitrification, denitrification, and N$_2$O flux

Ecological processes
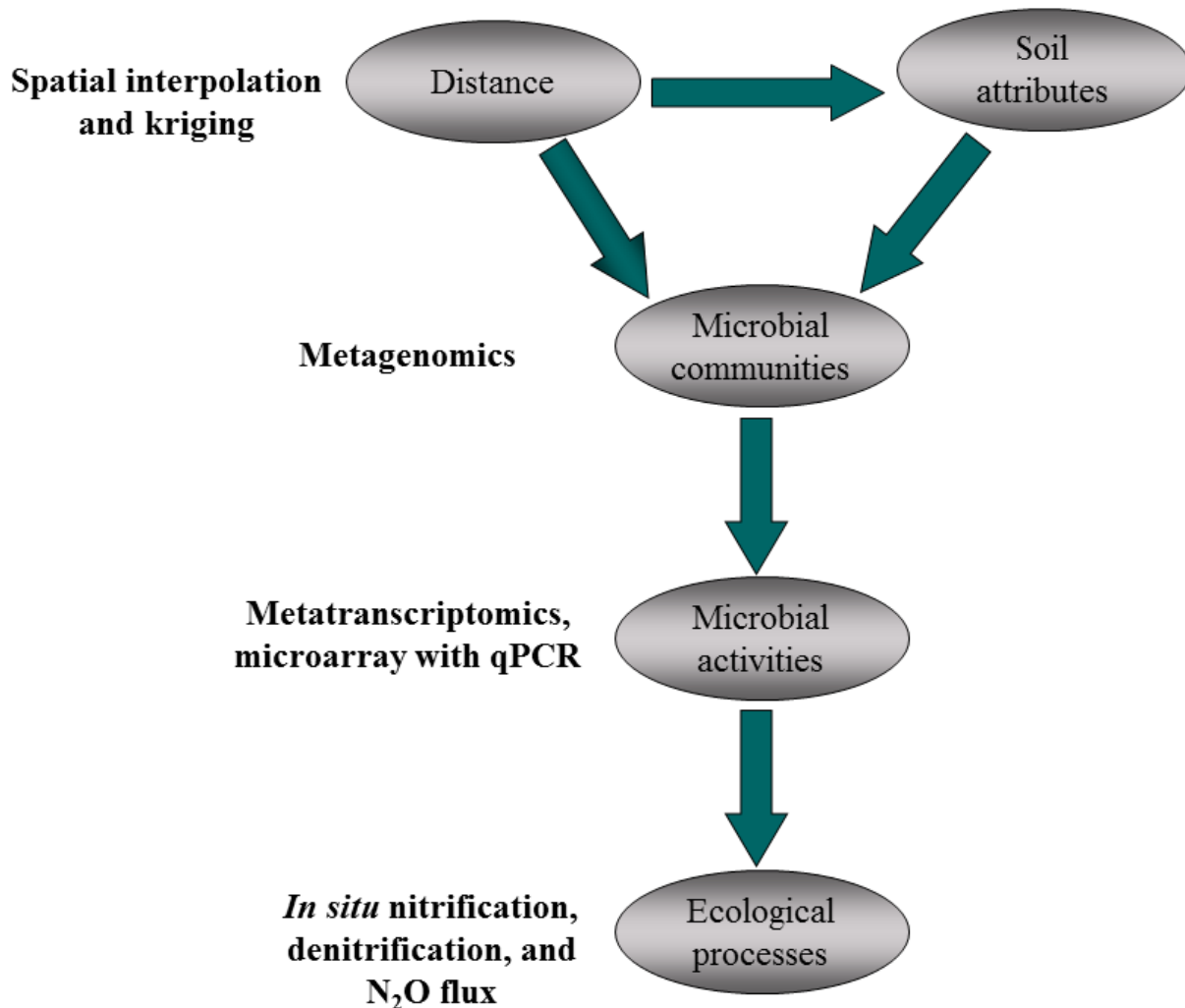
Figure 7.1 Conceptual model showing how relationships among distance, soil resources, and microbial communities may eventually drive microbial activities and ecosystems functioning. It also shows how future studies may focus on these processes by incorporating new techniques and field based measurements.

environment. While heterogeneity in space is very important, vertical heterogeneity is also crucial especially in Cryosols where a distinct permafrost table is present. The abundance, community composition, and distribution pattern of N cycling functional groups in a vertical profile may ultimately control the gas flux though permafrost. Future studies may wish to corroborate this nascent area of research.

This study assessed the abundance of overall microbial, ammonia oxidizing and denitrifying communities in Arctic soils. However, it would also be interesting to examine their community composition and diversity. Future studies may wish to employ new high throughput sequencing technologies of metagenomics such as 454 pyrosequencing, Illumina/Solexa Genome Analyzer and the Applied Biosystems SOLiD^{TM} System to comprehensively characterize microbial diversity in Cryosols. Moreover, this study shows the high functional potential of different types Cryosols, specifically Organic Cryosols. However, DNA based methodologies cannot intricately reveal current or *in situ* status of soil microbial system. Gene expression patterns of ammonia oxidizing and denitrifying functional guilds in Cryosols can explain differences in those biogeochemical processes in a soil ecosystem. Microarray in combination with qPCR may serve this purpose. With the advent of metatranscriptomics, a direct cDNA cloning technique that reveals the composition of RNA transcripts and identifies active genes undergoing transcription (Morales and Holben, 2011), functional potential of Arctic can go through enhanced elucidation.

The role of microbial communities in biogeochemical cycling and ecosystem functioning is imperative, such that they can be easily called 'the drivers of ecosystems'. Microorganisms are also known for their strong resilience to ever-increasing changes in ecosystems and thus they can play a pivotal role in constituting inertia of Arctic ecosystems to global climate change. Arctic regions have been predicted to experience 4°C increase in temperature by 2020. This study presented a multilevel assessment of the current status of N cycling processes and the associated functional communities and as such may serve as a foundation that future studies may wish to build on.

8.0 LIST OF REFERENCES

ACIA. 2005. Arctic climate impact assessment. Cambridge University Press, pp-1042.

Amann, R.I., W. Ludwig, and K.H. Schleifer. 1995. Phylogenetic identification and in-situ detection of individual microbial-cells without cultivation. Microbiol. Rev. 59:143-169.

Attard, E., S Recous, A. Chabbi, C. De Berranger, N. Guillaumaud, J. Labreuche, et al. 2011. Soil environmental conditions rather than denitrifier abundance and diversity drive potential denitrification after changes in land uses. Global Change Biol. 17:1975-1989.

Avrahami, S., W. Liesack, and R. Conrad. 2003. Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers. Environ. Microbiol. 5:691-705.

Banerjee, S., and S.D. Siciliano. 2012. What factors drive potential ammonia oxidation in Canadian Arctic ecosystems: Does spatial scale matter? Appl. Environ. Microbiol. (doi: 10.1128/AEM.06132-11)

Banerjee, S., A. Bedard-Haughn, B.C. Si, and S.D. Siciliano. 2011a. Soil spatial dependence in three Arctic ecosystems. Soil Sci. Soc. Am. J. 75:591-594.

Banerjee, S., B.C. Si, and S.D. Siciliano. 2011b. Evidence of high microbial abundance and spatial dependency in three Arctic soil ecosystems. Soil Sci. Soc. Am. J. 75:2227-2232.

Bardgett, R.D., R. van der Wal, I.S. Jonsdottir, H. Quirk, and S. Dutton. 2007. Temporal variability in plant and soil nitrogen pools in a high-Arctic ecosystems. Soil Biol. Biochem. 39:2129-2137.

Baxter S.J., M.A. Oliver, and J. Gaunt. 2003. A geostatistical analysis of the spatial variation of soil mineral nitrogen and potentially available nitrogen within an arable field. Precis. Agric. 4: 213-226.

Becker, J.M., T. Parkin, C.H. Nakatsu, J.D. Wilbur, and A. Konopka. 2006. Bacterial activity, community structure, and centimeter-scale spatial heterogeneity in contaminated soil. Microbiol. Ecol. 51:220-231.

Beer C. 2008. The Arctic carbon count. Nat. Geosci. 1: 569-570.

Bengtson P, N. Basiliko, C.E. Prescott, and S.J. Grayston. 2007. Spatial dependency of soil nutrient availability and microbial properties in a mixed forest of Tsuga heterophylla and Pseudotsuga menziesii, in coastal British Columbia, Canada. Soil Biol. Biochem. 39: 2429-2435.

Berg, P., and T. Rosswall. 1989. Abiotic factors regulating nitrification in Swedish arable soil. Biol. Fertil. Soils. 8:247-254.

Billings, W.D. 1973. Arctic and alpine vegetations: similarities, differences, and susceptibility to disturbances. BioScience. 23:697-704.

Bjork, R.G., H. Majdi, L. Klemedtsson, L. Lewis-Jonsson, and U. Molau. 2007. Long-term warming effects on root morphology, root mass distribution, and microbial activity in two dry tundra plant communities in northern Sweden. New Phytol. 176: 862-873.

Bliss, L.C. (ed.) 1977. Truelove Lowland, Devon Island, Canada A High Arctic Ecosystem. Edmonton: University of Alberta Press, Edmonton, Canada.

Bockheim J.G., and C. Tarnocai. 1998. Recognition of cryoturbation for classifying permafrost-affected soils. Geoderma 81: 281-293.

Bohlen, P.J., P.M. Groffman, C.T. Driscoll, T.J. Fahey, and T.G. Siccama. 2001. Plant-soil-microbial interactions in a northern hardwood forest. Ecology 82: 965-978.

Boyd, W.L. 1958. Microbiological studies of Arctic soils. Ecology 39:332–336

Bragato, G., and F. Primavera. 1998. Manuring and soil types influence spatial variation of soil organic matter properties. Soil Sci. Soc. Am. J. 62:1313-1319.

Braker G, Fesefeldt A, Witzel KP. 1998. Development of PCR primer systems for amplification of nitrite reductase gene (nirK and nirS) to detect denitrifying bacteria in environmental samples. Appl. Environ. Microbiol. 64:3769-3775.

Braker, G., H.L. Ayala-del-Rio, A.H. Devol, A. Fesefeldt, and J.M. Tiedje. 2001. Community structure of denitrifiers, Bacteria, and Archaea along redox gradients in pacific northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (nirS) and 16S rRNA genes. Appl. Environ. Microbiol. 67:1893-1901.

Brankatschk, R., S. Towe, K. Kleineidam, M. Schloter, and J. Zeyer. 2011. Abundances and potential activities of nitrogen cycling microbial communities along a chronosequence of a glacier forefield. ISME J. 5:1025-1037.

Bremner, J.M. 1997. Sources of nitrous oxide in soils. Nutr. Cycl. Agroecosys. 49:7-16.

Bru, D., A. Ramette, N.P.A. Saby, S. Dequidt, L. Ranjard, C. Jolivet et al. 2010. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME J. 5: 532-542.

Bruns, M.A., J.R. Stephen, G.A. Kowalchuk, J.I. Prosser, and E.A. Paul. 1999. Comparative diversity of ammonia oxidizer 16S rRNA gene sequences in native, tilled, and successional soils. Appl. Environ. Microbiol. 65:2994-3000.

Buckeridge K.M., E. Zufelt, H. Chu, and P. Grogan. 2010. Soil nitrogen cycling rates in low Arctic shrub tundra are enhanced by litter feedbacks. Plant Soil 330:407-421.

Calvo, L., M. Cortey, J.L. Garcia-Marin, and L.J. Garcia-Gil. 2005. Polygenic analysis of ammonia-oxidizing bacteria using 16S rDNA, amoA, and amoB genes. Int. Microbiol. 8:103-110.

Cambardella CA, T.B. Moorman, J.M. Novak, T.B. Parkin, D.L. Karlen, R.F. Turco, and A.E. Konopka. 1994. Field-scale variability of soil properties in Central Iowa soils. Soil Sci. Soc. Am. J. 58:1501-1511.

Campbell, N.E.R. and M.I.H. Aleem. 1965. The effect of 2-chloro,6-(trichloromethyl) pyridine on the chemoautotrophic metabolism of nitryfing bacteria. A. van Leeuw. 31:124-136.

Carney, K.M., P.A. Matson, and B.J.M. Bohannan. 2004. Diversity and composition of tropical soil nitrifiers across a plant diversity gradient and among land-use types. Ecol. Lett. 7:684-694.

Casciotti, K.L., and B.B. Ward. 2005. Phylogenetic analysis of nitric oxide reductase gene homologues from aerobic ammonia-oxidizing bacteria. FEMS Microbiol. Ecol. 52:197-205.

Cavigelli, M.A., and Robertson, G.P. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology 81:1402-1414.

Cerri CEP, M. Bernoux, V. Chaplot, B. Volkoff, R.L. Victoria, J.M. Melillo, K. Paustian, and C.C. Cerri. 2004. Assessment of soil property spatial variation in an Amazon pasture: basis for selecting an agronomic experimental area. Geoderma 123:51-68.

Chancy, H. F., and E. J. Kamprath. 1987. Effect of Nitrapyrin rate on nitrification in soils having different organic-matter contents. Soil. Sci. 144:29-35.

Chapin D.M. 1996. Nitrogen mineralization, nitrification, and denitrification in a high Arctic lowland ecosystem, Devon Island, NWT, Canada. Arct. Alp. Res. 28: 85-92.

Chapuis-Lardy, L., N. Wrage, A. Metay, J.L. Chotte, and M. Bernoux. 2007. Soils, a sink for N_2O? A review. Global Change Biol. 13:1-17.

Choi M, J.M. Jacobs, and M.H. Cosh. 2007. Scaled spatial variability of soil moisture fields. Geophys. Res. Lett. 34 (2007):L01401.

Christensen, T.R. 1999. Potential and actual trace gas fluxes in Arctic terrestrial ecosystems. Polar Res. 18:199-206.

Chu, H., and P. Grogan. 2010. Soil microbial biomass, nutrient availability and nitrogen mineralization potential among vegetation-types in a low Arctic tundra landscape. Plant Soil 329:411-420.

Chu, H., N. Fierer, C. L. Lauber, J. G. Caporaso, R. Knight, and P. Grogan. 2010. Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. Environ. Microbiol. 12:2998-3006.

CMDL. 2001. Climate Monitoring and Diagnostic Laboratory (CMDL) of the National Oceanographic and Atmospheric Administration, Boulder, CO, USA. N_2O data from: http://ftp.cmdl.noaa.gov/hats/n2o/insitu Gcs/global/. Hall, B.D. et al., Halocarbons and other Atmospheric Trace Species Group, CMDL Summary Report 1989–1999, NOAA/CMDL, in preparation 2000.

Coolen, M.J.L., E.C. Hopmans, W.I.C. Rijpstra, G. Muyzer, S. Schouten, J.K. Volkman, and J.S.S. Damste. 2004. Evolution of the methane cycle in Ace Lake (AntArctica) during the Holocene: Response of methanogens and methanotrophs to environmental change. Org. Geochem. 35:1151-1167.

Cuhel, J., M. Simek, R.J. Laughlin, D. Bru, D. Cheneby, C.J. Watson, and L. Philippot. 2010 Insights into the Effect of Soil pH on N_2O and N_2 Emissions and Denitrifier Community Size and Activity. Appl. Environ. Microbiol. 76:1870-1878.

Corstanje, R., R. Schulin, and R.M. Lark. 2007. Scale-dependent relationships between soil organic carbon and urease activity. Eur. J. Soil. Sci. 58:1087-1095.

Curtis, T. P., W. T. Sloan, and J. W. Scannell. 2002. Estimating prokaryotic diversity and its limits. Proc. Nat. Acad. Sci. USA 99:10494-10499.

Dalal, R.C., and Allen, D.E. 2008. Greenhouse gas fluxes from natural ecosystems. Aust. J. Bot. 56:369-407.

Dale, M.B., M. Anand, and R.E. Desrochers R. 2007. Measuring information-based complexity across scales using cluster analysis. Ecol. Inform. 2:121-127.

Dandie, C.E., S. Wertz, C.L. Leclair, C. Goyer, D.L. Burton, C.L. Patten, et al. 2011. Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. FEMS Microbiol. Ecol. 77:69-82.

DeBoer, W., and G.A. Kowalchuk. 2001. Nitrification in acid soils: microorganisms and mechanisms. Soil Biol. Biochem. 33:853-866.

Dumonceaux, T. J., J.E. Hill, S.A. Briggs, K.K. Amoaka, S.M. Hemmingsen, and A.G. Van Kessel. 2006. Enumeration of specific bacterial populations in complex intestinal communities using quantitative PCR based on the *chaperonin*-60 target. J. Microbiol. Methods 64:46-62.

Enwall, K., I.N. Throback, M. Stenberg, M. Soderstrom, and S. Hallin. 2010. Soil Resources Influence Spatial Patterns of Denitrifying Communities at Scales Compatible with Land Management. Appl. Environ. Microbiol. 76:2243-2250.

Environment Canada. 2011. www.weatheroffice.gc.ca (data accessed on 30th November, 2011)

Erguder, T. H., N. Boon, L. Wittebolle, M. Marzorati, and W. Verstraete. 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiol. Rev. 33:855-869.

Ettema, C.H., and D.A. Wardle. 2002. Spatial soil ecology. Trends Ecol. Evol. 17:177-183.

Firestone, M.K., R.B. Firestone, and J.M. Tiedje. 1980. Nitrous oxide from soil denitrification-factors controlling its biological production. Science 208:749-751.

Fortin, M. J., P. Drapeau, and P. Legendre. 1989. Spatial autocorrelation and sampling design in plant ecology. Vegetatio 83:209-222.

Franklin, R.B., and A.L. Mills. 2003. Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. FEMS Microbiol. Ecol. 44:335-346.

Franklin, R., and Mills, A. 2007. Statistical analysis of spatial structure in microbial communities. In Franklin, R., and Mills, A. (eds) The spatial distribution of microbes in the environment. Springer, Netherlands, pp 31-60.

Franklin, R.B., L.K. Blum, A.C. McComb, and A.L. Mills. 2002. A geostatistical analysis of small-scale spatial variability in bacterial abundance and community structure in salt marsh creek bank sediments. FEMS Microbiol. Ecol. 42:71-80.

Franklin, R.B., and A.L. Mills. 2007. The spatial distribution of microbes in the environment, Springer, New York.

French, H.M. 1996. The Periglacial Environment. Longman, London.

Gardner, W.H. 1986. Water content, p. 493-544. *In* A. Klute, (ed.) Methods in Soil Analysis. Part 1. Physical and Mineraligical Methods, Soil Science Society of America, Madison, WI.

Gardes, M., and T.D. Bruns. 1993. ITS primers with enhanced specificity for Basidiomycetes-Application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113-118.

Geets, J., de Cooman, M., Wittebolle, L., Heylen, K., Vanparys, B., De Vos, P., Verstraete, W., Boon, N. 2007. Real-time PCR assay for the simultaneous quantification of nitrifying and denitrifying bacteria in activated sludge. Appl. Microbiol. Biotechnol. 75:211-221.

Giblin, A.E., K.J. Nadelhoffer, G.R. Shaver, J.A. Laundre, and A.J. Mckerrow. 1991. Biogeochemical cycling diversity along a riverside toposequence in Arctic tundra. Ecol. Mongr. 61:415-435.

Gong, P., S.D. Siciliano, C.W. Greer, L. Paquet, J. Hawari, and G.I. Sunahara. 1999. Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field-contaminated soils to indigenous microorganisms. Environ. Toxicol. Chem. 18:2681-2688.

Gonzalez, O.J., and Zak D.R. 1994. Geostatistical analysis of soil properties in a secondary tropical dry forest, St Lucia, West Indies. Plant Soil 163:45-54.

Goovaerts, P. 1997. Geostatistics for natural resources evaluation. First ed. Oxford University Press, New York.

Goovaerts, P. 1998. Geostatistical tools for characterizing the spatial variability of microbiological and physico-chemical soil properties. Biol. Fertil. Soil 27:315-334.

Green, J., and B.J.M. Bohannan. 2006. Spatial scaling of microbial biodiversity. Trends Ecol. Evol. 21:501-507.

Griffiths, R.I., A.S. Whiteley, A.G. O'Donnell, and M.J. Bailey. 2000. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl. Environ. Microbiol. 66:5488-5491.

Grundmann, G.L., and D. Debouzie. 2000. Geostatistical analysis of the distribution of NH_4^+ and NO_2^- -oxidizing bacteria and serotypes at the millimeter scale along a soil transect. FEMS Microbiol. Ecol. 34:57-62.

Grundmann, G.L., A. Dechesne, F. Bartoli, J. P. Flandrois, J. L. Chasse, and R. Kizungu. 2001. Spatial modeling of nitrifier microhabitats in soil. Soil Sci. Soc. Am. J. 65:1709-1716.

Hallin H, Lindgren PE. 1999. PCR detection of genes encoding nitrite reductase in denitrifying bacteria. Appl. Environ. Microbiol. 64:1652-1657.

Hallin, S., C.M. Jones, M. Schloter, and L. Philippot. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J. 3:597-605.

Hanson, R.S., and T.E. Hanson. 1996. Methanotrophic bacteria. Microbiol. Rev. 60:439-471.

Harvey, A.N. 2011. Toxicity of petroleum hydrocarbons in polar soil. PhD Thesis. Dept. Soil Science, University of Saskatchewan, Saskatoon, Canada.

He, J., J. Shen, L. Zhang, Y. Zhu, Y. Zheng, M. Xu, and H.J. Di. 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. Environ. Microbiol. 9:2364-2374.

Van Der Heijden, M.G.A., R.D. Bardgett, and N.M. Van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11:296–310.

Heid, C.A., J. Stevens, K.J. Livak, and P.M. Williams. 1996. Real time quantitative PCR. Genome Res. 6:986-994.

Henry, S., E. Baudoin, J.C. Lopez-Gutierrez, F. Martin-Laurent, A. Brauman, and L. Philippot. 2004. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. J. Microbiol. Meth. 59:327-335.

Henry, S., D. Bru, B. Stres, S. Hallet, and L. Philippot. 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, narG, nirK, and nosZ genes in soils. Appl. Environ. Microbiol. 72:5181-5189.

Hynes, R.K. and R. Knowles. 1983. Inhibition of chemoautotrophic nitrification by sodium chlorate and sodium chlorite: a reexamination. Appl. Environ. Microbiol. 45:1178-1182.

Iqbal J, J.A. Thomasson, J.N. Jenkins, P.R. Owens, and F.D. Whisler. 2005. Spatial variability analysis of soil physical properties of alluvial soils. Soil Sci. Soc. Am. J. 69: 1338-1350.

Intergovernmental Panel on Climate Change. 2007. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning (eds.)].

Islam, A, D. Chen, and R.E. White. 2007. Developing a technique to quantify heterotrophic and autotrophic nitrification in acidic pasture soils. Comm. Soil. Sci. Plan. 38:2309-2321.

Jackson, R.B., and M.M. Caldwell. 1993. Geostatistical patterns of soil heterogeneity around individual perennial plants. J. Ecol. 81:683-692.

James, N., and M.L. Sutherland. 1942. Are there living bacteria in permanently frozen subsoil? Can. J. Res. Sect. Bot. Sci. 20:228–235.

Jenkins, S.N., I.S. Waite, A. Blackburn, R. Husband, S.P. Rushton, D.C. Manning, and A.G. O'Donnell. 2009. Actinobacterial community dynamics in long term managed grasslands. Ant. Leeuw. 95:319-334.

Jetten, M.S.M., P. deBruijn, and J.G. Kuenen. 1997. Hydroxylamine metabolism in Pseudomonas PB16: Involvement of a novel hydroxylamine oxidoreductase. Antonie Van Leeuwenhoek International J. Gen. Mol. Microbiol. 71:69-74.

Jonasson, S., A. Michelsen, and I.K. Schmidt. 1999. Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. Appl. Soil Ecol. 11:135-146.

Journel, A.G., and C.J. Huijbregts. 1978. Mining geostatistics, Academic Press, London, UK.

Jung, W.K., N.R. Kitchen, K.A. Sudduth, and S.H. Anderson. 2006. Spatial characteristics of claypan soil properties in an agricultural field. Soil Sci. Soc. Am. J. 70:1387-1397.

Junior V.V., M.P. Carvalho, J. Dafonte, O.S. Freddi, E.V. Vazquez, and O.E. Ingaramo. 2006. Spatial variability of soil water content and mechanical resistance of Brazilian ferralsol. Soil Till. Res. 85:166-177.

Kandeler, E., K. Deiglmayr, D. Tscherko, D. Bru, and L. Philippot. 2006. Abundance of narG, nirS, nirK, and nosZ genes of denitrifying bacteria during primary successions of a glacier foreland. Appl. Environ. Microbiol. 72:5957-5962.

Kang, S. 2005. Spatial structures of soil microbial communities and their controlling factors. PhD Thesis, Dept. of Environmental Sciences, University of Virginia, Charlottesville, Virginia.

Keil, D., A. Meyer, D. Berner, C. Poll, A. Schutzenmeister, H.P. Piepho et al. 2011. Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils. FEMS Microbiol. Ecol. 77:95-106.

Kemnitz, D., S. Kolb, and R. Conrad. 2007. High abundance of Crenarchaeota in a temperate acidic forest soil. FEMS Microbiol. Ecol. 60:442-448.

King, R.H. 1969. Periglaciation on Devon Island, NWT. PhD Thesis, Dept. of Geography, Univ. of Saskatchewan, Canada.

King, A.J., K.R. Freeman, K.F. McCormick, R.C. Lynch, C. Lozupone, R. Knight, and S.K. Schmidt. 2010. Biogeography and habitat modelling of high-alpine bacteria. Nat. Commun. 1:1–6.

Kloos, K., A. Mergel, C. Rosch, and H. Bothe. 2001. Denitrification within the genus Azospirillum and other associative bacteria. Aust. J. Plan. Physiol. 28:991-998.

Knowles, R. 1982. Denitrification. Microbiol. Rev. 46:43-70.

Kris, A.E. 1940. Microorganisms in permafrost. Microbiology 9:879–886 (in Russian)

Lal, R., and J.M. Kimble. 2000. Soil C pool and dynamics in cold ecoregions. In: Global Climate Change and Cold Regions Ecosystems. Advances in Soil Science (eds Lal R, Kimble JM, Stewart BA), pp. 91–103. CRC Press LLC, New York.

Lamb, E.G., S. Han, B.D. Lanoil, G.H.R. Henry, M.E. Brummell, S. Banerjee et al. 2011. A high Arctic soil ecosystem resists long-term environmental manipulations. Global Change Biol. 17:3187-3194.

Lamb, E.G., N. Kennedy, and S.D. Siciliano. 2011. Effects of plant species richness and evenness on soil microbial community diversity and function. Plant Soil 338:483-495.

Leininger, S., T. Urich, M. Schloter, L. Schwark, J. Qi, G. W. Nicol, et al. 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806-809.

Lev, A., and R.H. King. 1999. Spatial variation of soil development in a high Arctic soil landscape: Truelove Lowland, Devon Island, Nunavut, Canada. Permafrost Periglac. 10:289-307.

Levy-Booth, D.J., and R.S. Winder. 2010. Quantification of Nitrogen Reductase and Nitrite Reductase Genes in Soil of Thinned and Clear-Cut Douglas-Fir Stands by Using Real-Time PCR. Appl Environ Microbiol 76: 7116-7125.

Ludwig W, Schleifer KH (1999) Phylogeny of bacteria beyond the 16S rDNA standard. ASM News 65:752-757.

Lui, B., Morkved, P.T., Frostegrad, A., and Bakken, L.R. (2010) Denitrification gene pools, transcription and kinetics of NO, N2O, and N2 production as affected by soil pH. FEMS Microbiol Ecol 72: 407-417.

Ma, W.K., R.E. Farrell, and S.D. Siciliano. 2011. Nitrous oxide emissions from ephemeral wetland soils are correlated with microbial community composition. Front Microbiol. 2:1-11.

Ma, W. K., A. Bedard-Haughn, S.D. Siciliano, and R.E. Farrell. 2008. Relationship between nitrifier and denitrifier community composition and abundance in predicting nitrous oxide emissions from ephemeral wetland soils. Soil. Biol. Biochem. 40:1114-1123.

Ma, W.K., A. Schautz, L.A.E. Fishback, A. Bedard-Haughn, R.E. Farrell, and S.D. Siciliano. 2007. Assessing the potential of ammonia oxidizing bacteria to produce nitrous oxide in soils of a high Arctic lowland ecosystem on Devon Island, Canada. Soil. Biol. Biochem. 39:2001-2013.

Mayor, S.J., J.A. Schaefer, D.C. Schneider, and S.P. Mahoney. 2009. The spatial structure of habitat selection: A carbou's eye-view. Acta Oecolog. 35:253-260.

McCune B., and J.B. Grace. 2002. Analysis of Ecological Communities MjM Software, Gleneden Beach, Oregon.

McLain, J.E.T., and D.A. Martens. 2006. N_2O production by heterotrophic N transformations in a semiarid soil. Appl. Soil. Ecol. 32:253-263.

McTavish, H., J.A. Fuchs, and A.B. Hooper. 1993. Sequence of the gene coding for ammonia monooxygenase in *Nitrosomonas europaea*. J. Bacteriol. 175:2436-2444.

Mercer, W.B., and A.D. Hall. Experimental error of field trials. J. Agr. Sci. 4:107-132.

Miller, M.N., B.J. Zebarth, C.E. Dandie, D.L. Burton, C. Goyer, and J.T. Trevors. 2008. Crop residueinfluence on denitrification, N2O emissions and denitrifier community abundance in soil. Soil Biol. Biochem. 40:2553-2562.

Miller, M.N., B.J. Zebarth, C.E. Dandie, D.L. Burton, C. Goyer, and J.T. Trevors. 2009. Denitrifier community dynamics in soil aggregates under permanent grassland and arable cropping systems. Soil Sci Soc Am J 73:1843-1851.

Moir, J.W.B., J.M. Wehrfritz, S. Spiro, and D.J. Richardson. 1996. The biochemical characterization of a novel non-haem-iron hydroxylamine oxidase from Paracoccus denitrificans GB17. Biochem. J. 319:823-827.

Morales, S.E., T. Cosart, and W.E. Holben. 2010. Bacterial gene abundances as indicators of greenhouse gas emission in soils. ISME J. 4:799-808.

Morales, S.E., and W.E. Holben. 2011. Linking bacterial identities and ecosystem processes: can 'omic' analyses be more than the sum of their parts? FEMS Microbiol. Ecol. 75:2-16.

Morris, S.J. 1999. Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils: fine scale variability and microscale patterns. Soil Biol. Biochem. 31:1375-1386.

Mosier, A., C. Kroeze, C. Nevison, O. Oenema, S. Seitzinger, and O. van Cleemput. 1998. Closing the global N2O budget: nitrous oxide emissions through the agricultural nitrogen cycle - OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology. Nutr. Cycl. Agroecosyst. 52:225-248.

Mzuku, M., R. Khosla, R. Reich, D. Inman, F. Smith, and L. MacDonald. 2005. Spatial variability of measured soil properties across site-specific management zones. Soil Sci. Soc. Am. J. 69:1572-1579.

Natural Resources Canada:http://atlas.nrcan.gc.ca 2006.

Neufeld, J.D., and R. Knowles. 1999. Inhibition of nitrifiers and methanotrophs from an agricultural humisol by allylsulfide and its implications for environmental studies. Appl. Environ. Microbiol. 65:2461-2465.

Nicol, G.W., L.A. Glover, and J.I. Prosser. 2003. Spatial analysis of archaeal community structure in grassland soil. Appl. Environ. Microbiol. 69:7420-7429.

Nicol, GW, S. Leininger, C. Schleper, and J.I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia-oxidizing archaea and bacteria. Environ. Microbiol. 10:2966-2978.

Nielsen, D.R., J.W. Biggar, and K.T. Erh. 1973. Spatial variability of field-measured soil-water properties. Hilgardia 42:215-259.

Nogva, H.K., and K. Rudi. 2004. Potential influence of the first PCR cycles in real-time comparative gene quantifications. Biotechniques 37:246-253.

Nunan, N., K. Wu, I. M. Young, J. W. Crawford, and K. Ritz. 2002. In situ spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. Microb. Ecol. 44:296-305.

Oliver, M.A., and R. Webster. 1991. How geostatistics can help you. Soil Use Manag. 27:206-217.

Omelyansky, V.L. 1911. Bacteriological investigation of the Sanga mammoth and surrounding soil. *Arkhiv Biologicheskikh Nauk (Scientific Biological Archive)* 16:335–340 (in Russian)

Ovreas, L., L. Forney, F.L. Daae, and V. Torsvik. 1997. Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl. Environ. Microbiol. 63:3367-3373.

Pan, Y.X., and X.P. Wang. 2009. Factors controlling the spatial variability of surface soil moisture within revegetated-stabilized desert ecosystems of the Tengger Desert, Northern China. Hydrol. Proc. 23:1591-1601.

Pare, M.C. 2011. Organic matter quality in Cryosols: effect on soil nitrogen dynamics and greenhouse gas emissions. PhD Thesis. Dept. Soil Science, University of Saskatchewan, Canada.

Paré, M.C., and A. Bedard-Haughn. 2011. Optimum liquid density in separation of the physically uncomplexed organic matter in Arctic soils. Can. J. Soil Sci. 91:65-68.

Park, H.D., G.F. Wells, H. Bae, C.S. Criddle, and C. A. Francis. 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Appl. Environ Microbiol. 72:5643–5647.

Parkin, T.B. 1987. Soil microsites as source of denitrification variability. Soil Sci. Soc. Am. J. 51:1194-1199.

Parkin, T.B. 1993. Spatial variability of microbial processes in soil- A Review. J. Environ. Qual. 22:409-417.

Parkin, T.B. and J.L. Hatfield. 2010. Influence of Nitrapyrin on N2O Losses from Soil Receiving Fall-Applied Anhydrous Ammonia. Agr. Ecosyst. Environ. 136:81-86.

Pennock, D.J. 2004. Designing field studies in soil science. Can. J. Soil Sci. 84:1-10.

Pester, M., C. Schleper, and M. Wagner. 2011. The Thaumarchaeota: an emerging view of their phylogeny and ecophysiology. Curr. Opin. Microbiol. 14:300-306.

Petrone R.M., J.S. Price, S.K. Carey, and J.M. Waddington. 2004. Statistical characterization of the spatial variability of soil moisture in a cutover peatland. Hydrol. Proc. 18: 41-52.

Pfaffle, M.W. 2001. A new mathematical model for relative quantification real-time PCR. Nucl. Acid Res. 29:e45.

Philippot, L., and S. Hallin. 2005. Finding the missing link between diversity and activity using denitrifying bacteria as a model functional community. Curr. Opin. Microbiol. 8:234-239.

Philippot, L, J. Cuhel, N.P. Saby, D. Cheneby, A. Chronakova, D. Bru, et al. 2009. Mapping field-scale spatial patterns of size and activity of the denitrifier community. Environ. Microbiol. 11:1518-1526.

Philippot, L., J. Andert, J., C.M. Jones, D. Bru, and S. Hallin. 2011. Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. Global Change Biol. 17:1497-1504.

Purkhold, U., A. Pommerening-Roser, S. Juretschko, M.C. Schmid, H.P. Koops, and M. Wagner. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: Implications for molecular diversity surveys. Appl. Environ. Microbiol. 66:5368-5382.

Rich, J.J., and D.D. Myrold. 2004. Community composition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA. Soil Biol. Biochem. 36: 1431-1441.

Ritz, K., W. McNicol, N. Nunan, S. Grayston, P. Millard, D. Atkinson et al. 2004. Spatial structure in soil chemical and microbiological properties in an upland grassland. FEMS Microbiol. Ecol. 49:191-205.

Robertson, L.A., and J.G. Kuenen. 1988. Heterotrophic nitrification in Thiosphaera – Pantotropha-oxygen-uptake and enzymes studies. Journal of General Microbiology 134:857-863.

Robertson L, A. Treisman, S. FriedmanHill, and M. Grabowecky. 1997. The interaction of spatial and object pathways: Evidence from Balint's syndrome. J. Cog. Neurosci. 9:295-317.

Ramette, A., and J.M. Tiedje. 2007. Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. Proc. Nat. Acad. Sci. USA 104:2761-2766.

Rodriguez A, J. Duran, J.M. Fernandez-Palacios, and A. Gallardo. 2009. Spatial variability of soil properties under Pinus canariensis canopy in two contrasting soil textures. Plant Soil 322:139-150.

Rösch, C., Mergel, A., and Bothe, H. (2002) Biodiversity of denitrifying and distribution-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68: 3818-3829.

Rossello-Mora, R., and R. Amann. 2001. The species concept for prokaryotes. FEMS Microbiol. Rev. 25:39-67.

Rossi, R.E., D.J. Mulla, A.G. Journel, and E.H. Franz. 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence.Ecol. Monogr. 62:277-314.

Rotthauwe, J. H., K. P. Witzel, and W. Liesack. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63:4704-4712.

Rover M, and E.A. Kaiser. 1999. Spatial heterogeneity within the plough layer: low and moderate variability of soil properties. Soil Biol. Biochem. 31:175-187.

Russo D, and M. Bouton. 1992. Statistical-analysis of spatial variability in unsaturated flow parameters. Water Resour. Res. 28:1911-1925.

Ruth B., and B. Lennartz. 2008. Spatial variability of soil properties and rice yield along two catenas in southeast China. Pedosphere 18:409-420.

Saetre P, and E. Baath E. 2000. Spatial variation and patterns of soil microbial community structure in a mixed spruce-birch stand. Soil Biol. Biochem. 32:909-917.

Sayavedra-Soto, L. A., and D. J. Arp. 2011. Ammonia-oxidizing bacteria: their biochemistry and molecular biology. pp 11-38. *In*: Ward, B. B., D. J. Arp, and M. G. Klotz (eds). Nitrification. ASM Press, Washington, DC.

Sayavedra-Soto, L. A., N. Hamamura, C. W. Liu, J. A. Kimbrel, J. H. Chang, and D. J. Arp. 2011. The membrane-associated monooxygenase in the butane-oxidizing Gram-positive bacterium Nocardioides sp. strain CF8 is a novel member of the AMO/PMO family. Environ. Microbiol. Reports 3:390-396.

Schafer, A.N., I. Snape, and S.D. Siciliano. 2007. Soil biogeochemical toxicity end points for sub-AntArctic islands contaminated with petroleum hydrocarbons. Environ. Toxicol. Chem. 26:890-897.

Schafer, A.N., I. Snape, and S.D. Siciliano. 2009. Influence of liquid water and soil temperature on petroleum hydrocarbon toxicity in AntArctic soil. Environ. Toxicol. Chem. 28:1409-1415.

Schimel, J.P., and F.S. Chapin. 1996. Tundra plant uptake of amino acid and NH4+ nitrogen in situ: Plants compete well for amino acid N. Ecology 77:2142-2147.

Schimel, J.P., and J. Gulledge. 1998. Microbial community structure and global trace gases. Global Change Biol. 4:745-758.

Schimel, J.P., M.K. Firestone, and K.S. Killham. 1984. Identification of heterotrophic nitrification in a sierra forest soil. Appl. Environ. Microbiol. 48: 802-806.

Schmidt, I.K., S. Jonasson, G.R. Shaver, A. Michelsen, and A. Nordin. 2002. Mineralization and distribution of nutrients in plants and microbes in four Arctic ecosystems: responses to warming. Plant Soil 242:93-106.

Schleper, C. 2010. Ammonia oxidation: different niches for bacteria and archaea? ISME J. 4:1092-1094.

Schleper, C., and G. W. Nicol. 2010. Ammonia-oxidising archaea - physiology, ecology and evolution. Adv. Microb. Physiol. 57:1-41.

Scott, G.A.J. 1995. Canada's vegetation: A world perspective. McGill-Queen's University Press. Canada.

Shen, J.P., L.M. Zhang, Y.G. Zhu, J.B. Zhang, and J.Z. He. 2008. Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. Environ. Microbiol. 10:1601-1611.

Si, B.C., R.G. Kachanoski, and W.D. Reynolds. 2003. Analysis of soil variability. *In* Soil sampling and methods of analysis. *eds.* Carter, M. and E. Gregorich. CRC Press, USA.

Siciliano, S.D., W.K. Ma, S. Ferguson, and R.E. Farrell. 2009. Nitrifier dominance of Arctic soil nitrous oxide emissions arises due to fungal competition with denitrifiers for nitrate. Soil Biol. Biochem. 41:1104-1110.

Siciliano, S.D., W. Ma, and S. Powell. 2007. Evaluation of quantitative polymerase chain reaction to assess nosZ gene prevalence in mixed microbial communities. Can. J. Microbiol. 53:636-642.

Smith, C.J., and A.M. Osborn. 2009. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS Microbiol. Ecol. 67:6-20.

Smith, J.L., and J.W. Doran. 1996. Measurement and use of pH and electrical conductivity. p. 169–185. *In* J.W. Doran and A.J. Jones (ed.). Methods for assessing soil quality. SSSA Spec. Pub. 49. SSSA, Madison, WI.

Smith, S.M., and J.M. Tiedje. 1979. Phases of denitrification following oxygen depletion in soil. Soil. Biol. Biochem. 11:261-267.

Soil Classification Working Group. 1998. The Canadian System of Soil Classification, 3rd ed. NRC Research Press, Ottawa.

Sorensen, P.L., Jonasson, S., and Michelsen, A. 2006. Nitrogen fixation, denitrification, and ecosystem nitrogen pools in relation to vegetation development in the subArctic. Arct Antarct Alp Res 38: 263-272.

Stark, S. 2007. Nutrient cycling in the tundra. *In*: Marschner, P. and Rengel, Z. (*eds*), Nutrient cycling in terrestrial ecosystems. Soil Biol. 10: pp. 309–331.

Stephen, J. R., Y. J. Chang, S. J. Macnaughton, G. A. Kowalchuk, K. T. Leung, C. A. Flemming, et al. 1999. Effect of toxic metals on indigenous soil β-subgroup Proteobacterium ammonia oxidizer community structure and protection against toxicity by inoculated metal-resistant bacteria. Appl. Environ. Microbiol. 65:95-101.

Stewart, K.J., D. Coxson, and P. Grogan. 2011a. Nitrogen inputs by associative cyanobacteria across a low Arctic tundra landscape. Arct. Antarct. Alp. Res. 43:267-278.

Stewart, K.J., D. Coxson, and S.D. Siciliano. 2011b. Small-scale spatial patterns in N2 fixation and nutrient availability in an Arctic hummock-hollow ecosystem. Soil Biol. Biochem. 43: 133-140.

Stres, B., L. Philippot, J. Faganeli, and J.M. Tiedje. 2010. Frequent freeze-thaw cyclesyield diminishedyet resistant and responsive microbial communities in two temperate soils: a laboratory experiment. EFMS Microbiol. Ecol. 74:323-335.

Steven, B., R. Leveille, W.H. Pollard, and L.G. Whyte. 2006. Microbial ecology and biodiversity in permafrost. Extremophiles 10:259-267.

Su Y.Z., Y.L. Li, and H.L. Zhao. 2006. Soil properties and their spatial pattern in a degraded sandy grassland under post-grazing restoration, Inner Mongolia, northern China. Biogeochemistry 79:297-314.

Tarnocai, C. 2006. The effect of climate change on carbon in Canadian peatlands. Global Planet Change 53:222-232.

Tarnocai, C., and J.G. Bockheim. 2011. Cryosolic soils of Canada: Genesis, distribution, and classification. Can. J. Soil Sci. doi:10.4141/CJSS102020.

Tarnocai, C., J.G. Canadell, E.A.G. Schuur, P. Kuhry, G. Mazhitova, and S. Zimov. 2009. Soil organic carbon pools in the northern circumpolar permafrost region. Global Biogeochem. Cy. 23.

Tarnocai, C., J. Kimble, and G. Broll. 2003. Determining carbon stocks in Cryosols using the Northern and Mid Latitudes Soil Database.

Torstensson, L. 1993. Ammonium oxidation, a rapid method to estimate potential nitrification in soils, pp. 40–47. *In* L. Torstensson (*eds*.) Guidelines: Soil Biological Variables in Environmental Hazard Assessment. Swedish Environmental Protection Agency, Solna, Sweden.

Vlaeminck, S.E., Geets, J., Vervaeren, H., Boon, N., and Verstaete, W. (2007) Reactivation of aerobic and anaerobic ammonia oxidizers in OLAND biomass after long-term storage. Appl Microbiol Biotechnol 74: 1376-1384.

Voorhees, E.B. 1902. Studies in denitrification. J. Am. Chem. Soc. 24:785-804.

Wagner, D. 2008. Microbial communities and processes in Arctic Permafrost Environment. p. 133-154, *In*: Dion, P., and C.S. Nautiyal (eds.), Microbiology of extreme soils, Volume 13. Springer, New York.

Wallenstein, M.D., D.D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecol. Appl. 16:2143-2152.

Wang, D., and D.W. Anderson. 1998. Direct measurement of organic carbon content in soils by the Leco CR-12 Carbon Analyzer. Commun. Soil Sci. Plan. 29:15-21.

Wang, H.Q., C.A.S. Hall, J.D. Cornell, and M.H.P. Hall. 2002. Spatial dependence and the relationship of soil organic carbon and soil moisture in the Luquillo Experimental Forest, Puerto Rico. Landscape Ecol. 17:671-684.

Ward, B. B., D. J. Arp, and M. G. Klotz. 2011. Nitrification. ASM Press, Washington, DC.

Webster, R., and M.A. Oliver. 1992. Sample adequately to estimate variograms of soil properties. J. Soil Sci. 43:177-192.

Wehrfritz, J.M., J.P. Carter, S. Spiro, and D.J. Richardson. 1996. Hydroxylamine oxidation in heterotrophic nitrate-reducing soil bacteria and purification of a hydroxylamine-cytochrome c oxidoreductase from a Pseudomonas species. Arch. Microbiol. 166:421-424.

Western A.W., R.B. Grayson, and G. Bloschl. 2002. Scaling of soil moisture: A hydrologic perspective. Annu. Rev. Earth Pl. Sci. 30: 149-180.

Wickland K.P., J.C. Neff, and G.R. Aiken. 2007. Dissolved organic carbon in Alaskan boreal forest: Sources, chemical characteristics, and biodegradability. Ecosystems 10:1323-1340.

Wiens, J.A. 1989. Spatal scaling in ecology. Funct. Ecol. 3:385-397.

Wilhem, R.C., T.D. Niederberger, C. Greer, and L.G. Whyte. 2011. Microbial diversity of active layer and permafrost in an acidic wetland from the Canadian High Arctic. Can. J. Microbiol. 57: 303-315.

Williams, P.J., Smith M.W. 1989. The frozen earth: fundamentals of geocryology. Cambridge University Press, Cambridge, UK.

Woese, C.R. 1987. Bacterial evolution. Microbiological. Reviews. 51:221-271.

Wu, G., J. Wei, H.B. Deng, and J.Z. Zhao. 2006. Nutrient cycling in an alpine tundra ecosystem on Changbai mountain, northeast China. Appl. Soil Ecol. 32:199-209.

Wright, A.F., and J.S. Bailey. 2001. Organic carbon, total carbon, and total nitrogen determinations in soils of variable calcium carbonate contents using a Leco CN-2000 dry combustion analyzer. Commun. Soil Sci. Plan. 32:3243-3258.

Yanai J, A. Mishima, S. Furakawa, K. Akshalov, and T. Kosaki. 2005. Spatial variability of organic matter dynamics in the semi-arid croplands of northern Kazakhstan. Soil Sci. Plant Nutr. 51:261-269.

Yates, T.T., Si, B.C., Farrell, R.E., and Pennock, D.J. 2006. Probability distribution and spatial dependence of nitrous oxide emission: Temporal change in hummocky terrain. Soil Sci. Soc. Am. J. 70:753-762.

Yergeau,E., H. Hogues, L.G. Whyte, and C.W. Greer. 2010. The functional potential of high Arctic permafrost revealed by metagenomic sequencing, qPCR and microarray analyses. ISME J. 4:1206-1214.

Yuan, J.S., A. Reed, F. Chen, and C.N. Stewart. 2006. Statistical analysis of real-time PCR data. BMC Bioinformats 7:85.

Zeleke, T.B., and B.C. Si. 2005. Scaling relationships between saturated hydraulic conductivity and soil physical properties. Soil Sci. Soc. Am. J. 69:1691-1702.

Zhang, T., J.A. Heginbottom, R.G. Barry, and J. Brown. 2000. Further statistics on the distribution of permafrost and ground ice in the Northern Hemisphere. Polar Geo. 24:126-131.

Zhao, Y., S. Peth, J. Krummelbein, R. Horn, Z.Y. Wang, M. Steffens, C. Hoffmann, and X.H. Peng. 2007. Spatial variability of soil properties affected by grazing intensity in Inner Mongolia grassland. Ecol. Model. 205: 241-254.

Zhu, L., J.M. Chen, Q.M. Qin, J.P. Li, and L.X. Wang. 2009. Optimization of ecosystem model parameters using spatio-temporal soil moisture information. Ecol. Model. 220:2121-2136.

Zimmerman, G.M., H. Goetz, and P.W. Mielke. 1985. Use of an improved statistical method for group comparisons to study effects of prairie fire. Ecology 66:606-611.

Zinger, L., B. Shahnavaz, F. Baptist, R.A. Geremia, and P. Choler. 2009. Microbial diversity in alpine tundra soils correlates with snow cover dynamics. ISME J. 3:850-859.

Zinger L., D.P.H. Lejon, F. Baptist, A. Bouasria, S. Aubert, R.A. Geremia et al. 2011. Contrasting diversity patterns of crenarchaeal, bacterial and fungal soil communities in an alpine landscape. PLoS ONE 6:e19950.

Zumsteg, A., Bernasconi, S.M., Zeyer, J., and Frey, B. 2011. Microbial community and activity shifts after soil transplantation in a glacier forefield. Appl. Geol. 26:S326-S329.