RESPONSE AND VARIABILITY OF ARCTIC SOILS EXPOSED TO NITROGENOUS COMPOUNDS

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ABSTRACT

Increased development in Canada's northern environments has increased the need for accurate methods to detect adverse impacts on tundra ecosystems. Ammonium nitrate is a common water pollutant associated with many industrial and municipal activities, including diamond mining, and is of special concern due to the toxicity of ammonia in aquatic systems. One solution to reduce exposure of sensitive aquatic systems to nitrogenous compounds is to atomize (atmospherically disperse in fine particles) contaminated water over the arctic tundra which will reduce N loading to surface water. However, the toxicity of ammonium nitrate to arctic soils is poorly understood. In this study I investigate the potential toxicity of ammonium nitrate solutions to arctic soil functions such as carbon mineralization, nitrification and plant growth, to determine concentrations that can be applied without causing significant inhibition to these processes.

Arctic ecosystems are based on a soil type termed a cryosol that has an underlying permafrost layer. Often these soils are subject to cryoturbation, a process which heaves and mixes the soil, bringing the mineral horizons to the surface. I hypothesized that phytotoxicity test results in arctic soils would be highly variable compared to other terrestrial ecosystems due to the cryoturbation process and subsequent range of soil characteristics. The variability associated with phytotoxicity tests was evaluated using Environment Canada's standardized plant toxicity test in three cryoturbated soils from Canada's arctic exposed to a reference toxicant, boric acid. The phytotoxicity of boric acid to northern wheatgrass (*Elymus lanceolatus*) in cryosols was much greater than commonly reported in other soils, with less than 150 ug boric acid g^{-1} soil needed to inhibit root and shoot growth by 20%. There was also large variability in the phytotoxicity test results, with coefficients of variation for 10 samples ranging from 160 to 79%. Due to this variability in cryoturbated arctic soils, more than 30 samples should be collected from each control and potentially impacted area to accurately assess contaminant effects, and ensure that false negatives of toxicant impacts in arctic soils are minimized.

To characterize the toxicity of ammonium nitrate I exposed a variety of arctic soils and a temperate soil to different concentrations of ammonium nitrate solution over a

90 day time period. Dose responses of carbon mineralization, nitrification and phytotoxicity test parameters were estimated for ammonium nitrate applications. In addition to direct toxicity, the effect of ammonium nitrate on ecosystem resistance was investigated by dosing nitrogen impacted soils with boric acid. Ammonium nitrate solutions had no effect on carbon mineralization activity, and affected nitrification rates in only one soil, a polar desert soil from Cornwallis Island. In contrast, ammonium nitrate applications (43 mmol N L^{-1} soil water) significantly impaired seedling emergence, root length and shoot length of northern wheatgrass. Concentrations of ammonium nitrate in soil water that inhibited plant parameters by 20% varied between 43 to 280 mmol N L^{-1} soil water, which corresponds with 2,100 to 15,801 mg L^{-1} in the application water. Arctic soils were more resistant to ammonium nitrate toxicity than the temperate soil under these study conditions. However, it is not clear if this represents a general trend for all polar soils, and because nitrogen is an essential macro-nutrient, nitrogenous toxicity should likely be considered a special case for soil toxicity. As soil concentrations could be maintained under inhibitory levels with continual application of low concentrations of ammonium nitrate over the growing season, atomization of wastewater contaminated with ammonium nitrate is a promising technology for mitigation of nitrogen pollution in polar environments.

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This thesis is dedicated to everyone who believed that I could do it.

You know who you are.

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LIST OF ABBREVIATIONS

ºC = centigrade

µg g^{-1} = micrograms per gram

µg L^{-1} = micrograms per litre

µL = microlitre

µm = micrometer

µmol = micromoles

ANOVA = analysis of variance

CAPP = Canadian Association of Petroleum Producers

cm = centimetre

cm^2 = centimetres squared

CV = coefficients of variation

d = day

EC = Environment Canada

EC_{50} = median effect concentration

ft = foot

g = gram

h = hour

H_3BO_3 = boric acid

IC_{20} = 20% inhibition concentration

IC_{50} = 50% inhibition concentration

IC*p* = concentration that inhibits the response relative to the control by a chosen percentage, *p*)

IQ = interquartile range

kg = kilogram

km = kilometer

km^2 = kilometers squared

KCl = potassium chloride

L = litre

LC_{50} = median lethal concentration

LD = lack of data

LFS = lower fore slope

LOEC = lowest observed effect concentration

m = metre

m^2 = metres squared

M = moles per litre

MeHg = Methyl mercury

MFO = mixed function oxygenase

mg = milligram

mg kg^{-1} = milligrams per kilogram

mg/L = milligrams per litre

MHC = moisture holding capacity

MDD = minimum detectable difference

ml = millilitre

mm = millimeter

mmol = millimoles

n = number of samples

N = nitrogen

N_2 = dinitrogen gas

NW = Northwest

NaOH = sodium hydroxide

ND = non-detectable

ng =nanogram

ng/L = nangrom per litre

NH_3 = un-ionized ammonia

NH_4^+ = ionized ammonia, ammonium

NH_4NO_3 = ammonium nitrate

nm = nanometre

NM = not measured

NO_2^- = nitrite

NO_3^- = nitrate

NOEC = no observed effect concentration

NT = Northwest Territories

NU = Nunuvut

O_2 = oxygen

OH^- = hydroxide ion

OECD = Organisation for Economic Co-operation and Development

s = second

SE = standard error

TOC = total organic carbon

USEPA = United States Environmental Protection Agency

UV = ultra violet

WFPS = water-filled pore space

1.0 INTRODUCTION

Ammonium nitrate is not normally thought of as a terrestrial toxicant. Commonly applied as fertilizer to agricultural crops in temperate areas, it has been known to create problems such as eutrophication once it enters aquatic environments via groundwater or surface runoff (Camargo and Alonso, 2006; Smith, 2003). In cold regions where soils are often deficient in nitrogen, nitrogen sources such as ammonium nitrate have been applied to increase biodegradation rates (Walworth et al, 1997), and remediate hydrocarbon contaminated sites. However, the amount of ammonium nitrate that tundra soils can tolerate without significant inhibition of important soil processes has not been investigated.

Recent industrial and economic growth in northern Canada has resulted in increased human activity and impact, and of course, environmental pollution. A problem of particular concern is the treatment and disposal of human and industrial wastewater. Wastewater contaminated with ammonium nitrate for example, cannot be returned to natural surface water systems without treatment, as ammonia is toxic to fish and other aquatic organisms (Russo, 1985). The treatment and storage of wastewater is a significant challenge in this relatively pristine ecosystem, with its short growing season, low precipitation and reduced microbial activity due to sub-zero temperature conditions for most of the year.

This thesis project stemmed from an investigation of a novel approach to wastewater disposal proposed by BHP Billiton, operators of the Ekati Diamond Mine. They propose to atomize wastewater contaminated with ammonium nitrate over the tundra during the summer months, volatilizing the majority of the ammonia and allowing the tundra ecosystem to utilize any residual ammonium and nitrate that is deposited on the tundra surface. In this thesis, we identified concentrations of ammonium nitrate solutions that can be applied to specific arctic soil ecosystems without causing significant harm to or change in critical soil functions. This information is important for enabling the use of this economical method of waste treatment.

This thesis is presented in six chapters. This introduction (Chapter 1.0) is followed by a review of relevant literature in Chapter 2.0, which covers the toxicity of

nitrogenous compounds in various forms, and the unique conditions of the arctic landscape. Knowledge gaps will be identified, as well as the objectives of this research.

Chapter 3 focuses on obtaining adequate sample numbers in order to accurately detect impacts in the highly variable arctic soils of Ekati. We also determine appropriate concentrations of a reference toxicant, boric acid, to be used in arctic soil toxicity tests.

In Chapter 4 we investigate the biogeochemical toxicity and phytotoxicity of ammonium nitrate in arctic soils. Soil samples collected from the Ekati diamond mine site, as well as three other arctic locations are assessed. Soils from a temperate landscape are also included in all experiments for comparison. Soils were exposed to increasing ammonium nitrate concentrations in the laboratory at the University of Saskatchewan, and analyzed to determine the effects of exposure on microbial activity and plant growth.

Chapter 5 discusses the findings of all the experiments, and their applications and relevance to current situations in northern Canada. Recommendations for continuing and enhancing this research area are also given.

Finally, Chapter 6 is a list of all references cited throughout the thesis.

2.0 LITERATURE REVIEW

2.1 Diamond Mining in Canada

Natural diamonds are most often found in rare deposits of ultrabasic igneous rock called kimberlite. Originating more than 150 km deep, this rock was brought to the Earth's surface millions of years ago in a molten form during volcanic eruptions, where it hardened into carrot shaped pipes. A crystallized form of carbon that had become stable under pressure and time was contained within these pipes, and these diamonds were initially believed to have mystical healing powers as well as decorative purposes. Diamonds have become popular across the world for their beauty and their industrial value.

Although diamonds have been mined in other parts of the world for centuries, Canada has only recently joined the fray, becoming one of the top producers of gem-quality diamonds in less than ten years. The first kimberlite pipe in Canada was discovered in the Northwest Territories by geologists Charles Fipke and Stuart Blusson in 1991. This was followed by the largest staking rush in Canadian history, and the subsequent construction of several diamond mines. The Ekati Diamond Mine was Canada's first diamond mine, officially opening in the fall of 1998. Located near Lac de Gras, 300 kilometres north-east of Yellowknife, NWT, and about 200 kilometres south of the Arctic Circle, Ekati is operated by BHP Billiton Diamonds Inc., a part of the BHP Billiton Group, the world's largest diversified resources company (BHP, 2002a; BHP, 2002b).

2.1.1 Open pit mining process

Diamonds at the Ekati site are found in 45 to 62 million year old kimberlite pipes (Creaser, 2004), making them younger and therefore less eroded than similar deposits in South Africa and Russia. Open pit mining is the most economical way to mine these deposits. As kimberlite is a relatively soft rock, it was easily eroded by glaciers to form depressions, leaving the kimberlite pipes underneath shallow lakes. Once the lakes are fished out and dewatered, lake bottom sediments and overburden are cleared away to expose the pipe, composed of diamond containing ore and waste rock, which is mostly granite (BHP, 2000a). Explosives are used to remove the kimberlite from the ground.

Ore and waste rock are separated and removed from the pits by truck (BHP, 2002a). Ore is transported to a processing plant where diamonds are extracted using chemical free processes that reduce the ore to fine particles (</=0.5 mm). One carat of diamonds is extracted from roughly one ton of kimberlite, which itself is extracted from ten thousand tons of rock.

2.1.2 Contamination of surplus water by ammonium nitrate

The explosives used in the open pit mining process at Ekati are an emulsion of ammonium nitrate and fuel oil, typically 6% diesel fuel. Residues from these explosives remain in the waste rock piles and the walls of the pit. Ammonium nitrate (NH_4NO_3) dissociates readily into water that seeps through the waste rock piles and pit walls, and accumulates in the water that collects in the sump at the bottom of the pit (BHP, 2000b). The level of ammonia in this contaminated water must be monitored before it can be returned to the natural lake system, as ammonia in its unionized form (NH_3) is toxic to fish and other aquatic organisms.

2.1.3 Storage and treatment of surplus water at the Ekati Diamond Mine

Water quality in the Mackenzie Valley region of the Northwest Territories (NWT) is monitored by the Mackenzie Valley Water Board. Mining companies such as the Ekati Diamond Mine are required to ensure ammonia levels meet water quality criteria specified in their Water License before being discharged into lakes (EBA, 2002). Current practice at the Misery pit involves pumping water out of the sump and storing it temporarily in King Pond. High ammonia levels are treated naturally in this sedimentation pond by a combination of volatilization, biological uptake and conversion to nitrate, until the water is suitable for release to the downstream receiving environment. A pilot project at the Misery pit hopes to reduce the amount of ammonia in its surplus water more quickly by discharging it over the tundra using tall (12 m) towers during the growing season. When water is discharged as a fine mist, more than 98% of the ammonia present is expected to be volatilized at the spray nozzles, with residual nitrogenous compounds being deposited onto the tundra surface and subject to plant uptake and soil processes. It is not known how sensitive tundra ecosystems are to

additional nitrogen inputs, or what concentrations can be applied over the course of the arctic summer without disrupting the natural soil processes.

2.2 Toxicity of Nitrogenous Compounds

2.2.1 The nitrogen cycle

The most abundant chemical in the Earth's atmosphere, nitrogen (N) is required by living organisms as it is an essential component of many complex organic molecules such as amino and nucleic acids. It is the fourth most common chemical element in living tissue, behind carbon, oxygen and hydrogen. It is also the element most often in short supply for plant nutrition (Paul and Clark, 1989). Nitrogen is present in various forms, primarily dinitrogen gas (N_2), organic nitrogen in living and dead tissue, and as ammonia in its unionized (NH_3) and ionized (NH_4^+) forms (Figure 2.1). Microbially mediated processes transform the N atom between its different physical and oxidation states. Increased availability of inorganic nitrogen in soil or aquatic systems usually boosts production, but high concentrations in soil and surface waters resulting from anthropogenic inputs may be detrimental, as inorganic nitrogen pollution can have significant effects on aquatic and terrestrial organisms (NRC, 2000).

2.2.2 Aquatic toxicity

Ammonium nitrate residues are problematic when they enter water systems for several reasons. First, ammonia is usually oxidized to nitrite (NO_2^-) and then nitrate (NO_3^-) by aerobic bacteria in a process referred to as nitrification. Both nitrite and nitrate can be toxic to aquatic species (Russo, 1985; Scott and Crunkilton, 2000). Secondly, ionized ammonia (NH_4^+), referred to as ammonium, establishes equilibrium with unionized ammonia and hydroxide ions (OH^-) in water. The relative concentrations of ammonium and ammonia depend on the pH and the temperature of the water, with increased pH and temperature shifting the equilibrium toward ammonia (Emerson et al, 1975). Unfortunately, the unionized form of ammonia is generally more toxic to aquatic animals than the ionized form (Russo, 1985). Accumulated NH_3 can also inhibit the

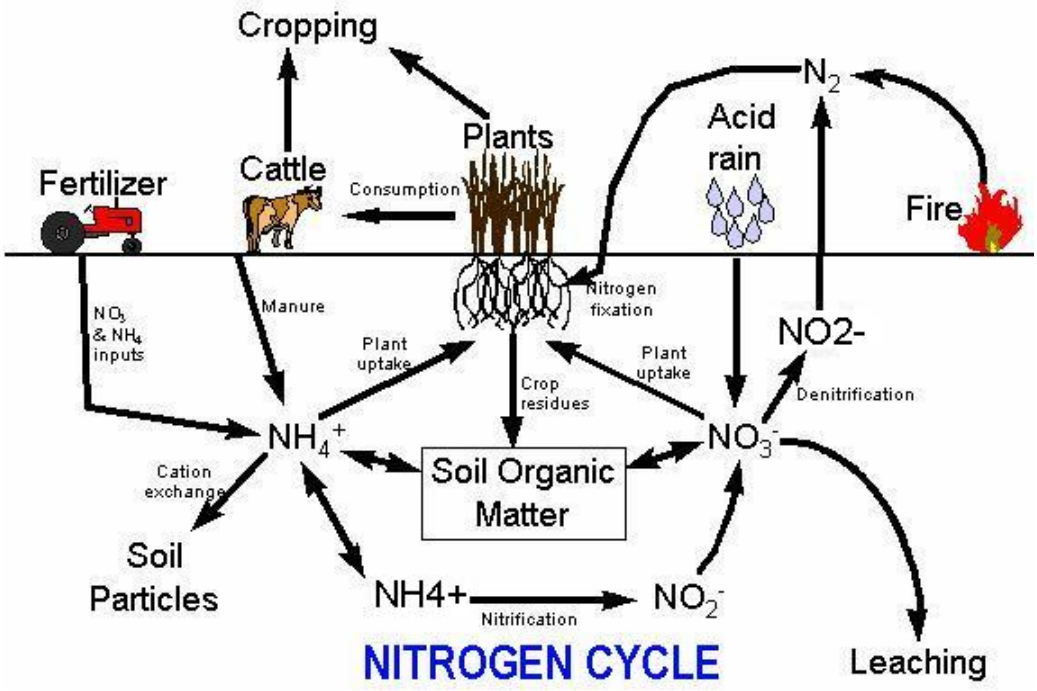

Figure 2.1 The nitrogen cycle

nitrification process by causing toxicity to the *Nitrosomonas* and *Nitrobacter* bacteria (Russo, 1985). Inhibition of this process maintains levels of NH_4^+, and therefore NH_3, which is its most toxic form. Thirdly, nitrogen is often limited in freshwater lakes, and increased input can result in proliferation of primary producers and eutrophication (Smith, 2003). Excess inorganic nitrogen will impact those organisms with low tolerance when the ecosystem can no longer assimilate the additional amounts (Camargo and Alonso, 2006).

Transformations of Ammonia

Nitrogen compounds occur naturally in freshwater and soil environments, resulting from organic matter degradation. Ammonia in its ionized form (NH_4^+), nitrite, and nitrate can all be taken up from solution as nitrogen sources for bacteria, algae, aquatic macrophytes and plants. Ammonia may undergo transformation by several processes, including volatilization, by which the unionized form is lost and returned to the atmosphere (Figure 2.1). Or, ammonia can be used as an energy source by specific bacteria in the nitrification process.

Nitrification

Nitrification is an important process in preventing the accumulation and persistence of ammonia in lakes and other slow moving waters receiving sewage effluent or runoff (Constable et al, 2003). Nitrification is a two stage process in the nitrogen cycle whereby reduced inorganic nitrogen (NH_4^+) is oxidized by chemolithotrophic bacteria, first to nitrite, and then to nitrate. Each stage is performed by a different group of bacteria, as no single bacterium is capable of transforming ammonia to nitrate on its own (Abeliovich, 1992). In the first step ammonia is oxidized to nitrite by the genus *Nitrosomonas*. There are two key reactions, each catalyzed by a different enzyme.

$$NH_3 + 2H^+ + 2e^- + O_2 \rightarrow NH_2OH + H_2O \qquad (2.1)$$

(ammonia monooxygenase)

$$NH_2OH + H_2O \rightarrow HNO_2 + 4H^+ + 4e^- \quad (2.2)$$

(hydroxylamine oxidoreductase)

The second step is carried out by bacteria in the genus *Nitrobacter* and *Nitrospira*, and provides energy for the bacteria. In most habitats these organisms are closely associated and nitrite is rapidly converted to nitrate (Paul and Clark, 1989).

$$HNO_2 + H_2O \rightarrow HNO_3 + 2H^+ + 2e^- \quad (2.3)$$

The nutritional requirements of these bacterial are minimal, and they can be found in any aerobic environment where ammonia is present. They have been proven to exist and function in arctic soils, although they can only be cultured in low numbers at many study sites (Chapin, 1996).

Toxicity of Nitrite

The nitrite ion is highly toxic to aquatic organisms. In fish, nitrite crosses gill epithelium in the same manner as Cl^-, and accumulates in the body fluids. Nitrite oxidizes the iron of fish hemoglobin to methemoglobin, causing anoxia and death, because methemoglobin is unable to transport oxygen (Jensen, 2003). This is similar to the effects seen in crayfish, where nitrite oxidizes hemocyanin, and the resulting methemocyanin cannot bind properly to oxygen atoms (Jensen, 2003). Other toxic effects in fish and crayfish include depletion of Cl^- ions causing severe electrolyte imbalances, damage to mitochondria in liver cells causing energy shortages in the tissue, and immune system depression, among others (Jensen, 2003). It has been suggested by Alonso (2005), that water concentrations of 0.08 – 0.35 mg NO_2^- L^{-1} are required to adequately protect sensitive species.

Toxicity of Nitrate

Nitrate itself is significantly less toxic than nitrite, and it must be transformed to nitrite within an organism to cause adverse effects. Uptake of nitrate is limited in aquatic animals (Scott and Crunkilton, 2000), which reduces the risk of it being converted into a

toxic form. Human infants are susceptible to methemoglobinemia from ingestion of nitrates as they can be converted to nitrites under the anaerobic conditions of the gut. The Canadian Council of Ministers of the Environment (CCME, 2003) recommended a range of 2.9 – 3.6 mg NO_3^- L^{-1} water in 2003, and more recently Camargo et al (2005) recommend that 2.0 mg NO_3^- L^{-1} was the maximum safe concentration for protecting sensitive aquatic animals.

Toxicity of Ammonia

Generally accepted as the most toxic form of inorganic nitrogen, unionized ammonia (NH_3) has several modes of action. All vertebrates are subject to ammonia toxicity because NH_3 can displace K^+ and depolarize neurons, leading to convulsions, coma and death (Randall and Tsui, 2002). Fish are particularly sensitive because gill epithelium are damaged directly, leading to asphyxiation (Russo, 1985). Ammonia also disrupts the glycolysis and Krebs cycles, causing acidosis and reduced blood oxygen-carrying capacity. Oxidative phosphorylation can be uncoupled, causing inhibition of ATP production, and depletion of ATP in the brain (Environment Canada, 2001). Laboratory studies have revealed that freshwater invertebrates such as mollusks and planarians are particularly sensitive to unionized ammonia (Alonso and Camargo, 2004). Concentrations of 0.05 -0.35 mg NH_3 L^{-1} water have been recommended as maximum thresholds for short term exposures (Constable et al, 2003; Environment Canada, 2001) of various aquatic animals.

2.2.3 Terrestrial toxicity

Being a volatile gas, 98% of the ammonia (NH_3) present in waters contaminated with ammonium nitrate is projected to dissipate to the atmosphere upon atomization. Only residual ionized ammonia (NH_4^+) and nitrate would be deposited onto the tundra surface (EBA, 2002), similar to the addition of a fertilizer. However, in terrestrial arctic ecosystems, fertilization and enhanced growth are not necessarily desired, and can cause detrimental impacts if the normal functioning of the soil is altered significantly. While few studies have focused on application amounts of fertilizer that cause toxicity to

northern soil functions, several authors confirm that concentrations above 250 mg N kg^{-1} (Walecka-Hutchison and Walworth, 2007) or 125 mg N kg^{-1} (Walworth et al, 2007) are not linked to increased activity. Inhibition of microbial functions in soil has recently been reported at concentrations of approximately 1200 mg N kg^{-1} soil (Walworth et al, 2007).

Effect of Salinity

Ammonium will be converted during the nitrification process in agricultural soils to nitrate, resulting in low soil concentrations of NH_4^+ compared to NO_3^- (Robertson, 1997). Most fertilizers are composed of ammonium and/or nitrate salts which dissolve quickly in soil pore water. This increases the salt concentration of the soil water and lowers the soil osmotic potential (the portion of the soil water potential energy attributable to dissolved solutes), which can inhibit microbial activity (Walworth et al, 2007). Even when low concentrations of fertilizer are added, salt concentration can increase quickly and become toxic, especially in coarse soils with limited capacity to retain water (Braddock et al, 1997).

2.3 The Arctic Ecosystem

While the tundra appears to be a vast, barren landscape, this is not the case. The inspiring spaciousness is due in part to the shortness of the plants, the living biomass of which can be over 90% underground (Pielou, 1994). Microbial activity in the soil occurs even under the snow, and the soil itself is subject to activity and movement as it thaws and freezes. Changes in nutrient and water availability may have dramatic effects on these ecosystems, which are adapted to nutrient limitations and exhibit low annual productivity. Few, if any, studies exist on the application of fertilizer to arctic soil or its effects on tundra plants.

2.3.1 Arctic Plants

Artic plants endure many hardships, including constant cold temperatures and poor soil conditions. A majority of the plants are perennials, and establish deep extensive root systems that help bind them to the soil despite harsh winds and frost heaves. Most plants are low growing, with their leaves close to the ground, and many have semi-evergreen leaves that survive through the winter and begin photosynthesis early in the spring, while new leaves are still developing (Pielou, 1994). The photosynthesis reaction is slow due to relatively cool temperatures in the growing season, as is the rate of decomposition, reducing the amounts of available nutrients in the soil and contributing to the limited plant growth. Enhancing the growth of arctic plants by fertilizer application has not been of interest, likely due to the short growing season, and subsequently little to no research has been done in this area.

2.3.2 Arctic Soil

The soils at Ekati, NWT, have been described as polar desert soils, as this region receives less than 10 cm of precipitation each year (BHP, 2002b). Based on these criteria, the soils of Resolute, Cornwallis Island, NWT, and most of the other islands in the Canadian Arctic Archipelago are also considered polar desert soils (Figure 2.2). Truelove Lowland, Devon Island, NWT is one exception, classified as a "Polar Oasis" by Bliss in 1977, as it has enhanced moisture retention due to its topography. According to the Canadian System of Soil Classification, Third Edition (NRC, 1998), soils of the high arctic generally belong to the Cryosolic Order, and are further classified into three great groups: Turbic, Static or Organic. All crysols have permafrost within 2 m of the soil surface and a mean annual temperature of 0°C. Turbic Cryosols usually develop in fine-textured mineral soils and are subject to cryoturbation processes during the repeated freezing and thawing of soil. They can often be identified by the presence of patterned ground, such as at the Ekati site (see Figure 3.1). Cryoturbation affects the arrangement of soil particles and pores as the surface layers are mixed into underlying horizons. Horizon structure, physical and chemical properties are therefore affected (Bockheim and Tarnocai, 1998). Static Cryosols exist in well-drained coarse parent materials, and have

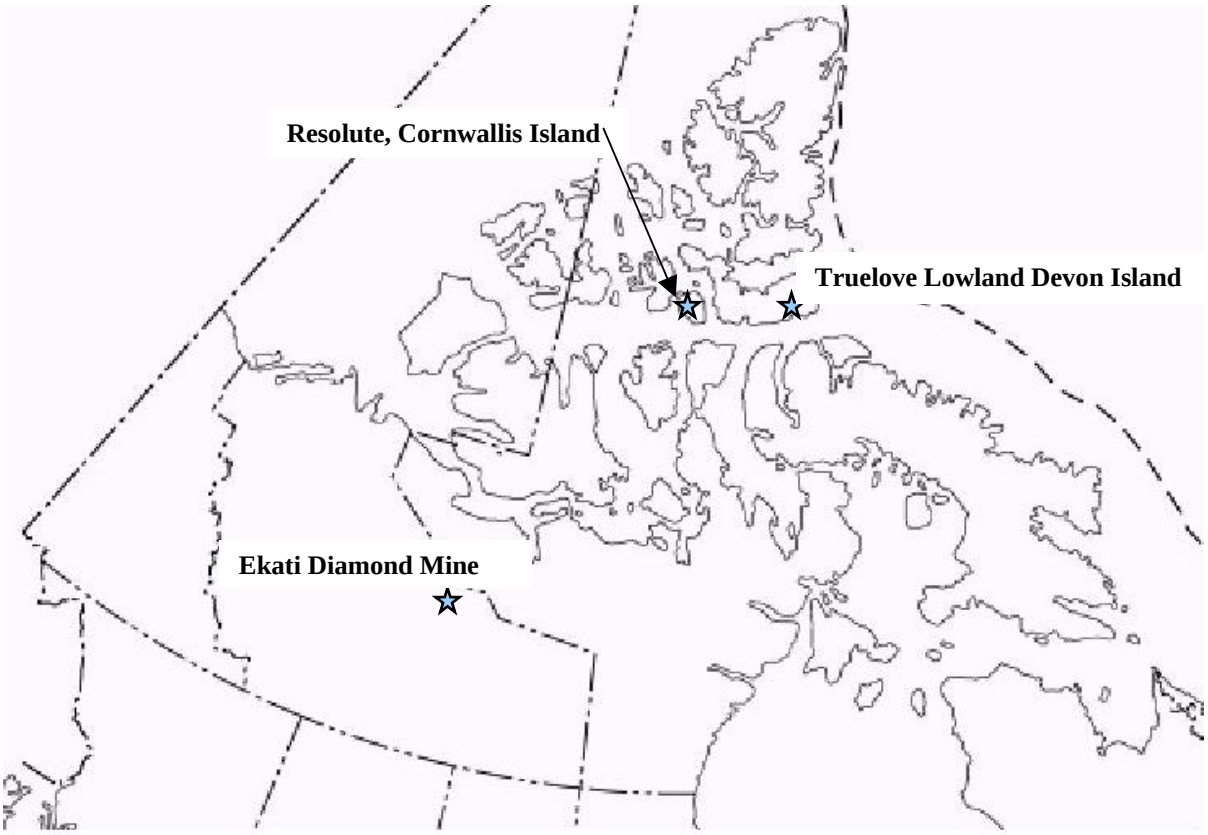

Figure 2.2 Sampling locations in the Canadian arctic

little evidence of cryoturbation. They may have an organic layer up to 40 cm thick. Once the organic layer exceeds 40 cm, the soil is termed an Organic Cryosol.

2.4 Toxicity Testing Standards

The Organisation for Economic Co-operation and Development (OECD) is an international organization of 30 countries, working together to provide a setting where governments can seek answers to common problems, and co-ordinate policies based on good practice. The organization provides a collection of the most relevant and internationally agreed test methods used by government, industry and independent laboratories.

2.4.1 Testing for effects on soil microorganisms

Microorganisms play an important role in the breakdown and transformation of organic matter and nutrient cycling in fertile soils. Any long-term interference with these biochemical processes can potentially alter soil fertility. Therefore, determining the effects of soil contaminants on soil microbial activities are important components of risk assessment. Although the microbial communities responsible for essential soil processes differ from soil to soil, the pathways of transformation are essentially the same, and the transformation of carbon and nitrogen occurs in all soils (OECD, 2000a; 2000b). The OECD recommends carbon and nitrogen transformation tests be carried out to determine the effects of chemicals on soil microflora (OECD, 2000a; 2000b). With such an important part in the nitrogen cycle, the nitrification process becomes an obvious choice for monitoring. Performed by very limited genera of bacteria, impairment of this sensitive function can indicate harmful levels of a toxicant in a timely fashion, especially as analytical methods for determining nitrate, nitrite, and ammonium are quick and accurate (Wentsel et al, 2003). Soil respiration is another excellent indicator of overall biological activity in soil. Carbon mineralization potential can be readily determined by the substrate induced respiration method (Wentsel et al, 2003). Addition of glucose as a substrate to soil samples induces a maximal response from soil microbial biomass, and can be measured by the amount of CO_2 respired.

The OECD has published guidelines (#'s 216 and 217) which describe laboratory test methods designed to investigate potential effects of a single exposure of chemicals on carbon and nitrogen transformation activity by soil microorganisms (OECD, 2000a; 2000b). Due to time constraints and the large number of samples, we chose laboratory methods that required less incubation time, but followed the principles outlined by the OECD.

2.4.2 Testing for effects on plants

The OECD has also published guidelines designed to assess potential effects of substances on terrestrial plants, focusing on seedling emergence and growth (OECD, 2003). Environment Canada has developed a more specific standardized biological test method based on the same criteria and a comprehensive review of existing methods used globally (Environment Canada, 2005a). We chose to follow Environment Canada's procedures, as they are meant to be applicable to diverse types of Canadian soil and use relevant terrestrial plants species to determine sub-lethal toxicity of contaminated soils to plants. However, this criterion was not designed for arctic soils, and may require modifications.

2.4.3 Resistance to Toxicants

In soils, resistance is defined as the capacity of the soil to continue to function without change throughout a disturbance (Seybold et al, 1999). Application of fertilizers is a common disturbance in temperate soils. While the capacity of the soil to function cannot be measured directly, it can be measured indirectly through indicators of specific essential functions. Although the endpoints being measured may not be affected by the initial disturbance or toxicant, the system may be weakened and unable to withstand further stresses from additional toxicants or environmental stresses. We chose to test if soil exposed to ammonium nitrate was more sensitive to a reference toxicant than soil that had not been exposed.

2.5 Statistical Analysis

Toxicity tests are standardized methods to evaluate potential adverse effects of soil contaminants (Stephenson et al, 2000). Detecting toxic effects at low concentrations is difficult, and best estimated by regression techniques (Moore and Caux, 1997). Non-linear regression was used to analyze data from the toxicity tests in this thesis. This involved fitting the data mathematically to selected models, and then calculating the IC*p* (concentration that inhibits the response relative to the control by a chosen percentage, *p*) using the model that best described the exposure-concentration response relationship. Not only did this choice address the non-linear relationship between the data, it also suited the heteroscedasticity of the data that could not be reduced by transformation, and accommodated several concentration response curves (Stephenson et al, 2000). We chose to calculate a more conservative IC*p* of 20% rather than the more typical IC_{50}. This was done to avoid calculating a guideline that would fall within the variance of the control, and still provide a conservative estimate of a site specific protection guideline (Environment Canada, 2005b).

2.6 Research Goal and Objectives

The main goal of this research was to investigate the potential impacts to arctic soils when atomizing surplus water high in ammonium nitrate over the tundra.
Due to the lack of pertinent literature involving arctic soils and fertilization effects, our objective was to characterize the biogeochemical toxicity and phytotoxicity of ammonium nitrate at three different arctic sites. The first challenge in assessing this application was to ensure the arctic sites were accurately represented by samples in a laboratory setting. Furthermore, we wished to determine if soil resistance to additional stressors was compromised by the ammonium nitrate deposition. A more detailed description of our specific objectives by chapter is provided in Table 2.1.

Table 2.1 Research objectives by chapter

Chapter	Objectives	Description of Chapter
1	Introduction	o Brief overview of project and layout of thesis contents
2	Literature review and research objectives	o Background information on diamond mining as a source of nitrogenous compounds in northern environments, transformations, forms and toxicity of nitrogenous compounds, arctic ecosystems and soil processes, toxicity tests and endpoints, the importance of resistance
3	To determine the appropriate sampling intensity for cryoturbated arctic sites	o Variability of physical and chemical characteristics of three arctic soils o Results of a standard phytotoxicity test o Calculation of MDD and CV
4	To determine the potential effects of NH_4NO_3 in arctic soils To determine the resistance of arctic soils after exposure to NH_4NO_3.	o Comparison of important soil functions such as rates of nitrification and carbon utilization, plant growth and emergence in five soils exposed to a range of NH_4NO_3 concentrations over time o Calculation of IC_{20} concentrations of NH_4NO_3 for each soil o After exposure to NH_4NO_3, all soils were challenged with an expected EC_{20} concentration of boric acid, and important soil functions were compared to control soils
5	General discussion and conclusions	o Summary and synthesis of results, important contributions of research, future directions

3.0 VARIABILITY OF ARCTIC SOILS AND THE RESULTING VARIABILITY IN TOXICITY TEST RESPONSES: HOW MANY SAMPLES SHOULD BE TAKEN FROM AN ARCTIC SITE?

3.1 Introduction

Potentially contaminated areas are often sampled to predict or assess the effects of a toxicant to the natural soil ecosystem. The soil samples collected are intended to provide information representative of the larger area or landscape. Therefore an appropriate amount of sampling must be done to accurately describe the system in question. For northern landscapes, the sorting processes that occur in turbic cryosols, referred to as cryoturbation, affect not only the structure of the soil horizons, but also the physical and chemical properties as the surface layers are mixed into the subsoil (Bockheim and Tarnocai, 1998) . The active layer can vary remarkably in its content of organic and mineral material. Biological parameters that are linked to soil fertility will also be highly variable due to this mixing of soil horizons (Bockheim and Tarnocai , 1998). Organic content, soil texture and soil nutrient status are important modulators of plant toxicological response. Thus, it is likely that cryoturbation occurring in northern soils will increase phytotoxicity test variability. Consequently, it is not clear what level of sampling intensity is needed to precisely estimate toxicant effects in northern landscapes.

The ability to detect differences between control and treated samples is dependent on the power of a statistical test as well as the variability of the response variable (Sokal and Rohlf, 1995). Statistical power increases with sample size, but decreases with variability (Sokal and Rohlf, 1995). This relationship can be best visualized by evaluating the minimum detectable difference (MDD). The MDD is the smallest percentage difference between control and treatment means that can be detected for a given endpoint variability and sample size. The coefficient of variation (CV), which is the expression of standard deviation as a percentage of the mean (Sokal and Rohlf, 1995), can be used to calculate MDDs between control and treatment means;

$$MDD = \frac{\sqrt{2} \times (t_{\alpha,v} + t_{\beta,v}) \times CV)}{\sqrt{n}} \quad (3.1)$$

where $t_{\alpha,v}$ is the two-tailed value from a *t*-distribution with *v* degrees of freedom corresponding to a significance level of α, $t_{\beta,v}$ is the *t*-value for β at *v* degrees of freedom, CV is the coefficient of variation, and n is the number of replicates (Brain et al, 2005; Kraufvelin, 1998; Conquest, 1983). For example, a CV of 10% implies that a 25% deviation from control at a significance level of 5% and a statistical power of 80% will require only 4 replicates. In contrast, a CV of 20% would require that 12 replicates would be needed to detect a 25% deviation from the control (Kraufvelin, 1998).

There are several soil toxicity tests that can be employed to assess the impact of a chemical on the soil ecosystem. Most assessment programs consist of several tests, forming a 'battery', which together indicate the total potential toxicity (Dutka and Bitton, 1986). As the predominant primary producer in terrestrial ecosystems vascular plants are commonly included in these toxicity test batteries (Siciliano et al, 1998; Wang and Freemark, 1994; Freemark & Boutin, 1994). Typical phytotoxicty tests measure emergence, shoot and root length and total plant mass after a relatively short growing period such as 14 or 21 days (Stephenson et al, 1997). A phytotoxicity test designed for soil conditions and plant species of southern Canadian environments has been shown to be a robust and sensitive measure when applied to soils with a variety of contaminants (Environment Canada, 2005; Stephenson et al, 1997).

The objective of this chapter was to estimate sample numbers required to assess toxicant effects in two different cryoturbated landscapes in the Canadian arctic. Increasing economic and community development in northern areas will demand rigorous monitoring to assess impacts to these unique areas. Feasible sampling practices to detect small but significant changes are therefore required. Soil physical characteristics known to influence toxicity were also assessed to explore the possibility of using these soil physical characteristics to reduce unexplained variation in phytotoxicity responses in cryoturbated landscapes.

3.2 Materials and Methods

3.2.1 Study sites

Ekati site

The Ekati diamond mine claim, located in the NT northeast of Lac de Gras (64° 42' N 110° 36' W) was selected as one cryoturbated landscape for evaluation. Located in the Mackenzie District Climatic Region, this area is subject to short summers and long winters, with a mean annual temperature of -10°C and annual precipitation of 345 mm of which half is snow (BHP, 2000b). In the summer of 2003, soil samples were taken from the proposed site of a waste water atomization pilot project, which was located near the Misery Pit at the southeast end of the claim block. At this time, two 12 m atomization towers had been erected adjacent to the main haul road, approximately 100 m apart and 50 m from the road itself. Four 100 m transects originating from the towers (two from each tower) were laid out and marked, representing areas of potentially high and low deposition from the towers when in operation. Two additional 100 m transects were marked outside the expected range of deposition, and designated as controls. Grids (10 m x 10 m) were marked at 20 m intervals along each transect, creating sampling areas 20, 40, 60, and 80 m from the towers (Figure 3.1). These 24 10 m^2 grids were sampled at the beginning of June and again at the beginning of July for a total of 48 samples. These samples were intended to represent the pre-impact landscape, and I expected to return to re-sample the area after 1 and 2 years of tower operation. However, the pilot project was discontinued, and no additional samples were collected.

The land surrounding the atomization towers slopes gently in a NW direction, and is mostly covered in ground moraine with an active layer of 2-3 meters over permafrost (EBA, 2002). There are some areas of exposed bedrock, and an abundance of stones and boulders. Soils in the active layer are a mixture of silt, sand and gravel (EBA, 2002). Vegetation was comprised mainly of communities of low shrubs and lichens, with some moss and sedges occurring in the lower, wetter areas (EBA, 2002).

Truelove site

Truelove Lowland consists of a 43 km^2 wetland located along the northeastern coast of Devon Island, NU (75° 33' N 84° 40' W). This portion of the island is designated as a "Polar Oasis" (Bliss, 1977), as the depressed landscape is able to retain surface water due to protection from wind by escarpments. Thus the area is able to support greater biological diversity than other islands in the Canadian Arctic Archipelago, and provides habitat for a range of arctic species (Bliss, 1977), in spite of a mean annual temperature of -16°C (Lev and King, 1999). In 2004, 100 soil samples were collected from one of the main landscapes of the island, referred to as a lower fore slope (LFS) of the raised beaches. These samples were included in the experiment to assess the degree of variability of another cryoturbated arctic landscape having different weather, topography, and parent material. The LFS consists of microhummocks originating from the decomposition of both cushion plant-lichen and cushion-plant moss communities (Bliss, 1977) existing on well drained, alkaline mineral soils (Lev and King, 1999).

3.2.2 Soil sampling and preparation

Ekati site

At the Ekati site, samples were taken of both the O and C horizon because the C horizon was present on the soil surface due to cryoturbation (Figure 3.1). Soil was sampled using a soil auger with a diameter of five cm, to a maximum depth of 15 cm. Sample depth was <15 cm in areas where the O horizon was relatively thin. Four cores of each horizon were taken from within each grid, and then the samples of each horizon were hand-mixed together to form one composite sample of each horizon per grid. Composite samples were stored in sealed plastic bags and frozen at –20° C prior to being shipped from the mine site to the soil science laboratory in Saskatoon. Samples remained at –20° C until used. Prior to analysis samples were thawed, air dried, and passed through a 2 mm sieve.

Truelove site

On Truelove Lowland samples were collected from along a north/south transect located on a lower fore slope, which was one of the largest landscape types on the island.

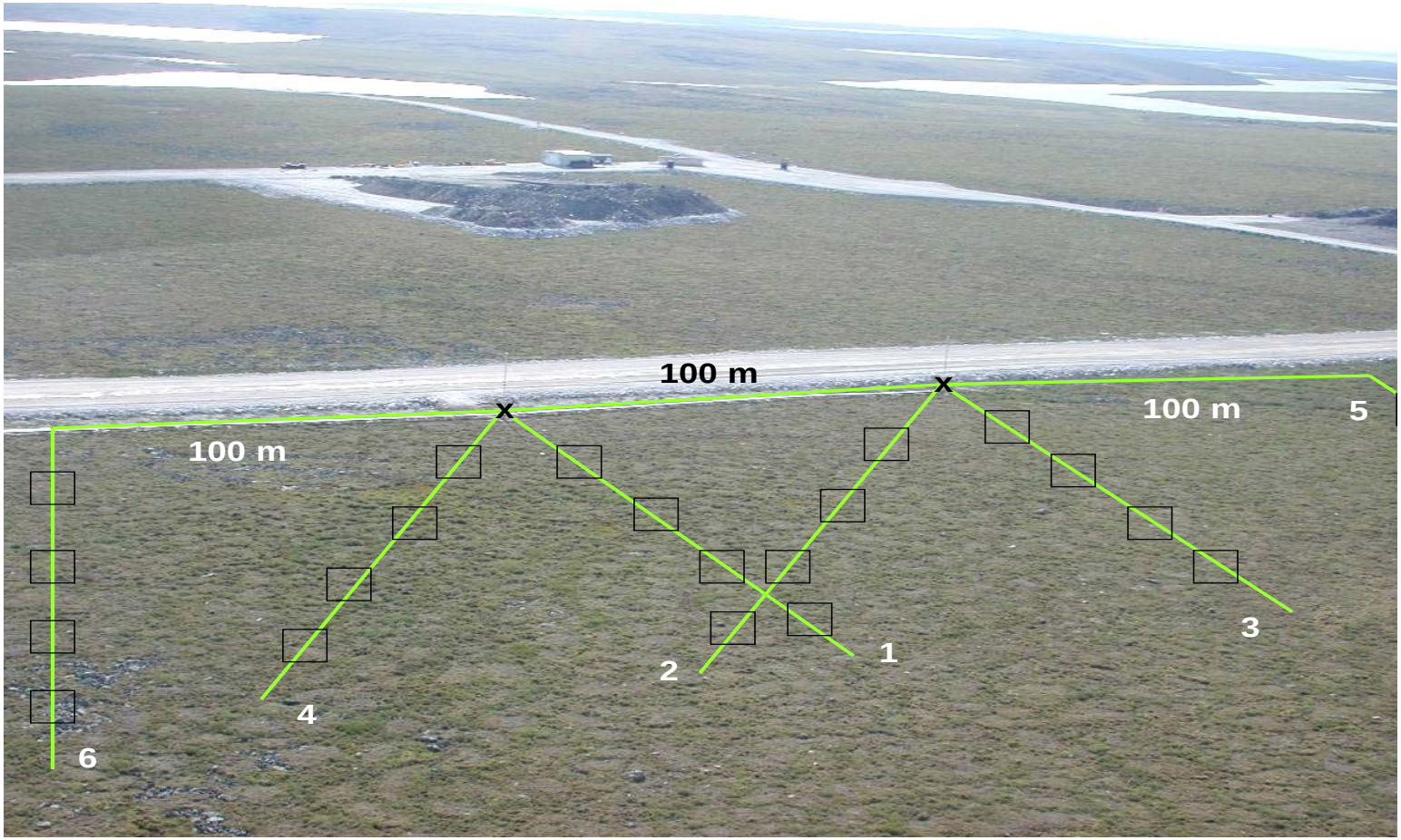

Figure 3.1 Sampling design at Misery site, Ekati diamond mine, NT

Figure 3.2 Sampling transect on Truelove Lowland, Devon Island, NU

Although this area was a turbic cryosol, the Ahky/Ahk layer of sandy loam was greater than 20 cm and there was no B/C horizon present on the surface. Consequently all 100 samples were from the Ah horizon. Samples were taken about 1 meter apart and within five meters of the transect line (Figure 3.2). The soil was sampled with a trowel, to a depth of 10 – 15 cm, with only one sample per location. Samples were bagged immediately and shipped to the University of Saskatchewan in coolers, where they were frozen at –20°C upon arrival. Each sample was air dried and sieved to 2 mm before analysis.

3.2.3 Soil analysis

pH

Soil pH was determined by addition of a 0.01M $CaCl_2$ solution to each sample in a 1:2 soil to solution ratio (1:4 for organic soils) (Kalra and Maynard, 1991). After allowing time for absorption, the solution was stirred several times over 30 minutes, and allowed to settle before the pH electrode was immersed in the supernatant.

Organic carbon

Organic carbon content of each soil was determined using the Leco CR-12 carbon analyzer following procedures outlined by Wang and Anderson (1998). Samples were ground with a mortar and pestle, and approximately 0.2 g was weighed into a crucible and placed in the carbonator at 841°C and ignited. The organic carbon content is expressed as a percentage of the total amount of soil.

Ammonium (NH_4^+) and Nitrate (NO_3^-)

Soil concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) were assessed by extraction with 2 M KCl (Kalra and Maynard, 1991). Soil samples (5 g) were placed in a flask with 50 ml of 2 M KCl (or any 1:10 ratio) and shaken for 30 minutes on a reciprocating shaker at approximately 160 strokes per minute. The slurry from each flask was passed through Whatman 42 μm paper filter (Whatman, New Jersey, USA) into a

separate dram vial, capped and refrigerated until analysis. Both ammonium and nitrate were determined colorimetrically from each sample at the same time.

Texture

Soil texture was determined for the C horizon of the Ekati soils as well as several of the Truelove samples by the hydrometer method (Gee and Bauder, 1986). Ekati O horizon soils had no detectable clay content with this method.

3.2.4 Phytotoxicity test

Phytotoxicity of soil was determined using a 21 day early seedling growth test (Environment Canada, 2005a). This method is more sensitive than the shorter seedling emergence test, and includes growth metrics (root and shoot lengths) as endpoints. Briefly, 5 seeds were planted in test units containing either untreated site soil or site soil spiked with a reference toxicant. All test units were grown simultaneously in an environmental chamber. Relevant arctic conditions were used, with 20 hours of daylight at an intensity of 400 μmoles m^{2-1} s^{-1}, and 4 hours of darkness. The daylight temperature was set at 16°C and the night temperature at 9°C (+/- 1%), with the relative humidity constant at 70% (+/- 5%). These conditions were based on average historic Ekati and Truelove weather patterns during June and July, when plants are actively growing.

Northern wheatgrass (*Elymus lanceolatus*) was chosen as the plant species. This species is commonly used in phytotoxicity tests, and has a well-defined dose response curve for exposure to the reference toxicant boric acid (Environment Canada, 2005a; Stepenson et al., 2000). Northern Wheatgrass occurs naturally as far north as Alaska. It is well adapted for low fertility soils such as those found in the north and is commonly used for the re-vegetation of oil and gas well sites and other construction areas due to its tolerance of severe soil conditions. Seedling percent emergence and seedling root and shoot length were measured and averaged for each test unit.

Boric acid (H_3BO_3) was chosen as a reference toxicant by Environment Canada and the Canadian Association of Petroleum Producers (CAPP) when they developed phytotoxicty tests specifically for Canadian soils (Stephenson et al, 1997). Boric acid is a

soluble compound with a low occupational hazard, yet persistent over time and readily absorbed and taken up by plant roots. Boric acid concentrations of 536 μg g^{-1} typically cause 20% inhibition (IC_{20}) of plant growth in temperate soils (Environment Canada, 2005a). In the present study, soils were maintained at 60% moisture holding capacity (MHC) for several days before the test began, to allow the soil microbial communities to stabilize. Each soil sample was divided into two portions and after stabilization, one portion was dosed with an aqueous boric acid solution to achieve a concentration of 536 μg g^{-1} soil. The remaining portion (control) was also watered but the solution did not contain boric acid. The damp soil was mixed thoroughly, and maintained at 60% MHC for a few more days before seeds were planted.

3.2.5 Dose-response test

A dose-response test using the same test conditions was conducted on the Ekati C horizon and Truelove soils with decreasing concentrations of boric acid as the treatment (there was not enough Ekati O soil remaining to perform this dose response experiment). There were seven doses of 536, 268, 134, 67, 34, 17, 8 and 0 μg boric acid g^{-1} soil. Each dose was replicated four times.

3.2.6 Statistical analysis

Soil parameters were not normally distributed with heterogeneous variances between soil types. Mood's median test was used to analyze for significant differences between medians of each soil type. The relationship between the standardized standard deviation (otherwise known as the coefficient of variation) and sample number was developed by randomly re-sampling the soil data set thirty times, and estimating the coefficient of variation for increasing number of samples. Scatter plots were used for comparison between soil parameters (non-parametric) and observed toxicity responses, but no correlations were performed.

The concentration of boric acid required to inhibit plant growth by 20% (IC_{20}) was estimated for each soil's growth endpoints by using re-parameterized logistic or exponential dose response relationships (Environment Canada, 2005b; Stepenson et al.,

2000). Boric acid dose was expressed as a logarithm of concentration, and hormetic effects were assessed. After hormesis was evaluated, data was checked for normality and the homoscedasticity of residuals from the dose response curves evaluated.

$$\text{Logistic: } Y = \frac{t}{1 + \left(\frac{p}{1-p}\right) \times \left(\frac{C}{IC_p}\right)^b} \tag{3.2}$$

$$\text{Exponential: } Y = t \times e^{\left(\log\left(\frac{t - t\times p - b\times(1-p)}{t}\right) \times \frac{C}{IC_p}\right)} + b \tag{3.3}$$

In the above equations, Y is the organism response, t is the control response, p is the desired effective concentration percentile, C is the dose or concentration, IC_p is the inhibitory concentration for percentile p, and b is a fitting parameter (Environment Canada, 2005; Stepenson et al., 2000; Van Ewijk and Hoekstra, 1993).

3.3 Results

3.3.1 Soil analysis

High variability was seen within each soil's characteristics (Table 3.1). The soils differed significantly (Moods Median Test: $p \leq 0.001$) in their percent clay, pH, percent organic carbon, and ammonium and nitrate levels. Truelove soil had the highest pH with a median of 7.27 (interquartile range (IQ) of 0.55) which was three pH units greater than Ekati C or O horizon. There was no detectable clay in Ekati O horizon but 1.4% in Truelove O horizon and 4.5% in Ekati C horizon. The Ekati O horizon had the highest amount of organic carbon with a median value of 17%, which was double that of the Truelove soil. The amount of organic carbon in the Ekati C horizon was considerably lower with a median of 0.5%. Ammonium was detected in all soils, but was significantly lower in the Ekati C horizon, the median values being 0.2 for Ekati O and 0.7 for Truelove O, while only 0.04 for Ekati C. Nitrate was detected only in the Truelove soil, with 2.0 being the median value.

The three soils had different degrees of variability with regard to their chemical parameters (Figures 3.3, 3.4). For example, Ekati C horizon required over 20 soil samples to achieve a coefficient of variation of less than 10% in organic carbon content. In contrast, the other soil types required less than 10 soil samples to have a similar level of variability in this parameter. This difference likely reflects differences in the total organic carbon content of the three soils, with Ekati C having a median organic carbon content of 0.5% (IQ 0.6) compared to median organic carbon contents of 18% for Ekati O and 8% for Truelove O horizons. Capturing this variability would require a large number of soil samples (Table 3.2). We estimated how many soil samples by interpolating the number of samples required for a coefficient of variation of < 10%. The Ekati C horizon required the greatest amount of sampling on average.

3.3.2 Phytotoxicity test

Remarkably, in Ekati C horizon, there was uniform 100% inhibition following exposure to a dose of boric acid reported to only cause 20% inhibition in temperate soils (Figure 3.5). The minimum and maximum inhibition of seedling emergence was -150 and 100% in Truelove O horizon soils, and in Ekati O horizon soils it was -200 and 100%. Negative inhibition occurs when addition of boric acid stimulates plant growth, rather than reducing it. This variability in seedling emergence is reflected in the non-normality of the data, with a coefficient of variation of 210% for Truelove O horizon and 590% for Ekati O horizon. Despite this variability, the average amount of inhibition on Day 21 median emergence caused by a 536 $\mu g\ g^{-1}$ dose of boric acid was 10% (IQ 0 to 50) in Ekati O horizon soil and 25% (IQ 0 to 60) in Truelove O horizon soil. These values are well within the expected range of plant responses. Due to the lack of emergence in Ekati C horizon soils, inhibition of root and shoot growth could not be calculated and was considered to be 100%.

Root growth inhibition varied widely in the soils, with root length in treated soils ranging from severely inhibited (as much as 96% in Truelove soils and 82 % in Ekati O soils) to being over 200% longer in treated soils than control soils. This variability in root length is reflected in the non-normality of the data, with a coefficient of variation of 190% for Truelove O horizon and 650% for Ekati O horizon. Median inhibition of root

growth was 8% (IQ -8 to 41) in Ekati O horizon soil and 32% (IQ 11 to 68) in Truelove O horizon soil.

Shoot growth inhibition also varied widely, with the maximum inhibition of shoot length being 75% for both Truelove and Ekati O horizon soils, while the minimum inhibition was -72% in Truelove soils and -266% in Ekati O soils, again resulting in longer shoots in treated soils than in control soils in some instances. This variability in shoot length is reflected in the non-normality of the data with a coefficient of variation of 190% for Truelove horizon and 4880% for Ekati O horizon. The median shoot inhibition was 23% (IQ -2 to 38) in the Truelove soil and -1.2% (IQ -16 to 14) in the Ekati O soil.

The variability in inhibition was much greater than the variability observed for soil properties (Table 3.3). For example, the coefficient of variation for root length for Ekati O was 654%, which is almost 20 times greater than the coefficient of variation for organic matter (33%) for the same soil. This variability is reflected in the relationship between sample number and the standardized standard deviation of root and shoot length inhibition (Figure 3.6). Coefficients of variation for shoot and root inhibition for all soils remained well above 10% despite having >30 independent samples.

To partially explain the variability observed in the root and shoot inhibition, the ability of soil parameters to predict root and shoot inhibition was explored (Figures 3.7, 3.8, 3.9, 3.10). Organic carbon content (Figure 3.7) appeared to be very weakly correlated with inhibition; however, as inhibition results were non-normally distributed, correlation analysis was not performed. The remaining soil parameters were clearly not related to inhibition results. Percent clay was not investigated because there was no detectable clay in Ekati O horizon. Further, only nine Truelove O horizon samples were analyzed for clay content, and while every Ekati C sample had clay content, there was 100% inhibition in all Ekati C samples.

3.3.3 Dose-response test

As the reported IC_{20} of 536 μg g^{-1} boric acid used in the standard phytotoxicity test caused 100% inhibition in the Ekati C horizon, it was necessary to determine a more appropriate concentration. The log dose of boric acid versus the responses of each growth endpoint was plotted for the two soils, and an appropriate curve was fitted using

non-linear regression. Ekati C horizon soils required exponential curves, while logistic curves fit best to the Truelove data (Figures 3.11 and 3.12). There was a strong relationship between the dose of boric acid and emergence in the Ekati C soil; as dose increased, emergence decreased ($r^2 = 0.75$) (Figure 3.11). However the Truelove soil did not exhibit a clear dose-response relationship for this endpoint (Figure 3.12). While appropriate curves were fitted to data for the root length and shoot length endpoints of both soils, these were not strong relationships and the r^2 value was less than 0.3 in all cases.

The IC_{20} of boric acid in these arctic soils ranged from 55 – 3257 mg kg^{-1}, with Ekati C horizon soils being the most sensitive for emergence and shoot length (Figure 3.13). We did not have enough soil to incorporate Ekati O horizon soil into the dose response curves. Thus, Ekati O horizon IC_{20}'s were calculated using data from the original phytotoxicity study by taking the average responses for control and boric acid treated samples, using the following calculations for each endpoint:

$$mg\_boric\_acid\_per\_\%inhibition = \frac{536 mg\_boric\_acid\_kg^{-1} soil}{100 - (\frac{Treated}{Control} \times 100)} \quad (3.4)$$

This value was then multiplied by 20 to estimate the boric acid concentration required to cause inhibition of 20% in plant growth parameters. We acknowledge that this is not the preferred method to estimate percent inhibition, but the values calculated by this technique appear to correspond with the field observations in which Ekati O horizon was the least sensitive to boric acid inhibition.

The IC_{20} differed significantly between soil types, with the Ekati C horizon being the most sensitive to boric acid with an IC_{20} for emergence of 55 mg kg^{-1} soil. In contrast, the Ekati O horizon had an IC_{20} for emergence of 554 mg kg^{-1} soil. Non-linear regression was unable to determine the IC_{20} for the Truelove soils in the second experiment, but calculations based on means from the single point dosing experiment estimate the IC_{20} for emergence to be 402 mg kg^{-1} soil in the Truelove soil.

Table 3.1 Variability in soil characteristics of three arctic soils. (EC = Ekati C horizon, EO= Ekati O horizon, TL= Truelove Lowland O horizon). ND = not determined.

Soil		*n*	*Median*	*Minimum*	*Maximum*	*Mean*	*Std. Deviation*	*Skewness*	*Coefficient of Variation (%)*
EC	pH	48	4.4	3.6	5.0	4.4	0.28	-0.36	6
	Clay (%)	46	4.5	0.7	15.6	5.0	3.12	1.07	62
	Organic carbon (%)	48	0.5	0.2	3.2	0.8	0.61	2.30	76
	Ammonium (mg kg^{-1})	14	0.04	0.0	0.4	0.1	0.10	2.44	100
	Nitrate (mg kg^{-1})	14	0.0	0.0	0.0	0.0	0.00	/	/
EO	pH	40	4.0	3.6	4.6	4.1	0.22	0.30	5
	Clay (%)	0	ND	ND	ND	ND	/	/	/
	Organic carbon (%)	41	17.8	7.0	29.9	17.4	5.71	-0.02	33
	Ammonium (mg kg^{-1})	41	0.2	0.1	1.4	0.3	0.21	3.87	70
	Nitrate (mg kg^{-1})	41	0.0	0.0	0.1	0.0	0.01	5.46	/
TL	pH	97	7.3	6.4	8.0	7.3	0.37	-0.13	5
	Clay (%)	9	1.3	0.0	2.8	1.4	1.16	0.13	83
	Organic carbon (%)	97	7.8	2.8	18.1	8.1	3.04	0.82	38
	Ammonium (mg kg^{-1})	39	0.7	0.3	1.4	0.7	0.21	0.87	30
	Nitrate (mg kg^{-1})	39	2.0	0.6	8.5	2.8	1.86	1.08	66

Table 3.2 Numbers of samples required from three arctic soils in order to reduce the coefficient of variation to 10%. (EC = Ekati C horizon, EO= Ekati O horizon, TL= Truelove Lowland O horizon). NC = not calculated

Soil	*pH*	*Clay*	*Organic Carbon*	*Ammonium*	*Nitrate*
EC	2	22	25	13	NC
EO	2	NC	5	21	NC
TL	2	7	9	8	17

Table 3.3 Variability in inhibition of three growth endpoints (day 21 emergence, root length and shoot length,) measured in Northern Wheatgrass after exposure to an IC_{20} concentration of boric acid (536 µg g^{-1} soil) in a standard phytotoxicity test applied to arctic soils (EO= Ekati O horizon, TL= Truelove Lowland O horizon).

Soil		*n*	*Median*	*Minimum*	*Maximum*	*Mean*	*Std. Deviation*	*Skewness*	*Coefficient of Variation (%)*
EO	Day 21 Emergence (%)	41	0.0	-200	100	10.4	61.95	-1.39	596
	Root Length (mm)	37	20.5	-240	82	8.5	55.62	-2.70	654
	Shoot Length (mm)	40	-2.0	-267	100	-1.2	58.55	-2.11	4879
TL	Day 21 Emergence (%)	90	29.0	-150	100	25.1	53.42	-0.62	213
	Root Length (mm)	90	30.9	-374	100	32.0	59.83	-3.49	187
	Shoot Length (mm)	90	13.0	-72	100	23.3	43.19	0.55	185

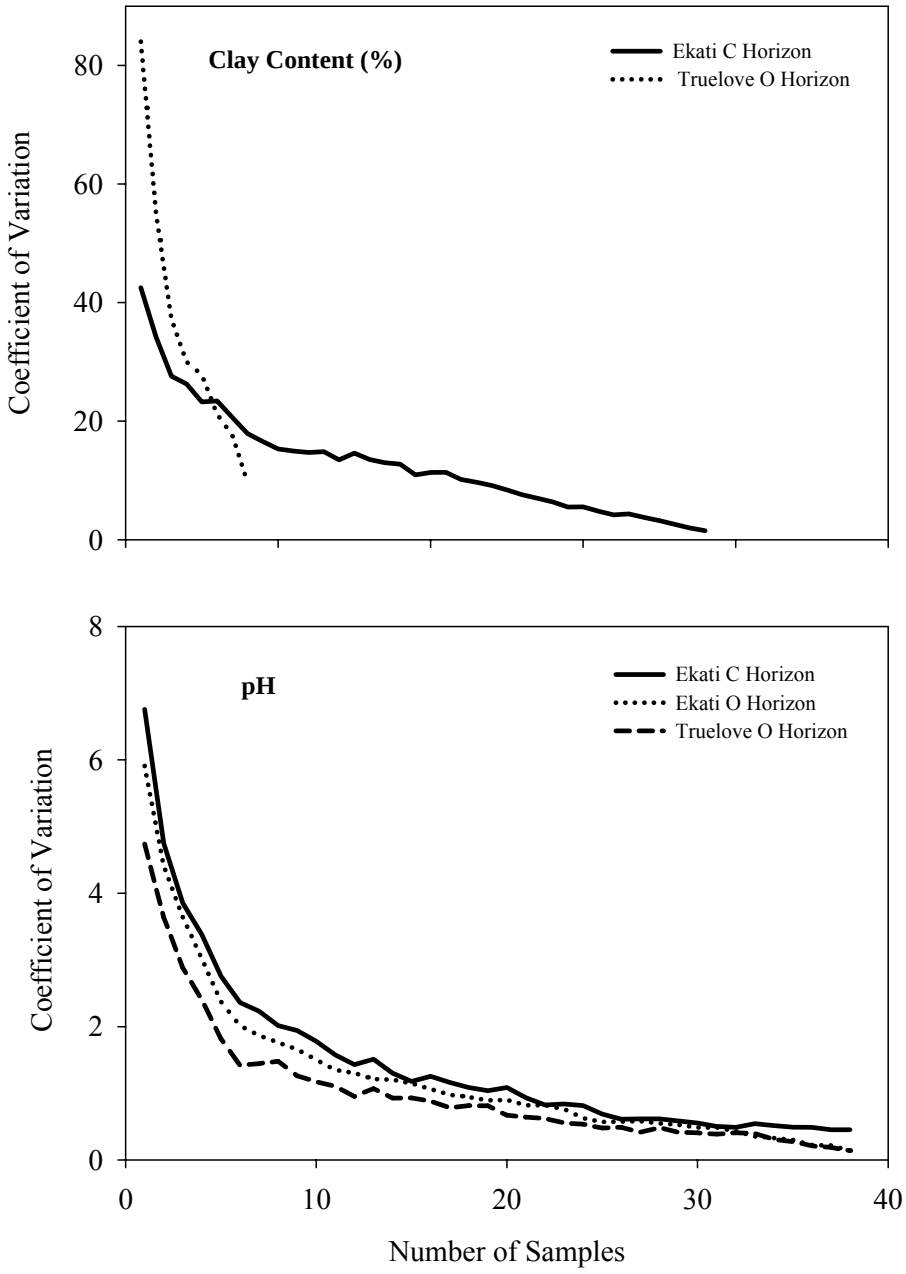

Figure 3.3 Variability of soil biological parameters percent clay and pH present in arctic soils as a function of the number of soil samples analyzed from the same area. Approximately 35 independent estimates of each parameter for each soil were randomly re-sampled 30 times and the coefficient of variation of increasing sample numbers estimated from each re-sampling.

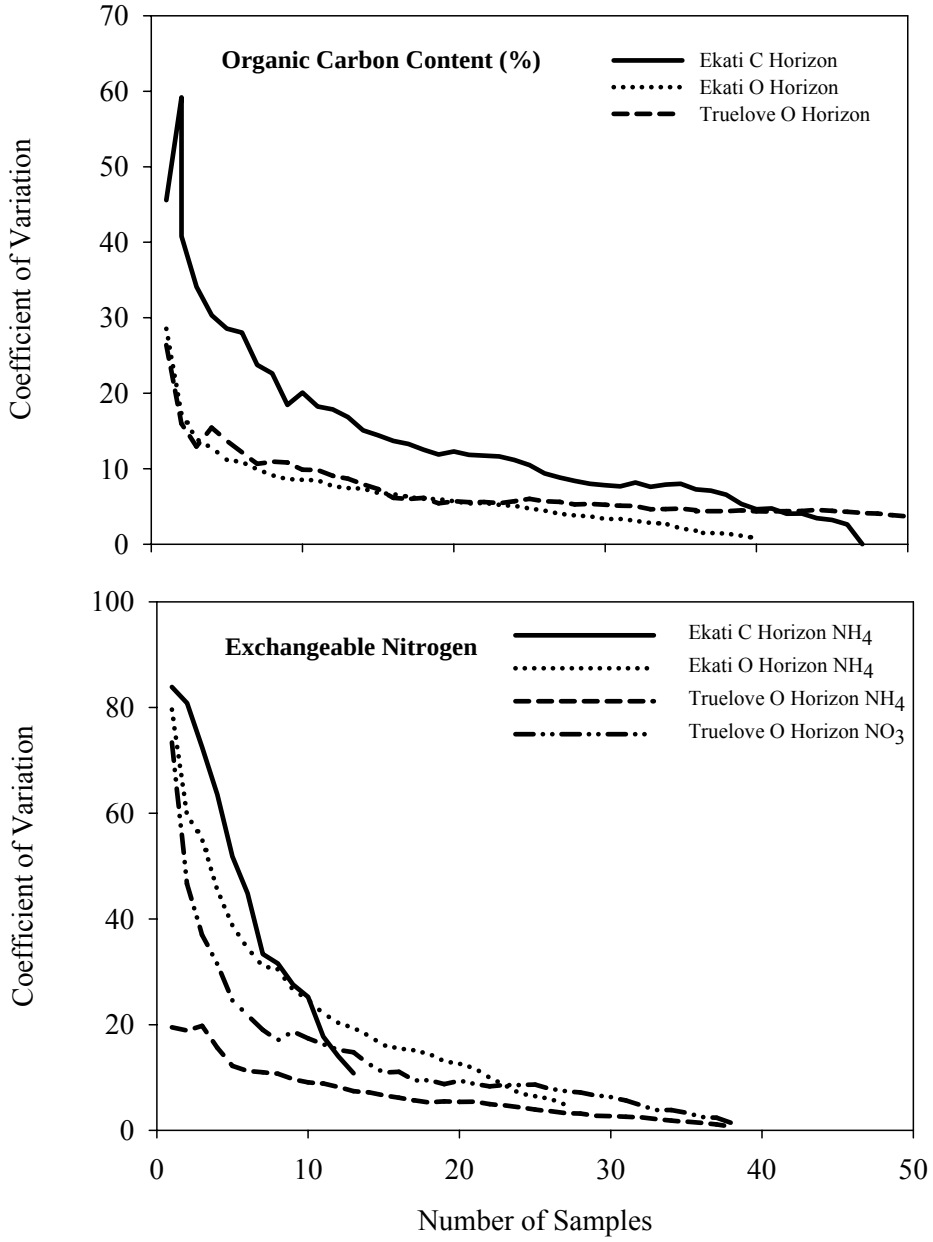

Figure 3.4 Variability of soil biological parameters organic carbon, ammonia and nitrate present in arctic soils as a function of the number of soil samples analyzed from the same area. Approximately 35 independent estimates of each parameter for each soil were randomly re-sampled 30 times and the coefficient of variation of increasing sample numbers estimated from each re-sampling.

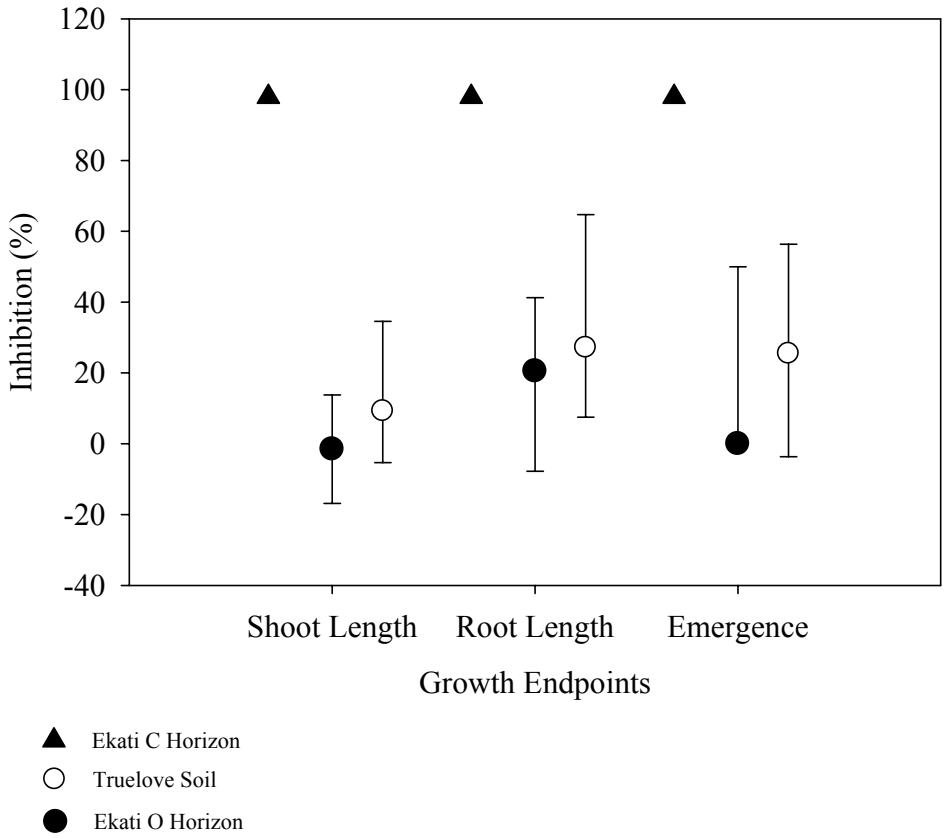

Figure 3.5 Inhibition of three growth endpoints (shoot length, root length, day 21 emergence) measured in Northern wheatgrass after exposure of three arctic soils (EC= Ekati C horizon, EO= Ekati O horizon, TL= Truelove Lowland O horizon) to boric acid at 536 µg g^{-1} soil in a standard phytotoxicity test. Each symbol represents the median value (EC n=46, EO n= 41, TL n=90), and error bars represent the first and third quartiles of the data range.

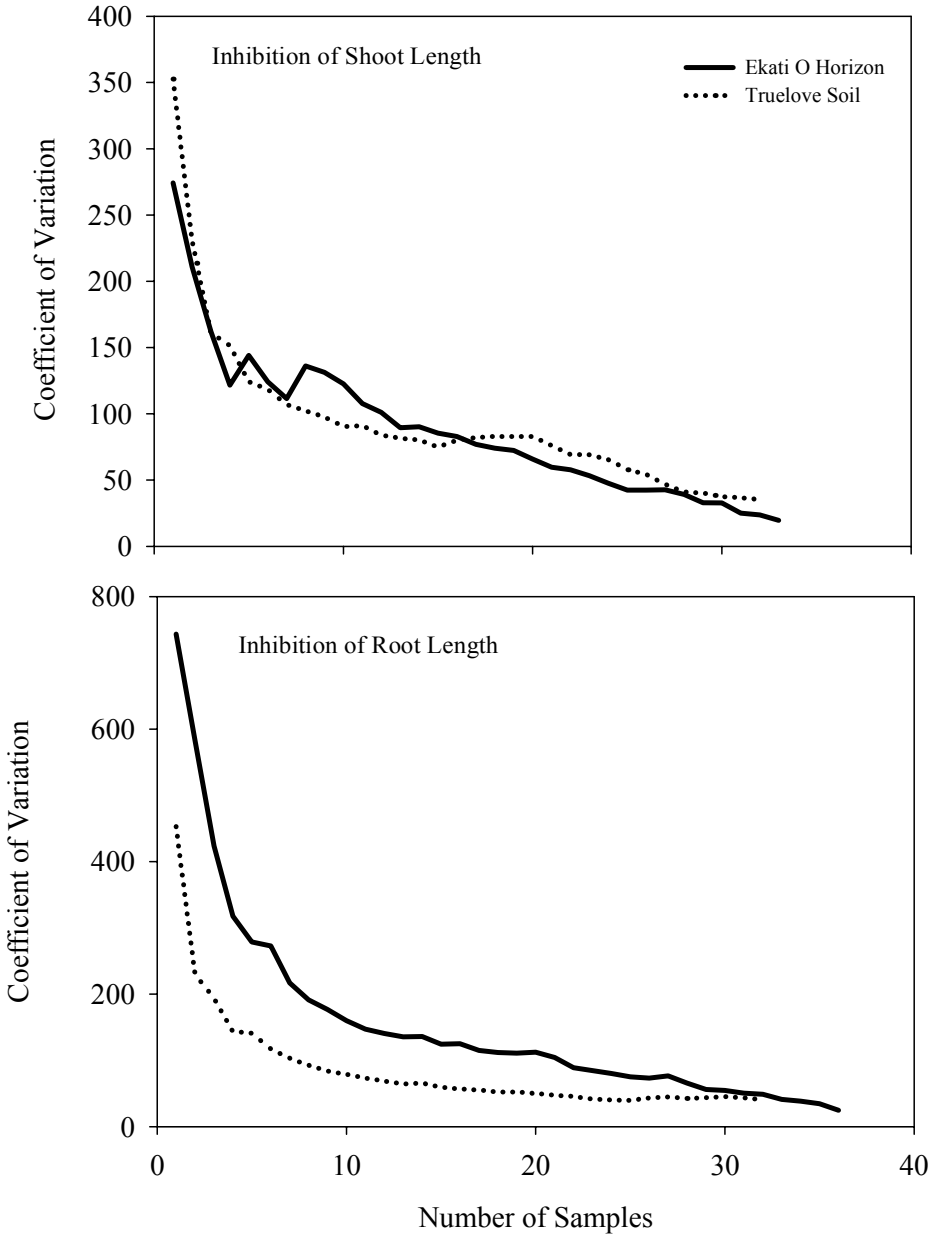

Figure 3.6 Variability of shoot or root inhibition by 536 µg boric acid g^{-1} soil after 21 days in a standard phytotoxicity assay as a function of the number of soil samples analyzed from the same area. Approximately 35 independent estimates of shoot or root inhibition for each soil were randomly re-sampled 30 times and the coefficient of variation of increasing sample numbers estimated from each re-sampling.

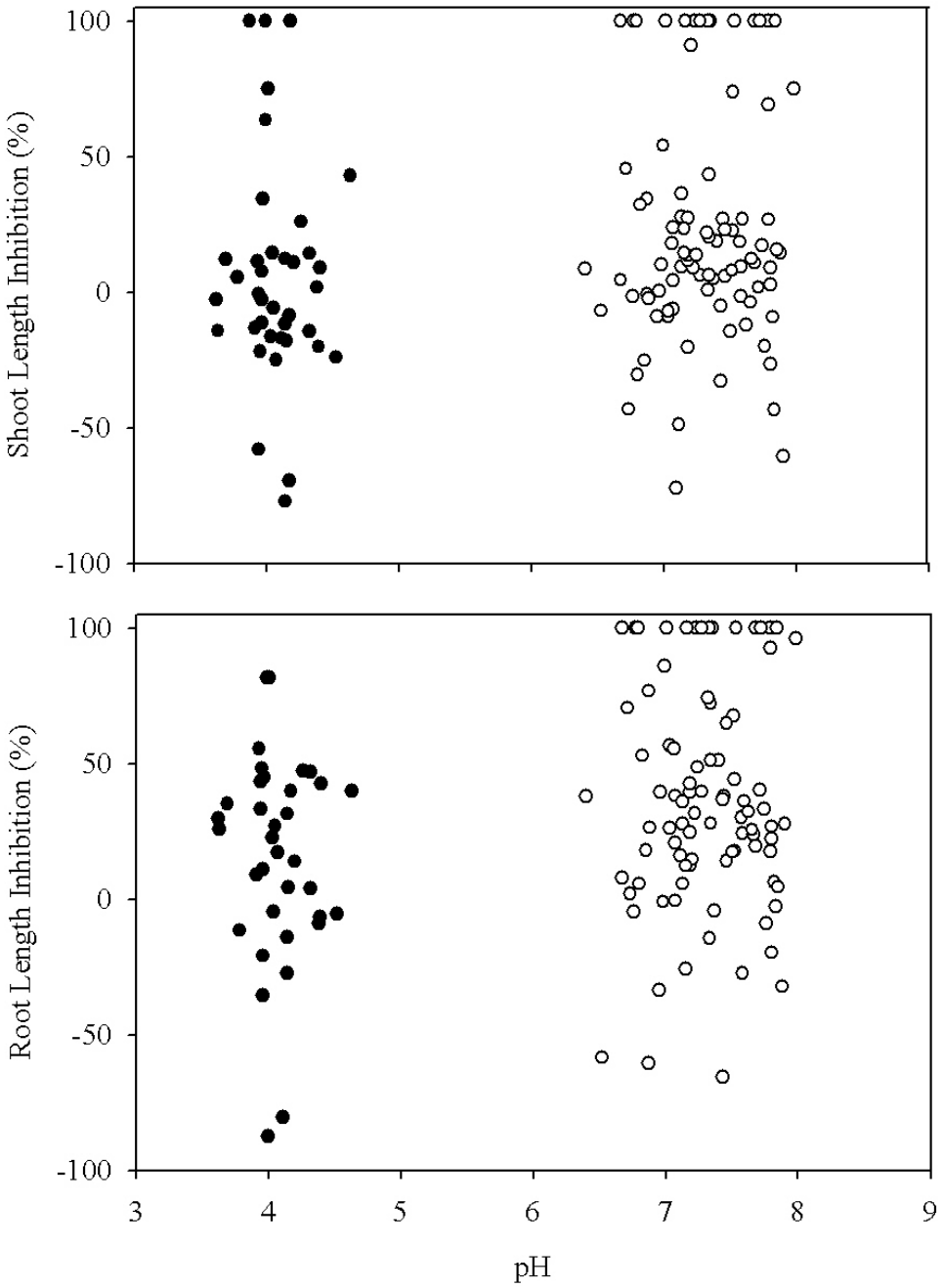

Figure 3.7 Inhibition of shoot and root growth in Northern wheatgrass exposed to 536 µg boric acid g^{-1} soil for 21 days in a standard phytotoxicity test compared to the soil pH (0.1 M $CaCl_2$) in two arctic soils. Open symbols are Truelove Lowland O horizon and closed symbols are Ekati O horizon.

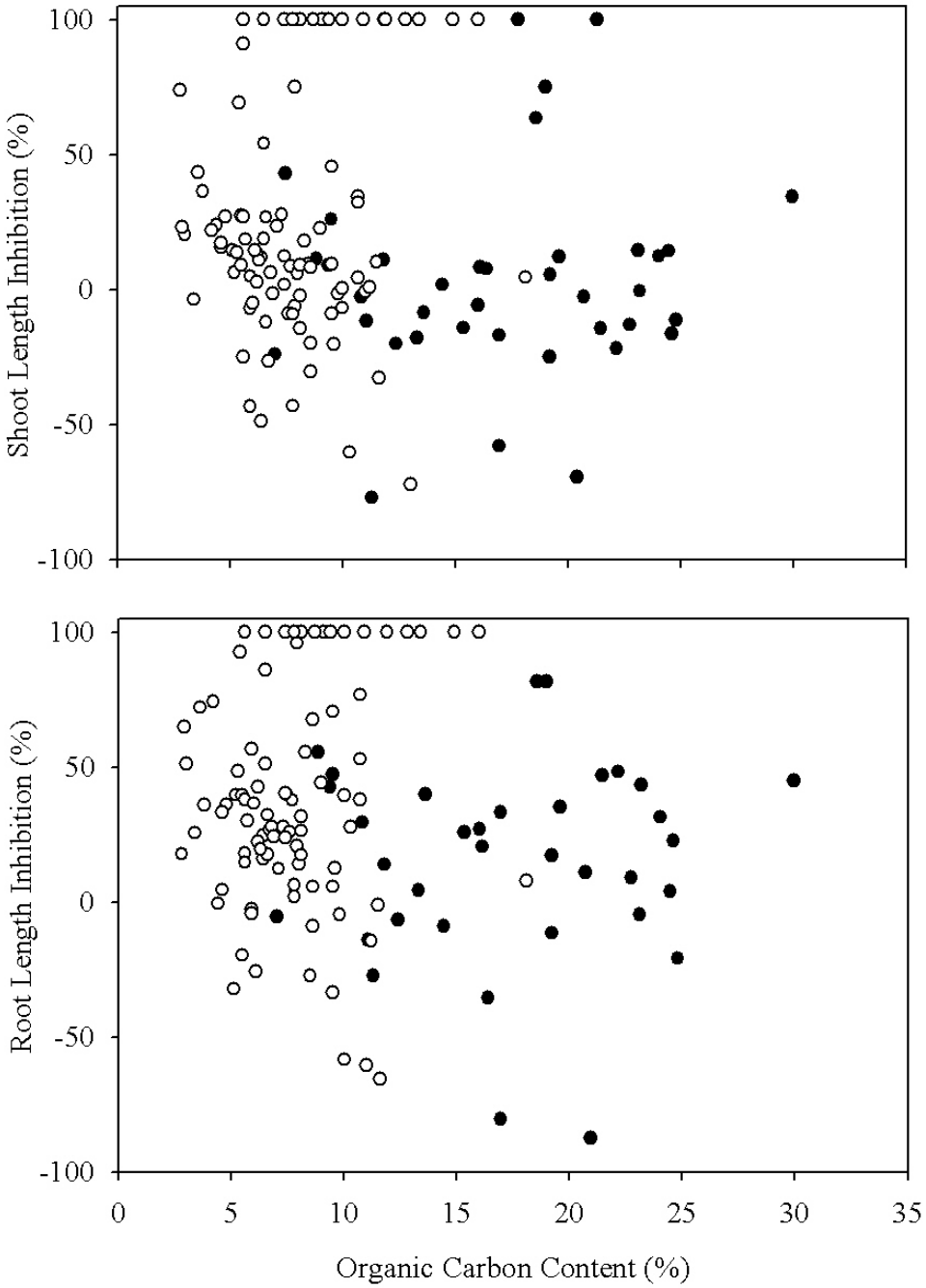

Figure 3.8 Inhibition of shoot and root growth in Northern wheatgrass exposed to 536 µg boric acid g^{-1} soil for 21 days in a standard phytotoxicity test compared to the amount of organic carbon present in two arctic soils. Open symbols are Truelove Lowland O horizon and closed symbols are Ekati O horizon.

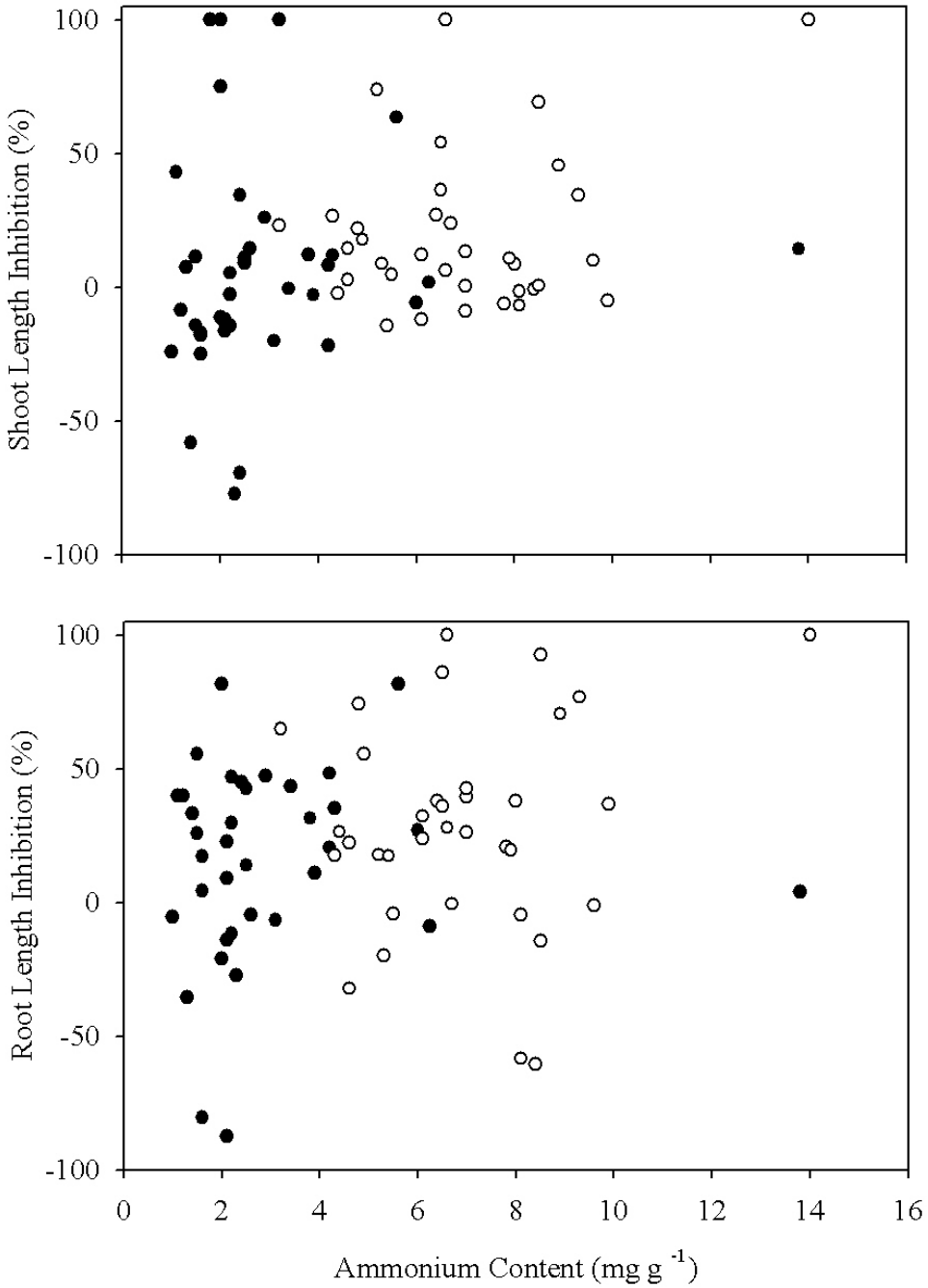

Figure 3.9 Inhibition of shoot and root growth in Northern Wheatgrass exposed to 536 µg boric acid g^{-1} soil for 21 days in a standard phytotoxicity test compared to the exchangeable ammonia in two arctic soils. Open symbols are Truelove Lowland O horizon and closed symbols are Ekati O horizon.

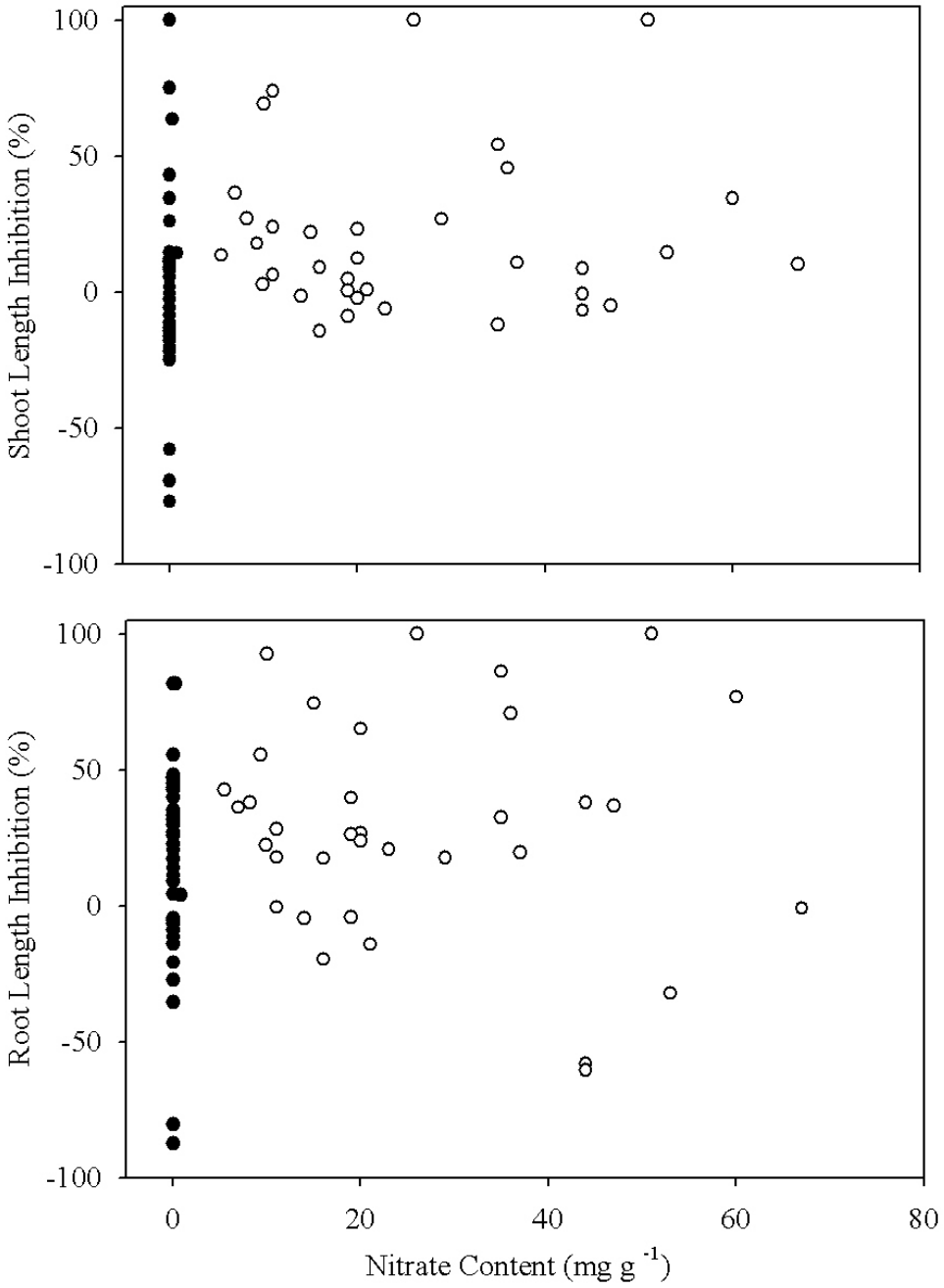

Figure 3.10 Inhibition of shoot and root growth in Northern Wheatgrass exposed to 536 µg boric acid g^{-1} soil for 21 days in a standard phytotoxicity test compared to the exchangeable nitrate in two arctic soils. Open symbols are Truelove Lowland O horizon and closed symbols are Ekati O horizon.

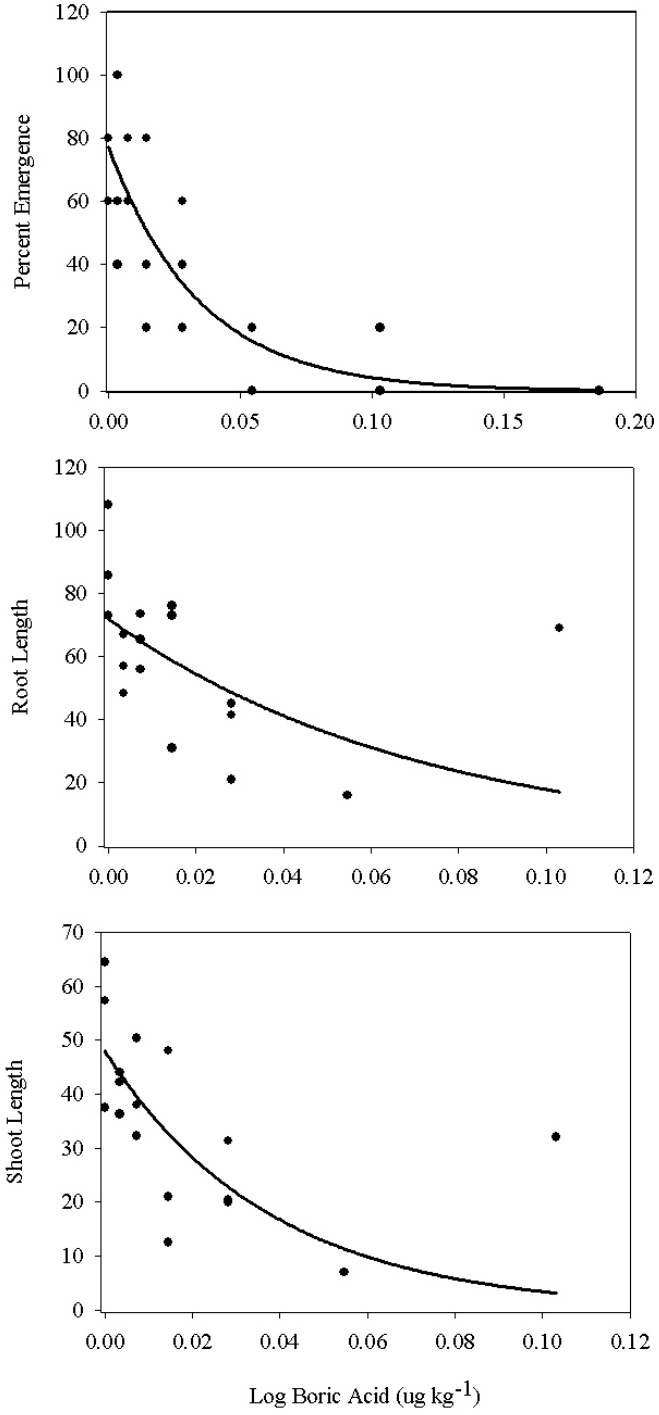

Figure 3.11 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in Northern wheatgrass after exposure to increasing concentrations of boric acid in a standard phytotoxicity test applied to Ekati C horizon soil.

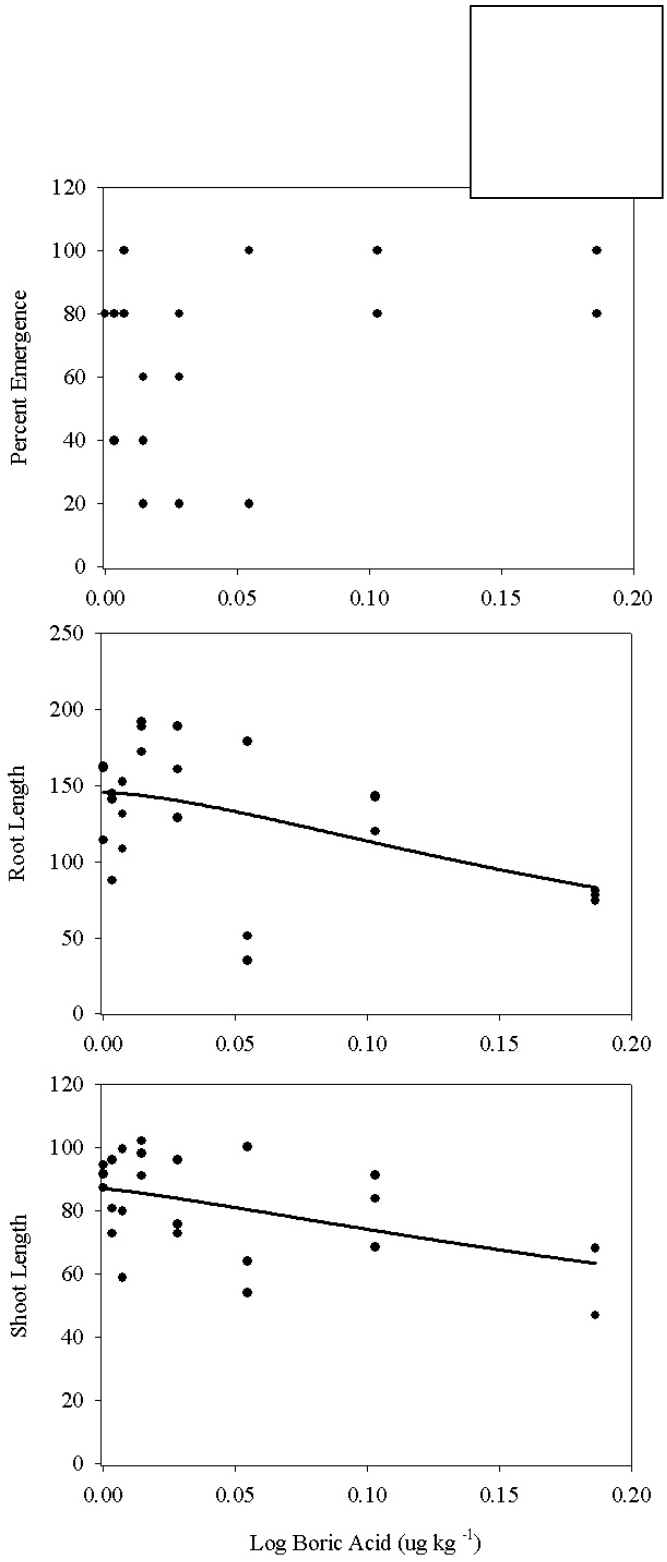

Figure 3.12 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in Northern wheatgrass after exposure to increasing concentrations of boric acid in a standard phytotoxicity test applied to Truelove O horizon soil.

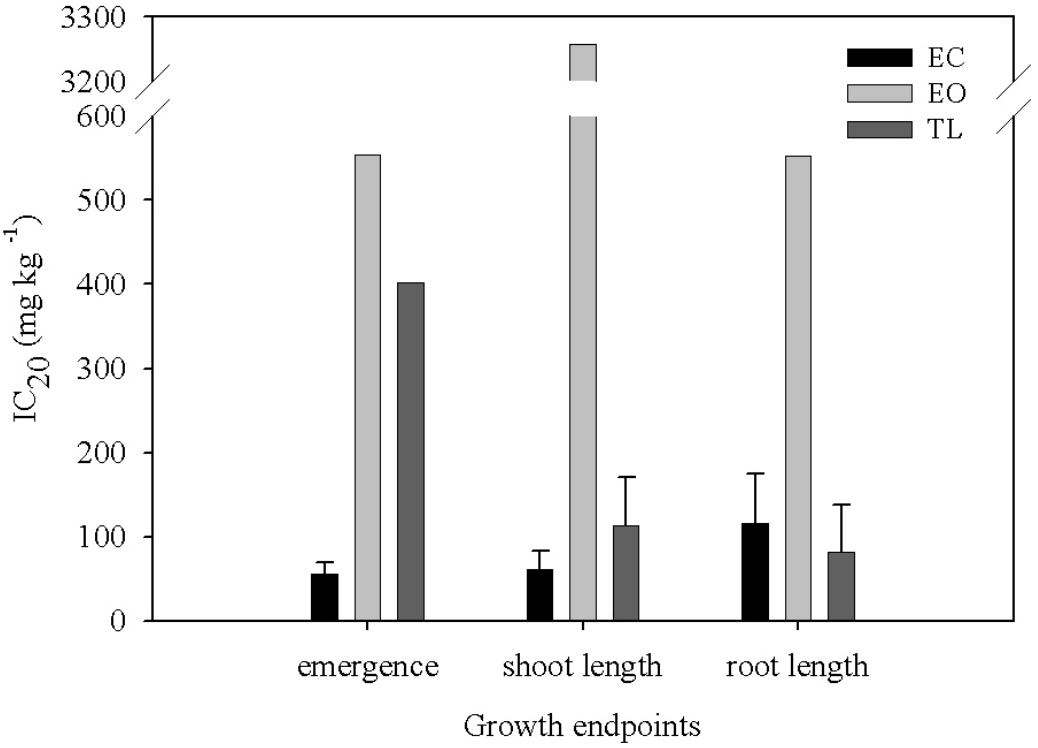

Figure 3.13 Sensitivity of growth endpoints of Northern wheatgrass exposed to boric acid in a standard phytotoxicity test applied to three arctic soils (EC = Ekati C horizon, EO = Ekati O horizon, TL = Truelove O horizon). Bars represent the amount of boric acid (mg kg^{-1} soil) required to cause 20% inhibition of each endpoint. All EO amounts and TL emergence were interpolated from field data results.

3.4 Discussion

In order to accurately detect impacts in arctic regions, we first needed to ensure that toxicity tests initially developed for temperate soils would be applicable and effective in the arctic environment. Furthermore, we were interested in determining the sampling intensity necessary to detect impacts using these standardized tests. We found that the phytotoxicity of boric acid to northern wheatgrass in cryosols was much greater than that commonly reported in other soils. For example, in the technical report by Stephenson et al. (1997), there was little to no observed toxicity on emergence for other monocotyledonous plants such as wheat, barley and corn below 300 ug g^{-1} boric acid, whereas we observed significant effects on emergence with boric acid concentrations well below this value. Further, the IC_{20} of boric acid for red clover in artificial soil was reported as 677 ug g^{-1} for shoot length and 585 ug g^{-1} for root length in a report outlining the development of plant phytotoxicty tests for use in assessing contaminated soils in Canada (Environment Canada, 2005a). Unfortunately in the round robin validation of that particular study, no other soils were dosed with boric acid, nor were any other plant species used. In contrast, in our arctic soils, only the O horizon of the Ekati site was able to tolerate values of over 500 ug g^{-1}. Our dose response curves indicate IC_{20}'s for the Ekati C and Truelove soils were less than 150 ug g^{-1} for both root and shoot length inhibition, indicating that plants grown in these soils were considerably more sensitive to boric acid. These differences may be due to the use of northern wheatgrass, but previous investigators did not observe that northern wheatgrass was especially sensitive to boric acid compared to red clover (Stephenson et al, 1997). There have been no other reports on the toxicity of a reference toxicant such as boric acid in arctic soils to test plant species, despite the fact that it is an acceptable method to compare soil and plant sensitivity. Comparative toxicity studies between temperate and polar soils are rare for other assessment endpoints as well. Schafer et al. (2007) recently reported that biogeochemical endpoints in sub-antarctic islands were similar to that seen in temperate soils. However, no phytotoxicity data was reported in that study.

In addition to high sensitivity to boric acid, there was large variability in the phytotoxicity test results. Coefficients of variation for 10 samples ranged from 160% down to 79%, while 5 samples yielded CVs well over 100% for all endpoints, with a CV of 279% for

Table 3.4 Coefficients of variation (CV) and minimum detectable differences (MDD) (%) of impact site compared to control site for test responses (root length inhibition and shoot length inhibition) measured in northern wheatgrass after exposure to an IC_{20} concentration of boric acid (536 µg g^{-1} soil) in a standard phytotoxicity test applied to arctic soils (EO= Ekati O horizon, TL= Truelove Lowland O horizon).

	Ekati O Horizon				*Truelove O horizon*			
	Root Length Inhibition		*Shoot Length Inhibition*		*Root Length Inhibition*		*Shoot Length Inhibition*	
n	*CV*	*MDD*	*CV*	*MDD*	*CV*	*MDD*	*CV*	*MDD*
5	279	484	144	250	141	245	124	216
10	160	186	123	142	79	91	90	105
15	125	116	85	80	59	55	75	70
21	104	82	60	47	47	37	76	59
31	51	32	25	15	43	28	37	23

root length in the Ekati O horizon. In contrast, typical phytotoxicity data with boric acid has coefficients of variation for 5 samples that range between 10 and 25% (Environment Canada, 2005a) in tests using temperate soil. Other inorganic and organic toxicants such as nickel (Rooney et al, 2007) and allelochemicals (Kong et al, 2007) have phytotoxicity variability of approximately 20% in temperate soils. As a result of the high variability of toxicity responses in cryosols, the detection of a subtle toxic effect using a phytotoxicity test will require increased sample numbers. For example, the number of samples required for a minimum detection difference of 20% from the control will require more than 30 samples at both Ekati and at Truelove (Table 3.4) when using a phytotoxicity test. By way of comparison, using the coefficients of variation of approximately 14% reported by Environment Canada for an Alberta Chernozem soil (J Princz, personal communication), only 7 samples would be required to detect a 25% effect (Kraufvelin, 1998).

The increased toxicity of boric acid in these cryosols was not explained by the characteristics of the soil. Soil properties such as organic matter and clay content are commonly used to estimate toxicity of metals to plants and other soil organisms (Rooney et al, 2007; Bradham et al, 2006; Feisthauer et al, 2006), but in the case of the three soils studied here, these parameters did not explain phytotoxicity responses to boric acid. Soil properties not only failed to explain the phytotoxicity results but also were highly variable in and of themselves. High variability in basic soil properties of a cryosol has been observed before, with CVs of 25-33% calculated for organic carbon content of six samples collected from each of three tundra ecosystems at Daring Lake, NT. (Nobrega and Grogan, 2006). Daring Lake is one of the control sites for nearby diamond mine development. In these northern soils, the high variability of soil characteristics can be attributed to cryoturbation, as surface materials are mixed into the subsoil, affecting both physical and chemical properties (Bockheim and Tarnocai, 1998). Similar variability is often seen in soil properties of temperate agricultural soils (Zeleke and Si, 2005) as well as forest soils (Be´langer and Van Rees, 2008). It is not clear why boric acid toxicity was not correlated to soil properties in these cryosols when this correlation has been seen in temperate soils for other toxicants.

As plants grown in these crysols were not only more sensitive to toxicants but their response to toxicants was also more variable than plants grown in temperate soils, experimental designs commonly used in soil ecotoxicology studies may not be sufficient to detect a realistic

toxicological effect in the arctic**.** Because of the increased variability in plant response, it is very likely that typical experimental designs would not detect an impact unless it was severe. For example, in our case, having only ten replicates of a control versus potentially impacted site would only detect a toxicological effect that reduced plant growth by 160%. We assume that other commonly used endpoints such as nitrification or carbon utilization would have variable toxicity responses as well. Based on our, admittedly, limited data set of three different arctic soils, we would recommend that more than 30 samples be taken from each control and potentially impacted area to accurately assess contaminant effects at sites in northern Canada. Such intensive sampling will ensure that false negatives of toxicant impacts in arctic soils are minimized.

4.0 RESPONSE AND RESISTANCE OF ARCTIC SOILS EXPOSED TO NITROGENOUS COMPOUNDS

4.1 Introduction

Increased resource development and industrial activity in northern Canada, including the opening of several diamond mines, has created many new opportunities for economic growth. However, these operations have also created new challenges to the sustainability of these northern environments. In particular, surplus water from open pit mining, which is employed at many diamond mines, accumulates residues from the ammonium nitrate explosives used in rock blasting. Ammonia is toxic to aquatic organisms at relatively low concentrations therefore surplus water must be treated before it can be returned to the natural lake system. An innovative approach to reducing ammonia loading of aquatic systems is to discharge contaminated surplus water over the tundra using tall (12 m) atomization towers. When water is discharged as a fine mist, >98% of the ammonia is volatilized at the spray nozzles. However, residual ammonium nitrate, ammonia, and other nitrogenous solutes are deposited onto the tundra surface, and subject to plant uptake and denitrification processes. It is not known how sensitive tundra soil ecosystems are to ammonium nitrate toxicity. This uncertainty is a potential impediment to the application of the atomization technology in arctic environments.

Arctic soils generally have low nutrient concentrations and microbial activity due to constant cold temperatures. Consequently, the dominant plant and microbial community species are typically those with low nutrient requirements, and soil functions are highly sensitive to changes in nutrient status (Jonasson et al, 1999). While microbial activity and plant growth is often limited by soil nutrient concentrations of nitrogen, the addition of nitrogen does not always result in increased microbial respiration and plant growth. Low levels of other nutrients, such as carbon and phosphorus, can then become the limiting factors (Yoshitake et al, 2007). Excess N application in arctic soils can decrease and even inhibit microbial respiration at relatively low applications (Rayner et al, 2007; Braddock et al, 1997). This suggests that arctic soils are sensitive to nitrogen inputs and that optimum levels are soil and even site specific.

Soil ecosystems provide critical ecosystem functions by (1) cycling carbon, (2) cycling nitrogen and (3) supporting primary producers such as plants. Carbon utilization refers to the conversion of organic carbon to inorganic forms by microbial decomposition. This conversion involves respiration, a very important part of the carbon cycle, as CO_2 is released back to the atmosphere. Monitoring soil respiration rates is a sensitive and practical method for testing the effects of contaminants at the community level (Salminen et al., 2002). The cycling of nitrogen involves a large number of different organisms and processes. Nitrification is one step in the soil nitrogen cycle which results in the oxidation of NH_4^+ to NO_2^- and then NO_3^- by two groups of bacteria, *Nitrosomonas* and *Nitrobacter sp.*. Nitrification is often used as a toxicological endpoint because of the limited number of organisms that participate in the process and their established sensitivity to toxicants (Gong et al, 1999). Vascular plants are commonly used in terrestrial toxicity test batteries (Siciliano et al, 1998; Wang and Freemark, 1995) as endpoints such as emergence and root and shoot length can be readily assessed. Carbon mineralization, nitrification, and plant growth are potentially affected by soil nitrogen levels, and can be assessed in the laboratory by measuring their respective endpoints over time.

Here we investigate the toxicity of ammonium nitrate to arctic soil ecosystem functioning at concentrations relevant to diamond mining activities in Canada's North. In addition to investigating direct toxicological effects, we also evaluate the potential for ammonium nitrate to have indirect toxic effects on the soil ecosystem. The term "resistance" is used to refer to the ability of a soil system to withstand immediate impacts by a disturbance, such as a toxicant, and continue to function without change (Griffiths et al, 2001; Seybold et al, 1999). This is important in soil ecosystem health because although the endpoints being measured may not be affected by the initial disturbance or toxicant, the system may be weakened and unable to withstand further stresses from additional toxicants or environmental stresses. We used boric acid, a reference toxicant, as an additional toxicant to determine if the soil system was more sensitive to its effects after exposure to ammonium nitrate.

4.2 Materials and Methods

4.2.1 Study soils

In addition to the Ekati and Truelove sites (Sec 3.2.1), two additional soils were selected to provide a range of soil characteristics (Table 4.1). The Saskatchewan soil was also chosen to allow for comparison between arctic and temperate soils.

Resolute soil

Resolute is also located in the high arctic, on the southern shore of Cornwallis Island, Nunavut (74° 44'N 94° 55' W). While the majority of Cornwallis Island consists of plateaus and rolling hills reaching altitudes of 359 m, approximately 15% of the island is comprised of low relief areas that support wetlands such as one existing just North of Resolute Bay (Washburn, 1997). Low relief areas have relatively consistent vegetation cover as a result of delayed snowmelt that provides water for the growing season, and the vegetation of the Resolute wetland site was moss covered with sedge and grass species (Edlund, 1992). The mean temperature of the short summers is only 4°C, while the long winters average -30 to -40 °C (Edlund, 1992). Due to theses harsh climate conditions, extensive cryoturbation, and high carbonate content, the soils present have little soil horizon development and are composed of mostly gravel and sandy loam (Cruickshank, 1971). Typically these soils are referred to as polar desert soils.

Saskatchewan soil

Ardill is a hamlet in southern Saskatchewan (49° 59' N 105° 51' W), which is part of Canada's prairie region Summers are much warmer, with average daily temperatures between 9 and 25°C, often reaching the mid to high 30s. This area generally receives 175 – 215 mm of precipitation over the summer months. Of medium texture and overlaying glacial till, this soil is classified as an Orthic Brown Chernozem under the Canadian system of classification (NRC, 1998).

4.2.2 Soil sampling and storage

Resolute site

In 2002 soil cores were collected from soil surrounding a wetland site north of Resolute Bay. The wetland was approximately 18500 m^2, and cores were taken from along a transect line (Loseto et al, 2004). The soil was sampled just after snowmelt at the end of June.

Table 4.1 Soil characteristics of five Canadian soils

Soil[a]	*pH*	*% OC*	*% clay*	*Texture*	*NO_3^- ($\mu g\ g^{-1}$ dry soil)*	*NH_4^+ ($\mu g\ g^{-1}$ dry soil)*
EC	6.01	0.6	0.0	sandy loam	5.03	10.06
EO	3.87	8.9	0.0	sandy loam	0.00	19.49
R	7.45	2.2	0.0	sandy loam	3.52	5.93
SK	7.43	2.0	0.2	silt loam	30.21	4.90
TL	6.73	6.2	0.1	sandy loam	23.76	4.75

[a] EC = Ekati C horizon, EO = Ekati O horizon, R = Resolute, SK = Saskatchewan, and TL = Truelove

Individual cores were placed in separate plastic bags and shipped back to the U of S, where they were frozen and maintained at –20°C.

Saskatchewan site:

Soil was collected from a wheat stubble field on a farm near Ardill, Saskatchewan in the summer of 2004. A shovel was used to remove the top 15 cm of soil and transfer it to a 5 gallon pail. The pail was covered and stored at room temperature.

4.2.3 Soil preparation

Individual samples from each location and soil horizon were thawed, removed from sample bags, air dried, sieved to 2 mm, and then hand-mixed together to form a homogenous bulk sample. Soil characteristics such as pH, % organic carbon, and ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined for each of the five bulk samples (Table 4.1) as detailed in Section 3.2.3.

Texture

The texture of each bulk sample was determined by a laser scattering particle size distribution analyzer (Horiba Partica LA-950). Organic matter was removed prior to analysis using a modified pipette method (Sheldrick and Wang, 1993).

Bulk Density and Total Porosity

The mass of each soil to be used in each treatment group was lightly packed into a cylinder to determine its volume. The gravimetric water content was used to calculate the mass of dry soil in the cylinder, and the bulk density (g/m^3), determined as the dry sample mass divided by the volume. Assuming a particle density of 2.65 Mg/m^3, total porosity was calculated as:

$$total\_porosity\_(\%) = \frac{1}{(\frac{bulk\_density}{2.65} \times 100)} \qquad (4.1)$$

This value was multiplied by the total volume to get the volume of pore space in that soil. Once the amount of pore space occupied by water in the air dried soil was determined, we calculated the amount of water necessary to bring the water-filled pore space (WFPS) up to 55%.

4.2.4 Ammonium nitrate exposure

Each of the five bulk soil samples was divided into equal sub-samples of 500g (Ekati O horizon, Resolute and Saskatchewan) or 300g (Ekati C horizon and Truelove) and placed in clean plastic planting trays 12 cm^2 and 5 cm deep. Soils were maintained in an environment designed to simulate average summer arctic conditions of Ekati, Truelove and Resolute. The temperature was 10°C, with 24 hours of daylight at light intensity of 400 μmoles $m^{2\ -1}$ s^{-1}, and a relative humidity of 70% (+/-5%). Soils were in the chamber for 95 days: 5 days to acclimatize and stabilize the microbial community, followed by 90 days of exposure (the average length of an arctic summer is 75 days).

Ekati C horizon and Truelove soils were exposed to nine different concentrations of ammonium nitrate (Table 4.2). Only five different concentrations of ammonium nitrate were used to expose Ekati O horizon, Saskatchewan and Truelove soils because there was less of these soils available. The ammonium nitrate (NH_4NO_3, VWR ACS Grade, 95% purity) dissolved in Millipore water was applied every second or third day via a 2 gallon polyethylene tank pressure sprayer (RL Flo-Master®, model # 1102HC) to ensure even application and mimic the deposition from atomization towers. Each soil received its treatment in the amount of water necessary to maintain 55% WFPS. This value was chosen because soil respiration and nitrification are known to increase up to approximately 60% WFPS (Linn and Doran, 1984). The amount of water or nitrate solution each soil received was recorded every application, and the amount of nitrate applied was then calculated for each treatment concentration for each soil at every sampling time. Samples were taken from all soils after the 5 day acclimatization period but before any exposure to nitrogen, and then after every eight applications. Soils were mixed thoroughly with a clean metal spoon before sampling. At each sampling time 25 g of soil was weighed into a clean dram vial, labeled, and immediately frozen at -80 °C until needed for analysis. After being sampled, soils were repacked and received their next ammonium nitrate exposure. Once the last round of sampling was completed (after 32 applications), the

Table 4.2 Concentrations of ammonium nitrate solutions used to water soils

Treatment Group	*Ammonium nitrate concentration (mg L^{-1})*	*Amount of N supplied (mg L^{-1})*	*Soils in treatment group*[a]
C	0	0	EC, EO, R, SK, TL
1	73.4	27	EC, TL
2	147	54	EC, EO, R, SK, TL
3	294	107	EC, TL
4	588	214	EC, EO, R, SK, TL
5	1180	429	EC, TL
6	2350	855	EC, EO, R, SK, TL
7	4700	1709	EC, TL
8	9400	3418	EC, EO, R, SK, TL

[a] EC = Ekati C horizon, EO = Ekati O horizon, R = Resolute, SK = Saskatchewan, and TL = Truelove

remaining soil in each treatment group was used in a phytotoxicity assay. A total of 165 samples were collected to be used in nitrification and carbon mineralization toxicity tests.

The ammonium nitrate concentration range reflected the tested nitrogen concentrations in a holding pond (King Pond) at the Ekati diamond mine. This holding pond was the source of the water that was to be used in the proposed atomization project. Previous studies by Walworth et al (1997) indicated that respiration is maximized in soil when soil water nitrogen is 800 mg L^{-1} and depressed at 2000 mg L^{-1}. Therefore, treatments well above these concentrations were included in the present study to ensure nitrogen had negative effects at some point. Very low concentrations were also included to investigate if NO_3^- and NH_4^+ would accumulate in the soil over time, enabling these upper limits to be reached even if the actual treatments were considerably lower.

4.2.5 Boric acid exposure

In addition to exposure to nitrate and ammonia, the ability of soils to withstand an additional toxicant, boric acid, was evaluated. After exposure to ammonium nitrate, soils were exposed to boric acid concentrations of either 554 µg g^{-1} soil (Ekati O horizon, Saskatchewan and Truelove soils) or 55 µg g^{-1} soil (Ekati C horizon and Resolute soils) as previously determined in Section 3.4, to evaluate if ammonium nitrate altered the resistance of the soils.

4.2.6 Methods of analysis

4.2.6.1 Phytotoxicity test

The phytotoxicty test described in Section 3.2.4 was modified slightly to evaluate phytotoxicity of ammonium nitrate or ammonium nitrate plus boric acid. For each soil type and exposure concentration there were 6 test units, 3 replicates containing the appropriate concentration of boric acid, and three without any boric acid addition. Chamber conditions were the same as the exposure experiment (Section 4.2.4).

4.2.6.2 Potential nitrification assay

The potential activity of ammonium-oxidizing bacteria in the soil was estimated by determining the amount of nitrite produced over a period of time (Gong et al, 1999). Briefly, 10 g from each soil sample was thawed and divided into two 5 g samples, and each was placed

in a 50 ml Erlenmeyer flask. Twenty-five milliliters of test media (4 mM $(NH_4)_2SO_4$, 15 mM $NaClO_3$, 1 mM $KHPO_4$) was added to each flask, with one flask receiving the appropriate dose of boric acid added directly to its media. Flasks were topped with foam plugs, and shaken on a rotary shaker at 125 rpm at 10°C for 36 hours. Slurry (2 ml) was drawn from each flask after 12, 24, and 36 hours of incubation, and pipetted into a 15 ml Falcon® tube containing 2 ml 4 M KCl to stop the reaction. The samples were then centrifuged at 5000 g for 5 minutes, and filtered through a Whatman® 0.45 μm syringe-type filter into a clean conical bottom, propylene Falcon® tube. These aliquots (3 ml) were pipetted into cuvettes, and 0.12 mls of color reagent was added to each. Samples were analyzed by colorimetry using a UV-VIS spectrophotometer, with an absorbance of 543 nm (Clesceri et al, 1995). The concentration of nitrite in each sample was calculated using a standard curve of nitrite absorbance.

4.2.6.3 Carbon utilization assay

A carbohydrate source (sucrose) was added to each soil at each nitrogen concentration to serve as a substrate in the assay. Two soil samples (5 g) were each placed in 125 ml Erlyenmeyer flasks and 7.5 mls of sucrose solution (1 M) was added. One flask also received boric acid in the test media. A sodium hydroxide trap (5 ml of 1.0 M NaOH) was quickly inserted into each flask to capture any CO_2 produced. The flask was immediately sealed with a rubber stopper and incubated for 24 hours at 10°C while shaking at 125 rpm. After incubation, the NaOH traps were quickly removed and capped, and stored for <24 hours at 4°C before analysis. The amount of CO_2 produced and captured in the trap was determined by titration using a 4.0 M HCl titrant (716 DMS Titrino autotitrator, Brinkmann) with endpoints set at pH 8.6 and 4.9 (Clesceri et al, 1995).

4.2.7 Statistical analysis

Accumulated ammonium and nitrate concentrations in soil were log-normally distributed. After checking data for normality and homogeneity of variance, analysis of variance was used to estimate the effect of boric acid on mineralization, nitrification and plant parameters. A full factorial design was used with soil, applied dose and boric acid as factors, and all interactions were evaluated. Estimating the concentration of ammonia nitrate at which

nitrification, mineralization and plant growth were inhibited by 20% was carried out using re-parameterized equations as described in Section 3.3.

4.3 Results

Accumulation of ammonium or nitrate in the soil was not linearly related to the applied ammonium nitrate dose (Figure 4.1). Furthermore, soil concentrations of nitrate and ammonium were not correlated (Figure 4.2) to one another. After exposure, a significant amount of ammonium and nitrate had accumulated in all the soils, and all soils had significantly more nitrate than ammonium, with the exception of the Saskatchewan soil exposed to the highest ammonium nitrate dose in which nitrate and ammonium concentrations were equivalent. The ammonium nitrate doses were expressed as the amount of nitrate or ammonium present in soil, or alternatively, the amount of total inorganic nitrogen in the soil or soil pore water.

Boric acid concentrations of 554 and 55 ug g^{-1} did not cause the intended 20% inhibition of soil functions (Figure 4.3), nor did they have a significant effect on nitrification (p=0.364), carbon mineralization (p=0.341), or plant growth parameters (emergence p=0.180, root length p=0.258, shoot length p=0.319). Therefore both the boric acid and non-boric acid treated responses were treated as replicates, and the average response to ammonium nitrate has been presented in Figures 4.6 – 4.15.

Carbon mineralization was not significantly affected by the ammonium nitrate dose (p=0.852). Rates of mineralization did differ (p<0.001*)* between soils (Figure 4.4) with the Ekati O horizon soil having the highest rate of carbon utilization and Ekati C the lowest.

Nitrification rates were also significantly different between soils (p<0.001), but surprisingly both Ekati O and Ekati C soil had much lower rates of nitrification compared to the other soils (Figure 4.5). Applied nitrogen did have an effect on nitrification (p=0.00), but the effect was soil-dependent with only Resolute soil being sensitive to nitrogen addition (Figures 4.8 & 4.9).

Plant parameters were inhibited by increased nitrogen in all soils, with emergence, root length, and shoot length responding in a similar fashion to increases in accumulated nitrate and ammonium (Figures 4.6 – 4.15). There was a significant difference in phytotoxicity between

soils caused by applied nitrogen when expressed as accumulated log ammonium, log nitrate, and log total N (Table 4.3). The calculated IC_{20} concentrations for accumulated ammonium and nitrate were similar for all plant parameters in the Ekati C horizon, Ekati O horizon and Truelove soils, while Resolute and Saskatchewan soils appeared to be more sensitive to accumulated ammonium than nitrogen (Table 4.3). Growth parameters of northern wheatgrass appeared to be the most affected by total nitrogen addition when planted in the Ekati C, Resolute and Saskatchewan soils, while plants in the Ekati O horizon were relatively insensitive (Table 4.3). Interestingly, there was not a significant difference in phytotoxicity between the Saskatchewan temperate and arctic soils. Normalizing the data and expressing it as mmol N L^{-1} soil water (Table 4.3) indicates that phytoxicity in Saskatchewan soil is actually significantly different than Ekati C and Ekati O soils with respect to seedling emergence, as Ekati C and Ekati O can tolerate higher concentrations of N in their pore water than the other soils. This suggests that although Saskatchewan soil accumulates N more slowly in pore water (as there is more water per gram of soil) plants are more sensitive to the accumulation.

The average concentration of nitrate expected to cause 20% inhibition of emergence in all soils was 575 μg g^{-1} soil. In contrast, only 215 μg ammonium g^{1} soil inhibited emergence by 20%. When expressed as μmols of total inorganic N, the average amount of N expected to cause 20% inhibition in emergence was calculated to be 42 μmol g^{-1} soil, with Ekati C, Resolute, and Saskatchewan soils tolerating less than half the amount of total N as Truelove, while Ekati O could tolerate more than 10x the amount of the Saskatchewan soil. However, the average concentration of total N in soil water causing 20% inhibition was 125 mmol L^{-1} soil water, with Ekati C horizon now tolerating more than 4 times the amount of N as the Saskatchewan soil (Table 4.3).

The lowest concentrations of applied ammonium nitrate (Treatment Groups 1 and 2) increased emergence but at application concentrations of approximately of 588 mg L^{-1} (Treatment Group 4) plant parameters began to be inhibited in all soils. To determine the applied ammonium nitrate dose per gram of each soil that would create a 20% inhibition in function and plant growth when applied over the course of the tundra growing season, the log of the accumulation of total N (μmol) occurring in the soil pore water was plotted against the log of the applied dose and a curve was fitted for each soil (data not shown). Using the previously established soil water concentrations that caused a 20% inhibition, we interpolated

the maximum concentrations of nitrogen that should be present in the treatment water (Table 4.4). The values ranged from 2100 to 15,801 mg L^{-1} of ammonium nitrate in the application water. Ekati C horizon accumulated total nitrogen much more quickly than the other soils, but its IC_{20} of ammonium nitrate was similar to that of the Saskatchewan soil. Ekati O horizon accumulated nitrogen at a rate similar to Truelove soil, but could withstand more than twice the amount of ammonium nitrate concentration before 20% inhibition occurred.

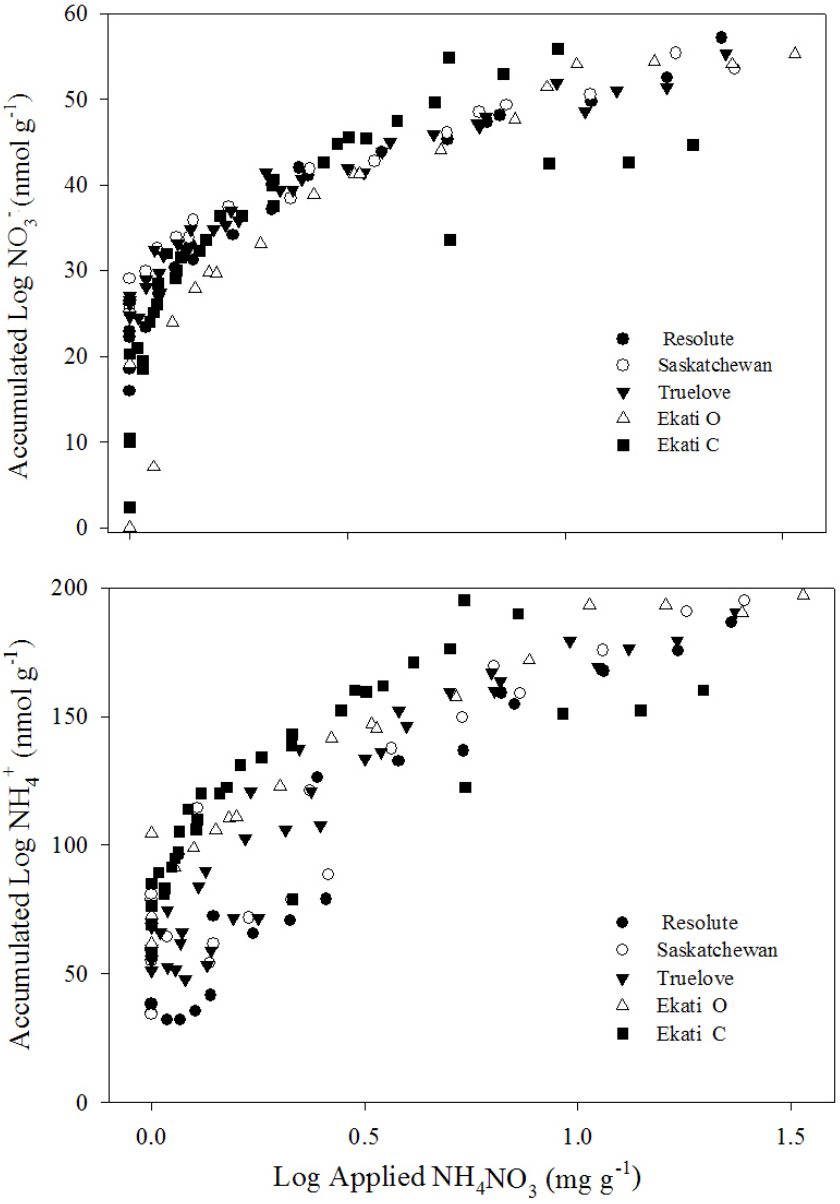

Figure 4.1 Accumulated log concentrations of nitrate (NO_3^{-}) and ammonium (NH_4^{+}) in four arctic soils and one temperate soil after 32 exposures of increasing concentrations of ammonium nitrate (NH_4NO_3) solution.

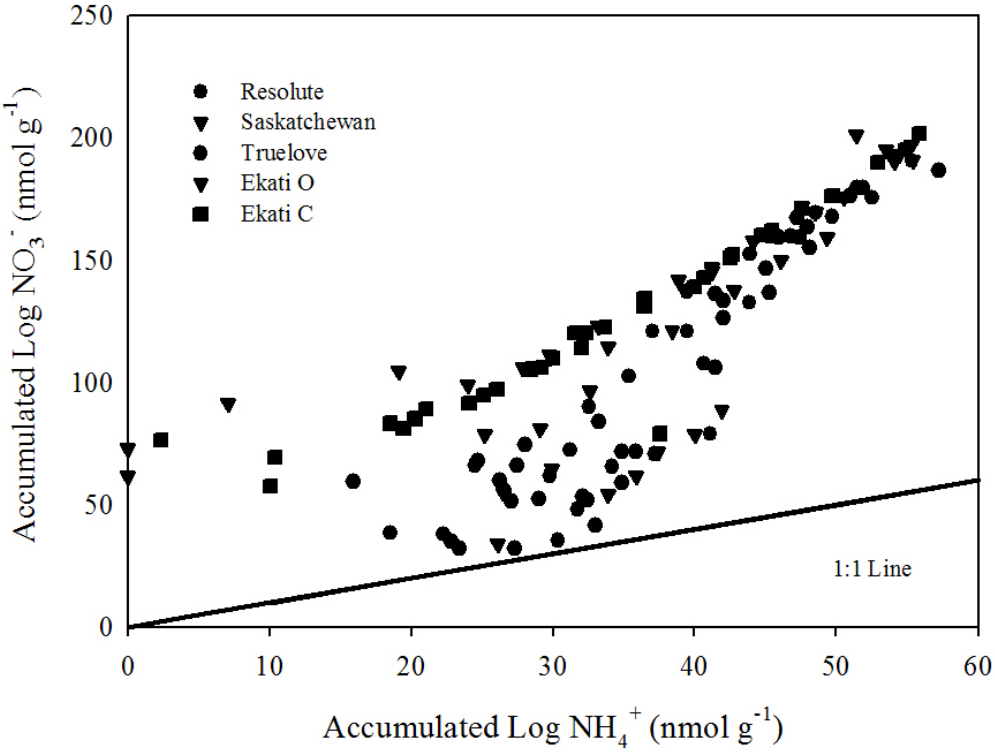

Figure 4.2 Log concentration of accumulated nitrate (NO_3^{-}) compared to the log concentration of accumulated ammonium (NH_4^{+}) in soils exposed to 32 exposures of increasing concentrations of ammonium nitrate solution.

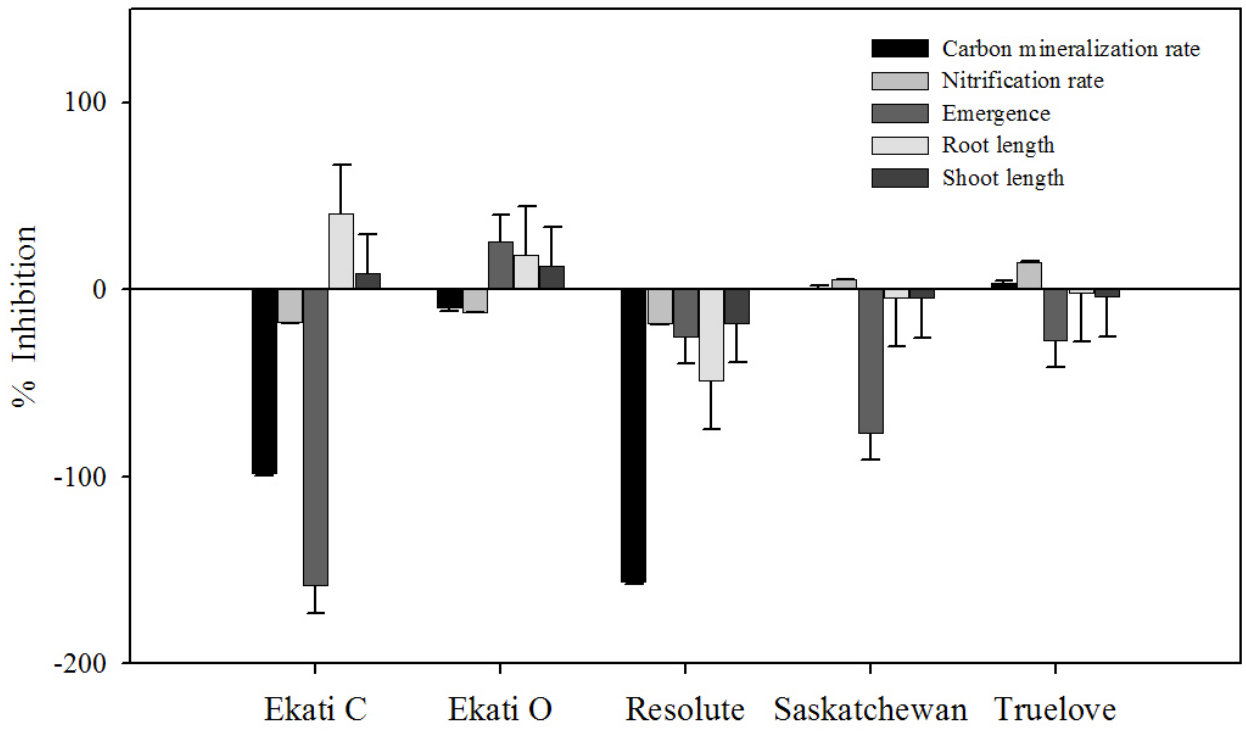

Figure 4.3 Average amount of inhibition (%) of soil functions and growth of northern wheatgrass caused by an application of boric acid expected to cause 20% inhibition (55 ug g^{-1} for Ekati C and Resolute soils, 554 ug g^{-1} for Ekati O, Saskatchewan and Truelove soils). Error bars represent the standard deviation from the mean.

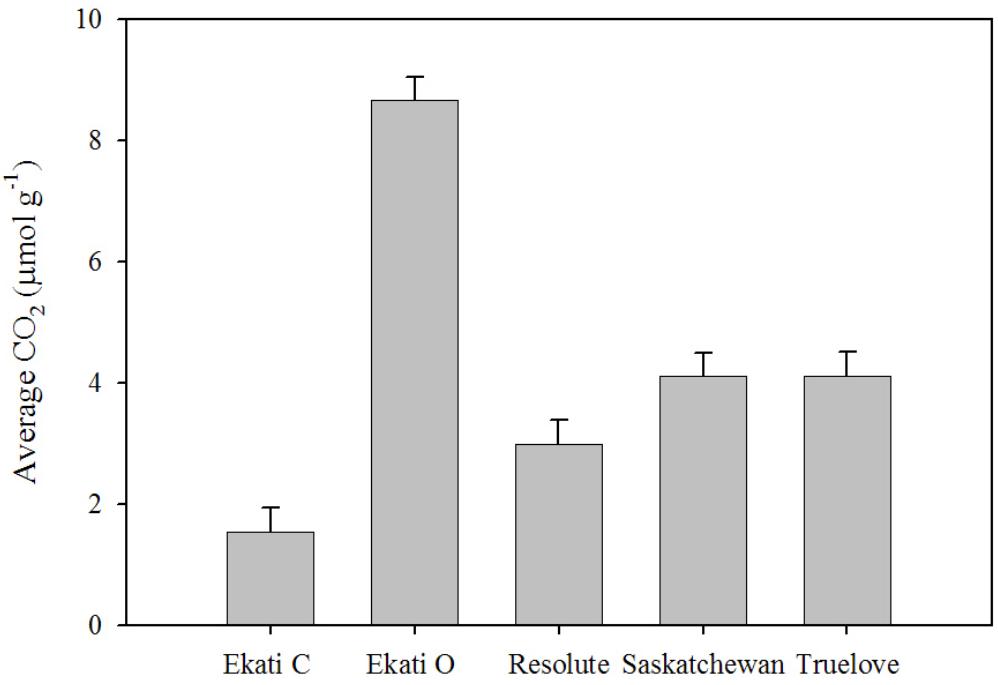

Figure 4.4 Average carbon mineralization occurring in four arctic soils and one temperate soil over 24 hours. Error bars represent the standard deviation from the mean.

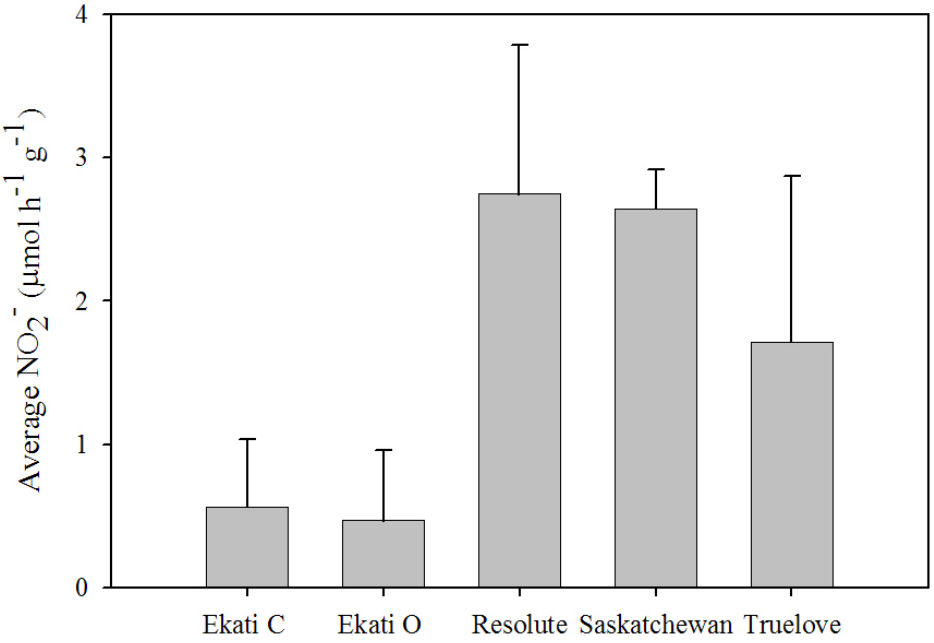

Figure 4.5 Average rate of nitrification occurring in four arctic soils and one temperate soil. Error bars represent the standard deviation from the mean.

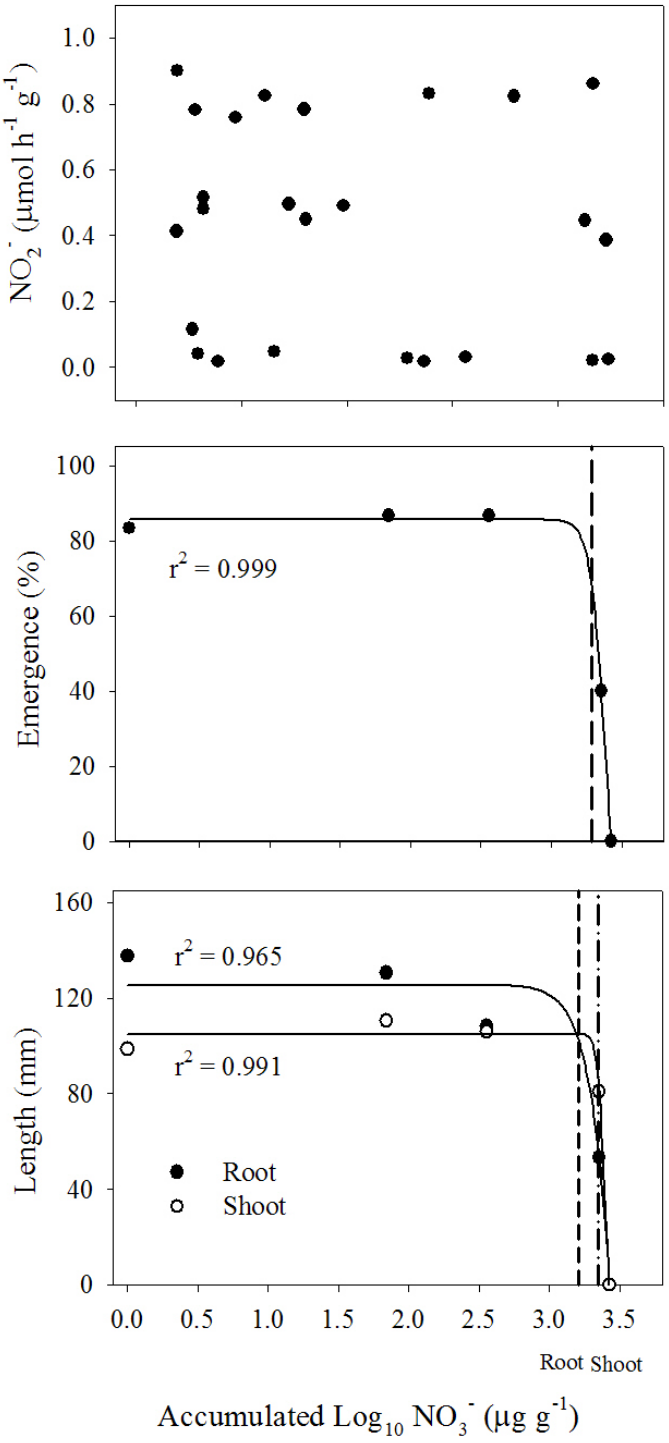

Figure 4.6 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Ekati O horizon soil. Applied ammonium nitrate is expressed as accumulated nitrate (NO_3^-).

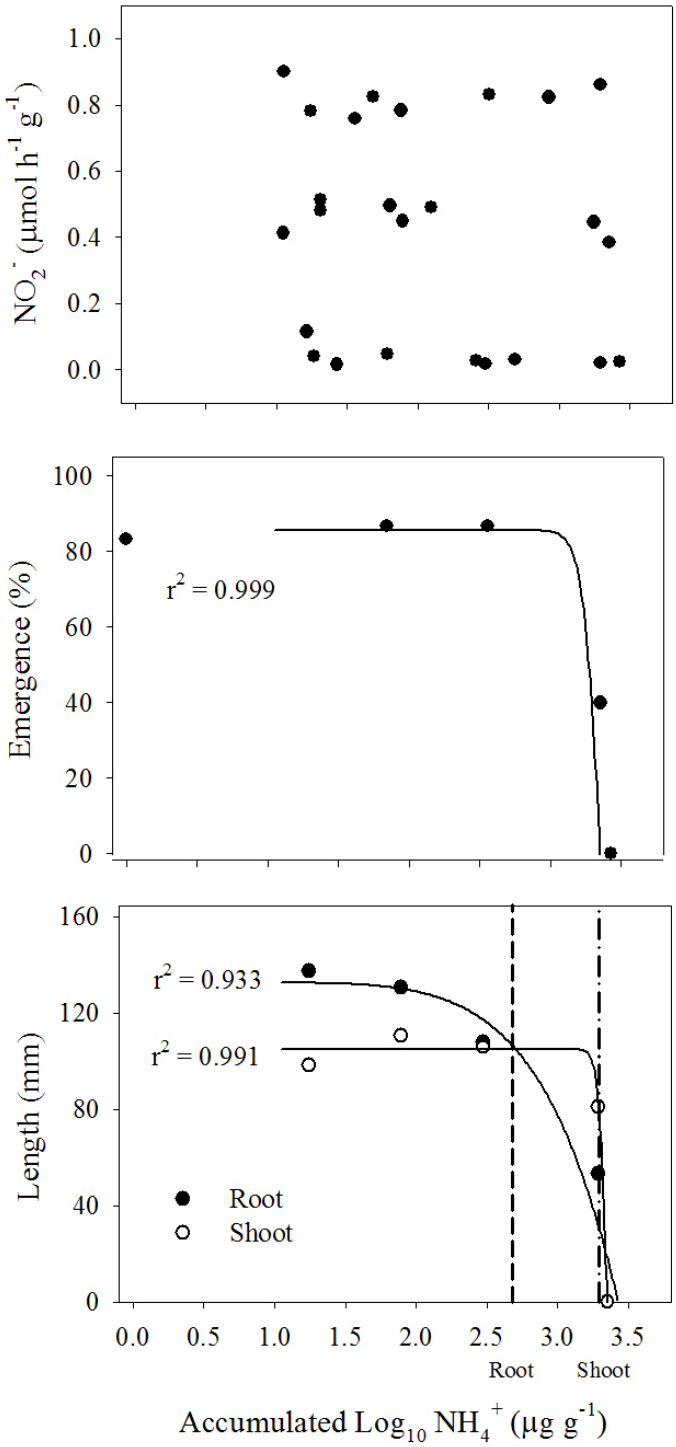

Figure 4.7 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Ekati O horizon soil. Applied ammonium nitrate is expressed as accumulated ammonium (NH_4^+).

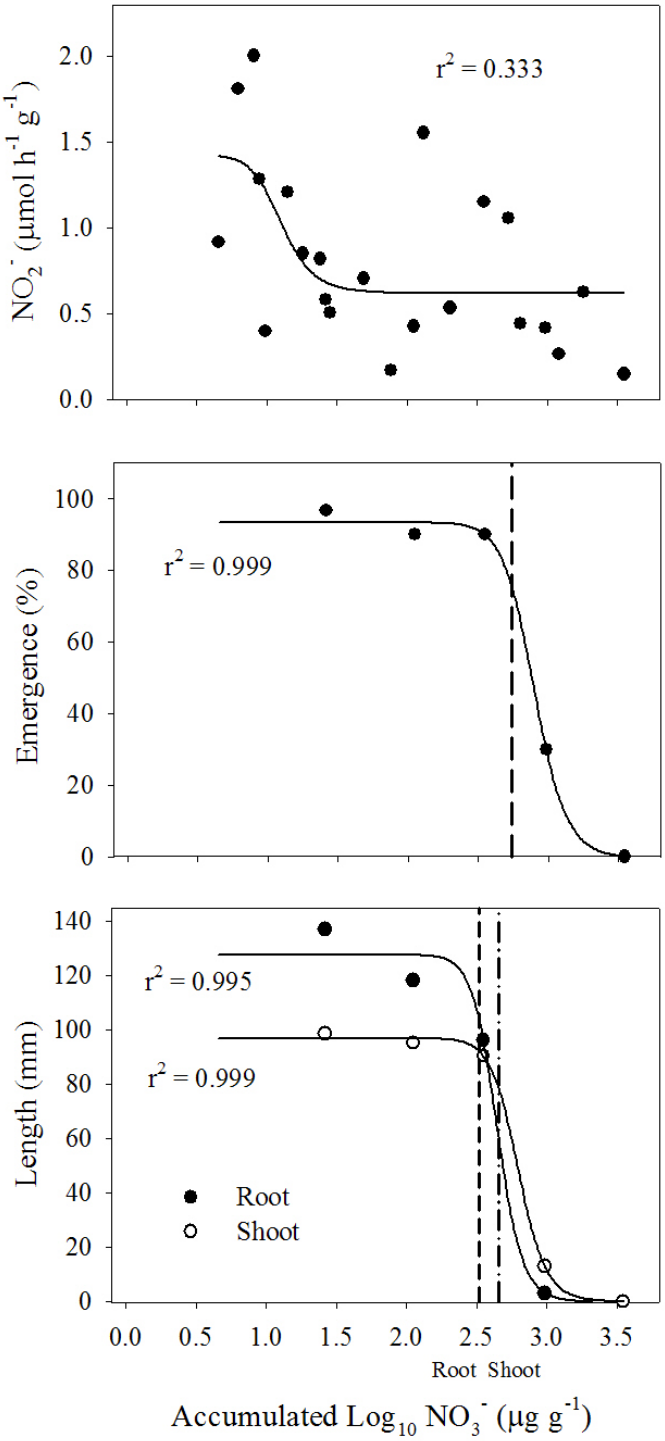

Figure 4.8 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Resolute soil**.** Applied ammonium nitrate is expressed as accumulated nitrate (NO_3^-).

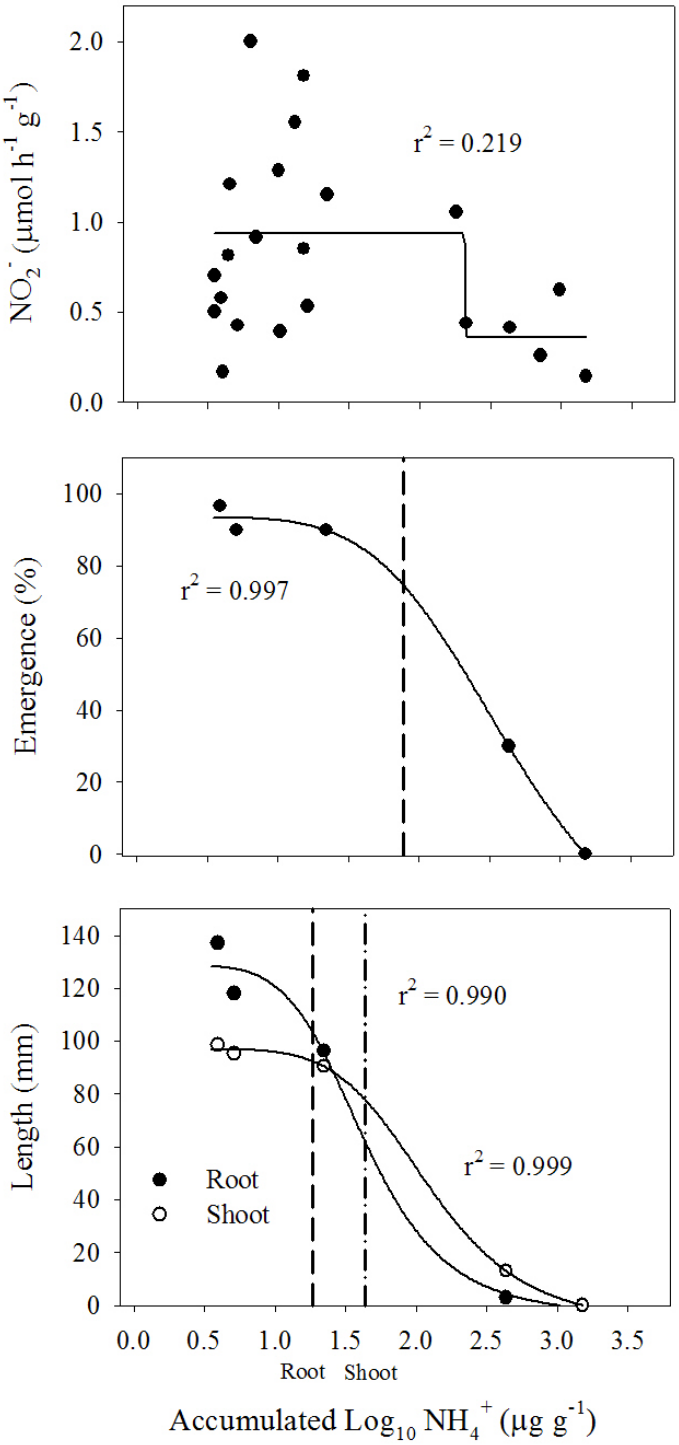

Figure 4.9 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Resolute soil. Applied ammonium nitrate is expressed as accumulated ammonium (NH_4^+).

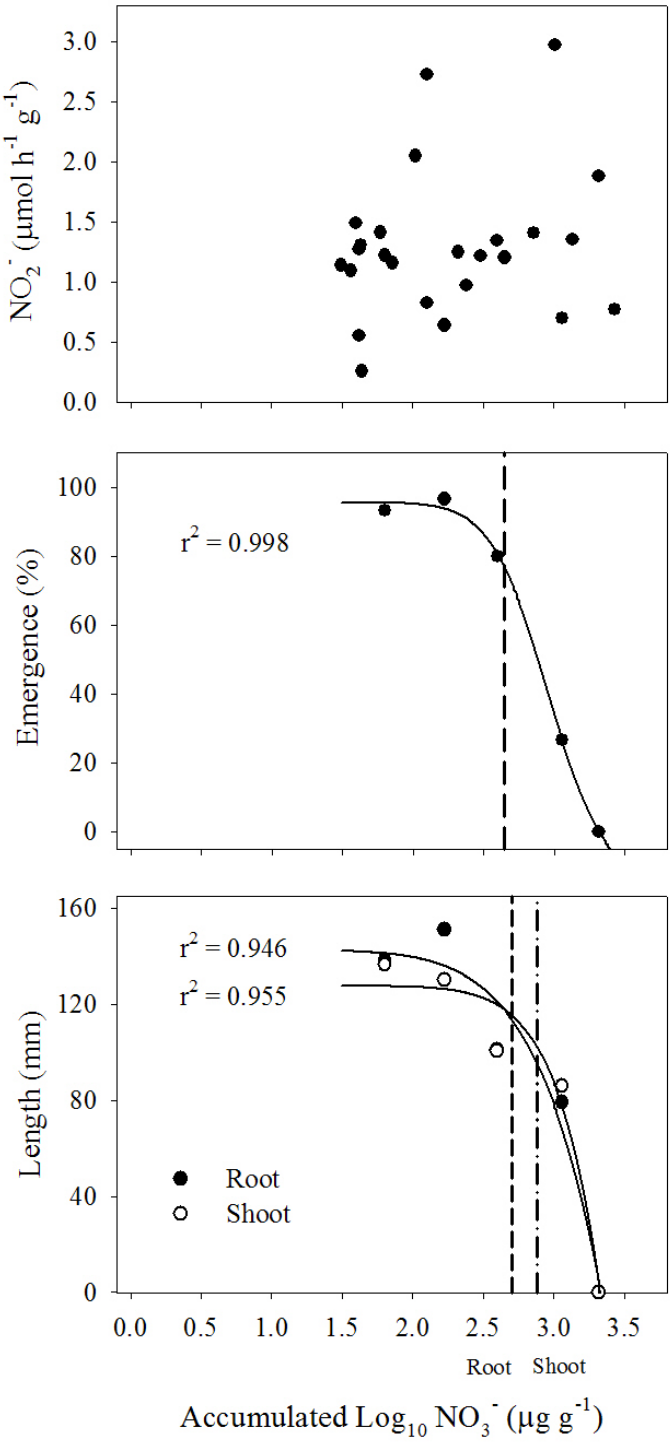

Figure 4.10 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Saskatchewan soil. Applied ammonium nitrate is expressed as accumulated nitrate (NO_3^-).

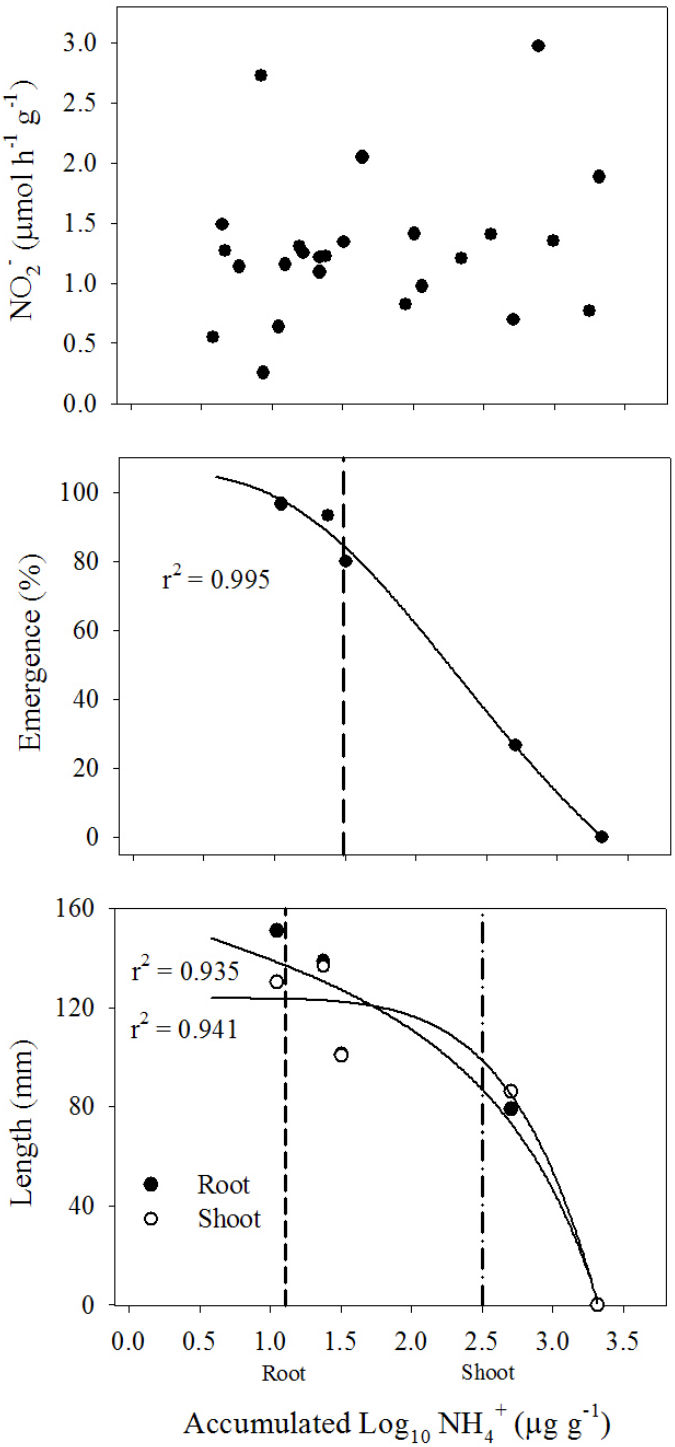

Figure 4.11 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Saskatchewan soil. Applied ammonium nitrate is expressed as accumulated ammonium (NH_4^+).

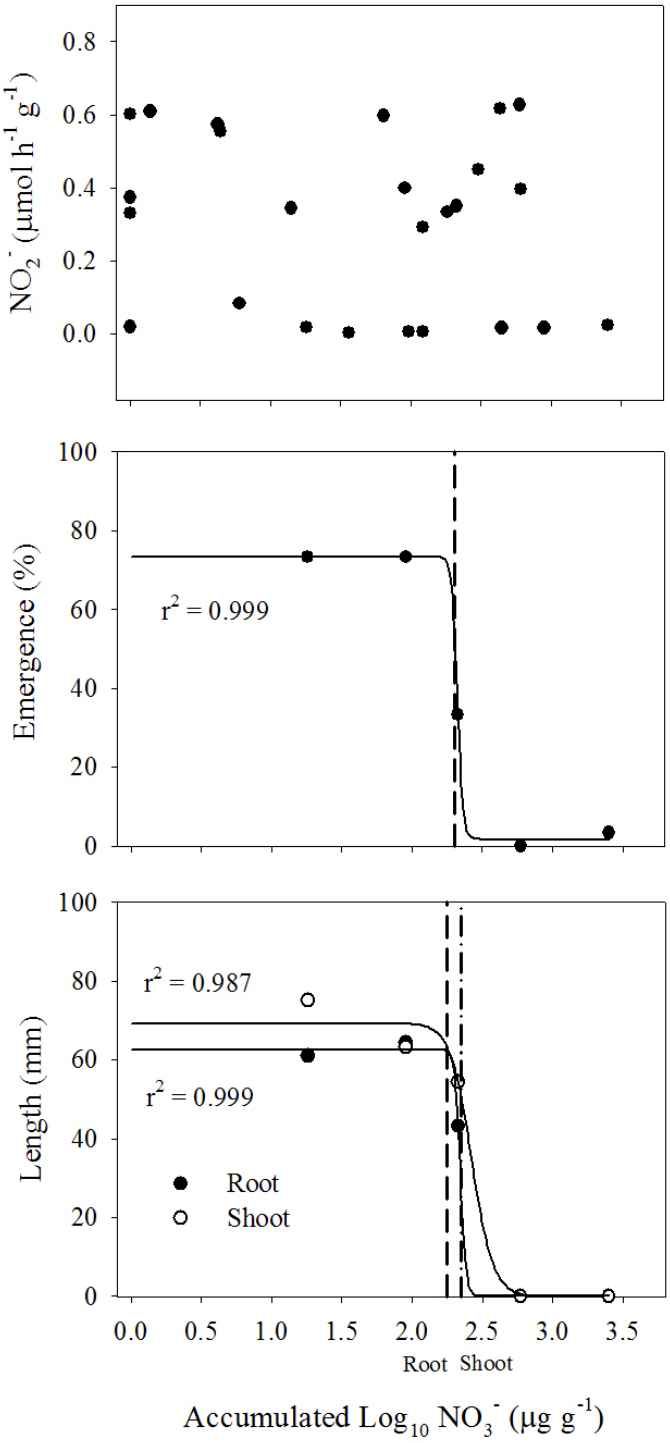

Figure 4.12 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Ekati C horizon soil. Applied ammonium nitrate is expressed as accumulated nitrate (NO_3^-).

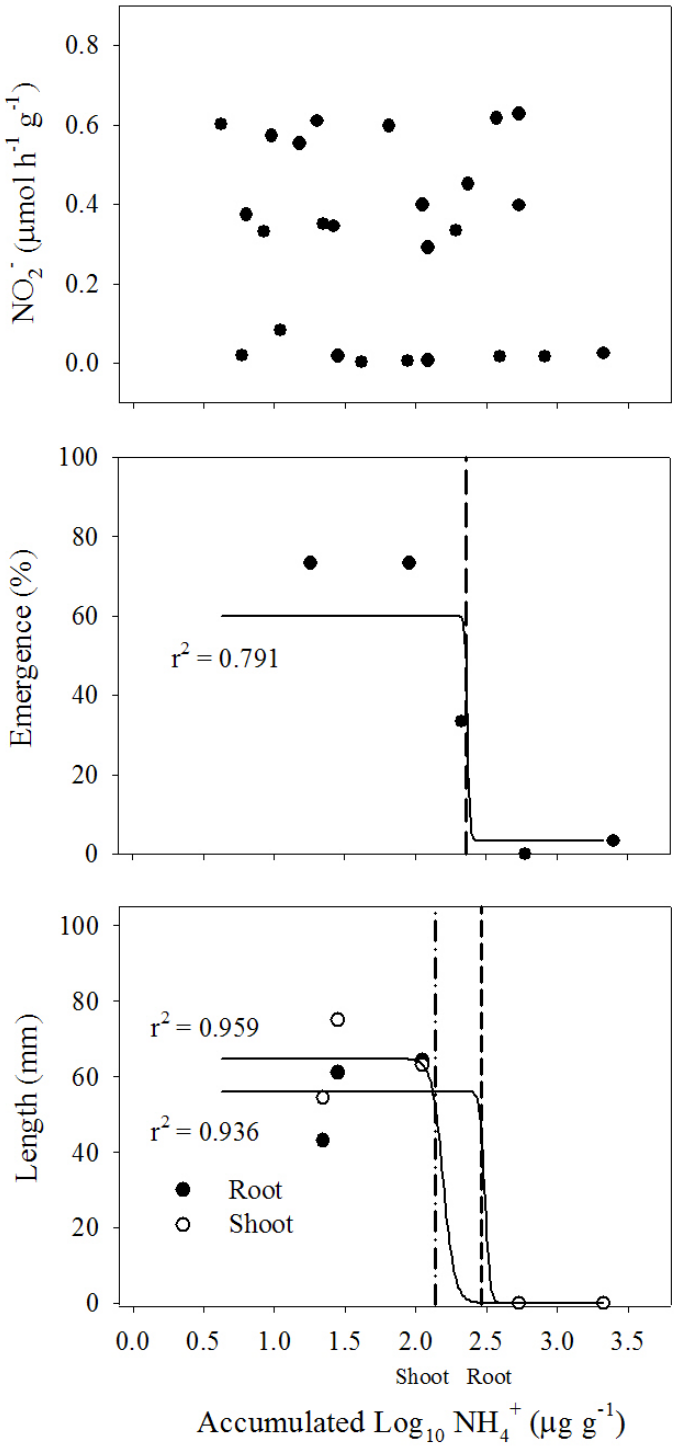

Figure 4.13 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Ekati C horizon soil. Applied ammonium nitrate is expressed as accumulated ammonium (NH_4^+).

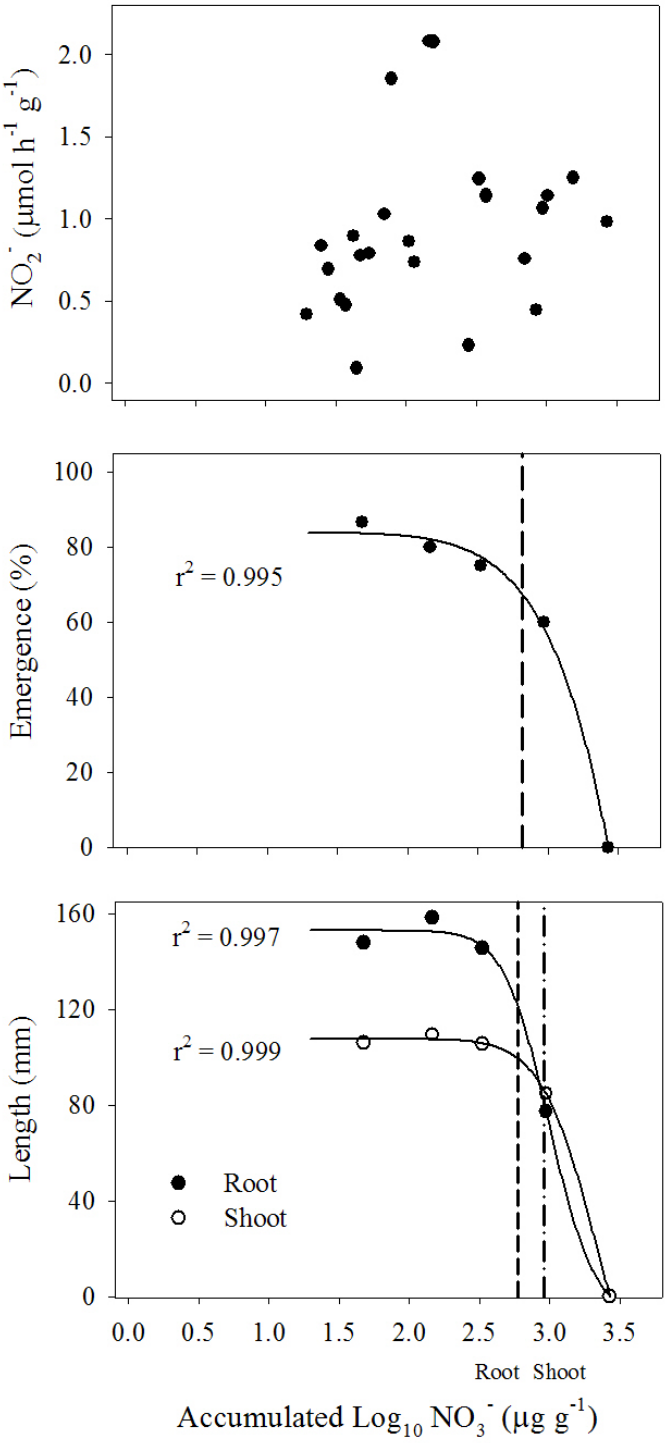

Figure 4.14 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Truelove horizon soil. Applied ammonium nitrate is expressed as accumulated nitrate (NO_3^-).

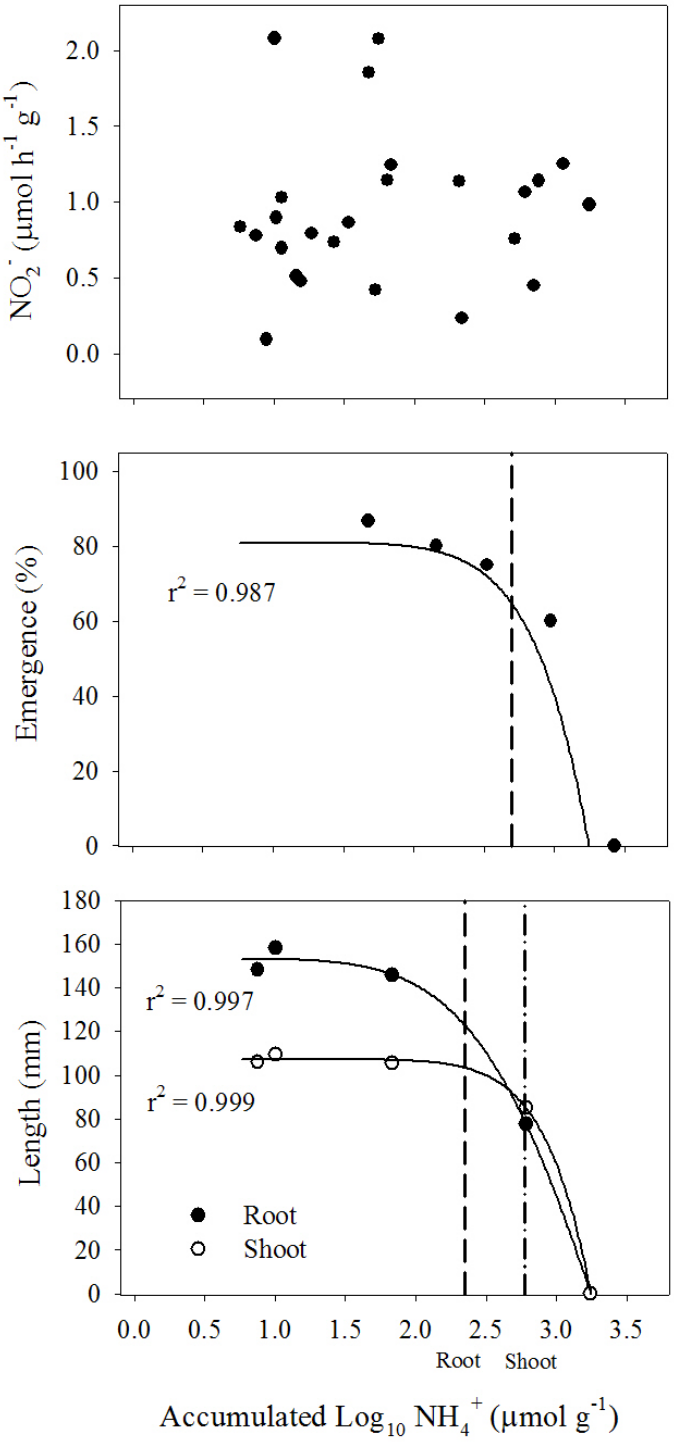

Figure 4.15 Response of three growth endpoints (day 21 emergence, root length, shoot length) measured in northern wheatgrass after exposure to increasing concentrations of ammonium nitrate in a standard phytotoxicity test applied to Truelove horizon soil. Applied ammonium nitrate is expressed as accumulated ammonium (NH_4^+).

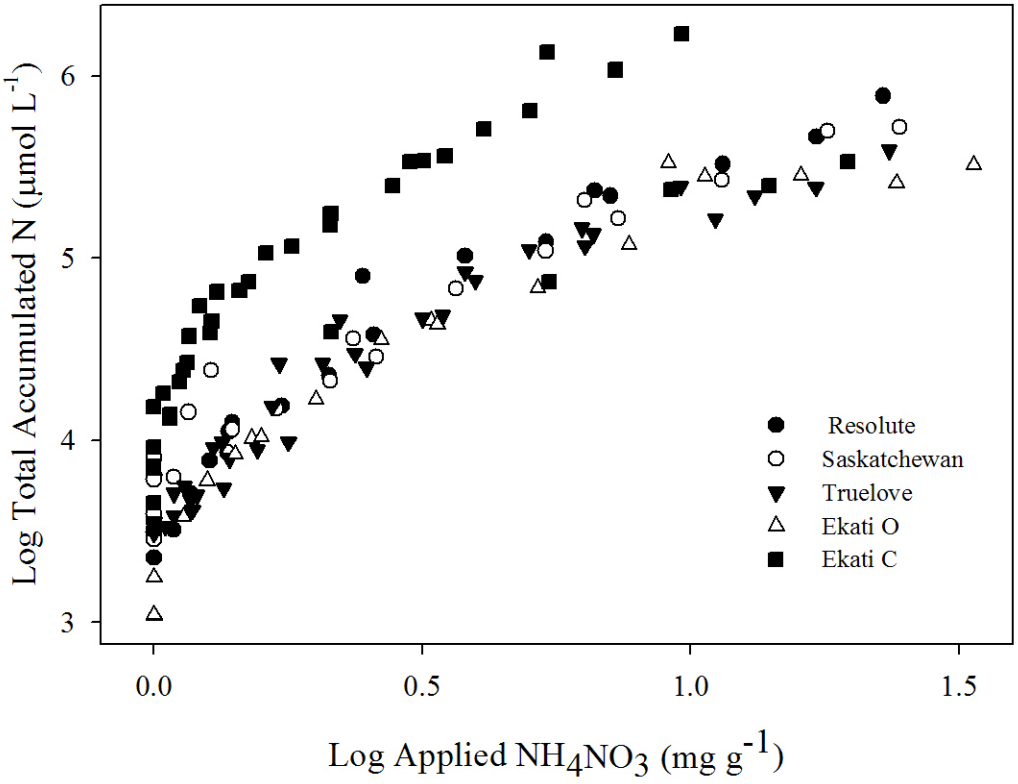

Figure 4.16 Accumulated log concentrations of total nitrogen (N) in pore water of four arctic soils and one temperate soil after 32 exposures of increasing concentrations of ammonium nitrate (NH_4NO_3) solution.

Table 4.3 Concentrations of nitrate (NO_3^-), ammonium (NH_4^+), and total inorganic nitrogen (N) predicted to cause a 20% inhibition (IC_{20}) in growth parameters of northern wheatgrass in four arctic soils and one temperate soil

Soil[a]	*Log IC_{20} of NO_3^-. (ug g^{-1})*			*Log IC_{20} of NH_4^+ (ug g^{-1})*			*Log IC_{20} of N (umol g^{-1})*			*IC_{20} of N (mmol L^{-1} soil water)*		
	Emergence	*Root Length*	*Shoot Length*	*Emergence*	*Root Length*	*Shoot Length*	*Emergence*	*Root Length*	*Shoot Length*	*Emergence*	*Root Length*	*Shoot Length*
EC	2.3	2.3	2.3	2.3	2.5	2.1	1.2	1.3	1.1	149	176	99
EO	3.3	3.2	3.3	3.2	2.7	3.3	2.1	1.5	2.2	243	67	280
R	2.7	2.5	2.7	1.9	1.3	1.6	1.2	0.9	1.1	84	40	63
SK	2.7	2.7	2.9	1.5	1.1	2.5	1	1.1	1.4	36	43	93
TL	2.8	2.8	3	2.7	2.4	2.8	1.6	1.4	1.7	113	65	136

[a] EC = Ekati C horizon, EO = Ekati O horizon, R = Resolute, SK = Saskatchewan, and TL = Truelove

Table 4.4 Rates of N accumulation of an applied ammonium nitrate (NH_4NO_3) solution in four arctic soils and one temperate soil. A two parameter logarithmic equation was used (y = ln (a + bx)) to determine the applied dose of ammonium nitrate expected to accumulate to the IC_{20} of total N for emergence of northern wheatgrass where y = the log IC_{20} of accumulated N for emergence.

Soil	*Log IC_{20} of Accumulated N (µmol L^{-1} soil water)*	*r^2*	*a*	*b*	*Applied NH_4 NO_3 (mg kg^{-1} dry soil)*	*Amount of N delivered (mg kg^{-1} dry soil)*
EC	5.2	0.799	61.30	305.80	2402	873
EO	5.4	0.953	26.61	160.67	15801	5746
R	4.9	0.981	30.31	200.92	3450	1255
SK	4.6	0.941	39.64	166.39	2169	789
TL	5.0	0.974	31.21	160.19	6014	2187

[a] EC = Ekati C horizon, EO = Ekati O horizon, R = Resolute, SK = Saskatchewan, and TL = Truelov

4.4 Discussion

The objective of this chapter was to determine application concentrations of ammonium nitrate that would not significantly affect normal microbial activity and plant growth in soils from four arctic sites. This information will assist in determining whether atomization of contaminated surplus water over the tundra is a practical and effective method of disposal. While there is substantial evidence that excessive nitrogen addition to arctic soils can have deleterious effects on microbial functions such as nitrification (Walecka-Hutchison and Walworth, 2007) and carbon mineralization (Braddock et al, 1997; Walecka-Hutchison and Walworth, 2007), the majority of the research has focused on identifying optimal fertilizer levels for remediation of contaminated sites in tundra regions.

Recent studies carried out in sub-Antarctic regions report optimal nitrogen levels of 600 mg L^{-1} soil water to achieve maximum respiration rates in hydrocarbon contaminated soils, with inhibition of carbon mineralization starting at 1200 mg L^{-1} soil water, which is equivalent to 80 mmol N L^{-1} soil water (Walworth et al, 2007). Earlier research by Walworth et al. (1997) stated that levels of N twice as high (above 2500 mg L^{-1} or 166 mmol L^{-1}) caused inhibition of the same function in hydrocarbon contaminated arctic soils in Alaska. These studies did not investigate effects on plant growth parameters as enhancing respiration was their primary concern. In our study, phytotoxicity was observed in arctic soils at much lower levels of approximately 10 mmol N L^{-1} soil water.

The addition of nitrogen itself did not have a significant effect on respiration in our soils at any concentration we applied. Perhaps, since our soils were not contaminated with hydrocarbons, microbial activity may have been limited by deficiencies of carbon or other essential nutrients. In a study on a High Arctic glacier foreland, only addition of both carbon and nitrogen was able to increase respiration, while addition of nitrogen alone had no effect (Yoshitake et al, 2007). The Ekati O horizon soil had the highest percentage of organic carbon, which may explain why the rate of respiration was significantly higher in this soil, although it too was unaffected by nitrogen addition. Supporting this, only in the Resolute soil was nitrification significantly influenced by nitrogen addition, suggesting nitrification may have been limited by lack of nutrients such as available carbon rather than nitrogen in the other soils. Microbial activity has been stimulated by additions of both nitrogen and phosphorus (Braddock et al, 1997) in other arctic soils.

Toxicity to plants may not be due to the addition of N itself, but rather to changes in soil water osmotic potential. As noted by Walworth et al (2007), fertilizers are commonly water soluble and dissolve into pore water, creating osmotic stress as the salt concentration increases. Both Braddock et al (1997) and Walworth et al (1997) agree that this lowering of osmotic potential can reduce populations and activity of microbes. Studies have shown that dry soils are more susceptible to N toxicity, as wetter soils effectively dilute the fertilizer salts into a larger volume of water (Walworth et al, 1997; Braddock et al, 1997). Based on this finding, we would expect Ekati C horizon to be the most sensitive to the addition of fertilizer as it had the smallest percentage of water, but in fact Ekati C soil was able to tolerate higher amounts of nitrogen than Resolute, Saskatchewan, and Truelove soils. The fact that our nitrogen applications occurred only every second or third day reduced the rate of nitrogen accumulation, and this may have prevented inhibition caused by a sudden change in osmotic potential (Braddock et al, 1997).

The toxicity of the ammonium nitrate may also have been influenced by the pH of the soil. Ammonium may be present in its ionized (NH_4^+) or unionized form (NH_3), and its equilibrium is affected by both pH and temperature. As pH increases the concentration of NH_3 also increases, and NH_3 is known to be the more toxic of the two forms (Constable et al, 2003). As the Ekati O horizon soil had a considerably lower pH than the other soils, more ammonia may have remained in the less toxic ionized form, allowing a greater accumulation of ammonium nitrate to be tolerated.

Recently, Walworth et al (2007) recommended smaller applications of nitrogen to maintain concentrations under inhibitory levels. Findings in this present study are consistent with this recommendation, as IC_{20} concentrations were reached in the soils only after 32 exposures of Treatment 4 (588 mg L^{-1} NH_4NO_3) or in the case of Truelove and Ekati O after 24 exposures of Treatment Group 6 (2350 mg L^{-1} NH_4NO_3). It is possible that the presence of plants could maintain low concentrations in the soil for even longer periods, as inorganic nitrogen increases the plant available nutrient pool, and generally results in increased plant productivity (Jonasson et al, 1999). Thus, the use of these concentrations can be considered a conservative estimate of the N concentrations likely to cause toxicity.

The addition of nitrogen to soil on the tundra surface is being considered here as a method of wastewater disposal. It is not confined to a particular area such as a remediation tool

would be. It will be possible to minimize accumulation in the soil by simply increasing or changing the area that receives ammonium nitrate solution. Even so, it is important to consider site specific factors such as moisture content and texture as the low moisture soils are likely to be sensitive to changes due to weather, and safe doses could quickly become toxic in the field. Other factors such as carbon and phosphorus levels may be limiting soil functions, and previous adaptations to these limitations may make the system vulnerable to sudden changes in nutrient availability (Jonasson et al, 1999).

5.0 SUMMARY AND CONCLUSIONS

5.1 Fulfillment of Research Objectives

The overall purpose of this thesis project was to assess atomization of surplus water contaminated with ammonium nitrate as an effective and acceptable means of disposal a diamond mine located in the Canadian arctic. Due to the toxicity of ammonia to aquatic organisms, water release back to the natural system is strictly regulated and monitored. Current methods of natural volatilization and conversion in holding ponds are not able to treat large volumes of contaminated water collected from open mining pits quickly enough to allow for timely disposal. New methods are therefore required to meet the increasing demand as the number and depth of the pits increases.

Many challenges are associated with applying atomization technology in the north, with the construction and operation of the atomization towers themselves in such a cold, remote region being an obvious one. In the absence of an operating pilot system to assess on-site effects, we attempted to mimic the exposure scenario in a controlled laboratory setting, in hopes of obtaining information that would be useful for the set up and operation of a site specific pilot operation. As factors such as the weather and terrain are highly influential, in no way do we believe that our laboratory exposure can be considered a replacement for specific on site field testing. Rather, we feel our choice of several sites and average weather conditions provides a general idea of the sensitivity of arctic soils to ammonium nitrate, and identifies important aspects to be considered when choosing to apply this, or other similar technologies, in the arctic.

Before we could begin to investigate the impacts of ammonium nitrate addition, we felt it was important to address other challenges presented by the arctic landscape, and confirm that the arctic sites chosen were adequately represented in a laboratory experiment. At the time this project began, standardized soil toxicity testing was based on temperate soils, and we expected the arctic sites to have more variability in their characteristics due to the cryoturbation process. Samples taken from the Ekati site, in which cryoturbation is highly evident by the patterned ground, confirmed this high variability in soil characteristics. The results from a standardized

phytotoxicity test (Environment Canada, 2005a) using a reference toxicant, boric acid, revealed high variability in responses (root and shoot length) as well. Our first specific objective was to determine the appropriate sampling intensity for cryoturbated arctic sites, to ensure that subtle differences from control samples could be detected. As physical parameters and microbial activity at our four sites varied widely, we concluded that each area of assessment should be considered individually in order to adequately sample and detect effects. The CV proved to be a useful tool for determining the amount of sampling required to prevent variability of toxicity test responses from masking subtle toxic effects at low concentrations.

Our next specific objective was to investigate the potential effects of ammonium nitrate in arctic soils, and determine application concentrations that would not significantly affect normal microbial activity and plant growth. We applied increasing concentrations of ammonium nitrate to four different arctic soils to see how much the soils could tolerate before a significant (>20%) inhibition in soil functions such as nitrification, carbon mineralization, and plant growth parameters was seen. We chose to calculate an IC_{20} concentration to give a conservative estimate of safe application concentrations. IC_{20}s differed among soils when expressed as total N kg^{-1} soil water, due in part to the fact that soils had different bulk densities and pore space, and accumulated N at different rates. As in the previous experiments, this resulted in responses, and therefore calculated IC_{20}s, that were site specific. The calculated IC_{20}s were useful for applications on the specific sites, and the confirmation that soil pore water and pH played an important role in moderating toxicity of ammonium nitrate will help determine appropriate application concentrations for other locations. Overall, we were able to conclude that arctic soil nitrogen concentrations could be maintained under inhibitory levels with continued application of low concentrations of ammonium nitrate over the arctic summer. This indicates that atomization of wastewater contaminated with ammonium nitrate is a reasonable method of disposal in arctic environments when managed properly.

5.1 Future Directions of Research

While the objectives of this particular study were met, further questions were raised about appropriate methods of testing and analysis in the Canadian arctic. There are several findings in this thesis that have important implications for future research directed towards

detection and prevention of impacts in arctic environments. Clearly, such a vast landscape which encompasses areas with significantly different temperatures, weather patterns, topography and soil parent materials, all of which strongly influence the rate of nutrient cycling and plant growth, requires its own set of guidelines. The high variability of arctic soil characteristics coupled with the high variability of toxicity test responses demands that intensive sampling be done on a site specific basis. But the fact that plants grown in some artic soils were significantly more sensitive to a reference toxicant than when grown in temperate soils suggests that arctic soil processes may be affected by lower concentrations of other toxicants than previously believed. Identifying the cause of this increased sensitivity to plants, whether it be the toxin's mechanism of action or a particular soil characteristic is a priority for predicting impacts of other contaminants in the future.

Although we could not determine a direct relationship between the variability of individual soil parameters and resulting toxicity test variability, high organic carbon content did appear to mitigate toxic effects of both boric acid and ammonium nitrate in the Ekati O horizon and Truelove soils to some extent. Certainly there is evidence that soil properties such as organic matter, clay content and pH do affect the bioavailability of other contaminants such as metals (Van Gestel and Koolhaas, 2004), and further studies could be focused in this area. Also, as soil levels of other nutrients such as carbon and phosphorus were not measured in this study, additional work could clarify critical relationships between soil nutrients and microbial functions in the arctic. Understanding the roles that C and nutrient amendments play in contaminant fate is crucial for waste management. Could supplying carbon and/or phosphorus along with the nitrogen maintain the nutrient balance and allow soil functions to continue with greater N applications?

One definite limitation of our research was that all experiments were conducted in the laboratory. Any on site testing would be beneficial, as so many more factors could be taken into consideration. Testing in the field with actively growing plants could further reduce the amount of nitrogen accumulation in the soil as plants could uptake ammonia and nitrate from the soil solution, allowing greater concentrations of ammonia nitrate to be applied without harm. Further investigation in to temperature effects would also be useful as temperatures at Ekati often reach 20°C for a few weeks in the summer. Temperature increases and longer exposure to sunlight increase microbial activity and photosynthesis reactions, which could also

allowing higher rates of nitrogen application. Another aspect that was not investigated in this study was the effect of constant moisture addition, and its effects on a desert-like ecosystem such as the tundra. Because all soils were maintained at 55% WFPS, additional studies in which soils are maintained at their natural field capacity could provide even more accurate IC_{20}s. More specifically, for the implementation of an atomization project, there needs to be some assessment of the amount of ammonium nitrate that would actually reach the tundra surface after atomization, as well as the dispersion pattern, factors which would be heavily influenced by wind and accurately measured only in a field situation. Long term impacts could also be investigated in the field; if the towers were turned off for the winter months, would the soils utilize enough accumulated nitrogen to be able to withstand similar deposition concentrations in the spring?

Another drawback to our study was that our investigation into resistance did not yield any tangible results. In fact, boric acid had no significant effect on any of our endpoints. The reason for this is not evident, as the boric acid concentrations were calculated specifically for the soil types, and early experiments indicated that plants grown in arctic soils were sensitive to boric acid. Microbial activity may not be as sensitive to boric acid, and it is possible that any effects were masked by the variability in the toxicity test responses. Soil resistance is definitely an area that warrants additional research, as it is a very important aspect when determining appropriate exposure concentrations. Other studies have shown that nitrifying bacteria in previously contaminated soils were more susceptible to toxicity of contaminants than previously uncontaminated soils (Maliszewska-Kordybach et al, 2007). Obviously more appropriate reference contaminants could be selected.

Finally, while results of this study would allow generation of site-specific guidelines that allow only a small amount of change in function, such as 20%, no specific threshold for acceptable change in these endpoints has been established for arctic soils. There is an opportunity (and challenge) for researchers and government agencies to set appropriate guideline criteria that will protect the arctic environment from changes that may cause irreparable damage.

It is important to remember that no environments are static or immune to changes caused by human activities or natural cycles. Polar regions are undergoing dramatic changes as temperatures rise and unprecedented amounts of pack ice melts. The arctic is not as remote as

it once was, and unfortunately, we cannot guarantee that any of it will remain a pristine, untouched wilderness. We can recognize that it is able to resist some impacts and adapt to changes, and attempt to minimize the amount of permanent damage from activities related to economic growth. This research demonstrates that technological innovations such as wastewater atomization offer promise of our ability to manage that impact in cold regions.

6.0 REFERENCES

Abeliovich A. 1992. Transformations of ammonia and the environmental impact of nitrifying bacteria. *Biodegradation 3, 255-264*

Alonso A. 2005. Valoración de la degradación ambiental y efectos ecotoxicológicos sobre la comunidad de macroinvertebrados bentónicos en la cabecera del río Henares. Doctoral Dissertation. Universidad de Alcalá. Alcalá de Henares (Madrid), Spain.

Alonso A and JA Camargo. 2004. Toxic effects of unionized ammonia on survival and feeding activity of the freshwater amphipod *Eulimnogammarus toletanus* (Gammaridae, Crustacea). *Bulletin of Environmental Contamination and Toxicology 72, 1052-1058*

Be´langer N and KCJ Van Rees. 2008. Sampling Forest Soils. *In* MR Carter and EG Gregorich, eds. Soil Sampling and Methods of Analysis. CRC Press, Boca Raton FL.

BHP Billiton Diamonds Inc. 2000a. Environmental Assessment Resport for Sable, Pigeon and Beartooth Kimberlite Pipes. Submitted to Mackenzie Valley Environmental Impact Review Board, Yellowknife, NT.

BHP Billiton Diamonds Inc. 2000b. Environmental Agreement Annual Report for Ekati Diamond Mine.

BHP Billiton Diamonds Inc. 2002a. Ekati Daimond Mine 2001 Annual Report and Action Plan for Greenhouse Gas Emissions, Yellowknife, Northwest Territories.

BHP Billiton Diamonds Inc. 2002b. Ekati Diamond Mine Facts.

Bliss LC. 1977. Truelove Lowland, Devon Island, Canada: A High Arctic Ecosystem. The University of Alberta Press, Edmonton.

Bockheim JG and C Tarnocai. 1998. Recognition of cryoturbation for classifying permafrost-affected soils. *Geoderma 8, 281-293*

Braddock JF, ML Ruth, PH Catterall, JL Walworth and KA McCarthy. 1997. Enhancement and inhibition of microbial activity in hydrocarbon-contaminated arctic soils: Implications for nutrient-amended bioremediation. *Environmental Science & Technology 31, 2078-2084*

Bradham KD, EA Dayton, NT Basta, J Schroder, M Payton and RP Lanno. 2006. Effect of soil properties on lead bioavailability and toxicity to earthworms. *Environmental Toxicology and Chemistry 25, 769-775*

Brain RA, K Bestari, H Sanderson, ML Hanson, CJ Wilson, DJ Johnson, PK Sibley and KR Solomon. 2005. Aquatic microcosm assessment of the effects of tylosin on *Lemna gibba* and *Myriophyllum spicatum*. *Environmental Pollution 133, 389-401*

Camargo, JA, A Alonso and A Salamanca. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere 58, 1255-1267*

Camargo, JA, and A Alonso. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International 32, 831-849*

Chapin, FS, RL Jeffries, JF Reynolds, GR Shaver and J Svoboda. 1993. Arctic ecosystems in a changing climate: An ecophysiological perspective. *Arctic and Alpine Research 25(2), 160-161*

Clesceri LS, Greenberg, AE, and AD Eaton, eds. 1995. Standard Methods for the Examination of Water and Wastewater. NO_2^- nitrogen (nitrite). United Book Press, Baltimore, MD, 112 -114

Conquest LL. 1983. Assessing the statistical effectiveness of ecological experiments - utility of the coefficient of variation. *International Journal of Environmental Studies 20, 209-221*

Constable M, M Charlton, F Jensen, K McDonald, G Craig and KW Taylor. 2003. An ecological risk assessment of ammonia in the aquatic environment. *Human and Ecological Risk Assessment 9, 527-548*

Creaser RA. 2004. Macrocrystal phlogopite Rb-Sr dates for the Ekati property kimberlites, Slave Province, Canada: evidence for multiple intrusive episodes in the Paleocene and Eocene. Proceedings, 8th International Kimberlite Conference June 22-27, 2003. Victoria BC, Canada, 399-414

Cruickshank JG. 1971. Soils and terrain units around Resolte, Cornwallis Island. *Arctic 24, 195-209*

Dutka BJ and G Bitton. 1986. Toxicity Testing Using Microorganisms. CRC Press, Boca Raton, Florida, USA.

EBA. 2002. Misery Site Surplus Water Atomization Project, Yellowknife, NT.

Edlund, SA. 1992. Vegetation of Cornwallis and adjacent islands, Northwest Territories: relationships between vegetation and surficial materials. Geological Survey of Canada Report 89-26. 24pp

Emerson K, RC Russo, RE Lund and RV Thurston. 1975. Aqueous ammonia equilibrium calculations - effect of pH and temperature. *Journal of the Fisheries Research Board of Canada 32, 2379-2383*

Environment Canada. 2001. Priority substances assessment report: ammonia in the aquatic environment. Minister of Public Works and Government Services Canada, Ottawa ON Canada.

Environment Canada. 2003. Canadian Water Quality Guidelines for the Protection of Aquatic Health: Nitrate Ion. Ecosystems Health: Science-based solutions Report No. 1-6. National Guidelines and Standards Office, Water Policy and Coordination Directorate, Environment Canada. 115pp.

Environment Canada. 2005a. Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil. Environmental Technology Centre Environment Canada, Ottawa ON.

Environment Canada. 2005b. Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environmental Technology Centre Environment Canada, Ottawa, ON.

Feisthauer NC, GL Stephenson, JI Princz and RP Scroggins. 2006. Effects of metal-contaminated forest soils from the Canadian shield to terrestrial organisms. *Environmental Toxicology and Chemistry 25, 823-835*

Freemark K and C Boutin. 1994. Non-target plant risk assessment for pesticide registration. *Environmental Management 18, 841-854*

Gee GW and JW Bauder. 1986. Particle-size Analysis, *In* AK ed. Methods of Soil Analysis, 2nd ed. Soil Science Society of America, Madison WI.

Gong P, SD Siciliano, CW Greer, L Paquet, J Hawari, and GI Sunahara. (1999). Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field-contaminated soils to indigenous microorganisms. *Environmental Toxicology and Chemistry, 18, 2681-2688*

Griffiths BS, M Bonkowski, J Roy and K Ritz. (2001). Functional stability, substrate utilisation and biological indicators of soils following environmental impacts. *Applied Soil Ecology 16, 49-61*

Jensen FB. 2003. Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology- Part A:Molecular & Integrative Physiology 135, 9-24*

Jonasson S, A Michelsen and IK Schmidt. (1999). Coupling of nutrient cycling and carbon dynamics in the Arctic, integration of soil microbial and plant processes. *Applied Soil Ecology 11), 135-146*

Kalra YP, and DG Maynard. 1991. Methods manual for forest soil and plant analysis Forestry Canada, Edmonton, AB Canada.

Kong CH, P Wang and XH Xu. 2007. Allelopathic interference of *Ambrosia trifida* with wheat (*Triticum aestivum*). *Agriculture Ecosystems & Environment 119, 416-420*

Kraufvelin P. 1998. Model ecosystem replicability challenged by the "soft" reality of a hard bottom mesocosm. *Journal of Experimental Marine Biology and Ecology 222, 247-267*

Lev A and RH King. 1999. Spatial variation of soil development in a High Arctic soil landscape: Truelove Lowland, Devon Island, Nunavut, Canada. *Permafrost and Periglacial Processes 10, 289-307*

Linn DM and JW Doran. (1984). Effect of water-filled pore-space on carbon-dioxide and nitrous-oxide production in tilled and nontilled soils. *Soil Science Society of America Journal, 48, 1267-1272*

Loseto LL, DRS Lean and SD Siciliano. 2004. Snowmelt sources of methylmercury to high arctic ecosystems. *Environmental Science & Technology 38, 3004-3010*

Maliszewska-Kordybach B, Klirnkowicz-Pawlas A, Smreczak B and Janusauskaite D. 2007. Ecotoxic effect of phenanthrene on nitrifying bacteria in soils of different properties. *Jouurnal of Environmental Quality 36, 1635-1645*

Moore DRJ and P Caux. 1997. Estimating low toxic effects. *Environmental Toxicology and Chemistry 16, 794-801*

NRC (National Research Council). 2000. Clean coastal waters: understanding and reducing the effects of nutrient pollution. National Academic Press, Washington, DC.

Nobrega S and P Grogan. In Press. Landscape and ecosystem-level controls on net carbon dioxide exchange along a natural moisture gradient in Canadian low arctic tundra. *Ecology*

OECD (Organisation for Economic Co-operation and Development). 2000a. Soil microorganisms: Nitrogen transformation test 216.

OECD (Organisation for Economic Co-operation and Development). 2000b. Soil microorganisms: Carbon transformation test 217.

OECD (Organisation for Economic Co-operation and Development). 2003. Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test 208.

Paul AE and FE Clark. 1989. Soil Microbiology and Biochemistry. Academic Press Inc, San Diego, California.

Pielou E. 1994. Plant Life - A Naturalist's Guide to the Arctic. The University of Chicago Press

Randall DJ and TKN Tsui. 2002. Ammonia toxicity in fish. *Marine Pollution Bulletin 45, 17-23*

Rayner JL, I Snape, JL Walworth, PM Harvey and SH Ferguson. (2007). Petroleum-hydrocarbon contamination and remediation by microbioventing at sub-Antarctic Macquarie Island. *Cold Regions Science and Technology, 48, 139-153*

Robertson PG. 1997. Nitrogen use efficiency in row-crop agriculture: crop nitrogen use and soil nitrogen loss. *In* LE Jackson, ed, Ecology in Agriculture. *347-365* Academic Press, San Diego CA.

Rooney CP, FJ Zhao SP McGrath. 2007. Phytotoxicity of nickel in a range of European soils: Influence of soil properties, Ni solubility and speciation. *Environmental Pollution 145, 596-605*

Russo R. 1985. Ammonia, nitrite and nitrate. *In* G Rand and S Petrocelli, eds. Fundamentals of Aquatic Toxicology: Methods and Applications. *455-471* Hemisphere, Washington DC.

Salminen J, M Liiri and J Haimi. 2002. Responses of microbial activity and decomposer organisms to contamination in microcosms containing coniferous forest soil. *Ecotoxicology and Environmental Safety 53, 93-103*

Schafer AN, I Snape and SD Siciliano. 2007. Soil biogeochemical toxicity end points for sub-Antarctic islands contaminated with petroleum hydrocarbons. *Environmental Toxicology and Chemistry 26, 890-897*

Scott G and RL Crunkilton. 2000. Acute and chronic toxicity of nitrate to fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia*, and *Daphnia magna*. *Environmental Toxicology and Chemistry 19, 2918-2922*

Seybold CA, JE Herrick and JJ Brejda. 1999. Soil resilience: A fundamental component of soil quality. *Soil Science 164, 224-234*

Sheldrick BH and Wang C. 1993. Particle Size Distribution. *In* MR Carter, ed, Soil Sampling and Methods of Analysis *499-511* Lewis Publishers.

Siciliano SD, CM Theoret, JR de Freitas, PJ Hucl and JJ Germida. 1998. Differences in the microbial communities associated with the roots of different cultivars of canola and wheat. *Canadian Journal of Microbiology 44, 844-851*

Smith VH. 2003. Eutrophication of freshwater and coastal marine ecosystems - A global problem. *Environmental Science and Pollution Research 10, 126-139*

Sokal RR, Rohlf FJ. 2003. Biometry: the principles and practice of statistics in biological research, 3rd edition. W. H. Freeman & Company, New York, USA.

Soil Classification Working Group. 1998. *The Canadian System of Soil Classification*. Agric. and Agri-Food Can. Publ. 1646 (Revised) 187 pp. NRC Research Press, Ottawa ON

Stephenson GL, KR Solomon, B Hale, BM Greenberg and RP Scroggins. 1997. Development of Suitable Test Methods for Evaluating the Toxicity of Contaminated Soils to a Battery of Plant Species Relevant to Soil Environments in Canada. *In* FJ Dwyer et al, eds. Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment Volume 6, 474-489. American Society for Testing and Materials, Philadelphia, PA.

Stephenson GL, N Koper, GF Atkinson, KR Solomon and RP Scroggins. 2000. Use of nonlinear regression techniques for describing concentration-response relationships of plant species exposed to contaminated site soils. *Environmental Toxicology and Chemistry 19, 2968-2981*

Van Ewijk, PH and JA Hoekstra. 1993. Calculation of the EC50 and its confidence interval when subtoxic stimulus is present. *Ecotoxicology and Environmental Safety 25, 25-32*

Van Gestel CAM and JE, Koolhaas. 2004. Water-extractability, free ion activity, and pH explain cadmium sorption and toxicity to *Folsomia candida* (Collembola) in seven soil-pH combinations. *Environmental Toxicology and Chemistry 23, 1822-1833*

Walecka-Hutchison CM and JL Walworth. 2007. Evaluating the effects of gross nitrogen mineralization, immobilization, and nitrification on nitrogen fertilizer availability in soil experimentally contaminated with diesel. *Biodegradation 18,133-144*

Walworth J, A Pond, I Snape, J Rayner, S Ferguson, and P Harvey. 2007. Nitrogen requirements for maximizing petroleum bioremediation in a sub-Antarctic soil. *Cold Regions Science and Technology 48, 84-91*

Walworth JL, CR Woolard, JF Braddock and CM Reynolds. (1997). Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: An approach to determining optimum levels. *Journal of Soil Contamination, 6 (5), 465-480*

Wang W and K Freemark. 1994. The use of plants for environmental monitoring and assessment. *Ecotoxicology and Environmental Safety 30, 289-301*

Washburn, AL. 1997. Plugs and plug circles: a basic form of patterned ground, Cornwallis Island, Arctic Canada - origin and implications. *Geological Society of America Memoir 190, 1-11*

Wentsel R, W Beyer, C Edwards, L Kapustka and R Kuperman. 2003. Effects of Contaminants on Soil Ecosytem Structure and Function. *In* RP Lanno, ed, Contaminated Soils: From soil-chemical interactions to ecosystem management. 117-150. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola, FL.

Yoshitake S, M Uchida, H Koizumi and T Nakatsubo. (2007). Carbon and nitrogen limitation of soil microbial respiration in a High Arctic successional glacier foreland near Ny-angstrom lesund, Svalbard. *Polar Research, 26, 22-30*

Zeleke TB and BC Si. 2005. Scaling relationships between saturated hydraulic conductivity and soil physical properties. *Soil Science Society of America Journal 69, 1691-1702*