Project Completion Report
Rocky Mountains Cooperative Ecosystem Studies Unit (RM-CESU)

Project Title: Measurement of Nitrogen and CO2 Production from Seasonally Frozen Alpine Tundra Soils

Project Code (such as UMT-72 and/or the "J" or "P" number):
Agreement Number H1200-09-0004
PR Number: R1526115100
Award Number: P11AT00223 MET-01

Type of Project (Research, Technical Assistance or Education): Research

Funding Agency: National Park Service

Partner University: Metropolitan State College (University) of Denver

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Start Date of Project: 5/1/2011

End Date of Project: 12/31/2012

Funding Amount: $17,894

<u>Project Summary</u>
(see attached)

Number of students participating in this project: 35 undergraduates from 5/1/2011 to 12/13/2012

Lessons Learned from this project:
(see attached)

Other RM-CESU agencies or research partners who participated in this project:
(none)

"Preliminary Report: Measurement of soil nutrient concentration and carbon dioxide production from seasonally frozen alpine tundra and forest soils"

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Abstract

With increased nitrogen deposition and changing alpine climatic conditions, alpine soils will be exposed to environmental stress. The ability of alpine soil to support existing and new plant communities will be a concern. In addition, it is important to identify additional sources of carbon dioxide (CO_2) that may be emitted from warming alpine soils. Soil nutrient and CO_2 concentrations were tracked from mid-June to late-September 2011 at two tundra soil sites (a mineral soil and an organic soil) as well as an Engelmann spruce forest soil. Greater concentrations of nutrients were found at the forested site compared to the alpine tundra. Of nitrogen forms, ammonia was the greatest; nitrite and nitrate levels were insignificant. Each nitrogen form shows decreasing abundance through summer to fall. Calcium was the most abundant nutrient and was "very high" on average at the forested site. Low concentrations of nitrogen, phosphorus, and other elements combined with soils that are becoming increasingly acidic make it highly unlikely that in the future the tundra ecosystem will be able to support succeeding coniferous vegetation as well as existing wildflowers or tundra grasses. Carbon dioxide average about 2500 ppm at the forest site compared to 1000 ppm at the mineral tundra soil site and 1600 ppm at the organic tundra soil site. Carbon dioxide at the forest site was highest during June (≈ 4000 ppm), and later decreased and plateaued during the summer and fall (≈ 2000 ppm). Carbon dioxide production in the Engelmann spruce forest soil is roughly double that of the alpine tundra soil and the area of the forest is roughly 3 times the area of the alpine tundra. As a result, the coniferous forest soil has significant potential for adding additional CO_2 to atmosphere.

Introduction

Human activities have more than doubled amount of nitrogen in the biosphere. In the Green Lakes Valley and nearby Niwot Ridge, CO, precipitation has shown an increase since 1967, which corresponds with a doubling of inorganic nitrogen deposition (Williams *et al.*, 2002). In Rocky Mountain National Park, this has resulted in enhanced phytoplankton growth in lakes such that phosphorous has become the limiting variable (Elser *et al.*, 2009). According to algal chlorins in Sky Pond, Colorado, a substantial shift in nitrogen cycling has occurred over the past 60 years, which is thought to be the result of development along the Front Range corridor (Enders *et al.*, 2008). In Rocky Mountain National Park, it appears a threshold has been crossed (Baron *et al.,* 2009). In the Loch Vale watershed, mean wet nitrogen deposition has not increased, but mean annual net export has increased, suggesting that melting ice in glaciers and rock glaciers has exposed sediments from which nitrogen can be flushed (Baron *et al.,* 2009).

The Colorado Front Range is a dynamic system in which anthropogenic inputs can be transported and redistributed from terrestrial to aquatic ecosystems to alter ecosystem health (Hood *et al.*, 2005; Seastedt *et al.*, 2004). In particular, nitrogen deposition has acidified soils through leaching of basic cations. With a reduced pH, increased availability of elements toxic to plants have reduced plant biodiversity (Bowman *et al.*, 2008). In the mountain west, modeling results indicate that the most sensitive lakes to acidic deposition are in Rocky Mountain National Park and Grand Teton National Park (Nanus *et al.*, 2009). The concentration of inorganic nitrogen in snowpack is high, is converted by biological and geochemical reactions, and is released as an ionic pulse (Williams *et al.*, 2009). As a result, water quality remains an issue because water stored in mountains often supply drinking water to nearby urban areas (Ley *et al.*, 2004).

It is important to determine areas of significant nitrogen production in seasonally frozen alpine soils. As a soil that was previously frozen thaws from a warming climate, new locations for microbial activity are exposed, increasing the potential for nitrogen transport to alpine lakes and streams. In addition, species can be affected by additional nitrogen input, causing grasses or other sedge species to outcompete other species, potentially reducing tundra species diversity or reducing stunning summer blooms that are unique to Rocky Mountain National Park. Increased nitrogen deposition might also cause plants to grow more, which would initially remove some carbon from the atmosphere, but in the long term, it also could cause soils to lose more of their carbon, adding carbon dioxide (CO_2) to the atmosphere.

Arctic permafrost soils contain nearly twice as much carbon as the atmosphere. When these soils thaw, large quantities of carbon are lost, mainly in the form of methane and CO_2 through decomposition (Lee *et al.*, 2010; Maslin *et al.*, 2010). The alpine tundra shows lower total CO_2 uptake over shorter periods compared to forested sites; however, the alpine tundra has greater total respiration over longer periods (Blanken *et al.*, 2009). Forests uptake CO_2 longer than tundra vegetation, but tundra loses CO_2 due to respiration over a longer time, which suggests tundra has the potential to release possibly significant amounts of CO_2 back to the atmosphere. According to Waldrop *et al.* (2010), respiration is higher in permafrost soils compared to active layer soils; this suggests that a positive feedback to warming exists. Findings in Siberia, Alaska, and northern Sweden imply that soil carbon that was once stored in deep permafrost is being released (Kuhry *et al.* 2010). According to climatic data from Europe and Western US, an increase in moisture availability has resulted in a six-fold increase in CO_2 respiration, despite only a 0.3 to 0.5° C temperature increase (Monson *et al.*, 2006). Recently, permafrost soils have also been shown to release nitrous oxides (Elberling *et al.*, 2010). Helmig *et al.* (2009) found that nitrogen oxide concentrations were highest at the bottom of the snowpack and experienced an upward flux of nitrogen oxide, which suggests that subnival soils are the origin.

Soil Nutrients

Nutrients in the soil provide a foundation for healthy plant growth. Six essential nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) are needed in large amounts (more than 500 ppm) and are termed macronutrients. Nitrogen is a constituent of all plant proteins, chlorophyll, and nucleic acids. Nitrogen exists in many forms; it is regularly cycled through the environment. The nitrogen cycle consists of a biochemical changes when nitrogen is used by organisms, transformed upon death, and converted to its original state. For plants, the reservoir of nitrogen is essentially from atmospheric gas. Dinitrogen fixation occurs when molecular nitrogen (N_2) is converted to ammonia (NH_3) though lightening or biological processes. About 99% of the soil nitrogen is organic nitrogen; of that, about 1-2% is decomposed and mineralized to produce ammonia. A fertile soil will have low amounts of ammonia unless there has been a recent application of nitrogenous fertilizer. In forest soils, ammonia is the most abundant form of nitrogen, particularly in a humus rich layer. Some ammonia volatizes from animal droppings, others react with water to form ammonium (NH_4^+).

Nitrification occurs when ammonium is converted to nitrite (NO_2^-) and then nitrate (NO_3^-). Nitrites are formed as in intermediate step in the production of nitrate. Well-drained and aerated soils contain small amounts of nitrite. Excessive nitrites may be toxic to plants. Nitrates give plants their dark green color, as they are a component of chlorophyll. Nitrate is subject to leaching whereas ammonium is adsorbed onto cation exchange sites on soil colloids. Both ammonium and nitrate are available forms of nitrogen for roots and microorganisms; their conversion to an organic form of nitrogen is known as immobilization. Soil nitrogen is subject to repeat cycling through mineralization (conversion into inorganic nitrogen), nitrification, and immobilization. Denitrification is the reduction of nitrate or nitrite to molecular nitrogen or nitrogen oxides. Many of these products are gaseous and escape from the soil.

Three additional macronutrients produced from weathering of the soil were analyzed in this study. Phosphate is necessary for hardy plant growth. It increases grain development, hastens maturity, encourages root development, increases cell development to reduce stresses such as disease, and stimulates the formation of fats. Calcium is an important component of cell walls in plants; it affects the permeability of membranes, stimulates root and leaf development, and activates reactions involved in plant metabolism. Potassium is found in minerals that weather. Potassium enhances the synthesis and translocation of carbohydrates, encouraging cell wall development, and many other functions that promote healthy plants.

Objectives

The objective of this study is to provide a seasonal analysis of soil nutrient concentrations and CO_2 production from soils at high elevation sites. It is important to assess potential impacts of a changing climate on these ecosystems. Excess nitrogen can affect species diversity and abundance as well as tundra blooms, an important Visitor attraction that makes Rocky Mountain National Park unique. Additional carbon released from previously frozen alpine soils is an unaccounted source of CO_2 that could enhance the effects of warming in the alpine.

Specifically, the objectives of this project are as follows:

1. Determine seasonal production of Nitrogen (in the form of Nitrite, Nitrate, and Ammonia) and CO_2 from high elevation soils;
2. Evaluate the effects of soil type, soil temperature, soil moisture, and site location on rates of CO_2 production;
3. Compare phosphorus, calcium, and potassium concentrations at tundra and forest sites; and
4. Examine the diversity of soil microbes that are predominantly found in alpine soils.

Study Area

Three field sites were selected for analysis (Figure 1). Two sample sites are located in the alpine tundra; one site is located at a lower elevation in the coniferous Engelmann spruce forest along Trail Ridge Road. One tundra site consists of a mineral soil; the other site contains organic soils. Characteristics of the field sites are listed in Table 1.

Table 1 – Topographic and environmental characteristics of the field sites.

Site	Soil Type	Elevation (ft)	Slope (°)	Aspect	Mean Annual Soil Temperature (°C)
Site 3	Organic	12060	4	SW	0.13
Site 4	Mineral	12067	9	SE	0.03
Forest	Organic	10558	24	NE	2.38

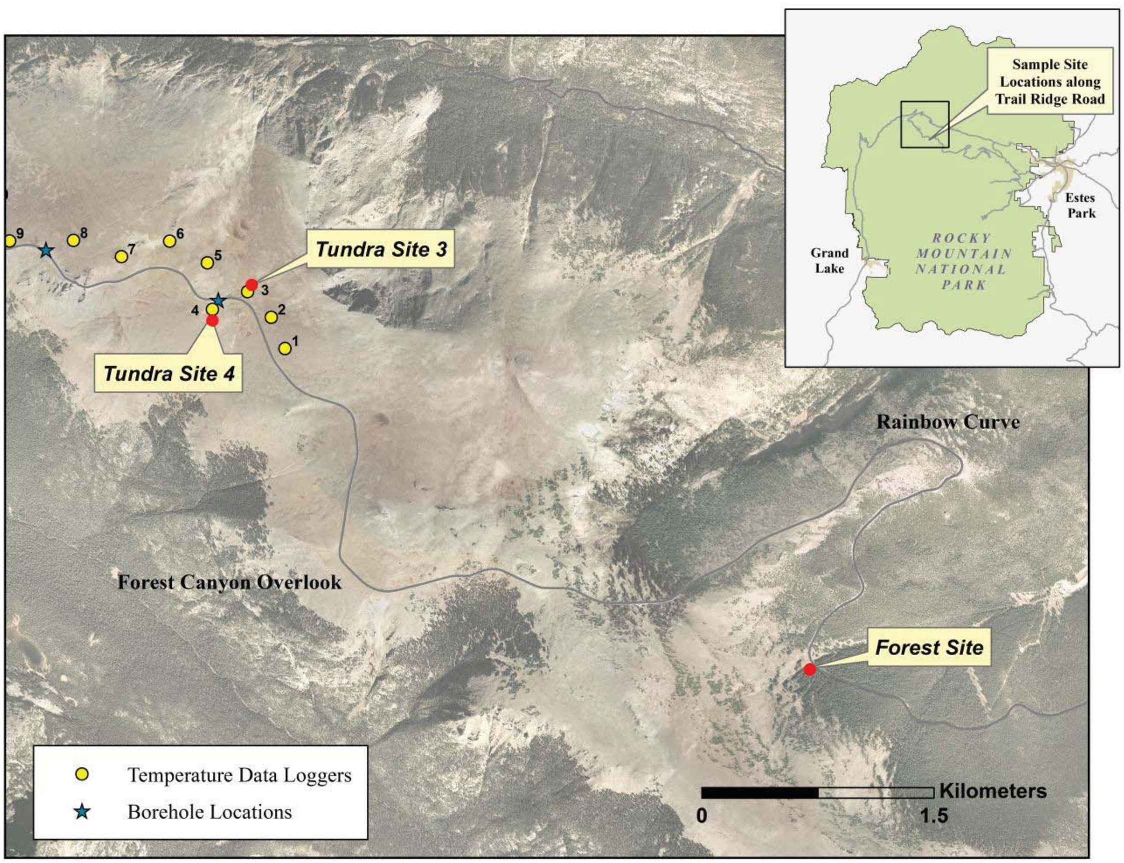

Figure 1 – Location of sample sites for analysis of soils.

Methods

From June 2011 through September 2011, CO_2 concentration in the soil was measured monthly at each field site, and soil samples were obtained for nutrient measurements and DNA analysis of soil microbes. Carbon dioxide in the soil was measured using a probe with a soil adapter manufactured by Vaisala, Inc. at mid-morning to early afternoon on the following dates: 6/10, 7/7, 8/17, and 9/30. The probes were installed and left in the upper 30 cm of soil at a sample site. The sensor was designed to measure concentrations from 0 to 2000 ppm with an accuracy of ±1.5 % of range or within 2% of an individual reading. In the field, a digital adapter was connected to the probe to obtain readings. Readings were recorded once a value stabilized, which usually took about 30 minutes per site. Percent soil moisture was measured using a Venier Probe at each site. Accuracy of the instrument is typically ± 4%.

Soil samples were taken to the laboratory for further analysis. Soils were kept frozen and then processed using a LaMotte SMART3 soil kit that utilizes a colorimetric method in which a reacted test sample is photoelectrically compared to a blank sample. The concentration in ppm was measured for nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), phosphorus, calcium, and potassium.

Simulations were also run in an environmental freeze-thaw chamber to determine CO_2 production of the soil samples under -10, -1.5, and -0.5° C temperature scenarios. Soil samples were isolated in a 6 inch long by 1.5-inch wide PVC pipe. The CO_2 probe was placed at the surface of the soil sample in the PVC pipe, sealed with a rubber gasket, and then connected to a data logger, which recorded measurements every 30 minutes. The freezer was set at each temperature regime for a 12-hour period.

Soil samples were collected for each site on June 10, 2011, July 7, 2011, and August 17, 2011 for DNA analysis. The employed multi-step procedure uses state-of-the-art molecular biology studies. To identify microbes present in the soil, the first step is to extract all DNA from the soil. This initial extraction presumably contains DNA from many sources, including bacteria, fungus, plant, and animal. To obtain specific bacterial DNA, we used a molecular technique, Polymerase Chain Reaction (PCR). Since soil typically contains many different bacterial sources, each individual piece of DNA must be isolated. This is done through a cloning procedure. Then the cloned DNA is transferred into a laboratory strain of E. coli bacteria, through a process known as transformation. This is an important step to store the DNA within the E. coli and then allow the future steps. A successful transformation procedure might yield 50 to 100 different E. coli cells, each potentially containing a different piece of bacterial DNA. To determine which exact type(s) of bacteria were in the original soil sample, we must then prepare DNA from each E. coli clone and screen it to be sure it has incorporated new bacterial DNA from the soil. The final step is to sequence the DNA from clones of interest. The sequenced DNA will allow us to identify which bacteria were present at each soil site.

Results and Discussion

Nutrient concentrations, CO_2 levels, and soil moisture values are listed in Table 2. Of the nitrogen forms, ammonia concentrations were highest at all sites. In comparison with agricultural soils, ammonia concentrations were at “medium” levels. Generally, nitrogen (ammonia, nitrite, and nitrate) was highest in the forest and decreased at the tundra sites (Figures 2 – 4; Table 2). Ammonia in the organic soil was higher than the mineral soil in the tundra, but nitrite and nitrate levels were comparable at the tundra sites. Nitrate levels were higher than nitrite concentrations at all sites. In comparison with agricultural soils, nitrite and nitrate levels are “low.” Low nitrite and nitrate values in comparison with ammonia indicate the slowness and ineffectiveness of the nitrification process in the alpine, which is perhaps due to the cold environment.

Table 2 – Seasonal change and average concentrations of nutrients, CO_2, and soil moisture at field sites.

	6/10/2011	**7/7/2011**	**8/17/2011**	**9/30/2011**	***Average***	***Standard Deviation***
Nitrate						
Tundra (mineral)	0.00	0.18	0.01	0.01	*0.05*	*0.09*
Tundra (organic)	0.06	0.06	0.07	0.03	*0.06*	*0.02*
Forest	0.09	0.13	0.08	0.04	*0.09*	*0.04*
Ammonia						
Tundra (mineral)	0.91	0.52	0.72	0.48	*0.66*	*0.20*
Tundra (organic)	1.90	1.13	1.05	0.68	*1.19*	*0.51*
Forest	1.54	1.11	1.12	1.14	*1.23*	*0.21*
Nitrite						
Tundra (mineral)	0.013	0.000	0.000	0.011	*0.006*	*0.007*
Tundra (organic)	0.008	0.000	0.013	0.000	*0.005*	*0.006*
Forest	0.012	0.027	0.019	0.008	*0.017*	*0.008*
Phosphate						
Tundra (mineral)	0.48	0.16	0.23	0.47	*0.34*	*0.16*
Tundra (organic)	0.97	0.32	0.26	0.35	*0.48*	*0.33*
Forest	0.40	0.67	0.80	0.58	*0.61*	*0.17*
Potassium						
Tundra (mineral)	3.2	2.3	4.8	1.4	*2.9*	*1.5*
Tundra (organic)	10.1	3.5	2.7	3.7	*5.0*	*3.4*
Forest	7.7	7.5	9.5	9.9	*8.7*	*1.2*
Calcium						
Tundra (mineral)	701.8	660.5	1279.7	619.2	*815.3*	*311.4*
Tundra (organic)	1279.7	1279.7	949.4	619.2	*1032.0*	*316.2*
Forest	1444.8	1816.3	2476.8	2972.2	*2177.5*	*680.3*
Carbon Dioxide						
Tundra (mineral)	750	1150	1340	850	*1023*	*271*
Tundra (organic)	1780	2680	920	1110	*1623*	*796*
Forest	4100	1990	1900	2030	*2505*	*1065*
Soil Moisture						
Tundra (mineral)	ND	26	4	26	*19*	*13*
Tundra (organic)	ND	32	2	8	*14*	*16*
Forest	ND	18	1	6	*8*	*9*

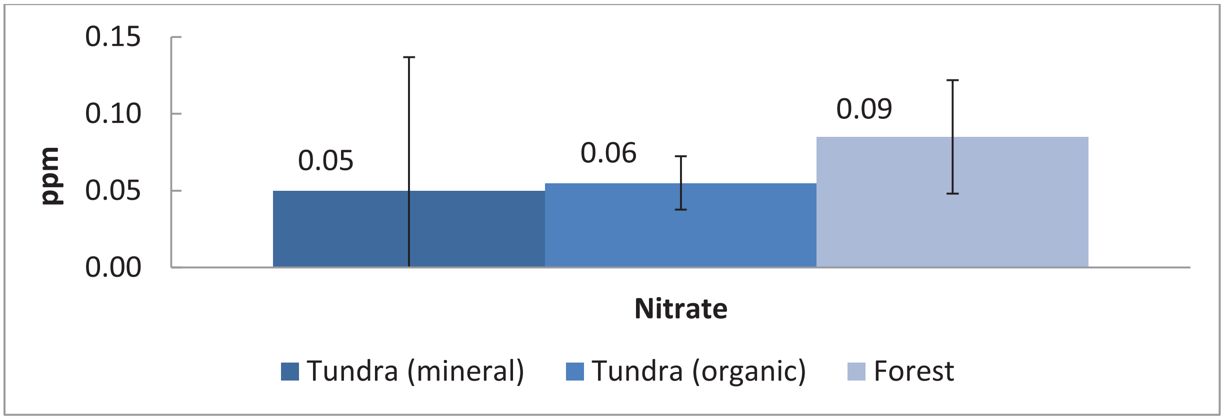

Figure 2 – Average nitrate concentrations at each site.

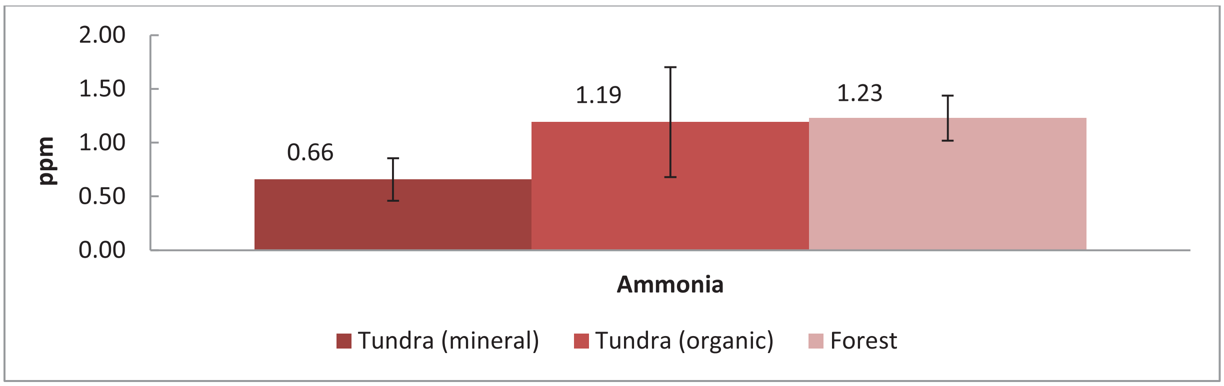

Figure 3 – Average ammonia concentration at each site.

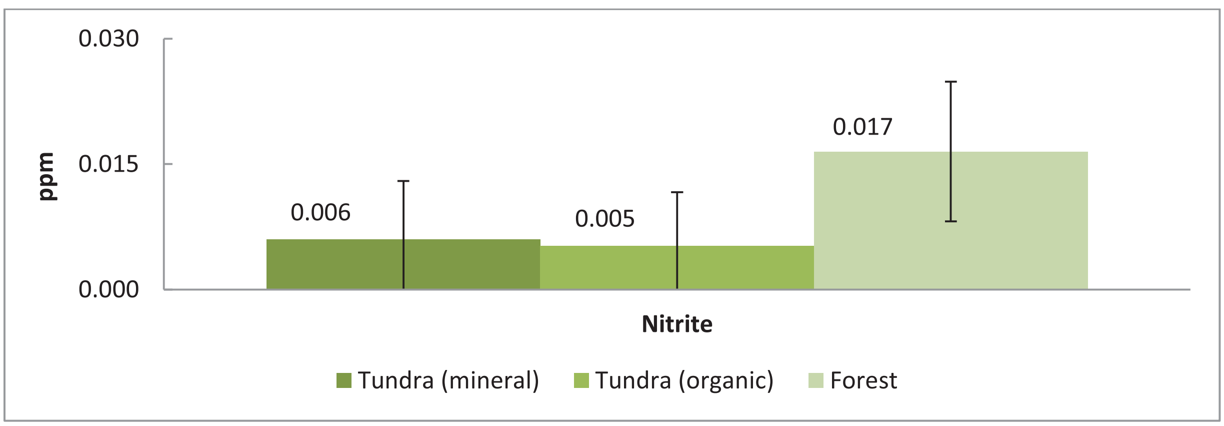

Figure 4 – Average nitrite concentration at each site.

Potassium, phosphate, and calcium were highest on average at the forest site as well (Figures 5 – 7; Table 2). Calcium was the most abundant nutrient according to the soils tests (Table 2). Calcium concentrations at the forest site were doubled that of the tundra sites, perhaps because of different parent materials or an indicator of the effectiveness of pine needles at "combing" the air to filter out calcium rich dusts (citation). Compared with agricultural soils, phosphate levels at all sites are "very low to low." Potassium at the tundra sites is "high," whereas it is "very high" at the forest site. "Medium" levels of calcium were observed on the tundra, but "very high" levels are common in the forest.

On average, CO_2 concentrations were about 2500 ppm at the forested site and decreased to 1000 - 1600 ppm on average at the tundra sites (Figure 8). The average concentration of the organic tundra soil was 600 ppm greater than the tundra mineral soil. This suggests that decaying organic matter greatly adds CO_2 to the soil. Tundra sites had greater soil moisture contents in comparison to forest soils (Figure 9). The mineral soil was wetter on average because of better-developed snowpack at this site. The wetter soils likely elevated CO_2 production at the mineral soil site.

Nitrate levels showed a general depletion from July through September as plants consume the available nitrogen (Figure 10). Ammonia also shows a decreasing trend from June through September (Figure 11). This also suggests that snowfall, which contains nitrogen, is an important nutrient input to the soil. The concentrations, however, are "very low" for most soils (Table 2). At the forest site, nitrite peaked in July, but was undetectable at the tundra sites (Figure 12). The tundra sites showed more variability in their nitrite concentrations over the field season (perhaps a limitation of LaMotte test kit), whereas the forest site showed a consistent decrease since July. Most nitrite levels, however, were insignificant.

Potassium remained consistent at the forest site, which had greater potassium levels compared to the tundra sites (Figure 13). The organic tundra site showed decreased levels after June, but a similar trend was not observable at the mineral tundra soil site. Phosphate at the forest site increased from June to August, and then decreased through September (Figure 14). At the tundra sites, greatest phosphate was observed in June, decreased through the mid-summer and eventually showed a slight increase though September. Greatest phosphate levels in early June at the tundra sites suggests that some phosphate is originating in the snowpack. At the forest site, calcium increased through the season (Figure 15). Calcium at the tundra site did not display an observable trend.

Carbon dioxide at the forest site was highest during June ($\approx$ 4000 ppm), and later decreased and plateaued during the summer and fall ($\approx$ 2000 ppm) (Figure 16). High CO_2 levels in the spring were likely the product of snow, which trapped CO_2 and increased concentrations. At the mineral tundra soil site, CO_2 gradually rose from June until August and decreased through September. At the organic tundra soil site, CO_2 rose until July, decreased through August, and gradually rose through September (Figure 16). Carbon dioxide levels did not show a positive correlation with water content (Figure 17); however, elevated CO_2 at the organic tundra site during July may be the result of high moisture content (> 25%).

Result of CO_2 production under various temperature regimes are listed in Table 3. Colder temperatures result in lower CO_2 production for all soil types. As temperatures warm, CO_2 concentrations increase (Figure 18 – 20). The background concentration of CO_2 in an empty chamber was about 600 ppm. According to the simulations, CO_2 levels in the forest were greater than in the tundra soils (Table 3). Both organic and mineral soils showed comparable rates of CO_2 production. Engelmann spruce covers about 146 mi^2 of the park, whereas tundra covers only 52 mi^2. Carbon dioxide production in the Engelmann spruce forest soil is roughly double that of the alpine tundra soil. The area of the forest is roughly 3 times the area of the alpine tundra. As a result, the coniferous forest soils have significant potential for

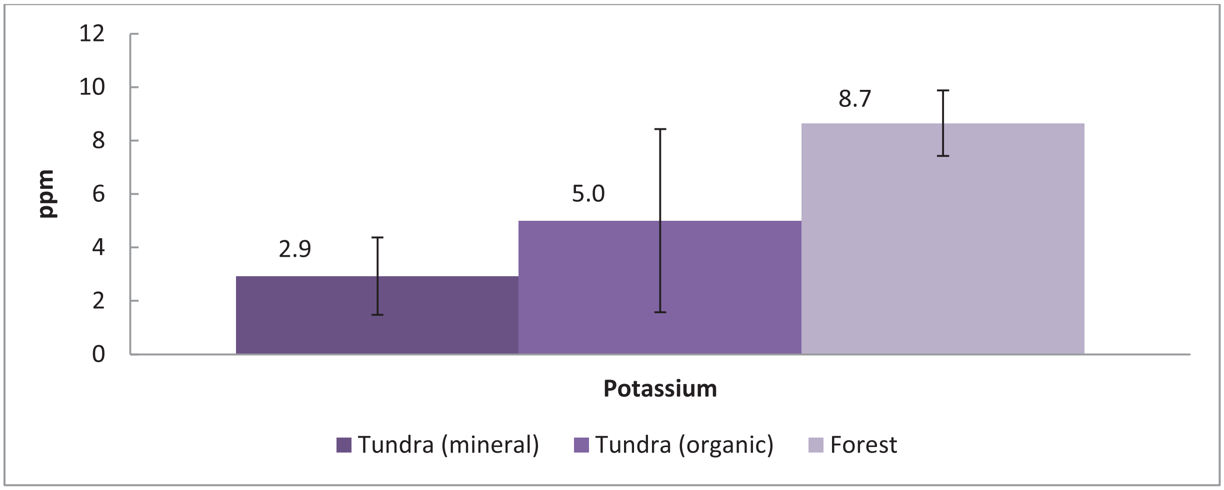

Figure 5 - Average potassium concentration at each site.

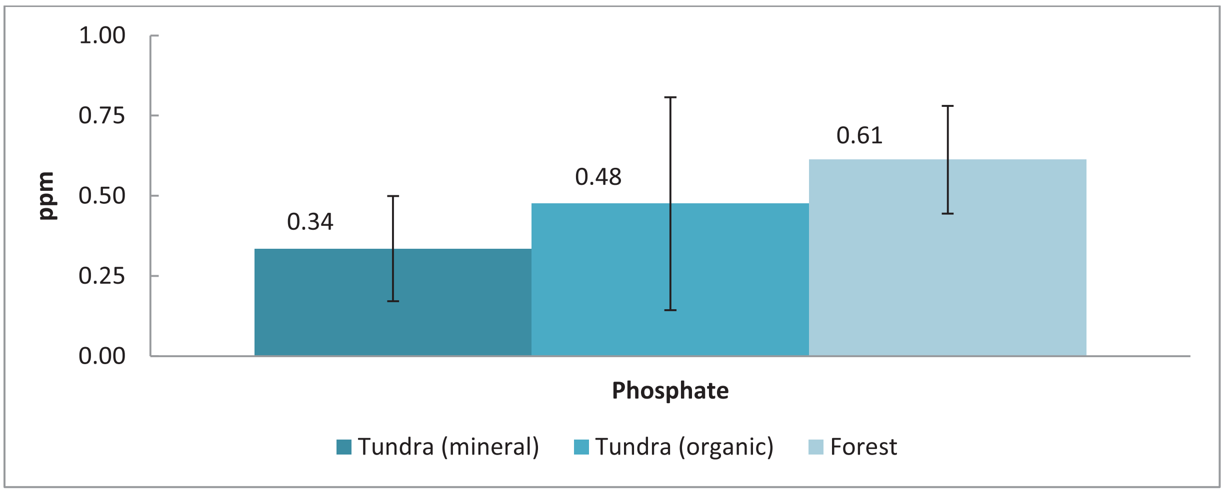

Figure 6 - Average phosphate concentration at each site.

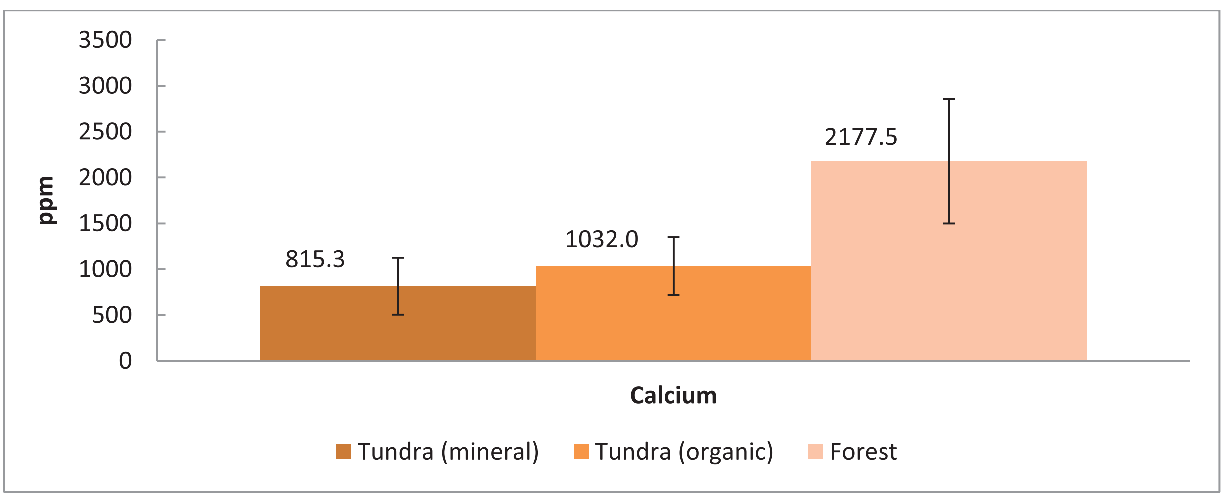

Figure 7 - Average calcium concentration at each site.

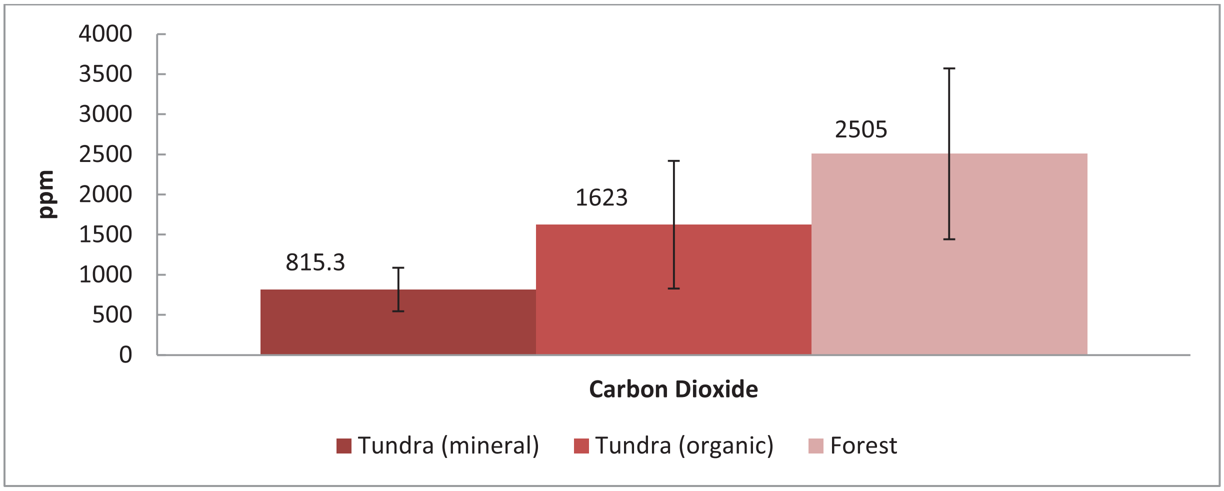

Figure 8 - Average CO_2 concentration at each site.

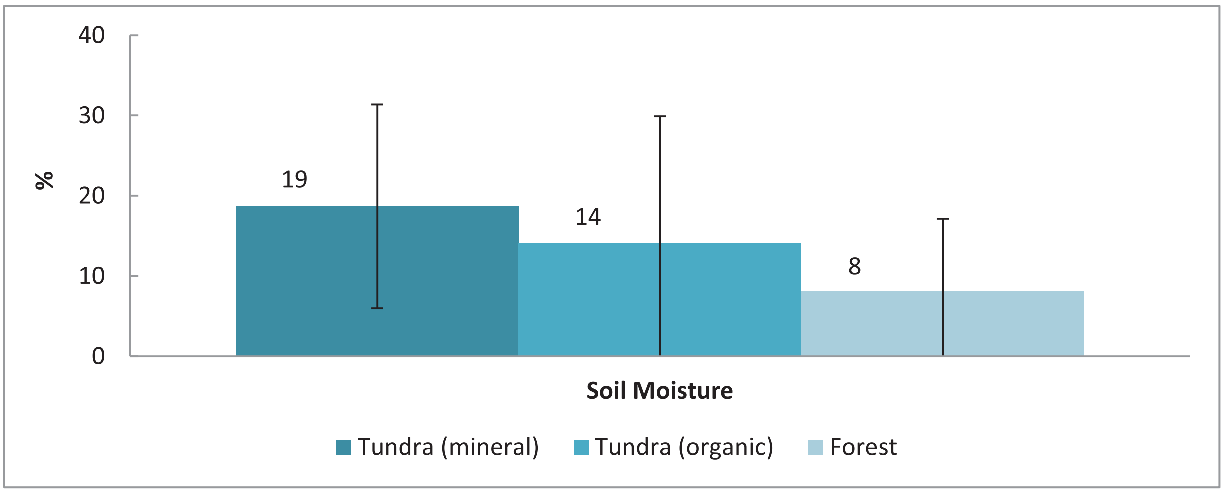

Figure 9 - Average soil moisture concentration at each site.

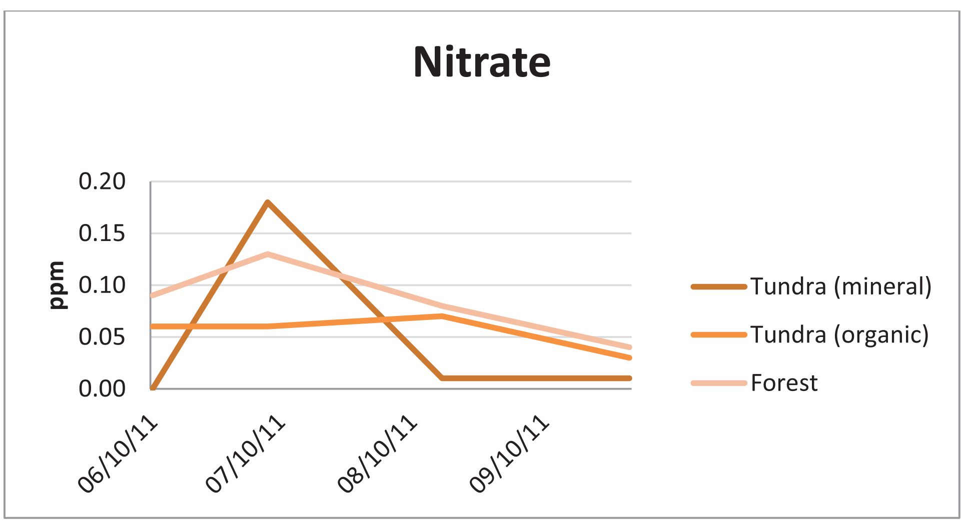

Figure 10 – Change in nitrate concentration over time.

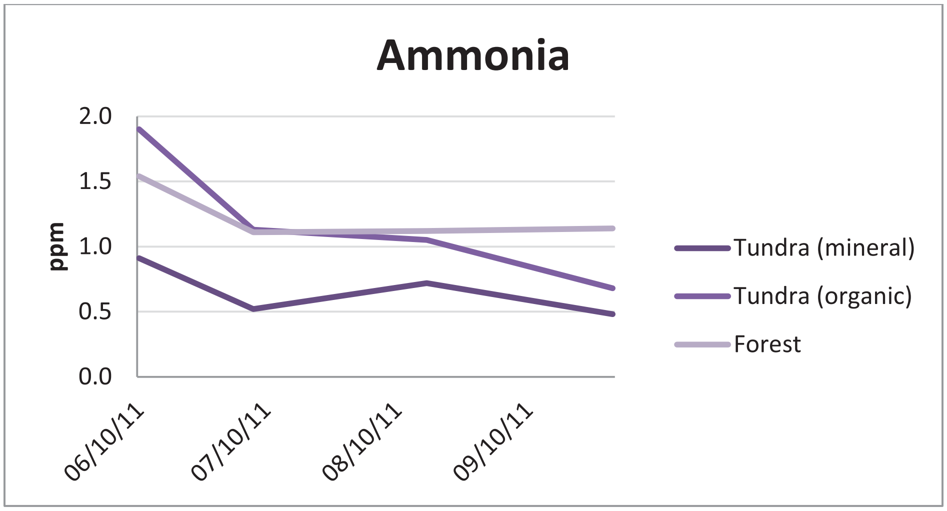

Figure 11 – Change in ammonia concentration over time.

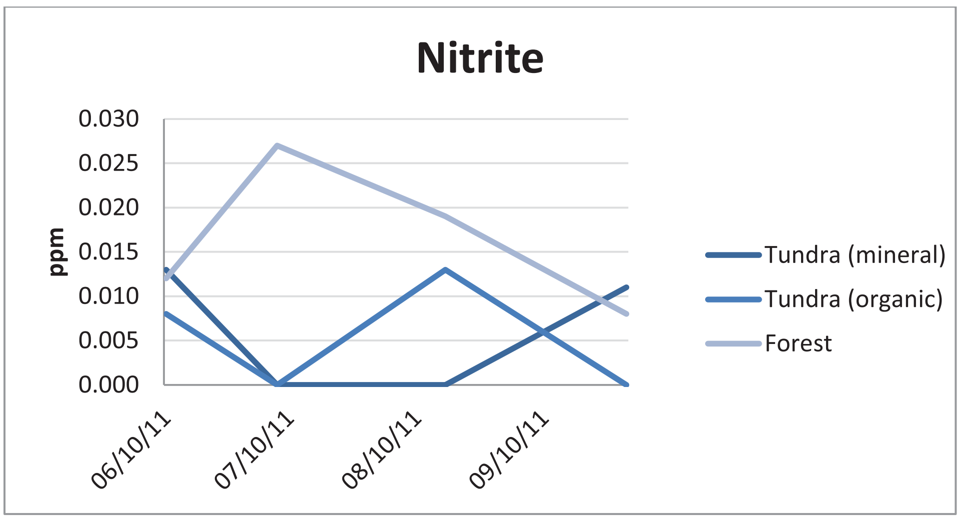

Figure 12 – Change in nitrite concentration over time.

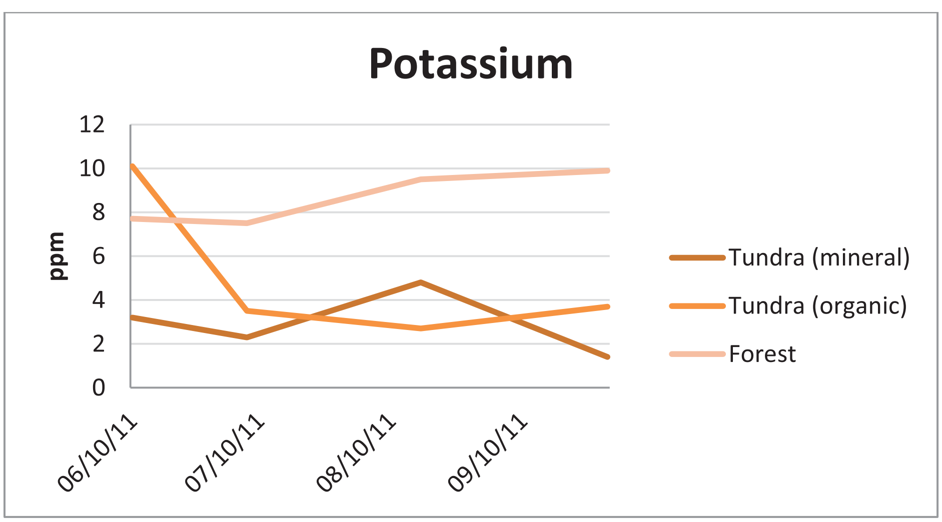

Figure 13 – Change in potassium concentration over time.

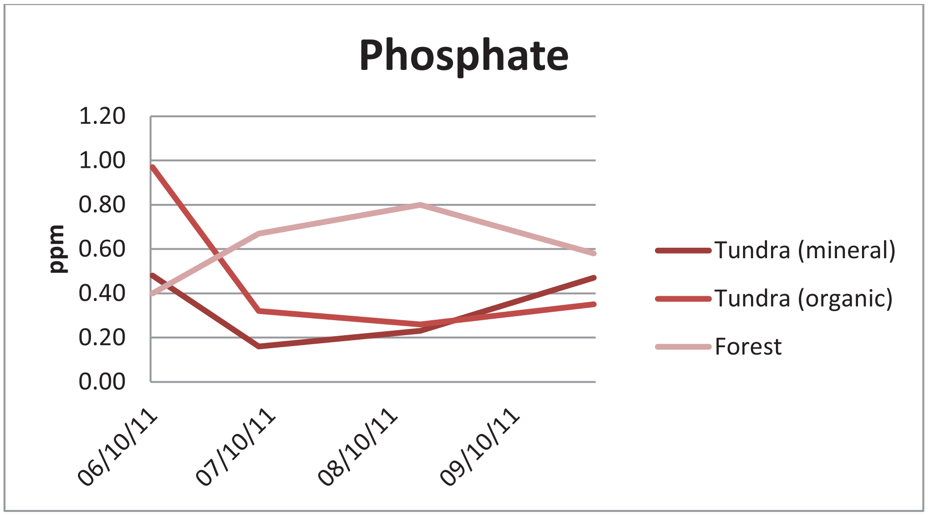

Figure 14 – Change in phosphate concentration over time.

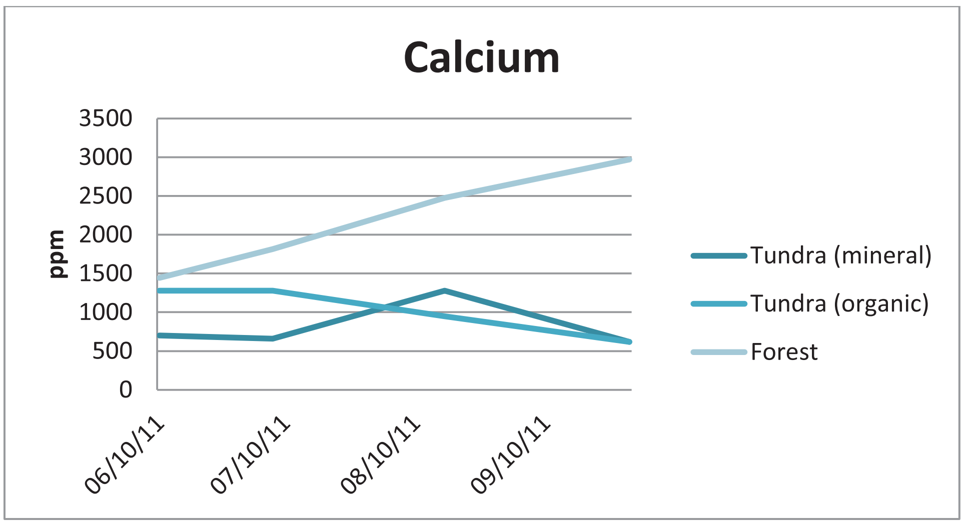

Figure 15 – Change in calcium concentration over time.

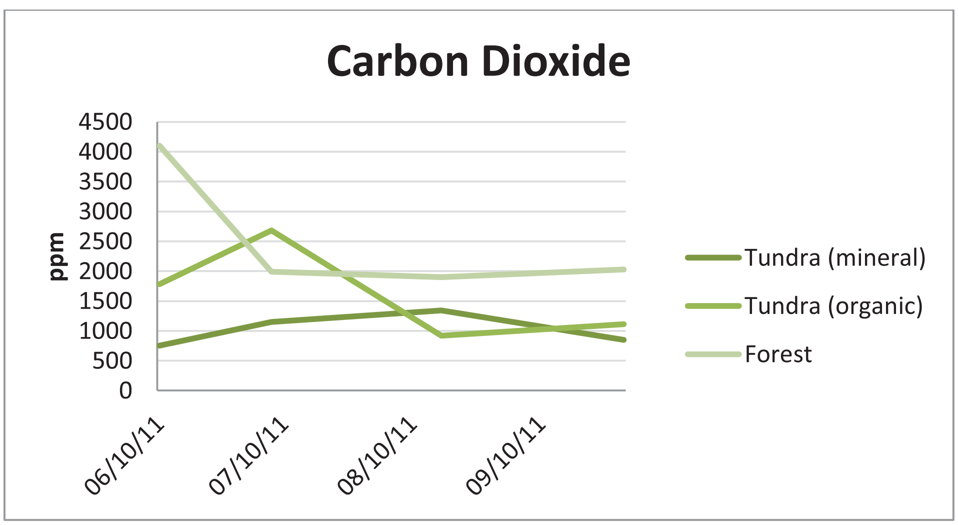

Figure 16 – Change in CO_2 concentration over time.

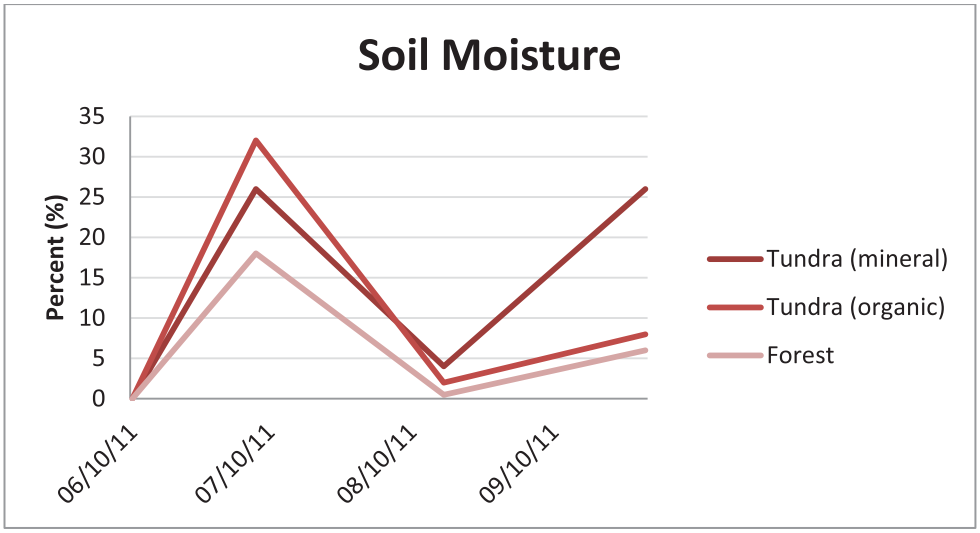

Figure 17 – Change in nitrate concentration over time.

Table 3 – Carbon dioxide production under temperature scenarios in a freeze-thaw chamber.

		Tundra: Organic	Tundra: Mineral	Forest
-0.5° C	Average	939	938	1377
	Standard Deviation	*51*	*114*	*167*
-1.5° C	Average	814	847	1139
	Standard Deviation	*110*	*36*	*232*
-10° C	Average	171	586	499
	Standard Deviation	*97*	*122*	*401*

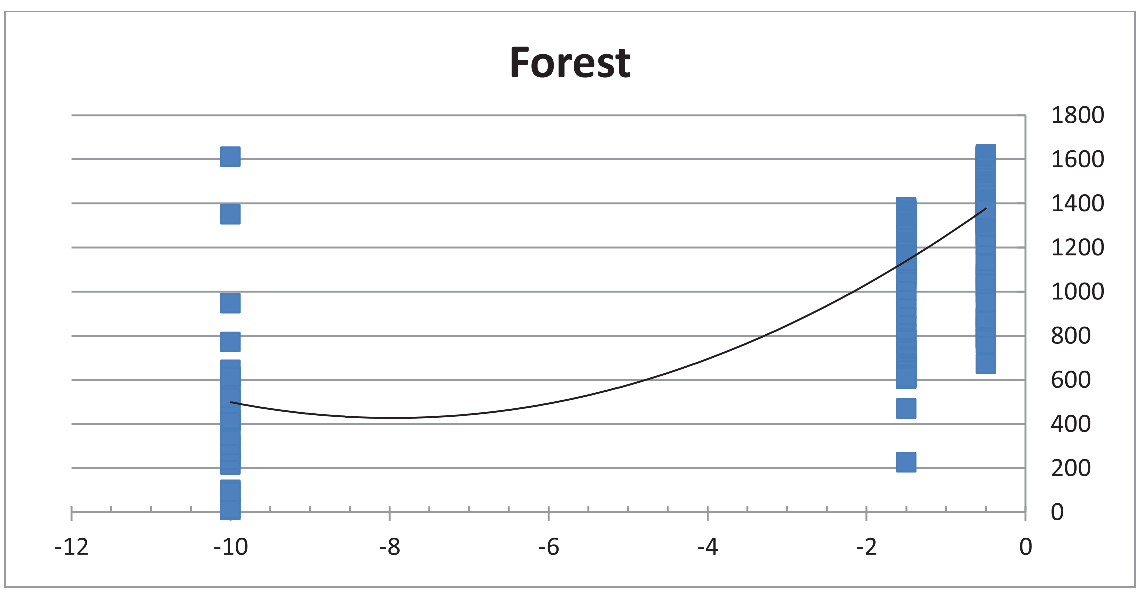

Figure 18 – Carbon dioxide production at various frozen temperatures for a forest soil sample.

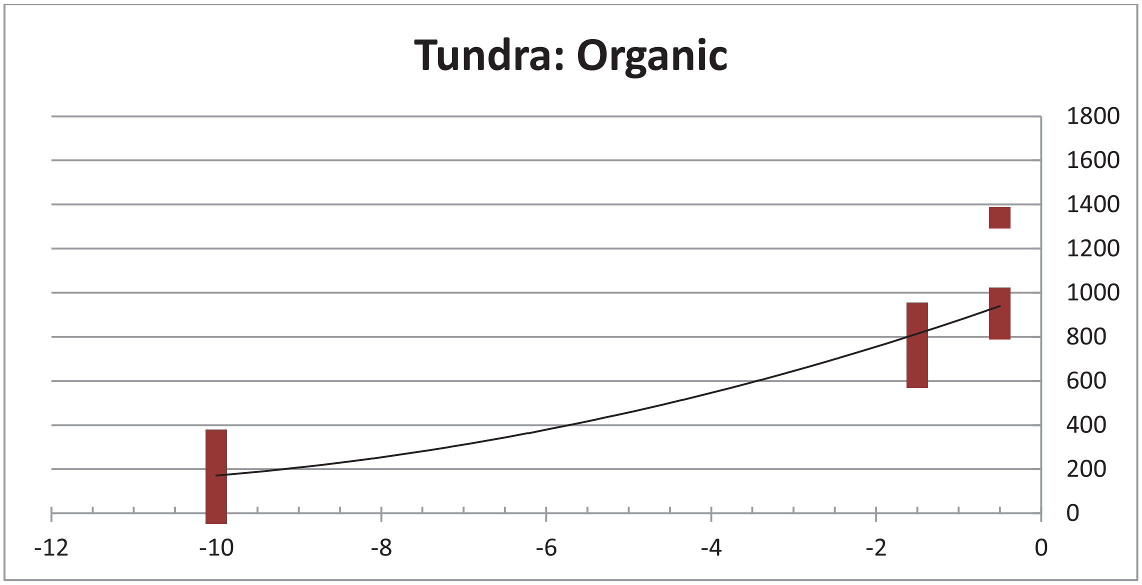

Figure 19 – Carbon dioxide production at various frozen temperatures for a tundra, organic soil sample.

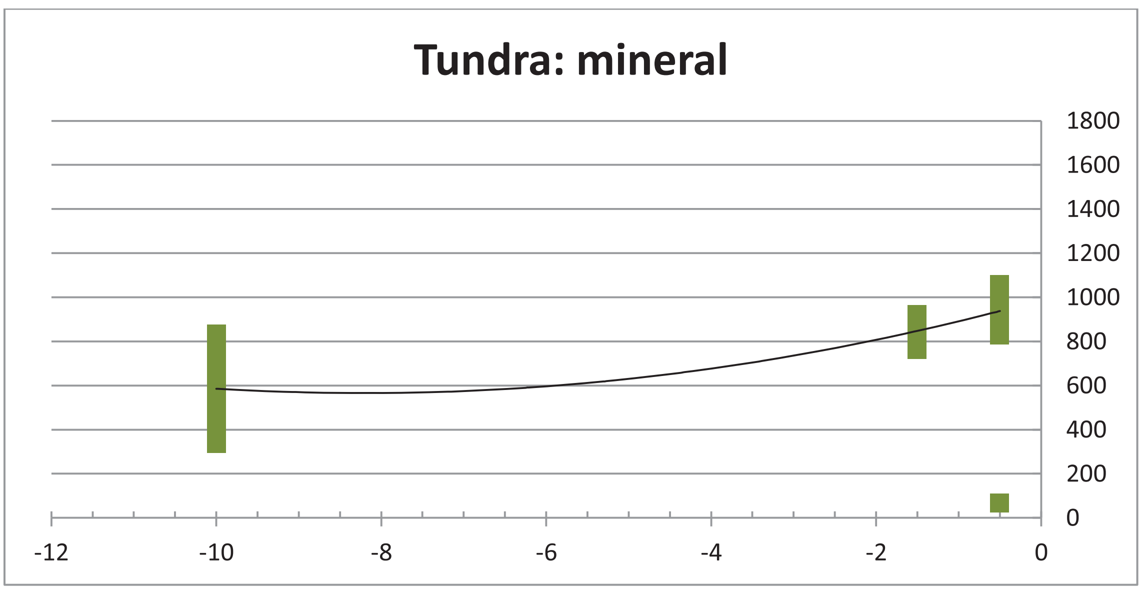

Figure 20 – Carbon dioxide production at various frozen temperatures for a tundra, mineral soil sample.

adding additional CO_2 to atmosphere. However, CO_2 production in the winter months needs to be measured and the interchange of CO_2 at the soil/atmosphere boundary needs to be assessed. In addition, Blanken *et al.* (2009) found that forest vegetation can assimilate more carbon, but tundra vegetation has a longer period of respiration. The interaction between soil, vegetation, and the atmosphere needs to be monitored to better assess its impact on the carbon cycle.

Undergraduate students Maggie Balas and Tessa Arends have completed the soil DNA extractions for all nine samples, and successfully performed PCR on five of those samples. Of the five samples that have been PCR'ed, all have been transformed, producing about 100 colonies for screening for each sample. have Multiple colonies were screened for all 3 soil sites collected on August 17, 2011, and the forest site collected on June 10, 2011. We have confirmed inserts for eight of the various samples that have been screened so far. The next step will be to sequence the samples with the confirmed inserts, and the goal is to sequence 10 transformants for each soil sample collected giving us a sample of all the genes present in the microbial community. From this, we should be able to assess both phylogenetic and metabolic diversity from these microbial communities.

Conclusions

Greater concentrations of nutrients were found at the forested site compared to the alpine tundra. Of nitrogen forms, ammonia was the greatest; nitrite and nitrate levels were insignificant. Each nitrogen form shows decreasing abundance through the late spring to the late fall, which indicates a large percentage of nitrogen is being supplied by the snowpack. With excess nitrogen deposition in the alpine tundra, it has been predicted that in 50 years, curly leaf sedges will dominate wildflowers. Biomass will not increase with increasing nitrogen deposition, but species diversity will increase because species that were previously not getting enough nitrogen now have enough. Realistically, however, the soil pH will become very acidic 3.0 - 3.5 with increased nitrogen deposition. At these pH levels, aluminum is soluble, but iron also reaches an extreme state of solubility. These elements are toxic to plants making it difficult for them to survive. Calcium was the most abundant nutrient and was "very high" on average at the forested site, which may have the ability to buffer or resist change in pH. Potassium was also "high," but other essential nutrients were only slightly detectable or considered "low levels." Low concentrations of nitrogen, phosphorus, and other elements combined with increasingly acidic soils raises the question of whether the tundra ecosystem will be able to support succeeding trees as well as existing wildflowers or tundra grasses.

Carbon dioxide was about 2500 ppm at the forest site soil compared to 1000 ppm at the mineral tundra soil site and 1600 ppm at the organic tundra soil site. Carbon dioxide at the forest site was highest during June ($\approx$ 4000 ppm), and later decreased and plateaued during the summer and fall ($\approx$ 2000 ppm). At the mineral tundra soil site, CO_2 gradually rose from June until August and decreased through September. At the organic tundra soil site, CO_2 rose until July, decreased through August, and gradually increased through September. Colder temperatures result in lower CO_2 production for all soil types. Tundra soils have the potential to release CO_2, but measurements must be obtained through the winter months and compared with the intake and respiration rates of vegetation to understand impacts on the carbon cycle.

Acknowledgements

Several MSCD students participated in this project. Becky Brice assisted with collection of field data and analysis of soil samples. Tessa Arends and Maggie Balas assisted Dr. Joanne Odden with the DNA analysis of soil microbes. Dr. Joanne Odden trained two Metro State undergraduate students on the multi-step procedure. Both students plan to apply for doctoral programs in molecular biology. Performing research of this caliber will be a key part of their applications for graduate school. Sarah Heller, Mike Kozub, Judson Zevin, Sam Rooney, and Cassandra Shenefelt helped interpret and present results of this study. Andy Streeter, Bryce Faliske, and Billy Boucher conducted freeze-thaw laboratory experiments on the soil samples.

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