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ASSESSING SOIL RESPIRATION AS AN INDICATOR OF SOIL MICROBIAL ACTIVITY IN RECLAIMED METAL CONTAMINATED LANDS

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ABSTRACT

Mining, roasting as well as smelting of these elements have caused sulphur dioxide fumigations and metal particulate depositions which have led to various detrimental effects on the overall environmental quality of the Greater Sudbury region. Soil amendment and revegetation within the Greater Sudbury Region were initiated to restore the damage land. Several methods have been used to assess the progress made toward full ecosystem recovery. Soil respiration rates are particularly critical in the assessment of soil health. They reflect the complete extent of biological activity of living microorganisms in the soil. Bacterial and fungal the main biological soil components are functionally important and must be properly determined. The objective of the present study was to measure soil respiration and health in limed and unlimed areas in the Northern Ontario (Canada) region. The results confirm that the liming did maintain an increase in soil pH from extremely acid to slightly acid, even 30 to 40 years after dolostone applications. Fungi were more abundant in limed sites compared to unlimed areas. Soil respiration based on CO_2 rate followed the same trend. Respiration rates for the reference sites were similar to those documented for the limed areas. Summer soil respiration rates were associated ($r = 0.50$) with total fungal abundance in the targeted sites. Overall, the Solvita test of assessing soil respiration and determining microbial mass and soil quality is a reliable and cost effective method.

Keywords: Soil Respiration, Liming, Metal Contamination, Northern Ontario, Soil Quality

1. INTRODUCTION

For decades, the Greater Sudbury Region has thrived economically because of its nickel, copper and other metal and mineral deposits. Mining, roasting as well as smelting of these elements have caused sulphur dioxide fumigations and metal particulate depositions which have led to various detrimental effects on the overall environmental quality of the Greater Sudbury region (Winterhalder, 1996; Amiro and Courtin, 1981). Concentrations of metals such as nickel and copper have been detected in higher concentrations in areas around the smelters compared to distal sites (Amiro Courtin, 1981; Nkongolo *et al*., 2008; 2013; Mehes-Smith *et al*., 2013). The increased metal-binding capacity of the soil coupled with its' lessened degree of organic matter were extremely damaging to the Sudbury landscape.

Soil amendment and revegetation within the Greater Sudbury Region were initiated to restore the damage land. Between 1978 and 2011, 3400 ha of the Greater Sudbury land were limed and over 7 million trees were planted (Lautenbach *et al*., 1995). This has resulted in improved soil fertility and plant growth.

Soil fertility is an important indicator of overall soil quality within an ecosystem (Doran *et al*., 1997). It defines the ability of a soil to function by examining the

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degree of biological activity which is linked to soil aggregation, plant nutrient and energy cycling and general soil tilth (Doran *et al*., 1997). Physical, chemical and biological properties of soils must be well evaluated to determine their fertility and quality. Physical soil properties include bulk density, water content, infiltration rate, aggregate stability, slacking and morphological estimations. Soil chemical properties consist of pH, Electrical Conductivity (EC) and soil nitrate levels. Soil biological properties include soil respiration and the presence of earthworms. Soil respiration rates are particularly critical in the assessment of soil health, primarily in cases where soil amendment strategies have been applied. This is because they indicate the complete extent of biological activity of living microorganisms available in the soil (Doran *et al*., 1997). Consequently, bacteria and fungi, the main soil biological components, are functionally important and must be properly characterized.

Currently, laboratory and field methods for measuring soil respiration prove to be somewhat impractical for a variety of reasons. Many techniques are time consuming, labour intensive, require specialized knowledge and equipment and involve rather extensive calculations (Doran *et al*., 1997). The Solvita Soil Test is a simple, inexpensive and a relatively quick method of measuring soil biological activity and/or respiration (Doran *et al*., 1997). This system offers great promise as a substitute for more refined methods of quantitatively measuring soil respiration in the laboratory and the field (Doran *et al*., 1997). This technology can assess accurately soil CO_2 respiration that provides an adequate estimation of microbial biomass in the soil.

The objective of this component is to measure soil respiration that is an indicator of microbial activities and soil health in limed and unlimed areas in Northern Ontario (Canada).

2. MATERIALS AND METHODS

2.1. Sampling

Six areas in Northern Ontario were selected for the present study. They included three areas consisting of paired limed and unlimed sites (Wahnapitae Hydro-Dam, Kelly Lake and Kingsway) and three reference sites (Capreol, St. Charles and Onaping Falls) (**Fig. 1**). For each area 10 sub-samples were collected and bulked. The liming of these sites was previously performed from 1981 to 1995 through the Sudbury's Regional Land Reclamation Program using dolostone (Lautenbach *et al*., 1995).

2.2. Metal Analysis in Soil

Soil pH was measured in water (Carter and Gregorich, 1993) Total metal analysis was performed as described by (Abedin, *et al*., 2012) or the estimation of total metal concentrations, a 0.5 g soil sample was treated with 10 mL of 10:1 ratio HF: HCl, heated to 110°C for 3.5 h in open 50 mL Teflon™ tube in a programmable digestion block to dry down samples, followed by addition of 7.5 mL of HCl and 7.5 mL of HNO_3 and heating to 110 ^{0}C for another 4 h to dry gently. The samples were then heated to 110°C for 1 h following addition of 0.5 mL of HF, 2 mL of HCl and 10 mL of HNO_3 to reduce sample volume to 8-10 mL. These samples were analyzed by plasma spectrometry.

Bioavailable metals were estimated by extracting 5 g of soil with 20 mL of 0.01M $LiNO_3$ in a 50mL centrifuge tubes in a shaker under ambient lighting conditions for 24 h at 20°C (Abedin *et al*., 2012). The pH ($LiNO_3$) of the suspension was measured prior to centrifugation at 3000 rpm for 20 minutes, with filtration of the supernatant through a 0.45 µm filter into a 20 mL polyethylene tube and made to volume with deionized water. The filtrate was preserved at approximately 3 ^{0}C for analysis by ICP-MS. The quality control program completed in an ISO 17025 accredited facility (Elliot Lake Research Field Station of Laurentian University) included analysis of duplicates, Certified Reference Materials (CRM's), Internal Reference Materials (IRM's), procedural and calibration blanks, with continuous calibration verification and use of internal standards (Sc, Y, Bi) to correct for any mass bias. All concentrations were calculated in mass/mass dry soil basis. The data obtained for all elements of interest in analyzed CRM soil samples were within ± 12% of the certified level.

2.3. Assessment of Fungi Abundance and Soil Respiration

Soil samples were collected in summer and fall 2013 from the LFH layer of each targeted site. Fungi isolation and abundance were conducted following established protocols using the Sabourand Dextrose Agar (SDA) medium (Zimbro *et al*., 2009). Soil respiration was assessed using the Solvita Soil Test. Soil samples were dried in an incubator at 45°C for 24 h prior to the test. Dried soil samples were weighed into a capillary cup after placing a fiber filter in the bottom of the cup. Each beaker, containing 40 g of dried soil, was placed into a glass jar with the use of forceps. Following this, 20 mL of distilled water was placed into the glass jar with special attention to not spill on the soil sample. A CO_2 probe was then inserted into the glass jar using forceps. The lid of the glass jar was then screwed on tightly.

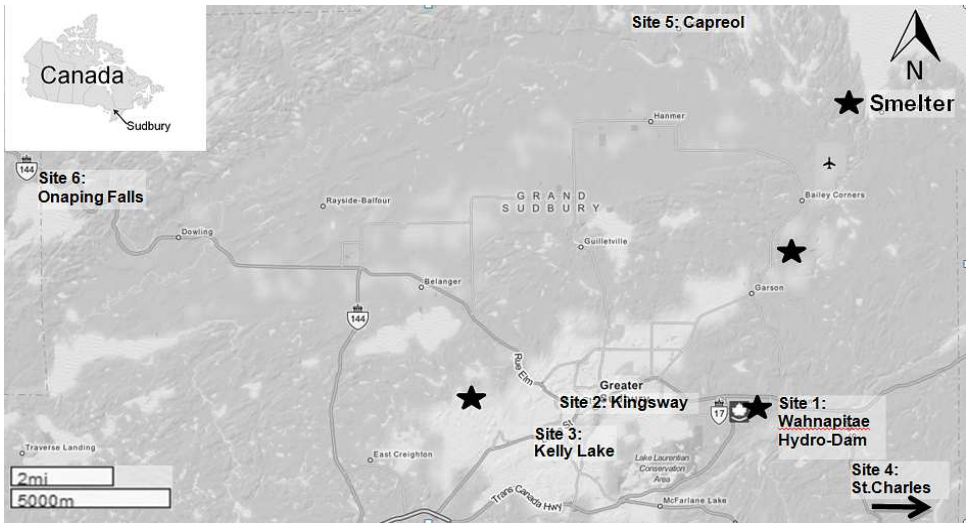

Fig. 1. Location of sampling area from the greater Sudbury region. Site 1: Wahnapitae Hydro-Dam; Site 2: Kingsway; Site 3: Kelly Lake; Site 4: St. Charles; Site 5: Capreol and Site 6: On aping falls. Sites 4, 5 and 6 were used as reference sites. St. Charles is outside the map as a reference site and is about 50-60 km from Sudbury

Jars were opened 24 h later to remove the probes. Probe color was determined by inserting the probe face-up into the Digital Color Reader (DCR) and by selecting the CO_2-Low mode. The DCR reads the color number on the first line as well as the CO_2-C in mg/kg (ppm) on the second line. Interpretations of the DCR provided data were based on Solvita's overall guidelines (Solvita, 2013). Temperature and rainfall were recorded daily for one month prior to sampling.

2.4. Statistical Analysis

The data for the metal levels in soil samples were analyzed using SPSS 7.5™ for Windows, with all the measures being transformed using a $\log_{10}$ to achieve a normal distribution. Variance-ratio test was done with an assumption of data normality in the underlying population distributions of the data. ANOVA, followed by Tukey's HSD multiple comparison analysis, were performed to determine significant differences ($p<0.05$) among the sites. Data from analysis of samples from limed and no limed areas were compared using the Student T-test. There were three replicates for each group (limed and unlimed sites).

Summer and fall soil respiration data for limed, unlimed and reference sites were compared using One-Way ANOVA. Association between respiration data and fungal abundance was determined using Spearman Correlation analysis. CO_2-Cdata from the analysis of summer and fall data were also compared using Spearman Correlation and Student T-test.

3. RESULTS

3.1. Metal Analysis

Soil acidity and metal content data are described in **Table 1 to 3**. The pH in unlimed sites was consistent with that documented for soils on coarser textured soils with coniferous vegetation on the Canadian Shield of at < 4, classified as extreme acid (Spiers *et al*., 2012). The pH in limed sites was significantly higher ranging from 4.12 to 6.75 in the top organic layer (**Table 3**). The results of soil acidity analysis confirmed that liming did maintain an increase in soil pH from extremely acid to slightly acid, even 30 to 40 years after dolostone applications.

Overall, the portion of total metals that was available to biota was very small. In fact, only 1.1% and 0.8% of total Cu and Ni respectively were bioavailable. As expected, the limed samples contained higher levels of total and bioavailable Ca and Mg compared to unlimed sites. There were also higher levels of total and bioavailable P in limed samples compared to unlimed (**Table 1 and 2**). On the other hand, higher contents of bioavailable Al, Co, Fe, Ni, Sr and Zn were observed in unlimed sites compared to limed areas (**Table 2**).

Soil samples were analyzed using Sabouraud Dextrose Agar (SDA) to determine fungi abundance. A

summary of fungi abundance data is presented in **Fig. 2**. There was a higher abundance of fungi in limed samples compared to unlimed soils.

Mean temperature for the 30 days prior to summer sampling was significantly higher (14°C) compared to 4.6°C observed in fall (October-November). Likewise, mean rainfall in the summer (May-June) was 85.8 mm while in fall, 49.3 mm rainfalls were recorded in the 30 days prior to sampling.

Significantly higher soil respiration rates were recorded for limed sites compared to unlimed sites in fall (**Fig. 3**). Respiration rates for the reference sites were very similar to those documented for the limed sites. Although variations were observed between summer and fall soil respiration within some sites, there was no in general significant differences between summer and fall soil data. Summer soil respiration rates was associated with total fungal abundance ($r = 0.50$) in the targeted sites.

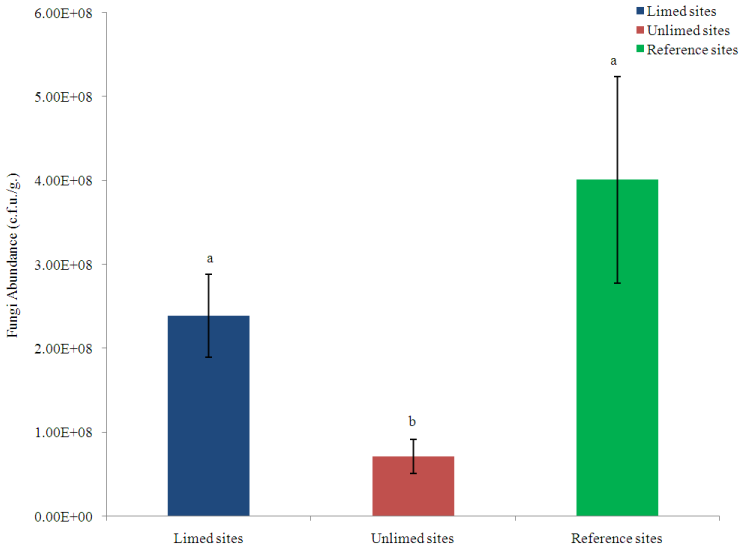

Fig. 2. Mean fungi abundance in limed, unlimed and reference sites in the greater Sudbury region. Limed and unlimed sites include: Wahnapitae Hydro-Dam, Kingsway and Kelly Lake. Reference sites include: St. Charles, on aping falls and Capreol

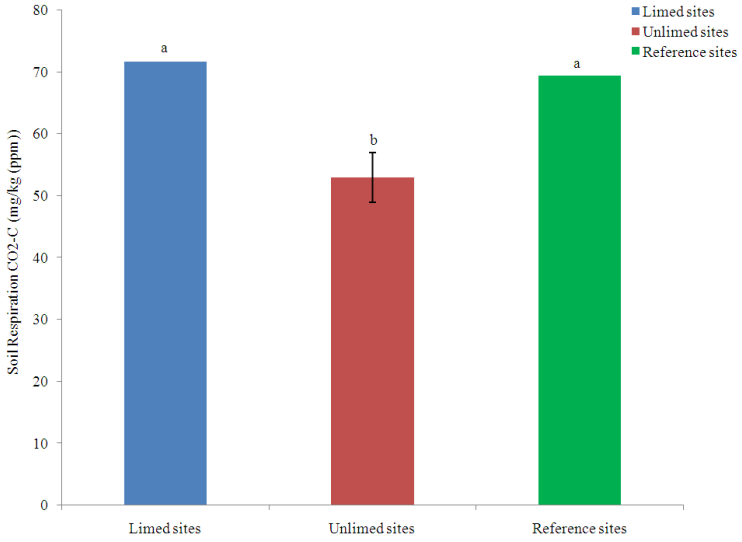

Fig. 3. Fall soil respiration rates for limed, unlimed and reference sites in the greater Sudbury region. Limed and unlimed sites include: Wahnapitae Hydro-Dam, Kingsway and Kelly Lake. Reference sites include: St. Charles, on aping falls and Capreol

Table 1. Total concentrations of nutrients and metals in top layer (0 –5 cm) of soil from the Sudbury region sites, concentrations are in mg kg^{-1}, dry weight

Elements															
Sampling sites	Al*	As	Ca*	Cd	Co	Cu	Fe*	K	P*	Pb	Mg*	Mn	Ni	Sr	Zn
Unlimed sites	24000	45.53	5010	1.12	56.17	1255	34333	8846	738	141.0	2176	216.0	1363	76.47	76.63
	±4303	±20.20	±298	±0.56	±10.2	±359	±1655	±141	±115	±44.4	±458	±16.7	±392	±10.60	±18.50
Limed sites	17733	31.96	14526	1.71	60.17	1304	24066	7210	2432	135.0	3276	261.0	1552	75.47	83.10
	±2677	±29.80	±5153	±0.84	±22.2	±491	±7716	±277	±1578	±61.4	±213	±36.8	±642	±3.61	±24.10

Table 2. Bio-available concentrations of nutrients and metals in top layer (0-5 cm) of soil from the Sudbury region sites, concentrations are in mg kg^{-1}, dry weight

Elements															
Sampling sites	Al*	As	Ca*	Cd	Co*	Cu	Fe*	K	P*	Pb	Mg*	Mn	Ni*	Sr*	Zn*
Unlimed sites	44.23	< DL	57.11	< DL	0.27	10.59	71.6	87.92	2.67	0.16	26.57	7.05	6.75	0.25	1.09
	±21.5		±24.3		±0.09	±5.07	±40.45	±48.61	±1.36	±0.16	±12.20	±3.23	±3.09	±0.13	±0.58
Limed sites	26.8	0.1	278.0	< DL	0.09	6.19	31.08	80.13	6.64	0.2	165.80	3.53	3.03	0.13	0.32
	±11.2	±0.10	±102.0		±0.09	±1.38	±13.23	±39.90	±2.45	±0.15	±84.10	±3.01	±2.21	±0.07	±0.32

Table 3. Total fungi abundance and soil respiration for limed, unlimed and reference sites in the Greater Sudbury Region

			CO_2-C (mg/kg (ppm))	
Site	pH H_2O	Total Fungi Abundance (c.f.u./g)	Summer	Fall
Kelly Lake limed	6.41	8.22×10^{8}	71	71
Kelly Lake unlimed	3.75	3.83×10^{8}	50	50
Kingsway limed	4.67	3.50×10^{8}	65	71
Kingsway unlimed	3.87	2.68×10^{8}	56	69
Wahnapitae Hydro-Dam limed	6.75	1.50×10^{8}	72	71
Wahnapitae Hydro-Dam unlimed	3.82	2.41×10^{7}	65	38
Capreol (reference site)	3.92	3.65×10^{9}	81	70
St. Charles (reference site)	3.5	7.44×10^{8}	-	68
On aping falls (reference site)	4.84	9.12×10^{8}	68	69

4. DISCUSSION

4.1. Liming, Soil Metal Content and Fungi Abundance

Metal analysis revealed significantly lower concentrations of total as and bioavailable Al, Fe and Sr in limed sites compared to unlimed sites. These findings are consistent with (Winterhalder, 1996) reports concerning the occurrence of toxic levels of Al in the soil when soil pH decreases. Furthermore, the high levels of bioavailable Ca and Mg in the limed sites reflects the addition of the dolomitic and calcitic lime having been applied 30-40 years ago.

Smith and Doran (1996) described that fungi growth is generally observed in the soil with a pH between 2 and 7 with an optimal of 5. These pH conditions are similar to the levels of soil acidity in the sampling sites. However, some fungi have a better growth rate in alkaline soils compared to acidic soils (Kendrick, 2000). In fact, soil pH affects many processes. For example, organic matter mineralization is slowed down or stopped at highly acidic or alkaline pH levels, which is attributed to poor microbial activity linked to bacteria (Smith and Doran, 1996). At low pH levels, nitrification and nitrogen fixation will also be inhibited (Smith and Doran, 1996). Furthermore, the solubility of heavy metals as well as the mobility and degradation of herbicides and insecticides are pH dependant (Smith and Doran, 1996). Soil pH affects cation availability which in turn influences aggregate stability because multivalent cations, like calcium ions, act as bridges between organic colloids and clays (Smith and Doran, 1996). All these factors play a key role in fungi diversity and abundance in a specific site or region.

In the present study, accumulation of bioavailable Al, Fe, Mg, Ni, Sr, Zn and some nutrients were higher in unlimed sites compared to limed areas. But the levels of these bioavailable elements are too small to have a significant impairment on fungi growth and activities. Higher fungi abundance was observed in the limed sites compared to the unlimed areas based

on growth on the SDA medium. These results support (Weyman-Kaczmarkowa and Pedziwilk, 2000) findings documenting the effects of liming ($Ca(OH)_2$) on the numbers of fungal Colony-Forming Units (CFU). They recorded elevated fungal CFU numbers when soil pH reached 4.5 (Weyman-Kaczmarkowa and Pedziwilk, 2000).

Metal effects in fungi can also vary a great deal between organisms, strains, the stage of growth and different vegetative and reproductive forms of the same organism (Gadd and Mowll, 1985; Sabie and Gadd, 1990). This was observed in the present study since some fungi species were found in less metal contaminated sites but were not observed in highly metal contaminated sites.

4.2. Soil Respiration and Weather Conditions

The major factors influencing soil respiration include soil moisture affecting microbial activity bacterial decomposition as a source of CO_2 efflux, soil diffusion kinetics and the existing correlation of CO_2 production with the rate of diffusion through the soil. The mean rates of soil respiration (g C m^{-2} yr^{-1}) vary among global biomes. However, climate change has caused a substantial increase in terrestrial CO_2 flux to the atmosphere particularly in temperate and tropical biomes compared to high latitude biomes (Anderson, 2011). Laboratory studies examining the effects of global warming on soil respiration have also indicated that the microbial respiration responses to warming as assessed by Q_{10} measurements can fluctuate significantly in soils from different latitudes (Bekku *et al*., 2003). Variations in temperature and precipitation patterns brought on by climate change will ultimately produce major boundary shifts in the biomes, particularly in transitions between grasslands and forests (Anderson, 2011). Consequently, these transitions will affect fungi communities in many regions and ultimately, soil respiration rates occurring in these environments.

Precipitation patterns coupled with soil moisture significantly affects soil microbial communities and their respiratory behaviours (Anderson, 2011). Some studies have examined the relevance of this association to better understand the possible effects of climate change, among other natural phenomenon's, on soil respiration and soil health as well as to fully comprehend the extent of the role of microbial communities in soil respiration. For instance, (Cook and Orchard, 2008) have examined the relationships between soil respiration and soil moisture in recent decades.

It has been established that microbial populations have adapted to various unfavourable environmental conditions around the world such as stresses from repeated cycles of precipitation and drying (Anderson, 2011). Microbial activity will be reduced or will cease below critical levels of soil moisture. This stimulates the formation of desiccation-resistant dormant stages such as spores or cysts in some microbial species (Anderson, 2011). Soil fungi have the ability to produce hyphal strands to bridge across air-filled pores and they are active at a water potential as low as -15 MPa (Swift *et al*., 1979). In comparison, bacteria are inactive below -1.0 to -1.5 MPa (Swift *et al*., 1979). This clearly demonstrates some of the key differences between fungal and bacterial communities inhabiting the soil. Other microorganisms such as protests are also known to have developed this adaptability to unfavourable climatic conditions. For example, one of the most common protests' in the soil, the naked amoebae, is known to encyst at low levels of soil moisture but will excyst when satisfactory levels of soil moisture are present (Anderson, 2011).

Soil respiration on a global scale (g C m^{-2} yr^{-1}) is linearly related to mean annual precipitation (mm), with a slope of ~0.5 (Raich and Schlesinger, 1992). Additionally, (Risch and Frank, 2006) have been able to identify a positive correlation between soil respiration and seasonal moisture ($r = 0.65$, $p<0.05$) in a temperate grassland in North America. Overall seasonal moisture levels will thus dictate soil respiration rates for particular regions. Consequently, variations in summer and fall moisture levels will cause variations in microbial structure and soil respiration outputs for these same seasons. In fact, the correlation between soil respiration in the summer and fall in the present study was only $r = 0.21$. Key factors to this seasonal variation in soil respiration include seasonal variations in mean temperature (°C), total rain (mm), total snow (cm) and total precipitation (mm). Mean monthly temperatures from May 11th to June 11th 2012-2013 were higher than the mean monthly temperatures from October 9th to November 9th 2013 during the present study. Total rainfall (mm) followed the same trend. It is expected that bacteria and fungi dynamics in the soil will be greatly modified during seasonal changes. Additionally, the complexity of the relationship between soil respiration and soil moisture is partially dictated on soil porosity, soil aeration levels in relation to soil water content as well as the differential physiological responses of the microbial community (Luo and Zou, 2006). Field observations have also revealed a curvilinear relation between soil CO_2 efflux and soil moisture with CO_2 efflux being mainly limited at the lowest and highest moisture levels and optimal in the optimum soil moisture ranges (Bowden *et al*., 1998; Xu *et al*., 2004). This is

also consistent with previous experimental reports (Ino and Monsi, 1969).

With increasing attention towards the global warming issue in recent years, the significance of the temperature dependence of soil microorganisms has become crucially important since these microorganisms are the main group producing CO_2 via the decomposition of organic material in the soil (Pietikåinen *et al*., 2005).At present, little is known concerning the temperature dependence of different groups of soil microorganisms such as bacteria and fungi. However, it has been reported that soil respiration is strongly linked to temperature. Instantaneous soil respiration rates will often increase when temperature reaches up to around 40°C or more; this phenomenon occurring even in soils from cold climates (Pietikåinen *et al*., 2005). Interestingly, this is not due to the growth of thermophilic organisms which would occur at higher temperatures since the same effect is observed even in short incubation times which would not allow for substantial thermophilic growth (Pietikåinen *et al*., 2005). Contrarily, soil bacterial and fungal growth rates in cold climates typically have optimum temperatures below 30°C; this is coupled by the fact that activity values decrease at higher temperatures (Pietikåinen *et al*., 2005). In the present study, samples were analyzed in summer and fall. Mean temperatures a month before soil sampling was 14°C and 4.6°C for the summer and fall, respectively. Because these temperatures were warm but below 30°C, bacterial and fungal growth rates were considered to have been optimal in the summer. In fact, total fungal abundance was strongly correlated with summer soil respiration rates ($r = 0.50$). But to clearly establish the association between soil microbial abundance and soil respiration in Northern Ontario, several measurements of respiration over few years will be required.

4.3. Soil Respiration and Liming

In general, summer soil respiration rates in the reference sites were similar or higher than those documented for the limed sites. Although soil respiration measurements do not differentiate between bacterial and fungal metabolic activity, we can conclude that via the addition of lime, fungi have returned to the ecosystem as prime decomposers and that they are actively participating in soil respiration.

Our results are consistent with Rajapaksha *et al*., (2004) who reported that the addition of lime in soil resulted in a short increase in respiration rate in the contaminated soils (Rajapaksha *et al*., 2004). Therefore, this soil amendment promotes optimal soil respiration outputs.

5. CONCLUSION

The present study confirms that the portion of total soil metals that is available to biota is very small. The dolomitic liming applied more than 30 years ago still has a significant effect on soil acidity. Fungi were more abundant in limed sites compared to unlimed areas. Likewise, soil respiration based on CO_2 rate followed the same trend. Surprisingly, no significant differences were found between summer and fall soil respiration rates. Limitation of the study, implication and future studies

The fungi abundance was analyzed using the SDA medium. Therefore only a portion of all the fungi available in soil were determined. This study showed the significant association between fungi abundance and soil respiration in the present study suggests that fungi activities are important in soil respiration. Soil microbial abundance based on molecular analysis would provide an accurate estimate of soil microbial diversity and abundance.

6. ACKNOWLEDGEMENTS

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