Assessing soil metal concentrations, microbial biomasses and enzymatic activities in aerial limed lands impacted by mining pollution in the City of Greater Sudbury

By

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Abstract

Mining pollution in the City of Greater Sudbury led to acid rain and metal contamination of soils, which ultimately caused massive environmental and ecological damage. Soil liming, in addition to tree planting and fertilizing, have been the techniques used to restore these damaged ecosystems. The objective of this study was to assess the effects of aerial liming on pH, and metal contamination microbial biomass and abundance, enzymatic activities. Soil pH was higher in limed sites compared to unlimed at Baby Lake and Wahnapitae sites but not at HWY 80 N site. Organic matter was higher in limed areas compared to reference site only at Baby Lake site. Metal concentrations of iron (Fe) and arsenic (As) were significantly higher at the unlimed sites. Enzyme assays and Phospholipid fatty acid (PLFA) analysis were used to assess three limed soil sites and their adjacent unlimed soils. β-N-acetylglucosaminidase (BG), aryl sulfatase (AS), and glycine aminopeptidase (GAP) activity levels were significantly increased in the limed soils compared to the unlimed soils. Total microbial biomasses, gram negative bacterial, fungal, and eukaryotic biomasses were all increased in the limed soils compared to the unlimed soils. Microbial biomass ratios of gram negative/gram positive, saturated/unsaturated, and mono/poly microorganisms were all significantly increased at the unlimed sites compared to the limed sites, indicating that the unlimed sites are still undergoing environmental stresses.

Keywords: City of Greater Sudbury; Land Reclamation; Liming; Microbial Enzyme Activity; PLFA Analysis; Soil Microbial Diversity and Abundance; Heavy Metal Contamination; Soil pH.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

1.1 Soil Ecosystem

Ecosystems and their environment are constantly changing because of human activities and natural causes. These changes can be beneficial or detrimental to the flora and fauna in that ecosystem. The productivity of plants and soil microbes are associated with overall health of a given ecosystem (Thomsen et al., 2012). Environmental changes such as an increase or decrease in heavy metals, water, air, sunlight, and pH changes can be beneficial to certain organisms and detrimental to others, sometimes fatally (Turkyilmaz et al., 2018).

Soil composition and environmental factors of an ecosystem play a large role in the ability of plants to absorb the crucial nutrients and water necessary for their survival, and if they cannot get these nutrients, some species may not survive in that ecosystem. There are different types of soil with different characteristics – certain soil types retains more or less water, are harder or softer (Passioura, 2002), have varying pH, and are composed of different amounts of important molecules; these all affect various plant species differently (Turkyilmaz et al., 2018).

An increasingly important environmental factor for ecosystems is global warming, due to the increase in ambient temperature, which in turn increases enzymatic activities and causes change in plant communities and their survival (Kardol et al., 2010).

In addition to environmental and anthropogenic factors, relationships between organisms in an ecosystem are important since they affect the health of an ecosystem as well as the growth of plants and other organisms. Some beneficial relationships between plants and microorganisms include symbiotic relationships like mycorrhizal fungi and nitrogen-fixing *Rhizobium* bacteria, which help supply nutrients to their hosts, as well as plant-growth-promoting bacteria that provide carbon compounds to their plant hosts (Bloem et al., 2006).

1.2 Remediation Techniques for improving Plant Development

Ecosystems that have been destroyed by heavy metal contamination, anthropogenic activities, and natural disasters have been restored by a variety of means, such as soil remediation (Dobson, 1997; Khalid et al., 2017; Narendrula-Kotha and Nkongolo, 2017b). Some heavy metal contamination can be naturally occurring from the weathering of metal-rich bedrock, however the majority of heavy metal contamination comes from human activities such as waste disposal, fertilizers, and the by-products of mining (Khalid et al., 2017). Ecological destruction can occasionally result in acidifying the soil, leading to plant damage and death other time, which is what happened in the City of Greater Sudbury (CGS). Acidic soil can damage plants by causing a decrease in plant development and species diversity and sometimes plant death (Kasemodel et al., 2019). Many restoration techniques are utilized to repair ecosystem damage, with the most efficient and cost-effective methods for heavy metal remediation being chemical and biological techniques (Khalid et al., 2017).

1.2.1 Chemical Remediation

Chemical remediation techniques, including precipitation, ion exchange, chelation, leaching, and chemical fixation/soil amendment are used to help improve soil quality in ecosystems contaminated with large amounts of heavy metals and inorganic compounds (Sharma et al., 2018).

Soil amendments, such as biochar, are used to immobilize heavy metals in contaminated soil. Biochar is a porous charcoal product that is created during pyrolysis of organic materials such as human and animal fecal matter. When it is added to heavy metal contaminated soil, it increases pH causing metal ion precipitation (Khalid et al., 2017). Due to the carbon-rich nature of biochar, it is also beneficial to plant health and growth as carbon is important in many biological mechanisms. Amending contaminated soil with biochar provides a rich source of carbon for plant growth and development causing an increase in their biomass, while also helping to suppress the toxic effects of heavy metal contamination on plant species (Kumar et al., 2020).

1.2.2 Biological Remediation

Biological remediation, or bioremediation is more environmentally friendly and cost-effective than physical and chemical remediation techniques are for remediating heavy metal contaminated soil (Li et al., 2019). Bioremediation is when biological processes in plants and microorganisms such as precipitation, biosorption and the conversion of heavy metals to enzymes are utilized to alter soil pH, absorb contaminants, and generally lessen the toxicity of heavy metals in the environment (Ojuederie and Babalola, 2017). Organisms used for bioremediation are chosen based on their ability to successfully remove or reduce the harmful contaminant from the environment (Ojuederie and Babalola, 2017).

Bioremediation also includes a process known as phytoremediation, where plants are used to reduce the toxicity and concentration of contaminants in the environment (Li et al., 2019). For example, the sunflower (*Helianthus annuus*) is a good phytoremediator because of its ability to take up heavy metals from its environment, including Pb, Zn, and Cd (Ojuederie and Babalola, 2017). There are some phytoremediators, such as those in the *Brassica* genus like *B. napus, B.*

juncea, and *B. rapa*, that are hyperaccumulator plants, meaning they can absorb large quantities of heavy metals with little to no adverse effects (Ebbs and Kochain, 1997).

Microbial bioremediation is another key part of bioremediation, where microorganisms that have the ability to precipitate, sequester, or reduce heavy metal ions are utilized (Ojuederie and Babalola, 2017). Many bacterial, fungal, yeast, and algal species are commonly used in bioremediation, with the most environmentally friendly options being species native to the contaminated environment. Kang *et al.* (2016) showed that using a mixture of microbial species is more effective than using only one species for bioremediation. A mixture of four bacterial species (*Viridibacillus arenosi, Sporosarcina soli,* and two *Enterobacter cloacae* strains) were found to be more resistant to heavy metal toxicity and more efficient in heavy metal remediation than when each species was used separately (Kang et al., 2016).

1.3 Soil Microbial Communities

Diversity of species within a soil microbial community is essential for a healthy ecosystem, because of their range of enzymes and functions, including carbon, nitrogen, and phosphorous cycling, which are necessary for the production of amino acids and proteins (Aislabie and Deslippe, 2013). Some microorganisms help to provide plants with necessary nutrients through symbiotic interactions when environmental factors limit plant growth and productivity (Van Der Heijden et al., 2008). For instance, symbiotic relationships between plants and nitrogen-fixing bacteria help provide the plant with nitrogen, because although plants cannot obtain nitrogen from the atmosphere, N-fixing bacteria can, allowing the plant to survive even when nitrogen in the soil is scarce (Van Der Heijden et al., 2008).

Many factors can affect soil microorganisms positively and negatively, which can then affect plants and other organisms in the ecosystem. For example, Kandeler et al. (1996) found that soil contaminated with Cu, Ni, and Cd lead to a decrease in phosphatase activity, which is crucial to the phosphorous cycle and for the bioavailability of phosphorous for plant uptake. Some of the factors that affect soil enzyme activities is warmer weather and plant abundance, since these cause an increase in chitin in the soil, which then causes an increase in the enzymatic activity of β-N-acetylglucosaminidase (Stark et al., 2014).

1.4 Effects of Soil Liming on the Microbial Community and Terrestrial Ecosystem

Soil liming is the addition of a calcium-based carbonate rock (limestone) as either calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$). Lime is added to acidic soil to increase the pH, and is often used in remediation programs because it is cost-effective, relatively easy to implement, and effective at improving and maintaining soil health (Khalid et al., 2017). By increasing soil pH with the addition of lime, an increase in microbial population and diversity can be observed, which in turn increases enzyme activity, leading to a general increase in carbon and nitrogen availability for plants and other organisms to use (Ekenler and Tabatabai, 2003a). Additionally, lime can act as a fertilizer, increasing N-mineralization and nitrification, which favors microbial growth (Fuentes et al., 2006). For example, Narendrula-Kotha and Nkongolo (2017) found that in the GCS, many enzymatic activities were increased in areas previously limed compared to adjacent unlimed areas. The increase in microbial enzymatic activities in the limed areas indicate an improvement in soil health, decomposition, and nutrient cycling compared to the unlimed areas (Narendrula-Kotha and Nkongolo, 2017b). Overall, soil pH is important for solubility and movement of minerals, soil respiration, microbial activity, biodegradation of toxic materials, nitrification and denitrification, and the mineralization of organic matter (Neina, 2019), and the addition of lime aids in remediating acidic soil.

1.5 Effects of Heavy Metals on the Microbial Community and Terrestrial Ecosystem

Soils contain varying levels of metals due to natural activities such as the weathering of rocks and minerals, or as a result of anthropogenic activities such as mining, smelting, wastewater irrigation, sewage, and agriculture practices (Li et al., 2019; Liu et al., 2018). Over 5 million sites globally have been reported to be contaminated with metals and/or metalloids (He et al., 2015). Metal contamination of soil and water is an important environmental problem across the globe, affecting agriculture, terrestrial and aquatic ecosystems, and human health (He et al., 2015; Narendrula-Kotha et al., 2020).

Regular monitoring of heavy metal concentrations in soils should be performed in any areas known or suspected to be contaminated due to the persistence and long half-life of many metals, as well as the potential for bioaccumulation (Kasemodel et al., 2019; Sherameti and Varma, 2015). There are a number of metals such as Fe, Cu, Zn, and Mn are essential for metabolic functions in microorganisms, plants, animals, and humans, but at high concentrations, even the essential metals can become toxic (Kamal et al., 2010; Narendrula-Kotha and Nkongolo, 2017b; Sherameti and Varma, 2015). The toxicity of these metals depends on the metal itself, the organism in question, and on the bioavailable concentration of that metal for the specific organism (Kamal et al., 2010; Sherameti and Varma, 2015). Metal toxicity can occur by affecting different biological functions and processes, including the binding of macromolecules (DNA, RNA and proteins), the formation of radicals, and the disruption of enzymatic activity (Kamal et al., 2010; Sherameti and Varma, 2015).

1.6 Microbial Community Assessment Methods

Microorganisms are extremely important to an ecosystems function and survival, and soil fertility depends upon the diversity of microbial species and their abundance. There are a variety of techniques used to examine soil microbial community structures (SMCS), diversity, and abundance including various gel electrophoresis methods, DNA fingerprinting, length heterogeneity PCR, and commonly phospholipid fatty acid (PLFA) analysis (Nkongolo and Narendrula-Kotha, 2020).

1.6.1 Phospholipid Fatty Acid (PLFA) Analysis

Phospholipid fatty acid (PLFA) analysis is commonly used for assessing microbial communities, particularly for estimating total microbial biomass and broad changes in soil microbial composition (Buyer and Sasser, 2012). This technique relies on identifying primary lipids (phospholipids) that make up the cellular membranes of microorganisms in order to broadly categorize the microbial community (Hinojosa et al., 2010). PLFA analysis uses organic solvents to extract lipids from a soil sample before separating the phospholipids using solid phase extraction techniques (Findlay, 1996). Once extracted, the phospholipids are then converted into fatty acid methyl esters (FAME's), which can be used to determine the types and quantities of bacteria (gram negative, gram positive, and anaerobes), fungi (Am fungi and other fungi), and other eukaryotes in the soil sample (Buyer and Sasser, 2012; Hinojosa et al., 2010).

Certain types of fatty acids are known to be markers for different groups of microorganisms. For example, lipopolysaccharides, specifically hydroxy-substituted fatty acids (OHFAs), make up a large portion of the outer membrane of gram negative bacteria, which makes them useful as a biomarker of gram negative bacteria in PLFA analysis (Hinojosa et al., 2010). Fungi are also known to have more long-chain polyunsaturated fatty acids in their cell membrane composition compared to bacteria (Hinojosa et al., 2010). Fatty acids 15:0, i15:0, a15:0, c17:0, i17:0, a17:0 and c19:0 are commonly used as biomarkers for bacteria, while 16:1ω5, 18:1ω7, 18:1ω9c, and 18:2ω6c are common biomarkers for fungi, and 10Me16:0, 10Me17:0, and 10Me18:0 are commonly used biomarkers for actinomycetes.

1.7 Rationale

Physical, chemical, and biological properties of soil are indicators of soil quality, while soil fertility is determined by the biological activity. Soil is a natural habitat for many different microorganisms, all of which require favourable conditions to function optimally. When there is an imbalance in soil microorganisms, a nutrient deficiency, and unideal changes in soil physiochemistry, the soil quality and fertility is reduced along with the overall health of the ecosystem.

To date there have been many studies focused on soil toxicity, soil acidification, plant metal accumulation, physical and chemical remediation techniques, landscape degradation, and terrestrial ecosystem composition in Northern Ontario. However, there is still a lack of knowledge on microbial community composition and soil health. The effects of metal contamination and liming on microbial biomass, relative abundance, and microbial diversity in the CGS also need to be further investigated. Moreover, most of the previous studies investigated the effects of manual liming on land recovery. Analysis of aerial-limed lands is limited.

1.8 Objectives

The main objective of this study was to assess the long-term effects of areal liming and heavy metal contamination on soil ecosystems in the CGS. The specific objectives were:

1) To determine the effects of long-term metal contamination and soil aerial liming on microbial biomass based PLFA analysis.
2) To assess the long-term effects of aerial liming and metal contamination on soil enzymatic activities.

We hypothesize that metal contamination will decrease microbial biomass and enzymatic activity. We also hypothesize that an increase in microbial biomass, relative and enzyme activity will be observed in the aerial limed soils.

Chapter 2: Metal analysis in aerial limed and unlimed lands in the City of Greater Sudbury

2.1 Introduction

Mining activities in the CGS in Northern Ontario, Canada started well over a century ago, and caused serious damages to the surrounding terrestrial and aquatic ecosystems. Ore smelting led to the release of large amounts of SO_2 (sulfur dioxide) emissions and metal particulates (Cu, Zn, Fe, Ni) into the air (Narendrula et al., 2012; Narendrula-Kotha and Nkongolo, 2017b; Winterhalder, 1996). These emissions damaged ecosystems in the CGS by reducing plant growth and population diversity of plants, animals, and microorganisms (Narendrula et al., 2012; Winterhalder, 1996). To help rectify this, remediation projects that focused on reducing industrial emissions and restoring the affected lands and waterways were initiated. In 1978, Sudbury developed the Regreening Program which focused on remediating these affected areas. To decrease the acidity caused by the SO_2 emissions, dolomitic limestone has been applied at a rate of 10 tons per hectare (Winterhalder, 1996). Following liming, fertilizer and grass seed were spread, and over 10 million trees and shrubs have been planted to help reclaim the land (Winterhalder, 1996; Narendrula et al., 2012; Nkongolo et al. 2016).

Several studies have examined the effects of adding lime to metal contaminated soils. The addition of lime lowers acidity, reduces soil erosion and metal mobility, and helps to increase organic matter (Narendrula et al., 2012; Narendrula and Nkongolo, 2015). Previous studies conducted in the CGS found that soil liming increased plant species richness and abundance, overall plant ecosystem health, and has led to improved soil fertility in limed areas compared to unlimed areas (Narendrula-Kotha and Nkongolo, 2017b; Nkongolo et al., 2013, 2016).

Organic carbon (OC) is stored in the soil organic matter (SOM), and enters the soil through decomposition of plants, animals, microorganisms, and soil biota. Many factors can affect OC content (Also known as percent organic carbon, or % OC) such as temperature, time, soil type (rich in clay, sand, etc.) and precipitation (*Soil Quality Indicators*, 2009). The addition of lime increases microbial activity, organic matter decomposition, and therefore increases % OC (*Soil Quality Indicators*, 2009). However, low % OC is known to reduce microbial biomass, activity, and diversity, as well as a reduction in nutrient mineralization due to a lack of energy sources (*Soil Quality Indicators*, 2009).

There are many studies that focus on soil pH and soil liming, and how it affects microbial communities and enzyme activities, but there are fewer studies that examine heavy metal contamination and the impacts on soil microbial communities and ecosystem health, with and without the addition of dolomitic lime.

2.2 Methods and Materials

2.2.1 Soil Sampling

Soil samples were collected from three sites around the CGS, and each site was composed of a reclaimed (limed) site and an adjacent undisturbed (unlimed) area. The selected sites were aerial limed by the Sudbury Regreening Program using dolomitic limes (10 t/ha). They include Wahnapitae, Baby Lake, and Highway 80 North, that were limed in 2009, 2018, and 2004 respectively. The coordinates for these sites are HWY 18 N: 460 33’17’’N 800 58’50” W; Baby Lake: 460 28’6”N 800 51’32” W; Wahnapitae: 460 28’59”N 800 48’18”W. See Figure 1 for a detailed map depicting the specific locations of each site. At each limed and unlimed areas, soil samples were collected at three transets each representing a replicate. Each replicate consisted of 10 subsamples from the organic (top) layer (0 – 5 cm). These soils were sieved to remove plant and other debris materials.

2.2.2 Soil pH, Organic Matter, Population Health, and Metal Analysis

Soil pH, organic matter, and metal analyses were performed at Testmark Laboratories Inc. (Sudbury, ON, Canada). The pH was measured in water and the organic matter content was determined using the loss on ignition (LOI) analysis. Metal analysis was performed as described in Nkongolo et al. (2013). The health quality of the aboveground plant population was b assessed using a health index ranging from 1 to 10, with 1 representing a population without trees or a herbaceous layer (all vegetation below 50 cm in height) and 10 representing a complexed and dense population with a high species diversity, with no apparent disturbances.

2.2.3 Statistical Analysis

All the statistical analyses were performed with the SPSS version 20 for windows (IBM, NY, USA). All the data were tested for normality using the Shapiro-Wilk test ($P \leq 0.05$). Student t-tests were performed on metal concentration data, soil pH, and organic matter content to determine statistical significance ($P \leq 0.05$) between limed and unlimed areas.

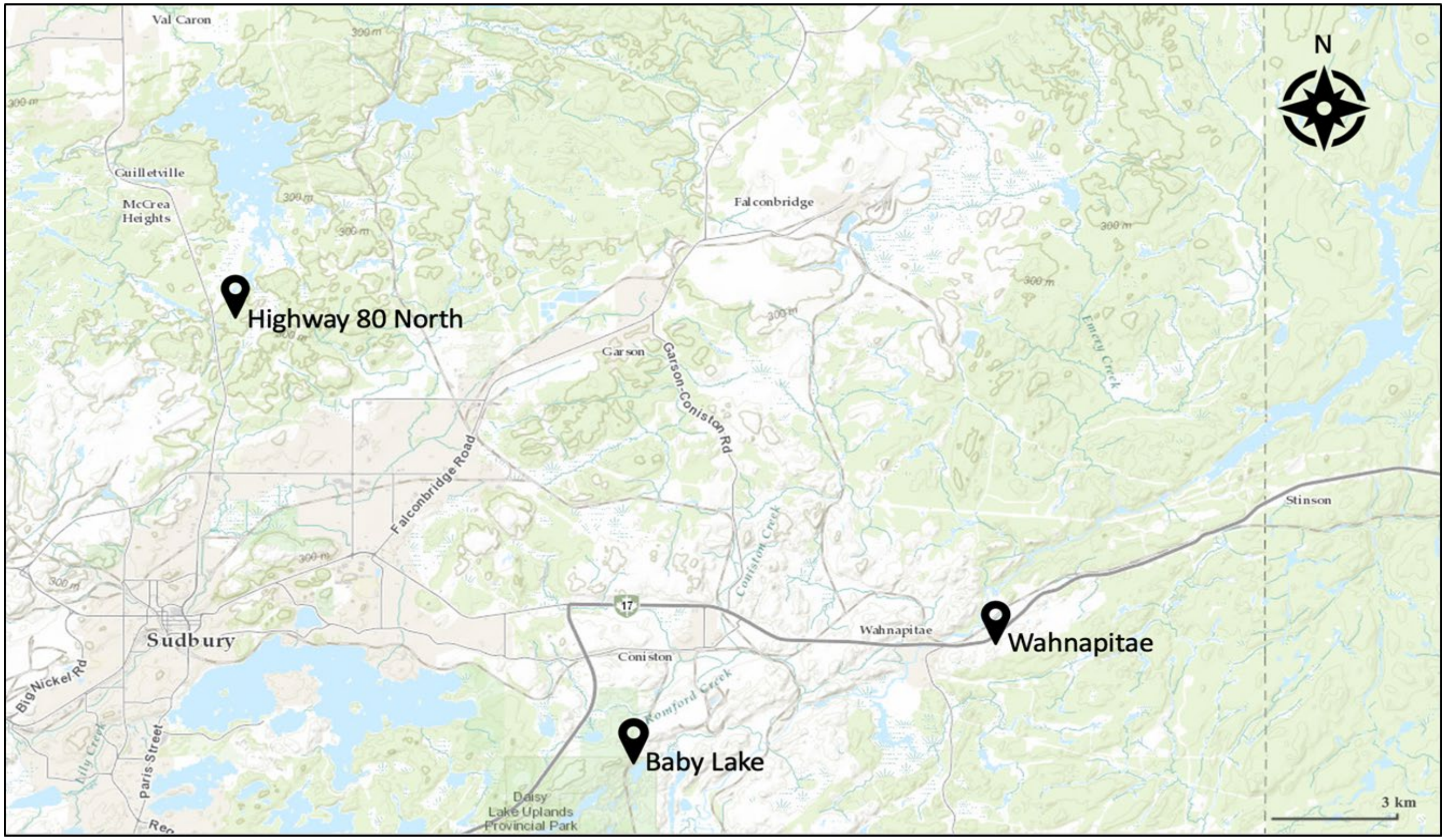

Figure 1. Geographical locations of the three sites chosen for this study in the City of Greater Sudbury.

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

Coordinates:
HWY 18 N: 46^0 33’17’’N 80^0 58’50” W
Baby Lake: 46^0 28’6”N 80^0 51’32” W
Wahnapitae: 46^0 28’59”N 80^0 48’18”W

2.3 Results

2.3.1 Soil pH, Percent Organic Matter, and Above-ground Population Health

As expected, liming increased soil pH at all three sites, however only Baby Lake and Wahnapitae had significantly higher ($P \leq 0.05$) soil pH at the limed sites compared to the unlimed sites (Fig. 2).

Organic matter content was significantly higher ($P \leq 0.05$) at Baby Lake's limed site compared to the unlimed area (Fig. 3). The level of organic matter at the limed area at Highway 80 North's location also increased (though not significantly) compared to the unlimed site, while the percentage of organic matter at Wahnapitae's limed site was slightly decreased compared to the unlimed site.

Overall, the forest population health index revealed a significant improvement in the limed sites compared to the unlimed areas. The population health index was higher in sites reclaimed 19 years ago (Highway 80 North) compared to more recently limed areas (Wahnapitae and Baby Lake).

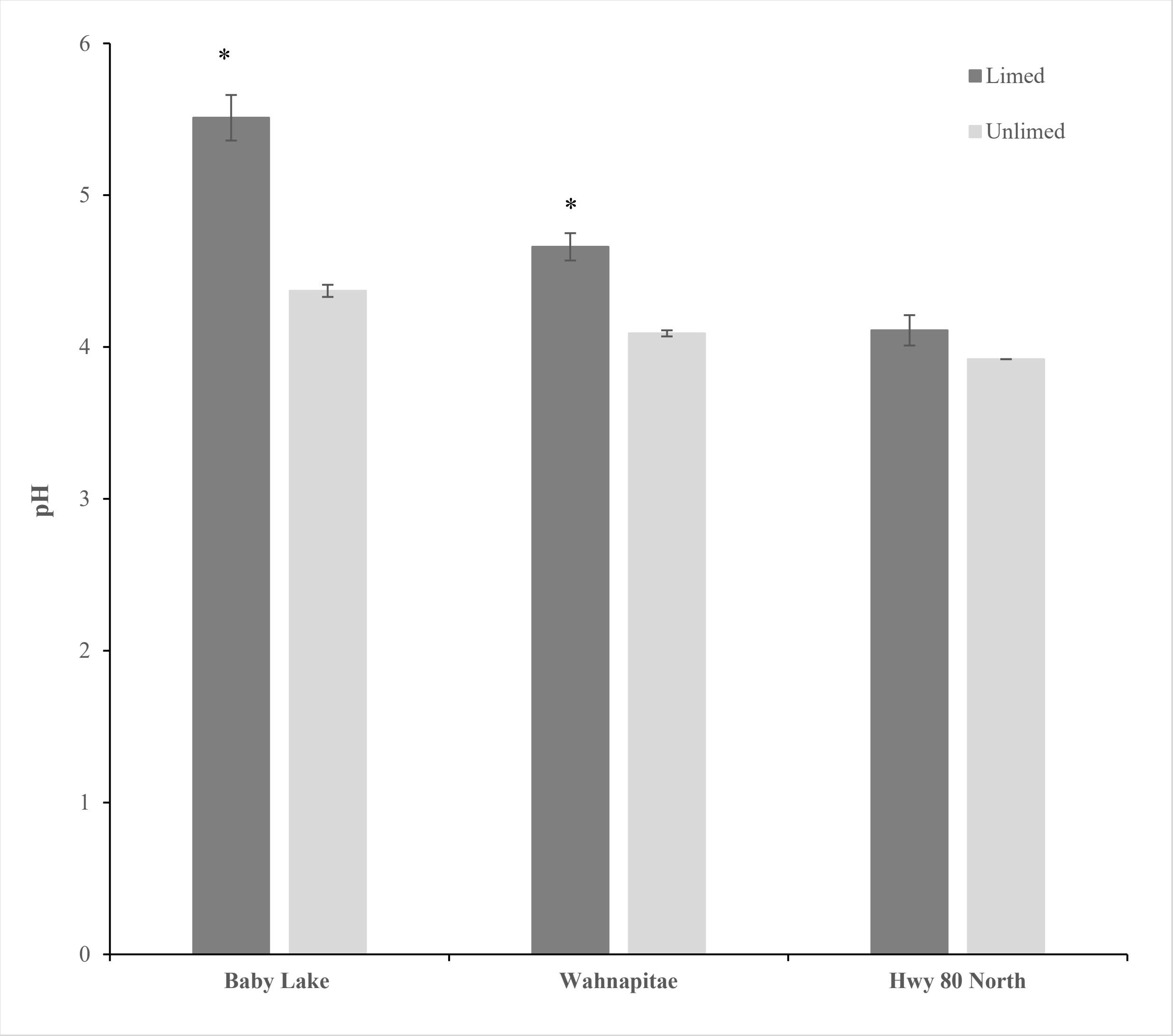

Figure 2. Soil pH of each limed and unlimed site sampled in the CGS.

* Represents significant differences between limed and unlimed sites based on student t-tests ($P \leq 0.05$). Bars represent standard errors (SE).
Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

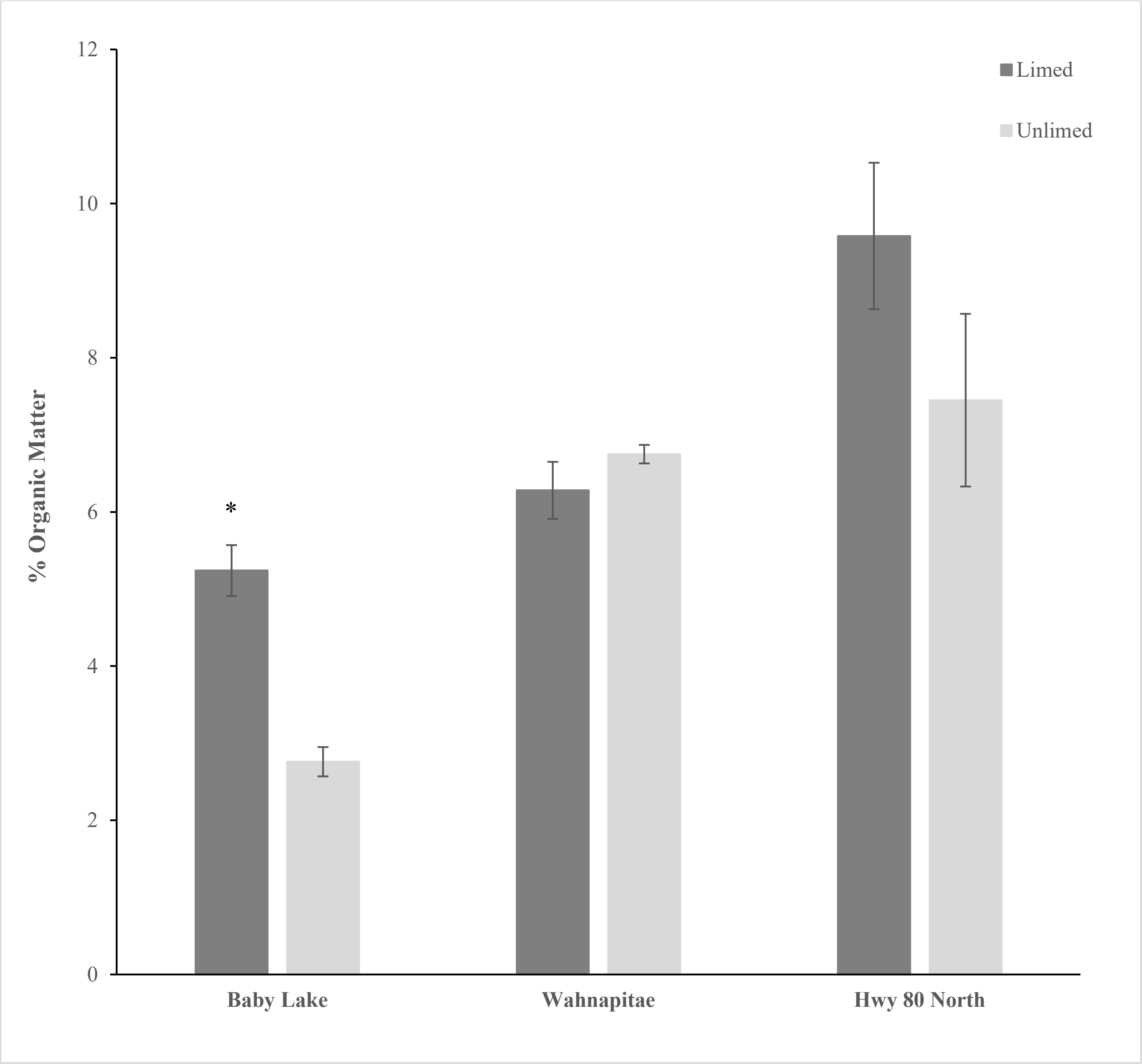

Figure 3. Percent (%) organic matter within the soil for each limed and unlimed site sampled in the CGS.

* Represents significant differences between limed and unlimed sites based on t-test (P ≤ 0.05). Bars represent standard errors (SE).

Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

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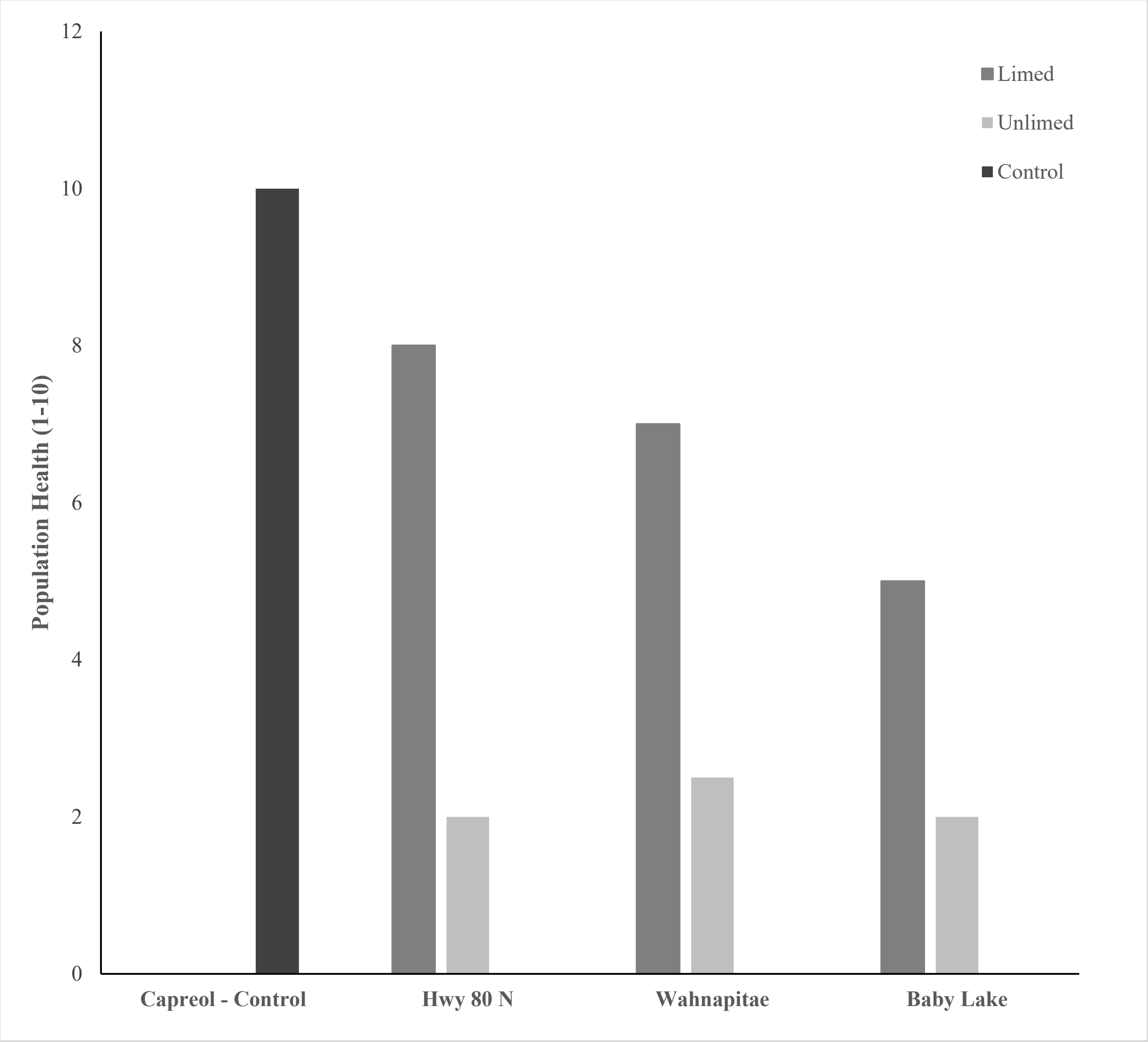

Figure 4. Population health indices of plant populations at Capreol (control), HWY 80 N, Wahnapitae, and Baby Lake

Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

2.3.2 Heavy Metal levels

Data of overall metal analysis for the three limed sites compared to the three unlimed sites are described in Figures 5-7. As expected, significant differences ($P \leq 0.05$) between the limed sites and the unlimed (control) sites were observed for arsenic (As), calcium (Ca) and iron (Fe).

However, there was no significant differences seen in aluminum (Al), magnesium (Mg), copper (Cu), manganese (Mn), nickel (Ni), phosphorous (P), cobalt (Co), lead (Pb), strontium (Sr) or zinc (Zn). The differences in metal concentrations between each limed site and its adjacent unlimed (control) site is described in Table 1. Lead (Pb) and strontium (Sr) were significantly different ($P \leq 0.05$) at each location between each limed and unlimed site, while magnesium was not significantly different ($P \leq 0.05$) at any location between the limed and unlimed sites.

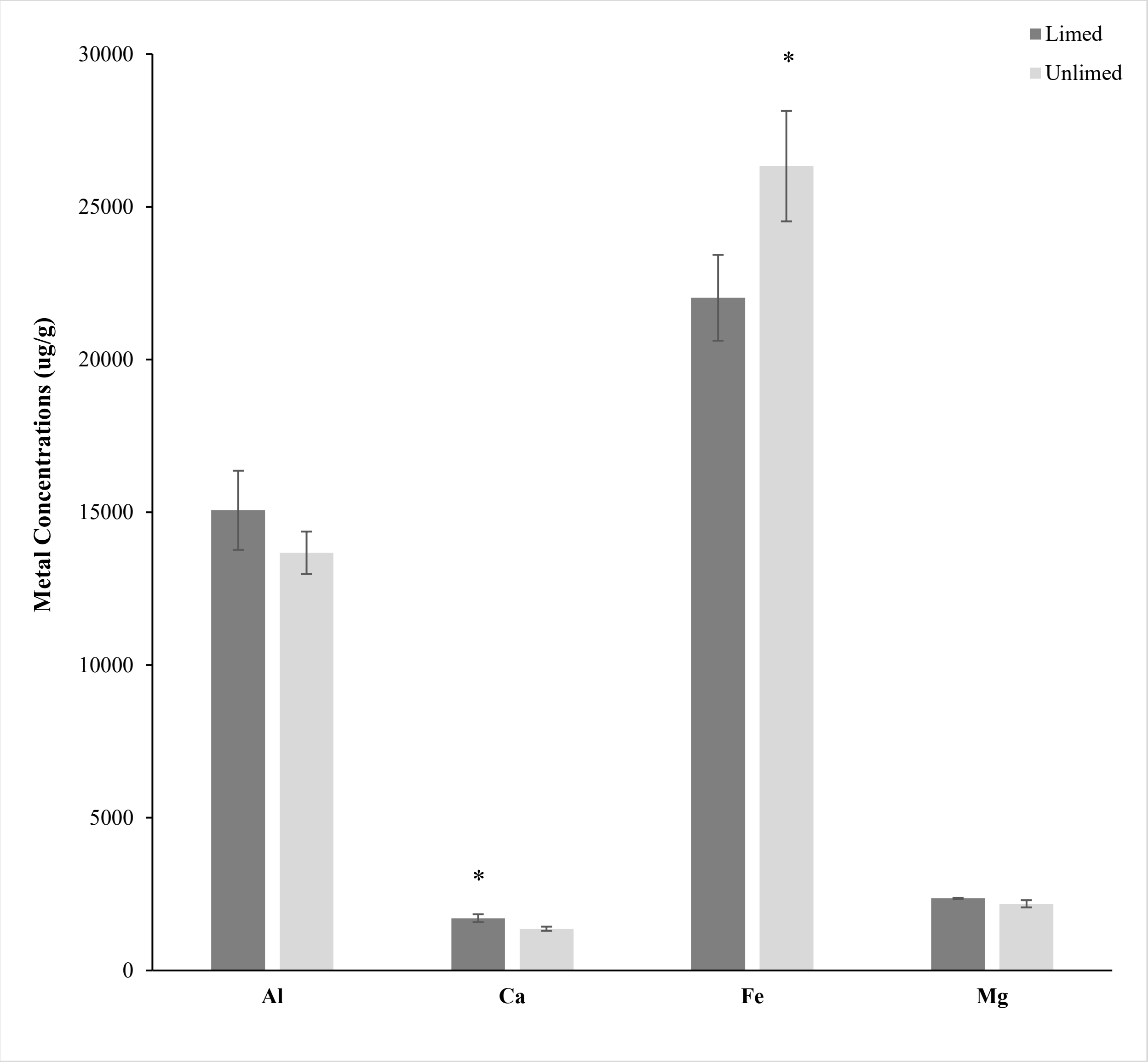

Figure 5. The overall metal concentrations from limed and unlimed soil samples from the Greater Sudbury Region (n = 27) for aluminum, calcium, iron, and magnesium.

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on student t-tests ($p \leq 0.05$). Bars represent standard errors (SE).

Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

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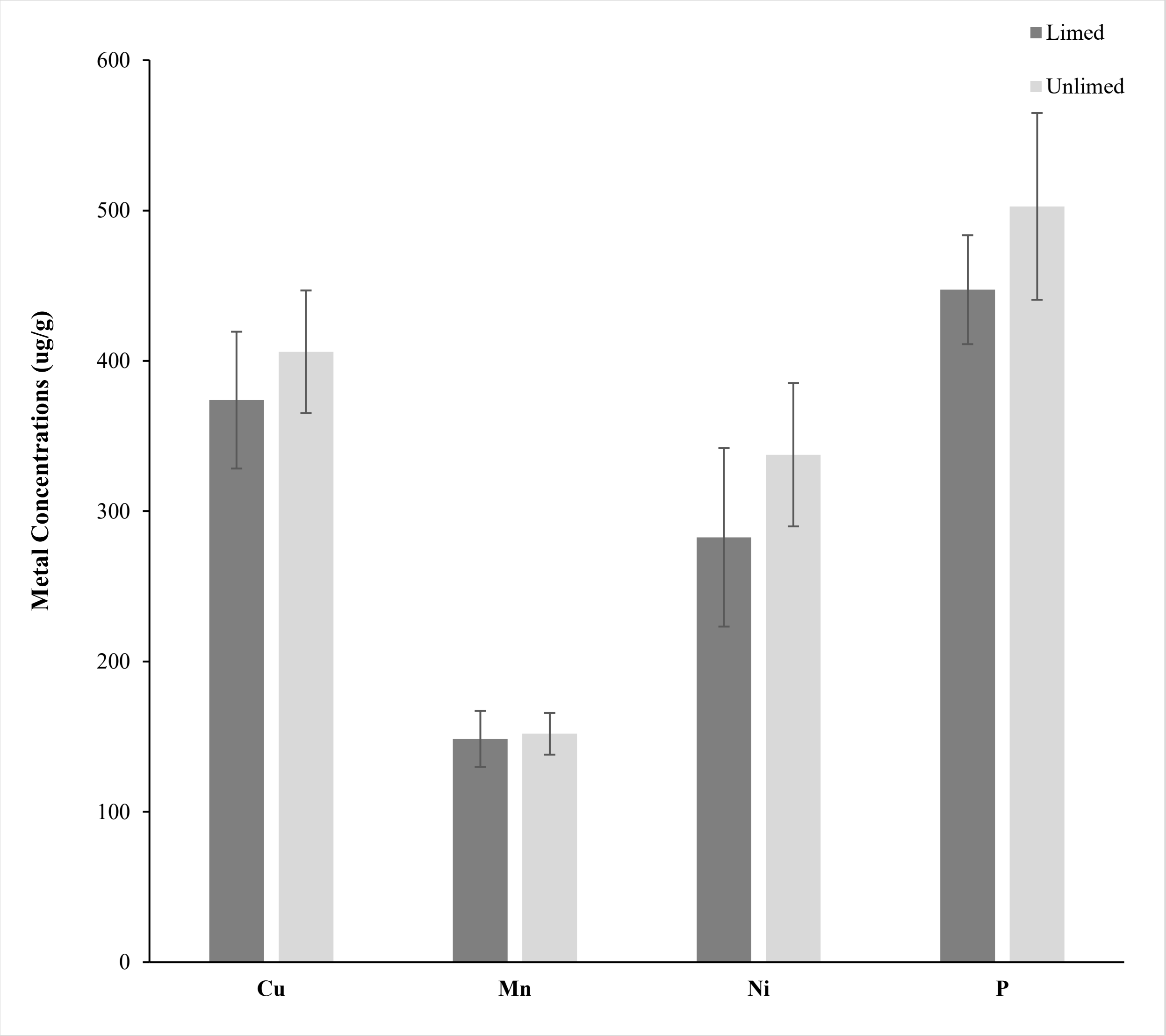

Figure 6. The overall metal concentrations from limed and unlimed soil samples from the Greater Sudbury Region (n = 27) for copper, manganese, nickel, and phosphorous.

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on student t-tests ($p \leq 0.05$). Bars represent standard errors (SE).

Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

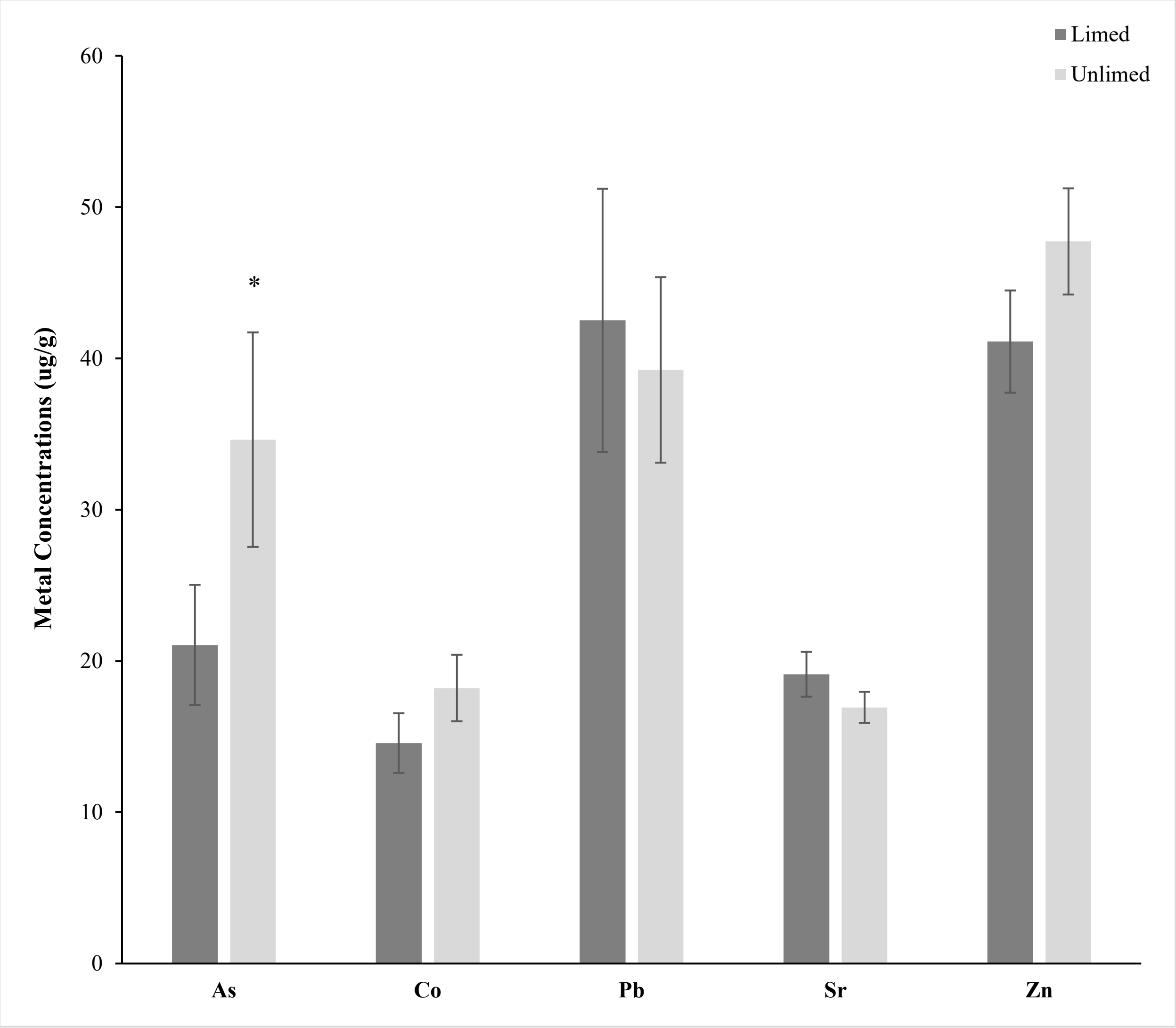

Figure 7. The overall metal concentrations from limed and unlimed soil samples from the Greater Sudbury Region (n = 27) for arsenic, cobalt, lead, strontium, and zinc. Bars represent standard errors (SE).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on student t-tests ($p \leq 0.05$).

Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

Table 1: Metal concentration in ug/g in soil samples collected from the CGS.

	Al*	**As**	**Ca**	**Co**	**Cu**	**Fe**	**Pb***	**Mg**	**Mn**	**Ni**	**P***	**Sr***	**Zn**
Baby Lake Limed	20200 ±778	16.13 ±3.74	1476.67 ±137.14	14.23 ±3.77	377.67 ±27.01	25333 ±1882	30.90 ±0.88	2233.33 ±108.56	142.10 ±40.04	190.00 ±52.67	488.67 ±20.58	14.73 ±0.64	41.80 ±6.40
Baby Lake Unlimed	14833 ±448	10.63 ±1.99	1533.33 ±33.44	11.97 ±0.71	274.67 ±34.39	20600 ±1172	17.40 ±3.23	2560.00 ±142	152.00 ±22.05	157.67 ±16.88	339.67 ±30.04	21.00 ±0.49	37.27 ±2.05

	Al	**As***	**Ca***	**Co***	**Cu***	**Fe***	**Pb***	**Mg***	**Mn**	**Ni***	**P***	**Sr***	**Zn***
Wahnapitae Limed	13433 ±859	10.50 ±1.47	2153.33 ±108.87	9.50 ±0.70	217.33 ±21.52	16800 ±424	19.30 ±2.08	2946.67 ±72.16	144.67 ±11.41	144.00 ±18.06	359.00 ±18.67	24.93 ±0.64	32.37 ±0.54
Wahnapitae Unlimed	13433 ±1085	57.03 ±8.58	1141.33 ±111.18	26.70 ±1.91	501.00 ±67.02	32400 ±2040	45.10 ±6.57	2006.67 ±108.46	186.67 ±17.84	461.33 ±46.56	574.67 ±77.11	14.97 ±0.95	55.57 ±5.88

	Al	**As**	**Ca**	**Co***	**Cu**	**Fe**	**Pb***	**Mg**	**Mn**	**Ni***	**P**	**Sr***	**Zn**
HWY 80 North Limed	11566 ±108	36.53 ±0.67	1503.33 ±157.93	19.97 ±1.45	526.67 ±38.55	23933 ±288	77.33 ±6.84	1896.67 ±112.48	158.67 ±36.62	514.00 ±39.74	494.33 ±84.48	17.70 ±0.65	49.17 ±3.77
HWY 80 North Unlimed	12750 ±1491	36.22 ±3.92	1423.33 ±42.77	15.95 ±0.90	442.50 ±8.59	26000 ±834	55.22 ±5.38	1981.67 ±138.65	117.12 ±11.26	393.83 ±32.83	593.83 ±120.39	14.83 ±0.35	50.37 ±3.59

Results are expressed as mean values ± standard error.

* Represent significance differences between limed and unlimed site based on t-test ($P \leq 0.05$)

Baby Lake limed in 2018; Wahnapitae limed in 2009; Highway 80 North limed in 2004.

2.4 Discussion

2.4.1 pH

As expected, soil pH values were higher in limed soil compared to the adjacent unlimed areas for each of the three sites. However, only Baby Lake and Wahnapitae showed a significant ($P \leq 0.05$) increase in pH at the limed sites compared to the unlimed areas (Fig. 2). One possible explanation for the lack of significant increase in pH at the Highway 80 N site could be the duration of time since liming. In fact, this site was limed in 2004 before the Wahnapitae and Baby Lake sites that were limed in 2009 and 2018, respectively.

2.4.2 Percent Organic Matter and Above-ground Population Health

The organic matter level was significantly higher ($P \leq 0.05$) at the Baby Lake limed site compared to the unlimed area, however there was no significant differences seen at the Wahnapitae or Highway 80N sites. The significant difference ($P \leq 0.05$) between the limed and unlimed sites at Baby Lake could be due to the addition of lime increasing pH, which in turn increases microbial activity, decomposition, and nutrient mineralization (*Soil Quality Indicators*, 2009). More research is needed to understand why there is no significant difference in organic matter content between the limed and unlimed sites at Wahnapitae and Highway 80 N. One possible explanation could be that the effects of liming on organic matter decrease as the period since liming (12 and 17 years respectively) increases.

The most significant differences between limed, unlimed, and control sites were observed in the forest population health. There was a noticeable improvement of the population health in limed compared to unlimed sites (Figure 4). However, there is still a significant gap between limed areas and the reference site (Capreol). This difference can be reduced thorough further tree planting or seeding.

2.4.3 Heavy Metal Concentrations

Some metals are required for biological processes; however, large amounts can be detrimental. On the other hand, a number of metals are not necessary for physiological processes and can have deleterious effects on living organisms at very small concentrations (Nkongolo et al., 2013). Overall, studies have shown that metal contamination decreases soil microbial diversity, and adversely affects various chemical and nutrient cycling processes (Kandeler et al., 1996; Sherameti and Varma, 2015). As a result, soil microbial composition is often used as an indicator of the level of metal contamination, soil quality, and ecosystem health. A number of studies on metal contaminated soils have reported reduced microbial activity and community abundance (Frostegård and Bååth, 1996; Narendrula-Kotha and Nkongolo, 2017a and b; Rajapaksha et al., 2004).

In this study, the levels of arsenic (As) and iron (Fe) were found to be significantly ($P \leq 0.05$) higher overall within the unlimed samples than the limed soils (Fig. 5 and 7), while calcium (Ca) concentrations were significantly ($P \leq 0.05$) higher in the limed soils compared to the unlimed soils (Fig. 5). This increase in calcium concentrations in the limed soils is likely due to the dolomitic lime [$CaMg(CO_3)_2$] application. Most significant ($P \leq 0.05$) differences between metals examined were found in Wahnapitae soils, with Ca, Mg, and Sr significantly ($P \leq 0.05$) higher in the limed soil samples, while As, Co, Cu, Fe, Pb, Ni, and Zn significantly ($P \leq 0.05$) higher in the unlimed soils compared to the limed soils (Table 1). The level of Al, Pb, and P at Baby Lake were significantly ($P \leq 0.05$) higher in the limed soils compared to unlimed samples. However, Sr soil content was significantly ($P \leq 0.05$) lower in the limed soils compared to the unlimed samples (Table 1). Meanwhile, Highway 80 North's limed soils had significantly ($P \leq 0.05$) higher concentrations of Co, Pb, Ni, and Sr than the unlimed soils. The differences seen in the concentrations of metals among each of the three locations and within each location could be attributed to many factors, such as microbial and plant diversity, soil pH, metal solubility, soil respiration rates, enzymatic activities, soil organic matter contents, time since liming occurred, and environmental factors.

2.5 Conclusions

A comparison of the limed and unlimed soils sampled from the three sites shows that aerial liming increases soil pH, somewhat increases percent organic carbon, and increases Ca concentrations, while decreasing the concentrations of other metals such as As and Fe. Other metals, such as Al, Pb, P, Sr, Mg, Co, Cu, Ni, Mn, and Zn varied in concentrations between each location and their limed and unlimed areas.

Chapter 3: Microbial biomass and function in aerial limed and unlimed lands in the City of Greater Sudbury

3.1 Introduction

Almost 150 years of mining activities in Northern Ontario, Canada, have led to a decline in soil quality. Large smelters released enormous amounts of SO_2 (sulfur dioxide) and heavy metal particulates into the air, resulting in contamination and acidification of soils and bodies of water (Narendrula et al., 2012; Narendrula-Kotha and Nkongolo, 2017b; Winterhalder, 1996). Legislation has been put in place in within the last 50 years to control the amount of toxic pollution that can be released into the environment. Locally, the CGS started the Regreening Program with the aim of remediating these damaged terrestrial and aquatic ecosystems. The application of dolomitic limestone, along with fertilizer and grass seeds have been crucial to restoring the CGS area to a healthy, green area once again (Narendrula et al., 2012; Winterhalder, 1996).

Several studies have investigated the effects of metal contamination on soil microorganisms and biology, and the addition of lime to metal contaminated soils. Dolomitic lime is added to soils to neutralize the acidity, allowing more plants and organisms to grow in the environment (Narendrula et al., 2012; Narendrula and Nkongolo, 2015). Previous investigations conducted in the CGS reported that soil liming increased soil microbial biomass, abundance, and enzymatic activity, leading to better soil fertility in these limed areas (Narendrula-Kotha and Nkongolo, 2017b; Nkongolo et al., 2013, 2016).

Enzymatic activity and PLFA results from this study will provide researchers with information on activities of microbial enzymes in aerially limed soils in addition to microbial biomasses in sites soils compared to untreated soils. These results are useful for further restoration efforts within the CGS and provide crucial information on aerial liming treatments as a part of restoration efforts that can be adapted for use globally.

3.2 Methods and Materials

3.2.1 Soil Sampling and Study Sites

Soil samples were collected from three sites around the CGS, and each site was composed of a reclaimed (limed) area and an adjacent undisturbed (unlimed) area to serve as the control site. The selected sites were limed by the Sudbury Regreening Program using dolomitic lime (10 t/ha). They include Wahnapitae, Baby Lake, and Highway 80 North, which were limed in 2009, 2018, and 2004 respectively. For each site's limed and unlimed areas, three replicate samples consisting of 10 subsamples were collected from the organic (top) layer (0 – 5 cm). These soils were sieved to remove plant and other debris materials. The enzymatic activities of each site were analyzed using fresh (≤ 24 hour old) samples.

3.2.2 Enzyme Activities

Nine different enzymes were analyzed in this study and were chosen because of their catalytic abilities as well as for their roles in ecological processes (Table 2). They include β-glucosidase (BG), cellobiohydrolase (CBH), β-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), acid phosphatase (AP), alkaline phosphatase (ALP), glycine aminopeptidase (GAP), leucine aminopeptidase (LAP), and peroxidase (PER). Each enzyme was analyzed at their optimal pH value and assayed using the Fluostar Optima 96-well plate reader from BMG Technologies. The substrates used in the assays for BG, CBH, NAGase, AS, AP, and ALP was p-nitrophenol (pNP), while GAP and LAP were assayed using p-nitroanilide substrates. PER and polyphenol oxidase (PPO) activities were assayed using the substrate L-DOPA (L-3,4-dihydroxyphenylalanine). The substrate concentrations used for all assays was 5 mM, except for CBH and NAGase, whose substrate concentrations were limited to 2mM because of their solubility and cost. The original protocols for each of these assays can be found on the Environment RCN website.

To determine enzymatic activities, a homogenate was created by adding 4.0 g of soil to 40.0 mL of 5 0mM sodium acetate buffer (pH 5.0), before being vortexed at a high speed for two minutes. Three replicates of each enzyme analysis were conducted on each sample. For all enzymes except for the control and peroxidases, three labelled 1.5 mL Eppindorph tubes were filled with 450 µL of their appropriate substrates, followed by 450 µL of the homogenate. For the control tubes, three labelled 2.0 mL Eppindorph tubes were filled with 900 µL of the homogenate and 900 µL of the buffer. For the peroxides, PPO, POD, and a control with H_2O_2 (hydrogen peroxide) were used. For PPO, three 1.5 mL Eppindorph tubes were each filled with 450 µL of the homogenate, 450 µL of the substrate, and 30 µL of 0.3% H_2O_2, while the POD tubes were filled in a similar fashion but replacing the substrate with 450 µL of buffer. For the control with H_2O_2, the three 1.5 mL tubes were filled with 450 µL of homogenate, 450 µL of buffer, and 30 µL of 0.3% H_2O_2. Next, all the PNP and peptidase tubes (BG, CBH, NAGase, AP, ALP, AS, GAP, and LAP) were incubated for two hours on a rotary shaker at 255 RPM (at room temperature), while the nine peroxidase tubes were incubated on a spinning wheel for two hours at 4°C. Following the incubation period, the tubes were centrifuged at 4000 RPM for three minutes, then 100 µL of the supernatant was deposited into each of the three replicate wells on a 96-well plate. Then 5 µl of

1.0 M NaOH (sodium hydroxide) was added to the pNP and p-nitroanilide substrates to stop the ongoing reaction. The microplate containing the pNP and p-nitroanilide substrates were then read at 405 nm, while the microplate with the PER substrates was read at 450 nm. All assays were performed in triplicates, and substrate and control samples were used for accuracy. To properly analyze the absorbencies of the assays, they were adjusted by subtracting the combined absorption results for the sample and substrate controls. The enzymatic activity of each sample was expressed in mmol h^{-1} g $soil^{-1}$.

Table 2: Enzyme assays, their function, and the substrate used (with their corresponding pH).

Assayed Enzymes	**Enzyme Function**	**Substrate Used**
β-glucosidase (BG)	Cellulose degradation used in carbon cycling	pNP β-D-glucopyranoside
Cellobiohydrolase (CBH)	Cellulose degradation and other beta-1,4 glucans used in carbon cycling	pNP-β-D-cellobioside
β-N-acetylglucosaminidase (NAGase)	Chitin degradation used in carbon and nitrogen cycling	pNP-N-acetyl-β-D-glucosaminide
Aryl sulfatase (AS)	Produces plant-available sulfates used in sulfur cycling	pNP sulfate
Acid phosphatase (AP)	Produces plant available phosphates that are used in phosphorus cycling	pNP phosphate (Buffer pH 5.0)
Alkaline phosphatase (ALP)	Releases ester bound phosphates that are used in phosphorous cycling	pNP phosphate (Buffer pH 9.0)
Glycine aminopeptidase (GAP)	Degrades protein into amino acids which are used in nitrogen cycling	Glycine-p-nitroanilide
Leucine aminopeptidase (LAP)	Degrades leucine and other hydrophobic amino acids which are involved in nitrogen cycling	L-Leucine-p-nitroanilide
Peroxidase (PER)	Lignin and tannin (polyphenols) degradation used in carbon cycling	L-3,4-dihydroxyphenylalanine (DOPA)

* pNP represents 4-nitrophenyl.

3.2.3 Phospholipid Fatty Acid (PLFA) Analysis

Soil samples (from the three limed and three unlimed sites) were analyzed following the protocol described by (Narendrula-Kotha and Nkongolo, 2017b; Nkongolo et al., 2013, 2016). Mole percentage of each PLFA were used to determine the bacterial and fungal biomasses in each soil sample. The total PLFA extracted from each sample was used as an index of total living microbial biomass (Buyer and Sasser, 2012). The selected PLFA's for bacterial biomass include i15:0, a15:0, i16.0, 16:1ω9, 16:1ω7c, cy17:0, i17:0, a17:0, 18:1ω7 and cy19:0, and the fungal biomass PLFA's used were 18:2ω6 and 18:1ω9. PLFA 10Me was used for the identification of actinomycetes, while 20:3ω6 and 20:4ω6 was used for protozoa, and 12:0, 16:1ω7, 18:2ω9, 18:2ω12, 18:3ω9, 18:3ω12, 18:3ω15 and polyunsaturated fatty acids were used for the identification of eukaryotes.

3.2.4 Statistical Analysis

Statistical analyses were performed using SPSS version 20 for windows (IBM, NY, USA). Normality testing of all the data was performed using the Shapiro-Wilk test ($p \leq 0.05$). Mann-Whitney tests were conducted on paired data to determine the significance of the enzymatic activities between the limed and unlimed sites. T-tests were performed to determine the significance of the mean PLFA data between the limed and unlimed sites.

3.3 Results

3.3.1 Soil Enzyme Activities

The enzymatic activity data from this study are illustrated in Figures 8-19. The data were analyzed for the overall enzymatic activity of the three sites, as well as for each individual (limed and unlimed) site. Overall, soil samples from the three limed sites combined show significantly higher ($P \leq 0.05$) β-N-acetylglucosaminidase (NAGase), aryl sulfatase (AS), and glycine aminopeptidase (GAP) activity levels when compared to the unlimed sites (Figs. 8 and Fig. 9). The remaining enzymes, β-glucosidase (BG), cellobiohydrolase (CBH), leucine aminopeptidase (LAP), alkaline phosphatase (ALP), acid phosphatase (AP), and peroxidase (PER) showed no significant ($P \leq 0.05$) difference in their activity levels between the unlimed and limed sites (Figs. 8, 9, and 10).

Variation in enzymatic activity levels found at the three sites was observed and data are depicted in figures 11-19. For BG, only one site (Baby Lake) showed a significant increase ($P \leq 0.05$) in the limed area compared to the adjacent unlimed site, while the other two sites did not show any significant differences (Fig. 11). CBH and LAP both showed a significant increase ($P \leq 0.05$) in the limed site at Baby Lake compared to the unlimed site, while Highway 80's limed site showed a significant decrease ($P \leq 0.05$) in both enzyme activities compared to the unlimed site (Fig. 12 and 18). In contrast, NAGase activity was significantly increased ($P \leq 0.05$) at all three limed sites (Wahnapitae, Baby Lake and Hwy 80) compared to their adjacent unlimed areas (Fig. 13). AP and ALP activities both showed a significant increase ($P \leq 0.05$) at Baby Lake's limed site compared to the unlimed site, and a significant decrease ($P \leq 0.05$) at Wahnapitae's limed site compared to its unlimed site (Fig. 14 and 15). Both Wahnapitae and Baby Lake showed a significant increase ($P \leq 0.05$) in AS activity at their limed sites compared to their unlimed sites, with Hwy 80 showing no significant differences between the two sites (Fig. 16). Finally, both GAP and PER activities were significantly increased ($P \leq 0.05$) at Baby Lake's limed site compared to the unlimed site, with no significant changes to either enzyme activity seen at Wahnapitae and Hwy 80 (Fig. 17 and 19).

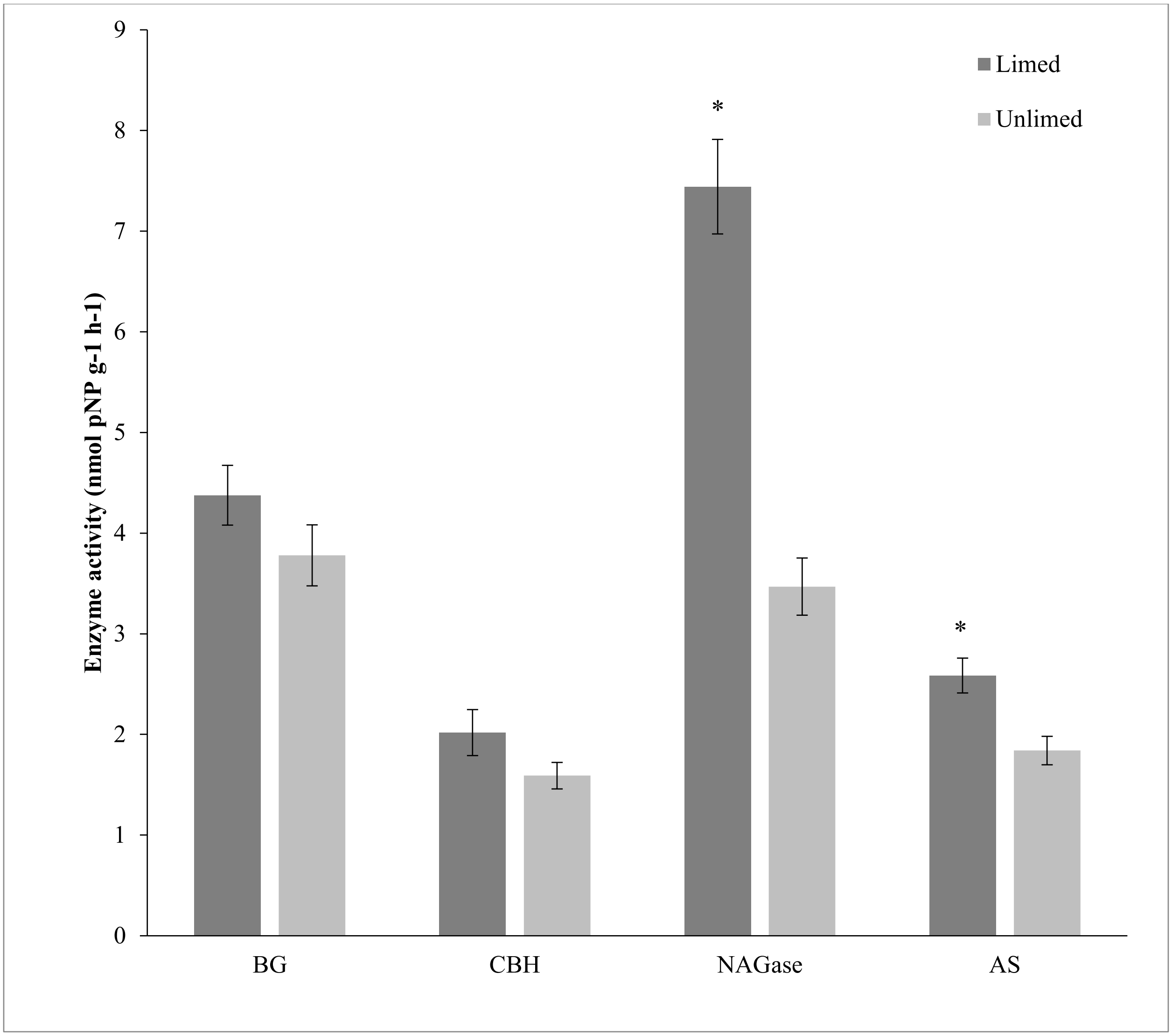

Figure 8. The activities of hydrolytic enzymes from limed and unlimed soil samples from the Greater Sudbury Region (n = 27) using p-nitrophenol (pNP) linked substrates. BG = β-glucosidase; CBH = Cellobiohydrolase; NAG = β-N-acetylglucosaminidase; AS = Aryl sulfatase.

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

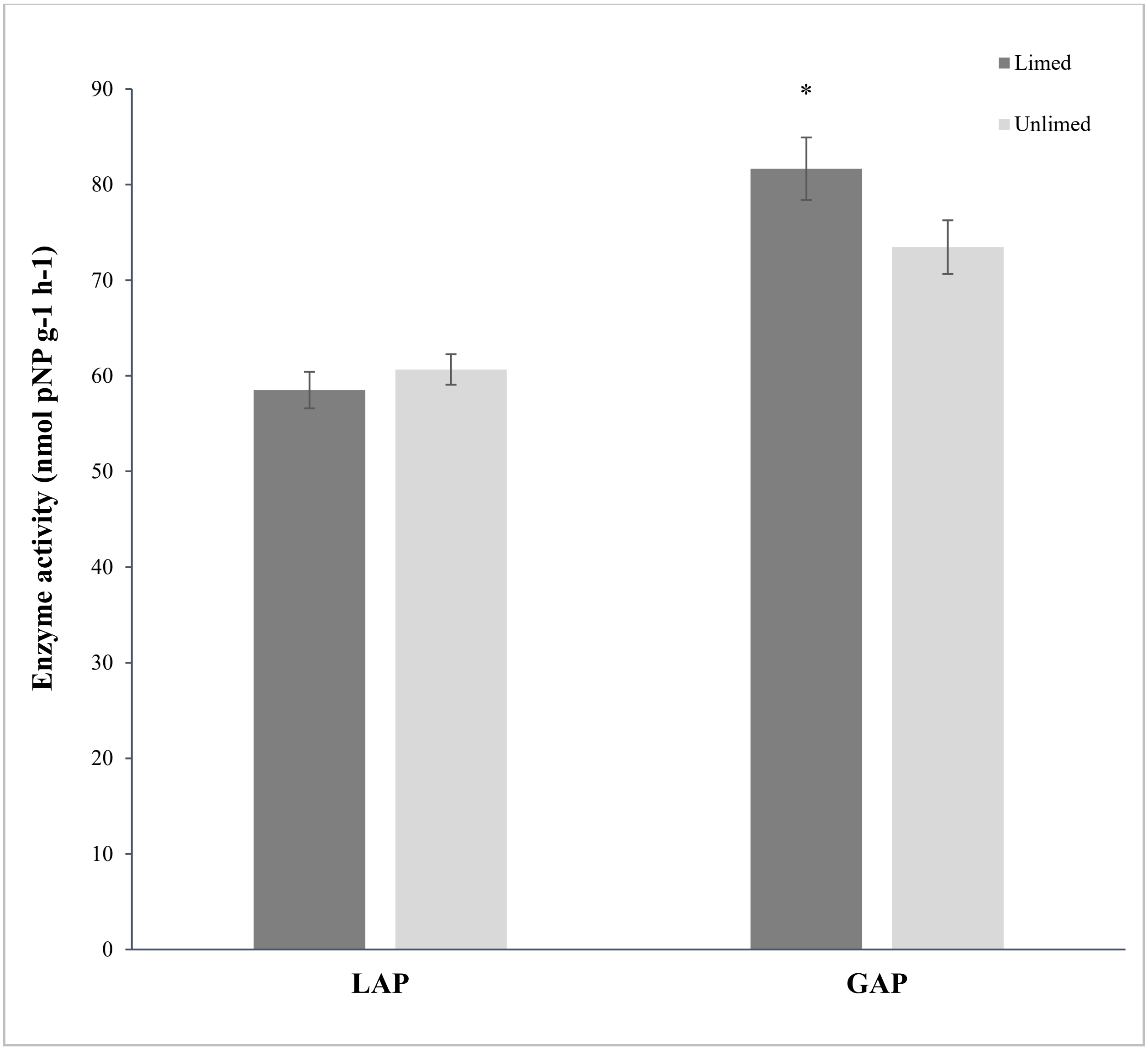

Figure 9. The activities of hydrolytic enzymes from limed and unlimed soil samples from the Greater Sudbury Region (n = 27) using p-nitroanilide linked substrate. GAP = glycine aminopeptidase, LAP = leucine aminopeptidase.

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

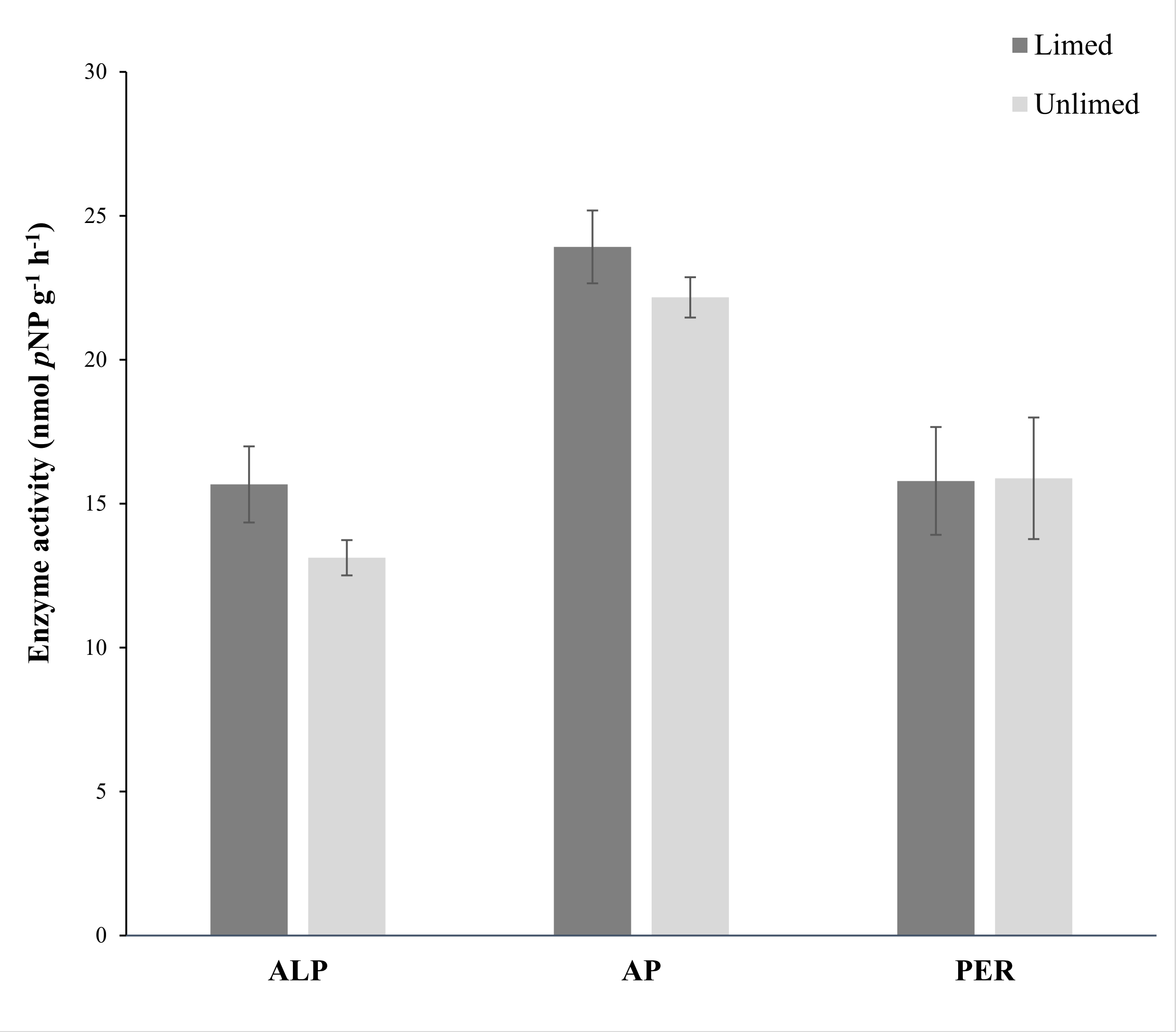

Figure 10. The activities of hydrolytic enzymes from limed and unlimed soil samples from the Greater Sudbury Region (n = 27) using p-nitrophenol (pNP) linked substrates for acid phosphatase (AP) and alkaline phosphatase (AlP) , and L-3, 4-dihydroxyphenylalanine (DOPA) linked substrates for peroxidase (PER) activity.

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

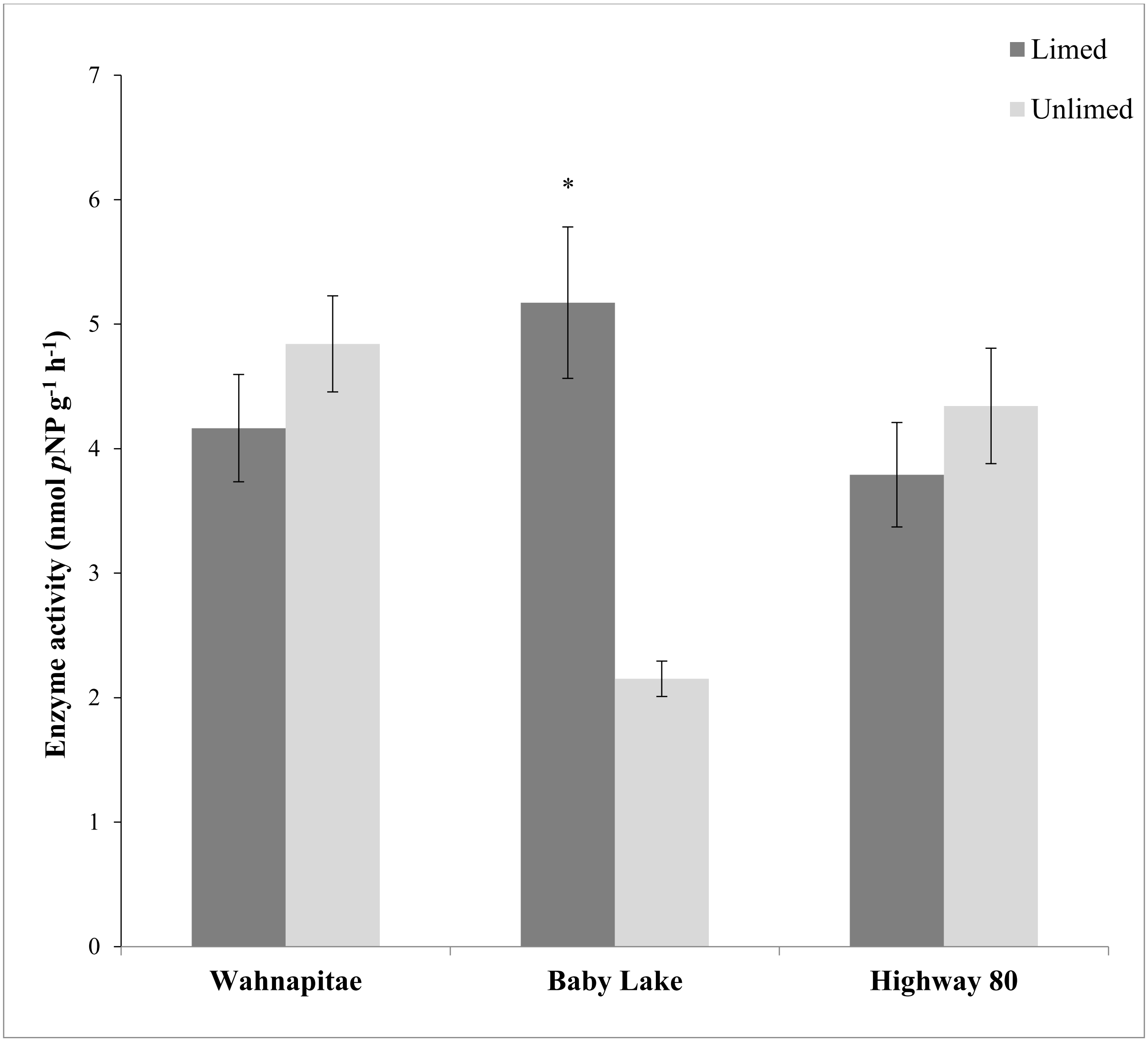

Figure 11. Activity of enzyme β-glucosidase (BG) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing $P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

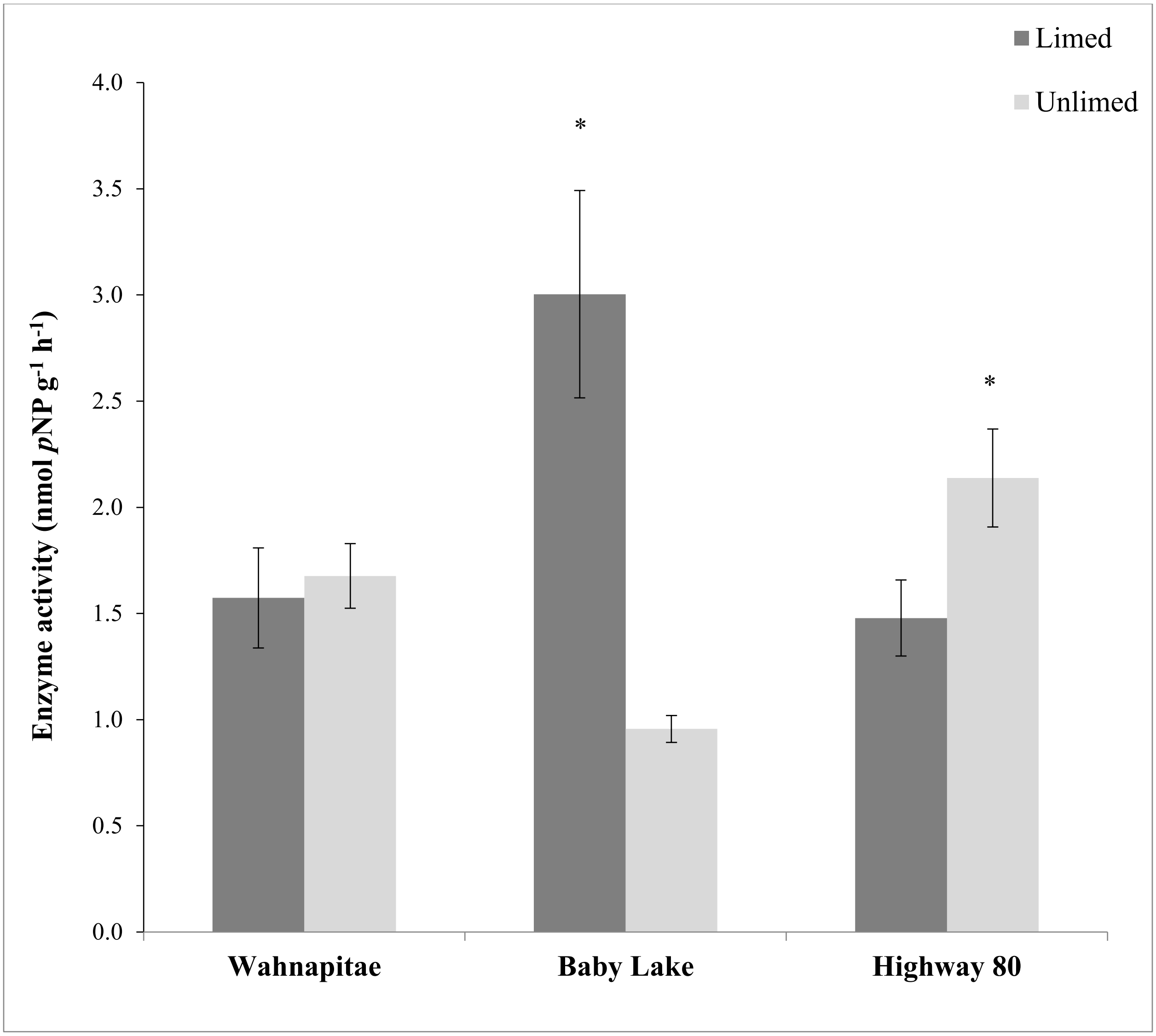

Figure 12. Activity of enzyme Cellobiohydrolase (CBH) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

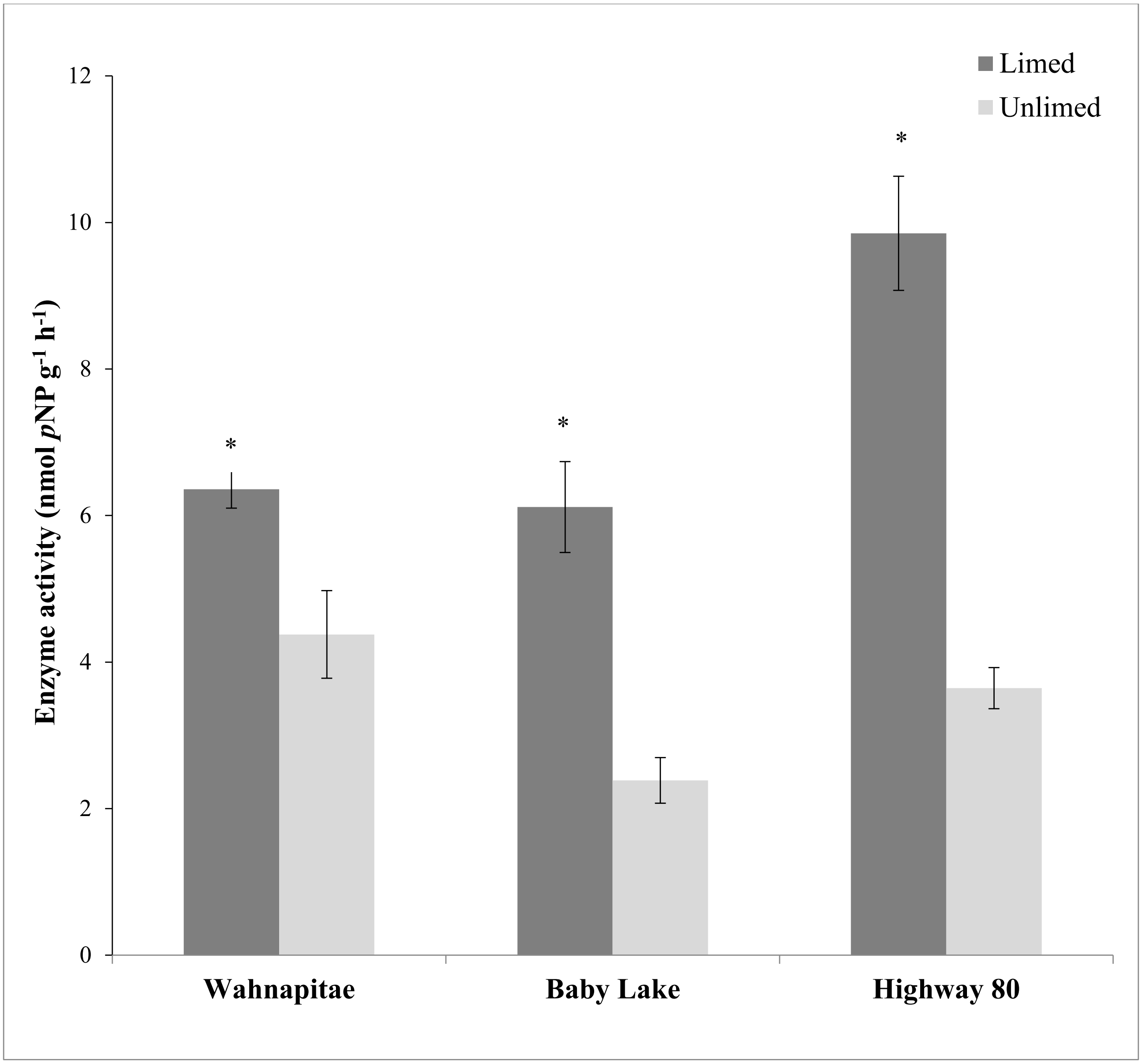

Figure 13. Activity of enzyme β-N-acetylglucosaminidase (NAGase) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

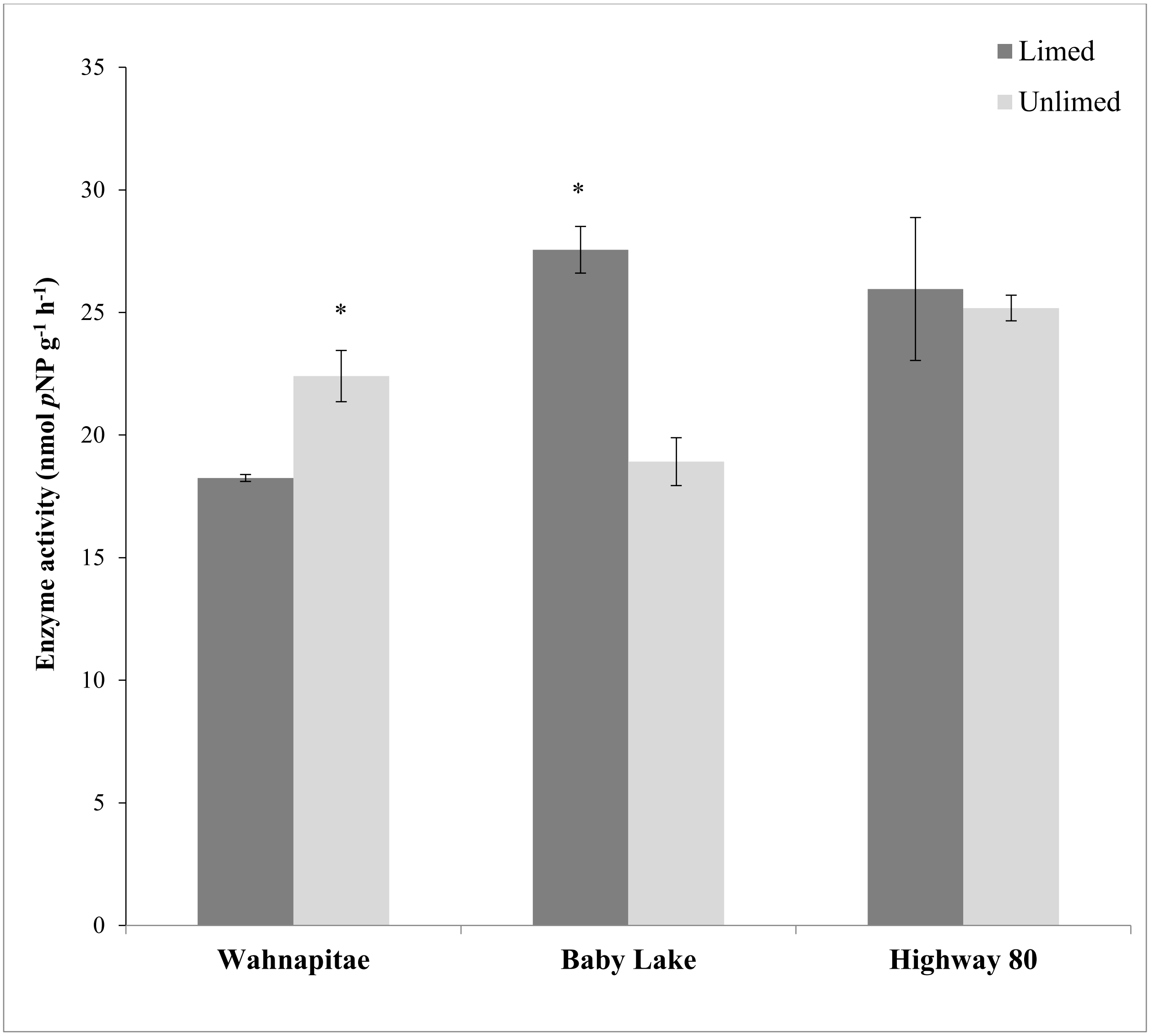

Figure 14. Activity of enzyme acid phosphatase (AP) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

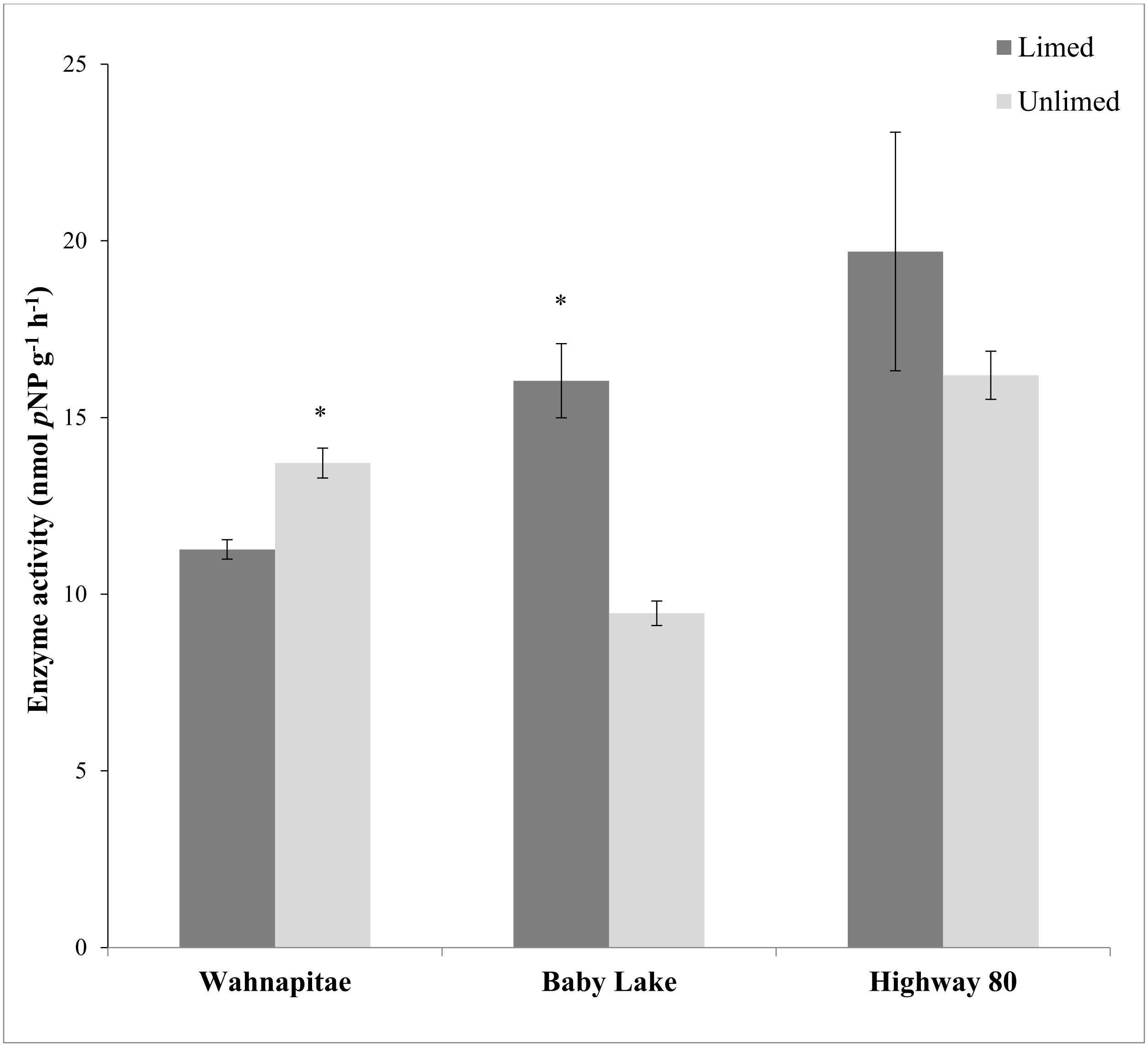

Figure 15. Activity of enzyme alkaline phosphatase (ALP) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

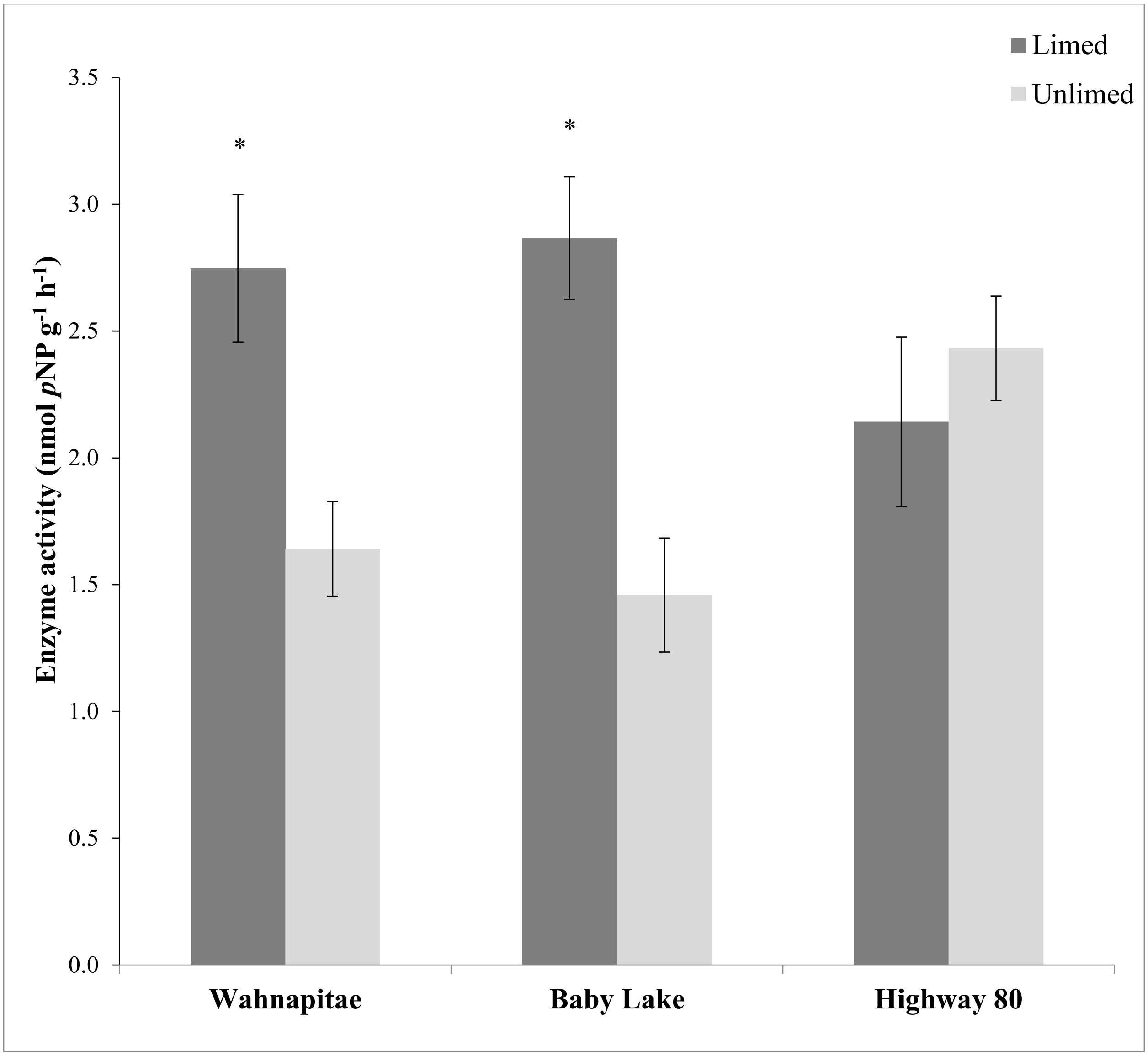

Figure 16. Activity of enzyme Aryl sulfatase (AS) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

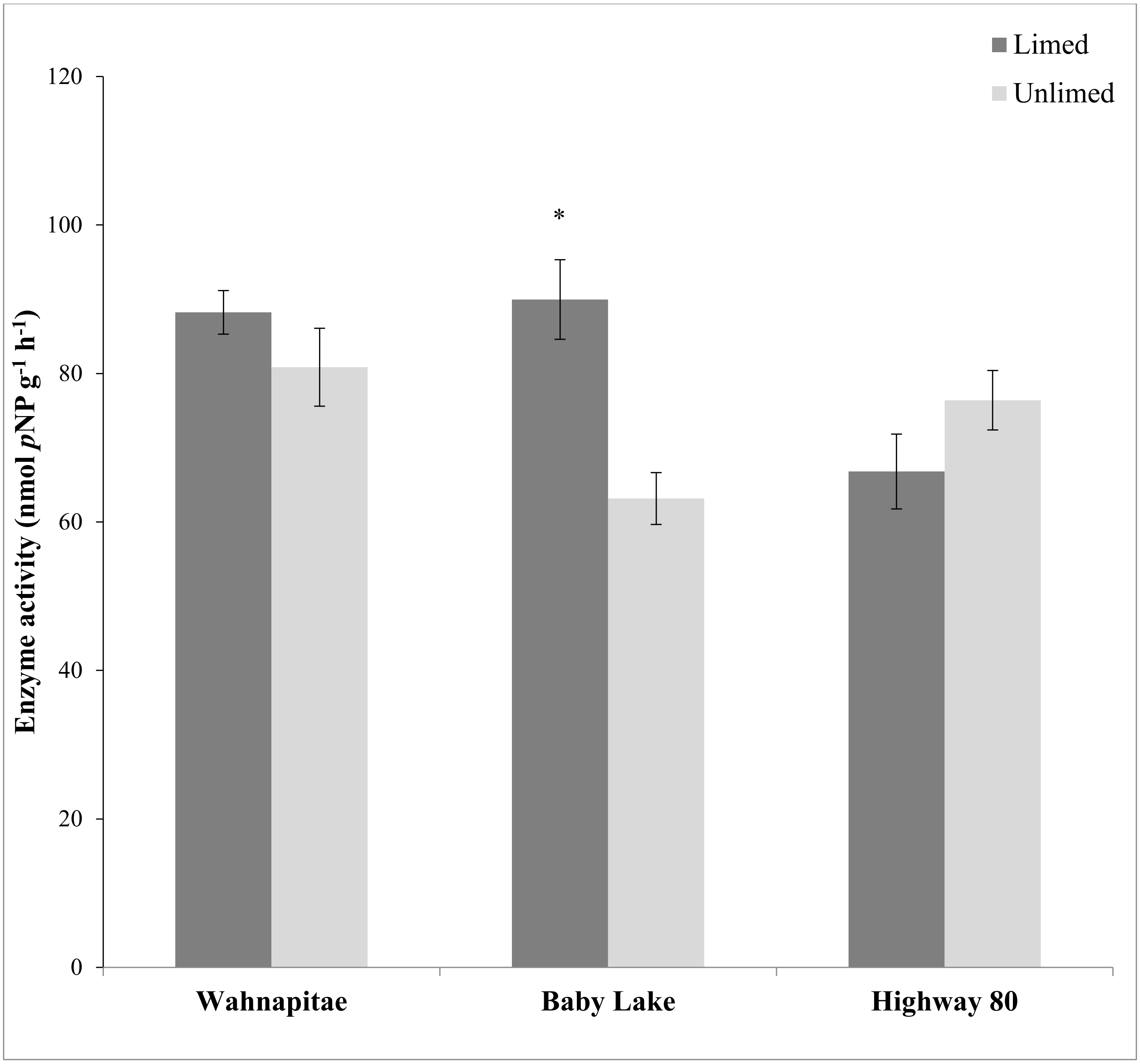

Figure 17. Activity of enzyme glycine aminopeptidase (GAP) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

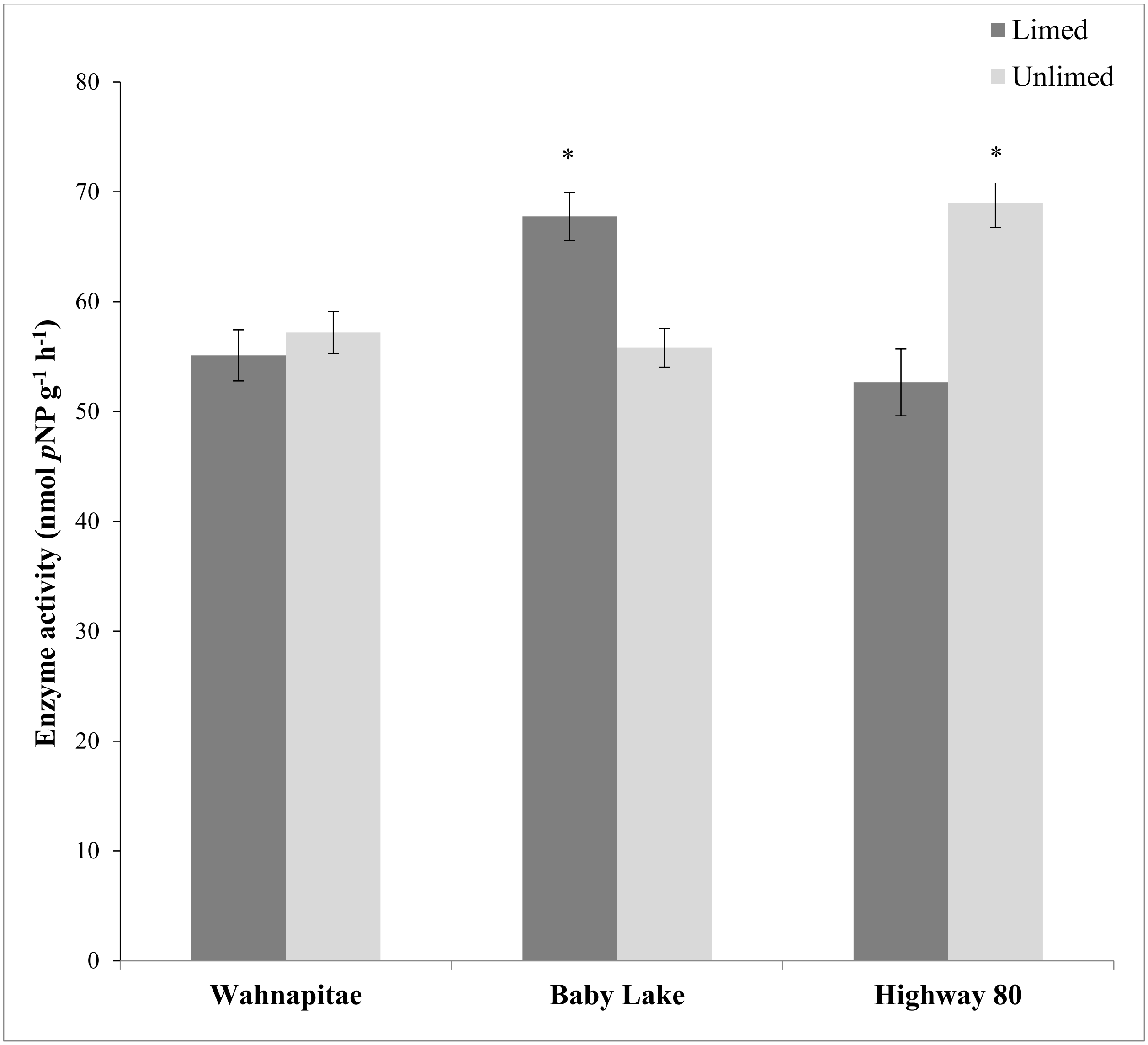

Figure 18. Activity of enzyme leucine aminopeptidase (LAP) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

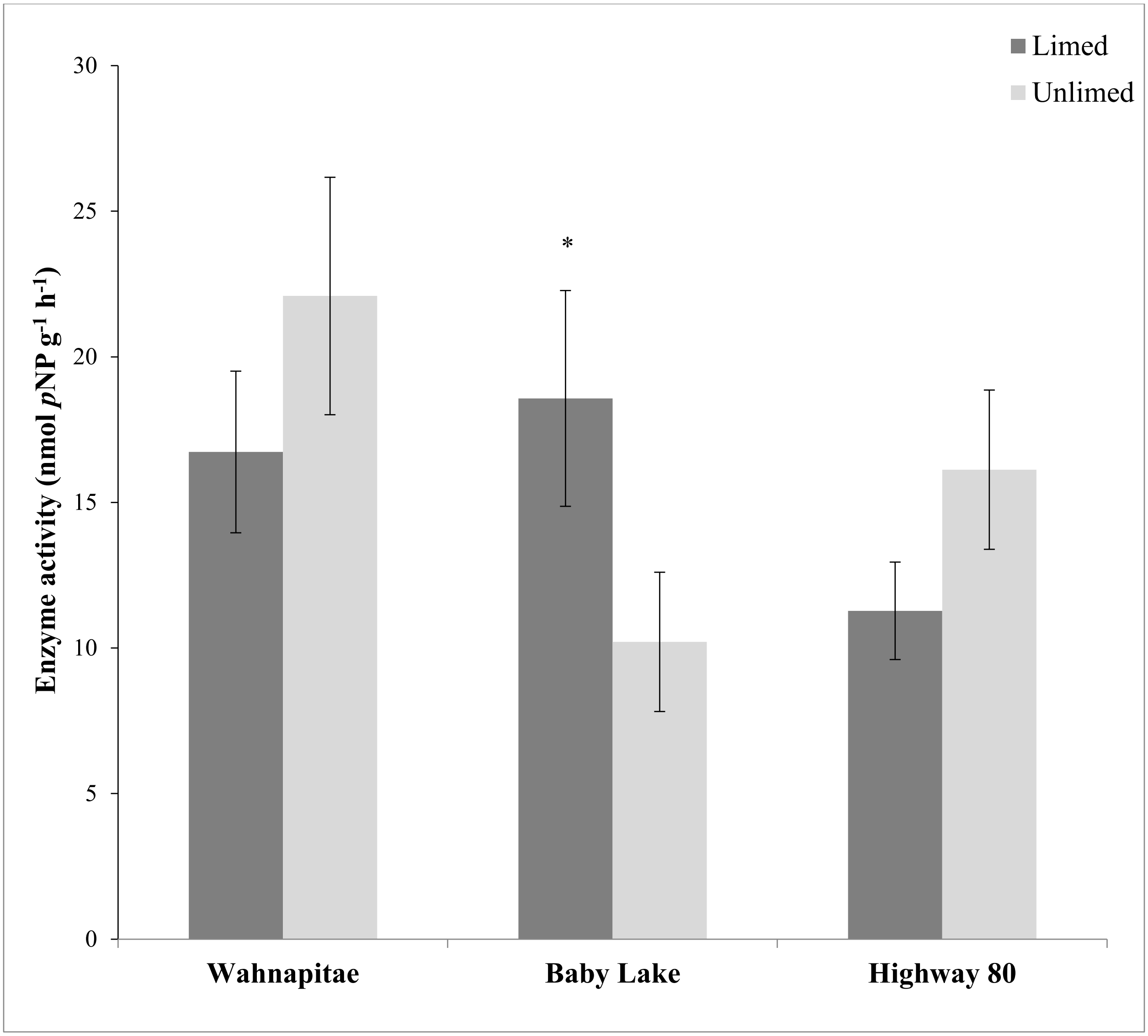

Figure 19. Activity of enzyme peroxidase (PER) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 9).

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

3.3.2 PLFA Analysis

PLFA analysis showed that the total microbial biomass was significantly increased ($P \leq 0.05$) at the Highway 80 limed site compared to the unlimed site, while there was no significant difference between the limed and unlimed sites for Baby Lake and Wahnapitae (Fig. 20). There was also no significant difference ($P \geq 0.05$) in total microbial biomass overall between the three limed and unlimed sites (Table 3). The relative abundance levels of fungi, gram negative bacteria, and eukaryotes were significantly increased ($P \leq 0.05$) overall at limed sites compared to unlimed areas, while arbuscular mycorrhizal (AM) fungi, gram positive bacteria and actinomycetes showed no overall significant differences (Table 3). There were no significant differences ($P \geq 0.05$) in relative abundances, between Baby Lake's limed and unlimed sites. A significant increase ($P \leq 0.05$) in the gram-negative abundance was observed between limed and unlimed sites at Wahnapitae (Table 4). Highway 80 N showed significant increases ($P \leq 0.05$) in the abundances of gram-negative bacteria, gram-positive bacteria and fungi at the limed sites compared to the unlimed sites (Table 4). The ratios of gram positive to gram-negative bacteria, saturated to unsaturated and mono to poly were all significantly decreased ($P \leq 0.05$) overall in the limed sites compared to the unlimed areas, while gram-negative stress and the predator to prey ratio were significantly increased ($P \leq 0.05$) in the limed sites (Table 5). The fungi to bacteria ratio was not significant ($P \geq 0.05$) different between limed and unlimed sites (Table 5). There was a significant increase ($P \leq 0.05$) observed for gram-negative stress at the Baby Lake and Wahnapitae limed sites compared to their respective unlimed sites. There was a significant decrease ($P \leq 0.05$) in the gram positive to gram-negative ratio between limed and unlimed sites at Wahnapitae and Highway 80 N (Table 6). A significant decrease was also recorded between limed and unlimed samples the mono to poly ratio, associated with a significant increase ($P \leq 0.05$) in the fungi to bacteria ratio for the Highway 80 N site when limed areas were compared to the unlimed sites. (Table 6).

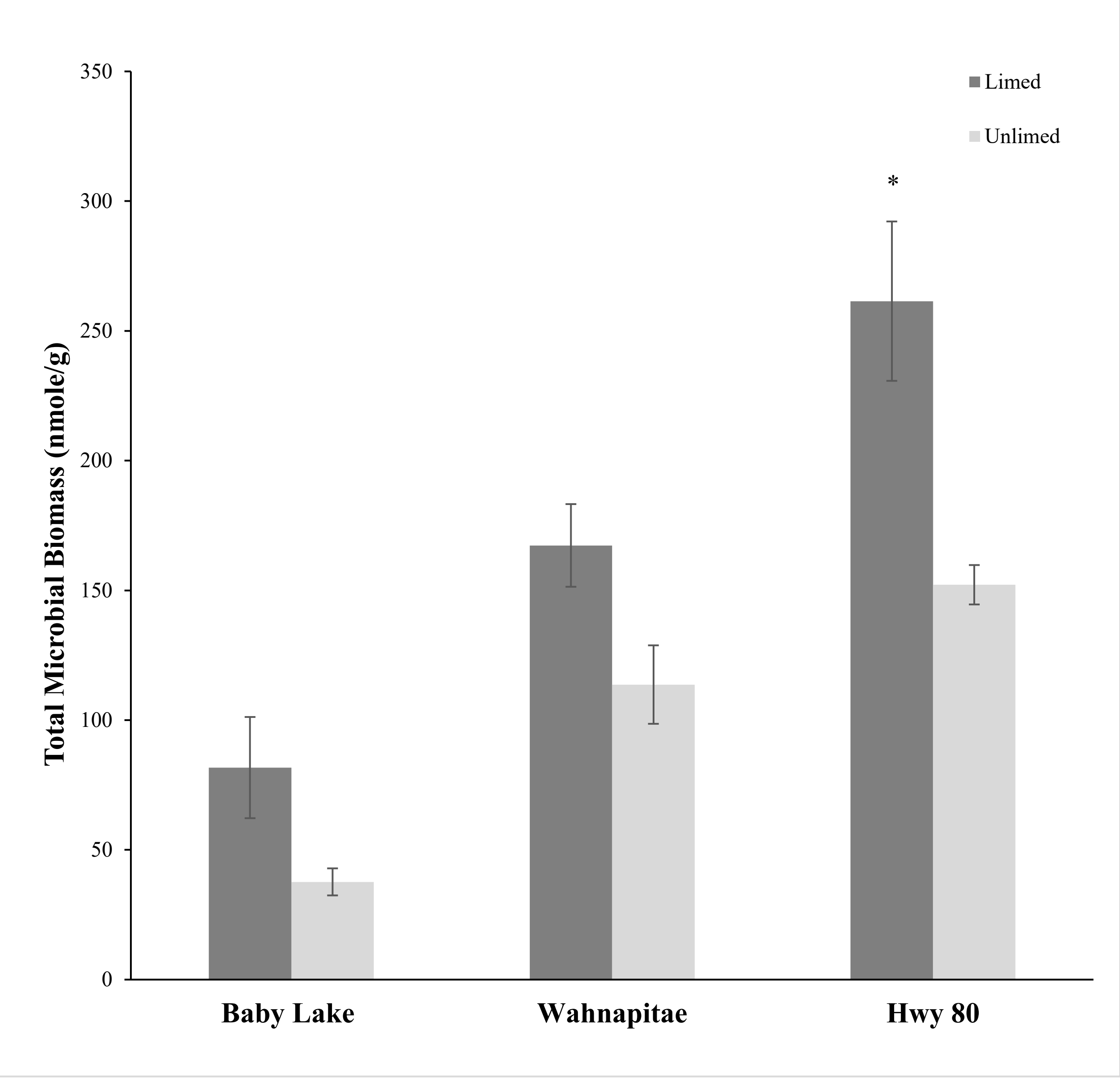

Figure 20. Total Microbial Biomass (in nmol/gram) in soils from limed and unlimed sites in the CGS. Means (±SE) are given (n = 3).

* Represents significant differences between limed and unlimed sites based on t-test ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

Table 3: Various organisms identified using phospholipid fatty acid (PLFA) analysis in soil samples from various limed and unlimed sites in the City of Greater Sudbury. Data is in nmole/gram.

Sites	**Total Microbial Biomass**	**AM Fungi**	**Fungi ***	**Gram Negative ***	**Gram Positive**	**Eukaryote ***	**Actinomycetes**
Limed	170.16 ± 28.37	5.39 ± 0.60	13.25 ± 2.37	69.25 ± 13.63	56.18 ± 8.53	6.73 ± 1.08	19.35 ± 2.51
Unlimed	101.18 ± 17.58	4.42 ± 0.82	4.65 ± 0.70	34.20 ± 6.43	37.67 ± 6.39	3.04 ± 0.65	17.10 ± 3.01

Data is reported as mean values ± standard error.

* Represent significance differences between limed and unlimed site based on t-test ($p \leq 0.05$)

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

Table 4: Various organisms identified using phospholipid fatty acid analysis (PLFA) in soil samples from various limed and unlimed sites in the City of Greater Sudbury. Data is in nmole/gram.

Sites	Total Microbial Biomass	AM Fungi	Fungi	Gram Negative	Gram Positive	Eukaryote	Actinomycetes
Baby Lake Limed	81.71 ± 19.50	3.55 ± 0.67	6.91 ± 2.92	25.76 ± 5.58	28.84 ± 11.32	4.89 ± 1.98	11.85 ± 1.87
Baby Lake Unlimed	37.66 ± 5.21	1.95 ± 0.37	3.15 ± 0.68	11.07 ± 1.33	14.07 ± 1.51	0.90 ± 0.35	6.51 ± 1.14
Wahnapitae Limed	167.32 ± 15.93	5.59 ± 0.59	12.52 ± 1.89	* 68.17 ± 5.70	57.08 ± 5.71	5.74 ± 0.84	18.22 ± 1.40
Wahnapitae Unlimed	113.70 ± 15.12	3.94 ± 0.49	6.15 ± 1.58	39.12 ± 6.45	42.72 ± 4.55	3.29 ± 0.60	18.18 ± 1.83
Hwy 80 N Limed	* 261.46 ± 30.71	7.03 ± 0.72	* 20.33 ± 3.16	* 113.82 ± 14.99	* 82.62 ± 8.59	9.63 ± 1.66	28.00 ± 2.04
Hwy 80 N Unlimed	152.17 ± 7.59	7.38 ± 0.39	4.64 ± 0.86	52.41 ± 2.74	56.22 ± 2.09	4.93 ± 0.68	26.60 ± 1.48

Data is reported as mean values ± standard error.

* Represent significance differences between limed and unlimed site based on t-test ($p \leq 0.05$)

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

Table 5: Phospholipid fatty acid (PLFA) ratios analyzed in soil samples from various limed and unlimed sites in the City of Greater Sudbury.

Sites	**Fungi/ Bacteria**	**Predator/ Prey** *	**Gram positive/ Gram negative** *	**Saturated/ Unsaturated** *	**Mono/ Poly** *	**Gram Negative Stress** *
Limed	0.15 ± 0.006	0.06 ± 0.004	1.00 ± 0.089	1.28 ± 0.062	3.02 ± 0.183	1.00 ± 0.071
Unlimed	0.15 ± 0.016	0.05 ± 0.004	1.39 ± 0.050	1.63 ± 0.135	4.05 ± 0.397	0.75 ± 0.050

Data is reported as mean values ± standard error.

* Represent significance differences between limed and unlimed site based on t-test ($p \leq 0.05$)

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

Table 6: Phospholipid fatty acid (PLFA) ratios analyzed in soil samples from each limed and unlimed site sampled in the City of Greater Sudbury.

Sites	**Fungi/ Bacteria**	**Predator/ Prey**	**Gram positive/ Gram negative**	**Saturated/ Unsaturated**	**Mono/ Poly**	**Gram negative Stress**
Baby Lake Limed	0.18 ± 0.02	0.09 ± 0.02	1.34 ± 0.05	1.45 ± 0.11	2.56 ± 0.39	* 1.04 ± 0.10
Baby Lake Unlimed	0.20 ± 0.02	0.04 ± 0.01	1.52 ± 0.05	2.00 ± 0.27	2.94 ± 0.41	0.63 ± 0.10
Wahnapitae Limed	0.14 ± 0.01	0.05 ± 0.00	* 0.91 ± 0.02	1.27 ± 0.06	3.37 ± 0.20	* 1.16 ± 0.08
Wahnapitae Unlimed	0.13 ± 0.01	0.05 ± 0.01	1.33 ± 0.12	1.58 ± 0.15	3.88 ± 0.30	0.72 ± 0.03
Hwy 80 N Limed	* 0.14 ± 0.00	0.06 ± 0.00	* 0.76 ± 0.05	1.13 ± 0.07	* 3.14 ± 0.19	0.82 ± 0.12
Hwy 80 N Unlimed	0.11 ± 0.01	0.05 ± 0.01	1.33 ± 0.04	1.31 ± 0.05	5.31 ± 0.45	0.90 ± 0.03

Data is reported as mean values ± standard error.

* Represent significance differences between limed and unlimed site based on t-test ($p \leq 0.05$)

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

3.4 Discussion

3.4.1 Effects of Liming

Soil properties such as pH and microbial enzymatic activities have previously shown to improve with the addition of dolomitic lime in the Greater Sudbury area (Narendrula-Kotha and Nkongolo, 2017b). In the present study, results also show that liming significantly ($P \leq 0.05$) improves pH, total microbial biomass, and overall GAP and NAGase activities in soil compared to unlimed soil. Additionally, the results of this study show increases in enzyme activities and bacterial and fungal biomasses at specific sites. The results of this study are consistent with previous findings from different soil conditions in Greater Sudbury (Goupil and Nkongolo, 2014; Narendrula-Kotha and Nkongolo, 2017b; Nkongolo et al., 2016).

3.4.2 Soil Enzyme Activity

3.4.2.1 Activity of β-glucosidase

β-glucosidase (BG) is a well-studied soil microbial enzyme that is important to a number of biochemical processes, such as the degradation of starch and the nutrient cycling of carbon molecules during cellobiose hydrolysis (Xue et al., 2018). BG is a catalyst for the hydrolysis of glycosidic linkages which aids in the degradation of organic carbon compounds and their products within the soil (Acosta-Martinez and Tabatabai, 2000; Nielsen and Winding, 2002). It has been shown that an increase in soil pH from acidic to neutral levels leads to an increase in BG activity (Acosta-Martinez and Tabatabai, 2000), and in addition to favouring a neutral pH, BG activity is related to organic matter content, biological activity, and carbon cycling of the soil ecosystem, making BG activity a favourable indicator for soil and ecosystem health (Adetunji et al., 2017).

In this study, BG was not found to be significantly increased across all three limed sites, however at Baby Lake, the limed soil samples showed a significant ($P \leq 0.05$) increase in BG activity compared to the unlimed soils (Fig. 11). These findings are somewhat consistent with previous studies in the CGS area, which have reported an increase in BG activities in limed soils compared to unlimed soils (Narendrula-Kotha and Nkongolo, 2017b).

3.4.2.2 Activity of Cellobiohydrolase

Cellobiohydrolase (CBH), similar to BG, is important for carbon cycling, where it aids in the breakdown of carbohydrates and polysaccharides, the products of which can be used as an energy source for soil microorganisms (Kardol et al., 2010). More specifically, CBH breaks down cellulose through hydrolysis of the glycosidic bonds in cellulose chains (Verchot and Borelli, 2005). Studies have shown that soils with a high organic N content have an increased CBH activity (R. Chen et al., 2013; Saiya-Cork et al., 2002). Previous investigations in the CGS reported that limed soil contained greater amounts of organic N than unlimed sites (Narendrula-Kotha and Nkongolo, 2017a).

In this study, CBH activity was found to be significantly ($P \leq 0.05$) higher at Baby Lake's limed sites compared to the unlimed areas, however at Highway 80 North, CBH activity were found to have significantly ($P \leq 0.05$) greater in unlimed sites than the limed sites (Fig. 12). This contrasts with previous studies reported by (Narendrula-Kotha and Nkongolo, 2017b).

3.4.2.3 Activity of β-N-acetylglucosaminidase

β-N-acetylglucosaminidase (NAGase) is an enzyme that degrades chitin through hydrolysis of glycosidic bonds into N-acetylglucosamine (Kardol et al., 2010; Verchot and Borelli, 2005). Chitin is an important source of nitrogen in terrestrial ecosystems where it can be found in the walls of fungi and some arthropod species' exoskeletons. Chen et al. (2011) reported that NAGase activity is the highest in a pH 7 environment.

In this study, NAGase activity was found to be significantly ($P \leq 0.05$) increased at all three limed sites compared to their unlimed areas (Fig. 8 and 13), which is consistent with previous studies' results from limed soils the CGS area (Narendrula-Kotha and Nkongolo, 2017b). This is also consistent with increased soil pH at all the limed sites.

3.4.2.4 Activity of Arylsulfatase

Arylsulfatase (AS) is an important enzyme in catalyzing the hydrolysis of aryl sulfate esters in soil (Acosta-Martinez and Tabatabai, 2000; Spencer, 1958). Inorganic and organic forms of sulfur can be found in soils, with ester sulfates accounting for up to 70% of total sulfur content found in soil surface layers (Klose et al., 1999). AS activity has been found to increase with greater amounts of soil organic matter (Xian et al., 2015). Increases in soil pH towards neutral has also been found to increase AS enzymatic activity (Acosta-Martinez and Tabatabai, 2000), and similar findings have been observed in limed soils as well (Ekenler and Tabatabai, 2003b).

The results of this study are consistent with these trends, as AS was significantly ($P \leq 0.05$) increased overall at the three limed sites compared to the three unlimed areas (Fig. 8) and was significantly ($P \leq 0.05$) increased at the limed Wahnapitae and Baby Lake sites compared to their respective unlimed sites (Fig. 16). These AS activities increase at the Wahnapitae and Baby Lake limed sites are also consistent with significant ($P \leq 0.05$) pH increases at both limed sites (Fig. 2) and a significant ($P \leq 0.05$) increase in % organic carbon at Baby Lake's limed site (Fig. 3).

3.4.2.5 Activity of Acid and Alkaline Phosphatase

Acid and alkaline phosphatase (AP and ALP respectively) are enzymes that catalyze the hydrolysis of phosphate ester bonds, releasing phosphate (P) into the soil which can then be taken up by plants and microorganisms (Adetunji et al., 2017; Ekenler and Tabatabai, 2003b). Soil pH has been found to affect the activity of phosphatases (Adetunji et al., 2017), where AP activity increases in acidic soils while ALP activity increases in alkaline soils (Acosta-Martinez and Tabatabai, 2000). A study by Weintraub (2011) found that arbuscular mycorrhizal (AM) fungi stimulate the release of AP in soils, with plants and bacteria contributing to AP activity, while ALP activity mainly comes from bacterial species.

In this study, the activity of both AP and ALP enzymes were found to have significantly ($P \leq 0.05$) increased at the Baby Lake limed sites compared to the unlimed sites. An opposite trend was observed at Wahnapitae where the activities of these enzymes decreased significantly in limed sites compared to the unlimed areas (Fig. 14 and 15). More research is needed to understand why AP and ALP levels have increased with the addition of lime in some areas and decreased in others.

3.4.2.6 Activity of Aminopeptidase

Aminopeptidases are important enzymes in nitrogen cycling and in the degradation of intracellular and extracellular peptides into amino acids for use in protein synthesis (Norman et al., 2020; Stark et al., 2014). Leucine aminopeptidase (LAP) is involved in the release of hydrophobic amino acids from polypeptides (Hagmann et al., 2015), while glycine aminopeptidase (GAP) hydrolyzes glycine-bonded amino acids, peptides, or arylamide bonds (GLY-X bonds) (Ito et al., 2003).

In this study, LAP activity was found to be significantly ($p \leq 0.05$) increased at the Baby Lake limed sites compared to the unlimed sites, however it was also found to be significantly ($p \leq 0.05$) decreased at the Highway 80 North limed sites compared to the unlimed sites (Fig. 18). GAP activity was found to be significantly ($p \leq 0.05$) increased overall at the limed sites compared to the unlimed sites (Fig. 9), which is consistent with the results from previous studies in the CGS area (Narendrula-Kotha and Nkongolo, 2017b).

3.4.2.7 Activity of Peroxidase

Peroxidases (PER) are a well-researched class of enzyme that uses H_2O_2 to degrade aromatic compounds (Johnsen and Jacobsen, 2008; Sinsabaugh, 2010). Fungal species such as white rot (*Basidomycetes)*, as well as bacterial species in the phylum *actinomycetes* are amongst some of the soil microorganisms known to produce extracellular peroxidases (Sinsabaugh, 2010). PER activity levels have been found to increase in acidic conditions, as well as in N-amended soils (Sinsabaugh, 2010).

In this study, PER activity was only increased significantly ($P \leq 0.05$) at the Baby Lake limed sites (compared to the unlimed sites) (Fig. 19), which is not consistent with previous studies of the CGS area (Narendrula-Kotha and Nkongolo, 2017b).

3.4.3 PLFA Analysis

PLFA analysis was used in this study to determine soil microbial responses to liming at a broad level. The results of this study show a significant ($P \leq 0.05$) increase in fungi, gram negative, and eukaryotic biomasses at the three limed sites compared to the unlimed sites (Table 3), and although not significant ($P \leq 0.05$), an increase in total microbial biomass at the limed sites compared to the unlimed sites was also seen (Fig. 20). These differences between limed and unlimed sites in their microbial biomasses can be partially attributed to changes in soil pH with the addition of lime.

Studies have shown that acidic soil conditions enhance the overall activity and development of fungal species compared to bacteria (Kaur et al., 2005; Narendrula and Nkongolo, 2015). In this study PLFA's 16:0, 18:1ω9, and 18:2ω6 are considered reliable indicators of fungal biomass, and significantly ($p \leq 0.05$) greater concentrations were found in the limed soils compared to the unlimed soils (Table 3). However, PLFA's 18:1ω9 and 18:2ω6 are not exclusively found in fungi; they are also present in plants and other eukaryotic organisms (Frostegård and Bååth, 1996; Narendrula and Nkongolo, 2015), so it is possible that some of these PLFA's that were identified at the limed sites are not from fungal species, but rather from plants or other eukaryotes. The PLFA results of this study show significantly ($P \leq 0.05$) greater amounts of gram negative PLFA's were present in the limed soils compared to the unlimed soils (Table 3). The PLFA's used as indicators of gram negative bacteria include 16:1ω5, 16:1ω9, 17:1ω9, cy17:0, 18:1ω7, and cy19:0 (Frostegård and Bååth, 1996; Kaur et al., 2005).

In addition to microbial biomasses and abundances, PLFA analysis can also be useful in analyzing the physiological state of the microorganisms present in an ecosystem. Significant ($P \leq 0.05$) increases in saturated/unsaturated and Gram positive/gram negative ratios in the unlimed soils in this study (Table 5) are consistent with stressed environmental conditions (Åkerblom et al., 2007; Kaur et al., 2005). An increase in the mono/poly ratio in unlimed sites compared to the limed sites in addition to the saturated/unsaturated and gram positive/gram negative ratios help confirm that these sites are still under environmental stress.

3.5 Conclusions

In the present study, microbial biomass, relative abundances, and community structure were analyzed in three aerially limed sites and compared to adjacent unlimed areas. Metal concentrations of iron (Fe) and arsenic (As) were significantly higher at the unlimed sites. As expected, (Ca) was significantly increased at the limed sites. Enzyme assays and Phospholipid fatty acid (PLFA) analysis were used to assess three limed soil sites and their adjacent unlimed soils. β-N-acetylglucosaminidase (BG), aryl sulfatase (AS), and glycine aminopeptidase (GAP) activity levels were significantly increased in the limed soils compared to the unlimed soils. PLFA analysis revealed an increase in total microbial biomass, fungal, gram negative, and eukaryotic biomasses at the limed sites in comparison to the unlimed sites. This research also shows that the unlimed sites in particular are still undergoing environmental stress from acidified soil and metal contamination.

Chapter 4. General Conclusions

The aim of this study was to analyze soil biochemical properties in metal contaminated and reclaimed land within the CGS. This is a novel study in that the three sites chosen for analysis are aerially limed and have not been studied before.

Soil pH analysis revealed that the addition of lime significantly increased soil pH, even years later. The level organic content was generally not significantly different between the limed areas and the unlimed areas, however a significant increase was seen at one of the limed sites compared to its adjacent unlimed site. Heavy metal concentrations varied between sites, however iron and arsenic concentrations were significantly increased in unlimed soil samples compared to the limed soil samples. Calcium was found to be significantly higher in limed soils, likely due to the addition of dolomite. The lack of a significant increase of Mg in limed sites suggests that the type of lime used was solely calcium-based.

The relative abundances of fungi, gram negative bacteria, and eukaryotic microorganisms were significantly increased at the limed sites in addition to a slight increase in total microbial biomass. The ratios of mono/poly, saturated/unsaturated, and gram negative/gram positive are all significantly higher in the unlimed soils, which confirms that the unlimed sites are still experiencing environmental stress in comparison to the limed sites.

Enzyme activity data for the three sites provide valuable information on the health, activity, and function of the ecosystems at each location. Detailed analysis showed that aerial liming has led to changes in soil enzyme activities, pH, microbial biomass, and metal concentrations. Liming increased β-N-acetylglucosaminidase, aryl sulfatase, and glycine aminopeptidase activity levels between all three sites compared to the unlimed sites.

Overall, the results of this study demonstrated the effectiveness of aerial liming to improve metal contaminated, acidified soil in the CGS. This method of liming is practical for large areas, can be performed for regions not easily accessible such as hills, deep valleys, rocky lands where manual liming will be challenging to apply. Long-term exposure to contamination and acidic soils has led to reduced microbial biomass and abundance and decreases in enzymatic activity. Aerial liming increases soil pH, percent organic carbon, microbial biomass and abundance, enzymatic activities, and decreases the concentrations of heavy metals.

Further research can be performed on the specific microbial community structure using other advanced techniques such as Illumina MiSeq sequencing to determine the microbial species abudance and diversity. Research on the bioavailable nutrients and heavy metals within these limed and unlimed soils would also be a valuable area to study further. This information would be useful for a more complete understanding of factors affecting microbial diversity, abundance, and the effects of limestone on reclaimed terrestrial ecosystems.

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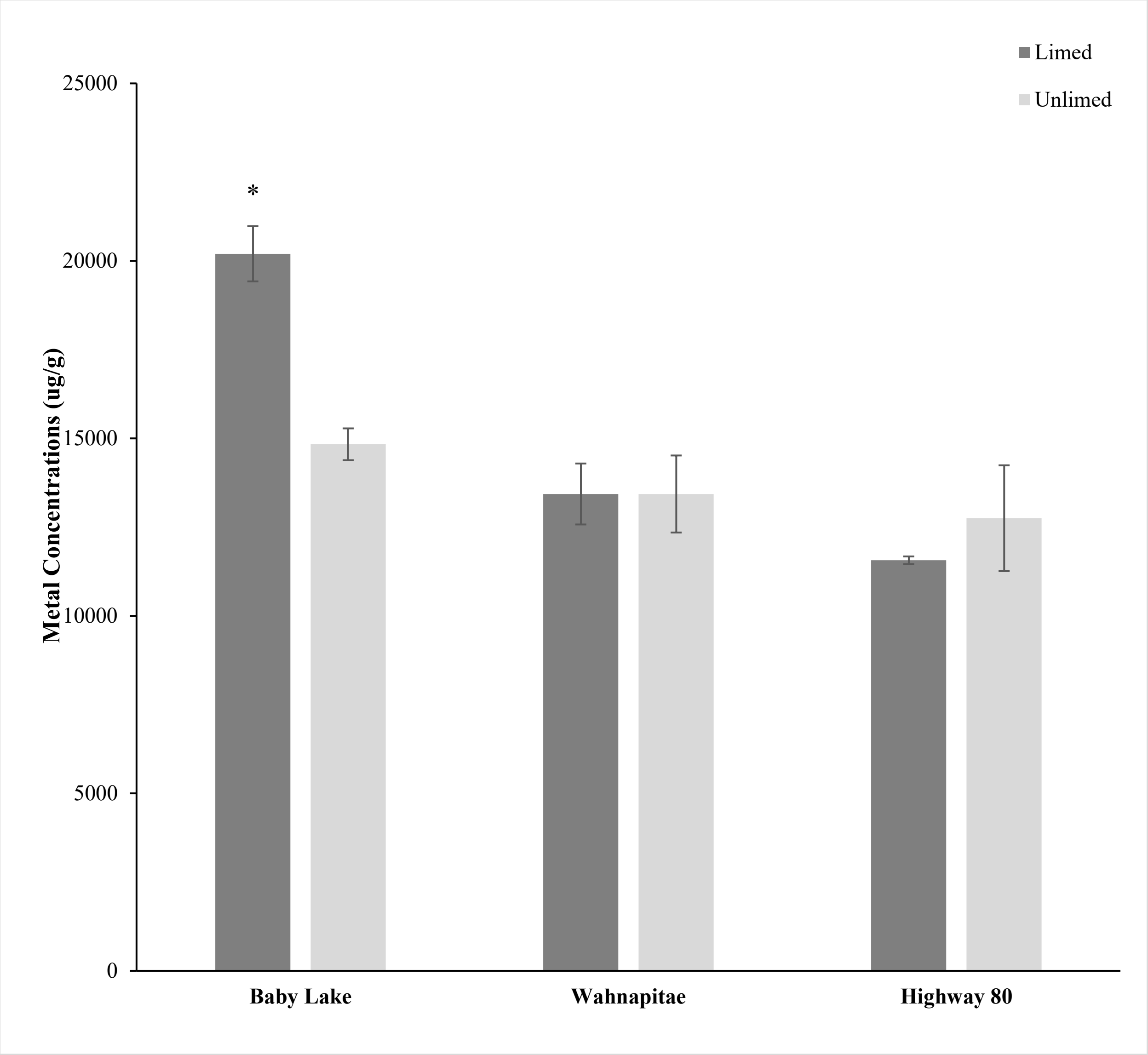
Appendices

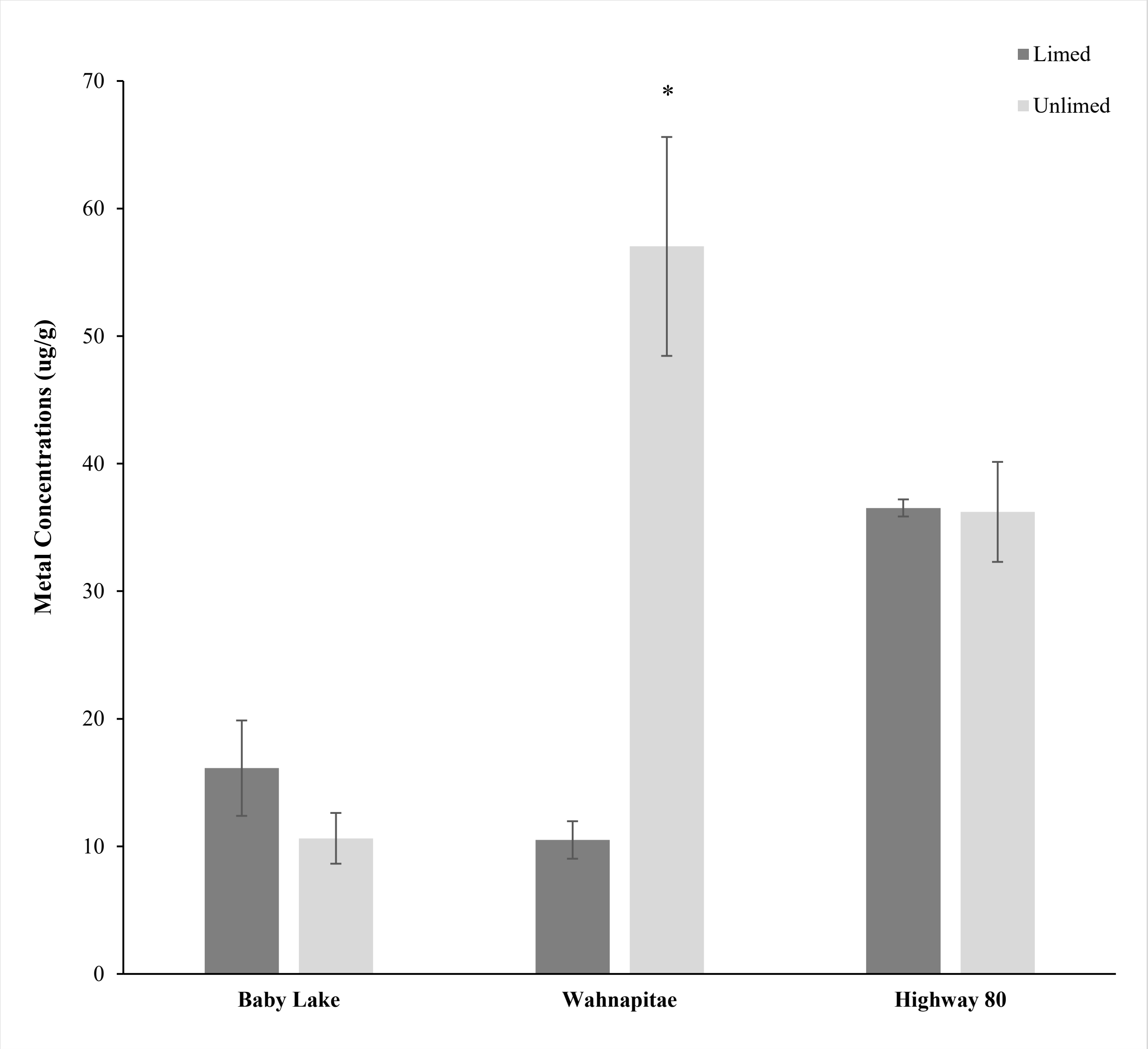


Appendix 1. The metal concentration of aluminum in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

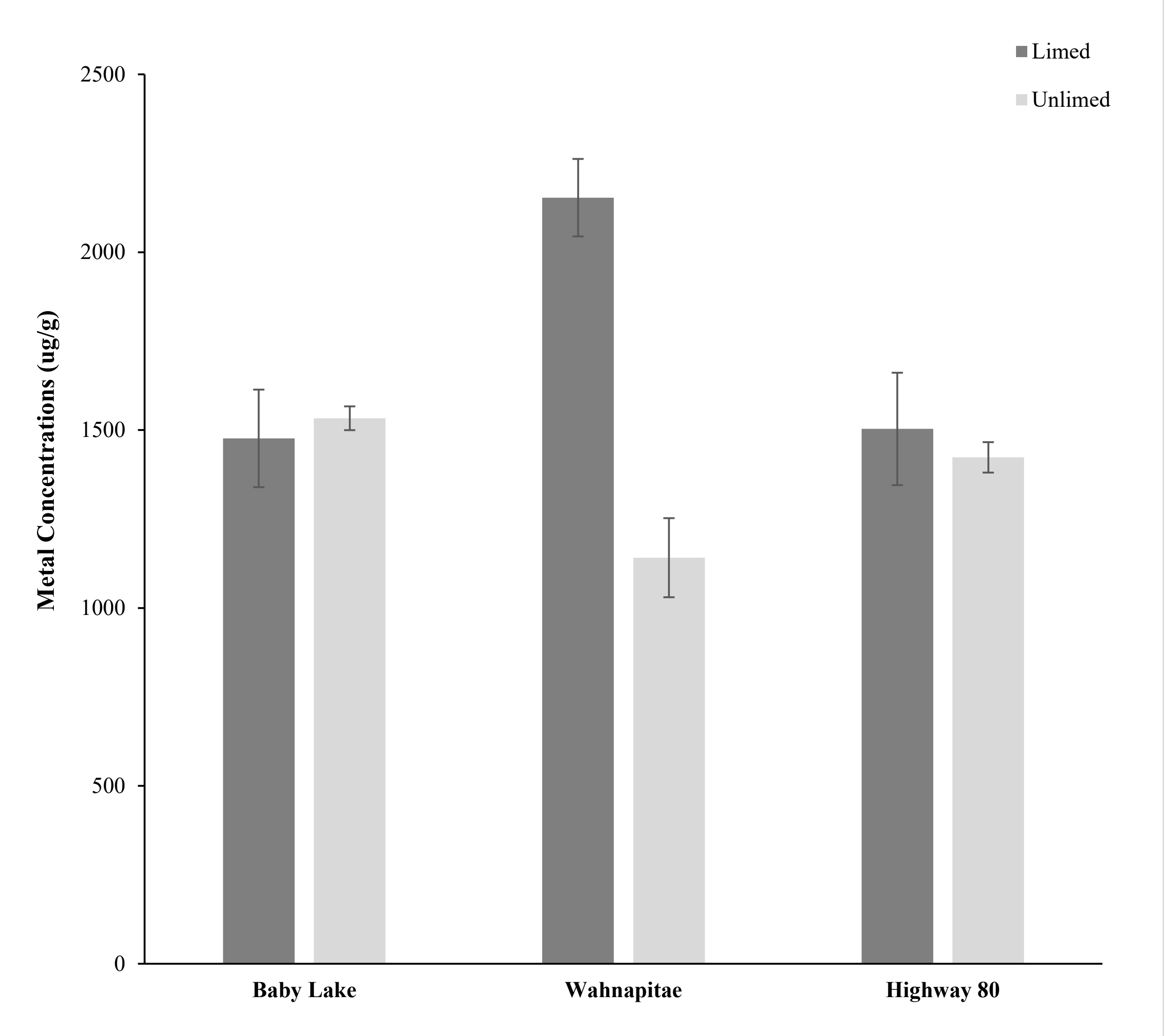


Appendix 2. The metal concentration of arsenic in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

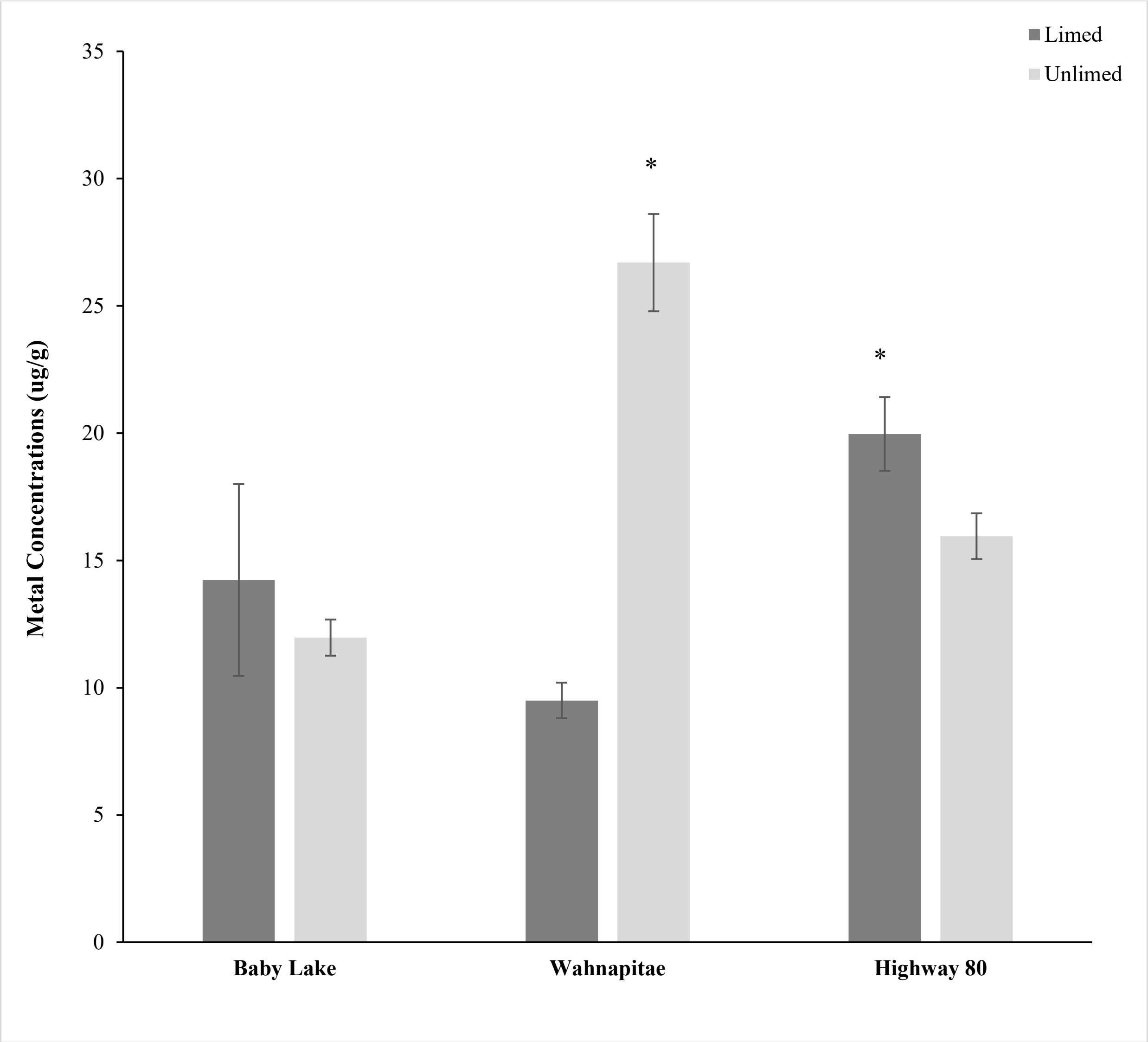


Appendix 3. The metal concentration of calcium in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

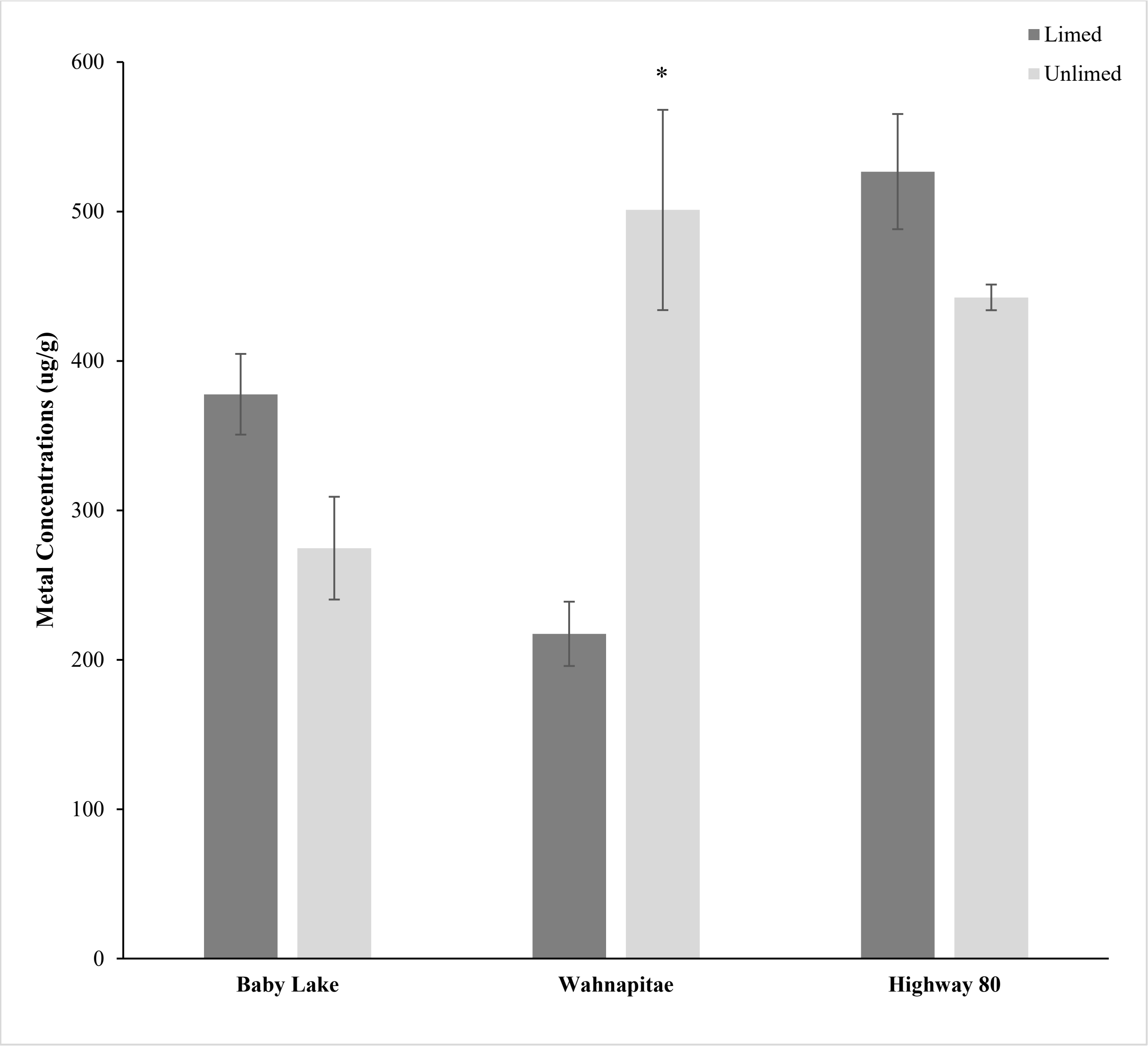


Appendix 4. The metal concentration of cobalt in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

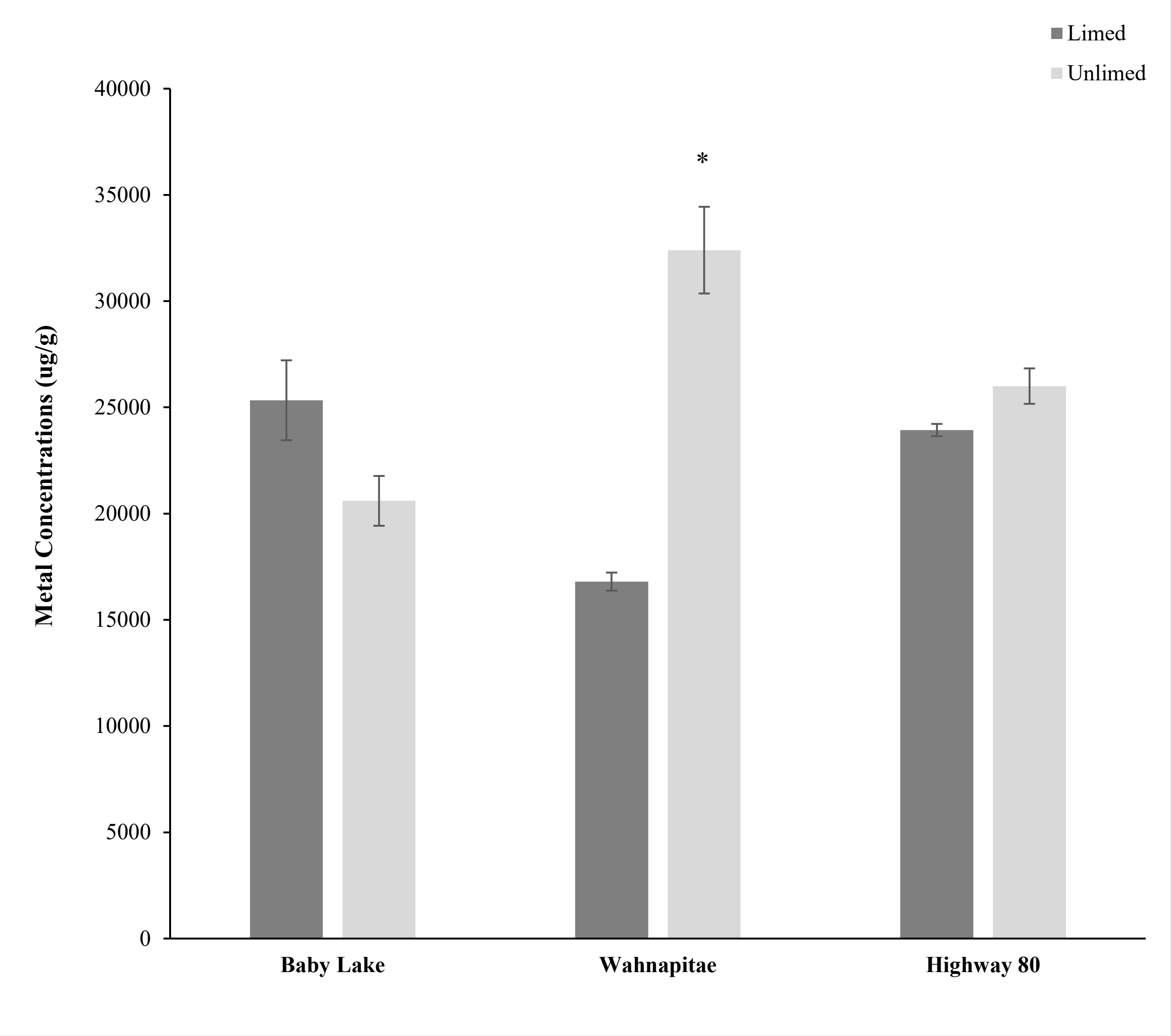


Appendix 5. The metal concentration of copper in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

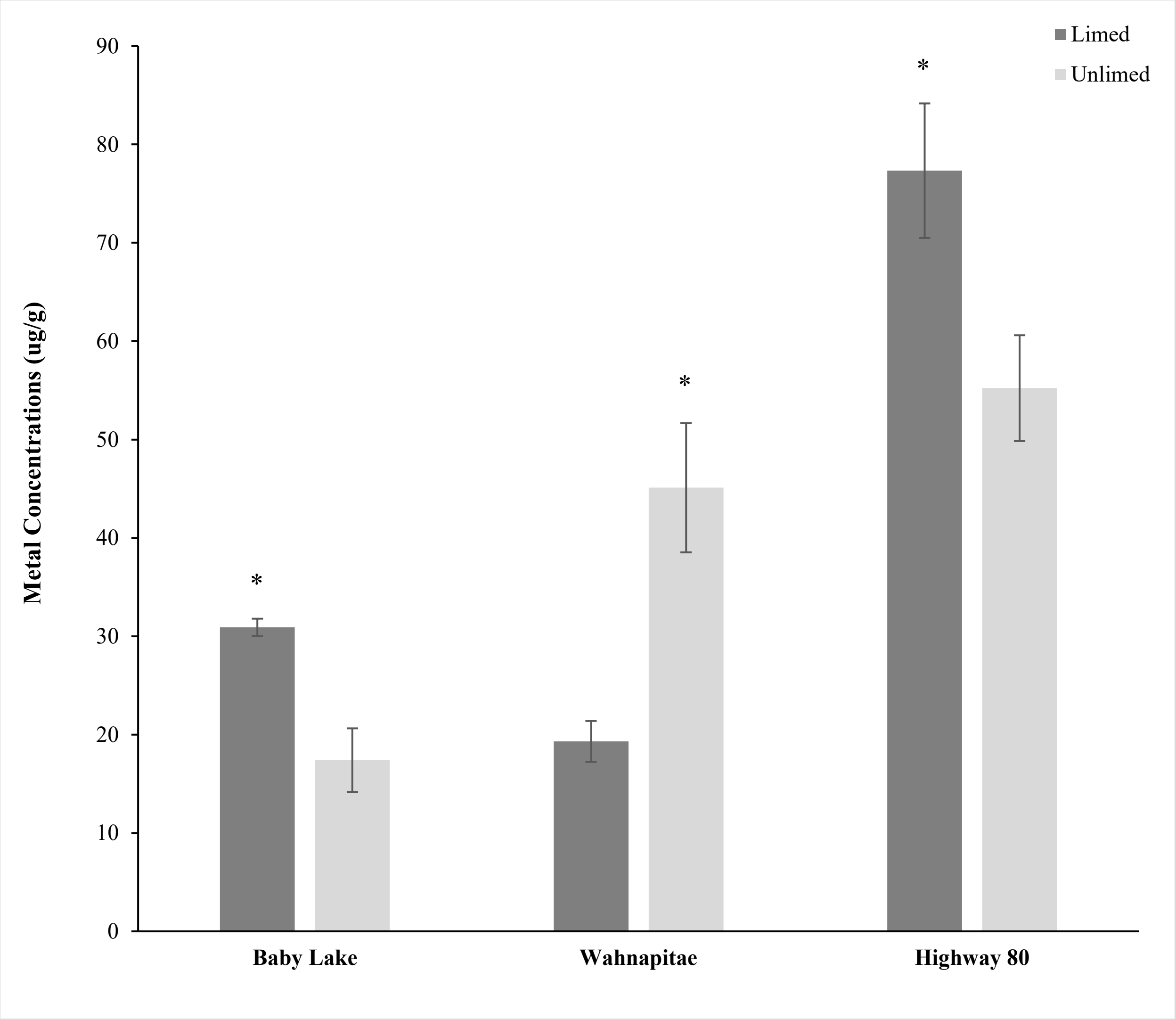


Appendix 6. The metal concentration of iron in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

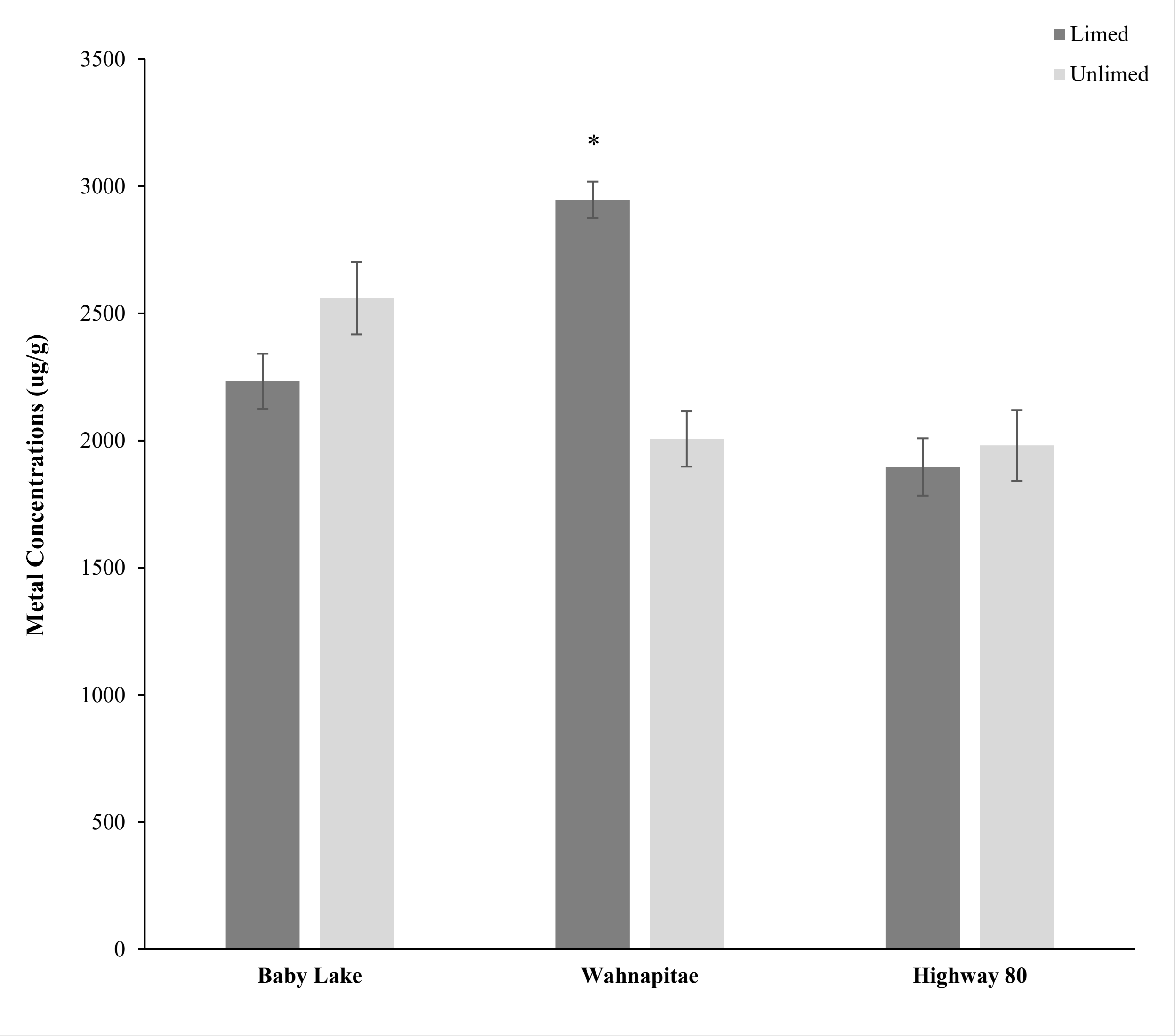


Appendix 7. The metal concentration of lead in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

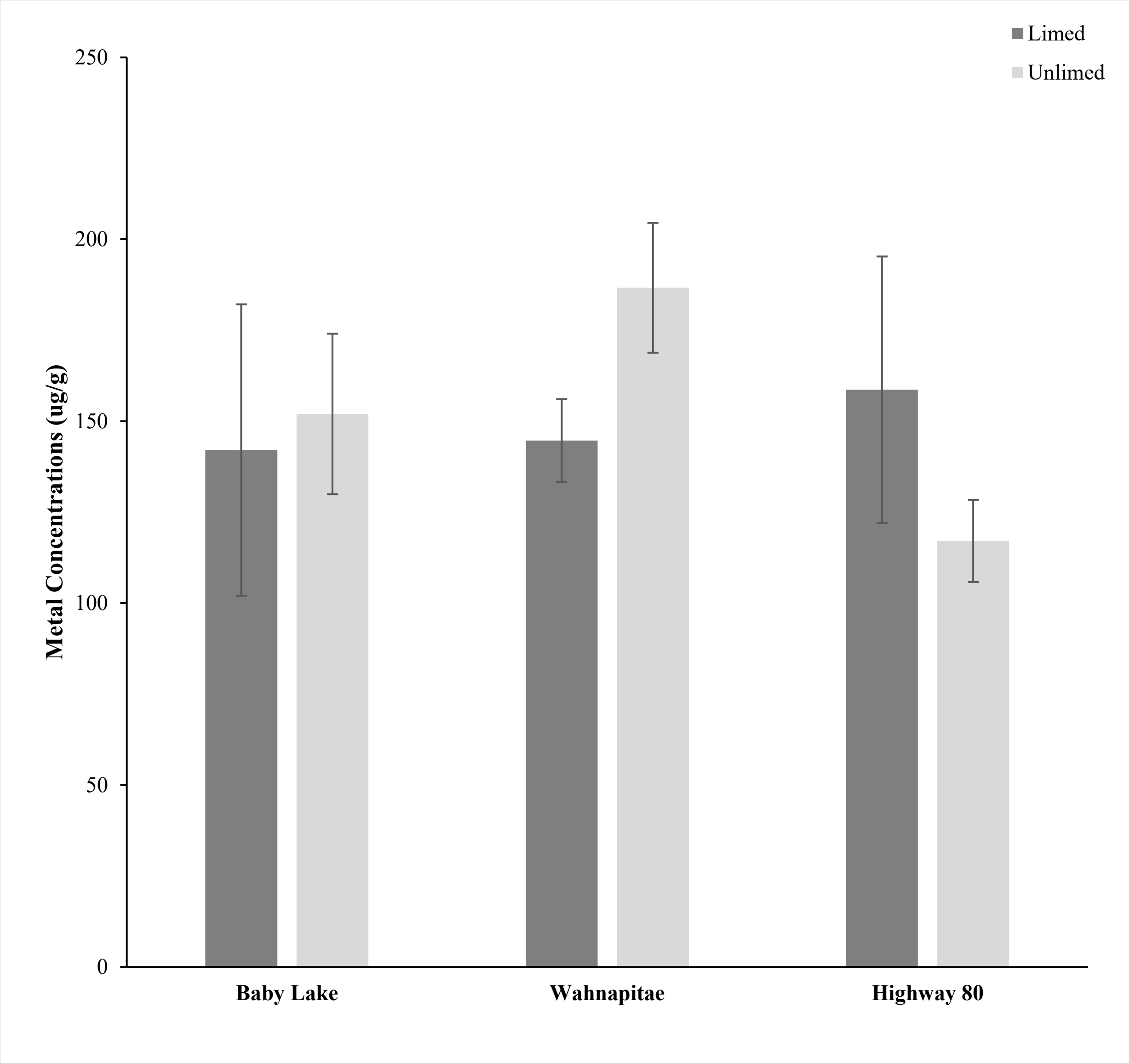


Appendix 8. The metal concentration of magnesium in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

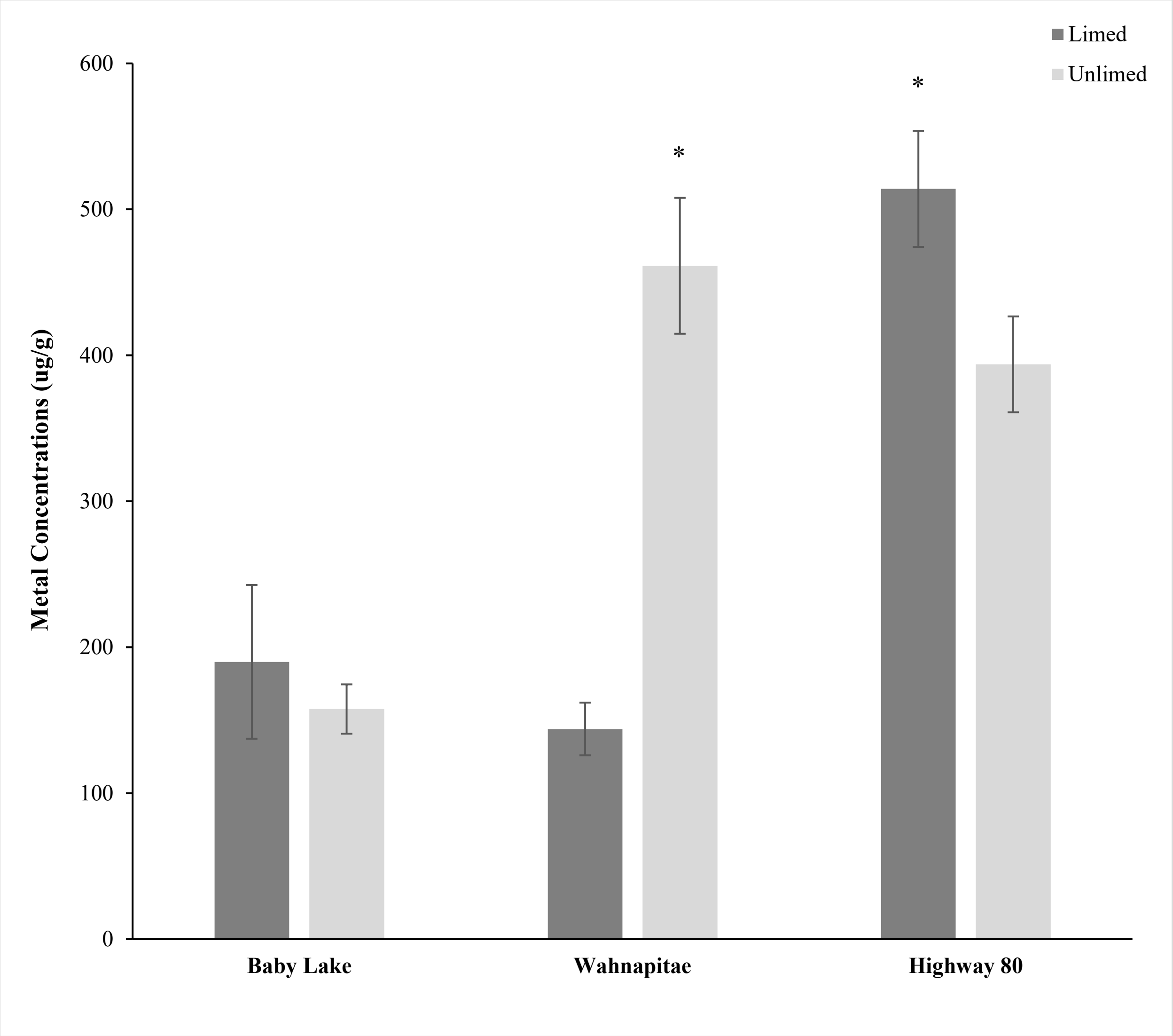


Appendix 9. The metal concentration of manganese in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

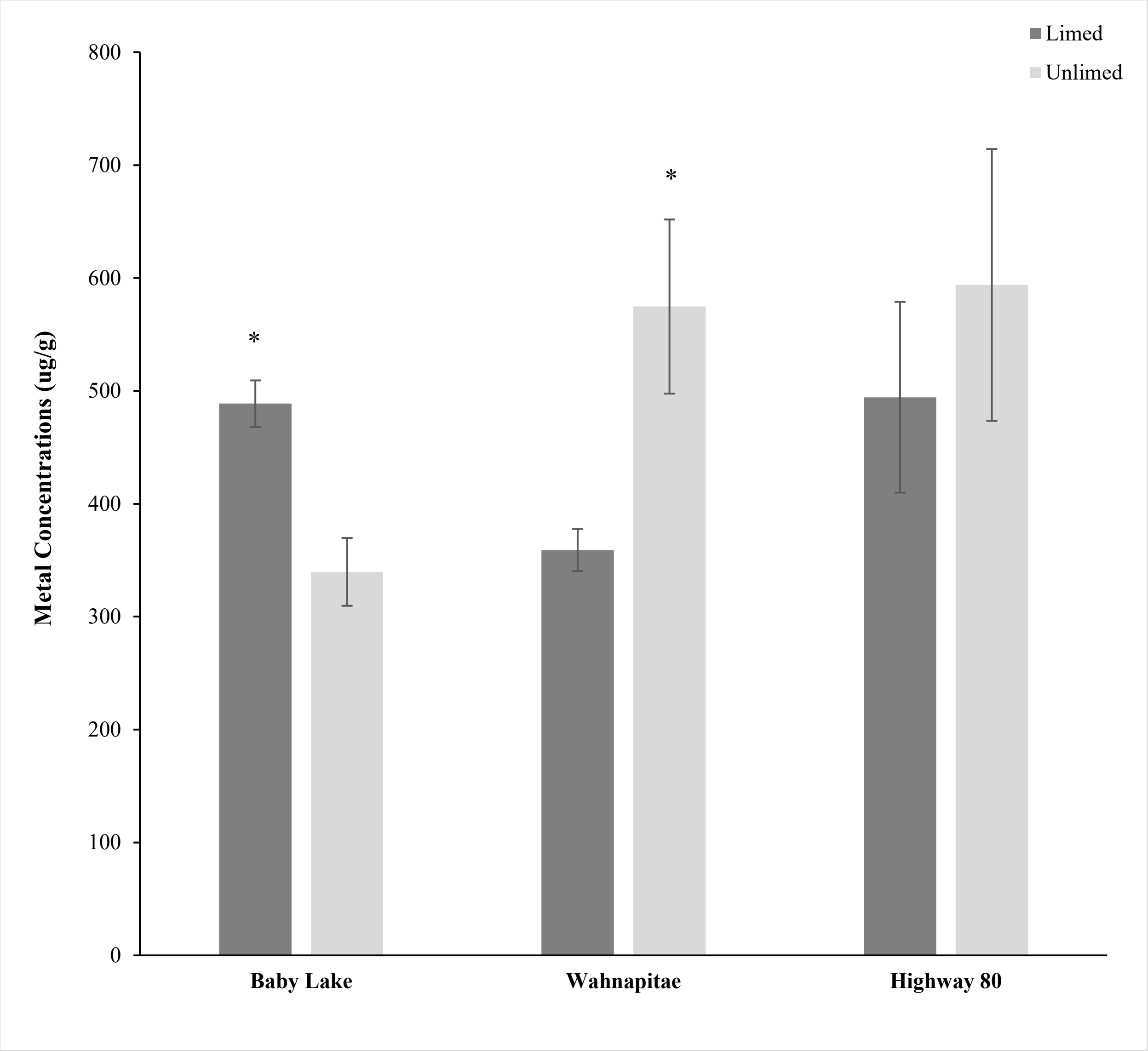


Appendix 10. The metal concentration of nickel in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

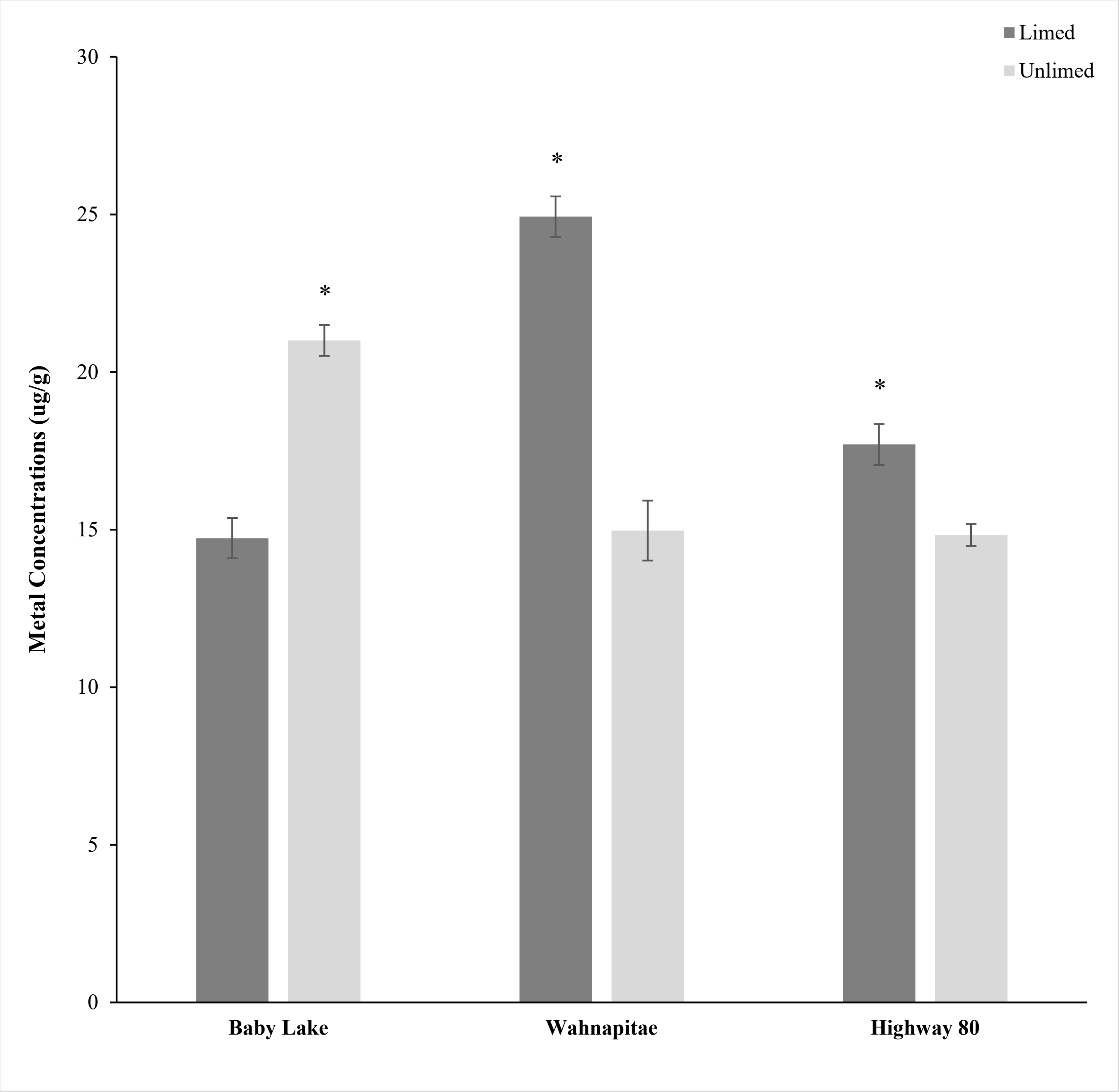


Appendix 11. The metal concentration of phosphorous in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

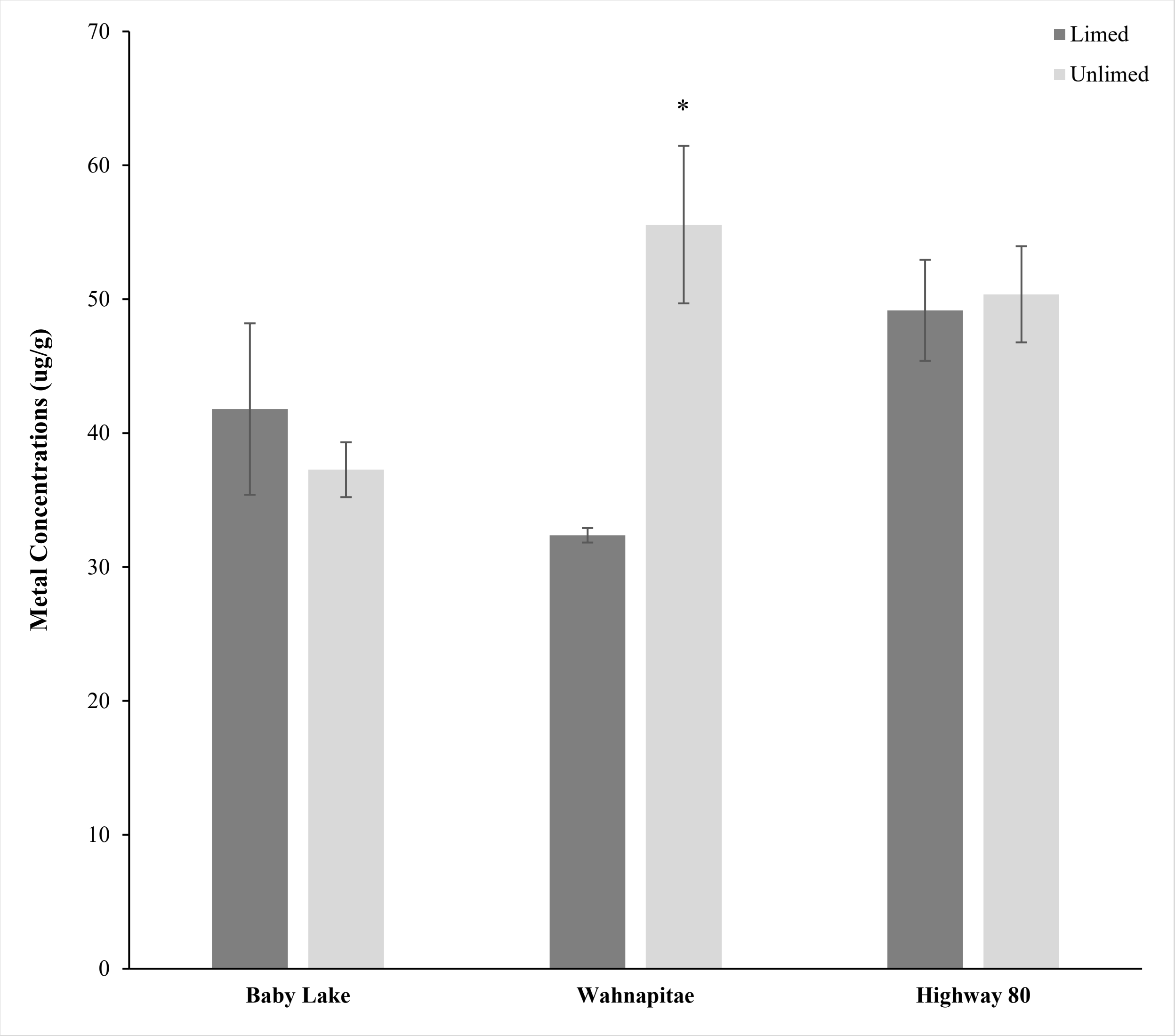


Appendix 12. The metal concentration of strontium in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.



Appendix 13. The metal concentration of zinc in soils from limed and unlimed sites in the Greater Sudbury Region (n = 27). Means (±SE) are given (n = 9).

Limed and Unlimed sites: Highway 80 North, Baby Lake, Wahnapitae

* Represents significant differences between limed and unlimed sites based on Mann-Whitney testing ($P \leq 0.05$). Bars represent standard errors (SE).

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.

Appendix 14: Metal concentration in ug/g for all limed sites compared to all unlimed sites.

	Al	As*	Ca*	Co	Cu	Fe*	Pb	Mg	Mn	Ni	P	Sr	Zn
Limed sites	15066 ±1295	21.06 ±3.97	1711.11 ±130.60	14.57 ±1.97	373.89 ±45.50	22022 ±1405	42.51 ±8.70	2358.89 ±18.64	148.48 ±18.64	282.67 ±59.43	447.33 ±36.23	19.12 ±1.48	41.11 ±3.38
Unlimed sites	13672 ±695	34.63 ±7.09	1366.00 ±68.77	18.21 ±2.20	406.06 ±40.76	26333 ±1810	39.24 ±6.13	2182.78 ±116.67	151.93 ±13.89	337.61 ±47.70	502.72 ±62.09	16.93 ±1.03	47.73 ±3.51

Results are expressed as mean values ± standard error.

* Represent significance differences between limed and unlimed site based on t-test ($p \leq 0.05$)

Year sites were limed: Wahnapitae limed in 2009; Baby Lake limed in 2018; Highway 80 North limed in 2004.