Lowbush Blueberries in Northwestern Ontario as a Commercial Crop Option in Post-Harvest Peatlands

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Abstract

Wild blueberries (*Vaccinium angustifolium* Ait.) were investigated as a possible reclamation tool for post harvest peatlands in Northwestern Ontario, by genetic fingerprinting using microsatellite markers, an examination of native blueberry soils in comparison with the GG3 peatland near Upsala, ON and identification of optimal soils in the GG3 peatland using GIS-based approach and multivariate statistics.

The genetic diversity of natural populations of *V. angustifolium* Ait. was investigated using four microsatellite markers developed for *Vaccinium corymbosum* L. and compared to nineteen commercial wild blueberry plants. Fifty blueberry plants collected from across Northwestern Ontario were selected to obtain genetic fingerprints and to estimate genetic similarity by microsatellite analysis. The microsatellite data set was analyzed using POPGENE. Each of the microsatellite primers was polymorphic creating a total of 133 bands with the proportion of polymorphic loci at 97%, with none of them in all of the plant samples. The number of alleles per locus was 33.35 with a range of 13 to 51. The average genetic similarity was 97%. The corresponding nonmetric multidimensional scaling analysis supported the clusters and showed the separation of the commercial plants from the wild plants from Northwestern Ontario and distinct outliers, plants from Nipigon and Sioux Lookout. Low gene flow could be attributed to fragmented habitat due to their need for open sunlight and acid soils. Microsatellite analysis is a beneficial tool for genetic fingerprinting and identification, as well as useful for investigating genetic similarities and differences.

We studied the soil chemistry of eight different native blueberry stands across Northwestern Ontario and compared them to 210 peat soil samples from the GG3

peatland near Upsala, ON. Comparing these soils to the Canadian Soil Quality Guidelines revealed that arsenic levels for the site near Wawa could present phytotoxicity in blueberry plants. Nonmetric multidimensional scaling showed a distinct separation of blueberry soils from the peat soils along the second axis. Bulk density was one of the main differences and could be increased by tilling the mineral subsoil into the peat in the preparation for blueberry planting. Layers within the peat bog were difficult to separate in the statistical analysis. Leaf tissue analysis would help identify nutrient deficiencies better than soils analysis due to the perennial nature of the blueberry plants.

Identification of optimal areas of soil within the GG3 peatland would ensure the success of using blueberries for reclamation. Using the GIS software, ArcGIS, nonmetric multidimensional scaling values for axis two were mapped for the seven individual layers of the peatland. A band, running from east to west in the middle of the peatland showed soil chemistry values closest to the native blueberry soils. An upper layer of the peat should be reserved for use in the blueberry reclamation as it would have the least issues with phytotoxicity due to high elemental content.

Acknowledgements

Necessity is the mother of invention. – Jonathan Swift

Necessity, thou best as peacemakers, as well as surest prompter of invention. – Sir Walter Scott.

Necessity is the mother of invention, it is true – but its father is creativity and knowledge is the midwife. – Jonathon Schattke

I don't think necessity is the mother of invention -- invention . . . arises directly from idleness, possibly also from laziness. To save oneself trouble. – Agatha Christie

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General Introduction

Review of Peat Harvesting Project

Peat has long been a source of energy in various cultures in areas across the Northern Hemisphere including Scandinavia, Ireland and Russia (Holmgren et al. 2008). Traditionally peat has been harvested in a "dry" method which includes draining the wetland for a few years by digging ditches throughout the area and then removing the dried peat from the land by using several different methods (Lindstrom 1980). This traditional method has many issues when it comes to rehabilitate the former wetland. A new wet harvest method of peat removal has been proposed for peat lands in Northern Ontario, followed by pelleting the peat for use as fuel for the Atikokan Generating Station. The wet harvest method would not drain water from the wetland, but would harvest the wet peat, squeeze the water from the peat through the pelleting process, and filter this water through an existing wetland (Peat Resources Ltd 2008). In some instances, the water table may be altered following the peat harvest activities. Habitat could be dry or flooded depending on the bottom surface of the peat land and the amount of water contained in the peat. The drier areas could be potentially rehabilitated using lowbush blueberries, *Vaccinium angustifolium* Ait. Primarily, possibly combined with some *Vaccinium myrtiloides* Michx., which are native succession species in Northern Ontario.

Peat lands refer to an area with or without vegetation with a naturally accumulated peat layer at the surface. The rate of decomposition of the plant and animal species in these areas is exceeded by the rate of accumulation causing a buildup of layers at various levels of decomposition. These layers could be a few centimeters to several meters deep and be ranked by decompositions using several different methods

(Table 0-1). Various countries relate to various classification terms so that it is important to understand which terms are used by a range of researchers. In this thesis, the Von Post classification (Andriesse 1988) will be used to classify the humified layers of peat.

Peat land Conversion

Canada's peat land covers approximately 113 million ha which equates to 11% of Canada's surface area (Daigle and Gautreau-Daigle 2001). Peat has a multitude of uses such as fuel, animal bedding, growing medium and for numerous horticultural and agricultural practices. Currently on a worldwide level, greater than 99.8% of peat lands are drained specifically for agriculture and forestry and less than 0.2% are being mined for fuel (Joosten and Clarke 2002). Agriculture, aforestation and the return to peat land are three accepted management options following a peat mining operation. Each of these choices represents a conflict between conservation and exploitation of the natural resources and the different values placed on these areas by the public. Restoration is the reestablishment of Sphagnum mosses following peat land disturbance in temperate and boreal zones (Grosvernier et al. 1995; Girard et al. 2002; Gorham and Rochefort 2003), while rehabilitation refers to the recreation of bog habitat including a high biodiversity with other bog plant and moss species. On the other hand, conversion of a peat bog is the transition of a harvested peat land to another use such as agriculture or forest. Rehabilitation options of harvested peat lands should be evaluated on both global and local levels to determine the best choice for each situation taking into account economic, social and environmental aspects.

Table 0-1 Comparison between Determination Methods for Degree of Peat Decomposition

Von Post Degree	USSR Humification Degree R	Rubbed Fiber Content			Germany No. in DIN 19682/12	Poland Humification Degree R
		ASTM	USDA-FAO	Canada (CSSC)		
1	R_1	Fibric	Fibric	Fibric	1	R_1
2	R_2	Hemic				
3					2	
4			Hemic	Mesic		R_2
5	R_3	Sapric			3	
6			Sapric			
7					4	R_3
8				Humic		
9					5	
10						
Source: From Malterer, TJ, Verry ES, and Erjavec, J. 1992. Proc. 9th Int. Peat Congr., Uppsala, Sweden, 1:310-318.						

Re-establishment of Natural Peat lands

Peat lands are a unique habitat for various species that cannot survive without the wet environment with low rates of decomposition, such as Sphagnum mosses. Many countries are trying to reestablish areas of wetlands to prevent the loss of these habitats. Natural peat lands are a carbon sink as well as a filter for water (Gorham 1991). They also act as a sink for particulates present in the atmosphere such as mercury (Heyes et al. 2000). Unfortunately, disturbance can make it hard to reinstate these habitats to their original state. Traditional peat harvest would alter the water situation of the area so much that it is nearly impossible to reintroduce the native species and achieve the diversity of the original site (Charman 2002). Invasion of non-native species is quite common and they tend to dominate the native flora and fauna.

Gasses, such as methane and carbon dioxide, emitted by the harvested peat land are also an environmental concern due to their contribution to climate change.

The length of time for the same amount and type of peat to be reestablished can take centuries due to the slow rate of peat formation.

Use for Annual Agricultural Crops

Historically, many peat land areas in Europe have been drained to become farmland due to the many positive aspects of the growing material (Chapman et al. 2003). High levels of organic matter which tend to supply and retain high amounts of nutrients are common on peat soils. There is also a high water holding capacity which can provide a higher crop tolerance for drought. Some downsides with harvested peat land would be the depth to bedrock, location of the water table, nutrient sustainability, erosion, and the possibility of spontaneous burning. Annual crops have a relatively short growing season that only encompasses a few months of the year which would leave a large period of time when the land could be exposed to wind and water erosion. Some crops also have a large nutrient requirement which would require a high amount of supplemental fertilization. Some crops also need a large depth to the bedrock so as to have a good root bed.

Kreshtapova et al. (2003) have developed criteria for cutover peat lands in Russia using the following characteristics: thickness of the arable peat layer, degree of peat decomposition, C/N ratio, ash content, bulk density, pH, and cationic base saturation (Table 0-2).

Table 0-2 Grouping of Soils Peat lands by the Degree of their Agricultural Improvement

Parameters	Assessment of Soil Quality			
	Poor	Medium	Good	Very Good
Thickness of the arable layer (cm)	<15	15-20	20-30	30-40
Thickness of the residual peat layer (cm)	<20	20-30	30-40	>40
C/N Ratio	>25	18-25	14-18	10-14
pH	<4.5	4.5-5.5	5.5-6.0	>6.0
Bulk Density (g/cm^3)	<0.20	0.20-0.30	0.30-0.40	>0.40
Ash Content (g/kg)	<100	100-200	200-400	>400
Degree of peat decomposition (v/v)	<20	20-35	35-50	>50
Cationic base saturation (%)	<0.50	0.50-0.60	0.60-0.75	>0.75
Extractable Fe (%)	>140	84-140	56-84	42-56
Extractable P (%)	<9	9-15	15-26	>26
Extractable K (%)	<25	25-42	42-58	>58
Source: Kreshtapova, VN and Krupov, RA. 1998. Genetic peculiarities and basics of reclamation of cutover peat lands in Central Russia. Peat land Restoration and Reclamation: Proc. Of the 1998 International Peat Symposium. Malterer TJ., Johnson, K, and Stewart, J. Eds. OPS Publ., Duluth, MN. P115-119.				

Aforestation

The use of drained peat lands for forestry is a relatively new type of conversion as it has begun in the middle of the twentieth century (Chapman at al. 2003, Vasander et al. 1998, Aro and Kaunisto 1998). Trees have a high carbon sequestering ability when they are actively growing. The location of the water table could be a possible issue and could prevent large trees from being established in wetter areas. Also, trees can take a long time to establish and need help from competition of weeds and other pests. Since the studied area is far from cities and surrounded by forested areas, planting trees would be an option in reclamation

Human Utilization of *Vaccinium* spp.

While the genus *Vaccinium* contains over 400 species, there are several species that are economically valuable small fruit crops: Highbush Blueberry (*V. corymbosum* L.), Rabbiteye Blueberry (*V. ashei*), Lowbush Blueberry (*V. angustifolium* Ait. and *V. myrtiloides*), and American Cranberry (*V. macrocarpon* Ait.). Lignonberry (also known as red berries, partridge berries, *V. vitis-idea*) are popular in European markets, especially for preserves. Lowbush Blueberries are native to Northwestern Ontario and have played an important role ecologically as well as culturally to the indigenous people of the region (Kuhnlein and Turner 1991). Additionally, the fruit of the blueberry have been shown to provide medicinal benefits to human health by fighting cancer, improving memory and combating against heart disease (Mainland and Tucker 2002). Managed stands of wild lowbush blueberries are being farmed commercially in Maine and Atlantic Canada (Trehane 2004). Other commercially farmed blueberries include highbush (in British Columbia and Southern Ontario, as well as parts of the United States) and rabbiteye (in the deep south of the United States) (Trehane 2004). Increasing demands for blueberries from consumers has created a market opportunity for economic development and improved management of native stands in Northwestern Ontario. Blueberries are in high demand in many food products from baked goods, jams, and liqueurs to yogurts and milkshakes and are considered to be a very healthy supplement to many diets.

Natural Habitat and Taxonomy

The genus *Vaccinium* is native to most continents of the world, with 40% in Asia, 25% in North America and 10% in South and Central America. Many of the cultivated or extensively harvested native plants of these are perennial shrubs with a large range

across North America restricted to mainly acidic soils, due to their ability to tolerate higher concentrations of metal cations at pH levels lower than 5.0, with optimal growth at 4.5-4.8 (Eck, 1988). The term lowbush refers to blueberry plants that have stems less than 1.0 meter in height and produce stolons or rhizomes. A comprehensive review of many Vaccinium species in terms of ecology, production and anatomy has been produced by Luby et al. (1991). Taxonomy has been in debate for lowbush blueberries by Camp (1945), Darrow et al. (1944), Vander Kloet (1978) and Hall et al. (1979). Some of the taxonomy for certain *Vaccinium* species has been troublesome due to polyploidy as well as much hybridization within sections, making it hard to classify plants, but this has not been much of an issue for plant breeding (Luby et al. 1991). The main boreal species are *V. angustifolium* and *V. myrtilloides* as well as six other species indigenous to Eastern North America (*V. boreale, V. pallidum, V. tenellum, V. darrowi*, *V. hirsutum,* and *V. myrsinites*). *V. angustifolium* is considered a tetraploid (2n=48), averages about 0.1-0.4m in height with green or glaucous leaves with a serrate leaf margin and black to bright blue berries. It ranges from Southern Manitoba and Minnesota and east through Northern Ontario and Quebec to Newfoundland and south to Delaware, Virginia and northern Illinois and Indiana. *V. myrtilloides* are diploid (2n=24) and average in height from 0.2-0.4 m, have green, pubescent leaves with an entire leaf margin, and slightly smaller light blue berries. The range is wider than *V. angustifolium* and spreads from British Columbia to the Northwest Territories, across the north to Labrador and Nova Scotia, and south to New York, Pennsylvania, Indiana and West Virginia. Both species grow in open, well drained soils as well as on moist bog sites, peaty barrens, dry sandy areas, harvested forests, and abandoned farmland.

Production of Blueberries

Commercial production of lowbush (wild) blueberries is only done in Maine, USA, Quebec and the Atlantic provinces of Canada (Luby et al. 1991). Native stands are managed by fire, and chemical weed control. Some are mechanically harvested for freezing or secondary processing.

Blueberries are considered a unique crop in terms of soil requirements compared to other grain, fruit or vegetable crops. First, like many species in the rhododendron family, they require a pH that is in the 4.5 to 5.5 range which is considered very acidic for other field crops (Blasing 1989). Below 4.5, there are issues with toxicity from manganese and above 5.5, there is iron chlorosis (Martens and Westerman 1991). Also, they have a low requirement for nitrogen, possibly due to their low pH needs. They seem to have a preference for the ammonium form of N, while most other crops prefer the nitrate form (Sugiyama and Ishigaki 1994). In acidic soils, the ammonium N is dominant and is held in the soil profile for availability for blueberry roots. In neutral or high pH conditions, ammonium N is converted to the nitrate form which is leached easily through the soil and lost. Organic materials, such as peat, tend to buffer soil and keep a regulated pH and prevent too many changes to the acidity levels. High organic matter environment also encourages mycorrhizal fungi associations to form with the fine net of blueberry roots augmenting the absorption of nutrients and water (Yang et al. 2002). Mycorrhizae associations are also responsible for the ability of the blueberries to tolerate metals and also increase uptake of the ammonium form of nitrogen (Litten and Smagula 2002). High levels of phosphorus have been linked to iron chlorosis. High levels of calcium are also undesirable as it would increase the soil pH

above optimal levels. At the low pH levels, competition from other plant species is greatly reduced and weeds are less likely to be a major issue.

Some questions about the water table after harvest could limit the planting of blueberries to select locations with suitable dryness. Peat's water holding capacity also lessens the need for irrigation as it retains and supplies a high amount of water. Time and method of establishment of a marketable blueberry crop would need to be determined as it could take up to 5 years for seedlings to enter the profitable stage. Blueberries are fairly frost hardy and can handle winter temperatures to -30^{o}C (which can be amplified by insulation from a good snow cover), but frost in the spring can damage flowers and reduce yield. Warmer winter temperatures can also have an impact on cold hardiness and can incur damages if colder periods happen later in the season.

Blueberry DNA Profiling and Genetics

Molecular genetic studies can be used to improve the use of plant genetic resources. Another application of fingerprinting is to assist plant breeders in choosing parent stock and ensuring a wide genetic base for the species. Moreover, the identification of markers and genomic linkages that can be used as fingerprints can aid in identifying potential cultivars with ideal genetic traits. DNA fingerprinting cultivars of blueberry species is a useful tool to identify species as it can be quite difficult to identify specific cultivars as they lack distinguishing morphological characteristics and can be misnamed (Polashock and Vorsa, 1997).

Wild lowbush blueberries in Northwestern Ontario are an untapped genetic source that would be beneficial to a cultivar breeding program. Although breeding

programs for lowbush blueberries have existed in Maine, Michigan, West Virginia, Wisconsin, Minnesota, and Nova Scotia since the 1970's, most of the production is still based on wild cultivars and native stands. An increasing demand for high quality blueberries for the fresh market and the large variety of secondary blueberry products has built up the need to select beneficial wild candidates for horticulture. Like many eukaryotes, plant genomes contain a variety of highly repetitive DNA sequences. A subclass is known as Microsatellites or Simple Sequence Repeats (SSR), which are very short repeats of one to five base pairs. SSRs have been created for highbush blueberry (Boches et al. 2005) but need to be tested on lowbush plants so as to reduce the cost of the fingerprinting process. Randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) primers has been successfully used on *Vaccinium* spp. for cultivar identification and relatedness studies (Burgher-MacLellan and MacKenzie, 2004; Arce-Johnson et al. 2002; Aruna et al. 1995; Levi and Rowland, 1997; Polaschock and Vorsa, 1997). As newer DNA fingerprinting procedures are discovered, fewer steps, lower costs, and a reduced amount of genetic contamination from other organisms are associated with the new techniques.

In this thesis the objectives were to 1) fingerprint lowbush blueberry plants from across Northwestern Ontario using SSR markers that would be useful to discover possible blueberry cultivars for reclamation potential on harvested peatlands, 2) identify optimal soil characteristics of native lowbush blueberry stands that would help to classify optimal lowbush blueberry sites, and 3) examine the GG3 peat land using GIS analysis to identify most favorable soil layers and sites to set aside for rehabilitation using lowbush blueberries.

1. Microsatellite polymorphisms and genetic diversity in wild populations of *Vaccinium angustifolium* Ait. in Northwestern Ontario

1.1 Introduction

V. angustifolium is one of the woody perennials that we know as wild blueberry. It occurs naturally across the Northeastern United States and much of Canada throughout much of the boreal forest areas. Their fruit is important to wildlife (Usui 1994) as well as an increasing demand as an agricultural crop. They are commonly found on acidic soils with good drainage in areas with full sunlight (Eck and Childers 1966) although some stands can be found in darker, old forests (Usui et al. 2005). Lowbush blueberries are mostly pollinated with insects and there is little self pollination which increases the fruit quality and seed set.

Although much of the current lowbush or wild blueberry production is from native stands, increasing demand for this flavorful and nutritious fruit is leading to an increasing need for tamed cultivars. *Vaccinium angustifolium* composes the bulk of the lowbush blueberry species being harvested in commercial stands. Capturing the genetic diversity of wild populations is a useful tool in expanding genebanks of agriculturally important species. Genetic conservation 1) helps to improve the current cultivars, 2) keeps traits accessible and 3) expands the genetic range (Richards et al. 2007).

Microsatellite or Short Sequence Repeat (SSR) primers that have been developed for one species can sometimes be used to detect polymorphisms in closely related species. However, this depends upon the evolutionary distance between the original species and the target species (Rossetto, 2001). SSR primers form an ideal marker system for creating fingerprints due to the display of complex banding patterns

which also shows multiple DNA loci. Microsatellites are five times less abundant in plants compared to animals, but still show amplified products with polymorphisms (Langercrantz et al. 1993). The development of microsatellite markers can be difficult, time intensive and costly so using SSR markers on multiple species may be a useful alternative. Boches et al. (2005) developed 30 SSR primers for highbush blueberry (*Vaccinium corymbosum*) using two existing expressed sequence tag (EST) libraries and a microsatellite-enriched genomic library and tested other close species to see if they would show polymorphisms. While lowbush blueberry (*V. angustifolium*) was not tested, other close species such as American cranberry (*V. macrocarpon*) and lignonberry (*V. vitis-idaea)* were positive for cross amplification and polymorphisms.

In this study, we investigate the genetic diversity of stands of lowbush blueberry throughout Northwestern Ontario using four microsatellite markers created for highbush blueberry and also studied polymorphisms in American cranberry (*V. macrocarpon*) and lignonberry (*V. vitis-idaea*).

1.2 Methods

1.2.1 Plant Materials

Native lowbush blueberry plants were selected from across Northwestern Ontario including the following locations: Aroland (16U 501052 5562646), Atikokan (15 U 580173 5412843), Dryden (15 U 514564 5526352), Ignace (15 U 599806 5475151), Lac Des Milles Lac (15 U 686986 5427300), Nipigon (16 U 443396 5440585), Sioux Lookout (15 U 581910 5552250), and Wawa (16 U 670499 5325867) (Figure 1-1). One bush representative of the area was chosen for a sample of leaves to be sent for SSR genetic fingerprinting at the Guelph Molecular Centre, University of Guelph. Native lowbush

samples were compared to two commercial varieties (Dominion Seed House, Georgetown, Ontario) purchased in April 2008.

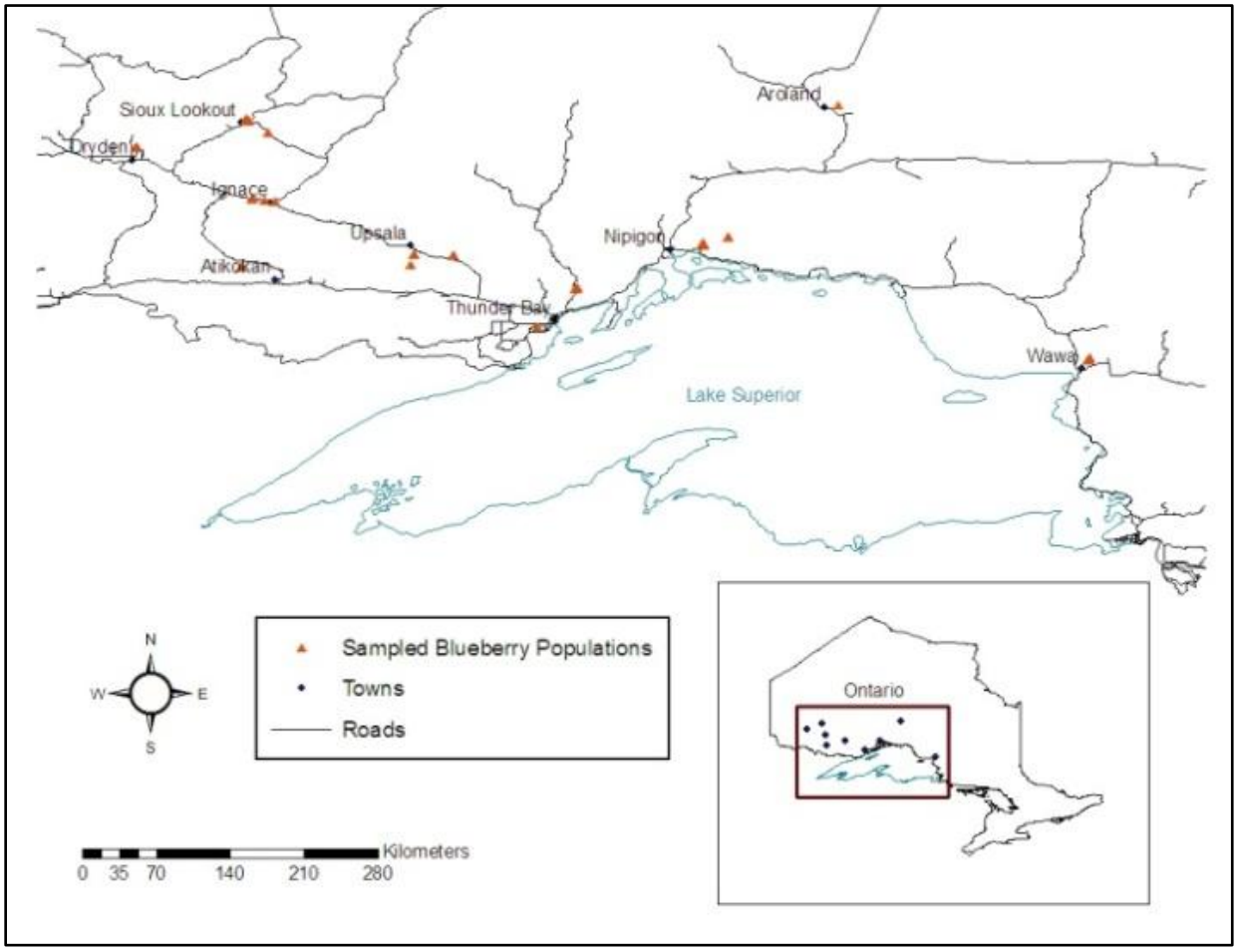

Figure 1-1 A map of the natural populations of Vaccinium angustifolium investigated in this thesis.

1.2.2 SSR Analysis of the Berry (Vaccinium) Leaf Samples

The genomic DNA was isolated from ~ 80 mg leaf using the DNeasy™ Plant Mini kit (Qiagen, Mississauga, ON) according to the manufacturer's protocol for plant tissue. The purified DNA was eluted in 100 µL AE Buffer (Qiagen) and stored at -20°C until PCR.

The four pairs of SSR primers, CA169F, NA800, NA1040 and VCC_S10, were as described by Boches et al. (2005) (Table 1-1), and synthesized at the Laboratory

Services, University of Guelph (Guelph, ON) using an ABI 3900 HT DNA synthesizer (Applied Biosystems, Foster City, CA). The forward primers were labeled with the fluorescent dye FAM. The PCR reaction mixtures (10 μL) contained 1× HotStarTaq Master Mix (Qiagen), 0.4 μM of each primer, 1.5 mM additional $MgCl_2$, and approximately 15 ng template DNA. Thermal cycling conditions were 1 cycle of 10 min at 95°C; 5 touchdown cycles of 15 sec at 95°C, 30 sec at 65°C decreased by one degree per cycle to 61°C, 1 min at 72°C; 32 more cycles of 15 sec at 95°C, 30 sec at 60°C, 1 min at 72°C; and a final extension time at 72°C for 30 min. All amplifications were performed using a thermal cycler GeneAmp PCR System 9700 (Applied Biosystems).

Amplified products were diluted 10 times in double distilled water. To 0.7 μL of the diluted PCR products, 11 μL formamide and 0.5 μL of GeneScan ROX500 internal size standard (Applied Biosystems) were added, and the mixture was denatured for 3 min at 94^{o}C, cooled for 5 min on ice and resolved by automated capillary electrophoresis using ABI 3730 Genetic Analyzer (Applied Biosystems) with POP-7 polymer and 3730 Buffer with EDTA (Applied Biosystems). The PCR fragments were sized by using the GeneMapper v4.0 software (Applied Biosystems). The fragment data were exported to Excel files for further analysis.

Table 1-1 Locus name, GenBank Accession no., repeat motif, primer sequences (F = forward, R = reverse), annealing temperature (*Ta*), allele size range and number of alleles per locus of 30 *Vaccinium* SSR loci evaluated in 11 *Vaccinium corymbosum* cultivars ('Grover', 'Pioneer', 'Rancocas', USDA-72, 'Cabot', 'Bluecrop', 'Georgiagem', 'Earliblue', 'Flordablue' and 'Toro') and one accession of wild blueberry (PI 55880). Locus name prefixes indicate source: CA, cold acclimated EST; NA, nonacclimated EST; VCC, genomic enriched (Boche et al. 2005)

Locus	GenBank accession no.	Repeat motif	Primer Sequence (5'-3')	$T_a(C^o)$	Allele Size Range (bp)	Allele no.
CA169F	CF811071	$(GAT)_4$	F:TAGTGGAGGGTTTTGCTTGG R:GTTTATCGAAGCGAAGGTCAAAGA	62	109-130	5
NA800*	CF811589	$(TC)_{13}$	F: CAATCCATTCCAAGCATGTG R: GTTTCCCTAGACCAGTGCCACTTA	60	230-290	31
NA1040	CF811165	$(TC)_{11}$	F: GCAACTCCCAGACTTTCTCC R: GTTTAGTCAGCAGGGTGCACAA	60	180-270	15
VCC_S10*	AY762685	$(CT)_{22}$	F: ATTTGGTGTGAAACCCCTGA R: GTTTGCGGCTATATCCGTGTTTGT	60	200-300	29
*amplifies multiple loci as determined by capillary electrophoresis.						

1.2.3 Allele scoring and evaluation of polymorphism

Genetic differences were scored on presence (1) or absence (0) of polymorphisms generated by 4 primers for 53 wild lowbush blueberry clones and 2 different purchased lowbush blueberry cultivars (19 plants). Stutter was interpreted following Bakker et al. (2005) and Harker (2003).

1.2.4 Data Analysis

Using the POPGENE program version 1.32 (Yeh et al. 1997), the following parameters of genetic variation were assessed for each population: the observed number of alleles per locus (*A*), the effective number of alleles (N_e), and percentage of polymorphic loci (99% criterion) (*P*). Shannon's Information Index (Lewontin 1972) (i) and Nei's (1973) gene diversity (h) were also calculated to show the level of diversity. Rare alleles were defined by a frequency of less than 0.05. The significance of these deviations was analyzed using a chi-square test (Workman and Niswander 1970). Linkage disequilibrium was explored using Ohta's two-locus analyses of population subdivision (D-statistics) for multiple populations ($P<0.05$) (Ohta 1982). In addition to the previous tests, Wright's (1978) analysis of hierarchical F-statistics was performed.

An estimation of gene flow was calculated using Nm=0.25(1-Fst)/Fst. Two phylogenetic trees were constructed using Nei's (1978) unbiased genetic distances on population pairs and using cluster analysis with Sorenson's distance in PC-ORD (McCune and Mefford, 1999). To estimate the relationships among the individuals, nonmetric multidimensional scaling (Mather 1976 and Kruskal 1964) was performed using Sorenson's distance measure at random staring configurations and 250 runs were performed with real data.

1.2 Results

Each of the primers was successful in amplifying products in most of the individuals. Three of the plant samples did not produce any fragments for one of the primers but successfully produced product for the other three. None of the primers were monomorphic but were successful in producing polymorphic peaks.

1.3.1 Allele Comparison among *V. angustifolium*

Total number of alleles per locus, their actual size ranges, and expected size ranges for the alleles identified in *V. angustifolium* are given in Table 1-2. Each of the SSR primers showed a high level of polymorphisms with the number of alleles ranging from 13 for CA169F to 51 for VCC_S10. The distribution for each of the SSR primers is shown in Figure 1-2. Rare alleles (frequency less than 0.05%) were determined to be approximately 42% of the total number of alleles amplified by the SSRs.

Table 1-2 Characterization of SSR loci

Locus	Repeat Type	Expected Size Range (bp)	Number of Alleles	Range of Sizes (bp)	Rare Alleles
CA169F	$(GAT)_4$	109-130	13	109-132	1
NA800	$(TC)_{13}$	230-290	43	179-250	16
NA1040	$(TC)_{11}$	180-270	26	180-220	7
VCC_S10	$(CT)_{22}$	200-300	51	176-255	32

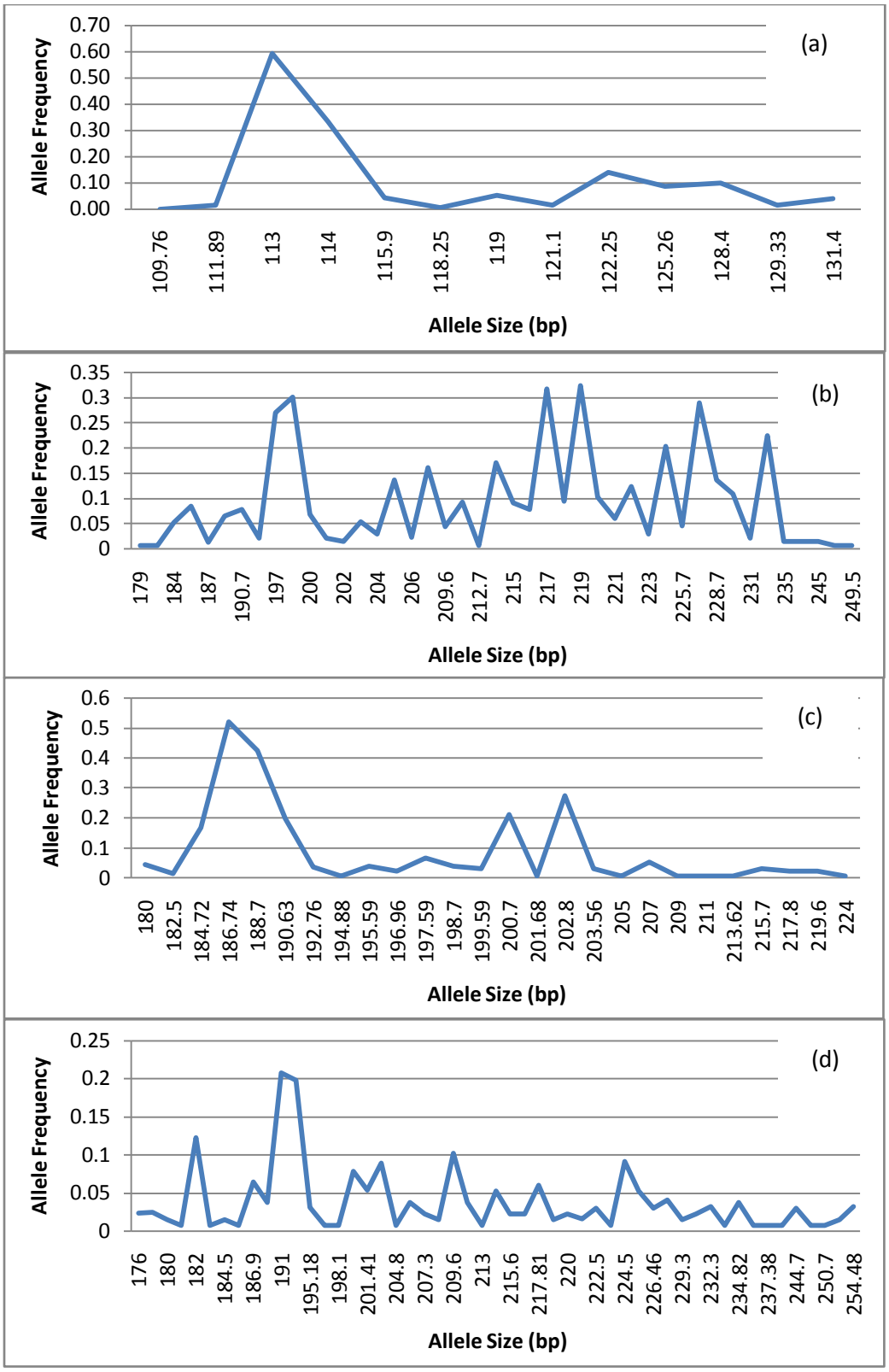

Figure 1-2 The distribution of allele frequencies at the loci CA169F (a), NA800 (b), NA1040 (c), and VCC_S10 (d)

Three of the SSRs used in this investigation were dinucleotide and one was a trinucleotide repeat, producing a range of product repeat sizes from 12 to 44 bp. All of the microsatellite loci used in this study was polymorphic across each of the 12 populations sampled. Table 1-3 is a summary of the populations and the number of alleles present for each of the SSR primers. A total of 133 alleles were identified with the average number per locus being 33.25. Twenty one percent of the alleles were detected in one of the wild clones, but not in any of the purchased cultivars, making them unique to the total population. None of the alleles were shared with all of the population. One of the alleles was shared by 65.2% and most of the alleles were only shared by 10.7% of the population.

Table 1-3 Allelic Variability at the four SSR loci in the 12 different Vaccinium angustifolium populations

Population	Number of Alleles				
	CA169F	NA800	NA1040	VCC_S10	Total
Aroland	4	15	8	13	40
Dryden	6	24	9	17	56
Hook Road	5	18	7	12	42
Ignace	6	19	6	17	48
Lac des Milles Lacs	8	13	12	20	53
Nipigon	10	31	13	26	80
Sioux Lookout	6	12	10	11	39
Atikokan	8	17	3	11	39
Wawa	6	18	8	17	49
Purchased cultivars	1	11	3	3	18
Total	13	43	26	51	133

1.3.2 Genetic Variation within populations

The genetic diversity within the *V. angustifolium* populations based on the frequency of alleles is complied in Table 1-4. The average number of individuals sampled for each population is 6. The Hogarth population only accounted for one

individual so there was no measurement of diversity for this population. The observed number of alleles per locus (*A*) had a mean of 1.29 and ranged from 1.09 to 1.41. The effective number of alleles per locus (N_e) had a mean value of 1.02 and a range from 1.04 to 1.16. Since the Hardy-Weinburg equation is unknown for this tetraploid population, the Shannon's Information Index (*i*) can provide some measure of gene diversity, ranging from 0.041 in the purchased cultivars with the lowest amount of differences to 0.18 in the plants from Dryden at 0.18 with the highest amount of diversity. Nei's diversity (*h*) provided the similar results to the Shannon's. Each of these diversity indices shows that the purchased cultivars have a lower level of diversity than the native plant populations.

Table 1-4 Genetic Variation within the population based on four SSR loci (*N*= number of individuals per population, *A*= observed number of alleles per locus, N_e = effective number of alleles per locus, *P* = percentage of polymorphic loci (99% criterion), *h* = Nei's gene diversity and *i* = Shannon's information index)

Population	*N*	*A*	N_e	*P*	*h*	*i*
Aroland	5	1.28±0.45	1.15±0.29	28.57	0.092±0.16	0.14±0.24
Dryden	5	1.41±0.49	1.16±0.24	41.35	0.11±0.15	0.18±0.23
Hook Road	5	1.29±0.46	1.10±0.20	29.32	0.070±0.12	0.12±0.19
Ignace	5	1.35±0.48	1.15±0.25	35.34	0.097±0.15	0.16±0.23
Lac des Milles Lacs	5	1.39±0.49	1.12±0.19	39.10	0.093±0.13	0.15±0.20
Nipigon East	5	1.32±0.47	1.14±0.25	32.33	0.093±0.15	0.15±0.22
Nipigon West	5	1.42±0.50	1.15±0.23	42.11	0.10±0.14	0.17±0.21
Sioux Lookout	5	1.29±0.46	1.10±0.19	29.32	0.070±0.12	0.12±0.19
Atikokan	5	1.27±0.45	1.09±0.18	27.07	0.066±0.12	0.11±0.19
Wawa	5	1.35±0.48	1.13±0.22	35.34	0.092±0.14	0.15±0.21
Purchased cultivars	19	1.09±0.29	1.04±0.16	9.02	0.027±0.096	0.041±0.14
Mean	6	1.29±0.40	1.02±0.20	28.82	0.082±0.13	0.14±0.20
Species	68	1.98±0.15	1.16±0.22	97.74	0.11±0.13	0.20±0.18

1.3.3 Population Genetic Structure

This study of genetic analyses showed high levels of genetic diversity among the different populations. Table 1-5 shows the relative differences in genetic differentiation and the estimates of gene flow among the populations by individual SSR markers. CA169F showed the greatest amount of diversity among the total population, while CA169F and NA800 had the greatest variation within the subpopulations. The gene differentiation among the subpopulations had the highest value with the NA1040 SSR marker.

The overall gene flow (N_m) within these populations was 1.3992. This value gives an idea of the average number of migrants per generation between the different populations investigated. This is quite a low value meaning that there is little migration between subpopulations.

Table 1-5 Relative measurements of genetic differentiation and the estimates of gene flow among populations of *Vaccinium angustifolium*

Locus	H_t	H_s	G_{st}	N_m
CA169F	0.16±0.02	0.09±0.005	0.411	0.7164
NA800	0.14±0.01	0.09±0.003	0.3977	0.7572
NA1040	0.11±0.02	0.07±0.005	0.4212	0.6870
VCC_S10	0.08±0.01	0.06±0.003	0.2927	1.2080
Mean	0.11±0.01	0.08±0.005	0.2633	1.3992
H_t gene diversity in the total populations, H_s average gene diversity within subpopulations, G_{st} gene differentiation among subpopulations, N_m Estimate of gene flow from G_{st}				

1.3.4 Genetic Relationships

Table 1-6 shows the genetic distances and identities calculated for each pairing of populations so as to provide an estimate of their variance. Genetic distance represents the measure of dissimilarity of the genetic material between the regions, while the genetic identity displays the level of similarity. The average distance between individual populations is 0.029 with the lowest genetic distance between Atikokan and

Wawa at 0.0122 and the greatest genetic distance between the purchased cultivars and Nipigon East at 0.1062. The same information can be seen using the genetic identities.

Table 1-6 Genetic distances and genetic identities among populations of *Vaccinium angustifolium*

Population	Purchased Cultivars	Aroland	Dryden	Hook Road	Ignace	Lac Des Milles Lac	Nipigon East	Nipigon West	Sioux Lookout	Atikokan	Wawa
Purchased Cultivars	****	0.9018	0.9216	0.9081	0.9141	0.9203	0.8992	0.9128	0.9344	0.9057	0.9140
Aroland	0.1034	****	0.9647	0.9559	0.9651	0.9671	0.9573	0.9564	0.9690	0.9649	0.9725
Dryden	0.0817	0.0360	****	0.9757	0.9819	0.9838	0.9759	0.9798	0.9826	0.9831	0.9810
Hook Road	0.0964	0.0451	0.0246	****	0.9782	0.9773	0.9803	0.9638	0.9780	0.9845	0.9775
Ignace	0.0898	0.0355	0.0182	0.0221	****	0.9791	0.9824	0.9724	0.9856	0.9866	0.9849
Lac des Milles Lacs	0.0830	0.0334	0.0163	0.0230	0.0212	****	0.9732	0.9781	0.9867	0.9805	0.9774
Nipigon East	0.1062	0.0436	0.0244	0.0198	0.0178	0.0272	****	0.9769	0.9717	0.9846	0.9840
Nipigon West	0.0912	0.0446	0.0204	0.0368	0.0280	0.0221	0.0233	****	0.9721	0.9742	0.9783
Sioux Lookout	0.0678	0.0315	0.0175	0.0222	0.0145	0.0134	0.0287	0.0283	****	0.9816	0.9817
Atikokan	0.0990	0.0357	0.0171	0.0157	0.0135	0.0197	0.0155	0.0261	0.0185	****	0.9879
Wawa	0.0899	0.0279	0.0192	0.0228	0.0152	0.0229	0.0162	0.0219	0.0184	0.0122	****

Above diagonal: genetic identities; below diagonal: genetic distances

Three separations occurred in each of the phylogenic trees. The dendrograms differentiated the purchased cultivars (LB) from the rest of the populations (Figure1-3). The rest of the populations were fairly closely related and were grouped closer, with a separation of Aroland and Nipigon West from the remaining populations.

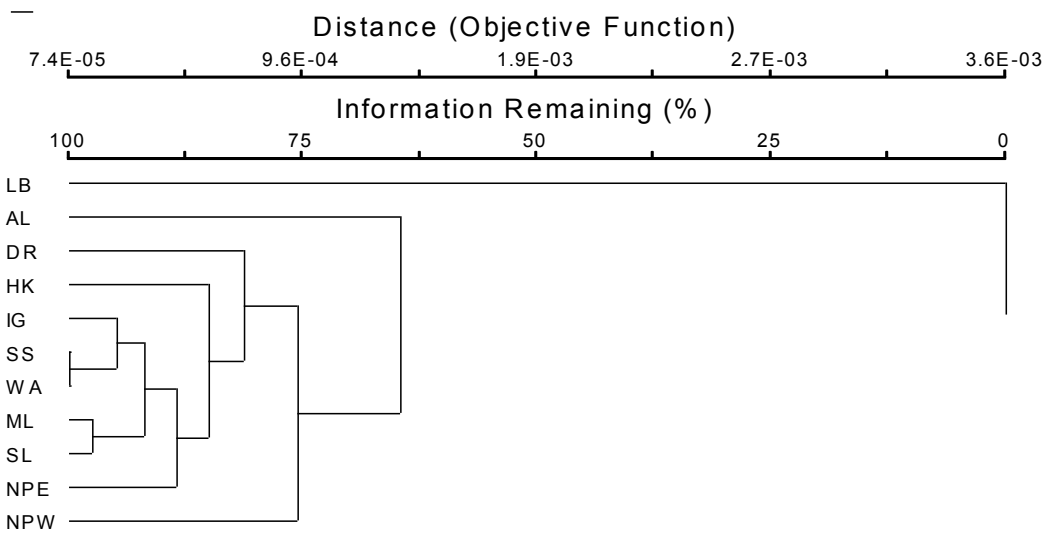

Figure 1-3 UPGMA dendrogram of *Vaccinium angustifolium* populations using Nei's original genetic distances. LB- purchased cultivars, AL- Aroland, DR- Dryden, IG- Ignace, HK- Hook Road, ML- Lacs des Milles Lacs, NPE- Nipigon East, NPW – Nipigon West, SL- Sioux Lookout, SS- Atikokan, WA- Wawa.

The total population cluster analysis (Figure 1-4) provided a slightly different picture than the earlier (Figure 1-3). Two of the plant samples (Nipigon East 2 and Sioux Lookout 4) are separated out and then the first large cluster of all the purchased plants (LB) is the next cluster followed by the wild clones in the third cluster. The NMS ordination also shows this trend (Figure 1-4). The final stress of the best solution with three axes and 202 iterations was 15.89, which is a little high, but still presents a usable picture. The final stability was 0.0000, which is what we are aiming for in the solution. The Monte Carlo test result is p=0.0040 which is significant. The graph of the ordination reflects the results of the total population cluster analysis seen in Figure 1-4 with the two plant samples (Nipigon East 2 and Sioux Lookout 4) and the purchased cultivars separating from the other wild clones. There is a small clustering of the plants from Aroland as well as Hook Road and Nipigon.

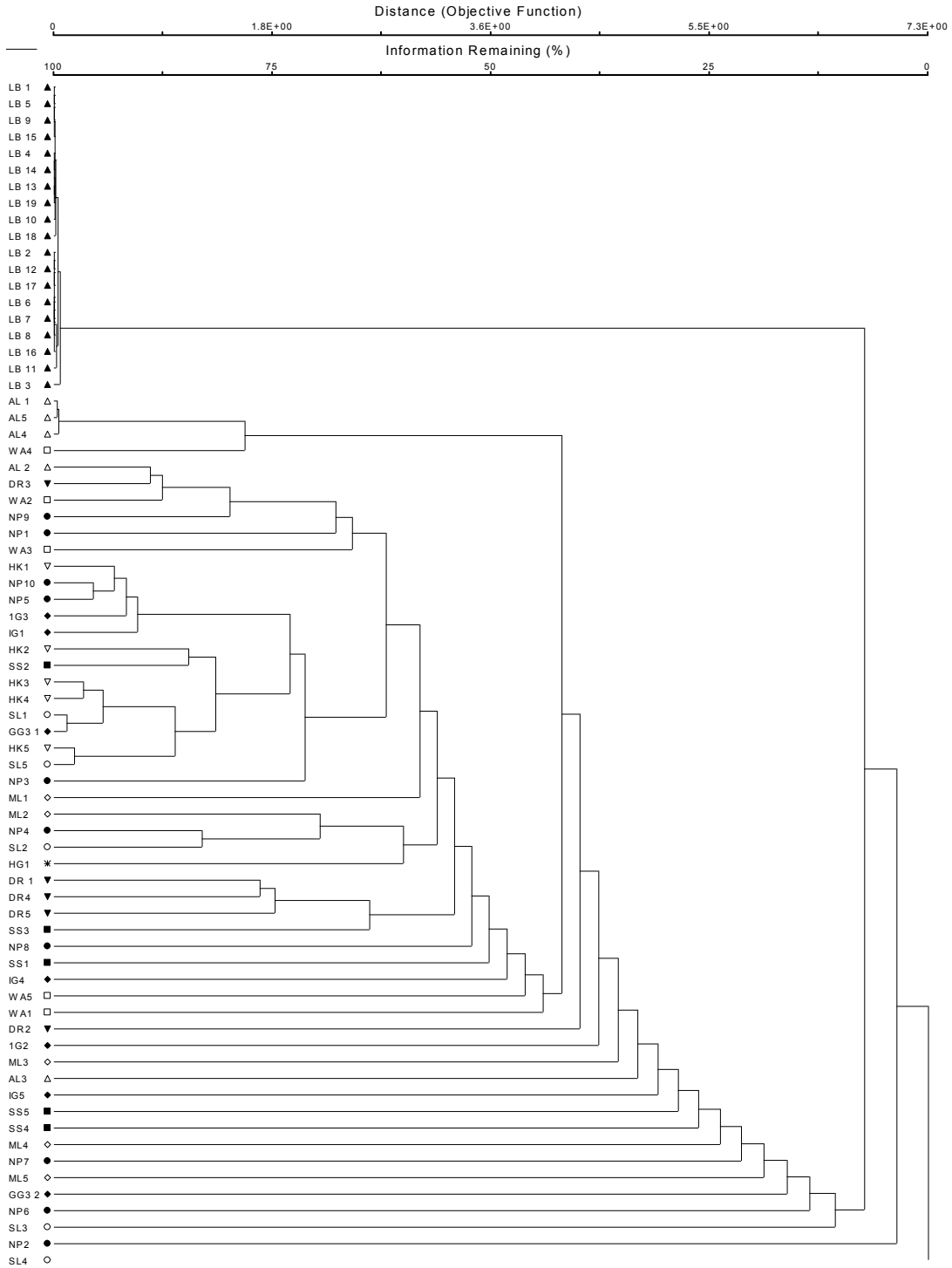

Figure 1-4 Cluster dendrogram of all plant samples created using Sorenson's distance measure. LB- purchased cultivars, AL- Aroland, DR- Dryden, IG- Ignace, HK- Hook Road, ML- Lacs des Milles Lacs, NPE- Nipigon East, NPW – Nipigon West, SL- Sioux Lookout, SS- Atikokan, WA- Wawa.

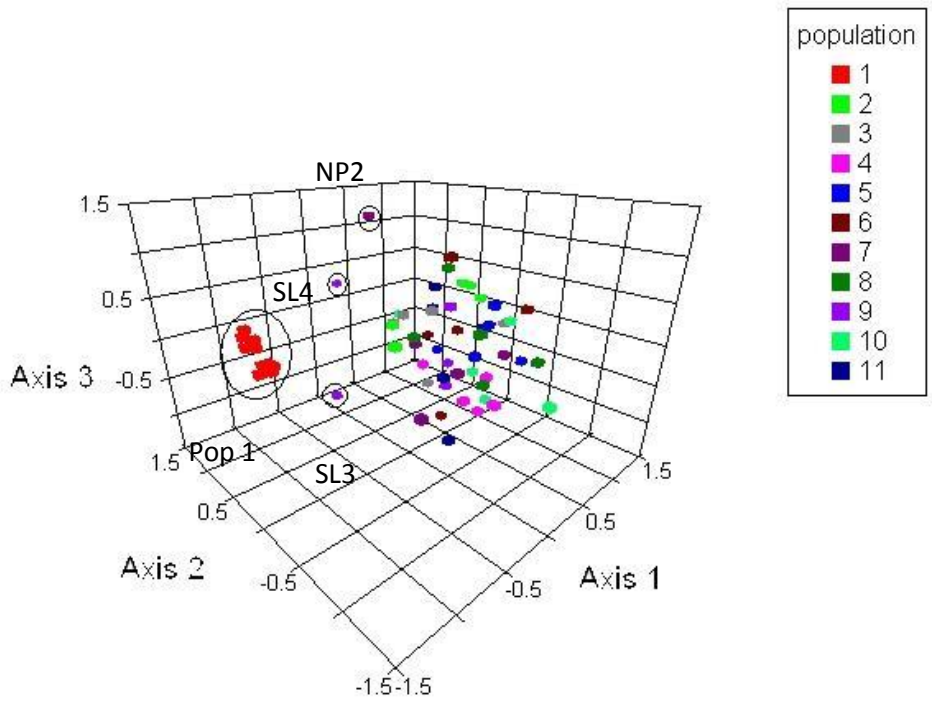

Figure 1-5 Nonmetric multidimensional scaling analysis of Sorenson's distance between 72 individuals of *Vaccinium angustifolium* from 12 populations found in Northwestern Ontario. 1- purchased cultivars, 2- Aroland, 3- Dryden, 4- Ignace, 5- Hook Road, 6- Lacs des Milles Lacs, 7- Nipigon East, 8- Nipigon West, 9- Sioux Lookout, 10- Atikokan, 11- Wawa. Population 1 –purchased cultivars, NP2 – plant from Nipigon 2 site, SL3 and SL4 – plants from Sioux Lookout 3 and 4

1.4 Discussion

There is increased interest in the commercial production of lowbush blueberries in Northwestern Ontario. An understanding of the area's native population germplasm is vital in the process to utilize potential clones. No genetic work has been previously published on *V. angustifolium* in Northwestern Ontario and data from this study show that some commercial cultivars have a genetic distance from the local populations. The results (Table 1-2) also demonstrated that microsatellite markers developed for *V. corymbosum* work well on *V. angustifolium* based on the four markers used in this study. They produced a high level of polymorphisms (Table 1-3) just as in Boches et al.

(2005) seen in Table 1-3, but they did not seem to separate individual populations (Figures 4 and 5). CA169F and NA1040 were very similar in size range, while the NA800 and VCC_S10 markers were shifted about 25 base pairs to the left. For each of the markers, a higher number of alleles were present in comparison to the original report for the 12 other *Vaccinium* spp (Boches et al. 2005). This may be due to inclusive identification of peaks due to the high amount of stutter produced by these SSRs (Figure 1-6). The stutter was interpreted by including the alleles with the highest peak, with no large spaces or declines. Figure 1-6 shows one example with a possible 9 peaks.

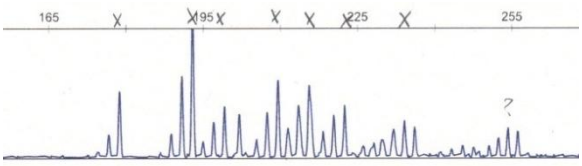

Figure 1-6 Microsatellite Stutter from VCC_S10 on a plant sample from Dryden

Other genetic fingerprinting studies have been conducted successfully on *Vaccinium* spp. including *V. angustifolium* genotypes from Nova Scotia, New Brunswick and Maine using randomly amplified polymorphic DNA (RAPD) (Burgher et al. 2002 and Burgher-MacLellan and MacKenzie 2004), *V. macrocarpon* using RAPD and amplified fragment length polymorphism (Polaschock and Vorsa 1997), various blueberry species using EST-PCR markers (Rowland et al. 2003), various blueberry species using RAPD and SSRs (Levi and Rowland 1997) and *V. macrocarpon*, *V. angustifolium*, and *V. vitis-ideae* using RAPD markers (Debnath, 2005). Other types of markers tested include Arbitrary Primer-PCR (AP-PCR), inter-simple sequence repeat (ISSR), cleaved amplified polymorphic sequences (CAPS) (Ratnaparkhe 2007). Blueberry derived EST-PCR markers have also been tested on other related Ericaceae species with varying success with *V.*

macrocarpon at 89% of primer pairs working effectively and *Rhododendron* spp. at 74% (Rowland et al. 2003). Bassil and Hummer (2009) have also tried 46 different blueberry SSR markers on *V. macrocarpon* with 16 being successful.

Recently a microsatellite diversity study was performed by Boches et al. (2006) on 69 *V. corymbosum* L. accessions consisting of 13 wild accessions and 56 cultivars (one half high, 18 southern highbush and 37 northern highbush). A decrease of genetic diversity was observed between wild clones and cultivated types of blueberries, as observed by number of alleles, number of unique alleles and Shannon's Index, while no difference was found between the cultivated types of blueberries. The clustering dendrogram separated their cultivars into three groups: southern highbush, northern highbush and wild clones. Their genetic information also corresponded with known pedigree information for the cultivated plants. These results matched with the findings of this study: a separation of the cultivated plants in the cluster dendrogram and NMS graph (Figure 1-4 and Figure 1-5) and also a lack of unique alleles in the cultivated plants sampled. Wider diversity of wild plants could be attributed to a wider variety of selection pressures (predation, water conditions, temperature, soil type, etc) than those used for cultivated plants which tend to be selected for fruit yield.

Early acting inbreeding depression can be a factor that contributes to a reduction in self fertility (Hokanson and Hancock 2000). Self sterility produces a higher reduction in wild lowbush blueberries (*V. myrtilloides* and *V. angustifolium)* in comparison to the highbush types (*V. corymbosum*). This could be from a dense rhizomatous growth habit compared to *V. corymbosum* and the stem densities of the other lowbush species.

There was a low amount of migration between populations (Table 1-4). This could be due to a fragmentation of the optimal growing environment between these areas. There are expansive distances among these areas of boreal forest and wetlands for these blueberry populations which create barriers for pollen spread between plants. Forest stand management may also factor into the ability for migration of blueberry genetic diversity to occur due to such factors as true seedling stocking levels following forest harvest and the use of herbicides to keep planting areas devoid of fruit-bearing shrubs (Noyce and Coy 1989).

1.4.1 Future Management Options

Early breeding programs for all types of blueberries dealt with factors relating to fruit quality, size and productivity (Ratnaparkhe 2007). Our northern wild clones could also provide factors that have need for further improvement such as winter hardiness and drought resistance. Ontario plants have been found to have a greater amount of seed set compared with plants found in Newfoundland and Nova Scotia (VanderKloet 1985) and could provide a diverse addition to any breeding program. They also experience greater spread in daily environmental conditions compared to plants in more temperate climates such as in the Maritimes.

In conclusion, SSRs developed for *V. corymbosum* are excellent tools for genetic fingerprinting of *V. angustifolium*. Ongoing research into the lowbush blueberry would determine if other SSR markers developed for highbush blueberry could help to describe this species in more detail. This could lead to identifying more of the genetic diversity of the species (for example, yield and maturity) and help accelerate a breeding program for the species that would be specific to Northwestern Ontario.

2. Multivariate statistical and GIS-based approach to understanding soil chemical properties of different layers of a peat bog in comparison to blueberry soils of Northwestern Ontario

2.1 Introduction

In a peatland, there are various layers of decomposing organic material that accumulate over time, with each of these layers holding different nutrient values. Agricultural use of cutover peatlands is a practice that has been undertaken for centuries in The Netherlands, Germany, and other countries. These harvested peatlands are also being afforested for softwood lumber, hardwood forest, grassland, and return to wetlands and natural landscapes (McNally 1995). These options are chosen based on biophysical and economic conditions. For example, characteristics such as depth of arable layer, depth of peat, C/N ratio, pH, bulk density, ash content, and extractable iron, potassium and phosphorus are compared from various peatlands to assess the soil quality following peat harvest for various agricultural crop requirements (Krestapova et al. 2003). Some of the factors that need to be addressed are the depth of the residual peat layer, nutrient imbalance, spatial and temporal variations in crop yield and quality, water logging or deficits due to imperfect contact between peat and mineral subsoil (Krestapova et al. 2003). Mixing of peat with the subsoil could help to increase the pH, reduce wind erosion, increase bulk density and available potassium and phosphorus (Krestapova et al. 2003). There is a high rate of cereal crop failure on peatlands and are connected to aquifers, have low pH, and high extractable iron content.

Lowbush blueberries (*V. angustifolium* Ait.) have specific soil requirements for optimal levels of growth that vary from most annual and perennial agricultural crops.

They are a slow growing woody perennial that spread by underground rhizomes, which also serve to store nutrients. Hall et al. (1964) established that optimal soil pH is a major factor in blueberry growth with an optimal range of 4.2-5.2. Lowbush blueberries have optimized their nutrient intake to this low range of pH as various nutrients are more or less available at different pH levels. Townsend et al. (1968) determined that lowbush blueberries tend to make use of the ammonium form of nitrogen, ensuring an adequate supply on sandy acidic soils, where leaching occurs under moderate rainfall. The ammonium form of nitrogen is able to be adsorbed to the sand particles, while the nitrate form leaches from the soil matrix. Hall et al. (1972) have determined that nitrogen is the only element that varies significantly during different growing seasons, while other nutrients are fairly comparable. Korcak et al. (1982) found that it was possible to select certain blueberry seedlings that grew well in a range of soil types. Korcak (1986) also found that blueberry plant growth and rooting over the first two seasons can have a significant effect due to soil types but the addition of peat moss produced an increase in fruit acidity. Peat tends to have a low pH which optimizes the nutrient availability to the blueberry roots and has a substantial establishment and flowering benefit (McArthur, 2001). In these low pH conditions, some micronutrient deficiencies can be seen such as manganese, iron and magnesium (Dale 1999).

Often visual observations of leaf colour can indicate nutrient deficiencies (Smagula 1987). Nitrogen deficiencies can be seen through a chlorosis of the leaves due to the nitrogen's role in chlorophyll synthesis. This tends to be seen in the lower leaves of the plant first. Phosphorus deficiencies are reflected by the production of small dark green leaves; in advanced stages can show large purple patches on the leaves appear. Potassium can also appear as chlorosis, but in the leaves can also develop a red margin

around the leaf which can turn into leaf scorch, drying dead tissue starting at the leaf tip (Smagula 1987).

Peat is commonly harvested for fuel in many areas of the world (Holmgren et al. 2008). These wetlands are typically drained for a few years and then cut/extracted by machine, and making the wetland almost impossible for reclamation (Wind-Mulder et al. 1996). In Canada, peatlands have been estimated to be approximately 12% of the land area totaling 1.13 million km^2 (Zoltai and Pollett 1983, Tarnocai 1998). Less than 0.02% or 17000 ha are currently being used for extracting horticultural peat moss and other uses but fuel (Daigle JY and Gautreau-Daigle H 2001). A proposed wet harvest of a peat bog (known as GG3) and covering an area of 1080 ha near Upsala, Ontario is to be used for fuel at Ontario Power Generation in Atikokan, Ontario (Figure 2-1).

Wet harvest is the method in which the peat is harvested without any prior drainage of the wetland (Peat Resources Ltd 2008). The living layer (acrotelm) is removed and the decomposed layers (catotelm) are removed to be pelletized for fuel. The water removed in the pelleting process is released onto a living part of the bog, which acts as a natural filter. The acrotelm is replaced after harvest. The catotelm layers of peat that accumulate in the bogs have several different layers of peat rated by humification, the degree of decomposition of the plant material in the peat. Humification is measured via the Von Post humification scale for *Sphagnum* and *Carex* peats (Andriesse 1988) and is subjectively ranked from 1 to 10 with 1 being the least decomposed and 10 being the most humic. The GG3 bog has peat humification ratings from 1 to 8. Humification layer 1 is also referred to as the blond layer, the living layer or the acrotelm, while humification layers 2 to 10 are referred to as the catotelm. These

layers correspond with various depths of the peatland with the more humified peat being at the lower depths of the peatland.

Geographic Information Systems (GIS) is increasingly being used to interpret environmental data, more specifically for identification of non point source contamination (Corwin and Wagenet, 1996), air pollution (Sengupta and Venkatachalam, 1994; Moragues and Alcaide, 1996) and other urban pollution (Ebbinghaus et al. 1997). Other GIS related studies have examined soil contamination (Adamus and Bergman (1995), Meinardi et al. (1995) and Facchinelli et al. (2001)). Wind-Mulder et al. (1996) investigated peat water chemistry in peatlands across Canada for peatland restoration. However, no studies have used GIS to plan the harvest and remediation of peat at a specific site.

The high demands for blueberries has brought to mind questions about what makes optimal soil conditions for establishing a stand of lowbush blueberries. Most soils with native lowbush blueberry stands tend to be sandy or gravelly, with low levels of organic matter and are well drained. However, their potential growth on highly organic peatlands is not known. In this study, we investigate the nutrient levels and soil characteristics in various native blueberry habitats to see if these soils are similar in soil nutrient levels. Additionally to those found in peatlands we will determine if lowbush blueberries can be successfully established on a harvested peat bog and if there is an optimal layer in the bog for blueberry plant growth.

A plan, that is dependant of the hydrology of the site, must be put in place to maximize both the peat harvested for fuel as well as the remaining soil left behind for reclamation opportunities which include berry or wild rice production. This study will

use topographical data and information from boreholes in the GG3 peat bog, coupled with multivariate statistics to develop such a plan.

2.2 Methods

2.2.1 Field Procedures

Native lowbush blueberry plants were studied across Northwestern Ontario at the following locations: Aroland, Atikokan, Dryden, Ignace, Lac Des Milles Lac, Nipigon, Sioux Lookout, and Wawa. Five sites in each location were analyzed and 10 sites were analyzed in Nipigon. Sites of 25 m by 25 m with high population of blueberries were identified. In each site, twenty-five sampling quadrats of 0.025m^2 were measured for number of stems and colour following the procedure of Nams (1994). One of the stems had the number of flowers and/or berries counted as well as measured for height. Soil sampling of blueberry sites followed Smagula and DeGomez (1987). Soil samples were taken to a depth of 2.5 to 12.5 cm from each sampling site, avoiding leaf and plant litter from the soil surface. Each soil sample was a composite of soils taken from the blueberry stand area. Visual leaf nutrient deficiencies were identified by the following symptoms: slight nitrogen deficiency = light green or yellow leaves in the older leaves, low nitrogen deficiency = slight reddish tinted older leaves, moderate nitrogen deficiency = red green older leaves, severe nitrogen deficiency = deep red older leaves, phosphorus deficiency = leaves with purple patches, potassium deficiency = leaves with red or scorched margins (Smagula 1987).

2.2.2 Laboratory Procedures

In addition to the blueberry soils collected in the field, peat soil cores were obtained from Peat Resources Limited from the GG3 bog area. There were a total of 210 samples from various layers of the bog. The soil was dried, screened through a 2mm

soil sieve to remove rocks and large plant material, and homogenized. Soil pH was measured with a 1:1 soil to water ratio. Loss on ignition was converted to % organic matter (OM) with the formula: % OM = (0.618 ×LOI) + 0.69 (Donald and Harnish, 1993). Soil nutrient concentrations of P were extracted using 0.5 M sodium bicarbonate extraction (Olsen et al. 1954). Zn and Mn extracted using the DPTA method (Lindsay and Norvell 1978). K, Ca, Mg were extracted with 1M neutral ammonium acetate (Simard 1993). Soil nitrate was extracted using 1M of KCl method. Zn, Fe, Mn, and Cu were also extracted using the phosphoric acid extractant (Hoff and Mederski 1958). These extractions were measured using ICP. Other soil properties measured are C/N ratio, bulk density and concentrations of total metals. Blueberry potential per quadrat was calculated by multiplying the number of flowers by 35 percent (Barker et al. 1964) or number of berries by the stem density.

2.2.3 Data Analyses

Skewness and kurtosis statistics were calculated using SPSS for all variables to determine whether or not the data followed normal distribution. If the data was non-normal, it was corrected with a logarithmic transformation.

Non-metric multidimensional scaling (NMS), an ordination technique, was performed on the data to explore similarities of the soil and blueberry characteristics and to link the physical and chemical properties of the environment to the blueberry community characteristics using PC-ORD v5.10 (Mather 1976 and Kruskal 1964). This statistical method's advantages and disadvantages are described by Clarke (1993). The NMS was performed using the Sorenson distance measure. Ordinations began using a random starting method with 6 initial axes and 250 runs with real data to minimize stress. Ninety-nine iterations were performed to create the final solution; with a final

stress of 9.90. The final instability was less than 0.00. The next statistical operation performed was Multi-response Permutation Procedures (MRPP) in PC-ORD as described by Mielke (1984) and Mielke and Berry (2001) using the Sorenson distance measure. The MRPP provided a measure of size and distance between the bog layers and blueberry soil areas. This procedure tests whether there is no difference between 2 or more groups. The final statistical technique used was Discriminant Analysis (DA) in SPSS v16.0 to examine the differences among the various bog layers and the blueberry soils (Horton et al. 1968). The DA will provide the opportunity to discover specific variables that predict group membership. Box's Test of Equality of Covariance Matrices was performed to test the assumption of homogeneity of covariance matrices.

2.2.4 Data Sources

The GIS 3D model of the GG3 peatland was constructed using information compiled in previous report (Fabius, 2005), which included a shapefile with the boundaries of 32 individual peatbogs, pdf maps of 73 boreholes in the peatlands, and peat characteristics from boreholes such as depths, pH, and bulk density. Other peat soil characteristics including total Al, Ba, Ca, Cr, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, Ti, V, Zn, and extractable Ca, K, Mg, Na, Cu, Fe, Mn, Zn and P (for 36 boreholes) were determined from stored borehole samples. Reference maps were downloaded from the Ontario Base Maps, including layers for pipelines, railway, lakes, roads and other wetland areas.

2.2.5 ArcGIS Procedure

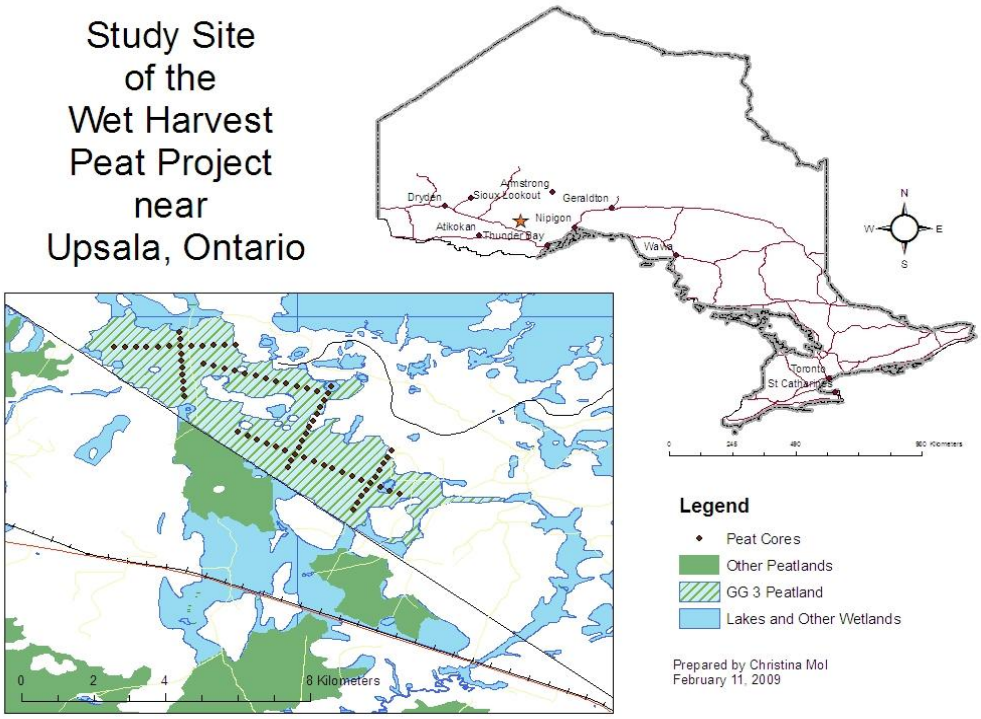

Figure 2-1 Study Site of the West Harvest GG3 Peatland near Upsala, ON

A pdf map from the Fabius' (2005) report (Figure 2-1) that showed the borehole locations was loaded into ArcGIS as a .tif file. The Georeferencing tool was used to ensure that the correct coordinate system, NAD_1983_UTM_Zone_15N, was used. This map showed a few different landmarks that allowed to line up the map with an Ontario Base Map, including a pipeline, railway, lakes and roads. It also contained a scale to determine the proper proportions. Five control points were chosen to ensure that the error was kept to a minimum. Then, using the Editor toolbar, a new shape file was created of the borehole locations, named *boreholes*. A table of borehole information was added to the project and called GISGG3. This table contained a column called boreholes that matched the newly created shape file in on ArcMap. A join was created to load the information into the borehole shape file. Inverse distance weighted

interpolation was used to create a raster of the peat thickness and was performed using the Geostatistical Wizard in the Geostatistical Analyst Tool in ArcGIS. This raster was then converted to fit the outline of the GG3 bog by using the Extract to Mask tool in the Spatial Analyst/Extraction Toolset. A contour can also be created in the Spatial Analyst/Surface Analysis toolset by choosing the contour option. ArcScene was used to create a 3D display of the layers created.

2.3 Results

2.3.1 Blueberry Soils

Soil property values are shown in Table 2-1 and Table 2-2. The pH in the various blueberry soils ranged from 4.4 to 5.9 with the lowest being in Nipigon and the highest at Aroland. The organic matter of the soils ranged from 2.3% at Aroland to 27.8% at Atikokan. Bulk density was between 0.33 g cm^{-3} at Nipigon to 0.68 g cm^{-3} at Dryden. The C/N ratio varied from 0.62 at Aroland to 8.74 at Wawa. Although not displayed in the tables, detectable levels of arsenic were evident at the sites in Wawa ranging from 14.7 to 154.1 ppm.

Table 2-1 pH, Organic Matter Content, Bulk Density and Extractible Concentrations of Blueberry Soils

Soil Material	pH	OM %	Bulk Density	Extractable elements (ppm)							
				Ca	P	K	Mg	Fe	Zn	Cu	Mn
Aroland	5.9	2.3	0.72	167	16	30	16	94	1.0	0.6	21.7
Dryden	5.3	3.2	0.68	175	20	47	38	112	0.9	0.6	9.9
Hook Rd	4.5	13.6	0.35	393	6	170	148	189	9.6	1.5	25.0
Ignace	5.2	5.2	0.60	164	3	60	31	138	1.7	1.0	11.4
Lac Des Milles Lacs	5.3	5.2	0.64	243	6	97	69	200	3.2	1.2	7.7
Nipigon	4.4	22.2	0.33	526	4	177	148	188	12.0	1.0	40.1
Sioux Lookout	4.7	11.6	0.56	182	9	75	60	128	3.8	0.6	20.2
Atikokan	4.6	27.8	0.31	844	14	259	194	128	13.7	1.8	73.1
Wawa	4.8	4.7	0.54	43	3	44	8	329	2.0	2.6	6.1
Mean	4.9	11.8	0.51	326	8	113	86	169	6.0	1.2	25.5
Standard Deviation	0.5	9.1	0.16	247	6	78	67	71	5.0	0.7	21.3

Table 2-2 Carbon to Nitrogen Ratio and Total Concentrations of Blueberry Soils

Soil Material	C/N Ratio	Total Concentration (% of dry weight of soil)					
		Al	Ca	Fe	Mg	P	K
Aroland	0.62	0.76	0.17	1.29	0.22	0.059	0.035
Dryden	0.82	0.82	0.12	1.03	0.15	0.042	0.037
Hook Rd	2.42	1.05	0.16	2.21	0.24	0.029	0.054
Ignace	5.68	0.97	0.13	1.31	0.15	0.020	0.030
Lac Des Milles Lacs	3.20	2.00	0.28	3.14	0.49	0.042	0.058
Nipigon	6.39	0.82	0.23	1.05	0.16	0.034	0.063
Sioux Lookout	1.45	0.41	0.07	0.78	0.07	0.029	0.027
Atikokan	0.90	0.43	0.22	0.72	0.07	0.039	0.046
Wawa	8.74	1.70	0.08	3.50	0.53	0.039	0.034
Mean	2.14	0.98	0.17	1.61	0.22	0.037	0.045
Standard Deviation	2.92	0.54	0.07	1.04	0.17	0.01	0.01

Various properties of the blueberry populations are summarized in Table 2-3. Height of the plants was highest in Dryden at 32.2 cm and shortest in the Hook Road population at 19.2 cm. The stem density ranged from 1.9 stems per 15 cm^2 quadrat in Dryden to 4.9 stems per 15 cm^2 quadrat in Nipigon. Berries per stem were the highest

in Lac des Milles Lac at 31.4 and lowest in Sioux Lookout at 14.8. Calculated blueberry potential per quadrat ranged from the highest in Nipigon at 148.9 to the lowest in Dryden at 32.1.

Table 2-3 Properties of Blueberry Plants

Plant Material	Height (cm)	Blueberry Potential per quadrat	Stem Density	Berries per stem
Dryden	32.2	32.1	1.9	16.6
Hook Rd	19.2	89.2	3.2	27.9
Ignace	21.6	46.5	2.8	16.6
Lac Des Milles Lacs	21.6	104.3	3.3	31.4
Nipigon	19.1	148.9	4.9	30.9
Sioux Lookout	20.3	32.6	2.2	14.8
Atikokan	22.3	66.1	2.9	22.6
Wawa	23.4	70.6	3.9	18.3
Average	22.5	73.8	3.1	22.4
All values are averages of 25 quadrats at 5 sites except for Nipigon which is at 10 sites. NA stands for not available. Blueberry potential per quadrat was calculated by multiplying the number of flowers by 35 percent or number of berries by the stem density. Berries per stem Information for Wawa and Sioux Lookout collected in 2009 while the rest was collected in 2008.				

The percentage of visually healthy and nutrient deficient leaves are tabulated in Table 2-4 and visualized in Figure 2-2. The site with the healthiest population was in Dryden while the site with the least healthy leaves was in Lac des Milles Lac, which had a high level of potassium deficiency and a moderate nitrogen deficiency showing in the leaves. Potassium deficiency was least evident with most populations showing less than 5.6% of the symptoms. The area with the highest potassium deficiency was in Sioux Lookout. The area with the most advanced nitrogen deficiency was on the Hook Road.

Table 2-4 Visual Nutrient Deficiencies in Blueberry Leaves

Location	Healthy Green	Slight Nitrogen Deficiency	Low Nitrogen Deficiency	Moderate Nitrogen Deficiency	Advanced Nitrogen Deficiency	Potassium Deficiency	Phosphorus Deficient	Visible Symptoms
Dryden	83.2	9.6	0.0	7.2	0.0	0.0	0.0	16.8
Hook	66.4	0.0	0.0	22.4	3.2	8.0	0.0	33.6
Ignace	72.0	4.8	0.0	21.6	0.8	0.0	0.8	28
Milles Lac	23.2	0.0	3.2	18.4	2.4	49.6	3.2	76.8
Nipigon East	40.0	1.6	8.8	25.6	1.6	20.0	2.4	60
Nipigon West	52.8	0.8	3.2	26.4	4.8	11.2	0.8	47.2
Sioux Lookout	60.0	9.6	0.0	16.8	0.8	7.2	5.6	40
Atikokan	72.8	0.8	0.0	13.6	0.0	12.0	0.8	27.2
Wawa	57.6	0.8	0.0	28.0	2.4	9.6	1.6	42.4
Total	57.5	3.0	1.7	21.2	2.2	12.8	1.7	42.5

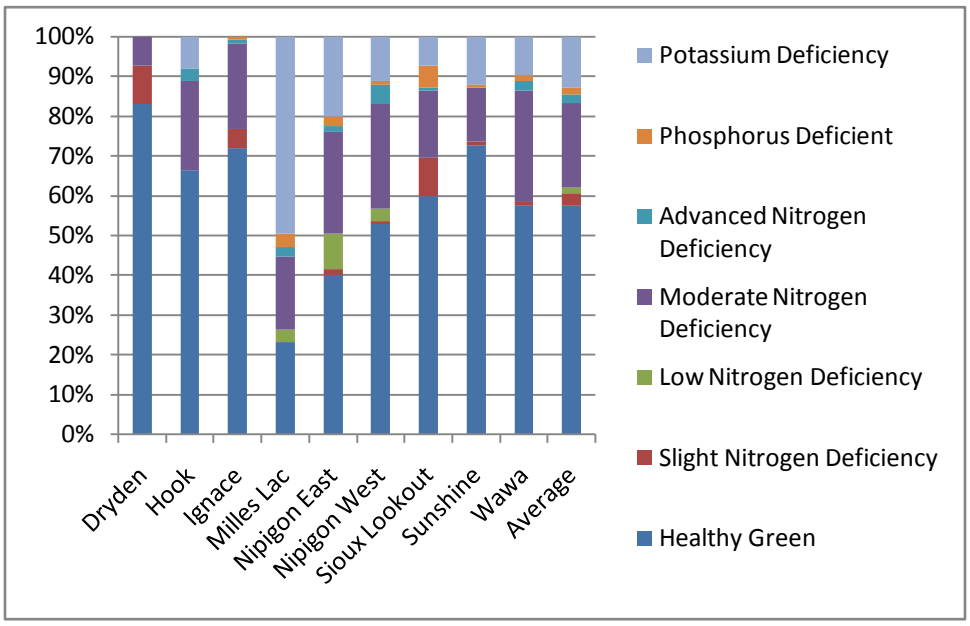

Figure 2-2 Percentages of Leaves with Nutrient Deficiencies at Sites across Northwestern Ontario

2.3.2 Peat Soils

Below detectable limits were found for beryllium, cadmium, selenium. Arsenic was below detectable limits for all samples except for 10 samples, which ranged from 10.2 to 31.5 (not shown). Molybdenum was below detectable limits for all samples

except one, which was at 5.9, just above the detectable limit of 5.0 (not shown). Table 2-5 contains the mean values of the extractable concentrations, pH, average humification level and bulk density for various depths of the peat soils. Ninety-four percent of the peat samples had a bulk density below 0.20 g cm^{-3}, four percent were between 0.20-0.30 g cm^{-3}, and 1.5 percent was in the range 0.49 to 1.04 g cm^{-3}. Of the peat samples, 11 % had a pH below 4.5, 25 % were between a pH of 4.5 to 5.5, 43 % were between 5.5 and 6.0 and 19% were between 6.0 and 7.0. One peat samples contained less than 140 ppm of extractable iron. Of peat samples, 73 % were less than 9ppm of extractable phosphorus, 15 % were between 9 and 15 ppm, 7 % of peat samples were between 15 and 26 ppm, and 3 % were between 26 ppm and 148 ppm. Forty seven percent of the peat samples fell below 25ppm of extractable potassium, 14 % were in the range of 25 to 42 ppm, 6 % were in the range of 42 to 58 ppm and 32 % were above 58 ppm.

Table 2-5 Mean values of extractable elements, bulk density, pH and humification classification level at different depths of the GG3 peatland

Soil Layer (m)	H	pH	Bulk Density	Extractable elements (ppm)							
				Ca	P	K	Mg	Fe	Zn	Cu	Mn
0.0-0.5	3	5.27	0.08	5915.94	14.96	1056.37	1540.40	2112.27	34.05	6.38	195.61
0.5-1.0	4	5.27	0.10	5427.93	14.38	95.74	1000.34	1365.85	11.83	5.01	85.13
1.0-1.5	5	5.53	0.11	7123.28	5.72	37.30	1131.28	1366.98	8.08	5.71	92.98
1.5-2.0	6	5.32	0.16	7125.71	5.73	21.96	1016.41	1448.23	9.25	5.91	67.03
1.5-2.5	6	5.65	0.11	8699.84	7.37	23.05	1419.44	1654.10	6.20	12.75	114.95
2.0-2.5	6	5.62	0.17	9164.41	6.74	19.87	1278.90	1120.10	11.37	8.90	113.57
2.5-3.0	6	5.62	0.13	10570.27	6.20	20.41	1318.14	1069.88	32.34	10.47	70.63
2.5-3.5	7	5.76	0.29	7268.99	4.50	20.09	1236.98	862.06	3.82	4.66	62.58
3.0-3.5	7	5.85	0.12	11855.62	5.33	20.81	1623.78	731.29	12.19	3.56	89.06
3.5-4.0	6	5.39	0.08	8965.55	5.38	23.21	1020.56	834.76	22.75	9.63	80.07
3.5-4.5	7	5.82	0.10	9570.40	4.91	26.96	1524.05	730.54	14.12	76.03	85.64
4.0-4.5	6	5.83	0.09	10657.77	5.15	35.59	1876.58	854.04	28.33	4.11	83.84
H – Humification rating in the Von Post Classification from 1 being the least decomposed to 10 being highly decomposed (Soil Classification Working Group 1998).											

Table 2-6 shows the total concentrations of select elements at different depths. Total iron had the greatest concentrations of all the elements tested. Potassium and phosphorus were the least prevalent element of all the nutrients.

Table 2-6 Total Concentrations of different layers of the GG3 Peatland

Soil Layer (m)	Total Concentration (%)					
	Al	Ca	Fe	Mg	P	K
0.0-0.5	0.2856	1.9686	3.9692	0.3501	0.1388	0.1837
0.5-1.0	0.3669	1.8160	2.0911	0.2524	0.1166	0.0346
1.0-1.5	0.3353	2.3626	2.0423	0.2686	0.0854	0.0216
1.5-2.0	0.4571	1.7652	1.4860	0.2036	0.0651	0.0193
1.5-2.5	0.4206	3.1542	2.0846	0.3719	0.0648	0.0298
2.0-2.5	0.6068	3.1453	2.0240	0.3595	0.0623	0.0311
2.5-3.0	0.4141	2.3051	1.1852	0.2423	0.0395	0.0256
2.5-3.5	0.6236	3.2441	1.7579	0.4398	0.0593	0.0443
3.0-3.5	0.5443	4.5046	1.8270	0.4682	0.0603	0.0342
3.5-4.0	0.6960	2.4504	1.2845	0.5072	0.0507	0.0456
3.5-4.5	1.0885	4.5425	2.2827	1.0229	0.0550	0.0887
4.0-4.5	0.3588	3.6474	1.5636	0.5050	0.0373	0.0274

Table 2-7 contains the chemical characteristics of the peat soils defined by the humification level within the peatland. Table 8 displays the total concentrations of elements by humification level. For the some of the extractable and total elements concentrations (Al and Ca), pH and bulk density the values increase with the increase in humification level. The bottom mineral layer does not follow this trend. Potassium, phosphorus, zinc and manganese values decrease with increasing humification level. Other elements do not show a noticeable trend.

Table 2-7 Mean values of extractable elements, bulk density, pH and depths at different humification levels of the GG3 peatland

H	Average Range of Depth (m)	pH	Bulk Density	Extractable elements (ppm)							
				Ca	P	K	Mg	Fe	Zn	Cu	Mn
2	0.2 - 0.6	5.14	0.073	6321.38	16.98	696.69	1310.21	1900.16	34.62	6.14	193.30
3	0.6 - 1.0	5.20	0.069	5162.33	11.16	399.41	1370.39	1745.49	27.66	4.09	135.49
4	1.2 - 1.7	5.55	0.108	7404.36	8.30	96.25	1245.53	1396.77	19.09	4.78	101.64
5	1.2 - 1.7	5.54	0.112	7463.96	12.31	38.32	1199.75	1106.90	8.76	5.52	106.43
6	1.6 - 2.2	5.46	0.115	7850.66	7.40	39.82	1138.17	1053.05	9.89	7.03	99.50
7	2.0 - 2.5	5.61	0.145	8225.61	5.80	22.68	1085.72	1215.47	10.55	7.96	74.89
8	2.6 - 3.1	5.75	0.182	10800.65	5.20	16.49	1735.23	1242.20	7.93	10.18	105.36
bottom layer (mineral)	2.5 - 2.8	NA	1.036	4939.83	9.38	54.02	402.17	1092.39	8.36	22.42	41.11
H – Humification rating in the Von Post Classification from 1 being the least decomposed to 10 being highly decomposed., NA – Not Available											

Table 2-8 Total Concentrations of significant elements at different humification levels in the GG3 peatland

H	Total Concentration (%)					
	Al	Ca	Fe	Mg	P	K
2	0.2546	1.9302	3.6275	0.3299	0.1273	0.1544
3	0.2656	1.8117	3.0210	0.2918	0.1178	0.1157
4	0.3305	2.0664	2.0459	0.2688	0.0917	0.0399
5	0.3948	2.9019	2.0587	0.3292	0.0909	0.0266
6	0.4471	2.8842	1.9900	0.3242	0.0845	0.0306
7	0.5362	2.6438	1.7485	0.2951	0.0786	0.0301
8	0.6659	4.0939	2.4279	0.4971	0.0603	0.0367
bottom layer (mineral)	1.4857	2.3645	2.0312	0.9051	0.0547	0.0681

2.3.3 A comparison of blueberry and peat soils using NMS procedure

NMS gave a solution on two axes for the different blueberry and peat soils (Figure 2-3 and Figure 2-4). Proportion of variance represented by axis one is 0.66 and 0.275 for axis 2. The cumulative variance of the two equations is 0.933 and the r-value for axis 1X2 is 0.194. The blueberry soils and peat soils are separated by a distinct line for the most part except for a few sites (Nipigon 5, 9 and 10 and Atikokan 2 and 3 in circles in Figure 2-3) that are mixed with some of the peat layers. The soil characteristics

that helped determine this separation are titanium, bulk density, chromium, vanadium, aluminum and lead (Figure 2-4). The peat soils do not seem to be grouped into separate layers but scattered on the top two thirds of the graph, while the lower third of the graph with the blueberry soils has some groupings such as Wawa and Ignace. MRPP comparison of the nine different blueberry soils and the eight different peat layers provided the chance-corrected within-group agreement (A), which was determined to be 0.23 with a p value of less than 0.00. The discriminant function analysis was performed using the blueberry soils with five sites per group for Aroland, Dryden, Hook Road, Ignace, Milles Lacs, Sioux Lookout, Sunshine Road and Wawa. Ten sites were in the group for Nipigon. Peat was grouped by humification layer with the following number of samples per Von Post classification, 2 – 18, 3 – 24, 4 – 40, 5 – 29, 6- 31, 7- 39, 8 – 23, mineral layer – 6. Using the soil properties as variables and the stepwise method, axes 1 and 2 accounted for 81% of the variance, with axes 1 accounting for 69% (Figure 2-5). The Box's Test of Equality of Covariance Matrices of Canonical Discriminant Functions gave a result of 1794 with a p of 0.000 and thus there was no violation for the assumption of homogenous variances among the groups. The most influential variables identified were bulk density, and total concentrations of lead, titanium, aluminum, chromium, sodium and nickel. 56.2% of cases were correctly classified (Table 2-9). The majority of the groups are centered within the plot of the first two axes (Figure 2-5), with the exception of the bottom mineral layer, Hook Road and Milles Lacs.

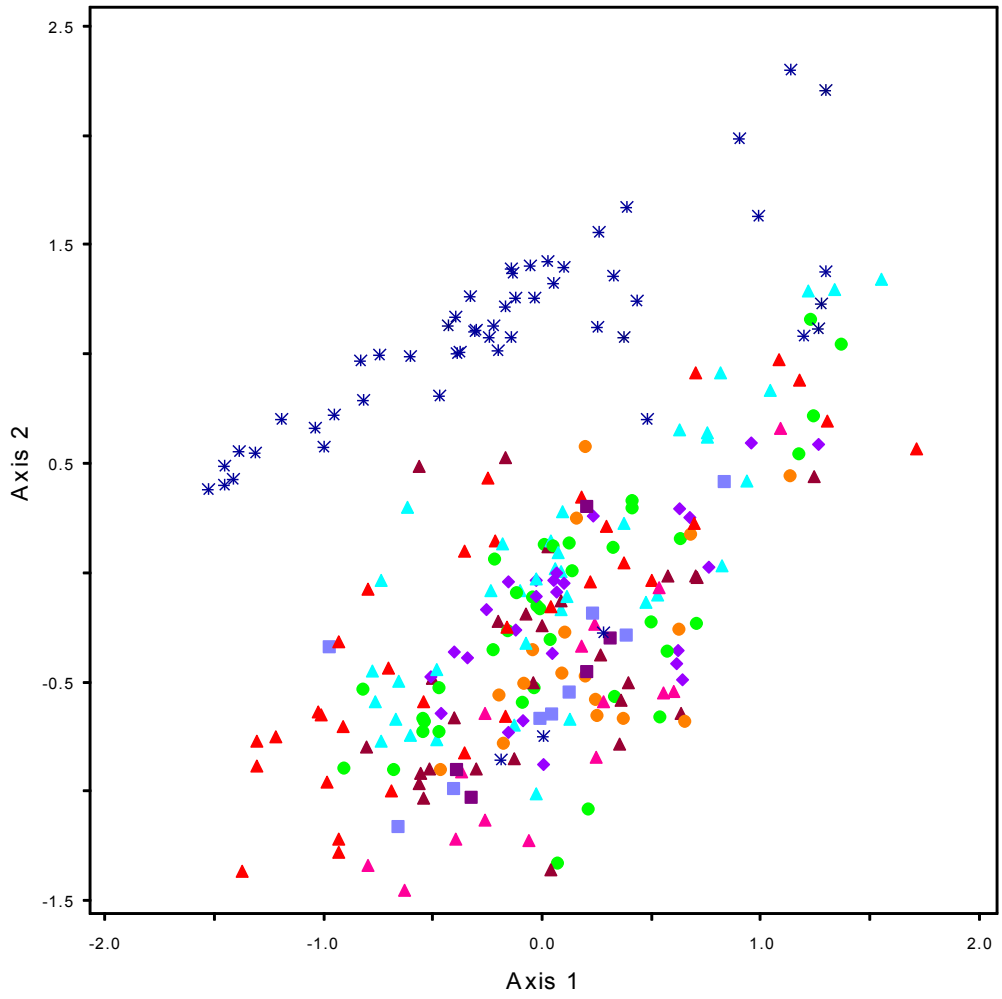

Figure 2-3 Nonmetric Multidimensional Scaling Graph Ordination of Axis One and Axis Two showing blueberry (asterix) and peat soils (solid shapes representing different peat layers).

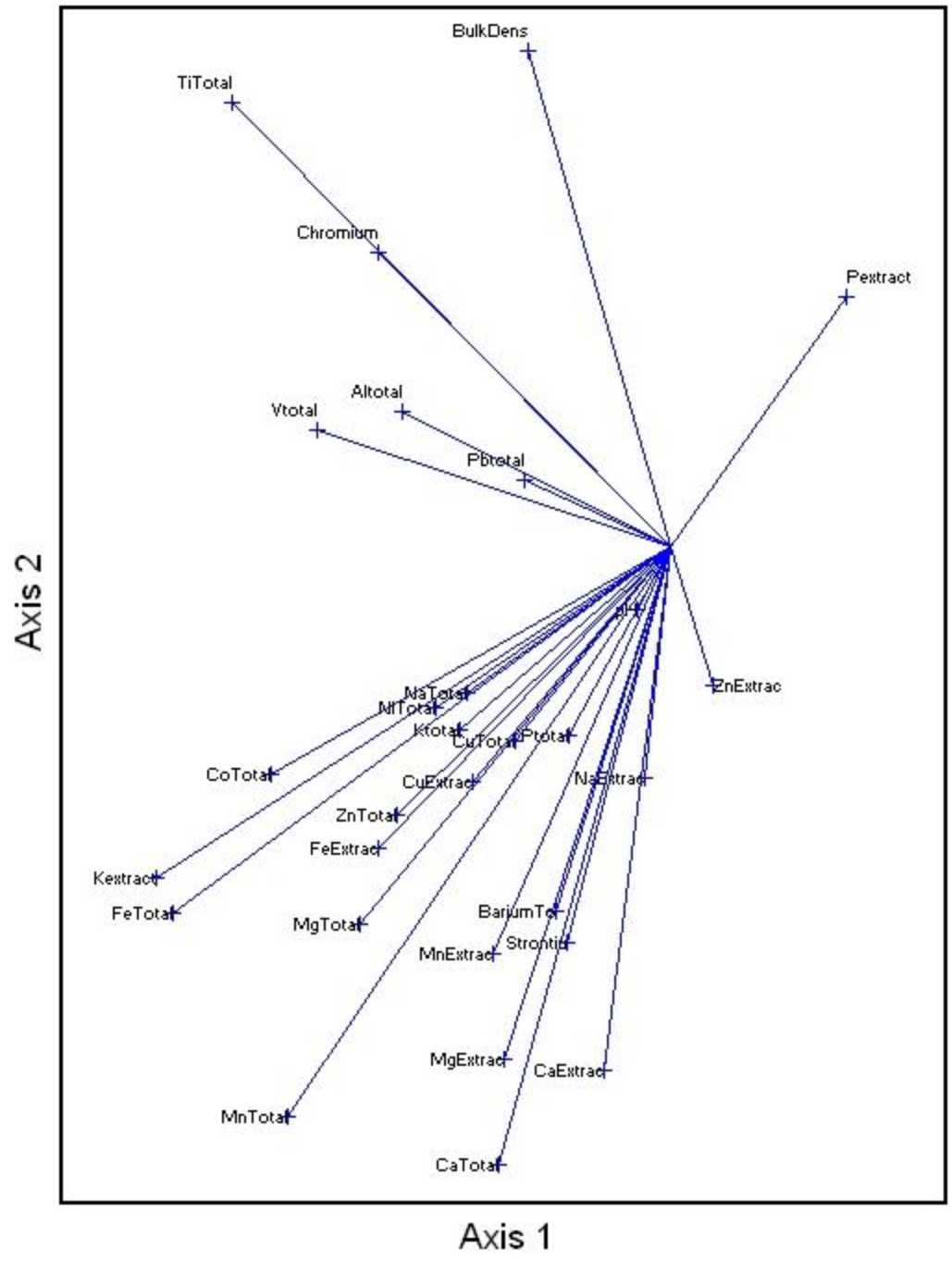

Figure 2-4 Nonmetric Multidimensional Scaling Graph Ordination of Axis One and Axis Two showing variables.

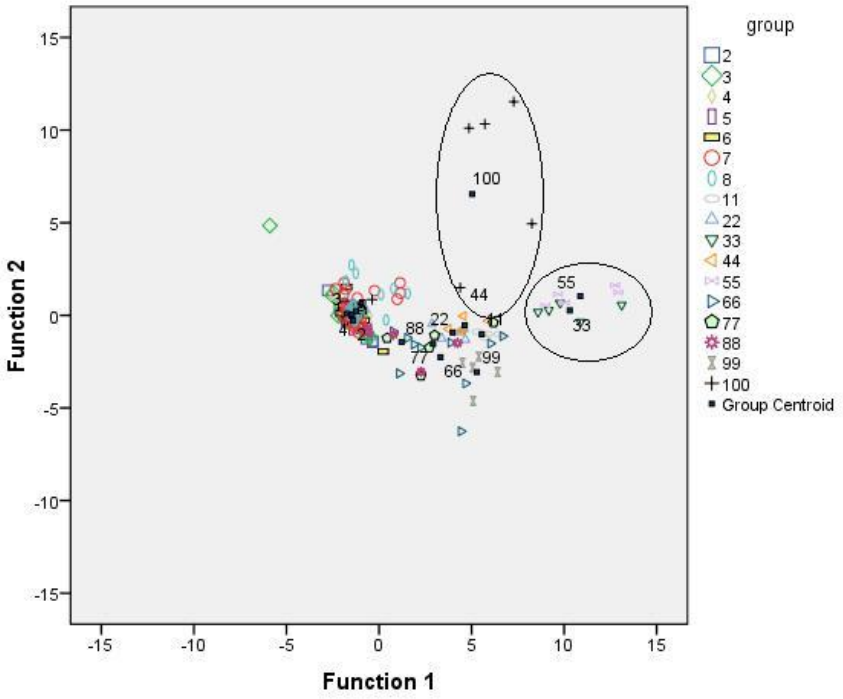

Figure 2-5 Canonical Discriminant Functions of the Discriminant Function Analysis. Groups 2 through 8 represent Humification layers of the GG3 Bog. 11 - Aroland, 22- Dryden, 33 - Hood Road, 44 - Ignace, 55 - Milles Lac, 66 - Nipigon, 77 - Sioux Lookout, 88 - Atikokan, 99 – Wawa, 100 – Mineral layer beneath the GG3 Peat bog.

Table 2-9 Characteristics of discriminant functions (DF1, DF2) used to separate the peat layers and the blueberry soils.

Variable	DF1	DF2
	Standardized Coefficient	Standardized Coefficient
Bulk Density	0.512	-0.147
Total Lead	0.291	-0.319
Total Titanium	1.490	0.367
Total Aluminum	-0.588	-0.099
Total Chromium	0.033	-0.744
Total Sodium	-0.579	0.692
Total Nickel	-0.103	0.497
Relative % of Sampling Variance Explained	69.2	12.0
Cumulative %	69.2	81.3
% correct classification of soils		56.2

The blueberry soils in Northwestern Ontario had a greater range in comparison to the soils from Nova Scotia while the peat had an extreme range compared to the blueberry soils. Toxicity of some of the micronutrients might be an issue due to their higher availability at low pH levels, especially iron, manganese and copper.

Table 2-10 Northwestern Ontario blueberry extractable soil nutrients as compared to soils in Nova Scotia and Peat from the GG3 bog (ppm for all variables except %OM and pH)

	Nova Scotia			Northwestern Ontario			Peat		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
%OM	9.0	6.7	11.3	11.8	0.7	58.4	NA	NA	NA
pH	4.7	4.4	5.0	4.9	3.8	6.6	5.5	3.6	6.5
P	65	18	111	8.5	0.3	31.9	8.8	1.2	148
K	143	86	200	114	14	427	183	0.3	10504
Ca	427	187	670	326	23	1594	7704	315	18568
Mg	81	33	139	86	4	377	1252	75	9019
Fe	286	184	389	169	29	763	1343	132	8386
Mn	59	14	105	26	1	145	105	3	601
Cu	0.9	0.5	1.4	1.2	0.1	6.2	7.1	0.3	76
Zn	3.4	2.3	4.6	6.0	0.3	27.3	15.6	0.8	243

2.3.4 Visualizing NMS using GIS

In order to examining the interrelationships of the data, descriptive statistics (Min, Max, Mean, and Standard Deviations) were calculated (Table 2-11). Pearson Correlations were calculated for the data. Using the NMS values as attributes in ArcGIS, a clear representation of the peat data is displayed (Figure 2-6, Figure 2-7, Figure 2-8, Figure 2-9, Figure 2-10, Figure 2-11, and Figure 2-12). Each of the layers is mapped using the NMS values from the soil chemistry statistical analysis. Areas that are blue have positive values on axis two just like the blueberry soils, with darker blue having the highest NMS values. These areas tend to be concentrated along a band running east to west through the centre of the bog, except for the bottom layer. A 3D representation of

these layers (with an elevation profile exaggerated by a factor of 100) can be seen in Figure 2-13, which shows that the blue areas are stacked in generally the same place.

Table 2-11 Descriptive Statistics for Peat Soils.

	Min	Max	Mean	SD
Bulk Density	0.03	1.04	0.193	0.201
Al total	344.6	31641.5	5442.470	4679.927
Ba Total	9.3	570.9	111.651	79.718
Ca Total	176.5	99071.6	20804.927	18015.560
Co Total	0.6	24.9	5.953	4.548
Cr Total	0.6	105.6	11.767	13.365
Cu Total	1.5	292.2	16.364	23.038
Fe Total	2028.1	142074.0	20888.154	19004.876
K total	82.1	10475.1	529.330	942.771
Mg Total	135.3	21377.7	3115.435	2432.659
Mn Total	10.6	3961.4	450.374	601.399
Na Total	1.3	3156.4	178.734	342.838
Ni Total	1.3	71.3	12.507	9.834
P Total	43.8	3181.8	769.003	521.295
Pb total	1.5	76.2	10.777	13.960
Sr Total	1.3	123.8	33.157	20.025
Ti Total	9.9	2584.2	296.834	473.681
V total	1.3	168.3	24.634	30.609
Zn Total	0.6	339.7	36.403	46.975
Ca Extract	22.8	18568.2	6278.885	4605.856
K Extract	0.3	10504.3	183.033	734.499
Mg Extract	3.7	9019.1	1036.916	869.495
Na Extract	13.7	1564.4	130.535	120.617
Cu Extract	0.1	76.0	3.668	7.546
Fe Extract	28.7	8386.3	1124.617	1164.427
Mn Extract	1.1	601.0	90.478	88.188
Zn Extract	0.3	242.7	13.925	21.119
P Extract	0.0	148.2	8.276	11.251
pH	3.6	6.6	5.383	0.656

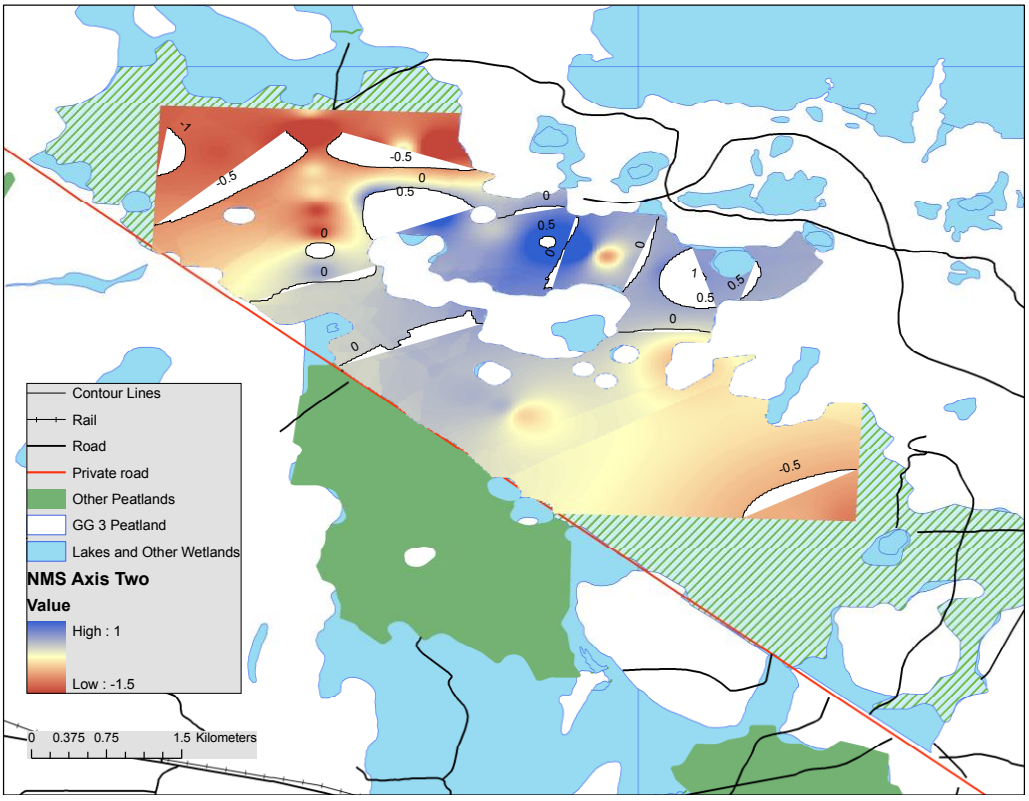

Figure 2-6 The upper layer (0-0.5m) of the GG3 peatland plotted with NMS axis two.

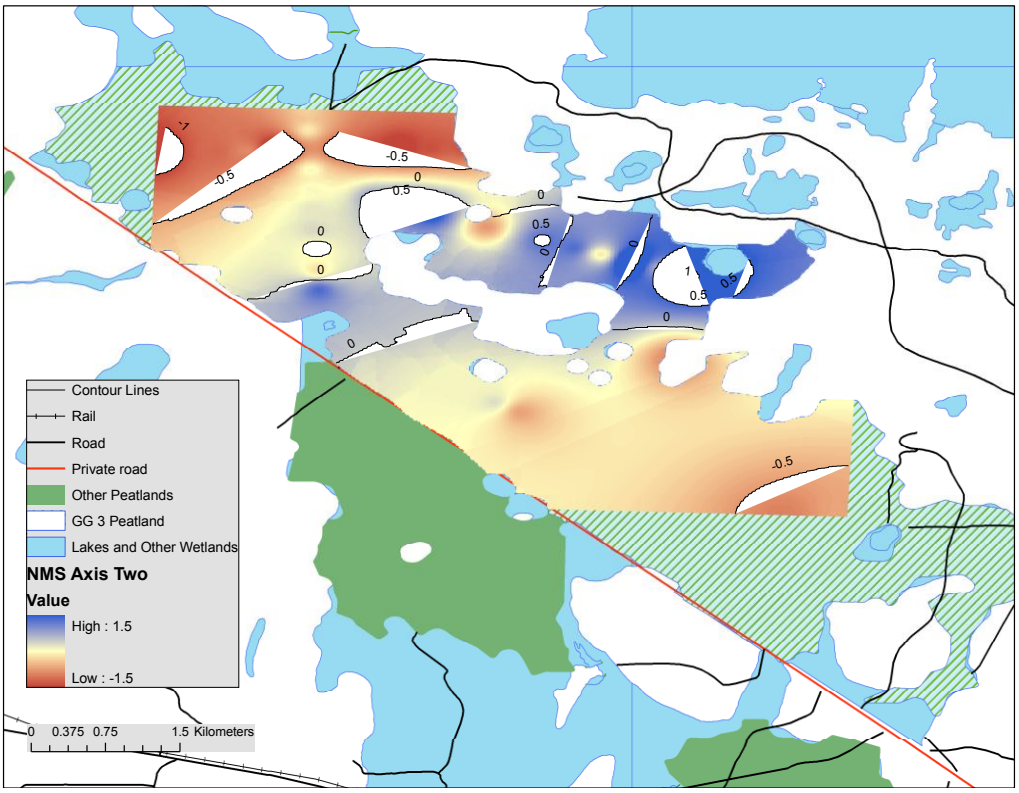

Figure 2-7 The second layer (0.5-1.0m) of the GG3 peatland plotted with NMS axis two.

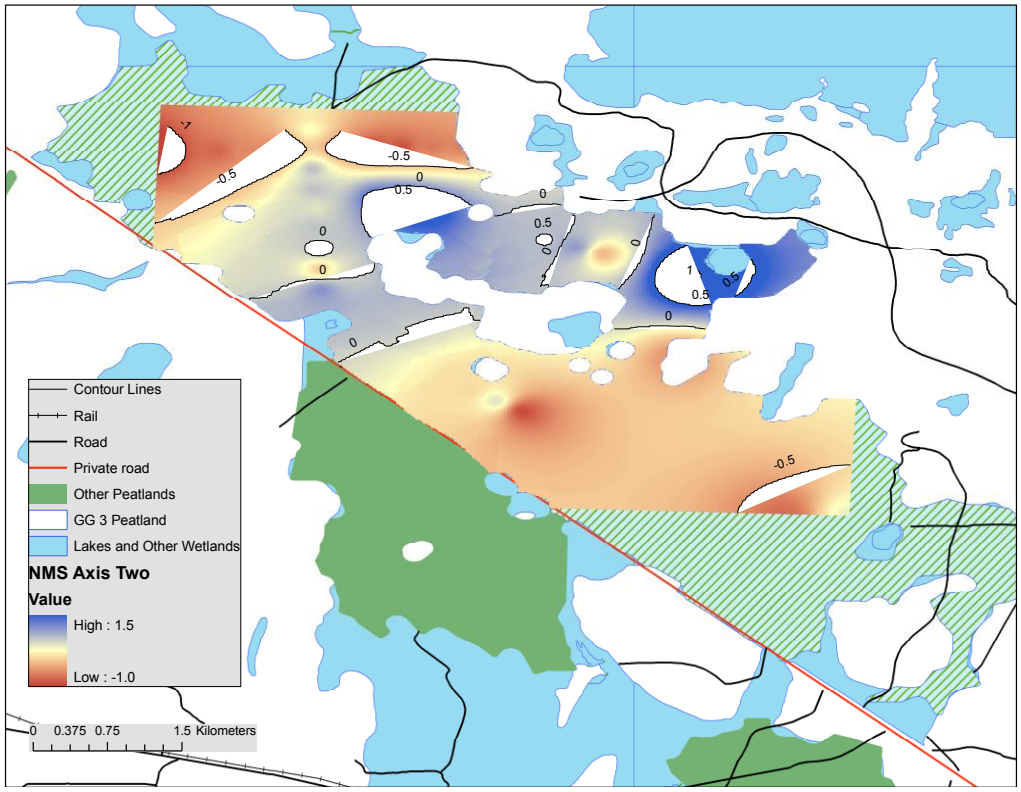

Figure 2-8 The third layer (1.0-1.5m) of the GG3 peatland plotted with NMS axis two.

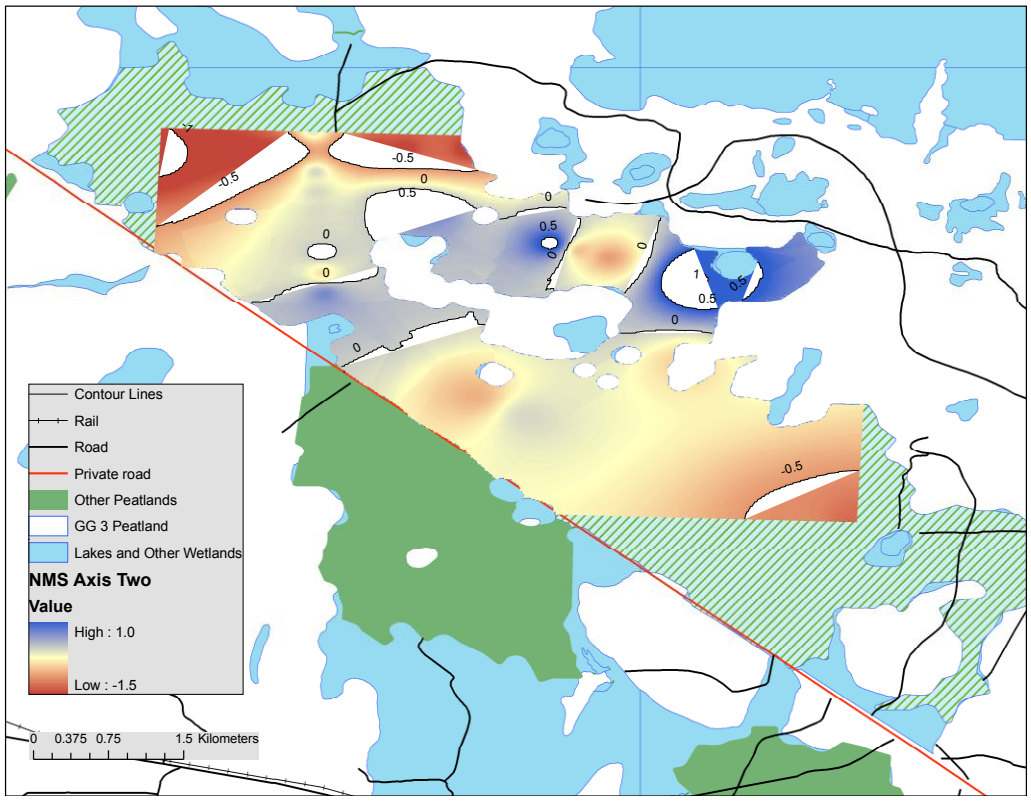

Figure 2-9 The fourth layer (1.5-2.0m) of the GG3 peatland plotted with NMS axis two.

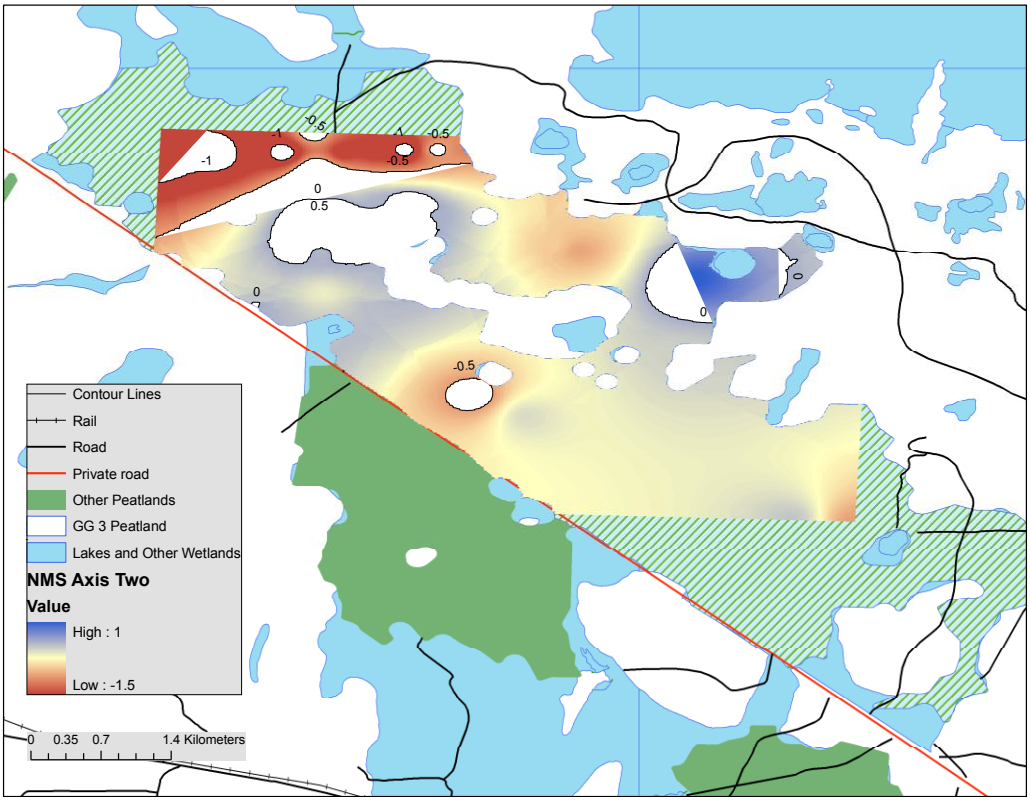

Figure 2-10 The fifth layer (2.0-2.5m) of the GG3 peatland plotted with NMS axis two.

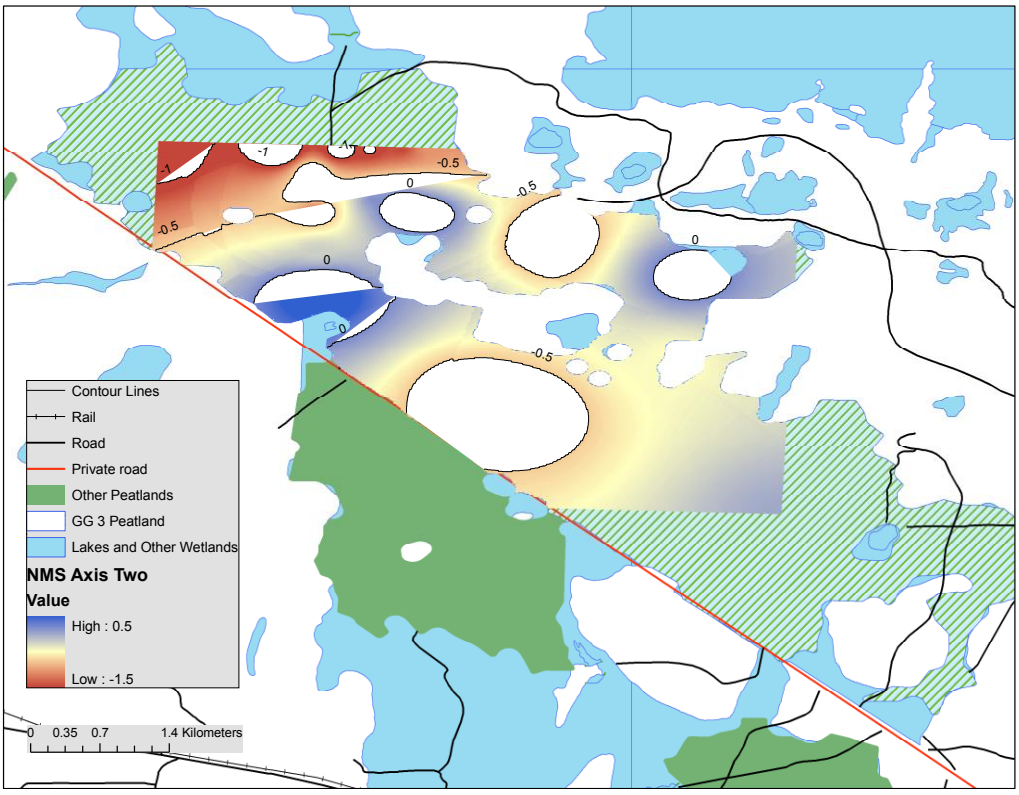

Figure 2-11 The sixth layer (2.5-3.0m) of the GG3 peatland plotted with NMS axis two.

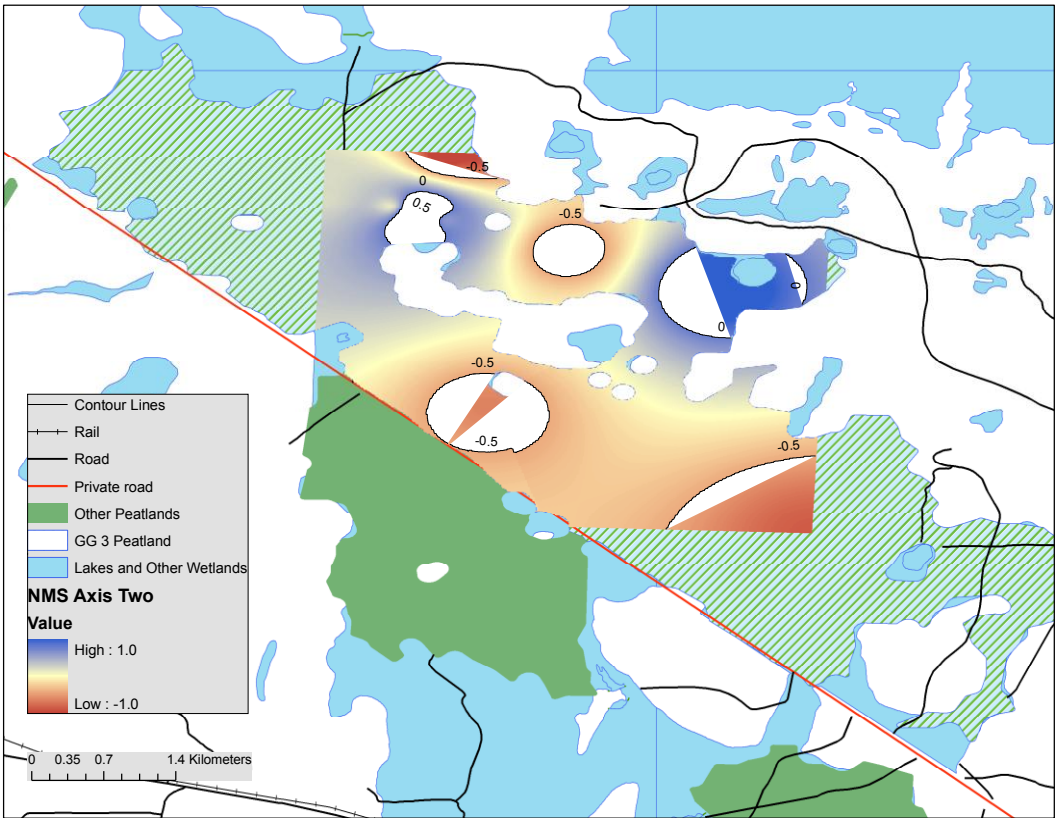

Figure 2-12 The bottom layer (3.0-4.5m) of the GG3 peatland plotted with NMS axis two.

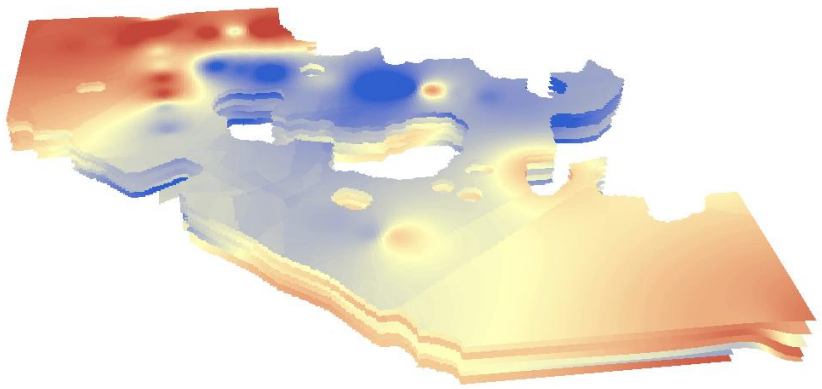

Figure 2-13 3D representation of the seven layers of the GG3 peatland plotted with NMS axis two.

2.4 Discussion

2.4.1 Soil Characteristics of Blueberry Populations

The native blueberry populations were within the accepted limits of metals for agricultural soil recommendations. The exception was for Wawa where the arsenic level was above 12 ppm which is the Canadian Soil Quality Guidelines (CSQG) (2002). Anastasia and Kender (1973) found that arsenic at levels above 69.5 ppm have the potential to decrease plant growth and productivity, but the arsenic limits itself mainly to the plant roots, stems and leaves. This high level around Wawa could be due to the mining activity in the region (Gizyn 2003).

Blueberries have different criteria for soil quality in comparison to the average agricultural crop. As they are a slow growing perennial crop, much of the nutrients are stored underground in rhizomes. Visual symptoms can give a general idea of possible nutrient deficiencies. While Lac de Milles Lac showed the most visual symptoms of potassium deficiency, Ignace, Dryden, Sioux Lookout and Wawa all had lower levels of extractable potassium. Possible cation interference with other nutrients could be one explanation for this site. The Hook Road population showed the highest amount of advanced nitrogen deficiency, but this also could have been due to forestry herbicide treatments, which can show similar symptoms of yellowing leaves.

The blueberry soils also show on average a poor rating for the C/N ratio, extractable iron and phosphorus, medium ranking for pH, and a very good level of extractable potassium and bulk density. Soil nutrient values can be a problem for determining potential nutrient deficiencies in wild blueberries (Argall et al. 1998), but Eaton's et al. (2009) results are used as a general guideline (Table 2-10). Since

blueberries are a perennial shrub, deficiencies can be a chronic problem and can develop over a period of time.

2.4.2 Peat Characteristics versus Agricultural Soils

Peat from the GG3 bog seems to meet most of the criteria for soil quality for most agricultural soils. The peat samples had a few examples that showed higher than accepted levels of metals. One of the samples registered a level above the CSQG for lead, another at a higher level for nickel and one above the level for vanadium (not shown), but these were not seen in the average values of the peat samples. Three peat samples were at higher than CSQG for zinc, but only one of the samples had extractable levels above the limit. Observed toxicity to these elements is rarely seen in field conditions. Vanadium can cause reduced yield though apical chlorosis and abnormal root growth, but the effects are reduced in the presence of high iron levels (Morrell et al. 1986). Zinc may also cause leaf chlorosis in blueberries at high concentrations (Gupton and Spiers 1996). Some metals in high amounts can also lead to phytotoxicity such as Cu, Cr, Mn, and Ni. The values of these elements have a tendency to increase with depth within the peat profile.

In terms of soil quality, Kreshtapova and Krupnov (1998) have classified harvested peatlands by various parameters for the average agricultural crop like cereals, legumes, potatoes, turnips and grasses. Using this scale, the GG3 peatland is rated as poor for pH, extractable iron, and bulk density (Table 2-5, Table 2-6, Table 2-7, and Table 2-8). The upper layers of peat in terms of humification levels 2, 3 and 4 are rated as very good for extractable potassium, levels 5, 6, and 7 are medium to good and level 8 is poor. Only humification layer 2 is rated good for extractable phosphorus, while the layers 3 and 5 are medium and the rest of the layers are poor. The upper layers of peat

in terms of depth show a very good rating for the upper layers until a depth of 1.5 m for extractable phosphorus, and a ranking of medium for the depth until 1.0 m. The layer of mineral below the peatland has a very good bulk density level, but poor rankings of the other criteria, except for extractable potassium with a good assessment.

2.4.3 Peat versus Blueberry soils

There is a distinct separation of the blueberry soils and the peat soils with the subsoil mineral layer of the peatland separating the two soil types as seen in the NMS ordination (Figure 2-3). Since the matrix of environmental variables used in the NMS was a similarity matrix, the points on the graph that are clustered are more alike than sites that are more distant. Some of the blueberry soils groups are clustered showing soils that are similar e.g. Wawa and Ignace (Figure 2-3).

MRPP describes within-group homogeneity compared to random chance. If A =1, then the sites are completely identical. According to McCune and Grace (2002), a value below 0.1 is common in ecological circumstances and a value of 0.3 is quite high. A value of 0.23 shows that the groups are more homogeneous than the common ecological circumstance but still have some separation between groups.

Discriminant function analysis failed to classify many of the peat samples by humification level but the majority of the blueberry soils were correctly classified by the soil chemical properties (Table 2-9). These layers are misclassified by one or two levels of humification. Peat could be misidentified by the Von Post humification rating due to the relativity of the degree of decomposition of the plant matter in the sample and also due to the lack of rigid decomposition boundaries. None of the blueberry soils are misclassified as peat layers or the mineral subsoil. Most of the factors that separate the

blueberry soils from the peat soils are metals that are not typically considered in plant nutrition but could be factors in plant toxicity, such as sodium, aluminum and chromium. Bulk density is the one factor that could be influenced by mixing the peat with the mineral subsoil so as to improve blueberry plant establishment and success.

2.4.4 Management of Peatlands for Blueberry Production

Harvested peatland shows promise for rehabilitation using lowbush blueberries if planted in the upper levels of the peat in the blue areas as seen in Figure 2-13. GIS mapping of the NMS values provides a useful picture of the areas within the GG3 peatland that are similar to the native blueberry soils in Northwestern Ontario. The results of the NMS performed on the soil chemistry of the peat and blueberry soils identified a separation of the soils on axis two, which is influenced by bulk density, total titanium, chromium, vanadium, aluminum and lead as well as extractable phosphorus. Of these variables, bulk density and extractable phosphorus could be altered to improve the success of establishing blueberries. Mixing the residual peat with the mineral layer below the peat land could improve the bulk density but would have to be tested for pH level to ensure optimal conditions for the blueberries. Some fertilizers may be required as based on a leaf tissue analysis to ensure proper plant nutrition. Other adverse factors that should be investigated should be the hydrology of the harvested peat land to understand where the water table location and quality following peat extraction, the uneven thickness of the residual peat and its impact of the blueberry rhizome growth, and fertilization to correct the nutrient variations across the peat bog.

The values found for this bog are also comparable to Peat margin swamp containing *Sphagnum*-wood-*Pleurozium* found near Cochrane in Northeastern Ontario for total Ca, Mg, Fe, P and K as opposed to Raised bogs or Basin Fens which show lower

nutrient values (National Wetlands Working Group 1988). Peat also has a high cation exchange capacity which means an ability to hold onto a high number of elements such as H, K, Na, Ca, Mg, and Al. Wind-Mulder et al. (1996) also found that nutrient levels increase in exposed areas of harvested bogs in comparison to natural, undisturbed bogs. P and K show variable values and Fe has a decreasing trend as the depth increases.

2.4.5 Future Research

Generally leaf tissue analysis could provide a better understanding of nutrients. Future research that would complement the soil analysis of Northwestern Ontario blueberry soils would be to do a foliar nutrient analysis of the plant leaves to determine if the plant is deficient in any of the nutrients. Another soil analysis could look at additional nutrients such as sulfur and boron. Other soils could be compared to the peat soils to identify areas that could be used for other plant, such as cranberries or trees. Areas of the peatland not ideal for blueberry growth could be rehabilitated with supplementary plants or crops. An understanding of the hydrology of the peatland following the wet harvest could improve the rehabilitation of the GG3 peatland, ensuring that the areas with optimal soil chemistry for blueberry growth would also have the ideal water conditions.

Summary and Conclusions

Understanding whether wild lowbush blueberries would be an optimal reclamation option in post wet harvest peatlands can be advanced by this thesis report. First, the diversity of genetics of *V. angustifolium* demonstrates a wide genetic foundation to initiate a breeding program for commercial cultivars for managed plantations of lowbush blueberries. Next, a comparison of soils in existing wild populations of blueberries from across Northwestern Ontario to the various layers of peat within the GG3 bog near Upsala provide a baseline for potential success of planting blueberries in the post harvest peatland. Finally, a GIS-based investigation of potential areas with optimal soil characteristics provide a visual of which layer of the peatland should be reserved for most advantageous soil for reestablishment of plant life.

Microsatellite markers developed for highbush blueberry (*V. corymbosum*) can be successfully used as a tool in lowbush blueberry (*V. angustifolium*), providing a method of identifying promising wild cultivars. The transferability of this technology to the closely related species can save much time and money in the development and expansion of the lowbush blueberry industry in Northwestern Ontario. Microsatellites are highly reproducible, and show high discrimination. Unfortunately the gene function of these markers is currently unknown and the stutter produced from the short sequence repeat can be somewhat confusing to interpret, especially for the dinucleotide repeats.

The layers within the GG3 peatland have large variability in soil chemistry and do not create distinct classes like the wild blueberry soils. The main factor that separated the peat soils from the blueberry soils is the bulk density of the soil. One

possible method of improvement could be to incorporate the mineral layer from below the peatland into the residual peat layer. Monitoring for pH and other nutrients should be done through soil tests and foliar leaf testing to ensure most favorable soil chemistry for the best blueberry establishment and production.

The use of GIS to visualize the multivariate statistics of the soil chemistries provides a visual of the peatland and can pinpoint the best locations for blueberry establishment. Areas that overlap with the native blueberry soils can be shaded, showing the best layer and location for planting.

Some questions on which reclamation option would be the best emerge after investigating the previous conclusions. The location of the water table and general hydrology of the site need to be investigated. If the area has a high water table or is too wet, blueberries would not be a viable option for this area. If the area is too dry, establishment of any crop would be difficult without some irrigation. Other crops to investigate include lignonberries (also known as red berries or partridge berries, *V. vitis-ideae*) and American cranberry (*V. macrocarpon*) which can handle wetter soil conditions. Aforestation could be another option considering the forestry industry in the region, but this option could take more time for establishment. Some sort of agroforestry opportunity with a combination of berries and trees could create a balanced choice.

Another area of concern is the survivability of the blueberry plants through the winter season. Snow cover is vital for the plants to be able to maintain the best microclimate in order to stay alive with little damage to the branches. As the bog is an open area, snow cover maybe reduced due to high winds and leave the plants open to

the elements. This issue could be rectified using snow fences and straw cover but still could be an issue for plants from other areas.

Future Research and Planning

As layers of the peatland are removed for fuel, soil chemistry and bulk density will be affected in the lower layers by the exposure to the air as well as the peat harvest machinery and method. Continued monitoring of the site would need to occur as this thesis shows a static picture of the situation. The area with optimal soil characteristics (coloured in blue in Figure 2-13) would have to be spread along the harvested areas in order to prepare a planting area for the blueberry plants. This region should also be tested to determine if the best blueberry soil is also optimal for fuel.

Also, as it is currently unknown as to what traits the microsatellite markers identify, more research has to be completed to understand which blueberries would survive best in peat soils and which genetic markers would identify these plants. Genetic variations from these blueberries in Northwestern Ontario could improve the commercial blueberry cultivars and preserve the global blueberry genetic resources. Long term monitoring of the blueberries planted at the peat harvest site could provide genetic data as well as yield information.

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Appendix 1: Wild Lowbush Blueberry and Lignonberry Planting at the GG3 peatland

Wild blueberry plants from across Northwestern Ontario were collected over the summer of 2008. These plants were grown in a greenhouse in 15 inch pots until planted at the GG3 peatland in September 2008 (Figure 0-1). Two purchased cultivars of lowbush blueberries and 13 plants of a lignonberry cultivar were also planted. Plants were covered in straw in October before the snowfall so as to trap as much snow as possible to prevent winterkill (Figure 0-2). The straw was cleared from the plants in May 2009 and used as mulch around the plantings to reduce weeds. These plants could be used as a source for tissue culture and cuttings as well as a seed source.

Lignonberry Planting	Open Space with Pizometers	Blueberry Planting
Harvested Peat Area		Boardwalk

NP10	NP8	HK5	NP1	IG4	Control	NP4	IG3
SS5	DR1	ML3	Control	WA4	IG2	NP3	WA2
HK2	WA3	NP5	NP9	Control	HK1	IG5	ML4
SS3	ML5	Control	SL1	SL4	NP7	ML1	DR2
	SL3	HK3	HG	HK4	SS2	DR5	NP6
		Control	SS1	SL2	Control	WA5	SS4

Region codes: NP – Nipigon, HK – Hook Road, IG – Ignace, Control – purchased cultivar from Dominion Seed House in Georgetown, ON, SS – Atikokan near Atikokan, DR – Dryden, ML – Lac Des Milles Lacs, WA – Wawa, SL – Sioux Lookout, HG – Hogarth Plantation, near Thunder Bay.

Figure 0-1 Blueberries directly after planting at the GG3 peat bog.

Figure 0-2 Covering blueberries with straw prior to snowfall

Appendix 2: Land Type and Climate Data for the populations of *Vaccinium angustifolium*

Table: *Vaccinium angustifolium* populations surveyed and their geographical parameters

Population	Land Type	Longitude	Latitude	Altitude	Annual Precipitation (mL)	Annual Average Temperature (^{o}C)	January Average Temperature (^{o}C)	July Average Temperature (^{o}C)
Aroland	Harvested Forest	50° 11.000' N	86° 42.000' W	324.60 m	M	M	-20.3	15.3
Dryden	Forested Area	49°47' N	92°50' W	372 m	705.5	1.8	-18.2	18.5
Hook Road	Harvested Forest	48° 38.000' N	88° 46.000' W	323.10 m	M	M	M	M
Ignace	Gravel Pit, Abandoned Airport, Harvested Forest	49° 29.000' N	92° 0.000' W	473.00 m	803.9	0.0	-18.8	16.7
Lac des Milles Lacs	Harvested Forest	48° 58.000' N	90° 12.000' W	459.00 m	671.0	-0.3	-16.9	16.6
Nipigon	Harvested Forest	49° 9.000' N	88° 21.000' W	228.60 m	816.6	1.7	-16.6	17.0
Sioux Lookout	Harvested Forest, Gravel Pit	50° 7.200' N	91° 54.000' W	383.40 m	716.1	1.6	-18.6	18.6
Atikokan	Harvested Forest	48° 45.000' N	91° 37.200' W	395.30 m	739.6	1.6	-18.1	17.7
Wawa	Surrounding Mines	47° 58.200' N	84° 46.800' W	287.10 m	1002.2	1.7	-14.8	14.8
Hogarth	Burned Forest	48° 22.200' N	89° 19.800' W	199.00 m	711.6	2.5	-14.8	17.6
Purchased cultivars	NK	NK	NK	NK	NK	NK	NK	NK
GG3	Peatland	49° 3.000' N	90° 28.000' W	483.70 m	844.8	0.1	-14.6	21.3

M = Missing data, NK = unknown

Data from Environment Canada National Climate Data and Information Archive www.climate.weatheroffice.ec.gc.ca [Accessed June 4, 2009]

Appendix 3: Correlation Matrix for the Soil Chemistry of the Blueberry Soils and the GG3 Peatland

Correlation Matrix	Bulk Density	Zn Total	P extract	pH	Ca Extract	K Extract	Mg Extract	Na Extract
Bulk Density	1.00	-0.12	0.01	-0.11	-0.49	-0.11	-0.46	-0.35
Zn Total	-0.12	1.00	0.06	0.10	0.12	0.14	0.18	0.08
P Extract	0.01	0.06	1.00	-0.03	-0.17	0.06	-0.12	0.03
pH	-0.11	0.10	-0.03	1.00	0.67	0.04	0.54	0.16
Ca Extract	-0.49	0.12	-0.17	0.67	1.00	-0.01	0.72	0.23
K Extract	-0.11	0.14	0.06	0.04	-0.01	1.00	0.58	0.79
Mg Extract	-0.46	0.18	-0.12	0.54	0.72	0.58	1.00	0.64
Na Extract	-0.35	0.08	0.03	0.16	0.23	0.79	0.64	1.00
Cu Extract	-0.11	0.15	0.02	0.21	0.21	-0.04	0.11	0.06
Fe Extract	-0.31	0.11	0.02	0.36	0.27	0.20	0.39	0.30
Mn Extract	-0.38	0.38	-0.05	0.28	0.39	0.22	0.45	0.27
Zn Extract	-0.26	0.71	0.03	-0.01	0.08	0.23	0.18	0.28
Al Total	0.51	0.01	-0.10	-0.11	-0.34	-0.14	-0.37	-0.32
Mg Total	-0.09	0.39	-0.16	0.34	0.37	0.05	0.34	-0.01
Mn Total	-0.23	0.46	-0.07	0.27	0.32	0.13	0.40	0.13
Na Total	-0.08	0.21	0.05	0.00	-0.05	0.11	-0.05	0.13
Ni Total	0.10	0.01	-0.15	0.13	0.12	-0.13	0.06	-0.14
P Total	-0.37	0.33	0.04	-0.02	0.00	0.25	0.20	0.22
Pb Total	-0.10	0.40	0.10	-0.31	-0.23	0.43	0.07	0.21
Sr Total	-0.48	0.23	-0.16	0.30	0.59	0.05	0.55	0.12
Ti Total	0.53	0.05	-0.02	-0.14	-0.41	-0.08	-0.41	-0.30
V Total	0.35	0.05	-0.08	-0.01	-0.17	-0.09	-0.22	-0.17
Ba Total	-0.38	0.21	-0.14	0.25	0.47	-0.02	0.41	0.07
Ca Total	-0.41	0.21	-0.18	0.50	0.77	-0.01	0.60	0.10
Co Total	0.07	0.32	-0.12	0.25	0.09	0.04	0.17	-0.05
Cr Total	0.48	0.02	-0.10	-0.23	-0.39	-0.10	-0.38	-0.29
Cu Total	-0.07	0.18	0.05	0.10	0.17	-0.04	0.07	0.01
Fe Total	-0.10	0.37	-0.05	0.34	0.21	0.36	0.47	0.25
K Total	-0.11	0.44	0.07	-0.03	-0.02	0.58	0.25	0.36

Correlation Matrix	Cu Extract	Fe Extract	Mn Extract	Zn Extract	Al Total	Mg Total	Mn Total
Bulk Density	-0.11	-0.31	-0.38	-0.26	0.51	-0.09	-0.23
Zn Total	0.15	0.11	0.38	0.71	0.01	0.39	0.46
P Extract	0.02	0.02	-0.05	0.03	-0.10	-0.16	-0.07
pH	0.21	0.36	0.28	-0.01	-0.11	0.34	0.27
Ca Extract	0.21	0.27	0.39	0.08	-0.34	0.37	0.32
K Extract	-0.04	0.20	0.22	0.23	-0.14	0.05	0.13
Mg Extract	0.11	0.39	0.45	0.18	-0.37	0.34	0.40
Na Extract	0.06	0.30	0.27	0.28	-0.32	-0.01	0.13
Cu Extract	1.00	0.28	0.10	0.10	0.06	0.30	0.04
Fe Extract	0.28	1.00	0.49	0.17	-0.32	0.03	0.22
Mn Extract	0.10	0.49	1.00	0.36	-0.34	0.27	0.76
Zn Extract	0.10	0.17	0.36	1.00	-0.21	0.05	0.21
Al Total	0.06	-0.32	-0.34	-0.21	1.00	0.41	-0.11
Mg Total	0.30	0.03	0.27	0.05	0.41	1.00	0.45
Mn Total	0.04	0.22	0.76	0.21	-0.11	0.45	1.00
Na Total	0.38	-0.01	0.07	0.09	0.35	0.55	0.08
Ni Total	0.13	-0.01	-0.10	-0.12	0.57	0.54	0.06
P Total	-0.11	0.17	0.37	0.24	-0.11	0.28	0.46
Pb Total	-0.05	0.09	0.27	0.33	-0.02	0.14	0.29
Sr Total	-0.01	0.09	0.38	0.10	-0.07	0.61	0.49
Ti Total	0.14	-0.29	-0.34	-0.18	0.83	0.33	-0.17
V Total	0.15	-0.09	-0.26	-0.07	0.73	0.30	-0.14
Ba Total	0.03	0.06	0.46	0.10	-0.05	0.44	0.68
Ca Total	0.05	0.08	0.39	0.02	-0.10	0.66	0.49
Co Total	0.20	0.30	0.33	0.03	0.45	0.60	0.55
Cr Total	0.06	-0.27	-0.33	-0.16	0.77	0.26	-0.16
Cu Total	0.71	0.03	-0.05	0.08	0.31	0.51	0.00
Fe Total	0.07	0.51	0.45	0.11	0.07	0.44	0.60
K Total	-0.02	0.08	0.41	0.33	-0.03	0.29	0.36

Correlation Matrix	Na Total	Ni Total	P Total	Pb Total	Sr Total	Ti Total	V Total
Bulk Density	-0.08	0.10	-0.37	-0.10	-0.48	0.53	0.35
Zn Total	0.21	0.01	0.33	0.40	0.23	0.05	0.05
P Extract	0.05	-0.15	0.04	0.10	-0.16	-0.02	-0.08
pH	0.00	0.13	-0.02	-0.31	0.30	-0.14	-0.01
Ca Extract	-0.05	0.12	0.00	-0.23	0.59	-0.41	-0.17
K Extract	0.11	-0.13	0.25	0.43	0.05	-0.08	-0.09
Mg Extract	-0.05	0.06	0.20	0.07	0.55	-0.41	-0.22
Na Extract	0.13	-0.14	0.22	0.21	0.12	-0.30	-0.17
Cu Extract	0.38	0.13	-0.11	-0.05	-0.01	0.14	0.15
Fe Extract	-0.01	-0.01	0.17	0.09	0.09	-0.29	-0.09
Mn Extract	0.07	-0.10	0.37	0.27	0.38	-0.34	-0.26
Zn Extract	0.09	-0.12	0.24	0.33	0.10	-0.18	-0.07
Al Total	0.35	0.57	-0.11	-0.02	-0.07	0.83	0.73
Mg Total	0.55	0.54	0.28	0.14	0.61	0.33	0.30
Mn Total	0.08	0.06	0.46	0.29	0.49	-0.17	-0.14
Na Total	1.00	0.22	0.15	0.13	0.13	0.49	0.31
Ni Total	0.22	1.00	0.03	-0.08	0.36	0.37	0.56
P Total	0.15	0.03	1.00	0.48	0.51	-0.26	-0.11
Pb Total	0.13	-0.08	0.48	1.00	0.09	0.03	-0.03
Sr Total	0.13	0.36	0.51	0.09	1.00	-0.28	-0.09
Ti Total	0.49	0.37	-0.26	0.03	-0.28	1.00	0.77
V Total	0.31	0.56	-0.11	-0.03	-0.09	0.77	1.00
Ba Total	0.03	0.31	0.41	0.02	0.74	-0.24	-0.07
Ca Total	0.10	0.35	0.29	-0.09	0.87	-0.26	-0.10
Co Total	0.27	0.59	0.32	0.17	0.36	0.37	0.45
Cr Total	0.18	0.56	-0.19	0.05	-0.25	0.69	0.63
Cu Total	0.48	0.45	0.01	0.04	0.19	0.29	0.41
Fe Total	0.09	0.24	0.45	0.37	0.38	0.02	0.09
K Total	0.27	-0.06	0.45	0.63	0.19	0.00	-0.03

Correlation Matrix	Ba Total	Ca Total	Co Total	Cr Total	Cu Total	Fe Total	K Total
Bulk Density	-0.38	-0.41	0.07	0.48	-0.07	-0.10	-0.11
Zn Total	0.21	0.21	0.32	0.02	0.18	0.37	0.44
P Extract	-0.14	-0.18	-0.12	-0.10	0.05	-0.05	0.07
pH	0.25	0.50	0.25	-0.23	0.10	0.34	-0.03
Ca Extract	0.47	0.77	0.09	-0.39	0.17	0.21	-0.02
K Extract	-0.02	-0.01	0.04	-0.10	-0.04	0.36	0.58
Mg Extract	0.41	0.60	0.17	-0.38	0.07	0.47	0.25
Na Extract	0.07	0.10	-0.05	-0.29	0.01	0.25	0.36
Cu Extract	0.03	0.05	0.20	0.06	0.71	0.07	-0.02
Fe Extract	0.06	0.08	0.30	-0.27	0.03	0.51	0.08
Mn Extract	0.46	0.39	0.33	-0.33	-0.05	0.45	0.41
Zn Extract	0.10	0.02	0.03	-0.16	0.08	0.11	0.33
Al Total	-0.05	-0.10	0.45	0.77	0.31	0.07	-0.03
Mg Total	0.44	0.66	0.60	0.26	0.51	0.44	0.29
Mn Total	0.68	0.49	0.55	-0.16	0.00	0.60	0.36
Na Total	0.03	0.10	0.27	0.18	0.48	0.09	0.27
Ni Total	0.31	0.35	0.59	0.56	0.45	0.24	-0.06
P Total	0.41	0.29	0.32	-0.19	0.01	0.45	0.45
Pb Total	0.02	-0.09	0.17	0.05	0.04	0.37	0.63
Sr Total	0.74	0.87	0.36	-0.25	0.19	0.38	0.19
Ti Total	-0.24	-0.26	0.37	0.69	0.29	0.02	0.00
V Total	-0.07	-0.10	0.45	0.63	0.41	0.09	-0.03
Ba Total	1.00	0.74	0.38	-0.19	0.12	0.33	0.06
Ca Total	0.74	1.00	0.27	-0.23	0.19	0.33	0.08
Co Total	0.38	0.27	1.00	0.31	0.27	0.69	0.16
Cr Total	-0.19	-0.23	0.31	1.00	0.26	0.04	-0.02
Cu Total	0.12	0.19	0.27	0.26	1.00	0.06	0.06
Fe Total	0.33	0.33	0.69	0.04	0.06	1.00	0.29
K Total	0.06	0.08	0.16	-0.02	0.06	0.29	1.00