NITROGEN CYCLING, OPTIMIZATION OF PLANT NUTRITION AND REMOTE SENSING OF LEAF NUTRIENTS IN WILD BLUEBERRIES (*VACCINIUM ANGUSTIFOLIUM* AIT.)

by

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DEDICATION PAGE

Naqshbandia Owaisiah Order

For whom I am forever indebted for every positive in me
All shortcomings pertain to me

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ABSTRACT

This thesis consists of three sections that provide detailed knowledge of nutrient estimation and management in wild blueberry production. The first section investigated the main and interactive effects of long term fertilizer (NPK) enrichments on soil mineral nitrogen, organic nitrogen and carbon, microbial biomass nitrogen and carbon, net mineralization and net nitrification in wild blueberry soils. The second section studied the optimization of wild blueberry growth, development, foliar nutrients and harvestable yields by using response surface methodology. The third section examined nutrient estimation technologies using field spectroscopy. The remote sensing data was analysed with a combination partial least squares regression and variable selection algorithms (Chemometric analysis).

The results indicated elevated nitrification activity under nitrogen enrichments, mainly performed by heterotrophs, report unusually high levels of dissolved organic carbon (> 150 C ha^{-1}), a fungal dominated soil system and high concentration of soluble organic nitrogen in the crop year of production. Nitrification and high dissolved organic carbon levels were observed in connection with possible nitrogen saturation and potential environmental hazards. The results imply a need for nitrification inhibition measures.

Results from field studies examining the main and interactive effects of soil applied N, P and K suggested that applications of nitrogen (35 kg ha^{-1}), phosphorus (40 kg ha^{-1}) and potassium (30 kg ha^{-1}) were required to optimize growth, development and harvestable yields of wild blueberry. Under these fertilizer rates, the corresponding predicted harvestable yield was 4126 kg ha^{-1} that is as much as 13% higher than would be produced by commonly used fertilizer rate in the industry. This study presented new leaf nutrient ranges for sprout and crop years for wild blueberry fields in Atlantic Canada. Hyperspectral remote sensing technologies were used for estimating macro and micro nutrients. This study provides critical information on wavelengths important for nutrient estimation in reflectance spectra (400-2500 nm).The results and inferences from this thesis may be employed to improve crop production, increase economic returns and health of soil and sustainability of wild blueberry production in Nova Scotia.

LIST OF ABBREVIATIONS AND SYMBOLS USED

$	Dollar
%	Percentage
°C	Celsius
3D	Three dimensional
ANN	Artificial neural networks
ANOVA	Analysis of variance
AOA	Ammonia oxidizing archaea
AOB	Ammonia oxidizing bacteria
ASD	Analytical spectral devices
ASTER	Advanced space borne thermal emission and reflection radiometer
AVIRIS	Airborne visible/infrared imaging spectrometer
B	Boron
C	Carbon
Ca	Calcium
CCD	Central composite design
CEC	Cation exchange capacity
Chl	Chlorophyll
Chls	Chlorophylls
cm	Centimeter
CSAI	Compact airborne spectrographic imager
CV	Coefficient of variation
DAF	Days after fertilization
DOC	Dissolved organic carbon
Eqn	Equation
Fe	Iron
FOV	Field of view
FS-PLS	Full spectrum-PLS

g	Gram
GA	Genetic algorithm
GA-PLS	Genetic algorithm-PLS
GER	Geophysical and environmental research
GPS	Global positioning system
ha	Hectare
ICAP-AES	Inductively coupled argon plasma atomic emission spectroscopy
K	Potassium
K_2O	Potassium oxide
kg	Kilogram
L	Liter
LVs	Latent vectors
m	Meter
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
Mg	Magnesium
mm	Millimeter
Mn	Manganese
mol	Mole
N	Nitrogen
Na	Sodium
NASA	National aeronautics and space administration
NIR	Near infrared
NIRS	Near-infrared spectroscopy
nm	Nanometer
NOB	Nitrite oxidizing bacteria
OM	Organic matter
P	Phosphorus

P_2O_5	Phosphorus oxide
PCR	Principal component regression
pH	Power of hydrogen
PLS	Partial least squares
PLSR	Partial least squares regression
ppm	Parts per million
r	Pearson correlation coefficient
R^2	Coefficient of determination
RCBD	Randomized complete block design
REP	Red edge position
RMSE	Root mean square error
RMSECV	Root mean square error of cross validation
RSM	Response surface methodology
RubisCo	Ribulose1,5-bisphosphate carboxylase-oxygenase
S	Sulfur
SD	Standard deviation
SFS-PLS	Stepwise forward selection-PLS
SMLR	Stepwise multiple linear regression
SOM	Soil organic matter
SON	Soluble organic nitrogen
SWIR	Shortwave infrared
TSN	Total soluble nitrogen
VIP	Variable importance in projection
VIS	Visible
VNIR	Visible-to-near infrared
VSWIR	Visible-to-shortwave infrared
Zn	Zinc

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Chapter 1 Introduction

1.1 Description of the Wild Blueberry Crop

The wild or lowbush blueberry (*Vaccinium angustifolium* Ait.) is native to northeastern North America. The wild blueberry belongs to Ericaceae family. It is a calcifuge (i.e. acid-loving) that thrives in acidic soil pH (4.0-5.0) environment. The wild blueberry soil conditions (natural habitats) are infertile that are characterized as acidic, fungal dominated, high in recalcitrant organic matter, low mineralization and nitrification, and subsequent low mineral nitrogen (N) content, low availability of soil phosphorous (P), low base cations, high aluminium availability (Korcak 1988). These conditions do not suit the production of most of the other field crops and fruits. However, the wild blueberry plant is well adapted to these harsh edaphic conditions. The wild blueberry cope with this soil environment by naturally built in characteristics such as extensive root and rhizome system that accounts for 75-85% of the total plant dry weight, ericoid mycorrhizae association, slow growth dynamics, uptake of organic N forms (Ericaceae family) and ability to tolerate high aluminium levels (Korcak 1988). The rhizome system serves as nutrient and carbon reservoir. The nutrients are transferred between above and below ground parts according to need of plant. The nutrient uptake notably N and P is assisted through the symbiotic association withericoid mycorrhizae (Korcak 1988). Recently mutualistic ericoid association has been found to acquire organic N forms. The ericoid mycorrhizal association have also been involved in the mobilization and release of N and P from the natural substrates of plant and microbial origin (Read and Perez-Moreno 2003). Wild blueberries are also known for their ability to tolerate relatively high soil aluminium levels and/or accumulate manganese that is toxic to most of the calciphile plants (Korcak 1988).

Wild blueberry grows naturally in forests and competing vegetation is removed from native stands to develop commercial fields. The wild blueberry plant slowly spreads by its rhizomes and can fully cover a commercial field in about ten years. The commercial wild blueberry field follows a two year production cycle (sprout and crop year). In first year, plants grow vegetative in early spring (sprout year), floral bud initiation occurs in late summer along with leaf drop during fall. In the subsequent year (crop year), the plant grow leaves, flowers, pollination occurs, fruit set, berry ripens and fruit are harvested. Commercial fields are harvested mainly by mechanical harvester and small proportions of berries are hand raked. Most of the harvested berries are frozen in processing plants and sold in domestic markets or exported to the United States, Europe and Japan. Fields can be mowed after harvest or in early spring of sprout year and that will re-initiate the two year production cycle. The alternate year pruning practice maximizes floral bud formation, fruit set, yield, and ease of mechanical harvest (Percival and Sanderson 2004).

1.2 Economic Importance

Northeastern North America is the world's leading producer of wild blueberries.In total, there are 158,108 acres of wild blueberries in Maine, Atlantic Canada and Quebec (Agriculture and Agri-Food Canada 2012; United States Department of Agriculture 2013). Maine, Nova Scotia, Quebec, New Brunswick and the remainder of eastern Canada accounts for 36%, 24%, 21%, 13%, and 6%, respectively of the acreage in production. Total wild blueberry production now exceeds 100 million kg (Agriculture and Agri-Food Canada 2012; United States Department of Agriculture 2013). Nova Scotia has over 1,100 producers and 800 wild blueberry farms that generate a $60 million market value (Agriculture and Agri-Food Canada 2012).

1.3 Soil Nitrogen in Wild Blueberry Production

Wild blueberry soils contain large amounts of soil organic matter (SOM) that undergoes decomposition and provide nutrients to soil fauna and flora. Plant litter/organic matter/humus are of low quality having variable carbon to nitrogen ratios (C:N ratio) due to low foliage N content in the natural habitat of wild blueberries (unfertilized stands). The mineralization rates are considered relatively slow and subsequent low mineral N and high organic N are available for plants and microorganisms. The dissolved organic nitrogen (DON) is a particularity important component of plant nutrition in infertile and/or organic soils (Chapin 1995). Wild blueberries can also potentially take up organic N forms. More recently, it is recognized that the plant can take up simple, soluble organic forms of N directly by roots or through mycorrhizae associations. Fungal organisms present in the mycorrhizal association absorb amino acids, sugars, peptides, proteins which are then used by host plants. This short circuit or bypassing of the N mineralization has been demonstrated in boreal forest plants, especially important in *Vaccinium* shrubs that absorbed 90% of the added organic N (Näsholm et al. 1998).

The oxidation of ammonium, the first stage and rate limiting step in nitrification, is considered curtailed mainly due to sensitivity of autotrophic ammonia oxidizing bacteria to low soil pH conditions. The low nitrate production results in low nitrate leaching and denitrification losses. The nitrification capacity in wild blueberries may be further reduced by release of phenolic by Ericaceous plant roots (Korcak 1988). Consequently soil mineral N is believed to be predominantly present in ammonium form of N (Korcak 1988). Wild blueberry plants have displayed preferences for ammonium over the nitrate forms of nutrition and produced more biomass and leaf N when grown with ammonium based products (Cain 1952; Korcak 1989; Percival and Privé 2002). The two forms of mineral N, when acquired by

plants, may have distinct genetic and metabolic consequences (Britto and Kronzucker 2013). The natural preference of wild blueberry for ammonium ions makes soil N transformations critical. Any disturbance in N cycle may adversely affect the growth and development of wild blueberry production system.

1.4 N Fertilization of Wild Blueberry

N is a major plant nutrient that is required in large quantities. N is often limited in most native and commercial vegetation ecosystems. Likewise, N has long been recognized potentially deficient in commercial production of wild blueberry. N deficiency may be attributed to naturally low N mineralization rates, and gradual N depletion by reducing in organic matter layer was attributed to intensive burning practices over the past 80 years (Lafond2009). Approximately 30-35 kg N ha^{-1} loss has been estimated in a 2-yr production cycle by burning (Eaton 1986). After the 1980's, large amounts of low quality plant debris were repeatedly deposited by mowing the fields this has widened the C:N ratio. This may have caused a lag in the initiation of the mineralization process and release of N early in the vegetative year of production which is the period when plants grow actively. Wild blueberry plants acquired up 64% of the labeled fertilizer N within 3 months of fertilizer applied in early spring (Eaton and Patriquin 1990). The nutritional demands of the wild blueberry plant may have increased given the extensive pruning practices and the resulting growth of new shoots in each production cycle. Thus, it is generally thought that mineralization and organic sources do not fulfill the N requirement of the commercial wild blueberry crop, consequently, fertilization is practiced. Commercial applications of N have increased from 20 to 30 kg N ha^{-1} to well in excess of 60 kg ha^{-1} in some fields and have resulted in generally better growth and yield potentials (Percival and Privé 2002). The response of wild blueberries to N fertilization may indirectly imply that soil N supply is limiting but we also know that

repeated N enrichments have altered the soil N cycle in northern temperate forest systems (Aber et al. 1998; Aber et al. 2003). However, there are no detailed studies that account for the effects of repeated fertilizer enrichment on soil N and C cycles and processes in wild blueberry fields and soils.

In addition to disturbance in soil N cycle, N additions have been found to shift the abundance and composition of soil organism and vegetation. Particularly fungal dominated soil ecosystem may change to bacteria dominated systems under N enrichments (Marcel et al. 2008). The fauna and flora naturally occurring in the low N environments may be replaced by others that are more adapted to higher N levels. For example, the species diversity (richness) of mycorrhizal fungi changed in N limited coniferous forests, even under small external N inputs (Boxman et al. 1998). Wild blueberry stands have evolved under N-limited soil conditions. Therefore, the wild blueberry plant naturally grows slowly and requires low soil N concentrations. Similarly, the fauna and flora inhabiting wild blueberry soils are also adapted to low N conditions. Fertilization practices may disturb the soil organism and vegetation community structure, coupled with disturbance of soil processes driven by soil microbes (Allison et al. 2007). However, plant species also resist changing their soil environment through feedback mechanism between plant and soil communities. Plants of fungal dominated food web may retain their soil environment by phenolic rich litter production and root exudates (Marcel et al. 2008).

Soil N retention and nitrogen saturation (N saturation) are important concepts under external soil N addition. A small amount of N is removed from wild blueberry fields through the harvest of berries. Most of plant N is returned to soil in the form of plant litter or transferred to underground rhizome systems (Townsend et al. 1968). In commercial fields, SOM is generally of low quality with wide C:N ratios and high phenolic content. N retention

can be increased by adopting strategies that produce low mineral N (ammonium and nitrate) or high mineral N production combined with efficient N immobilization and assimilation. Although wild blueberry soils theoretically possess huge N retention capacity, the repeated application of N may gradually saturate inherited soil-plant-microorganisms N retention capacity. Such surplus N may leach out of soil and cause water and atmospheric pollution.

1.5 Soil N Cycle Processes in the Wild Blueberry

Wild blueberry soils and N processes most closely matches northern temperate broad leaf and mixed forest systems (Agriculture Canada 1991). These processes include foliage production, foliage loss, subsequent decomposition, mineralization, nitrification, immobilization by microbes and plant uptake. Without external N supply, temperate forests have been considered N limited with almost all N internally cycled within the soil-microbe-plant system. With external N supplies, N cycling in temperate forests is accelerated (Aber et al. 1989). Although the N pools may be small in an unmanaged vegetation system the N flux could be large. N flux has been reported from 15 to 150 kg N ha^{-1} yr^{-1} through closed internal cycling (Gundersen et al. 2006). N gains (N fixation and atmospheric N deposition) have approximately equaled N losses through leaching and denitrification in closed N cycle (Matson et al. 2002). The increased N turnover in response to N enrichments may exceed biological uptake potential in temperate forests (Aber et al. 1989).

Commercial practices in wild blueberry production may have changed the closed internal N cycle. These practices include the use of a forced two-year production cycle through flail mowing, external N supply through fertilization, and weed management. Such management practices have been reported to disturb N cycle in forest ecosystems (Martikainen et al. 1993; Pietikäinen and Fritze 1995; Paavolainen and Smolander 1998; Smolander et al. 2005; Wang et al. 2010).

There is a long standing acknowledgment that plant, soil, and microbial activity and processes can interact to modify soil N dynamics and availability within ecosystems (Vitousek 1982; Perakis and Sinkhorn 2011). The atmospheric N additions and fertilizer N enrichments can influence both above and below ground production. The increased soil N additions may lead to increased soil N turnover and subsequent high plant and microbial uptake thus increasing N cycling rates, above ground biomass and mineralization rates. N additions influence the activities of extracellular enzymes. It increases activity of enzymes involved in P and C acquisition during cell wall breakdown, decreases the activity of soil organic N degrading enzymes (Allison et al. 2007) and inhibits the degradation of lignin (Sinsabaugh et al. 2005).

N saturation may be defined as "where soil N availability exceeds the capacity of the plants and soil microbes to assimilate all N" (Aber et al. 1989). N saturation has been defined in a number of different ways, but all include an increase in N availability over time that results in the removal of N limitation on all biological processes in the ecosystem (Aber et al. 1998). Lower atmospheric N deposition has been reported in Atlantic Canada compared to Central Europe deposition (5-10 kg N ha^{-1} yr^{-1} vs. 20-30 kg ha^{-1} yr^{-1}; Gauger et al. 2008). However, when fertilizer additions (20 kg N ha^{-1}in alternate years) are added to atmospheric N inputs, they may exceed critical loads. The atmospheric N depositions may lead temperate forests to N saturation (Aber et al. 1989), a condition when soil N exceeds the total biological nutrition demands of microbes and plants. And ultimately N will be lost through leaching. The N saturation poses serious threats to sustainability and environmental stewardship through leaching and emission of greenhouse gasses, microbial and community shifts and loss of biological diversity.

N saturation has multiple stages; the plant responses depend on the severity of N saturation. Added to that soil mineralization and nitrification processes depend on soil temperature, water and microbial activity. Plant and microbe N demand also varies with growth stage and different times of year (temporal variations). Therefore, soil ammonium and nitrate levels vary at different times of the year (temporal variations). This implies, that soil N supply may be in excess to biological demand at one point of time, could be deficient either due to low N cycling rates or higher plant demand at other times. Wild blueberry plant is known to quickly acquire added N within 2-3 months of fertilizer application and store it in its rhizome system to fulfill plant N demands. This means that wild blueberry may still be requiring an external supply of N even if wild blueberry fields experience N saturation conditions.

Repeated N additions (atmospheric and fertilizers) may cause significant changes in the wild blueberry production system including shifts from N limited to N saturated. Early signs of N saturation include elevated nitrification, subsequent reduction in microbial biomass and ultimately slow mineralization. Repeated N depositions of 30 kg N ha^{-1} yr^{-1}have been reported to induce N saturation in temperate forests. Wild blueberry fields receive similar N amounts; therefore, there is a justification to study soil N processes in wild blueberry agroecosystems.

Possible adverse effects of N saturation implies a change in N cycling from closed internal cycle to an open N cycle, with increased nitrification and emissions of nitrous oxides from the soil, leaching of highly soluble nitrates accompanied by the loss of positively charged alkaline minerals such as K and Mg which may reduce the overall fertility of soils (Nihlgård 1985; Gundersen and Bashkin 1994; Aber et al. 1998). Due to calcium (Ca) depletion and acidification of soil, aluminum ions are mobilized and can eventually reach

toxic concentrations (Vitousek et al. 1997). Plants and microbes may become limited in P, Mg, or Ca and thus develop nutrient imbalances or experience aluminum toxicity (Vitousek et al. 1997). Other harmful effects include plant physiological disruptions, the risk of nitrate pollution of surface and ground waters, eutrophication of aquatic systems (Aber 1989).

Increased aluminum (Al) availability has been reported to potentially damage tree roots under soil acidification (Schaedle et al. 1989). Many wild blueberry soils in Nova Scotia contain high Al and magnesium (Mg) levels due to leaching of Ca under high precipitation conditions and are very acidic (pH 4.0-5.0) (Agriculture Canada 1991). The wild blueberry is indigenous to these conditions and has adapted reasonably well to these conditions (Korcak 1988). Calcifuges avoid Al and manganese toxicity by lowering root cation exchange capacity (Korcak 1988). Additionally, organic matter is known to regulate the Al and Ca supplies in acid soils (Korcak 1988). However, the wild blueberry plant may observe Al toxicity under N saturation (increased nitrate production). Al has been found more toxic to calcifuges in the presence of nitrate than ammonium N (Korcak 1988).

Wild blueberry fields are highly susceptible to NO_3^- leaching due to factors including sandy soils, steep topography, a high proportion of bare patches and a relatively short growing season. Recently, Saleem (2012) reported higher N leaching from uniform fertilizer application compared to variable rates in a comparative study of NO_3^-leaching from wild blueberry fields. A vast number of studies in forest ecosystems have provided knowledge of how these systems responded to increased N inputs through atmospheric deposition or fertilization (Vitousek et al. 1997). There is a need to gain insight into how NPK fertilization affects soil N pools in wild blueberry production system (Chapter 2).

1.5.1 Mineralization/Immobilization

N is the only major nutrient that is not weathered from soil minerals. Conversely, N originates from the atmosphere where it exists as N_2 (78% of atmospheric gasses). Conversion of N_2 to reactive N (N bonded to hydrogen, oxygen or carbon) is termed N fixation. Habitats depend on N_2 fixation by lightning (5.4 Tg $year^{-1}$), natural biological (symbiotic and asymbiotic) fixation(110 Tg $year^{-1}$), industrial fixation with the Haber-Bosch process in the form of N based fertilizers (100 Tg $year^{-1}$), and deposition to continents of reactive N species including NO_3^-, NH_4^+, NH_3, HNO_3 and organic N deposition (aerosols, organic nitrates and particulate N) from atmosphere to biosphere (64 Tg $year^{-1}$) which are mainly derived from NH_3 and NO_x emissions (Galloway et al. 2008). The nitrogen fixation requires lot of energy to break the triple bond that is provided through biological and physical processes. Biological nitrogen fixation is catalyzed by complex enzyme nitrogenase to reduce N_2 to $2NH_3$that is naturally found only in certain specialized free living, close association with plants and symbiotic microorganisms-plant use most of fixed N in exchange for a carbon source (Postgate 1982). Mineral N forms (NH_4^+ and NO_3^-) have been known to suppress biological N fixation. Therefore, in undisturbed vegetated systems, N is mainly supplied through decomposition of soil organic matter (SOM) which is the largest pool in the plant root zone. Decomposition of SOM is mainly performed by soil microorganism which release mineral nutrients into soil.

Galloway et al. (2003) estimated total N stocks in forest (soils and plant biomass) as high as 500 g N m^{-2} and over 80% of which is present in organic forms (Schulten and Schnitzer 1998). Wild blueberry fields contain high SOM content (~10%) that may support huge microbial populations and potentially provide N needs of crop. Degradation of SOM involves the mineralization and immobilization which are simultaneous and complementary

processes. Mineralization is a process where microorganisms convert organic compounds into inorganic forms (nitrates, phosphates, sulfates). Similarly, N mineralization involves the conversion of organic N to inorganic N (NH_3 or NH_4^+) through microbial activity. Microorganisms acquire inorganic N and make part of microbial tissue that is called N immobilization. Gross mineralization is the total N produced and gross immobilization is the total N consumed by microorganism and net is the difference between these two processes.

The organic matter decomposition is ecosystem and eco-zone (climate) specific. The substrate quality and N additions and enzymatic activity influence the degradation of organic matter (Hobbie 2005). N additions increases degradation of cellulose and glycosidase activities enrichments and reduce the decomposition of lignin, humus and secondary metabolites (Gallo et al. 2004; DeForest et al. 2004). The organic matter decomposition rate also varies with stage under N additions, at start it increases and declines at later stages when lignin is degraded (Fog 1988; Berg and Matzner 1997). Carreiro et al. (2000) found that N deposition alteration of litter decomposition rates were closely linked to changes in phenol oxidase activity.

N mineralization involves a diverse group of heterotrophic soil microorganisms including bacteria, fungi, and actinomycetes under aerobic and anaerobic conditions. Soil fauna plays an important role in SOM degradation by preliminary breaking down of detritus (dead biomass), thus regulating the population of fungi and bacteria (Robertson and Groffman 2007). These microorganisms can utilize plant litter, SOM, humus and dead microbial tissue. Mineralization mainly occurs in biologically top soil surface (0-10 cm) that contains most of detritus. Microorganism activity and efficiency is influenced by soil conditions such as pH, temperature, water content and aeration (Robertson and Groffman 2007).

Ammonification is the first step in mineralization. It is involves the enzymatic conversion of organic N to ammonium (NH_4^+) and release into the soil solution. Ammonium can also be produced by the dissimilatory reduction of nitrate (NO_3^-). Ammonium may be released in soil through leakage or excretion from bacterial cells (mobilization) and/or from fine roots (Bengtson 2004). Heterotrophic microorganisms exclusively completes ammonification step through the utilization of C substances including polysaccharides as an energy source. Ammonium released during the ammonification process is either taken up by microbial heterotrophs (microbial immobilization), or by plants, known as assimilation, and non- biological fixation in clay lattices or adsorption to clay or organic matter. Microbes immobilize N primarily from the ammonium pool (Luce et al. 2011) and to lesser extent from nitrate pool. However, microbe can also uptake significant fraction of free amino acids (Finzi and Berthrong 2005). Immobilized and assimilated N is incorporated into living tissues are rendered temporarily unavailable. However, upon mineralization it may be available again to plants and microorganisms. Once ammonium is acquired by plants, it is assimilated into amino acids in the plant root. The glutamine ($C_5H_{10}N_2O_3$) is the first product in assimilation which is transported to other plant parts and subsequently converted to amino acids. Recent studies suggested that ammonium may be translocated from roots to shoots within plant xylem system (Schjoerring et al. 2002).

N mineralization and immobilization occur simultaneously but are inversely related processes. Soil microorganisms are often considered C limited (Högberg et al. 2003). Therefore, microbial N immobilization is strongly controlled by the amount of available C in the soil (Booth et al. 2005; Laungani and Knops 2012). N immobilization has also been inversely related to soil inorganic N availability (Bengtsson and Bergwall 2000). The relative availability of C and N in substrate (decomposing matter) and the metabolic needs of

microbes determine the balance between these two competing processes (mineralization and immobilization). Decomposition rates are often correlated positively with litter N content (Hobbie 2005) and microbial biomass and microbial N content. Mineralization-immobilization is also affected by relative populations of fungi and bacteria. Fungi have a wider C:N in its tissues than bacteria, thus can utilize low quality substrate due to lower needs of N. Generally net mineralization occurs at C:N of 25:1, whereas decomposing matter with > 25:1 exhibit net immobilization (Myrold and Bottomley 2008). One exception to this generalization is highly decomposed humus that has low C:N ratio due to depletion of labile C and remaining C are complex forms. Net mineralization rate are usually measured with some kind of soil incubation and subsequent periodic leaching while gross rates may be measured by labeled isotopes and dilution methods (Davidson et al. 1991).

The C and N compounds in SOM can be placed in two pools consisting of a labile, and a stabilized pool. The labile pool of organic matter is composed of readily decomposable compounds, microbial biomass, and particulate organic matter (fine plant residues). Soil microbes prefer to use labile forms such as root exudates, root litter and leaf litter (Knops et al. 2002). The stable pool (recalcitrant) is composed of complex organic compounds that are resistant to microbial decomposition (Kleber 2010). The SOM carbon is often highly stable. The stable pool mineralizes slowly and provides food to soil microorganisms and vegetation over the long term.

Top-soil (~10 cm) supplies most of the inorganic N to plant roots in coniferous forests and rhizome systems run in the top 10 cm of soil for wild blueberry plants (Kinsman 1993). Therefore, N supply by mineralization may be easily tapped by wild blueberry roots. In undisturbed, temperate and boreal forests a minimum of about 5-10 kg and a maximum of about 100 kg N $ha^{-1}$are mineralized each year (Attiwill and Adams 1993).

1.5.2 Nitrification-Denitrification in Acidic Soils

Nitrification in acidic soils was first reported a century ago in late 19th century (Houzeau 1872). Later studies presented increasing evidence of widespread nitrification occurrence in acidic soils (Fred and Grual 1916; Noyes and Conner 1919). At that time nitrification in acid soils was believed to occur in calcium carbonate particle (Hall et al. 1908). For many years, it was widespread view that nitrification was insignificant in acidic soils. However, during the second of half of the 20th century nitrification was recorded in acidic soils sometimes even in significant amounts. Numerous studies reported nitrification in a wide range low pH soils including forest soils, heath lands, natural grass lands, tea plantation, agriculture fields, wild blueberry soils (e.g. Weber and Gainey 1962; Williams 1972; Remacle 1977; Walker and Wickramasinghe 1979; Van Miegrot and Cole 1985; Eaton and Patriquin 1988; Becquer et al. 1990; Killham 1990; Troelstra et al. 1990).However, there were also many acidic soils from where nitrification was not detected (Robertson 1982). During this period, the active nitrification in acidic soil environments was attributed to heterotrophic nitrifiers (Focht and Verstraete 1977). Chemolithotrophic bacteria dominance in acidic soil conditions was widely accepted with substantial evidence (De Boer and Kowalchuk 2001). Heterotrophic nitrification has also been reported in acidic soils (Jordan et al. 2005). Some recent studies also suggested the potential involvement of ammonia oxidizing archaea (AOA) in acidic soils (Leininger et al. 2006; He et al. 2007; Nicol et al. 2008; Stopnisek et al. 2010). AOA has been found predominantly responsible for nitrification in some acidic soils (Zhang et al. 2012).

Nitrification is the aerobic oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and of nitrite (NO_2^-) to nitrate (NO_3^-). Classically these reactions are catalyzed by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), respectively. The nitrifiers

AOB and NOB are obligate autotrophs exemplified by *Nitrosomonas* and Nitrobacter bacteria species. Some AOB are known to possess limited heterotrophic capability (can take up and assimilate organic carbon) mostly in anoxic conditions. However, in aerobic conditions, organic carbon uptake is not sufficient to fulfill the carbon needs of cells thus AOB does not use organic carbon compounds as a sole source of energy (Arp and Bottomley 2006). AOB derives the majority of its carbon from carbon dioxide (CO_2) to satisfy cell carbon needs. Autotrophic nitrifiers can also derive C from carbonates. Autotrophic bacterial oxidation of ammonia to hydroxylamine (NH_2OH) utilizes membrane bound enzymes namely ammonia monooxygenase (AMO), which can also oxidize organic compounds such as phenol, methanol and methane. This first reaction (endergonic) in the oxidation is inhibited by acetylene therefore it used to differentiate autotrophic from heterotrophic nitrification. Second reaction (exergonic) is mediated by hydroxylamine oxidoreductase in the periplasmic space and four electrons are produced, two are required in the NH_3 oxidation. The remaining two electrons are used to synthesize NADH from ATP in a reverse electron flow. The energy produced is utilized by autotrophs for cell growth and metabolism. Most of the bacteria nitrifiers descend from a photosynthetic proteobacterial ancestor (Ward 2011). Nitrifiers obtain organic carbon by carbon dioxide fixation. The CO_2 assimilation takes place by the Calvin-Benson-Bassham (CBB) cycle and ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) catalyzes the carboxylation reaction. In AOB, ammonia assimilation and transport appear to via glutamate dehydrogenase. The pathway for the synthesis of amino acids and other N-containing compounds with established pathways in other organisms.

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$$

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

Autotrophic nitrifiers have long been known to be inhibited by low pH, high soil solution CI^- concentrations, and certain organic chemicals either produced naturally or synthetically (Roseberg et al. 1986). Laboratory batch growth of ammonia oxidizers (Family Nitrobacteraceae) does not occur at pH below 6.5 and their acid sensitivity is well documented (Watson 1974). Additionally, ionization of ammonia in acidic conditions significantly reduces the availability of substrate for ammonia oxidizers and inhibition by nitric and nitrous acids whose production increases due to absence of nitrite oxidization in acidic conditions. Nevertheless, autotrophic ammonia oxidation occurs in some acidic soils at pH levels far below the reported acid tolerance of the chemoautotrophic nitrifiers. However, acid sensitive ammonia oxidizers have been frequently isolated from acidic soils and active autotrophic ammonia oxidation has been demonstrated in acidic soils by use of specific inhibitors of autotrophic ammonia oxidation and stable isotope studies. Nitrate production in acidic soils may be explained by suggestions of nonenzymatic oxidation (Bartlett 1981), autotrophic nitrifying bacteria, active only in micro sites at pH values higher than that of the bulk of the surrounding soil (Hankinson and Schmidt 1984;), acidophilic ammonia oxidizers, biofilm and aggregate formation (De Boer et al. 1991; Allison and Prosser 1993), release of pH limitation on acid sensitive autotrophic bacteria by localized buffering capacity of soils (Bazin et al. 1991) and acid tolerant autotrophs (De Boer and Kowalchuk2001). Nitrification in acidic soil may also be attributed to the fertilization of the soil that may increase the autotrophic nitrifiers population (Martikainen 1985), diversity of autotrophic nitrifying species (Woldendorp and Laanbroek 1989), methylotrophic bacteria capable of oxidizing ammonium to nitrate (Whittenbury et al. 1970), ammonia oxidizers of urease activity, uptake by passive diffusion and growth independent of pH in the range 4-8 and using urea as sole nitrogen source (Burton and Prosser 2001), and heterotrophic microorganisms may be contributing factor or even exclusive agents of nitrification in some environments (Ishaque

and Cornfield 1976; Johnsrud 1978; Killham 1987; Killham 1990; Duggin et al.1991; Papen and von Berg 1998) and by ammonia oxidizing archaea (Zhang et al. 2012).

The oxidation of nitrite to nitrate reaction is membrane associated and mediated by nitrite oxidoreductase enzyme. This reaction is reversible and can result in nitrate reduction to nitrite. Nitrite oxidizers fix carbon dioxide for growth but also possess the ability to grow on simple organic substrates (mixotrophic and heterotrophic). Under anaerobic conditions, nitrite oxidoreductase enable heterotrophic growth and produce nitrite, and ammonia, and nitrogen oxides. Growth efficiency of nitrifies are low due to respiration energy up to 80%, produced in nitrification. This partially explains their poor ability to compete with heterotrophs for ammonium.

$$2NO_2^- + 2H_2O \rightarrow 2NO_3^- + 4H + 2e^-$$

Nitrite (NO_2^-) is highly reactive (both chemically and biologically), short lived (autotrophic oxidation of nitrite proceeds at faster rate than the oxidation of ammonium), thus keeping its concentration low in the soil environment. Therefore, nitrification processes mainly leave nitrate (NO_3^-) in the environment. Nitrate (NO_3^-) produced can either be assimilated by plants, immobilized by soil microorganisms (NO_3^- immobilization), in anaerobic environments nitrate loss to atmosphere in the form of N_2 through reduction to gaseous forms(NO and N_2O) dominantly by heterotrophic bacteria and fungi under restricted oxygen availability (conventional denitrification), nitrifier denitrification (reduce NO_2^- to N_2O and N_2) by autotrophic ammonia oxidizers, dissimilatory reduction to NH_4^+ by fermentative bacteria (NO_2^- to N_2O) under readily available carbon and low oxygen, or leached from the soil out of plant root zone and accumulate in surface or ground water that may cause algal, weed blooms and water safety issues (Groffman and Rosi-Marshall

2012).Unlike ammonium in plant, nitrate is not usually toxic in plants and can be transported to other parts before assimilation. However, nitrate assimilation requires a lot more energy than ammonium assimilation. Nitrate is stored in vacuoles and reduced to NO_2^- to NH_4^+ by enzyme nitrate reductase and NH_4^+ assimilation by glutamine. The nitrification reduces the ammonia based fertilizer use efficiency and up to 70% loss has been reported from agricultural systems (Prosser 2011). The nitrification and denitrification processes are often linked via oxic and anoxic conditions. These two processes mainly lead the N loss from the soils. Nitrification can reduce the ammonia volatilization losses from agriculture systems and promote denitrification losses by providing primary substrate, thus nitrification can contribute to fixed N inventory.

Soil particles (organic matter, clay surfaces) mostly possess net negative charges. Therefore, nitrate (NO_3^-) is repelled from soil exchange sites. Negative charges make it much more mobile than ammonium (NH_4^+) which can be held on exchange sites associated with organic matter, clay surfaces, and variable charge minerals and adsorbed or fixed on soil particles. Nitrification is important regulatory process as some plants and microbes appear to prefer one form of mineral N to another (NH_4^+ and NO_3^-) thus affecting the plant, soil fauna and flora structure.

While the classical nitrifiers aerobically oxidize ammonia to nitrite, an anaerobic ammonia-oxidizing bacteria (ANAOB) was discovered in 1995 (van de Graaf et al. 1995). This process is called anammox and it consumes 1:1 of ammonium and nitrite. Anammox produces and releases N_2 and its pathway is distinctively different than conventional denitrification (Klotz and Stein 2011). The production of N_2 gas as an end product in anammox processes makes it a form of denitrification (Ward 2011). The term used to describe anammox is “denitrifying ammonia oxidation” (Klotz and Stein 2011).

More recently ammonia-oxidizing archaea (AOA) have been discovered (Venter et al. 2004) which can derive energy for growth from oxidation processes. AOA has also been shown to possess the capability of mixotrophic or heterotrophic growth. Jia and Conrad (2009) reported heterotrophic growth with incorporation of labeled carbon dioxide or archaea populations without ammonia oxidation activity. AOA apparently outnumbers their bacterial counterparts (AOB) by up to two orders of magnitude in most soil environments (Nicol et al. 2011). The relative contribution of AOA to AOB does not correlate to their abundance in ammonia oxidation activity. The correlation of AOA abundance with their activity is also not clear, and different studies have provided contrasting results (Nicol et al. 2011).

AOA physiology studies propose their adaptation to low ammonia concentrations, extreme temperature, acidity and salinity environments (Hatzenpichler et al. 2008). Nicol et al. (2008) studied the influence of pH variations in soil on relative abundance of AOA and AOB through quantification of *amo*A gene. They found AOA relatively more active in acidic soils while AOB bacteria activity increased in neutral pH soil conditions (Nicol et al. 2008). Archaea may be a major contributor to nitrification in soils that lack AOB and where nitrification takes place and they may be the sole agents performing this process in some extreme environments (Nicol et al. 2011).

Methanotrophic bacteria possess the capability to oxidize ammonia to nitrite in toxic conditions coupled with the aerobic (nonconventional) denitrification ability that yields and release NO and N_2O (Nyerges and Stein 2009). Additionally, recently methane oxidizing bacteria (MOB) has shown potential to oxidize ammonia in aerobic conditions and coupled to denitrification. Methanotrophic bacteria uses membrane bound methane monooxygenase, similar to ammonia monooxygenase in ammonia oxidizers.

Aside from obligate chemolithoautotrophs (aerobic and anaerobic) such as AOB, AOA and ANAOB, more heterogeneous groups of microorganism (chemoorganotrophic) including bacteria and many eukaryotes possess the heterotrophic nitrifying ability. The heterotrophic nitrification has broader definition "oxidation of any reduced form of nitrogen to more oxidized form" (Stein 2011). The heterotrophs can utilize relatively wide ranges of substrates (both inorganic and organic) including ammonia, hydroxylamine, various organic, and/or nitrite. There is evidence of two distinct pathways for heterotrophic ammonia oxidation. First path way, heterotrophic bacteria use similar pathway for ammonia oxidation as those of autotrophic bacteria and many strains are found to have the capability of simultaneous aerobic denitrification (heterotrophic nitrifier denitrify their nitrification products to nitrogenous gas simultaneously). The second pathway, termed fungal nitrification, fungi uses a distinctive pathway, linked to lignin degradation by fungi that involve reduced organic substrates in a series of oxidation reactions from amines to a nitro compound as follows:

$$RNH_2 \rightarrow RNHOH \rightarrow RNO \rightarrow RNO_3 \rightarrow NO_3^-$$

-3 -1 +1 +3 +5

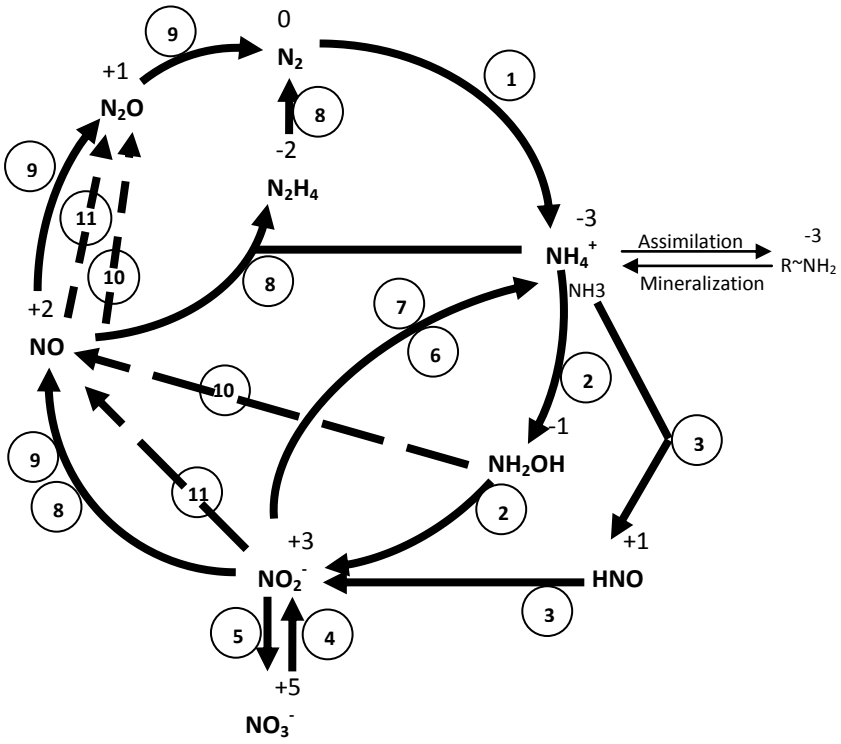
These reactions did not couple to ATP and gain energy. Heterotrophic oxidation is carried out by bacteria including *Paracoccus denitrificans*, *Thiosphaera pantotropha*, *Pseudomonas putida* and *Alcaligenes faecalis* (Kuenen and Robertson1994, Moir et al. 1996, Daum et al. 1998; Nishio et al. 1998) and fungi including *Aspergillus Flavus* ATCC, substrate ammonia and peptone in acidic forest soil (Schimel et al. 1984), *Absidia cylindrospora*, substrate ammonia and organics in acidic forest soil (Stroo et al. 1986). Fungus was isolated that formed nitrite in pure culture from an actively nitrifying acid forest

soil (Stroo et al. 1986). This was the first fungal isolate shown to nitrify in acid media. Heterotrophic fungal nitrification predominates in acidic coniferous forest soils (Killham 1986; Jordan et al. 2005). The arguments in favor of fungal nitrification in these environments include fungi that are not sensitive to low pH conditions, massive fungal biomass, abundance of organic N to nitrify and carbon to metabolize. However, acidic soils not in coniferous forests exhibit AOB nitrification (De Boer and Kowalchuk 2001) or Archaea (Nicol et al. 2008).

Unlike chemolithoautotrophs which uses ammonia as sole energy source and reductant/reducer for cellular growth, heterotrophs perform nitrification for other reasons. Heterotrophic nitrification is linked to reoxidation of NAD(P)H, under low oxygen conditions in bacteria, endogenous respiration in fungi, and utilizes certain oxidation intermediates as biocides or growth factors to gain competitive advantages or forms of defense mechanisms against other soil organisms (Focht and Verstraete 1977; Stein 2011). Nevertheless, heterotrophic nitrification may be significant in some acidic soils where heterotrophic biomass is high. High microbial biomass may compensate the relative low growth rate of the heterotrophs.

Many heterotrophic nitrifiers are capable of simultaneous nitrification–denitrification (SND) in aerobic conditions. The SND involves the enzymatic reduction of nitrite and nitrate produced via nitrification to N-oxides or N_2. Chemolithotrophic ammonia oxidizers reduce nitrite to nitric acid, nitrous oxide or dinitrogen.

Figure 1.1 Processes in the microbial nitrogen cycle (Adapted from/Figure redrawn from Klotz and Stein 2011). 1, Dinitrogen gas fixation; 2, aerobic dissimilatory ammonia oxidation to nitrite by bacteria; 3, aerobic ammonia dissimilatory oxidation to nitrite by archaea; 4, aerobic dissimilatory nitrite oxidation to nitrate by bacteria; 5, assimilatory or dissimilatory nitrate reduction to nitrite by microbes; 6, respiratory ammonification as the second step of dissimilatory nitrate reduction to ammonia (DNRA, 5 and 6); 7, assimilatory ammonification as the second step of assimilatory nitrate reduction of ammonia (ANRA, 5 and 7); 8, denitrifying anaerobic ammonia oxidation (anammox, typified by ANAOB); 9, classic (anaerobic) denitrification by mixotrophs and heterotrophs; 10, aerobic oxidation by hydroxylamine to nitrous oxide to nitrous oxide by AOB and Aerobic ammonia-oxidizing nonlithotrophic bacteria (ANB); 11, aerobic denitrification by AOB and ANB.



1.6 The Need and Scope for Remote Sensing of Foliar Nutrients in Wild Blueberry

Fertilizer practices, including timing, dose, type and formulation have intensified in the recent 20 years. This has coincided with increased plant coverage, harvestable berry yields and greater farm profitability (Percival and Sanderson 2004). However, excessive fertilization has caused extensive vegetative growth, delays in visual signs of tip dieback and floral bud development, increased stem heights, reduced yield potential (floral bud and flower number) and reduced harvestable berry yields. In addition, the floral buds on these tall stems are prone to winter injury under inadequate snow cover in winter. The tall stems are prone to lodging in crop year of production and cause substantial reductions in harvester efficiency. Unfortunately, available soil extraction techniques do not accurately estimate soil available nutrients (Ring 2001). Therefore, present nutrient assessment techniques are reliant on leaf tissue sampling (Trevett 1972). The procedure for leaf tissue analysis consists of collecting leaf tissue samples at tip dieback stage of development, drying and grinding the samples. Thus, analytical techniques, including use of LECO CNS and ICAP are time consuming (more than one week to complete), labour intensive (extensive sampling is required to capture the widespread variability in blueberry fields) and expensive (approximately \$20 sample^{-1}) to conduct.

The wild blueberry has extensive rhizome system which accounts for approximately 85% of the total plant dry weight (Hall 1957; Jeliazkova and Percival 2003). The rhizome system serves as carbohydrate and nutrient reservoir and source for early stem and root growth (Kaur et al. 2012). N has accumulated in plant root system after continuous N-based fertilizer additions (D. C. Percival, personal communication). N additions have sometimes

resulted in lack or variable response to added fertilizers. More recently, a survey of forty commercial wild blueberry fields in Nova Scotia was completed in 2008, indicated that the majority of wild blueberry fields had excess leaf tissue N levels (D. C. Percival, personal communication and unpublished survey data collected from Nova Scotia).

Soil-plant N dynamics and in-field variability present the major challenge for nutrient management in wild blueberries. Hainstock (2002) indicated that leaf tissue N levels can vary by as much as 89% within a field, and 52% throughout the course of a growing season, resulting in spatial and temporal variability. Foliar nutrient status differs significantly in the sprout and crop year (Percival and Sanderson 2004).In conjunction with this are results from Penney and MacRae (2000), which stress the importance of monitoring leaf tissue N levels throughout the production cycle of plant.Combined with the time (sampling at tip dieback stage) and cost constraints, producers are unable to accurately, precisely or efficiently determine foliar nutrient levels and cannot practically assess spatial variability occurring within fields resulting in increased interest in remote sensing technologies. By assessing precise foliar nutrient levels, both over and under the application of fertilizer can be avoided ensuring optimal harvestable yields, increased economic returns and the reduced likelihood of environmental concerns such as leaching and erosion of nutrients. To date, optical sensor-based remote sensing of fresh canopies has proven to be an excellent approach for on-the-go site specific fertilizer applications in various field crops due to its capability to sense crop canopies compared to a single leaf and its flexibility of use with farm machinery. For example, Raun et al. (2001) successfully demonstrated the benefits of an on-the-go N sensing system in winter wheat.

Such field systems are commercially available including GreenSeeker®, NADIR viewing system, uses two diodes arrays emitting light source alternatively for VIS And NIR

bands, detected by double photodetector system, eliminates chance of signal mixing but area under illumination differs between bands, maintain constant foot print which is independent of distance between sensor and target, recommended sensor height is 0.86 m (NTech Industries, Inc. Ukiah, CA), Crop Circle ACS-210 equipped with single LED light source that simultaneously emits light for VIS and NIR, mimic natural light conditions, drawback of single light source is signal mixing, uses auto calibration in real time sensing eliminating need to establish reference strips in field(Holland Scientific, Lincoln, NE), CropScan Inc. Rochester, MN and Yara N-Sensor, available with both active and passive (one sensor measure object of interest and second sensor faces sky, to measure the incoming light and corrects reflectance signal) active (own light source), uses oblique angle of °64 with zenith that reduces soil reflectance and eliminate the necessity to mount sensor exactly above canopy in wide row crops (Yara International ASA, Oslo, Norway).

These systems are commercially used in field crops (maize, wheat, barley, cotton, rice, vegetable crops) for monitoring leaf N status, but their utility in a wild blueberry production system may not be as successful considering unique plant and soil characteristics (woody material, leathery leaves and understory litter layer) of wild blueberry plant. The normalized difference vegetation index (NDVI) was inadequate in estimation leaf N levels in wild blueberry (Bourguignon 2006). The NDVI along with other vegetation indices are commonly employed with commercial sensors.

Hyperspectral remote sensing provides additional bands within the visible, NIR and shortwave infrared (SWIR) spectrum compared to broad band sensors. Most hyperspectral sensors have produced semi continuous spectra consisting of over 100 contiguous bands, each 10 nm or less between 350 nm to 2500 nm (Goetz 2009). Leaf biochemicals (chlorophylls, carotene, anthocyanin, amino acids, proteins, nitrogen, lignin, cellulose, starch,

sugar and water) absorb electromagnetic radiation (Curran 1989; Matson et al. 1994) in specific wavelength regions, which may be masked by the broadband satellite images. Hyperspectral systems with high numbers of narrow bands provide the ability to detect small changes in narrow absorption features. This high sensitivity makes it possible to try to estimate the biochemicals from fresh vegetation (Goetz 2009).

In order to develop an on-the-go N sensing system in wild blueberry, we require knowledge of wavebands/spectral regions that are correlated to foliar N content. This goal can be achieved collecting the field spectra at various growth and development stages from pre-planned field experiments that contain a wide range of leaf tissue nutrient contents. This then proceeds with knowledge of the biochemical compounds of interest, their influence on the reflectance properties of light and the subsequent full spectrum statistical techniques to find the related wavebands that will be used in estimating nutrient content. For the purpose, we need a field portable radiometer that can rapidly acquire data. The companies such as Analytical Spectral Devices (ASD) of Boulder, CO and Geophysical Environmental Research (GER) of Millbrook, NY develop such field spectrometers.

Bourguignon (2006) conducted preliminary work on the potential use of remotely sensed reflectance spectra to estimate wild blueberry foliar nutrients. Results from this work illustrated the need for portable field radiometer and multivariate data analysis techniques. Field radiometers produce semi continuous spectrum consisting of over 100 contiguous bands, each 10 nm or less between 350 to 2500 nm. The instrument cost approximately $53,000, needs to be continually cleaned and calibrated and can only operate under sunny conditions which has limited its use at the farm level. For commercial use, a user friendly and tough sensor with its own light source is required that can operate under cloudy conditions. However, the field radiometer can provide information about specific wavebands potentially

useful for nutrient estimation and further sensor development. The hyperspectral data are often collinear. However, partial least squares regression (PLSR) solves collinear data analysis issues typically encountered with classical multiple linear regression techniques.

1.6.1 Vegetation Reflectance

Vegetation reflectance is primarily a function of plant constituents (e.g. photosynthetic pigments, proteins, lignin, cellulose, sugar, starch, etc.), vegetation optical properties, plant biophysical attributes, soil properties, illumination conditions and viewing geometry. Leaf reflectance in visible region (VIS; 400-700 nm) is mainly affected by photosynthetic pigment absorptions. The near-infrared region (NIR; 750-1350 nm) reflectance is affected primarily by leaf structure. The shortwave infrared (SWIR) region (1350-2500 nm) is influenced by organic molecular bonds comprising proteins and water content (Gates et al. 1965).

1.6.2 Visible (VIS) Range Reflectance Spectrum

The reflectance spectrum of the VIS region (400-700 nm) is dominated by the absorption features of leaf photosynthetic pigments (Curran et al. 1991). Each photosynthetic pigment absorbs light more efficiently at specific wavelengths. For example, the chlorophyll a (Chl *a*) absorbs strongly at wavelengths ~430 nm and ~660 nm. The chlorophyll b (Chl *b*) absorbs at wavelengths ~460 nm and ~640 nm. Pigments absorb less light in the green region (~550nm).Relatively high reflectance forms a peak in green region of the vegetation spectrum thus giving the leaves their green colour.

Chl *a* is the most common of the five photosynthetic pigments present in plants. Typically, plants contain two to three times higher concentration of Chl *a* compared to Chl *b*. However, this ratio differs between different plant species. Chlorophylls (Chls; Chl *a* and *b*)

are necessary photosynthetic pigments. These pigments are required to convert light energy into chemical energy. Chls also determine the photosynthetic potential and primary production by controlling the amount of solar radiation absorbed by leaves. Under low concentrations of Chls, plants fail to achieve full (maximize) photosynthetic potential, which results high reflectance in the VIS spectrum. Leaf reflectance in VIS range is influenced by the ratio of the concentration of Chl *a* and Chl *b* (Curran et al. 1991; Asner 1998), and Chl fluorescence (Blackburn and Milton 1995).

N is as an integral part of Chl molecule structure. Chl molecule contains four N atoms in the central tetrapyrrole head which establishes a close link between leaf Chl and N content. The Chl molecule directly holds only small proportion (approximately 1.7%) of total leaf N. Other light harvesting complexes holds approximately 19% in C3 plants (Kokaly et al. 2009). Leaf N and Chl content moderately correlated at ecosystem level (Kokaly et al. 2009). However, several field scale studies have found Chls an accurate estimator of plant N status and soil N supply in various plant species (e.g. Schepers et al. 1992; Filella et al. 1995; Denuit et al. 2002; Zebarth et al. 2002; Zhang et al. 2008).

1.6.3 Leaf Structure and NIR Region

The internal leaf structure mainly governs vegetation spectral signature in NIR range (701-1300 nm). The NIR spectral region also possesses two minor water-related absorption bands (centered at 970 and 1200 nm). Water-related absorption bands (centered at 1400 nm) are located at the transition between NIR and SWIR spectral regions. Vegetation reflectance intensity in NIR region is commonly greater than most non-vegetated surfaces. This property allows discrimination of the vegetation from other materials of lesser reflectance (soil, urban areas etc.).

The internal cellular arrangement in leaf plays an important role in enhancing the interception of light. The leaf is sheathed with upper and lower epidermis cells, whose primary function to these tough cells are to protect the leaf tissue. All wavelengths penetrate epidermis layer. The middle layer between two epidermis layers, photosynthetic tissues are located, known as mesophyll tissues. The upper layer of mesophyll cells are elongated, cylindrical, stacked tightly together and long axis perpendicular to top leaf surface and are called palisade mesophyll cells. Bottom end of palisade cells is connected to the spongy mesophyll cells that are loosely packed, irregular shape and have numerous air spaces between the cells, allowing rapid exchange of gasses.

Chls absorb the light energy used in photosynthesis. The absorbing pigments/Chls are confined to the chloroplast. Palisade cells generally have a larger number of chloroplasts than spongy mesophyll cells. Palisade cells absorb strongly across entire visible range of spectrum but relatively less at green wavelengths, reflecting approximately 10-30% of the total visible incident light at the leaf surface.

Infrared wavelengths penetrate the palisade cells into the underlying mesophyll. The mesophyll cells scatter much of the NIR radiation (approximately 60%) reaching the leaf layer (Woolley 1971). Upon entering the leaf, light scatter at interfaces of cell wall and intercellular air spaces. Scattering occurs due to a large change in the refractive index from 1.00 (vacuum) to 1.33 (water) (Slaton et al. 2001). The light scattering gives strong reflectance in the NIR region of the spectrum.

1.6.4 Red Edge

The leaf reflectance greatly increases at the transition from VIS (red) to NIR region which producing a distinct spectral feature known as the red edge. The red edge arises due to combination of strong Chl absorption in the red wavelengths and increased NIR reflectance due to the internal light scattering (Mutanga and Skidmore 2007). The maximum slope (inflection point) in red edge region is generally termed as red edge position (REP). The positioning of this edge contains valuable information about vegetation (Horler et al. 1983). The REP has been found correlated to the leaf Chl (Lamb et al. 2002), plant N (Mutanga and Skidmore 2007), plant phenological stages and plant stress (Smith et al. 2004). REP may provide information that is not available in VIS and NIR range (Mutanga and Skidmore 2007).

Increase in Chl concentration results in broadening and deepening of Chl absorption feature approximately located at (670 nm). This causes a shift of maximum slope towards longer wavelengths and is known as red edge shift. Conversely, low Chl concentrations cause red edge move towards shorter wavelengths (i.e. blue shift) (Hare et al. 1984).

1.6.5 Shortwave Infrared (SWIR)

The plant tissue consists of organic compounds which are made of C-H, N-H, O-H, C-N and C-C bonds. The organic bonds absorb radiation at various peaks in the SWIR (1300-2500 nm). The absorptions arise from the energy transition of the molecular vibrations (Elvidge 1990). The amount and the type of organic molecules determine the chemical composition of plant tissue and amount of light absorbed at various wavelengths in SWIR (Rabkin 1987).

Laboratory near-infrared spectroscopy (NIRS) was developed using light and matter interaction knowledge. NIRS methods are commercially used to the chemical composition of dried plant samples (Norris et al. 1976). Laboratory NIRS methods have estimated biochemical accurately. NIRS technique has provided advantages over wet chemical methods with its greater speed, safety, simplicity of sample preparation, and the nondestructive nature of the sample analysis.In recent last 20 years, laboratory NIRS has been extended to field scale foliar biochemical estimation using hyperspectral sensors (Hansen and Schjoerring 2003; Stuth et al. 2003; Huang et al. 2004;Mutanga, et al. 2004; Zhao et al. 2005; Ferwerda and Skidmore 2007; Cho et al. 2008; Darvishzadeh et al. 2008;Herrmann et al. 2010; Rambo et al. 2010).

Proteins are primary nitrogenous compounds in leaves and typically hold 70-80% of total N in plant. Ribulose1,5-biphosphatec carboxylase-oxygenase (RubisCO) is a plant protein that holds about 30-50% of total leaf N. The leaf N is a small component on dry weight basis typically ranging from 0.26 % to 3.5% in different type of vegetation. However, leaf N can be estimated by remote sensing using absorption features in NIR-SWIR (Kokaly et al. 2009). Remote sensing has also been used successfully to estimate other foliar nutrients (Clark et al. 1987; Mutanga and Skidmore 2004; Ferwerda and Skidmore 2007; Pimstein et al. 2011).

The estimation of leaf biochemicals at the canopy level is a complex problem. SWIR spectrum of fresh canopies is dominated by water absorptions that may mask N related absorption peaks particularly in the SWIR (Elvidge 1990).However, water perturbations did not interfere with the estimation of leaf N (Mutanga et al. 2003).The leaf architecture, atmospheric absorption, soil background effects and environmental conditions complicate field scale remote sensing of biochemicals (Asner 1998). Therefore, field scale remote

sensing has sometimes yielded inconsistent results (Grossman et al. 1996). The challenge is, therefore, to develop techniques that can accurately predict leaf biochemical content.

1.7 Sensors and Potential Use in Fertilizer Management

Remote sensing systems (Air borne and satellite mounted) provide bands within VIS, NIR and SWIR range to remotely estimate foliar nutrients. Examples of such systems include Airborne Visible/Infrared Imaging Spectrometer (AVIRIS-NASA), HyMap Imaging Spectrometer (HyMap^{TM}-Integrated Spectronics Pty Ltd, Australia), Compact Airborne Spectrographic Imager (CSAI-ITRES Research Limited, Alberta, Canada) and satellite mounted systems e.g. Hyperion-NASA. Other satellite sensors include Ikonos, Quick Bird and ASTER (Advanced Space borne Thermal Emission and Reflection Radiometer). The hyperspectral sensors provide 10 to 100's narrow bands in visible-shortwave infrared range that could potentially be used in wide range vegetated system application including detection of weeds, disease, insect, stress and foliar chemistry. The absorption features may be masked in multispectral systems (typically 3 to 7 bands). Example multispectral system is the Landsat Program of satellite missions jointly managed by NASA and the U.S. The high sensitivity of sensors makes it possible to estimate the biochemical composition of vegetation using spectral data (Goetz 2009). Air borne and satellite based sensors can cover large areas. However, their use on small farms has been limited due to the high image cost, infrequency of satellite revisit time, atmospheric correction requirements (Laune and Guerif 2001), weather conditions (Grenzdörfer 2001), the necessity of GPS-based fertilizer application, ground referencing (Bennedsen and Guiot 2001) and time lag that may affect time-sensitive agrochemical applications. The farm vehicle mounted sensors has been developed commercially to solve the logistic limitations encountered with satellite/air born platforms.

These on-the-go farm vehicle mounted systems have proven successful in vegetation management in various field crops (Samborski et al. 2009).

1.8 Empirical Techniques

Statistical approaches continue to dominate the field of hyperspectral remote sensing. These techniques are used to find a relationship between the target parameter and its spectral reflectance or some transformation of reflectance (Dorigo et al. 2007). Stepwise multiple linear regression (SMLR) was most commonly used technique in estimating foliar nutrients. SMLR have been affected by multicollinearity among wavelengths in spectrum (Curran 1989; Dorigo et al. 2007). Recently more studies are using principal component regression (PCR), partial least square regression (PLSR) and artificial neural networks (ANN). These techniques use several spectral bands for estimating the parameter of interest by exploring the full potential of several available bands in hyperspectral signature.

Statistical techniques have been reported to lack robustness and portability of models. Others have developed deductive or physical-based approaches involving leaf and canopy radiative transfer models (Jacquemoud et al. 2009). However, physical-based models require large computations, many leaf and canopy variables, which are often difficult to estimate and/or obtain in real situations (Fang et al. 2003). The focus of this study shall be on empirical methods for estimating vegetation parameters.

Chapter 2 Soil Mineral Nitrogen, Organic Nitrogen and Carbon, Microbial Biomass, and Net Mineralization and Net Nitrification in Response to Soil Applied Nitrogen, Phosphorus and Potassium in an Acidic Wild Blueberry Soil

2.1 Abstract

This study used response surface methodology to optimize in situ soil NH_4^+, NO_3^-, total soluble nitrogen, soluble organic nitrogen, microbial biomass nitrogen, microbial biomass carbon and dissolved organic carbon concentration over the whole production cycle (sprout and crop year of production) within a wild blueberry field located near Kemptown, Nova Scotia, Canada. Five levels each of soil applied nitrogen (0, 12, 30, 48, 60 kg N ha^{-1}), phosphorous (0, 44, 110, 176, 220 kg P_2O_5 ha^{-1}) and potassium (0, 12, 30, 48, 60 kg K_2O ha^{-1}) were combined in central composite design. The experiment was a continuing trial and same treatments were applied for 6 production cycles from 2000 to 2012. Soil samples were collected on10-May, 7-July, 26-July and 9-September in sprout year and 22-May, 15-July, 31-July and 24 August in crop year of production. We also determined the soil net mineralization and net nitrification through an aerobic incubation experiment conducted in a laboratory. Soil was sampled during the sprout year from the same field experiment located at Kemptown. Nine leachates were collected during six months and analyzed for soil NH_4^+ and NO_3^- concentration. Our results illustrated significant main and interactive effects of soil applied (N, P_2O_5 and K_2O) on soil ammonium (NH_4^+), nitrate (NO_3^-), soluble organic nitrogen (SON), microbial biomass nitrogen (MBN), microbial biomass carbon (MBC), dissolved organic carbon (DOC), net ammonification and net nitrification. Soil NH_4^+ concentration to soil added N was dependent on the length of time from fertilizer application

event. Soil NH_4^+ significantly increased with soil applied nitrogen following the fertilizer application. NH_4^+ concentration then proceeded to decrease for the next two samplings (9-Sep-Sprout and 22- May-Crop) and increased again from mid-crop year to early sprout before fertilizer application (15-July-Crop, 24-Aug-Crop and 10-May-Sprout). Soil NH_4^+ concentration, in most cases, increased with phosphorus addition and quadratic effects were found significant for nitrogen, phosphorus and potassium but shape of the response curve differed among samplings. Soil NO_3^- concentration response to soil applied nitrogen, phosphorus and potassium was convex in sprout year and reverse (concave) was found in crop year. Soil NH_4^+, and SON concentration was greater in the crop year than the sprout year. In comparison, soil NO_3^- concentrations were similar in both cycles. SON and MBN significantly decreased with addition of nitrogen fertilizer. MBC increased on 10-May-Sprout and it decreased on 9-Sep-Sprout, with nitrogen addition. Reverse was found for DOC, where it decreased on 10-May-Sprout and it increased on 9-Sep-Sprout, with nitrogen addition.

In the lab mineralization experiment, the quadratic terms of soil applied nitrogen and potassium were significant in most leachates. The net mineralization response was concave and net nitrification response was convex. We found elevated nitrification activity that may suggest early sign of nitrogen saturation. Excess nitrogen of plant and microbial demand may be at risk of leaching to water bodies, runoff to low lying areas and gaseous emissions from wild blueberry fields. Our results indicated that wild blueberry soils are fungal dominated and heterotrophs play an important role in nitrogen mineralization and particularly nitrification process. The lower concentrations of soil NH_4^+ and SON in the sprout year insinuate a preference by the wild blueberry plant for NH_4^- over $NO_3^-$and also uptake of SON. I further hypothesize for the future studies that the wild blueberry plant has special nitrogen needs and part of the whole nitrogen demand has to be fulfilled from the soil organic fractions of

nitrogen. Therefore, we suggest measuring and considering soil SON in future studies and nutrient management programs.

Repeated fertilizer enrichments generally reduced the microbial biomass. We found N fertilizer have a positive effect on soil DOC concentration mainly through increase in above ground biomass production. DOC concentrations (> 100 kg C ha^{-1}) were higher than typically reported in northern forest ecosystems. The abundance of labile carbon strengthen our suggestion that heterotrophic microorganism (no C limitation on their metabolism), and their contribution in N cycling may be greater than autotrophs in wild blueberry productions system.

2.2 Introduction

Wild blueberries grow well on Nova Scotia's Podzolic soils. These soils are typically sandy, acidic, highly leached and poorly buffered. Podzols are not naturally fertile. However, these soils can become quite productive with fertilization. Commercial fields are routinely fertilized with nitrogen, phosphorous and potassium in early May of the sprout year of production. Fertilization generally promotes the formation of floral nodes and harvestable berry yields. For example, the beneficial effects of ammonium sulfate and sulfur coated urea fertilizers has been demonstrated by increased plant growth and yields (Percival and Privé 2002). However, repeated and/or over application may alter nitrogen (N) cycling, induce N saturation condition that ultimately lead to ground water pollution and greenhouse gasses emissions into atmosphere.

A sprout year application of 20-40 kg N ha^{-1} is generally recommended for wild blueberries. Phosphorous (P_2O_5) and potassium (K_2O) are applied in a 1:2:0.7 ratio of N, P_2O_5 and K_2O fertilizer. However, no detailed study in wild blueberry has examined the N cycling, N and carbon (C) pools and microbial biomass response to long term fertilization.

Therefore, soil C and N response are largely unknown. Additionally, an understanding of the interactions between soil applied fertilizers (N, P_2O_5 and K_2O), and optimization of soil added fertilizers are needed to develop sustainable fertilizer practices.

Fertilizer enrichment has been found to alter the microbially driven N and C cycle, coupled with microbial biomass abundance and composition in various vegetated systems. Generally, N mineralization and nitrification rates increase in N-limited system during early stages of N additions and, decreases when soils become N-saturated (Aber et al. 1989). Since soil N and C transformations are performed mainly by soil bacteria and fungi, N enrichment could reduce microbial biomass in many vegetated systems (Treseder 2008). Soil microbial biomass is important to plant productivity, diversity and sustainability because of its potential role in nutrient cycling, competition and retention (Marcel et al. 2008). Soil microbes have also been known to immobilize and buffer the soil nutrients including N, P, K and magnesium (Díaz-Raviña et al. 1993). Soil microbial biomass abundance and activity is influenced by availability of carbon substrates in soil. The microbial biomass abundance and C:N ratios change with season and cropping system (Wardle 1998). The relative abundance of fungi and bacteria in soil are controlled by soil faunal component of the food web (Marcel et al. 2008). Fungal dominated microbial communities often occur in acidic soils that are rich in organic matter, have closed and slow nutrient cycles, a low nutrient availability, low leaf litter quality and slow growing plant species (Marcel et al. 2008).The opposite is true for bacterial dominated food webs (Marcel et al. 2008). The relative abundance of soil microorganism communities may shift following soil disturbances, nutrient enrichments and intensive farming (De Vries et al. 2006).

Soil soluble organic nitrogen (SON) has been identified as an important N pool in N poor ecosystems such as arctic, boreal, temperate forests and unfertilized grasslands

(Kielland 1994; Näsholm et al.1998; Bhogal et al. 2000; Murphy et al. 2000; Zhong and Makeschin 2003). Ericaceae plant species, with or without mycorrhiza association and other microorganism have been found to acquire a wide array of organic N forms ranging from free amino acids to polymeric N forms such as proteins (Näsholm et al. 2009). SON may be an important pool of N in wild blueberry soils but it has not been previously studied in the wild blueberry production system.

Dissolved organic carbon (DOC) is a small and reactive fraction of soil organic matter in soil. The major sources are plant canopies, fresh plant biomass (surface litter, root litter) rhizosphere activity (root exudates and decomposition of dead roots) and/or decay of soil organic matter (Kalbitz et al. 2000). DOC is comprised of a mixture of organic compounds ranging from simple (short-chain) to complex humic substances. DOC influences microbial activity, toxicity and transport of metals, and DOC fluxes can facilitate soil nutrient availability (Kalbitz et al. 2000). DOC serves as carbon source for soil microbes (Högberg and Högberg 2002). DOC production and flux are influenced by amount and quality of fresh plant material, older organic matter (humus as substrate), microbial community composition, fertilizer additions and abiotic factors such as temperature and water flux (McDowell 2003). Mycorrhizal roots have been reported to discharge large quantities of DOC in soil. N enrichments increase (Rappe-George et al. 2013) have no effect (Sjöberg et al. 2003; McDowell et al. 2004), or decrease (Vestgarden et al. 2001) concentrations of DOC in soil.

DOC is released from microbial decomposition of the native organic matter (Malik and Gleixner 2013), surface litter, fresh plant material, fine root turnover, root exudates, soluble organic nitrogen and soil organic matter being the most important source (Filep and Rékási 2011). DOC pool is consumed through microbial respiration decay and exchanged

with soil exchange sites, and leached out of soil system further reducing the total DOC concentrations (Bengtson and Bengtsson 2007).

Response surface methodology (RSM) was employed in this study to simultaneously consider several factors at different levels and allow the examination of interactive effects using a smaller number of treatments. The single-factor techniques do not account for the combined effect of the factors involved by keeping all other factors at fixed levels. RSM eliminates single-factor limitations by considering all the factors collectively. RSM can also estimate the optimum conditions for the factors (soil applied fertilizers) that provide best possible response (soil parameters).

The first objective of this study was to investigate the effects of soil applied N, P_2O_5 and K_2O and their interactions, on concentrations of soil ammonium (NH_4^+), nitrate (NO_3^-), SON, microbial biomass nitrogen (MBN), microbial biomass carbon (MBC) and DOC content during the whole production cycle (sprout and crop year of production). The second objective was to study the effects of soil applied N, P_2O_5 and K_2O and their interactions on net N ammonification and net nitrification in wild blueberry soil. The third objective was to determine the rates of soil applied fertilizers that optimize soil NH_4^+ and NO_3^- and soil organic fractions.

Based on the literature to date in natural wild blueberry habitats, temperate and boreal forest soils we hypothesized that:

(1) Soil applied fertilizers (N, P_2O_5 and K_2O) and their interactions would influence concentrations of soil NH_4^+, NO_3^-, SON, DOC, MBN, net ammonification and net nitrification.

(2) Heterotrophic microorganism would have important role in driving N transformation.

(3) The concentrations of soil NH_4^+ would be low in sprout year (active growth phase and plant uptake) compared that of crop year of production. The concentrations of soil NO_3^- would be similar in both years of production. Wild blueberry plant is known to prefer NH_4^+ over NO_3^- form of N. NH_4^+ is the dominant form of N present under natural soil conditions of wild blueberry habitats.

(4) Considering the high organic matter in wild blueberry soils, soluble organic nitrogen may be important N pool in wild blueberry soils.

(5) Repeated soil N additions would reduce microbial biomass.

(6) Soil applied N would increase concentrations of DOC by increasing above ground biomass production.

(7) Soil applied fertilizers (N, P_2O_5 and K_2O) would influence Net ammonification and increase net nitrification in wild blueberry soil.

2.3 Materials and Methods

2.3.1 Field Experiment

The field experiment was established in spring 2001 on a commercial field (N 45°30' 7.91", W -63° 7'27.72", elevation 223 m) in Kemptown, Nova Scotia. This site was continuously managed under industry standards except fertilization. The soil parent material was a mixture of igneous and metamorphic rocks. The soil pH was 4.3 ± 0.1 SD (0- to 15-cm depth). The soil organic matter was 10.2 % ± 1.4 SD (0-15 cm depth). The soil contained 575 g kg^{-1} sand, 84 g kg^{-1} silt, and 341 g kg^{-1} clay. The land was very stony and moderately rocky. The soil was included in the Cobequid soil association, classified either as gleyed sombric ferro-humic or gleyed humo-ferric podzol (Agriculture Canada 1991).

A plot size of 6 x 8 m was used. The experimental site was fertilized in the form of urea/ammonium sulfate, triple-superphosphate and potassium chloride in early May of sprout year of production with same treatment combinations provided in 2000, 2003, 2005, 2007 and 2009 (Table 2.1 and 2.2). Fertilizers were applied using a Scotts SR2000 rotary fertilizer spreader (Marysville, Ohio). Six response variables were studied at various samplings data in sprout and crop year of production: NH_4^+, NO_3^-, SON, DOC, MBC and MBN.

2.3.2 Soil Sampling

Soil samples were collected from each plot at four times in each sprout (2010) and crop year (2009) of production (Table 2.3). Seven soil cores of 3.0 cm diameter were randomly collected at depth of 12 cm from each plot. Soil cores from each plot were bulked and thoroughly mixed within a plastic bag. Plastic bags were kept in a cooler with ice packs before transporting to laboratory. All soil samples were brought to lab for further analysis. The soil samples were sieved (3 mm diameter) manually which removed the stones, gravel, and visible dead or living plant material. Soil samples were kept at 4°C in a refrigerator prior to extractions. The extractions were performed within 24 hours of soil sampling. The extractions were stored at -20 °C in a freezer for further determination of soil N and C pools. Soil pH was determined in a 1:2.5 soil:water suspension (McLean 1982). Particle size distribution was determined by the hydrometer method (Day 1965). Soil organic matter was measured using loss on ignition method (Davies 1974). Moisture was determined after drying at 105 °C for 48 hours. Measurements units were converted to units of kg ha^{-1} using soil bulk density which was measured by soil core method (Blake and Hartge 1986).

2.3.3 Sample Measurements

2.3.3.1 Soil ammonium (NH_4^+), nitrate (NO_3^-) and Total Soluble Nitrogen (TSN)

Twenty-five grams of moist field (not air dried) soil was extracted with 100 mL of 2.0 mol L^{-1} KCl by shaking for 1 hour on a reciprocating shaker. Extracts were filtered through Whatman Grade No. 42 paper into Nalgene bottles. Extracts were frozen at -20 °C until further analysis. The NH_4^+, NO_3^- and TSN were analyzed using Technicon® flow injection autoanalyzer II (Technicon Instruments, Terrytown, NY) following the Technicon® industrial methods 487-77A and 791-86T for NO_3^- and NH_4^+, respectively (Technicon Industrial Systems 1977; Technicon Industrial Systems 1986). TSN in extracts was measured as NO_3^- after oxidation of aliquots of extracts with alkaline persulfate (Rutherford et al. 2008). NO_3^- in the oxidized solutions was determined colorimetrically after reduction to NO_2^- using copperized cadmium (Maynard et al. 2008). SON was calculated as SON = TSN – ($NH_4^+ + NO_3^-$). This method occasionally was resulted in small negative values because of the errors associated with the measurement of each of component (Bhogal et al. 2000).

2.3.3.2 Dissolved Organic Carbon (DOC), Microbial Biomass Carbon (MBC) and Microbial Biomass Nitrogen (MBN)

The MBC and MBN were determined by chloroform fumigation-extraction method (Voroney et al. 2008). Twenty-five grams of moist soil was fumigated with ethanol-free chloroform for 36 hours. Both fumigated and non-fumigated soils were extracted with 50 mL of 0.5 mol L^{-1} K_2SO_4. Soil-extract mixture were shaken for 1 hour, filtered through Whatman® GF 934-AH). Soil extracts were stored at -20 °C before laboratory analysis. Extracts were analyzed for dissolved DOC (Technicon Industrial systems 1976), NH_4^+ and NO_3^- using by Technicon® flow injection Autoanalyzer II (Technicon Instruments,

Terrytown, NY). Extractable DOC was defined as the total organic C of the unfumigated extracts. DOC pool sizes have been found greater in salt extracts than water extracts (Jones and Willett 2006). However, 0.5 M K_2SO_4 and 2 M KCl extracts remove very similar DOC (Jones and Willett 2006). The MBC and MBN values were calculated from the flushes of extractable C and N using the recovery factors of 0.35 for C and 0.50 for N (Joergensen 1996).

The MBC was calculated as follows:

$$MBC = \frac{C\ in\ fumigatedsoil - C\ in\ unfumigated\ soil}{k_{EC}} \qquad (2.1)$$

where k_{EC} = 0.35, the factor used here to convert the extracted C to MBC.

The MBN was calculated as follows:

$$MBN = \frac{N\ in\ fumigatedsoil - N\ in\ unfumigated\ soil}{k_{EN}} \qquad (2.2)$$

where k_{EN} = 0.50, the factor used here to convert the extracted N to MBN.

2.3.3.3 Net N Ammonification and Nitrification

The long-term (24 weeks) aerobic incubation procedure was used to measure N mineralized at different time intervals (Curtin and Campbell 2008). The protocol involved the incubation of 25 g field-moist soil mixed with 25 g acid-washed sand in leaching tube for 24 weeks. A thin pad of glass wool is placed on top of sand-soil mixture and sandwich of glass wool/Whatman glass microfiber filter/glass were put on the bottom of leaching tube. The incubation temperature was varied between 17 and 25 °C. The temperatures were selected to better represent the mean temperature of the tested soil during the growing season. The soils

were leached periodically (every 2 week for the first 12 weeks and every 4 weeks thereafter) with 0.01 mol L^{-1} $CaCl_2$ followed by the N-free nutrient solution. The leachate was first allowed to drain at room pressure, then a vacuum (-80 k Pa) was applied to remove excess water. The leaching tubes were placed in the incubator with tube in the bottom and top covered by plastic film with holes to facilitate aeration. The leachates were filtered through prewashed Whatman No. 42 filter paper. Leachates were stored at -20 °C before laboratory analysis. Concentrations of NH_4^+ and NO_3^- were measured colorimetrically using a Technicon® flow injection Autoanalyzer 11 (Technicon Instruments, Terrytown, NY).

2.3.3.4 *Experimental Design and Statistical Analysis*

A three factor central composite design (CCD) (Myers et al. 2009) was used to study the response surfaces. The three factors were: N (kg N ha^{-1}), P (kg P_2O_5 ha^{-1}) and K (kg K_2O ha^{-1}), and the levels for the CCD were provided (Table 2.1 and Table 2.2). The factors were coded according to equation (Eqn.).

$$x_i = \frac{X_i - X_0}{\Delta X_i} \qquad i = 1, 2, 3, \ldots\ldots, k \tag{2.3}$$

Where x_i is the factor coded value, X_i the factor real value, X_0 is the real value of factor at center point and ΔX_i the step change. The relationship between the coded and the real values of independent variables are described by Eqn. (2.3).

Within each sampling, all design settings were replicated four times, and to improve the precision of parameter estimations, the averages of the four replications were used, except the center point, which the CCD calls for the replications to allow lack of fit tests. The data from various samplings in sprout and crop year production cycle were analyzed separately to study seasonal trends. The lack of fit test measures the adequacy of the quadratic response

surface model. We tested models for lack of fit test to make sure there is no lack of second order model and proceeded to response surface analysis (Myers et al. 2009). The Proc RSREG of SAS (SAS Inc. 2010) was used to do complete response surface analysis of the data. The second order model used for the CCD response surface analysis was:

$$Y = \beta_0 + \sum_{1=1}^{3} \beta_i X_i + \sum_{1=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon \qquad (2.4)$$

where Y is the response, X_i are the coded levels of the factors, and β_0, β_i, β_{ii} and β_{ij} are the regression coefficients, and ε was the error term assumed to be independent and have normal distribution with constant variance (Myers et al. 2009). The regression coefficients of the response surface model were estimated using the Least Squares method. Interpretation of the data was based on the signs (positive or negative effect on the response) and statistical significance of coefficients ($P < 0.05$). Interactions between two factors could appear as an antagonistic effect (negative coefficient) or a synergistic effect (positive coefficient). The R^2 value (coefficient of determination) expressed the variation of Y explained by the model (three factors studied, X_i). The adjusted R^2 were reported instead of R^2 to describe the percentage of the total variance explained by the model because the adjustment is needed when there are more than one variable. Three dimensional (3D) surface and contour plots were generated using Minitab 16 software (Minitab Inc. State College, PA).

The optimum values of the factors were obtained by completing canonical analysis (Myers et al. 2009). The estimated second order approximations can be written in a matrix form as follows: The second-order response surface model (fitted) in matrix notion as

$$\hat{y} = b_0 + x^T b + x^T \hat{B} X \quad (2.5)$$

where b_0, b and $\hat{B}$ are the estimates of the intercept, linear, and second-order coefficients, respectively. The location of stationary point X_S, $\hat{y}$ is differentiated in Eqn. 1 with respect to x and obtain:

$$\frac{\partial \hat{y}}{\partial x} = b + 2\hat{B}x \quad (2.6)$$

Setting the derivative equal to 0, solve for the stationary point of system:

$$X_S = -\frac{1}{2}\hat{B}^{-1}b \quad (2.7)$$

The nature of the stationary point is defined by the eigen values λ of the matrix B and by solving the following equation:

$$\det(\hat{B} - \lambda E) = 0 \quad (2.8)$$

where λ is also known as canonical coefficients and E is the identity matrix. The canonical analysis was carried out using the RSREG procedure of SAS (SAS Inc. 2010).

2.4 Results and Discussion of Field Study

In order to explore for the optimum combination of the soil applied N, P_2O_5 and K_2O, experiments were performed according to the CCD experimental plan (Table 2.1 and Table 2.2). The experimental data were fitted and explained with a second order polynomial equation. The ANOVA results of the second-order response surface fitted model are illustrated (Table 2.4). The lack of fit of the regression models was not significant indicating that the quadratic model adequately fit for all responses, and *P*-values of the Fisher's *F* test

demonstrated significance at 5% level for the regression (Table 2.4). The fitness of the model was checked by the coefficient of determination (adjusted R^2), which provides a measure of how much variability in the observed response is explained by experimental factors and their interactions (Table 2.4). The R^2 value is always between 0 and 1. The closer the R^2 value to 1.00, the stronger the model is and the better it predicts the response. For example, on 7-July for ammonium, the value of $R^2 = 0.849$ indicates that 15.1% of the total variation was not explained by the model (Table 2.4). The *P*-values for all regression models were listed (Table 2.4). The smaller the magnitude of *P*-value, the more significant is the corresponding regression model. Linear and quadratic effects N, P_2O_5 and K_2O, as well as some of the corresponding interactive effects, were significant, although at different levels (Table 2.4 and Eqn. 2.4). However, to minimize the errors, all the coefficients were included in the model. The significant factors and interactions were provided (Table 2.4). The three dimensional (3D) response surface plots described by regression model were generated to illustrate the effects of each independent variable, and interactive effects of each independent variable on the response variables. The surface plots for each pair of variables by keeping the third variable constant at its middle level have been provided (Figure 2.1-Figure 2.8). The contour plots for all significant terms have been provided in Appendix-B. From the 3D response surface and contour plots, the optimal values of the independent variables and the corresponding response could be predicted. The stationary points/optimal levels of N, P_2O_5 and K_2O application rates were calculated by canonical analysis, solving the system of partial derivatives for the different independent variables. The optimal values of the soil applied fertilizers (N, P_2O_5 and K_2O) in actual units are provided for field measured responses (Table 2.5).Percent change in response (increase or decrease) was calculated by changing one factor and keeping other two at mid values unless otherwise mentioned.

2.4.1 Soil ammonium (NH_4^+)

Table 2.4, Table 2.5, Figures 2.1-2.2 and Appendix B.1-B.3are associated with this section.

In the sprout year of production, the concentration of NH_4^+ was increased by 339% when the N dose was increased from 0 to 60 kg ha^{-1} on 7-July-Sprout (Figure 2.1b). These soil samples (7-July-Sprout) were collected 53 days after fertilization (DAF). Soil NH_4^+ concentration also increased by 93% on 26-July-Sprout (72 DAF) when the N dose was increased from 0 to 30 kg ha^{-1} and NH_4^+ concentration decreased at high N doses (Figure 2.1c). The addition of N fertilizer in the spring of the sprout year of production was found to increase soil NH_4^+ content. The external supply of N induced the decomposition of newly added plant material with subsequent release of NH^{4+} in the soil (Kristensen and McCarty 1999; Raun et al. 1998). The acceleration of soil organic matter decomposition through N fertilizers may be caused by lowering C:N ratio, increase in microbial activity (easily available organic substances), increase in microbial turn over, N release through lysis and dying (salts from mineral fertilizers) (Kuzyakov et al. 2000). Soil NH_4^+ concentration decreased by 29% and 48% on 9-Sep-Sprout (117 DAF) and 22-May-Crop (372 DAF), respectively at N dose between 0 to
30 kg ha^{-1} but soil NH_4^+ concentration increased at higher N application rates (Figure 2.1d, Figure 2.2a and Figure 2.2c). The decrease in soil NH_4^+ to added N may be attributed to switching of microbial biomass from soil organic matter to the easily available C and N sources (which are now available from early season microbial work), N immobilization by microorganism, phenolic substances released by wild blueberry (direct inhibition of microorganism or their enzymes) (Kuzyakov et al. 2000). In middle of the crop year (July onwards) soil NH_4^+ increased again by 24% and 40% on 15-July-Crop and 24-August-Crop

with soil applied N dose while keeping P_2O_5 at low and K_2O at middle rate (Figure 2.2b and 2.2d). Soil NH_4^+ concentration increased by 71% with N on 10-May-Sprout in beginning of the sprout year which was before fertilizer application of current production cycle. These increases may be attributed to microorganism may switched back again to work mode as explained earlier in the paragraphs. Soil NH_4^+ concentration increased with soil applied N within first two months of fertilizer application, then soil NH_4^+ concentration decreased for next two samplings (late in sprout year and early crop year) and soil NH_4^+ concentration started to increase again from mid of crop year to beginning of the sprout year. Response of soil NH_4^+ concentration to soil applied N resembled concave shape around production cycle assuming date of fertilizer application as center point. These differences may be attributed to increased activity or amount of microbial biomass, pool substitution, competition of nutrients plant roots and soil microorganism and preferred uptake (Kuzyakov et al. 2000). Soil NH_4^+ concentration increased by 97% and 51% on 7-July-Sprout (53 DAF) and 26-July-Sprout (72 DAF), respectively when P_2O_5 application was increased from 0 to 110 kg ha^{-1} but soil NH_4^+ concentration decreased (51% and 42%) from middle to higher P_2O_5 rates. Similarly in crop year, soil NH_4^+ concentration increased by 19% and 18% on 31-July-Crop (442 DAF) and 24-Aug-Crop (466 DAF) respectively, when the P_2O_5 application was increased from 0 to 110 kg ha^{-1} but soil NH_4^+ concentration decreased (7.5% and 19%) at higher P_2O_5 application rates. The soil NH_4^+ concentrations were lower in the sprout year than the crop year of production (Table 2.5) despite fertilizer application in the sprout year. I take these NH_4^+concentration differences as an indication of soil NH_4^+ usage by wild blueberry plant in the sprout year. Further discussion related to this can be found in soil nitrate and soluble organic nitrogen section.

2.4.2 Soil nitrate (NO_3^-)

Tables 2.4- 2.5, Figure 2.3 and Appendix B.4-B.5 are associated with this section.

In spout and crop year of production, the *P*-values of the Fisher's *F* test demonstrated significance at 5% level for the regression except on 10-May-Crop, 31-July-Crop and 24-Aug-Crop (730, 442 and 466 DAF) (Table 2.4). The R^2 values of soil NO_3^- models were 0.729, 0.811 and 0.748 for 7-July-Sprout, 26-July-Sprout and 15-July-Crop, respectively.

In sprout year, concentration response of soil NO_3^- was convex (Figure 2.3a, Figure 2.3b) on 7-July-Sprout (53 DAF) and 26-July-Sprout (72 DAF). It suggested maximum soil NO_3^- was produced at middle levels of soil applied N, P_2O_5 and K_2O (Table 2.5). The depressing influence of soil applied N on nitrification may due to the combined effect of low pH and increase in salt content with increased soil applied N (ammonium sulfate) from 30 to 60 kg ha^{-1} (Malhi and McGill 1982). In crop year, soil NO_3^- response was opposite (Concave) to that of the sprout year (Figure 2.3c and Figure 2.3d). For example, minimum response of soil NO_3^- concentration was found on 15-July-Crop (Figure 2.3d; Table 2.5). The difference in sprout and crop year nitrification patterns may be attributed to temporal variations in nitrification in soil related to difference availability of organic substrates, nitrogen inputs, carbon availability (Wheatley et al. 2001) and soil C:N ratio (Ross et al. 2004). The addition of ammonium based fertilizer in the sprout year may have provided the substrate for nitrification (Zhao et al. 2007). In spout year of production, the fertilizers may have triggered microbial activity and high decomposition rates were expected from newly added plant material by mowing in fall of the previous year.

Higher nitrate production was observed than initially expected because the wild blueberry soils have naturally low pH and by release of phenolic compounds into soil by

Ericaceous plant roots (Korcak 1988), thus nitrification activity is generally considered to be limited in such soil environments (Ward 2011). In the sprout and crop year, concentration of soil NO_3^- indicated nitrification activity in blueberry soil (~ 5 kg N ha^{-1}), raising concerns of ground and surface water contaminations, green-house gas emissions through denitrification, soil acidification, plant physiological disturbance through changing the relative concentration of soil N pools and subsequent uptake. This concentration of soil NO_3^- was calculated by taking average of all stationary points (Table 2.5).

In the spout year of production, the addition of ammonium based fertilizer increased the soil NH_4^+ concentration on 7-July-Spourt (1^{st} sampling after fertilizer application) compared to sampling before fertilization. But the fertilizer application did not increase the concentration of soil NO_3^- (stationary point) which was similar before and after fertilizer application (Table 2.5). This indicated that the production of soil NO_3^- did not appear to be limited by soil ammonium supply. If the addition of soil ammonium did not increase the soil NO_3^- production, it may be assumed that soil NO_3^- is produced directly from soil organic N which may indirectly suggest heterotrophic nitrification pathway in wild blueberry soil. In a study at Debert (R. Maqbool, unpublished data), production of soil NO_3^- production was not significantly affected by the addition of 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), an inhibitor of autotrophic nitrifiers, or ammonium. These findings suggest that the active nitrifying microorganisms may be mainly heterotrophs in these soils. The application of inhibitors is not exact science because some soils may bind or remove Nitrapyrin (Stein 2011). Molecular and stable isotope studies may be required to find conclusive evidence for this observation.

In the sprout year, concentration of soil NH_4^+ (8.5 kg N ha^{-1}) was less compared to the crop year concentration (~15.3 kg N ha^{-1}). However, concentration of soil NO_3^- did not

vary between sprout (~5.5 kg N ha^{-1}) and crop year (4.2 kg N ha^{-1}) of production. I interpret these concentration differences as an indication that the wild blueberry plant may preferably absorb NH_4^+ compared to NO_3^- in the sprout year of production (the period when blueberry plant grows actively). The wild blueberry has been shown to exhibit better growth under NH_4^+ supply compared to NO_3^- nitrogen (Korcak 1988).

Wild blueberry soils in northeastern North America have been receiving nitrogen applications as they were considered nitrogen limited. Fertilizer experiments generally have shown positive growth and yield responses mainly to the nitrogen component. Rates of nitrogen addition to wild blueberry system range from 20 kg N ha^{-1} to 60 kg N ha^{-1}. Continuous applications may alter nitrogen cycling and crop productivity. The added nitrogen may be sequestered in wild blueberry rhizomes and soil organic matter. Over time, these N additions may exceed the capacity for N uptake and retention by soil, plants (wild blueberry and weeds) and microbes.

In this study, we found excess mineral N in spout year and, mineral N and SON in crop year to plant and microbial needs at our experimental site at Kemptown (Table 2.5). The excess N may be at a high risk of leaching and runoff to low lying areas and out to water bodies. Wild blueberry fields are typically located on, sandy soils, steep slopes and regions of significant precipitation. Elevated nitrification rates (field and lab studies) may suggest early indicators of N saturation. The implications could be serious for overall soil health and sustainability of the wild blueberry production system. Possible negative effects may include further increases in concentrations of NO_3^- in soil, which may be toxic to wild blueberry plants, would decrease water quality, physiological disruptions such as delayed transition from vegetative to reproductive phase and frost damage that would reduce the productivity of wild blueberry. Other effects may include increased nitrous oxide emissions, increased cation

leaching and increased acidity. This situation demands continuous research on soil nitrogen dynamics to monitor and cope with the possible adverse effects of nitrogen additions. This emphasizes the need to tailor N applications doses that fulfill the crops needs while maintaining the soil health and the sustainability of wild blueberry production system.

2.4.3 Soluble Organic Nitrogen (SON)

Tables 2.4-2.5, Figure 2.4 and Appendix B.6-B.8 are associated with this section.

The *P*-values of the Fisher's *F* test of soil SON demonstrated significance at 5% level for the regression for all samplings (Table 2.4). The 3D surface plots of soil SON evaluating two variables were generated by keeping the third variable constant at its middle level (Figure 2.4 and Appendix B.6-B.8). Soil SON decreased when the N rate was increased from 0 to 60 kg ha^{-1} (Figure 2.4a and Figure 2.4 b). Soil SON exhibited concave (minimum) response to soil applied N, P_2O_5 and K_2O fertilizer in sprout year (Figure 2.4c and Figure 2.4d) and convex (maximum) response in crop year to soil applied N ,P_2O_5 and K_2O (Figure 2.4e and Figure 2.4f).

Soil SON concentration in the sprout year was close to zero or even negative in comparison to greater soil SON concentration of ~ 18 kg N ha^{-1} that was mean of stationary points across four samplings in crop phase of production. These lower concentrations of SON in the sprout year may be an indication of uptake of N organic fractions by the wild blueberry plant. Wild blueberry fields are pruned in the fall of the crop year or in the early spring of the sprout year that deposits substantial amounts of plant material to the soil in each production cycle and gradually builds soil organic nutrient reserves. Organic N adhering to organic matter is slowly available to plants upon mineralization.

There is strong case of wild blueberry (ericaceous plant) using organic N forms considering the symbiotic association with ericoid mycorrhizal fungi and reduced assimilatory cost associated with uptake of amino acids (organic N) relative to inorganic N (Allen et al. 2003). Ericaceous plants lack root hairs but have epidermal cells that are occupied by mycorrhizal fungi. The fungi proliferate within the epidermal cells of roots to form ericoid mycorrhiza. The ericoid mycorrhiza fungi produce extracellular enzymes that have good proteolytic and litter degrading capabilities (Read et al. 2004). These enzymes degrade soil organic matter to simple organic forms such as amino acids that are potentially available for plant uptake either ericoid mycorrhiza fungi association or directly by plants or microorganism (Read et al. 2004).

SON concentration during the mid-season (7-July-Sprout and 26-July-Sprout) was close to zero in comparison to soil NH_4^+ concentration (10 kg N ha^{-1}) from same samplings dates. Based on these results I further hypothesize that wild blueberry SON consumption in presence of adequate soil NH_4^+supply may indicate an inherited mandatory N demand that has to be fulfilled from organic N supply. Wild blueberry dependence on organic N sources may be linked to natural wild blueberry habitats which resembles undisturbed northern heathland, temperate and boreal forests where predominantly organic forms are present with little inorganic N (Finzi and Berthrong 2005; Jones et al. 2005; Kielland 2007; Näsholm et al. 2009). In this study, soil SON emerged as an important soil N pool in wild blueberry soils. Therefore, soil SON measurements may be included in future nutrient management program of wild blueberry production.

2.4.4 Dissolved Organic Carbon (DOC)

Tables 2.4- 2.5, Figure 2.5 and Appendix B.9 are associated with this section.

Concentration of DOC in temperate forest ecosystems of North America and Europe have been reported below 100 kg C ha^{-1} (Michalzik et al. 2001; Höberg and Höberg 2003; Moore et al. 2008). In this study, concentrations of DOC were greater than 100 kg C $^{-1}$ ha^{-1} (Table 2.5; Figure2.5a and Figure 2.5b). The unusually high concentration of DOC may reflect the unique wild blueberry soil system characterized by extensive root/rhizome system, bulk addition of plant material through flail mowing, shedding of leaves in autumn (Cellulose) and fungi dominated soil environment. Yano et al. (2000) reported correlation between root biomass and DOC concentrations in soil. Fungi have been reported to be the most important agents in dissolved organic matter production because of incomplete decay of organic matter. In addition, higher fluxes of DOC are reported in sandy and organic soils (Moore and Jackson 1989; Hope et al. 1994).

The concentration of DOC varied between two samplings in sprout year of production. The concentration of DOC in early season was 319 kg C ha^{-1} (stationary point) on 10-May-Sprout in comparison to 183 kg C ha^{-1} (stationary point) on 9-Sep-Sprout (Table 2.5). Our finding of a seasonal pattern for DOC (higher values in spring than late summer) is consistent with the results of Yano et al. (2000) who also measured higher DOC in summer (72 mg L^{-1}) than fall (66 mg L^{-1}) in pine forest floor. The greater concentration of DOC in spring of the sprout year (10-May-Sprout) may be explained through release of DOC through addition of plant material in previous fall. The high concentration of DOC early in the season may be built up in the soil during the long winter by slow continued decay and release of a dissolved organic matter fractions then production of DOC in the early spring. The DOC concentration difference could be the balance among a wide range of production and

consumption processes, including biotic and abiotic (temperature, moisture and abiotic leaching) factors (McDowell 2003). Release of DOC generally increased with temperature, soil moisture (Michalzik et al. 2001) and frequency of leaching (Gödde et al. 1996). The microbial biomass carbon of soil has declined with N additions on 9-Sep-Sporut (Figure 2.5b), suggesting reduced uptake of the DOC needed to fuel microbial respiration and a net increase in DOC concentrations. On10-May-Sprout, the reverse was found with microbial C component of soil, giving less profound net increase in DOC.

The *P*-values of the Fisher's *F* test of soil DOC demonstrated significance at 5% level for the regression on 10-May-Sprout (Table 2.4). The R^2 of soil DOC was 0.739 for 10-May-Sprout (Table 2.4). Soil DOC model for 9-Sep-Spourt was marginally significant (*P*-value =0.06). However, soil DOC model for 9-Sep-Sprout did not fit the data well and accounted only 59% of the total variation (Table 2.4). Soil DOC increased by 25% on 10-May-Sprout, when N was increased from 0-60 kg ha^{-1} and keeping P_2O_5 low and K_2O at middle levels (Figure 2.5a).

DOC increased significantly with applied N at our experimental site. This trend has been reported in temperate forests which are N limited under natural conditions (Pregitzer et al. 2004; Sinsabaugh et al. 2004; Rappe-George et al. 2013). A recent metal analysis in various ecosystems provided that DOC concentration was increased by 18% under N additions (Liu and Greaver 2010). Hypothetically, several possible reasons have been suggested in the literature for increase in DOC pool with N additions. These include: (1) increased above ground productivity subsequently high leaf and foliage fall, and increased C leaching when water passes through vegetation (Kalbitz et al. 2000), (2) increased DOC mobilization through stimulated microbial activity under N enrichments (Kalbitz et al. 2000); (3) N induced increases in degradation of cellulose through increases in the activity of

cellulolytic enzymes; (4) N-induced suppression of ligninase, which results in an incomplete decomposition of lignin, accumulation of partially degraded and highly water soluble products (Kalbitz et al. 2000); (5) decrease in DOC decomposition rates because N-induced reduction in oxidative enzyme activity (Sinsabaugh et al. 2004) and (6) net loss from soil C stable reserves in a form of DOC to the labile pool (Findlay 2005; Monteith et al. 2007; Evans et al. 2008). In our study, increased DOC production with N additions is most likely through the increased plant biomass production, subsequent decomposition including increased cellulolytic enzyme activity and fungi mediated partial decomposition. The net DOC concentration in soil is the balance between the production and consumption of DOC in soil. The microbial biomass C concentration was reduced with added N on 9-Sep-Sprout (Figure 2.7b). Thus, a decrease in microbial DOC consumption could have partially contributed in the increased DOC concentrations on 9-Sep-Sprout (Figure 2.5b).

Overall, we found the addition of N to have a positive impact on DOC concentration by increasing organic C pool through increased above ground productivity. N fertilizer addition may alter C output by changing the dynamics of DOC leaching. These elevated levels may pose a threat of increased C export to water bodies particularly in sandy soils with little adsorption capacity (Aitkenhead-Peterson et al. 2007). In wild blueberry production system, study of the retention capacity and turnover of DOC may be useful in quantification of organic carbon and C storage capacity of soils.

2.4.5 Microbial Biomass Nitrogen (MBN)

Tables 2.4-2.5, Figure 2.6 and Appendix B.10 are associated with this section.

The *P*-values of the Fisher's *F* test demonstrated significance at 5% level for the regression for both samplings. The R^2 values were 0.719 (good fit) and 0.557 (weak fit) for

10-May-Sprout and 9-Sep-Sprout, respectively (Table 2.4). MBN decreased (79%) on 10-May-Sprout when N is increased from 0 to 60 kg $ha^{-1}$and keeping K_2O dose low and P_2O_5 at middle levels (Figure 2.6a). MBN also decreased on 9-Sep-Sprout with increasing N (Figure 2.6b).

We found MBN reduction on both sampling with soil applied N fertilizer. Soil microbial biomass has consistently reported to decrease with N fertilization in forest ecosystems (Bowden et al. 2004; Smolander et al. 2005). Microbial biomass reduction has also been found along natural soil fertility gradients. The low soil fertility conditions promote abundance and activity of the soil microbial biomass (Yeates et al. 1997). Demoling et al. (2008) reported 40% reduction of microbial biomass in fertilized plots.

2.4.6 Microbial Biomass Carbon (MBC)

Tables 2.4-2.5, Figure 2.7 and Appendix B.11 are associated with this section.

The *P*-values of the Fisher's *F* test demonstrated significance at 5% level for the regression (Table 2.4). The R^2 were 0.809 and 0.778 for 10-May-Sprout and 9-Sep-Sprout, respectively (Table 2.4). MBC increased (108%) on 10-May-Sprout when increasing N dose from 0-60 kg ha^{-1} and keeping P_2O_5 and K_2O dose at low (Figure 2.7a) .MBC decreased (58%) on 9-Sep-Sprout (117 DAF) when N dose was increased from 0-60 kg ha^{-1} (Figure 2.7b). MBC also decreased on 10-May-Sprout and 9-Sep-Sprout with increasing N and P_2O_5 (Figure 2.7a and Figure 2.7 b). These results indicated different MBC response to soil applied N before (long term effect) and after fertilizer (short term effect) application.

A recent meta-analysis provided that N addition decreased MBC by an average of 20% across all studies (Treseder 2008). In our study, MBC significantly decreased in September and a non-significant increase pattern with N fertilization was observed in May of

the sprout year of production. The soils with low fertility had more pronounced reduction in microbial biomass C and N than soil with high fertility (Arnebrant et al. 1996). It has been suggested that fertilizer N additions repress enzyme activity and accumulate recalcitrant and toxic compounds thus directly inhibit microbial biomass (Arnebrant et al. 1996).

DOC may serve as both an energy source and a potential source of N (as DON) to heterotrophic microorganisms. It appears that wild blueberry plants have retained their fungal-dominated response to external N enrichments through production of DOC (leaching of litter, decomposition of organic matter, release of organic compounds from roots (exudates, mucilage and mucigel). In wild blueberry production system, heterotrophic microorganisms may play significant role in N cycling, and that their metabolism is not restricted by the availability of C in the soil.

2.4.7 Microbial Biomass Carbon to Microbial Biomass Nitrogen (MBC: MBN) Ratio

Figure 2.8 and Appendix B.12 are associated with this section.

Microbial biomass carbon to nitrogen (MBC: MBN) ratio (~ 15:1 kg ha^{-1}) was found in wild blueberry soil (Figure 2.8). Typically fungi have higher C:N ratio than bacteria (10 vs 4). This (MBC: MBN) value of 15 is higher than typically found in bacterial biomass (de Vries 2006). This observation suggested fungal dominance in wild blueberry soil system. Wild blueberry production systems are no-till and fungal-dominance was expected under no-till agricultural practices instead of the bacterial-dominance that would prevail with crops grown under conventional tillage practices (Strickland and Rousk 2010). This outcome is controlled by the difference in the growth pattern of fungi and bacteria. Most fungi exhibit a hyphal growth form but bacteria typically grow as individual cells. The fungi exhibit a

competitive advantage under low fertility system by translocation of nutrients and resources using hyphal growth from microsites where they are abundant to sites where they are limiting (Frey et al. 2003). Fungal-dominated soil communities may enhance C storage and slow soil organic matter (SOM) turnover due both to fungal alteration of soil physical properties and to fungal physiology (Strickland and Rousk 2010). Thus, ecosystems with soils dominated by fungi may sequester more C than systems with lower fungal abundance (Six et al. 2006). This has been demonstrated indirectly in our study through high MBC values (~ 200 kg C ha^{-1}) than typically found in other managed vegetation systems.

Fungi take a lead role in decomposition of recalcitrant litter using their lignin degrading ability whereas bacteria are less capable of decomposing that component of litter (de Boer et al. 2005; Marcel et al. 2008; Esperschütz et al. 2013). Fungi also dominate the decomposition of cellulose and hemi-cellulose, which are important constituents of wild blueberry organic matter (woody stem). Mineral fertilization is reported to increase the lignin content of foliage. Inorganic fertilizer generally has been reported to reduce fungal biomass (Bittman et al. 2005) and decrease in the F:B biomass mainly due to reduction in fungal biomass (deVries et al. 2006). Van Groenigen et al. (2007) reported a decrease in fungal-derived residues following the application of N fertilizer, while organic matter with a high C:N ratio is believed to stimulate the fungal contribution to decomposition (Thiet et al. 2006). Our results indirectly suggested that fungal biomass increased with increased N rates for both samplings. The May samplings had considerably higher MBC: MBN ratio than in September. Our results seem to partially contradict the literature where N fertilization in low fertile soils has been found to favor bacterial pathways of decomposition. However, our results indicated that N additions promoted fungal pathways of decomposition (Yeates et al. 1997; Bardgett and McAlister 1999). In September, MBC:MBN were considerably lower than the ratios in

May but still suggested fungal dominance. It appeared that fertilizers caused small shift in favor of bacterial decomposition. These results contradict most of the fungal:bacteria fertilizer responses found in the literature. In our study, very high MBC:MBN was observed suggesting very high carbon in early spring but that fertilizer additions reduced the MBC:MBN ratio. Additionally, fungal dominance could be dependent on both the microbial community in question and the litter being decomposed. This agrees with Güsewell and Gessner (2009), who reported fungi were more important at wider N:P and when P was limited, therefore, soil N:P supply to decomposers may be important determinant of relative abundance and activity of fungi and bacteria. However, direct measurements of bacterial and fungal biomass are needed to confirm the relationship between soil applied fertilizers and F:B ratio and its effect on sustainability of wild blueberry production system.

2.5 Results and Discussion of Lab study (Net Ammonification and Nitrification)

We found significant main and interactive effects of soil applied (N, P_2O_5 and K_2O) on net ammonification and net nitrification (Table 2.6). Here after in this section soil applied P_2O_5 and K_2O will be written as P and K, respectively. The stationary points/optimal levels of N, P and K application rates were calculated by canonical analysis and optimal values of the soil applied fertilizers (N, P and K) in actual units are provided for net ammonification and nitrification (Table 2.7). Soil NH_4^+ present in 1st leachate at 0 days (30 days after fertilization-DAF) exhibited a significant linear response to increased N applied to soil and a marginally significant convex quadratic response to levels of K (Figure 2.9a; Table 2.6). Soil applied P had no effect on NH_4^+ content in 1st leachate (Table 2.6).

Net ammonification (NH_4^+) in 2nd leachate at 15 days (45 DAF) was a concave quadratic function of the soil applied N, P and K levels (Figure 2.9b; Table 2.6). The

significant N x K and P x K interactions indicated that response to each of these fertilizers was dependent on the levels of other fertilizer (Figure 2.9b; Figure 2.9c and Figure 2.9d; Table 2.6). The net ammonification response to soil applied N and K of 2nd leachate resembles with that of 3rd leachate at 30 days (60 DAF) and 9th leachate at 165 days (195 DAF) (Figure 2.9b; Appendix B.13). The soil applied P and K effect on net ammonification of 2n leachate is similar that of 3rd leachate, 8th leachate at 135 day (165 DAF) and 9th leachate (Figure 2.9c; Appendix B.14). Net ammonification (NH_4^+) at 6th leachate at 85 days (105 DAF) and was a complex function of N, P and K (Table 2.6). The N x P and N x K interactions were significant for net ammonification (Table 2.6; 2.16c). The net ammonification at 7th leachate at 105 days (135 DAF) was significant concave quadratic response of soil applied K was significant (Table 2.6). Similar to 2nd, 3rd and 9th leachate, the N x K and P x K interactions were significant for net ammonification (Table 2.6; Figure 2.9d; Appendix B.13-B.14). The net ammonification in 8th leachate was significant concave quadratic response of soil applied K was significant (Table 2.6; Appendix B.14). The P x K interaction was also significant for net ammonification at 8th leachate (Table 2.6; Appendix B.14).

The NO_3^- present in the 1st leachate showed a significant convex quadratic response to soil applied N, P and K (Figure 2.10a; Table 2.6). The P x K interaction was also significant for NO_3^- in 1st leachate (Table 2.6). The net nitrification in the 2nd leachate indicated a significant quadratic response to the soil applied P levels (Figure 2.10c; Table 2.6). The significant N x K and P x K interactions for net nitrification in 2nd leachate indicated that the response to each of these fertilizers was dependent on levels of other fertilizer (Table 2.6; Figure 2.10d; Appendix B.15-B.16). The net nitrification in the 3rd leachate was a convex quadratic function of the soil applied N, and K fertilizer levels. The N

x K and P x K interactions were significant as before in 2nd leachate (Table 2.6; Figure 2.10b; Appendix B.15-B.16). The N x K interaction of the net nitrification was also significant for 2nd, 4th and 9th leachates (Table 2.6; Appendix B.15). The N x K interaction plots were presented in Appendix B.15 and shape of 3rd, 4th, 5th and 9th leachate plots were similar (Appendix B.15). The net nitrification in the 3rd leachate was a convex quadratic function of the soil applied N, and K fertilizer levels. The N x K and P x K interactions for net nitrification were significant as before in 2nd leachate (Table 2.6). The P x K interaction for net nitrification was also significant for 6th and 7th leachates and shape of the net nitrification response were similar for 2nd, 3rd, 6th and 8th leachates (Table 2.6; Appendix B.16).

The 1st leachate (40 DAF) contained 47 kg NH_4^+ and 44 kg NO_3^- ha^{-1}. The high mineral N (combination of ammonium and nitrate), may be explained by residual effects of N fertilizer added to soil, sieving effects (release of small amounts of previously protected N sources) and dead microbial cells. The ammonification rate in 2nd to 5th leachate was 0.61 kg N ha^{-1} day^{-1} and the last four leachates (6th to 9th leachate) were 0.24 kg N ha^{-1} day^{-1}. The decline in ammonification rates may be explained by the diminishing organic sources (organic substrates may be utilized by heterotrophs in organic pathways of nitrification) and/or low temperatures (14-20 ˚C) from the last 4 (6th to 9th) samplings. However, nitrification rates did not drop as much as ammonification rates. The early nitrification rates (2nd to 5th leachate) and later (6th to 9th leachate) samplings averaged 1.46 and 0.62 kg N ha^{-1} day^{-1}, respectively. The lack of reduction in nitrification rates may suggest organic pathways of nitrification where heterotrophic nitrifiers oxidize organic substrates directly instead using NH_4^+ as substrate. In nitrification process, heterotrophs apparently did not gain energy from the nitrification process and utilize organic compounds both as a carbon and an energy source. There is abundance of organic compounds and carbon (DOC) in this

soil (Table 2.4), Therefore, heterotrophic nitrification pathway is not limited by substrate or energy source. The other explanation may be that nitrification process is less sensitive to low temperatures than ammonification. Given the larger numbers of fungi (heterotrophs) present in wild blueberry soils, heterotrophs might make a significant contribution to total nitrification under Maritime conditions.

The NO_3^- was double that of NH_4^+ in the lab incubation experiments. In contrast, more NO_3^- (laboratory) was found than nitrate N (field). Field and laboratory results should be compared with caution due to different *in situ* conditions. For example, in the field there was potential for nitrate leaching and subsequent accumulation of NH_4^+ that eventually converted to nitrate during the course of the experiment while in the laboratory there was no vegetation uptake and there was an absence of roots that may exudates allelochemicals and/or or phenolic that potentially reduce the nitrification directly or by altering the soil microbial composition.

Higher nitrification rates were found than previously thought to exist in the wild blueberry production systems (Eaton and Patriquin 1988). It seems that heterotrophic nitrification is important in wild blueberry soils and that the nitrifiers are adapted to acidic conditions. The high heterotrophic microbial biomass may compensate the low nitrification rates per unit of biomass typically associated to heterotrophs. The proliferation of nitrifying bacteria could be another contributing factor in the high nitrification observed in this study. Proliferation processes have been reported earlier as a major process in some acidic soils (De Boer et al. 1991). Proliferation did not appear to depend on the acid sensitive autotrophic nitrification that requires microsites of high pH for function (De Boer et al. 1991). In summary, potential contribution of heterotrophic nitrification is greater (fungal dominated

system, abundance of organic matter, dissolved organic C) than autotrophic nitrification in wild blueberry soil.

2.6 Conclusions

Our results illustrated significant main and interactive effect of soil applied fertilizers (N, P_2O_5 and K_2O) on concentrations of soil NH_4^+, NO_3^-, SON, DOC, MBN, net ammonification and net nitrification. The concentration of soil NH_4^+ and SON differed and NO_3^- remained similar between sprout and crop year of production. These lower concentrations of soil NH_4^+ and SON in the sprout year insinuate a preference by the wild blueberry plant for NH_4^- over $NO_3^-$and also uptake of SON. The concept SON being a significant factor in supporting wild blueberry growth and development is a new and important concept. Based on these results, it appears that wild blueberry plants may acquire organic N fractions and some part of total N demand of the wild blueberry plant may be supplied by the by soil organic N pool. We also found higher nitrification activity than initially hypothesized. Results indicated that mainly heterotrophs were involved in nitrification activity in wild blueberry soils. The incubation laboratory study results suggest that N enrichments reduced net ammonification and increased net nitrification.

Results from this study also supported our hypothesis that soil applied N reduced microbial biomass. This was consistent with previous studies of soil microbial biomass in various soils and vegetation systems. High nitrification activity along with reduced microbial may indicate early signs of N saturation. Higher than anticipated concentrations of DOC (up 319 kg C ha^{-1})were also observed in this study which were also higher than those normally found in temperate forests of northeastern North America(Moore et al. 2008). The high concentration of DOC could be attributed to high above ground productivity and fertilizer induced changes in leaf chemistry. There is a hint that fertilizer additions changed soil

bacteria and fugal balance in favor of fungal communities. This contradicts to general response of soil microorganisms to nitrogen enrichments. I further hypothesize that wild blueberry plants were able to retain its fungal dominated soil environment through release of organic compounds and the presence of high concentration of DOC in soil. The concentration of NO_3^- from leachate laboratory experiment was greater than concentration of NO_3^- from field samplings, indicating NO_3^- leaching and gaseous (denitrification) losses in wild blueberry soils. The N enrichments increased nitrification activity, concentration of DOC and reduced the microbial biomass. It may raise concerns of soil health and sustainability. N fertilization improved plant biomass production, subsequent deposition of plant materials to soil by prune mowing, potential increase in carbon sequestration and retention of fungal dominated soil system. These positive effects would keep wild blueberry soil in a healthy state.

Table 2.1 Description of the eight sampling dates, crop phase and fertilizer event

Sprout-year			Crop-year		
Sampling Dates	*DAF	Phase	Sampling Dates	*DAF	Phase
10-May	730	Pruned to ground	22-May	372	Bud development
7-July	53	Actively growing	15-July	426	Fruit development
26-July	72	Tip dieback	31-July	442	Fruit maturation
9-September	117	Shedding leaves	24-August	466	After harvest

* DAF: days after fertilization. Fertilizer was applied on 15-May-Sprout (sprout year of production)

Table 2.2 Experimental range and levels of the independent variables

	Range and levels				
Original factors	-1.68	-1	0	1	1.68
[z]N (kg ha^{-1}): X_1	0	12	30	48	60
P_2O_5 (kg ha^{-1}): X_2	0	44	110	176	220
K_2O (kg ha^{-1}): X_3	0	12	30	48	60

[z]The source of soil applied N was ammonium sulfate, P was triple superphosphate and K was potassium chloride

Table 2.3 Real and coded values of the central composite experimental design

	Coded values			Real values		
	N	P	K	N	P_2O_5	K_2O
					(kg ha^{-1})	
Factorial points	-1.00	-1.00	-1.00	12	44	12
	1.00	-1.00	-1.00	48	44	12
	-1.00	1.00	-1.00	12	176	12
	1.00	1.00	-1.00	48	176	12
	-1.00	-1.00	1.00	12	44	48
	1.00	-1.00	1.00	48	44	48
	-1.00	1.00	1.00	12	176	48
	1.00	1.00	1.00	48	176	48
Star points	-1.68	0.00	0.00	0	110	30
	1.68	0.00	0.00	60	110	30
	0.00	-1.68	0.00	30	0	30
	0.00	1.68	0.00	30	220	30
	0.00	0.00	-1.68	30	110	0
	0.00	0.00	1.68	30	110	60
Center points	0.00	0.00	0.00	30	110	30
	0.00	0.00	0.00	30	110	30
	0.00	0.00	0.00	30	110	30
	0.00	0.00	0.00	30	110	30

Table 2.4 *P*-values from Analysis of variance (ANOVA) for the response surface model for ammonium

N form	Source of variation	Sprout year				Crop year			
		10-May	7-July	26-July	9-Sep	22-May	15-July	31-July	24-Aug
NH_4^+	Regression	0.02	<0.01	<0.01	0.02	<0.01	0.03	0.01	0.04
	LOF	0.81	0.63	0.74	0.69	0.70	0.67	0.85	0.99
	Adjusted R^2	0.67	0.85	0.79	0.68	0.84	0.63	0.76	0.60
	Significant terms	P, NP, PK	N, PP, KK,	N, NN, PP, KK	K, NN, NP	K, NN, PP, KK	KK	NN, PP	PP
NO_3^-	Regression	0.07	<0.01	<0.01	0.04	0.02	<0.01	0.08	0.07
	LOF	0.59	0.89	0.70	0.74	0.55	0.74	0.86	0.94
	Adjusted R^2	0.51	0.73	0.81	0.57	0.67	0.75	0.54	0.51
	Significant terms	NK	N, NN, PP, KK, PK	N, NN, PP, KK	K, PP, NK	K, NN, KK, NP, PK	NN, PP, KK, PK	N, PP	NN
SON	Regression		<0.01	0.03		0.03	0.02	0.02	0.01
	LOF		0.68	0.72		0.23	0.65	0.59	0.42
	Adjusted R^2		0.75	0.62		0.63	0.68	0.69	0.72
	Significant terms		N, PP, KK	N, PP, KK		N, K, NN, PP, KK	N, P, KK	NN, PP	N, NN, PP, PK

Table 2.4 continued

N form	Source of variation	Sprout year				Crop year			
		10-May	7-July	26-July	9-Sep	22-May	15-July	31-July	24-Aug
MBN	Regression	0.02			0.05				
	LOF	0.24			0.60				
	Adjusted R^2	0.72			0.56				
	Significant terms	N, K, NK			N, P				
DOC	Regression	<0.01			0.06				
	LOF	0.92			0.86				
	Adjusted R^2	0.74			0.59				
	Significant terms	PP			PP, NP				
MBC	Regression	<0.01			<0.01				
	LOF	0.86			0.63				
	Adjusted R^2	0.81			0.78				
	Significant terms	PP, NP, PK			N, P, K, PP				

Table 2.5 Canonical analysis performed for each response surface model

Soil variable	Responses	Stationary points in real units (kg ha^{-1})			Y_S (kg ha^{-1})	Nature of stationary point
		N	P_2O_5	K_2O		
NH_4^+ (Sprout)	10-May	30	112	42	12	Saddle
	7-July	-10	119	21	14	Saddle
	26-July	35	97	30	6	Maximum
	9-Sep	38	18	21	2	Saddle
NH_4^+ (Crop)	22-May	30	118	26	10	Minimum
	15-July	17	47	27	18	Saddle
	31-July	27	123	38	15	Saddle
	24-Aug	40	79	41	18	Saddle
NO_3^- (Sprout)	10-May	28	115	31	4	Saddle
	7-July	33	115	27	5	Maximum
	26-July	34	110	30	5	Maximum
	9-Sep	37	106	23	8	Saddle
NO_3^- (Crop)	22-May	37	145	31	6	Saddle
	15-July	29	110	28	3	Minimum
	31-July	22	122	26	4	Saddle
	24-Aug	34	144	31	4	Saddle
SON (Sprout)	7-July	237	53	49	-12	Minimum
	26-July	61	71	26	-2	Minimum
SON (Crop)	22-May	25	116	27	5	Maximum
	15-July	-48	-247	13	54	Saddle

Table 2.5 continued

Soil variable	Responses	Stationary points in real units (kg ha^{-1})			Y_S (kg ha^{-1})	Nature of stationary point
		N	P_2O_5	K_2O		
MBN (Sprout)	10-May	38	154	28	14	Saddle
	9-Sep	39	112	46	17	Saddle
DOC (Sprout)	10-May	24	115	37	319	Saddle
	9-Sep	28	115	28	183	Saddle
MBC (Sprout)	10-May	31	115	30	278	Saddle
	9-Sep	56	125	39	110	Minimum

Table 2.6 *P*-values from analysis of variance (ANOVA) for the response surface model for ammonium and nitrate

	Source of variation	1st leachate	2nd leachate	3rd leachate	4th leachate	5th leachate	6th leachate	7th leachate	8th leachate	9th leachate
NH_4^+	Regression	0.04	<0.01	<0.01	0.01	<0.01	0.01	<0.01	0.03	<0.01
	LOF	0.52	0.53	0.69	0.66	0.49	0.96	0.65	0.92	0.35
	Adjusted R^2	0.60	0.84	0.80	0.70	0.77	0.72	0.77	0.61	0.79
		N	NN, PP, KK, NK, PK	NN, NK, PK	N, NN, KK, NP	N, P, NN, PP, KK, NP	N, P, NP, NK	P, KK, NK, PK	P, KK, PK	NN, KK, NK, PK
NO_3^-	Regression	0.02	0.02	0.01	0.03	<0.01	0.03	0.03	<0.01	0.08
	LOF	0.67	0.44	0.82	0.56	0.87	0.56	0.56	0.68	0.29
	Adjusted R^2	0.67	0.44	0.82	0.56	0.87	0.56	0.56	0.68	0.81
		K, NN, PP, KK, PK	PP, NK, PK	NN, KK, NK, PK	N, NN, KK, NK	N, P, NN, KK	KK, PK	P, PP, PK	N, P, K, PP, KK	KK, NK

Table 2.7 Canonical analyses performed for each response surface model

	Responses	Stationary points in real units (kg ha^{-1})			Y_S (kg ha^{-1})	Nature of stationary point
		N	P_2O_5	K_2O		
NH_4^+	1st leachate	46	262	41	47	Saddle
	2nd leachate	31	110	31	8	Saddle
	3rd leachate	31	111	33	9	Saddle
	4th leachate	27	104	31	11	Minimum
	5th leachate	29	112	30	8	Minimum
	6th leachate	27	120	31	10	Saddle
	7th leachate	38	162	22	9	Minimum
	8th leachate	32	103	32	8	Saddle
	9th leachate	31	107	32	2	Saddle
NO_3^-	1st leachate	30	105	34	44	Maximum
	2nd leachate	34	117	33	21	Saddle
	3rd leachate	30	104	31	22	Saddle
	4th leachate	27	106	30	26	Maximum
	5th leachate	26	82	31	17	Saddle
	6th leachate	25	111	29	17	Saddle
	7th leachate	28	113	35	18	Saddle
	8th leachate	39	113	33	21	Saddle
	9th leachate	29	104	30	19	Maximum

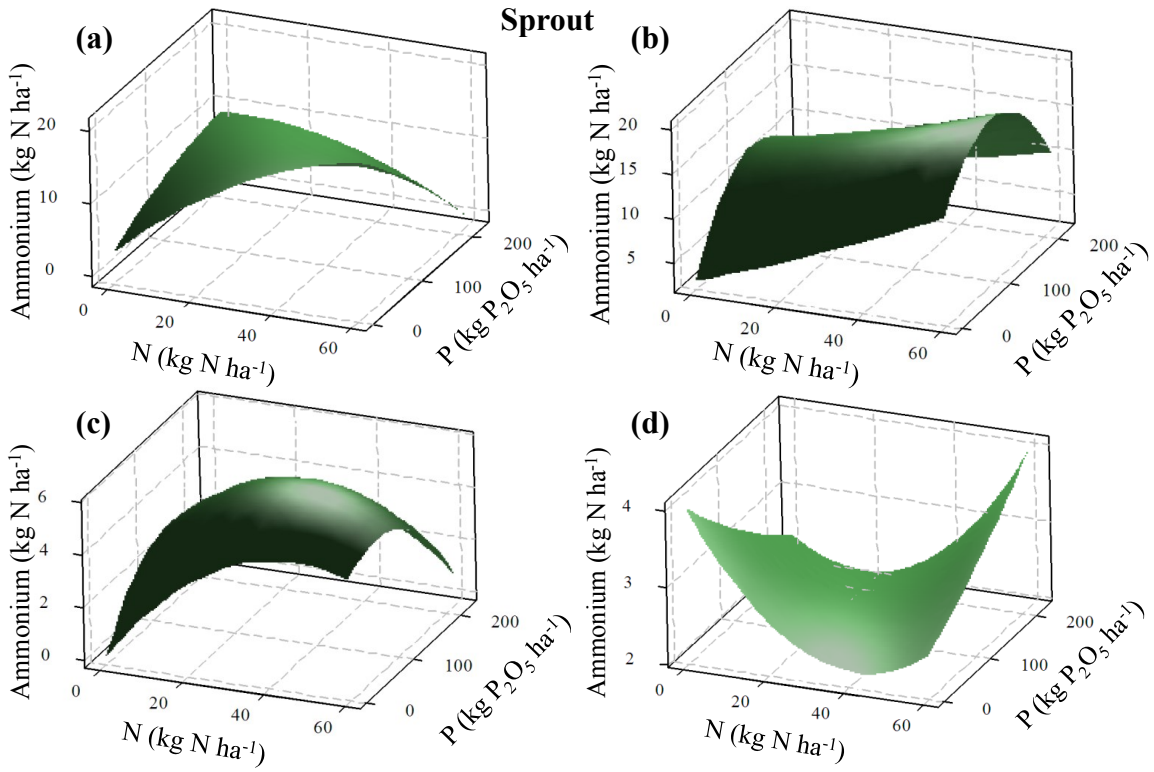


Figure 2.1 Response surface of ammonium in the sprout year of production on the 10-May (a), 7-July (b), 26-July (c) and 9-September (d) for K_2O = 30 kg ha^{-1}

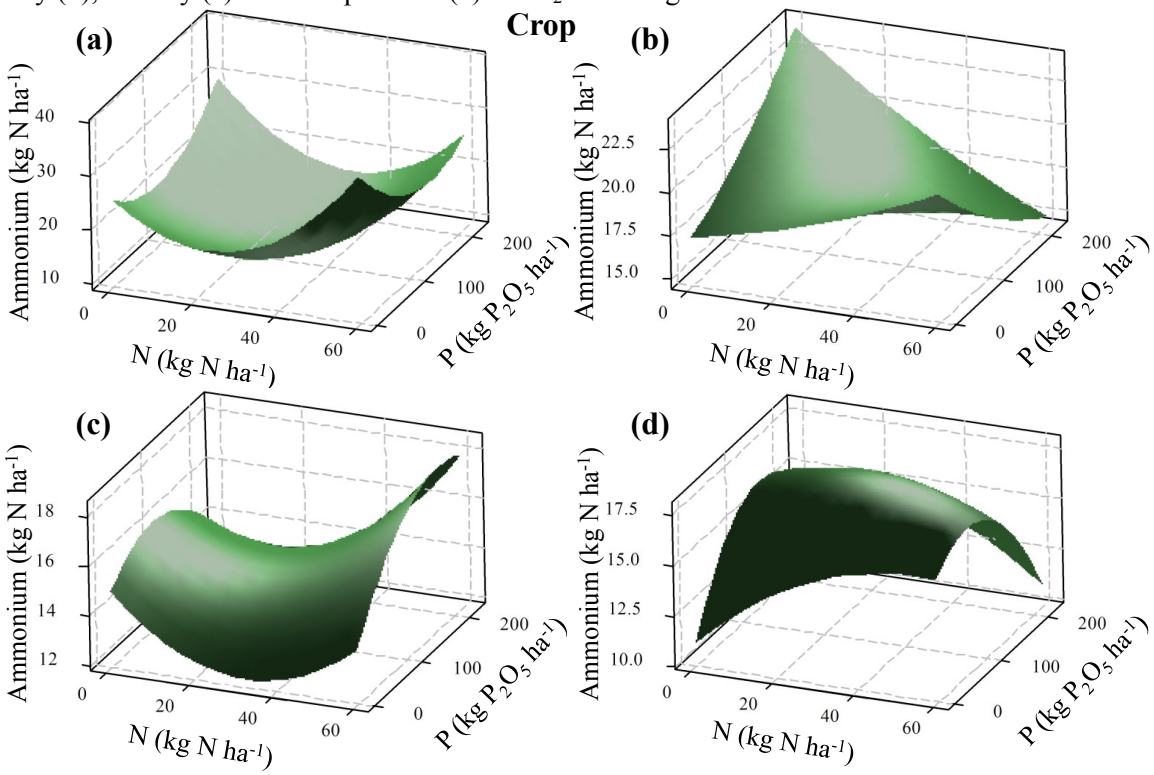


Figure 2.2 Response surface of ammonium in the crop year of production on the 22-May (a), 15-July (b), 31-July (c) and 24-August (d) for K_2O = 30 kg ha^{-1}

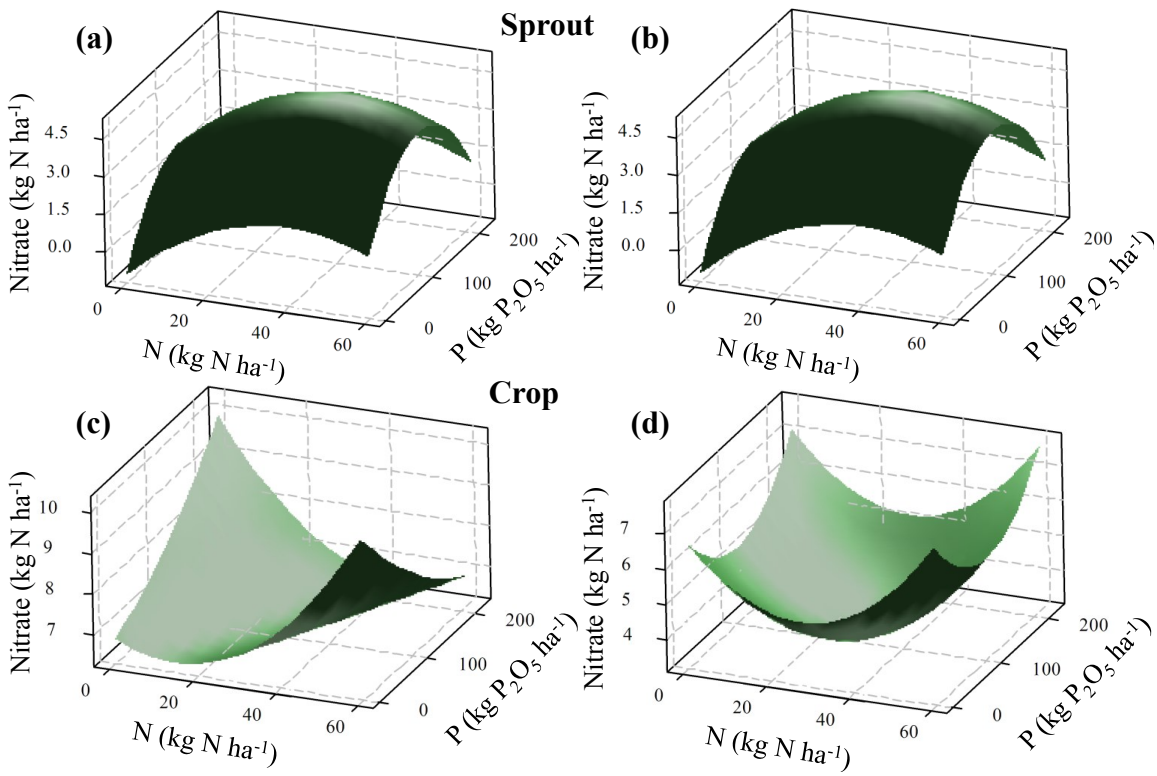


Figure 2.3 Response surface of nitrate in the sprout year of production on the 7-July (a), 26-July (b) and crop year of production on 22-May (c) and 15-July (d) for K_2O = 30 kg ha^{-1}

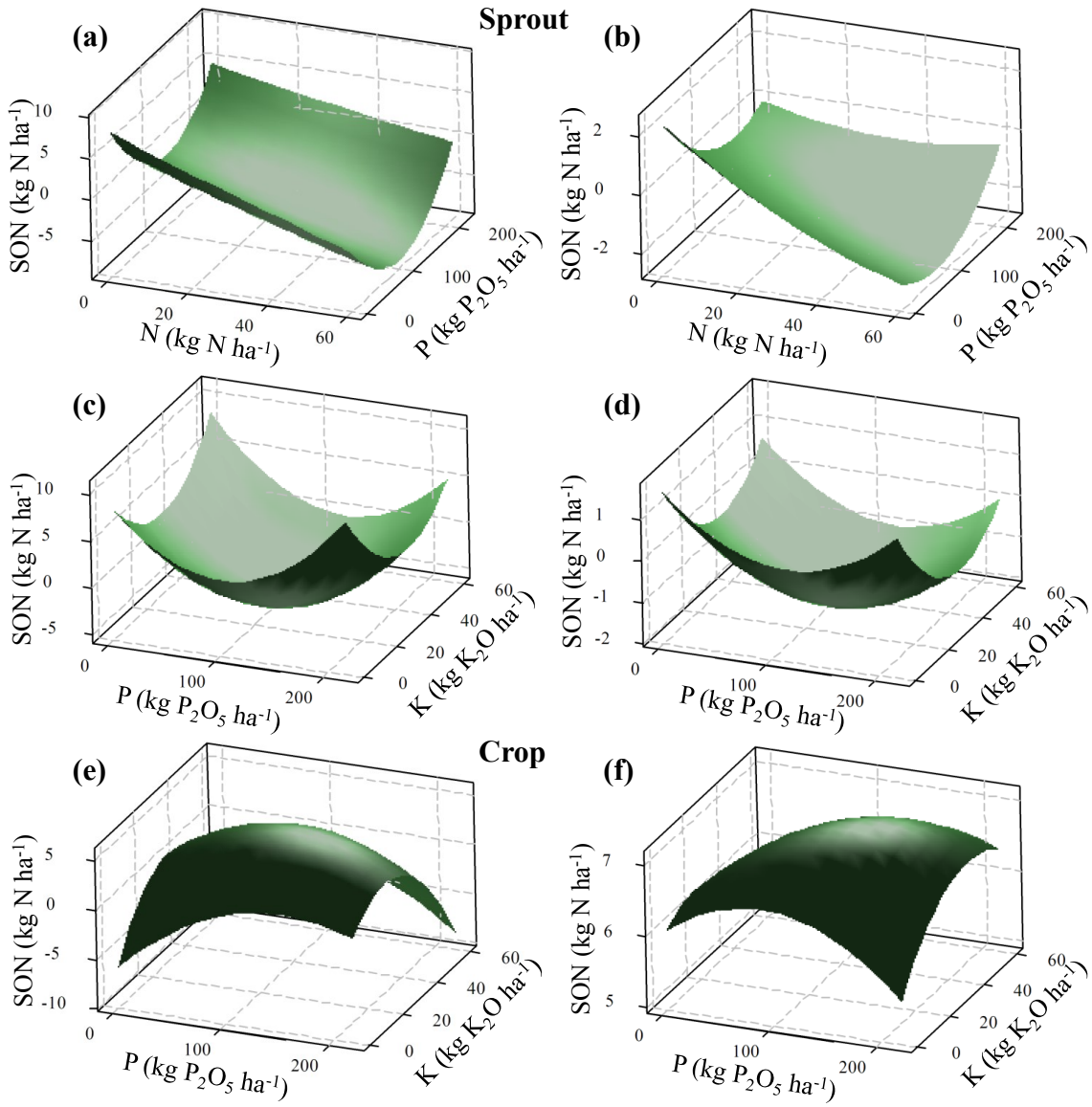


Figure 2.4 Response surface of soluble organic N in the sprout year of production on the 7-July (a), 26-July (b) for K_2O = 30 kg ha^{-1} and 7-July (c), 26-July (d) for N = 30 kg ha^{-1}.Response surface of soluble organic N in the crop year of production on the 22-May (a), 31-July (b) for N = 30 kg ha^{-1}

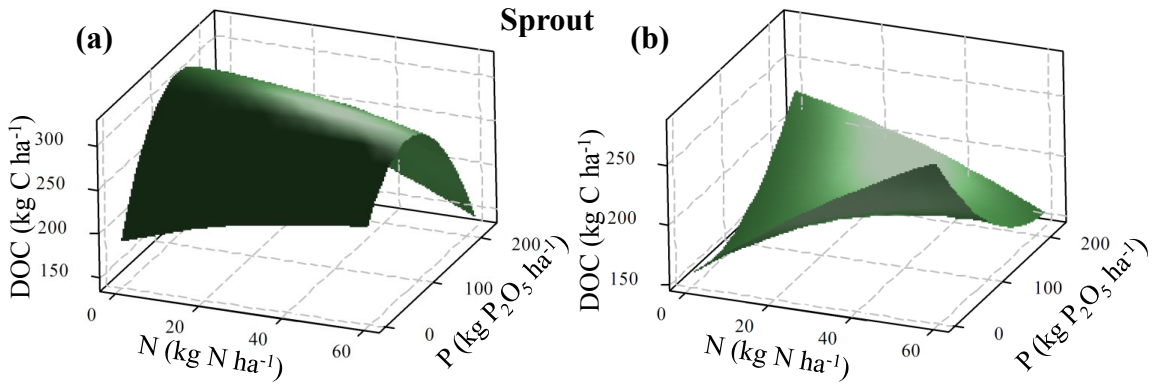


Figure 2.5 Response surface of dissolved organic C in the sprout year of production on the 10-May (a) and 9-Sep (b) for K_2O = 30 kg ha^{-1}

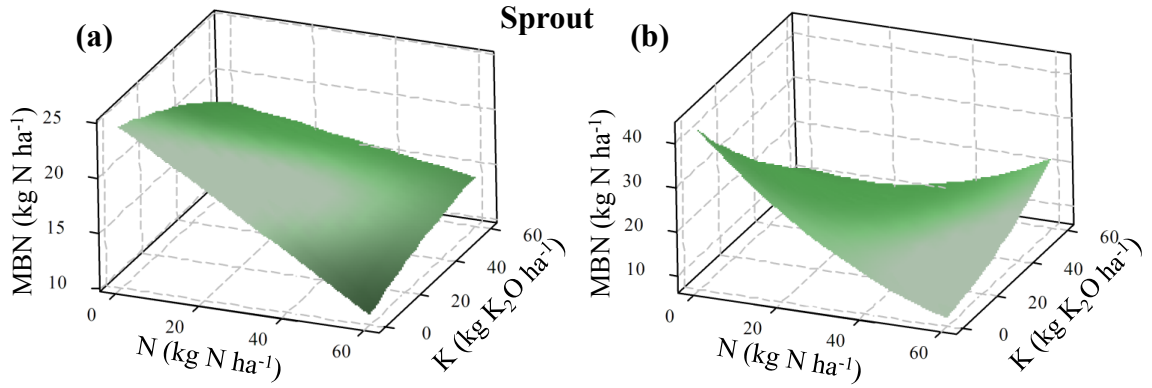


Figure 2.6 Response surface of microbial biomass N in the sprout year of production on the 10-May (a) and 9-Sep (b) for P_2O_5 = 110 kg ha^{-1}

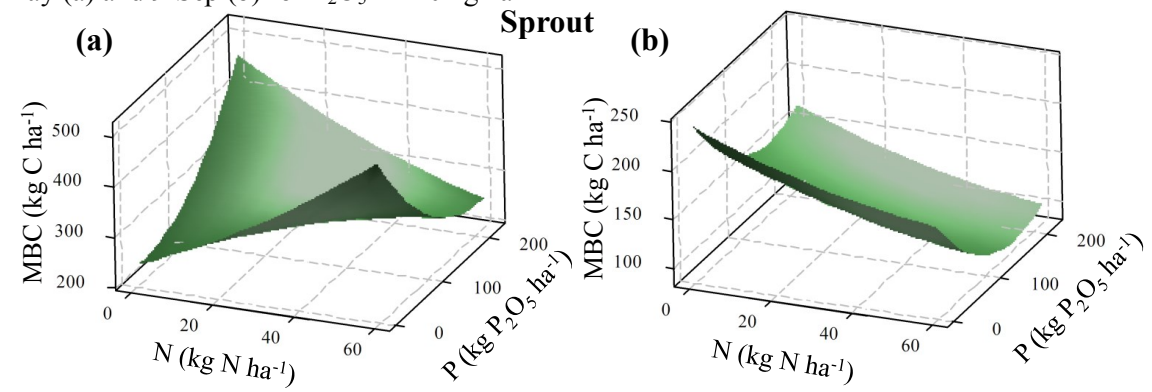


Figure 2.7 Response surface of microbial biomass carbon in the sprout year of production on the 10-May (a) and 9-Sep (b) for K_2O = 30 kg ha^{-1}

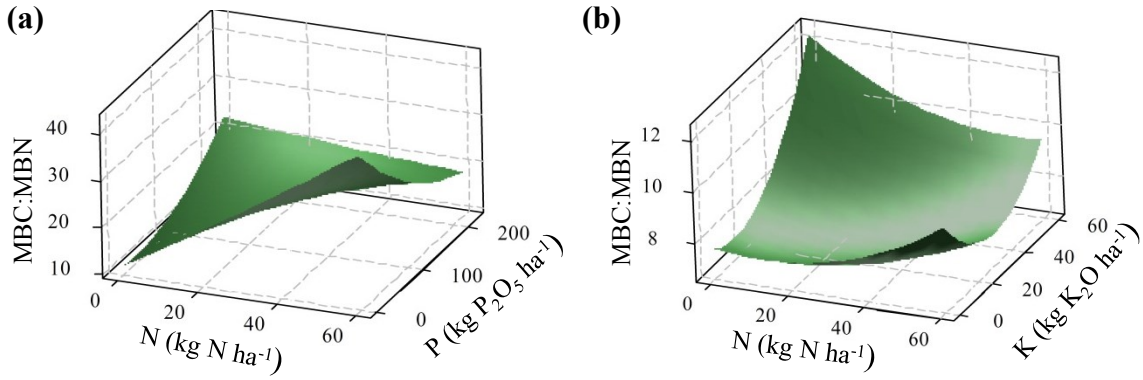


Figure 2.8 Response surface of microbial biomass carbon: microbial biomass nitrogen (MBC:MBN) in the sprout year of production on the 10-May (a) for K_2O = 30 kg ha^{-1} and 9-Sep (b) for P_2O_5 = 110 kg ha^{-1}

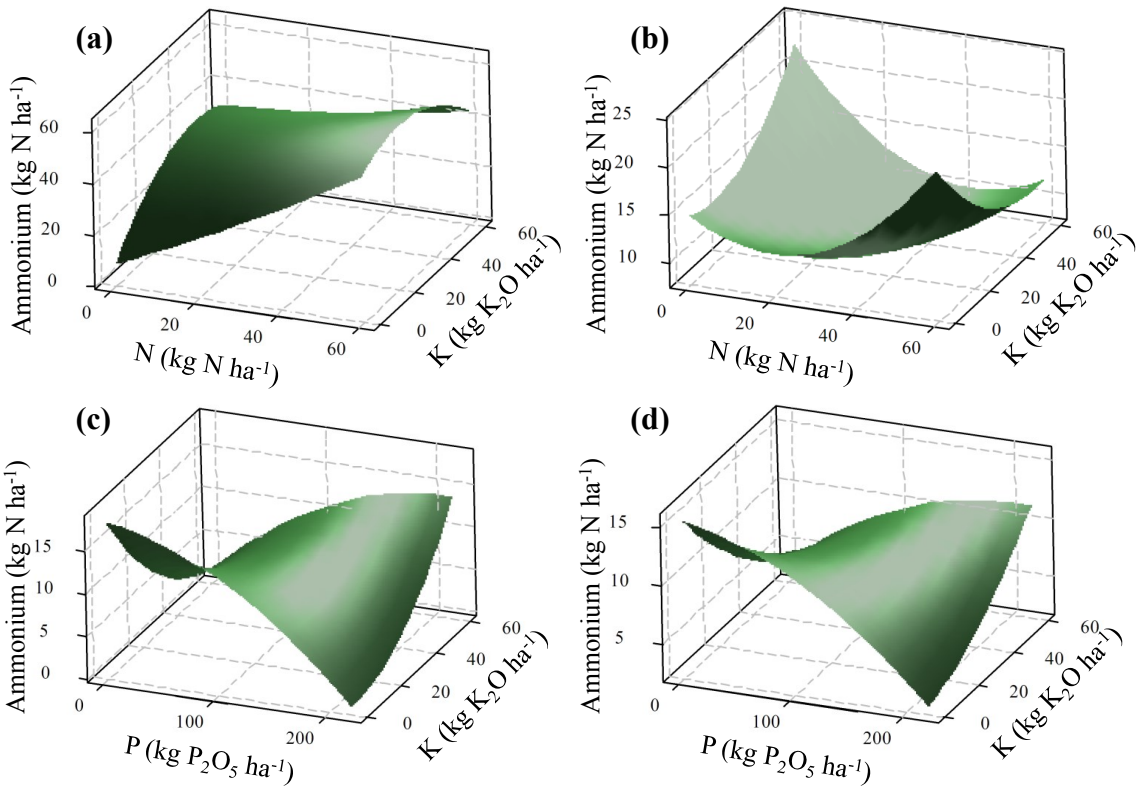


Figure 2.9 Response surface of **net ammonification** in soil by periodic leaching (a) 1st leachate and (b) 2nd leachate for. P_2O_5 fixed at 75 kg ha^{-1}. Response surface of net ammonificationin soil by periodic leaching (c) 2nd leachateand (d) 3rd leachatefor N fixed at 30 kg ha^{-1}

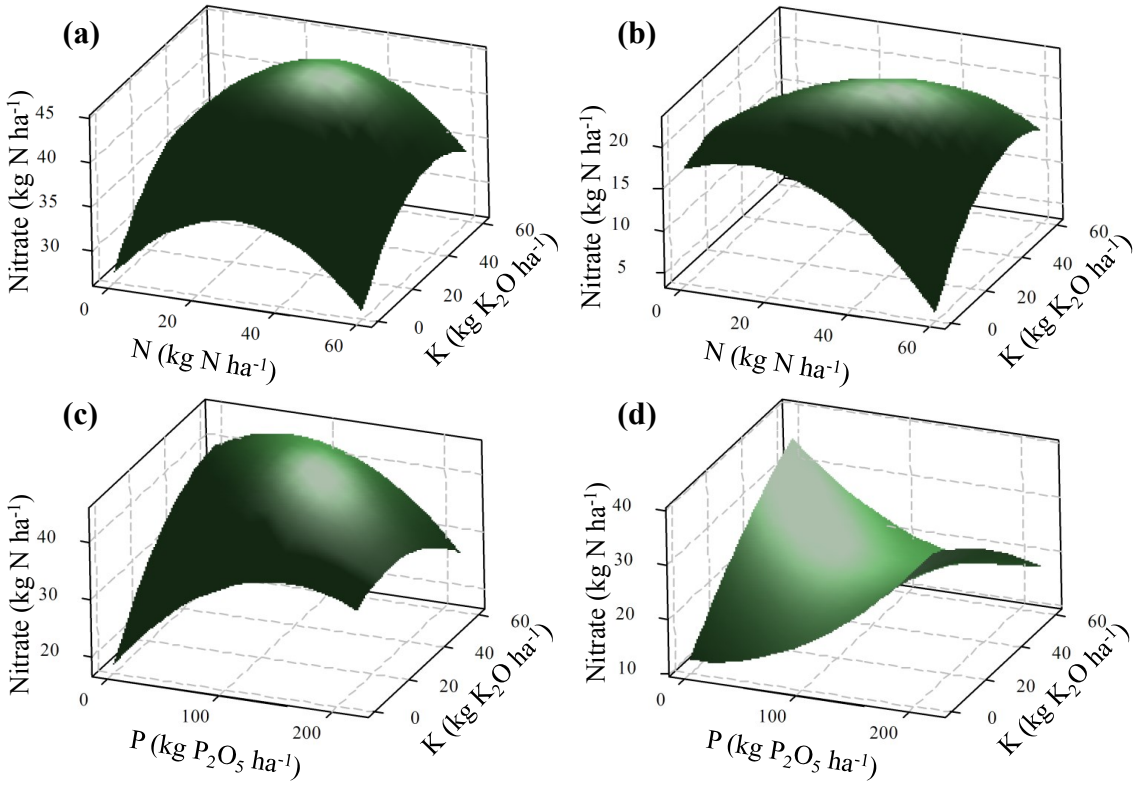


Figure 2.10 Response surface **net nitrification**in soil by periodic leaching (a) 1st leachate and (b) 3rd leachate for P_2O_5 fixed at 75 kg ha^{-1}. Response surface of net nitrification in soil by periodic leaching (c) 1st leachateand (d) 2nd leachate for N fixed at 30 kg ha^{-1}

Chapter 3 Improved Growth and Harvestable Yields Through Optimization of Fertilizer Rates of Soil-Applied Nitrogen, Phosphorus and Potassium in Wild Blueberry (*Vaccinium angustifolium* Ait.)

3.1 Abstract

The study examined the main and interactive effects of soil applied fertilizers (nitrogen, phosphorous, and potassium)and recommends the optimum rate for the improvement in the growth and harvestable yield of wild blueberry (*Vaccinium angustifolium* Ait.) from a 12-yr (6-production cycles) field experiment conducted at Kemptown, Nova Scotia. The experiment was laid out in central composite design. The treatment combinations consisted of five levels of fertilizer nitrogen (0, 12, 30, 48 and 60 kg N ha^{-1}), phosphorous (0, 18, 45, 78 and 90 kg P ha^{-1}), and potassium (0, 12, 30, 48 and 60 kg K ha^{-1}). The responses studied were namely stem length, vegetative nodes, floral nodes, fruit set and harvestable berry yields. Response surface analysis and canonical analysis provided means to determine optimum fertilizers rates and expected change in yield and yield contributing components. We obtained 54%, 25% and 13% more floral buds, fruit set and berry yield while keeping stem length below 20cm (> 20cm considered too tall) with the optimized over the commonly used fertilizer rates (20N, 10P and 15K kg ha^{-1}) at ratio (4:2:3). We recommend 35N, 40P and 30K kg ha^{-1}(7:8:6) at the onset of shoot emergence from rhizomes in early spring of the sprout year of the production. These optimum fertilizer (NPK) rates improved growth, development, berry yields, and economic returns. A finding of this study contribute towards better farm profitability and suggest that modifications to existing fertilizer rates be made for

Central Nova Scotia wild blueberry fields and suggests that it be tried in other areas with similar growing conditions.

3.2 Introduction

The wild blueberry (*Vaccinium angustifolium* Ait.) plant is well adapted to orthic humo-ferric podzols. These soils are typically sandy, acidic (pH 3.9-5.5), highly leached, poorly buffered with well-developed organic horizons. Podzols are not naturally fertile. However, these soils can become quite productive with proper fertilizer applications. In Nova Scotia, growers apply fertilizer in a form of diammonium phosphate or ammonium sulfate in combination with phosphorous and potassium at rate of 20 kg nitrogen (N)10 kg phosphorus (P) and 15 kg potassium (K) ha^{-1}at the onset of shoot emergence from rhizomes in the sprout year of the production cycle. Fertilized fields are treated with selective herbicides in order to realize full fertilizer benefits by controlling competing vegetation.

Nutrient management is complex and dynamic in wild blueberry fields. Although fertilizer applications generally improve growth and yield potential in wild blueberry fields, many indigenous factors and human-induced management practices can also affect their soil and plant environment. These factors cause significant temporal and spatial variations in harvestable yields. These factors include a fungal dominated soil environment, mutualistic fungi, acidic soil, organic matter rich soil, slow nutrient cycling under natural settings, a slow growing plant, an extensive underground rhizome system, inherent phenotypic variability, no till system, non-irrigated (generally), deposition of organic plant material from mowing every other year, repeated fertilizer enrichments, nitrate production, potential soil N saturation, high organic N forms (Chapter 2), uptake of organic N (Chapter 2) and physiological disturbances and imbalances in soil nutrient availability under N rich soil environment (Nilsson and Wiklund 1995). Hence, soil and plant nutrient status under repeated fertilizer enrichments

warrants a revisiting of the fertilizer application rates, the examination of their main and interactive effects on growth responses and the possible development of new fertilizer recommendations.

Wild blueberry is a slow growing perennial calcifuge (i.e. acid-loving), spread by underground stems called rhizomes. The extensive root and rhizome system occurs within the top 10-cm of soil and accounts for 75-85% of the total plant dry weight (Jeliazkova and Percival 2003). The rhizomes serve as a reservoir for nitrogenous compounds as well as carbohydrates and some inorganic constituents particularly N, P and magnesium (Mg) (Townsend et al. 1968). The numerous fine 'root hairs' are heavily colonized by indigenous ericoid mycorrhizae fungi. The ericoid mycorrhizae fungi assist nutrient uptake notably N and P (Korcak 1988). In addition, ericoid mycorrhizae fungi also assist with the acquisition of nutrients from organic sources that are normally unavailable to host plant roots. The plants of the Ericaceae family have been known to uptake organic N forms directly through roots. In the present project, indirect evidence has been presented that wild blueberry consumes organic N forms as part of its nutrition demand (Chapter 2).

Wild blueberry nutrient management varies considerably compared to typical tilled crop systems. Berries are removed from the fields every two years (cropping cycle) while extensive plant debris deposition to fields occurs in every production cycle in the form of leaf drop in fall and every two years in the form of pruning when the plants are mowed after harvest. As a result, wild blueberry soils contain as high as 10% organic matter (Kinsman 1993). The wild blueberry fields have a fungal dominated soil system that promotes a slow cycling of nutrients and a low availability of nutrients. Nutrients tied in the organic matter are slowly available to plants through mineralization and nitrification is considered curtailed under low pH conditions, which is typical of wild blueberry soils (average pH = 4.7).

Therefore, ammonium is the dominant form of N present in wild blueberry soils and P may not be readily available to plants due to the soils high acidity. Significant temporal and spatial harvestable yield variations occur in wild blueberry that have been attributed to several factors, including intra-field soil variability (Farooque 2012), plant-related variability (genetic, coverage and other vegetation) (Hepler and Yarborough 1991), pollinator diversity and intensity (Eaton and Nams 2012), temporal plant and soil nutrient status (Chapter 2). Additionally, the majority of the industry does not use irrigation so it is reliant upon rainfall and subsequently may be occasionally (1-3 years in a decade) exposed to drought which may significantly reduce berry yields by affecting floral bud development, berry weight, mineralization rates and fertilizer response. The dynamic nature of interactions among plant and soil factors results in tremendous amount of uncertainty in wild blueberry nutrient management (Percival and Sanderson 2004).

Past nutrient management research has provided valuable information on nutrient dynamics (Trevett et al. 1968a; Trevett et al. 1968b), optimum leaf tissue nutrient levels (Trevett 1972), the influence of soil pH (Hall et al. 1964), nitrification potential and inorganic nitrogen levels (Eaton and Patriquin 1988), N formulation (Percival and Privé 2002), P fertilizer (Eaton et al. 1997; Smagula and Dunham 1995), and NPK fertilization (Penney and MacRae 2000; Percival et al. 2003). The form of N in plant nutrition is of special interest for wild blueberry growth and development. Previous research demonstrated that improved blueberry growth occurs with the ammonium form of N nutrition (Townsend 1966). Plant growth and development (shoot number, and fruit development) can be about double with ammonium than with nitrate N (Cain 1952; Townsend 1969). Studies have even reported toxic effects of nitrate N on blueberries (Cain 1952).

Fertilization generally promotes floral nodes, fruit set and berry yield (Percival and Sanderson 2004). For example, N application (43 kg ha^{-1}) in the form of urea produced 22% more flower buds $stem^{-1}$ and 25% yield over on unfertilized control (Smagula and Hepler 1978).However, an excess of N applied in the sprout year may reduce yields by promoting vegetative growth, increasing weed growth, causing micronutrient imbalances, increasing susceptibility to winter injury (excessively tall stems), or stimulating an over- production of flower buds relative to the nutrient budget in the crop year (Benoit et al. 1984;Penney and McRae 2000; Smagula 1999; Yarborough et al. 1986;). Yet, in Maine, high N application rates (19-78 kg ha^{-1}) increased stem length, total berry number and yields (D. Percival, personal communication). Therefore, the impact of N applications upon berry yield have been inconsistent, with studies reporting yield gains (Ismail et al. 1981; Percival et al. 2003;Smagula and Hepler 1978), yield reductions (Smagula and Ismail 1981;Penney and McRae 2000), or no effect (Benoit et al. 1984; Blatt 1993).

Variable responses in soil applied P and K have also been reported. Phosphorus can either significantly increase berry yields (Smagula and Dunham 1995) or have no effect on yield potential (buds $stem^{-1}$; Eaton et al. 1997). Potassium was found to increase yield and berry size up to 40 kg of K ha^{-1} with no additional response occurring at higher rates (Eck 1983). Percival and Sanderson (2004) found significant effects of soil-applied N and K on fruit set despite large levels of inherent phenotypic variability. They also reported that the harvestable yields of the unfertilized treatments were as much as 36% lower than the other soil-applied NPK treatments. One limitation from the previous studies was that fertilizer treatments were not varied across the entire range and mostly one or two nutrients were studied thus ignoring the full spectrum of interactions when applying nutrients from deficiency to over saturation levels.

We chose to use response surface methodology (RSM) and canonical analysis as aids in modeling and examining the relationships between fertilizer rates and plant responses. The central composite design (CCD), the most efficient design for response surface analysis, considers several factors simultaneously (Myers et al. 2009). CCD also allows the determination of the interactions among factors using a smaller number of experiments. This methodology has been used by others to describe the effects of fertilization on plant growth. For example, Lippke et al. (2006) evaluated soil applied fertilizers (N and P) on annual ryegrass (*Lolium multiflorum* Lam.). The fitted response surface models provided optimum N and P levels for maximum dry matter yields. Sanchez (2000) used response surface methods with quadratic models to examine the effect of water and nitrogen on lettuce (*Lactuca sativa* L.). In this study, it was used to determine the optimum levels of soil applied fertilizers (NPK) that could maximize yield and potential yield factors.

The first objective of this research was to determine the main and interactive effects of soil applied fertilizers (NPK) on wild blueberry growth, development and berry yields while the second objective was to recommend fertilizer rates that optimize these same factors. The study provides the much needed information on fertilizer (NPK) requirement and nutrition management of wild blueberry crop.

3.3 Materials and Methods

1.1.1 Experimental Design, Field Experiment and Data Collection

A three factor central composite design (CCD) (Myers et al. 2009) was used to study the response surfaces. The three factors were: nitrogen (kg N ha^{-1}), phosphorus (kg P ha^{-1}) and potassium (kg Kha^{-1}), and the levels for the CCD are provided in Table 3.1. Within each year, all design settings were replicated four times.

The field experiment was established in spring 2000 on a commercial field (N 45°30' 7.91", W -63° 7'27.72", elevation ~223 m) in Kemptown, Nova Scotia, Canada. The soil parent material was mixture of igneous and metamorphic rocks. The soil was included in the Cobequid soils association, classified either as gleyed sombric ferro-humic or gleyed humo-ferric podzol (Agriculture Canada 1991). The soil pH was 4.3 ± 0.1 SD (0- to 15-cm depth). The soil organic matter was 10.2 % ± 1.4 SD (0 to 15 cm depth). The soil contained 575 g kg^{-1} sand, 84 g kg^{-1}silt, and 341 g kg^{-1} clay. The land was very stony and moderately rocky.

The N was applied in the form of ammonium sulfate, P in the form of triple super phosphate and K in the form of chloride of potassium. The fertilizers were applied at the onset of shoot emergence from rhizomes in early spring of the sprout year of the production. Fertilizer treatment combinations were applied using a Scott SR2000 rotary fertilizer spreader (Marysville, Ohio). This site was continuously managed under commercial industry standards for Nova Scotia (D. C. Percival, personal communication).

Data were collected between 2000and 2011. Stems were collected in July of the crop year of production using a 7.5 m long rope was extended diagonally in each plot and marked at fifteen equally spaced points. One stem was randomly collected at each mark. Stem samples were stored in plastic bags in a cooler and transported to the laboratory. Stem length (cm) was measured from ground level to the apical bud. In case of branching, the longest distance was recorded. Vegetative nodes, floral nodes and fruit set were counted both on main stems, including those of any branches. Stem length, vegetative nodes, floral nodes were determined for each collected stem (15 stems per plot) and averaged to get a single value for each plot. Harvests occurred in mid-to-end August. Berries were harvested with a forty-tine commercial wild blueberry hand rake from four, randomly selected 1 m^2 quadrants

in each plot (Kinsman 1993; Percival and Sanderson 2004).Harvested berry yields were recorded using a digital balance (Mettler PE 6000, Burlington, ON).

3.3.1 Statistical Analysis

In order to improve the precision of parameter estimations, the averages of the four replications were used, except the center point, which the CCD calls for the replications to allow lack of fit tests (Myers et al. 2009).The data from all years were analyzed together using Year as a blocking factor to account for year to year variability. Minitab 16 software (Minitab Inc. State College, PA) was used for response surface analyses of the data. The lack of fit test measures the adequacy of the quadratic response surface model. We tested models for lack of fit test to make sure there is no lack of second order model and proceeded to surface plots and canonical analysis to pin point the optimum settings (Myers et al. 2009). Three dimensional (3D) surface plots were drawn to illustrate the interactive effects of the factors on the response variables. The optimum settings of the factors were obtained by completing canonical analysis (Myers et al. 2009).

The second-order response surface model (fitted) in matrix notion as

$$\hat{y} = b_0 + x^T b + x^T \hat{B} X \qquad (3.1)$$

where, b_0, b and $\hat{B}$ are the estimates of the intercept, linear, and second-order coefficients, respectively. The location of stationary point X_S, $\hat{y}$ is differentiated in equation 1 with respect to x and obtain

$$\frac{\partial \hat{y}}{\partial x} = b + 2\hat{B}x \qquad (3.2)$$

Setting the derivative equal to 0, solve for the stationary point of system:

$$X_S = -\frac{1}{2}\hat{B}^{-1}b \qquad (3.3)$$

The nature of the stationary point is defined by the eigen values λ of the matrix B and by solving the following equation:

$$\det(\hat{B} - \lambda E) = 0 \qquad (3.4)$$

where λ is also known as canonical coefficients and E is the identity matrix.

Matlab 7.10 (The Mathworks, Inc. Natick, MA) was used to complete canonical analysis of the data.

3.4 Results and Discussion

The analysis of variance (ANOVA) *P*-values for all responses are provided (Table 3.2). For all responses, the lack of fit test results were not significant, suggesting that the quadratic model adequately fit for all responses and the design settings are in the neighborhood of the optimum setting. The regression models for stem length, vegetative and floral nodes, fruit set and berry yield were all significant ($P< 0.05$) (Table 3.2).

3.4.1 Stem Length

The goal was to devise a fertilizer dose that maintained a minimum stem length (< 20 cm) without negatively affecting yield potentials. The optimal value of 17.8cmfor stem length was obtained with fertilizer rates of 50N, 48P and 30K kg ha^{-1}, respectively (Figure3.1 and Table 3.3). The stem length (17.8 cm) is not excessively high considering high rates of N (50 kg N ha^{-1}), this may be the effect (regulation of N uptake and/or timely transition to reproductive phase) of complete fertilization (NPK), or genetic potential of the clones at our

experimental site. The P has been known to counteracting the adverse effects (excessive vegetative growth) of over applications of N. The rapid flush of growth in corn has been attributed to excessive N that is out of balance with K. Percival and Sanderson (2004) reported maximum of 17.1cm stem length under similar NPK fertilization from same site. The fertilizer N rate that correlated best for stem length in this study was higher than those typically observed in the highly productive fields of Nova Scotia under favorable conditions (D. C. Percival, personal communication). The soil applied N (0-60 kg ha^{-1}) and P (0-50 kg ha^{-1}) both significantly increased stem length (Figure3.1a and Figure 3.1b). However, higher P rates (> 65 kg P ha^{-1}) reduced stem length (Figure3.1a and Figure 3.1b). The P x K interaction was significant (Table 3.2) as depicted by the saddle nature of the 3D surface plot (Figure3.1b). Increased stem length by fertilization (N or NP) has been consistently reported in earlier studies (e.g. Smagula and Dunham 1995; Percival and Sanderson 2004) but interestingly, stems never increased above the danger zone of >20 cm in this study. Excessively taller stems may (> 20 cm) may lodge and hinder mechanical harvesting, therefore can result in substantial yield losses.

3.4.2 Vegetative Nodes

Vegetative nodes increased with as function of increased N and P rates (Figure3.2). The increase in vegetative nodes in response to fertilization is consistent with earlier studies (e.g. Jeliazkova and Percival 2003). The K had no significant effect on vegetative nodes (Table 3.2). The increase in vegetative nodes can produce a higher number of side branching and this has been reported to negatively affect berry yields (DeGomez 1988). The goal was to maximize the reproductive output while limiting the excessive vegetative growth. The maximum number of vegetative nodes per stem occurred at fertilizer rates of 49N, 48P and 65K kg ha^{-1} (Table 3.3). The NPK rates for maximum vegetative nodes were higher than

required to maximize yield components (floral nodes, fruit set) with K even exceeding the experimental range. The higher fertilizer rates for maximum number of vegetative nodes per stem are required than to maximize yield components serve the final goal of limiting the vegetative nodes and simultaneously increasing the yield contributing factors. The high N rates gave the best stem length (50 kg N ha^{-1}) and vegetative nodes (49 kg N ha^{-1}) but N rates (30-40 kg N ha^{-1}) at a much lower levels provided the best response in reproductive variables (Table 3.3).

Phosphorus rate had convex quadratic effect on vegetative nodes (Table 3.2 and Figure3.2). Under acidic soil conditions (average 4.7 in wild blueberry fields), optimal phosphorus is required for leaf expansion, leaf surface area and number of leaves during vegetative growth (Marschner 1995). Excessively higher concentration may also cause P toxicity. Thus, optimal P supply through fertilization is critical in wild blueberry. P is not readily available because it forms insoluble compounds and may become unavailable for plant uptake. Eaton et al. (1997) reported that as much as 31% of applied P accumulated within the organic horizon. Slow P turnover has been reported in short term incubation study along with immobilization of P from soluble to insoluble forms in wild blueberry soil system (English 2007).

3.4.3 Floral Nodes

Floral nodes had significant convex quadratic response to N-P-K (Table 3.2; Figure3.3a and Figure 3.3b). The maximum number of floral nodes $stem^{-1}$ was found with fertilizer rates of 34N, 45P and 30K kg ha^{-1}, respectively. The 3.7 nodes $stem^{-1}$ that was obtained at current fertilizer recommendations (20N-10P-15K kg ha^{-1}) increased to 5.7 nodes $stem^{-1}$ at our optimum levels (Table 3.3). Thus, 54% more floral nodes$stem^{-1}$were obtained by switching with these new fertilizer rates. The response surface plots also depicted similar

optimal values, for other fertilizer associations (Figure3.3b). A similar fertilizer response on floral nodes has been reported in previous studies (e.g. Smagula and Dunham 1995; Percival and Sanderson 2004) although at lower soil P rates. Our results confirm that high levels of soil phosphorus may retard the formation of reproductive organs (Marschner 1995) while inadequate levels can delay flower initiation (Rossiter 1978) and decrease the number of flowers (Bould and Parfitt 1973).

3.4.4 Fruit Set

Fruit set increased linearly with N rate as it increased from 0 to 40 kg ha^{-1} (Figure3.4a). A significant negative P x K interaction was found for fruit set (Table 3.2). At low P rates (0-20 kg ha^{-1}) fruit set increased when K was increased linearly (Figure3.4b), however at high P levels (> 60 kg ha^{-1}), fruit set decreased with increasing K fertilization (Figure3.4b). The optimal fruit set (11.9 berries $stem^{-1}$) was obtained at N, P and K rates of 40, 38 and 33 kg ha^{-1}, respectively. Fruit set was 25% greater with these optimal levels as compared to the current fertilizer recommendations (20N-10P-15K kg ha^{-1}). Glass et al. (2005) reported the most significant positive correlation (R=0.82) in her analysis which resulted from the relationship between floral nodes and fruit set. This relationship is evident from floral nodes and fruit set plots (Figure3.3a and Figure3.4a) where optimal fertilizer rates were very similar for these two yield components.

3.4.5 Berry Yield

The rates of NPK calculated for optimal berry yield were 30, 45 and 32kg ha^{-1}, respectively (Table 3.3). The berry yield at optimum fertilizer levels was 4126 kg ha^{-1} which is 14% higher compared to the commonly used industry standard (20N-10P-15K kg ha^{-1}). This maximum yield of 4126 kg ha^{-1} was greater than values reported from commercial fields in Nova Scotia over the past 12 years (D. C. Percival, personal communication). However,

the increase in berry yield is not as high as those found for floral nodes (54%) and fruit set (25%). The smaller increase in berry yield may be attributed to uncontrollable factors such as climatic (temperature, precipitation, wind, etc.), inadequate bee population and pollination (Eaton and Nams 2012), disease pressures or controllable factors such as poor harvesting techniques (Kinsman 1993).

N was the most important fertilizer increasing berry yield (Figure3.5a and Figure 3.5b) and agrees with previous reports (Percival and Sanderson 2004).A significant negative N x K interaction was depicted by 3D surface plots (Figure3.5a) whereat low N rates (0-20 kg N ha^{-1}) berry yield increased linearly with K (0-60 kg ha^{-1}) where as it decreased with medium to high N rates (>30 kg N ha^{-1}; Figure3.5a). The negative N x K interaction may be explained by soil ammonium (NH_4^+) and K^+ competition for exchange sites. NH_4^+ and K^+ have the same affinity for exchange sites and also can remove each other from soil colloid surfaces when high concentrations are present, such as when fertilizers are applied (Pugh 2008). The high rate of K fertilizer (> 40 kg K ha^{-1}) may have increased concentration of K^+ ions in the soil solution and displaced NH_4^+ ions from exchange sites. The increased NH_4^+ availability at high K fertilizer rates (>40 kg K ha^{-1}) in soil solution and subsequent increased plant uptake, may have induced imbalance in the plant nutrients (N and K). This imbalance may have delayed the transition from vegetative to reproductive phase in the plant. A prolonged vegetative phase (late transition to reproductive phase) may cause a reduction in the development of floral buds and thus their resulting berry yields. However, application of K may be necessary to avoid flush of growth when N is applied without K, stem strength to avoid lodging and to guard plant against fungal pathogens.

Considering the significant quadratic (convex) effect of soil applied P on floral nodes and positive P and K interaction effect on fruit set (Table 3.2; Figure3.5a; Figure3.4b), the

non-significant P effect on berry yield was somewhat surprising. Previous studies have both reported significantly enhanced yield of wild blueberry from increased P (Litten et al. 1997; Smagula and Dunham 1995) while others report no effect (Sanderson and Eaton 2008). Percival and Sanderson (2004) noted a reduction in stem density to soil applied P and this may explain the lack of linear significant yield increase. Additionally, N has been reported to be an active diluter of P in wild blueberries since continued use of N applications may decrease leaf P content and induce an imbalance and/or deficiency (Trevett et al.1968a). The P deficiency may also cause reduction in berry yields (Figure3.5b). Fertilizer P application may be critical to keep foliar P levels in optimal range required for optimal reproductive organ growth without direct berry yield gains. This study reported the effect of NPK in sprout year of production. Further potential increases in harvestable yields may be tested in future studies by splitting fertilizer doses between sprouting and cropping years. Percival et al. (2003) showed that multiple fertilizer applications can improve the nutrient status, growth, and harvestable yields of wild blueberries. Multiple fertilizer applications (initial fertilizer dose of 28N-12P-23K kg ha^{-1} in sprout year + 10 kg N ha^{-1} was added prior to bloom of the crop year) increase berry yields by 51% over single application of initial fertilizer dose of 28N-12P-23K kg ha^{-1} in the sprout year.

Wild blueberry fields possess huge organic nutrient reserves in the form of organic matter (~ 10%) in Nova Scotia. The role of organic nutrients in plant nutrition especially when wild blueberries have been known to take up organic forms directly (short circuiting of nutrients) by aid of ericoid mycorrhizal fungi, large mineralization potentials of organic to inorganic forms (ammonium and nitrate) which results in the total amount to nitrogen applied (~35 kg ha^{-1}) to be quite small compared to what is potentially available in the soil system, the carry-over/ residual supply of nutrients within plant system (rhizomes), the minimal

removal of nutrients by harvesting the berries, and the small nutrient requirements of the plant combined with narrow optimal window of synthetic fertilizers application. In this whole blueberry nutrition picture, the tailoring of soil applied nutrients to crop demand by simultaneously optimization the growth and yield of wild blueberry is discussed in the next section.

3.4.6 Simultaneous Optimization of Growth and Berry Yield

The optimum settings to optimize stem length, floral nodes, fruit set and berry yield was obtained by overlaying the plots and looking at the Sweet Spots (Myers et al. 2009). The primary objective of this study was to maximize reproductive output while avoiding excessive vegetative growth. This was achieved by maximizing the floral nodes, fruit set and berry yield while maintaining a reasonable stem length (<20 cm). This contour plot overlaid the response surface models for our four variables (stem length, floral nodes, fruit set and berry yield) over all the ranges of applied fertilizers (Figure3.6). The sweet spot (white area) was the area where all responses were optimized (Figure3. 6). The optimal range obtained from the overlay plot was found to be between 30-40(kg N ha^{-1}), 25-60 (kg P ha^{-1}), and 20-30 (kg K ha^{-1}). The graphically derived values (sweet spot) matched closely to the numerically derived value through canonical analysis (Table 3.3).The balanced fertilization (NPK) may have synergistically enhanced uptake of nutrient and plant metabolic functions; therefore, it would reduce gaseous losses, leaching and runoff of soil nutrients and improving overall health and sustainability of wild blueberry stands.

3.5 Conclusions

This study confirms previous research showing significant main and interactive effects of soil applied fertilizers (NPK) on growth, yield components and final berry yields. In wild blueberry production systems, there are significant year to year variations in soil nutrient supplies, nutrient reserves within the rhizome system, ranges in abiotic stresses, pollinator densities, weed and disease pressures that may affect the final yield of wild blueberries. Data used in the RSM analysis were collected from 6-production cycles (12-years) and effectively accounted for the year to year variations typically found in wild blueberry fields.

Our results demonstrated beneficial effects of soil applied fertilizers (NPK) on yield components and berry yields. The new fertilizer rates of 35N, 40P and 30K kg ha^{-1}(7:8:6), when applied in spring of sprout year, increased floral bud formation by 54%, fruit set by 25% and berry yield by 13% while keeping stem length below the danger zone (< 20cm). The simultaneous growth and optimization and adjustments of soil applied fertilizer doses may increase economic returns, reduced likelihood of environmental concerns such as leaching and erosion of nutrients, thus improving the overall sustainability of the wild blueberry production system.

Table 3.1 Experimental range and levels of the independent variables

	Range and levels				
Original factors	-1.68	-1	0	1	1.68
[z]N (kg ha^{-1}): X_1	0	12	30	48	60
P (kg ha^{-1}): X_2	0	18	45	72	90
K (kg ha^{-1}): X_3	0	12	30	48	60

[z]The source of soil applied N was ammonium sulfate, P was triple superphosphate and K was potassium chloride

Table 3.2 Analysis of variance (ANOVA), *P*-values of the central composite design for stem length, vegetative nodes, floral nodes and berry yield

Source of variation	Degrees of freedom	Stem length	Vegetative nodes	Floral nodes	Fruit set	Berry yield
Blocks ([z]years)	5	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Regression	9	< 0.01	0.05	<0.01	0.02	<0.01
Lack of fit	75	0.88	0.89	0.56	0.99	0.99
Adjusted R^2		0.78	0.88	0.89	0.94	0.89
Significant effects		N, P, PK	N, PP	N, NN, PP, KK	N, PK	N, NK

[z]Years when wild blueberry crop was harvested in alternate years following two-year production cycle (2001, 2003, 2005, 2007, 2009 and 2011). Years was used as blocking factor to account for year to year variability

Table 3.3 Canonical analysis performed for each response surface model

Responses (units)	Stationary points in real units (kg ha^{-1})			Y_S	Nature of stationary point
	N	P	K		
Stem length (cm)	50	48	30	17.8	Saddle
Vegetative nodes (nodes stem^{-1})	49	48	65	16.9	Maximum
Floral nodes (nodes stem^{-1})	34	45	30	5.7	Maximum
Fruit set (berries stem^{-1})	40	38	33	11.9	Saddle
Berry yield (kg ha^{-1})	30	45	32	4126	Saddle

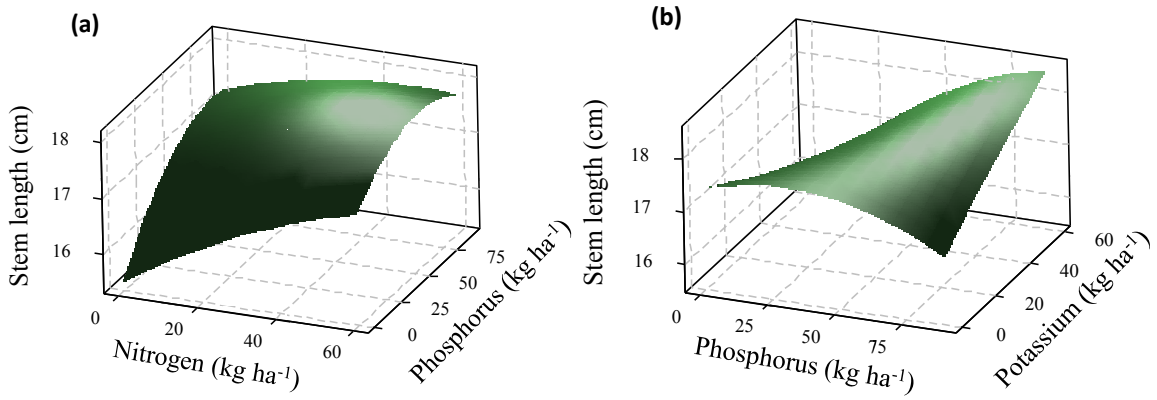


Figure 3.1 Response surface of stem length: (a) the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}, and (b) the effect of phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 kg N ha^{-1}

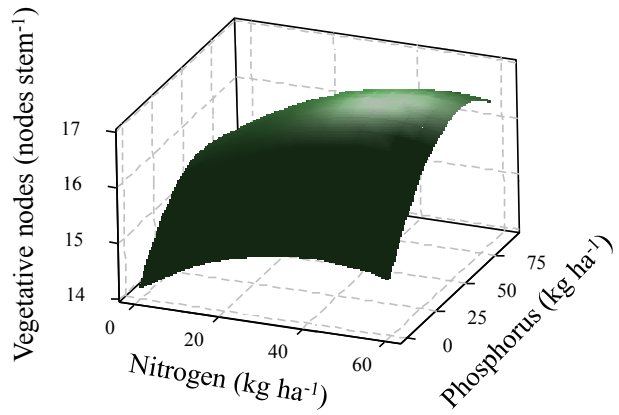


Figure 3.2 Response surface of vegetative nodes, the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}

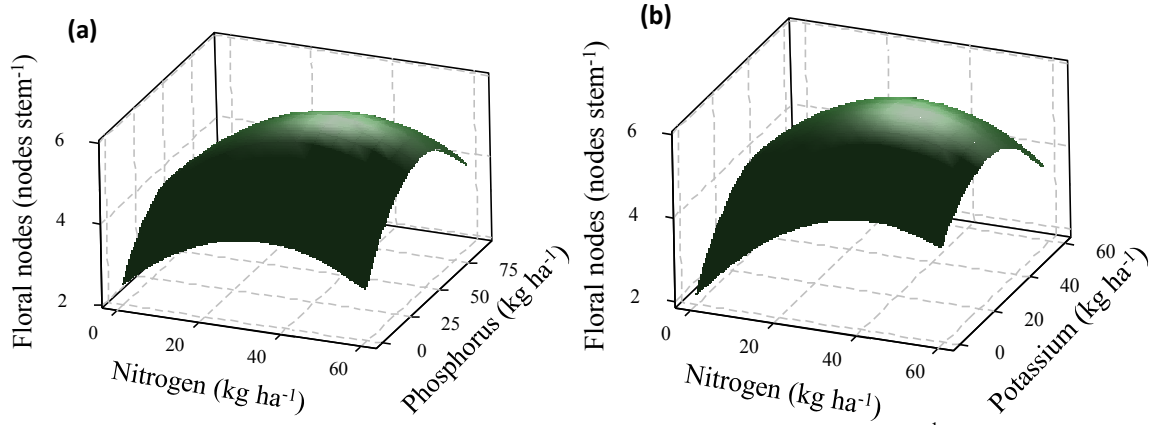


Figure 3.3 Response surface of floral nodes: (a) the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}, and (b) the effect of nitrogen (kg N ha^{-1}) and potassium (kg K ha^{-1}) for phosphorous fixed at 45 kg P ha^{-1}

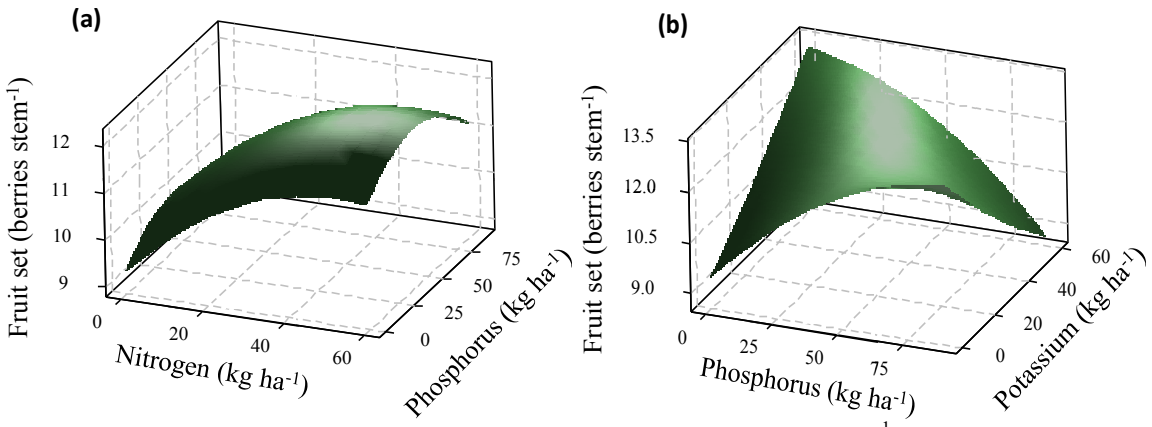


Figure 3.4 Response surface of fruit set: (a) the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1},and (b) the effect of phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 N kg ha^{-1}.

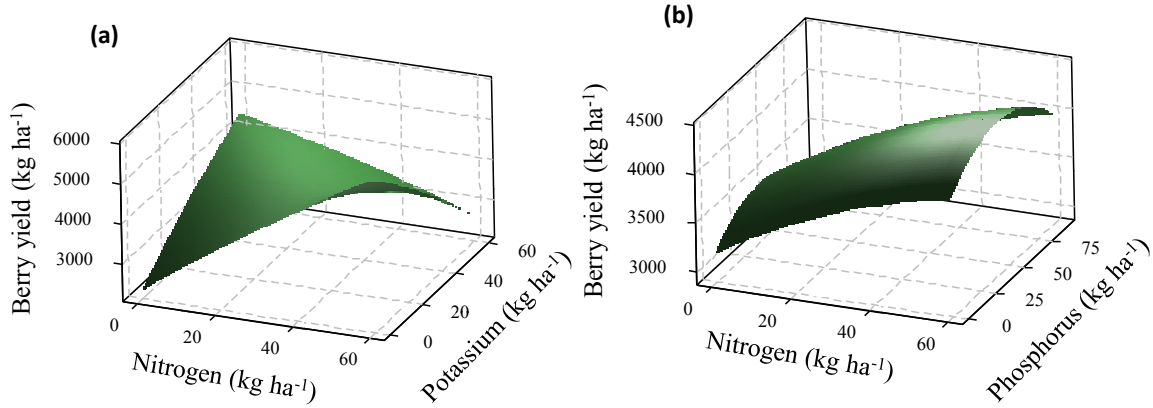


Figure 3.5 Response surface of berry yield(a) the effect of nitrogen (kg N ha^{-1}) and potassium (kg K ha^{-1}) for phosphorus fixed at 45 kg K $ha^{-1}$and, (b) the effect of phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 N kg ha^{-1}

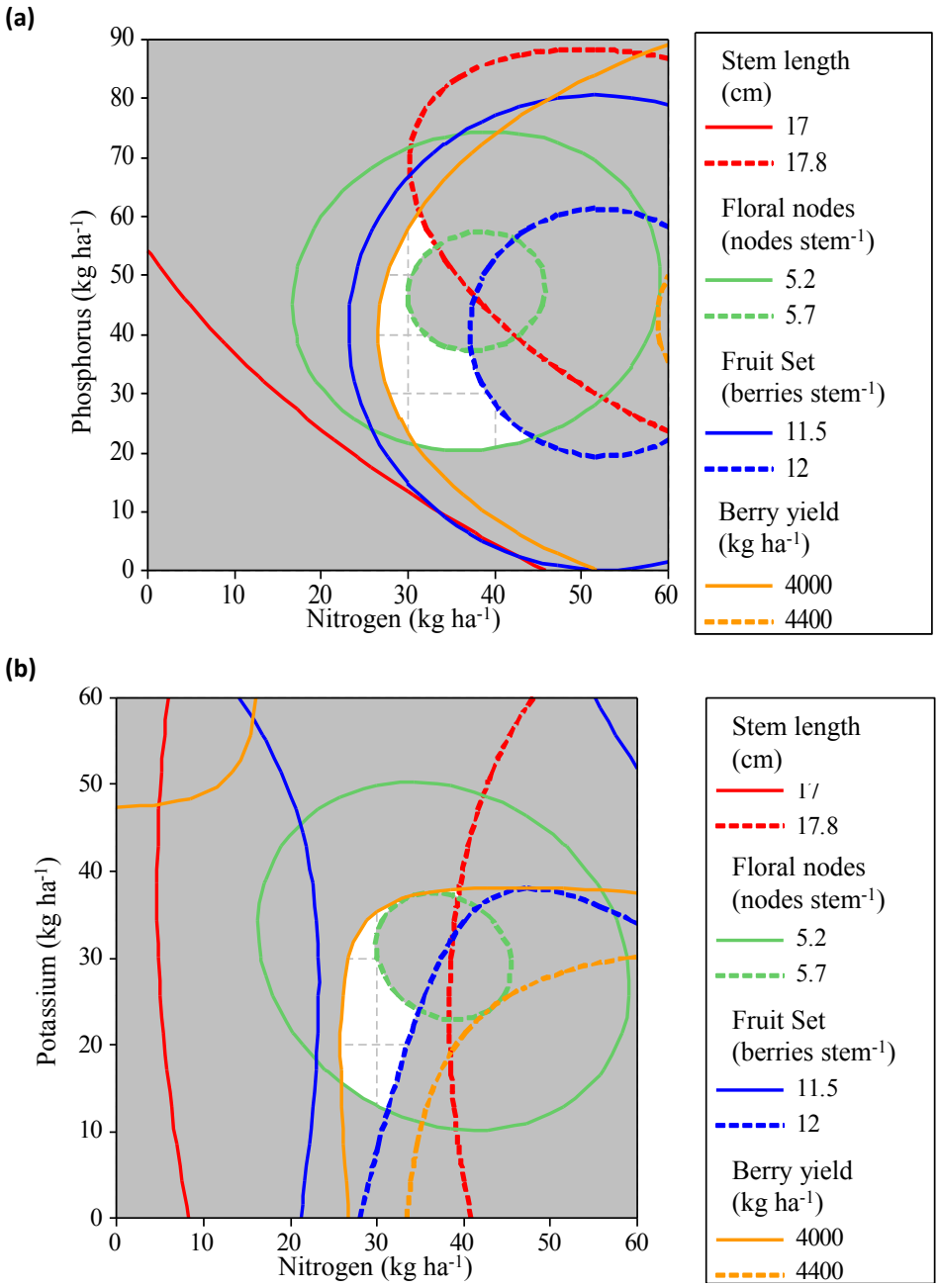


Figure 3.6 Overlaid contour plot of four response surface models: (a) the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1} on stem length, floral nodes, fruit set, and berry yield, and (b) the effect of nitrogen (kg N ha^{-1}) and potassium (kg Kha^{-1}) for phosphorus fixed at 45 kg P ha^{-1} on stem length, floral nodes, fruit set, and berry yield. The white area is the Sweet Spot where the criteria of all four responses are fulfilled

Chapter 4 Leaf Nutrients Ranges and Berry Yield Optimization in Response to Soil-Applied Nitrogen, Phosphorus and Potassium in Wild Blueberry (*Vaccinium angustifolium* Ait.)

4.1 Abstract

The study examined the main and interactive effects of soil applied fertilizers (nitrogen, phosphorous, and potassium) and optimum fertilizer rate for the spout and crop phase leaf nutrients, and berry yields of wild blueberry (*Vaccinium angustifolium* Ait.). Data were collected between 2004 and 2010 from previous and ongoing nutrition studies that used a central composite design (CCD). Experimental sites were located at Kemptown and Mount Thom, Nova Scotia, and Brantville, New Brunswick. Treatments consisted of five levels of soil applied nitrogen (0, 12, 30, 48 and 60 kg N ha^{-1}), phosphorous (0, 18, 45, 78 and 90 kg P ha^{-1}), and potassium (0, 12, 30, 48 and 60 kg K ha^{-1}). Leaf nitrogen (N) and phosphorus (P) concentrations significantly increased with addition of soil applied N and P fertilizers, respectively. Leaf potassium (K) content in sprout year had a significant quadratic response (convex shape) to soil applied N and P. Leaf calcium (Ca) and magnesium (Mg) concentration exhibited significant concave quadratic (depression) response to soil added N, P and K. Nitrogen fertilizer significantly increased berry yields while soil applied P and K had quadratic effects on berry yield. We recommend soil fertilizer rates of 30N, 40P and 30K kg ha^{-1} applied at pre emergence of shoots in the sprout year of production to optimize foliar nutrient levels and berry yields. Leaf concentration levels for N, P and K were higher than current foliar standards by Eaton (Nova Scotia), suggesting that a separate set of nutrient ranges may be adopted for fertilized fields in Nova Scotia. We suggest new optimal sprout year leaf nutrient ranges N (1.80-2.03%), P (0.155-0.160%), K (0.53-0.55%),

Ca (0.44-0.46%), Mg (0.115-0.13%) and B (24-26 ppm) for fertilized wild blueberry fields in Atlantic Canada.

4.2 Introduction

Wild blueberry plants have low nutrient requirements and exhibit slow growing patterns. Their natural habitats are characterized by nutritionally marginal soils (Korcak 1988) so in commercial fields, fertilizers are routinely applied (20-40 kg NPK ha^{-1}) in early spring of the sprout year of production with the aim to sustain berry yields in the following crop year. Fertilization generally improves growth and yield of wild blueberries (Percival and Sanderson 2004).

Fertilizer programs are often guided by leaf tissue analyses due to the inability of presently available soil analyses to accurately determine the wild blueberry nutritional demands (Ring 2001). Leaf tissue nutrient standards have been developed for blueberry growing regions of Nova Scotia, Maine, New Brunswick and Quebec (Lockhart and Langille 1962; Townsend and Hall 1970; Trevett 1972; Lafond 2009; Eaton et al. 2009; Santiago 2011). Recently, Eaton et al. (2009) proposed leaf tissue ranges from data collected in 1998-99 from 44 high yield farmer fields across Nova Scotia. This study surveyed fields across Nova Scotia but fields received several different combinations of NPK fertilization and no statistical design was used thus, it may not account for potential main (N, P and K)quadratic (NN, PP and KK) and interactive effects (NP, NK, PK) effects of soil applied fertilizers (NPK). Additionally, this study reported 42%, 12% and 48% of surveyed fields were deficient in leaf phosphorus (P), potassium (K) and boron (B), respectively when compared to standards from Maine (Trevett1972). One criticism of this study was Nova Scotia fields may not have attained their full yield potentials and that the optimum ratio of leaf nutrient concentrations to yield needed to be determined by experiment rather than by survey.

Nutrient management of wild blueberries is dynamic with at least 12 nutrients involved and is complicated by the fact that this plant is naturally adapted to low soil nutrient environments (Korcak 1988). The wild blueberry plant is a slow growing perennial calcifuge (i.e. acid-loving) that spreads by underground stems called rhizomes. The extensive root system occurs within top 10 cm of soil and accounts for 85% of the total plant dry weight (Jeliazkova and Percival 2003). The rhizomes serve as a reservoir for nitrogenous compounds as well as carbohydrates and some inorganic constituents, particularly P and Mg (Townsend et al. 1968). The numerous fine 'root hairs' are heavily colonized by indigenous ericoid mycorrhizae. These ericoid mycorrhizal fungi assist in nutrient uptake, notably N, P and organic forms of nutrients (Korcak 1988).

Wild blueberry fields are pruned in the fall of the crop year or in the early spring of the sprout year. Pruning the wild blueberry plant is necessary to encourage new vigorous shoot growth from the rhizomes and to maximize the production of floral buds and yields (Kinsman 1993). Until the early 1980's, the fields were pruned by burning but this has largely been replaced with flail mowing due to environmental risks (carbon footprint), increasing oil prices and organic matter loss caused by burning. The ash left after burning has been reported to supply nutrients (Smith and Hilton 1971) but medium to long-term burning can gradually reduce soil fertility by depleting the organically bound nutrient reserves (Lafond2009). The amount of N lost from blueberry fields by burning was estimated at 35-50 kg N ha^{-1} (Eaton 1986). Conversely, pruning by mowing deposits substantial amounts of plant material to the soil in each production cycle and gradually builds soil organic nutrient reserves. Nutrients adhering to organic matter are slowly available to plants upon mineralization especially N. In acidic conditions, soil P precipitates with soil aluminum (Al) and iron (Fe) to form relatively insoluble compounds. This slow mineralization,

immobilization and formation of insoluble forms of P have been reported in short term incubation studies in wild blueberry (English 2007). Therefore, continuous P supply may be required to meet crop demands in wild blueberry production systems.

Although wild blueberry nutrition has been studied extensively, earlier studies have focused mainly on the main effects of soil applied fertilizers (NPK), leaf tissue nutrient standards, the effects of fertilizer formulations, timings for application and nutrient estimation (Lockhart and Langille 1962; Townsend and Hall 1970; Jackson 1976; Trevett 1972; Smagula and Ismail 1981; Warman 1987; Warman 1988; Eaton 1994;Smagula and Dunham 1995; Penney and MacRae 2000; Percival and Privé 2002; Percival et al. 2003; Percival and Sanderson 2004; Lafond 2009; Eaton et al. 2009; Maqbool et al. 2012; Santiago and Smagula 2012). However, quadratic (NN, PP and KK) and interactive effects (NP, NK and PK) of soil applied NPK remains unknown. We also suspect continuous mowing, repeated fertilizer applications, pest and weed management inputs have all contributed to the changes in soil and plant nutrient dynamics and ultimately crop demands. These management changes may have left a gap in understanding the effect of soil applied NPK on leaf nutrient concentrations. A nutrition management trial was started in early 2000 in order to better examine the long-term main and interactive effects of N, P, and K fertilizer applications, with one of the goals to determine the rates that provide the necessary leaf nutrient ranges in leaf nutrients to maximize yields under NS growing conditions.

Response surface methodology (RSM) and canonical analysis in combination with central composite design (CCD) has been used by others to describe the effects of fertilization on foliar nutrients (Myers et al. 2009). Chintala (2012) used CCD and RSM to examine the effect of water potential and pH on N and P fertilizer additions in order to optimize Kentucky bluegrass (*Poa pratensis* L.) herbage yield and foliar nutrients.

The objectives of this research were to: (1) determine the main and interactive effects of soil applied fertilizers (NPK) on wild blueberry leaf nutrients and berry yields, and (2) provide new leaf nutrient ranges, pin point optimum leaf nutrient levels and corresponding fertilizer rates that maximize berry yield.

4.3 Materials and Methods

4.3.1 Experimental Design

A three factor central composite design (CCD) was used to study the response surfaces between soil applied fertilizers and their impact on leaf nutritional levels (Myers et al. 2009). The three factors were: soil-applied N, P and K (kg ha^{-1}), and their levels for provided in Table4.1. Design settings were replicated four times. A plot size of 6 m x 8 m was used. These sites were fertilized in the form of Urea/ammonium sulfate (source of N), triplesuperphosphate (source of P) and potassium chloride(source of K) in early May every year at the pre-emergence of shoots from the rhizomes in the vegetative (i.e. "sprout") stage of production as listed in Table. 4.1. Fertilizers were applied using a Scotts SR2000 rotary fertilizer spreader (Marysville, Ohio).

4.3.2 Field Experimental Sites

The field experiments are established on commercial fields located at Kemptown, NS, MountThom, NS and Brantville, New Brunswick, Canada. The Kemptown experiment site (N 45°30' 7.91", W -63° 7'27.72", elevation ~ 214 m) was established in spring 2001. The soil was typically sandy loam, acidic, imperfectly drained, very stony and moderately rocky (Cobequid soil association). The soil pH was 4.3 ± 0.1 SD (0-15 cm depth). The soil organic matter was 10.23 % ± 1.34 SD (0-15 cm depth). The Mount Thom experimental site (N 45°29' 32.10", W -62° 59' 30.65", elevation ~ 220 m) was established in May 2004. The

soil was typically silt loam, strongly acidic, poorly drained, slightly to moderately stony and non-rocky (Thom soil association). The soil pH at 0-15 cm depth was 4.17 ± 0.13 SD (± 0.1 SD). The organic matter was 9.59% (± 1.21 SD). The Brantville (47° 22' 22.74" N, 64° 58' 17.48" W, elevation ~ 12 m) experiment site was established in 2006. The soil was typically sandy, very acidic, poorly drained, non stony and non rocky (Acadie Siding).

4.3.3 Leaf Sampling and Foliar Nutrient Analysis

Leaf tissue samples were obtained in sprout and crop years of production in 2004, 2005, 2006, 2007, 2009 and 2010. The site and sampling dates are listed in Table. 4.2. A 7.5 m long rope was extended diagonally in each plot, marked at fifteen equally spaced points. One stem was randomly selected at each mark. The stem was grasped from its base, and pulled gently to collect the leaves. Leaf samples were stored in paper bags, placed in a cooler and transported to the laboratory. The leaves were dried by placing the samples in an oven at 65 ℃ for 8-10 hours. Samples were then ground using a Wiley Mill (Arthur H. Thomas Co, Philadelphia, PA, USA) with 2 mm mesh. Foliar N was measured by combustion using LECO CNS-1000 elemental auto analyzer (Leco Corporation, St. Joseph, MI). The system combusted the sample in a stream of pure O_2 at high temperatures (950 ℃), producing NO_x and N_2. An aliquot of this gas was carried out by pure He into a reduction zone where elemental Cu reduced NO_x to N_2, which was subsequently measured by a thermal conductivity detector (Rutherford et al. 2008). For P, K, Ca, Mg, and B, one gram of the ground leaf sample was placed in crucibles. These samples were then burnt at 550℃ for 4 hours, 5 ml of 30% (v.v.) of concentrated hydrochloric acid (HCl) was added, and swirled gently to ensure the sample was completely wet. Resulting mixtures were transferred to 50 ml centrifuge tubes and filled with deionized water. Samples were centrifuged using an International Centrifuge (IEC), model K (International Equipment Co. Massachusetts) at

3000 rpm for 15 minutes (Percival and Privé 2002; Bourguignon 2006). Supernatants were sent to the Soil and Feed Testing Laboratory, Agriculture Resource Division (Prince Edward Island Department of Agriculture and Forestry) for testing. Each sample was analyzed using a Thermo Jarrell Ash ICAP61-1100. Berries were hand raked(mid to end of August) from four randomly selected 1 m^2 quadrats in each plot and weighed using a digital balance (Mettler PE 6000, Burlington, ON) to calculate harvestable berry yield. Berry yield was measured from sites sampled for sprout year and crop year leaf nutrients (Table 4.2). In the results and discussion section, sprout year berry yield means the yield obtained from sites that were sampled for the sprout year leaf nutrients and similarly crop year berry yield means yield obtained from sites sampled for the crop year leaf nutrients.

4.3.4 Statistical Analysis

The averages of the four replications (within each sampling date) were used in order to improve the precision of the parameter estimations, except the center point which the CCD calls for all replications to allow for a lack of fit test. The lack of fit test measures the adequacy of the quadratic response surface model. We tested models for lack of fit test to make sure there was no lack of second order model and proceeded to response surface analysis of the data (Myers et al. 2009).Foliar nutrient data for sprout and crop year were analyzed separately. Within each production cycle, the data from all samplings dates were analyzed together using sampling date as a blocking factor to account for date to date variability. Berry yield data from sites sampled for sprout and crop year foliar nutrients were analyzed separately using sites as a blocking factor to account for site to site variability. Minitab 16 software (Minitab Inc. State College, PA) was used for regression and graphical analyses of the data. The second order model was used for the CCD response surface analysis.

$$Y = \beta_0 + \sum_{1=1}^{3} \beta_i X_i + \sum_{1=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \varepsilon \qquad (4.1)$$

where Y is the response, X_i are the coded levels of the factors, and β_0, β_i, β_{ii} and β_{ij} are the regression coefficients, and ε is the error term assumed be independent and have normal distribution with constant variance (Myers et al. 2009). The regression coefficients of the response surface model were estimated using the Least Squares method. Three dimensional (3D) surface plots were drawn to illustrate the interactive effects of factors on response variables. The overlaid contour plots were drawn for joint optimization of foliar nutrients and berry yield. Based on second order response surface models the canonical analysis was performed to determine the location and the nature of the optimum setting of the factors of the response functions (Myers et al. 2009). Matlab 7.10 (The Mathworks, Inc. Natick, MA) was used to complete the canonical analysis of the data. The details about canonical analysis can be found in Chapter 2 and Chapter 3. The canonical analysis optimizes one response at a time, so canonical analysis results may be used if interest is optimizing single response (e.g. berry yield). The overlaid surface plots simultaneously or jointly optimize (sweet spot) of number of responses, the overlaid contour plot results are more relevant for joint optimization.

4.4 Results and Discussion

All results reported were for fertilization (NPK) that was applied in early spring of sprout year and hereafter is written as "soil applied fertilizers". The analysis of variance (ANOVA) and *P*-values for all responses were provided in sprout (Table4.3) and crop year (Table4.4). For all responses, the lack of fit test results were not significant, suggesting that the quadratic model adequately fit for all responses and the design settings are in the

neighborhood of the optimum setting. All leaf elements were significant in both sprout and crop year except for leaf K in the crop year (Table 4.3 and Table 4.4).

4.4.1 Leaf nutrients

Overall significant main and interactive effects were found between the soil-applied N, P, and K for leaf nutrient content (Table 4.3 and Table 4.4). Leaf N content increased linearly in both years of production (sprout and crop) with the soil applied N but was found to be higher in the year of application (sprout, Figure 4.1). The linear increase in leaf N agrees with previous findings (Trevett 1972; Smagula and Ismail 1981; Penney and McRae 2000). Fertilizer N had a beneficial effect on wild blueberry yield potential and berry yields (Chapter 3). Fruit set increased linearly with soil applied N rate as it increased from 0 to 40 kg ha^{-1}(Chapter 3). The response foliar N (Figure 4.1) and fruit set (Chapter 3; Figure 3.4a) are similar to soil applied N and P fertilizers.

The sprout and crop year leaf P content increased linearly with soil applied P (Figure 4.2). A significant linear increase in leaf P with soil applied P fertilizer rate (0-195 kg P ha^{-1}) was reported in Maine (Smagula and Dunham 1995; Smagula and Ismail 1981). Leaf K concentration was explained by convex quadratic effects only in the sprout year (Figure 4.3a). The quadratic response of sprout year leaf K is consist with finding of Smagula and Ismail (1981), they reported significant quadratic response sprout year leaf K, initially leaf K was increased applying fertilizer 1:1:1 (0-135 kg ha^{-1}) and leaf K decrease at higher fertilizer rates (180 kg ha^{-1}). In our experiment, the fertilizers were applied in sprout year of production, it seems that P remained available to the wild blueberry plant through P mineralization in the longer term (crop year) or alternatively wild blueberry plants have taken up all the P required during whole production cycle (two-year), when fertilizers (sprout year) were applied, nutrients were stored in their rhizome system and utilized when required

(sprout and crop) to maintain foliar P and K levels. Repeated applications of fertilizer to wild blueberry stands resulted in increased levels of soil P and K (Eaton and Patriquin 1988). Phosphorus concentrations in rhizome tissue were increased in fertilized plots (NPK) but K content of rhizome was not (Eaton 1994). This may explain the lack of K effect on leaf K levels (Table 4.4). Therefore, the residual effect of P fertilizer on leaf P can possibly attributed to efficient nutrient cycling, retention of K (soil colloids and hums) and special ability of wild blueberry plant to store nutrients in rhizome system (Eaton 1994). The leaf K in sprout year benefited from soil applied N and P when applied at optimum levels (Figure 4.3). So, as limiting nutrients (N or P) are available through external input or mineralization, the leaf K levels increased. Under no till systems, the availability of K is reduced and thus the addition of K fertilizer is required in blueberry production systems.

The leaf tissue Ca response was illustrated by concave quadratic effect of soil applied P and K and response was similar in both sprout and crop year of production (Figure 4.4a and 4.4b). Smagula and Ismail (1981) reported quadratic trend of leaf Ca levels 0.387, 0.369, 0.344, 0.361 and 0.35%at 0, 45, 90, 135, and 180 kg ha^{-1} fertilizer (1:1:1). The concentrations of Ca are low in the acidic soil. The wild blueberries (ericaceous plants) grow in an acidic, low pH environment, were efficient in Ca uptake (Korcak 1989) and foliar leaf Ca were not affected by pH over the range of 4.1-6.8 (Korcak 1989).

Leaf Mg content varies significantly with concave quadratic (K) and interactive (N x P and P x K) effects in sprout year of production (Figure 4.5a and 4.5b). Significant concave quadratic effects of N, P and K were also found for leaf Mg concentrations in crop year (Figure 4.5c and 4.5d). These complex interactions found in the sprout year may be partially explained by soil pH shifts, CEC, cation competition and soil K^+ concentrations (Brady and Weil 2007). The uptake of cations such as K^+ and NH_4^+, and the naturally low soil pH

conditions found in blueberry fields may depress the uptake of Mg^{+2} from soil. Ammonium build up in plant tissue can suppress uptake of cationic nutrients such as Ca^{+2} and Mg^{+2} (Britto and Kronzucker 2013) and the suppression effect can be viewed by combining leaf N, Ca and Mg results (Figures 4.1; 4.4 and 4.5). The significant liner decrease in leaf Mg levels were also found by Smagula and Ismail (1981) when they applied N fertilizer (0-180 kg ha^{-1}). However, in their study the fertilizer treatments were laid out in a randomized complete block design and did not appear to consider any possible interactions between the nutrients. Ammonium based fertilizers are reported to reduce the soil pH, which is reported to reduce the leaf Mg levels (Trevett 1968a; Warman 1988).

We found significant NP interaction for sprout year leaf B (Figure 4.6a). Soil applied K linearly (0-60 kg ha^{-1}) reduced foliar B (Figure 4.6b). The complex NP interaction (shape of Figure 4.6a) may be explained by NP effect on soil organic matter decomposition. Among the soil B pools (soluble, weathering of minerals, adsorbed on clay and/or iron hydroxides, soil organic matter), the soil organic matter pool tends to contain the largest amount of B which is released into soil solution on decomposition (Marschner1995).

Crop year foliar B was explained by quadratic effect (concave) of the soil applied N and P rates (Figure 4.6c). This concave shape of NP may be explained by the allocation of B towards beneficial reproductive growth during the crop year. Maximum reproductive growth (floral nodes) occurred at mid to high soil applied fertilizers (N30-40, P40-60, K30 kg ha^{-1}) (Chapter 3), so utilizing plant available B to maximum extent and creating concave shape of foliar B in the crop year of production. The low leaf B concentration at middle values of NPK in crop year may indicate the allocation of leaf B to the developing inflorescence (Lahav and Whiley 2002). B is an essential micronutrient for plants and plays a vital role in reproductive growth (Marschner 1995).

4.4.2 Berry Yield

Commercial wild blueberry production follows a two year production cycle with the crop harvested in the second year of production. In this section, sprout year berry yield means the yield obtained from sites those were sampled for the sprout year leaf nutrients and similarly crop year berry yield means yield obtained from sites sampled for the crop year leaf nutrients. The soil applied N linearly increased berry yields on sites sampled for foliar nutrients in the sprout year. These results are in agreement with previous studies (Percival and Sanderson 2004; Chapter 3). We recommended 30N 45P and 30 K kg ha^{-1} to optimize yield potentials and berry yields by analyzing a 6-production cycle data (Chapter 3). In this study, we found 20 kg N ha^{-1} for sprout sites which is 10 N ha^{-1} lower than that of Chapter 3 to optimize yields. The P and K fertilizer rates to optimize berry yields were similar in both studies. This difference in N rate to optimize berry yields may be due to year to variation in N supplying capacity of soils (mineralization) that is affected by climatic factors such as variations in temperature and soil moisture. However, crop sites had similar N rates (28 kg N ha^{-1}) to Chapter 3 (30 kg N ha^{-1}).

Quadratic effect (convex) of soil applied P and K was found for berry yield on sites sampled for sprout year foliar nutrients (Table 4.3; Table 4.4; Figure 4.7b and 4.7d). Studies have reported significant linear increases in berry yield with soil applied P (0-195 kg P ha^{-1}) in Maine (Smagula and Dunham 1995). The reason for the higher rates of P necessary in Maine is because this soils are sandy in nature but with a lower organic matter than NS soils. Wild blueberry plants in NS are better able to acquire P from the high soil organic matter decomposition, therefore, comparatively low P fertilizer (40-60 kg ha^{-1}) may be adequate in NS soils. The quadratic effect of soil applied N, P and was also significant for sites sampled for crop year foliar nutrients (Table 4.4). The same trend held true for foliar nodes (yield

potential) (Chapter 3). Optimal berry yields were different between sites sampled for sprout (3620 kg ha^{-1}) and crop (3170 kg ha^{-1}) year foliar nutrients. These site to site optimal berry yield differences may be attributed to uncontrollable factors such as inherited yield potentials due to clone variably among blueberry fields, weed and disease pressures and bee pollinator densities.

4.4.3 Simultaneous Optimization of Leaf Nutrients and Berry yield

In order to simultaneously optimize all the foliar nutrients and berry yield, all response surface plots were overlaid. We used stationary points for each response from canonical analysis to guide ourselves to get a common optimal point (Sweet Spot) for all the responses. By doing so, we get fertilizer rates (25-30 kg N, 30-55 kg P and 25-45 kg K ha^{-1}) that simultaneously optimized sprout year leaf nutrients and berry yields (sites sampled for sprout leaf nutrients).For crop year leaf nutrients, fertilizer rates were 15-35 kg N, 15-50 kg P and 20-40 kg K ha^{-1}. From overlaid plots, we obtained optimal leaf nutrient ranges for sprout and crop year of production, reported in Table 4.7. The optimum settings to optimize leaf N, P, K, Ca, Mg, B and berry yield was obtained by overlaying the plots and looking at the various sweet spots (Figures 4.8 and Figure 4.9) (Myers et al. 2009). The primary objective of this study was to determine the optimal leaf nutrient ranges that resulted in producing maximum berry yields. The canonical analysis was used to pin point the optimum levels for the leaf nutrients and berry yields whereas sweet spot analyses provided optimum ranges (Table 4.5 and 4.6).

The sprout year leaf N (dotted dark green) and berry yield (dotted purple) followed the upward trend suggesting leaf N (sprout) and berry yield are closely related and nitrogen fertilization increased leaf N maximized berry yields when supplied with P (40 kg P ha^{-1}) and K (30 kg K ha^{-1}) fertilizers (Figure 4.8a). The relationship of crop year leaf N with berry

yields followed the similar pattern as of sprout year leaf N and berry yields (Figure 4.9a and 4.9b) but optimum levels in crop year leaf N (1.5-1.7%) were lower that of sprout year leaf N (1.8-2.03%). The lower leaf N in the crop year may be explained by that wild blueberry plant may acquire N (mainly NH_4^+ and organic N fractions) in the sprout year of production (Chapter 2), store N in rhizomes and use during the whole production cycle (2-year commercial cycle). These lower leaf N levels in the crop year may have been due to allocation of recourses towards reproductive organs (flowering, berry production). Fertilizers were applied in the sprout year of production and earlier studies have suggested an increase in berry yields by splitting fertilizer doses between the sprout and crop phases of production. In our sites, the fertilizers and soil N supply seemed to fulfill the crop demands during both the sprout and crop year of production. Soil N data from the Kemptown site suggested that the wild blueberry crop uptake soil N mainly in the form of ammonium and organic fractions in the sprout year of production and store it in their rhizomes for later use in the crop year of production. Eaton (1994) found N concentration in leaf and rhizome tissues were elevated in fertilized (NPK) plots for 5-production cycles. However, N based fertilizer applications may be required in crop years where wild blueberry plants do not acquire enough N in the sprout year of production due to low N supplying capacity of the soil (e.g. sandy soils and low organic matter).

The decision to make split fertilizer applications (sprout and crop) could be guided through local soil knowledge and comparing leaf N status in the sprout and the crop year with the suggested leaf N nutrition ranges reported in this study. The soil applied N rates (14 kg N ha^{-1}) required to optimize foliar N in sprout year were lower than that required to optimize other foliar nutrients, yield potentials (Chapter 3) and berry yields (Table 4.5 and Table 4.6). These lower N rates (14 kg N ha^{-1}) may be enough to optimize foliar N, because some of the

leaf N nutrient demands are coming from the stored reserves in the rhizomes, however, higher N applications (30 kg N ha^{-1}) may be required to replenish this rhizome pool of N, and/or perform other metabolic process in the plant to achieve optimal yield potentials. Relatively high N requirement in the crop year may indicate that higher rates of N fertilizer in the sprout year are required to keep crop year leaf N within the recommended range due to competition for nutrients by the berries (Penney and MacRae 2000).

The amount of fertilizer N required to optimize other sprout year foliar nutrients (P, K, Ca, Mg and B) ranged from 27-30 kg N ha^{-1} (Table 4.5 and Table 4.6), which is very similar to the requirement to optimize yield potentials and berry yields (Chapter 3). The optimal dose of N ranged from 22-36 kg N ha^{-1} with average of 30 kg N ha^{-1} required to optimize foliar nutrients (P, K, Ca, Mg and B), again this N dose agreed with those required to optimize yield components and berry yields (Chapter 3).

Contrary to N, where a lower level of fertilizer N was required to optimize foliar N than that of yield and harvestable yields, higher P (59 and 50 kg P ha^{-1}) was required to optimize sprout and crop year leaf P levels than the amount needed to optimize berry yields (Chapter 3). The amount of K fertilization required to optimize sprout (27 kg K ha^{-1}) and crop year (33 kg K ha^{-1}) berry yield were similar (Table 4.5 and Table 4.6).The amount of K fertilizer K required to optimize foliar nutrients and berry had the least spread from the average (SD = 4.5 for sprout and SD =3.6 for crop year) than soil applied N and P (Table 4.5 and Table 4.6).

Our sprout year leaf N, P and K nutrient ranges (N =1.8-2.03%, P =0.155-0.160% and K = 0.53-0.55%) concentrations were higher than existing nutrient ranges (Eaton et al. 2009) in Nova Scotia (N = 1.6-2.0%, P = 0.110- 0.144% and K =0.41-0.52%). Our sprout

year leaf Ca and B nutrient range (Ca = 0.44-0.46% and B = 24-26 ppm) fell within similar ranges (Eaton et al. 2009) but ranges were narrower in scope (Ca = 0.32-0.47% and B = 19-31 ppm). The optimal sprout year leaf nutrient levels obtained in this study may be due to enhanced nutrient uptake through a more balanced fertilization (NPK). These relatively high N, P and K leaf nutrient levels may also be required for better growth, development and maximize yield potentials. For example availability of ammonium was increased after fertilization (Chapter 2) and enhanced uptake of soluble organic nitrogen was also observed in sprout year of production (Chapter 2). These optimum nutrient ranges also reflect the effect of fertilization on soil N pools and N uptake.

Our sprout year leaf Mg nutrient ranges (0.115-0.130%) were lower than existing nutrient ranges for NS (0.15-0.19%). Optimum leaf Mg content (0.11-0.13%) in sprout year was also lower compared to ranges obtained in earlier studies (Lockhart and Langille 1962, Townsend and Hall 1970; Trevett 1972; Lafond 2009). Lower leaf Mg content could be due to the suppression effect of ammonium build up (Britto and Kronzucker 2013) from our increased N applications and/or due to application of K fertilizer (antagonistic effect) (Mengel et al. 2001; Milošević et al. 2013) but these lower foliar Mg levels were not correlated to reduced berry yields. However, plants require specific ratio of nutrients depending on life cycle, genetic characteristics and environment to realize full genetic yield potentials. Therefore, ratio of nutrients may be more critical than actual nutrient levels (Marschner 1995).

Optimum leaf B concentration in sprout (24-26 ppm) and crop year (18-22 ppm) were below the Maine critical values for sprout (24 ppm) and crop (30 ppm) proposed by Trevett, (1972), but within NS optimal ranges of 19-31 ppm (Eaton et al. 2009). B deficiency occurs mostly on coarse textured soils with low organic matter content and high rainfall conditions

like those found in Maine, USA. The optimal leaf B content (18-22 ppm) in the crop year supplied enough B to the inflorescences for good fruit set and optimum yields, so the wild blueberry plants may not require the high B levels (30-70 ppm) proposed by Trevett (1972). The additional B supply from external sources may not be required in low pH soils with high soil organic matter until foliar B content falls below a critical level (<18ppm) that affect berry yields.

Our crop year optimal leaf N (1.5-1.7%), K (0.535-0.545%), Ca (0.465-0.495%) and B (18-22 ppm) ranges agrees with those previously reported by Trevett (1972) (Table 4.7). However, our crop year leaf P (0.158-0.164%) ranges were higher (due to balanced fertilization) while leaf Mg (0.115-0.125%) ranges were lower (antagonistic effect of K application or ammonium built up) than previously suggested by Trevett (1972) (Table 4.7). Interestingly, our results differ from the literature and suggest a narrower range of leaf nutrient concentrations for foliar N, P, K, Ca, Mg and B be used as standards during the crop year. Our crop year leaf nutrient ranges were narrower (Figure 4.7 and Figure 4.8) than suggested by Trevett (1972) (Table 4.7).

In Chapter 3, we recommended 35N 45P and 30 K kg ha^{-1} to maximize yield potentials and optimize berry yields, while in this study based on our canonical analysis and overlaid surface plots recommends a slight reduction in N (30 kg N, 45 kg P and 30 kg K ha^{-1} fertilizer application in the sprout year of production). This reduction may be due to the supplying capacity of the soils through mineralization and can be affected by climatic factors such as variations in temperature and soil moisture. The new proposed leaf nutrient (N, P, K, Ca, Mg and B) ranges for sprout and crop year are presented (Table 4.7).

4.5 Conclusions

Results from this study have provided insight into the main and interactive effects of soil applied N, P and K, their optimum rates of application, and the corresponding predicted foliar nutrient contents for optimum berry yields of wild blueberries grown in areas of Atlantic Canada. The results of this study emphasize the importance of applying balanced fertilization. Based on the canonical analysis and overlaid surface plots, we recommend 30N, 45P and 30K kg ha^{-1} soil applied fertilizer at shoot pre-emergence in the spring of the sprout year. We also present the optimal sprout year leaf nutrient ranges N (1.8-2.03%), P (0.155-0.160%), K (0.53-0.55%), Ca (0.44-0.46%), Mg (0.115-0.13%) and B (24-26 ppm) for Nova Scotia wild blueberry fields. We suggest optimum leaf N (1.5-1.7%), P (0.158-0.164%), K (0.535-0.545%), Ca (0.465-0.495%), Mg (0.115-0.125%) and B (18-22 ppm) for crop year leaf nutrient levels. The leaf nutrient ranges in response to optimum fertilizer doses may be used as guidelines for wild blueberry production in Atlantic Canada.

Table 4.1 Experimental range and levels of the independent variables

	Range and levels				
Original factors	-1.68	-1	0	1	1.68
[z]N (kg ha^{-1}): X_1	0	12	30	48	60
P (kg ha^{-1}): X_2	0	18	45	72	90
K (kg ha^{-1}): X_3	0	12	30	48	60

[z]The source of soil applied N was ammonium sulfate, P was triple superphosphate and K was potassium chloride

Table 4.2 Schedule of wild blueberry sampling dates and cropping cycles at three study sites

Sampling Dates	Site	Cropping cycle	Foliar nutrient
20th July 2004	Mount Thom	Sprout	N
27th June 2005	Kemptown	Crop	N, P, K, Ca, Mg and B
27th June 2005	Mount Thom	Crop	N, P, K, Ca, Mg and B
5th August 2005	Kemptown	Crop	N, P, K, Ca, Mg and B
8th August 2005	Mount Thom	Crop	N, P, K, Ca, Mg and B
30th June 2006	Kemptown	Sprout	N, P, K, Ca, Mg and B
3rd August 2006	Brantville	Sprout	N, P, K, Ca, Mg and B
14th August 2006	Kemptown	Sprout	N, P, K, Ca, Mg and B
15th September 2006	Kemptown	Sprout	N, P, K, Ca, Mg and B
25th June 2007	Kemptown	Crop	N
15th July 2009	Kemptown	Crop	N
25th June 2010	Kemptown	Sprout	N
16th August 2010	Kemptown	Sprout	N

Table 4.3 Analysis of variance (ANOVA) *P*-values of the central composite design in sprout year of production

Source of variation	N	P	K	Ca	Mg	B	[z]Berry yield
Blocks (samplings)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Regression	0.03	0.03	< 0.01	0.01	< 0.01	0.01	< 0.01
Lack of fit	0.88	0.98	0.96	0.84	0.99	0.99	0.53
Adjusted R^2	0.95	0.96	0.70	0.95	0.91	0.75	0.85
Significant terms	N	P	NN, PP	K, PP, KK,	KK, NP, PK	K, NP	N, K, PP, KK

[z]Commercial blueberry fields follow two year production cycle and berries are harvested in alternate years (crop year). Berry yield results presented in table are from sites that are sampled sprout year leaf nutrients. Berries yields are measured from same sites in the crop year

Table 4.4 Analysis of variance (ANOVA) *P*-values of the central composite design in crop year of production

Source of variation	N	P	K	Ca	Mg	B	Berry yield
Blocks (samplings)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Regression	0.03	0.04	0.57	0.04	< 0.01	0.02	< 0.01
Lack of fit	0.99	0.99	0.99	0.94	0.73	0.96	0.73
Adjusted R^2	0.90	0.94	0.87	0.92	0.75	0.64	0.75
Significant terms	N	P		P, KK	K,NN, PP, KK	NN, PP	N, P, K, NN, PP, KK

Table 4.5 Canonical analysis performed for each response surface model in sprout year

Responses	Stationary points in real units (kg ha^{-1})			Y_S	Nature of stationary point
	N	P	K		
N (%)	14	41	36	2.011	Saddle
P (%)	27	59	27	0.159	Saddle
K (%)	35	43	27	0.548	Maximum
Ca (%)	34	38	34	0.454	Minimum
Mg (%)	32	50	34	0.116	Saddle
B (ppm)	32	50	31	24.7	Saddle
Berry yield (kg ha^{-1})	20	37	24	3620	Saddle
SD	7.9	7.9	4.5		

Table 4.6 Canonical analysis performed for each response surface model in crop year

Responses	Stationary points in real units (kg ha^{-1})			Y_S	Nature of stationary point
	N	P	K		
N (%)	24	47	27	1.698	Saddle
P (%)	36	50	37	0.163	Maximum
K (%)	22	51	33	0.541	Maximum
Ca (%)	36	32	34	0.484	Minimum
Mg (%)	27	45	37	0.119	Minimum
B (ppm)	31	45	34	20.77	Minimum
Berry yield (kg ha^{-1})	27	38	30	3170	Saddle
SD	5.6	6.8	3.6		

Table 4.7 Mineral concentration of wild blueberry leaves in sprout year and crop year of production

Nutrient	Sprout year				Crop year			
	Optimal ranges obtained through overlaying surface plots		Optimal concentrations reported by Trevett 1972		Optimal ranges obtained through overlaying surface plots		Optimal concentration reported by Trevett 1972	
	Min	Max	Min	Max	Min	Max	Min	Max
N (%)	1.8	2.03	1.60	2.00	1.5	1.7	1.50	1.75
P (%)	0.155	0.160	0.125	0.222	0.158	0.164	0.119	0.158
K (%)	0.53	0.55	0.400	0.900	0.535	0.545	0.300	0.700
Ca (%)	0.44	0.46	0.270	0.517	0.465	0.495	0.440	0.644
Mg (%)	0.115	0.130	0.130	0.249	0.115	0.125	0.150	0.330
B (ppm)	24	26	24	60	18	22	30	70

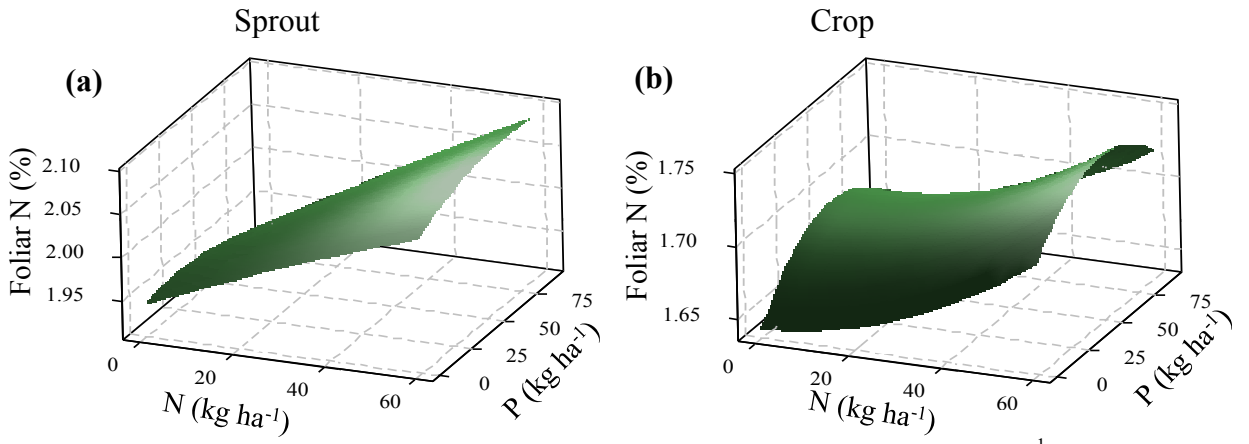


Figure 4.1 Response surface of foliar N (%), the effect of soil applied nitrogen (kg N ha^{-1}) and Phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}, (a) in sprout year and (b) crop year

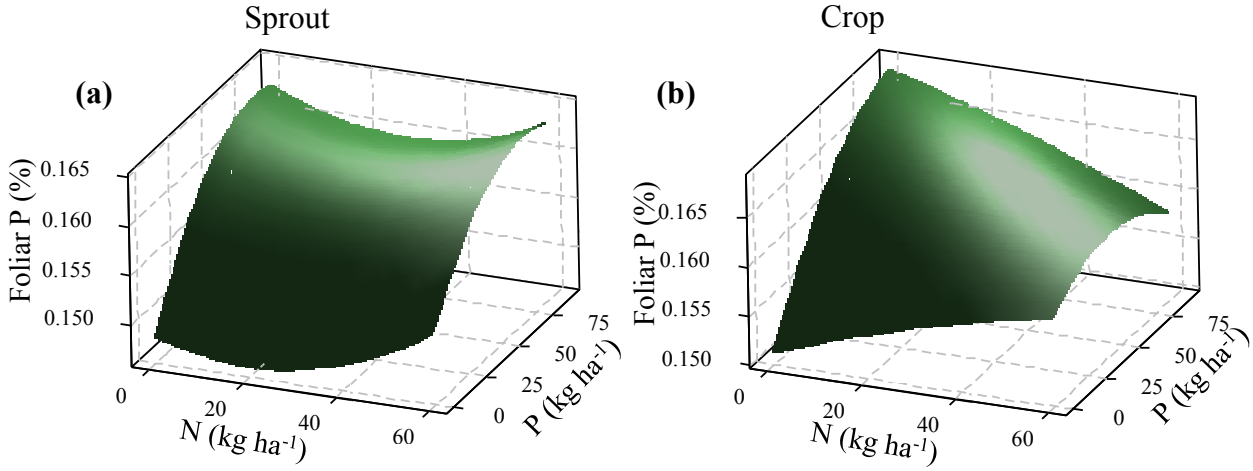


Figure 4.2 Response surface of foliar P (%) in sprout year, the effect of soil applied nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}, (a) in sprout year and (b) crop year

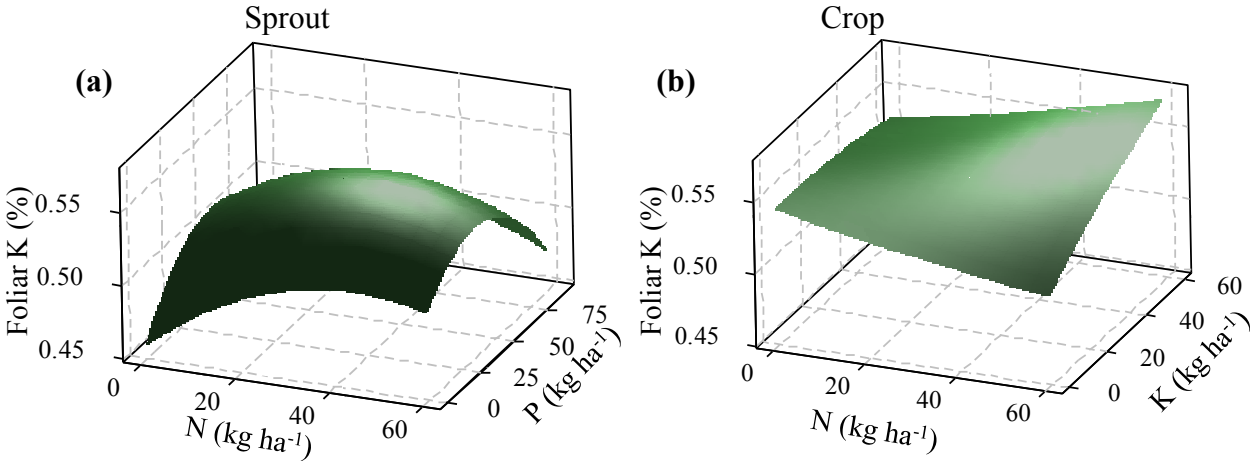


Figure 4.3 Response surface of foliar K (%) in sprout year (a), the effect of soil applied nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}. Response surface of foliar K (%) in crop year (b), the effect of soil applied nitrogen (kg N ha^{-1}) and potassium (kg K ha^{-1}) for phosphorus fixed at 45 kg P ha^{-1}

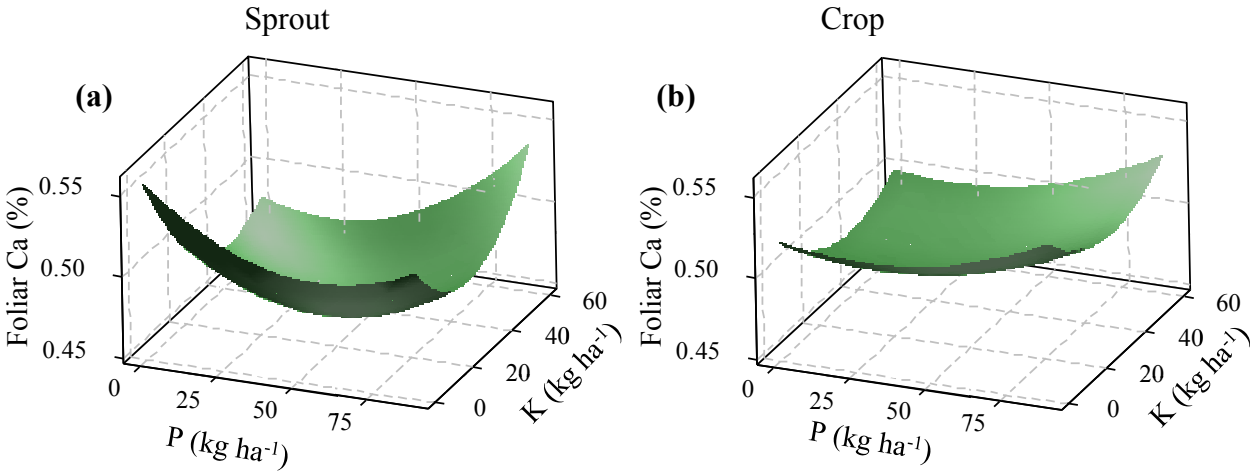


Figure 4.4 Response surface of foliar Ca (%), the effect of soil applied phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 kg N ha^{-1}, (a) in sprout year and (b) crop year

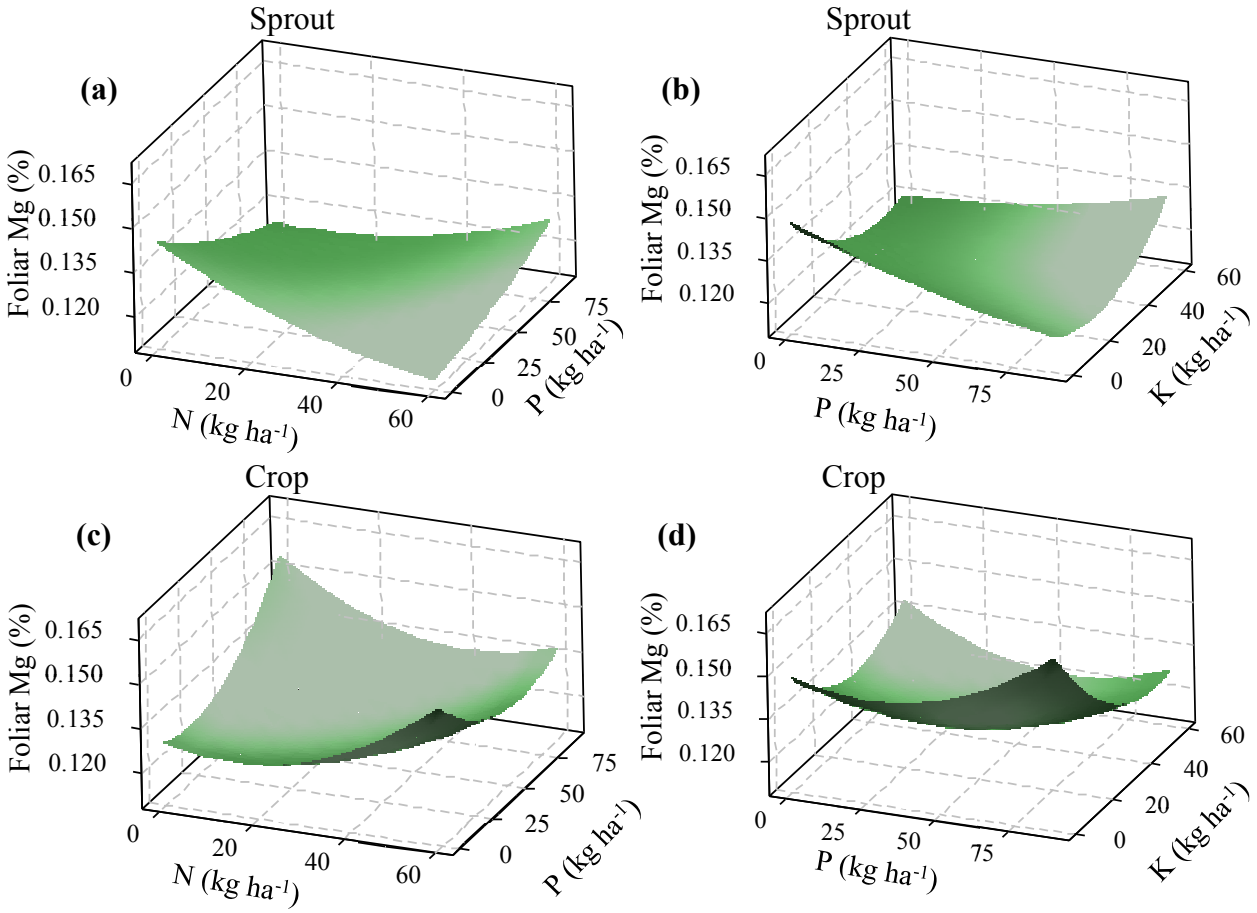


Figure 4.5 Response surface of foliar Mg (%) in sprout year: (a) the effect of soil applied nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1} and (b), the effect of soil applied phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 kg ha^{-1}. Response surface of foliar Mg (%) in crop year: (c) the effect of soil applied N (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1} and (d), the effect of soil applied phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 kg ha^{-1}

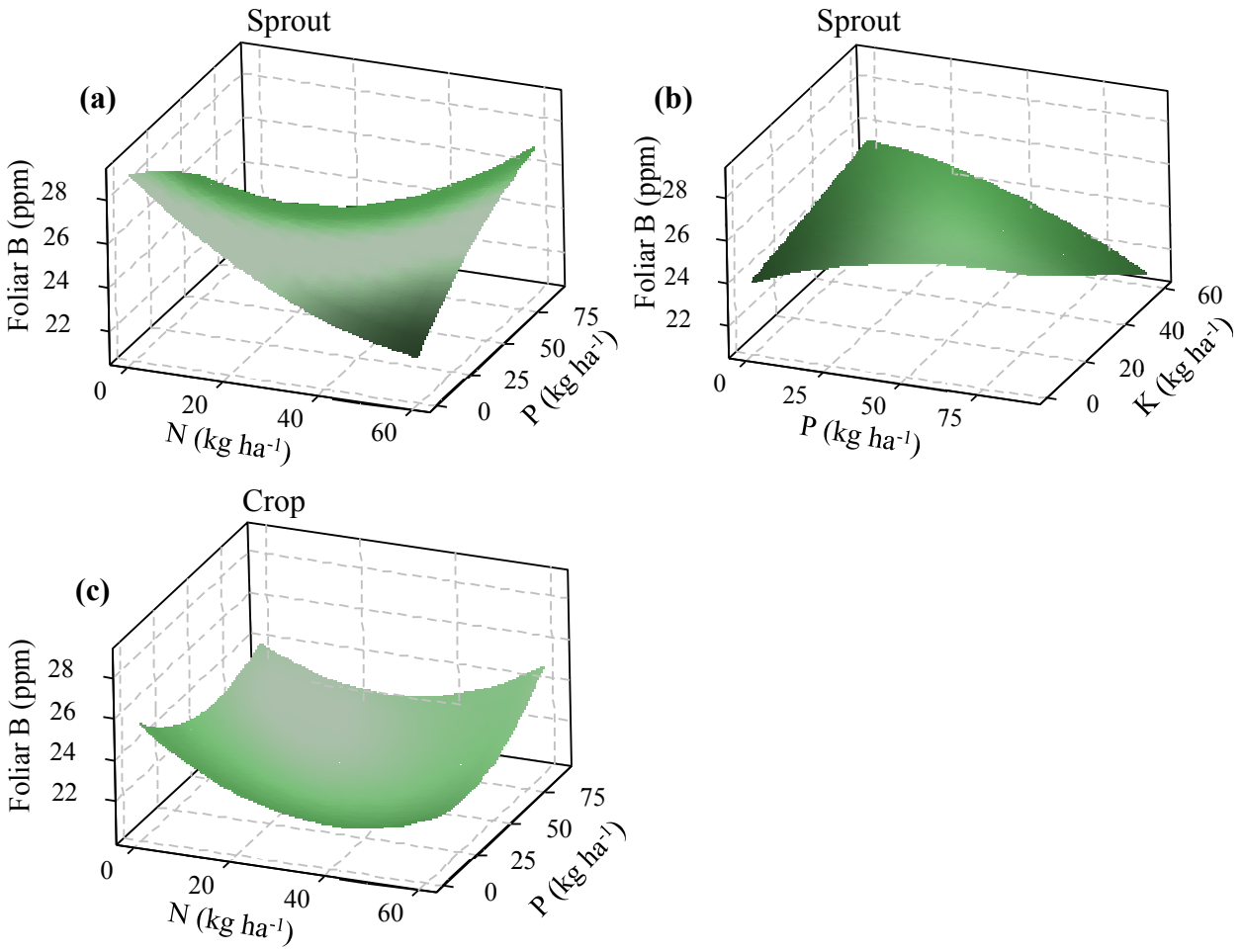


Figure 4.6 Response surface of foliar B (ppm) in sprout year: (a) the effect of soil applied nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1} and (b), the effect of soil applied phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 kg ha^{-1}. Response surface of foliar B (ppm) in crop year, (c) the effect of soil applied (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}

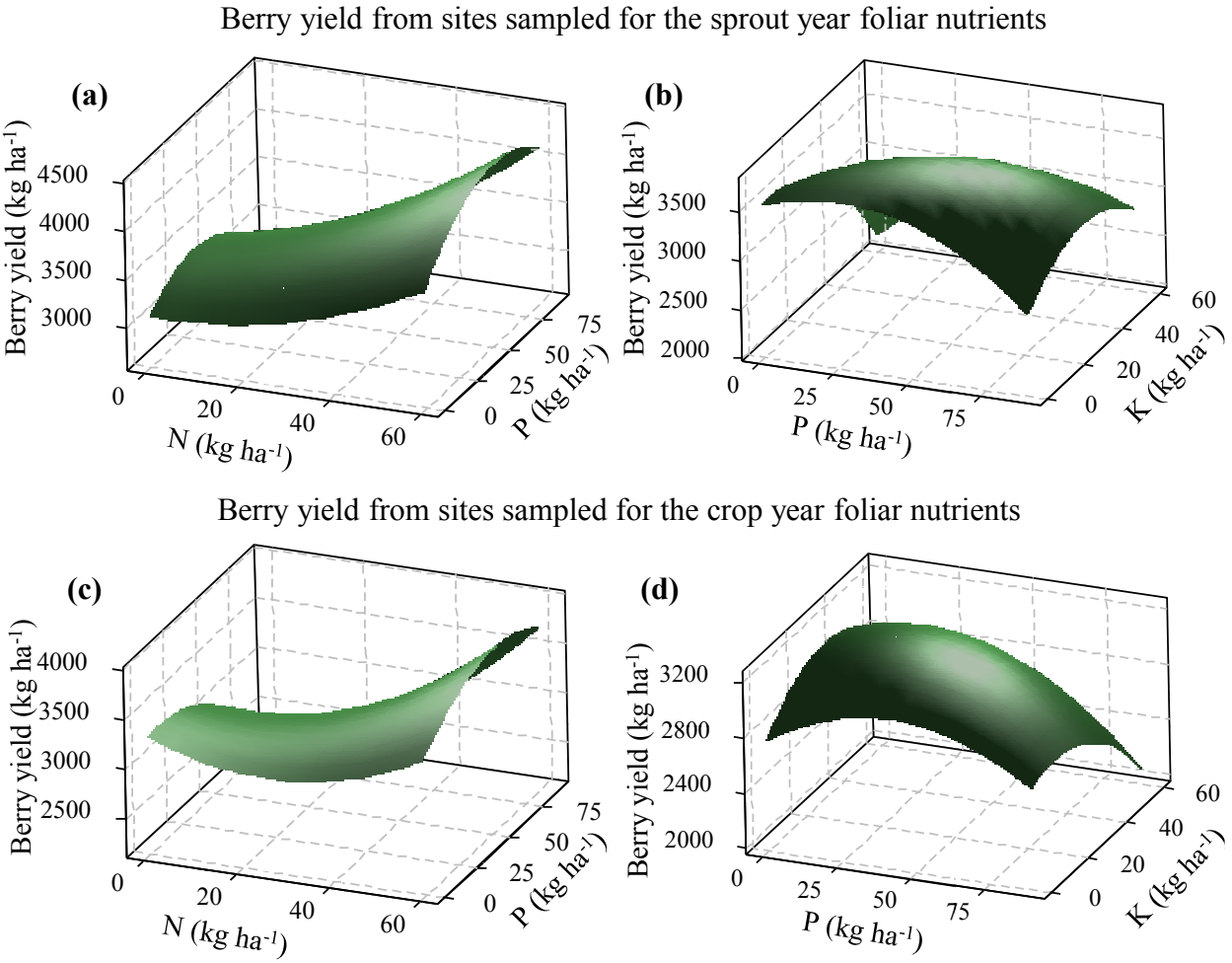


Figure 4.7 Response surface of berry yield (a) the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1} and, (b) the effect of phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 N kg ha^{-1} for sites sampled for foliar nutrients in sprout year of production. Response surface of berry yield (a) the effect of nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K $ha^{-1}$and, (b) the effect of phosphorus (kg P ha^{-1}) and potassium (kg K ha^{-1}) for nitrogen fixed at 30 N kg ha^{-1} for sites sampled for foliar nutrients in crop year of production

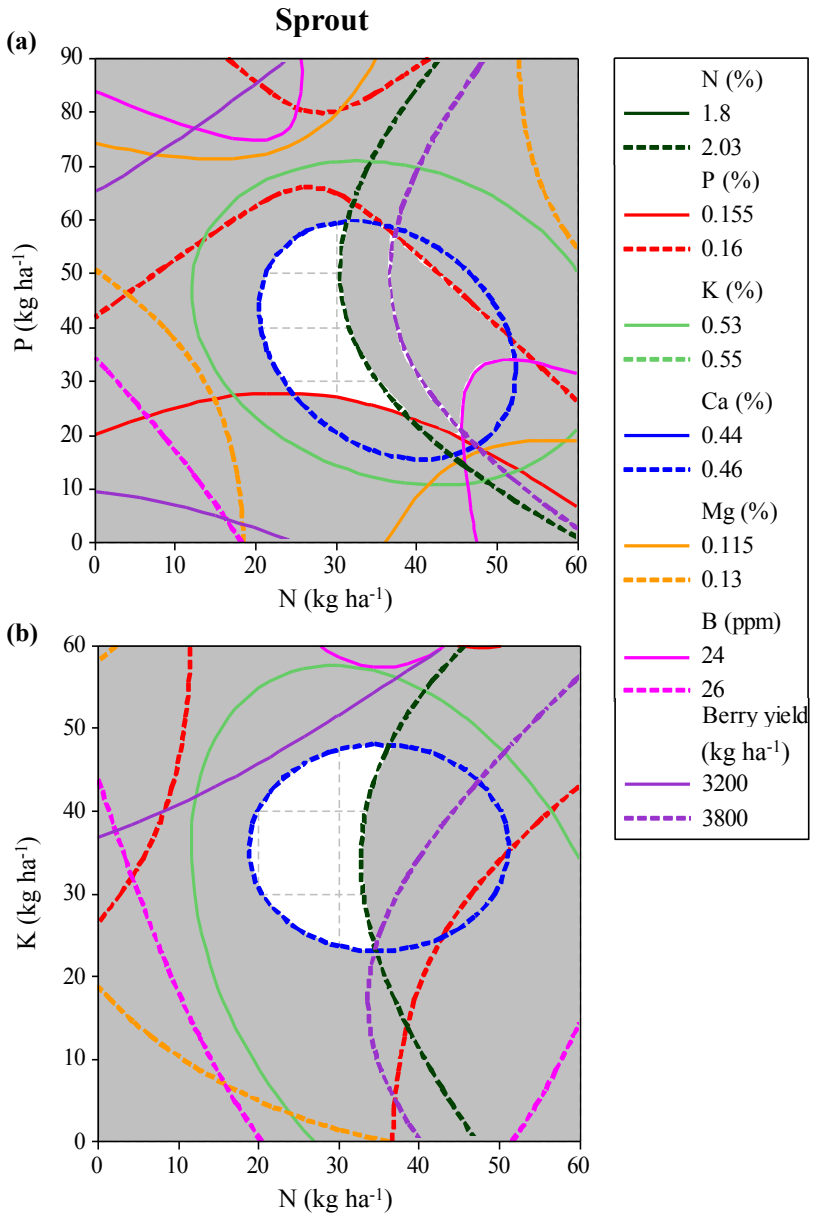


Figure 4.8Overlaid contour plots of response surface models for sprout year: (a) the effect of soil applied N (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg ha^{-1}),and (b) the effect of soil applied nitrogen (kg N ha^{-1}) and potassium (kg Kha^{-1}) for phosphorus fixed at 45 kg ha^{-1} on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg ha^{-1}). The white area is the Sweet Spot where the criteria of all responses are fulfilled

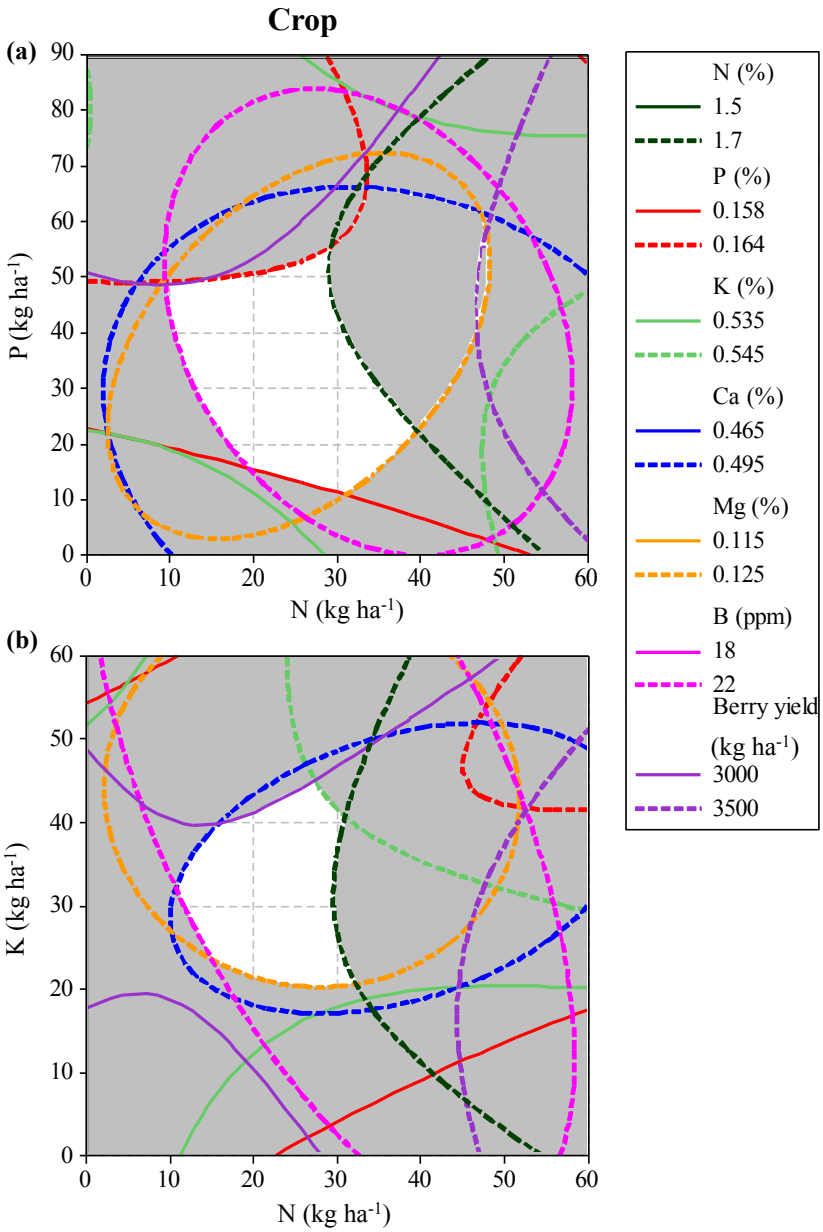


Figure 4.9 Overlaid contour plot of response surface models for crop year: (a) the effect of soil applied nitrogen (kg N ha^{-1}) and phosphorus (kg P ha^{-1}) for potassium fixed at 30 kg K ha^{-1}on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg ha^{-1}),and (b), the effect of soil applied nitrogen (kg N ha^{-1}) and potassium (kg Kha^{-1}) for phosphorus fixed at 45 kg ha^{-1} on foliar N (%), P (%), K (%), Ca (%), Mg (%), B (ppm), and berry yield (kg ha^{-1}). The white area is the Sweet Spot where the criteria of all responses are fulfilled

Chapter 5 Remote Sensing of Leaf Macro and Micro Nutrients in Wild Blueberry Stands

5.1 Abstract

The ability to remotely assess the nutrient status of the canopy is of interest to the wild blueberry industry with over 90,000 ha in production scattered throughout Maine, Quebec and the Atlantic provinces of Canada. Despite the potential of hyperspectral remote sensing to estimate foliar chemicals, field spectroscopy has been limited by scarce field data, expense of field missions and lack of suitable analytical methods (Martin et al. 2008). The goal of this study was to investigate the performance of chemometrics methods namely; standard full-spectrum partial least squares regression (FS-PLS), genetic algorithms partial least squares regression (GA-PLS), interval partial least squares regression (iPLS), and stepwise forward selection partial least squares regression (SFS-PLS) analyses for estimating wild blueberry foliar nutrients. We analyzed reflectance spectra taken from 709 wild blueberry canopies in sprout phase to estimate 12- foliar nutrients (N, P, K, S, Ca, Mg, Mn, B, Fe, Cu, Zn and Na) from 3-sites (located in Nova Scotia and New Brunswick) in 2006 andencompassing4 years for N (2006-2010). The intent was to develop an estimation method using a combination of partial least squares regression (PLS) methods and variable selection techniques (FS-PLS, GA-PLS, iPLS and SFS-PLS). By integrating these steps, we found that the accuracy and precision of multi-nutrient remote sensing of wild blueberry differed among nutrients, spectral range and chemometric method. Nitrogen, P, K, Ca, Mg and B were accurately estimated using visible-to-shortwave infrared spectroscopy (VSWIR; 400-2500 nm) while S, Na and transitional metals (Mn, Fe, Cu and Zn) were estimated with lower accuracy. We also found that foliar nutrients were retrieved with more

precision and accuracy in VSWIR range than the limited visible-to-near infrared range (VNIR; 400-1050 nm). The GA-PLS model best estimated foliar nutrients among all tested techniques. GA-PLS improved the estimation accuracy over FS-PLS by 8.2% and reduced wavelengths by 85% to VSWIR range. We reduced the wavebands to 10 using the SFS-PLS approach. The wavelengths selected by SFS-PLS are presented in Table 5.6. SFS-PLS yielded comparable results to VSWIR range with FS-PLS. These results proved to be independent of site conditions, varying growth conditions, thus they may be broadly applicable to multi-nutrient mapping in wild blueberry. Results from this study provide critical information with respect to the potential wavelengths that can be used in accurately and precisely estimating the nutrient levels of wild blueberries. Ongoing research however, is required to utilize this information in the development of a suitable nutrient estimation sensor for wild blueberry production.

5.2 Introduction

Wild blueberry nutrition involves at least 12 elements along with interactions with the soil environment making nutrition management complex and challenging task. Unfortunately, soil extraction techniques including Bray I, Mehlich I, Mehlich III, Olson, Modified Morgan and Anion Exchange membrane fail to accurately estimate soil nutrients that are available for plant uptake in wild blueberry production system (Ring 2001; Ring et al. 2005). The soil extracted phosphorus (P) and the leaf tissue P had non-significant correlations except at higher P application rates (Ring et al. 2005).The blueberry plant appeared to acquire sufficient amount of P through mycorrhiza that has the ability to release phosphate enzymes at the surface of the hyphae. The phosphate enzymes can mineralize organic P in the close vicinity of root-mycorrhiza association. Therefore, present P nutrient assessment techniques rely on leaf tissue sampling and subsequent traditional laboratory

analysis (Trevett 1972). Nutrient ranges have been developed for the tip-dieback stage of wild blueberry shoot growth (cessation of growth associated with the shoot apical meristem) which typically occurs in late July of the sprout year (Trevett 1972; Chapter 4). Optimal leaf nutrient ranges are provided (Table 4.7). Analytical techniques including use of LECO combustion CNS auto-analyzer and inductively coupled argon plasma atomic emission spectroscopy (ICAP-AES) require more than one week to complete, cost approximately \$20 sample^{-1} and fail to capture natural intra-field variability typical of wild blueberry stands. Time and cost constraints force most growers to apply fertilizers uniformly at recommended rates without taking into account crop demand. Additionally, the wild blueberry plant has a narrow optimal nutrient window for application and plant need. Wild blueberry has exhibited sensitivity to both excess and deficiency of nutrient(s) that resulted in nutrient imbalances and subsequent yield loss. Particularly, excessive N fertilization promotes vegetative growth, increases stem heights, delays transition to reproductive phase that results in reduced yield potential (floral bud and flower number), reductions in harvest efficiency and harvestable berry yields. Nutrients in excess of crop requirements may be prone to leaching and erosion that also jeopardizes environmental stewardship and sustainability of the system.

Remote sensing technologies can provide non-destructive and instant (i.e. "on-the-go ") estimation of nutrient levels within grower's fields. Remotely sensed vegetation spectra contain wide range of biochemical and biophysical information (Kokaly and Clark 1999). During past the 20 years, great technical advancements have been made in remote sensing with field, airborne and space-based technologies now existing that include estimating and mapping plant nutrients/chemicals (reviewed by Dorigo et al. 2007; Kokaly et al. 2009).

Remote sensing of foliar chemistry started in the early 1960's with work of Gates et al. (1965). Early studies discussed the mechanism by which radiant energy interacted with the leaf pigments and focused on relating spectra to the leaf photosynthetic pigments, N, water, leaf structural components, and spectral features associated with leaf chemicals (Curran 1989).Later, researchers had used imaging spectroscopy to estimate foliar N and other chemicals in forest canopies (Peterson et al. 1988; Wessman et al. 1988). During last 15 years imaging spectroscopy and field spectroscopy were widely used in estimation foliar chemicals using the visible-to-near infrared (VSWIR: 400-2400 nm) spectrum (Herrmann et al. 2010; Rambo et al. 2010).

Chlorophyll (Chl) and protein absorptions in VSWIR provide the basis for foliar chemical estimation (Kokaly et al. 2009). VSWIR spectra possess specific absorption features for foliar biochemicals such as Chl, protein, N, starch, lignin, cellulose and water. Chl is strongly absorbed in the visible (VIS) range at peaks 460 nm and 660 nm. A direct link exists between leaf N content and pigments that may be used to estimate foliar nutrients. This link results from the fact that four N atoms in the central tetrapyrrole head of the Chl molecule act to stabilize the central Mg ion (Kokaly 2009). However, Chl can directly hold a small proportion of total leaf N (1.7%) with about 19% of leaf N in C_3 plants allocated to light harvesting complexes (Evans 1983). High N fertilizer dose scan increase the Chl content thereby causing a deepening and widening of the absorption feature centered near 680 nm (Mutanga et al. 2003). A large proportion of leaf N (ca. 30-50%) is present in the form of a single protein, ribulose-1, 5-biphosphate carboxylase-oxygenase (RuBisCO) in green leaves (Evans 1983).The protein is made of N-containing amide bonds that give absorptions in the near-infrared (NIR; 750-1300 nm) and shortwave infrared (SWIR; 1500-2500 nm) ranges.

Multiple linear regression (MLR) procedures have been the most commonly used empirical method for building regression models in field spectroscopy studies. However, models constructed with MLR using hyperspectral data consist of hundreds of wavebands, with the end result often over fitting of the regression models and resulting in the uncertainty of the predictions that tend to increase with the relation k/n (where k and n are the number of predictor wavebands and observations used in regression, respectively). Therefore, the use of large number of wavebands may lead to a model with poor predictive ability. This problem can be further aggravated by the presence of multicollinearity between wavebands (typical of hyperspectral data) which degrade the conditioning of the regression, if k>n, it may not be possible to obtain MLR model because of singularity issue. Thus, MLR models had often limited usage with hyperspectral data particularly when using whole spectrums (Grossman et al. 1996). Regularization methods (ridge regression) or regression techniques based on latent variables, namely principal component regression and partial least squares regression (PLS) can be employed to avoid the MLR related problem described above (Martens and Næs 1989). Unlike MLR, latent vector methods such as PLS use all available wavebands simultaneously control collinearly among wavebands and take advantage of all the information present in different parts of spectrum (Martens and Næs 1989). For example, Asner et al. (2011) estimated 21 phytochemicals from 6136 humid tropical forest canopies using visible-to-shortwave near infrared (VSWIR; 400-2400 nm) with PLS method.

It is widely accepted that proper variable selection can improve the predictive ability of PLSR models in field spectroscopy (Kawamura et al. 2010). Variable selection decreases the cost and time involved in sensor measurements which is particularly important under routine field use. Fewer wavelengths are often easier to understand in terms of spectral band attributions (Galvão and Araújo 2009; Martens and Næs 1989). Several theoretical and

applied studies reported the improvement in the predictive ability of principal component regression (PCR)/PLS models through use of variable selection (Goicoechea and Olivieri 2003; Kawamura et al. 2010; Spiegelman et al. 1998; Zhao et al. 2013). Several approaches have been developed to select the most relevant and informative wavebands for regression models (see review by Galvão and Araújo 2009). Based on a few informative wavebands, field remote sensing has evolved over the years and benefits of these on-the-go systems have been demonstrated in winter wheat, corn, cotton, rice, citrus and other field crops (Raun et al. 2002; Schumann 2010). Such systems are commercially available from NTech Industries Inc. Ukiah, CA; Holland Scientific, Lincoln, NE; CropScan Inc. Rochester, MN and Yara International ASA, Oslo, Norway for use in field crops.

Despite the significant advancement to correlate leaf foliar N and canopy spectral data using PLSR (Maqbool et al. 2012), it remained unknown whether the spectral data could be used for other foliar nutrients and how the variable selection algorithms would improve the precision and accuracy of PLSR models.

Model portability is one of the major challenges in sensor development. We aimed to address model portability through methodology used in this study. Previous studies have suggested using large observations (calibration objects) for chemometric analysis (Martens and Næs 1989). Large calibration data sets stabilize the calibrations and build most general models (Martens and Næs 1989). Large sets of observations make models portable to new settings by spanning the wide spectral and foliar chemical variations expected to appear in future samples. The large observations are also significant because of the vast and inherent variability (phenotypic and genotypic) of wild blueberry fields. However, obtaining large sets of observations is particularly challenging in our wild blueberry producing region considering our Maritime climate such as a relatively short growing season (i.e. < 7 months),

high cloud cover, high humidity and windy conditions. Data acquisition in this study was managed by carefully planning field visits on clear sunny days.

The objectives of this investigation were to: (1) test the precision and accuracy of full-spectrum partial least squares regression (FS-PLS) models for various foliar nutrients based on VSWIR and VIS spectral ranges using field spectroscopy and (2) compare the performance of standard FS-PLS, genetic algorithms partial least squares regression (GA-PLS), interval partial least squares regression (iPLS), and stepwise forward selection-partial least squares regression (SFS-PLS) models for estimating foliar nutrient concentrations and improve precision and accuracy of models using a reduced set of wavelengths. To achieve these objectives, FS-PLS techniques using VSWIR and VIS spectral ranges were used due to FS-PLS techniques being reported to be the most successful regression technique for estimating foliar nutrients from field spectral data (Asner and Martin 2008; Kawamura 2010; Asner et al. 2011).

5.3 Materials and Methods

5.3.1 Experimental Sites

The experimental sites were fertilizer management trials which represent the typical wild blueberry fields of eastern Canada. The first commercial site was located near Kemptown, Nova Scotia (45° 30' 7.91" N, 63° 7' 27.72" W). The soil was typically sandy loam, acidic, imperfectly drained, stony and moderately rocky (Cobequid association). The second site was located near Brantville, New Brunswick (47° 22' 22.74" N, 64° 58' 17.48" W). The soil was typically sandy, acidic, poorly drained, non stony and non rocky (Acadie siding). The third site was located at Highland Village, Nova Scotia. A plot size of 6 x 8 m was used with a 2 m buffer between plots to reduce movement of fertilizer between plots. At

Kemptown and Brantville, a three factor (soil applied N, Phosphorous and Potassium) circumscribed centrally composite design (CCD) with four replications was used with sixteen treatment combinations with five levels (0%, 20%, 50%, 80%, and 100% of full application rate) including a no applied fertilizer treatment. (Table 5.1) Application rate consisted of N at 0-60 kg ha^{-1} in the form of ammonium sulfate, Phosphorous (P) at 0-90 kg P ha^{-1} in the form of triple superphosphate, and potassium (K) at 0-60 kg K ha^{-1} in the form of potassium chloride (Table 5.1). At Highland Village, randomized complete block design (RCBD) with 7 treatments consisted of 0, 10, 20, 35, 50, 70 and 100 kg N ha^{-1} in the form of ammonium sulfate. All treatments were replicated four times. At all three sites, fertilizer treatments were applied early May in the sprout year of production using a Scotts SR2000 rotary fertilizer spreader (Marysville, Ohio). This design resulted in the wide range of foliar nutrient values. The experimental area was managed by Bragg Lumber Company (Collingwood, Nova Scotia) under standard industry practices with provisions for pruning, agrochemical applications and introduction of pollinators (honey bees) (Maqbool et al. 2012).

5.3.2 Canopy Spectral Measurements

To develop a general approach to spectroscopic mapping of foliar chemistry in wild blueberry production system, we analyzed plant reflectance data, 12 inorganic nutrients, acquired from 709 and 255 plots for N and other nutrients respectively, and developed simple models using a combination variable selection methods and PLS regression. Spectral measurements dates were provided (Table 5.2).The canopy was closed at time of spectral measurements. The under story consisted of plant litter mainly due to leaf drop in fall of sprout year and mowed plant material after harvesting in crop year. The sampling dates were chosen to avoid the spectral signature of plant litter, soil and rocks. Spectra were acquired with an Analytical Spectral Devices (ASD) FieldSpec®3spectroradiometer (ASD Inc.

Boulder, CO). The instrument samples a spectral range of 350–2500 nm. The instrument optimization, white reference measurements, and settings such as number of scans per measurement were controlled through RS^3 6.0 software (ASD Inc. Boulder, CO). A fore optic lens of field of view (FOV) 10° was kept approximately 1 m above the crop canopy at nadir position. The ground field of view was approximately 17.5 cm in diameter. Spectra were recorded at seven spots from each plot along the diagonal encompassing the clone variability typical characteristic of blueberry fields. Each individual spectral measurement was average of 30 scans (Figure5.1). After fifteen minutes, the instrument was recalibrated taking scans of a white Spectralon® reference card (Labsphere Inc. North Sutton, NH) measuring 4.6 cm radius and an internal (dark) voltage measurement. The ratio of reflected radiance of the observed sample to the reflectance radiance of a known reference card was calculated. It allowed the adjustments to changing light conditions. All measurements were taken under clear sky conditions within 2 hours of solar noon to minimize the effects of the sun angle.

5.3.3 Plant Sampling and Nutrient Content Analysis

Stems were collected from the same seven spots on same day, used for spectral data (Table 5.2). Stems were kept in labeled plastic bags. The leaves were removed gently by grasping the stem at the base and pulling it. The leaf tissue samples were oven dried for 36 h at 60 °C. The samples were ground with a Wiley mill (Arthur H. Thomas Co. Philadelphia, PA) to pass through a 1 mm mesh screen. The samples were placed in dry, labeled bottles and stored for chemical analysis. N content was determined by combustion analysis using LECO CNS-1000 elemental auto analyzer (LECO Corp. St. Joseph, MI). Concentrations of other foliar nutrients (P, K, S, Ca, Mg, Mn, B, Fe, Cu, Zn and Na) were determined from

samples taken in 2006 at 2 sites using a Thermo Jarrell Ash ICAP61-1100 (Thermo Jarrell Ash Corp. Franklin, MA).

5.3.4 Foliar Nutrient Data

We summarized the range, mean, and coefficient of variation for foliar nutrient (Table 5.3). Data were collected from sites located at Kemptown, Brantville and Highland Village, first two sites were laid in CCD and Highland Village was laid in RCBD. Soil applied N, P and K were varied across wide range. This data set consisted of multiple years, growth stages, and sites of contrasting soil properties. It is also important to note that these settings resulted in broad range of foliar nutrient values, meeting and often exceeding the reported in previous studies (e.g. Trevett 1972; Chapter 4). We compiled foliar data with a range of 1.33-2.87% for N and 0.103-0.281% for P. Our coefficient of variations range for S, Ca, Mg, Zn and Na were 26, 29, 31, 40 and 46%, respectively. As a result, this foliar nutrient data and associated spectral data were typical of wild blueberry fields. Thus, we contend that the results from this study can be extrapolated to wild blueberry producing fields of northeastern North America with respect to foliar nutrient ranges and resulting spectral variations.

5.3.5 Data Analysis

5.3.5.1 Preprocessing of Spectra

ViewSpec (ASD Inc. Boulder, CO) was used to view and average the seven reflectance spectra of each plot to minimize the noise. Wavebands below 400 nm, above 2400 nm, between 1350-1450 nm and between 1850-1975 nm were removed due to high noise or lack of absorption features. The mean reflectance spectra of the wild blueberry used for foliar N (n = 709) and other nutrients (n = 255) have been provided (Figure5.1). The

question whether sample size of 256 is enough in remote sensing discipline. After examining different nutrient estimation studies where PLS and waveband selection method were used from wide range of vegetation system. The studies used sample size of 100 in single species studies. For example, n =100 (Kawamura 2010), n = 98 (Grossman et al. 1996), heather: n= 10, willow: n= 10, olive: n =18 (Ferwerda and Skidmore 2007), n = 90 (Mutanga et al. 2004), n = 137 (Martin et al. 2008), n = 6136 (Asner et al. 2011). Asner et al. (2011) was an international scale study in humid topical forests including reflectance spectra of hundreds of plant species from trees, liana, palm, hemi-epiphyte and vine. Other similar studies had sample size: n =72 (Hansen and Schjoering 2003), n = 342 (Nguyen and Lee 2006), n = 133 (Næsset et al. 2005) and n = 60 (Huang et al. 2004) and Huang et al. 2004 (n = 60). Considering the natural variability in wild blueberry fields, the sample size of 256 used in this study was adequate to run the further analysis. Spectral data was examined in Matlab 7.10 (The Mathworks, Inc. Natick, MA).

5.3.5.2 Chemometric Analyses (Partial Least Squares Regression (PLS)

Chemometric analyses were used to determine which foliar nutrient could be remotely sensed using reflectance spectroscopy. The compression of large number of collinear variables (e.g. hyperspectral data is commonly practiced in chemometrics (Martens 2001). Partial least square regression (PLS) is one of those techniques, which addresses collinear and high dimensional data (e.g. hyperspectral data). PLS belongs to a bilinear class of calibration methods. The bilinear modeling reduces/compresses a large number of measured collinear spectral variables to a few non-correlated (independent/orthogonal) factors/latent variables (LVs)/principal components in such a way that latent variables correlate with the response variable(s). Hence, high collinear data require few orthogonal latent variables to describe/account relevant variance (variance-covariance structure). The

PLS is used to parameterize a predictive model based on latent variables. The PLS provides the insight of data and predictive ability together. For example, Hansen and Schjørring (2003) modeled canopy biomass of wheat crops from hyperspectral reflectance data by employing PLS method.

PLS analyses were performed to relate reflectance spectra to field measured foliar nutrients.

The PLS equation is described as

$$Y = \beta w\, X + \varepsilon \qquad (5.1)$$

where Y is a vector with the measured foliar nutrients, X is a matrix of reflectance value per wavelength, ε is the error vector, βw is the matrix of estimated weighted coefficients. A large absolute weighted coefficient indicates an important/informative wavelength. The βw is calculated directly from the PLSR loadings corresponding to the model with the optimum number of PLSR latent vectors, based on the following equation

$$\beta w = W \times (P^T \times W)^{-1} \times Q^T \qquad (5.2)$$

where W is the X-weight loading matrix, P is the X loading matrix, Q is the Y loading matrix, and T indicates matrices that have been transposed. The loadings P are not orthogonal like W but usually W and P are usually similar, so it suffices to study one of them graphically (Martens and Næs 1989).More details pertaining to the PLSR algorithm can be found in Martens and Næs (1989). In the present study, optimal number of latent variables was determined based on the lowest root mean square error of cross validation (RMSECV). The details were provided in the validation static section (5.3.7).The setting used to run PLSR models in PLS tool box were as follows: analysis = PLS, algorithm = SIMPLS,

preprocessing = mean centered (X block), cross-validation, method = random subsets, Maximum number of LVs = 10, number of data splits = 10, Number of iterations = 20. Mean centering was used to obtain better linear approximation of possible slight non-linearities. We repeated PLSR analysis to test two common configurations in imaging spectroscopy, VNIR (visible to near-infrared; 400-1050 nm) such as the compact Air-borne Spectrographic Imager or CASI; and the VSWIR (visible to shortwave infrared; 400-2500) such as Airborne Visible/Infrared Imaging Spectrometer or AVIRIS.

In PLS modelling, a variable (x_k) may be important for modelling of *Y* (leaf nutrient). Such variables are identified by large PLS regression coefficients, However, a variable may also be important for modelling of X (reflectance spectrum) which is identified by large loadings. A summary of the importance of an X- variable for both Y and X is given by VIP (variable importance projection). The VIP value is namely a weighted sum of squares of the PLS weights (w), with the weights calculated from the amount of Y-variance of each PLS component which takes into account the explained variance of each PLS dimension (Indahl et al. 2009).

$$VIP_j = \sqrt{\frac{p}{\sum_{m=1}^{M} SS\,(b_m.t_m)} . \sum_{m=1}^{M} w_{mj}^2 \,.\, SS(b_m.t_m)}. \qquad (5.3)$$

Within this, p is the number of variables, M the number of retained latent vectors, w_{mj} the PLS weight of the j^{th} variable for the m^{th} latent vector and $SS(b_m \,.t_m)$ is the percentage of y explained by the m^{th} latent variable, w is the PLS weights. The "greater than one" rule is generally used as a criterion for variable selection because the average of squared VIP scores is equal to 1. The wavelengths were selected by visual inspection of VIP score plots and PLS models were built using selected wavelengths (Appendix C.1).

5.3.6 Variable Selection

5.3.6.1 Genetic Algorithm-Partial Least Squares Regression (GA-PLS)

Genetic algorithm (GA) is a biologically inspired global search method based on genetic principals and evolution by natural selection. In the GA, the first step is to generate a large number (e.g. 32, 64 and128) of random selections of the variables. Each subset of variables is called an individual. The personal traits of each individual are encoded in chromosomes, made by a very high number of genes (as many as the variables) and typically stored as binary strings in computer memory. Each gene (binary integer) being 1 bit long, 'zero' or 'one' (no/yes flag, 0 = variable absent, 1 = variable present) represent one gene, indicating which variables are used by that individual. The pool of all tested individuals is called population. GA iteratively calculates the root mean square error of cross validation (RMSECV) using subset of variables using any of regression methods (e.g. MLR or PLSR). The RMSECV values (fitness of the individual) indicate predictive ability of each individual in the selection. GA discards the individuals with fitness greater than the median fitness. At this point, the population has been shrunk to half to its original size. To replace the discarded variables (individuals), the GA mates (breeds) the retained individuals. At each iteration of GA, mating pool is formed by taking couples of individuals from the population. According to the principles of natural selection, GA favors the individuals with lower RMSECV (fitness function). Therefore, useful features of these individuals (i.e. variables subsets that lead to good model) are passed on to their descendants. After successive generations (iterations), the search narrows down to combination of variables that have been found with higher fitness. During this iteration computation (evolutionary process), one or more genes (bits) are swapped within or between individuals (crossover). The purpose of mutation is to increase the genetic diversity in the population. Briefly, selection and mutation (crossover) are applied

to initial populations to generate new populations. Further details and practical applications about GA can be found elsewhere (Leardi2001; Galvão and Araújo 2009).

GA is inspired by the biological mechanism of evolution by natural selection and is well suited for variable selection problems (Galvão and Araújo 2009). GA methods use a population of individuals to explore the space of possible solutions, while direct optimization methods (e.g. steepest ascent) can be unreliable, since they can be caught by a local optimum and give a solution far away from the global optimum (Galvão and Araújo 2009).Now it is widely recognized that the GA method is especially useful when the solution is searched from high dimensional data and has many local optima. Leardi et al. (1992) used simulated data set demonstrated that a GA can always find global solution of a simple problem in much less time than required by full search algorithms. In a comparative study, Lucasius et al. (1994) reported that GA generally performs better than stepwise elimination while simulated annealing was least efficient in finding global solution.

Over-fitting is the greatest risk when applying GA variable selection because of the large number of variables in hyperspectral data set. Studies have taken into account this aspect and suggested parameter settings with the highest possible "elitism", small population sizes and high mutation rates (Leardi and Lupiáñez González 1998).They suggest that the final product (sensor) should use a model which examines a high number (100) of independent runs, a moving average window (window size 3) that takes into account autocorrelations of spectral bands, that are less dispersed, provides better interpretability and contiguous blocks. GA was employed using the PLS toolbox 5.8 (Eigenvector Research Inc. Wenatchee, WA) by projecting reflectance spectra (VSWIR; 400-2400 nm) to GA and parameters and their settings are provided in Table 5.4.GA settings were closely matched to those suggested by Leardi (2000) to avoid over fitting except number of independent runs.

We used 5 instead of 100 independent runs that were not possible due to size of data set and speed of the computing resources. Parameter conditions were also set to match settings used in full spectrum, iPLS techniques for comparison purposes. For example, maximum allowable latent vector of 10 was used to provide even ground for all techniques and target as % of total variables (wavebands) was set to 2.5-3.0 to force GA-PLS to select ~ 250 wavebands, similar to that selected via iPLS method.

5.3.6.2 Interval-Partial Least Squares Regression (iPLS)

Interval-partial least squares (iPLS) is an interval/window based selection algorithm specifically designed for sequentially arranged variables such that VSWIR spectra. iPLS splits the spectra into non-overlapping equidistant regions, and then PLS models are built for each sub-interval using the latent vectors. Root mean square error (RMSE) is calculated for every sub-interval. The most useful sub-interval (variable ranges) are determined based on lowest of RMSE values. Then, the center point and width of intervals that gives best models were also adjusted following the predefined ranges to optimize results. The concept used in iPLS was proposed by Norgaard et al. (2000). iPLS algorithm was used in forward wavelength (intervals) selection mode. PLS models were built using successively improving intervals with respect to RMSE measurements, i.e. the first model is based on interval that has the best performing model, the second model is based on the next best interval, and the process continues until stopping criteria reaches. The other algorithm settings were: (1) the interval size was set to 5 wavebands to split data into non-overlapping and equidistant intervals; (2) the number of intervals in the model was 50 (Total wavebands in model: 5 x 50 = 250 wavebands; and (3) the maximum number of latent vectors allowed was set to 10. Adjacent variables such as hyperspectral data were likely to be highly correlated, so interval

based approaches such as iPLS was not recommended for MLR. Interval based techniques best suited for use with the latent variable methods such as PLS.

5.3.6.3 Stepwise Forward Selection-Partial Least Squares Regression (SFS-PLS)

Stepwise forward selection-PLS (SFS-PLS) is an iterative model wise addition technique. It is based on algorithm developed by Brown et al. (1991) and included in PLS toolbox 5.8. First, the PLS regression model is developed using the best single variable/interval on the basis of largest empirical correlation of predictor with the dependent variable in regression model. In each addition cycle, the predictor with the maximum importance is added, and the model is computed iteratively using the remaining variables/intervals. SFS step-by-step adds more variables to model until stopping criteria is met, which was the number of intervals in our study. The final model is that with the maximum predictive ability under given parameter conditions, defined by minimum value of RMSE. SFS-PLS does not account for multicollinearity. Another limitation of forward selection is that once a variable is included in a model, it cannot be removed. This means that there is also no verification of how other selected variables may perform in other possible combinations. SFS-PLS algorithm was applied as follow: the number of intervals used was ten from the entire spectrum, interval size of 1variale (wavelength) and auto step size (search from entire spectrum without gap or overlap as algorithm moves across spectrum). The automatic setting assured that there were neither overlaps nor gaps between intervals. These settings force the algorithm to select 10 wavebands. The maximum latent vectors of 10 were used to match with GA-PLS and iPLS and FS-PLS techniques.

5.3.7 Validation Statistics

To avoid over-fitting, optimal number of LVs retained in the full spectrum PLS models was determined by minimizing the RMSE statistic (Martens and Næs 1989).RMSE was used to evaluate the predictive ability of the FS-PLS, SFS-PLSR, iPLS and GA-PLS models. RMSE statistic was calculated through a cross-validation prediction for each model. A common way to estimate best/optimal number of latent variables by "leave-one-out" cross validation but "leave-one-out" cross validation has been found to over fit models (Shao 1993). Therefore, we used a random-subset cross-validation method. This procedure iteratively generates regression models while randomly reserving 10 disjoint groups from the input data until the RMSE statistic is minimized (Cruciani et al. 1992). The RMSE statistic provides direct estimate of the model error expressed in original measurement units. RMSE measures how well the model fits the data while RMSECV measures model's ability to predict new samples (Martens and Næs 1989). Both together (RMSE and RMSECV) gives the accuracy of models. The coefficient of determination (R^2) assesses the precision of models developed. The RMSE and R^2 statistics were applied to compare the precision and accuracy of models. According to Mutanga et al. (2004), RMSE values were standardized to the percentage mean (%RMSE). It provides simple comparisons across foliar nutrients and models.

The RMSE was calculated as:

$$RMSECV = \sqrt{\frac{\sum_{v=1}^{v} (y_i - \hat{y}_i)^2}{v}} \qquad (5.4)$$

where y_i and $\hat{y}_i$ were the measured and predicted foliar nutrients, respectively, and v was the number of samples. All data handling, variable selection and regression calculations

were performed using PLS toolbox 5.8 (Eigenvector Research Inc. Wenatchee, WA) in Matlab 7.1 (The Mathworks Inc. Natick, MA).

5.4 Results and Discussion

5.4.1 Plant Optical Properties

Broad variation was found in reflectance spectra among the 709 for N and 256 for other foliar nutrients in wild blueberry samples (Figure5.1a). We observed widest reflectance range in the near-infrared (NIR; 750-1300 nm) (Figure5.1a). We found largest coefficients of variation in shortwave-infrared (SWIR; 1900-2500 nm), followed by the visible spectral region (VIS: 400-750) (Figure 5.1b). This agrees previous findings that, although the reflectance range is lowest in the VIS and SWIR, the spectral variation is proportionally greater in these spectral regions (Asner et al. 2011). This wide variation is a requisite to develop general calibration models. The peaks at 1400 nm, 1850-1950 nm and 2350-2500 nm represent water absorptions and water induced/related excessive noise in the spectrum (Kumar et al. 2001).

5.4.2 Full-Spectrum Partial Least Squares Regression (FS-PLS)

5.4.2.1 FS-PLS with VSWIR and VNIR Spectral Regions

FS-PLS modeling using VSWIR reflectance results indicated that foliar N, P, and Ca can be estimated accurately and precisely (Table 5.5). For these foliar nutrients, regression R^2-values and %RMSE ranged from 0.74 to 0.82 and 9.5-15.7%, respectively. The foliar K and B predicted with high accuracy (%RMSE = 7.2 and 12.4%) but medium precision (R^2 = 0.55 and 0.50). The K and B results deviated from normal pattern (nutrients predicted with high accuracy normally have high precision also and vice versa (Table 5.5 and Figure

5.2).The exact reason for this deviation is not known but it could be due to low variability K and B (CV = 10.8 and 18.1%) compared to average CV 31.7% of other nutrients (Table 5.3).

Other well-predicted foliar nutrients included Mg, Mn, Fe and Zn (R^2 = 0.51-0.67; %RMSE = 16.2-26.2%). The remaining nutrients (S, Cu and Na) displayed weaker relationship with VSWIR spectra, with R^2-values ranging from 0.25 to 0.44, and %RMSE remain below 24% except for Na (%RMSE = 31.9%). These less well predicted results, particularly Cu, are useful because they demonstrated that PLSR does not over-fit any given parameter, a concern sometimes associated with this approach. Variable importance in projection (VIP) scores and latent vector analysis were provided (Figure 5.3). The VIP scores of foliar N were similar in shape and magnitude as those reported by Maqbool et al. (2012). The latent vector and VIP scores indicated that important wavebands in predictions were distributed across the entire spectral range (400-2400 nm). The shortwave infrared range (1050-2400 nm) and red edge position (~690 nm) generally took high weights and VIP scores for all foliar nutrients. The wavelength selection based on VIP scores is presented in Table 5.6 and further discussion may be found in section 5.4.4.

Prediction estimate accuracy was reduced for all foliar chemicals when data was limited to the VNIR spectral range. Overall, the R^2-value and %RMSE was decreased by 17.0 and 9.1%, respectively (Table 5.5). These results agree with those of found by Asner et al. (2011) using similar technique in humid tropical forests. They reported that lower and less consistent precision and accuracy in foliar nutrient estimation was found using the limited spectral range of VNIR (400-1050 nm) than using the VSWIR range (400-2500 nm). Foliar nutrients most negatively affected by only using VNIR spectra included S, Mn, Cu and Na, the precision dropping by 30% on average. P and K showed decreases in R^2-value by 18.3% and 12.7%, respectively while N and Mg had their R^2-values decreased by 5.4 and 8.9%,

respectively (Table 5.5). The foliar nutrients N, Ca and Mg that are associated with chlorophyll molecules were least negatively affected because chlorophyll holds specific absorption features in the visible spectral range (Kokaly et al. 2009).

5.4.3 Variable Selection

5.4.3.1 Genetic Algorithm Partial Least Squares Regression (GA-PLS)

GA-PLS estimated foliar nutrients with the best precision and accuracy. When GA-PLS was employed, R^2-value and %RMSE increased on an average by 11.9 and 8.2%, respectively compared to VSWIR samplings (Table 5.5). Best predicted foliar nutrients were N and P (R^2 = 0.78 and 0.85, respectively) and %RMSE less than 10%. Overall, prediction accuracy and precision for different nutrients followed the similar pattern as that of VSWIR spectrum. GA-PLS diagnostic plots were provided indicating the wavebands and their frequency of inclusion in calibration and prediction model (Figure 5.4). GA-PLS plots confirm VIP score results that the entire spectrum possesses useful information and contributed to final predictions. Additionally, SWIR section was found more important than other spectral regions.GA-PLS can also be used as an aid to spectral interpretation, to understand which spectral regions are correlated with a specific characteristic of the product. The waveband selection using GA plots is discussed in section 5.4.4. GA-PLS analysis was performed using 5 runs instead of 100 runs due to limited commuting resources available. This could be the possible limitation and GA may not have provided the best possible wavelength selection, this limitation can be overcome in future by employing high computing machines.

5.4.3.2 Interval Partial Least Squares Regression (iPLS)

Foliar N, P and Ca were best predicted with R^2-values ranged from 0.72-0.80 and %RMSE below 17% with iPLS (Table 5.5). iPLS results were comparable to FS-PLS (VSWIR, VNIR spectral ranges) and GA-PLS. Other nutrients predicted with greater than 50% precision were K, S, Mg, B, Fe, and Zn and accuracy range for these nutrients ranged from 7.2-25.1% (Table 5.5). Using iPLS selected wavebands compared to VSWIR spectral range; R^2-values were increased for K, S, B, Cu Zn and Na, reduced for N, P, Mg, Mn and Fe while R^2-value was same for Ca, accuracy (%RMSE) as increased for S, Cu and Zn and decreased for all other nutrients. Including all nutrients, R^2-values iPLS prediction results increased by 1.8% and RMSE dropped by 0.9% compared to VSWIR spectral range. These mixed improvements over full spectrum (VSWIR) could be attributed to the limitation of forward selection. Forward iPLS algorithm starts selecting wavebands (intervals) from the first portion of the spectrum (VNIR). Once a waveband (interval) was included in the model it could not be removed in subsequent iterations. Additionally, we forced it to select 50 intervals, so it may have missed some informative wavebands located in the SWIR region. Graphical output from iPLS contributed towards the interpretation of the chemical system. The iPLS figures were provided showing the selected intervals, corresponding RMSE and full spectrum RMSE (Appendix C.4).The main drawback iPLS is that this procedure does not test all possible intervals, and may result local solution.

5.4.3.3 Stepwise Forward Selection-Partial least Squares Regression (SFS-PLS)

Although SFS-PLS estimated foliar nutrients with the least precision and accuracy among the tested techniques it performed surprisingly well despite its local search heuristic. Cross validated results between SFS-PLS selected wavelengths and foliar nutrients are

provided R^2 values ranged from 0.18 to 0.77 (Table 5.5). Although the wavebands were reduced to 10 by imposing restriction on selection for the estimation of each nutrient, the macro nutrients (N, P, Ca and Mg) were predicted with adequate precision and accuracy. The best predicted accuracy with SFS-PLS was obtained for foliar N and P (R^2 = 0.69 and 0.77, respectively) and % RMSE were 11.9% and 10.3%, respectively. Wavebands selected by SFS-PLS and related priori information for each of the 12 nutrients are presented (Table 5.6).

The initial plan was to use stepwise forward selection with MLR, but examining the VIF statistic (consistently greater than 20), we used PLSR instead. MLR analysis has been widely used to regress spectral reflectance measurements against biochemical concentrations (Grossman et al. 1996) but it is well known that the stepwise regression method suffers from the multicollinearity issue and the selection of bands that fail to correspond with known absorption bands (Grossman et al. 1996).By examining results of stepwise procedure, 55% of selected wavebands were agreed to known absorption bands (Priori information-Table 5.6). These results were contrary to the commonly held view that SFS selected wavebands generally did not correspond to known absorption features (Grossman et al. 1996). We had used Brown's algorithm that was different algorithm than commonly used stepwise multiple linear regression method.

5.4.4 Wavelength selection

The selection of wavelengths is an important step towards the development of general models and ultimate development of sensor for predicting/mapping foliar nutrient in wild blueberry plants. The methods presented in this study were: 1) variable importance in projection (VIP) and wavelengths were selected on basis of high VIP scores and their correspondence to known absorption wavelengths through visual inspection of VIP plots (Figure-Appendix C.2).A total of 10 wavelengths were selected for each nutrient (Table 5.6),

PLS models were built using VIP selected wavebands and results are presented in Appendix C.1. The precision and accuracy of foliar nutrient estimation by VIP selected wavebands closely match to GA and iPLS and SFS-PLS (Table 5.5 and AppendixC.1). iPLS selected wavelengths (250) by the algorithm (Table 5.6 and Appendix. C.4) and SFS-PLS selected wavelengths (10) by the algorithm (Table 5.6). GA algorithm selected wavelengths (~250) presented in plots (Appendix C.3), for GA emphasis would on important spectral regions rather than specific wavelengths because of two reasons, First I was unable to extract the wavelength vector to present specific wavelengths and second selected wavelengths (250) are scattered in the entire spectrum not stacked together as was in iPLS (interval selection).Therefore, emphasis will be placed on examining wavelengths that corresponded to N and protein related absorption bonds. In future, it may be more useful to run GA with settings that select wavelengths as intervals, results will be more interpretable and useful for sensor development. The foliar nutrients were divided into macro (N, P, K, S, Ca and Mg) and micro (Mn, B, Fe, Cu, Zn and Na) nutrient for discussion on particular absorption feature and important wavelengths.

5.4.4.1 Macro nutrients

For N, the most consistently influential reflectance wavelengths occur in VIS, REP and SWIR region of reflectance spectrum. The selected wavelengths were related to leaf Chl (430, 429, 421-430, 436-490, 460 and 631-640), green peak (550 and 552), REP (691-710, 693 and 700), structural components (cellulose and lignin), starch and sugar (1526-1540, 1780, 1736-1740, 2080, 2100) and protein and nitrogen (1029@N-H stretch, 1507, 1510 @N-H stretch, 1st overtone, 1686-1690@C-H stretch, 1st overtone, 1725@C-H stretch, 1975-1980@N-H asymmetry, 2060@N-H stretch, N=H rotation, 2300@C-H rotation, C=O stretch, N-H stretch). Most of the selected wavelengths for leaf N related to N-H and C-H bonds

found in proteins (Kumar et al. 2001; Maqbool et al. 2012), Chls a and b absorptions (Kokaly et al. 2009; Maqbool et al. 2012), Green peak (Mutanga et al. 2004; Maqbool et al. 2012) and REP (Mutanga and Skidmore 2007; Maqbool et al. 2012). By examining the GA plots (Figure 5.4), one can see 430 and 460 (Chls), 690 (REP), 1020-1030, 1730, 2180 and 2350 (Protein and N related absorptions).Based on wavelength selection results from all four methods, 430, 460, 640, 550, 690-710, 1020-1030, 1510, 1690, 1725-1730, 1975-1980, 2060, 2181, 2300 and 2350 (Chl, N and protein related) were important wavelengths in leaf estimation. It is also suggested that adding few wavebands from our selection related to structural component to final sensor development, in order to explain the variability in leaf N not captured by N and protein related wavelengths.

The N is structural component of Chl molecules and N is involved in the protein synthesis that promotes the photosynthesis (Kokaly et al. 2009). Leaf Chls a and b are the primary light absorbing (VIS range) pigments in photosynthetic process, therefore, Chls determines the spectral reflectance in VIS range and relationship between concentration of leaf N and Chls absorption band is expected. The strong correlation between the content of leaf N and concentration of Chl a and b have reported by Peñuelas et al. 1994; Mutanga et al. 2004; Kokaly et al. 2009; Maqbool et al. 2012). Green peak is the expression of Chl absorptions of either side of the peak and Gitelson et al. (1996) reported the importance of Green peak in estimation leaf N. Maqbool et al. 2012 found green peak ($R^2 = 0.85$) among tested regions in VSWIR reflectance range for estimating leaf N in wild blueberry. A combination of strong Chl absorptions at red wavelengths and high reflectance in NIR region due to internal leaf scattering forms the REP (Mutanga and Skidmore 2007). Because REP is related to Chl absorptions, it has been found relation to leaf N (Maqbool et al. 2012; Mutanga and Skidmore 2007).

The selection of wavelengths in the SWIR region within ±10 nm of known protein and N absorption bands are connected to the absorption of electromagnetic radiation by leaf biochemicals. These absorptions originate from the energy transition of the molecular vibrations specifically rotation, bending and stretching of the C-H, N-H, O-H, C-N and C-C bonds in leaf tissues (Kokaly 2009; Rabkin 1987). The chemical composition of leaf tissues determines the type and number of bonds present that partially affect the amount of light reflected at any specific wavelength. Therefore, spectral signature of the leaf tissue possesses the information about the leaf chemical composition (Kokaly 2009).

Some selected wavelength for leaf N were related to leaf structural components, starch and sugars. This could be explained by the role of N in synthesis of these compounds, plant growth and development. The starch is formed in chloroplasts as a result of photosynthesis during the day and sugar and starch synthesis rates are related to photosynthetic rates, starch is remobilized at night to support continued respiration, and growth in the dark. In this context, starch has been identified as a major integrator in the regulation of plant growth to cope with continual changes in carbon availability.

Although there are no specific absorption features exist in VSWIR spectrum for other foliar nutrients than N, but mineral nutrients are required for photosynthetic electron transport as well as for ATP formation (N, P, Mg, Fe, S, Cu, Zn, Mn) (Marschner 1995).The same apply to the other macronutrients (P, K Ca and Mg) and micronutrients are involved (directly or indirectly) in the photosynthetic process, leaf tissue components and other metabolic functions, therefore, relationship between leaf macro nutrients and leaf reflectance, especially to those wavebands related to Chls, protein, N, cellulose, lignin, sugar and starch is expected. Following discussing on functions of leaf nutrients (macro, micro and beneficial) is based on Marschner (1995).

The wavelength selection results for leaf P suggested 430, 460, 670, 553 (Chl), 906-910, 1016-1020, 1506-1510, 2060 (N and protein), 1526-1530, 1580, 1686-1695, 1965, 1996 (Structural components, starch, sugars) are important wavelengths in leaf estimation. The Leaf P estimation information seems to equally distribute among Chl, N, protein and other constituents of leaf. In contrast to N results, no band was selected in REP of spectrum, probably indicating leaf P is less related to plant biomass than leaf N. In leaves, inorganic phosphate (Pi) strongly affects photosynthesis and carbon portioning in the light-dark reactions (Marschner 1995).P is involved in starch synthesis through ATP that is energy rich phosphate required for starch synthesis. The P function in plant provides the basis of relationship between leaf P content and Chl, protein and starch absorption wavelengths.

For leaf K, wavelength length selection methods selected 908, 1022, 1500-1510, 1520, 1980, 2180 (Protein) and 989, 1525-1555, 2080 (starch, sugar, cellulose). The K is required for protein synthesis in higher plants (Marschner 1995) hence the relationship between leaf K and protein absorption features was expected, The leaf K is not related to any of the wavebands in VIS spectrum in contrast to N and P, Although, K affects photosynthesis (Marschner 1995). The selection of starch and cellulose related bands may be explained by K role sugar and starch production and it role in cell wall development (expansion) in conjunction with Ca and B (Marschner 1995). The protein, starch and cellulose related absorption bands emerged as importation in leaf K estimation.

Leaf S content is also closely related to leaf Chl absorption features (430, 460, 546, 661-685, 689), protein (1009, 1980, 1971-1980, 2160-average expression of 2 protein absorption peaks located at 2130 and 2180, 2300, 2166-average expression of 2 protein bonds located at 2130 and 2180).S is a constituent of amino acids and hence proteins (Marschner 1995). S is a catalyst in Chl production and a decrease in Chl content of leaf

tissues is typical feature of S deficiency (Marschner 1995). The protein and Chl related function of S in plants may explain the association between leaf S content and Chl and protein related absorption bands. Leaf S concentration is related to oil related absorptions features in the VSWIR spectrum (930, 1040 and 2310). This may indicate the role of S in leaf oil content. Other wavelengths important in S estimation are 1120, 1526, and 2271-2345.

The important wavelengths for leaf Ca estimation are 430, 460, 554, 665(Chl), 1422, 1580, 2004, 1531-1560 (Starch lignin, cellulose and sugar), 1021, 2065, 2231(Protein and N). By examining the leaf Ca wavelength results (Table 5.6), wavelength related to Chl, cellulose, lignin and sugar selected more often than wavelengths related to leaf protein. Ca involved in starch metabolism and has structural roles in cell wall and cell membrane. Ca bound as pectate in the middle lamella is required for strengthening the cell walls (Marschner 1995).The may explain the association between leaf Ca content and wavelengths related to cell wall structural components absorptions. Ca is also reported to affect Chl content and net photosynthesis in forest trees (Liu et al. 2011), this relation may explain the association of leaf Can and Chl absorption bands.

The important wavelengths for leaf Mg estimation are 436-560, 635, 699 (Chl) 1490, 1566-1580, 1690, 1997, 2081-2130 (lignin, cellulose, starch, sugar), 1971-1990, 2140, 2350 (N, protein). The wavelengths related to Chl and REP appeared multiple times, indicating the role of Mg in photosynthesis and biomass production. Mg is central atom of Chl molecule (light absorbing pigment) in green plants and thus essential for photosynthesis. Mg and K are required for efficient use of absorbed light energy in photosynthetic CO_2 fixation to avoid light damage. Mg is required for synthesis of amino acids thus protein synthesis, sugar synthesis, carbohydrate partitioning (Marschner 1995). This may explain wavebands associated with protein and carbohydrate absorption in the spectrum.

5.4.4.2 Micronutrients

Leaf Mn content was correlated to wavebands 440, 460,553, 700 (Chl), 1550, 2080, 2100 (Starch, sugar, cellulose), and 1690, 1980, 2130, 2240 (protein and nitrogen). The results may be explained by the direct role of Mn in photosynthesis by aiding in the Chl formation and Mn is constituent of the water splitting enzyme (hill reaction) within the electron transport chain for photosynthesis and assimilation of CO_2 in photosynthesis. Mn is a constituent of enzyme that helps the synthesis of chloroplasts.

Leaf B concentrations are also correlated to leaf Chl absorption features (430, 640, 667), protein absorption features (1024, 1690, 1730, 2240 2352). This correlation may be explained by the B involvement in nitrogen metabolism and protein synthesis. Other wavelength found important in estimation leaf B content are 1490, 1690, 2000, 2247, 2280, 2352 (cellulose, lignin, starch, sugar). B has been known to maintain a balance between sugar and starch (regulation of carbohydrate metabolism by increasing the rate of transport of sugars which are produced by photosynthesis from mature leaves to actively growing regions), translocation of sugar (sugar-borate complexes) and starch, constituents of cell walls (involved with Ca in cell wall structure) and membranes.

The informative wavelength to estimate leaf Fe are 421-452, 430, 440, 636, 700, (Chl), 1690, 2126, 2351-2355 (protein, N), 1481-1490, 2270, 2341-2345, 2351-2355 (Cellulose, sugar Starch) and (931-935, 2311-2320) oil. Fe is linked to photosynthesis process by biosynthesis of chlorophyll, formation of chloroplast protein (iron containing-heme- proteins in plants example of which are cytochromes).Fe is also part of proteins such as ferredoxin. Cu is constituent of enzyme involved of electron transport in photosynthesis. Cytochromes are found in the electron transfer systems in chloroplasts and mitochondria. The function of Cu function is to accept and donate electrons and plays important roles in the

electron-transport chains of photosynthesis and respiration. Cu is also involved in lignin formation.

Wavelengths found important in leaf Cu estimation are 430, 439, 444, 452, 460, 545, 670, (Chl) and 1730, 2137 2178 (protein, N). This relationship may be explained as Cu is a constituent of amino acids and proteins found in the several enzyme systems. Cu functions as a catalyst in photosynthesis and involved in electron transport in photosynthesis. It functions as electron carrier in enzymes during oxidation reduction reactions in plants. Cu is also involved in Chl synthesis by aiding in Fe utilization. Other important wavelengths (1200, 1580) related cellulose, lignin, starch and sugar. Cu is important to the formation of lignin in plant cell wall, Cu affects the storage and sugar contents of fruits.

The wavelength found influential in estimation leaf Zn are 430, 450, 460, 548, 702 (Chl), 1716, 2191 (protein) and 1736, 2106 (Cellulose, Starch). The selected wavelength and their association specific absorption feature may be explained by the role of Zn in plants. Zn plays a both functional (catalyst) and structural role in enzyme reactions. Zinc an important component of various enzyme systems (carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, and protein involved in DNA replication) that are responsible for driving many metabolic reactions in plants including energy production, carbohydrate metabolism, protein synthesis, and cytochrome synthesis, formation of Chl and growth regulation.

The wavelengths found important for leaf Na are 440, 460, 553, 702, (Chl) 1720, 1978, 2030, 2240 (protein) and 1537, 2080, 2100 (starch, cellulose). Na is not essential nutrient but only reported to promote growth in certain C4 plant species, is usually categorized as beneficial nutrient and Na can substitute for K in non-specific functions, such as being an osmoticum during cell enlargement (Marschner 1995).

The selected wavelengths were generally around the known absorption features. The wavelength selected by four different methods differs due to different criteria used by each wavelength selection method and matches because of common biochemical basis of each foliar nutrient. By closely examining the wavelength results of each foliar nutrient, there are wavebands that are selected from the waveband regions with no prior known absorption features, with low signal-to-noise, regions of strong atmospheric water absorptions generally produced models with lower precision and accuracy. I suggest not using such wavebands in future sensor development.

5.5 Conclusions

Remote sensing of foliar chemistry has the potential to accurately and precisely estimate the nutrient content of the wild blueberry. The accurate nutrient mapping depends on the development of general and reliable approach, using field spectroscopy. Here we found that macro and micro foliar nutrients can be estimated using a combination of consistently measured spectral and chemical data from multi-years representing wide nutrient and growth ranges, FS-PLS analysis, and variable selection techniques those chose informative wavebands as well as reduce model complexity. Even with careful implementation of these steps and using advanced chemo metric analysis, our ability to predict multi-nutrient with high precision and accuracy depends on the foliar nutrient and spectral wavelengths. Under these conditions, we are best able to determine macronutrients. Micronutrients relatively less well estimated, but they do remain measurable with somewhat less accuracy. The best prediction results follows this order; GA-PLS, VSWIR (FS-PLS), iPLS, VNIR (FS-PLS) and SFS-PLS. Our data represent wide nutrient ranges, multi-years, multi-growth phases and contrasting site conditions, and thus we believe this is an approach that will be broadly applicable to multi-nutrient remote sensing in wild blueberry producing region.

Finally, we conclude that foliar nutrients are estimated far more consistently, and with greater precision and accuracy, using the VSWIR (400-2400 nm) spectral range than with the VNIR (400-1050 nm) samplings. Differences in prediction efficiency are varied by foliar nutrient, macro nutrients generally estimated with greater accuracy than the micronutrients. Foliar nutrients are predicted best using GA-PLS selected wavebands. And comparable results are obtained using iPLS and stepwise forward selection methods. We found latent vector technique such as PLSR is required with collinear spectral data even after wavelength reduction. Wavelengths are generally chosen across the entire VSWIR spectrum (400-2400 nm), utilizing SWIR and visible spectral range more frequently than the near infrared observations, using variable selection methods. Selected wavelengths correspond to known spectral absorption features particularly those located in SWIR range. The full range (SWIR and VNIR) results provide the possibly of mapping nutrients with imaging spectrometers such as CASI, AVIRIS and a recently launched next generation imaging sensor CAO-AToMS (http://cao.ciw.edu). The wavelength selection results a major step towards reaching the goal of senor development utilizing informative wavelengths, nutrient mapping in wild blueberry production region.

Table 5.1 Real and coded values of the central composite experimental design

	Coded values			Real values		
	N	P	K	[z]N	P_2O_5	K_2O
					(kg ha^{-1})	
Factorial points	-1.00	-1.00	-1.00	12	44	12
	1.00	-1.00	-1.00	48	44	12
	-1.00	1.00	-1.00	12	176	12
	1.00	1.00	-1.00	48	176	12
	-1.00	-1.00	1.00	12	44	48
	1.00	-1.00	1.00	48	44	48
	-1.00	1.00	1.00	12	176	48
	1.00	1.00	1.00	48	176	48
Star points	-1.68	0.00	0.00	0	110	30
	1.68	0.00	0.00	60	110	30
	0.00	-1.68	0.00	30	0	30
	0.00	1.68	0.00	30	220	30
	0.00	0.00	-1.68	30	110	0
	0.00	0.00	1.68	30	110	60
Center points	0.00	0.00	0.00	30	110	30
	0.00	0.00	0.00	30	110	30
	0.00	0.00	0.00	30	110	30
	0.00	0.00	0.00	30	110	30
Zero Fertilizer				0	0	0

[z]The source of soil applied N was ammonium sulfate, P was triple superphosphate and K was potassium chloride

Table 5.2 Schedule of spectral measurement, corresponding foliar sampling were performed ± 3 days of spectral measurements

Year	Site	Cropping cycle	Foliar nutrient
1st July 2006	Kemptown	Sprout	N and other nutrients
3rd August 2006	Brantville	Sprout	N and other nutrients
14th August 2006	Kemptown	Sprout	N and other nutrients
18th September 2006	Kemptown	Sprout	N and other nutrients
25th June 2007	Highland village	Sprout	N
29th June 2007	Kemptown	Crop	N
13th July 2007	Brantville	Crop	N
3rd August 2007	Highland village	Sprout	N
6th September 2007	Highland village	Sprout	N
15th July 2009	Kemptown	Crop	N
25th June 2010	Kemptown	Sprout	N
19th July 2010	Kemptown	Sprout	N
16th August 2010	Kemptown	Sprout	N

Table 5.3 Leaf foliar nutrients properties (n = 709 in case of N; n = 255 for all other nutrients) for wild blueberry

Nutrient	Mean	Range	CV %	Units of measure
N	1.868	1.33-2.87	19	%
P	0.157	0.103-0.281	28	%
K	0.505	0.362-0.632	11	%
S	0.093	0.051-0.169	26	%
Ca	0.491	0.187-0.844	29	%
Mg	0.123	0.059-0.227	31	%
Mn	704.894	295.7-1662	37	ppm
B	24.854	15.29-37.73	18	ppm
Fe	28.203	16.13-57.9	32	ppm
Cu	3.494	1.612-6.615	29	ppm
Zn	15.101	6.431-40.491	40	ppm
Na	0.007	0.002-0.019	46	ppm

Table 5.4 Parameter settings of the genetic algorithms partial least squares (GA-PLS) regression

Parameter	Condition
Population size	64 chromosomes
Regression method	PLSR
Maximum number of latent variables	10
Window width	5
Target as % of variables	2.5-3.0%
Maximum generations	50
Mutation rate (probability of mutation)	0.5%
Cross-validation settings	Random subsets with 10 splits and 5 iterations
Replicate runs	10

Table 5.5 Prediction results using full-spectrum partial least squares (FS-PLS) regression with reflectance data representing visible-to-shortwave infrared (VSWIR; 400-2400 nm), visible-to-near infrared (VNIR; 400-1050 nm), Genetic algorithm (GA) -PLS (250-265 wavelengths selected from VSWIR), interval partial least squares (iPLS) (250 wavelength intervals selected from VSWIR) and Stepwise forward selection (SFS)-PLS (10 wavelength intervals selected from VSWIR). Regression coefficient of determination is the R^2-value of the prediction, and %RMSE is normalized as percentage of the parameter mean

Parameter	FS-PLS (VSWIR)		FS-PLS (VNIR)		GA-PLS		iPLS		SFS-PLS	
	R^2	%RMSE	R^2	%RMSE	R^2	%RMSE	R^2	%RMSE	R^2	%RMSE
N	0.74	9.5	0.70	10.4	0.78	8.2	0.72	9.7	0.69	10.3
P	0.82	10.8	0.67	14.7	0.85	9.6	0.80	11.4	0.77	11.9
K	0.55	7.2	0.48	7.2	0.58	7.0	0.56	7.2	0.56	7.2
S	0.41	19.2	0.29	21.2	0.59	16.0	0.58	16.5	0.34	20.3
Ca	0.74	15.7	0.70	16.9	0.78	14.4	0.74	16.1	0.60	15.7
Mg	0.67	16.2	0.61	17.9	0.68	14.6	0.63	17.1	0.60	16.2
Mn	0.57	22.9	0.39	27.1	0.58	22.2	0.38	27.4	0.47	25.1
B	0.50	12.4	0.45	13.5	0.55	12.0	0.51	12.6	0.41	10.3
Fe	0.53	19.9	0.49	20.7	0.54	19.6	0.51	20.2	0.53	19.8
Cu	0.25	23.8	0.18	24.9	0.35	22.5	0.28	23.5	0.18	22.6
Zn	0.51	26.2	0.42	28.7	0.54	25.4	0.56	25.1	0.42	25.4
Na	0.44	31.9	0.31	34.6	0.52	29.1	0.45	31.9	0.29	30.5

Table 5.6Selected wavelengths form VIP, iPLS and SFS-PLS algorithms to estimate foliar nutrients in wild blueberry fields using reflectance spectra, with information on wavelengths and absorbing compounds

Nutrient	Method	Wavelengths selected (nm) and [Z]Interpretation in brackets
N	VIP	**430**(Chla@430), **460**(Chlb@460), **550**(Gp@550), **700**(REP@700),**1510**(Pr,N@1510&St@1530), **1780**(Clu,Sg,St,@1780), 2030, **2060**(Pr, N@2060), **2080**(St,Sg@2080), **2100**(St,Clu@2100), **2300**(Pr,N@2300)
	iPLS	**421:430**(Chl a@430),**436:490**(Chl b@460), 501:505(U),**631:640**(Chl b@640),**691:710**(REP@700), 946:950(U), 1131:1155(Lg@1120), 1166:1170(U), 1311:1315(U), 1326:1330(U), 1351:1355(U),**1526:1540**(St@1530&St,Clu@1540),**1546:1550**(St,Clu@1540),**1571:1575**(St,Su@1580), 1651:1670,**1676:1680**(Lg,St,Pr@1690),**1686:1690**(Lg,St,Pr@1690),**1736:1740**(Pr@1730&Clu@1736),**1766:1770**(Clu,Sg,St@1780),**1791:1795**(Clu,Sg,St@1780),**1971:1975**(Pr@1980)
	SFS-PLS	403, 419, **429**(Chla@430), **552**(Gp@550), **693**(REP@700), **1029**(Pr@1020), **1507**(Pr,N@1510), **1725**(Pr@1730), 2038 (expression of 2 Pr bands@1980&2060), **2300**(Pr,N@2300)
P	VIP	**430**(Chla@430), **460**(Chlb@460), 674(Chla@660), 1087(U), **1450**(St,Sg,W,Lg@1450), **1580**(St,Sg@1580), **1965**(St,Sg@1960), **1996**(St@2000), **2060**(Pr,N@2060), **553**(Gp@550)
	iPLS	581:590(U), 721:730(U), 751:760(U), 766:770(U), 776:790(U), 801:830(U), 836:845(U),**906:910**(Pr@910), 931:935(Ol@930), 1**016:1020**(Pr@1020), 1026:1030(U), 1051:1100(U), 1106:1110(U), 1201:1205(W,Clu,St,Lg@1200), 1221:1230(U), 1236:1270(U),**1506:1510**(Pr@1510), 1511:1520(Pr@1510), **1526:1530**(St@1530),**1686:1695**(Lg,St,Pr@1690) 1701:1705(U)
	SFS-PLS	675(Chla@660), 1172(U), 1318(U), 1168(U), 1428(Lg@1420), 1164(U), 1497(Clu,Sg@1490&Pr,N@1510), **1580**(St,Sg@1580), **1499**(Clu,Sg@1490&Pr,N@1510), 1176(U)
K	VIP	680(U), 780(U), **1040**(Ol@1040), **1520**(Pr,N@1510&St@1530), **1445**(St,Sg,W,Lg@1450), **1980**(Pr@1980), 2032(U), **2080**(St,Sg@2080), **2180**(Pr,N@2180), **930**(Ol@930)
	iPLS	511:520(U), 701:765(U), 771:785(U), 886:890(U),**1041:1080**(Ol@1040), 1296:1300(U),**1500:1510**(Pr,N@1510),**1526:1555**(St,Clu@1540), 1601:1630(U), 1666:1670(U), 1826:1830(U), 2386:2390(U), 2406:2420, 2441:2450(U)
	SFS-PLS	1978(U), 780(U), **989**(St@990), 1112(Lg@1120), 835(U), **908**(Pr@910), 2216(U), **1022**(Pr@1020), 870(U), 1549(St,Clu@1540)

Table 5.6 Continued

Nutrient	Method	Wavelengths selected (nm)
S	VIP	**430**(Chla@430), **460**(Chlb@460), **546**(Gp@550), 780(U), **930**(Ol@930), **1120**(Lg@1120), **1526**(St@1530), **1980**(Pr@1980), 2030(U), **2080**(Sg,St@2080), 2160(Over all expression of 2 Pr bands@2130&2180)
	iPLS	**661:685**(Chl@660), 751:755(U), 766:795(U), 811:815(U), 826:830(U),**926:930**(Ol@930), 936:940(U), **1040**(Ol@1040), 1076:1090(U),**1171:1200**(W,Clu,St,Sg@1200) 1291:1305(U),**1971:1990**(Pr@1980),**2271:2345**(St,Clue@2280, Pr,N@2300, Ol@2310, St@2320, Clu@2340)
	SFS-PLS	762(U), **929**(Ol@930), 755(U), 1179(U) 1009(Pr@1020), 1227(U), 2144(Pr@2130), **657**(Chla@660), 689(REP@690-710), 2166(Over all expression of 2 Pr bands@2130&2180)
Ca	VIP	403(U), **430**(Chla@430), **460**(Chlb@460), **554**(Gp@550), **665**(Chla@660), **1422**(Lg@1420), **1580**(St,Sg@1580), **2004**(Sg@2000), **2231**(Pr@2240), **2065**(Pr,N@2060)
	iPLS	586:590(U), 736:760(U), 771:845(U), 931:935(U),**1021:1025**(Pr@1020), 1096:1100(U),**1176:1240**(W,Clu,St,Sg@1200),**1531:1560**(St,Clu@1540), 1946:1950(U), 1956:1960(St,Su@1960), 2001:2005(U), 2021:2025(U),**2241:2245**(Pr@2240), 2376:2380(U), 2436:2440(U)
	SFS-PLS	**666**(Chla@660), 1175(U), 1320(U), 1296(U), **1421**(Lg@1420), 1083(U), **556**(Gp@550), **1532**(St@1530&Sr,Clu@1540), **2002**(St@2000), 1238(U)
Mg	VIP	583(U), 607(U), **635**(Chlb@640), **699**(REP@700), **1455**(St,Sg,W,Lg@1450), **1490**(Clu,Sg@1490), **1690**(Lg,St,Pr@1690), **1997**(St@2000), 2158(Over all expression of 2 Pr bands@2130&2180), 440(Chla@430)
	iPLS	**436:560**(Chl@460), 1316:1320(U),**1566:1580**(St,Su@1580), 1636:1640(U),**1971:1990**(Pr@1980), 2026:2050(U),**2081:2130**(St,Su@2080, St,Clu@2100, Pr@2130), 2131:2135(Pr@2130)
	SFS-PLS	693(REP@700), 1308(U), **645**(Chlb@640), 1665(U), **2350**(Clu,N,Pr@2350), 2175(Pr,N@2180), 2016(U), 1329(U), 1748(Clu@1736), **2140**(Pr@2130)

Table 5.6 Continued

Nutrient	Method	Wavelengths selected (nm)
Mn	VIP	**440**(Chla@430), **460**(Chlb@460), **553**(Gp@550), 609(U), 626(Chlb@640), **700**(REP@700), 1090(U), 1140(U), **1550**(St,Clu@1540), 2215(U)
	iPLS	596:610(U), 1236:1255(U), 1276:1280(U), 1606:1625(U),**1686:1700**(Lg,St,Pr@1690)**1966:1995**(Pr@1980), 2026:2050(U),**2066:2160**(St,Su@2080, St,Clu@2100, Pr@2130),**2221:2245**(Pr@2240)
	SFS-PLS	1170(U), 406(U), 612(U),576(U), 1304(U), **2100**(St,Clu@2100), **2181**(Pr,N@2180), **456**(Chlb@460), **2240**(Pr@2240), 2153(expression of 2 Pr bands@2130&2180)
B	VIP	585(U), 603(U), 675(Chla@660), **1490**(Clu,Sg@1490), **1690**(Lg,St,Pr@1690), **2005**(St@2000), 2145 (Pr@2130), **2240**(Pr@2240), 1089(U), **2280**(St,Clu@2280)
	iPLS	**416:500**(Chl@430, Chl@460), 531:575(U), 606:610(U), **626:640**(Chl@640), **1111:1120**(Ol@1120), 1151:1155(U), **1691:1735**(Lg,St,Pr@1690, Pr@1730), 1796:1800(U), **1946:1965**(St,Sg@1960), **2001:2015**(St@2000)
	SFS-PLS	**2247**(St@2250&Pr@2240), **2352**(Clu,N,Pr@2350), 1084(U), 1671(U), 1324(U), 1302(U), **1024**(Pr@1020), 1173(U), **667**(Chla@660), 1338(U)
Fe	VIP	**440**(Chla@430), 485(U), 584(U), 603(U), **636**(Chlb@640), **700**(REP@700), **1690**(Lg,St,Pr@1690), 2150(Over all expression of 2 Pr bands@2130&2180), 2205(U), **2270**(Clu,Sg,St@2270)
	iPLS	406:415(U),**421:455**(Chl@430), 561:575(Gp@550), 681:685(U), 746:760(U), 771:785(U), 796:895(U),**931:935**(Ol@930), 1326:1330(U), 1396:1400(W@1400),**1481:1490**(Clu,Sg@1490),**2311:2320**(Ol@2310, St@2320), 2331:2335(U),**2341:2345**(Clu@2340),**2351:2355**(Clu,N,Pr@2350), 2371:2375(U)
	SFS-PLS	**696**(REP@700), **635**(Chlb@640), **2126**(Pr@2130), **648**(Chlb@640), 2143(Pr@2130), 2151(Over all expression of 2 Pr bands@2130&2180), 2157(Over all expression of 2 Pr bands@2130&2180), 1178(U), **431**(Chla@430), 1307(U)

Table 5.6 Continued

Nutrient	Method	Wavelengths selected (nm)
Cu	VIP	**430**(Chla@430), **439**(Chla@430), **444**(Chla@430), **452**(Chlb@460), **460**(Chlb@460), 485(U), **545**(Gp@550), 1140(U), **1580**(St,Sg@1580), 2145(Pr@2130)
	iPLS	706:715(U),**1036:1095**(Ol@1040), 1126:1150(U),**1196:1265**(W, Clu, St,lg@1200)**1691:1755**(Pr@1730), 2216:2235 (U)
	SFS-PLS	1181(U), **453**(Chlb@460), 1337(U), 1323(U), **2137**(Pr@2130), 1296(U), **2178**(Pr,N@2180), 609(U), 857(U), 887(U)
Zn	VIP	**430**(Chla@430), **450**(Chlb@460), **460**(Chlb@460), **548**(Gp@550), 672(Chla@660), **702**(REP@700), 1075(U), 1554(St,Clu@1540)
	iPLS	401:420(Chla@430), 706:760(U), 766:775(U), 781:850(U), 1221:1260(U), 1701:1705(U),**1716:1760**((Pr@1730,Clu@1736), 1766:1770(U)
	SFS-PLS	1083(U), **1044**(Ol@1040), 755(U), 1108(Lg@1120), 1153(U), 1049(Ol@1040), **670**(Chla@660),2191(Pr,N@2180), **2106**(St,Clu@2100), 2212(U)
Na	VIP	**440**(Chla@430), **460**(Chlb@460), 1200(W,Clu,St,Lg@1200), **1720**(Pr@1730), **470**(Chlb@460), 485(U), **553**(Gp@550), 610(U), 677(U), **702**(REP@700)
	iPLS	571:580(U), 606:610(U),**1036:1085**(Ol@1040), 1451:1455(St,Su,W,Lg@1450), 1636:1640(U), 1646:1655(U), 1676:1680(U), 1906:1910(U), 1941:1945(W,Pr,Lg,Clu@1940),**1971:1990**(Pr@1980), 2021:2035(U), 2041:2055(U), **2071:2135**(St,Sg@2080, St,Clu@2100, Pr@2030, 2236:2240(Pr@2240), 2261:2265(U), 2361:2370(U), 2381:2385(U), 2396:2405(U)
	SFS-PLS	**551**(Gp@550), **710**(REP@700), 1227(U), 1258(U), **538**(GP@550), **454**(Chlb@460), 1135(Lg@1120), 1148(U), **1978**(Pr@1980), **1537**(St,Clu@1540)

[Z]Interpretation in brackets, unattributed (U), Chlorophyll a (Chl a), Chlorophyll b (Chl b), Green peak (Gp), Red edge position (REP), Protein (Pr), Nitrogen (N), Starch (St), Sugar (Sg), Lignin (Lg), Cellulose (Clu), Oil (Ol) and Water (W)

The bold face wavelengths referred that selected wavelength (VIP, SFS-PLS) or range (iPLS) was within ± 10 nm of known absorption feature (foot note of Table 5.6), italic face meant that selected wavelength (VIP, SFS-PLS) or range (iPLS) was within ± 20 nm of known absorption feature and normal font meant that selected wavelength was unidentified

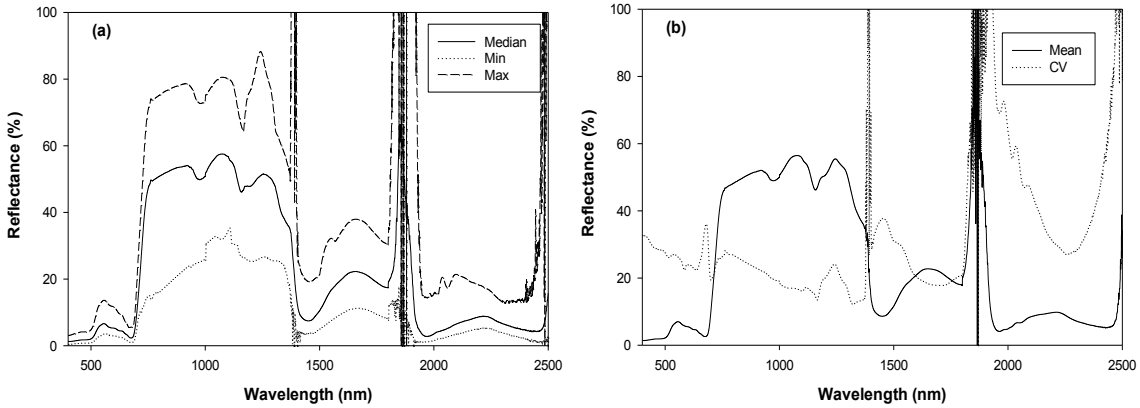
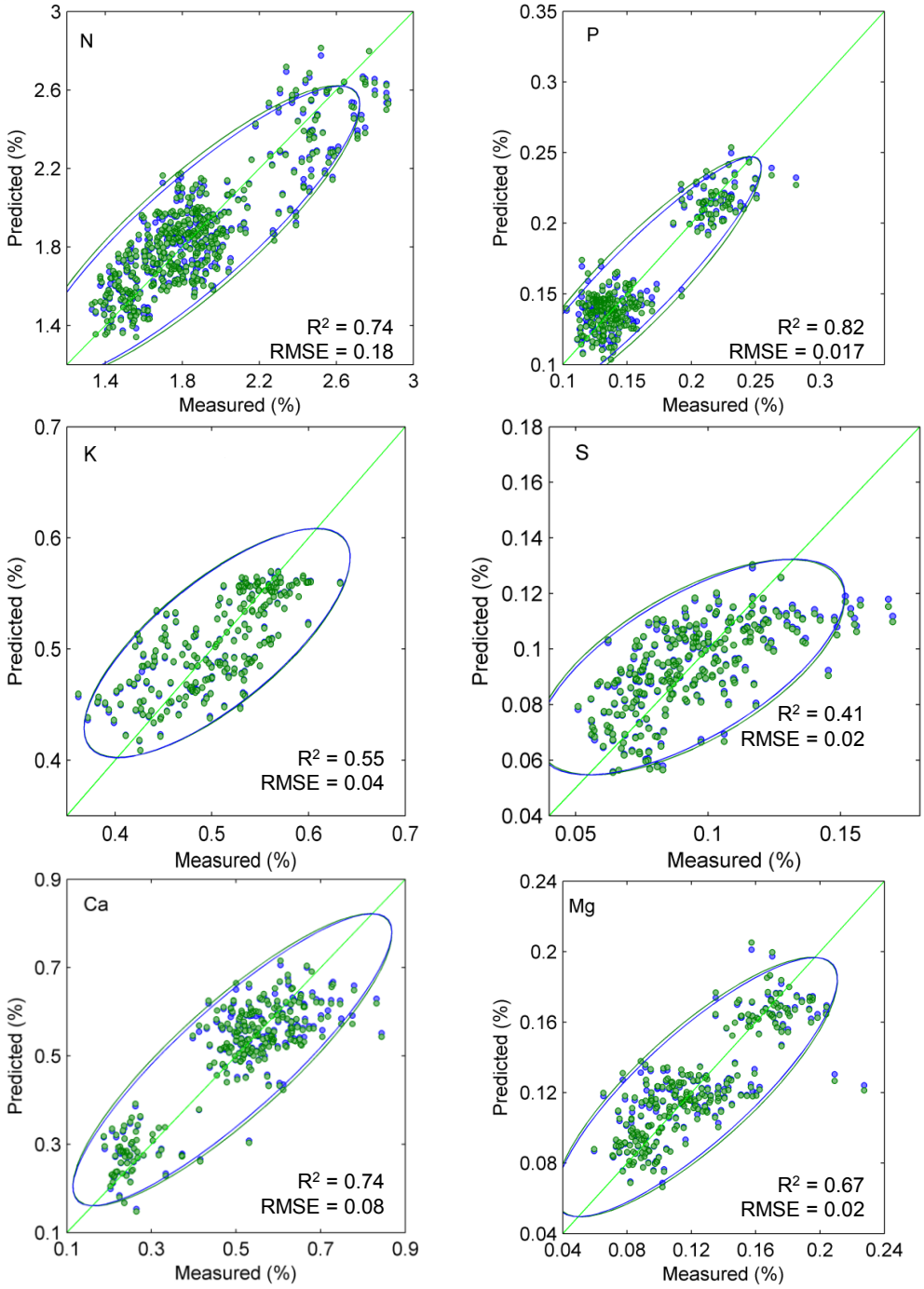
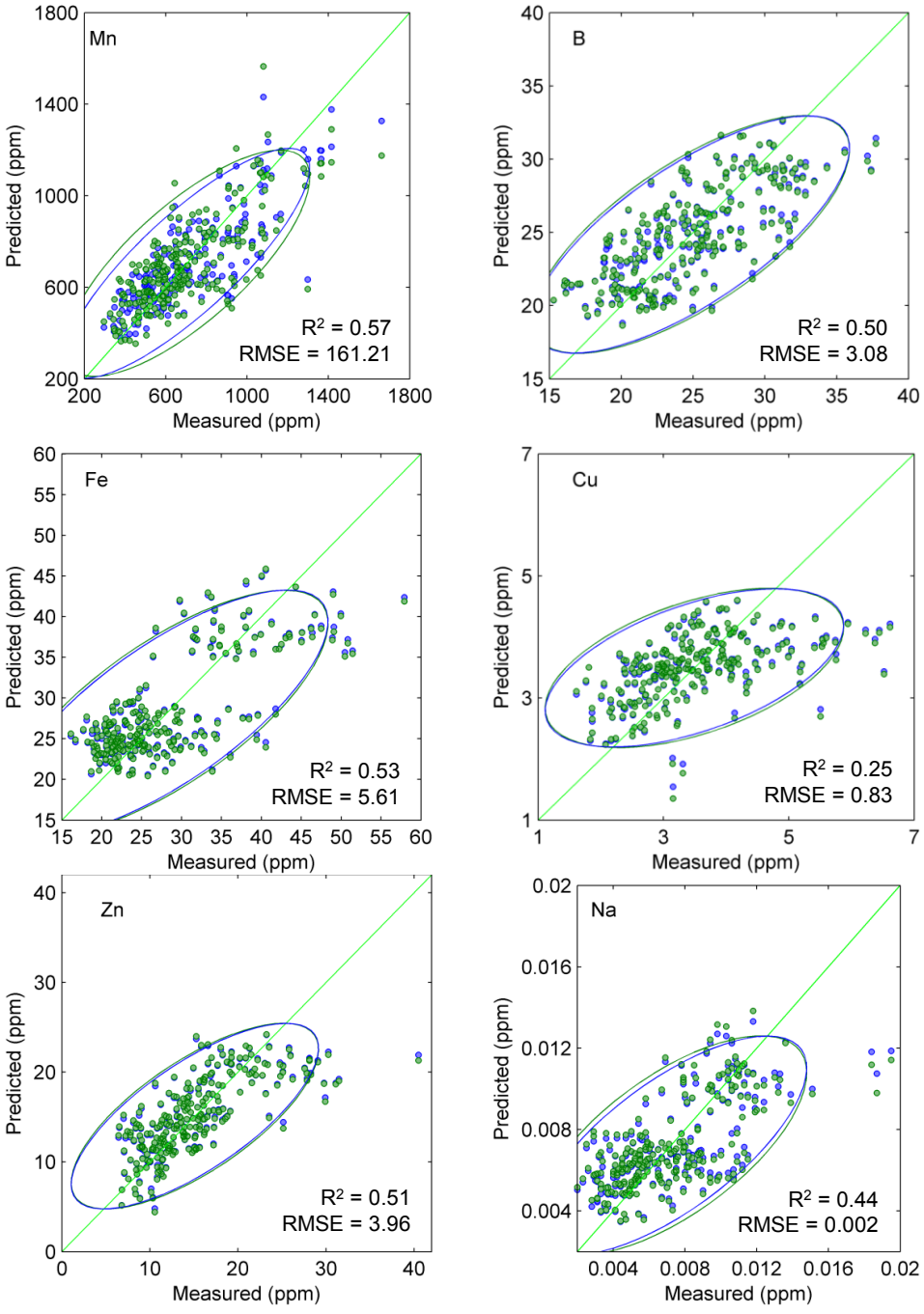


Figure 5.1 Median and range of canopy reflectance collected form 709 individual canopies over 4-years from sites located in Nova Scotia and New Brunswick (a); mean and coefficient of variation (CV) of same spectral data (b)

Figure 5.2 Relationship between measured and cross-validated predicted foliar nutrients using full-spectrum partial least squares (FS-PLS) regression models with visible-to-shortwave infrared (400-2400 nm) reflectance range. The green line depicts the linear fit. Blue solid circle represents calibrated samples. Green solid circle circles represents cross-validated predicted sample. Blue ellipse indicates 95% confidence interval of regression. Green ellipse indicates 95% confidence interval of prediction. The coefficient of determination (R^2) and root mean square error for prediction are also provided in each plot. The results are presented for foliar N, P, K, S, Ca, Mg, Mn, B, Fe, Cu, Zn and Na




Mn
R² = 0.57
RMSE = 161.21
Predicted (ppm)
Measured (ppm)
B
R² = 0.50
RMSE = 3.08
Fe
R² = 0.53
RMSE = 5.61
Cu
R² = 0.25
RMSE = 0.83
Zn
R² = 0.51
RMSE = 3.96
Na
R² = 0.44
RMSE = 0.002

Chapter 6 Summary and Conclusions

This study was undertaken to examine the response of the wild blueberry plant to soil applied fertilizers and encompasses soil nitrogen and carbon pools, plant growth and development, leaf nutrient concentrations and harvestable yields. In addition, given the vast area in which wild blueberry fields are located, the study also examined the feasibility of assessing plant nutrient status through the use of remote sensing hyperspectral technologies. Our results emphasize the importance of monitoring for soil nitrogen and carbon pools in the context of accelerated nitrogen cycling, nitrogen saturation, the fine-tuning of current leaf nutrient ranges in Atlantic Canada in connection to fertilizer rates, the possibility of estimating leaf nutrient contents by remote sensing technologies all with the aim of optimizing wild blueberry yields.

In terms of statistical techniques, this thesis used response surface methodologies with a central composite design as a means of discovering, the main and interactive effects of soil applied fertilizers to determine the most appropriate soil nitrogen levels and leaf nutrient ranges that correlate to the highest harvestable yields. The remote sensing data used to estimate leaf nutrients concentrations, various models that combined chemometrics and response surface methodologies for determining model efficiencies with aim of getting informative wavelengths in wild blueberry fields.

The results of this project can be summarized and concluded as follows:

6.1 Effect of Soil Fertilizers on Soil and Plant Reponses

Our results suggested that fertilizer additions alter the ammonium and nitrate production in wild blueberry soils possibly via one or more mechanism including change in soil pH, soil Mg, Ca, Al levels, ligninase activity, dissolved carbon production, below ground

productivity, above ground productive, biomass quality, microbial biomass and microbial community structure. In the field study, N rate affected soil ammonium and nitrate levels, with soil levels depending on growth stage of crop (plant demand and uptake) and time distance from fertilizer application event (seasonal trends and residual effect of fertilizers) with soil ammonium and nitrate levels remain raised for 2-3 months and declined thereafter. Results from laboratory incubation experiment illustrated that soil microorganisms involved in ammonification process are N limited (initial dose of N may be required to trigger ammonification process by lowering C:N ratios) and K limited. However, these organisms are not P limited, and nitrifying organisms are generally P and K limited but not N limited. From a plant response perspective, the N and P applications benefited vegetative growth (stem length and vegetative nodes) which is an indication that vegetative shoot growth is N and P limited. However, from a reproductive perspective, floral node growth and development and resulting fruit set were N, P and K limited and an optimum soil applied fertilizer level existed consisting of 35N, 40P and 30K. Harvestable berry yield was observed to decrease at high fertilizer rates and final berry yield was benefited mainly by N (Chapter 3). Leaf N and P concentration were N and P limited respectively, but leaf K was not K limited. The observed difference in soil microorganism and plant nutrient requirements may be partially explained by different requirement of NPK for their metabolic functions.

Fungi:bacteria ratio increased following soil applied N applications suggesting an alteration in microbial community. This implies that microorganisms may respond differently to N. The increase in fungi:bacteria ratio was somewhat unexpected considering previous studies indicated that fungi become scarcer under N enrichments although published reports of N increasing the fungi:bacteria ratio do exist. This may be due to the root system of the wild blueberry exuding allelochemicals that keep fungal biomass unchanged or increased to

retain its natural habitats which are characterized with fungal dominated soil environment. The increase in fungal:bacteria ratio especially under a scenario of decreasing overall microbial biomass levels may have decreased the bacterial populations. However, these results do not provide any separate insight into how free living and mycorrhizal fungi in particular may have responded to N fertilization nor do they provide an indication of how soil applied fertilizers influence microbial diversity. Generally, under N enrichment plants tend to allocate less C to mycorrhizal fungi when N is more available (Treseder 2008) but the wild blueberry plant may be not following these general trends as supported by a previous review illustrating to fungal dominance could be dependent on both the microbial community in question and the quality of litter being decomposed (Marcel et al. 2008). The wild blueberry may be more accommodative a host or the plant requires the fungal association for the acquisition of other nutrients or other metabolic functions.

N enrichment caused an increase in above ground biomass production which may increase C sequestration in wild blueberry soils. Most of the biomass was returned to the soil through mowing and leaf fall. This may have contributed to the very high dissolved organic carbon concentrations in the soil (~ 300 kg C ha^{-1}) which could have alleviated microbial (eubacteria, archaea and fungi) C limitation. However, microbial biomass declined with N application rates which indicates these microorganisms were not N limited. Therefore, microbial biomass might be limited by other factors including potassium, calcium and magnesium. Examining the MBN and MBC results from this study and the frequent precipitation obtained in Nova Scotia, it does appear that microorganisms are not limited by water, N or P. N saturation can decrease soil pH, leading to leaching of calcium and magnesium, availability of aluminum. Microorganisms may become magnesium, calcium or potassium limited, or may experience aluminum toxicity.

Microorganism abundance and plant community composition and growth can be influenced by N availability. Under natural environment conditions, the wild blueberry plant and soil fauna and flora may be limited by the same elements. However, management practices such as pruning by mowing and herbicide applications help keep the wild blueberry plant as the dominant plant species and limit possible changes in plant community composition. Conversely, N additions increased above ground biomass production with greater biomass quality including higher N contents and easily decomposable cellulose. When returned to the soil, these compounds may have induced shifts of soil microorganism community which require different nutrition requirements than microorganisms in natural habitats. These microbial community shifts in tandem with the facilitated dominance of the wild blueberry and alleviation of microbial N limitation attributed to soil fertilizer applications. These man induced changes may be responsible for the difference in wild blueberry plant and microorganism nutrition requirements under managed system. These changes in microbial community abundance and structure under fertilizer enrichments have consequences for production of nitrate greenhouse gases (CO_2, N_2O and CH_4).

By examining the changes in soil nitrogen levels between the sprout and crop years of production, it appears as though there may be a preference by the wild blueberry plant for the ammonium form of nitrogen and that the bulk of the nitrogen used by the plant was acquired in the sprout year of production. Based on the results from this study, there exists the possibility that the wild blueberry assimilates organic forms of N even in presence of adequate soil mineral N and the N demand (partially) of the plant may be fulfilled through these organic sources. The possible use of organic N by wild blueberry has not been examined, and is an area that needs to be examined in more detail in future studies.

Accelerated soil nitrogen cycling, through nitrification activity was reported to be much higher than previously reported by Eaton and Patriquin (1988) in wild blueberry soils. Our results indicated that nitrification was predominately undertaken by heterotrophic microorganisms (fungal pathway). Although elevated nitrate production and nitrate losses were detected in wild blueberry fields, these losses may be small relative to the N additions. This was in part due to the N retention capacity and efficiency that lied in wild blueberry plant growth and berry yields were limited by N (mainly ammonium). Hence, less substrate should have been available for nitrification, although heterotrophs can use organic sources as substrate for nitrate production. Although microbial biomass did not appear to N limited in this study, it is feasible that microbial biomass does not reflect microbial turnover (i.e. growth rate and death rate) and could have increased even while overall microbial biomass dropped, especially considering the high levels of carbon supply (DOC) present in wild blueberry soils. Therefore, it is possible that with high microbial turnover present in wild blueberry soils, a continuous supply of N (nitrate) is required resulting in less nitrate available for leaching.

Based on the net mineralization, net nitrification, nitrate leaching, foliar N concentrations, wild blueberry vegetative growth and reproductive output results from this study, it is inconceivable that current N application practices could lead to N-saturation and an accompanying decline in wild blueberry biomass production (net primary production) could occur in the near future. This is further supported with characteristics of the production system including elevated N cycling, increased C availability, a fungal dominated soil system, low Ca and Mg requirements and tolerance to high Al levels. However, not all wild blueberry stands will move towards saturation or within saturation stages at the same rate. The rate towards and passing through different N saturation stages is dependent on the initial

fertility status of field, management history, and the amount and duration for which fertilizers were applied. It is also appears that the wild blueberry plant is maintaining its fungal dominated soil system, However detailed studies may be required to make statement about soil food web and health.

Most of the wild blueberry fields respond to increased N applications with increased shoot biomass production. Compared to other commercial fields examined, the N application rates used and plant N levels observed did not reach threshold levels where plant growth declined. The accelerated soil N cycle and N-saturation is an entirely new area of study in wild blueberry production. How the plant may cope or adapt to new soil N regimes especially with increasing atmospheric carbon dioxide levels is largely unknown and will be an interesting subject for future investigations.

6.2 Growth, Development and Leaf Nutrients

Soil fertilizer rates were determined for producing optimum stem length, fruit nodes, fruit set and harvestable yields. The optimum dose of 35 kg nitrogen (ammonium sulfate), 40 kg phosphorous (triple super phosphate) and 30 kg potassium (potassium chloride) per hectare provided approximately 500 kg ha^{-1} additional berries over the current industry fertilizer rates (20-10-15 kg NPK ha^{-1}). This optimum dose alone could increase farm gate value in Nova Scotia by \$430 ha^{-1} assuming berry price of 1\$ kg^{-1} and \$70 in additional fertilizers cost. Leaf nutrient levels were at their optimum ranges at similar fertilizer application rates as those reported for growth, development and yield. Leaf nutrients were optimum at fertilizer rates of 28-45-32 kg NPK ha^{-1}compared to 35-40-30- NPK ha^{-1} for the yield components. The results indicated decreased leaf Mg contents under nitrogen additions but leaf Mg content remained within the zone recommended by Trevett (1972). The optimal nutrient ranges that simultaneously maximized berry yields, were narrower than previous

studies. Optimum leaf ranges in sprout year of production were N (1.8-2.03%), P (0.155-0.160%), K (0.53-0.55%) and Ca (0.44-0.46%). These were higher than optimum levels reported in other studies in Quebec (Lafond 2009) and Maine (Santiago 2011). However, these optimal leaf N, P, K and Ca concentrations were consistent with previous studies in Nova Scotia and Maine (Eaton et al. 2009; Trevett 1972). Upon examining leaf tissue Mg. levels though, the optimum concentration (0.116%) from this study was lower reported in the previously mentioned studies. These lower levels may be due to that the wild blueberry prefers uptake of ammonium N (ammonium also dominate on exchange sites of in wild blueberry soils) and for every ammonium ion acquired one hydrogen ion produced in the cell is released from the plant root into the soil solution. Hydrogen ions are also added from the nitrification process which is acidifying process. They cause leaching of Mg and other cations including Ca and K from the upper soil horizon. This leaching and resulting deficiency becomes more important when high soil ammonium levels compete with uptake of Mg. Mg is central atom of chlorophyll molecule and also required in protein synthesis. In our experiment, leaf Mg levels did not appear to affect yields but, Mg based fertilizers may be tested in future studies on sites where considerable nitrification is taking place.

Mineral nutrients including P, K and Mg are needed for ammonium assimilation or protein synthesis. When available in sufficient concentrations, the plant can function and photosynthesize at an increased rate and final growth, development and production may be enhanced. The wild blueberry plant can meet its N requirements from multiple sources such external inorganic N supply, soil mineralization and or organic sources (Chapter 2). However, this increased N supply also requires other nutrients (P and K) for vital plant metabolic process including protein synthesis. P availability in wild blueberry soil is curtailed due to acidic conditions and precipitation with aluminum (Al) and iron (Fe) to form relatively

insoluble compounds and additionally slow P mineralization in wild blueberry soils. This limited supply and uptake of P by the plant may cause a P deficiency within the plant and subsequent reductions in photosynthetic rate and carbohydrate production due to an increased supply of other nutrients especially N. The increased N supply, in particular with increased nitrate levels would be detrimental to mycorrhizal function and from a decreased supply of carbohydrates. This may further decrease the phosphorus uptake which is needed to sustain protein synthesis. Although, the commonly held view is that application of P to the wild blueberry is not beneficial, mainly due to its unavailability, it is vital for the above mentioned physiological functions and to also balance crop nutrition demands. Sole application of N may increase yield for short term due to the ability of the plant to use residual nutrients within the plant and in the surrounding root zone but as those reserve deplete with time, yield would decrease. Combined with the phenotypic variability observed in wild blueberry fields and need for large sample sizes, this may also explain inconsistent and variable fertilizer responses.

In wild blueberry production in Maine, 1:1:1 ratios are recommended (Smagula and Dunham 1995; Smagula et al. 2004). But in our study, we suggest using (7:8:6) NPK, these higher application of P may be justified through enhanced availability of N through external supply, increased rate of mineralization, organic N availability and its use due high organic matter (~10%). Therefore, a slightly higher supply of P may be required to balance N supply and meet the nutritional needs of the wild blueberry.

6.3 Remote Sensing Technologies

Precise and accurate mapping of plant nutrient within a field is essential to optimizing wild blueberry growth and development and is an important component of wild blueberry cultural management practices. With this in mind, our study determined that a general and reliable nutrient estimation method could be developed using field visible and near-infrared hyperspectral remote sensing technologies. Macro and micro foliar nutrients can be estimated by full spectrum-PLS (FS-PLS) and variable selection algorithms that reduce number of wavebands to provide simple and easily interpretable calibration models. Spectral and chemical data can encompass a wide nutrient range and development stages. The precision and accuracy of the estimation varies with spectral range, foliar nutrient, and chemometrics method. Generally, macro nutrients were better predicted than micronutrients and VSWIR (400-2400 nm) provided greater precision and accuracy than VNIR (400-1500 nm). The chemometrics models in order of preference were GA-PLS, VSWIR (FS-PLS), iPLS, VNIR (FS-PLS) and SFS-PLS. Informative wavelengths and waveband regions have been identified for the macro and micro leaf nutrients that will enable the eventual development of "on-the-go"(i.e. "real time") systems that apply fertilizers where needed in wild blueberry fields. Although leaf nutrients were successfully estimated by remote sensing technologies, this technique only provides leaf nutrient status and does not reflect the overall N-status of the soil-plant environment. With ~80% of the wild blueberry plant biomass being located beneath the soil surface, these roots and rhizomes providing a reservoir of nutrients, carbohydrates and photosynthates, there is also a need to consider the nutrient content on a whole-plant and biomass basis.

6.4 Future Research

Results from this study illustrated the dynamic nature of the wild blueberry system and the shifts in microbial populations that occur with soil applied fertilizer applications. However, there is a need to scrutinize these changes that occur in wild blueberry soils to also identification of the specific microbial species involved, their temporal changes and variability and the impact of intensified management practices. As more wild blueberry fields continue to be developed from a native, forest-style habitat to an intensively managed wild blueberry fields, there also needs to be an improved understanding of the consequences of chemical fertilizer additions, the resulting shift in microbial communities, their abundance, structure and function as wild blueberry soils move toward N saturation. This could be augmented at a molecular level by obtaining genome sequences and enzyme activities from blueberry soils. The molecular techniques may also provide conclusive evidence of the type of microorganisms involved in nitrification in these wild blueberry soils.

Future research activities could also utilize stable isotope techniques to assess the wild blueberry's preference for and the relative contribution from inorganic (ammonium and nitrate) and organic N forms (amino acids), and to also examine the competition between the plant, its clones and the soil microbes. Preferential uptake may be examined by using a range of ^{13}C and ^{15}N-labeled amino acids (glycine, serine, and phenylalanine),^{15}N-labeled inorganic N in combination with detection of intact labels by gas chromatography–mass spectrometry (GC-MS).

In the future, enhanced internal N cycle may compensate in part for N demand, so conducting fertilizer experiments should be continued to assess wild blueberry demands and possible reduction in fertilizer rates. N enrichment experiments may also be set at other sites with different soil conditions in Maritimes to monitor soil N cycling and possible N

saturation because N saturation condition depend on the management history of sites and initial fertility status of sites and duration of and amount of N additions. The fertilizers rates of approximately 35, 40 and 30 kg N, P and K ha^{-1} provided optimized yield components and harvestable yields but similar fertilizer rates also maximized the nitrate production in the Kemptown soil. Therefore, increased nitrate production and approaching soil N-saturation condition may compromise the overall sustainability of wild blueberry production systems, so in addition to reversibility efforts, studies may be designed to curtail nitrification and maintenance of the ammonium form through the use of nitrification inhibitors. Alternatively, the use of foliar-applied nutrients may also be a means of more effectively providing nutrients to the wild blueberry. Previous studies using foliar nutrients were largely unsuccessful due to increases in fungal leaf spot diseases and the reluctance by producers to make additional agrochemical applications. However, with more emphasis being placed on minimizing various leaf spot diseases (e.g. Septoria, Rust and Valdensinia) and the ability to incorporate foliar nutrient applications into this, the use of foliar applied nutrients may be a more practical and cost effective means of supplying nutrients including phosphorus and micronutrients to the wild blueberry.

With respect to the growth and development characteristics of the wild blueberry plant, N applications have been reported to promote better and earlier growth but can also defer the plant's transition from the vegetative to the reproductive stage causing delays in the induction and initiation. Future studies could focus on the induction of post tip die back stress that may divert available resources to the reproduction organs. The re-growth of vegetative buds has been observed in fertilized plots during wet periods particularly in low lying areas and could be the result of N pool accumulation carried by the rain water. Thus, variable rate technologies may be employed to apply less N in low lying areas. This study showed

negative impact of potassium chloride on harvestable yields. In the future potassium sulfate may be explored as a potassium source.

This study has identified important wavebands for nutrient estimation by remote sensing techniques under natural sunlight (i.e. passive remote sensing). The wavelengths we obtained in this study would be equally useful with sensor containing its own light source (active sensor) but would remove climatic (i.e. erratic light source) conditions often encountered in a maritime climate and would also allow operation of the sensor at night. One other possible solution to cloudy conditions with natural sun light could consist of employing an additional sensor facing the sky that collected incoming light, calculate light corrections and apply these corrections under varying light intensities. Active sensors containing their own light source could be explored but the height of a sensor is critical because at higher heights artificial light does not penetrate the foliage that may affect the nutrient estimation ability of sensing system.

6.5 Recommendations for Wild Blueberry Industry

We found soluble organic nitrogen (SON) to be an important pool in wild blueberry soil. In the future, SON should be considered as part of nutrient management programs. Nitrogen enrichments have increased nitrification acidity, elevated concentrations of dissolved organic carbon (leaching of carbon) and reduced the microbial biomass. Although this may raise concern for soil health, N fertilization also increased above ground productivity, increased return of plant materials to soil and may potentially increase carbon sequestration. All these factors are important in the retention of a fungal dominated and healthy soil system.

Our results demonstrated beneficial effects of soil applied fertilizers (NPK) on yield components and berry yields. We recommend fertilizer rates of 35N, 40P and 30K kg ha^{-1} (7:8:6) in the form of ammonium sulfate, triple superphosphate and potassium sulfate in the spring of the sprout year at the onset of shoot from rhizomes.

We also present the optimal sprout year leaf nutrient ranges N (1.8-2.03%), P (0.155-0.160%), K (0.53-0.55%), Ca (0.44-0.46%), Mg (0.115-0.13%) and B (24-26 ppm) for wild blueberry fields in Atlantic Canada. We suggest optimum leaf N (1.5-1.7%), P (0.158-0.164%), K (0.535-0.545%), Ca (0.465-0.495%), Mg (0.115-0.125%) and B (18-22 ppm) for crop year leaf nutrient levels. Recently, Eaton et al. (2009) proposed leaf tissue ranges for Nova Scotia. The study surveyed fields and reported 42%, 12% and 48% of surveyed fields were deficient in leaf phosphorus (P), potassium (K) and boron (B), respectively (Eaton et al. 2009) when compared to standards from Maine (Trevett 1972). One criticism of this study was that the Nova Scotia fields examined may not have attained their full yield potentials and that the optimum ratio of leaf nutrient concentrations to yield needed to be determined by pre-planned experiment rather than by survey.

In our study the sprout year leaf N, P and K nutrient ranges concentrations were higher than existing nutrient ranges (Eaton et al. 2009) in Nova Scotia (N = 1.6-2.0%, P = 0.110- 0.144% and K =0.41-0.52%). Our sprout year leaf Ca and B nutrient range fell within similar ranges (Eaton et al. 2009) but ranges were narrower in scope (Ca = 0.32-0.47% and B = 19-31 ppm). The optimal sprout year leaf nutrient levels obtained in this study may be due to enhanced nutrient uptake through a more balanced fertilization (NPK). These relatively high N, P and K leaf nutrient levels may also be required for better growth, development and maximize yield potentials. Optimum leaf B concentration in sprout (24-26 ppm) and crop year (18-22 ppm) were below the Maine critical values for sprout (24 ppm) and crop (30

ppm) proposed by Trevett, (1972), but within NS optimal ranges of 19-31 ppm (Eaton et al. 2009). These leaf nutrient ranges in response to optimum fertilizer doses may be used as guidelines for wild blueberry production in Atlantic Canada.

Therefore, results from this study have illustrated that simultaneous growth and yield optimization, adjustments of soil applied fertilizer nutrient sources and doses, remote sensing of leaf nutrient and subsequent need based applications may increase economic returns, reduced likelihood of environmental concerns such as leaching and erosion of nutrients, thus improving the overall sustainability of the wild blueberry production system. Acquisition of this knowledge and development of the applicable technology based products, processes and services is of the utmost importance if the wild blueberry industry is to remain prosperous in a global economy.

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Appendix A Manuscript Published in Canadian Journal of Plant Science

In situ estimation of foliar nitrogen in wild blueberry using reflectance spectra

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Maqbool, R., Percival, D. C., Adl, M. S., Zaman, Q. U. and Buszard, D. 2012. **In situ estimation of foliar nitrogen in wild blueberry using reflectance spectra**. Can. J. Plant Sci. **92**: 1155–1161. Remote sensing techniques have the potential to serve as an important nutrient management tool in wild blueberry. The potential of visible (VIS), near infrared (NIR) and shortwave infrared (SWIR) spectroscopy was evaluated during 2006 (sprout/vegetative phase of production) to estimate foliar nitrogen (N). Canopy reflectance measurements were taken from two nutrient management experimental sites located in Nova Scotia (NS) and New Brunswick (NB). Partial least squares regression (PLSR) estimated foliar N, giving the coefficients of determination (R^2) values ranging from 0.69 to 0.85, and root mean square errors of cross validation (RMSECV) from 0.16% (±8.29% of mean) to 0.24% (±12.43% of mean) for different spectral ranges used in this study. The green peak region located in the VIS region best estimated foliar N. The tested spectral ranges differed in their predictive ability, but generally followed the biochemical basis. Variable importance in projection scores (VIP), regression vector coefficients and PLSR loading weights (LWs) plots highlight the importance of wavebands (~550 nm, ~610 nm, 1510 nm, ~1690 nm, ~1730 nm, ~1980 nm and ~2030 nm) for in situ foliar N estimations. Thus, it was concluded that reflectance spectra may be used to estimate and ultimately map foliar N in wild blueberry production. The results illustrated the ability of multivariate techniques, such as PLSR to explore hyperspectral data and estimate leaf tissue nutrient content.

Key words: Reflectance spectra, wild blueberry, foliar nitrogen, partial least squares regression

Maqbool, R., Percival, D. C., Adl, M. S., Zaman, Q. U. et Buszard, D. 2012. **Estimation in situ de la concentration d'azote dans les feuilles du bleuet sauvage grâce au spectre de réflectance**. Can. J. Plant Sci. **92**: 1155–1161. Les techniques de télédétection pourraient faciliter la gestion des éléments nutritifs dans les peuplements de bleuet sauvage. En 2006 (germination/phase végétative), les auteurs ont évalué dans quelle mesure la spectroscopie dans le spectre visible (VIS), dans le proche infrarouge et dans l'infrarouge ondes courtes permet d'estimer la teneur en azote (N) des feuilles. Ils ont mesuré la réflectance du feuillage à deux endroits où l'on procédait à des expériences sur la gestion de la nutrition, soit un en Nouvelle-Écosse (N.-É.) et l'autre au Nouveau-Brunswick (N.-B.). La méthode de régression des moindres carrés partiels (RMCP) permet d'estimer la concentration foliaire de N, avec des coefficients de détermination (R^2) de 0,69 à 0,85 et une contrevalidation de l'écart-type de 0,16 % (± 8,29 % de la moyenne) à 0,24 % (± 12,43 % de la moyenne) pour les fourchettes du spectre employées dans l'étude. Le pic vert dans la partie VIS du spectre donne l'estimation la plus précise de la concentration foliaire de N. L'utilité des diverses fourchettes testées varie sur le plan des prévisions, mais ses fondements reposent sur la biochimie. Les courbes traçant l'importance variable des résultats obtenus par projection, les coefficients des vecteurs de régression et les facteurs de pondération de la RMCP illustrent combien certaines longueurs d'onde (~550 nm, ~610 nm, 1510 nm, ~1690 nm, ~1730 nm, ~1980 nm et ~2030 nm) importent pour estimer la concentration foliaire du N in situ. On en déduit que les spectres de réflectance pourraient servir à estimer et éventuellement à cartographier la concentration du N dans les feuilles du bleuet sauvage. Les résultats de l'étude prouvent l'utilité des techniques à variables multiples comme la RMCP pour explorer les données hyperspectrales et estimer la teneur des tissus foliaires en éléments nutritifs.

Mots clés: Spectre de réflectance, bleuet sauvage, azote foliaire, régression des moindres carrés partiels

Wild blueberry (*Vaccinium angustifolium* Ait.) is native to northeastern North America. Approximately 112 million kg of fruit valued at $470 million are produced annually (Yarborough 2009). Fields are developed from native stands or abandoned farmland by clearing competing vegetation. Commercial fields are managed on a 2-yr production cycle with pruning done in alternate years to sustain optimal floral bud initiation, fruit set, yield, and ease of mechanical harvest (Hall et al. 1979). Fertilizers generally containing nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O)

Abbreviations: **Chl**, chlorophyll; **LW**, loading weight; **NIR**, near infrared; **PLSR**, partial least squares regression; **REP**, red edge position; **SWIR**, shortwave infrared; **VIP**, variable importance in projection; **VIS**, visible

are applied in early spring of the sprout year. Optimum leaf N levels range from 1.6 to 2.0% N on a dry weight basis at the tip-dieback stage of shoot growth (cessation of growth associated with the shoot apical meristem), which typically occurs in late July of the sprout year (Trevett 1972).

Fertilizer practices, including timing, dose, type and formulation, have intensified in the recent 20 yr. Increased plant coverage, harvestable berry yields and greater farm profitability have been observed (Percival and Sanderson 2004). However, excessive fertilization has caused extensive vegetative growth, increased stem heights, reduced yield potential (floral bud and flower number) and reduced harvestable berry yields. Unfortunately, soil extraction techniques do not accurately estimate available soil nutrients in wild blueberry production system (Ring 2001). Therefore, present nutrient assessment techniques are reliant on leaf tissue sampling (Trevett 1972). Analytical techniques including use of LECO combustion CNS auto-analyzer and inductively coupled argon plasma atomic emission spectroscopy (ICAP-AES) are time consuming (more than 1 wk to complete), labour intensive, expensive (approximately \$20 sample^{-1}) to conduct and do not capture intra-field variability in plant nutrient content.

Soil-plant N dynamics and in-field variability present the major challenge for nutrient management in wild blueberries. Combined with the time and cost constraints, producers are unable to accurately, precisely or efficiently determine foliar nutrient levels resulting in increased interest in remote sensing technologies. By assessing the precise foliar nutrient levels, both over and under application of fertilizer can be avoided ensuring optimal harvestable yields, increased economic returns and reduced likelihood of environmental concerns such as leaching and erosion of nutrients. The benefits of an on-the-go nitrogen sensing and application system have been demonstrated in crops including winter wheat (Raun et al. 2001) and are now commercially available.

The absorption features in visible (VIS), near infrared (NIR) and short wave infrared (SWIR) spectral ranges have been used to predict foliar N (Herrmann et al. 2010; Rambo et al. 2010). About 30–50% leaf N is present in the form of a single protein, ribulose-1, 5-biphosphate carboxylase-oxygenase (RuBisCO) in green leaves (Kokaly et al. 2009). Chlorophyll (Chl) *a* and *b* are the primary absorbing pigments present in leaf tissue, and actively absorb radiation in the VIS spectra (Kokaly et al. 2009). Between the VIS spectra and NIR there exists the red edge position (REP). It is caused by combination of strong Chl absorption in the red portion of spectra and strong reflectance due internal leaf scattering in the NIR region (Dawson and Curran 1998). An increase in leaf Chl concentration, biomass or leaf N content results in deepening and, more importantly, broadening of the Chl absorption feature ($\sim$680 nm). Studies have used this phenomenon of reflectance spectra to estimate the leaf Chl (Curran et al. 1990) and foliar N (Mutanga and Skidmore 2007). Previous work has reported REP less sensitive than the normalized difference vegetation index to soil background, atmospheric absorptions, and sensor view angle (Mutanga and Skidmore 2007).

Bourguignon (2006) conducted preliminary work on the potential use of remotely sensed reflectance spectra to estimate foliar nutrients in wild blueberry. Results from this work illustrated the need for an Analytical Spectral Devices (ASD) FieldSpec®3 VIS/NIR/SWIR spectroradiometer (ASD Inc., Boulder, CO) and multivariate data analysis techniques. Field spectrometers (including radiometers) produce semi-continuous spectrum consisting of over 100 contiguous bands, each 10 nm or less between 350 to 2500 nm. The hyperspectral data retain information present in specific narrow bands. The instrument costs approximately \$50 000, which limits its farm level use. However, it can provide information about specific wavebands potentially useful for nutrient estimation. The hyperspectral data are often collinear and partial least squares regression (PLSR) analysis techniques can be successfully used as an empirical technique for estimating foliar nutrients (Ollinger et al. 2002). To test the strength and accuracy of the relationship between canopy reflectance spectra and foliar N, reflectance spectra were divided into different biochemically meaningful regions. In doing so, we tested the following hypotheses: (i) foliar N in wild blueberry can be estimated from canopy reflectance spectra; and (ii) the strength and accuracy of relationship between canopy reflectance spectra and foliar N depends on the specific spectral region (VIS/NIR/SWIR).

MATERIALS AND METHODS

Experimental Sites

The experimental sites were fertilizer management trails that represent the typical wild blueberry fields of eastern Canada. The first commercial site was located near Kemptown, Nova Scotia (lat. 45°30′7.91″N, long. 63°7′27.72″W). The soil was typically sandy loam, acidic, imperfectly drained, stony and moderately rocky (Cobequid association). The second site was located near Brantville, New Brunswick (lat. 47°22′22.74″N, long. 64°58′17.48″W). The soil was typically sandy, acidic, poorly drained, non-stony and non-rocky (Acadie siding). A three-factor (soil-applied N, P_2O_5 and K_2O) circumscribed centrally composite design with four replications was used with 16 treatment combinations including a no applied fertilizer treatment (Table 1). A plot size of 6×8 m was used with 1 m between plots. Ammonium sulphate, triple-superphosphate, and muriate of potash were applied early May in the sprout year of production using a Scotts SR2000 rotary fertilizer spreader. This design

Table 1. Central composite design (CCD) of independent variables (soil applied nitrogen, phosphorous and potassium)

	Soil applied fertilizer (kg ha^{-1})		
	N	P_2O_5	K_2O
Factorial points	12	30	12
	48	30	12
	12	120	12
	48	120	12
	12	30	48
	48	30	48
	12	120	48
	48	120	48
Star points	0	75	30
	60	75	30
	30	0	30
	30	150	30
	30	75	0
	30	75	60
Center point	30	75	30
Control	0	0	0

resulted in the wide range of foliar nutrient levels, giving more realistic samples for model building. The experimental area was managed by Bragg Lumber Company (Collingwood, Nova Scotia) under standard industry practices with provisions for pruning, agrochemical applications and introduction of pollinators (honey bees).

Canopy Spectral Measurements

Spectral measurements were taken on 2006 Jun. 30, Aug. 14 and Sep. 15 from Kemptown, NS, and on 2006 Aug. 03 from Brantville, NB. The canopy was closed at the time of spectral measurements. The understory consisted of plant litter mainly due to leaf drop in the fall of the sprout year and mowed plant material after harvesting in the crop year. The sampling dates were chosen to avoid the spectral signature of plant litter, soil and rocks. Spectra were acquired with an Analytical Spectral Devices (ASD) FieldSpec®3 spectroradiometer (ASD Inc., Boulder, CO). The instrument samples a spectral range of 350–2500 nm. The instrument optimization, white reference measurements, and settings, such as number of scans per measurement, were controlled through RS^3 6.0 software (ASD Inc., Boulder, CO). A foreoptic lense of field of view (FOV) 10° was kept approximately 1 m above the crop canopy at nadir position. The ground field of view was about 17.5 cm in diameter. Spectra were taken at seven spots from each plot along the diagonal encompassing the clonal variability typical characteristic of blueberry fields. Each individual spectral measurement was average of 30 scans (Fig. 1). After 15 min, the instrument was recalibrated taking scans of a white Spectralon reference card (Labsphere Inc., North Sutton, NH) measuring 4.6 cm radius and an internal (dark) voltage measurement. The ratio of reflected radiance of the observed sample to the reflectance radiance of a known reference

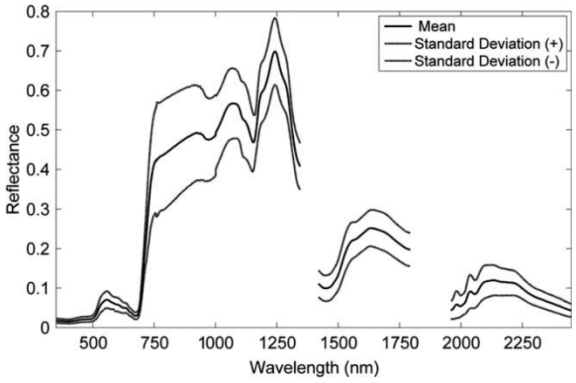

Fig. 1. Mean and ± standard deviation of wild blueberry reflectance spectra ($n = 256$) collected in the vegetative phase of production from fertilizer management trials located in Kemptown, NS, and Brantville, NB.

card was calculated. It allowed the adjustments to changing light conditions. All measurements were taken under clear sky conditions within 2 h of solar noon to minimize the effects of sun angle.

Plant Sampling and Nitrogen Content Analysis

Stems were collected from the same seven spots used for spectral data. Stems were kept in labeled plastic bags. The leaves were removed gently by grasping the stem at the base and pulling it. The leaf tissue samples were oven dried for 36 h at 60°C. The samples were ground with a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm mesh screen. The samples were placed in dry, labeled bottles and stored for chemical analysis. The nitrogen content was determined by combustion analysis using LECO CNS-1000 elemental auto analyzer (LECO Corp., St. Joseph, MI).

Data Analysis

Preprocessing of Spectra

The spectral ranges were selected based on canopy spectral properties, biochemical basis, sensor specifications and previous research in field crops, grassland and forests (Kumar et al. 2001). ViewSpec (ASD Inc., Boulder, CO) was used to view and average the seven reflectance spectra of each plot to minimize the noise. Wavebands below 400 nm, above 2400 nm, between 1341 and 1420 nm and between 1791 and 1960 nm were removed due to high noise or lack of absorption features. The sensor-specific discontinuities were excluded. The mean reflectance spectra of the wild blueberry ($n = 256$) have been provided (Fig. 1). Spectral and nitrogen content data handling were made in Matlab 7.10 (The Mathworks, Inc., Natick, MA).

Partial Least Squares Regression

Partial least squares regression is a widely used technique suitable for analyzing continuous spectrum and collinear data. PLSR belongs to a bilinear class of methods. The bilinear modeling reduces the large number of measured collinear spectral variables to a few non-correlated latent variables (LVs) in a way that they correlate with the variable(s) of interest. Further details pertaining to the PLSR algorithm can be found in Martens and Næs (1989). Partial least squares regression analysis was used to estimate foliar N from reflectance spectra collected under field conditions. To avoid over-fitting, the number of LVs retained PLSR models was determined by root mean square error of cross validation (RMSECV) statistic (Martens and Næs 1989). Leave-one-out cross validation was not used because it has been found prone to over fit models (Shao 1993). The RMSE statistic was calculated through a cross-validation for each model. This cross-validation procedure iteratively generates regression models while randomly reserving 10 disjoint groups from the input data until the RMSE statistic is minimized (Cruciani et al. 1992). The root mean square error (RMSE) is a measure of how well the model fits the data, while root mean square error of cross validation (RMSECV) is measure of a model's ability to predict new samples (Martens and Næs 1989). The RMSE statistic provides a direct estimate of the model error expressed in original measurement units. The PLSR analysis was performed using the PLS toolbox 5.8 (Eigenvector Research Inc., Wenatchee, WA).

RESULTS AND DISCUSSION

Foliar N data cover the wide range of values represented in the forthcoming PLSR analyses. The foliar N data were summarized as mean (1.93%), standard deviation (0.43%), maximum (2.93%), minimum (1.27%) and range (1.66%), which includes optimum, below and above optimum values (Trevett 1972). The foliar N data set matches most wild blueberry fields in northeastern North America. A summary of field reflectance spectra ($n=256$) of wild blueberry is provided (Fig. 1). The visual inspection of plotted spectra exhibited highest reflectance variation in the NIR (750–1300 nm), followed by SWIR (1500–2500 nm). The NIR range is dominated by variation in leaf water concentration and leaf thickness and variations in SWIR are caused by leaf water content, protein, N, cellulose and lignin.

Partial least squares regression analysis illustrated that foliar N was highly correlated with tested spectral regions with R^2 ranging from 0.69 to 0.85 and RMSECV from 0.16 to 0.24% (Table 2). Upon examining specific spectral regions, the green peak provided the best estimation of foliar N ($R^2=0.85$) and RMSECV value of 0.16% (Table 2). Studies have reported the importance of green region for Chl and N estimation (Gitelson et al. 1996). Although not as effective as the green region, the VIS-range, Chl *a*, Chl *b* and Chl ($a+b$) regions yielded comparable results (Table 2). The predictive ability of Chl *a* region was probably due to a higher proportion of Chl *a* in blueberry leaf tissues (Percival et al. 2012), and a resulting stronger Chl *a* correlation with foliar N.

Table 2. Statistics for estimated foliar nitrogen (N) obtained using partial least squares regression (PLSR) technique from twelve spectral ranges tested in this study

			Calibration			Cross-validation		
Spectral ranges[z]	No. of wavelengths	LVs[y]	R^2	RMSEC[x]	±% mean	R^2	RMSECV[w]	±% mean
VIS-range	270	7	0.85	0.16	8.29	0.84	0.17	8.80
Chl *a*	40	4	0.78	0.20	10.36	0.77	0.20	10.36
Chl *b*	40	3	0.70	0.23	11.92	0.69	0.24	12.43
Chls ($a+b$)	80	6	0.83	0.18	9.33	0.82	0.18	9.33
Green-peak	60	8	0.86	0.16	8.29	0.85	0.16	8.29
REP range	25	4	0.72	0.22	11.40	0.71	0.23	11.92
Chls ($a+b$)+REP range	105	4	0.73	0.22	11.40	0.72	0.23	11.92
Mutanga et al. 2004 range	250	5	0.77	0.20	10.36	0.75	0.21	10.88
NIR plateau	550	5	0.81	0.18	9.33	0.81	0.18	9.33
SWIR range	810	4	0.79	0.20	10.36	0.78	0.20	10.36
Full spectrum[v]	1750	5	0.84	0.17	8.80	0.82	0.18	9.33
Known absorptions	380	4	0.81	0.18	9.33	0.80	0.19	9.84

[z]Spectral ranges (nm) consisting of VIS range (401–670), Chl *a* (421–440 and 651–670), Chl *b* (451–470 and 631–650), Chls $a+b$ (421–440, 451–470 and 631–670), REP range (696–720), Chls ($a+b$)+REP range (421–440, 651–670 and 681–720), Mutanga et al. (2004) range (470–518 and 550–750), NIR plateau (781–1330), SWIR range (1421–1790 and 1961–2400), full spectrum (401–1340,1421–1790,1961–2400), known absorptions (420–440, 450–470, 630–670, 900–920, 1010–1030, 1500–1520, 1680–1700, 1720–1740, 1970–1990, 2050–2070, 2120–2140, 2170–2190, 2230–2250, 2290–2310, 2340–2360).
[y]Number of latent vectors (LVs) used in the partial least squares regression (PLSR) model.
[x]Root mean square error of calibration.
[w]Root mean square error of cross-validation.
[v]Full spectrum after removing noisy wavebands.

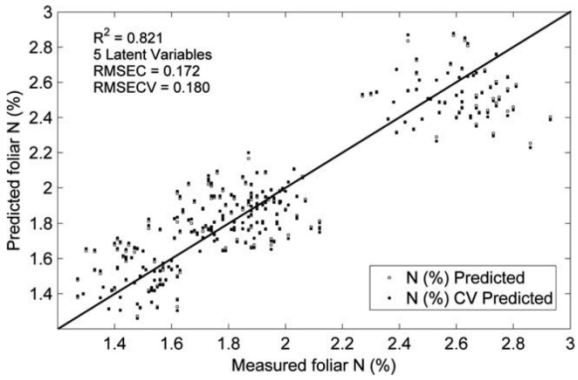

Fig. 2. Relationship between measured and predicted (calibration and cross-validation) foliar nitrogen (N) based on partial least squares regression (PLSR) model using full spectrum (401–1340, 1421–1790 and 1961–2400 nm) range. The solid line depicts a linear fit through the data.

Results from this study also indicate that foliar N could be accurately predicted using REP-range ($R^2 = 0.71$, RMSECV = 0.23%) and NIR-plateau ($R^2 = 0.81$ and RMSECV = 0.18%). Given that leaf water variations in NIR-plateau, we think that this good correlation simply results from the two protein-absorbing bands centered at 910 nm and 1020 nm. The SWIR contains protein, N, lignin and leaf water absorption features. Lower than expected predictive power of SWIR-range ($R^2 = 0.78$, RMSECV = 0.20%) could have been due to the presence of water content in canopies (Kokaly and Clark 1999) and/or to relatively low signal to noise ratios of the instrument in this range.

These results show the difference in relationship strength and predictive accuracy among tested spectral regions (Table 2). All tested sub-ranges produced comparable results to full spectrum or even better results (Table 2, Fig. 2) highlight the benefits of selecting biochemically meaningful regions. In order to extend these techniques to practical applications, the absolute error of estimation is an important consideration. In this study we found small RMSECV that were comparable with other studies in cultivated crops, woody species, or grassland (e.g., Curran et al. 2001; Mutanga et al. 2004). Overall, reflectance spectra may be used to estimate foliar N in wild blueberry production system.

Partial least squares regression regression vector coefficients indicate the importance of the wavelengths in the model for foliar N estimation. The VIS-range and SWIR-range regression coefficients were evaluated for their correspondence to a priori absorption features (Kumar et al. 2001). In general, good relationship was found between regression coefficients and known absorption features (Fig. 3). For example, peaks were observed at wavelengths 430 nm (Chl *a*), 460 and 640 nm (Chl *b*). The regression coefficients were high at various peaks 520, 550, 575, 590, 610 nm pertains to green peak region in VIS spectra (Fig. 3a). The large regression coefficients were observed in far red region, indicating REP contribution (Fig. 3a).

The variable importance in projection (VIP) scores of PLSR provide the summary of wavelength importance for both perspectives of foliar N and reflectance spectra modeling. The VIP scores may serve as a guide to highlight the absorption features and their relative contribution towards final model. A VIP score greater than one is generally considered a significant contribution (Chong and Jun 2005). The VIP scores of each wavelength in VIS-range were plotted (Fig. 4a). Particularly strong VIP scores (>2) obtained for wavelengths centered at 550 nm and 610 nm. The VIP scores above than minimum threshold limit (>1) of wavelengths between 520 nm and 620 nm illustrate the importance the green peak region. The prominent VIP score peaks in the SWIR-range were obtained at 1510 (protein and

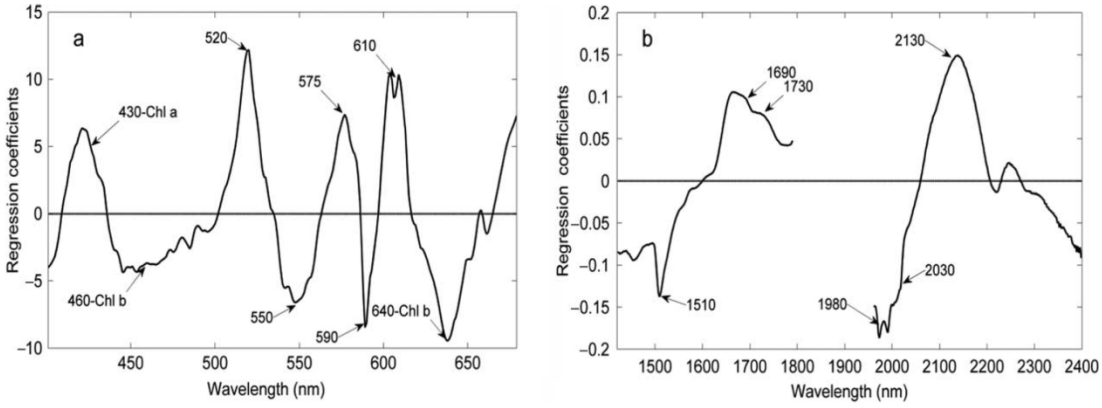

Fig. 3. The regression vector obtained from partial least squares regression (PLSR) models based on reflectance spectra: (a) visible-range and (b) short wave infrared (SWIR)-range. Values in graphs are wavelengths with high-regression coefficients and their association to a priori nitrogen related absorption features.

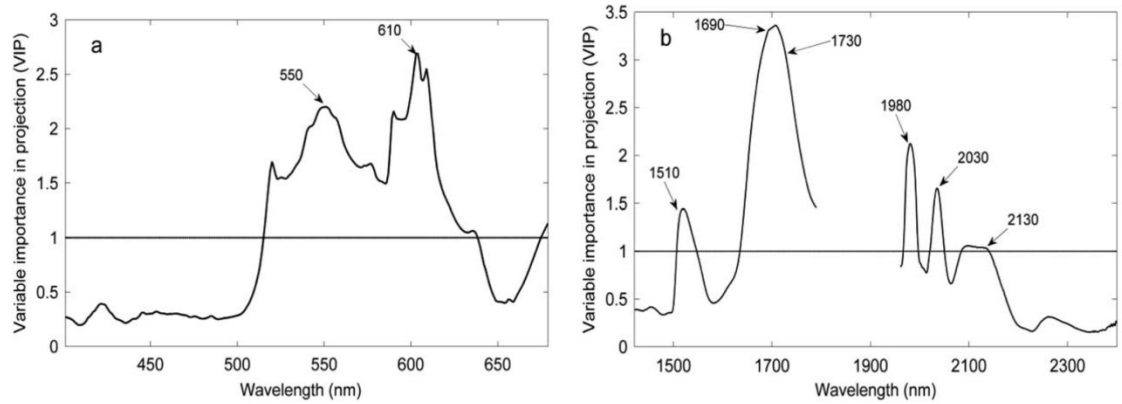

Fig. 4. Variable importance in projection (VIP) scores obtained from partial least squares regression (PLSR) models: (a) visible-range and (b) short wave infrared (SWIR)-range. Horizontal line represents the VIP threshold value (>1) of significant contribution. Values in graphs are wavelengths with high VIP scores and their association to a priori absorption features.

N absorption; N–H stretch, 1st overtone), 1690 (protein absorption; C–H stretch, 1st overtone), 1730 (protein absorption; C–H stretch), 1980 (protein absorption; N–H asymmetry), 2030 (protein and N absorption @ 2060; N–H rotation, N–H stretch) and 2130 nm (protein absorption; N–H stretch) (Fig. 4b). Some of the known absorption features were missing, possibly due to weak absorptions and/or because they were masked by leaf water content (Kokaly and Clark 1999). The PLSR loading weights (LWs) plot indicated N-related chemical

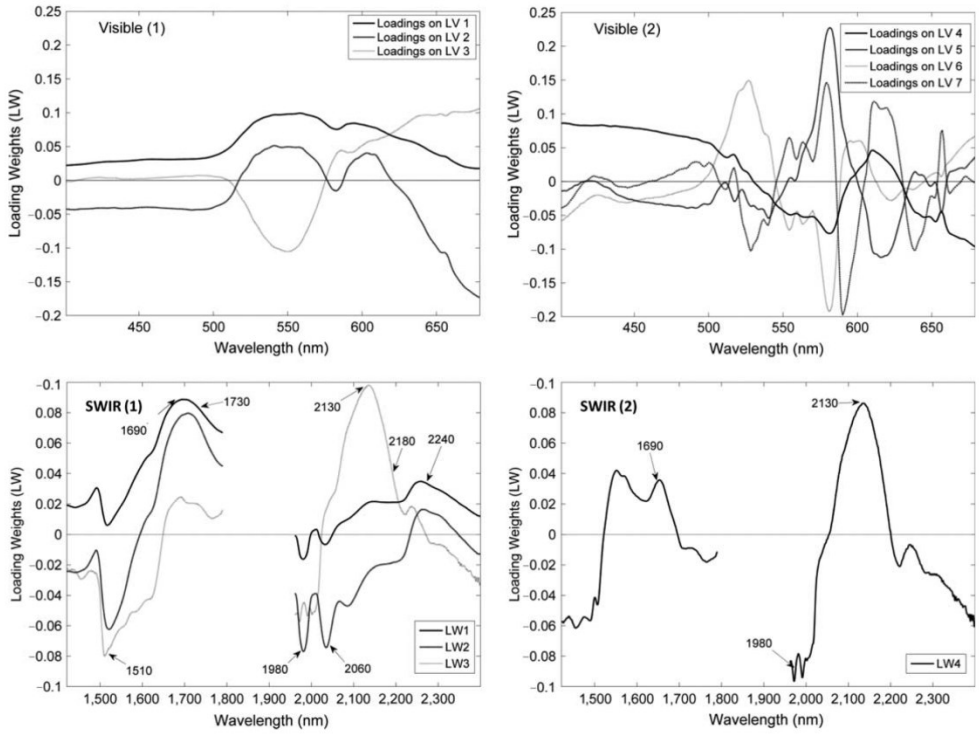

Fig. 5. Partial least squares (PLSR) models loading weights (LWs) calibrated to estimate foliar nitrogen (N) for visible-range and short wave infrared (SWIR) range of reflectance spectra. Visible (1) and Visible (2) showed the relevant loadings from 1 to 3 and from 4 to 8, respectively. SWIR (1) and SWIR (2) showed the relevant loadings from 1 to 3 and 4, respectively. Large LW values indicate high importance of wavelength in foliar N estimation.

contributions throughout much of the wavelength range (Fig. 5). The LWs, regression vector coefficients and VIP scores mostly correspond to known absorption features (Figs. 3, 4 and 5).

CONCLUSIONS

The following conclusions can be drawn from this study. First, the high R^2 and small RMSECV indicate that reflectance spectra can be used successfully to assess foliar N levels at field level. Second, the accuracy with which foliar N content was estimated using spectral regions in decreasing order from green peak 0.16 ($\pm$8.29% of mean), VIS-range 0.17 ($\pm$8.80% of mean), Chl ($a+b$) 0.18 ($\pm$9.33% of mean), full spectrum 0.18 ($\pm$9.33% of mean), NIR-plateau 0.18 ($\pm$9.33% of mean), known absorption features 0.19 ($\pm$9.84% of mean) and SWIR-range 0.20 ($\pm$10.4% of mean). Third, VIP scores, PLSR LWs and PLRS regression coefficient plots highlight the importance of wavebands ($\sim$550 nm, $\sim$610 nm, 1510 nm, $\sim$1690 nm, $\sim$1730 nm, $\sim$1980 nm and $\sim$2030 nm) for in situ foliar N estimations in wild blueberry production system. Considering the spectral data were collected under field conditions and encompassing different growth stages, this study has shown the potential for using reflectance spectra for in situ foliar N estimation in wild blueberry production.

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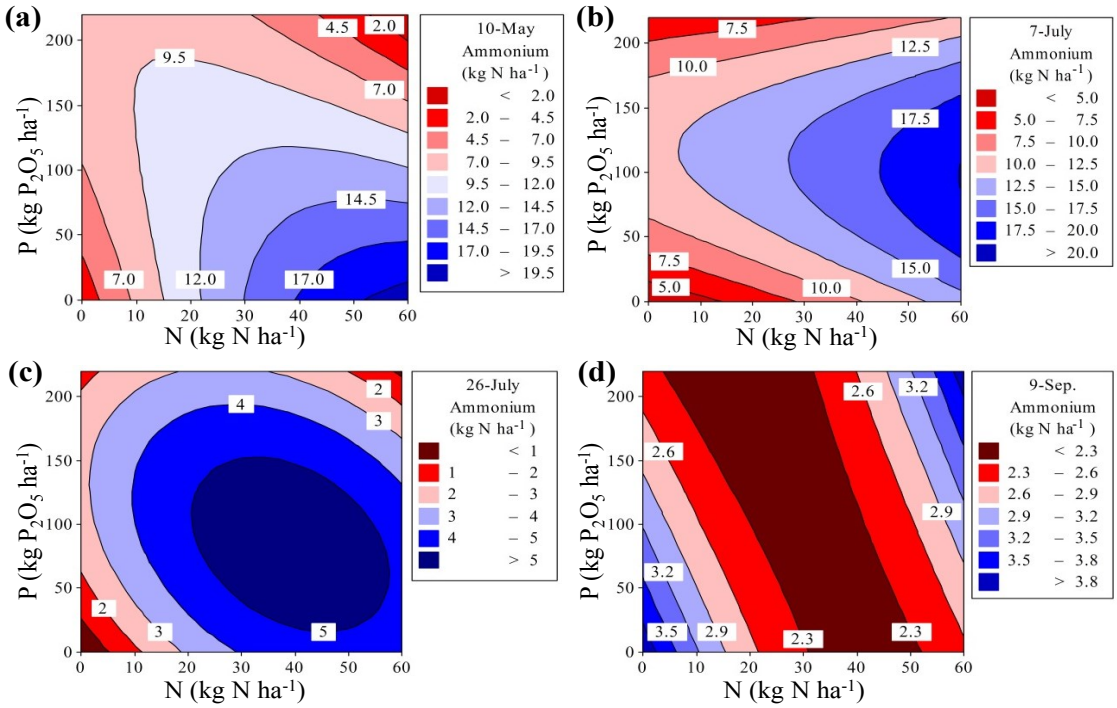
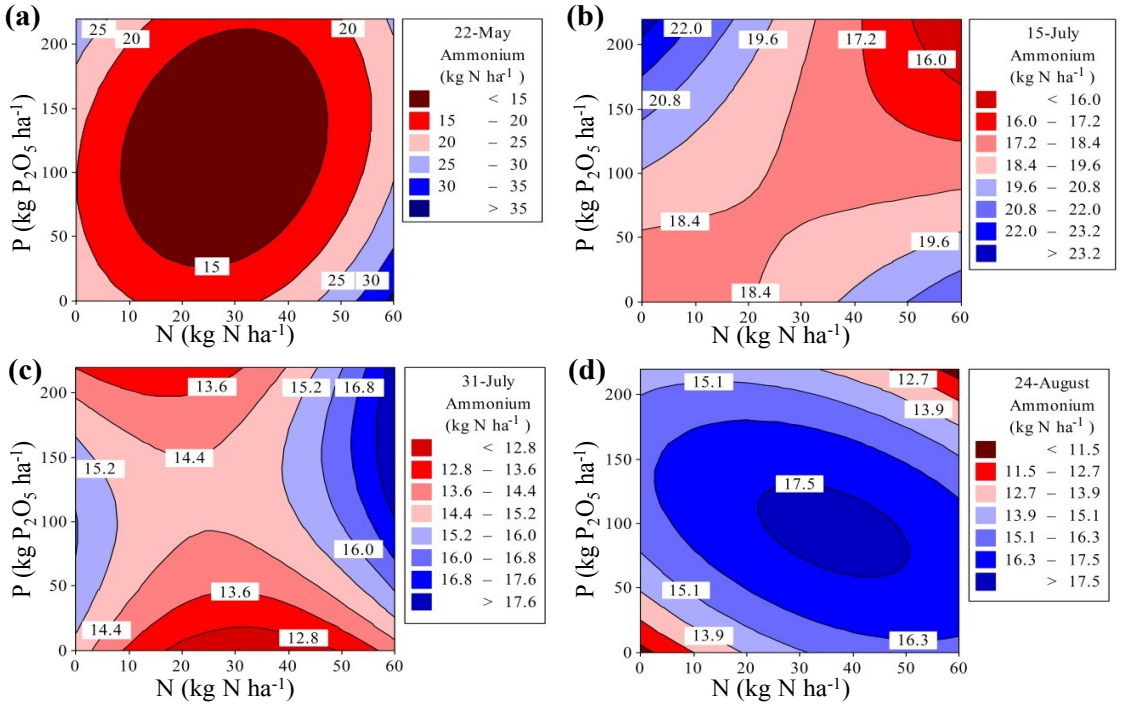
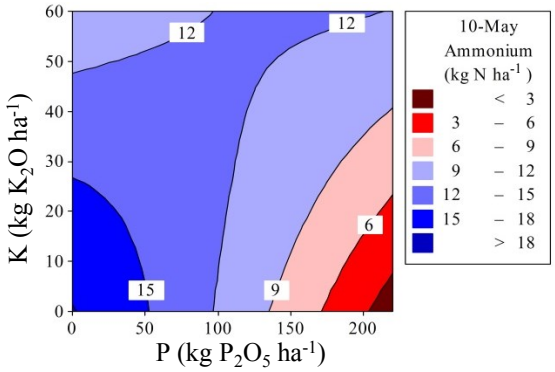
Appendix B Contour Plots Associated with Chapter 2



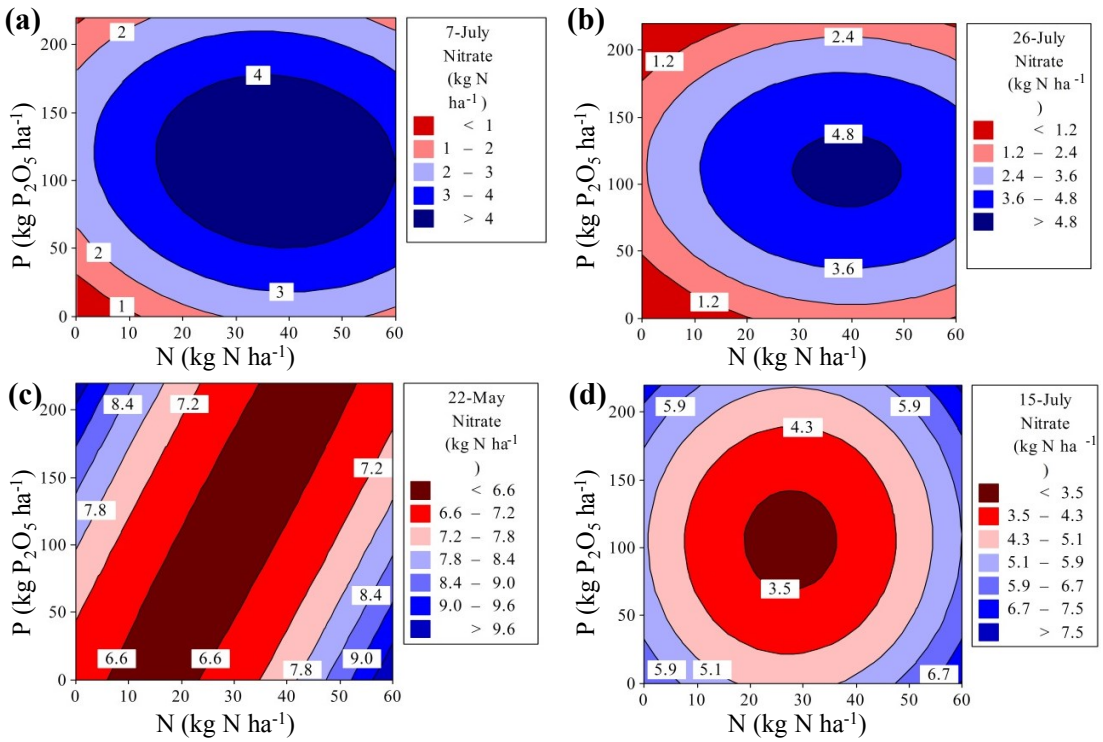
Appendix B.1 Contour plots of **ammonium** in the **sprout year** of production on the 10-May (a), 7-July (b), 26-July (c) and 9-September (d) for K_2O = 30 kg ha^{-1}



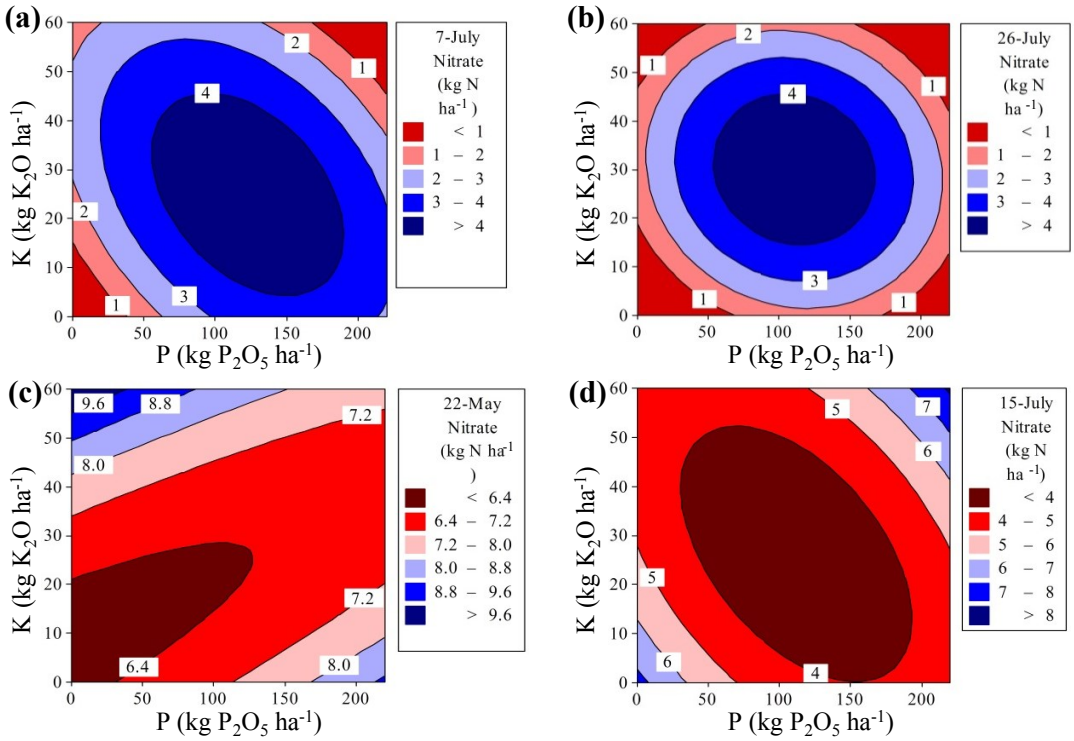
Appendix B.2 Contour plots of **ammonium** in the **crop year** of production on the 22-May (a), 15-July (b), 31-July (c) and 24-August (d) for K_2O = 30 kg ha^{-1}



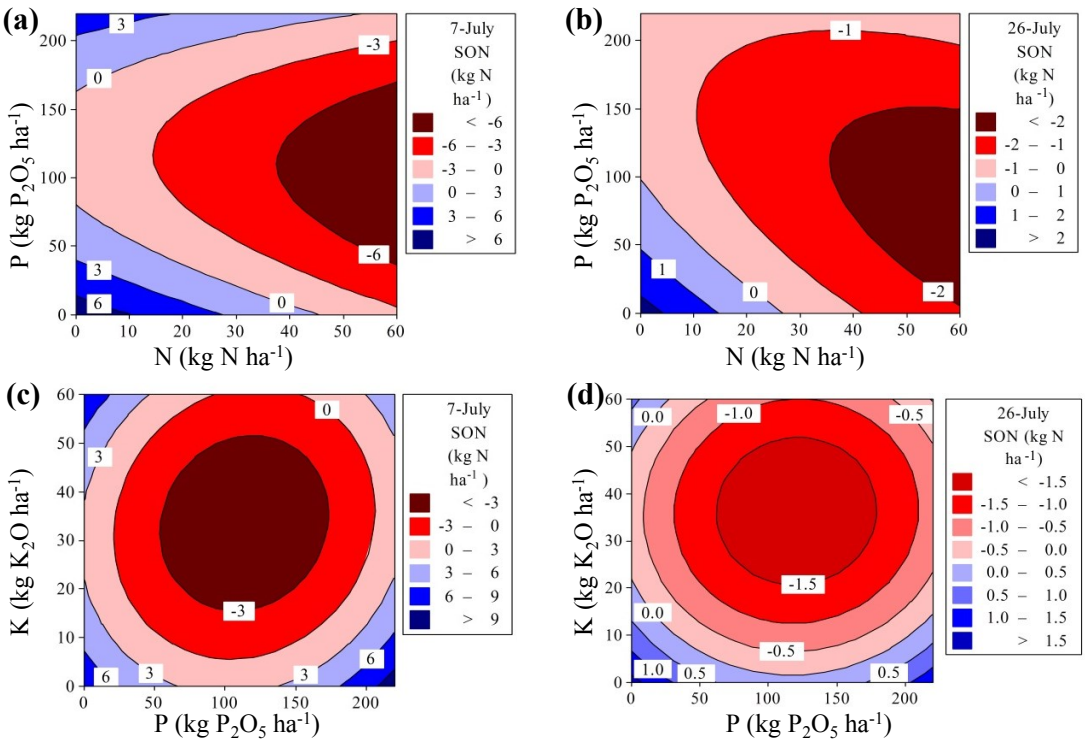
Appendix B.3 Contour plots of **ammonium** in the **sprout** year of production on the 10-May for N = 30 kg ha^{-1}



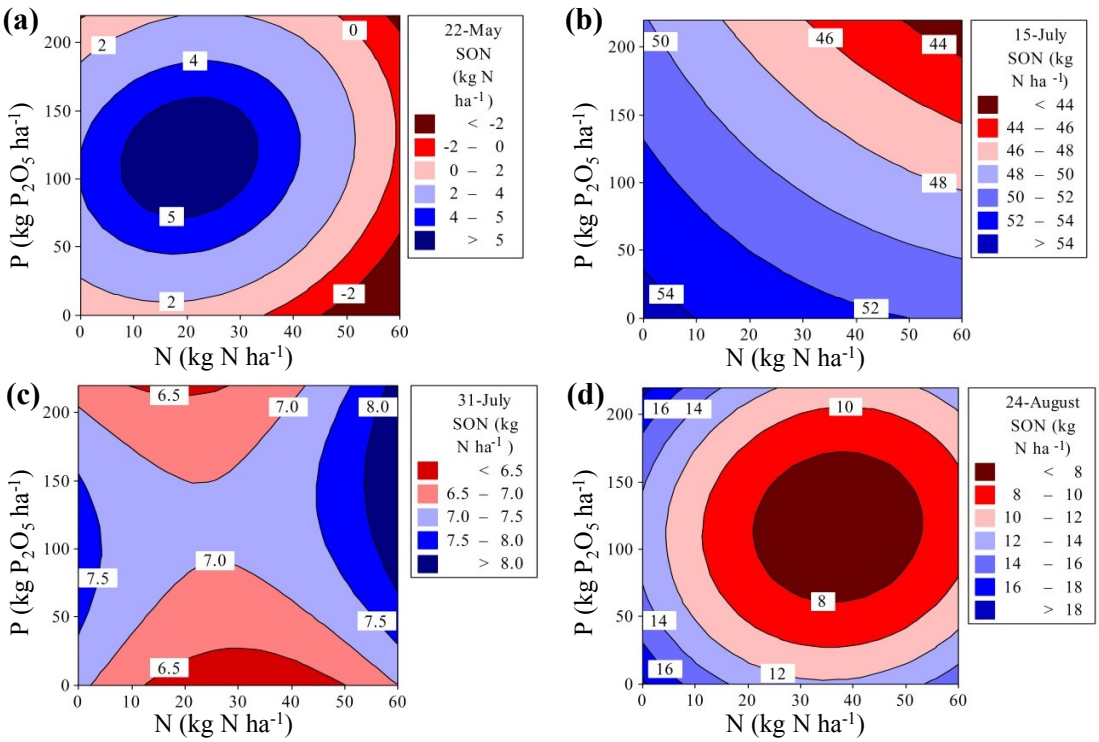
Appendix B.4 Contour plots of **nitrate** in the **sprout year** of production on the 7-July (a), 26-July (b) and crop year of production on 22-May (c) and 15-July (d) for K_2O = 30 kg ha^{-1}



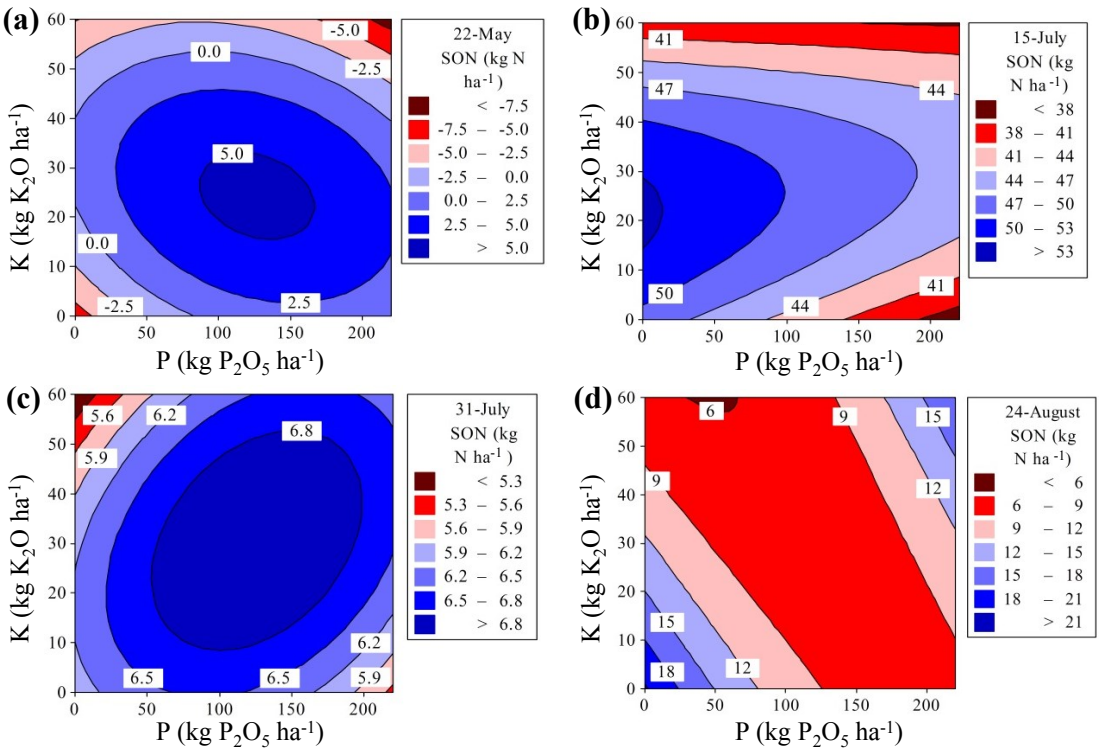
Appendix B.5 Contour plots of **nitrate** in the **sprout year** of production on the 7-July (a), 26-July (b) and crop year of production on 22-May (c) and 15-July (d) for N = 30 kg ha^{-1}



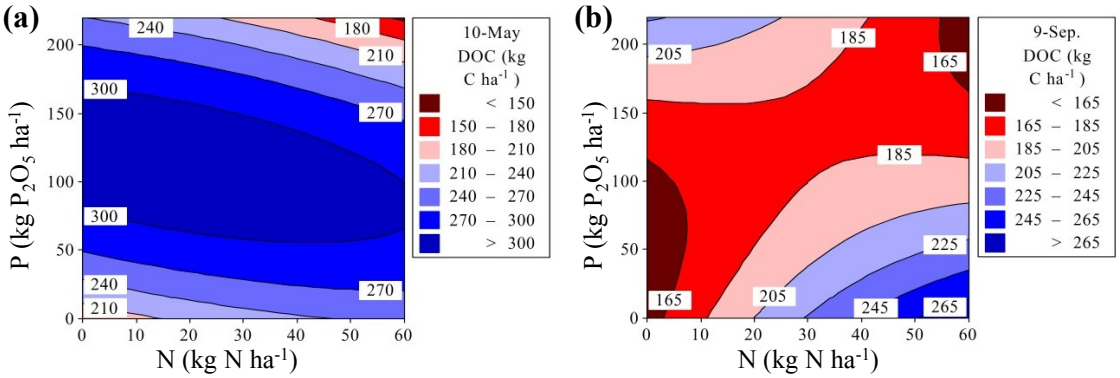
Appendix B.6 Contour plots of **soluble organic nitrogen** (SON) in the **sprout year** of production on the 7-July (a), 26-July (b) for K_2O = 30 kg ha^{-1} and 7-July (c), 26-July (d) for N = 30 kg ha^{-1}



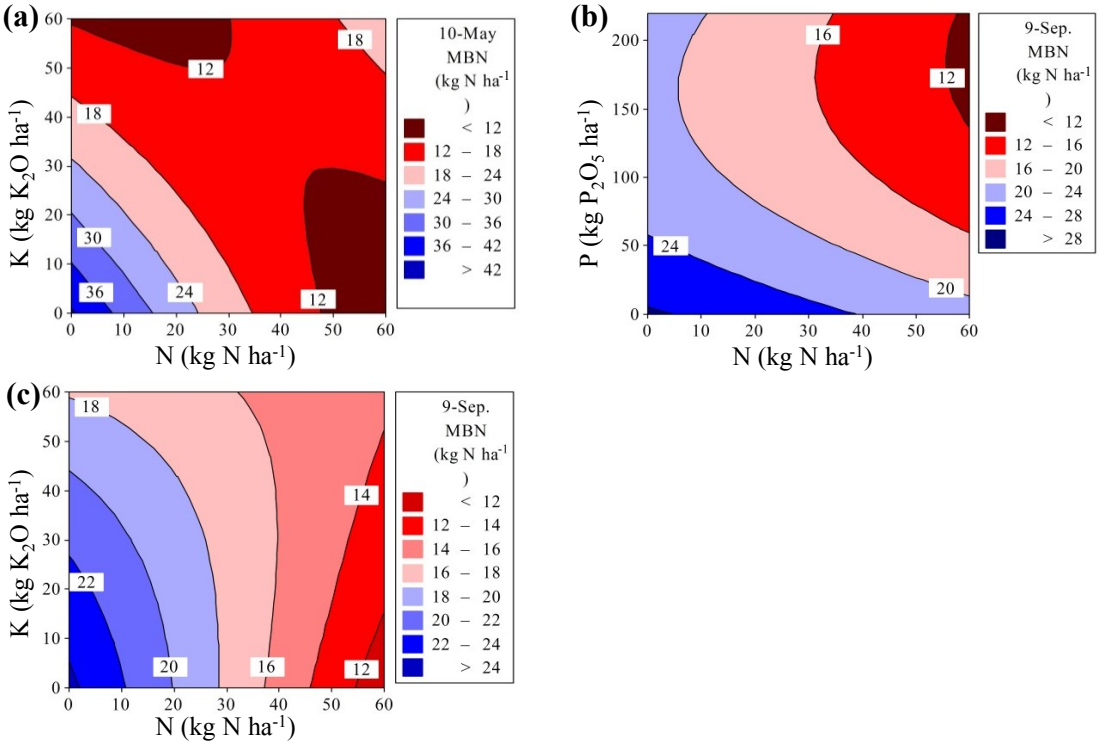
Appendix B.7 Contour plots of **soluble organic nitrogen** in the **crop year** of production on the 22-May (a), 15-July (b), 31-July (c) and 24-August (d) for K_2O = 30 kg ha^{-1}



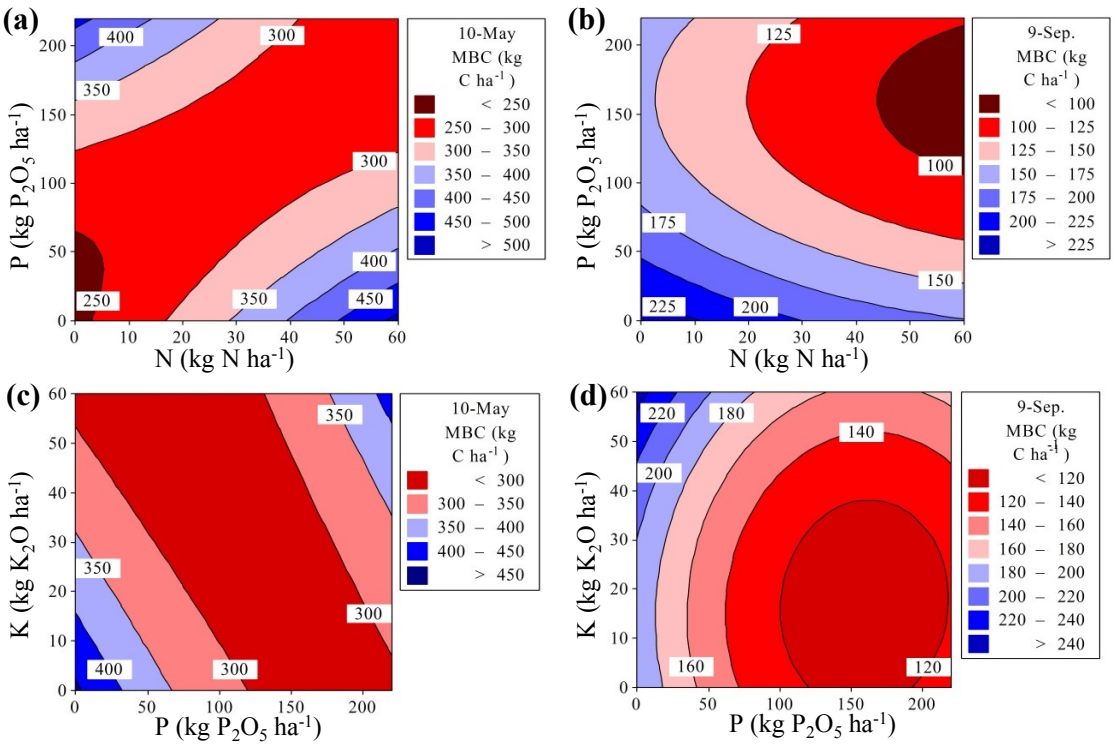
Appendix B.8 Contour plots of **soluble organic nitrogen** (SON) in the **crop year** of production on the 22-May (a), 15-July (b), 31-July (c) and 24-August (d) for N = 30 kg ha^{-1}



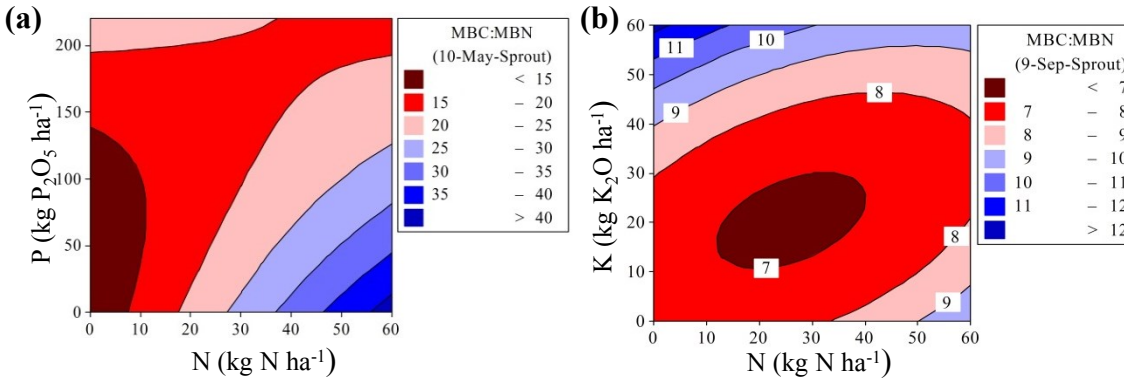
Appendix B.9 Contour plots of **dissolved organic carbon** (DOC) in the **sprout year** of production on the 10-May (a) and 9-Sep (b) for K_2O = 30 kg ha^{-1}



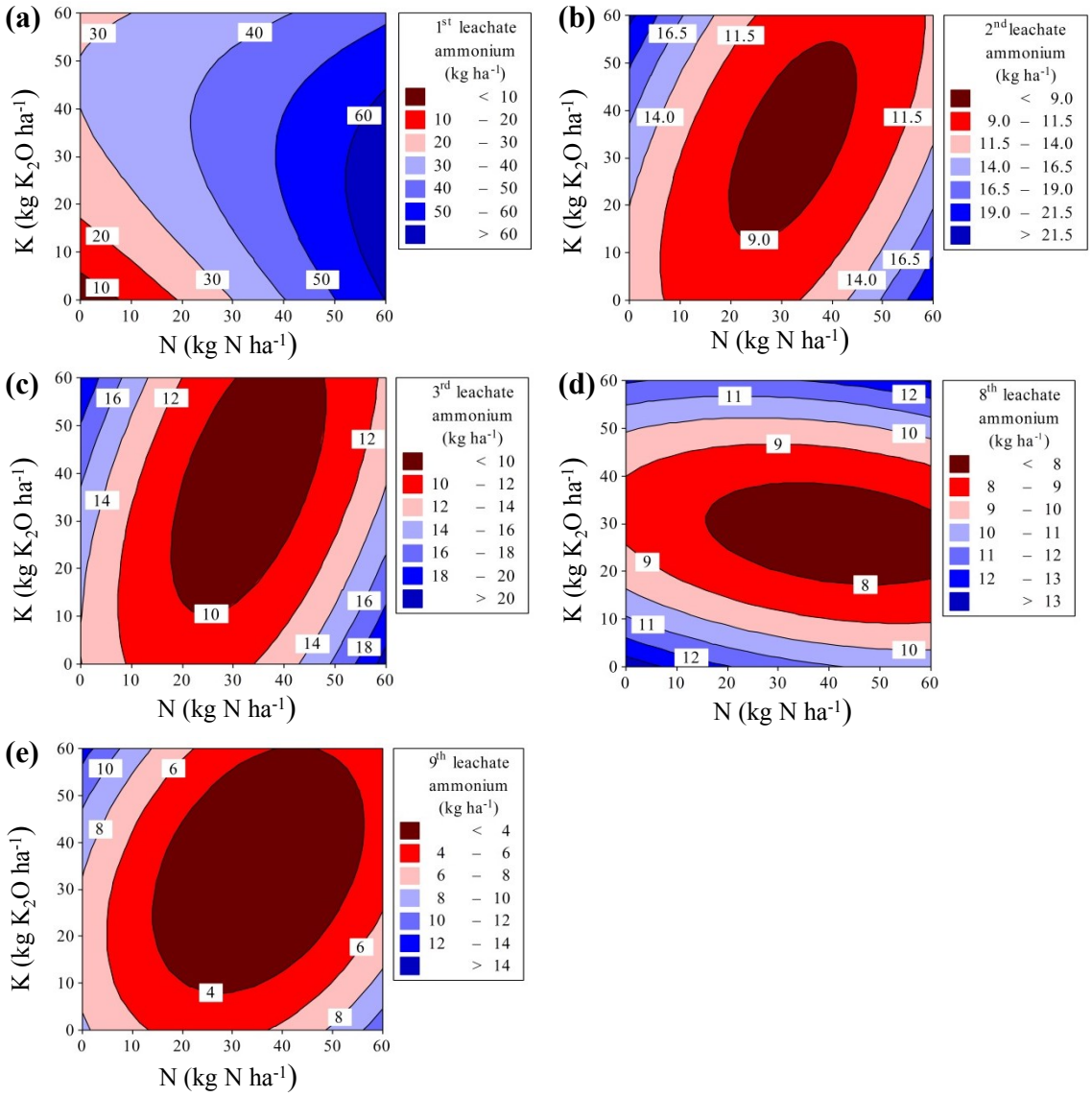
Appendix B.10 Contour plots of **microbial biomass nitrogen** (MBN) in the **sprout year** of production on the 10-May (a) for P_2O_5 = 110 kg ha-^{1}, 9-Sep (b) for K_2O = 30 kg ha^{-1}, and 9-Sep (d) for P_2O_5 = 110 kg ha^{-1}



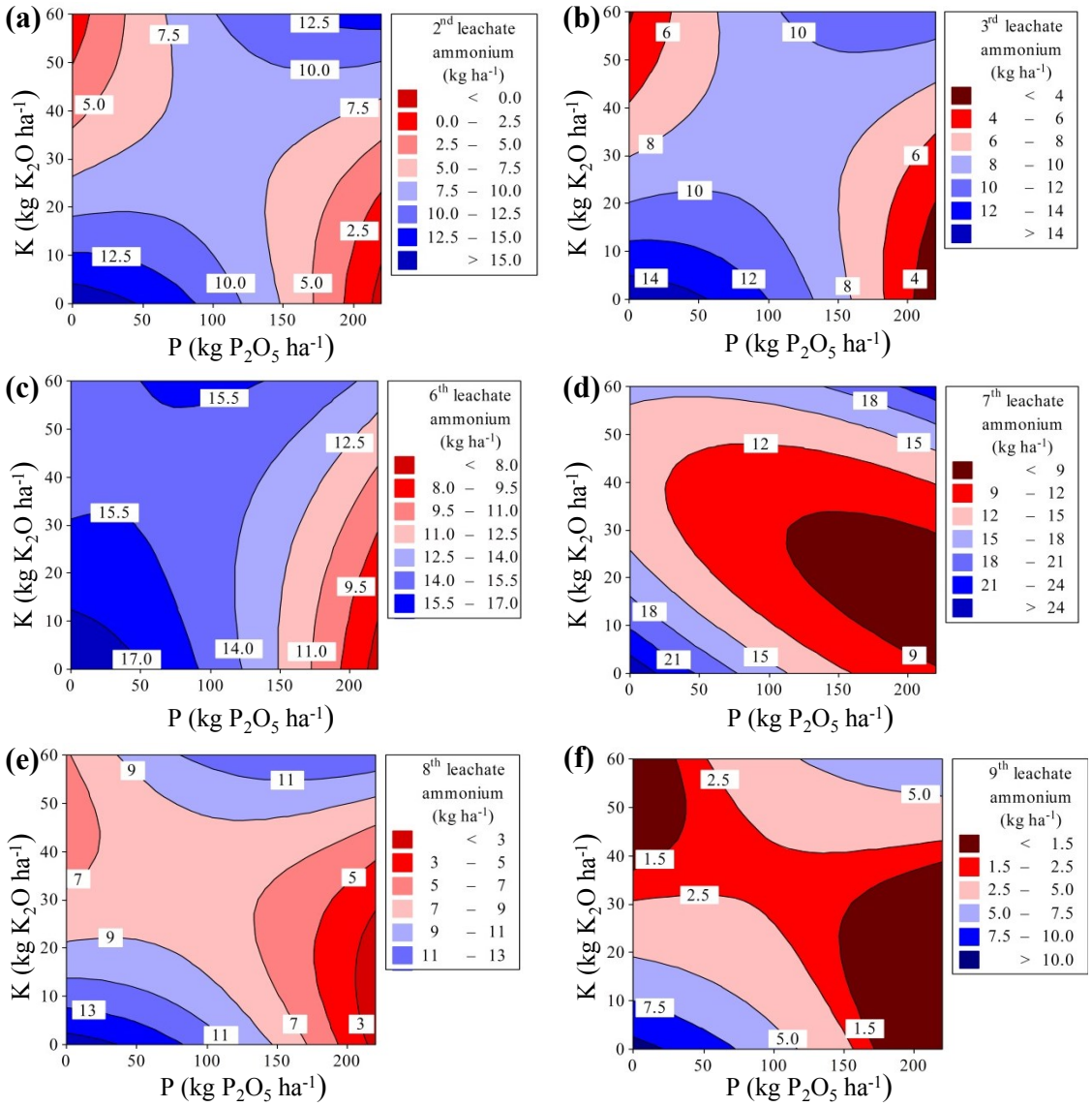
Appendix B.11 Contour plots of **microbial biomass carbon** (MBC) in the **sprout year** of production on the 10-May (a), 9-Sep (b) for K_2O = 30 kg ha^{-1} and 10-May (c), 9-Sep (d) for N = 30 kg ha^{-1}



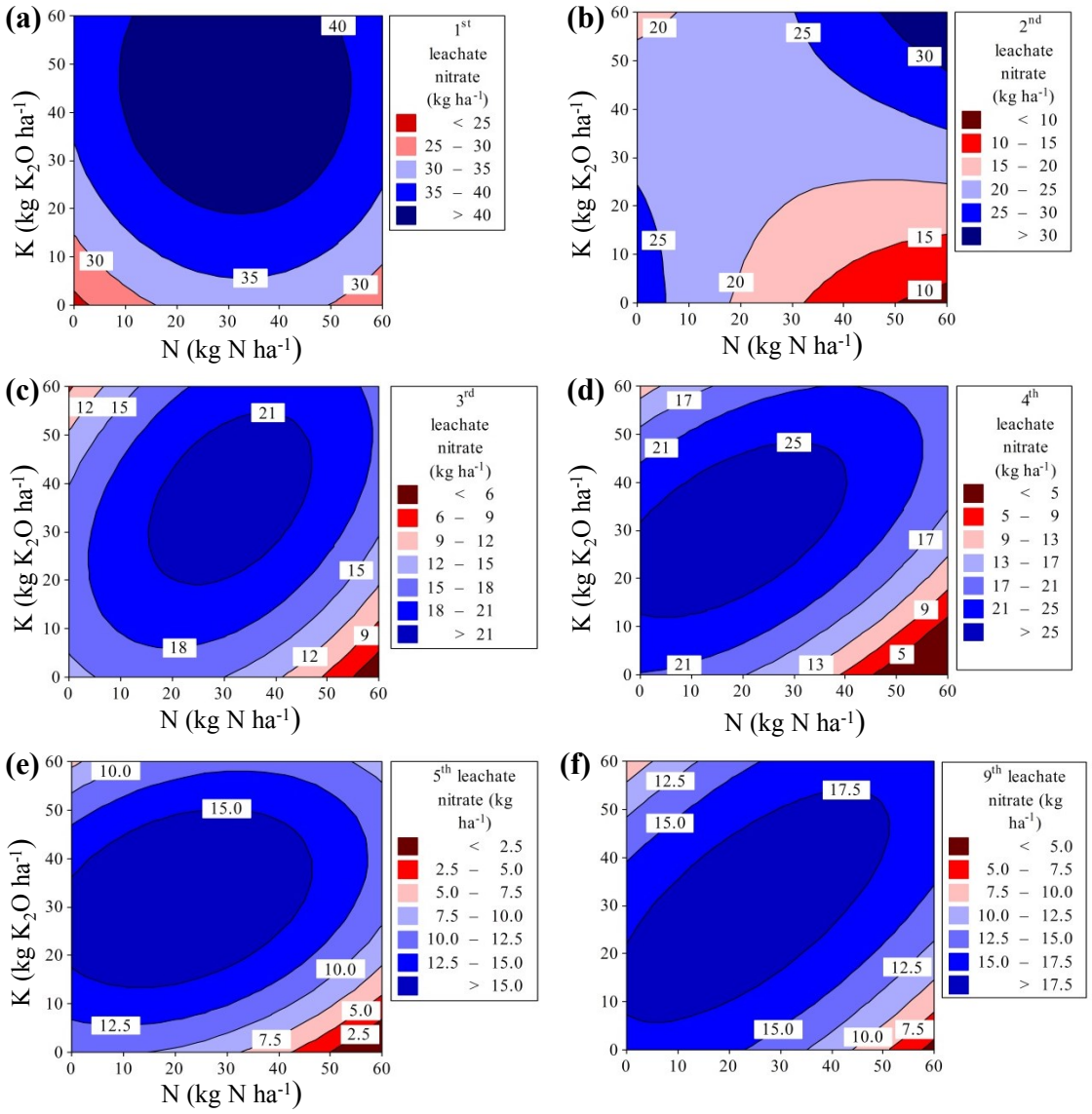
Appendix B.12 Contour plot of microbial biomass carbon: microbial biomass nitrogen **(MBC:MBN)** in the **sprout year** of production on the 10-May (a) for K_2O = 30 kg ha^{-1} and 9-Sep (b) for P_2O_5 = 110 kg ha^{-1}



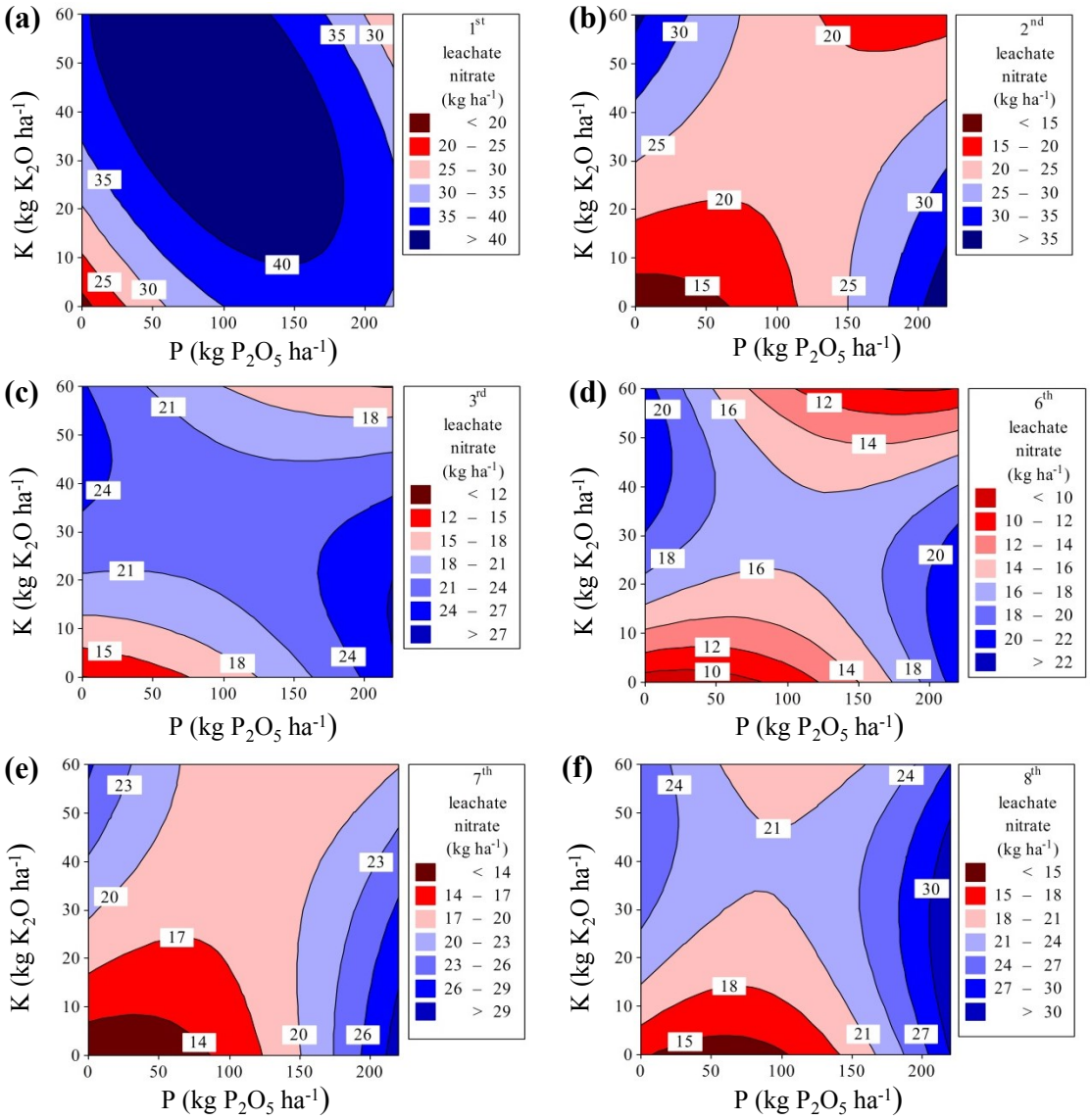
Appendix B.13 Contour plot of **net ammonification**in soil by periodic leaching; (a) 1st leachate, (b) 2nd leachate, (c) 3rd leachate, (d) 8th leachateand (e) 9th leachate for P_2O_5 fixed at 75 kg ha^{-1}



Appendix B.14 Contour plot of **net ammonification** in soil by periodic leaching; (a) 1st leachate, (b) 2nd leachate, (c) 3rd leachate, (d) 8th leachate and (e) 9th leachate for. P_2O_5 fixed at 75 kg ha^{-1}



Appendix B.15 Contour plot of **net nitrification** in soil by periodic leaching; (a) 1st leachate, (b) 2nd leachate, (c) 3rd leachate, (d) 4th leachate, (e) 5th leachateand (f) 9th leachate for P_2O_5 fixed at 75 kg ha^{-1}



Appendix B.16 Contour plot of **net ammonification** in soil by periodic leaching; (a) 1st leachate, (b) 2nd leachate, (c) 3rd leachate, (d) 6th leachate, (e) 7th leachate and (f) 8th leachate for N fixed at 30 kg ha^{-1}

Appendix C Table and Graphs Associated with Chapter 5

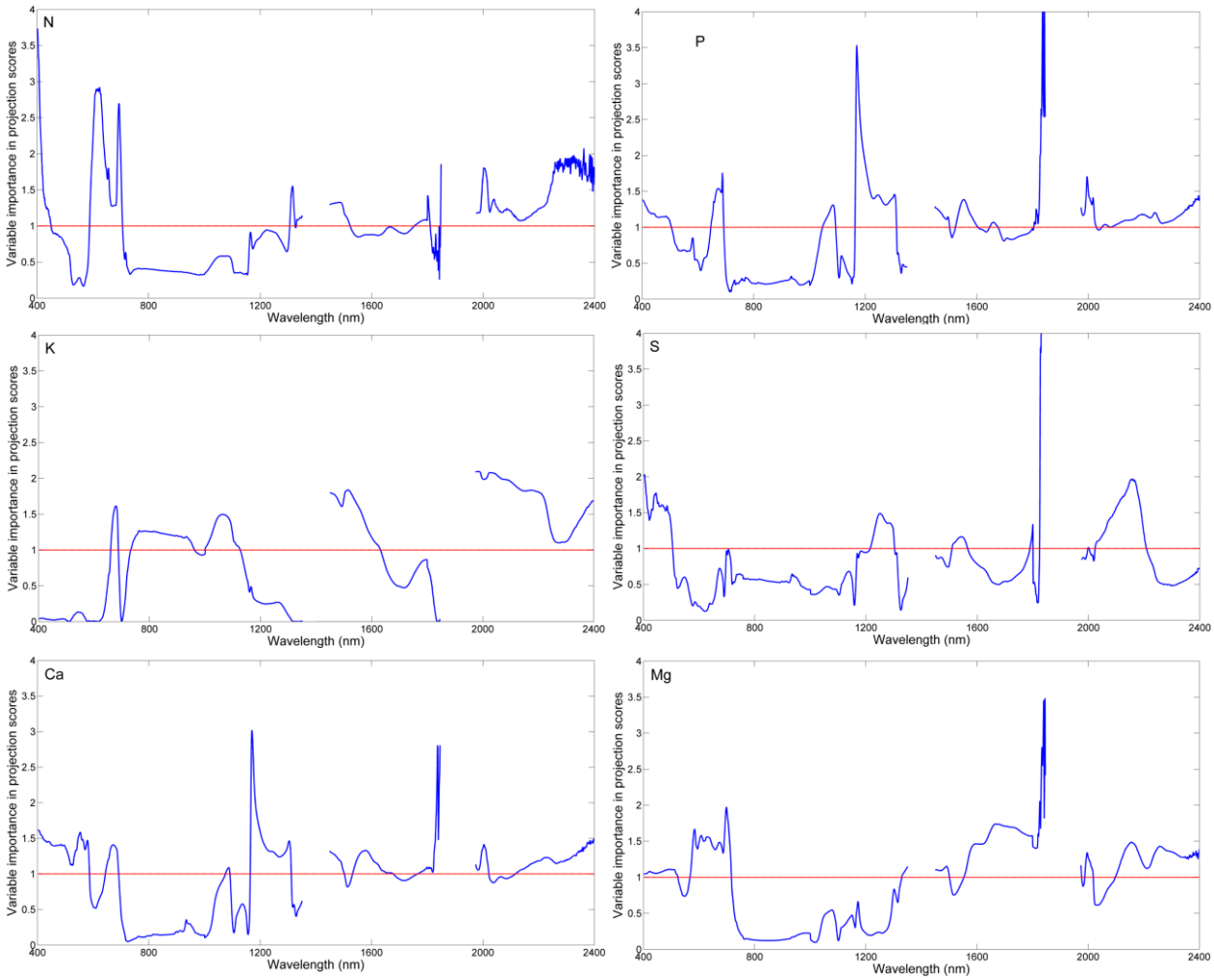
Appendix C.1 Variable Importance in Projection (VIP) Selected Wavelengths Models

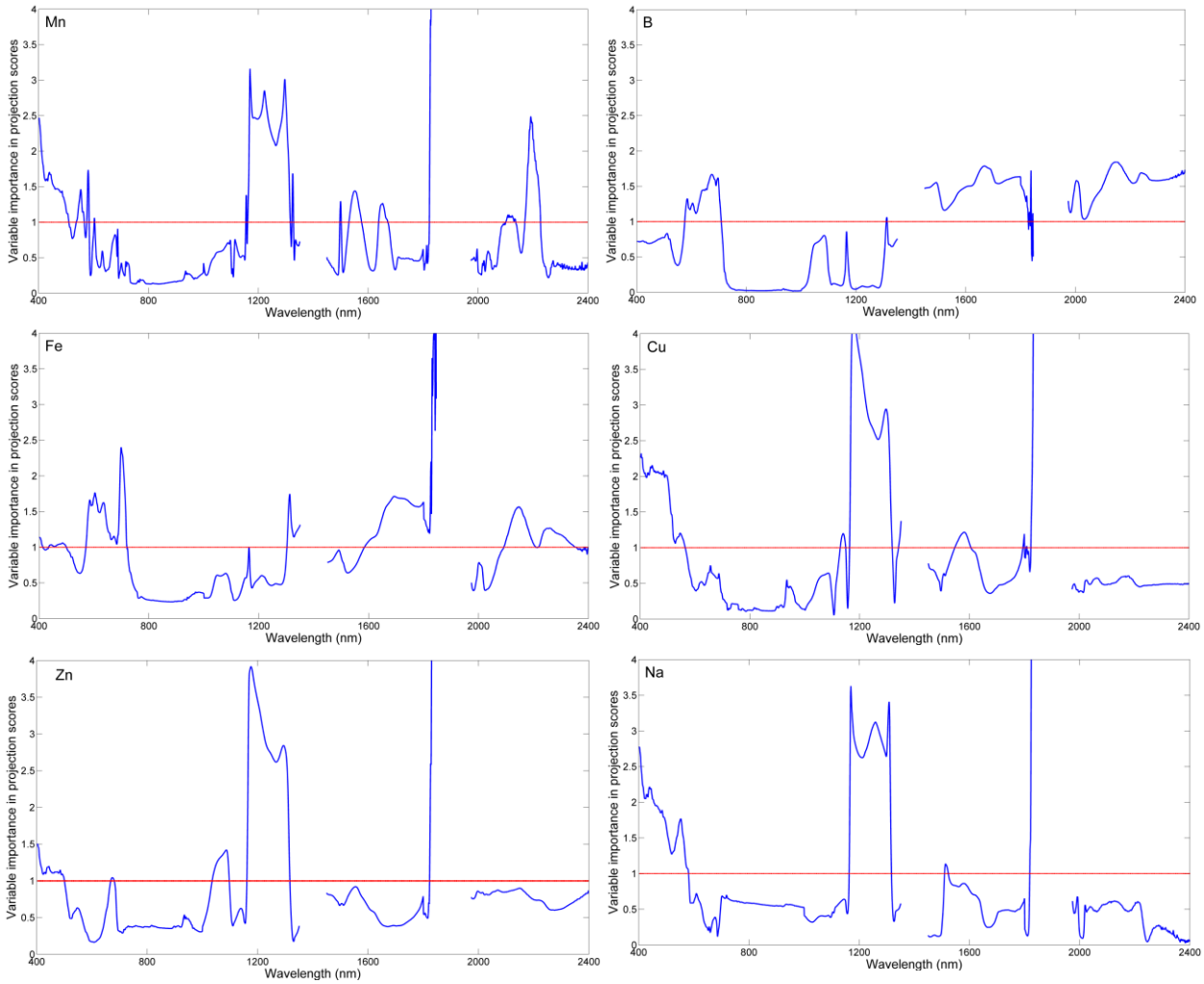
Prediction results using variable importance in projection (VIP) wavelengths from full spectrum partial least squares (FS-PLS) regression models

Parameter	VIP (VSWIR)			
	Calibration		Cross-validation	
	R^2	%RMSE	R^2	%RMSE
N	0.73	9.8	0.71	10.1
P	0.84	10.1	0.82	10.6
K	0.52	7.89	0.47	8.34
S	0.58	13.06	0.54	13.7
Ca	0.72	16.2	0.70	17.0
Mg	0.80	12.5	0.78	13.3
Mn	0.63	18.3	0.59	19.1
B	0.61	9.8	0.57	10.4
Fe	0.67	17.4	0.63	18.5
Cu	0.40	16.1	0.34	16.9
Zn	0.58	21.9	0.55	22.8
Na	0.62	20.7	0.56	22.18

Appendix C.2 Variable Importance in Projection (VIP) Plots

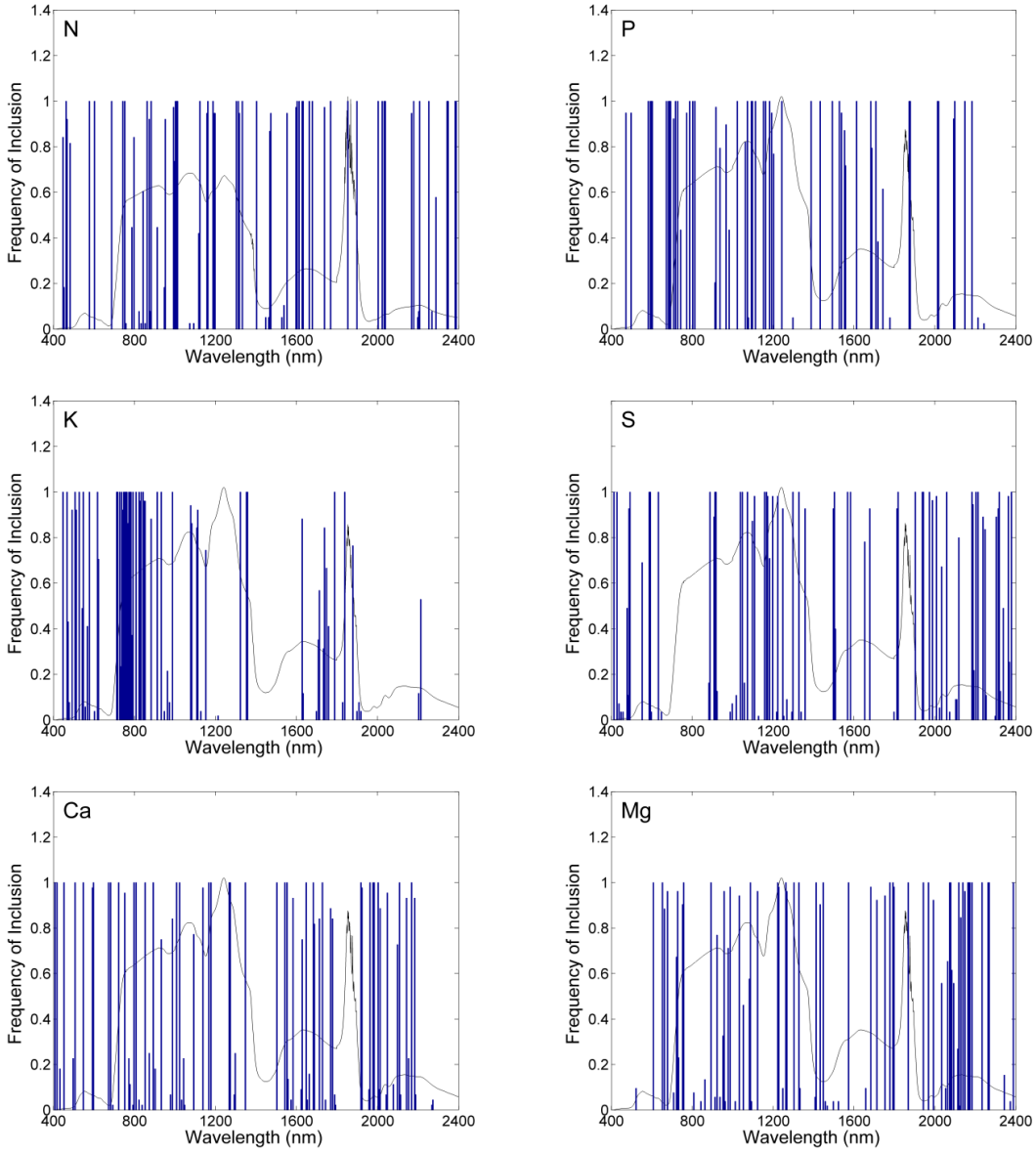
Variable importance in projection (VIP) scores obtained from full-spectrum partial least squares (FS-PLS) regression models using visible-to-shortwave infrared (VSWIR) spectral range. Horizontal line represents the VIP threshold value (> 1) of significant wavelength contribution in prediction. Each plot represents one foliar nutrient. The results are presented for foliar N, P, K, S, Ca, Mg, Mn, B, Fe, Cu, Zn and Na

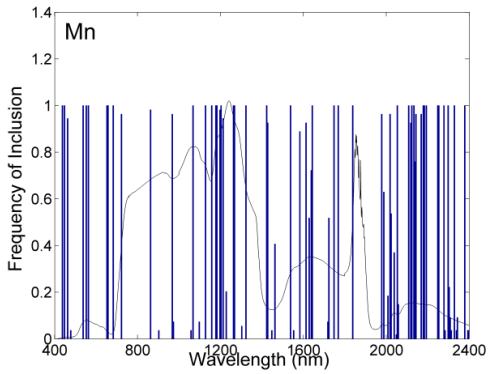


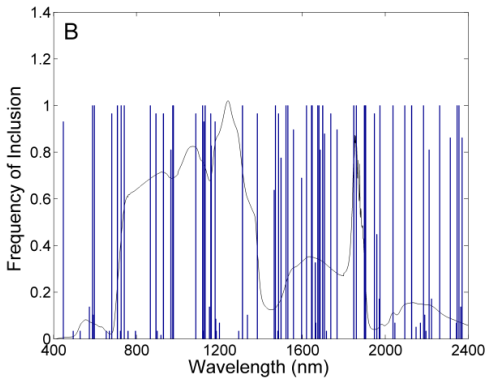

Mn
B
Fe
Cu
Zn
Na
Variable importance in projection scores
Wavelength (nm)
0
0.5
1
1.5
2
2.5
3
3.5
4
400
800
1200
1600
2000
2400

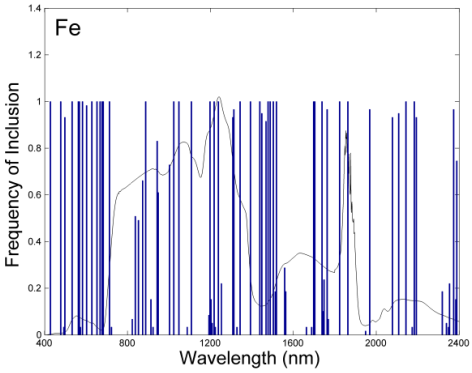
Appendix C.3 Genetic Algorithm-Partial Least Squares (GA-PLS) Plots

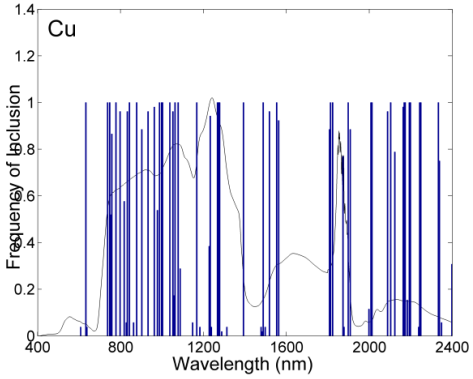
Wavelengths selected by genetic algorithm-partial least squares regression (GA-PLS) using reflectance spectra to estimate foliar nutrients. Frequency waveband inclusion in regression models is represented by blue horizontal bars from 100 GA-PLS runs. More frequently selected spectral regions suggest potentially informative wavelengths in the regression. The results are presented for foliar N, P, K, S, Ca, Mg, Mn, B, Fe, Cu, Zn and Na

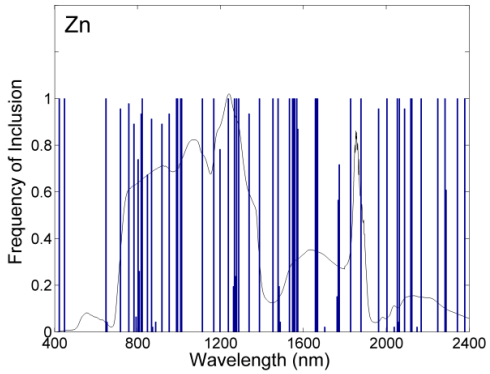


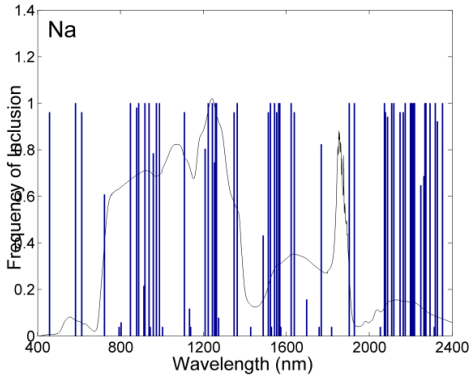

Mn
Frequency of Inclusion
Wavelength (nm)


B
Frequency of Inclusion
Wavelength (nm)


Fe
Frequency of Inclusion
Wavelength (nm)

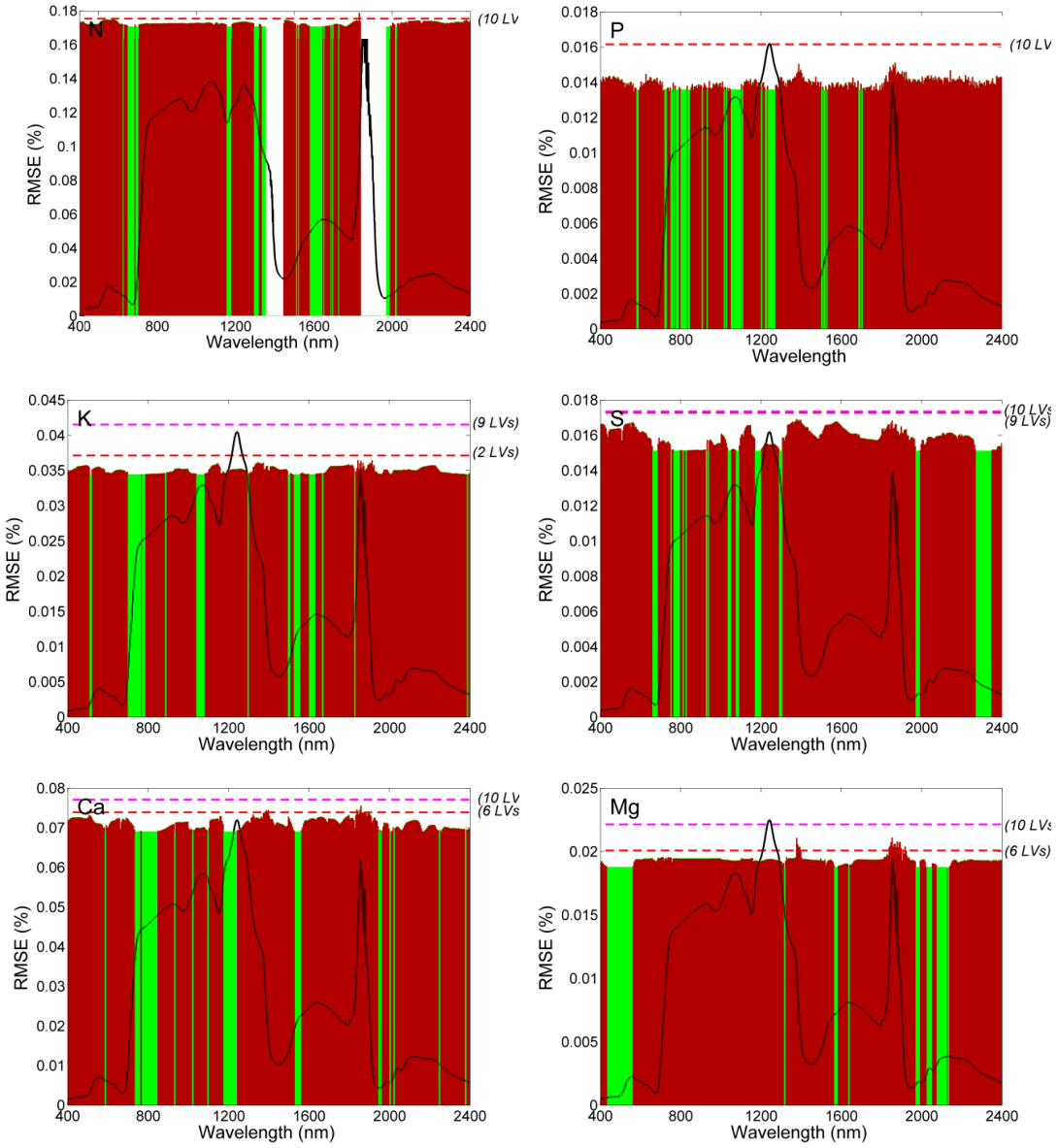

Cu
Frequency of Inclusion
Wavelength (nm)

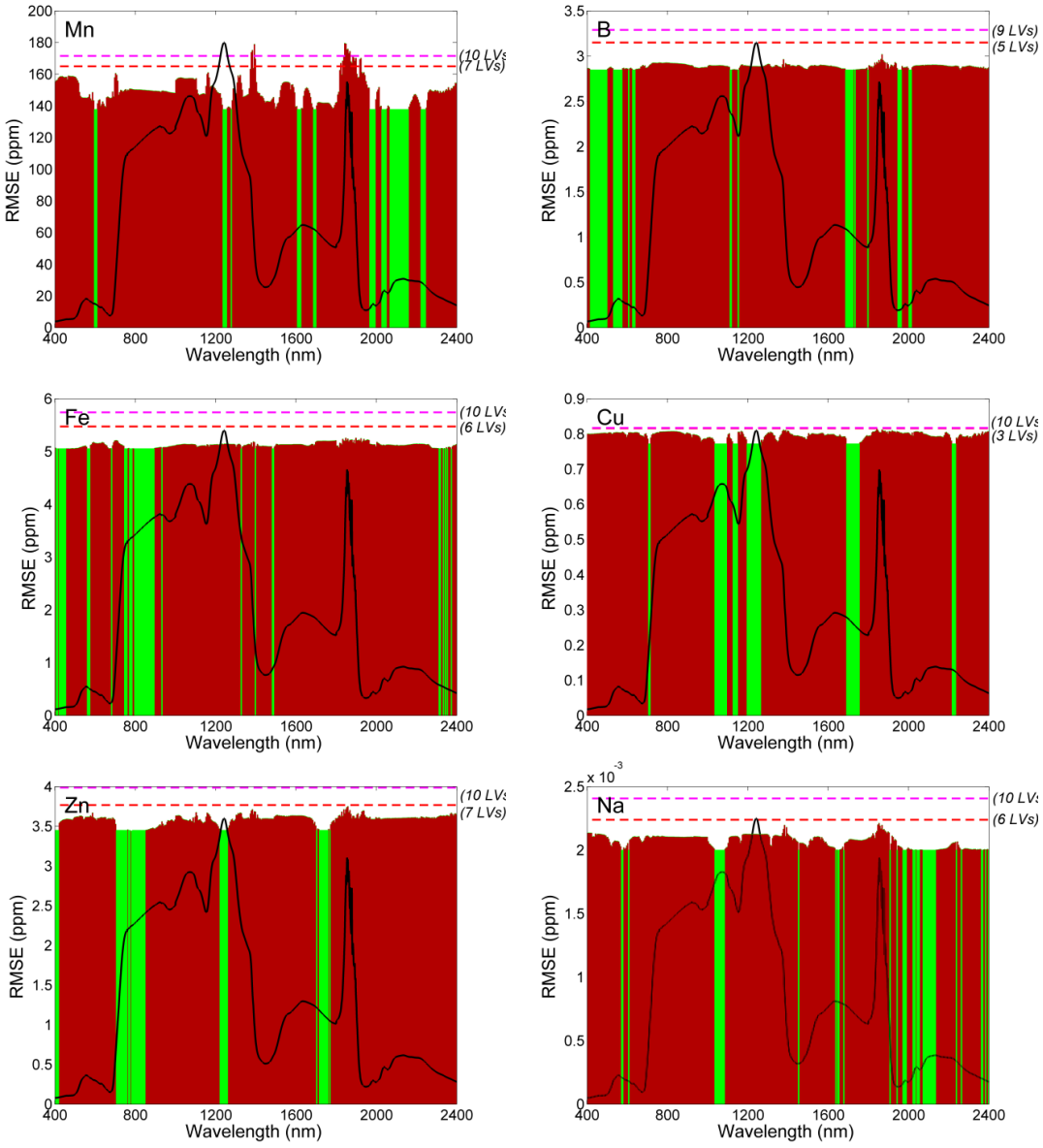

Zn
Frequency of Inclusion
Wavelength (nm)


Na
Frequency of Inclusion
Wavelength (nm)

Appendix C.4 Interval Partial Least Squares (iPLS) Plots

Selected wavebands in interval partial least squares regression (iPLS) based on cross-validated root mean square error (RMSE) using reflectance spectra to estimate foliar nutrients. The figure shows the root mean square of cross validation (RMSE) obtained for each interval with the average spectrum superimposed as a black line. The green vertical bars are the selected intervals. The horizontal dashed lines (red or magenta) indicate the RMSE obtained when using all variables (full spectrum) and the number of latent variables (LVs) used to obtain the given RMSE with maximum up to 10 LVs. Note that selected interval on its own generally gave a better prediction models than did the model using all full spectrum, including all the wavelengths (400-2400 nm) and iPLS did not removed the water related noisy bands and full spectrum model efficiency of prediction and precision was reduced, therefore, these full spectrum results did not agree to FS-PLS results present in Table 5.5. However, these results emphasize the removing of noisy bands




Mn
(10 LVs)
(7 LVs)
B
(9 LVs)
(5 LVs)
Fe
(10 LVs)
(6 LVs)
Cu
(10 LVs)
(3 LVs)
Zn
(10 LVs)
(7 LVs)
Na
x 10^-3
(10 LVs)
(6 LVs)
RMSE (ppm)
Wavelength (nm)