Effects of Intensive Fertilization on Soil Nutrient Cycling in Lodgepole Pine and Interior Spruce Forests in the Central Interior of British Columbia

by

Daniel Harrison
BSc, University of Victoria, 2008

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Supervisory Committee

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ABSTRACT

The growth and productivity of British Columbia's interior forests is largely limited by soil nutrient availability. Fertilization has been shown to be an effective silvicultural tool for increasing the development of immature stands throughout the region. This has lead to increased interest in long-term, repeated fertilization as a means of addressing timber-supply shortfalls as a result of the current mountain pine beetle (*Dendroctonus ponderosae*) outbreak. However, there is little information related to the impacts of repeated fertilization on the cycling of nutrients in many of these stands. This study makes use of a long-term (13-15 year) fertilization experiment in two lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm) and two interior spruce (*Picea glauca* [Moench] Voss and *Picea engelmannii* Parry) forests in the central interior of British Columbia subject to two levels (periodic and annual) of nitrogen(N)-based fertilization. The primary goal of the project was to examine the effects of different fertilizer regimes on aspects of soil chemistry. Specifically, this project was concerned with the impacts of repeated fertilization on: 1) soil carbon (C) and N cycling, and 2) soil base cation (e.g.,

Ca, Mg & K) availability. Soil and foliar nutrient regimes were quantified throughout the 2008 and 2009 growing seasons using ion-exchange membrane (IEM) plant root simulator (PRS) probes and traditional soil and foliar analyses. Fertilization increased N cycling at all sites, with generally elevated soil and foliar N and significant soil-foliar N relationships in several cases. Nitrate (NO_3^-) increased in the fertilized plots in several cases; however, there was minimal evidence of NO_3^- leaching. Greater than 90% of fertilizer-N inputs were retained onsite, suggesting these forests are not N-saturated. Soil, tree and total ecosystem C generally increased in response to fertilization, with the spruce sites exhibiting greater C accrual per unit of fertilizer N than the pine sites. Further, significant linear relationships between soil C and N were evident at all sites. At sites with poorly buffered soils (pH < 4), fertilizer treatments generally led to increased soil acidification and decreases in soil and foliar Ca. Decreases in soil Ca may have been due to significant increases in sulfate leaching; whereas foliar Ca decreases appear to be related to compromised uptake systems, potentially from increased soil aluminum. Buffering capacities, rather than forest type, appear to be the best predictor of soil and foliar Ca responses to fertilization. Despite significant changes in soil chemistry at all four sites, it does not appear that current fertilization rates are detrimentally affecting tree growth.

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CHAPTER 1 – General Introduction and Literature Review

Earth's population is expected to rise in upwards of 9 billion by 2050, with concomitant increases in the demand for forest products (Cown 2007). The majority of the forests required to meet these demands are already growing; however, global forest cover continues to decrease (United Nations FAO 2006). In response, intensive forest management has increased in recent decades in an attempt to improve the productivity of the forest land base (Bowyer 2001). In British Columbia, the timber harvesting land base is shrinking, largely due to land withdrawals for non-timber forest uses (Brockley and Simpson 2004). In addition, British Columbia's pine forests are currently experiencing the largest mountain pine beetle (*Dendroctonus ponderosae*) outbreak in recorded history. As a result, the mature pine forests on which much of the forest industry in the Interior of British Columbia is based is expected to reach 80% mortality by 2013 (British Columbia Ministry of Forests and Range 2006). In an attempt to increase the operability of remaining forest lands, the B.C. Ministry of Forests and Range has proposed widespread intensive fertilization as an intervention strategy to accelerate the growth of immature stands and increase short and mid-term timber supply.

Fertilization is the most proven silvicultural method for accelerating tree and stand development in existing immature stands (Brockley and Simpson 2004; Fisher and Binkley 2000). Although a single fertilizer application generally produces only short-term increases in stand development, long-term growth responses are possible with repeated fertilization (Albaugh et al. 2004; Ringrose and Neilson 2005; Brockley 2007a). For example, research in Sweden has found that intensive fertilization is capable of

shortening rotation periods of boreal spruce *(Picea spp.*) by as much as 20-60 years (Bergh et al. 2005). Similar growth responses in B.C. would be valuable for addressing mountain pine beetle-related timber shortages and increasing the productivity of forest lands.

Extensive research throughout the interior of British Columbia has confirmed widespread nutrient deficiencies and favourable growth responses to a range of fertilizer treatments in interior spruce (*Picea glauca* [Moench] Voss and *Picea engelmannii* Parry, or naturally occurring hybrids of these species) and lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm) forests (Brockley & Simpson 2004; Brockley 2007a). While both species appear to respond reasonably well to large nutrient additions in the short-term, there is concern that repeated nutrient additions may affect long-term site productivity through alterations in soil chemistry (Ringrose and Neilsen 2005).

Fertilization has been found to induce changes in the species composition of forest vegetation (Kellner 1993; Brockley 2007b), which can affect the quantity and quality of litter inputs to the soil and alter patterns of organic matter decay (Laiho and Prescott 2004; Prescott et al. 2004; Magill et al. 2004). For example, Magill and Aber (1998) found that nitrogen (N)-fertilization increased the lignin content of forest litter and reduced long-term decomposition rates. Alterations in soil organic matter can significantly affect soil nutrient cycles because the size and composition of the organic matter pool controls soil microbial populations (Scott and Binkley 1997; Webster et al. 2001) and the supply of plant-available nutrients (Attiwill and Weston 2001; Hart et al. 1994). Long-term N-fertilization has been found to alter the soil organic matter pool (Hyvonen et al. 2008; Mack et al. 2004), negatively affect soil microbial populations

(Frey et al. 2004; Bowden et al. 2004; Berch et al. 2006) and decomposition rates (Micks et al. 2004; Fog 1988).

Aside from alterations in microbial populations and the soil organic matter pool, fertilization can directly affect mineral soil chemistry and associated nutrient supply rates. For example, N-fertilization often results in soil acidification due to increased rates of nitrification (Aber et al. 1989, 1998). Nitrification is the biological oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and subsequently nitrate (NO_3^-). Hydrogen (H^+) ions produced during nitrification reduce soil pH and have been found to replace metallic cations (e.g., Ca^{2+}, Mg^{2+} and K^+) from exchange sites, making them susceptible to leaching losses (Homann et al., 2001). In fact, substantial soil calcium (Ca), magnesium (Mg) and potassium (K) losses in fertilized forests are predominantly attributed to intensified nitrate leaching induced by fertilization (Likens et al. 1996; Kolling et al. 1997; Aber et al. 1989; Perakis et al. 2006). This can result in foliar and soil nutrient imbalances (e.g., N:Ca, N:Mg) that are a primary cause of forest decline (Attiwill and Adams 1993). Further, the production of NO_3^-, which is not adsorbed by the negatively charged exchange sites that dominate most soils, leads to nitrogen losses that can result in forest nutrient impoverishment as well as negatively affect downstream aquatic ecosystems (Vitousek et al. 1997; Galloway et al. 2003; Venterea et al. 2004).

In addition to the effects of fertilization on soil nutrient cycling, elevated N inputs have been shown to significantly affect carbon (C) storage in forest ecosystems (Högberg 2007). Forest ecosystems represent approximately half of the terrestrial C reservoir (Dixon et al. 1994); thus, any changes in C dynamics in these systems can have enormous implications on atmospheric C concentrations and global climate. Aboveground forest C

dynamics generally respond positively to elevated N inputs (LeBauer and Treseder 2008); however, belowground (forest floor and mineral soil) C responses are less predictable. Elevated N has been associated with increased (Mäkipää 1995; Pregitzer et al. 2008), decreased (Mack et al. 2004; Allison et al. 2010), or no change (Neff et al. 2002; Johnson et al. 2003; Leggett and Kelting 2006; Sartori et al. 2007) in belowground C. Approximately two thirds of forest C is stored in soils (Dixon et al. 1994); thus, understanding how N inputs affect belowground C accumulation is essential for predicting whether forest ecosystems will act as sinks or sources of C as anthropogenic N inputs increase.

As outlined above, the effects of fertilization on forest ecosystems can be both positive (e.g., increased timber production, elevated C storage) and negative (e.g., soil acidification, base cation depletion); however, responses vary greatly by forest type and cannot be accurately predicted based on current knowledge (Aber and Magill 2004). As forest fertilization programs continue to increase in response to forest disturbances and increased demand for wood products (Cown 2007), it is essential to understand whether such practices will significantly affect long-term forest productivity.

Forest fertilization research in British Columbia has focused primarily on the impacts of intensive fertilization on aboveground timber and non-timber resources (e.g., Brockley and Simpson 2004; Brockley 2007a, 2007b, 2010a, 2010b); however, belowground processes have received limited attention. While the effects of fertilization on aspects of soil biota have been examined to a limited degree (Berch et al. 2006, 2009), research focused on soil chemistry and nutrient cycling has been minimal (Brockley and Sanborn 2009). The goal of this masters project is to address this research gap by

studying the effects of repeated fertilization on aspects of soil nutrient cycling in immature lodgepole pine and interior spruce stands in the central interior of British Columbia.

Beginning in 1994, the B.C. Ministry of Forests and Range established two lodgepole pine and two interior spruce "maximum productivity" field installations (Experimental Project 886.13) to assess the impacts of repeated fertilization on aspects of forest productivity (see Chapter 2 for complete description of field sites). These research sites were studied in 2008 and 2009 to address two main research objectives: 1) assess whether repeated fertilization has affected soil pH and base cation cycling; and 2) determine whether repeated N-loading has affected C and N cycling in these stands. These objectives were addressed by integrating traditional soil assays with innovative technologies in field and laboratory experiments to assess changes in key nutrient cycling processes at these sites. The details of these experiments make up the remainder of this thesis.

This thesis is designed as three distinct research papers (Chapters 3-5). Chapter 2 provides a thorough description of the field sites used in this study. Chapter 3 compares a traditional N mineralization assay with ion-exchange membrane N measurements to assess how each method quantifies the N mineralization activity of the soil. Chapter 4 assesses the impacts of fertilization on soil C and N dynamics. Chapter 5 examines the effects of fertilization on soil base cation cycling and soil acidification. A general discussion of the results is provided in Chapter 6.

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CHAPTER 2 – Site Description and Experimental Design

SITE DESCRIPTION

Research was carried out at two lodgepole pine (*Pinus contorta* Dougl. Var. *latifolia* Engelm.) and two interior spruce (*Picea glauca* [Moench] Voss and *Picea engelmannii* Parry, or naturally occurring hybrids of these species) fertilizer trials in the central interior of British Columbia. All four sites were established as part of the BC Ministry of Forests "maximum productivity" field installations (Experimental Project 886.13; Brockley and Simpson 2004) to assess the effects of repeated fertilization on aspects of forest productivity (Brockley 2007). The study sites, Lodi Lake (S1), Crow (S2) Creek, McKendrick Pass (P1) and Crater Lake (P2), are referred to as S1, S2, P1 and P2 throughout the remainder of the text. However, in this chapter only, study sites are referred to by both their full title and abbreviation. The abbreviations allow for easy distinction between the spruce (S1 & S2) and pine (P1 & P2) sites. In chapters 4 and 5, site abbreviations are also accompanied by sampling years in several cases (e.g., S1 2008 = S1-08).

Spruce Sites

The Lodi Lake (S1) site is located approximately 40 km southeast of Hixon, BC, in the Prince George forest district. The site is situated within the wet cool subzone of the Sub-Boreal Spruce Biogeoclimatic Zone (SBSwk) with 641 mm of mean annual precipitation (32% of which is snow) and a mean annual temperature of 4.9 ºC (Environment Canada 2011). The soils are derived from morainal parent material on an

east facing mid-slope (<5%) and are moderately well drained and relatively stone-free. Soil textures in the lower- and mid-slope plots are predominantly loams and sandy loams while the upper-slope plots are dominated by loams and clay loams. Soils in all plots are classified as Eluviated Dystric Brunisols (Soil Classification Working Group 1998). The previous stand was clearcut harvested and broadcast burned in 1985 and subsequently replanted in 1987. All treatment plots were thinned to 1100 stems per hectare during site establishment in 1995. In June 2008, the beginning of this study, the stand was 24 years old (Brockley and Simpson 2004). For additional site and stand characteristics, see Table 2.1.

The Crow Creek (S2) site is located approximately 60 km southeast of Houston, BC, in the Nadina forest district. The site is situated within the moist cold subzone of the Sub-Boreal Spruce Biogeoclimatic Zone (SBSmc) with 461 mm of mean annual precipitation (40% of which is snow) and a mean annual temperature of 2.8 ºC (Environment Canada 2011). Soil parent material is morainal in origin and deposited as a relatively flat geomorphological feature. Soils are well-drained loams and clay loams with approximately 25% gravels in the upper horizons. Soils are classified as Orthic Dystric Brunisols (Soil Classification Working Group 1998). The previous stand was clearcut harvested and broadcast burned in 1985 and subsequently replanted in the spring of 1986. All treatment plots were thinned to 1100 stems per hectare during site establishment in 1994. In June 2008, the beginning of this study, the stand was 24 years old (Brockley and Simpson 2004). For additional site and stand characteristics, see Table 2.1.

Pine Sites

The McKendrick Pass (P1) lodgepole pine site is located approximately 23 km north of Smithers, BC, in the Skeena Stikine forest district. The site is situated within the moist cold subzone of the Engelmann Spruce-Subalpine Fir Biogeoclimatic Zone (ESSFmc) with 513 mm of mean annual precipitation (40% of which is snow) and a mean annual temperature of 3.9 ºC (Environment Canada 2011). Site geomorphology suggests the soils were derived from morainal parent material deposited to form a gentle east-south-east facing slope. Soils and vegetation suggest the upper slope is slightly drier than the mid and lower slope positions; however, soils at all slope positions are classified as Orthic Humo-Ferric Podzols (Soil Classification Working Group 1998). Soils are loamy in texture and have approximately 60% coarse fragments in the upper horizons. The previous stand was clearcut harvested and broadcast burned in 1987 and subsequently replanted in June 1988. All treatment plots were thinned to 1100 stems per hectare during site establishment in 1995. In June 2008, the beginning of this study, the stand was 22 years old (Brockley and Simpson 2004). For additional site and stand characteristics, see Table 2.1.

The Crater Lake (P2) lodgepole pine site is located approximately 85 km west of Quesnel, BC, in the Quesnel forest district. The site is situated within the very dry very cold subzone of the Montane Spruce Biogeoclimatic Zone (MSxv) with 540 mm of mean annual precipitation (33% of which is snow) and a mean annual temperature of 3.9 ºC (Environment Canada 2011). The soils are derived from morainal parent material along a south facing hill-slope. Soils are well drained with predominantly loam and sandy loam textures and approximately 60% coarse fragments within the upper surface horizons.

Soils at all slope positions were classified as Eluviated Dystric Brunisols (Soil Classification Working Group 1998). The previous stand was clearcut harvested in 1978, chain dragged in the fall of 1979 and left to regenerate naturally. All treatment plots were thinned to 1100 stems per hectare during site establishment in 1996. In June 2008, the beginning of this study, the stand was approximately 27 years old (Brockley and Simpson 2004). For additional site and stand characteristics, see Table 2.1.

In addition to precipitation averages outlined in the site descriptions above, rainfall was monitored for two 6-week samplings during the 2008 and 2009 growing seasons (see Table 2.2 for sampling dates). At each site, 2 standard tipping bucket rain gauges equipped with Hobo data loggers (Onset Computer Corp., Bourne, MA) were installed at approximately 1.5 m above the soil surface immediately adjacent the treatment plots. Rainfall data can be found in Figures 2.1 & 2.2.

Table 2.1. Site and stand descriptions of six "maximum productivity" installations (adapted from Brockley & Simpson, 2004).

Site	Latitude	Longitude	Species	Year Established	Age @ Establishment
Lodi Lake (S1)	53˚ 22'	122˚ 06'	Spruce	1995	11
Crow Creek (S2)	54˚ 20'	126˚ 17'	Spruce	1994	10
McKendrick Pass (P1)	54˚ 49'	126˚ 48	Pine	1995	9
Crater Lake (P2)	52˚ 50'	123˚ 44'	Pine	1996	15

Table 2.2. 2008 and 2009 rain gauge sampling dates.

Site	2008			2009		
Lodi Lake (S1)	June 9	July 23	August 31	June 17	July 29	September 16
Crow Creek (S2)	June 11	July 25	September 3	June 18	July 30	September 20
McKendrick Pass (P1)	June 12	July 26	September 2	June 19	July 31	September 19
Crater Lake (P2)	June 13	July 27	September 4	June 21	August 1	September 15

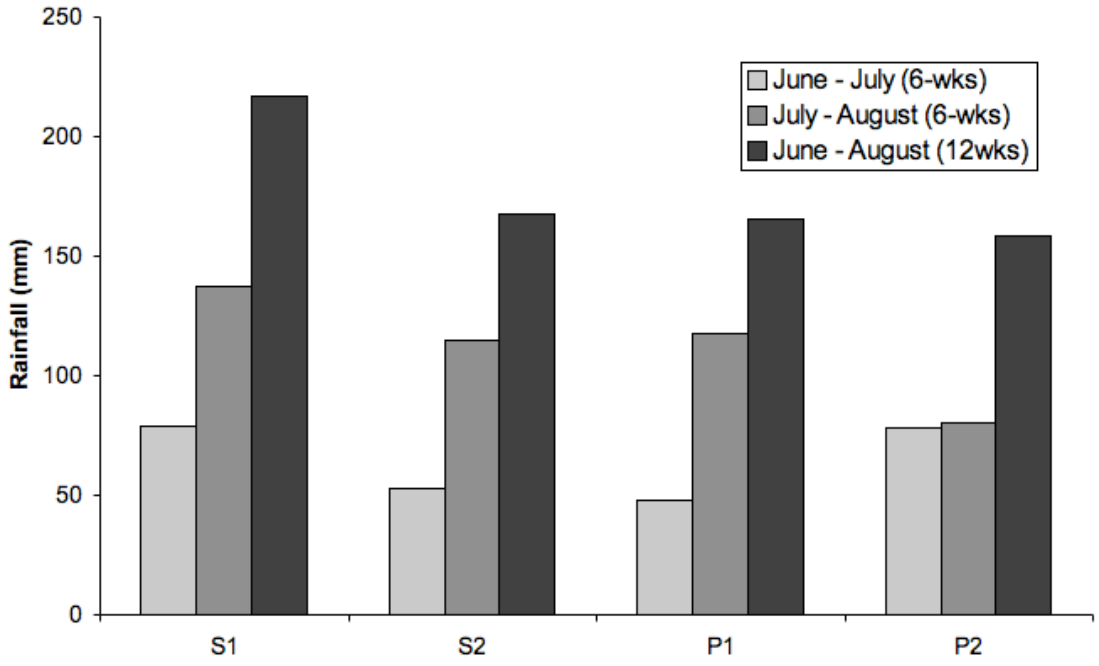

Figure 2.1. 2008 rainfall by site and sampling period.

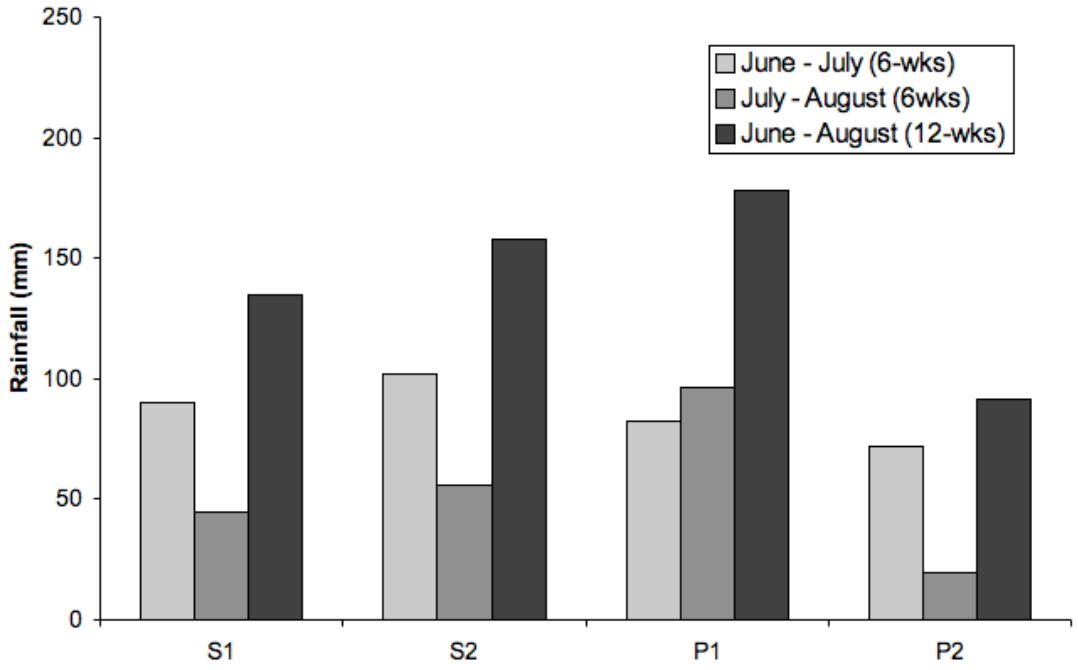

Figure 2.2. 2009 rainfall by site and sampling period.

EXPERIMENTAL DESIGN

At each of the 4 sites, two fertilizer treatments and an unfertilized control were replicated three times for a total of 9 X 0.164 ha treatment plots (see Table 2.3 for description of fertilizer treatments and Table 2.4 & 2.5 for fertilization histories). Outer boundaries of adjacent treatment plots were separated by a minimum distance of 5 m. Fertilization was carried out by hand immediately following spring snowmelt on the dates outlined in Tables 2.4 and 2.5. To minimize within-site geographical differences, the Lodi Lake (S1), McKendrick Pass (P1) and Crater Lake (P2) sites were laid out in a randomized complete block experimental design. The Crow Creek (S2) site was arranged as a completely randomized experimental design (Brockley and Simpson 2004). For site maps, see Figures 2.3 – 2.6.

In the periodic treatment, urea ($CO(NH_2)_2$) was the major source of N. A small amount of N (24% of the total) was added as monoammonium phosphate (11-52-0; N-P-K), which also serves as the P source. Potassium was delivered as potassium chloride (0-0-60; N-P-K) and sulfate potash magnesia (0-0-22-22-11; N-P-K-S-Mg). The latter fertilizer was also the source of S and Mg. Boron was added as granular borate (Brockley and Sanborn 2009).

The annual treatment also received urea as the primary N source, with additional sources of N as monoammonium phosphate and ammonium nitrate (34-0-0; N-P-K). Phosphorus was always added as monoammonium phosphate. Sulphate potash magnesia was the primary source of K, S and Mg. Potassium chloride, ammonium sulphate and ProMag 36 (36% Mg) were used to supply additional K, S and Mg, respectively. Boron was supplied as granular borate (Brockley and Sanborn 2009).

Table 2.3. Description of Fertilizer Treatments (Brockley and Sanborn 2009).

Treatment Code	Treatment
Control	Not Fertilized
Periodic	Fertilized every 6 years with 200N, 100P, 100K, 50S, 25Mg, 1.5B
Annual	Fertilized annually to maintain foliar N concentration at 1.3% and other nutrients in balance with foliar N*

Note: Numbers preceding each nutrient symbol represent nutrient application rate (kg/ha). Nutrient abbreviations: N = nitrogen, P = phosphorus, K = potassium, S = sulfur, Mg = magnesium, B = boron.
*Upper thresholds for foliar nutrient ratios are as follows (Ingestad 1979): N/P = 10, N/K = 3, N/S = 14.5, N/Mg = 20, N/Ca = 20, N/B = 1000, N/Fe = 500, N/Cu = 5000.

Table 2.4. Lodi Lake (S1) and Crow Creek (S2) Fertilization History.

Lodi Lake (S1)			Crow Creek (S2)		
Date	Annual	Periodic	Date	Annual	Periodic
			May 5, 1995	100N, 100P, 100K, 50S, 25Mg, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B
May 7-8, 1996	100N, 100P, 100K, 50S, 25Mg, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B	May 13, 1996	100N, 100P, 100K, 50S, 25Mg,	
May 21, 1997	50N, 50P, 50K, 100Mg, 50S		May 22, 1997	50N, 50P, 50K, 100Mg, 50S, 1.5B	
May 5, 1998	50N, 50P, 50K, 50Mg, 49S, 1.5B		June 15, 1998	50N, 50P, 50K, 50Mg, 49S, 1.5B	
May 20, 1999	50N		May 17, 1999	50N	
May 16, 2000	100N, 50K, 63S, 32Mg		May 12, 2000	100N, 50K, 63S, 32Mg	
May 16, 2001	100N		May 13-14, 2001	100N, 10Fe, 3Cu, 2S, 2Z	200N, 100P, 100K, 50S, 25Mg, 1.5B
May 30-31, 2002	50N, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B	June 4, 2002	50N, 1.5B	
May 16, 2003	50N, 50S		May 20, 2003	100N, 50S	
May 13, 2004	75N, 50P, 50K, 3S, 1.5B, 5Cu, 10Fe, 3Zn		May 17, 2004	100N, 50P, 50K, 3S, 1.5B, 5Cu, 10Fe, 3Zn	
May 13, 2005	75N, 50S		May 18, 2005	75N, 50S	
May 9, 2006	50N, 50P, 50K, 52S, 25Mg, 5Cu, 10Fe, 3Zn, 1.5B		May 16, 2006	50N, 50P, 50K, 52S, 25Mg, 5Cu, 10Fe, 3Zn	
May 22, 2007	75N, 50P, 50K, 50S, 25Mg		May 30-31, 2007	75N, 50P, 50K, 50S, 25Mg, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B
May 30-31, 2008	75N, 50S	200N, 100P, 100K, 50S, 25Mg, 1.5B	June, 2008	75N, 50S	
May 25, 2009	50N, 50P, 50K, 50S, 25Mg, 1.5B		May 28, 2009	50N, 50P, 50K, 1.5B	
TOTAL	**950N, 400P, 450K, 517S, 282Mg, 9B, 10Cu, 20Fe, 6Zn**	**600N, 300P, 300K, 150S, 75Mg, 4.5B**	**TOTAL**	**1125N, 500P, 550K, 519S, 282Mg, 10.5B, 13Cu, 30Fe, 8Zn**	**600N, 300P, 300K, 150S, 75Mg, 4.5B**

Table 2.5. McKendrick Pass (P1) and Crater Lake (P2) Fertilization History.

McKendrick Pass (P1)			Crater Lake (P2)		
Date	Annual	Periodic	Date	Annual	Periodic
June 5-6, 1996	100N, 100P, 100K, 50S, 25Mg, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B			
May 27, 1997	50N, 50P, 50K, 100Mg, 50S		May 13-16, 1997	100N, 100P, 100K, 107Mg, 64S, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B
May 16, 1998	50N, 50P, 50K, 50Mg, 49S, 1.5B		May 7, 1998	50N, 50P, 50K, 50Mg, 49S	
June 11, 1999	50N		May 28, 1999	50N, 50P, 50K, 50Mg, 51S, 1.5B	
May 25, 2000	100N, 50K, 63S, 32Mg		May 17, 2000	100N, 50K, 63S, 32Mg	
May 25, 2001	50N, 50Mg, 8S		May 15, 2001	50N	
June 5-7, 2002	50N, 1.5B	200N, 100P, 100K, 50S, 25Mg, 1.5B	May 28, 2002	50N, 1.5B	
June 5, 2003	50N, 50S		May 17-18, 2003	50N, 50S	200N, 100P, 100K, 50S, 25Mg, 1.5B
May 18, 2004	75N, 50P, 50K, 3S, 1.5B, 5Cu, 10Fe, 3Zn		May 12, 2004	75N, 50P, 50K, 3S, 1.5B, 5Cu, 10Fe, 3Zn	
May 19, 2005	50N, 50P, 50K, 50S, 25Mg		May 12, 2005	50N, 50P, 50K, 50S, 25Mg	
May 17, 2006	50N, 50P, 50K, 54S, 25Mg, 10Cu, 10Fe, 6Zn		May 10, 2006	50N, 50P, 50K, 50S, 25Mg	
June 14, 2007	75N, 100P, 100K, 50S, 25Mg, 1.5B		May 23, 2007	75N, 100P, 100K, 50S, 25Mg, 1.5B	
June, 2008	50N, 50P, 50K, 50S, 25Mg	200N, 100P, 100K, 50S, 25Mg, 1.5B	June, 2008	50N, 50P, 50K, 50S, 25Mg	
May 28, 2009	50N, 50P, 50K, 50S, 25Mg, 1.5B		May 26-27, 2009	50N, 50P	200N, 100P, 100K, 50S, 25Mg, 1.5B
TOTAL	**850N, 550P, 600K, 527S, 382Mg, 9B, 15Cu, 20Fe, 9Zn**	**600N, 300P, 300K, 150S, 75Mg, 4.5B**	**TOTAL**	**800N, 550P, 550K, 480S, 339Mg, 7.5B, 5Cu, 10Fe, 3Zn**	**600N, 300P, 300K, 150S, 75Mg, 4.5B**

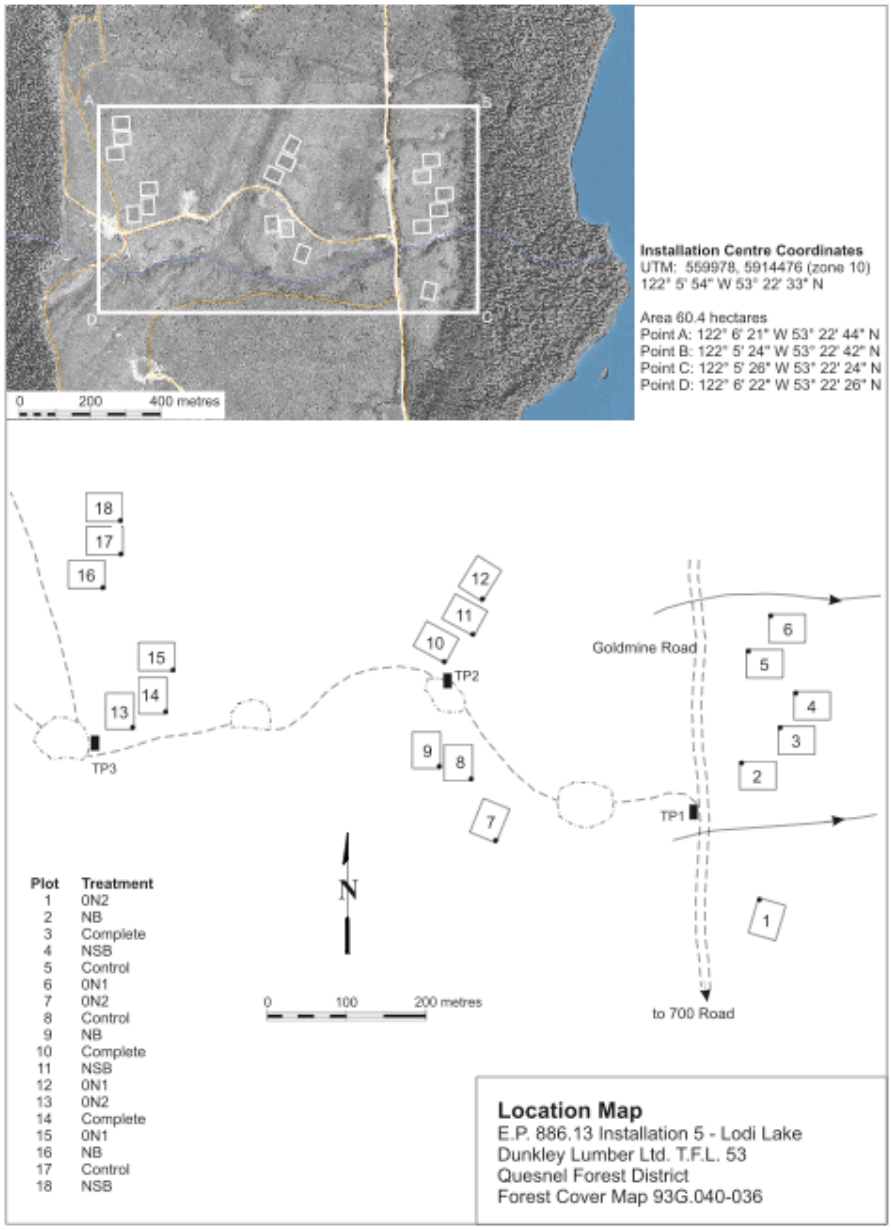

Figure 2.3. Lodi Lake (S1) installation map. Treatment legend (on map = this study): control = control, complete = periodic, ON1 = annual. Treatments ON2, NB and NSB were not included in this study (map provided by Rob Brockley, BC Ministry of Forests, 2010).

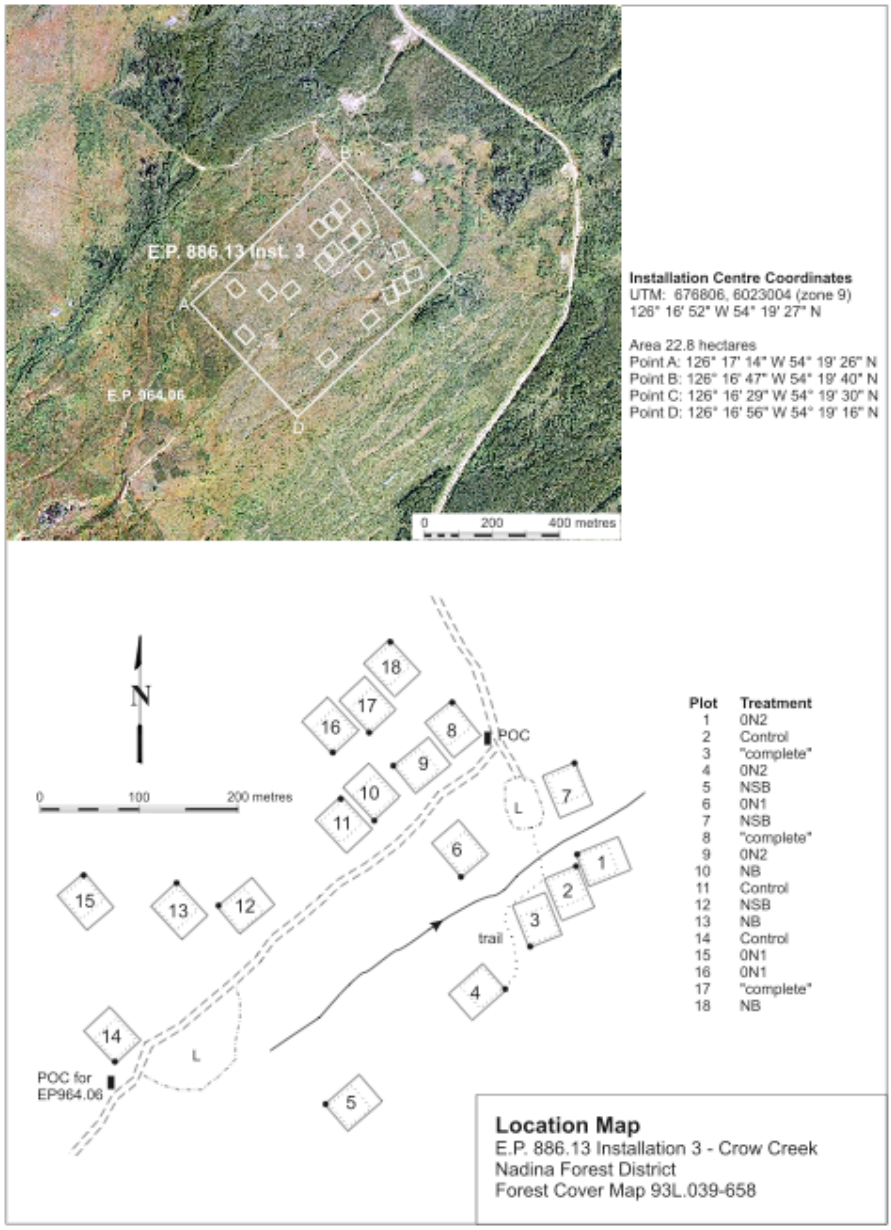

Figure 2.4. Crow Creek (S2) installation map. Treatment legend (on map = this study): control = control, complete = periodic, ON1 = annual. Treatments ON2, NB and NSB were not included in this study (map provided by Rob Brockley, BC Ministry of Forests, 2010).

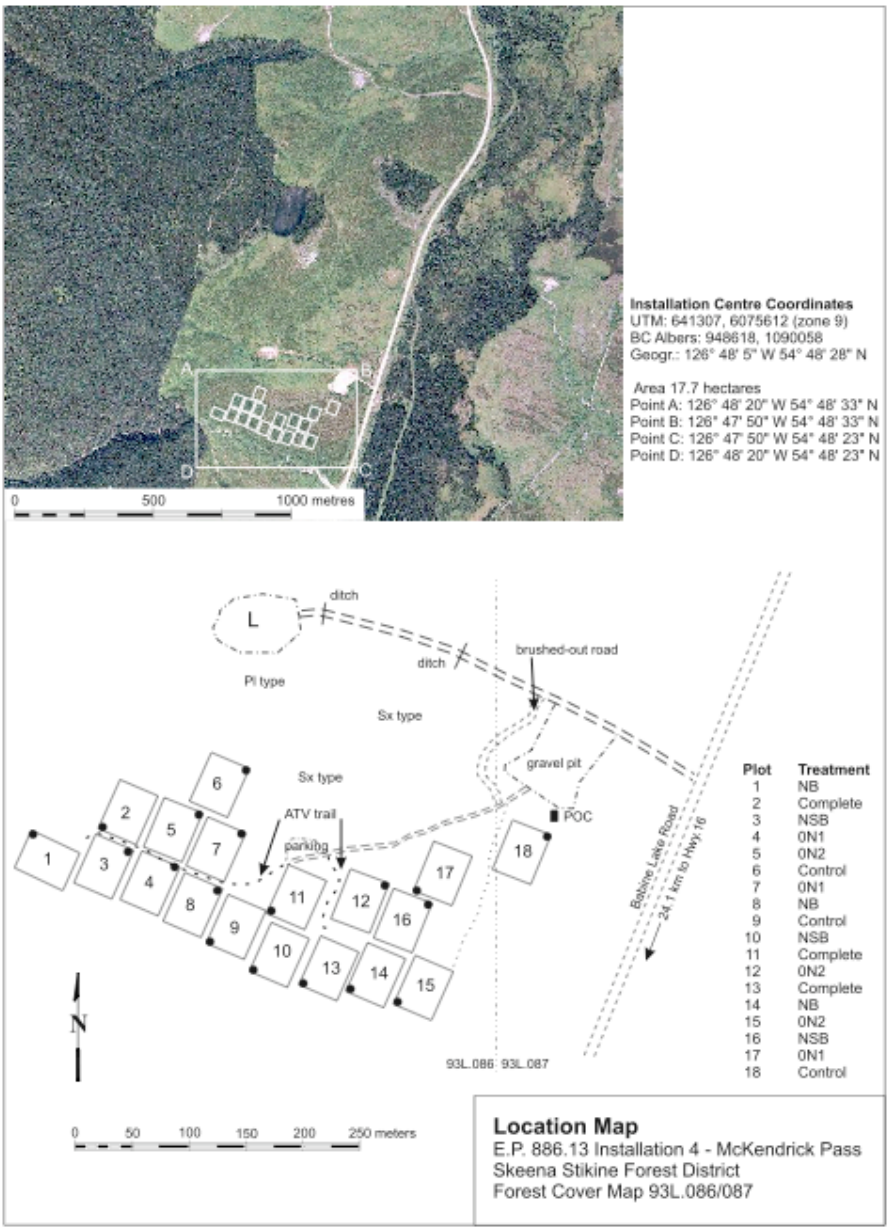

Figure 2.5. McKendrick Pass (P1) installation map. Treatment legend (on map = this study): control = control, complete = periodic, ON1 = annual. Treatments ON2, NB and NSB were not included in this study (map provided by Rob Brockley, BC Ministry of Forests, 2010).

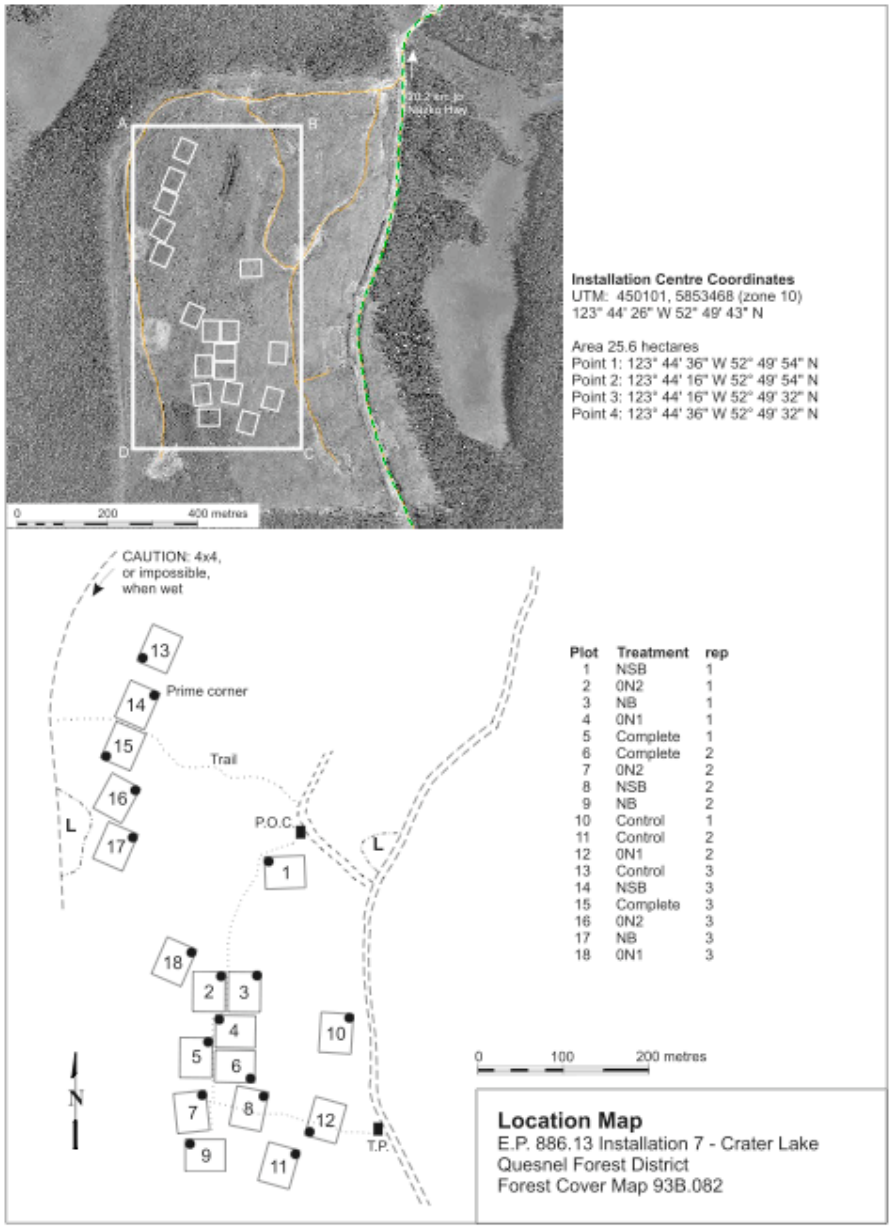

Figure 2.6. Crater Lake (P2) installation map. Treatment legend (on map = this study): control = control, complete = periodic, ON1 = annual. Treatments ON2, NB and NSB were not included in this study (map provided by Rob Brockley, BC Ministry of Forests, 2010).

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CHAPTER 3 – Comparison of N-mineralization rates in fertilized and unfertilized pine and spruce soils using ion-exchange membranes and soil extractions

INTRODUCTION

Mineralizable nitrogen (N) represents the labile fraction of soil N that is converted into mineral forms through microbial processes (Curtin and Campbell 2008). Mineralizable N is a common measure of the potential N supply power of soils and has been reasonably successful in refining agricultural fertilizer prescriptions (Qian and Schoenau 1995; Stanford et al. 1977; Carter et al. 1974, 1976) and to a lesser degree predicting forest productivity (Powers 1980). Several techniques have been developed for assessing mineralizable N (e.g., Keeney and Bremner 1966; Stanford and Smith 1972; Paul et al. 2002; Curtin and McCallum 2004), though currently there is no standardized method for determining the N supply power of the soil.

The majority of N mineralization studies have examined mineralization processes by repeated samplings of incubated soils and subsequent chemical extractions to determine N concentrations on a soil mass basis (e.g., Maynard et al. 1983; Maynard 1993). Chemical extractions measure the quantity of ions in a given soil sample; those ions may be in solution or adsorbed on exchange sites (Maynard et al. 2008). However, inorganic N pools are extremely dynamic, with rapid turnover rates ranging from hours to days (Binkley and Hart 1989; Stark and Hart 1997; Fisher and Binkley 2000; Booth et al. 2005). Thus, traditional chemical extractions do not adequately assess the temporal fluxes of these pools and some researchers have recently begun exploring more temporally-sensitive measures of mineralizable N through the use of diffusion-sensitive ion-

exchange resins (IERs) and membranes (IEMs; e.g. Qian and Schoenau 1995; Huang and Schoenau 1997; Johnson et al. 2005).

Ion-exchange resins are synthetic, electrostatically-charged organic polymers designed to mimic root-exchange properties in soils (Qian and Schoenau 2005; Qian et al. 2008). Ion exchange resins are either positively charged (i.e., cation resins) or negatively charged (i.e., anion resins) and as a pair (cation + anion) are able to adsorb a range of essential soil nutrients. Charged IER surfaces are neutralized with counter-ions of opposite charge and when exposed to soil solution, readily adsorb soluble ions to the membrane surface through mass flow and diffusion. Adsorbed ions can then be eluted by a second counter-ion with a stronger attraction to the resin surface and eluted nutrients can be used as an index of mineralization (Qian and Schoenau 2002; Drohan et al., 2005).

Conventional use of IERs in mineralization assays have focused predominantly on IERs in bead form, often deployed in mesh bags within the soil matrix (Schaff and Skogley 1982; Binkley and Matson 1983). More recently, ion exchange resins in membrane form have become more popular (Qian and Schoenau 1995, 2005; Huang and Schoenau 1997; Hangs et al. 2004; Johnson et al. 2001, 2005). Ion exchange membranes have several practical advantages over IERs in bead form: they can be easily inserted in the soil with minimal disturbance, they can facilitate direct soil-membrane contact and long-term mineralization estimates can be achieved through the repeated insertion of IEMs in the same soil location (Hangs et al. 2004; Huang and Schoenau 1997).

Ion exchange membranes are designed to act as an ion sinks, constantly accumulating ions as they become available in solution. However, when the exchange capacity of the membrane becomes saturated, its function changes from that of a sink to a

dynamic exchanger (Qian and Schoenau 2002). Thus, a principal requirement for the effective use of IEMs in mineralization studies is to avoid membrane saturation. The length of IEM mineralization studies in fertilized agricultural soils has predominantly been less than 2 weeks (Qian and Schoenau 1995, 1996, 2000, 2007; Nguyen et al. 2001; Thavarajah et al. 2003); whereas IEMs have been incubated in unfertilized forest soils for 2 weeks (Huang and Schoenau 1997; Johnson et al., 2001, 2003), 4 weeks (Johnson et al. 2004; Man et al. 2008), 6 weeks (Jerabkova and Prescott 2007; Hope 2009), 8 weeks (Bengston et al. 2007) and 10 weeks (Cortini and Comeau 2008). Longer burials (8 months) have also been attempted under frozen conditions (Hope 2009). Ion-exchange membranes have only been used to a limited extent in fertilized tropical (Meason and Idol 2008, Meason et al. 2009) and temperate (Hangs et al. 2004) forest soils, with only the Meason and Idol (2008) study exceeding 2-week incubations. In that specific case, the authors found IEMs exhibited saturation behavior after 4 weeks, at which time maximum ion capacities for several nutrients had been exceeded. A few select cases have also been reported where nutrients have been desorbed from unsaturated IER/IEM surfaces under strongly immobilizing conditions (Giblin et al. 1994; Subler et al. 1995), suggesting microbial removal of sorbed nutrients.

In addition to the uncertainty regarding optimal IEM incubation times and the potential of N desorption, a major challenge associated with the adoption of IEM technology is the inability to directly compare IEM nutrient measures with traditional soil extractions. Traditional soil extractions measure nutrient concentrations on a soil mass basis (e.g. mg kg^{-1}); whereas IEMs assess the mass of ions adsorbed per resin surface area per unit burial time (e.g. μg cm^{-2} burial $^{-1}$; Qian and Schoenau, 2002). Thus, soil

extractions and IEMs do not necessarily measure the same components of the available soil N pool. Regardless of these differences, both assays are widely used in soil mineralization assessments.

Varying attempts have been made to correlate IER mineralization rates with soil extractions; some authors found significant relationships (Binkley 1984; Binkley and Matson 1983; Binkley et al. 1986; Lajtha 1988; Subler et al. 1995), while others found no relationships (Hart and Binkley 1985; Giblin et al. 1994). Comparisons have also been made between IEM and soil extractable N measures (Qian and Shoenau 1995; Pare et al. 1995; Johnson et al. 2005; Ziadi et al. 1999, 2006) in agricultural/prairie soils, though we are not aware of any study that has compared these assays in forest soils. The objective of this study was to explore the relationships between N-mineralization rates in fertilized and unfertilized forest soils using IEMs and soil extractions to examine how each method quantifies the mineralization activity of the soil. By pairing IEM and extraction samplings over a 12-week incubation, we attempted to determine whether these measures co-varied over time and if they did not, at what point they differed and what may have contributed to the differences. In addition, we attempted to determine optimal incubation periods for IEMs in fertilized forest soils and to assess whether N desorption may affect mineralization estimates in long-term incubations.

MATERIALS AND METHODS

Site Description

Soils used in this study were collected from the S1 and P1 interior spruce and lodgepole pine field installations in 2009 (see Chapter 2 for complete description of field

sites). The abbreviated site names allow for easy distinction between spruce (S1) and pine (P1) study sites as well as comparison with other experiments in this thesis. In this specific chapter, the study sites are referred to as "spruce" and "pine," rather than the abbreviated site names. Three treatments were studied at each site: control, periodic and annual (see Chapter 2, Table 2.3 for description of fertilizer treatments and Tables 2.4 & 2.5 for detailed fertilization history).

Mineralization Study Experimental Design

Mineral soil samples (0-10cm) were collected in June 2009 at the S1 and P1 sites. Four soil samples per plot were randomly collected and combined to form one composite sample per plot (3 treatments x 3 reps = 9 samples per site). Samples were kept on ice while in the field and wet sieved (<2mm) immediately upon arrival at the lab. Each composite sample made up one experimental unit consisting of 7 x 200 mL pots and one 11 L pot.

Ion-Exchange Membrane Nitrogen

Soil N supply was measured using ion exchange membrane (IEM) plant root simulator (PRS) probes (Western Ag Innovations Inc., Saskatoon, SK). Each IEM probe consists of an ion exchange membrane encapsulated in a flat plastic frame that can be easily inserted into the soil. Ion exchange membranes are either positively (i.e. cation membranes) or negatively (i.e. anion membranes) charged, and as a pair (cation + anion) are able to adsorb essential soil nutrients (e.g. NH_4^+, NO_3^-).

Seven Plant Root Simulator IEM probe (Western Ag Innovations, Saskatoon, SK) pairs (cation + anion) were inserted vertically into the soil of the 11 L pots and sampled after one week, and two weeks thereafter (1, 2, 4, 6, 8, 10 and 12 weeks). At each sampling, IEM probes were immediately rinsed with pressurized de-ionized water to remove all residual soil, packaged on ice and sent overnight to Western Ag Innovations laboratory (Saskatoon, SK, Canada) for analysis. Ion-exchange membrane probes were eluted in Ziploc® plastic bags containing 17.5 mL of 0.5 M HCl solution. The IEM elution process requires a 1-hr equilibration period to ensure that ≥95% of adsorbed ions are removed for elemental determination. Soil nitrogen supply rates were expressed as μg $N/10cm^2$/burial period. PRS-probe maximum ion capacities are as follows (μg 10 cm^{-2}): NO_3^- = 1050, NH_4^+ = 3014 (Western Ag Innovations Inc., 2006).

Extractable Soil Nitrogen

The 7 small pots were destructively sampled at the same sampling intervals as the IEM probes. Initial (t=0) and destructively sampled soils were extracted for ammonium (NH_4^+) and (NO_3^-) as outlined by Kalra and Maynard (1991); NH_4^+ and NO_3^- extractions were also done on the soils of the larger pots after 12 weeks to determine whether mineralization rates in the smaller destructively sampled pots differed from the larger IEM pots. Five gram sub-samples were oven dried at 105°C for 48 hours to determine soil moisture content. At the same time, 5 g sub-samples of sieved field moist soil was weighed into 250 mL sealable polyethylene Nalgene bottles. Fifty mL of 2.0 M KCl solution was added to each bottle containing 5 g field moist soil and the bottles were then shaken for 30 minutes at 200 strokes per minute. During shaking, Whatman No. 42 filter

papers were rinsed once with 40 mL 2.0 M KCl and twice with 20 mL de-ionized water. Shaken samples were gravity filtered through the rinsed Whatman filters and collected in 60 mL sealable polyethylene Nalgene bottles. Following 1 hour of filtering, extractions were frozen immediately. All samples drained completely within one hour. Ammonium and NO_3^- determination of soil extracts was done using an Alpkem flow solution IV segmented flow auto-analyzer.

Total Carbon and Nitrogen

Air-dried sub-samples of pre and post incubated soils were pulverized using a Seibtechnic concentric ring grinder and analyzed for total carbon and nitrogen by dry combustion using a LECO CNS-2000. Elemental concentrations were recorded as percent of the sample dry weight.

Effective Cation Exchange Capacity and pH

Sub-samples of pre-incubated soils were also sieved (<2mm), air-dried and analyzed for effective cation exchange capacity (CECe) and pH. Each sample was combined with 20 mL 0.01 M $CaCl_2$ to form a 1:2 ratio (soil:solution). Samples were agitated and left to equilibrate before inserting a combination electrode to determine pH (Kalra and Maynard 1991). Effective CEC was determined through a three-step extraction process using a Centurion mechanical vacuum extractor (Kalra and Maynard 1991). Step one involved the displacement of exchangeable cations by saturating cation exchange sites with NH_4^+ using unbuffered 1.0 M NH_4Cl solution. Step two involved the

removal of excess NH_4^+ from the soil sample with an ethanol wash procedure. The remaining NH_4^+ ions adsorbed on exchange sites following the ethanol wash was considered the effective cation exchange capacity. The final step involved the displacement of NH_4^+ by saturating cation exchange sites with Na^+ using 10% NaCl. Ammonium concentration in the leachate solution was determined by Alpkem flow solution IV segmented flow auto-analyzer.

Incubation Conditions

Soil moisture was maintained at 80% field capacity (± 10%; watered every second day) in all pots for the duration of the study and air temperatures were kept constant at 21° C; these conditions were considered optimal for mineralization processes as well as the diffusion of NH_4^+ and NO_3^- to IEM surfaces. Total N was assessed at t=0 and after 12-weeks in both the destructively sampled pots and the larger IEM pots to assess denitrification N-losses; leaching losses were avoided by the use of pots with sealed bases.

Data Reporting

Extractable NH_4^+ and NO_3^- are expressed throughout the study as: 1) mineralized N (NH_4^{MIN} & NO_3^{MIN}), the concentration of NH_4^+ and NO_3^- in the soil at each sampling interval; and 2) net mineralized N (NH_4^{NET} & NO_3^{NET}), the concentration of NH_4^+ and NO_3^- at each sampling interval minus the initial (t=0) NH_4^+ and NO_3^- concentrations. Mineralized N as recorded by the IEM-probes is expressed as NH_4^{IEM} and NO_3^{IEM}. By

reporting both N^{MIN} and N^{NET} we were able to assess the immobilization activity of the soil while also considering the initial mineral N as part of the labile N pool. It was hypothesized that IEM probes, having not been reduced by any initial N values, would relate more closely to N^{MIN} than N^{NET}. By examining all three measures of the N mineralization activity of the soil, we were able to assess relationships between the different assays and attempt to determine some of the factors contributing to any differences.

Data Analysis

Treatment effects on mineralization rates by sampling date and differences between extractable NH_4^+ and NO_3^- in the large and small pots after 12 weeks were assessed by analysis of variance (ANOVA) using the general linear model (GLM) procedure. Repeated measures analysis GLM was used to assess differences in total soil N before and after the incubation. Where significant differences were found, Tukey's HSD post-hoc test was used to determine significance between individual treatment means. Coefficients of variation (CV = standard deviation/mean) for each assay (extractions and IEMs) were calculated to assess differences in the variability of the measures. Pearson's correlation was used to assess relationships between soil extractions and IEM N by soil type, treatment and sampling; correlations including all samples from a given soil were reported as "bulk" correlations. A level of significance of $\alpha = 0.05$ was used for inferring statistical significance throughout the study. Statistical analyses were completed using PASW 18 (SPSS Inc., 2009).

RESULTS

Total Carbon and Nitrogen

Both soils received nearly identical N additions for 14 consecutive years (Chapter 2, Tables 2.4 & 2.5); however, intersite differences in total C and N pools reveal that each soil processed these inputs differently (Table 3.1). Spruce total C and N values were higher, on average, compared to the pine soil. Total N in the spruce soil was not significantly different in any of the treatments at the beginning and the end of the 12 weeks (data not shown); however, total N in the pine soil was significantly lower after 12 weeks than at the beginning of the study in all of the treatments (control: $p = 0.008$; periodic: $p = 0.032$; annual: $p = 0.004$). Mean total N in the pine soils decreased from 0.07 to 0.062% in the control, 0.081 to 0.067% in the periodic and 0.12 to 0.097% in the annual treatment, indicating some N may have been lost from the pine soil during the 12-week incubation period. It is unclear how this may have occurred as soil moisture was kept well below saturation and all pots were sealed to preclude leaching losses.

Table 3.1. Initial (t=0) mean soil carbon, nitrogen, C:N ratios, effective cation capacity (CECe), pH ($CaCl_2$), NH_4^+, NO_3^- and corresponding ANOVA *p*-values. Different letters for means of the same variable indicate differences at the $\alpha = 0.05$ level. Significant *p*-values are marked by an asterisk. Standard errors are shown in parentheses.

	Treatment			
	Control	Periodic	Annual	*p*-value
SPRUCE				
%C	2.36 (0.66)	3.40 (1.39)	3.30 (1.43)	0.404
%N	0.10 (0.035)	0.17 (0.085)	0.18 (0.097)	0.339
C:N	23.98 (1.43)a	21.89 (1.95)ab	20.06 (2.02)b	0.029*
CECe	5.43 (0.9)	4.94 (0.8)	5.67 (1.1)	0.605
pH	3.94 (0.1)a	3.65 (0.2)ab	3.53 (0.2)b	0.037*
NH_4^+	2.95 (0.36)	8.38 (2.01)	31.77 (13.56)	0.139
NO_3^-	2.26 (0.36)	4.25 (2.11)	6.74 (4.42)	0.448
PINE				
%C	1.9 (0.04)b	2.21 (0.22)b	3.1 (0.14)a	0.020*
%N	0.07 (0.003)b	0.08 (0.008)ab	0.12 (0.009)a	0.028*
C:N	27.44 (1.82)	27.30 (0.7)	26.04 (1.07)	0.750
CECe	9.08 (0.02)	9.24 (0.4)	9.7 (0.2)	0.367
pH	3.39 (0.08)	3.26 (0.02)	3.32 (0.04)	0.218
NH_4^+	1.47 (0.26)	24.35 (18.34)	13.27 (4.49)	0.350
NO_3^-	2.38 (0.17)	2.12 (0.38)	2.03 (0.04)	0.655

Note: C:N ratios were calculated prior to rounding of C and N values.

Effect of pot size on nitrogen dynamics

Extractable NH_4^+ and NO_3^- in the spruce soil and extractable NH_4^+ in the pine soil did not differ between the larger and smaller pots after 12-weeks (data not shown). Extractable nitrate values in the pine soil were significantly higher in the larger pot compared to the smaller pots in the control ($p = 0.009$) treatment only; however, nitrate values were extremely low (≤ 2 mg/kg) in both pot types. Because there were no differences in the mineralization activity of the soils between pot types, differences in extractable and IEM N mineralization rates were attributed to differences in the ability of each assay to quantify the mineralization activity of the soil.

Mineralization Rates

Ammonium mineralization rates in the spruce soils did not differ significantly by treatment in any of the assessments (Figure 3.1a, c, e). Significant differences in NH_4^+ mineralization rates in the pine soils were evident in the extractions, though only to a limited degree on the IEM probes (Figure 3.1b, d, f). Extractable NH_4 indices (NH_4^{MIN} and NH_4^{NET}) in both soils revealed an initial immobilization period within the first two weeks of the incubation (Figure 3.1a-d); these measures differed greatly from NH_4^{IEM} in both soils in the first week. Following the first week, NH_4^{MIN}, NH_4^{NET} and NH_4^{IEM} generally increased throughout the incubation with NH_4^+ mineralization rates in the fertilized treatments generally higher than the control. Mineralized NH_4^+ (NH_4^{MIN}; Figure 3.1c, d) was very similar in both soils; however, NH_4^{IEM} was approximately 3 times higher in the spruce soil than the pine soil (Figure 3.1e, f).

Nitrate production occurred mainly in the fertilized spruce soils; there was minimal NO_3^- measured by either of the assays in the pine soil (Figure 3.2). Nitrate mineralization rates in the spruce soils were not significantly different by treatment. Similar to NH_4^+, the first week of the incubation was dominated by immobilizing conditions, though this was only observed by the soil extractions (Figure 3.2a, c).

Mean NH_4^{IEM} in the pine and spruce soils generally increased throughout the incubation period, suggesting desorption of NH_4^+ ions did not substantially affect mineralization estimates. The only apparent NH_4^{IEM} decrease occurred in the annual treatment at the spruce site after 12 weeks; however, mean NH_4^{IEM} after 12 weeks was well within the error estimates of the previous sampling and was ~4% of the maximum ion capacity. Mean NO_3^{IEM} in the spruce soil generally increased until week 8, at which

time mineralization rates in the fertilized treatments plateaued (even decreased in the periodic treatment in week 10) with maximum ion capacities well within one standard error of the means. Nitrate production in the fertilized treatments beyond 6-weeks may have saturated the IEMs and altered the behaviour from that of a sink to a dynamic exchanger.

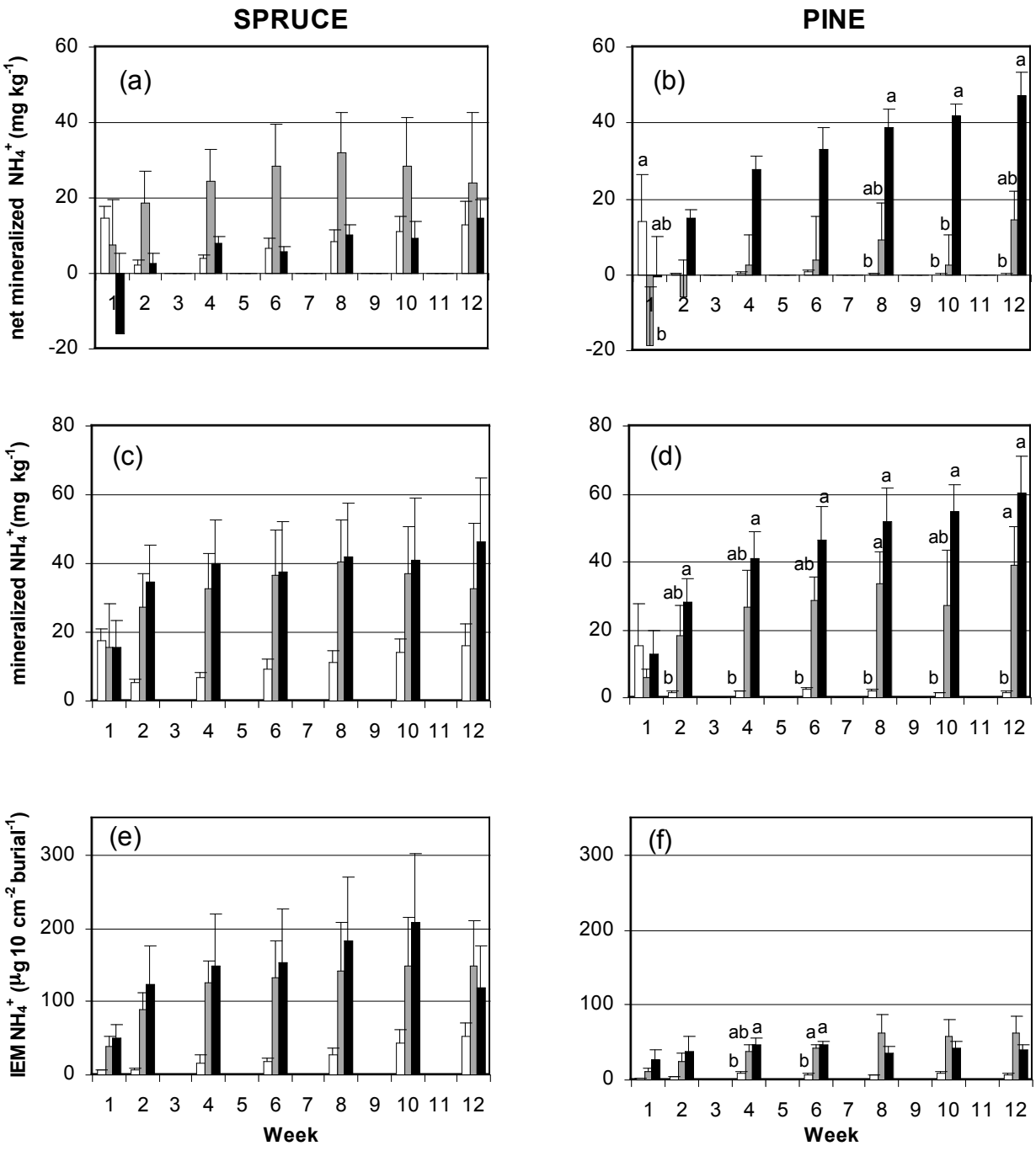

Figure 3.1. Mean soil NH_4^+ by sampling. Treatment legend: white = control, grey = periodic, black = annual. Site legend: spruce = a, c, e; pine = b, d, f. Means with different letters are significantly different at $p < 0.05$. Error bars indicate ±1 SE.

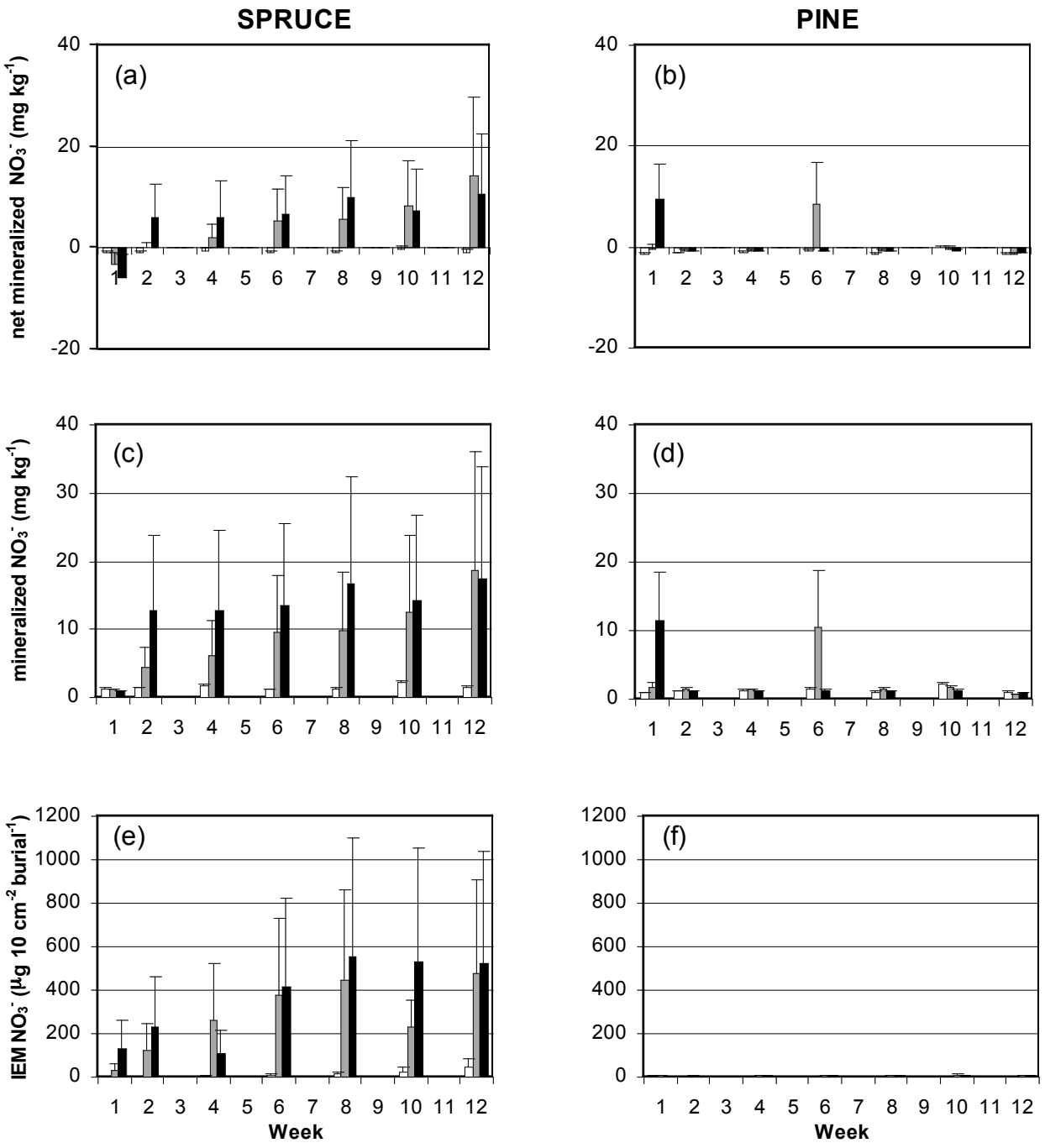

Figure 3.2. Mean soil NO_3^- by sampling. Treatment legend: white = control, grey = periodic, black = annual. Site legend: spruce = a, c, e; pine = b, d, f. Error bars indicate ±1 SE.

Coefficients of Variation

Both extraction and IEM measures of N mineralization were highly variable (Table 3.2). Ion-exchange membrane NH_4^+ was less variable than NH_4^{MIN} in both soils and NH_4^{IEM} variability was less affected by the treatments than NH_4^{MIN}. There was no apparent difference in the variability of NO_3^- with either measure. Ion-exchange membrane NO_3^- was, in general, more variable than NH_4^{IEM}; whereas the variability of NH_4^{MIN} did not appear to differ from NO_3^{MIN}.

Table 3.2. Coefficient of variation ranges for N^{MIN} and N^{IEM} over the incubation period.

	Coefficients of Variation (%)		
	Control	Periodic	Annual
SPRUCE			
NH_4^{MIN}	33.4 – 69.3	52.1 – 139	55.1 – 85.5
NO_3^{MIN}	1.3 – 47.2	14.6 – 163.9	16.8 – 162.3
NH_4^{IEM}	40.6 – 105	42.2 – 82.4	62.4 – 84
NO_3^{IEM}	25 – 133.6	91.4 – 170.4	165.7 – 171.9
PINE			
NH_4^{MIN}	5.4 – 140.7	43.1 – 103.9	24.4 – 99
NO_3^{MIN}	9.5 – 47.7	9.2 – 134	6.2 – 107.3
NH_4^{IEM}	13.9 – 62.9	17.7 – 77	18.4 – 88
NO_3^{IEM}	53.9 – 121.2	48.4 – 105	6.7 – 29.3

Correlation Analysis

Bulk relationships between extractable and IEM N measures differed by site and by ion species (Figures 3.3 and 3.4). Significant relationships were found for NH_4^{MIN} and NH_4^{IEM} in both soils, though the strength of the relationship and the general trendlines suggest site-specific relationships (Figure 3.3). The relationship for NH_4^+ in the spruce soil was largely driven by the highly significant correlation in the annual treatment ($r = 0.896$, $p = 0.000$, $n = 21$); bulk relationships between spruce NH_4^{MIN} and NH_4^{IEM} were not

significant in the control or periodic treatments. The only significant treatment-specific relationship between NH_4^{MIN} and NH_4^{IEM} in the pine soil was in the periodic treatment ($r = 0.867$, $p = 0.000$, $n = 21$), though a positive relationship in the annual treatment was evident ($r = 0.425$, $p = 0.055$, $n = 21$). Thus, any relationships between extractable and soluble NH_4^+ in these soils was driven exclusively by the fertilizer treatments.

Relationships between extractable and IEM NO_3^- were only evident in the spruce soil; the limited NO_3^- production in the pine soil precluded any correlation. The bulk NO_3^- relationship in the spruce soil was highly significant ($r = 0.916$, $p = 0.000$, $n = 63$), suggesting both assays similarly quantify the NO_3^- mineralization activity of the soil. The strength of the relationship between spruce NO_3^{MIN} and NO_3^{IEM} followed the trend control < periodic < annual ($r = 0.508$, $p = 0.019$; $r = 0.881$, $p = 0.00$; $r = 0.923$, $p = 0.00$). Significant relationships were also found for spruce NO_3^{NET} and NO_3^{IEM} in the periodic and annual treatments ($r = 0.840$, $p = 0.00$; $r = 0.881$, $p = 0.00$); these were the only significant relationships between N^{NET} and N^{IEM} in this study.

There were no relationships between IEM and extractable N in either soil during the first week of the incubation. By the second week, extractable and IEM NH_4^+ and NO_3^- were significantly correlated in both soils. The duration of relationships were highly variable, with significant NH_4^+ correlations in the spruce soil for weeks 2 and 4 only, and weeks 2, 4, 6, 10 at the pine site. Nitrate relationships at the spruce site were highly significant in weeks, 2, 6, 8, 10 and 12, though only a 2-week NO_3 relationship was significant at the pine site.

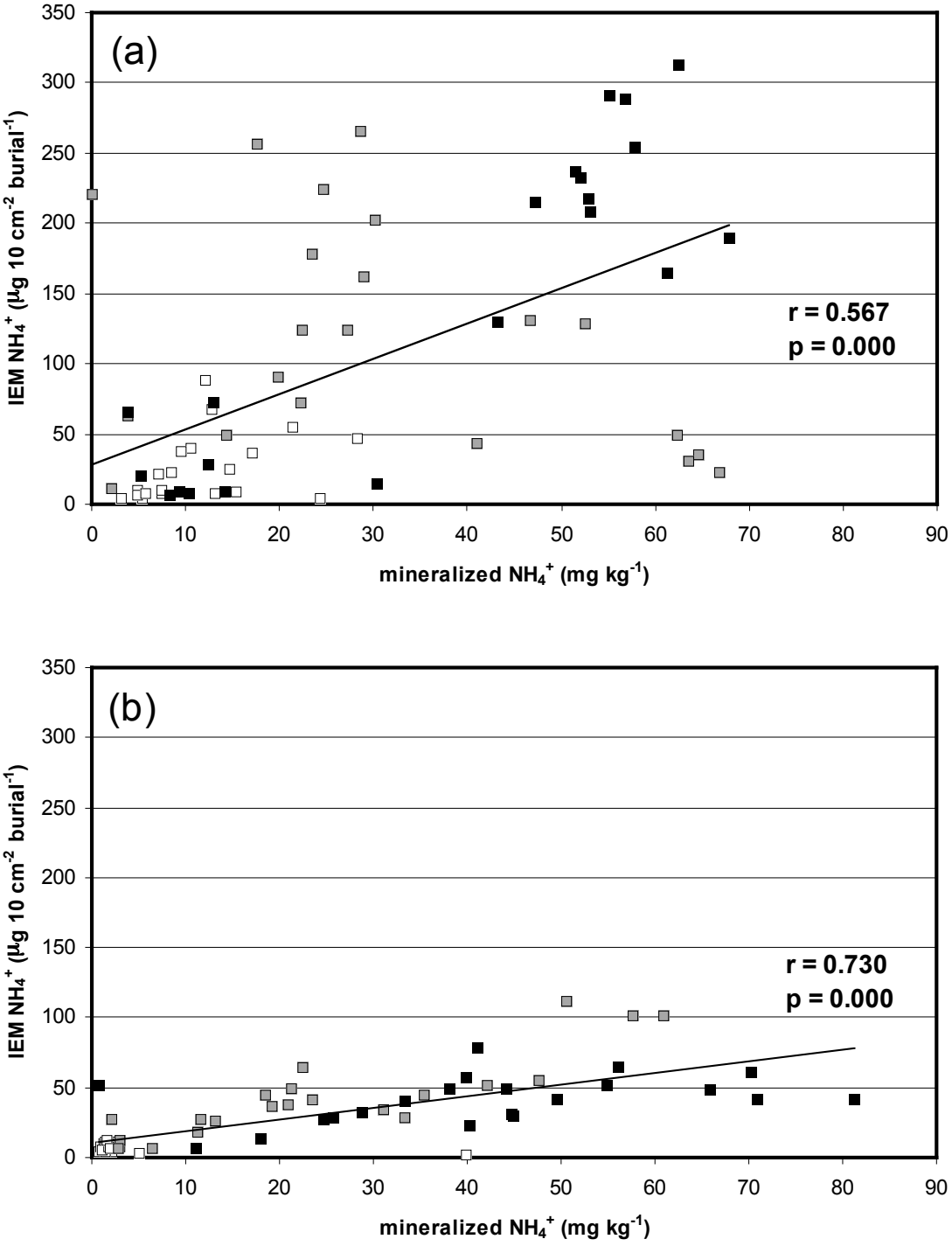

Figure 3.3. (a) Spruce and (b) Pine correlations for mineralized and IEM NH_4^+. Pearson's r is reported for bulk correlation only. Treatment legend allows for differentiation between points: white = control, grey = periodic, black = annual. Linear trendlines (a) y = 2.5313x + 26.651 and (b) y = 0.8323x + 10.156 are displayed for interpretive purposes only.

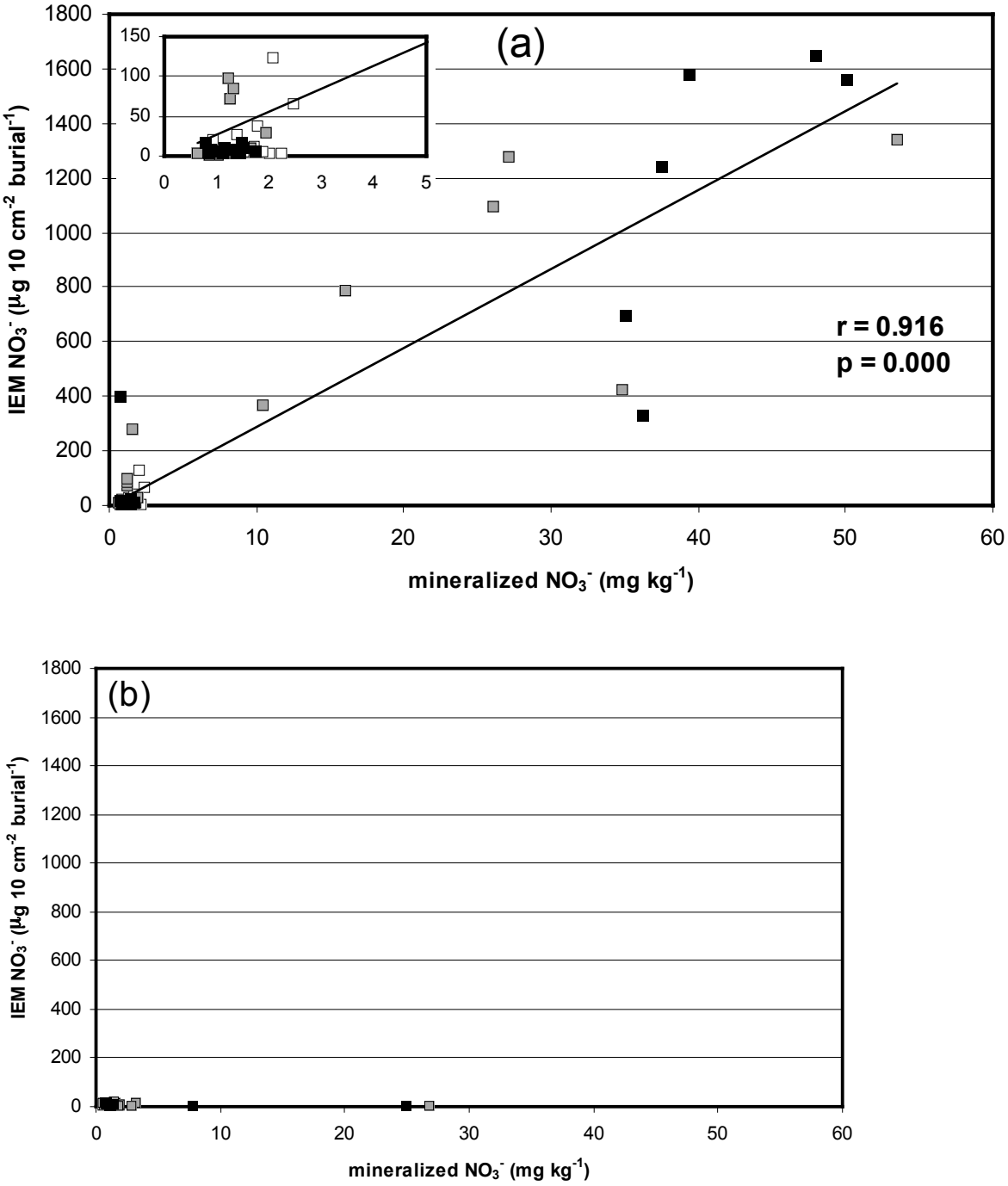

Figure 3.4. (a) Spruce and (b) Pine correlations for mineralized and IEM NO_3^-. Pearson's r is reported for bulk correlation only. Treatment legend allows for differentiation between points: white = control, grey = periodic, black = annual. Inset graph in (a) is a magnification of the data cluster in the lower left hand corner of the graph. Linear trendline (a) y = 28.885x - 4.7607 is displayed for interpretive purposes only.

DISCUSSION

Comparison of N-Mineralization Assays

The supply of N ions to plant roots is affected by the size of the labile N pool, microbial activity (N-mineralization/immobilization), ion mobility and transport processes (mass flow and diffusion; Fisher and Binkley 2000; Binkley 1984). The comparison of N-mineralization rates using IEMs and soil extractions provides a useful indication of how each method differs in its ability to quantify these interacting factors and assess available soil N.

The initial week of the incubation revealed some of the key distinctions between the two assays; the soil extractions were able to characterize the immobilizing conditions of the soil, while the IEMs were not. Positive IEM N values indicate that at some point during the first week there was some available N in solution, whereas the extractions suggest that after one week the majority of the mineralized N had been consumed by the microbial population. These different perspectives reflect the cumulative nature of the IEMs compared to the static nature of the extractions. Clearly, IEM mineralization rates were affected by the immobilizing conditions, as N adsorbed to the IEM surfaces was lower during the first week burial compared to subsequent weeks; however, the IEMs were unable to describe the degree of immobilization as effectively as the extractions.

By the second week of the incubation, once immobilizing conditions had waned, both measures co-varied positively, at least in the fertilized treatments. Significant NH_4^+ correlations between the two assays persisted for varying durations, presumably due to differing exchange properties affecting NH_4^+ mobility. Nitrate relationships in the spruce soil persisted for much of the incubation, suggesting that the higher mobility and minimal

exchange phases of this ion allowed both IEMs and extractions to measure a similar soil pool. This supports the findings of other researchers who have found stronger IER/IEM relationships with soil extractable NO_3^- than NH_4^+ (Binkley 1984; Binkley et al. 1986; Johnson et al. 2005). It has also been suggested that NO_3^-, with higher mobility than NH_4^+, accumulates at a disproportionately higher rate on the IERs than NH_4^+ (Binkley 1984; Hart and Binkley 1985; Binkley et al. 1986; Giblin et al. 1994). Our results do not show this trend, as interpretive linear equations in Figures 3 and 4 indicate that in the spruce soil (where NH_4^+ and NO_3^- were both produced) NH_4^{IEM} and NO_3^{IEM} supply rates were similarly proportional to soil NH_4^+ and NO_3^- concentrations. However, it is possible that our result differs from others simply as a result of soil moisture. Soil moisture has been shown to be a significant factor affecting IER/IEM N measures (Binkley 1984; Johnson et al. 2005); thus, by maintaining optimal moisture conditions for ion transport, our incubation may have facilitated different conditions for ion diffusion than the incubations of other researchers. This clearly has implications for field-based incubations where soil moisture is highly variable.

Factors Affecting Ion Mobility

By assessing NH_4^+ and NO_3^- separately we were able to determine how differences in ion mobility affected the relationship between both assays. Site-specific relationships for NH_4^{MIN} and NH_4^{IEM} mineralization rates were largely driven by differences in soluble NH_4^+. Ion-exchange membrane NH_4^+ mineralization rates were substantially lower in the pine soil than the spruce soil, whereas NH_4^{MIN} values were nearly identical in both soils. We offer two potential explanations for this: 1) The greater

CECes in the pine soils—nearly twice those of the spruce soils (Table 3.1)—may have reduced ion mobility through increased adsorption, thus reducing the relative proportion of soluble NH_4^+ compared to the spruce soil. This is consistent with other researchers who have suggested soil exchange properties as a confounding factor affecting the relationships between extractable and IER NH_4^+ (Binkley 1984; Hart and Binkley 1985; Binkley and Hart 1989). 2) Higher C:N ratios in the pine soil (>26 in all treatments; Table 3.1) suggest microbial competition for soluble NH_4^+ may be more fierce than in the spruce soil (C:N <24 in all treatments; Hart et al. 1994; Booth et al. 2005). While NH_4^{NET} data do not indicate that overall immobilization rates in the pine soil were substantially higher than the spruce soil, the NH_4^{IEM} data force us to consider that differences in the soluble NH_4^+ pool may be related to microbial uptake. As mentioned previously, IEMs have been shown to compete poorly for available N under immobilizing conditions; thus, the striking difference in NH_4^{IEM} may, in part, be due to such factors. The only other potential consumptive fate of soluble NH_4^+ in an aerobic, plant-free environment would be the oxidation to NO_3^-; however, this is unlikely as there was minimal NO_3^- produced in the pine soil. This disparity between NH_4^+ indices in the different soils raises the question as to which measure provides the most plant-relevant estimate of available NH_4^+. Both assays measured NH_4^+ mineralization in the absence of plants; therefore, it is not possible to conclude with any certainty which measure is more plant-relevant. However, based on the assumption that IEMs largely adsorb ions in solution, as do plant roots, it is anticipated that the differences in NH_4^{IEM} between both soils reflect differences in plant-available NH_4^+.

Ion Desorption

There was minimal evidence of ion desorption throughout the incubation. Only in two cases did mean IEM N values decrease from previous samplings, though in both cases they were within one standard error of the mean from the previous sampling. In addition, NH_4^{IEM} values did not approach IEM saturation after 12-weeks; the highest value was <10% of the maximum ion capacity. Conversely, NO_3^{IEM} values appear to have reached IEM saturation beyond the 6-week sampling in the fertilized spruce soils.

Relationships Between Ion-Exchange Membranes and Soil Extractions

All significant relationships between extractions and IEM mineralization rates were driven by the fertilizer treatments. The higher rates of mineralized N in the fertilized treatments compared to the controls highlights the need for substantial labile N to facilitate comparisons between IEM and extractable N indices; therefore, any differences between the two mineralization assays apparently become more prominent in N-poor soils. This supports the model proposed by Schimel and Bennett (2004) that N-mineralization assays can be very effective in soils with high N availability, yet largely ineffective in N-poor soils.

Variability

The variability of N-mineralization measures was, on average, greater in the extracted samples than the IEM samples. This may be due to the fact that IEM probes access ions in solution, which are presumably in greater equilibrium than the N pool

measured by extractions. Further, the large variability in extractable-N in the fertilized soils may be due to fertilizer pellet contamination as extractable-N indices in the fertilized treatments were more variable compared to the control; a phenomenon not observed by the IEMs. Despite these findings, there appears to be no discernable consensus in the literature on this issue. Some researchers have found higher variability in extractable N assays compared to IEMs (Binkley and Matson 1983; Sharifi et al. 2009), while some have found the opposite (Hart and Binkley 1985) and others have found no trend (Binkley et al. 1986; Giblin 1994). This variability made it difficult for either assay to significantly distinguish between the individual treatments.

Nitrification

The most striking difference in N-mineralization patterns in these soils was the degree of nitrification. Nitrification in the spruce soils was substantial, at least in the fertilized treatments, whereas in the pine soil NO_3^- production was minimal. It is unclear what factors may have contributed to these differences, though it has been suggested that differences in the structure of the microbial community, differences in nitrifying inhibitors or differences in ammonium availability are key drivers affecting nitrification (Johnson et al. 1980; Robertson 1982; DeBoer and Kowalchuk 2001). We did not assess differences in the microbial community or nitrifying inhibitors in this study; however, the IEMs suggest inadequate NH_4^+ may have limited nitrification in the pine soil. Nitrification differences may also be related to the N-losses experienced in the pine soil during the incubation. While moisture levels were maintained well below field capacity, denitrification losses can occur in soils with as low as 60% water-filled pore space

(Bateman and Baggs 2005), suggesting denitrification may have occurred in the pine soils, potentially affecting nitrification rates. It is also important to note that both soils have received nearly identical N additions over the past 14-years, though total N pools are strikingly different (Table 3.1); thus, differences in N-mineralization patterns appear to be related to differences in the incorporation of fertilizer-N into the total N pool.

CONCLUSION

The comparison of N-mineralization rates using IEMs and soil extractions suggests that the nature of each assay provides unique opportunities for assessing available soil N. Significant correlations were found between the two assays in several cases, though these relationships varied by soil type, sampling and ion species. This indicates that there is clearly some overlap in how the two assays quantify the mineralization activity of the soils; however, it also suggests differing sensitivities to the dynamic conditions affecting N-mineralization. The static nature of soil extractions appears to be especially applicable for assessing fluxes between immobilizing and mineralizing conditions, whereas IEMs appear to be more integrative and sensitive to factors affecting ion mobility. Further, correlations between the two assays appear to be directly related to the availability of sufficient labile N, as all significant relationships were driven exclusively by the fertilized treatments. This indicates that differences between the assays are further exacerbated in low N environments. Ion exchange membranes did not appear to reach saturation in the unfertilized soils, though it appeared as though NO_3^- may have saturated the IEMs beyond 6-weeks in the fertilized spruce

soils. Ion desorption also did not appear to affect IEM N-mineralization estimates in either of the soils.

Ion-exchange membrane N estimates have shown significant relationships with plant uptake and crop yield in agricultural systems (e.g. Qian and Schoenau 1995, 2005, 2007); however, this type of research has only been done to a limited degree in forest soils (e.g. Hangs et al. 2004). Further investigation of IEM relationships with tree growth and foliar nutrition is essential to address some of the knowledge gaps exposed in this experiment. Regardless, the results from this study suggest that IEM N estimates are not only comparable to traditional N-mineralization assays, but may present additional opportunities for characterizing N dynamics in fertilized forest soils. This is promising, as traditional nitrogen mineralization indices in forest soils have been largely unreliable for predicting the N supply power of the soil (Landsberg et al. 1991).

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CHAPTER 4 – Effects of repeated fertilization on carbon and nitrogen dynamics in immature pine and spruce forests in British Columbia

INTRODUCTION

The biogeochemical cycles of carbon (C) and nitrogen (N) are intrinsically linked through a multitude of natural processes affecting all life on earth (Galloway et al. 2003). Unprecedented alteration of global C and N cycles (largely from fertilizer and fossil fuel sources) has drastically affected the coupling of these elements in several ecosystems (Norby 1998). The global nitrogen cycle has been modified to such an extent that annual inputs of anthropogenic N into the environment now exceeds that of natural processes (Millenium Ecosystem Assessment 2005; Vitousek 1994). Nitrogen is a key factor controlling the functioning of several ecosystem processes and any changes in N invariably elicit changes in the ability of ecosystems to store C and regulate C fluxes to the atmosphere (Vitousek et al. 1997; Liu and Greaver 2010).

Forest ecosystems represent approximately half of the terrestrial C reservoir (Dixon et al. 1994); thus, any changes in C dynamics in these systems can have enormous implications on atmospheric C concentrations and global climate. With increased anthropogenic N inputs forecast to continue well into the future (Lamarque et al. 2005), it is necessary to understand how elevated N affects C dynamics in forest ecosystems. Net primary production (NPP) of many northern forests is limited by available N and increases in N inputs often result in significant increases in aboveground biomass (Nohrstedt 2001; LeBauer and Treseder 2008); such increases invariably increase aboveground C accumulation (Van Miegroet and Jandl 2007). In fact, elevated

anthropogenic N inputs have been widely suggested as a primary factor responsible for increased aboveground C in northern forests (Högberg 2007; Magnani et al. 2007).

Aboveground forest C dynamics respond rather consistently to elevated N inputs (LeBauer and Treseder 2008); however, belowground (forest floor and soil) C responses are less predictable. Elevated N has been associated with increased (Mäkipää 1995; Pregitzer et al. 2008), decreased (Mack et al. 2004; Allison et al. 2010), or no change (Neff et al. 2002; Johnson et al. 2003; Leggett and Kelting 2006; Sartori et al. 2007) in belowground C. Several possible mechanisms (e.g., increased litter inputs, reduced decomposition, shifting biomass allocation) have been suggested to explain belowground C responses to elevated N; however, such mechanisms are not understood well enough to adequately predict soil C responses to N inputs. Approximately two thirds of forest C is stored in soils (Dixon et al. 1994); thus, understanding how N inputs affect belowground C accumulation is essential for predicting whether forest ecosystems will act as sinks or sources of C as anthropogenic N inputs increase.

The belowground C pool of a forest can be viewed simply as the difference between C inputs and C losses; the residence time of C in the soil pool is ultimately determined by the quantity (mass) and quality (ease of degradation) of the material deposited and the ability of the microbial community to process it (Prescott 2005; Treseder 2008). The quantity of carbon inputs to the soil generally increases in response to N-induced increases in aboveground biomass (e.g. Mäkipää 1995; McFarlane et al. 2009); however, the relative amounts of C deposited as litter and root turnover may be altered in a high-N environment (Högberg 2007). While N-induced increases in aboveground biomass generally produce concomitant increases in litterfall (Smaill et al.

2008; Leggett and Kelting 2006; McFarlane et al. 2009), root transfers of C to the soil are less predictable. For example, several authors have found reduced fine root production under elevated N (Shan et al. 2001; Berch et al. 2006; Will et al. 2006; Leggett and Kelting 2006), a phenomenon that is often coupled with increased accumulation of C in aboveground woody tissues (Fisher and Binkley 2000; Högberg 2007) and coarse roots (Albaugh et al. 1998; Retzlaff et al. 2001). A decrease in fine root production may arise as nutrient limitations are met by fertilizer inputs and less C is required for maintaining nutrient acquisition structures (Janssens and Luyssaert 2009). While decreases in fine root production are widely documented in response to excess N, the opposite has also been reported (King et al. 2002; Berch et al. 2009). For example, King et al. (2002) found that elevated N increased fine-root production and fine-root turnover, which enriched the soil C pool. These results were largely due to increases in non-mycorrhizal fine-roots, which turn over more rapidly than roots with mycorrhizal associations (King et al. 2002). Thus, differential root C responses to N additions may be largely related to the effects of N on root symbionts.

Once detrital plant biomass reaches the soil, either through litterfall or root turnover, it undergoes decomposition processes by soil microbial communities. Some substrates are completely mineralized to CO_2 and thus lost from the system, while others are stored within the soil as labile or recalcitrant organic matter (Van Miegroet and Jandl 2007). Thus, the fate of C inputs to the soil is ultimately determined by the degree of microbial processing (Prescott 2005). Nitrogen additions have been found to disrupt microbial processes in several ways. For example, in a meta-analysis of 82 studies subject to N additions, Treseder (2008) found that N enrichment reduced microbial

biomass and soil CO_2 respiration. In a similar meta-analysis of N-manipulation studies, Janssens et al. (2010) found that soil C increased as microbial biomass decreased, suggesting the enrichment of soil C is related to N-induced suppression of the microbial community. Indeed, elevated N inputs have been widely linked to reduced microbial decomposition (e.g. Fog 1988; Berg and McClaugherty 1987), though it is not clear whether this is directly or indirectly related to excess N.

Nitrogen-enriched forest soils can directly inhibit the growth of soil microbes by reducing soil pH and altering the availability of other nutrients (e.g. Ca, Mg, K; Homann et al. 2001). Thus, elevated N may constrain microbial growth by inducing secondary deficiencies within the microbial community. Soil microbes can also be negatively affected by the toxic effects of increased soluble aluminum associated with decreases in soil pH (Wood 1995). Indirect effects of elevated N on soil C are related to either the quality of the litter inputs or the ability of the microbial community to degrade such inputs with litter-degrading enzymes (Prescott 2005; Pregitzer et al. 2008). Lignin and cellulose are the most abundant biochemicals in litter material and N additions have been found to alter both the production and decomposition of both compounds (Waldrop et al. 2004). For example, long-term additions of N have been found to increase the lignin content of litter and subsequently reduce decomposition (Magill and Aber 1998). Further, several authors have found that N-inputs may inhibit the degradation of lignin by suppressing the production of lignin-degrading enzymes (Sinsabaugh et al. 2002; Deforest et al. 2004; Waldrop et al. 2004; Pregitzer et al. 2008). Alternatively, cellulose degradation may be stimulated in N-enriched soils, thereby increasing decomposition of cellulosic inputs (Sinsabaugh et al. 2002). Therefore, N inputs appear to differentially

affect litter decomposition processes suggesting the variability in soil C responses to elevated N may be related to site-specific differences in the composition and function of microbial communities and the influence of soil chemistry on those communities (Waldrop et al. 2004).

Clearly, C responses to N inputs are complex and not well understood. However, much of this variability is presumably due to differences in N dynamics, which ultimately drive forest C responses. For example, N used in the production of plant tissues will eventually be returned to the soil as litter, thereby contributing to soil C cycling; whereas N lost as leachate or immobilized abiotically may contribute little to soil C dynamics. Thus, the effects of N on soil C processes are ultimately determined by how the added N is cycled and where it accumulates (Janssens and Luyssaert 2009).

Soils are the major sink for N inputs in forest ecosystems (Gunderson et al. 1998; Davidson et al. 2003). Forests accumulate soil N either through the incorporation into plant or microbial biomass or abiotically by stabilizing N in organic matter or within 2:1 clays (Johnson 1992). Biological processes are often considered to dominate N cycling in forest soils, with competition between microbes and plants determining the amount of N retained within the soil. However, there is increasing evidence that abiotic immobilization may play a major role in soil N retention, especially in high N environments (Johnson et al. 2000; Dail et al. 2001; Barrett and Burke 2002; Colman et al. 2007; Davidson et al. 2008). Physical condensation reactions between nitrogen inputs and phenols have been shown to form relatively stable N compounds, known as “brown, nitrogenous humates” (Mortland and Wolcott 1965; Nommik 1965; Berg et al. 1984). Such abiotic reactions can immobilize substantial soil N following fertilization (Foster et

al. 1985) as well as in acid (pH < 4.5) forest soils (Axelsson and Berg 1988; Schimel and Firestone 1989). While the specific reactions involved are unclear, abiotic N immobilization has been shown to play a major role in the retention of N in a range of forest soils (Johnson et al. 2000). Thus, understanding whether N is cycled through biotic or abiotic pathways is important for determining how N inputs interact with soil C cycles.

We studied the effects of 13-15 years of repeated, N-based fertilization on N and C dynamics in 2 interior spruce (*Picea glauca* [Moench] Voss and *Picea engelmannii* Parry) and 2 lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm) stands in the interior of British Columbia. Specifically, the objectives were to: 1) assess changes in N cycling patterns and determine how the added N was incorporated into the ecosystem; and 2) determine how changes in N dynamics have affected C storage and allocation at these sites.

MATERIALS AND METHODS

Site Description

This study was carried out at the S1, S2, P1 and P2 interior spruce and lodgepole pine field installations in 2008 and 2009 (see Chapter 2 for complete description of field sites). The abbreviated site names allow for easy distinction between spruce (S1 & S2) and pine (P1 & P2) study sites. On occasion, site abbreviations are also accompanied by sampling years (e.g., S1 2008 = S1-08). Three treatments were studied at each site: control, periodic and annual (see Chapter 2, Table 2.3 for description of fertilizer treatments and Tables 2.4 & 2.5 for detailed fertilization history).

Soil Sampling

Soil N supply was measured using ion exchange membrane (IEM) plant root simulator (PRS™) probes (Western Ag Innovations Inc., Saskatoon, SK). In each treatment plot, 4 IEM-probe pairs (cation + anion) were inserted vertically into the top 10 cm of the mineral soil. Probes were buried for two 6-week sampling intervals over each of the 2008 and 2009 growing seasons (see Table 4.1 for sampling dates). Following the first 6-weeks of burial, all four probe pairs were collected from each plot and replaced with recharged probes in the same soil slots. After both the first and second 6-week sampling periods, all 4 probe pairs from each plot were combined to form one composite sample per plot. Following field collection, probes were immediately rinsed with pressurized de-ionized water to remove all residual soil, packaged on ice and sent overnight to Western Ag Innovations laboratory (Saskatoon, SK) for analysis (see chapter 3 for analysis details). Soil N supply rates from the first and second samplings were combined and reported as μg N/10 cm^2/12-weeks.

Table 4.1. 2008 and 2009 PRS-Probe Sampling Dates.

Site	2008			2009		
S1	June 9	July 23	August 31	June 17	July 29	September 16
S2	June 11	July 25	September 3	June 18	July 30	September 20
P1	June 12	July 26	September 2	June 19	July 31	September 19
P2	June 13	July 27	September 4	June 21	August 1	September 15

Forest floor and mineral soil samples were collected in September 2007 at the pine sites (P1 & P2) and September 2008 at the spruce sites (S1 & S2) for ^{13}C and ^{15}N analysis. Ten forest floor samples (10 cm x10 cm x forest floor depth) were collected in

each plot and combined to make one composite sample per plot. Ten soil samples were also collected from the upper 10 cm of the mineral soil and combined to make one composite sample per plot. Each composite sample was then analyzed for total C and N as well as ^{13}C and ^{15}N at the UC Davis Stable Isotope facility using an EA-IRMS (PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer, Sercon Ltd., Cheshire, UK). Forest floor C and N concentrations were used in nutrient pool calculations; however, mineral soil C and N pools were calculated based on 2009 soil samples (see below). Isotope data were reported as the difference between the atom (%) in the sample and the atom (%) in the official reference standard (atom % of ^{13}C in Pee Dee Belemnite standard = 1.1112328; atom % of ^{15}N in air standard = 0.3663033). Data were expressed as $\Delta^{13}C$ and $\Delta^{15}N$ and used as an index of microbial activity. Increased $\Delta^{13}C$ and $\Delta^{15}N$ were assumed to indicate increases in microbial activity and decreases were assumed to indicate the opposite (i.e., microbial discrimination against heavier isotopes; Griffith 2004; Dijkstra et al. 2006); however, this assumption does not factor in differences in litter inputs. For example, if litter inputs increase with fertilization and there is no difference in $\Delta^{13}C$ and $\Delta^{15}N$ values, it can be assumed that microbial activity has increased concomitantly in response to the added litter. Thus, litter data (below) will be central to interpreting $\Delta^{13}C$ and $\Delta^{15}N$ responses to fertilization. Forest floor C and N, forest floor and mineral soil ^{13}C and ^{15}N, as well as litter input data (below) have all been generously provided by Lori Phillips (Post-Doctoral Fellow, University of British Columbia - Okanagan).

Four mineral soil samples per plot were randomly collected at each site in June 2009. Samples were collected from two depths: 0-10cm and 10-20cm. All four samples at

each depth were combined to form one composite sample at each depth in each plot (9 plots x 2 depths = 18 samples per site). Samples were kept on ice while in the field and wet sieved (<2mm) immediately upon arrival at the lab. Air-dried sub-samples were pulverized using a Seibtechnic concentric ring grinder and analyzed for total carbon and nitrogen by dry combustion using a LECO CNS-2000. Elemental concentrations were recorded as percent of the sample dry weight and used in nutrient pool calculations (see below).

Soil bulk density was assessed at all four sites by sampling two randomly selected locations immediately adjacent the treatment plots. Bulk density was determined using the sand-cone replacement method as outlined by Maynard and Curran (2008). At each sampling location, a 30 cm x 30 cm flat metal plate with a 10 cm diameter hole in the centre was placed on the soil surface. Soil material within the hole was removed in a cylindrical pattern down to the desired depth: forest floor; 0-10 cm; 10-20 cm. Excavated soil was weighed immediately and bagged for further determination of specific soil fractions. The excavated hole was then filled with Ottawa sand (mined in Ottawa, Illinois) to determine the volume of the hole and calculate total bulk density and bulk density of the fines as outlined by Maynard and Curran (2008). Bulk density of the fines was used to calculate forest floor and mineral soil C and N pools on a per hectare basis.

Foliar and Litter Sampling

Samples of the current year's foliage were collected from 10 healthy trees in each treatment plot in September 2008 and 2009. Foliar samples were collected from the lower portion of the top third of the live crown and combined to make 1 composite sample per

plot. Composite samples were then ground using a coffee grinder and analyzed for total N by dry combustion using a Fisons NA-1500 elemental analyzer (Brockley 2007). Foliar data was generously provided by Rob Brockley (Research Scientist, BC Ministry of Forests and Range).

Litter trays were placed on top of the forest floor and left in the field over a one-year period. At the pine sites (P1 & P2), 4 litter trays per plot were installed from September 2007 until September 2008. At the spruce sites (S1 & S2), 5 litter trays per plot were installed from September 2008 until September 2009. Following sample collection, litter was brought back to the lab and sorted into needles, other conifer (cones), and herbaceous (sorting done by hand with forceps). Sorted litter was allowed to air-dry for two weeks, then it was weighed. Foliar litter was ground through a 60 mesh screen, ball ground to a fine powder and analyzed for total C and N at the UC Davis Stable Isotope Laboratory. Each tray was 1326 cm^2; total tray area was converted to hectares and leaf litter biomass was reported on a kg/ha basis. Nitrogen concentration from the foliar litter was subtracted from the current year's foliage to determine the amount of foliar N retained by the trees prior to senescence. In addition, N content (kg/ha/yr) of the foliar litter was calculated to assess the amount of foliar N returned to the soil.

Tree Growth

Diameter at breast height (DBH) and total height (HT) were measured for all 64 trees within each assessment plot in the fall of the following years (site): 2007 (P1 & S1); 2008 (P2); 2009 (S2). Diameter measurements were taken using a steel diameter tape at

1.3 m above the ground and height was measured with a Forester Vertex hypsometer. Diameter at breast height and HT measurements were then used to calculate aboveground tree biomass (wood, bark, foliage and branches) using species-specific biomass equations (Lambert et al. 2005). Root biomass was calculated as 0.222 of aboveground tree biomass as suggested by Li et al. (2003). Both aboveground tree and root biomass estimates were combined and as "tree biomass;" 50% of the tree biomass was reported as "tree C" in Mg/ha (Leggett and Kelting 2006). Tree growth data was generously provided by Rob Brockley (Research Scientist, BC Ministry of Forests and Range).

Data Analysis

Paired t-tests were used to assess differences in soil and foliar variables in control plots in 2008 and 2009. Analysis of variance (ANOVA) using the general linear model (GLM) procedure was used to assess treatment effects on the following variables: soil N supply rates, N concentrations of current and litter foliage, forest floor and mineral soil (0-10 and 10-20cm) C and N pools, forest floor and mineral soil (0-10cm) $\Delta^{13}C$ and $\Delta^{15}N$, annual litter inputs as well as tree and total ecosystem C. Where significant differences were found, Tukey's HSD post-hoc test was used to test for significant differences between individual treatment means. Nonlinear regression was used to assess relationships between soil N supply rates and foliar N within the same year. Simple linear regression was used to examine relationships between soil C and N pools by depth at individual sites as well as combined (all sites pooled by depth). A level of significance of $\alpha = 0.05$ was used for inferring statistical significance throughout the study. Statistical analyses were completed using PASW 18 (SPSS Inc., 2009).

RESULTS

Soil N Supply Rates

Soluble inorganic N (NH_4^+ and NO_3^-) supply rates as measured by IEM probes were very responsive to spring fertilizer additions (Figures 4.1 & 4.2). Nitrogen supply rates in the control plots were not significantly different in 2008 and 2009 at all sites except S2, where 2008 N supply rates were significantly higher than 2009. Differences in control N supply rates at the S2 site were presumably due to warmer and drier conditions in 2009 affecting ion mobility to the IEM surfaces (see Chapter 2, Figures 2.1 & 2.2 for rainfall data). Ammonium was the dominant N form in the control plots at all sites, with NO_3^- supply rates never exceeding 8 μg N/10 cm^2/12-weeks or 45% of total inorganic N.

Periodic fertilization significantly elevated N supply rates over the controls during the growing season in the year of fertilizer application at P1-08 and P2-09 (Figures 4.2a & d). Residual N did not significantly affect N supply rates in subsequent years. In the year of fertilizer application, the periodic treatment resulted in minimal NO_3^- production at the P2 site, whereas, at the S1 and P1 sites, NO_3^- increased substantially compared to the controls. At the S2 site, periodic N fertilization did not affect soil N supply 1 and 2 years following fertilization and at P2 there was no effect on N supply 5 years after fertilization.

Annual fertilization significantly increased N-supply rates compared to the controls at S2-08, S2-09 and P1-09; however, mean N-supply rates were elevated above the controls at all sites. The S1 and P1 sites both appear to process the annual fertilizer inputs similarly, in that both NH_4^+ and NO_3^- supply rates were elevated, with NO_3^- representing the majority of the soluble inorganic N (Figures 4.1a & b, 4.2a & b).

Conversely, increases in N supply rates in the annual treatments at the S2 and P2 sites were largely dominated by NH_4^+ (Figures 4.1c & d, 4.2c & d). Annual fertilization produced the highest NO_3^- levels of the two fertilizer treatments.

Fertilizer-N inputs in both the periodic and annual treatments varied greatly in their contributions to N supply rates. For example, in 2009, additions of 50 kg N/ha at the S2 site resulted in N supply rates of 17.5 μg N/10 cm^2/12-weeks, whereas at the P1 site the same contribution produced 320.6 μg N/10 cm^2/12-weeks. This was presumably due to the sensitivity of the IEM probes to conditions affecting ion mobility (e.g. root and microbial competition, abiotic immobilization, moisture, etc.). Therefore, differences in soil conditions between the sites appear to drastically affect the amount of N available in soil solution (see Chapter 3 for more detail on the effects of soil properties on N supply rates). There were no discernable trends in the seasonality (1^{st} versus 2^{nd} sampling) of NH_4^+ or NO_3^- supply rates (data not shown).

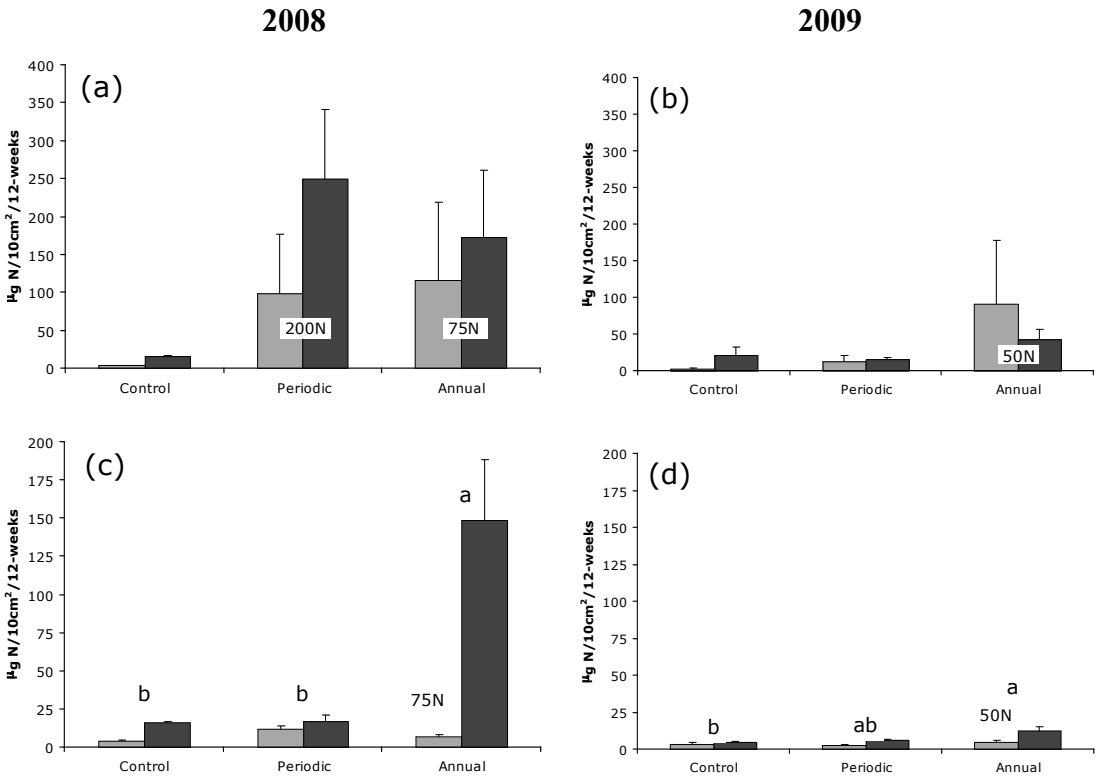


Figure 4.1. Spruce N ($NH_4^+ + NO_3^-$) 2008 (a & c) and 2009 (b & d) supply rates. S1 = a & b; S2 = c & d. Treatment legend: light grey = NO_3^-, dark grey = NH_4^+. Different letters above bars indicate significant differences ($p < 0.05$) in total inorganic N ($NH_4^+ + NO_3^-$) between treatment means. Error bars indicate ±1 SE. Values above or embedded in bars indicate the amount of N fertilizer (kg/ha) added in that particular treatment in the spring of the given year.

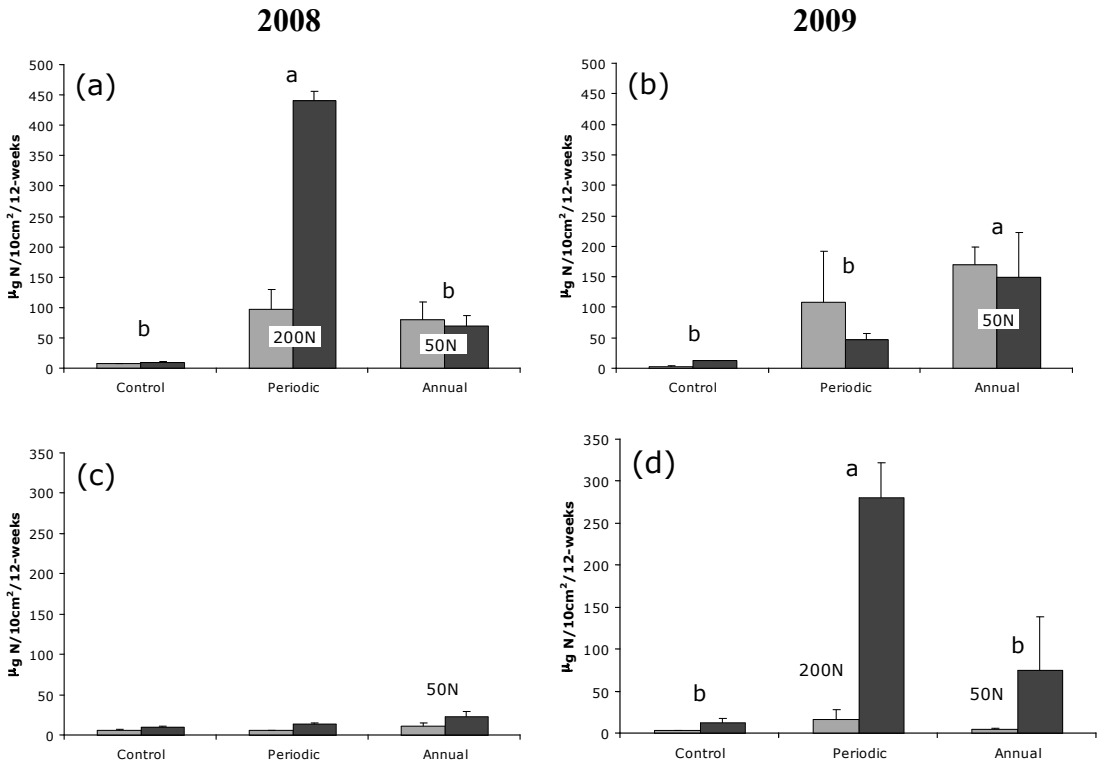


Figure 4.2. Pine N ($NH_4^+ + NO_3^-$) 2008 (a & c) and 2009 (b & d) supply rates. P1 = a & b; P2 = c & d. Treatment legend: light grey = NO_3^-, dark grey = NH_4^+. Different letters above bars indicate significant differences ($p < 0.05$) in total inorganic N ($NH_4^+ + NO_3^-$) between treatment means. Error bars indicate ±1 SE. Values above or embedded in bars indicate the amount of N fertilizer (kg/ha) added in that particular treatment in the spring of the given year.

Foliar Nitrogen

Nitrogen concentrations in the current foliage were higher in the annual treatments than the controls at all sites for at least one of the two years (Table 4.2). The same was true of the periodic treatments, indicating that both species are capable of maintaining elevated foliar N beyond the year of fertilizer application. The N concentration of the foliar litter suggests species differ in the degree of N recycled from senescing foliage (Table 4.2). A greater percent of foliar N was retained in the trees at the pine sites compared to the spruce sites; P1 and P2 retained 62-63% and 63-70%, whereas

S1 and S2 retained 18-30% and 24-42%. Concentrations of foliar N internally recycled were not significantly affected by treatment at any of the sites. Further, the quantity of N deposited to the soil as foliar litter in the fertilized plots was higher at the spruce sites (4-20 kg/ha/yr) compared to the pine sites (4-7 kg/ha/yr) in all but one sampling (data not shown).

Table 4.2. Mean (n = 3) N concentrations (%) of current and litter foliage in 2008 and 2009. Means with different letters are significantly different ($p < 0.05$). Numbers in brackets indicate ± 1 SE.

			2008			2009		
Species	**Site**	**Foliage**	**Control**	**Periodic**	**Annual**	**Control**	**Periodic**	**Annual**
Spruce	S1	Current	1.20b (0.01)	1.51a (0.04)	1.41a (0.07)	1.26 (0.07)	1.39 (0.05)	1.35 (0.05)
		Litter				0.88 (0.03)	0.98 (0.07)	1.1 (0.00)
	S2	Current	1.04b (0.004)	NA	1.31a (0.05)	1.10b (0.01)	1.25a (0.02)	1.31a (0.02)
		Litter				0.64 (0.03)	0.95 (0.07)	0.8 (0.09)
Pine	P1	Current	1.20b (0.02)	1.47a (0.02)	1.39ab (0.06)	1.36 (0.01)	1.61 (0.11)	1.63 (0.03)
		Litter	0.37 (0.01)	0.41 (0.03)	0.49 (0.03)			
	P2	Current	1.21b (0.01)	1.12c (0.02)	1.36a (0.02)	1.17c (0.02)	1.47a (0.05)	1.27b (0.03)
		Litter	0.44 (0.02)	0.55 (0.07)	0.52 (0.02			

Soil-Foliar Nitrogen relationships

Soil N supply rates ($NH_4^+ + NO_3^-$) over the growing season were significantly related to foliar N levels at the end of the growing season when both the periodic and annual fertilizer treatments were applied in the same year (S1-08, P1-08 & P2-09; Table 4.3). However, in the year immediately following both periodic and annual fertilizer inputs (S1-09 & P1-09), soil-foliar relationships were no longer significant. Significant

relationships were also found in 2008 and 2009 at the S2 site, which was not subject to periodic fertilization. Further, soil-foliar N relationships appear to be differentially affected by ion species. For example, at the S1 and P2 sites, soil N relationships with foliar N were driven almost equally by NH_4^+ and NO_3^-; whereas, at the S2 and P1 sites, relationships were driven largely by NH_4^+ (Table 4.3).

Table 4.3. Non-linear relationships between soil N (NH_4^+; NO_3^-; total N = $NH_4^+ + NO_3^-$) supply rates and foliar N (n = 9 at all sites, except n = 6 for S2-08). Ion-specific relationships were examined separately to assess the contribution of each N form to overall N uptake. Asterisks indicate significant relationships ($p < 0.05$).

		2008			2009		
		R^2	*p*-value	Equation	R^2	*p*-value	Equation
SPRUCE							
S1							
	NH_4^+	0.70*	0.005	y = 0.097Ln(x) + 0.954	0.05	0.578	y = 0.029Ln(x) + 1.247
	NO_3^-	0.71*	0.005	y = 0.077Ln(x) +1.148	0.13	0.348	y = 0.024Ln(x) + 1.285
	Total N	0.85*	0.000	y = 0.101Ln(x) + 0.900	0.13	0.342	y = 0.036Ln(x) + 1.207
S2							
	NH_4^+	0.75*	0.026	y = 0.110Ln(x) + 0.753	0.66*	0.008	y = 0.162Ln(x) + 0.901
	NO_3^-	0.31	0.249	y = 0.230Ln(x) + 0.787	0.08	0.465	y = 0.057Ln(x) + 1.143
	Total N	0.73*	0.029	y = 0.176Ln(x) + 0.680	0.50*	0.032	y = 0.166Ln(x) + 0.820
PINE							
P1							
	NH_4^+	0.69*	0.005	y = 0.067Ln(x) + 1.070	0.44	0.053	y = 0.101Ln(x) + 1.162
	NO_3^-	0.48*	0.040	y = 0.072Ln(x) + 1.099	0.13	0.339	y = 0.031Ln(x) + 1.428
	Total N	0.67*	0.007	y = 0.072Ln(x) + 1.013	0.35	0.093	y = 0.064Ln(x) + 1.046
P2							
	NH_4^+	0.40	0.071	y = 0.171Ln(x) + 0.768	0.46*	0.044	y = 0.057Ln(x) + 1.085
	NO_3^-	0.32	0.111	y = 0.153Ln(x) + 0.918	0.49*	0.035	y = 0.126Ln(x) + 1.084
	Total N	0.39	0.070	y = 0.118Ln(x) + 0.703	0.49*	0.036	y = 0.071Ln(x) + 1.219

Soil N pools

Mean fertilizer-N retained belowground in 2009 (forest floor + mineral soil 0-20cm) represented more than 90% of the fertilizer-N inputs at all sites. In fact, belowground N increases exceeded N inputs in several cases; however, this was presumably due to the large variability in belowground N pools (Table 4.4). For example, each kilogram of fertilizer-N added per hectare contributed to varying changes in belowground (forest floor + soil 0-20cm) N pools (ranges in kg N/ha): S1 = 2.16 – 2.18; S2 = 1.1 – 1.4; P1 = 0.97 – 1.6, P2 = 0.91 – 0.94 (Table 4.5). The annual treatments were more effective at increasing mean soil N pools compared to the periodic treatments at all sites. Further, belowground N increases per unit of fertilizer-N added were greater at the spruce sites than the pine sites.

Table 4.4. 2009 mean (n = 3) nitrogen and carbon pools. Different letters for means of the same variable indicate significant differences ($p < 0.05$). Numbers in brackets indicate ± 1 SE.

			Nitrogen (kg/ha)			Carbon (Mg/ha)		
Species	**Site**	**Pool**	**Control**	**Periodic**	**Annual**	**Control**	**Periodic**	**Annual**
Spruce	**S1**	Forest Floor	1057.43b (109.94)	1481.59ab (62.34)	1932.43a (338.74)	30.00b (2.37)	37.41ab (2.86)	45.30a (3.66)
		0-10 cm	989.87 (338.13)	1642.66 (817.32)	1756.00 (936.76)	22.77 (6.33)	32.81 (13.45)	31.82 (13.80)
		10-20 cm	611.60b (182.72)	829.82ab (182.09)	1042.76a (160.15)	13.88 (3.36)	17.50 (2.99)	21.81 (3.35)
		Total Soil	2658.90 (544.08)	3954.06 (978.88)	4731.19 (1397.46)	66.65b (9.93)	87.73ab (13.69)	98.93a 18.15)
		Tree				20.14b (2.82)	31.61ab (6.52)	42.85a (4.77)
		Ecosystem				86.79b (11.83)	119.34ab (19.73)	141.77a (22.73)
	S2	Forest Floor	599.76b (62.47)	1038.64ab (220.61)	1504.37a (261.63)	21.66 (3.80)	36.14 (10.82)	40.98 (7.08)
		0-10 cm	741.11b (45.60)	897.24a (94.74)	1208.19a (117.89)	23.40b (1.18)	25.77ab (2.44)	35.87a (3.67)
		10-20 cm	711.24 (190.25)	776.46 (29.94)	917.09 (90.21)	18.27 (4.43)	20.57 (0.63)	22.07 (2.87)
		Total Soil	2052.10b (268.04)	2712.34ab (241.99)	3629.65a (96.15)	63.33b (5.69)	82.47ab (12.25)	98.92a (0.66)
		Tree				19.10b (2.55)	37.68a (1.66)	49.37a (3.61)
		Ecosystem				82.43b (5.33)	120.15a (13.18)	148.29a (3.21)
Pine	**P1**	Forest Floor	666.50 (90.91)	1104.43 (55.17)	1222.93 (328.14)	23.51 (0.56)	32.23 (1.94)	36.26 (8.75)
		0-10 cm	588.75b (26.06)	683.71ab (65.72)	1009.30a (75.43)	16.06b (0.37)	18.67ab (1.87)	26.14a (1.14)
		10-20 cm	627.16 (104.82)	676.86 (79.66)	1019.49 (205.84)	15.26 (1.84)	17.19 (2.44)	28.17 (5.45)
		Total Soil	1882.40b (174.00)	2465.01b (111.76)	3251.72a (51.48)	54.83b (2.35)	68.10b (4.76)	90.57a (3.69)
		Tree				25.90b (2.53)	34.01a (2.03)	39.79a (1.03)
		Ecosystem				80.73c (4.03)	102.10b (4.75)	130.36a (2.95)
	P2	Forest Floor	593.93 (134.87)	712.17 (69.09)	840.68 (11.25)	16.90 (3.52)	23.81 (2.57)	31.18 (10.23)
		0-10 cm	584.21b (106.38)	796.73ab (96.53)	925.16a (94.00)	19.15b (2.79)	20.12b (2.55)	24.41a (3.56)
		10-20 cm	676.26 (159.93)	715.68 (58.94)	833.75 (152.96)	16.81 (1.53)	16.81 (0.37)	19.93 (4.94)
		Total Soil	1854.40 (165.12)	2224.58 (268.10)	2599.59 (412.35)	52.85 (0.97)	60.74 (3.06)	75.53 (10.21)
		Tree				17.45b (2.36)	25.58a (2.30)	30.49a (3.75)
		Ecosystem				70.33b (2.90)	86.33ab (3.45)	106.01a (6.53)

Table 4.5. Forest floor, soil 0-10cm, soil 10-20cm and total soil (forest floor + soil 0-20cm) N increases in the fertilized treatments over the controls per unit (kg/ha) of fertilizer N added (total N inputs).

		Periodic (kg/ha)				Annual (kg/ha)			
Species	Site	Forest Floor	Soil 0-10cm	Soil 10-20cm	Total Soil	Forest Floor	Soil 0-10cm	Soil 10-20cm	Total Soil
Spruce	S1	0.71	1.09	0.36	2.16	0.92	0.81	0.45	2.18
	S2	0.73	0.26	0.11	1.1	0.80	0.42	0.18	1.4
Pine	P1	0.73	0.16	0.08	0.97	0.65	0.49	0.46	1.6
	P2	0.49	0.35	0.07	0.91	0.31	0.43	0.20	0.94

Carbon inputs and microbial activity

Litter inputs were significantly elevated in the annual treatments compared to the controls at 2 of the 4 sites, with the periodic treatments intermediate (Figure 4.3). While litter inputs were generally elevated in the fertilized plots, there were no differences in $\Delta^{13}C$ in the fertilized or control plots in the forest floor or mineral soil (data not shown), indicating that microbial processing was not adversely affected by any of the treatments and, therefore, increased concurrently with detrital C inputs. Similarly, $\Delta^{15}N$ of the forest floor was not significantly different in 3 of the 4 sites, though $\Delta^{15}N$ was significantly lower in the annual treatment compared to the control at the S1 site. In the mineral soil, $\Delta^{15}N$ was significantly lower in the annual treatment compared to the control at the S2 site, though $\Delta^{15}N$ was not affected in the mineral soil at any of the other sites. Therefore, microbial processing of C inputs was not adversely affected by fertilization; however, in two cases, microbial incorporation of N inputs was reduced at the spruce sites (data not shown), though not enough to alter the overall microbial processing of forest floor and mineral soil C.

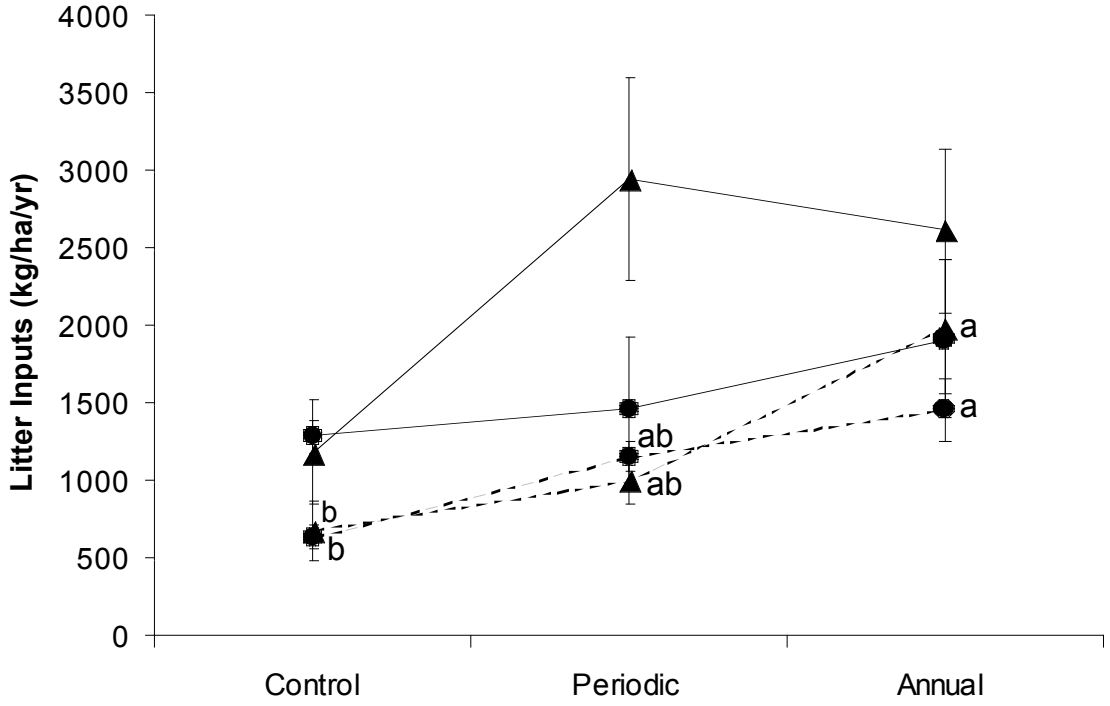


Figure 4.3. Annual litter inputs from 2008 (pine) and 2009 (spruce). Legend: S1 = triangle with solid line; S2 = triangle with dotted line; P1 = circle with solid line; P2 = circle with dotted line. Means with different letters are significantly different ($p < 0.05$). Error bars indicate ± 1 SE.

Carbon Pools

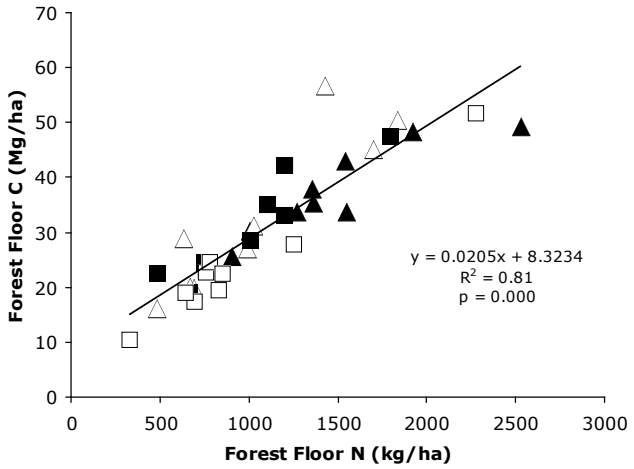
Annual fertilization significantly increased total ecosystem carbon (TEC) at all sites, with 63%, 80%, 61% and 51% increases compared to the controls at the S1, S2, P1 and P2 sites (Table 4.4). Periodic fertilization also significantly increased TEC at the S2 and P1 sites. Total ecosystem carbon increases in the periodic treatments were more pronounced at the spruce sites, with increases of 38% and 46% over the controls at the S1 and S2 sites compared to 26% and 23% increases at the P1 and P2 sites. Increases in TEC were greater in the annual treatment compared to the periodic at all sites, though only significantly greater at the P1 site.

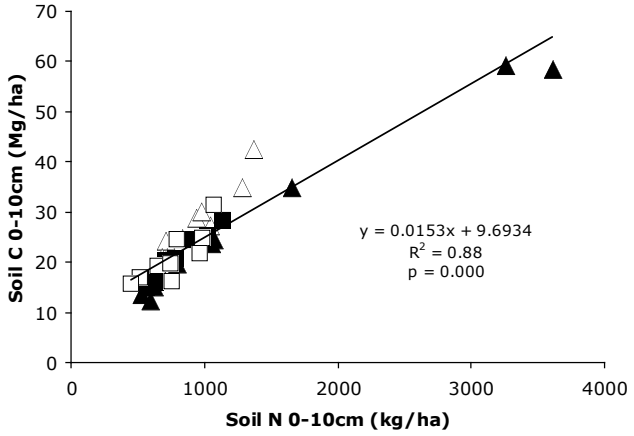
Tree C was significantly higher in both fertilizer treatments than in the controls at all sites except S1 (where only the annual treatment was significantly higher; Table 4.4). Tree growth responses to the fertilizer treatments were substantial (for detailed growth-specific results see Brockley and Simpson 2004; Brockley 2010a, 2010b); however, the proportional aboveground contribution to TEC did not significantly change at any of the sites, with tree C representing between 23-33% of TEC at all sites.

Soil C increased significantly in the annual treatments compared to the controls at 3 of 4 sites (Table 4.4); periodic fertilization resulted in intermediate soil C. Changes in soil C accumulation by sampling depth varied by site and treatment. In general, the majority of soil C increases in the periodic treatments occurred in the forest floor, whereas the annual treatment resulted in similar C increases in both the forest floor and mineral soil.

Soil Carbon-Nitrogen Relationships

Total soil C pools were significantly and positively related to soil N pools at all sampling depths when data were pooled for all sites and treatments (Figure 4.4); the strength of the relationships (R^2) were very similar by depth, especially when the two outliers were removed from the soil 0-10 cm C and N regression (R^2 was reduced slightly to 0.80, $p = 0.000$). Thus, approximately 80% of the variation in soil C pools was explained by soil N pools. Positive soil C and N relationships were also significant at all individual sites and depths (data not shown).





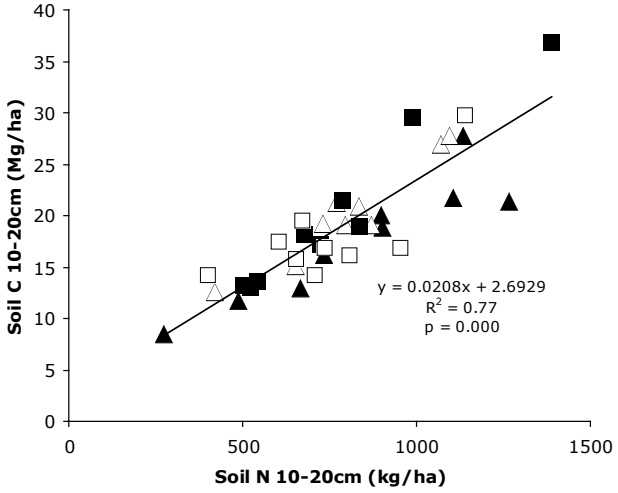


Figure 4.4. Linear regressions between soil C and N pools by sampling depth (n = 36). Legend: S1 = solid triangle, S2 = hollow triangle, P1 = solid square, P2 = hollow square.

DISCUSSION

Nitrogen cycling

Soluble and total soil N pools suggest there are stark differences in the way N is processed and stored in the different soils and the different fertilizer treatments. The annual treatment was very effective at maintaining elevated soil N supply, whereas the periodic treatment appeared to stimulate soil N supply only in the growing season immediately following fertilization. Foliar N was very responsive to elevated soil N following fertilization; however, when N-supply rates were low, it appears foliar N concentration may have been more affected by factors other than soluble soil N (e.g. internal cycling). Thus, the transfer of fertilizer inputs from the soil to tree roots may largely occur as pulses of elevated soil N following fertilization, especially in the periodic treatment.

The cycling of fertilizer N from soil to trees also appeared to be affected by the ionic form of N in soil solution. Both fertilizer treatments increased NO_3^- production compared to the controls at all sites; however, regression analyses suggest sites differ in their utilization of the available NO_3^-. For example, in 2008, the S1 and P1 sites showed significant relationships between soil and foliar NO_3^-, whereas in 2009, only the P2 site exhibited a significant soil-foliar NO_3^- relationship (Table 4.3). Several authors have raised concern over fertilizer-induced increases in nitrification as the highly mobile NO_3^- ion can be easily leached from the soil leading to nitrogen losses and potentially impacting downstream aquatic ecosystems (Vitousek et al. 1997; Galloway et al. 2003; Venterea et al. 2004). Overwinter burials of IEM probes at depth in the spruce soils (see Chapter 5) suggest NO_3^- was not leached below the rooting zone, though it is unclear

whether any leaching occurred in the pine soils, as comparable experiments could not be performed at the pine sites. Total soil N pools at all sites indicate that the majority of the added N was retained in the forest floor and top 20 cm of the mineral soil; thus, we assumed leaching losses from these sites were negligible. While volatile N losses are common with urea-based fertilizers (Kissel et al. 2009), we also assumed such losses to be minimal based on the fact that the majority of the fertilizer N remained onsite.

Despite similarities in fertilizer N inputs at all 4 sites, the contributions of fertilizer N to soil N supply rates differed greatly by site, treatment and year (Figures 4.1 & 4.2) indicating differential demand for available soil N. The degree to which fertilizer N inputs increase soil N supply is largely determined by competition between trees, microbes and abiotic processes that stabilize N in soil organic matter (Johnson 1992). Tree growth and soil-foliar relationships suggest trees were successfully competing for elevated soil N in all of the fertilized plots. While rates of fertilizer N recovery by trees are often quite low, ranging from 5-25% of total inputs (Ballard 1984; Nadelhoffer et al. 1999), tree uptake may consume a substantial portion of the soluble N pool (Fisher and Binkley 2000). Microbial demand for soluble N was also elevated in the fertilized plots, as microbial activity paralleled increased litter inputs. Elevated microbial processing requires concomitant increases in microbial N to facilitate decomposition reactions (Van Miegroet and Jandl 2007). Thus, biotic (plant and microbial) immobilization of soluble N appears to have increased in response to fertilization, thereby retaining a portion of the N-inputs to the ecosystem. However, the bulk of the fertilizer N inputs were retained in the forest floor and upper mineral soil. It is unlikely that increased microbial immobilization could have accounted for the belowground retention of all the fertilizer inputs, suggesting

some of the N may have been immobilized abiotically. Rapid abiotic immobilization following N fertilization has been shown to reduce the soluble N pool in several cases (Foster et al. 1985; Johnson 1979; Johnson et al. 1980; Dail et al. 2001). In fact, Johnson et al. (2000) suggest that abiotic immobilization can play a major role in overall soil immobilization, representing up to 90% of total N immobilized in some forest soils. Thus, it appears as though biotic and abiotic immobilization were both responsible for the high N retention in these stands.

Total N pools in all of the fertilized plots have increased after 13-15 years of repeated fertilization. However, species-specific differences in N cycling within trees appear to affect the amount of N cycled back to the soil. For example, the spruce trees returned more N to the soil in needle fall than the pine trees, which was presumably related to the generally larger soil N pools at the spruce sites. The greater recovery of N from senescing needles in the pine trees, suggests that more fertilizer N is stored within the pine trees than the spruce trees. Soil N pools were also differentially affected by the two fertilizer treatments, with a greater percentage of fertilizer N inputs retained belowground in the annual treatment compared to the periodic treatment. This suggests that the rate and the quantity of fertilizer N applied affected the degree of N incorporation into soil pools. The majority of the N pool increases in the periodic treatment occurred in the forest floor, whereas, the annual treatment was more effective at enriching mineral soil pools. This may have been related to the fact that the annual treatment was able to sustain elevated soil NO_3^- levels and facilitate greater N movement down the soil profile. In fact, at the S1 and P2 sites, where NO_3^- supply rates were highest, the N pool at the 10-

20 cm depth in the annual treatment was 70% and 63% higher than the control, compared to 29% and 23% at the S2 and P2 sites.

Effects of Nitrogen Inputs on Carbon Dynamics

Fertilizer inputs effectively stimulated tree C accumulation. This was expected as N-based fertilizers have been shown to increase tree growth in several pine and spruce stands in British Columbia (Brockley and Simpson 2004; Brockley 2007, 2010a, 2010b). Aboveground C represented approximately 1/3 of TEC at all sites and the relative proportion of aboveground TEC was not affected by the N-inputs. Thus, above and belowground C in the fertilized treatments increased at similar rates. While positive aboveground C responses to N inputs are common, soil C responses tend to be more variable (e.g., Mäkipää 1995; Sartori et al. 2007; Mack et al. 2004). However, at all 4 sites in this study, soil C increased with fertilization and soil C accumulation was positively and linearly related to soil N pools.

Nitrogen inputs can increase soil C pools in two major ways: 1) Increasing the photosynthetic capacity of the trees, resulting in increased litter inputs to the soil; 2) decreasing decomposition of soil C by suppressing the microbial community (or a combination of the two such that inputs accumulate at a higher rate than decomposition; Janzen 2005; McFarlane et al. 2009). Our findings suggest trees in the fertilized plots were successfully competing for fertilizer N inputs, increasing their photosynthetic capacity and depositing more litter to the forest floor. Once the litter was deposited, microbial processing increased concomitantly, resulting in the incorporation of C (and N) into microbial biomass. Litter inputs increased both recalcitrant (lignin-humic) and labile

(cellulose-hemicellulose) organic matter fractions; however, microbial processing of litter inputs focused largely on labile soil C, resulting in the buildup of recalcitrant soil organic matter (Lori Phillips personal communication, 2011). Long-term N inputs have been shown to stimulate cellulose degradation, resulting in a buildup of lignin-enriched litter (Knorr et al. 2005). Thus, it appears that N-induced increases in soil C pools may be primarily due to the build-up of recalcitrant organic matter following microbial processing of increased litter.

Up until now, our discussion has focused exclusively on aboveground litter inputs; however, belowground litter inputs may have also played a major role in soil C accrual at our sites. Fertilization has been shown to significantly affect belowground C dynamics in several forest types (Albaugh et al. 1998; Retzlaff et al. 2001; Shan et al. 2001; King et al. 2002), including pine and spruce stands in British Columbia (Berch et al. 2006 & 2009). For example, Berch et al. (2009) found that fine root length increased after 10 years of annual fertilization at the S2 site. Conversely, at a pine site within the same region, annual fertilization reduced fine root production (Berch et al. 2006). Thus, species-specific differences in fine root inputs may partially explain the generally greater increases in soil C at the spruce sites compared to the pine sites.

Relationships between soil N and C suggest there is a link between soil N retention and C accrual at these sites. Nitrogen inputs increased soil, tree and ecosystem C at all sites (Table 4.4). Soil, tree and ecosystem response ratios (kilogram of C increase per kilogram of N added) were generally higher at the spruce sites than the pine sites (kg C per kg N): spruce soil 32-35, tree 27-31, ecosystem 59-64; pine soil 13-42, tree 17-20, ecosystem 33-61 (Table 4.6). However, all sites were well within C:N response ratios

found by other researchers for similar forest types (Sutton et al. 2008; de Vries et al. 2008; Janssens et al. 2010).

Table 4.6. Tree C, soil C and total ecosystem carbon (TEC) increases in the fertilized treatments over the controls per unit (kg/ha) of fertilizer N added (total N inputs).

		Periodic (kg/ha)			Annual (kg/ha)		
Species	**Site**	**Tree C**	**Soil C**	**TEC**	**Tree C**	**Soil C**	**TEC**
Spruce	S1	29	35	64	28	34	62
	S2	31	32	63	27	32	59
Pine	P1	20	22	42	19	42	61
	P2	20	13	33	17	28	46

The major uncertainties in our analysis are related to tree C estimates, which were calculated based on tree height and diameter at breast height measurements using species-specific equations for unfertilized forests (Li et al. 2003; Lambert et al. 2005). These equations were unable to account for differences in tree C allocation between treatments. While our aboveground C estimates may suffer from the variability associated with biomass equations, all of our soil C data was collected, processed and analyzed based on established methodologies to ensure utmost accuracy. Thus, our soil C findings should be interpreted as a credible assessment of soil C responses to repeated N-based fertilization in several immature stands in the interior of British Columbia.

Positive C responses to fertilization at all of our sites were largely driven by significant increases in aboveground biomass production. However, high rates of biomass production require repeated fertilizer treatments as single fertilizer additions often produce only temporary increases in stand growth (Brockley 2007). It is also important to note that while N was the focus of our discussion, N was not the only nutrient added in the fertilizer treatments (see Chapter 2, Table 2.2). Additional nutrients were added in

conjunction with N to alleviate secondary deficiencies and maintain positive growth responses; thus, results must be interpreted in the context of the other nutrients in the fertilizer mix. Further, when considering these findings in the context of C modeling it is important to consider that while these stands have been very responsive to fertilizer additions, canopy closure has only recently been achieved in the most rapidly growing stands. Nutrient uptake and aboveground growth increments generally decline following crown closure, at which point fertilizer responses also tend to be less pronounced as internal nutrient cycling becomes increasingly important (Miller 1981); therefore, it is not clear whether C accumulation will continue at the same rates beyond the initial stage of canopy development. Additionally, even though it appears that long-term repeated fertilization may be a feasible option for increasing C storage in these stands, depending on the time scale and remoteness of the site, net C gains may be nullified by the fossil fuel emissions required in fertilizer production and application (Markewitz 2006).

CONCLUSION

Findings from our study suggest C increases in some immature pine and spruce forests in British Columbia can be achieved by alleviating nutrient limitations on tree growth. At all of our sites, fertilization increased aboveground C, which drove soil C accrual by increasing litter inputs and facilitating the build-up of recalcitrant soil C. While aboveground C increases may be valuable from a timber supply perspective, in managed forests, the majority of the biomass accumulated over the course of a rotation will be harvested and never incorporated into the soil (Van Miegroet and Jandl 2007). Thus, the major means of C accrual in managed forests occurs through the build-up of

soil C. Strong positive relationships between soil N and C pools suggest that the amount of C accumulation in these soils was largely determined by how the N inputs were incorporated into the ecosystem. Similar relationships between total ecosystem C and soil N have also been found along soil fertility gradients in the interior of BC (Kranabetter 2009), suggesting soil N concentrations play an important role in regulating C dynamics in both fertilized and unfertilized forests within the region.

Our results suggest that repeated fertilization may be a viable means of building up soil C stocks in immature pine and spruce stands in British Columbia; however, it is important to consider whether these systems will store this C for a duration that is sufficient to mitigate the effects of atmospheric C on global climate. For example, we need to understand whether increases in soil C are related to the buildup of stable soil organic matter or whether we are simply stockpiling easily decomposable C that may be rapidly decomposed in a warmer climate (Fissore et al. 2008 & 2009; Janzen 2005). The boreal forest is expected to experience the greatest warming of all forest biomes (Olsson et al. 2005); thus, considerations of the nature of the soil C being stored in these systems is of utmost importance.

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CHAPTER 5 – Effects of repeated fertilization on base cation cycling in immature lodgepole pine and interior spruce forests in British Columbia

INTRODUCTION

British Columbia's pine forests are currently experiencing the largest mountain pine beetle (*Dendroctonus ponderosae* Hopkins) outbreak in recorded history. As a result, the mature pine forests on which much of the forest industry in the Interior of British Columbia is based is expected to reach 80% mortality by 2013 (British Columbia Ministry of Forests and Range, 2006). This will create serious timber supply shortages in the immediate future and threaten the economies of several forestry-dependent communities. Virtually all trees to be harvested from the interior of B.C. within the next 50 years are already growing; therefore, the B.C. Ministry of Forests and Range has proposed widespread intensive fertilization as an intervention strategy to accelerate the growth of immature stands and increase short and mid-term timber supply (Brockley, 2006).

Fertilization is the most proven silvicultural method for accelerating tree and stand development in existing immature stands (Brockley, 2006; Fisher and Binkley 2000). Although a single fertilizer application generally produces only short-term increases in stand development, long-term growth responses are possible with repeated fertilization (Albaugh et al. 2004; Ringrose and Neilson 2005; Brockley 2007). For example, research in Sweden has found that intensive fertilization is capable of shortening rotation periods of boreal spruce *(Picea spp.*) by as much as 20-60 years

(Bergh et al. 2005). Similar growth responses in B.C. would be valuable for addressing mountain pine beetle-related timber shortages.

Extensive research throughout the Interior of British Columbia has confirmed widespread nutrient deficiencies and favourable growth responses to a range of fertilizer treatments in interior spruce (*Picea glauca* [Moench] Voss and *Picea engelmannii* Parry, or naturally occurring hybrids of these species) and lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm) forests (Brockley & Simpson 2004; Brockley 2007). While both species appear to respond reasonably well to large nutrient additions in the short-term, there is concern that repeated nutrient additions may affect long-term site productivity through alterations in soil chemistry (Ringrose and Neilsen 2005).

Studies on the impacts of the long-term nutrient loading in forest ecosystems have primarily identified base cation (e.g., Ca^{2+}, Mg^{2+} and K^{+}) depletion and soil acidification as the major belowground responses affecting forest productivity (e.g. Fox 2004; Ringrose and Neilsen 2005; Högberg et al. 2006; Ring et al. 2011). For example, following 30 years of N-based fertilization of a Swedish boreal forest, Högberg et al. (2006) found up to 70% decreases in soil exchangeable base cations, accompanied by increased soil acidity and soluble aluminum (Al). The mechanisms identified as driving these responses are largely related to increases in N cycling and nitrate (NO_3^-) production. Hydrogen (H^+) ions produced during nitrification tend to reduce soil pH and replace metallic cations from exchange sites, making them susceptible to leaching losses (Aber et al. 1989, 1998). Nitrate is an extremely mobile anion in temperate forest soils and tends to leach rapidly when in excess of plant and microbial demand. By charge balance, NO_3^- (and other anions) will remove base cations as they leach down the soil

profile, which leads not only to acidification of the exchange complex (as base cations are replaced by H^+ and Al^{3+}), but a general loss of essential plant nutrients from the ecosystem (Johnson et al. 1991; Binkley and Högberg 1997; Fenn et al. 2006).

In addition to N-induced leaching of base cations, soil acidification can negatively affect base cation cycling by increasing the mobilization of Al, which can directly inhibit root uptake of base cations (Shortle and Smith 1988; Cronan and Grigal 1995; McLaughlin and Wimmer 1999; deWit et al. 2010). For example, increases in soluble Al have been shown to inhibit calcium (Ca) and magnesium (Mg) root uptake in several forest types (Schröder et al. 1988; Cronan 1991; deWit et al. 2010). It has been suggested that Al^{3+} ions may accumulate on root cell walls and block the uptake of base cations (Sverdrup et al. 1992; Godbold and Jentschke 1998; Kinraide 2003). Based on this hypothesis, Cronan and Grigal (1995) proposed the use of Ca:Al molar ratios in soil solution as a means of assessing the antagonistic effects of soluble Al on the root uptake of Ca. Below a Ca:Al molar ratio of 1, forest health is expected to be compromised (i.e., reduced tree vigor or mortality; Cronan and Grigal 1995). However, the underlying mechanisms of this hypothesis and its effects on forest productivity may not be appropriate in all forest types (Högberg and Jensen 1994; Lökke et al. 1996; Binkley and Högberg 1997; Högberg et al. 2006).

An alternative assessment of forest soil acidification, which builds on many of the mechanisms outlined above (e.g., base cation leaching, reduced root uptake), is the use of critical foliar nutrient thresholds. Forests exhibiting symptoms of base cation depletion can lead to foliar nutrient imbalances (e.g., N:Ca, N:Mg) that have been suggested as a primary cause of forest decline (Attiwill and Adams 1993). For example, excessive N-

loading in a Scots pine forest resulted in widening N:Mg foliar ratios, premature needle drop and reduced tree vigor (Van Dijk and Roelofs 1988). Further, foliar Ca decreases following N-deposition in red spruce forests resulted in decreased cold tolerance (DeHayes et al. 1999). Based largely on work by Ingestad (1979), which established "optimum foliar nutrient ratios" for pine and spruce trees, foliar nutrition analysis has been essential in identifying base cation deficiencies in several northern forest ecosystems (e.g., Tamm 1985; Brockley 2010a, 2010b). Ingestad's "optimal ratios" have since been tailored to British Columbian forests to account for local conditions (Ballard and Carter 1986); however, both of these ratios continue to be used in identifying base cation deficiencies in a range of forest types (e.g., Wang and Klinka 1997; Brockley 2010a, 2010b).

Accelerated base cation depletion following forest fertilization has emerged as a major forest management concern in recent decades (Fenn et al. 2006). The long-term sustainability of forest soils may be affected by increased losses of base cations, leading to nutrient deficiencies and detrimental soil acidification. As a result, several researchers have identified Ca as a major indicator of the soil response to elevated N-loading and soil acidification in forest ecosystems (DeHayes et al. 1999; Lawrence et al. 1999; Huntington 2000; Huntington et al. 2000). Calcium is an essential plant nutrient that is involved in a range of physiological processes, such as the growth and development of cell walls, carbohydrate metabolism, stomatal regulation, pathogen resistance and cold tolerance (DeHayes et al. 1999). Further, the fact that Ca provides a range of physiological services and that it is highly immobile within plants makes it a sensitive

indicator of base cation dynamics in forests subject to soil acidification (Attiwill and Weston 2001; Fenn et al. 2006).

Previous research in repeatedly fertilized pine and spruce forests in British Columbia has shown general decreases in soil pH following a range of different N-based fertilizer treatments (Berch et al. 2006; Brockley and Sanborn 2009). In some cases, shifts in pH have been accompanied by decreases in soil Ca (Brockley and Sanborn 2009), suggesting continued N-fertilization may negatively affect Ca nutrition in these stands and compromise long-term site productivity. To address these concerns, we studied base cation dynamics in immature interior spruce and lodgepole pine stands in the central interior of British Columbia subject to 13-15 years of repeated N-based fertilization. Our study was specifically concerned with the effects of long-term fertilization on forest Ca dynamics, as Mg and K were both included in fertilizer mixtures, while Ca was not. However, Ca, Mg and K dynamics were all studied to assess changes in base cation chemistry following repeated fertilization in both forest types.

MATERIALS AND METHODS

Site Descriptions

This study was carried out at the S1, S2, P1 and P2 interior spruce and lodgepole pine field installations in 2008 and 2009 (see Chapter 2 for complete description of field sites). The abbreviated site names allow for easy distinction between spruce (S1 & S2) and pine (P1 & P2) study sites. On occasion, site abbreviations are also accompanied by sampling years (e.g., S1 2008 = S1-08). Three treatments were studied at each site:

control, periodic and annual (see Chapter 2, Table 2.3 for description of fertilizer treatments and Tables 2.4 & 2.5 for detailed fertilization history).

Soil Nutrient Supply Rates

Soil base cation (Ca^{2+}, Mg^{2+} & K^{+}) supply rates were measured using ion exchange membrane (IEM) plant root simulator (PRS) probes (Western Ag Innovations Inc., Saskatoon, SK). Ion exchange membrane probes were also used to measure the supply rates of other soil elements to assess changes in soil chemistry. These additional measurements include soil supply rates of: NH_4^{+}, NO_3^{-}, SO_4^{2-} and Al^{3+}.

In each treatment plot, 4 IEM-probe pairs (cation + anion) were inserted vertically into the top 10 cm of the mineral soil. Probes were buried for two 6-week sampling intervals over the 2008 and 2009 growing season (see Chapter 4, Table 4.1 for sampling dates). Following the first 6-weeks of burial, all four probe pairs were collected from each plot and replaced with recharged probes in the same soil slots. After both the first and second 6-week sampling periods, all 4 probe pairs from each plot were combined to form one composite sample per plot. Following field collection, probes were immediately rinsed with pressurized de-ionized water to remove all residual soil, packaged on ice and sent overnight to Western Ag Innovations laboratory (Saskatoon, SK) for analysis (see chapter 3 for analysis details). Soil nutrient supply rates from the first and second samplings were combined and reported as $\mu g/10\ cm^2$/12-weeks.

At both spruce sites (S1 & S2), leaching potential was assessed by burying IEM-probes in the upper 10 cm of the mineral soil and at depth (20-25 cm) from the fall of 2008 (September) until the spring of 2009 (June). It was anticipated that overwinter

burials would assess the movement of nutrient ions down the soil profile during the spring snowmelt of 2009.

Mineral Soil Sampling

Four soil samples per plot were randomly collected at each site in June 2009. Samples were collected from two depths: 0-10 cm and 10-20 cm. All four samples at each depth were combined to form one composite sample at each depth in each plot (9 plots x 2 depths = 18 samples per site). Samples were kept on ice while in the field and wet sieved (<2 mm) immediately upon arrival at the lab.

Effective Cation Exchange Capacity (CECe) and Extractable Cations

Sieved (< 2 mm), air-dried sub-samples were extracted in a three-step process using a Centurion mechanical vacuum extractor as outlined by Kalra and Maynard (1991). Step one involved the displacement of exchangeable cations by saturating cation exchange sites with NH_4^+ using unbuffered 1.0 M NH_4Cl solution. Exchangeable cations (Ca, Mg, K, Na, Al, Fe and Mn) in the NH_4Cl leachate were determined by Leeman Prodigy Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Step two involved the removal of excess NH_4^+ from the soil sample with an ethanol wash procedure. The remaining NH_4^+ ions adsorbed on exchange sites following the ethanol wash was considered the effective cation exchange capacity. The final step involved the displacement of NH_4^+ by saturating cation exchange sites with Na^+ using

10% NaCl. Ammonium concentrations in the leachate solution were determined by Alpkem flow solution IV segmented flow auto-analyzer.

When comparing the CECe as determined by NH_4^+ displacement to the sum of exchangeable cations, the CECe was, on average, 38% lower. As a result, percent base saturation (%BS; exchangeable Na+K+Mg+Ca/CEC) values often exceeded 100%, which is inconceivable in acidic forest soils (pH 3-4.5). It was hypothesized that this overestimation of percent base saturation resulted from the initial 1.0 M NH_4Cl extraction, which may have leached substantial cations in solution in addition to cations associated with exchange sites. This would explain why the sum of exchangeable cations exceeded the CECe and how the %BS could exceed 100% in an acidic soil. Maynard and Fairbarns (1994) experienced similarly high %BS values in forest floor and mineral soils in Saskatchewan and Alberta and Van Cleve and Noonan (1971) found that a substantial portion (up to 91%) of the cations of a forest floor were leachable in distilled water. Therefore, CECe was reported as determined by NH_4^+ concentration following NaCl extraction. Cations were reported as extractable, rather than exchangeable, as it was assumed that the cations removed in the initial extraction included soluble and exchangeable pools. Extractable cations are reported with an "ex" subscript (e.g., extractable calcium = Ca_{ex}).

Acid Digests

Acid digests were performed on mineral soils to determine organically-bound elemental concentrations. Air-dried sub-samples were pulverized using a Rocklabs concentric ring grinder with tungsten carbide head. Ground samples were microwave

digested with concentrated nitric acid (70% HNO_3), concentrated hydrochloric acid (37% HCl) and hydrogen peroxide (30% H_2O_2; Kalra and Maynard 1991). Digests were analyzed using a Leeman Prodigy Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) to determine elemental concentrations of Al, B, Ca, Cu, Fe, K, Mg, Mn, P, S and Zn. It was assumed that all organic material was digested by acid oxidation (Kalra and Maynard 1991); thus, "digestible," as reported in this study, represents the organically-bound+exchangeable+soluble nutrient fraction of the soil. Digestible cations are reported with a "dig" subscript (e.g., digestible calcium = Ca_{dig}).

pH Determination

Sieved (<2 mm), air-dried sub-samples were weighed out at 10 g apiece. Each sample was then combined with 20 mL 0.01 M $CaCl_2$ to form a 1:2 ratio (soil:solution). Samples were agitated and left to equilibrate before inserting the combination electrode to determine soil pH (Kalra and Maynard 1991).

Foliar Sampling

Samples of the current year's foliage were collected from 10 healthy trees in each treatment plot in September 2008 and 2009. Foliar samples were collected from the lower portion of the top third of the live crown and combined to make 1 composite sample per plot. Composite samples were then ground using a coffee grinder and analyzed for total N, Ca, Mg, K. Foliar N was determined by dry combustion using a Fisons NA-1500 elemental analyzer (Brockley 2007). For foliar Ca, Mg and K determination, ground

foliage underwent closed-vessel microwave digestion using HNO_3-NCl acid and hydrogen peroxide (Kalra and Maynard 1991). The digest solutions were diluted with HCl and individual nutrients were determined by Leeman Prodigy Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Foliar data was generously provided by Rob Brockley (Research Scientist, BC Ministry of Forests and Range).

Data Analysis

Treatment effects at individual sites were assessed by analysis of variance (ANOVA) using the general linear model (GLM) procedure for the following variables: soil N, Ca, Mg, K, S and Al supply rates; extractable Ca, Mg and K; digestible Ca, Mg and K; foliar Ca, Mg and K; soil CECe; soil pH. Where significant differences were found, Tukey's HSD post-hoc test was used to test for significant differences between individual treatment means. Pearson's correlation analysis was used to assess relationships between soil base cations (IEM-probe and extractable Ca, Mg and K) and foliar base cation concentrations within the same year. Treatment effects on soil nutrient supply rates at the surface and at depth from overwinter burials at the spruce sites were tested individually (i.e., differences between surface and depth were not tested). Ion exchange membrane probes buried at depth were between 1.5 and 3 meters away from IEM-probes buried at the surface to ensure samples were independent. A level of significance of $\alpha = 0.05$ was used for inferring statistical significance throughout the study. Statistical analyses were completed using PASW 18 (SPSS Inc., 2009).

RESULTS

Effective Cation Exchange Capacity and Soil pH

Effective cation exchange capacity (CECe) was not significantly different between treatments at any of the sites in the top 10 cm of the mineral soil (Table 5.1). At depth (10-20 cm), CECe was significantly higher in the annual treatments compared to the controls at the pine sites (P1 & P2); however, there were no CECe differences between treatments at the 10-20 cm depth at the spruces sites. Soil pH (0-10 cm) was significantly lower in the annual treatment compared to the control at the S1 site (Table 5.1). At the 10-20 cm depth, soil pH was significantly lower in the annual treatments compared to the controls at the pine sites as well as between the periodic and control treatment at the P1 site. In addition, mean soil pH at both depths showed a general decrease in the fertilized plots compared to the controls at all sites (Table 5.1).

Table 5.1. 2009 mean (n = 3) soil effective cation exchange capacity (CECe) and pH. Means with different letters are significantly different ($p < 0.05$). Numbers in brackets indicate ± 1 SE.

			CECe (cmol+/kg)			**pH** (0.01 M $CaCl_2$)		
Species	**Site**	**Depth (cm)**	**Control**	**Periodic**	**Annual**	**Control**	**Periodic**	**Annual**
Spruce	S1	0-10	5.43 (0.90)	4.94 (0.79)	5.67 (1.13)	3.94**a** (0.12)	3.65**ab** (0.19)	3.53**b** (0.16)
		10-20	3.60 (0.55)	3.81 (0.43)	4.80 (0.67)	4.27 (0.08)	3.85 (0.16)	3.97 (0.02)
	S2	0-10	10.24 (0.89)	9.90 (0.40)	13.14 (0.89)	4.39 (0.03)	4.32 (0.09)	4.38 (0.19)
		10-20	8.50 (0.90)	8.70 (0.51)	9.56 (0.33)	4.67 (0.12)	4.64 (0.06)	4.54 (0.11)
Pine	P1	0-10	9.08 (0.02)	9.24 (0.37)	9.70 (0.18)	3.38 (0.08)	3.26 (0.02)	3.32 (0.04)
		10-20	4.29**b** (0.49)	6.17**ab** (1.05)	6.91**a** (0.14)	4.48**a** (0.12)	4.02**b** (0.04)	3.82**b** (0.05)
	P2	0-10	7.37 (0.23)	7.92 (0.38)	8.36 (0.49)	4.59 (0.08)	4.54 (0.13)	4.30 (0.07)
		10-20	6.82**b** (0.20)	7.19**ab** (0.36)	8.25**a** (0.18)	4.66**a** (0.08)	4.57**ab** (0.08)	4.53**b** (0.08)

Soil Cation Supply Rates

Calcium supply rates were not significantly affected by fertilization at any of the sites in 2008 or 2009 (Figure 5.1). Magnesium supply rates were significantly higher in the annual treatments compared to the controls at the S2 site in 2008 and at both pine sites (P1 & P2) in 2008 and 2009 (Figure 5.2). Following periodic fertilization, Mg supply rates were significantly higher than the controls at the P1 site in 2008 and 2009. Potassium supply rates were significantly elevated compared to the controls following annual fertilization in 2008 and 2009 in all but two cases (not S2-08 or P1-09; Figure 5.3). Periodic fertilization significantly increased K supply rates over the controls at 3 of the 4 sites in 2008 (not S2) though only at S1 in 2009. While differences in Mg and K supply rates between fertilized and unfertilized soils were not always significant, mean Mg and K supply rates were elevated compared to the controls at all sites in 2008 and 2009 (Figures 5.2 & 5.3). In addition to changes in base cation supply rates, Al supply rates were significantly higher in the annual treatment compared to the control at the P1 site in 2008 and 2009 (data not shown). Further, mean Al supply rates were higher in the fertilized plots compared to the controls in all but one case (P2-08).

Ion exchange membrane probes buried over the 2008-09 winter in the spruce soils showed no significant differences in K and Mg in upper 10 cm of mineral soil; however, at the S1 site, soil Ca in the upper 10 cm was significantly lower in the annual treatment compared to the control (data not shown). At depth (20-25 cm), Ca, Mg and K were not significantly different by treatment, nor were any patterns evident (data not shown). Nitrate and NH_4^+ supply rates were not significantly affected by any of the treatments in

the surface or at depth following the overwinter burial (data not shown); however, at depth, SO_4^{2-} was significantly higher in both fertilizer treatments compared to the control at S1 and significantly higher in the annual compared to the control at S2 (data not shown), suggesting SO_4^{2-} ions may have leached down the soil profile during the spring snowmelt of 2009.

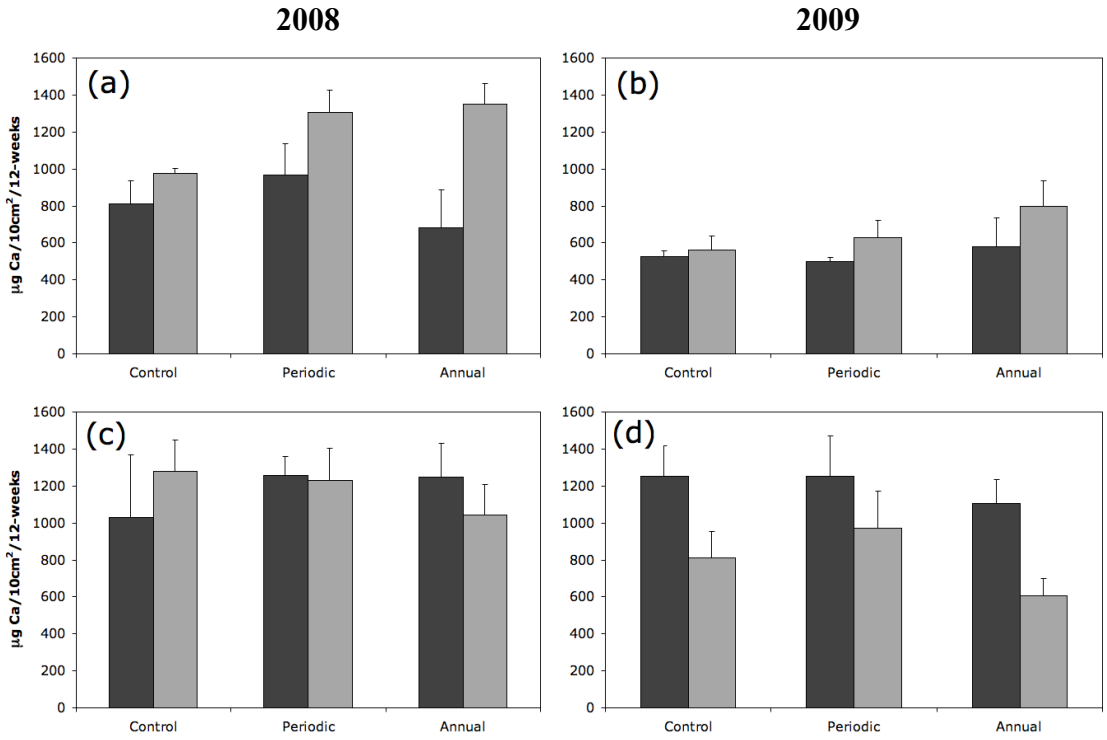

Figure 5.1. 2008 (a & c) and 2009 (b & d) spruce and pine calcium supply rates. Legend: spruce (a & b) dark grey = S1, light grey = S2; pine (c & d) dark grey = P1, light grey = P2. Error bars indicate ±1 SE.

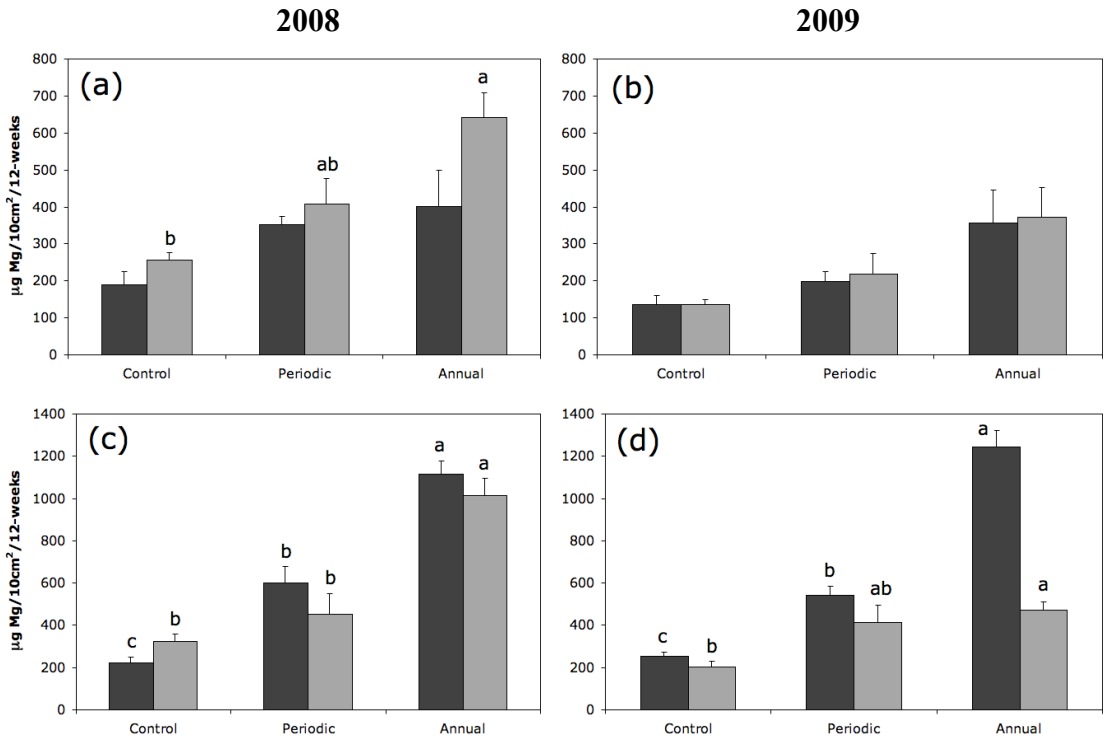

Figure 5.2. 2008 (a & c) and 2009 (b & d) spruce and pine magnesium supply rates. Legend: spruce (a & b) dark grey = S1, light grey = S2; pine (c & d) dark grey = P1, light grey = P2. Different letters above bars of the same site indicate significant differences ($p < 0.05$) between treatment means. Error bars indicate ±1 SE.

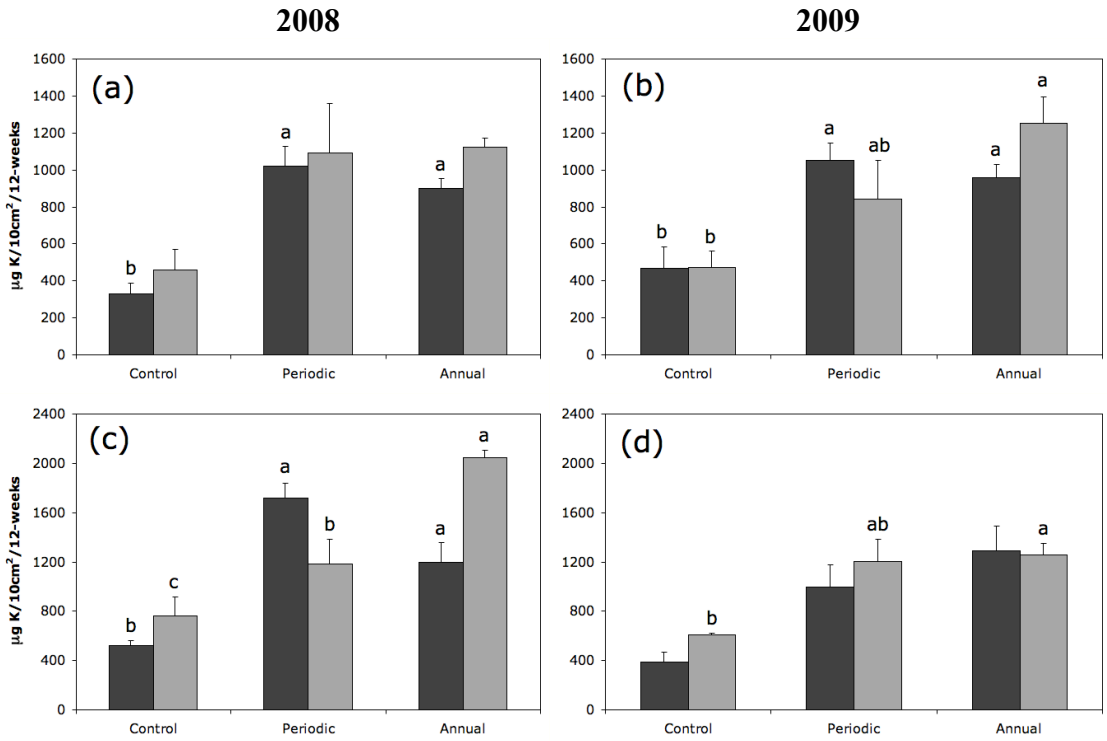

Figure 5.3. 2008 (a & c) and 2009 (b & d) spruce and pine potassium supply rates. Legend: spruce (a & b) dark grey = S1, light grey = S2; pine (c & d) dark grey = P1, light grey = P2. Different letters above bars of the same site indicate significant differences ($p < 0.05$) between treatment means. Error bars indicate ±1 SE.

Extractable cations

Extractable Ca (Ca_{ex}) in the upper soil (0-10 cm) was significantly lower in the annual treatment compared to the control at the S1 site (Table 5.2); mean Ca_{ex} at the pine sites also decreased in the fertilized plots, though differences were not significant. At the 10-20 cm depth, mean Ca_{ex} showed a general decreasing trend with fertilization at the S1, S2 (periodic only) and P1 sites (Table 5.2); however, none of these decreases were significantly different than the controls. Annual fertilization significantly increased Mg_{ex} in the upper soil (0-10 cm) at the P1 site and at depth (10-20 cm) at the P1 and P2 sites (Table 5.2). Periodic fertilization did not significantly affect Mg_{ex} at any of the sites; however, mean Mg_{ex} was higher in the fertilized treatments than the controls at all sites

and depths. Annual fertilization significantly increased K_{ex} compared to the controls in the upper 10 cm of the mineral soil at all sites (Table 5.2). At the 10-20 cm depth, annual fertilization significantly increased K_{ex} compared to the control at the S1 site. Periodic fertilization did not significantly affect K_{ex} at any of the sites or depths; however, mean K_{ex} was elevated in the fertilized treatments compared to the controls at all sites and depths.

Table 5.2. 2009 mean (n = 3) extractable and digestible Ca^{2+}, Mg^{2+} and K^{+} concentrations by depth. Different letters for means of the same variable indicate significant differences ($p < 0.05$). Numbers in brackets indicate ± 1 SE.

				Extractable (mg/kg)			**Digestible** (mg/kg)		
Species	**Site**	**Cation**	**Depth** (cm)	**Control**	**Periodic**	**Annual**	**Control**	**Periodic**	**Annual**
Spruce	**S1**	Ca^{2+}	0-10	609.14**a** (127.86)	317.86**ab** (153.57)	290.44**b** (94.64)	2991.48 (188.11)	2339.63 (802.75)	2202.83 (352.41)
			10-20	290.96 (138.35)	87.80 (19.27)	189.07 (21.71)	2845.89 (319.01)	2271.48 (704.62)	2223.74 (317.85)
		Mg^{2+}	0-10	80.35 (11.85)	64.62 (20.43)	93.77 (8.44)	2473.76 (661.38)	2180.18 (772.97)	2468.54 (1251.63)
			10-20	50.82 (16.19)	27.13 (7.06)	64.71 (6.26)	5292.08 (1163.23)	3388.12 (821.70)	3544.32 (381.83)
		K^{+}	0-10	68.17**b** (7.97)	93.32**ab** (18.77)	120.21**a** (0.44)	2977.94 (555.32)	2623.36 (436.94)	3733.86 (1314.64)
			10-20	55.15**b** (4.69)	59.42**b** (2.42)	88.10**a** (6.89)	3222.30**a** (744.31)	3784.82**a** (274.77)	1670.90**b** (317.41)
	S2	Ca^{2+}	0-10	1114.93 (243.52)	1174.59 (275.51)	1851.95 (431.76)	4896.81 (369.94)	3955.05 (498.83)	5373.64 (809.11)
			10-20	1192.46 (164.53)	1138.59 (139.20)	1238.73 (141.84)	4828.24 (349.13)	5067.77 (360.91)	4540.98 (229.97)
		Mg^{2+}	0-10	227.74 (59.83)	242.43 (73.42)	438.37 (9.67)	5865.82**ab** (321.18)	4505.78**b** (288.07)	6011.87**a** (396.20)
			10-20	272.80 (50.00)	257.02 (51.73)	290.38 (24.21)	6487.44 (537.74)	5969.17 (234.93)	6126.06 (192.87)
		K^{+}	0-10	234.31**b** (20.21)	313.25**ab** (14.25)	465.75**a** (81.82)	2445.47 (213.55)	1836.26 (201.14)	2711.08 (507.99)
			10-20	215.48 (28.34)	249.48 (18.71)	341.29 (75.42)	1943.49 (296.79)	2521.29 (128.71)	1812.79 320.47
Pine	**P1**	Ca^{2+}	0-10	462.88 (80.94)	297.45 (54.35)	255.84 (45.26)	1052.15 (45.56)	1176.03 (278.71)	1287.42 (128.51)
			10-20	130.62 (16.09)	91.62 (32.42)	123.07 (47.93)	1215.10 (199.32)	1079.40 (79.59)	976.46 (165.00)
		Mg^{2+}	0-10	52.90**b** (3.17)	77.37**b** (8.16)	192.75**a** (16.08)	1171.31 (91.01)	1520.15 (196.06)	1827.01 (139.84)
			10-20	15.67**b** (1.41)	21.47**b** (5.05)	96.63**a** (14.03)	4797.89 (35.52)	4485.65 (648.96)	4363.71 (159.89)
		K^{+}	0-10	76.25**b** (2.37)	141.28**ab** (19.27)	213.64**a** (43.75)	1508.10 (765.38)	2099.86 (803.39)	3380.50 (772.74)
			10-20	67.54 (3.16)	102.96 (14.92)	144.60 (17.72)	2861.94 (910.66)	2846.73 (737.84)	2492.00 (971.52)
	P2	Ca^{2+}	0-10	912.65 (71.00)	907.62 (121.28)	637.75 (50.04)	3693.53 (357.80)	2787.04 (557.58)	2701.91 (455.30)
			10-20	1061.10 (54.21)	1124.17 (73.14)	1074.96 (50.11)	3617.98 (195.12)	2843.90 (359.89)	3202.11 (760.86)
		Mg^{2+}	0-10	147.93 (24.91)	204.85 (47.78)	345.02 (77.15)	3567.58 (283.26)	2646.43 (571.86)	3340.56 (560.54)
			10-20	166.54**b** (24.59)	227.81**ab** (44.36)	315.75**a** (31.08)	3778.81 (275.47)	3387.82 (876.27)	3778.21 (1128.93)
		K^{+}	0-10	265.15**b** (5.50)	392.81**ab** (23.47)	556.70**a** (69.41)	2670.39 (474.51)	2094.91 (38.71)	2563.08 (37.32)
			10-20	285.57 (12.10)	342.95 (51.88)	490.11 (88.79)	2376.54 (322.44)	1795.88 (332.08)	2253.55 (252.76)

Extractable cation concentrations were also assessed as a percentage of total extractable cations on a charge basis (cmol+/kg) to examine treatment-induced changes in the extractable cation pool (Figures 5.4 & 5.5). Extractable Ca at the 0-10 cm depth at the S1 site (Figure 5.4a) decreased from 42% of the extractable cation pool in the control treatment to 25% and 21% in the periodic and annual treatments. This shift was accompanied by an increase in Al from 42% to 58% and 60% in the control, periodic and annual treatments, suggesting that increases in soil Al concentrations were largely related to decreases in soil Ca availability. Similarly, at the P1 site (Figure 5.4c), Al dominated soil (0-10 cm) cation chemistry, representing 74%, 78% and 73% (control, periodic, annual) of the extractable pool; however, unlike S1, Ca decreases (from 18% to 12% to 9%; control, periodic, annual) were accompanied by increases in Mg and K rather than Al. Conversely, at the S2 and P2 sites, cation pools were dominated by Ca, Mg and K, with Al never exceeding 34% of the extractable cation pool (Figure 5.4b & d). In fact, at S2, the percent of soil (0-10 cm) Ca, Mg and K all increased with fertilization and Al decreased from 34% to 30% to 21% in the control, periodic and annual treatments. At the P2 site, soil (0-10cm) Ca concentrations decreased from 49% in the control to 47% and 27% in the periodic and annual treatments; these decreases were accompanied concomitant increases in Mg, K and Al.

Extractable cation pools at the 10-20 cm depth followed similar patterns as the upper mineral soil (Figure 5.5); however, at the S1 and P1 sites, shifts in the extractable cation pool were more pronounced, whereas at the S2 and P2 sites, cation pools were

largely unchanged. For example, at the S1 and P1 sites (Figure 5.5a & c), soil Al in the fertilized plots occupied a greater percentage of the 10-20 cm cation pool than the overlying horizon, with decreases in soil Ca accompanying these shifts. At the S2 and P2 sites, shifts in the soil (10-20 cm) extractable cation pool were minimal, with minor (<10%) decreases in Ca availability in the fertilized plots associated with increases in K and Mg. Thus, at depth (10-20 cm), the S1 and P1 cation pools appeared to be more affected by fertilization than the S2 and P2 sites.

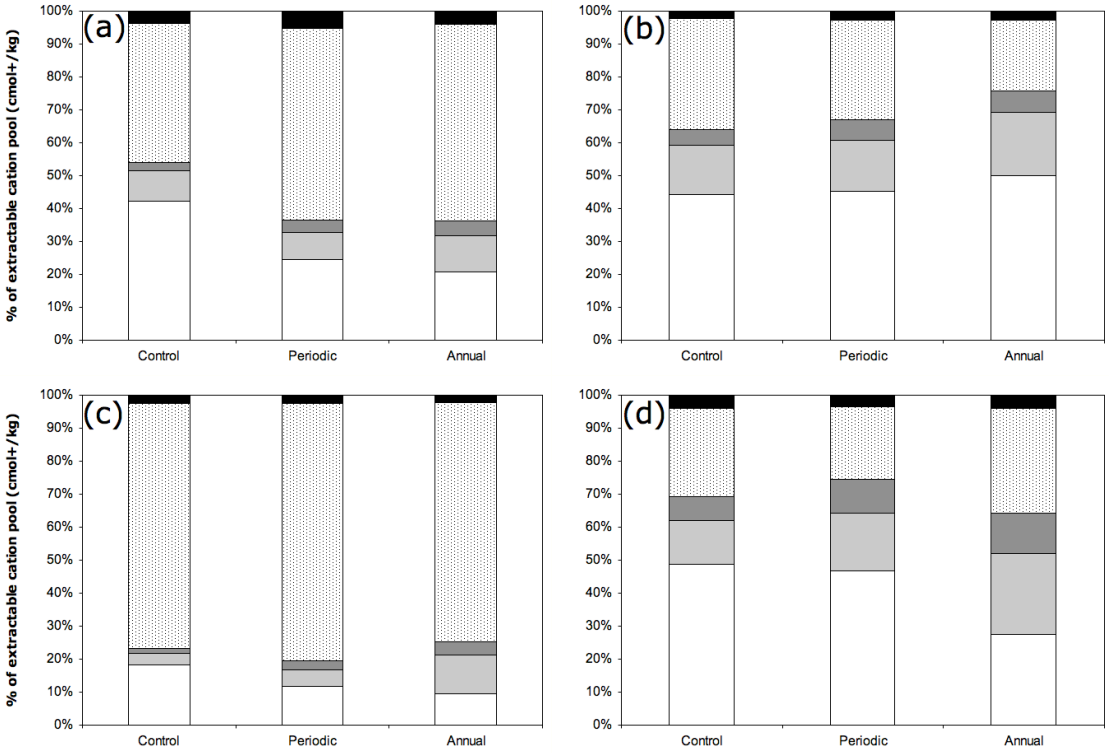

Figure 5.4. 2009 percent of soil 0-10cm extractable cation pool occupied by individual cations. Cation legend: white = Ca, light grey = Mg, dark grey = K, white with black dots = Al, black = Na+Fe+Mn. Site legend: a = S1, b = S2, c = P1, d = P2.

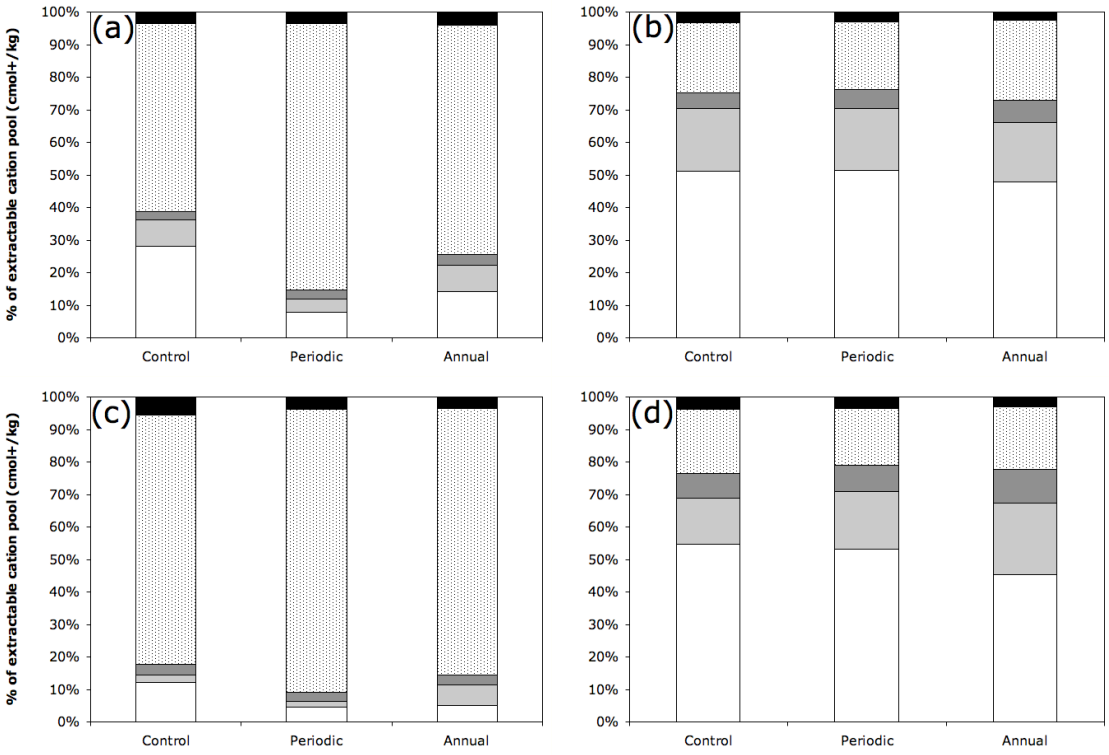

Figure 5.5. 2009 percent of soil 10-20cm extractable cation pool occupied by individual cations. Legend: white = Ca, light grey = Mg, dark grey = K, white with black dots = Al, black = Na+Fe+Mn. Site legend: a = S1, b = S2, c = P1, d = P2.

Digestible Base Cations

Digestible Ca, Mg and K in the soil 0-10 cm depth of the fertilized plots did not significantly differ from the controls at any site (Table 5.2). At the 10-20 cm depth, K_{dig} in the annual treatment was significantly lower than the control and periodic treatments at the S1 site; however, there were no other differences in Ca_{dig}, Mg_{dig} or K_{dig} at the 10-20 cm depth at any of the sites. Despite the lack of significant treatment effects, mean Ca_{dig} decreased in the fertilized plots compared to the controls at both soil depths at the S1 and P2 sites and at the 10-20 cm depth at the P1 site (Table 5.2).

Foliar Cations

Foliar Ca in the fall of 2008 was significantly lower in the annual treatments compared to the controls at the S1 and P1 sites (Table 5.3). In 2009, foliar Ca was significantly lower in the annual treatment compared to the control at the S1 site; however, mean foliar Ca at the P1 site showed a decreasing trend. Foliar Ca was unaffected by fertilization at the S2 and P2 sites. Significant differences in foliar Mg and K were only evident at the S2 site, with Mg elevated in the annual treatment over the control in 2008 and 2009 and higher foliar K in the annual treatment compared to the control in 2009. Mean foliar Mg and K showed a general increasing trend with fertilization at all sites (Table 5.3). Correlations between soil base cations (IEM-probe and extractable Ca, Mg and K) and foliar base cations did not reveal any significant relationships.

Table 5.3. 2008 and 2009 mean (n = 3) foliar Ca^{2+}, Mg^{2+} and K^{+} concentrations (%). Means with different letters are significantly different ($p < 0.05$). Numbers in brackets indicate ± 1 SE. NA = data not available.

			2008			2009		
Species	**Site**	**Cation**	**Control**	**Periodic**	**Annual**	**Control**	**Periodic**	**Annual**
Spruce	S1	Ca^{2+}	0.42**a**	0.40**a**	0.31**b**	0.35**a**	0.33**ab**	0.26**b**
			(0.04)	(0.03)	(0.04)	(0.01)	(0.02)	(0.03)
		Mg^{2+}	0.11	0.12	0.11	0.11	0.12	0.12
			(0.00)	(0.01)	(0.01)	(0.00)	(0.00)	(0.00)
		K^{+}	0.78	0.80	0.88	0.75	0.85	0.90
			(0.06)	(0.03)	(0.05)	(0.05)	(0.02)	(0.06)
	S2	Ca^{2+}	0.46	NA	0.45	0.36	0.41	0.42
			(0.01)		(0.02)	(0.01)	(0.03)	(0.01)
		Mg^{2+}	0.11**b**	NA	0.12**a**	0.11**b**	0.12**ab**	0.12**a**
			(0.00)		(0.00)	(0.00)	(0.00)	(0.01)
		K^{+}	0.68	NA	0.73	0.66**b**	0.80**a**	0.78**a**
			(0.03)		(0.00)	(0.01)	(0.03)	(0.02)
Pine	P1	Ca^{2+}	0.14**a**	0.13**ab**	0.11**b**	0.13	0.12	0.11
			(0.00)	(0.00)	(0.01)	(0.01)	(0.01)	(0.00)
		Mg^{2+}	0.10	0.11	0.10	0.10	0.11	0.11
			(0.00)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)
		K^{+}	0.56	0.54	0.58	0.52	0.53	0.57
			(0.01)	(0.02)	(0.01)	(0.01)	(0.04)	(0.02)
	P2	Ca^{2+}	0.16	0.18	0.17	0.13	0.14	0.13
			(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
		Mg^{2+}	0.10	0.10	0.11	0.10	0.10	0.11
			(0.01)	(0.01)	(0.01)	(0.01)	(0.00)	(0.01)
		K^{+}	0.51	0.50	0.54	0.52	0.52	0.53
			(0.01)	(0.03)	(0.02)	(0.03)	(0.02)	(0.02)

DISCUSSION

Soil Buffering Capacities

Repeated fertilization significantly affected base cation dynamics at these sites in several ways; however, responses were largely site specific and related to differences in the buffering capacities of the different soils. The buffering capacity of a soil refers to its ability to resist rapid changes in pH through reactions that either consume or produce H^{+} ions (Brady and Weil 2002). Sites with large cation exchange capacities (CECs) and considerable reserves of base cations, in general, are more capable of resisting the detrimental effects of soil acidification than poorly buffered soils with small CECs and

low base cation concentrations. Poorly buffered soils are especially susceptible to soil acidification and base cation depletion in high-N environments as acid-neutralizing mechanisms can be easily overwhelmed (Fenn et al. 2006).

In this study, the S1 and P1 sites were rather poorly buffered in comparison to the S2 and P2 sites (Table 5.1). For example, soil (0-10 cm) pH was < 4 at the S1 & P1 sites, whereas it was > 4 at the S2 and P2 sites. Further, CECe at the S2 site was approximately twice that of the S1 site, further highlighting the differing buffering capacities at the spruce sites. At the pine sites, CECe was not substantially different between sites; however, soil (0-10 cm) pH was approximately 1 unit lower at the P1 site than the P2 site, suggesting varying buffering capacities despite similarities in exchange properties. The most striking illustration of the buffering capacities of individual soils can be seen in Figures 5.4 and 5.5, which describe the extractable cation pools in terms of the percent charge occupied by individual cations. In the less buffered soils, S1 and P1 (Figures 5.4a & c; 5.5a & c), the extractable cation pool is dominated by Al, whereas the more buffered soils, S2 and P2 (Figures 5.4b & d; 5.5b & d), are dominated by Ca, Mg and K. Further, extractable and digestible cation concentrations (Table 5.2), especially for Ca, were generally higher at the S2 and P2 sites, thereby increasing the ability of these soils to counteract acidifying processes. Buffering capacities at the different sites were strongly related to differing responses to the fertilizer treatments; therefore, much of the discussion will consider individual site responses with regard to soil buffering capacities.

Soil Mg and K dynamics

Soil base cation supply rates during the growing season were highly responsive to nutrients included in the fertilizer treatments (i.e., Mg and K), with significantly elevated Mg and K supply rates in the fertilized plots in several cases (Figures 5.2 & 5.3) and generally greater mean Mg and K supply rates in the fertilized plots compared to the controls. Similarly, foliar Mg and K concentrations generally increased in the fertilized plots compared to the controls, with significant increases in several cases (Table 5.2), suggesting fertilization was very effective at elevating soil Mg and K supply rates to meet the nutritional requirements of faster growing trees (for growth responses, see Brockley and Simpson 2004; Brockley 2010a, 2010b; and Chapter 4, Table 4.4 for tree carbon responses). Extractable Mg and K responses were generally positive following fertilization; however, trends in digestible Mg and K were less clear. Thus, it appears that fertilization was most effective at stimulating labile Mg and K pools (soluble and extractable), with fertilizer inputs showing only minor effects on organically-bound nutrients.

Soil Ca dynamics

Soil Ca supply rates did not significantly differ by treatment; however, mean Ca did decrease in the annual treatments compared to the controls at S1-08, P2-08, P1-09 and P2-09, suggesting annual fertilization may be affecting soluble Ca at these sites. There was more evidence of fertilizer effects on soil Ca in the extractable soil pool, with significantly lower Ca_{ex} in the annual treatment compared to the control at the S1 site, and a general decreasing trend in Ca_{ex} with fertilization at the pine sites (Table 5.2). At

the S2 site, the most highly buffered site in this study, mean Ca_{ex} increased with fertilization; however, in treatment plots subject to higher levels of annual fertilization (see Brockley and Sanborn 2009, ON2 treatment), Ca_{ex} significantly decreased compared to the control after 12 years of treatment. This suggests that nutrient additions at levels used in this study were not high enough to affect Ca_{ex} at the S2 site, though at higher rates there was evidence of Ca_{ex} depletion. At the less buffered sites (S1 & P1), the annual treatment did exhibit signs of Ca_{ex} depletion, indicating a greater sensitivity to lower rates of nutrient inputs than the S2 site. Extractable cation pools (Figures 5.4 & 5.5) further illustrate a general decrease in Ca as a percent of the total cation pool at 3 of the 4 sites (not S2). Shifts in the extractable cation pool varied greatly between sites, with increases in Mg and K largely driving Ca decreases at the P2 site, whereas Al appeared to be the major contributor to Ca decline at the S1 and P1 sites.

Foliar Ca concentrations exhibited similar trends as soil Ca pools, with the poorly buffered sites (S1 & P1) showing signs of Ca decline in the fertilized plots, while the S2 and P2 sites were largely unaffected. For example, foliar Ca concentrations at the S1 and P1 sites in 2008 and the S1 site in 2009 were significantly lower in the annual treatments compared to the controls (Table 5.3). Conversely, foliar Ca concentrations at the S2 and P2 sites did not differ by treatment. While foliar Ca levels at the S1 and P1 sites both exhibited significant decreases in plots receiving annual fertilization, foliar Ca levels approached but did not cross provincial foliar Ca concentration thresholds (spruce %Ca = 0.20, pine %Ca = 0.10; Ballard and Carter 1986). Similarly, foliar N:Ca ratios at both sites approached N:Ca thresholds (spruce N:Ca = 7.75, pine N:Ca = 15.5; Ballard and Carter 1986), with foliar N:Ca at the S1 site increasing from 2.9 to 3.8 to 4.6 in the

control, periodic and annual treatments in 2008 and from 3.6 to 4.2 to 5.3 in 2009. At the P1 site, foliar N:Ca ratios increased from 8.7 to 11.3 to 12.3 in the control, periodic and annual treatments in 2008 and from 10.4 to 14.7 to 14.4 in 2009. Thus, at both the S1 and P1 sites, foliar Ca did not keep pace with foliar N increases in the fertilized plots (see Chapter 4, Table 4.2 for foliar N concentrations), suggesting soil Ca was unable to meet the increased nutritional demands of the fertilized trees, or that Ca uptake from soil pools was somehow affected by the fertilizer treatments.

Fertilization effects on Ca uptake

Fertilization has been shown to negatively affect root uptake of base cations (deWit et al. 2010), largely as a result of decreases in soil pH and increased mobilization of Al (Van Breemen et al. 1982; McLaughlin and Wimmer 1999; Kinraide 2003). As pH decreases following fertilization (as was the general trend in this study) monomeric Al (Al^{3+}) becomes increasingly soluble and can accumulate on root cell walls, thereby blocking the uptake of other cations (Sverdrup et al. 1992; Godbold and Jentschke 1998). Soil Al supply rates were significantly higher in the annual treatment compared to the control at the P1 site in 2008 and 2009, and mean Al generally increased in the fertilized plots at all sites (data not shown). This trend of increasing soil Al was further illustrated in the extractable cation pools (Figures 5.4 & 5.5), which suggest that Al is generally increasing as a percentage of the extractable pool at the S1, P1 and P2 sites. At a pH of 4, more that 90% of ionic Al is in Al^{3+} form, with minimal hydrox-Al species, $Al(OH)^{2+}$ and $Al(OH)_2^{+}$, present in solution (Ross et al. 2008). Thus, at the S1 and P1 sites, increased

soil Al^{3+} may be accumulating on root cell walls, thereby blocking the uptake of soil Ca, resulting in lower foliar Ca concentrations.

Extensive research on the mechanisms and effects of forest acidification has focused on the role of Al in soil solution. Based on a literature review of dozens of forest acidification experiments, Cronan and Grigal (1995) proposed the Ca:Al molar ratio in soil solution as an indicator for assessing detrimental forest acidification. Below a Ca:Al molar ratio of 1, the authors suggest there is a significant risk of forest damage from Al stress and nutrient imbalances. Despite significant decreases in soil and foliar Ca at the S1 and P1 sites, Ca:Al molar ratios (calculated from IEM-probe supply rates) did not exceed threshold levels. In fact, at all sites, Ca:Al molar ratios were well above 1; ratios ranged between 5-11.5 in the control plots and between 3.5-12.4 in the fertilized plots. At the S1, P1 and P2 sites, Ca:Al molar ratios decreased with fertilization, with the lowest Ca:Al molar ratios at the S1 and P1 sites (data not shown). Therefore, the poorly buffered sites may be at risk of approaching critical Ca:Al thresholds with continued fertilization, potentially affecting forest productivity. While there is certainly indication that increases in soluble Al may be affecting Ca uptake at the poorly buffered sites (reduced foliar Ca at S1 and P1), there is no indication of forest decline at any of the sites in this study (Brockley and Simpson 2004; Brockley 2010a, 2010b; Chapter 4, Table 4.4), suggesting the Ca:Al molar ratio of 1 may be an appropriate threshold for assessing the negative effects of soluble Al on forest productivity at these sites.

Factors contributing to soil acidification and Ca depletion

Soil acidification and the potential antagonistic effects of soluble Al on root Ca uptake provide a reasonable explanation for declining foliar Ca concentrations in the fertilized plots at the S1 and P1 sites; however, it is less clear which mechanisms are responsible for the general decreasing trend in soil pH and how these mechanisms may be related to decreases in extractable and digestible soil Ca. Increased rates of nitrification following N-based fertilization is considered a major contributing factor to soil acidification following fertilization (Johnson and Todd 1988, Aber et al. 1989, 1998; McNulty and Aber 1993; Binkley and Högberg 1997). At all 4 sites, NO_3^- production in the fertilized plots was generally higher than the controls (see Chapter 4, Figures 4.1 & 4.2), suggesting hydrogen (H^+) ions produced during increased nitrification may have contributed to reductions in soil pH in the fertilized treatments. Aside from the direct pH effects of increased H^+ ion production, nitrification can lead to accelerated NO_3^- and base cation leaching (Aber 1992). However, NO_3^- losses only occur when NO_3^- production exceeds the demands (biotic and abiotic) of the ecosystem. Total N pool increases in the fertilized plots suggest that the majority of N inputs were retained in the forest floor and top 20 cm of the mineral soil (see Chapter 4, Table 4.4). Further, IEM-probes buried at depth (20-25 cm) in the spruce soils did not reveal any differences in NO_3^- supply rates between treatments following the spring snowmelt of 2009 (data not shown). Therefore, there was little indication of significant NO_3^- leaching in this study, especially at the spruce sites.

Sulfur is another major component of the fertilizer treatments that may have affected soil pH and Ca depletion at these sites. In fact, S fertilizers have been widely

used to reduce soil pH for experimental purposes (Tamm and Popovic 1995; Fisher and Binkley 2000), suggesting S inputs may have contributed to increased soil acidification at these sites. Further, mean soil SO_4^{2-} supply rates were elevated in the fertilized plots compared to the controls at all sites (significantly higher in the annual compared to the control at P1-08, P2-08, P1-09; data not shown), suggesting S-inputs increased S cycling in these soils. In fact, IEM-probe burials at depth (20-25 cm) in the spruce soils (S1 & S2) found significantly higher SO_4^{2-} levels in the annual treatments compared to the controls following the spring snowmelt of 2009 (data not shown). Soil S levels in the interior of British Columbia are considered extremely low for temperate and boreal forests (Kishchuk and Brockley 2002; Sanborn et al. 2005); therefore, elevated S cycling may have exceeded the ability of the ecosystem to retain the added S, resulting in leaching losses. Thus, significant increases in SO_4^{2-} leaching following spring snowmelt may have been one possible loss pathway for Ca_{ex} at the S1 site (Ryan et al. 1989; Johnson et al. 1993).

In addition to N and S effects on soil pH, increases in soil organic matter can contribute to soil acidity through the addition of organic acids (Skyllberg 1995). Soil C generally increased with fertilization at all sites in this study (see Chapter 4, Table 4.4 for soil C data), suggesting increases in soil C stocks may have contributed to soil acidification. Further, dissolved organic C can form soluble complexes with base cations and leach them from soil solution (Brady and Weil 2002); thus, soil C at the S1 site may have leached beyond the 10-20 cm sampling depth, thereby removing Ca_{ex} in the process. While the data in this study does not allow for adequate assessment of this Ca loss pathway, the soil C data available does not contradict it either.

Implications of Ca depletion

The S1 and P1 sites exhibited the most apparent signs of Ca depletion, with significant decreases in soil (Ca_{ex}, S1 only) and foliar Ca between the annual and control treatments. Further, fertilization generally decreased soil pH, Ca_{ex} and Ca_{dig} at both of these sites. There was little evidence of Ca depletion at the more highly buffered sites (S2 & P2), suggesting a greater ability to counteract fertilizer-induced leaching and acidification. However, there is evidence that high rates of fertilization can lead to soil Ca_{ex} depletion at even the most highly buffered site (S2; Brockley and Sanborn 2009), suggesting that all sites in this study may be susceptible to Ca depletion with continued N-inputs. Forest Ca depletion has been shown to negatively affect forest productivity in several forest types (Van Dijk and Roelofs 1988; Eklund and Eliasson 1990; DeHayes et al. 1999; McLaughlin and Wimmer 1999); therefore, any indication of fertilizer-induced Ca depletion should be seriously considered.

The weathering of primary minerals is the major Ca source in most forest soils (Schlesinger 1997). Weatherable minerals such as feldspars are abundant in soils of the interior of British Columbia, thereby providing a large potential source of slowly available Ca (Arocena and Sanborn 1999). However, evidence of soil Ca depletion at the poorly buffered sites suggests weathering is not re-supplying Ca at the same rate it is being depleted. Trees are unable to recycle Ca from senescing foliage (Van den Driessche 1985); therefore, significant changes in soil Ca can rapidly affect tree nutrition. Applications of lime have been shown to alleviate Ca losses induced by elevated N-inputs (Derome 1990; Misson et al. 2001). Should repeated fertilization continue at these

sites, lime application may be a necessary intervention strategy to re-supply depleted soil Ca reserves to ensure long-term site productivity.

CONCLUSION

Following 13-15 years of repeated N-based fertilization, soil base cation dynamics were significantly affected at all sites. Magnesium and K included in the fertilizer treatments effectively increased Mg and K cycling above control levels in several cases. Conversely, Ca, which was not included in the fertilizer treatments, responded negatively to fertilization in several cases at the poorly buffered sites. Buffering capacities, rather than forest type, appeared to be the best predictor of Ca response, with highly buffered sites showing very few signs of Ca depletion. At the poorly buffered sites, fertilization led to decreases in soil pH, extractable soil Ca and foliar Ca. Losses of exchangeable Ca may have been related to increased SO_4^{2-} leaching; however, there was little evidence of NO_3^- leaching at any of these sites. Decreases in foliar Ca were presumably related to compromised uptake systems (potentially from increased Al), as Ca supply rates in soil solution were not affected by fertilization.

Despite some indication of increased Ca depletion at the poorly buffered sites, it does not appear that current fertilization rates are leading to Ca loss or pH decreases that are detrimentally affecting tree growth. However, any evidence of Ca decline should be taken seriously, with possible intervention methods (e.g., liming) to counteract Ca depletion should long-term fertilization continue. As forest fertilization programs increase in response to forest disturbances and increased demand for wood products (Cown 2007), Ca depletion has emerged as a major concern in several forest ecosystems

(Fenn et al. 2006). Studies such as this should serve as an early warning of the effects of long-term N-fertilization on soil Ca dynamics in poorly buffered forest soils.

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CHAPTER 6 – General Conclusions

British Columbia contains Canada's largest forestry sector. While considerable swaths of BC's mature forests have fallen victim to the mountain pine beetle, the landscape of the BC interior is rapidly being redefined by healthy, immature stands dominated by lodgepole pine and interior spruce. These forests represent the majority of the forests that will be harvested in the next 60 years (Rob Brockley personal communication, 2010). Management decisions being made in response to the pine beetle epidemic will have a lasting effect on Canada's forests for generations to come. Intensive fertilization has been identified as one of the key approaches to addressing timber shortages in the face of the mountain pine beetle epidemic; however, it is essential to understand the risks of such silvicultural practices to ensure long-term site productivity is not jeopardized.

Maintaining soil productivity is central to sustainable forest management (Nambiar 1996). The availability of nutrients in soil solution largely determines the growth and vigor of forests (Smethurst et al., 2001); therefore, changes in soil chemistry can directly affect soil productivity. Accurate measurements of soil chemical properties are essential to understanding the impacts of forest management practices on soil productivity. Chapter 3 compared traditional soil extraction assays with ion-exchange membrane (IEM) probe measurements to assess the utility of each method in forest fertilization research. Findings from Chapter 3 suggest both assays provide unique opportunities for assessing N-mineralization processes in forest soils; however, IEM probes appear especially promising for improving soil N-mineralization estimates.

Further, IEM probes offer a simple, affordable and reliable alternative to traditional indicators of soil chemistry.

Findings from Chapters 4 and 5 of this project suggest 13-15years of repeated fertilization can significantly affect soil chemistry, both positively and negatively. For example, results from Chapter 4 suggest repeated fertilization may be a viable means of building up soil C pools in British Columbian forests. In addition to its role as a master variable in determining soil productivity (Brady and Weil 2002), soil C also plays a major role in regulating global C cycles. Soils are the largest carbon sink of all terrestrial ecosystems; therefore, even small changes in soil C cycles could potentially affect atmospheric carbon concentrations and global climate (Janzen 2005). Our findings suggest soil C increases are largely limited by available soil nutrients (especially N). Thus, building up soil nutrients in pine and spruce forests in the interior of British Columbia may increase the ability of these stands to store C and regulate C fluxes to the atmosphere (Liu and Greaver 2010).

Potential negative effects of repeated fertilization were highlighted in Chapter 5, which indicated that repeated fertilization may pose significant risks to long-term site productivity through alterations in soil base cation cycling and soil pH. For example, there was substantial evidence of soil and foliar Ca depletion coupled with increased soil acidification in soils with poor buffering capacities. In fact, soil buffering capacities, rather than forest type, was the best predictor of soil Ca and pH decreases at these sites. Therefore, an understanding of inherent soil properties is essential to accurately predicting soil base cation and pH responses to long-term forest fertilization.

Despite some indications that 13-15 years of repeated fertilization may be negatively affecting aspects of soil chemistry (e.g., Ca, pH), there is no indication that such effects are detrimentally impacting forest productivity at any of the four sites in this study. Experiments carried out in this project have addressed some of the major uncertainties related to the effects of intensive fertilization on soil chemistry (e.g., Högberg et al. 2006; Van Miegroet and Jandl 2007; Ring et al. 2011). Further, research approaches used in this study were generally successful in achieving research goals. Therefore, such approaches could be valuable for researchers as well as forest managers attempting to monitor the impacts of silvicultural fertilization on aspects of soil chemistry. It is anticipated that findings from this project will improve our understanding of forest soils in British Columbia and provide forest managers with the necessary information to effectively implement sustainable forest management practices.

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