

WINNERS AND LOSERS IN A CHANGING CLIMATE:
CAUSES AND CONSEQUENCES OF SHIFTING VEGETATION DYNAMICS AFTER
LONG-TERM NUTRIENT ENRICHMENT IN A BOREAL FOREST UNDERSTORY

by

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ABSTRACT

Nitrogen levels are predicted to rise dramatically in the coming decades as a result of increased deposition from the burning of fossil fuels and the large-scale conversion of nitrogen into a useable form for agriculture. Plant communities react strongly to increases in soil nitrogen, particularly in northern ecosystems where nitrogen levels are naturally very low. An experiment in northern Canada that began in 1990 has been investigating the effects of long-term nutrient enrichment (fertilizer addition) and release from herbivory (fencing). After 22 years of treatment, plant community composition has been substantially altered in fertilized plots, whereas exclusion of herbivores has had almost no detectable effects. I used this experiment to address two questions about the causes and consequences of long-term nitrogen enrichment in the boreal forest.

In the first data chapter I focused on four understory species that had different levels of success in fertilized plots to investigate why some species are more successful than others under nutrient enrichment. I hypothesized that successful species would be taller, have higher specific leaf area, sprout earlier in the growing season and be more morphologically plastic than their less successful counterparts. I demonstrated that each of the two species that came to dominate fertilized plots has a different combination of traits and responses that likely gave them a competitive advantage; *Mertensia paniculata* has the highest specific leaf area of the four species, and *E. angustifolium* is tallest and exhibits morphological plasticity when fertilized by increasing biomass allocation to leaf tissue.

In the second data chapter I assessed how increasing nitrogen levels has affected investment in sexual reproduction in four herbaceous understory species. Whereas plants in northern ecosystems reproduce mainly through clonal growth, rapidly changing environmental conditions and warmer temperatures will likely result in increased benefits of sexual reproduction. Fertilization increased the probability of flowering for *Achillea millefolium* and resulted in a higher allocation of biomass to flower parts for *E. angustifolium* but did not affect investment in reproduction for *Festuca altaica* or *M.*

paniculata. Increased investment in sexual reproduction could give a competitive advantage to the former two species in the future.

PREFACE

Chapters 2 and 3 were co-authored by Roy Turkington and will be submitted for publication. The experiment and sampling protocol for vegetation composition were determined by Roy, and he has maintained and monitored this experiment since 1990. I designed and determined all other sampling protocols in consultation with my supervisory committee, collected and analyzed the data, and wrote the manuscripts. Roy also provided editorial assistance during the revision of the manuscripts.

Results included in Chapter 3 are reported in:

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CHAPTER 1

Introduction

Human activities increasingly dominate the nitrogen cycle, as the anthropogenic conversion of unreactive N_2 into useable reactive forms of nitrogen now accounts for more than twice as much nitrogen mobilization as all natural processes combined (Galloway et al. 2008). Levels of reactive nitrogen levels are predicted to rise dramatically in the coming decades due to increased deposition from the burning of fossil fuels and the industrial conversion of inorganic nitrogen into fertilizer for agricultural use (Galloway et al. 2004). In addition, higher temperatures associated with global climate change will result in faster mineralization rates of this limiting nutrient, further exacerbating the influx of useable nitrogen (Chapin et al. 1995, Rustad et al. 2001). This increased availability of nitrogen is a contributing factor in several serious environmental issues including smog, freshwater acidification, anoxic plumes and the eutrophication of coastal waters (Howarth 1998, Rabalais 2002, Galloway et al. 2004). As an essential nutrient for plant growth and development, nitrogen also plays a major role in shaping terrestrial plant communities. As such, altering levels of this nutrient has the potential to impact plant communities worldwide.

Variable Success of Species with Nutrient Enrichment

Increasing soil nitrogen levels promotes productivity in plant communities. However, addition of this nutrient does not affect all species equally. In greenhouse experiments where plants are isolated from competition, response to nitrogen addition is highly variable between species in everything from allocation to roots and shoots (Muller et al. 2000) to growth rates (Chapin 1980) and reproductive effort (Van Andel and Vera 1977, Chapin 1980). Interspecific differences in response to fertilizer can become even more pronounced when nutrients are added to natural communities and plants are able to compete for resources. Results from many fertilization experiments confirm that species diversity declines in fertilized plots as certain species increase in abundance and come to dominate when plant communities are released from nutrient limitation (Rajaniemi 2002, Turkington et al. 2002, Clark et al.

2007, Bobbink et al. 2010). Interspecific differences in plant species' ability to compete under high nutrient conditions have been cited as the principal mechanism for the observed decline in species richness in fertilizer experiments (Diekmann and Falkengren-Grerup 2002, Bobbink et al. 2010). For example, the presence of certain functional traits such as tall stature and fast growth rates, or the ability to be morphologically plastic, can confer a great advantage in high nutrient conditions (Bret-Harte et al. 2001, Diekmann and Falkengren-Grerup 2002, Laliberte et al. 2012). Which species succeed under fertilized conditions, however, is highly ecosystem-dependent (Dormann and Woodin 2002, Bobbink et al. 2010). In herbaceous ecosystems, grasses often respond strongly to fertilization and are able to outcompete neighbours (Dormann and Woodin 2002, Nordin et al. 2005, Suding et al. 2005, Clark and Tilman 2008). In fertilization experiments in the low Arctic, shrubs often gain a competitive advantage and become dominant (Chapin et al. 1995, Bret-Harte et al. 2001), whereas in the high Arctic an increase in forbs and graminoids has been reported with fertilization (Henry et al. 1986). In the boreal forest, an increase in graminoids and nitrophilic forbs has been reported in plots treated with fertilizer (Turkington et al. 2002, Manninen et al. 2009).

The consistently observed loss of species under nutrient enrichment and the variability between ecosystems makes it imperative to investigate, in a variety of ecosystems, why some species are able to outcompete others in high nutrient conditions.

Allocation to Sexual Reproduction

In addition to altering the competitive environment for established plant communities, nitrogen enrichment, combined with increased temperatures, could influence the manner by which species become established. Such a shift may take place in plant reproduction in northern biomes. Most northern ecosystems are dominated by plants that reproduce primarily through clonal growth because a lack of opportunities for seedling establishment in this harsh and competitive landscape and a highly heterogeneous environment both favor the foraging ability of clonal offshoots (Jónsdóttir et al. 1996). However, climate change could increase the adaptive importance of sexual reproduction in northern

biomes, for several reasons. Firstly, increased temperatures can cause plants to produce more seeds of better quality that have a higher likelihood of germinating successfully (Klady et al. 2011). Secondly, climate change could create a warmer, more favorable germination environment, further increasing successful germination (Wookey et al. 1993, Moulton and Gough 2011). Thirdly, changing environmental conditions will make range shifts necessary for survival for many species, leading to increased reliance on the successful dispersal and germination of seeds to colonize habitable areas (Dyer 1995). Finally, intraspecific variation resulting from genetic recombination during sexual reproduction may give species that frequently reproduce sexually an advantage in adapting to rapidly changing environmental conditions (Wookey et al. 1993, Hedhly et al. 2008, Steltzer et al. 2008). Nutrient enrichment affects investment in sexual reproduction in plants, and these effects vary between species (Doorman and Woodin 2002, Burkle and Irwin 2009, Moulton and Gough 2011). This disparity in species' reproductive response to nutrient addition can lead long-term changes in the seed bank and the plant community, with those species that increase investment to sexual reproduction when fertilized becoming better represented (Moulton and Gough 2011). It is therefore critical to investigate how such disparities in investment in sexual reproduction might influence the composition of future boreal forest understory plant communities by favoring some species over others.

Study Site Background

The effect of nitrogen enrichment could be most notable in northern ecosystems such as the boreal forest, where temperature increases are expected to be especially pronounced and nitrogen levels have historically been very low (Aerts et al. 2006). This research used a long-term study near Kluane Lake, Yukon, that has examined the effects of fertilizer addition and herbivore exclusion on plant community composition and dynamics in a boreal forest ecosystem (Turkington et al. 2002, DeKoning 2011). Plots at this site have been fertilized with NPK fertilizer and/or fenced to exclude herbivores since 1990.

Although grazing pressure by snowshoe hares has historically been substantial in this region (Krebs et al. 1986), and herbivory can have compounding effects on plants treated with fertilizer (Nordin et al. 1998,

Bobbink et al. 2010, Gilliam 2006), the effects of herbivore exclusion have been minimal at this site (Turkington et al. 2002, DeKoning 2011). In contrast, increasing nutrient levels caused several marked changes in the understory vegetation community including a loss of species diversity, a decline in grasses, prostrate woody species, mosses and lichens (Turkington et al. 2002, DeKoning 2011) and an advancement of phenological stages (Fremlin et al. 2011). As such, this study focuses on fertilizer effects, although the herbivore exclusion treatment is also included in analysis and discussion of results.

Thesis Overview

In this study, I focus on four herbaceous understory species that vary in their ability to compete when fertilized to ask two central questions about the causes and consequences of long-term nutrient enrichment in a boreal forest ecosystem. The first chapter looks at how vegetation composition has been altered by fertilizer addition and attempts to explain the differing levels of success of each of the four species. Drawing from previous work demonstrating that certain traits and responses make some species superior competitors in nutrient-enriched environment, I investigate whether functional traits and allocation to tissues can explain species' differential success when fertilized. The second chapter focuses on how vegetation dynamics could shift if a changing climate increases the benefits of sexual reproduction in northern biomes. In order to determine whether some species may gain an advantage in a changing climate by increasing investment to sexual reproduction, I assess how investment in sexual reproduction is altered by long-term nutrient enrichment for each species. These avenues of investigation will help shed light on some of the consequences of nitrogen enrichment in northern plant communities.

CHAPTER 2

Mechanisms for Success After Long-term Nutrient Enrichment for Four Boreal Forest Understory Species

Introduction

Plant ecologists have long attempted to relate types of vegetation to the abiotic conditions in which they live and to determine why some plants outcompete others in a given environment (Grime 1977, Tilman 1988, Southwood 1988). Although such theories were originally used to describe the natural world as it is, they have recently been applied to predict how vegetation will respond to changing environmental conditions in the anthropocene (Bret-Harte et al. 2001, Lavorel and Garnier 2002, Suding et al. 2005, Laliberte et al. 2012). For example, the current dramatic worldwide increase nitrogen availability makes it imperative to understand how increased levels of this key nutrient will alter vegetation composition and dynamics. Atmospheric deposition of nitrogen from burning fossil fuels, inputs from industrial fertilizers and faster mineralization rates resulting from rising temperatures are predicted to dramatically increase nitrogen availability globally in the coming decades (Galloway et al. 2004). In addition, experimental evidence from a wide array of ecosystems including grasslands, temperate forests and arctic tundra has revealed that nutrient enrichment leads to species loss when some species fail to compete in an altered environment (Gough et al. 2000, Turkington et al. 2002, Suding et al. 2005, Aerts et al. 2006, Bobbink et al. 2010). Classic theory, along with recent experimental evidence, provides several potential mechanisms for the observed variability in species' success under altered nutrient regimes.

Functional Traits

Grime (1977) and Chapin (1980) described several characteristics that affect a plant's ability to utilize soil nutrients and are indicative of whether a species will have greater success under high or low nutrient conditions. They agreed that plants that thrive in high nutrient conditions tend to be taller,

faster-growing species with thin leaves, and high leaf turnover (acquisitive strategy), whereas slower-growing, shorter species with thicker leaves tend to succeed under low nutrient conditions (conservative strategy). More recently, there has been a focus on specific functional traits as quantifiable indicators of plant strategies (Keddy 1992, Wright et al. 2004, Shipley et al. 2006). When functional trait theory is applied in experiments investigating the effects of nutrient addition on plant communities, the earlier theories are corroborated; plants with traits such as high specific leaf area (SLA), faster growth rates and taller stature are found to be more prevalent in communities treated with fertilizer (Diekmann and Falkengren-Grerup 2002, McIntyre 2008, Laliberte et al. 2012). However, most research relating functional traits to shifts in community composition after fertilization has been conducted in grasslands (Quetier et al. 2007, McIntyre 2008, Laliberte et al. 2012) and the relative importance of different traits in determining a plant's success under nutrient enrichment depends on local context and species assemblage. Therefore, research on the importance of various traits in determining success in non-grassland ecosystems is needed in order to increase the generality and applicability of the current models and predict species and ecosystem responses to global environmental change.

Morphological Plasticity

Harper (1967) recognized that a plant's investment in its various tissue types is not static, and introduced to plant ecology the concept of variable allocation to different tissues as a strategy to cope with changing environmental conditions. Chapin (1980) posited that species in nutrient-limited environments increase allocation to roots, whereas those growing where light is limiting allocate more biomass into shoots. This concept of relative allocation to roots and shoots under different nutrient conditions is also central to Tilman's (1990) predictive models. Recent experimental evidence indicates that when environmental conditions change, species vary in their ability to alter allocation to tissue types such as stems and leaves (Muller et al. 2000, Bret-Harte et al. 2001). The ability to be morphologically plastic in a changing environment can give some species an important advantage under

nutrient-enriched conditions and may even determine which species are successful when soil nutrient levels increase (Bret-Harte et al. 2001).

A long-term experiment started in 1990 in the boreal forest of northern Canada provides the ideal arena in which to test the importance of functional traits and morphological plasticity in determining success under an altered nutrient regime. The experiment was originally set up in 1990 to investigate bottom up (nutrient addition) vs. top-down (grazing by herbivores) controls on the understory vegetation (John and Turkington 1997, Turkington et al. 2002). When data for this study were collected, plots had been fenced to exclude herbivores and/or fertilized to add macronutrients for 22 years. Over these 22 years of treatment, marked changes in vegetation composition have been observed in fertilized plots, while fencing has had little effect on the vegetation in this system (Turkington et al. 2002). While some species have been able to thrive in enriched nutrient conditions and now dominate fertilized plots, others have declined to local extinction. Here, I focus on four understory species, including species both successful and unsuccessful in fertilized conditions, and ask the following two questions:

1. Which plant traits are associated with success under fertilized conditions?
2. Are species that succeed under high nutrient conditions those that are able to be most morphologically plastic when fertilized?

Methods

Study Site

The study site is in the boreal forest near Kluane Lake in southwestern Yukon Territory, and is described in detail by Turkington et al. (2002). The site is in the Shawkwak Trench, in the rain shadow of the St. Elias Mountains. The area receives an annual mean precipitation of ca. 290 mm and daily mean temperatures range from 4.7°C - 18.0°C on average during the June - August growing season (Environment Canada 2012). The soil at the study site has low fertility, with levels of key nutrients in mineral soil falling within the ranges of: total N 0-5 ppm, P 3-32 ppm, K 53-824 ppm (Turkington, Wippich and Seccombe-Hett unpublished data). The forest is moderately open (45-60% canopy cover, 160-220 stems/ha) and is dominated by white spruce (*Picea glauca* (Moench) Voss) interspersed with stands of trembling aspen (*Populus tremuloides* Michx.) and balsam poplar (*Populus balsamifera* L.). In 1995 an outbreak of spruce bark beetle killed off many trees in this area, allowing for more penetration of light into the understory. There is a well-developed shrub layer consisting primarily of willows (*Salix glauca* (L.) and other *Salix* spp.), dwarf birch (*Betula glandulosa* Michx.) and soapberry (*Shepherdia canadensis* (L.) Nutt.). Common understory species at the study site include arctic lupine (*Lupinus arcticus* S. Wats.), northern rough fescue (*Festuca altaica* Torr.), twin-flower (*Linnaea borealis* L.), bluebell (*Mertensia paniculata* (Aiton) G. Don), fireweed (*Epilobium angustifolium* L. s.l.), bearberry (*Arctostaphylos uva-ursi* (L.) Spreng.) and yarrow (*Achillea millefolium* L. var. *borealis* (Bong) Farwell). The snowshoe hare (*Lepus americanus* Erxleben) is the main herbivore in the system, and experiences an 8-12 year population cycle (Krebs et al. 2001). During population peaks in 1990 and 1998, snowshoe hare density in this region reached 148 hares/km² and 198 hares/km² respectively (Boutin et al. 1995, Hodges et al. 2001). However, hare density during the most recent peak in 2006 was only 92 hares/km². In 2011 when the current study was conducted, snowshoe hares were at a low point in their cycle with a density of 10 hares/km² (Krebs et al. 2011).

Experimental Design

Experimental plots were set up in 1990. Fertilizer application served to increase soil nutrients and fences were erected to exclude snowshoe hares. The site has sixteen 5 m X 2.5 m plots with fully crossed fertilizer and fencing (exclosure) treatments (+/0 fertilizer, +/-0 fencing) for a total of four treatments. Experimental plots were placed in semi-open areas within the understory with no rooted trees, and treatments were randomly assigned to each plot for a total of four replicates of each treatment.

Since 1990, granular fertilizer (N:P:K 35:10:5) has been applied to all fertilized plots each year after snow melt, between mid-May and early June. Fertilizer was applied at a rate of 0.625 kg per 5 m X 2.5 m plot per year, resulting in a total addition of 17.5 g N/m²/year, 5 g P/m²/year and 2.5 g K/m²/year. In contrast, mineralization rates of nitrogen in natural conditions in white spruce forests are approximately 4.7 g/m²/year (Binkley and Hart 1989). This level of application falls within the range of fertilization rates used by other long-term fertilization experiments (Kellner 1993, Gough et al. 2002). Each plot is further divided into five subplots, between which lie walkways approximately 20 cm wide to reduce trampling of plants within the plots. To prevent the spread of clonal species between the inside and outside of plots, the soil is cut to a depth of 20 cm around all plots in early June of each year to sever rhizomes and roots.

Study Species

Four common understory species were selected for this study: *Mertensia paniculata*, *Epilobium angustifolium*, *Festuca altaica* and *Achillea millefolium* var. *borealis*. These species are all native herbaceous perennials. These species were selected because they are the only species that are still present in sufficient numbers in all treatments. *Mertensia paniculata*, *E. angustifolium* and *A. millefolium* are all capable of reproducing asexually through the underground spread of rhizomes, whereas *F. altaica* is a densely-tufted bunchgrass that grows from fibrous roots. Due to the clonal

nature of these species, an “individual” may include ramets of the same clone, as identification of individuals was not genetically-based (as in Klady et al. 2011, Fremlin et al. 2011).

Achillea millefolium and *M. paniculata* have two distinct growth forms: a rosette form consisting of several leaves and no stem (not sexually reproductive), and an erect form (sexually reproductive). *Epilobium angustifolium* has only an erect form, and flower parts, although often stunted, are almost always present.

Treatment Effects

Effects of fertilizer and fencing treatments on soil nutrient levels were assessed in 2009 (DeKoning 2011). Soil nutrient supply rates were measured using ion exchange membranes (Plant Root Simulator (PRS) TMprobes; Western Ag Innovations Inc., Saskatoon, SK). Four anion and four cation probes were randomly placed in each subplot at the beginning of the growing season in early June and left until mid-August when they were removed and sent to Western Ag Innovations Inc. for analysis.

Incidences of herbivory were recorded every three days on plants being monitored for growth rates (five individuals of each species in each plot). Plants were classified as attacked by herbivores if any evidence of herbivory (chewed leaves) was detected on any day.

Abundance

Treatment effects on abundance of focal species were measured by recording the percent cover of each species in all plots during surveys conducted between July 11th – 13th 2011. In all plots, a sampling pin was dropped every 10 cm along five 2 m long transects and each of the focal species was recorded as present or absent at each pin drop location. Percentage cover for each species was the percentage of points at which that species was present within each plot. Relative success of each species in fertilized conditions was assessed by calculating the percent change between percent cover in unfertilized and fertilized plots using the formula:

$$\text{Percent Change} = \left(\frac{C_{\text{Fert}} - C_{\text{Unfert}}}{\left(\frac{C_{\text{Unfert}} + C_{\text{Fert}}}{2} \right)} \right) \times 100$$

where C_{Unfert} is the average percent cover of a species in unfertilized plots and C_{Fert} is the average percent cover of that species in fertilized plots. Here, percent change is calculated by taking the difference between percent cover of a species in fertilized and unfertilized plots, dividing by the mean of the two numbers and multiplying by 100 to get a percentage. Species that declined in abundance with fertilizer treatment therefore had a negative value for percent change.

Selection of Individuals for Trait and Biomass Allocation

Five individuals of each growth form (reproductive and non-reproductive) of *E. angustifolium* and *M. paniculata* were randomly selected from each plot for the measurement of traits. However, due to an absence of reproductive *F. altaica* and *A. millefolium* in fertilized plots, only non-reproductive (rosette form) individuals of these species were collected. For biomass allocation measurements, only reproductive *E. angustifolium* and *M. paniculata* (the same five individuals per plot collected for trait data) were used.

To avoid edge effects, plants were not sampled within 20 cm of the edge of the plot. Plants were also excluded if they were within 15 cm of a previously selected individual of the same species to minimize the chances of selecting two individuals of the same clone. Sampling was conducted during the period of peak flowering for each species. *Mertensia paniculata* was harvested on June 26th, *F. altaica* on July 6th and *A. millefolium* on July 19th. Because fertilization advances the phenology of *E. angustifolium* by seven days in these plots (Fremlin et al. 2011), *E. angustifolium* was counted in fertilized plots on August 1st and in unfertilized plots on August 8th.

Trait Measurements

Differences in growth form made it impossible to measure all traits on all species, so methods to measure some traits differed between species. Sampling techniques depended upon the morphology of each species, and especially whether the plant being sampled had a rosette or an erect form (Table 1.1).

Table 2.1. Summary of traits measured for four boreal forest understory species.

	<i>Mertensia paniculata</i>	<i>Epilobium angustifolium</i>	<i>Achillea millefolium</i>	<i>Festuca altaica</i>
Height (cm)	•	•	•	•
Specific leaf area (cm ² /g)	•	•	•	•
Growth rate (cm/day)	•	•	•	•
Total biomass of plant (g)	•	•	•	•
Biomass of leaves and stems (g)	•	•		
Leaf revoluteness				•

Festuca altaica Revoluteness

Festuca altaica leaves are rolled in upon themselves (revolute), and there is variation in their degree of revoluteness. The revoluteness of the longest leaf of each individual was assessed by measuring, at the centre of each leaf, the width of the leaf when rolled and when pressed flat. “Revoluteness” was defined as the rolled width divided by the unrolled width, so the closer this ratio approached a value of 1.0, the flatter the leaf.

Specific Leaf Area (SLA)

Leaves were collected from each individual for calculating SLA. To consistently select young, fully expanded leaves at approximately the same location on all *M. paniculata* and *E. angustifolium* individuals, the first two fully expanded leaves just below the lowest flower were selected. For those plants with no erect stem (*F. altaica* and *A. millefolium*), the longest leaf was collected for SLA measurements. Any leaves exhibiting obvious herbivory or pathogen damage were excluded, and in those rare cases, the next leaf down on the stem or the next longest leaf was used. Harvested leaves

were immediately placed between damp paper towels and transported back to the lab. Leaves were re-wetted upon return to the lab, placed between wet paper towels in a cooler and left for 18 hours in a refrigerator (4°C) for re-hydration (Garnier et al. 2001). Leaves were re-wetted every six hours during this re-hydration period. Re-hydrated leaves were patted dry, weighed and scanned to obtain leaf area. Scanned images were converted into black and white and analyzed for surface area using ImageJ software that calculated area of black space in each leaf image (Rasband 2012). Due to the fineness and rolled morphology of *F. altaica* leaves, obtaining an accurate leaf area using a scanner was not possible. Therefore 20 cm sections were cut at the centre of all *F. altaica* leaves and the width of the unrolled leaf was measured at its centre. Assuming a roughly uniform width for the 20 cm centre section of the leaf, the area of the leaf section was calculated. After scanning, all leaves were dried for 48 hours at 60°C and weighed again. SLA was calculated as the area per unit dry mass of each leaf.

Biomass Allocation to Leaves

To obtain dry above ground biomass measurements, all focal plants (individual ramets) were harvested, oven dried at 60°C for 48 hours and weighed. Erect, reproductive individuals (*E. angustifolium* and *M. paniculata*) were divided into leaves, stems and flowers and each tissue type was weighed separately. The dry mass of those leaves that had been removed for specific leaf measurements were added to total leaf mass for each plant.

Growth Rates and Herbivory

At the beginning of the growing season, just after snowmelt (June 3rd 2011), five emerging individuals of each species were randomly selected (one from each subplot) in each of the sixteen plots (wherever present) for a maximum of 20 individuals of each species for each of the four treatments. For *F. altaica*, an individual was a ramet within the larger clump, identified by the sheath at the base of the ramet.

Target individuals were tagged using an aluminum tag with a unique ID number attached to the base of the plant. Every three days between June 3rd and July 24th (18 surveys) the heights and any herbivory was recorded on all selected individuals.

Data Analysis

Species were analyzed separately except tests for effects of fertilization and fencing on herbivory (species were pooled) and tests for differences between species in height and SLA for unfertilized plants (species were compared using pairwise comparisons and Bonferroni's correction). Analyses of traits and biomass allocation were based on mean values of five individuals in each plot. Some plots did not contain five individuals of one or several species, and those plots containing fewer than three individuals of a species were excluded from analysis. Two-way ANOVAs were used to test for effects of fertilizer and fencing treatment on herbivory, percent cover, SLA and leaf revoluteness (*F. altaica* only). Fertilizer and fencing effects on biomass allocation in leaves and stems in *M. paniculata* and *E. angustifolium* were assessed using two separate ANCOVAs (one for fertilizer effects and one for fencing effects) with total plant biomass used as a covariate (Muller et al. 2000, Huang et al. 2010). A two-way repeated measures ANOVA was used to test for effects of fertilizer and fencing on growth rates of each species, with analysis based on averages of five individuals in each plot and individuals that died excluded. The Greenhouse-Geisser correction was used because the assumption of sphericity was violated. Two-way ANOVAs were performed on height of each species at each date, and the first date at which fertilized plants were significantly taller than unfertilized plants was defined as the growth spurt date. Data were log or rank transformed to meet model assumptions. Repeated measures ANOVAs were performed using the SPSS statistical package (SPSS Statistics 2008) and all other data analyses were performed using the R statistical package (R Development Core Team, 2010).

Results

Treatment Effects

Fertilization increased the levels of NO_3^- ($F_{1,12}=255.43$, $p<0.001$), NH_4^+ ($F_{1,12}=18.12$, $p<0.001$), P ($F_{1,12}=7.43$, $p=0.018$) and K ($F_{1,12}=5.05$, $p=0.044$) in the soil (Table 2.2) (DeKoning 2011).

Table 2.2. Mean (1 SE) levels of nutrient in control and fertilized plots in 2009, in $\mu\text{g}/10\text{cm}^2/61$ days. Bold p-values are significant at $p<0.05$. From DeKoning (2011).

	Control		Fertilizer				Fencing			
	Mean	SE	Mean	SE	F	p	Mean	SE	F	p
NO_3^-	1.55	0.66	847.00	127.86	255.43	<0.001	2.40	0.54	0.35	0.565
NH_4^+	6.60	1.43	63.60	19.56	18.12	<0.001	7.15	1.53	0.06	0.814
P	13.10	2.79	46.35	11.94	7.43	0.018	11.75	4.87	0.57	0.464
K	122.60	31.33	273.70	46.33	5.05	0.044	126.90	27.57	0.01	0.934

The level of herbivory (total incidences of individuals showing signs of herbivory for all species combined) was not affected by fertilizer ($F_{1,12}=3.05$, $p=0.106$) or fencing ($F_{1,12}=0.048$, $p=0.831$). Incidence of herbivory was much higher for *E. angustifolium* than for any other species ($F=1.00$, $p<0.001$ for all pairwise comparisons with *E. angustifolium*) (Figure 2.1) and thus the observed lack of treatment effects on herbivory was primarily driven by the data for this species. Most herbivory appeared to be primarily insect-caused (personal observation).

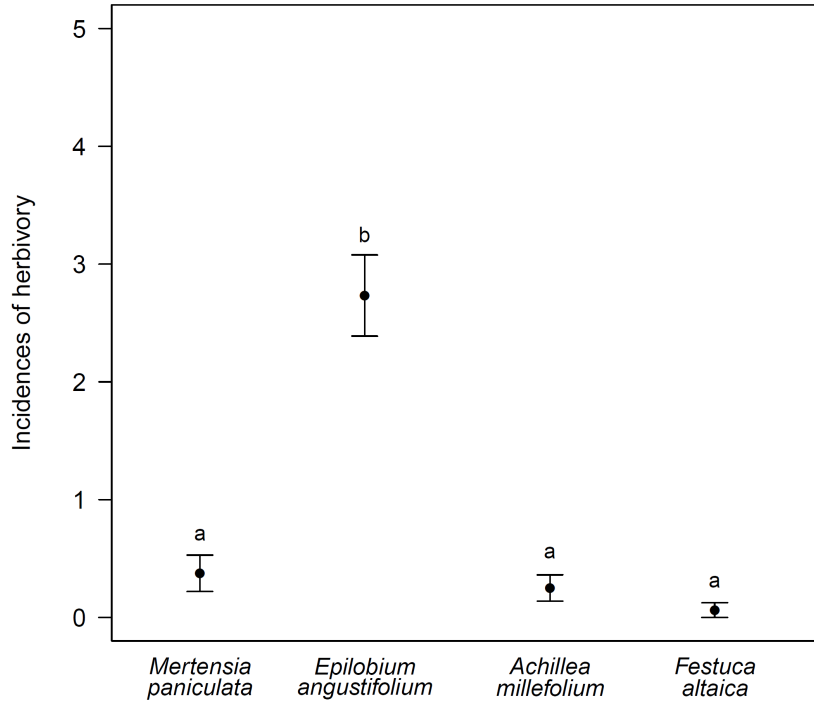


Figure 2.1. Number of individuals (\pm SE) showing signs of herbivory for four understory boreal species. Means are based five plants per plots and are averaged over all 16 plots for each species, for a total of 80 individuals per species. Species with the same letter are not significantly different ($p < 0.05$) using pairwise comparisons of species' means.

Abundance

Fertilization resulted in significantly higher percent cover of *M. paniculata* ($F_{1,12}=12.06$, $p=0.005$) and *E. angustifolium* ($F_{1,12}=41.80$, $p<0.001$) and lower percent cover of *F. altaica* ($F_{1,12}=40.76$, $p<0.001$) (Table 2.3; Figure 2.2). Fenced plots had significantly higher percent cover of *A. millefolium* than unfenced plots ($F_{1,12}=4.87$, $p=0.048$). There was no interaction between fertilizer and fencing treatments for any species.

Table 2.3. Summary of ANOVAs testing for effects of fertilizer and fencing on percent cover of four boreal forest understory species. Bold values are significant at $p < 0.05$. Positive values for percent difference unfertilized to fertilized indicate a higher abundance in fertilized plots.

Species	d.f.	Fertilizer		Fencing		Interaction		Percent Difference Unfertilized to Fertilized
		F	p	F	p	F	p	
<i>Mertensia paniculata</i>	1,12	12.06	0.005	0.25	0.629	2.89	0.115	+ 161 %
<i>Epilobium angustifolium</i>	1,12	41.80	<0.001	1.47	0.249	0.16	0.693	+ 125 %
<i>Achillea millefolium</i>	1,12	3.58	0.083	4.87	0.048	0.90	0.363	-23 %
<i>Festuca altaica</i>	1,12	40.76	<0.001	1.20	0.294	0.16	0.693	-165 %

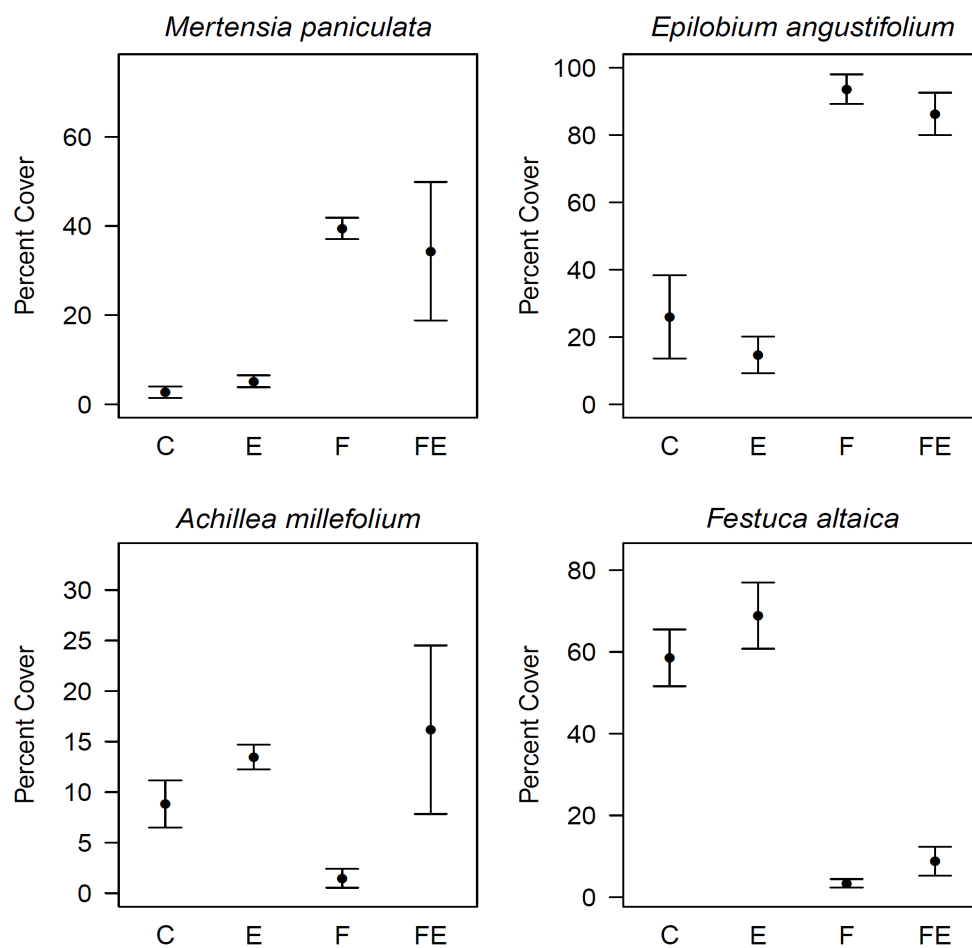


Figure 2.2. Percent cover (± 1 SE) of four boreal forest understory species in control (C), exclosure (E), fertilized (F) and fertilized and exclosure (FE) plots. Note the different scales on the Y-axes.

Functional Traits

In unfertilized plots, *E. angustifolium* was the tallest species and *M. paniculata* had the highest SLA (Table 2.4; Figure 2.3).

Table 2.4. Summary of pairwise comparisons of heights (cm) and specific leaf area (cm²/g) of unfertilized plants for four boreal forest understory species using Bonferroni's correction.

	Height (cm)		
	<i>Mertensia paniculata</i>	<i>Epilobium angustifolium</i>	<i>Achillea millefolium</i>
<i>Epilobium angustifolium</i>	p<0.001	---	---
<i>Achillea millefolium</i>	p=1.00	p<0.001	---
<i>Festuca altaica</i>	P=1.00	p<0.001	P=1.00

	Specific leaf area (cm ² /g)		
	<i>Mertensia paniculata</i>	<i>Epilobium angustifolium</i>	<i>Achillea millefolium</i>
<i>Epilobium angustifolium</i>	p<0.001	---	---
<i>Achillea millefolium</i>	p<0.001	P=1.00	---
<i>Festuca altaica</i>	p<0.001	p=1.00	P=0.96

Fertilization caused an increase in SLA for *M. paniculata*, *A. millefolium* and *F. altaica*, and an increase in height for *M. paniculata*, *E. angustifolium* and *A. millefolium* (Table 2.5; Figure 2.3). *Achillea millefolium* also had a higher SLA in fenced plots ($F_{1,12}=5.42$, $p=0.042$).

Table 2.5. Summary of two-way ANOVAs testing for effects of fertilizer and fencing on height (cm) and SLA (cm²/g) of four boreal forest understory species.

Species	Trait	d.f.	Fertilizer		Fencing		Interaction	
			F	p	F	p	F	p
<i>M. paniculata</i>	Height	1,8	7.67	0.021	1.92	0.204	0.0098	0.924
	SLA	1,8	9.66	0.014	0.012	0.917	1.33	0.282
<i>E. angustifolium</i>	Height	1,10	16.33	0.002	0.27	0.615	1.93	0.195
	SLA	1,10	4.53	0.059	0.011	0.919	0.74	0.411
<i>A. millefolium</i>	Height	1,10	10.91	0.008	0.00	0.995	0.11	0.743
	SLA	1,10	30.99	0.000	5.42	0.042	4.16	0.069
<i>F. altaica</i>	Height	1,12	3.51	0.086	0.01	0.917	0.00	0.958
	SLA	1,12	58.28	<0.001	0.83	0.380	0.84	0.376

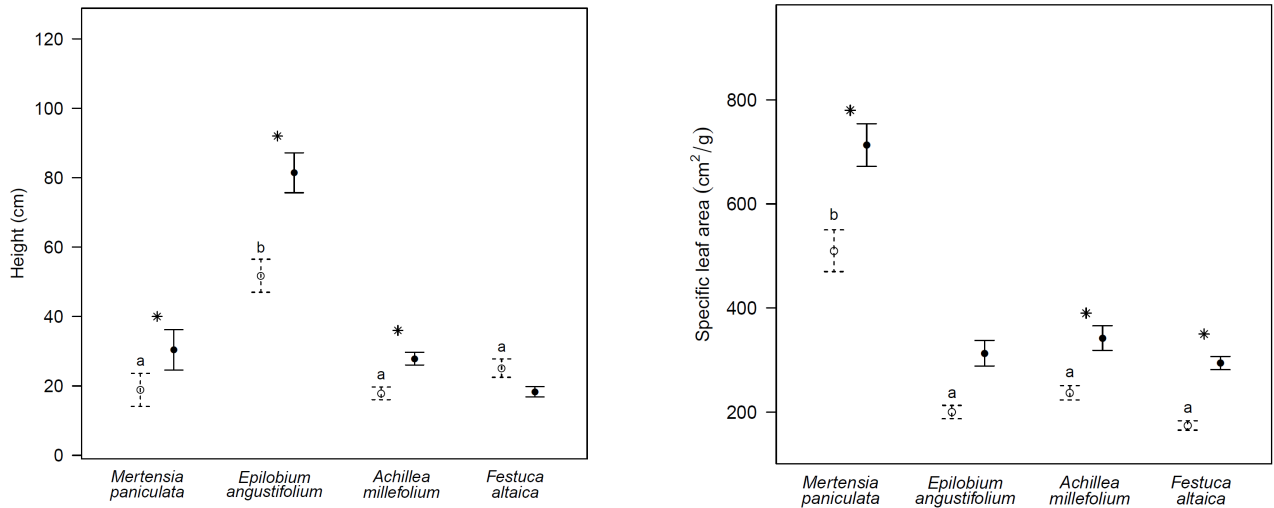


Figure 2.3. Height (± 1 SE) and specific leaf area (± 1 SE) of four boreal forest understory species grown in unfertilized (dashed lines) and fertilized (solid lines) conditions. Asterisks indicate significant difference between unfertilized and fertilized treatments, within a species. Species with the same letter are not significantly different ($p < 0.05$) using pairwise comparison of species' means for unfertilized plants. Data for each species are from five individuals in each of eight unfertilized plots.

Festuca altaica plants in fertilized plots had flatter (less revolute) leaves ($F_{1,12}=27.92$, $p < 0.001$), whereas fencing had no effect on *F. altaica* leaf revoluteness ($F_{1,12}=0.23$, $p=0.640$) (Figure 2.4).

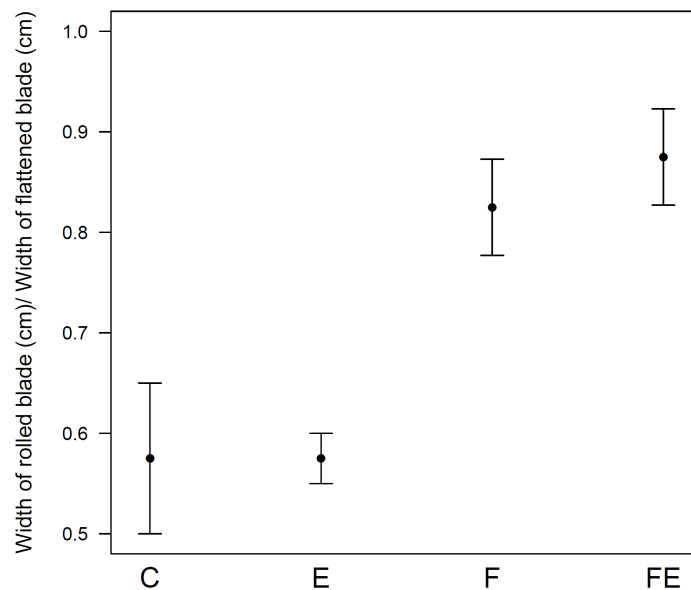


Figure 2.4. Leaf revoluteness of *F. altaica* (± 1 SE) in control (C), exclosure (E), fertilized (F) and fertilized and exclosure (FE) plots. Higher values indicate a flatter blade. Five individuals were sampled in each plot.

Biomass Allocation to Leaves

There was no effect of fencing on proportional allocation of biomass to leaves or stems for *E.*

angustifolium or *M. paniculata*, nor did fertilizer affect biomass allocation for *M. paniculata*. There was a significant relationship between allocation to stems and total plant biomass ($F_{1,8}=375.14$, $p<0.001$) for *M. paniculata*, with proportional allocation to stems increasing with plant size (Table 2.6). *Epilobium angustifolium* showed a negative relationship between proportional allocation to leaves and total plant biomass ($F_{1,10}=16.28$, $p=0.002$), and the reverse trend for allocation to stems ($F_{1,10}=9.95$, $p=0.01$).

Therefore larger plants had proportionally less biomass in leaves and more in stems. Therefore fertilized *E. angustifolium*, which were larger than their unfertilized counterparts, had lower proportional biomass in leaves as a result of the inherent relationship between plant size and investment to leaves for this species (Figure 2.5). However, fertilizer also weakened the negative relationship between allocation to leaves and total plant size ($F_{1,10}=6.40$, $p=0.033$) so that the largest fertilized plants invested more biomass in leaves than they would have had they not been fertilized (Figure 2.5).

Table 2.6. Summary of ANCOVA testing effects of fertilization on the proportional allocation to leaves and stems for *M. paniculata* and *E. angustifolium* with total plant biomass used as a covariate. Bold values are significant at $p<0.05$. Total Biomass refers to whether there is a significant relationship between total plant biomass and proportional allocation to stems or leaves, Fertilizer refers to treatment effects of fertilizer on proportional allocation to stems or leaves with total plant biomass as a covariate and Interaction refers to whether fertilizer treatment changes the relationship between total plant biomass and proportional allocation to stems or leaves.

Species	Trait	d.f.	Total Biomass		Fertilizer		Interaction	
			F	p	F	p	F	p
<i>Mertensia paniculata</i>	Leaves	1,8	1.08	0.328	0.0068	0.936	0.017	0.899
	Stems	1,8	375.14	<0.001	0.027	0.873	0.030	0.866
<i>Epilobium angustifolium</i>	Leaves	1,10	16.28	0.002	6.05	0.033	6.40	0.030
	Stems	1,10	9.95	0.010	1.54	0.243	4.78	0.054

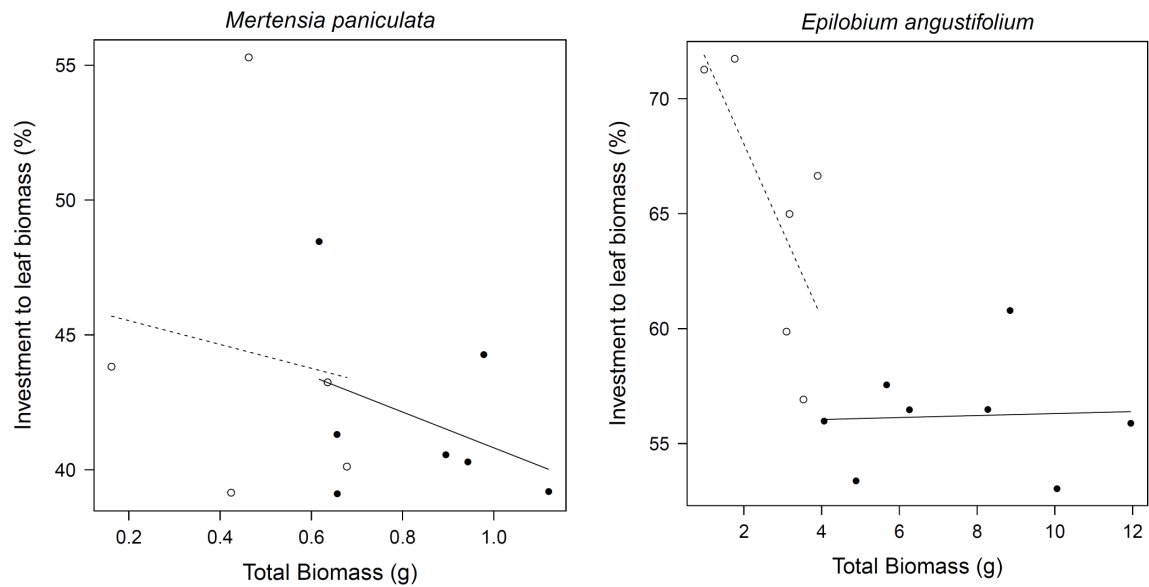


Figure 2.5. The relationship between proportional allocation to leaves (leaf biomass/total biomass) and total plant biomass for *E. angustifolium* and *M. paniculata* in unfertilized (open symbols, dashed line) and fertilized plots (closed symbols, solid line). Each point represents a plot average taken from five individual plants.

Growth Rates

Mertensia paniculata, *E. angustifolium* and *A. millefolium* grew faster under fertilized conditions, whereas fertilized *F. albaica* grew more slowly (Table 2.7; Figure 2.6). There was a significant interaction between fertilizer and fencing treatments for *F. albaica* growth rate ($F_{17,204}=3.48, p=0.037$): under fertilized conditions, fenced plants grew faster, whereas under unfertilized conditions, unfenced plants grew faster. *Mertensia paniculata* and *A. millefolium* had their growth spurts on June 27th and *E. angustifolium* had its growth spurt on July 15th. *Festuca albaica* did not have a growth spurt date because there was no date at which fertilized plants were significantly taller than unfertilized plants for this species.

Table 2.7. Summary of two-way repeated measures ANOVA testing for effects of fencing and fertilizer treatments on the rate of increase of plant height measured on four boreal forest understory species every three days between June 3rd and July 24th 2012. P-values were adjusted using the Greenhouse-Geisser correction to account for violation of the sphericity assumption.

	d.f.	Time* Fertilizer		Time* Fencing		Time* Fertilizer* Fencing		Growth Spurt Date
		F	p	F	p	F	p	
<i>Mertensia paniculata</i>	17,187	16.81	<0.001	0.49	0.527	0.076	0.827	June 27
<i>Epilobium angustifolium</i>	17,187	6.23	0.023	0.41	0.563	1.25	0.294	July 15
<i>Achillea millefolium</i>	17,170	7.61	0.006	0.28	0.721	0.50	0.580	June 27
<i>Festuca altaica</i>	17,204	16.81	<0.001	0.69	0.535	3.48	0.037	NA

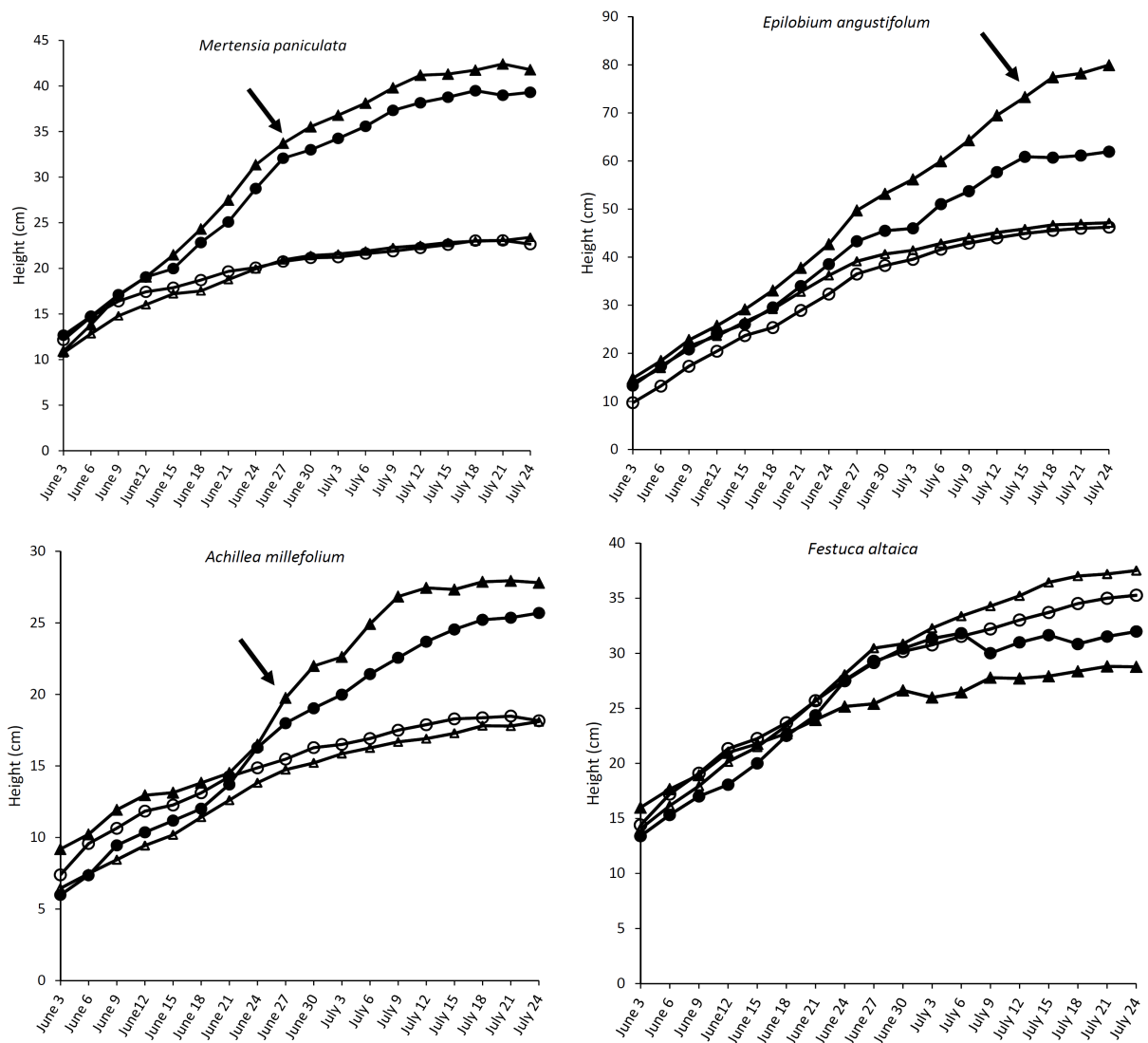


Figure 2.6. Height of four boreal forest understory species measured every three days between June 3rd and July 24th 2012. Solid symbols show fertilized treatments, open symbols are unfertilized. Triangles show fenced treatments, circles are unfenced. Arrows indicate for each species the date at which plants in fertilized treatments were significantly taller than plants in unfertilized treatments

Discussion

Overall, fertilization had a much greater effect than herbivore exclusion on the four species considered in this study. Twenty two years of nutrient addition has substantially altered the competitive environment in fertilized plots; as expected, fertilization caused an increase in levels of key nutrients, with NO_3^- levels increasing the most (DeKoning 2011). This influx of nutrients has increased biomass production in these plots, and more crowding combined with the dominance of two tall species has led to fertilized plots having a more light-limited environment than control plots. Strengbom et al. (2004) reported that light can be a limiting factor in the boreal forest understory, and DeKoning (2011) also reported that less light was penetrating the herbaceous layer in these fertilized plots. Observed shifts in community composition with fertilization have often been attributed to competition effects, as species that are more competitive in these new high-nutrient and low-light conditions outcompete neighbours and dominate fertilized plots (Diekmann and Falkengren-Grerup 2002, Gilliam 2006). In this study, *M. paniculata* had the highest relative increase in abundance in fertilized plots, followed by *E. angustifolium*, whereas *A. millefolium* showed little change and *F. altaica* declined. By investigating several possible mechanisms underlying the disparity in success for the four focal species in fertilized plots, we determined that although all four species displayed some advantageous response to high nutrient conditions that allowed them to persist in fertilized plots, the superior combination of traits and responses displayed by *M. paniculata* and *E. angustifolium* allowed them to thrive when fertilized.

Under control conditions, *M. paniculata* had a higher SLA than any other species, indicating that it had large thin leaves, and these leaves became even thinner when fertilized. High SLA has been associated with an acquisitive life history strategy adapted to high nutrient, light-limited conditions (Poorter and De Jong 1999, Wright et al. 2004, Laliberté et al. 2012). A switch from dominance by species with a conservative strategy to those with an acquisitive strategy with nutrient enrichment is predicted by theoretical reasoning (Grime 1977, Chapin 1980) and has been observed experimentally (Lavorel and Garnier 2002, Suding et al. 2005, Laliberté et al. 2012). Although *M. paniculata* emerges

early in the growing season (Fremlin et al. 2011) and was predicted to have the earliest growth spurt date, the heights of fertilized plants were not significantly taller than their unfertilized counterparts until June 27th. Heights of fertilized and unfertilized *A. millefolium* also diverged on June 27th and *E. angustifolium* diverged on July 15th. There was thus no evidence that growth spurt date was related to success in fertilized plots.

Epilobium angustifolium had neither a high SLA nor an early growth spurt date. However, *E. angustifolium* was the tallest of the four species under control conditions and grew even taller in fertilized plots, which likely allowed it access to light and contributed to its competitive dominance in fertilized conditions (Diekmann and Falkengren-Grerup 2002). This species also demonstrated morphological plasticity when fertilized. Although fertilized plants appeared to allocate proportionally less biomass to leaves than unfertilized plants, this was likely due to the negative relationship between plant size and allocation to leaves observed for this species, rather than a treatment effect (Coleman et al. 1994, Weiner 2004). Fertilized *E. angustifolium* allocated proportionally less biomass to leaves simply because fertilized plants were larger. However, fertilization did have an effect on allocation to leaves: it changed the relationship between proportional allocation to leaves and plant size. By alleviating the negative relationship between size and allocation to leaves, fertilization resulted in large plants allocating proportionally more biomass to leaves than they would have had they not been fertilized. Increased allocation to leaves in nutrient-rich, light-limited conditions is a strategy adopted by plants to optimize light capture, and is a recognized characteristic of more acquisitive species (Muller et al. 2000, Bret-Harte et al. 2001, Huang et al. 2010).

Achillea millefolium has a patchy distribution and an especially high variation in abundance was recorded in plots treated with both fertilizer and fencing (FE). Rajaniemi (2011) reported a high foraging precision for *A. millefolium*, meaning that this species is able to grow roots preferentially in patches with high resources. This could enable *A. millefolium* to aggressively increase clonal expansion into some fertilized areas, leading to the observed variability in abundance between plots. Due to the dominance

of the shorter, non-reproductive growth form of this species, *A. millefolium* had a short average height. Although a lack of erect, reproductive *A. millefolium* precluded analysis of morphological plasticity in this growth form, the presence of tall reproductive individuals exclusively in fertilized plots (discussed in Chapter 3) may have been a response to the light-limited conditions in these plots and an attempt to increase light capture when surrounded by tall neighbours. However, the short stature and low SLA seems to have limited *A. millefolium*'s ability to dominate in high nutrient conditions.

Festuca altaica had the least success in high nutrient conditions of the four species considered here; it was less abundant and plants grew more slowly in fertilized plots. The observed decline of *F. altaica* in fertilized plots contrasts with other studies that describe graminoids as one of the more successful functional groups in fertilizer experiments (Shaver and Chapin 1986, Dormann and Woodin 2002, Suding et al. 2005, Clark and Tilman 2008). However, these studies were conducted in herbaceous rather than forested ecosystems, where grasses are often the tallest species present and light is less limiting (but see Nordin et al. 2005, Manninen et al. 2009). In addition, results for most of these studies were reported after a shorter treatment period (often 1-5 years) than we report here. In the early years of this experiment both *F. altaica* and *A. millefolium* increased in abundance in fertilized plots, and only in years 10-15 when *E. angustifolium* and *M. paniculata* had come to dominate the fertilized plots did *F. altaica* and *A. millefolium* decline. Nams et al. (1993) also reported an increase in *F. altaica* abundance in a 2 year study in the same region. However, as a shorter species with a low SLA it was not as competitive as its more acquisitive neighbours. *Festuca altaica* had no growth spurt date because fertilized plants never grew taller than their unfertilized counterparts, and as this species consists entirely of leaves (or blades), proportional biomass allocation to leaves is not a meaningful metric for this species. However, evidence of morphological plasticity was observed in the unrolling of *F. altaica* leaves with fertilizer treatment. In the light-limited environment of fertilized plots, leaves with the greatest possible surface area are advantageous as they allow for maximum light capture. Evidently, this unrolling of leaves was not sufficient to give *F. altaica* a competitive advantage over its neighbours. In

addition, *F. altaica* has a bunched growth form, which contrasts with the rhizomatous form of the other three species considered in this study. An inability to rapidly expand by rhizomatous growth when fertilized could also have contributed to the lack of success in fertilized plots observed for *F. altaica*.

Several other herbaceous species not examined in this study were almost entirely eliminated from fertilized plots in the first ten years of the study (Turkington et al. 2002). These species were mostly short, slow-growing woody species with low SLA such as *Arctostaphylos uva-ursi* and *Linnaea borealis* (Turkington et al. 2002). Although morphological plasticity with fertilization could not be measured for these species due to lack of replicates in fertilized plots, the competitive exclusion of species with more conservative strategies supports the conclusion that only those species that are able to compete in a light-limited environment, by having a high SLA, tall stature and/or morphological plasticity, will be competitive when nitrogen becomes readily available (Grime 1977).

Ideally competition would have been isolated as a factor from the beginning of this experiment to separate direct response to fertilizer from responses to an altered competitive environment, but unfortunately it is too late in the experiment for this to be possible. Another experiment in this region found no effect of reduced above- and below-ground competition on biomass production on *F. altaica*, *A. millefolium* or *M. paniculata* (Arii and Turkington 2002). Isolation from above-ground competition was also attempted for *E. angustifolium* in the present study, and no effect was found for any parameter measured (data not reported). Plants in these studies were isolated for only one growing season, however, and the lack of results demonstrate the difficulty of detecting competition effects in a field environment.

In contrast to the notable effects of fertilizer on this plant community, fencing had almost no effect on the species considered here. The majority of herbivory that was detected was insect-caused (personal observation), and *E. angustifolium* was the most commonly eaten of the four focal species. Locally abundant species are often targeted by herbivores, so *E. angustifolium* in fertilized plots may be attracting insect herbivores that are specialists on this species (Kennedy and Southwood 1984).

Herbivore exclusion likely had little effect on the four focal species because snowshoe hare levels were at a low when data for this study were collected (Krebs et al. 2011). In addition, feeding trials in these plots showed that the summer diet of snowshoe hares consists mainly of woody shrubs (*Salix spp.*, *Shepardia canadensis* and *Betula glandulosa*) and *F. altaica* (Seccombe-Hett and Turkington 2008). As such, herbivore exclusion had little effect on any of the plant species; the few fencing effects that were detected were only marginally significant. *Achillea millefolium* was slightly more abundant ($p=0.048$) and had slightly higher SLA in fenced plots ($p=0.042$), which was likely an artifact of presence of two fenced and fertilized plots in which *A. millefolium* was very successful and abundant.

Conclusion

A recent focus on plant traits and their usefulness in assessing the causes and consequences of ecosystem change has led to several studies investigating which traits determine species success in high nutrient conditions (Suding et al. 2005, McIntyre 2008, Laliberte et al. 2012). A separate body of work has investigated how plants alter allocation to different tissue types in response to nutrient enrichment (Muller et al. 2000, Bret-Harte et al. 2001, Huang et al. 2010), but rarely have both mechanisms for success been considered in tandem. This research reveals that rather than one strategy prevailing as the most successful under fertilized conditions, success in a nitrogen-enriched boreal forest understory is determined by a combination of traits and strategies, and that some strategies are more successful than others in this ecosystem. This study also highlights the importance of long-term experiments, as some of the shifts in vegetation dynamics observed during the first ten years of treatment were reversed in subsequent years. Only through continued monitoring of this and other long-term environmental change experiments will we be in a position to understand and predict ecosystem response to global change.

CHAPTER 3

Long-term Nutrient Enrichment Differentially Affects Investment in Sexual Reproduction in Four Boreal Forest Understory Species

Introduction

Anthropogenic impacts on the global nitrogen cycle have caused a dramatic increase in available nitrogen since pre-industrial times (Galloway et al. 2004). Elevated deposition rates of atmospheric nitrogen from fossil fuel combustion, combined with the large-scale production of fertilizer for agricultural use, have led to humans currently mobilizing more nitrogen than all natural processes combined (Vitousek et al. 1997, Galloway et al. 2004). In addition, warmer temperatures due to global climate change are predicted to result in faster mineralization rates of key nutrients (Chapin and Shaver 1996, Rustad et al. 2001). Northern ecosystems, which are often severely nutrient-limited, may be especially impacted by an influx of soil nutrients (Aerts et al. 2006) and are projected to experience the greatest warming as a result of climate change (IPCC 2007). As nitrogen is the major limiting nutrient for plants in most temperate ecosystems, nutrient enrichment has the potential to have major effects on plant communities by increasing in biomass production and causing a shift in species composition (Dormann and Woodin 2002, Bobbink et al. 2010). Of particular concern is an observed decline in plant species diversity following nitrogen-enrichment, a trend that has been reported in many long-term fertilization experiments and summarized in several meta-analyses (Gough et al. 2000, Suding et al. 2005, Aerts et al. 2006, Bobbink et al. 2010). Species loss could be widespread across many ecosystems worldwide if nitrogen levels continue to rise at the current rate (Bobbink et al. 2010).

In recent years, several conceptual frameworks have been proposed by which to assess the impacts of global climate change on terrestrial ecosystems (Shaver et al. 2000, Suding et al. 2008, Smith et al. 2009). Such frameworks emphasize the importance of considering the potential for interactions between multiple natural processes shaping vegetation communities (Shaver et al. 2000, Smith et al. 2009). For example, the effects of increased nutrient availability may be compounded by interactions

with other natural processes, such as herbivory. High foliar nitrogen content in plants grown in high nutrients can lead to increased grazing pressure by herbivores (Nordin et al. 1998, Bobbink et al. 2010, Gilliam 2006). In addition, fertilization has been shown to reduce phenolic production in some species, which could make such species even more susceptible to attack (Koricheva et al. 1998). Herbivory could be a particularly important driver of vegetation dynamics in those northern regions with high and fluctuating herbivore densities (Krebs et al. 1986).

Although many studies focus on effects solely at the community level, the theoretical frameworks also emphasize the importance of scaling down to the level of the individual plant (Shaver et al. 2000, Smith et al. 2009). Responses at the individual (e.g. physiological processes or mortality) are often the first to be detected, and can have far-reaching effects as drivers of shifts at the community level (e.g. species reordering) (Smith et al. 2009). It is essential to consider interactive effects and multiple scales of response if we hope to mitigate species loss under altered temperature and nitrogen regimes.

Although plants in northern regions are mostly long-lived perennials that rely heavily on clonal growth (Jónsdóttir et al. 1996), an increased reliance on sexual reproduction in plants may be observed in this region in the future (Wookey et al. 1993). Environmental change could cause sexual reproduction to become more important for plants at high latitudes. As ranges shift, the genetic recombination and dispersal associated with sexual reproduction will likely give sexually reproductive plants an advantage in colonizing and adapting to new areas (Wookey et al. 1993, Dyer 1995, Arft et al. 1999, Aerts et al. 2006, Hedhly et al. 2008, Steltzer et al. 2008). More soil nitrogen and higher temperatures may also improve conditions in a previously harsh germination environment, further enhancing the role of sexual reproduction (Moulton and Gough 2011).

Soil nutrient availability has a known influence on investment in sexual reproduction (Harper and Ogden 1970). However, attempts to model how altering soil nutrients affects the trade-off between sexual and vegetative reproduction in clonal species have produced conflicting predictions, as allocation

to reproduction may depend on species identity, competition intensity and patchiness of resources (Loehle 1987, Sakai 1995, Gardner and Mangel 1999, Fu et al. 2010). One line of reasoning is that plants should decrease sexual reproduction in favor of clonal growth when soil nutrients are abundant because clonal growth produces proximate and genetically identical offspring with a proven genotype that can take advantage of favorable local conditions (Sakai 1995, Fu et al. 2010). Conversely, plants may invest more energy into sexual reproduction when conditions improve because flower and seed production are useful but nutrient-intensive, and plants can afford make them when nutrients are abundant (Loehle 1987, Gardner and Mangel 1999). Experimental evidence is similarly conflicting; some researchers have reported increased reproductive effort in plants when fertilized (Van Andel and Vera 1977, Kawano and Nagai 1986, Dormann and Woodin 2002, Burkle and Irwin 2009) while others reported decreased reproductive effort (Bai et al. 2009, Huang et al. 2010).

Herbivory also influences allocation to sexual reproduction. Perennial plants generally reduce allocation of biomass to reproductive parts when attacked and divert resources to survival to ensure future reproductive opportunities (Michaud 1991, Obeso 1993). However, this response varies by species (Obeso 1993), and increased allocation to reproductive parts under herbivore pressure has been demonstrated for some species (Paige and Witham 1987).

The measured effects of soil nutrients and herbivory on investment in sexual reproduction may vary by study in part due to differences in methods used to assess reproductive effort. Accepted proxies for reproductive effort include probability of flowering (Van Andel and Vera 1977, Bai et al., 2009, Moulton and Gough 2011), number of buds, flowers, seeds or reproductive tillers produced per plant (Burkle and Irwin 2009, Hautier et al. 2009), mass and percent germination of seeds (Fenner 1991, Burkle and Irwin 2009) and biomass allocated to reproductive parts (Van Andel and Vera 1977, Sugiyama and Bazzaz 1998, Huang et al. 2010). Although there is no consensus on the best method for assessing investment in sexual reproduction, biomass allocation to reproductive parts has a long history of use since it was introduced to plant ecologists by Harper (1967). Since this method was introduced as

an effective tool for assessing plant strategies, the importance of also considering how allocation to various tissues varies with plant size has been emphasized (Coleman et al. 1994, Samson and Werk 1986). Because there is a demonstrated relationship between plant size and allocation to reproduction, observed differences in biomass allocated to reproduction between resource treatments could be simply a product of plants being larger when fertilized (Samson and Werk 1986, Weiner 1988). Consequently, it is more informative to examine how the relationship between allocation to reproductive parts and total plant size is altered by nutrient addition (allometric approach) than simply testing for differences in ratios of reproductive and vegetative biomass (Samson and Werk 1986, Sugiyama and Bazzaz 1998, Weiner 2004, Huang et al. 2010). In this way, it is possible to distinguish between apparent plasticity between treatments (due to size differences) and true plasticity (due to treatment effects) (Muller et al. 2000, Huang et al. 2010).

This study uses a long-term fertilizer experiment in Yukon to investigate the effects of 22 years of nutrient enrichment and herbivore exclusion on investment in sexual reproduction in four boreal forest understory species. Experimental plots treated with fertilizer have undergone major shifts in community composition, and plant species richness has declined as plots became dominated by a few species (Turkington et al. 2002, DeKoning 2011). Almost no effects of herbivore exclusion on the plant community have thus far been detected, despite the presence of three peaks in densities of snowshoe hares (the major herbivore in the system) during the study period (Turkington et al. 2002, Fremlin et al. 2011, DeKoning 2011). Here, we use a snap-shot dataset collected in the 22nd year of the experiment to address two questions:

1. Have fertilization or herbivore exclusion treatments altered investment in reproduction for any of four focal species (*Mertensia paniculata*, *Epilobium angustifolium*, *Achillea millefolium* and *Festuca altaica*)?
2. Are previous assessments of responses of these species to the treatments consistent with current findings or do long-term trends differ from short-term responses?

Methods

Study Site and Experimental Design

The study site and experimental design are described in detail in Chapter 2.

Study Species

Four common understory species were selected for this study: *M. paniculata*, *E. angustifolium*, *F. altaica* and *A. millefolium* var. *borealis*. These species are all native herbaceous perennials. These species were selected because they are the only species that are still present in sufficient numbers in all treatments. *Mertensia paniculata*, *E. angustifolium* and *A. millefolium* are all capable of reproducing asexually through the underground spread of rhizomes, whereas *F. altaica* is a densely-tufted bunchgrass that grows from fibrous roots. Due to the clonal nature of these species, an “individual” may include ramets of the same clone, as identification of individuals was not genetically-based (as in Klady et al. 2011, Fremlin et al. 2011).

Achillea millefolium and *M. paniculata* have two distinct growth forms: rosettes (not sexually reproductive) consisting of several leaves and no stems, and erect (sexually reproductive). Reproductive *M. paniculata* has several branched, terminal flower clusters, with tubular, bell-shaped corollas and *A. millefolium* has numerous flower heads in a short, flat or round-topped cluster (Douglas et al. 2002). *Epilobium angustifolium* has only an erect form and flower parts (a many-flowered terminal raceme), although often stunted, are usually present. *Festuca altaica* is a bunch grass with flowers in an open panicle, and each bunch may or may not include a reproductive ramet.

Due to a lack of reproductive individuals of all species in all plots and differences in morphology between the species, all metrics of assessment of reproductive effort could not be measured for all species (Table 3.1).

Table 3.1. Summary of reproductive traits measured for four boreal forest understory species.

	<i>Mertensia paniculata</i>	<i>Epilobium angustifolium</i>	<i>Achillea millefolium</i>	<i>Festuca altaica</i>
Density of reproductive individuals	•	•	•	•
Number of buds	•	•		
Number of flowers	•	•		
Number of fruits		•		
Biomass of reproductive parts (g)	•	•		
Biomass of plant (g)	•	•		
Height (cm) ¹		•		
Seed biomass (g)	•			
Percent germination	•	•		

¹ Measured on both reproductive and non-reproductive individuals to assess minimum size for reproduction.

Treatment Effects

The effects of fencing and fertilizing on herbivory levels and soil nutrients were assessed using methods described in Chapter 2.

Abundance

The effects of fencing and fertilizing on percent cover (abundance) of each focal species were assessed using methods described in Chapter 2.

Density of Reproductive Individuals

The number of reproductive stems of each species was counted in each plot during that species' period of peak flowering (*M. paniculata* on June 26th, *F. altaica* on July 9th, *A. millefolium* on July 19th). Because fertilization advances the phenology of *E. angustifolium* by seven days (Fremlin et al. 2011), *E. angustifolium* was counted in fertilized plots on August 1st and in unfertilized plots on August 8th. Stems were considered reproductive if any buds, flowers, fruits or seeds were visible. Abundance data were used to obtain a measure of the density of reproductive individuals.

Biomass Allocation to Reproductive Parts

Due to an absence of reproductive individuals of *F. altaica* and *A. millefolium* in many plots, reproductive individuals could not be analyzed for these species. At peak flowering for *M. paniculata* and *E. angustifolium*, five reproductive individuals were randomly selected from each plot for the measurement of reproductive traits. To avoid edge effects, plants were not sampled within 20 cm of the edge of the plot. Plants were also excluded if they were within 15 cm of a previously selected individual of the same species to minimize the chances of selecting two individuals of the same clone. Sampling was conducted during the period of peak flowering for each species; *Mertensia paniculata* were harvested on June 26th, fertilized *E. angustifolium* were harvested on August 1st and unfertilized *E. angustifolium* were harvested on August 8th. All target plants were oven dried at 60°C for 48 hours, individuals were divided into vegetative tissues (leaves and stems) and reproductive parts and each tissue type was weighed separately. Reproductive parts included all petals, sepals, bracts, open or closed achenes (*E. angustifolium*) or nutlets (*M. paniculata*).

Number of Buds, Flower and Fruits

All buds and flowers on each individual of *M. paniculata* and *E. angustifolium* were counted prior to harvest. On *E. angustifolium*, fruits, flowers and buds appeared simultaneously on the same plant and therefore fruits were also counted. *Epilobium angustifolium* was considered to be in fruit when the petals had fallen off a developed achene.

Minimum Size for Reproduction

Minimum size for reproduction could only be assessed for *E. angustifolium* because this species has one growth form (erect) that can be either reproductive or non-reproductive, whereas *M. paniculata* has two distinct forms: a rosette and erect form. To determine whether fertilizer treatment altered the minimum height at reproduction, height data from harvested reproductive (5 individuals per plot) and

non-reproductive individuals (5 individuals per plot) were combined into one dataset and analyzed.

Individuals displaying any sign of buds, flowers or fruits were classified as reproductive.

Seeds

Mertensia paniculata and *E. angustifolium* seeds were collected to assess effects of fertilizer on seed weight and percent germination. However, there was a lack of individuals producing seeds in unfertilized plots for both of these species. To obtain sufficient seeds from plants that had not been fertilized but that were in a similar environment to plants in the fertilized plots, the area surrounding each fertilized plot (defined as being between 2 m – 10 m from the plot) was used for seed collection. Plants were chosen haphazardly from within this area, and seeds were collected from a minimum of ten individuals in each plot and in each “outside plot” area. Seven out of eight fertilized plots had *M. paniculata* that set seed and were sampled (along with a corresponding, surrounding unfertilized area), and five out of eight fertilized plots contained *E. angustifolium* that set seed. Seeds were collected from a minimum of ten individuals in each plot and in each “outside plot” area. Only individuals with mature seeds that appeared ready for dispersal were selected for collection and the entire seed head was collected from these individuals. Seeds from each plot were combined and were left to air dry for one week before being placed in a refrigerator (4°C) for one week. Seeds were then stored in a freezer (-15°C) for one month to break dormancy before being transferred back to the refrigerator for a final week.

Percent Germination

From the pool of seeds from each plot or “outside plot” area, 90 *M. paniculata* seeds and 90 *E. angustifolium* seeds were haphazardly selected, divided into three groups of 30. *Mertensia paniculata* seeds were weighed, however the extremely low mass of *E. angustifolium* seeds made obtaining an accurate seed mass for this species impossible with the available equipment, and these seeds were not weighed.

Each group of 30 seeds was then placed on damp filter paper in a 9 cm diameter petri dish. *Mertensia paniculata* seeds were scarified by making a small hole in the seed coat with a needle. Petri dishes were placed in a greenhouse at the University of British Columbia for four weeks and kept at an ambient temperature of 20°C - 24°C. Seeds were misted for five seconds every 20 minutes to keep filter paper moist. Petri dishes were placed on a heating pad to facilitate evaporation and prevent seeds from becoming too damp. Seeds were checked for germination every five days and germinated seeds (cotyledon >2 mm) were counted and removed.

Data Analyses

Each species was analyzed independently for all analyses. A two-way ANOVA was used to test for effects of fertilizer and fencing treatments on percent cover of the four focal species. Fertilizer and fencing effects on the number of reproductive individuals per plot of the four species was assessed using two ANCOVAs (one for fertilizer effects and one for fencing effects) with species abundance used as a covariate. Analyses of biomass allocation and number of buds, flowers and fruits of *M. paniculata* and *E. angustifolium* were based on mean values for five individuals in each plot. Some plots did not contain five reproductive individuals of one or both species, and those plots containing fewer than three reproductive individuals of a species were excluded from analysis. Fertilizer and fencing effects on biomass allocation to reproductive parts was assessed using two separate ANCOVAs (one for fertilizer effects and one for fencing effects) for each species, with total plant biomass used as a covariate (Muller et al. 2000, Huang et al. 2010). Count data (number of buds, fruits, flowers) were analyzed using a two-way ANOVA fitted with a generalized linear model, and a quasipoisson distribution was used because of overdispersion. A logistic regression was used to assess the effects of fertilizer and fencing on the minimum height at reproduction for *E. angustifolium*; individuals were considered separately rather than averaging by plot in order to preserve the height-reproductive relationship for each individual. One-way ANOVAs were used to assess the effect of fertilizer on seed mass and percent germination.

Data were log or rank transformed to meet model assumptions. All data analyses were conducted using the R statistical package (R Development Core Team, 2010).

Results

Treatment Effects

The effects of fencing and fertilization on incidences of herbivory and levels of soil nutrients are described in Chapter 2 (Table 2.2, Figure 2.1).

Abundance

The effects of fencing and fertilization on the abundance of the four focal species are described in Chapter 2 (Table 2.3, Figure 2.2).

Density of Reproductive Individuals

There was no effect of herbivore exclusion on the density of reproductive individuals in for any species. *Achillea millefolium* was reproductive only in fertilized plots, therefore fertilization both altered the relationship between percent cover and number of reproductive individuals (creating a positive relationship) ($p < 0.001$) and increased the number of reproductive individuals with percent cover considered as a covariate ($p < 0.001$) (Table 3.2, Figure 3.1). Although fertilizer increased percent cover of *M. paniculata* and *E. angustifolium* in fertilized plots, leading to more reproductive individuals, when percent cover was considered as a covariate there was no effect of fertilizer on number of reproductive individuals for either species (Table 3.2, Figure 3.1). Likewise, there was no significant effect of fertilization on the number of reproductive individuals of *F. altaica* (Table 3.2, Figure 3.1).

Table 3.2. Summary of ANCOVA testing for effects of fertilization on the number of reproductive individuals in a plot for four boreal forest understory species, with percent cover as a covariate. Bold values are significant at $p < 0.05$. Percent cover refers to whether there is a significant relationship between percent cover and number of reproductive individuals, Fertilizer refers to treatment effects of fertilization on the number of reproductive individuals in a plot with percent cover as a covariate and Interaction refers to whether fertilization changes the relationship between percent cover and number of reproductive individuals.

Species	d.f.	Percent Cover		Fertilizer		Interaction	
		F	p	F	p	F	p
<i>Mertensia paniculata</i>	1,12	44.47	<0.001	1.42	0.256	0.65	0.436
<i>Epilobium angustifolium</i>	1,12	49.49	<0.001	1.34	0.269	0.22	0.647
<i>Achillea millefolium</i>	1,12	252.81	<0.001	153.97	<0.001	29.75	<0.001
<i>Festuca altaica</i>	1,12	2.32	0.015	0.59	0.459	0.013	0.911

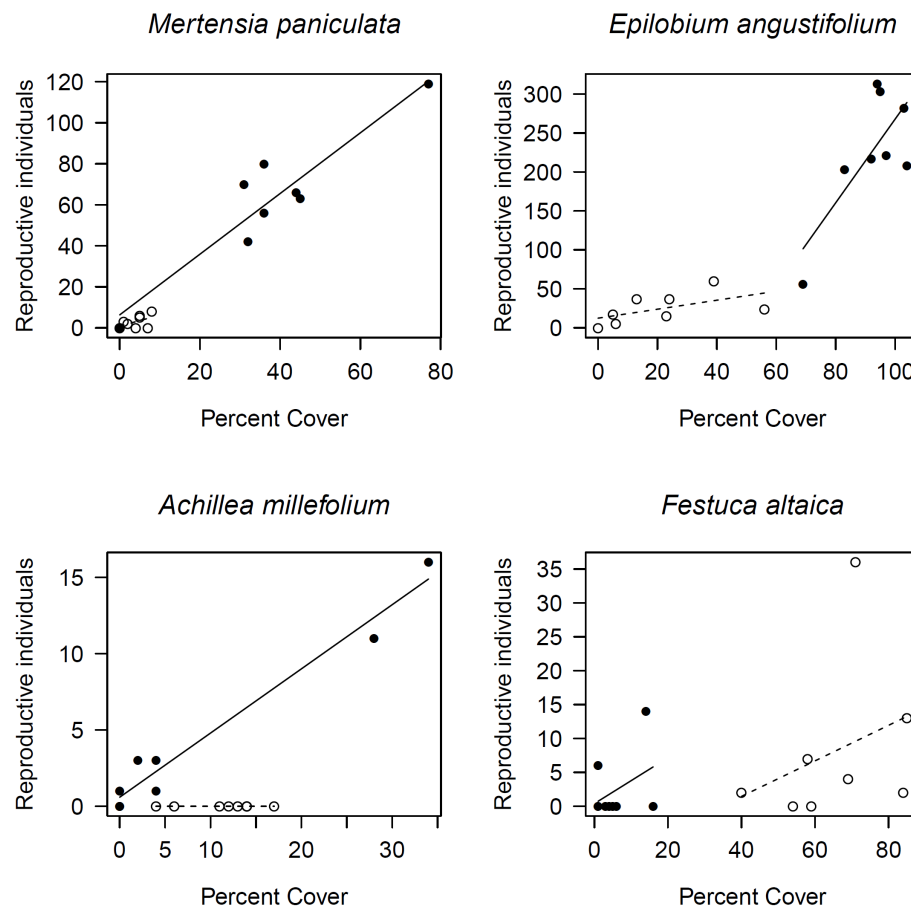


Figure 3.1. The number of reproductive individuals per plot, plotted against percent cover of that species in unfertilized (open symbols, dashed lines) and fertilized plots (closed symbols, solid lines) for four boreal forest understory species. Each point represents a plot.

Biomass Allocation to Reproductive Parts

There was no effect of herbivore exclusion on allocation to reproductive parts for either *M. paniculata* or *E. angustifolium*. There was also no relationship between proportional allocation to reproductive parts and total plant biomass for either species, and treatment with fertilizer did not affect this relationship (Table 3.3). Fertilization caused an increase in proportional allocation to reproductive parts in *E. angustifolium* ($F_{1,10}=6.90$, $p=0.025$), but had no effect on *M. paniculata* ($F_{1,8}=0.24$, $p=0.637$) (Table 3.3, Figure 3.2).

Table 3.3. Summary of ANCOVA testing for effects of fertilization on the proportional allocation to reproductive parts (mass of reproductive parts/total plant mass) for *M. paniculata* and *E. angustifolium* with total plant biomass used as a covariate. Total Biomass refers to whether there is a significant relationship between total plant biomass and proportional allocation to reproductive parts, Fertilizer refers to treatment effects of fertilization on proportional allocation to reproductive parts with total plant biomass as a covariate and Interaction refers to whether the fertilizer treatment changes the relationship between total plant biomass and proportional allocation to reproductive parts. The bold value are significant at $p<0.05$.

Species	d.f.	Total Biomass		Fertilizer		Interaction	
		F	p	F	p	F	p
<i>Mertensia paniculata</i>	1,8	0.46	0.519	0.24	0.637	0.12	0.736
<i>Epilobium angustifolium</i>	1,10	2.63	0.136	6.90	0.025	0.061	0.809

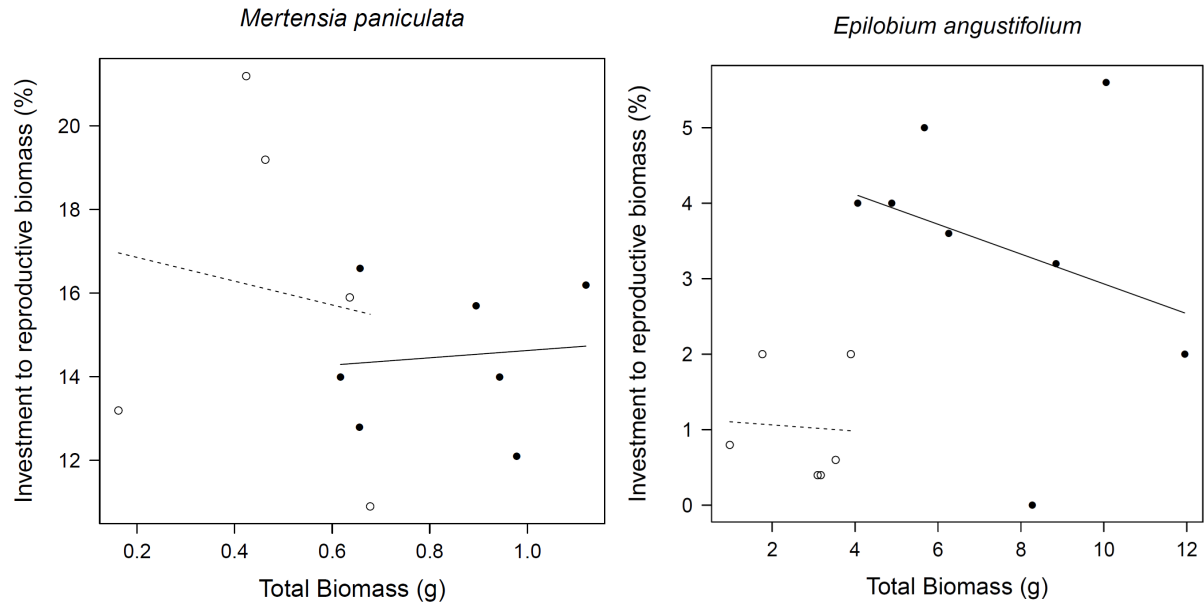


Figure 3.2. The relationship between proportional allocation to reproductive parts (as a percentage of total biomass) and total plant biomass for *M. paniculata* and *E. angustifolium* in unfertilized (open symbols, dashed lines) and fertilized (closed symbols, solid lines) plots. Reproductive biomass includes petals, sepals, bracts and nutlets (*M. paniculata*) or achenes (*E. angustifolium*). Each point represents a plot average taken from five individual plants.

Number of Buds, Flowers and Fruits

Fertilization resulted in an increased number of flower buds on *M. paniculata* ($F_{1,8}=6.89, p=0.030$), and more buds ($F_{1,10}=12.01, p=0.00607$) and flowers ($F_{1,10}=24.37, p<0.001$) on *E. angustifolium* (Table 3.4, Figure 3.3). *Epilobium angustifolium* in fenced plots also had more fruits ($F_{1,10}=6.59, p<0.028$).

Table 3.4. Summary of two-way ANOVA testing for the effects of fertilization and fencing on the number of buds and flowers for *M. paniculata* and buds, flowers and fruits for *E. angustifolium*. Bold values are significant at $p < 0.05$.

<i>Mertensia paniculata</i>							
	d.f.	Fertilizer		Fence		Fertilizer x Fence	
		F	p	F	p	F	p
Buds	1,8	6.89	0.030	0.014	0.908	0.136	0.722
Flowers	1,8	2.60	0.146	4.41	0.0688	0.28	0.610

<i>Epilobium angustifolium</i>							
	d.f.	Fertilizer		Fence		Fertilizer x Fence	
		F	p	F	p	F	p
Buds	1,10	12.01	0.006	0.69	0.424	0.22	0.649
Flowers	1,10	24.37	<0.001	0.27	0.618	1.93	0.19
Fruits	1,10	0.95	0.353	6.59	0.028	0.99	0.341

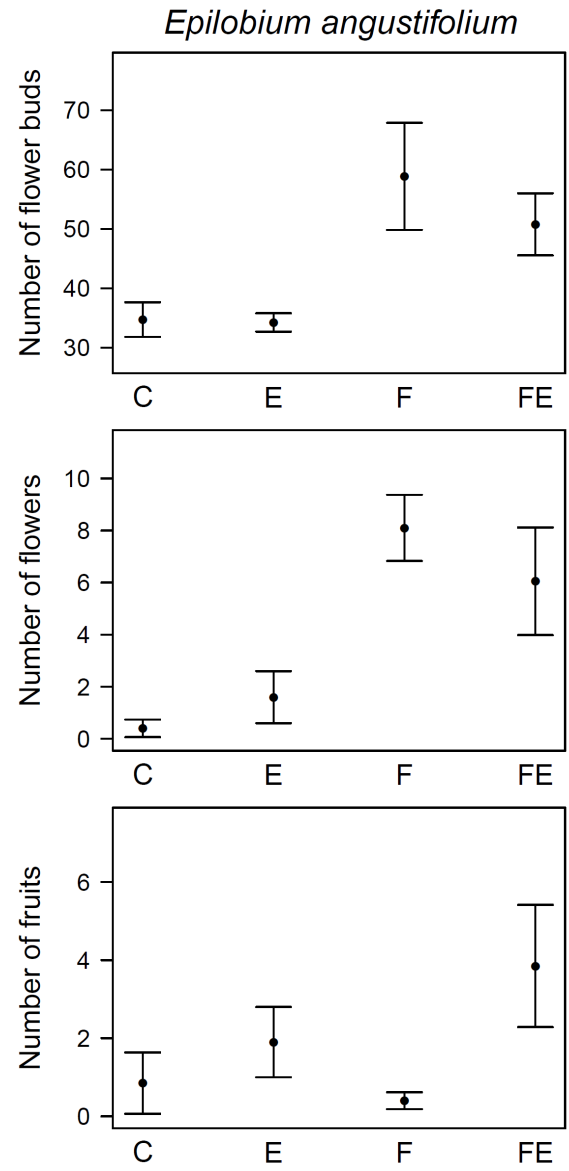
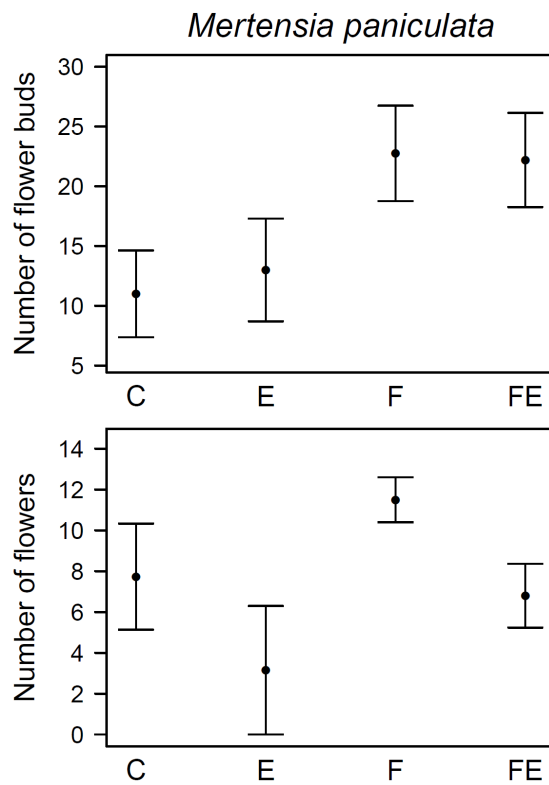


Figure 3.3. Mean number (± 1 SE) of flower buds, flowers and fruits per plant on *M. paniculata* and *E. angustifolium* in control (C), exclosure (E), fertilized (F) and fertilized and exclosure (FE) plots. Five individuals of each species were sampled in each plot. See Table 3.4 for ANOVA results.

Minimum Size for Reproduction

There was a significant relationship between height and probability of reproducing for *E. angustifolium* ($p < 0.001$) (Table 3.5). Fertilization increased the probability of reproducing ($p < 0.001$) and unfertilized plots had a marginally weaker relationship between height and probability of reproducing ($p = 0.053$). Fertilization also increased the threshold height for reproduction: whereas 50% of unfertilized plants were reproductive at 53.6cm (± 3.9 cm), this threshold was increased to 79.7cm (± 2.5 cm) for fertilized plants (Figure 3.4). There was a significant interaction between height and fencing treatment on the probability of reproducing ($p < 0.001$) with unfenced plots having a weaker relationship ($p < 0.001$) between height and probability of reproducing.

Table 3.5. Summary of logistic regression testing for effects of plant height, fertilization and fencing on the probability of *E. angustifolium* being reproductive. Bold values are significant at $p < 0.05$.

Term	d.f.	p
Height	1,143	<0.001
Fertilizer	1,142	<0.001
Fencing	1,141	0.594
Height: Fertilizer	1,140	0.053
Height: Fencing	1,139	<0.001
Fertilizer: Fencing	1,138	0.745
Height: Fertilizer: Fencing	1,137	0.863

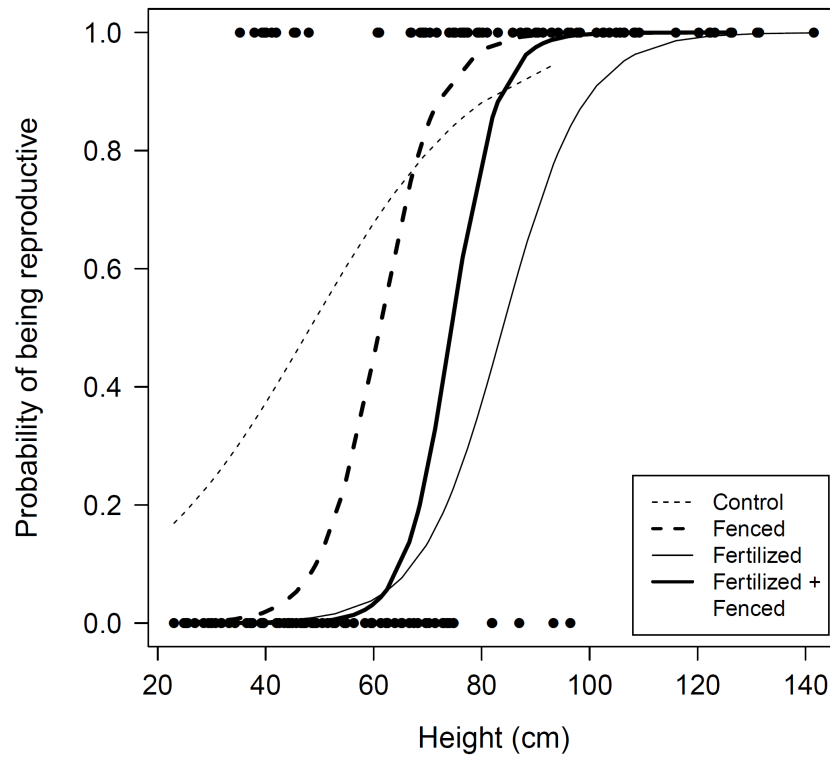


Figure 3.4. Relationship between the height of *E. angustifolium* and its probability of reproducing when grown in four treatments. Each dot represents an individual plant.

Seeds

There was no significant effect of fertilization on *M. paniculata* seed mass ($F_{1,12}=0.96$, $p=0.761$). All *M. paniculata* seeds failed to germinate, therefore only *E. angustifolium* germination was analyzed. There was no effect of fertilization on percent germination of *E. angustifolium* seeds ($F_{1,8}=1.25$, $p=0.292$) (Grainger and Turkington 2012).

Discussion

The four species in this study system vary widely in their ability to compete in high nutrient conditions. Thus far, most of the observed increase in percent cover of the species that have come to dominate has occurred by clonal expansion. However, future conditions will likely increase the role of sexual reproduction in successful colonization of northern ecosystems, and differences in species' investment in sexual reproduction in a changing environment could dictate which species dominate this region in the future. Both *A. millefolium* and *E. angustifolium* increase investment in sexual reproduction when fertilized, the former by producing more reproductive individuals and the latter by increasing biomass allocation to reproductive parts. Conversely, reproductive effort for *M. paniculata* and *F. altaica* was largely unaffected by the treatments. Overall, fertilization had a much greater effect than herbivore exclusion on the reproductive ecology of the species examined here (John and Turkington 1997, Fremlin et al. 2011). The lack of fencing effects is likely due to a lack of herbivores in the system in the year the data was collected (Krebs et al. 2011) and a preference by snowshoe hares for plants other than the focal species (Seccombe-Hett and Turkington 2008), as described in Chapter 2.

Treatment Effects

As expected, twenty years of fertilizer treatment resulted in increased levels of key soil nutrients, with NO_3^- experiencing the most drastic increase. However, there was no detectable effect of fencing on the incidence of herbivory. Snowshoe hares were at a low in their population cycle during 2011 when data for this experiment were collected, and were likely not present in high enough densities for effects of grazing to be detected (Krebs 2011). In addition, feeding trials in these plots have revealed that the summer diet of snowshoe hares consists mainly of woody shrubs (*Salix spp.*, *Shepardia canadensis* and *Betula glandulosa*), and of the four focal species considered here, only *F. altaica* was consumed (Seccombe-Hett and Turkington 2008). The majority of herbivory detected in this study was from insects, and *E. angustifolium* was the most commonly consumed of the four species. The abundance of

E. angustifolium in fertilized plots could be attracting herbivores that are specialists on this species, as locally abundant species are often targeted by herbivores (Kennedy and Southwood 1984).

Reproductive Investment at the Plot Level

Due to an increase in abundance of *M. paniculata* and *E. angustifolium* with fertilization, there were more reproductive individuals of these two species in fertilized plots. However, when abundance was taken into consideration in analysis, fertilizer had no effect on the number of reproductive individuals for either of these species, and the observed increase in number of reproductive individuals was simply a result of increased abundance. This contrasts with John and Turkington's (1997) finding during a demographic study conducted in these plots between 1990 and 1994 that *M. paniculata* had a higher probability of flowering in fertilized plots. This could indicate that there has been a decline in reproductive effort for *M. paniculata* in the seventeen years between that study and this study. Similarly, there were fewer reproductive individuals of *F. altaica* in fertilized plots as a result of a decline in abundance for this species, which contrasts with the initial increase in abundance for this species reported by Turkington et al. (2002).

It is evident that *A. millefolium* has a patchy distribution; it is abundant in some fertilized plots but virtually absent in others. Rajaniemi (2011) reported that *A. millefolium* has a high foraging precision or ability to grow roots into areas of high resource availability. This trait is advantageous in soils with patchy resource distribution, so *A. millefolium* may have been able to colonize fertilized plots by elongating roots into the high nutrient soil in areas where it was originally present at a sufficient density. *Achillea millefolium* was also the only species that had more flowering individuals in fertilized plots even when abundance was accounted for; *A. millefolium* only flowered in fertilized plots and no flowering individuals were seen even in the surrounding forest (personal observation). Nams et al. (1993) also reported an extremely low density of reproductive *A. millefolium* in unfertilized plots and an increased number of flowering individuals with fertilizer treatment. This species may have a minimum nutrient requirement for reproduction that is only attained in fertilized plots. *Achillea millefolium* was also

slightly more abundant in fenced plots than in unfenced plots, however this was only marginally significant ($p=0.048$) and DeKoning (2011) did not detect this difference when plots were surveyed in 2009.

Allocation to Reproduction at the Individual Plant Level

Due to a scarcity of reproductive *A. millefolium* in unfertilized plots and reproductive *F. altaica* in fertilized plots, we were unable to assess biomass allocation to reproductive parts at the individual level for these species. The observed lack of relationship between plant size and allocation to reproduction for *M. paniculata* has been reported for other species (Samson and Werk 1986) although a negative relationship is more common for perennial species (Kawano and Nagai 1986). Despite having more buds in fertilized plots, there was no effect of fertilization on allocation to reproduction for *M. paniculata* when plant size was taken into account. These results highlight the importance of considering the effect of plant size on allocation to sexual reproduction; if we had only counted buds and flowers or used ratios of tissues types here (as in Abrahamson 1975, Van Andel and Vera 1977, Kawano and Nagai 1986, Burkle and Irwin 2009) we could have erroneously concluded that allocation to reproduction had increased in *M. paniculata*. In contrast, fertilized *E. angustifolium* allocated more biomass to reproductive parts when fertilized, even when plant size was taken into account. Although plants in fertilized plots were larger than their unfertilized counterparts, it is unlikely that the observed increase in allocation to reproductive tissue was apparent plasticity as a result of increased plant size, as there was no relationship between plant size and allocation to reproductive parts for this species. This increased allocation was also reflected in higher numbers of both buds and flowers on fertilized plants. *Epilobium angustifolium* also had more fruits in fenced plots, which is an unexpected result. Of studies that took size into account when assessing nutrient effects on allocation to reproduction, Sugiyama and Bazzaz (1998) reported that nutrient addition resulted in increased allocation to reproduction in *Abutilon theophrasti*, whereas Huang et al. (2010) found decreased allocation to reproductive parts under high nutrient conditions in *Corispermum macrocarpum*. Response to fertilization appears to be

species-specific, and increased reproductive output of a widespread species such as *E. angustifolium* could have important impacts on the boreal forest.

A minimum size at which plants are reproductive has been demonstrated for many species (Silvertown 1991, Weiner 2004). The finding that *E. angustifolium* was reproductive at shorter heights in unfertilized control plots is supported by previous research demonstrating that plants reproduce at smaller sizes in poor conditions (Bonser and Aarssen 2009). For plants grown in low nutrient conditions, it is more beneficial to reproduce at a smaller size even though this could result in reduced fecundity and quality of offspring, because plants may never reach the optimal size for reproduction. However plants in high nutrient conditions can afford to delay reproduction, divert resources to growth and reproduce only at large sizes (Bonser and Aarssen 2009). The weaker relationship between height and probability of reproducing in control plots than in fenced plots was unexpected and could simply be an artifact of size distributions of the plants in each of the treatments.

There was no effect of fertilization on germinability of *E. angustifolium* seeds (Grainger and Turkington 2012). In a review of experiments investigating the effects of soil nutrient on seed germination, Baskin and Baskin (2001) reported substantial interspecific variation in responses, with several species showing no response to nutrient treatment. However no study has investigated this effect on *E. angustifolium*, so it is possible that *E. angustifolium* is a species for which fertilizer treatment does not affect germinability. The lack of difference in *M. paniculata* seed mass between control and fertilized plots further confirms the unresponsiveness of this species' reproductive effort to high nutrient conditions.

The Role of Competition

Adding key soil nutrients increased productivity in fertilized plots, which led to increased competition from densely packed neighbours (Turkington et al. 2002). Competition is an important and possibly confounding variable in this study because neighbour density can affect a plant's productivity, abundance and allocation to reproduction (Waite and Hutchings 1982, Goldberg and Barton 1992, Van

Kleunen et al. 2001, Treberg and Turkington 2010). The low abundance and small size of *F. altaica* in fertilized plots was likely a result of competition with neighbours (Chapter 2). This result contrasts with most other studies of nutrient enrichment, which report that graminoid species tend to increase in abundance in fertilizer experiments (Shaver and Chapin 1986, Dormann and Woodin 2002, Bobbink et al. 2010). In a different fertilization experiment in this region, Nams et al. (1993) reported an increase in both the abundance and reproductive effort of *F. altaica* after two years of fertilizer treatment. In fact, Turkington et al. (2002) reported an increase in *F. altaica* abundance in the first ten years of this experiment, after which time this species began to decline. The decline of *F. altaica* in fertilized plots reported here is likely a result of competition from tall neighbours (*M. paniculata* and *E. angustifolium*) which took several years to dominate the fertilized plots, rather than a direct effect of fertilizer (Turkington et al. 2002). However, when plants were isolated from above-ground competitive interactions with neighbours, no effects of competition was found on allocation to reproduction for *E. angustifolium* (Grainger unpublished data). Other studies have likewise failed to detect an effect of density on allocation to reproduction (Zhang et al. 1996, Huang et al. 2010). Although competition intensity likely varies between fertilized and unfertilized plots, the resulting effect on allocation to reproduction is likely not as substantial as the direct effects of treatment with fertilizer.

Conclusions

This study and comparisons with earlier results from the same area highlight the need for long-term experiments. Some earlier results, such as an increase in reproductive *A. millefolium* in fertilized plots and the lack of any effects of herbivore exclusion, were corroborated in the current study and indicate long-term trends (Nams et al. 1993, Turkington et al. 2002). However other findings, such as the early increase in *F. altaica* abundance and *M. paniculata* probability of flowering in fertilized plots, were not confirmed and may reflect the shifting dynamics of this community during more than two decades of treatment (John and Turkington 1997, Turkington et al. 2002). Use of an analysis that takes plant size

into consideration has further clarified the effects of increased nutrients on the reproductive ecology of these four species and highlighted subtleties that may previously have been overlooked.

If global nutrient enrichment and higher temperatures increase the benefits of sexual reproduction and the likelihood of successful germination in this northern ecosystem, *E. angustifolium* and *A. millefolium* could become increasingly dominant while *M. paniculata* and *F. altaica* could lose the competitive advantage that were initially reported at this study site. Continued monitoring of the response of such widespread and abundant species to environmental change is essential. By using long-term monitoring sites, embracing new methods when available and incorporating past findings into our research, we improve our ability to predict vegetation response to environmental change.

CHAPTER 4

Conclusions and Future Directions

As ecosystems continue to be transformed on a global scale by human influences, it is becoming increasingly necessary to assess how natural systems respond to the myriad changes imposed on them. Three steps can be taken to study ecosystem responses to environmental change in a meaningful way that will allow us to predict potential changes and mitigate environmental impacts. In this thesis I have addressed these three steps in the context of plant species' responses to elevated nitrogen levels. The steps are: (1) assess experimentally how vegetation changes in an altered environment, (2) determine the mechanisms behind these changes, and (3) take other environmental factors (such as warming) into account in an effort to predict future shifts in plant communities. For the first step, elevated nitrogen levels have been shown, both here and in previous studies in these plots, to cause a shift in community composition and a loss of species. The second step is addressed in chapter two through an investigation of possible reasons why some species are more successful than others when fertilized. Chapter three addresses the third step by considering how increased nutrient supply alters the reproductive ecology of understory species. Altered investment in sexual reproduction may determine the future success of these species as sexual reproduction becomes increasingly advantageous in a warmer climate. By considering these consequences of nutrient enrichment on boreal forest understory species, this study has contributed to our understanding of the impacts of what is predicted to be a major environmental change on a widespread ecosystem. Here I will discuss how realistic the treatments were relative to actual climate change projections, address some of benefits and challenges of using long-term experiments, outline potential future directions and summarize the main conclusions of the study.

How Realistically Does Fertilizer Application Simulate Environmental Change?

Results described here may never be observed in natural systems affected by climate change if levels of nitrogen available to plants do not approach levels applied in this experiment. Although the nitrogen

level applied in this experiment is comparable to treatments used in other fertilization experiments (Kellner 1993, Gough et al. 2002), these application rates are more than two orders of magnitude higher than deposition rates predicted for this region, even in 2050 (Galloway et al. 2004). The present estimate for critical nitrogen loads for the boreal forest (the level below which this ecosystem will not experience harmful effects) is between 0.6 g and 1.5 N/m²/year, in contrast to the 17.5 g N/m²/year applied in this study (Nordin et al. 2005). The high rate of application used here is justified in experimental approaches in order to determine whether a system will respond to treatment at any level. Having detected major responses at this high level of application, it would be beneficial in future studies to measure responses to nitrogen applied at a level that more realistically approximates future conditions.

In addition, the form of nitrogen applied may affect how plant communities respond. Nitrate (NO₃⁻) is normally rare in boreal forests and boreal plants get nitrogen primarily in the form of NH₄⁺ (Nordin et al. 2001). Recent evidence also suggests that organic nitrogen may also provide a substantial portion of the nitrogen requirements of boreal forest plants (Nasholm et al. 1998). However, because airborne nitrogen deposition from anthropogenic sources consists only of inorganic nitrogen, in approximately equal parts reduced form (NH₄⁺) and oxidized form (NO₃⁻), there could be a shift in the dominant nitrogen form in this system in the future if airborne deposition becomes a major source of nitrogen (Nasholm et al. 1998, Bobbink et al. 2010). An increase in NO₃⁻ could thus have a major impact on plant communities, as both total nitrogen availability and the dominant form of nitrogen are altered (Bobbink et al. 2010). As such, the excessive amounts of NO₃⁻ input in this experiment and the shift in dominant nitrogen form in fertilized plots from NH₄⁺ to NO₃⁻ may be an exaggerated version of predicted changes. If the dominant source of nitrogen for boreal plants shifts from NH₄⁺ and organic forms to NO₃⁻, then species that are better able to take up NO₃⁻ would gain an advantage (Gilliam 2006). This may have been a factor in this experiment; however data on the ability of each of the four focal species to take up various forms of nitrogen is not available.

Finally, this experiment included application of phosphorus or potassium, which may not accurately represent future conditions. Although phosphorus levels may increase slightly due to faster mineralization rates associated with warmer temperatures (Chapin et al. 1995), neither phosphorus nor potassium are predicted to increase to near the same degree as nitrogen. To more accurately mimic future conditions, this experiment should have only included nitrogen addition. However, because nitrogen is much more limiting in northern forests than phosphorus or potassium and fertilizer increased nitrogen levels in these plots far more than potassium or phosphorus, we feel justified in concluding that the effects of fertilizer addition observed here are primarily due to the nitrogen component of the fertilizer.

Limitations and Benefits of a Using a Long-term Study

As mentioned previously, the use of a long-term experiment in its 22nd year of treatment to answer new questions precluded several interesting avenues of investigation. Four main difficulties arose because treatments are already in place, plots cannot be disturbed and treatments have already substantially altered the plant community. First, fencing is no longer a relevant treatment due to the absence of evidence of herbivory effects on this understory community. Although it was still possible to draw valuable conclusions about the effects of fertilizer even given this second treatment, the presence of a fencing treatment unnecessarily complicated the analysis without adding useful information. Second, isolation of competition as a factor to separate the direct effects of fertilizer from indirect effects of shifts in competitive environment was not possible this far into treatment. Ideally, a subset of individuals of each species would have been isolated from above and below-ground competition for the duration of the experiment treatment using methods described in Arian and Turkington (2002). Third, harvesting roots of each species would have allowed for an examination of changes in allocation to root tissue as a result of fertilizer treatment. It is likely that some species are better able to divert resources away from roots and allocate biomass to above-ground growth when released from nitrogen limitation, which would give these species an advantage (Chapin 1980). However, the experimental plots used in

this study continue to be treated and monitored, so harvesting roots would have been too destructive to the long-term integrity of the plots. Finally, sampling after twenty-two years of treatment meant that it was not possible to include those species which were the least successful in fertilized conditions. These species (*Arctostaphylos uva-ursi*, *Lupinus arcticus* and *Linnaea borealis*) were the true “losers” of this experiment, as they were almost entirely eliminated from fertilized plots (Turkington et al. 2002). It is likely that conclusions drawn in chapter two regarding explanations for the success or failure of each species would have been even more apparent had these unsuccessful species been included in the analysis.

Despite the challenges of working in a long-term experiment, the benefits of using a well-designed ecological experiment maintained over multiple decades outweigh any drawbacks. The body of work conducted in these experimental plots provided invaluable information during the formulation of research questions and hypotheses. The observed changes in community composition and the disparity in success of various species that have been recorded over the past two decades were the starting point for this study (Turkington et al. 2002, DeKoning 2011). Invaluable insight into the competition dynamics (Arii and Turkington 2002) and the effects of fertilizer on the reproductive ecology of understory species in these plots were also provided by previous researchers (Nams et al. 1993, John and Turkington 1997, Fremlin et al. 2011). Furthermore, long-term studies also allow for an assessment of ecosystem response after a sustained, or “press” treatment, which can be a more realistic approximation of actual environmental change than short-term “pulse”-type treatments (Shaver et al. 2000). Responses detected on short time scales may be equalized or even reversed after years of treatment, and such long-term trends can only be observed by maintaining and monitoring experiments over many years (Shaver et al. 2000).

Future Directions

Moving forward from this study and from previous research at this site, there are several interesting avenues of investigation yet to be explored. In addition to increased nitrogen supply, northern

ecosystems are predicted to experience substantial warming in future decades (IPCC 2007).

Experimental warming has been shown to affect plant community composition and investment in sexual reproduction in northern ecosystems (Arft et al. 1999, Klady et al. 2011). In addition, warmer temperatures can also affect plants indirectly by increasing mineralization rates of soil nutrients, thereby increasing nutrient availability (Chapin et al. 1995, Rustad et al. 2001). Therefore, combining nutrient addition and warming treatments would more accurately mimic future climate change impacts (Chapin et al. 1995).

This community could also be analyzed for changes in the community values of functional traits over the 22 years of treatment (Laliberte et al. 2012). By assessing changes in average community values of functional traits such as height, SLA and growth rates, it would be possible to quantify the importance of these traits in determining species success under high nutrient conditions (Laliberte et al. 2012). This would provide a more complete assessment of how important various traits are in a nutrient-enriched environment by allowing for the inclusion of those species that have been eliminated from fertilized plots.

Conclusions

In this study I used a long-term fertilizer experiment to investigate why some species are more successful than others under nutrient enrichment and to determine how long-term fertilizer treatment affects investment in sexual reproduction in four herbaceous understory species. Although this study also included a herbivore exclusion treatment, almost no effects of excluding herbivores were detected, so the focus here will be on fertilizer effects. The main results of this study were:

1. *Mertensia paniculata* and *E. angustifolium* were the most successful species under fertilized conditions, while *A. millefolium* did not change in abundance when fertilized and *F. altaica* declined.
2. All four focal species displayed some traits or responses that were beneficial in fertilized (nutrient-rich, light-limited) conditions.

3. Rather than one strategy or trait determining success under fertilized conditions, the two successful species displayed different combinations of advantageous traits that allowed them to succeed: *M. paniculata* had high SLA that increased when fertilized whereas *E. angustifolium* had a tall stature and increased allocation of biomass to leaves when fertilized.
4. Fertilizer increased the probability of flowering for *A. millefolium*.
5. Of the two species that could be analyzed for changes in allocation to sexual reproduction at the individual plant level, only *E. angustifolium* was affected by fertilizer, increasing allocation to flower parts.

Overall *E. angustifolium* appears to have the best combination of traits and responses to compete in nutrient-enriched conditions; it has successfully outcompeted neighbours in fertilized, light-limited areas by growing taller and increasing allocation to leaves more than other species, and it will potentially be able to colonize new areas in the future by increased investment in sexual reproduction.

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