SEXUAL REPRODUCTIVE PROCESSES OF PLANTS IN AN ALPINE TUNDRA ENVIRONMENT

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ABSTRACT

Sexual reproduction is an important mechanism shaping plant community composition that will likely be affected by unprecedented rates of climate change in Canada's North. To anticipate potential changes in plant communities, I aim to understand how changing environmental conditions affect the processes of seed production and seedling emergence, and determine the overall impacts on the reproductive potential of alpine tundra vegetation in Yukon, Canada. I tested the effect of soil warming and nitrogen addition treatments on the timing and success of sexual reproduction of the six tundra species; *Dryas octopetala* M. Vahl, *Salix arctica* Pall, *Salix reticulata* L., *Lupinus arcticus* L., *Carex microchaeta* Holm, and *Hierochloë alpina* (Sw.) R. & S. A summer snow event occurred on 2 July 2012, and I considered the impacts of such an event on the reproductive timing and success of the study species. I also examined the influence of seed availability and soil conditions on initial seedling emergence of three tundra species and three boreal species. I applied seed to natural disturbance sites with bare substrate exposed, and to plots with altered soil temperature and nitrogen availability. Results indicated that reproductive phenology, seed production, and seed viability of tundra species were not affected by increases in soil temperature and/or nitrogen availability but were impacted by the snowfall event. In addition, changes in soil temperature and nitrogen did not affect seedling emergence. Seedling emergence of both boreal and tundra species increased on bare substrates, indicating that surface disturbance creates opportunities for seedling establishment. Overall, my study shows that factors affecting seed production and local disturbance will have greater impact on the success of sexual reproduction in tundra plant communities than changes in soil temperature or nutrients caused by climate change.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

This thesis is a compilation of two manuscript-style chapters based on data collected and analyzed throughout my degree. The two data chapters are preceded by a general introduction and are followed by a concluding chapter.

1.0 INTRODUCTION

1.1 Plant population dynamics and the importance of environmental conditions

The dynamics of a plant population are driven by individual births and deaths along with some degree of spread or movement. The processes and mechanisms used in a plant population to ensure long-term survival are unique due to the sessile nature of plants. First, the immobility of individual plants leaves the population susceptible to environmental disturbances. These disturbances can alter plant population dynamics by causing death, inducing plant recruitment, or altering abiotic conditions. Second, vegetative growth can affect processes such as reproduction, competition, and relative abundance of an individual within a community. Vegetative growth can also include asexual reproduction, which is a reproductive strategy in plants. Third, the sessile nature of plants creates interesting challenges in how genetically distinct individuals are introduced into the population. Sexual reproduction is a key mechanism responsible for producing new individuals and includes the processes of flowering, pollination, and seed production. Seeds contribute new genetic diversity to the population and seed dispersal allows colonization into new areas (Crawley and Ross 1990).

Environmental conditions strongly influence the success of each stage of the sexual reproductive cycle in arctic and alpine plants (Arft *et al.* 1999). For example, environmental cues such as temperature and photoperiod trigger the initiation of flowering in many species at the beginning of the season (Körner and Basler 2010). Early season snow melt with its impacts on the plant's reproductive organs, can affect the abundance of flowering during the summer season (Inouye 2008). In later stages of reproduction, in order to germinate and successfully emerge, seeds have strong requirements for temperature and moisture of the seedbed (Eriksson and Ehrlén 1992).

Therefore, changes to environmental conditions involving temperature, moisture, and nutrient availability, such as those expected with continued climate change, can strongly influence reproductive success.

Seed production, availability, and dispersal strongly control individual establishment and the reproductive potential of a plant population (Fenner and Thompson 2005). Seed availability is controlled by the soil seed bank and seed dispersal. Seeds can be stored in the soil and soil disturbances can expose stored seeds, making them available for germination (Cooper *et al.* 2004). For example, following a large environmental disturbance such as fire, the post-fire regeneration will mostly rely on seed availability in the surrounding environment (Turner *et al.* 1999). Smaller disturbances such as the burrowing of small mammals can increase seed availability through disrupting the soil seed bank (Cooper *et al.* 2004) or can alter local seed production due to changes in abiotic conditions (Thorn 1982, Chambers 1995). Local seed dispersal ensures the maintenance of a population through the establishment of new individuals, whereas long distance dispersal allows the introduction of individuals to new areas and can facilitate species' range expansion across the landscape (Klanderud and Totland 2007, Hampe 2011). It is critical that we understand the processes of seed production and availability in order to understand how changes in environmental conditions may affect plant population dynamics.

1.2 Climate change and its impacts on plant populations

It is estimated that global mean annual temperatures have increased by approximately 0.18 °C per decade over the last 50 years (Hartmann *et al.* 2013). However this global increase has not been uniform and climate has changed most

rapidly in northern latitudes where an increase of approximately 0.40 °C per decade (1966-2003) in mean annual air temperature has been experienced. In northwest regions of the arctic, including the Yukon, rates of increase have been recorded up to approximately 2.2 °C per decade (McBean *et al.* 2005, Prowse *et al.* 2009). This change in climate has brought about changes in the carbon balance of tundra ecosystems (Oberbauer *et al.* 2007), the frequency of permafrost disturbances (Walker *et al.* 2008), and arctic and alpine species diversity (Sala *et al.* 2000). The growth and reproduction of arctic and alpine vegetation have been observed to be highly sensitive to changes in climate especially increases in temperature and nutrient availability (Chapin *et al.* 1995). Increases in temperature can have direct affect on a plant's physiological processes and thereby increase photosynthesis, growth rate, and nutrient uptake (Bazzaz *et al.* 2000). Additionally, increases in temperature can indirectly affect a plant's growth by increasing the availability of limiting nutrients in the soil by stimulating increases in microbial decomposition and mineralization (Nadelhoffer *et al.* 1992, Jonasson *et al.* 1999). Consequently, arctic and alpine plants are expected to continue responding to climate change through altering their vegetative growth, phenology, sexual reproduction, and ultimately their range distributions.

Projected temperature increases in northern ecosystems are also expected to be accompanied by increases in unusual or extreme weather events (Hartmann *et al.* 2013). Current research and knowledge of basic ecological and physiological processes provides clear evidence that natural systems should be strongly influenced by extreme weather events (Easterling *et al.* 2000). Short-term extreme events, such as drought, temperature extremes, and frost, can be mechanistic drivers of broad ecological responses (Parmesan *et al.* 2000). Responses to extreme events first felt at the

population level can drive gradual range shifts of species, as has been well documented for species of Lepidoptera (butterflies and moths) and migratory birds (Parmesan *et al.* 2000). Significant and detrimental impacts of frost and snowmelt on subalpine flower phenology have been reported and have the potential to result in demographic changes in populations (Inouye 2008). While focused research is difficult because impacts of extreme events on ecosystems are disproportionate relative to their short duration, impacts of extreme events need to be documented. Including extreme events in predictive models will improve predictions of future impacts of climate change on ecosystems (Jentsch *et al.* 2007).

1.3 Tundra vegetation in a changing climate – potential impacts

The importance of sexual reproduction in tundra plant populations is underestimated because of the generalization that tundra species primarily reproduce vegetatively. This oversimplification suggests that cold, harsh tundra environments constrain successful sexual reproduction by keeping seed production low and limiting the establishment of new individuals from seed (Chambers 1995, Körner 2003). However, it has been shown that allocation to sexual reproduction, particularly seed output, in tundra plants is similar to that in temperate species (Chester and Shaver 1982). A study of population demographics on the long-lived alpine species *Geum reptans* (Rosaceae) found that the overall contribution to population growth was equal between vegetative and sexual reproduction (Weppler *et al.* 2006). The contribution that each reproductive strategy makes to overall population growth is strongly influenced by weather conditions, with sexual reproduction occurring with favourable weather, and vegetative reproduction dominating in less favourable weather conditions (Weppler *et al.*

2006). These observations highlight the influence that weather conditions in a given year have on plant reproduction, and that we need to recognize the dynamics of sexual reproduction in order to understand how climate conditions may affect population dynamics.

Sexual reproduction in arctic and alpine tundra plants has adapted to short growing seasons, low summer temperatures, and low soil nutrients (Billings and Mooney 1968, Billings 1974). The trait of perennial growth is common in tundra species as it allows for rapid growth and enables plants to take advantage of good conditions early in the growing season, even when temperatures remain low (Billings and Mooney 1968, Billings 1974). Preformation, the development of flower buds in the years prior to emergence, is also widespread and allows plants to compensate for unfavourable conditions in the current year (Billings and Mooney 1968). However, tundra plants are still living in conditions well below their temperature optima for reproduction and even small changes in the physical environment can lead to significant changes in reproductive success (Svoboda and Henry 1987).

Plants' responses to changes in environmental conditions will not be similar across all species (CaraDonna *et al.* 2014). Changes in the physical environment, such as a longer growing season, extension of the snow-free period, and increased nutrient availability, could alter species competitive abilities resulting in changes in community composition (Klanderud and Totland 2007) or local species extinctions (Danby *et al.* 2011). Moreover, it has been observed that species endemic to the upper boreal zone can survive in arctic and alpine tundra especially where increases in temperature are experienced (Holtmeier and Broll 2005).

We still lack a comprehensive understanding of the relative impacts that long-term climate change and extreme events will have on the reproductive potential of tundra species or on the potential invasion of boreal species. Understanding the dynamics of both long-term warming and short-term unusual events on arctic and alpine ecosystems will enhance our ability to predict changes in primary production, nutrient cycling, and local species diversity (Jentsch *et al.* 2007, CaraDonna *et al.* 2014).

1.4 Objectives

The goal of the research presented in this thesis was to investigate how climate-driven environmental changes may affect sexual reproduction in an alpine tundra plant community. This research was conducted through field observations of an experimental study in the alpine region of the Wolf Creek drainage basin in Yukon, Canada. The site is part of a long-term study monitoring plant responses to manipulations in soil temperature and nitrogen availability (Johnstone *et al.* 2013). Currently, this is the only study conducted at this experimental site that looks at sexual reproduction in detail. Research presented in this thesis will contribute to the growing scientific knowledge of how climate driven changes affect reproduction and productivity of vegetation in the local area (Pieper *et al.* 2011, Allen 2012).

My research focused on two questions of how environmental factors may control sexual reproduction in an alpine tundra community. Firstly, how do soil warming and nitrogen addition treatments affect the timing and success of sexual reproduction? In Chapter 2, I summarize observations of flowering and seed production of six alpine tundra species; *Dryas octopetala* M. Vahl (mountain avens), *Salix arctica* Pall (arctic willow), *Salix reticulata* L. (net–leaved willow), *Lupinus arcticus* L. (arctic lupine), *Carex*

microchaeta Holm (alpine tundra sedge), and *Hierochloë alpina* (Sw.) R. & S (sweet grass). I hypothesized that under the experimental treatments, species would advance or prolong their stages of reproductive phenology and would increase their reproductive outputs. A summer snow event occurred on 2 July 2012, and I considered the impacts of such an event on the timing and success of the study species. Secondly, how do seed availability and changes in soil conditions affect initial seedling emergence in alpine tundra? In this study I refer to initial seedling emergence as the presence of a seedling within its first growing season (*i.e.* the seed has germinated, the initial cotyledons have emerged, and the seedling has not yet experienced a winter season). In Chapter 3, I summarize observations of initial seedling emergence on naturally occurring disturbance sites (frost boils) and experimental manipulations of soil warming and nitrogen addition. I hypothesized that if initial seedling emergence was affected by soil conditions related to disturbance, temperature, or nitrogen availability, then I would observe increased seedling densities in these treatments. I monitored seedling occurrence under natural seed rain and with experimental seed addition of the tundra species *Dryas octopetala*, *Salix arctica*, *Lupinus arcticus* and the boreal species *Pinus contorta* Dougl. Ex Loud. (lodgepole pine), *Picea glauca* (Moench) Voss s.l. (white spruce), and *Alnus crispa* (Drylander ex Ait.) (green alder). Finally, in the concluding chapter, I summarize findings from Chapter 2 and 3 to assess the role climate and environmental factors had on influencing the reproductive potential of *Dryas octopetala*, *Salix arctica*, and *Lupinus arcticus* in this tundra environment.

This research provides much needed information on sexual reproduction and the reproductive potential of species in alpine tundra ecosystems. Results from these field studies provide information on how climate and environmental factors influence sexual

reproduction and the complexities of reproductive ecology in tundra ecosystems. By better understanding the dynamics of sexual reproduction in tundra vegetation we will be better at predicting outcomes of climate change.

2.0 FLOWERING AND SEED PRODUCTION OF ALPINE TUNDRA PLANTS

2.1 Introduction

Climate is predicted to change rapidly in the next century in northern latitudes (McBean *et al.* 2005, Hartmann *et al.* 2013). Warming air temperatures along with changes in environmental conditions, such as increased soil temperatures and nutrient availability, have the potential to impact alpine and arctic species diversity (Sala *et al.* 2000). In fact, significant changes in arctic and alpine vegetation have been observed in northern locations, such as Alaska and Yukon, where substantial warming has been observed (Chapin *et al.* 1995, Danby *et al.* 2011). The environmental conditions found on the tundra, such as short growing seasons and low nutrient availability, have limited the diversity of local species and strongly influenced life history and physiological traits of the vegetation (Billings 1974, Chambers 1995). The flora of the tundra consists mostly of perennial cushion and rosette plants, forbs, graminoids, and dwarf deciduous and evergreen shrubs (Billings 1974). However, these species face many challenges as environmental conditions. Changes in the physical environment may also create opportunities for non-native species to invade and expand their ranges into the tundra. Sexual reproduction is a key mechanism that enables plants to respond to new environmental conditions and can play a strong role in changing community composition (Bruun and Ejrnæs 2006, Weppler *et al.* 2006, Walck *et al.* 2011). In order to better predict potential changes in tundra plant communities, it is important that we gain a better understanding of how sexual reproduction may allow plants to respond to changes in climate and environmental conditions.

Reproductive phenology is an important component of a species' life history that has evolved to rely directly on environmental cues, particularly temperature, to detect

optimal conditions for successful reproduction (Inouye 2008, Chuine 2010, Körner and Basler 2010). These environmental cues have proven reliable, but as climate and weather conditions change plants must be able to respond accordingly. Increased air temperatures have caused earlier snow pack melt resulting in advanced onset of bud burst and flowering and an extended duration of flowering in numerous tundra species (Arft *et al.* 1999, Inouye 2008). However, flowering too early in the growing season increases the risk of frost damage to the highly sensitive reproductive organs. Frost damage to flowers and loss of seed production can lead to demographic changes in the population due to lack of recruitment from seed (Inouye 2008). Understanding the effects associated with frost events may affect reproductive phenology and seed production of tundra species will allow us to better predict changes in community composition on the tundra.

Climatic and environmental conditions strongly affect reproductive effort (the investment in reproductive tissues) and reproductive success (the final outcome of that investment) of tundra species (Bell and Bliss 1980, Molau 1993). Temperatures can limit resource availability, such as nitrogen, and allocation of these resources to reproduction. The allocation of limited resources to reproductive tissue reflects evolved reproductive strategies and the physiological compromises that occur within the plant (Lovett Doust 1989). These compromises are also known as ‘trade-offs’ and are important considerations when looking at the reproductive potential of species. One of the most studied trade-offs is between seed mass and number of seeds produced (Moles and Westoby 2004b). For example, increases in air temperature and soil nitrogen availability have lead to increased flower production, pedicel lengths, seed set and viability of the seeds produced by the species *Dryas octopetela* (Wookey *et al.* 1995, Klady *et al.*

2011). Changing environmental conditions can affect how a plant allocates its resources to reproductive effort, however these changes will not occur uniformly across all species (Arft *et al.* 1999, Klady *et al.* 2011). Changes to a plants' ability to allocate resources, especially those related to ensuring seed viability, will likely alter species-specific reproductive success, resulting in changes in population demography and potentially community composition in a rapidly changing climate.

In this study, I investigated how climate-induced environmental changes may affect reproductive success of six plant species in the alpine tundra of the Wolf Creek watershed, southwest Yukon. I used manipulations of soil temperature and nitrogen availability to test how direct effects of warming on the soil environment may alter reproductive phenology and seed production given predicted long-term changes in climate. My fieldwork coincided with an unusual snow event on 2 July 2012, and I used this as an opportunity to assess the effects of an extreme snow event on reproductive timing and success of the study species. The six species were representative of a variety of life forms and I examined how the trade-offs of their reproductive strategies may play a role in determining their success in future environmental conditions.

2.2 Methods

2.2.1 Study site

This study was conducted in an alpine region in southern Yukon, Canada (60°33′46.4″ N, 135°07′55.0″ W; elevation 1565 m.a.s.l.), approximately 20 km south of Whitehorse (Figure 2.1). The vegetation at low elevations is dominated by boreal forest. At high elevations, including the study site location, vegetation is dominated by low-shrub alpine tundra. Plants grow close to the ground to escape harsh winter winds.

Vascular plants, composed of woody and deciduous shrubs along with various forb and herbaceous species, cover approximately 40% of the area (Pieper *et al.* 2011). Lichens, mosses, rock and bare soil areas make up the remaining area. Common species include the dwarf evergreen *Dryas octopetala* M. Vahl (mountain avens), the dwarf deciduous shrubs *Salix arctica* Pall (arctic willow) and *Salix reticulata* L. (net–leaved willow), the herbaceous species *Lupinus arcticus* L. (arctic lupine), and the graminoids *Carex microchaeta* Holm (alpine tundra sedge), and *Hierochloë alpina* (Sw.) R. & S (sweet grass).

The area experiences a subarctic climate characterized by large seasonal variations in temperatures and low precipitation (Wahl *et al.* 1987). Temperatures in the summer months (June-August) range from monthly means of 5 to 15°C and in the winter months (December-February) range from monthly means of -10 to -20 °C (Environment Canada 2013). Mean annual precipitation is 300 to 400 mm with approximately 40% of it falling as snow (Janowicz 1999).

2.2.2 Experimental manipulations

The study area was located on a gently sloping (~3°), south-facing ridge, and covered a total area of 30 m x 50 m across homogeneous low-shrub tundra vegetation. Experimental manipulations at this field site began in 2011. Soil warming and nitrogen fertilization treatments were applied as a factorial design with 6 replicates of each treatment (including control conditions). The 24 plots (1.0 m x 1.0 m) were arranged in a randomized block design (Figure 2.2). Blocks (n= 6) were arranged parallel to the slope contours to account for any effect due to slope (Johnstone *et al.* 2013). Blocks have not been seen to have any significant effect (Pieper *et al.* 2011, Allen 2012), therefore they

were not considered in this study.

Warming of near-surface soil temperatures was done using 100 vertical electrocuting probes placed in each of the warming plots (Johnstone *et al.* 2013). The soil heating probes were inserted to a depth of 15 cm. The heating system was operational only during the summer snow-free period, starting 24 May until 1 September 2012. Average soil temperatures in six warmed and six control plots were collected from thermocouples and recordings were made every 15 minutes during the season using a Campbell Scientific CR1000 data logger (Johnstone *et al.* 2013).

Nitrogen fertilizer was applied as granular pellets of ammonium nitrate (NH_4NO_3) at a rate of 2 g N/m^2 of nitrogen. This rate of nitrogen application reflects a doubling of average nitrogen mineralization found in control plots (1.05 g N/m^2 as described by Rustad *et al.* 2001) and the potential increase in nitrogen mineralization in tundra soils as a result of climate warming (Hartley *et al.* 1999, Rustad *et al.* 2001). Fertilizer was sprinkled over the fertilization plots and then gently shaken off the vegetation to land on the ground surface. Application of the fertilizer treatment was done once a year at the beginning of the snow-free season (early- to mid-June). The effectiveness of the fertilization treatment to add available nitrogen to the soil was assessed using resin beads (R276-500 Rexyn 300 (H-OH) Beads, Fisher Chemical). These resin beads act as cation and anion exchangers in the soil, taking in a measure of the available nitrogen present (Binkley *et al.* 1986). Two bags of resin beads were carefully buried in each plot (causing as little disruption to the vegetation as possible) at the beginning of the summer season (9 June 2012) and pulled out late in the summer (9 August 2012). All resin bags were processed and analyzed for NH_4 and NO_3 concentrations by the British Columbia Ministry of Environment laboratory in Victoria, B.C.

At the nitrogen dosage site, granular pellets of ammonium nitrate fertilizer were added at different rates; 1 g/m^2, 2 g/m^2, 4 g/m^2, 8 g/m^2, and control (no nitrogen addition). Application of the fertilizer was carried out in the same manner as at the experimental site (see above). Twenty 1 m x 1 m plots were arranged in a randomized block design (Figure 2.2A). Blocks (n= 4) were arranged in two lines parallel to the slope contours to account for any effect due to slope (Johnstone *et al.* 2013). The effectiveness of these treatments to add available nitrogen to the soil was assessed in the same manner as at the experimental site (see above).

2.2.3 Target species, field measurements, seed collection and germination trials

I studied the reproductive ecology of six alpine tundra species. Species examined were: *Salix arctica* Pall (arctic willow), *Salix reticulata* L. (net–veined willow), *Carex microchaeta* Holm (alpine tundra sedge), *Lupinus arcticus* S. Wats. (arctic lupine), *Dryas octopetala* L. (mountain avens), and *Hierochloë alpina* (Sw.) R. & S. (alpine sweet grass). In selecting the target species, I considered the following: a) capture the most widespread or dominant species that would have representation in the majority of plots at my study site, b) encompass a range of plant growth form types, and c) keep in mind logistic constraints and only select a manageable number of species for measurement.

Salix arctica and *Salix reticulata* (Figure 2.3A and B) are circumpolar in distribution across arctic and alpine tundra, and are frequent throughout Yukon (Cody 2000). They are prostrate deciduous dwarf shrubs with branches growing underneath or along the ground surface (Cody 2000). Vegetative reproduction occurs through clonal growth and the spread of vegetative ramets. *S. arctica* and *S. reticulata* are dioecious – distinct male and female plants. Sexual reproduction occurs through the formation of

male and female catkins. Female catkins of *S. arctica* and *S. reticulata* are dense and many-flowered and grow on leafy peduncles (Molau and Edlund 1996). Ovaries swell once fertilized and seed dispersal occurs when capsules split open. Many tiny seeds attached to a wreath of fine hairs are released and dispersed via wind (Molau and Edlund 1996). Seeds of *Salix* species are usually the first of the tundra species to be dispersed and natural dispersal has been observed to occur in mid-August (Densmore and Zasada 1983).

Carex microchaeta (Figure 2.3C) is common in alpine and subalpine zones across the circumpolar arctic. It is a perennial, tufted graminoid that grows from short, stout rhizomes. An individual produces 2 to 5 spikes, the terminal one with male flowers and the lower spikes with female flowers. Seeds are narrow egg-shaped perigynia that are reddish-brown to purplish-black (Tande and Lipkin 2003). Seed maturation period normally lasts 6-8 weeks or 42-56 days (Wagner and Reichegger 1997). Dispersal of seed is often facilitated by strong winds.

Dryas octopetala (Figure 2.3D) is circumpolar in distribution and is a dominant vascular plant species at many arctic and alpine tundra locations (Molau and Edlund 1996), including locations across Yukon (Cody 2000). *D. octopetala* is a woody dwarf evergreen shrub that grows in dense vegetative matts. Vegetative reproduction occurs through colonial growth and the spread of vegetative ramets (Wookey *et al.* 1995). The species reproduces sexually via showy white (or pale yellow) flowers that occur on a solitary, bare pedicel (Cody 2000). Seeds are plumed achenes. Dispersal occurs in late fall once seeds have fully matured and are usually dispersed by wind (Molau and Edlund 1996).

Lupinus arcticus (Figure 2.3E) is a native endemic species to north-west North America and is found throughout Yukon (Cody 2000). It is found in alpine tundra environments but also makes up a component of the understory vegetation in boreal forests. *L. arcticus* is in the family Fabaceae (legumes). Plants of *L. arcticus* produce nodules on their roots and have the capabilities to fix nitrogen in symbiosis with bacteria. However, the species' ability to fix nitrogen and the rate of fixation in tundra soils are not well understood. *L. arcticus* is an herbaceous perennial and grows in clonal clumps. One to several racemose inflorescences emerge from the clonal clump surrounded by many basal leaves. Each inflorescence bears ten to twenty flowers that yield legume pods that each contains five to ten seeds and seed numbers can differ between years. When seeds are mature, pods dry out and explode to disperse seeds. Natural seed dispersal is reported to occur from late July till late August. Seed pods of *L. arcticus* are an important part of the diets of many local herbivores (Fremlin *et al.* 2011) and this is the context in which it is most often studied.

Hierochloë alpina (Figure 2.3F) is common in alpine and subalpine zones across the circumpolar arctic, including throughout Yukon (Cody 2000). *H. alpina* is a perennial, tufted graminoid that grows from short rhizomes. Stems are erect, hollow, and individuals are usually 15-40 cm tall. The inflorescence of *H. alpina* is a contracted panicle. Each spikelet is 3-flowered with a single central female floret with 2 sterile or male lower florets (Cody 2000). Seeds are wind dispersed when mature. Natural seed dispersal can occur in the fall, but partially developed inflorescences have been shown to survive through the winter. This would indicate that continued seed maturation and dispersal could occur the following spring (Hodgson 1966).

The reproductive variables that I collected for each species can be found in Table 2.1. I collected phenology and reproductive output measurements following standardized protocols for the species *S. arctica*, *S. reticulata*, and *D. octopetala* (Molau and Edlund 1996). The species *L. arcticus, C. microchaeta*, and *H. alpina,* were not included in these protocols, so I made slight modifications (see below) to the protocols set out for other forbs, sedges, and grasses*,* respectively. Other studies have used similar modifications (Pieper *et al.* 2011). All data collected on the reproductive variables in the control plots can be found in the TRY plant trait database (www.try-db.org).

I observed reproductive phenology at the experimental site for *S. arctica*, *S. reticulata*, *C. microchaeta, L. arcticus*, and *D. octopetala*. The phenology of *H. alpina* was not followed due to the inherently difficult nature of observing flowering in grass species. I observed plots at least every two days when possible. A snowfall event occurring on 2 July (day 184), created difficulties in obtaining observations over a 4 day period at the beginning of July. For each species, I noted the reproductive stages that were present among individuals in each plot (Molau and Edlund 1996). I counted individuals and assigned them to the corresponding reproductive phenological stage. The number of individuals at each stage was counted at least every two days, creating frequency counts and allowing for determination of peak event dates. The key phenological stages were then used to define onset, peak, and end dates and duration of two key periods; flowering period and seed maturation period. Key phenological stages were summarized into flowering and seed maturation periods to facilitate comparison among species (Table 2.2).

For each species, I counted the number of inflorescences in a 1.0 m x 1.0 m plot (density). I then standardized the count value using percent cover data for that plot and

recorded a relative density value. The percent cover data used was collected by point-intercept method by Jill Johnstone in the summer of 2010. I defined an inflorescence for each species as follows: catkins for *S. arctica* and *S. reticulata* (only female catkins are reported because they produce the seeds), an individual flower for *D. octopetala*, full flowering individual for *C. microchaeta* and *H. alpina*, and an entire racemose inflorescence for *L. arcticus*.

I measured inflorescence length for each species in every plot where present. I randomly selected five individuals per plot and measured each to the nearest millimetre. I defined inflorescence length as follows: the length of the maturing female catkin from the axil of the subtending leaf for *S. arctica* and *S. reticulata*, the length of the pedicel (from the axil to the base of the flower) for *D. octopetala*, the length of the flowering stem to the base of terminal spike for *C. microchaeta*, the length of the flowering stalk for *H. alpina*, and the length of the inflorescence from the base of the individual to the top of the flowering section for *L. arcticus*.

Seeds were collected from each plot for all six species. The exact collection day differed between species because time to maturity is different for each. However, collection of seed for a single species was done from every plot on the same day regardless of whether the maturity of seed differed between plots. When I observed natural seed dispersal, I determined seed to be mature and collected them on or as near to that time as I could. All seeds were collected by the end of August. Seeds were air dried for 24 hours and then oven dried at 40 ºC for 24 hours. They were then frozen at approximately -18 ºC until further processing could take place.

Seeds were processed in January 2013 in the lab at the University of Saskatchewan. I removed the plumes and hairs from the seeds of *D. octopetala*, *S.*

reticulata and *S. arctica,* respectively. *L. arcticus* seeds were removed from their pods. *C. microchaeta* and *H. alpina* were removed from spike and husk, respectively. Once processed, seeds were weighted (using a Mettler Tuledo PB303 scale) and counted. Seeds were placed back into the freezer until germination trial could be run.

Germination trials were conducted on a per plot basis. Seeds were placed in a 9 cm plastic Petri dish with two layers of filter paper. The filter paper was initially moistened with de-ionized water and was moistened as needed through the duration of the trials. The number of seeds in each dish differed between plots, but no more than 100 seeds of the small seeded-species were placed in a single Petri dish. Seeds were arranged so that they were not touching each other or the edge of the dish. I randomly arranged dishes under fluorescent lights (18 hours light, 6 hours dark) and randomly re-shuffled them at least every second day. The room was not temperature controlled, but was approximately 20 ºC. I considered a seed to have germinated when the radicle was approximately twice the length of the seed coat. Germinated seeds were counted and removed at least every second day. I terminated germination trials when no germinated seeds were found for five consecutive days (trials lasted approximately two weeks).

2.2.4 Data analysis

All statistical analyses and graphing were performed in R (R Development Core Team 2013). An α-level of 0.05 was used to assess statistical significance. All graphing was done using the package ‘ggplot2’ (Wickham 2009).

To assess the effectiveness of the warming treatment, I analyzed temperature data from six warmed and six controlled plots. For each plot, I used the daily mean, maximum and minimum temperatures and averaged each measurement for the summer

period (1 June – 24 August). Analysis of variance (ANOVA) was used to assess if soil temperature had changed significantly with the warming treatment. This procedure was done using the base R package ‘stats’ (R Development Core Team 2013).

Nitrogen concentrations from the two resin bags were averaged per plot. NH_4 and NO_3 concentrations were log transformed to improve normality. To assess the effectiveness of the nitrogen treatment, NH_4 and NO_3 concentrations were analyzed using ANOVA in a mixed-effects model with block as a random factor. This procedure was done using the R package ‘nlme’ (Pinheiro *et al.* 2011).

Examining the effects of the experimental manipulations on plant responses was inherently difficult in this study. The small number of replicates of each manipulation (maximum replicates per treatment were n= 6 at experimental site, and n= 4 at the nitrogen dosage site), along with the low occurrence of individuals of each species in the plots limited my ability to statistically detect effects. I only attempted a statistical test when there were data for at least half the replicates per treatment (n= 3 at experimental site, n= 2 at nitrogen dosage site). I used the conservative non-parametric Kruskal-Wallis rank sum analysis to test for significant plant responses in phenology and reproductive outputs (relative density, inflorescence length, number of seeds per inflorescence, seed weight, and germination). All relationships between the reproductive outputs were tested using linear regression. The Kruskal-Wallis tests and the linear regression models were conducted in the base R package ‘stats’ (R Development Core Team 2013).

Table 2.1: Measurements of reproductive outputs collected for six tundra species in this study.

Species	Phenology	Inflorescence length	# seeds per inflorescence	Seed weight	Germination	Relative reproductive density
Dryas octopetala	X	X	X	X	X	X
Lupinus arcticus	X	X	X	X	X	X
Salix arctica	X	X	X	X	X	X
Salix reticulata	X	X	X	X	X	X
Carex microchaeta	X	X	X	X	X	X
Hierochloë alpina		X	X	X		X

Table 2.2: Definitions of flowering and seed maturation phenoperiods of five tundra species based on dates featuring key observable phenological stages.

Species	Date	Flowering Period		Seed Maturation Period
		Female	Male	
Salix arctica	*Onset*	<176*	<176*	First female catkin with swollen capsule
	Peak	Maximum # female catkins with stigmas visible	Maximum # male catkins with pollen visible	Onset of seed dispersal (capsules dehiscing)
	Finish	Maximum # catkins with capsules swollen	Maximum # males with all pollen shed	*Collection date* +
Salix reticulata	*Onset*	First female catkin with stigmas visible	<176*	First female catkin with swollen capsule
	Peak	Maximum # female catkins with stigmas visible	Maximum # male catkins with pollen visible	Onset of seed dispersal (capsules dehiscing)
	Finish	Maximum # catkins with capsules swollen	Maximum # males with all pollen shed	*Collection date* +
Carex microchaeta	*Onset*	First individual with stigmas visible	First individual with anthers exposed	First individual with fruit developing
	Peak	All individuals with stigmas visible	All individuals with stigmas visible	All individuals with fruit developing
	Finish	All individuals with fruit developing	All individuals with fruit developing	*Collection date* +
Lupinus arcticus	*Onset*	First inflorescence with at least 1 open flower		First inflorescence with first pod developing
	Peak	Maximum # of inflorescences with at least 1 open flower		Maximum # of inflorescences with at least 1 pod developing
	Finish	Maximum # of inflorescences with final petal lost		*Collection date* +
Dryas octopetala	*Onset*	First open flower		First view of twisting styles
	Peak	Maximum flower count		Maximum view of twisting styles
	Finish	Maximum petal loss		*Collection date* +

* I did not observe the onset of this event as it occurred prior to the first observations made on Julian day 176

+ Collection date occurred on or near natural seed dispersal in the surrounding area and defined the end of the seed maturation period

2.3 Results

2.3.1 Experimental manipulations

Soil temperatures recorded at 10 cm depth during the summer months of 2012 (1 June to 24 August) were significantly higher in warmed plots compared to control plots. Mean daily temperatures were on average 1.16 ± 0.23 ºC (mean ± S.E.) higher ($F= 10.88$, $p= 0.008$, $n= 6$), while maximum temperatures were 1.51 ± 0.34 ºC higher ($F= 6.83$, $p= 0.026$, $n= 6$) and minimum temperatures were 0.77 ± 0.15 ºC higher ($F= 12.58$, $p= 0.004$, $n= 6$) in warmed plots compared to controls. NO_3 concentrations in the soil were significantly increased in the fertilized plots but fertilization had no significant effect on NH_4 concentrations (Figure 2.4, Table 2.3). The warming treatment did not have a detectable effect on NH_4 or NO_3 in the plots meaning there was no direct or interactive effect of warming on nitrogen availability (Table 2.3).

At the Nitrogen dosage site, NO_3 concentrations in 2012 were significantly increased with all levels of fertilization and NH_4 concentrations were significantly increased only in the highest fertilization dosage level of 8 g/m^2 (Figure 2.5; Table 2.4). Observations on plant responses (outputs) were pooled together for the two sites to increase replication for within- and among-species comparisons.

2.3.2 Flowering and seed maturation phenoperiods

Flowering dates varied among species (Figure 2.6), but most were not clearly affected by experimental soil warming and nitrogen fertilization (Table 2.5). *Salix* species were the earliest to flower with average peak flowering of both males and females occurring in the last week of June and the first week of July for *S. arctica* and *S. reticulata*, respectively. *Carex microchaeta* was also an early flowering species, with

average peak flowering of both males and females occurring in the first week of July. *Lupinus arcticus* and *Dryas octopetala* were later flowering species with average peak flowering occurring in the second week of July.

Flowering success for most species was not clearly affected by plot-level variations in the timing of peak flowering (Figure 2.9). *D. octopetala* was the only species to show significant increase in flowering success when peak flowering occurred later in the season (Figure 2.9E; linear regression). *L. arcticus* experienced a low flowering success regardless of timing of peak flowering. The earlier flowering species, *S. arctica*, *S. reticulata* and *C. microchaeta,* had high flowering success regardless of timing of peak flowering.

Timing and duration of seed maturation periods varied between species (Figure 2.6) and were also not clearly affected by experimental soil warming and nitrogen fertilization (Table 2.5). The onset of seed maturation began within a week after peak flowering for all species, except *D. octopetala*, where onset began approximately two weeks after peak flowering. The duration of the seed maturation period (onset date to seed dispersal date) was between 26-32 days for all species studied, with the exception of *C. microchaeta* (44 days). Seed dispersal of *S. arctica* and *S. reticulata* occurred earliest in the season with average onset of seed dispersal occurring in the last week of July and the first week of August, respectively. Seed dispersal occurred for all of *L. arcticus*, *D. octopetala,* and *C. microchaeta* during the last week of August.

Success of the seed maturation phenoperiod was not clearly affected by timing of peak seed maturation (Figure 2.10). Most species did not show any change in the number of seeds per inflorescence or germination success given timing of peak seed maturation. *D. octopetala* was the only species to show a significant decrease in the

number of seeds produced per inflorescence when peak seed maturation date occurred later in the season (Figure 2.10E, linear regression). *S. arctica* was the only species to show a significant decrease in seed germination success with later peak seed maturation dates (Figure 2.10A, linear regression). Regardless of peak seed maturation dates, seeds of *L. arcticus* exhibited the highest germination rates (often above 90%; Figure 2.10D) and seeds of *S. reticulata* exhibited low germination rates (often below 20%; Figure 2.10B). Seeds of *C. microchaeta* failed to germinate in the laboratory.

The unusual snow event occurred on 2 July 2012 and covered the study sites and any flowers that were present at the time with a layer of snow (Figure 2.7 and 2.8). The snow remained on the ground for no longer than 3 days because when the site was re-visited 3 days after there were no signs of any snow. Success of both flowering and seed maturation was also observed in relation to the July snow event (Figure 2.9 and Figure 2.10). In the early-season flowering species *S. arctica*, flowering success was unrelated to peak flower date and seed viability decreased with later peak seed maturation date (when the snow event occurred). *L. arcticus*, a late-season flowering species, had low flowering success over the entire flowering period (the snow event occurred early in the period), and seed maturation success was unrelated to peak seed maturation date. I observed a high amount of bud and flower kill early in the flowering period of *D. octopetala*, during the time of the snow event (personal observation; Figure 2.8), and flowering success increased through the flowering period (after the snow event). The number of seeds produced per flower of *D. octopetala* decreased with later peak seed maturation date. No relationships between the snow event, phenology and success could be discerned for the other two species I examined, *C. microchaeta* and *S. reticulata*.

2.3.3 Within- and among-species comparisons of reproductive outputs

The reproductive outputs of inflorescence length, number of seeds per inflorescence, seed mass, germination success, and density of reproductive structures were not clearly affected by the experimental manipulations of soil warming and nitrogen fertilization (Table 2.6, Appendix A). There was no relationship between inflorescence length and number of seeds produced within individual species (Figure 2.11, linear regression). However, when comparing among the species studied, long inflorescence lengths produced the fewest number of seeds and the shortest inflorescence lengths produced greatest number of seeds. For example, of the 6 species, *L. arcticus* on average produced few seeds per inflorescence (4.9 ± 0.5; Table 2.7) but had on average a long inflorescence length (149.6 mm ± 5.1; Table 2.7). In contrast, *S. arctica* had on average a short inflorescence length (49.3 mm ± 1.7; Table 2.7, but produced high numbers of seeds per inflorescence (144.1 ± 21.3; Table 2.7).

Although each species produced seeds that varied in mass and number, there was no clear relationship between seed mass and number of seeds produced within individual species (Figure 2.12, linear regression). However, among species, there was a very clear negative relationship between seed mass and number of seeds per inflorescence, with the exception of *S. reticulata* (Figure 2.12). For example, *L. arcticus* had heavy seeds ($140.30 \pm 6.76 \times 10^{-4}$ g; Table 2.7) with few seeds per inflorescence (4.9 ± 0.5 seeds; Table 2.7) whereas *S. arctica* had light seeds ($1.12 \pm 0.04 \times 10^{-4}$ g; Table 2.7) and a large number of seeds per inflorescence (144.1 ± 21.3 seeds; Table 2.7).

The relationship between average seed mass and germination rate across plots was species specific (Figure 2.13). Germination rate of *D. octopetala* and *L. arcticus* significantly increased with increased seed mass. In contrast, germination in *S. arctica* significantly decreased as seed mass increased and *S. reticulata* did not show any significant relationship. However, when comparing among all six species, seed germination was positively related to seed mass. For example, of all the species studied, *L. arcticus* had the heaviest seed mass ($140.30 \pm 6.76 \times 10^{-4}$ g; Table 2.7) and correspondingly the highest average germination rate (82.5%; Table 2.7). Similarly, *S. reticulata* had the lightest seed mass ($0.62 \pm 0.04 \times 10^{-4}$ g; Table 2.7) and correspondingly the lowest average germination rate (10.6%; Table 2.7). Seeds of *H. alpina* and *C. microchaeta* failed to germinate in the laboratory trials. *H. alpina* seeds were observed to have produced mature seed that simply failed to germinate, however seeds of *C. microchaeta* were mostly empty perigynia devoid of a mature embryo.

Table 2.3: Results from mixed-effects ANOVA models estimating the effects of the experimental fertilization on available soil nitrogen (n = 6 replicates per treatment) at the Experimental site. Block was included as a random effect in the NH_4 and NO_3 models and accounted for 21.4% and <0.1% of the variation, respectively. Available soil nitrogen concentrations were detected by ion exchange resins deployed in the soil from 9 June – 9 August 2012. Significant effects are shown in bold font (α = 0.05).

Nitrogen	Treatment	numDF	denDF	F-value	p-value
NH_4	Fertilizer	1	15	1.54	0.23
	Warming	1	15	0.28	0.60
	Fertilizer*Warming	1	15	1.67	0.22
NO_3	Fertilizer	1	15	296.07	**<0.01**
	Warming	1	15	0.19	0.67
	Fertilizer*Warming	1	15	1.99	0.18

Table 2.4: Results from mixed-effects models estimating the effects of experimental fertilization on available soil nitrogen (n=4) at the Nitrogen dosage site. There were significant main effects of the fertilizer treatment on NH_4 ($F_{4,12} = 3.50$, p=0.04) and NO_3 ($F_{4,12} = 27.38$, p<0.01). Block was included as a random effect in the NH_4 and NO_3 models and accounted for 8.4% and 51.9% of the variation, respectively. Parameter estimates (and standard error) are given for all treatments including the controls. Available soil nitrogen concentrations were detected by ion exchange resins deployed in the soil from 9 June – 9 August 2012. Significant effects are shown in bold font (α = 0.05).

Nitrogen	Treatment	Parameter estimate *(SE)*	t-value	p-value
NH_4	C	4.68 *(0.68)*		
	1g	0.09 *(0.92)*	0.10	0.92
	2g	0.84 *(0.92)*	0.91	0.38
	4g	1.54 *(0.92)*	1.67	0.12
	8g	2.97 *(0.92)*	3.22	**<0.01**
NO_3	C	4.11 *(0.48)*		
	1g	1.19 *(0.47)*	2.50	**0.03**
	2g	2.71 *(0.47)*	5.71	**<0.01**
	4g	3.18 *(0.47)*	6.70	**<0.01**
	8g	4.51 *(0.47)*	9.50	**<0.01**

Table 2.5: Average Julian date (±SE) of flowering and seed maturation periods for 5 tundra species in 2012 at the Experimental site. Statistical results are shown for the Kruskal Wallis rank sum test (H; with 1 degree of freedom) testing the null hypothesis that phenological dates were equal among experimental treatments (C = control, N = nitrogen fertilizer, W = warming, NW = nitrogen fertilizer and warming) All = average across all treatments. Significant values are shown in bold (α = 0.05).

		Flowering Period			Seed Maturation Period			
Species	**Treatment (n)**	**Onset**	**Peak**	**Finish**	**Onset**	**Peak**	**Seed dispersal**	**Coll. date**
Dryas octopetala	C (6)	191 (0.9)	197 (2.0)	208 (3.1)	213 (3.3)	228 (7.6)		
	N (6)	191 (2.9)	197 (1.8)	206 (1.2)	208 (1.3)	215 (6.6)		
	W (6)	191 (2.6)	198 (1.9)	207 (3.0)	212 (3.3)	221 (0.0)		
	NW (5)	187 (1.7)	196 (1.9)	202 (2.2)	208 (0.8)	211 (3.1)		
	All	190 (1.2)	197 (1.0)	206 (1.3)	210 (1.4)	218 (2.8)		237
	H	1.00	0.29	1.81	1.69	**4.75**		
	p-value	0.32	0.59	0.18	0.19	**0.03**		
Lupinus arcticus	C (3)	182 (1.9)	192 (2.4)	202 (3.3)	202 (0.8)	206 (1.4)		
	N (5)	186 (3.7)	198 (1.8)	205 (0.7)	204 (1.3)	208 (0.7)		
	W (4)	186 (2.6)	194 (2.5)	203 (1.4)	203 (0.0)	206 (0.5)		
	NW (3)	189 (3.1)	195 (1.2)	204 (0.5)	203 (0.0)	204 (0.5)		
	All	186 (1.7)	195 (1.2)	204 (0.9)	203 (0.5)	206 (0.6)		235
	H	0.66	2.22	0.92	0.93	0.54		
	p-value	0.42	0.14	0.34	0.33	0.46		
Salix arctica (M)	C (3)		177 (0.5)	186 (1.7)				
	N (4)		177 (0.4)	182 (1.7)				
	W (4)		178 (0.7)	184 (1.3)				
	NW (4)		178 (0.8)	182 (2.1)				
	All		177 (0.4)	183 (0.9)				
	H		1.16	2.41				
	p-value		0.28	0.12				
Salix arctica (F)	C (5)		177 (0.7)	186 (1.7)	180 (0.7)	186 (1.7)	216 (3.8)	
	N (5)		176 (0.0)	183 (2.0)	180 (1.0)	183 (2.0)	213 (5.3)	
	W (3)		176 (0.0)	186 (0.0)	179 (1.4)	186 (0.0)	208 (0.7)	
	NW (4)		176 (0.0)	188 (1.1)	184 (2.6)	188 (1.1)	208 (0.7)	
	All		176 (0.1)	186 (1.0)	180 (0.9)	186 (1.0)	210 (1.6)	224
	H		1.13	0.13	0.88	1.47		
	p-value		0.29	0.72	0.35	0.23		

Table 2.5 continued…

Species	Treatment (n)	Flowering Period			Seed Maturation Period			
		Start	Peak	End	Start	Peak	Seed dispersal	Coll. date
Salix reticulata (M)	C (1)		186 (0.0)	198 (0.0)				
	N (3)		180 (0.9)	195 (1.4)				
	W (3)		184 (2.8)	195 (3.3)				
	NW (3)		184 (1.6)	195 (1.2)				
	All		183 (1.2)	195 (1.2)				
	H		0.97	**3.89**				
	p-values		0.32	**0.05**				
Salix reticulata (F)	C (3)	178 (0.0)	189 (1.2)	199 (2.4)	191 (0.0)	199 (2.4)	217 (8.2)	
	N (5)	180 (2.0)	184 (2.1)	193 (0.7)	193 (0.4)	193 (0.7)	222 (6.6)	
	W (5)	180 (2.0)	187 (2.2)	200 (2.0)	194 (1.5)	200 (2.0)	221 (0.0)	
	NW (6)	178 (0.9)	183 (1.5)	197 (1.7)	191 (0.3)	197 (1.7)	228 (7.6)	
	All	179 (0.8)	185 (1.1)	197 (1.0)	192 (0.5)	197 (1.0)	218 (1.9)	224
	H	0.62	3.10	0.05	0.01	0.68		
	p-value	0.43	0.09	0.83	0.92	0.41		
Carex microchaeta (M)	C (1)	178 (0.0)	178 (0.0)	193 (0.0)				
	N (4)	179 (1.5)	188 (1.7)	197 (2.6)				
	W (5)	180 (2.1)	186 (3.1)	198 (3.5)				
	NW (5)	179 (1.8)	185 (1.7)	201 (1.6)				
	All	179 (1.0)	186 (1.4)	198 (1.4)				
	H	0.14	0.43	0.33				
	p-value	0.71	0.51	0.57				
Carex microchaeta (F)	C (1)	176 (0.0)	176 (0.0)	193 (0.0)	193 (0.0)	193 (0.0)		
	N (4)	178 (0.4)	182 (2.6)	197 (2.6)	194 (2.5)	197 (2.6)		
	W (4)	178 (1.3)	186 (3.6)	198 (3.5)	188 (1.9)	198 (3.5)		
	NW (6)	177 (0.4)	184 (2.0)	201 (1.6)	192 (1.6)	201 (1.6)		
	All	177 (0.4)	183 (1.5)	198 (1.4)	192 (1.2)	198 (1.4)		236
	H	0.50	0.04	0.33	1.76	0.33		
	p-value	0.48	0.85	0.57	0.18	0.57		

Table 2.6: Results from Kruskal Wallis rank sum (H) testing the null hypothesis that experimental treatments did not differ in reproductive outputs at the Experimental site (factorial; df = 1) and Nitrogen dosage site (df = 4). Species are: *Dryas octopetala* (D.oct), *Lupinus arcticus* (L.arc), *Salix arctica* (S.arc), *Salix reticulata* (S.ret). Two species (*Carex microchaeta* and *Hierochloë alpina*) were excluded from analysis because of insufficient data. Significant effects are shown in bold font (α = 0.05).

Reproductive Output	**Species**	**Experimental site**		**Nitrogen dosage site**	
		H	**p-value**	**H**	**p-value**
Inflorescence length	D.oct	0.01	0.92		
	L.arc	0.05	0.82	1.51	0.82
	S.arc	0.02	0.87	2.37	0.67
	S.ret	0.24	0.63		
Number seeds per inflorescence	D.oct	2.49	0.11		
	L.arc	0.60	0.44		
	S.arc	0.35	0.56	3.83	0.43
	S.ret	1.18	0.28		
Seed weight	D.oct	3.99	0.05		
	L.arc	3.27	0.07		
	S.arc	0.13	0.72	3.82	0.43
	S.ret	0.07	0.78		
Germination	D.oct	6.11	**0.01**		
	L.arc	0.29	0.59		
	S.arc	2.53	0.11	2.41	0.66
	S.ret	0.41	0.52		
Relative Density	D.oct	1.69	0.19	4.07	0.40
	L.arc	<0.01	0.98	6.19	0.19
	S.arc	1.28	0.26	2.61	0.63
	S.ret	0.40	0.53	5.85	0.21

Table 2.7: Plot level mean, standard error and range for reproductive outputs (inflorescence length (mm), number of seeds per inflorescence, seed mass (g x 10^{-4}), and germination rate (%)) in 2012 for six tundra species at Wolf Creek, Yukon.

Reproductive output	**Value**	***Dryas octopetala***	***Lupinus arcticus***	***Salix arctica***	***Salix reticulata***	***Carex microchaeta***	***Hierochloë alpina***
Inflorescence length (mm)	*Mean (± SE)*	57.1 *(3.3)*	149.6 *(5.1)*	49.3 *(1.7)*	29.2 *(1.1)*	56.8 *(2.8)*	194.2 *(5.1)*
	Range	38-115	98-208	30-70	21-44	30-89	154-235
	*n**	25	31	30	27	27	21
# seeds per inflorescence	*Mean (± SE)*	61.4 *(2.8)*	4.9 *(0.5)*	144.1 *(21.3)*	21.4 *(2.2)*	34.3 *(4.4)*	9.4 *(0.4)*
	Range	42-92	1-13	16-414	0-40	5-102	6-14
	*n**	25	26	29	22	24	20
Seed mass (g x 10^{-4})	*Mean (± SE)*	1.81 *(0.09)*	140.30 *(6.76)*	1.12 *(0.04)*	0.62 *(0.04)*	3.97 *(0.22)*	11.23 *(0.40)*
	Range	1-3	45-200	1-2	0-1	3-5	9-16
	*n**	25	26	29	20	9	20
Germination (%)	*Mean (± SE)*	41.0 *(4.7)*	82.5 *(4.5)*	33.5 *(3.7)*	10.6 *(1.7)*	0.2 *(0.1)*	0.0 *(0.0)*
	Range	0-86	0-100	6-80	0-27	0-2	0
	*n**	25	26	28	20	26	20

*Represents number of plots; within plot measurements were pooled or averaged at the plot level.

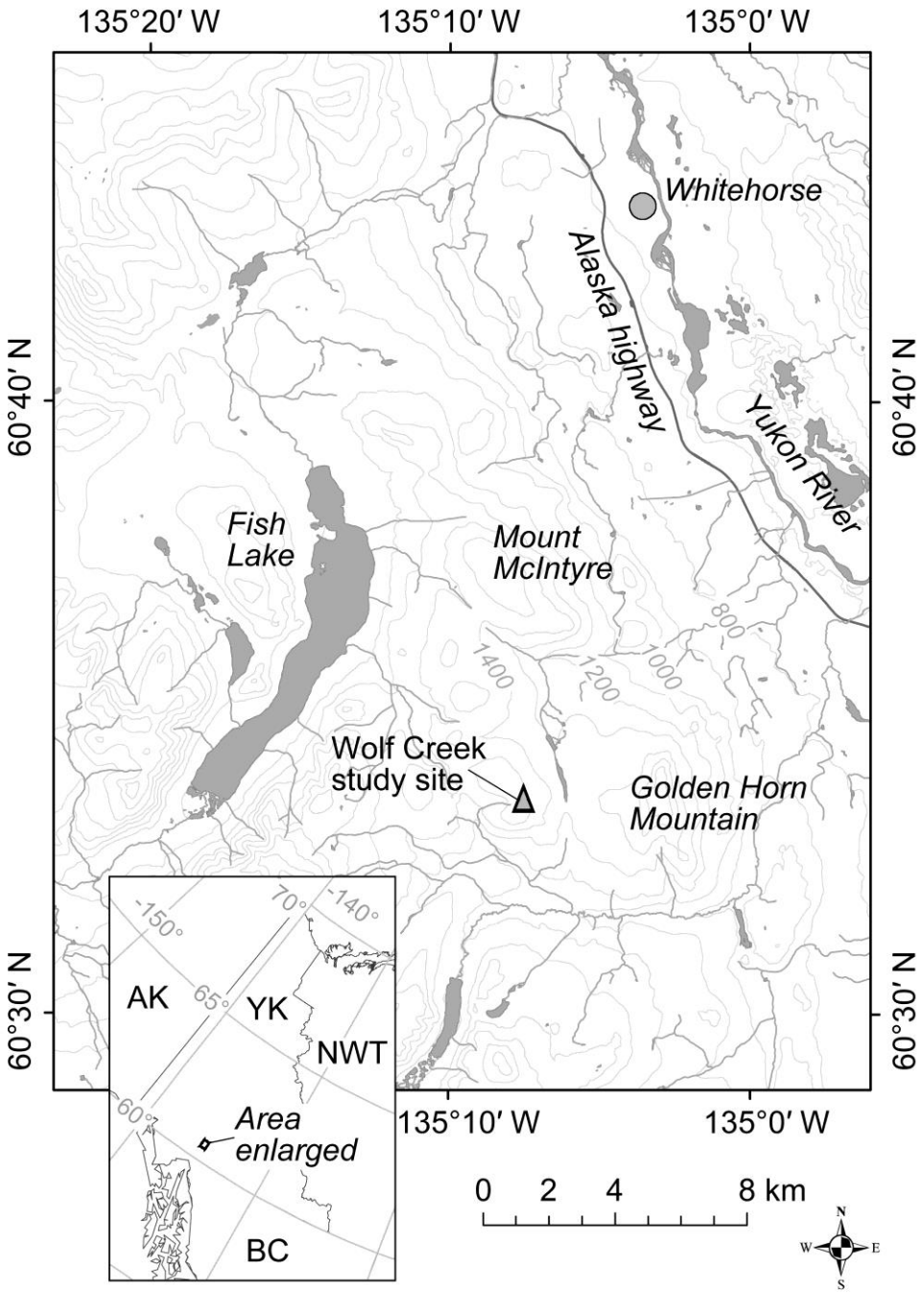
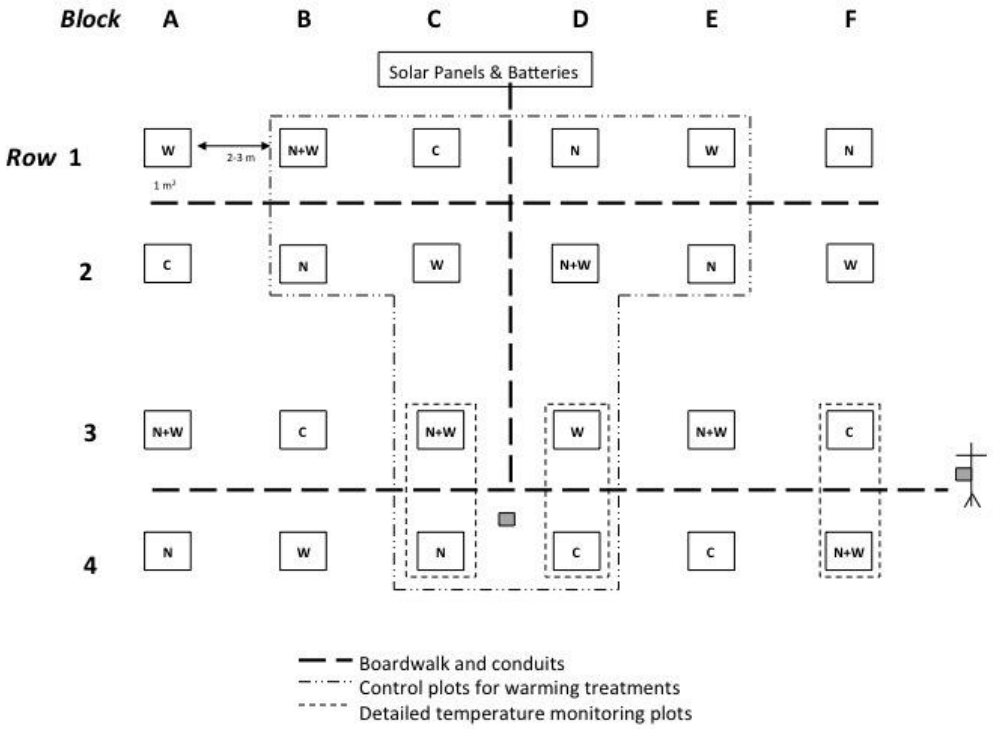


Figure 2.1: Map of study sites found in the alpine region of the Wolf Creek watershed in southern Yukon, Canada

A)



B)

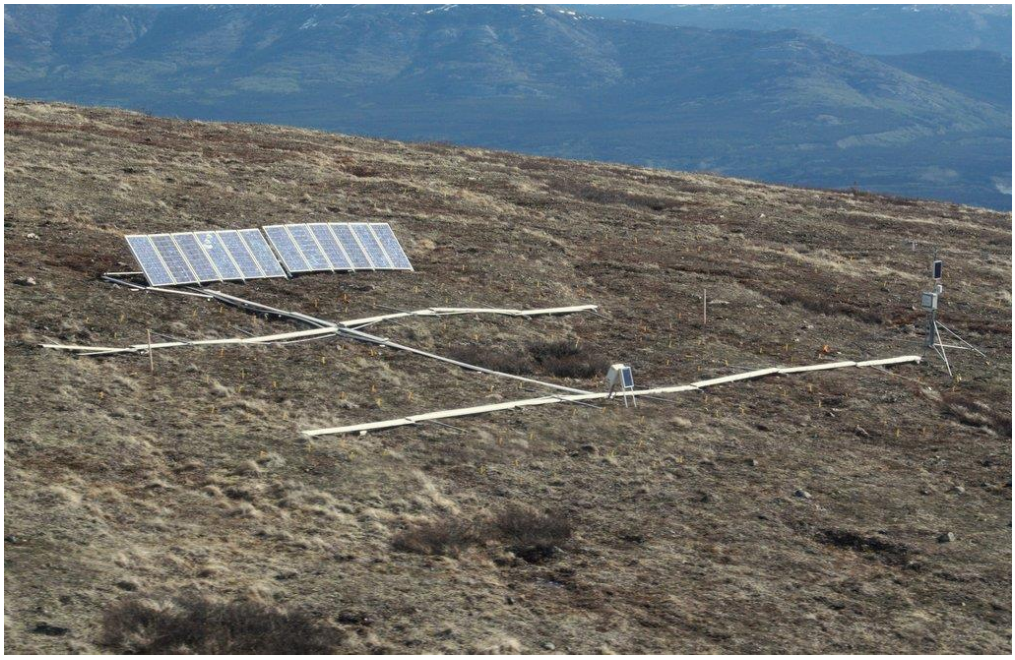


Figure 2.2: A) Diagram of experimental site layout at Wolf Creek. 1m x 1m plots are arranged within experimental blocks (A-F) and treatments were applied as a 2 x 2 factorial design within blocks (W = warming, N = fertilizer, N+W = warming and fertilizer, C = control). Twelve plots were used for monitoring of the warming treatment. Modified from Allen, 2012. B) Aerial photograph of experimental site at Wolf Creek taken in June 2012 (photo credit: J.F. Johnstone)

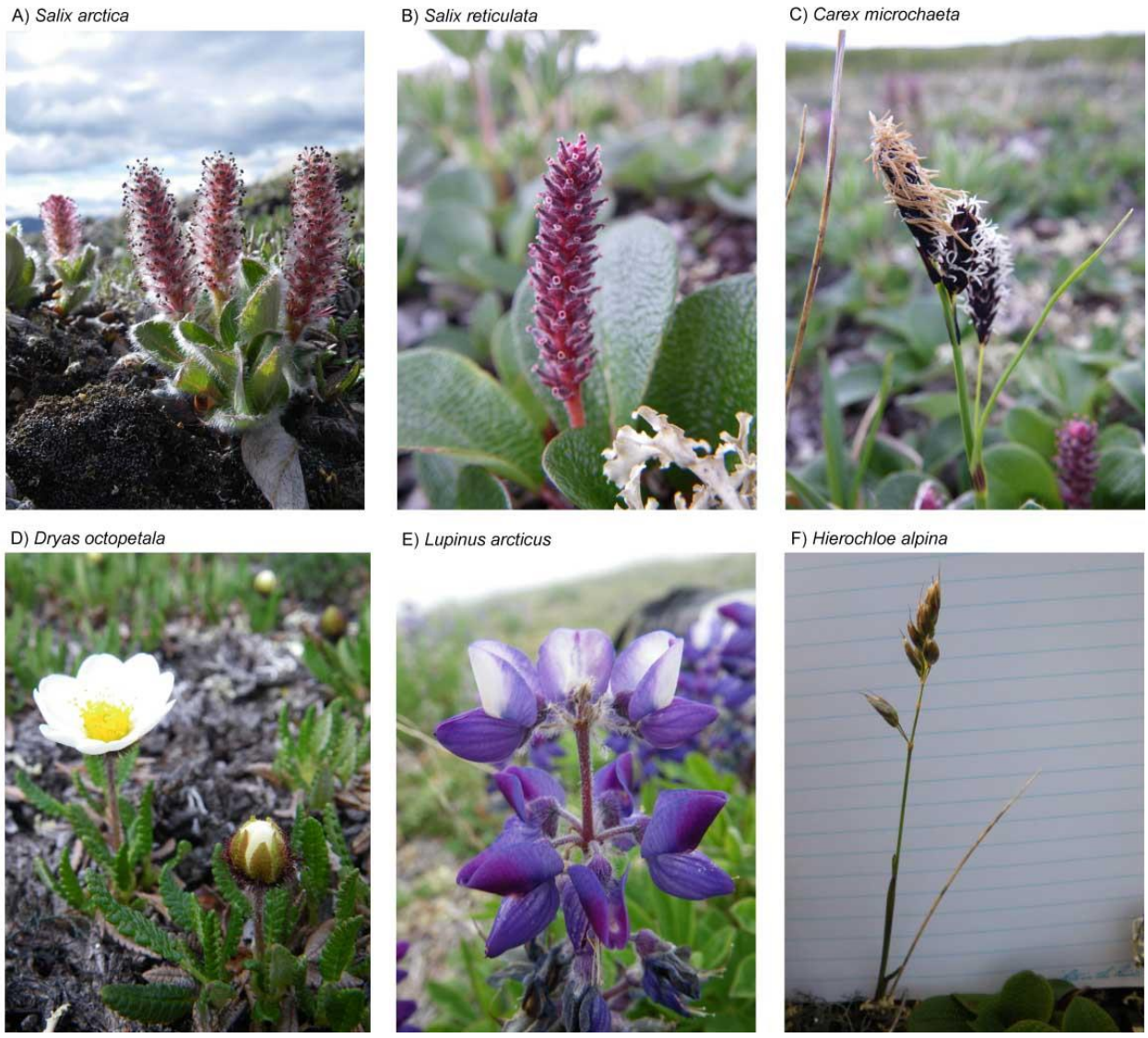


Figure 2.3: Tundra study species A) *Salix arctica*, B) *Salix reticulata*, C) *Carex microchaeta*, D) *Dryas octopetala*, E) *Lupinus arcticus*, and F) *Hierochloë alpina* found at the Wolf Creek study sites, Yukon, Canada.

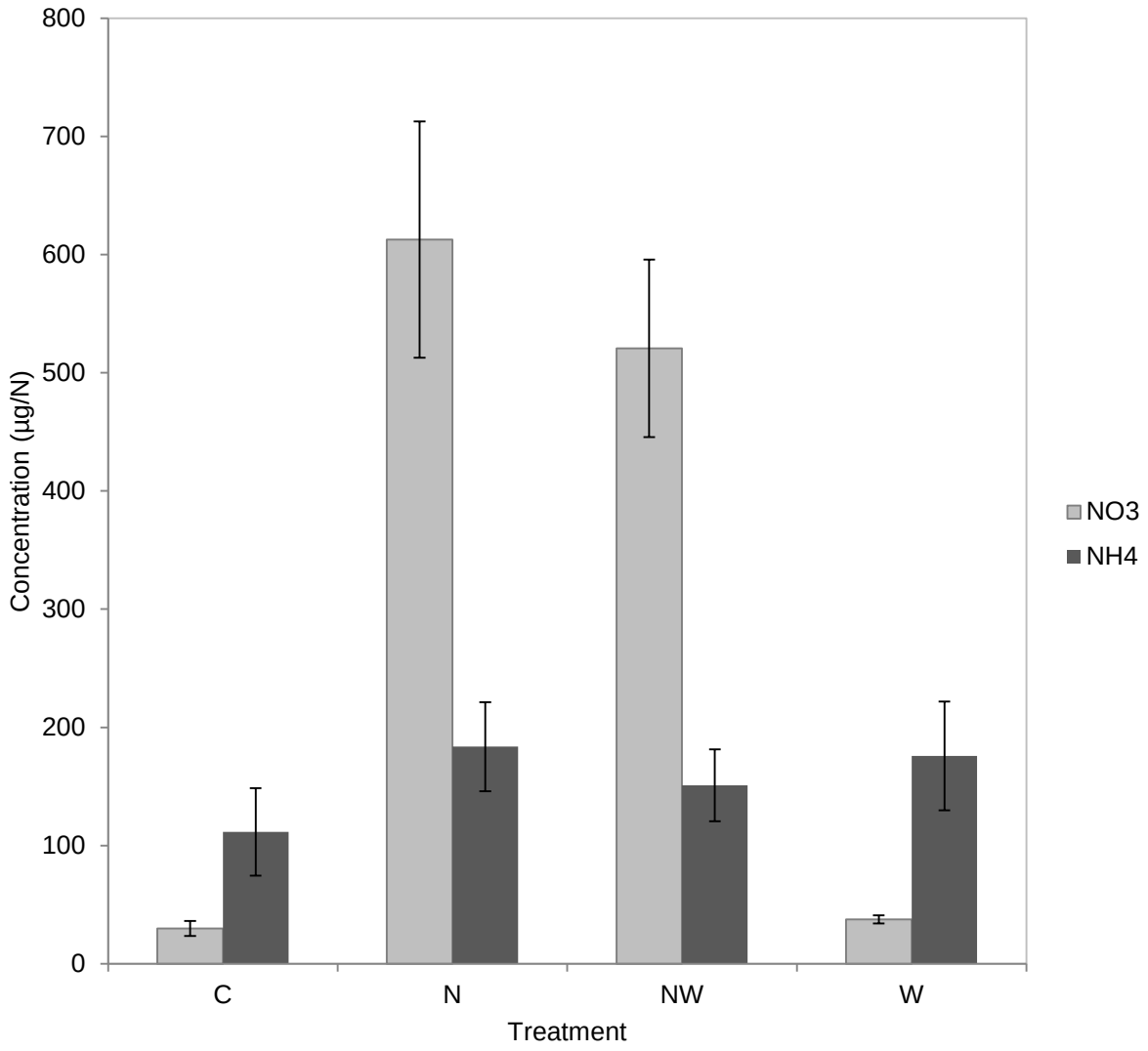


Figure 2.4: Mean NH_4 and NO_3 concentrations (µg) by treatment (C= control, N= 2 g nitrogen fertilizer, NW= nitrogen fertilizer and warming, W= warming) as detected by ion exchange resins in plots at the Experimental site (n=48). Error bars show ± 1SE.

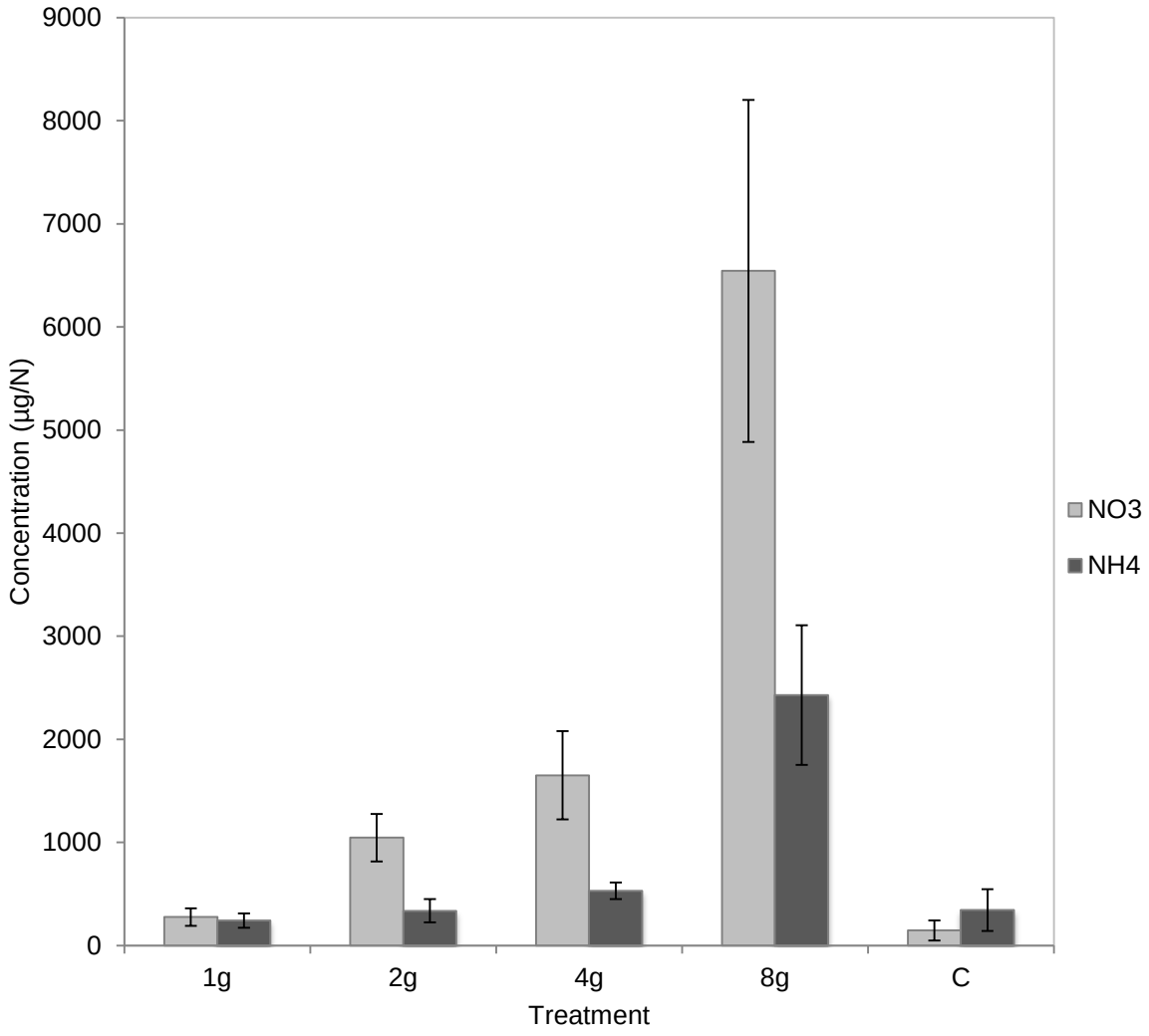


Figure 2.5: Mean NH_4 and NO_3 concentrations (µg/N) by nitrogen fertilizer treatment as detected by ion exchange resins in plots at the N dosage site (n=40). Error bars show ± 1SE.

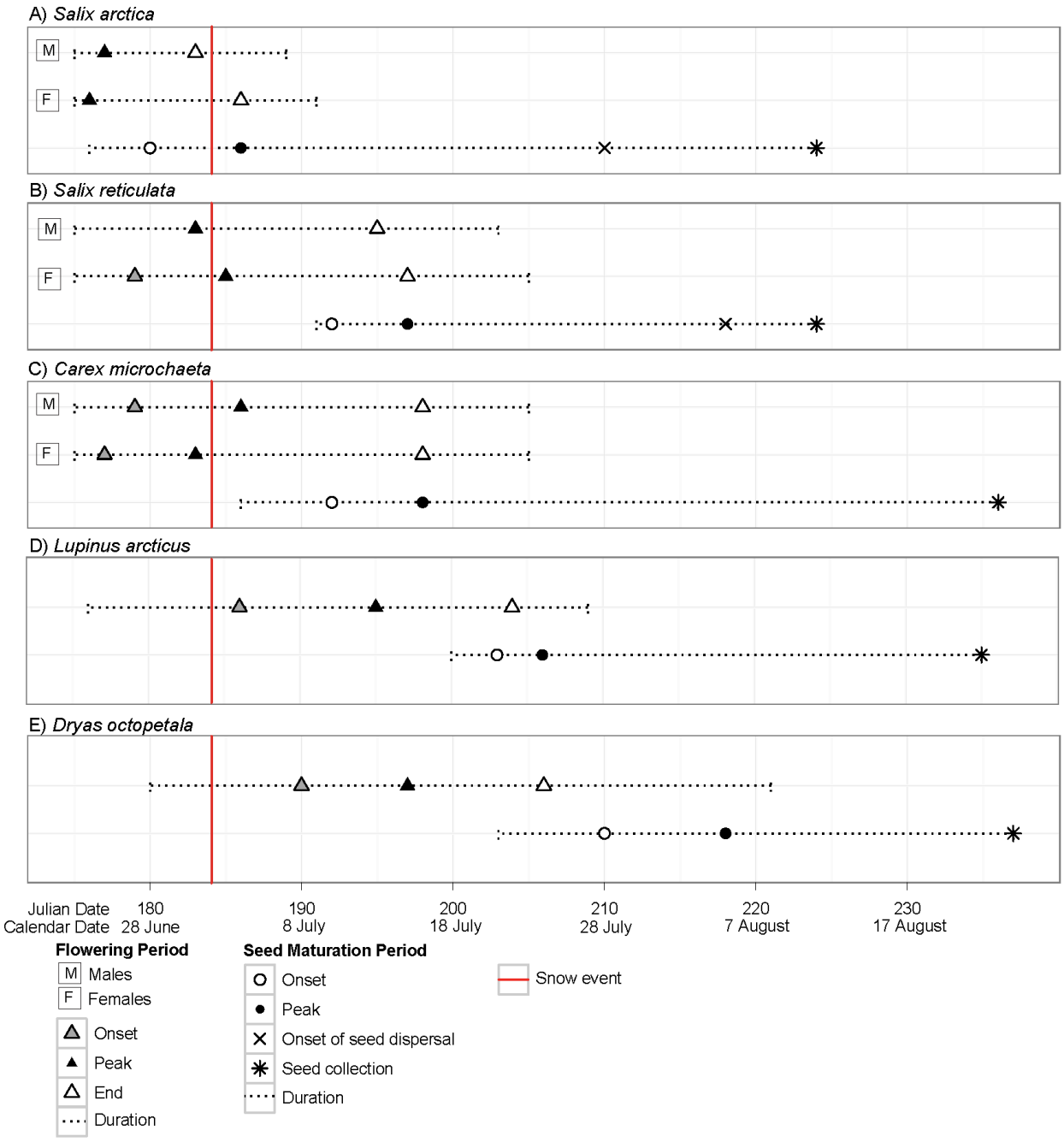


Figure 2.6: Observations of flowering and seed maturation phenology of five tundra species during summer of 2012. Species include A) *Salix arctica*, B) *Salix reticulata*, C) *Carex microchaeta*, D) *Lupinus arcticus*, and E) *Dryas octopetala*. Key phenological dates are indicated by symbols and represent average dates (n~24, but varies based on species presence in plots). Full duration of a phenoperiod is shown with dotted lines. Snow event occurred on July 2 (day 184) and is indicated by vertical line.

Figure 2.7: Snow event at Wolf Creek, Yukon on 2 July 2012. A) and B) Snow accumulation at experimental site. C) Snow accumulation on a frost boil.

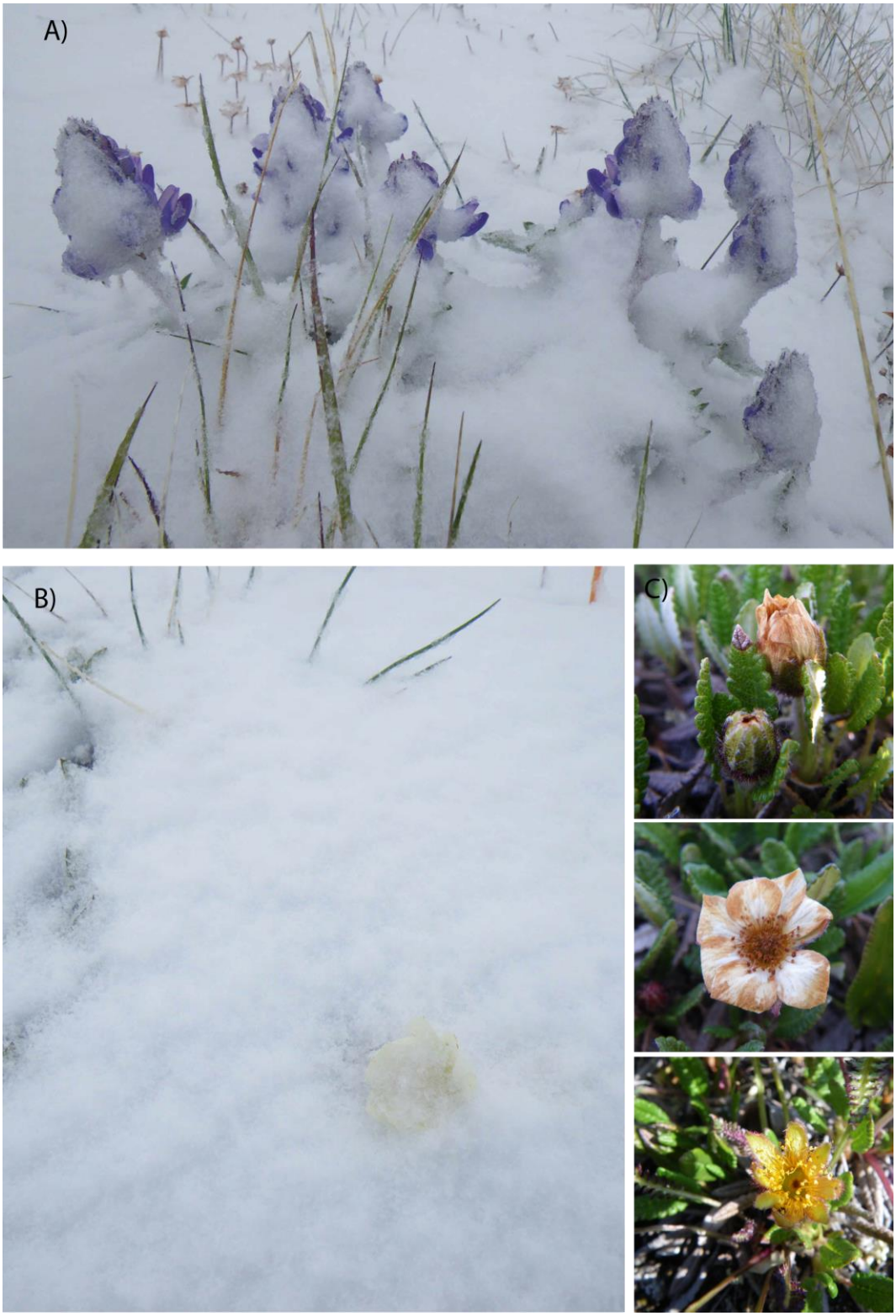


Figure 2.8: Snow event at Wolf Creek, Yukon on 2 July 2012. A) Flowers of *Lupinus arcticus* covered in snow. B) Flower of *Dryas octopetala* lost under the snow cover. C) Range of destruction felt by the flowers of *D. octopetala* from browned buds and flower petals, to complete loss of flower petals.

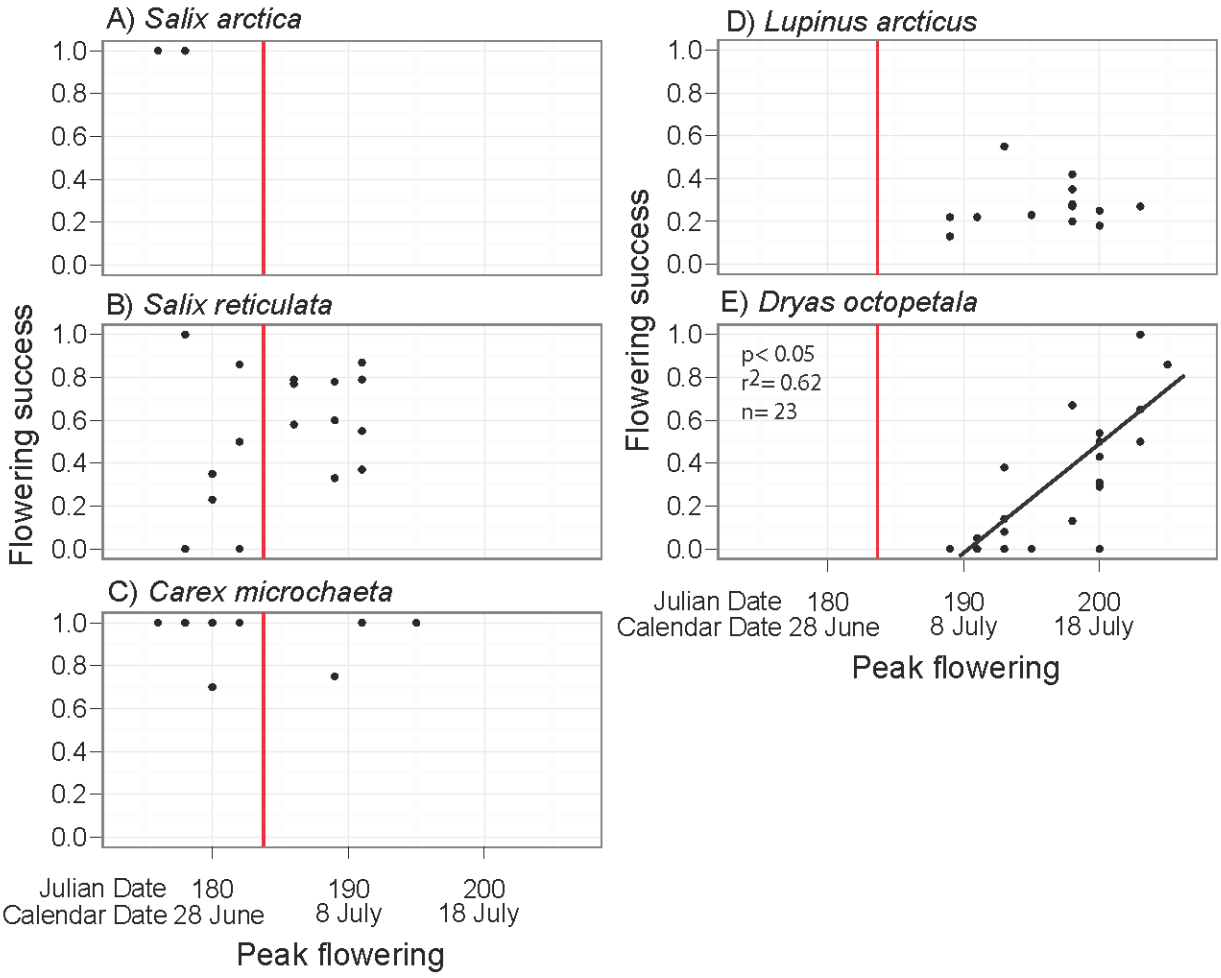


Figure 2.9: Flowering success (ratio of inflorescences that produced seed to total inflorescences per plot) based on date of peak flowering during summer 2012 for five tundra species. Species include A) *Salix arctica*, B) *Salix reticulata*, C) *Carex microchaeta*, D) *Lupinus arcticus*, and E) *Dryas octopetala*. Snow event occurred on July 2 (day 184) and is indicated by vertical line. Regression line added only when significant relationship exists. Points represent plot-based observations. Points are not jittered; therefore, points may represent multiple plots (such as in *S. arctica*).

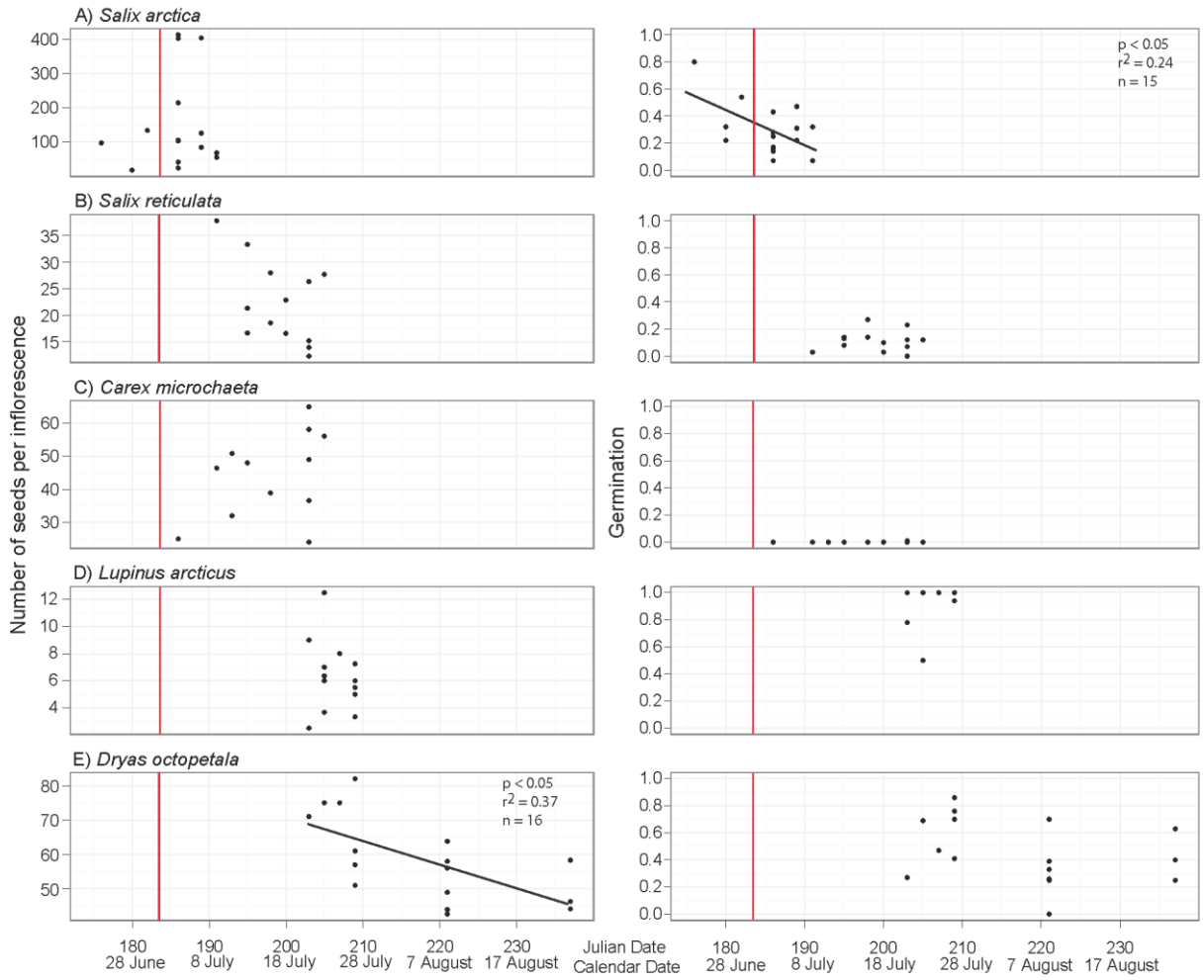


Figure 2.10: Seed maturation success as measured by number of seeds per inflorescence and germination rate (proportion of seeds that germinated) based on date of peak seed maturation in summer 2012 for five tundra species. Species include A) *Salix arctica*, B) *Salix reticulata*, C) *Carex microchaeta*, D) *Lupinus arcticus*, and E) *Dryas octopetala*. Snow event occurred on July 2 (day 184) and is indicated by vertical line. Regression line added only when significant relationship exists. Points represent plot-based observations. Points are not scattered, therefore points may represent multiple plots.

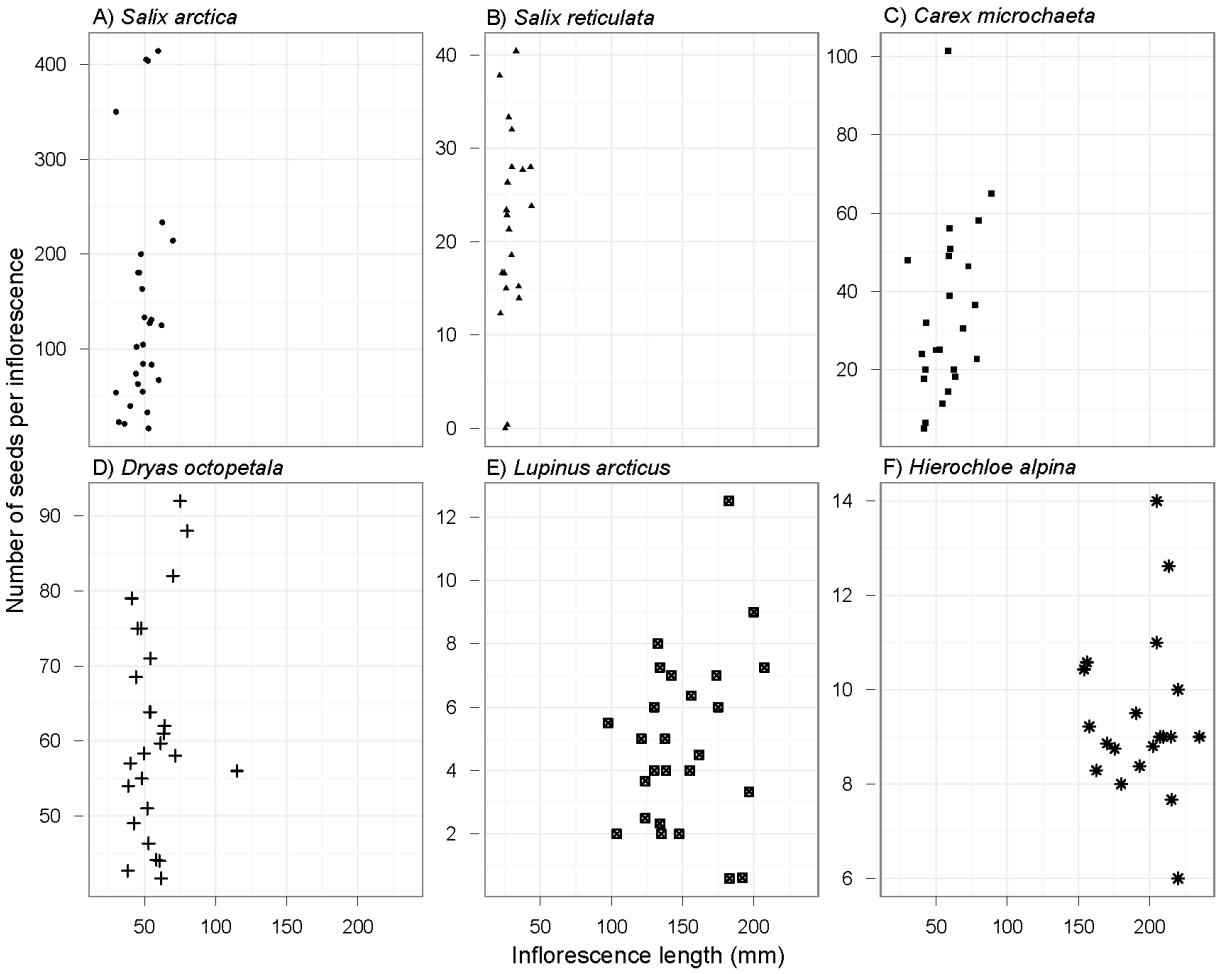


Figure 2.11: Relationship between average inflorescence length (mm) and average number of seeds per inflorescence for six tundra species. Species include: A) *Salix arctica*, B) *Salix reticulata*, C) *Carex microchaeta*, D) *Dryas octopetala,* E) *Lupinus arcticus*, and F) *Hierochloë alpina*. Dots represent plots. Data is from the Experimental site and the Nitrogen dosage site. Note the change in y-axis.

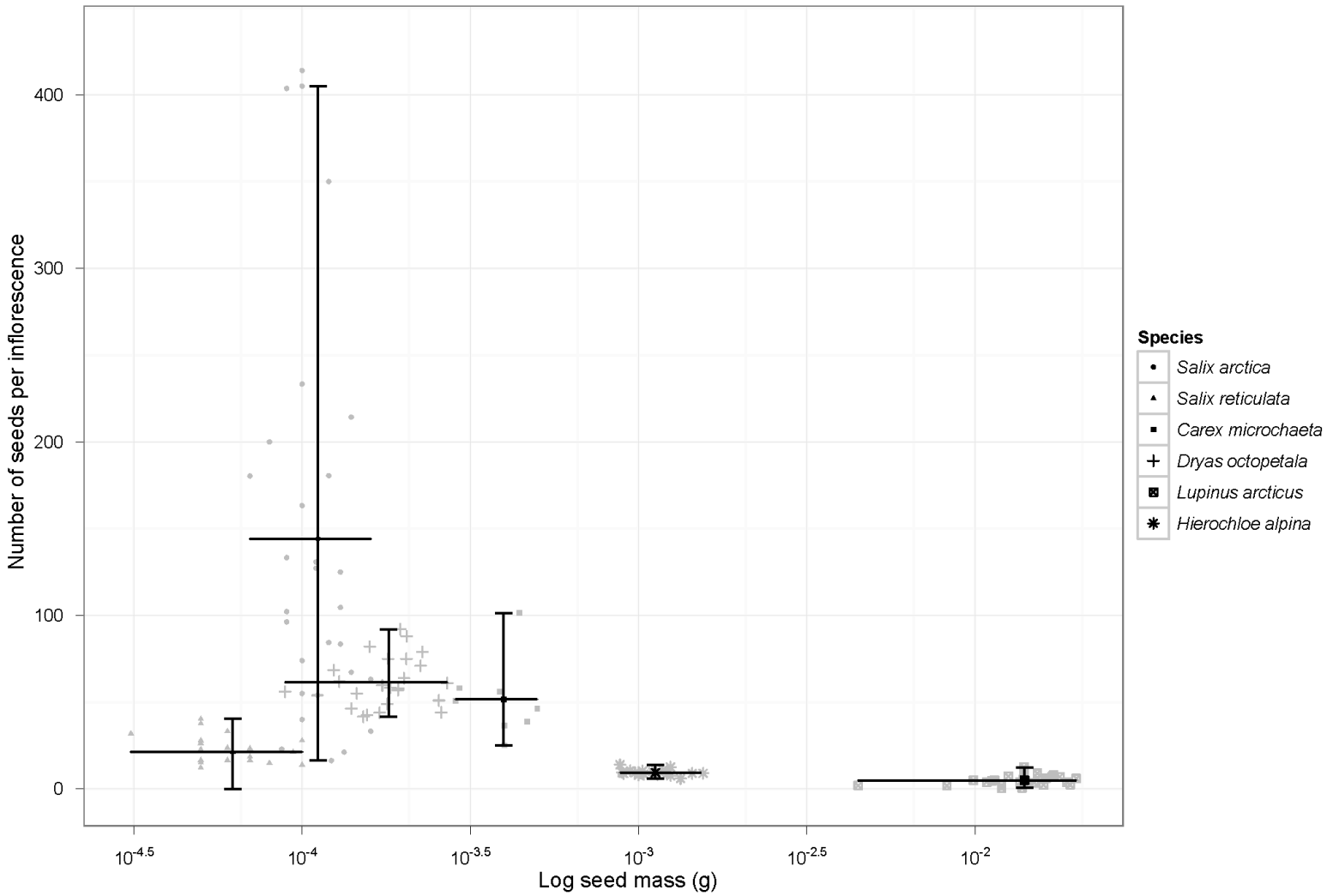


Figure 2.12: Relationship between log average seed mass (g) and average number of seeds per inflorescence for six tundra species. Species include *Salix arctica*, *Salix reticulata*, *Carex microchaeta*, *Dryas octopetala, Lupinus arcticus*, and *Hierochloë alpina*. Points represent plots. Solid lines represent range (of both variables) around the mean value. Data are from the Experimental site and the Nitrogen dosage site.

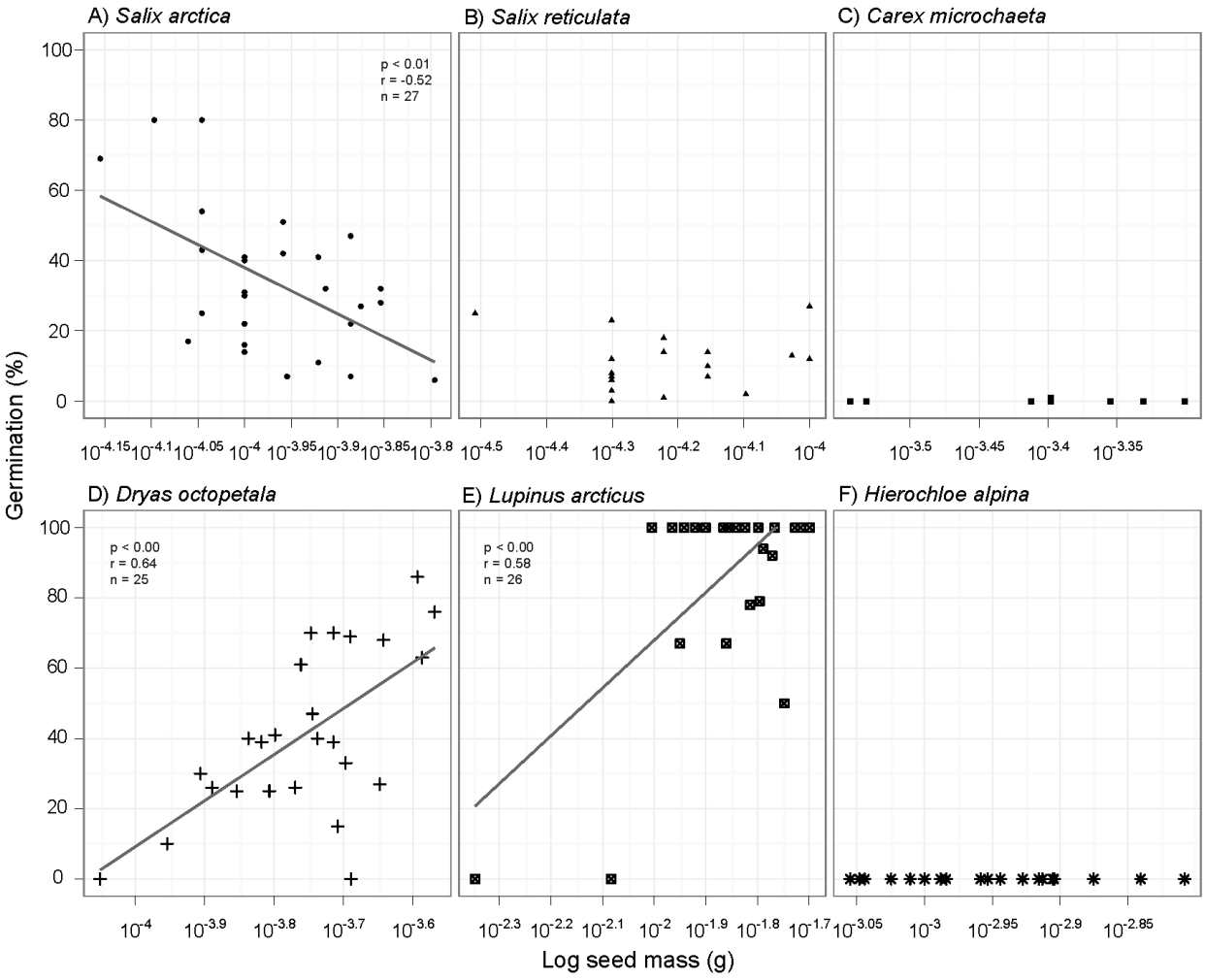


Figure 2.13: Relationship between log mean seed mass (g) and germination rate (%) for six tundra species. Species include A) *Salix arctica*, B) *Salix reticulata*, C) *Carex microchaeta*, D) *Dryas octopetala,* E) *Lupinus arcticus*, and F) *Hierochloë alpina*. Points represent plots. Data are from the Experimental site and the Nitrogen dosage site. Note the variation in x-axis scales. Regression lines indicate significant relationship ($p<0.05$).

2.4 Discussion

2.4.1 Experimental manipulations

Soil warming in the range of 1-3 ºC is predicted as a result of air temperature increases in northern latitudes in the next century due to a changing climate (Hartmann *et al.* 2013). In the growing season of 2012, mean soil temperatures in the experimentally warmed plots were significantly increased by 1.16 ºC over the temperatures in control plots. This increase falls short of the objective to reach a consistent increase of 2 ºC above ambient soil temperature, however, the increase does fall well within the predicted range. Therefore, the solar-powered heating system was an effective way to heat soil temperatures. Also, an advantage of this system is that it limits secondary physical changes in the soil environment, such as soil moisture, therefore allowing any observed plant responses to be attributed to the warmed conditions (Johnstone *et al.* 2013).

Fertilization using granular pellets of ammonium nitrate (NH_4NO_3) has been determined to be an effective way to increase available nitrogen in the soil (Johnstone *et al.* 2013). At both the experimental and nitrogen dosage site, concentrations of NO_3 (nitrate) were increased in fertilization plots, whereas little effect was seen on NH_4 (ammonium) concentrations. Similar observations were made in Toolik, AK (Chapin *et al.* 1995). The limited variation in ammonium concentrations between treatments can be attributed to the uptake of ammonium within the soils by microbes (nitrification), or negatively charged particles (organic matter and clay particles) before being attracted to the resin beads (Chapin *et al.* 1995). Increases in nitrate concentrations were experienced in the fertilization treatments most likely due to the ability of nitrate to readily move through the soil and reach the resin beads.

Increased soil temperatures had no significant effect on the availability of nitrogen in the soils. The interaction between soil temperature and nutrient availability in tundra soils is very complex. It is expected that warmed soil conditions will promote increased microbial activity and increased rates of decomposition and nutrient turn over (Nadelhoffer *et al.* 1992), however the time-scale on such responses is not well understood. After eight years of experimental warming conducted in Alaska, nitrogen availability increased under warmed soil conditions (Chapin *et al.* 1995). However, many other studies conducted in arctic soils have reported a lack of response of nitrogen availability due to warming over various time frames ranging from 2 to 16 years of experimentation (Jonasson *et al.* 1993, Rinnan *et al.* 2007, Lamb *et al.* 2011). The heating system used in this study was operational for 2 years but it may require more time for full effects of warming on total nitrogen availability to be detected.

2.4.2 Effects of experimental treatments on phenology and seed production

Other observations of phenological shifts and changes in seed production have been attributed to increased air temperatures (Wookey *et al.* 1993, Arft *et al.* 1999, Klady *et al.* 2011). Increased air temperatures cause an indirect warming of the soil surface and the increase in soil temperatures is often thought to have an additive effect on the overall reproductive responses of plants. However, my results indicate that without air temperature warming, increases in soil temperature and nitrogen availability have no impact on sexual reproduction. Overall, I observed no alteration in onset, peak, or end dates for flowering or seed maturation phenoperiods for the five study species. I also observed no changes in the density of reproductive structures, inflorescence length, number of seeds produced per inflorescence, seed mass, or germination rate. This

suggests that a plant's control of flowering and seed development are directly affected by changes in air temperatures and have less influence from soil temperature and nutrient limitations on overall plant growth. In addition, I present evidence in the next section describing the impact of the July snow event on reproductive success to further support the strong influence of air temperatures on a plant's physiological processes.

Although changes in soil temperature and nitrogen availability were not observed to influence sexual reproduction in 2012, I suggest that as climate continues to change, it will be important to monitor how soil conditions interact with the sexual reproduction of plants. Reproductive phenology and development place great demands on soil resources early in the growing season, usually during soil thaw (Nord and Lynch 2009). As climate interacts with thawing processes of soils, the timing and amount of resources a plant is able to acquire will change. I would suggest that the seasonal availability and fluctuations in nutrients would also be impactful on reproduction. Plant and soil interactions are complex and my one year study does not provide enough evidence to rule out the impacts of changing soil conditions on reproduction. More studies that look at the interactions between soil conditions and reproductive phenology and seed production of tundra vegetation are needed in order to gain better understanding of the dynamics.

2.4.3 Snow event in relation to timing and success of reproduction

In this study, the patterns observed for *S. arctica*, *L. arcticus,* and *D. octopetala* highlight some potentially interesting trends on the connection between reproductive phenology and how timing of a snow event may alter a plant's reproductive success. The impact of frost events on reproductive phenology and success of different tundra

species is not well known (Inouye 2008). It is possible that the July snow event directly affected seed production of *S. arctica* (decreased seed viability with seed maturation date), flowering of *L. arcticus* and *D. octopetala* (overall reduced flowering and seed production), and also indirectly affected seed production of *D. octopetala* (delayed peak flowering). What remains unclear is if the patterns I observed were a result of the early July snow event or if they are reflective of what would be observed in other years for these species. Multiple years of data and understanding of long-term phenological trends are needed to make any conclusive statements. However, this study is at least consistent with the hypothesis that snow events can have strong impacts (both directly and indirectly) on the success of a variety of flowering species on the tundra by first affecting a plant's phenology.

The timing of the snow event affected the success of reproduction by affecting tissue development and causing damage to highly sensitive reproductive tissues. The early July snow event occurred during the onset of flowering for *D. octopetala* and *L. arcticus*. It is reasonable to suggest that the snow caused direct damage to the tissues of flower buds and flowers (such as browning). Flower buds were left damaged and did not produce flowers. The petals on flowers were damaged leaving the flower unable to attract pollinators. The early July snow event also occurred during the development of seed for the *Salix* species and could be directly responsible for the low seed production in *S. reticulata* and the decreased seed success in *S. arctica*. The snow event may also have indirectly reduced the seed-set and success of *D. octopetala* by delaying it's peak flowering, which in turn, delayed peak seed development to occur later in the fall when tissues were susceptible to cooling temperatures. With both direct and indirect potential effects of cool temperatures on the reproductive success of tundra species, it will be

important to continue paying close attention to the timing of phenology events throughout the season.

It is expected that the frequency of unusual weather events, such as snowfall and frost in the summer season, will increase as climate changes, and there is a growing call for studies to look at how the timing and frequency of these events will influence growth and survival of vegetation in both the short and long terms (Jentsch *et al.* 2007, Jentsch *et al.* 2009). As my study has indicated, weather conditions in a given year can strongly influence phenology and development and this is collaborated by long-term studies that have observed large differences between years (Inouye 2008, CaraDonna *et al.* 2014). These events will differentially affect species' reproductive potential and competitive balances resulting in changing composition of tundra vegetation communities (Aerts *et al.* 2006). For example, if frost events become more frequent at the beginning of the growing season it could be detrimental for reproductive success of species, especially the *Salix* species, causing a potential decline in population success. It will be important to study these short term events in the context of long term change.

2.4.4 Within- and between-species variation in reproductive strategies

To understand the variation of sexual reproductive strategies on the tundra, I looked at trade-offs in the reproductive outputs for each of the study species and compared the trade-offs between the study species. The trade-offs I examined were i) inflorescence length and number of seeds produced, ii) number of seeds produced and seed mass, and iii) seed mass and germination rate. From these trade-offs we can learn how allocation of resources differs between strategies and how these strategies impact

reproductive success. With this insight we can better understand how changes in any of these outputs may affect survival of species in the tundra.

The non-destructive measure of inflorescence length has been widely used as an indicator of reproductive investment in many long-term studies (Molau and Edlund 1996). It has not been well documented if information on inflorescence length can provide reliable, species-specific information in regards to reproductive success. While knowledge of reproductive investment can be important, we ultimately need information on reproductive success if we wish to understand how communities may change with environmental conditions. In this study, I observed no relationship between average inflorescence length and the average number of seeds produced within a plot for the species *S. arctica*, *S. reticulata, C. microchaeta, L. arcticus, D. octopetala,* or *H. alpina*. It appears that within these species, inflorescence length is an unreliable predictor of seed production. Among the study species, it appeared that the longest inflorescence lengths (for example *H. alpina* and *L. arcticus*) produced the smallest number of seeds, and the shortest lengths (for example *S. arctica* and *S. reticulata*) produced the largest number of seeds. This is likely reflective of the species studied and not conducive to application across all the reproductive strategies in this particular community. Less dominant species in this community such as *Silene acaulis*, *Sassurea angustifolia*, and *Saxifraga flagellaris* display short inflorescence lengths and produce only a few seeds and as such do not fit into this generalized observation. Therefore, inflorescence length appears to be an unsuitable measurement for inferring seed production or potential reproductive success of tundra vegetation. This suggests that there are stronger influences on the number of seeds produced, such as: individual flower structure,

pollination potential, or seed mass. These are likely better estimates of reproductive success than simply inflorescence length.

A trade-off between number of seeds produced and seed mass is the result of resource allocation, as the plant devotes resources to seeds of a given mass (Henery and Westoby 2001, Westoby *et al.* 2002). This trade-off has been widely studied in plants and there is an expected negative relationship between seed production and seed mass. Within each of the species studied, I did not observe any relationship between the number of seeds produced and seed mass. This is similar to what other studies looking at individual species have found (Schaal 1980, Wulff 1986, Winn and Werner 1987). Each species' seed mass is often constrained within a narrow range and the variability within that range often produces too much scatter for a relationship to be seen (Henery and Westoby 2001). However, among all six of my study species, I observed a clear negative relationship. This has also been observed in tree and shrub communities (Greene and Johnson 1994), grassland communities (Turnbull *et al.* 1999, Jakobsson and Eriksson 2000) and Australian plant communities (Henery and Westoby 2001). A variety of seed sizes and mass can be found in tundra plant communities (Westoby *et al.* 1996). This indicates that there is a wide range of different strategies to maintain the community and/or to take advantage of new conditions.

While the number of seeds a plant produces is central to understanding the potential colonization ability of plant species within a community (Henery and Westoby 2001), seed mass is also important in understanding the regeneration potential of a plant. Small-seeded species are at an advantage with the production of high quantities of seed, however, it is hypothesized that large-seeded species gain a competitive advantage by increasing the chance of seedling emergence under hazardous conditions

because it provides the seedling with more initial resources (Westoby *et al.* 2002, Moles and Westoby 2004a). Among the study species, I observed that the species with a large seed mass had a higher lab germination rate than small-seeded species. This pattern was not always present within an individual species and was species-specific. For the species *L. arcticus* and *D. octopetala,* an increase in germination rate was observed with heavier seeds. *S. arctica* had the opposite relationship, where seed germination rate decreased with increasing mass. This observed pattern in *S. arctica* is perplexing and needs further investigation (Densmore and Zasada 1983).

In this study, I was unable to obtain seed germination data for the species *C. microchaeta* and *H. alpina.* Others have also been unsuccessful in germinating *H. alpina* seeds in the lab (Moulton 2009). There are a number of potential reasons for this. For one, my lab methods may not have been conducive to germination in these species. Warm temperatures and moisture were provided, but not well controlled. If there was too much moisture the seeds were vulnerable to fungi. A cold period (freezer storage) was provided but perhaps there are other dormancy breaking techniques these two species require that I did not provide. Secondly, maturity of seeds is hard to detect in the field. It is possible that these seeds needed a longer period in the fall season to reach full maturity (Wagner and Reichegger 1997).

The number of seeds, seed mass, and germination rate all play a role in the reproductive potential of a species. Changes in allocation patterns to sexual reproduction of tundra species under future environmental conditions will likely alter species-specific reproductive success and result in changes in plant community composition.

2.5 Conclusions

There is still much that needs to be studied about how changes in environmental conditions will impact reproductive phenology, reproductive success of tundra vegetation. In this chapter, I provided evidence that increases in soil temperatures and nitrogen availability did not have any clear influences on the reproductive phenology or reproductive success (such as seed viability) of six tundra species. Instead, a snowfall event at the beginning of July had a much greater impact on the success of the species' flowering and seed production. Additional research in this area will need to focus observations towards the need to understand short-term variability in weather, which is expected to increase as climate changes, as well as the longer term consequences of warming. In combination with the research presented in this chapter, further evidence is needed to support the importance of sexual reproduction as a mechanism that contributes to species diversity across the tundra and also across landscapes.

3.0 SEEDLING EMERGENCE OF TUNDRA AND BOREAL SPECIES IN AN ALPINE TUNDRA ENVIRONMENT

3.1 Introduction

Climate is predicted to change most rapidly in the next century in northern latitudes (McBean *et al.* 2005, Hartmann *et al.* 2013). This warming has the potential to impact the carbon balance of tundra ecosystems (Oberbauer *et al.* 2007), the occurrence of permafrost disturbances (Walker *et al.* 2008), and arctic and alpine species diversity (Sala *et al.* 2000). Increased air temperatures have directly affected the phenology (Wookey *et al.* 1993, Körner and Basler 2010) and growth (Arft *et al.* 1999, Walker *et al.* 2006, Elmendorf *et al.* 2012) of tundra vegetation. In fact, substantial warming in many northern locations has already been observed to have significant effects on arctic and alpine vegetation communities (Chapin *et al.* 1995, Danby *et al.* 2011). In addition to warmer air temperatures, it is also expected that increased soil temperatures and microbial activity will indirectly effect on vegetation growth (Jonasson *et al.* 1999). Changes in environmental conditions can also lead the way for invasion and expansion of other species' ranges into areas that were once unsuitable. In order to predict potential changes in tundra plant communities, it is important that we gain a better understanding of the mechanisms that allow plants to respond to changes in climate and environmental conditions.

A key mechanism that enables plants to take advantage of new environmental conditions is sexual reproduction (Bruun and Ejrnæs 2006, Weppler *et al.* 2006). The establishment of new individuals plays a vital role in determining the abundance and distribution of species across a landscape (Zobel *et al.* 2000, Levine and Rees 2002). In addition, seed production, seed dispersal and the establishment of new individuals

allows plants to expand their current population range as suitable habitat becomes available (Theoharides and Dukes 2007). Consequently, responses of tundra communities to climate change over decadal to century scales will likely depend on how environmental changes interact with the sexual reproduction cycle. While slow growing, long lived, colonially propagating plants routinely inhabit arctic and alpine tundra (Bell and Bliss 1980), the importance of sexual reproduction has been increasingly examined in these plant populations in recent years (Chambers 1995, Walck *et al.* 2011). However, there still remains a large gap in understanding of the factors that affect the different stages of the reproduction cycle, particularly controls over seed availability and initial emergence and how these stages will respond to environmental changes.

Emergence of a seedling depends on both the availability of seed and the suitability of surface conditions, and the limitations of these two factors act along a continuum (Eriksson and Ehrlén 1992, Clark *et al.* 2007). If seed availability was the most limiting to emergence then it would be expected that seed addition experiments would result in higher seedling counts. On the other hand, if suitable surface conditions were most limiting to emergence then it would be expected that changes in various surface conditions would alter success. Suitable surface conditions require a combination of substrate, soil temperature and nutrients to support rapid early growth and continued survival (Graae *et al.* 2011). Any changes to the surface conditions, such as changes to substrate type, warmer soil temperatures, or greater nitrogen availability may result in an increase in the potential for seedling emergence on the tundra. In fact, it has been observed that disturbance sites that experience a change in substrate types, such as exposed mineral soil, often act as important microsites (Freedman *et al.* 1982, Chambers *et al.* 1990). It is not well understood how new soil conditions brought on by

climate change will affect the emergence and establishment potential of tundra and boreal seedlings, but it will ultimately shape the structure of future tundra environments.

In this study, I investigate how environmental changes may affect initial seedling emergence of tundra and boreal species in an alpine tundra environment. I examine the influence of seed availability and the suitability of surface conditions on initial seedling emergence of tundra and boreal species by applying seed to naturally occurring disturbance sites with bare substrate exposed, and to plots with altered soil temperature and nitrogen availability. Specifically, I tested the hypothesis that if initial seedling emergence was affected by soil conditions related to disturbance, temperature, or nitrogen availability, then I would observe increased seedling densities in these treatments. Seeding trials with both tundra and boreal species provides information on whether there are differences in emergence potential between tundra and boreal seeds, helping us anticipate future changes in tundra plant communities.

3.2 Methods

3.2.1 Study site

This study was conducted in southern Yukon, Canada (60°33′46.4″ N, 135°07′55.0″ W; elevation 1565 m.a.s.l.), approximately 20 km south of Whitehorse (see Chapter 2, Figure 2.1). This area experiences a subarctic climate that is characterized by large seasonal variations in temperatures and low precipitation (Wahl *et al.* 1987). Monthly mean temperatures in the summer months (June-August) range from 5 to 15°C and in the winter months (December-February) range from -10 to -20 °C (Environment Canada 2013). Mean annual precipitation is 300 to 400 mm with approximately 40% of it falling as snow (Janowicz 1999).

The study sites were located in the alpine region of the Wolf Creek watershed. Vegetation at low elevations is dominated by boreal forest species including *Abies lasiocarpa* Hook. Nutt. (subalpine fir), *Pinus contorta* Dougl. Ex Loud. (lodgepole pine), *Picea glauca* (Moench) Voss s.l. (white spruce), and *Alnus crispa* (Drylander ex Ait.) (green alder). Vegetation at high elevations is characteristic of alpine low shrub tundra where vegetation often grows close to the ground surface. Total coverage by vascular plants is approximately 40% (Pieper *et al.* 2011) and common species include the dwarf evergreen *Dryas octopetala* M. Vahl (mountain avens), the dwarf deciduous shrub *Salix arctica* Pall (arctic willow), the herbaceous species *Lupinus arcticus* L. (arctic lupine), and the graminoids *Carex microchaeta* Holm (alpine tundra sedge) and *Hierochloë alpina* (Sw.) R. & S (sweet grass). Other ground cover includes various mosses and lichens, as well as plant litter and bare soil. Soils in the area are classified as Orthic Eutric Brunisols (Soil Classification Working Group 1998) with a sandy loam texture that is a result of the weathering of the underlying sedimentary bedrock and of surface deposits of glacial till (Janowicz 1999). These soils are subject to seasonal freezing and thawing and there is evidence of surface disturbance caused by cryoturbation in the form of the periglacial features known as frost boils (mud boils, non-sorted circles). These features expose mineral soils to the surface through active freeze and thaw action. The active section of the frost boil is devoid of vegetation and composed of bare soil and rock (Overduin *et al.* 2003, Boike *et al.* 2008).

3.2.2 Experimental design

At the main experimental site, soil warming and nitrogen fertilization were applied to experimental plots (1.0 x 1.0 m) as part of a larger study (Johnstone *et al.* 2013).

Further details on the methods for experimental soil warming and nitrogen fertilization application are given in Chapter 2 (section 2.2.2).

Areas of surface disturbance and intact vegetation were also used to test the effects of disturbance on initial seedling emergence. To accomplish this, I choose at random twelve frost boil sites (approximately 1.0 m^2 in size) in close proximity to the experimental site (n=12; Figure 3.1). Each frost boil was divided into adjacent vegetated and un-vegetated sections.

The basic experimental unit at both the experimental site and the frost boil sites were 15 x 15 cm subplots. Each of the 24 plots at the experimental site were divided into nine 15 x 15 cm subplots, with a minimum 5 cm buffer between subplots used in the seeding trials. Each frost boil was divided into eight 15 x 15 cm subplots, four located on vegetated substrate (edge of the frost boil) and four on un-vegetated substrate (center of the frost boil). Seeding trials were applied to randomly selected subplots (as described below). Vegetation cover of the subplots was estimated using visual percent cover. Canopy cover of the dominant species was estimated to the nearest 1% and to the nearest 0.5% for the rare species.

3.2.3 Seed collection, storage, and germination

Seeds from the tundra species *D. octopetala*, *S. arctica*, *and L. arcticus* were collected in early August 2011 from multiple individuals located within a 50m radius of the study site. Seeds were air dried for approximately 24 hours. *D. octopetala* and *S. arctica* seeds had plumes and hairs removed, respectively. *L. arcticus* seeds were removed from their pods. Seeds were then frozen (approximately -18ºC) until the time of germination trials and sowing. *D. octopetala* and *S. arctica* seeds did not require

dormancy breaking (other than a cold period). Seeds of *L. arcticus* required specific dormancy breaking techniques (Baskin and Baskin 1998). Seeds of this species were scarified using 50 grade sandpaper and soaked in de-ionized water for 48 hours immediately prior to germination trials or sowing (Baskin and Baskin 1998).

Cones from the boreal species *P. contorta*, *P. glauca*, and *A. crispa* were collected in August 2010 from multiple individuals around the Whitehorse area. After collection, boreal cones were stored in a refrigerator until further processing could occur. Cones were dried in an oven at approximately 40°C for 48 hours, enough time to open bracts. Seeds were then removed from the cone using tweezers and by shaking the cones vigorously inside a tin can. For *P. contorta* and *P. glauca* seeds, “winged” material was removed to lessen the likelihood that they would blow away during and after sowing. De-winged seeds of *P. contorta* and *P. glauca* along with seeds of *A. crispa* were stored in a freezer (approximately -18°C) until time of germination trials and sowing.

Germination trials were run prior to sowing in order to help determine the amount of seed that would be needed in sowing trials (Table 3.1). Boreal species were tested in both 2011 and 2012. Tundra species were only tested in 2012 prior to sowing. Seeds were placed in a 9 cm plastic Petri dish with two layers of filter paper. The filter paper was initially moistened with de-ionized water and was moistened as needed through the duration of the trials. It was often difficult to keep humidity high throughout the day, so to help maintain a more consistent humidity the dishes were placed inside plastic bags (unzipped). Seeds were arranged so that they were not touching each other or the edge of the dish. Seeds of each species were placed in separate Petri dishes. Dishes of *P. glauca* contained 20 seeds, *P. contorta* contained 16 seeds and dishes of *A. crispa*

contained 88 seeds. Three replicate dishes were done for each species (n=60 seeds, 48 seeds, 264 seeds total). For the tundra species, 25 seeds of each species were placed in their own dish. Four replicate dishes were done of each species (n=100 seeds per species). Dishes were placed on the lab bench next to the window and exposed to natural light variation during the month of May in Saskatoon, SK (approximately 16 light-hours per day). Dishes were re-shuffled at least every second day for the duration of the trials. The room was not temperature controlled but was approximately 20ºC. A seed was considered to have germinated when the radicle was approximately twice the length of the seed coat. Germinated seeds were counted and removed from each dish at least every second day. Germination trails were terminated when no germinated seeds were found for five consecutive days (trials lasted for approximately 2 weeks for tundra species and 3 weeks for boreal species).

3.2.4 Seeding trials

Two subplots were randomly selected within each experimental or frost boil plot for the sowing trials. The three boreal species were sown together into a single subplot and the three tundra species were sown together in a different subplot. Species were sown one at a time and seeds were spread as equally as possible over the subplot. The vegetation cover was gently shaken to ensure that the seeds fell to the ground surface. Tundra seeds were sown in subplots on 26 June 2012, following collection in fall 2011 and processing during winter 2011-12. Without prior knowledge of the local seed-rain, seeding densities of tundra species were arbitrarily chosen at 100 seeds per subplot of *D. octopetala* and *S. arctica* (small seeded species) and 10 *L. arcticus* (large seeded species). Estimates of viable seeds sown were derived from the laboratory viability trials

(Table 3.1). Boreal seeds were sown into subplots on 16 August 2011, close to the time of natural dispersal in early fall. Seeding densities of boreal species were based on seed weight (~0.040 g per subplot), which equated to additions of 20, 16, and 88 seeds per subplot of *P. glauca, P. contorta,* and *A. crispa,* respectively (Table 3.1).

All subplots were examined for presence of emerged seedlings on 12 August 2012, near the end of the growing season. An emerged seedling was defined as a seedling showing initial growth (cotyledons) arising from a seed or bulbil. Initial growth had to be visibly alive (green) and above the soil or lichen surface. Subplots that were not used as part of the sowing trials (n=8 at experimental site, and n=3 at frost boil sites) were surveyed to provide estimates of naturally occurring seedling emergence.

3.2.5 Data analysis

All statistical analyses were performed in R (R Development Core Team 2013) and an α-level of 0.05 was used to assess statistical significance. Analysis the effectiveness of the warming and nitrogen addition treatments can be found in Chapter 2.

A multi-response permutation procedure (MRPP) was performed on the visual percent cover classes to test for differences in vegetation composition between seeded and non-seeded subplots (tundra and boreal) at the experimental and frost boil sites. Visual percent cover was divided into classes of lichen, moss, rock, bare soil, litter, live vegetation, and roots. Bray Curtis distances measures and a maximum of 1000 permutations were used. This procedure was conducted using the using the R package 'vegan' (Oksanen *et al.* 2011).

To examine the effects of sowing, experimental treatments, and substrate on seedling emergence, the observed number of seedlings in seeded subplots and the average number of seedlings observed in the non-seeded subplots were subtracted, to give a single value per plot. Values close to zero indicate no difference in the number of seedlings between seeded and non-seeded subplots. A positive difference indicates that seeding increased the number of seedlings and a negative difference indicates that seeding had no effect on the number of seedlings in each plot. This difference was referred to as the response to seeding. A generalized linear model was used to test the effect of experimental manipulations and substrate on response to seeding at both the experimental and frost boil sites. The overall effect of sowing was interpreted based on whether the intercept values in these models was significantly different from zero, and parameter estimates for experimental or frost boil treatments provided a test of substrate effects. The generalized linear model was conducted using the using the base R package ‘stats’ and the function ‘glm’ (R Development Core Team 2013).

Table 3.1: Characteristics of seeding treatments used at the experimental and frost boil sites at Wolf Creek, Yukon in 2012, by species. Results are presented as mean ± 1 SE (n).

	Alnus crispa	***Picea glauca***	***Pinus contorta***	***Dryas octopetala***	***Lupinus arcticus***	***Salix arctica***
Seed viability	86 ± 1% (2)	57 ± 7% (2)	58 ± 7% (2)	38 ± 1% (2)	73% (1)	76 ± 3% (2)
No. seeds sown*	88	20	16	100	10	100
Estimated # of viable seed*	75.7	11.4	9.3	38.0	7.3	76.0
Total weight of seeds sown (g)*	0.040	0.040	0.041	-	1.7 ± 0.01 (22)	0.01
Average seed mass ($g \times 10^{-4}$)	4.54	20.00	25.60	1.81^{+}	140.30^{+}	1.21^{+}
Weight of viable seeds sown ($g \times 10^{-4}$)*	343.7	228.0	238.1	68.8	1024.2	92.0

***** Numbers are presented per subplot (0.0225 m^2)

$^{+}$ Average seed mass from Chapter 2, Table 2.7

3.3 Results

3.3.1 Experimental treatments

For results on the experimental treatments of soil warming and nitrogen fertilization, please see Chapter 2, Section 2.3.1.

3.3.2 Vegetation cover

Un-vegetated areas of the frost boils were dominated by bare soil and rock whereas the adjacent vegetated areas were dominated by lichen, litter, and live vegetation (Figure 3.2). For each substrate type (vegetated or un-vegetated), there was no significant difference in the composition of vegetation cover classes between the seeded and non-seeded subplots (Table 3.2, Figure 3.2). Similarly, there was no significant difference between the seeded and non-seeded subplots for the sown tundra species or the sown boreal species at the experimental site (Table 3.2). Lichen, litter and live vegetation dominated visual cover at the experimental site, and areas of bare soil and rock were relatively rare (Figure 3.3).

3.3.3 Seedling emergence

Observations of natural seedling emergence in 2012 identified newly emerged seedlings of 10 native tundra species plus two seedlings of unknown species. *Polygonum viviparum* and *Dryas octopetala* were the most frequently observed seedlings at the frost boil and experimental sites and showed the highest average seedling densities (Figure 3.4). *Lupinus arcticus* and *Salix arctica* were also frequently observed as seedlings at the frost boil and experimental sites. Natural seedling densities of *L. arcticus* tended to be higher at the frost boil sites than at the experimental site and

natural seedling densities of *S. arctica* were generally lower at the frost boil sites than at the experimental site. Seedlings of *Campanula rotundifolia*, *Stellaria longipes*, and *Rhodiola rosea* were observed less frequently. *C. rotundifolia* and *S. longipes* had low seedling densities observed only at the experimental site and *R. rosea* had low seedling densities observed only at the frost boil sites (Figure 3.4).

Sowing treatments significantly increased seedling densities for 5 of the 6 species sown in the frost boil plots (Table 3.3) and 3 of the 6 species in the experimental warming and nitrogen plots (Table 3.4). Seedling densities in the non-seeded subplots (0.0225 m^2) were on average ≤1.0 seedling per subplot for the tundra species *D. octopetala, L. arcticus,* and *S. arctica* (Figure 3.5 and Figure 3.6) due to a high frequency of zero counts. Zero counts were also observed at a high frequency in seeded subplots. In the seeded subplots, *S. arctica* showed the single highest observed density of seedlings, with 14 seedlings in one subplot in the un-vegetated substrate (Figure 3.5C). There was a significant effect of the un-vegetated substrate on the seeding response for *S. arctica* (Table 3.3, Figure 3.9C). At the experimental site, all three tundra species showed a maximum seedling density of three seedlings per subplot in at least one seeded subplot, but this was not consistent by treatment type (Figure 3.6). There was no clear effect of the experimental treatments of soil warming and fertilization on the seeding response for any of the seeded tundra species (Table 3.4, Figure 3.9).

In the sowing trials with boreal seeds, no seedlings were observed in the non-seeded subplots for *Alnus crispa*, *Picea glauca*, and *Pinus contorta* (Figure 3.7 and Figure 3.8). In the seeded subplots, *A. crispa* showed the single highest observed density of seedlings, with 11 seedlings in one subplot in the un-vegetated substrate (Figure 3.7A). There was a significant effect of the un-vegetated substrate on the

seeding response for *A. crispa* (Table 3.3, Figure 3.10A). At the experimental site, the frequency of seedlings was highest for *P. glauca* and *P. contorta* (Figure 3.8). *P. contorta* often had high seedling densities in seeded subplots, regardless of treatment or substrate (Figure 3.7C and Figure 3.8C). Of the approximately 9 viable seeds sown of *P. contorta* per subplot (Table 3.1), there were three observations of a maximum seedling density of 7 at the experimental site, while many observations were made with densities at 4-6. There was no clear effect of the experimental treatments of soil warming and fertilization on the seeding response for any of the seeded boreal species (Table 3.4, Figure 3.10).

Average seedling densities (seeded subplots only) between the frost boil sites and the experimental site were similar for the species *D. octopetala*, *L. arcticus*, and *P. glauca* (Table 3.5 and 3.6). Average seedling densities were higher for *A. crispa* and *S. arctica* at the frost boil sites compared to the experimental site (Table 3.5 and 3.6), as explained by the treatment effect above. *P. contorta* experienced higher averaged seedling densities at the experimental site compared to the frost boil sites (Table 3.5 and 3.6). When comparing across boreal and tundra species types, average seedling densities for the boreal species were higher overall than the tundra species (Table 3.5 and 3.6). However, the mean number of seedlings per gram of viable seeds, does not show a consistent pattern in relation to seed weight (Table 3.5 and 3.6, and see Table 3.1). The mean number of seedlings per 100 seeds was far less in the field trials than the lab germination and viability tests (Table 3.5 and 3.6, and see Table 3.1).

Table 3.2: Results from multi-response permutation procedure (MRPP) tests looking at differences in vegetation cover between groups of seeded and non-seeded subplots (tundra and boreal) at the frost boil and experimental sites. Vegetation cover classes of lichen, moss, rock, bare soil, litter, live vegetation, and roots were used. n= number of plots, A= chance-corrected within-group agreement.

Site	Species	n	A	p-value
Frost boil	Tundra	12	-0.003	0.387
Un-vegetated	Boreal	12	-0.002	0.391
Frost boil	Tundra	12	-0.022	0.883
Vegetated	Boreal	12	-0.003	0.429
Experimental site	Tundra	24	-0.008	0.849
	Boreal	24	-0.004	0.603

Table 3.3: Results from generalized linear models estimating the effects of disturbance on response to seeding at the frost boil sites. Results show effects of sowing treatments (Intercept), where positive intercept indicates an increase in seedlings with sowing, and substrate condition (Veg), where a negative number indicates fewer seedlings in vegetated than unvegetated plots. Significant effects are shown in bold font (α ≤0.05).

Species	Treatment	Estimate	Std. error	t value	p-value
Alnus crispa	Intercept	4.31	0.68	6.38	**0.000**
	Veg	-3.64	0.95	-3.81	**0.001**
Picea glauca	Intercept	1.50	0.33	4.51	**0.000**
	Veg	-0.75	0.47	-1.59	0.125
Pinus contorta	Intercept	1.92	0.54	3.57	**0.002**
	Veg	-0.42	0.76	-0.55	0.588
Dryas octopetala	Intercept	-0.20	0.14	-1.35	0.191
	Veg	0.42	0.20	2.04	0.053
Lupinus arcticus	Intercept	0.45	0.16	2.72	**0.012**
	Veg	-0.42	0.23	-1.80	0.085
Salix arctica	Intercept	3.50	0.83	4.22	**0.000**
	Veg	-2.92	1.17	-2.48	**0.021**

Table 3.4: Results from generalized linear models estimating the effects of experimental treatment on response to seeding at the experimental site. Results show effects of sowing treatments (intercept) and experimental treatments (N = fertilization, W = warming, NW = fertilization and warming). Significant effects are shown in bold font (α ≤0.05).

Species	Treatment	Estimate	Std. error	t value	P-value
Alnus crispa	Intercept	0.83	0.56	1.48	0.153
	N	0.00	0.79	0.00	1.000
	W	-0.17	0.79	-0.21	0.836
	NW	-0.50	1.12	-0.45	0.661
Picea glauca	Intercept	1.67	0.47	3.51	**0.002**
	N	-1.00	0.67	-1.49	0.152
	W	-0.50	0.67	-0.75	0.465
	NW	0.33	0.95	0.35	0.729
Pinus contorta	Intercept	3.33	1.07	3.13	**0.005**
	N	0.33	1.51	0.22	0.827
	W	-0.50	1.51	-0.33	0.744
	NW	-1.00	2.13	-0.47	0.644
Dryas octopetala	Intercept	-0.11	0.29	-0.36	0.724
	N	0.06	0.42	0.15	0.883
	W	0.40	0.42	0.96	0.351
	NW	-0.27	0.59	-0.46	0.649
Lupinus arcticus	Intercept	0.65	0.29	2.23	**0.037**
	N	-0.71	0.41	-1.74	0.098
	W	-0.52	0.41	-1.28	0.217
	NW	0.71	0.58	1.23	0.234
Salix arctica	Intercept	0.27	0.37	0.72	0.480
	N	0.86	0.53	1.63	0.120
	W	-0.21	0.53	-0.39	0.699
	NW	-0.52	0.75	-0.70	0.491

Table 3.5: Mean number of seedlings *(± standard error)* from frost boil sites i) observed per subplot or 0.0225 m^2 (n=24), ii) expected per gram of viable seeds sown, and iii) expected given 100 seeds.

	Alnus crispa	***Picea glauca***	***Pinus contorta***	***Dryas octopetala***	***Lupinus arcticus***	***Salix arctica***
Mean number of seedlings per subplot *(SE)*	2.50 *(0.59)*	1.13 *(0.24)*	1.71 *(0.37)*	0.17 *(0.08)*	0.33 *(0.10)*	2.08 *(0.64)*
Mean number seedlings/ g of viable seed	72.74	49.34	71.40	24.27	3.35	226.41
Mean number seedlings/ 100 seeds	2.84	5.63	10.63	0.17	3.30	2.08

Table 3.6: Mean number of seedlings *(± standard error)* from experimental site i) observed per subplot or 0.0225 m^2 (n=24), ii) expected per gram of viable seeds sown, and iii) expected given 100 seeds.

	Alnus crispa	***Picea glauca***	***Pinus contorta***	***Dryas octopetala***	***Lupinus arcticus***	***Salix arctica***
Mean seedling density per subplot *(SE)*	0.63 *(0.26)*	1.00 *(0.24)*	3.00 *(0.05)*	0.25 *(0.15)*	0.29 *(0.14)*	0.54 *(0.19)*
Mean number seedlings/ g of viable seed	18.18	43.86	125.99	36.33	2.85	58.88
Mean number seedlings/ 100 seeds	0.71	5.00	18.75	0.25	2.92	0.54

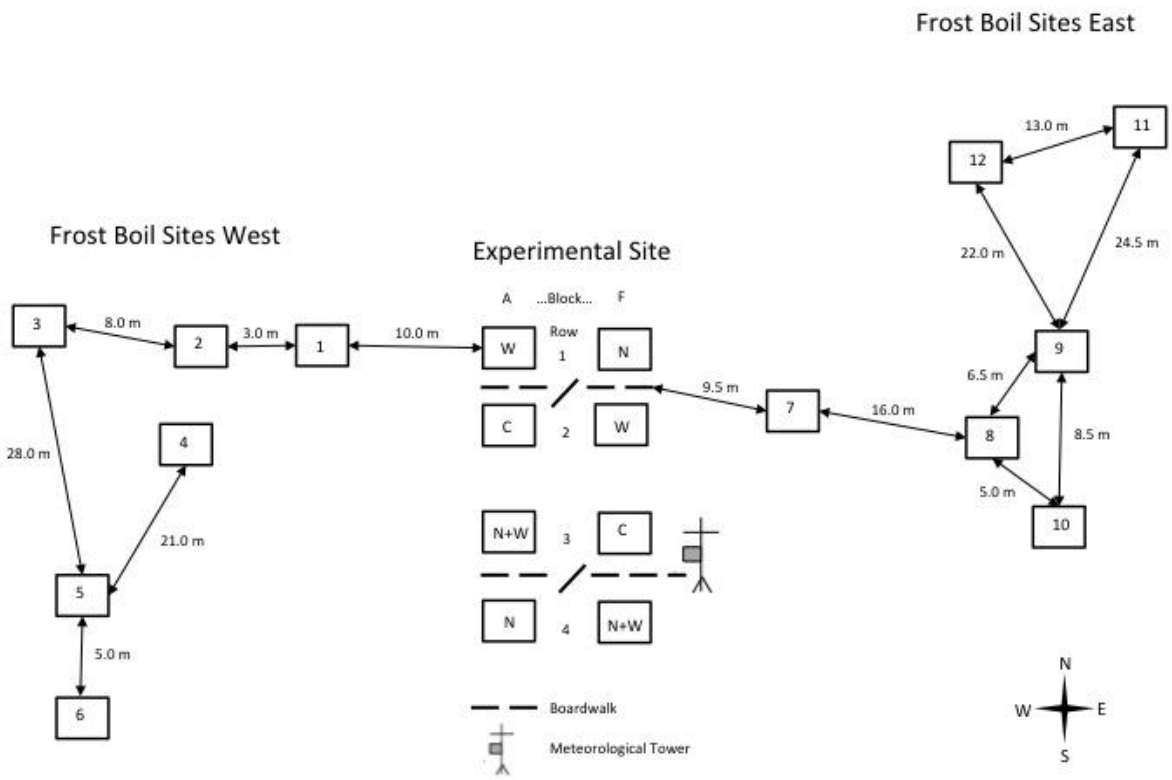


Figure 3.1: Diagram of frost boil site layout in relation to experimental site at Wolf Creek. Frost boils were randomly selected. Plots were approximately 1m x 1m. Each frost boil was divided into adjacent vegetated and un-vegetated sections.

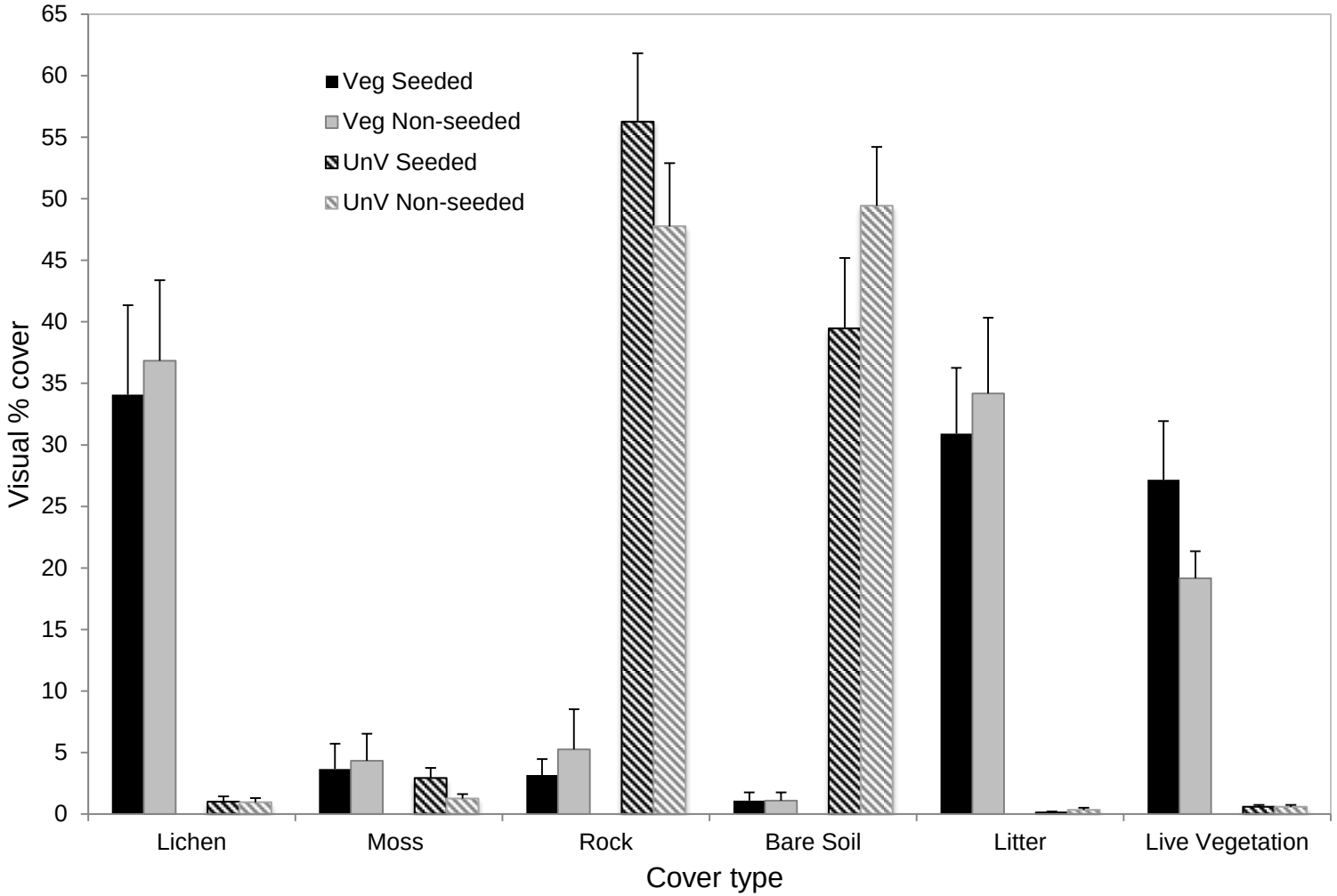


Figure 3.2: Variation in vegetation cover between seeded and non-seeded plot for the vegetated and un-vegetated substrates at the frost boil sites. Bar heights indicate average percent cover (± 1 SE) of lichen, moss, rock, bare soil, litter, and live vegetation. Data are visual percent cover estimates of the subplots for tundra sown species.

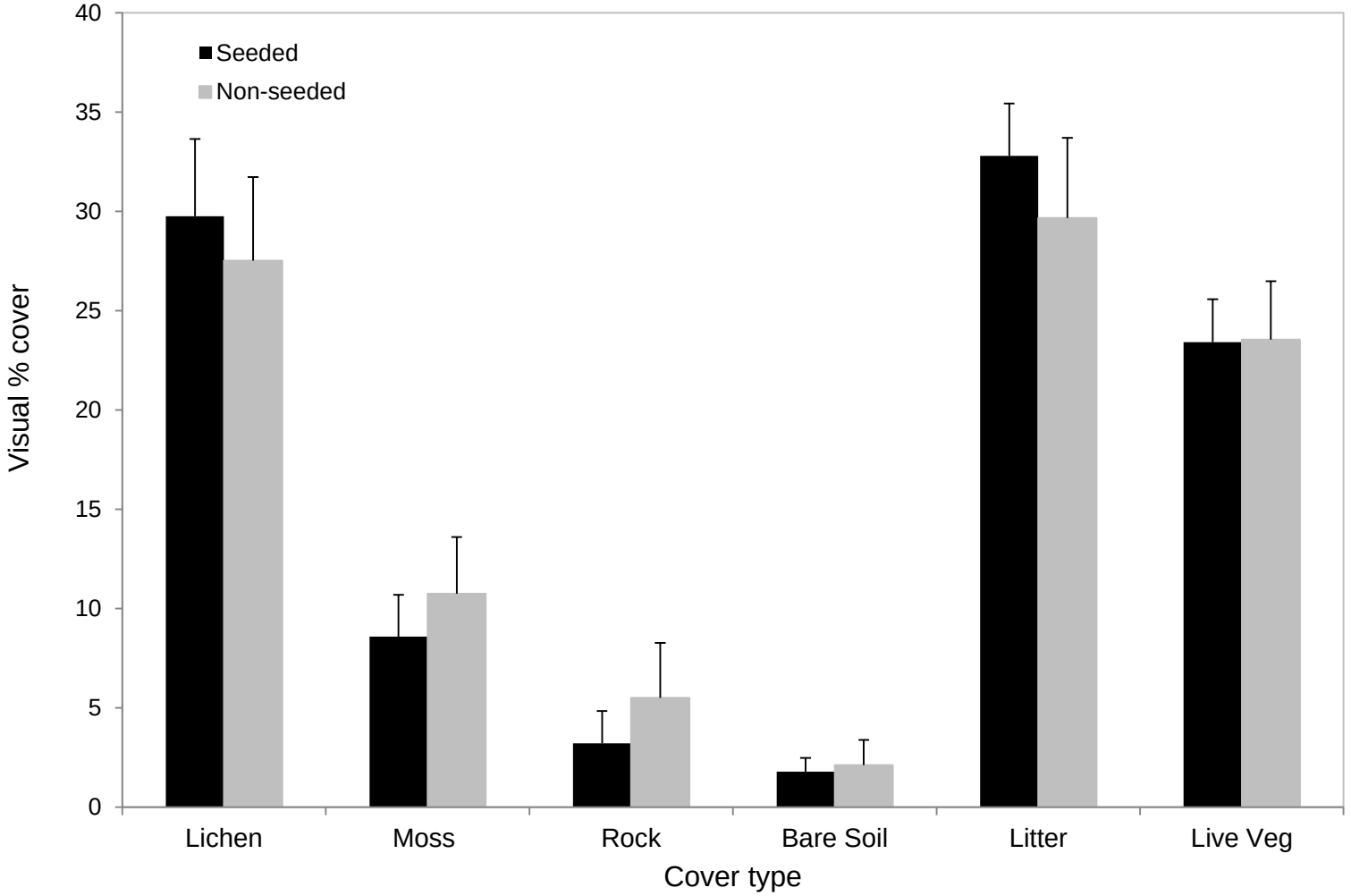


Figure 3.3: Variation in vegetation cover between seeded and non-seeded plot for the experimental site, with experimental warming and nitrogen treatments pooled. Bar heights indicate average percent cover (± 1 SE) of lichen, moss, rock, bare soil, litter, and live vegetation. Data are visual percent cover estimates of the subplots for tundra sown species.

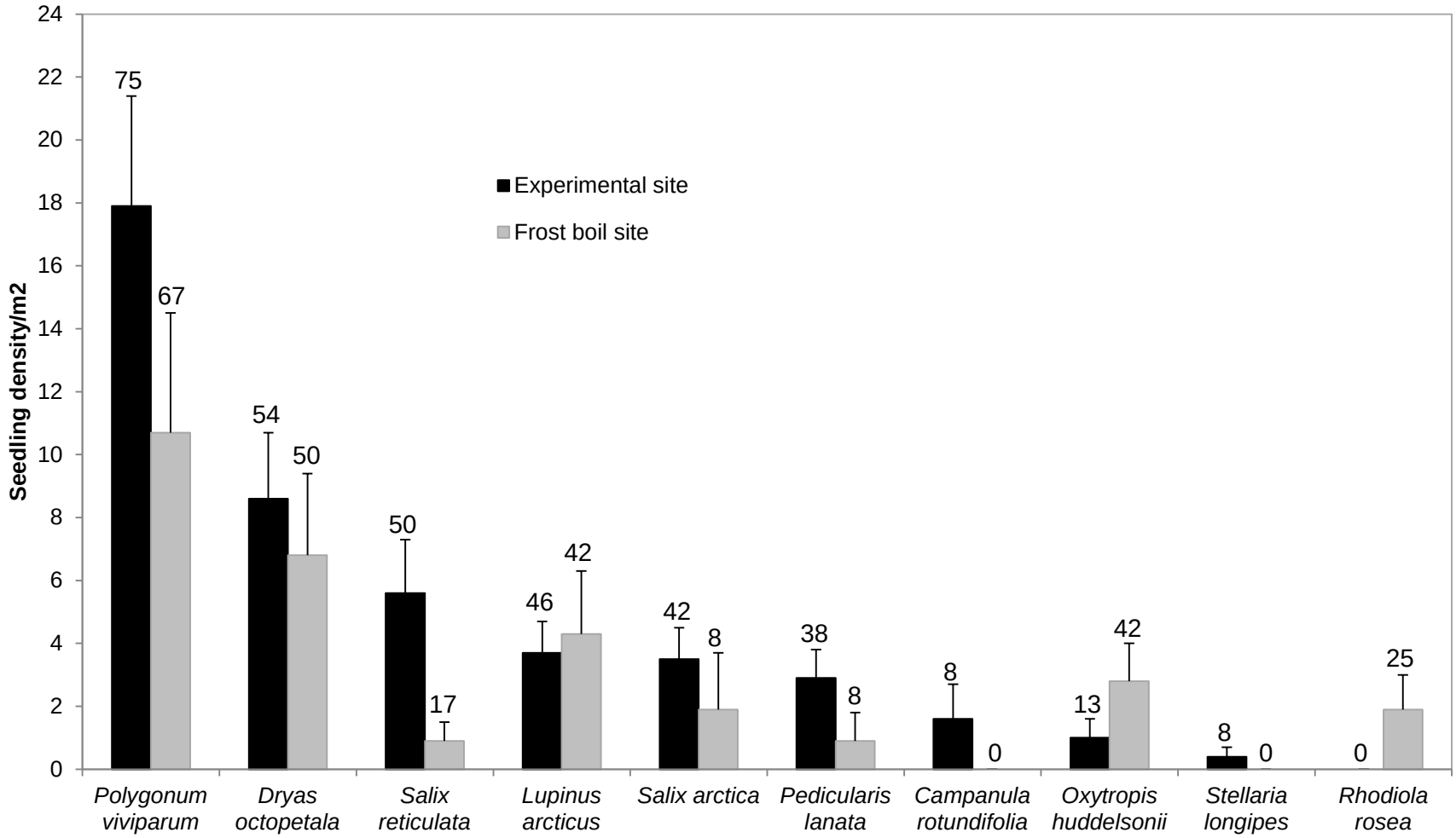


Figure 3.4: Seedling density (number per m^2) of native tundra species observed in non-seeded plots at the experimental site (black bars, n=24) and the frost boil sites (gray bars, n=12). Bars show mean seedling density ± 1 SE. Total area observed was 0.180 m^2 for each experimental plot and 0.135 m^2 for each frost boil plot. Numbers above each bar indicate the percentage of plots where seedlings occurred.

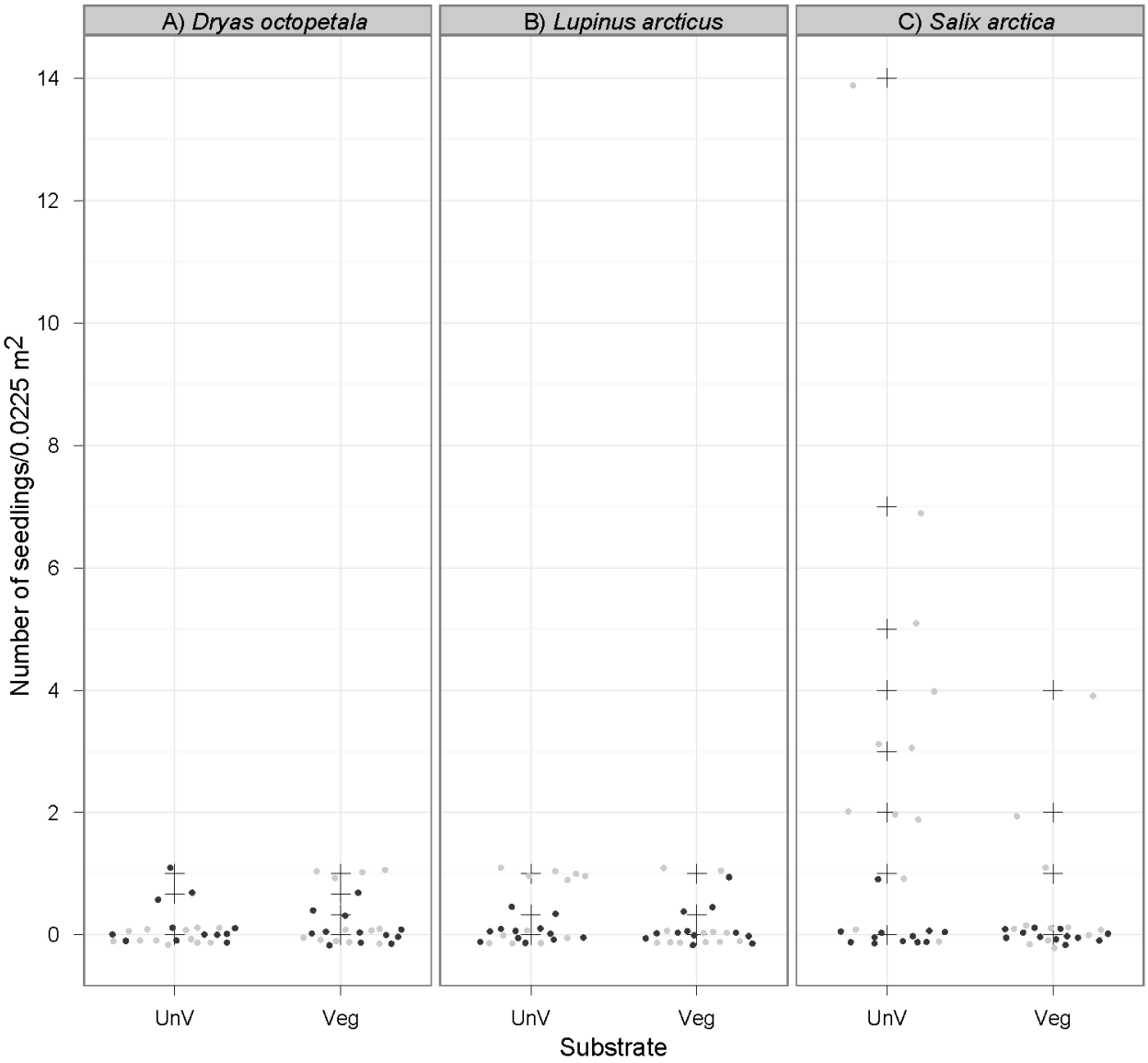


Figure 3.5: Observed seedling densities at frost boil sites for tundra species A) *Dryas octopetala* (mountain aven), B) *Lupinus arcticus* (arctic lupine), and C) *Salix arctica* (arctic willow), on un-vegetated (UnV) and vegetated substrates (Veg). Gray dots represent actual densities observed in seeded subplots (n=1 per pot) and black dots represent the average densities observed in the non-seeded subplots (n=3 per plot). Observed seedling densities (n=12 per treatment) have been randomly offset to avoid overlapping points around the central cross hairs that represent the actual density value.

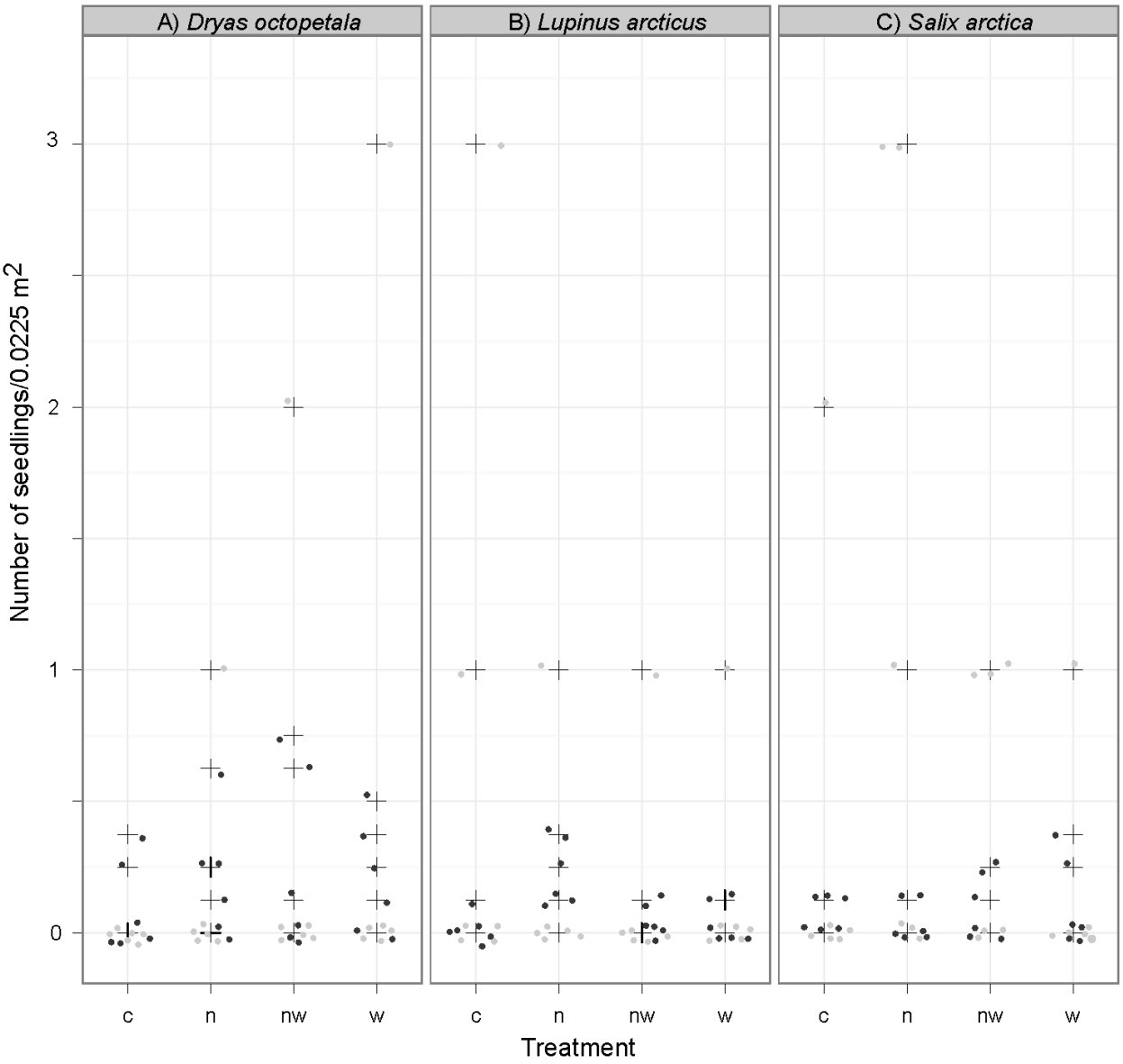


Figure 3.6: Observed seedling densities at experimental site for tundra species A) *Dryas octopetala* (mountain aven), B) *Lupinus arcticus* (arctic lupine), and C) *Salix arctica* (arctic willow), in control plots (c), nitrogen fertilization plots (n), nitrogen fertilization and warming plots (nw), and warming plots (w). Gray dots represent actual densities observed in seeded subplots (n=1 per plot) and black dots represent the average densities observed in non-seeded subplots (n=8 per plot). Observed seedling densities (n=6 per treatment) have been randomly offset to avoid overlapping points around the central cross hairs that represent the actual density value.

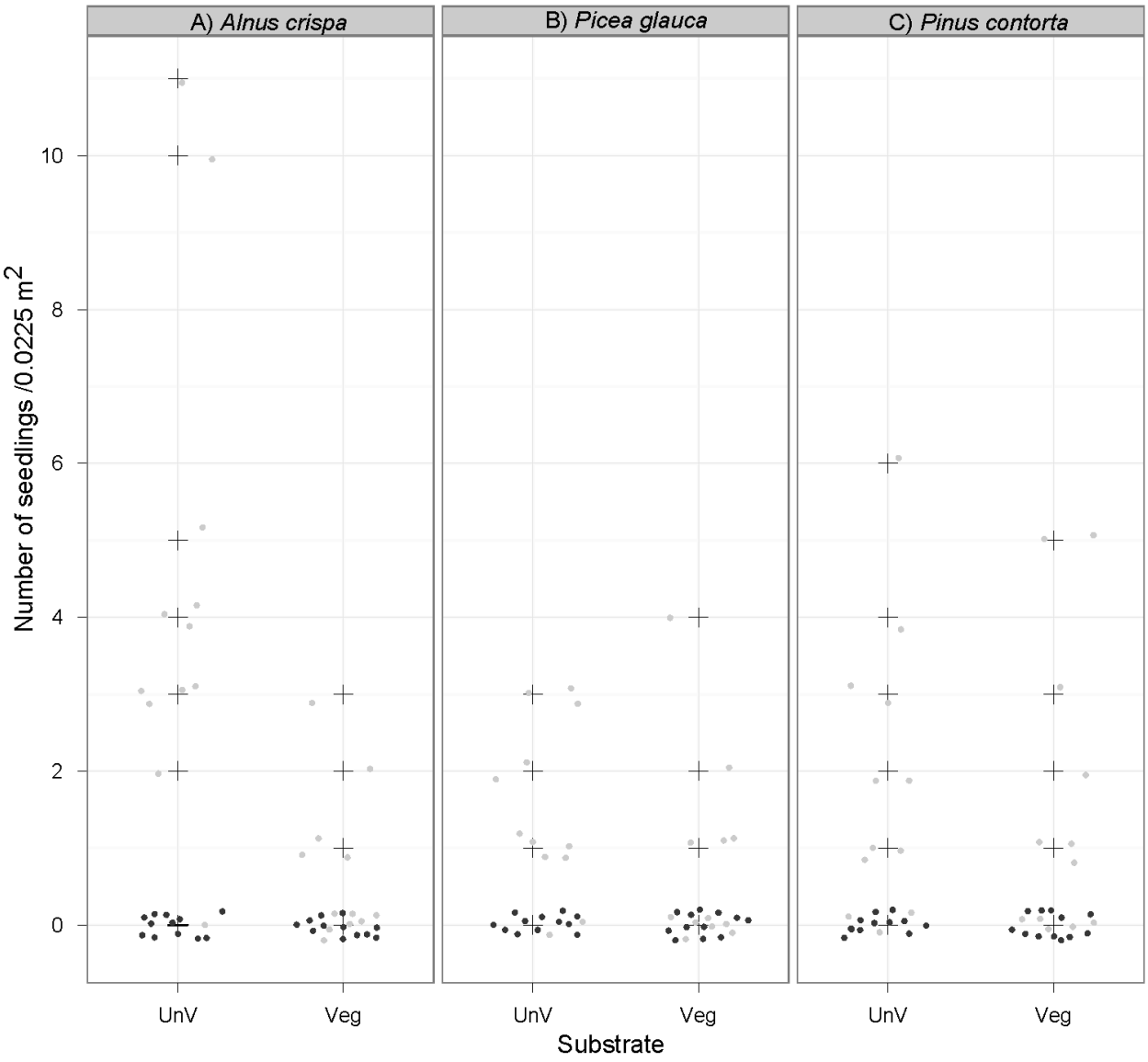


Figure 3.7: Observed seedling densities at frost boil sites for boreal species A) *Alnus crispa* (green alder), B) *Picea glauca* (white spruce), and C) *Pinus contorta* (lodgepole pine), on un-vegetated (UnV) and vegetated substrates (Veg). Gray dots represent actual densities observed in seeded subplots (n=1 per pot) and black dots represent the average densities observed in the non-seeded subplots (n=3 per plot). Observed seedling densities (n=12 per treatment) have been randomly offset to avoid overlapping points around the central cross hairs that represent the actual density value.

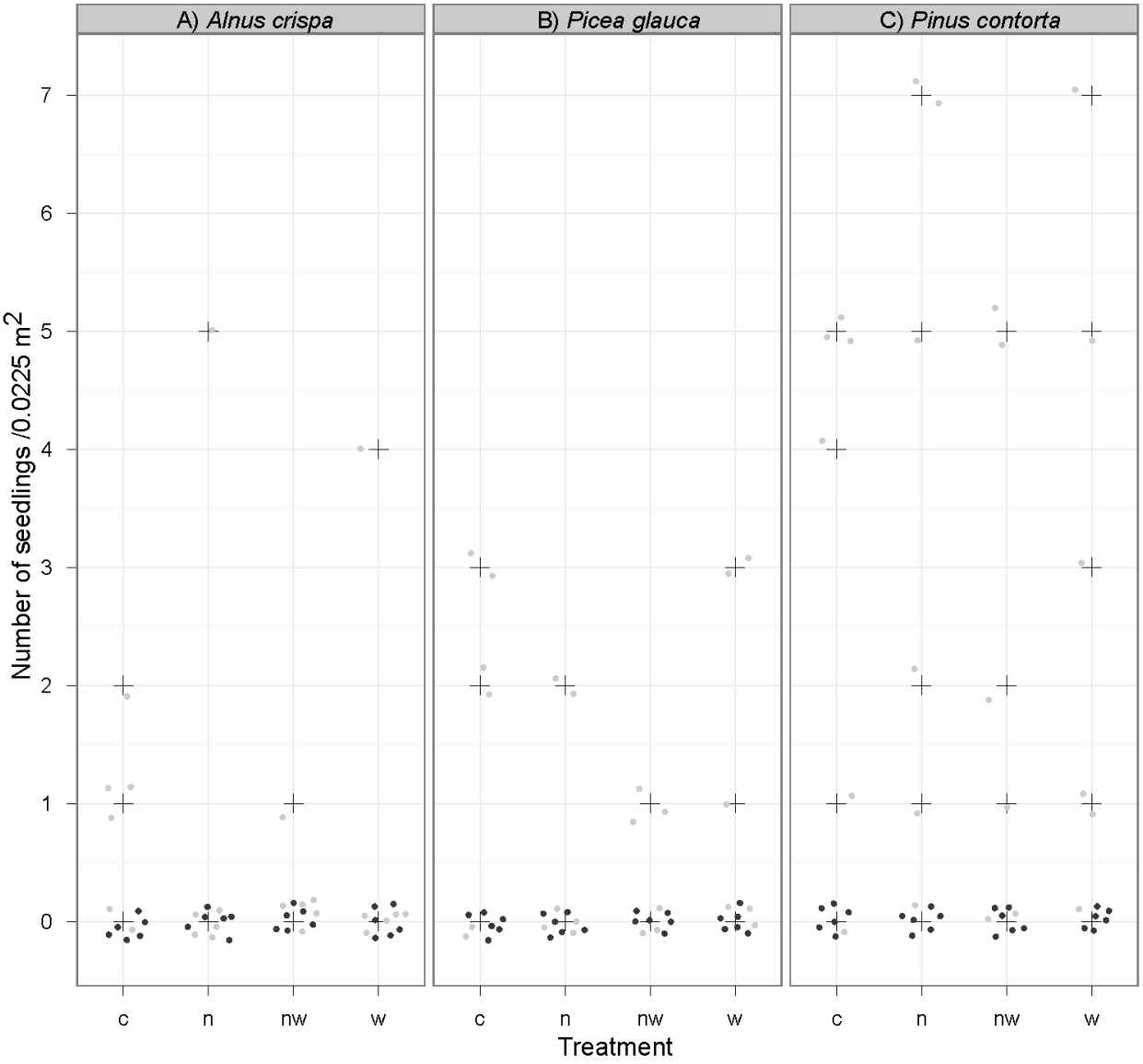


Figure 3.8: Observed seedling densities at experimental site for boreal species A) *Alnus crispa* (green alder), B) *Picea glauca* (white spruce), and C) *Pinus contorta* (lodgepole pine), in control plots (c), nitrogen fertilization plots (n), nitrogen fertilization and warming plots (nw), and warming plots (w). Gray dots represent actual densities observed in seeded subplots (n=1 per plot) and black dots represent the average densities observed in non-seeded subplots (n=8 per plot). Observed seedling densities (n=6 per treatment) have been randomly offset to avoid overlapping points around the central cross hairs that represent the actual density value.

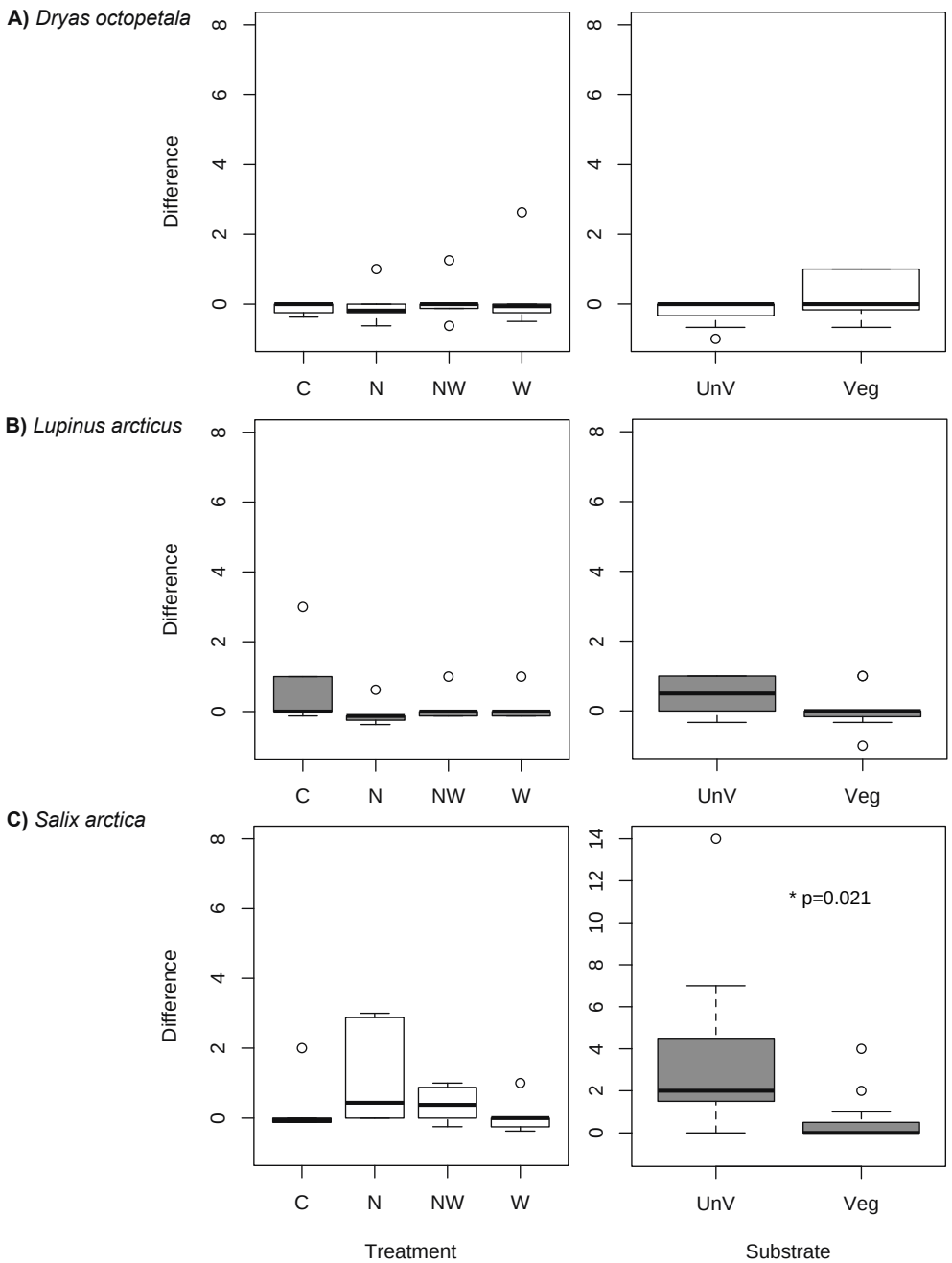


Figure 3.9: The response to seeding (difference between observed seedling densities in seeded subplots and average densities non-seeded subplots) across the different experimental treatments and substrate types at experimental and frost boil sites for tundra species A) *Dryas octopetala* (mountain aven), B) *Lupinus arcticus* (arctic lupine), and C) *Salix arctica* (arctic willow). A gray box indicates a significant seeding effect (See Table 3.3 and 3.4 for p-values). Box represents 25%-75% quartiles with the median difference shown as a line in the middle. Whiskers extending from the box encompass the 95% quartiles and dots represent outlying values. A median difference around 0 indicates no difference in seedling densities between seeded and non-seeded subplots. A positive difference indicates that seeding increased densities and a negative difference indicates that seeding had no effect on densities. A significant substrate effect is indicated by (*) p-value. There were no significant treatment effects.

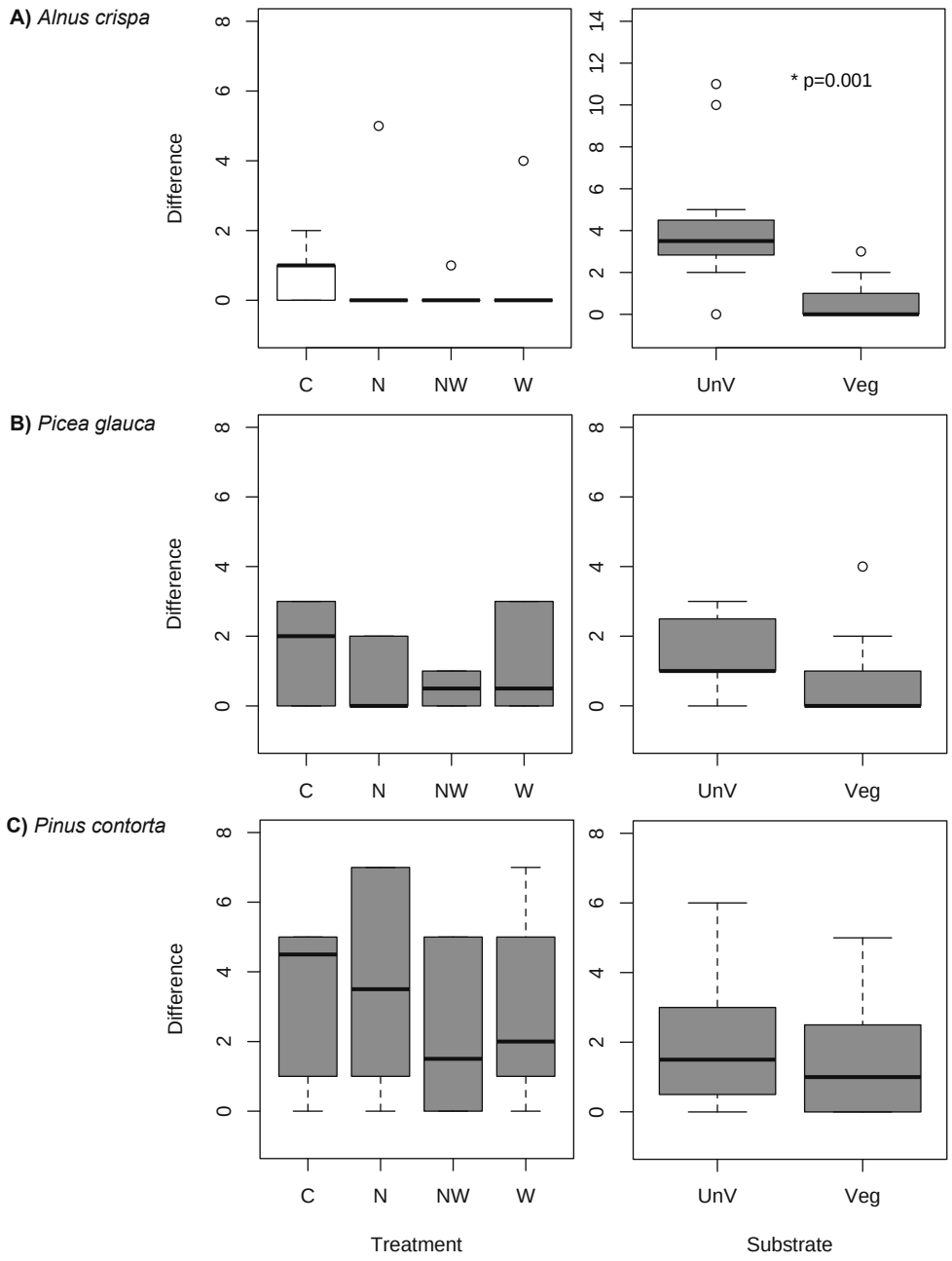


Figure 3.10: The response to seeding (as given by the difference between observed seedling densities in seeded and average non-seeded subplots) across the different experimental treatments and substrate types at experimental and frost boil sites for boreal species A) *Alnus crispa* (green alder), B) *Picea glauca* (white spruce), and C) *Pinus contorta* (lodgepole pine). A gray box indicates a significant seeding effect (See Table 3.3 and 3.4 for p-values). Box represents 25%-75% quartiles with the median difference shown as a line in the middle. Whiskers extending from the box encompass the 95% quartiles and dots represent outlying values. A median difference around 0 indicates no difference in seedling counts between seeded and non-seeded subplots. A positive difference indicates that seeding increased seedling counts and a negative difference indicates that seeding had no effect on seedling counts. A significant substrate effect is indicated by (*) p-value. There were no significant treatment effects.

3.4 Discussion

3.4.1 Experimental treatments

For a discussion on the experimental treatments of soil warming and nitrogen fertilization, please see Chapter 2, section 2.4.1.

3.4.2 Seedling emergence

In this study, I observed natural seedling emergence for 10 identifiable native vascular plant species representing nine different families and three growth forms: perennial, evergreen, and deciduous. The 10 vascular plant species I observed represent about 26% of the known vascular plant species at the study site. This diversity suggests that potential regeneration from seed in this alpine tundra environment is not limited to a single species, family or growth form. This notion is corroborated by similar observations of extensive coverage of naturally emerging seedlings in arctic (Freedman *et al.* 1982, Cooper *et al.* 2004) and alpine environments (Chambers 1995). Overall, the frequency of natural seedling emergence suggests that seeds are an important resource for regeneration in tundra environments.

Observed emergence in the field is lower (Müller *et al.* 2011) than what is observed in emergence studies done using soil seed banks (Cooper *et al.* 2004). In years with favourable conditions for germination and emergence, a depletion of the seed bank will be observed, whereas in years unfavourable to emergence, seed will be added to the seed bank (Welling and Laine 2002). Cold temperatures during the initial growth stages could limit natural seedling emergence.

Seed limitation is thought to be widespread in many plant populations (Clark *et al.* 2007). Increased seedling density after seed addition experiments for both tundra and

boreal species indicate that a seed limitation exists in this alpine tundra plant community (Austrheim and Eriksson 2003, Lindgren *et al.* 2007). A seed limitation in a population may be attributed to a combination of factors including: the amount of seeds produced, the viability of seeds, the dispersal of seeds, and the suitability of surface conditions for germination. The production and viability of seeds is important in determining seed limitations and not considering the information regarding the background seed rain can lead to an underestimation of micro-site limitations (Clark *et al.* 2007). In Chapter 2, I demonstrated seed production and viability of *Dryas octopetala*, *Lupinus arcticus*, and *Salix arctica*. Therefore, the seed limitation experienced by these tundra species can likely be attributed to the suitability of surface conditions. No natural regeneration of the boreal species *Pinus contorta*, *Alnus crispa*, or *Picea glauca* was observed in non-seeded plots. This indicates that natural regeneration is uncommon for the boreal species at this alpine site and is likely due to a lack of seeds. Individuals of *P. glauca* have been identified close to the site (personal observation) but have not been observed as sexually mature. Therefore, the seed source from full-stature sexually mature trees for these species would occur near the tree-line which is between 675 and 1170 m in distance from the study site, depending on slope aspect (NE-S). The seed limitation these species experience can be attributed to a dispersal barrier preventing long distance dispersal from the parent tree into the tundra environment (Hampe 2011).

The initial emergence of a seedling is dependent on the breaking of dormancy and the germination of the seed, which are two processes that are strongly regulated by temperature (Baskin and Baskin 1998). Thus, the cold temperatures found on the tundra limit regeneration from seed (Bell and Bliss 1980, Billings 1987) and it would be expected that any alleviation of these limits would increase emergence. Therefore, my

observation that warmed soil temperatures did not influence initial seedling emergence is in direct contrast to what I expected. Increases in air temperature of 1-4 °C, often with inconclusive effects on surface soil conditions, can cause an increase in the overall germination percentage of tundra species (Wookey *et al.* 1995, Cooper *et al.* 2004). Additionally, increased air temperature of just 1 °C can double germination of boreal species, even without increases in soil temperatures 10cm below the surface (Hobbie and Chapin 1998). These studies and results from my study demonstrate that germination and emergence are likely more sensitive to air temperatures than they are to soil temperatures. Mean air temperatures in Whitehorse, YK for June and July 2012 were 0.8 °C cooler than historical (1981-2010) mean temperatures (Environment Canada 2013). In addition to the cold temperatures, a snow event occurred at the beginning of July and may have come at a detrimental time for emergence. Pulses of soil warming, seen as 'extreme events', at the beginning and middle of the summer season have significantly affected the chance for survival of early germinating species, such as *Dryas octopetala* (Graae *et al.* 2009, Shevtsova *et al.* 2009). I postulate that similar results would be observed for cold temperature 'extreme events' if occurring at the critical germination periods, as was possibly the case in this study.

Initial emergence of a seedling relies on carbohydrate and other macromolecule reserves that are provided by the seed and could explain why I did not detect any effects from the soil nitrogen addition treatment. A seed must provide sufficient resources to meet the nutrient demands of initial germination and emergence and these reserves are reflected intraspecifically by seed size and mass (Fenner and Thompson 2005). Larger, heavier seeds have a higher success of emergence and establishment than that of smaller, lighter seeds, especially when considering the many hazards encountered by

the seed in early stages (Moles and Westoby 2004b). The influence of seed size and mass on emergence and establishment success has been observed in grassland environments (Jakobsson and Eriksson 2000). However in this study, while seedling densities were generally higher for heavy boreal seeds than for the light tundra seeds, seed mass did not always provide a good predictor of seedling densities. This highlights that often the species producing higher quantity of small seeds have a greater chance to produce seedlings especially when bare ground was involved (Leishman 2001). Once the seedling has consumed it's initial reserves, the advantage of seed size expires (Moles and Westoby 2004b) highlighting that increased nitrogen availability may become more important in the long term growth and survival of seedlings.

Soil warming and nitrogen addition treatments address abiotic condition in the tundra environment, however, new individuals face many biotic interactions as well. Established vegetation creates difficulties for seedlings to acquire light and moisture for growth. The established vegetation at the experimental site may have contributed to low seedling emergence. Previous studies looking at regeneration from seed on the tundra have shown that emergence and success of seedlings have occurred more often on disturbed sites that interrupt the established vegetation (Freedman *et al.* 1982, Gough 2006, Sullivan and Sveinbjörnsson 2010). Similarly to these studies, I observed some increases in seedling densities of both tundra and boreal species on disturbed sites where bare mineral substrate was left exposed.

Local disturbances, such as those created by animals and frost action, are likely to become important microsites for colonization by species that put energy into regenerating by seed on the tundra (Freedman *et al.* 1982, Chambers 1995, Landhäusser *et al.* 2010). Exposed bare mineral soils provide good quality surface

conditions for germination and initial seedling emergence during the summer. Bare mineral soils allow for the direct contact of seed and soil surface and fine soil texture adequately supply needed resources such as moisture and warmth (Fenner and Thompson 2005, Sullivan and Sveinbjörnsson 2010, Graham *et al.* 2012). While some disturbance sites may provide new individuals with safe sites that are highly sheltered and high in nutrients, such as those of burrowing animals (Freedman *et al.* 1982), other sites may provide more challenges. Frost disturbances are likely to increase in the future (Walker *et al.* 2008); however, due to their active nature, survivorship will likely be an issue on these features. My results do not include the potential effects of surface frost heave or needle ice on mortality, and this disturbance effect may negate the potential for increased establishment on these surfaces. Thus the net potential for recruitment on frost boils is a balance between increased germination rate (affected by seed availability) and increased mortality rate (Gartner *et al.* 1986).

3.5 Conclusions

There is still much that needs to be learned about initial seedling emergence in tundra ecosystems and how this stage of the reproductive cycle will be influenced by changes in environmental conditions, including changes in seedbed quality. In this chapter, I found that increased soil temperature or nitrogen availability had no impact on initial seedling emergence. However, substrate quality was more influential and bare mineral substrate led to higher seedling densities. The sowing of seeds led to an increase in the number of seedlings present providing evidence that the quantity of seed available in this environment is a compounding factor. This study also showed that regardless of treatment, boreal species, when their seeds were present, were able to

successfully produce initial seedlings. Substrate quality and seed availability limit seedling emergence, with little evidence that changes in soil temperature or nutrients affect initial emergence. Therefore, additional research should be focused on understanding the effects of environmental change on seed production, dispersal, and/or seedbed conditions because they will have a stronger effect on seedling emergence in this environment than the warming effects on soil conditions alone. Such studies, in combination with the research presented in this chapter will provide greater understanding of the importance of sexual reproduction as a mechanism that contributes to species diversity across the tundra and also across landscapes.

4.0 SYNTHESIS

The tundra is a globally important biome, due in part to its large role in climate-ecosystem feedbacks and its large carbon storage found frozen in the permafrost (Tarnocai *et al.* 2009). The tundra is home to human settlements and unique animal populations that rely on plant production and growth as a food source. There is a need to study it not only because of its local role in supporting human and animal populations, but also because of its larger role in global climate.

Sexual reproduction is the mechanism that allows plants to maintain or migrate their populations, given changes in environmental conditions. With the increasing impacts of climate driven environmental changes on species distribution, a greater understanding of the role of sexual reproduction and the influence of reproductive ecology on population dynamics in tundra ecosystems is needed (Chambers 1995, Arft *et al.* 1999, Inouye 2008). Climate is changing most rapidly at northern latitudes (Hartmann *et al.* 2013) and the increases in temperatures are having profound effects on the growth and reproduction of arctic and alpine vegetation (Chapin *et al.* 2005). In addition to increases in temperature, changes in the frequency of disturbance, such as those caused by permafrost degradation, and extreme weather events, such as drought or frost, are also anticipated (Hartmann *et al.* 2013). Better understanding of the effects that these climate driven environmental changes will have on tundra plants is needed (Parmesan *et al.* 2000, Jentsch *et al.* 2007).

The goal of the research presented in this thesis was to investigate how climate-driven environmental changes may affect sexual reproduction in an alpine tundra plant community. The results from this research contribute to our understanding of how increases in soil temperature and nitrogen availability, changes in substrate conditions,

and the occurrence of an extreme weather event (summer snowfall) influence sexual reproduction in tundra vegetation. Four main conclusions can be made: 1) the reproductive phenology and overall seed production of tundra plants has the potential to be influenced by extreme weather conditions, 2) seed viability in tundra plants is not affected by increases in soil temperature or nitrogen availability, 3) bare substrate is an important microsite and open sites will be important locations for future regeneration, and 4) initial seedling emergence is possible for southern boreal species regardless of soil conditions.

I was unable to clearly identify soil conditions that place constraints on sexual reproduction. However, with detailed observations I was able to highlight potential bottlenecks in the process of sexual reproduction when considering climatic and environmental conditions. I tried to identify how the reproductive potential of tundra species may be altered, beyond just soil conditions, by looking closely at the interaction of flowering, seed production, seed viability, and seedling emergence within environmental conditions. As environmental conditions (such as soil conditions and seedbed quality) change, a shift in communities across the landscape is expected. In the next section, I synthesize the four main conclusions to examine broader implications for sexual reproduction on the tundra.

4.1 Reproductive potential of tundra species

In this section, I summarize the data collected using the concept of reproductive potential. Reproductive potential is the relative capacity of an individual to reproduce itself under given conditions (Crawley and Ross 1990). In plant species, reproductive potential incorporates both seed production and seedling emergence. Thus, the two key

values used in the calculations are: 1) seed production, the number of viable seeds produced per m^2; and 2) germination success, the number of initial seedlings emerged per viable sown seeds. I calculated the reproductive potential for the species *D. octopetala*, *L. arcticus*, and *S. arctica* (see details provided in Appendix B). In using this approach, I provide insights on the potential consequences that change in environmental conditions could have on individual species and species composition across the landscape.

The reproductive potentials for the studied species, *L. arcticus* and *S. arctica*, strongly increased on bare mineral substrate of the frost boils compared to vegetated tundra (Table 4.1). The reproductive potentials were doubled for *L. arcticus* and were almost 100 times higher for *S. arctica* on bare mineral substrate. However, the mat forming species, *D. octopetala* experienced a higher reproductive potential in vegetated tundra than on bare substrate of the frost boils. It is possible that the sheltering effect of neighbouring plants improves the reproductive success of *D. octopetala*. It appears that the colonisation potential of tundra plants onto new substrates may be species specific. This is an important consideration as it is expected that under continued climate change an increase in disturbances related to permafrost will occur in arctic and alpine regions (Walker *et al.* 2008). This increase in disturbances will create new patches of available bare mineral substrates and facilitate colonization.

The production of viable seeds within a population is a key factor influencing a species' reproductive potential. The reproductive potentials (seedling densities) calculated for the species *D. octopetala* and *L. arcticus* in seeded subplots were much lower than naturally occurring seedling densities (see Figure 3.4; Table 4.1). However, I would have expected that the seedling densities would have been similar if not higher in

the seeded subplots because of the significant sowing effect detected in the seeding trials (Chapter 3). The snow event in 2012 caused low seed production estimates (viable seeds/m^2) for *D. octopetala* and *L. arcticus* (Chapter 2), which then produced underestimated reproductive potentials (see Appendix B). Reproductive potential of *S. arctica* was similar in both seeded and non-seeded subplots meaning that the seed source produced in 2012 is similar to what would be produced in 2011 or earlier. This further corroborates my observations that the flowering and seed production of *S. arctica* was less affected by the snow event than was that of *D. octopetala* and *L. arcticus*. These observations emphasize the importance of seed production, viability, and weather conditions on reproductive potential.

Calculating reproductive potential in the natural environment is a challenging task. There are many factors that I have not been able to explicitly measure. I suggest that predation and loss of seeds (due to wind, water, or other vectors) are important factors related to reproductive potential. In particular at this Yukon site, I feel predation of *L. arcticus* seeds by arctic ground squirrels (*Spermophilus parryii*) and local bird populations could be very important factors influencing the reproductive potential (personal observation). Future studies need to take a more explicated approach to measuring these factors if good estimates of reproductive potential are desired.

4.2 Challenges in reproductive ecology data collection

As the reproductive potential of plant species is influenced by many complex and interacting factors, studying this concept is laden with challenges. However, studying reproductive ecology can provide powerful insights into the impacts of environmental change on plant communities. We therefore need to overcome the challenges

associated with studying plant reproduction in field settings. In the section that follows, I suggest where data collection needs to be improved in order to enhance our ability to predict (based on empirical data) the impacts of environmental change on tundra plant communities.

Firstly, collecting observations throughout the course of the growing season, from initial spring snow melt to the first fall frost, will enable better inferences regarding how reproductive potential links to longer term survival. Although observing reproductive ecology in a shortened period of time is more financially and logistically reasonable, it heightens the probability of missing important phenological stages in plant reproduction (ex. bud burst or senescence). Furthermore, each stage of a plant's reproductive cycle can be differently affected by environmental conditions, so valuable insight into the impacts of environmental change will be lost when observations occur over a short period.

Secondly, better field methods need to be developed to capture the effects of short term weather extremes when they occur. Standardized field methods repeatable over long timeframes, such as those that I used to monitor phenology, have been widely used but are insufficient at capturing the effects of short term unexpected extreme events, such as the July snowfall event. Extreme events are predicted to increase in frequency due to climate change (Easterling *et al.* 2000), making it important to incorporate better methods in the sampling protocol so the impact of these events can be supported by empirical evidence. Better field methods would also need to be combined with additional study on the recovery of a plant's reproductive organs after a frost event. Additional understanding of this physiological process would provide guidance in developing methods to best capture the extent of damage done and its

overall impact on a plant's reproductive success. My study highlights that more focus needs to be made on developing methods that are able to capture shorter term changes and that data collection on both long term and short term can work together for more insight into reproductive potential.

Thirdly, my study highlights key areas within the reproductive cycle where more sampling and effort needs to be made in order to improve our understanding of species' reproductive potential. Firstly, seed germination and viability of alpine and arctic tundra species' requires additional detailed study. In particular, my study highlights the need to better understand the germination requirements for the grass (*Hierochloë alpina)* and sedge (*Carex microchaeta*) species. Increased distribution of grasses and sedges are expected in the arctic (Gough *et al.* 2002) therefore it is critical to further our understanding of these species' reproductive potential. In order to collect better germination data, it would require close attention to natural seed dispersal dates to ensure the collection of fully developed seeds and additional samples over multiple years. Secondly, seed dispersal is a key area that is a very challenging process to study. Seeds, by nature, blow everywhere and get carried away by movable vectors. The ability of a plant to disperse its seed is imperative for the spread, invasion, or even maintenance of a population. To provide valuable estimations of reproductive potential, more studies looking at seedling emergence and survival are needed in order to increase our understanding of the dynamics of viability and dispersal, both locally and across the landscape.

Finally, my study highlights the importance of data collection across multiple years. One year of data collection is not adequate enough to draw concrete conclusions from. I have done my best to provide insight into how the summer of 2012 may have

been an “unusual” year at the field site in Wolf Creek, YK. But just how unusual was it? Without long term observations and data collection, this question cannot fully be answered.

4.3 Future research

There is still much that we need to learn about how climate and environmental change will influence sexual reproduction and the complexities of reproductive ecology in tundra ecosystems. Future research should include more empirical studies that address both how immediate and long term changes in climate and environmental conditions will impact sexual reproduction. Additional research should work towards illustrating how each process involved in reproduction influences the subsequent processes and thus, focused ecological observations should be made over the duration of the reproductive cycle. Such studies, in combination with the research presented in this thesis, will help us in better understanding the dynamics of reproduction in tundra vegetation and will aid us in predicting outcomes of climate change.

Table 4.1: Summary table displaying seed production and germination success data (average value, pooled across plots) used to estimate reproductive potential for *Lupinus arcticus*, *Dryas octopetela*, and *Salix arctica* based on seeding treatments in vegetated tundra and bare substrate (frost boils).

Species	**Seed production** (No. viable seeds/m^2)	Treatment	**Germination success** (No. seedlings / No. viable seeds sown)	**Reproductive potential** (No. seedlings / m^2)
Lupinus arcticus	13.75	Vegetated	0.03	0.47
		Bare substrate	0.07	0.94
Dryas octopetela	470.18	Vegetated	0.01	3.48
		Bare substrate	0.00	0.00
Salix arctica	203.96	Vegetated	0.01	1.51
		Bare substrate	0.05	96.07

5.0 LITERATURE CITED

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6.0 APPENDIX A

Table A1: Summary of plot level reproductive output variables for *Carex microchaeta*. Table shows plot level data for both Nitrogen dosage and Experimental sites. Summary averages (with standard error) and total number of plots where species occurred can also be found in Table 2.7. Description of reproductive output variables can be found in section 2.2.3. Data for control plots can be found in the TRY plant trait database (www.try-db.org).

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	Proportion of seeds filled	# seeds per catkin	# catkins per inflor.	abundance	Inflor. density
Nitrogen dosage	1G	53		42.4	6.4	0.31	0.0	0.0	-	-	8.0	5.0
	1G	56		-	-	-	-	-	-	-	0.5	0.0
	1G	65		54.3	11.3	0.59	0.0	0.0	-	-	6.0	3.0
	1G	67		58.6	14.4	0.95	0.0	0.1	-	-	14.0	11.0
	2G	52		-	-	-	-	-	-	-	0.0	0.0
	2G	59		-	-	-	-	-	-	-	0.0	0.0
	2G	62		69.0	30.5	3.77	0.0	0.4	-	-	6.0	2.0
	2G	68		-	-	-	-	-	-	-	0.0	0.0
	4G	51		41.3	17.7	0.57	0.0	0.0	-	-	6.0	3.0
	4G	58		-	-	-	-	-	-	-	0.0	0.0
	4G	63		62.4	20.0	0.63	0.0	0.0	-	-	14.0	11.0
	4G	66		78.6	22.8	0.70	0.0	0.0	-	-	10.0	6.0
	8G	55		-	-	-	-	-	-	-	1.0	0.0
	8G	57		-	-	-	-	-	-	-	0.0	0.0
	8G	64		63.4	18.2	1.38	0.0	0.1	-	-	14.0	6.0
	8G	69		-	-	-	-	-	-	-	0.0	0.0
	C	54		41.6	5.0	1.71	0.0	0.3	-	-	2.0	7.0
	C	60		-	-	-	-	-	-	-	0.0	0.0
	C	61		58.6	101.4	1.48	0.0	0.1	-	-	2.0	5.0
	C	70		52.4	25.1	1.38	0.0	0.3	-	-	20.0	15.0

Table A1 continued: Reproductive outputs of *Carex microchaeta*.

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	Proportion of seeds filled	# seeds per catkin	# catkins per inflor.	abundance	density
Experimental	C	A2	A	-	-	-	-	-	-	-	2.0	0.0
	C	B3	B	-	-	-	-	-	-	-	-	-
	C	C1	C	-	-	-	-	-	-	-	2.0	0.0
	C	D4	D	-	-	-	-	-	-	-	2.0	0.0
	C	E4	E	-	-	-	-	-	-	-	4.0	0.0
	C	F3	F	43.0	32.0	0.94	0.0	0.0	16.0	2.0	10.0	1.0
	N	A4	A	-	-	-	-	-	-	-	-	-
	N	B2	B	59.4	56.1	1.37	0.0	0.1	25.5	2.2	11.0	11.0
	N	C4	C	-	-	-	-	-	-	-	1.0	0.0
	N	D1	D	47.3	-	0.78	0.0	0.0	-	1.7	0.5	3.0
	N	E2	E	72.6	46.4	1.42	0.0	0.1	23.2	2.0	2.0	10.0
	N	F1	F	30.0	48.0	-	-	0.0	24.0	2.0	1.0	1.0
	NW	A3	A	59.6	38.9	0.95	0.0	0.1	17.7	2.2	7.0	12.0
	NW	B1	B	89.0	65.0	1.23	0.0	0.0	26.0	2.5	4.0	2.0
	NW	C3	C	79.8	58.1	0.81	0.0	0.1	22.4	2.6	3.0	8.0
	NW	D2	D	60.0	50.9	1.50	0.0	0.2	28.3	1.8	2.0	2.0
	NW	E3	E	59.0	49.0	1.53	0.0	0.7	24.5	2.0	2.0	2.0
	NW	F4	F	40.0	24.0	0.83	0.0	0.0	12.0	2.0	4.0	2.0
	W	A1	A	77.4	36.6	1.00	0.0	0.1	18.3	2.0	3.0	8.0
	W	B4	B	-	-	-	-	-	-	-	0.5	0.0
	W	C2	C	63.3	-	1.27	0.0	0.1	-	2.3	6.0	4.0
	W	D3	D	42.5	20.0	0.25	0.0	0.0	10.0	2.0	3.0	3.0
	W	E1	E	50.0	25.0	0.40	0.0	0.0	12.5	2.0	4.0	1.0
	W	F2	F	36.8	-	0.94	0.0	0.1	-	2.0	5.0	4.0
	Average (std err)			56.8 (2.8)	34.3 (4.4)	1.10 (0.13)	0.0	0.1 (0.0)	20.0 (1.6)	2.1 (0.1)	4.3 (0.7)	3.5 (0.6)
	Count			27	24	26	26	27	13	16	42	42

Table A2: Summary of plot level reproductive output variables for *Dryas octopetala*. Table shows plot level data for both Nitrogen dosage and Experimental sites. Summary averages (with standard error) and total number of plots where species occurred can also be found in Table 2.7. Description of reproductive output variables can be found in section 2.2.3. Data for control plots can be found in the TRY plant trait database (www.try-db.org).

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	abundance	density
Nitrogen dosage	1G	53		-	-	-	-	1.0	0.0
	1G	56		-	-	-	-	20.0	33.0
	1G	65		41.0	79.0	2.28	0.7	30.0	40.0
	1G	67		-	-	-	-	14.0	23.0
	2G	52		80.0	88.0	2.05	0.0	20.0	25.0
	2G	59		-	-	-	-	38.0	54.0
	2G	62		-	-	-	-	22.0	30.0
	2G	68		64.0	62.0	1.29	0.3	42.0	18.0
	4G	51		38.7	54.0	1.11	0.1	38.0	43.0
	4G	58		-	-	-	-	14.0	40.0
	4G	63		48.0	55.0	1.46	0.4	6.0	8.0
	4G	66		-	-	-	-	12.0	27.0
	8G	55		-	-	-	-	14.0	0.0
	8G	57		61.7	41.7	1.52	0.4	22.0	70.0
	8G	64		44.0	68.5	1.24	0.3	20.0	11.0
	8G	69		61.3	59.7	1.73	0.6	12.0	46.0
	C	54		75.0	92.0	1.96	0.2	24.0	26.0
	C	60		-	-	-	-	22.0	10.0
	C	61		-	-	-	-	56.0	39.0
	C	70		-	-	-	-	18.0	4.0

Table A2 continued: Reproductive outputs of *Dryas octopetala*.

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	abundance	density
Experimental	C	A2	A	-	-	-	-	20.0	17.0
	C	B3	B	-	-	-	-	38.0	15.0
	C	C1	C	52.8	46.3	1.40	0.3	32.0	17.0
	C	D4	D	40.0	57.0	1.93	0.7	36.0	28.0
	C	E4	E	49.7	58.3	1.83	0.4	42.0	61.0
	C	F3	F	-	-	-	-	35.0	55.0
	N	A4	A	54.0	71.0	2.25	0.3	31.0	55.0
	N	B2	B	52.0	51.0	2.55	0.9	28.0	5.0
	N	C4	C	-	-	-	-	30.0	48.0
	N	D1	D	-	-	-	-	36.0	11.0
	N	E2	E	70.0	82.0	1.59	0.4	25.0	30.0
	N	F1	F	58.3	44.1	2.59	0.6	30.0	38.0
	NW	A3	A	47.5	75.0	1.80	0.5	11.0	9.0
	NW	B1	B	-	-	-	-	33.0	21.0
	NW	C3	C	42.5	49.0	1.79	0.7	31.0	36.0
	NW	D2	D	45.0	75.0	2.04	0.7	26.0	20.0
	NW	E3	E	63.5	61.0	2.70	0.8	26.0	41.0
	NW	F4	F	-	-	-	-	12.0	4.0
	W	A1	A	-	-	-	-	9.0	18.0
	W	B4	B	53.9	63.8	2.01	0.3	27.0	103.0
	W	C2	C	115.0	56.0	0.89	0.0	13.0	8.0
	W	D3	D	71.6	58.0	1.93	0.4	15.0	24.0
	W	E1	E	38.3	42.7	1.56	0.3	36.0	59.0
	W	F2	F	60.5	44.0	1.70	0.3	29.0	48.0
	Average (std err)			57.1 (3.3)	61.4 (2.8)	1.81 (0.09)	0.4 (0.0)	24.9 (1.7)	30.0 (3.2)
	Count			25	25	25	25	44	44

Table A3: Summary of plot level reproductive output variables for *Hierochloë alpina*. Table shows plot level data for both Nitrogen dosage and Experimental sites. Summary averages (with standard error) and total number of plots where species occurred can also be found in Table 2.7. Description of reproductive output variables can be found in section 2.2.3. Data for control plots can be found in the TRY plant trait database (www.try-db.org).

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	Proportion of seeds filled	abundance	density
Nitrogen dosage	1G	53		-	-	-	-	-	0.5	0.0
	1G	56		215.0	9.0	14.44	-	0.7	2.0	1.0
	1G	65		202.4	8.8	11.14	-	0.3	4.0	7.0
	1G	67		207.3	9.0	12.22	-	0.3	4.0	3.0
	2G	52		-	-	-	-	-	0.5	0.0
	2G	59		220.0	6.0	13.33	-	-	4.0	1.0
	2G	62		-	-	-	-	-	0.0	0.0
	2G	68		235.0	9.0	15.56	-	0.4	0.0	2.0
	4G	51		-	-	-	-	-	2.0	0.0
	4G	58		-	-	-	-	-	0.5	0.0
	4G	63		-	-	-	-	-	0.0	0.0
	4G	66		220.0	10.0	11.00	-	0.0	4.0	1.0
	8G	55		-	-	-	-	-	0.5	0.0
	8G	57		-	-	-	-	-	0.5	0.0
	8G	64		209.6	9.0	10.00	-	0.2	4.0	5.0
	8G	69		-	-	-	-	-	0.0	0.0
	C	54		-	-	-	-	-	0.0	0.0
	C	60		215.4	7.7	12.46	-	-	2.0	9.0
	C	61		-	-	-	-	-	0.0	0.0
	C	70		-	-	-	-	-	0.0	0.0

Table A3 continued: Reproductive outputs of *Hierochloë alpina*.

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	Proportion of seeds filled	abundance	density
Experimental	C	A2	A	190.0	-	-	-	-	0.5	1.0
	C	B3	B	175.4	8.8	12.14	-	0.5	0.5	9.0
	C	C1	C	-	-	-	-	-	1.0	0.0
	C	D4	D	-	-	-	-	-	-	-
	C	E4	E	-	-	-	-	-	1.0	0.0
	C	F3	F	-	-	-	-	-	-	-
	N	A4	A	162.6	8.3	11.38	-	0.4	6.0	21.0
	N	B2	B	-	-	-	-	-	-	-
	N	C4	C	154.0	10.4	10.27	-	0.3	7.0	7.0
	N	D1	D	205.0	11.0	11.82	-	0.4	1.0	1.0
	N	E2	E	190.5	9.5	8.95	-	0.3	2.0	2.0
	N	F1	F	170.0	8.9	9.03	-	0.0	9.0	8.0
	NW	A3	A	193.0	8.4	10.37	-	0.4	8.0	17.0
	NW	B1	B	205.0	14.0	8.81	-	0.2	2.0	3.0
	NW	C3	C	156.0	10.6	9.45	-	0.0	5.0	12.0
	NW	D2	D	213.4	12.6	12.44	-	0.7	2.0	12.0
	NW	E3	E	180.0	8.0	10.00	-	0.1	1.0	1.0
	NW	F4	F	-	-	-	-	-	1.0	0.0
	W	A1	A	-	-	-	-	-	2.0	0.0
	W	B4	B	157.6	9.2	9.76	-	0.3	6.0	8.0
	W	C2	C	-	-	-	-	-	4.0	0.0
	W	D3	D	-	-	-	-	-	2.0	0.0
	W	E1	E	-	-	-	-	-	2.0	0.0
	W	F2	F	-	-	-	-	-	4.0	0.0
	Average (std err)			194.2 (5.1)	9.4 (0.4)	11.23 (0.40)	-	0.3 (0.0)	2.3 (0.4)	3.2 (0.8)
	Count			21	20	20	-	18	41	41

Table A4: Summary of plot level reproductive output variables for *Lupinus arcticus*. Table shows plot level data for both Nitrogen dosage and Experimental sites. Summary averages (with standard error) and total number of plots where species occurred can also be found in Table 2.7. Description of reproductive output variables can be found in section 2.2.3. Data for control plots can be found in the TRY plant trait database (www.try-db.org).

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	Proportion of seeds filled	# seeds per pod	# pods per inflor.	abundance	density
Nitrogen dosage	1G	53		134.0	2.3	160.00	0.8	1.0	2.3	1.0	4.0	6.0
	1G	56		183.0	0.6	120.00	1.0	1.0	1.5	0.4	2.0	6.0
	1G	65		137.5	5.0	99.00	0.5	0.5	3.3	1.5	0.5	2.0
	1G	67		175.0	-	-	-	-	-	-	6.0	1.0
	2G	52		155.0	4.0	136.25	0.6	0.6	1.3	3.0	4.0	2.0
	2G	59		115.0	-	-	-	-	-	-	4.0	5.0
	2G	62		-	-	-	-	-	-	-	2.0	0.0
	2G	68		138.3	4.0	108.33	1.0	1.0	1.5	2.7	4.0	3.0
	4G	51		103.8	2.0	45.00	0.0	0.0	2.0	1.0	8.0	4.0
	4G	58		161.7	4.5	112.22	0.7	0.7	1.8	2.5	4.0	3.0
	4G	63		173.8	7.0	169.29	0.9	0.9	2.0	3.5	6.0	4.0
	4G	66		207.5	7.3	140.00	0.9	0.9	2.4	3.0	6.0	4.0
	8G	55		133.8	-	-	-	-	-	-	14.0	4.0
	8G	57		192.0	0.6	138.00	0.4	0.6	1.0	0.6	6.0	8.0
	8G	64		147.5	2.0	82.50	0.0	0.5	1.0	2.0	2.0	2.0
	8G	69		-	-	-	-	-	-	-	2.0	0.0
	C	54		-	-	-	-	-	-	-	2.0	0.0
	C	60		165.0	-	-	-	-	-	-	4.0	2.0
	C	61		130.0	4.0	187.50	1.0	1.0	4.0	1.0	6.0	2.0
	C	70		-	-	-	-	-	-	-	0.5	0.0

Table A4 continued: Reproductive outputs of *Lupinus arcticus*.

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	Proportion of seeds filled	# seeds per pod	# pods per inflor.	abundance	density
Experimental	C	A2	A	175.0	6.0	162.50	0.9	0.9	1.4	4.3	22.0	14.0
	C	B3	B	-	-	-	-	-	-	-	4.0	0.0
	C	C1	C	-	-	-	-	-	-	-	1.0	0.0
	C	D4	D	123.8	2.5	192.00	1.0	0.9	1.4	1.8	4.0	4.0
	C	E4	E	135.0	2.0	120.00	1.0	1.0	2.0	1.0	5.0	1.0
	C	F3	F	-	-	-	-	-	-	-	5.0	0.0
	N	A4	A	121.0	5.0	114.33	1.0	1.0	2.5	2.0	11.0	7.0
	N	B2	B	-	-	-	-	-	-	-	2.0	0.0
	N	C4	C	123.8	3.7	150.00	1.0	1.0	1.4	2.7	6.0	4.0
	N	D1	D	134.0	7.3	125.75	1.0	0.7	1.7	4.2	12.0	22.0
	N	E2	E	196.7	3.3	144.00	1.0	0.6	1.1	3.0	6.0	3.0
	N	F1	F	97.5	5.5	139.00	1.0	0.9	2.2	2.5	5.0	2.0
	NW	A3	A	182.5	12.5	140.00	1.0	0.9	1.8	7.0	5.0	2.0
	NW	B1	B	-	-	-	-	-	-	-	6.0	0.0
	NW	C3	C	200.0	9.0	153.33	0.8	1.0	3.0	3.0	3.0	1.0
	NW	D2	D	-	-	-	-	-	-	-	2.0	0.0
	NW	E3	E	-	-	-	-	-	-	-	4.0	0.0
	NW	F4	F	142.0	7.0	178.57	0.5	0.9	1.2	6.0	5.0	8.0
	W	A1	A	-	-	-	-	-	-	-	2.0	0.0
	W	B4	B	130.0	6.0	200.00	1.0	0.8	2.4	2.5	9.0	2.0
	W	C2	C	-	-	-	-	-	-	-	2.0	-
	W	D3	D	132.5	8.0	171.25	1.0	0.9	2.0	4.0	2.0	2.0
	W	E1	E	134.0	-	-	-	-	-	-	2.0	5.0
	W	F2	F	156.0	6.4	159.00	1.0	0.9	2.0	3.2	10.0	14.0
Average				149.6 (5.1)	4.9 (0.5)	140.30 (6.76)	0.8 (0.1)	0.8 (0.0)	1.9 (0.1)	2.7 (0.3)	5.0 (0.6)	3.5 (0.7)
Count				31.0	26.0	26.00	26.0	26.0	26.0	26.0	44.0	43.0

Table A5: Summary of plot level reproductive output variables for *Salix arctica*. Table shows plot level data for both Nitrogen dosage and Experimental sites. Summary averages (with standard error) and total number of plots where species occurred can also be found in Table 2.7. Description of reproductive output variables can be found in section 2.2.3. Data for control plots can be found in the TRY plant trait database (www.try-db.org).

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	abundance	density
Nitrogen dosage	1G	53		-	-	-	-	2.0	0.0
	1G	56		48.3	163.3	1.00	0.4	14.0	3.0
	1G	65		44.0	74.0	1.00	0.2	26.0	10.0
	1G	67		45.4	63.2	1.60	0.1	12.0	18.0
	2G	52		62.5	233.3	1.00	0.4	8.0	2.0
	2G	59		54.8	130.8	1.10	0.5	14.0	19.0
	2G	62		30.0	350.0	1.20	-	62.0	1.0
	2G	68		-	-	-	-	46.0	0.0
	4G	51		53.6	127.3	1.10	0.4	4.0	15.0
	4G	58		46.3	180.6	1.20	0.4	2.0	4.0
	4G	63		-	-	-	-	0.5	0.0
	4G	66		36.0	21.3	1.33	0.3	8.0	11.0
	8G	55		47.5	200.0	0.80	0.8	4.0	4.0
	8G	57		49.0	84.4	1.20	0.1	12.0	8.0
	8G	64		52.0	33.3	1.60	-	30.0	40.0
	8G	69		-	-	-	-	12.0	0.0
	C	54		48.8	55.0	1.00	0.3	2.0	4.0
	C	60		-	-	-	-	8.0	0.0
	C	61		45.6	180.4	0.70	0.7	24.0	11.0
	C	70		-	-	-	-	1.0	0.0

Table A5 continued: Reproductive outputs of *Salix arctica*.

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	abundance	density
Experimental	C	A2	A	-	-	-	-	1.0	0.0
	C	B3	B	60.0	67.3	1.40	0.3	14.0	14.0
	C	C1	C	59.6	414.0	1.00	0.1	12.0	5.0
	C	D4	D	62.0	125.0	1.30	0.2	9.0	8.0
	C	E4	E	55.0	-	-	0.2	3.0	0.0
	C	F3	F	32.0	23.0	0.87	0.2	4.0	1.0
	N	A4	A	52.8	16.4	1.22	0.3	8.0	11.0
	N	B2	B	50.0	133.3	0.90	0.5	1.0	1.0
	N	C4	C	-	-	-	-	2.0	0.0
	N	D1	D	49.0	104.6	1.30	0.1	13.0	5.0
	N	E2	E	-	96.3	0.90	0.8	5.0	5.0
	N	F1	F	51.3	405.0	1.00	0.3	14.0	4.0
	NW	A3	A	-	-	-	-	4.0	0.0
	NW	B1	B	-	-	-	-	8.0	0.0
	NW	C3	C	55.0	83.5	1.30	0.5	8.0	14.0
	NW	D2	D	30.0	54.0	1.11	0.1	2.0	1.0
	NW	E3	E	52.4	403.7	0.90	0.4	13.0	7.0
	NW	F4	F	70.0	214.3	1.40	0.3	-	-
	W	A1	A	-	-	-	-	4.0	0.0
	W	B4	B	-	-	-	-	7.0	0.0
	W	C2	C	40.0	40.0	1.00	0.2	4.0	2.0
	W	D3	D	-	-	-	-	1.0	0.0
	W	E1	E	44.4	102.2	0.90	0.3	12.0	5.0
	W	F2	F	50.2	-	-	-	8.0	5.0
	Average			49.2 (1.7)	144.1 (21.4)	1.12 (0.04)	0.3 (0.0)	10.4 (1.8)	5.5 (1.1)
	count			30.0	29.0	29.00	28.0	43.0	43.0

Table A6: Summary of plot level reproductive output variables for *Salix reticulata*. Table shows plot level data for both Nitrogen dosage and Experimental sites. Summary averages (with standard error) and total number of plots where species occurred can also be found in Table 2.7. Description of reproductive output variables can be found in section 2.2.3. Data for control plots can be found in the TRY plant trait database (www.try-db.org).

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	abundance	density
Nitrogen dosage	1G	53		-	-	-	-	32.0	0.0
	1G	56		-	-	-	-	0.0	0.0
	1G	65		-	-	-	-	6.0	0.0
	1G	67		30.0	28.0	0.50	0.1	28.0	26.0
	2G	52		33.0	40.4	0.50	0.1	48.0	53.0
	2G	59		26.0	15.0	0.80	0.0	8.0	25.0
	2G	62		-	-	-	-	0.0	0.0
	2G	68		24.6	16.7	0.60	0.2	6.0	15.0
	4G	51		43.8	23.8	0.60	0.0	32.0	10.0
	4G	58		-	-	-	-	0.0	0.0
	4G	63		-	-	-	-	56.0	0.0
	4G	66		-	-	-	-	14.0	0.0
	8G	55		-	-	-	-	4.0	0.0
	8G	57		-	-	-	-	8.0	0.0
	8G	64		25.4	0.0	-	-	24.0	27.0
	8G	69		26.8	0.4	-	-	12.0	31.0
	C	54		-	-	-	-	38.0	0.0
	C	60		-	-	-	-	0.5	0.0
	C	61		27.0	-	-	-	0.5	1.0
	C	70		26.2	23.4	0.70	0.1	24.0	54.0

Table A6 continued: Reproductive outputs of *Salix reticulata*.

site	tmt	plot	block	Inflor. length (mm)	# seeds per inflor.	Seed mass (g x 10^{-4})	Proportion of seeds germinated	abundance	density
Experimental	C	A2	A	22.0	12.3	0.50	0.2	22.0	41.0
	C	B3	B	30.0	32.0	0.31	0.3	3.0	1.0
	C	C1	C	-	-	-	-	-	-
	C	D4	D	33.8	-	-	-	4.0	9.0
	C	E4	E	24.4	16.6	0.70	0.1	26.0	118.0
	C	F3	F	-	-	-	-	-	-
	N	A4	A	21.4	37.8	0.50	0.0	8.0	26.0
	N	B2	B	23.2	16.7	0.50	0.1	5.0	11.0
	N	C4	C	-	-	-	-	10.0	0.0
	N	D1	D	-	-	-	-	9.0	0.0
	N	E2	E	27.8	33.3	0.60	0.1	7.0	6.0
	N	F1	F	-	-	-	-	1.0	0.0
	NW	A3	A	26.6	22.9	0.50	0.0	22.0	60.0
	NW	B1	B	24.0	-	-	-	2.0	2.0
	NW	C3	C	33.0	-	-	-	4.0	1.0
	NW	D2	D	34.8	15.2	0.50	0.1	13.0	32.0
	NW	E3	E	43.2	28.0	1.00	0.3	10.0	33.0
	NW	F4	F	28.0	21.3	0.94	0.1	13.0	15.0
	W	A1	A	29.8	18.6	0.70	0.1	35.0	120.0
	W	B4	B	-	-	-	-	20.0	0.0
	W	C2	C	27.0	26.4	0.50	0.0	17.0	27.0
	W	D3	D	35.0	14.0	1.00	0.1	30.0	53.0
	W	E1	E	25.0	-	-	-	3.0	1.0
	W	F2	F	37.6	27.7	0.50	0.1	10.0	12.0
	Average			29.2 (1.1)	21.4 (2.2)	0.62 (0.04)	0.1 (0.0)	14.6 (2.1)	19.3 (4.4)
	count			27	22	20	20	42	42

7.0 APPENDIX B

In the following, I explain the calculations used in determining the reproductive potential of the species *Dryas octopetala*, *Lupinus arcticus*, and *Salix arctica*. The data used in these calculations have already been given in chapters 2 and 3.

In chapter 2, Table 2.7, I averaged across all plots from both sites to compute overall averages of density (number of flowers per m^2), number of seeds per flower, germination (proportion of viable seeds per number of seeds produced). Using these data, I calculated estimates of seed production (number of viable seeds produced per m^2) as follows:

$$\frac{\#\ of\ flowers}{m^2}\ x\ \frac{\#\ of\ seeds}{flower} x\ \frac{\#\ of\ viable\ seeds}{\#\ of\ seeds} = \frac{\#\ of\ viable\ seeds}{m^2}$$

In the seeding trials of chapter 3, I seeded subplots and observed initial seedling emergence in these subplots. Using these data, I calculated the germination success (number of initial seedlings emerged per number of viable seeds sown) as follows:

$$\frac{\#\ of\ viable\ sown\ seeds}{m^2} x\ germination\ success = \frac{\#\ of\ seedlings}{m^2}$$

$$germination\ success = \frac{\#\ of\ seedlings}{\#of\ viable\ sown\ seeds}$$

Reproductive potential for each of the species was then calculated using the values of seed production and germination success as follows:

$$Seed\ production\ x\ germination\ success = Reproductive\ potential$$

$$\frac{\#\ of\ viable\ seeds}{m^2} x \frac{\#\ of\ seedlings}{\#\ of\ viable\ seeds} = \frac{\#\ of\ seedlings}{m^2}$$

Reproductive potentials were calculated for vegetated tundra and bare substrate (frost boils) using the seeded subplots pooled all other treatments. Observations of initial seedling emergence (seedling densities) in the non-seeded subplots gave estimates of reproductive potentials under natural conditions.

Table B1: Table displaying the data that were used in calculations of seed production and germination success to estimate reproductive potential for the species *Lupinus arcticus*, *Dryas octpetela*, and *Salix arctica* based on seeding treatments in vegetated tundra and bare substrate (frost boils).

	Seed production					Seedling emergence in seeding trials			
Species	No. of inflorescences per m^2	No. of seeds per inflorescence	Proportion of viable seeds	No. of viable seeds per m^2	Tmt	Seedlings per m^2	Viable seeds sown per m^2	No. of seedlings per No. of viable seeds sown	Reproductive potential (seedlings per m^2)
Lupinus arcticus	3.5	4.9	0.86	13.75	Vegetated	11.1	324.4	0.03	0.47
					Bare substrate	22.2	324.4	0.07	0.94
Dryas octopetela	30.0	61.4	0.41	470.18	Vegetated	12.4	1688.9	0.01	3.48
					Bare substrate	0.0	1688.9	0.00	0.00
Salix arctica	5.5	144.1	0.33	203.96	Vegetated	24.7	3377.8	0.01	1.51
					Bare substrate	159.3	3377.8	0.05	96.07