8-26-2019 10:30 AM

The Cost of Protection: Frost Avoidance and Competition in Herbaceous Plants

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biology

Recommended Citation

Lubbe, Frederick Curtis, "The Cost of Protection: Frost Avoidance and Competition in Herbaceous Plants" (2019). *Electronic Thesis and Dissertation Repository*. 6398.
https://ir.lib.uwo.ca/etd/6398

Abstract

Perennial herbaceous plants in regions that experience winter freezing must survive using belowground structures that can tolerate or avoid frost stress. Soil and plant litter can insulate plant structures from frost exposure, but plants must invest into growth to penetrate through these layers to reach the surface in the spring. The overall goal of my thesis was to test the hypothesis that the protection of overwintering clonal structures by soil or plant litter (frost avoidance) comes at the expense of subsequent reduced growth and competitive ability in absence of freezing stress. I first explored this trade-off with a suite of experiments using plants with bulbs and stem tubers - storage-focused organs that are typically located below the soil surface. Seven plant species were subjected to different burial and frost exposure treatments (via snow removal) to disentangle the relationship between frost avoidance and the cost of organ depth. I then examined frost avoidance trade-offs for species with shallow bud placement (rhizomes). Rhizome fragments of six species were subjected to different soil depth and litter cover treatments combined with frost exposure treatments. There was a general trend of increased growth with depth under snow removal (increases soil freezing), but decreased growth with depth under ambient snow cover. These results were consistent with the mortality and growth trends observed for the species in controlled environment freezing trials. Responses to litter thickness were more variable. I also examined the freezing responses of mature plants within a self-assembled, old field community over three separate winters using snow removal. Species responses were pooled based on recruitment, organ of perennation, and life form (bud placement). Snow removal decreased total plant cover, primarily in species with shallow bud recruitment. Snow removal responses also varied based on recruitment depth and organ of perennation. These are the first studies to explore the trade-off between frost avoidance and competitive ability with growing depth in herbaceous species. In northern temperate regions, the balance of this trade-off may be altered by future increases in soil freezing intensity caused by declining snow cover and increased temperature variability in a warmer climate.

Keywords

belowground, bulb, clonal, frost, herbaceous, litter, resource allocation, rhizome, soil depth, tuber

Summary for Lay Audience

Non-woody plants that live in areas with seasonal freezing temperatures must survive under the ground over winter. Plants can avoid freezing stress by overwintering deep within the soil, under dead plant material, and under snow, all of which act as insulation. However, plants deep in the soil have the added cost of growing to reach the soil surface in spring. In this project, I explored the strength and generality of this trade-off using a range of species. I first used seven plant species adapted for high storage and deep growth. They were planted at different times and exposed to different depths and levels of frost stress. Generally, plants grew more if they overwintered deep when winter temperatures were severe, but grew more if they overwintered shallow when winter soil temperatures were milder. I then studied six species with belowground stems near the soil surface. They were planted at different times and exposed to different depths, cover of dead plant material, and levels of frost stress. For half of the species deep soil placement was a cost when winter soil temperatures were mild, and the response to the thickness of dead plant matter cover was highly variable. Finally, I exposed plants in a mature plant community to freezing stress and compared how the responses varied among species with different types of belowground structures. Freezing stress reduced plant growth, with tap-rooted species being the most sensitive. These trade-offs with respect to the depth of overwintering are particularly important to consider in the context of future changes in winter soil temperatures caused by climate warming.

Co-Authorship Statement

A version of Chapter 2 was accepted by Plant and Soil with Dr. Hugh Henry as a co-author. Dr. Henry supplied the funding, was involved in conception and study design, and contributed to writing of the manuscript.

A version of Chapter 3 was submitted to Perspectives in Plant Ecology, Evolution, and Systematics with Dr. Hugh Henry as a co-author. Dr. Henry supplied the funding, was involved in conception and study design, and contributed to writing of the manuscript.

A version of Chapter 4 was submitted to the Journal of Ecology with Dr. Hugh Henry as a co-author. Dr. Henry supplied the funding, was involved in conception and study design, and contributed to writing of the manuscript.

Acknowledgments

First, I would like to thank my supervisor, Dr. Hugh Henry for his support and guidance through the years. I showed up rather suddenly with a lot of my own interests and Hugh patiently worked with me to combine our research goals and make something new and interesting.

Thank you to my committee members, Dr. André Lachance, Dr. Greg Thorn, and Dr. Dani Way for your advice and sitting through my rants about clonal plant structures.

A special thanks to Peter Duenk and Caroline Rasenberg at the Environmental Sciences Western Field Station for their guidance, humor, and commitment to making sure I always had the right shoes.

I would like to thank my labmates (Thea Courtial, Spencer Heuchan, Ricky Kong, Will Laur, Mathis Natvik, Samuel Rycroft, Kyra Simone, Dr. Holly Stover, and Dr. Yanxue Zhou) for your support and camaraderie; without Holly, I would have never survived snow removal during the blizzard of 2016.

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List of Abbreviations

°C = degrees Celsius

2B = 2 cm soil depth and bare ground treatment

2H = 2 cm soil depth and high litter treatment

2L = 2 cm soil depth and low litter treatment

5B = 5 cm soil depth and bare ground treatment

ANOVA = analysis of variance

cm = centimeters

g = grams

m = meters

mm = millimeters

NA = not applicable

spp. = species

Chapter 1

1. General introduction

1.1 Plant trade-offs

From the morphological scale down to the molecular scale, plants encounter trade-offs with respect to the allocation of limited resources among different structures. There also are trade-offs regarding the design and dimensions of individual structures, and trade-offs based on phenology (e.g. the timing of leaf out and flowering). Variation in resource allocation, design and timing can in turn result in functional trade-offs (Bazzaz, Ackerly, & Reekie, 2000). For plants, commonly observed suites of allocation patterns have been described in terms of alternative plant strategies, and commonly cited examples of the latter were those proposed by Grime (1977) as part of the C-S-R Model. The latter distinguishes among plant strategies based on the allocation patterns that are most successful in response to different combinations of stress (i.e. factors that reduce plant growth) and disturbance (i.e. events that result in plant mortality). These strategies are characterized based on variation in key plant traits, such as growth rate, seed production (number, size and longevity), root versus shoot allocation, protection against herbivores, investment in storage, and nitrogen and water use efficiency.

Grime’s model distinguishes between allocation patterns that favour individuals in stressful environments (where disturbance in low), versus those that favour individuals in competitive environments (where both stress and disturbance are low). However, in northern temperate regions, plants experience portions of the annual cycle when competitive ability is favoured (e.g. the late spring through early fall, excluding periods of drought), whereas other times of the year favour stress tolerance (e.g. the winter). Therefore, perennial plants in these systems may encounter trade-offs with respect to plant traits that maximize competitive ability versus those that maximize stress tolerance.

1.2 Plant functional traits

Plant functional traits help explain plant distribution based on responses to environmental conditions (McGill, Enquist, Weiher, & Westoby, 2006; Ackerly & Cornwell, 2007).

Plant traits can be more informative than species assignment, because they provide information regarding the role of a plant in the ecosystem (Fukami, Bezemer, Mortimer, & Putten, 2005; Cardinale et al., 2011); this is especially relevant when working with plants that are difficult to identify (Medina, Lara, Goffinet, Garilleti, & Mazimpaka, 2012). Plant traits can be categorized based on their ubiquity, with some held by many species (i.e. trait convergence), and which are important to possess for continued presence in a given environment (α traits), and those held by fewer species, and which can provide them with a competitive advantage (β traits) (Ackerly & Cornwell, 2007). An α trait in a productive, temperate old field may be having buds near the soil surface (e.g. as is typical of hemicryptophytes) for early emergence under favourable conditions and thus greater competitive ability. Variation in those bud-bearing structures would be β traits, and may include short internodes and a phalanx growth strategy (Lovett Doust, 1981) to better compete with neighboring plant species (Fukami et al., 2005; Ackerly & Cornwell, 2007; Fischer, von der Lippe, & Kowarik, 2013).

1.3 Clonal growth in plants

Clonal structures are important and versatile organs; as part of vegetative growth they are capable of photosynthesis, vascular conduction, and structural enhancement (Bazzaz, Ackerly, & Reekie, 2000). However, they also can become fully-formed, independent plants. Vegetative reproduction can occur in tandem with sexual reproduction, and many plants reproduce asexually more readily than sexually (Schmid, Bazzaz, & Weiner, 1995: Vallejo-Marín, Dorken, & Barrett, 2010). For example, many plants will only produce sexual structures upon reaching a certain height, but there is no such limit for clonal structures (Hartnett, 1990; Schmid, Bazzaz, & Weiner, 1995). Clonal propagules may have advantages over seeds; they often perform a storage function and are better supplied with nutrients than seeds. Parent and daughter plants may remain connected for years in some species (Latzel et al., 2011) and this connection can help mitigate the effects of stressors (e.g. UV-B (Liu et al., 2015) and drought (Zhang, Zhang, & Sammul, 2012)). Clonal reproduction also can be advantageous for species that are not necessarily clonal by nature (Martínková & Klimešová, 2016). However, clonal growth increases population size, but not the gene pool, and thus with the proliferation of clones there is the increased likelihood of loss of genetic diversity and possibly inbreeding depression (Vallejo-Marín, Dorken, & Barrett, 2010). These negative consequences may be of particular concern for threatened species.

Clonal structures can be positioned to avoid stress or disturbance (e.g. fire or frost), but their positioning also determines the expansion pattern of the plant as a whole. Total clonal plant size is often determined by ‘lateral spread’ (i.e. the expansion of the plant with respect to surface area), as opposed to the size of any individual ramet (i.e. the physiologically distinct plant units produced by vegetative reproduction), because it may contain any number of individual ramets, depending upon the persistence of the connecting structure (Latzel et al., 2011). Bud bank and clonal traits are directly linked to adaptations to disturbances (such as fire or frost) that necessitate regular resprouting (Klimešová, Tackenberg, & Herben, 2015) and are better for predicting vegetation trends than foliar traits (Bittebiere, Clément, & Mony, 2013).

1.4 Plant belowground structures in herbaceous plants

Plant belowground structures are important for nutrient and water uptake, conduction, support, and reproduction (Bazzaz, Ackerly, & Reekie, 2000). The herbaceous habit evolved after woodiness, primarily in areas that did not experience freezing temperatures, but many lineages increased in their number of herbaceous species after expanding their territories into regions with freezing temperatures (Zanne et al., 2014). Temperate herbaceous species depend on belowground structures for perennation (Klimešová, Takenberg, & Herben, 2015), and belowground organs of perennation can be clonal or non-clonal. Non-clonal structures are often thick, vertical, and with buds at or below the soil surface (e.g. taproots, caudices, and root crowns (Raunkiær, 1934; Stewart-Wade Neumann, Collins, & Boland, 2002; Chmielewski & Semple, 2003)). Clonal structures present belowground include bulbs, stem tubers, rhizomes, and buds from lateral and adventitious roots (Raunkiær, 1934; Klimešová, Danihelka, Chrtek, de Bello, & Herben, 2017).

Belowground clonal structures adapted for high storage capabilities include bulbs and stem tubers (Kamenetsky, 2013; Klimešová, 2018). Bulbs form as truncated stems (basal plate) with leaves that are thickened for storage purposes (Kamenetsky, 2013; Klimešová, Danihelka, Chrtek, de Bello, & Herben, 2017). These structures are present only in some lineages of monocots and *Oxalis* (Meerow, 2013). Stem tuber presence is not limited by phylogenetic lineage and can arise independently. They are short-lived, tuberously thickened stems that can either function as the primary plant body (corms), propagules adjacent to a primary stem tuber (cormel), or on the ends of hypogeogenous rhizomes (Suzuki and Steufer, 1999; Kamenetsky, 2013; Klimešová et al. 2017). Both of these structures are commonly located deep in the soil (i.e. for geophytes (Raunkiær, 1934)), but there are exceptions. Rhizomes are belowground horizontal stems that are often utilized for maintaining consistent apical bud depth (epigeogenous stems) or commonly as spacers (hypogeogenous stems), with increased internode length and capability for lateral spread (Klimešová et al., 2017). Rhizomes can be part of the geophyte life form (Raunkiær, 1934; Kamenetsky, 2013), but are frequently present closer to the surface of the soil (Raunkiær, 1934; Komac, Pladevall, Peñuelas, Conesa, & Domènech, 2015) Although not specifically adapted for storage (unless tuberously thickened), rhizomes are important carbon stores and a component of plant architecture as well as connectivity (Elgersma et al. 2015; Klimešová, Martíncová, & Ottaviani, 2018). While not inherently clonal, the adventitious and lateral roots of some species can develop stem buds that also can provide a method of recruitment from deep within the soil (Bartušková, Malíková, & Klimešová, 2017). Nonclonal organs of perennation includes taproots as well as rootstocks with adventitious roots, and both have buds primarily at the soil surface (Raunkiær, 1934). Taproots are prone to degradation through damage and age, which can cause clonal splitting (called rootsplitting) (Chmelíková & Hejcman, 2012; Klimešová et al., 2017).

An important feature of a given belowground clonal structure is its placement along the soil depth profile. The lack of geophytes and prevalence of the hemicryptophyte life form in temperate regions (Kamenetsky, 2013; Klimešová, 2018) implies an advantage for herbaceous species that risk damage from surface stress or disturbance as opposed to investing extra tissue solely for the purpose of positioning structures deep in the soil. Early emergence in spring may be an added benefit of positioning clonal structures at shallow depth. However, there are clearly benefits to deep positioning of organs under specific environmental conditions; for example, some hemicryptophyte species grow deeper than normal in response to a changes in moisture regime (Craig and Buckley, 2013; Baseggio, Newman, Sollenberger, Fraize, & Obreza, 2015), and bulbs (or other belowground organs with large storage capabilities) are often a common trait in arid species (Appezzato-da-Glória et al., 2008; Qian et al. 2017).

The importance of organs of perennation was recognized in the creation of the life form classification system, first published in 1907 (Raunkiær, 1934). Although belowground functional traits and organs of perennation are important components of plant persistence (Benson & Hartnett, 2006), belowground traits are especially difficult to study (Lavorel & Garnier, 2002). Interest has increased recently, but most of the focus has been on root traits, and much is left to be learned regarding perennation traits and the ecological responses of these structures (Klimešová, Martíncová, & Ottaviani, 2018). Although the herbaceous habit developed in many lineages as a response to frost stress (Zanne et al., 2014), little is known about herbaceous plant response to frost damage beyond injury and decreased growth (Farrell, Clifton-Brown, Lewandowski, & Jones, 2006; Reinmann, Susser, Demaria, & Templer, 2019).

1.5 Soil freezing

Ice formation can occur below 0 °C, because at this temperature ice is more stable than water (Iwata, Tabuchi, & Warkentin, 1995), although the freezing point of soil water is usually below 0 °C, because of pore water chemistry and dissolved ions (Barnes, 2010). When pressure or temperature within a system of soil and water changes, and the chemical potential of water becomes larger than that of ice, ice is formed (Iwata, Tabuchi, & Warkentin, 1995). Freezing begins with a thin layer of water on the soil surface, and this forms a gradient between the sub-zero air and the unfrozen ground along which latent heat travels from the soil to the air (Jefferies, Walker, Edwards, & Dainty, 2010). Water within the soil continues to freeze as the latent heat travels from progressively deeper within the soil (Jefferies et al., 2010). Frozen soils contain a mixture of ice, unfrozen water, and air (Kutilek & Nielson, 1994). The depth of the freezing within soil can be increased by moisture or lower clay content (Barnes, 2010) and can range from only a few centimeters to greater than 2 m (Federal Highway

Administration, 2008). Clay soils have low water permeability, and this decreases the flow of liquid water to join forming ice (Barnes, 2010). Although the penetration of freezing in soil may be very deep, the severity of the freezing is reduced with depth, such that shallow plant structures within the soil typically experience the greatest exposure to freezing stress (Boydston et al., 2006). Under some circumstances, ice lenses will form surrounded by otherwise unfrozen soil and the soil will expand, creating heaving pressure (Iwata, Tabuchi, & Warkentin, 1995), which can lift buried structures from their original location in the soil (Barnes, 2010). Water within the soil moves from the unfrozen zone to the freezing zone, and then to the ice lens (Iwata, Tabuchi, & Warkentin, 1995). Frost heave, and the subsequent shrinking of soil back to the original size, can unearth sensitive plant structures and increase plant exposure to freezing air temperatures (Perfect, Miller, & Burton, 1987).

1.6 Plant freezing

Freezing is an important stress and disturbance in temperate regions, and it affects the distributions of many plant species (Box, 1996). For herbaceous species, the structures that persist during times of frost exposure are primarily belowground organs (Klimešová, Tackenberg, & Herben, 2015). Plants can survive freezing air temperatures through frost tolerance (Pearce, 2001) or spatial frost avoidance (Boydston et al., 2006; Groffman, Hardy, Driscoll, & Fahey, 2006; Wang et al., 2017). Frost tolerance is physiological and protects important cellular structures from ice crystal penetration through membrane stabilization and/or molecular components within the cytosol (Pearce, 2001; Wisnieski, Willick, & Gusta, 2017). Physiological frost avoidance (deep supercooling) is the manipulation within cells of ice nucleation, causing the freezing point of water to decrease to well below zero (as low as -50 °C), and it occurs in some temperate woody plants (Wisnieski, Willick, & Gusta, 2017). In contrast, spatial frost avoidance involves insulating structures from freezing temperatures via physical barriers such as snow (Groffman et al., 2006), senesced leaf and stem tissue (McKinney, 1929), and/or the soil (Boydston et al., 2006).

Ice formation within plant cells or penetration by ice crystals from outside the cells is generally lethal, depending upon the freezing process and crystal size (Slováková, Matušíková, Salaj, & Hudák 2010). Ice formation requires a nucleation site for water molecule orientation and thus ice crystallization (Burke & Lindlow, 1990). Frost damage from ice formation occurs between -2 and -5 °C in frost-sensitive species, although ice nucleation is usually uncommon in plant tissues and they can resist freezing until -8 °C (McKersie & Lesham, 1994). Leaves may be more sensitive than other plant organs because of the presence of nucleation-prone bacteria (McKersie & Lesham, 1994); however, these structures are generally absent in herbaceous plants during cold winters (Klimešová, Tackenberg, & Herben, 2015). Cell membranes are likely to be the most sensitive plant structure and the main site of frost injury (McKersie & Lesham, 1994; Slováková et al. 2010). Apical buds can be the most sensitive tissue in belowground overwintering structures (Livingston III & Tuong, 2013). The effects and extent of frost damage in plants can be difficult to identify and quantify (Wisnieski, Willick, & Gusta 2017). The infiltration of freezing temperatures into plant tissues is not necessarily indicative of frost damage and measures such as electrolyte leakage and cell damage do not necessarily relate to the recovery of these tissues (Palta, Levitt, & Stadelmann, 1977). Tissue repair and the response of adjacent, undamaged tissue is vital for frost survival (Palta, Levitt, & Stadelmann, 1977; Livingston III & Tuong, 2013), and thus the measurement of regrowth is often used to assess frost damage. For example, root damage can decrease foraging ability, impairing subsequent regrowth (Wisnieski, Willick, & Gusta, 2017). Herbaceous plants are most commonly tolerant to frost and desiccation because of the low threshold for freezing of soil (0 °C) and the readiness for ice nucleation in soil surrounding the belowground organs (McKersie & Lesham, 1994). Frost tolerance not only varies among species (Wisnieski, Willick, & Gusta, 2017), but it also varies within species, and certain genotypes have greater tolerance than others (Farrell et al., 2006). Frost tolerance also varies along the annual cycle and for individual plants based on internal and external cues (Wisnieski, Willick, & Gusta, 2017).

1.7 Spatial frost avoidance

Snow, litter and soil can act as insulation to sensitive plant structures and thus contribute to spatial frost avoidance. Snow cover insulates soil from freezing air temperatures, and snow cover alone can be enough to prevent any soil freezing under conditions where bare soil freezes (Groffman et al. 2006). The frost avoidance provided by snow cover protects plants near and below the soil surface, and the loss of snow cover can increase the frequency and intensity of soil freeze-thaw cycles, damaging plants (Henry, 2008). Similarly, litter can modify soil temperature; litter cover can delay soil freezing and decrease frost penetration (McKinney, 1929). The presence of litter such as leaf/residue cover and upright plant stems (such as corn stubble) also can trap and retain snow, thus increasing insulation (Sharratt, 2002; Wang et al. 2017). The penetration of freezing temperatures into the soil from the air typically declines with increasing soil depth, and so deeper organ placement can allow the avoidance of frost stress (Boydston et al. 2006). Apical buds are some of the most sensitive overwintering plant structures (Livingston III & Tuong, 2013) and the bud bank is vital for perennial growth (Benson & Hartnett, 2006). Therefore, these structures may be especially dependent upon spatial frost avoidance for protection from freezing stress.

Plants growing from deep within the soil profile may be protected from frost, but also emerge later (Pan, Geng, Li, & Chen, 2009), which can place them at a competitive disadvantage. Although spatial frost avoidance is important for plant survival, its costs are not known, and resource allocation has never been measured under different insulation scenarios. Future climate predictions indicate changes in temperature variability, which may increase the likelihood of frost exposure when plants are otherwise not properly acclimated (e.g. in late spring) (Henry, 2013). During the winter, a general decrease in consistent freezing temperatures and a lack of snow cover as insulation could increase frost exposure and penetration (Groffman et al., 2001). It is not known how plant resource allocation and growth may change in plants that had adapted to survival through spatial frost avoidance, and the trade-off of the spatial frost avoidance strategy and possible investment costs have not been studied experimentally.

1.8 Objectives and Thesis organization

The overall goal of my thesis was to test the hypothesis that the protection of overwintering clonal structures by soil or plant litter (frost avoidance) comes at the expense of subsequent reduced growth and competitive ability. My three main objectives were to:

1. use a suite of temperature and soil depth manipulations to examine the trade-off between frost avoidance and growth/competitive ability in a range of species that have overwintering bulbs or stem tubers (Chapter 2).

2. use a suite of temperature and soil/litter depth manipulations to examine the trade-off between frost avoidance and growth/competitive ability in a range of species that have overwintering rhizomes (Chapter 3).

3. examine trade-offs between frost avoidance and growth/competitive ability for plants in an intact, established plant community (Chapter 4).

In Chapter 1 I introduced the topics of plant resource allocation trade-offs, functional traits, and freezing stress, and in Chapter 5 I shall synthesize the results from Chapters 2-4 and suggest future avenues of study for my research topic.

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Chapter 2

2. The cost of depth: frost avoidance trade-offs in herbaceous plants

2.1 Introduction

Freezing is one of the primary stresses that limits plant distribution at a global scale (Box, 1996; Pearce, 2001). In regions that experience cold winters, plants must have structures and phenology that enable them to tolerate or avoid freezing stress. Plant structures located at or above the soil surface have the highest risk of frost exposure, whereas frost effects typically decrease with increasing soil depth (Sharratt, 2002), much like fire damage (Vesk, Warton, & Westoby, 2004). Herbaceous species therefore often overwinter as seeds or belowground structures that can avoid or otherwise tolerate the frost, perennial species resprout when conditions improve (Klimešová, Tackenberg, & Herben, 2015).

Freezing can be tolerated through physiological mechanisms ((e.g. via antifreeze proteins and vitrification (Pearce, 2001)), whereas frost avoidance is achieved through spatial organization and temporal activity, accomplished respectively through the positioning of organs and tissues within the soil profile, or by the avoidance of growth and bud exposure during periods of frost risk (Raunkiær, 1934; Komac, Pladevall, Peñuelas, Conesa, & Domènech, 2015). Factors other than soil depth can reduce soil frost exposure in plants. As herbaceous plants enter dormancy for the winter, senesced aboveground structures create a layer of litter that functions as insulation for underlying plant structures (Raunkiær, 1934; Sharratt, 2002). In addition, snow cover provides insulation from freezing temperatures for plants that overwinter at or below the soil surface (Bertrand & Castonguay, 2003; Komac et al., 2015). Reductions in snow cover can harm plants that depend upon this insulation, resulting in habitat invasion by non-native plants and shifts in vegetation type (Simons, Goulet, & Bellehumeur, 2010; Komac et al., 2015).

Although the positioning of plant structures deep in the soil can protect from stress and disturbance of both environmental origin (e.g. frost (Boydston, Seymour, Brown, & Alva, 2006), fire (Vesk & Westoby, 2004), and drought (Baseggio et al., 2015)) and biotic origin (e.g. herbivory (Santamaría & Rodríguez-Gronés, 2002) and pathogens (Porter, Dasgupta, & Johnson, 2005)), plants employing this strategy must increase investment in belowground stem at a later date to emerge from the soil. Plants positioned far below the soil surface also may emerge late, and hence be at a competitive disadvantage relative their neighbours (Pan, Geng, Li, & Chen, 2009). Therefore, while in many stressful environments, deep bud placement in the soil may be advantageous as part of the stress tolerance strategy, and in low-stress environments, shallow bud placement may be advantageous as part of the competitive strategy (Grime, 1977), in seasonally stressful environments, these traits may form the basis of a potential plant trade-off.

Bulbs and stem tubers are storage organs that are often part of the geophyte life form, and they are a repository of buds below the ground during unfavourable seasons (Raunkiær, 1934). Their high tolerance of soil depth makes them well-suited to investigate the possible trade-off between frost avoidance and the costs associated with deep soil positioning. Only a few studies (e.g. Cavins & Dole, 2002; Qodliyati, Supriyono, & Nyoto, 2018; both conducted in the context of horticulture) have investigated how the positioning of these organs below the soil affects plant resource allocation, and with the exception of a single study conducted on potato (*Solanum tuberosum* (Boydston et al., 2006)), the interaction between soil depth and frost avoidance has not been examined for geophytes/tuberous species. Moreover, while studies of plant belowground responses to freezing often have focused on mortality (de Melo Peixoto, Friesen, & Sage, 2015), the temperatures imposed in these experiments frequently are much lower than those encountered by sub-surface plant structures in the field (Henry, 2007). Such an approach does not address the potential importance of sub-lethal freezing damage, which can affect subsequent plant growth and competitive ability (Weih & Karlsson, 2002; Malyshev & Henry, 2012). In the event that bulbs survive winter frost, the positioning of overwintering plant structures along the soil depth profile may be optimal at the depth where the cost of sublethal damage is balanced by the cost of growing to the surface and any subsequent reductions in competitive ability.

I performed a combination of controlled environment and field transplantation experiments using a range of geophyte species to examine the extent to which the protection of overwintering clonal structures, specifically bulbs, by soil (i.e. frost avoidance) comes at the expense of subsequent reduced growth or delayed emergence. First, I planted a set of bulbs at different depths in the field to overwinter, and these treatments were combined with snow removal and ambient snow treatments to vary frost exposure. Based on the expectation that frost damage would be most severe near the surface, I predicted that the shallowest bulbs would not produce the largest plants, despite the reduced cost of growing to the surface and the potential for early emergence. I also predicted that snow removal would increase frost penetration, and thus favour the growth of plants positioned at greater depths more than in the ambient snow plots. To isolate the direct effects of soil bulb depth on plant growth from the confounding effects of variation in soil frost exposure, I conducted two additional experiments. First, I incubated a set of bulbs over winter at a mild temperature (1 °C) in growth chambers, and then transplanted these bulbs in the spring to different soil depths in the field. Given that these plants did not experience freezing damage, I predicted that increased planting depth would be negatively correlated with subsequent growth. Second, I isolated the effects of freezing intensity (minimum temperature) from soil planting depth by exposing another set of bulbs to a range of temperature treatments in freezing chambers and subsequently transplanting them in the spring to the field at a uniform depth.

2.2 Methods and Materials

2.2.1 Field site and study species

The experiments were conducted at the Environmental Sciences Western field station in Ilderton, Ontario, Canada (43°04´29´´N, 81°20´18´´W), in plowed research plots. The soil was characterized as a London clay loam (Hagerty & Kingston, 1992). The seven study species (*Allium cernuum* Roth, *Apios americana* Medikus, *Crocus vernus* (Linnaeus) Hill (flower record cultivar), *Helianthus tuberosus* Linnaeus, *Muscari armeniacum* Leichtlin ex Baker, *Narcissus pseudonarcissus* Linnaeus (yellow trumpet cultivar), and *Scilla sibirica* Haworth) all naturalize in the study region. The plants were purchased from commercial growers in or near the study region or (in the case of *Allium* *cernuum* and *Helianthus tuberosus*) locally harvested near the study site. All produce specialized storage organs (bulbs or stem tubers) and are geophytes (buds positioned below the soil surface during unfavourable seasons) (Raunkiær, 1934). True bulbs are composed of a shortened stem (with buds) and leaves modified for storage while stem tubers are stem-derived organs modified for storage (Klimešová, 2018). Stem tubers can either be what are traditionally called 'corms,' which have a defined orientation in the soil (dorsal/ventral), or tubers, which are also stem derived storage organs, but typically grow from the nodes of hypogeogenous rhizomes. Both are consumed during the annual cycle and are thus sometimes referred to as semi-annual (Suzuki & Steufer, 1999). *Crocus vernus* forms a corm, while *Helianthus tuberosus* and *Apios americana* form stem tubers on rhizomes. Bulbs and stem tubers (clonal organs) are not independently capable of spread, and storage is the major function (Raunkiær, 1934; Suzuki & Steufer, 1999; Vallejo-Marín, Dorken, & Barrett, 2010).

2.2.2 Overwintering field experiment

Clonal organs were weighed and planted 3-4 November 2016 at one of three soil depths (2, 5 or 15 cm). The soil depth treatment was fully combined with a snow removal treatment (snow removal or ambient snow). The propagules were placed 20 cm apart in twelve 80 × 1600 cm plots (six for snow removal and six for control, all positioned randomly). The plots were spaced 2 m apart and each contained three rows. Each row in a plot corresponded with a single burial depth (positioned randomly) and contained one randomly-positioned specimen from each species. Starting in November 2016, snow was removed after heavy snowfall events that were likely to be followed by below freezing temperatures. Snow removal ceased after 14 March 2017 to minimize possible snow removal effects on post-snow melt soil moisture levels (i.e. all plots became saturated with water as a result of the final snow melt). Two soil temperature probes (LogTag TRIX-8, MicroDAQ, NH, U.S.A.) were placed at each soil depth (2, 5, and 15 cm deep) for each snow removal treatment, and temperatures were logged hourly.

2.2.3 Spring clonal organ depth experiment

Another set of propagules (six replicates per treatment) was overwintered at 1°C in an incubator from 1 December 2016 to 20 April 2017. In the spring the propagules were weighed and planted in a common garden at the field site at either 2, 5 or 15 cm soil depth, with the propagules spaced 20 cm apart and positioned randomly. These methods were repeated for *Crocus vernus*, *Narcissus pseudonarcissus*, and *Scilla siberica* in 2017/2018 (because of leaf growth during incubation) with overwintering from 7 December 2017 to 23 April 2018, and planting on 7 May 2018. Unusually warm spring temperatures caused all plants to eventually die or to cease growth, but initial growth data were recorded for *Crocus vernus*.

2.2.4 Minimum freezing temperature experiment

A separate set of propagules (six replicates per treatment) also was overwintered in an incubator at 1 °C from 1 December 2016 to 20 April 2017, except for during 16 March 2017 to 23 March 2017, when the propagules were removed and subjected to freezing treatments for three days at one of 6 temperatures (0, -2, -4, -6, -8, and -10 °C). After the freezing treatments, the propagules were weighed and planted in a common garden at the field site, with 20 cm spacing. Each species was planted in a single plot with bud depth at 6 cm (*Muscari armeniacum* and *Scilla siberica*), 8 cm (*Crocus vernus*), or 10 cm (*Allium cernuum*, *Apios americana*, *Helianthus tuberosus*, and *Narcissus pseudonarcissus*) to best reflect natural propagule depth, distributor's planting instructions and standard gardening practice (Rockwell & Grayson, 1953; Okubo & Sochacki, 2013; Breck's, 2013; and field observations).

2.2.5 Data collection and analyses

Height measurements were conducted as applicable beginning 10 April 2017 and up to five times before harvest for all experiments. Survival (whole plant) was assessed, and final biomass was harvested according to the phenology (peak growth) of each species. Harvesting began 15 May 2017 for *Narcissus pseudonarcissus*, *Crocus vernus*, and *Scilla siberica*. *Helianthus tuberosus* was the final species collected, and harvesting was completed on 31 October 2017. Specimens were then dried at 70 °C for 72 hours, separated into reproductive structures, aboveground leaf, belowground leaf and stem, clonal organ, and others where applicable (roots, rhizome, tubers) and weighed. For the overwintering field experiment, the effects of snow removal, soil depth (both fixed effects) and their interaction, along with initial propagule weight as a co-variate, were analyzed using a general linear model. The data for the minimum temperature and spring clonal organ depth experiments were analyzed using linear regression. All categorical data (e.g. survival) were analyzed using chi-squared tests. The data were log transformed when positively skewed and reflected (e.g. 500 - x) then log transformed when negatively skewed to meet the assumption of normality when applicable. Analyses were conducted using JMP version 13 (SAS Institute).

2.3 Results

2.3.1 Overwintering field experiment

Frost severity and freeze thaw event frequency increased both with decreasing soil depth and with snow removal (Table 2.1). All species increased in height with increased clonal organ depth (Fig 2.1; effect not significant for *Allium cernuum*), but total biomass was not affected significantly by clonal organ depth, with the exception of *Crocus vernus*, which exhibited increased growth with increased depth (Table 2.2). Belowground stem biomass increased significantly with soil depth for all species except *Helianthus tuberosus* (Fig. 2.1, Table 2.2), but the latter species decreased in stem diameter with increasing depth. Root biomass and number of tubers also increased with decreasing clonal organ depth for *Helianthus tuberosus* (P=0.02 and P=0.01, respectively). *Apios americana* grew earlier with decreasing clonal organ depth (P =0.005), but mid-season height increased with clonal organ depth and under ambient snow (P=0.023). In addition, horizontal belowground stems (rhizomes) increased with decreasing clonal organ depth for this species (P=0.002) and the number of horizontal tubers (when present) decreased with snow removal (P=0.023). The number of vertical tubers for *Apios americana* increased with bulb depth (P<0.0001). *Crocus vernus* and *Scilla siberica* flowering increased with clonal organ depth (P=0.002 and P=0.006, respectively) and both species exhibited an interaction between snow removal and depth, with the greatest likelihood of flowering for the deepest individuals in the ambient snow plots (P=0.026 and P=0.015, respectively). *Narcissus pseudonarcissus* exhibited an interaction between snow removal and clonal organ depth with respect to early height, with it being smallest at the shallow depth and under snow removal (P=0.019). Snow removal decreased early season height for *Narcissus pseudonarcissus* (P=0.038; averages of 216 mm in control plots and 188 in snow removal plots), decreased mid-season and final height for *Muscari armeniacum* (P=0.001 and P=0.003, respectively, 117 mm and 152 mm in control plots and 102 mm and 138 mm in snow removal plots) and decreased final height for *Helianthus tuberosus* (P=0.040, 2618 mm in control plots and 2328 mm in snow removal plots). *Scilla siberica* and *Apios americana* survival decreased in response to snow removal (P=0.012 for both species, 95% in control plots and 63% and 83% in snow removal plots, respectively).

Table 2.1. Means and standard errors of temperatures and freeze thaw cycles under the different depth and snow removal treatments (during winter and early spring). Freeze thaw cycles were defined as any drop below 0 °C followed by an increase to above 0 °C.

	Control			Snow Removal		
Depth	Minimum (°C)	Average (°C)	Cycles	Minimum (°C)	Average (°C)	Cycles
2 cm	-5.6±0	1.4±0.2	23±7	-6.5±0	1.1±0	38±0
5 cm	-3.1±0.1	1.6±0	7±3	-3.9±0.4	1.4±0.2	10±2
15 cm	-0.8±0.3	1.9±0	3±1	-1±0.7	1.8±0	4±1

Table 2.2. P values for the linear regression analysis of morphological measurements for the overwintering field experiment. Depth responses indicate increasing size with depth, with the exception of the bold values, which indicate a trend of smaller size with greater depth. Snow removal responses indicate decreases with snow removal. Depth*snow removal interactions indicate decreases under snow removal and at shallow depths. *P<0.05 **P<0.01 ***P<0.001

Depth Response	*Allium cernuum*	*Apios americana*	*Crocus vernus*	*Helianthus tuberosus*	*Muscari armeniacum*	*Narcissus pseudonarcissus*	*Scilla siberica*
early height (mm)	0.97	0.47	0.002**	**0.001*** **	**0.001*** **	0.71	0.33
mid height (mm)	0.16	0.03*	<0.0001***	**0.004** **	0.88	0.03*	0.01*
full height (mm)	0.12	0.19	0.003**	0.86	0.02*	0.001***	0.001**
leaf (g)	**0.04* **	0.77	0.01**	0.35	0.84	0.12	0.01**
below-ground stem (g)	0.001***	0.002**	<0.0001***	0.72	<0.0001***	<0.0001***	<0.0001***
clonal organ (g)	0.10	0.85	< 0.0001***	0.09	0.01*	0.83	0.29
total above-ground (g)	0.11	0.10	0.01**	0.37	0.07	0.18	0.35
total below-ground (g)	0.61	0.26	< 0.0001***	0.09	0.07	0.43	0.42
total biomass (g)	0.39	0.41	< 0.0001***	0.15	0.17	0.83	0.37

Snow Removal Response							
early height (g)	0.64	0.45	0.61	0.56	0.11	0.04*	0.29
mid height (g)	0.59	0.78	0.10	0.87	0.001***	0.30	0.29
full height (g)	0.20	0.19	0.39	0.04*	0.003**	0.80	0.41
leaf (g)	0.63	0.20	0.70	0.67	0.88	0.84	0.59
below-ground stem (g)	0.78	0.15	0.60	0.97	0.94	0.13	0.74
clonal organ (g)	0.82	0.25	0.50	0.11	0.61	0.42	0.80
total above-ground (g)	0.68	0.67	0.70	0.65	0.92	0.91	0.82
total below-ground (g)	0.84	0.69	0.47	0.15	0.45	0.50	0.71
total biomass (g)	0.79	0.97	0.49	0.28	0.46	0.86	0.70
Depth*Snow Removal Interaction							
early height (g)	0.12	0.63	0.15	0.36	0.78	0.43	0.37
mid height (g)	0.12	0.02*	0.23	0.23	0.36	0.33	0.10
full height (g)	0.19	0.78	0.47	0.87	0.95	0.75	0.46
leaf (g)	0.77	0.30	0.06	0.77	0.10	0.41	0.28
below-ground stem (g)	0.71	0.96	0.58	0.97	0.64	0.02*	0.94

clonal organ (g)	0.70	0.88	0.15	0.54	0.24	0.66	0.62
total above-ground (g)	0.87	0.99	0.06	0.76	0.62	0.22	0.23
total below-ground (g)	0.60	0.55	0.19	0.60	0.23	0.88	0.20
total biomass (g)	0.65	0.66	0.13	0.88	0.11	0.94	0.19

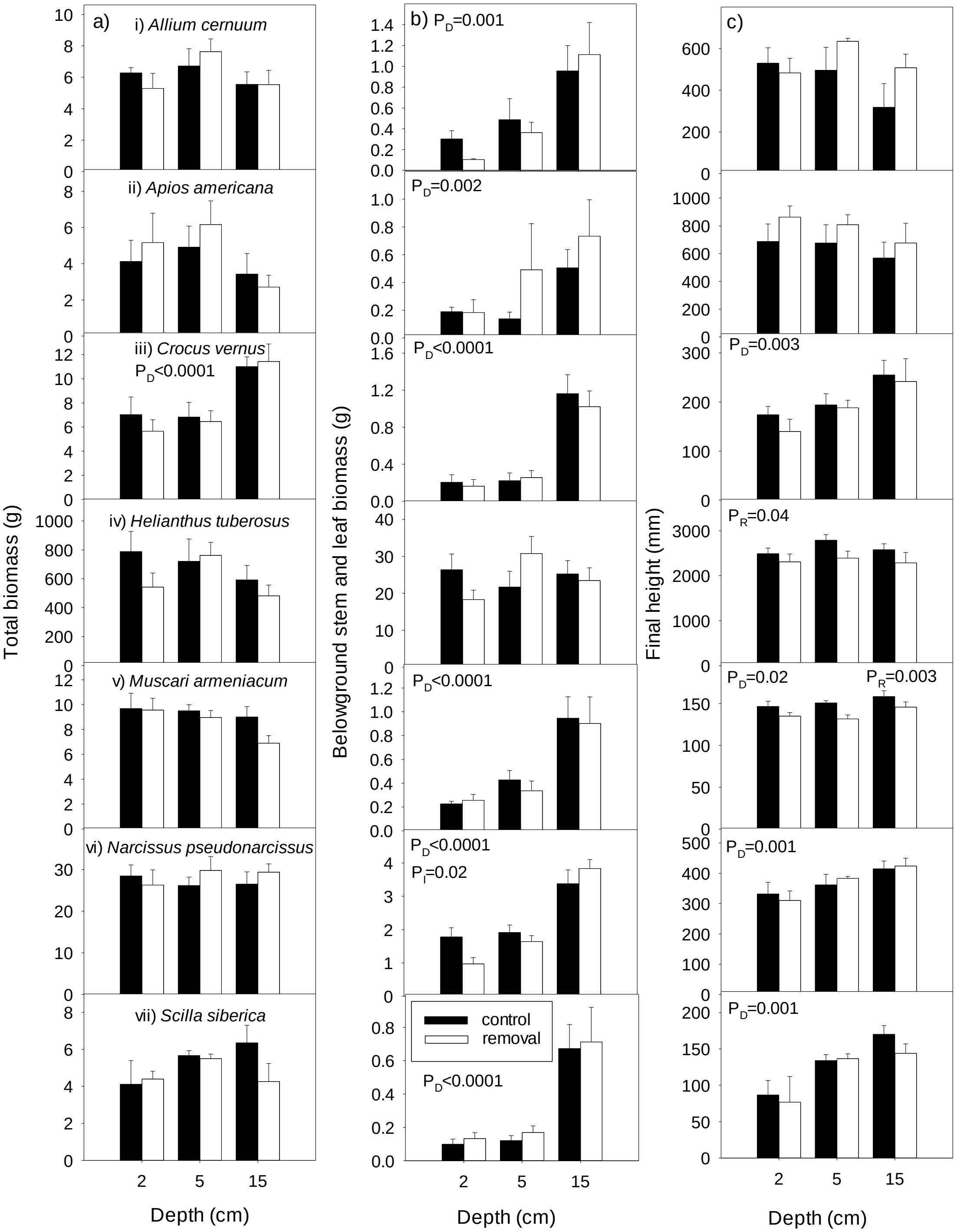
a)
i) *Allium cernuum*
ii) *Apios americana*
iii) *Crocus vernus*
P_D<0.0001
iv) *Helianthus tuberosus*
v) *Muscari armeniacum*
vi) *Narcissus pseudonarcissus*
vii) *Scilla siberica*
Total biomass (g)
Depth (cm)
2
5
15
b) P_D=0.001
P_D=0.002
P_D<0.0001
P_D<0.0001
P_D<0.0001
P_I=0.02
P_D<0.0001
control
removal
Belowground stem and leaf biomass (g)
Depth (cm)
c)
P_D=0.003
P_R=0.04
P_D=0.02
P_R=0.003
P_D=0.001
P_D=0.001
Final height (mm)
Depth (cm)

Figure 2.1. Mean values and standard error for a) total biomass, b) belowground stem and leaf biomass, and c) final height in the overwintering field experiment. Rows represent data for the species named in the first column. P values were obtained through linear regression (only the significant and marginally significant P values are displayed). Subscripts are for effects of snow removal (R), depth (D), and snow removal*depth interaction (I).

2.3.2 Spring soil depth experiment

Increased spring clonal organ depth decreased clonal organ size and total biomass in *Allium cernuum* and *Muscari armeniacum* (Table 2.3). *Apios americana* and *Muscari armeniacum* exhibited increased belowground stem biomass with increasing clonal organ depth (Fig. 2.2), and no effect was present for *Allium cernuum* or *Heliantus tuberosus*. For *Apios americana*, the number of vertical tubers and total tubers increased with increasing clonal organ depth (P=0.003 and P=0.001, respectively), and total stem weight also increased with increasing clonal organ depth (P=0.028). Initial growth for *Crocus vernus* was greatest for the shallowest depth (P =0.001).

Table 2.3. P values for the linear regression analysis of morphological measurements for the depth treatment in the spring soil depth experiment. All effects were in the direction of decreased biomass with greater depth with the exception of the bold values, which indicate a trend of smaller size with greater depth. *P<0.05 **P<0.01 ***P<0.001

	Allium cernuum	*Apios americana*	*Helianthus tuberosus*	*Muscari armeniacum*
height (mm)	0.35	0.99	0.86	0.97
leaf (g)	0.13	0.23	0.74	0.05
belowground stem (g)	0.64	**0.0002*****	0.98	**0.001*****
clonal organ (g)	0.01**	0.2	0.87	0.009**
total aboveground (g)	0.17	0.24	0.74	0.05
total belowground (g)	0.04*	0.07	0.84	0.03*
total biomass (g)	0.04*	0.08	0.79	0.02*

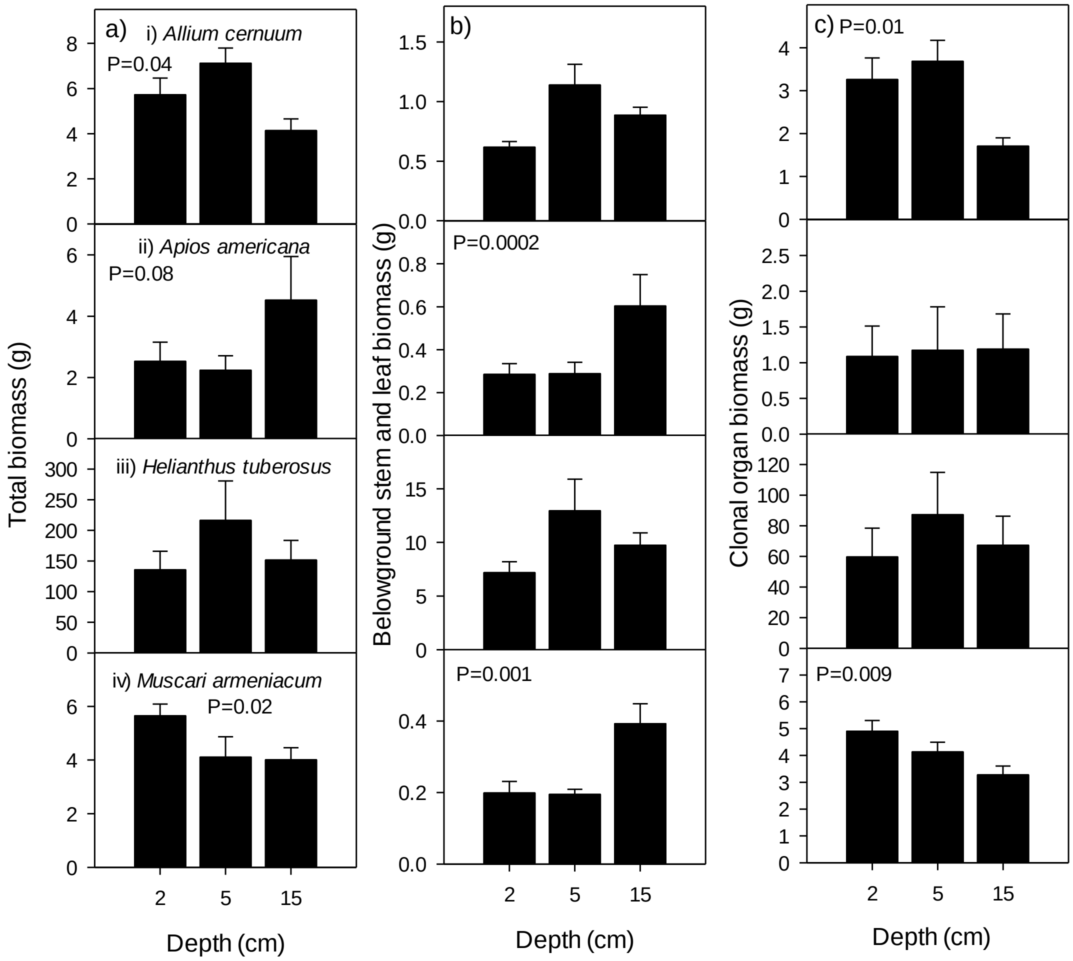

Figure 2.2. Mean values and standard error for a) total biomass, b) belowground stem and leaf biomass, and c) clonal organ in the spring soil depth experiment. Rows represent data for the species named in the first column. P values were obtained through linear regression (only the significant and marginally significant P values are displayed).

2.3.3 Minimum temperature experiment

While survival and growth responses to minimum freezing temperature varied among species (Table 2.4), all species except *Helianthus tuberosus* experienced increased mortality with increased freezing severity (Fig. 2.3). *Helianthus tuberosus*, *Apios americana*, and *Crocus vernus* did not exhibit significant increases in sublethal freezing effects with increasing freezing severity, unlike *Muscari armeniacum*, which experienced decreases in biomass, flowering and reproduction, and *Narcissus pseudonarcissus*, which decreased in biomass (Fig. 2.3).

Table 2.4. P value responses for the linear regression analysis of morphological measurements for the minimum temperature experiment. Effects were in the direction of decreased biomass and survival with decreasing temperature. NA indicates a lack of data. ***Helianthus tuberosus*** **did not experience mortality.**

	Allium cernuum	*Apios americana*	*Crocus vernus*	*Helianthus tuberosus*	*Muscari armeniacum*	*Narcisus pseudo-narcisus*
early height (mm)	0.69	0.73	0.92	0.34	0.93	0.01*
mid height (mm)	0.08	NA	0.74	0.45	NA	< 0.0001***
full height (mm)	0.76	0.99	0.1	0.81	0.01**	0.01*
leaf (g)	0.03*	0.4	0.14	0.7	0.0004***	0.03*
below-ground stem (g)	0.52	0.17	0.6	0.18	0.02*	0.15
clonal organ (g)	0.68	0.76	0.15	0.93	0.02*	0.06
total above-ground (g)	0.38	0.43	0.14	0.69	< 0.0001 ***	0.02*
total below-ground (g)	0.8	0.92	0.16	0.94	0.01**	0.03*
total biomass (g)	0.38	0.67	0.13	0.84	0.002**	0.002**
survival	<0.0001 ***	0.02*	0.0004 ***	NA	0.26	0.002**

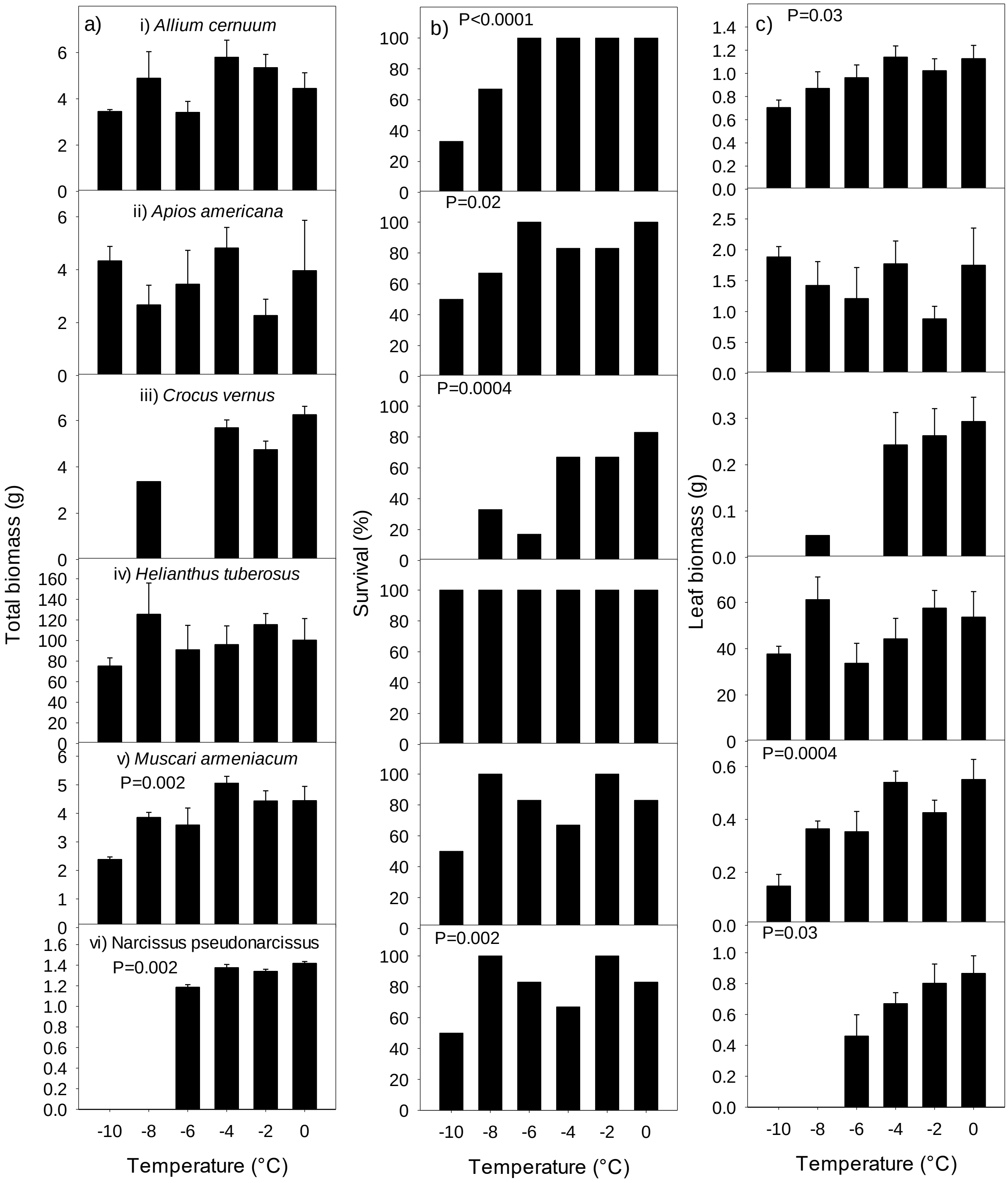

Figure 2.3. Mean values and standard error for a) total biomass, b) percent survival, and c) belowground stem and leaf biomass in the minimum temperature experiment. Rows represent data for the species named in the first column. P values

were obtained through linear regression except for survival which was analyzed through chi-square tests (only the significant and marginally significant P values are displayed).

2.4 Discussion

Overall, while the responses to freezing and depth varied among species, there was a general trend of a trade-off between the avoidance of frost stress with greater soil depth and the extra investment in belowground stem and leaf tissue needed to reach the soil surface. Half the species studied decreased in total biomass as a result of deep clonal organ placement in the absence of frost stress. In contrast, for most species, there was no growth penalty for deep clonal organ placement when clonal organs overwintered in the field and thus risked exposure to soil frost. Moreover, snow removal, which increased frost intensity, reduced the survival and growth of several species. These results demonstrate that freezing can play an important role in modulating the effects of clonal organ depth on plant growth. Such a result is consistent with the work of Boydston et al. (2006), who reported the avoidance of frost stress by the deep soil placement of tubers in *Solanum tuberosum*.

The growth chamber freezing results indicated that the survival and/or growth of most species were reduced when their clonal organs were exposed to temperatures that corresponded with the minimum temperatures observed at the shallowest clonal organ depth (i.e. -5 to -6 °C, observed at 2 cm depth). Nevertheless, in addition to increasing with freezing intensity, plant freezing damage can vary based on the timing, duration, rate or frequency of freezing exposure (Malyshev & Henry, 2012). Moreover, in the field, freezing damage is not restricted to the direct effects of cold temperature. Specifically, frost heave can physically damage plants and increase their frost exposure by moving them towards or past the soil surface (Goulet, 1995). In this study, *Allium cernuum* was particularly vulnerable to heave; ten out of 36 specimens were present at shallower depths than their planting depth at harvest, and one individual was ejected from the soil entirely.

While deep clonal organ placement can minimize frost damage, it comes at the cost of increased resource investment in belowground stem. Unlike photosynthetic tissues (leaves and stems), which provide a return on plant carbon investment, and roots, which enhance nutrient and water acquisition, belowground stem provides no direct return to the plant in terms of resource acquisition. Belowground stem and leaves can be a site of carbohydrate storage, much like belowground horizontal stems (rhizomes) (Kleyer & Minden, 2014), although the recovery of this carbon would depend on the lifespan of the stem and leaf tissue. For example, the long, thin bulbs of *Allium cernuum* are surrounded by long-lived leaves, and they remain green through winter, which implies that they could be meaningful for carbon storage (although the leaf also was the primary organ affected by increased frost exposure). Similarly, the ability of *Apios americana* to produce tubers from vertical belowground stem allows the plant to utilize investment for storage, and can gain recruitment from this structure.

The responses of the bulb-bearing species (*Allium cernuum*, *Muscari armeniacum*, *Narcissus pseudonarcissus*, and *Scilla siberica*) as well as *Crocus vernus* (stem tuber-bearing of the corm-type) were more similar to each other than to the stem tuber-bearing species (*Apios americana* and *Helianthus tuberosus*). The stem tuber-bearing species may have been unique compared to the other species because of the annual nature of their original propagules, the presence of rhizomes, a greater spreading belowground habit, and a longer growing season. For example, *Apios americana* and *Helianthus tuberosus* had long growing seasons (from early May until late October for *Helianthus tuberosus*, which also grew much larger than the other species; roughly 3 m in height). A long growing season may allow for greater recovery from frost and depth effects. When the benefit of frost avoidance for deep clonal organ placement was removed (i.e. via spring planting), both *Muscari armeniacum* and *Allium cernuum* exhibited decreased resource allocation to their storage organs (i.e. bulbs), which could have strong repercussions on fitness in subsequent years.

Although the planting of some clonal organs in the fall and the others in the spring allowed us to disentangle the effects of frost and depth, the spring planted and fall planted clonal organs differed in size (the former were generally smaller) and in maturation time (the former usually matured later; however, this was not the case for the *Helianthus tuberosus* and *Apios americana*). *Apios americana* did not produce horizontal stems and tubers in the spring-planted specimens, which may have been related to temporal growth cues such as photoperiod (Way & Montgomery, 2015). An early onset of rhizome growth may explain the decrease in horizontal tuber investment under colder conditions (i.e. snow removal) in *Apios americana*. *Allium cernuum* did not exhibit a belowground stem response to planting depth for the spring planting, which may have been caused by a difference in resource allocation among the winter and spring planted individuals. Depth did not affect belowground stem investment in *Helianthus tuberosus*, nor was there a significant difference in stem circumference. This observation may be explained by differences in material composition and investment into stems or the lack of dorsal/ventral orientation in tubers, with sprouting occurring from any point on the tuber (i.e. some shallow tubers did sprout from the ventral portion of buried tubers). Therefore, the results from winter-planted individuals would most likely provide the best insight into true clonal organ condition and function under field conditions. Moreover, growth during the overwintering period in the chambers was minimal and may have been greater in fall-planted specimens. Specifically, temperature fluctuated greatly in the field over the experimental period and was often above zero degrees, even during winter months, which could have allowed more growth and development during that time. Even though harvest was at peak aboveground biomass, substantial investment in clonal organs had occurred, and they appeared fully matured by this time.

2.5 Conclusions

This study demonstrates that the cost of frost exposure can outweigh the cost of greater depth, but alone depth can be a cost. In general, belowground traits are poorly understood relative to aboveground traits. Understanding how plants, both economically relevant ornamental species and native species, are affected by frost, may be particularly important in the context of global climate change. In particular, despite the occurrence of shorter and warmer winters, reduced snow cover may increase the vulnerability of over-wintering geophyte clonal organs to soil frost during cold spells (Groffman et al., 2001), which could alter the balance of the trade-off between deep bulb placement to avoid frost stress and shallow bud placement to maximize growth and competitive ability.

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Chapter 3

3. Plant rhizome positioning in the soil and under litter: trade-offs of frost avoidance versus growth

3.1 Introduction

In regions that experience freezing temperatures, herbaceous plants typically enter dormancy over winter, and then resprout and grow further when conditions improve (Klimešová, Tackenberg, & Herben, 2015). To survive winter, they must develop structures and strategies either to tolerate or to avoid frost stress. Freezing can be tolerated or avoided at the cellular level, through physiological means (e.g. the production of proteins and sugars, Pearce, 2001) and enzymes (Davik et al., 2013)). At the level of plant organs and tissues, freezing can be avoided through temporal means such as the avoidance of growth when frost risk is likely (Raunkiær, 1934; Komac, Pladevall, Peñuelas, Conesa, & Domènech, 2015). Plant organs and tissue also can be positioned spatially to avoid frost; frost severity is limited by resistance to its penetration into the soil; plant structures above or at the soil surface are most vulnerable (Sharratt, 2002; Boydston, Seymour, Brown, & Alva, 2006). Frost stress thus can be avoided by herbaceous plants through the placement of their frost-sensitive tissues (buds) deep in the soil (Raunkiær, 1934). In addition, senesced leaf and stem tissue can form a layer of litter that insulates vulnerable tissues (Sharratt, 2002). Snow cover also decreases the level of frost exposure for plant structures positioned at or below the soil surface (Bertrand & Castonguay, 2003; Komac et al., 2015). Decreased snow cover can therefore harm plant species that depend upon snow cover for frost avoidance (Simons, Goulet, & Bellehumeur, 2010; Komac et al., 2015).

Although bud positioning deep in the soil can protect from many stresses (Vesk & Westoby, 2004; Boydston et al., 2006; Baseggio et al., 2015), the protection afforded by deep bud positioning may come at the cost of delayed stem and leaf emergence, and a reduction in subsequent growth in the spring (Pan, Geng, Li, & Chen, 2009). Therefore, on balance, bud positioning deep in the soil may be an advantage under stressful conditions, but a disadvantage in low-stress environments, where shallow bud placement may provide a competitive advantage (Grime, 1977). While surface litter, similar to soil depth, may offer frost protection, shading from litter cover also can hinder regeneration in the spring (Hartnett & Keeler, 1995; Benson & Hartnett, 2006). Therefore, trade-offs between frost avoidance and competitive ability in response to soil bud positioning may be modulated by the thickness of the surface litter layer.

Rhizomes are belowground stems utilized in perennation, clonal growth, and lateral spread to varying degrees, depending on species and rhizome type (Klimešová, Danihelka, Chrtek, de Bello, & Herben, 2017). These structures can be epigeogenous or hypogeogenous. Epigeogenous stems originate above ground and are further adjusted to maintain growing depth, while hypogeogenous stems originate below ground and grow horizontally at a maintained depth. Hypogeogenous stems are generally characterized by greater internode length and thus capacity for lateral spread (Klimešová et al., 2017). Rhizomes are often part of the hemicryptophyte life form (Raunkiær, 1934; Komac et al., 2015) and thus have their buds close to or at the soil surface. Clonal structures can improve the vascular conduction and growth of the parent plant (Bazzaz, Ackerly, & Reekie, 2000); however, they also can become fully-formed, independent daughter plants. Vegetative reproduction can occur in tandem with sexual reproduction and many plants can reproduce asexually more readily than sexually (Schmid, Bazzaz, & Weiner, 1995; Vallejo-Marín, Dorken, & Barrett, 2010). Clonal propagules often perform a storage function and are thus better equipped with nutrients than are seeds. The connectivity and nutrient storage of clonal offspring also may aid in the ability to compete and colonize new space after a heavy frost. However, the placement of rhizomes in relation to soil depth, litter and snow thickness may be governed by trade-offs between frost avoidance and competitive ability.

I conducted a suite of experiments to examine the extent to which the protection of overwintering rhizomes by soil and litter (i.e. frost avoidance) comes at the expense of subsequent reduced resource allocation or delayed emergence. To explore these effects, I planted a set of rhizome fragments from a range of locally-occurring species at different soil depths and under different amounts of litter; these treatments were further combined with a snow removal treatment to vary frost exposure. I predicted that frost damage would be most severe for the rhizome fragments positioned near the soil surface in the absence of litter, and that the latter fragments would exhibit decreased resource allocation to vital structures (e.g. leaves and belowground storage organs), despite the reduced cost of growing to the surface and the potential for early emergence. I also predicted decreased resource allocation to vital structures in response to snow removal. To disentangle the direct effects of soil depth and litter cover on plant growth from the confounding effects of variation in frost exposure, I conducted two additional experiments. For the first, rhizome fragments were incubated at a mild temperature (1 °C) over winter, and then transplanted at varying soil depths under varying amounts of litter cover in the field in the spring. Given that these plants did not experience freezing damage, I predicted that increased planting depth and litter cover would be negatively correlated with subsequent growth. For the second experiment, I isolated the effects of freezing intensity (minimum temperature) from soil planting depth by exposing another set of rhizome fragments to a range of controlled temperature treatments in freezing chambers and compared their growth after spring planting at a uniform depth.

3.2 Materials and Methods

3.2.1 Field site and study species

The experiments were conducted at the Environmental Sciences Western field station in Ilderton, Ontario, Canada (43°04´29´´N, 81°20´18´´W) in plowed research plots. The soil was characterized as a London clay loam (Hagerty & Kingston, 1992). All six study species grow in the region. Three are native (*Anemone canadensis* Linnaeus, *Solidago canadensis* Linnaeus, and *Viola sororia* Willdenow) while the other three are exotic (*Convallaria majalis* Linnaeus, *Hemerocallis fulva* (Linnaeus) Linnaeus, and *Iris* x *germanica* Linnaeus). All species produce rhizomes (belowground horizontal stems). All are hypogeogenous rhizomes, except those of *Viola sororia*, which are epigeogenous rhizomes. The rhizomes of *Iris* x *germanica* are tuberously thickened, and *Hemerocallis fulva* forms tuberous belowground structures along with rhizomes.

Plants were collected in spring and summer of 2016 (depending on the phenology of each species) from within London, Ontario (primarily from the Western University rock garden, with permission from the Friends of the Garden Society of Western University) or north of London at the Environmental Sciences Western field station (*Anemone canadensis*). Adequate plant material to acquire roughly 150 rhizome segments of each species was collected, divided, and planted in pots with growing medium (Pro-Mix, BX M, Premier Horticulture Inc., PA, USA) supplemented with a fertilizer solution (20-20-20 Classic, Plant Products, ON, Canada) applied after planting. The rhizome fragments (propagules) were stored in a greenhouse and allowed to form calluses before experimentation. All of the propagules had intact roots.

3.2.2 Overwintering field experiment

The propagules were buried at 2 cm or 5 cm soil depth from 7 November 2016 to 9 November 2016. In addition, for the propagules buried at 2 cm depth, there were three litter layer thickness treatments (bare, low - 110 g per m^2, and high - 250 g per m^2)), with the litter composed of hay. There were six replicates for each species in each different treatment group. Thus, the treatment pairings for the litter and soil depth treatments were as follows: bare/2 cm (2B), low/2 cm (2L), high/2 cm (2H), and bare/5 cm (5B). The rhizomes were buried to overwinter in twelve 80 × 1600 cm plots spaced 2 m apart. Each of the four rows in a plot corresponded with a litter and depth treatment pair (positioned randomly) and contained one randomly-positioned replicate from every species (i.e. the depth treatment was nested within the stress treatment). The response variables were height and reproduction (presence or number of inflorescences, depending on species anatomy). Half (6) of the plots experienced a snow removal treatment to increase soil freezing intensity, and the other half were an ambient snow cover control. Snow was removed after any heavy snowfall that was likely to be followed by below freezing temperatures, and was ceased after 14 March 2017 to minimize possible snow removal effects on post-snow melt soil moisture levels (i.e. all plots became saturated with water as a result of the final snow melt). Two soil temperature probes (LogTag TRIX-8, MicroDAQ, NH, U.S.A.) were placed adjacent to each depth treatment, and one was present for each litter treatment.

3.2.3 Spring soil depth and litter thickness experiment

Another set of propagules was overwintered at 1 °C in an incubator from 1 December 2016 to 20 April 2017. There were six replicates for each species in each different treatment group. In the spring the propagules were planted in a common garden at the field site at one of each of the litter/depth treatment pairings described above, with the propagules spaced 20 cm apart and positioned randomly.

3.2.4 Minimum freezing temperature experiment

A different set of propagules also was overwintered in an incubator at 1 °C from 1 December 2016 to 20 April 2017. From 16 March 2017 to 23 March 2017 the propagules were removed and subjected to freezing treatments for three days at one of 6 temperatures in an incubator (0, -2, -4, -6, -8 or -10 °C). After the freezing treatments, the propagules were planted in a common garden at the field site, with 20 cm spacing. There were six replicates for each species in each different treatment group. Each species was planted in a single plot with bud depth at the soil surface to best reflect their natural propagule depth, based on field observations.

3.2.5 Data collection and analyses

Survival (whole plant) and flowering (whole plant) were assessed, and height measurements were conducted as applicable beginning 10 April 2017 and up to five times before harvest for all experiments. Final biomass was harvested according to the phenology (peak growth) of each species. Harvesting began 13 June 2017 with *Convallaria majalis* and *Viola sororia* and ended 9 November 2017 with *Solidago canadensis*. Specimens were then dried at 70 °C for 48 hours, separated into reproductive structures, aboveground leaf, belowground leaf and stem, clonal organ, and other structures where applicable (roots, rhizome, stolons), and weighed. Belowground stem is vertical stem tissue used to reach the surface, originating from stem buds on rhizomes and is not rhizome tissue. Because of difficulty with organ identification upon drying, all belowground structures of *Hemerocallis fulva* were labeled as belowground organ.

The response variables were height, organ biomass, and reproduction (presence or number of inflorescences, depending on species anatomy). For the overwintering field experiment, the effects of snow removal, soil depth (both fixed effects), and their interaction, along with initial propagule size as a co-variate, were analyzed for each species using general linear models. The data for the minimum temperature and spring rhizome depth experiments were analyzed using linear regression. All categorical data (e.g. survival) were analyzed using chi-square tests. Analyses were conducted using JMP version 13 (SAS Institute).

3.3 Results

3.3.1 Overwintering field experiment

Frost intensity and the number of freeze-thaw cycles were greatest for the bare soil and shallow soil depth with snow removal, and both freezing metrics increased in response to snow removal, except for freeze thaw cycles under low litter; high litter cover provided greater insulation than low litter cover, and for minimum temperature, high litter had a greater effect than soil depth (Table 3.1). All species except for *Iris* x *germanica* responded to the treatments (Table 3.2). For *Anemone canadensis*, early season growth (P=0.03), mid-season height (P=0.02), flowering (P=0.02), and final height (P=0.06) all were greater or more likely in response to low litter, as opposed to high litter or burial at 5 cm depth (Fig. 3.1). For *Convallaria majalis*, organ investment (P=0.02) was greatest in response to high litter and lowest for low litter and 5 cm soil depth, while there was a trend of greater early season growth (P=0.06) in the 2 cm soil bare and low litter treatments compared to high litter. For *Hemerocallis fulva*, there were trends of greater mid-season height (P=0.06), flowering (P=0.06), and survival (P=0.08) under high litter compared to 2 cm soil depth with either no or low litter. For *Solidago canadensis*, there were trends of greater early season height (P=0.07) and final season height (P=0.07) in high litter and low litter, respectively. For *Viola sororia*, there was a trend (P=0.07) of increased belowground stem growth at 5 cm soil depth.

No native plant species exhibited a response to snow removal. *Hemerocallis fulva* decreased in mid-season height (P=0.02) in response to snow removal, and there was a trend of decreased final height (P=0.06), but it also flowered more frequently (P=0.007). *Convallaria majalis* decreased in belowground organ (rhizome) investment (P=0.05) in response to snow removal, and *Iris* x *germanica* exhibited a trend of decreased height for the early and mid-season measurements (P=0.1 and P=0.09, respectively).

For *Anemone canadensis,* there was an interaction between treatment and snow removal, with the greatest biomass and height in response to low litter under ambient snow, and the lowest at 5 cm under ambient snow (Table 3.2). For flowering likelihood in *Hermerocallis fulva,* there was a trend towards an interaction (P=0.09), with a decrease in response to snow removal at 2 cm depth and no litter compared to 5 cm depth, and an increase at 2 cm soil depth under ambient snow with either no litter or high litter. For *Viola sororia*, there was a trend of an interaction (P=0.08) for horizontal stem biomass, with increased biomass under high litter in response to snow removal, and 2 cm and 5 cm soil depth with no litter were always lowest.

Table 3.1. Mean temperatures (minimum and average) and number of freeze thaw cycles (± standard error) under the different soil depth, litter thickness and snow removal treatments (during winter and early spring). Freeze thaw cycles were defined as any drop below 0 °C followed by an increase to above 0 °C. The treatment pairings are: bare/2 cm (2B), low/2 cm (2L), high/2 cm (2H) and bare/5 cm (5B). There were no replicates for 2L and 2H.

	Ambient snow			Snow removal		
Depth	Minimum	Average	Cycles	Minimum	Average	Cycles
2B	-5.6±0	1.4±0.2	23±7	-6.5±0	1.1±0	38±0
2L	-4.4	1.1	35	-3.4	1.1	26
2H	-2.6	1.4	20	-2.5	1.4	12
5B	-3.1±0.1	1.6±0	7±3	-3.9±0.4	1.4±0.2	10±2

Table 3.2. P values for the analysis of morphological measurements for the overwintering field experiment (soil depth/litter treatment, snow removal and their interaction). Snow removal responses indicate decreases with snow removal except for bold values.

Treatment Response	*Anemone canadensis*	*Convallaria majalis*	*Hemerocallis fulva*	*Iris* x *germanica*	*Solidago canadensis*	*Viola sororia*
early height (mm)	0.22	0.3	0.15	0.35	0.07^{+}	0.71
full height (mm)	0.06^{+}	0.7	0.92	0.99	0.07^{+}	0.8
flowered (%)	0.02*	NA	0.06^{+}	NA	NA	0.74
leaf (g)	0.12	0.24	0.48	0.52	0.27	0.18
below-ground stem (g)	0.59	0.27	0.17	0.62	0.67	0.07^{+}
clonal organ (g)	0.61	0.02*	0.68	0.93	0.13	0.78
total above-ground (g)	0.13	0.2	0.21	0.52	0.35	0.32
total below-ground (g)	0.5	0.35	0.44	0.87	0.23	0.72
total biomass (g)	0.25	0.33	0.32	0.87	0.31	0.51
Snow Removal Response						
early height (mm)	NA	NA	0.65	0.1^{+}	0.42	0.43
full height	0.26	0.45	0.06^{+}	0.62	0.79	0.7

(mm)						
flowered (%)	0.99	NA	**0.01***	NA	NA	0.99
leaf (g)	0.27	0.17	NA	0.28	0.43	0.24
Below-ground stem (g)	0.32	0.79	0.57	0.55	0.83	0.72
clonal organ (g)	0.56	0.05*	0.49	0.23	0.87	0.83
total above-ground (g)	0.33	0.26	0.42	0.28	0.28	0.2
total below-ground (g)	0.41	0.24	0.58	0.88	0.47	0.25
total biomass (g)	0.33	0.23	0.5	0.21	0.31	0.2
Treatment*Snow Removal Interaction						
early height (mm)	0.77	0.39	0.99	0.25	0.44	0.82
full height (mm)	0.02*	0.34	0.77	0.33	0.67	0.96
flowered (%)	0.05*	NA	0.09^{+}	NA	NA	0.02*
leaf (g)	0.03*	0.64	0.9	0.49	0.49	0.62
below-ground stem (g)	0.07^{+}	0.67	0.93	0.85	0.49	0.14
clonal organ (g)	0.01*	0.39	0.68	0.79	0.97	0.62

total above-ground (g)	0.02*	0.58	0.65	0.49	0.49	0.69
total below-ground (g)	0.01*	0.52	0.76	0.87	0.56	0.47
total biomass (g)	0.01*	0.57	0.75	0.75	0.48	0.64

$^{+}P<0.1$ $*P<0.05$

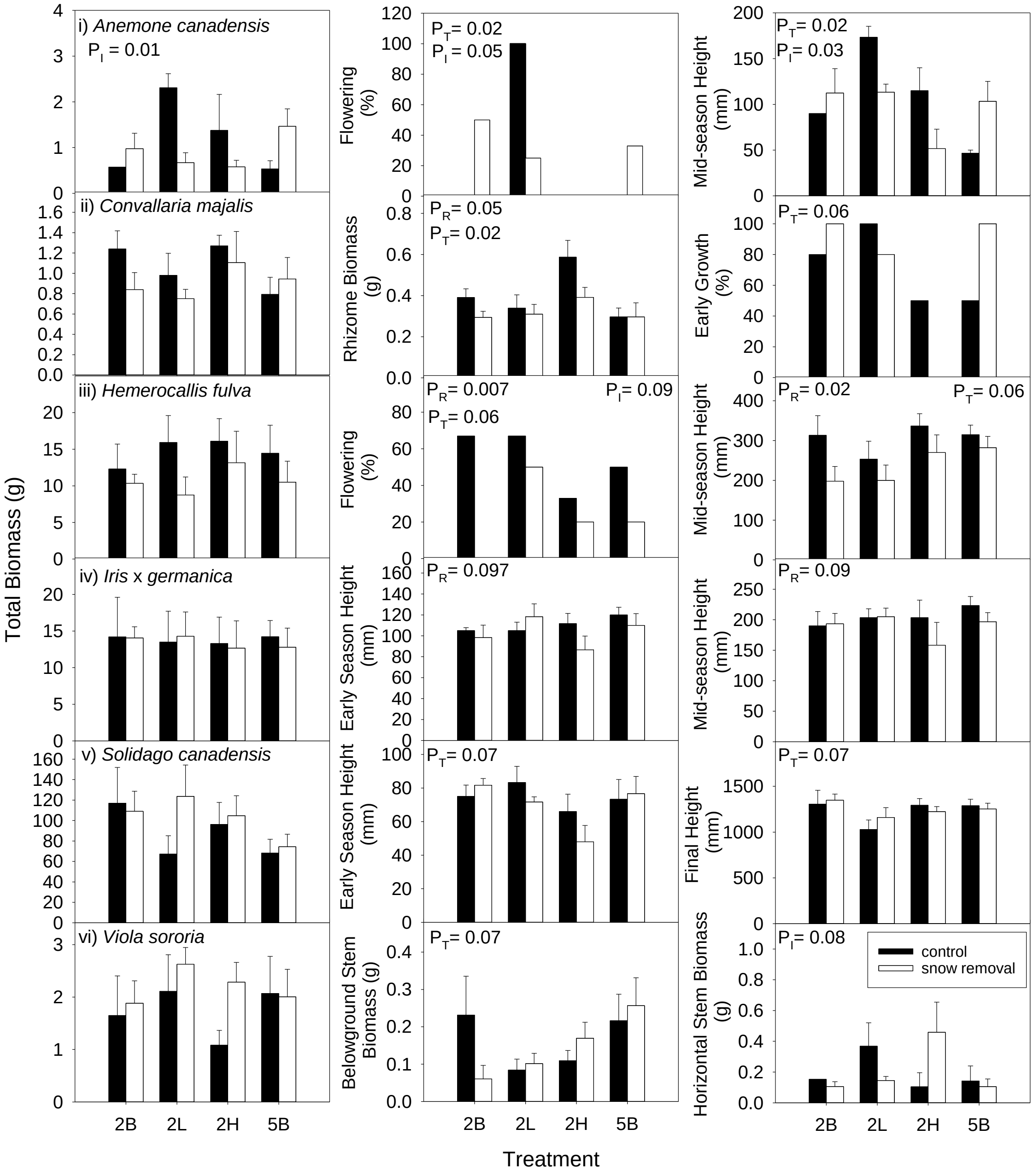

Figure 3.1. Mean values and standard error for total biomass and other notable response variables for each species in the overwintering field experiment. P values were obtained through linear regression except for flowering and early growth which were analyzed through chi-square tests (only the significant and marginally significant P values are displayed). Subscripts are for effects of snow removal (R), treatment (T), and snow removal*treatment interaction (I).

3.3.2 Spring soil depth and litter thickness experiment

Half the species exhibited at least a trend of response to the spring soil depth and litter thickness treatments (Table 3.3, Fig. 3.2). For *Convallaria majalis*, survival (P=0.02) decreased with greater depth and litter thickness. For *Viola sororia*, there was a trend of decreased biomass with greater soil depth and litter thickness, particularly for the aboveground structures. For *Hemerocallis fulva*, there was a trend of decrease mid-season height (P=0.07) with greater soil depth and litter thickness and flowering (P=0.09) with greater depth. *Solidago canadensis* exhibited an opposite response to the other species, with an increase in most belowground structures with greater soil depth (Table 3.3).

Table 3.3. P values for morphological measurements for soil depth and litter depth treatment effects in the spring soil depth and litter thickness experiment. NA indicates a lack of data.

	Anemone canadensis	*Convallaria majalis*	*Hemerocallis fulva*	*Iris* x *germanica*	*Solidago canadensis*	*Viola sororia*
early height (mm)	0.68	0.7	0.1^{+}	0.48	0.21	NA
full height (mm)	0.23	0.67	0.99	0.51	0.65	0.66
leaf (g)	0.26	0.49	0.36	0.96	0.18	0.05*
below-ground stem (g)	0.59	0.79	0.89	0.56	0.02*	0.47
clonal organ (g)	0.42	0.54	0.54	0.88	0.03*	0.16
roots (g)	NA	0.61	NA	0.82	0.09	0.08^{+}
total above-ground (g)	0.26	0.71	0.36	0.96	0.4	0.05*
total below-ground (g)	0.45	0.72	0.55	0.89	0.08^{+}	0.09^{+}
total biomass (g)	0.41	0.79	0.48	0.91	0.27	0.06^{+}

$^{+}P<0.1$ $^{*}P<0.05$

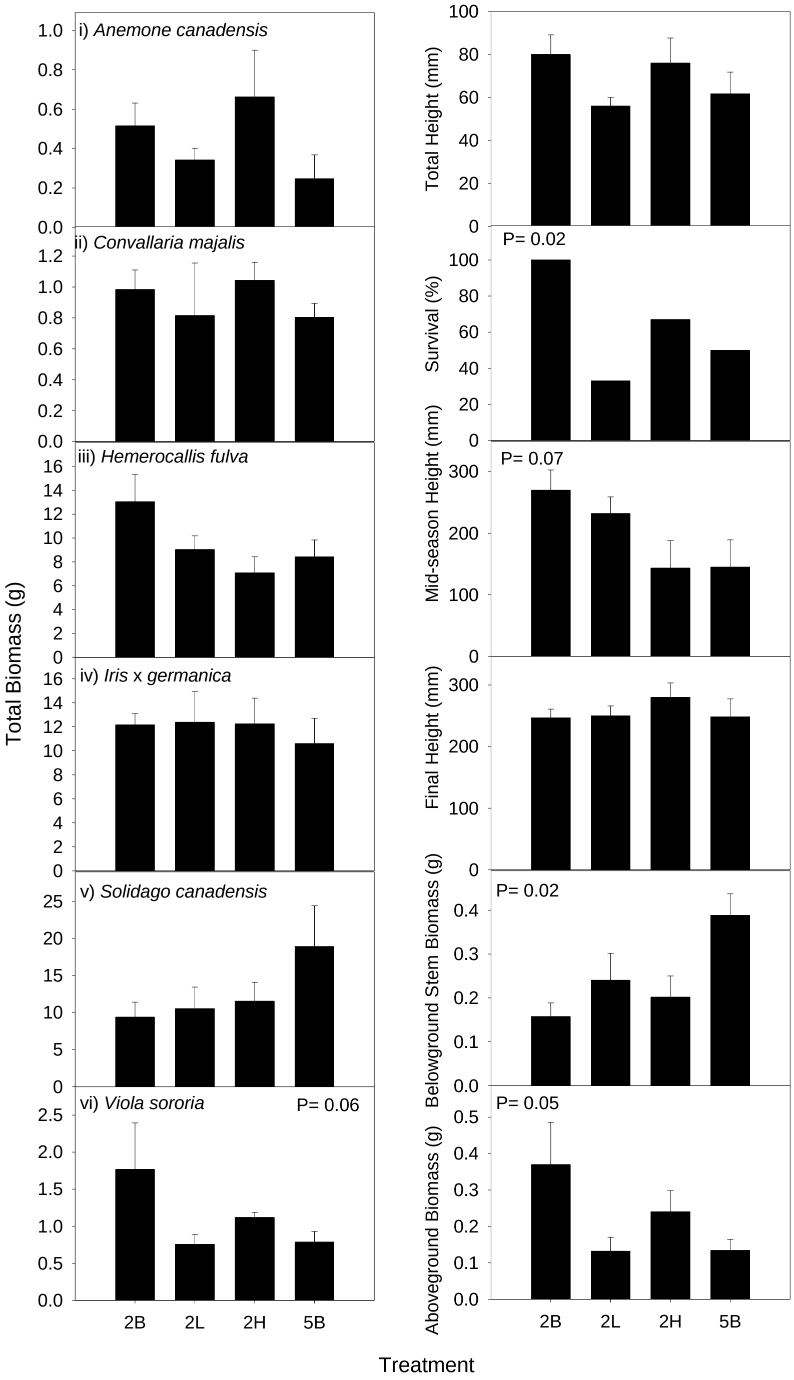

Figure 3.2. Mean values and standard error for total biomass and other notable response variables for each species in the spring soil depth and litter thickness experiment. P values were obtained through linear regression except for survival

which was analyzed through chi-square tests (only the significant and marginally significant P values are displayed).

3.3.3 Minimum temperature experiment

Early height decreased with increasing freezing intensity for most species (Table 3.4, Fig. 3.3), and *Anemone canadensis*, *Hemerocallis fulva*, and *Viola sororia* experienced a decrease in survival. Except for *Anemone canadensis*, all species experienced a decrease in at least one response variable with increasing freezing intensity; *Convallaria majalis* and *Hemerocallis fulva* exhibited decreases in root biomass (P=0.07) and early height (P=0.001), respectively; *Solidago canadensis* exhibited decreases in reproductive structures (P=0.009), mid season height (P=0.004), rhizome mass (P=0.02) and number (P=0.03); *Viola sororia* exhibited decreased sexual reproduction (P=0.007), and there was a trend of a decrease in horizontal stem (P=0.08).

Table 3.4. P values for morphological measurements for the minimum temperature experiment. Effects were in the direction of decreased biomass and survival with decreasing temperature. NA indicates a lack of data. *Iris* x *germanica* and *Solidago canadensis* did not experience mortality.

	Anemone canadensis	*Convallaria majalis*	*Hemerocallis fulva*	*Iris* x *germanica*	*Solidago canadensis*	*Viola sororia*
early height (mm)	0.24	0.85	0.001**	0.003**	0.001***	0.08^{+}
full height (mm)	0.14	0.41	0.36	0.12	0.06^{+}	0.08^{+}
survival (%)	0.09	0.48	<0.0001***	NA	NA	0.0004***
leaf (g)	0.31	0.26	0.74	0.06^{+}	0.001***	0.61
below-ground stem (g)	0.72	0.49	0.41	0.98	0.07^{+}	0.67
clonal organ (g)	0.93	0.25	0.54	0.66	0.002**	0.83

roots (g)	NA	0.07^{+}	NA	0.63	0.01**	0.29
total above-ground (g)	0.31	0.49	0.74	0.06^{+}	0.001**	0.36
total below-ground (g)	0.98	0.08^{+}	0.51	0.76	0.006**	0.38
total biomass (g)	0.42	0.18	0.56	0.4	0.001**	0.36

$^{+}$P<0.1 *P<0.05 **P<0.01 ***P<0.001

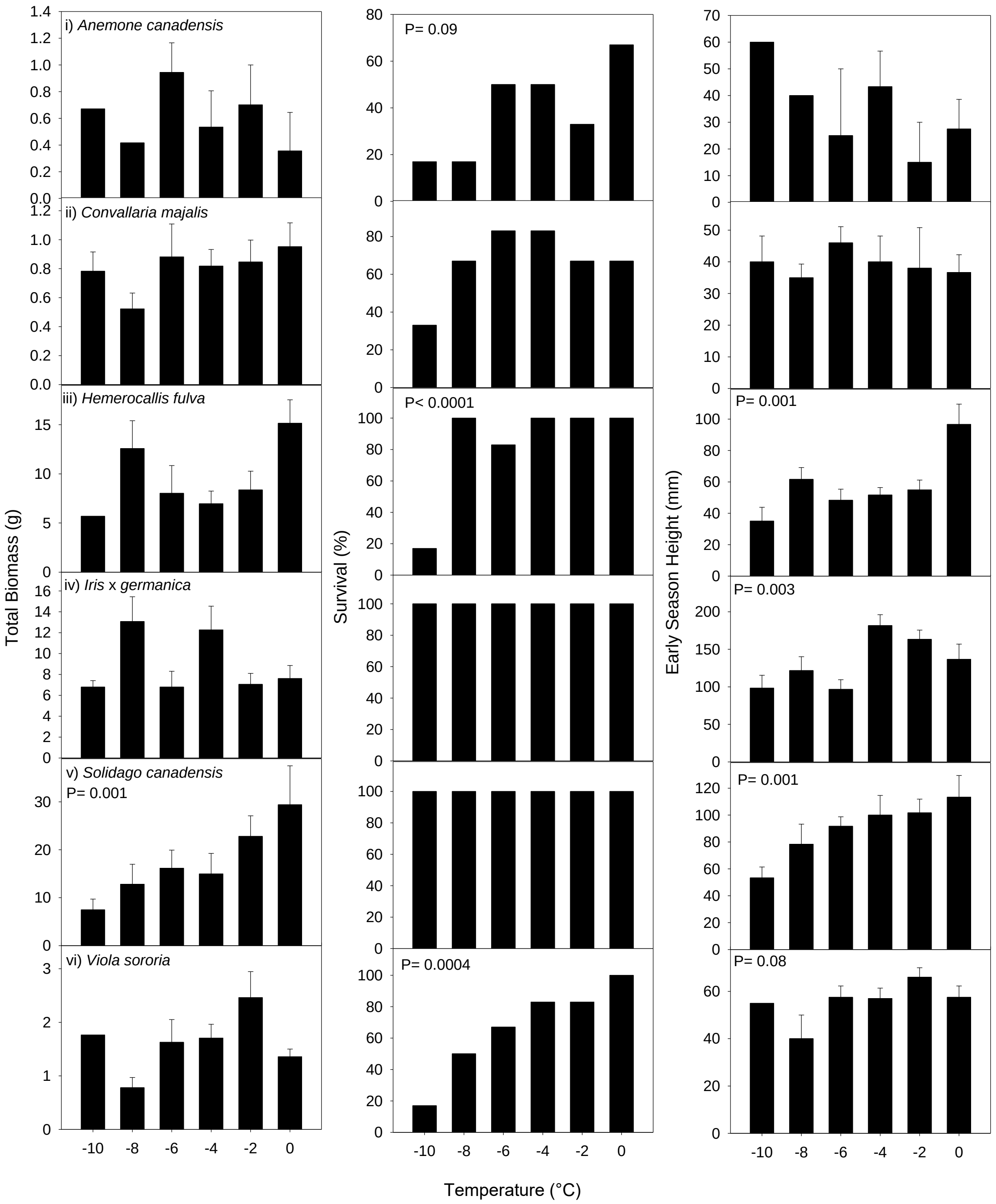

Figure 3.3. Mean values and standard error for total biomass, percent survival, and early season height in the minimum temperature experiment. P values were obtained through linear regression except for survival data which was analyzed with chi-square tests (only the significant and marginally significant P values are displayed).

3.4 Discussion

Overall, our results demonstrated that while the positioning of rhizome fragments deep in the soil or under plant litter can reduce subsequent plant growth and reproduction, there can be indirect benefits of deep soil positioning or litter cover potentially enabled by frost avoidance. Half of the species exhibited an interaction between snow removal and the soil depth/litter treatment, whereby increased soil depth or litter cover modulated the responses of the rhizomes to snow removal. The latter interactions generally were consistent with the respective effects of soil depth, litter cover and snow removal on the intensity of soil freezing, and the results of the minimum temperature experiment also provided support for freezing damage being the main driver of this response. In the absence of frost (i.e. for rhizome fragments overwintered under controlled, mild conditions, then transplanted to the field in the spring), increased soil depth and litter cover were a cost for rhizomes; growth or survival were reduced for most species. This result was consistent with the majority of responses documented in the literature, where accelerated emergence and increased growth typically have been observed for rhizomes positioned at shallow depths (Klimeš, Klimešová, & Osbornová, 1993; Ivany, 1997; Cushman, Maqbool, & Gerard, 2005; Thomas et al., 2006). By favoring vertical growth to reach the soil surface, the positioning of rhizomes deep in the soil also may result in a cost with respect to lateral spread, and as a result, the number of stem buds that sprout may need to be minimized to avoid excessive crowding and self-shading.

In contrast to the majority of previous experiments, the positioning of rhizome fragments deep in the soil in some cases favoured emergence and increased growth; this response has been linked to increased moisture availability at greater depth (Baseggio et al., 2015; Ishimine, Hossain, Ishimine, & Murayama, 2003). In our study, *Solidago canadensis* also exhibited increased growth (i.e. via allocation to structures to reach the surface, as well as to storage) when rhizomes transplanted in the spring were positioned deep in the soil. While the mechanism underlying this response remains unclear, for this species growth during warm spells occurs during the winter, with the tips from the shallow rhizomes often visible in early spring. Provided that the rhizome fragments possessed adequate frost tolerance (and the lack of mortality for *Solidago canadensis* in the minimum temperature experiment suggests that they do), this growth over winter could allow *Solidago canadensis* to overcome the potential cost of deep soil positioning delaying spring emergence. In a previous study (Weber, 2011), high tolerance of rhizome fragmentation and deep burial was noted for *Solidago gigantea*, although the tolerance was not as high for *Solidago canadensis*. It is worth noting that the use of rhizome fragments does not address the potential benefits of connectivity to a parent plant, which could mitigate the response to deep soil positioning in new rhizomes (Bazzaz, Ackerly, & Reekie, 2000) or convey parental costs (Zhang, Zhang, & Sammul, 2012).

Among the native species I studied, none responded to the enhanced freezing temperatures imposed via snow removal. The reason for this may be that these species (or the specific ecotypes that I examined) have been selected for high physiological freezing tolerance (Pearce, 2001) through adaptation to the local climate. Such an inference was not wholly supported by the results from the minimum temperature experiment, although the timing, duration and frequency of freezing, all of which can affect plant responses (Malyshev & Henry, 2012) would have differed among the overwintering and minimum temperature experiments.

Previous studies of rhizome growth have reported interactions between the effects of litter (mulch) cover type, soil depth, and year of study (Cushman & Maqbool, 2005), but the effects of winter insulation of rhizomes by litter cover have not been explored. In our experiment the effect of litter cover varied greatly (i.e. the treatment effects on soil temperature did not fully align with the plant responses to the respective soil depth and litter cover treatments), and in some cases, litter cover conveyed advantages to the rhizomes that were transplanted in the spring and thus not exposed to potential frost. This positive response to litter cover may have occurred because of increased moisture retention, similar to the previously reported response of *Anemone nemorosa* (Craig & Buckley, 2013), the congeneric species with *Anemone canadensis*, which was examined in our study. The lack of response by *Anemone canadensis* to the soil depth/litter cover treatments may have occurred because of its unique rhizome architecture, which branches at an angle as opposed to forming horizontal stems, thus allowing recruitment from buds at different areas along the depth profile. Buds could then be selected from deeper along the rhizome to compensate for frost damaged buds.

3.5 Conclusions

This study demonstrates that for overwintering rhizomes, optimal positioning with respect to soil depth and litter cover can be influenced by the trade-off between frost avoidance and the capacity for early emergence, and the maximal growth that otherwise could be achieved in the absence of frost. Resprouting is an important component of plant re-establishment in strongly seasonal environments (Benson & Hartnett, 2006), and further understanding of how rhizomes overwinter can better reveal the mechanisms explaining the responses of relative species abundance and plant community composition to stress. These responses will likely be of further relevance in a changing climate, where reduced snow cover over winter caused by warming or reductions in precipitation are projected to increase the frequency and intensity of soil frost exposure in some regions (Groffman et al., 2001).

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Chapter 4

4. The role of perennation traits in plant community frost stress responses

4.1 Introduction

Frost is an important stress that limits both the seasonal activity and the global distribution of many plant species (Box, 1996). During periods of frequent or extended frost, perennial herbaceous plants often enter dormancy, at which point many of their aboveground tissues senesce (Raunkiær, 1934; Klimešová, Tackenberg, & Herben, 2015). Upon breaking dormancy, subsequent regeneration of tissue in a plant, can depend highly on the survival and condition of perennial belowground structures (Klimešová et al. 2015). Plants can circumvent freezing stress through physiological tolerance and avoidance of freezing at the cellular level (Pearce 2001; Davik et al. 2013) or through spatial and temporal avoidance of freezing at the plant organ level (Raunkiær, 1934; Klimešová et al., 2015). Snow cover can be an important source of surface insulation from cold air temperatures during winter (Bertrand & Castonguay, 2003), and reductions in snow cover can therefore cause plant community shifts (Komac, Pladevall, Peñuelas, Conesa, & Domènech, 2015). Similarly, senesced stem and leaf tissue (plant litter) can decrease frost penetration into soil, thereby protecting vulnerable buds (Sharrat, 2002). The positioning of vulnerable tissue deep in the soil also can decrease its exposure to frost (Boydston, Seymour, Brown, & Alva, 2006).

Despite the potential benefits of plant structures being positioned deep in the soil or under thick litter for minimizing exposure to a range of biotic and abiotic stresses (Vesk & Westoby, 2004; Boydston et al., 2006; Baseggio, Newman, Sollenberger, Fraisse, & Obreza, 2015), these scenarios can result in delayed emergence and impaired aboveground growth in the spring, potentially reducing competitive ability (Hartnett & Keeler, 1995; Pan, Geng, Li, & Chen, 2009). Therefore, there may be a trade-off between protection from frost and competitive ability that is mediated by the thickness of soil and litter cover. Moreover, the balance of this trade-off can be dynamic; stress avoidance may be important for survival in one year, but in another year that features milder conditions, a riskier, more competitive strategy may be advantageous (Grime, 1977).

Life form classification categorizes plants based on the location of their vulnerable tissues during stressful seasons (Raunkiær, 1934), and in many temperate regions, winter frost stress is used to determine this category. Hemicryptophytes have buds at or just below the soil surface, and this is the most common life form for perennial temperate herbs, especially in fields (Benson & Hartnett, 2006; Klimešová, 2018). Hemicryptophytes can be clonal or nonclonal, and they often have rhizomes (belowground horizontal stems) (Raunkiær, 1934; Komac et al., 2015). Geophytes have buds positioned deep in the soil, and they often produce clonal structures, such as bulbs, stem tubers, and rhizomes, with high storage capacity, (Raunkiær, 1934). In temperate regions these species are most common in woodlands (Kamenetsky, 2013). Therophytes are annuals and do not overwinter as vegetative structures, but instead use seed (Raunkiær, 1934).

Perennial structures are important for plant persistence, but also for clonal spread and recruitment (Klimešová & Klimeš, 2013; Klimešová et al., 2015). Rhizomes are an important organ of clonality and can achieve large amounts of growth and lateral spread via extension of internode length (Cornelissen, Song, Yu, & Dong, 2014). Taproots are generally thickened, vertical roots that can be branched to varying degrees (Chmelíková & Hejcman, 2012), and stem bud recruitment from taproots occurs at the soil surface. The shallow base is prone to damage and ageing, which can induce root-splitting, a form of clonal growth with minimal lateral spread (Chmelíková & Hejcman, 2012; Klimešová, Danihelka, Chrtek, de Bello, & Herben, 2017). Plants also can develop a thickened perennial stem base while forming fibrous roots. In eudicots, this structure can produce annual rings and can thicken like in woody plants (Schweingruber & Poschlod, 2005); the structure and terminology varies, but here I refer to this structure as a rootstock. Plants with monopodial branching or epigeogenous rhizomes with very short internodes are included, because of their similarity in structure. Some plants also can induce stem buds from lateral and adventitious roots (Bartušková, Malíková, & Klimešová, 2017). These root bud banks allow for the recruitment of buds deep in the soil profile.

As described above, seed production is another overwintering strategy employed by plants. Seed response to frost varies, in that while seeds can be damaged by severe freezing, moderate freezing can be required for germination (Chouard 1960). Unlike clonal growth, which is limited by the speed of lateral spread, seed dispersal and seed banks can be particularly beneficial for the colonization of large soil patches denuded of live vegetation by disturbance. Although seeds are often a relatively tolerant life stage, newly germinated plants can be particularly vulnerable to frost, and seedlings often emerge in the spring, at a time when the risk of potentially damaging freeze/thaw cycles is high (Walck, Hidyati, Dixon, Thompson, & Poschlod, 2011; Connolly & Orrock, 2015).

I performed snow removal experiments for three years in an intact, self-assembled, old field plant community to determine the effects of increased frost stress, both at the species level and with respect to groups based on categorical belowground functional traits relevant to perennation. In the third year I also combined snow removal with a litter removal treatment, with the prediction that the latter would increase plant frost exposure. Plant responses were quantified using three cover surveys per growing season (a new set of plots was treated and assessed each year), and the cover data were then pooled based on their categorical functional traits with respect to recruitment method, organ of perennation, and life form. Overall, I predicted that increased frost stress would decrease total plant cover, and more specifically, it would decrease disproportionately for plants with traits associated with shallow bud depth and vulnerable belowground structures. Susceptible groups were expected to include hemicryptophytes, particularly taproot and rhizome bearing species. I also predicted that plants dependent on seeds would be the most resistant to frost stress.

4.2 Materials and Methods

4.2.1 Site

Experiments were conducted in an old field at the Western University Environmental Sciences Western field station (ESW), near Ilderton, ON, Canada (43°04'37.6"N 81°20'16.1"W). The soil at the site was characterized as Bryanston silt loam, which is a

Brunisolic Gray Brown Luvisol (Hagerty and Kingston 1992), and it had a mean pH of 7.5. The field, which was bordered by a woodland, a cropland, and maintained grass pathways and lawns, had been left fallow and permitted to naturalize since 2013. Dominant plant species were goldenrod (*Solidago* spp.) and red clover (*Trifolium pratense*) with a notable presence of asters (*Symphyotrichum* spp.), and thistles (*Cirsium* spp. and *Sonchus* spp.). Wild carrot (*Daucus carota*) and birdsfoot trefoil (*Lotus corniculatus*) also were abundant in patches.

4.2.2 Snow removal (Year 1)

Six pairs of 1 m × 2 m plots (12 plots total) were laid out, with the pairings based on proximity and vegetation similarity. Over the winter of 2015/2016, I administered either snow removal via shovelling or control (no snow removal) to one plot in each pair. Before treatment, all plots were overlain with white plastic netting with 1 cm openings (Protective Winter Wrap; Quest Plastics, Mississauga, ON, Canada) to denote the shovelling depth and to prevent the removal of litter and disturbance of the soil surface. Soil temperature probes (LogTag TRIX-8, MicroDAQ, NH, U.S.A.) were placed 2 cm deep in the centre of three plots from each treatment to record soil temperature hourly. Snow was removed opportunistically after heavy snowfall events that were forecast to be followed by below freezing temperatures, which resulted in nine snow removal events total from 12 January 2016 until 17 February 2016. Snow removal was halted before the end of winter to minimize potential snow removal effects on soil moisture over the subsequent growing season. The plastic netting was removed 18 April 2016 to avoid interference with aboveground plant growth. Plant cover was surveyed to the nearest 5% (but also including 1%) for each species present. *Solidago altissima* and *Solidago canadensis* were combined as *Solidago* spp., because of the inability to distinguish between them during the first two surveys of the year. I conducted cover surveys on 11-17 May, 8 June, and 14-16 September 2016 to capture initial and peak growth of the common species and focal functional groups.

4.2.3 Snow removal (Year 2)

The experiment was repeated during the winter of 2016/2017 for a new set of plots (12 pairs of 1 m × 1 m plots - 24 plots total). Snow was removed four times from 13 December 2016 until 14 March 2017. Winter wrap was removed on 24 April 2017. I conducted plant cover surveys on 23 May, 14 June, and 12 September 2017.

4.2.4 Snow and winter litter removal (Year 3)

The experiment was repeated during the winter of 2017/2018, with the addition of a winter litter removal component (applied as a full factorial experiment in combination with the snow removal treatment; 10 blocks of 1 m × 1 m plots - 40 plots total). For winter litter removal, all aboveground biomass was cut on 27 November 2017 and placed adjacent to the plot to allow it to undergo decomposition under field conditions. For the remaining plots, litter was cut and removed, but placed immediately back onto the plot to produce the same disturbance effect as the litter removal plots. Snow was removed seven times, from 26 December 2017 until 12 February 2018. The plastic netting was removed, and the litter placed back on the winter removal plots on 11 April 2018. I conducted plant cover surveys on 14-17 May, 21-22 June, and 11-12 September 2018.

4.2.5 Plant functional group categorization

The plant functional type of each species present (Appendix 1) was assessed with respect to three different categories: recruitment method, organ of perennation, and life form. Total percent cover for the species exhibiting each trait was totalled. Species trait data were acquired from databases (Fitter & Peat, 1994; Klimešová et al., 2017; Native Plant Trust, 2019), the literature (Lemieux, Cloutier, & Leroux, 1993; Bhowmik & Bandeen, 1976; Turkington & Cavers, 1978; Werner, Bradbury, & Gross, 1980; Lemna & Messersmith, 1990; Chmielewski & Semple, 2001a, 2001b, 2003; Stewart-Wade, Neumann, Collins, & Boland, 2002; Klimešová, 2018), and personal observation. Obligate biennials were assessed by emergence and size. Recruitment method included 1) germination (genets) that overwintered as seed, and 2) resprouting (ramets) that overwintered as vegetative structures. Organs of perennation included 1) rhizomes (plant belowground horizontal stems of the hypogeogenous type that have long internodes and high potential for lateral spread), 2) rootstocks (concentrated belowground stem bases with greater likelihood of woodiness, including epigeogenous stems that have very short internodes), 3) taproots, 4) root buds (stem bud-bearing roots), and 5) none (overwintering as seeds). Rhizome and rootstock bearing plants both exhibited fibrous roots. *Symphyotrichum lanceolatum* has hypogeogenous rhizomes, but these have low conductivity and short lifespans (Chmielewski & Semple, 2001a), thus this species functions more similarly to a rootstock species and is classified as one in this system. Life form was based on the location of sensitive tissue (buds) during the harshest season (winter in this region) (Raunkiær, 1934), and included 1) hemicryptophytes (buds at or just below the soil surface), 2) geophytes (buds deep in the soil), and 3) therophytes (overwintering as seeds).

4.2.6 Data analyses

Total cover and species cover, both individual, and summed for each functional trait group, were the response variables for each treatment. Total, species and functional trait cover for years 1 and 2 were analyzed with paired one-tailed t-tests (for the species-level analyses, only the dominant species, which were present in the majority of plots, were examined). Species and functional trait cover for Year 3 was analyzed with two-way ANOVAs and further analyzed with paired one-tailed t-tests for each treatment variable (snow removal or litter removal) when no interaction was present. Analyses were conducted using JMP version 13 (SAS Institute).

4.3 Results

4.3.1 Soil temperature

The snow removal experiments were conducted in three contrasting winters; the first was relatively mild (average daily temperature 0 ºC) with regular snow cover late in the season (3.5 cm average snow cover from December through March), the second was colder (average daily temperature -1 °C) but with relatively low snowfall later in the season (4.0 cm average snow cover from December through March), and the third was cold (average daily temperature -4 °C) but with relatively high snow cover (6.9 cm average snow cover from December through March) (Environment Canada, 2019). Snow removal decreased minimum soil temperature and increased the number of soil freeze-thaw cycles, although the effect sizes varied by year (Table 4.1). Litter removal also decreased minimum temperature and increased the number of freeze-thaw cycles, both combined with and separate from snow removal (Table 4.1).

Table 4.1. Means and standard errors of minimum and average temperatures and number of freeze thaw cycles at 2 cm soil depth under the different litter and snow removal treatments (during winter and early spring). Freeze thaw cycles were defined as any drop below 0 °C followed by an increase to above 0 °C.

	Control			Snow Removal		
	Minimum (°C)	Average (°C)	Cycles	Minimum (°C)	Average (°C)	Cycles
Year 1	-1.2±0.1	1.9±0.1	10±2	-2.0±0.2	1.9±0.1	11±3
Year 2	-3.5±0.3	1.8±0.1	21±1	-6.0±0.7	2.5±0.1	66±13
Year 3 (with litter)	-1.6±0.8	1.2±0.1	8±4	-5.3±0.1	0.5±0.1	16±1
Year 3 (without litter)	-3.1±0.4	0.8±0.1	21±4	-6.7±1	0.4±0.1	22±4

4.3.2 Total cover and dominant species responses

Total cover decreased with snow removal significantly in May in year 1 (P=0.004) and there was a marginally significant decrease in September (P=0.06) (Fig. 4.1i). *Trifolium pratense* cover decreased in response to snow removal for all three cover surveys (P=0.0006. P=0.001, and P=0.0006, respectively; Table 4.2). There were no significant snow removal effects on total cover in year 2 (Fig. 4.1ii), although *Solidago* spp. cover decreased significantly with snow removal for the May survey (P=0.03) and there were marginally significant decreases for this species (P=0.09) and for *Taraxicum officinale* (P=0.06) for the September survey (Table 4.2). There also were marginally significant decreases in *Trifolium pratense* cover in May and June in year 2 (P=0.06 and P=0.05, respectively; Table 4.2). In year 3, snow removal decreased total cover for all three surveys (P=0.002, P=0.004, and P=0.01; Fig. 4.1iii), with a significant decrease in *Trifolium pratense* cover in May (P=0.004), and decreases in *Taraxicum officinale* cover in May (P=0.07) and June (P=0.04) (Table 4.2). There was a marginally significant decrease in total cover in response to litter removal in May of year 3 (P=0.07).

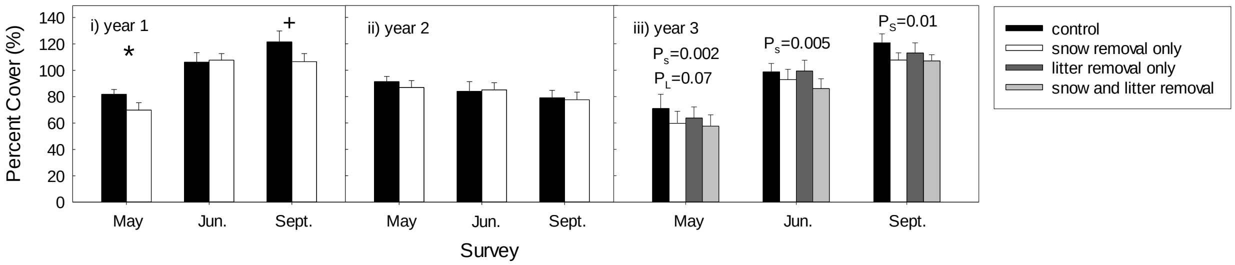

Figure 4.1. Mean values and standard error for total plant percent cover for years i) 1, ii) 2, and iii) 3. Subscripts for P values in year 3 are snow removal (S), litter removal (L), and snow removal*litter removal interaction (I). +P<0.1 *P<0.05 for years 1 and 2.

Table 4.2. Mean values and standard error for percent cover for the dominant species in response to snow and litter removal. +P<0.1 *P<0.05 **P<0.01 ***P<0.001

		May		June		September	
	Species	Control	Removal	Control	Removal	Control	Removal
Year 1	*Solidago* spp.	35±4	31±2	47±6	53±7	50±3	53±7
	Taraxicum officinale	18±5	13±4	19±5	17±4	19±6$^+$	13±3
	Trifolium pratense	10±3***	2±1	10±3***	3±1	20±5***	4±2
Year 2	*Solidago* spp.	57±3*	50±4	38±5	37±5	43±5$^+$	38±5
	Taraxicum officinale	13±2	13±2	12±2	13±2	2±1	2±1
	Trifolium pratense	9±3$^+$	5±2	20±6$^+$	12±3	25±5	28±5
Year 3 with litter	*Solidago* spp.	21±2$^+$	20±2$^+$	30±6	31±5	30±7	27±3
	Taraxicum officinale	14±2$^+$	11±2	21±3*	16±2	14±3	15±3
	Trifolium pratense	27±8***	21±6	34±9	31±8	43±7	38±5
Year 3 without litter	*Solidago* spp.	17±2	19±2	31±6	27±5	26±4	30±6
	Taraxicum officinale	12±2$^+$	12±3	17±3	17±2	12±3	14±3
	Trifolium pratense	27±7***	19±6	36±9	28±8	43±6	40±6

4.3.3 Recruitment method

The cover of resprouted plants decreased in response to snow removal in May of each year (P=0.002, P=0.0003, and P=0.001, respectively), and there was a marginally significant decrease in response to litter removal in May (P=0.1) (Fig. 4.2a.). The cover of germinated plants exhibited a marginally significant decline in response to snow removal in June of year 1 (P=0.08), but it increased in May and June in year 2 (P=0.005 and P=0.05 respectively; Fig. 4.2b). Snow and litter removal had interactive effects on the cover of germinated plants in May of year 3 (P=0.021) and these plants decreased in response to snow removal in June and September (P=0.007 and P=0.002 respectively) and increased in response to litter removal in June (P=0.05).

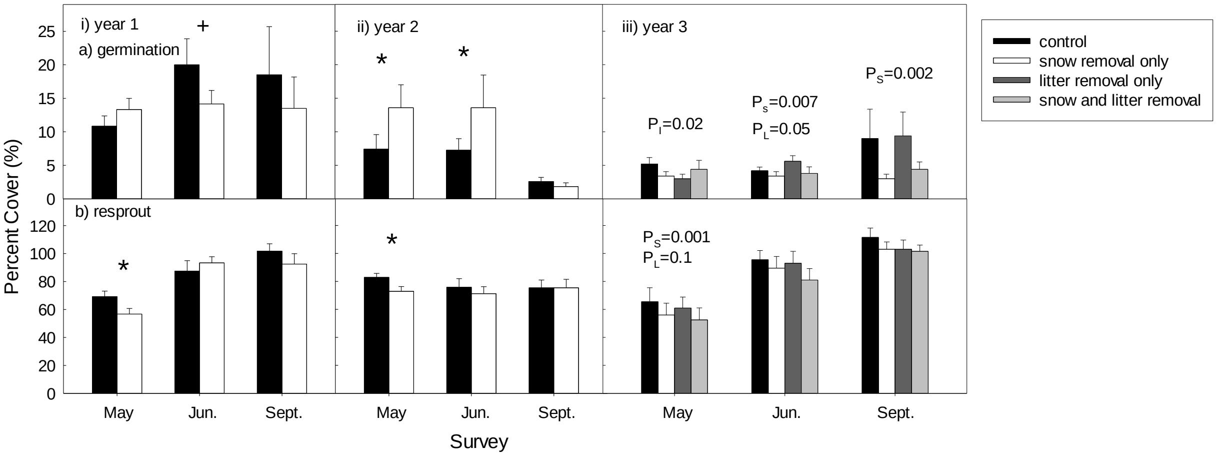

Figure 4.2. Mean values and standard error for trait groups based on recruitment method: a) germination and b) resprouted, for years i) 1, ii) 2, and iii) 3. Subscripts for P values in year 3 are snow removal (S), litter removal (L), and snow removal*litter removal interaction (I). +P<0.1 *P<0.05 for years 1 and 2.

4.3.4 Organ of perennation

The cover of plants that produce root buds increased in response to snow removal in year 1 in all surveys (P=0.02, P=0.01, and P=0.006) and in year 2 there was a marginally significant increase in May in response to snow removal (P=0.09; Fig. 4.3a). Cover of these plants decreased in May year 3 (P=0.004). The cover of plants that produce rhizomes decreased marginally significantly with snow removal in May of year 1 (P=0.07) and there was significant decrease in the cover of these species in year 2 and 3 (P=0.02). Rhizomatous species decreased in cover in May year 3 with litter removal (P=0.02) (Fig. 4.3b). The cover of plants that produce rootstocks increased in response to snow removal in June of year 1 (P=0.02) and May of year 2 (P=0.03) and there was a marginally significant increase in May of year 3 (P=0.07; Fig. 4.3c). The cover of plants that produce taproots generally decreased with snow removal. The cover of these species decreased in response to snow removal in all 3 May surveys (P=0.008, P=0.03, and P=0.0004 respectively), with marginally significant decreases in June in years 1 and 2 (P=0.06 and P=0.09 respectively). Cover responded significantly in June year 3 (P=0.009) and September year 1 (P<0.0001). Totals and results for cover of plants with no organ of perennation was the same as for the cover of germinated plants described above (i.e. these groups contained the same set of species).

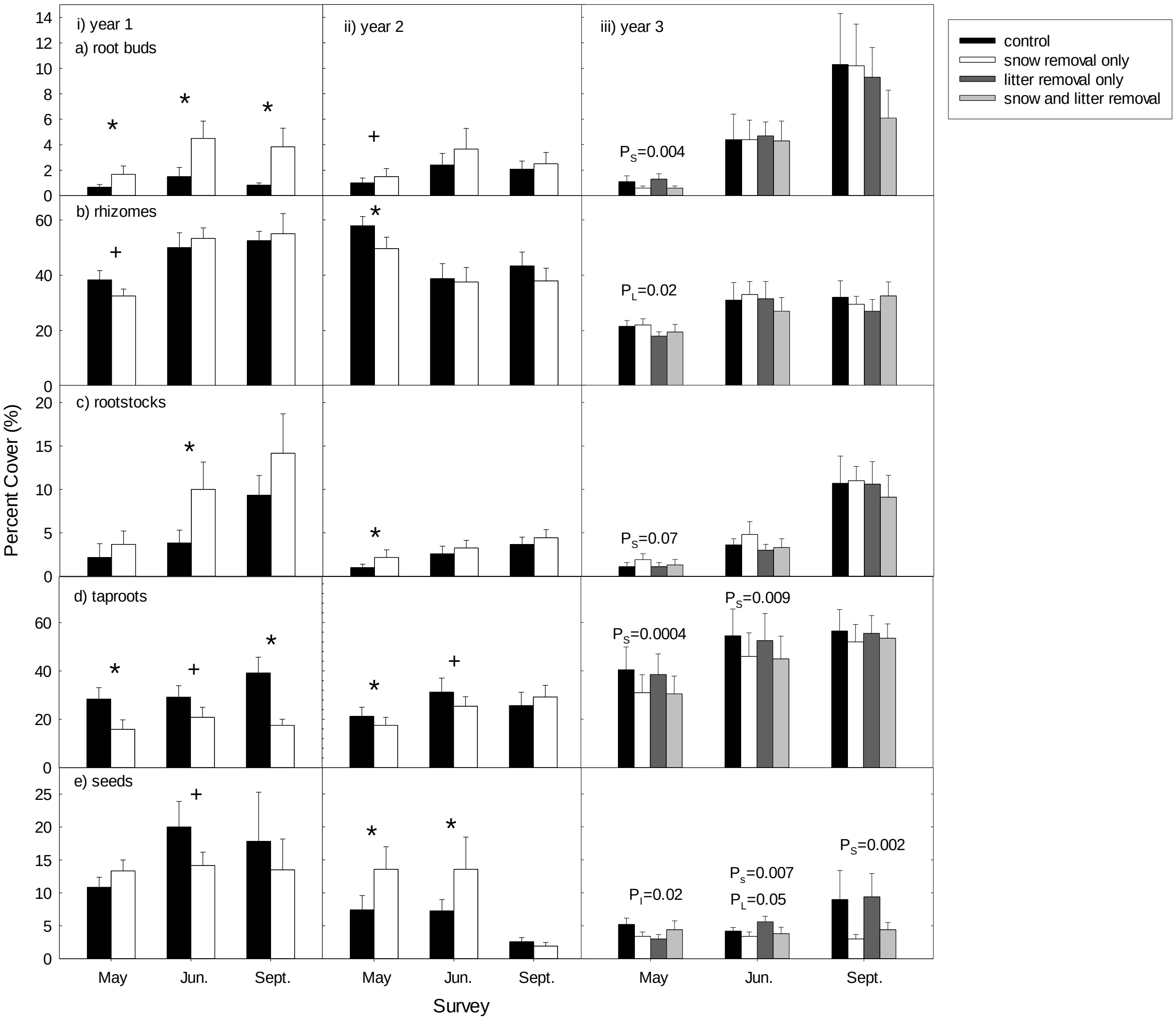

Figure 4.3. Mean values and standard error for trait groups based on organ of perennation: a) root buds, b) rhizomes, c) rootstock, d) taproot, and e) seed (no organ) for years i) 1, ii) 2, and iii) 3. Subscripts for P values in year 3 are snow removal (S), litter removal (L), and snow removal*litter removal interaction (I). +P<0.1 *P<0.05 for years 1 and 2.

4.3.5 Life form

Hemicryptophyte cover decreased with snow removal in every May survey (P=0.002, P=0.0002, and P=0.005, respectively), in the June survey in years 2 and 3 (P=0.0002 and P=0.01, respectively), and in the September survey in year 1 (P=0.04; Fig. 4.4a). Litter removal decreased the cover of these species in May (P=0.07). Geophyte totals and results are the same as those for plants bearing root buds; this was the only organ represented by geophytes in the study. Totals and results for therophytes were the same as for the cover of plants with no organ of perennation and germinated plants described above (i.e. they represented the same species).

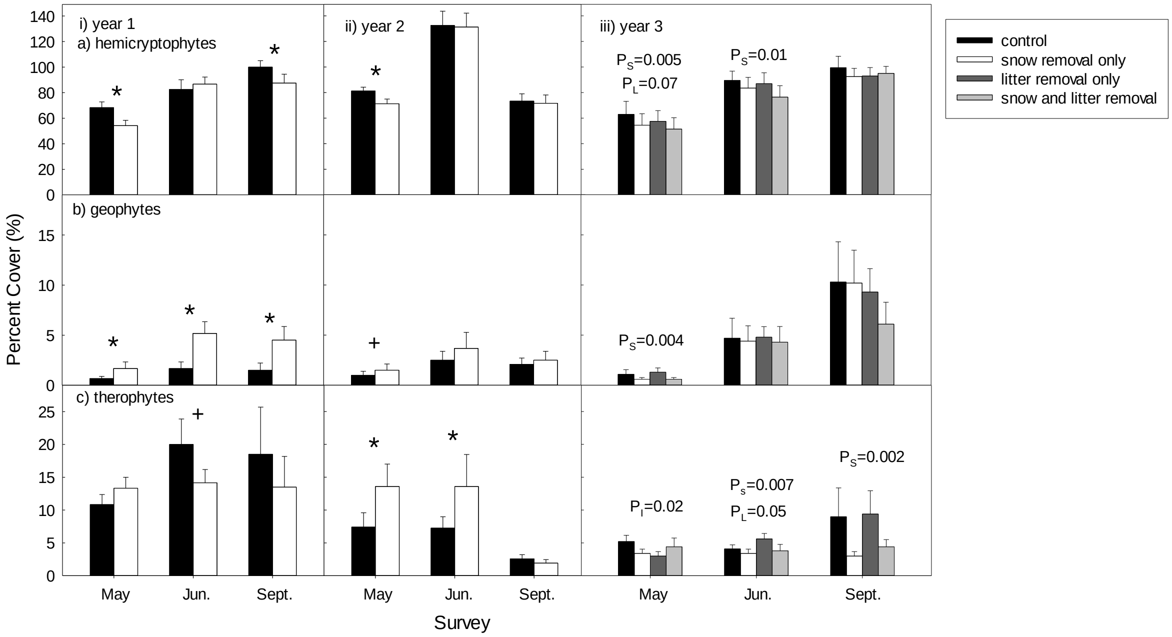

Figure 4.4. Mean values and standard error for life form: a) hemicryptophyte, b) geophyte, and c) therophyte for years i) 1, ii) 2, and iii) 3. Subscripts for P values in year 3 are snow removal (S), litter removal (L), and snow removal*litter removal interaction (I). +P<0.1 *P<0.05 for years 1 and 2.

4.4 Discussion

Consistent with our prediction and previous results in the literature (Malyshev & Henry, 2012; Henry et al., 2018; Reinmann, Susser, Demaria, & Templer, 2019), snow removal generally had a negative effect on total plant cover, although both the magnitude and significance of this effect varied based on year and survey date. In particular, in year 2, which featured both cold temperatures and low snow cover, there was no snow removal effect on total plant cover, and plant cover did not increase appreciably across survey dates, unlike the other two years. My interpretation here is that although very cold temperatures were experienced in the snow removal plots in year 2, the ambient plots also experienced substantially cold temperatures (i.e. colder than the snow removal plots in year 1) as a result of the low ambient snow cover. This could have resulted in the threshold temperature for plant damage (e.g. Malyshev & Henry, 2012) being exceeded in all plots. In addition, decreased total cover later in the growing season as a result of drought stress could have masked any snow removal effect (this year experienced abnormally high rainfall in May - 133 mm as opposed to 31 mm and 54 mm in years 1 and 3, respectively - followed by a drought the next four months - 192 mm as opposed to 395 mm and 335 mm in years 1 and 3, respectively (Environment Canada, 2019)). Litter cover also affected plant cover, but it was most influential for species overwintering as either seed or rhizomes.

With respect to recruitment method, resprouting was decreased consistently by snow removal in each year. Although such an effect also was observed for germination in year 3, and there was no significant snow removal effect on germination in year 1, in year 2 the germinated plants increased in response to snow removal. The latter response could have been driven by competitive release as a result of the decrease in resprouted plants, and thus an indirect response to snow removal. In support of this hypothesis, I observed a general delay of emergence via germination compared to resprouting from vegetative structures, which could have provided a competitive advantage to resprouting plants in the absence of frost damage. In year 3, the recruitment of seeds in general was very low compared to the other years, whereas functional groups with vegetative structures for recruitment were much more successful. In May, germination recruitment was greatest when both snow and litter were present, but the effects of litter became more complicated in June when presence increased in both the control plots and the litter removal plots.

Snow removal effects also differed among life form categories and organ of perennation categories, with the cover of species with bud-bearing organs at or near the soil surface (hemicryptophytes) being most sensitive to snow removal, which was consistent with the relatively high intensity of frost exposure at the soil surface. Hemicryptophytes represent the majority of herbaceous species in many temperate habitats (Komac et al. 2015; Hameed, Uzun, & Saeed, 2016; Klimešová, 2018), and they feature substantial structural diversity. Secondary thickening, composition, and structure persistence all can vary among and within species, especially with age (Klimešová & Klimeš, 1996; Klimeš, Klimešová, & Čížková, 1999). Species with rootstocks generally had an early growth advantage in snow removal plots, as opposed to the other hemicryptophytes. This result likely occurred because of organ structure, with increased woodiness possibly offering greater protection from the cold (Wisniewski, Bassett, and Gusta, 2003). Rhizomatous species responded roughly the same as hemicryptophytes as a whole during years 1 and 2, likely because of the presence of buds near the surface, as well as their horizontal stems. In year 3, rhizomatous cover decreased with litter removal, suggesting that litter cover is important for insulation from cold air in hypogeogenous rhizomatous species. Soil surface temperatures may be particularly relevant for rhizomatous species, because of their high concentration of sensitive structures near the surface, and branching occurs parallel to the surface, as opposed to downward. Taproots have a concentration of stem buds near the soil surface, and these structures are often quite vulnerable to damage (Chmelíková & Hejcman, 2012; Klimešová, et al. 2017) including frost (Perfect, Miller, & Burton, 1987). These structures are vertically long and cylindrical, and this root structure also may contribute to increased vulnerability to frost heave, which could push these structures even higher and increase exposure to freezing air temperatures, as compared to fibrous root systems (Perfect et al., 1987). Recruitment by vegetative structures in general was vulnerable to reduced growth in response to snow removal in the emergence and early growth stage, but it recovered later in the growing season. Species dependent upon recruitment from buds closer to the surface varied in their responses among years, but they remained negatively affected by snow removal in June in year 3 and even in September of year 1. Aside from cover, the effects on reproductive structures and investment in storage organs (not investigated here) also could reveal the potential for long term legacy frost effects on plant community structure.

Species bearing buds from roots (the only geophytes present) were most successful in year one under snow removal, which may have occurred because these species could regenerate from deeper in the soil profile than their competitors, and thus avoid frost stress to a greater extent. The decrease in the cover of root budding species in the first season of year 3 may have been driven by increased frost exposure of their shallow structures; successful root budding would then occur deeper in the soil, and this increased growing depth could come at the cost of later emergence. Soil heave also may have fragmented roots and severed connectivity to the parent plant, thus slowing growth.

4.5 Conclusions

Studies of plant belowground traits generally have been under-represented in plant trait studies, and although interest has increased in recent years, much of this has been specifically for root traits. In contrast, there is still relatively little known about plant storage organs and perennation traits (Klimešová, Martínková, & Ottaviani, 2018), and to our knowledge, our study is the first to examine the response of these traits to frost stress. Knowledge of these responses at the functional trait level with respect to regeneration and perennation will provide a better understanding of how plant communities may respond to changes in soil frost in the coming decades, which is predicted based on decreased snow cover and increased temperature variability (Groffman et al., 2001). Rare taproot bearing species may be at greatest risk and thus possibly an important focus for conservation efforts.

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Chapter 5

5. General discussion

5.1 Major trends

Belowground traits have important consequences for plant and ecosystem function, and yet they are difficult to examine and are thus studied infrequently (Lavorel et al., 2002). In recent years, studies into belowground traits have increased, but these have primarily focused on root traits, little is known about traits pertaining to storage and perennation (Klimešová, Martíncová, & Ottaviani, 2018). Previously, the avoidance of frost stress through soil depth positioning has been examined, but without exploring the potential costs (Boydston et al., 2006), and in other studies the costs of soil depth positioning have been measured without considering the potential benefits (Cavins & Dole, 2002; Qodliyati et al., 2018). Therefore, my studies were the first to explicitly investigate the interaction between the responses of herbaceous plant species to frost stress and the depth of belowground structures, as well as the response of perennation traits to frost. I also extended these studies to enhance the understanding of how plant belowground traits affect stress responses and competition among multiple species within a sample community.

In a broad sense, the positioning of organs deep in the soil conveys many potential benefits (Santamaría & Rodríguez-Gronés, 2002; Vesk, Warton, & Westoby, 2004; Porter et al., 2005; Boydston et al., 2006; Baseggio, Newman, Sollenberger, Fraize, & Obreza, 2015). Buds themselves cost very little to produce (Vesk & Westoby, 2004), but when buds are initiated, the belowground stem investment to reach the surface is a cost. In Chapter 2, I examined bulbs and tubers positioned at different depths, with and without frost stress, and showed that the advantage of being shallow was negated if the risk of stress (i.e. via freezing) was high. Chapter 2 explored this trade-off using species specifically adapted to grow and emerge from deep within the soil, and that utilize structures specially adapted for high storage capability (Kamenetsky, 2013). In Chapter 3, I explored this trade-off with species that were more representative of locally common structures and growth (i.e. rhizomatous hemicryptophytes) (Werner, Bradbury, and Gross, 1980; personal observation). The response to frost was then explored in an intact old field community, and assessed based on relevant recruitment and perennation traits (Chapter 4). In addition, in Chapter 4 I examined plants that had established naturally and grown for multiple years *in situ*.

A further distinction between Chapters 2 and 3 was that while the hemicryptophyte growth form (belowground stem bud bank, primarily from rhizomes; Chapter 3) is common under mesic to moist conditions, the geophyte habit (bulbs and stem tubers; Chapter 2) is most common under low moisture conditions (Qian et al., 2017). In the study region, the geophyte habit is mostly restricted to spring ephemerals in woodlands, where their large storage capacity can be used to overcome frost stress, as well as stress from shade when the tree leaves emerge (Kamenetsky, 2013). The use in Chapter 2 of belowground structures adapted for deep positioning in the soil allowed for a greater depth gradient to be explored than for the species in Chapter 3. In contrast, as described above, Chapter 3 explored a more widespread life form (hemicryptophyte) with greater relevance to the study region. In addition, warm summers and variable spring conditions make shallow buds a more competitive strategy than bulbs and tubers (Grime, 1977), which further explains why rhizomes are a common structure among temperate herbs (Ott & Hartnett, 2014; Komac, Pladevall, Peñuelas, Conesa, & Domènech, 2015; Klimešová, 2018). Rhizomes also provide a high concentration of buds at the surface through their horizontal growth, and they can support a great number of stems because of the connectivity between these structures (Latzel et al., 2011; Liu et al., 2015). Compared to the rhizome fragments used in Chapter 3, Chapter 4 provided a more realistic understanding of stress responses for hemicryptophyte species, because these structures were able to mature for multiple years, which would have altered their structural qualities (Klimešová, & Klimeš, 1996; Klimeš, Klimešová, & Čížková, 1999). Rhizomes from ramets also remain connected to one another and the parent plant, which allows the whole plant to mediate its response, and is thus more realistic than analyzing the responses of unconnected rhizome fragments (Elgersma et al., 2015).

In general, soil and litter modulated the frost responses for the species studied in Chapter 3, and depth was a cost with respect to reduced growth or survival when this stress was removed, but these responses were more complicated and nuanced than the clearer trade-off demonstrated in Chapter 2. In Chapter 4, where the importance of litter was examined *in situ*, I identified a greater sensitivity of rhizomes to litter cover than other organs of perennation. Because of the proximity of rhizomes to the surface, their local edaphic conditions are more strongly affected by factors other than just soil depth, and the insulation properties of snow and litter become more important (Raunkiær, 1934; Komac et al. 2015). In addition, plant recruitment is not limited to clonal structures; it includes seed and non-clonal organs of perennation (Klimešová, Tackenberg, & Herben, 2015). For all of these structures, in addition to litter providing insultation from cold air, it may be important for moisture retention (Baseggio, Newman, Sollenberger, Fraisse, & Obreza, 2015), which can provide a benefit during the growing season, and potentially counteract the negative shading effects of litter at that time.

5.2 Future directions

Soil frost is an important and yet often underacknowledged factor in temperate systems (Kreyling, 2010; Henry et al., 2018). With respect to frost avoidance vs. soil depth positioning trade-offs, further study is needed to examine how responses may vary with differences in soil type and soil qualities (e.g. clay content and pore size), or how they are affected by different moisture regimes (Barnes, 2010). Moreover, all of the plant species I studied naturalize in southern Ontario, and it would be informative to expand knowledge of frost avoidance trade-offs to other regions and habitats, such as forest, alpine, and tundra. Even within temperate regions, there can be substantial variation in freezing responses and freezing exposure along latitudinal or elevational gradients (Michalski, Malyshev, & Kreyling, 2017; Henry et al. 2018) that could have an important bearing of frost avoidance trade-offs. In addition, Chapters 2 and 3 only detailed the responses of thirteen species, whereas a more comprehensive study focused on variation within and among phylogenetic lineages could further broaden our understanding of frost avoidance trade-offs.

My experiments also only examined responses to soil depth positioning and frost stress over individual winters followed by a single growing season, whereas it would be informative to examine responses to repeated freezing events or legacy effects from a single event in the following years (Blume-Werry, Kreyling, Laudon, & Milbau, 2016). For example, some species decreased or altered tuber and rhizome placement in response to freezing stress, which could limit future lateral spread, or cause possible crowding or a lack of stem bud recruitment. Allocation to storage organs also decreased in some species during the single season of observation, and this response could cause possible detrimental legacy effects beyond a year after the initial stress. While such legacy effects may be long-lasting, alternatively, there may be complete recovery by the next growing season (Blume-Werry et al., 2016); these responses also may be species or structure dependent. Repeated freezing may cause either acclimation or cumulative weakening of plants (Kong & Henry, 2017), and responses to a single or repeated events may be further altered by the timing of the events (Malyshev & Henry, 2012; Kong & Henry, 2017). In particular, if a frost event occurs at a time when vulnerable reproductive structures are present, this could decrease sexual reproduction and recombination (Vallejo-Marín, Dorken, & Barrett, 2010) and thus decrease genetic diversity.

The variation in response to litter cover in Chapter 3, with litter functioning as insulation or as a barrier depending upon the season and species, is another area that merits further study. Future studies should investigate the effects of litter cover with varying decomposition rates, carbon:nitrogen ratios, and qualities as insulation and as a barrier. For example, the benefit of litter (Chapter 4) to rhizomatous species may have been due to the insulation of these structures by the litter directly or by the capture and retention of snow by the litter (Sharratt, 2002; Wang et al., 2017). There also is much to learn about the effect of the structural qualities of litter, such as how stem components and plant architecture (e.g. branching type) may trap snow and retain heat. Such changes in soil insulation caused by litter would be altered by changes in the relative dominance of plant species in response to climate change.

The variation in response and frost susceptibility based on organ of perennation, and especially the resilience of plants in the rootstock category demonstrated in Chapter 4, is an area where much more can be learned. The age of a structure alters its composition (Schweingruber & Poschlod, 2005) and carbohydrate storage (Klimeš, Klimešová, & Čížková, 1999). Therefore, an examination of frost response based on structure age, woodiness, and other stem or root characteristics could shed further light on the variation in responses among different structures I observed or even be used to elucidate trends that my studies were not able to identify, such as with respect to stem composition. Specifically, while limitations of woody plant tolerance to freezing stress are usually caused by xylem conduit cavitation (Sperry & Sullivan, 1992; Wisnieski, Willick, & Gusta 2017), it is unknown what benefits or costs of woodiness may be conveyed to the belowground structures of herbaceous plants.

There also is much room to study depth, storage and perennation traits in response to stresses and gradients other than soil frost (Klimešová, Martíncová, and Ottaviani, 2018). There are many advantages to greater soil depth (Raunkiær, 1934; Porter, Dasgupta, & Johnson, 2005) and these protection versus investment trade-offs have also yet to be explored. Herbivory, especially of belowground storage organs (Santamaría & Rodríguez-Gronés, 2002), is an example of a stress that can be alleviated with depth, but would still theoretically convey a cost with respect to increased belowground stem. The increased reliance on bulb buds with decreased moisture (Qian et al., 2017), as well as increased size and woodiness of perennial structures (Appezzato-da-Glória et al., 2008), also is an interesting candidate area for further study. In addition, future study should explore investment costs associated with recruitment from greater depths when multiple stressors are present, and especially those associated with the growing season, such as fire (Vesk, Warton, &Westoby, 2004) and moisture (Baseggio, Newman, Sollenberger, Fraize, & Obreza, 2015).

Many plants can recruit buds from along a soil depth gradient (Ott, Klimešová, & Hartnett, 2019); for example, *Anemone canadensis* features abundant branching and angled rhizomes. Future studies should assess the location and degree of bud mortality (using appropriate staining techniques to identify live or dead tissue) (Livingston III & Tuong, 2013) along soil depth gradients. Plant recruitment efforts also can be examined through careful excavation to determine the location of recruitment and the level of lateral spread based on the location of bud initiation. Plants also have the ability to change their location in the soil by growing in different directions (Ott, Klimešová, & Hartnett, 2019) or through the use of contractile roots (Putz & Sukkau, 1995); it would be informative to explore how organ positioning is changed based on initial placement, and as a frost stress response after a single event (short or long term) or multiple events. Furthermore, there is much to be learned about the storage organs of herbaceous plants. The presence of bulbs is subject to phylogenetic constraints (Li, Zhou, He, & Wei, 2012; Meerow, 2013), but like root buds (Bartušková, Malíková, & Klimešová, 2017), the presence of stem tubers and other tuberously thickened structures occurs in many plant families and genera. It is unknown what stressors and gradients may be linked to this adaptation or of any phylogenetic limitations.

5.3 Significance

The responses of plants to frost stress, and the interaction of the latter with soil depth positioning and perennation traits, will be of particular relevance in northern temperate regions in the coming decades, because snow cover is anticipated to decrease with climate warming (Groffman et al. 2001), which is expected to increase the severity of freezing experienced by important belowground overwintering structures (Zhang, 2005). All species studied were acclimated to cold winters, yet all but two species discussed in Chapters 2 and 3 incurred damage and mortality upon exposure to temperatures no greater than -10 °C. This study highlights the vulnerability of plant belowground structures to frost and the importance of the adaptation of spatial frost avoidance. The use of functional trait groups allows for the identification of frost-vulnerable groups without specifically studying a species (Lavorel et al. 2007). The functional groups used in Chapter 4 can be applied to many different species and systems. From this study, I found that taprooted species may be of greatest concern under increased soil freezing, but any hemicryptophyte species with peak growth in the spring may be especially vulnerable.

Species that produced new rhizome growth during the studies (Chapters 2 and 3) did so without increasing investment in rhizomes with greater depth, which would increase the amount of vertical space traveled and decrease the horizontal space, thus limiting lateral spread. A decrease in spread also may decrease the number of buds initiated and stems present during the growing season. Burial of plants may be an option to decrease the growth and spread of invasive or weedy species when other methods may be too destructive. Burial can delay emergence and allow vulnerable species to emerge earlier than weedy competitors (Dalbato, Alfredsson, Karlsson, & Andersson, 2014) and deplete the weeds' carbohydrate reserves (Klimeš, Klimešová, & Obornová, 1993). Snow and litter removal also may be used to deter the growth of undesirable species; this method has been used to control unharvested potato tubers (Yazaki et al. 2013). Burial also can be a way of protecting vulnerable target species (Santamaría & Rodríguez-Gronés, 2002) that may be highly sensitive to frost.

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Appendices

Appendix 1. Species and functional groups for the old field experiment

Species	Regeneration	Organ of perennation	Life form
Ambrosia artemisiifolia	germinate	none	therophyte
Asclepias syriaca	sprout	root buds	geophyte
Carduus nutans	sprout/germinate	taproot/none	hemicryptophyte/therophyte
Cirsium arvense	sprout	root buds	geophyte
Cirsium vulgare	sprout/germinate	taproot/none	hemicryptophyte/therophyte
Cornus sericia	sprout	aboveground	phanerophyte
Dactylis glomerata	sprout	rhizome	hemicryptophyte
Daucus carota	germinate	none	therophyte
Elymus repens	sprout	rhizome	geophyte
Epilobium ciliatum	sprout	other	hemicryptophyte
Erigeron anuus	germinate	none	therophyte
Erigeron pulchellus	sprout	rhizome	hemicryptophyte
Fragaria virginiana	sprout	rhizome	hemicryptophyte
germinating species	germinate	none	therophyte
Geum canadense	sprout	other	hemicryptophyte
Geum urbanum	sprout	rhizome	hemicryptophyte
grass species	sprout	rhizome	hemicryptophyte
Holosteum umbellatum	germinate	none	therophyte
Leucanthemum vulgare	sprout	rhizome	hemicryptophyte
Medicago lupulina	germinate	none	therophyte
Onopardum acanthium	sprout/germinate	taproot/none	hemicryptophyte/therophyte
Plantago major	sprout	rootstock	hemicryptophyte
Poa compressa	sprout	rhizome	hemicryptophyte
Poa pratensis	sprout	rhizome	hemicryptophyte
Prunella vulgaris	sprout	rootstock	hemicryptophyte
Rubus sp.	sprout	aboveground	phanerophyte
Solidago spp.	sprout	rhizome	hemicryptophyte
Sonchus arvensis	sprout	root buds	geophyte
sprouting species	sprout	taproot	hemicryptophyte
Symphyotrichum ericoides	sprout	rootstock	hemicryptophyte
Symphyotrichum lanceolatum	sprout	rootstock	hemicryptophyte
Symphyotrichum novae-angliae	sprout	rootstock	hemicryptophyte
Symphyotrichum pilosum	sprout	rootstock	hemicryptophyte
Syphyotrichum lateriflorum	sprout	rootstock	hemicryptophyte
Taraxacum officinale	sprout	taproot	hemicryptophyte

Trifolium pratense	sprout	taproot	hemicryptophyte
Trifolium repens	germinate	none	therophyte
Veronica persica	germinate	none	therophyte

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