



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

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Received: 25 April 2019 / Accepted: 15 August 2019 / Published online: 28 August 2019
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Abstract

Aims The positioning of bulbs and stem tubers deep in soil can decrease frost exposure, yet it can come at the cost of increased investment to reach the surface, and delayed emergence. We explored the strength and generality of this trade-off for a range of herbaceous, temperate species.

Methods To isolate the direct effects of soil depth on plant growth and survival from the effects of variation in frost exposure, one set of plants buried over winter at three depths experienced either ambient or increased frost (via snow removal), another set sheltered from winter frost stress was buried at three depths in spring, and a third set was exposed to freezing under controlled conditions.

Results Growth generally increased with organ soil depth under increased frost. However, for many species, spring-planted organs exhibited a trade-off of decreased aboveground leaf biomass with greater organ depth. Increased freezing exposure alone generally decreased survival, with sub-lethal effects (particularly decreased leaf biomass) observed for some species.

Conclusions Our results highlight that optimal bud depth is influenced by trade-offs between frost avoidance and plant growth. The balance of this trade-off may shift in coming decades with reduced snow cover and resulting increases in soil frost exposure.

Keywords Belowground · Clonal · Depth · Frost avoidance · Geophyte · Herbaceous

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 et al. 2004). Herbaceous species therefore often overwinter as seeds or belowground structures that can avoid or otherwise tolerate the frost, and perennial species then re-sprout when conditions improve (Klimešová et al. 2015).

Freezing can be tolerated through physiological mechanisms ((e.g. via antifreeze proteins and vitrification (Pearce 2001), and alcohol dehydrogenase (ADH) (Davik et al. 2013))), whereas frost avoidance is achieved through spatial presence and temporal activity, accomplished respectively through the positioning of organs and tissues within the soil profile, or by avoiding growth and bud exposure during periods of frost risk (Raunkiaer 1934; Komac et al. 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 (Raunkiaer

Responsible Editor: W Richard Whalley.

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1934; Sharratt 2002). In addition, snow cover provides insulation from freezing temperatures for plants that overwinter at or below the soil surface (Bertrand and Castonguay 2003; Komac et al. 2015). Reductions in snow cover can harm plants that depend upon this insulation, resulting in invasion and shifts in vegetation type (Simons et al. 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 et al. 2006), fire (Vesk and Westoby 2004), and drought (Baseggio et al. 2015)) and biotic origin (e.g. herbivory (Santamaría and Rodríguez-Gronés 2002) and pathogens (Porter et al. 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 et al. 2009). Therefore, while in many stressful environments, deep bud depth in the soil may be advantageous as part of the stress tolerance strategy, and in low-stress environments, shallow bud depth 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 (Raunkjær 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 and Dole 2002; Qodliyat and Supriyono 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 focussed on mortality (de Melo Peixoto et al. 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 (Weihs and Karlsson 2002; Malyshev

and 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.

We 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, we planted a set of bulbs at different depths in the field over winter, 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, we 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. We 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, we conducted two additional experiments. First, we incubated a set of bulbs over winter at a mild temperature (1 °C) in growth chambers, then transplanted these bulbs in the spring to different soil depths in the field. Given that these plants did not experience freezing damage, we predicted that increased planting depth would be negatively correlated with subsequent growth. Second, we 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.

Materials and methods

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 on level ground. The soil was characterized as a Bryanston silt loam, which is a Brunisolic Gray Brown

Luvisol (Hagerty and Kingston 1992), and it had a mean pH of 7.5. . All seven study species were selected because they naturalize in the region, produce specialized clonal storage organs, and can grow from deep within the soil profile. Three species are native (*Allium cernuum* Roth, *Apios Americana* Medikus, and *Helianthus tuberosus* Linnaeus) and four are exotic ornamental species (*Crocus vernus* (Linnaeus) Hill (flower record cultivar), *Muscari armeniacum* Leichtlin ex Baker, *Narcissus pseudonarcissus* Linnaeus (yellow trumpet cultivar), and *Scilla sibirica* Haworth) (USDA 2019). The native species are present throughout the United States of America and Canada, although *Apios Americana* is limited to the east (USDA 2019). *Allium cernuum* is listed as threatened in three states (USDA 2019). *Helianthus tuberosus* can be considered weedy in both the United States of America and Canada (Swanton et al. 1992; USDA 2019). The exotic species have been introduced throughout the eastern United States of America and Canada, except for *Muscari armeniacum* which is only present in three states and the province of Ontario (USDA 2019). The exotic species are native to Europe (*Crocus vernus* and *Narcissus pseudonarcissus*), or both Europe and Asia (*Muscari armeniacum* and *Scilla sibirica*) (Native Plant Trust 2019). *Muscari armeniacum*, *Narcissus pseudonarcissus*, *Scilla sibirica* can both spread to disturbed and anthropogenic habitats (Native Plant Trust 2019; personal observation). Both cultivars selected are commonly present in local gardens and landscaping. 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) (Raunkiaer 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 and Stuefer 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 (Raunkiaer 1934; Suzuki and Stuefer 1999; Vallejo-Marín et al. 2010).

Overwintering field experiment

Clonal organs were weighed and planted 3–4 November 2016 at one of three soil depths (2, 5 or 15 cm). We chose these depths to bracket the typical range of soil burial depths for bulbs of the study species and to expose the bulbs to a range of different soil temperatures. The 2 cm and 5 cm depths are on the shallow end of the range observed for these species and the 15 cm depth was selected to extend the range of the natural growing depths (Rockwell and Grayson 1953; Okubo and Sochacki 2013; Breck’s 2013; and field observations) as a means of examining the protection vs. delayed emergence trade-off, while at the same time allowing for emergence (which was facilitated by the presence of storage-adapted structures). 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.

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, then in the spring the propagules were weighed and planted in a common garden along with propagules from the minimum freezing temperature experiment (100 cm × 160 cm, one species per plot and 2 m between plots) 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. For *Crocus vernus*, unusually warm spring temperatures caused all plants to eventually die or to cease growth, but initial growth data were recorded.

Minimum freezing temperature experiment

A different set of propagules (six replicates per treatment) 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 3 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 along with propagules from the spring clonal organ depth experiment, 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 and Grayson 1953; Okubo and Sochacki 2013; Breck's 2013; and field observations).

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, a principal component analysis (PCA) was performed to reveal the interrelationships between the response variables and to provide

a global analysis of the treatment effects averaged over all species. Early, mid- and final height, leaf mass, belowground stem and clonal organ mass were the factors included in the PCA, and each factor was standardized within species prior to the PCA to remove the effect of variation in size among species. Subsequently, 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 covariate, were analysed using a general linear model. The data for the minimum temperature and spring clonal organ depth experiments were analysed 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. *P* values less than 0.05 were considered significant. Analyses were conducted using JMP version 13 (SAS Institute).

Results

Overwintering field experiment

Frost severity and freeze thaw event frequency increased both with decreasing soil depth and with snow removal (Table 1). While PCA axis 1 was associated primarily with plant size (i.e. all factors loaded positively and approximately equally for this axis), PCA axis 2 was associated with a trade-off between belowground stem and early height (Fig. 1a). Averaged over all species, the treatments associated with the warmest and most stable overwinter soil temperatures (15 cm depth and ambient snow) corresponded with the highest PCA axis 1 loadings (i.e. high plant biomass), whereas snow removal and 2 cm burial depth had the lowest PCA axis 1 loadings (Fig. 1b). The highest PCA axis 2 loadings (i.e. high belowground stem allocation) corresponded with the 15 cm burial depth, whereas the lowest PCA axis 2 loadings (i.e. high early height) corresponded with the 2 and 5 cm burial depths (Fig. 1b).

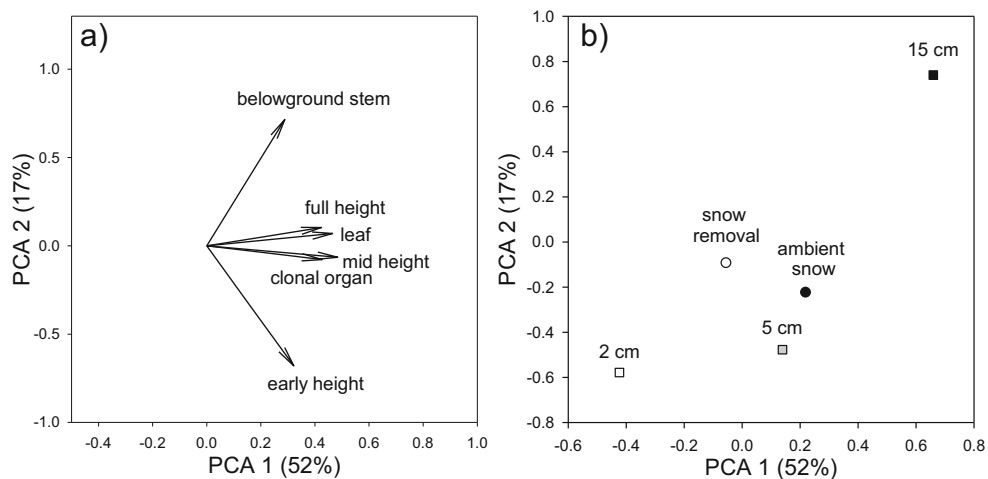
For the within species GLM analyses, all species increased in height with increased clonal organ depth (Fig 2; effect not significant for *Allium cernuum*), but total biomass was not affected significantly by clonal organ depth, with the exception of *Crocus vernus*, which exhibited increased growth with increased depth

Table 1 Temperatures and freeze thaw cycles under the different depth and snow removal treatments. Freeze thaw cycles were defined as any drop below 0°C followed by an increase to above 0°C

Depth	Control			Snow Removal		
	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). Belowground stem biomass increased significantly with soil depth for all species except *Helianthus tuberosus* (Fig. 2, Table 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).

**Fig. 1** Principal component analysis (PCA) ordination axes 1 and 2, displaying a) factor loadings (arrows) and b) treatment loadings averaged over all species (trait data standardized within species

prior to the analysis). The percent variances explained by each PCA axis are displayed in parentheses in the axis labels

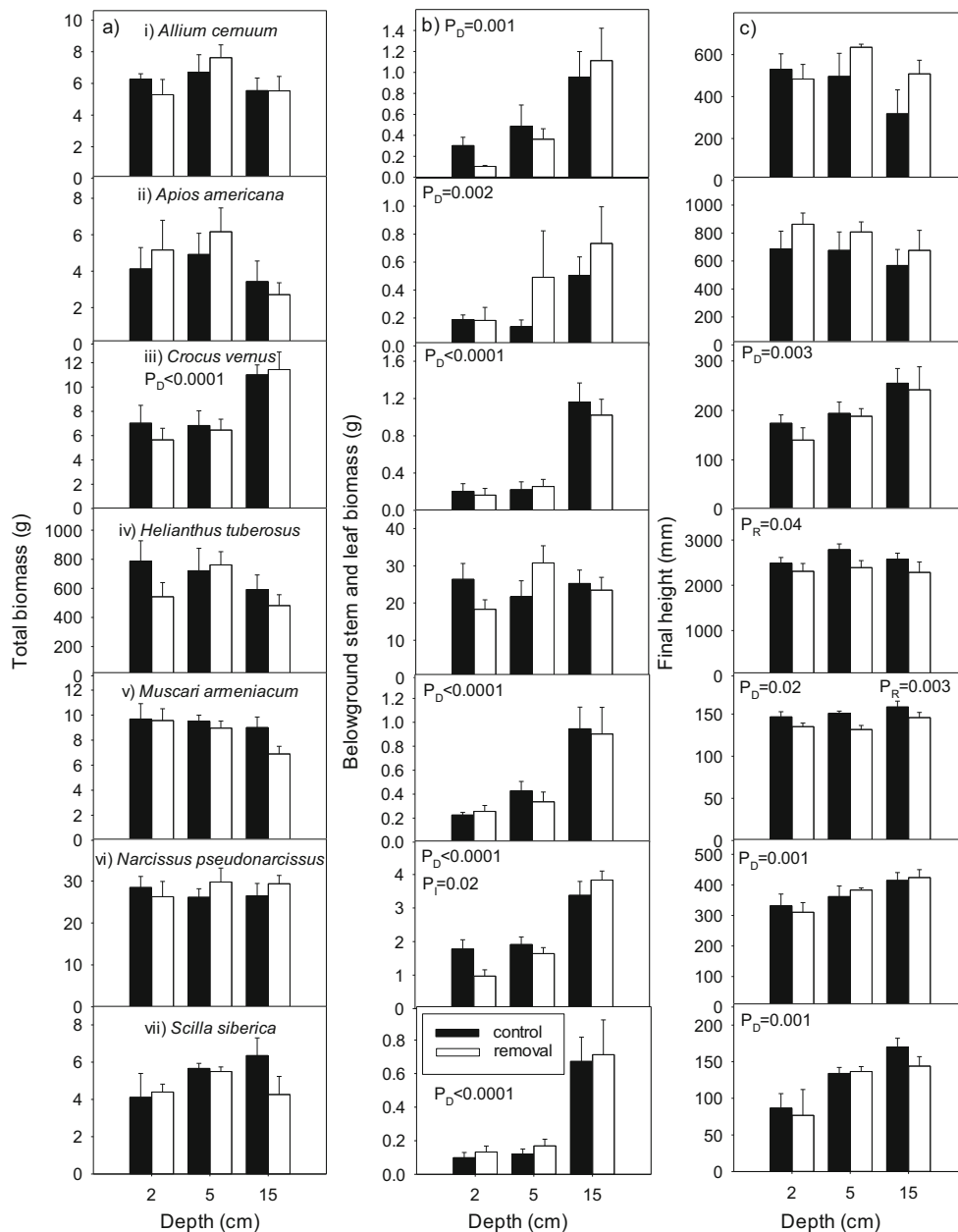


Fig. 2 Mean values and standard error for (a) total biomass, (b) belowground stem and leaf biomass, and (c) final height in the overwintering field experiment. Subscripts are for effects of snow removal (R), depth (D), and snow removal*depth interaction (I)

Spring soil depth experiment

Increased spring clonal organ depth decreased clonal organ size and total biomass in *Allium cernuum* and *Muscari armeniacum* (Table 3). *Apios americana* and *Muscari armeniacum* exhibited increased belowground stem biomass with increasing clonal organ depth (Fig. 3), and no effect

was present for *Allium cernuum* or *Helianthus 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 *P* values for the GLM analyses 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. For survivor #, c = control, sr = snow removal. **P*<0.05 ***P*<0.01 ****P*<0.001

	<i>Allium cernuum</i>	<i>Apios americana</i>	<i>Crocus vernus</i>	<i>Helianthus tuberosus</i>	<i>Muscari armeniacum</i>	<i>Narcissus pseudonarcissus</i>	<i>Scilla siberica</i>
Depth Response							
survivors (out of 12) (2, 5, 15 cm)	(10, 11, 11)	(12, 10, 10)	(10, 11, 12)	(all)	(all)	(all)	(9, 8, 11)
early height	0.97	0.47	0.002**	0.001***	0.001***	0.71	0.33
mid height	0.16	0.03*	< 0.0001***	0.004***	0.88	*0.03*	0.01*
full height	0.12	0.19	0.003**	0.86	0.02*	0.001***	0.001**
leaf	0.04*	0.77	0.01**	0.35	0.84	0.12	0.01**
belowground stem	0.001***	0.002**	< 0.0001***	0.72	< 0.0001***	< 0.0001***	< 0.0001***
clonal organ	0.10	0.85	< 0.0001***	0.09	0.01*	0.83	0.29
total aboveground	0.11	0.10	0.01**	0.37	0.07	0.18	0.35
total belowground	0.61	0.26	< 0.0001***	0.09	0.07	0.43	0.42
total biomass	0.39	0.41	< 0.0001***	0.15	0.17	0.83	0.37
Snow Removal Response							
survivors (out of 24) (c, sr)	(20, 24)	(23, 21)	(22, 23)	(all)	(all)	(all)	(23, 17)
early height	0.64	0.45	0.61	0.56	0.11	0.04*	0.29
mid height	0.59	0.78	0.10	0.87	0.001***	0.30	0.29
full height	0.20	0.19	0.39	0.04*	0.003**	0.80	0.41
leaf	0.63	0.20	0.70	0.67	0.88	0.84	0.59
belowground stem	0.78	0.15	0.60	0.97	0.94	0.13	0.74
clonal organ	0.82	0.25	0.50	0.11	0.61	0.42	0.80
total aboveground	0.68	0.67	0.70	0.65	0.92	0.91	0.82
total belowground	0.84	0.69	0.47	0.15	0.45	0.50	0.71
total biomass	0.79	0.97	0.49	0.28	0.46	0.86	0.70
Depth* <i>Snow</i> Removal Interaction							
early height	0.12	0.63	0.15	0.36	0.78	0.43	0.37
mid height	0.12	0.02*	0.23	0.23	0.36	0.33	0.10
full height	0.19	0.78	0.47	0.87	0.95	0.75	0.46
leaf	0.77	0.30	0.06	0.77	0.10	0.41	0.28
belowground stem	0.71	0.96	0.58	0.97	0.64	0.02*	0.94
clonal organ	0.70	0.88	0.15	0.54	0.24	0.66	0.62
total aboveground	0.87	0.99	0.06	0.76	0.62	0.22	0.23
total belowground	0.60	0.55	0.19	0.60	0.23	0.88	0.20
total biomass	0.65	0.66	0.13	0.88	0.11	0.94	0.19

Table 3 *P* values for the GLM analyses of morphological measurements for the depth treatment in the spring planting 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

	<i>Allium cernuum</i>	<i>Apios americana</i>	<i>Helianthus tuberosus</i>	<i>Muscari armeniacum</i>
survivors (6) (2 cm, 5 cm, 15 cm)	(5, 6, 6)	(6, 6, 5)	(all)	(all)
height	0.35	0.99	0.86	0.97
leaf	0.13	0.23	0.74	0.05
belowground stem	0.64	0.0002***	0.98	0.001***
clonal organ	0.01**	0.2	0.87	0.009**
total aboveground	0.17	0.24	0.74	0.05
total belowground	0.04*	0.07	0.84	0.03*
total biomass	0.04*	0.08	0.79	0.02*

Minimum temperature experiment

While survival and growth responses to minimum freezing temperature varied among species (Table 4), all species except *Helianthus tuberosus* experienced increased mortality with increased freezing severity (Fig. 4). *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. 4).

Discussion

Overall, while the responses to freezing and depth varied among species, there was a general trend of a trade-

Fig. 3 Mean values and standard error for (a) total biomass, (b) belowground stem and leaf biomass, and (c) clonal organ in the spring planting depth experiment

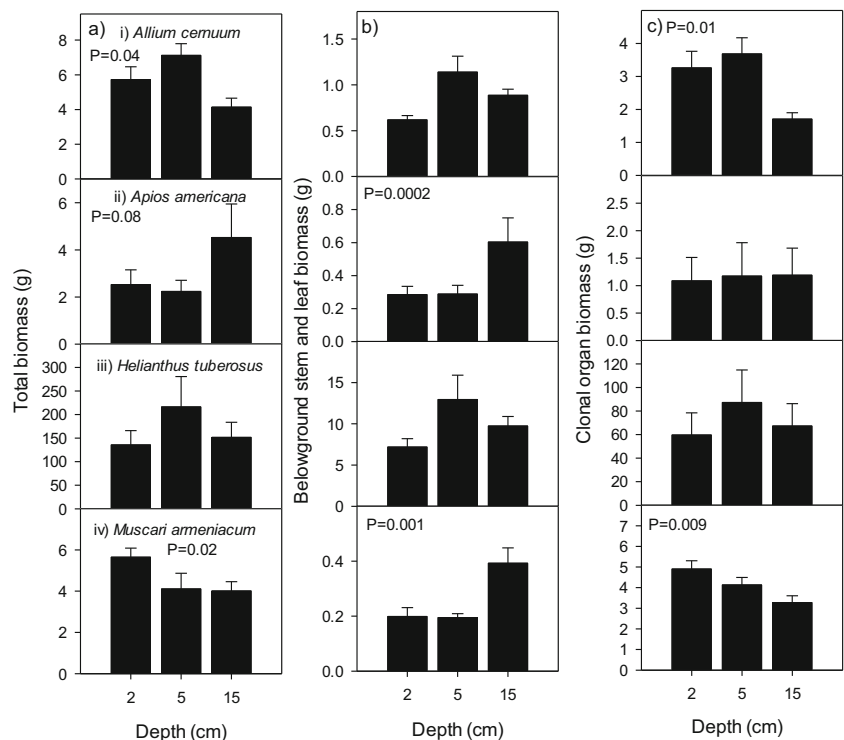


Table 4 *P* value responses for the GLM analyses of morphological measurements for the minimum temperature experiment. Effects were in the direction of decreased biomass and survival withdecreasing temperature. NA indicates a lack of data. *Helianthus tuberosus* did not experience mortality

	<i>Allium cernuum</i>	<i>Apios americana</i>	<i>Crocus vernus</i>	<i>Helianthus tuberosus</i>	<i>Muscari armeniacum</i>	<i>Narcissus pseudonarcissus</i>
early height	0.69	0.73	0.92	0.34	0.93	0.01*
mid height	0.08	NA	0.74	0.45	NA	< 0.0001***
full height	0.76	0.99	0.1	0.81	0.01**	0.01*
leaf	0.03*	0.4	0.14	0.7	0.0004***	0.03*
belowground stem	0.52	0.17	0.6	0.18	0.02*	0.15
clonal organ	0.68	0.76	0.15	0.93	0.02*	0.06
total aboveground	0.38	0.43	0.14	0.69	< 0.0001***	0.02*
total belowground	0.8	0.92	0.16	0.94	0.01**	0.03*
total biomass	0.38	0.67	0.13	0.84	0.002**	0.002**
survival	< 0.0001***	0.02*	0.0004***	NA	0.26	0.002**

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 Boydston et al. (2006), who reported the avoidance of frost stress by the deep soil placement of tubers in *Solanum tuberosum*. As predicted, increased growing depth alone was a cost in the form of increased belowground vertical stem and leaf tissue, but this cost could not be detected when the exposure to freezing temperatures increased near the soil surface. Although the snow removal treatment increased frost exposure, even under ambient conditions there was a response of decreased growth for the shallow burial depth for most species.

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 and 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 and Minden 2015), 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 present and green through winter, which implies 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

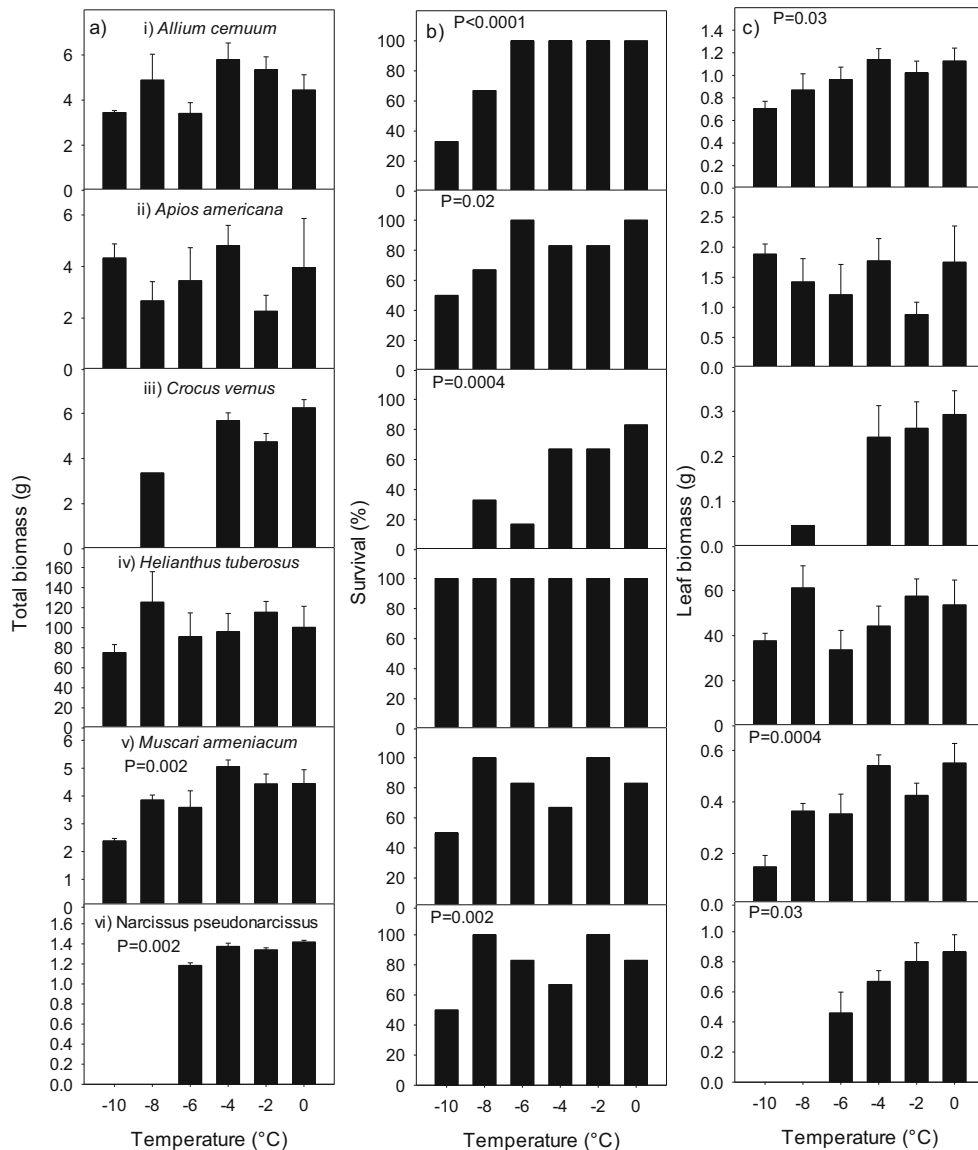


Fig. 4 Mean values and standard error for (a) total biomass, (b) percent survival, and (c) belowground stem and leaf biomass in the minimum temperature experiment

from its vertical belowground stem allows it to utilize this investment for storage, and it 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 and 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, although 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 and 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. 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. 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). Biomass measurements reflect carbon input and allocation, but do not fully explain differences in tissue structure and nutrient content (e.g. nitrogen). Changes in nutrient acquisition in response to freezing stress may occur as a result of root damage decreasing foraging ability (Wisniewski et al. 2017), and this is an interesting area of study for future research. Although the soils of the overwintering plots were equally saturated with water

after the spring thaw, it is possible that differences in soil moisture with depth could alter soil freezing damage (Barnes 2010). However, the treatment likely to have experienced the highest soil moisture (15 cm) also experienced the least intense freezing, and in general the soil moisture from 0–15 cm soil depth was high throughout winter at the study site.

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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