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LETTER

Deepened winter snow increases stem growth and alters stem $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in evergreen dwarf shrub *Cassiope tetragona* in high-arctic Svalbard tundra

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Daan Blok¹, Stef Weijers², Jeffrey M Welker³, Elisabeth J Cooper⁴, Anders Michelsen^{1,5}, Jörg Löffler² and Bo Elberling¹¹ Center for Permafrost (CENPERM), Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark² Department of Geography, University of Bonn, Bonn, Germany³ Department of Biological Sciences, University of Alaska, Anchorage, AK, USA⁴ Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway⁵ Department of Biology, University of Copenhagen, Copenhagen, DenmarkE-mail: be@ign.ku.dk**Keywords:** *Cassiope tetragona*, climate change, stable isotope, High Arctic, snowSupplementary material for this article is available [online](#)**Abstract**

Deeper winter snow is hypothesized to favor shrub growth and may partly explain the shrub expansion observed in many parts of the arctic during the last decades, potentially triggering biophysical feedbacks including regional warming and permafrost thawing. We experimentally tested the effects of winter snow depth on shrub growth and ecophysiology by measuring stem length and stem hydrogen ($\delta^2\text{H}$), carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$) and oxygen ($\delta^{18}\text{O}$) isotopic composition of the circumarctic evergreen dwarf shrub *Cassiope tetragona* growing in high-arctic Svalbard, Norway. Measurements were carried out on *C. tetragona* individuals sampled from three tundra sites, each representing a distinct moisture regime (dry heath, meadow, moist meadow). Individuals were sampled along gradients of experimentally manipulated winter snow depths in a six-year old snow fence experiment: in ambient (c. 20 cm), medium (c. 100 cm), and deep snow (c. 150 cm) plots. The deep-snow treatment consistently and significantly increased *C. tetragona* growth during the 2008–2011 manipulation period compared to growth in ambient-snow plots. Stem $\delta^{15}\text{N}$ and stem N concentration values were significantly higher in deep-snow individuals compared to individuals growing in ambient-snow plots during the course of the experiment, suggesting that soil N-availability was increased in deep-snow plots as a result of increased soil winter N mineralization. Although inter-annual growing season-precipitation $\delta^2\text{H}$ and stem $\delta^2\text{H}$ records closely matched, snow depth did not change stem $\delta^2\text{H}$ or $\delta^{18}\text{O}$, suggesting that water source usage by *C. tetragona* was unaltered. Instead, the deep insulating snowpack may have protected *C. tetragona* shrubs against frost damage, potentially compensating the detrimental effects of a shortened growing season and associated phenological delay on growth. Our findings suggest that an increase in winter precipitation in the High Arctic, as predicted by climate models, has the potential to alter the growth and ecophysiology of evergreen shrub *C. tetragona* through changes in plant mineral nutrition and frost damage protection.

1. Introduction

The Arctic has generally warmed during the last decades, especially during the winter period (Serreze and Barry 2011). Winter precipitation in the Arctic is expected to increase during this century as a

consequence of higher atmospheric temperatures, sea-ice loss and associated increasing air humidity, especially in the High Arctic (IPCC 2013, Bintanja and Selten 2014). Changes in winter precipitation patterns may affect vegetation composition in arctic tundra. During the last decades, shrub expansion has been

observed across many arctic and alpine tundra sites (Tape *et al* 2006, Callaghan *et al* 2011, Myers-Smith *et al* 2011, Elmendorf *et al* 2012, Frost and Epstein 2014). Snow trapping by shrubs, resulting in higher soil winter temperatures and potentially enhanced plant mineral nutrition, has been suggested as a possible mechanism for shrub expansion in the Arctic (Sturm *et al* 2005). This snow-shrub hypothesis is supported in part by recent dendroecological studies, indicating a link between winter precipitation/snow cover and shrub growth, although the direction and magnitude of the responses differed from positive (Hallinger *et al* 2010) to negative (Schmidt *et al* 2010, Mallik *et al* 2011). We studied the growth response and underlying physiological changes in *Cassiope tetragona* (Arctic bell heather) to experimental snow depth changes in high-arctic Svalbard tundra to improve our mechanistic understanding of the controls of snow depth on shrub growth. *Cassiope tetragona* is an evergreen shrub species with a circumarctic distribution and great latitudinal spread (Callaghan *et al* 1989, Rayback and Henry 2005), and is dominant in hemi-prostrate dwarf shrub tundra regions (Walker *et al* 2005). Therefore, the potential impacts of changing patterns of snow coverage on *C. tetragona* growth could be of particular importance for high-arctic tundra ecosystem functioning now and in the future.

An increase in winter snow precipitation may lead to higher winter soil temperatures due to the insulating capacity of snow, resulting in higher microbial activity and N mineralization rates (Schimel *et al* 2004). This may increase plant N availability during the growing season (Natali *et al* 2012) and increase leaf N concentrations (Welker *et al* 2005a). Experimental studies have shown that mainly shrubs may benefit from higher nutrient availability by growing denser and taller (Shaver and Chapin 1980, Bret-Harte *et al* 2004). However, the timing of nutrient-release by winter soil mineralization may not coincide with the start of the plant growing season. Thus, deeper snow may not lead to enhanced nutrient availability for shrubs in all cases (Buckeridge *et al* 2010, Vankoughnett and Grogan 2014).

Deeper snow can also increase the availability of water during the growing season (Morgner *et al* 2010, Leffler and Welker 2013), unless snow melt water runs off the frozen surface during early spring before soil thaw commences (Löffler 2005). Ehleringer and Dawson (1992) suggested that plants growing in areas with low summer precipitation inputs, as in high-arctic Svalbard, may rely mostly on water from deeper soil layers to ensure stable provision of water and maintenance of growth during dry summers. In the High Arctic, growth of *C. tetragona* is generally restricted to winter snow-covered areas, which apart from being a strategy to prevent frost damage during the extreme cold winter (Billings and Mooney 1968), may prevent desiccation during the growing season (Callaghan

et al 1989). A summer water manipulation study in high-arctic Svalbard did however fail to observe an effect of additional water supply on *C. tetragona* stem length growth (Weijers *et al* 2013a), in contrast to the positive growth response to increased summer precipitation observed for *Betula nana* in the Siberian tundra (Blok *et al* 2011, Keuper *et al* 2012).

Finally, deeper snow may also affect growth positively by providing protection against frost after episodic winter warming events (Bokhorst *et al* 2011), as *C. tetragona* growth has been found to be negatively affected by winter warming events (Weijers *et al* 2012). At the same time, increased snow depth may result in a delay of the growing season onset (Borner *et al* 2008), potentially reducing *C. tetragona* growth (Mallik *et al* 2011). The complexities of snow depth effects on *C. tetragona* growth thus appear to be operating across a range of processes that may be both indirect by altering the ecohydrological and nutritional conditions for growth, as well as directly via physical protection by snow from harsh winter climate conditions (Cooper 2014).

Despite the correlative evidence for a snow depth-shrub growth linkage, an experimental approach is needed to assess the direct effects of winter snow depth on shrub growth. Moreover, to unravel the underlying mechanisms, it is essential to go beyond analyses of growth metrics and utilize information on physiological responses to snow cover changes, as contained in stable isotope data (McCarroll and Loader 2004, Pattison and Welker 2014). To estimate the impacts of snow depth changes on *C. tetragona* growth, leaf gas exchange, nitrogen (N) acquisition and water source usage, we manipulated winter snow depth in a field experiment conducted in high-arctic Svalbard and measured *C. tetragona* stem length growth, stem carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$) isotopes. *Cassiope tetragona* individuals were sampled along winter snow depth gradients in a six-year old snow fence experiment: in ambient (*c.* 20 cm), medium (*c.* 100 cm), and deep snow (*c.* 150 cm) plots located in three tundra sites, each representing a distinct moisture regime (dry heath, meadow, moist meadow). The aim of our study was to improve the mechanistic understanding of the controls of winter snow depth on *C. tetragona* growth and ecophysiology in a high-arctic tundra ecosystem. We tested our hypotheses that *C. tetragona* stem length growth increases with deeper winter snow, with the magnitude of the responses depending on the contrasting effects of increased snow depth on growth: negative through shortening of the growing season and positive through frost protection. Secondly, we hypothesized deeper snow would alleviate both water and nitrogen limitations for *C. tetragona* growth.

Table 1. Average maximum winter snow depth, snow-free date, and winter soil temperature (mid-September to mid-June) measured at 2 cm depth in ambient-snow, medium-snow, and deep-snow plots.

	Ambient snow	Medium snow	Deep snow
Average maximum winter snow depth 2008–2012 (cm) ^a	20	100	150
Average snow-free date 2008–2012 ^a	June 1	June 14	June 18
Average 2010–2011 winter (mid-September to mid-June) soil temperature, 2 cm depth (°C)	−6.1	−4.1	−2.6

^a From Semenchuk *et al* (2013).

2. Materials and methods

2.1. Site description and experimental setup

To understand the impacts of winter snow depth on annual growth and stem isotopic characteristics ($\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of the evergreen shrub *Cassiope tetragona*, individuals were harvested from a snow manipulation experiment in the Adventdalen Valley in Spitsbergen, Svalbard (78.17°N, 16.27°E). Mean July temperatures (1975–2012) of 6.4 °C were recorded at the closest meteorological station at Svalbard Airport, Longyearbyen (WMO station 99849), located approximately 15 km from the research site. The coldest month is March with an average temperature of −13.7 °C. Annual precipitation sums are *c.* 190 mm, most of which falls as snow during the November–May period (*c.* 120 mm).

Snow fences were used to create snowdrifts on the leeward side of the fences. Nine fences of 1.5 m height and 6.2 m length were established during autumn 2006. Three fences were located within each of three sites (heath, meadow, moist meadow), characterized by differences in plant species composition, soil moisture regimes and landscape types. A detailed vegetation description of sites is given in Cooper *et al* (2011). All fences were located within an area of 1.5 by 2.5 km (Morgner *et al* 2010). The heath site is relatively dry due to its topography and is dominated by *C. tetragona*, whereas the meadow site is wetter due to its location in a drainage area of an adjacent hill and is dominated by *Salix polaris*. The moist meadow site is similar to the meadow site, but has undergone surface collapse behind one of the three snow fences during recent years and has on average higher soil moisture (Semenchuk *et al* 2015). Snow drifts raised winter soil temperatures in comparison to ambient snow areas, maintaining top-permafrost temperatures just below freezing point (Semenchuk *et al* 2013), which presumably led to deeper thaw during summer. Thaw of deeper ice-rich permafrost layers resulted in surface collapse, thus increasing topographical differences on the snowdrift side of the fences at the moist meadow site during the course of the experiment. A similar phenomenon was observed in a snow fence experiment in Northern Sweden (Johansson *et al* 2013).

A transect was laid out perpendicular to each snow fence to study the impacts of two incremental snow depths on soil temperature and soil moisture. Snow

depth measurements were carried out during winter 2010 and 2011 using a probing stick. Snow depth decreased with distance from each snow fence (Semenchuk *et al* 2013). Soil moisture measurements (Theta Probe ML2x; Delta-T Devices, Cambridge, UK) were recorded at irregular intervals during carbon flux measurement campaigns between 2007 and 2011. Soil moisture was generally higher in snow drift plots (5–10 vol%) compared to ambient snow early in the growing season, with differences declining to similar values by the end of the growing season (Morgner *et al* 2010, Semenchuk *et al* 2015). Soil temperature was measured at an hourly interval at a depth of *c.* 2 cm in ambient (8 out of 9 fences), medium (1 fence per site) and deep snow plots (all 9 fences) using temperature probes (Tiny Tag Plus 2, Gemini Data Loggers, UK). Daily average soil temperature data from 2008–2012, measured in the snow fences, are presented in Semenchuk *et al* (2013). Plots for *C. tetragona* collection (see next section for details) were selected under three different snow depth regimes, at a distance of approximately 5 m (deep-snow, *c.* 150 cm) and 10 m (medium-snow, *c.* 100 cm) from the leeward site of the fences. In addition, plots were selected within 100 m of each snow fence from areas receiving ambient-snow levels (control, *c.* 20 cm). Table 1 lists winter snow depths, snow-free dates and winter soil temperatures in the three different plot types.

2.2. *Cassiope tetragona* collection

Cassiope tetragona (L.) D. Don. (Ericaceae) is a long-lived (>150 years, Weijers *et al* 2012) evergreen shrub species with a circumpolar distribution (Callaghan *et al* 1989, Rayback and Henry 2005). *Cassiope tetragona* is associated with ericoid mycorrhizal fungi, utilizing organic nitrogen (N) sources (Michelsen *et al* 1996b) and maintaining a slow-growth strategy typical for plant species in nutrient-poor ecosystems (Berendse and Jonasson 1992).

Five samples of complete aboveground parts of *C. tetragona* individuals were collected during early July 2012 at all three plots (ambient, medium and deep snow) from each snow fence at all three sites. The hemi-prostrate *C. tetragona* individuals at our research site were, on average, between 5 and 10 cm tall. At the heath site, collection took place along two transects behind one of the three snow fences to compensate for another snow fence at the heath site that

had fallen over and laid flat during two years of the experiment and where no *C. tetragona* collections were made. Behind the fence that was sampled twice, transects were laid out 5 m apart to prevent sampling of individuals within an identical clonal patch. Care was taken to select only healthy individuals with green active meristems. Samples were packed in separate paper bags and air-dried before shipment to the lab for analysis.

2.3. Annual stem length measurements

Leaves of *C. tetragona* grow in opposite pairs and form rows on four sides of the branches. A wave-like pattern in leaf lengths and leaf scar distances allows for the reconstruction of annual leaf and stem growth rates for several decades (Callaghan *et al* 1989, Havström *et al* 1995). Recently, a novel method has been developed allowing reliable reconstructions of growth on even longer time scales by the detection of dark marrow tissue bands between growth years, termed the *wintermarksepta method* (Rozema *et al* 2009, Weijers *et al* 2010). Annual stem length growth is measured as the distance between wintermarksepta (Rozema *et al* 2009). Wintermarksepta are considered to be small bands of meristem tissue in the pith, formed at the end of the growing season, and thereby demark the end of one year of stem growth. Distances between wintermarksepta have proven to be a reliable proxy for past climate (Weijers *et al* 2010, Weijers *et al* 2013b). Of each set of five individual aboveground plant individuals sampled from each plot, the two presumably oldest individuals were selected for further analysis. Thus, 54 individuals, spread evenly across sites and snow depth treatments, were measured in total. Annual growth was measured on all individuals using the wintermarksepta method with a precision of 0.01 mm from the latest year of growth (2012) up to the oldest year of growth (the oldest individual measured was 35 years old) on the dominant stem from each individual, defined as the oldest stem containing a green meristem. Growth records of individuals were visually crossdated and further checked for errors with the software program COFECHA (Holmes 1983). The crossdated raw stem increment growth records were detrended by applying a linear regression line with flexible slope to each individual growth curve using the program ARSTAN v6.05 (Cook 1985, Cook and Holmes 1986) to remove age-related growth trends, a common procedure in shrub dendroecology (Myers-Smith *et al* 2015). The final chronology was developed by averaging the residual growth records from ambient snow plots of all three sites by biweight robust mean ($n=19$ individuals). The residual chronology was correlated with a standard *C. tetragona* chronology for the overlapping period 1998–2008, developed from nearby Endalen (figure S2 in supporting information) located within 5 km of our research site (Weijers *et al* 2010).

Residual growth curves were averaged across the two individuals collected per snow depth locality to avoid spatial pseudo-replication in the subsequent mixed-model analyses (see statistical analysis section below).

2.4. Isotopic analyses

Of the two *C. tetragona* individuals analyzed per plot for annual stem length growth, one individual was allocated to $\delta^{13}\text{C}/\delta^{15}\text{N}$ analysis, and the other individual to $\delta^2\text{H}/\delta^{18}\text{O}$ analysis. *Cassiope tetragona* increments were weighed on a fine balance (precision 1 μg), cut, and packed (*c.* 2 mg) into tin ($\delta^{13}\text{C}/\delta^{15}\text{N}$) or silver capsules ($\delta^2\text{H}/\delta^{18}\text{O}$). Annual growth increments from the year before the establishment of the experiment in 2006 up until 2011 (growth increments of 2011 were bulked with underdeveloped 2012 growth increments) were analyzed for isotopic composition for one individual per plot for each snow fence. In addition, growth increments of samples from ambient snow plots were analyzed for the years 2000–2005 to study annual isotopic variations in response to fluctuations in local climate parameters. We measured isotopes on whole stem wood, as stem mass was limited, preventing extraction of sufficient amounts of cellulose for isotopic analysis. Earlier work showed that $\delta^{13}\text{C}$ records derived from whole wood may show greater climate sensitivity than $\delta^{13}\text{C}$ records from extracted cellulose or lignin tissue (Loader *et al* 2003), but this has not yet been validated for arctic shrubs.

Isotopic ratios of carbon ($^{13}\text{C}:^{12}\text{C}$) and nitrogen ($^{15}\text{N}:^{14}\text{N}$), nitrogen concentration (%N) and carbon concentration (%C) were determined on an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, Cheddle Hulme, UK) coupled to a CN elemental analyzer (Eurovector, Milan, Italy) using continuous flow, with helium used as a carrier gas. Samples were combusted at a temperature of 1050 °C for C and 700 °C for N. Isotopic C and N ratios are presented in δ -notation ($\delta = [R_{\text{sample}}/R_{\text{standard}} - 1] * 1000$; R depicting $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$ ratios), relative to isotopic ratios of Vienna Pee Dee Belemnite and atmospheric N_2 , respectively. Samples were analyzed with reference gas calibrated against international standards IAEA C5, CH6, CH7, N1, N2 and USGS 25, 26, 32, and drift corrected using peach leaves (NIST 1547) as working standard. The standard deviation of isotope measurements of the standards was ± 0.2 parts per mille (‰) for $\delta^{15}\text{N}$ and ± 0.1 ‰ for $\delta^{13}\text{C}$.

Isotopic analysis of hydrogen ($^2\text{H}:^1\text{H}$) and oxygen ($^{18}\text{O}:^{16}\text{O}$) were determined by combustion in the absence of oxygen using pyrolysis technique on a ThermoFinnigan Temperature Conversion Elemental Analyzer (TC/EA; Thermo Scientific, Bremen, Germany), connected to a ThermoFinnigan Delta Plus XP (Thermo Scientific, Bremen, Germany) isotope ratio mass spectrometer. The instrument was calibrated against international reference standards from the International Atomic Energy Agency (IAEA) (IAEA-

601, IAEA-602, IAEA-CH7, and IAEA-C3). Internal standards of purified methionine (Alfa Aesar, $\delta^2\text{H} = -59.34 \pm 1.8$, $\delta^{18}\text{O} = 3.14 \pm 0.16$), homogenized peach leaf (NIST 1547, $\delta^2\text{H} = -96.49 \pm 1.8$, $\delta^{18}\text{O} = 26.55 \pm 0.16$), and homogenized Labrador tea leaf (UAA, $\delta^2\text{H} = -196.76 \pm 2.0$, $\delta^{18}\text{O} = 18.52 \pm 0.25$) were included with all samples for in-run calibration and as quality controls. Stable isotope values are reported in standard δ notation ($\delta = [R_{\text{sample}}/R_{\text{standard}} - 1] * 1000$; R depicting $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratios) in ‰, referenced to Vienna Standard Mean Ocean Water (VSMOW). Long-term records of internal standards yielded an analytical precision of 0.2‰ for $\delta^{18}\text{O}$ and 1.8‰ for $\delta^2\text{H}$.

2.5. Meteorological and precipitation isotope data

Daily air temperature and precipitation data (1975–2012) were obtained from the meteorological station at Svalbard Airport (WMO station 99849; www.eklima.no), approximately 10 km from the research location. Average monthly temperature and monthly precipitation sums were calculated for months with complete data records.

Monthly mean precipitation (1990–2011) $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values from a meteorological station (WMO station 100 400) in Ny-Ålesund, Svalbard (78.92°N, 11.92°E), located approximately 130 km northwest of the experimental snow fence site, were obtained from the Global Network of Isotopes in Precipitation (GNIP; <http://www.iaea.org/water>). Monthly composite precipitation samples were collected and analyzed for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ at the IAEA lab in Vienna, Austria. Precipitation source was recorded as snow, rain, or mixtures of snow and rain. Isotope contents are expressed as ‰ deviation from the standard VSMOW isotope ratio.

2.6. Statistical analyses

The effects of snow depth and site on annual *C. tetragona* stem length, stem %N, stem N pools, and isotopic composition ($\delta^2\text{H}$, $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for years 2008–2011, as well as for the pre-treatment year 2006, were analyzed using the PROC MIXED procedure in SAS Enterprise Guide 6.1. We excluded growth and isotopic data from 2007 fragments from the mixed models as this may have been affected by pre-treatment conditions through storage of plant resources during the end of the 2006 growing season, when snow treatments were not yet in effect. In addition, stem %N, stem N-pool and stem isotope data from 2011 growth increments were excluded from the mixed models, as these values strongly deviated due to inclusion of underdeveloped 2012 increments with the 2011 fragments. Data were natural log-transformed where needed to achieve normal distributions. *Snow depth*, *site*, and *snow depth*site* were selected in the models as fixed factors, with *snow fence* nested within *site* as random factor.

Fixed effects were determined using the Restricted Maximum Likelihood method, with degrees of freedom estimated by the Kenward and Rogers method. *Growth year* was selected as repeated factor to constrain model degrees of freedom. Toeplitz, autoregressive or heterogeneous autoregressive structures were used as covariance structure for the repeated effect to take into account potential autocorrelation of stem growth, stem N pools and isotopic composition of stems within individuals between years, depending on which structure yielded lowest Akaike Information Criterion statistics. One-way Analysis of Variance post hoc tests were performed to compare differences in stem growth, stem N pools and isotopic composition of stems between individuals growing under medium- and deep- versus ambient-snow conditions.

3. Results

3.1. *Cassiope tetragona* annual growth and isotopic composition variability in relation to climate

We developed a growth chronology from the residuals of detrended growth curves of 18 individuals from ambient-snow plots sampled across all three sites for the period 1998–2011. Our residual chronology had an expressed population signal over 0.85 for the entire chronology period, indicating the sampled pool of individuals from ambient-snow plots expressed a growth signal that was likely representative for the regional *C. tetragona* population. The mean sensitivity of our residual chronology was 0.31, indicating the relative change in stem increment length from one growth year to the next. Our residual chronology correlated strongly with a growth chronology developed by Weijers *et al* (2010) from *C. tetragona* individuals collected at nearby Endalen ($r = 0.85$, $P < 0.001$, $n = 11$ years; figure S2 in supporting information).

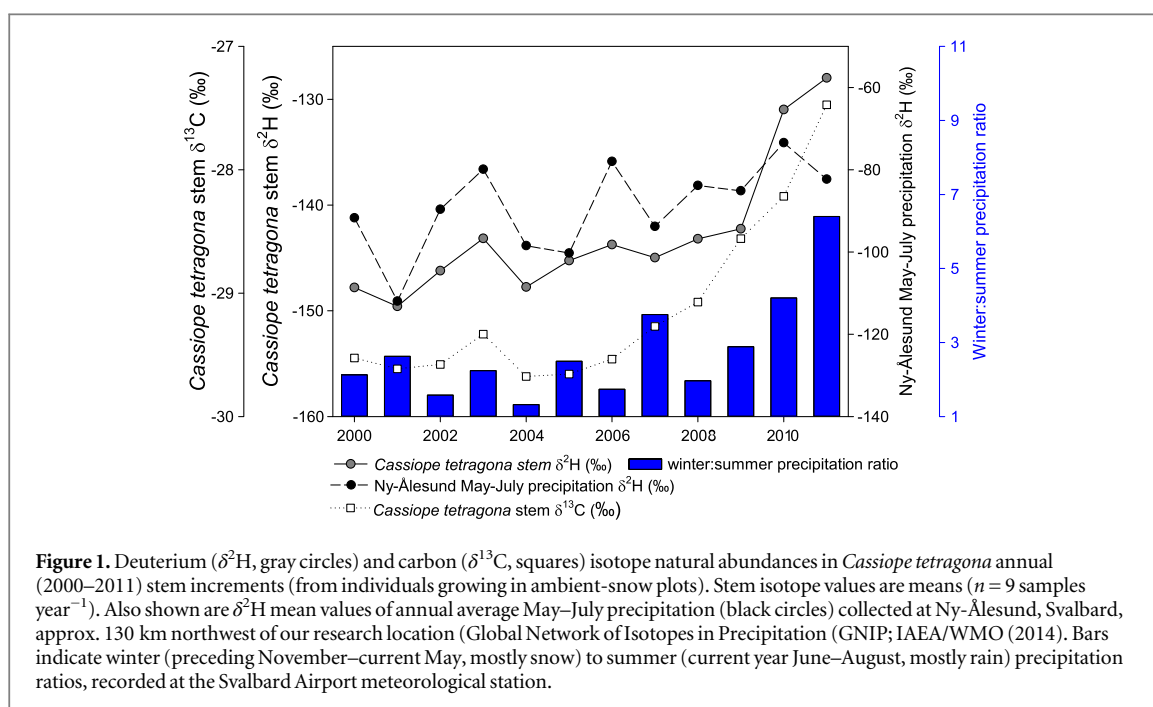
Growth of ambient-snow individuals (1998–2011), measured as annual stem growth, was negatively correlated to summer (June–September) precipitation, but positively correlated to summer (June–August) air temperatures (table 2). The strongest, positive, correlation was observed between growth and mean July temperature ($r = 0.82$, $P < 0.001$, $n = 12$ years). Summer precipitation and summer temperature were negatively correlated ($r = -0.61$, $P < 0.05$, $n = 14$ years; data not shown). No significant relationships between annual stem growth and winter monthly temperature or winter monthly precipitation sums were observed (data not shown).

May–July average precipitation $\delta^2\text{H}$ values closely matched $\delta^2\text{H}$ values in annual *C. tetragona* stem increments from ambient-snow plots between 2000 and 2009 ($r = 0.82$, $P < 0.01$, $n = 10$ years; figure 1). From 2010 onwards this relationship became less apparent, with stem $\delta^2\text{H}$ values sharply increasing and deviating from the precipitation $\delta^2\text{H}$ temporal trend. The ratio

Table 2. Pearson's correlation coefficients (r), regression slopes and corresponding P -values between climate parameters and the *Cassiope tetragona* stem length residual growth chronology (1998–2011) constructed from individuals growing in ambient-snow plots ($n = 14$ years). Significant correlations are marked with asterisks.

Climate variable	Pearson's correlation coefficient	Regression slope stem growth chronology versus climate variable	
Temperature			
July	0.82***	0.19	
August	0.59*	0.11	
Summer (June–August)	0.58*	0.14	
Precipitation			
May	0.25	0.01	
June–September	−0.67**	−0.004	
Winter:summer precipitation ratio	0.14	0.01	

Stem length residual data are averages of 18 *C. tetragona* individuals. Daily average climate data were derived from the Svalbard Airport meteorological station. Regression slopes were calculated by plotting the residual growth chronology against each climate variable. Significant correlations are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



between annual winter (preceding year November–current year May) and summer (current year June–August) precipitation, hereafter referred to as the ‘winter:summer precipitation ratio’, was closely related to *C. tetragona* stem $\delta^{13}\text{C}$ ($r = 0.88$, $P < 0.001$, $n = 12$ years) and stem $\delta^2\text{H}$ values ($r = 0.84$, $P < 0.001$, $n = 12$ years; figure 1) for the period 2000–2011. This relationship became non-significant when excluding the last two years with relatively high *C. tetragona* $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values. Stem $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values were significantly correlated in ambient-snow individuals for the period 2000–2011 ($r = 0.94$, $P < 0.001$, $n = 12$ years; figure 1).

3.2. Snow manipulations effects on stem length growth, isotopic composition and nitrogen content

Average *C. tetragona* individual age was 19 years (SE = 0.6 year), with similar mean ages across snow depths and sites. Snow depth had a significant effect on

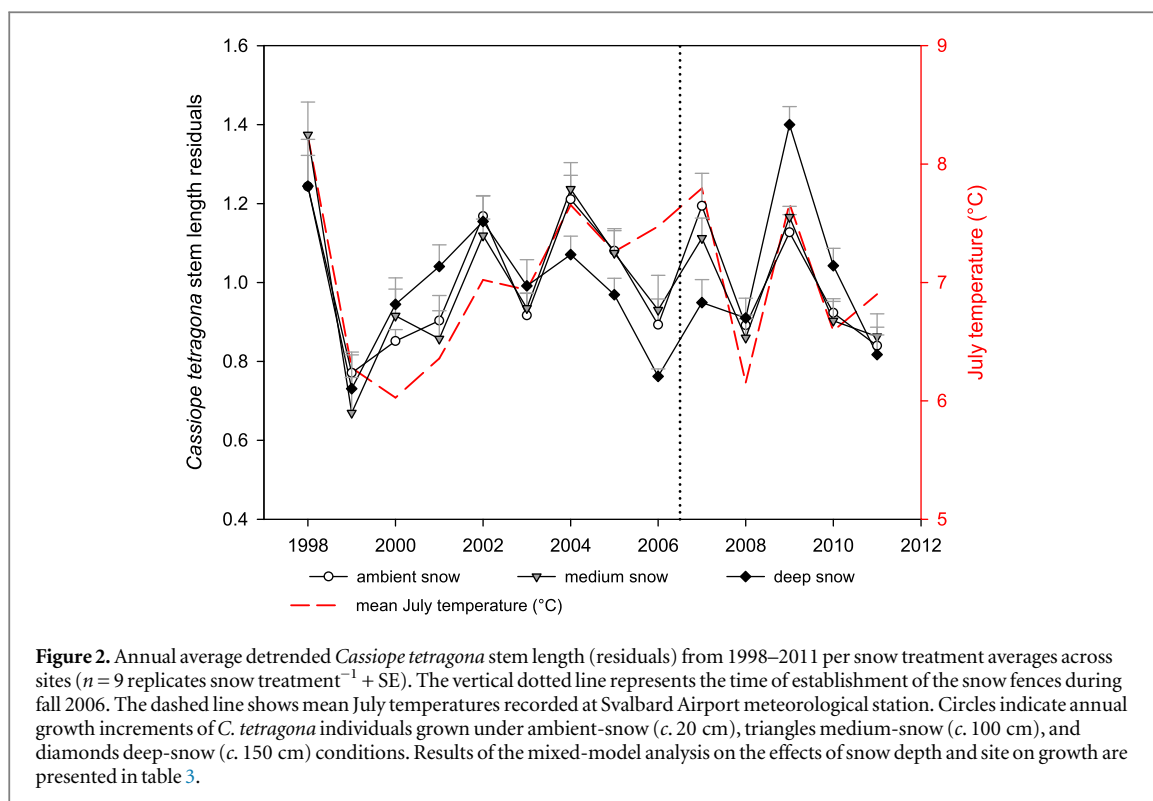
C. tetragona annual stem growth during the snow manipulation period 2008–2011, with higher growth observed in individuals growing in deep-snow plots compared to individuals growing in ambient-snow plots (table 3, figure 2). The strongest positive shrub growth response to the deep-snow treatment was observed in all three sites during the most productive growth year 2009. During time of sampling in July 2012, annual *C. tetragona* stem growth was uncompleted, with snow-manipulated plots showing lower growth than ambient-snow plots (figure S1 in supporting information). Growth was not significantly different between the snow depth treatments during the pre-treatment growth period 1998–2006. Growth did also not differ significantly between sites during both pre-treatment and treatment periods ($P > 0.05$).

Snow depth had a significant effect on stem $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the period 2008–2010, but not on $\delta^2\text{H}$ or $\delta^{18}\text{O}$ values (table 3, figure 3). Stems from

Table 3. Mixed model repeated analyses of the effects of ‘snow depth’ and ‘site’ on annual *Cassiope tetragona* stem length growth (mm), stem nitrogen content (%N), stem total nitrogen pool (mg N), carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$) stable isotope abundances measured on annual stem increments.

	Stem length growth residuals (no units)			Stem nitrogen content (%)			Stem increment total nitrogen pool (mg)			$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)			$\delta^{18}\text{O}$ (‰)			$\delta^2\text{H}$ (‰)		
	Df	F	Pr > F	Df	F	Pr > F	Df	F	Pr > F	Df	F	Pr > F	Df	F	Pr > F	Df	F	Pr > F	Df	F	Pr > F
Snow depth	2, 45	3.2	0.05	2, 23	3.5	0.05	2, 22	2.8	0.08	2, 21	4.4	0.03	2, 22	6.1	0.01	2, 21	0.9	0.42	2, 18	2.4	0.12
Site	2, 45	3.0	0.06	2, 23	3.9	0.04	2, 22	0.6	0.56	2, 21	6.8	<0.01	2, 22	18.9	<0.001	2, 22	16.3	<0.001	2, 18	0.1	0.91

Differences between stem %N, N-pools, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$ were analyzed for 2008–2010 growth increments (stem N and isotope values in 2011 increments deviated strongly due to inclusion of under-developed 2012 increments). Effects of snow depth and site on stem length growth were analyzed for the period 2008–2011. Values are based on averages of nine individuals snow depth⁻¹, with three individuals analyzed snow depth⁻¹ site⁻¹. Significant effects ($P < 0.05$) are marked in bold. Differences were assessed between individuals growing under three different snow depth regimes (ambient-, medium- and deep-snow) at three different sites (heath, meadow, moist meadow). The interaction term snow ‘depth*site’ was not significant for any parameter and thus removed from the models.



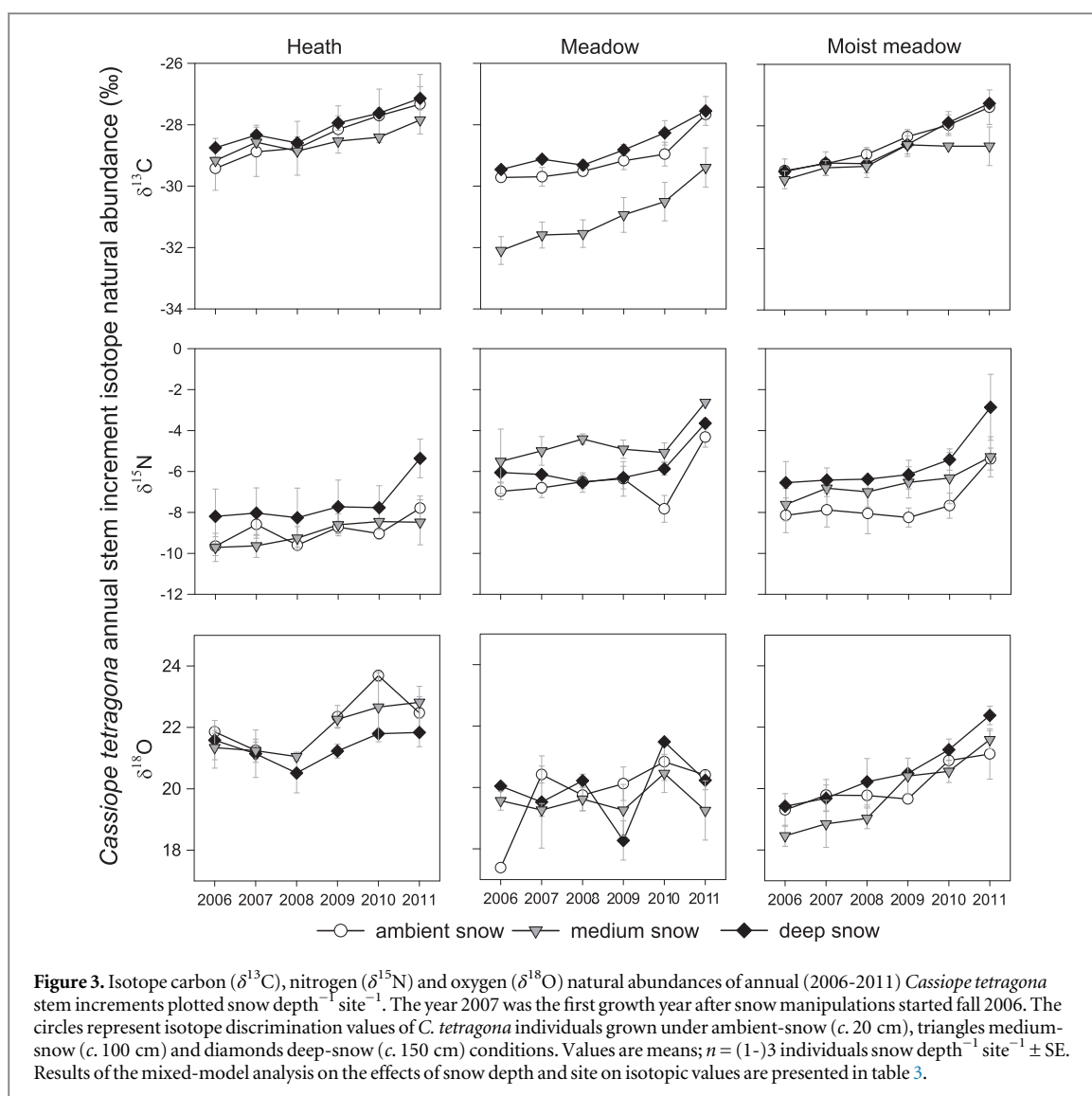
medium-snow plots exhibited depleted $\delta^{13}\text{C}$ values compared to ambient-snow stems during the experiment ($P < 0.05$, $n = 9$ stem increments snow depth⁻¹; figure 3), as well as in the pre-treatment year 2006 ($P < 0.05$, $n = 9$ stem increments snow depth⁻¹; figure 3). On average, stem $\delta^{15}\text{N}$ values were enriched in deep-snow and medium-snow plots ($P < 0.01$, $n = 9$ individuals snow depth⁻¹; figure 4) compared to ambient-snow individuals during the experiment from 2008–2010, but not during pre-treatment year 2006. Stem $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values differed between sites between 2008 and 2010 (table 3, figure 3), but stem $\delta^2\text{H}$ values did not (table 3, not shown). Individuals from the meadow site had depleted $\delta^{13}\text{C}$ values compared to *C. tetragona* individuals growing in the heath site ($P < 0.01$, $n = 27$ stem increments site⁻¹ year⁻¹; figure 3). Annual *C. tetragona* increments from individuals growing in heath site showed consistently depleted $\delta^{15}\text{N}$ values compared to the meadow and moist meadow site ($P < 0.01$, $n = 27$ stem increments site⁻¹ year⁻¹; figure 3). Stem $\delta^{18}\text{O}$ values in the heath site were enriched compared to stem $\delta^{18}\text{O}$ values from the other two sites ($P < 0.001$, $n = 27$ stem increments site⁻¹ year⁻¹; figure 3). Total stem N-pools (segment mass* nitrogen concentration) were overall not affected by snow depth during the manipulation period 2008–2010, neither did stem N-pools differ between sites (table 3). In contrast, stem N-concentrations (%N) were affected by both snow depth and site during the snow manipulation period (table 3, figure 4). Stem %N was significantly higher in individuals from deep-snow plots compared to individuals from ambient-snow plots ($P < 0.05$, $n = 9$

individuals per snow depth⁻¹), whereas stem %N was not significantly altered by the medium-snow treatment. On average, stem %N was lower in the heath compared to the meadow site ($P < 0.05$, $n = 27$ stem increments site⁻¹ year⁻¹, data not shown). A significant relationship between *C. tetragona* stem $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (years 2007–2011) was observed in medium- ($r = 0.45$, $P < 0.01$, $n = 34$ growth increments), ambient- ($r = 0.53$, $P < 0.01$, $n = 34$ growth increments), and deep-snow ($r = 0.65$, $P < 0.001$, $n = 34$ growth increments) individuals, with regression slopes increasing in subsequent order (figure 5).

4. Discussion

4.1. *Cassiope tetragona* growth responses to deepened snow

Our results show that the deep-snow treatment enhanced stem length growth of *C. tetragona* compared to ambient-snow individuals, despite the delayed snowmelt and reduction in growing season length. Individuals from the medium-snow plots did not show differences in growth compared to ambient-snow individuals, suggesting a snow depth threshold needs to be crossed before snow depth has a significant effect on growth (figure 2). Wipf and Rixen (2010) analyzed 39 snow manipulation studies conducted at arctic and alpine tundra sites with ambient snowmelt dates between day of year (DOY) 120–180, showing that delayed snowmelt generally decreased above-ground plant productivity, except at sites with intermediate snowmelt dates. This is consistent with our



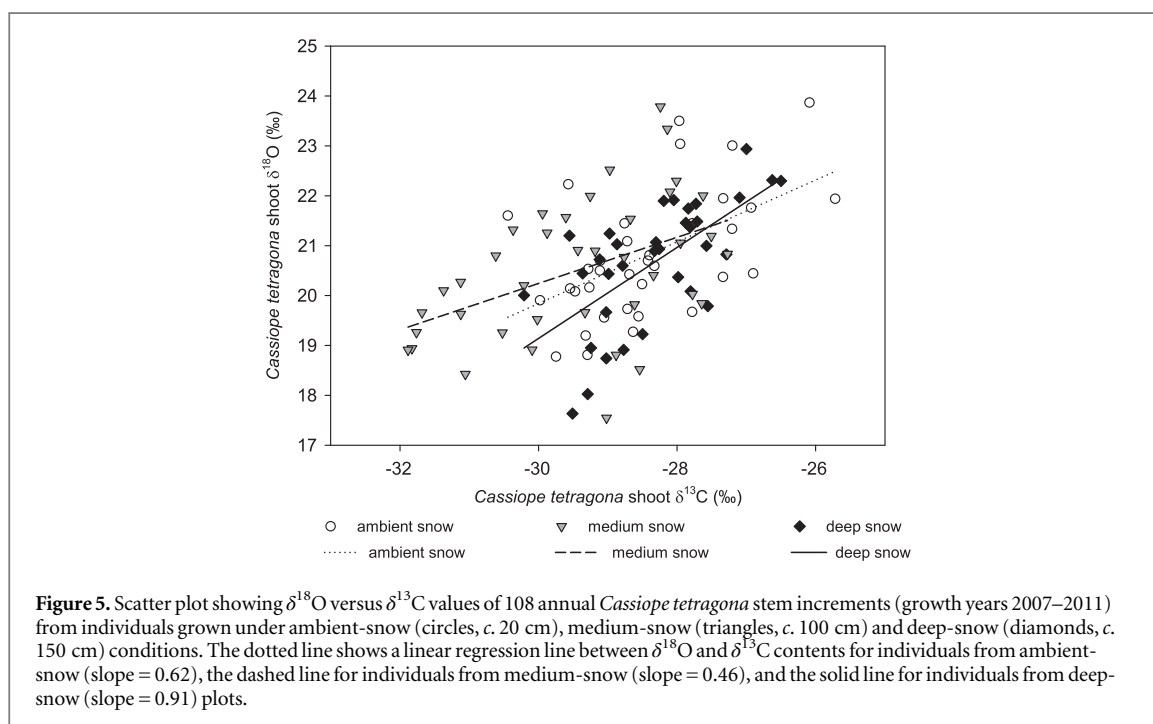
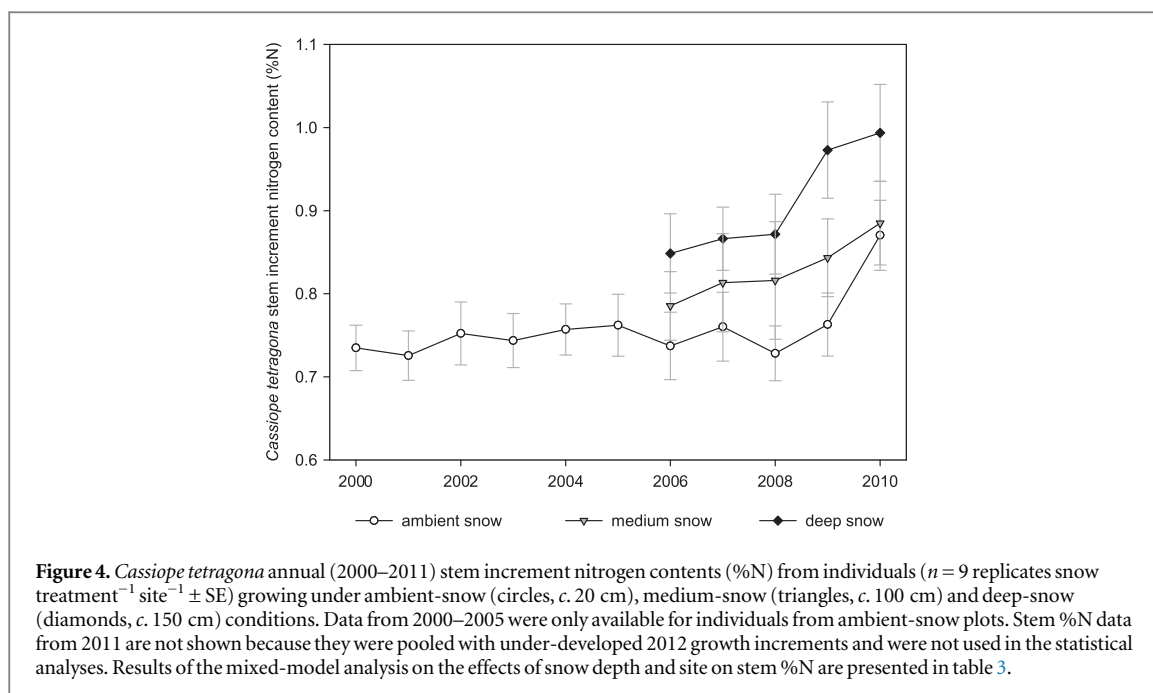
observation of increased *C. tetragona* stem length growth under deep snow at our site with average ambient snowmelt dates around DOY 152 (June 1st; table 1), roughly falling in the category of ‘intermediate ambient snowmelt dates’, as defined by Wipf and Rixen (2010). Stem length of the underdeveloped 2012 growth fragments was similar between medium- and deep-snow individuals during early summer 2012, consistent with roughly equal snow-free dates between medium- and deep-snow plots. This suggests that other factors than growing season length may be responsible for the observed differences in growth response between the medium- and deep-snow individuals.

Soil temperatures during early winter (November–December) in medium-snow plots were roughly similar to soil temperatures in ambient-snow plots, whereas deep-snow plots showed on average 5–10 °C significantly higher early winter top-soil temperatures compared to ambient-snow soils (Semenchuk *et al* 2013). As snowdrifts start building up close to the snow fences, deep-snow plots are covered earlier with

snow than the medium-snow plots which are located further away from the fences. Individuals growing in the medium-snow plots may thus not have the same level of frost protection against extreme early-winter warming events as deep-snow individuals (Bokhorst *et al* 2011), as it takes a longer time for a deep snow pack to completely melt out. Other snow fence experiments performed in arctic and subarctic tundra also measured increased plant productivity in snow-accumulation plots, which was attributed to the combined effects of higher moisture and nutrient availability during the growing season (Schimel *et al* 2004, Natali *et al* 2012, Bosiö *et al* 2014).

4.2. Responses of photosynthesis and stomatal conductance to deepened snow

We observed a strong positive *C. tetragona* growth response to deep snow in 2009 at all our three sites (figure S1 in supporting information), despite this being a year with a relative thick and stable ambient snowpack of approximately 30 cm deep (Semenchuk *et al* 2013). We did not observe a growth response to



the medium-snow treatment, suggesting that a snow depth threshold exists for the snow treatment to positively affect *C. tetragona* growth. Although the medium- and deep-snow treatments initially reduced stem growth early in the growing season, as observed in the 2012 growth segments (figure S1 in supporting information), this growth delay was offset in the deep-snow individuals later during the growing season. Starr and Oberbauer (2003) reported that *C. tetragona* starts photosynthesizing when still snow-covered during spring, although light levels received by *C. tetragona* under snow may be lower compared to snow-free conditions, reducing the photosynthetic

capacity. Corresponding with this observation, Wipf (2010) showed that the dormancy period of *C. tetragona* is unaffected by snow depth. This suggests that for *C. tetragona*, the photosynthetic active period may not have been affected by the snow treatments, which could indicate that frost protection is an important control positively influencing growth at sites with deep-snow winter conditions (Wipf et al 2009).

We observed depleted stem- $\delta^{13}\text{C}$ values in *C. tetragona* plants growing in medium-snow plots (figure 3). Depleted stem- $\delta^{13}\text{C}$ values may be due to either higher rates of stomatal conductance or lower

photosynthetic rates (higher internal CO₂: ambient air CO₂ ratios) when plants are growing under sufficient water supply (Farquhar *et al* 1989, Welker *et al* 2005b). We observed higher stem %N values, a surrogate for changes in leaf photosynthesis (Welker *et al* 1993), in individuals from deep-snow plots but not in medium-snow individuals. This suggests that the observed lower stem- $\delta^{13}\text{C}$ values in medium-snow individuals are not related to lower photosynthesis rates but are most likely associated with higher rates of stomatal conductance.

Since *C. tetragona* leaves have a lifespan of approximately four years (Weijers *et al* 2012), C assimilation and discrimination against ¹³C may have continued for a few years in segments formed before establishment of the experiment, as suggested by the lower $\delta^{13}\text{C}$ values in medium-snow 2006 stem segments from the meadow site. A similar observation was made in a water addition experiment nearby our research location, in which *C. tetragona* leaves and stem increments showed depleted $\delta^{13}\text{C}$ values in water addition plots both before and after the start of the experiment (Weijers *et al* 2013a). Although radial growth may contribute to the isotopic values of previous years' growth, the strong linkage between the isotope time-series and the large inter-annual variations within isotope series suggest that the annual isotope signal is largely preserved in these relatively young branches. The increase in $\delta^{13}\text{C}$ values (becoming less negative, figure 1) from older to recent growth segments is not coherent with trends in stem length growth over time, but may be explained by the observed declines in the relative amount of summer precipitation during the last years of growth, reducing stomatal conductance and intercellular CO₂ partial pressure. The latter will lead to a shift towards less depleted $\delta^{13}\text{C}$ in newly synthesized plant tissue, closer to atmospheric $\delta^{13}\text{C}$ values (Farquhar *et al* 1989). The observed strong correlation between stem $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ (figure 5) supports the suggestion that inter-annual variations in stomatal conductance are reflected in the observed $\delta^{13}\text{C}$ trend over time (Saurer *et al* 1997, Scheidegger *et al* 2000). An alternative explanation for the $\delta^{13}\text{C}$ increase with stem age could be related to lignification of cellulose compounds during secondary radial growth, since lignin is more ¹³C-depleted than cellulose (Hobbie and Werner 2004). Similar stem age- $\delta^{13}\text{C}$ patterns have previously been observed for *C. tetragona* by Weijers *et al* (2013a) and Rayback *et al* (2012).

4.3. Deep snow increases *Cassiope tetragona* stem nitrogen concentrations

Stem $\delta^{15}\text{N}$ values were less depleted in medium-snow and deep-snow individuals compared to ambient-snow individuals (figure 3), suggesting a higher uptake of inorganic-N over organic-N compounds under deep-snow than under ambient-snow conditions

(Michelsen *et al* 1996b, Yano *et al* 2010). This potential shift in inorganic-N over organic-N may be due to greater winter decomposition of organic matter in relatively warm deep-snow covered soils (Schimel *et al* 2004, Natali *et al* 2012). Higher soil-extractable ammonium and nitrate concentrations were observed in deep- compared to ambient-snow plots at our site (Semenchuk *et al* 2015), which were reflected in higher *C. tetragona* stem %N values in deep-snow individuals in our study. Our findings suggests that soils in deep-snow plots had a higher soil N-availability resulting from greater winter soil microbial activity under deep snow (Schimel *et al* 2004). In line with our findings of higher *C. tetragona* stem %N under deep snow, *Salix polaris* leaves collected at our site in deep-snow plots also showed increased leaf %N (Semenchuk *et al* 2015). Similar findings were reported for *Salix arctica* leaf tissue in a snow fence experiment in the high-arctic of NW-Greenland (Rogers *et al* 2011, Lefler and Welker 2013).

Evergreen species like *C. tetragona* have the advantage of long (leaf) life spans and low nutrient loss rates, at the cost of low growth rates and limited responsiveness to environmental changes (Aerts 1995). A long-term fertilization experiment in a subarctic heath of Northern Sweden showed that higher N-availability initially stimulated *C. tetragona* growth (Michelsen *et al* 1996a). However, after 22 years of fertilization, *C. tetragona* cover declined, with graminoid species increasing in cover (Capioli *et al* 2012). These findings highlight the importance of long-term studies to understand plant community responses to environmental changes in slow-growing arctic tundra environments. *Cassiope tetragona*'s poor competitive ability for nutrient acquisition in comparison with fast-growing deciduous shrub or graminoid species may eventually lead to an incremental decline in *C. tetragona* growth rates under higher winter-released soil N (Havström *et al* 1993, Kielland 1994, Molau 1997, Aerts and Chapin 2000). However, large differences may exist in growth limitations for *C. tetragona* between high-, low-, and sub-arctic regions, with presumably greater temperature limitation for growth in the high-arctic and greater N-limitation in the sub-arctic (Havström *et al* 1993).

4.4. Source water use of *Cassiope tetragona* not affected by snow depth

We observed a strong correlation between May–July precipitation $\delta^2\text{H}$ and *C. tetragona* stem $\delta^2\text{H}$ (figure 1), suggesting that most of the water supply to *C. tetragona* originated from precipitation falling during the growing season. Neither $\delta^2\text{H}$ nor $\delta^{18}\text{O}$ *C. tetragona* stem values were affected by snow depth, suggesting that source water usage by *C. tetragona* was unaffected, similar to observations by Lefler and Welker (2013) for *Salix arctica* in high-arctic NW-Greenland. However, we cannot exclude the possibility that local

differences in source water usage were canceled out by differences in *C. tetragona* transpiration rates under different snow regimes, providing an alternative explanation for similar $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. Stem $\delta^{18}\text{O}$ values were enriched in the heath site compared to the meadow and moist meadow sites, suggesting that *C. tetragona* individuals growing in the relatively dry and elevated heath site are more dependent on water derived from rain than snow precipitation, in contrast to the meadow and moist meadow sites which likely have a greater inflow of more $\delta^{18}\text{O}$ -depleted snowmelt water from a nearby ridge.

The marked enrichment in $\delta^2\text{H}$ during 2010 and 2011 in *C. tetragona* stems growing under ambient-snow conditions coincided with a shift towards greater precipitation inputs during winter versus summer. The summers of 2009–2011 were remarkably dry with average June–August precipitation sums of 26 mm. Greater winter snow precipitation inputs normally lead to more $\delta^2\text{H}$ -depleted source water, since snow has depleted $\delta^2\text{H}$ compared to rain (Welker *et al* 1995, Welker 2000, Welker 2012). This contradicts our observation of enriched stem $\delta^2\text{H}$ values during the last two years. One explanation for this offset between stem- and precipitation- $\delta^2\text{H}$ may be the incremental depletion in ^2H during wood formation in *C. tetragona* stems, with more recently formed wood containing less ^2H -depleted lignified compounds than old wood (White 1989). An alternative explanation for ^2H -enriched stem tissue during these years with low summer precipitation could be the result of stomatal closure in response to drier summer conditions, leading to reduced transpiration rates and associated leaf heating, causing leaf water evaporation and enrichment of newly synthesized leaf and stem tissue. Both factors are considered to lead to leaf water ^2H -enrichment (Farquhar *et al* 2007). This explanation is supported by observed enrichment in stem $\delta^{13}\text{C}$ during the last two years of growth (figure 1), which may indicate higher water-use-efficiency with lower internal CO_2 vapor pressure after stomatal closure (Farquhar and Sharkey 1982) and reduced discrimination against $^{13}\text{CO}_2$ by enzymes involved in the photosynthesis pathway (Farquhar *et al* 1989). In agreement with our conclusion that growth was indeed water-limited during the last two growth years, we observed relatively low annual stem growth during 2010 and 2011, especially in the medium- and ambient-snow plots (figure 2). However, *C. tetragona* growth in a heath site close to our research location in Svalbard did not respond to an experimental doubling of ambient summer precipitation over four years (Weijers *et al* 2013a), suggesting that *C. tetragona* growth is generally not water-limited in our study area (Callaghan *et al* 1989). Instead, we observed a negative relationship between summer precipitation and growth under ambient snow conditions, which has been noted in an earlier study comparing inter-annual climate and *C. tetragona* growth patterns (Callaghan *et al* 1989). We

conclude, however, that the correlation between precipitation and growth in our study may be corroborated by the inverse relationship observed between summer precipitation and air temperature between 1998 and 2011.

5. Conclusions

We show that our experimental deep-snow treatment increased *C. tetragona* stem growth and stem %N. Enriched stem $\delta^{15}\text{N}$ values in deep-snow individuals suggest that an increased N-availability from higher soil winter mineralization rates may have contributed to the observed increase in growth under deep snow. Deeper snow may also have increased frost protection and thereby compensated for the potential negative effects of a shortened growing season, providing an additional explanation for the observed positive growth response to our deep-snow treatment. The importance of both optimal winter and summer conditions for *C. tetragona* growth may partly explain the frequently observed lack of direct correlations between winter climate conditions and shrub growth. Combined, our results show the importance of multi-year experimental snow manipulation studies to improve our understanding of the complex interactions between the factors regulated by snow depth changes affecting shrub growth: growing season length, winter frost protection, and water- and N-availability. A better understanding of the impacts and interactions of these complex factors is needed to predict the effects of changing snow conditions on shrub growth in the High Arctic.

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DB and BE conceived the project, BE collected *Cassiope tetragona* samples, EJC established the snow fence experiment, SW measured stem growth, AM measured *C. tetragona* stem carbon and nitrogen isotopes, DB performed data analyses, DB wrote the manuscript with contributions from all authors. We kindly thank the anonymous reviewers for providing helpful suggestions that greatly improved our paper. We are grateful to Philipp Semenchuk for providing soil temperature, soil moisture and snow depth data. We thank Stefan Terzer from the International Atomic Energy Agency (IAEA), Vienna for providing precipitation deuterium data. We thank Matthew C Rogers from the University of Alaska Anchorage (UAA) Stable Isotope Laboratory for measurements of oxygen and hydrogen isotopes in *C. tetragona* stems. We thank Annelein Meisner for constructive comments on a previous version of the manuscript. This work was funded by a grant from the Danish National Research Foundation (CENPERM DNR100), with further financial support from a Fulbright Distinguished US Arctic Chairship-Norway awarded to JMW. In

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