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Master's Thesis 2024   60 ECTS
Faculty of Environmental Sciences and Natural Resource Management

The Impact of Environmental Stress on the Plant Sex Expression and Reproduction of *Silene acaulis* at Finse, Norway

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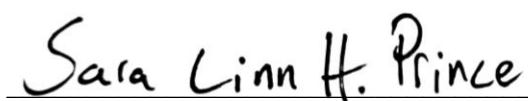
Master of Science in Ecology

Preface

This thesis is the final result of my MSc in Ecology at the Norwegian University of Life Sciences (NMBU). I have been interested in plants for as long as I can remember, so it has been incredibly rewarding to be able to study and write about plants for my master's degree. Throughout the year I have gained many new experiences, but I particularly loved soldering the DIY sensors and being out in the field at beautiful Finse.

I would like to thank my supervisors for the opportunity to do this project, and for their support and guidance. To my main supervisor, Erik Trond Aschehoug, for all the ideas, experience, and patient guidance throughout this process. To my co-supervisor, Kari Klanderud, for great help and advice in the field, for feedback and guidance, and for letting me use her OTC experiment as an experimental setup in my project. I would also like to thank Siri Lie Olsen for helpful advice in the field at Finse, and the people at Finse Research Station for the nice accommodation during my field trips. Thanks to Knut Asbjørn Solhaug for letting me use his Fluorometer, and for all he taught me about plant physiology. Thanks to the great people at Eik Lab for letting me use their equipment and experience, especially Lars Øvergård who showed great patience when teaching me how to code and solder. And to the R-club for all statistical help and inspiration.

Lastly, thank you to my friends and family for their encouragement throughout this entire project. A particularly big thank you to my parents, for their enthusiastic support and motivation.

Norwegian University of Life Sciences

Ås, May 15th, 2024

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Abstract

Arctic and alpine zones are low productivity systems characterized by extreme conditions such as low temperatures, heavy snow loads, and short growing seasons. Plants in these ecosystems have evolved specific adaptations, including compact forms, genetic freeze resistance, and reproductive strategies. However, human-induced climate change has dramatically increased global annual average temperatures over the last century, leading to cascading effects on the primary environmental stressors of Arctic and alpine ecosystems. Such widespread changes to the fundamental abiotic conditions can lead to a mismatch between plant evolutionary adaptations and altered environmental conditions. For instance, earlier snowmelt can alter the timing of water availability causing water stress, while changes in temperature can disrupt the synchronization between flower time and pollinator activity.

Silene acaulis, a cushion plant common in alpine and Arctic habitats, commonly exhibits a gynodioecy breeding system, consisting of females and hermaphrodites. Interestingly, this study also identified gynomonoecious, or mixed, individuals within the population. This thesis investigates how *Silene acaulis* responds to temperature by examining plant sex distribution, soil moisture levels, and their effect on plant health and reproductive outputs. The study used a natural elevational gradient and a long-term warming experiment with open top chambers (OTCs) at Finse, Norway. The warmest conditions were at the mid elevation. While there was no significant difference in plant sex distribution across groups, there was a non-significant 32% increase in female frequency from the low to high elevation, and a significantly higher proportion of females at the highest elevation. Surprisingly, higher soil moisture levels led to lower plant health, whereas plants inside the OTCs showed greater plant health, suggesting an initial positive response to increased temperatures.

The study revealed a complex relationship between environmental stress and reproductive strategies. Hermaphrodites had minimal pollination success, potentially due to an evolutionary shift towards male function. In contrast, females exhibited greater pollination success, possibly benefitting from outcrossing. A trade-off was observed between seed quantity and quality: the plants at the coldest site had the most seeds per capsule, while those at the warmest sites had the largest seeds. These findings suggest that *S. acaulis* demonstrates genotype x environment interactions and possible evolutionary shifts in response to climate change. The presence of mixed individuals may indicate an adaptive strategy to optimize reproduction under shifting environmental conditions, underscoring a dynamic relationship between the variation in climate and reproductive outcomes.

Understanding plant responses to shifts in environmental stress, particularly long-term effects on reproduction, is crucial for predicting how alpine species may adapt to ongoing changes to climate.

Table of Contents

Introduction

Global climate change has resulted in dramatic increases of annual average temperatures over the last century (IPCC, 2023). The warming of ecosystems can drastically alter the abundance and distribution of plant species (Inouye, 2020), and in some cases lead to local extinction (Nomoto & Alexander, 2021).

Arctic and alpine environments are particularly susceptible to increases in temperature. Higher temperatures can alter key environmental conditions, such as snow loads, timing of snowmelt, and the amount of winter rain (Gobiet et al., 2014). In addition, Arctic and alpine plant species face disruptions in biotic interactions, including increased competition, changes in pollinator communities, and mismatches between flowering times and peak insect activity (Renner & Zohner, 2018). Given these challenges, understanding the ways in which climate change affects the long-term persistence of Arctic and alpine species is important.

Alpine ecosystems cover about 15% of the total land surface globally (Inouye, 2020). The environmental conditions are characterized by cold temperatures, strong winds, and short growing seasons, with snow cover for much of the year (Körner, 2021). Plants in alpine ecosystems have evolved distinct adaptations to the harsh conditions, including lower height, compact forms, and genetic freeze resistance, such as supercooling or antifreeze substances (Hacker et al., 2011; Inouye, 2020; Körner, 2021). However, some of these adaptations make them sensitive to changes in their environment, particularly increases in temperature.

Environmental changes, such as shifts in temperature and soil moisture, can alter plant physiological responses. Soil moisture is crucial for photosynthesis and nutrient uptake and can be significantly impacted by earlier snowmelt (Harpold & Molotch, 2015). This can lead to a mismatch between the timing of water availability and plant needs, potentially exacerbating water stress and directly impacting plant health (Harpold & Molotch, 2015). Additionally, studies on Arctic plants have shown changes in key photosynthetic parameters in response to temperature and water stress. Li et al. (2013) found that plants respond to stress with a notable decrease in photosynthetic efficiency (Fv/Fm ratio), and under extreme stress, some plants stop photosynthesis completely. This immediate physiological response highlights the capacity of plants to adapt at the cellular level, and while important for short-term survival, differs from the long-term evolutionary adaptations seen in reproductive strategies.

One such evolutionary adaptation is gynodioecy, a breeding system characterized by the co-existence of hermaphrodite and female individuals (Hermanutz & Innes, 1994). Gynodioecious systems are common in stressful environments such as alpine and Arctic regions (Delph, 2003). It promotes outcrossing in females while allowing hermaphrodites to self-pollinate, thus promoting genetic diversity and resilience. In gynodioecious systems, female plants are often over-represented and can have higher reproductive outputs (Delph & Carroll, 2001; Keller & Schwaegerle, 2006; Reid et al., 2014; Shykoff, 1988; Spigler & Ashman, 2012). This breeding system may offer an evolutionary advantage, allowing plants to better withstand and adapt to the accelerated environmental changes ecosystems are facing.

Silene acaulis, a cushion plant common in alpine and Arctic regions, generally exhibits a gynodioecious breeding system. *S. acaulis* primarily consists of pure female and hermaphrodite individuals, but it is not uncommon to find mixed cushions exhibiting female and hermaphrodite flowers on the same plant. These mixed cushions, intermediates between females and hermaphrodites, are often referred to as gynomonoecious individuals (Baskin & Baskin, 2020). However, the specific ecological role of these mixed cushions remains unclear. Additionally, populations with male plants have also been observed (Alatalo & Molau, 1995; Jones & Richards, 1962; Philipp et al., 2009).

Silene acaulis is considered an ecologically important species. It is widely regarded as a nurse plant, increasing local biodiversity by harboring smaller species within its cushions (Antonsson et al., 2009; Larcher et al., 2010). The cushions not only provide microhabitats, but also offer resting places for insects and other small fauna (Antonsson et al., 2009; Bonanomi et al., 2016; Molenda et al., 2012). As such, *S. acaulis* acts as an alpine foundation species; with effects across several trophic levels, thereby having a crucial role in the stability of alpine communities (Molenda et al., 2012). Therefore, changes in phenology, abundance, or distribution of *S. acaulis* may have cascading impacts on alpine ecosystems.

Some plants can respond to environmental changes, such as high temperatures, by altering their physical or physiological traits within a single generation without genetic changes, a phenomenon known as phenotypic plasticity (Dorken & Mitchard, 2008). For example, *S. acaulis* demonstrates this adaptability through changes in flowering time and variation in their flower sex expression (Alatalo & Molau, 1995; Anderson et al., 2012; Delph, 2003; Hermanutz & Innes, 1994). While this allows for quick responses to environmental changes, long-term shifts in traits, such as sex ratio of plants, often involve evolutionary processes

over several generations. Research has suggested that increasing temperatures could shift the sex ratio of *S. acaulis* towards more females (Dorken & Mitchard, 2008), however, it is important to differentiate between phenotypic responses, and genetic adaptations that occur over time. The extent of these responses is not yet fully understood.

Various studies have used different methods to understand how *S. acaulis* responds to climate change (Alatalo & Little, 2014; Alatalo & Totland, 1997; DeMarche et al., 2018; Reid et al., 2014). One common experimental approach uses open top chambers (OTCs) to increase temperature and simulate warming scenarios. For example, using OTCs, *S. acaulis* has demonstrated earlier flowering, faster flower development, and faster maturation when exposed to warmer conditions compared to control groups (Alatalo & Totland, 1997). In contrast, several studies have found that elevated temperatures can have a direct negative impact on the fitness, survival and performance of cushion plants (Alatalo & Little, 2014; Doak & Morris, 2010; Gauslaa, 1984).

Another method to study climate involves analyzing elevational gradients, which are used as a natural proxy for climatic variation, with higher elevations typically experiencing colder temperatures (Alatalo & Little, 2014; Alatalo & Molau, 1995; Delph & Carroll, 2001). Studies using this method have shown shifts in sex distribution, such as Alatalo & Molau (1995) finding an increase in female frequency at higher elevations.

While a lot is known about the adaptations of *Silene acaulis* to the alpine environment, the effects of temperature on the plant sex distribution and health remains largely unknown. Investigating the relationship between temperature and the expression of sex in *S. acaulis* could help shed light on how alpine plants adapt to climate change. This study aims to contribute to this area by examining the effect of temperature on the sexual expression and distribution in *S. acaulis*, its plant health (Fv/Fm ratio), and their impact on reproduction.

Research questions and hypotheses

To examine how temperature affects *Silene acaulis* sex expression and reproduction, I used two different methodologies. The first evaluates the effects of temperature variation on *S. acaulis* using a natural elevational gradient, comparing populations at low, mid, and high elevations. The second assesses the impact of experimental warming by comparing *S. acaulis* plants inside and outside OTCs.

With these frameworks in place, I explored the following research questions:

1) How do changes in temperature across an elevational gradient and long-term experimental warming influence the plant sex distribution frequencies of *S. acaulis*?

2) How do temperatures and soil moisture levels affect the plant health (Fv/Fm ratio) of *S. acaulis*, as measured by maximum quantum yield?

3) What is the relationship between temperature, soil moisture levels, plant health (Fv/Fm ratio), flower- and plant sex, and reproductive outputs in *S. acaulis*?

The following hypotheses aim to provide testable predictions to each research question:

1) Rooted in the theory that higher environmental severity results in a higher frequency of female plants in gynodioecious systems, I hypothesize that the extreme conditions at the high elevation site will result in an increased proportion of female plants.

2) I hypothesize that relatively low soil moisture levels will negatively impact the plant health (Fv/Fm ratio) of *S. acaulis*. Further, within the elevation gradient setup, I expect the relatively low temperatures at the high elevation site to impair plant health. Additionally, in the experimental warming setup, I expect the warming stress and increased competition inside the OTCs to have a negative effect on the plant health of *S. acaulis*.

3) I hypothesize plants characterized by a low Fv/Fm ratio will have lower reproductive outputs than plants with a high Fv/Fm ratio. Additionally, I predict that female plants will exhibit higher seed quality and greater pollination success than hermaphrodites.

Materials and methods

Study species

Silene acaulis is a common, low-growing cushion plant in the family *Caryophyllaceae.* The species is known for its adaptation to extreme climatic conditions, surviving in temperatures from -80°C to +60°C, and is found in alpine and Arctic regions in the northern hemisphere (Jones & Richards, 1962; Larcher et al., 2010).

Silene acaulis has small and pink flowers that bloom during the summer months, approximately from June to August (Figure 1) (Alatalo & Totland, 1997). Its leaves are densely packed, forming a dome-like cushion that protects it from the harsh and unpredictable environmental conditions. *S. acaulis* reproduces sexually and can form large mats (Jones & Richards, 1962). The seed capsules can contain a large quantity of seeds, open apically, and the seeds are predominantly dispersed by gravity and wind (Gehring & Delph, 1999). *Silene acaulis* is long-lived and slow growing, with the oldest recorded individuals being over 300 years old (Morris & Doak, 1998).

Figure 1: Hermaphrodite S. acaulis *individuals. Left photo: Sara Linn H. Prince. Right photo: Gaute Eiterjord. All photos are credited to Sara Linn H. Prince unless otherwise stated.*

Study area

This study was conducted at Finse in Ulvik municipality in western Norway (Figure 2). The study sites were at Sanddalsnuten, a mountain in the north-west of Hardangervidda mountain plateau, characterized by its alpine tundra ecosystem (Figure 2). The region has a mildly oceanic climate, with the average annual temperature and precipitation at the Finsevatn weather station, recorded from 2003 to 2023, at -0.84°C and 913.39 mm, respectively (MET,

2024). During the summer season (June-August), the average temperature is 7.64°C, and the average precipitation 266.02 mm (MET, 2024).
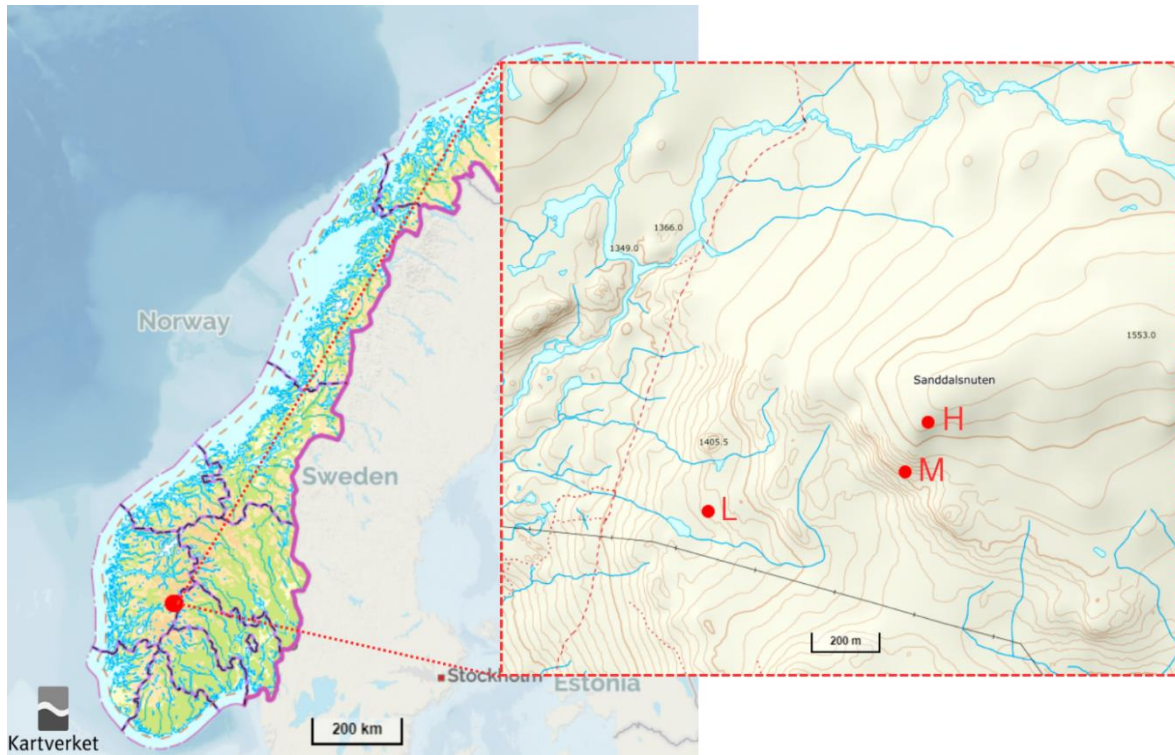

Figure 2: Geographical location of Finse in Ulvik, Norway, denoted by a red dot on the Norway map. Additionally, a close-up inset of Sanddalsnuten with the elevational gradient marked by red dots labelled L (low), M (mid), and H (high). Source: Norgeskart (2023).

Experimental design

For this study, sites at three elevations were established: low (L), mid (M), high (H), along with open top chambers (OTCs) at the highest elevation (Figure 2; Table 1).

Table 1: Site placement information. The high elevation/treatment site utilized already established experimental warming chambers, and the control plots were also used as the highest elevation in the elevational gradient.

Site	Starting coordinates (N, E)	Ending coordinates (N, E)	Elevation (m a.s.l.)
Low	60.612257, 007.510323	60.612402, 007.510150	1387
Mid	60.613877, 007.520373	60.613855, 007.520660	1459
High / OTCs	60.615200, 007.521270	No transect*	1530 - 1550

The experimental warming site, established in 2000, has 40 OTCs, as well as 40 control plots of 1x1 m, to experimentally increase the temperature. The open top chambers (OTCs) act as miniature greenhouses and simulate climate change (Figure 3) (Klanderud, 2005). They are

used to study the response of high-latitude and elevation ecosystem warming, with a mean air temperature increase of 1.2°C-1.8°C inside the chambers (Marion et al., 2003).

At the low (L) and mid (M) elevation sites, a 30-meter measuring tape was laid out in straight transects, and the 30 first flowering individuals within 1 m on each side of the measuring tape were chosen and marked (Figure 3).

In the OTCs, all flowering individuals on the first field trip, 29th of June 2023, were included, resulting in 21 OTCs with 44 total individual plants (Figure 3). Control plants, similar in size to those inside the OTCs, were selected from areas 1-4 m away from paired OTC plots and additionally constituted the highest elevation in the natural elevation gradient.

All individual plants were marked with the plant ID and a marker for visibility (Figure 3).

Figure 3: From left to right: Transect at the lowest elevation, experimental setup with OTCs, and the tag marking a S. acaulis *individual.*

PLANT CHARACTERISTICS AND SAMPLING

To describe the plant characteristics, cushion size was measured, the number of flowers counted, and the sex of the plants (female, hermaphrodite, or mixed) determined. In mixed cushions (Figure 4), the flower sex with the fewest flowers was marked to distinguish between the sexes. Buds were marked and evaluated later.

To measure cushion size, the longitudinal length (a1) and the longest perpendicular width (a2), were measured along the ground plane. The percentage of missing components (PM) within the elliptical shape, including factors such as dead plant material, litter, rocks, or missing chunks due to plant die-back, was estimated. To calculate cushion area, I followed the method of Doak & Morris (2010), using the formula:

$$A = \frac{a1}{2} \times \frac{a2}{2} \times (1 - PM)$$

In early September, seed capsules (Figure 4) were sampled and collected in paper envelopes. The number of capsules on each plant, and the number of capsules of different sex on mixed cushions, were registered. A maximum of 10 seed capsules were collected of both sexes from each individual plant. If the seed capsules were open, they were placed in separate smaller envelopes within the main envelope.

The envelopes were brought to the laboratory at the Norwegian University of Life Sciences (NMBU) where all the capsules were opened, and the seeds were counted and weighed (Figure 4). The complete field and lab work took place from the 26th of June to the 29th of September 2023.

Figure 4: From left to right: Mixed S. acaulis *individual with hermaphrodite and a single female flower, seed capsules, and seeds from a capsule in the lab.*

PHOTOSYNTHETIC EFFICIENCY AND SOIL MOISTURE

The maximum quantum yield of photosystem II (Fv/Fm ratio) was measured using the Chlorophyll Fluorescence Induction Kinetics (OJIP) setting on the FluorPen FP100 (Figure 5) (Photon Systems Instruments, 2013). The measurements were done in the field after the sun went down for dark adaptation and repeated for every plant five times over the field season.

The soil moisture levels were also measured five times over the field season (Figure 5). This was done with the Soil moisture set SM150T with HH2 meter (Delta-T Devices, 2015) inside the cushion, except on one individual growing on rock where it was marked as N/A. Soil moisture measurements resulting in the "Under" or "Above" error was measured again. If the message persisted, "Under" was changed to N/A, and "Above" to 100. The "Above"

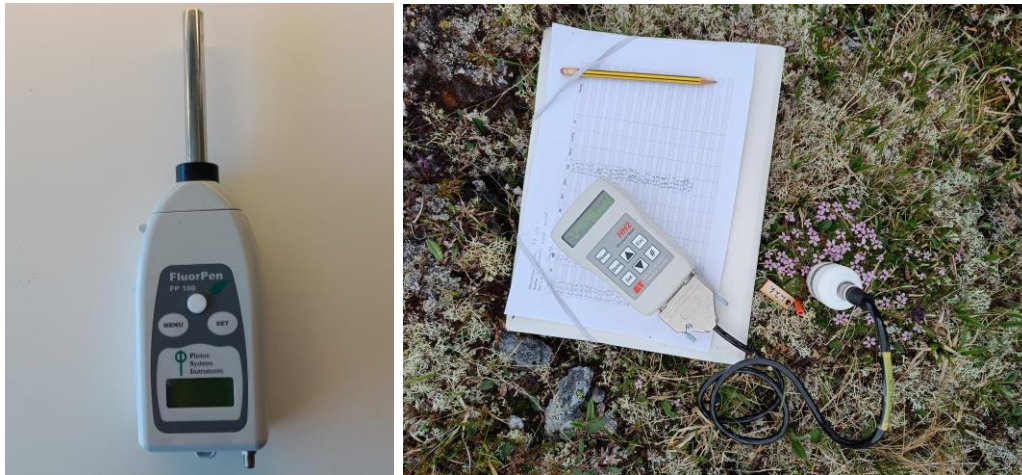measurements occurred mostly in marsh-like areas, or if there had been heavy rainfall a few days prior.

Figure 5: FluorPen (left) used to measure plant health (Fv/Fm ratio), and soil moisture meter (right).

TEMPERATURE DATA

To measure the surface temperature of the *S. acaulis* cushions, I designed, constructed, and programmed do-it-yourself (DIY) temperature sensors, specifically customized for the challenging field conditions at Finse in Norway. I named them "TempBox" followed by an ID number.

The basis for each TempBox was the "Rocketscream Mini Ultra" board, with the microcontroller ATmega4808, chosen for its efficiency and compact size. The infra-red thermometer "MLX90614" sensor was added to measure surface- and ambient temperature, and the "Adafruit MicroSD SPI or SDIO Card Breakout Board" to store the measurement data. Every temperature reading was stored on a 16 GB SD card, and they were powered using two Energizer Ultimate Lithium (L91) AA batteries.

The initial phase of making the TempBoxes consisted of prototyping on a breadboard and exploring a variety of set-ups and coding options (Figure 6). The programming was conducted in C++ in Arduino IDE (Arduino, 2024), where they were programmed to measure and log the surface- and ambient temperature twice, in quick succession, every 15 minutes, and sleep between measurements to preserve power. After prototyping, the sensors were soldered and mounted in robust and waterproof junction boxes, and silica gel packets were placed inside each box to mitigate moisture (Figure 6). Once assembled, the sensors were deployed in the field, placed randomly by plants in OTCs and control plants (Figure 7). Ten

boxes were placed in each group to have a comprehensive collection of temperature data, ensuring redundancy in case some of the boxes malfunctioned. The batteries were changed once a month during the field season.
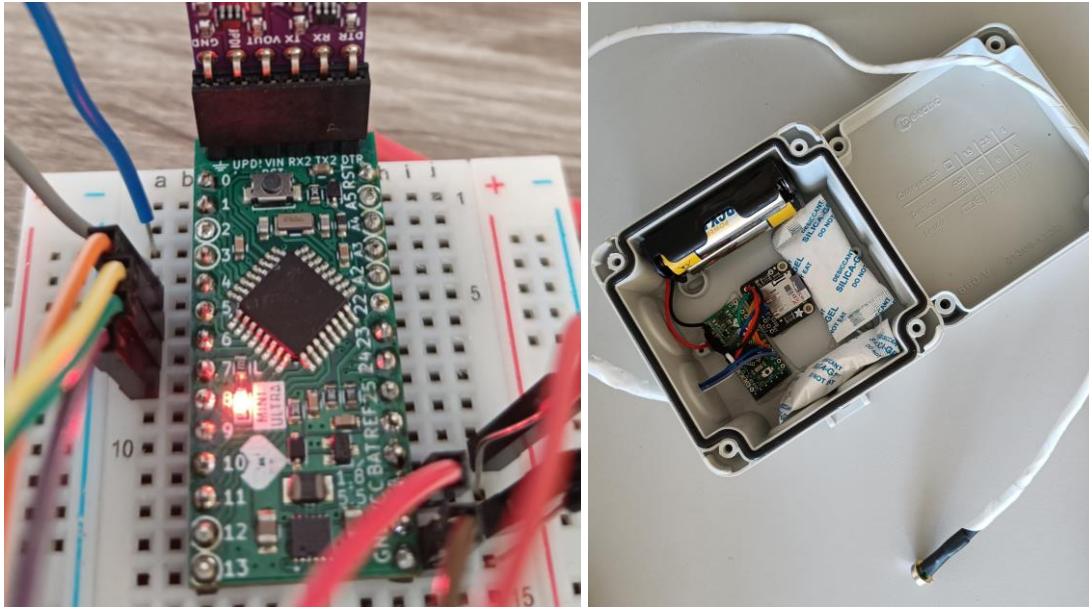
Figure 6: DIY Science. Prototyping (left) and constructing (right) the TempBoxes. Made using the Rocketscream Mini Ultra.

Tinytag temperature loggers (Gemini Data Loggers, 2019) were used to measure air temperature and the temperature inside the cushion (Figure 7). The ambient air temperature loggers were placed randomly with three at the low elevation, three at the mid elevation and four at the high elevation (control plants). One was also placed in an OTC as a backup and comparison to the "TempBoxes". For the in-cushion Tinytags, the *S. acaulis* individuals with large cushion areas were chosen so the thermistor probes would fit inside the plant cushion. For these, two were placed at the low elevation, two at the mid elevation, three at the high elevation (control plants), and four inside the OTCs.

All the temperature sensors measured and logged the temperature every fifteen minutes, with initial deployment between June 28 and July 1. Additional Tinytags were placed two weeks later for backup and supplementary readings at the different elevations and inside the OTCs. The loggers remained in the field throughout the field season and were collected on September 10, 2023.

Figure 7: Ambient- and in-cushion Tinytags (left) and DIY TempBox (right) in placed by S. acaulis *plants in the field.*

Statistical analysis

All statistical analyses were conducted using R (version R-432) in R studio (version RStudio/2023.12.0+369) (R Core Team, 2024). Field and lab data were formatted in Microsoft Excel and split into two separate datasets for the setups. Daily mean, and average daily minimum and maximum, were calculated for all temperature data. The tidyverse package (Wickham et al., 2019), including ggplot2, dplyr, tidyr, readr, and tibble, was used to design and organize all plots, tallies, and tables in my analyses. Model fits were selected based on Akaike Information Criterion (AIC) tests, detailed in Table A1.

TEMPERATURE ANALYSIS

Temperature variation across the elevations and inside and outside the OTCs was illustrated with time-line plots for each temperature type. Nested ANOVAs were conducted using the base function, with dates nested within each elevation or treatment group. Tukey's Honest Significant Difference (TukeyHSD) was performed to compare elevations. The different temperature parameters were tested with one-way ANOVAs, with results shown in boxplots using the gridExtra library (Auguie, 2017).

PLANT SIZE, FLOWER DENSITY AND PLANT SEX PROPORTIONS

The impact of experimental warming and elevation on the plant sex distribution of *S. acaulis* was investigated in three parts. First with plant sex as the response variable and

elevation/treatment as the explanatory variable, then with cushion area (cm^2) as the response and plant sex and elevation/treatment as the explanatory variables, and lastly with flower density as the response and plant sex and elevation/treatment as explanatory variables. Means and standard errors were calculated for cushion area and flower density for the different groups and plant sexes. Variation in flower density was analyzed using the MASS package's (Venables & Ripley, 2002) negative binomial model (glm.nb). Cushion size was log-transformed and analyzed with a two-way ANOVA, followed by a TukeyHSD test, and results presented in boxplots. Plant sex distribution was tested with Pearson's Chi-squared test, and differences within groups with Fisher's test and Bonferroni correction.

SOIL MOISTURE AND PLANT HEALTH

The effect of an elevational gradient and long-term experimental warming, and soil moisture levels on plant health (Fv/Fm ratio) was analyzed in a time-line plot using the scales and zoo libraries (Wickham et al., 2023; Zeileis & Grothendieck, 2005). To make the plot, the values were normalized using the formula (x – min_value) / (max_value – min_value). A linear mixed model was fitted using the lme4 library (Bates et al., 2015) to analyze the effect of time, cushion area (cm^2), flower density, vegetation cover and soil moisture on the plant health, with p values found with the lmerTest library (Kuznetsova et al., 2017). Further analysis on plant health (Fv/Fm ratio) used the betareg library (Cribari-Neto & Zeileis, 2010) for a beta regression model, with results illustrated in a boxplot. To analyze the soil moisture in the different sites and treatments, the variable was log-transformed, and a one-way ANOVA was performed.

RELATIONSHIP BETWEEN TEMPERATURE, SOIL MOISTURE, PLANT HEALTH, SEX, AND REPRODUCTIVE OUTPUTS

To explore the relationship between different environmental conditions, plant health (Fv/Fm ratio), plant sex, and reproduction for *S. acaulis,* pollination success was calculated as the ratio of seed capsules to flowers per plant. Beta-regression analyses tested the effect of plant sex and elevation or treatment on the pollination success, with results illustrated in boxplots. The impact of plant health (Fv/Fm ratio) and soil moisture on pollination success was also analyzed using beta regression models. Mean seed mass and mean number of seeds per capsule were fitted as linear models, and prediction plots were made and organized using the patchwork package (Pedersen, 2024). The interaction between pollination success and mean number of seeds per capsule at the mid elevation was illustrated in a contour plot using the ggpubr package (Kassambara, 2023).

Results

Temperature

Elevational gradient

The ambient air temperature was significantly coldest at the high elevation compared to the low and mid elevation (Figure 8, Table 2). However, the temperature did not differ significantly between the low and mid elevations. Inside the plant cushions, the temperature was significantly warmest at the mid elevation (Figure 8, Table 2). The low elevation was colder than the highest elevation, although this difference was only marginally significant (Table 2).
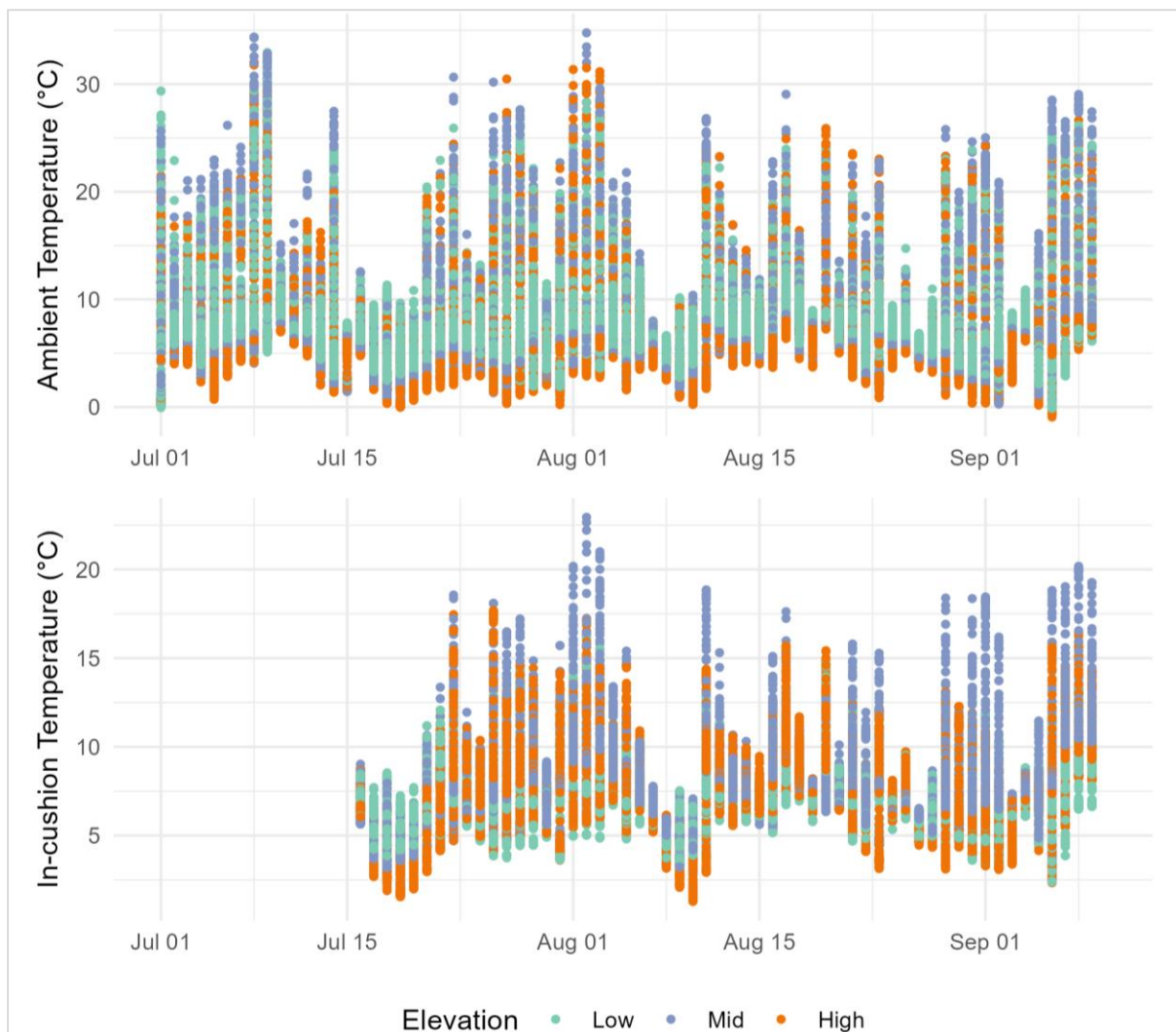

Figure 8: Daily variation of ambient- (top) and in-cushion (bottom) temperature for S. acaulis *across an elevational gradient at Finse, Norway. Measured by Tinytags.*

Table 2: Nested ANOVA and Post-Hoc (Tukey HSD) results for ambient- and in-cushion temperature with the factors "Elevation" and "Date". The nested model accounts for the multiple logs within each day throughout the measurement period, organized by elevation.

Temperature Type	Factor	DF	Sum Sq	Mean Sq	F value	p-value
Ambient	Elevation	2	2947	1473.5	47.818	**<0.001**
	Elevation:Date	3	539	179.8	5.834	**<0.001**
	Residuals	20442	629936	30.8		
In-cushion	Elevation	2	2592	1295.9	161.93	**<0.001**
	Elevation:Date	3	2090	696.8	87.07	**<0.001**
	Residuals	16122	129017	8		

Temperature Type	Group Comparison	Difference	Lower	Upper	p-Value
Ambient	Low-High	0.795	0.572	1.018	**<0.001**
	Mid-High	0.815	0.593	1.038	**<0.001**
	Mid-Low	0.021	-0.202	0.243	0.974
In-cushion	Low-High	-0.125	-0.253	0.002	*0.056*
	Mid-High	0.781	0.653	0.909	**<0.001**
	Mid-Low	0.906	0.778	1.034	**<0.001**

When analyzing the different temperature parameters for ambient air temperature, only the highest elevation showed significantly colder average daily minimum temperatures of 2.81°C compared to 3.95°C at the low elevation. For the daily mean and average daily maximum parameters, there were no significant differences. Inside the plant cushion, temperatures at the mid elevation were significantly warmer, with an average daily maximum of 13.01°C compared to 10.53°C at the low elevation and 11.00°C at the high elevation. The daily mean temperature in the cushion was also warmer at mid elevation, registering at 8.12°C compared to 7.22°C at low and 7.34°C at high (Figure 9, Table A2). Full ANOVA results can be found in Table A3.
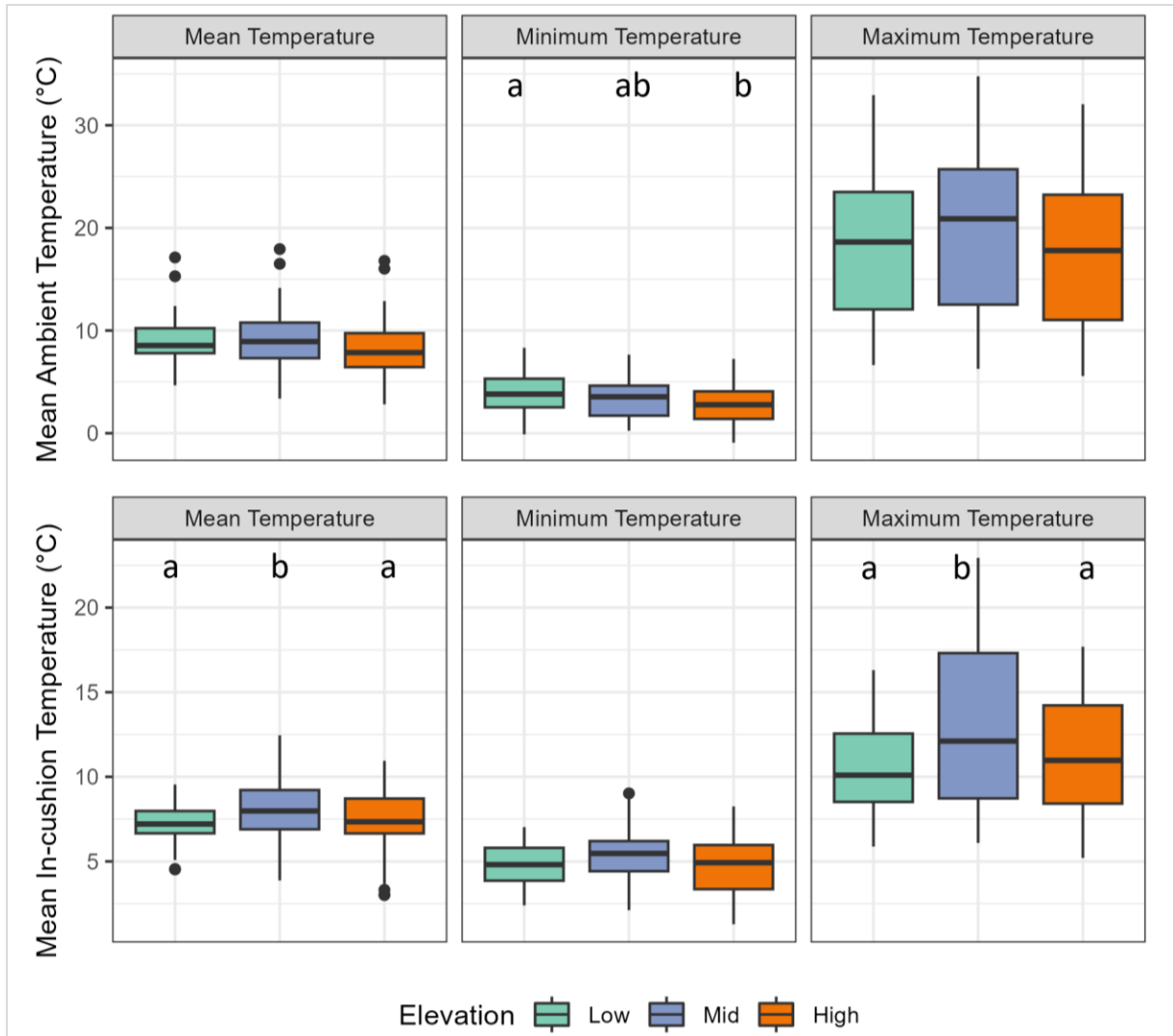
Figure 9: Boxplots illustrating the temperature over the season at the different elevations, comparing different parameters for ambient- and in-cushion temperature. Significance at p<0.05, illustrated by letters within each temperature parameter.

Long-term experimental warming

In the long-term experimental warming setup, ambient-, surface- and in-cushion temperature were significantly warmer in the open top chambers (OTCs) than in the control plants (Figure 10; Table 3). The interaction between treatment and date also significantly differed between OTCs and controls, but not for surface temperature (Table 3).
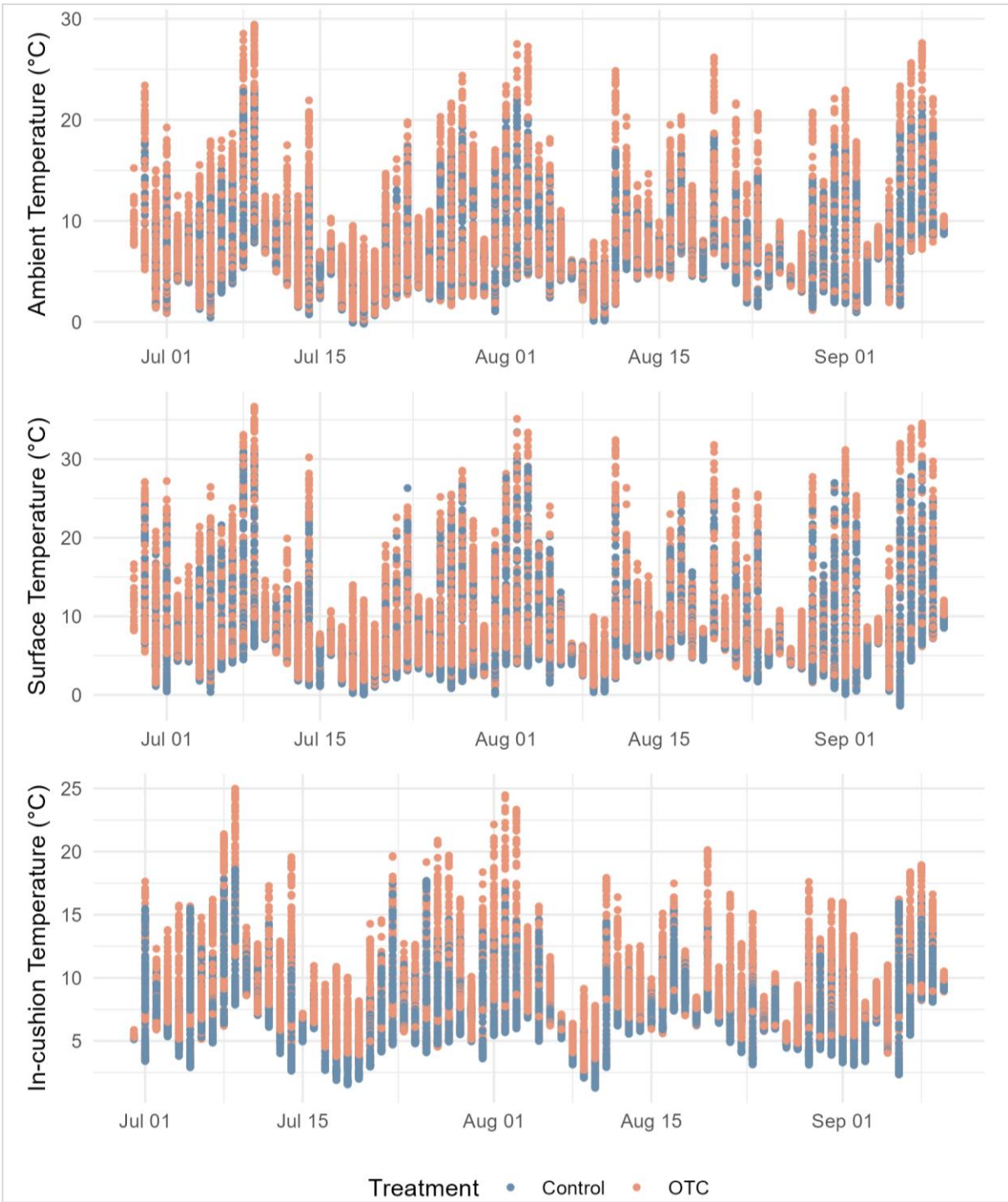
Figure 10: Daily variation of ambient- and surface temperature (TempBox) and in-cushion temperature (Tinytag) for S. acaulis *inside and outside OTCs at Sanddalsnuten, Finse, Norway, through the growing season.*

Table 3: Nested ANOVA results for ambient-, in-cushion-, and surface temperature with the factors "Treatment" and "Date". The nested model accounts for the multiple logs within each day throughout the measurement period, organized by treatment (OTC/Control).

Temperature Type	Factor	DF	Sum Sq	Mean Sq	F value	p-value
Ambient (Tinytag)	Treatment	1	1140	1139.7	33.27	**<0.001**
	Treatment:Date	2	1967	983.5	28.71	**<0.001**
	Residuals	12304	421502	34.3		
In-Cushion	Treatment	1	4350	4350	384.94	**<0.001**
	Treatment:Date	2	455	227	20.12	**<0.001**
	Residuals	13734	155209	11		
Surface	Treatment	1	4465	4465	128.81	**<0.001**
	Treatment:Date	2	127	64	1.836	0.159
	Residuals	14071	487732	35		
Ambient (TempBox)	Treatment	1	3339	3339	154.9	**<0.001**
	Treatment:Date	2	736	368	17.06	**<0.001**
	Residuals	14071	303309	22		

The daily mean and average daily maximum temperature were significantly warmer inside the OTCs compared to control plants for ambient-, surface-, and in-cushion temperatures. There was no difference in minimum temperature inside the OTCs compared to control. The temperature inside the OTCs were approximately 1.1°C-1.2°C times as warm as for the control plants (Figure 11, Table A4). ANOVA results in Table A5.

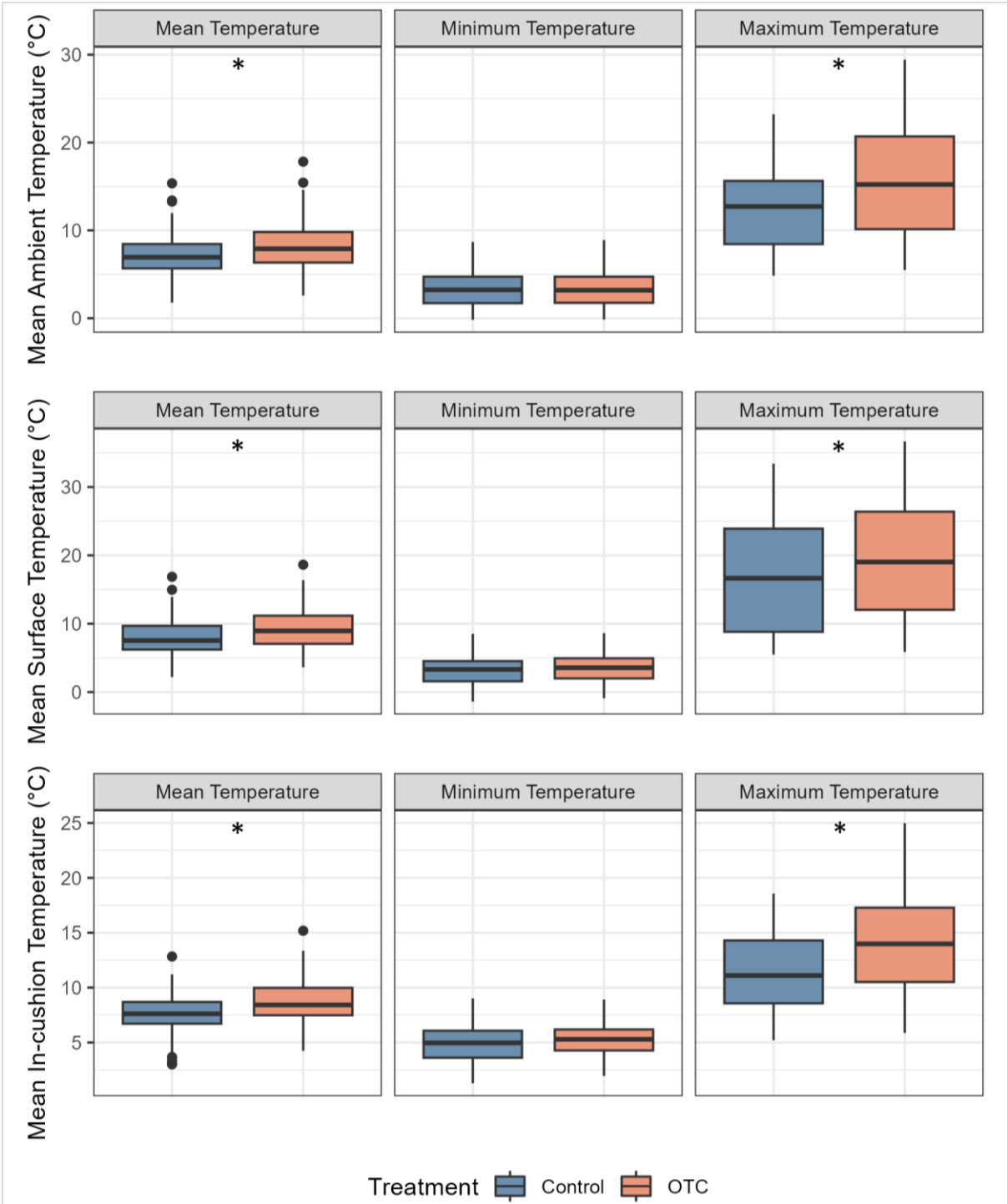*Figure 11: Boxplots illustrating the temperature over the season inside and outside OTCs, comparing different parameters for ambient-, surface-, and in-cushion temperature. Significance at p<0.05, illustrated with an asterisk within each temperature parameter.*

Plant sex proportions

There were no significant differences for plant sex proportion across the elevations and inside versus outside the OTCs. Within the low elevation there was a significantly higher proportion of female plants compared to hermaphrodites, and within the mid elevation there was a significantly higher proportion of female plants compared to mixed (Table 4). At the high elevation and control site female plants had a significantly higher proportion than both hermaphrodite and mixed plants (Table 4). Within the OTCs as well, the proportions of female plants were significantly higher than both mixed and hermaphrodite plants (Table 4).

Table 4: Proportions of the plant sexes (female, hermaphrodite, mixed cushion) at the different elevations, and for the control and OTCs. Significant differences within sites denoted with letters. Significance at p<0.05.

Low			Mid			High and Control			OTCs		
Plant sex	**Prop**		**Plant sex**	**Prop**		**Plant sex**	**Prop**		**Plant sex**	**Prop**	
Female	0.500	a	Female	0.600	a	Female	0.659	a	Female	0.705	a
Herm	0.167	b	Herm	0.267	ab	Herm	0.205	b	Herm	0.205	b
Mixed	0.333	ab	Mixed	0.133	b	Mixed	0.136	b	Mixed	0.090	b

Plant size and flower density

The *S. acaulis* cushions at the high elevation were significantly smaller than at the low elevation (Figure 12, left). The cushion size was also significantly larger for mixed plants with both female and hermaphrodite flowers (Figure 12, right). Plant sex and elevation did not individually have a significant impact on flower density. However, there was a significant interaction effect as mixed-sex plants exhibited a higher flower density at the highest elevation, compared to the low elevation (Table A6). Means and standard errors for plant size and flower density for elevation and plant sex can be found in Table A7.
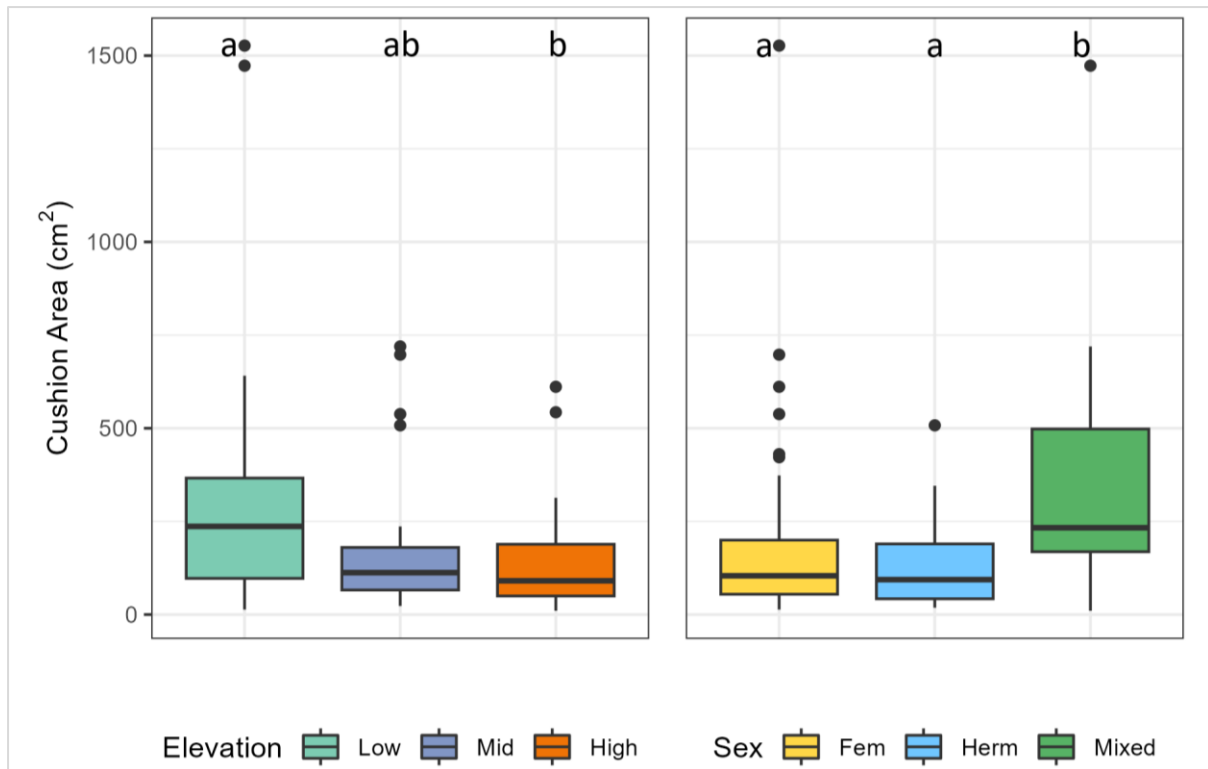
Figure 12: Cushion size (cm²) by elevation (left) and plant sex (female, hermaphrodite and mixed) (right). Significance at p<0.05, denoted by letters within each facet.

There was no effect of experimental warming on cushion size. The flower density was higher for mixed plants, but the combined effect of warming (OTCs) and mixed plant sex, reduced flower density (Table A8). Calculated means and standard errors for plant size and flower density are illustrated in Table A9.

The effect of soil moisture and plant sex on plant health

The soil moisture levels were lowest at the highest elevation, significantly different from the mid elevation. There was no significant difference in soil moisture between the low and mid elevation (Table A10).

In the elevational gradient, the plant health (Fv/Fm ratio) of *S. acaulis* significantly declined over time (number of days in the growing season) (Figure 13, Table 5). Plant health also seems to have decreased with increasing soil moisture, but this was not significant in the elevational gradient (Table 5). The plants at the high elevation had significantly lower plant health compared to the low elevation (Figure 14, Table 5, Table A11).
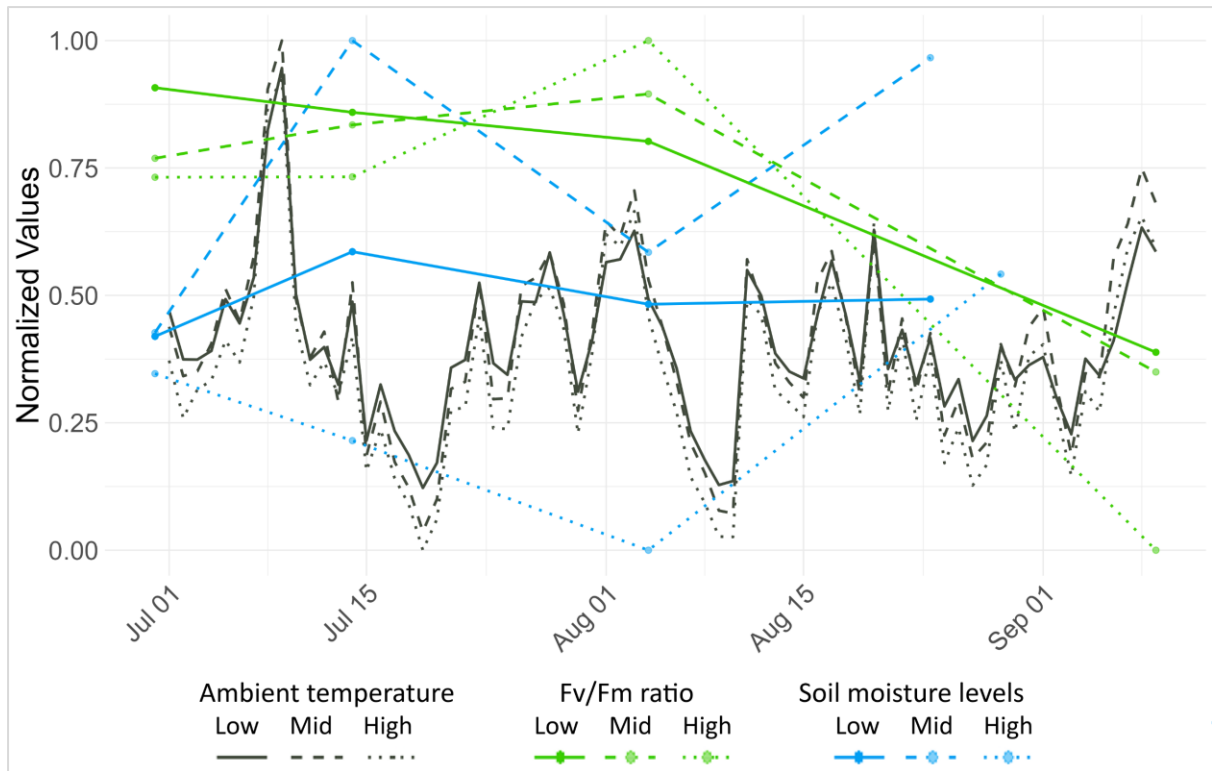
Figure 13: Relative changes in temperature, soil moisture levels, and Fv/Fm ratio over the growing season for S. acaulis *at the different elevations at Sanddalsnuten, Finse, Norway.*

Table 5: Results from a linear mixed effect model the effect of time (Date), elevation, area, flower density, vegetation cover, and soil moisture on the plant health (Fv/Fm ratio) of S. acaulis. *Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001. Trends marked with .p<0.1.*

Variable	Estimate	Std. Error	Degrees of Freedom	t-value	p-value	
(Intercept)	1.598	0.066	129.1	24.164	**<0.001**	***
Date (numeric)	-0.005	0.001	313	-7.655	**<0.001**	***
Elevation Mid	-0.036	0.052	97.17	-0.684	0.495	
Elevation High	-0.129	0.049	97.2	-2.648	**0.009**	**
Soil Moisture (%)	-0.002	0.001	204.5	-1.762	*0.080*	.
Area (cm^2)	<-0.001	<0.001	96.57	-1.685	*0.095*	.
Flower density (cm^2)	-0.052	0.055	96.47	-0.950	0.344	
Vegetation cover (%)	<-0.001	0.001	96.49	-0.238	0.812	
Random Effect	**Variance**	**Std. Dev.**				
Plant ID (Intercept)	0.009	0.097				
Residual	0.108	0.329				

21
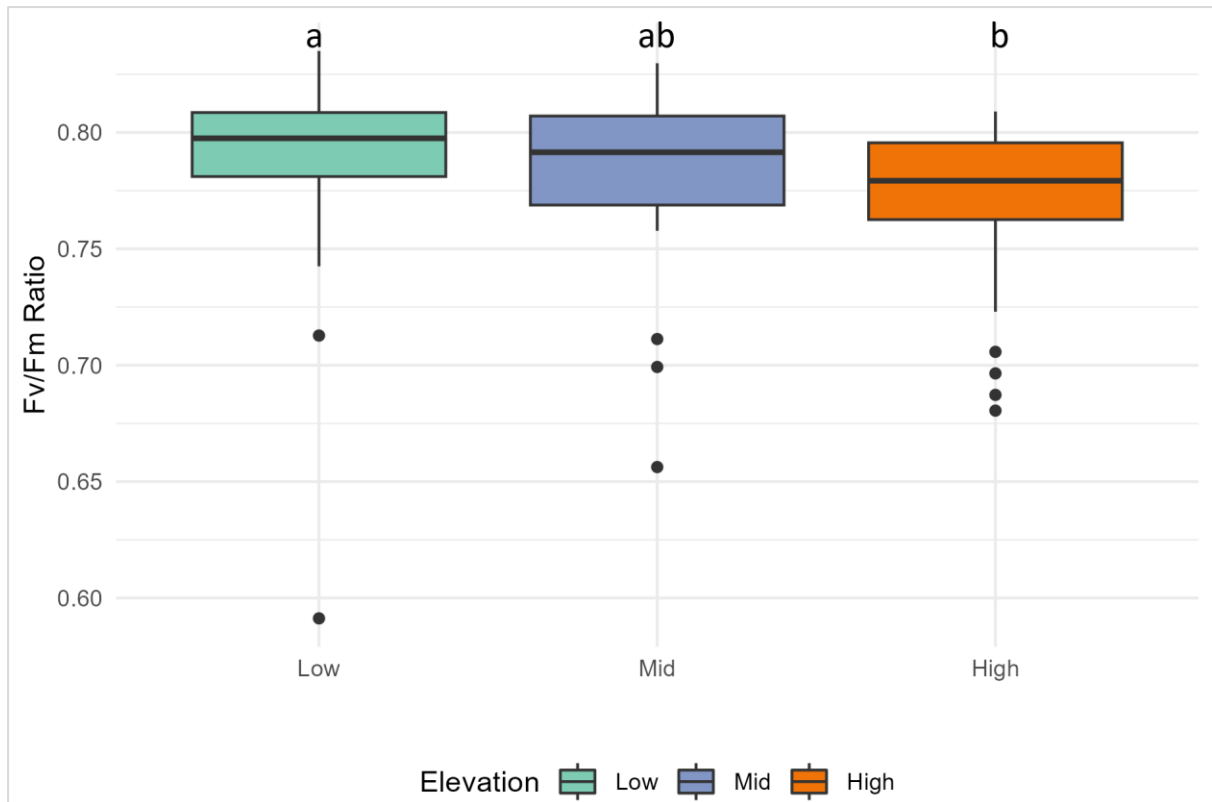
Figure 14: Boxplot showing the difference in the plant health (Fv/Fm ratio) at different elevations. Significance at p<0.05, denoted by letters.

The soil moisture was significantly lower inside the OTCs compared to the control plants (Table A12).

In the OTCs, the plant health (Fv/Fm ratio) of *S. acaulis* significantly declined over time (Figure 15, Table 6). Higher soil moisture had a significant negative relationship with plant health (Fv/Fm ratio), and bigger cushion size (cm^2) had a small positive effect (Table 6). The plants inside the OTCs had better plant health compared to the control plants (Figure 16, Table 6, Table A13).

There was no significant relationship between sex and the Fv/Fm ratio at the different elevations, or for plants inside and outside the OTCs (Table A14).
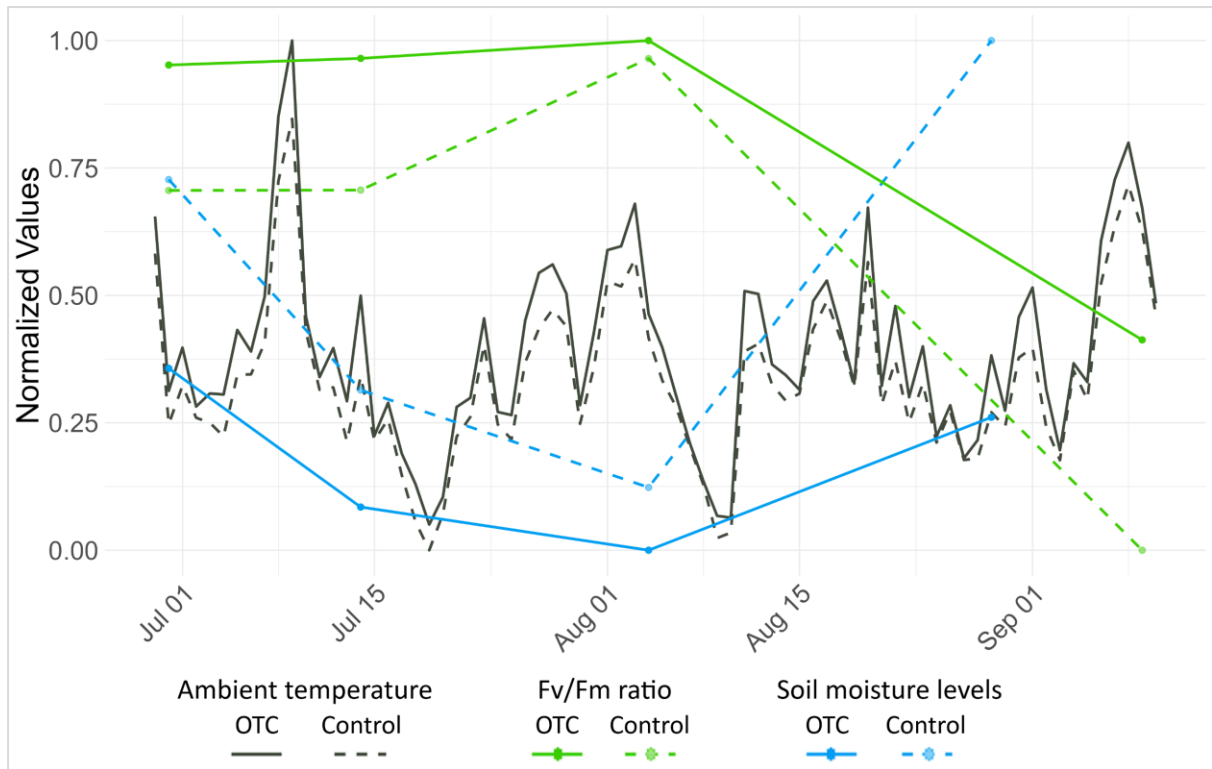
Figure 15: Relative changes in temperature, soil moisture levels, and Fv/Fm ratio over the growing season for S. acaulis *inside and outside OTCs at Sanddalsnuten, Finse, Norway.*

Table 6: Results from a linear mixed effect model the effect of time (Date), treatment, area, flower density, vegetation cover, and soil moisture on the plant health (Fv/Fm ratio) of S. acaulis. *Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.*

Variable	Estimate	Std. Error	Degrees of Freedom	t-value	p-value	
(Intercept)	1.442	0.053	143	27.282	**<0.001**	***
Date (numeric)	-0.005	0.001	256.6	-10.153	**<0.001**	***
Treatment OTC	0.134	0.035	83.6	3.780	**<0.001**	***
Soil Moisture (%)	-0.003	0.001	264.1	-2.680	**0.008**	**
Area (cm^2)	<0.001	<0.001	77.7	2.269	**0.026**	*
Flower density (cm^2)	0.008	0.049	75.8	0.163	0.871	
Vegetation Cover (%)	0.001	0.001	79.1	0.834	0.407	
Random Effect	**Variance**	**Std. Dev.**				
Plant ID (Intercept)	0.008	0.091				
Residual	0.063	0.252				
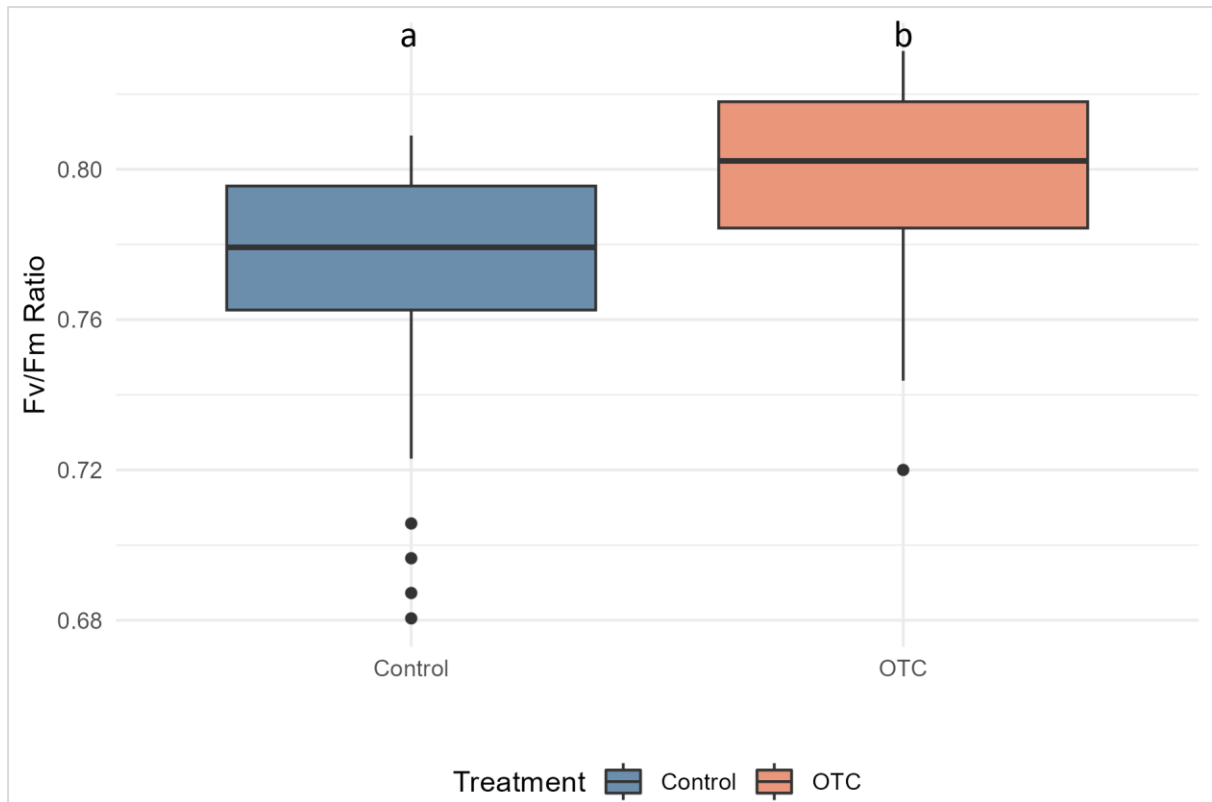
Figure 16: Boxplot showing the difference in plant health (Fv/Fm ratio) between S. acaulis *inside and outside OTCs. Significance at p<0.05, denoted by letters.*

Pollination success

Elevation

In the elevational gradient, there was no significant relationship between the plant health (Fv/Fm ratio) and pollination success (nr seed capsules / nr flowers) of *S. acaulis* (Table A15). However, there may have been a small negative effect of higher soil moisture level, but this was not statistically significant (p 0.076, Table A15).

There was no significant relationship between elevation and pollination success of *S. acaulis* (Figure 17, Table A16). The plant sex categories had more impact, with hermaphrodite plants having very low pollination success compared to female plants (Figure 17). Mixed plants also had lower pollination success compared to female plants, but with a lot more variation than hermaphrodite plants (Figure 17).
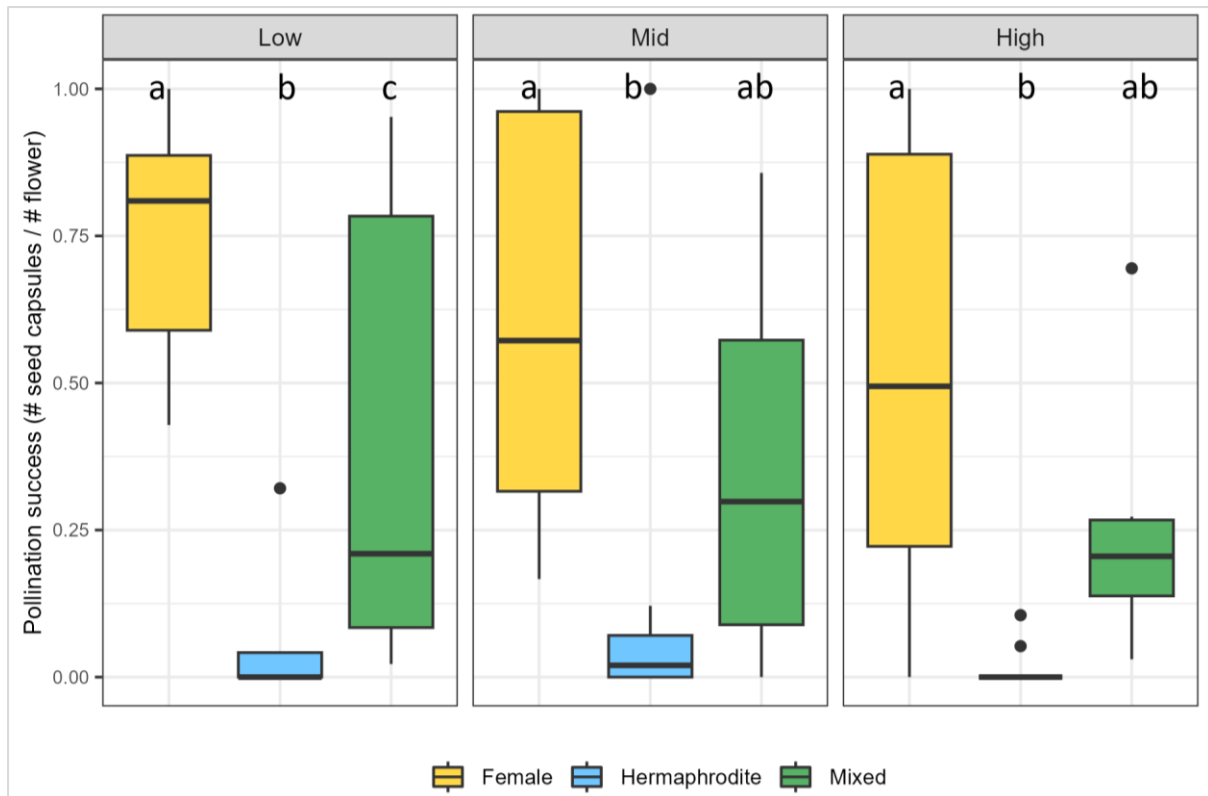
Figure 17: Pollination Success by plant sex across elevations. Pollination success is calculated as the seed capsule to flower ratio. Significance at p<0.05, denoted by letters within each site.

Long-term experimental warming

In the long-term warming experiment, there was a slight negative relationship between higher plant health (Fv/Fm ratio) and pollination success (p-value of 0.084, Table A17). There was no relationship between soil moisture and plant health inside or outside the OTCs (Table A17).

The pollination success varied greatly between plant sexes, with female plants having the highest success for control plants, but with a lot of variation, and mixed plants having the highest success in OTCs (Figure 18). The effect of sex was only significant for hermaphrodites compared to females (Figure 18, Table A18). The hermaphrodite plants had extremely low pollination success for both groups, but in the OTCs none of the flowers of the hermaphrodite plants produced seed capsules (Figure 18). The warming effect of the OTCs did not have a significant effect on the pollination success.

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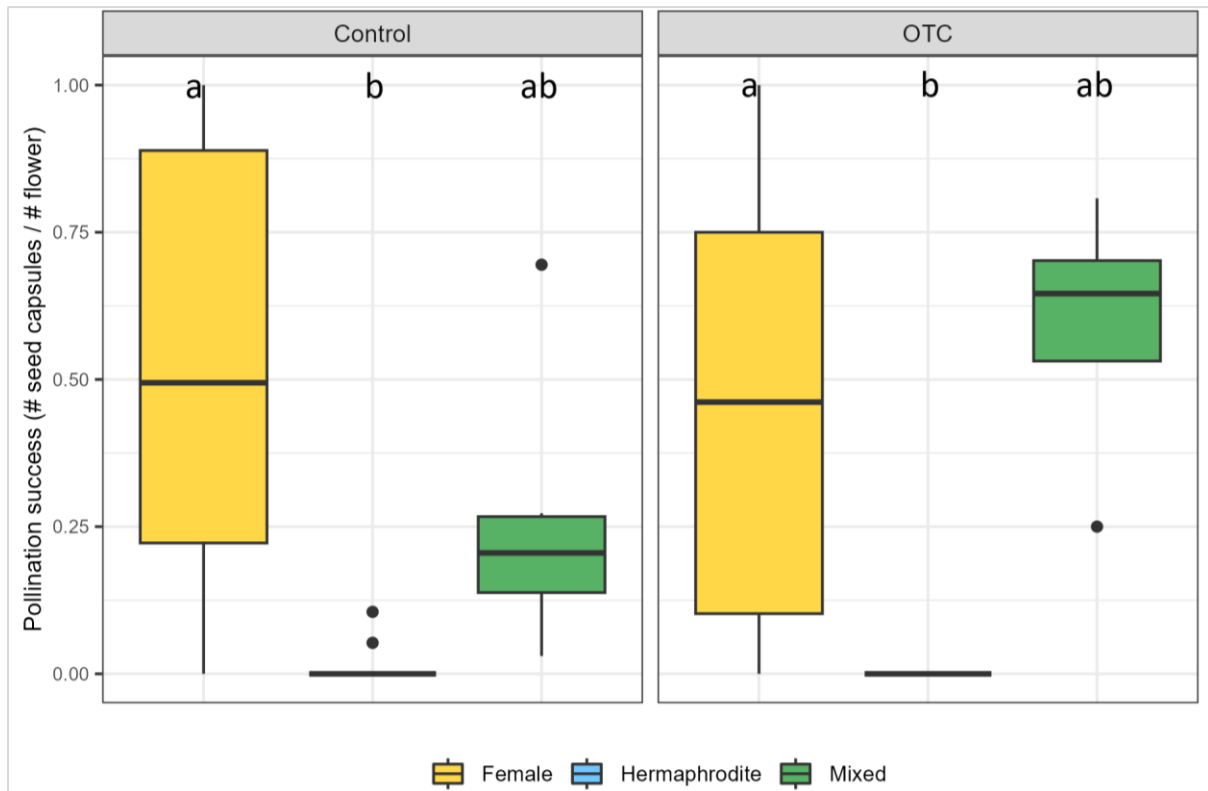
Figure 18: Pollination Success by plant sex inside and outside OTCs. Calculated as the seed capsule to flower ratio. Significance at p<0.05, denoted with letters within each treatment group.

Reproductive outputs

The pollination success for hermaphrodites was too low to yield enough seed capsules for sufficient sample size. Therefore, the analyses on average seed mass and number of seeds per capsule does not separate by sex, but focuses on the effect of soil moisture, Fv/Fm ratio, pollination success, along with treatment or elevation.

Elevational gradient

The mean seed mass for plants at the mid elevation was significantly heavier compared to the low elevation (Figure 19, Table A19). Pollination success may also have a slightly positive effect on mean seed mass, although not significant ($p = 0.089$). Further analysis revealed some complex interaction effects at the mid elevation. The positive relationship between pollination success and seed mass was significantly reduced, and a higher number of seeds led to a more pronounced decrease in the mean seed mass, compared to the low elevation (Figure A1). However, the combination of higher pollination success and a greater number of seeds per capsule, surprisingly had a positive effect on seed mass (Figure A1, Figure A2).
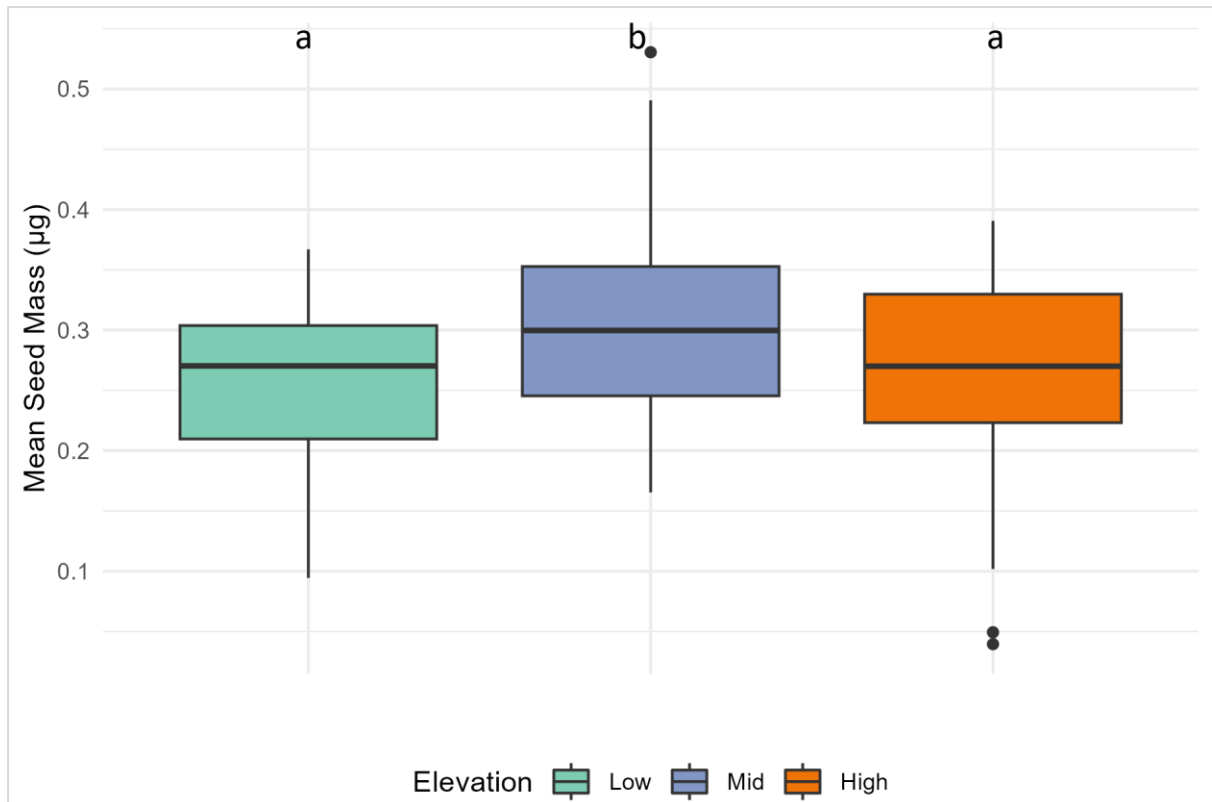
Figure 19: Mean seed mass per seed (μg) at the different elevations. Significance at p<0.5, denoted with letters.

For the mean number of seeds per capsule (log-transformed), a higher pollination success led to significantly more seeds, and there were also significantly less seeds per capsule at the highest elevation compared to low (Figure 20, Table A20).
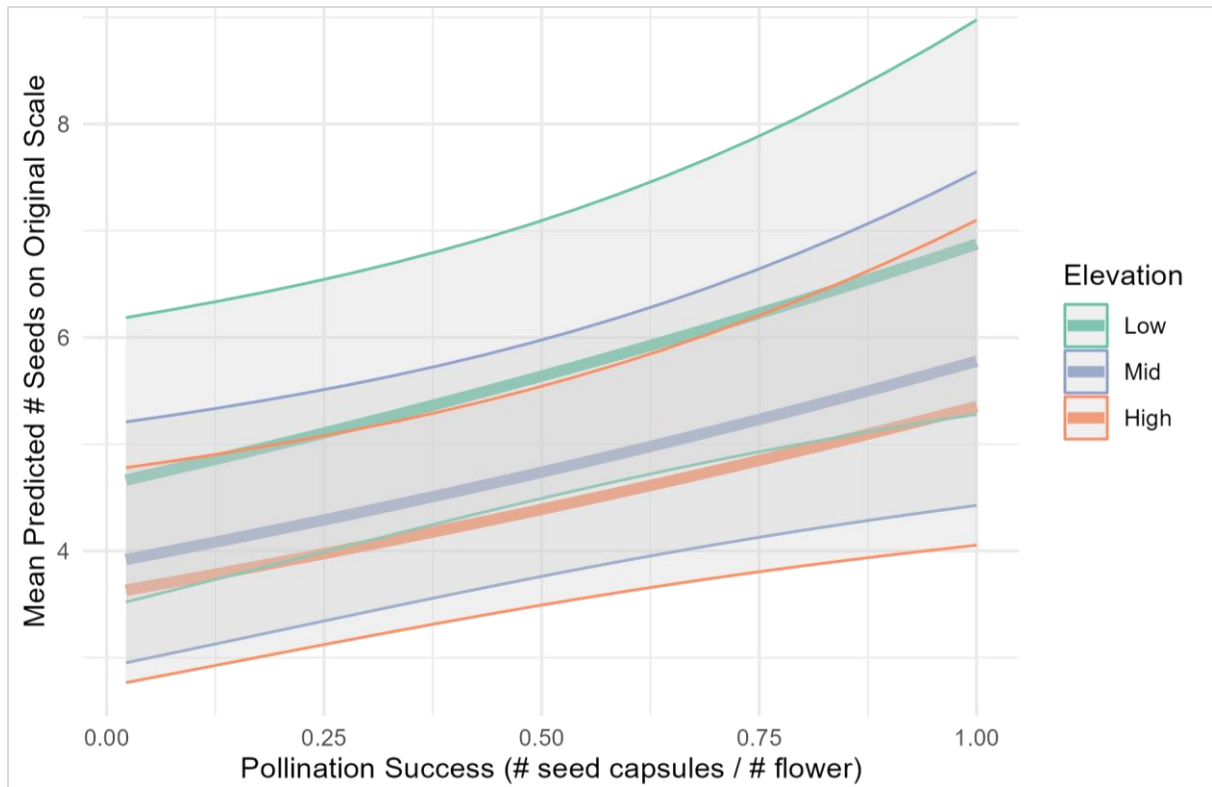
Figure 20: Relationship between plant pollination success and the number of seeds per capsule, analyzed across elevation. Response variable is back-transformed to the original scale.

Long-term experimental warming

For the long-term experimental warming, a higher pollination success significantly increased the mean seed mass for *S. acaulis*. There was also a trend (p = 0.079), suggesting that the increasing effect of pollination success was less pronounced for plants inside OTCs compared to control plants (Figure 21, Table A21). The average number of seeds per capsule was significantly lower inside the OTCs compared to the control plants (Figure 22, Table A 22).
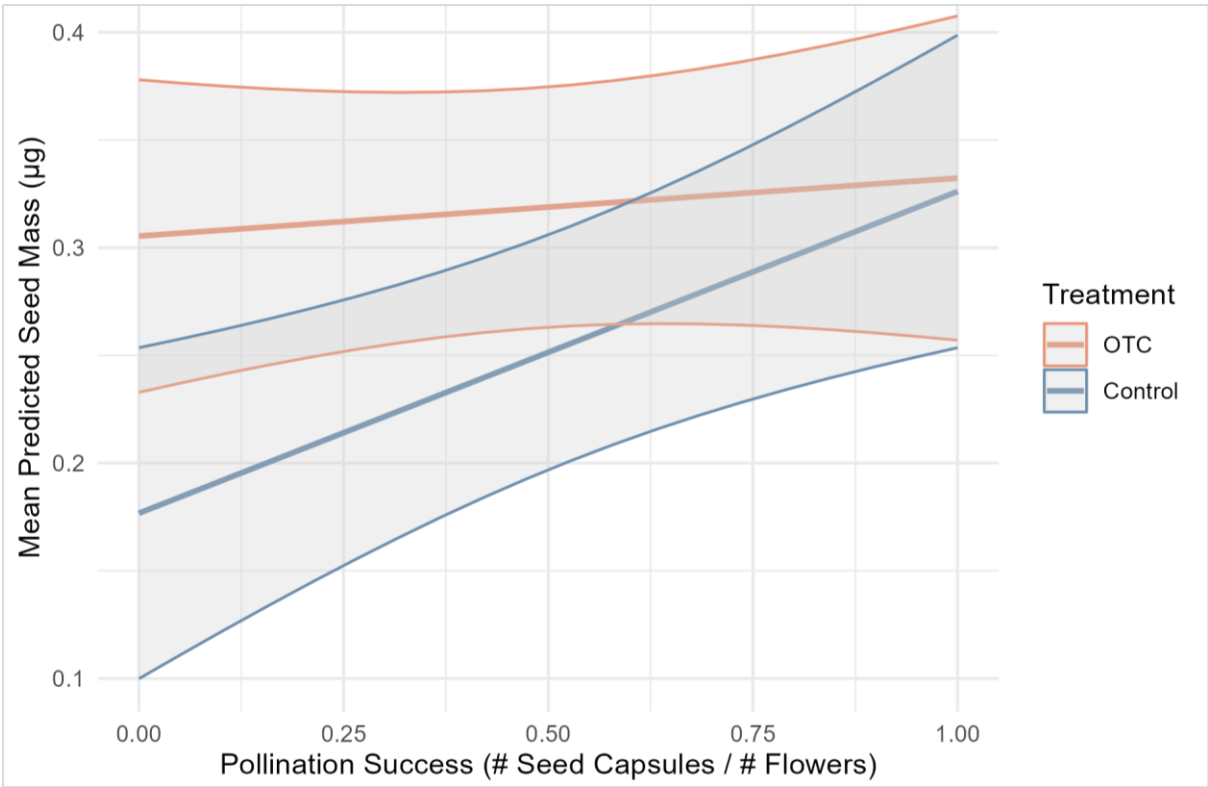
Figure 21: Prediction plot showing the relationship between pollination success and mean seed mass per seed (μg) across OTC treatment and control plants.
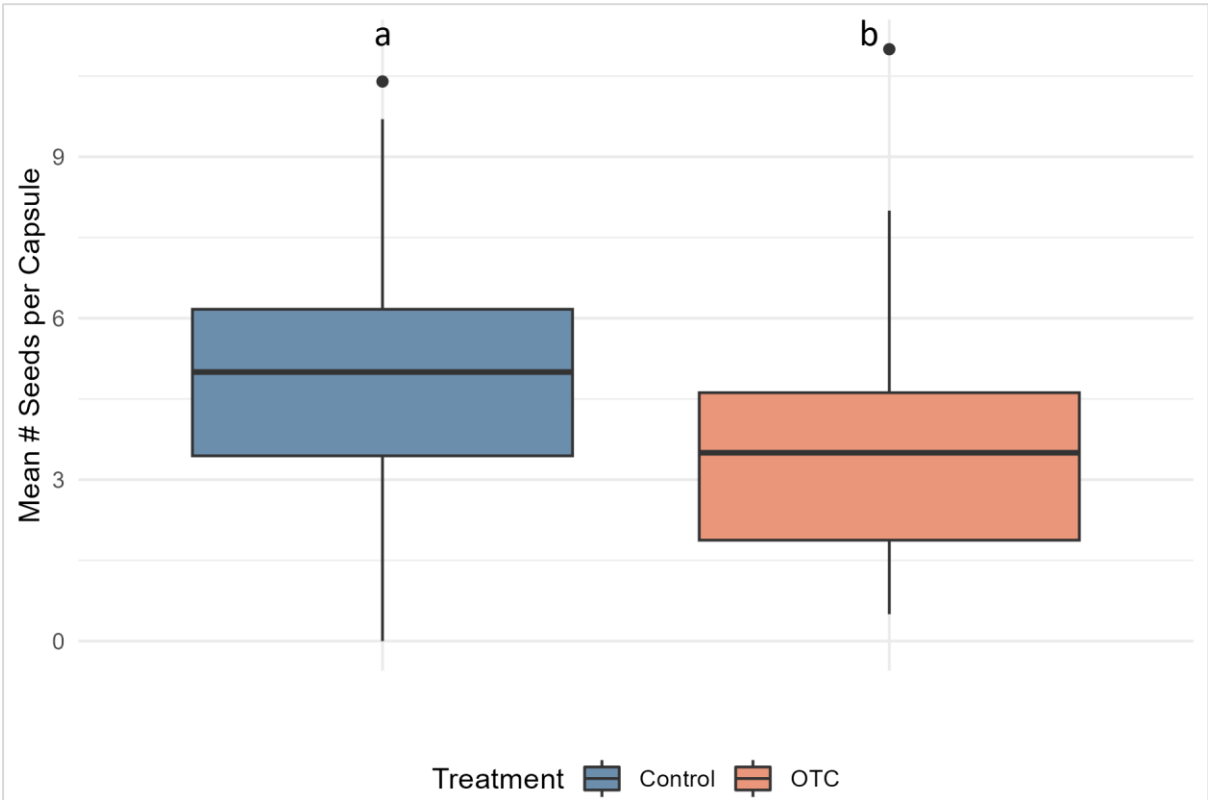
Figure 22: Mean number of seeds per capsule for control plants and inside OTCs. Significance at p<0.05, denoted by letters.

Discussion

This study explores the responses of *Silene acaulis* to a changing climate, with a specific focus on the effects of increased temperature on plant sex expression, reproduction, and plant health. Two methodologies were used to study the interaction between temperature and *S. acaulis*: a natural elevation gradient, and an experimental warming treatment with open top chambers (OTCs).

Exploring the impact of temperature on *S. acaulis* across an elevation gradient and within the OTCs revealed complex interactions between climatic factors and reproductive strategies. Initially, it was hypothesized that stressful conditions, through either higher elevation or relatively high temperatures, would lead to an increase in the proportion of female plants. However, I found a non-significant increase of 32% in females from the low to high elevation sites, and no differences between plants inside the warmed chambers and control plants outside. Although an increase was observed, my results suggest that temperature does not have a significant role in shifting the plant sex distribution towards a higher female frequency. The second hypothesis, exploring how variations in soil moisture and temperature impacts the plant health (Fv/Fm ratio) of *S. acaulis* was partly supported. The cushions at the high elevation site had the lowest plant health on the elevation gradient, supporting the hypothesis. However, the greater plant health of the plants inside the OTCs compared to the control plants did not. Additionally, contrary to the hypothesis, my findings indicated that higher soil moisture levels, instead of lower levels, negatively affected plant health (Fv/Fm ratio). The relationship between environmental stressors and reproductive outputs was examined in the third hypothesis. Stressful conditions were expected to increase female plant seed quality and pollination success, but the results were varied. No relationship was found between the plant health and pollination success along the elevational gradient, and in the warming setup, only a weak negative trend was observed. There was a significant difference in the number of seed capsules produced, with hermaphrodite plants producing almost none, strongly supporting part of the hypothesis. Consequently, the low number of seeds from hermaphrodite plants made it impossible to analyze seed quality by sex.

The effect of temperature on plant sex distribution patterns

The change in sex distribution was not significant, however, an increase in female frequency from 50% to 66% was observed between the low and high sites. This observation aligns with Alatalo & Molau (1995) who found that the percentage of female *S. acaulis* plants increased

from 42% to 59% with higher elevation. Further, my results are consistent with theory suggesting that female individuals have a fitness advantage in gynodioecious systems. The outcrossing hypothesis (Sun & Ganders, 1986) proposes that higher selfing rates among hermaphrodites leads to increased inbreeding depression, while female seeds have higher fitness due to outcrossing (Alatalo & Molau, 1995; Inouye, 2020; Reid et al., 2014). Such differences in fitness may ultimately result in higher proportions of females in the population (Alatalo & Molau, 1995), particularly at high elevations where pollinators are often in low abundance (Reid et al., 2014). My findings further support this idea, as the highest elevation site, characterized by relatively cold and poor conditions, had significantly more of female individuals, compared to mixed and hermaphrodites.

Tushabe et al. (2023), in a study of four *Silene* species, found that hermaphrodite individuals were considerably more susceptible to damage from environmental stressors than female individuals. During the gametophytic stage, environmental stress negatively affected hermaphrodite flower bud formation, flowering, and fruit ripening at a considerable higher degree than in females (Tushabe et al., 2023). This supports the speculation that *Silene* hermaphrodites are less equipped to handle the extreme conditions at the highest elevations, possibly leading to long-term changes in their distribution patterns. Although *S. acaulis* was not included in this study, the results are consistent with my findings and may point to a larger, generalizable conclusion that hermaphrodites are more likely to be limited by environment than female plants.

Experimental warming using open top chambers (OTCs) did not show a significant difference in plant sex proportions within (70%) and outside (66%) the OTCs. This suggests that if there is an evolutionary shift in plant sex distribution due to the outcrossing hypothesis, it may not yet be observable in this experiment. Given that the OTCs have been in use for 23 years and *Silene acaulis* can live up to 300 years, the duration of the OTC treatment may be too short to detect any long-term changes due to increased inbreeding depression.

Plant health responses to soil moisture and temperature

Interestingly, the Fv/Fm ratio was lower for plants with higher soil moisture levels, which strongly contrasts with the hypothesis of drier soils leading to lower plant health. A possible explanation for this could be that the field summer was characterized by heavy rainfall and relatively cold temperatures, leading to waterlogged conditions in some areas. Waterlogged soil can significantly impact plant physiological processes, impairing the root system's ability

to take up nutrients and exchange gases, something that is crucial for maintaining a healthy plant (Sairam et al., 2008). Increased soil moisture could also exacerbate cold stress (Singh et al., 2023). However, to better understand the relationship between soil moisture and plant health, studies across years in which variation in temperature and precipitation occur are required; something beyond the scope of this study. Nonetheless, my results can serve as a starting point for longer-term evaluations of the physiological responses of *S. acaulis* to different soil moisture stressors.

Silene acaulis cushions inside the OTCs had better plant health (a higher Fv/Fm ratio) than nearby control plants. The increase in the plant health (Fv/Fm ratio) suggests that *S. acaulis* has the ability to respond positively to elevated temperatures by enhancing its photosynthetic efficiency. This finding aligns with the concept that alpine plants are capable of adjusting their physiological processes to cope with the gradually changing climate (Körner & Hiltbrunner, 2021).

However, there may be a potential threshold where the positive effect of warming abruptly turns negative. *Colobanthus quitensis,* like *S. acaulis*, is a cushion plant in the Caryophyllaceae family and adapted to extremely cold and harsh environments. Xiong et al. (1999) found clear temperature thresholds for *C. quitensis:* a depression in photosynthetic rates at temperatures above 26°C or below -2°C, and a critical threshold at approximately 42°C where the photosynthetic apparatus was directly impaired. The relatively cool growing season conditions observed during this study suggest it's unlikely that *S. acaulis* experienced temperatures sufficient to negatively affect photosynthetic function.

However, prolonged exposure to favorable conditions has the potential to turn detrimental. Alatalo & Little (2014) found that increased warming and nutrients had an initial positive effect on *Silene acaulis* growth, but after five years, the effect switched to negative. Thus, even though I observed higher plant photosynthetic health inside the OTCs, there may be an upper limit that is yet to be reached. This limit could either result from short-term extreme weather events, like heatwaves, or longer-term ecological pressures, such as modest increases in temperature over time.

While initial observations indicate a positive response to higher temperatures, my findings underscore the complexity of plant adaptations to multiple environmental stressors. As climate change escalates, both the frequency and intensity of extreme climatic events, such as

drought, heatwaves, and heavy rainfall, are expected to increase, resulting in significant challenges to plant health and the survival of alpine species.

The relationship between environmental stressors and reproduction

The observed variation in pollination success among the different plant sexes in *Silene acaulis* could signify differing adaptive strategies based on environmental context. Hermaphrodites, despite their ability to self-pollinate, exhibited significantly lower pollination success. This could be because under conditions of limited resources, hermaphrodites may re-allocate resources towards pollen production, which is more energy-efficient than producing seed. This theory aligns with Dorken & Mitchard (2008), suggesting that under unfavorable conditions, plants optimize their reproductive strategy to maximize fitness, potentially driving evolution towards separate sexes.

Further, the limited pollination success of hermaphrodites in my study is consistent with the findings of Hermanutz & Innes (1994), who reported that 75% of hermaphrodites and 50% of females failed to produce seed capsules. This variability in seed production among hermaphrodites may reflect an evolutionary trend towards male function, particularly in pollen-limited areas with few pollinators, such as alpine summits.

Resource limitation in harsh environments may render it challenging for hermaphrodites to maintain both sexual functions, while females, investing solely in seed production, represent a less costly reproductive strategy (Dorken & Mitchard, 2008). Therefore, resource-limited environments may drive a plastic response of reduced seed production in hermaphrodites, favoring females because of their relatively higher seed fitness (Dorken & Mitchard, 2008).

The evolutionary transition from hermaphroditism in gynodioecious systems to dioecy, where females and males coexist, has long fascinated scientists (Dorken & Mitchard, 2008; Yang et al., 2014). This transition, believed to have occurred numerous times in flowering plants, offers insight into the potential advantages of dioecy, such as avoidance of inbreeding, as proposed by Darwin (1877). Notably, the existence of nearly dioecious *S. acaulis* populations in certain areas like Greenland (Philipp et al., 2009) could inspire research to examine whether this evolutionary shift is already underway.

Another interesting finding was the observable trade-off between seed mass and number of seeds per capsule in *Silene acaulis*. The control plants produced significantly more seeds per capsule but with lower mass compared to those inside the OTCs. This suggests that under

warmer conditions, larger seeds may offer a competitive advantage (Leishman, 2001). In contrast, under the harsher and colder conditions experienced by the control plants, there is a shift towards a greater quantity of smaller seeds to potentially maximize the reproductive opportunities. Similarly, within the elevational gradient, I observed a trade-off between seed number and seed size; plants at the mid, and warmest, elevation produced the largest seeds, while at the highest, and coldest, elevation they produced the most seeds per capsule.

Further, plants with higher pollination success seemed to have greater reproductive performance in general. It appears that the studied population has not yet reached its reproductive limit, where an increase in number of capsules begins to negatively impact seed mass and seed count. Interestingly, the positive effect of pollination success on seed mass was less pronounced for the plants inside the OTCs. This suggests that the plants may already be near their optimal energy investment in seed mass, and thereby limiting the potential for further improvements. Similar findings were revealed in the elevational gradient, where the mid and warmest elevation also had a smaller positive effect of pollination success on seed mass. Additionally, a significant three-way interaction was discovered: at the mid elevation, plants with high pollination success and a greater number of seeds per capsule exhibited increased seed mass. This indicates that the local environmental conditions at the mid elevation may provide an optimal environment for *S. acaulis* to maximize resource allocation towards both seed mass and number of seeds, without being negatively impacted by the energy demands of seed capsule production. This interaction highlights *S. acaulis* ability to exploit favorable conditions to optimize reproduction, demonstrating its nuanced adaptations in reproductive strategies.

The notable variability in reproductive strategies highlights the importance of considering the role of mixed, or gynomonoecious, individuals in *S. acaulis* populations. It has been proposed that mixed plants represent a shift from hermaphrodites rather than females (Maurice, 1999). This plasticity in sexual expression could be an adaptive strategy to maximize reproductive success under stressful abiotic and biotic conditions, such as those found in pollinator-poor alpine zones. The presence of both flower morphs may increase pollination success by exploiting different pollinator preferences. Sexual dimorphism can influence flower scent, leading to visits from different pollinators based on distinct scent profiles (Ashman, 2009). Furthermore, Galen et al (1987), demonstrated that plants with different scent morphs attracted different pollinators, with some attracting bumblebees and others flies. In *S. acaulis* populations, pollinators have been found to spend significantly more time on female,

compared to hermaphrodite individuals (Shykoff, 1992), which could potentially be attributed to differences in scent emission and the type of pollinator.

Following this theory, my finding that the largest cushions are mostly mixed could be explained by hermaphrodites developing female flowers as an adaptive strategy to attract more pollinators. This adaptation may be particularly beneficial for large cushions by reducing the risk of inbreeding depression. Although this theory remains speculative for *Silene acaulis*, it suggests a potential link between scent emission and pollination success, representing a promising area for further research.

The role of DIY sensors in climate research

By implementing the use of do-it-yourself (DIY) science to create surface- and ambient temperature sensors, I have introduced a novel methodology that addresses the need for real-time data in future climate research. Global climate change is leading to increasingly higher frequencies of extreme weather events, such as heatwaves, severe storms, and droughts. The use of low-cost, DIY environmental sensors in the field of ecology opens for the possibility for larger, globally distributed datasets, particularly in geopolitical areas with low levels of science funding. The TempBoxes I developed could serve as a starting point for more elaborate DIY sensor networks, with the potential to integrate additional functionalities like soil moisture sensors, CO_2 sensors, without adding substantial cost.

Sources of uncertainty

There are several caveats to the findings presented in this study. First, it was conducted over a single growing season, which limits its scope of inference. In addition, some aspects of reproduction in *Silene acaulis* may be governed by prior year stresses or the accumulation of stress over longer periods than assessed here. The open top chambers used in this experiment, while creating warmer growing conditions, also affect plants in different ways, such as reducing herbivory and wind, resulting in more resources being available for growth and reproduction compared to control plants. Further, the Fv/Fm ratio was measured using the FluorPen FP100 in an unconventional manner, measuring a part of the cushion instead individual leaves, and relying on nighttime readings for dark adaptation, which could potentially be influenced by moonlight. Consequently, the Fv/Fm ratio readings may not be comparable with other studies but are consistent within this study.

Conclusion

The results of this thesis shed light on the temperature dynamics of alpine ecosystems and the adaptability of *S. acaulis* in the face of climate change. Understanding how plants respond to variation in temperature is important for predicting how alpine species may respond to ongoing climate changes, especially physiological stress responses and reproductive strategies. This study revealed a significant difference in pollination success between the different plant sexes. Hermaphrodites had extremely low pollination success compared to females, suggesting a possible re-allocation of resources towards pollen production under conditions where resources are limited. This may indicate an evolutionary trend towards male function due to inbreeding depression in hermaphrodites. The higher fitness of female seeds from outcrossing suggests that dioecy may offer an evolutionary advantage in alpine areas, where pollinators are scarce, hinting at a potential shift towards separate sexes.

Additionally, this study revealed a potential temperature-dependent trade-off between seed quality and quantity in alpine flora. At the control site, plants produced significantly more seeds per capsule than those inside the warmed OTCs. Further, across the elevational gradient, the highest and coldest elevation produced the greatest number of seeds per capsule, while the mid and warmest elevation produced the largest seeds. This indicates a strategy favoring seed quantity in harsher, colder conditions and seed quality in warmer conditions.

Plant health, measured by maximum quantum yield (Fv/Fm ratio), was significantly higher for plants inside the OTCs, indicating a positive response to the current level of warming. This suggests that in this experimental setup, *S. acaulis* may not be experiencing a temperature that crosses the threshold for negative effects on photosynthesis.

Given the significant challenges posed by climate change, it is important to focus research on long-term data collection that captures both gradual adaptations and more immediate responses of alpine plants. Further studies should explore the impact of climate change on plant sex proportions, particularly focusing on genotype and gene x environment interactions in sex determination and differentiation. Additionally, plant ecologists should examine the biotic interactions that influence sex-specific pollination success, such as changes in pollinator populations and pollinator mismatch. By focusing on these areas, we can better predict and mitigate the impacts of climate change on biodiversity and ecosystem stability.

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Appendix

Table A1: All models included in AIC tests (lowest scores chosen, marked as bold).

Response variable	Predictor variables	Df	AIC score	Model type
Mean number of flowers per plant	Plant sex + Elevation + offset(Area cm2)	6	969.0818	Negative binomial
	Plant sex * Elevation + offset(Area cm2)	10	**965.5019**	
Mean number of flowers per plant	Plant sex + Treatment + offset(Area cm2)	5	727.9664	Negative binomial
	Plant sex * Treatment + offset(Area cm2)	7	**724.1479**	
Mean Fv/Fm ratio	Elevation + Mean soil moisture levels + Area cm2 + Vegetation cover	7	-384.6703	Beta regression
	Elevation + Mean soil moisture levels + Vegetation cover	6	-384.8358	
	Elevation + Mean soil moisture levels + Area cm2	6	-386.6522	
	Elevation + Area cm2	5	**-392.2204**	
Mean Fv/Fm ratio	Treatment + Mean soil moisture levels + Area cm2 + Vegetation cover	6	-378.0785	Beta regression
	Treatment + Mean soil moisture levels + Vegetation cover	5	-376.1646	
	Treatment + Mean soil moisture levels + Area cm2	5	-379.2863	
	Treatment + Area cm2	4	**-380.6365**	
Pollination success	Mean Fv/Fm ratio + Mean soil moisture levels + Elevation + Plant sex	8	**-108.00484**	Beta regression
	Mean Fv/Fm ratio * Mean soil moisture levels * Elevation * Plant sex	37	-76.71029	
	Mean Fv/Fm ratio * Elevation + Mean soil moisture levels * Elevation	10	-69.22305	
	Mean Fv/Fm ratio + Elevation + Sex * Mean soil moisture levels	10	-104.08427	
	Mean Fv/Fm ratio + Mean soil moisture levels + Elevation	6	-73.31385	
	Mean Fv/Fm ratio * Mean soil moisture levels * Elevation	13	-69.40408	
Pollination success	Mean Fv/Fm ratio + Mean soil moisture levels + Treatment + Plant sex	7	**-101.80578**	Beta regression
	Mean Fv/Fm ratio * Mean soil moisture levels * Treatment * Plant sex	25	-72.41863	
	Mean Fv/Fm ratio * Treatment + Mean soil moisture levels * Treatment	7	-75.15109	
	Mean Fv/Fm ratio + Treatment + Sex * Mean soil moisture levels	9	-98.54532	
	Mean Fv/Fm ratio + Mean soil moisture levels + Treatment	5	-78.80613	

	Mean Fv/Fm ratio * Mean soil moisture levels * Treatment	9	-75.29127	
Mean seed mass	Mean Fv/Fm ratio + Mean soil moisture levels + Pollination success + Mean number of seeds per capsule + Elevation	8	-168.0470	Linear model
	Mean Fv/Fm ratio + Mean soil moisture levels + Pollination success + Mean number of seeds per capsule * Elevation	10	-174.3354	
	Mean Fv/Fm ratio + Mean soil moisture levels + Pollination success * Elevation + Mean number of seeds per capsule * Elevation	12	-185.3539	
	Pollination success * Elevation + Mean number of seeds per capsule * Elevation	10	-191.4404	
	Pollination success * Mean number of seeds per capsule * Elevation	13	**-199.6705**	
Mean seed mass	Mean Fv/Fm ratio + Mean soil moisture levels + Pollination success + Mean number of seeds per capsule + Treatment	7	-115.9975	Linear model
	Mean Fv/Fm ratio + Mean soil moisture levels + Pollination success + Mean number of seeds per capsule * Treatment	8	-114.9505	
	Mean Fv/Fm ratio + Mean soil moisture levels + Pollination success * Treatment + Mean number of seeds per capsule * Treatment	9	-116.9007	
	Pollination success * Treatment + Mean number of seeds per capsule * Treatment	7	**-120.1637**	
	Pollination success * Mean number of seeds per capsule * Treatment	9	-117.9041	
Mean number of seeds per capsule (log transformed)	Mean soil moisture levels + Mean Fv/Fm ratio + Pollination success + Elevation	7	110.3430	Linear model
	Mean soil moisture levels + Pollination success + Elevation	6	**108.8734**	
	Mean Fv/Fm ratio + Pollination success + Elevation	6	115.7294	
	Pollination success + Elevation	5	114.1741	
	Pollination success * Elevation	7	117.5176	
	Mean Fv/Fm ratio * Pollination success * Elevation	13	127.1640	

Mean number of seeds per capsule (log transformed)	Mean soil moisture levels + Mean Fv/Fm ratio + Pollination success + Treatment	6	128.7812	Linear model
	Mean soil moisture levels + Pollination success + Treatment	5	127.8630	
	Mean Fv/Fm ratio + Pollination success + Treatment	5	128.0411	
	Pollination success + Treatment	4	**126.8847**	
	Pollination success * Treatment	5	127.6166	

Table A2: Descriptive temperature statistics for the elevational gradient in Celsius. Measured from July 1st to September 9th, 2023.

	Ambient temperature (Tinytag)				In-cushion temperature (Tinytag)			
Elevation	*Average Daily Mean*	*Standard Error*	*Average Daily Minimum*	*Average Daily Maximum*	*Average Daily Mean*	*Standard Error*	*Average Daily Minimum*	*Average Daily Maximum*
Low	8.93	± 0.41	3.95	17.81	7.22	± 0.18	4.89	10.53
Mid	8.95	± 0.46	3.41	19.30	8.12	± 0.23	5.34	13.01
High	8.13	± 0.43	2.81	17.58	7.34	± 0.20	4.78	11.00

Table A3: Results from the one-way ANOVAs for the effect of elevation on each temperature parameter, for ambient- (top) and in-cushion (bottom) temperatures. Includes results from post hoc tests.

Ambient temperature		
Parameter	**ANOVA results**	**TukeyHSD Post Hoc**
Daily mean	Elevation: Df: 2 Sum Sq: 30.7 Mean Sq: 15.346 F-value: 2.213 p-value: 0.112 Residuals: Df: 210, Sum Sq: 1456.3, Mean Sq: 6.935	Not significant
Average daily min	Elevation: Df: 2 Sum Sq: 46.6 Mean Sq: 23.313 F-value: 6.533 **p-value: 0.002** Residuals: Df: 210, Sum Sq: 749.3, Mean Sq: 3.568	**Low vs. High: diff = -1.145, p = 0.0011**
Average daily max	Elevation: Df: 2 Sum Sq: 125 Mean Sq: 62,29 F-value: 1.203 p-value: 0.302 Residuals: Df: 210, Sum Sq: 10872, Mean Sq: 51.77	Not significant

In cushion temperature		
Parameter	ANOVA results	TukeyHSD Post Hoc
Daily mean	Elevation: Df: 2 Sum Sq: 27 Mean Sq: 13.509 F-value: 4.609 **p-value: 0.011** Residuals: Df: 165, Sum Sq: 483.6, Mean Sq: 2.931	**Mid vs. Low: diff = 0.907, p = 0.0156** **High vs. Mid: diff = -0.781, p = 0.0444**
Average daily min	Elevation: Df: 2 Sum Sq: 9.6 Mean Sq: 4.794 F-value: 2.276 p-value: 0.106 Residuals: Df: 165, Sum Sq: 347.6, Mean Sq: 2.106	Not significant
Average daily max	Elevation: Df: 2 Sum Sq: 193.9 Mean Sq: 96.96 F-value: 7.204 **p-value: 0.001** Residuals: Df: 165, Sum Sq: 2220.8, Mean Sq: 13.46	**Mid vs. Low: diff = 2.478, p = 0.0013** **High vs. Mid: diff = -2.006, p = 0.0120**

Table A4: Descriptive temperature statistics for treatment OTC and control plants in Celsius. Measured from July 1st to September 9th, 2023.

Ambient temperature (TempBox)				
Treatment	Average Daily Mean	Standard Error	Average Daily Minimum	Average Daily Maximum
Control	7.19	± 0.28	3.41	12.70
OTC	8.12	± 0.38	3.45	15.90
Surface temperature (TempBox)				
Treatment	Average Daily Mean	Standard Error	Average Daily Minimum	Average Daily Maximum
Control	7.99	± 0.40	3.21	16.70
OTC	9.07	± 0.48	3.52	19.65
In-cushion temperature (Tinytag)				
Treatment	Average Daily Mean	Standard Error	Average Daily Minimum	Average Daily Maximum
Control	7.56	± 0.20	4.94	11.24
OTC	8.66	± 0.28	5.34	14.09

Table A5: Results from the one-way ANOVAs for the effect of treatment (OTC) on each temperature parameter, for ambient- (top), surface- (middle) and in-cushion (bottom) temperatures.

Ambient temperature

Parameter	ANOVA results
Daily mean	Treatment: Df: 1, Sum Sq: 34.2, Mean Sq: 34.19, F-value: 4.41, **p-value: 0.037** Residuals: Df: 147, Sum Sq: 1139.8, Mean Sq: 7.75
Average daily min	Treatment: Df: 1, Sum Sq: 0.3, Mean Sq: 0.317, F-value: 0.069, p-value: 0.793 Residuals: Df: 147, Sum Sq: 672.2, Mean Sq: 4.473
Average daily max	Treatment: Df: 1, Sum Sq: 380, Mean Sq: 380.3, F-value: 11.36, **p-value: 0.001** Residuals: Df: 147, Sum Sq: 4919, Mean Sq: 33.5

Surface temperature

Parameter	ANOVA results
Daily mean	Treatment: Df: 1, Sum Sq: 45.8, Mean Sq: 45.75, F-value: 5.242, **p-value: 0.024** Residuals: Df: 147, Sum Sq: 1283,1, Mean Sq: 8.73
Average daily min	Treatment: Df: 1, Sum Sq: 5.2, Mean Sq: 5.161, F-value: 1.17, p-value: 0.281 Residuals: Df: 147, Sum Sq: 648.3, Mean Sq: 4.41
Average daily max	Treatment: Df: 1, Sum Sq: 316, Mean Sq: 315.65, F-value: 4.553, **p-value: 0.035** Residuals: Df: 147, Sum Sq: 10191, Mean Sq: 69.33

In-cushion temperature

Parameter	ANOVA results
Daily mean	Treatment: Df: 1, Sum Sq: 44.9, Mean Sq: 44.88, F-value: 11.17, **p-value: 0.001** Residuals: Df: 144, Sum Sq: 578.5, Mean Sq: 4.02
Average daily min	Treatment: Df: 1, Sum Sq: 5.8, Mean Sq: 5.81, F-value: 2.18, p-value: 0.142 Residuals: Df: 144, Sum Sq: 3838, Mean Sq: 2.665
Average daily max	Treatment: Df: 1, Sum Sq: 298.3, Mean Sq: 298.32, F-value: 18.15, **p-value: <0.001** Residuals: Df: 144, Sum Sq: 2366.8, Mean Sq: 16.44

Table A6: Results from the negative binomial linear model on flower density for the elevational gradient setup. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-0.984	0.191	-5.137	**<0.001**	***
Hermaphrodite	0.125	0.386	0.325	0.745	
Mixed	-0.406	0.300	-1.350	0.177	
Mid elevation	-0.090	0.260	-0.347	0.729	
High elevation	-0.370	0.238	-1.553	0.120	
Hermaphrodite : Mid elevation	-0.372	0.501	-0.743	0.457	
Mixed : Mid elevation	0.100	0.505	0.199	0.842	
Hermaphrodite : High elevation	-0.095	0.482	-0.197	0.844	
Mixed : High elevation	1.378	0.449	3.072	**0.002**	**
Null deviance	124.68 on 103 degrees of freedom				
Residual deviance	111.79 on 95 degrees of freedom				
AIC	965.5				
Theta	1.928				
Std. Err.	0.267				

Table A7: Calculated means and standard error (SE) on cushion size and flower density for the different elevations.

Elevation	Average Plant Size (cm^2) ± SE				Average Flower Density (cm^2) ± SE			
	Total	Females	Herm.	Mixed	Total	Females	Herm.	Mixed
Low	315.7	289.56	164.07	430.77	0.348	0.377	0.448	0.254
	± 65.8	± 95.8	± 62.6	± 129	± 0.039	± 0.037	± 0.155	± 0.066
Mid	175.40	157.63	137.45	331.30	0.314	0.348	0.268	0.251
	± 34.2	± 41.7	± 54.7	± 130	± 0.050	± 0.077	± 0.063	± 0.099
High	132.75	119.64	118.16	218.00	0.336	0.266	0.275	0.767
	± 19.3	± 22.1	± 33.6	± 76.7	± 0.072	± 0.030	± 0.071	± 0.477

Table A8: Results from the negative binomial linear model on flower density for the long-term experimental warming setup. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Coefficient	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-1.351	0.148	-9.120	**<0.001**	***
Hermaphrodite	0.031	0.303	0.102	0.919	
Mixed	0.980	0.349	2.807	**0.005**	**
Treatment OTC	-1.66	0.206	-0.807	0.420	
Hermaphrodite : Treatment OTC	0.182	0.434	0.420	0.675	
Mixed : Treatment OTC	-1.672	0.570	-2.931	**0.003**	**
Null deviance	109.45 on 87 degrees of freedom				
Residual deviance	93.862 on 82 degrees of freedom				
AIC	724.15				
Theta	1.742				
Std. Err.	0.266				

Table A9: Calculated means and standard error (SE) on cushion size and flower density for the OTC treatment and control plants.

Treatment	Average Plant Size (cm²) ± SE				Average Flower Density (cm²) ± SE			
	Total	Females	Herm.	Mixed	Total	Females	Herm.	Mixed
Control	132.75	119.64	118.16	218.00	0.336	0.266	0.275	0.767
	± 19.3	± 22.1	± 33.6	± 76.7	± 0.072	± 0.030	± 0.071	± 0.477
OTC	123.96	147.64	41.10	126.81	0.227	0.229	0.271	0.119
	± 20.2	± 26.0	± 5.77	± 74.2	± 0.027	± 0.036	± 0.031	± 0.029

Table A10: Results from the one-way ANOVA exploring the relationship between soil moisture and elevation. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001. Trends marked with .p<0.1.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	3.358	0.066	51.219	**<0.001**	***
Elevation Low	0.199	0.103	1.935	0.055	.
Elevation Mid	0.297	0.104	2.853	**0.005**	**
Residual standard error	0.4349 on 100 degrees of freedom				
Multiple R-squared	0.08159				
Adjusted R-squared	0.06322				
F-statistic	4.442 on 2 and 100 DF				
p-value	0.01418				

Table A11: Results from the Beta Regression model showing the effect of elevation and area on the Fv/Fm ratio of S. acaulis. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	1.223	0.0323686	37.963	**<0.001**	***
Elevation Mid	-0.037	0.054	-0.683	0.495	
Elevation High	-0.109	0.051	-2.142	**0.032**	*
Area (cm²)	<-0.001	<0.001	-1.512	0.131	
(phi)	138.67	19.18	7.229	<0.001	***

Table A12: Results from the one-way ANOVA exploring the effect of OTC treatment on soil moisture. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	3.358	0.057	58.685	**<0.001**	***
Treatment OTC	-0.348	0.081	-4.302	**<0.001**	***
Residual standard error	0.3796 on 86 degrees of freedom				
Multiple R-squared	0.1771				
Adjusted R-squared	0.1675				
F-statistic	18.51 on 1 and 86 DF				
p-value	<0.001				

Table A13: Results from the Beta Regression model showing the effect of treatment and area on the Fv/Fm ratio of S. acaulis. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	1.176	0.029	40.527	**<0.001**	***
Treatment OTC	0.168	0.034	4.988	**<0.001**	***
Area (cm^2)	<0.001	<0.001	2.216	**0.027**	*
(phi)	235.58	35.47	6.643	**<0.001**	***

Table A14: Results from the Beta Regression exploring the effect of sex on the Fv/Fm ratio for both experimental setups. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Setup 1: Elevational gradient					
Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	1.259	0.027	47.44	**<0.001**	***
Hermaphrodite	-0.023	0.052	-0.454	0.65	
Mixed	0.003	0.054	0.049	0.961	
(phi)	131.6	18.2	7.23	<0.001	***
Setup 2: Long-term experimental warming					
Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	1.302	0.024	55.4	**<0.001**	***
Hermaphrodite	-0.034	0.048	-0.692	0.489	
Mixed	0.005	0.062	0.082	0.934	
(phi)	178.18	26.81	6.646	<0.001	***

Table A15: Results from the Beta Regression model showing the effect of the Fv/Fm ratio, soil moisture levels, elevation, and sex on the pollination success of S. acaulis. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001. Trends marked with .p<0.1.

Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	2.846	2.534	1.123	0.261	
Mean Fv/Fm ratio	-2.133	3.144	-0.678	0.498	
Mean Soil Moisture levels	-0.013	0.007	-1.777	*0.756*	.
Elevation Mid	0.012	0.319	0.039	0.969	
Elevation High	-0.631	0.299	-2.112	**0.035**	*
Hermaphrodite	-1.989	0.319	-6.248	**<0.001**	***
Mixed	-1.070	0.327	-3.276	**0.001**	**
(phi)	1.305	0.157	8.296	<0.001	

*Table A16: Results from the Beta Regression model showing the effect of elevation and sex on the pollination success of S. acaulis. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.*

Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	0.728	0.272	2.677	**0.007**	**
Elevation Mid	-0.015	0.331	-0.045	0.964	
Elevation High	-0.478	0.306	-1.560	0.119	
Hermaphrodite	-2.058	0.328	-6.270	**<0.001**	***
Mixed	-0.992	0.349	-2.844	**0.004**	**
(phi)	0.790	0.092	8.556	<0.001	***

*Table A17: Results from the Beta Regression model showing the effect of the Fv/Fm ratio, soil moisture levels, treatment, and sex on the pollination success of S. acaulis. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001. Trends marked with .p<0.1.*

Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	6.401	3.534	1.811	*0.070*	.
Mean Fv/Fm ratio	-7.967	4.607	-1.729	*0.084*	.
Mean Soil Moisture levels	-0.008	0.013	-0.587	0.557	
Treatment OTC	0.105	0.321	0.325	0.745	
Hermaphrodite	-0.717	0.327	-5.251	**<0.001**	***
Mixed	-0.267	0.422	-0.630	0.529	
(phi)	1.215	0.155	7.826	<0.001	***

*Table A18: Results from the Beta Regression model showing the effect of treatment and sex on the pollination success of S. acaulis. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.*

Variable	Estimate	Std. Error	z-value	p-value	
(Intercept)	0.125	0.223	0.559	0.576	
Treatment OTC	-0.073	0.275	-0.265	0.791	
Hermaphrodite	-1.812	0.340	-5.326	**<0.001**	***
Mixed	-0.300	0.456	-0.659	0.510	
(phi)	0.685	0.084	8.213	<0.001	***

Table A19: Results from linear model on the mean seed mass. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001. Trends marked with .p<0.1.

| Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.173	0.054	3.230	**0.002**	***
Pollination Success (#seed capsules/ #flowers)	0.175	0.101	1.727	*0.089*	.
Mean Number of Seeds per Capsule	-0.001	0.007	-0.147	0.883	
Elevation Mid	0.398	0.075	5.342	**<0.001**	***
Elevation High	-0.053	0.083	-0.647	0.520	
Pollination Success : Mean Number of Seeds	-0.003	0.013	-0.235	0.815	
Pollination Success : Elevation Mid	-0.540	0.147	-3.676	**<0.001**	***
Pollination Success : Elevation High	0.125	0.142	0.877	0.384	
Mean Number of Seeds : Elevation Mid	-0.038	0.010	-3.635	**<0.001**	***
Mean Number of Seeds : Elevation High	0.013	0.014	0.935	0.353	
Pollination Success : Mean Number of Seeds : Elevation Mid	0.051	0.020	2.614	**0.011**	*
Pollination Success : Mean Number of Seeds : Elevation High	-0.025	0.021	-0.162	0.249	
Residual standard error	0.066 on 70 degrees of freedom				
Multiple R-squared	0.513				
Adjusted R-squared	0.436				
F-statistic	6.699 on 11 and 70 DF				
p-value	<0.001				
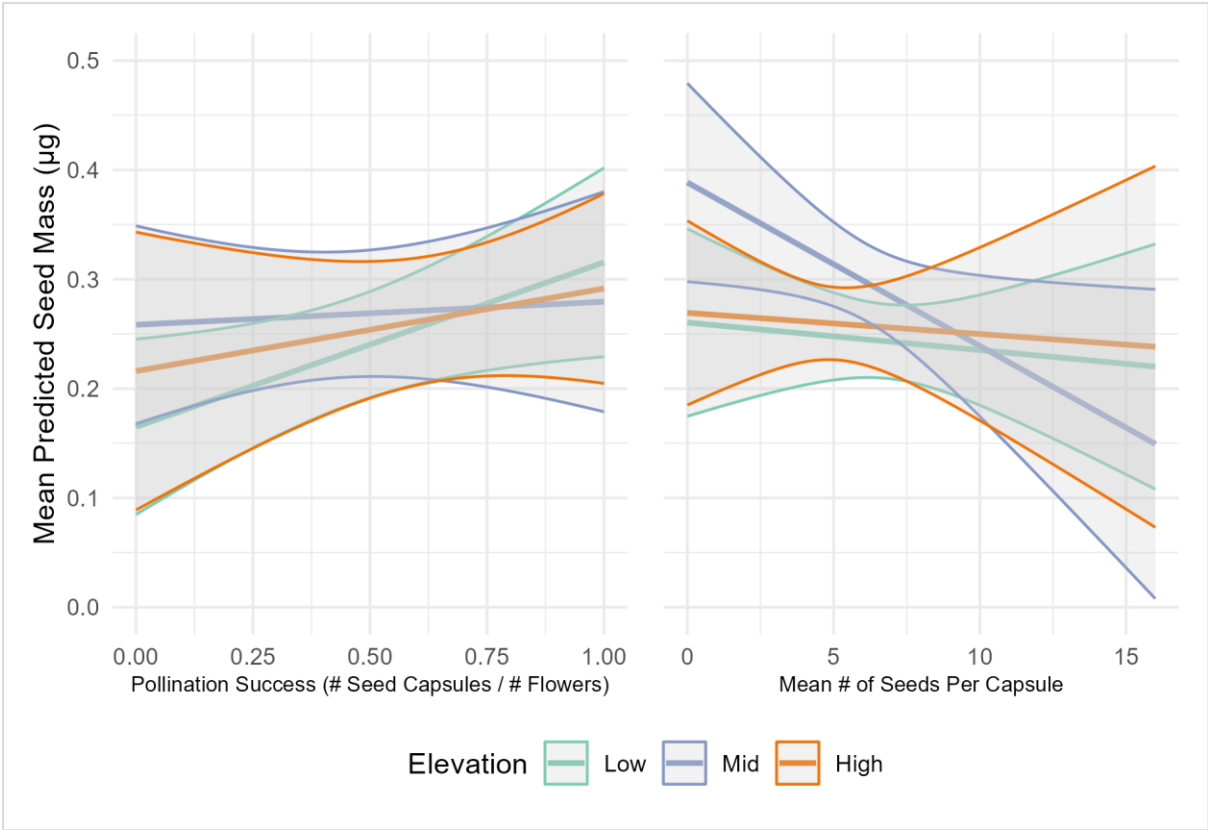
Figure A1: Mean Seed Mass (μg) in relation to Plant Pollination Success and Seed Production across different elevation levels (Low, Mid, High).
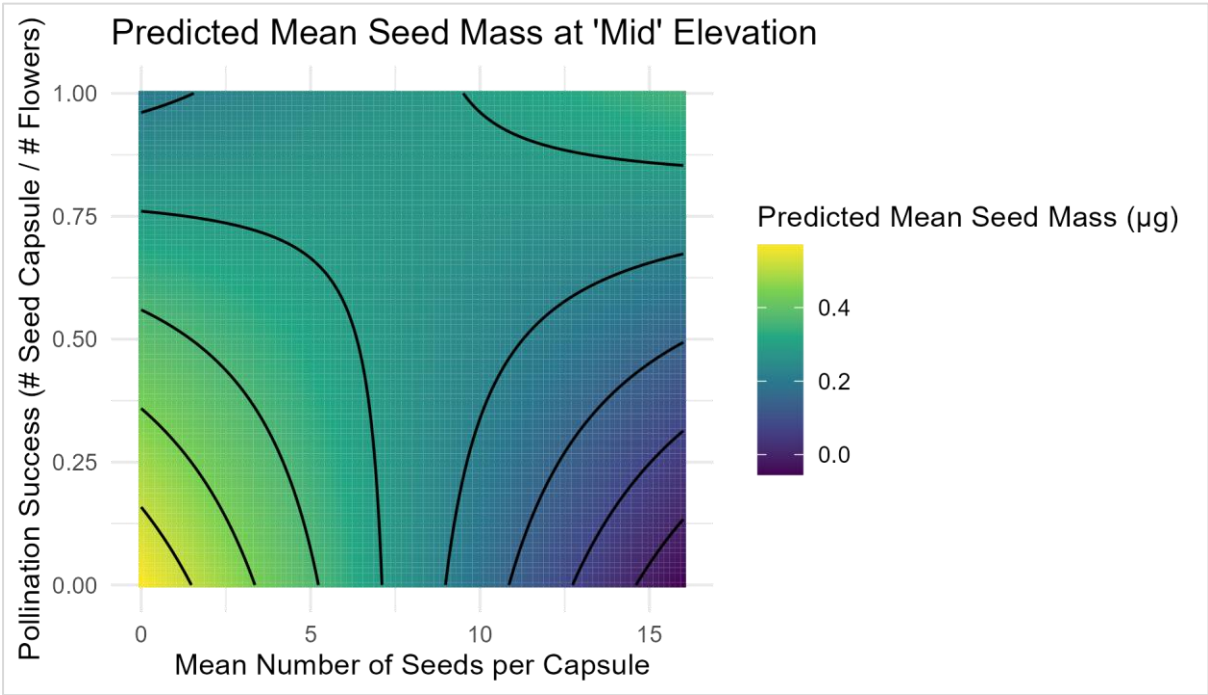

Figure A2: Heatmap with contours showing the predicted mean seed mass at the mid elevation.

Table A20: Results from linear model exploring the effect of soil moisture, pollination success and elevation on the average number of seeds per capsule (log transformed). Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.687	0.167	10.093	**<0.001**	***
Soil Moisture Levels	-0.003	0.003	-1.152	0.253	
Pollination Success	0.397	0.153	2.595	**0.011**	*
Elevation Mid	-0.174	0.129	-1.344	0.183	
Elevation High	-0.250	0.123	-2.042	**0.045**	*
Residual standard error	0.4543 on 76 degrees of freedom				
Multiple R-squared	0.1524				
Adjusted R-squared	0.1078				
F-statistic	3.415 on 4 and 76 DF				
p-value	0.013				

Table A21: Results from linear model exploring the effect of pollination success, mean number of seeds per capsule and treatment on the mean seed mass. Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001. Trends marked with .p<0.1.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	0.208	0.042	4.956	**<0.001**	***
Pollination Success	0.149	0.050	2.973	**0.004**	**
Treatment OTC	0.068	0.058	1.182	0.242	
Mean Number of Seeds	-0.006	0.007	-0.785	0.436	
Pollination Success : Treatment OTC	-0.122	0.069	-1.788	*0.079*	.
Treatment OTC : Mean Number of Seeds	0.011	0.010	1.124	0.266	
Residual standard error	0.086 on 56 degrees of freedom				
Multiple R-squared	0.2158				
Adjusted R-squared	0.1458				
F-statistic	3.082 on 5 and 56 DF				
p-value	0.01582				

Table A 22: Results from linear model exploring the effect of pollination success and treatment on the average number of seeds per capsule (log transformed). Significance levels indicated with asterisks: *p<0.05, **p<0.01, ***p<0.001.

Coefficient	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.5	0.176	8.535	**<0.001**	***
Pollination Success	0.097	0.249	2.388	0.699	
Treatment OTC	-0.515	0.164	-3.129	**0.003**	**
Residual standard error	0.647 on 59 degrees of freedom				
Multiple R-squared	0.1447				
Adjusted R-squared	0.1157				
F-statistic	4.992 on 2 and 59 DF				
p-value	0.0099				