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Effects of long-term experimental warming on fitness, morphology and species interactions of *Silene acaulis*

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Preface

This thesis is the final product of my MSc in Biology at the Norwegian University of Life Sciences (NMBU). This year has brought me new experiences, knowledge and friendships. The most memorable part has definitely been my field work in Finse, with its astounding landscapes and unpredictable weather. I wish to thank my wonderful supervisors for the opportunity to do this project and for all their support. Particularly, I want to thank my main supervisor Erik Aschehoug for great advice, patient guidance, and for always finding time for conversations. Further, I want to thank my co-supervisor Kari Klanderud for her feedback and guidance, as well as for letting me base my thesis on her OTC experiment. I also want to thank my fellow *Silene acaulis* enthusiast Maria Elisa Pierfederici for her help during field work and for interesting conversations. Thanks to Ross Wetherbee for the statistics advice and to Finse research station for nice accommodations during the field work. Last but not least, I wish to thank my family for their support and a special thanks to my daughter Ronja for her team spirit, joyfulness and patience.

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Abstract

The global annual average surface temperature has increased significantly during the past century and is predicted to continue rising. Studies have shown that alpine ecosystems can be particularly sensitive to climate change. However, due to the complexities of interactions between biotic and abiotic factors, a lot remains unknown regarding the magnitude of climate change impacts on alpine plant communities. Cushion plants are known to play a key role in alpine communities by creating micro-environments that are beneficial for other species. *Silene acaulis* is a long-lived cushion plant that is found in alpine and arctic habitats in the northern hemisphere. Several studies have explored the short-term effects of elevated temperatures on the fitness, performance and interactions of *S. acaulis* with other species, but less is known about the long-term effects of warming on *S. acaulis* despite its ecological importance. Thus, the objective of this thesis was to investigate how prolonged changes to climate will affect the fitness, morphology and species interactions of *S. acaulis*

The study was conducted at Finse, western Norway, in an ongoing long-term, open-top chamber (OTC) experiment. For all *S. acaulis* cushions found inside control plots and OTCs, I measured the size of the cushions, the number of fruits per cushion, morphological traits (colour, height and shape), and counted the number of individuals growing inside the cushions. In addition, air and soil temperature was measured during the summer of 2019 for a subset of control plots and OTCs.

Unusually low rates of reproduction were observed at the site with only eight out of 56 individuals containing fruits. Experimental warming was not a significant predictor for fruit production, but a correlation was found between the probability of fruits and cushion size, with larger cushion being more likely to contain fruits. Cushions inside warmed plots were found to have more discolouration, potentially due to heat damage caused by midday temperature spikes considerably above the species optimum. A shift in species interactions was found with a decline in the number of lichens and bryophytes per cushion inside warmed plots, while the number of vascular plants was not affected by warming.

Contents

Introduction

Alpine plants are primarily distributed beyond the high elevation timberline in areas where weather conditions are unstable and extreme both within and across seasons (Körner, 2003). Plant species in alpine environments often have specific adaptations, such as prostrate growth forms and well-developed root systems. Abiotic stress factors, such as temperature, wind, solar radiation, water availability, soil properties and snow duration limit plant growth and distribution in high altitudes. Some of these factors can interact to cause highly context dependent levels of stress, such as solar input and temperature which in combination determine growing season length. In contrast with other terrestrial ecosystems at lower altitudes where plants have a substantial effect on the microclimate at any given site, the microclimate in alpine sites are mainly controlled by their physical environment (Billings & Mooney, 1968; Körner, 2003). Therefore, alpine plants must cope with stress via three pathways: 1) individuals can cope with stress via acclimation (usually temporary and reversible); 2) individuals can also go through ontogenetic modification because of environmental effects on development; and 3) species can adapt via natural selection on heritable traits across generations (Körner, 2003).

The global annual average surface temperature has increased by approximately 0.9°C in the time period since 1880 and is expected to continue rising. Winters are milder while extreme heat waves during summers are becoming more frequent (NCAR, 2011). Alpine and arctic ecosystems are predicted to be particularly affected by climate change and there are a number of studies exploring the effects of climate change on alpine plant communities (Bellard et al., 2012; Grabherr et al., 2010; Sala, 2000). Recent studies substantiate that alpine ecosystems are changing at a rapid rate with shifts in species composition, species geographic ranges and interactions being recorded and predicted to continue taking place (Grabherr et al., 2010; Hall et al., 2018; Klanderud & Birks, 2003; Klanderud, 2005; Peterson et al., 2018), but see (Cotto et al., 2017).

Silene acaulis is an ecologically important alpine foundation species that is known to be a nurse plant. With its relatively large and dense cushions acting as a heat-trap, *S. acaulis* creates beneficial microenvironments for other plant species that would otherwise not survive outside the cushions. This allows more species to establish in the harsh alpine environment, thus increasing the biodiversity of alpine ecosystems (Callaway et al., 2002; Cavieres et al., 2014).

A number of studies have explored how facilitative interactions between *S. acaulis* and its neighbouring species respond to climate (Antonsson et al., 2009; Bonanomi et al., 2016; Peterson et al., 2018; Reid & Lortie, 2012). Facilitative plant interactions may be dependent on the severity of abiotic stress that plants experience, with facilitation becoming more frequent under high stress conditions, as stated in the stress-gradient hypothesis (SGH) (Bertness & Callaway, 1994; Callaway et al., 2002), but see (Cavieres & Sierra-Almeida, 2012; Holmgren & Scheffer, 2010). Benefactor plants, experiencing mild conditions have been shown shift from facilitative to neutral or competitive interactions (Antonsson et al., 2009; Kjaer et al., 2018) and the release of facilitation has been predicted to be one of the key drivers for the shifts in alpine plant communities (Anthelme et al., 2014).

In addition to shifts in species interactions, elevated temperatures have also been shown to have a direct negative effect on cushion plant performance, fitness and survival (Alatalo & Little, 2014; Doak & Morris, 2010; Gauslaa, 1984; Hall et al., 2018; Peterson et al., 2018; Villellas et al., 2018; Xiong et al., 1999). Doak & Morris (2010) reported a decline in most vital rates in *S. acaulis* in response to high mean summer temperatures, while Gauslaa (1984) observed immediate heat damage in response to temperatures slightly above species optimum. Alatalo and Little (2014) found that experimental warming combined with nutrient deposition resulted in an initial positive response (increased stem length, leaf elongation, increased fruit production, cushion size and general plant cover), while temperature alone only had a significant positive effect on the cover and biomass of *S. acaulis*. However, after a period of five years, the initial positive response to the temperature and nutrient combination was replaced by a strong negative response, with a significant decrease in *S. acaulis* cover as well as a decrease in fruit production, though not beyond the initial values. These negative medium-term effects might be explained by increased competitive interactions with species that are able to expand their altitudinal range over a long period of favourable conditions. Other potential explanations include changed trophic interactions with the soil microbiota due to an increase in soil temperature, freezing damage due to earlier snowmelt in the warming chambers or other stress factors (Alatalo & Little, 2014).

Still, little is known about how long-term warming affects the fitness, morphology, and species interactions of *S. acaulis*. With temperature being an important restrictive factor (Bellard et al., 2012) potentially determining the fitness and the performance and thus the future distribution and of *S. acaulis*, the objective of this thesis is to test the effects of long-term experimental warming on the fitness, morphology, and the interactions between *S. acaulis* and other plant species. I hypothesize that the consequences of long-term experimental warming on *S. acaulis* will be: 1) A decrease in fruit production (Doak & Morris, 2010). 2) A decrease in cushion colour/vigour, height and intactness (Gauslaa, 1984). 3) A decline in facilitative interactions with other species (Bertness & Callaway, 1994; Callaway et al., 2002).

Materials & methods

STUDY SPECIES

Silene acaulis is a perennial, cushion-forming plant that is commonly found in the arctic and alpine in Europe, North America and Asia, with a distribution gap in central Asia (Jones & Richards, 1962). The species is known for its resilience to extreme climatic conditions and it has been recorded to survive in temperatures from -80 up to +60ºC due to its dense, dome-shaped structure (Larcher et al., 2010). *S. acaulis* is a gynodioecious plant that reproduces sexually and forms seed capsules which contain large quantities of seeds. The seed capsules open apically, and the seeds are dispersed predominantly by wind. The plant has a single tap root, narrow, densely crowded leaves and radially symmetric, pink flowers. *S. acaulis* thrives on a variety of substrates (from acidic to calcareous) and requires snow protection during the colder months of the year (Jones & Richards, 1962; Mossberg, 2018).

STUDY SYSTEM

The study was conducted at the Sanddalsnuten mountain summit, in the north-western part of the Hardangervidda mountain plateau, in western Norway. The site is located at 1550 m.a.s.l., approximately 2,5 km north- east of Finse train station (60°36′N, 7°31′E). The climate in this region is mildly oceanic, with a mean annual temperature of -0.6°C, a monthly mean temperature of -7.5°C in January and 9.8°C in July (NCCS, 2019). The mean annual precipitation in 2018 at the Finse weather station was 142,6 mm, with highest precipitation levels in September (292 mm) and least precipitation in May (29.9 mm) (NCCS, 2019). Plant species that are characteristic to the site include cushion species, *Silene acaulis* and *Saxifraga oppositifolia,* grasses and sedges such as *Deschampsia, Poa* and *Carex* as well as several moss and lichen species. The site was initially established in 2000 with forty plots, fitted with open-top chambers (OTCs) to experimentally increase temperature and forty control plots of approximately 1x1 m. OTCs be used to partially simulate climate change, as they are known to act as miniature greenhouses, providing a mean air temperature increase of approximately 1,5 °C (Marion et al., 1997). Our experiment was limited to 24 OTCs and 14 control plots that were intact and contained *S. acaulis* individuals (Figure 1).

Figure 1: *Open-top chambers and control plots with temperature loggers at the study site on Sanddalsnuten, a mountain summit in Finse, Norway, September 2019.*

SAMPLING

In total, I sampled 55 cushions from within the OTCs, and 29 cushions from within the control plots. I did not include individuals close to the edges of the OTCs to avoid edge effects. Each plot at the site has a unique ID, but since some of the plots contain more than one *S. acaulis* individual (up to 10 individuals per plot), I marked each individual in order for the experiment to be reproducible in the future. I did this by marking each individual with a coloured plastic toothpick. In plots where there were more than five individuals, I used a combination of two toothpicks of different colours in order to distinguish the individuals from each other. After all individuals were marked, I collected size measurements, measuring the longest point of the cushion (major axis) as well as the widest point of the cushion (minor axis), and estimated the percentage of the cushion that was missing (not living plant tissue). Following the method of Doak and Morris (2010), I calculated the cushion area by using the formula for an ellipse and subtracting the percentage missing:

$$Area = \frac{a_1}{2} \cdot \frac{a_2}{2} \cdot \pi$$

In order to describe cushion morphology, I observed the colour, the shape (intactness) and the height of each cushion and created four categories for each trait (0 - 3), where a score of 0 in all three classes would indicate that the individual has completely withered while a score of 3 in all three classes would mean that the individual is green, intact and dome-shaped (Table 1). The height of the cushions was measured with a ruler, from ground level and to the highest point of the cushion. I decided to use cushion colour as a proxy for cushion vigour, meaning that cushions with a score of 3 would be green and vigorous, a score of 2 would indicate moderate discolouration/moderate vigour, and a colour score of 1 would indicate strong discolouration and low vigour.

To examine the potential effects of experimental warming on *S. acaulis* interactions with other species, I counted the total number of individuals growing inside *S. acaulis* cushions both in control plots and OTCs and divided them into two subgroups: 1) vascular plants, 2) lichens and bryophytes.

TEMPERATURE DATA

A total of 14 air temperature loggers and 10 soil temperature loggers were placed in randomly selected plots, recording air and soil temperature every 30 minutes in the time period between June 2019 and September 2019. I used the Tinytag temperature loggers (Gemini Data Loggers Ltd*,* *https://www.geminidataloggers.com*) both with and without thermistor probes, depending on whether soil or air temperature was measured.

DATA ANALYSIS

All statistical analyses were conducted in R-Studio in R version 3.6.1 (R Core Team, 2019). I estimated the effects of experimental warming on the following response variables: fruits present/absent, cushion colour/vigour and the number of individuals growing inside *Silene* cushions (total individuals, lichens and bryophytes, as well as vascular plants). To avoid fitting the models with too many parameters with regard to the data, I only included main effects for some of the responses, even though several "ecological" interactions might have been apparent.

For each of the models, I conducted a model selection process, based on AICc (Akaiki Information Criterion for small sample sizes), including a null model in the selection procedure (Zuur et al., 2009). The models were fitted using the “lme4” package (Bates et al., 2015), if not stated otherwise.

I assessed the probability of a cushion containing fruits by fitting generalized linear mixed models (GLMM) with cushion area and treatment as the only predictors and Plot.ID as a random effect. The random effect was included to account for the nested design of the experiment as well as to deal with spatial dependencies in the data. Only eight out of all of the sampled individuals contained fruits, so the model structure had to be simplified to avoid model convergence issues. Fruits were recorded as either present or absent, so a binomial error distribution was assumed for the model. When plotting the raw data, there appeared to be an indication of a relationship between cushion colour/vigour and the probability of fruits (Appendix 6), however, adding cushion colour into the model caused model convergence issues and significant underdispersion, and was thus excluded from the final model.

In addition, I wanted to explore the effects of treatment on cushion morphology. I performed a Welch two sample t-test, testing the difference in means (plot level) for cushion colour, shape and height. To further explore the relationship between warming treatment and cushion colour (used as a proxy for cushion vigour), I fitted a multinomial ordered model with cushion colour as a multilevel response and treatment and log(area) as fixed effects. This was done using the “nnet” package (Venables & Ripley, 2002). As the “polr” ordinal model function in the “nnet” package does not support mixed models, the model calculations are based on the individual/cushion level.

In order to confirm the temperature differences between the OTCs and the control plots, I performed a Welch two sample t-test, comparing the average air and soil temperatures and created a timeline temperature graph, using the “ggplot2” package (Wickham, 2016).

During data exploration, I observed a difference in cushion colour/vigour between the treatments, which indicated that it might be interesting to explore the relationship between treatment, colour/vigour and the interactions of *S. acaulis* with other species. In order to model the effects of warming on species interactions (i.e. number of individuals growing inside a cushion), I fitted GLMM with Plot.ID as a random effect. Due to the structure and the sparsity of the data, I chose to fit random intercept models instead of random slope models. As the “individuals per cushion” data only included positive integer values, with the majority of observations concentrated around zero, I assumed a Poisson error distribution for the model. I used the R package “emmeans” (Lenth et al., 2020) to determine *post hoc* differences between the levels of the categorical predictors as well as to compute predicted rates for the response variables, based on the GLMM´s. In addition, I conducted a posterior predictive simulation for the species interactions models to test whether the models match the characteristics of the data by looking at the distributions of the numbers of zero-shell outcomes in the simulated data (Zuur et al., 2016).

Results

EFFECTS OF EXPERIMENTAL WARMING ON MICROCLIMATE

The results from the Welch two sample T-tests showed a significant difference between treatments both for soil and air temperature, with the average soil temperature being 0.62°C higher in warmed plots, compared to control plots. The average air temperature was 1,03°C higher in warmed plots, compared to the control plots. (Table 1). Furthermore, the daily temperature variations were more pronounced in warmed plots, with frequently higher air temperature peaks inside the open top chambers. The difference in soil temperature variations were less pronounced, with temperature peaks present both inside OTCs and control plots (Figure 2).

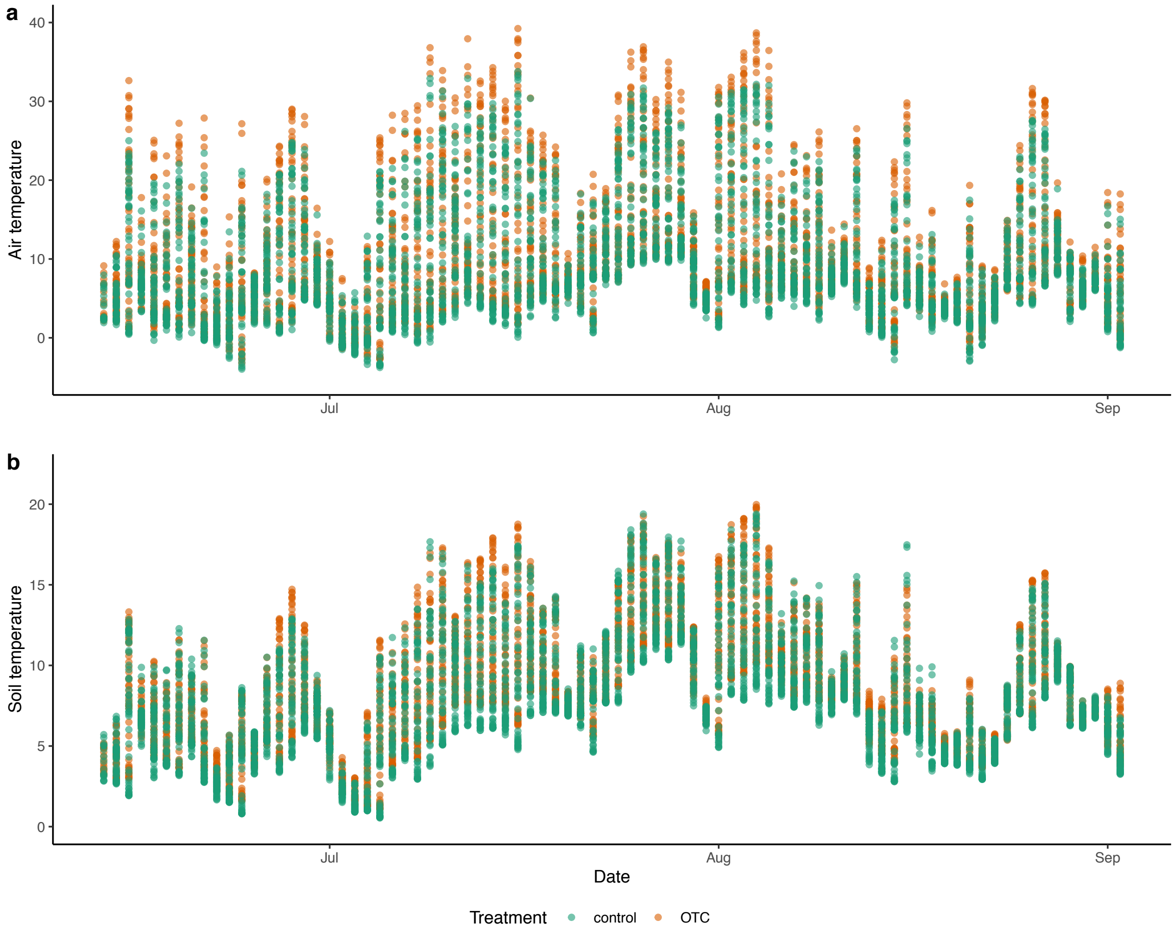

Figure 2: *The daily variations in a) air temperature and b) soil temperature in control plots vs. warmed plots. Based on hourly temperature measurements with temperature loggers in the time period from June 13th to September 2nd, 2019.*

Table 1: *Results from two sample T-tests, testing the effects of OTCs on soil and air temperature. Highlighted p-values are significant at a 1% test level.*

	Control	OTC	Df	T-value	p-value
Soil temperature, ℃	7.85	8.47	7805	-7.40	**<<0.001**
Air temperature, ℃	9.09	10.12	7736	-5.97	**<< 0.001**

EFFECTS OF EXPERIMENTAL WARMING ON FITNESS AND MORPHOLOGY

Results from the Welch two sample T-tests indicated that warming had a significant effect on cushion colour, while it had no effect on either cushion height or shape/intactness. The mean cushion colour per plot was significantly lower inside warmed plots, compared to control plots (Table 2).

Table 2: *Morphology class definitions, the number of individuals within each class by treatment and the mean class per plot. Highlighted p-values denote significant differences (< 0.05) between treatments. T-value gives the difference in units of se.*

Trait	Class	Definition	No. of individuals		Mean class/plot		T	df	p
			Control	OTC	Control	OTC			
Height	0	Withered	0	1	1.61	1.65	-0.23	24.4	0.822
	1	0-2 cm	14	23					
	2	2-3 cm	12	26					
	3	> 3 cm	3	6					
Shape/ intactness	0	Withered	0	1	2.70	2.38	1.87	35.8	0.070
	1	Strongly fragmented	1	12					
	2	Mostly intact, some fragmentation	8	14					
	3	No fragmentation	20	28					
Colour/ vigour	0	Withered	0	1	2.80	2.46	2.41	35.9	**0.021**
	1	Strong discolouration	0	3					
	2	Some discolouration	8	23					
	3	Bright green, no discolouration	21	28					

*During model fitting, colour classes 0 and 1 were merged to ensure model convergence.

The best model in the set of candidate models for fitness included cushion area as the only fixed effect and had a significantly lower (> 2) AICc value, compared to the null model and the model including treatment as the only predictor. Models with interaction terms between cushion colour/vigour, treatment and cushion area were attempted, but all caused convergence errors due to the sparsity of observations. The best candidate model for cushion colour/vigour was found to include treatment as the only fixed effect, based on the AICc value and correcting for degrees of freedom (Table 3).

Table 3: *GLMM binomial models with AICc values and ΔAICc values to estimate the probability of fruits and multinomial ordered regression models with AICc values and ΔAICc values to estimate the probability of a specific cushion colour class based on treatment. Df= degrees of freedom for the respective candidate model.*

Response	Explanatory variables	Random effect	df	AICc	AICc
Fruits ~	log(Area)	1|Plot.ID	3	44.08	0.00
	Treatment	1|Plot.ID	3	55.11	11.03
	1	1|Plot.ID	2	53.87	9.79
Colour ~	Treatment	-	3	140.78	0.00
	Treatment + log(Area)	-	4	142.70	0.00
	1	-	2	143.03	2.25

The following output is from the best binomial GLMM in the set of candidate models for estimating the probability of fruits. The output entails parameters selected through correlation analysis and stepwise model selection (Table 3).

Table 4: *Parameter estimates for the best model predicting the probability of a cushion containing fruits (presented in Table 3). Significant values at p < 0.05 are highlighted. Parameters are presented on the logit scale.*

	Estimate	Std. error	p-value
Fruits probability			
(Intercept)	-19.31	8.04	**0.02**
log(Area)	2.51	1.14	**0.03**

I found that an increase by unit log(area) and thus an increase in cushion area is associated with an increase in the probability of a cushion containing fruits (Table 4). The regression coefficient β_1=2.51 represents the log of the odds for fruits present for a given cushion area, meaning that an increase in log(area) by one unit will increase the probability of a cushion containing fruits 12.3 times. The wide confidence interval for larger cushions indicates the level of uncertainty for the prediction and is likely to stem from there being very few observations of fruits present (Figure 3).

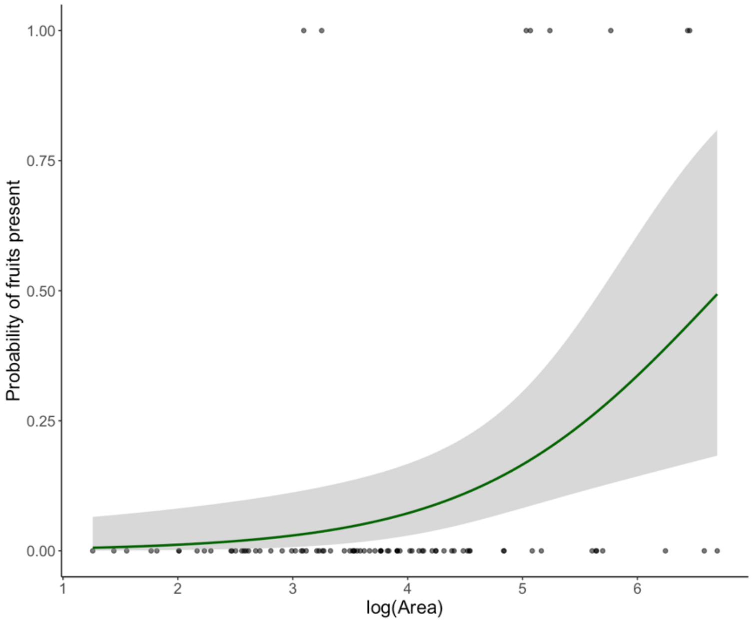

Figure 3: *The predicted probability of a cushion to contain fruits, based a binomial fitted GLMM with fruits present/absent as a response variable and log(area) as the only predictor variable.*

The following output is for the multinomial ordered regression model testing the effects of warming on cushion colour/vigour, and entails parameters selected through correlation analysis and stepwise model selection (Table 3).

Table 5: *Parameter estimates for the best of the multinomial ordered regression models in the set of candidate models (presented in Table 3), predicting the probability of a cushion belonging to a certain colour class, based on treatment. Significant values at p < 0.05 are highlighted. Parameters are presented on the logit scale.*

	Estimate	Std. error	t-value	p-value
Cushion colour				
(Intercept 1|2)	-3.72	0.64	-5.80	**<< 0.001**
(Intercept 2|3)	-1.00	0.41	-2.42	**<< 0.001**
TreatmentOTC	-1.00	0.49	-2.00	**<< 0.001**

Warming had a significant negative effect on cushion colour/vigour (Table 5). Probability for colour class 3 was 23% lower for warmed plots, compared to control plots, while probability for colour class 2 and 1 was higher for warmed plots (19% and 4% higher, respectively) (Figure 4).

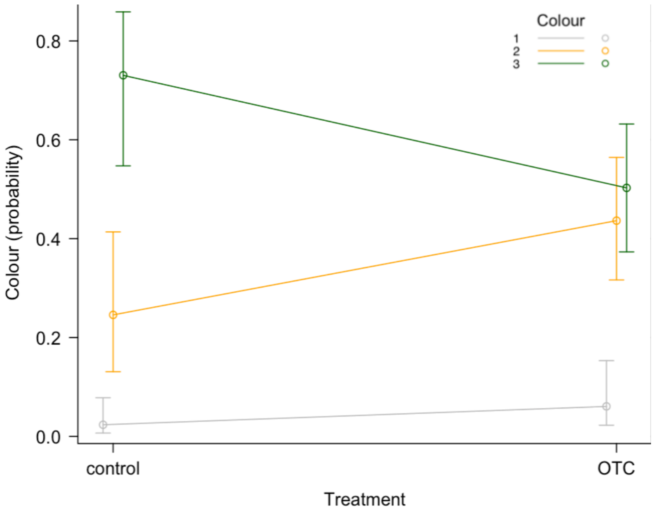

Figure 4: *Results from the multinomial ordered regression model (Table 5). Predicted probability of a cushion belonging to a specific colour class, with colour class as a multilevel response and treatment as an explanatory variable.*

SHIFTS IN PLANT INTERACTIONS IN RESPONSE TO EXPERIMENTAL WARMING

The raw data analyses indicated that the number of lichens and bryophytes per cushion decreased with warming treatment, while the number of vascular plants remained unaffected (Table 6**,** Appendix 10b and 10e). Both the number of vascular plants and lichens and bryophytes per cushion were positively correlated to cushion area (Figure 5a and 5b), while cushion area did not differ significantly between the treatments (Figure 5c, Appendix 1). Both vascular plants and lichens and bryophytes were most abundant in cushions with colour score 2 (Appendix 10c and 10f). However, when plotting the number of individuals per cushion by colour and treatment, it became apparent that the responses to treatment varied between colour classes, indicating that there might be an interaction present (Table 6, Appendix 10a and 10d).

Table 6: *The number of cushions in each colour class by treatment and mean individuals per cushion by treatment and colour class ± se. Raw data.*

Treatment	**Colour**	**n (%)**	**Total individuals**	**Lichens and bryophytes**	**Vascular plants**
			Mean individuals ± se		
control	1	0 (0)	-	-	-
control	2	8 (28)	10.37 ± 3.88	3.00 ± 1.63	7.37 ± 2.32
control	3	21 (72)	18.71 ± 5.84	5.62 ± 2.06	13.09 ± 4.4
OTC	1	4 (7)	9.75 ± 0.48	2.25 ± 0.95	7.50 ± 1.19
OTC	2	23 (42)	18.87 ± 5.73	4.30 ± 1.49	14.56 ± 4.83
OTC	3	28 (51)	10.79 ± 3.54	1.07 ± 0.23	9.71 ± 3.39

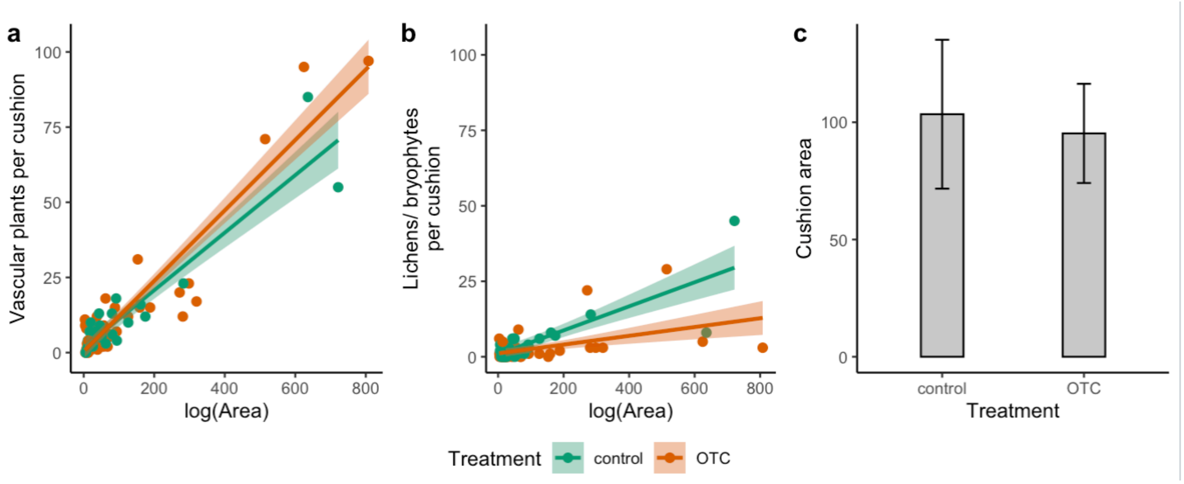

Figure 5: *Relationship between cushion area and the number of individuals per cushion by treatment (a, b), and mean cushion area per treatment ± se (c). Raw data*

The model with the lowest AICc score and thus the best model out of the candidate models for the number of lichens and bryophyte included treatment, cushion area and colour/vigour as main effects with a colour*treatment interaction. The best candidate model for number of vascular plants was found to include cushion area and cushion colour/vigour as fixed effects (Table 7).

Table 7: *GLMM models with AICc values and ΔAICc values to estimate the occurrence of lichens and bryophytes and vascular plants inside* S. acaulis *cushions. Df=degrees of freedom for the respective candidate model. Wt=model weights.*

Response	Predictors	Df	AICc	AICc	Wt
Total individuals ~	Treatment*Colour + log(Area)	7	573.96	0.00	0.77
	Treatment + Colour + log(Area)	6	576.93	2.98	0.17
	Treatment + log(Area)	4	579.05	5.09	0.06
Lichens/bryophytes ~	Treatment*Colour + log(Area)	7	357.28	0.00	0.96
	Treatment + Colour + log(Area)	6	363.79	6.51	0.04
	Treatment + log(Area)	4	367.17	9.89	0.001
Vascular plants ~	Colour + log(Area)	5	514.71	0.00	0.67
	Treatment + Colour + log(Area)	6	517.00	2.29	0.21
	Treatment + log(Area)	4	518.12	3.41	0.12

The following output is from the best of all considered GLMMs for species interactions and entails parameters selected through correlation analysis and stepwise model selection (Table 7).

Table 8: *Parameter estimates for the best species interactions model in the set of candidate models (presented in Table 7). Significant values at $p < 0.05$ are highlighted. Parameters are presented on the log-link scale.*

	Estimate	**Std. error**	**p-value**
Total individuals inside cushion			
(Intercept)	0.48	0.33	0.151
TreatmentOTC	0.36	0.16	**0.023**
Colour2	-1.10	0.32	**<0.001**
Colour3	-0.84	0.28	**0.003**
log(Area)	0.70	0.04	**<0.001**
TreatmentOTC:Colour2	0.41	0.18	**0.020**
Lichens and bryophytes			
(Intercept)	0.57	0.74	0.444
TreatmentOTC	-1.43	0.34	**<0.001**
Colour2	-1.67	0.72	**0.021**
Colour3	-1.49	0.66	**0.025**
log(Area)	0.53	0.08	**<0.001**
TreatmentOTC:Colour2	1.13	0.38	**0.003**
Vascular plants			
(Intercept)	-0.31	0.33	0.350
Colour2	-0.77	0.31	**0.012**
Colour3	-0.75	0.30	**0.013**
log(Area)	0.75	0.04	**<0.001**

In mixed models with non-linear link functions, as in this case with the Poisson mixed models, the estimated coefficients (Table 8) have an interpretation that is conditional on the random effect (Rizopoulos, 2018), in this case, the Plot.ID. Given the wide confidence intervals for the mean random effect variances for both species interactions models (Appendix 21, Appendix 22), I was interested in comparing the conditional means with the marginal means and the raw means. The marginal/population means were found to be the closest estimates to the raw data means for both species interactions models and were thus assumed to be the most accurate.

Table 9: *Predicted conditional, marginal and raw means with standard errors for lichens and bryophytes. Calculated, based on the best model out of the set of GLMMs for lichens and bryophytes and back-transformed from the log scale.*

Treatment	**N**	**No terms marginalized**	**All random effects marginalized**	**Raw data**
			Mean ± SE	
control	29	3.73 ± 0.56	4.85 ± 1.46	4.90 ± 1.56
OTC	55	1.65 ± 0.22	2.47 ± 0.52	2.51 ± 0.66

The predicted marginal mean (back-transformed from the log scale) for lichens and bryophytes per cushion was significantly lower in the OTCs, compared to the control plots (2.47 and 4.85, respectively) (Table 9, Figure 6c). Cushion colour alone was not a significant predictor for the number of lichens and bryophytes (Figure 6b, Appendix 3), but the colour*treatment interaction was significant at a 5% test level (Table 8).

Table 10: *Predicted conditional, marginal and raw means per colour/vigour class by treatment for lichens and bryophytes, with standard errors. Calculated, based on the best model out of the set of GLMMs for lichens/ bryophytes and back-transformed from the log scale.*

Treatment	Colour	N	No terms marginalized	All random effects marginalized	Raw data
				Mean ± SE	
control	1	0	-	-	-
control	2	8	2.96 ± 0.59	2.97 ± 1.19	3.00 ± 1.63
control	3	21	4.01 ± 0.74	5.57 ± 1.96	5.62 ± 2.06
OTC	1	4	2.09 ± 0.47	2.22 ± 0.60	2.25 ± 0.95
OTC	2	23	2.52 ± 0.44	4.26 ± 1.14	4.30 ± 1.49
OTC	3	28	0.87 ± 0.12	1.03 ± 0.14	1.07 ± 0.23

In control plots, the predicted marginal mean for lichens and bryophytes was highest in cushions with colour score 3 (Table 10), while in the OTCs, the marginal mean in cushions with equal colour score was significantly lower (5.57 and 1.03, respectively) (Appendix 4). The highest marginal mean for liches and bryophytes inside the OTCs was in cushions with colour score 2, which was significantly higher than the mean in cushions with colour score 3 (4.26 and 1.03, respectively) (Appendix 4, Figure 6a).

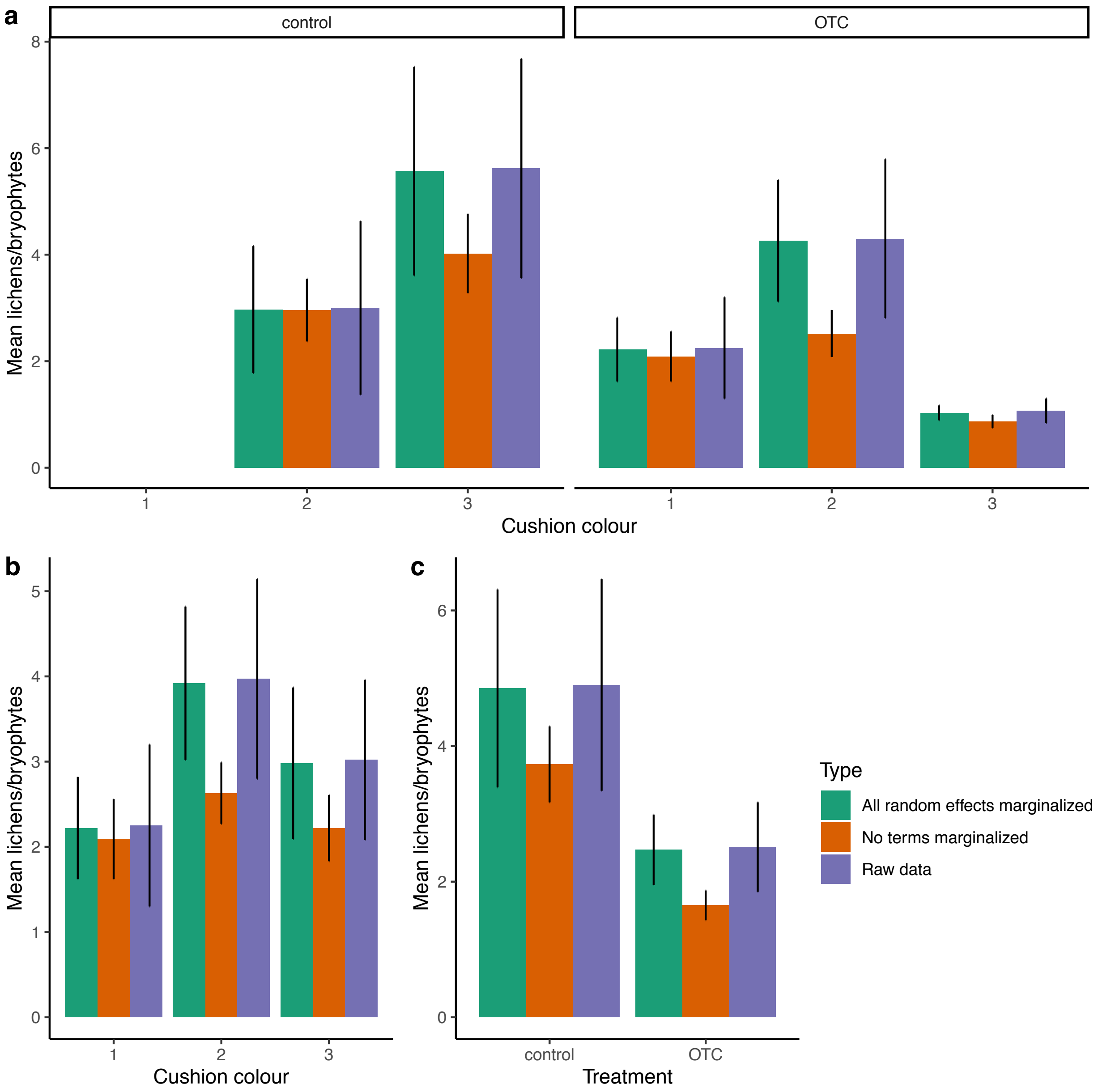

Figure 6: *Predicted conditional, marginal and raw means with standard errors for lichens and bryophytes per cushion. Means are calculated, based on the best models out of the set of GLMMs for lichens and bryophytes and back- transformed from the log scale.*

Table 11: *Predicted conditional, marginal and raw means for vascular plants per colour class, with standard errors. Calculated, based on the best model out of the set of GLMMs for vascular plants and back-transformed from the log scale.*

Colour	**N**	**No terms marginalized**	**All random effects marginalized**	**Raw data**
			Mean ± SE	
1	4	7.30 ± 2.16	7.47 ± 2.13	7.50 ± 1.19
2	31	9.90 ± 2.07	12.67 ± 3.61	12.71 ± 3.65
3	49	9.51 ± 1.58	11.12 ± 2.41	11.16 ± 2.68

Treatment was not a significant predictor for vascular plants and was not included in the final model to avoid overfitting (Table 7). However, cushion colour was a significant predictor for the mean rate of occurrence of vascular plants per cushion, with highest marginal mean for cushions in colour class 2 and the lowest mean for cushions in colour class 1 (12.67 and 7.47, respectively) (Table 11, Figure 7). The difference in means for colour classes 2 and 3 was not significant (Appendix 5).

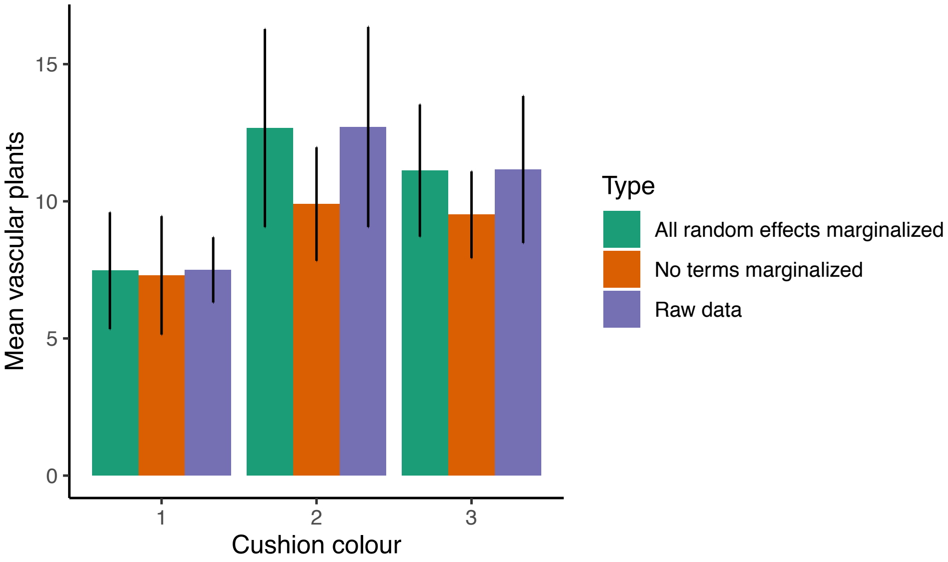

Figure 7: *Predicted conditional, marginal and raw means with standard errors for vascular plants. Means are calculated, based on the best models out of the set of GLMMs for vascular plants and back- transformed from the log scale.*

The predicted number for lichens and bryophytes per cushion in response to cushion area was found to be dependent on treatment and cushion colour/vigour (Figure 8a). In control plots, the slope for lichens and bryophytes was slightly higher for cushions in colour class 3, compared to colour class 2, indicating that for cushions of equivalent size, cushions with a higher colour score tended to contain more lichens and bryophytes. In warmed plots, the slope for colour class 1 was slightly higher, indicating that small cushions with colour score 1 contained more lichens and bryophytes. The slope for class 2 was higher than for colour class 3, indicating for medium to large cushions of equal size, cushions with colour score 2 contained more lichens and bryophytes (Figure 8a). For vascular plants, the slope for colour class 1 was slightly higher for small cushions, compared to colour classes 2 and 3, indicating that small cushions with colour score 1 contained more vascular plants. With increasing cushion size, no individuals were found in colour class 1, while the slopes for colour classes 2 and 3 were almost identical, indicating that for medium to large cushions, there was no difference in the number of vascular plants (Figure 8b).

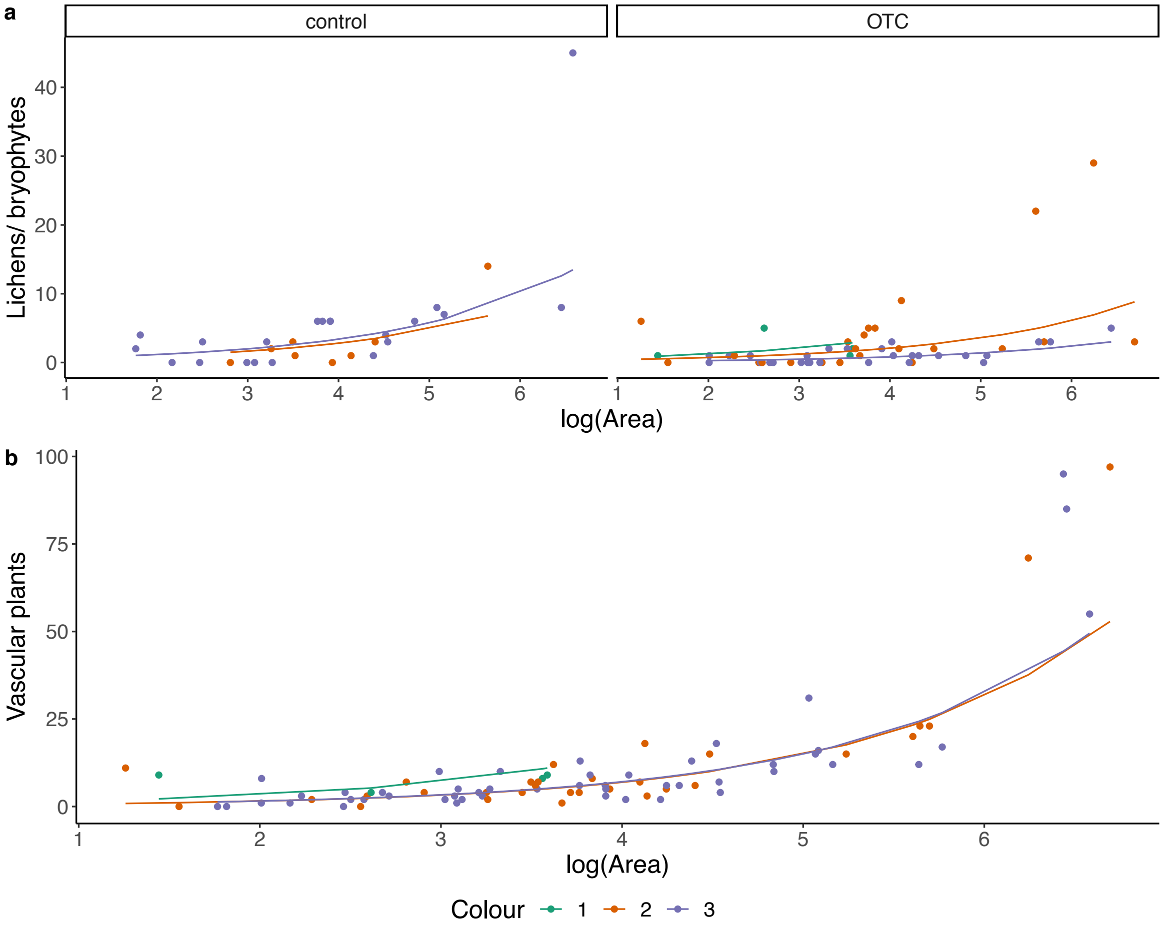

Figure 8: *Predicted number of individuals per cushion in response to cushion area and by treatment/colour. Predictions are calculated, based on the best models out of the set of GLMMs for lichens and bryophytes and vascular plants and back-transformed from the log scale. Dots represent the raw data, while lines represent model predictions.*

Discussion

In this thesis, I explored the effects of long-term experimental warming on the benefactor plant, *Silene acaulis* and its interactions with other species. In contrast to my first hypothesis, I found no significant effect of warming on the probability of fruits. The probability of a cushion containing fruits appears to be dependent on cushion size. Warming was expected to have a negative effect on cushion morphology, which was supported for cushion colour/vigour, but not for cushion height and intactness. The probability for a cushion found inside the warmed plots to be vigorous/green (colour class 3) was significantly lower (50%) compared to cushions in control plots (73%). Lastly, I found mixed results for my third hypothesis. The number of lichens and bryophytes per cushion was significantly lower inside warmed plots, which supports my hypothesis. However, the number of vascular plants appeared to be neither positively nor negatively affected by warming.

EFFECTS OF EXPERIMENTAL WARMING ON FITNESS

Warming treatment was not found to be a significant predictor for the probability of fruits and was thus excluded from the final model. However, it is important to add that the structure of the data as a function of sample size may have affected this result: only eight individuals out of the 56 individuals that were sampled in September 2019 had fruits, thus resulting in a low sample size. A possible explanation for the lack of reproduction observed in the plots at the Sanddalsnuten site might be the high mean and maximum temperatures, with the added effect of unusually low precipitation levels during the previous growing season (July 2018;NCCS, 2019). It is possible that supraoptimal temperature stress coupled with drought stress may have affected resource allocation in *S. acaulis,* resulting in prioritized growth and survival over reproduction (Bazzaz et al., 1987). Indeed, temperature appears to be an important factor, affecting growth, survival and reproduction in cushion plants. Xiong et al. (1999) found that the net photosynthetic rates of the Antarctic cushion plant *Colobanthus quitensis* were affected by temperature and not visible irradiance, with relatively high net photosynthetic rates (P_n) on cool days and negligible photosynthetic rates on warm, sunny days. The optimal air temperature for photosynthesis for many arctic and alpine plant species is within the 10 - 20°C range (Körner, 2003), and 14°C for *C. quitensis,* specifically (Xiong et al., 1999). Even minor increases in air temperature beyond 20°C were found to cause major depressions in P_n in *C. quitensis,* mainly due to high respiration rates (Xiong et al., 1999). Given the variations in air temperature measured at Sanddalsnuten during the summer of 2019, with extreme temperature peaks measured inside the OTCs, and the unusually warm/dry summer of 2018 (NCCS, 2019), it is quite likely that the cushions have been exposed to considerable heat and drought stress with a subsequent reduction in carbon uptake. Thus, the lack of reproduction found at Finse might be explained by a trade-off in resource allocation, with survival and growth being prioritized over reproduction (Bazzaz et al., 1987; Mooney, 1972). This possibility is supported by the findings of Doak and Morris (2010), who compared the vital rates of four different *S. acaulis* populations, across a latitudinal gradient over a six year period. Most vital rates, including fruit production, were shown to increase with low to moderate mean July temperatures, stabilize or increase at moderate temperatures and decline at high temperatures.

I found that the probability of a cushion containing fruits is correlated to cushion size, with bigger cushions being more likely to reproduce. This is in line with the findings of Morris and Doak (1998), who found that larger cushions produced more fruits, and that the first reproduction only commenced after reaching a minimum diameter of ≈3 and ≈4 cm for females and hermaphrodites, respectively. The probability of survival has also been found to increase with cushion size/age (Morris & Doak, 1998) as has the resistance to heat stress (Gauslaa, 1984), with younger leaves being more susceptible to heat damage. Interestingly, fruits were only found inside cushions with colour score 2 and 3 (Appendix 6), which further corroborates the point of non-stressed, more vigorous cushions being able to invest in reproduction.

EFFECTS OF EXPERIMENTAL WARMING ON CUSHION MORPHOLOGY

I found that cushion height and intactness were not affected by warming, which is in line with the findings of (Alatalo & Little, 2014), who found that warming treatment alone did not result in significant changes in *S. acaulis* biomass, while warming, in combination with nutrient treatment resulted in a significant decline in biomass. However, I found that warming had a negative effect on cushion colour/vigour, with a 23% lower probability for cushions found inside OTCs to be vigorous/green. These results are in line with the findings of Xiong (1999), who reported that the critical temperature for *C. quitensis* is ≈42°C, with temperatures beyond this threshold causing instability in the thylakoid membranes and an impairment of photosystem II. With *C. quitensis* and *S. acaulis* both belonging to the carnation family (Caryophyllaceae) and both being cold-adapted, cushion-forming plants, it is likely that the physiological responses to supraoptimal and critical temperatures observed in *C. quitensis* may also apply to *S. acaulis.* Interestingly, Gauslaa (1984), observed brown/discoloured leaves in

S. acaulis the day after measuring a season high midday air temperature of 21°C, with a corresponding leaf temperature of 45.5°C. Thus, extreme air temperature peaks measured during the summer of 2019, may have been the cause for the overall lower cushion vigour inside the warmed plots. Indeed, it has been shown that northern *S. acaulis* populations have lower temperature optima, compared to southern populations, meaning that temperatures in which the southern populations still are growing, might be detrimental to the northern populations (Doak & Morris, 2010; Peterson et al., 2018). Furthermore, the effects of rising temperatures have been shown to be exacerbated by drought, as transpiration cooling is a key factor in leaf temperature regulation (Buchner et al., 2017). Thus, when predicting future scenarios for *S. acaulis*, one should not only look at temperature effects, but also consider the potential effects of temperature and drought combined.

SHIFTS IN PLANT INTERACTIONS IN RESPONSE TO EXPERIMENTAL WARMING

Warming was expected to reduce facilitative interactions of *S. acaulis* with other species, which was supported by my findings for lichens and bryophytes, but not for vascular plants. I found that experimental warming had a negative effect on the number of lichens and bryophytes found inside *S. acaulis* cushions. While the number of lichens and bryophytes per cushion was found to be positively correlated to cushion size and vigour in the control plots, the opposite effect was observed in the OTCs. Inside the OTCs, the number of lichens and bryophytes per cushion was highest for cushions with lower vigour scores. A possible explanation for these results might be a shift from neutral or facilitative interactions to competitive interactions between *S. acaulis* and lichens and bryophytes, with different amounts of competition present in the OTCs, depending on cushion size and colour.

According to the stress-gradient hypothesis, positive interactions between plants increase with environmental stress. A number of studies have explored the degree of environmental stress that is required for the shift from competitive to facilitative plant interactions (Callaway et al., 2002; Cranston et al., 2012; Holmgren & Scheffer, 2010; Maestre et al., 2005), but the answer to the question of how much stress is enough for facilitation to occur still remains unclear. Schöb et al (2013) argued that facilitation in cushion plants is linked to cushion physiology and morphology, with cushions growing at higher elevations being more vigorous (larger, more compact) and thus creating more favourable microhabitats, while cushions growing in lower elevations probably experiencing suboptimal conditions and thus being too stressed for facilitation to occur, but see (Bonanomi et al., 2016). This supports my findings on the interactions of *S. acaulis* and lichens and bryophytes, as the highest number of lichens and bryophytes was found inside large and vigorous cushions in control plots, while a release from facilitation appears to have occurred inside the OTCs. Facilitation may also be an important temporal determinant of species diversity, with interactions between species shifting across the growing season. In this way, *S. acaulis* may serve as a temporary facilitator of species during times of abnormally high stress, such as seasonal heatwaves or spikes in maximum daytime temperatures by providing improved soil moisture conditions (Antonsson et al., 2009; Cavieres et al., 2014).

However, facilitation and competition are not mutually exclusive along an altitudinal gradient; both can occur together (Aschehoug & Callaway, 2015; Klanderud & Totland, 2005). This is in line with my findings on the interactions of *S. acaulis* and vascular plant species: while the number of vascular plants per cushion did not differ significantly between the treatments, similar to lichens and bryophytes, more individuals were found inside cushions with the lowest colour/vigour score and small area, indicating that interactions may be dependent on both cushion size and vigour. Small cushions appeared to engage in competitive interactions, while medium/large cushions appeared to interact in neutral or positive ways.

It is important to add that these results are based on field observations during one season and that factors other than competition, such as responses to environment, may have played a role in the decline in lichens and bryophytes. In order to gain a better understanding of the full suite of interactions occurring, it would be necessary to conduct additional experiments (e.g. removals) and obtain data from several consecutive growing seasons.

SOURCES OF UNCERTAINTY AND THE ECOLOGICAL SIGNIFICANCE OF THIS THESIS

This thesis is based on data, collected during one field season and thus might not give a complete picture of the effects of long-term warming on *S. acaulis* and its interactions with other species. In order to gain a more complete understanding of these effects and the mechanisms behind them, it would be necessary to obtain data for several consecutive growing seasons and from different sites to improve sample size. Studies have suggested that OTCs may pose unwanted ecological effects, such as lower soil moisture (Marion et al., 1997), reduced pollinator activity (Totland & Eide, 1999) and restricted species migration (Yang et al., 2018). Therefore, it might be beneficial to implement other methods, in addition to OTCs, such as whole-community transplantation, altitudinal or latitudinal gradient studies, and space-for-time substitution as these methods have been shown to yield different results (Yang et al., 2018). Thus, a comparative analysis, using two or more of these methods might potentially provide more robust results. Consequently, this thesis provides a baseline for future studies on the effects of long-term warming on alpine cushion plants.

Conclusion

S. acaulis is an ecologically important species due to its facilitative effects and its contribution to alpine ecosystem diversity. Most studies on the effects of elevated temperatures on *S. acaulis* and its interactions with other species have been based on short to medium-term warming experiments, leaving a gap in knowledge about the long-term effects. This thesis is based on a twenty-year warming experiment and the results highlight the susceptibility of *S. acaulis* to long-term warming.

I found surprisingly low reproduction rates at the site, indicating that cushions both inside the control plots and the OTCs were experiencing a considerable amount of stress. However, cushions inside the OTCs were found to have more discolouration, compared to control plots. In addition, cushions inside the OTCs contained significantly less lichens and bryophytes, while the number of vascular plants per cushion did not differ between the treatments, indicating a possible shift in interactions of *S. acaulis* and lichens and bryophytes from neutral or facilitative to competitive.

It is likely that other cushion plant communities growing in analogous environments will respond similarly to elevated temperatures. Thus, when predicting the future effects of climate change on alpine plant communities, it is essential to take into account both the direct and the indirect effects of elevated temperatures on benefactor species, as these species play a key role in alpine plant community dynamics.

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Appendix

Appendix 1. *Mean number of cushions per plot, mean cushion area (population mean) and mean cushion area per plot with standard errors.*

Treatment	**Cushions per plot** ± se	**Mean cushion area** ± se	**Mean cushion area per plot** ± se
control	2.07	103.44 ± 31.76	127.18 ± 51.76
OTC	2.29	95.26 ± 21.14	97.97 ± 23.66

Appendix 2. *Number of cushions with fruits, and mean fruits per plot by treatment with standard errors.*

Treatment	**Number of cushions containing fruits**	**Fruits per plot** ± se
control	1	1.64 ± 1.64
OTC	7	0.55 ± 0.24

Appendix 3. *Predicted population/marginal means per colour class, with standard errors for lichens and bryophytes. Calculated, based on the best model out of the set of GLMM´s for lichens and bryophytes and back-transformed from the log scale.*

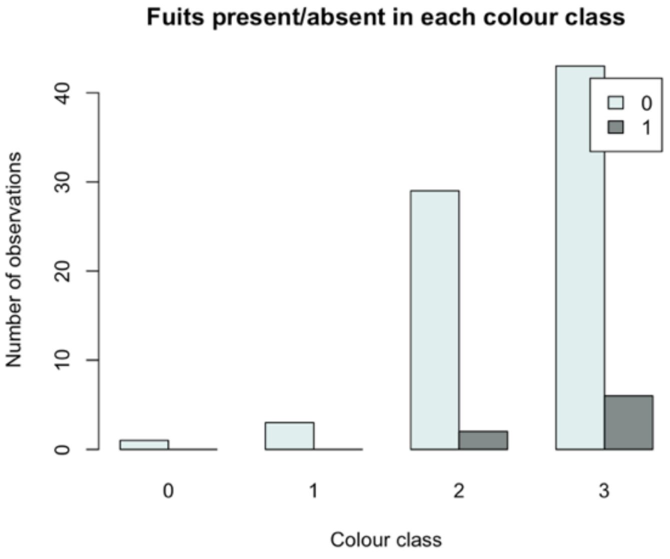
Colour	**N**	**No terms marginalized**	**All random effects marginalized**	**Raw data**
			Mean ± SE	
1	4	2.09 ± 0.47	2.22 ± 0.60	2.25 ± 0.95
2	31	2.63 ± 0.36	3.92 ± 0.90	3.97 ± 1.17
3	49	2.22 ± 0.39	2.98 ± 0.89	3.02 ± 0.94

Appendix 4. *Estimated differences in means for colour*treatment regarding number of lichens and bryophytes per cushion, and the corresponding standard error, and p value. The Tukey test was based on a GLMM with individuals per cushion as response and treatment and cushion colour as interacting explanatory variables variable (averaged over log(area)). *, **, and *** indicate increasingly stronger significant differences. The estimates are given on the log scale.*

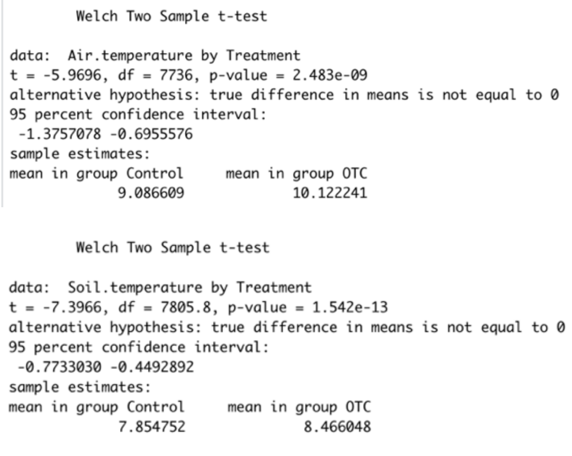
Contrast	**Estimate**	**Std. error**	**P- value**
OTC,1 - control,2	0.2448	0.705	0.9993
OTC,1 - OTC,2	0.5422	0.654	0.9623
OTC,1 - control,3	0.0572	0.666	1.0000
OTC,1 - OTC,3	1.4828	0.662	0.2195
control,2 - OTC,2	0.2974	0.397	0.9756
control,2 - control,3	-0.1876	0.282	0.9858
control,2 - OTC,3	1.2380	0.411	**0.0314***
OTC,2 - control,3	-0.4850	0.326	0.6716
OTC,2 - OTC,3	0.9406	0.254	**0.0030****
control,3 - OTC,3	1.4256	0.345	**0.0005*****

Appendix 5. *Estimated differences in means for the three colour classes regarding number vascular plants per cushion, and the corresponding standard error, and p value. The Tukey test was based on a GLMM with individuals per cushion as response and cushion colour as an explanatory variable (averaged over log(area)). *, **, and *** indicate increasingly stronger significant differences. The estimates are given on the log scale.*

Contrast	**Estimate**	**Std. error**	**P- value**
1-2	0.77	0.31	**0.033***
1-3	0.75	0.30	**0.036 ***
2-3	-0.02	0.08	0.974



Appendix 6. *Number of cushions with/ without fruits by colour class, raw data.*



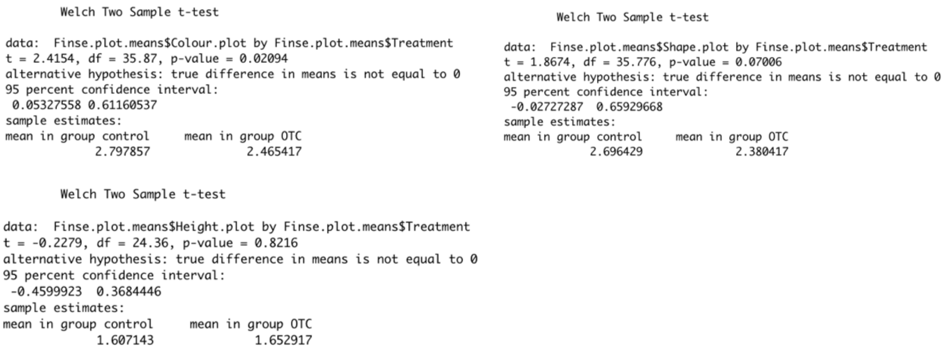
```
        Welch Two Sample t-test

data:  Air.temperature by Treatment
t = -5.9696, df = 7736, p-value = 2.483e-09
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -1.3757078 -0.6955576
sample estimates:
mean in group Control     mean in group OTC
             9.086609             10.122241
```

```
        Welch Two Sample t-test

data:  Soil.temperature by Treatment
t = -7.3966, df = 7805.8, p-value = 1.542e-13
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.7733030 -0.4492892
sample estimates:
mean in group Control     mean in group OTC
             7.854752              8.466048
```

Appendix 7. *Welch two sample t-tests, testing the difference in mean air and soil temperatures between the two treatments.*



```
        Welch Two Sample t-test

data:  Finse.plot.means$Colour.plot by Finse.plot.means$Treatment
t = 2.4154, df = 35.87, p-value = 0.02094
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.05327558 0.61160537
sample estimates:
mean in group control     mean in group OTC 
             2.797857              2.465417 
```

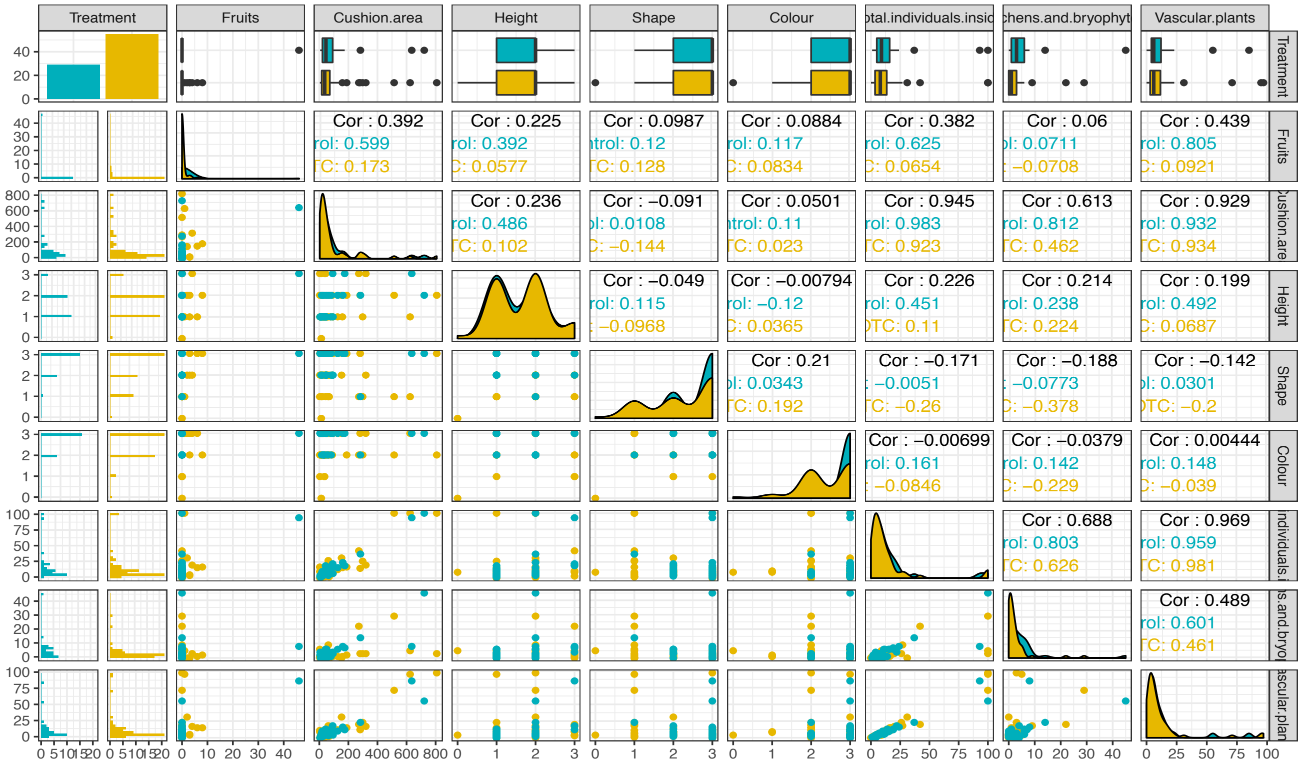
```
        Welch Two Sample t-test

data:  Finse.plot.means$Shape.plot by Finse.plot.means$Treatment
t = 1.8674, df = 35.776, p-value = 0.07006
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.02727287  0.65929668
sample estimates:
mean in group control     mean in group OTC 
             2.696429              2.380417 
```

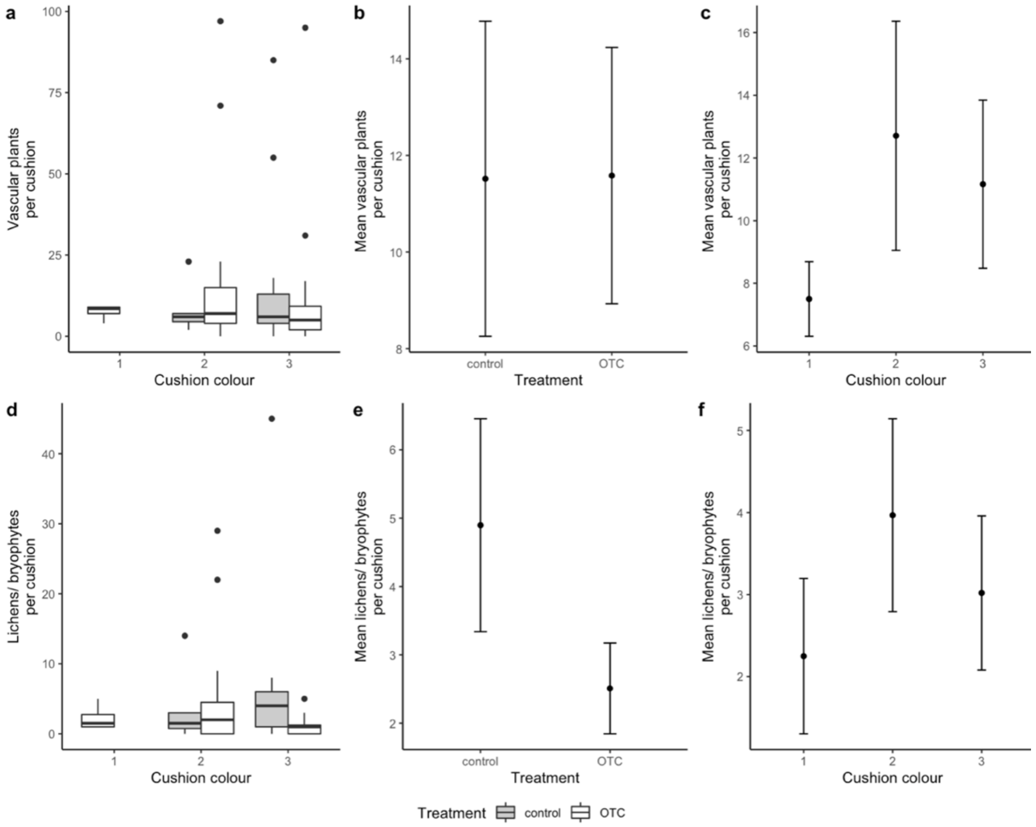
```
        Welch Two Sample t-test

data:  Finse.plot.means$Height.plot by Finse.plot.means$Treatment
t = -0.2279, df = 24.36, p-value = 0.8216
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.4599923  0.3684446
sample estimates:
mean in group control     mean in group OTC 
             1.607143              1.652917 
```

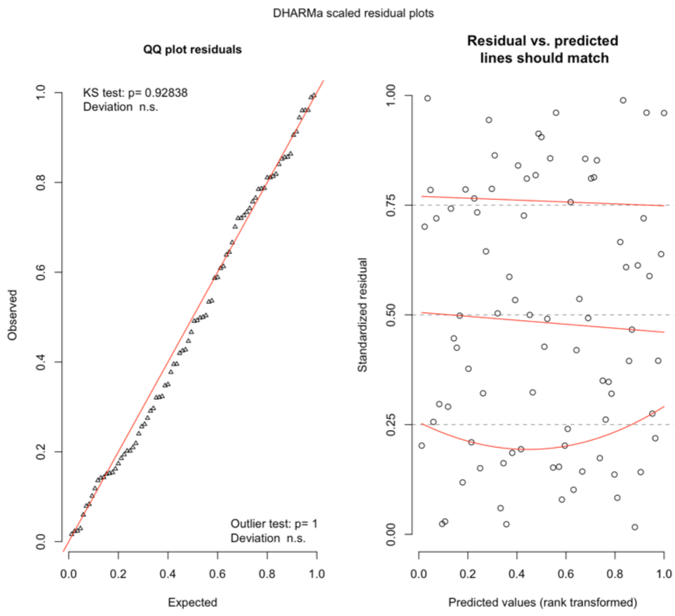
Appendix 8. *Welch two sample t-tests, testing the difference in mean morphology per plot class by treatment.*



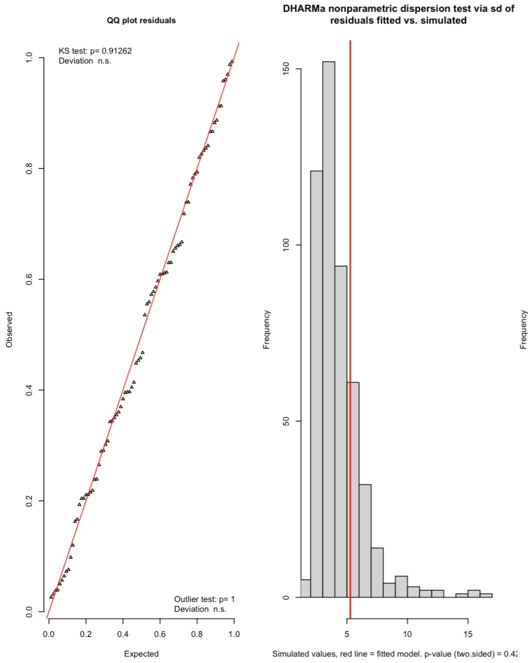
Appendix 9. *Correlation matrix with the response variables and covariates. The lower left triangle of the matrix exhibits scatterplots and bar charts with linear/non-linear trend lines, while the upper right triangle exhibits spearman correlation coefficients.*



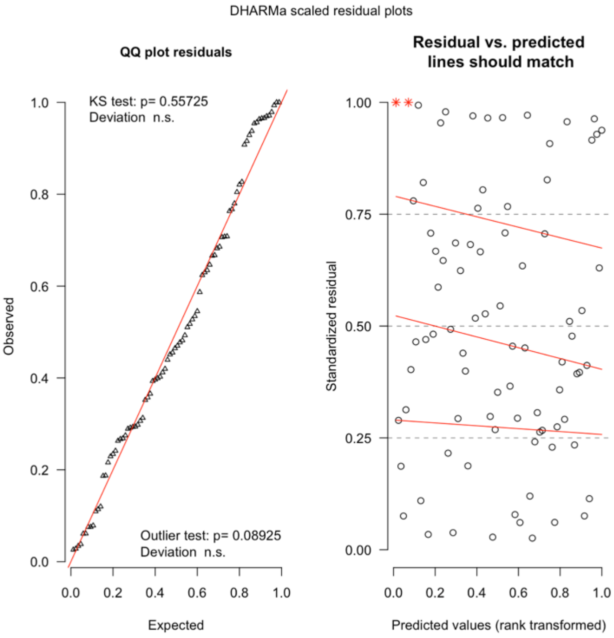
Appendix 10. *Relationship between treatment, cushion colour/vigour, and the number of individuals found inside* S. acaulis *cushions, raw data.*



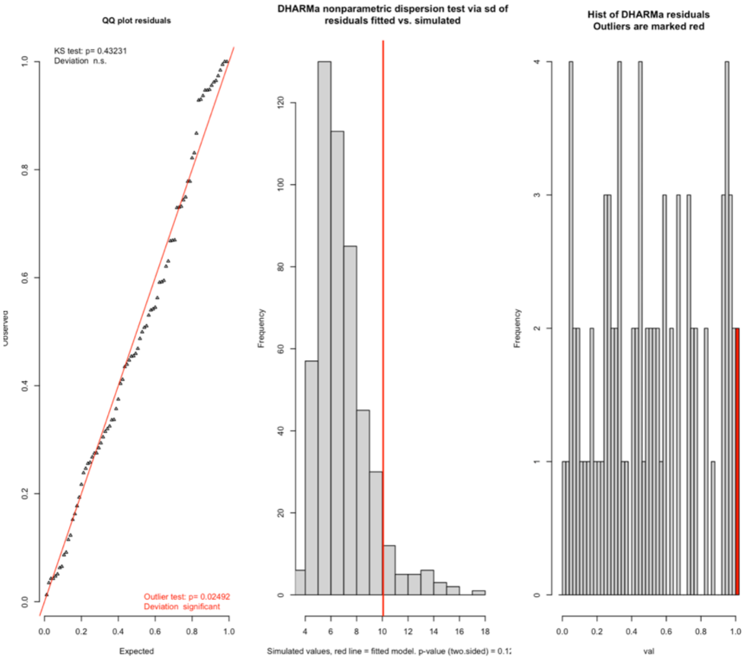
Appendix 11. *Dharma residual analysis for the best GLMM in the candidate set of models for lichens and bryophytes: glmer(Lich ~ Treatment*Colour + logArea + (1|Plot.ID), family=poisson(link = "log")). Predicted values vs standardizes residuals.*



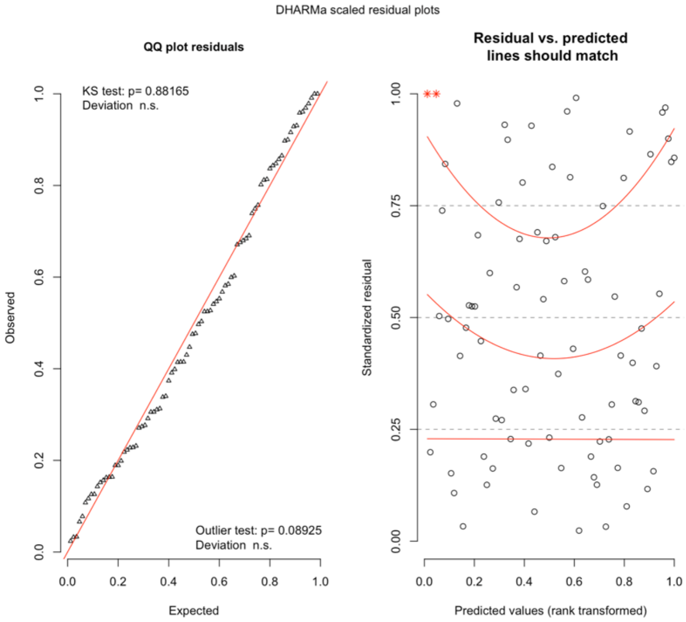
Appendix 12. *Dharma nonparametric residual tests for the best GLMM in the candidate set of models for lichens and bryophytes: glmer(Lich ~ Treatment*Colour + logArea + (1|Plot.ID), family=poisson(link = "log")). Testing uniformity, dispersion and outlier tests for simulated residuals (none significant)*



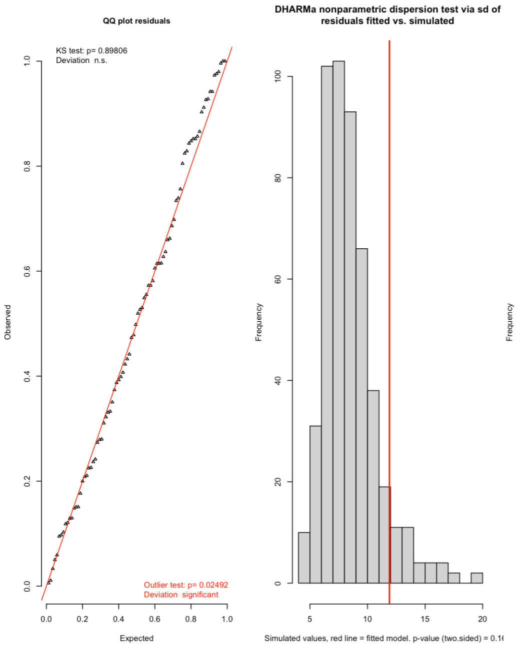
Appendix 13. *Dharma residual analysis for the best GLMM in the candidate set of models for vascular plants: glmer(Vasc ~ Colour + logArea + (1|Plot.ID), family=poisson(link="log")). Predicted values vs standardizes residuals.*



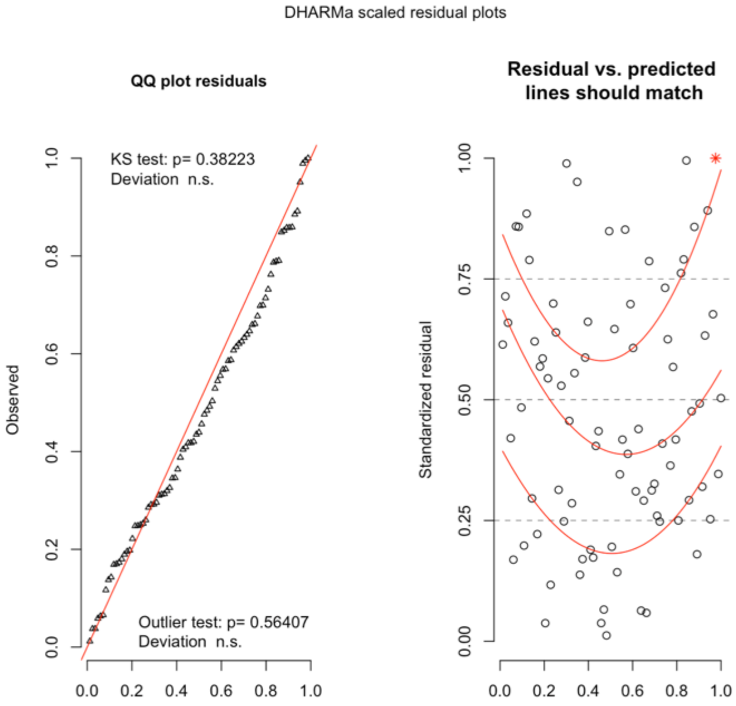
Appendix 14. *Dharma nonparametric residual tests for the best GLMM in the candidate set of models for vascular plants: glmer(Vasc ~ Colour + logArea + (1|Plot.ID), family=poisson(link="log"). Testing Uniformity, dispersion and outlier tests for simulated residuals (outlier test significant)*



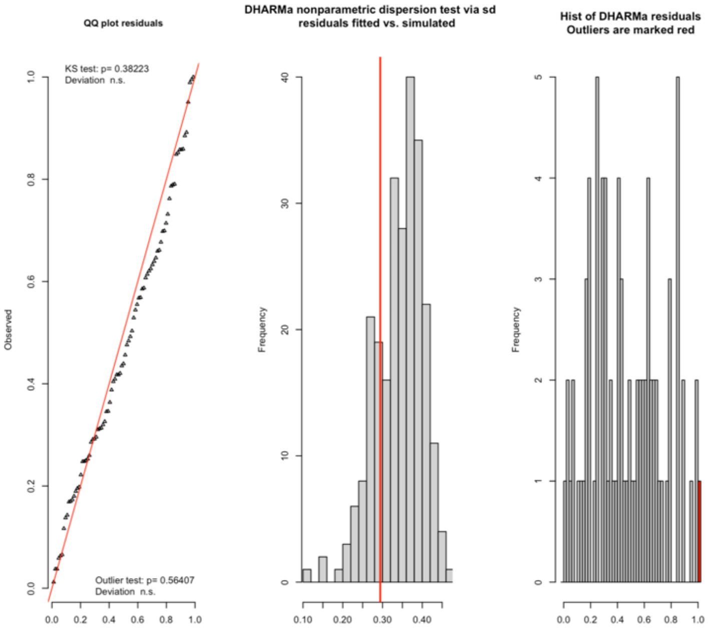
Appendix 15. *Dharma residual analysis for the best GLMM in the candidate set of models for total individuals: glmer(Tot.ind ~ Treatment*Colour + logArea + (1|Plot.ID), family=poisson(link="log")). Predicted values vs standardizes residuals.*



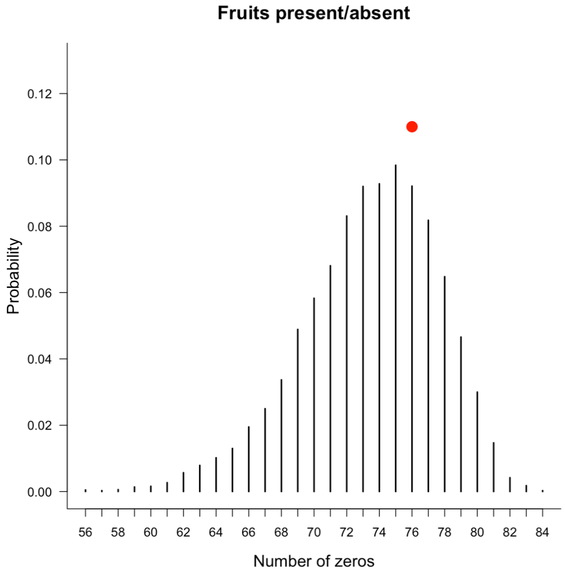
*Appendix 16. Dharma nonparametric residual tests for the best GLMM in the candidate set of models for total individuals: glmer(Tot.ind ~ Treatment*Colour + logArea + (1|Plot.ID), family=poisson(link="log")). Testing uniformity, dispersion and outlier tests for simulated residuals (outlier test significant)*



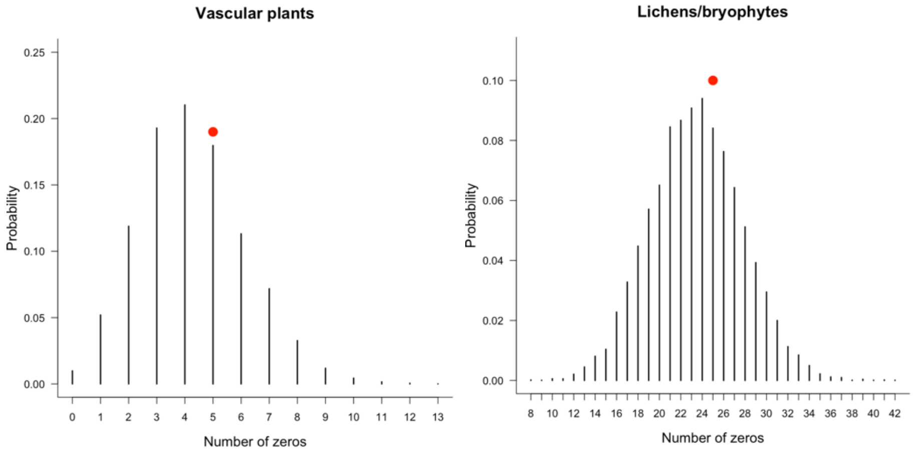
Appendix 17. *Dharma residual analysis for the best GLMM in the candidate set of models for probability of fruits: glmer(Fruits ~ logArea + (1|Plot.ID), family=binomial(link="logit")). Predicted values vs standardizes residuals.*



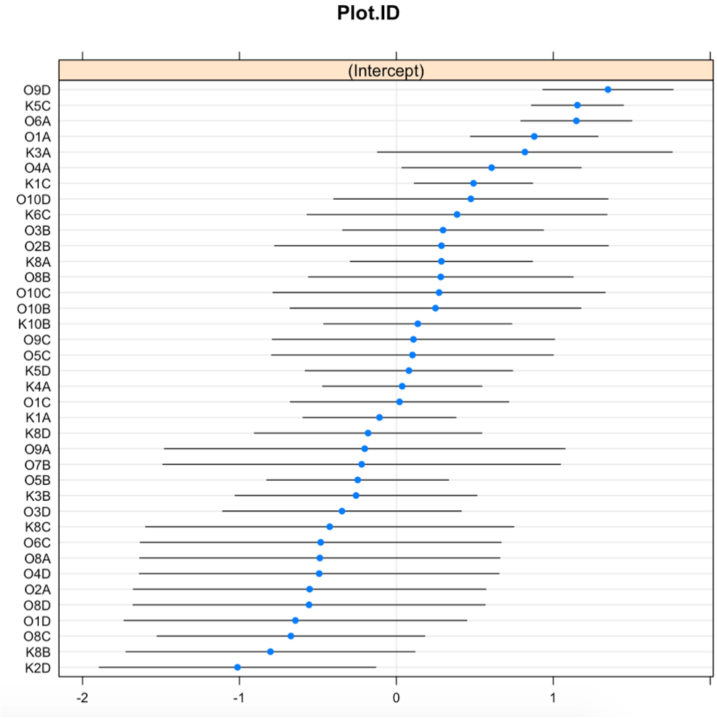
Appendix 18. *Dharma nonparametric residual tests for the best GLMM in the candidate set of models for probability of fruits: glmer(Fruits ~ logArea + (1|Plot.ID), family=binomial(link="logit")). Testing Uniformity, dispersion and outlier tests for simulated residuals (outlier test significant)*



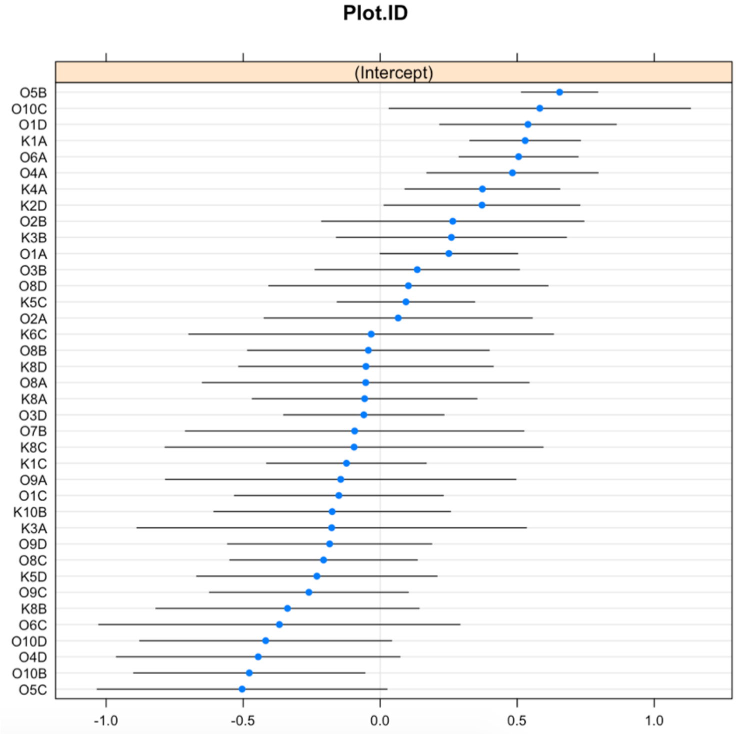
Appendix 19: *Results from a posterior predictive simulation for the fitness/fruits model, based on 10 000 simulations and showing the posterior distribution of the number of zeros. The red dot represents the number of zero-shells in the data. The test was based on 10 000 simulations and the p-value returned for the fitted model was 0.91, meaning the null hypothesis of the model matching the characteristic of the data could not be rejected.*



Appendix 20**:** *Results from a posterior predictive simulation for the species interactions models, based on 10 000 and showing the posterior distribution of the number of zeros. The red dots represent the number of zero-shells in the data. Both tests were based on 10 000 simulations and the p-values returned for the lichen and bryophyte model and the vascular plants model were 0.912 and 0.8227, respectively. Hence, the null hypothesis of the models matching the characteristic of the data could not be rejected.*



Appendix 21**:** *The mean random effect (plot) variances and prediction intervals for the final GLMM for lichens and bryophytes.*



Appendix 22**:** *The mean random effect (plot) variances and prediction intervals for the final GLMM for vascular plants.*

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