Warming responses of two native Icelandic species, *Ranunculus acris* and *Thymus praecox* ssp. *arcticus* in geothermal areas

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Abstract

Global average temperature is predicted to increase from 0.3 - 4.8ºC by 2100, resulting in higher soil temperatures. My objective was to determine 1) how native Icelandic plant species, *Ranunculus acris* and *Thymus praecox* ssp. *arcticus* may respond to soil warming in three geothermal sites, 2) if these responses affect plant fitness, and 3) whether responses are site specific. Geothermal areas provide natural temperature gradients within small geographic areas where soil temperature can be isolated from other variables. Each site has soil temperatures ranging from 9ºC to 49ºC, but they differ in elevation and the time since warming started. Plant phenology, fitness and functional traits were recorded for both species at each site. Linear mixed models were used to describe the relationship between plant traits and soil temperature, revealing that warming responses were species and site specific. At the low elevation/short-term warmed site, temperature affected fitness and height of both species. At the low elevation/long-term warmed site, temperature affected *R. acris* fitness, size and height, and *T. praecox* phenology. In the high elevation/long-term, warmed site (Hen) temperature affected *T. praecox* phenology and size. Temperature effects on fitness were partly mediated by the effect of temperature on two plant traits, height and size, where resource allocation to reproduction lead to a decrease in vegetative growth and *vice versa*. This study highlights the complexity of plant responses to warming in subarctic environments and encourages research into whole-ecosystem responses to warming using geothermal systems.

Keywords: Climate change, global change, phenology, fitness, *Ranunculus acris*, *Thymus praecox*, geothermal warming, natural gradients

Útdráttur

Því er spáð að lofthiti jarðar muna að meðaltali vera 0,3-4,8ºC hærri árið 2100 en nú. Mikilvægt er að skilja hvaða áhrif þessi hlýnun mun hafa á lífríki. Svæði þar sem jarðvegurinn er hitaður upp af gufum frá jarðvarma skapa einstakar aðstæður til að rannsaka þetta. Markmið þessa verkefnis var að ákvarða hvaða áhrif jarðvegshlýnun hefur á brennisóley (*Ranunculus acris*) og blóðberg (*Thymus praecox* ssp. *arcticus)* á þremur jarðvarmasvæðum á Íslandi. Innan hvers svæðis voru merktar 60 plöntur af hvorri tegund, sem uxu við hitastig frá 9 ºC til 49 ºC. Fyrir hverja merkta plöntu var skráð hver langt hún var komin í þroskunarferlinu og hæfni og stærð hennar mæld. Almennt þroskuðust plöntur fyrr við hærra hitastig, en áhrif jarðvegshita á hæð, stærð og hæfni voru mismunandi eftir tegundum og svæðum. Brennisóleyjarplöntur minnkuðu með hærri jarðvegshita á tveimur svæðum og blóðbergsplöntur urðu hærri á einu svæði en minnkuðu á öðru. Jarðvegshiti jók hæfni brennisóleyjar en dró úr hæfni blóðbergs, en aðeins á sumum svæðanna. Þessi rannsókn sýnir að viðbrögð plantna við hlýnun eru flókin og breytileg eftir tegundum, svæðisbundnum aðstæðum, t.d. hæð yfir sjávarmáli og því hversu lengi hlýnunin hefur hefur átt sér stað.

Table of Contents

List of Figures ii

List of Tables iii

Abbreviations iv

Acknowledgements v

1 Theoretical Background 1
- 1.1 The Arctic 1
- 1.2 The Arctic and Climate Change 2
- 1.3 Plant Responses to Arctic Warming 4
  - 1.3.1 Change in distribution patterns 5
  - 1.3.2 Change in plant phenology 6
  - 1.3.3 Phenotypic plasticity and adaptive evolution 7
- 1.4 Predicting Future Changes 8
  - 1.4.1 Warming Studies 8
  - 1.4.2 Transplant and Common Garden Studies 10
  - 1.4.3 Modeling 11
- 1.5 Geothermal Areas 12
- 1.6 Iceland 13

2 Warming responses of two native species, *Ranunculus acris* and *Thymus praecox* ssp. *arcticus* in geothermal areas, Iceland 17
- 3.1 Introduction 17
  - 3.1.1 Objectives 19
- 3.2 Methods 19
  - 3.2.1 Study Sites 19
  - 3.2.2 Study Species 21
  - 3.2.3 Plant Measurements 22
  - 3.2.4 Environmental Parameters 24
  - 3.2.5 Statistical Analysis 24
- 3.3 Results 25
  - 3.3.1 Plant response to soil temperature 25
  - 3.3.2 Soil warming and the selection for traits that will effect plant fitness 28
- 3.4 Discussion 28
  - 3.4.1 Trait responses to soil warming 29
  - 3.4.2 Site Conditions 32
- 3.5 Conclusions 34

References 35

Appendix A: Trait correlations 35

Appendix B: Effect of soil temperature on correlated traits for each site 48

Appendix C: Path analysis for all traits, including those that were correlated 50

List of Figures

Figure 1: High, Low and Sub - Arctic, and geographic Arctic circle, northern treeline and 10 ºC July isotherm. 1

Figure 2: Predicted changes in average surface temperatures between 1986-2005 to 2081-2100 under the most and least modest climate change scenario. 3

Figure 3: Schematic of the predicted changes within Arctic biomes as a result of climate change...4

Figure 4: Changes in flowering onset for six species between 1996 and 2005 in the high-Arctic of Greenland. 6

Figure 5: Map of geothermal areas around the world representing the hottest areas, including high-latitude ecosystems. 13

Figure 6: Map of Iceland´s main volcanic zones, systems and central volcanos. 14

Figure 7: Location of study sites within Iceland...19

Figure 8: The Thingvallavatn catchment of SW-Iceland, major faults, volcanic systems including Hengill, Hrómundartindur and Hveragerði, as well as active and extinct central volcanoes..20

Figure 9: Plant phenology, fitness, plant size, and plant height of R. acris across the three study sties; Hengill, GN and GO, and all three sites together. 26

Figure 10: Plant phenology, fitness, plant size, and plant height of T. praecox across the three study sties; Hengill, GN, and GO, and all three sites together...............27

Figure 11: Results of the likelihood ratio test comparing models. a) model with height and fitness for R. acris at GN, b) model for height and fitness for R. acris from GO, c) model for total plant size for R. acris from GO, d) model for plant height and fitness, measured with the average number of seeds for each flower for T. praecox from GN. 28

List of Tables

Table 1: Site conditions for GN, GO and Hengill including short-term versus long-term warming, elevation, GPS corrdinates, mean annual temperature, mean annnual precipitation, and the presense of sheep grazing. The sample size, and temperature gradients from control to T5 for both R. acris and T. praecox at each site. 21

Table 2: Phenological stages for R. acris. 23

Table 3: Phenological stages 23

Table 4: The best fit models for R. acris and T. praecox across the three geothermal sites combined. The models compared included one with interaction between sites, one without interaction but with temperature and site as fixed variables, and the models with only one of the two fixed variables. 25

Table 5: Results of linear mixed effect models for R. acris and T. praecox across each of the three sites individually as well as results from the comparison for the model using temperature as the fixed variables, measured plant traits as the response variables (phenology, fitness, size and height) and soil moisture as the random effect. 26

Abbreviations

ACIA -Arctic Climate Impact Assessment

CAFF -Conservation of Arctic Flora and Fauna

ENMs -Ecological Niche Models

GN -Grassland New

GO -Grassland Old

Hen -Hengill

IPCC -Intergovernmental Panel on Climate Change

ITEX -International Tundra Experiment

LMM -Linear mixed effect models

LT -Long-term

NDVI -Normalized Difference Vegetation Index

SEM -Structural Equation Models

ST -Short-term

STS -Space for time substitutions

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1 Theoretical Background

1.1 The Arctic

The Arctic encompasses areas from Eurasia to North America, with diverse land and seascapes that make up a single complex and integrated system. The definition of the Arctic is variable, with disciplines setting different southern limits (ACIA 2005). There are sociopolitical interpretations, including the Arctic Circle based on the latitudinal gradient (66º33'North) or the definition established by the Conservation of Arctic Flora and Fauna (CAFF). Transportation and shipping routes use a definition that is based on a marine context. For natural scientists the Arctic tundra can be defined as the area beyond the treeline which, in continental areas corresponds closely to the climatological interpretation which uses the 10 - 12ºC mean daily July isotherm (Löve 1970; ACIA 2005; Meltofte *et al.* 2013). The Arctic includes; the high Arctic in the north with sparsely vegetated lowland areas and Polar deserts; the low Arctic further south, with lush vegetation and tundra ecosystems and the transition into the subarctic below the treeline and the 10 ºC July isotherm that encompasses the boreal forest-tundra biome (Löve 1970; Meltofte *et al.* 2013; IPCC 2014; Figure 1).

Figure 1: Geographic extent of the high, low and subarctic, defined by CAFF, along with the boundary of the CAFF itself. High and low the Circumpolar Arctic Vegetation Mapping Project defined Arctic terrestrial boundaries - Bioclimatic subzones. Subarctic boundary was defined by the Arctic Biodiversity Assessment. Source: http://geo.abds.is/geonetwork/srv/eng/catalog.search#/home

Three distinct types of landforms dominate the Arctic landscape composition; (i) uplands shaped by ice sheets leading into fjords, (ii) flat plains or plateaus with glacial, alluvial or marine deposits, and (iii) mountain ranges in the forms of peaks or rounded slopes. Climate of the Arctic and subarctic regions are greatly influenced by absence of sunlight throughout the winter months and continuous sunlight in the summer. The region is highly dependent on the cryosphere, with snow and ice playing a prominent role in the determination of both regional and global climate (Dicks *et al.* 2013). This reliance on the cryosphere makes the Arctic particularly vulnerable to disturbances because of small changes in temperature (ACIA 2005). The environmental conditions of the Arctic are characterised by significant environmental variability on both spatial and temporal scales, with steeper temperature gradients than in any other biome (Wielgolaski 1997). For example, average summer temperatures as well as thawing soil layers will vary significantly over a small geographic area, with a large impact on the terrestrial plant composition. This is seen in northern Siberia where the average July temperature varies by 10 ºC over 900 km, a similar temperature change would require over 2000 km in the boreal zones (ACIA 2005). Instead of a homogenous region, the Arctic is composed of distinct regional climates with specific ecological conditions (Wielgolaski 1997). Unique local conditions, such as soil type and temperature, create microclimates within the larger macroclimate communities, creating the diverse ecosystem structure (Wielgolaski 1997). These variations in local conditions lead to abundant structural diversity in the landscape that follow a latitudinal gradient with temperature from the boreal-tundra transition of the subarctic, to the polar desserts in the high Arctic (Wielgolaski 1997; ACIA 2005). This small scale heterogeneity makes some Arctic environments more vulnerable to climate change as population size and range are relatively restricted by local conditions (ACIA 2005; Forbes 2015).

As a result of low mean summer temperatures and a short growing season, net primary productivity, net ecosystem productivity, decomposition rates, and species diversity are low in Arctic regions. Thus, mean summer temperature is one of the environmental variables that best predict plant diversity of the Arctic mosaic (ACIA 2005). Arctic communities have developed specific adaptations to Arctic conditions and can cope with freezing temperatures, ice encapsulation, low summer temperatures, short and late growing seasons, inter annual variability, and snow cover. However, despite these adaptations, the low species diversity of Arctic communities, means these ecosystems are sensitive to even minor departures from typical climatic conditions (ACIA 2005). Thus, climate change will likely have harsh effects on organisms in the Arctic.

1.2 The Arctic and Climate Change

Anthropogenic climate changes are expressed as increasing average temperatures, rising sea levels, altered precipitation/hydrological patterns, as well as more frequent and extreme weather events globally (IPCC 2014). These changes are amplified in the global poles where regional warming is nearly twice as high in the Arctic compared to the global average (ACIA 2005; IPCC 2014; Figure 2). In 2015 the average global land surface temperature was +0.7ºC (± 0.18ºC standard error) relative to the baseline between 1981 and 2010, while the Arctic experienced a 1.2ºC increase in average land surface temperatures in 2015 relative to the same base period (IPCC 2014). Changes to terrestrial ecosystems in the Arctic and subarctic are already apparent because of warming conditions. These changes range from general trends showing a greening of the Arctic, to specific species level changes, such as local extinction (IPCC 2014). These changes are highly dependent on site characteristics. For example, the advancement of woody and herbaceous plants is influenced by local factors such as warming, herbivory, precipitation and land use patterns, therefore the advancement has been heterogeneous across the Arctic (IPCC 2014).

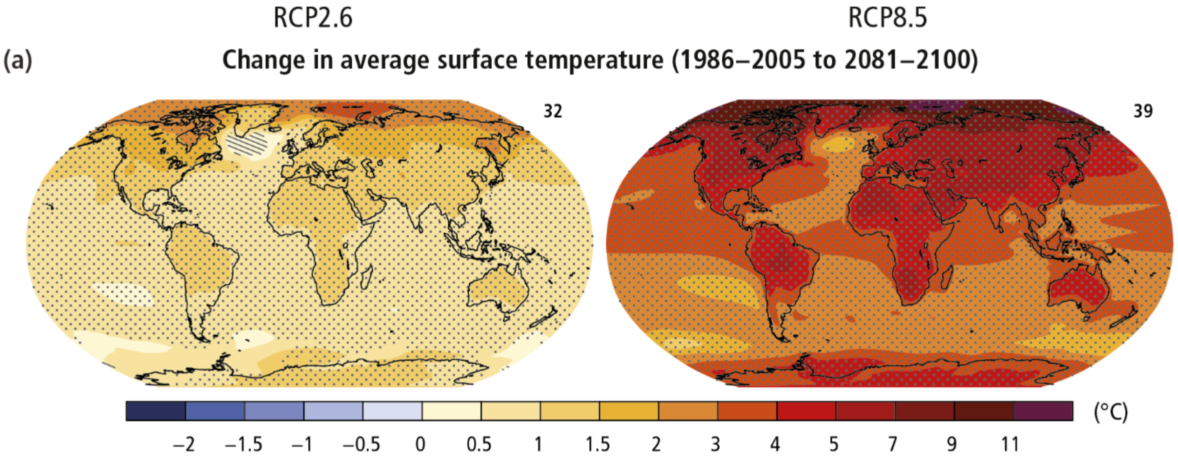

Figure 2: Change in average surface temperature based on projections for 2081–2100 relative to 1986–2005 under the RCP2.6 (left) and RCP8.5 (right) scenarios. (IPCC 2014).

Changes to the Arctic are of global concern because their impacts are not restricted to Arctic regions. The global climate regime is dependent on processes such as the earth's energy balance and the oceanic circulation system, both which are maintained by conditions in the Arctic (ACIA 2005). Climate modeling suggests that as a result of feedback processes warming will continue into the future, with greater changes occurring in the winter (ACIA 2005; Winton 2006; Bony *et al.* 2006; Koenigk *et al.* 2013). For example, decreases in annual snow cover in the Arctic mean that reflective surfaces will be replaced by darker land and water surfaces that have the ability to absorb more solar radiation, triggering a feedback mechanism and accelerating the warming process further (ACIA 2005; Winton 2006). It is predicted that Arctic surface temperature over land area will increase by 2.2 ºC during summers months and up to 5 ºC by 2100 (RCP4.5 Scenario, +1.8 ºC), while the global average is estimated to range from 1.1 to 2.6 ºC (RCP4.5 Scenario, +1.8 ºC; IPCC 2014; IPCC 2015).

This warming is likely to result in significant changes unique to Arctic environments, such as a reduction in sea ice cover, melting of glaciers, and thawing of permafrost, as well as general global changes, such as increases in soil temperatures (ACIA 2005; Hinzman *et al.* 2005). This warming will be accompanied by an increase in annual precipitation (25% in the winter, 15% in the summer; (ACIA 2005; Hinzman *et al.* 2005; IPCC & Stocker 2014). Arctic ecosystems will be influenced by these warming conditions with an extension of regional growing seasons, and potential shifts in the range of species distribution. However, the response of individual species is difficult to estimate (ACIA 2005; Hinzman *et al.* 2005; IPCC 2014; Franks, Weber & Aitken 2014; Figure 3). Some plant species may be ill adapted to cope with modern climate change, whereas other species may thrive.

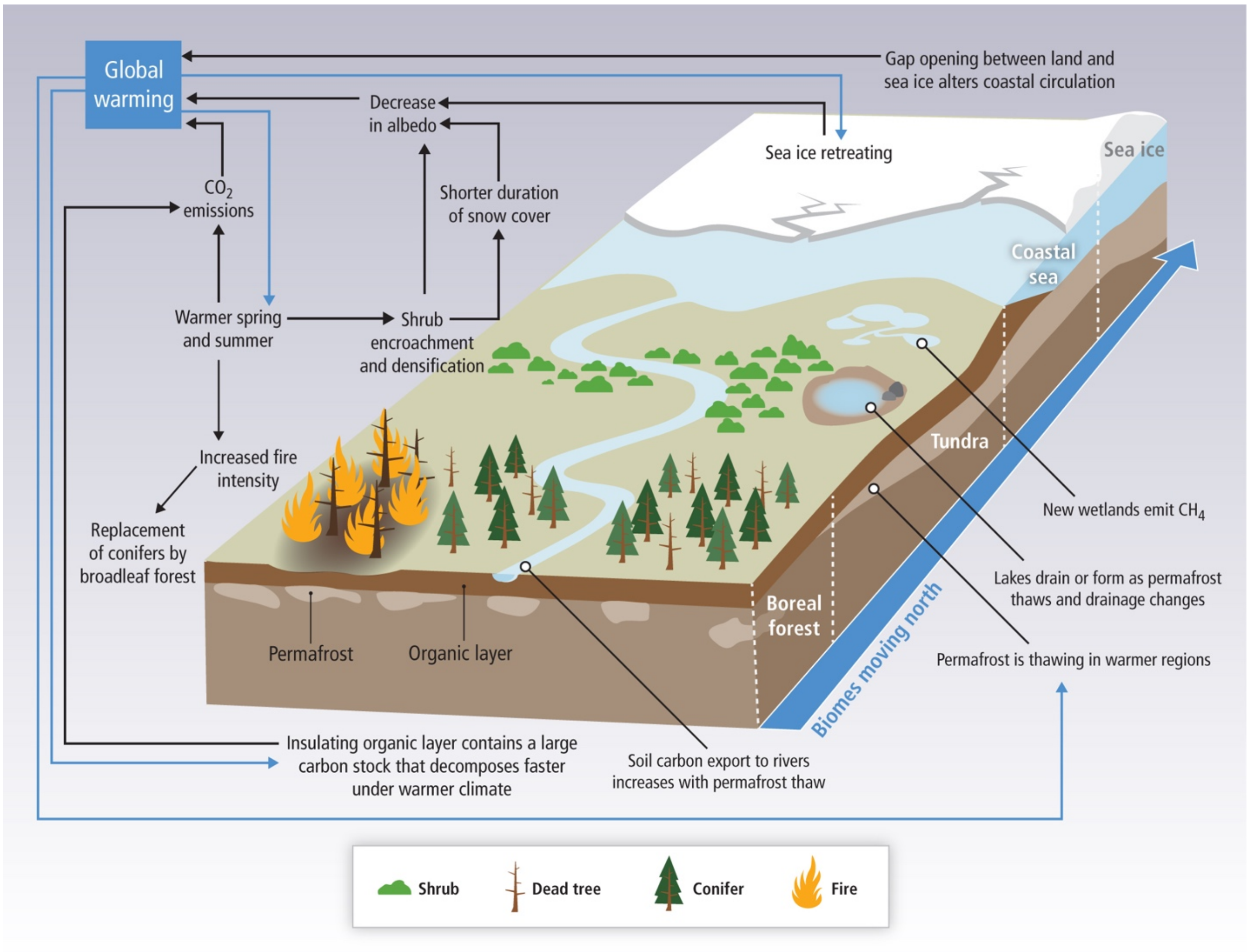

Figure 3: Changes in the boreal-tundra biome, as a result of global change and warming in Arctic and subarctic environments. This includes a northern shift of Arctic vegetation, with the boreal moving into tundra areas. There will also be changes in surface energy balance, carbon balance and emissions, leading to increased feedbacks and more climate change (IPCC 2014).

1.3 Plant Responses to Arctic Warming

Changing environmental conditions will have substantial effects on ecosystems in the Arctic. Terrestrial plant communities can be studied to determine how short term changes (years and decades) in local climate will influence species responses (Primack & Kang 1989). Primary production, or the abundance and diversity of plant species, are the building blocks for entire ecosystems and are vital for maintaining population dynamics (ACIA 2005). Warming temperature is likely to have a direct impact on species, as well as on the trophic relationships between populations. For example, herbivore populations could become dependent on climate responses in vegetative populations. A species ability to respond to warming could determine not only the vulnerability of the species, but also the vulnerability of entire ecosystems. Species responses could include, but are not limited to, changes in distribution patterns and species range, changes in plant phenology, and adaptive evolution.

1.3.1 Change in distribution patterns

Using the Normalized Difference Vegetation Index (NDVI), a general trend towards increased plant productivity, or a general greening in the Arctic has emerged (IPCC 2014). While over half the Arctic has shown no increase in productivity, at least one third has greened and only 4% has browned (greening refers to a general increase in vegetation cover throughout the growing season, while browning refers to a decrease; IPCC 2014). This greening has been associated with changes in vegetative communities at northern latitudes including, but not limited to species migration. In Iceland, greening has been observed, with increases in NDVI measurements between 1982-2010, likely due to warming climate as well as reductions in grazing activity, increased afforestation and soil reclamation, as well as glacial retreat (Raynolds *et al.* 2015).

According to paleoecological evidence through pollen analysis, plant communities have responded to historic climate change primarily through relocation (Huntley 1991). In past warming events that occurred early in the Holocene, species had the ability to migrate northward with increasing temperatures because sea levels were still low, extending the available areas around Arctic coastlines (Forbes 2015). Current global trends in species migration show similar movements, with a poleward range shift of terrestrial plant species (Parmesan 2006). General trends in Arctic and alpine vegetation studies suggest that warming is resulting in an increase of shrub cover along with an overall decrease in species richness and an upwards movement of plant species (Wilson & Nilsson 2009; Elmendorf *et al.* 2012). In Sweden a temperature increase of nearly 2.0 ºC over a 20 year period, resulted in an increase of the evergreen shrub *Empetrum hermaphroditum*, accompanied by a decrease in overall species richness –and increase in herbaceous vegetation within forest communities , with most of the changes occurring at the lower elevations (Wilson & Nilsson 2009). A short term vegetation study in Iceland looking at warming responses of tundra communities found no change in species richness as temperature increased, however there was an increase in deciduous (*Betula nana*) and evergreen dwarf (*Empetrum nigrum* ssp. *hermaphroditum*) shrub abundance (Jonsdottir *et al.* 2005). Multispecies studies have indicated that latitudinal species range expansion northward will occur at a rate between 6.1 km/decade in the Northern Hemisphere (Parmesan & Yohe 2003), to 16.9 km each decade within Europe, North America and Chile (Chen *et al.* 2011).

The rate of northward migration of new or novel species into Arctic regions could become problematic for native populations. As climate change influences the conditions in the Arctic, it is estimated that the abundance and distribution of boreal species, which by current estimates comprise of 40% of all the Arctic species, will continue to increase in Arctic areas (Forbes 2015). Arctic species having the largest abundance or widest ecological amplitude are well adapted to Arctic conditions and are widely distributed mainly due to lack of competition (Forbes 2015). However, with changing conditions, exposure to more stressful local climate conditions will be more likely for species at the trailing southern edge of their range, such as warmer and drier conditions, and increased competition from the southern neighbours. Species with slow growth, low plant stature and relatively low (and variable) flowering and seed set, risk being outcompeted by new species moving into a newly widened northern ecological niche (ACIA 2005). By contrast, species at the leading edge of their range have only limited potential to colonize new areas as a result of extreme and variable local climate events, and maritime encroachment with sea-level rise. Therefore, it is expected that the most significant decline and replacement of cold-adapted species by warm-adapted species, will occur at the southern and northern ecotones (Gottfried *et al.* 2012). However, populations at the leading and trailing edges of their habitat, are often better suited to respond to a warming climate because here the genetic variation is greater compared to the interior population (Parmesan & Yohe 2003; Parmesan 2006).

1.3.2 Change in plant phenology

Plant phenology is an important component of plant development. It describes the timing of specific reoccurring natural processes within a plant's life cycle that are influenced by the environment (Menzel *et al.* 2006; Cleland *et al.* 2007; Wolkovich *et al.* 2012; Schwartz 2013). Phenological characteristics include, but are not limited to plant emergence, bud emergence, flowering time, and senescence. The timing of these phases is critical for the entire life cycle (Cleland *et al.* 2007). Changes in plant phenology, particularly timing of phenological stages can play a role in ecosystem processes like nutrient capture, productivity, species composition of an ecosystem and in the global carbon intake balance (Cleland *et al.* 2007; Leblans 2016).

Phenological characterises are triggered by environmental factors, such as temperature, CO_2 concentration, and precipitation. All these factors are predicted to change considerably in Arctic and subarctic environments as a result of climate change (Menzel *et al.* 2006; Cleland *et al.* 2007; IPCC 2014), thus changes in plant phenology are expected (Molau, Mølgaard & ITEX 1996; Cleland *et al.* 2007; Oberbauer *et al.* 2013). Most Arctic terrestrial ecosystems are already showing signs of phenological responses to warming that vary in magnitude from nearly non-responsive, to drastically earlier (ACIA 2005; Cleland *et al.* 2007; Wolkovich *et al.* 2012; IPCC 2014; Figure 4). The trend emerging is showing later senescence associated with warmer fall temperatures and earlier spring flowering, or spring advancement occurring at a rate between 2.3 to 5.1 days earlier per decade (Cleland *et al.* 2007; Wolkovich *et al.* 2012). Studies have demonstrated that earlier spring flowering has been associated with recent temperature increases, especially increases in spring temperatures (Menzel *et al.* 2006; Cleland *et al.* 2007; Wolkovich *et al.* 2012). Species not considered early spring species are often less responsive to changes in temperature and have shown signs of delayed phenology when temperatures exceed their tolerance, potentially attributed to other factors such as photoperiod or soil moisture (Rathcke & Lacey 1985). This phenological advancement is estimated to increase by 2.3 days per decade (Parmesan & Yohe 2003).

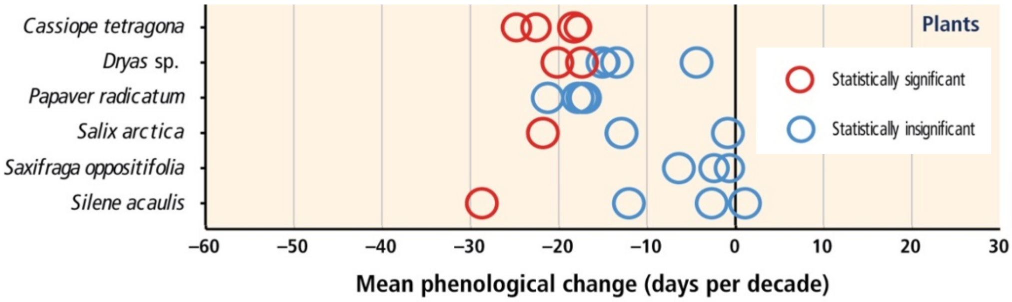

Figure 4: Changes in onset of flowering for six species based on weekly sampling across various plots (individual circles) between 1996 and 2005 in the high-Arctic of Greenland. Sampling lasted between eight and ten years for all species listed. Negative values indicate the earlier onset of flowering in number of days per decade. Adapted from (Høye et al. *2007; IPCC 2014).*

The shifts in spring flowering time and the longer growing seasons might not be the result of temperature increases alone. There is evidence to suggest that atmospheric CO_2 concentrations, and altered precipitation patterns may also play a role in the earlier spring start and general plant phenology (Cleland *et al.* 2007). For example, in Spain, experiments have been unable to rule out precipitation, specifically drier weather, as a contributing factor to changes in plant phenology (Llorens & Peñuelas 2005). Meanwhile, studies of crop species have documented increased levels of CO_2 concentrations leading to an acceleration of phenology stages (Kimball, Kobayashi & Bindi 2002).

1.3.3 Phenotypic plasticity and adaptive evolution

The plant responses that trigger the phenological change or changes in other plant traits as response to warming can be attributed to either phenotypic plasticity or adaptive evolution. Phenotypic plasticity refers to the ability of an identical genotype to display variation in plant traits (phenotypes) under different environmental conditions (Primack & Kang 1989; Conner & Hartl 2004; Franks *et al.* 2014). Phenotypic plasticity is a means for plant populations to react to environmental conditions as a result of climate change in both the short-term and the long-term.

Phenotypic plasticity can be simply a non-adaptive response to environmental conditions with no effect on overall plant fitness, or an adaptive response to environmental stimuli that increases fitness (Merilä & Hendry 2014). Fitness can be described as the ability for an individual to successfully grow, survive and reproduce (the output being the relative number of offspring in the next generation), or as the abundance and success of genes over multiple generations (Primack & Kang 1989). When phenotypic plasticity increases species fitness, adaptive evolution can occur. Adaptive evolution is the gradual natural selection of traits, that increase plant fitness because they are better suited for the new environmental conditions (Conner & Hartl 2004; Franks *et al.* 2014). Genetic diversity contributes to adaptive evolution by increasing the traits that can be influenced by natural selection. Compared to other biomes worldwide, ecosystem resilience of Arctic communities is highly dependent on genetic diversity because species diversity is relatively low (ACIA 2005).

To determine if global change is affecting plant populations and species through plastic or adaptive responses, it is crucial to identify which environmental factors are leading to specific changes. Temperature increases may favour certain plants groups, such as vascular plants however, the effect of temperature often coincides with other environmental changes, making it challenging to isolate the impact of changing temperatures alone (ACIA 2005; Merilä & Hendry 2014). Additionally, there are secondary effects of increased temperature which, when acting alongside increased competition, and changes to moisture and nutrient regimes, will likely have negative impacts on plant communities that could outweigh any impacts.

If species do not respond to climate change by means of phenotypic plasticity or adaptive evolution they will need relocate or expand their range into locations that are more suitable, otherwise they will become locally extinct (Nicotra *et al.* 2010). As a result, the relative importance of plant plasticity compared to other plant responses, such as adaptive evolution or species migration has been questioned. It is unknown if environmental conditions triggering plastic phenotypic responses will continue to be reliable or if at a certain point, species will have to migrate/ expand their ranges, or respond with adaptive evolution in order to cope with the new conditions (Nicotra *et al.* 2010).

1.4 Predicting Future Changes

In order to predict how Arctic terrestrial ecosystems will respond to climate change, we must first understand the way in which environmental variations, such as temperature increases, influence the life cycles of individual plant species. Scientists use various approaches to study this e.g. warming studies to determine how individual plants will respond, transplanting and common garden studies to determine if observed responses are caused by phenotypic plasticity or adaptive evolution, and modeling to predict future conditions. Ideally, these approaches should be combined to study the complex species and environmental dynamics (ACIA 2005).

1.4.1 Warming Studies

Warming studies typically use one of three approaches; long-term observational studies, space for time substitutions or small scale warming of natural plant communities to simulate future changes (Wolkovich *et al.* 2012).

Observational Studies

Long-term observational studies have been carried out at various locations in the Arctic. These studies typically use time series data to determine the change in terrestrial communities (Elmendorf *et al.* 2015). A study using satellite data and the normalized difference vegetation index (NDVI) for long term observations (21 years) of vegetation change, determined that there was an increase in peak vegetation greening by nearly 17% and an increase in aboveground biomass by 171g/m^2 in the Alaskan tundra, between 1981 and 2001 (Jia, Epstein & Walker 2003). Similar results were found when change in shrub cover over a 50-year-old period in Alaska was estimated from high-resolution oblique-aerial photographs. The photo comparisons showed an increase or no decrease in the height, diameter and infilling of three shrub species (Sturm, Racine & Tape 2001; Tape, Sturm & Racine 2006). Studies using long term field monitoring, such as the 42 year study in Yukon, Canada, determined that changes in community composition were occurring as a result of warming temperatures (up to 2 ºC; Danby *et al.* 2011).

Observational data can be used to predict plant responses however, these studies may be limited in their ability to evaluate the full range of a plant's response if the sites are located at the leading or trailing edges of species range (Reyer *et al.* 2013). There are also concerns that studies which only focus on these extreme ecosystems fail to capture the true species response across its population range and can negatively influence the understanding of mean climate impacts or responses of individual species (Reyer *et al.* 2013). These studies have even been criticized for being too opportunistic, referring to the inability to plan the environmental conditions, such as an unseasonably warm growing period, during the study (Reyer *et al.* 2013). The length of the study is also a crucial factor to consider when using observational studies, as the initial responses to warming can be significantly different, in both direction and magnitude, from the long-term responses. Factoring in time scale can be difficult given long term studies rarely have the ability to date back longer than 100 years and cannot determine future conditions (Shaver *et al.* 2000; Rustad 2008).

Space for Time Substitution

Space for time substitutions (STS), are an attempt to address problems that arise when long-term observations are not feasible. STS use existing gradients in plant distribution and climate conditions to predict the impact of future climate changes (Elmendorf *et al.* 2015). Individual species studied in space for time substitutions exist naturally across a range of conditions that simulate predicted changes. For example, the impact of climate change induced warming has been studied in plant species growing across a range of latitudinal or altitudinal gradients (Nicotra *et al.* 2010). In Niwot Ridge, Colorado, USA, slopes with varied elevation were used to represent different temperature conditions, revealing a significant change in community composition, diversity and distribution for herbaceous plant species correlated with the changes in temperature (Sproull *et al.* 2015). Space for time experiments are advantageous because they use natural features to simulate changes, they allow researchers to compare plants already present in a location, growing in natural conditions. However, these locations span large areas geographically, with variation in external environmental conditions that can confound the results related to temperature and plant responses, resulting in high variability in the results (Leblans 2016) and making site comparisons difficult (Rustad 2008).

Small Scale Warming Studies

Numerous small-scale warming studies have been performed in the Arctic and subarctic through research organizations such as the International Tundra Experiment (ITEX; (ITEX 1996)). Using passive open top warming chambers situated in the field, warming temperatures between 1 and 3 ºC were simulated during the growing season (Henry *et al.* 2013). Through long term monitoring, the ITEX initiative was able to demonstrate changes in vegetation with an increase in shrub cover and decrease of lichens and bryophytes that were a direct result of warming temperatures (Elmendorf *et al.* 2012). The experiment also revealed that experimental warming lead to early phenology events and increased vegetative growth, especially in Low Arctic locations (Henry & Molau 1997). The results indicated that Arctic ecosystems were more sensitive to smaller increases in air temperature compared to other climatic zones (Oberbauer *et al.* 2013).

Experimental warming, using passive open top chambers (ITEX 1996) or other techniques such as field greenhouses or active open-top chambers are simple to use and inexpensive, however they do not allow for control of the amount of temperature increase, leading to significant variability in the warming (Shaver *et al.* 2000). They also tend to alter other variables that could influence plant growth, such as wind, light, humidity and precipitation and evapotranspiration. They are also limited in their application, in that only relatively small areas can be manipulated simultaneously (Shaver *et al.* 2000). Warming experiments are useful for determining short term (years to decade) responses of vegetation to climate conditions but fail to identify the long term (decades to centuries) responses that are identified through other methods, such a space for time experiments (Shaver *et al.* 2000). As a result, they tend to under predict warming responses (Wolkovich *et al.* 2012).

Limitations of Warming Studies

To determine the strengths and weaknesses of both spatial gradients, long-term observations and manipulation experiments, Elmendorf *et al.* (2015) compared the shift from cold-adapted species to more warm-adapted species in tundra plant communities using the three approaches. The results indicated that each method was a suitable option for monitoring changes in tundra vegetation alongside climate change, as long as limitations for each method, such as community response lag times and underestimation of climate change impacts for manipulation experiments, high variability within elevation gradients, and the limited feasibility of long-term observations, were considered.

While warming experiments provide valuable information, they are often limited by monetary and/or logistical constraints and can examine only few factors over a restricted time period (Reyer *et al.* 2013). As a result, long-term monitoring studies of plant responses to climate change, including warming, are sparse. When the studies do exist, experimental studies underestimate the timing of phenology responses to warming temperatures compared to observational studies (Wolkovich *et al.* 2012). The response of species and populations to change is influenced by the rate in which the change is introduced, step changes, are a common approach in experimental design but can be an unreliable way of determining local responses to external variables (Rustad 2008). As a result, warming experiments may overestimate the influence of experimental variables by introducing sudden changes to a system, versus a gradual change that would be representative of the rate of change in a natural system. For example, experimental data predicts a 1.9 - 3.3 day change in flowering for every ºC increase, whereas observational data predicts between 2.5 – 5 days for every ºC increase (Wolkovich *et al.* 2012). For this reason, warming experiments should be used alongside other approaches, such as transplant sand common garden studies, and modeling techniques to eliminate bias from each study design (Reyer *et al.* 2013).

1.4.2 Transplant and Common Garden Studies

Transplant studies and common gardens can isolate individual processes by effectively identifying cause and effect relationships (De Boeck *et al.* 2015) and are thus an effective way of determining if and how genotypes are responding to environmental conditions through the expression phenotypes (Primack & Kang 1989). Transplant experiments allow researchers to investigate potential adaptation without taking away natural conditions, whereas common garden or greenhouse experiments can isolate specific controlled effects on individual plants (Primack & Kang 1989).

Transplant Studies

Transplant studies determine if there are genetic differences between different populations and if these changes are adaptive. Reciprocal transplantations of individuals into new environments are used to compare traits and fitness of resident and foreign individuals. If resident individuals have higher fitness, then it can be inferred that those individuals are different, perhaps even locally adapted to the specific set of environmental conditions (Primack and Kang 1989). A reciprocal transplant study planting seeds and seedlings of *Arabidopsis thaliana* originating from the northern extent of its range in Sweden in the southernmost part of the range (and vice versa) found that populations were adapted to local conditions (Ågren & Schemske 2012).

Transplant studies allow for interactions between natural variables but provide limited opportunities to determine causal relationships between variables due to the difficulty in isolating single environmental variables in the field (Leblans 2016). For that reason, common garden studies are a valuable complement to climate change studies.

Common Garden Studies

Common garden or greenhouse studies can also be used to determine genetic differentiation and plastic responses of plant communities by comparing individuals under standardized conditions (Nicotra *et al.* 2010). They are a tool used to evaluate cause-and-effect relationships between a limited number of controlled variables (Rustad 2008). Individual species with known genetic composition, or that have been tracked over several generations, are grown under managed conditions either in a laboratory or in the field. A common garden study of *Mimulus guttatus* in Yellowstone National Park, determined that individuals growing in geothermal areas had vegetative, floral mating and phenological responses to temperature that were significantly different from those in non-geothermal areas. The study was also able to determine that the local conditions (geothermal vs non-geothermal) was not related to the genetic variation present within the population therefore this adaptation was likely a result of phenotypic plasticity (Lekberg *et al.* 2012).

1.4.3 Modeling

Species responses to local climate changes can be important component of both global climate change modeling as well as species distribution modeling. Work has begun to demonstrate the correlation between species ranges and future climate scenarios using modeling techniques including Ecological Niche Models (ENMs) and processed based models (ACIA 2005; IPCC 2014). These models have suggested that Arctic species will see an increase in range if they are habitat generalists or a decrease in range for habitat specialists. General trends suggest a decrease in projected future ranges for Arctic species compared to present ranges however, the extent of this range reduction will depend on the physiological constraints that species experience at the southern extent of their range and on propagule dispersal at the northern extent of the plant range (ACIA 2005).

Modeling can be used to test hypothesis inferred through experimental analysis, for combining multiple observed responses and for scaling up responses in terms of time and space (Rustad 2008). Schwartz, Ahas, and Aasa (2006) performed this type of modeling to determine the relationship between warming of the Northern Hemisphere and the onset of spring (i.e. plant phenology/ spring greening). Modeled and derived measurements were used to test the hypothesis of earlier spring warming across a large spatial scale. Both the small-scale studies and the modeled results indicated that phenological events in plant growth were occurring earlier across the Northern Hemisphere.

Modeling can also be used to determine current and future plant distribution. Current range models, or species niche models, are often based on the known ecological range of a species and are assumed to represent the limits to their growth tolerance, or the ecological potential of the species. Distribution modeling was used to predict the occurrence and abundance of the dwarf shrub *Dryas octopetala* L. in Svalbard (Beck *et al.* 2005). Temperature, exposure and slope increased the occurrence of the shrub whereas snow and water cover decreased it. Using ground truthing, it was determined that the model was successful at identifying the actual distribution of the species and suggests models for Arctic species continue to be used, as long as they incorporate data such as snow cover (Beck *et al.* 2005).

By incorporating updated knowledge of species tolerance determined through warming or manipulation experiments, these models can be expanded beyond the realized niche of the plants and represent the fundamental or the potential niche of a species. Determining the ability for plants to grow in a range of temperatures, will allow modellers to produce visuals that can help to update our understanding of the niche in which plants can occupy and predict the likelihood of survival across larger geographic ranges (Nicotra *et al.* 2010; IPCC 2014).

As with the other techniques discussed, modeling has its disadvantages. Modeling is challenging because it needs to incorporate heterogeneity and disturbance into the design however it cannot always account for variables such as biodiversity or random events that influence the system under investigation (Rustad 2008). It is also difficult for models to fully incorporate in impacts of important processes such as genetic adaptation, phenotypic plasticity, and species interactions, nor are they able to fully account for the rates in which changes will occur (IPCC 2014).

1.5 Geothermal Areas

An alternative approach that draws from both manipulation experiments and observational studies is the use of natural gradients or naturally heated ecosystems to study the influence of warming soil temperatures. Natural gradients, unlike manipulation studies, have variables that have existed long-term and can be examined over prolonged periods at a relatively low cost (Leblans 2016). Geothermal areas constitute natural thermal gradients and have been used for climate change studies as the systems offer a unique and important cross between field and laboratory conditions, or the observational and experimental conditions (O’Gorman *et al.* 2014). Geothermal areas are found near tectonic plate boundaries, such as the mid-Atlantic ridge in Iceland (Figure 5), where water is heated from the upwelling of magma below the surface of the earth. As heated water accumulates under impermeable rock, it creates high-pressure areas. This high-pressure process keeps the heated water circulating while warming the rock and soil above (Barbier 2002). Where the warmed channels are close to the surface, temperatures can exceed 50ºC above ambient. As a result, geothermal system become locations where terrestrial plants grow in soil across a large and often gradual range of temperatures, displaying small scale environmental gradients that can be regarded (or exploited) as a natural experiment. This range is often of small biogeographical area therefore, site characterises remain fairly constant within the system itself and between the system and the external environmental (O’Gorman *et al.* 2014; De Boeck *et al.* 2015; Leblans 2016).

Geothermal areas provide researchers with the opportunity to study temperature changes on biotic systems without the confounding factors such as spatial scale and time. In theory, they are a way for researchers to isolate the influence of soil temperature from other variables, such as space, time and biological complexity, because the experiment is performed in the same geographic area (O’Gorman *et al.* 2014). These areas allow researchers to determine how terrestrial species behave in natural environments that are warmer than a species preferred growing conditions (Woodward *et al.* 2010). This soil temperature gradient from normal to >50ºC offer a space for researching the impact of climate change across the full range of modelled scenarios (IPCC 2014).

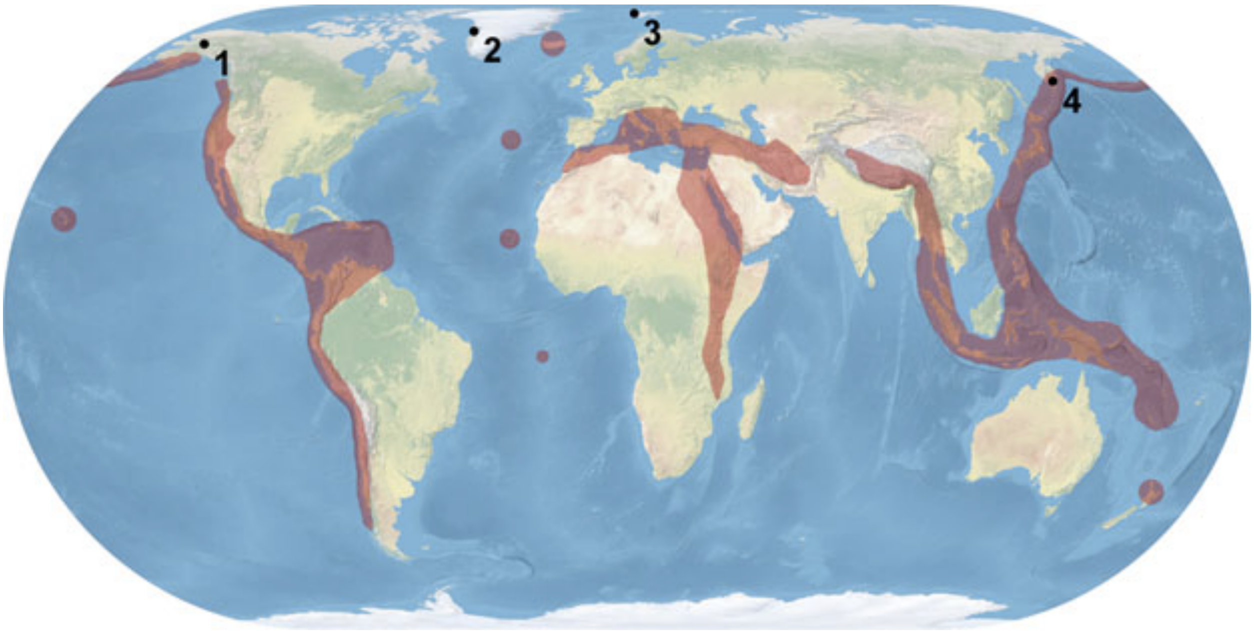

Figure 5: Map of geothermal areas around the world representing the hottest areas (in red; underlying data adapted from the US Geological Survey). The numbered black circles indicate high-latitude ecosystems (1: Alaska; 2: Greenland; 3: Svalbard; 4: Kamchatka). Source (O'Gorman et al. *2014)*

1.6 Iceland

Iceland is a volcanic island in the Atlantic Ocean, situated between the latitudes 62º23'N and 66º32'N and the longitudes 13º30'W and 24º32'W. Between 16 and 18 million years BP, the oldest parts of Iceland were formed by volcanic activity that resulted in a dome uplift situated at the intersection between the Mid-Atlantic mobile hydrogeological region and the Iceland-Faroe ridge. Through the gradual spreading of the ridges, the Iceland mantle plume reached the surface, with peaks now reaching as high as 2110 m (Zakharova & Spichak 2012). The tectonic plates continue to separate, maintaining the volcanic activity in the area and an annual mean divergence of 2 cm each year. As a result, the major geological drivers for Iceland are volcanic activity, with over 30 active volcanic systems (Figure 6) and the dynamic glaciers that represent approximately 11% of the country's surface area. The volcanic zones that span the length of the country have led to unique geological features and are the cause of active geothermal areas found throughout the country (Figure 6). With the abundance of these areas, Iceland provides a unique opportunity for climate change studies.

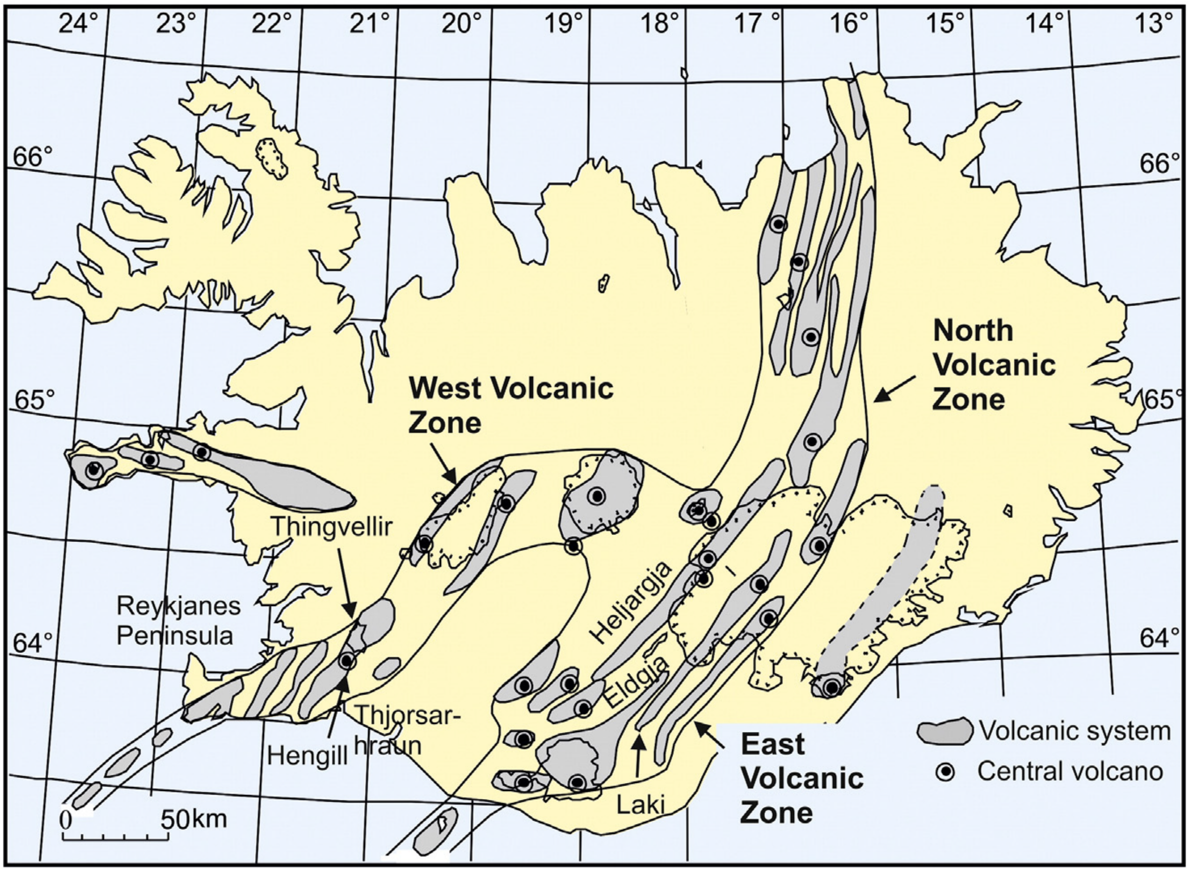

Figure 6: Map of Iceland showing the main volcanic zones (outlined in black) and systems (gray circles) with central volcanos (black points: (Gudmundsson 2016)

Volcanic activity, climate and grazing pressure are the main historic drivers for vegetation distribution across the country (Ólafsdóttir, Schlyter & Haraldsson 2001). Iceland straddles two biomes including the Arctic across the Highlands and northern coastlines where vegetation is sparse resulting in tundra and moss heath, and the subarctic lowlands of the coasts where vegetation types include meadow forbes, grasslands, wood/shrublands and wetlands (Jonsdottir *et al.* 2005; Kristinsson 2010). This boundary between climatic zones is another reason why Iceland is a suitable location for climate change studies, many of the geothermal areas are situated within ecotones that are expected to experience significant stress as a result of climate change (Callaghan *et al.* 2002).

Several of these climate change studies have already begun in the South West of the country, in the systems Hengill and Reykjadalur that fall within the West Volcanic Zone (Figure 6; Gudmundsson 2016). The climate change research coming out of these areas in the past has demonstrated that even newly warmed geothermal areas in Iceland can be used as a valuable proxy for short-term and long term climate studies (Woodward *et al.* 2010; O'Gorman *et al.* 2014; Leblans 2016).

In Hengill, studies have focused primarily on stream ecosystems. Here community structure and trophic level interactions in warmed streams were examined. The studies determined that even modest warming predictions will result in changes to organizational structure and functioning in these ecosystems, leading to severe ecosystem degradation similar to that in polluted stream ecosystems (Friberg *et al.* 2009; Woodward *et al.* 2010). In these studies, warming had an influence on the phenology of macrophytes, where warmed streams saw year round adult emerge compared to only seasonal emergence in cooler streams (Hannesdóttir, Gíslason & Ólafsson 2012). The research in this areas has also begun looking at the influence of warmed streams on multiple primary producers, including vascular plants, however studies of this type are limited and have demonstrated no significant response to warming (Gudmundsdottir *et al.* 2011a).

In Reykjadalur, also within the Hengill system, a research consortium (FORHOT) studies the effects of warming on ecosystems, with a particular focus on long term and short term warming. By comparing sites that have been warmed long term to those that have been warmed for a short period of time, researchers participating in the FORHOT initiative have been able to study the terrestrial ecosystem responses to warming (Sigurdsson *et al.* 2016). In her recent dissertation, Leblans (Leblans 2016) assessed the effectiveness of these study areas and found that they are suitable proxies for terrestrial ecosystem responses to warming. In her work with these new and old geothermal sites, Leblans determined that there existed significant relationships between organic litter decomposition rates, lengthening of the growing season, and soil organic carbon losses with warming temperatures (Leblans 2016). Other studies from the FORHOT research program have focused on how warming realtes to emission rates, soil preperties, vegetation and biomass (Sigurdsson *et al.* 2016; Poeplau *et al.* 2016; Maljanen *et al.* 2017).

2 Warming responses of two native species, *Ranunculus acris* and *Thymus praecox* ssp. *arcticus* in geothermal areas, Iceland

2.1 Introduction

Anthropogenic global climate changes are amplified in the Polar Regions, where increasing temperature has already affected local ecosystems (IPCC 2014). The harsh conditions of northern ecosystems, with low temperatures, short growing season and limited nutrient availability, makes plant communities vulnerable to even minor changes that are predicted under modest climate change scenarios (Kremers, Hollister, and Oberbauer 2015; IPCC 2014). Changes to the Arctic will occur at a local level, making it difficult to predict how individual species will respond (Franks *et al.* 2014).

With changing conditions, plants can migrate into new locations where conditions for growth and reproduction are optimal. These shifts occur both poleward and towards higher elevation, with species expanding north and upslope from their historic range (Parmesan and Hanley 2015). The movement of plant species is occurring primarily in the ecotones between biomes, a process known as thermopolization. In these ecotones, cold-adapted species become less abundant while warm-adapted species become more abundant (Gottfried *et al.* 2012). However, migration does not ensure that populations will find themselves in environments that fulfill their requirements for survival. Species could be limited by the lack of mutualistic relationships in newly colonised environments, or be limited by other external factors such as photoperiod and nutrient availability. However, plants can prevent both localized and general extinction through the processes of evolutionary adaptation or phenotypic plasticity (Franks *et al.* 2014).

Evolutionary adaptation occurs when an evolutionary change increases average population fitness within a specific environment (Conner & Hartl 2004; Franks *et al.* 2014). Studies indicate that plants have adapted locally to climate across a range of conditions in the past, which lead to evolutionary adaptation (Franks *et al.* 2014). Plants may also exhibit more plastic responses to their changing environments through the expression of varied phenotypes from a single genotype, with documented incidences of phenotypic plasticity becoming more common (Conner & Hartl 2004; Nicotra *et al.* 2010; Franks *et al.* 2014).

These phenotypic or adaptive responses of plants will be manifested in the expression or change of varying plant traits. Traits affected may include functional trais, such as leaf size, plant size, plant height, or inflorescence height, fitness traits such as number of inflorescences, number of seeds, or phenological traits such as time of leaf and flower emergence or senescence. Changes in the expression of these traits or in the timing of their development may provide insight into the factors limiting reproductive capacity, total growth and phenology of plants. These are important metrics to monitor as they can reflect changes in conditions, such as warming temperature (Kremers *et al.* 2015). If we can predict functional trait responses to changing conditions, we may be better placed to understand future composition of plant communities (Nicotra *et al.* 2010). Additionally, responses of these traits could indicate larger scale processes occuring at a population or ecosystem level, such as changes in carbon uptake associated with plant productivity (i.e. plant growth), or higher order trophic interactions (plant phenology; Kremers *et al.* 2015).

The expression of phenological stages throughout the life of a plant represent an individual's direct or indirect response to environmental stimuli, such as temperature and photoperiod (Cleland *et al.* 2007; Lessard-Therrien, Davies, and Bolmgren 2014). Warmer temperatures in spring and summer have been associated with earlier onset of phenological events, such as leaf emergence and flowering (Menzel *et al.* 2006). These shifts in flowering phenology could also signify shifts in species interactions, where earlier flowering individuals might experience lower fertilization because of mismatch with pollinators that are not yet active, or damage because of extreme cold events that are more common early in the growing season. Late flowering individuals could experience lower productive output as a result of shortened time for seed maturation (Cleland *et al.* 2007; Lessard-Therrien, Davies, and Bolmgren 2014).

The warming responses of both individual species and communities are being studied throughout the Arctic and subarctic, with particular attention being paid to experimental design in order to ensure adequate data collection (Wolkovich *et al.* 2012; Merilä & Hendry 2014; De Boeck *et al.* 2015). These studies have included short and long term field and experimental studies, space for time studies, including reciprocal transplants, common gardens and natural thermal gradients (Franks *et al.* 2014). Geothermal areas represent an example of natural thermal gradients that can be used to study the response of plants to different temperatures, specifically to increased soil temperatures (O'Gorman *et al.* 2014). These areas are often found near tectonic plate boundaries, such as the mid-Atlantic ridge in Iceland, where rock and soil at the surface of the earth is warmed via geothermal channels belowground (Barbier 2002). The warmed soil provides an area were plants grow in a range of temperatures within an isolated geographic area with no influence from confounding factors such as soil moisture or soil pH (Sigurdsson *et al.* 2016).

Geothermal heated systems are ideal for climate change research because they act as proxies for climate warming (O'Gorman *et al.* 2014; Sigurdsson *et al.* 2016). They provide an opportunity to investigate the impact of long term warming on whole ecosystems over a small area and are a valuable alternative to long-term studies of temperature variation. Long-term studies of warming on ecosystems are an effective way to determine plant response to environmental changes, such as increased temperature. These studies are essential because warming responses differ between the short term and the long term (Kremers *et al.* 2015). However, they are sparse, often expensive and require manipulated variables to simulate future conditions. By using geothermal areas for long-term studies, researchers have the opportunity to study temperature changes on biotic systems at a low cost and without the confounding factors such as spatial scale and time. Geothermal systems are *natural laboratories* in which the short term and long-term effect of warming can be investigated (O'Gorman *et al.* 2014).

2.1.1 Objectives

The objective of this project is to use three different sites, all within geothermal areas, to determine if plants have the ability to respond to increased soil temperatures. I aim to answer three specific questions: (i) are plants traits changing in response to soil temperature, (ii) does soil warming effect plant fitness and is this effect mediated by the effect of warming on other plant traits, and (iii) do these responses differ among population growing at different sites. To determine how plants are responding, two herbaceous plants, *Ranunculus acris* L. and *Thymus praecox* Opiz ssp. *arcticus,* were studied.

2.2 Methods

2.2.1 Study Sites

The effect of soil warming on plant traits was studied at three sites in Southwest Iceland. Two of the sites, Reykir (herein referred to as "GN" or "Grassland New", 64°.006'N, 21°.175'W; 102 – 110 m a.s.l.) and Grændalur (herein referred to as "GO" or "Grassland Old" 2.0-2.5 km NW of GN, 64°.026'N; 21°.196'W; 115-165 m a.s.l.) were located near the town Hveragerði (Figure 7). The third site, Hengill (64º.03'N; 021º.18'W, 350–420 m a.s.l.) was located approximately 8 km from the first two sites at a higher elevation. The GN, GO and Hengill grasslands are located at the base of the Hengill volcanic system, near to the Hrómundartindur and Hveragerði volcanic systems, where the Reykjanes Volcanic Zone, the West Volcanic Zone and the South Iceland Seismic Zone converge (Saemundsson, 1992; Zakharova & Spichak, 2012); Figure 8)).

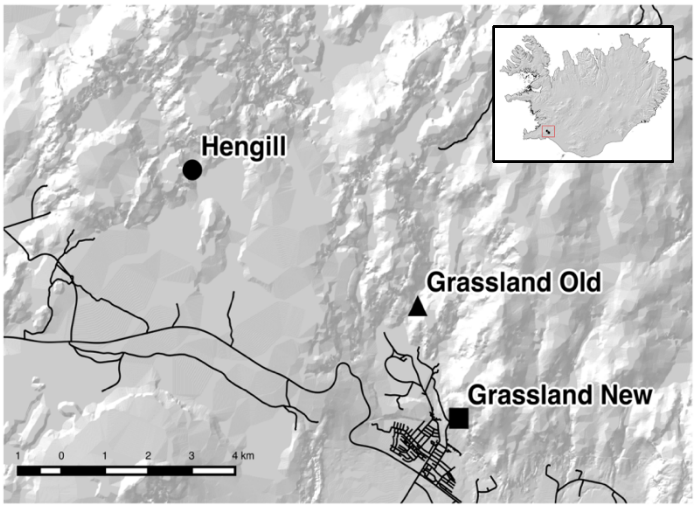

Figure 7: Location of study sites within Iceland.

The underlying bedrock of the study sites contain geothermal channels originating from high volcanic activity common at tectonic boundaries (Zakharova & Spichak 2012; Poeplau *et al.* 2016). These channels warm the water and soil through radiative heating (Sæmundsson 1995a; b; Gudmundsdottir *et al.* 2011b). The soil temperatures range from average ambient to over 50ºC above ambient in some areas with little to no warming on the air temperature (O'Gorman *et al.* 2014; Sigurdsson *et al.* 2016). Studies have determined that the soil warming in these areas has not resulted in significant changes to important soil chemistry characteristics such as soil pH and soil water content, making these sites suitable for studying the effect of soil warming on the ecosystem (O'Gorman *et al.* 2014; Sigurdsson *et al.* 2016).

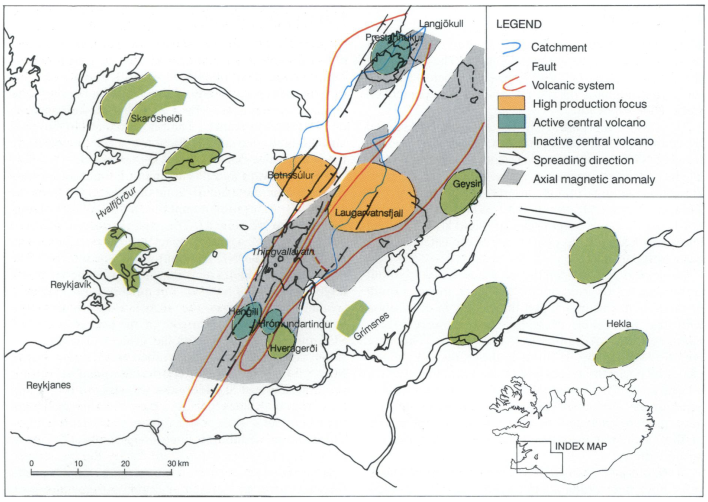

Figure 8: The Thingvallavatn catchment of SW-Iceland is outlined in blue, the major faults are outlined in black and the volcanic systems are outlined in orange. Active and extinct central volcanoes are indicated with blue and green shaded areas. The Hengill, Hrómundartindur and Hveragerði systems are indicated in green and blue in the middle of the image. From Saemundsson, 1992.

The Hengill and GO areas have been warmed for hundreds, potentially even thousands of years. Grændalur, the location of GO, means "Green Valley" which was a reference to the earlier spring greening, and persistent vegetation cover in the fall, typical in the valley compared to surrounding areas. The earliest reference of this area by the name Grændalur dates back to 1708 (Magnússon & Vídalín 1918; Sigurdsson *et al.* 2016). Empirical evidence from geological surveys, performed between 1963 and 1965 and in 1967 for GO and Hengill respectively, confirmed that these areas are warmed by geothermal heating (Sæmundsson 1967, 1995a). By contrast, GN grassland, has only been warmed since 2008, when an earthquake measuring 6.3 on the Richter scale shifted the shallow geothermal channels beneath Reykir, warming the soil in an area that had not previously experienced warming (Halldórsson & Sigbjörnsson 2009; Poeplau *et al.* 2016).

The GO and GN sites are part of a research initiative known as FORHOT, which focuses on terrestrial ecosystem responses to soil warming (Sigurdsson *et al.* 2016; Poeplau *et al.* 2016; Leblans 2016). Hengill, by contrast, is the center of stream ecosystem and climate change research (Friberg *et al.* 2009; Woodward *et al.* 2010; Demars *et al.* 2011; Gudmundsdottir *et al.* 2011c; b; Rasmussen *et al.* 2011; O'Gorman *et al.* 2012; Perkins *et al.* 2012; Adams *et al.* 2013; O'Gorman *et al.* 2014; Hannesdóttir *et al.* 2012). However, to date little work is published looking exclusively at the impact of natural soil warming on terrestrial plant species.

The GO site is a sloped valley, the GN is on a steep slope and Hengill is a flat plain. The dominant soil type of all sites is brown andosols with volcanic origins (Arnalds 1999, 2004) Steam vents, mud pools, steaming ground, and hot or tepid springs are all present in

GO and Hengill (Sæmundsson, 1995b), whereas GN contains only steam vents and steaming ground. Terrestrial vegetation for the three sites is very similar, with mosses and herbaceous flowering plant species. Anthropogenic disturbances are minimal at the study sites with the exception of sheep grazing and occasional hiking in both GO and Hengill.

The climate at the study sites is oceanic with cool summers and mild winters. Weather measurements for GO and GN were gathered from the Eyrabakki synoptic station, the closest weather station to the sites, 9 km south of Hveragerði. Mean annual temperature was 5.2ºC, mean growing season temperature (May to September) was 9.7 ºC and average annual precipitation 1374.4 mm (Data from 2009-2016; Icelandic Meteorological Office, 2016). This growing season does not take into account the influence of geothermal warming. Weather for the Hengill site was obtained from the Hellisskarð station 4 km southwest of the site. The mean 2009-2016 annual and growing season temperatures for Hengill were 2.60 ºC, and 8.22 ºC, respectively (Table 1). The average annual precipitation was 2101.7 mm during the same period (with 2014 omitted; Icelandic Meteorological Office, 2016). The Hengill site is often covered by snow as a result of the higher elevation however, permanent snow cover is not typical for the GN and GO areas as a result of lower elevation and the mild oceanic climate (Leblans 2016).

Table 1: Site conditions for GN, GO and Hengill (Hen) including short-term (ST) versus long-term warming (LT), elevation (m a.s.l.), GPS corrdinates, mean annual temperature (MAT) in °C, mean annnual precipitation in mm, and the presense of sheep grazing.

Site	ST/LT	Elevation	GPS	MAT	MAP	Grazing
GN	ST	102-110	64°.01'N; 21°.18'W	5.2	1374.4	NO
GO	LT	115-165	64°.03'N; 21°.20'W	5.2	1374.4	YES
Hen	LT	350-420	64°.03'N; 21°.18'W	2.6	2101.7	YES

2.2.2 Study Species

Ranunculus acris L.

Ranunculus acris L. is a circumpolar perennial that grows in meadows and pastures where the soil moisture is high and the substrate is either calcareous or neutral (Totland 1999; Jacobs, Graves & Mangold 2010). In Iceland, the *Ranunculus acris* subsp. *villosus,* also known as Brennisóley is found throughout the lowlands in meadows and pastures, whereas the subsp. *pumilus* is common growing in snow beds of the highlands up to 900 m a.s.l. (Dick; Kristinsson 2008). Each season, *R. acris* individuals produce four basal leaves directly from the rhizome, with the total number of leaves up to 40 or 50 (Totland 1999). Leaves vary in length from 2.5 to 8 cm. The leaves have three palmate lobes each divided into three distinct segments (Coles 1971; Jacobs *et al.* 2010). Flower stems are erect, typically 15 to 40 cm in height with multiple stems per plant. The plants are hermaphroditic and flowering occurs from May to June. *R. acris* produces self-incompatible flowers 2-2.5 cm in diameter, with five to eight petals (Kristinsson 2010). At the center of each flower is an orb shaped receptacle surrounded by 30 to 70 stamens and 15 to 40 pistils (Jacobs *et al.* 2010). The fruit develops as a small two to three-millimeter-long hooked achene containing a single seed. *R. acris* reproduces by seeds or clonally through rhizomes that divide into daughter plants at distances up to one meter (Totland 1999). The reproductive output of *R. acris* is dependent on the plant size, and thus temperature may influence reproductive output as result of its influences on plant size (Totland 1999).

Thymus praecox ssp. arcticus (Durand) Jalas

Thymus praecox is a small creeping shrub from the Laminaceae family found throughout Northern and Central Europe, eastern North America, Greenland and Iceland (Pigott 1954). In Iceland, the plant is distributed abundantly throughout both the lowlands and the central highland, where it grows at elevations up to +1000 m a.s.l. (Stahl 1982). *T. praecox* grows in a variety of locations, such as gravelly soils, lava fields, meadows and geothermal areas (Stahl 1982; Kristinsson 2010). The leaves of *T. praecox* are 3-5mm long, opposite, reverse paddle shaped and covered with fine hairs in the underside (Kristinsson 2010). *T. praecox* grows as a cushion-like plant with individual plants growing in close proximity to one another (Pigott 1955). Each individual *T. praecox* has multiple globose inflorescences growing from short erect stems 2-5cm long and appear in rows along the older parts of the plant runners. Many small pink flowers cover the shrubby plant and bloom in June and July (Kristinsson 2010). The arrangement of inflorescences can be extremely variable across the plant's range. *T. praecox* is gynodioecious and hermaphroditic individuals are more common than female plants (Pigott 1955). *T. praecox* either it persists by clonal growth or undergoes sexual reproduction, however, self-fertilization does not occur (Pigott 1954, 1955). Each flower will develop a calyx, and the fruit contain up to four spherical seeds, 0.7 to 0.8mm in diameter however, it is rare that all fours seeds will be viable. Seeds are either ejected from the calyx or dispersed by wind (Pigott 1955).

2.2.3 Plant Measurements

In June 2016, 60 individuals of both species were marked by placing a pin with a tag into the ground close to the plant at each of the three study locations. Individuals were sampled at intervals of 0.5 – 1 m along six N-S (or E-W) transects (10-20 m long) in GO and along three to five transects in GN. At Hengill, the population density was too low to sample individuals in transects so between two and fifteen individuals were selected randomly from six to seven areas within the site. The density of individuals determined the number of individuals sampled. Location of transects/individuals were chosen to cover the whole gradient of temperatures in which the species grew, which did vary somewhat between the two species and among the study sites (Table 2). Individuals were sampled only if they grew in similar soil conditions, therefore individuals growing in barren/open areas or those in close proximity to streams were avoided. Each plot and transect was marked with a GPS coordinate and flag.

Table 2: The sample size (SS), and temperature gradients (in °C) from lowest (LT) to highest temperature (HT) for both R. acris and T. praecox at each site.

Site	Species	SS	LT	HT
GN	*R. acris*	62	12.3	33.7
	T. praecox	60	13	49.5
GO	*R. acris*	62	10.6	38
	T. praecox	59	10	57
Hen	*R. acris*	64	9.1	22.5
	T. praecox	61	11	43

Plant phenology and plant traits (height, leaf area, flower size, and total plant area) were measured once during peak growing season for each marked individual. For each species within a site, individuals were sampled over three days. Phenological stage of individuals of *R. acris* (Table 3) were measured on the flowers of the tallest stem while for *T. praecox* five inflorescences were selected at random from the most advanced part of the plant (Table 4). Plant height was measured from the base of the stem until the base of the flower. Height of *R. acris* and *T. praecox* was measured for the tallest flower and from the five randomly selected inflorescences, respectively. For both species, length of basal leaves was measured from the point in which the leaf met the stem to the tip of the leaf, and leaf width was measured on the widest part of the leaf, perpendicular to the length. The total diameter of the plant was measured for *R. acris* using the length from the tip of the largest basal leaf to the tip of the leaf opposite, and the perpendicular width. The total diameter for *T. praecox* was measured based on the longest length of the entire plant and the perpendicular width.

Table 3: Phenological stages for R. acris used to estimate phenology. Adapted from Molau, Mølgaard, & ITEX, 1996 and Totland, 1999

Category	Description
RP1	Only leaves visible, flowers have not developed.
RP2	Bud formation has started but still completely shut.
RP3	Anthesis: half-opened buds, anthers are underdeveloped and closed.
RP4	Flower and anthers are open, pistils underdeveloped and closed.
RP5	Flowers, anthers and pistils are developed and open.
RP6	Achenes are developing from pistils, anthers are shedding and petals are beginning to drop.
RP7	The last petals are falling from the flower; achenes developing but still green, all anthers have dropped.
RP8	First seed dispersal. Achenes are fully developed, have darkened and begun to disperse.

Table 4: Phenological stages used to estimate phenology of T praecox. The estimation is based on the entire surface of the plant. Adapted from James, 2006.

Category	Description
TP0	100% pre-bud is the vegetative state of the plant, no inflorescences visible.
TP1	Sepals developed, identified by their colouring, buds are not developed.
TP2	Sepals apparent, buds starting to develop.
TP3	Large buds have formed but there are fewer than two flowers blooming.
TP4	50 to 90% of inflorescences budding while 10-50% are flowering, no flowers over seed head visible.
TP5	1-50% of inflorescences budding while 50-100% are flowers, no flowers over seed head visible.
TP6	1-50% inflorescences are flower over seed head, remaining are budding or flowering.
TP7	Over 50% flower over seed head.
TP8	100% flower over seed head.

Fitness for *R. acris* was estimated by counting the total number of flowers from each plant and the total number of flowers on each stem during peak growing season. Fitness for *T. praecox* was measured by counting the total number of inflorescences on each plant, the average number of flowers for each inflorescence, and the average number of seeds for each plant (Mauricio, Bowers & Bazzaz 1993; Noel *et al.* 2006). The total number of inflorescences was counted for each *T. praecox* plant, unless the total exceeded 200. In which case, inflorescences were counted for three 10 cm^2 quadrats on the most developed parts of the plant and total number was extrapolated (James 2006). The total number of flowers, buds, and seed heads were counted for the five randomly selected inflorescences. In addition, for *T. praecox*, in September, five seed heads from each individual were sampled (Thompson et al. 2004) and the number of fully developed seeds were recorded within each seed head.

2.2.4 Environmental Parameters

Near the base of each marked individual, soil temperature, moisture and elevation were measured at the same time as phenological stages. Soil temperature was taken at depth between 5 to 10 cm using a T–model Digital Multi-Purpose Thermometer; soil moisture was measured at 10 cm depth using the ML3 ThetaProbe Soil Moisture Sensor and the elevation using the Garmin GPSMAP 64s.

2.2.5 Statistical Analysis

There was a strong correlation among many of the measured traits for both *R. acris* and *T. praecox* (Appendix 1). Thus only flowering phenology, plant fitness, measured as the average number of flowers on each stem for *R. acris* and the average number of seeds per flower for *T. praecox*, plant size and plant height was used in the analysis. Analyses of omitted variables are available in Appendix 1. Before analysis, fitness and size measurements were log transformed to obtain normal distribution for *R. acris* while size was log transformed for *T. praecox*.

Linear mixed effect models (LMM), with soil temperature, site, and their interaction as fixed effects, plant traits as response variables, and soil moisture as random effects, were used to estimate if plant response to soil warming differed among sites and if soil temperature or/and site location affected the response. If the best model included the interaction term, species response to warming was site specific.

As plant responses were site specific for most variables, each site was also analysed separately to determine how and if plants responded to increase soil temperature. For each site LMM, with soil temperature as the fixed variable and soil moisture as the random effect and traits as the response variable were constructed. Before analysis, some response variables were log transformed to achieve normal distribution.

The most suitable model was selected in the above analyses for the sites in general and individually using model simplification. Models were compared to determine if there was a significant difference for each trait, the final model was the one with only significant variables. The linear mixed effect models were constructed and examined using the lmer package in R (Bates *et al.* 2015; R Core Team 2016).

To determine if the observed increase in plant fitness with soil warming was mediated through the effect of warming on plant traits, structural equation models (SEM) were constructed to perform path analysis. Path analysis is used to understand the relationships between several variables and can explain the causal relationship among them (Fan *et al.* 2016). This type of analysis can identify latent or hidden variables by assuming a single variable can influence an outcome either directly or indirectly through another variable

(Fan *et al.* 2016). SEM was only constructed for species when soil temperature had an effect on plant fitness within the site, using only the traits that were affected by temperature. Fully mediated SEM that examine the path relationship between soil temperature, measured traits and fitness, and partially mediated SEM that traced the path between soil temperature, measured traits and fitness, and between temperature and fitness were constructed. The models were then compared to determine if there was a significant difference between them. With the partially mediated models, we were able to identify which plant traits were having an indirect effect on overall plant fitness. These SEM use path analysis with the lavaan package in RStudio (Yves 2012; R Core Team 2016).

2.3 Results

2.3.1 Plant response to soil temperature

R. acris

The overall phenological stage of *R. acris* advanced as soil temperature increased, with GN having in general more advanced flowers than GO at the same temperature (Table 5; Figure 9). When each site was analyzed separately, there was no effect of phenology (Figure 9). Changes in plant fitness and height as a response to increased soil temperature differed among sites (Table 5; Figure 9). Plant fitness increased with soil temperature at GN, decreased at GO, while plant height decreased at both sites (Table 6; Figure 9). There was also a significant relationship between plant size and soil temperature at GO (Table 6; Figure 9). At Hengill, soil temperature did not affect plant traits of *R. acris*.

Table 5: Comparison of linear mixed models for R. acris *and* T. praecox *across all sites combined. The best fit model is shown. The models compared included interaction between sites (ResponseVariable ~ Site * Temp), without interaction but with temperature and site as fixed variables (ResponseVariable ~ Site + Temp), and with one of the two fixed variables (ResponseVariable ~ Temp or ResponseVariable~Site). For response variables with no site interaction, the p values are listed. For response variables with interation, the p-value is listed for the comparision of models with and without interaction. For resonsponse varibales without interaction but with a significant affect of site and temperature, the p-value is listed as the comparison of the models with and without the effect of both site and temperature.* R. acris *plant size is not listed because there was no site interaction, and no significance for any of the response variables.*

Ranunculus acris	**$R^2_{marginal}$**	**$R^2_{conditional}$**	**AIC**	**Estimate**	**Std Err**	**t**	**p**
Phenology ~ Site + Temp	0.19	0.19	697.9				<0.001
Temp				0.04	0.02	2.38	
GO-Hen				-0.52	0.31	-1.68	0.094
GO-GN				0.78	0.27	2.88	0.008
Fitness ~ Site * Temp	0.23	0.35	381.4				<0.001
Height ~ Site * Temp	0.32	0.51	1437.2				<0.001
Thymus praecox	**$R^2_{marginal}$**	**$R^2_{conditional}$**	**AIC**	**Estimate**	**Std Err**	**t**	**p**
Phenology ~ Site * Temp	0.44	0.44	532.5				0.006
Fitness ~ Temp	0.03	0.23	352.7				0.021
Temp				-0.01	0.01	-2.36	
Size ~ Site * Temp	0.14	0.14	613.6				0.016
Height ~ Site + Temp	0.06	0.14	1391.9				0.010
Temp				0.21	0.09	2.34	
GO-Hen				-5.03	2.06	-2.45	0.043
GO-GN				-4.79	2.07	-2.31	0.043

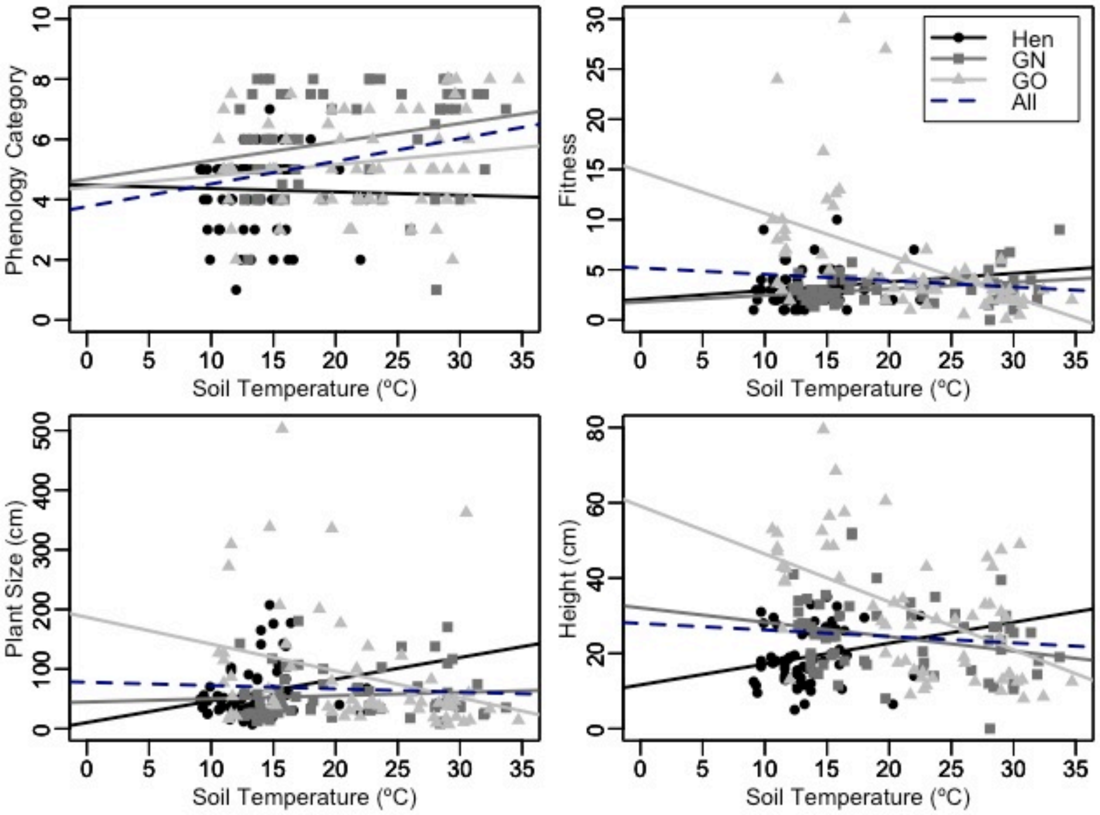

Figure 9: The effect of soil temperature on plant phenology (top left), fitness (top right), plant size (bottom right) and plant height (bottom left) of R. acris across the three study sites individually; Hengill (Hen), Grassland New (GN) and Grassland Old (GO), and all three sites examined together (All). Plant fitness was estimated as the average number of flowers on each stem.

T. praecox

The overall fitness of *T. praecox* decreased, and plant height increased with warmer soil temperatures, GO plants were in general taller than both GN and Hengill plants at the same temperature (Figure 10). Changes in plant phenology and size as a response to increased soil temperature differed among sites (Table 5; Figure 10). Plant phenology was more advanced with increased soil temperature at GO and less advanced at Hengill, while plant size increased with soil temperature at Hengill but did not have a significant affect at either GN or GO.

Table 6: Results of linear mixed effect models for R. acris *and* T. praecox *across each of the three sites individually as well as results from the comparison for the model using temperature as the fixed variables, measured plant traits as the response variables (phenology, fitness, size and height) and soil moisture as the random effect. Bold values indicate a significant main effect of the treatment at p≤ 0.05. * indicates log-transformed values.*

	Site	Est	Std Err	X^2	p		Site	Est	Std Err	X^2	p
Ranunculus acris						*Thymus praecox*					
Phen	GN*	0.01	0.007	1.67	0.197	Phen	GN	0.010	0.0159	0.38	0.537
	GO	0.04	0.028	1.84	0.176		GO	0.064	0.0092	35.82	**0.000**
	Hen*	-0.01	0.016	0.16	0.686		Hen	0.060	0.0177	10.58	**0.001**
Fit	GN	0.07	0.026	6.12	**0.013**	Fit	GN	-0.020	0.0070	7.27	**0.007**
	GO*	-0.08	0.014	25.27	**0.000**		GO	-0.012	0.0079	2.20	0.138
	Hen*	0.03	0.024	1.09	0.296		Hen	0.005	0.0119	0.15	0.696
Size	GN	0.54	0.763	0.50	0.481	Size	GN*	0.022	0.0173	1.43	0.231
	GO*	-0.06	0.016	11.41	**0.001**		GO*	0.032	0.0185	2.90	0.088
	Hen*	0.05	0.031	1.91	0.167		Hen*	-0.049	0.0132	9.45	**0.002**
Hght	GN	-0.38	0.179	4.44	**0.035**	Hght	GN	0.282	0.1275	4.70	**0.030**
	GO	-1.26	0.257	20.34	**0.000**		GO	0.227	0.1475	2.43	0.119
	Hen	0.55	0.322	2.80	0.094		Hen	0.029	0.2045	0.02	0.889

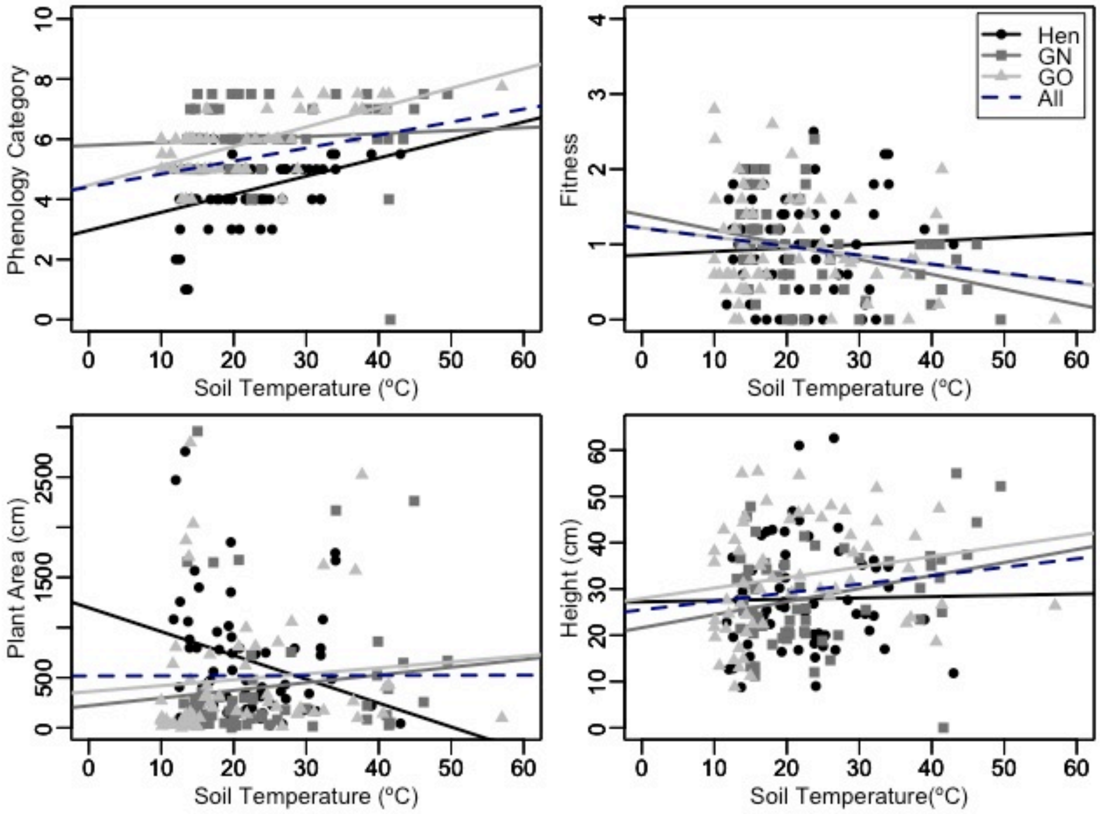

Figure 10: The effecto of soil temperature on plant phenology (top left), fitness (top right), plant size (bottom right) and plant height (bottom left) of T. praecox across the three study sties individually; Hengill (Hen), Grassland New (GN) and Grassland Old (GO), and all three sites examined together (All). The phenological categories ranged from 0 with 100% pre bud, plant in the vegetative state with no inflorescences visible, to 8 with 100% flower over seed head, and fitness was measures as the average number of seeds for each flower.

2.3.2 Soil warming and the selection for traits that will effect plant fitness

The increase in fitness due to temperature for *R. acris* was partially mediated by the influence of temperature on plant height and size at GN (Figure 11b and c) and height for GO (Figure 11a). For *T. praecox,* the influence of temperature on plant fitness was partially mediated by plant height at GN (Figure 11b).

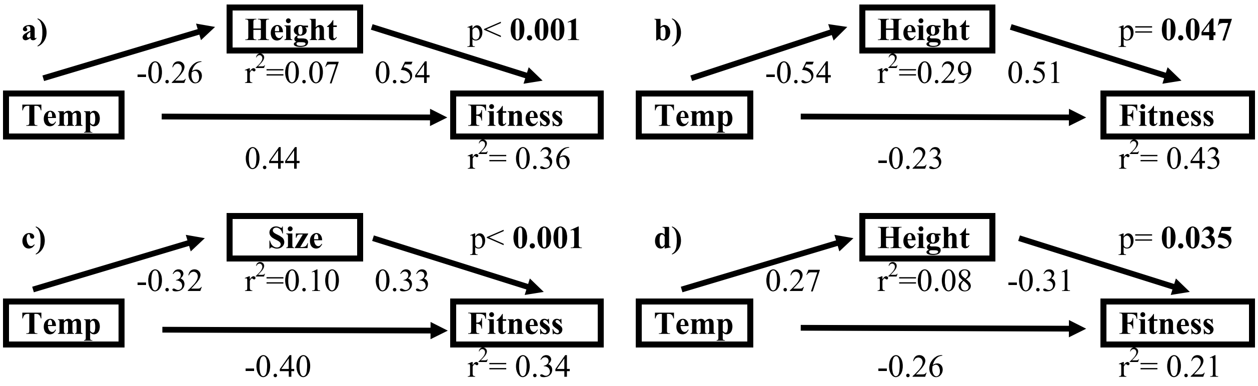

Figure 11: Results of the likelihood ratio test comparing the fully mediated (not pictured) and partially mediated models (pictured above, A, B, C and D).The partially mediated models were a better fit, p values indicated at the top right of each model. Standardized path coefficients between variables and r^2 for each variable is indicated. a) partially mediated path analysis model with height and fitness (measured with the number of flowers in each stem) for R. acris at GN, b) partially mediated path analysis model for height and fitness for R. acris from GO, c) partially mediated path analysis model for total plant size for R. acris from GO, d) partially mediated path analysis model for plant height and fitness, measured with the average number of seeds for each flower for T. praecox from GN.

2.4 Discussion

Geothermal systems can act as natural laboratories, where temperature is isolated from other drivers, with minimal loss of realism (O'Gorman *et al.* 2014). As a result, these systems are being used in climate change studies. However, geothermal areas are imperfect proxies in the context of plant responses and future warming. For example, soil warming in geothermal systems, like the one in this study, has a minimal effect on air temperature (Sigurdsson *et al.* 2016; Leblans *et al.* 2017). Therefore, any temperature increases within the system will be limited to the soil, which is not representative of how environmental changes of global warming will affect most areas. This decoupling of soil and surface temperatures could influence how plants respond to temperature increases (Leblans *et al.* 2017). Therefore, geothermal systems are a limited representation of future warming. Integration of laboratory, and manipulation experiments, such as common garden studies and open-top chambers (Molau *et al.* 1996), within geothermal systems can help address these concerns and would have been a valuable addition to this study (O'Gorman *et al.* 2014).

Geothermal areas alone cannot be used to determine how plants respond to sudden temperature changes. Only when temporal experiments are embedded into the geothermal system can the impact of both short and long-term warming be examined (O'Gorman *et al.* 2014). In this study, the temporal influence of warming was examined using sites that had been warmed for different amounts of time. However, there is some evidence to suggest that acclimation to warming occurs quickly in these systems, and the GN site may be approaching thermal response equilibrium (Leblans *et al.* 2017). Additionally, interannual variation in plant responses to soil warming, specifically phenological responses, have been observed in geothermal systems (Leblans *et al.* 2017).

It would be imprudent to assume that the results from this one-year study are truly representative of the relationship between plant responses and soil warming. However, this study does identify with confidence, a plant response to soil warming. Fitness, growth and phenology of *R. acris* and *T. praecox* were influenced by soil warming. The responses were species and sites specific indicating that there is a complex relationship between soil warming and plant responses. Extending this study over several years, sampling more individuals, would help better understand the relationship between trait responses and soil warming.

2.4.1 Trait responses to soil warming

Flowering Phenology

In this study, increased soil temperature was at times associated with advanced flower development, even though this differed among sites and between species. In subarctic systems where the growing season is short, the timing of phenological stages plays an important role in the reproductive success of plants (Molau 1993; Oberbauer *et al.* 2013). Typically flowering phenology is determined by a combination of photoperiod, rainfall (soil moisture), temperature, snow-cover and pollinator presence (Rathcke & Lacey 1985; Molau 1993). In tundra plants, prefloration timing, or the time between the spring thaw and flowering onset, can be in part determined by genetic factors, and the climate of the previous season (Molau 1993). Flowering phenology of Arctic plants is also highly correlated with temperatures at the start of the growing season (Panchen & Gorelick 2017). Perennial herbs in temperate locations rely on temperature, specifically cumulative heat sums, to determine the onset of flowering, while in tropical areas, rainfall is the primary driver of flowering time (Rathcke & Lacey 1985). With the exception of some alpine and wind-pollinated species that flower under snow cover or in freezing weather, spring frost is also a limitation to the flowering season (Rathcke & Lacey 1985). Often the interaction of these environmental cues determine an individual's flowering time (Panchen & Gorelick 2017). For example, some plants rely on cold temperatures followed by changes in the photoperiod to induce flowering (Rathcke & Lacey 1985).

In regions farther south than the study locations, one might predict that the photoperiod would be the factor limiting the induction of earlier phenological responses however, in the subarctic climates of the study sites, the amount of daylight in the early spring already approaches 20+ hours a day, suggesting that photoperiod was not a limiting factor (Kremers *et al.* 2015). Despite being a known driver of phenological change (Rathcke & Lacey 1985), it is unlikely that soil moisture influenced the phenology responses of either species because, as in similar studies in the same geothermal system, the variation in soil moisture between individuals was minimal (Leblans *et al.* 2017). Snowfall and snowmelt, which effects soil moisture, plays a crucial role in plant phenology for Arctic and subarctic plants, and could have been a factor in this study (Molau 1993). Years with cold spring and late ablation delay flowering phenology, for example, a combination of low temperatures and little snowfall have caused *R. acris* to flower sporadically during the early spring (Billings & Mooney 1968; Totland 1993).

For Arctic and alpine plants, temperature plays a key role in the timing of flowering phenology (Thórhallsdóttir 1998; Panchen & Gorelick 2017). Previous studies near GN and GO (referred to as ST and LT in that study) found that soil temperature had a direct effect on plant phenology, lengthening the growing season of a range of species within the entire population (Leblans *et al.* 2017). Using NDVI measurements, it was determined that soil warming advanced the onset of the growing season between 1.3 and 2.1 days per ºC, across both long term and short term warmed sites (Leblans 2016). Community level studies that examined plant response to warming alongside other relationships, have come to similar conclusions and have determined that earlier flowering species are found to be especially sensitive to warming, likely because of high temperature variation typical of in the spring (Thórhallsdóttir 1998; Arft *et al.* 1999; Menzel *et al.* 2006). Phenology studies in Iceland, which examined the flowering phenology of *T. praecox* alongside several other species, determined that warmer temperatures increased the number of species flowering in the early spring (Thórhallsdóttir 1998). Finally, studies of phenological advancement of European species, including plants, determined that most species showed signs of phenological advancement with warming temperature (Menzel *et al.* 2006).

While *T. praecox* generally followed this pattern, *R. acris* only showed phenological advances with higher temperature when all three sites were analysed together, indicating that species phenological responses to warming will vary (Arft *et al.* 1999). While few, if any studies have investigated the relationship between flowering phenology and temperature for *T. praecox*, studies of *Ranunculus* are common and reveal considerable variability in phenology responses to warming (Panchen & Gorelick 2017). In south-west Norway, warmed plots indicated only minor acceleration in flowering time of *R. acris* when compared to unwarmed control plots, suggesting that phenological advancement was limited due to the early flowering time (Totland 1999). Despite being an early spring plant, *R. acris* did not show signs of earlier phenology with warming temperatures. It is likely that flowering phenology cannot be accelerated because other physiological processes, such as petal development and ovule maturation, will not be able to advance at the same rate (Totland 1999).

Size and Height

Plant height and size were affected by soil temperature for both species. For *R. acris* there was a strong negative association between soil temperature and plant height and plant size at individual sites. At GO plants became shorter and smaller as temperatures increased while at GN total plant size decreased. For *T. praecox* this trend of smaller plants with increased temperature, was only apparent in individuals growing at Hengill, as plant height increased with temperature for individuals at GN and across all three sites combined.

In experimental warming studies, increased temperature is often associated with increased plant growth and therefore larger plants (Arft *et al.* 1999). Low Arctic plants have shown consistent vegetative growth responses through four years of experimental warming, including increases in leaf length, shoot length, biomass, and leaf width (Arft *et al.* 1999).

Increased plant height has also been related to warm night temperatures in some herbaceous plants (Jing *et al.* 2016). Meta-analysis of the ITEX experiment has identified this trend among several herbaceous species, including *R. acris*. Therefore, we expected *R. acris* size and height to increase with temperature instead of decrease as observed. However, other studies have found smaller individuals growing in warmed geothermal areas. In Yellowstone National Park, *Mimulus guttatus* individuals were shorter and had lower flower set in geothermal sites. It was suggested that lower flowering was genetic adaptation to the warmed areas, where flowering was closer to the ground to limit damage from cooler ambient air temperature above the surface of the warmed soil (Lekberg *et al.* 2012). Studies have also indicated that temperature increases are linked to earlier phenology but not an increase in vegetative biomass (a measurement of plant size) or leaf area (which was correlated with plant size for our study and considered within that variable; Hatfield and Prueger 2015).

Fitness

The general effect of soil warming on fitness for both *R. acris* and *T. praecox* was an overall decrease with temperature, although we found also evidence of increased fitness at individual sites. While evidence of decreased fitness does exist (Anderson 2016), fitness is more often found to increase with higher temperatures, as was observed for *R. acris* at GN (Totland 1999; Arft *et al.* 1999; Straka & Starzomski 2015). Warmer temperatures can accelerate photosynthesis in herbaceous species, allowing individuals to allocate more resources to reproduction. Warmer temperatures also speed up the processes of pollen tube development, fertilization, embryo development, and seed maturation in *R. acris*, enabling plants to produce both more and larger seeds, leading to overall increased plant fitness (Totland 1999). However, in this study plant fitness was not only influenced by soil warming directly, but also indirectly through the effect of soil warming on other plant traits.

There is high correlation between flowering phenology, habitat thawing and the reproductive output of tundra species, where early thaw and in turn earlier flowering can lead to increased relative reproductive success (Molau 1993). Fitness is increased because extended flowering duration increases the chances of flower pollination, while the lengthened growing season allows individuals to accumulate more resources needed for seed maturation (Rathcke & Lacey 1985). Species with flexible phenology timing are more likely to show these increases in performance with warming, indicating that fitness can be mediated by the effect of warming on plant phenology (Thórhallsdóttir 1998; Cleland *et al.* 2012; Parmesan & Hanley 2015). The relationship between flowering phenology, or lengthened growing season, and fitness was not apparent in this study. Here, phenology and fitness showed opposite responses for both species across the three sites combined, and responses in phenology to temperature did not occur at the same sites as responses in fitness. However, the effect of temperature on plant fitness was partly mediated by the responses of plant size and height. *R. acris* had reduced size and height with temperature, which had positive effect on fitness, even though the overall effect of temperature on fitness did vary (increased at GN, decreased at GO and had no response at Hengill). For *T. praecox* at GN, increased plant height led to reduced plant fitness.

These findings are constant with the notion that plants allocate resources to either vegetative or reproductive processes during development (Lekberg *et al.* 2012). *R. acris* responded to warming temperatures by reallocating resources to increase fitness at the cost of other traits (i.e. height and size). These results are similar plants growing in other geothermal systems, where plants allocated fewer resources to vegetative growth (biomass) and more to reproductive growth (flowers per gram of biomass), despite overall lower biomass (Lekberg *et al.* 2012). The fact that our results revealed the opposite relationship in *T. praecox*, where increased plant height with soil temperature lead to an indirect decrease of plant fitness, helps to validate the relationship. Indicating that if plants allocate resource to vegetative structures, their ability to allocate to reproductive output will be reduced.

It is worth mentioning that the most accurate relationship between plant fitness and soil temperature may not have been captured in this study due to sampling procedures. The measurement of fitness via number of seeds per flower may not represent the full reproductive potential of *T. praecox.* Reproduction of *T. praecox* occurs via both seeds and runners therefore, by using seed number as a proxy for fitness, we may have underestimated the response of warming on plant fitness (Pigott 1955). Additionally, in the northern limits of the species, individuals can be sterile or have irregular seed production during some years, with seeds only produced during exceptionally warm and sunny years (Pigott 1955). Fitness of *R. acris* may also be affected by the study method. *R. acris* fitness was estimated using flower number instead of average seed number as a result of sheep grazing at two of the three sites. Although flower number is a suitable estimation of fitness, there is evidence to suggest that resource allocation to bud formation and seed maturation are conflicting and therefore, may reveal difference in fitness estimates. *R. acris* individuals with more abundant and larger seeds will often have fewer flowers (Totland 1999). To improve the estimation of fitness for *R. acris*, plants should have been protected from grazing, and seeds collected alongside flower number.

2.4.2 Site Conditions

Plant responses to soil warming were site specific, fitness and height responses depended on site for *R. acris* while flowering phenology and size responses depended on site for *T. praecox*. This variation in responses could be the results of different local conditions.

Short-Term vs Long-Term Warming

The duration of time that systems have been exposed to warming can play a crucial role on the responses of plant traits to temperature, where short and long-term responses differ (De Boeck *et al.* 2015). This difference in early versus late-stage warming responses has been observed, for example, in the ITEX experiment where early years of warming showed a greater increase in vegetative growth compared to later years (Arft *et al.* 1999), while other studies revealed that nearly half of the observed traits changed responsiveness from short to long-term (Kremers *et al.* 2015).

In this study, the duration of warming between plants growing at GN and GO was considerably different, as were the plant responses to warming across these two sites. The *R. acris* population was more fit as temperatures increased at GN and less fit following the same temperature gradient and elevation at GO. Additionally, the *R. acris* population growing at GN responded to soil warming with a decrease in height but no change in size, whereas the GO population had plants that were smaller and shorter as temperatures increased. For the *T. praecox* population growing at GN, there was an increase in height but a decrease in fitness with warming soil temperatures. Height and fitness did not have a relationship with the same temperature gradient at GO. By contrast, at GO the *T. praecox* population had advanced flowering phenology as temperatures increased but was not responsive to the same temperature gradient at GN.

As plant growth increases initially, stimulated by warmer temperatures, the uptake of nutrients stored as biomass will increase, thereby decreasing the available nutrients in the soil over the long-term. This could in turn limit plant growth and affect flowering phenology (Molau 1993; Arft *et al.* 1999; Kremers *et al.* 2015). Similarly, if warming temperatures increase the species abundance or the species composition, the competition for these nutrients could increase in the long term (Sigurdsson *et al.* 2016). For example, under warmed conditions, grass and shrub cover has increased, reducing the density and fitness of herbaceous plants (Totland 1999). This could help to explain why *R. acris* size and height decreased more with temperature at GO compared to GN, the impact of warming temperature at the long-term warmed site could be mediated by limited nutrient availability and increased competition. This could also explain the differences in fitness and phenology responses observed in the short vs long-term sites here (Molau 1993).

Manipulation studies have found that traits were less responsive to warming over time as a result of increased competition and reduced nutrient availability (Kremers *et al.* 2015). In this study, decreased responsiveness to warming over time was evident for the phenology of *R. acris*, and plant size and height of *T. praecox.* For these traits, the response to soil warming was more sensitive (steeper slope) in GN compared to GO for phenology of *R. acris*, and plant size and height of *T. praecox.* However, responses for the remaining traits; fitness, size and height for *R. acris*, and phenology and fitness for *T. praecox*, were more sensitive (steeper slopes) to long-term warming at GO.

Influence of Elevation

Elevation has also played an important role in plant morphology (size and height), and plant fitness (Körner *et al.* 1989; Straka & Starzomski 2015). Two sites in this study, GO and Hengill, have been exposed to geothermal warming long-term but differ in elevation, (83-168 m a.s.l and 350–420 m a.s.l respectively), and response to warming. *R. acris* fitness, size and height all decreased with warming at GO but were unresponsive to the same temperature gradient at Hengill. While for *T. praecox,* phenology advanced with warming at both Hengill and GO, size decreased only at Hengill.

Elevation can effect snow accumulation throughout the winter as well as snowmelt in the summer (Körner 2007). Snowmelt timing has shown to have an influence on the leaf phenology of plants growing close to the Arctic Long-Term Ecological Research site in Alaska, where timing and rate of leaf emergence, and expansion advanced in response to earlier snowmelt (Livensperger *et al.* 2016). The relationship between elevation and snowmelt could explain why there were indications that plants at GO had more advanced phenology in general compared to Hengill (Figure 10), despite there being no difference in the phenological response to warming at the two sites.

Compared to their lowland counterparts, plants at higher elevations are often exposed to harsher conditions that influence plant growth. These factors, which include shorter growing seasons, lower mean temperatures, increased wind speed, and cloud cover, could influence ability of plants respond to temperature increases (Totland & Birks 1996; De

Frenne *et al.* 2013). Decomposition rates, which decrease with elevation in meadow ecosystems (Sundqvist, Giesler & Wardle 2011), could also influence the plant's ability to respond to warming by increased competition for limited resources. Increased competition could explain why *T. praecox* size only responded to temperature at Hengill. However, the opposite was found for *R. acris*, where plant size only responded to temperature at GO. Therefore, there could be additional factors, such as the capacity of different species to respond to soil warming or grazing pressure, that are also affecting plant responses at each site.

Grazing Pressure

Grazing has a variable effects on the growth form of herbaceous plants (Díaz *et al.* 2007; Damgaard *et al.* 2016), and in some cases can even mediate the response of plants communities to warming (Speed *et al.* 2012). Here, there were no signs of grazing on *T. praecox* at any of the sites however; *R. acris* was heavily grazed, particularly at Hengill but not at all in GN. The influence of grazing pressure could help explain why *R. acris* responds to warming at GO but not Hengill, and could account for some of the differences observed between GO and GN.

2.5 Conclusions

This study has highlighted the complexity of plant responses to warming in subarctic environments. By understanding how plant growth, fitness and phenology are affected by increased temperatures, we will be able to improve our predictions regarding species responses to future warming. This work can contribute to species range models, as well as models of ecosystem processes given the link between the measured traits and species interaction, nutrient availability, and carbon storage. The results from this project are by no means comprehensive, as I did not attempt to tackle ecosystem and community level responses to warming. However, this work does reveal that plant responses are significant, and suggests that research of this kind should be scaled up. Research looking into whole-ecosystem responses to warming should be prioritized and geothermal systems with embedded manipulation experiments can be used to do this. Continued research with these systems will help to address some of the questions that could not be answered throughout the course of this study. This would include species interactions, such as those between plants, herbivores and warming; as well as the below ground processes that could also affect the relationship between measured trait responses and soil warming. For example, the relationship between grazing animals and plant responses to warming opens the door to questions regarding preferential feeding of grazing animals that select individuals in warmer areas. Finally, there is potential to further the knowledge of the adaptive capacity of plants if studies such as these were incorporated with a genetic analysis over the long term and/or common garden and transplanting studies.

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Appendix A: Trait correlations

Pearson correlation coefficients between the four measured traits of R. acris across the three sites individually (GN, GO and HEN) and combined. Flowering phenology (Phen), fitness (Fit), plant size and plant height, and the additional traits measured, including flower area (Flw Area), leaf length (LL) and width (LW), and total number of inflorescence (Infl). Numbers in bold represent strong correlation (r from -1.0 to -0.5 or 1.0 to 0.5).

GN	Phen	Fit	Size	Height	Flw Area	LL	LW	Infl
Phen	-	0.09	-0.03	-0.09	**-0.53**	-0.26	-0.18	0.14
Fit		-	0.38	0.43	-0.12	0.22	0.35	**0.61**
Size			-	**0.60**	0.08	**0.53**	**0.55**	**0.55**
Height				-	0.41	**0.56**	**0.67**	0.49
GO	**Phen**	**Fit**	**Size**	**Height**	**Flw Area**	**LL**	**LW**	**Infl**
Phen		0.13	-0.04	-0.02	-0.26	-0.10	-0.16	-0.04
Fit			0.45	**0.63**	0.13	**0.55**	**0.63**	**0.51**
Size				**0.58**	0.11	**0.64**	**0.71**	**0.64**
Height					0.36	**0.78**	**0.81**	**0.58**
HEN	**Phen**	**Fit**	**Size**	**Height**	**Flw Area**	**LL**	**LW**	**Infl**
Phen		-0.13	0.07	-0.04	**0.59**	-0.07	0.05	-0.14
Fit			**0.50**	0.44	0.00	0.21	0.30	**0.98**
Size				0.42	0.01	0.47	**0.50**	**0.51**
Height					0.12	0.33	0.47	0.41
ALL	**Phen**	**Fit**	**Size**	**Height**	**Flw Area**	**LL**	**LW**	**Infl**
Phen		0.06	-0.01	0.04	-0.22	-0.12	-0.10	0.07
Fit			0.48	**0.60**	0.10	0.44	**0.55**	**0.52**
Size				**0.57**	0.11	**0.58**	**0.65**	**0.60**
Height					0.29	**0.65**	**0.72**	**0.56**

The square root of the Pearson correlation coefficients between the four measured traits of T. praecox. *Flowering phenology (Phen), fitness (Fit), plant size and plant height, and the additional traits measured, including leaf area (LA), total number of inflroescences per plant (Infl.), total number of buds, flowers and seed heads per stem (BFSH/Stem), and total number of buds, flowers and seed heads per plant (BFSH). Numbers in bold represent strong correlation (r from -1.0 to -0.5 or 1.0 to 0.5).*

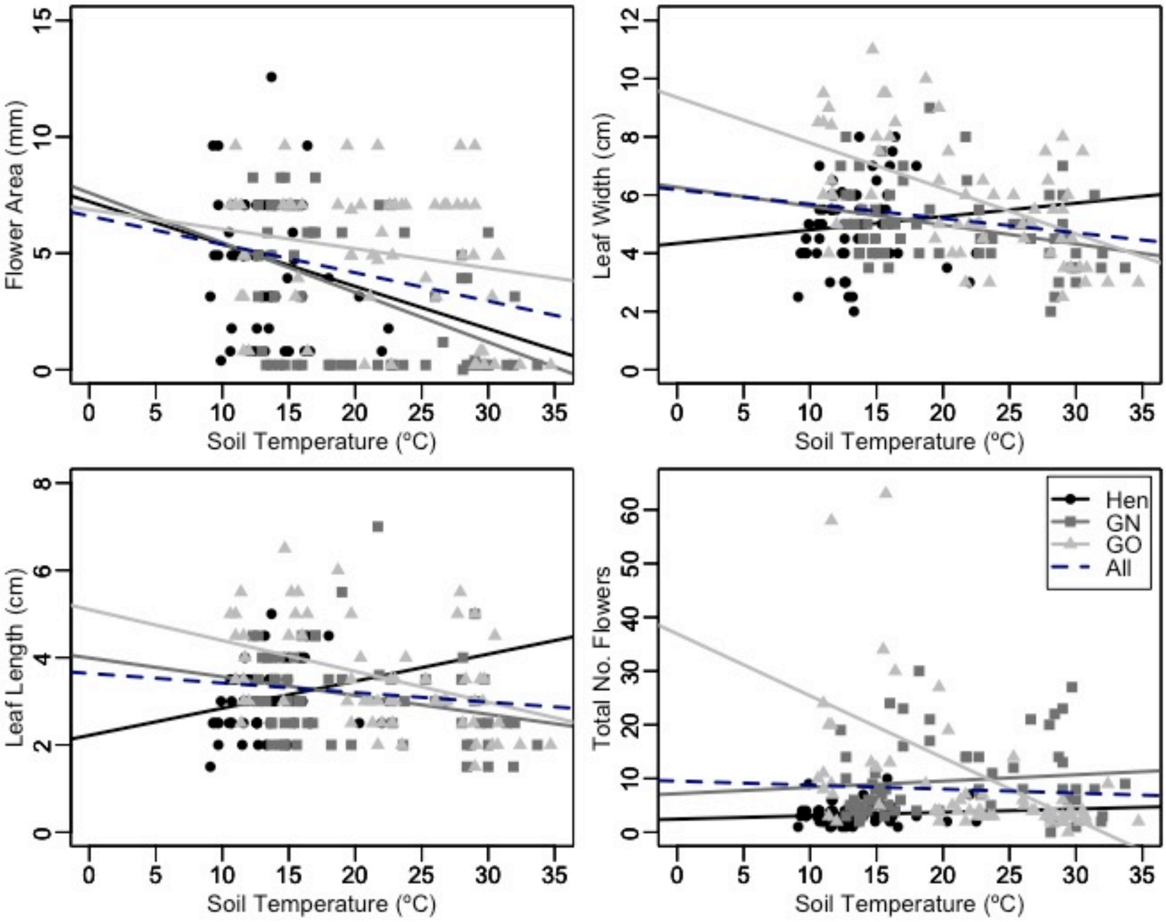
GN	**Phen**	**Fit**	**Size**	**Height**	**LA**	**Infl.**	**BFSH/Stem**	**BFSH**
Phen		-0.32	0.19	0.44	0.16	0.20	0.42	0.21
Fit			-0.14	-0.38	-0.09	-0.14	-0.17	-0.16
Size				0.42	**0.92**	**0.83**	0.36	**0.85**
Height					0.42	0.37	**0.55**	0.39

GO	**Phen**	**Fit**	**Size**	**Height**	**LA**	**Infl.**	**BFSH/Stem**	**BFSH**
Phen		-0.03	0.22	0.21	0.28	0.17	0.27	0.16
Fit			-0.16	-0.04	-0.13	-0.06	0.04	-0.06
Size				0.34	**0.90**	**0.86**	0.40	**0.86**
Height					0.29	0.30	**0.67**	0.34

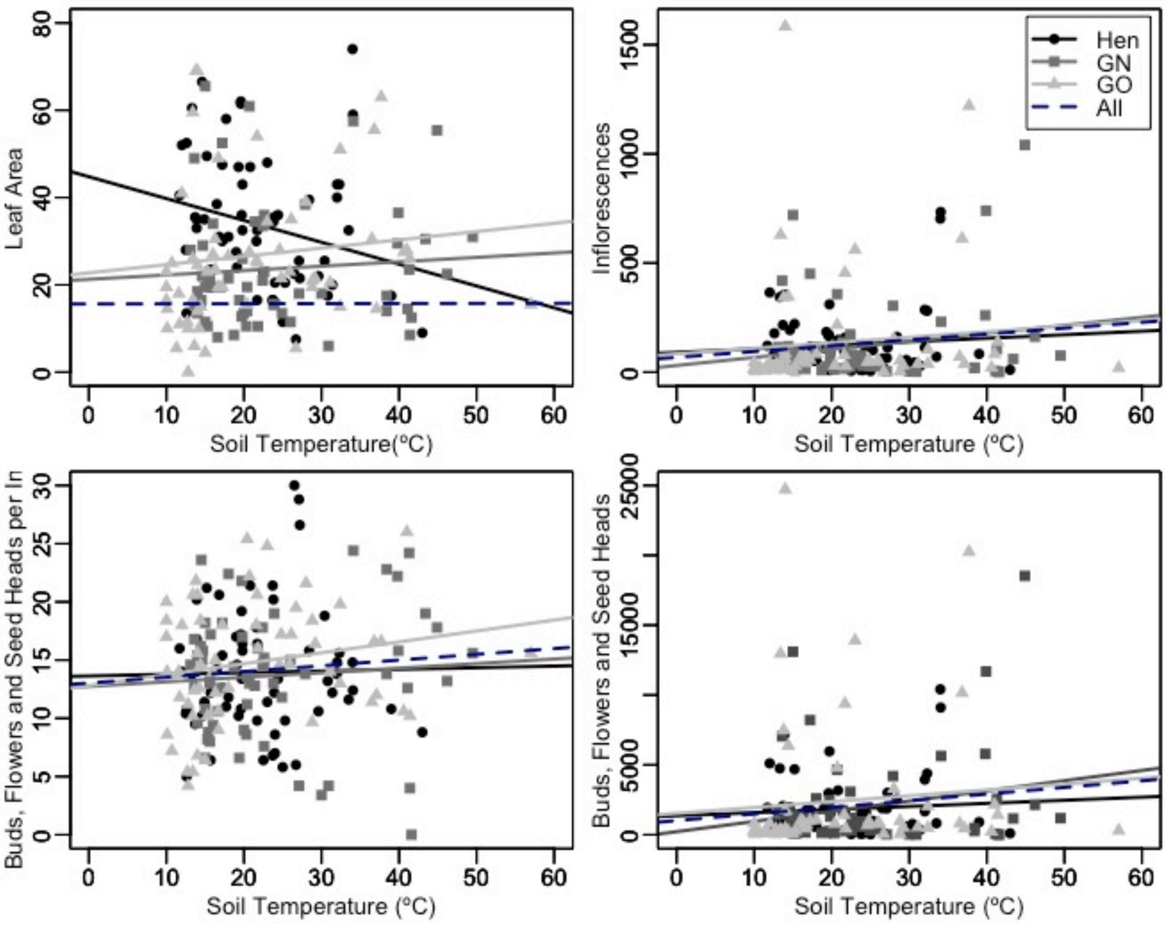
HEN	**Phen**	**Fit**	**Size**	**Height**	**LA**	**Infl.**	**BFSH/Stem**	**BFSH**
Phen		-0.11	-0.32	0.19	-0.11	-0.02	0.20	0.09
Fit			0.20	-0.23	0.22	0.29	-0.08	0.24
Size				0.00	**0.86**	**0.72**	0.06	**0.66**
Height					0.11	0.06	**0.56**	0.18

ALL	**Phen**	**Fit**	**Size**	**Height**	**LA**	**Infl.**	**BFSH/Stem**	**BFSH**
Phen		-0.14	0.07	0.28	0.06	0.11	0.26	0.15
Fit			-0.03	-0.20	0.04	0.00	-0.07	-0.02
Size				0.24	0.19	**0.79**	0.27	**0.77**
Height					0.46	0.26	**0.59**	0.32

Appendix B: Effect of soil temperature on correlated traits for each site

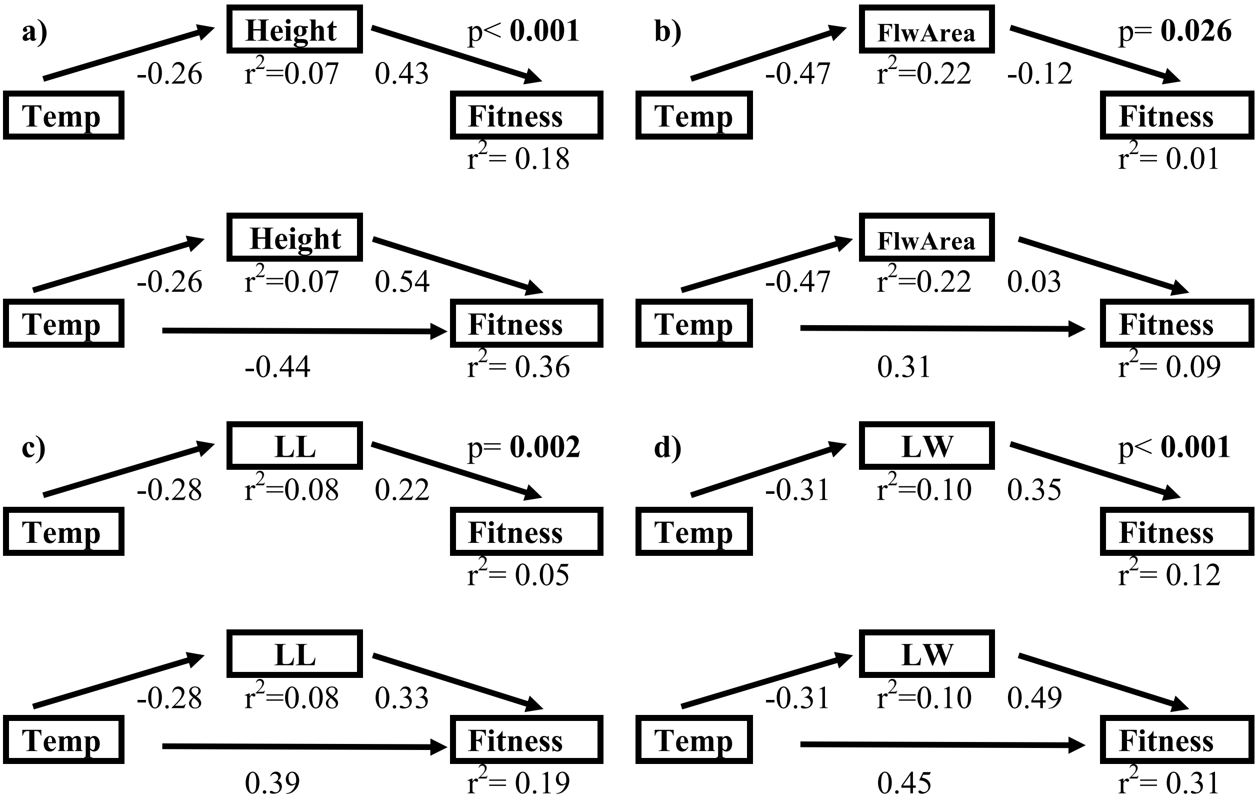


The effect of soil temperature on flower area (top left), leaf width (top right), leaf length (bottom right) and total number of flowers per plant (bottom left) of R. acris across the three study sites individually; Hengill (Hen), Grassland New (GN) and Grassland Old (GO), and all three sites examined together (All).

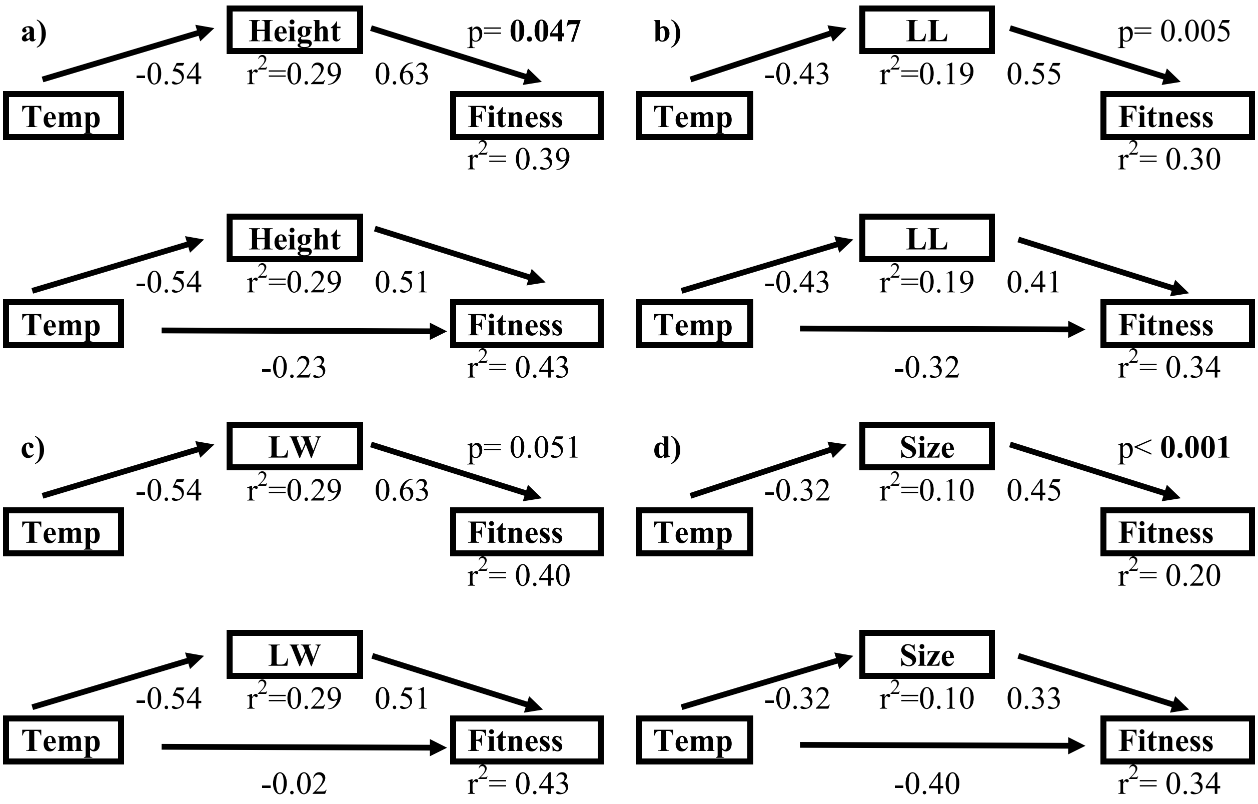


The effect of soil temperature on leaf area (top left), total number of inflorescences per plant (top right), total number of buds, flowers and seed heads per inflorescence (bottom right) and total number of buds, flowers and seed heads per plant (bottom left) of T. praecox across the three study sites individually; Hengill (Hen), Grassland New (GN) and Grassland Old (GO), and all three sites examined together (All).

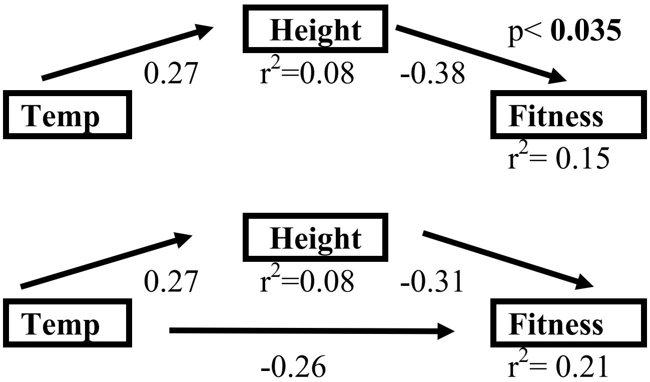
Appendix C: Path analysis for all traits, including those that were correlated



Results of the likelihood ratio test comparing the fully mediated and partially mediated models for R. acris traits, including height (a), flower area (FlwArea; b), leaf leagth (LL; c) and leaf width (LW; d) from GN. The partially mediated models were a better fit, p values indicated at the top right of each model.



Results of the likelihood ratio test comparing the fully mediated and partially mediated models for R. acris traits, including height (a), leaf leagth (LL; b) and leaf width (LW; c) and total plant size (Size; d) from GO. The partially mediated models were a better fit, p values indicated at the top right of each model.



Results of the likelihood ratio test comparing the fully mediated and partially mediated models for T. praecox Height from GN. The partially mediated models was a better fit, p value is indicated at the top right of the model.