**Detailed state of knowledge and aim**

Global warming is predicted to cause an increase in average surface temperatures by 1.0 – 3.7 °C by 2100 AD and the Arctic is expected to warm even more rapidly (IPCC 2014). Plant populations can adjust to these novel conditions through dispersal to colder sites, but this is not an option for Arctic populations, which are at the thermal edge of their range. Plants must therefore respond to these changes on site, either through phenotypic plasticity (the ability of the same genotype to produce different phenotypes under different environmental conditions) or through adaptive evolution (selection for traits that enhance fitness under warmer conditions, leading to changes in genetic structure) (Conner and Hartl 2004).

Numerous studies have examined the effects of warming on plants (Arft et al. 1999, Rustad et al. 2001), usually using either experimental manipulations (e.g. Arft et al. 1999, Hartley et al. 1999)) or natural climate gradients (e.g. Scheepens and Stöcklin 2013). Studies using experimental manipulation are usually short term, while evolutionary changes in plants usually take few generations (see review in Dunne et al. 2004). Studies have indeed indicated that long term effects of warming will be different than short term effects (Shaver et al. 2000). On the other hand, natural climate gradients, like differences in latitude, longitude or aspect, used to predict the long term effects of warming climate suffer from the limitation that also many other factors that can effect organisms differ along these gradients (see review in Dunne et al. 2004).

This project strives to overcome many of the limitations of previous studies by exploring plant responses at two sites where the soil is heated by steam from deep geothermal reservoirs. In these systems there is a broad gradient of soil warming (ambient to + 20 °C above ambient) in an area less than 500 m2 that does not differ in soil chemistry (see e.g. O'Gorman et al. 2014). As these systems have been heated for at least 50 years (and probably much longer) they offer unique natural experiments to study the long-term effects of warming on terrestrial ecosystems. In this study system, soil warming might influence trait expression (i.e. the genotype-phenotype relationship) and phenotypic selection for many traits. This form of warming has been shown to be a good proxy for climate change in previous studies, both in aquatic ecosystems at Hengill (e.g. Friberg et al. 2009, Woodward et al. 2010, O'Gorman et al. 2012) and in the terrestrial ecosystem in Grændalur (Leblans 2016, Sigurdsson et al. 2016).

The overall aim of this project is to investigate the responses of arctic plant populations to soil warming. The main research questions (RQ) are:

RQ1) Is soil warming associated with phenotypic changes in plant traits, such as phenology, and fitness?

RQ2) Does warming influence phenotypic selection on plant traits?

RQ3) Do plant response to warming depend differ between high and low elevation populations?

In the summers of 2015 and 2016 pilot studies were initiated in Hengill, where the plant phenology of the two of the study species was recorded once during the growing season. Results from these studies indicate that phenology differ with temperature; flowering time being later at lower soil temperatures.

**Detailed research plan**

*Study area*

The study will be conducted at two sub-arctic geothermal sites in the Ölfus municipality in SE-Iceland, Hengill (360 m.a.s.l) and Grændalur (100 m.a.s.l). Both sites exhibit a gradient of soil temperatures, from non-heated soils to soils heated over 20 °C above ambient, over a short distance, without significant changes in other abiotic factors (e.g. soil chemistry, elevation).

*Study species*

According to the 2017 application to Orkurannsóknasjóður the focal species were *C. fontanum* and *C. pratensis,* which are perennial herbs, commonly found in sub-arctic and arctic ecosystems. They are insect-pollinated, cross- and self-fertilized and flower between May and June in Iceland (Kristinsson 2010). As more funds could be secured from Stockholm University in 2017 and 2018 than initially planned, the project was expanded and three more perennial herbs, common in sub-arctic and arctic ecosystems included as focal species: *Bistorta vivipara*, *Viola palustris* and *Pinquicula vulgaris*. They were only marked and measured at Hengill as they only few plants of these species are found at Grændalur. The three species are all cross-fertilized and insect pollinated. *B. vivipara* mainly reproduces asexually with bulbils, and flowers from June to July. *V. palustris* flowers in May and *P. vulgaris* is a carnivorous plant that flowers in June.

*Study design*

In early May 2017 nine plots were established at each site, covering the soil temperature gradient over which the focal plants naturally occur. Within each plot 30 plants of the five focal species (in Grændalur only *Cerastum fontanum* and *Cardamine pratensis*) were marked within each plot, so that the location of the marked plants represented the temperature gradient in the plot. In addition, in Hengill five plots were established for each species in non-geothermally heated areas to establish a base line (control) for the species, due to lack of suitable sites, no control plots were established in Grændalur. For C. *pratensis* and C. *fontanum* around 690 plants of each species were marked, 270 at each of the geothermally heated site and 150 in the controls. For the other three species ca. 420 plants were marked. At the time of marking, the soil temperature was measured beside each plant at 10 cm depth. In addition, in each plot, 10 Thermochron iButtons (Maxim integrated) were buried at 10 cm depth in October 2017.

In 2017, once a week from May to middle of August, when the plants had started to wither, flowering phenology of each individual was estimated as the stage of the most advanced flower. From these data, first day of flowering, change in phenology over the summer and duration of flowering will be obtained for each individual and then associated with soil temperature. To examine the effect of soil warming on other plant traits, plant traits (plant size, flower size and flower number) of each individual plant was measured during peak growing season, defined as the time when over 50% of flowers of that individual had flowered. To estimate the effect of temperature on plant fitness, the number of fruits for each individual was counted at the end of the season after seeds had developed and before seed set and at least one intact fruit collected. The number of viable seeds per fruit were then counted for each individual. Plant fitness for *C. pratensis* and *B. vivipara* will be estimated from plant size, as sheep ate most of the *C. pratensis* flowers and seeds of *B. vivipara* were not collected. The seeds of *C. fontanum* and *P. vulgaris* will be divided up to two parts, one part is used for seed viability measurements and the other is used in a common garden experiment at Stockholm University. The common garden experiment will examine to what extent the differences in plant phenology observed in the field, are genetically based by growing plants originating from mother plants at sites differing in temperature under the same environmental conditions.

In 2018, methodology of 2017 will be repeated using the same marked plants, with some adjustments. *C. pratensis* will be omitted, as in 2017 most plants were eaten by sheep. The methodology will be repeated for C. *fontanum* at both sites and *P. vulgaris* and *B. vivipara* on Hengill. *Viola palustris* will only been measured during peak flowering and noted if plants flower and plant traits measured. In addition, some additional *V. palustris* will be measured. This is done as very few marked plants flowered in 2017, thus we need to increase the sample size, but due to time constrictions, it is not possible to both increase the sample size and have measurements every week.

Regression analyses will be used to assess the influence of soil temperatures on plant traits, phenology and individual fitness over the study period (RQ1). Standard selection analyses and path models will be applied to examine if warming induces selection towards traits that increase plant fitness (RQ2). Phenotypic selection analysis will be done using regression models (Barrett and Silander 1992) including soil temperature, plant traits and their interactions as fixed factors and fitness as the response variable. If a trait significantly affects plant fitness, it indicates that there is selection towards that trait; if temperature significantly affects fitness it implies that plant fitness is dependent on temperature. Finally, a significant interaction between soil temperature and plant traits would indicate that soil temperature influences selection on phenology. Since temperature might affect both trait expression (phenotypes) and selection, path models (Kingsolver and Schemske 1991) will be used to assess the direct, indirect and interactive effects of soil temperature and trait on plant fitness. To determine if plant response to warming differ between high and low elevation populations, results from Grændalur (low elevation) and Hengill (High elevation) will be compared (RQ3).

The amount of data and material collected in this project is extensive, and will form the base for further studies. The data is being used as background information in a study, in collaboration with London Imperial College, investigating if the intensity of insect herbivory on marked plants is influenced by soil temperature. Part of the seed collected for fitness measurements will be used in a common garden experiment at Stockholm University examining whether the differences in plant phenology seen in the field, will persist when plants from mothers growing at different temperatures, are grown under the same environmental conditions. More project build on the basis of the data collected in 2017 and 2018 are in the planning phase e.g. projects in collaborations with evolutionary biologist that look at the evolutionary basis of the plant response to temperature. In addition, our results indicate that for *C. pratensis* soil temperature influences when the plants are eaten by sheep, which might indicate that grazing impact the response of plants to global warming. This will be explored more thoroughly in future.

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