

MISS NIKI I.W. LEBLANS (Orcid ID : 0000-0001-6154-1538)

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Title: **Phenological responses of Icelandic subarctic grasslands to short-term and long-term natural soil warming.**

Running head: **Phenological responses to natural warming**

Leblans, N. I. W.^{1, 2}, Sigurdsson, B. D.², Vicca, S.¹ Fu, Y.^{1,3}, Penuelas, J.^{4, 5} and Janssens, I.

A.¹

¹ Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

² Agricultural University of Iceland, Hvanneyri, 311 Borgarnes, Iceland

³ Sino-French Institute for Earth System Science, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

⁴ CREAM, Cerdanyola del Vallès 08193, Catalonia, Spain

⁵ CSIC, Global Ecology Unit, Cerdanyola del Vallès 08193, Catalonia, Spain

Corresponding author: Niki I. W. Leblans; Tel: 0032 32651726; e-mail: niki.leblans@uantwerpen.be

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Abstract The phenology of vegetation, particularly the length of the growing season (LOS; i.e. the period from greenup to senescence), is highly sensitive to climate change, which could imply potent feedbacks to the climate system, e.g. by altering the ecosystem carbon (C) balance. In recent decades, the largest extensions of LOS have been reported at high northern latitudes, but further warming-induced LOS extensions may be constrained by too short photoperiod or unfulfilled chilling requirements. Here, we studied subarctic grasslands, which cover a vast area and contain large C stocks, but for which LOS changes under further warming are highly uncertain. We measured LOS extensions of Icelandic subarctic grasslands along natural geothermal soil warming gradients of different age (short-term, where the measurements started after 5 years of warming and long-term, i.e. warmed since ≥ 50 years) using ground-level measurements of normalized difference vegetation index (NDVI). We found that LOS linearly extended with on average 2.1 days per $^{\circ}\text{C}$ soil warming up to the highest soil warming levels (ca. $+10^{\circ}\text{C}$) and that LOS had the potential to extend at least one month. This indicates that the warming impact on LOS in these subarctic grasslands will likely not saturate in the near future. A similar response to short- and long-term warming indicated a strong physiological control of the phenological response of the subarctic grasslands to warming, and suggested that genetic adaptations and community changes were likely of minor importance. We conclude that the warming-driven extension of the LOSs of these subarctic grasslands did not saturate up to $+10^{\circ}\text{C}$ warming, and hence that growing seasons of high latitude grasslands are likely to continue lengthening with future warming

(unless genetic adaptations or species shifts do occur). This persistence of the warming-induced extension of LOS has important implications for the C-sink potential of subarctic grasslands under climate change.

Introduction

The phenology of vegetation (the timing of recurrent biological events and its biotic and abiotic causes; Lieth, 1974) is highly sensitive to climate change (Henry & Molau, 1997, Penuelas & Filella, 2001, Tucker *et al.*, 2001, Linderholm, 2006, Richardson *et al.*, 2013) and shifts in phenology could in turn induce strong feedbacks to the climate system by altering fluxes of CO₂, water, energy and biogenic organic compounds (Gu *et al.*, 2003, Cleland *et al.*, 2007, Penuelas *et al.*, 2009, Ahlstrom *et al.*, 2012, Richardson *et al.*, 2013). These potent phenological feedbacks to the climate system are mainly driven by changes in the length of the growing season (LOS) due to shifts in the start and/or the end of the growing season (SOS and EOS, respectively).

In this study, we use the ecological definition of LOS, i.e. the period between budburst (SOS) and leaf fall (EOS) (Linderholm, 2006), in contrast to the climatological LOS, which refers to daily minimum and maximum temperature thresholds (Menzel *et al.*, 2003). Changes in ecological LOS are generally assessed by ground-level phenology measurements (e.g. Richardson *et al.*, 2006) or by using remote sensing at ground-level (Ólafsdóttir & Óskarsson, 2014) or at satellite level (Zhou *et al.*, 2001, Beck *et al.*, 2006, White *et al.*, 2009, Jeong *et al.*, 2017). Remote sensing studies often use the normalized vegetation index (NDVI; a measure of the greenness of the vegetation). For evergreen vegetation, where the period of greenness does not correspond to the period of photosynthetic

activity (Gamon *et al.*, 1995), other measures have been developed to measure LOS (e.g. Zhao & Liu, 2014, Walther *et al.*, 2016, Jeong *et al.*, 2017), but for grasslands (our study system), the NDVI-based LOS is a reliable proxy for the period of photosynthetic activity (Gamon *et al.*, 1995, Richardson *et al.*, 2013, Ólafsdóttir & Óskarsson, 2014, Luo *et al.*, 2016) and has thus a close link to the carbon cycle.

Climate warming in recent decades has generally extended LOS by the combined responses of SOS and EOS (Linderholm, 2006, Jeong *et al.*, 2011), with the largest extensions at high northern latitudes (Raynolds *et al.*, 2015, Zhao *et al.*, 2015, Gonsamo & Chen, 2016) and high altitudes (Zhao *et al.*, 2015) where temperatures are rising fastest (IPCC, 2013). This warming-induced extension of LOS at high northern latitudes has primarily been driven by an advance in SOS, while EOS has generally been less responsive to temperature (Menzel *et al.*, 2006, Cleland *et al.*, 2007, Zhao *et al.*, 2015). The advance of SOS, however, might saturate under further climate warming, as the shorter photoperiod or incomplete chilling become more important for the local flora. Such a reduced sensitivity of the SOS response to warming was observed in a common garden experiment on broadleaf and evergreen tree seedlings (Morin *et al.*, 2010) and in long-term *in situ* observations of various mature broadleaf tree species across Europe (Fu *et al.*, 2015). The warming-induced advance of SOS (and thus the extension of LOS) for ecosystems at high northern latitudes, including grassland ecosystems, may also be slowing, despite the continuous increase in temperature (Zhao *et al.*, 2015, Jin *et al.*, 2016). However, how the extension of LOS will respond to future climate warming remains highly uncertain (Kimball *et al.*, 2007, Zhao *et al.*, 2013, Fu *et al.*, 2014b, Keenan & Richardson, 2015).

Grassland ecosystems cover a large area of the global terrestrial surface (ca. 40 %, whereof 25 % at northern high latitudes; Chapin *et al.*, 2011) and have a high C-sink capacity (Soussana *et al.*, 2007, Yoshitake *et al.*, 2015). Further, grassland phenology has been studied less than forest phenology (Steinaker & Wilson, 2008, Li *et al.*, 2016a), even if its C uptake, and thus the strength of the potential feedback to the climatic system, is highly sensitive to changes in phenology, more than that of deciduous and needle leaved forests (Richardson *et al.*, 2013). Limited previous research has shown that temperature is an important driver of subarctic and alpine grassland phenology (Cleland *et al.*, 2006, Frei *et al.*, 2014, Shen *et al.*, 2016).

This study investigates warming-induced changes in the LOS (SOS and EOS) of unmanaged subarctic grasslands exposed to gradients in soil temperature (from +0 to ca. +10°C) for different duration. The study site (www.forhot.is) is located in southwest Iceland, where natural geothermal soil warming gradients of different age (5-7 y vs. ≥ 50 y of continuous warming) occur. The vegetation at the study site is dominated by circumpolar species that have a wide distribution in boreo-arctic as well as temperate regions (*Agrostis capillaris*, *Ranunculus acris* and *Equisetum pratense*; Kristinsson & Sigurdsson, 2010, Sigurdsson *et al.*, 2016), improving the generalizability of the responses. Interestingly, no significant changes in dominant species occurred along the soil warming gradients (Gudmundsdóttir *et al.*, 2014, Michielsen, 2014). The grasslands of different warming duration enabled the elucidation of short- and long-term warming effects on plant phenology (an important uncertainty in phenology projections; Kimball *et al.*, 2007), while also being indicative of the mechanisms behind the phenological response (fast physiological changes vs. slower acting genetic or community changes).

Based on existing knowledge, we expected that the LOS of these subarctic grasslands would extend with warming, and that this extension would be mainly driven by an advance in SOS. We expected, however, that the extension of LOS would saturate at the highest warming levels due to a decrease in the temperature sensitivity of SOS at high warming levels. Furthermore, we hypothesized that the temperature responses would be similar for short-term and long-term exposure to warming, based on previous observations of rapid phenological responses to warming in alpine grasslands (Frei *et al.*, 2014) and in many other ecosystem types (e.g. Byers & Quinn, 1998, Williams *et al.*, 2008, Morin *et al.*, 2010, De Frenne *et al.*, 2011). In other words, we expected no additional long-term changes in the phenological response to temperature by genetic adaptations and/or community changes.

Materials and methods

Site description

The study sites were located in the Hengill geothermal area, 40 km east of Reykjavik, Iceland (64°00'01"N, 21°11'09"W; 100-225 m a.s.l.), and are part of the ForHot research site (www.forhot.is). The mean annual air temperature at the sites was 4.9 °C during the study period (2013-2015), and the mean temperatures of the coldest and warmest months were -1.0 and 11.7 °C, respectively. The mean annual precipitation was 1431 mm (Icelandic Meteorological Office; www.vedur.is), with variable monthly distribution, ranging from 23 to 255 mm per month during the study period, with no distinct rainy season. Important to note is that the spring of 2015 was both particularly cold and dry.

We studied two sets of grassland sites, within 2.5 km of each other, which had been subjected to geothermal soil warming for different periods of time. One set of sites, the short-term warmed grassland sites (hereafter “SWG”), had been warmed for 5-7 years, since 29

May 2008, when a major earthquake caused geothermal systems to shift to previously unwarmed areas. The other set of sites, the long-term warmed grassland sites (hereafter “LWG”), had been warmed for at least 50 years and probably for centuries (Sigurdsson *et al.*, 2016). The soil was warmed by heat conducted from the underlying bedrock, which was warmed by geothermally heated groundwater (Sigurdsson *et al.*, 2016). No signs of soil contamination by geothermal by-products were found. The degree of warming was relatively constant throughout the study period, and warming did not cause noteworthy changes in soil pH or soil moisture, with soil moisture rarely dropping below the permanent wilting point and no relation between soil temperature and the frequency of drought events (SI, Fig. S.1; Sigurdsson *et al.*, 2016). The main vegetation type at both sites was unmanaged subarctic grassland, dominated by *Agrostis capillaris*, *Ranunculus acris* and *Equisetum pratense*. Further description of the study sites can be found in O’Gorman *et al.* (2014), Michielsen (2014), Gudmundsdóttir *et al.* (2014), Poeplau *et al.* (2016) and Sigurdsson *et al.* (2016).

Study design

Twenty-five 2 × 2 m plots were established in autumn 2012 at both the SWG and LWG soil temperature gradients, ranging from ambient soil temperature to ca. +10 °C at five temperature levels (approximately +0, 1, 3, 5 and 10 °C), with five replicate plots per temperature level. Soil temperatures were measured hourly at a depth of 10 cm using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, Bourne, USA). Air temperature was measured at heights of 2 m (hereafter “air temperature”) and 2 cm (hereafter “surface temperature”) above the soil surface, using the same loggers and logging frequency. All air and surface temperature loggers were protected from direct sunlight, while allowing sufficient air circulation. The surface temperature was only measured at 10 out of the 25 plots per site, so to derive the surface temperature for all individual pots, we

determined the correlation between the surface warming (i.e. difference between surface temperature and air temperature) for the available data and the plot-specific soil temperature at a depth of 10 cm. This relationship between surface warming and soil warming was used to calculate plot-specific surface temperatures. Infrequent extreme deviations in surface temperature (i.e. differences between air and surface temperature of $> +5$ or -5 °C) caused by, for example, direct insolation or radiation frosts on clear nights, were set to $+5$ or -5 °C, because such episodes could not be excluded from the data since continuous temperature data were needed to calculate the number of growing degree days (GDD) (see § 2.5). The frequency of these extreme temperature deviations was not significantly correlated with average soil temperature (SI, Fig. S.2).

NDVI measurements

Plot-specific NDVI was measured weekly, except during periods of continuous snow cover, in 2013, 2014 and 2015 from April to November. The measurements were performed between 9 AM and 4 PM, selecting the clearest day possible, and no influence of timing or weather conditions on the NDVI measurements could be detected (possibly due to the long summer days at high latitudes and because of the technique used to measure the NDVI, which measures both incoming and reflected radiation close to the surface). We used a hand-held SpectroSense 2+ four channel sensor (Skye Instruments, Powys, UK) for the measurements and each measurement was recorded at a fixed location in each plot by placing the sensor pole in a pre-marked corner of the plot and tilting the pole in the direction of the opposite diagonal corner. A uniform tilt ($\sim 74^\circ$), corresponding to a height of 2 m and a measurement surface of 0.62 m^2 , was acquired by using a level bubble. The NDVI was calculated as described by Tucker (1979):

$$NDVI = \frac{\rho_{840} - \rho_{660}}{\rho_{840} + \rho_{660}} \quad \text{Eq. 1}$$

where ρ_{840} and ρ_{660} are the surface reflectances at the selected infrared wavelength (840 nm) and the visible red wavelength (660 nm), respectively.

Function fitting and determination of SOS and EOS

Each plot yielded three NDVI time series, one for each measurement year (Fig. 1), which were all scaled to a maximum value of one. The unscaled maximum NDVI is shown in the supporting information (Fig. S.3). Two phenological key dates were derived from each time series: (1) SOS and (2) EOS. These dates were obtained by fitting two logistical functions to the NDVI time series, one for greening and one for senescence, based on the approach of Zhang *et al.* (2003) (Fig. 1). All functions yielded good fits over the entire gradient of soil temperature ($R^2 \geq 0.88$, mean $R^2 = 0.96 \pm 0.01$ (SE)).

SOS was obtained by calculating the second derivative of the first part of the logistic greening function, thereby identifying the function's highest change in curvature, following Zhang *et al.* (2003). The timing of 10% senescence was used for obtaining EOS, because the real end of the growing season (the time point when the logistic senescence function levels off) could not be derived due to a lack of data in early winter. We selected the 10% senescence threshold because this point was reached in >90% of all data series and because the photosynthetic activity after this time point was expected to be negligible due to low light and temperature conditions. Moreover, Jeong *et al.* (2017) showed that the photosynthetic activity (measured as solar induced chlorophyll fluorescence) of high latitude forests decreased in the fall more than a month earlier compared to the NDVI, showing that the 'real' growing season was shorter than suggested by the NDVI. The EOS was calculated as:

$$EOS = \ln\left(\frac{a+d}{(a+d)*0.9}\right) - \left(\frac{b}{-c}\right) \quad \text{Eq. 2}$$

where EOS corresponds to the timing of 10% senescence, and a, b, c and d are parameters of the logistic senescence function (Zhang *et al.*, 2003).

Calculation of cumulative GDD at SOS

The cumulative GDD at SOS was calculated using both soil temperatures (measured at a depth of 10 cm) and surface temperatures (measured at 2 cm above the soil surface), i.e. soil GDD and surface GDD respectively. The cumulative GDDs were calculated as described by (McMaster & Wilhelm, 1997), where daily mean temperatures lower than the base temperature are set equal to the base temperature. GDD depends strongly on the chosen base temperature and on the starting date of GDD summation. We compared the calculated GDD of each unwarmed plot with its expected GDD (based on the average GDD of all other unwarmed plots) across all combinations of 16 base temperatures (from -5 to +10 °C at increments of 1 °C) and nine starting dates (from 1 January to 9 May, which is the date of the first observed SOS, at increments of 15 days). We then selected the combination of base temperature and starting date that yielded the smallest difference between actual and expected GDD across all unwarmed plots. The optimal base temperatures based on this procedure were -1 and -3 °C for soil and surface GDD, respectively, and 15 February was the optimal starting date in both cases (SI, Fig. S.4). These parameters were used to calculate the cumulative surface GDD of all plots (both unwarmed and warmed). The cumulative soil GDD could only be calculated for 2014 and 2015, because no data for soil temperature were available for the first months of 2013.

Data analyses

We tested the influence of soil warming on surface temperatures by determining the relationship between soil and surface warming (i.e. the difference between air and surface temperature) using a linear regression model. The relationships between LOS, SOS or EOS and average soil or surface temperature were tested with linear mixed models, with temperature, warming time (SWG and LWG) and year (2013, 2014 and 2015) as fixed factors. A logarithmic relationship for LOS, SOS and EOS vs. temperature was fitted when it yielded a better fit than a linear relationship (based on Akaike information criterion comparison with correction for finite samples). A linear mixed model was used to test for differences in cumulative soil GDD at SOS between the years, warming times and warming treatments (unwarmed and +1, +3, +5 and +10 °C). Non-significant interactions were excluded from the model, and a Tukey's test identified specific differences in the final model. Differences between cumulative surface GDD at SOS were identified in the same way. The relationship between SOS and EOS was determined using a linear mixed model, with temperature, warming time and year as fixed variables. The relationship between Δ SOS and Δ EOS was determined within the years with linear mixed models, with temperature and warming time as fixed variables. The requirements for normality and homoscedasticity were met in all cases. All tests were performed in R (R-core-team, 2014), and null hypotheses were rejected at $p < 0.05$.

Results

Effects of soil warming on surface temperature

Soil warming (at 10 cm soil depth) caused the surface temperature (at 2 cm above the soil surface) to warm slightly, by approximately 0.074 °C per °C soil warming (data not shown).

The effects of soil warming were even lower at a height of 15 cm (see Sigurdsson *et al.*, 2016 for more information about surface warming). Air temperature measured at 2 m above the soil surface was not affected by the soil warming, and did not differ significantly between the short-term and the long-term warmed grasslands.

Effects of soil warming on LOS, SOS and EOS of subarctic grasslands

Averaged over the full soil warming gradient (+0 vs. +10 °C), LOS increased by 32 days due to a 23.5 day advancement of SOS and a 9.5 day delay in EOS (Table 1). The LOS of the unwarmed plots was approximately 120 days (four months) for both SWG and LWG in 2014 and 2015 (Fig. 2; upper panels). The growing season for SWG in 2013, was about a month shorter, but this was likely initiated by a local pest and not by a climatological cue and should therefore be interpreted with caution. Soil warming significantly extended LOS in all cases, with an average extension of 2.1 ± 0.3 (SE) days per °C soil warming (Fig. 2; upper row), or 29 ± 5 (SE) days per °C surface warming (Table 2). There was no consistent difference in the responses of SWG and LWG, since the warming response was stronger (2013), weaker (2014) or the same (2015) for SWG compared to LWG.

SOS occurred in the unwarmed plots in late May in 2013 and 2014 and about a month later after the much cooler spring of 2015 (Fig. 2; middle panels). Soil warming significantly advanced SOS in all cases, following a logarithmic relationship. The temperature sensitivity of SOS did not differ significantly in 2013 between SWG and LWG, when both advanced with roughly 2.1 days per °C soil warming. Also in 2014, the temperature sensitivity of SOS did not differ significantly between SWG and LWG, but it was slightly lower than in 2013 (roughly 1.3 days per °C soil warming; Fig. 2) (corresponding to a sensitivity of 28 and 17

days per °C surface warming; Table 2). In 2015, the temperature response was slightly higher for SWG than for LWG (1.9 vs. 1.6 days per °C soil warming, or 23 vs. 19 days per °C surface warming, respectively; Fig. 2, Table 2).

The EOS of most of the unwarmed plots in 2013 and 2014 took place in late September (Fig. 2; lowest panels). EOS in 2013 was about a month earlier for SWG than LWG. The EOS of the unwarmed plots was half a month later in 2015 (when SOS was also late) than in 2013 and 2014. Warming had no significant effect on EOS in 2013 and 2014 but slightly delayed it in 2015 in a similar way for SWG and LWG (0.93 days per °C soil warming, or 12.6 days per °C surface warming).

Cumulative GDD at SOS

The cumulative soil GDD at SOS (calculated from soil temperatures at a depth of 10 cm) increased significantly with soil warming in all cases (Fig. 3, upper panels), except for the lowest warming treatment (+1 °C; Table 3). The warmest plots (ca. +10 °C soil warming) had received, for example, about three times the amount of soil GDD compared to the unwarmed plots. In addition, we found that the cumulative soil GDD at SOS was also significantly higher during the year with the coldest spring (2015). The cumulative surface GDD at SOS (calculated from air temperature at 2 cm above the soil surface), on the contrary, decreased significantly with soil warming (Fig. 3, lower panels), except for the lowest warming treatment. Again, the year with the coldest spring (2015) had a significantly higher surface GDD than the two warmer years. The surface GDD was slightly lower for SWG than LWG (Table 3).

Coupling between EOS and SOS

The relationship between EOS and SOS was determined for both the entire dataset and the data excluding SWG 2013 (because 2013 was likely an outlier year; Fig. 4, main panel). For the full dataset, warming treatment had no influence on the relationship between EOS and SOS, but warming time (SWG vs. LWG) and year (2013, 2014 and 2015) significantly influenced the relationship (both $p < 0.001$). The outcome was very similar when SWG 2013 was excluded, but then the significant interannual difference disappeared (Table 4).

The overall relationship between SOS and EOS was statistically significant (Fig. 4, main panel), both with and without SWG 2013, with regression-line slopes of 0.66 and 0.73, respectively. The relationship between EOS and SOS within years, however, disappeared (in 2013 and 2014) or was even reversed (in 2015) (Fig. 4, side panels) with a regression-line slope of -0.41 ($p < 0.01$). Warming time (SWG vs. LWG) did not influence the interannual relationships.

Discussion

LOS extension potential under further climate warming

The LOS extended consistently in response to warming, with limited interannual variation in the magnitude of the warming response, confirming that temperature is an important driver of LOS in these subarctic grasslands. This result is in line with earlier research on phenological drivers in northern ecosystems (Richardson *et al.*, 2013). The extension was linear up to the highest warming treatment (+10 °C soil warming), with 2.1 days per °C soil warming, averaged across the three measurement years and across the short-term and long-term warmed grassland. This shows that no saturation of the warming-induced extension of LOS

had been reached yet. More importantly, the maximum extension of LOS amounted to no less than 32 days over the whole soil warming range (+0 – 10 °C), indicating that the LOS of these subarctic grasslands can still extend by (at least) a month if climate warming continues.

The extension of LOS was mainly driven by an advance in SOS, with an average and maximum advance of 18 and 23.5 days respectively across all measurement years and across the two grasslands. EOS was only slightly delayed (with an average of 3 days and a maximum of 9 days), as expected, possibly to avoid a premature halt in the recovery of nutrients by early frosts (Estiarte & Penuelas, 2015). This result agreed with studies on grasslands in cold climates (Richardson *et al.*, 2013), although exceptions do occur (Yang *et al.*, 2015). It also agreed with other studies on a wide range of northern ecosystems, using ground observations, temperature manipulation experiments and remote-sensing techniques (Cleland *et al.*, 2007, Zeng *et al.*, 2011, Zhao *et al.*, 2015).

It is interesting to note the close similarity in the strong response of SOS to soil warming after short-term (SWG) and long-term (LWG) soil warming. This indicated that the phenological responses occurred relatively shortly (max. 5 years) after the onset of the warming and stabilized soon afterward (remaining similar for at least 50 years). This is an important finding with respect to the uncertainty of the long-term perspective of phenological responses to warming. Long-term changes in phenological responses have been difficult to predict due to their dependence on other variables that are also affected by long-term warming and that are affected by vegetation growth itself (e.g. the availability of adequate resources; Kimball *et al.*, 2007).

The delay of EOS with warming, albeit modest, indicated that these subarctic grasslands have the potential to extend their growth in autumn, if thermal conditions allow, thereby strengthening the warming-induced extension of LOS. These small changes in EOS might be partly related to the internal constraints from spring phenology, as recent studies found a positive correlation between SOS and EOS, which might be due to a fixed foliar longevity, a depletion of soil water reserves by early spring greening or a C sink saturation of the vegetation (Fu *et al.*, 2014a, Keenan & Richardson, 2015). This correlation became most obvious in 2015, when a late SOS (due to cold and dry conditions; see § 4.5) was followed by an exceptionally late EOS (Fig. 2). This late EOS could not be explained by a warm fall, as the fall temperatures were very similar to those in 2014 when EOS, was on average 27.5 days earlier. However, although this coupling may have mitigated the interannual differences in LOS in this study (Fig. 4), it did not restrict the warming-induced extension of LOS along the warming treatments within the years.

The linear extension of LOS with warming up to high warming levels in these subarctic grasslands and their large potential for LOS extension (at least one month) contrasts with the study of Zhao *et al.* (2015), which found a reduction (or even a reversing) of the warming-induced extension of LOS at middle and high northern latitudes over the past 10 years. This has important implications for the C-sink potential of these ecosystems under further climate warming.

Physiological control of the phenological response

Various mechanisms may have underlain the advance of SOS with warming (plant physiology, genetic adaptations and community changes), and we can only speculate about their relative importance based on the differences between the warming responses in SWG

and LWG. The strong similarity between SWG and LWG indicated that the response was relatively rapid (stabilized after maximum 5 years of warming), agreeing with the results from many short-term (1-3 years) common garden experiments and transplantation studies (e.g. Byers & Quinn, 1998, Williams *et al.*, 2008, De Frenne *et al.*, 2011, Frei *et al.*, 2014, Li *et al.*, 2016b). The rapid response to warming indicated a strong physiological control of subarctic grassland phenology (i.e. the ability of a given genotype to produce variable phenotypes in different environments; Agrawal, 2001), which can act quickly (Jump & Penuelas, 2005) and play a major role in the warming response. These physiological control mechanisms of SOS are for most northern plant species largely driven by temperature sums (e.g. GDD), more than by the phytochrome system (light regime) (Bennie *et al.*, 2010, Poikolainen *et al.*, 2016; see also § 4.3). This is also generally the case for temperate and subarctic grasslands (Xu *et al.*, 2017).

Genetic adaptations and community changes can also play a role in phenological responses (Høye *et al.*, 2007, Chen *et al.*, 2014), but the similarity between SWG and LWG indicated that such slower acting mechanisms (Jump & Penuelas, 2005) were likely not active in the warming response in this study. Unfortunately, no data were available to test the ‘genetic adaptations’ hypothesis, but surveys of vegetation have found little change in community composition for both SWG and LWG up to warming levels of +5 °C and even at our highest warming level (+10 °C), no changes in dominant plant species occurred (Gudmundsdóttir *et al.*, 2014, Michielsen, 2014).

SOS advance constraint by environmental factors other than temperature?

Even if LOS extended linearly up to the highest warming level, the temperature sensitivity of SOS of these subarctic grasslands declined at higher warming levels (as indicated by the non-linearity of the temperature response in Fig. 2, middle panel). This is in line with the

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deceleration of the warming-induced advance of SOS (or in some cases a delay in SOS) that has recently been found in some northern regions due to gradual climate warming (Zhao *et al.*, 2015) and with studies on the temperature sensitivity of SOS advance of broadleaf trees in Europe and North America (Morin *et al.*, 2009, 2010, Fu *et al.*, 2015). This declining temperature sensitivity has been linked to other environmental factors (i.e. day length, precipitation, snow cover and lack of chilling; Richardson *et al.*, 2013, Fu *et al.*, 2014b). Interannual comparisons allowed us to evaluate the importance of each of these potentially constraining environmental factors in the SOS of these subarctic grasslands.

Day length constrains SOS in some high-latitude ecosystems as a frost avoidance mechanism (Høye *et al.*, 2007). However, the considerable difference in the timing of earliest greening in 2013 and 2014 (beginning of May) versus 2015 (beginning of June), in combination with the clear decline in temperature sensitivity in 2015, indicated that day length was not the main driver of the SOS in these subarctic grasslands. Precipitation can play a role in SOS of subarctic and alpine grasslands, although its effect is not consistent, varying between non-existent (Piao *et al.*, 2011), positive (Fu *et al.*, 2014b), negative (Chen *et al.*, 2014, Sha *et al.*, 2016) and dependent on the specific situation (Shen *et al.*, 2011, Shen *et al.*, 2015, Zhang *et al.*, 2015). In this study, no major variation in soil water status occurred along the temperature gradients (Sigurdsson *et al.*, 2016), especially in early spring, so it is unlikely that precipitation was an important determinant of the decelerating advance of SOS. Overall differences in spring precipitation, however, affected all treatments equally, as discussed in § 4.5.

Snow cover delays plant growth until the timing of spring melt (Richardson *et al.*, 2013), but it did not limit the advance of SOS in our study, because these Icelandic sites do not have permanent snow cover due to their mild oceanic winter climate (even if separate

periods of snow can occur from late September until May; Sigurdsson *et al.*, 2016). The chilling requirements for SOS could also be compromised by warming (Li *et al.*, 2016a) but are generally believed to be of little importance in grasslands, because the phenology of grass leaves is likely opportunistic (Li *et al.*, 2016a).

Since there are no strong indications that any of the abovementioned environmental factors played an important role in the declining temperature sensitivity of SOS, we hypothesize that the decline was (at least partly) caused by an artifact common for soil warming experiments (Patil *et al.*, 2013): the increasing decoupling of soil and surface temperatures along the soil warming gradient. An earlier study on alpine grasslands (where this artifact was avoided by using a transplant approach where soil and air temperature are changed in parallel), did indeed find a linear advance of SOS over a warming range of 4°C (Frei *et al.*, 2014). A persistence of the advance in SOS with warming of the subarctic would have far-reaching consequences for the potential for C storage in this region under future warming conditions. This should be taken into account in the dynamic global vegetation models (DGVMs) to further improve our understanding of feedbacks between vegetation and climate change.

Does SOS respond to soil or surface temperature?

The magnitude of the SOS response to warming as well as the opposite warming response of the GDD requirement for soil (at 10 cm depth) versus surface temperature (Fig. 3) presumably indicated that the tissues that drove the SOS response (hereafter “responsive tissues”) were located somewhere in between the soil surface and 10 cm depth. Firstly, the SOS responsiveness to soil warming was in the lower range of previous reports for mid- and high northern latitudes (on average -1.7 days per °C vs. 0.2 to 16 days per °C; Zhao *et al.*,

2015), while the responsiveness to surface warming (on average -22 days per °C) was more extreme than any previous study that we are aware of. Secondly, the GDD requirement for SOS of the responsive tissues was assumed to be constant over the entire warming gradient (Sigurdsson, 2001, Liu *et al.*, 2014, Li *et al.*, 2016a, Cong *et al.*, 2017), or potentially increasing moderately (to avoid too early SOS). Contrary to these assumptions, the surface GDD requirement decreased along the warming gradient, while the soil GDD requirement did increase, but too drastic to be caused by a saturation of the SOS advance (a tripling over a gradient of 10°C). Thus, if the GDD requirement did indeed remain constant or increased moderately with warming, our data suggests that the temperatures measured at a soil depth of 10 cm were too high and at the surface too low to calculate the true GDD requirements for the responsive tissues.

This led to the hypothesis that grass meristems, which are the tissues where greening starts (Pautler *et al.*, 2013), are the primary driver of the phenological response to warming. Indeed, grass meristems are located in the layer with intermediate warming (the topsoil and litter layer; Benson *et al.*, 2004), and this was also true for these subarctic grasslands (personal observations). Moreover, meristem temperature has been shown to drive the SOS of maize grown in cold temperate climates (Stone *et al.*, 1999). Unfortunately, we could not verify this hypothesis because meristem temperature was not measured. We recommend that further phenological studies include this measurement, because a better understanding of meristem physiology could greatly enhance model performance, as a lack of information for physiological mechanisms that drive warming-induced phenological responses remains a key restraint for further model improvements (Zhao *et al.*, 2013).

Contribution of secondary effects to SOS and EOS

The interpretation of warming-induced changes in LOS can be confounded by secondary effects. We were able to detect two such events by comparing data from different years. Firstly, the temperature control of SOS could be confounded by spring drought in these subarctic grasslands (across all treatments). This was revealed by the higher cumulative GDD requirements during the coldest year (2015) (both for soil and surface GDD), which was counterintuitive because GDD requirements tend to be lower under colder conditions due to higher energy-use efficiency (Liang & Schwartz, 2014, Liu *et al.*, 2014). The unusually low precipitation during the late spring of 2015 (the average calculated PET from May to July was 0.7 ± 0.4), which induced a homogeneous drying of the soil across all soil warming levels (data not shown), thus likely delayed the greenup beyond the timing of ‘GDD fulfillment’. This can explain the higher cumulative GDD at SOS. This delaying effect of spring drought to the timing of SOS agreed with earlier studies of subarctic grasslands (Chen *et al.*, 2014) and of grasslands in general (Sha *et al.*, 2016).

Secondly, the earlier EOS for SWG than LWG in 2013, while climatic conditions were very similar for both sites, probably related to non-climatic factors. We presume that the discrepancy was caused by a local pest at the SWG site in 2013, such as a fungal infection or the mite *Penthaleus major* that is known to affect Icelandic perennial grasslands in summer (Gudleifsson *et al.*, 2002). The analyses in our study, however, were robust against the exclusion of SWG 2013. To conclude, SOS in 2015 and EOS in 2013 for SWG may not have been totally driven by warming, highlighting a potential caveat in studies of the effects of warming on phenology (and other ecosystem processes).

Consequences for the carbon balance of subarctic grasslands

Our observations of the large and linear warming-induced extension of the growing season reveal a substantial potential for prolonged photosynthetic activity and C uptake in subarctic grasslands under further climate warming. However, concerns have been raised on the correlation between NDVI and C uptake. This is especially true for regions with a high coverage of evergreen vegetation, where greenness is disconnected from photosynthetic activity (Gamon *et al.*, 1995). In such ecosystems, photosynthetic activity should be measured with other techniques, such as solar-induced chlorophyll fluorescence (SIF; Jeong *et al.*, 2011). Yet, for grasslands ecosystems in general (and certainly for subarctic grasslands, which show a clear seasonality in NDVI; Ólafsdóttir & Óskarsson, 2014), the NDVI-based LOS has been shown to be a reliable proxy for the period of photosynthetic activity (Gamon *et al.*, 1995, Richardson *et al.*, 2013, Ólafsdóttir & Óskarsson, 2014, Luo *et al.*, 2016, Vicca *et al.*, 2016).

Finally, one should be aware that the maximum annual NDVI was scaled to one in the present study to facilitate the comparison of the warming effect on LOS across different years and sites. This scaling makes the phenological changes clearer, but would have been suboptimal if NDVI was to be linked to productivity differences. Then, the LOS should optimally be combined with the (unscaled) maximum NDVI and, when possible, biomass harvests or photosynthetic measurements. Such an analysis is underway and it will allow to verify to which extent the strong warming-induced C-uptake potential (LOS extension) leads to increased productivity in these subarctic grasslands

Opportunities for using geothermal gradients in soil temperature for phenological research

Despite some drawbacks of natural geothermal gradients in soil temperature, such as the decoupling of soil and surface warming (§ 4.3 and 4.4), these ‘natural experiments’ are

highly valuable for investigating phenological responses to warming. Firstly, the presence of SWG and LWG, caused by the dynamic nature of geothermal systems (O'Gorman *et al.*, 2014), allowed us to observe the temporal dynamics of the response to warming and offered clues to the underlying mechanisms. The similarity in the phenological responses to warming between SWG and LWG indicated that the phenological response to warming occurred soon after the onset of the warming and was maintained for at least 50 years, allowing us to speculate that the response was mainly driven by phenotypic plasticity and not by genetic adaptations or community changes.

Secondly, the large gradual increase in soil temperature (in this case up to +10°C), typical for geothermal gradients in soil temperature, allowed us to detect nonlinearities in the response. Interestingly, we found that the warming-induced advance in SOS continued at soil warming levels higher than +5°C, but that EOS was mostly unresponsive to soil warming, even at the highest warming levels. Observing long-term effects of such broad soil warming gradient is often not possible in climate manipulation experiments, where the warming time and the number of warming treatments are strongly constrained by logistical and financial limitations (De Boeck *et al.*, 2015).

Thirdly, the typical small spatial scale of geothermal soil temperature gradients allowed us to keep most of the environmental variables (e.g. photoperiod, precipitation and soil type) largely constant along the warming gradients (Sigurdsson *et al.*, 2016), which allowed us to identify the contribution of secondary (non-warming) effects on LOS (see § 4.5). This limited complexity of the environmental factors in our study offered an advantage compared to space-for-time studies, which often have to deal with a multitude of confounding factors (De Boeck *et al.*, 2015).

The linear extension of LOS under warming – i.e. more than a month at the highest warming level - demonstrated that the warming-induced phenological responses in these subarctic grasslands were still far from thermally saturated. The extension of LOS was mainly driven by an advance of SOS, and no clear saturation of the advance (due to other environment constraining factors) was observed. The similarity in temperature response between the SWG and LWG responses suggests that phenotypic plasticity, rather than changes in community and genetic adaptations, likely regulates the phenological response of subarctic grasslands to warming. Furthermore, we hypothesize that meristem temperature might be the major determinant of the changes in SOS, and we urge for a better understanding of meristem physiology to improve projections of feedbacks from ecosystem phenology to the climate system. Finally, other environmental factors, such as drought, were found to play a role in the phenological process.

This study suggests that the warming-induced extension of LOS in subarctic grasslands could continue under future climate warming conditions (unless genetic adaptations or species shifts occur that would change that pattern). This has important implications for the C-uptake potential of these subarctic grasslands under future climate change, and can thus induce a powerful ecosystem climate change feedback.

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List of tables:

Table 1: Extension, advance and delay of the length of the growing season (LOS), the start of the growing season (SOS) and the end of the growing season (EOS), respectively, over the full soil warming range (+0 vs. +10 °C) during the different measurement years (2013, 2014 and 2015) in the short-term warmed grassland (SWG) and the long-term warmed grassland (LWG). The maximum change gives an indication of the potential of these subarctic grasslands to adjust the phenology under warming conditions.

<i>Change over the full warming range (+0 vs +10 °C)</i>	2013		2014		2015		Maximum
	SWG	LWG	SWG	LWG	SWG	LWG	
Extension LOS (days)	+22	+10	+14	+32	+24	+24	+32
Advance SOS (days)	-23.5	-23.5	-15	-15	-18	-13	-23.5
Delay EOS (days)	0	0	0	0	+9	+9	+9

Table 2: Changes in the length of the growing season (LOS), the start of the growing season (SOS) and the end of the growing season (EOS) in days per °C soil and surface warming. Soil temperatures were measured at a depth of 10 cm, and surface temperatures were measured 2 cm above the soil surface. The relationships were determined with linear mixed models, with surface temperature, short-term (SWG) and long-term (LWG) warming times and year (2013, 2014 and 2015) as fixed variables. The significance of the source variables ($p < 0.05$) is indicated in parentheses: $\circ p = 0.10-0.05$, $* p = 0.05-0.01$, $ p = 0.01-0.001$, $*** p < 0.001$. Errors indicate SEs. ns not significant.**

	Change in days per °C increase in temperature			
	2013	2014	2015	Average
<i>Soil warming</i>				
LOS				
SWG	+2.2 (**)	+1.4 (*)	+2.4 (***)	+2.1 ± 0.3
LWG	+1.0 (*)	+3.2 (***)		
SOS				
SWG	-2.1 (***)	-1.3 (***)	-1.3 (**)	-1.6 ± 0.2
LWG			-1.3 (***)	
EOS				
SWG	ns.	ns.	+0.93 (*)	-
LWG	ns.	ns.		
<i>Surface warming</i>				
LOS				
SWG	+28 (**)	+20 (*)	+33 (***)	+29 ± 5
LWG	+13 (\circ)	+48 (***)		
SOS				
SWG	-28 (***)	-17 (***)	-19 (***)	-22 ± 2
LWG			-23 (***)	
EOS				
SWG	ns.	ns.	+12 (*)	-
LWG	ns.	ns.		

Table 3: Results of the linear mixed models, with cumulative soil or surface GDD as the response variable, and year (2013, 2014 and 2015), short-term (SWG) and long-term (LWG) warming times and warming treatment (unwarmed, +1, +3, +5 and +10 °C soil warming) as fixed factors. None of the interactions were significant. Significant source variables ($p < 0.05$) are indicated with asterisks: * $p = 0.05-0.01$, ** $p = 0.01-0.001$, * $p < 0.001$.**

	Difference	Lower	Upper	Q	p
<i>Cumulative soil GDD</i>					
Year					
2014-2015	173.1	105.8	240.5	1.41	***
Warming time					
SWG-LWG	-41.4	-108.8	26.0	1.41	0.23
Warming treatment					
+1 °C - unwarmed	18.2	-131.1	167.5	0.05	1.00
+3 °C - unwarmed	188.5	39.2	337.7	0.52	**
+5 °C - unwarmed	361.2	211.9	510.4	1.00	***
+10 °C - unwarmed	880.8	731.6	1030.1	2.44	***
<i>Cumulative surface GDD</i>					
Year					
2013-2014	0.3	-34.5	35.2	0.01	1.00
2013-2015	62.6	27.6	97.4	1.74	***
2014-2015	62.1	27.3	97.0	1.73	***
Warming time					
SWG-LWG	38.6	14.9	62.4	1.41	**
Warming treatment					
+1 °C - unwarmed	-20.1	-72.6	32.4	0.39	0.83
+3 °C - unwarmed	-62.2	-114.7	-9.7	1.21	*
+5 °C - unwarmed	-91.6	-144.1	-39.1	1.79	***
+10 °C - unwarmed	-125.6	-178.1	-73.1	2.45	***

Table 4: Results of the linear mixed models, end of season (EOS) as the response variable, and start of season (SOS) as fixed factor and warming treatment (unwarmed, +1, +3, +5 and +10 °C soil warming), year (2013, 2014 and 2015) and warming time (short-term (SWG) and long-term (LWG)) as random factors. Significant source variables ($p < 0.05$) are indicated with asterisks: * $p = 0.05-0.01$, ** $p = 0.01-0.001$, * $p < 0.001$.**

<i>Relationship EOS and SOS</i>	Entire dataset			Dataset excluding SWG 2013		
	Df	F-value	P-value	Df	F-value	P-value
Interaction with warming treatment (+0, 1, 3, 5 and 10°C)	4	1.41	0.23	4	1.8	0.13
Interaction with year (2013, 2014 and 2015)	2	44.8	***	2	24.1	***
Interaction warming time (SWG and LWG)	1	22.9	***	1	0.3	0.59
Overall relationship between EOS and SOS (Taking significant interactions into account)	1	148	***	1	127	***

Figure captions:

Figure 1: An example of an NDVI measurement series (datapoints) with logistic function fits for greening (solid line) and senescence (dashed line). Arrows indicate (1) the start of the growing season (SOS) and (2) the end of the growing season (EOS), corresponding to the timing of 10% senescence. The data show the 2014 NDVI time series of a plot with ambient soil temperature from the long-term warmed grassland.

Figure 2: Length of the growing season (LOS; upper panels), start of season (SOS; middle panels) and end of season (EOS; lower panels) versus average soil temperature in 2013 (left column), 2014 (middle column) and 2015 (right column). The average soil temperature was calculated from May 2013 to May 2015. The short-term (SWG) and long-term (LWG) warmed grasslands are shown as open and solid circles, respectively. The colors indicate the soil warming treatments (blue, ambient; green, +1; yellow, +3; orange, +5 and red, +10 °C). The smaller symbols for LOS and EOS for SWG in 2013 indicate that EOS was likely initiated by a local pest and not by a climatological cue and should therefore be interpreted with caution. Error bars are SEs. Solid lines indicate significant relationships when SWG and LWG did not significantly interact. Dotted (SWG) and dashed (LWG) lines indicate significant interactions. The equations for SOS and EOS are expressed in days of the year.

Figure 3: Cumulative soil growing degree days (GDD) at the start of the growing season (SOS) versus SOS (upper panels) and cumulative surface GDD at SOS versus SOS (lower panels) for the short-term (SWG, open symbols; left panels) and long-term (LWG, solid symbols; right panels) warmed grassland for the three years (2013, circles; 2014, triangles; 2015, stars). Cumulative soil GDD could not be calculated for 2013, due to a lack of soil temperature measurements in early 2013. Cumulative soil GDD corresponds to the GDD at a depth of 10 cm (base temperature of -1 °C, starting date 15 February), and cumulative surface GDD corresponds to the GDD at 2 cm above the soil surface (base temperature of -3 °C, starting date 15 February). The colors indicate the soil warming treatments (blue, ambient; green, +1; yellow, +3; orange, +5 and red, +10 °C). The cumulative soil and surface GDD from January to June for the different soil warming treatments is shown in SI, Fig. S.5 and S.6. Error bars are SEs.

Figure 4: Main panel: relationship between the end of the season (EOS) and the start of the season (SOS) across all years (2013, circles; 2014, triangles and 2015, stars) for the short-term (SWG, open symbols) and long-term (LWG, solid symbols) warmed grasslands together. The relationship was determined both for the entire dataset and the data excluding SWG 2013 (marked in grey), because the EOS in SWG 2013 may have been caused by a local pest and not by a climatological cue. More statistical details can be found in Table 4. Side panels: relationships between the changes in the end of the season (Δ EOS) and the start of the season (Δ SOS) per year for SWG and LWG. Changes are expressed relative to the timing of SOS and EOS for the unwarmed plots of the same grassland and during the same year. Significant relationships are shown with solid lines. The equation for the relationship between Δ EOS and Δ SOS in 2015 was $y = -0.41 * x$, with $R^2 = 0.12$ and $p < 0.01$.







