Ecosystem warming extends vegetation activity but heightens cold temperature vulnerability

Andrew D. Richardson[1,2,3*], Koen Hufkens[1], Thomas Milliman[4], Donald M. Aubrecht[1], Morgan E. Furze[1], Bijan Seyednasrollah[1,2,3],Misha B. Krassovski[5], John M. Latimer[5], W. Robert Nettles[5], Ryan R. Heiderman[5], Jeffrey M. Warren[5], and Paul J. Hanson[5]

[1]Harvard University, Department of Organismic and Evolutionary Biology, Cambridge MA 02138 USA
[2]Northern Arizona University, School of Informatics, Computing and Cyber Systems, Flagstaff AZ 86011 USA.
[3]Northern Arizona University, Center for Ecosystem Science and Society, Flagstaff AZ 86011 USA.
[4]University of New Hampshire, Institute for the Study of Earth, Oceans and Space, Durham NH 03824 USA
[5]Oak Ridge National Laboratory, Climate Change Science Institute and Environmental Sciences Division, Oak Ridge TN 37831 USA

*Corresponding author; Andrew.Richardson@nau.edu

Shifts in vegetation phenology are a key example of the biological impacts of climate change[1–3]. However, there is substantial uncertainty about whether these temperature-driven trends will continue, or whether other factors – e.g. photoperiod – will become more important as warming exceeds the bounds of historical variability[4,5]. Using phenological transition dates derived from digital repeat photography[6], we show that experimental whole-ecosystem warming treatments[7] of up to +9 °C are correlated linearly with delayed autumn green-down and advanced spring green-up of the dominant woody species in a Boreal *Picea-Sphagnum* bog. Results were confirmed by direct observation of both vegetative and reproductive phenology of these and other bog plant species, and multiple years of observations. There was little evidence that the observed responses were constrained by photoperiod. Our results indicate a likely extension of the period of vegetation activity by 1-2 weeks under a "stabilization" climate scenario (+ 2.6 ± 0.7 °C), and 3-6 weeks under a "high emission" scenario (+ 5.9 ± 1.1 °C) through the end of the 21st

century. We also observed severe tissue mortality in the warmest enclosures following a severe spring frost event. A failure to cue to photoperiod resulted in precocious green-up and a premature loss of frost hardiness[8], suggesting increased vulnerability to spring frost damage in a warmer world[9,10]. While vegetation strategies evolved to balance tradeoffs associated with phenological temperature tracking may be optimal under historic climates, those strategies may not be optimized for future climate regimes. These *in situ* experimental results are of particular significance because Boreal forests have a circumpolar distribution and play a key role in the global carbon cycle[11].

In temperate and boreal regions, rising temperatures are advancing spring onset (e.g. budburst and flowering) and delaying autumn senescence (e.g. leaf coloration and leaf fall) [12,13]. Whether these trends will be maintained is an open question[4]. Warm and cold temperatures, photoperiod and insolation, precipitation and water availability, have been shown to influence plant phenology[2,5,14,15]. However, the future response of phenology to rising temperatures still remains largely unknown because of the high degree of uncertainty associated with interactions among these drivers[12]. Importantly, it has been proposed that photoperiod may constrain the phenological response to rising air temperatures[4,5,16]. While there is evidence for this in some species[8,15], the generality of these results – and whether there are robust patterns across functional types – has yet to be demonstrated[5].

Analyses of observational data sets to disentangle the effects of these drivers are challenged by the lack of variability in natural systems, the inherent correlation among drivers, and the realism of space-for-time assumptions[12]. Experimental approaches are thus required. However, there are sizable challenges associated with conducting realistic environmental manipulations, particularly for ecosystems with tall vegetation. Because of financial, logistical and technological hurdles, experimental warming treatments have not previously been applied to forest stands, and have only rarely been applied to single mature trees[17]. While experiments with seedlings and branch cuttings are relatively common[18,19], artifacts associated with these approaches may limit their broader applicability[20,21].

We are studying the impact of experimental whole-ecosystem warming treatments on vegetation phenology at the SPRUCE (Spruce and Peatland Responses Under Changing Environments) facility, a long-term, multi-factor, manipulative experiment situated in a Boreal peatland forest in the Upper Midwest of the US[7]. The experiment is unique in that the five levels of warming (from 0 to +9 °C, see Methods and Supplementary Analysis 1) are being applied to intact communities of native plants, including woody shrubs and mature trees. The dominant plant species at SPRUCE represent key genera that are found across the vast boreal forest, or taiga, which covers much of the northern hemisphere land surface from 45° to 70° N. Knowledge of the environmental controls on the phenology of these species is poor and does not, at present, provide a strong basis for making predictions about the capacity for phenological tracking of a warmer climate. Results from SPRUCE will therefore inform our understanding of climate change impacts on processes related to biogeochemical cycling and biosphere-atmosphere feedbacks for this globally extensive biome.

Our focus here is on the impact of the experimental ecosystem warming treatments on spring and autumn phenology in this forested peat bog. Specifically, we tested three competing hypotheses:

H1. Temperature is the dominant control on phenological events. *Prediction*: the observed phenological transition date is directly related to the degree of warming (Figure 1 a).

H2. Photoperiod is the dominant control on phenological events. *Prediction*: the observed phenological transition date is constant regardless of the degree of warming (Figure 1 b).

H3. Photoperiod constrains the phenological response to temperature. *Prediction*: the observed response to temperature is flat beyond a threshold temperature, t^* (Figure 1 c).

We tracked phenological responses to the experimental treatments in two ways. Since August 2015, we have monitored the vegetation within each enclosure using digital repeat photography (Figure 1 d, e)[6]. And, since April 2016, we have made weekly ground observations of vegetative and reproductive phenology on a variety of plant species.

For our analysis of camera imagery, we distinguished between three distinct vegetation types: evergreen conifer, *Picea mariana* (black spruce); deciduous conifer, *Larix laricina* (eastern tamarack or larch); and a mixed, ground-level shrub community dominated by *Rhododendron groenlandicum* (Labrador tea) and *Chamaedaphne calyculata* (leatherleaf). For each, green-down (as determined by G_{CC}, a color index derived from the digital images) in autumn 2015 was delayed with increasing warming (Figure 2, a-c). The response to warming was significantly stronger (interaction effect between temperature and species, $P < 0.001$) for the mixed shrub community (≈5 days delay per 1 °C warming) than for either of the tree species (1-2 days delay per 1 °C warming), but was in all cases highly linear. Our results unequivocally support H1, i.e., that temperature is the dominant control on the timing of autumn phenology. The fact that the temperature sensitivities were, in all cases, significantly different from zero allows us to reject H2. And, in no case did our breakpoint analysis (see Methods) identify a t^* value that substantially improved model fit (Extended Data Table 2), allowing us to reject H3. While the above results are for autumn 2015, comparable results were observed in autumn 2016 and 2017 (Supplementary Analysis 2).

Similarly, green-up in spring 2016 was advanced with increasing warming (Figure 2, d-f). The response to warming (1-2 days advancement per 1 °C warming) was not significantly different among vegetation types (interaction effect between temperature and species, $P = 0.34$). As in autumn, the fact that the temperature sensitivities were significantly different from zero allows us to reject H2. Breakpoint model analysis allowed us to reject H3, as in no cases was a t^* value identified that would improve model fit (Extended Data Table 2). In spring, as in autumn, H1 is best supported by the experimental results. Results in spring 2017 were generally consistent with those for spring 2016 (Supplementary Analysis 2).

The above results clearly indicate a continued extension of the period of vegetation activity in response to future warming. By combining downscaled climate projections (Extended Data Figure 2) from CMIP5 [22] with the phenological temperature sensitivities estimated from Figure 2 (Supplementary Analysis 3), we predict that the physiologically active season of the two conifer species may be extended by ≈1 week under a "stabilization" climate scenario (RCP 4.5, + 2.9 ± 0.7 °C), and up to 3 weeks under a "high emission" scenario (RCP 8.5, + 5.9 ± 1.1 °C) by 2100 (Extended Data Table 3). Active season extension for the shrub layer is projected to be roughly twice as large. These results are judged to be entirely plausible, given that future warming is not projected to exceed the levels of experimental warming at SPRUCE, and thus we are not extrapolating into unsampled climate space.

Previous work has shown that the seasonality of G_{CC} is a robust proxy for the seasonality of vegetation photosynthesis in both conifer forests and wetland ecosystems[23,24], and thus earlier plant green-up and delayed green-down at SPRUCE are almost certainly associated with a longer photosynthetically active period, and likely associated with enhanced annual photosynthetic uptake (though not necessarily increased vegetation growth). This result is consistent with analysis of long-term data from an FLUXNET (Supplementary Analysis 4, Extended Data Figure 3), as well as previous experimental[19] and observational[25] studies. However, this does not necessarily imply an increase in net carbon uptake or carbon sequestration under future warming, because the long-term C balance of this peatland forest ecosystem is likely dependent the stability of the underlying peat deposits[26].

Camera-based results are generally consistent with direct observation of spring (2016 and 2017) and autumn (2017 only) phenological transitions for plant species spanning a range of leaf habits and growth forms (Table 1; see also Supplementary Analysis 5). Spring phenophases advanced by just over 3 days per 1 °C warming, providing strong support for H1. Autumn phenophases related to leaf coloration or senescence were delayed by almost 3 days per 1 °C warming, again providing support for H1. Relatively little variation was observed in dates of fall bud set for *Chamaedaphne* and *Picea*, providing support for H2 for this particular phenophase of these species. While t^* breakpoints that improved model fit were commonly identified, we note that in most cases, ΔAICc > 0, meaning that the simpler, linear temperature model was better supported by the data. Furthermore, the identified breakpoint temperatures were generally quite high – below 4.5°C in only a few instances – indicating that future warming greatly exceeding RCP 4.5 projections would have to occur before photoperiod constraints begin to limit phenological shifts. The ground observations therefore robustly support H1 over H2 or H3, and are consistent with future extension of the active season at both ends.

There is abundant evidence in the literature that photoperiod plays a role in triggering phenological events[27,28]. In many species there has been local adaptation of phenology to both photoperiod and temperature cues[5,15]. In some species and environments, photoperiod sets a hard limit on the phenological response to rising temperatures[4,15]. But, with warming of up to +9 °C above current levels, we found little evidence for this in most of the species and phenophases studied here. Thus, photoperiod requirements are still getting met even during the shortened winter simulated by the warmest enclosures. In the few cases where there was evidence of a photoperiod effect, it was generally only a factor at temperatures well above current temperatures, again implying that substantial future warming would be required for photoperiod to become limiting. These findings are consistent with a recent analysis showing that for high-latitude species, spring leaf out was generally not photoperiod-sensitive[8].

The purported role of photoperiod as a phenological constraint is to prevent plants from responding to temperature signals at the "wrong" time of the year[4]. However, if photoperiod is not a strong constraint on spring phenological development, then a counterintuitive prediction is that continued warming coupled with increasing frequency of climate extremes may increase the likelihood of spring frost damage[9,10]. At SPRUCE, atypical weather in March (unusually warm) and April (extreme cold) 2016 showed that, in addition to triggering visually apparent phenological shifts, the warming treatments also advanced tissue de-hardening, thereby heightening the potential for spring frost damage (Supplementary Analysis 6, Extended Data Figure 4). Following a spring frost event where ambient temperatures dropped to –15 °C, we observed extensive foliar damage in the +9.0 °C enclosures (where temperatures dropped to about –4°C) and moderate damage in the +6.75 °C enclosures. Minimal damage occurred in the enclosures that received less warming and thus experienced colder minimum temperatures. This suggests that the transition from frost-hardy to frost-vulnerable is cued by warm temperatures[9], and is not constrained by photoperiod. Without photoperiod as a safety check on the de-hardening process, frost damage may be more severe and/or more frequent under future climate conditions. Woody plants generally have sufficient nonstructural carbon reserves to recover from occasional frost damage[10], but repeated damage could impair the competitive ability of susceptible species (Extended Data Table 6)[9,29].

Results from the first year of the SPRUCE experiment, conducted in a winter-dormant ecosystem, show decisively that warming treatments directly influence vegetation phenology at both the start and end of the annual period of vegetation activity. These phenological shifts will almost certainly influence photosynthesis and transpiration[3,16], as well as feedbacks to the climate system through impacts on the surface energy budget[12]. Future extension of the active season in most cases appears unlikely to be strongly constrained by photoperiod in this Boreal ecosystem. Potentially inopportune responses to environmental signals may occur as the climate moves beyond the range of historical variability, as demonstrated by the spring frost damage in the warmest enclosures. Thus, temperature-tracking strategies evolved to guide phenological responses to historical year-to-year variation in weather may be increasingly mis-matched to future conditions[5].

References

1. Settele, J. *et al.* Terrestrial and Inland Water Systems. in *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* 271–359 (2014).

2. Cleland, E. E., Chuine, I., Menzel, A., Mooney, H. A. & Schwartz, M. D. Shifting plant phenology in response to global change. *Trends Ecol. Evol.* **22,** 357–365 (2007).

3. Morisette, J. T. *et al.* Tracking the rhythm of the seasons in the face of global change: phenological research in the 21st century. *Front. Ecol. Environ.* **7,** 253–260 (2009).

4. Körner, C. & Basler, D. Phenology under global warming. *Science* **327,** 1461–1462 (2010).

5. Way, D. A. & Montgomery, R. A. Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant. Cell Environ.* **38,** 1725–1736 (2015).

6. Sonnentag, O. *et al.* Digital repeat photography for phenological research in forest ecosystems. *Agric. For. Meteorol.* **152,** 159–177 (2012).

7. Hanson, P. J. *et al.* Attaining whole-ecosystem warming using air and deep-soil heating methods with an elevated CO_2 atmosphere. *Biogeosciences* **14,** 861–883 (2017).

8. Zohner, C. M., Benito, B. M., Svenning, J.-C. & Renner, S. S. Day length unlikely to constrain climate-driven shifts in leaf-out times of northern woody plants. *Nat. Clim. Chang.* **6,** 1120–1123 (2016).

9. Augspurger, C. K. Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. *Ecology* **94,** 41–50 (2013).

10. Gu, L. *et al.* The 2007 Eastern US spring freeze: Increased cold damage in a warming world. *Bioscience* **58,** 253 (2008).

11. Bonan, G. B. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* **320,** 1444–1449 (2008).

12. Richardson, A. D. *et al.* Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agric. For. Meteorol.* **169,** 156–173 (2013).

13. Gill, A. L. *et al.* Changes in autumn senescence in northern hemisphere deciduous trees: A meta-analysis of autumn phenology studies. *Ann. Bot.* **116,** 875–888 (2015).

14. Laube, J. *et al.* Chilling outweighs photoperiod in preventing precocious spring development. *Glob. Chang. Biol.* **20,** 170–182 (2014).

15. Basler, D. & Körner, C. Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agric. For. Meteorol.* **165,** 73–81 (2012).

16. Migliavacca, M. *et al.* On the uncertainty of phenological responses to climate change, and implications for a terrestrial biosphere model. *Biogeosciences* **9,** 2063–2083 (2012).

17. Slaney, M., Wallin, G., Medhurst, J. & Linder, S. Impact of elevated carbon dioxide concentration and temperature on bud burst and shoot growth of boreal Norway spruce. *Tree Physiol.* **27,** 301–312 (2007).

18. Gunderson, C. A. *et al.* Forest phenology and a warmer climate - growing season extension in relation to climatic provenance. *Glob. Chang. Biol.* **18,** 2008–2025 (2012).

19. Stinziano, J. R., Hüner, N. P. A. & Way, D. A. Warming delays autumn declines in photosynthetic capacity in a boreal conifer, Norway spruce (*Picea abies*). *Tree Physiol.* **35,** 1303–1313 (2015).

20. Vitasse, Y., Basler, D. & Way, D. Is the use of cuttings a good proxy to explore phenological responses of temperate forests in warming and photoperiod experiments? *Tree Physiol.* **34,** 174–183 (2014).

21. Morin, X., Roy, J., Sonié, L. & Chuine, I. Changes in leaf phenology of three European oak species in response to experimental climate change. *New Phytol.* **186,** 900–910 (2010).

22. Brekke, L., Thrasher, B., Maurer, E. & Pruitt, T. *Downscaled CMIP3 and CMIP5 Climate Projections*. (U.S. Department of the Interior, Bureau of Reclamation, Technical Services Center, 2013).

23. Peichl, M., Sonnentag, O. & Nilsson, M. B. Bringing color into the picture: Using digital repeat photography to investigate phenology controls of the carbon dioxide exchange in a Boreal mire. *Ecosystems* **18,** 115–131 (2015).

24. Bowling, D. R. *et al.* Limitations to winter and spring photosynthesis of a Rocky Mountain subalpine forest. *Agric. For. Meteorol.* **252,** 241–255 (2018).

25. Richardson, A. D. *et al.* Influence of spring phenology on seasonal and annual carbon balance in two contrasting New England forests. *Tree Physiol.* **29,** 321–331 (2009).

26. Wilson, R. M. *et al.* Stability of peatland carbon to rising temperatures. *Nat. Commun.* **7,** 13723 (2016).

27. Singh, R. K., Svystun, T., AlDahmash, B., Jönsson, A. M. & Bhalerao, R. P. Photoperiod- and temperature-mediated control of phenology in trees - a molecular perspective. *New Phytol.* **213,** 511–524 (2017).

28. Zohner, C. M. & Renner, S. S. Perception of photoperiod in individual buds of mature trees regulates leaf-out. *New Phytol.* **208,** 1023–1030 (2015).

29. Hufkens, K. *et al.* Ecological impacts of a widespread frost event following early spring leaf-out. *Glob. Chang. Biol.* **18,** 2365–2377 (2012).

Acknowledgements This material is based upon work supported by the U.S. Department of Energy (DOE), Office of Science, Office of Biological and Environmental Research. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for DOE under contract DE-AC05-00OR22725. Support for PhenoCam has come from the National Science Foundation (EF-1065029, EF-1702697). David Hollinger, Mariah Carbone and Colleen Iverson provided feedback on a draft manuscript. Eric Ward assisted with litter collection. For CMIP, we acknowledge the World Climate Research Programme's Working Group on Coupled Modelling. We thank the climate modeling groups (listed in Supplementary Analysis 3) for making their

model output available. DOE's Program for Climate Model Diagnosis and Intercomparison additionally provides coordinating support and led development of software infrastructure for CMIP in partnership with the Global Organization for Earth System Science Portals.

Author Contributions A.D.R. designed the study with input from P.J.H. A.D.R., K.H., D.M.A., T.M., M.E.F., B.S., and M.B.K. contributed PhenoCam imagery and derived data. J.L., W.R.N., J..M.W., and R.R.H. contributed phenological observations. J.M.W. contributed data on frost damage. M.B.K., W.R.N., and P.J.H. maintained site infrastructure including warming treatments and meteorological observations. A.D.R. assembled data sets and conducted the analysis. A.D.R. drafted the manuscript. All authors commented on and approved the final manuscript.

Competing interests The authors declare no competing interests.

Additional Information

Extended data and Supplementary information are available in the online version of the paper. **Reprints and permissions information** is available online at www.nature.com/reprints. **Correspondence and requests for materials** should be addressed to A.D.R.

Notice This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world wide license to publish or

Figure 1. Testing competing hypotheses for phenological responses to warming using data from a whole-ecosystem warming experiment. a–**c**, Conceptual model of relationship between temperature and vegetation phenology, illustrating three competing hypotheses: **a**, temperature is the dominant control (H1); **b**, photoperiod is the dominant control (H2); **c**, photoperiod limits the temperature response above the temperature threshold *t** (H3). **d**–**e**, Sample digital camera imagery showing the inside of plot 19 (unheated control enclosure), **d**, and plot 17 (+9.0 °C warming treatment enclosure), **e**, on 6 April 2016. At the time the photographs were taken, the air temperature was 5 °C in plot 19 (note the last snow of the season), compared to 14 °C in plot 17.

Figure 2. Impact of whole-ecosystem warming treatments on dates of autumn green-down and spring-green up, as derived from digital camera imagery. Response of autumn green-down (**a**–**c**, 2015) and spring green-up (**d**–**f**, 2016) phenology to experimental warming treatments for *Larix laricina*, *Picea mariana*, and a mixed shrub layer community dominated by *Rhododendron groenlandicum* and *Chamaedaphne calyculata*, based on observations across *n* = 10 experimental enclosures (*n* = 9 for *Larix*, as in one enclosure this species was not within the camera field of view) Green-down and green-up are proxies for autumn senescence and spring onset, respectively. Error bars indicate 95% confidence interval around estimated phenological transition dates. Additional results are presented in Supplementary Analysis 2, and Extended Data Table 2.

Table 1. Impact of SPRUCE warming treatments on spring and autumn phenological events (phenophases) for a variety of woody and herbaceous species. Statistics (mean ± 1 standard deviation) are calculated across all observed species. Sample size (*N*) indicates the number of species observed. "T effect" is the temperature sensitivity, in days change per 1 °C warming, as estimated from the linear temperature model based on regression of transition date (*y*) on warming treatment (*x*). "Breakpoints found" indicates the number of species for which a *t** threshold was identified using the breakpoint temperature model (response is linear up to a temperature threshold *t**, and flat thereafter; see Methods). Species-level results are reported in Supplementary Analysis 5, and Extended Data Tables 4 and 5.

	2016				2017			
Phenophase	*N* (species)	T effect (d °C^{-1} ± 1 SD)	Breakpoints found	*t** (± 1 SD)	*N* (species)	T effect (d °C^{-1} ± 1 SD)	Breakpoints found	*t** (± 1 SD)
Leaves growing	5	-3.19 ± 0.69	3	6.34 ± 1.08	7	-3.19 ± 1.25	5	5.46 ± 1.36
Shoots elongating	4	-3.53 ± 0.92	2	6.14 ± 1.59	5	-3.69 ± 1.04	4	7.12 ± 1.66
Flowering (cones open)	6	-2.51 ± 0.89	5	6.09 ± 1.89	7	-2.91 ± 1.33	5	6.74 ± 2.34
Flowers terminated					6	-1.45 ± 1.17	1	4.65 ± N/A
Fruiting	1	-2.56 ± N/A			6	-2.09 ± 1.38	3	6.06 ± 1.52
Fall buds					3	-0.59 ± 1.03		
Fall coloration (senescence)					6	2.70 ± 1.45	2	4.73 ± 2.88

Methods

Study site and experimental design

The SPRUCE (Spruce and Peatland Responses Under Changing Environments) experiment is located within the S1 peat bog at the Marcell Experimental Forest (47° 30.171’ N, 93° 28.970’ W)[30], approximately 40 km north of Grand Rapids in north-central Minnesota, USA. The historic climate at the site is subhumid continental: mean annual temperature is 4°C, mean annual precipitation is 750 mm, and extreme temperatures range from –38 °C to +30 °C. Because this ecosystem is located at the southern edge of the Boreal zone, it is considered particularly vulnerable to climate change.

The S1 bog is an ombotrophic peatland with a perched water table. Trees are approximately 5-8 m in height. Canopy vegetation is dominated by the tree species *Picea mariana* (Mill.) B.S.P. (black spruce), with additional contributions from *Larix laricina* (Du Roi) K. Koch (eastern tamarack or larch). *P. mariana* and *L. laricina* both have a vast geographic range across North America, from Alaska east to Quebec and Labrador, and south to the Great Lakes and New England. A number of closely related *Picea* and *Larix* species are distributed across the Boreal zone of northern Europe, Scandinavia and much of Russia and Siberia, indicating the relevance of results of this experiment to our understanding of Boreal ecosystem processes globally.

The SPRUCE understory is dominated by the evergreen shrubs *Rhododendron groenlandicum* (Oeder) Kron & Judd (Labrador tea) and *Chamaedaphne calyculata* (L.) Moench. (leatherleaf), and is underlain by a bryophyte layer dominated by *Sphagnum* spp. moss. Other common plant species include the evergreen shrub *Kalmia polifolia* Wangenh. (bog laurel), the deciduous shrub *Vaccinium angustifolium* Aiton 1789 not Benth. 1840 (lowbush blueberry), the sedge *Eriophorum* spp. (cottongrass), and the perennial herb *Maianthemum trifolium* (L.) Sloboda (false Solomon's seal).

At SPRUCE, experimental temperature (+0 °C – "unheated control" – to +9.0 °C, in 2.25°C increments for both air and deep soil) and CO_2 (ambient and elevated, approximately 400 and 900 ppm, respectively) treatments are being applied through the use of large (approximately 12 m wide, 8 m high) open-topped octagonal enclosures[7]. Overall, five temperature treatments are paired with two CO_2 treatments, yielding a total of ten enclosures (additionally, there are two "ambient environment" plots without constructed enclosures). Each enclosure is hydrologically isolated from the rest of the bog by a sheet pile corral which has been driven 3-4 m through the peat into the underlying ancient lake sediments. Outflow pipes allow for lateral drainage from each enclosure. Within each enclosure, warming of the deep soil began in June 2014, while aboveground warming was initiated in August 2015 and at this time the phenological observations were commenced in each individual plot (note that pre-treatment observations were made in a common area, outside of the enclosures, beginning in 2010). CO_2 treatments were switched on in June 2016.

For context, the warmest enclosures (+9.0 °C) simulate current climate conditions of Wichita, Kansas (mean annual temperature 13 °C, mean annual precipitation 850 mm), located approximately 1100 km (10 ° of latitude) to the south. The SPRUCE experiment, with treatments that will exceed the historic range of climatic variability (Extended Data Figure 1), is intentionally planned to push the system past projected warming levels to approach or include tipping points for any number of ecosystem response variables. The regression-based experimental design facilitates the estimation of temperature response functions, which may be nonlinear[7].

The enclosure design, and detailed performance metrics for the above- and below-ground warming, along with a discussion of potential artifacts, are more fully described and assessed in a prior publication[7]. Observed temperature differentials were consistent with the nominal warming treatments for target enclosures. Warming was homogeneous within individual enclosures, and was sustained over time (see Supplementary Analysis 1, Extended Data Table 1).

Phenological observations

We are using two methods to track the phenological responses of vegetation to warming and elevated CO_2 in each enclosure. First, beginning in August 2015, we installed digital cameras[31], or phenocams[32], in each enclosure in order to track seasonal variation in vegetation "greenness", a proxy for vegetation phenology and associated physiological activity[6,33–35]. Second, beginning in April 2016, human observers have been directly tracking phenological events of both woody and herbaceous species.

PhenoCam imagery—Digital cameras (NetCam model SD130BN, StarDot Technologies, Buena Park, CA) were configured and installed following standard protocols of the PhenoCam network[36]. Cameras record sequential visible-light (red, green, blue; RGB) and visible+infrared images[37] every 30 minutes from 4 am to 10 pm, every day of the year. Minimally-compressed JPEG images, accompanied by a metadata file containing the current status of all camera settings and diagnostics, are uploaded via FTP (file transfer protocol) to the PhenoCam server for archiving and processing; a local copy is also maintained on a server running at SPRUCE. The filename of every image identifies the enclosure in which the picture was recorded, as well as a date and time stamp in local standard time.

The aluminum structural members of each enclosure provided convenient and consistent mounting points for the cameras. All cameras were mounted, at a height of 6 m, in the middle of the third horizontal structural member on the south wall of each enclosure. Cameras were enclosed in lightweight, compact weatherproof enclosures (model ENC-OUTD3, StarDot Technologies, Buena Park, CA). Network connectivity and DC power were delivered to each camera using a single Ethernet cable and standard power-over-Ethernet (POE) technology. To reduce the likelihood of lightning damage, an Ethernet surge protector (ProtectNet model PNET1GB, APC by Schneider Electric, West Kingston, RI) was installed on the camera end of each Ethernet cable, and grounded to the mounting point.

All imagery is posted in near-real time to the PhenoCam project web page (http://phenocam.sr.unh.edu/), where it is publicly available. Images are processed nightly, using standard PhenoCam routines[6,36]. Briefly, this consists of several steps. First, we defined three separate regions of interest (ROIs) for each camera field of view, demarcating (1) *Picea* trees; (2) *Larix* trees; and (3) the mixed shrub layer. The ROI definitions are converted to binary masks, so that image analysis can be completed separately for each vegetation type. Next, images were read in sequentially, and for each vegetation type the mean pixel value for each of the three color channels (red, green and blue; for the purposes of the present analysis we used only the visible-wavelength imagery) was calculated across the corresponding ROI, yielding a digital number (DN) triplet (R_{DN}, G_{DN}, B_{DN}). Then for each ROI in each image, we calculated the green chromatic coordinate, G_{CC}, which has been shown in numerous studies to be a reliable metric for characterizing the seasonal trajectory of vegetation color and activity[6,31,38]:

$$G_{\mathrm{CC}} = \frac{G_{\mathrm{DN}}}{R_{\mathrm{DN}}+G_{\mathrm{DN}}+B_{\mathrm{DN}}}$$

Basic quality control included eliminating images that were recorded when the sun was less than 5° above the horizon, images that were too dark, or images that were too bright. Additionally, because snow might obscure the vegetation of interest, for each day from late August 2015 through the end of December 2017, we visually inspected the mid-day image from each camera. We flagged images in which there was (1) snow on the ground; or (2) snow on trees. We excluded from further processing all days on which the camera's view of the vegetation of interest was potentially snow-contaminated. For the shrub layer, this meant eliminating images from days with snow on the ground; for *Picea* and *Larix*, this meant eliminating images from days with snow on trees. The frequency of snow decreased with increasing plot temperature, from over 100 days per year with snow on the ground in the unheated enclosures (from late October to early May), to less than 30 days per year in the +9.0 °C enclosures (from late November to early February). The longest period of continuous snow cover was almost three months in the unheated enclosures, compared with only 2 weeks in the +9.0 °C enclosures.

Next, we determined 3-day G_{CC} values using the 90th quantile method[6]. We then used a spline-based method to sequentially remove outliers in three iterative steps. Finally, we re-fit the spline, and used the summertime maxima and dormant-season minima to define the seasonal G_{CC} amplitude, from which we were then able to identify dates at which 10%, 25% and 50% of the seasonal amplitude were reached in autumn (senescent or "green-down" phase) and spring (onset or "green-up" phase). Uncertainties on these dates were then derived based on the uncertainty around the smoothing spline. Our analysis here focuses on the 25% amplitude threshold dates.

Ground observations—Ground observations of spring phenology were made at approximately weekly intervals by WRN and JL in 2016, and by RRH in 2017. The protocol used by WRN and RRH involved recording, on a pre-printed form for each of the 10 enclosures and the two ambient environment plots, whether or not (“Yes” or “No”) specific vegetative and reproductive phenophases were observed each week. Observations were conducted on a selection of woody species (the trees *Picea* and *Larix*; the evergreen shrubs leatherleaf, bog laurel, Labrador tea, and lowbush blueberry), as well as a sedge (cottongrass), and a perennial herb (false Solomon’s seal). We transcribed the data by taking as the observed date the first survey date on which an event was definitively observed (i.e., “No” through week 4, followed by “Yes” in week 5: the event occurred in week 5). Not all phenophases were observed for all species, and in some difficult-to-observe cases, the data were deemed not reliable because of some inconsistencies in the recorded data (e.g. blank cells rather than “No”, or “No” followed by “Yes” followed by “No” again) or poor representation of the species in question in some of the plots (e.g., bog laurel and lowbush blueberry are sparsely distributed). All transcribed data of questionable reliability were excluded from the analysis.

JL’s protocol involved recording the first date at which *Larix* leaf buds were observed to be just beginning to break (data recorded for all 10 enclosures, plus the two ambient environment plots), and the first date at which flowers of leatherleaf, bog laurel, and Labrador tea were observed in each enclosure (data recorded in only half of the treated enclosures, plus one or both of the ambient environment plots). Although data recorded by JL are not as complete as those recorded by WRN, they are included to demonstrate the robustness of the observed patterns.

On-site meteorological data

Air temperature and relative humidity were measured (model HMP-155, Vaisala, Vantaa, Finland) at four points above the peat surface within each enclosure (0.5, 1, 2 and 4 m), and 30-

minute mean values recorded. We used the measured air temperature at 2 m in our analyses. SPRUCE environmental data[39] are available through the Vista Data Vision portal (http://sprucedata.ornl.gov/vdv).

Historical perspective and future climate projections

To put the weather during winter and spring of 2016 in historical context (122 year record), we used data from NOAA's National Climatic Data Center (NCDC). Specifically, we used summary data from the State of the Climate report (https://www.ncdc.noaa.gov/sotc/national/), and 3-month divisional temperature rankings (https://www.ncdc.noaa.gov/temp-and-precip/climatological-rankings/). The SPRUCE site falls within Minnesota's Climate Division 2.

To place our results in the context of projected warming trends over the coming century, we used downscaled (1/8°) climate projections from a selection of ten models (see Supplementary Analysis 2) contributing to the CMIP5 multimodel ensemble dataset[22,40]. We used output for two Representative Concentration Pathway (RCP) scenarios: RCP 4.5 (CO_2 stabilization) and RCP 8.5 (rising CO_2)[41,42]. To quantify future trends, we calculated the projected decadal mean air temperature change relative to the 2006-2015 mean for each model.

Statistical analysis

To characterize the relationship between air temperature and phenological timing (H1 and H2), we used ordinary linear regression, with the observed phenological date as the dependent variable, y_i, and the measured air temperature differential for each plot (see Supplementary Analysis 1) as the independent variable, x_i. The regression slope, β, thus gives the temperature sensitivity in days per 1 °C warming for the "linear temperature model". We used a significance level of 0.05 to test the null hypothesis that $\beta = 0$. To account for potential effects of elevated CO_2 on phenology, we also analyzed data (where appropriate) using a "linear temperature and CO_2 model", which included temperature, CO_2 (elevated and ambient) and a temperature x CO_2 interaction effect.

For breakpoint analysis (H3), we fit a three-parameter (α, β, t^*) "breakpoint temperature model", which was specified as:

$$y_i = \alpha + \beta x_i + \epsilon_i \quad \text{for } x_i < t^*$$

$$y_i = \alpha + \beta t^* + \epsilon_i \quad \text{for } x_i \geq t^*$$

where x_i and y_i are as for the ordinary linear regression, ϵ_i is the regression residual, and t^* is the temperature breakpoint, as illustrated in Figure 1. We constrained t^* to fall in the range of 2–9 °C. An edge-hitting value of $t^* = 9$°C was obtained when the linear model fit the data every bit as well as the breakpoint model.

We used Akaike's Information Criterion (AIC)[43] to identify whether the linear model or the breakpoint model was best supported by the available data. AIC is typically calculated as:

$$\mathrm{AIC} = n\log(\sigma^2) + 2p$$

where n is the number of observations, p is the number of fit parameters plus one, and σ^2 is the residual sum of squares divided by n. When n is small relative to p, the small-sample corrected criterion, $\mathrm{AIC_C}$, is preferred[43]:

$$\mathrm{AIC_C} = \mathrm{AIC} + \frac{2p(p+1)}{n-p-1}$$

AIC effectively balances improving explanatory power (lower σ^2) against increasing complexity (larger p), and thus AIC selects against over-parameterized models. The model with the lowest AIC is considered the best model given the data, and the absolute difference in AIC_C scores between two models can be used to evaluate the weight of evidence in support of the better model. If the difference (ΔAIC) is small or zero then the two models are equally good. But, if $\Delta AIC \approx 2.0$, then the model with the lower AIC_C is almost three times more likely to be best[43].

Data Availability

PhenoCam imagery is publicly available through the project web page (http://phenocam.sr.unh.edu), and the phenological data sets used in this study are available through the ORNL DAAC[44,45].

References

30. Kolka, R., Sebestyen, S., Verry, E. S. & Brooks, K. *Peatland Biogeochemistry and Watershed Hydrology at the Marcell Experimental Forest*. (CRC Press, 2011).

31. Richardson, A. D. *et al.* Use of digital webcam images to track spring green-up in a deciduous broadleaf forest. *Oecologia* **152,** 323–334 (2007).

32. Brown, T. B. *et al.* Using phenocams to monitor our changing Earth: toward a global phenocam network. *Front. Ecol. Environ.* **14,** 84–93 (2016).

33. Keenan, T. F. *et al.* Tracking forest phenology and seasonal physiology using digital repeat photography: a critical assessment. *Ecol. Appl.* **24,** 1478–1489 (2014).

34. Richardson, A. D., Klosterman, S. & Toomey, M. Near-surface sensor-derived phenology. in *Phenology: An Integrative environmental science* (ed. Schwartz, M. D.) 413–430 (Springer Netherlands, 2013).

35. Toomey, M. *et al.* Greenness indices from digital cameras predict the timing and seasonal dynamics of canopy-scale photosynthesis. *Ecol. Appl.* **25,** 99–115 (2015).

36. Richardson, A. D. *et al.* Tracking vegetation phenology across diverse North American biomes using PhenoCam imagery. *Sci. Data* **5,** 180028 (2018).

37. Petach, A. R., Toomey, M., Aubrecht, D. M. & Richardson, A. D. Monitoring vegetation phenology using an infrared-enabled security camera. *Agric. For. Meteorol.* **195–196,** 143–151 (2014).

38. Richardson, A. D., Braswell, B. H., Hollinger, D. Y., Jenkins, J. P. & Ollinger, S. V. Near-surface remote sensing of spatial and temporal variation in canopy phenology. *Ecol. Appl.* **19,** 1417–1428 (2009).

39. Hanson, P., Riggs, J., Nettles, W., Krassovski, M. & Hook, L. *SPRUCE Whole Ecosystems Warming (WEW) Environmental Data Beginning August 2015*. (2016). http://dx.doi.org/10.3334/CDIAC/spruce.032

40. Maurer, E. P., Brekke, L., Pruitt, T. & Duffy, P. B. Fine-resolution climate projections enhance regional climate change impact studies. *Eos, Trans. Am. Geophys. Union* **88,** 504–504 (2007).

41. Riahi, K. *et al.* RCP 8.5—A scenario of comparatively high greenhouse gas emissions. *Clim. Change* **109,** 33–57 (2011).

42. Thomson, A. M. *et al.* RCP4.5: a pathway for stabilization of radiative forcing by 2100. *Clim. Change* **109,** 77–94 (2011).

43. Burnham, K. P. & Anderson, D. R. *Model Selection and Multimodel Inference: a Practical Information-theoretic Approach*. (Springer, 2002).

44. Richardson, A. D., Latimer, J. M., Nettles, W. R., Heiderman, R.R., Warren, J. M. & Hanson, P. J. SPRUCE Ground Observations of Phenology in Experimental Plots, 2016-2017. (2018). http://dx.doi.org/10.3334/CDIAC/spruce.044

45. Richardson, A. D. *et al.* SPRUCE Vegetation Phenology in Experimental Plots from Phenocam Imagery, 2015-2017. (2018). http://dx.doi.org/10.3334/CDIAC/spruce.045

Extended Data Table 1 | Mean daily air temperature, and temperature differentials, over the duration of the whole-ecosystem warming treatments (August 2015–December 2017).

Daily means are calculated based on the mean half-hour data for two temperature sensors mounted at 2 m height. Temperature differentials (ΔT) are calculated relative to the mean of the two unheated enclosures (Plots 19 and 06). Plots are arranged in order of increasing ΔT; overall mean ± 1 SD ΔT is calculated across *n* = 5 multi-month means.

Extended Data Table 2 | Impact of SPRUCE warming treatments on spring green-up and autumn green-down, as derived from PhenoCam imagery

Results are shown from the start of the whole-ecosystem warming experiment (autumn 2015), based on observations across *n* = 10 experimental enclosures (*n* = 9 for *Larix*, as in one enclosure this species was not within the camera field of view). Mean transition dates are reported ± 1 SD. Statistics for the "linear temperature model" are based on regression of transition date (*y*) on warming treatment (*x*), and the model slope is the phenological temperature sensitivity in days per 1°C warming. "T effect" column reports *P*-value for null hypothesis of no temperature effect. Statistics for the "breakpoint temperature model" are based on a model in which the response to warming treatment is assumed linear up to a temperature threshold *t**, and flat thereafter (see Methods for additional details). No statistics are reported for cases in which a *t** could not be identified or where the addition of *t** did not improve model fit. ΔAICc is the difference in Akaike's Information Criterion (corrected for small sample sizes) between the linear temperature model and breakpoint temperature model, with a positive value indicating that the linear temperature model is better supported by the data, and a negative value indicating that the beakpoint temperature model is better supported by the data. RMSE is root mean squared error. SE is standard error. Results not shown for the "linear temperature and CO_2 model" as the CO_2 effect and CO_2 x T interaction effect were generally not significant (see Supplementary Analysis 2 for additional information).

Extended Data Table 3 | Projected future extension of the period of vegetation activity, based on linear extrapolation of experimental results using CMIP5 climate projections.

Temperature sensitivities are derived from Figure 2; total projected active season extension is the product of the temperature sensitivity of total active season length multiplied by the mean projected temperature increase (decadal means, relative to 2006-2015). Uncertainties in active season extension represent the uncertainty in the climate projections (SD across ten models), not the uncertainty in the temperature sensitivities.

Extended Data Table 4 | Impact of SPRUCE warming treatments on visually observed vegetative and reproductive phenological transitions in 2016.

Data are from 2016 growing season, based on observations across *n* = 12 plots. Species are ordered by functional type, and within each species, phenophases are ordered according to the mean (± 1 SD) day of year (DOY) on which the event occurred. Statistics for the "linear temperature model" are based on regression of transition date (*y*) on warming treatment (*x*), and the model slope is the phenological temperature sensitivity in days per 1°C warming. "T effect" column reports *P*-value for null hypothesis of no temperature effect. Statistics for the "breakpoint temperature model" are based on a model in which the response to warming treatment is assumed linear up to a temperature threshold *t**, and flat thereafter (see Methods for additional details). No statistics are reported for cases in which a *t** could not be identified, or where the addition of *t** did not improve model fit. ΔAICc is the difference in Akaike's Information Criterion (corrected for small sample sizes) between the linear temperature model and breakpoint temperature model, with a positive value indicating that the linear temperature model is better supported by the data, and a negative value indicating that the breakpoint temperature model is better supported by the data. RMSE is root mean squared error. SE is standard error.

Extended Data Table 5 | Impact of SPRUCE warming treatments on visually observed vegetative and reproductive phenological transitions in 2017.

Data are from 2017 growing season, based on observations across *n* = 12 plots. Species are ordered alphabetically, and within each species, phenophases are ordered according to the mean (± 1 SD) day of year (DOY) on which the event occurred. Statistics for the "linear temperature model" are based on regression of transition date (*y*) on warming treatment (*x*), and the model slope is the phenological temperature sensitivity in days per 1°C warming. "T effect" column reports *P*-value for null hypothesis of no temperature effect. Statistics for the "breakpoint temperature model" are based on a model in which the response to warming treatment is assumed linear up to a temperature threshold *t**, and flat thereafter (see Methods for additional details). No statistics are reported for cases in which a *t** could not be identified, or where the addition of *t** did not improve model fit. ΔAICc is the difference in Akaike's Information Criterion (corrected for small sample sizes) between the linear temperature model and breakpoint temperature model, with a positive value indicating that the linear temperature model is better supported by the data, and a negative value indicating that the breakpoint temperature model is better supported by the data. RMSE is root mean squared error. SE is standard error. Results not shown for the "linear temperature and CO_2 model" as the CO_2 effect and CO_2 x T interaction effect were generally not significant (see Supplementary Analysis 5 for additional information).

Extended Data Table 6 | *Larix laricina* and *Picea mariana* litter nutrient content following premature foliar senescence in early May 2016, and natural senescence in late October 2016.

Following the April 9th 2016 spring frost event, damaged foliage from trees that had lost frost hardiness began a period of senescence, culminating in heavy leaf fall during early May as air temperatures frequently exceeded 30 °C in the +9.0 °C plots (temperatures over 40 °C were observed in plot 10 and plot 17 on May 5 and 6). Prematurely senescent litter was collected May 6 from the ground underneath damaged trees in the two warmest treatments (+6.75 and +9.0 °C) (n = 3 – 7 trees). Normally senescent litter was collected November 4 from ambient environment plots outside of the experimental treatments using litter baskets (n = 8 trees). Litter was analyzed for carbon and nitrogen by combustion using 0.1 g samples of oven-dried and finely ground tissue on a LECO TruSpec elemental analyzer (LECO Corporation, St. Joseph, MI). Data are presented on a percent dry matter basis.

Extended Data Figure 1 | Air temperature and precipitation in the SPRUCE S1 Bog (August 2015-December 2017) relative to long-term (1960-2016) means and variability. a, Long-term daily mean temperature (°C, ±1 SD indicated by shading), compared with daily mean temperature (calculated from 30-minute means, based $n = 2$ sensors mounted at 2 m height in each enclosure) in a +0 °C enclosure (unheated control) and a +9.0 °C enclosure. **b**, Long-term monthly mean temperature (mean daily maximum and mean daily minimum indicated by shaded bars), compared with monthly mean temperature (calculated from daily means, as in **a**) in different experimental treatments. **c**, Long-term monthly mean precipitation (mm, ±1 SD indicated by shading, with maxima and minima indicated by dotted lines), compared with measured monthly precipitation ($n = 1$ rain gage) in the S1 Bog.

Extended Data Figure 2 | Decadal mean temperature change (relative to 2006-2015 mean) projections from ten CMIP5 earth system models for the SPRUCE site. a, "Stabilization" climate scenario (RCP 4.5). **b**, "High emission" climate scenario (RCP 8.5).

Extended Data Figure 3 | Relationships between air temperature and the start and end of the photosynthetic uptake period, as derived from FLUXNET data for evergreen conifer-dominated sites. Across-site patterns in **a**, spring, and **b**, autumn, in relation to mean annual temperature ($n = 12$ sites); within-sites patterns in **c**, spring, and **d,** autumn, in relation to seasonal temperature anomalies ($n = 86$ site-years).

Extended Data Figure 4 | Unusually warm weather in late winter, followed by extreme cold in early April, resulted in severe frost damage in the warmest enclosures at SPRUCE in 2016. a, Time series of daily mean air temperature, comparing plot 17 (+9.0 °C warming) and plot 19 (unheated enclosure), during winter and spring 2016. By the time the frost event occurred (grey shading), daily mean temperature in plot 17 had been above freezing for over a month, but had repeatedly dropped below freezing in plot 19. **b**, Time series of 30-minute air temperature, again comparing plot 17 and plot 19, leading up to and immediately following the frost event which occurred on the morning of April 9 and again on April 12. The thin red lines indicate the variability (maximum and minimum) across $n = 5$ temperature sensors in plot 17. **c,** Time series of daily G_{CC}, the green chromatic coordinate, for *Picea* trees in plot 17 and plot 19. Arrows denote spring green-up dates (progressively larger arrows corresponding to 10%, 25% and 50% of seasonal amplitude) estimated from G_{CC}. The pronounced decline in G_{CC} in plot 17 following the frost event (grey shading) is readily apparent. Trees in plot 19 retained sufficient frost hardiness that they were undamaged, despite experiencing much colder temperatures. **d**, Brown, frost-damaged *Larix* foliage in plot 17. **e**, *Picea* branches in plot 17, showing loss of most foliage from previous years, with green foliage from the 2015 flush retained only at branch tips. **f**, *Picea* branches with frost-damaged foliage from previous years, but healthy green foliage from the 2016 flush.