Diverse responses of phenology to global changes in a grassland ecosystem

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Shifting plant phenology (i.e., timing of flowering and other developmental events) in recent decades establishes that species and ecosystems are already responding to global environmental change. Earlier flowering and an extended period of active plant growth across much of the northern hemisphere have been interpreted as responses to warming. However, several kinds of environmental change have the potential to influence the phenology of flowering and primary production. Here, we report shifts in phenology of flowering and canopy greenness (Normalized Difference Vegetation Index) in response to four experimentally simulated global changes: warming, elevated CO_2, nitrogen (N) deposition, and increased precipitation. Consistent with previous observations, warming accelerated both flowering and greening of the canopy, but phenological responses to the other global change treatments were diverse. Elevated CO_2 and N addition delayed flowering in grasses, but slightly accelerated flowering in forbs. The opposing responses of these two important functional groups decreased their phenological complementarity and potentially increased competition for limiting soil resources. At the ecosystem level, timing of canopy greenness mirrored the flowering phenology of the grasses, which dominate primary production in this system. Elevated CO_2 delayed greening, whereas N addition dampened the acceleration of greening caused by warming. Increased precipitation had no consistent impacts on phenology. This diversity of phenological changes, between plant functional groups and in response to multiple environmental changes, helps explain the diversity in large-scale observations and indicates that changing temperature is only one of several factors reshaping the seasonality of ecosystem processes.

Shifting plant phenology over the last several decades provides compelling evidence that natural ecosystems are already responding to human-caused environmental changes (1–8). Earlier flowering (3–5) and an earlier peak in primary productivity in satellite data (6, 7) in the northern hemisphere in recent decades are correlated with rising temperatures (8). Experimental warming leads to earlier flowering (9, 10), but data on the phenological impact of other cooccurring global changes are limited. Additionally, previous experimental studies have not linked species-level observations of flowering date with remote-sensing-based measures of the phenology of ecosystem primary productivity. As part of the Jasper Ridge Global Change Experiment (JRGCE), we monitored the timing of both flowering and primary productivity (estimated by canopy greenness) in an annual grassland ecosystem in response to four interacting global changes: warming, elevated CO_2, nitrogen (N) deposition, and increased precipitation.

The JRGCE is located on sandstone-derived soils within the Jasper Ridge Biological Preserve, on the campus of Stanford University, in coastal central California. The plant community is dominated by naturalized Eurasian annual grasses but also contains native and nonnative perennial grasses, forbs (herbaceous non-grass plants), and legumes (11). We observed experimental plots every 2–3 days from early February until mid-June of each year (2000–2002) and recorded the flowering date of the first flowering individual of five (2000) or nine (2001 and 2002) naturalized species. We monitored plant canopy development by using a standard index of canopy greenness, the Normalized Difference Vegetation Index (NDVI), frequently used for satellite studies (e.g., 6).

Results

Warming accelerated the onset of flowering of all annual species by 2–5 days ($P = 0.006$, Fig. 1), consistent with results of past experiments (9, 10). Only *Crepis vesicaria*, a biennial or short-lived perennial forb, was unresponsive to warming. Cumulative annual temperature sums predict flowering in many species (12), but perennial species with high storage allocation may require multiple growing seasons to respond to altered environmental conditions (13). The effects of elevated CO_2 differed between grasses and forbs (significant $CO_2 \times$ functional group interaction, $P < 0.0001$). The onset of flowering was accelerated in forbs (2–4 days) but delayed in grasses (2–6 days), with flowering in the most common grass species (*Avena spp*) delayed by up to 9 days (data for each year and species are presented in Fig. 4, which is published as supporting information on the PNAS web site). Experimental N deposition also caused accelerated flowering in forbs and delayed flowering in grasses (N $\times$ functional group interaction, $P = 0.0015$). As might be expected, *Vicia sativa* (N-fixing annual forb) was unresponsive to N addition.

Under ambient conditions, grasses flowered significantly earlier than forbs ($P < 0.0001$, Fig. 2*A*). Elevated CO_2 and N deposition reduced the difference in flowering date between these two groups, increasing temporal overlap, thus decreasing phenological complementarity (Fig. 2 *B* and *C*).

The combination of elevated CO_2 and warming produced additive responses (i.e., there were no higher order statistical interactions); as a consequence, the first flowering date for grasses in treatments with both elevated CO_2 and warming was not significantly different from in the controls (Fig. 2*C*). Increased precipitation had no effect on first flowering date for grasses or forbs, a finding in agreement with previous analyses finding either no response (14) or complex species-specific responses (15) to shifts in precipitation.

Changes in the timing of peak NDVI were similar to the changes in flowering phenology of the grasses that dominated primary productivity (Fig. 3). Elevated CO_2 delayed the timing of peak NDVI (+6.5 days, $P = 0.009$), consistent with previous seasonal observations at this site where peak canopy photosynthesis was delayed under an experimental doubling of CO_2 (16).

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Abbreviations: JRGCE, Jasper Ridge Global Change Experiment; NDVI, Normalized Difference Vegetation Index.

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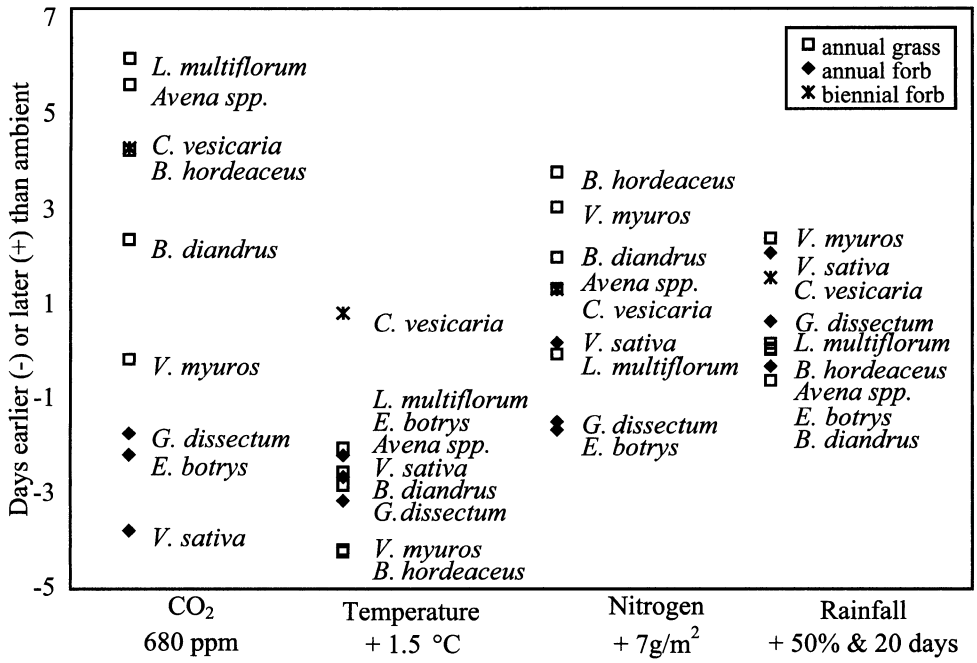

Fig. 1. Shift in date of flowering onset caused by the four simulated global changes, for nine common species observed in the JRGCE. The effect shown for each species is the difference in mean first flowering date for all plots where each aspect of environmental change is elevated ($n = 64$) versus ambient ($n = 64$), over 3 years of observation.

Although warming at ambient N advanced the date of peak NDVI (−9.3 days, $P = 0.005$), the effect was dampened in combination with N deposition, resulting in a significant statistical interaction (warming × N, $P = 0.014$). This interaction may reflect the combined effects of an increase in grass abundance caused by N deposition (17), plus the opposing flowering responses of grasses to warming and N deposition already discussed.

Discussion

Rising temperatures in recent decades are associated with accelerated phenology in many plant species (1–5). However, this pattern is not universal; a number of species have displayed delayed phenology in recent decades (3–5). For instance, the average flowering date of 385 British plant species was, on average, 4 days earlier in the 1990s as compared with the previous 45 years; 92 species, however, flowered later than in the previous decades, although the delay was statistically significant for only 10 species (5). The proportion of species with phenological delays across Europe (3) and around Washington, DC (4), over a comparable time period, is similar to that for Britain. The results of this study indicate that these phenological delays may not be unimportant exceptions. Instead, they may come from sites where environmental cues for delays are stronger than the cues for accelerated phenology, or from plant species that are more sensitive to the environmental cues that lead to phenological delays. In this study, warming caused the expected acceleration of flowering and greening, but elevated CO_2 and N deposition delayed the peak of canopy greenness, reflecting delayed flowering in the grasses, which dominate primary production in this system. These opposing responses to multiple global changes may dampen or eliminate phenological shifts in natural ecosystems. Thus, observations interpreted only as responses to warming may underestimate both the intrinsic sensitivity to temperature and the extent of current forcing of phenology by global environmental changes.

Researchers investigating crop species' responses to elevated CO_2 have found predominantly accelerated development (18). Hence, we were surprised to find that elevated CO_2 consistently delayed flowering for all of the grass species we observed. However, crop species respond more to elevated CO_2 than wild species for a number of other reproductive traits (19). Furthermore, flowering in two crops closely related to wild grasses, rice (20) and sorghum (20, 21), is delayed by elevated CO_2. In contrast to crop research, elevated CO_2 studies focusing on non-crop species reveal few generalizations. Budburst is delayed in poplar (22) and spruce (23), and flowering is accelerated in clover (24). Forbs can be either accelerated or delayed (25–28), but most studies have found no significant effects of elevated CO_2 on phenology (29–34).

In this case, the differences among species or functional groups in their phenological response to the global changes appear to reflect physiological differences in their cues for flowering, including the relative roles of photoperiod and resource availability (35) and the direction of the response to resource availability. When resources were abundant (e.g., elevated CO_2 and/or increased N deposition), the annual grasses that dominated production in the JRGCE delayed the switch in allocation from growth to reproduction that occurred in concert with flowering. Accelerated phenology in the forbs in response to abundant resources suggests that flowering in these species was more tuned to internal than external cues.

In plants, the onset of flowering occurs in concert with shifts in allocation and subsequent declines in nutrient uptake (36), making first-flowering date a simple metric for phenological complementarity, or temporal overlap in resource uptake, among species in a community. Phenological complementarity promotes coexistence in multispecies plant communities (37, 38) and is one of the main mechanisms by which species composition influences ecosystem processes such as nutrient capture and primary production (39). In Mediterranean-type ecosystems, plant growth is concentrated in a short growing season, so even small shifts in phenology can disrupt complementarity among species. At this site, grasses flower significantly earlier than forbs under ambient conditions, but elevated CO_2 and N deposition reduced the difference in flowering date between these two

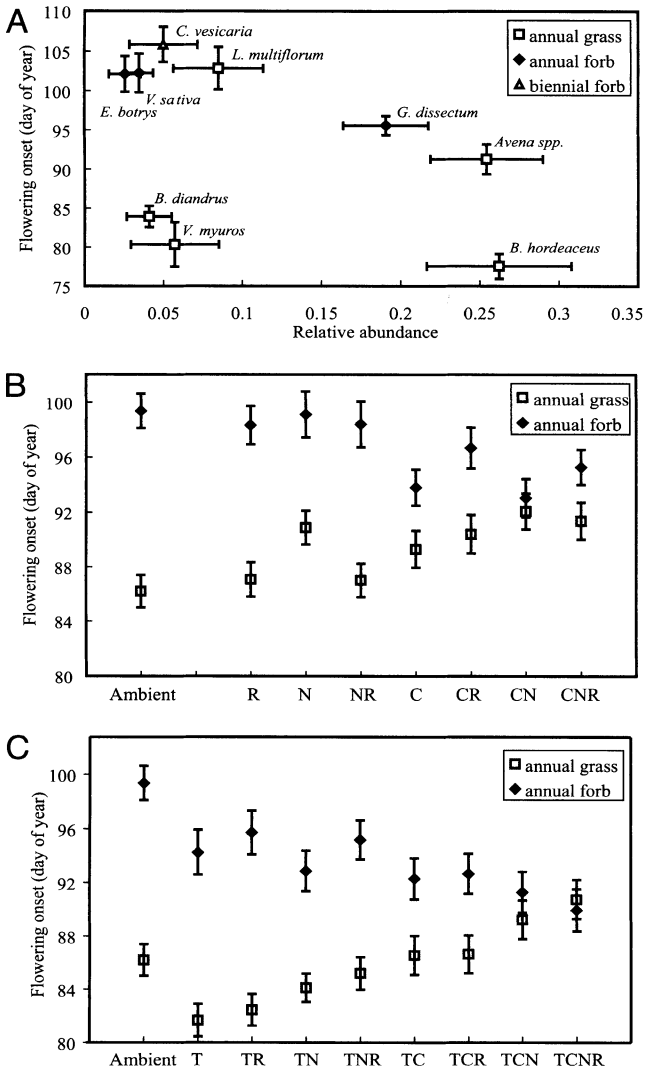

Fig. 2. Flowering onset data. (*A*) Mean day of first flowering in relation to proportional abundance for nine common species under ambient conditions. Mean dates of flowering onset averaged across the grass and forb species for all treatment combinations at ambient temperatures (*B*) and with warming (*C*). Treatment abbreviations are as follows: C, elevated CO_2; N, nitrogen deposition; R, increased rainfall; T, increased temperature. n = 8 plots per treatment, averaged across 3 years of observation. Error bars denote 1 SE of the mean.

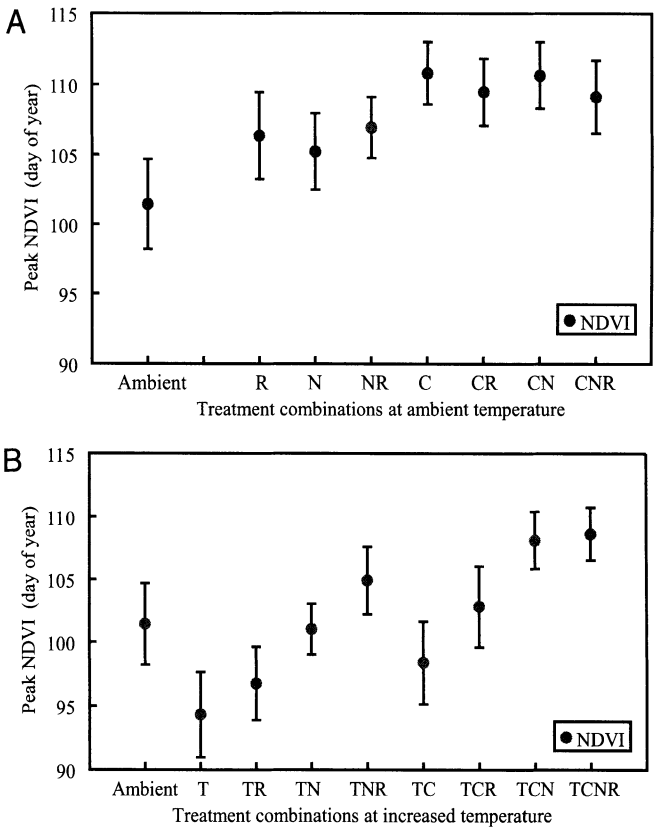

Fig. 3. Mean day of peak canopy greenness (NDVI) for all treatment combinations at ambient temperatures (*A*) and with warming (*B*). Treatment abbreviations are as follows: C, elevated CO_2; N, nitrogen deposition; R, increased rainfall; T, increased temperature. n = 8 plots per treatment, averaged across 4 years of observation. Error bars denote 1 SE of the mean.

groups, increasing temporal overlap, decreasing phenological complementarity, and potentially increasing competition for one or more resources. In the JRGCE, elevated CO_2 suppressed the tendency of other environmental changes to stimulate primary production (40, 41). Decreased phenological complementarity is one mechanism that could lead to such an effect, whereby delayed growth and resource uptake by grasses could overlap into the temporal window of forb activity, thus reducing the contribution of late-active forbs to overall production. Decreased phenological complementarity is also one mechanism that could explain the shifting species composition observed in the JRGCE; grasses have become increasingly dominant under N deposition, and elevated CO_2 has increased the grass/forb ratio through decreased growth and abundance of forbs (17).

Although the onset of flowering indicates an important phase of plant growth, the timing of senescence is likely to be equally important in shaping phenological complementarity among species, as well as the magnitude of primary production in this annual system. In an investigation of the influence of warming on ecosystem water balance in the JRGCE, Zavaleta *et al.* (42) determined that warming caused earlier senescence, as evidenced by a steeper decline in NDVI at the end of the growing season.

Many phenological studies investigate plants or ecosystems at high elevations (10) or high latitudes (3, 5, 9), in areas where warming is likely to advance flowering via earlier snowmelt and hence an earlier onset of the growing season. In this study, the influences of the global change treatments were not convolved with the start of the growing season, because the onset of the growing season depended on the first fall rains and was not altered by the experimental treatments. Additionally, because the growing season in this grassland ecosystem is not truncated by winter frosts, phenology can respond to a wide range of factors, and it can modulate a diverse set of community and ecosystem processes. Furthermore, our findings are likely to generalize to other locations because the dominant species in this study are widespread; for instance, *Avena fatua* (wild oat) is found in all 50 United States and is a major cereal weed worldwide (43). Future increases in atmospheric CO_2 will be relatively uniform across the globe (44), whereas the amount of N deposition (45) and warming (46) will vary by region. As a consequence, phenological responses to future global changes are likely to vary regionally. We would expect accelerated phenology at high latitudes and regions where the greatest amount of warming is predicted, but delayed phenology for species that are sensitive to the increase in elevated CO_2 or N deposition, particularly in regions that warm less.

Shifting phenology of whole ecosystems is evident in satellite studies showing recent trends toward earlier springs and longer growing seasons (6, 7). These trends combine responses of many species and responses to a variety of global changes. In ecosystems exposed to a range of global-change factors, the combined responses may be only part of the picture. Simultaneous responses in opposite directions tend to mask the sensitivity of phenology to warming, whereas opposite responses among groups of species can decrease phenological complementarity. The diversity of phenological responses we observed under controlled experimental conditions can provide a framework for reinterpreting large-scale patterns in the context of interacting plant species and interacting global changes.

Methods

The experimental design of the JRGCE (17, 40, 41) consists of plots arranged in a randomized block, split-plot design, with eight replicates of each treatment. Elevated CO_2 and warming were applied to whole circular plots (each 2-m diameter, 3.14-m^2 area), whereas N deposition and increased precipitation were applied in a factorial arrangement to four 0.78 m^2 quadrants within each plot. Ambient CO_2 concentrations averaged 380 ppm. Elevated CO_2 concentrations of 680 ppm were achieved via free air CO_2 enrichment. Warming of +1.5°C was achieved via overhead infrared heaters. An irrigation system increased precipitation by supplementing +50% to every rainfall event. In addition, we extended the wet season by 20 days with two supplemental irrigation events. N deposition was simulated by surface applications totaling 7 g of N per m^2 per year calcium nitrate. At the beginning of the growing season, 2 g of N per m^2 was added in a wet pulse, and the remaining 5 g of N per m^2 was added in slow-release pellets (Nutricote). Global change treatments were applied to undisturbed grassland plots beginning in November 1998, and are presently ongoing.

We observed experimental plots every 2–3 days from early February until mid-June of each year (2000–2002) and recorded the first date on which we observed a flowering individual of five (2000) or nine (2001 and 2002) naturalized species of European origin. A maximum of 1 min was spent looking for each species in each plot. The focal species included: five annual grasses: (two *Avena* spp., *Avena barbata* or *Avena fatua*, which are not distinguishable at the time of first flowering, *Bromus diandrus*, *Bromus hordeaceus*, *Lolium multiflorum*, *Vulpia myuros*), two annual forbs (*Erodium botrys*, *Geranium dissectum*), one biennial forb (*Crepis vesicaria*), and one annual N-fixing forb (*Vicia sativa*). Observations of *C. vesicaria*, *E. botrys*, *L multiflorum*, and *V. myuros* began in 2001. Due to variation in the abundances of these species, as well as the patchy nature of grasslands, we seldom observed all focal species within a given experimental plot. Thus, we could not analyze the flowering responses at the species-level. Instead, we chose to analyze responses within the *a priori* functional grouping of grasses versus forbs. All of the focal species use the C3 photosynthetic pathway.

To monitor plant canopy development (NDVI), we measured spectral reflectance under cloud-free conditions by using a portable spectroradiometer (Analytical Spectral Devices, Boulder, CO), normalized to a spectrally neutral reference panel (Spectralon, Labsphere, North Sutton, NH). Reflectance measurements were made throughout the growing season at intervals dictated by weather conditions, averaging every 15 days from October to mid-February, and 11 days (standard deviation = 7.05) from late February until early June. Each recorded spectrum was the mean of 10 spectra obtained with a fiber optic collector (25° field of view) held ≈1.2 m directly above each plot and oriented normal to the soil surface. On nearly all days, measurements were started and completed within 1 h of solar noon. NDVI was calculated as (reflectance at 775 nm − reflectance at 675 nm)/(reflectance at 775 nm + reflectance at 675 nm). For each plot, we determined the date of maximum NDVI during each growing season from 2000 to 2003. Across the 4 years, the earliest date of peak NDVI in a plot was in late February and the latest was in late May.

Statistical models were coded in SAS version 9.0 (SAS Institute, Cary NC). The four experimental fixed effects were analyzed by using a factorial split-plot design, using the PROC MIXED method of maximum likelihood estimation. Additional information is included in *Supporting Text* and Tables 1 and 2, which are published as supporting information on the PNAS web site.

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