Science of the Total Environment 745 (2020) 140999

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Enhanced spring temperature sensitivity of carbon emission links to earlier phenology

Fandong Meng [a], Lirong Zhang [a], Zhenhua Zhang [b], Lili Jiang [a], Yanfen Wang [d], Jichuang Duan [e], Qi Wang [a,d], Bowen Li [a,d], Peipei Liu [a,d], Huan Hong [a,d], Wangwang Lv [a,d], Wangmu Renzeng [a,d], Zhezhen Wang [f], Caiyun Luo [b], Tsechoe Dorji [a,c], Huakun Zhou [b], Mingyuan Du [g], Yiqi Luo [h], Shiping Wang [a,c,*]

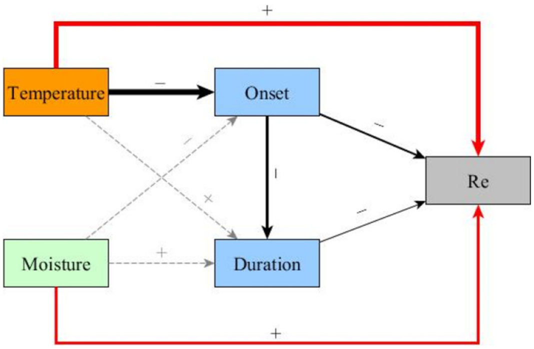
[a] Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China
[b] Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Qinghai Provincial Key Laboratory of Restoration Ecology of Cold Area, Xining 810008, China
[c] CAS Center for Excellence in Tibetan Plateau Earth Science, Chinese Academy of Sciences, Beijing 100101, China
[d] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[e] Binhai Research Institute in Tianjin, Tianjin 300457, China
[f] The University of Chicago Medicine and Biological Sciences Division, 5801 South Ellis Ave., Chicago, IL 60637, USA
[g] Institute for Agro-Environmental Sciences, NARO, Tsukuba 305-8604, Japan
[h] Center for Ecosystem Science and Society (Ecoss), Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA

HIGHLIGHTS

- Variation of onset of phenophases determines the duration of phenophases on the Tibetan Plateau.
- Reproductive stage has a higher temperature sensitivity of ecosystem respiration.
- Advanced early phenophases enhanced spring temperature sensitivity of carbon emission.

GRAPHICAL ABSTRACT

+ and − denote positive and negative relationships between two variables, onset, duration and Re indicate onset of phenology, duration of phenology and ecosystem respiration. Re is significantly affected by abiotic factors (temperature and moisture) and biotic factor (phenology). Effects of phenology on Re are mainly achieved by lengthening plant activity time and increasing growth rate.



ARTICLE INFO

Article history:
Received 18 May 2020
Received in revised form 9 July 2020
Accepted 13 July 2020
Available online 18 July 2020

Editor: G. Darrel Jenerette

Keywords:
Phenological sequences
Climate warming
Temperature sensitivity

ABSTRACT

Phenology has a great effect on the carbon cycle. Significant relationships have been well demonstrated between phenology and photosynthesis. However, few studies have been undertaken to characterize relationships between phenology and ecosystem respiration (Re). We conducted a reciprocal transplant experiment among three elevations for two-years to measure Re over six phenological sequences throughout the growing seasons. Our results showed that changes in phenological duration were mainly determined by the onset of phenology, as one day advance of phenological onset could lengthen 0.13 days of phenological duration. Advances in early spring phenophases (i.e., first leaf-out, first bud/boot-set and first flowering) under warming strengthened the temperature sensitivity of Re. However, the late phenophases (i.e., first seeding-set, first post-fruiting vegetation and first leaf-coloring) had non-significant relationships with Re. In total, after pooling all the data, one day advance of phenophases would increase Re by 2.23% under warming. In particular, Re would increase by 29.12% with an advance of phenophases by 8.46 days of under a 1.5 °C warming scenario. Our analysis of the coupling

---

[*] Corresponding author at: Key Laboratory of Alpine Ecology, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101, China.
E-mail address: wangsp@itpcas.ac.cn (S. Wang).

https://doi.org/10.1016/j.scitotenv.2020.140999

Carbon source
Reciprocal transplant

between temperature/moisture–phenology–Re may further supplement evidence that warmer spring temperature increases carbon emission by advancing early phenophases. This points to a faster and easier way to investigate how aboveground functional traits (phenology) affect unseen functional traits (Re) on the Tibetan Plateau.

1. Introduction

Ecosystem respiration (hereafter "Re") by all organisms in the ecosystem converts organic carbon to inorganic carbon (i.e., carbon dioxide) (Yvon-Durocher et al., 2012), and plants are the leading contributor among all organisms (Boone et al., 1998; Wan and Luo, 2003; Schmitt et al., 2013). Re is mainly affected by climate and biological factors (Hu et al., 2016; Niu et al., 2017; Zhao et al., 2018), among which climate change is considered as the main driving force. However, the biological mechanisms affecting Re are still less well researched (Niu et al., 2017). Transitions in plant phenology have large effects on ecosystem processes by altering plant activity periods (Richardson et al., 2013), because plant phenology greatly affects plant development and metabolism (Barr et al., 2009). Plant phenology has a particularly strong relationship with climate change (Richardson et al., 2013; Richardson et al., 2018). Therefore, climate change exerts a strong influence on terrestrial carbon balances mediated through plant phenology (Barr et al., 2009; Richardson et al., 2010). Most studies pay attention to relationships between phenology and photosynthesis or carbon sink (Richardson et al., 2010; Keenan et al., 2014), while few studies have focused on the relationships between phenology and Re. Plant photosynthetic activity could be enhanced by earlier phenology, and may also increase Re based on dynamic equilibrium relationships between them (Odum, 1969; Keenan and Williams, 2018). Some studies have found remarkable relationships between soil respiration and plant phenology (Fu et al., 2002; Curiel yuste et al., 2004; DeForest et al., 2006), because soil respiration largely relies on recent photosynthetic products transferred from aboveground plants (Högberg et al., 2001; Ryan and Law, 2005; Tang et al., 2005; Huang et al., 2012). Thus, Re may also have close relationships with plant phenology, because it is composed of soil respiration and aboveground plants' autotrophic respiration. A better understanding of the relationships between carbon emission and plant phenology may improve predictions of the terrestrial carbon budget under climate change.

Onset and duration are two key characteristics of phenology (Post et al., 2008; Haggerty and Galloway, 2011; Wang et al., 2014). A prolonged growing season is generally considered as an indicator of photosynthetic activity (Wu et al., 2012; Richardson et al., 2013; Xia et al., 2015). However, few studies have focused on the effects of the onset of phenology and variation in different phenophases on the carbon cycle. First, phenological duration is determined by the balance between changes in the onset and offset of a given phenophase, and some studies show that the onset of specific phenophases has a greater temperature response than phenological duration (Price and Waser, 1998; Post et al., 2008; Haggerty and Galloway, 2011; CaraDonna et al., 2014; Wang et al., 2014). Therefore, phenological onset may be a better indicator of the carbon cycle than its duration due to possible uniform advance in phenological onset and non-uniform variation in phenological offset in response to warming. One reason is that the advanced onset of phenology could lengthen plant activity time. Another is that advanced onset of phenology may lead to a higher growth rate of height or biomass in the community (Sun and Frelich, 2011). Better separation of these two phenological components is needed. We therefore hypothesize that phenological onset rather than its duration exerts a stronger effect on Re. Second, plant respiration varies between phenophases (Fu et al., 2002; Balogh et al., 2019). This may be attributed to different phenophases having divergent nutrient-consuming and metabolic strategies (Körner, 2003; Wang et al., 2014). For example, a higher rhizosphere respiration rate has been observed at flowering stage at the species-level (Fu et al., 2002). The reproductive stage has a higher temperature sensitivity due to it being dominated by the growing season under warming (Li et al., 2016). We therefore hypothesize that Re in the early reproductive stages may have a higher temperature sensitivity than during other phenophases at the community level.

To investigate the effects of phenological variation on Re, we conducted a reciprocal transplant experiment on the Tibetan Plateau over two years (i.e., 2008 and 2009). We applied reciprocal transplanting along three elevational gradients (i.e., 3200 m, 3400 m and 3800 m), because it is an important method involving space-for-time substitution to examine the effects of global changes on plants (Körner, 2003). Firstly, the reciprocal transplant experiment included warming (downward transplant) and cooling (upward transplant) treatments, as global warming is not in a trend of continuous warming but consists of warming and cooling spells (Menzel et al., 2011; Li et al., 2016). Secondly, most current warming experiments are designed with less than two or three gradients (e.g., warming or elevational gradients), which may lead to erroneous conclusions because one single gradient is insufficient to describe future phenological trends (Wolkovich et al., 2012; Kreyling et al., 2018). We therefore simulated six temperature change gradients by reciprocal transplant of plants downhill and uphill among three elevations. We then continuously observed community phenological sequences (i.e., six phenophases from leaf-out to leaf-coloration) and monitored Re across the whole growing season in each year. The objective of our study was to address how the transitions in the onset and duration of phenological sequences control Re, especially under a scenario of 1.5 °C warming.

2. Materials and methods

2.1. Experimental design

In early May 2007, we conducted a reciprocal transplant experiment along three elevations near Haibei Station on the Tibetan Plateau, China (3200 m in 37.62° N, 101.31° E, 3400 m in 37.67° N, 101.33° E and 3800 m in 37.70° N, 101.37° E). The research area has a typical continental climate. The long-term average annual temperature is −1.7 °C. Annual precipitation is from 420 to 860 mm. In general, the growing season lasts from May to September. All sites at each of the three elevations were fenced since 2007 to avoid grazing by yak and goats. The dominant species at 3200 m are *Elymus nutans*, *Stipa aliena*, *Poa pratensis*, *Thalictrum alpinum*, *Medicago ruthenica*, *Gentiana straminea* and *Kobresia humilis*, the total coverages of which account for ~60% of coverage in the community. At 3400 m, the dominant species are *Kobresia humilis*, *Potentilla fruticosa*, *Poa pratensis*, *Elymus nutans*, *Polygonum viviparum*, *Carex scabrirostris* and *Anemone cathayensis*, the total coverages of which account for ~50% of coverage in the community. At 3800 m, the dominant species are *Leontopodium pusillum*, *Poa pratensis*, *Potentilla nivea*, *Carex scabrirostris*, *Rheum pumilum*, *Kobresia humilis* and *Carex atrofusca*, the total coverages of which account for ~70% in the community (Meng et al., 2016).

We first dug out 9 cubic soil blocks (i.e., 1 m^2 (base area) × 0.3/0.4 m (depth), with a shallower depth at 3800 m due to the shallower soil layer) at each elevation with minimum damage to belowground roots. We then randomly chose 6 cubic soil blocks at each elevation, and reciprocally transplanted them to two other elevations, leaving 3 cubic soil blocks in situ. For example, 9 blocks from 3200 m were placed at

3200 m, 3400 m and 3800 m (i.e., 3 blocks per elevation), and 3200 m also received 6 blocks from 3400 m and 3800 m. Therefore, the site at 3200 m had 9 blocks, including 3 blocks from 3200 m, 3 blocks from 3400 m and 3 blocks from 3800 m. To insulate each block against the effects of soil fertility and soil moisture from nearby soil, the four vertical sides of each block were sealed by plastic. We started our field observations and measurements in 2008 to eliminate to the extent possible the effects of disturbance caused by transplanting.

2.2. Soil temperature and soil moisture

Soil temperature and soil moisture at 20 cm depth were continuously monitored at a frequency of 1 min by HOBO (Onset Computer Corporation, Bourne, Massachusetts, USA) weather stations at each site, and the logger automatically calculated and stored data on the half-hour average. Averaging across the two experimental years, annual mean soil temperature at 20-cm depth was 3.44 ± 1.27 °C, 2.17 ± 1.14 °C and 0.18 ± 1.19 °C at 3200 m, 3400 m and 3800 m, respectively, and annual mean soil moisture at 20-cm depth was 27 ± 2%, 21 ± 2% and 8 ± 1% at each elevation (Fig. S1).

2.3. Community phenological observations and calculations

In 2008 and 2009, community phenological sequences were observed every 3–5 days across the whole growing season. We used a quadrat method (1 m × 1 m) with 100 cross points (10 cm × 10 cm) to monitor phenological variation from April to October. The state of plant growth was observed under each cross point. Onset and duration of phenological sequences were calculated based on field observations of individuals at each of the 100 cross points, with the onset of the community phenophase defined as emergence of 15% of individuals at each given phenophase, and the end of the community phenophase defined as achievement by 95% individuals of the given phenophase (Meng et al., 2016; Meng et al., 2017). Therefore, the duration of the community phenophase was defined as the difference between the date of the end and the date of the onset of the community phenophase. Onset of phenological sequences included first leaf-out (FL), first bud/boot-set (FB), first flowering (FF), first seeding-set (FS), first post-fruiting vegetation (FP), first leaf-coloring (FC). The corresponding duration of phenological sequences included duration of leaf-out (DL), duration of bud/boot-set (DB), duration of flowering (DF), duration of seeding-set for graminoids or fruit-set for forbs (DS), duration of post-fruiting vegetation (DP) and duration of leaf-coloring (DC). The temperature sensitivity of the onset and duration of community phenophases (d $°C^{-1}$) was calculated as the difference in a given phenophase between the transferred elevation and the original elevation divided by annual soil temperature change (°C) at 20-cm depth.

2.4. Re measurements and calculations

In 2008 and 2009, Re was measured at each elevation every 7–10 days depending on weather conditions during the whole growing season from early May to late September. A static chamber with dimensions of 40 cm × 40 cm × 40 cm was chosen to monitor Re. Chambers were not opened until the end of each 30 min measurement event. Four 100 ml plastic syringes were used to extract air from the closed static chamber at an interval of 10 min (i.e., 0, 10, 20 and 30 min) at each plot. Each measurement event was finished on the same day between 9 a.m. and 11 a.m. All gas samples were protected from exposure to light. The CO_2 concentration was analyzed by gas chromatography (HP Series 4890D, Hewlett Packard, USA), which was completed within 24 h after sampling. The coefficient of variation of CO_2 concentration was less than 1% for four repeats. More details are given in Hu et al. (Hu et al., 2016).

The temperature sensitivity of Re (% $°C^{-1}$) was defined as the percentage change in Re divided by current soil temperature change (°C) at a 20-cm depth between the transferred elevation and the original elevation. The percentage change in Re was calculated as the increase or decrease in Re between the transferred elevation and the original elevation divided by Re at the original elevation.

2.5. Data analysis

The time series of Re were classified by the duration of each phenophase across the growing season and Re observations were distributed to six intervals corresponding to the duration of phenological sequences (i.e., DL, DB, DF, DS, DP and DC). LSD of one-way ANOVAs and *t*-tests were performed to make multiple comparisons among the temperature sensitivities of Re in different phenophases. The analysis was conducted in SPSS v.23. Simple linear regressions were used to analyze relationships between phenological onset and duration, and between abiotic (i.e., soil temperature and soil moisture) or biotic factors (onset and duration of phenological sequences) and ecological respiration. Structural equation modeling (SEM) was used to determine how different pathways of abiotic and biotic factors affected Re. We first specified all possible linkages as a saturated SEM model (Figs. S2 and S3). Although this was not necessarily a bad model, the statistical results did not report goodness-of-fit statistics. We then sequentially deleted non-significant paths in the saturated model and obtained the final model with better goodness-of-fit statistics. We used the chi-square value (χ^2) and the root mean square error of approximation (RMSEA) together with their P value to evaluate the fit of the model to the data. The χ^2 test is used to perform a test of perfect fitness of the model. The null hypothesis of perfect fitness of the model is rejected (i.e., $P < 0.001$). RMSEA is an index of the goodness-of-fit of a model, whereby a smaller RMSEA is considered acceptable. However, if some pathway coefficients are greater than 1, this may either signify a strong relationship (Grace et al., 2016) or multicollinearity. To further verify the robustness of the SEM results, we also used partial correlation analysis to analyze relationships between Re and abiotic/biotic factors. For example, we executed partial correlations between Re and soil temperature, setting soil moisture, onset and duration of phenophases as the control variables.

We used phenological sequences as an indicator of plant activity time. We used leaf area index (hereafter "LAI", defined as the total green leaf area on one side per unit ground surface area, i.e., LAI = leaf area / ground area ($m^2 \cdot m^{-2}$)) as an indicator of community plant growth rate, due to a high relationship between LAI and vegetation primary biomass (Prince, 1991; Zhu et al., 2016). The duration between the date of first leaf-out and date of peak LAI was considered as the leaf growth stage, with a shorter duration indicating a faster growth rate. The duration between the date of peak LAI and the date of the end of leaf-coloring was considered as the leaf aging stage. Because we lacked directly measured LAI data, we modeled daily LAI dynamics to examine whether warming increased the community growth rate in early phenological stages and the lengthened leaf coloring stage across the entire growing season. We used empirical formulas (Eqs. (1) and (2)) fitted based on measured LAI data at a nearby site to simulate LAI at three elevations for two years (Sun et al., 2010). We then extracted the maximum LAI values as the peak LAI in the growing season (Fig. S6). To validate the reliability of this method, we used community first flowering date (FFD) to see whether the peak time of LAI had a similar trend with FFD. Results showed they had similar trends with different magnitudes (Fig. S7).

$$\mathrm{LAI} = 1.055 + 0.019 * GDD - 5.09 * 10^{-5} * GDD^2 + 3.451 * 10^{-8} * GDD^3 \tag{1}$$

$$GDD = \sum_1^{DOY} \mid T_t - T_{base} \mid, if\ T_t > T_{base} \tag{2}$$

where GDD is growing degree days (°C·d), which sums daily air temperature exceeding the base air temperature (T_{base}) of 3 °C

(Sun et al., 2010); DOY is the day of the year; and T_t is daily mean air temperature (°C).

3. Results

3.1. Relationship between temperature sensitivity of onset and duration of community phenological sequences

The temperature sensitivities of the onset and duration of community phenological sequences were significantly affected by the transfer treatments when all data was pooled (Linear mixed model: $P < 0.001$ and $P = 0.02$; Table S1). All temperature sensitivities of the onset and duration of phenophases had significant linear responses to temperature change ($P < 0.05$, Fig. S4) across the two years. There were significant relationships between the onset and duration of phenological sequences, except for FB and FF (Fig. 1 and Table S2). Meanwhile, all absolute values of the slope were smaller than 1, except for FC (from −0.33 to −0.15, Fig. 1 and Table S2).

3.2. Divergent temperature sensitivities of Re at different phenological stages

The temperature sensitivity of Re was significantly affected by transfer treatments when all data was pooled (Linear mixed model: $P = .10$ and *t*-test: $P < 0.001$; Fig. 2 and Table S1). All temperature sensitivities of Re were significantly increased by warming in each phenophase (Figs. 2 and S5). The temperature sensitivity of Re at DB was higher than at other phenological stages, but there were no significant differences among the other phenological stages (Figs. 2 and S5).

3.3. Relationships between abiotic/biotic effects and Re

Based on simple linear regressions, the temperature sensitivity of Re had significant relationships with soil temperature ($R^2 = 0.34$, $P < 0.001$) and soil moisture ($R^2 = 0.47$, $P < 0.001$), and with the temperature sensitivity of the onset of community phenological sequences ($R^2 = 0.41$, $P < 0.001$, Fig. 3a–c and Table S3). Although the duration of community phenological sequences significantly affected Re ($P < 0.001$), its coefficient of determination was very small ($R^2 = 0.03$, Fig. 3d and Table S3). In particular, the temperature sensitivity of Re was 29.12% $°C^{-1}$ with a phenological temperature sensitivity of −8.46 d $°C^{-1}$ under a 1.5 °C scenario (Fig. 3c).

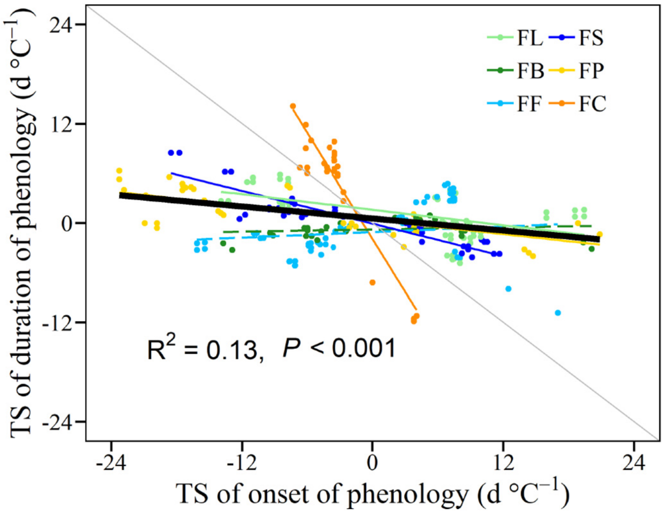


Fig. 1. Relationships between the temperature sensitivities (TS) of the onset and duration of phenology ($n = 82$). The grey diagonal indicates a negative 1:1 line. The dashed and solid lines indicate non-significance and significance at 0.05 level, respectively. Black lines indicate linear regressions on all merged data. Colored lines indicate linear regressions of grouped variables. Details of regression parameters are given in Table S2. The onset of phenology includes FL: first leaf-out, FB: first bud/boot-set, FF: first flowering, FS: first seeding-set for graminoids or fruit-set for forbs, FP: first post-fruiting vegetation and FC: first leaf-coloring. The duration of phenology indicates the duration of each corresponding phenophase.

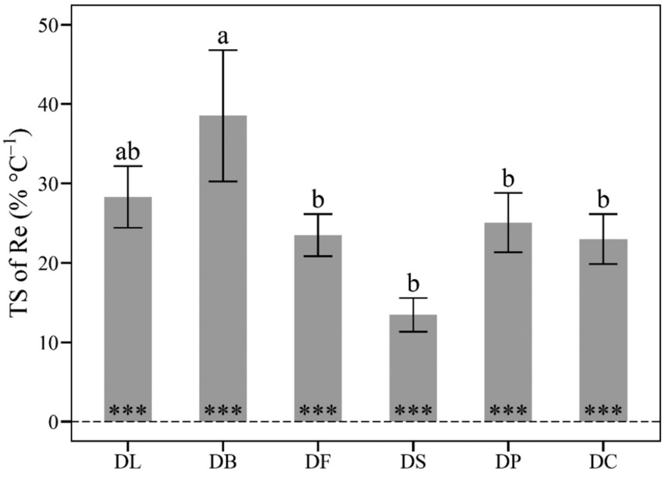


Fig. 2. The temperature sensitivity (TS) of Re at different phenological stages (replicates vary from 44 to 168 depending on the phenological duration length). DL: duration of leaf-out, DB: duration of bud/boot-set, DF: duration of flowering, DS: duration of seeding-set for graminoids or fruit-set for forbs, DP: duration of post-fruiting vegetation and DC: duration of leaf-coloring. ***$P < 0.001$.

The SEM showed that the temperature sensitivity of Re could be mainly explained by soil temperature and moisture together with the onset of community phenological sequences (Fig. 4). In general, soil temperature and moisture had direct positive relationships with Re, while the advanced onset of community phenological sequences caused by warming had a direct negative relationship with the temperature sensitivity of Re (Fig. 4). For separate phenophases, only the onset of early phenophases had direct negative effects on Re, while the duration of phenophases had non-significant effects on Re, except for DP (Fig. 5). To further verify the robustness of the SEM results, we found a similar trend when using partial correlation analysis (Table S4).

4. Discussion

4.1. Effects of phenological transitions on Re under temperature changes

The effects of warming have been well demonstrated by many studies (Rustad et al., 2001). Similar to previous studies in the same area (Hu et al., 2016; Zhao et al., 2018), we also found that soil temperature and soil moisture significantly affected Re, as soil temperature and soil moisture generally had significant positive relationships with Re when all data was merged (Fig. 4). However, for biotic factors, our results partially supported our aforementioned hypothesis, as we found that Re was significantly enhanced by advanced onset of community phenophases in spring (i.e., FL, FB and FF). This finding is similar to that of previous studies (Fu et al., 2002; Keenan et al., 2014). However, the onset of other phenophases and the duration of nearly all phenophases had non-significant influences on Re. The following mechanisms may explain these divergent effects of phenological changes on Re.

Effects of phenology on Re may be mainly achieved through photosynthesis. Because autotrophic respiration (leaf, root and mycorrizae) directly depends on carbohydrates transported from aboveground photosynthesis, as directly evidenced by large-scale forest girdling experiments (Högberg et al., 2001). It may be more so for grassland, as a large percentage of photosynthetic product is allocated to roots (Kuzyakov and Domanski, 2000; Robinson, 2007). Belowground heterotrophic respiration largely relies on carbohydrate transported from dead organic matter, and dead organic materials may be indirectly related to phenology over one or several years (Kuzyakov and

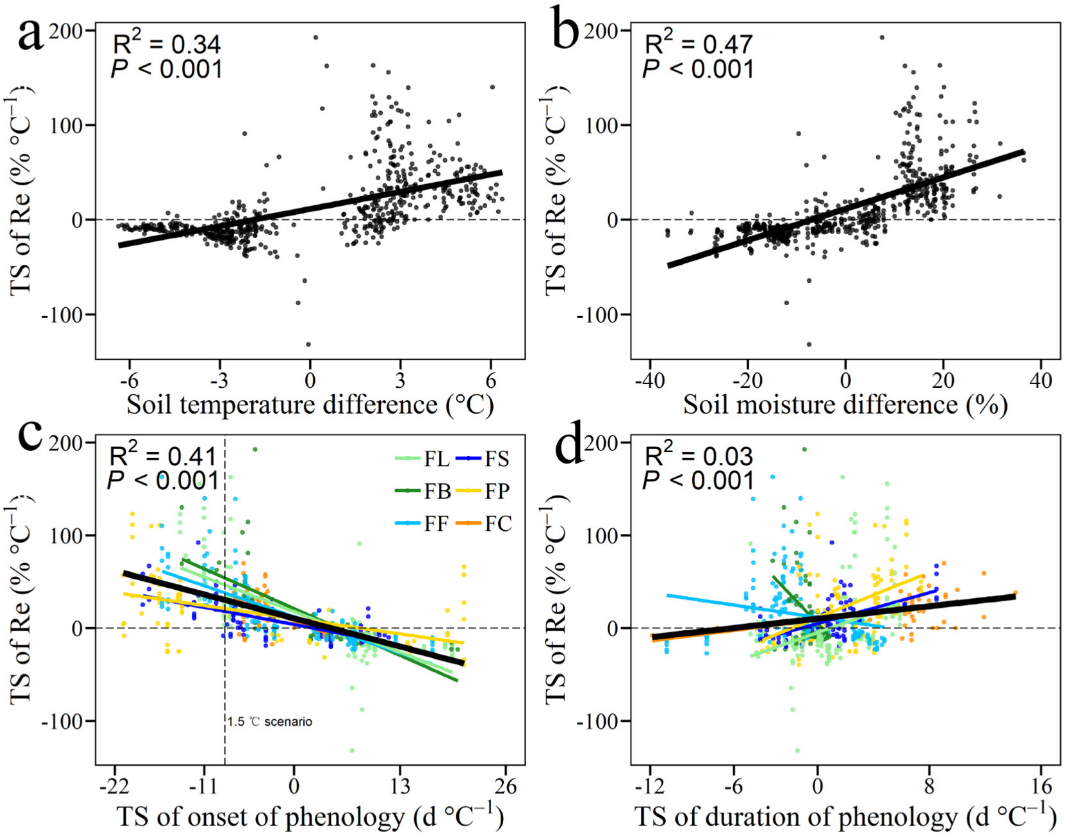
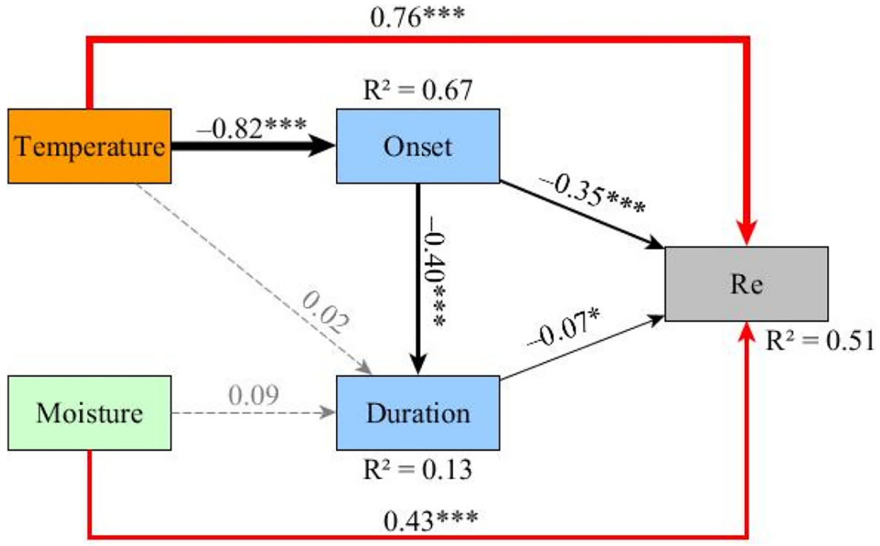


Fig. 3. Linear relationships between abiotic/biotic factors and temperature responses of Re (n = 601, a–d). Dashed and solid lines indicate non-significance and significance at 0.05 level. Black lines indicate linear regressions of all merged data. Colored lines indicate linear regressions of grouped variables. Details of regression parameters are given in Table S3. The onset of phenology includes FL: first leaf-out; FB: first bud/boot-set; FF: first flowering; FS: first seeding-set for graminoids or fruit-set for forbs; FP: first post-fruiting vegetation; and FC: first leaf-coloring. The duration of phenology includes DL: duration of leaf-out; DB: duration of bud/boot-set; DF: duration of flowering; DS: duration of seeding-set for graminoids or fruit-set for forbs; DP: duration of post-fruiting vegetation; and DC: duration of leaf-coloring. The vertical dashed line represents the temperature scenario under 1.5 °C warming in (c). (d) Shares the same legend with (c).



$\chi^2 = 3.91$, P = 0.05; RMSEA = 0.07, P = 0.23

Fig. 4. Structural equation model for the effects of different abiotic (soil temperature, soil moisture) and biotic (onset and duration of community phenological sequences) factors on Re. Red and black arrows indicate positive and negative direction of causation, respectively. Dashed arrows signify non-significant relationships. The numbers adjacent to arrows are standardized path coefficients, which reflect the effect size of the relationship. The R^2 given in each box is the proportion of variance explained for each response variable. Goodness-of-fit statistics for SEM are shown below the model. *P < .05, ***P < .001. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

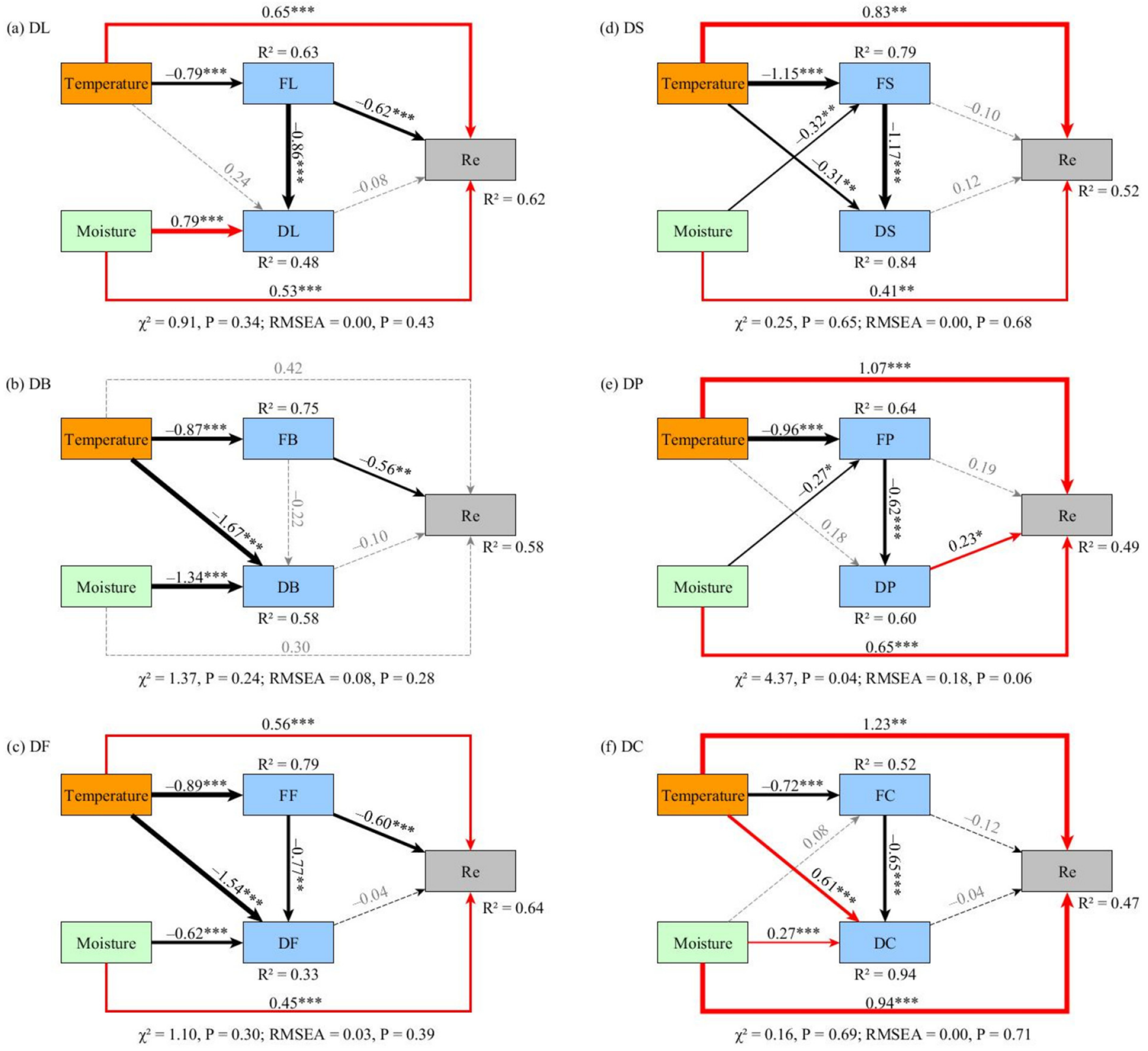


Fig. 5. Structural equation model for the effects of different abiotic (soil temperature, soil moisture) and biotic (onset and duration of community phenological sequences) factors on Re at different phenophases. Red and black arrows indicate positive and negative direction of causation, respectively. Dashed arrows signify non-significant relationships. The numbers adjacent to arrows are standardized path coefficients, which reflect the effect size of the relationship. The R^2 given in each box is the proportion of variance explained for each response variable. Goodness-of-fit statistics for the SEM are shown below the model. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Onset of phenology includes: FL: first leaf-out; FB: first bud/boot-set; FF: first flowering; FS: first seeding-set for graminoids or fruit-set for forbs; FP: first post-fruiting vegetation; and FC: first leaf-coloring. Duration of phenology includes: DL: duration of leaf-out; DB: duration of bud/boot-set; DF: duration of flowering; DS: duration of seeding-set for graminoids or fruit-set for forbs; DP: duration of post-fruiting vegetation; and DC: duration of leaf-coloring. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Domanski, 2000; Ryan and Law, 2005). Therefore, aboveground and soil respiration are driven by phenology which has strong relationships with photosynthesis (Richardson et al., 2010; Keenan et al., 2014). We found that all temperature sensitivities of the onset and duration of community phenophases were significantly advanced and lengthened under warming (Fig. 1). The length of community phenological duration was mainly determined by phenological onset, because their absolute slopes were less than 1, except for FC (lengthening 0.13 d per day advance, Fig. 1), which is similar to previous studies (CaraDonna et al., 2014; Wang et al., 2014). Therefore, onset of community phenophases determined changes in Re. However, our results showed that only early onset of community phenophases (i.e., FL, FB and FF) affected Re.

Onset of early phenophases has a large effect on Re, primarily through lengthening plant activity time and improving growth rates (Sun and Frelich, 2011). Warming decreases the low temperature limit of alpine plants, lengthening the greening period of leaf phenology. Therefore, longer growing seasons also have a longer growth respiration period. Similar to a previous study (Zhang et al., 2013), our results also demonstrated that advanced onset of early phenophases led to a longer duration (Fig. 3), and LAI reached maximum values quickly with warming in early phenophases (Fig. S6). This indicates that warming induced a fast growth rate or photosynthesis rate. Plants, therefore, could have a higher biomass and generate more recent photosynthates for higher maintenance and growth respiration during the early phenophases (Molau, 1993; Körner, 2003; Ernakovich et al., 2014; Collalti et al., 2020), such as the flowering stage (Körner, 2003). Our result also supports our hypothesis that Re in the reproductive stage (i.e., DB) would have a higher temperature sensitivity than at other stages (Fig. 2). This may indicate that plants consume more nutrients in the reproductive stage, which could be attributed to the need to maintain population stability in the harsh environment of the Tibetan Plateau (Jiang et al., 2016).

Changes in late phenophases had non-significant effects on Re. This may be caused by precocious plants in the early phenophases, as full leaf cover is generally completed in about one month in early spring (Fatichi et al., 2019). Our results also showed a similar trend in LAI, which quickly reached maximum values after less than two months, while the duration of leaf aging was longer (Fig. S6). This may indicate that warming not only advances early phenophases but also advances leaf coloration (Archetti et al., 2013; Fu et al., 2014; Li et al., 2016). Autotrophic respiration therefore decreases for recycling nutrients, and heterotrophic respiration may be the predominant contributor compared to autotrophic respiration in the late growing season (Savage et al., 2013). Another reason may be that coverage increases in the community and higher plant height could overshadow shorter plants (Körner, 2003), or it may be due to change in day length (Bauerle et al., 2012), which may also alleviate increased Re in the late growing season. Therefore, in the late season, changes in soil temperature and soil moisture mainly affect Re, not phenology. Non-significant relationships between nearly all durations and Re may be because the onset of phenology has a legacy effect on late phenophases (Fu et al., 2014; Li et al., 2016) and concurrently accelerates the ending of plant development (Halevy, 1985; Qiang et al., 2016). The duration of phenophases therefore may have a non-uniform trend compared with trend in the onset of phenology (Fig. S4). Hence, early onset of phenophases, not phenological duration, determines Re.

4.2. Limitations and implications of this study

In our reciprocal transplant experiments, continuously observing temporal variations in community phenological sequences and Re allowed us to explore their relationships across the growing season. However, there are some limitations that may be improved by future studies. Firstly, although a previous study using girdling experiments demonstrated that aboveground processes directly affect soil respiration, roots still have stored carbohydrates which may continue to be used for some time (Bhupinderpal-Singh et al., 2003). However, other studies consider that soil respiration may strongly depend on newly retrieved carbohydrates (Wertin and Teskey, 2008) or LAI dynamics (Bond-Lamberty and Thomson, 2010). Therefore, whether root carbohydrate stocks affect soil respiration, or how long they will affect soil respiration, still needs further investigation. This may be important because nutrient supply and environmental perception are controlled by roots due to dieback of aboveground leaves of herbs in winter or early spring. Secondly, changes in photosynthesis among different phenophases should also be studied. The balance between photosynthesis and Re determines the carbon sink or carbon source of an ecosystem (Piao et al., 2008), and they may also have interactions with each other (Zhao et al., 2018).

Phenological transitions, including coordinated variation of climate information, may alter Re by affecting other biotic factors, because advanced phenology is always accompanied by increased plant height, leaf area index, tiller and biomass (Dahlgren et al., 2006; Kolb et al., 2006; Richardson et al., 2013; Meng et al., 2017). In particular, biomass is considered to be an important indicator of ecosystem respiration (Hu et al., 2016; Zhao et al., 2018). However, biomass may non-uniformly change with Re, because carbon budgets are determined by two contrasting processes: carbon uptake through photosynthesis and carbon release through Re. These two processes are generally increased at the same time under warming, so changes in biomass depend on their relative difference. Mismatch of co-enhancement between photosynthesis and Re may not lead to increased biomass, such as when autumn warming leads to carbon losses due to increasing mismatch (Piao et al., 2008).

5. Conclusions

Our results showed that the temperature sensitivity of phenological onset mainly determined phenological duration due to a mutual offsetting effect between onset and offset of phenophases. Furthermore, advanced early phenophases significantly enhanced the temperature sensitivity of Re under warming in spring, but not the onset of late phenophases or the duration of nearly all phenophases. In a context of accelerated global warming to 1.5 °C (Hoegh-Guldberg et al., 2018), the generality of our study findings should be tested in alpine or arctic grasslands on a regional scale.

CRediT authorship contribution statement

Fandong Meng: Formal analysis, Writing - original draft, Writing - review & editing. **Lirong Zhang:** Formal analysis. **Zhenhua Zhang:** Investigation. **Lili Jiang:** Formal analysis. **Yanfen Wang:** Writing - original draft, Writing - review & editing. **Jichuang Duan:** Investigation, Formal analysis. **Qi Wang:** Formal analysis. **Bowen Li:** Formal analysis. **Peipei Liu:** Formal analysis. **Huan Hong:** Formal analysis. **Wangwang Lv:** Formal analysis. **Wangmu Renzeng:** Formal analysis. **Zhezhen Wang:** Formal analysis. **Caiyun Luo:** Investigation. **Tsechoe Dorji:** Formal analysis. **Huakun Zhou:** Writing - original draft, Writing - review & editing. **Mingyuan Du:** Investigation. **Yiqi Luo:** Writing - original draft, Writing - review & editing. **Shiping Wang:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

There is no conflict of Interest Statement.

Acknowledgements

This work was supported by projects from the National Science Foundation of China (41731175, 31672470 and 41988101), the Strategic Priority Research Program A of the Chinese Academy of Sciences (XDA20050101), the China Postdoctoral Science Foundation (2017LH033 and 2018M640187), the National Natural Science Foundation for the Youth of China (31702162) and the State Scholarship Fund of the China Scholarship Council (201804910175). The authors declare no conflicts of interest in this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140999.

References

Archetti, M., Richardson, A.D., O'Keefe, J., Delpierre, N., 2013. Predicting climate change impacts on the amount and duration of autumn colors in a New England forest. PLoS One 8, e57373.

Balogh, J., Foti, S., Papp, M., Pinter, K., Nagy, Z., 2019. Separating the effects of temperature and carbon allocation on the diel pattern of soil respiration in the different phenological stages in dry grasslands. PLoS One 14, e0223247.

Barr, A., Black, T.A., McCaughey, H., 2009. Climatic and phenological controls of the carbon and energy balances of three contrasting boreal forest ecosystems in western Canada. Phenology of Ecosystem Processes. Springer, pp. 3–34.

Bauerle, W.L., Oren, R., Way, D.A., Qian, S.S., Stoy, P.C., Thornton, P.E., Bowden, J.D., Hoffman, F.M., Reynolds, R.F., 2012. Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. Proc. Natl. Acad. Sci. U. S. A. 109, 8612–8617.

Bhupinderpal-Singh, Nordgren, A., Ottosson Löfvenius, M., Högberg, M., Mellander, P.E., Högberg, P., 2003. Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. Plant Cell Environ. 26, 1287–1296.

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature 464, 579–582.

Boone, R.D., Nadelhoffer, K.J., Canary, J.D., Kaye, J.P., 1998. Roots exert a strong influence on the temperature sensitivityof soil respiration. Nature 396, 570–572.

CaraDonna, P.J., Iler, A.M., Inouye, D.W., 2014. Shifts in flowering phenology reshape a subalpine plant community. Proc. Natl. Acad. Sci. U. S. A. 111, 4916–4921.

Collalti, A., Tjoelker, M.G., Hoch, G., Mäkelä, A., Guidolotti, G., Heskel, M., Petit, G., Ryan, M.G., Battipaglia, G., Matteucci, G., Prentice, I.C., 2020. Plant respiration: controlled by photosynthesis or biomass? Glob. Chang. Biol. 26, 1739–1753.

Curiel yuste, J., Janssens, I.A., Carrara, A., Ceulemans, R., 2004. Annual Q10 of soil respiration reflects plant phenological patterns as well as temperature sensitivity. Glob. Chang. Biol. 10, 161–169.

Dahlgren, J.P., Eriksson, O., Bolmgren, K., Strindell, M., Ehrlén, J., 2006. Specific leaf area as a superior predictor of changes in field layer abundance during forest succession. J. Veg. Sci. 17, 577–582.

DeForest, J.L., Noormets, A., McNulty, S.G., Sun, G., Tenney, G., Chen, J., 2006. Phenophases alter the soil respiration–temperature relationship in an oak-dominated forest. Int. J. Biometeorol. 51, 135–144.

Ernakovich, J.G., Hopping, K.A., Berdanier, A.B., Simpson, R.T., Kachergis, E.J., Steltzer, H., Wallenstein, M.D., 2014. Predicted responses of arctic and alpine ecosystems to altered seasonality under climate change. Glob. Chang. Biol. 20, 3256–3269.

Fatichi, S., Pappas, C., Zscheischler, J., Leuzinger, S., 2019. Modelling carbon sources and sinks in terrestrial vegetation. New Phytol. 221, 652–668.

Fu, S., Cheng, W., Susfalk, R., 2002. Rhizosphere respiration varies with plant species and phenology: a greenhouse pot experiment. Plant Soil 239, 133–140.

Fu, Y.S.H., Campioli, M., Vitasse, Y., De Boeck, H.J., Van den Berge, J., AbdElgawad, H., Asard, H., Piao, S., Deckmyn, G., Janssens, I.A., 2014. Variation in leaf flushing date influences autumnal senescence and next year's flushing date in two temperate tree species. Proc. Natl. Acad. Sci. 111, 7355–7360.

Grace, J.B., Anderson, T.M., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hautier, Y., Hillebrand, H., Lind, E.M., Pärtel, M., 2016. Integrative modelling reveals mechanisms linking productivity and plant species richness. Nature 529, 390–393.

Haggerty, B.P., Galloway, L.F., 2011. Response of individual components of reproductive phenology to growing season length in a monocarpic herb. J. Ecol. 99, 242–253.

Halevy, A., 1985. Handbook of Flowering. vol. 1–6. CRC Press, Boca Raton, Florida.

Hoegh-Guldberg, O., Jacob, D., Taylor, M., Bindi, M., Brown, S., Camilloni, I., Diedhiou, A., Djalante, R., Ebi, K., Engelbrecht, F., 2018. Impacts of 1.5 °C Global Warming on Natural and Human Systems.

Högberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Högberg, M.N., Nyberg, G., Ottosson-Löfvenius, M., Read, D.J., 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411, 789.

Hu, Y.G., Jiang, L.L., Wang, S.P., Zhang, Z.H., Luo, C.Y., Bao, X.Y., Niu, H.S., Xu, G.P., Duan, J.C., Zhu, X.X., Cui, S.J., Du, M.Y., 2016. The temperature sensitivity of ecosystem respiration to climate change in an alpine meadow on the Tibet plateau: a reciprocal translocation experiment. Agric. For. Meteorol. 216, 93–104.

Huang, N., Niu, Z., Zhan, Y., Xu, S., Tappert, M.C., Wu, C., Huang, W., Gao, S., Hou, X., Cai, D., 2012. Relationships between soil respiration and photosynthesis-related spectral vegetation indices in two cropland ecosystems. Agric. For. Meteorol. 160, 80–89.

Jiang, L.L., Wang, S.P., Meng, F.D., Duan, J.C., Niu, H.S., Xu, G.P., Zhu, X.X., Zhang, Z.H., Luo, C.Y., Cui, S.J., Li, Y.M., Li, X.E., Wang, Q., Zhou, Y., Bao, X.Y., Li, Y.N., Dorji, T., Piao, S.L., Ciais, P., Penuelas, J., Du, M.Y., Zhao, X.Q., Zhao, L., Zhang, F.W., Wang, G.J., 2016. Relatively stable response of fruiting stage to warming and cooling relative to other phenological events. Ecology 97, 1961–1969.

Keenan, T.F., Williams, C.A., 2018. The terrestrial carbon sink. Annu. Rev. Environ. Resour. 43, 219–243.

Keenan, T.F., Gray, J., Friedl, M.A., Toomey, M., Bohrer, G., Hollinger, D.Y., Munger, J.W., O'Keefe, J., Schmid, H.P., Wing, I.S., 2014. Net carbon uptake has increased through warming-induced changes in temperate forest phenology. Nat. Clim. Chang. 4, 598–604.

Kolb, A., Barsch, F., Diekmann, M., 2006. Determinants of local abundance and range size in forest vascular plants. Glob. Ecol. Biogeogr. 15, 237–247.

Körner, C., 2003. Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems. Springer Science & Business Media.

Kreyling, J., Schweiger, A.H., Bahn, M., Ineson, P., Migliavacca, M., Morel-Journel, T., Christiansen, J.R., Schtickzelle, N., Larsen, K.S., 2018. To replicate, or not to replicate–that is the question: how to tackle nonlinear responses in ecological experiments. Ecol. Lett. 21, 1629–1638.

Kuzyakov, Y., Domanski, G., 2000. Carbon input by plants into the soil. Review. J. Plant Nutr. Soil Sci. 163, 421–431.

Li, X., Jiang, L., Meng, F., Wang, S., Niu, H., Iler, A.M., Duan, J., Zhang, Z., Luo, C., Cui, S., Zhang, L., Li, Y., Wang, Q., Zhou, Y., Bao, X., Dorji, T., Li, Y., Penuelas, J., Du, M., Zhao, X., Zhao, L., Wang, G., 2016. Responses of sequential and hierarchical phenological events to warming and cooling in alpine meadows. Nat. Commun. 7, 12489.

Meng, F.D., Cui, S.J., Wang, S.P., Duan, J.C., Jiang, L.L., Zhang, Z.H., Luo, C.Y., Wang, Q., Zhou, Y., Li, X.N., Zhang, L.R., Dorji, T., Li, Y.N., Du, M.Y., Wang, G.J., 2016. Changes in phenological sequences of alpine communities across a natural elevation gradient. Agric. For. Meteorol. 224, 11–16.

Meng, F.D., Jiang, L.L., Zhang, Z.H., Cui, S.J., Duan, J.C., Wang, S.P., Luo, C.Y., Wang, Q., Zhou, Y., Li, X.E., Zhang, L.R., Li, B.W., Dorji, T., Li, Y.N., Du, M.Y., 2017. Changes in flowering functional group affect responses of community phenological sequences to temperature change. Ecology 98, 734–740.

Menzel, A., Seifert, H., Estrella, N., 2011. Effects of recent warm and cold spells on European plant phenology. Int. J. Biometeorol. 55, 921–932.

Molau, U., 1993. Relationships between flowering phenology and life history strategies in tundra plants. Arct. Alp. Res. 391–402.

Niu, S., Fu, Z., Luo, Y., Stoy, P.C., Keenan, T.F., Poulter, B., Zhang, L., Piao, S., Zhou, X., Zheng, H., 2017. Interannual variability of ecosystem carbon exchange: from observation to prediction. Glob. Ecol. Biogeogr. 26, 1225–1237.

Odum, E.P., 1969. The Strategy of Ecosystem Development. Boletín CF+ S.

Piao, S., Ciais, P., Friedlingstein, P., Peylin, P., Reichstein, M., Luyssaert, S., Margolis, H., Fang, J., Barr, A., Chen, A., 2008. Net carbon dioxide losses of northern ecosystems in response to autumn warming. Nature 451, 49–52.

Post, E.S., Pedersen, C., Wilmers, C.C., Forchhammer, M.C., 2008. Phenological sequences reveal aggregate life history response to climatic warming. Ecology 89, 363–370.

Price, M.V., Waser, N.M., 1998. Effects of experimental warming on plant reproductive phenology in a subalpine meadow. Ecology 79, 1261–1271.

Prince, S., 1991. Satellite remote sensing of primary production: comparison of results for Sahelian grasslands 1981-1988. Int. J. Remote Sens. 12, 1301–1311.

Qiang, L., F. Y. H., Zaichun, Z., Yongwen, L., Zhuo, L., Mengtian, H., J. I. A., Shilong, P., 2016. Delayed autumn phenology in the Northern Hemisphere is related to change in both climate and spring phenology. Glob. Chang. Biol. 22, 3702–3711.

Richardson, D., Andy Black, T., Ciais, P., Delbart, N., Friedl Mark, A., Gobron, N., Hollinger David, Y., Kutsch Werner, L., Longdoz, B., Luyssaert, S., Migliavacca, M., Montagnani, L., William Munger, J., Moors, E., Piao, S., Rebmann, C., Reichstein, M., Saigusa, N., Tomelleri, E., Vargas, R., Varlagin, A., 2010. Influence of spring and autumn phenological transitions on forest ecosystem productivity. Philos. Trans. R. Soc. B Biol. Sci. 365, 3227–3246.

Richardson, A.D., Keenan, T.F., Migliavacca, M., Ryu, Y., Sonnentag, O., Toomey, M., 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agric. For. Meteorol. 169, 156–173.

Richardson, A.D., Hufkens, K., Milliman, T., Aubrecht, D.M., Furze, M.E., Seyednasrollah, B., Krassovski, M.B., Latimer, J.M., Nettles, W.R., Heiderman, R.R., Warren, J.M., Hanson, P.J., 2018. Ecosystem warming extends vegetation activity but heightens vulnerability to cold temperatures. Nature 560, 368–371.

Robinson, D., 2007. Implications of a large global root biomass for carbon sink estimates and for soil carbon dynamics. Proc. R. Soc. Lond. B Biol. Sci. 274, 2753–2759.

Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., Gurevitch, J., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia 126, 543–562.

Ryan, M.G., Law, B.E., 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73, 3–27.

Savage, K., Davidson, E.A., Tang, J., 2013. Diel patterns of autotrophic and heterotrophic respiration among phenological stages. Glob. Chang. Biol. 19, 1151–1159.

Schmitt, A., Pausch, J., Kuzyakov, Y., 2013. Effect of clipping and shading on C allocation and fluxes in soil under ryegrass and alfalfa estimated by 14C labelling. Appl. Soil Ecol. 64, 228–236.

Sun, S., Frelich, L.E., 2011. Flowering phenology and height growth pattern are associated with maximum plant height, relative growth rate and stem tissue mass density in herbaceous grassland species. J. Ecol. 99, 991–1000.

Sun, J., Li, Y., Song, C., Wang, J., Zhang, F., Wang, Q., 2010. Seasonal dynamics model of aboveground biomass and leaf area index on alpine Kobresia humilis meadow in Qinghai Tibet plateau. Chin. J. Agrometeorol. 31, 230–234.

Tang, J., Baldocchi, D.D., Xu, L., 2005. Tree photosynthesis modulates soil respiration on a diurnal time scale. Glob. Chang. Biol. 11, 1298–1304.

Wan, S., Luo, Y., 2003. Substrate regulation of soil respiration in a tallgrass prairie: results of a clipping and shading experiment. Glob. Biogeochem. Cycles 17.

Wang, S.P., Wang, C.S., Duan, J.C., Zhu, X.X., Xu, G.P., Luo, C.Y., Zhang, Z.H., Meng, F.D., Li, Y.N., Du, M.Y., 2014. Timing and duration of phenological sequences of alpine plants along an elevation gradient on the Tibetan plateau. Agric. For. Meteorol. 189, 220–228.

Wertin, T.M., Teskey, R.O., 2008. Close coupling of whole-plant respiration to net photosynthesis and carbohydrates. Tree Physiol. 28, 1831–1840.

Wolkovich, E.M., Cook, B.I., Allen, J.M., Crimmins, T.M., Betancourt, J.L., Travers, S.E., Pau, S., Regetz, J., Davies, T.J., Kraft, N.J., Ault, T.R., Bolmgren, K., Mazer, S.J., McCabe, G.J., McGill, B.J., Parmesan, C., Salamin, N., Schwartz, M.D., Cleland, E.E., 2012. Warming experiments underpredict plant phenological responses to climate change. Nature 485, 494–497.

Wu, C., Gonsamo, A., Chen, J.M., Kurz, W.A., Price, D.T., Lafleur, P.M., Jassal, R.S., Dragoni, D., Bohrer, G., Gough, C.M., 2012. Interannual and spatial impacts of phenological transitions, growing season length, and spring and autumn temperatures on carbon sequestration: a North America flux data synthesis. Glob. Planet. Chang. 92, 179–190.

Xia, J., Niu, S., Ciais, P., Janssens, I.A., Chen, J., Ammann, C., Arain, A., Blanken, P.D., Cescatti, A., Bonal, D., 2015. Joint control of terrestrial gross primary productivity by plant phenology and physiology. Proc. Natl. Acad. Sci. 112, 2788–2793.

Yvon-Durocher, G., Caffrey, J.M., Cescatti, A., Dossena, M., Giorgio, P.d., Gasol, J.M., Montoya, J.M., Pumpanen, J., Staehr, P.A., Trimmer, M., Woodward, G., Allen, A.P., 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487, 472.

Zhang, G., Zhang, Y., Dong, J., Xiao, X., 2013. Green-up dates in the Tibetan Plateau have continuously advanced from 1982 to 2011. Proc. Natl. Acad. Sci. U. S. A. 110, 4309–4314.

Zhao, J., Luo, T., Li, R., Wei, H., Li, X., Du, M., Tang, Y., 2018. Precipitation alters temperature effects on ecosystem respiration in Tibetan alpine meadows. Agric. For. Meteorol. 252, 121–129.

Zhu, Z.C., Piao, S.L., Myneni, R.B., Huang, M.T., Zeng, Z.Z., Canadell, J.G., Ciais, P., Sitch, S., Friedlingstein, P., Arneth, A., Cao, C.X., Cheng, L., Kato, E., Koven, C., Li, Y., Lian, X., Liu, Y.W., Liu, R.G., Mao, J.F., Pan, Y.Z., Peng, S.S., Penuelas, J., Poulter, B., Pugh, T.A.M., Stocker, B.D., Viovy, N., Wang, X.H., Wang, Y.P., Xiao, Z.Q., Yang, H., Zaehle, S., Zeng, N., 2016. Greening of the Earth and its drivers. Nat. Clim. Chang. 6, 791.