Diverging shifts in spring phenology in response to biodiversity loss in a subtropical forest

Yanjun Du¹,²,³ | Bo Yang⁴ | Si-Chong Chen⁵ | Keping Ma³

¹Key Laboratory of Genetics and Germplasm Innovation of Tropical Special Forest Trees and Ornamental Plants, Ministry of Education, College of Forestry, Hainan University, Haikou, China
²Wuzhishan National Long Term Forest Ecosystem Research Station, College of Forestry, Hainan University, Haikou, China
³State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, China
⁴Key Laboratory of Plant Resources and Biodiversity of Jiangxi Province, Jingdezhen University, Jingdezhen, Jiangxi, China
⁵Royal Botanic Gardens, West Sussex, UK

Correspondence
Keping Ma, State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, China.
Email: kpma@ibcas.ac.cn

Abstract
Questions: Despite our increased understanding of how climate change influences plant phenology, it remains poorly understood whether diversity loss could alter phenology as well. Here we investigated the following: (a) do changes in plant diversity affect leaf-out and flowering dates for woody species; (b) which group of variables are more important in influencing leaf-out and flowering dates, abiotic variables (soil variables) or biotic variables (plant diversity)?

Study site: A subtropical forest in the Biodiversity–Ecosystem Functioning Experiment of China, located in Jiangxi Province, China.

Methods: Species were planted in various combinations to create plots with varying tree richness: 1, 2, 4, 8, 16 or 24 species. We monitored leaf-out and flowering dates of eight randomly selected species in 17 plots in 2018. A linear model was used to test whether species diversity was a predictor of leaf-out/flowering times for each species. We then fit linear mixed-effects models to assess the combined influence of species diversity, soil Total Carbon and Total Nitrogen on the overall community.

Results: In the low-diversity plots, we found two species leafing out earlier, one leafing out later and four showing no significant difference. Leaf-out date advanced an average of 0.3 days per species lost. Of all the biotic and abiotic predictor variables, Total Nitrogen was the only one significantly correlated with leaf-out date. No significant correlation was found between species diversity and flowering date for any of the species.

Conclusions: Our study provides the first empirical evidence concerning the effects of biodiversity loss on plant spring phenology for woody species. Our findings illustrate that fluctuation in plant diversity could alter the timing of leaf-out and that abiotic variables may be more important than biotic variables in determining leaf-out dates in subtropical forest. Overall, declining diversity may exacerbate the phenological changes attributed to rising global temperatures.

KEYWORDS
BEF-China, biodiversity–ecosystem functioning, flowering, leaf-out, species interactions, species loss, spring phenology, Total Carbon, Total Nitrogen
Evidence of climate change has been widely documented in the Northern Hemisphere, particularly linked with anthropogenic forces. It is well recognized that anthropogenic climate change has already begun to affect phenology (Inouye, 2008; Polgar & Primack, 2011). Numerous studies have shown that spring phenologies, such as leafing out and flowering, have generally advanced in the northern high- and mid-latitudes, with median advances of 4–5 days per degree Celsius (Du et al., 2017; Ge, Wang, Rutishauser, & Dai, 2015; Menzel et al., 2006; Miller-Rushing & Primack, 2008; Shen, Tang, Chen, Zhu, & Zheng, 2011). On the other hand, autumn events, such as leaf coloring or leaf fall, have usually become delayed, although with more variability than spring events (Bertin, 2008; Ge et al., 2015; Menzel et al., 2006). Changes in plant phenology that are brought about by the current anthropogenic scenario of global climate change have had major impacts on ecosystem productivity, species interactions, community patterns and interactions with heterotrophic organisms (Badeck et al., 2004; Chuine & Beaubien, 2001). However, we understand much less about climate-driven phenological changes in subtropical and tropical regions than about those in temperate regions, mainly because of a paucity of long-term field observations of plant phenology (Chen, Wang, & Inouye, 2017; Park & Schwartz, 2015; Primack & Miller-Rushing, 2011).

It is evident that plant phenology could be affected or modified by an array of environmental factors in addition to temperature, including photoperiod length (Saxe, Cannell, Johnsen, Ryan, & Voritiis, 2001), precipitation (Reich, 1995; Shen et al., 2011), soil physical properties (Borchert, 1994; Cardoso, Marques, Botosso, & Marques, 2012), urban air pollution (Honour, Bell, Ashenden, Cape, & Power, 2009), nitrogen deposition (Power, Green, Barker, Bell, & Ashmore, 2006) and drought (Dai, 2011). Most of these environmental variables have been altered by human activities. Despite our increased understanding of how abiotic factors influence plant phenology, we know very little about the phenological impact of an ecosystem’s biotic properties and about how plants interact with each other. This is a significant knowledge gap given the ongoing human-caused alteration of biodiversity and plant community structure at a global scale.

Biodiversity has been observed to positively affect ecosystem function in numerous experiments. Forests with higher levels of biodiversity grow faster, produce more biomass, store more carbon and maintain plant–insect interactions and functional stability better than forests with lower levels of biodiversity or monoculture tree plantations (Brueelheide et al., 2014; Duffy, 2009; Hooper et al., 2005; Loreau, Naem, & Inchausti, 2002). Most biodiversity–ecosystem functioning experiments have employed small model systems with fast-growing primary producers, often herbaceous plants. However, the relationship between biodiversity and ecosystem function has rarely been investigated in forests, and therefore it is not well understood (Ma et al., 2017). Plant phenology underpins a variety of aspects of ecosystem function (Miller-Rushing & Primack, 2008; Piao, Friedlingstein, Ciais, Viovy, & Demarty, 2007; Richardson et al., 2010; Scheffers et al., 2016), but it remains poorly studied whether the loss of diversity could alter the phenology. Among the few studies showing how plant density affects phenology, only one experimentally investigated whether flowering phenology shifted in response to biodiversity loss. Wolf, Zavaleta, and Selmants (2017) found that many species flowered earlier in response to diversity loss, with peak flowering dates (PFD) advancing an average of 0.6 days per species lost. This study was performed in a grassland ecosystem, but it is unclear whether the same findings, that plant diversity loss could affect leaf-out and flowering timing, would hold true in other ecosystems, like the subtropical evergreen forest.

In this study, we used phenology data collected in a subtropical forest ecosystem and investigated the following questions: (a) do changes in plant diversity affect leaf-out and flowering dates for woody species; (b) which group of variables are more important in influencing leaf-out and flowering dates, abiotic variables (soil variables) or the biotic variable (plant diversity)?

## 2 | METHODS

### 2.1 | Study site and plot selection

The study was conducted on the platform of the “Biodiversity-Ecosystem Functioning Experiment of China” (BEF-China), located in Xingangshan, Jiangxi Province, China (117°55′ E, 29°60′ N). The BEF-China project is the first BEF forest experiment in the species-rich subtropics. The study area was comprised of two sites established in 2009 (Site A) and 2010 (Site B) that are located 100–300 m above sea level. The study area is characterized by a subtropical monsoon climate, with an average annual temperature of 17.4°C and a mean annual precipitation of 1,635 mm (Yang et al., 2017). January is the coldest month with a mean temperature of 0.4°C, and July is the hottest with a mean temperature of 34.2°C (Yang et al., 2017). The dominant vegetation type is subtropical evergreen broad-leaved forest dominated by Castanopsis spp., Cyclobalanopsis spp. and Schima superba.

The BEF-China experiment is comprised of 566 experimental plots over approximately 50 ha (Brueelheide et al., 2014). Horizontally, each plot is equivalent to one mu, a traditional Chinese measurement unit, which corresponds to a size of 25.8 m × 25.8 m. In total, 40 different native broad-leaved tree species were planted in various combinations to create plots with tree richness levels of 1, 2, 4, 8, 16 and 24 species (Figure 1). Each plot contained 400 tree individuals, planted in a grid of 20 × 20 regularly arranged columns and rows, with 1.29 m between trees. More descriptive details regarding the BEF-China can be found in Brueelheide et al. (2014).

### 2.2 | Soil variables

Nine soil samples were collected in each plot in 2010 by taking soil cores at a depth of 0–5 cm. This depth range includes the mineral soil layer and corresponds to the A horizon. The nine soil samples from each plot were combined and fully mixed. We then used the
FIGURE 1  Tree species richness in plots at Site A at the BEF-China study site [Colour figure can be viewed at wileyonlinelibrary.com]
resulting bulk sample for analyzing the Total Carbon (TC) and Total Nitrogen (TN) content of each plot using gas chromatography (Vario EL, Elementar, Hanau, Germany); for details see Scholten et al. (2017).

2.3 Phenology monitoring

Phenology was monitored in Site A (Figure 1). Nine years after the trees were planted, many had grown taller than 3 m. We randomly selected eight of the 40 species using the “sample” function in R. These species were Daphniphyllum oldhamii, Choerospondias axillaris, Diospyros glaucifolia, Quercus glandulifera, Quercus glauca, Castanopsis carlesii, Sapium discolor and Sapium sebiferum (Table 1). Due to time constraints, one representative 1-m² plot was selected for each combination of species and plot diversity level from which to sample and monitor. Therefore, phenology was monitored in 17 total plots, including eight plots with a monoculture, four plots with a richness level of two species, two plots with four species, one plot with eight species, one plot with 16 species and one plot with 24 species. Because the Castanopsis carlesii failed to grow in almost every plot, we excluded this species from further analyses.

We selected five individuals of each species present in each plot for phenology monitoring. In total, 149 individuals of seven species were monitored for leaf-out and flowering phenology. We made observations twice a week in spring and early summer 2018 for both leaf-out time (LD) and PFD. We defined LD as the first date on which the final shape of the expanding young leaves was partially visible on at least three branches of an individual plant. This definition has been used in other phenology studies as well (Du et al., 2017; Panchen et al., 2014). This definition is comparable to the definitions used by the USA National Phenology Network (USA-NPN National Coordinating Office, 2012) and the International Phenological Gardens of Europe (IPGE, 2013). PFD is the date when an individual plant of a given species unfolds 50% of the flower buds.

Only individuals from four species reached flowering age during the monitoring study; these were Quercus glandulifera, Quercus glauca, Sapium discolor and Sapium sebiferum. Their flowering data were collected from at least three levels of richness plots.

2.4 Statistical analyses

We performed the two-sample Wilcoxon test using the “wilcox.test” function in R (R Core Team, 2017) to compare the LD and PFD between deciduous and evergreen species. Boxplots were created using the “ggboxplot” function in the R “ggpubr” package (Kassambara, 2018). The “ggplot” function in the R “ggplot2” package was used to make graphs plotting LD and PFD against species diversity for all species studied.

We first tested whether target species diversity was predictive of overall LD and PFD using linear models. Second, we fit linear mixed-effects models to assess the combined influences of both biotic (target species diversity) and abiotic (TN, TC) predictor variables on the overall community. Both plot ID and species ID were included as random factors. Before fitting models, all exploratory variables were standardized using the “scale” function in R (R Core Team, 2017); that is, the mean was subtracted from each variable and then it was divided by its standard deviation.

3 RESULTS

3.1 Leaf-out and flowering phenology

Mean leaf-out dates for the seven species included in the analyses ranged from the 12th of March (Quercus glauca) to the 23rd of April (Diospyros glaucifolia), spanning 42 days (Table 1). Mean LDs were similar for species in the same genus: Sapium discolor (100) and Sapium. sebiferum (98). Two species in the Fagaceae family also had similar LDs (Table 1), with Cyclobalanopsis glauca (80) leafing out one week earlier than Quercus glandulifera (87). LDs were significantly earlier in the evergreen than in the deciduous species (W = 3,395, p < 0.001; Figure 2a).

The flowering period ranged from the 2nd of April (Daphniphyllum oldhamii, Cyclobalanopsis glauca) to the 5th of July (Sapium sebiferum), spanning 94 days (Table 1). The two species in the genus Sapium had similar flowering periods that were clearly later than those of the other four species. PFDs were significantly earlier in the evergreen than in the deciduous species (W = 425, p < 0.001; Figure 2b).

### TABLE 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Deciduousness</th>
<th>Mean LD</th>
<th>Mean PFD</th>
<th>Range of LD</th>
<th>Range of PFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daphniphyllum oldhami</td>
<td>Daphniphyllaceae</td>
<td>Evergreen</td>
<td>85</td>
<td>100</td>
<td>76–94</td>
<td>92–114</td>
</tr>
<tr>
<td>Choerospondias axillaris</td>
<td>Anacardiaceae</td>
<td>Deciduous</td>
<td>88</td>
<td>109</td>
<td>83–95</td>
<td>207–223</td>
</tr>
<tr>
<td>Diospyros glaucifolia</td>
<td>Ebenaceae</td>
<td>Deciduous</td>
<td>102</td>
<td>NA</td>
<td>95–116</td>
<td>NA</td>
</tr>
<tr>
<td>Quercus glandulifera</td>
<td>Fagaceae</td>
<td>Deciduous</td>
<td>87</td>
<td>100</td>
<td>76–94</td>
<td>97–104</td>
</tr>
<tr>
<td>Cyclobalanopsis glauca</td>
<td>Fagaceae</td>
<td>Evergreen</td>
<td>80</td>
<td>93</td>
<td>71–96</td>
<td>92–93</td>
</tr>
<tr>
<td>Sapium discolor</td>
<td>Euphorbiaceae</td>
<td>Deciduous</td>
<td>99</td>
<td>159</td>
<td>95–107</td>
<td>148–170</td>
</tr>
<tr>
<td>Sapium sebiferum</td>
<td>Euphorbiaceae</td>
<td>Deciduous</td>
<td>98</td>
<td>171</td>
<td>95–100</td>
<td>158–186</td>
</tr>
</tbody>
</table>

Note: LD and PFD are represented by mean Julian day. The ranges of LD and PFD represent the differences between different individuals and plots.
3.2 | Phenology in plots of different species richness

Both evergreen species, *Daphniphyllum oldhamii* and *Cyclobalanopsis glauca*, leafed out significantly earlier at lower diversity levels, and *Diospyros glauca* was marginally affected by diversity loss (Figure 3). The average slope of the seven species analyzed was 0.30, ranging from an advancement of 1.0 days per species lost to a delay of 0.5 days per species lost. Only one species, *Quercus glandulifera*, leafed out significantly later at lower diversity levels, with a slope of −0.52 (p < 0.05; Figure 3). Reduced species diversity did not significantly affect the leaf-out phenology for the two species in the *Sapium* genus.

Only four species reached flowering age for at least three levels of species richness. No significant relationship was found between flowering date and richness level for the following four species: *Quercus glandulifera*, *Quercus glauca*, *Sapium discolor* and *Sapium sebiferum* (Figure 4).

Of all the biotic and abiotic predictor variables, TN was significantly correlated with leaf-out date (Table 2), with declining soil TN contributing to earlier LD. TC was marginally significantly correlated with leaf-out date, with increasing soil TC leading to earlier LD. For flowering time, neither biotic nor abiotic variables were significant predictors (Table 2).

4 | DISCUSSION

We experimentally manipulated plant diversity in a subtropical forest and explored how abiotic variables and biotic interactions affect spring phenology. Our study is the first to explore whether
biodiversity could help advance plant spring phenology for woody species. In general, experimentally reducing plant diversity resulted in earlier leaf-out dates, with an average advancement in leaf-out date of 0.3 days per species lost. We found diverging shifts in spring phenology in response to biodiversity loss, with leaf-out dates advancing for two species, one species delaying leaf-out and four species showing no significant change in low diversity plots. However, no significant correlation was found between species diversity and flowering date for any of these species. Our finding is at odds with another similar study conducted in the grassland BEF experiment, which showed five of nine plant species flowering earlier in response to reductions in diversity (Wolf et al., 2017). These findings may be explained by the higher soil surface temperature and declining soil moisture in plots of lower species diversity. This difference may indicate that the effects of biodiversity on spring phenology differ between forest and grassland ecosystems. The significant advancement or delay of phenology under diversity loss demonstrates the important role of biotic processes and plant community interactions, which should be considered alongside the more commonly addressed abiotic processes, such as increasing temperatures and altered precipitation regime, that also affect plant phenology (Wolf et al., 2017).

For these seven species native to subtropical China, leaf-out dates ranged from mid-March to late April and PFD ranged from early April to early July, indicating that flowering phenology may be more variable than leafing-out in subtropical forests. Within a family or a genus, mean leaf-out and flowering dates tended to be similar, which supports the hypothesis of “phylogenetic conservatism of phenology” that has been widely demonstrated at community (Chang-Yang, Lu, Sun, & Hsieh, 2013; Davies et al., 2013; Hu, Chang-Yang, Mi, Du, & Chang, 2015; Panchen et al., 2014) and regional levels (Du et al., 2015).

Deciduous species are widespread across subtropical evergreen forests, and even in tropical rainforests (Richards, 1957), but it remains unclear by which mechanism deciduous species coexist with evergreen species in these evergreen broad-leaved forests. Within the context of evolution, the difference in the timing of the phenology between deciduous and evergreen species has been poorly investigated. In our study, evergreen species have earlier leaf-out
and flowering dates than deciduous species, which may indicate that evergreen and deciduous species occupy different temporal niches as a resource usage strategy. This is consistent with other studies on both herbaceous and woody species (Du et al., 2015). Evergreen species may begin their spring phenologies earlier because they are already photosynthesizing. Therefore, they are able to allocate resources to growth and reproduction earlier than deciduous species, which need to produce leaves before starting photosynthesis. Thus, evergreen species can leaf out and flower when the temperature is warm, and the rainy season is approaching. However, caution should be taken because we only observed two evergreen species in this study.

Interestingly, we found that the leaf-out dates of both evergreen species, *Daphniphyllum oldhamii* and *Cyclobalanopsis glauca*, were affected by declining biodiversity, which indicates that evergreen species change their leaf-out phenology under diversity loss. However, this may be a side effect of these two species being the earliest to leaf-out, which is consistent with the previous finding that phenologies of species with earlier spring leaf-out and flowering dates are the most accelerated by warming (Dunne, Harte, & Taylor, 2003). In contrast, phenologies of species leafing out in late spring/summer can be unresponsive or even be delayed with warming (Sherry et al., 2007), particularly if temperatures rise above their physiological tolerances.

In addition, species in the same family, *Cyclobalanopsis glauca* and *Quercus glandulifera*, showed the opposite pattern. It remains to be tested whether the phenologies of closely related species tend to have similar or opposite responses to diversity loss. Understanding which species will exhibit phenological shifts in response to biodiversity loss is important in light of climate change.

We found an average advancement in leaf-out date of 0.3 days per species lost. The magnitude of this change in leaf-out timing is less than the magnitude of phenological change previously attributed to global warming. Phenological advancement in plants has been estimated at 2.11–2.29 days per decade for woody species in China, according to long-term observations (Ge et al., 2015), and this phenological shift is largely attributed to global warming. The magnitude of the advancement in leaf-out date in our study is equivalent to the loss of seven species in the subtropical region. Our findings illustrate that biotic interactions, or how plants interact with each other, influence phenology in a way that is critical to understanding the combined anthropogenic effects on leaf-out dates. Thus, biotic interactions ought to be considered when studying global climate change because declining diversity could be contributing to or exacerbating phenological changes attributed to rising temperatures. In addition, previous work has found that warming experiments could underestimate plant phenological responses to climate change (Wolkovich et al., 2012). Our findings indicate that shifts in community diversity and plant composition may account for some of this difference because warming experiments that occur over short time scales are unlikely to capture the full effects of community shifts (Wolf et al., 2017).

Diversity level appears to have no significant effect on either leaf-out or flowering dates in models where abiotic and biotic effects were considered together. The positive correlation between increasing soil TN and later leaf-out date is consistent with previous studies (Wolf et al., 2017). This may be because the TN favors root growth over shoot growth (Nord & Lynch, 2009). Plants tend to leaf-out earlier with increasing soil Total Carbon. Root and shoot phenology may be closely related because of physiological coupling, where shoots depend on roots for soil resources. Thus, rich soil nutrients can accelerate new leaf growth (Steinaker & Wilson, 2008). Although nitrogen availability is one of the cues most often associated with human-caused phenological changes, phenology may also respond to other abiotic variables (e.g., solar radiation, other nutrient concentrations) that we did not measure. For example, soil surface temperature and water availability were found to correlate with the PFD of most species in the BEF grasslands in California (Wolf et al., 2017) and in other soil-warming experiments (Lupi, Morin, Deslauriers, Rossi, & Houle, 2012). Our findings indicate that abiotic variables may be more important than biotic variables (species diversity) in determining LD in subtropical forests.

Nonetheless, caution should be taken. First, it would have been better to have more replicates for each diversity level and for each species to test whether our results are robust. However, in the overall design of this study, the plots featuring the climate sensors comprised only one replicate of tree species composition, and thus did not allow the separation of species richness effects from species composition. A second limitation of this study is that we only had one year of phenology data, but the regression trend of phenology date is expected to be robust across different diversity plots, even with one year of phenology data (Wolf et al., 2017). Previous studies have revealed considerable interspecific variation in spring phenology, but consistent order in the timing of leaf out and flowering in various years and among sites (Bennie, Kubin, Wiltshire, Huntley, & Baxter, 2010; Lechowicz, 1984; Panchen et al., 2014; Wesolowski & Rowinski, 2006), which suggests that our results should be robust over several years. However, multiple years of data should be collected in the future. Because our data are limited, our study best serves as an exploratory study to determine hypotheses or questions for further testing, with more replications. Ideally, for each diversity level under each species, there would be a range of N and C values, with each of these replicated. Several additional questions should be explored in the future using more replicates. Can the finding that evergreen species begin their spring phenologies earlier than deciduous species be generalized across subtropical evergreen forests? What is the impact of different species compositions on the response of phenology to diversity change (i.e., does it matter with which species a plant co-occurs, or is it just a number)?

The lack of replication of diversity-level data for each species makes it challenging to separate the influence of abiotic and biotic factors. Because the influence of diversity on leaf-out date varies, with different taxa showing different responses, and there is no significant effect of diversity when abiotic effects are modeled in, we should be careful to draw the conclusion that diversity fluctuation definitely influences phenology. Future research should also address
the importance of biotic effects on woody phenology across a range of global ecosystems, including tropical and temperate forests.

5 | CONCLUSIONS

Overall, our findings demonstrate that changes in plant diversity may alter leaf-out phenology in a subtropical region. We found diverging shifts in spring phenology in response to biodiversity loss, with the magnitude of the advancement in leaf-out date equivalent to the loss of seven species in this region. Secondarily, abiotic variables may be more important than biotic variables (species diversity) in determining leaf-out dates in subtropical forest. Altogether, our findings indicate that declining diversity could exacerbate the phenological changes attributed to rising global temperatures.

ACKNOWLEDGEMENTS

We would like to thank YuLiang Wang for assisting with data collection in the field, and BEF-China for permitting us to carry out this experiment. We would like to thank Elizabeth Tokarz at Yale University for her assistance with English language and grammatical editing. We are grateful to three anonymous reviewers for many helpful suggestions.

DATA AVAILABILITY STATEMENT

The phenology data and R codes can be downloaded at GitHub: https://github.com/YanjunDu/JVS-BEF_phenology

ORCID

Yanjun Du https://orcid.org/0000-0002-7311-0265
Si-Chong Chen https://orcid.org/0000-0002-6855-2595
Keping Ma https://orcid.org/0000-0001-9112-5340

REFERENCES


Chen, Keping Ma


