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This is the **accepted version** of the article:

Fu, Yongshuo H.; Piao, Shilong; Delpierre, Nicolas; [et al.]. «Nutrient availability alters the correlation between spring leaf-out and autumn leaf senescence dates». *Tree physiology*, Vol. 39, issue 8 (Aug. 2019), p. 1277-1284.

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1 **Title:** Nutrient availability alters the correlation between spring leaf-out  
2 and autumn leaf senescence dates

3

4 **Authors:** Yongshuo H. Fu<sup>1,2\*</sup>, Shilong Piao<sup>3,4,5</sup>, Nicolas Delapierre<sup>6</sup>, Fanghua Hao<sup>1</sup>, Heikki Hänninen<sup>7</sup>,  
5 Xiaojun Geng<sup>1</sup>, Josep Peñuelas<sup>8,9</sup>, Xuan Zhang<sup>1</sup>, Ivan A. Janssens<sup>2</sup>, Matteo Campioli<sup>2</sup>

6

7 <sup>1</sup>College of water sciences, Beijing Normal University, Beijing, China.

8 <sup>2</sup>Centre of Excellence Plants and Ecosystems, Department of biology, University of Antwerp, Antwerp,  
9 Belgium.

10 <sup>3</sup>Sino-French Institute for Earth System Science, College of Urban and Environmental Sciences, Peking  
11 University, Beijing 100871, China

12 <sup>4</sup> Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy  
13 of Sciences, Beijing 100085, China

14 <sup>5</sup> Center for Excellence in Tibetan Earth Science, Chinese Academy of Sciences, Beijing 100085, China

15 <sup>6</sup> Ecologie Systématique Evolution, Univ. Paris-Sud, CNRS, Agro Paris Tech, Université Paris, Saclay,  
16 91400 Orsay, France

17 <sup>7</sup> State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture & Forestry University, Hangzhou  
18 311300, China

19 <sup>8</sup> CREAM, Cerdanyola del Vallès, Barcelona 08193, Catalonia, Spain

20 <sup>9</sup> CSIC, Global Ecology Unit CREAM -CSIC-UAB, Cerdanyola del Vallès, Barcelona 11 08193, Catalonia,

21

22 **Corresponding author:**

23 Yongshuo H. Fu, Tel: 32-651721, email: [Yongshuo.fu@uantwerpen.be](mailto:Yongshuo.fu@uantwerpen.be); [yfu@bnu.edu.cn](mailto:yfu@bnu.edu.cn)

24 **Keywords:** Climate change; leaf senescence; fertilization; spring leaf-out; legacy effect

25

26 **Revised manuscript for *Tree physiology***

27

**15 February 2019**

28 **Abstract**

29 Leaf senescence affects tree fitness, species distribution, and ecosystem structure and  
30 functioning. The drivers of leaf senescence and the processes underlying it have been studied,  
31 but the studies have mainly focused on environmental cues and have mainly been based on  
32 statistical analyses using in situ datasets. Experimental investigation and field verification of the  
33 processes and drivers are thus urgently needed. We conducted a nutrient-addition-experiment  
34 after a spring-warming-experiment in which an approximately 40 days range of leaf-out dates  
35 was induced in horse chestnut and beech saplings. We found that both increased nutrient supply  
36 and advanced leaf-out date significantly affected the timing of leaf senescence, but their effects  
37 were opposite, as the former delayed and the latter advanced the senescence. The effects of  
38 nutrient supply and leaf-out interacted species-specifically. In chestnut the delay of senescence  
39 caused by fertilization increased with the delay of leaf-out and was thus stronger for individuals  
40 that flushed late in the spring. On the contrary, in beech the delay of senescence caused by  
41 fertilization decreased with the delay of leaf-out and was insignificant for individuals with the  
42 latest leaf-out. The experimental findings for beech were confirmed with mature trees at a  
43 regional scale. The interactive effect between nutrients and leaf-out on senescence may be  
44 associated with variable sensitivity to photoperiod, growth sink limitation, and/or direct effect of  
45 foliar nutrition on the timing of senescence. Our novel results show that the interactive effects of  
46 leaf-out and nutrient supply on the timing of leaf senescence should be further addressed  
47 experimentally in forthcoming studies. It would also be interesting to consider our results in the  
48 further development of phenological models used in assessing effects of climatic change. The  
49 differences found in the present study between horse chestnut and beech suggest that the results

50 found for one species cannot necessarily be generalized to other species, so studies with different  
51 temperate tree species are called for.

## 52 **Introduction**

53 Plant phenology is sensitive to the ongoing anthropogenic climate change (Fu *et al.*, 2015,  
54 Menzel *et al.*, 2006b, Peñuelas & Filella, 2001, Walther *et al.*, 2002). Changes in plant  
55 phenology can strongly affect tree fitness, species distribution, ecosystem structure, functioning,  
56 and feedback to the climatic system (Peñuelas & Filella, 2009, Piao *et al.*, 2007, Piao *et al.*,  
57 2017, Thackeray *et al.*, 2016). The number of phenological studies has increased drastically in  
58 recent decades (Richardson *et al.*, 2013), but these studies have concentrated mainly on spring  
59 phenological events, whereas autumn phenology, such as timing of leaf senescence (LS), has  
60 been less intensively studied (Gallinat *et al.*, 2015). Nonetheless, due to its crucial role in  
61 determining nutrient recycling, leaf senescence also plays a key role in the nutrient and carbon  
62 cycles of ecosystems (Killingbeck, 1996, Maillard *et al.*, 2015); and in regulating the length of  
63 the photosynthetic period (Garonna *et al.*, 2014). A better understanding of LS and its drivers is  
64 therefore needed.

65

66 Previous studies have investigated the roles of various environmental cues, such as photoperiod,  
67 temperature, and precipitation, in controlling the timing of LS (Delpierre *et al.*, 2016, Hänninen,  
68 2016). The gradually decreasing photoperiod during autumn has traditionally been believed to  
69 trigger LS (Perry, 1971), thus enabling trees to re-translocate nutrients from their leaves prior to  
70 frost damage (Borthwick & Hendricks, 1960, Lagercrantz, 2009, Welling & Palva, 2006).

71 However, using both in situ and remotely sensed observations, substantial delays in LS in the  
72 Northern Hemisphere have been reported in recent decades of climatic warming (Gill *et al.*,  
73 2015, Liu *et al.*, 2016). These findings suggest that air temperature may also be an important  
74 driver of LS (Čufar *et al.*, 2012, Delpierre *et al.*, 2009, Olsson & Jönsson, 2015, Sparks &

75 Menzel, 2002), possibly even more important than photoperiod. Whether temperature and/or  
76 photoperiod dominates the timing of LS remains largely unclear and is currently under debate  
77 (Estiarte & Peñuelas, 2015). In addition to these two environmental cues, nutritional status and  
78 fertilization can also affect the LS date with a general delay following fertilization (Schaberg *et*  
79 *al.*, 2003, Sigurdsson, 2001, Thomas & Ahlers, 1999, Weih, 2009). One study even found that  
80 deciduous trees heavily fertilized in late autumn maintained green leaves until they were killed  
81 by frost (Sakai & Larcher, 1987). In line with this, N-fixing species also maintain green leaves  
82 longer than non-N-fixers (Koike, 1990). In all, the interactive effects of photoperiod,  
83 temperature, and nutrient supply on LS are still unclear.

84

85 In addition to environmental cues, internal physiological factors also regulate LS. For example,  
86 the genetic control of foliar longevity modifies the delay in LS induced by warming (Badenoch-  
87 Jone *et al.*, 1996, Kikuzawa *et al.*, 2013). Recent studies have reported that earlier leaf-out  
88 induced by spring warming advances autumn LS dates (Fu *et al.*, 2014, Keenan & Richardson,  
89 2015, Panchen *et al.*, 2015, Signarbieux *et al.*, 2017). The opposite effects of increased nutrient  
90 supply (delay) and earlier leaf-out (advance) on LS dates indicate that their relative importance  
91 and interactive effects on LS need to be investigated. To our knowledge, however, these  
92 interactive effects between spring leaf-out date and nutrient supply (and their relationship with  
93 photoperiod) on the timing of LS have not been investigated, so experimental studies are  
94 required.

95

96 We therefore conducted a nutrient-addition experiment after a spring-warming experiment  
97 during which a large range of leaf-out dates was induced in two widely distributed temperate

98 species of European deciduous trees: *Fagus sylvatica* (European beech), a typical late leaf-out  
99 species, and *Aesculus hippocastanum* (horse chestnut), an early leaf-out species. The main aims  
100 of this research were to study (i) the interactive effect between spring leaf-out and nutrient  
101 addition on the timing of autumn leaf senescence, and (ii) whether this interactive effect differs  
102 between species. In order to examine the validity of our experimental results for beech, we  
103 explored the relationship between phenological timing and the nutrient status of mature trees  
104 using long-term phenological observations of a French forest-monitoring network. We also  
105 discuss the possible impacts of photoperiod on the relationship between leaf-out, LS and nutrient  
106 supply.

107

## 108 **Materials and methods**

### 109 **Experimental design**

110 The experiment was conducted in 2016 in the Drie Eiken campus of the University of Antwerp,  
111 Belgium (51 °19'N, 4 °21'E). The long-term mean annual air temperature at the study site is  
112 9.6 °C and the mean monthly air temperature varies from 2.2 °C in January to 17.0 °C in July.

113 The mean air temperature during the growing season in 2016 (from March to October) was about  
114 1.2 °C higher than the long-term average (16.3 °C, Fig. S1a). The average annual precipitation is  
115 780 mm and is equally distributed throughout the year (Fu *et al.*, 2016). We used 1.5-m saplings  
116 of beech and horse chestnut bought from a local nursery where they had been grown in a  
117 common field from seeds collected in a nearby forest (Sonian Forest, Brussels). The saplings  
118 were transplanted into pots filled with a soil substrate with a pH of 6.0 and 20% of organic  
119 matter (Universal potting soil, Viano, Aalst, Belgium). The transplanted saplings were placed in

120 transparent, temperature-controlled growth chambers on 1 January 2016. To avoid any effects of  
121 drought the saplings were irrigated as needed (1-3 times a week) during the growing season.  
122 We conducted a gradient warming experiment in late winter and spring 2016, with five warming  
123 treatments (+1, +2, +3, +4 and +5 °C, two chambers per treatment) and a control treatment  
124 (+0 °C) (Fu et al., 2019, submitted). All saplings were removed from the chambers after leaf-out  
125 and put into a ‘common garden’ in natural conditions outdoors. The warming experiment had  
126 induced large differences in leaf-out dates among the treatments (FigS.1a and b). The saplings of  
127 both species were divided into two equal groups of 45 saplings, both groups having the same  
128 mean leaf-out date (FigS.1b). One of the groups was subjected to a fertilizing treatment (AD,  
129 nutrient addition), and the other was used as a control without nutrient addition (NO) (for details  
130 of the fertilization, see below). Within both groups (AD or NO), in order to determine the  
131 correlation between leaf-out and LS, we classified the 45 saplings into three subgroups with ~10  
132 days difference in the mean leaf-out dates, i.e., subgroups with early, intermediate and late leaf-  
133 outs were established, with 15 saplings per subgroup. Slow-release fertilizer (100 g m<sup>-2</sup>,  
134 Potgrond-terreau, Aalst, Belgium) was then added to the saplings of the treatment with nutrient  
135 addition (AD) on 31 May 2016, whereas the other treatment remained unfertilized (NO). The  
136 percentage composition of the fertilizer was 13-10-20 for N, P and K, respectively. We thus used  
137 180 saplings, 90 saplings per species, with two nutrient treatments (AD and NO, 45 saplings per  
138 treatment per species) and three classes of leaf-out dates per nutrient treatment per species (15  
139 saplings per subgroup per species).

140

141 Leaf senescence (LS) was monitored from 1 September every three days. LS was defined as  
142 described by Vitasse *et al.* (2009). In brief, the percentages of autumn colored leaves and of



143 dropped leaves of the saplings were assessed visually, and the senescence date was recorded as  
144 the date when 50% of the leaves were either colored or had dropped. Only the LS date was  
145 recorded, not the time series of the leaf coloring and leaf fall.

#### 146 **Testing the effect of foliar nutrient content on the correlation between leaf-out and** 147 **senescence in mature trees**

148 We further explored the effect of foliar nutrition on the relationship between leaf-out and LS in  
149 mature beech trees using phenological data from the RENECOFOR network (Delpierre *et al.*,  
150 2009). The RENECOFOR network recorded the leaf-out and LS dates and autumn temperatures  
151 (monthly temperature for September, October and November) for 1997-2014 over 18 mature  
152 Beech forests located throughout France, representing at least 10 years of leaf-out and LS  
153 observations collected during the same year. At each RENECOFOR site, leaf-out and leaf  
154 senescence were monitored weekly over 36 trees. Leaf-out was defined as the date (Day of Year)  
155 when 90% of the 36 observed trees showed open buds over 25% to 50% of the tree crown. LS  
156 was defined as the date (DoY) when in 90% of the 36 observed trees leaves had colored or fallen  
157 over 25% to 50% of the crown. The average dates of leaf-out and senescence for each site are  
158 provided in Table S1. The foliar N, P and K mass concentrations were quantified every other  
159 year over 1997-2011 in mature, non-senescent leaves sampled (from July 15 to August 31) in the  
160 upper-third of the crown of eight dominant trees per site (Jonard *et al.*, 2009). Damages caused  
161 over the RENECOFOR network by the 1999 winter storm and 2003 summer drought prevented  
162 the collection of leaf samples for nutrient concentration measurements during those years. This  
163 resulted in an actual average collection interval of 2.5 years for leaf nutrient concentrations over  
164 the 18 Beech forests. The average foliar nutrient contents of the sampled trees at each site were  
165 calculated to provide a proxy of the nutritional status of the trees. We removed the covariate  
166 effects of autumn temperatures by applying a partial correlation analysis to calculate the partial  
167 correlation coefficient between leaf-out and LS (Fu *et al.*, 2014). This was necessary because the  
168 relationship between LS and autumn temperature is well established for beech and was recently  
169 found to be stronger than the relationship between spring temperature and leaf-out in this species  
170 (Fu *et al.*, 2018). The correlations between the average foliar nutrient contents and the partial

171 correlation coefficients were then analyzed by linear regression across all phenological sites  
172 separately for N, P and K.

173

## 174 **Results**

175 In both horse chestnut and beech, the timing of LS was significantly delayed ( $P < 0.05$ ) in  
176 saplings supplied with additional nutrients (AD), as compared with saplings without nutrient  
177 addition (NO) (Fig. 1). This was despite the fact that the saplings were grown in a common  
178 garden (same climate) and had the same mean leaf-out dates. LS was delayed more in horse  
179 chestnut (9 days) than in beech (6 days). The delayed senescence indicated that the supply of  
180 nutrients during the growing season had substantial impacts on the leaf senescence process.

181

182 LS date was positively and linearly correlated with leaf-out date in both horse chestnut and beech  
183 (Fig. 2a and 2b). On average, a 10-days advance in leaf-out induced a 5 to 7-days advance in LS  
184 in both species. Nutrient application, AD, affected this correlation species-specifically. In detail,  
185 AD did not substantially affect the correlation between LS and leaf-out in horse chestnut ( $r =$   
186  $0.71$  vs.  $0.64$ ,  $p = 0.006$  vs.  $< 0.001$ , for the control and AD, respectively; the slopes of the  
187 regression lines did not differ significantly; Fig. 2b). In beech the effect of AD on the  
188 relationship between LS and leaf-out was significant; as no correlation was any more found after  
189 nutrients were added ( $r = 0.60$  vs.  $0.14$  and  $p = < 0.001$  vs.  $0.32$ , for the control and AD,  
190 respectively, Fig. 2a), resulting in a significant difference in the slopes of the regression lines,  
191  $0.58$  vs.  $0.14$  for the control and AD, respectively ( $P < 0.05$ ).

192

193 Among the subgroups of horse chestnut, the significant delaying effect of AD on LS dates  
194 increased from the early sub-group (6 days), via the middle subgroup (8 days) to the late

195 subgroup (12 days) (Fig. 2d). On the contrary, in beech the delaying effect decreased from the  
196 early (9 days) to the intermediate subgroup (5 days); and in the late subgroup the effect of AD on  
197 LS was not significant (Fig. 2c).

198  
199 Consistent with the experimental results, data from the French forest monitoring network  
200 (RENECOFOR) indicated a general positive partial correlation between spring leaf-out and LS  
201 dates, with a mean partial correlation coefficient after removing the covariate effect of autumn  
202 temperature of  $0.27 \pm 0.34$  across all the phenological sites. The distribution of the partial  
203 correlations was not symmetric and for this reason the standard deviation was large. Despite this  
204 the mean partial correlation was significant (meta-analytic  $P < 0.001$ , Fig. S2, Laliberté 2011).  
205 Furthermore, the partial correlation coefficient between leaf-out and LS dates was significantly  
206 negatively correlated with foliar K content (Fig. 3a), but no such correlation was found for foliar  
207 N (Fig. 3b) or P (Fig. 3c) content. This suggests that foliar potassium content, but not nitrogen or  
208 phosphorus content, affects the relationship between spring leaf-out and LS, with the two  
209 phenophases being more strongly correlated at sites with low levels of foliar K. The negative  
210 correlation between leaf-out and senescence vs. foliar K levels indicated that the relationship  
211 between spring and autumn phenology was weaker on more fertile sites.

212

## 213 **Discussion**

214 Consistent with previous studies (Fu *et al.*, 2014, Leblans *et al.*, 2017, Signarbieux *et al.*, 2017),  
215 we found a significant positive correlation between the dates of spring leaf-out and LS for both  
216 horse chestnut and beech. The addition of nutrients during the growing season, however,  
217 significantly altered this correlation species-specifically. Adding nutrients delayed horse chestnut

218 LS in all saplings, and the delay increased from 5 days in the saplings with early leaf-out to 12  
219 days in those with late leaf-out (Fig. 2d). In contrast, adding nutrients delayed beech LS only in  
220 saplings with early and intermediate leaf-out, and the delay decreased from 9 days in the saplings  
221 with early leaf-out to 2 days in the sapling with late leaf-out (Fig. 2c).

222

223 Previous studies have suggested that a photoperiodic threshold controls the timing of LS in  
224 temperate and boreal trees (Lagercrantz, 2009, Way & Montgomery, 2015, Welling & Palva,  
225 2006). Other studies have reported that autumn temperature controls LS (Chung *et al.*, 2013,  
226 Delpierre *et al.*, 2009, Fu *et al.*, 2018, Liu *et al.*, 2018, Menzel *et al.*, 2006a, Tanino *et al.*, 2010,  
227 Vitasse *et al.*, 2011, Xie *et al.*, 2015). Gallinat *et al.* (2015), however, found that autumn  
228 temperature only explains less than half of the variation in the LS date. The results regarding the  
229 environmental regulation of LS therefore remain inconclusive. Our experimental results suggest  
230 that the supply of nutrients during the growing season and the date of spring leaf-out both  
231 significantly affect LS and, as a true novelty, that these factors interact species-specifically. In  
232 this way our results add to the continuing efforts to better understand of the drivers of the LS  
233 timing.

234

235 Given the novelty of our results concerning the species-specific effects of nutrient supply, spring  
236 leaf-out date and their interaction on the LS date, no single and direct mechanism for the causal  
237 phenomena involved can be identified. However, we propose three potential and mutually  
238 nonexclusive hypotheses for the causal phenomena: (i) foliar nutrient content and photoperiod  
239 interact to determine the date of LS, (ii) nutrient deficiencies limit the tree carbon sinks, reducing

240 foliar life-span, and (iii) foliar and tree nutritional status affects LS by affecting the dynamics of  
241 nutrient resorption.

242

243 Our first hypothesis states that photoperiod triggers the onset of LS, but the sensitivity of trees to  
244 photoperiod might not be constant but increases during autumn. For instance, for beech, in early  
245 autumn, its sensitivity to photoperiod is low and the growth is highly dependent on the nutrient  
246 status, so that fertilization (reducing the need of nutrient resorption) delays the date of LS.

247 However, later in the autumn, the sensitivity of beech to photoperiod gradually increases, and LS  
248 is triggered even in fertilized saplings. The typical date of LS of naturally growing beech trees at  
249 the experimental site is near the end of October (~DOY 300) (Fu et al, 2012), and the sensitivity  
250 to photoperiod in beech can be high at this time (corresponding to 9.6 h of daylight in Antwerp).

251 In our study, we found that adding nutrients significantly delayed LS to DOY 300 for saplings  
252 with early spring leaf-out but no such delay was observed in saplings with late leaf-out, which  
253 also senesced around DOY 300. These findings thus support our first hypothesis. Accordingly,  
254 photoperiod would help the trees to balance the trade-off between adaptation for survival

255 (Hänninen 2016), i.e., preventing early frost damage, and adaptation for capacity, i.e., maximal  
256 use of the growth resources of the site. The flexible and variable sensitivity to photoperiod may  
257 represent a behavior common in nature, with organisms taking risks (e.g. early frost) when the  
258 risk is low (e.g. early autumn) but not when the risk is high (e.g. late autumn). This hypothesis  
259 therefore suggests that photoperiod represents a measure of risk, at least for the beech trees.

260 However, the different dynamics shown by horse chestnut suggests that this hypothesis might be  
261 valid only for late-flushing species; or that, for horse chestnut, the photoperiodic threshold was  
262 not reached during the experiment. Finally, note that the photoperiod effect could not be directly

263 investigated from the present in situ dataset because the exact photoperiodic cue (e.g. night  
264 length, radiation sum etc.) affecting senescence has not yet been determined (Liu et al, 2016).  
265 Further experimental investigations are thus needed to test this hypothesis.

266

267 An alternative hypothesis suggests that LS is triggered when sinks of tree resources are no longer  
268 active (Wingler *et al.*, 2005). In other words LS occurs when tree growth ceases, or is strongly  
269 down-regulated. Fertilization may amend nutrient deficiencies even under low temperature and  
270 low radiation in early autumn and promote growth through the root system (Delpierre *et al.*,  
271 2016, McCormack *et al.*, 2014); or it can reduce the rate of cellular maturation, e.g. reduce  
272 lignification of stem cells (Cuny *et al.*, 2015), and in this way postpone the date of LS (Wingler  
273 *et al.*, 2005). However, in the late autumn, growth is likely limited by other factors, such as low  
274 temperature (Körner, 2015), so that fertilization may not play a role any more in the growth  
275 process and thus in the leaf senescence process.

276

277 Our third hypothesis suggests that the nutritional status of the leaves and trees influences the  
278 timing of LS, with a better nutritional status being associated with delayed senescence, possibly  
279 with less efficient nutrient resorption (Millard, 1996, Ono *et al.*, 2001, Withington *et al.*, 2006).

280 The mature beech trees from the RENECOFOR network shown that the foliar K content was  
281 significantly correlated with the partial correlation coefficients between spring leaf-out and leaf  
282 senescence, indicating that the foliar K content interacts with spring leaf-out to influence the LS  
283 dates. The mechanism of foliar K in determining LS needs further investigation, but evidence  
284 suggests that the availability of K can alter foliar longevity (Laclau *et al.*, 2009). K deficiency  
285 may indeed be involved in triggering early LS, fed back by sugar signaling (Wingler *et al.*,

286 2005), even though K deficiency has negative impacts on both photosynthesis and the export of  
287 sucrose from the leaves through the phloem (Cakmak, 2005). According to the third hypothesis,  
288 horse chestnut seems more sensitive to fertilization than beech as the delay of senescence caused  
289 by fertilization increased with the delay of leaf-out and was stronger for individuals that flushed  
290 late in the spring. The rate of LS of horse chestnut can therefore be further slowed by fertilization  
291 also in late autumn, because fertilization may increase foliar nutrient contents (Ordoñez *et al.*,  
292 2009) and that increased carbon assimilation would still benefit the horse chestnut saplings in the  
293 latest part of the season (Capioli *et al.*, 2012).

294

## 295 **Conclusions**

296 We combined experimental results and in situ observations to provide empirical evidence of a  
297 positive correlation between spring leaf-out and autumn LS dates and their interaction with  
298 nutrient availability. The novelty of our results should stimulate further research on this topic.  
299 The results should also be incorporated into phenological models to improve model performance  
300 and to improve our understanding of the responses of ecosystems to the ongoing climate change.  
301 Considerable effort, however, will be needed to obtain species-specific model parameters for  
302 more woody plant species, because the effect of nutrient availability and leaf-out date on LS (and  
303 thus on growing-season length) varied in our study considerably between the two species  
304 examined. This difference between horse chestnut and beech suggests that the results found for  
305 one species cannot necessarily be generalized to another species, so studies with different  
306 temperate tree species are called for.

307

## 308 **Acknowledgments**

309 We acknowledge Dr. Hang Gao, Dr. Maarten Op de Beeck, and Marc Wellens for their  
310 assistance. This study was supported by the National Key Research and Development Program  
311 of China (2017YFA06036001), the general program of national nature science foundation of  
312 China (No.31770516), the Beijing Normal University (No. 310430002) and the Thousands  
313 Talents Program for Young Professionals. Ivan A Janssens and Josep Peñuelas acknowledge  
314 support from the European Research Council (ERC) through Synergy grant ERC-2013-SyG-  
315 610028 “P-IMBALANCE” as well as support from the University of Antwerp. Nicolas Delpierre  
316 acknowledges support from the GIP-Ecofor (SACROBOQUE project, grant # 2016.013). The  
317 authors thank the Office National des Forêts and the RENECOFOR team, particularly Manuel  
318 Nicolas, Sébastien Cecchini and Sébastien Macé for providing access to their database.

319

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470 **Figure legends**

471

472 Figure 1. Mean dates ( $\pm 1$  SE) of (a) leaf-out and (b) leaf senescence in horse chestnut and beech  
473 saplings without (control, NO) and with (AD) added nutrients (n=45 for each group). Different  
474 letters denote a statistically significant difference between the NO and AD saplings at  $P < 0.05$ .

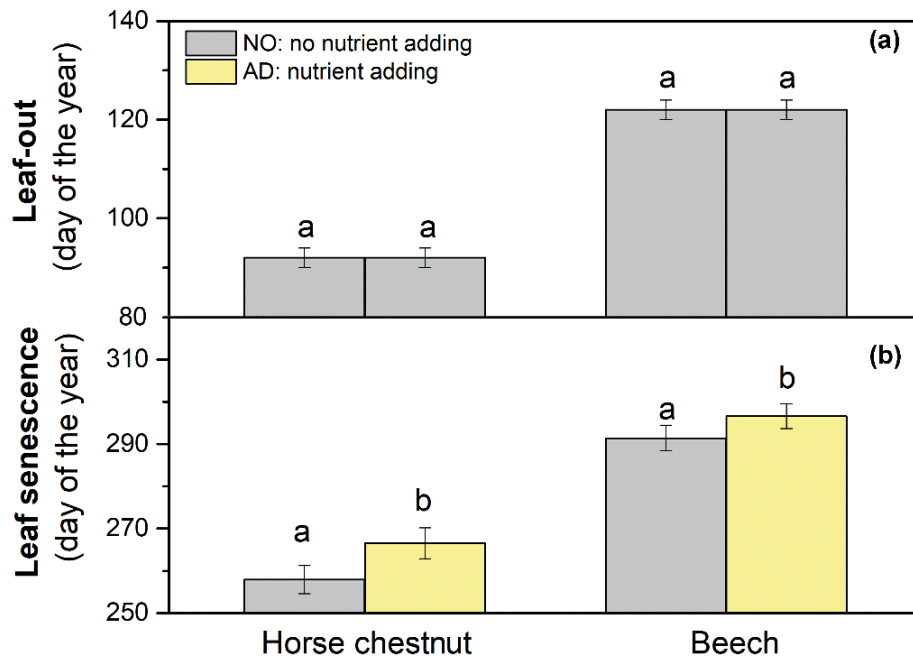
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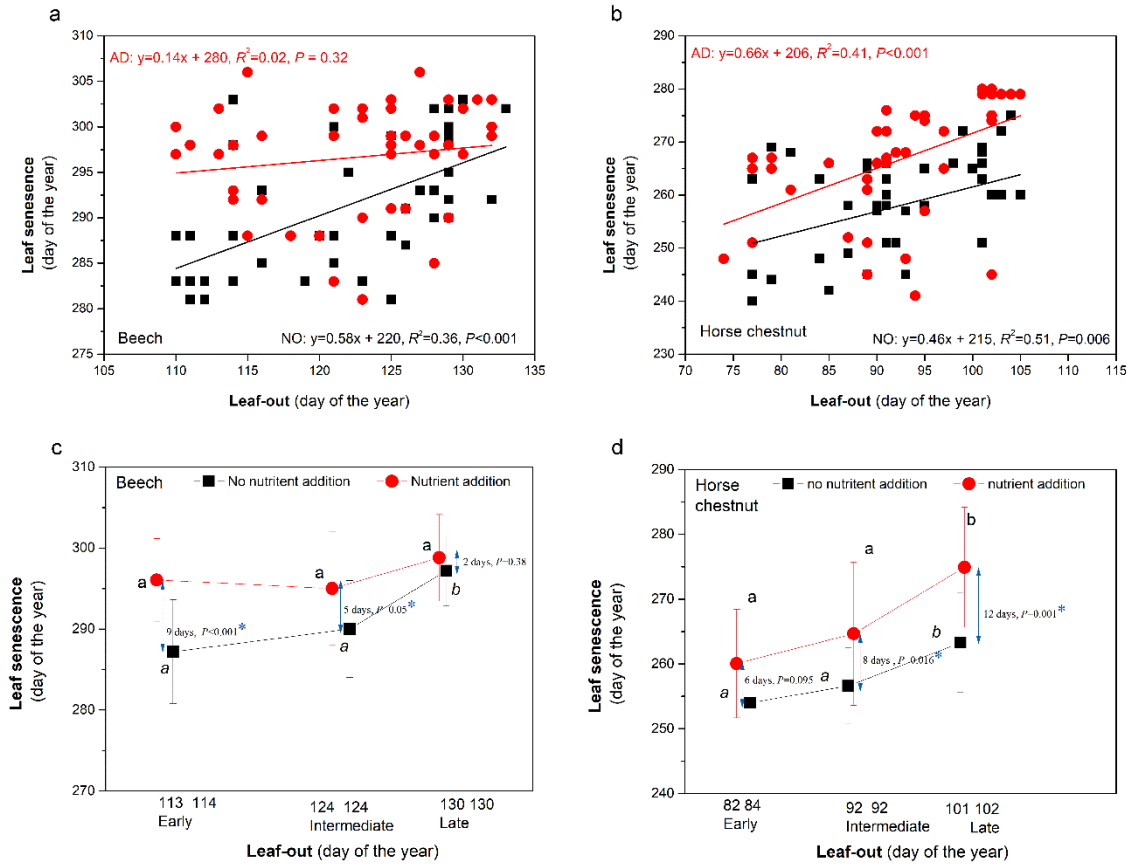
476 Figure 2. (a-b) Linear regression between leaf senescence and leaf-out dates for (a) horse  
477 chestnut and (b) beech. The red dots and the solid squares represent saplings with (AD) and  
478 without (control, NO) nutrient addition during the growing season, respectively. The slope,  
479 intercept and  $R^2$  of the regression lines are provided. The 95% confidence bands are presented as  
480 shadows around the linear regression lines. (c-d) Mean date of leaf senescence for the three leaf-  
481 out subgroups (early, intermediate, and late) for (c) horse chestnut and (d) beech. Different  
482 letters denote statistically significant differences at  $P < 0.05$  among the three subgroups. The  
483 double-headed arrows indicate the difference of leaf senescence dates between the AD and NO  
484 saplings. The corresponding asterisks denote a statistically significant difference between the AD  
485 and NO saplings at  $P < 0.05$ .

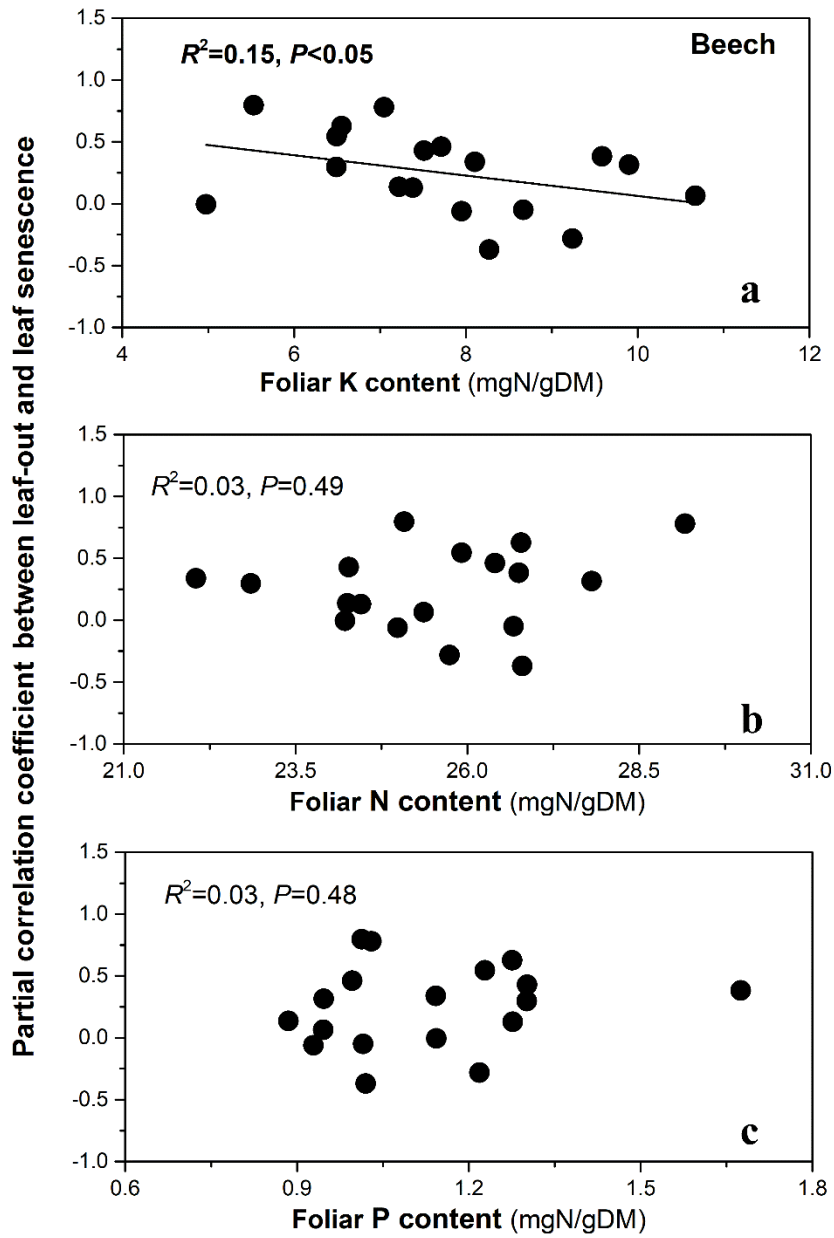
486

487 Figure 3. Correlation between partial correlation coefficient between leaf-out and senescence  
488 dates with foliar contents of (a) potassium (K), (b) nitrogen (N), and (c) phosphorus (P) in  
489 mature beech trees. The partial correlation between leaf-out and leaf senescence dates was  
490 determined after removing the effect of autumn temperatures. The results are based on data from  
491 the French forest monitoring network (RENECOFOR) (Delpierre et al., 2009); one point  
492 represents one phenological site.

493 **Figure 1**









496

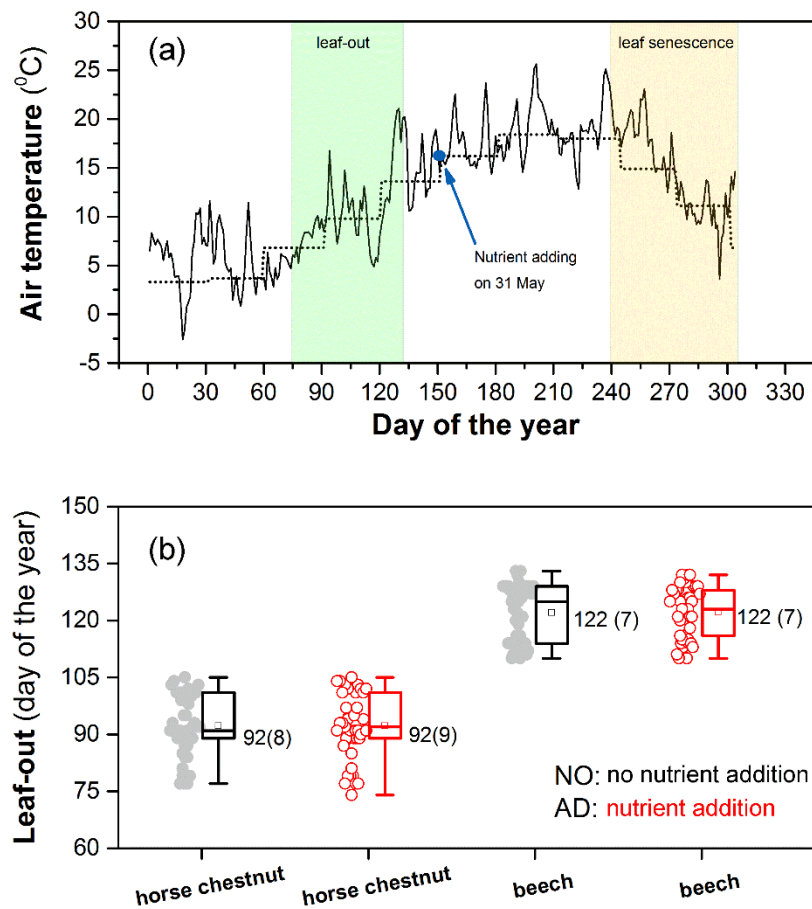
**Supporting tables and figures**

497 Table S1. The average dates (Day of Year) of leaf-out and leaf senescence (LS) in mature beech  
 498 trees for 18 phenological sites in France during 1997-2011. The phenological data was obtained  
 499 from the RENECOFOR network, as indicated by the site acronyms used in the network. Leaf-out  
 500 was defined as the date (Day of Year) when 90% of the 36 observed trees showed open buds  
 501 over 25% to 50% of the tree crown. LS was defined as the date (DoY) when in 90% of the 36  
 502 observed trees leaves had colored or fallen over 25% to 50% of the crown.

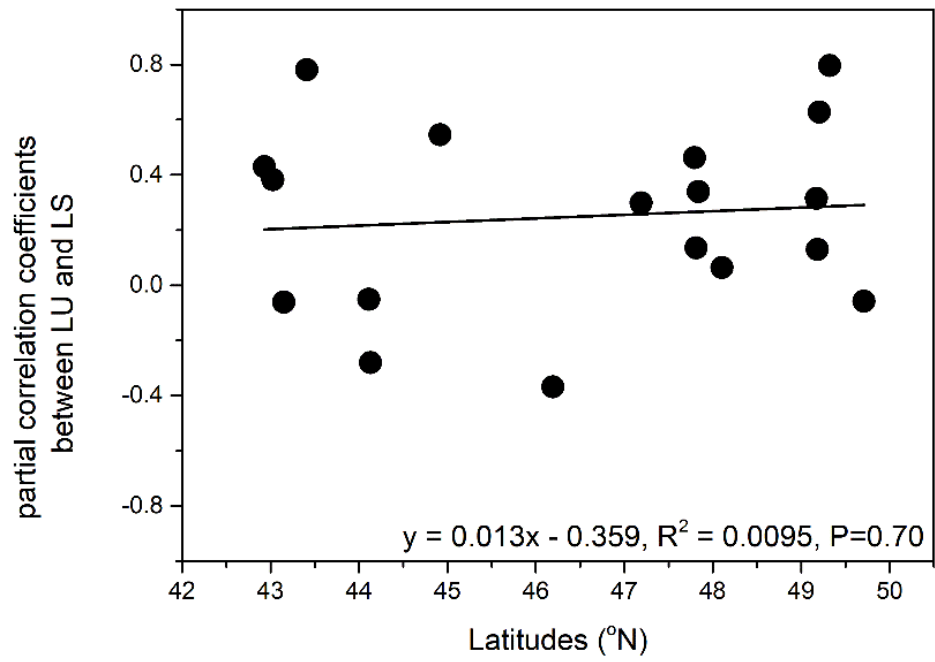
503

Site	Acronym	Latitude	Longitude	Leaf-out	LS
1	HET02	49.21	3.13	120	293
2	HET03	46.19	3.00	121	295
3	HET04	44.13	5.80	122	292
4	HET09	42.93	1.28	120	297
5	HET14	49.18	-0.86	118	291
6	HET21	47.81	4.85	120	302
7	HET25	47.19	6.28	129	292
8	HET26	44.92	5.29	133	276
9	HET29	47.84	-3.54	113	287
10	HET30	44.11	3.54	132	280
11	HET52	47.80	5.07	119	289
12	HET55	49.17	5.00	115	307
13	HET60	49.32	2.88	116	298
14	HET64	43.15	-0.66	112	317
15	HET65	43.03	0.44	116	292
16	HET76	49.71	1.33	119	304
17	HET81	43.41	2.18	115	286
18	HET88	48.10	6.25	119	271

504 Figure S1. (a) The daily mean air temperature (solid line) and the ranges of the leaf-out and leaf  
 505 senescence dates of the studied saplings (green and yellow shading, respectively) during the  
 506 study period. The dotted line represents the long-term mean monthly temperature at the  
 507 experimental site. Nutrients were added to the trees on 31 May 2016. (b) Distribution and plot  
 508 boxes of the leaf-out dates for saplings of horse chestnut and beech in treatments with (AD, open  
 509 circles) and without (control, NO, solid circles) nutrient addition during the growing season.



510 Figure S2. The correlation between latitude of each site and corresponding partial correlation coefficient.



511 Figure S3. Partial correlation coefficients of leaf senescence and leaf out data, controlling for the  
512 impact of autumn temperature, established for each study site of RENECOFOR. The  
513 acronym of each site is provided on the left. The diamond symbol at the bottom of the  
514 figure ("summary") indicates the mean and standard deviation of the distribution of  
515 correlation coefficient

