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.

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

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

Revised manuscript for *Tree physiology*

15 February 2019

Abstract

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

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

Introduction

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

Previous studies have investigated the roles of various environmental cues, such as photoperiod, temperature, and precipitation, in controlling the timing of LS (Delpierre *et al.*, 2016, Hänninen, 2016). The gradually decreasing photoperiod during autumn has traditionally been believed to trigger LS (Perry, 1971), thus enabling trees to re-translocate nutrients from their leaves prior to frost damage (Borthwick & Hendricks, 1960, Lagercrantz, 2009, Welling & Palva, 2006). However, using both in situ and remotely sensed observations, substantial delays in LS in the Northern Hemisphere have been reported in recent decades of climatic warming (Gill *et al.*, 2015, Liu *et al.*, 2016). These findings suggest that air temperature may also be an important driver of LS (Čufar *et al.*, 2012, Delpierre *et al.*, 2009, Olsson & Jönsson, 2015, Sparks &

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

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

We therefore conducted a nutrient-addition experiment after a spring-warming experiment during which a large range of leaf-out dates was induced in two widely distributed temperate species of European deciduous trees: *Fagus sylvatica* (European beech), a typical late leaf-out species, and *Aesculus hippocastanum* (horse chestnut), an early leaf-out species. The main aims of this research were to study (i) the interactive effect between spring leaf-out and nutrient addition on the timing of autumn leaf senescence, and (ii) whether this interactive effect differs between species. In order to examine the validity of our experimental results for beech, we explored the relationship between phenological timing and the nutrient status of mature trees using long-term phenological observations of a French forest-monitoring network. We also discuss the possible impacts of photoperiod on the relationship between leaf-out, LS and nutrient supply.

Materials and methods

Experimental design

The experiment was conducted in 2016 in the Drie Eiken campus of the University of Antwerp, Belgium (51°19′N, 4°21′E). The long-term mean annual air temperature at the study site is 9.6 ℃ and the mean monthly air temperature varies from 2.2 ℃ in January to 17.0 ℃ in July. The mean air temperature during the growing season in 2016 (from March to October) was about 1.2 ℃ higher than the long-term average (16.3 ℃, Fig. S1a). The average annual precipitation is 780 mm and is equally distributed throughout the year (Fu *et al.*, 2016). We used 1.5-m saplings of beech and horse chestnut bought from a local nursery where they had been grown in a common field from seeds collected in a nearby forest (Sonian Forest, Brussels). The saplings were transplanted into pots filled with a soil substrate with a pH of 6.0 and 20% of organic matter (Universal potting soil, Viano, Aalst, Belgium). The transplanted saplings were placed in transparent, temperature-controlled growth chambers on 1 January 2016. To avoid any effects of drought the saplings were irrigated as needed (1-3 times a week) during the growing season. We conducted a gradient warming experiment in late winter and spring 2016, with five warming treatments (+1, +2, +3, +4 and +5 ℃, two chambers per treatment) and a control treatment (+0 ℃) (Fu et al., 2019, submitted). All saplings were removed from the chambers after leaf-out and put into a 'common garden' in natural conditions outdoors. The warming experiment had induced large differences in leaf-out dates among the treatments (FigS.1a and b). The saplings of both species were divided into two equal groups of 45 saplings, both groups having the same mean leaf-out date (FigS.1b). One of the groups was subjected to a fertilizing treatment (AD, nutrient addition), and the other was used as a control without nutrient addition (NO) (for details of the fertilization, see below). Within both groups (AD or NO), in order to determine the correlation between leaf-out and LS, we classified the 45 saplings into three subgroups with ~10 days difference in the mean leaf-out dates, i.e., subgroups with early, intermediate and late leaf-outs were established, with 15 saplings per subgroup. Slow-release fertilizer (100 g m^{-2}, Potgrond-terreau, Aalst, Belgium) was then added to the saplings of the treatment with nutrient addition (AD) on 31 May 2016, whereas the other treatment remained unfertilized (NO). The percentage composition of the fertilizer was 13-10-20 for N, P and K, respectively. We thus used 180 saplings, 90 saplings per species, with two nutrient treatments (AD and NO, 45 saplings per treatment per species) and three classes of leaf-out dates per nutrient treatment per species (15 saplings per subgroup per species).

Leaf senescence (LS) was monitored from 1 September every three days. LS was defined as described by Vitasse *et al*. (2009). In brief, the percentages of autumn colored leaves and of dropped leaves of the saplings were assessed visually, and the senescence date was recorded as the date when 50% of the leaves were either colored or had dropped. Only the LS date was recorded, not the time series of the leaf coloring and leaf fall.

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

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

Results

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

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

Among the subgroups of horse chestnut, the significant delaying effect of AD on LS dates increased from the early sub-group (6 days), via the middle subgroup (8 days) to the late subgroup (12 days) (Fig. 2d). On the contrary, in beech the delaying effect decreased from the early (9 days) to the intermediate subgroup (5 days); and in the late subgroup the effect of AD on LS was not significant (Fig. 2c).

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

Discussion

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

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

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

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

Our first hypothesis states that photoperiod triggers the onset of LS, but the sensitivity of trees to photoperiod might not be constant but increases during autumn. For instance, for beech, in early autumn, its sensitivity to photoperiod is low and the growth is highly dependent on the nutrient status, so that fertilization (reducing the need of nutrient resorption) delays the date of LS. However, later in the autumn, the sensitivity of beech to photoperiod gradually increases, and LS is triggered even in fertilized saplings. The typical date of LS of naturally growing beech trees at the experimental site is near the end of October (~DOY 300) (Fu et al, 2012), and the sensitivity to photoperiod in beech can be high at this time (corresponding to 9.6 h of daylight in Antwerp). In our study, we found that adding nutrients significantly delayed LS to DOY 300 for saplings with early spring leaf-out but no such delay was observed in saplings with late leaf-out, which also senesced around DOY 300. These findings thus support our first hypothesis. Accordingly, photoperiod would help the trees to balance the trade-off between adaptation for survival (Hänninen 2016), i.e., preventing early frost damage, and adaptation for capacity, i.e., maximal use of the growth resources of the site. The flexible and variable sensitivity to photoperiod may represent a behavior common in nature, with organisms taking risks (e.g. early frost) when the risk is low (e.g. early autumn) but not when the risk is high (e.g. late autumn). This hypothesis therefore suggests that photoperiod represents a measure of risk, at least for the beech trees. However, the different dynamics shown by horse chestnut suggests that this hypothesis might be valid only for late-flushing species; or that, for horse chestnut, the photoperiodic threshold was not reached during the experiment. Finally, note that the photoperiod effect could not be directly investigated from the present in situ dataset because the exact photoperiodic cue (e.g. night length, radiation sum etc.) affecting senescence has not yet been determined (Liu et al, 2016). Further experimental investigations are thus needed to test this hypothesis.

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

Our third hypothesis suggests that the nutritional status of the leaves and trees influences the timing of LS, with a better nutritional status being associated with delayed senescence, possibly with less efficient nutrient resorption (Millard, 1996, Ono *et al.*, 2001, Withington *et al*, 2006). The mature beech trees from the RENECOFOR network shown that the foliar K content was significantly correlated with the partial correlation coefficients between spring leaf-out and leaf senescence, indicating that the foliar K content interacts with spring leaf-out to influence the LS dates. The mechanism of foliar K in determining LS needs further investigation, but evidence suggests that the availability of K can alter foliar longevity (Laclau *et al.*, 2009). K deficiency may indeed be involved in triggering early LS, fed back by sugar signaling (Wingler *et al.*, 2005), even though K deficiency has negative impacts on both photosynthesis and the export of sucrose from the leaves through the phloem (Cakmak, 2005). According to the third hypothesis, horse chestnut seems more sensitive to fertilization than beech as the delay of senescence caused by fertilization increased with the delay of leaf-out and was stronger for individuals that flushed late in the spring. The rate of LS of horse chestnut can therefore be further slowed by fertilization also in late autumn, because fertilization may increase foliar nutrient contents (Ordoñez *et al.*, 2009) and that increased carbon assimilation would still benefit the horse chestnut saplings in the latest part of the season (Campioli *et al.*, 2012).

Conclusions

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

Acknowledgments

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

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Figure legends

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

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

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

493 **Figure** 1

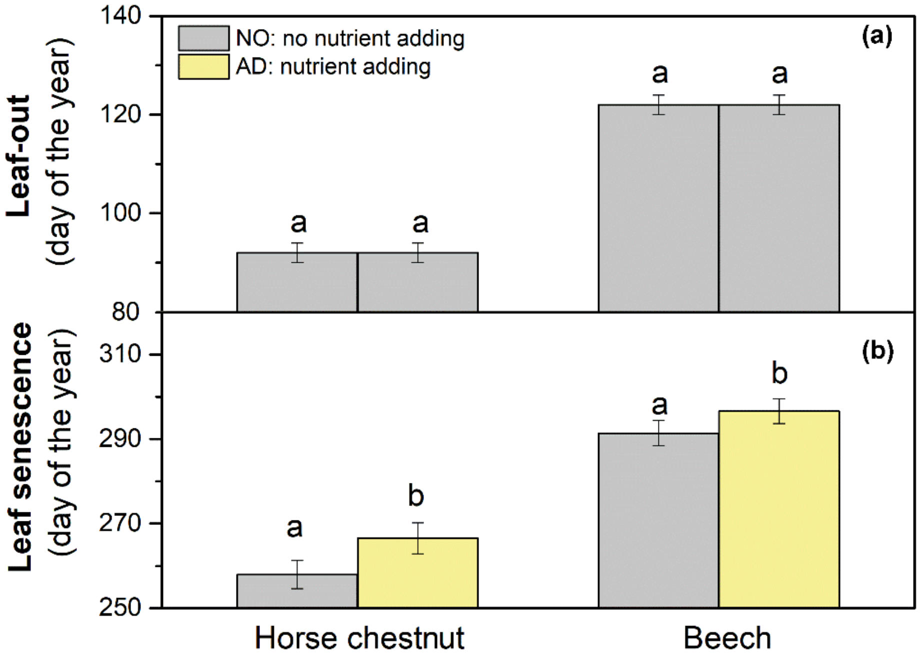

140
120
100
80
310
290
270
250
Leaf-out
(day of the year)
Leaf senescence
(day of the year)
NO: no nutrient adding
AD: nutrient adding
(a)
(b)
a
a
a
a
a
b
a
b
Horse chestnut
Beech

Figure 2

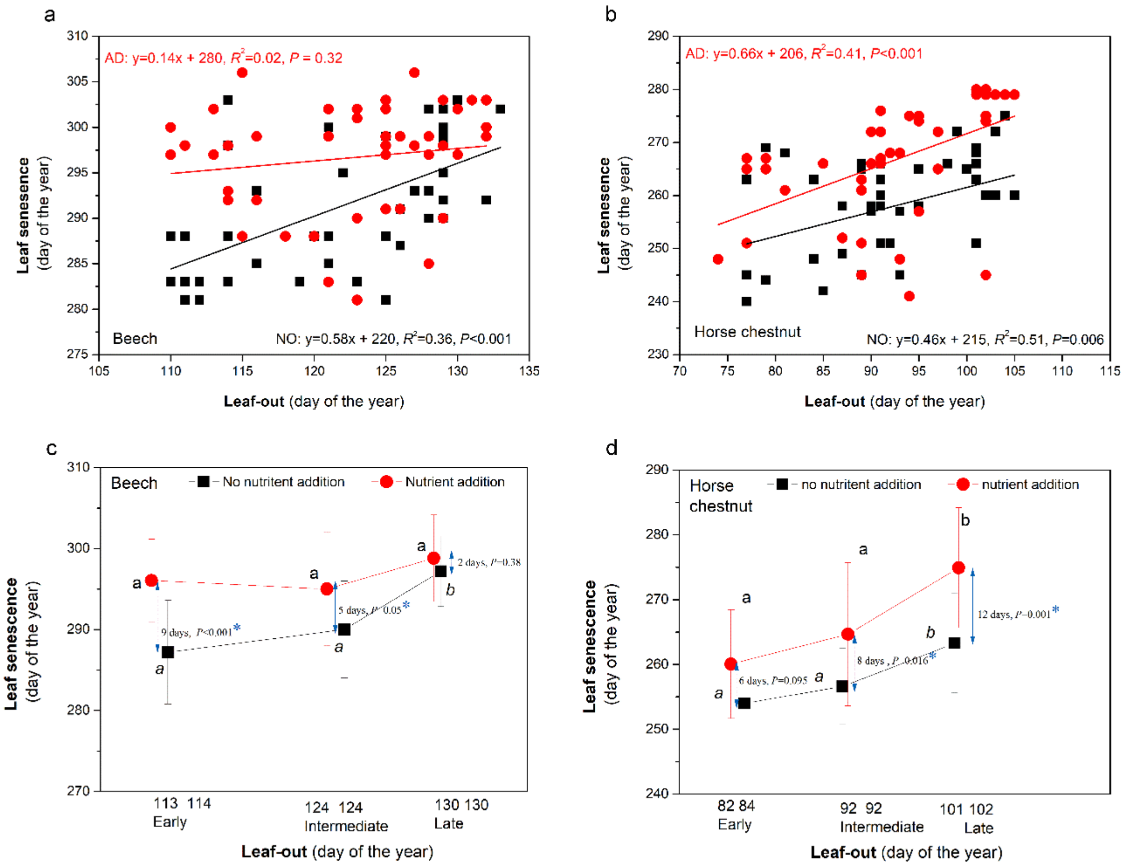
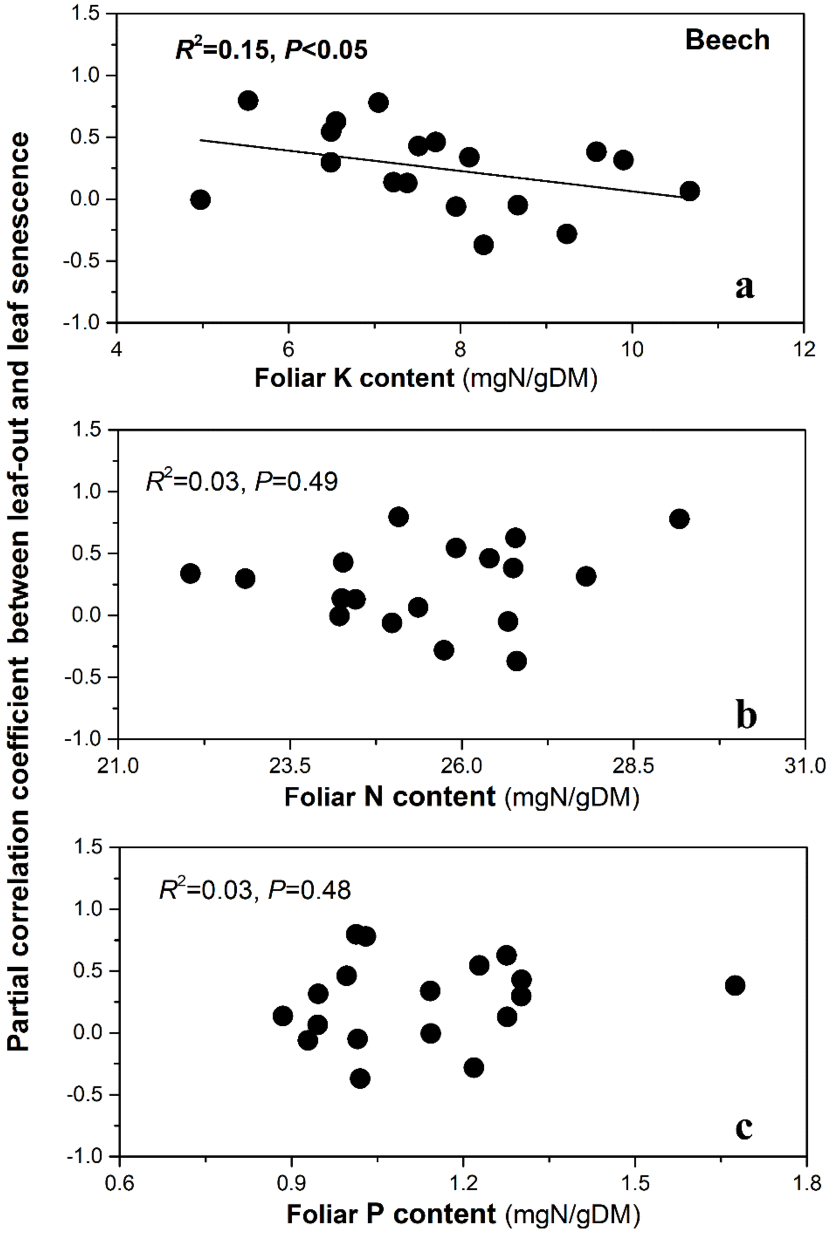

a
AD: y=0.14x + 280, R^2=0.02, P = 0.32
Beech
NO: y=0.58x + 220, R^2=0.36, P<0.001
Leaf senescence (day of the year)
310
305
300
295
290
285
280
275
105
110
115
120
125
130
135
Leaf-out (day of the year)
b
AD: y=0.66x + 206, R^2=0.41, P<0.001
Horse chestnut
NO: y=0.46x + 215, R^2=0.51, P=0.006
Leaf senescence (day of the year)
290
280
270
260
250
240
230
70
75
80
85
90
95
100
105
110
115
Leaf-out (day of the year)
c
Beech
■ No nutritent addition
● Nutrient addition
a
a
a
a
a
b
9 days, P<0.001*
5 days, P<0.05*
2 days, P=0.38
Leaf senescence (day of the year)
310
300
290
280
270
113 114
Early
124 124
Intermediate
130 130
Late
Leaf-out (day of the year)
d
Horse chestnut
■ no nutritent addition
● nutrient addition
a
a
b
a
a
b
6 days, P=0.095
8 days, P=0.016*
12 days, P=0.001*
Leaf senescence (day of the year)
290
280
270
260
250
240
82 84
Early
92 92
Intermediate
101 102
Late
Leaf-out (day of the year)

Figure 3

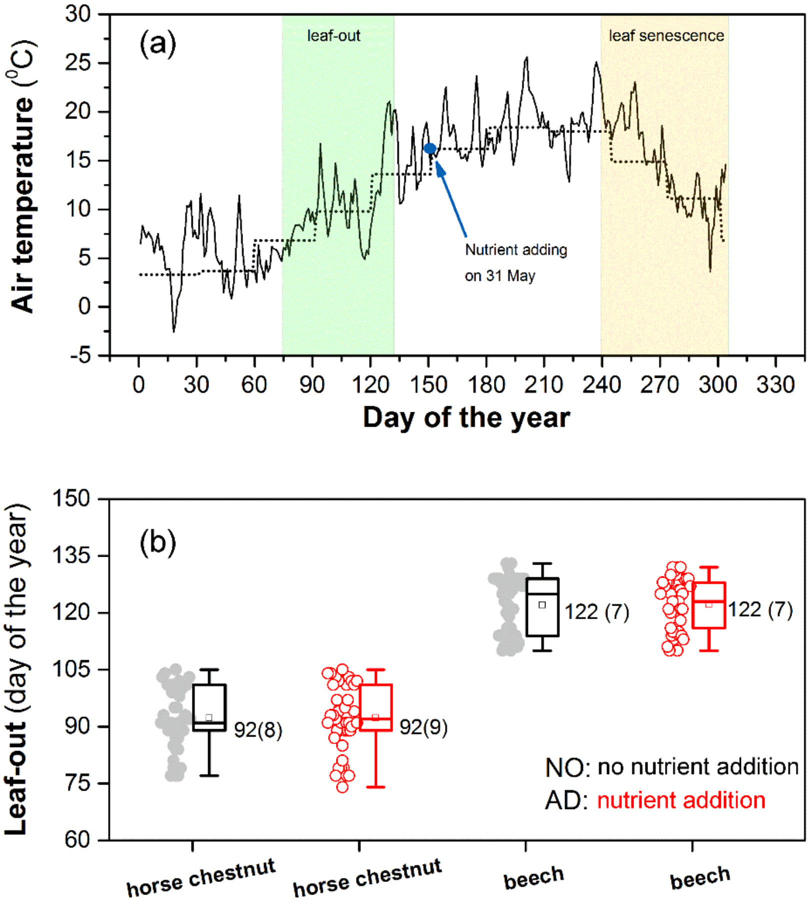

Partial correlation coefficient between leaf-out and leaf senescence
Beech
R^2=0.15, P<0.05
a
Foliar K content (mgN/gDM)
4
6
8
10
12
1.5
1.0
0.5
0.0
-0.5
-1.0
R^2=0.03, P=0.49
b
Foliar N content (mgN/gDM)
21.0
23.5
26.0
28.5
31.0
R^2=0.03, P=0.48
c
Foliar P content (mgN/gDM)
0.6
0.9
1.2
1.5
1.8

Supporting tables and figures

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

Site	Acronym	Latitude	Lontitude	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

Figure S1. (a) The daily mean air temperature (solid line) and the ranges of the leaf-out and leaf senescence dates of the studied saplings (green and yellow shading, respectively) during the study period. The dotted line represents the long-term mean monthly temperature at the experimental site. Nutrients were added to the trees on 31 May 2016. (b) Distribution and plot boxes of the leaf-out dates for saplings of horse chestnut and beech in treatments with (AD, open 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.

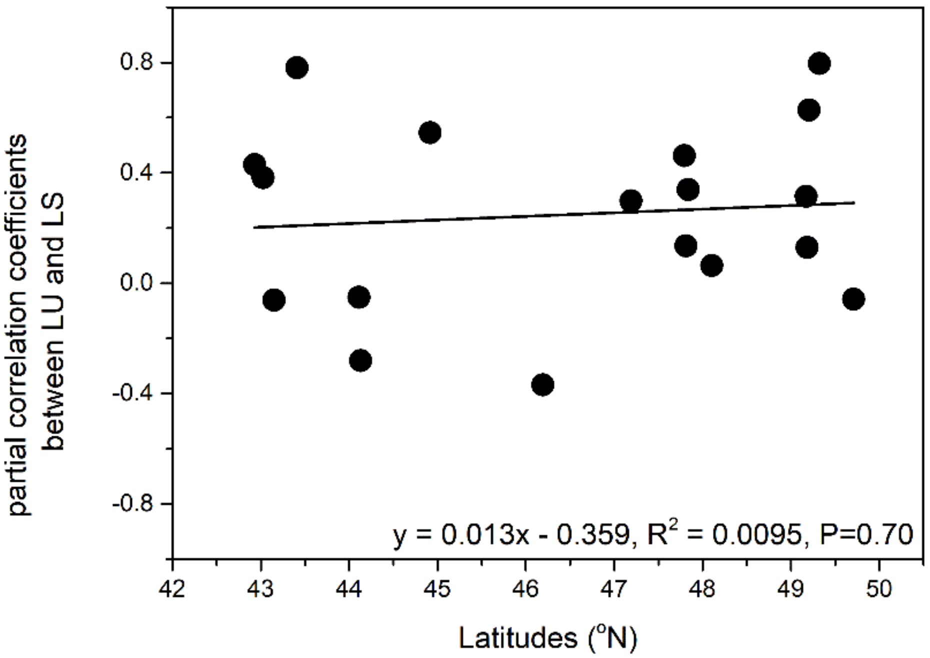


Figure S3. Partial correlation coefficients of leaf senescence and leaf out data, controlling for the impact of autumn temperature, established for each study site of RENECOFOR. The acronym of each site is provided on the left. The diamond symbol at the bottom of the figure ("summary") indicates the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