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

1 **Title:** Nutrient availability alters the correlation between spring leaf-out
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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.

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 &

4

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^{-2},

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.

Testing the effect of foliar nutrient content on the correlation between leaf-out and

146

senescence in mature trees

147

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

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 ± 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 (Campioli *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**

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469

470   **Figure legends**

471

472   Figure 1. Mean dates (± 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 $P<0.05$.

475

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.
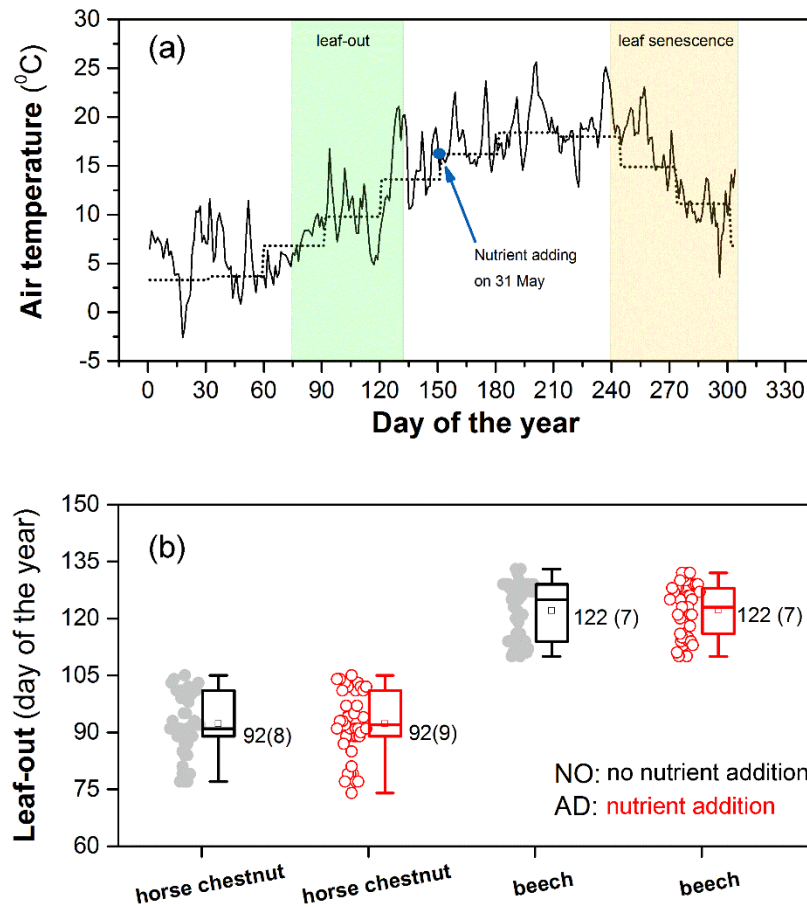
Figure 1

494 **Figure 2**
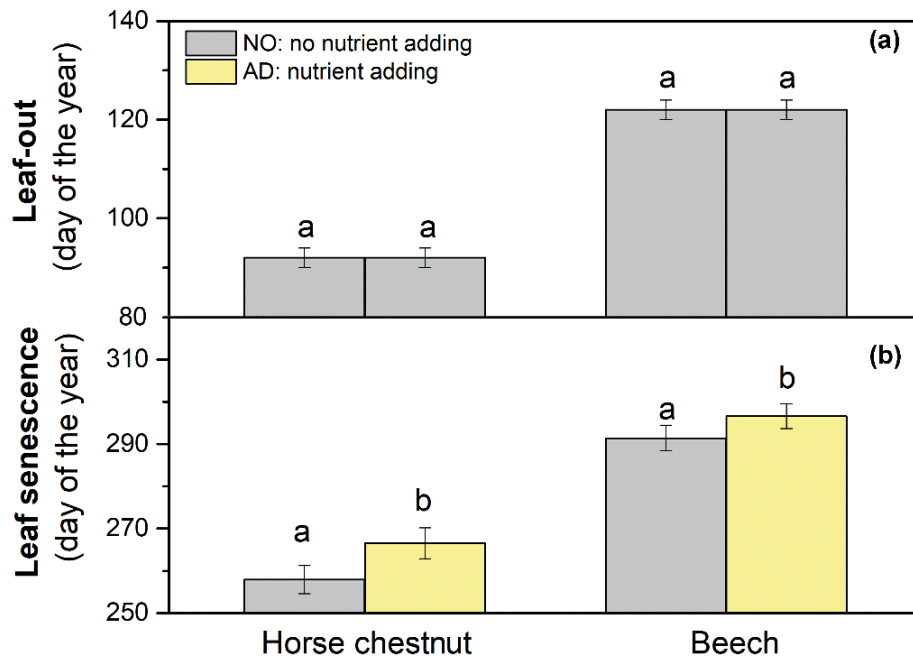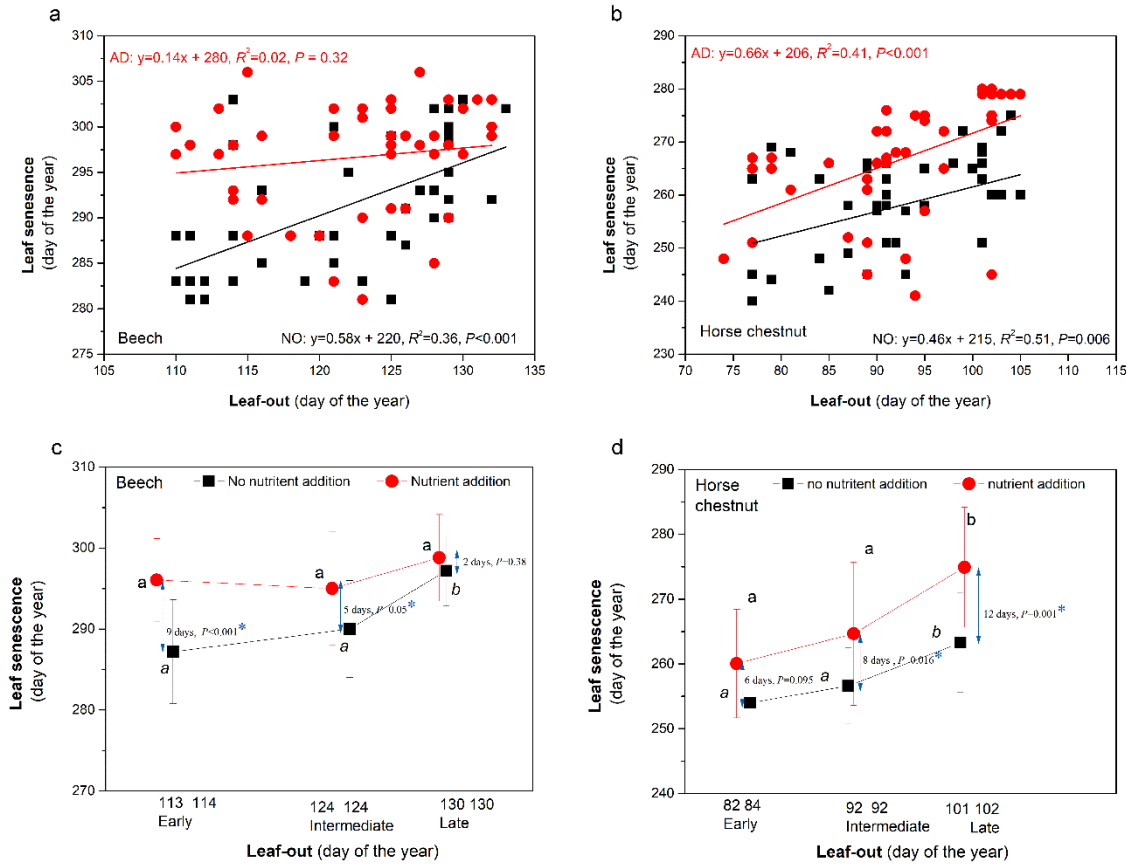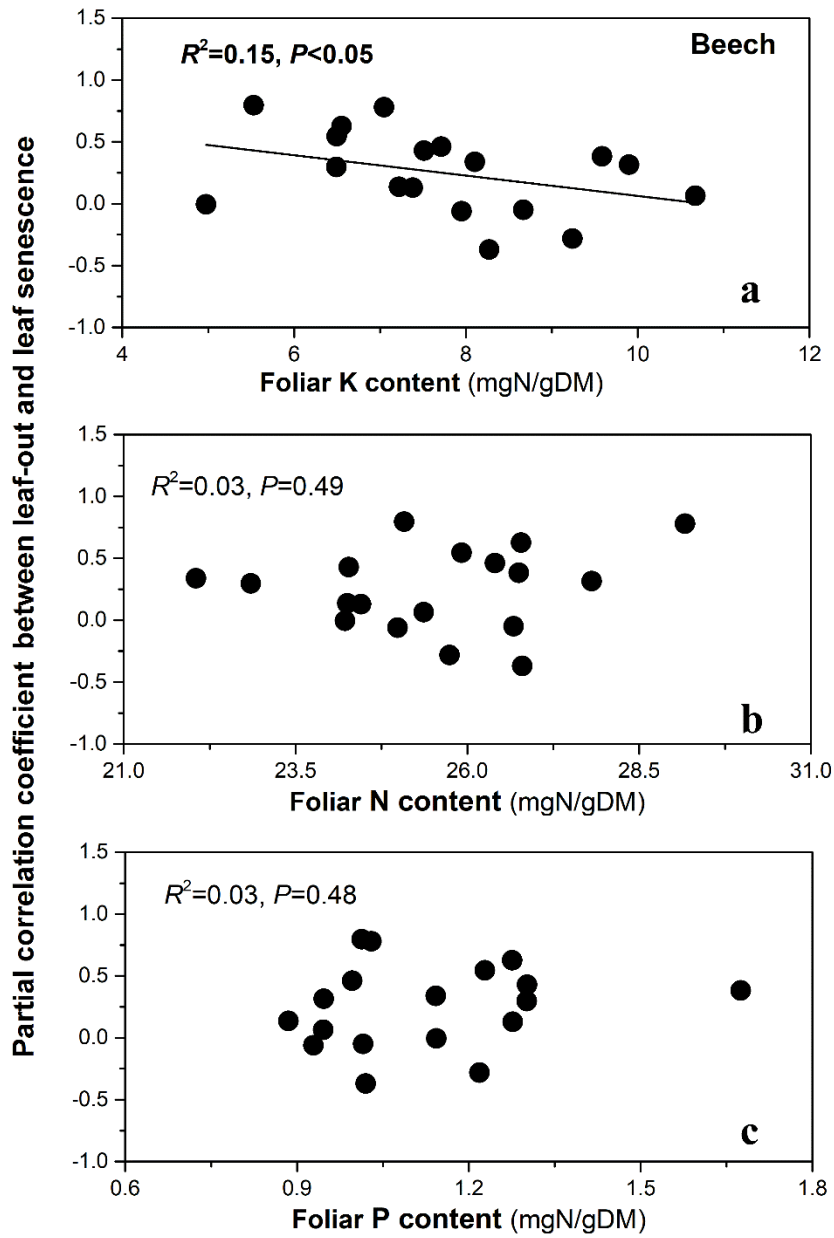
Supporting tables and figures

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	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

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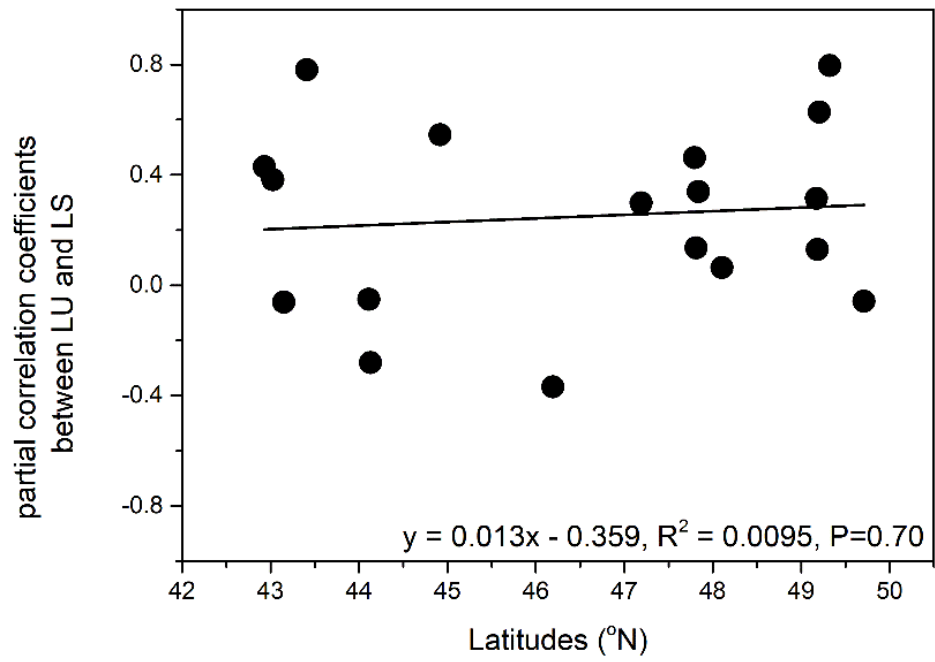
511   Figure S3. Partial correlation coefficients of leaf senescence and leaf out data, controlling for the
512   impact of autumn temperature, established for each study site of RENECOFOR. The
513   acronym of each site is provided on the left. The diamond symbol at the bottom of the
514   figure ("summary")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