This item is the archived peer-reviewed author-version of:

Larger temperature response of autumn leaf senescence than spring leaf-out phenology

Larger temperature response of autumn leaf senescence than spring leaf-out phenology

Yongshuo H. Fu[1,2*], Shilong Piao[3,4], Nicolas Delpierre[5], Fanghua Hao[1],

Heikki Hänninen[6], Yongjie Liu[2], Wenchao Sun[1], Ivan A. Janssens[2], Matteo Campioli[2]

[1] College of water sciences, Beijing Normal University, Xinjiekouwaidajie 19, 100875, Beijing, China

[2] Centre of Excellence PLECO (Plant and Vegetation Ecology), Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

[3] Sino-French Institute for Earth System Science, College of Urban and Environmental Sciences, Peking University, Beijing 100871, China

[4] Institute of Tibetan Plateau Research, Center for Excellence in Tibetan Earth Science, Chinese Academy of Sciences, Beijing 100085, China

[5] Ecologie Systématique Evolution, Univ. Paris-Sud, CNRS, Agro Paris Tech, Université Paris, Saclay, 91400 Orsay, France

[6] Zhejiang A&F University, State Key Laboratory of Subtropical Silviculture, Huanbei Road No. 88, 311300 Hangzhou, China

*Corresponding author: Yongshuo H. Fu, Tel: +32-651721, Email: Yongshuo.fu@uantwerpen.be

Manuscript for *Global Change Biology*

Abstract

Climate warming is substantially shifting the leaf phenological events of plants, and thereby impacting on their individual fitness and also on the structure and functioning of ecosystems. Previous studies have largely focused on the climate impact on spring phenology, and to date the processes underlying leaf senescence and their associated environmental drivers remain poorly understood. In this study, experiments with temperature gradients imposed during the summer and autumn were conducted on saplings of European beech to explore the temperature responses of leaf senescence. An additional warming experiment during winter enabled us to assess the differences in temperature responses of spring leaf-out and leaf senescence. We found that warming significantly delayed the dates of leaf senescence both during summer and autumn warming, with similar temperature sensitivities (6 - 8 days delay per °C warming), suggesting that, in the absence of water and nutrient limitation, temperature may be a dominant factor controlling the leaf senescence in European beech. Interestingly, we found a significantly larger temperature response of autumn leaf senescence than of spring leaf-out. This suggests a possible larger contribution of delays in autumn senescence, than of the advancement in spring leaf-out, to extending the growing season under future warmer conditions.

Introduction

Plant phenology is the study of periodic plant life cycle events, and how these are influenced by seasonal and interannual variations in climate (Lieth, 2013). Plant phenology is one of the most reliable biological indicators of anthropogenic climate change (Parmesan & Yohe, 2003, Root *et al.*, 2003, Walther *et al.*, 2002), and changes in plant phenology impact on individual fitness, species distribution, interspecific interactions, ecosystem structure and function, as well as on feedbacks to the climate system (Chuine *et al.*, 2010, Peñuelas & Filella, 2009, Piao *et al.*, 2007, Thackeray *et al.*, 2016, Zeng *et al.*, 2017). Therefore, understanding the processes underlying plant phenology is essential to improve our understanding of plant and ecosystem responses to the ongoing climate change.

Plant phenology research has grown tremendously over the past four decades (Hänninen, 2016, IPCC, 2014, Wolkovich & Ettinger, 2014). Most studies, however, have addressed spring phenological events, such as budburst, leaf-out and flowering (Fu *et al.*, 2015, Richardson *et al.*, 2013), while autumn phenological events, such as leaf senescence, have been paid comparatively less attention (Gallinat *et al.*, 2015, Panchen *et al.*, 2015). One probable reason for this is the difficulty to accurately acquire leaf senescence observations in natural conditions. However, as the final stage of the leaf's life cycle and as adaptive strategy to unfavorable environmental conditions of temperate and boreal plant species (Chabot & Hicks, 1982), leaf senescence is critical to plants' fitness as well as to ecosystem functions (Estiarte & Peñuelas, 2015, Piao *et al.*, 2008, Rohde & Bhalerao, 2007).

The main function of autumn leaf senescence is to recycle nutrients from senescing leaves and transport them to other plant tissues to support growth during the following spring (Chapin III,

69   1980, Killingbeck, 1996, Maillard *et al.*, 2015). This nutrient-conservation mechanism increases

70   the fitness of individual plants, especially in nutrient poor environments (Chapin III, 1980, May

71   & Killingbeck, 1992). Generally, more than half of the leaf macro-nutrients, such as N and P,

72   are being resorbed during the leaf senescence process, although the nutrient resorption efficiency

73   varies widely among species and elements (Aerts, 1996, Freschet *et al.*, 2010, Wright &

74   Westoby, 2003). Apart from its influence on nutrient cycles, the timing of leaf senescence

75   influences the ecosystem carbon balance by modulating the length of the photosynthetically

76   active period (Myneni *et al.*, 1997, Richardson *et al.*, 2010). Leaf senescence may even play a

77   more critical role than spring phenology in determining the length of the photosynthetically

78   active period (Garonna *et al.*, 2014, Wu *et al.*, 2013). Understanding the response of leaf

79   senescence to climate change is therefore important. However, to date, the processes underlying

80   autumn leaf senescence, their associated environmental controls and the response of leaf

81   senescence to climate change are still poorly understood.

82

83   Photoperiod has generally been proposed as the primary driver of leaf senescence, with a critical

84   photoperiodic threshold, i.e., a critical day length below which leaf senescence is triggered

85   (Lagercrantz, 2009, Wareing, 1956, Way & Montgomery, 2015, Welling & Palva, 2006). For

86   example, the autumnal senescence in mature European aspen occurs every year on almost the

87   same date (Fracheboud *et al.*, 2009). However, photoperiod is not consistently important across

88   species and even sites. For example, a recent study reported that leaf senescence is not triggered

89   by photoperiod across 116 European aspen natural genotypes (Michelson *et al.*, 2017),

90   suggesting that other environmental factors must be involved in driving the leaf senescence

91   process. In line with this finding, many studies have suggested that temperature interacts with

92   photoperiod to control the leaf senescence process (Chung *et al.*, 2013, Hänninen & Tanino,

93   2011, Heide & Prestrud, 2005, Liu *et al.*, 2016a, Tanino *et al.*, 2010), and that temperature can

94   be even the main controlling factor of leaf senescence (Chmielewski & Rötzer, 2001, Estiarte &

95   Peñuelas, 2015, Heide & Prestrud, 2005, Xie *et al.*, 2015), in particular autumn temperature

96   (Delpierre *et al.*, 2009). However, these results are not conclusive because other studies have

97   shown that autumnal senescence is only weakly affected by air temperature (Čufar *et al.*, 2012,

98   Olsson & Jönsson, 2015, Sparks & Menzel, 2002). In addition to photoperiod and temperature,

99   also other environmental factors have been reported to influence the leaf senescence process.

100 These include light intensity (Liu *et al.*, 2016b), precipitation and soil water conditions (see

101 review in Estiarte and Peñuelas (2015)) and soil nutrient status (Sigurdsson, 2001, Weih, 2009).

102 Furthermore, a positive correlation was recently reported between spring leaf-out dates and

103 autumn leaf senescence dates, suggesting that the factors regulating the leaf-out days are carried

104 over to affect leaf senescence in the following autumn (Fu *et al.*, 2014, Signarbieux *et al.*, 2017).

105 Thus, the literature remains inconsistent about the determinants of autumn leaf senescence

106 (Estiarte & Peñuelas, 2015), so that well-designed experiments are needed to investigate and

107 better understand the leaf senescence process.

108

109 Current studies of leaf senescence are generally based on either species-specific long-term in situ

110 observations (Menzel *et al.*, 2006, Panchen *et al.*, 2015), or on remote-sensing based

111 observations (Garonna *et al.*, 2014, Julien & Sobrino, 2009, Liu *et al.*, 2016b, Shen *et al.*, 2015,

112 Xie *et al.*, 2015, Jeong *et al.*,2011). While manipulation experiments have been conducted, only

113 few have studied the autumn phase in relation to climate change, as opposed to spring

114 (Wolkovich *et al.*, 2012). Furthermore, these few-experimental autumn phenology studies were

115   designed with only one or two warming treatments   (Gunderson *et al.*, 2012, Marchin *et al.*,

116   2015, Morin *et al.*, 2010, Norby *et al.*, 2003). How leaf senescence responds to a temperature

117   gradient, whether summer and autumn warming influence leaf senescence differently, and

118   whether autumn phenology has the same temperature sensitivity as spring phenology, to our

119   knowledge, has not yet been experimentally investigated.

120

121   In the present study, we therefore carried out gradient-warming/cooling manipulation

122   experiments using two-year old and one-meter-high saplings of *Fagus sylvatica* L. (European

123   beech), a widespread deciduous forest tree species in temperate Europe. Specifically, we exposed

124   the saplings to either summer or autumn warming. The primary objectives of this study were (1)

125   to quantify the temperature sensitivity of leaf senescence date (St, changes in days per °C

126   warming); (2) to investigate the effect of summer versus autumn warming on leaf senescence

127   dates, and (3) to compare St of autumn senescence with that of spring leaf-out.

128

129   **Materials and methods**

130   **Study site and climate chambers**

131   The experiment was conducted in 12 climate-controlled transparent chambers at the Drie Eiken

132   campus of the University of Antwerp (Belgium, 51º19′N, 4º21′E). The long-term mean annual

133   air temperature is 9.6 ºC, and mean monthly air temperatures vary from 2.2 ºC in January to 17.0

134   ºC in July. Annual precipitation averages 780 mm, being uniformly distributed throughout the

135   year (Campioli *et al.*, 2012). The chambers could be artificially warmed/cooled by a centralized

136   heating/cooling system ensuring different levels of continuous (day and night) warming or

137   cooling above/below the fluctuating ambient air temperature (Fu *et al.*, 2016). Each chamber

138   could accommodate 12 saplings. Temperature sensors (Siemens, type QFA66, Berlin, Germany)

139   were used to continuously monitor the air temperature inside each chamber, logging every 30

140   minutes and storing hourly data. Here, we combine the results from three different experiments

141   performed in the climate chambers using beech saplings of the same provenance and size.

142

143   **Experimental design and leaf senescence measurements**

144   *Tree material.* The experiments used 2-year-old and one-meter-high saplings of *Fagus sylvatica*

145   L. grown from seeds of the same origin and cultivated in the same field for one year at a

146   commercial nursery. We transplanted the saplings into plastic pots (diameter 25 cm, depth 40

147   cm). The pots were moved into the climate-controlled chambers during early summer, late

148   summer or winter (see below). The pots were filled with a substrate that was created by

149   combining potting soil and Lommel sand (grain size <1 mm diameter), bought from commercial

150   suppliers (Van den Broeck and Jos Meeussen & Zoon bvba) in Belgium. In experiment 1 and 2,

151   slow release fertilizer (100 g·m^{-2}, 13–10–20 for N, P, and K, respectively, all in percentage) was

152   added in end of May to each sapling. Over the growing period, the saplings were watered at least

153   three times per week to ensure no water limitation.

154

155   *Experiment 1 (temperature treatment during summer, targeting leaf senescence)* (Fig. 1). In this

156   experiment, one control treatment (+0°C) and three temperature treatments were applied: +1°C,

157   +3°C and +4°C. Two to four replicate chambers were used, except for the +4°C treatment, for

158   which data from only one chamber were available because one chamber failed. At summer

159   solstice (21 June 2016), four saplings were placed in each chamber. The saplings were moved

160 out at the "end of summer", i.e., on 15 August 2016. In total, 9 chambers and 36 saplings were

161 used in this experiment.

162

163 *Experiment 2 (temperature treatment during autumn, targeting leaf senescence)* (Fig. 1). In this

164 experiment, we applied one control treatment (+0°C), one cooling treatment (-1°C) and one

165 warming treatment (+1°C), using three replicated chambers for each treatment. Four saplings

166 were exposed to the treatment in each chamber during the autumn period, i.e. from 15 Aug 2016

167 to leaf senescence. In total, 9 chambers and 36 saplings were used in this experiment.

168

169 *Experiment 3 (temperature treatment during winter-spring, targeting leaf-out)*. In winter-spring

170 2016, we conducted a separate warming experiment on the spring leaf-out phenology. Four

171 saplings were moved into each of the twelve climate-controlled chambers on 1 January 2016.

172 During the experiment, five warming temperature treatments, i.e. +1°C, +2°C, +3°C, +4°C and

173 +5°C (two chambers per treatment) and a control treatment (+0°C) were applied. In total, 12

174 chambers and 48 saplings were used in this experiment.

175

176 The warming/cooling provided was generally stable for the experiments 1 and 3 (actual warming

177 was on average ± 10% of the prescribed value; see below for description of the experiments).

178 However, for experiment 2 (see below) the warming during autumn was less stable (within ±

179 20%). This inaccuracy was not considered crucial, because our main analyses were based on the

180 actually realized warming/cooling (e.g. regression analysis, see below), not the envisaged

181 warming.

182

Observation of leaf senescence and leaf-out

Leaf senescence was monitored following Vitasse *et al.* (2009). In detail, the number of leaves with autumn colour was determined visually and the percentage of them, out of the total number of leaves, was calculated. The senescence date was defined as the date when 50% of the leaves had autumn colour. Leaf-out date was defined as the day when the entire leaf blade and the leaf stalk were visible on the terminal buds (Fu *et al.*, 2016).

Cooling degree hours

To evaluate the relationships between leaf senescence and air temperature, we calculated cooling degree hours (CDH) as the sum of hourly temperature below a temperature threshold (T_{base}) during the study period, i.e., from summer solstice (21 June) to the day of leaf senescence (LS) (Dufrêne *et al.*, 2005). The T_{base} was set at 25°C, according to a previous study on *Fagus sylvatica* (Delpierre *et al.*, 2009):

$$CDH = \sum_{start}^{LS} (T_{base} - T_{hourly}) \text{ if } T_{hourly} < T_{base}$$

We also tested the use of a higher T_{base}, i.e. 30°C, but found very similar results, and therefore only reported the results based on 25°C in the main text.

Data analysis

The temperature responses of leaf senescence and leaf-out were evaluated using linear regression based on the average dates obtained from the four saplings per chamber. The temperature sensitivity of leaf senescence and leaf-out were defined as the slopes of the linear regression between dates and the actual temperature change in the chambers. Independent samples *t*-tests were used to evaluate the difference between leaf senescence, or leaf-out dates, as well as the

205 accumulated CDH, among different temperature treatments. Differences in the temperature

206 sensitivity of leaf senescence between autumn cooling and autumn warming, and between

207 summer and autumn, as well as in the temperature sensitivity between leaf senescence and leaf-

208 out, were tested using ANCOVA, i.e. testing the slopes and intercepts. All statistical analyses

209 were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

210

211 **Results**

212 **Leaf senescence response to experimental warming and cooling**

213 Leaf senescence dates were significantly delayed by the warming treatments, but were

214 significantly advanced by cooling (both $P<0.05$), as compared to the control treatment (Fig. 1a).

215 Although both summer and autumn warming significantly delayed the leaf senescence dates,

216 compared to the control, a larger delay (14 days on average) was found under summer warming

217 treatments than under autumn warming treatments (11 days on average) (Fig. 1a). As opposed to

218 the warming treatments, cooling significantly advanced the dates of leaf senescence, by 3 days

219 on average ($F=9.8$; $P=0.005$; Fig. 1a).

220

221 **Temperature sensitivity of leaf senescence and leaf-out**

222 In the previous paragraphs the phenology responses were given, independent of the intensity of

223 the warming or cooling. This paragraph aims to render these treatments more comparable by

224 expressing all phenology changes on a per °C basis. By using a relative variable, we aim to

225 removing the influence of different periods (summer vs. autumn) and exposure times to warming.

226 On average, a rise of air temperature by one degree delayed the leaf senescence date by 6.4 ± 1.1

227 days, and the difference in the temperature sensitivity of leaf senescence (St, delay in days per °C

228 warming) between autumn warming treatment (St_autumn, 8.3 ± 1.1 days °C-1) and summer

229 warming treatment (St_summer, 6.1 ± 0.8 days °C^{-1}) was not statistically significant (P=0.75,

230 Fig. 1b). The absolute St values of leaf senescence during autumn warming and autumn cooling

231 (-6.7 ± 1.0 days °C^{-1} for cooling treatments) also did not differ statistically significantly (Fig. 2).

232

233 Warming significantly advanced the date of leaf-out in spring, with 4.5 ± 0.5 days advancement

234 per degree Celsius warming (Fig. 3a). Compared to the autumn leaf senescence (delay of 8.3 ±

235 1.1 days °C^{-1}), the temperature sensitivity of leaf-out was thus significantly lower (Fig. 3b),

236 suggesting a larger effect of climate warming on autumn leaf senescence than on spring leaf-out

237 phenology.

238

239 **Correlation between leaf senescence and cooling degree hours**

240 No significant correlation was found between leaf senescence dates and CDH that were

241 accumulated from 21 June to the day of leaf senescence across the temperature treatments

242 (R^2=0.09, P=0.12, Fig. 4). In addition, the CDH requirement was not significantly different

243 between the two autumn treatments, i.e., 14677K and 13067K for autumn warming treatment

244 and autumn cooling treatment, respectively. However, the CDH requirement of saplings in the

245 summer warming treatment was statistically significantly lower than in the autumn warming and

246 autumn cooling treatments (P<0.05).

247

248 **Discussion**

249 Previous studies have highlighted the ambiguous nature of the warming response of leaf

250 senescence (Gunderson *et al.*, 2012, Heide & Prestrud, 2005, Menzel *et al.*, 2006), and

251   attributed this to the limited availability of long-term datasets, the difficulty of quantifying the

252   exact date of leaf senescence under natural conditions, and the lack of focused experimental

253   studies designed to understand the leaf senescence response to temperature. For temperate trees

254   under favorable conditions, i.e., without water or nutrient stress, it is generally assumed that the

255   leaf senescence process is mainly triggered by photoperiod and temperature (Lieth, 2013, Way &

256   Montgomery, 2015). Some studies reported a delayed trend of leaf senescence with climate

257   warming, based on in situ observations (Delpierre *et al.*, 2009, Vitasse *et al.*, 2011), remote

258   sensing observations (Liu *et al.*, 2016b, Reed *et al.*, 2009, Stöckli & Vidale, 2004), as well as

259   open top chamber-based field warming experiments (Gunderson *et al.*, 2012). In contrast, other

260   studies reported insignificant responses or even advanced senescence with climate warming

261   (Norby *et al.*, 2003, Xie *et al.*, 2015), which may be related to warming-induced drought stress

262   (Xie *et al.*, 2015). Based on gradient warming experiments, in which drought was excluded, our

263   study clearly revealed that warming significantly delays the timing of leaf senescence in

264   European beech saplings in both summer and autumn warming, with even more than 30 days

265   delay under the +4°C treatment (4°C higher than ambient). This suggested that, under sufficient

266   water and nutrient conditions, temperature may be more important than photoperiod in

267   controlling the leaf senescence process under temperate latitudes. In fact, if there was a

268   photoperiod threshold, this would have been overpassed by up to 30 days. Note that we found a

269   larger delay (14 days on average) under summer warming treatments than under autumn

270   warming treatments, this was, however, maybe largely attributed to the more intensive warming

271   treatment that was applied during summer (warmed up to ca. 4°C) than during autumn (warmed

272   up to ca. 1°C) (see Fig. 1b). We also did not find a statistical difference of the temperature

273   sensitivity of leaf senescence between autumn warming and autumn cooling treatments. This

274   may be due to the limited sample size in this study, i.e. only one cooling treatment and one

275   warming treatment during autumn, and therefore this conclusion needs to be further studied. In

276   addition, we found a larger temperature sensitivity of leaf senescence, with 6-8 days delay per

277   degree Celsius warming, as opposed to 2-7 days delay per °C warming found in earlier

278   experimental studies (Gunderson *et al.*, 2012, Han *et al.*, 2014, Nakamura *et al.*, 2010). This

279   difference might be related to species differences, to differences in the local environment, as well

280   as to the different experimental designs, i.e., only one or two warming treatments in the previous

281   studies versus gradient warming/cooling in the present study.

282

283   Surprisingly, there were no statistically significant differences in the sensitivity of the leaf

284   senescence process to summer and autumn warming. This implies that the positive impacts of

285   warming on leaf physiology, such as delayed chlorophyll degradation (Fracheboud *et al.*, 2009),

286   leading to delayed leaf senescence at the end of the growing period, does not depend on the

287   seasonal timing of the temperature elevation. Nonetheless, warming may affect different

288   processes during summer (e.g. predominantly cell division and expansion) than during autumn

289   (cell maturation and lignification). Furthermore, warming might affect different phases of the

290   leaf senescence process when applied in summer versus autumn. During summer, warming

291   might delay the leaf senescence onset, whereas autumn warming might slow down the

292   progression rate of the leaf senescence (Fracheboud *et al.*, 2009). These different aspects (delay

293   in leaf senescence onset vs. slowdown of leaf senescence rate) cannot be independently assessed

294   with the coloration method we used.

295

296 The lower CDH requirement associated with the summer treatment should be related to more

297 intense warming in summer than in autumn. We do not believe that the low CDH requirement in

298 summer is related to differences in the leaf senescence date as summer warming elicited, on

299 average, later leaf senescence than autumn warming, which should have caused a larger CDH.

300 The timing of leaf senescence simulated by cooling degree days-based models has been

301 compared in earlier studies with in situ observations (Archetti *et al.*, 2013, Delpierre *et al.*, 2009,

302 Jolly *et al.*, 2005, Vitasse *et al.*, 2011). Consistent with the assumption of degree days-based

303 models, we found that the differences in the cooling degree hours (CDH) required for leaf

304 senescence between the two autumn treatments were statistically insignificant. Furthermore,

305 good model performances were found in boreal tree species (Koski & Selkäinaho, 1985,

306 Partanen, 2004, Viherä-Aarnio *et al.*, 2005). However, contrary to the results of the present study,

307 these studies found that warming during summer and autumn would advance, not delay, the

308 timing of leaf senescence. Possibly, this opposite temperature response is attributable to

309 differences among boreal and temperate-zone species. Jeong and Medvigy (2014) reported a

310 nonlinear temperature sensitivity of leaf senescence using many ground observations and

311 suggested that warmer regions may have a larger temperature sensitivity than cooler regions. In

312 addition, recent studies have reported a positive correlation between spring leaf-out and leaf

313 senescence dates in trees (Fu *et al.*, 2014, Signarbieux *et al.*, 2017), delayed senescence

314 following exceptionally late spring greening in sub-arctic grasslands (Leblans *et al.*, 2017), and

315 the performance of senescence models was substantially improved by incorporating this legacy

316 effect.

317

318   Interestingly, we found a larger temperature sensitivity (St) of autumn leaf senescence than

319   spring leaf-out using European beech saplings of the same age. Contrasting conclusions were

320   obtained from a meta-analysis of observations on mature trees from the European phenology

321   network, which reported a larger St of spring leaf-out (4.6 ± 0.07 days $^{\circ}C^{-1}$) than of autumn leaf

322   colouring (1.0 ± 0.4 days $^{\circ}C^{-1}$) across plant species (Menzel *et al.*, 2006). This difference may be

323   attributable to the species-specific differences in the phenology response to temperature

324   (Panchen *et al.*, 2015, Richardson *et al.*, 2006, Vitasse *et al.*, 2009). However, similar finding

325   was reported on *Quercus variabilis* seedlings in an open-field warming experiment (Han *et al.*,

326   2014), and on mature beech trees in an altitude gradient (Vitasse *et al.*, 2009). The difference in

327   temperature sensitivity of leaf-out and leaf senescence are likely related to the differential

328   processes between spring and autumn phenology. Concerning spring leaf-out (particularly for

329   diffuse porous species like beech), temperature impacts the end of bud dormancy and the speed

330   of leaf unfolding but no other trees organ (which are inactive before budburst) (*Delpierre et al*.,

331   2016). On the other hand, in autumn, temperature impacts both the leaf physiological status

332   (chlorophyll content, photosynthesis, pigment degradation etc) and tree growth (e.g. wood

333   lignification, fine root growth). The latter reduction of carbon sink activity at the tree scale may

334   be an additional, overlooked trigger of leaf senescence. These interactions might affect leaf

335   senescence onset, in other words not only leaf status but also (and maybe primarily) a lack of

336   sink activity might trigger leaf senescence (see hypothesis in Fu et al., 2014). Therefore, it is

337   logical that temperature has a strong effect on autumn phenology, which, as showed by our data,

338   and even can be stronger than that on spring phenology.

339

340 Given the larger warming response of leaf senescence than of spring leaf-out found in the present

341 study, under future climate warming conditions we can expect a larger contribution of the delay

342 in autumn senescence dates to the extension of photosynthetic season than of the earlier spring

343 leaf-out. Thus, warming induced changes in leaf senescence could play an important role in the

344 ecosystem carbon balance (Keenan *et al.*, 2014, Piao *et al.*, 2008). However, delayed leaf

345 senescence in response to warmer summers-autumns may increase the risk of extreme events

346 such early-frost damage to leaves (Augspurger, 2013, Hänninen, 2016, Inouye, 2008), which

347 would hamper the nutrient resorption. This can lead to reduced nutrient reserves to support next

348 season's growth, and subsequentially impact the ecosystem carbon and nutrient cycles (Estiarte

349 & Peñuelas, 2015, Fracheboud *et al.*, 2009). Finally, note that considering the legacy effect of

350 leaf-out on the leaf senescence dates (Fu *et al.*, 2014), the delays in leaf senescence as observed

351 in our experiments might be partially offset by the earlier spring leaf-out in response to warmer

352 winters.

353

354 The underlying physiological processes of leaf senescence and their environmental cues,

355 especially the interactive effect of temperature and photoperiod, are still unclear. Moreover,

356 warming responses of leaf senescence largely differ between natural observations and warming

357 experiments (Wolkovich *et al.*, 2012), ontogenetic differences have been reported between

358 saplings and mature trees (Mediavilla *et al.*, 2014, Vitasse, 2013), and species variability exists

359 in response to warming (Parmesan & Hanley, 2015, Primack *et al.*, 2015). Nonetheless, our

360 study provides important insights. Taking advantage of temperature manipulative experiments,

361 we found that, in the absence of water and nutrient limitation, temperature is a dominant factor

362 controlling the leaf senescence process in European beech, and warming during summer and

16

363 autumn both significantly delay the date of leaf senescence. Furthermore, we found a larger

364 temperature response of leaf senescence than spring leaf-out. These findings enhance our

365 understanding of leaf phenology response to the climate change, and potentially improve our

366 understanding of phenological impacts on ecosystem carbon and nutrient cycles.

367

368 **Acknowledgments**

376

Reference

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? Journal of Ecology, 597-608.

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

Augspurger CK (2013) Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. Ecology, **94**, 41-50.

Campioli M, Vincke C, Jonard M, Kint V, Demarée G, Ponette Q (2012) Current status and predicted impact of climate change on forest production and biogeochemistry in the temperate oceanic European zone: review and prospects for Belgium as a case study. Journal of forest research, **17**, 1-18.

Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. Annual review of ecology and systematics, **13**, 229-259.

Chapin Iii FS (1980) The mineral nutrition of wild plants. Annual review of ecology and systematics, **11**, 233-260.

Chmielewski F-M, Rötzer T (2001) Response of tree phenology to climate change across Europe. Agricultural and Forest Meteorology, **108**, 101-112.

Chuine I, Morin X, Bugmann H (2010) Warming, Photoperiods, and Tree Phenology. Science, **329**, 277-278.

Chung H, Muraoka H, Nakamura M, Han S, Muller O, Son Y (2013) Experimental warming studies on tree species and forest ecosystems: a literature review. Journal of plant research, **126**, 447-460.

Čufar K, De Luis M, Saz MA, Črepinšek Z, Kajfež-Bogataj L (2012) Temporal shifts in leaf phenology of beech (Fagus sylvatica) depend on elevation. Trees, **26**, 1091-1100.

Delpierre N, Dufrêne E, Soudani K, Ulrich E, Cecchini S, Boé J, François C (2009) Modelling interannual and spatial variability of leaf senescence for three deciduous tree species in France. Agricultural and Forest Meteorology, **149**, 938-948.

Delpierre, N., Vitasse, Y., Chuine, I., Guillemot, J., Bazot, S., & Rathgeber, C. B. (2016). Temperate and boreal forest tree phenology: from organ-scale processes to terrestrial ecosystem models. Annals of Forest Science, 73(1), 5-25.

Dufrêne E, Davi H, François C, Le Maire G, Le Dantec V, Granier A (2005) Modelling carbon and water cycles in a beech forest: Part I: Model description and uncertainty analysis on modelled NEE. Ecological Modelling, **185**, 407-436.

Estiarte M, Peñuelas J (2015) Alteration of the phenology of leaf senescence and fall in winter deciduous species by climate change: effects on nutrient proficiency. Global Change Biology, **21**, 1005-1017.

Fracheboud Y, Luquez V, Björkén L, Sjödin A, Tuominen H, Jansson S (2009) The control of autumn senescence in European aspen. Plant Physiology, **149**, 1982-1991.

415 Freschet GT, Cornelissen JH, Van Logtestijn RS, Aerts R (2010) Substantial nutrient resorption
416   from leaves, stems and roots in a subarctic flora: what is the link with other resource
417   economics traits? New Phytologist, **186**, 879-889.

418 Fu YH, Campioli M, Vitasse Y *et al.* (2014) Variation in leaf flushing date influences autumnal
419   senescence and next year's flushing date in two temperate tree species. Proceedings of
420   the National Academy of Sciences, **111**, 7355-7360.

421 Fu YH, Liu Y, De Boeck HJ *et al.* (2016) Three times greater weight of daytime than of night-
422   time temperature on leaf unfolding phenology in temperate trees. New Phytologist, **212**,
423   590-597.

424 Fu YH, Zhao HF, Piao SL *et al.* (2015) Declining global warming effects on the phenology of
425   spring leaf unfolding. Nature, **526**, 104-+.

426 Gallinat AS, Primack RB, Wagner DL (2015) Autumn, the neglected season in climate change
427   research. Trends in ecology & evolution, **30**, 169-176.

428 Garonna I, Jong R, Wit AJ, Mücher CA, Schmid B, Schaepman ME (2014) Strong contribution
429   of autumn phenology to changes in satellite-derived growing season length estimates
430   across Europe (1982–2011). Global Change Biology, **20**, 3457-3470.

431 Gunderson CA, Edwards NT, Walker AV, O'hara KH, Campion CM, Hanson PJ (2012) Forest
432   phenology and a warmer climate–growing season extension in relation to climatic
433   provenance. Global Change Biology, **18**, 2008-2025.

434 Han S, Chung H, Noh NJ *et al.* (2014) Effect of open-field experimental warming on the leaf
435   phenology of oriental oak (Quercus variabilis) seedlings. Journal of Plant Ecology, **7**,
436   559-566.

437 Hänninen H (2016) Boreal and temperate trees in a changing climate. Springer Science Business
438   Media, Dordrect.

439 Hänninen H, Tanino K (2011) Tree seasonality in a warming climate. Trends in Plant Science,
440   **16**, 412-416.

441 Heide O, Prestrud A (2005) Low temperature, but not photoperiod, controls growth cessation and
442   dormancy induction and release in apple and pear. Tree physiology, **25**, 109-114.

443 Inouye DW (2008) Effects of climate change on phenology, frost damage, and floral abundance
444   of montane wildflowers. Ecology, **89**, 353-362.

445 Ipcc (2014) Climate change 2013: the physical science basis: Working Group I contribution to
446   the Fifth assessment report of the Intergovernmental Panel on Climate Change,
447   Cambridge University Press.

448 Jeong, S. J., HO, C. H., GIM, H. J., & Brown, M. E. (2011). Phenology shifts at start vs. end of
449   growing season in temperate vegetation over the Northern Hemisphere for the period
450   1982–2008. Global Change Biology, **17**(7), 2385-2399.

451 Jeong, S. J., & Medvigy, D. (2014) Macroscale prediction of autumn leaf coloration throughout
452   the continental United States. Global ecology and biogeography, **23**(11), 1245-1254.

453 Jolly WM, Nemani R, Running SW (2005) A generalized, bioclimatic index to predict foliar
454   phenology in response to climate. Global Change Biology, **11**, 619-632.

455   Julien Y, Sobrino J (2009) Global land surface phenology trends from GIMMS database.
456   International Journal of Remote Sensing, **30**, 3495-3513.

457   Keenan TF, Gray J, Friedl MA *et al.* (2014) Net carbon uptake has increased through warming-
458   induced changes in temperate forest phenology. Nature Climate Change, **4**, 598.

459   Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption
460   and resorption proficiency. Ecology, **77**, 1716-1727.

461   Koski V, Sievänen R (1985) Timing of growth cessation in relation to the variations in the
462   growing season. In: Tigerstedt PMA, Puttonen P, Koski V (Eds) Crop physiology of
463   forest trees. Helsinki University Press. Helsinki, Finland. p. 167-193.

464   Lagercrantz U (2009) At the end of the day: a common molecular mechanism for photoperiod
465   responses in plants? Journal of experimental botany, **60**, 2501-2515.

466   Leblans N, Sigurdsson B, Vicca S, Fu Y, Penuelas J, Janssens I (2017) Phenological responses of
467   Icelandic subarctic grasslands to short-term and long-term natural soil warming. Global
468   Change Biology.

469   Lieth, H. (1974). Purposes of a phenology book. In Phenology and seasonality modeling (pp. 3-
470   19). Springer Berlin Heidelberg.

471   Liu Q, Fu YH, Zeng Z, Huang M, Li X, Piao S (2016a) Temperature, precipitation, and
472   insolation effects on autumn vegetation phenology in temperate China. Global Change
473   Biology, **22**, 644-655.

474   Liu Q, Fu YH, Zhu Z *et al.* (2016b) Delayed autumn phenology in the Northern Hemisphere is
475   related to change in both climate and spring phenology. Global Change Biology, **22**,
476   3702-3711.

477   Maillard A, Diquélou S, Billard V *et al.* (2015) Leaf mineral nutrient remobilization during leaf
478   senescence and modulation by nutrient deficiency. Frontiers in plant science, **6**.

479   Marchin RM, Salk CF, Hoffmann WA, Dunn RR (2015) Temperature alone does not explain
480   phenological variation of diverse temperate plants under experimental warming. Global
481   Change Biology, **21**, 3138-3151.

482   May JD, Killingbeck KT (1992) Effects of preventing nutrient resorption on plant fitness and
483   foliar nutrient dynamics. Ecology, **73**, 1868-1878.

484   Mediavilla S, Herranz M, González-Zurdo P, Escudero A (2014) Ontogenetic transition in leaf
485   traits: a new cost associated with the increase in leaf longevity. Journal of Plant Ecology,
486   **7**, 567-575.

487   Menzel A, Sparks TH, Estrella N *et al.* (2006) European phenological response to climate
488   change matches the warming pattern. Global Change Biology, **12**, 1969-1976.

489   Michelson IH, Ingvarsson PK, Robinson KM, Edlund E, Eriksson ME, Nilsson O, Jansson S
490   (2017) Autumn senescence in aspen is not triggered by day length. Physiologia
491   Plantarum.

492   Morin X, Roy J, Sonié L, Chuine I (2010) Changes in leaf phenology of three European oak
493   species in response to experimental climate change. New Phytologist, **186**, 900-910.

494   Myneni RB, Keeling C, Tucker CJ, Asrar G, Nemani RR (1997) Increased plant growth in the
495   northern high latitudes from 1981 to 1991. Nature*, **386**, 698.

496   Nakamura M, Muller O, Tayanagi S, Nakaji T, Hiura T (2010) Experimental branch warming
497   alters tall tree leaf phenology and acorn production. Agricultural and Forest Meteorology*,
498   **150**, 1026-1029.

499   Norby RJ, Hartz-Rubin JS, Verbrugge MJ (2003) Phenological responses in maple to
500   experimental atmospheric warming and CO2 enrichment. Global Change Biology*, **9**,
501   1792-1801.

502   Olsson C, Jönsson AM (2015) A model framework for tree leaf colouring in Europe. Ecological
503   Modelling*, **316**, 41-51.

504   Panchen ZA, Primack RB, Gallinat AS, Nordt B, Stevens A-D, Du Y, Fahey R (2015)
505   Substantial variation in leaf senescence times among 1360 temperate woody plant species:
506   implications for phenology and ecosystem processes. Annals of botany*, **116**, 865-873.

507   Parmesan C, Hanley ME (2015) Plants and climate change: complexities and surprises. Annals
508   of botany*, **116**, 849-864.

509   Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across
510   natural systems. Nature*, **421**, 37.

511   Partanen J (2004) Dependence of photoperiodic response of growth cessation on the stage of
512   development in Picea abies and Betula pendula seedlings. Forest Ecology and
513   Management*, **188**, 137-148.

514   Peñuelas J, Filella I (2009) Phenology feedbacks on climate change. Science*, **324**, 887-888.

515   Piao S, Ciais P, Friedlingstein P *et al.* (2008) Net carbon dioxide losses of northern ecosystems
516   in response to autumn warming. Nature*, **451**, 49.

517   Piao S, Friedlingstein P, Ciais P, Viovy N, Demarty J (2007) Growing season extension and its
518   impact on terrestrial carbon cycle in the Northern Hemisphere over the past 2 decades.
519   Global Biogeochemical Cycles*, **21**.

520   Primack RB, Laube J, Gallinat AS, Menzel A (2015) From observations to experiments in
521   phenology research: investigating climate change impacts on trees and shrubs using
522   dormant twigs. Annals of botany*, **116**, 889-897.

523   Reed BC, Schwartz MD, Xiao X (2009) Remote sensing phenology. In: *Phenology of ecosystem
524   processes.* pp Page., Springer.

525   Richardson AD, Bailey AS, Denny EG, Martin CW, O'keefe J (2006) Phenology of a northern
526   hardwood forest canopy. Global Change Biology*, **12**, 1174-1188.

527   Richardson AD, Black TA, Ciais P *et al.* (2010) Influence of spring and autumn phenological
528   transitions on forest ecosystem productivity. Philosophical Transactions of the Royal
529   Society of London B: Biological Sciences*, **365**, 3227-3246.

530   Richardson AD, Keenan TF, Migliavacca M, Ryu Y, Sonnentag O, Toomey M (2013) Climate
531   change, phenology, and phenological control of vegetation feedbacks to the climate
532   system. Agricultural and Forest Meteorology*, **169**, 156-173.

Rohde A, Bhalerao RP (2007) Plant dormancy in the perennial context. Trends in plant science, **12**, 217-223.

Root TL, Price JT, Hall KR, Schneider SH (2003) Fingerprints of global warming on wild animals and plants. Nature, **421**, 57.

Shen M, Piao S, Cong N, Zhang G, Jassens IA (2015) Precipitation impacts on vegetation spring phenology on the Tibetan Plateau. Global Change Biology, **21**, 3647-3656.

Signarbieux C, Toledano E, Sanginés De Carcer P, Fu YH, Schlaepfer R, Buttler A, Vitasse Y (2017) Asymmetric effects of cooler and warmer winters on beech phenology last beyond spring. Global Change Biology.

Sigurdsson BD (2001) Elevated [CO 2] and nutrient status modified leaf phenology and growth rhythm of young Populus trichocarpa trees in a 3-year field study. Trees-Structure and Function, **15**, 403-413.

Sparks TH, Menzel A (2002) Observed changes in seasons: an overview. International Journal of Climatology, **22**, 1715-1725.

Stöckli R, Vidale PL (2004) European plant phenology and climate as seen in a 20-year AVHRR land-surface parameter dataset. International Journal of Remote Sensing, **25**, 3303-3330.

Tanino KK, Kalcsits L, Silim S, Kendall E, Gray GR (2010) Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant molecular biology, **73**, 49-65.

Thackeray SJ, Henrys PA, Hemming D *et al.* (2016) Phenological sensitivity to climate across taxa and trophic levels. Nature.

Viherä-Aarnio A, Häkkinen R, Partanen J, Luomajoki A, Koski V (2005) Effects of seed origin and sowing time on timing of height growth cessation of Betula pendula seedlings. Tree physiology, **25**, 101-108.

Vitasse Y (2013) Ontogenic changes rather than difference in temperature cause understory trees to leaf out earlier. New Phytologist, **198**, 149-155.

Vitasse Y, François C, Delpierre N, Dufrêne E, Kremer A, Chuine I, Delzon S (2011) Assessing the effects of climate change on the phenology of European temperate trees. Agricultural and Forest Meteorology, **151**, 969-980.

Vitasse Y, Porté AJ, Kremer A, Michalet R, Delzon S (2009) Responses of canopy duration to temperature changes in four temperate tree species: relative contributions of spring and autumn leaf phenology. Oecologia, **161**, 187-198.

Walther G-R, Post E, Convey P *et al.* (2002) Ecological responses to recent climate change. Nature, **416**, 389-395.

Wareing P (1956) Photoperiodism in woody plants. Annual Review of Plant Physiology, **7**, 191-214.

Way DA, Montgomery RA (2015) Photoperiod constraints on tree phenology, performance and migration in a warming world. Plant, Cell & Environment, **38**, 1725-1736.

572   Weih M (2009) Genetic and environmental variation in spring and autumn phenology of biomass
573   willows (Salix spp.): effects on shoot growth and nitrogen economy. Tree physiology, **29**,
574   1479-1490.

575   Welling A, Palva ET (2006) Molecular control of cold acclimation in trees. Physiologia
576   Plantarum, **127**, 167-181.

577   Wolkovich EM, Cook BI, Allen JM *et al.* (2012) Warming experiments underpredict plant
578   phenological responses to climate change. Nature, **485**, 494.

579   Wolkovich EM, Ettinger AK (2014) Back to the future for plant phenology research. New
580   Phytologist, **203**, 1021-1024.

581   Wright IJ, Westoby M (2003) Nutrient concentration, resorption and lifespan: leaf traits of
582   Australian sclerophyll species. Functional Ecology, **17**, 10-19.

583   Wu C, Chen JM, Black TA *et al.* (2013) Interannual variability of net ecosystem productivity in
584   forests is explained by carbon flux phenology in autumn. Global Ecology and
585   Biogeography, **22**, 994-1006.

586   Xie Y, Wang X, Silander JA (2015) Deciduous forest responses to temperature, precipitation,
587   and drought imply complex climate change impacts. Proceedings of the National
588   Academy of Sciences, **112**, 13585-13590.

589   Zeng Z, Piao S, Li LZ *et al.* (2017) Climate mitigation from vegetation biophysical feedbacks
590   during the past three decades. Nature Climate Change, **7**, 432-436.
591

Figure captions

594 Fig 1. (a) The distribution, mean, and standard deviation (plot box) of the leaf senescence dates

595 of European beech saplings under different temperature manipulations and the control. Each grey

596 dot indicates the result for one sapling. (b) Relationship between leaf senescence dates of

597 European beech saplings and the mean temperature change in the treatments, as compared with

598 the ambient temperature. Open circles: Experiment 1, i.e. temperature treatment over the summer

599 period (from summer solstice to 15 August 2016); Grey squares: Experiment 2, temperature

600 treatment over the autumn period (from 15 August 2016 to the date of leaf senescence). The grey

601 line and shaded areas represent linear regression fits (with 95% confidence intervals) across

602 summer and autumn treatments.

603

604 Fig 2. Temperature sensitivities of leaf senescence to autumn (from 15 August 2016 to the date

605 of leaf senescence) cooling and warming. The temperature sensitivity was calculated using

606 simple linear regression. The difference in the sensitivity between autumn cooling and warming

607 is not statistically significant

608 .

609 Fig 3. (a) Experiment 3: temperature treatment during winter-spring 2016. The temperature

610 sensitivity of leaf-out, in relation to the mean temperature change in the treatments, as compared

611 with the ambient temperature. (b) A comparison of the temperature sensitivity of leaf-out and

612 leaf senescence. The asterisk indicates a statistically significant difference ($P < 0.05$).

613

614 Fig 4. Correlation between leaf senescence dates and cooling degree hours accumulated in the

615 different treatments. Each dot denotes one climate-controlled chamber. The color bar shows the

616   temperature anomalies in the temperature treatments (blue is control). Treatment of summer

617   warming (squares) refers to the period from summer solstice to 15 August 2016; autumn

618   warming (circles) and autumn cooling (diamonds) refer to the period from 15 August 2016 to the

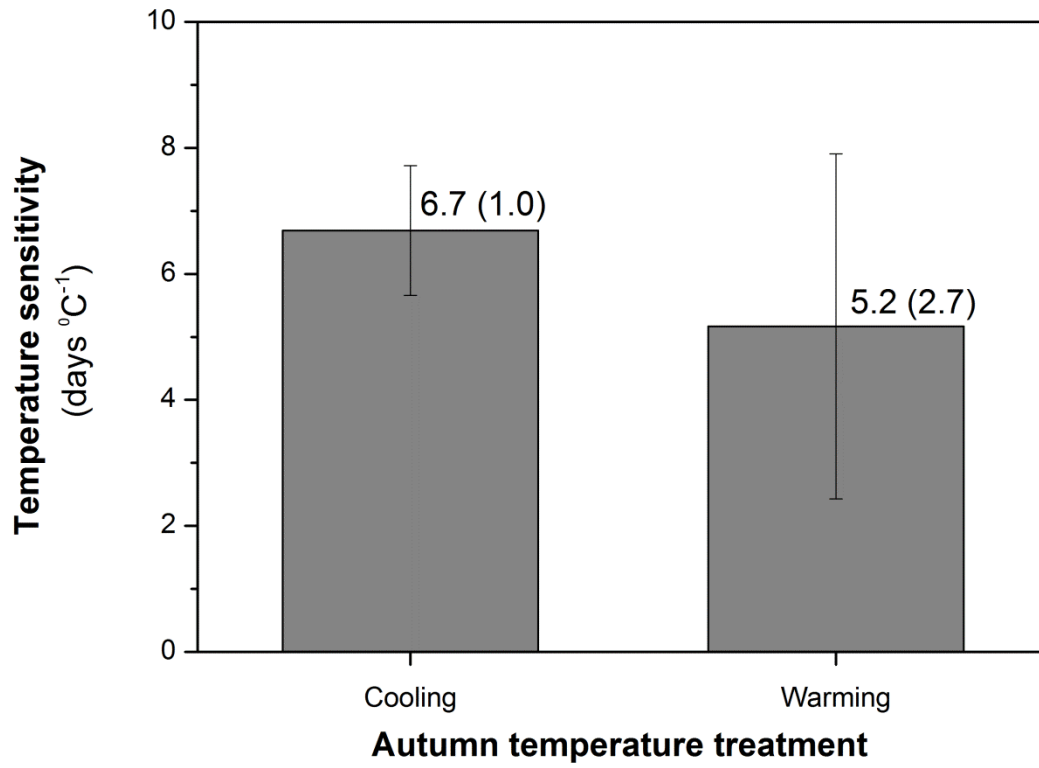619   date of leaf senescence.

620

621   FIGURE 1

622
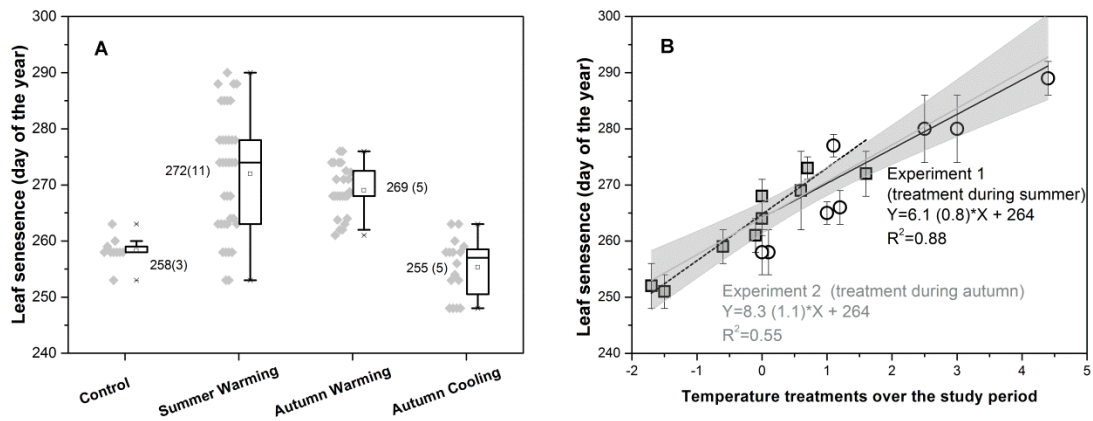
623
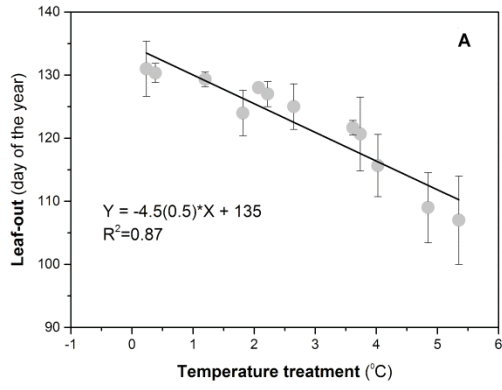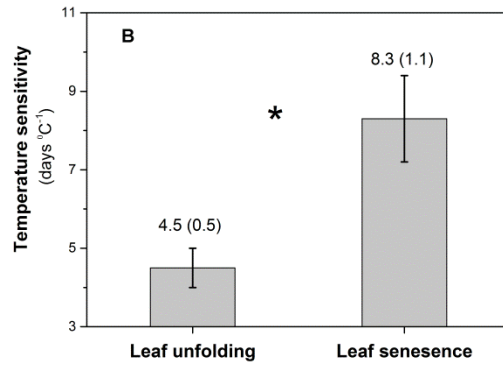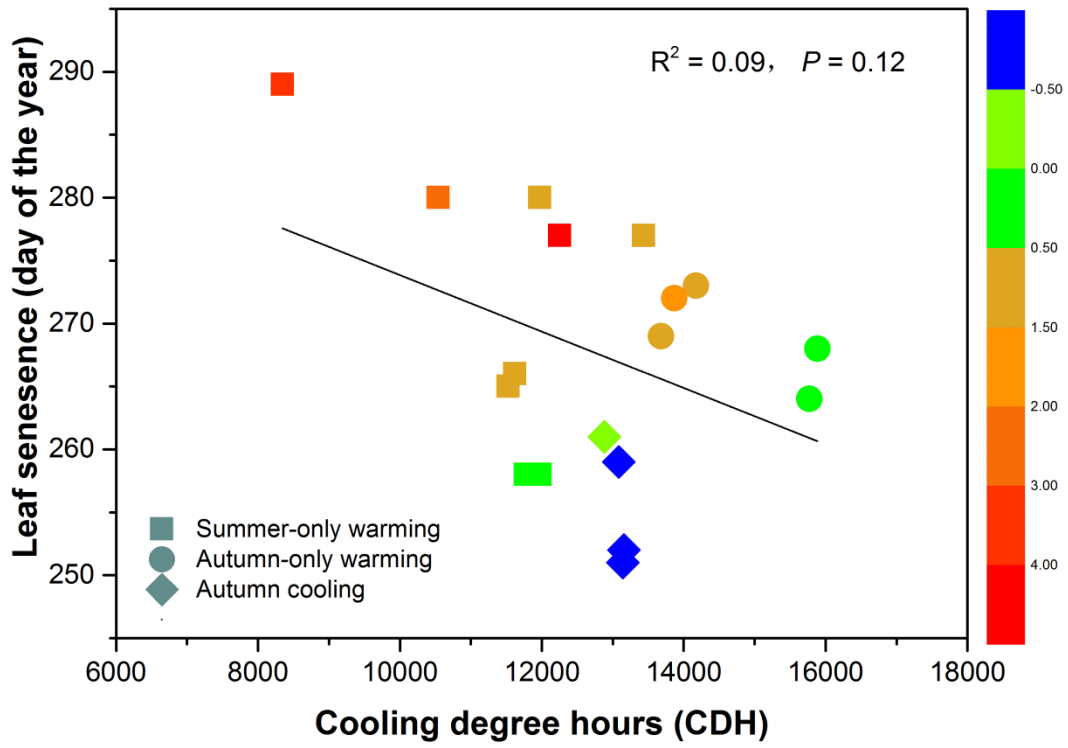
624

625

626   FIGURE 2

627

628

629

630   FIGURE 3

631

632

633

634

635   FIGURE 4

636

637