Does drought advance the onset of autumn leaf senescence in temperate deciduous forest trees?

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Abstract

- Severe droughts are expected to become more frequent and persistent. However, their effect on autumn leaf senescence, a key process for deciduous trees and ecosystem functioning, is currently unclear. We hypothesized that (I) severe drought advances the onset of autumn leaf senescence in temperate deciduous trees and that (II) tree species show different dynamics of autumn leaf senescence under drought.

- We tested these hypotheses using a manipulative experiment on beech saplings and three years of monitoring mature beech, birch and oak trees in Belgium. The autumn leaf senescence was derived from the seasonal pattern of the chlorophyll content index and the loss of canopy greenness using generalized additive models and piece-wise linear regressions.

- Drought and associated heat stress and increased atmospheric aridity did not affect the onset of autumn leaf senescence in both saplings and mature trees, even if the saplings showed a high mortality and the mature trees an advanced loss of canopy greenness. We did not observe major differences among species.

- Synthesis: The timing of autumn leaf senescence appears conservative across years and species, and even independent on drought, heat and increased atmospheric aridity. Therefore, to study autumn senescence and avoid confusion among studies, seasonal chlorophyll dynamics and loss of canopy greenness should be considered separately.

Key words

Autumn leaf senescence, *Betula pendula*, Drought, Heat stress and increased atmospheric aridity , *Fagus sylvatica*, Generalized additive mixed models, Leaf coloration and fall, *Quercus robur*, Rainfall deficit

1. Introduction

Autumn leaf senescence is a developmental stage of the leaf cells. The core function of this process is the remobilization of nutrients and death is its consequence (Medawar, 1957;Keskitalo et al., 2005). Its evolutionary purpose is likely stress resistance and, as such, the process dynamics are affected by different forms of environmental stress (e.g. high temperatures, water logging) (Benbella and Paulsen, 1998;Leul and Zhou, 1998;Munné-Bosch and Alegre, 2004). The process of autumn leaf senescence is highly coordinated and characterized by a tight control over its timing. Furthermore, its most manifest feature, the detoxification of chlorophyll, allows the degradation of leaf macromolecules and subsequent nutrient remobilization -the essence of autumn leaf senescence- (Hörtensteiner and Feller, 2002;Munné-Bosch and Alegre, 2004;Matile, 2000). In addition, chlorophyll degradation allows for the typical leaf coloration during autumn. However, autumn leaf senescence is also an important process at the ecosystem scale because it affects multiple ecological processes, such as trophic dynamics, tree growth or the exchange of matter and energy between the ecosystem and atmosphere (Richardson et al., 2013).

Despite its relevance, literature on autumn senescence has maintained a wide variety of definitions and observational methods (Gill et al., 2015;Fracheboud et al., 2009;Gallinat et al., 2015). This has hampered our understanding of the effects of drought stress on the timing of the onset of autumn leaf senescence, as opposed to the timing of leaf abscission or accelerated leaf senescence. For example, Estiarte and Penuelas (2015) reported that leaf senescence advances due to drought stress, while Vander Mijnsbrugge et al. (2016) reported a delay in the leaf senescence of young trees subjected to drought. After the summer drought in central Europe of 2003, Leuzinger et al. (2005) even reported that the leaf longevity (measured as a delay in the leaf discoloration and fall) of five deciduous tree species was on average prolonged by 22 days.

Droughts are expected to occur more frequently and become more intensive due to global warming and changes in precipitation patterns (IPCC, 2014;Crabbe et al., 2016). Extended periods with lower than average rainfall are often associated with higher air temperatures and higher vapor pressure deficits, which can negatively affect the functioning of trees in the temperate zone (Novick et al., 2016;De Boeck and Verbeeck, 2011). Belgian forests are thought to be especially vulnerable to droughts as they typically have sandy soils with low soil field capacities (Vander Mijnsbrugge et al., 2016;van der Werf et al., 2007).

To examine the effects of drought stress on the onset of autumn leaf senescence, we hypothesized that:

(I)   the timing of the onset of autumn leaf senescence in temperate deciduous trees is advanced by severe drought stress. The leaves of a tree that experiences drought will accumulate the consequences of stress exposure and lose functionality. Therefore, it is likely not beneficial for a tree to maintain active leaves late in the season after severe drought. Instead, to maximize nutrient recovery, trees probably prefer an earlier leaf senescence. In addition, drought would reduce the tree's wood growth and increase its fine root mortality (Brunner et al., 2015;Campioli et al., 2013). Consequently, the tree's carbon sink strength will decline, causing a reduced demand for carbon from the sources (e.g. the leaves) and advance the onset of autumn leaf senescence.

(II)   different tree species show different dynamics in their onset of autumn leaf senescence under drought. We hypothesized that, under drought stress, species with continuous flushing (e.g. birch) will have a more stable timing onset of autumn leaf senescence than species with only one or two leaf flushes during spring-summer (e.g. beech and oak) (Koike, 1990).

112 We tested these hypotheses by subjecting young trees to treatments comprising less irrigation and
113 warming, and by examining the effect of years with different drought intensities (2017, 2018 and 2019)
114 on mature trees in natural forest stands. Both young and mature trees experienced not only drought, but
115 also heat and increased atmospheric aridity.

2. Materials and methods

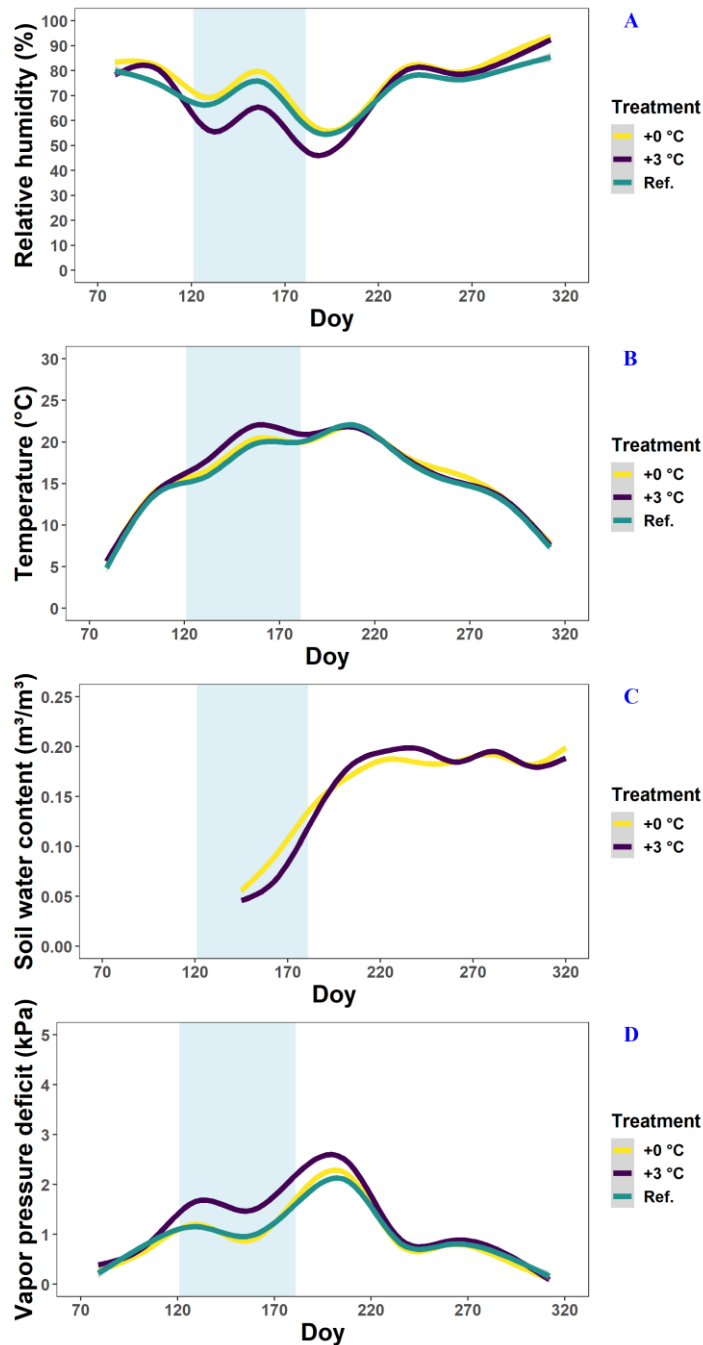
2.1. Study sites and experimental setting

2.1.1. Manipulative experiment

119 In 2018, we carried out a manipulative experiment at the Drie Eiken Campus in Wilrijk, Belgium (51°09'N,
120 4°24'E). In early March, 128 individuals of three-year-old beech (*Fagus sylvatica*) saplings, from a local
121 nursery and with the same local provenance, were planted in pots with a volume of 35 liters and a surface
122 area of 0.07 m². The pots were filled with 20% peat and 80% white sand. Eight beech saplings were placed
123 in each of twelve climate-controlled glasshouses with a ground surface of 1.5 x 1.5 m and a height at the
124 north and south side of 1.5 m and 1.2 m, respectively. The glasshouses had a roof of colorless
125 polycarbonate (a 4 mm thick plate) reducing the incoming light by ± 20% and modifying the spectral
126 quality only in the UV range (Kwon et al., 2017). The glasshouses had three sides that could be opened or
127 closed and were equipped with a combined humidity-temperature sensor (QFA66, Siemens, Erlangen,
128 Germany) to monitor the relative humidity and air temperature (Fig. 1, panel A and B) (Kwon et al., 2017).
129 One pot per glasshouse was also equipped with a soil moisture smart sensor (HOBO S-SMD-M005, Onset,
130 MA, USA) to monitor the soil water content (Fig. 1, panel C). The latter sensors became available only at
131 the time the drought stress was alleviated (see below). More details on the set-up of the glasshouses can
132 be found in the literature (Van den Berge et al., 2011;De Boeck et al., 2012;Fu et al., 2014). Two treatments
133 were organized (n = 48 per treatment; see below). In addition to the saplings in the glasshouses, eight
134 beech saplings were placed in each of four reference plots outside of the glasshouses (n = 32, Ref.). The
135 relative humidity and air temperature of the outside reference plots were monitored by a pocket weather
136 meter (Kestrel 3000, Nielsen, PA, USA). Once in April and once in July, all saplings received 35 g of NPK
137 slow-release fertilizer (DCM ECO-XTRA 1) and 1.8 g of micro elements (DCM MICRO-MIX). Using the
138 relative humidity and air temperature data between 7 a.m. and 7 p.m., the vapor pressure deficit was
139 calculated for both treatments (see below) and the reference plots using the formulas of Buck (1981) (Eq.
140 1; Fig. 1, panel D).

142 Equation 1

$$e_0 = 613.75 \times \exp((17.502 \times T)/(240.97 + T))$$
$$e = (RH/100) \times e_0$$
$$VPD = e_0 - e$$

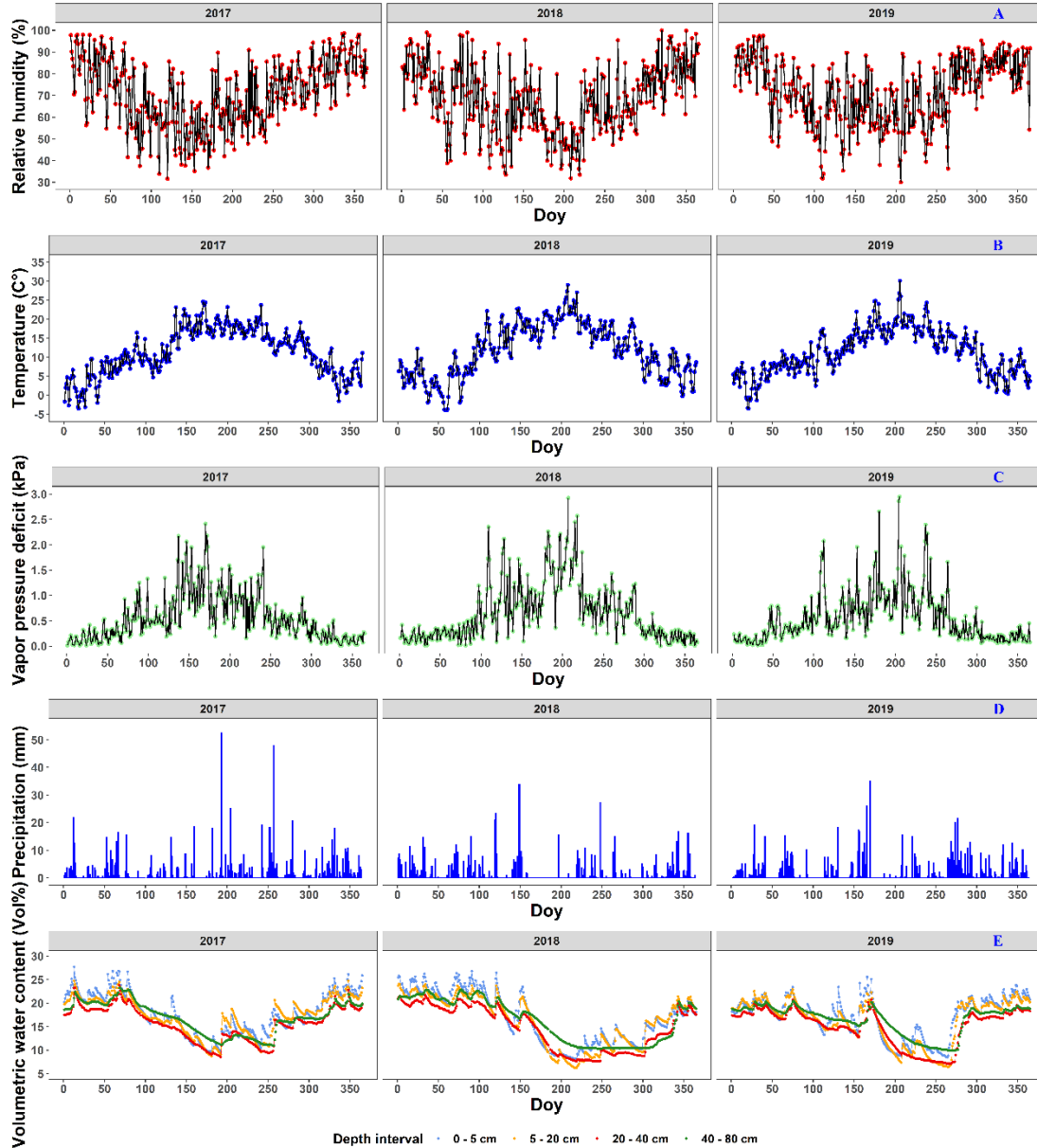
147 where e_0 is the saturation vapor pressure (in Pa), T is the temperature (in °C), e is the actual vapor pressure
148 deficit (in Pa), RH is the relative humidity (in %) and VPD is the vapor pressure deficit (in Pa).

150
151 Fig. 1: The relative humidity (panel A), temperature (panel B), soil water content (panel C) and vapor
152 pressure deficit (panel D) in the glasshouses and outside plots at the Drie Eiken Campus in Wilrijk. Solid
153 lines represent regressions of half-hourly measurements of the relative humidity (%), temperature (°C),
154 and soil water content (m³/m³). The vapor pressure deficit (kPa) was calculated using the formulas of Buck
155 (1981) using data of the relative humidity and air temperature between 7 a.m. and 7 p.m. Green, blue and
156 red lines represent the conditions in the reference plots (Ref.), glasshouses that follow the outside
157 ambient air temperature (+0 °C) and glasshouses that are three degrees warmer than the outside ambient
158 air temperature (+3 °C), respectively. The light blue band represents the treatment-period.
159 kPa).
160

161 From planting until April, the saplings were all irrigated two to three times a week until the pots
162 overflowed. The reference plots outside were maintained with abundant irrigation during the whole
163 growing season. On the other hand, at the start of the treatment, in early May, we shielded all the
164 glasshouses using polyethylene film (200 μm thick) and irrigated the saplings only once a week with circa
165 2.5 liter of water. In addition, we enhanced the drought in six glasshouses by raising the air temperature
166 three degrees compared to the ambient air temperature (+3 °C). The air temperature in the other six
167 glasshouses followed the ambient air temperature (+0 °C). There were no significant differences in the
168 temperature, relative humidity and vapor pressure deficit among the glasshouses with the reference and
169 +0 °C treatment (Fig. 1). Although no data on the soil water content was available for the reference plots
170 (due to sensor malfunctioning), we did not expect major drought stress due to their abundant irrigation
171 and lack of stress signals. Based on this information, the +0 °C treatment can be considered a 'less-
172 irrigation/drought' treatment. On the other hand, during the treatment, the daily soil water content and
173 the daily relative humidity in the glasshouses with the +3 °C treatment were significantly lower ($P < 2$ x
174 10^{-16}) in comparison to the glasshouses with the +0 °C treatment. After statistical testing following Rose
175 et al. (2012), the difference was found to be around 0.025 m³/m³ for the soil water content and 20% for
176 the relative humidity (Fig. 1). The +3 °C treatment can therefore be considered a combined 'less-
177 irrigation/drought, warming and increased atmospheric aridity' treatment. In fact, this treatment should
178 simulate natural drought conditions, which are often associated with heat stress and increased
179 atmospheric aridity. The plan was to continue the treatment till the end of June but, due to the significant
180 mortality rate, we were obliged to alleviate the drought already from the 20th of June by increasing the
181 irrigation to the level of the reference plots. From July, the glasshouses were opened again and the
182 saplings were irrigated four to five times a week until the end of the season.
183
184 A draw-back of the experiment is that the saplings in the reference plots received more incoming light
185 (i.e. ± 20%) than the saplings in the glasshouses (Van den Berge et al., 2011). However, as beech is a shade
186 tolerant species, reduced light is unlikely to have limited tree growth. In addition, preliminary tests
187 suggested that the ratio of light in different wavelengths (e.g. R/FR) during civil twilight (i.e. what is
188 required for phytochrome to detect the photoperiod) does not change seasonally significantly in our study
189 area (Chelle et al., 2007).
190
191 *2.1.2. Field observations in deciduous forests*
192 From 2017 to 2019, we monitored dominant mature trees in two forests near Antwerp: the Klein
193 Schietveld in Kapellen (KS; 51°21'N, 4°37'E) and the Park of Brasschaat (PB; 51°12'N'', 4°26'E). In the KS,
194 we monitored eight beech trees and eight birch (*Betula pendula*) trees. In the PB, we monitored eight
195 beech trees and eight oak (*Quercus robur*) trees (thus 32 trees in total). The two forests and their
196 meteorological conditions are described in detail by Mariën et al. (2019), which also showed a lack of site
197 effects on the autumn chlorophyll dynamics for the tree species studied here. To have a larger statistical
198 sample, the data of the two beech stands (also of similar age and stem diameter) were aggregated.
199
200 For summer and autumn, we report here the average values for the temperature, precipitation, number
201 of rainy days, relative humidity, sunshine duration and global solar radiation for the meteorological station
202 of the Royal Meteorological Institute (KMI) in Ukkel, Belgium (Table 1). For these data, long-term averaged
203 data was available. The temperature, relative humidity, vapor pressure deficit (see Eq. 1), precipitation
204 and volumetric soil water content from 2017 to 2019 are presented in more detail using daily values that
205 were measured at Brasschaat and, whenever necessary, gap-filled with data from the meteorological
206 station in Woensdrecht, Netherlands (Fig. 2, panel A − B; panel D). The meteorological data from
207 Brasschaat was provided by the Flemish Institute for Nature and Forest (INBO) and the Integrated Carbon

208 Observation System (ICOS), while the data from Woensdrecht was provided by the Royal Dutch
209 Meteorological Institute (KNMI).

Table 1: Overview of the meteorological conditions perceived by the mature trees in the study region in 2017, 2018 and 2019. All data is measured by the meteorological station of the Royal Meteorological Institute (KMI) in Ukkel, Belgium (KMI, 2018a, b, 2017b, c, 2019a, b). The degree of abnormality of the values is represented by (a; abnormal values that happen on average once every 6 years) and (e; exceptional values that happen on average once every thirty years). Since 2019, the KMI uses a new system to show the degree of abnormality. Values that are with the five highest values since 1981 are marked by (+), while values within the three highest values are marked by (++).

	Normal (1981-2010)		2017		2018		2019	
	summer	autumn	summer	autumn	summer	autumn	summer	autumn
Average temperature (°C)	17.6	10.9	18.6 (a)	11.3	19.8 (e)	11.8	19.1 (++)	11.3
Total precipitation (mm)	224.6	219.9	179.9	226.5	134.7 (a)	168.5	198.6	209.3
Average number of rainy days	43.9	51	44	63 (a)	20 (e)	32 (e)	33	53
Relative humidity (%)	73	82	67.7 (e, June)	62	62.3 (e, July)	75 (e, July)	70	83
Sunshine duration (h:m)	578:20	322:00	573:21	322:00	693:06 (a)	471:12 (e)	714:38 (++)	322:23
Global solar radiation (kWh/m²)	429.6	168.2	447.1 (a, June)	233.8	498.6 (e, July)	213.4 (e, October)	487.9 (+)	178.4

219

220   Fig. 2: The meteorological conditions near the Klein Schietveld and Park of Brasschaat. The line plots
221   represent the daily average relative humidity (%; red), temperature (°C; blue) and vapor pressure deficit
222   (kPa; green). The bar plots represent the daily precipitation (mm; light blue). The volumetric soil water
223   content (Vol%) at depth intervals of 0 – 5 cm, 5 – 20 cm, 20 – 40 cm and 40 – 80 cm is presented as line
224   plots in cornflower blue, orange, red and green, respectively. The relative humidity, temperature, vapor
225   pressure deficit and precipitation data was measured every half hour and provided by the Flemish
226   Institute for Nature and Forest (INBO), the Integrated Carbon Observation System (ICOS) and the Royal
227   Dutch Meteorological Institute (KNMI). The vapor pressure deficit (kPa) was calculated using the formulas
228   of Buck (1981) using data of the relative humidity and air temperature between 7 a.m. and 7 p.m. The
229   volumetric soil water content data was first measured every six hours but after 03/07/2018
230   measurements were made every hour. The volumetric soil water content data was provided through
231   courtesy of INBO.

232

233 The distance from Ukkel and Woensdrecht to our sites is 60 km and 20 km, respectively. However, both
234 locations show no major climatological differences with the KS and PB, and are representative for the
235 inter-annual variability experienced by the forests. The station of Ukkel is located within a green area in
236 the suburb of Brussels (thus, classifiable as "urban park"). The microclimate is expected to be different
237 than at our study sites. However, data from Ukkel were used to describe the intra-annual variability and
238 long-term trends, which are less affected by the microclimate. The meteorological station of Brasschaat
239 is very close to our sampling site in the Park of Brasschaat and in the Klein Schietveld (± 3 km and ± 4 km,
240 respectively). The meteorological station in Brasschaat is a 40 m high scaffolding tower, at which
241 measurements are taken at various heights, and stands in a patch of mixed forest covered mainly by Scots
242 pines and deciduous tree species, such as oak and birch (see Carrara et al. (2003) for more information).
243 Data of the temperature, precipitation and humidity were taken at the top of the tower. Concurrently,
244 the volumetric soil water content was measured near the scaffolding tower using twelve water
245 reflectometers (CS616 Water Content Reflectometer, Campbell Scientific, UT, USA) connected to a central
246 data logger (CR1000 data logger, Campbell Scientific, UT, USA). The water reflectometers were equally
247 divided over three sampling pits at an 8 m distance from the central data logger. In 2010 and in each pit,
248 the water reflectometers were installed in pedogenetic horizons at four depth intervals (i.e. 0 – 5 cm, 5 –
249 20 cm, 20 – 40 cm and 40 – 80 cm). The volumetric soil water content data was first measured every six
250 hours but after 03/07/2018 measurements were made every hour. The volumetric soil water content was
251 calibrated following De Vos (2016) and averaged per day and depth interval. The station of Woensdrecht
252 is located in an open field at a local airport surrounded by heathland and urban area. It is located near the
253 Markiezaatsmeer, an enclosed swamp ecosystem, within the river mouth of the Schelde. The
254 measurements in both Ukkel and Woensdrecht are taken at a height of 1.5 m. However, these data were
255 only used as gap-filling in case of short term gaps in the long-term Brasschaat series.
256
257 *2.1.3. The rainfall deficit: an indicator of drought stress for 2017 - 2019*
258 To indicate the magnitude of the droughts, we computed the rainfall deficit from 2017 to 2019 using data
259 on the relative humidity, solar radiation, wind speed, temperature and precipitation from the
260 meteorological station in Ukkel. Here, the meteorological records go back the longest in Belgium. The
261 rainfall deficit is computed on a daily basis by accumulating the daily potential evapotranspiration minus
262 the daily amount of precipitation. This was done in two ways: (I) per hydrological year, starting from a
263 zero deficit at the start of the hydrological year (1st of April) and (II) continuous computation, so no restart
264 from 0 at the start of each hydrological year. The latter method has the benefit that the long-term effect
265 of accumulated droughts from successive years is accounted for.
266
267 The potential evapotranspiration was computed by means of the method of Bultot et al. (1983), which is
268 similar to the method of Penman (1948), but has parameters that are calibrated specifically for the local
269 Belgian conditions. Unlike for the rainfall deficit starting from a zero deficit, we accounted in the
270 calculation of the continuously computed rainfall deficit for the hydrological fraction in wet periods that
271 does not contribute to building up ground water reserves. At the station of Ukkel, daily precipitation and
272 potential evapotranspiration data are available since more than 100 years. The precipitation data are
273 collected since 1898 on the same location, and is measured using the same instrument. For this study, the
274 data for the 100-year period 1901-2000 was considered as the reference period for the computation of
275 long-term statistics on the rainfall deficit.
276

2.2. Measuring autumn leaf senescence: the chlorophyll content index and the loss of canopy greenness

In the manipulative experiment from late-July until late-November, we measured the chlorophyll content index (CCI; a proxy for the chlorophyll concentration) of each tree sapling weekly by randomly selecting one leaf from the outer, middle and inner layer of the upper part of the crown. The CCI was measured using a chlorophyll content meter, which measures the optical absorbance in the 653 nm and 931 nm wavebands (CCM-200 plus, Opti-Sciences Inc., Hudson, NH, USA). Concurrently, we visually estimated the loss of canopy greenness (LOCG; scaled between 0 and 1) of each sapling following the method of Vitasse et al. (2011), which accounts for both the percentage of leaves that have changed color and the percentage of leaves that have fallen.

For half of the monitored mature trees in the two forests and from the end of July to the end of November, tree-climbers collected leaves on eight occasions per year separated by two to three weeks. During each measurement day, they collected five sun-leaves and five shade-leaves from each tree. Afterwards, the CCI was immediately measured on the harvested leaves using the same chlorophyll content meter as described above. From early September to late November, the loss of canopy greenness was estimated in a similar fashion to the manipulative experiment for the 32 mature trees (Vitasse et al., 2011).

Following the method of Mariën et al. (2019), we validated the CCI values by measuring also the chlorophyll concentrations (Fig. A1). In 2017 and 2018, on one occasion per month and using a 10-mm diameter cylinder, we collected samples of leaf tissue from the leaves of the mature trees for which we also measured the CCI. After storage at -80 °C, the samples were grounded using glass beads and a centrifuge. The result was dissolved in ethanol and the absorption of the solution was measured using a spectrophotometer (Smart Spec Plus Spectrophotometer, Bio-Rad) at different wavelengths for Chlorophyll a (662 nm) and chlorophyll b (644 nm). The chlorophyll concentrations could then be derived from the absorption values using the formulas described in Holm (1954) and Vonwettstein (1957).

2.3. Tree mortality in the manipulative experiment

In this study, we only considered those trees that defoliated due to autumn leaf senescence. Other tree saplings have died or defoliated completely due to accelerated leaf senescence during or just after the treatment period. Since chlorophyll degradation is a common feature of both senescence processes and nutrient remobilization was only measured indirectly by CCI, we did not consider (I) tree saplings that showed an early or abrupt defoliation (without gradual coloration) before the 18th of August (n = 20) and (II) tree saplings with constant CCI values lower than three, the limit at which the values of the CCI meter can be interpreted, for the whole period from August to November (n = 18). Like in other studies, some defoliated tree saplings produced a few new leaves as last attempt to prevent death (Vander Mijnsbrugge et al., 2016;Turcsan et al., 2016). However, there were not enough of such leaves for meaningful analyses.

2.4. Statistical analyses

All statistical analyses were performed using R v.3.6.1. (R Core Team, 2020). The model assumptions were tested following Zuur et al. (2010) and using R/ggpubr (Kassambara, 2019). All graphical output is built using the packages R/GGPLOT2, R/VIRIDIS and R/COWPLOT (Wickham, 2009;Wilke, 2019;Garnier, 2018).

320 ### 2.4.1. Assessing the patterns of CCI and loss of canopy greenness using generalized additive
321 mixed models

322 The patterns of the CCI and loss of canopy greenness data from both our tree saplings and mature trees
323 were assessed using generalized additive mixed models (GAMMs) built using the packages R/MGCV,
324 R/GRATIA and R/DPLYR (Wood, 2011;Wickham et al., 2018;Simpson, 2020;Hastie and Tibshirani,
325 1986;Pedersen et al., 2019). We used GAMMs because they allow more flexibility than other models (e.g.
326 generalized linear models) to model the distribution parameter μ (i.e. the mean of the observed random
327 variable) and the continuous explanatory variables (Rigby and Stasinopoulos, 2005).

329 To model the CCI of both our tree saplings and mature trees as a function of their covariates, Gaussian
330 GAMMs with the identity link function were used (Table 2). To model the loss of canopy greenness of both
331 our tree saplings and mature trees as a function of their covariates and because the loss of canopy
332 greenness is scaled between 0 and 1, Binomial GAMMs with the logistic link function were used (Table 2).
333 The GAMMs were chosen with the lowest AIC value (Akaike information criterion) and all factor-smooth
334 interaction terms were smoothed using P-splines to address the large gap in data (i.e. from November to
335 June) between the yearly sampling periods.

337 For the CCI of the beech saplings, the fixed covariates were the *treatment* (categorical with three levels),
338 *leaf place* (categorical with three levels) and *day of the year* (continuous; model 1). The interaction term
339 was modelled as a factor-smooth interaction between the covariates *day of the year* and *treatment*. The
340 dependency among observations of the same individual tree was incorporated by using *individual tree* as
341 random intercept.

342 Model 1

343 $$Y_{ij} \sim Gaussian(\mu_{ij},\ cst.)$$
344 $$g(\mathbb{E}(Y_{ij})) = g(\mu_{ij})$$
345 $$g(\mu_{ij}) = Treatment_{ij} + Leaf\ place_{ij} + f(Day\ of\ the\ year_{ij}, Treatment_{ij}) + Individual\ tree_i$$

347 where g is the identity link function, μ_{ij} is the conditional mean, Y_{ij} is the *j*th observation of the response
348 variable (i.e. the CCI) in Individual tree *i*, and *i* = 1,…, 128, and Individual tree*i* is the random intercept (Zuur
349 et al., 2007;Zuur et al., 2016).

350 For the loss of canopy greenness of the beech saplings, the fixed covariates were the *treatment*
351 (categorical with three levels) and *day of the year* (continuous; model 2). The interaction term and the
352 dependency among observations of the same individual tree were treated as in model 1.

353 Model 2

354 $$Y_{ij} \sim B(n_{ij}, \pi_{ij})$$
355 $$g(\mathbb{E}(Y_{ij})) = g(\mu_{ij})$$
356 $$g(\mu_{ij}) = Treatment_{ij} + f(Day\ of\ the\ year_{ij}, Treatment_{ij}) + Individual\ tree_i$$

358 where n_{ij} is the number of observations, π_i is the probability of 'success', g is the logit link function, μ_{ij} is
359 the conditional mean, Y_{ij} is the *j*th observation of the response variable (i.e. the loss of canopy greenness)
360 in Individual tree *i*, and *i* = 1,…, 128, and Individual tree*i* is the random intercept.

361 For the CCI of the mature beech, birch and oak trees, the fixed covariates were the *year* (categorical with
362 three levels), *leaf type* (categorical with two levels) and *day of the year* (continuous; model 3). The
363 interaction term was modelled as a factor-smooth interaction between the covariates *day of the*

364   *year* and *Year*. The dependency among observations of the same individual tree was incorporated
365   using *individual tree* as random intercept.

366   Model 3

367    $$Y_{ij} \sim \text{Gaussian}(\mu_{ij}, \text{cst.})$$
368    $$g(\mathbb{E}(Y_{ij})) = g(\mu_{ij})$$
369    $$g(\mu_{ij}) = \text{Year}_{ij} + \text{Leaf type}_{ij} + f(\text{Day of the year}_{ij}, \text{Year}_{ij}) + \text{Individual tree}_i$$
370

371   where g is the identity link function, μ_{ij} is the conditional mean, Y_{ij} is the *j*th observation of the response
372   variable (i.e. the CCI) in Individual tree *i*, and *i* = 1,…, 8 for beech, *i* = 1,…, 4 for birch and *i* = 1,…, 4 for oak,
373   and Individual tree$_i$ is the random intercept.

374   For the loss of canopy greenness of the mature beech, birch and oak trees, the fixed covariates were the
375   *Year* (categorical with three levels) and *day of the year* (continuous; model 4). The interaction term and
376   the dependency among observations of the same individual tree were treated as in model 3.

377   Model 4

378    $$Y_{ij} \sim B(n_{ij}, \pi_{ij})$$
379    $$g(\mathbb{E}(Y_{ij})) = g(\mu_{ij})$$
380    $$g(\mu_{ij}) = \text{Year}_{ij} + f(\text{Day of the year}_{ij}, \text{Year}_{ij}) + \text{Individual tree}_i$$
381

382   where n_{ij} is the number of observations, π_{ij} is the probability of 'success', g is the logit link function, μ_{ij} is
383   the conditional mean, Y_{ij} is the *j*th observation of the response variable (i.e. the loss of canopy greenness)
384   in Individual tree *i*, and *i* = 1,…, 16 for beech, *i* = 1,…, 8 for birch and *i* = 1,…, 8 for oak, and Individual
385   tree$_i$ is the random intercept.

386

Table 2: Adjusted R², effective degrees of freedom (edf) and F-test values of the GAMM smooth terms (*Day of the year*). All smooth terms were significant, with p-values < 0.001. $\mathbb{E}(y_i)$ are the expected values of the response variable y_i, $f(x_i)$ is the smooth function of the covariate x_i, β_i is the intercept of the covariate x_i, ζ is the random effect and ε_i are the errors. All smooth functions were fitted using P-splines. The chlorophyll content index, loss of canopy greenness, day of the year and tree individual are abbreviated by CCI, LOCG, Doy and ID, respectively.

Site	Species	Y_i	Model equation	Family distribution	Link function	Adjusted R^2	Smooth term	Treatment	Edf	F or Chi.sq
Wilrijk	*Fagus sylvatica*	CCI	(1) $g(\mathbb{E}(y_i)) = f_1 Treatment_i(Doy_i) + \beta_1 Treatment_i + \beta_2 Leaf\_place_i + \zeta_{ID} + \varepsilon_i$	Gaussian	Identity	0.61	Day of the year	Reference	4.8	337.5
								+0 °C	5.8	175
								+3 °C	6.1	34.4
Wilrijk	*Fagus sylvatica*	Loss of canopy greenness	(2) $g(\mathbb{E}(y_i)) = f_1 Treatment_i(Doy_i) + \beta_1 Treatment_i + \zeta_{ID} + \varepsilon_i$	Binomial	Logit	0.76	Day of the year	Reference	3.6	112.6
								+0 °C	1.1	105.9
								+3 °C	1	53.7
								Year		
KS & PB	*Fagus sylvatica*	CCI	(3) $g(\mathbb{E}(y_i)) = f_1 Year_i(Doy_i) + \beta_1 Year_i + \beta_2 Leaf\_type_i + \zeta_{ID} + \varepsilon_i$	Gaussian	Identity	0.7	Day of the year	2017	4.6	197.8
								2018	5.3	221.6
								2019	5.2	193.2
KS & PB	*Fagus sylvatica*	Loss of canopy greenness	(4) $g(\mathbb{E}(y_i)) = f_1 Year_i(Doy_i) + \beta_1 Year_i + \zeta_{ID} + \varepsilon_i$	Binomial	Logit	0.87	Day of the year	2017	2.4	44.8
								2018	2.5	70.6
								2019	2.7	66
KS	*Betula pendula*	CCI	(5) $g(\mathbb{E}(y_i)) = f_1 Year_i(Doy_i) + \beta_1 Year_i + \beta_2 Leaf\_type_i + \zeta_{ID} + \varepsilon_i$	Gaussian	Identity	0.44	Day of the year	2017	3.2	25.9
								2018	5	56.9
								2019	3.1	14.7
KS	*Betula pendula*	Loss of canopy greenness	(6) $g(\mathbb{E}(y_i)) = f_1 Year_i(Doy_i) + \beta_1 Year_i + \zeta_{ID} + \varepsilon_i$	Binomial	Logit	0.89	Day of the year	2017	1	20.6
								2018	1	36
								2019	1.6	48.2
PB	*Quercus robur*	CCI	(7) $g(\mathbb{E}(y_i)) = f_1 Year_i(Doy_i) + \beta_1 Year_i + \beta_2 Leaf\_type_i + \zeta_{ID} + \varepsilon_i$	Gaussian	Identity	0.52	Day of the year	2017	3.3	62.5
								2018	5.1	84.4
								2019	4.3	30.7
PB	*Quercus robur*	Loss of canopy greenness	(8) $g(\mathbb{E}(y_i)) = f_1 Year_i(Doy_i) + \beta_1 Year_i + \zeta ID + \varepsilon_i$	Binomial	Logit	0.85	Day of the year	2017	1.2	12.5
								2018	1.9	33.6
								2019	2.4	32

2.4.2. Using breakpoints to indicate the onset of autumn leaf senescence and the onset of the loss of canopy greenness

In principle, the onset of autumn leaf senescence could be derived from the CCI or loss of canopy greenness. However, Mariën et al. (2019) recently showed that the latter method cannot be used under severe drought stress. Therefore, two phenological variables were considered to describe the autumn canopy dynamics: the onset of autumn leaf senescence derived from the CCI (the onset of autumn leaf senescence) and the onset of the loss of canopy greenness. For each tree, we defined the onset of autumn leaf senescence and the onset of loss of canopy greenness as the date by which the variable of interest started to decline substantially in early autumn. These dates were calculated using piecewise linear regressions and are represented by the breakpoints resulting from these analyses (Menzel et al., 2015;Mariën et al., 2019;Xie and Wilson, 2020). The piecewise linear regressions were performed using R/SEGMENTED (Vito and Muggeo, 2008). The uncertainty reported represents the inter-tree variability. Trees that did not show a clear breakpoint (13 in the manipulative experiment) were not considered in the analysis. These trees did not show a different pattern of CCI or loss of canopy greenness than the other trees (Fig. A2).

2.4.3. Comparing the onset of autumn leaf senescence among tree saplings exposed to different treatments

We tested whether the beech saplings exposed to the three treatments in 2018 differed in their onset of autumn leaf senescence using a linear model with the onset of autumn leaf senescence as response variable and *treatment* (categorical with three levels) as fixed covariate. The residuals of the model were approximately normally distributed and a Breusch-Pagan test, the R/ncvTest and R/bptest in the R/CAR and R/LMTEST packages, showed no evidence of heteroscedasticity ($P > 0.05$) (Fox and Weisberg, 2019;Zeileis and Hothorn, 2002). A one-way ANOVA was used to detect significant differences in the onset of autumn leaf senescence among the treatments.

2.4.4. Comparing the onset of autumn leaf senescence and the onset of loss of canopy greenness in mature trees among species and years

To model the onset of autumn leaf senescence and the onset of the loss of canopy greenness as a function of their covariates, Gaussian linear mixed models were used. These models were built with the package R/LME4 (Bates et al., 2015).

The effect of the year on the onset of autumn leaf senescence and the onset of the loss of canopy greenness was assessed using two linear mixed effect models with the onset of autumn leaf senescence and the onset of the loss of canopy greenness from the mature beech, birch and oak trees as response variable. The fixed covariate in these two models was the *Year* (categorical with three levels; model 5). To incorporate the dependency among observations of the same species, we used *species* as random intercept.

Model 5

$$Y_{ij} \sim \text{Gaussian}(\mu_{ij}, \text{cst.})$$
$$g(\mathbb{E}(Y_{ij})) = g(\mu_{ij})$$
$$\mu_{ij} = \text{Year}_{ij} + \text{Species}_i$$

where g is the identity link function, μ_{ij} is the conditional mean, Y_{ij} is the jth observation of the response variable in Species i, and $i = 1,…, 3$ and Species_i is the random intercept.

440 The effect of the species on the onset of autumn leaf senescence and the onset of the loss of canopy
441 greenness was assessed using two linear mixed effect models with the onset of autumn leaf senescence
442 and the onset of the loss of canopy greenness from the mature beech, birch and oak trees as response
443 variable. The fixed covariate in these two models was the *Species* (categorical with three levels; model 6).
444 To incorporate the dependency among observations of the same year, we used *Year* as random intercept.

445 Model 6

$$Y_{ij} \sim \text{Gaussian}(\mu_{ij},\ \text{cst.})$$
$$g(\mathbb{E}(Y_{ij})) = g(\mu_{ij})$$
$$\mu_{ij} = \text{Species}_{ij} + \text{Year}_i$$

450 where g is the identity link function, μ_{ij} is the conditional mean, Y_{ij} is the *j*th observation of the response
451 variable in Year *i*, and *i* = 1,…, 3 and Year_i is the random intercept.

452 The residuals of the models were approximately normally distributed and showed no heteroscedasticity
453 (tested using diagnostic plots). Therefore, we used Pearson's chi-square test, R/drop1 in the R/LME4
454 package, to detect significant differences in the onset of autumn leaf senescence and the onset of the loss
455 of canopy greenness among the predictor variables. A multiple comparison test, the R/glht test with
456 method Tukey in the R/MULTCOMP package, was used to test for significant differences among the means
457 of the levels in the predictor variables (Hothorn et al., 2008).

3. Results

3.1. Magnitude of the drought stress in 2017, 2018 and 2019

460 The weather in 2018 and 2019 was exceptional, as can be seen in the overview of the meteorological
461 conditions from 2017 to 2019 against the long-term reference values in Table 1 and Figure 2. In 2017, the
462 weather during spring was dry and warm but the weather during summer and autumn was relatively
463 normal (KMI, 2017b, c, a). In contrast, the warm and dry summer of 2018 was marked by abnormal (with
464 an average return time of 6 years) to exceptional (with an average return time of 30 years or more) values
465 (KMI, 2018b). Furthermore, the autumn of 2018 was abnormally dry and all precipitation fell on relatively
466 few days (32) (KMI, 2018a). In the summer of 2019, the average air temperature and the total amount of
467 sunshine were both among the three highest values recorded since 1981. In fact, the absolute maximum
468 air temperature record for Belgium was broken in 2019 (KMI, 2019b). On the other hand, the autumn of
469 2019 was considered normal (KMI, 2019a).

471 The rainfall deficit for each day in the hydrological year (from the 1st of April until the 31st of March) and
472 different return times are shown in Figure 3 (panel A & B). This demonstrates that in the late spring of
473 2017, the summer of 2018 and the summer of 2019 the rainfall deficit reached a return time between 20
474 and 50 years, 50 years, and 20 years, respectively. The hydrological summers of 2017, 2018 and 2019 had
475 therefore moderate to extremely dry conditions, which led to accumulated rainfall deficit conditions over
476 time (see Figure 3; panel A). Especially the hydrological year starting in 2018 ended with a strong rainfall
477 deficit of about 150 mm, which was not reduced during 2019. The effects of this strong rainfall deficit are
478 also apparent in the lower volumetric soil water content values (ca. 5% less) measured at the beginning
479 of 2019, compared to the same measurements in 2017 and 2018.

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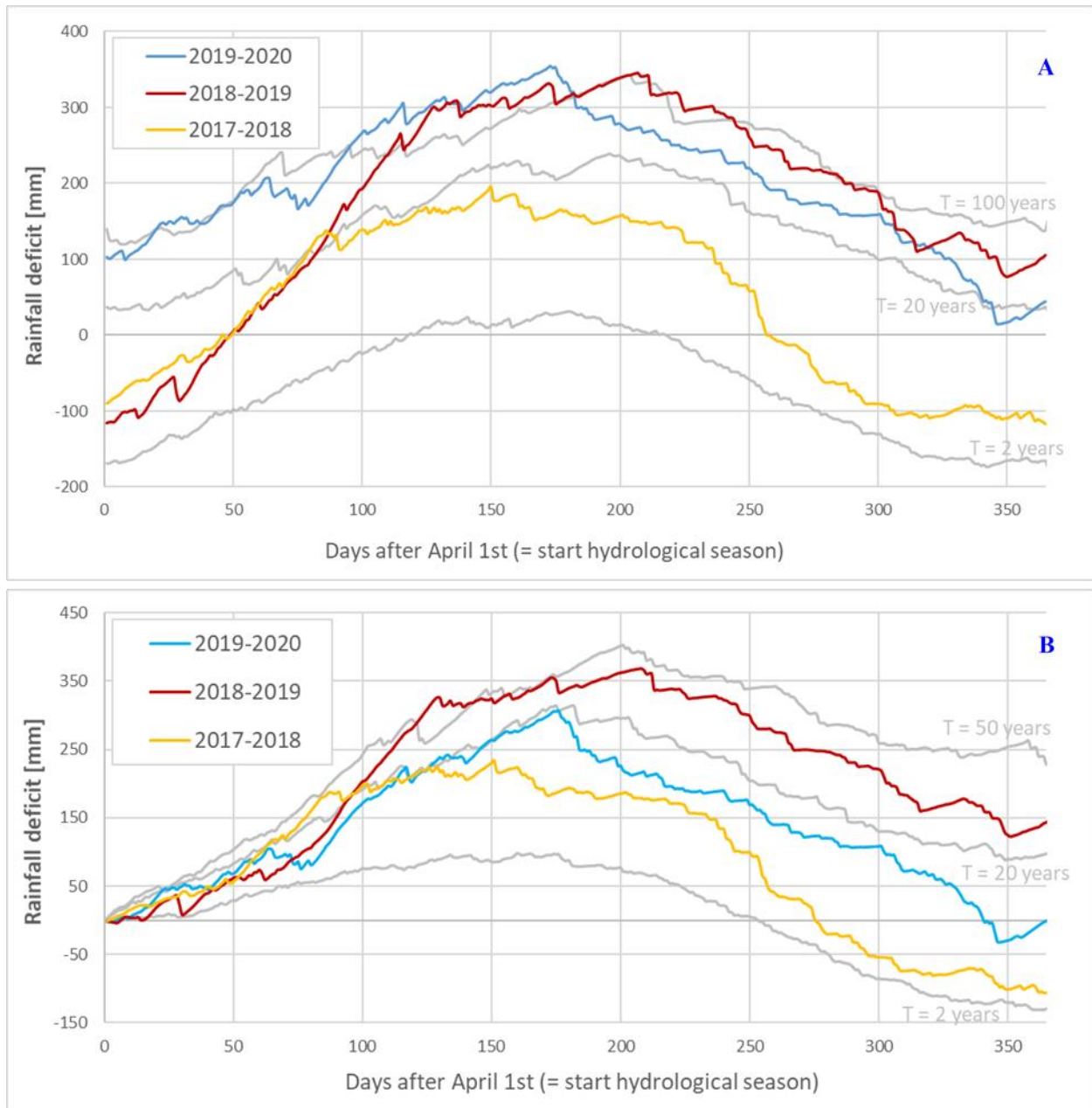
Fig. 3: The rainfall deficit for the meteorological station of the Royal Meteorological Institute (KMI) in
Ukkel, Belgium. The colored solid lines represent the rainfall deficit for the hydrological years in the period
2017-2020, while the grey solid lines represent the long-term reference statistics (computed for the 100-
year period 1901 - 2000) with T as the return period, which represents the mean time between two
successive exceedances of a given deficit value and is computed in an empirical way (Willems, 2000, 2013).
Panel A uses a continuous computation, while panel B starts from a zero deficit on the first of April (the
start of the hydrological year). The colors represent the rainfall deficit in 2017 (light blue), 2018 (red) and
2019 (yellow).

490

3.2. The effect of drought, heat stress and increased atmospheric aridity on the onset of autumn leaf senescence in tree saplings in the manipulative experiment

For all treatments, the CCI values of the beech saplings showed an overall moderate decrease until the beginning of October. Afterwards, this decrease accelerated (Fig. 4; panel A & C; Table 2). In the +0 °C and especially the +3 °C treatment, an abnormal CCI decline was observed in early August with only a partial recovery later on. As a result, from the beginning of August until mid-September, the CCI values of the beech saplings in the reference plots were significantly higher than the CCI values of the beech saplings in the glasshouses. However, no significant difference was detected in the timing of the onset of autumn leaf senescence among the beech saplings exposed to the three different treatments, as the mean onset of autumn leaf senescence was between the 21st (DOY = 260 ± 5) and 25th (DOY = 264 ± 4) of September (P = 0.7; Fig. A3).

The canopy greenness for the beech saplings showed a stable decline from early August until the end of autumn (Fig. 4; panel B & D; Table 2). Nevertheless, during September, the canopy greenness of the beech saplings in the reference plots was significantly higher than the canopy greenness of the beech saplings in the glasshouses with the +3 °C treatment.

The tree saplings in the glasshouses of both treatments were exposed to a high mortality with 14% and 26% of the tree saplings in the glasshouses with the +0 °C and +3 °C treatment, respectively, considered 'dead' along our criteria (see §2.3.). In the reference plots, no beech saplings died.

511
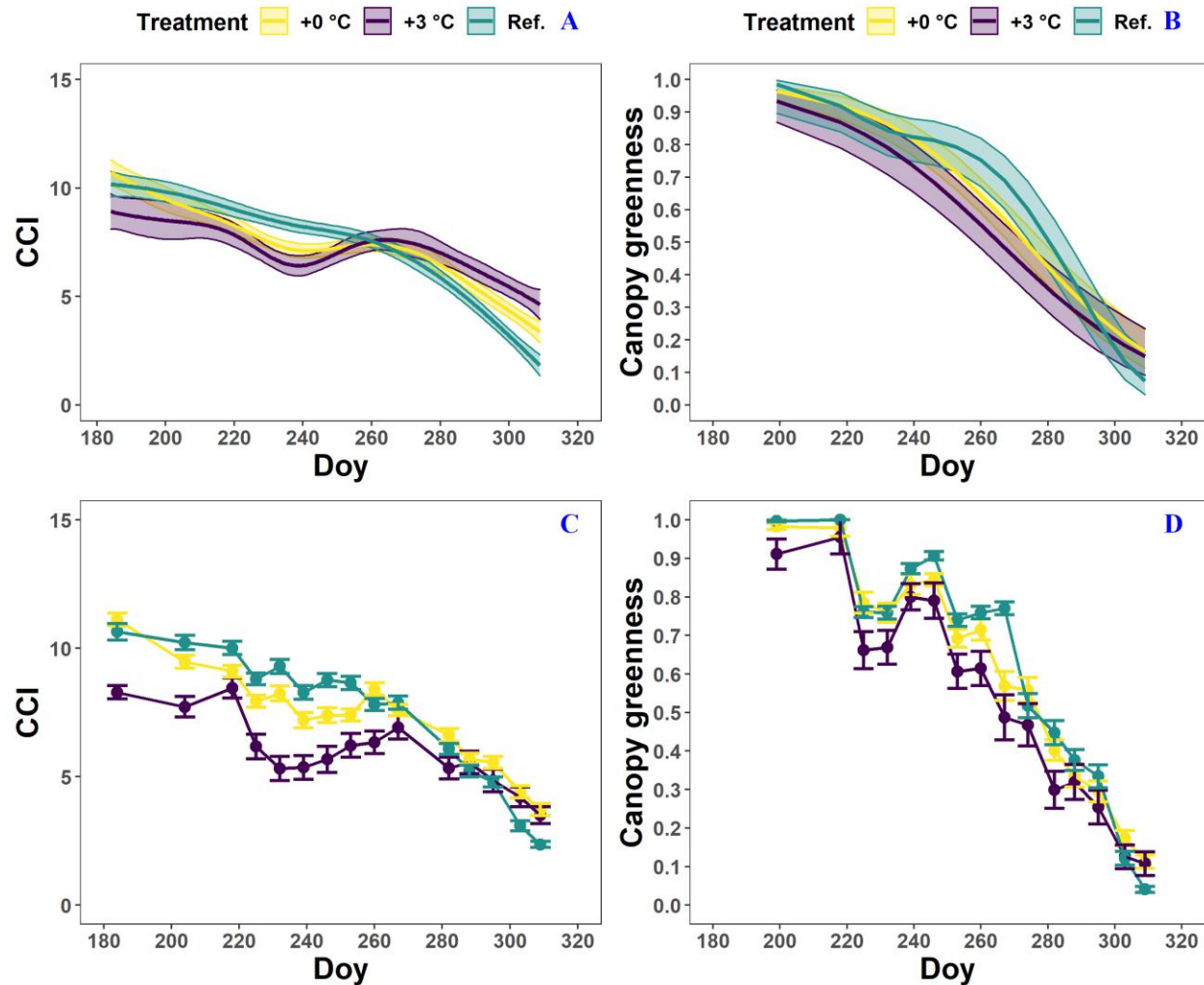
Fig. 4: The generalized additive mixed model fits for the chlorophyll content index (CCI; panel A) and loss
of canopy greenness (panel B) of the *Fagus sylvatica* saplings at the Drie Eiken Campus in Wilrijk. The
colored solid lines represent smooth terms, while the colored shaded bands around the smooth terms
approximate the 95% simultaneous confidence intervals (panel A) and 95% pointwise confidence intervals
(panel B). The dots and error bars represent the mean CCI (panel C) and mean canopy greenness (panel
D) with standard errors. The colors represent the CCI or the loss of canopy greenness of the beech saplings
in the reference plots (green; Ref.), the glasshouses that followed the outside ambient air temperature
(yellow; +0 °C) and the glasshouses that were three degrees warmer than the outside ambient air
temperature (purple; +3 °C), respectively.

521

3.3. Inter-annual and inter-species variability in the timing of the onset of autumn leaf senescence and the onset of the loss of canopy greenness in mature trees

The pattern in the CCI values for the mature beech, birch and oak trees seems consistent throughout the years with stable values in summer and a rapid decline around late October (Fig. 5 - 7; panel A & C; Table 2). We also observed no significant difference in the onset of autumn leaf senescence among the years (P = 0.09) and species (P = 1). The mean onset of autumn leaf senescence among the years was from the 8th (DOY = 281 ± 6) to the 19th (DOY = 292 ± 6) of October (Fig. A4; panel A), while the mean onset of autumn leaf senescence among the species was around the 13th of October (DOY = 286 ± 6; Fig. A4; panel B). The CCI correlated linearly with the chlorophyll concentrations but the data showed more variation in 2018 than 2017 (see Fig. A1).

The pattern in the canopy greenness for the mature beech, birch and oak trees seemed less consistent throughout the years (Fig. 5 - 7; panel B & D; Table 2). The loss of canopy greenness showed a very similar pattern between 2017 and 2019 for birch and beech, with the start of the decline in canopy greenness values around late September for birch and late October for beech. Like beech and birch, oak showed a standard pattern in 2019 with the start of the seasonal decline in late October. However, in 2017, oak showed an earlier loss of canopy greenness with the start of the seasonal decline in mid-September. In all cases, a rapid decline in the canopy greenness was observed in late autumn. In 2018, all species showed an earlier and steeper decline in their canopy greenness values. This effect was also reflected by a significant difference in the onset of the loss of canopy greenness among the years (P = 5 x 10^{-11}). Across species, the onset of the loss of canopy greenness did not differ significantly (P = 0.9) between 2017 (DOY = 292 ± 9) and 2019 (DOY = 290 ± 4), while it occurred 26 and 25 days earlier in 2018 (DOY = 266 ± 4) compared to 2017 (P = 1 x 10^{-5}) and 2019 (P = 1 x 10^{-5}), respectively (Fig. A5; panel A). However, all tree species differed significantly in their onset of the loss of canopy greenness across years (P = 6 x 10^{-9}). Compared to birch (DOY = 268 ± 9; Fig. A5; panel B), the onset of the loss of canopy greenness for beech was on average 16 days later (P = 1 x 10^{-4}; DOY = 284 ± 4), while for oak this was 30 days later (P = 1 x 10^{-4}; DOY = 298 ± 4). The onset of the loss of canopy greenness for beech was also 14 days earlier than that for oak (P = 7 x 10^{-4}).

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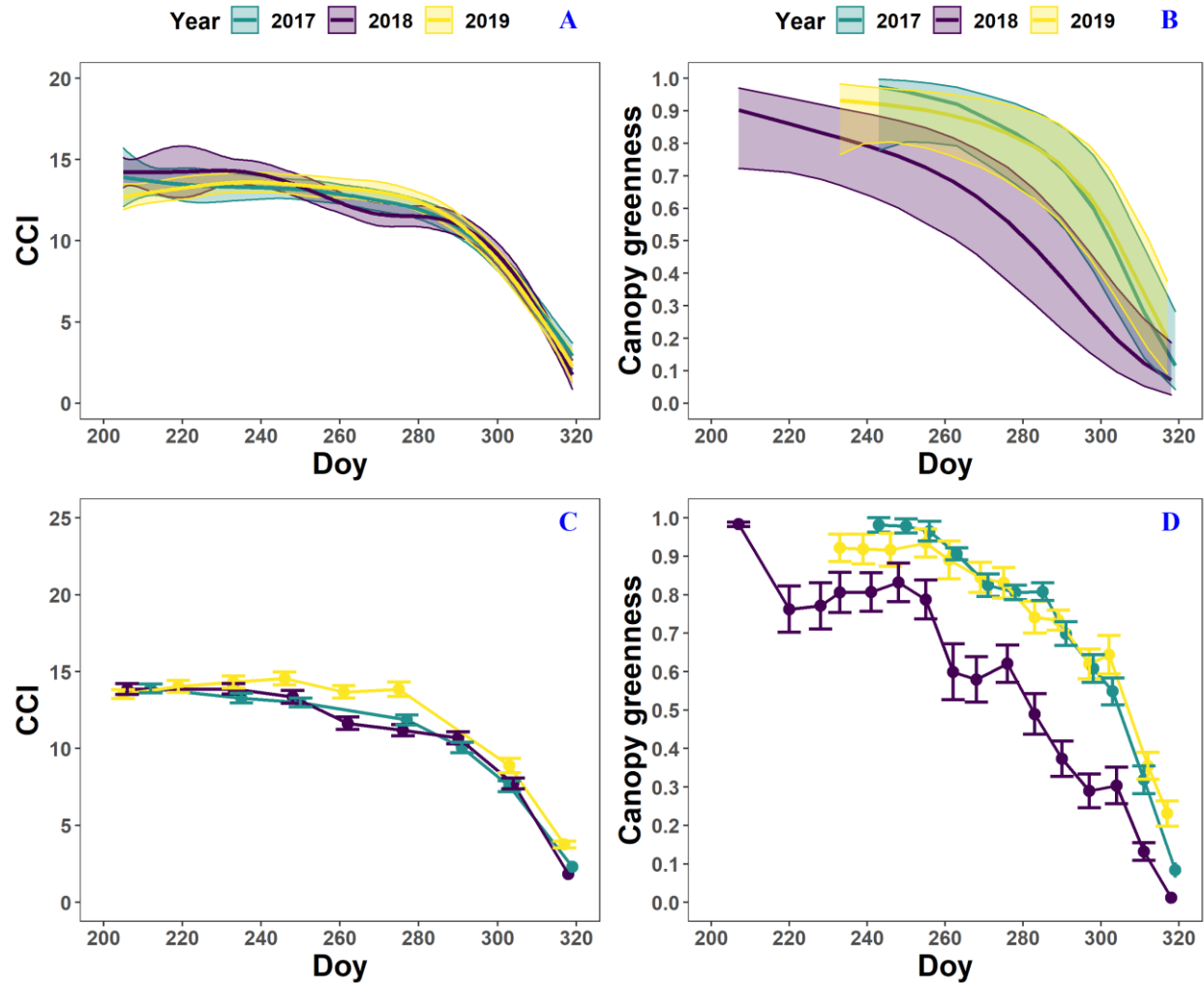
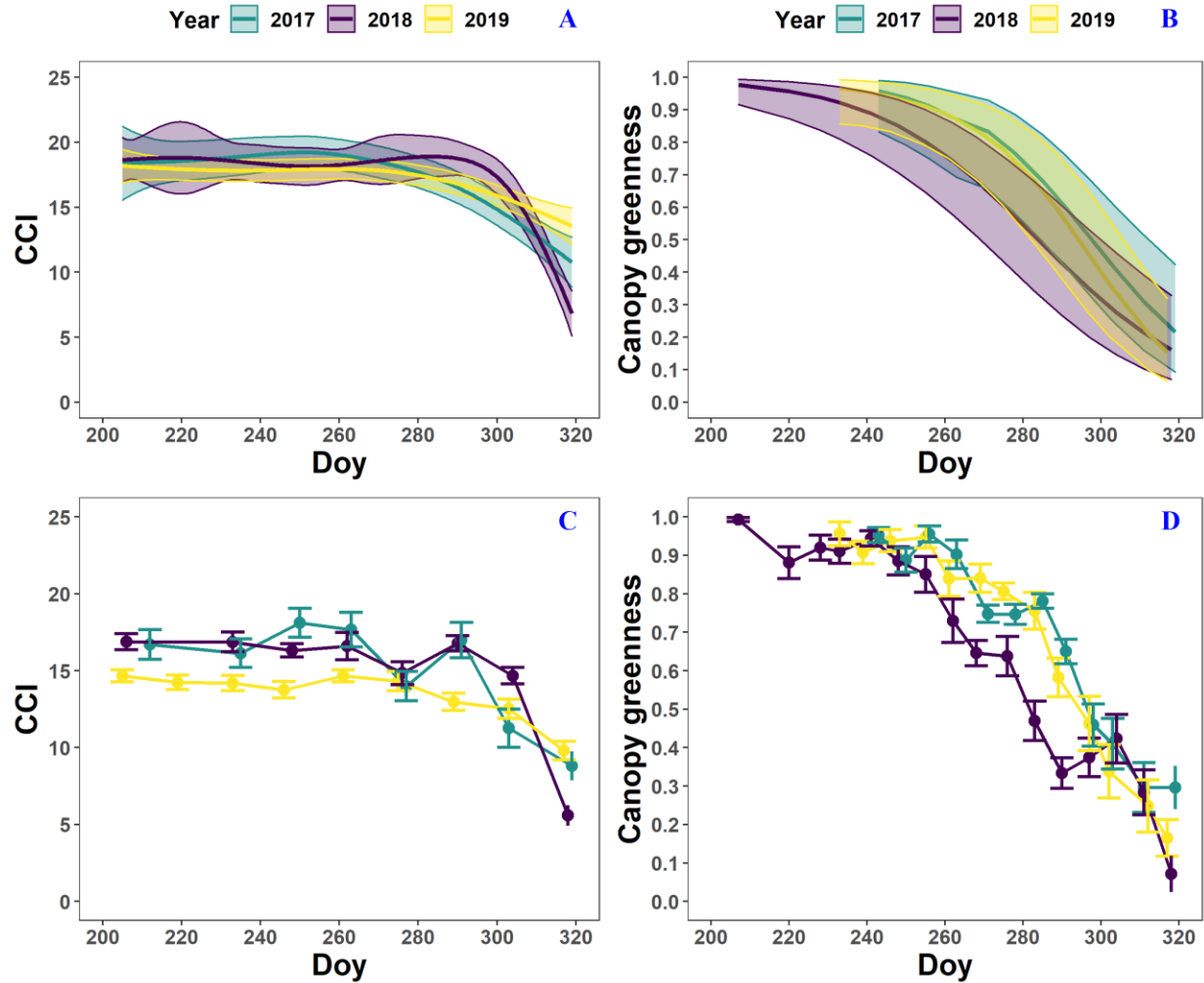
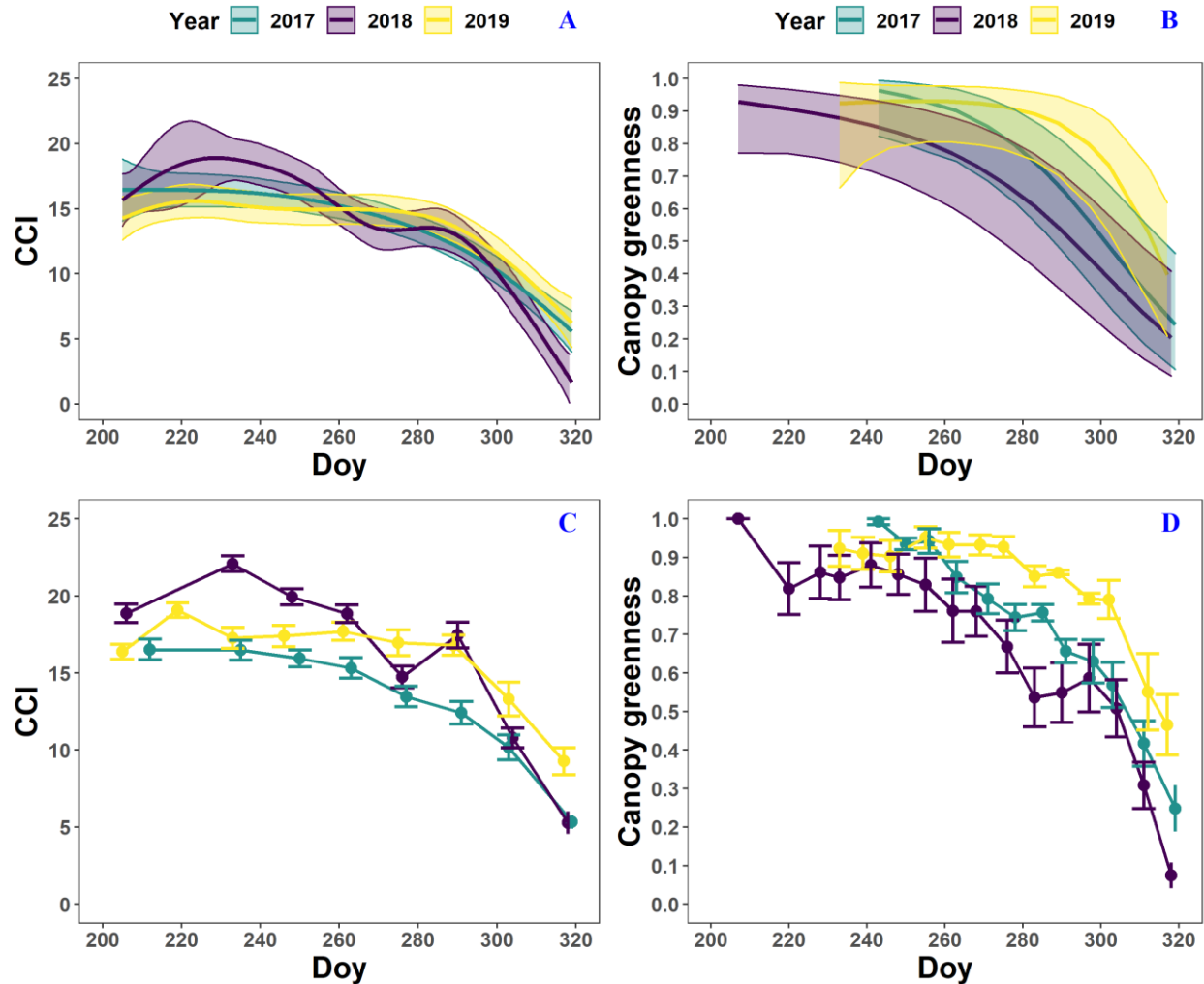


Fig. 5: The generalized additive mixed model fits for the chlorophyll content index (CCI; n = 8; panel A) and
loss of canopy greenness (n = 16; panel B) of the mature *Fagus sylvatica* trees at the Klein Schietveld and
Park of Brasschaat. The colored solid lines represent smooth terms, while the colored shaded bands
around the smooth terms represent approximate 95% simultaneous confidence intervals (panel A) and
95% pointwise confidence intervals (panel B). The dots and error bars represent the mean CCI (panel C)
and mean canopy greenness (panel D) with standard errors. The colors represent the CCI or the loss of
canopy greenness of the mature beech trees in 2017 (green), 2018 (purple) and 2019 (yellow).

559

560
561 Fig. 6: The generalized additive mixed model fits for the chlorophyll content index (CCI; n = 4; panel A) and
562 loss of canopy greenness (n = 8; panel B) of the mature *Betula pendula* trees at the Klein Schietveld. The
563 colored solid lines represent smooth terms, while the colored shaded bands around the smooth terms
564 represent approximate 95% simultaneous confidence intervals (panel A) and 95 % pointwise confidence
565 intervals (panel B). The dots and error bars represent the mean CCI (panel C) and mean canopy greenness
566 (panel D) with standard errors. The colors represent the CCI or the loss of canopy greenness of the mature
567 birch trees in 2017 (green), 2018 (purple) and 2019 (yellow).
568

569
570 Fig. 7: The generalized additive mixed model fits for the chlorophyll content index (CCI; n = 4; panel A) and
571 loss of canopy greenness (n = 8; panel B) of the mature *Quercus robur* trees at the Park of Brasschaat. The
572 colored solid lines represent smooth terms, while the colored shaded bands around the smooth terms
573 represent approximate 95% simultaneous confidence intervals (panel A) and 95% pointwise confidence
574 intervals (panel B). The dots and error bars represent the mean CCI (panel C) and mean canopy greenness
575 (panel D) with standard errors. The colors represent the CCI or the loss of canopy greenness of the mature
576 oak trees in 2017 (green), 2018 (purple) and 2019 (yellow).
577

4. Discussion

Our results showed that the timing of the onset of autumn leaf senescence in both tree saplings and mature trees was not significantly altered by severe drought, heat stress and increased atmospheric aridity induced by a decline in the soil moisture, relative humidity, and an increase in the air temperature and vapor pressure deficit. These results are in contrast to other studies reporting, for example, that drought stress delays the onset of autumn leaf senescence (determined using remote sensing indices or visual assessment) (Wang et al., 2016;Vander Mijnsbrugge et al., 2016;Zeng et al., 2011;Gárate-Escamilla et al., 2020;Seyednasrollah et al., 2020). However, in our study, drought, heat stress and increased atmospheric aridity did affect the loss of CCI and canopy greenness of our beech saplings, their mortality, and the onset of the loss of canopy greenness in our mature trees. The effect of the drought, heat stress and increased atmospheric aridity on the loss of canopy greenness might be due to an early leaf abscission in response to hydraulic failure of the branches (Wolfe et al., 2016;Munné-Bosch and Alegre, 2004). The manipulation experiment on the beech saplings also revealed that the 'drought/less irrigation' treatment alone (the + 0°C treatment) had less impact (e.g. lower tree mortality, lower premature degradation of chlorophyll in summer) than the combined 'drought/less irrigation, warming and increased atmospheric aridity' treatment (the + 3°C treatment). Our experimental design did not allow disentangling the effect of the three different stressors within the + 3°C treatment. However, Fu et al. (2018) found that summer warming delayed senescence in beech. In addition, Kint et al. (2012) found that growth in birch is primarily controlled by the water deficit and low relative humidity values during summer. Therefore, the effects observed in the + 3°C treatment might be mainly related to the atmospheric aridity. For the mature trees, the different drought response of the autumn pattern of chlorophyll (no effect) and the loss of canopy greenness (advanced and enhanced) is probably an important reason of confusion still present today in the literature on the relationship between drought and autumn senescence. While the detoxification of chlorophyll is a prerequisite for the expression of different coloration values, chlorophyll does not degrade at the same speed as other leaf pigments. In fact, not even all leaf pigments degrade (or are formed) at the same velocity throughout the senescence process (Keskitalo et al., 2005). Consequently, observations of changing coloration levels are difficult to interpret. Moreover, note that coloration measurements also take into account leaf yellowing and mortality due to hydraulic failure.

The continuously computed rainfall deficit was similar between 2018 and 2019. Nevertheless, the loss of canopy greenness suggests that the drought of 2019, which coincided with several heat waves and increased atmospheric aridity, might have been less damaging for the late-summer leaf dynamics than the drought of 2018 (which lasted longer). The rainfall deficit starting from a zero deficit supports the observation that, despite the accumulated drought effect, the drought of 2019 was less severe in the growing season than the drought of 2018. Perhaps, the conditions of 2018 (i.e. sunny and warm with high vapor pressure deficits, and a long period with a low soil moisture starting earlier than in 2019) triggered the damaging process of cavitation in the trees, while this might have occurred less intensively in 2019 if the stomatal conductance was lower (Barigah et al., 2013;Bolte et al., 2016;Banks et al., 2019). Alternatively, the difference in the timing of the drought peaks (i.e. the drought of 2018 peaked around one month and half earlier than the drought of 2019, Fig. 3A) could have led to divergent responses due to differences in drought sensitivity along the growing season (Banks et al., 2019).

The drought (but also the heat stress and increased atmospheric aridity) did not affect the onset of autumn leaf senescence of both the beech saplings and the mature trees. Deciduous trees therefore seem to have a conservative strategy concerning the timing of their autumn leaf senescence that might be under the control of a constant variable (e.g. the day-length or spectral quality) (Michelson et al., 2018;Chiang et al., 2019). Such a strategy prioritizes carbon uptake over nutrient remobilization, as a fixed onset of

625 autumn leaf senescence would not allow an advanced nutrient remobilization when required (Keskitalo
626 et al., 2005;Brelsford et al., 2019). Moreover, such a strategy makes the trees vulnerable against the
627 effects of early frost. In case of early frost, the trees might not complete their nutrient resorption. Possible
628 consequences of an incomplete nutrient resorption over a longer time period might include a decline in
629 the overall fitness of the trees and negative feedbacks on the growth dynamics of the next season, such
630 as less buds (Fu et al., 2014;Vander Mijnsbrugge et al., 2016;Crabbe et al., 2016). Although Fu et al. (2014)
631 suggested a correlation between the bud burst and the onset of autumn leaf senescence, we have found
632 no relationships for 2018 and 2019 in birch and beech, and a positive relationship in oak (every delay of
633 one day in the bud burst corresponded to a delay of ± two days in the onset of autumn leaf senescence).
634
635 Surprisingly, the onset of autumn leaf senescence did not differ significantly among the different tree
636 species, which supports the idea that the onset of autumn leaf senescence in different deciduous trees
637 might be controlled by the same (light related) signal. Perhaps the onset of leaf senescence is timed in a
638 manner similar to flowering, as put forward by the external coincidence model (i.e. clock-regulated gene
639 expression and light both determine the perception of photoperiodism) (Böhlenius et al., 2006;Kobayashi
640 and Weigel, 2007;Koornneef et al., 1991;Yanovsky and Kay, 2002). Other explanations for the lack of
641 significant differences in the onset of autumn leaf senescence among the species could have been the
642 small sample size (i.e. eight beech, four birch and four oak trees for the CCI measurements) or the
643 inaccuracies related to the method of piece-wise linear regressions. Given our results, the drought in 2017,
644 2018 and 2019 had little impact on the CCI trend and onset of autumn leaf senescence in mature beech,
645 birch and oak trees. Other explanations for this result could be the small sample size (i.e. 8 beech, 4 birch
646 and 4 oak trees for the CCI) or the inaccuracies related to the method of piece-wise linear regressions.
647 Given our results, the drought in 2018 and 2019 had little impact on the CCI trend and onset of autumn
648 leaf senescence in mature beech, birch and oak trees.
649
650 In this regard, the exact impact of the light quantity and spectral quality on the trigger for the onset of
651 senescence (directly or indirectly through photoperiodic detection), is not well known in deciduous trees
652 (Michelson et al., 2018). If phytochrome only responds to the presence of red wavelengths, the effect of
653 the polycarbonate in the glasshouses must have been minimal. However, experimental biases might be
654 caused if cryptochrome, which is sensitive to UV light and active at low fluency rates, played a significant
655 role in the onset of senescence (Schulze et al., 2019;Smith, 1982). Because very low light intensities are
656 required by plants to generate a photosynthetic potential (a minimum scalar irradiance of $\pm$ 1 µmol/m²)
657 and very low fluencies (starting from 0.1 µmol/m²) are required for phytochrome action, we assumed the
658 decrease in the incoming light intensity would not have had a significant effect (Legris et al., 2019;Poorter
659 et al., 2019;Franklin and Quail, 2010;Legris et al., 2016;Neff et al., 2000;Mancinelli and Rabino, 1978).
660
661 Although the onset of autumn leaf senescence in both the tree saplings and the mature trees was not
662 advanced by drought, heat stress and increased atmospheric aridity, the onset of autumn leaf senescence
663 in beech saplings was around 22 days earlier than mature beech trees. Such difference could be due to
664 the different growing conditions (pots versus normal soil), environmental conditions at the different sites,
665 the difference in the average leaf age (tree saplings have an earlier bud-burst than mature trees) or the
666 different ecophysiological response of tree saplings and mature trees (e.g. tree saplings are more
667 vulnerable than mature trees and therefore are likely to use different functional strategies) (Niinemets,
668 2010;Vander Mijnsbrugge et al., 2016;Pšidová et al., 2015). As there is very little difference in the light
669 conditions among the different sites, the difference in the day length is unlikely to have affected the
670 difference in the timing of the onset of autumn leaf senescence between the beech saplings and mature
671 trees. However, it is possible that the beech saplings have a different sensitivity to the light cues, as they

672   usually grow in the understory and therefore under a different light regime than mature trees (Brelsford
673   et al., 2019;Michelson et al., 2018;Chiang et al., 2019).
674
675   Concerning the onset of the loss of canopy greenness for all species and opposed to 2017 (i.e. a year with
676   normal environmental conditions in late-summer and autumn) and 2019 (i.e. a year with high
677   temperatures in summer, relatively normal precipitation in summer and autumn, but suffering from the
678   accumulated effects of the rainfall deficit), the onset of the loss of canopy greenness in 2018 was around
679   three-and-a-half weeks earlier. The canopy greenness metric had been declining earlier in 2018 because
680   the leaves have likely been shed earlier due to an advanced leaf abscission process to protect the tree
681   from hydraulic failure (Munné-Bosch and Alegre, 2004;Wolfe et al., 2016). There was also a difference in
682   the onset of the loss of canopy greenness among the species. This might be due to two reasons. First,
683   birch (the species with the earliest onset of the loss of canopy greenness) has an indeterministic growth
684   pattern, which also means continuous leaf mortality. Second, the fact that oak (the species with the latest
685   onset of the loss of canopy greenness) has typically a second leaf flush, which might connect the difference
686   between beech and oak to differences in leaf longevity.

687   5. Conclusion

688   The different environmental conditions of three years (comprising a severe dry year and a severe warm
689   year) did not affect the timing of the onset of autumn leaf senescence in mature beech, birch and oak
690   forest trees in Belgium. This suggests that deciduous trees have a conservative strategy concerning the
691   timing of their senescence. Like our mature beech trees, beech saplings exposed to drought, heat stress
692   and increased atmospheric aridity also did not show any advancement in their onset of autumn leaf
693   senescence compared to beech saplings in normal conditions. Although the drought, heat stress and
694   increased atmospheric aridity did not affect the timing of the onset of autumn leaf senescence, it is clear
695   from our results that they affect the mortality rate in tree saplings and the leaf mortality in mature trees.

Appendix A

697 Figures

698

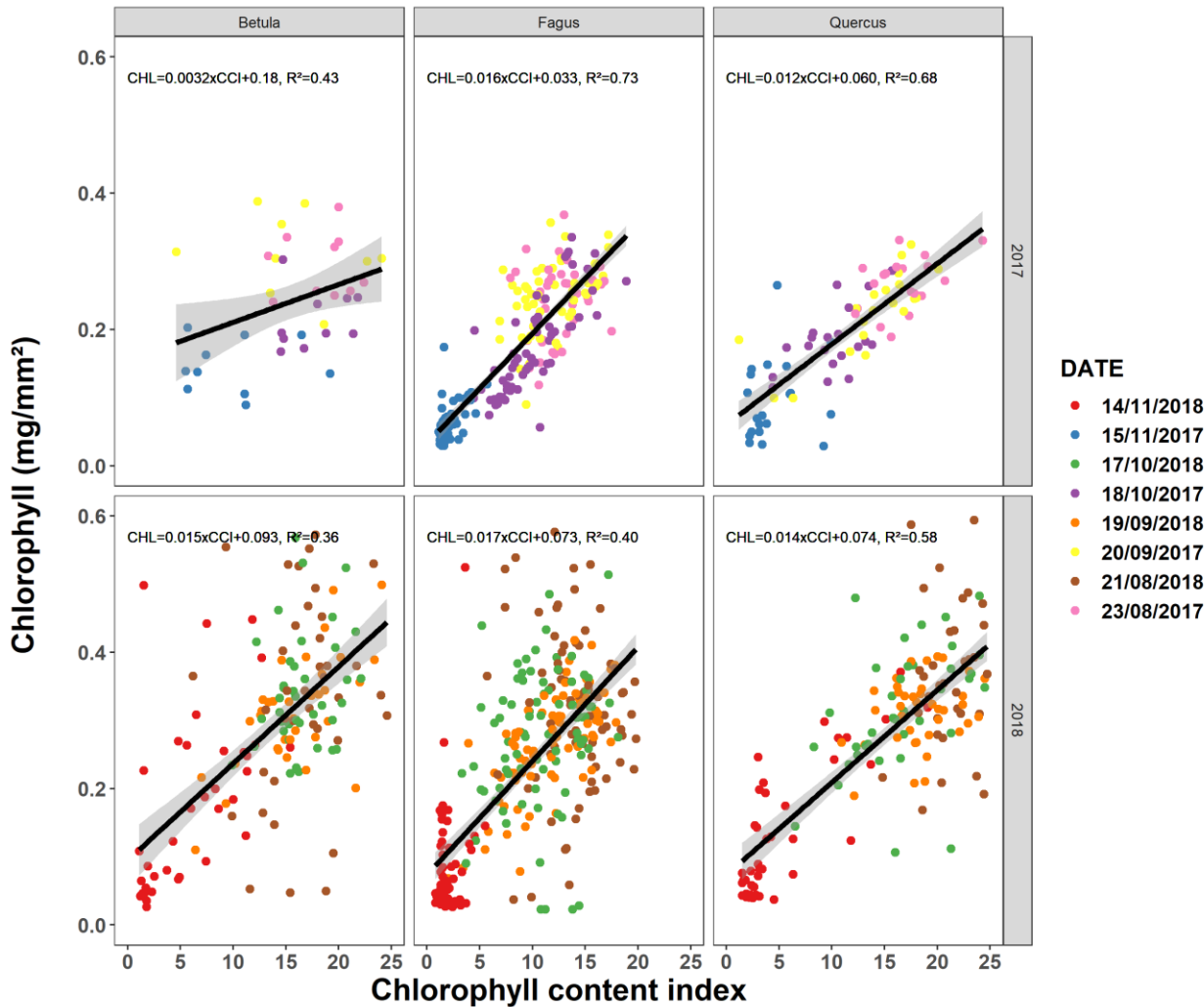



Fig. A1: Relationship between the chlorophyll content index measured using a chlorophyll content meter
(CCM-200 plus, Opti-Sciences Inc., Hudson, NH, USA) and the chlorophyll concentration measured using
spectrophotometric analysis (Mariën et al., 2019). Between late August and late November 2017-2018,
we sampled every month 20-40 leaves (five leaves for four to eight trees) for beech and 10 to 20 leaves
(five for two to four trees) for birch and oak. The different colors represent different sampling dates.

705

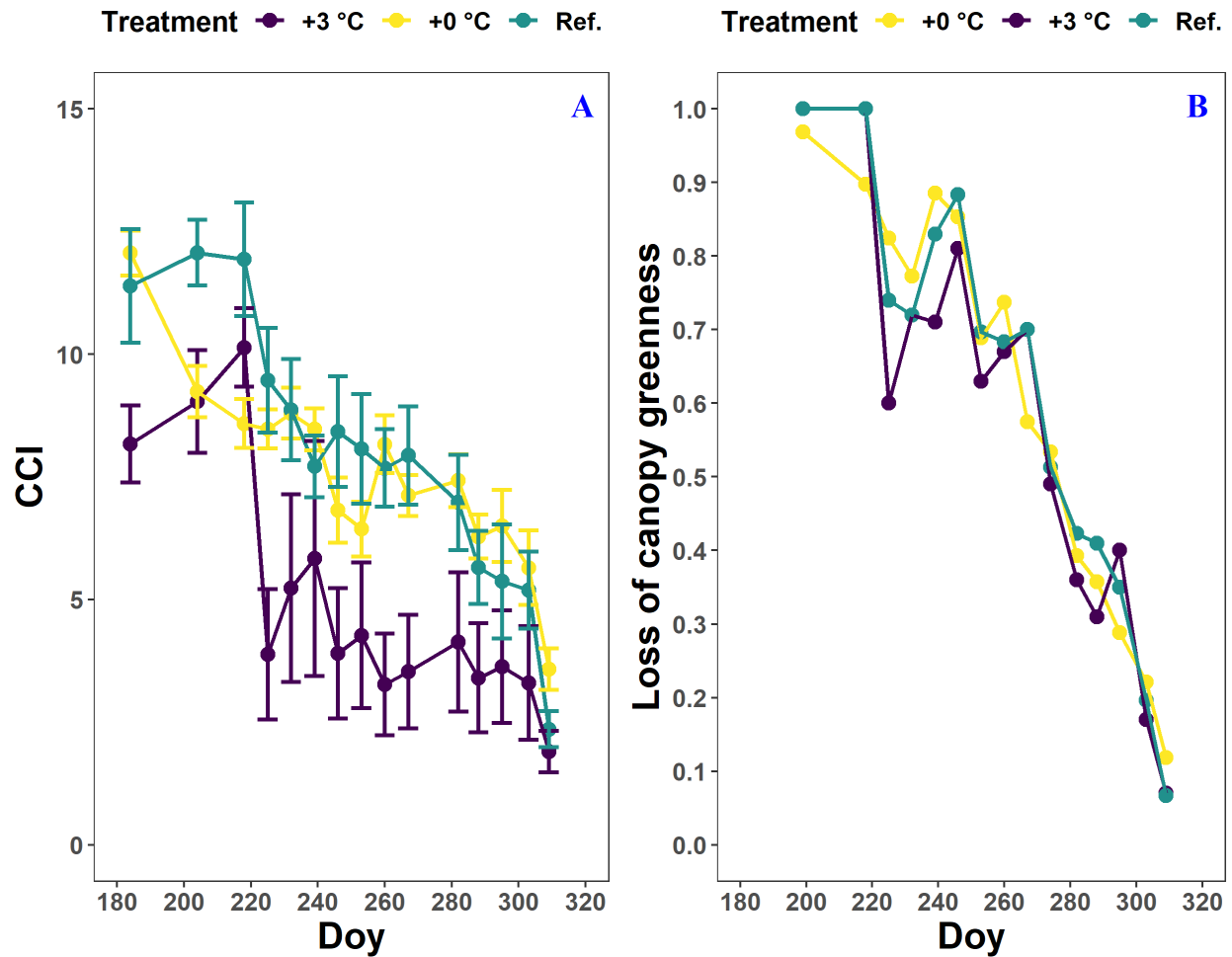



Fig. A2: The chlorophyll content index (CCI; panel A) and loss of canopy greenness (panel B) of the *Fagus*
sylvatica saplings at the Drie Eiken Campus in Wilrijk for which no breakpoint could be calculated. The
dots and error bars represent the mean CCI (panel A) and mean loss of canopy greenness (panel B) with
standard errors. The colors represent the CCI or the loss of canopy greenness of the beech saplings in the
reference plots (green; Ref.), the glasshouses that followed the outside ambient air temperature (yellow;
+0 °C) and the glasshouses that were three degrees warmer than the outside ambient air temperature
(purple; +3 °C), respectively.

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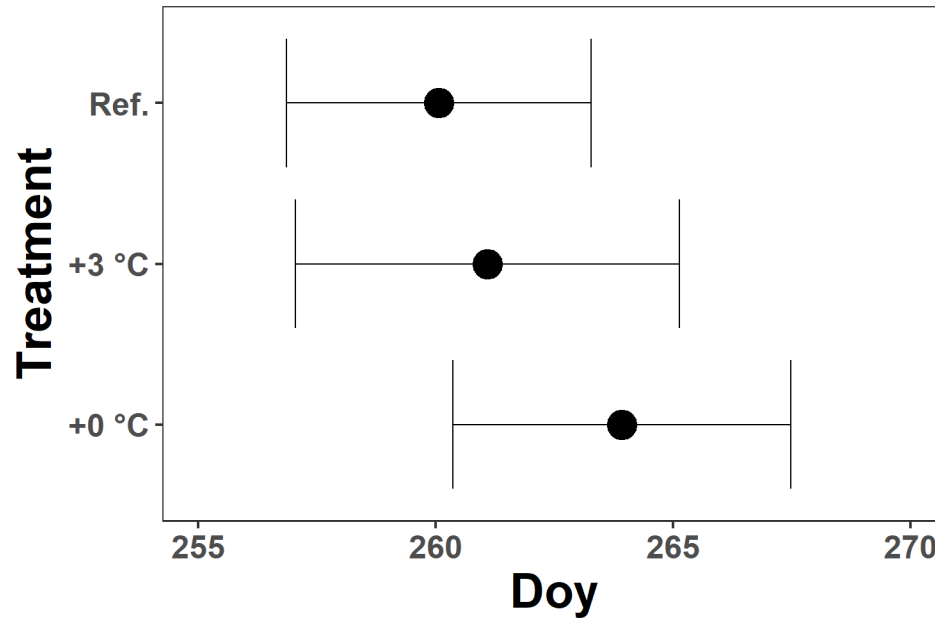
Fig. A3: The mean onset of autumn leaf senescence per treatment for all *Fagus sylvatica* saplings at the Drie Eiken Campus in Wilrijk. Black dots represent the mean onset of autumn leaf senescence, while the error bars represent standard errors that indicate the inter-individual variability. All breakpoints are calculated using the chlorophyll content index data and piecewise-linear regressions ($n_{Ref.}$ = 29; $n_{+0\,°C}$ = 26; $n_{+3\,°C}$ = 22). : Ref. represents the breakpoints of the trees in the reference plots, while +0 °C and +3 °C represents the breakpoints of the trees in the glasshouses under the 'drought/less irrigation' and 'drought/less irrigation, warming and increased atmospheric aridity' treatments, respectively.

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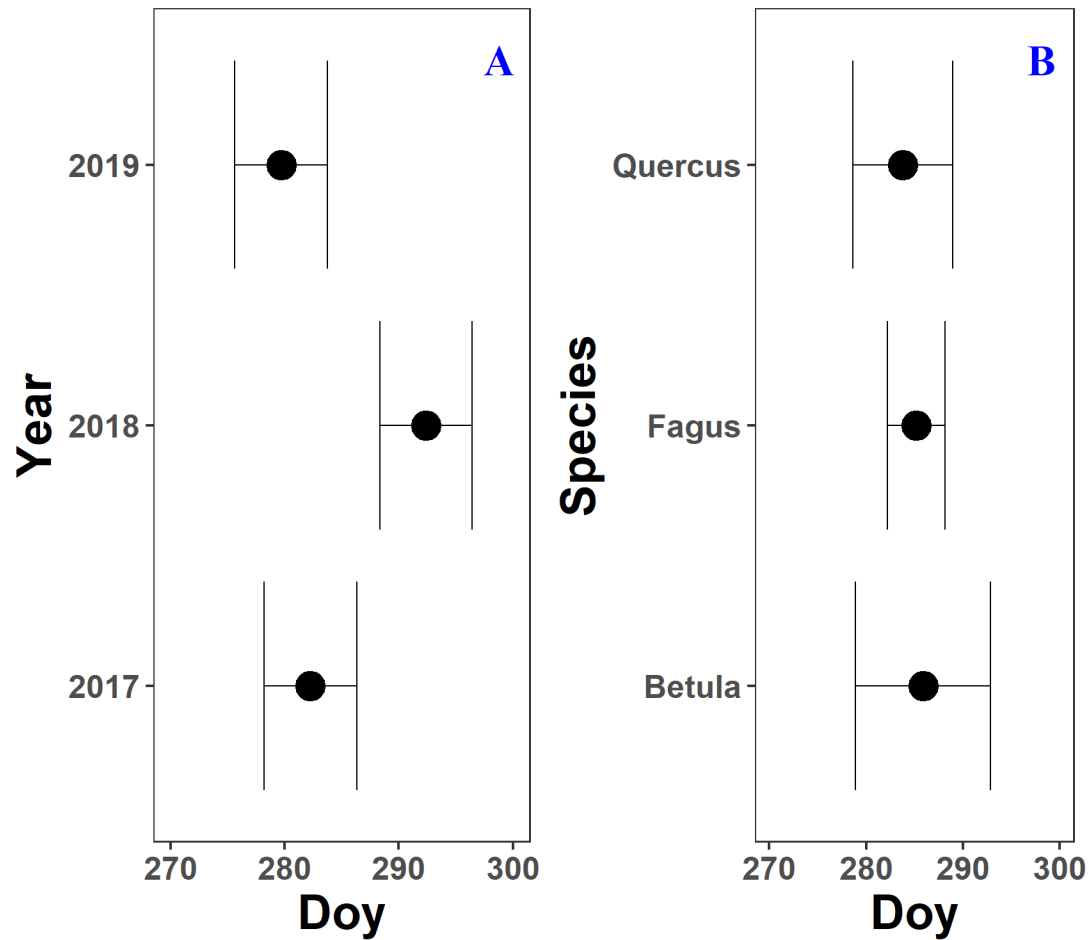
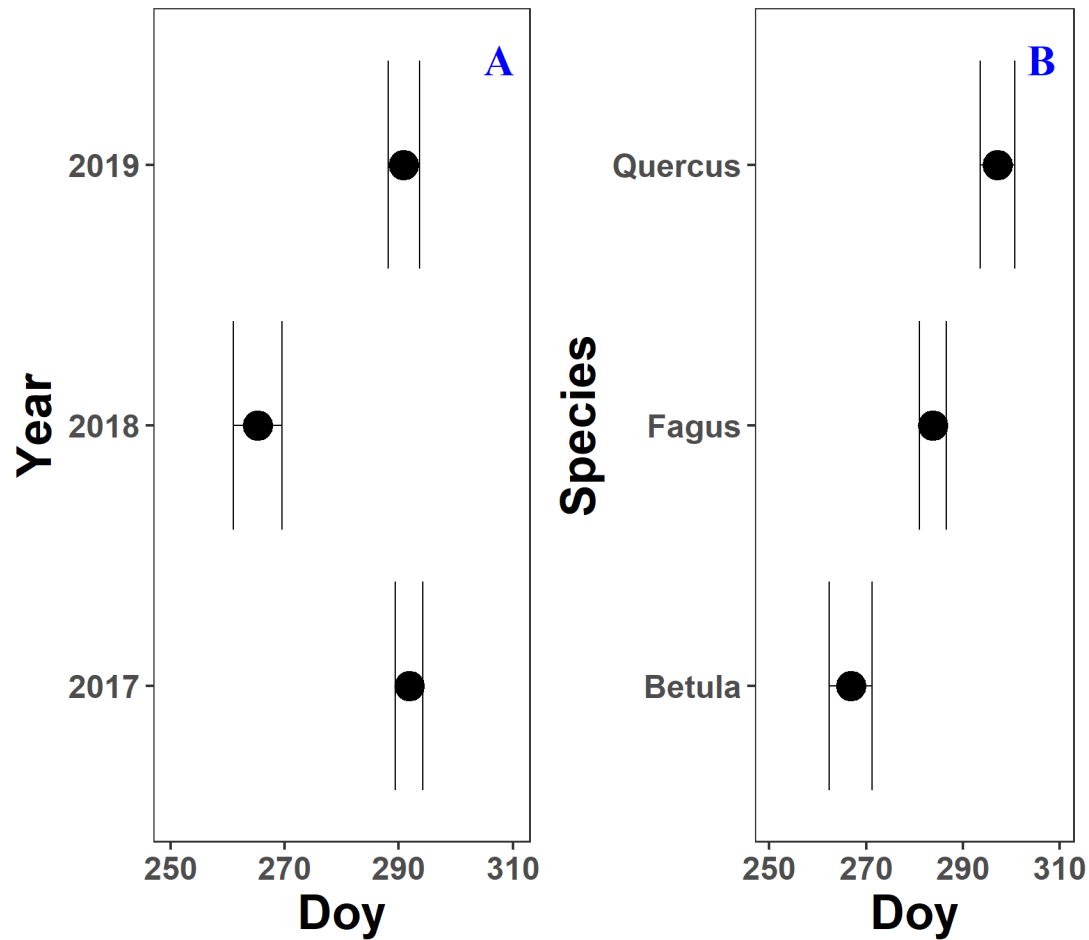


Fig. A4: The mean onset of autumn leaf senescence for three years (panel A; 2017 - 2019) and the three species (panel B; *Fagus sylvatica*, *Betula pendula* and *Quercus robur*) measured on all mature trees at the Klein Schietveld and Park of Brasschaat. Black dots represent the mean onset of autumn leaf senescence, while the error bars represent standard errors that indicate the inter-individual variability. All breakpoints are calculated using the chlorophyll content index data and piecewise-linear regressions (n_{Fagus} = 8; n_{Betula} = 4; $n_{Quercus}$ = 4).

729
Fig. A5: The mean onset of the loss of canopy greenness for three years (panel A; 2017 – 2019) and the
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three species (panel B; *Fagus sylvatica*, *Betula pendula* and *Quercus robur*) measured on all mature trees
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at the Klein Schietveld and Park of Brasschaat. Black dots represent the mean onset of the loss of canopy
732
greenness, while the error bars represent standard errors that indicate the inter-individual variability. All
733
breakpoints are calculated using the loss of canopy greenness data and piecewise-linear regressions
734
(nFagus = 16; nBetula = 8; nQuercus = 8).
735

Data availability

The code and data corresponding to the work presented in this article is available at Zenodo as doi: 10.5281/zenodo.4559535

Author contributions

MC and HDB designed the experiment. ID, SL, PW and BM collected the data. PW computed the rainfall deficit, while BM performed all other analyses. BM, PW and MC wrote the text. All authors contributed to the discussions.

Competing interests

The authors declare that they have no conflict of interest.

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