



*Elevated atmospheric CO₂ and humidity delay leaf fall in *Betula pendula*, but not in *Alnus glutinosa* or *Populus tremula* × *tremuloides**

Article

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1 **Elevated atmospheric CO₂ and humidity delays leaf fall in *Betula pendula*,**
2 **but not in *Alnus glutinosa* or *Populus tremula* × *tremuloides*.**

3

4 **Executive Summary**

5 The effects of both elevated atmospheric CO₂ and increased air humidity on
6 autumn leaf fall were assessed using free air systems. Both factors delayed leaf
7 litter fall in *Betula pendula*, but not in *Populus tremula* × *tremuloides* or *Alnus*
8 *glutinosa*.

9

10 **Abstract**

11 Context: Anthropogenic activity has increased the level of atmospheric CO₂,
12 which is driving an increase of global temperatures and associated changes in
13 precipitation patterns. At Northern latitudes, one of the likely consequences of
14 global warming is increased precipitation and air humidity.

15 Aims: In this work, the effects of both elevated atmospheric CO₂ and increased air
16 humidity on trees commonly growing in northern European forests were assessed.

17 Methods: The work was carried out under field conditions by using Free Air
18 Carbon dioxide Enrichment (FACE) and Free Air Humidity Manipulation
19 (FAHM) systems. Leaf litter fall was measured over 4 years (FACE) or 5 years
20 (FAHM) to determine the effects of FACE and FAHM on leaf phenology.

21 Results: Increasing air humidity delayed leaf litter fall in *Betula pendula*, but not
22 in *Populus tremula* × *tremuloides*. Similarly, under elevated atmospheric CO₂,
23 leaf litter fall was delayed in *Betula pendula*, but not in *Alnus glutinosa*. Increased

24 CO₂ appeared to interact with periods of low precipitation in summer and high
25 ozone levels during these periods to effect leaf fall.

26 Conclusions: This work shows that increased CO₂ and humidity delay leaf fall,
27 but this effect is species specific.

28

29 **Keywords:** climate change, Free Air CO₂ Enrichment (FACE), Free Air Humidity
30 Manipulation, leaf fall, ozone

31

32 **Introduction**

33 Anthropogenic activities since the industrial revolution have increased
34 atmospheric CO₂ concentrations (IPCC 2013), leading not only to climate
35 warming, but also to direct effect of elevated CO₂ on forest net primary
36 productivity (NPP, Norby et al. 2005). In addition, climate change is predicted to
37 increase precipitation at Northern latitudes (IPCC 2013), likely leading to an
38 increase in air humidity. For example, in the Baltic region climate change
39 scenarios for the year 2100 predict an increase in air temperature (by 2.3–4.5 °C),
40 precipitation (by 5–30%), cloudiness (by 2%), but also higher wind speeds and
41 vapour pressure (Kont et al. 2003). Studies investigating the impact of global
42 environmental change on terrestrial ecosystems have identified a consistent
43 pattern of phenological change in the Northern hemisphere (IPCC 2013). Analysis
44 of normalised difference vegetation index (NDVI) remote sensing data gathered
45 during 1985-1999 has revealed an 18 day extension of the growing season in
46 Eurasia (Zhou et al. 2001). Multiple drivers have been shown to differentially

47 influence plant phenophases, earlier bud break has been correlated with
48 atmospheric warming and delayed senescence (Menzel et al. 2006) and
49 interactions between temperature and elevated atmospheric CO₂ concentrations
50 have been described (Taylor et al. 2008). The process of senescence is governed
51 by developmental age, but also influenced by various integrated endogenous and
52 environmental signals (Lim et al. 2007). Environmental factors influencing leaf
53 senescence can be grouped into: (i) abiotic factors that include drought, nutrient
54 limitation, extreme temperatures, ozone induced oxidative stress, and (ii) biotic
55 factors including, pathogen infection or shading by other plants (Li et al. 2000).
56 Endogenous factors influencing senescence include carbon source-sink
57 relationships, phytohormones, particularly jasmonic (JA) and abscisic acid
58 (ABA), ethylene and salicylic acid (SA). The aforementioned phytohormones
59 initiate senescence through cellular signalling pathways in response to various
60 abiotic and biotic stresses that promote the expression of senescence inducing
61 genes (Morris et al. 2000).

62 Elevated atmospheric CO₂ has shown been to increase long term forest net
63 primary productivity (Zak et al. 2011), if nutrients are not limiting (Leuzinger
64 and Hätenschwiler 2013). However studies of the effects of elevated atmospheric
65 CO₂ on tree autumnal phenophase have produced conflicting results. For example,
66 elevated CO₂ advanced senescence in two varieties of *Pinus ponderosa* (Houpis et
67 al. 1988) and also in *Populus trichocarpa* (Sigurdsson 2001), yet delayed
68 senescence of *Quercus myrtifolia* (Li et al. 2000) and *Populus* species grown in
69 freely rooted field conditions during the AspenFACE and POPFACE studies

70 (Taylor et al. 2008). At the DukeFACE experiment, however, no effect on leaf
71 phenology was observed in *Liquidambar styraciflua* (Herrick and Thomas 2003).
72 Air water vapour content determines the vapour pressure difference between
73 ambient air and leaf interior (VPD_L), a gradient which drives the transpiration
74 process of plant foliage. At higher relative humidity, both VPD_L and
75 transpirational flux decrease, which has been demonstrated in the Free Air
76 Humidity Manipulation (FAHM) experiment in both *Betula pendula* Roth and
77 *Populus tremula* L. \times *P. tremuloides* Michx. in rainy summers when soil water
78 content is not limiting in ambient conditions (Kupper et al. 2011; Tullus et al.
79 2012a). It has been shown that elevated humidity diminishes nutrient supply to the
80 leaves and photosynthetic capacity, altering foliar and fine-root properties and tree
81 growth rate (Tullus et al. 2012a; Hansen et al. 2013; Parts et al. 2013; Sellin et al.
82 2013). However, the effect of air humidity changes on leaf fall in trees has not
83 been studied to date.

84 Natural autumnal senescence is regulated by the interaction of a number of factors
85 including day length and temperature, nitrogen and water supply, as well as sink
86 strength within the plant (Wingler et al. 2006). Thus, changes in the timing of leaf
87 senescence are governed by, amongst other factors, assimilation during the
88 vegetation period and sugar accumulation in leaves (Swartzberg et al. 2010).
89 Several studies utilising molecular genetic approaches have indicated that high
90 concentrations of leaf sugars reduce photosynthetic activity, which in turn induces
91 leaf senescence (Swartzberg et al. 2010). In *Acer saccharinum*, girdling resulted in
92 increased sugar accumulation in leaves, and subsequent formation of anthocyanins

93 (Murakami et al. 2008), whilst increased anthocyanin content in another study
94 utilising the same species was associated with a delay in leaf senescence
95 (Schaberg et al. 2008). Furthermore, transcriptome analysis of *Populus* trees
96 grown under elevated CO₂ in field conditions revealed up-regulation of genes
97 determining anthocyanin production during delayed senescence (Tallis et al.
98 2010). These authors suggest that anthocyanins may play a protective role in leaf
99 metabolism and increase leaf longevity.

100 In the work presented here we investigated the effect of two factors of global
101 climate change, atmospheric CO₂ and humidity, on autumn leaf fall. We
102 speculated the effects of both of these factors were tree species specific. Thus, we
103 hypothesised that (i) elevated CO₂ delays and (ii) elevated atmospheric humidity
104 anticipates leaf senescence in broadleaved species.

105

106 **Material and Methods**

107 The investigation was carried out at two sites, a Free Air Carbon dioxide
108 Enrichment experiment (BangorFACE) and a Free Air Humidity Manipulation
109 (FAHM) experiment.

110 **The FACE facility**

111 The BangorFACE experimental site was established in March 2004 on two former
112 agricultural fields with a total area of 2.36 ha at the Bangor University research
113 farm (53°14'N, 4°01'W) in North Wales, UK. Both fields were originally
114 pastures, one field was used for small scale forestry experiments for the last 20
115 years, the other field was ploughed and planted with oil seed rape in 2003.

116 Climate at the site is classified as Hyperoceanic, with a mean annual temperature
117 in 2005 through 2008 of 11.5 °C and an annual rainfall of 1034 mm (Figure 1a).
118 Soil is a fine loamy brown earth over gravel (Rheidol series) and classified as
119 Fluventic Dystrochrept (Smith et al. 2013a). Soil texture is 63% sand, 28% silt
120 and 9% clay. The topography consists of a shallow slope of approximately 1–2°
121 on a deltaic fan. The site aspect is northwesterly, with an altitude of 13 to 18m
122 a.s.l. The depth of the water table ranges between 1 and 6 m.

123 At the BangorFACE site eight octagonal plots, four ambient and four CO₂
124 enriched were established, creating a 2 × 4 factorial block design across the two
125 fields. Three tree species (*Alnus glutinosa* [L.] Gaertner, *Betula pendula* Roth. and
126 *Fagus sylvatica* L.) were selected due to their contrasting shade tolerance,
127 successional chronology and to represent a range of taxonomic, physiological and
128 ecological types. Each plot was divided into seven planting compartments and
129 planted in a pattern creating areas of one, two and three species mixtures. The
130 present study makes use of observations originating from three single species
131 subplots of *B. pendula* and *A. glutinosa*. The site was planted with 60 cm saplings
132 of each species. Within each treatment, the planting pattern was rotated by 90 °
133 between the four plots to avoid potential artefacts introduced by microclimate, soil
134 and uneven growth rates of the different species. Each plot was surrounded by a
135 10 m border of *B. pendula*, *A. glutinosa* and *F. sylvatica* planted at the same
136 density. The remaining field was planted at a 1 m hexagonal spacing with a
137 mixture of birch (*B. pendula*), alder (*A. glutinosa*), beech (*F. sylvatica* L.), ash
138 (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.), chestnut (*Castanea*

139 *sativa* Mill.) and oak (*Quercus robur* L.). To protect the saplings, the entire
140 plantation was fenced.

141 Carbon dioxide enrichment was carried out using high velocity pure CO₂
142 injection, with a target concentration in the FACE plots as ambient plus 200 ppm
143 (Smith et al. 2013a). The elevated CO₂ concentrations, measured at 1 minute
144 intervals, were within 30% deviation from the pre-set target concentration of 580
145 ppm CO₂ for 75-79% of the time during the photosynthetically active part of 2005
146 – 2008 (Smith et al 2013a). Vertical profiles of CO₂ concentration measure at 50
147 cm intervals through the canopy showed a maximum difference of 7%.

148 Air temperature and precipitation were monitored using an automatic weather
149 station (Campbell Scientific, Logan, UK) sampling at 3 m above the ground at
150 hourly intervals Ground level ozone concentration was measured at a DEFRA air
151 quality monitoring station at Aston Hill (52°30'N, 3°02'W) ca. 50 km from
152 BangorFACE at hourly intervals, and was matched to measurements made at the
153 Centre for Ecology and Hydrology ozone research facility directly next to the
154 BangorFACE site (53°14'N, 4°01'W).

155

156 **FAHM facility**

157 The Free Air Humidity Manipulation (FAHM) experimental facility is located at
158 Järvselja Experimental Forest District in South-East Estonia (58°14'N, 27°18'E).
159 The study area lies in the northern part of the temperate climate zone in the
160 transition zone between maritime and continental climate. The study period
161 comprised two growing seasons with drought conditions (2010 and 2011) and

162 three with average precipitation conditions (2008, 2009 and 2012) (Figure 1b).
163 Soil is classified as Endogleyic Planosol (Hansen et al. 2013). The FAHM site is a
164 2.7 ha fenced area, previously used for agriculture, where nine experimental circle
165 plots are situated. Three experimental plots act as control plots. In three plots the
166 relative air humidity (RH) is elevated by approximately 7% over ambient level
167 using a misting technique (water is vaporized to a droplet size ca 10 μm) and
168 FACE-like technology to mix humidified air inside the plots (for more detailed
169 technical description see Kupper et al. 2011 and Tullus et al. 2012a).
170 Humidification is applied when ambient RH < 75%, air temperature > 10 °C and
171 wind speed < 4 m/s. Three experimental plots were “open-top” plots from 2009-
172 2011 and are not included in the current study. Half of each plot was planted with
173 silver birch (*Betula pendula* Roth) and another half with hybrid aspen (*Populus*
174 *tremula* L. \times *P. tremuloides* Michx.) in 2006. The experimental plots are
175 surrounded by a buffer zone, composed of hybrid aspen. Humidity manipulation
176 experiment started in 2008 and has been running during all growing seasons
177 (May-Oct) since then. The first experimental period with *Betula pendula* ended in
178 2011, after that a new birch generation was established with planted seedlings.
179 Hybrid aspens were cut in 2012 and a new generation emerged as regrowth roots
180 and stumps.
181 Air temperature and precipitation were monitored using an automatic weather
182 station (Campbell Scientific, Logan, UK) collecting in 10 minute intervals at 6 m
183 above the ground. Temperature data were collected in 10 minute intervals. Winter

184 precipitation (snow) data was obtained from the Estonian Environment Agency's
185 weather station, situated ca. 70 km from the FAHM site.

186

187 **Litter collection**

188 ***BangorFACE***

189 Following observation of leaf fall, fallen leaf litter was collected at weekly
190 intervals using litter baskets with an area of 0.11 m² until all leaves had abscised
191 (September to December). A litter basket was located in each of the single species
192 subplots. Litter was returned to the laboratory on the day of collection, washed
193 and sorted into individual species, and then dried at 80 °C for 24 hours. The dry
194 weight of each species was determined and recorded for each species subplot
195 within each ambient and elevated CO₂ plot. *Fagus sylvatica* was not used as
196 senesced leaves remained attached until bud burst the following spring. Leaf
197 retention was calculated by subtracting fallen litter at each sampling collection
198 from the total fallen litter after all the leaves had abscised.

199

200 ***FAHM***

201 Litter was collected from three control (C) and three humidified (H) plots. Under
202 both *Betula pendula* and hybrid aspen, two litter baskets (0.21 m²) per species
203 were installed. Litter collection started in the end of July/beginning of August and
204 continued in ca 2-week interval until all leaves had abscised (usually by mid-
205 November). Birch litter was collected during four experimental years (2008-
206 2011), after that the first generation of birch trees was harvested. *Populus tremula*

207 × *tremuloides* litter was collected during five years (2008-2012), after which the
208 first generation of aspen was removed. Litter samples were dried at 70 °C to
209 constant weight and dry mass of the samples was determined. Leaf retention was
210 calculated as described above.

211

212 **Data analysis**

213 Generalized additive mixed models (GAMMs; Zuur et al., 2007; Wood, 2008)
214 were used to describe the percentage change in remaining leaf mass at each
215 collection date between ambient and treatment plots. Visual assessments of
216 variograms and residuals vs. fitted values found weak evidence of temporal
217 autocorrelation. However, as the time series consisted of <20 data points, it was
218 more appropriate to model the variance structure, rather than the autocorrelation
219 structure (Zuur et al. 2009). For *Alnus glutinosa* and *Populus tremula* data
220 exploration indicated violation of homogeneity of variances as a result of
221 differences between FACE rings and precipitation respectively. As a result, we
222 used a random effects model to model variability caused by the factor “Ring” (for
223 *A. glutinosa*) and the variable “precipitation” (for *P. tremula*). The additive
224 (GAM; *Betula pendula*) and additive mixed models (GAMM; *A. glutinosa*, *P.*
225 *tremula*) were modelled with a binomial distribution and a logistic link function
226 (Zuur et al. 2009). For both the FAHM and FACE analyses, the initial models of
227 the GAMs and GAMMs included a smoother over “Collection Day” (s(Days)),
228 the factors “Treatment” (ambient or elevated), “Year”, as well as “Precipitation”
229 and “Ozone” for the FACE analyses and “Precipitation” and “Temperature” for

230 the FAHM analyses. To estimate the optimal amount of smoothing for each
231 smoother, we used cross-validation (Zuur et al., 2009) and alternative models
232 were compared using the Akaike information criterion (AIC). Once the optimal
233 model was identified, the residuals were re-examined to ensure that model
234 assumptions were met. Analyses were conducted in R (R Development Core
235 Team 2014) and the “mgcv” library for additive (mixed) models (Wood, 2014).

236

237 **Results**

238 *Environmental factors*

239 At BangorFACE during the four-year experiment period, in the summers of both
240 2006 and 2008 there were two consecutive months with extremely low
241 precipitation (Figure 1a). These months were June and July in 2006, and May and
242 June in 2008. In 2006 the highest summer temperature of the period under
243 observation was reached. The highest temperature of 34.3°C (Table 1) was
244 recorded in July 2006 during a week long period of very high temperatures.
245 Accumulative ozone over the threshold of 40 ppb (AOT₄₀) was highest during
246 2006, with daily peaks in excess of 210 ppb. In 2008, over the year neither
247 cumulative precipitation was very low nor was cumulative AOT₄₀ very high.
248 However, during the low rainfall months of May and June, 50 % of the total
249 annual AOT₄₀ exceedance occurred and levels of over 170 ppb were reached.
250 Based on the growing degree days (GDD) and maximum temperature, 2007 was
251 the coolest of the 4 years (Table 1).

252 At the FAHM site, the five-year experiment period also included two consecutive
253 years with conditions of drought in the middle of the growing season; 2010 and
254 2011. The year 2010 was the warmest of the 5 years of the investigation, with ca.
255 double the number of growing degree days compared to 2008 and 2012 (Table 1).
256 The year 2011 was the driest year for plant growth as spring precipitation was low
257 (Figure 1b).

258

259 *Leaf fall*

260 At both the FAHM and the BangorFACE sites, based on weekly observations the
261 timing of budburst was not affected by either elevated humidity or CO₂,
262 respectively. The autumn leaf fall at the FAHM site was modelled using a GAMM
263 for *Populus tremula* × *tremuloides* and a GAM for *Betula pendula*. The curves of
264 the measured data (Figure 2) and the modelled data (Figure 3) showed a high
265 degree of agreement. In *Populus tremula* × *tremuloides*, the r^2 for the GAMM fit
266 was 97%, and in *Betula pendula* the r^2 for the GAM fit was 95%. At the FAHM
267 site, different patterns of leaf fall were observed between *Betula pendula* and
268 *Populus tremula* × *tremuloides* (Figure 2). In *Betula pendula* fall began earlier
269 and continued over an 8-9 week period, where as in *Populus tremula* ×
270 *tremuloides* ca 80% of the leaves were lost within a two week period. In all study
271 years the leaf fall of *Betula pendula* was significantly delayed (Figure 3, Table 2)
272 and slower in the increased humidity plots ($p < 0.0001$), while such a consistent
273 trend was not detected in *Populus tremula* × *tremuloides* ($p < 0.0001$). In 2010, in
274 *Populus tremula* × *tremuloides* leaf fall was significantly earlier in the increased

275 humidity plots ($p < 0.0001$, Figure 3). Generally, in hybrid aspen, leaf fall started
276 later and lasted for a shorter period. In control plots, leaf fall of *Betula pendula*
277 began in the first half of August, whereas in the increased humidity plots leaves
278 started to fall almost 4 weeks later (Figure 2). Litter fall dynamics in both *Populus*
279 *tremula* × *tremuloides* and *Betula pendula* appeared to be dependent on annual
280 weather conditions. Litter fall started earlier and more vigorously in the years
281 2010 and 2011 with dry summers (Figure 1b). But *Betula pendula* litter dynamics
282 were also affected by increased humidity even in wet years (Figures 1a, 2 and 3).
283 However, in the modelled data, inclusion of the treatment factors temperature and
284 precipitation did not improve the GAM, and both variables were removed during
285 the backward selection procedure. The prolonged leaf retention in *Betula pendula*
286 meant that the time of 50% leaf fall was reached ca. 21 days later in the increased
287 humidity plots (Table 2). However, the duration to 100% leaf fall did not differ
288 between the ambient and humidity treatment.

289 At BangorFACE, a similar pattern of leaf loss was observed in *Betula pendula*
290 and *Alnus glutinosa*. Again the curves of the measured data (Figure 4) and the
291 modelled data (Figure 5) showed a high degree of agreement, with the exception
292 of *Betula pendula* in 2007. In *Alnus glutinosa*, the r^2 for the GAMM fit was 95%,
293 and in *Betula pendula* the r^2 for the GAM fit was 89%. Inclusion of the factors
294 temperature, precipitation and ozone did not improve the GAM or GAMM, and
295 again these variables were removed during the backward selection procedure. In
296 *Alnus glutinosa*, in 2007 leaf loss was significantly earlier in both ambient and
297 elevated atmospheric CO₂ compared to the other years (Figures 4 and 5, Online

298 Resource 1). In *Alnus glutinosa*, leaf fall was not significantly affected by
299 elevated atmospheric CO₂ (Figure 5, Online Resource 1). In contrast in *Betula*
300 *pendula* leaf fall was delayed by elevated atmospheric CO₂ in the years 2006 and
301 2008 based on the measured data (Figure 4), and in all years based on the
302 modelled data (Figure 5, Online Resource 1). In 2006, litter collection was
303 initiated on the 20th September (day 263). Under ambient CO₂, 3 weeks later on
304 the 11th October (day 283), 61% of the *Betula pendula* leaf canopy was still
305 retained in the crowns. In comparison under elevated CO₂, 80% of the leaf canopy
306 was still present in the crowns of the trees on the same date. Under elevated CO₂,
307 *Betula pendula* still had 61% of the total canopy 14 days later on the 25th October
308 (day 298), thus extending the life span of the canopy (Table 1). In 2008, litter
309 collection started on the 26th September (day 269), and by the 24th October (day
310 297) in the ambient plots 96% of the leaf canopy had fallen. Under elevated CO₂,
311 on the 24th October 89% of the canopy had fallen, and to reach a level of 96% a
312 further 12 days were required.

313

314 **Discussion**

315 Plant leaf senescence is a complex process predominantly influenced by
316 environmental factors such as temperature, light, nitrogen availability and soil
317 moisture. An example of this was seen in *Alnus glutinosa*, where early leaf fall in
318 2007 occurred in the coolest of the four years. In addition, plant physiological
319 interactions which affect leaf senescence include phytohormones, leaf sugar
320 content and source-sink status of the plant (Winger et al. 2006; Taylor et al.

321 2008). The data presented here show that elevated CO₂ and increased humidity
322 both result in two to three weeks longer leaf retention in *Betula pendula*. This
323 effect was not seen in either *Alnus glutinosa* under elevated CO₂ or in hybrid
324 aspen (*Populus tremula* × *tremuloides*) under increased humidity. On the contrary,
325 in one year, 2010, in *Populus tremula* × *tremuloides* under increased humidity leaf
326 fall was earlier. However, the effect of elevated CO₂ on leaf retention in *Betula*
327 *pendula* also appears modified by interactions with other environmental factors,
328 such as periods of drought, high temperature and high levels of ozone. Also in
329 *Populus tremula* × *tremuloides* the shorter retention occurred in the warmest year
330 (2010).

331 Plant growth in an elevated CO₂ atmosphere is often associated with increased
332 accumulation of leaf starch and sugars, whilst leaf N is reduced (Ainsworth and
333 Long 2005). Studies of *Arabidopsis* have demonstrated that leaf senescence can
334 be induced by low N availability, and that N deficiency can result in leaf sugar
335 accumulation (Pourtau et al. 2004). In support of this, leaf N of *Quercus*
336 *myrtifolia* in summer was lower under elevated CO₂ than under ambient CO₂, but
337 higher in autumn (Li et al. 2000). The higher autumn leaf N contents were related
338 to delayed leaf fall. At BangorFACE, N contents of *Betula pendula* and *Alnus*
339 *glutinosa* leaves were not changed under elevated CO₂ (Smith et al. 2013a) during
340 the summer, and in *Betula pendula* in the autumn (Ferreira et al. 2010). No
341 autumnal leaf N data are available for *Alnus glutinosa*. In contrast, N content in
342 both *Betula pendula* and hybrid aspen leaves were significantly lower in increased
343 humidity plots in rainy summers (Tullus et al. 2012a; Sellin et al. 2013). This

344 indicates that in species under consideration, a change in leaf N status is not a
345 common factor related to longer leaf retention. A generally consistent response to
346 the process of leaf senescence is an increase in sugar content (Quirino et al. 2001).
347 Complex interactions during sugar metabolism could help to explain these
348 observations, which are supported by the results of a sugar maple (*Acer*
349 *saccharum*) girdling experiment where leaf sugar accumulation initiated the
350 formation of anthocyanin, a molecule associated with delayed senescence
351 (Murakami et al. 2008). Furthermore, using *Populus* spp., specific cDNA
352 microarrays up-regulated gene expression of leucoanthocyanidin dioxygenase
353 (LDOX) and dihydroflavonol reductase (DRF), two enzymes involved in the
354 biosynthesis of anthocyanin were observed, in addition to increased autumnal leaf
355 sugar accumulation (Tallis et al. 2010). At BangorFACE, *Betula pendula* glucose
356 and total soluble sugars leaf content were increased in leaves collected during
357 2006 under elevated CO₂, whereas only the contents of glucose increased in *Alnus*
358 *glutinosa* (Ahmed 2006).

359 Cytokinins are known to delay leaf senescence (Yong et al. 2000), and usually an
360 excellent negative correlation between leaf cytokinin content and autumnal
361 phenophase exists during senescence (Buchanan-Wollaston 1997). However, the
362 physiology and biochemistry relating to the production of cytokinins and their
363 interactions with senescence processes are poorly understood. Many researchers
364 consider cytokinins to be predominantly root-sourced plant hormones, which are
365 translocated from the roots through the xylem (Dong et al. 2008). The supposition
366 that cytokinin synthesis occurs primarily in roots was supported by the discovery

367 of IPT-genes that control cytokinin synthesis in plants (Chang et al. 2003). As
368 elevated CO₂ has been shown to increase carbon allocation to roots and
369 mycorrhizal symbionts (Iverson et al. 2010), elevated CO₂ may also raise
370 cytokinin production and subsequently increase leaf cytokinin concentrations. In
371 the BangorFACE experiment the leaf area index was not different between
372 ambient and elevated CO₂ (Smith et al. 2013a), but the numbers of root tips in
373 *Betula pendula* were increased by 31 and 41% in 2006 and 2008 under elevated
374 CO₂, and in *Alnus glutinosa* a decrease or a 20% increase were found in 2006 and
375 2008 respectively (Smith et al. 2013b). Similarly, under FAHM, in *Betula*
376 *pendula* the root tip frequency per DW was 20 % and 7% higher in 2009 and
377 2010, respectively (Parts et al. 2013), and the number of root tips m⁻² was
378 increased by 42% compared to ambient in 2011 (Ostonen, unpublished), but no
379 data are available for hybrid aspen. A feedback mechanism involving a higher
380 number of root tips and thus greater cytokinin production has the potential to
381 explain the longer leaf retention under FACE and FAHM. An increase in fine root
382 growth is a common feature in trees under elevated CO₂, and has been suggested
383 to be due to high C allocation to roots, but also as a mechanism to increase
384 nutrient uptake to meet the demand of increased aboveground growth (Smith et al.
385 2013a). Similarly, elevated humidity increased specific fine-root length (SRL)
386 increase in *Betula pendula* and was interpreted as a morphological adaptation
387 leading to an increase in the absorptive area to facilitate nutrient uptake (Parts et
388 al. 2013).

389 At BangorFACE, the years of longer leaf retention, 2006 and 2008, were
390 characterised by periods of low precipitation for 2 successive months in the
391 summer and high tropospheric O₃ concentration during this period. The
392 physiological mechanisms behind this effect can only be speculated upon. Both
393 O₃ (Yendrek et al. 2013) and elevated CO₂ (Eamus and Jarvis 1989) have been
394 shown to reduce stomatal conductance, and thus reduce instantaneous leaf water
395 loss. Further, as O₃ has been reported to directly contribute to earlier leaf
396 senescence (Yendrek et al. 2013), lower stomatal conductance under elevated CO₂
397 may reduce O₃ exposure. Common to both FACE and FAHM is the potential to
398 lower transpiration loss either through lower stomatal conductance (in FACE) or
399 through lower water vapour pressure gradient (in FAHM). Higher water retention
400 by the ecosystem throughout the growing season may lead to lower cumulative
401 water stress in dry summers. Alternatively, the higher root biomass as discussed
402 above may be beneficial in drier periods and also contribute to lower cumulative
403 water stress. However, it should also be noted that both *Alnus glutinosa* and
404 *Populus tremula* × *tremuloides* displayed varying leaf fall pattern compared to
405 *Betula pendula*.

406

407 **Conclusions**

408 Two separate experiments, one increasing atmospheric CO₂ whilst the other
409 increasing air humidity, have both shown that deciduous tree species can respond
410 to changing atmospheric conditions by prolonging their growing season. This
411 effect, however, is not universal and appears species-specific. Further, the ability

412 of trees to respond to changing atmospheric composition by retaining their foliage
413 for longer may be modified by interaction with other factors. This research shows
414 that the recently observed increasing duration of foliage cover in forests may not
415 only be an effect of increasing tropospheric temperature, but also be driven
416 directly by changing atmospheric composition.

417

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428

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548

549 **Table 1.** Environmental variables and the lifespan of the leaf canopy (bud-burst to
550 final leaf fall) in *Betula pendula* at BangorFACE throughout the four years of CO₂
551 enrichment. The effect of elevated CO₂ on canopy lifespan is shown in
552 parenthesis in days. T_{min} and T_{max} are based on the daily minimum and maximum
553 temperatures. GDD = growing degree days. $GDD = \left(\frac{T_{min}+T_{max}}{2}\right) - 10$.

554

Year	T _{min} (°C)	T _{max} (°C)	GDD (base 10°C)	Rain (mm)	Ozone (AOT40)	Ambient CO ₂ canopy lifespan (days)	Elevated CO ₂ canopy lifespan (days)
2005	-3.5	27.0	1910	726	9058	201	201 (+0)
2006	-5.5	34.3	2065	1111	12931	176	190 (+14)
2007	-3.3	24.3	1672	705	3783	172	172 (+0)
2008	-4.5	25.4	1788	1077	7561	165	177 (+12)

555

556

557 **Table 2.** Environmental variables and the lifespan of the leaf canopy (bud-burst to final leaf fall) at FAHM throughout the five years
 558 of relative humidity (RH) manipulation. The effect of FAHM on canopy lifespan is shown in parenthesis in days. T_{min} and T_{max} are
 559 based on the average annual minimum and maximum temperatures. $GDD = \text{growing degree days. } GDD = \left(\frac{T_{min}+T_{max}}{2}\right) - 10$

560

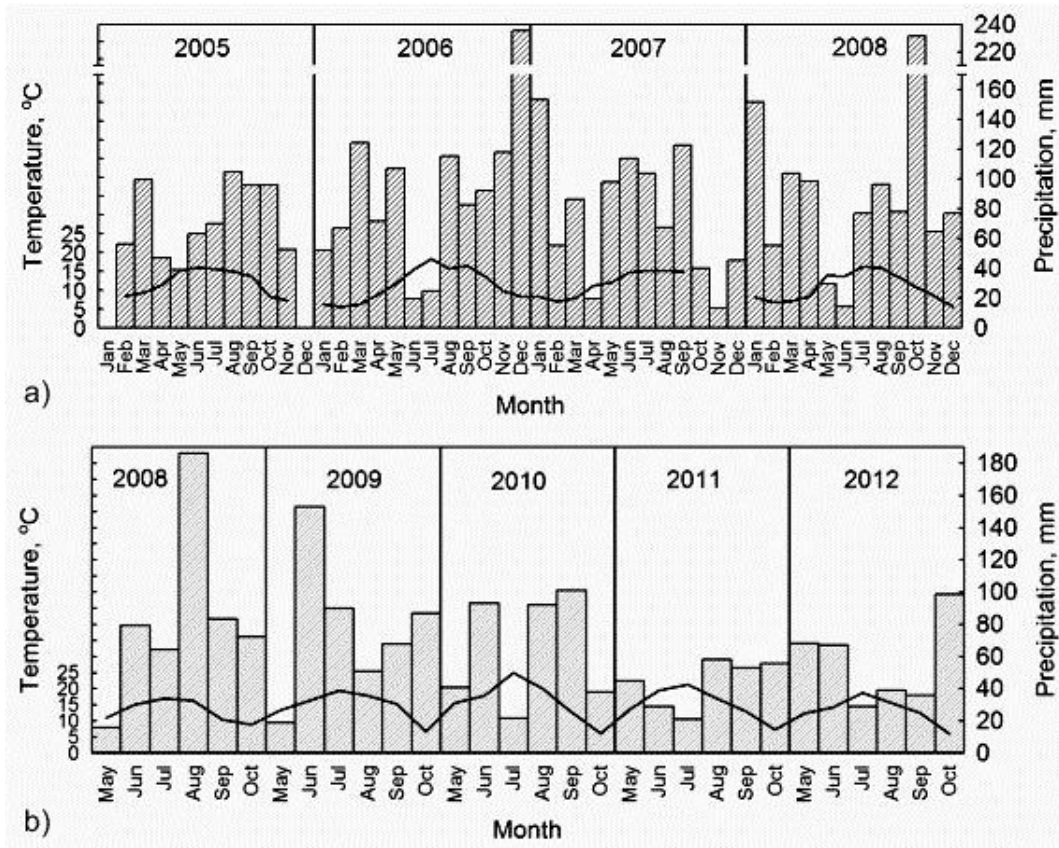
Year	T_{min} (°C)	T_{max} (°C)	GDD (base 10°C)	Rain (May-Oct) (mm)	*Total precip. (mm)	Ambient RH canopy lifespan (days)		Elevated RH canopy lifespan (days)	
						50% fallen	100% fallen	50% fallen	100% fallen
2008	-17.1	30.8	619	502	853	Aspen: 156	170	156 (+0)	170 (+0)
						Birch: 168	205	177 (+9)	205 (+0)
2009	-20.7	30.7	1015	468	696	Aspen: 160	190	160 (+0)	190 (+0)
						Birch: 145	211	166 (+21)	211 (+0)
2010	-27.6	36.9	1321	387	828	Aspen: 151	193	137 (-14)	193 (+0)
						Birch: 123	205	163 (+40)	205 (+0)
2011	-28.8	32	1043	261	669	Aspen: 154	178	154 (+0)	178 (+0)
						Birch: 141	192	153 (+12)	192 (+0)
2012	-31.3	32.9	753	339	756	Aspen: 140	171	140 (+0)	171 (+0)
						Birch: -**	-**	-**	-**

561 *total annual precipitation recorded by the Estonian Environment Agency's weather station, situated ca 70 km from FAHM

562 **birches were harvested in dormant season of 2011/2012

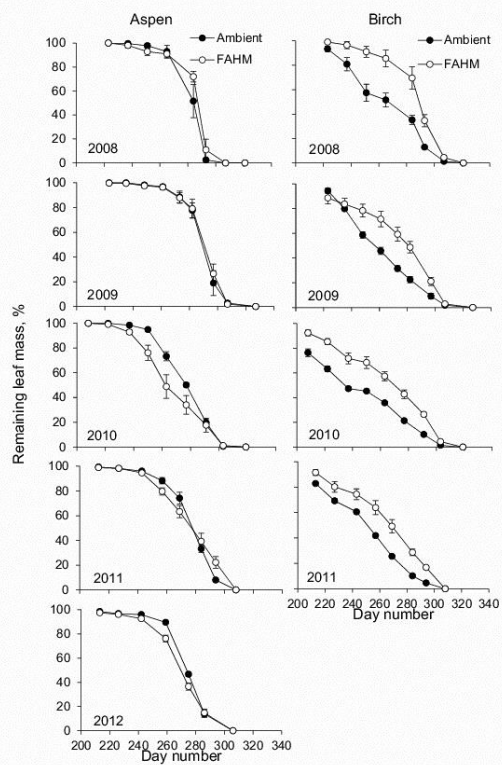
563 **Figure legends**

564 **Fig. 1.** Monthly mean air temperature (line) and total precipitation (columns) at
565 (a) BangorFACE during the years 2005-2008 and at (b) FAHM during the
566 growing seasons 2008-2012.



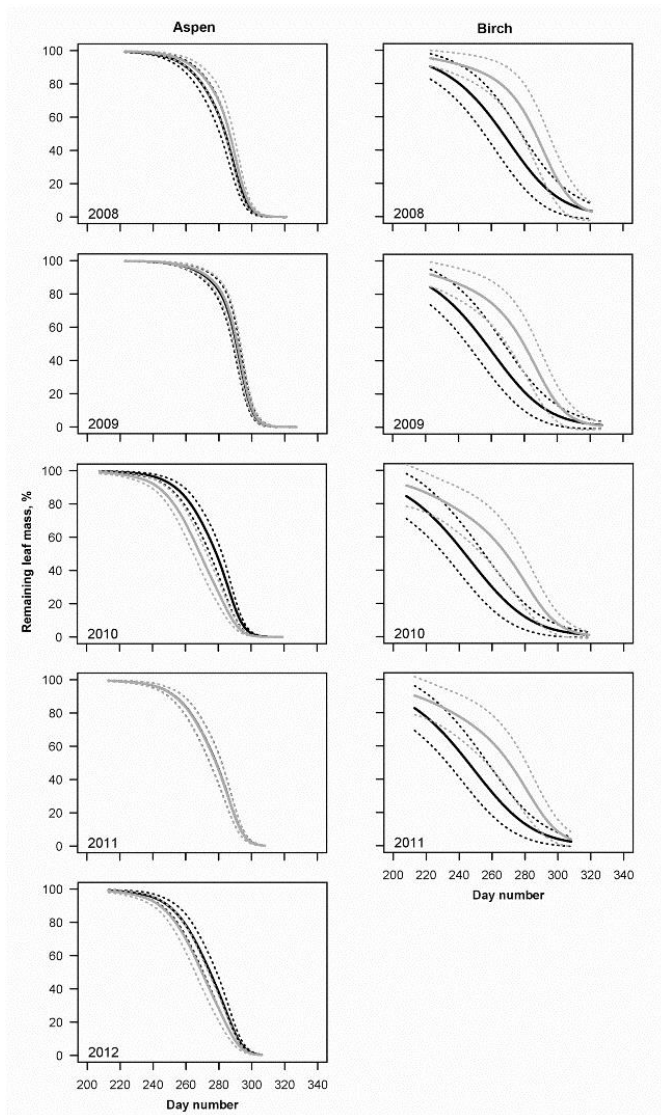
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569 **Fig. 2.** Percentage leaf mass remaining in the canopy of birch (*Betula pendula*)
570 and hybrid aspen (*Populus tremula* × *tremuloides*) grown at ambient humidity or
571 increased humidity (FAHM). Data points show mean ± SE. n=3.
572



573

574 **Fig. 3.** Percentage leaf mass remaining in the canopy of birch (*Betula pendula*)
575 and hybrid aspen (*Populus tremula* × *tremuloides*) grown at ambient humidity or
576 increased humidity (FAHM). Model predictions (solid lines) and 95% confidence
577 intervals (dashed lines) are shown for leaf mass remaining over time for
578 individual years in the ambient (black) and elevated (grey) humidity treatments.

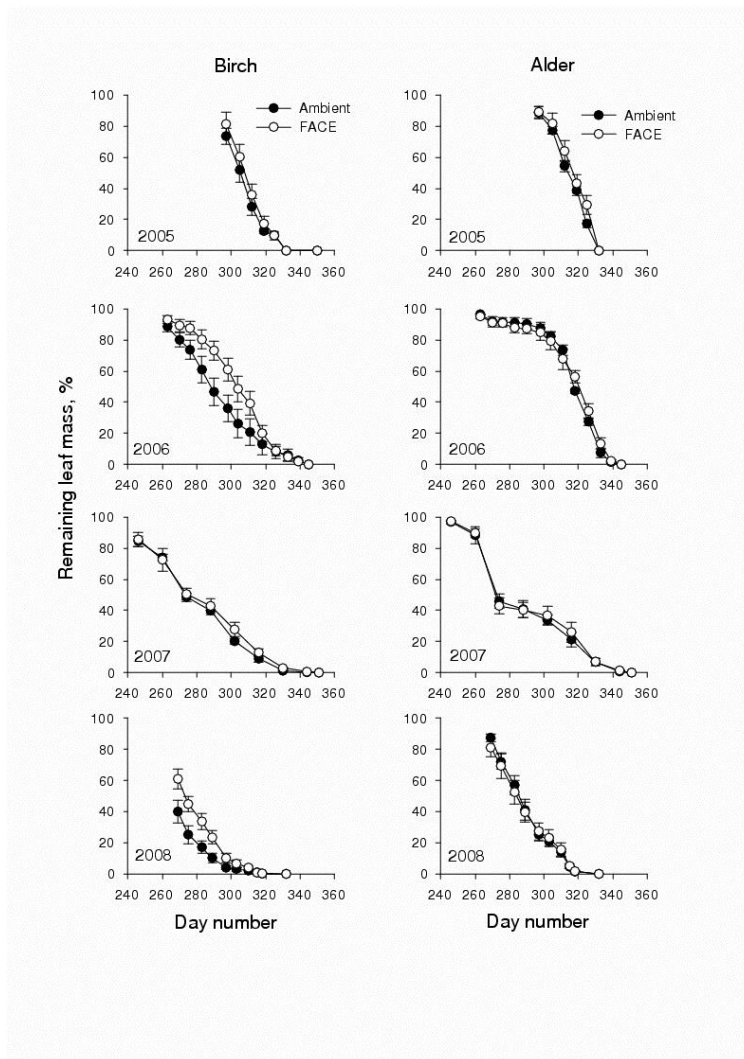


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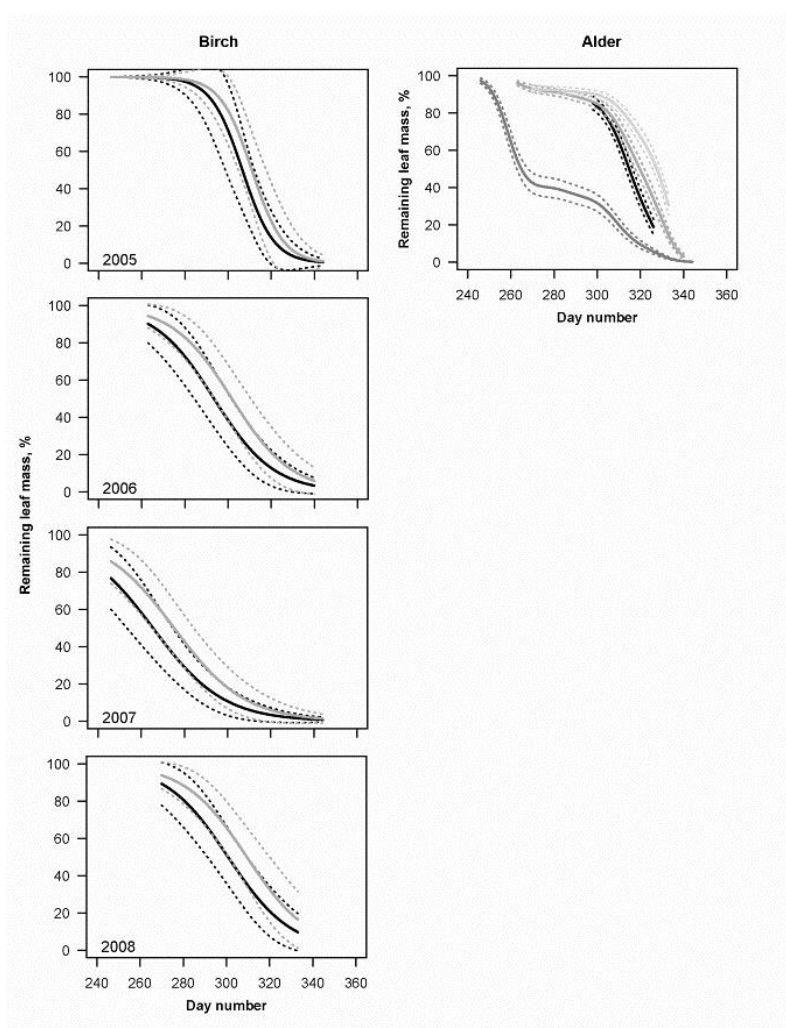
582 **Fig. 4.** Percentage leaf mass remaining in the canopy of birch (*Betula pendula*)
 583 and alder (*Alnus glutinosa*) grown at ambient or elevated atmospheric CO₂
 584 (FACE). Data points show mean ± SE. n=4.



585

586

587 **Fig. 5.** Percentage leaf mass remaining in the canopy of birch (*Betula pendula*)
588 and alder (*Alnus glutinosa*) grown at ambient or elevated atmospheric CO₂
589 (FACE). Model predictions (solid lines) and 95% confidence intervals (dashed
590 lines) are shown for leaf mass remaining over time. In *Betula pendula* this is for
591 the individual years in the ambient (black) and elevated (grey) CO₂ treatments. In
592 *Alnus glutinosa* shown are the individual years with the treatments combined, as
593 there are no treatment effects, but a significant difference between 2007 and the
594 other years.
595



596