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

Elevated atmospheric CO_2 and humidity delays leaf fall in *Betula pendula*, but not in *Alnus glutinosa* or *Populus tremula* × *tremuloides*.

Executive Summary

The effects of both elevated atmospheric CO_2 and increased air humidity on autumn leaf fall were assessed using free air systems. Both factors delayed leaf litter fall in *Betula pendula*, but not in *Populus tremula* × *tremuloides* or *Alnus glutinosa.*

Abstract

Context: Anthropogenic activity has increased the level of atmospheric CO_2, which is driving an increase of global temperatures and associated changes in precipitation patterns. At Northern latitudes, one of the likely consequences of global warming is increased precipitation and air humidity.

Aims: In this work, the effects of both elevated atmospheric CO_2 and increased air humidity on trees commonly growing in northern European forests were assessed.

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

Results: Increasing air humidity delayed leaf litter fall in *Betula pendula*, but not in *Populus tremula* × *tremuloides.* Similarly, under elevated atmospheric CO_2, leaf litter fall was delayed in *Betula pendula*, but not in *Alnus glutinosa*. Increased

CO_2 appeared to interact with periods of low precipitation in summer and high ozone levels during these periods to effect leaf fall.

Conclusions: This work shows that increased CO_2 and humidity delay leaf fall, but this effect is species specific.

Keywords: climate change, Free Air CO_2 Enrichment (FACE), Free Air Humidity Manipulation, leaf fall, ozone

Introduction

Anthropogenic activities since the industrial revolution have increased atmospheric CO_2 concentrations (IPCC 2013), leading not only to climate warming, but also to direct effect of elevated CO_2 on forest net primary productivity (NPP, Norby et al. 2005). In addition, climate change is predicted to increase precipitation at Northern latitudes (IPCC 2013), likely leading to an increase in air humidity. For example, in the Baltic region climate change scenarios for the year 2100 predict an increase in air temperature (by 2.3–4.5 °C), precipitation (by 5–30%), cloudiness (by 2%), but also higher wind speeds and vapour pressure (Kont et al. 2003). Studies investigating the impact of global environmental change on terrestrial ecosystems have identified a consistent pattern of phenological change in the Northern hemisphere (IPCC 2013). Analysis of normalised difference vegetation index (NDVI) remote sensing data gathered during 1985-1999 has revealed an 18 day extension of the growing season in Eurasia (Zhou et al. 2001). Multiple drivers have been shown to differentially

influence plant phenophases, earlier bud break has been correlated with atmospheric warming and delayed senescence (Menzel et al. 2006) and interactions between temperature and elevated atmospheric CO_2 concentrations have been described (Taylor et al. 2008). The process of senescence is governed by developmental age, but also influenced by various integrated endogenous and environmental signals (Lim et al. 2007). Environmental factors influencing leaf senescence can be grouped into: (i) abiotic factors that include drought, nutrient limitation, extreme temperatures, ozone induced oxidative stress, and (ii) biotic factors including, pathogen infection or shading by other plants (Li et al. 2000). Endogenous factors influencing senescence include carbon source-sink relationships, phytohormones, particularly jasmonic (JA) and abscisic acid (ABA), ethylene and salicylic acid (SA). The aforementioned phytohormones initiate senescence through cellular signalling pathways in response to various abiotic and biotic stresses that promote the expression of senescence inducing genes (Morris et al. 2000).

Elevated atmospheric CO_2 has shown been to increase long term forest net primary productivity (Zak et al. 2011), if nutrients are not limiting (Leutzinger and Hätenschwiler 2013). However studies of the effects of elevated atmospheric CO_2 on tree autumnal phenophase have produced conflicting results. For example, elevated CO_2 advanced senescence in two varieties of *Pinus ponderosa* (Houpis et al. 1988) and also in *Populus trichocarpa* (Sigurdsson 2001), yet delayed senescence of *Quercus myrtifolia* (Li et al. 2000) and *Populus* species grown in freely rooted field conditions during the AspenFACE and POPFACE studies

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

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

(Murakami et al. 2008), whilst increased anthocyanin content in another study utilising the same species was associated with a delay in leaf senescence (Schaberg et al. 2008). Furthermore, transcriptome analysis of *Populus* trees grown under elevated CO_2 in field conditions revealed up-regulation of genes determining anthocyanin production during delayed senescence (Tallis et al. 2010). These authors suggest that anthocyanins may play a protective role in leaf metabolism and increase leaf longevity.

In the work presented here we investigated the effect of two factors of global climate change, atmospheric CO_2 and humidity, on autumn leaf fall. We speculated the effects of both of these factors were tree species specific. Thus, we hypothesised that (i) elevated CO_2 delays and (ii) elevated atmospheric humidity anticipates leaf senescence in broadleaved species.

Material and Methods

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

The FACE facility

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

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

At the BangorFACE site eight octagonal plots, four ambient and four CO_2 enriched were established, creating a 2 × 4 factorial block design across the two fields. Three tree species (*Alnus glutinosa* [L.] Gaertner, *Betula pendula* Roth. and *Fagus sylvatica* L.) were selected due to their contrasting shade tolerance, successional chronology and to represent a range of taxonomic, physiological and ecological types. Each plot was divided into seven planting compartments and planted in a pattern creating areas of one, two and three species mixtures. The present study makes use of observations originating from three single species subplots of *B. pendula* and *A. glutinosa*. The site was planted with 60 cm saplings of each species. Within each treatment, the planting pattern was rotated by 90 º between the four plots to avoid potential artefacts introduced by microclimate, soil and uneven growth rates of the different species. Each plot was surrounded by a 10 m border of *B. pendula*, *A. glutinosa* and *F. sylvatica* planted at the same density. The remaining field was planted at a 1 m hexagonal spacing with a mixture of birch (*B. pendula*), alder (*A. glutinosa*), beech (*F. sylvatica* L.), ash (*Fraxinus excelsior* L.), sycamore (*Acer pseudoplatanus* L.), chestnut (*Castanea*

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

Carbon dioxide enrichment was carried out using high velocity pure CO_2 injection, with a target concentration in the FACE plots as ambient plus 200 ppm (Smith et al. 2013a). The elevated CO_2 concentrations, measured at 1 minute intervals, were within 30% deviation from the pre-set target concentration of 580 ppm CO_2 for 75-79% of the time during the photosynthetically active part of 2005 – 2008 (Smith et al 2013a). Vertical profiles of CO_2 concentration measure at 50 cm intervals through the canopy showed a maximum difference of 7%.

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

FAHM facility

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

three with average precipitation conditions (2008, 2009 and 2012) (Figure 1b). Soil is classified as Endogleyic Planosol (Hansen et al. 2013). The FAHM site is a 2.7 ha fenced area, previously used for agriculture, where nine experimental circle plots are situated. Three experimental plots act as control plots. In three plots the relative air humidity (RH) is elevated by approximately 7% over ambient level using a misting technique (water is vaporized to a droplet size ca 10 μm) and FACE-like technology to mix humidified air inside the plots (for more detailed technical description see Kupper et al. 2011 and Tullus et al. 2012a). Humidification is applied when ambient RH < 75%, air temperature > 10 °C and wind speed < 4 m/s. Three experimental plots were "open-top" plots from 2009-2011 and are not included in the current study. Half of each plot was planted with silver birch (*Betula pendula* Roth) and another half with hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) in 2006. The experimental plots are surrounded by a buffer zone, composed of hybrid aspen. Humidity manipulation experiment started in 2008 and has been running during all growing seasons (May-Oct) since then. The first experimental period with *Betula pendula* ended in 2011, after that a new birch generation was established with planted seedlings. Hybrid aspens were cut in 2012 and a new generation emerged as regrowth roots and stumps.

Air temperature and precipitation were monitored using an automatic weather station (Campbell Scientific, Logan, UK) collecting in 10 minute intervals at 6 m above the ground. Temperature data were collected in 10 minute intervals. Winter

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

Litter collection

BangorFACE

Following observation of leaf fall, fallen leaf litter was collected at weekly intervals using litter baskets with an area of 0.11 m^2 until all leaves had abscised (September to December). A litter basket was located in each of the single species subplots. Litter was returned to the laboratory on the day of collection, washed and sorted into individual species, and then dried at 80 ºC for 24 hours. The dry weight of each species was determined and recorded for each species subplot within each ambient and elevated CO_2 plot. *Fagus sylvatica* was not used as senesced leaves remained attached until bud burst the following spring. Leaf retention was calculated by subtracting fallen litter at each sampling collection from the total fallen litter after all the leaves had abscised.

FAHM

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

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

Data analysis

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

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

Results

Environmental factors

At BangorFACE during the four-year experiment period, in the summers of both 2006 and 2008 there were two consecutive months with extremely low precipitation (Figure 1a). These months were June and July in 2006, and May and June in 2008. In 2006 the highest summer temperature of the period under observation was reached. The highest temperature of 34.3°C (Table 1) was recorded in July 2006 during a week long period of very high temperatures. Accumulative ozone over the threshold of 40 ppb (AOT_{40}) was highest during 2006, with daily peaks in excess of 210 ppb. In 2008, over the year neither cumulative precipitation was very low nor was cumulative AOT_{40} very high. However, during the low rainfall months of May and June, 50 % of the total annual AOT_{40} excedance occurred and levels of over 170 ppb were reached. Based on the growing degree days (GDD) and maximum temperature, 2007 was 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 CO_2,
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

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

At BangorFACE, a similar pattern of leaf loss was observed in *Betula pendula* and *Alnus glutinosa.* Again the curves of the measured data (Figure 4) and the modelled data (Figure 5) showed a high degree of agreement, with the exception of *Betula pendula* in 2007. In *Alnus glutinosa*, the r^2 for the GAMM fit was 95%, and in *Betula pendula* the r^2 for the GAM fit was 89%. Inclusion of the factors temperature, precipitation and ozone did not improve the GAM or GAMM, and again these variables were removed during the backward selection procedure. In *Alnus glutinosa,* in 2007 leaf loss was significantly earlier in both ambient and elevated atmospheric CO_2 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 CO_2 (Figure 5, Online Resource 1). In contrast in *Betula*
300 *pendula* leaf fall was delayed by elevated atmospheric CO_2 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 CO_2, 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 CO_2, 80% of the leaf canopy
306 was still present in the crowns of the trees on the same date. Under elevated CO_2,
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 CO_2,
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*, were 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.

2008). The data presented here show that elevated CO_2 and increased humidity both result in two to three weeks longer leaf retention in *Betula pendula*. This effect was not seen in either *Alnus glutinosa* under elevated CO_2 or in hybrid aspen (*Populus tremula* × *tremuloides*) under increased humidity. On the contrary, in one year, 2010, in *Populus tremula* × *tremuloides* under increased humidity leaf fall was earlier. However, the effect of elevated CO_2 on leaf retention in *Betula pendula* also appears modified by interactions with other environmental factors, such as periods of drought, high temperature and high levels of ozone. Also in *Populus tremula* × *tremuloides* the shorter retention occurred in the warmest year (2010).

Plant growth in an elevated CO_2 atmosphere is often associated with increased accumulation of leaf starch and sugars, whilst leaf N is reduced (Ainsworth and Long 2005). Studies of *Arabidopsis* have demonstrated that leaf senescence can be induced by low N availability, and that N deficiency can result in leaf sugar accumulation (Pourtau et al. 2004). In support of this, leaf N of *Quercus myrtifolia* in summer was lower under elevated CO_2 than under ambient CO_2, but higher in autumn (Li et al. 2000). The higher autumn leaf N contents were related to delayed leaf fall. At BangorFACE, N contents of *Betula pendula* and *Alnus glutinosa* leaves were not changed under elevated CO_2 (Smith et al. 2013a) during the summer, and in *Betula pendula* in the autumn (Ferreira et al. 2010). No autumnal leaf N data are available for *Alnus glutinosa*. In contrast, N content in both *Betula pendula* and hybrid aspen leaves were significantly lower in increased humidity plots in rainy summers (Tullus et al. 2012a; Sellin et al. 2013). This

indicates that in species under consideration, a change in leaf N status is not a common factor related to longer leaf retention. A generally consistent response to the process of leaf senescence is an increase in sugar content (Quirino et al. 2001). Complex interactions during sugar metabolism could help to explain these observations, which are supported by the results of a sugar maple (*Acer saccharum*) girdling experiment where leaf sugar accumulation initiated the formation of anthocyanin, a molecule associated with delayed senescence (Murakami et al. 2008). Furthermore, using *Populus* spp., specific cDNA microarrays up-regulated gene expression of leucoanthocyanidn dioxygenase (LDOX) and dihydroflavonol reductase (DRF), two enzymes involved in the biosythesis of anthocyanin were observed, in addition to increased autumnal leaf sugar accumulation (Tallis et al. 2010). At BangorFACE, *Betula pendula* glucose and total soluble sugars leaf content were increased in leaves collected during 2006 under elevated CO_2, whereas only the contents of glucose increased in *Alnus glutinosa* (Ahmed 2006).

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

of IPT-genes that control cytokinin synthesis in plants (Chang et al. 2003). As elevated CO_2 has been shown to increase carbon allocation to roots and mycorrhizal symbionts (Iverson et al. 2010), elevated CO_2 may also raise cytokinin production and subsequently increase leaf cytokinin concentrations. In the BangorFACE experiment the leaf area index was not different between ambient and elevated CO_2 (Smith et al. 2013a), but the numbers of root tips in *Betula pendula* were increased by 31 and 41% in 2006 and 2008 under elevated CO_2, and in *Alnus glutinosa* a decrease or a 20% increase were found in 2006 and 2008 respectively (Smith et al. 2013b). Similarly, under FAHM, in *Betula pendula* the root tip frequency per DW was 20 % and 7% higher in 2009 and 2010, respectively (Parts et al. 2013), and the number of root tips m^{-2} was increased by 42% compared to ambient in 2011 (Ostonen, unpublished), but no data are available for hybrid aspen. A feedback mechanism involving a higher number of root tips and thus greater cytokinin production has the potential to explain the longer leaf retention under FACE and FAHM. An increase in fine root growth is a common feature in trees under elevated CO_2, and has been suggested to be due to high C allocation to roots, but also as a mechanism to increase nutrient uptake to meet the demand of increased aboveground growth (Smith et al. 2013a). Similarly, elevated humidity increased specific fine-root length (SRL) increase in *Betula pendula* and was interpreted as a morphological adaptation leading to an increase in the absorptive area to facilitate nutrient uptake (Parts et 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 O_3 concentration during this period. The
392 physiological mechanisms behind this effect can only be speculated upon. Both
393 O_3 (Yendrek et al. 2013) and elevated CO_2 (Eamus and Jarvis 1989) have been
394 shown to reduce stomatal conductance, and thus reduce instantaneous leaf water
395 loss. Further, as O_3 has been reported to directly contribute to earlier leaf
396 senescence (Yendrek et al. 2013), lower stomatal conductance under elevated CO_2
397 may reduce O_3 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 CO_2 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

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

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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 CO_2
551 enrichment. The effect of elevated CO_2 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)

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556

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

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: -**	-**	-**	-**

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

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

Figure legends

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

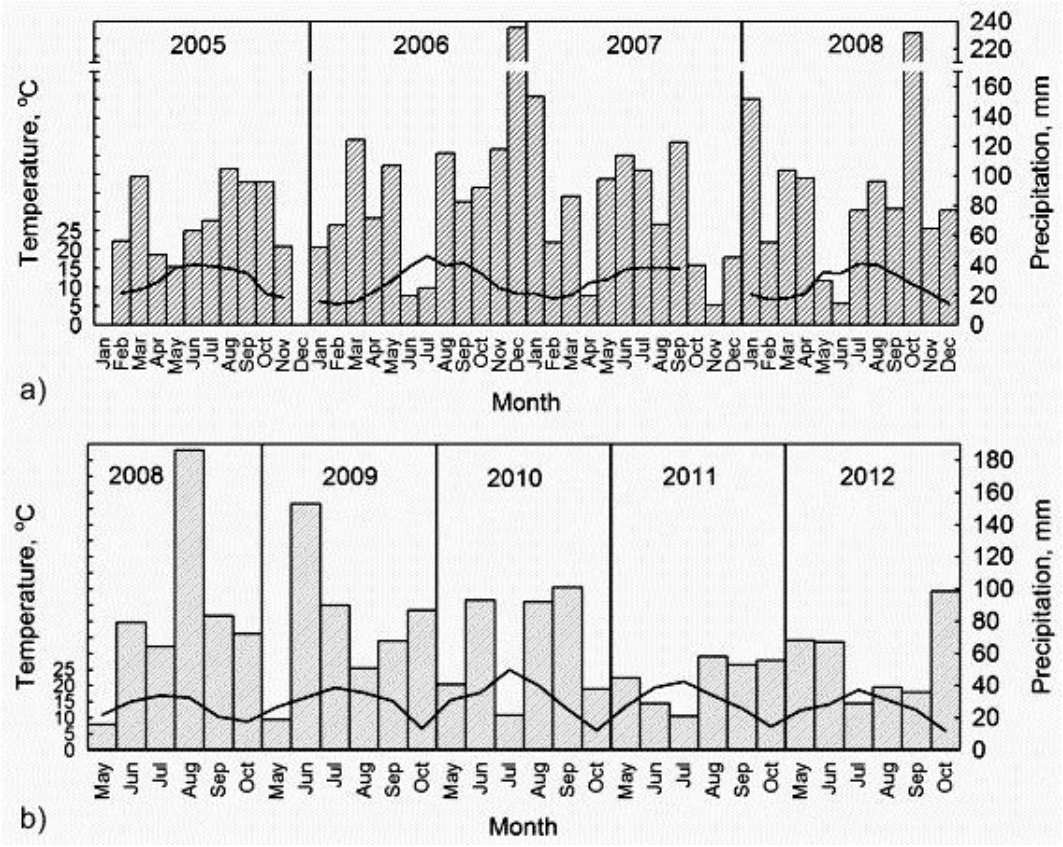


Fig. 2. Percentage leaf mass remaining in the canopy of birch (*Betula pendula*) and hybrid aspen (*Populus tremula* × *tremuloides*) grown at ambient humidity or increased humidity (FAHM). Data points show mean ± SE. n=3.

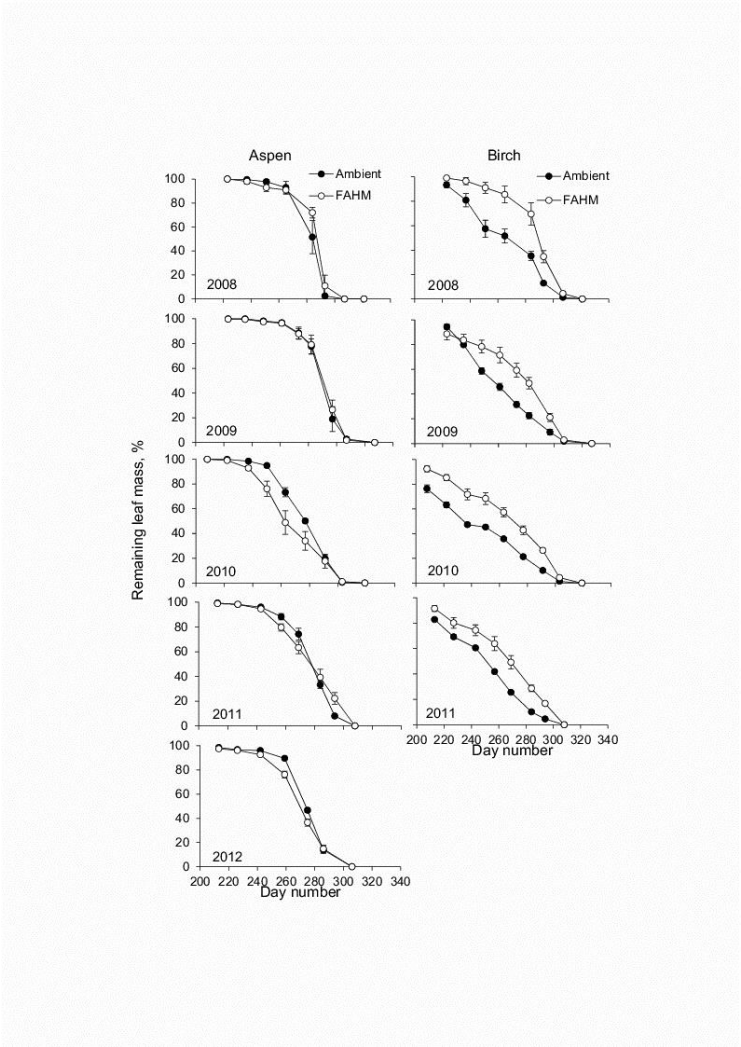
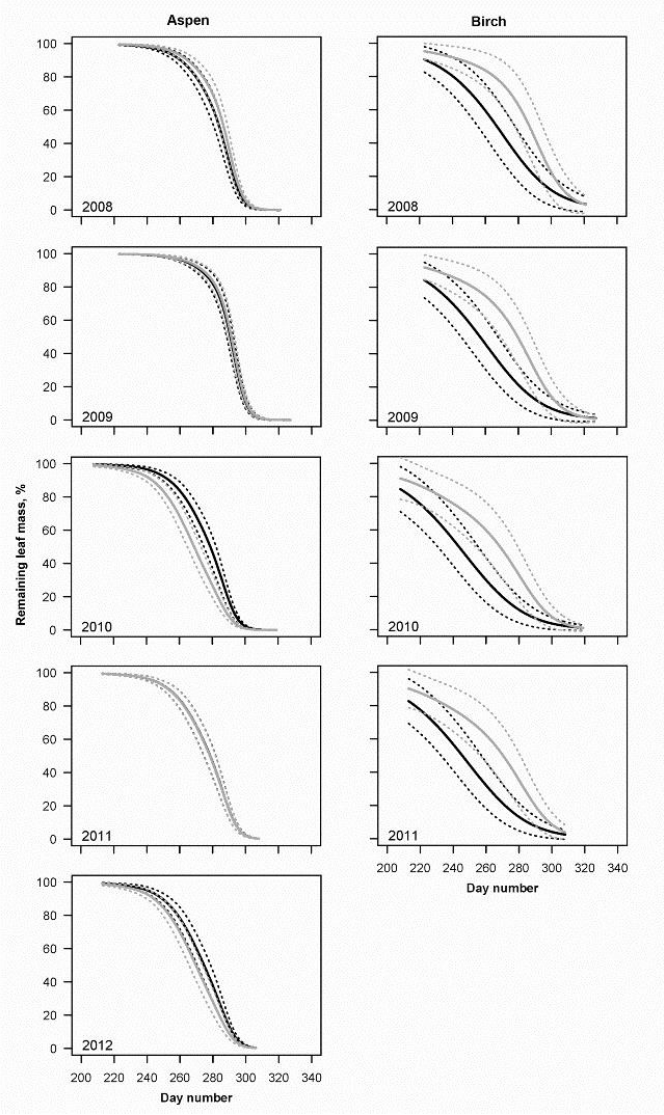


Fig. 3. Percentage leaf mass remaining in the canopy of birch (*Betula pendula*) and hybrid aspen (*Populus tremula* × *tremuloides*) grown at ambient humidity or increased humidity (FAHM). Model predictions (solid lines) and 95% confidence intervals (dashed lines) are shown for leaf mass remaining over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



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 CO_2

584 (FACE). Data points show mean ± SE. n=4.

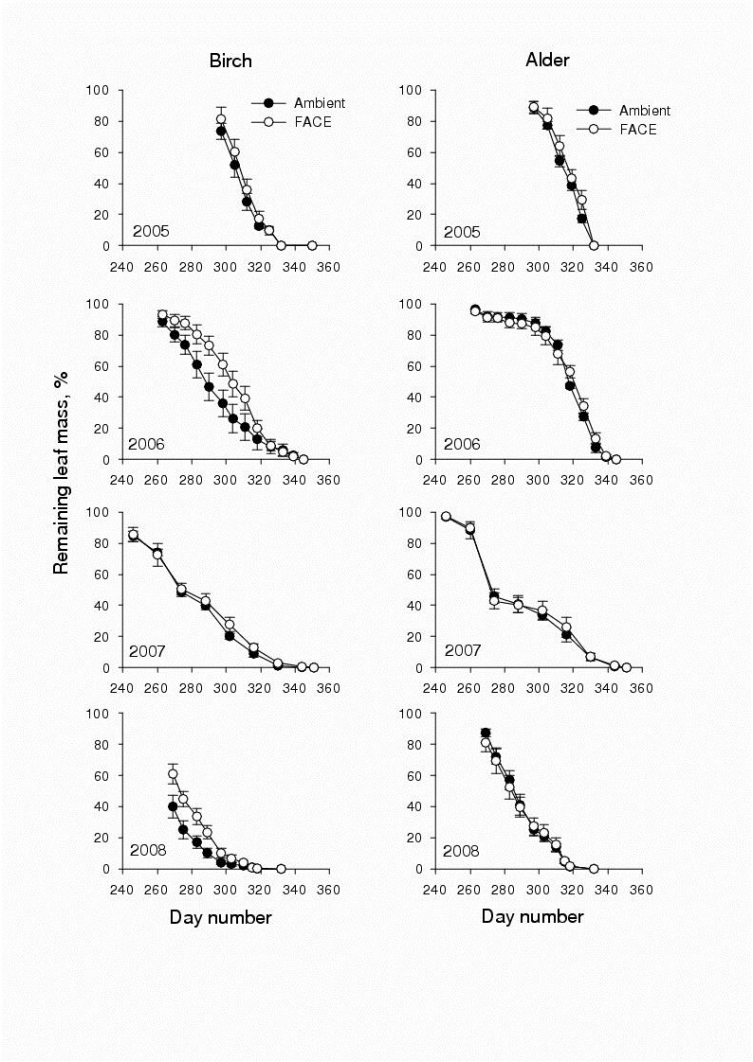


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Fig. 5. Percentage leaf mass remaining in the canopy of birch (*Betula pendula*) and alder (*Alnus glutinosa*) grown at ambient or elevated atmospheric CO_2 (FACE). Model predictions (solid lines) and 95% confidence intervals (dashed lines) are shown for leaf mass remaining over time. In *Betula pendula* this is for the individual years in the ambient (black) and elevated (grey) CO_2 treatments. In *Alnus glutinosa* shown are the individual years with the treatments combined, as there are no treatment effects, but a significant difference between 2007 and the other years.