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KATRE KETS

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Department of Botany, Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, Estonia

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications referred to in the text by their Roman numerals:

I **Kets K**, Darbah JNT, Sõber A, Riikonen J, Sõber J, Karnosky DF. 2010. Diurnal changes in photosynthetic parameters of *Populus tremuloides*, modulated by elevated concentrations of CO_2 and/or O_3 and daily climatic variation. *Environmental Pollution* 158: 1000–1007.

II Riikonen J, **Kets K**, Darbah J, Oksanen E, Sõber A, Vapaavuori E, Kubiske ME, Nelson N, Karnosky DF. 2008. Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO_2 and O_3. *Tree Physiology* 28: 243–254.

III Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Sharma P, Gioli B, Calfapietra C, Gielen B, Kubiske ME, Scarascia-Mugnozza GE, **Kets K**, Long SP, Karnosky DF. 2008. Future atmospheric CO_2 leads to delayed autumnal senescence. *Global Change Biology* 14: 264–275.

IV Darbah JNT, Kubiske ME, Nelson N, **Kets K**, Riikonen J, Sõber A, Rouse L, Karnosky DF. 2010. Will photosynthetic capacity of aspen trees acclimate after long-term exposure to elevated CO_2 and O_3? *Environmental Pollution* 158: 983–991.

The participation of the author in preparing the listed publication is as follows:

Paper I – collected the data, analysed the data and was lead author in writing the paper;

Paper II – collected gas exchange data for *Populus tremuloides*, participated in manuscript preparation;

Paper III – collected and analysed gas exchange data for *Populus tremuloides* at the Aspen FACE experiment, participated in manuscript preparation;

Paper IV – collected gas exchange data in 2004 and 2005, participated in manuscript preparation.

LIST OF ABBREVIATIONS

A/C_i	instantaneous photosynthetic rate and internal CO_2 partial pressure response curve
Chl	leaf chlorophyll content
C_i	intercellular [CO_2] (μmol CO_2 mol^{-1})
[CO_2]	concentration of carbon dioxide (μmol CO_2 mol^{-1} air)
FACE	free air CO_2 enrichment experiment
g_s	leaf stomatal conductance (mol H_2O m^{-2} s^{-1})
[O_3]	concentration of ozone (nmol CO_2 mol^{-1} air)
PAR	photosynthetically active radiation (μmol photons m^{-2} s^{-1})
Pn	light-saturated net photosynthesis (μmol CO_2 m^{-2} s^{-1})
Pn_{sum}	daily sum of absorbed CO_2 (g CO_2 m^{-2})
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP	ribulose-1,5-bisphosphate
T_L	leaf temperature (C°)
VPD_L	vapor-pressure difference between leaf and air (kPa)
Vc_{max}	maximum carboxylation rate of Rubisco (μmol CO_2 m^{-2} s^{-1})
J_{max}	RuBP regeneration capacity (μmol CO_2 m^{-2} s^{-1})
Ψ_L	leaf water potential (MPa)

1. INTRODUCTION

1.1 Increasing concentrations of CO_2 and O_3

The consequences of human activities have rapidly increased the concentrations of the main greenhouse gases, atmospheric CO_2 ($[CO_2]$) and tropospheric ozone ($[O_3]$). A new report by the Intergovernmental Panel on Climate Change (IPCC) shows that global emissions of greenhouse gases have risen to unprecedented levels despite a growing number of policies to reduce climate change. Emissions grew more quickly between 2000 and 2010 than in each of the three previous decades (IPCC 2013). Carbon dioxide concentrations have increased by 40% since pre-industrial times at the end of the 18th century, primarily from fossil fuel emissions and secondarily from net land use change emissions (Keeling et al. 1995, IPCC 2013). In 2011 the concentration of carbon dioxide was 391 ppm (parts per million). Atmospheric CO_2 concentrations will be higher in 2100 relative to present day as a result of a further increase in cumulative emissions of CO_2 to the atmosphere during the 21st century (IPCC 2007, 2013). According to IPCC 2013, different models predict the $[CO_2]$ to rise up to 421 ppm, 538 ppm, 670 ppm or even 936 ppm by the year 2100.

Tropospheric ozone is a secondary pollutant created by chemical reactions between oxidized nitrogen (NO_x) and volatile organic compounds (VOC), produced by automobiles and biomass burning, in the presence of sunlight (Wang et al. 1986, Kull et al. 1996, Karnosky 2007B, Wittig et al. 2007). Unlike atmospheric $[CO_2]$, which is well mixed in the atmosphere, the concentration of ozone varies across regions and occurs in "hot spots", depending on the proximity to sources of pollutants and time of day and year. This complicates accurate projections of current regional trends or future concentrations. However, it has been suggested that daytime $[O_3]$ in the temperate latitudes of the northern hemisphere range between 20 and 65ppb (parts per billion) with an average of about 40 ppb (Wittig et al. 2009). Tropospheric $[O_3]$ is projected to rise globally by 20–25% between 2015 and 2050, and by 40–60% by 2100 (Wittig et al. 2007). Ozone pollution is a concern especially during the summer months because of strong sunlight and hot weather. The largest $[O_3]$ increases are projected for the northern hemisphere because of both increasing precursor concentrations and climatic conditions, which are more favorable to ozone formation (Wittig et al. 2007).

1.2 Effects of CO_2 and/or O_3 on photosynthesis and plant growth

Elevated $[CO_2]$ is generally beneficial for plants, because CO_2 is a substrate for photosynthesis and causes increases in light-saturated net photosynthesis, Pn (Drake et al. 1997, Noormets et al. 2001A,B, Karnosky et al. 2003A, 2007A, Long et al. 2004, 2006, Riikonen et al. 2005, Špunda et al. 2005, Bernacchi et

al. 2005, 2006, Ainsworth & Rogers 2007, Calfapietra et al. 2008, Lindroth et al. 2010, Ellsworth et al. 2012). Elevated [CO_2] increases Pn by increasing the carboxylation rate of Ribulose-1,5-bisphosphate (RuBP), catalyzed by Rubisco, because this reaction is substrate limited at the current atmospheric [CO_2] (Drake et al. 1997, Ellsworth et al. 2004, Ainsworth & Rogers 2007). Pn increases despite the negative effect of CO_2 on stomatal conductance (g_s), that is often found (Field et al. 1995, Paoletti & Grulke 2005, Ainsworth & Rogers 2007, Riikonen et al. 2008, Lindroth et al. 2010). The intensity of photosynthesis is related not only to the substrate concentration, but also to the amount and efficiency of the photosynthetic apparatus (Evans et al. 1989, Niinemets & Tenhunen 1997, Niinemets 1999, Noormets et al 2010.) Trees grown under elevated [CO_2] generally but not always (Noormets et al. 2010) have lower Rubisco concentration (Drake et al. 1997, Noormets et al. 2001A, Wustman et al. 2001, Eichelmann et al. 2004) and lower chlorophyll concentration (Centritto & Jarvis 1999, Lütz et al. 2000, Wustman et al. 2001, Eichelmann et al. 2004, Noormets et al. 2010), which is usually described as negative acclimation of photosynthetic apparatus under elevated [CO_2] (Long et al. 2004). Also, the reduced availability of nitrogen (N) causes lower levels of Rubisco and chlorophyll, and the rise in Pn under elevated [CO_2] can be smaller (Drake et al. 1997).

Plant growth generally increases with exposure to elevated [CO_2] (Isebrands et al. 2001, King et al. 2005, Kubiske et al. 2006, 2007), although the growth stimulation by CO_2 may be decreasing over time (Kubiske et al. 2006). It is argued that competition among individuals may compromise the growth and survival of individual trees under elevated [CO_2] by decreasing availability or acquisition of limiting resources (McDonald 2002). Earlier results from the Aspen FACE (free-air carbon dioxide enrichment) experiment indicate that competitive status strongly influenced tree growth, and the positive growth response to elevated [CO_2] was greater for competitively advantaged individuals than for disadvantaged individuals of most aspen clones (McDonald et al. 2002, Kubiske et al. 2006).

Tropospheric ozone is known to have a negative effect on plant growth, affecting productivity of crops and forests (Kull et al. 1996, Coleman et al. 1995A,B, Dickson et al. 1998, Matyssek & Innes 1999, Kaakinen et al. 2004, Karnosky et al. 2003B, 2005, 2007B, King et al. 2005, Kontunen-Soppela et al. 2007, Wittig et al. 2007, 2009, Oksanen et al. 2007, Feng et al. 2008, Matyssek et al. 2010, Lindroth 2010, Zhu et al. 2011, Ainsworth et al. 2008A,B, 2012). The early symptoms of ozone injury are decreased Rubisco concentration (Noormets et al. 2001A, 2010, Häikiö et al. 2009, Wittig et al. 2009), chlorophyll content (Wustman et al. 2001, Häikiö et al. 2009, Noormets et al. 2010), degradation of chloroplasts (Oksanen et al. 2001) and oxidative damage to cell membranes and light harvesting processes (Noormets et al. 2001B, 2010), which in turn lead to decreased photosynthetic capacity (Long & Naidu 2002, Wittig et al. 2007, 2009). Ozone is entering leaves through stomata and the reaction of ozone with water and solutes in the apoplasm leads to the formation

of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), which can damage cell membranes and the photosynthetic apparatus (Oksanen et al. 2003). Several studies of crop and tree species have shown that light-saturated photosynthesis was significantly decreased by elevated [O_3] (Kull et al. 1996, Karnosky et al. 2003B, 2005, Kontunen-Soppela et al. 2007, Matyssek et al. 2010, Ainsworth et al. 2012). For example, a meta-analytic review of northern temperate and boreal forests has shown that, relative to preindustrial levels, current levels of tropospheric O_3 account on average for an 11% depression in leaf photosynthetic CO_2 uptake, a 13% reduction in stomatal conductance and a 7% reduction in biomass growth in trees (Wittig et al. 2007, 2009). However, H_2O_2 is also suggested to function as a primary signal molecule, leading to cell death (Oksanen et al. 2003), accelerated leaf senescence and early leaf fall (Pell et al. 1997, Karnosky et al. 2003B, Riikonen et al. 2004, Lindroth 2010, Ainsworth et al. 2012), which are additionally decreasing total carbon accumulation and productivity (Percy et al. 2007). Results from Aspen FACE have also shown that the species composition of forest stands can be altered by elevated [O_3]. For example, aspen trees with a competitive disadvantage were strongly affected by elevated [O_3], although that effect was also controlled by clonal effects (Kubiske et al. 2007). However, as O_3 levels continue to rise, further decreases in biomass growth of 11% and 17% are predicted for 2050 and 2100, respectively (Wittig et al. 2009).

The flux of ozone into leaves depends on stomatal conductance (the higher the conductance, the greater the amount of O_3 entering the leaves). Ozone may directly decrease stomatal conductance, which reduces ozone uptake and protects leaves from damage (Reich & Lassoie 1984, Niinemets 2010). However, various analyses suggest that decreased stomatal conductance is considered to be a secondary response as a result of decreasing carboxylation capacity and hence declining intercellular [CO_2] (C_i) (e.g. Farage et al. 1991), differing in different species. If O_3 causes a large-scale decrease in stomatal conductance in forests, there may arise additional implications for regional hydrology (Sellers et al. 1996).

When combined, elevated [CO_2] may partially ameliorate the negative effects of elevated [O_3] in plants (Donelly et al. 2000, Percy et al. 2002, Karnosky et al. 2003B, Kubiske et al. 2007, Noormets et al. 2010, Lindroth et al. 2010). Elevated [CO_2] may reduce ozone damage to trees both by decreasing g_s (and consequent oxidative stress) and thus the potential O_3 flux into leaves (Paoletti & Grulke 2005) and by increasing carbohydrate pools for the synthesis of antioxidant compounds (Lindroth et al. 2010). However, one study from Aspen FACE suggests that it is not possible to explain combined effects only by stomatal closure under elevated [CO_2], as stomatal flux of O_3 was not reduced by elevated [CO_2]. Instead, there was a significant but unexplained CO_2xO_3 interaction on accumulated stomatal flux of O_3 (Uddling et al. 2010). Also, CO_2 enrichment seems to ameliorate the impact of oxidative stress, as harmful H_2O_2 accumulation was not found from the chloroplasts of aspen and paper birch leaves when elevated [CO_2] was present (Oksanen et al. 2003), leading to higher

NADPH concentrations and increased activity of enzymatic detoxification (Podila et al. 2001). Earlier Aspen FACE studies have shown no change (Sharma et al. 2003, Karnosky 2003B), decreases (Noormets et al. 2010) or small increases (Noormets et al. 2001A) in Pn, whereas tree growth was completely annulled or greatly reduced in the combination treatment (King et al. 2005, Kubiske et al. 2006).

1.3 Factors modulating the extent of CO_2 and/or O_3 responses

Both, CO_2 and ozone enrichment have repeatedly been shown to affect photosynthesis and growth, but the degree of stimulation has varied widely between different studies. Differences also occur between dates within each study (Gunderson et al. 2002, Bernacci et al. 2003). For example, previous Aspen FACE studies with trembling aspen have shown 0 (Noormets et al. 2010) to 48% increases in Pn under elevated [CO_2] (Noormets et al. 2001A, Takeuchi et al. 2001, Sharma et al. 2003, Ellsworth et al. 2004, Calfapietra et al. 2008). The effect of O_3 on Pn in *Populus* trees varied from -60% (Sharma et al. 2003) to no change (Calfapietra et al. 2008). Studies on factors that alter the sensitivity of plants to CO_2 and O_3 are scarce and were practically absent when this study was initiated.

The effects of CO_2 and O_3 can differ in different genotypes (Drake et al. 1997, Ellsworth et al. 2004, 2012, Häikiö et al. 2007, Lindroth et al. 2010, Ainsworth et al. 2012). Additionally, all processes in plants can change rapidly, but also acclimate under changing environmental conditions (Niinemets 2010). We know that Pn increases rapidly when CO_2 concentration is raised (A/C_i response curve), but the acclimation of different processes that determine sensitivity of Pn and g_s to elevated concentrations of CO_2 and/or O_3 can take hours, weeks or even years.

A meta-analytic review from FACE experiment across different C_3 and C_4 plant species indicate that trees are generally more responsive to elevated [CO_2] (showing an average 47% stimulation in Pn) compared to grass, forbs, legumes and crops (Ainsworth & Long 2004, Ainsworth & Rogers 2007). The analysis of the A/C_i response curves showed that at current [CO_2], Pn is Rubisco limited in all functional groups, whereas photosynthesis in trees and grasses are Rubisco limited at both current and elevated [CO_2] (Ainsworth & Rogers 2007). Therefore, trees and grasses have the largest potential for stimulation at elevated [CO_2] (ca. 50%), because rising [CO_2] increases carboxylation. In contrast, there is lower potential stimulation of photosynthesis in shrubs, legumes and crops (ca 30%), because, as [CO_2] rises, Pn becomes limited by the capacity for RubP regeneration (Ainsworth & Rogers 2007).

It is argued that conifers can be less sensitive to O_3 compared to broadleaved trees, possibly due to their lower average stomatal conductance (Nunn et al. 2006). While this may be the case, a meta-analysis has shown that major angio-

sperms and gymnosperms showed a similar reduction in Pn under elevated [O_3] (Wittig et al. 2007). However, *Populus* taxa in general have rapid growth, high photosynthetic rates and stomatal conductance, and a competitive growth strategy designed to take advantage of favorable environmental conditions. Because of these characteristics, *Populus* may be quite sensitive to environmental stresses such as O_3 exposures (Karnosky et al. 1996, Dickson et al. 2001). Indeed, Wittig et al. 2007 have shown in a meta-analytic review that *Populus* species were among the most sensitive angiosperms impacted by ozone. Pn decreased under ozone by 26%, 24%, 20%, 14%, 11% and 7% in *Populus, Prunus, Acer, Betula, Fagus and Querqus* families, respectively. However, there is considerable clonal variation in the photosynthetic sensitivity to elevated [O_3] (Coleman et al. 1995B, Karnosky et al. 2003B, 2005, 2007B, Häikiö et al. 2007, 2009, Matyssek et al. 2010).

Environmental stress has been proposed to play an important role in shaping the response of plants to elevated [CO_2] and [O_3] (Kirchbaum 2004, Nowak et al. 2004, Leakey et al. 2006, Ainsworth & Roger 2004, Niinemets 2010, Mäenpää et al. 2011, Ellsworth et al. 2012). Plant growth responses can be altered by environmental factors such as drought (Gunderson et al. 2002, Niinemets 2010), soil moisture (Grulke et al. 2003), temperature (Darbah et al. 2010, Matyssek et al. 2010, Mäenpää et al. 2011), light intensity (Fredericksen et al. 1996), and nutrient (such as nitrogen) availability (Oren et al. 2001, Häikiö et al. 2007, 2009). Plants grown in elevated [CO_2] have been shown to be less vulnerable to drought (Niinemets 2010). Stimulation of photosynthesis at elevated [CO_2] is theoretically predicted to be greater at higher temperatures (Drake et al. 1997, Ainsworth & Long 2004, Kirchbaum 2004) and under water-limited conditions (Gunderson et al. 2002, Bernacchi et al. 2006, Ellsworth et al. 2012). A meta-analytic overview of FACE data among woody and herbaceous plants showed a 30% increase of Pn when plants were grown at temperatures above 25ºC and a 19% increase in the case of lower temperatures (below 25ºC) (Ainsworth & Long 2004).

The magnitude of the effect of elevated [CO_2] on stomatal conductance also varies considerably with environmental factors (Medlyn et al. 2002, Gunderson et al. 2002, Ainsworth & Rogers et al. 2007), particularly when temperature is high and humidity is low (vapor pressure difference between leaf-air, VPD_L, is high) and therefore absolute rates of g_s are low (Sõber 1980, Gunderson et al. 2002). Stressful conditions (low N and drought) have been shown to exacerbate the decrease in g_s under elevated [CO_2] (Ainsworth & Long 2004).

It has been suggested that the negative effect of elevated [O_3] on Pn is ameliorated by any environmental condition that reduces stomatal conductance, such as water stress, drought, low humidity (high VPD_L), high temperature, elevated [CO_2] or nutrient deficiency, thus reducing O_3 uptake and lessening the potential for oxidant damage (Volin et al. 1998, Long & Naidu 2002, Wittig et al. 2007, 2009, Niinemets 2010). In Mäenpää et al. 2011, the ozone effect on Pn in silver birch and European aspen was partly compensated for at elevated temperature, showing an interactive effect of the treatments. However, Wittig et

al.'s 2009 meta-analysis suggests the need for caution in assuming that increasing [CO_2] and drought incidence will provide protection against rising [O_3] – because, as shown in their meta-analysis, field evidence is lacking. Taken together, there is much evidence that combined stresses can influence the survival of large trees even more than chronic exposure to a single predictable stressor, such as CO_2 or ozone.

Photosynthetic activity is also subject to diurnal changes, which are mainly influenced by environmental conditions. Under common field conditions, where many stress factors occur, the assimilatory apparatus is exposed to variable intensities of photosynthetically active radiation (PAR) causing bell-shaped daily curves of photosynthesis, although high temperature and low air humidity may result in a typical midday depression of Pn during summer days (Singsaas et al. 2000, Špunda et al. 2005, Ribeiro et al. 2009). This afternoon decline is attributed to stomatal closure and/or photoinhibitory damage (Muraoka et al. 2000), as well as to increased VPD_L (Singsaas et al. 2000), or to subsequent decreases in C_i (Špunda et al. 2005) and Rubisco carboxylation efficiency (Singsaas et al. 2000). Partial stomatal closure is expected around midday (Singsaas et al. 2000, Ribeiro et al. 2009), when VPD_L and air temperature reach their highest values. If diurnal curves are changing under elevated [CO_2] and [O_3], their effects might change during the day. Nevertheless, in comparison with ambient air, elevated [CO_2] treatment led to the diminution of midday photosynthesis depression that was predominantly caused by stomatal closure and the subsequent decrease of C_i (Špunda et al. 2005). This means that a more pronounced positive effect of CO_2 would be expected in the case of a comparison of daily sums, than in the case of a comparison of daily maximum values of Pn. However, not much is known about the impacts of elevated [O_3] alone or in combination with elevated [CO_2] on diurnal patterns of Pn.

It is generally assumed that CO_2 response is highest in the middle of the growing season, because sink activity is highest during that period (Riikonen et al. 2008, Herrick and Thomas 2001, Ellsworth et al. 2004) and that elevated [CO_2] might improve the C balance of leaves in autumn, thus increasing leaf lifespan (Riikonen et al. 2008, Jach et al. 2001). It has been hypothesized that later season positive leaf carbon balance (increased C:N ratio) will result in delayed leaf senescence when stimulated photosynthetic uptake in elevated [CO_2] is sustained (Herrick & Thomas 2003). However, studies of leaf senescence in response to atmospheric CO_2 enrichment in a variety of herbaceous and woody species have produced conflicting results. For example, in an open-top field chambers experiment with maple trees (*Acer rubrum* and *A. saccharum*), elevated [CO_2] had no consistent effect on leaf abscission in autumn (Norby et al. 2003). Experiments using free-air carbon dioxide enrichment with fast growing *Populus* species have shown a delay in leaf abscission (Tricker et al. 2004, Karnosky et al. 2003A,B) but those with sweetgum (Herrick & Thomas 2003) have shown no effect on leaf longevity or abscission date. Previous findings have shown that elevated [CO_2] increased Pn of sweetgum trees by 63% during the middle portion of the growing season, but when leaves

were senescing, from late September until early November, the magnitude of the stimulation varied between 51 and 96% in sun leaves (Herrick and Thomas 2003), indicating increased sensitivity in Pn in autumn.

Tree growth and photosynthetic responses to ozone are likely to vary with age (Karnosky et al. 2007B, Noormets et al. 2010) and the capacity of the trees to detoxify O_3 (Matyssek et al. 2008). Some individual studies have shown that O_3-induced damage on leaf morphology and physiology is most pronounced on older leaves undergoing a longer exposure to elevated [O_3] (Matyssek et al. 1993, Coleman et al. 1995B, Morgan et al. 2003, Noormets et al. 2010). Results from Aspen FACE have shown that older aspen leaves had a greater accumulation of excess H_2O_2 in chloroplasts (Oksanen et al. 2003), indicating that oxidative stress in chloroplasts increases during leaf ageing. If the O_3 exposure is long, repair mechanisms are turned on. Because chemical defense is energetically demanding and carbon uptake is reduced, leaf senescence is activated earlier under elevated [O_3] (Kontunen-Soppela et al. 2010). Alternatively, the senescence associated processes, and remobilization and storage of carbohydrates and nutrients may not be completed (Kontunen-Soppela et al. 2010, Uddling et al., 2005). However, there is also evidence that the negative effect of O_3 on Pn diminished as leaves aged (Häikiö et al. 2007, 2009).

While the shorter-term stimulation of Pn in elevated [CO_2] and elevated [O_3] is well documented, it is not clear whether the leaf carboxylation capacity of dominant species will be down-regulated (acclimated) or not in the longer term. Acclimation is mostly defined as (those) physiological changes in photosynthetic apparatus that occur when plants are grown in high [CO_2] (i.e. the photosynthetic properties of leaves that have developed at elevated [CO_2] differ from those developing at current [CO_2], Drake et al. 1997). The implication of this is that after medium or long term growth in elevated [CO_2], the initial stimulation of Pn from elevated [CO_2] might be reduced (Drake et al. 1997). Thus, only the results of long-term experiments can indicate whether changes in leaf morphology and biochemistry are a common response to elevated [CO_2] and whether they preface increases or decreases in photosynthetic capacity (Ellsworth et al. 2004). Previous findings reveal that the magnitude of stimulation in Pn at elevated [CO_2] and the occurrence of acclimation appeared to be both growth-form and environment specific (Nowak et al. 2004, Ainsworth & Rogers 2007). A meta-analytic review with findings from FACE experiments has demonstrated that all functional groups acclimated to growth at elevated [CO_2] (maximum carboxylation rate of Rubisco, Vc_{max} and RuBP regeneration capacity, J_{max} were significantly reduced in all functional groups), with trees having smaller reduction in Vc_{max} than shrubs, grasses and crops (Ainswoth Rogers 2007). Furthermore, negative acclimation of the photosynthetic apparatus under elevated [CO_2] can be caused by limitations in the supply of nutrients (such as nitrogen). When the supply of nitrogen is limited, the rise in photosynthesis is smaller (Drake et al. 1997). Trees grown under elevated [CO_2] generally have lower N concentrations in their foliage (Lindroth et al. 2001, Karnosky 2003A, Riikonen et al. 2005). However, if soil nitrogen is not limited,

photosynthetic capacity will increase with increasing N content without any negative acclimation. Previous findings from Aspen FACE also showed that changes in nitrogen per leaf area drives the change in carboxylation efficiency (Sharma et al. 2003). Structural acclimation to elevated [O_3] has been reported in birch trees grown in open-top chamber experiment (Pääkkonen et al. 1995) and in container-grown beech and spruce trees (Luedemann et al. 2005).

1.4 Aims of the thesis

The general aim of this thesis was to find out how and why the long-term effects of elevated [CO_2] and/or [O_3] on photosynthetic responses vary in trembling aspen clones with different O_3 sensitivity. As reviewed in 1.3, there are several factors that can change the photosynthetic responses to elevated [CO_2] and/or [O_3]. The changes in sensitivity of plants to higher concentrations of CO_2 and O_3 may be caused by changing meteorological conditions and other environmental stresses, but diurnal, seasonal and interannual differences can also occur. In the latter case, the sensitivity of photosynthetic parameters to elevated [CO_2] and/or [O_3] can follow the dynamics of environmental factors or acclimate in a different time scale to new conditions. We hypothesized that the responses of elevated [CO_2] and/or [O_3] on photosynthesis would change daily, seasonally and interannually. We proposed that the degree of CO_2 and ozone effects can be influenced by the degree of environmental stress (such as drought and high temperature). We expected elevated [CO_2] to lengthen the growing season by delaying leaf abscission, and elevated [O_3] to shorten the growing season by accelerating leaf senescence.

The specific aims of this doctoral thesis were:

1) To study the effects of elevated [CO_2] and [O_3] alone and in combination on light-saturated net photosynthesis (Pn) and stomatal conductance (g_s) in *Populus tremuloides* Michx. (clones 42E and 271, differing in O_3 tolerance) in a long-term Aspen FACE (free-air carbon dioxide enrichment) experiment (I).
2) To examine how environmental constraints, such as drought and high temperature, impact the primary responses of elevated [CO_2] and/or [O_3] on Pn and g_s (I).
2) To study the daily, seasonal and interannual variability in the effects of elevated [CO_2] and/or [O_3] on Pn and g_s (I-IV).
3) To analyze the possible causal role of environmental stress and acclimation in generating variability in the effects of elevated [CO_2] and/or [O_3] (I and IV).

2. MATERIALS AND METHODS

2.1 Experimental design and plant material

A field experiment was performed at the Aspen FACE experimental site (I–IV) in Wisconsin, USA (45º6'N, 89º5'W) in the summers 2004–2005 (I–III) and 2004–2008 (IV), where the effects of elevated [CO_2] (ambient and 200 ppm above ambient; ca. 360 and 560 ppm, respectively) and elevated [O_3] (ambient and 1.5 x ambient O_3, ca. 35–45 and 52–67 ppb, respectively) on leaf physiological parameters was studied. In 1997, saplings of aspen (five clones), birch (*Betula papyrifera*) and maple (*Acer saccharum*) were planted in 30m diameter treatment rings, representing the dominant species in northern hardwood forests. The experimental site consists of 12 treatment rings, with three replicate rings per treatment (ambient [CO_2] and [O_3], elevated [CO_2], elevated [O_3] and a combination of elevated [CO_2] and elevated [O_3]). The current thesis studies two trembling aspen (*Populus tremuloides* Michx) clones 42E and 271. Clone 271 had been previously determined to be relatively tolerant to O_3, and Clone 42E to be more O_3 sensitive (Dickson et al. 2000). The trees had been fumigated continuously since spring 1998 during daylight hours from bud burst (mid-May) to leaf fall (mid- to late-October) (Dickson et al. 2000). The complete design of the Aspen FACE and a summary of responses are available elsewhere (Karnosky et al. 1999).

2.2 Environmental conditions

The microclimatic conditions on the gas exchange measurement days were typical for summer months in northern regions of the USA (Fig. 1 in I and II). Generally, July was the warmest month and October was the coolest month of the growing season in terms of both air and soil temperatures (Kubiske et al. 2006). Photosynthetically active radiation (PAR) ranged from 1300 to 1700 μmol m^{-2} s^{-1} during daylight hours and under clear sky conditions, approaching 2000 μmol m^{-2} s^{-1} on some days. Leaf temperature (T_L) increased from a low of about 17–28 Cº around 09:00 h to near 26–39 Cº in the afternoon between 15:30–17:00 h (Fig. 1 in I). Vapor pressure difference between leaf and air (VPD_L) exhibited a strong diurnal variation, climbing from 0.8–2.6 kPa at 09:00 h to 1.3–4.8 kPa at 15:30–17:00 h, coinciding with the maximum daily air temperatures (Fig. 1 in I).

2.3 Gas exchange, chlorophyll and water potential measurements

Gas exchange was measured on sun-exposed upper canopy short-shoot leaves (three leaves per clone) throughout the growing seasons 2004–2008 (I, II, IV). For diurnal curve measurements, gas exchange was measured six times during

the day from sunrise to sunset under saturating light conditions (>1000 μmol m^{-2} s^{-1}), Paper I. Measurements were taken while leaves were still attached to the trees. Light-saturated net photosynthesis (Pn) was measured with a Li-6400 (Li-Cor, Lincoln, NE) portable gas exchange apparatus, using growth CO_2 concentrations (360 ppm for control and elevated [O_3] treatments and 560 ppm for elevated [CO_2] and combination treatments), in the leaf chamber (I–IV). Leaf temperature and relative humidity (RH) were not controlled. This system also measured leaf stomatal conductance (g_s), intercellular CO_2 (C_i), transpiration rate, vapor pressure difference between leaf and air (VPD_L), leaf and air temperatures and relative humidity. Leaf water potential (Ψ_L) was measured after each gas exchange measurements using a portable Scholander type pressure chamber (Model 600, PMS Instruments, Corvallis, OR) (I). To measure leaf water potential, we detached a leaf from the branch immediately after measuring gas exchange and put it in the pressure chamber (n=3). Leaf chlorophyll was measured during gas exchange measurements throughout the growing seasons 2004–2005 using SPAD-meter 502 (Minolta Camera Co., Osaka, Japan) (I). To test changes in carboxylation efficiency, photosynthetic CO_2 response (A/C_i) was measured before midday (9 AM) and in the late afternoon (3 PM) (I, IV). A/Ci curves were then analyzed by computing the Vc_{max} (maximum carboxylation rate of Rubisco) and J_{max} (RuBP regeneration capacity) using the model described by Farquhar et al. (1980).

For late season photosynthetic behavior, gas exchange was measured until the 12th of October, 2004 (III) and until the 7th October, 2005 (II). From the 23rd of September, 2004 onwards, the gas exchange measurements were continued only in elevated [CO_2] and control rings (III). Late season gas exchange was measured from six (II) to nine (III) time points during the senescence period (n=9 in 2004 and n=6 in 2005).

To study whether photosynthesis and stomatal conductance acclimates to long-term [CO_2] and/or [O_3] exposures, gas exchange measurements were taken throughout the 2004–2008 growing seasons (IV).

2.4 Data processing

The overall mean comparison between treatments was calculated using Factorial Analysis of Variance (ANOVA) (I–IV). To elucidate whether environmental stress affects the relative impact of elevated [CO_2] and/or [O_3] treatment on trees, we considered days with a daily average $g_s > 0.15$ mol m^{-2} s^{-1} to be environmentally unstressed and days with a daily average $g_s < 0.15$ mol m^{-2} s^{-1} to be environmentally stressed (I). To assess the main factors responsible for afternoon depression in Pn, we calculated the relative changes in individual parameters across the whole dataset, between 9:00 h and 16:30 h, and then used linear regression analysis to find coefficient of determination (R^2) and P values (I). Treatment effects on diurnal decline in Pn were compared using General Linear Models (GLM). The maximum rate of Rubisco carboxylation (Vc_{max})

was calculated by fitting A/Ci curve data to the model described by Farquhar et al. (1980) (I, IV). In all cases, P values <0.05 were considered significant. Treatment effects on seasonal CO_2 uptake were calculated according to the area under the vegetational curves of Pn.

3. RESULTS AND DISCUSSION

3.1 Treatment effects on leaf-level photosynthetic parameters

Treatment effects on photosynthetic parameters of *Populus tremuloides* clones followed the already known pattern for different species with different tolerance to ozone. Elevated [CO_2] on average increased light-saturated net photosynthesis (Pn) by 33% in Clone 42E and by 46% in Clone 271 ($P < 0.001$, Fig. 1), reduced stomatal conductance (g_s) by 21% in Clone 42E and by 16% in Clone 271 ($P < 0.001$, Fig. 1) and increased leaf chlorophyll content ($P < 0.001$, Fig. 3 in I) compared to the control treatment. Elevated [O_3] decreased Pn by 21% ($P = 0.03$) and g_s by 12% ($P = 0.03$) in ozone sensitive Clone 42E (Fig. 1). In Clone 271, we found a slight but non-significant decrease in Pn and no change in g_s (Fig. 1). Leaf chlorophyll content decreased by 14% ($P < 0.001$) for both clones under elevated [O_3] compared to the control (Fig. 3 in I).

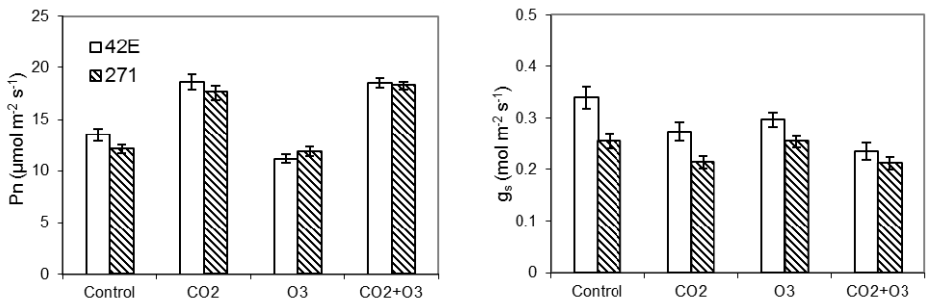

Figure 1. Light-saturated net photosynthesis (Pn) and stomatal conductance (g_s) in aspen Clones 42E and 271 exposed to elevated [CO_2] and/or [O_3]. Measurements were taken in June, July and August, 2005, between 9:00 and 10:00 h at the Aspen FACE site. Data shown are means ± SE (n = 27, ANOVA).

It has been previously shown that *Populus* species in general are often found to be the most sensitive angiosperms impacted by ozone, with an average reduction in Pn of 26% (Wittig et al. 2007) and an average reduction in total biomass of 22% (Wittig et al. 2009). In contrast, Häikiö et al. 2007, 2009 did not find very strong evidence of the ozone sensitivity of *Populus* species in a short-term experiment, but the concentration of O_3 was relatively low in this study compared to other studies. In the combination treatment, photosynthetic rates remained about similar to the rates in the elevated [CO_2] treatment. Pn increased ~ 32% in Clone 42E and ~ 50% in Clone 271 compared to the control ($P < 0.05$), Fig. 1. Leaf chlorophyll content exhibited a slight (4–5%) but significant ($P < 0.04$) decrease under elevated [CO_2+O_3] in both clones, but this decrease was smaller than that in the elevated [O_3] treatment (Fig. 3 in I).

Stomatal conductance was reduced by 29% in Clone 42E and by 16% in Clone 271 ($P < 0.05$, Fig. 1).

The magnitude of the effect of elevated [CO_2] and/or [O_3] on Pn varies considerably between studies. It has been shown in many experimental studies that C_3 photosynthesis responds strongly to CO_2 concentration (Bernacci et al. 2003, Karnosky et al. 2003A, 2005, Ainsworth & Rogers 2007, Calfapietra et al. 2008, Lindroth et al. 2010, Ellsworth et al. 2012). For example, a meta-analytic review, where data were averaged across all FACE experiments, has shown a 47% increase in Pn at elevated [CO_2] (Ainsworth and Long 2004). However, individual studies from Aspen FACE with various *Populus* genotypes have shown 25–73% enhanced Pn (Noormets et al. 2001A, Takeuchi et al. 2001, Karnosky et al. 2003A, Sharma et al. 2003, Ellsworth et al. 2004, Calfapietra et al. 2008, Papers I, II, IV) under elevated [CO_2]. More specifically, 33–73% increments in Pn for Clone 42E and 33–52% increments in Pn for Clone 271 under elevated [CO_2] were documented (Calfapietra et al. 2008, Papers I, II, IV). Elevated [O_3] has been shown to decrease Pn from –7% to –46% for Clone 42E and from no change to -40% for Clone 271 (Calfapietra et al. 2008, Papers I, II and IV). Results from Aspen FACE have also shown that the Pn under the combined treatment is either similar to control values or shows up to 58% increase in Pn (Noormets et al. 2001A, Karnosky et al. 2003B, Sharma et al. 2003, Calfapietra et al. 2008, Noormets et al. 2010, Papers I, II, IV). Together, we can see that there is huge variability in the effects of CO_2 and O_3 on Pn. In the current thesis we will search for factors and processes that can change the sensitivity of Pn to long-term elevation of CO_2 and/or O_3 concentrations.

3.2 Does environmental stress modify the responses of elevated [CO_2] and/or [O_3] on Pn and g_s?

To study whether the relative effects of elevated [CO_2] and/or [O_3] on Pn and g_s are changing under drought and high-temperature stress, we compared the differences in Pn, g_s and in the daily sum of absorbed CO_2 (Pn_{sum}, g CO_2 m^{-2}) between treatments in days with and without stress. As g_s is a good indicator of drought stress, the data were separated into two groups: data on days when daily average $g_s > 0.15$ mol m^{-2} s^{-1} was referred to as data from unstressed trees and data on days when daily average $g_s < 0.15$ mol m^{-2} s^{-1} was referred to as data from stressed trees (I).

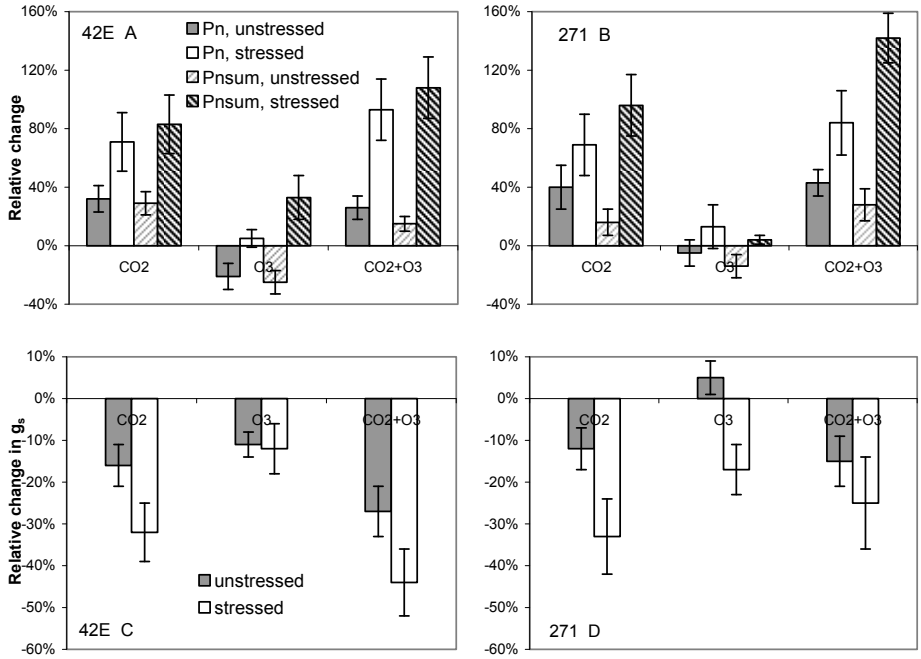

Figure 2. Relative difference of light-saturated net photosynthesis (Pn), absorbed CO_2 (Pn_{sum}) and stomatal conductance (g_s) measured under elevated [CO_2] and/or [O_3] from control treatment in aspen Clones 42E and 271. Percentages were calculated from measurements taken in June, July and August 2005. Pn and g_s were measured between 9:00–10:00 h, Pn_{sum} was calculated as summary CO_2 uptake between 9:00–17:00. Plants measured on days when daily average g_s was below 0.15 mol m^{-2} s^{-1} were defined as "stressed" and plants with g_s above 0.15 mol m^{-2} s^{-1} as "unstressed". A – relative difference of Pn and Pn_{sum} in Clone 42E in stressed and in unstressed trees. B – relative difference of Pn and Pn_{sum} in Clone 271 in stressed and in unstressed plants. C – relative difference of g_s in Clone 42E in stressed and in unstressed trees. D - relative difference of g_s in Clone 271 in stressed and in unstressed trees.

We found that exposure to elevated [CO_2] alone or combined with elevated [O_3] results in a greater relative impact on Pn and Pn_{sum} in plants exposed to stress, at least for stresses that reduce g_s (drought, high temperature), Fig. 2A,B. Similarly, the relative reduction in g_s under elevated [CO_2] and combined treatment was more pronounced in stressed trees compared to unstressed trees (Fig. 2C,D). The drought enhancement of the effect of CO_2 on Pn is in agreement with earlier predictions that greater photosynthetic enhancement by elevated [CO_2] is expected under dry conditions compared to wet conditions (Gunderson et al. 2002, Ainsworth & Long 2004, Kirchbaum 2004, Nowak et al. 2004, Ellsworth et al. 2012). These predictions are based on the studies of several authors who have reported a greater relative enhancement due to elevated CO_2 concentrations during drought (Dixon et al. 1995, Scarascia-Mugnozza et al.

1996) or in elevated temperatures (Kellomäki & Wang 1996). Some authors have, however, reported reduced enhancement during drought (Ellsworth 1999). One explanation for more pronounced relative responses in Pn is that drier conditions will shift the operational set-point for plant gas exchange to the more responsive part of the photosynthetic CO_2 response curve (e.g., the initial slope region of A/Ci curve), Nowak et al. 2004, Ellsworth et al. 2012. This explanation can be valid in cases where the saturating value of the A/Ci curve itself does not drop significantly under elevated [CO_2]. It is more complicated to analyze the relative differences in Pn when the saturation value of the A/Ci curve and Vc_{max} are decreasing, which is probably the case in studies where a decreased CO_2 effect under drought conditions was documented (Ellsworth 1999). However, there is also an explanation based on the strong temperature × CO_2 interaction (Kirchbaum et al. 2004). Rubisco can react either with CO_2, or with O_2, with CO_2 being released (Kirchbaum et al. 2004, Farquhar et al. 1980, Farquhar and von Caemmerer 1982). The relative reaction rates with O_2 and CO_2 depend on the relative concentrations of the two gases at the enzyme sites and on temperature, with higher temperatures favoring reactions with O_2 (Kirchbaum et al. 2004). This causes photosynthesis to be more under-saturated with CO_2 at higher temperatures, so that relative responses to increasing CO_2 concentration increase with temperature (Kirchbaum et al. 2004). Both explanations may be valid in Aspen FACE, where both drought and high temperature occurred.

More pronounced relative reductions in g_s under elevated [CO_2] alone and combined with elevated [O_3] were documented in stressed trees (Fig. 2C,D). The recalculation and meta-analysis of data of several studies (Curtis 1996, Saxe et al. 1998, Medlyn et al. 2001) also showed the greater relative effect of CO_2 on g_s under stress conditions for several European tree species (Medlyn et al. 2001). The negative effect of CO_2 on g_s can be partly mediated by vapor pressure difference between leaf and air (VPD_L): VPD_L was higher in stress conditions, and differences in VPD_L correlated with differences in g_s (R^2= 0.6, $p<0.001$, I).

We observed a positive effect of elevated [O_3] on Pn (5–13% increments) and Pn_{sum} (4–33% increments) in stressed trees, but a typical negative effect in unstressed trees (Fig. 2A,B). Elevated [O_3] decreased g_s similarly in stressed and unstressed trees in Clone 42E, but in Clone 271 the effect of ozone on g_s changed from negative to positive (Fig. 2C,D). Periods of high ozone are generally associated with hot, dry weather that reduces g_s and therefore can offset [O_3] impact (Ollinger et al. 1997, Wittig et al. 2009). Drought-driven reduced O_3 impact on Pn has also been reported for adult beech, silver birch and European aspen trees (Matyssek et al. 2010, Maenpaa et al. 2011). Other results of the interaction between ozone and abiotic stress effects on Pn are not so clear and seem to depend on O_3 concentrations used in experiments. For example, the study comparing the response of Pn in trees grown at ambient background [O_3] (44ppb) with Pn in trees grown under elevated [O_3] (81 ppb) found no significant effect of additional treatment (drought, high temperature) on Pn nor

g_s. But when the response of Pn in elevated [O_3] (78ppb) was compared to Pn in charcoal-filtered controls, the negative effect of elevated [O_3] on Pn and g_s was ameliorated by drought (Wittig et al. 2007). Growth concentrations in Aspen FACE were relatively low (average ambient [O_3] was 37 ppb and average elevated [O_3] was 53 ppb). The lower ozone concentrations compared to Wittig's group study, but also differences in tree age (Coleman et al. 1995A,B, Noormets et al. 2010) can explain the different results. However, our results showed that the negative effect of elevated [O_3] on Pn and Pn_{sum} was ameliorated by drought and high temperature stress and this was likely caused by reduced g_s and O_3 uptake in stressed trees (Fig. 2).

3.3 Do treatment effects change during the day?

Under all treatments, the highest light-saturated net photosynthesis and stomatal conductance was found in the morning, between 9:00 and 10:00 h (Fig. 3), when environmental conditions were less limiting to leaf gas exchange, relative to the rest of the day (vapor pressure difference between leaf and air, VPD_L and leaf temperature, T_L were lower in the morning than in the afternoon, Fig. 1 in I). Some diurnal studies with other species have observed peak values of Pn at different times (Singsaas et al. 2000, Špunda et al. 2005, Ribeiro et al. 2009). For example, Singsaas et al. 2000 found that Pn on leaves of sweetgum peaked at 12:00 h and then decreased. It is likely that the peak value of Pn and the time of its achievement is different for each field site, year and species, and must be evaluated on a case-by-case basis.

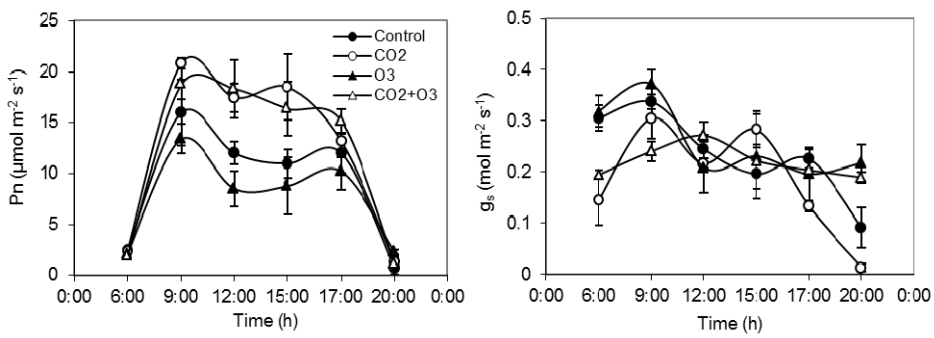

Figure 3. Example set of measured diurnal courses of light-saturated net photosynthesis (Pn) and stomatal conductance (g_s) for Clone 42E in days with no environmental stress (daily average $g_s > 0.15$ mol m^{-2} s^{-1}). Measurements were taken six times a day throughout the growing seasons of 2004 and 2005. Data points are means ±SE.

We observed a decreasing pattern in Pn after midday, with some recovery in the afternoon between 14:00 and 15:00 h in some (but not most) days, which were

accompanied by trends in g_s (Fig. 3). The similar diurnal patterns of Pn and g_s suggest that both processes are tightly coupled and dependent: the high photosynthetic performance in the morning was supported by high stomatal conductance. However, absolute values of all parameters varied with daily climatic conditions but the diurnal patterns were qualitatively similar on all days and for both clones. Pn and g_s values of stressed and unstressed plants did not differ significantly in the morning hours, but soon these values started to decrease in stressed plants (Fig. 2B,D in I).

The changes in both g_s and maximum carboxylation efficiency (Vc_{max}) together probably caused a significant decline in Pn in the afternoon (I). According to Singsaas et al. 2000, Pn can decrease by as much as 40–60% during the day as a result of varying environmental factors. Aspen clones exhibited 25–33% diurnal depression in Pn after 10:00 h. Overall, VPD_L remained the most significant environmental influence, explaining more of the variation than either leaf temperature or leaf water potential (Table 1 in I). Our findings suggest that increasing VPD_L in the afternoon caused the decreasing trend of g_s during the day ($P < 0.001$). Similar results with different species have been reported before by Koch et al. 1994 (lowland rain forest canopy), Špunda et al. 2005 (spruce trees), Ribeiro et al. 2009 (sweet orange trees). However, we cannot exclude the effects of higher T_L on g_s in the afternoon as it explained the additional portion ($R^2 = 0.2$, $P < 0.001$) of the variation in g_s, although R^2 for correlation of $\Delta g_s/g_s$ and $\Delta T_L/T_L$ was low (Table 1 in I). This probably indicates that increased transpiration, not the direct effect of temperature, was causing the stomatal closure. In addition, we found significant changes in Vc_{max} between mid-morning and afternoon in both clones, with Vc_{max} often lower in the afternoon (Fig. 5 in I). These findings are in agreement with Singsaas et al. 2000, who has shown that Vc_{max} in leaves of *Liquidambar styraciflua* decreased throughout the day. Others have shown no significant changes in Vc_{max} between mid-morning and afternoon (Ribeiro et al. 2009). However, the afternoon change in Pn was probably not caused only by stomatal closure, as intercellular $[CO_2]$ (C_i) correlated with the ratio of $\Delta g_s/g_s$ and $\Delta Pn/Pn$ ($R^2 = 0.7$, $P < 0.001$) better than with $\Delta g_s/g_s$ and $\Delta Pn/Pn$ singly (Table 1 in I). During the afternoon, Pn and g_s declined together in a coordinated way, which most likely allowed C_i to remain relatively constant.

Leaf water potential (Ψ_L) was more negative in the afternoon than in the morning (especially pronounced in stressed trees), indicating higher evaporative demand in the afternoon (Fig. 2E,F in I). More negative Ψ_L is commonly found when studying diurnal dynamics of gas exchange (Singsaas et al. 2001, Ribeiro et al. 2009) and Ψ_L was inversely related to the patterns of T_L and VPD_L (Koch et al. 1994). We found slight but significant correlation between $\Delta Pn/Pn$ and $\Delta\Psi_L/\Psi_L$ ($R^2 = 0.1$, $P = 0.036$), therefore, we cannot exclude the possibility that the low leaf water potential initiated the reduction of Pn (Table 1 in I).

In unstressed trees, we found a significant impact ($P < 0.001$) of the combined treatment on the slope of the diurnal decline of Pn compared to the control, with the effect more pronounced in Clone 42E than in Clone 271 (Fig.

6A,B in I). We also found a slight but significant impact (P=0.02) of the elevated [CO_2] treatment on the slope of the diurnal decline of Pn and the effect was more pronounced in Clone 271 (Fig. 6A,B in I). The trend to lower increments in the daily sum of absorbed CO_2, Pn_{sum}, than in morning values of Pn (Fig. 2A,B) are in agreement with the more significant diurnal decline of Pn under elevated [CO_2] alone and combined with elevated [O_3]. The more significant diurnal decrease in Pn under elevated [CO_2+O_3] was unrelated to more severe stress, as stomata showed the lowest decrease under this treatment (Fig. 6C,D in I), and the relative difference of Pn from control did not increase (as it happens under stress), but decreased in the afternoon. It is possible that the afternoon drop of Pn is caused by down-regulation of Pn in conditions of reduced growth and accumulation of unused photosynthetic assimilates in leaves (Ainsworth & Rogers 2007). We propose that the rapid reduction in Pn under the combined treatment was related to decreasing Vc_{max} and Rubisco activity in the afternoon (Fig. 5 in I). Hence, different results can be found in literature. Singsaas et al. 2000 studied three deciduous species (sweetgum, eastern redbud and red maple) and found that the overall pattern of Pn throughout the day was not significantly affected by the elevated [CO_2] treatment. However, Špunda et al. 2005 found that elevated [CO_2] treatment led to the diminution of midday photosynthesis depression, which was predominantly caused by stomatal closure and the subsequent decrease in C_i.

Contrary to our expectations, we did not find a significant impact of elevated [O_3] on the overall pattern of Pn (Fig. 6A,B in I), although in Clone 42E the negative impact of O_3 tended to decrease in the afternoon (Fig. 6A in I). We expected to see decreased photosynthetic sensitivity to O_3 in the afternoon, when many stress factors, such as higher VPD_L, that will lead to decreased g_s occur (Paper I), and because we have shown a less negative effect of elevated [O_3] on Pn in stressed trees, compared to unstressed trees (Fig. 2A,B). However, this expectation was not confirmed. Stomata in Clone 42E exhibited significantly slower afternoon closure ($P < 0.001$) under elevated [O_3], being less sensitive to ozone in afternoon hours. For Clone 271, the sensitivity of g_s to ozone decreased in the afternoon only under the combined treatment ($P < 0.001$, Fig. 6C,D in I).

3.4 Do the effects of elevated [CO_2] and/or [O_3] on Pn change during the seasonal course?

Pn measured over the growing season was highest in mid-season (June-August), when stomatal conductance was higher, and declined in late-season (September-October) as the leaf aged (I–III). Such age-related changes in Pn are common in a variety of plants, including deciduous trees (Häikio et al. 2007, 2009, Niinemets 2010, Noormets et al. 2010). Stomatal conductance followed the same seasonal pattern as did Pn (II). Leaf chlorophyll content exhibited almost constant values throughout the summer and declined in late-season (III).

Generally, elevated [CO_2] delayed senescence of leaves in autumn and Pn was maintained for longer, whereas elevated [O_3] accelerated leaf senescence (II, III) in agreement with our hypothesis and in accordance with earlier findings with *Populus* (Karnosky et al. 2003B, Sharma et al. 2003, Tricker et al. 2004, Kontunen-Soppela et al. 2010).

We compared treatment effects in summer and autumn of 2005 on the basis of seasonally summarized CO_2 uptake (Fig. 4) and found higher late-season CO_2 effect on carbon uptake (60% in autumn compared to 37% in summer) in Clone 42E. In Clone 271, however, the increment in carbon uptake was more equally distributed throughout the growing season of 2005 under elevated [CO_2] (Fig. 4). It is generally assumed that CO_2 response is highest during the middle of the growing season, because sink activity is highest during that period (Riikonen et al. 2008), however, this was not evident from our results. Similar seasonal differences in CO_2 effects were found for *Liquidambar styraciflua,* studied in the Duke Forest FACE facility (Herrick and Tomas 2003). Results from Paper I have shown that the effect of CO_2 was more pronounced on days with environmental stress (valid for at least those stresses that reduce g_s). The higher late-season CO_2 effect on carbon uptake in this study might also be explained through stressors that appear in the late season, when absolute rates in g_s are low (Fig. 3 in II).

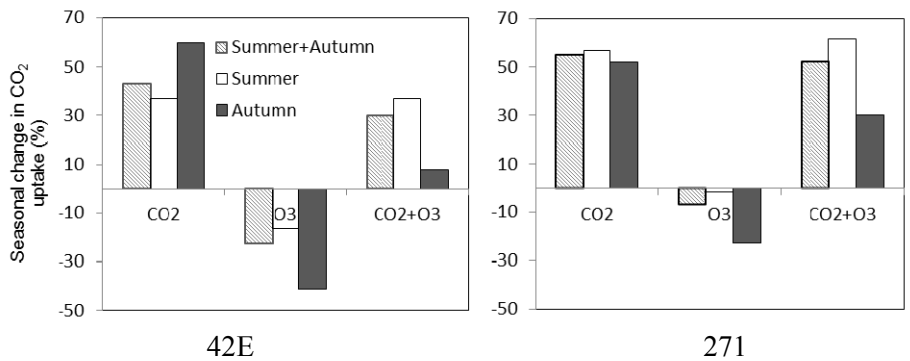

Figure 4. Relative treatment (elevated [CO_2], [O_3] and [CO_2+O_3]) effects on seasonally summarized CO_2 uptake in 2005. Seasons were defined as summer (15.06–29.08) and autumn (29.08–07.10). Seasonal CO_2 uptake in different treatments was calculated from data presented in Paper II, figure 2 (as area under seasonal curve of Pn).

When both CO_2 and O_3 concentrations were elevated, then the negative effect of elevated [O_3] prevailed over the positive effect of elevated [CO_2] in autumn, but not in summer (Fig. 4) in both clones. The effect was especially pronounced in O_3-sensitive Clone 42E, in which the positive effect of CO_2 almost disappeared in autumn (Fig. 4). This result is in agreement with Noormets et al 2010, who demonstrated that the magnitude of the effect of elevated [CO_2+O_3] on all photosynthetic parameters increased with time.

Under elevated [O_3], the response in summary CO_2 uptake was severely affected in autumn (23–41% decreases in seasonally summarized Pn) compared to summer (0–16% decreases in seasonally summarized Pn), and these decreases were especially pronounced in the O_3-sensitive clone (Fig. 4). In agreement with our study, Noormets et al. 2010 found that the O_3 effect on aspen Pn and chlorophyll content grew progressively with time, especially in the O_3-sensitive clone. Furthermore, an open top chamber experiment with aspen Clones 216, 271 and 259, (Coleman et al. 1995B) and a FACE experiment with soybean (Morgan et al. 2004) have demonstrated that leaves grown in elevated [O_3] show damage only after prolonged exposure in older leaves. Similarly, Calfapietra et al. 2008 did not find significant reduction in Pn in Clone 42E in elevated [O_3] in 2006 most likely because the campaign was carried out quite early in the season. Ozone exposure is suppressing leaf chlorphyll content (Chl) and the effect on Chl increases progressively with time (Noormets et al. 2010). Hence, degradation of chlorophyll leads to generation of ROS that are normally removed by the constitutive antioxidative system in the apoplast and symplast of leaves (Häikiö et al. 2009, Foyer & Noctor 2005). In senescing leaves, the loss of antioxidative enzymes adds to the increase in ROS due to protein breakdown (Häikiö et al. 2009, Zimmermann & Zentgraf 2005) leading to further damage to the photosynthetic apparatus. However, there are also results showing that leaves are most sensitive to O_3 after full expansion early in the growing season (Häikiö et al. 2007, 2009). Häikiö et al. 2007, 2009 have demonstrated that the deleterious impact of ozone on photosynthesis in native European aspen (*Populus tremula* L.) and hybrid aspen (*P. tremula* L. x *Populus tremuloides* Michx) was more evident in young leaves (during the early growing season) and diminished as leaves aged. One explanation for differences in O_3 sensitivity is the different effect of ozone on g_s, as less ozone is entering leaves when stomata are more closed.

3.5 Do the effects of elevated [CO_2] and/or [O_3] on Pn change during successive years?

We observed significant enhancement of Pn under elevated [CO_2] and [CO_2+O_3] exposure in both clones of *P. tremuloides* in all study years from 2004 to 2008 (IV, Fig. 5). Under elevated [CO_2], the average increments in Pn were between 55% in Clone 42E and 56% in Clone 271, which were even higher than the average 47% increments suggested for trees by Ainsworth & Rogers 2007. However, in 1998–1999, results from Aspen FACE have shown that the clonal average of Pn increased only by approximately 33% (Karnosky et al. 1999, 2003B) under elevated [CO_2]. Therefore, the effect on Pn has increased rather than decreased in time. Our findings are in accordance with leaf responses from the Duke FACE experiment (Ellsworth et al. 2012), where sustained enhancement of Pn over ten years under elevated [CO_2] was found in a *Pinus taeda* canopy. At the beginning of the experiment, elevated [CO_2+O_3]

did not affect Pn and the rates were almost similar to the control (Karnosky et al. 2003B). Hence, we have seen on average 35% (Clone 42E) and 43% (Clone 271) increments in Pn under combined treatment in this study (Fig. 5). However, the relative increase in Pn was less pronounced under elevated [CO_2+O_3] than under elevated [CO_2] in ozone-sensitive Clone 42E, in which Pn was also significantly reduced under elevated [O_3] treatment, but not in ozone-tolerant Clone 271, in which the effect of ozone was not significant at both levels of [CO_2] (IV, Fig. 5). Average g_s did not change under elevated [CO_2] and [CO_2+O_3] in some years, while in some years g_s increased and in other years decreased, compared to the control treatment (IV).

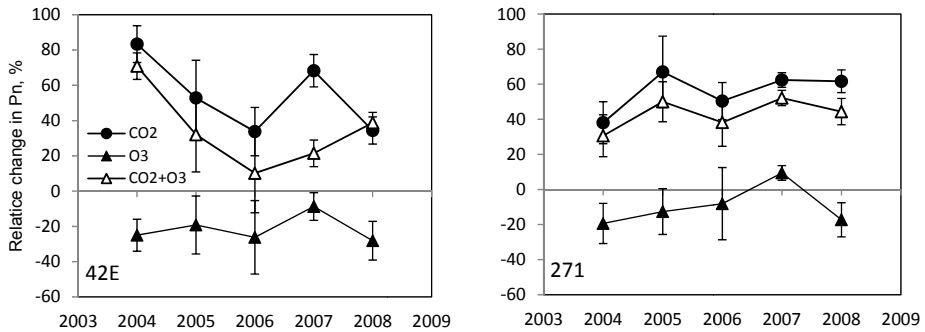

Figure 5. Photosynthetic sensitivity to elevated [CO_2], [O_3] and [CO_2+O_3] for the growing seasons 2004 through 2008 in aspen Clones 42E and 271, measured at the Aspen FACE site. Data are mean values during growing season ± standard errors. Relative changes of Pn were calculated from the data of Paper IV, Figure 1.

Rogers et al. 2004 reported that photosynthetic capacity can be lost during certain conditions under elevated [CO_2]. We observed a lower CO_2 effect during some growing seasons and a higher effect in other seasons. In Clone 42E (but not in Clone 271), the increment in Pn correlated negatively with the Pn in the control (R^2=0.7, data not shown). High values of Pn at elevated [CO_2] can be down-regulated because of sink limitation and excess of carbohydrates (Ainsworth & Rogers 2007). Poplars grown at elevated [CO_2] have high capacity for starch synthesis and carbon export (Davey et al. 2006). These traits usually enable poplars to maintain high photosynthetic rates at elevated [CO_2] and avoid a major source-sink imbalance that could lead to a deduction in the potential for carbon acquisition (Ainsworth & Rogers 2007, Davey et al. 2006). It is possible that the mentioned imbalance occurred in Clone 42E, but not in the best-growing Clone 271, as the increment of Pn due to elevated [CO_2] did not decrease with increasing Pn in the latter case (data not shown). On the other hand, environmental stresses such as drought and temperature differences most likely explain within-experiment variability (Gunderson et al. 2002, Kirchbaum et al. 2004, Ainsworth & Roger 2007, Ellsworth et al. 2012). We have shown

that exposure to high CO_2 alone or combined with high O_3 would have a greater relative impact on Pn in plants exposed to stresses that reduce g_s, seen in Fig. 4. But the average value of g_s indicated slight drought stress only for Clone 42E in 2004, when g_s was 0,12 mol m^{-2} s^{-1} (data not shown) and the relative increase in Pn under elevated [CO_2] was really very high (Fig. 5).

Our results indicate that both aspen clones have consistently sustained or even increased their enhanced photosynthetic rate and photosynthetic capacity under elevated [CO_2] and there is no indication of long-term photosynthetic down-regulation (IV). This was also true for the elevated [CO_2+O_3] treatment, which did not show any evidence of long-term negative acclimation in Pn during the growing seasons 2004 through 2008, and also compared to 1999 (Karnosky et al. 2003B). This finding is in disagreement with Ainsworth & Rogers 2007, who showed in their meta-analysis that all functional groups acclimated to elevated [CO_2], although trees had the smallest reduction in Vc_{max}. Photosynthetic down-regulation has frequently been related to plant N status, as N-containing amines are needed for synthesizing and maintaining photosynthetic proteins (Ellsworth et al. 2004, Bloom et al. 2010). Photosynthetic down-regulation in elevated [CO_2] has been reported to be more pronounced when plants are N-limited, and to be absent when N supply is adequate (Ellsworth et al. 2004, 2012). Soil fertility levels at the Aspen FACE site were within the range of natural aspen forest soil fertility (Karnosky et al. 2003B), but sufficient to ensure that tree growth was not limited by nutrient availability. Long-term stomatal acclimation was also not found under elevated [CO_2] or elevated [CO_2+O_3] treatment (IV).

On a long-term basis, elevated [O_3] has consistently and significantly reduced Pn, without any significant trend in this reduction (IV, Fig. 5). The reduction in Pn in ozone-sensitive Clone 42E was 21% (averaged in 2004–2008), which is similar to the reduction in Pn in 1999 (Karnosky et al. 2003B). In ozone-tolerant Clone 271, the average reduction in Pn in the measured years was 10% (Fig. 5). There was no evidence of positive photosynthetic acclimation (up-regulation of photosynthetic variables), but roughly similar damage to the photosynthetic apparatus by the reactive oxygen species probably occurred in all years (IV). The relative decrease in Pn varied annually, but the absolute change in Pn under elevated [O_3] correlated negatively with Pn in the control (R^2=0.6 for Clone 42E and R^2=0.5 for Clone 271, data not shown) and turned positive at low values of Pn (and g_s). This is in agreement with our findings that stressful conditions can protect trees from ozone (Fig. 2). Stomatal conductance increased in some seasons, decreased in others, and there were some seasons with no change compared to the control treatment, indicating that there was no evidence of long-term stomatal acclimation (IV).

4. CONCLUSIONS

Our findings demonstrate that the photosynthetic responses of *Populus tremuloides* to increasing [CO_2] and/or [O_3] are changing in diurnal, seasonal and interannual scales and depend on environmental constraints such as drought and high temperature. We have demonstrated that drought and high temperature stress can protect trees from ozone, and the effect of elevated [CO_2] overrode the effect of O_3 on photosynthesis in the case of the combined action of both gases. These findings highlight the importance of multiple factors in determining the future responses of trees to climate change. The key conclusion of this study is that exposure to combined factors can influence trees even more than exposure to a single factor. As changes in photosynthesis and stomatal conductance are likely to affect both the ability of plants to sequester carbon, and plant water use, these changes can affect ecosystem carbon- and hydrological cycles. Consequently, interactions discovered in this thesis should be taken into account in models that predict changes in productivity of forest ecosystems and the feed-backs from these changes on climate. These results also provide novel evidence that the [CO_2] effect has been increasing rather than decreasing in time, but the negative ozone effect has remained the same over the 11 years of the study.

Main results and conclusions in more detail:

1. Elevated [CO_2] and elevated [CO_2+O_3] increased and elevated [O_3] decreased significantly the light-saturated net photosynthesis (Pn) in both *P. tremuloides* clones compared to ambient concentrations, with the CO_2 effect being more pronounced in the ozone-tolerant and the O_3 effect in the ozone-sensitive clone. Stomatal conductance (g_s) decreased in both clones under elevated [CO_2] and [CO_2+O_3] treatments, but there was no change under elevated [O_3].
2. Forest trees are exposed to a variety of single and combined stresses differing in strength and duration throughout their lifetime, and many of the stress factors strongly interact. This study adds additional evidence that drought and high temperature stress interact strongly with the effects of elevated [CO_2] and/or [O_3] and modify the primary impact of those gases on Pn as well as on g_s. We demonstrated that drought and high temperature increased the relative impact of elevated [CO_2] and decreased the relative impact of elevated [O_3] on Pn in *P. tremuloides*, predicting CO_2 effects to prevail over O_3 effects in the case of the combined action of both gases in stress conditions.
3. We have demonstrated that *P. tremuloides* exhibited a significant afternoon reduction (25–33%) in Pn. This reduction in Pn was related to low g_s, which was a consequence of high vapor pressure difference between leaf and air (VPD_L), but also of a reduction in maximum carboxylation efficiency (Vc_{max}). Leaf water potential had only a limited influence on the diurnal pattern of Pn between 9:00 and 17:00.

4. We found that elevated [CO_2+O_3] resulted in more pronounced afternoon decline in Pn compared to ambient concentrations. This decline was unrelated to decreased stomatal conductance, but rather related to decreased Vc_{max} and Rubisco activity. Growth at elevated [CO_2] and elevated [O_3] had only little effect on the afternoon decline in Pn.
5. Elevated CO_2 and O_3 concentrations altered Pn in a similar way throughout the summer, but in autumn, CO_2 can delay and O_3 can accelerate senescence of leaves. We found that the relative effects of elevated [CO_2] and/or [O_3] on Pn were generally more pronounced in autumn compared to summer. The O_3 effect on Pn increased progressively during the late season under both ambient and elevated CO_2 concentrations, especially in the O_3-sensitive clone.
6. This study showed a significant interannual variation in Pn under all treatment. Elevated [CO_2] alone or combined with elevated [O_3] increased Pn in all years (2004–2008) of the study, whereas the [CO_2] effect has been increasing rather than decreasing in time compared to the first years (1998–1999) of the Aspen FACE experiment. No indication of Pn down-regulation by elevated [CO_2] or Pn up-regulation by elevated [O_3] was found after 11 years of fumigation.

5. REFERENCES

Ainsworth EA. 2008A. Rice production in a changing climate: a meta-analysis of responses to elevated carbon dioxide and elevated ozone concentration. *Global Change Biology* 14: 1642–1650.

Ainsworth EA, Long SP. 2004. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* 165: 351–71.

Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant, Cell and Environment* 30: 258–270.

Ainsworth EA, Rogers A, Leakey ADB. 2008B. Targets for crop biotechnology in a future high CO_2 and high O_3 world. *Plant Physiology* 147: 13–19.

Ainsworth EA, Rogers A, Nelson R, Long SP. 2004. Testing the 'source-sink' hypothesis of down-regulation of photosynthesis in elevated [CO_2] in the field with single gene substitutions in *Glycine max*. *Agricultural and Forest Meteorology* 122: 85–94.

Ainsworth EA, Yendrek CR, Sitch S, Collins WJ, Emberson LD. 2012. The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change. Annual *Review of Plant Biology* 63: 637–661.

Bernacchi CJ, Calfapietra C, Davey PA, Wittig VE, Scarascia-Mugnozza GE, Raines CA, Long SP. 2003. Photosynthesis and stomatal conductance responses of poplars to free-air CO_2 enrichment (PopFACE) during the first growth cycle and immediately following coppice. *New Phytologist* 159: 609– 621.

Bernacchi CJ, Leakey ADB, Heady LE, Morgan PB, Dohleman FG, McGrath JM, Gillespie JM, Wittig VE, Rogers A, Long SP, Ort DR. 2006. Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open-air field conditions. *Plant, Cell and Environment* 29: 2077–2090.

Bernacchi CJ, Morgan PB, Ort DR, Long SP. 2005. The growth of soybean under free-air CO2 enrichment (FACE) stimulates photosynthesis while decreasing in vivo rubisco capacity. *Planta* 220: 434–446.

Bloom AJ, Burger M, Asensio JSR, Cousins AB. 2010. Carbon Dioxide Enrichment Inhibits Nitrate Assimilation in Wheat and *Arabidopsis*. *Science* 328: 899–903.

Calfapietra C, Mugnozza GS, Karnosky DF, Francisco L, Sharkey TD. 2008. Isoprene emission rates under elevated CO2 and O3 in two field-grown aspen clones differing in their sensitivity to O3. *New Phytologist* 179: 55–61.

Centritto M, Jarvis PG. 1999. Long-term effects of elevated carbon dioxide concentration and provenance on four clones of Sitka spruce (*Picea sitchensis*). II. Photosynthetic capacity andnitrogen use efficency. *Tree Physiology* 19: 807–814.

Coleman MD, Dickson RE, Isebrands JG, Karnosky DF. 1995A. Carbon allocation and partitioning in aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiology* 15: 593–604.

Coleman MD, Isebrands JG, Dickson RE, Karnosky DF. 1995B. Photosynthetic productivity of aspen clones varying in sensitivity to tropospheric ozone. *Tree Physiology* 15: 585–592.

Curtis PS. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell and Environment* 19: 127–137.

Darbah JNT, Sharkey TD, Calfapietra C, Karnosky DF. 2010. Differential response of aspen and birch trees to heat stress under carbon dioxide. *Environmental Pollution* 158:1008–1014.

Davey PA, Olcer H, Zakhleniuk O, Bernacchi CJ, Calfapietra C, Long SP, Raines CA. 2006. Can fast growing plantation trees escape biochemical down-regulation of photosynthesis when growing throughout their complete production cycle in the open air under elevated carbon dioxide? *Plant, Cell and Environment* 29: 1235–1244.

Dickson RE, Coleman MD, Pechter P, Karnosky D. 2001. Growth and crown architecture of two aspen genotypes exposed to interacting ozone and carbon dioxide. *Environmental Pollution* 115: 319–334.

Dickson RE, Coleman MD, Riemenschneider DE, Isebrands JG, Hogan GD, Karnosky DF. 1998. Growth of five hybrid poplar genotypes exposed to interacting elevated CO2 and O3. *Canadian Journal of Forest Research* 28: 1706–1716.

Dickson RE, Lewin KF, Isebrands JG, Coleman MD, Heilman WE, Reimenschneider DE, Sober J, Host GE, Hendrey GR, Pregitzer KS, Karnosky DF. 2000. Forest atmosphere carbon transfer and storage-II (FACTS II). The aspen free-air CO2 and O3 enrichment (FACE) project: an overview. USDA Forest Service North Central Research Station, General Technical Report NC-214, 68 pp.

Dixon M, Le Thiec D, Garrec JP. 1995. The growth and gas exchange response of soil-planted Norway spruce [*Picea abies* (L.) Karst.] and red oak (*Quercus robur* L.) exposed to elevated CO2 and to naturally occurring drought. *New Phytologist* 129: 265–273.

Donelli A, Craigon J, Black CR, Colls JJ, Landon G. 2001. Does elevated CO_2 ameliorate the impact of O_3 on chlorophyll content and photosynthesis in potato (*Solanum tuberosum*)? *Plant Physiology* 111:501–511.

Drake BG, Gonzàlez-Meler MA and Long SP. 1997. More efficient plants: a consequence of rising atmospheric CO2? Annual Revew. *Plant Physiology. Plant Molecular Biology* 48: 609–639.

Eichelmann H, Oja V, Rasulov B, Padu E, Bichele I, Pettai H, Möls T, Kasparova I, Vapaavuori E, Laisk A. 2004. Photosynthetic parameters of birch (*Betula pendula* Roth) leaves growing in normal and in CO_2- and O_3- enriched atmospheres. *Plant, Cell and Environment* 27: 479–495.

Ellsworth DS. 1999. CO2 enrichment in a maturing pine forest: are CO2 exchange and water status in the canopy affected? *Plant, Cell and Environment* 22: 461–472.

Ellsworth DS, Reich PB, Naumburg ES, Koch GW, Kubiske ME, Smith SD. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Global Change Biology* 10: 2121–2138.

Ellsworth DS, Thomas R, Crous K, Palmroth S, Maier C, Delucia E, Oren R. 2012. Elevated CO_2 affects photosynthetic responses in canopy pine and subcanopy deciduous trees over 10 years: a synthesis from Duke FACE. *Global Change Biology* 18: 223–242.

Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9–19.

Farage PK, Long SP, Lechner EG, Baker NR. 1991. The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. *Plant Physiology* 95: 529–535.

Farquhar GD, von Caemmerer S. 1982. Modelling of photosynthetic response to environmental conditions. In Physiological Plant Ecology II. Water Relations and

Carbon Assimilation, Encyclopedia of Plant Physiology, New Series, Vol. 12 B (Lange, O. L., Nobel, P. S., Osmond, C. B., and Ziegler, H., eds.), Berlin, Heidelberg, New York: Springer-Verlag, pp. 549–588.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta* 149: 78–90.

Feng ZZ, Kobayashi K, Ainsworth EA. 2008. Impact of elevated ozone concentration on growth, physiology, and yield of wheat (*Triticum aestivum* L.): a meta-analysis. *Global Change Biology*, 14: 2696–2708.

Foyer CM, Noctor G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment* 28: 1056–1071.

Fredericksen TS, Kolb DE, Skelly JM, Steiner KC, Joyce BJ, Savage JE. 1996. Light environment alters ozone uptake per net photosynthetic rate in black cherry trees. *Tree Physiology* 16: 485–490.

Gunderson CA, Sholtis JD, Wullschleger SD, Tissue DT, Hanson PJ, Norby RJ. 2002. Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. *Plant, Cell and Environment* 25: 379–393.

Grulke NE, Johnson R, Monschein S, Nikolova P, Tausz M. 2003. Variation in morphological and biochemical O3 injury attributes of mature Jeffrey pine within canopies and between microsites. *Tree Physiology* 23: 923–929.

Herrick JD, Thomas RB. 2001. No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke Forest FACE experiment. *Plant, Cell and Environment* 24: 53–64.

Herrick JD, Thomas RB. 2003. Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations. *Tree Physiology* 23: 109–118.

Häikiö E, Freiwald V, Julkunen-Tiitto R, Beuker E, Holopainen T, Oksanen E. 2009. Differences in leaf characteristics between ozone-sensitive and ozone-tolerant hybrid aspen (*Populus tremula* x *Populus tremuloides*) clones. *Tree Physiology* 29: 53–66.

Häikiö E, Freiwald V, Silfver T, Beuker E, Holopainen T, Oksanen E. 2007. Impacts of elevated ozone and nitrogen on growth and photosynthesis of European aspen (*Populus tremula*) and hybrid aspen (*P. tremula* x *Populus tremuloides*) clones. *Canadian Journal of Forest Research* 37: 2326–2336.

IPCC 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex Vand Midgley PM (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IPCC. 2007. Climate change 2007: The physical science basis. Contribution of working group I. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds.), Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK, Cambridge University Press.

Isebrands JG, McDonald EP, Kruger E, Hendrey G, Pregitzer K, Percy K, Sober J, Karnosky DF. 2001. Growth responses of *Populus tremuloides* clones to interacting carbon dioxide and tropospheric ozone. *Environmental Pollution* 115: 359–371.

Jach ME, Ceulemans R, Murray MB. 2001. Impact of greenhouse gases on the phenology of forest trees. In The Impact of Carbon Dioxide and Other Greenhouse

Gases on Forest Ecosystems. Eds. D.F. Karnosky, R. Ceulemans, G.E. Scarascia-Mugnozza and J.L. Innes. *CABI Publishing,* Oxford, pp 193–223.

Kaakinen S, Kostiainen K, Saranpää P, Kubiske ME, Sober J, Karnosky DF, Vapaavuori E. 2004. Stem wood properties of *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* saplings after 3 years of treatments to elevated carbon dioxide and ozone. *Global Change Biology* 10: 1513–1525.

Karnosky DF. 2003A. Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. *Environment International* 29: 161–169.

Karnosky DF, Gagnon ZE, Dickson RE, Coleman MD, Lee EH, Isebrands, JG. 1996. Changes in growth, leaf abscission, and biomass associated with seasonal tropospheric ozone exposures of *Populus tremuloides* clones and seedlings. *Canadian Journal of Forestry Research* 26: 23–37.

Karnosky DF, Mankovska B, Percy K, Dickson RE, Podila GK, Sober J, Noormets A, Hendrey G, Coleman MD, Kubiske M, Pregitzer KS, Isebrands JG. 1999. Effects of tropospheric O3 on trembling aspen and interaction with CO2: Results from an O3-gradient and a FACE experiment. *Water, Air and Soil Pollution* 116: 311–322.

Karnosky DF, Pregitzer KS, Zak DR, Kubiske ME, Hendrey GR, Weinstein D, Nosal M, Percey KE. 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant, Cell and Environment* 28: 965–981.

Karnosky DF, Skelly JM, Percy KE and Chappelka AH. 2007B. Perspectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests. *Environmental Pollution* 147:489–506.

Karnosky DF, Tallis M, Darbah J, and Taylor G. 2007A. Direct effects of elevated CO2 on forest tree productivity. In: Freer-Smith, P.H., Broadmeadow, M.S.J. and Lynch, J.M. (eds), *Forestry and Climate Change*. CABI Publishing, Oxford, UK pp. 136–142.

Karnosky DF, Zak DR, Pregitzer KS, Awmack CS, Bockheim JG, Dickson RE, Hendrey GR, Host GE, King JS, Kopper BJ, Kruger EL, Kubiske ME, Lindroth RL, Mattson WJ, McDonald EP, Noormets A, Oksanen E, Parsons WFJ, Percy KE, Podila GK, Riemenschneider DE, Sharma P, Thakur R, Sõber A, Sõber J, Jones WS, Anttonen S, Vapaavuori E, Mankovska B, Heilman W, Isebrands JG. 2003B. Tropospheric O_3 moderates responses of temprate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* 17: 289–304.

Keeling CM, Whort TP, Wahlen M & Vander Plict J. 1995. International extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* 375: 666–670.

Kellomäki S, Wang KY. 1996. Photosynthetic responses to needle water potentials in Scots pine after a four-year exposure to elevated CO2 and temperature. *Tree Physiology* 16: 765–772.

King JS, Kubiske ME, Pregitzer KS, Hendrey GR, McDonald EP, Giardina CP, Quinn VS, Karnosky DF. 2005. Tropospheric O3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO2. *New Phytologist* 168: 623–636.

Kirchbaum MUF. 2004. Direct and Indirect Climate Change Effects on Photosynthesis and Transpiration. *Plant Biology 6*: 242–253.

Koch GW, Amthor JS, Goulden ML. 1994. Diurnal patterns of leaf photosynthesis, conductance and water potential at the top of a lowland rain forest canopy in Cameroon – measurements from the *Radeau des Cimes*. *Tree Physiology* 14: 347–360.

Kontunen-Soppela S, Ossipov V, Ossipova S, Oksanen E. 2007. Shift in birch leaf metabolome and carbon allocation during long-term open-field ozone exposure. *Global Change Biology* 13: 1053–1067.

Kontunen-Soppela S, Parviainen J, Ruhanen H, Brosche M, Keinänen M, Thakur RC, Kolehmainen M, Kangasjärvi J, Oksanen E, Karnosky DF, Vapaavuori E. 2010. Gene expression responses of paper birch (Betula papyrifera) to elevated CO2 and O3 during leaf maturation and senescence. *Environmental Pollution* 158: 959–968.

Kubiske ME, Quinn VS, Heilman WE, McDonald EP, Marquardt PE, Teclaw RM, Friend AL, Karnosky DF. 2006. Interannual climatic version mediates elevated CO2 and O3 effects on forest growth. *Global Change Biology* 12: 1054–1068.

Kubiske ME, Quinn VS, Marquardt PE and Karnosky DF. 2007. Effects of Elevated Atmospheric CO2 and/or O3 on Intra- and Interspecific Competitive Ability of Aspen. *Plant Biology* 9: 342–355.

Kull O, Sõber A, Coleman MD, Dickson RE, Isebrands JG, Gagnon Z, Karnosky DF. 1996. Photosynthetic responses of aspen clones to simultaneous exposures of ozone and CO_2. *Canadian Journal of Forest Research* 26: 639–648.

Leakey ADB, Ainsworth E, Bernacchi CJ, Rogers A, Long SP, Ort DR. 2006A. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Environmental Botany* 60: 2859–2876**.**

Lindroth RL. 2010. Impacts of elevated atmospheric CO_2 and O_3 on forests: phytochemistry, trophic interactions, and ecosystem dynamics. *Journal of Chemical Ecology* 36: 2–21.

Lindroth RL, Kopper BJ, Parsons WFJ, Bockheim JG, Karnosky DF, Hendrey GR, Pregitzer KS, Isebrands JG, Sõber J. 2001. Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environmental Pollution* **115: 395–404.**

Long SP, Ainsworth EA, Bernacchi CJ, Davey PA, Hymus GJ, Leakey ADB., Morgan P.B. & Osborne C.P. 2006. Long-term responses of photosynthesis and stomata to elevated [CO2] in managed systems. In Managed Ecosystems and CO2. Case Studies, Processes and Perspectives (eds J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey & H. Blum), pp. 253–270. Springer-Verlag, Heidelberg, Germany.

Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* 55:591–628.

Long SP, Naidu SL. 2002. Effects of oxidants at the biochemical, cell and physiological levels, with particular reference to ozone. *In Air Pollution and Plant Life* 69–88.

Luedemann G, Matyssek R, Fleischmann F, Grams TEE. 2005. Acclimation to ozone affects host/pathogen interaction and competitiveness for nitrogen in juvenile *Fagus sylvatica* and *Picea abies* trees infected with *Phytophthora citricola*. *Plant Biology* 7: 640–649.

Lütz C, Anegg S, Gerant D, Alaoui-Sosse B, Gerard J, Dizengremel P. 2000. Beech trees exposed to simulated summer ozone levels: Effectson photosynthesis, chloroplast components and leaf enzyme activity. *Physiologia Plantarum* 109: 252–259.

Matyssek R, Innes JR. 1999. Ozone – a risk factor for trees and forests in Europe? *Water, Air and Soil Pollution* 116: 199–226.

Matyssek R, Karnosky DF, Wieser G, Percy K, Oksanen E, Grams TEE, Kubiske M, Hanke D, Pretzsch H. 2010. Advances in understanding ozone impact on forest

trees: Messages from novel phytotron and free-air fumigation studies. *Environmental Pollution* 158: 1990–2006.

Matyssek R, Keller T, Koike T. 1993. Branch growth and leaf gas exchange of *Populus tremula* exposed to low ozone concentrations throughout two growing seasons. *Environmental Pollution* 79: 1–7.

Matyssek R, Sandermann H, Wieser G, Booker F. Cieslik S, Musselman D, Ernst D. 2008. The challenge of making ozone risk assessment for forest trees more mechanistic. *Environmental Pollution* 156: 567–582

McDonald EP, Kruger EL, Riemenschneider DE, Isebrands JG. 2002. Competitive status influences tree-growth responses to elevated CO2 and O3 in aggrading aspen stands. *Functional Ecology* 16: 792–801.

Medlyn BE, Barton CVM, Broadmeadow MSJ, Ceulemans R, De Angelis P, Forstreuter M, Freeman M, Jackson SB, Kellomäki S, Laitat E, Rey A, Roberntz P, Sigurdsson BD, Strassemeyer J, Wang K, Curtis PS, Jarvis PG. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO2 concentration: a synthesis. *New Phytologist* 149: 247–264.

Medlyn BE, Loustau D, Delzon S. 2002. Temperature response of parameters of a biochemically-based model of photosynthesis. I. Seasonal changes in mature maritime pine (*Pinus pinaster* Ait.). *Plant, Cell and Environment* 25: 1155–1165.

Meehl GA, Stocker TF, Collins WD et al. 2007. Global climate projections. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Inter-governmental Panel on Climate Change (eds Solomon S, Qin D, Manning Met al.), pp. 747–845. Cambridge University Press, Cambridge, UK/New York, NY, USA.

Morgan PB, Ainsworth EA, Long SP. 2003. How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant, Cell and Environment* 26: 1317–1328.

Morgan PB, Bernacchi CJ, Ort DR, Long SP. 2004. An In Vivo Analysis of the Effect of Season-Long Open-Air Elevation of Ozone to Anticipated 2050 Levels on Photosynthesis in Soybean. *Plant Physiology* 135: 2348–2357.

Muraoka, H., Tang, Y., Terashima, I., Koizumi, H., Washitani, I., 2000. Contributions of diffusional limitation, photoinhibition and photorespiration to the midday depression of photosynthesis in *Arisaema heterophyllumin* the natural high light. *Plant, Cell and Environment* 23: 235–250.

Mäenpää M, Riikonen J, Kontunen-Soppela S, Rousi M, Oksanen E. 2011. Vertical profiles reveal impact of ozone and temperature on carbon assimilation of *Betula pendula* and *Populus tremula*. *Tree Physiology* 31: 808–818.

Noormets A, Kull O, Sõber A, Kubiske ME, Karnosky DF. 2010. Elevated CO_2 response of photosynthesis depends on ozone concentration in aspen. *Environmental Pollution* 158: 992–999.

Noormets A, McDonald EP, Kruger EL, Sõber A, Isebrands JG, Dickson RE and Karnosky DF. 2001B. The effects of elevated carbon dioxide and ozone on leaf – and branch level photosynthesis and potential plant-level carbon gain in aspen. *Trees* 15: 262–270.

Noormets A, Sõber A, Pell EJ, Dickson RE, Podilla GK, Sõber J, Isebrands JG, Karnosky DF. 2001A. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (Populus tremuloides Michx.) clones exposed to elevated CO_2 and/or O_3. *Plant, Cell and Environment*. 24: 327–336.

Norby RJ, Hartz-Rubin JS, Verbrugge MJ. 2003. Phenological responses in maple to experimental atmospheric warming and CO_2 enrichment. *Global Change Biology* 9: 1792–1801.

Nowak RS, Ellsworth DS, Smith SD. 2004. Functional responses of plants to elevated atmospheric CO_2 – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* 162: 253–280.

Niinemets Ü. 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management* 260: 1623–1639.

Niinemets Ü, Tenhunen JD. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant Cell and Environment* 20: 845–866.

Nunn A, Weiser G, Reiter IM, Häberle KH, Grote R, Havranek WM, Matyssek R. 2006. Testing the unifying theory of ozone sensitivity with mature trees of Fagus sylvatica and Picea abies. *Tree Physiology* 26: 1391–1403.

Oksanen E. 2003. Physiological ozone responses of birch (*Betula pendula* Roth) differ between soil-grown trees in a multi-year exposure and potted saplings in a single-season exposure. *Tree Physiology* 23: 603–614.

Oksanen E, Kontunen-Soppela S, Riikonen J, Peltonen P, Uddling J, Vapaavuori E. 2007. Northern environment predisposes birches to ozone damage. *Plant Biology* 9: 191–196.

Oksanen E, Sôber J, Karnosky DF. 2001. Interactions of elevated CO2 and ozone in leaf morphology of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in aspen FACE experiment. *Environmental Pollution* 115: 437–446.

Ollinger SV, Aber JD, Reich PB. 1997. Simulating ozone effects on forest productivity: Interactions among leaf-, canopy-, and stand-level processes. *Ecological Applications* 7: 1237–1251.

Oren R, Ellsworth DS, Johnson KH, Phillips N, Ewers BE, Maier C, Schafer KVR, McCarthy H, Hendrey G, McNulty SG, Katul GG. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO2-enriched atmosphere. *Nature* 411: 469–472.

Paoletti E, Grulke NE. 2005. Does living in elevated CO_2 ameliorate tree response to ozone? A review on stomatal responses. *Environmental Pollution* 137: 483–493.

Pell EJ, Schlagnhaufer CD, Arteca RN. 1997. Ozone-induced oxidative stress: Mechanisms of action and reaction. *Physiologia Plantarum* 100: 264–273.

Percy KE, Awmack CS, Lindroth RL, Kubiske ME, Kopper BJ, Isebrands JG, Pregitzer KS, Hendrey GR, Dickson RE, Zak DR, Oksanen E, Sober J, Harrington R, Karnosky DF. 2002. Altered performance of forest pests under CO_2- and O_3-enriched atmospheres. *Nature* 420: 403–407.

Percy KE, Karnosky DF. 2007. Air quality in natural areas: Interface between the public, science and regulation. *Environmental Pollution* 149: 256–257.

Podila GK, Paolacci AR, Badiani M. 2001. The impact of greenhouse gases on antioxidants and foliar defence compounds. In Karnosky DF, Ceulemans R, Scarascia-Mugnozza GE, Innes JL, eds. The impact of carbon dioxide and other greenhouse gases on forest ecosystems. Vienna, Austria: *CABI Publishing*: 57–125.

Pääkkönen E, Holopainen T, Renlampi LK. 1995. Effects of ozone on birch (*Betula pendula* Roth.) clones. *Water, Air and Soil Pollution* 85: 1331–1336.

Reich PB, Lassoie JP. 1984. Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. *Plant, Cell and Environment* 7: 661–668.

Ribeiro RV, Machado EC, Santos MG, Oliveira RF. 2009. Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees. *Environmental and Experimental Botany* 66: 203–211.

Riikonen J, Holopainen T, Oksanen E, Vapaavuori E. 2005. Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO_2 and O_3 in the field. *Tree Physiology* 25: 549–560.

Riikonen J, Lindsberg MM, Holopainen T, Oksanen E, Lappi J, Peltonen J, Vapaavuori E. 2004. Silver birch and climate change: variable growth and carbon allocation responses to elevated concentrations of carbon dioxide and ozone. *Tree Physiology* 24: 1227–1237.

Riikonen J, Syrjala L., Tulva I, Mänd P, Oksanen E, Poteri M, Vapaavuori E. 2008. Stomatal characteristics and infection biology of Pyrenopeziza betulicola in Betula pendula trees grown under elevated CO_2 and O_3. *Environmental Pollution* 156: 536–543.

Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Dohleman FG, Heaton EA, Mahoney J, Zhu XG, Delucia EH, Ort DR, Long SP. 2004. Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment. *Plant, Cell and Environment* 27: 449–458.

Saxe H, Ellsworth D, Heath J. 1998. Tree and forest functioning in an enriched CO2 atmosphere. *New Phytologist* 139: 395–436.

Scarascia-Mugnozza G, De Angelis P, Matteucci G, Valenti R. 1996. Long-term exposure to elevated [CO2] in a natural *Quercus ilex* L. community: net photosynthesis and photochemical efficiency of PSII at different levels of water stress. *Plant, Cell and Environment* 19: 643–654.

Sellers PJ, Bounoua L, Collatz GJ, Randall DA, Dazlich DA, Los SO, Berry JA, Fung I, Trucker CJ, Field CB, Jensen TG. 1996. Comparison of radiative and physiological effects of doubled atmospheric CO_2 on climate. *Science* 271: 1402–1406.

Sharma P, Sober A, Sober J, Podila GK, Kubiske ME, Mattson WJ, Isebrands JG, Karnosky DF. 2003. Moderation of [CO_2]-induced gas exchange responses by elevated tropospheric O_3 in trembling aspen and sugar maple. *Ekologia 22* (Supplement 1): 304–317.

Singsaas E, Ort D, DeLucia E. 2001. Variation of photosynthetic quantum yield in ecophysiological studies. *Oecologia* 128: 15–23.

Singsaas EL, Ort DR, DeLucia EH. 2000. Diurnal regulation of photosynthesis in understory saplings. *New Phytologist* 145: 39–49.

Sõber A. 1980. Stomatal responses to changes of air humidity and participation of CO_2 in these reactions. *Russian Journal* of *Plant Physiology* 27: 383–85.

Špunda V, Kalina J, Urban O, Luis VC, Sibisse I, Puertolas J, Šprtova M, Marek MV. 2005. Diurnal dynamics of photosynthetic parameters of Norway spruce trees cultivated under ambient and elevated CO_2: the reasons of midday depression in CO_2 assimilation. *Plant Science* 168: 1371–1381.

Takeuchi Y, Kubiske ME, Isebrands JG, Pregitzer KS, Hendrey G, Karnosky DF. 2001. Photosynthesis, light and nitrogen relationships in a young deciduous forest canopy under open-air CO_2 enrichment. *Plant, Cell and Environment*. 24: 1257–1268.

Tricker PJ, Calfapietra C, Kuzminsky E, Puleggi E, Ferris R, Nathoo R, Pleasants M, Alston V, De Angelis, Taylor. 2004. Long-term acclimation of leaf production,

development, longevity and quality following 3 year exposure to free-air CO_2 enrichment during canopy closure in Populus. *New Phytologyst* 162: 413–426.

Uddling J, Hogg AJ, Teclaw RM, Carroll MA, Ellsworth DS. 2010. Stomatal uptake of O_3 in aspen and aspen-birch forests under free-air CO_2 and O_3 enrichment. *Environmental Pollution 158*: 2023–2031.

Uddling J, Karlsson PE, Glorvigen A, Sellde´n G. 2005. Ozone impairs autumnal resorption of nitrogen from birch (*Betula pendula*) leaves, causing an increase in whole-tree nitrogen loss through litter fall. *Tree Physiology* 26: 113–120.

Volin JC, Reich PB, Givnish TJ. 1998. Elevated carbon dioxide ameliorates the effects of ozone on photosynthesis and growth: species respond similarly regardless of photosynthetic pathway or plant functional group. *New Phytologist* 138: 315–325.

Wang D, Bormann FH, Karnosky DF. 1986. Regional tree growth reductions due to ambient ozone: evidence from field experiments. *Environmental Science and Technology* 20: 1122–1125.

Wittig VE, Ainsworth EA, Long SP. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments. *Plant, Cell and Environment* 30: 1150–1162.

Wittig VE, Ainsworth EA, Naidu SL, Karnosky DF, Long SP. 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. *Global Change Biology* 15: 396–424.

Wustman BA, Oksanen E, Karnosky DF, Noormets A, Isebrands JG, Pregitzer KS, Hendrey GR, Sober J, Podila GK. 2001. Effects of Elevated CO_2 and O_3 on aspen clones varying in O_3 sensitivity: can CO_2 Ameliorate the harmful effects of O_3? *Environmental Pollution* 115: 473–481.

Zhu XK, Feng ZZ, Sun TF, Liu XC, Tang H, Zhu J, Guo W, Shi K. 2011. Effects of elevated ozone concentration on yield of four Chinese cultivars of winter wheat under fully open-air field conditions. *Global Change Biology* 17: 2697–2706.

Zimmermann P, Zentgraf U. 2005. The correlation between oxidative stress and leaf senescence during plant development. *Cellular and Molecular Biology* 10: 515–534.

SUMMARY IN ESTONIAN

Kõrgendatud CO_2 ja O_3 kontsentratsioonide mõju fotosünteesi parameetritele ameerika haava lehestikus: päevased, sesoonsed ja aastatevahelised erinevused

Globaalse soojenemisega kaasneb süsinikdioksiidi kontsentratsiooni ([CO_2]) ja osooni kontsentratsiooni ([O_3]) suurenemine maalähedases atmosfäärikihis. CO_2 kontsentratsioon on industriaalajastueelse ajaga võrreldes suurenenud *ca* 40%. Osooni kontsentratsioonid on viimase 100 aasta vältel kahekordistunud. Käesoleval sajandil prognoositakse jätkuvat CO_2 ja O_3 kontsentratsioonide kasvu. Lisaks sellele prognoositakse kliima muutumist heitlikumaks ning esineda võib nii põua- kui liigniiskuse perioode. Muutused maalähedases atmosfäärikihis võivad otseselt mõjutada taimede kasvu, mis omakorda avaldab tagasimõju atmosfääris toimuvatele protsessidele.

Varasema kirjanduse põhjal on teada, et kõrgem [CO_2] üldjuhul soodustab taimede, sealhulgas puude kasvu, kuna [CO_2] tõus põhjustab netofotosünteesi (Pn) suurenemist. Kõrged osooni kontsentratsioonid on puude kasvu ja metsa produktsiooni seisukohalt aga kahjulikud, kuna osoon on tugev oksüdeerija, mis kahjustab fotosünteesiaparaati ning põhjustab Pn vähenemist. Pn väärtus näitab, kui palju lehe pinnaühik ajaühikus süsihappegaasi seob ning lehestiku pinnaga korrutatult iseloomustab see süsiniku hulka, mida taim ajaühikus kasvuks kasutada saab.

Suurenevate CO_2 ja/või O_3 kontsentratsioonide mõju taimede fotosünteesile ja kasvule ei pruugi olla erinevates tingimustes ühesugune. Tundlikkus nendele gaasidele võib erineda liigiti ja perekonniti ning ümbritsevad keskkonnatingimused võivad samuti reaktsioonide ulatust muuta. Suurenevate CO_2 ja O_3 kontsentratsioonide toimega samaaegselt mõjutavad taimi veel mitmed muud keskkonnategurid, kusjuures üks faktor võib tugevdada või vähendada teise faktori mõju. CO_2 ja/või O_3 mõju ulatus võib varieeruda ka päeva lõikes ning vegetatsiooniperioodi vältel. Kuna taimedel on võime keskkonnamuutustega kohaneda, siis võib juhtuda, et CO_2 ja O_3 esialgu esinev tugev mõju pikaajalises perspektiivis hoopis kaob. Erinevate stressifaktorite koosmõju taimedele on suhteliselt vähe uuritud ja seda tööd alustades ei olnud teada, kas ja kuidas Pn reaktsioon suurenevatele CO_2 ja/või O_3 kontsentratsioonidele erinevates tingimustes muutub. Samas on see aga oluline metsa produktsiooni täpsemaks prognoosimiseks.

Käesoleva doktoritöö üldine eesmärk oli välja selgitada, kuidas mõjutavad kõrgendatud CO_2 ja/või O_3 kontsentratsioonid kiirekasvuliste lehtpuude fotosünteesi iseloomustavaid parameetreid ning millest need mõjud sõltuvad.

Töö kitsamad eesmärgid olid järgmised.

1. Uurida, kuidas mõjutavad kõrgendatud CO_2 ja/või O_3 kontsentratsioonid ameerika haava (*Populus tremuloides* Michx.) osoonitundliku klooni (42E) ja osoonile tolerantse klooni (271) netofotosünteesi (Pn) ja õhulõhede juhti-

vust (g_s) pikaajalises välieksperimendis Aspen FACE (free-air carbon dioxide enrichment), **I**.

2. Analüüsida, kuidas muudab keskkonnastress kõrgendatud CO_2 ja/või O_3 kontsentratsiooni mõju ulatust netofotosünteesile ja õhulõhede juhtivusele, **I**.
3. Uurida, kuidas Pn ja g_s tundlikkus kõrgendatud CO_2 ja/või O_3 kontsentratsioonide suhtes muutub päeva jooksul, sesoonselt ja järjestikustel aastatel (**I–IV**).
4. Analüüsida CO_2 ja/või O_3 tundlikkuse muutusi erinevas ajaskaalas, nii stressi mõju kui ka kohanemise kontekstis (**I, IV**).

Püstitatud küsimustele vastuste leidmiseks mõõdeti lehtede gaasivahetust Aspen FACE´i katsealal (Rhinelander, WI, USA). Aspen FACE´i katsealal suurendati õhu süsinikdioksiidi ja osooni kontsentratsioone, imiteerides nii looduslikes metsakooslustes toimuvaid muutusi. Ameerika haava erinevate kloonide pistoksad istutati katseala viljakasse mulda 1997. a. Taimi fumigeeriti iga aasta vegetatsiooniperioodil alates 1998. aastast nelja erineva töötlusega: 1) kontrolltöötlus (ümbritsev [CO_2] ja ümbritsev [O_3]); 2) kõrgendatud CO_2 töötlus (kõrgendatud [CO_2], *ca* 560 ppm-i); 3) kõrgendatud O_3 töötlus (1,5 x ümbritsev [O_3]); 4) kombineeritud töötlus (kõrgendatud [CO_2] ja kõrgendatud [O_3]). Mõõtmisi tehti päikesele eksponeeritud võrastiku ülaosas ja alati Pn jaoks küllastuvatel valguse intensiivsustel (>1000 μmol m^{-2} s^{-1}). Andmed koguti aastatel 2004–2008. CO_2 ja/või O_3 mõjude hindamiseks Pn-i ja g_s-i päevastele käikudele tehti aastatel 2004 ja 2005 mõõtmisi kuuel korral päevas päikesetõusust päikeseloojanguni. Lisaks Pn-i ja g_s-i päevastele käikudele mõõdeti ka lehtede veepotentsiaali päevaseid käike. Võrastiku ülaosas mõõdeti veel lehtede klorofüllisisaldust. Pn sõltuvust intertsellulaarsest CO_2-st (A/C_i kõver) mõõdeti nii ennelõunal kui pärastlõunal. Stressi mõju hindamiseks võrreldi andmeid päevadel, mil keskkonnatingimused kas olid limiteerivad (esines põud või kõrge temperatuur) või ei olnud limiteerivad. Et teada saada, kas CO_2 ja/või O_3 mõjud Pn-ile muutuvad vegetatsiooniperioodi vältel, mõõtsime 2004. ja 2005. aastal lehtede gaasivahetust suve algusest kuni lehtede märgatava kolletumiseni. Selleks, et hinnata, kas vegetatsiooniperioodi Pn ja g_s kohanevad pikaajaliste CO_2 ja/või O_3 kontsentratsioonidega, tehti lehe gaasivahetuse mõõtmisi viie järjestikuse aasta (2004–2008) suvekuudel.

Uurimistöö tulemusena selgus, et kontrolltöötlusega võrreldes suurendasid kõrgendatud [CO_2] ja [CO_2+O_3] (kombineeritud töötlus) haava kloonide 42E ja 271 netofotosünteesi, kõrgendatud [O_3] põhjustas aga Pn-i vähenemise, võrreldуna kontrolltöötlusega. CO_2 efekt oli seejuures suurem osoonile tolerantsel kloonil (271) ja O_3 efekt osoonile tundlikul kloonil (42E). Õhulõhe juhtivus vähenes kõrgendatud [CO_2] ja [CO_2+O_3] korral, kuid ei muutunud kõrgendatud [O_3] tingimustes. Saadud tulemused on kooskõlas varem lehtpuude kohta avaldatud andmetega.

Põud ja kõrged temperatuurid muutsid CO_2 ja/või osooni poolt põhjustatud reaktsioonide ulatust. Kõrgendatud CO_2 mõju Pn-le ja g_s-le (nii eraldi kui

kombineeritult O_3-ga) suurenes stressi tingimustes. Põuaperiood ja kõrged temperatuurid vähendasid mõlemal haava kloonil osooni negatiivset toimet fotosünteesile.

Fotosünteesi päevaste käikude analüüs näitas, et pealelõunal vähenes Pn hommikusega võrreldes 25–33%, ja seda mitte valguse intensiivsuse muutumise tõttu. Pn vähenemise peamine põhjus oli õhulõhede juhtivuse vähenemine, mis oli omakorda tingitud suuremast veeauru rõhu defitsiidist (VPD_L). Pn vähenemine oli osaliselt põhjustatud ka CO_2 siduva ensüümi, Rubisco aktiivsuse (ja seda iseloomustava parameetri, Vc_{max}) vähenemisest pärastlõunal. Lehe veepotentsiaali muutused seletasid vaid väikese osa Pn-i päevastest muutustest.

Hindamaks, kas CO_2 ja/või O_3 kontsentratsioonide mõju ulatus hommikul ja õhtupoolikul erineb, võrreldi erinevates töötlustes sirgete tõuse, mis kirjeldasid Pn-i ja g_s-i langust päeva jooksul. Analüüsi tulemused näitasid, et kõrgendatud [CO_2] ja [O_3] avaldasid üksikult vaid minimaalset mõju. Kõrgendatud [CO_2+O_3] põhjustas aga kontrolltöötlusega võrreldes oluliselt kiirema Pn-i vähenemise, mis ei olnud seotud g_s-i kiirema vähenemisega, vaid pigem Vc_{max}-i ja Rubisco aktiivsuse vähenemisega õhtupoolikul.

Vegetatsiooniperioodi vältel tehtud mõõtmiste analüüs näitas, et kõrgendatud [CO_2] pikendas lehtede eluiga, kõrgendatud [O_3] aga, vastupidi, kiirendas lehtede sügisest vananemist. Üldiselt oli CO_2 ja/või O_3 mõju Pn-ile sügisel suhteliselt suurem kui suvel. Kuna valdav osa lehtede gaasivahetuse mõõtmisi viiakse läbi taimede kasvuperioodi keskel (suvel), siis CO_2 ja/või O_3 tegelik mõju Pn-le on pigem suurem, kui ainult suviste mõõtmiste põhjal otsustades.

Käesoleva töö tulemusena selgus, et CO_2 positiivne efekt Pn-le on võrreldes Aspen FACE´i eksperimendi algusaegadega (1998–1999) pigem kasvanud kui kahanenud. Kõrgendatud osooni mõju on aga jäänud 11 aasta jooksul praktiliselt samasuguseks.

Kokkuvõtvalt selgus doktoritööst, et ameerika haava Pn ja g_s reaktsioonid kõrgendatud CO_2 ja/või O_3 kontsentratsioonidele sarnanesid kvalitatiivselt teiste lehtpuude reaktsioonidega. Uudseks tulemuseks saadi, et tundlikkuses kõrgendatud CO_2 ja/või O_3 kontsentratsioonidele esinesid nii päevased, sesoonsed kui ka aastatevahelised erinevused. Samuti muutis neid tundlikkusi keskkonnastress (põud ja kõrged temperatuurid). Keskkonnastress leevendas osooni negatiivset mõju, aga suurendas CO_2 positiivset mõju Pn-ile. Kuna muutused netofotosünteesis ja õhulõhede juhtivuses mõjutavad nii süsiniku sidumist kui vee tarbimist taimede poolt, siis võivad need muutused avaldada märkimisväärset mõju kogu ökosüsteemi süsiniku- ja veeringele. Seetõttu tuleks käesolevas doktoritöös kirjeldatud interaktiivseid mõjusid võtta arvesse ökosüsteemide produktsiooni ja aineringet kirjeldavates mudelites.

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PUBLICATIONS

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Kets K, Darbah JNT, Sõber A, Riikonen J, Sõber J, Karnosky DF. 2010. Diurnal changes in photosynthetic parameters of *Populus tremuloides*, modulated by elevated concentrations of CO_2 and/or O_3 and daily climatic variation. *Environmental Pollution* 158: 1000–1007.

Riikonen J, **Kets K**, Darbah J, Oksanen E, Sõber A, Vapaavuori E, Kubiske ME, Nelson N, Karnosky DF. 2008. Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO_2 and O_3. *Tree Physiology* 28: 243–254.

Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Sharma P, Gioli B, Calfapietra C, Gielen B, Kubiske ME, Scarascia-Mugnozza GE, **Kets K**, Long SP, Karnosky DF. 2008. Future atmospheric, CO_2 leads to delayed autumnal senescence. *Global Change Biology* 14: 264–275.

Darbah JNT, Kubiske ME, Nelson N, **Kets K**, Riikonen J, Sõber A, Rouse L, Karnosky DF. 2010. Will photosynthetic capacity of aspen trees acclimate after long-term exposure to elevated CO_2 and O_3? *Environmental Pollution* 158: 983–991.

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- ETF9186 (Anu Sõber) Interactions between foliage formation, photosynthesis and wood production in deciduous canopy: experimental analysis of environmental impacts
- F11100PKTF Centre of Excellence in Environmental Adaptation (ENVIRON)
- SF0180025s12 (Anu Sõber) Multi-scale interactions between water, carbon and nitrogen fluxes in experimental and natural ecosystems: effects of climate change

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