Environmental Pollution 158 (2010) 1000–1007

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Diurnal changes in photosynthetic parameters of *Populus tremuloides*, modulated by elevated concentrations of CO_2 and/or O_3 and daily climatic variation

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Diurnal and seasonal patterns of environmental stress (drought, high air temperature) affects a relative impact of elevated concentrations of CO_2 and O_3 on trees.

ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form
24 August 2009
Accepted 1 September 2009

Keywords:
Photosynthesis
Stomatal conductance
Vapour-pressure difference
Environmental stress
CO_2/O_3

ABSTRACT

The diurnal changes in light-saturated photosynthesis (Pn) under elevated CO_2 and/or O_3 in relation to stomatal conductance (g_s), water potential, intercellular [CO_2], leaf temperature and vapour-pressure difference between leaf and air (VPD_L) were studied at the Aspen FACE site. Two aspen (*Populus tremuloides* Michx.) clones differing in their sensitivity to ozone were measured. The depression in Pn was found after 10:00 h. The midday decline in Pn corresponded with both decreased g_s and decreased Rubisco carboxylation efficiency, Vc_{max}. As a result of increasing VPD_L, g_s decreased. Elevated [CO_2] resulted in more pronounced midday decline in Pn compared to ambient concentrations. Moreover, this decline was more pronounced under combined treatment compared to elevated CO_2 treatment.

The positive impact of CO_2 on Pn was relatively more pronounced in days with environmental stress but relatively less pronounced during midday depression. The negative impact of ozone tended to decrease in both cases.

1. Introduction

The effects of elevated atmospheric [CO_2] on tree canopies are manifested by changes in photosynthesis. Studies of both deciduous and evergreen species have shown that elevated [CO_2] leads to increased photosynthesis (Dickson et al., 1998; Ellsworth et al., 2004; Noormets et al., 2001a; Long et al., 2004) and decreased stomatal conductance (Field et al., 1995; Wang et al., 2000; Noormets et al., 2001a; Gunderson et al., 2002; Ainsworth et al., 2003; Calfapietra et al., 2005). Photosynthesis is also affected by several other environmental changes, including increased concentrations of tropospheric O_3, a damaging phytotoxin inhibiting photosynthesis (Broadmeadow et al., 1998; Pell et al., 1994) and stomatal conductance (Noormets et al., 2001b; Sharma et al., 2003).

Intensity of photosynthesis is related to the amount of photosynthetic apparatus (often assessed by nitrogen, Rubisco and (or) chlorophyll) per leaf area (Evans, 1989; Niinemets and Tenhunen, 1997; Rosati et al., 2000), but also to the efficiency of photosynthetic apparatus (Rubisco activity).

Both the amount and efficiency of photosynthetic apparatus are strongly governed by environmental conditions. Trees grown under elevated [CO_2] generally have lower nitrogen concentrations (Moore et al., 1999; Lindroth et al., 2001; Long et al., 2004). Elevated [CO_2] increases Rubisco activity and decreases Rubisco concentration (Drake et al., 1997; Moore et al., 1999; Centritto and Jarvis, 1999; Eichelmann et al., 2004) and chlorophyll concentration (Centritto and Jarvis, 1999; Lütz et al., 2000; Eichelmann et al., 2004). Ozone decreases Rubisco activity (Oksanen and Saleem, 1999; Noormets et al., 2001b; Wustman et al., 2001; Yamaji et al., 2003). Ozone usually causes a reduction in chlorophyll content (Oksanen and Saleem, 1999; Wustman et al., 2001) and photochemical reaction in PSII (Lorenzini et al., 1999; Shavnin et al., 1999). However, when combined, elevated [CO_2] may partially ameliorate the negative effects in plants of elevated O_3 (Donnelly et al., 2000; Percy et al., 2002; Karnosky et al., 2003). It has been suggested that elevated levels of [CO_2] reduce stomatal conductance and ozone uptake, and might therefore reduce the potential for oxidant damage (Volin et al., 1998) despite reduced Rubisco content (Wustman et al., 2001; Noormets et al., 2001b), leaf nitrogen content (Karnosky et al., 2003) and carboxylation efficiency (Kull et al., 1996).

We can see that relative effects of [CO_2] and [O_3] on net photosynthesis and stomatal conductance are different in different

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doi:10.1016/j.envpol.2009.09.001

studies. To explain these differences, we propose that the degree of [CO_2] and ozone effects can be influenced by the degree of environmental stress during measurements. For example, responses of plant photosynthesis and growth to elevated concentrations of ozone were closely related to water stress (Pääkönen et al., 1998). Plants were more protected from ozone under drought conditions because stomata were more closed and ozone was unable to enter and damage mesophyll tissue. This can be true also in the case of high temperature and low humidity (high VPD_L), when stomatal conductance generally decreases (Sõber, 1980; Franks and Farquhar, 1999; Bunce, 2006). Under natural conditions, when many stress factors occur, interactions between these factors can influence the diurnal pattern of photosynthesis, but also patterns of different environmental effects (Singsaas et al., 2000).

Previous research on diurnal dynamics on different tree species have revealed afternoon declines in photosynthesis, even under unchanging light conditions (Singsaas et al., 2000; Špunda et al., 2005) when some other stress occurs. This afternoon decline is often attributed to stomatal closure and/or photoinhibitory damage (Muraoka et al., 2000), as well as VPD_L and light (Singsaas et al., 2000), subsequent decreases in intercellular CO_2, (Špunda et al., 2005) and Rubisco carboxylation efficiency (Singsaas et al., 2000). Elevated [CO_2] has no impact on afternoon decline in photosynthesis in understory saplings (Singsaas et al., 2000), but little is known of the impacts of elevated ozone, alone or in combination, with elevated [CO_2] on diurnal patterns of photosynthesis. The variability in relative effects of [CO_2] and [O_3] on photosynthetic parameters in published research is possibly a result of diurnal and seasonal patterns of environmental stress.

The aim of this study was to analyze diurnal patterns of photosynthesis and stomatal conductance on leaves of *P. tremuloides* trees (clones 42E and 271, differing in O_3 sensitivity), grown under elevated concentrations of CO_2 and/or O_3 in relation to water potential, intercellular CO_2, leaf temperature, VPD_L and photosynthetically active radiation.

It was hypothesized that diurnal and seasonal patterns of environmental stress (drought, high air temperature) would affect the relative impact of elevated concentrations of CO_2 and O_3 on trees and that elevated concentrations of CO_2 and O_3 would alter diurnal patterns of photosynthesis and stomatal conductance of aspen leaves.

2. Materials and methods

2.1. Experimental design and plant material

The 32-hectare Aspen FACE site was established in July 1997 near Rhinelander, Wisconsin (45.6°N, 89.5°W) on sandy loam soil. The project is a full factorial experiment consisting of three replicate 30 m diameter rings of four treatments: control (ambient CO_2, ambient O_3); elevated CO_2 (560 ppm); elevated O_3 (1.5 × ambient); elevated CO_2 and O_3. The rings are spaced 100 m apart to minimize drift of CO_2 and O_3. One-year-old plants of five trembling aspen (*Populus tremuloides* Michx.) genotypes of differing O_3 sensitivity were planted in July 1997 (Karnosky et al., 1999). We worked with aspen genotypes 42E and 271 as they exhibit different tolerance to ozone exposure; 42E is more sensitive than 271 (Isebrands et al., 2001; Karnosky et al., 2003). Aspen trees were up to 8 m tall (Kubiske et al., 2007) by the time of measurements in 2004 and 2005. The weather during both summers (2004 and 2005) was similar: warm (occasionally hot) with enough rain (one 1–2 weeks dry period in both years). Ambient air temperature, soil moisture content, wind speed and photosynthetic active radiation were logged every 30 min by the FACTS-II site control system. Relative humidity was ca 85% at sunrise and decreased in the afternoon. Minimum air temperature was 17 °C; maximum air temperature reached 38 °C in the afternoon. Maximum leaf temperature reached 39 °C.

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). Daytime CO_2 concentrations in elevated CO_2 treatments were about 525 ppm in 2004 and 527 ppm in 2005 and elevated [O_3] was about 43 ppb in 2004 and 53 ppb in 2005 (based on 8 h mean during June–August). No [O_3] fumigation was done on cool days (<15 °C) or during rain, fog, or dew events (Karnosky et al., 1999). More detailed information on the study site, plant material and the methodology of fumigation are available from the Aspen FACE website (http://aspenface.mtu.edu) and publications by Dickson et al. (2000), Karnosky et al. (2003) and Kubiske et al. (2006).

2.2. Gas exchange, chlorophyll and water potential measurements

Gas exchange measurements were made on sunny days from early June to the end of August, 2004 and 2005. Measurements were taken on three intact, sun-exposed leaves per clone (42E and 271) selected from the upper canopy. On sunny days photosynthetically active radiation (PAR) changed from 1500 to 2000 μmol m^{-2} s^{-1}, whereas net photosynthesis (Pn) saturated under 1000 μmol m^{-2} s^{-1}. We took measurements six times during the day from sunrise to sunset and data for one experimental ring was collected in one day. One round of measurements took 1–1.5 h. Photosynthetic measurements were made using portable open gas-exchange system (model LI-6400; LI-COR Inc., Lincoln, NE, USA) adjusting PAR to ambient level before each series of measurements. Leaf temperature and relative humidity were not measured. This system also measured leaf stomatal conductance (g_s), intercellular CO_2 (C_i), transpiration rate, vapour-pressure difference between leaf and air (VPD_L), leaf and air temperatures and relative humidity. Leaves were clamped in the standard 6 cm^2 cuvette, using growth CO_2 concentrations (360 ppm) for control and elevated O_3 treatments, and 560 ppm for elevated CO_2 and combination treatments. Leaf water potential (Ψ) measurements were made after each gas exchange measurements using a portable Scholander's type pressure chamber (Model 600, PMS Instruments, Corvallis, OR). For leaf water potential, we detached a leaf from the branch immediately after measuring gas exchange and put it in the pressure chamber. To test changes in carboxylation efficiency, photosynthetic CO_2 response (A-C_i) was measured on the same leaf, under saturating irradiance before midday (9 AM) and in late afternoon (3 PM). Leaf chlorophyll was measured during gas exchange measurements at 9:00–10:00 h using SPAD-meter 502 (Minolta Camera Co., Osaka, Japan). SPAD was converted into units of chlorophyll per leaf area (μmol m^{-2}) using calibration curves made for both clones (chlorophyll content of leaves with known SPAD was analyzed as described by Inskeep and Bloom, 1985).

2.3. Data analyses

To elucidate weather environmental stress affects, a relative impact of elevated CO_2 and O_3 treatment on trees we considered days with daily average g_s > 0.15 mol m^{-2} s^{-1} being environmentally unstressed (referred as unstressed trees) and days with daily average g_s < 0.15 mol m^{-2} s^{-1} being environmentally stressed (referred as stressed trees). To assess the main factors responsible for midday depression in photosynthesis, the relative changes of different parameters measured at 9:00 h and 16:30 h were calculated and correlated. Correlation coefficients (R^2) and P values were found using correlation analysis. Treatment effects on diurnal decline in photosynthesis were compared using General Linear Models (GLM). The overall mean comparison between treatments was calculated using Factorial Analysis of Variance (ANOVA). All data were checked for normality (Shapiro–Wilk test). In all cases, P values < 0.05 were considered significant. The maximum rate of Rubisco carboxylation (Vc_{max}) was calculated by fitting A-C_i curve data to the model described by Farquhar et al. (1980).

3. Results

3.1. Diurnal changes in microclimatic conditions and parameters of tree photosynthesis and water relations

The typical diurnal variation of main microclimatic factors such as incident photosynthetically active radiation (PARi), leaf temperature (T_L) and VPD_L in the growing season of 2005 are presented in Fig. 1. Microclimatic conditions on the days of gas exchange measurements changed within the typical range for summer months common to northern regions of USA. During daylight hours (09:00–17:00) PAR ranged from 1300 to 1700 μmol m^{-2} s^{-1} in clear sky conditions achieving 2000 μmol m^{-2} s^{-1} on some days. T_L increased during the day from a low at 09:00 h (17–28 °C) to a maximum (26–39 °C) between 15:30 and 17:00. VPD_L was 0.8–2.6 kPa at 09:00 h and maximized (1.3–4.8 kPa) between 15:30 and 17:00 h. The peak in VPD_L in the afternoon (15:30–17:00 h) was probably caused by higher air temperatures at that time.

Two sets of typical diurnal trends (for stressed and unstressed trees) of net photosynthesis, stomatal conductance and leaf water potential under all treatments are presented on Fig. 2. Absolute values of all parameters changed over a wide range with climatic conditions but the diurnal patterns were qualitatively similar on all days and for both clones. Diurnal photosynthesis typically

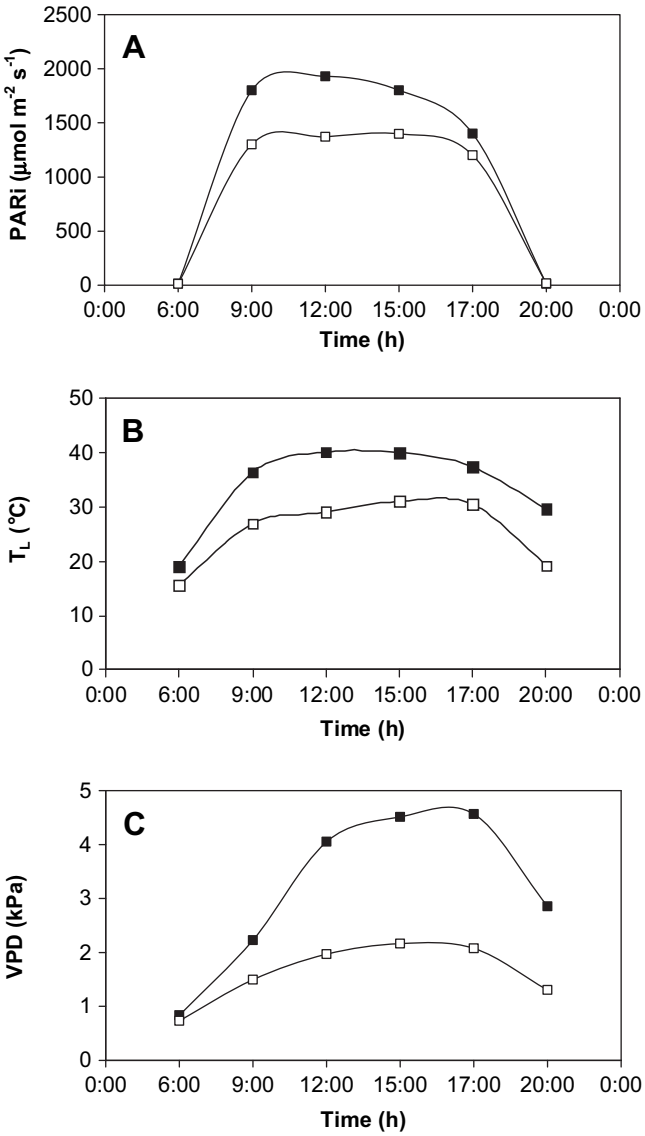

Fig. 1. Minimum and maximum values of typical diurnal trends of the incident photosynthetically active radiation, PARi (A), leaf temperature, T_L (B) and vapour-pressure difference between leaf and air, VPD_L (C), measured at the Aspen FACE site in the growing season of 2005. Data points were collected six times a day during the gas exchange measurements. Trends represent measurements made in control rings on different days.

maximized between 9:00 and 10:00 h and decreased thereafter (Fig. 2A,B). In some cases, there was a slight recovery in Pn between 14:00 and 15:00 h. The lowest values of light-saturated Pn were observed between 15:00 and 17:00 h. Diurnal variation in stomatal conductance followed the trend of Pn reaching its maximum at 9:00–10:00 h and decreasing thereafter (Fig. 2C,D). Leaf water potential dropped in the morning hours, being quite constant during the day in unstressed trees, but declined in stressed trees (Fig. 2E,F). Pn and stomatal conductance of stressed and unstressed plants did not differ significantly in the morning hours, but in stressed plants the daytime values were much lower (right column on Fig. 2).

3.2. Overview of gas exchange and chlorophyll measurements under elevated [CO_2] and/or [O_3](9:00–10:00)

The mean values of Pn, g_s and chlorophyll per leaf are (Chl) are presented in Fig. 3. Generally, trees grown in elevated [CO_2] exhibited about 33 and 46% higher Pn in clones 42E and 271 ($P < 0.001$), respectively, and significantly ($P < 0.001$) lower stomatal conductance (21% in clone 42E and 16% in clone 271) compared to the control treatment.

The response of photosynthesis to elevated [O_3] varied among clones and reflected their tolerance to ozone. Therefore, significantly lower Pn (21%, $P = 0.03$) was measured under elevated O_3 concentration in clone 42E. In clone 271 Pn was similar to the control values. Furthermore, there was no significant ozone effect on stomatal conductance in clone 271, but g_s decreased significantly (about 12%, $P = 0.03$) in ozone sensitive clone 42E. The decrease in Chl (14%, $P < 0.001$) was observed under elevated ozone treatment in both clones.

Under combined treatment, Pn enhanced ~32% in clone 42E and ~50% in clone 271 ($P < 0.05$), whereas stomatal conductance decreased about 29% in clone 42E and 16% in clone 271. Chl decreased significantly ($P = 0.04$) in both clones in combined treatment, although not as much as under O_3 treatment.

3.3. Relative differences between treatments of net photosynthesis, stomatal conductance and amount of absorbed CO_2 in stressed and unstressed trees

We compared the difference 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 no environmental stress (daily average $g_s > 0.15$ mol m^{-2} s^{-1}) and in days with environmental stress (daily average $g_s < 0.15$ mol m^{-2} s^{-1}), Fig. 4A–D. In stressed trees we observed 69–71% higher Pn in elevated [CO_2] and 84–93% higher Pn in elevated [$CO_2 + O_3$] than in control. These increases were much greater compared to unstressed trees, in which Pn increased only 32–40% under elevated [CO_2] and 26–43% under elevated [$CO_2 + O_3$] (Fig. 4A,B). Difference in CO_2 response between stressed and unstressed trees was even more striking for Pn_{sum} (Fig. 4A,B). The increase in Pn in unstressed trees under elevated [CO_2] and [$CO_2 + O_3$] was greater than that in Pn_{sum}.

In contrast, we observed a positive effect of elevated [O_3] on Pn and Pn_{sum} in stressed trees, but a typical negative effect in unstressed trees (Fig. 4C,D). The g_s decreased much more under elevated [CO_2] and [$CO_2 + O_3$] in stressed trees compared to unstressed trees.

3.4. The main physiological factors responsible for the midday suppression in photosynthesis

To assess the main factors responsible for midday depression in photosynthesis, we calculated the relative changes in different parameters (denoted as $\Delta Pn/Pn$, $\Delta g_s/g_s$, $\Delta\Psi/\Psi$, $\Delta VPD_L/VPD_L$, $\Delta C_i/C_i$, $\Delta T_L/T_L$, $\Delta PARi/PARi$), between 9:00 h and 16:30 h. These parameters were correlated to determine the main reason(s) for diurnal declines in Pn. Correlation coefficients and P values between the parameters are presented in Table 1. Clearly, the diurnal drop in light-saturated photosynthesis was not limited by photosynthetically active radiation as the correlation coefficient (R^2) between $\Delta Pn/Pn$ and $\Delta PARi/PARi$ is not significant ($R^2 = 0.03$, $P = 0.821$). However, an important factor responsible for the decline in Pn seemed to be reduced stomatal conductance (as a result of high VPD_L) as it followed the pattern of light-saturated Pn. We found a significant correlation ($R^2 = 0.54$, $P < 0.001$) between $\Delta Pn/Pn$ and $\Delta g_s/g_s$. In turn, reduced stomatal conductance accompanied decreases in C_i ($P < 0.001$); R^2 between $\Delta g_s/g_s$ and $\Delta C_i/C_i$ was 0.62 but changes in C_i did not correlate with changes in photosynthesis ($R^2 = 0.02$, $P = 0.358$). Rather, C_i was a result of simultaneously declined Pn and g_s (R^2 between the ratio of $\Delta g_s/g_s$ to $\Delta Pn/Pn$ and $\Delta C_i/C_i$ was 0.7, $P < 0.001$). To estimate the role of Rubisco activity,

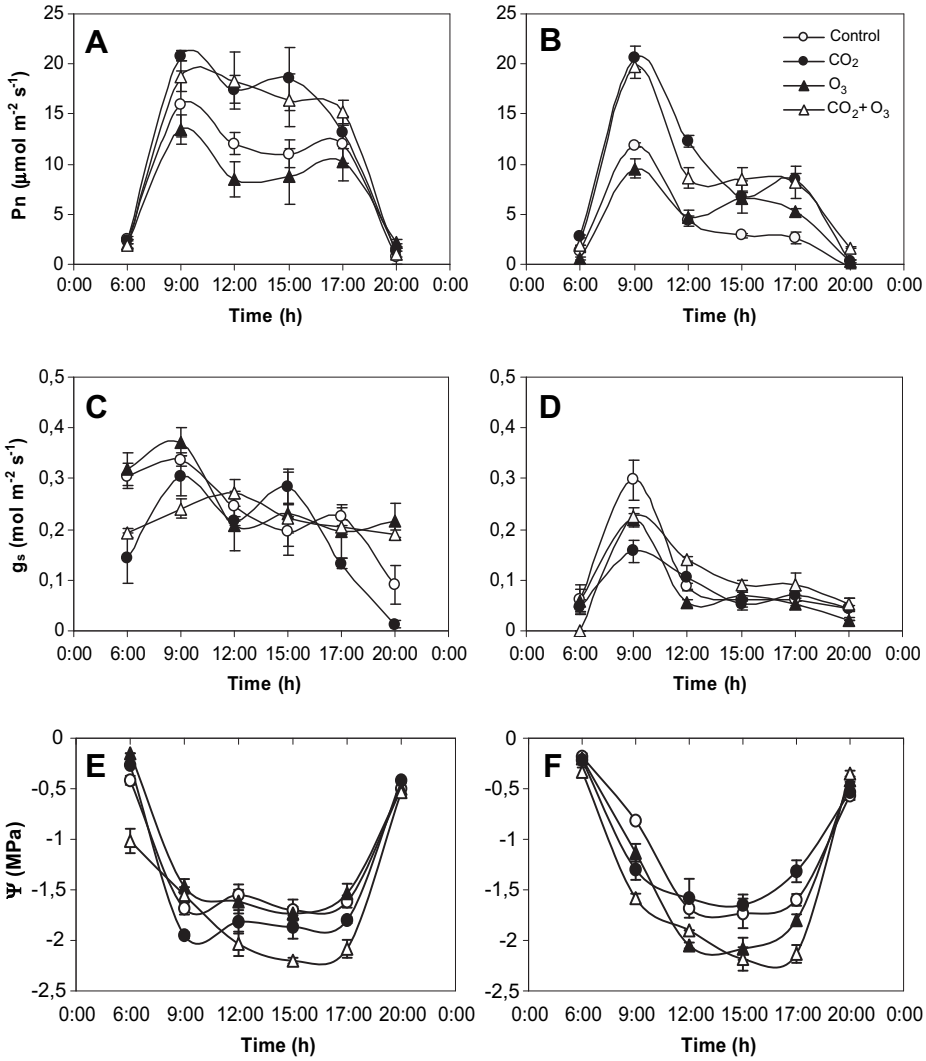

Fig. 2. One (example) set of measured diurnal courses of Pn, g_s and Ψ in days with no environmental stress (daily average $g_s > 0.15$ mol m^{-2} s^{-1}) and in days with environmental stress (daily average $g_s < 0.15$ mol m^{-2} s^{-1}) for clone 42E. Measurements were made six times a day throughout the growing seasons of 2004 and 2005. Data points are means ± SE. A – Pn, no environmental stress. B – Pn, environmental stress. C – g_s, no environmental stress. D – g_s, environmental stress. E – Ψ, no environmental stress. F – Ψ, environmental stress.

we calculated Vc_{max}, using A-C_i curves. Vc_{max} was often less in the afternoon (Fig. 5).

3.5. Treatment effects to diurnal decline of light-saturated photosynthesis and stomatal conductance

According to differences of treatment effects in stressed and unstressed plants (Fig. 4), we propose that declines in Pn should be more pronounced in CO_2 treatment and less pronounced in O_3 treatment than in the control treatment. No significant treatment effect was found for diurnal data (Pn and g_s) in days with environmental stress. It may be that treatment effects on diurnal curves of stressed plants were not found significant due to low values of Pn and g_s (values in afternoon were close to measurement errors), and (or) due to limited number of measurements (there were two times less days with environmental stress than normal days). However, in unstressed trees, a significant impact ($P < 0.001$) of combined treatment on diurnal decline of Pn was found. The decline was more rapid in combined treatment and more pronounced in clone 42E compared to clone 271 (Fig. 6A,B). We also found a slight but significant impact ($P = 0.02$) of CO_2 treatment to diurnal decline. In clone 42E, stomata closed significantly slower under elevated O_3 ($P < 0.001$, Fig. 6C), in contrast to clone 271 (Fig. 6D), which exhibited the slowest decrease in g_s under combined treatment ($P < 0.001$).

4. Discussion

4.1. Daily trends of light-saturated net photosynthesis in changing environmental conditions

Our measurements showed a significant diurnal decline (25–33%) in light-saturated photosynthesis. Environmental factors changing concurrently were temperature, light, water potential and VPD_L. Light-saturated Pn of sweetgum (*Liquidambar styraciflua*), eastern redbud (*Cersis Canadensis*) and red maple (*Acer rubrum*) can decrease as much as 40–60% during the day as a result of varying environmental factors (Singsaas et al., 2000). However, Singsaas et al. (2000) found that Pn peaked at 12:00 h and then decreased; maximum Pn in our study occurred at 9:00–10:00 h. Singsaas suggested that one signal responsible for reduction in Pn is VPD_L (Singsaas et al., 2000) as VPD_L usually affects stomatal conductance

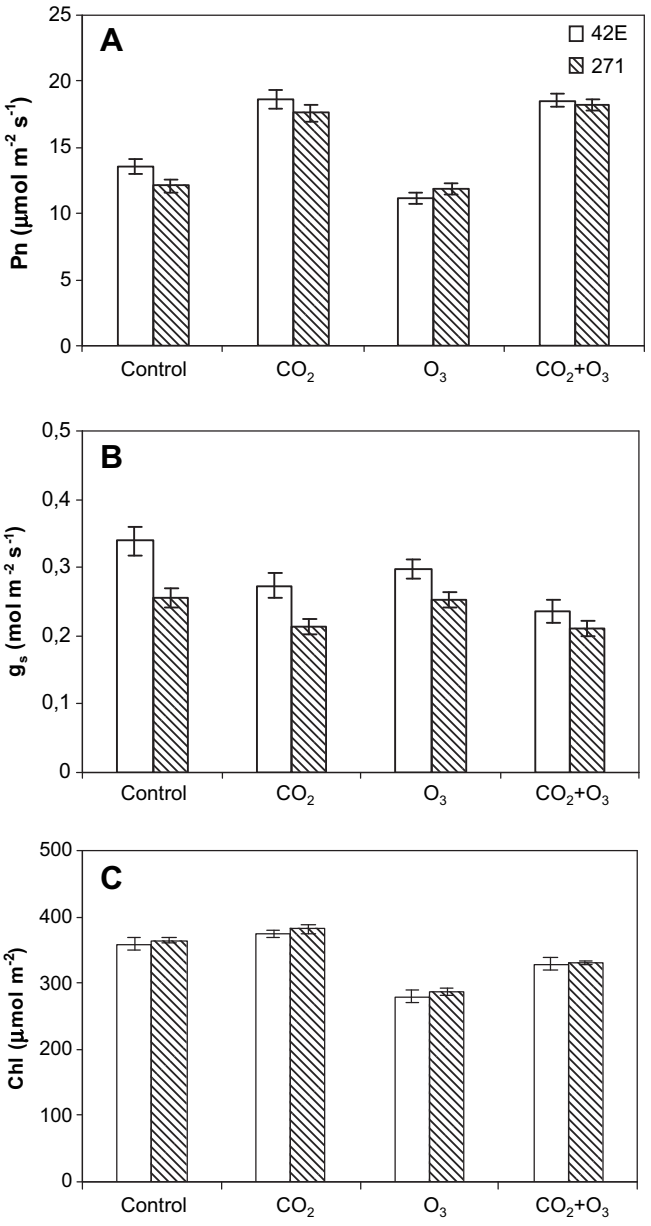

Fig. 3. Light-saturated photosynthesis, Pn (A), stomatal conductance, g_s (B) and chlorophyll content per leaf area, Chl (C) in aspen clones 42E and 271 exposed to elevated [CO_2] and/or [O_3]. Measurements were done in June, July and August, 2005, between 9:00 and 10:00 h. Data shown are means ± SE ($n = 27$, ANOVA).

(Sõber, 1980; Bunce, 1996; Franks and Farquhar, 1999). VPD_L was really on its daily minimum at 9:00–10:00 h in our study and a strong correlation between $\Delta VPD_L/VPD_L$ and $\Delta g_s/g_s$ ($R^2 = 0.63$, $P < 0.001$) was found. Consequently, afternoon decline in stomatal conductance seemed to be caused by higher VPD_L in the afternoon. Similar results have been reported previously with cotton (Yong et al., 1997) and spruce trees (Špunda et al., 2005). Our work revealed a significant positive correlation between $\Delta VPD_L/VPD_L$ and $\Delta T_L/T_L$, but R^2 for correlation of $\Delta g_s/g_s$ and $\Delta T_L/T_L$ was low, showing that increased transpiration, not the direct effect of temperature, was causing the stomatal closure.

In contrast to Singsaas et al. (2001), a slight but significant correlation between ΔPn/Pn and $\Delta\Psi/\Psi$ was also found. Daytime changes in Ψ were small – probably Ψ was up regulated by stomatal closure after its initial drop.

As the ratio of $\Delta g_s/g_s$ and ΔPn/Pn correlated with $\Delta C_i/C_i$ ($R^2 = 0.7$) better than $\Delta g_s/g_s$ and ΔPn/Pn singly, it was likely that g_s and Pn had combined influence on C_i. Possibly decreased stomatal conductance reduced C_i whereas simultaneous decrease in RuBP carboxylation caused increase in C_i. The changes in both g_s and Vc_{max} together probably caused significant midday decline in net photosynthesis but only relatively small change in C_i.

4.2. Effects of elevated [CO_2] and/or [O_3] on photosynthesis, stomatal conductance and chlorophyll content, compared to previous studies at same place

Enhancement in net photosynthesis was found under elevated CO_2 treatment (33–46%), as in many studies (Nowak et al., 2004; Ainsworth and Long, 2005; Calfapietra et al., 2008; Riikonen et al., 2008). Surprisingly, we observed a small but significant ($P < 0.05$) increase in Chl in both clones under elevated concentration of CO_2 in contrast to previous studies (Mulholland et al., 1997; Lütz et al., 2000; Eichelmann et al., 2004). It remains speculative whether increase in photosynthesis would continue for several growing seasons in the field conditions. Previous Aspen FACE studies have reported 25–36% increases in Pn (Noormets et al., 2001a; Takeuchi et al., 2001; Sharma et al., 2003; Ellsworth et al., 2004); therefore, the effect in Pn has rather been increasing in time than decreasing. This may be caused by a slight but significant increase in leaf chlorophyll content per leaf area, which is rather positive acclimation in photosynthetic apparatus than negative acclimation (Centritto and Jarvis, 1999; Lütz et al., 2000; Eichelmann et al., 2004). Another reason for increased CO_2 effect may be smaller reduction in g_s than found in younger trees (Sharma et al., 2003).

Under elevated O_3 treatment, a decline in Pn occurred in clone 42E but not in clone 271. This confirms the relative tolerance of 271 to ozone (Karnosky et al., 2003). However, it is well known that Chl in aspen leaves exposed to elevated ozone is subject to degradation (Farage, 1996; Oksanen and Saleem, 1999). We found a reduction in Chl under elevated ozone in both clones, as in earlier studies at Aspen FACE (Wustman et al., 2001). Reduction in Chl accounts for the decrease in Pn in clone 42E. However, as we observed no significant decrease in Pn in clone 271, it is likely that chlorophyll content is not limiting Pn in this clone. Stomata were more open in clone 42E than in clone 271, and this may determine differences in ozone tolerance of clones. We observed a significant decrease in g_s in clone 42E but not in clone 271. Despite of decreased g_s, stomata remained more open under ozone in clone 42E than in clone 271 (Fig. 3).

Previous studies of aspen have shown that the effect of combined treatment on Pn is either similar to control values (Sharma et al., 2003; Karnosky, 2003) or results in a small increase (Noormets et al., 2001a). Pn under combined treatment in this study was similar to Pn under elevated CO_2 treatment. Because stomata were closed compared to other treatments, ozone influx and its effect was probably smaller than in earlier studies. In addition, we observed only a slight decrease in leaf chlorophyll in contrast to Sharma et al. (2003).

4.3. Relative effects of elevated CO_2 and O_3 on photosynthesis and stomata are changing under drought and high-temperature stress

Days with environmental stress revealed much higher relative increments of Pn and its daily sum (Pn_{sum}) under elevated [CO_2] alone and combined with [O_3]. The relative difference from control is often used to show impact of elevated CO_2. Relative change is constant only when Pn depends exponentially on [CO_2]. This is not the case, because the shape of A-C_i curve is far from exponential and the relative change in Pn is much higher when C_i is lower due to lower g_s. Consequently, relative change in Pn must be different,

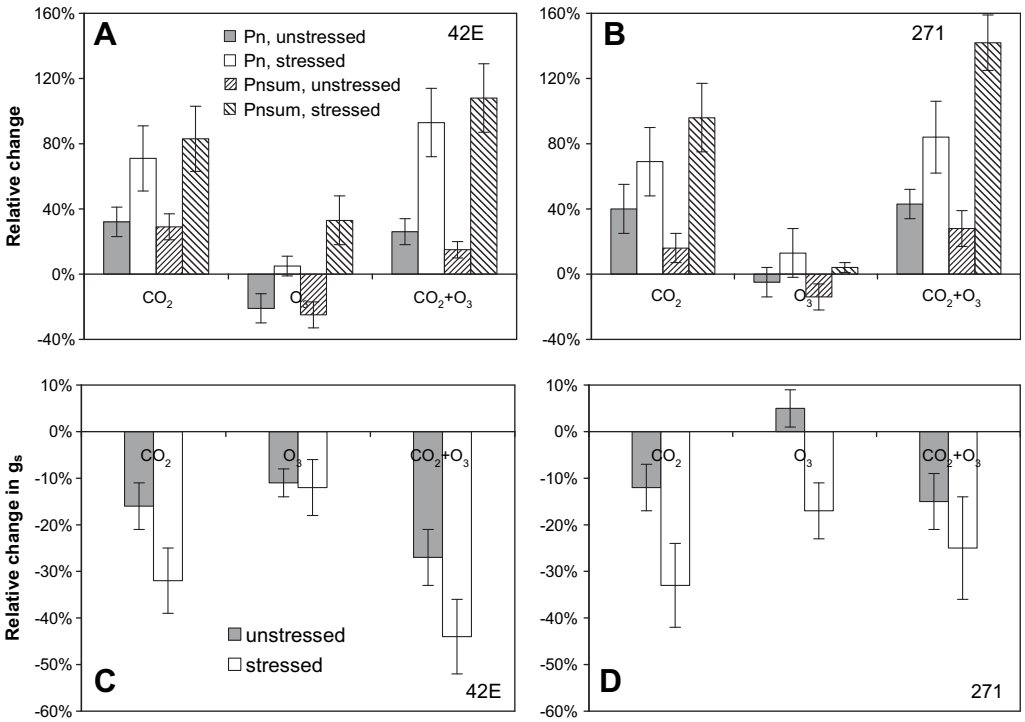

Fig. 4. Relative difference of light-saturated photosynthesis (Pn), absorbed CO_2 (Pn_{sum}) and stomatal conductance (g_s) measured under elevated [CO_2] and/or [O_3] from control treatment in aspen clones 42E and 271. Percentages were calculated from measurements done in June, July and August 2005. Pn and g_s were measured between 9:00 and 10:00 h, Pn_{sum} was calculated as summary CO_2 uptake between 9:00 and 17:00. Plants measured in 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 plants. 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 plants. D – relative difference of g_s in clone 271 in stressed and in unstressed plants.

when C_i is different and we cannot compare relative increments when initial C_i differs significantly. We can get variable relative increments dependent on number of days with environmental stress. It is complicated to analyze relative differences in Pn when A-C_i curve and Vc_{max} are also changing.

The direction of ozone effect on Pn changes from negative to positive in stressed trees (Fig. 4A,B). This demonstrates that stress protects trees against ozone via more closed stomata and via increased sensitivity to ozone (Fig. 4C,D).

4.4. Effects of elevated CO_2 and O_3 concentrations on diurnal decline of Pn and g_s

We found no significant treatment effects when comparing data measured in stressed trees. Nonetheless, we found more pronounced diurnal drop in Pn under elevated CO_2 and $CO_2 + O_3$ treatments than in control treatment in unstressed trees. The effect was even more evident under combined treatment (Fig. 6), especially in clone 42E. The lower increments in Pn_{sum} than in Pn (Fig. 4) are in agreement with more significant decline of Pn under elevated CO_2. For example, under combined treatment and in clone 42E, increments of Pn were 26% compared to increments in Pn_{sum}, which were only 15%. Significant diurnal decrease in Pn under elevated $CO_2 + O_3$ was unrelated to decreased stomatal conductance as stomata showed the least decrease under this treatment (Fig. 6C,D). We assume that the rapid reduction in Pn was related to decreased Vc_{max} and Rubisco activity. This is also because of stress, but relative difference of Pn from control did not increase (as in Fig. 4), but decreased in afternoon. It is possible that afternoon drop of Pn is not a situation of Pn – affecting stress, but

Table 1
Correlation coefficients (R^2) and P values between $\Delta Pn/Pn$, $\Delta g_s/g_s$, $\Delta\Psi/\Psi$, $\Delta VPD_L/VPD_L$, $\Delta C_i/C_i$, $\Delta T_L/T_L$ and $\Delta PAR/PAR$. The data represent all treatments and both clones (42E and 271).

Parameter	R^2	P
$\Delta Pn/Pn$ and $\Delta g_s/g_s$	0.54	**<0.001**
$\Delta VPD_L/VPD_L$ and $\Delta Pn/Pn$	0.03	0.808
$\Delta VPD_L/VPD_L$ and $\Delta g_s/g_s$	0.63	**<0.001**
$\Delta C_i/C_i$ and $\Delta Pn/Pn$	0.02	0.358
$\Delta C_i/C_i$ and $\Delta g_s/g_s$	0.62	**<0.001**
$[(\Delta g_s/g_s)/(\Delta Pn/Pn)]/(\Delta C_i/C_i)$	0.7	**<0.001**
$\Delta T_L/T_L$ and $\Delta Pn/Pn$	0.006	0.489
$\Delta T_L/T_L$ and $\Delta g_s/g_s$	0.2	**<0.001**
$\Delta\Psi/\Psi$ and $\Delta Pn/Pn$	0.1	**0.036**
$\Delta\Psi/\Psi$ and $\Delta g_s/g_s$	0.02	0.12
ΔVPD_L and $\Delta T_L/T_L$	0.7	**<0.001**
$\Delta PAR/PAR$ and $\Delta Pn/Pn$	0.03	0.821
$\Delta PAR/PAR$ and $\Delta g_s/g_s$	0.02	0.439

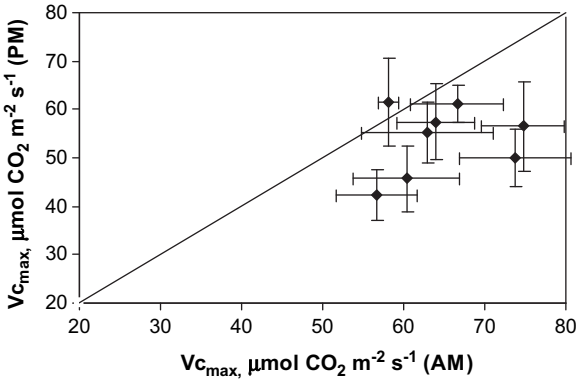

Fig. 5. Maximum carboxylation efficiency (Vc_{max}) in AM hours and in PM hours estimated from CO_2-response (A-C_i) curves.

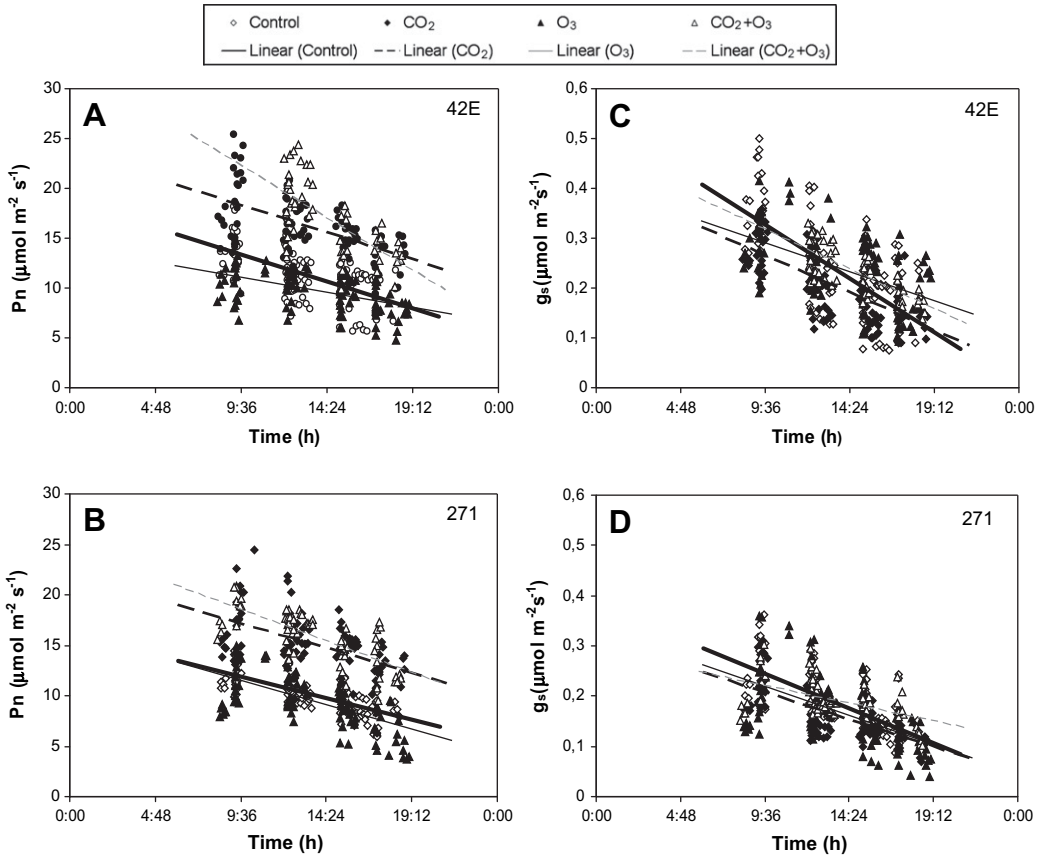

Fig. 6. Light-saturated Pn (A, B) and g_s (C, D) measured from 9:00 h to 17:00 h under elevated [CO_2], [O_3] and [$CO_2 + O_3$] in clones 42E (A, C) and 271 (B, D). Data points represent diurnal measurements from unstressed trees made in the growing season of 2004 and 2005. Linear trends show the decline in Pn and in g_s for each treatment. Trends were analyzed using General Linear Model (GLM).

down-regulation of Pn and g_s in conditions of reduced growth and accumulation of unused photosynthetic assimilates in leaves. This hypothesis is in agreement with slow afternoon drop of Pn in clone 42E under elevated [O_3] in conditions when Pn was low and when less assimilates were accumulated.

5. Conclusions

In conclusion, this work supports previous findings about effects of elevated CO_2 and O_3 concentrations on photosynthetic parameters of tree leaves. Daily courses of photosynthesis (measured during 2 vegetation periods) showed additionally that long-term effects of both CO_2 and O_3 can be less, than effects, measured in short-term experiments. Drought and high-temperature stress increased relative impact of CO_2 and decreased relative impact of O_3, predicting CO_2 effects to prevail over O_3 effects in the case of combined action of both gases in stress conditions.

Acknowledgements

This study was supported by the U.S. Department of Energy's Office of Biological and Environmental Research (Grant No. DE-FG02-95ER62125), the USDA Forest Service Northern Global Change Program, Michigan Technological University, and the Estonian Science Foundation (Grant No. 6969).

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