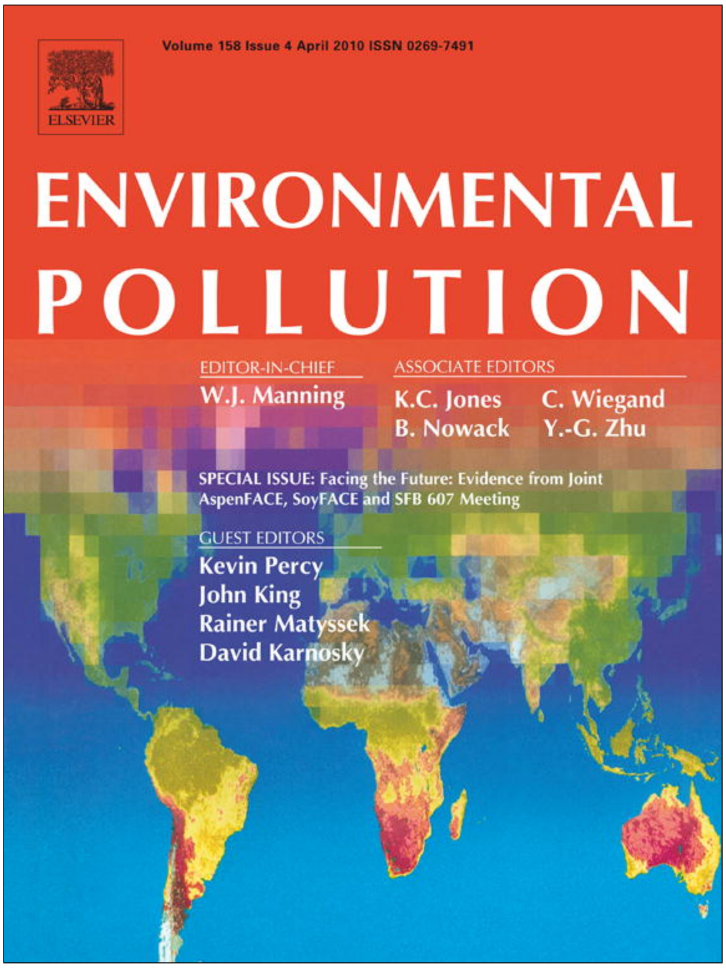

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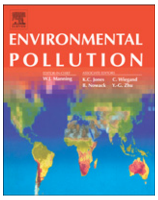

Will photosynthetic capacity of aspen trees acclimate after long-term exposure to elevated CO_2 and O_3?

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We report of no evidence of photosynthetic and stomatal acclimation in aspen trees grown under elevated CO_2 and O_3 after over a decade of exposure.

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ABSTRACT

Photosynthetic acclimation under elevated carbon dioxide (CO_2) and/or ozone (O_3) has been the topic of discussion in many papers recently. We examined whether or not aspen plants grown under elevated CO_2 and/or O_3 will acclimate after 11 years of exposure at the Aspen Face site in Rhinelander, WI, USA. We studied diurnal patterns of instantaneous photosynthetic measurements as well as A/C_i measurements monthly during the 2004–2008 growing seasons. Our results suggest that the responses of two aspen clones differing in O_3 sensitivity showed no evidence of photosynthetic and stomatal acclimation under either elevated CO_2, O_3 or $CO_2 + O_3$. Both clones 42E and 271 did not show photosynthetic nor stomatal acclimation under elevated CO_2 and O_3 after a decade of exposure. We found that the degree of increase or decrease in the photosynthesis and stomatal conductance varied significantly from day to day and from one season to another.

1. Introduction

Levels of atmospheric CO_2 and O_3 are increasing rapidly (IPCC, 2007). These two gases affect plants physiologically in diametrically opposite ways. There have been many discussions as to whether or not plants will acclimate to long-term exposure to elevated CO_2 and/or O_3.

Studies have documented the impacts of elevated CO_2 on tree gas exchange variables such as increases in maximum instantaneous photosynthesis (A_{max}) (Will and Ceulemans, 1997; Tissue et al., 1997, 1999; Bernacchi et al., 2005, 2006), decrease in stomatal conductance (g_s) (Medlyn et al., 2001; Bernacchi et al., 2006; Leakey et al., 2006; Ainsworth and Rogers, 2007; Paoletti et al., 2007), increased maximum carboxylation capacity (V_{cmax}) (Ainsworth et al., 2003a,b; Bernacchi et al., 2005), and increased maximum electron transport (J_{max}) (Ainsworth et al., 2003a; Rogers et al., 2004). Other studies have reported decreases in V_{cmax} and J_{max} under elevated CO_2 as reported in the review by Ainsworth and Long (2005).

Some researchers have reported photosynthetic and/or stomatal acclimation (down-regulation) of A_{max} enhancement under long-term CO_2 exposure (Moore et al., 1999; Tissue et al., 1999; Griffin et al., 2000; Rogers et al., 2004; Bernacchi et al., 2005), whereas others report no down-regulation or acclimation of photosynthesis and/or stomatal conductance (Nijs et al., 1997; Medlyn et al., 2001; Ainsworth et al., 2003b; Leakey et al., 2006; Darbah, 2007; Ainsworth and Rogers, 2007; Paoletti et al., 2007). Hence, it is not clear as to whether or not there is down-regulation of A_{max} enhancement after long-term exposure to elevated CO_2.

Some researchers report that down-regulation of photosynthesis under elevated CO_2 is strongly linked to an increased carbon:nitrogen ratio of the photosynthesizing leaves, when the increased uptake of CO_2 cannot be matched by a sufficient nutrient supply (Liberloo et al., 2007).

Wand et al. (1999), Wullschleger et al. (2002), Long and Bernacchi (2003) and Ainsworth and Long (2005) have reported a decrease in g_s after long-tern exposure to elevated CO_2. According

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to Ainsworth and Rogers (2007), across all plant species, elevated CO_2 decreases g_s by 22%, but they did not find any significant long-term change in this decrease in g_s in trees under elevated CO_2 in contrast to the report of Saxe et al. (1998). Also, Medlyn et al. (2001) found no stomatal acclimation in six tree species they investigated and Nijs et al. (1997) found no independent stomatal acclimation under elevated CO_2 either. Hence, the literature is still somewhat mixed as to whether or not stomatal acclimation occurs under long-term exposure to elevated CO_2.

In their review of the effects of greenhouse gases on gas exchange, Eamus and Ceulemans (2001) noted that there is no reason to expect down-regulation of A_{max} (photosynthetic acclimation) following long-term exposure to CO_2 enrichment when root volume is not restricted based on the work done by Curtis (1996), but when root volume is limited, downward acclimation is observed (Gunderson and Wullschleger, 1994; Sage, 1994; Will and Teskey, 1997).

Here, we define photosynthetic acclimation as a significant decrease in the stimulation of A_{max}, V_{cmax} and J_{max} relative to ambient CO_2 concentrations (control) as used by Ainsworth et al. (2003a), Rogers et al. (2004), Bernacchi et al. (2005), Leakey et al. (2006) and Darbah (2007). We also define stomatal acclimation as the significant decrease in stomatal conductance relative to control as used by Leakey et al. (2006). Photosynthetic acclimation under elevated O_3 will be defined as the up-regulation of A_{max} after long-term exposure of plants to elevated O_3 levels.

Less information is available on the effects of elevated O_3 on these gas exchange variables. Elevated O_3 is reported to decrease maximum instantaneous photosynthesis (Volin and Reich, 1996, 1998; Tjoelker et al., 1998; Noormets et al., 2001), alter stomatal conductance (Noormets et al., 2001; Paoletti, 2005; Paoletti et al., 2007), decrease maximum carboxylation capacity, and maximum electron transport (Eichelmann et al., 2004). Structural acclimation to elevated O_3 has been reported in birch trees grown in open-top chamber experiment (Günthard-Goerg et al., 1993; Paakkonen et al., 1995) and in container grown beech and spruce trees (Luedemann et al., 2005).

Few studies have considered the interacting effect of both elevated CO_2 and O_3 gases on plant gas exchange variables such as photosynthesis (Kull et al., 1996; Kellomäki and Wang, 1997, 1998; Noormets et al., 2001; Sharma et al., 2003; Eichelmann et al., 2004) and stomatal conductance (Volin and Reich, 1996; Volin et al., 1998; Noormets et al., 2001; Sharma et al., 2003). It has been reported that elevated CO_2 ameliorates the negative effects of elevated O_3 on photosynthesis (Volin et al., 1998). However, this does not occur in all situations (Kull et al., 1996; Wustman et al., 2001).

In this paper, we sought to examine if photosynthetic and stomatal acclimation occurred following long-term exposure to elevated CO_2 and/or O_3 for trembling aspen (*Populus tremuloides* Michx.) growing at the Aspen FACE experiment.

The objectives of this study were to: (1) determine whether or not photosynthetic and/or stomatal acclimation is occurring in 11-year-old aspen trees grown under elevated CO_2, O_3, and $CO_2 + O_3$ and (2) evaluate clonal differences in aspen with respect to photosynthetic and stomatal acclimation.

2. Materials and methods

2.1. Study site and planting material

This experiment was conducted at the Aspen FACE site in Wisconsin, USA, which was established in 1997 as the first open-air facility to examine the responses of forest trees to interacting CO_2 and O_3 (Dickson et al., 2000). The Aspen FACE facility is located at the United States Department of Agriculture (USDA) Forest Service, Northern Research Station Harshaw Experimental Farm near Rhinelander, WI, USA (45.6°N, 89.5°W) (Karnosky et al., 1999, 2003). The experimental site consists of four treatments each of control (ambient air), elevated CO_2 (target of 560 ppm), elevated O_3 (1.5 times ambient) and elevated CO_2 plus elevated O_3 conditions in triplicate rings of 30-meter diameter each; details of this can be found in Karnosky et al. (2005).

Planting material of the Aspen FACE project was chosen to represent northern hardwood forests. Aspen, maple and birch are the dominant species naturally growing in northern hardwood forests. Seed sources were from local northern Michigan sources and 3 aspen clones (including 271) were selected on the basis of sensitivity to O_3 (Karnosky et al., 1996). Seedlings were planted in 1 m × 1 m spacing. These rings were fumigated from 1998 onwards and average concentrations of the CO_2 and O_3 over the experiment are detailed in (Karnosky et al., 2005).

2.2. Measurements of gas exchange and environmental variables

Nine leaves per treatment (3 leaves per plot) were selected from fully expanded short shoot leaves from the upper canopy of trees (sun leaves) for aspen clones 42E and 271. Aspen clones 42E (O_3 sensitive) and 271 (O_3 tolerant) were selected because of their sensitivities to O_3 levels (Isebrands et al., 2001; Karnosky et al., 2003). Measurements were taken while leaves were still attached to the plants. Instantaneous photosynthetic rate and internal CO_2 partial pressure (A/C_i) response curves were measured with the leaf placed in a 6 cm^2 chamber of an infrared gas analyzer (IRGA) system equipped with CO_2 control modules and LED light sources (Li-Cor photosynthetic system, model Li 6400 version 5.02 from Li-Cor, Inc. Lincoln, Nebraska, USA).

A/C_i curves were measured at 9 am and also at 3 pm to allow us to infer changes in the underlying photosynthetic capacity of leaves at these two maximum points on the bimodal daily diurnal curve. For each A/C_i curve, the procedure described by Long and Bernacchi (2003) was followed by allowing each leaf to attain a steady-state CO_2 and water vapor exchange at the growth CO_2 concentration (360 $\mu mol\ mol^{-1}$ or 560 $\mu mol\ mol^{-1}$), and then CO_2 concentration was decreased stepwise to 50 $\mu mol\ mol^{-1}$. The CO_2 concentration was then set again to the growth concentration and increased stepwise to 1800 $\mu mol\ mol^{-1}$ all at a saturating photosynthetic active radiation of 1200 $\mu mol\ m^{-2}\ s^{-1}$ for aspen. Photosynthetic response curves (A/C_i) were analyzed by computing the V_{cmax} (maximum carboxylation rate of rubisco), J_{max} (RuBP regeneration capacity mediated by maximum electron transport rate) using the model described by Farquhar et al. (1980).

Instantaneous photosynthetic rate measurements were made from pre-dawn to dusk (measured from 5 h before sunrise, 9 am, 12 pm, 3 pm, 5 pm and 8:30 pm after sunset) on photosynthetic characteristics including A_{max}, vapor pressure gradient (VPG), stomatal conductance, photosynthetic photon flux density, intercellular CO_2 concentration (C_i), leaf temperature (Tlf), transpiration rate, relative humidity, etc. measurements were taken throughout the growing seasons of 2004–2008. Target growth concentrations used was 360 ppm and 560 ppm for ambient and elevated CO_2 respectively and ambient O_3 levels were 45 ppb and elevated levels were 1.5 times the ambient.

Reciprocal light saturated instantaneous photosynthetic rate measurements were taken between 8:30 am and 11:30 am during the peak photosynthetic period (period of maximum photosynthetic activity) to test for evidence of photosynthetic acclimation in the summer of 2007 and 2008. Reciprocal photosynthetic measurement were taken by taking instantaneous photosynthetic measurements on trees grown under ambient CO_2 concentration at 360 ppm followed by a second measurement at CO_2 concentration at 560 ppm on the same leaves. Photosynthetic measurements were also taken from trees grown under elevated CO_2 at 560 ppm followed by a second measurement at CO_2 concentration of 360 ppm on the same leaves. These sets of measurements allowed us to evaluate whether the trees grown under elevated CO_2 have acclimated or not. Five leaves from each tree were sampled from short shoot at the top canopy for measurements and replicated three times.

2.3. Meteorological data

An on-site weather station measured the air temperature (T_{air}), relative humidity, wind speed, and photosynthetic photon flux density at the top of the canopy, in addition to precipitation and soil water content (at 5, 50 and 100 cm depth) measured with water content reflectometer (CS616-L, CS Campbell Scientific Inc.). These measurements were taken at every 30 min throughout the day and year. Details of the meteorological data from this weather station can be assessed from http://www.aspenface.mtu.edu.

2.4. Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's-test significant at $P < 0.05$ level (Sokal and Rolph, 1995) was computed for determination of significant differences between treatments with respect to A_{max}, V_{cmax}, J_{max}, g_s etc. there were four treatments with 3 replicates in this experiment. Average values computed ± standard errors (SE) were presented and different letters were used to indicate significant differences. The PROC GLM component of the SAS statistical software (by SAS Inc.) was used in carrying out this analysis.

3. Results

3.1. Is photosynthetic and stomatal acclimation occurring in aspen trees grown under elevated CO_2?

Fig. 1 shows that A_{max} rate measured in all five years were inconsistent in the magnitude of elevated CO_2 stimulation from 2004 through 2008 (85%, 55%, 35%, 69% and 30%, respectively), in clone 42E. Clone 271 did not show any evidence of photosynthetic acclimation either, as seasonal A_{max} stimulation varied and did not follow any particular pattern in the years 2004 through 2008 (38%, 67%, 51%, 62% and 65%, respectively). There is no evidence of photosynthetic acclimation as the photosynthetic stimulation under elevated CO_2 was measured to be between 33 and 43% in 2000 at canopy closure by Noormets et al. (2001), as seen in Table 1.

Fig. 2 shows no evidence of acclimation in both clones of aspen comparing the percentage increase in V_{cmax} in 2006 and 2008. We observed an increase of 52% and 48% in 2006 and 2008, respectively, in V_{cmax} of clone 42E. Also, J_{max} increased by 90% in 2006 and 34% in 2008 indicating that photosynthetic capacity is still being maintained after 11 years of exposure. In clone 271, V_{cmax} was increased by 64% in 2006 and 85% in 2008, while J_{max} was increased by 108% in 2006 and 23% in 2008.

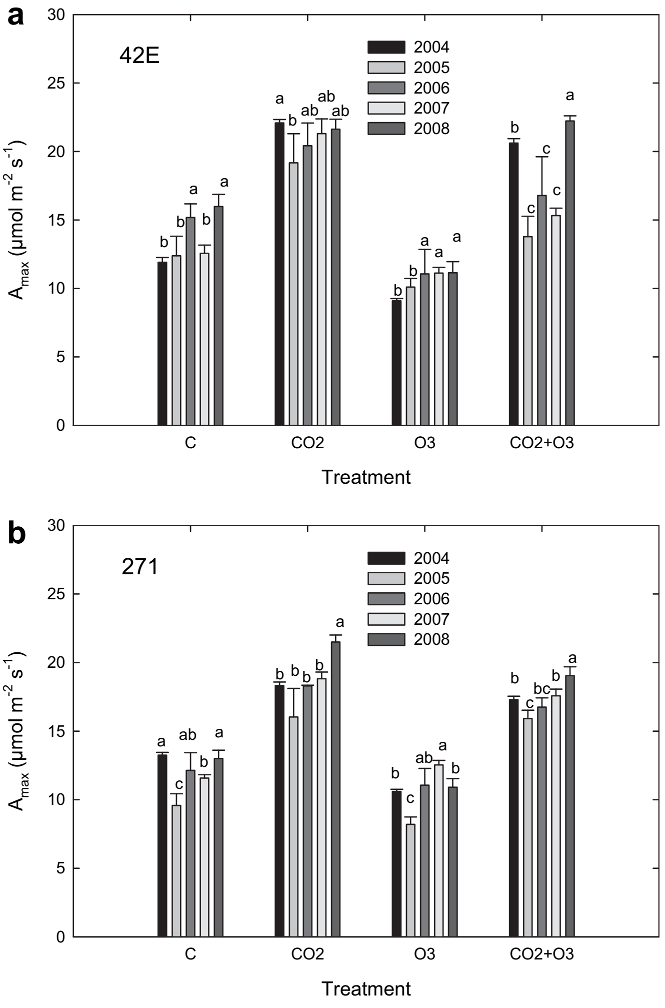

Fig. 1. Average seasonal maximum assimilation rate (A_{max}) for the growing seasons 2004 through 2008 in aspen clones 42E and 271 showing significant differences between seasons in each of the four treatments. Measurements were taken from the same trees each year at the Aspen FACE site, Rhinelander, WI, USA.

The results of the reciprocal instantaneous photosynthetic measurements taken in 2007 (Fig. 3) show that when CO_2 concentration was increased from 360 ppm to 560 ppm for trees grown under ambient conditions (360 ppm), photosynthetic rates increased significantly (34.3% with P value of 0.002 in clone 42E and 66% with P value of <0.001 in clone 271) in both clones in 2007. Photosynthetic rates decreased significantly in both clones (−43% with P value of <0.001 in clone 42E and −35% with P value of <0.001) when CO_2 concentration was decreased from the growth concentration of 560 ppm to 360 ppm.

Reciprocal instantaneous photosynthesis measured in 2008 showed similar patterns. When CO_2 concentration was increased from 360 ppm to 560 ppm for trees grown under ambient conditions (360 ppm), photosynthetic rates increased significantly (54% with $P < 0.001$ in clone 42E and 64% with $P < 0.001$ in clone 271) in both clones in 2008. Photosynthetic rates decreased significantly in both clones (−53% with $P < 0.001$ in clone 42E and −64% with $P < 0.001$) when CO_2 concentration was decreased from the growth concentration of 560 ppm to 360 ppm. Fig. 4a shows no evidence of photosynthetic acclimation in aspen after 11 years of exposure to elevated CO_2.

Stomatal conductance was observed to be quite variable between growing seasons, as seen in Table 2. Also, Fig. 5 shows that stomatal conductance was significantly low in 2006 compared to 2007 across all the four treatments. Our observations also show that there are days that stomatal conductance decreased under elevated CO_2 in each year and there are days that it increased relative to control (data not shown). Considering Fig. 4b, it can be seen that stomatal conductance was decreased under elevated CO_2 in the reciprocal measurement. Furthermore, Fig. 4b shows that stomatal conductance did not change as CO_2 concentration levels were changed for the same leaves after Licor readings had stabilized.

3.2. Is aspen acclimating to O_3 treatment effects?

Reduction in A_{max} was consistently significant (between 33% and 46%, Fig. 1 and Table 1) in both clones for the growing seasons 2004, 2005 and 2006. In 2007, elevated O_3 effect was not pronounced as there was no fumigation from June 15 to July 19, 2007 (due to equipment failure). Noormets et al. (2001) observed a decrease of 38–50% under elevated O_3 in these same aspen trees at the FACE site at canopy closure in 1999. This shows that aspen trees have not acclimated after 11 years of exposure to elevated O_3. We observed no change in V_{cmax} as well as J_{max} in clone 42E under elevated O_3 in both 2006 and 2008. In clone 271, which is O_3 tolerant, there was no change in 2006 in both V_{cmax} and J_{max}, but an increase of 60% ($P = 0.001$) and 47.5% ($P = 0.03$) in V_{cmax} and J_{max} respectively, in 2008 (Fig. 2).

Stomatal conductance under elevated O_3 was not consistent either throughout the five seasons that measurements were taken. In some season, we recorded an increase in stomatal conductance (with $P < 0.001$ in 42E and $P = 0.01$ in 271 in 2004), while in other seasons stomatal conductance decreased ($P = 0.03$ in 42E and $P = 0.01$ in 271 in 2008) and yet still, some seasons with no change, as in 2006 (Fig. 5) relative to control (Table 2). We, therefore, have no evidence of stomatal acclimation under elevated O_3 after 11 years of exposure.

3.3. Is photosynthetic and stomatal acclimation occurring in aspen trees grown under elevated $CO_2 + O_3$?

Percentage A_{max} enhancement values for both clones did not show any evidence of photosynthetic acclimation for 2004 through 2008 growing seasons with percentage enhancement values of

Table 1
Percentage change in photosynthetic rate in aspen trees exposed to elevated CO_2 and/or O_3 since 1997 at the Aspen Face site in Rhinelander, WI.

Year	2000*	2004	2005	2006	2007	2008
Treatment						
CO_2	(+) 39–43	(+) 38–85	(+) 55–67	(+) 35–51	(+) 45–63	(+) 30–65
O_3	(−) 38–50	(−) 37–41	(−) 33–42	(−) 40–46	(−) 06–12	(−) 16–34
$CO_2 + O_3$	(+) 05–30	(+) 50–58	(+) 23–49	(+) 51–52	(+) 52–53	(+) 32–47

Measurements were taken on nine different sunny days during the growing seasons. *Data from 2000 was from Noormets et al. (2001).

(58%, 23%, 52%, 21.4% and 39%) and (50%, 49%, 51%, 52% and 47%) for clones 42E and 271, respectively (Table 1). We observed no significant change in V_{cmax} and J_{max} in clone 42E in both 2006 and 2008 (although there was a slight positive change in 2008) despite the increase in A_{max} in these years. In clone 271, there was no change in V_{cmax} and J_{max} in 2006, but in 2008 we observed an increase of 87% ($P < 0.001$) in V_{cmax} and 62% ($P = 0.001$) in J_{max}. Both clones 42E and 271 did not show any evidence of acclimation under elevated $CO_2 + O_3$ after 11 years of exposure.

Reciprocal instantaneous photosynthesis measured in 2008 showed that, when CO_2 concentration was increased from 360 ppm to 560 ppm for trees grown under ambient conditions (360 ppm), photosynthetic rate increased significantly (54% with $P < 0.001$ in clone 42E and 64% with $P < 0.001$ in clone 271) in both clones in 2008. Photosynthetic rate decreased significantly in both clones (−56% with $P < 0.001$ in clone 42E and −60% with $P < 0.001$) when CO_2 concentration was decreased from the growth concentration of 560 ppm to 360 ppm. Fig. 4a shows no evidence of photosynthetic acclimation in aspen after 11 years of exposure to elevated CO_2.

We recorded a decrease of 48% ($P = 0.035$) in clone 42E in 2005 in stomatal conductance but no significant change in 2006, 2007 and 2008 (data not shown). In clone 271, there was no significant change in stomatal conductance (despite the 33% decrease in 2006) under elevated $CO_2 + O_3$ in all the years. Table 2 and Fig. 5 help us appreciate how stomatal conductance varies seasonally. Fig. 4b also shows that stomatal conductance was not affected by the sudden change in the CO_2 concentration during the reciprocal photosynthetic measurements.

4. Discussion

4.1. *Is photosynthetic and stomatal acclimation occurring in aspen trees grown under elevated CO_2?*

We report of sustained enhanced maximum photosynthetic rate (30–63% for clone 42E and 271 in 2008), J_{max} (23–34% for clones 42E and 271 in 2008) and V_{cmax} (48–85% for clone 42E and 271 in 2008) in two clones of aspen trees after 11 years of exposure to elevated CO_2. According to Noormets et al. (2001) elevated CO_2 stimulated photosynthesis by 39–43% in different clones of aspen from these same trees in the summer of 1999. Our results show that both clones of aspen trees have consistently sustained their enhanced photosynthetic capacity and rate from 2004 to 2008.

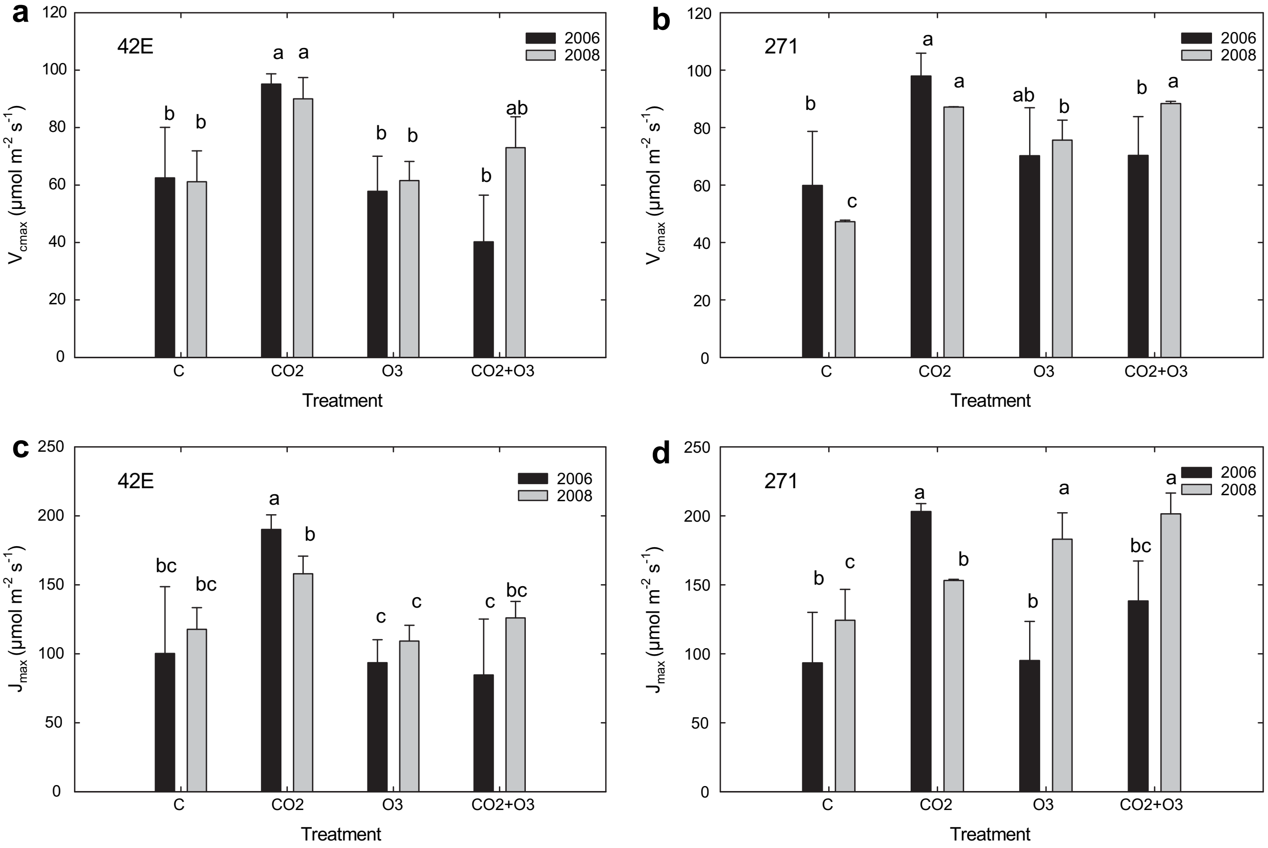

Fig. 2. Seasonal average V_{cmax} and J_{max} in the four treatments for the growing seasons 2006 and 2008 in clones 42E and 271. Measurements were taken from the same trees each year at the Aspen FACE site, Rhinelander, WI, USA.

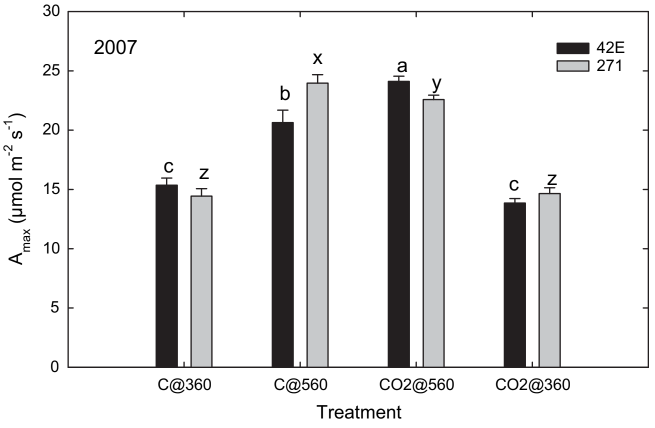

Fig. 3. Reciprocal maximum photosynthetic rate measurements in aspen clones 42E and 271. Two sets of measurements were taken under ambient growth concentration ([C360 ppm] and then [C560 ppm]) and two sets of measurements under elevated CO_2 growth concentration ([CO_2 560 ppm] and then [CO_2 360 ppm]). These reciprocal instantaneous measurements were taken at the Aspen FACE site Rhinelander, WI, in the summer of 2007.

Furthermore, our results support Liberloo et al. (2007) who reported 49% and 15% significant increases in net photosynthetic rate and J_{max}, respectively, while V_{cmax} was unaffected (−1.6%) in poplar after 6 years of exposure, and that there is no indication of photosynthetic down-regulation in agreement with our findings. Sholtis et al. (2004) reported a 44% stimulation of net photosynthesis in sweet gum trees after 3 years of exposure to elevated CO_2. Also, Crous and Ellsworth (2004) found a significant photosynthetic enhancement of 51–69% in *Pinus taeda* trees after 6 years of exposure. Davey et al. (2006) suggested that poplar trees “escape” from long-term acclamatory down-regulation of photosynthesis through a high capacity for starch synthesis and carbon export. This result agrees with Paoletti et al. (2007) who reported that there was no photosynthetic acclimation (down-regulation) occurring in *Quercus ilex* under long-term CO_2 enrichment.

Finally, when CO_2 concentration was lowered in elevated CO_2 treatment after 11 years of exposure (from 560 μmol mol^{-1} back to 360 μmol mol^{-1}), the percentage decrease (53% and 64% for clone 42E and 271) was about the same as the percentage increase (54% and 64% for clone 42E and 271) in photosynthesis when CO_2 was increased from 360 to 560 μmol mol^{-1} in the control treatment in 2008 (Figs. 3 and 4a). This confirms that photosynthetic enhancement has not been lost in both clones of aspen after more than a decade of exposure. Our observations support the findings of Ainsworth et al. (2003b) who found that photosynthetic stimulation in *Trifolium repens* remained after nine years of exposure to elevated CO_2.

Our results also show that the magnitude of elevated CO_2 enhancement varies appreciatively from year to year (for example 85% in 2004 and 35% in 2006) and not necessarily a gradual decrease resulting in acclimation. Rogers et al. (2004) reported that photosynthetic capacity can be lost during certain conditions under elevated CO_2. We did not observe a total loss of enhanced photosynthetic rate but a decrease in magnitude during some growing seasons depending on climatic conditions. Our findings show that there is no down-regulation of A_{max} when there is no root volume limitation.

Stomatal acclimation was not observed in any of the aspen clones we studied. Table 2 shows no significant change in stomatal conductance between control and elevated CO_2 treatments in some seasons, while other seasons saw some changes; some increased, and others decreased. This result suggests that there is no stomatal acclimation occurring, as the stomatal responses are not consistent throughout the five year study period. Our results are in harmony with the findings of Leakey et al. (2006) who found no evidence of stomatal acclimation in soybeans grown under FACE conditions. Also, Saxe et al. (1998) and Medlyn et al. (2001) reported no stomatal acclimation under elevated CO_2 in their reviews.

Fig. 5 helps us appreciate the difference in stomatal conductance values between 2004 and 2008 growing seasons possibly due to the micrometeorological conditions (Fig. 6). The mean g_s values for the 2006 growing season were much lower than that of 2005 and 2007, possibly because of the unusually high temperatures and drought (air temperatures were commonly between 34 and 38 °C in July and

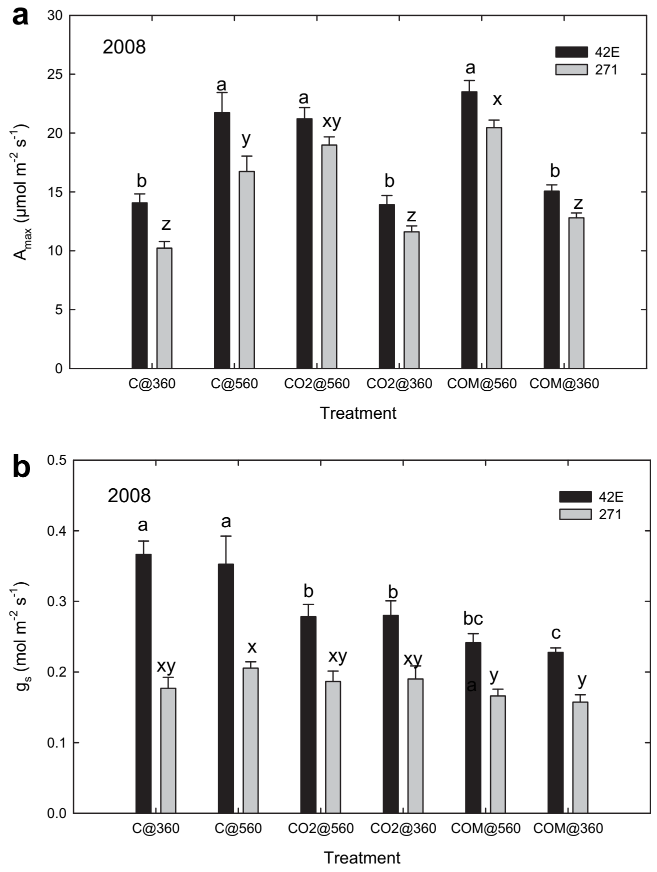

Fig. 4. Reciprocal maximum photosynthetic rate measurements in aspens clone 42E and 271. Two sets of measurements were taken under ambient growth concentration ([C@360 ppm] and then [C@560 ppm]), two sets of measurements under elevated CO_2 growth concentration ([CO_2@560 ppm] and then [CO_2@360 ppm]) and two sets in elevated $CO_2 + O_3$ ([COM@560 ppm] and then [COM@360 ppm]). These reciprocal instantaneous measurements were taken at the Aspen FACE site Rhinelander, WI, in the summer of 2008.

Table 2

Percentage (%) seasonal average change in stomatal conductance for each treatment relative to control (ambient). These measurements were taken during the summers of 2004 through 2008 at the Aspen FACE site in Rhinelander WI, USA.

Treatment	2004	2005	2006	2007	2008
Clone 42E					
CO_2	(+) 152	(+) 02	(+) 03	(+) 06	(−) 22
O_3	(+) 92	(−) 12	(+) 06	(+) 22	(−) 30
$CO_2 + O_3$	(+) 112	(−) 48	(−) 24	(−) 06	(−) 06
Clone 271					
CO_2	(+) 02	(−) 15	(−) 13	(−) 01	(+) 14
O_3	(+) 18	(−) 17	(−) 07	(+) 08	(−) 28
$CO_2 + O_3$	(−) 03	(−) 28	(−) 33	(−) 02	(−) 18

a drought severity index of −3 according to NOAA (2006) in Rhinelander, WI, USA). This lowered stomatal conductance in 2006 is likely a mechanism to conserve water by cutting down on transpirational losses to prevent wilting. Our results emphasize the need to be careful in making general statements about plant responses to elevated CO_2, as micrometeorological conditions play a key role in controlling g_s and, hence, difficult to determine stomatal acclimation.

Our findings from the reciprocal photosynthetic measurements reveal something unusual. We observed that stomatal conductance was higher in clone 42E under the elevated CO_2 (only in 2008) but lower in clone 271 (Fig. 4b and Table 2); hence, we do not have any evidence of stomatal acclimation occurring. Rather, we can say that although the two clones behaved similarly, there is a difference in the stomatal conductance. Calfapietra et al. (2008) also reported of no significant difference in stomatal conductance between trees grown in elevated CO_2 and ambient CO_2 after nine years of exposure in 2006 at the Aspen FACE site in Rhinelander in the same aspen clones 42E and 271. Significant differences in stomatal conductance between these two aspen clones (42E and 271) were reported by Calfapietra et al. (2008). This implies that caution must be taken in making general statements about stomatal acclimation, even within species, as it is problematic across species (Paoletti and Grulke, 2005).

Also, our observations from the reciprocal measurements did not show any decrease in stomatal conductance between the two CO_2 growth concentrations (Fig. 4b) as reported by Mott (1988). Neither does our observation agree with idea that stomata are able to acclimate quickly to elevated CO_2 during reciprocal transfer measurements from low to high CO_2 and vice versa, as stated by Paoletti and Grulke (2005). Our results suggest no stomatal acclimation under elevated CO_2.

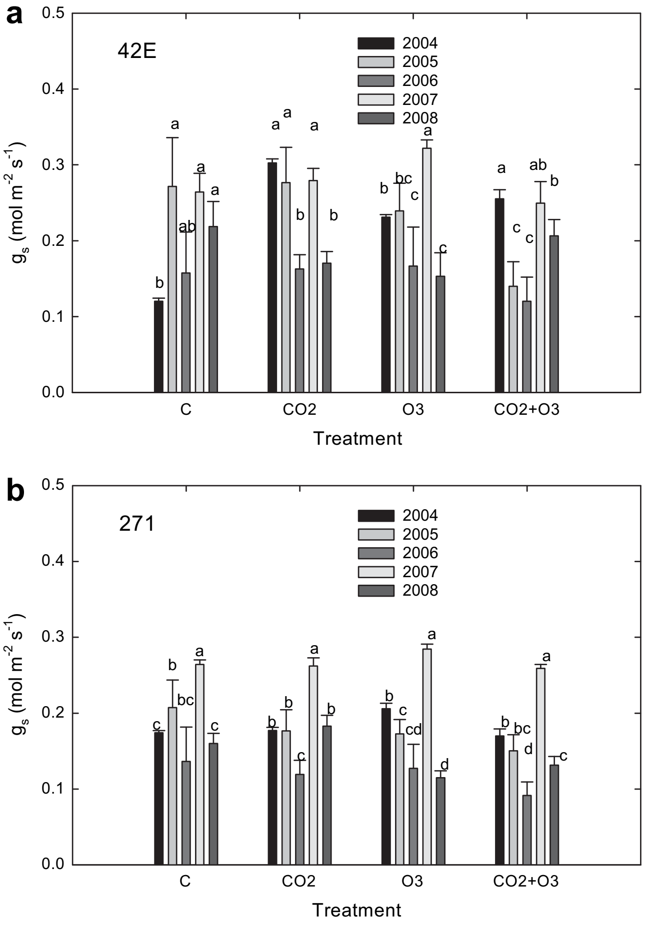

Fig. 5. Seasonal average g_s in the four treatments for the growing seasons 2004 through 2008 in clones 42E and 271 showing significant seasonal variations. These measurements were taken from the same trees each season. Data were collected at the Aspen FACE site, Rhinelander, WI, USA.

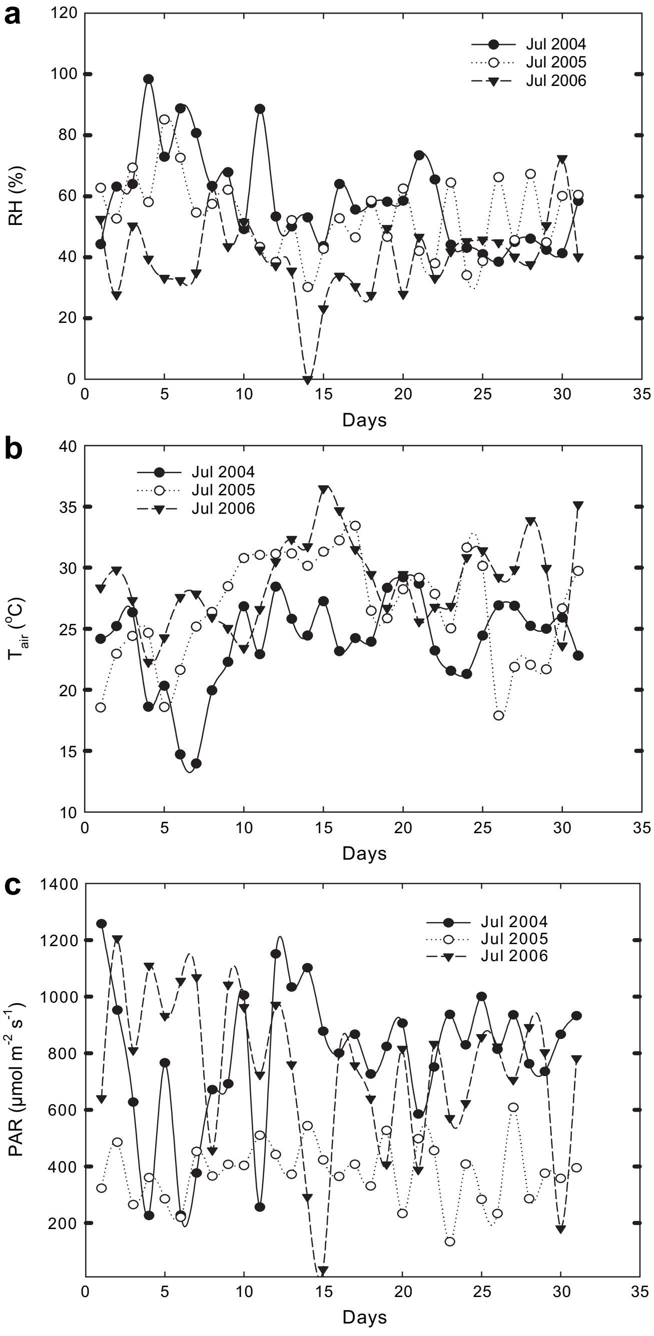

Fig. 6. Daily average relative humidity, temperature and photosynthetic active radiation in July of 2004, 2005 and 2006 as recorded at the Aspen FACE site in Rhinelander WI.

4.2. Is aspen acclimating to O_3 treatment effects?

Much attention has been given to the effect of elevated O_3 on plants, but relatively not much has been done on neither photosynthetic nor stomatal acclimation of plants exposed to elevated O_3

on a long-term basis. The decrease in A_{max} in both clones is consistent over the three-year period, 2004, 2005 and 2006 (-41% to -46%, for clone 42E and -33% to -40%, for clone 271). These percentage decreases that we recorded 11 years after exposure are quite consistent with the 38–50% decrease reported by Noormets et al. (2001) in 1999 on the same trees at the Aspen FACE site after only 3 years of exposure. Our findings agree with Morgan et al. (2004), who reported that A_{max} decreases with O_3 after prolonged exposure.

Despite the recorded 40–46% decrease in A_{max} under elevated O_3, we observed no significant change in either V_{cmax} nor J_{max} in clone 42E in both 2006 and 2008. But in clone 271, which is O_3 tolerant, there was a significant increase in V_{cmax} and J_{max} in 2008 but not in 2006. Our results suggest that in 2007 when there was no fumigation from June 15 through July 19, aspen trees under elevated O_3 made good recovery and, hence, the small magnitude of decrease in A_{max}. This finding is explained by Matyssek and Sandermann (2003), Wieser et al. (2003), Matyssek et al. (2004) and Löw et al. (2007) who reported that it is the actual dose of O_3 uptake through leaf stomata rather than exposure that determines the O_3 stress and drives the stress response in plants. O_3 reacts with water in the apoplast mesophyll and it solutes to form different reactive oxygen species (ROS). These ROS in turn initiate damage response upon interaction with the plasma membrane (Bernacchi et al., 2006), and so it is reasonable to expect that the damage done to the photosynthetic apparatus will continue as long as the plant is exposed to elevated O_3, and O_3 enters the stomata resulting in significant decrease in A_{max}, as observed in this study. This constant decrease in A_{max} shows that there is no evidence of photosynthetic acclimation (up-regulation of photosynthetic variables [nor increased tolerance]), as there is no shift nor is there an adjustment in photosynthetic apparatus, but rather continuous damage to photosynthetic apparatus by the reactive oxygen species formed which decrease the photosynthetic capacity of the plants.

There was no evidence of stomatal acclimation under elevated O_3 after 11 years of exposure, as there was no significant difference in the mean g_s in both the O_3 sensitive and O_3 tolerant clones of aspen in 2004 through 2008 growing seasons.

4.3. *Is photosynthetic and stomatal acclimation occurring in aspen trees grown under elevated $CO_2 + O_3$?*

The magnitude of A_{max} stimulation under elevated $CO_2 + O_3$ in the growing seasons 2004 through 2008 did not show any evidence of acclimation in both clones. The least magnitude of A_{max} stimulation in 2008 is above the highest observed in 1999 by Noormets et al. (2001) in these same aspen trees (Table 1). The significant increase in A_{max} under elevated $CO_2 + O_3$ is in agreement with the findings of Volin et al. (1998) who reported of similar findings in aspen seedlings. Our findings are supported by the fact that others have reported that elevated CO_2 does not always ameliorate the negative effects of elevated O_3 (Kull et al., 1996; Oksanen et al., 2001; Sharma et al., 2003). Our reciprocal photosynthetic measurement (Fig. 4a) showed no sign of photosynthetic acclimation, as the percentage change in photosynthesis between the two CO_2 levels were not different. We report that there is no photosynthetic acclimation occurring under elevated $CO_2 + O_3$ treatment and that aspen trees have sustained their A_{max} stimulation for over a decade.

We observed no significant change in V_{cmax} and J_{max} in clone 42E in both 2006 and 2008 despite the significant increases in the A_{max} in these two years. In clone 271, there was no change in V_{cmax} and J_{max} in 2006 also, but there was a significant increase in both V_{cmax} and J_{max} in 2008. Our observations on the V_{cmax} and J_{max} values from the elevated $CO_2 + O_3$ are not surprising because many researchers have reported of the ameliorative effects elevated CO_2 have on elevated O_3 (Volin and Reich, 1996; Volin et al., 1998) and this shows that this ameliorative effect is being sustained after 11 years of exposure. It is obvious that photosynthetic response to elevated $CO_2 + O_3$ also varies seasonally depending on climatic conditions. This shows that there is no evidence of photosynthetic acclimation in clone 271 under elevated $CO_2 + O_2$ and neither is there any solid conclusive evidence in clone 42E to say acclimation is occurring.

With respect to stomatal acclimation, we found that there was a decrease in clone 42E only in 2005 and in both clones in 2006 relative to control, but no change in 2007 and no significant change in 2008 either. Our finding of no consistent change in stomatal conductance agrees with Volin and Reich (1996) who reported high photosynthetic response under elevated $CO_2 + O_3$, but with no effect of stomatal conductance. In the review of Paoletti and Grulke (2005), it was stated that change in stomatal conductance is a secondary response which maintains stable internal CO_2 concentration under the given treatment and that indicates a close coupling between stomatal and mesophyll processes.

The decrease in stomatal conductance in 2006 could possibly be due to the heat wave experienced in the mid-eastern part of United States in July 2006 and its associated drought severity index of -3. These decreases may well be in the range expected in the year-to-year differences due to several differences in micrometeorological conditions. The stomatal conductance observed in the reciprocal measurement followed the similar pattern as those under elevated CO_2, as discussed previously giving no evidence of acclimation.

5. Conclusion

In conclusion, our results suggest no long-term photosynthetic and stomatal acclimation to elevated CO_2, O_3 or $CO_2 + O_3$ in aspen trees exposed to elevated CO_2 and/or O_3 gases for 11 years. It also shows that the magnitude of photosynthetic stimulation under elevated CO_2 is being sustained but not consistent in all seasons, but varies from one season to another depending on climatic conditions. It also suggests that researchers need to be cautious in making general statements about plant responses to long-term exposure to elevated CO_2 and O_3 on acclimation issues, especially when their study covers one or two growing seasons, as wrong conclusions may be drawn based on inadequate data collected. Furthermore, different clones within the same species have different stomatal sensitivities to environmental conditions and, hence, their response to elevated CO_2 and O_3.

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