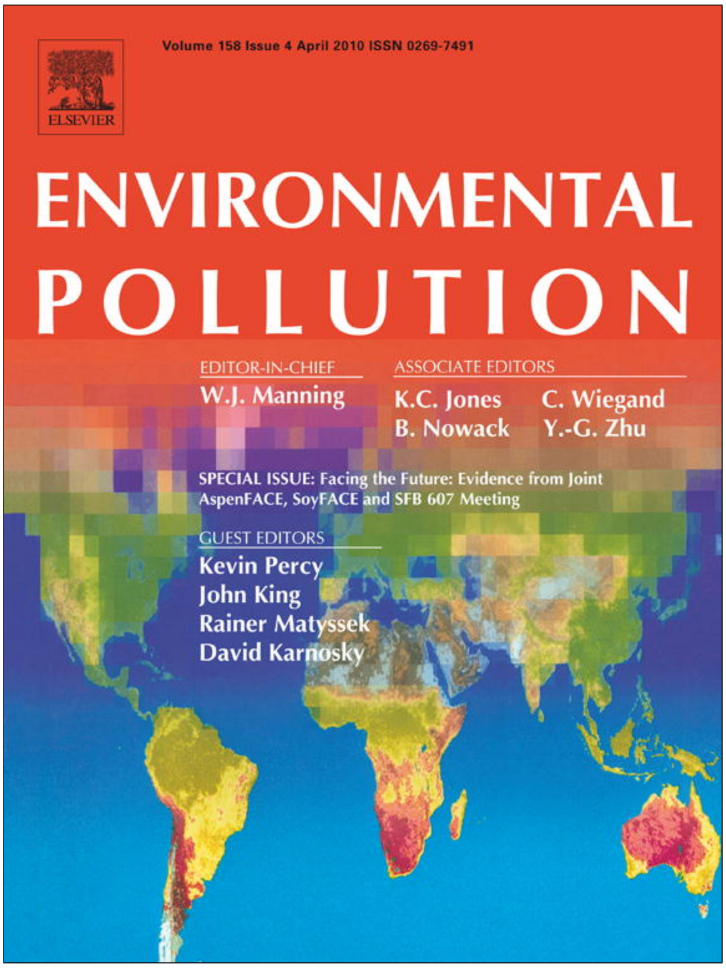
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Elevated CO_2 response of photosynthesis depends on ozone concentration in aspen☆

Asko Noormets[a,*,1], Olevi Kull[b], Anu Sôber[b], Mark E. Kubiske[c], David F. Karnosky[a]

[a] Michigan Technological University, School of Forest Resources and Environmental Science, Houghton, MI 49931, USA
[b] University of Tartu, Institute of Botany and Ecology, Tartu, Estonia, USA
[c] US Forest Service, Northern Research Lab, Rhinelander, WI 54501, USA

Photosynthetic acclimation to elevated CO_2 depends on the background oxidant levels.

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ABSTRACT

The effect of elevated CO_2 and O_3 on apparent quantum yield (ϕ), maximum photosynthesis (P_{max}), carboxylation efficiency (V_{cmax}) and electron transport capacity (J_{max}) at different canopy locations was studied in two aspen (*Populus tremuloides*) clones of contrasting O_3 tolerance. Local light climate at every leaf was characterized as fraction of above-canopy photosynthetic photon flux density (%PPFD). Elevated CO_2 alone did not affect ϕ or P_{max}, and increased J_{max} in the O_3-sensitive, but not in the O_3-tolerant clone. Elevated O_3 decreased leaf chlorophyll content and all photosynthetic parameters, particularly in the lower canopy, and the negative impact of O_3 increased through time. Significant interaction effect, whereby the negative impact of elevated O_3 was exaggerated by elevated CO_2 was seen in Chl, N and J_{max}, and occurred in both O_3-tolerant and O_3-sensitive clones. The clonal differences in the level of $CO_2 \times O_3$ interaction suggest a relationship between photosynthetic acclimation and background O_3 concentration.

1. Introduction

Human activities have led to a steady increase in atmospheric CO_2 and tropospheric O_3 concentrations (Houghton et al., 2001). These pollutants, in addition to being radiatively active and contributing to global warming, also have direct influence on plants. The stimulating effect of elevated CO_2 level results from improved substrate availability for assimilation, as well as from reduced water loss due to lower stomatal conductance (Noormets et al., 2001). The negative effect of O_3 on plants results form its highly oxidative properties that damage cell membranes, denature critical enzymes and give rise to other oxidatively active species (Samuelson and Kelly, 2001; Karnosky et al., 2005). While the individual effects of CO_2 and O_3 are relatively well understood, the effect of combined exposure of plants to them is still being debated. The intuitive hypothesis that the contrasting effects of CO_2 and O_3 on plants would cancel each other and lead to an intermediate response (Allen, 1990) has received support in a number of experiments focusing on integrative parameters like plant growth or total photosynthetic production (Grams et al., 1999; McKee et al., 2000; Cardoso-Vilhena et al., 2004; Rebbeck et al., 2004; Karnosky et al., 2005). Other studies, however, have revealed several peculiarities of plant physiological responses showing that the responses of individual processes may be much more complex than straightforward amelioration of O_3 stress by CO_2 (Kull et al., 1996; Kytöviita et al., 1999; Paoletti and Grulke, 2005).

In many experiments the increase in leaf net photosynthesis in C3 plants has not been proportional to CO_2 increase because stomata tend to close and often leaf photosynthetic capacity is down-regulated (Ceulemans et al., 1999; Moore et al., 1999; Nowak et al., 2004). Such down-regulation seems to be greater when plants encounter stresses like nutrient deficiency or space limitation. Under elevated CO_2 the intrinsic limitation of photosynthesis shifts from CO_2 fixation in carboxylation towards energy capture by photochemical component of the photosynthesis, and therefore it has been hypothesized that it should be beneficial for a plant to invest relatively more resources into light harvesting pigments and electron transport related compounds at the expense of reduced carboxylation capacity (Long and Drake, 1992; Medlyn, 1996). Although the down-regulation of V_{cmax} at elevated CO_2 is a general phenomenon, and greater than the

☆ In memory of our beloved mentors and colleagues Olevi Kull (1951–2007) and David F. Karnosky (1949–2008).

* Corresponding author. Tel.: +1 919 515 7040.
E-mail address: anoorme@ncsu.edu (A. Noormets).
[1] Present address: North Carolina State University, Department of Forestry and Environmental Resources, Raleigh, NC 27606, USA.

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down-regulation of J_{max} (Ainsworth and Long, 2005), in individual studies it may often go unnoticed because not all species exhibit equally distinct response (Medlyn, 1996; Eichelmann et al., 2004).

Ozone can decrease photosynthesis even at relatively low concentration (Noormets et al., 2001; Samuelson and Kelly, 2001) and this decrease is often accompanied by decreased leaf chlorophyll or nitrogen content (Retzlaff et al., 1992; Kellomaki and Wang, 1998; Bäck et al., 1999; Eichelmann et al., 2004). However, the exact mechanism of the photosynthesis damage by chronic ozone exposure is not clear. In some studies dark and light reactions have been found to be suppressed to a similar extent (Bortier et al., 2000; Eichelmann et al., 2004), suggesting that both carboxylation (Soja et al., 1998; Bortier et al., 2000; Polle et al., 2000), and light harvesting (Samuelson and Edwards, 1993; Samuelson, 1994; Shavnin et al., 1999) are adversely affected.

Despite some controversy between the results of different experiments it is evident that both CO_2 and O_3 may affect components of the leaf photosynthetic machinery differently. Often the magnitude of these effects depends on light conditions (Tjoelker et al., 1995; Crous and Ellsworth, 2004). Therefore, to understand canopy level responses to elevated CO_2 and O_3 it is vital to understand the role of light intensity and leaf position in the canopy on leaf level responses. In general, ozone damage is greater at low light or lower canopy leaves despite lower stomatal conductance and lower ozone uptake than in well illuminated upper canopy leaves (Tjoelker et al., 1995; Bäck et al., 1999; Samuelson and Kelly, 2001). This has been hypothesized to be due to loosely packed mesophyll cells in the shade leaves which are more exposed to O_3 in shade than in sun leaves (Topa et al., 2001). It has also been proposed that higher photosynthesis-to-ozone uptake ratio of sun than shade leaves may confer increased resistance to the oxidative damage (Fredericksen et al., 1996). At the same time, elevated CO_2-induced enhancement of leaf photosynthesis is also greater in the shaded foliage of lower canopy (Idso et al., 1993; McDonald et al., 1999). In addition, when observed, the acclimation of photosynthetic capacity to elevated CO_2 (i.e. the down-regulation of photosynthetic capacity) is often greater in the upper canopy (Crous and Ellsworth, 2004). Consequently, because of interactions between leaf position and ozone sensitivity as well as acclimation to elevated CO_2, the entire range of leaves in the canopy light profile should be studied to allow upscaling of CO_2 and O_3 responses from leaf to canopy.

The goal of the current study was to partition the O_3-sensitivity between the light harvesting, electron transport and carboxylating components of photosynthesis along the canopy light gradient at ambient and elevated CO_2 using two aspen clones previously shown to exhibit different levels of sensitivity to O_3 (Kull et al., 1996; Noormets et al., 2001).

Table 1
Average and standard error of tree height (m) and LAI ($m^2\ m^{-2}$) in 2000 for the two aspen clones and for five-clone average throughout the treatment rings.

Height	Clone 216	Clone 259	Canopy average
Control	3.50 ± 0.16	2.87 ± 0.09	3.71 ± 0.08
CO_2	3.73 ± 0.33	3.07 ± 0.28	3.91 ± 0.23
O_3	3.34 ± 0.10	2.94 ± 0.14	3.51 ± 0.10
$CO_2 + O_3$	3.74 ± 0.08	2.96 ± 0.11	3.80 ± 0.17
LAI			
Control	2.45 ± 0.12	2.19 ± 0.16	1.79 ± 0.14
CO_2	1.90 ± 0.15	2.68 ± 0.25	1.88 ± 0.18
O_3	1.48 ± 0.38	1.60 ± 0.09	1.50 ± 0.30
$CO_2 + O_3$	1.51 ± 0.31	2.04 ± 0.20	1.52 ± 0.21

Data from Isebrands et al. (2001).

Table 2
Monthly ambient and elevated O_3 exposures for 2000 growing season ($\mu l\ l^{-1} \times h$).

Month	May	June	July	August	September	Total
Ambient						
AOT 0	8.5	13.3	12.6	12.3	11.5	58.2
AOT 40	1.21	0.94	0.71	0.48	0.78	4.12
AOT 60	0.00	0.13	0.00	0.02	0.03	0.18
Elevated						
AOT 0	9.2	17.3	17.4	19.6	16.4	80.0 (1.4×)
AOT 40	2.4	4.92	4.27	6.57	5.44	23.60 (5.7×)
AOT 60	0.69	1.59	1.00	2.91	2.13	8.28 (46×)

AOT 0, AOT 40 and AOT 60 are the total hourly O_3 concentrations over 0, 40 and 60 $\mu l\ l^{-1}$, respectively.

2. Materials and methods

2.1. Experimental site and plant material

Two aspen (*Populus tremuloides* Michx.) clones (#216 – O_3 tolerant; #259 – O_3 sensitive), were grown in a free-air carbon dioxide enrichment (Aspen FACE) facility (Karnosky et al., 1999; Dickson et al., 2000) near Rhinelander, Wisconsin, USA. The experimental site is located at 45°30' N and 89°30' W, on sandy loam soil with high nutrient levels (average N concentration 0.12%). There were no significant differences between individual treatment rings in C:N ratio, NH_4^+ and NO_3^- content, pH, soil texture parameters or water holding capacity (Dickson et al., 2000).

The differential O_3 tolerance of these two clones has been characterized based on the visual foliar symptoms, gas exchange and growth parameters (Coleman et al., 1995; Karnosky et al., 1996, 1998). The plant material was propagated from greenhouse-grown stock plants. The rooted cuttings were 6-months-old at the time of planting in July 1997. By the time of this experiment the trees were about 2.9–3.8 m tall (Isebrands et al., 2001) with the LAI in the fumigation rings ranging from 1.5 to 2.5 (Table 1). The clonal differences in growth potential and treatment responses were clearly expressed.

The treatments – control (C), elevated CO_2 ($+CO_2$), elevated O_3 ($+O_3$) and elevated CO_2 and O_3 ($+CO_2 + O_3$) – are triplicated, at least 100 m apart and arranged in a randomised complete block design. Each ring is 30 m in diameter and the trees are planted at the density of one tree per square meter. The detailed description of the experimental set-up and conditions can be found elsewhere (Dickson et al., 2000).

2.2. Fumigation

Control plants were exposed to ambient air ([CO_2] averaged 350 $\mu l\ l^{-1}$ between 0700 h and 1900 h and 390 $\mu l\ l^{-1}$ between 1900 h and 0700 h for the season and ambient [O_3] averaged 35 $nl\ l^{-1}$ between 07:00 h and 19:00 h for the season). Elevated CO_2 and O_3 were applied from bud break (1 May in 1998 and 10 May in 1999) to leaf abscission (15 October in 1998 and 30 September in 1999). Elevated CO_2 treated plants (alone and in combination with O_3) were exposed to 550 $\mu l\ l^{-1}$ CO_2 from 0700 h to 1900 h. Elevated O_3 treated plants (alone and in combination with CO_2) received approximately 1.5× the ambient dose (56 $\mu l\ l^{-1} \times h$ vs. 80 $\mu l\ l^{-1} \times h$ seasonal sum 0 for 12 h fumigation) with an average daytime (0700 h to 1900 h)

Table 3
Significance of factors (O_3, CO_2, Clone) and a covariate (%PPFD) on leaf chlorophyll content (Chl), apparent quantum yield (ϕ), leaf nitrogen content (N) and maximum photosynthetic capacity (P_{max}) using GLM procedure.

Factor	Chl		ϕ		*N*		P_{max}	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
O_3	57.8	***	38.4	***	12.2	***	14.1	***
CO_2	3.09	*	0.49	n.s.	3.70	*	0.81	n.s.
Clone (C)	0.36	n.s.	12.9	***	0.71	n.s.	5.86	**
%PPFD	75.9	***	27.6	***	508	***	80.8	***
$O_3 \times CO_2$	12.8	***	0.14	n.s.	6.48	**	0.07	n.s.
$O_3 \times$ C	8.94	***	0.05	n.s.	1.53	n.s.	0.00	n.s.
$CO_2 \times$ C	0.25	n.s.	2.25	n.s.	0.24	n.s.	0.46	n.s.
$O_3 \times$ %PPFD	6.69	**	9.76	***	0.02	n.s.	0.28	n.s.
$CO_2 \times$ %PPFD	1.52	n.s.	0.38	n.s.	2.03	n.s.	1.88	n.s.
C × %PPFD	11.9	***	2.28	n.s.	16.4	***	1.27	n.s.
$CO_2 \times O_3 \times$ C	1.51	n.s.	0.08	n.s.	3.84	*	1.87	n.s.
$CO_2 \times O_3 \times$ %PPFD	0.52	n.s.	0.09	n.s.	1.91	n.s.	0.18	n.s.
$CO_2 \times$ C × %PPFD	1.17	n.s.	4.57	*	1.10	n.s.	0.48	n.s.
$O_3 \times$ C × %PPFD	0.26	n.s.	0.29	n.s.	0.70	n.s.	3.89	*
$CO_2 \times O_3 \times$ C × %PPFD	0.13	n.s.	0.01	n.s.	0.88	n.s.	1.48	n.s.

Data from July and August are combined. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 4
Significance of factors (O_3, CO_2, Clone) and a covariate (%PPFD) on the capacities of electron transport (J_{max}) and carboxylation (V_{cmax}) as determined form leaf A–C_i curves using GLM procedure.

Factor	J_{max}		V_{cmax}		J_{max}:V_{cmax}	
	F	p	F	p	F	p
O_3	22.8	***	28.8	***	6.68	**
CO_2	0.81	n.s.	1.47	n.s.	6.74	**
Clone (C)	5.46	**	7.73	***	2.72	n.s.
%PPFD	33.6	***	34.8	***	1.95	n.s.
$O_3 \times CO_2$	7.72	***	2.79	*	0.72	n.s.
$O_3 \times$ C	0.36	n.s.	2.79	*	0.02	n.s.
$CO_2 \times$ C	1.42	n.s.	0.16	n.s.	17.7	***
$O_3 \times$ %PPFD	7.41	***	5.02	**	0.17	n.s.
$CO_2 \times$ %PPFD	0.90	n.s.	1.71	n.s.	4.17	**
C × %PPFD	0.35	n.s.	0.51	n.s.	0.90	n.s.

Data from July and August are combined. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

exposure concentration of 48 nl l^{-1} compared to the ambient concentration of 35 nl l^{-1}. These average O_3 concentrations, however, do not provide much information about the effective dose. Accumulated exposure over a threshold of 40 and 80 nl l^{-1} (AOT40 and AOT80, respectively) are being used to assess biologically significant dose, and these were considerably larger for $+O_3$ and $+CO_2+O_3$ treatments than the regional ambient exposures for each month of the 1999 (data not shown) and 2000 growing seasons (Table 2). The daily target peak concentration of elevated O_3 treatments for each day was calculated as the twice-ambient concentration at 0700 h (the base value). The target concentration was to be reached at noon, with sigmoidal increase and decrease, and O_3 concentration equal to the base level during the first and last hour of the daily fumigation. Ozone exposures varied by month and were as follows: June > July > August > September = May. The 1.5× ambient O_3 regimen was chosen for this experiment because it had been found to significantly increase visible foliar injury symptoms in the sensitive aspen clone (259) in open top experiments (Karnosky et al., 1996) whereas 2× ambient O_3 concentration caused significant injury even in the tolerant clone (216). Furthermore, the applied 1.5 × O_3 concentration caused significant increase in visible foliar symptoms even under FACE conditions (Karnosky et al., 1999). There were no O_3 fumigations during rain, fog, mist or dew conditions, which were about 30% of the time and were most frequent in September.

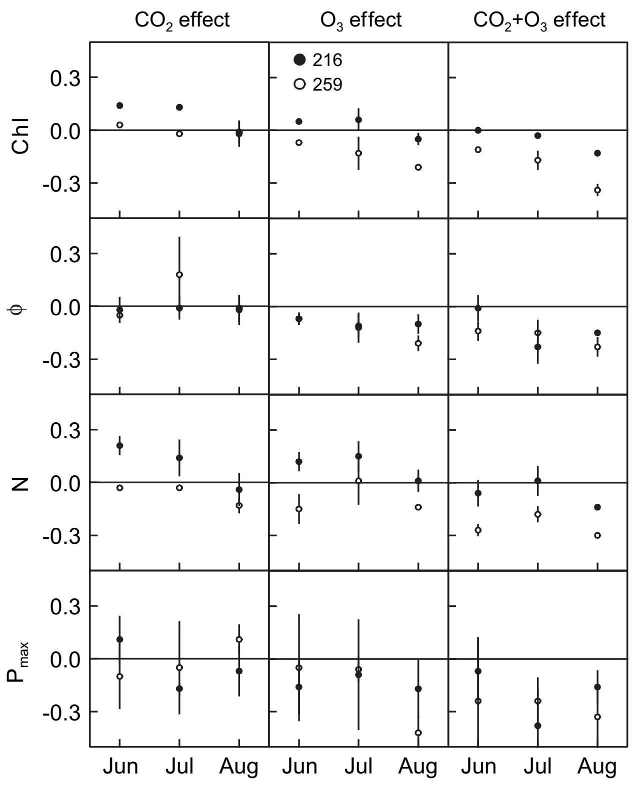

Fig. 1. Relative %PPFD-adjusted effect sizes of CO_2 and O_3 treatments on leaf chlorophyll content (Chl), apparent quantum yield (ϕ), N content (N), and maximum light-saturated photosynthesis (P_{max}) in two aspen clones (216: O_3-tolerant, 259: O_3-sensitive) throughout the 2000 growing season.

2.3. Leaf measurements

Photosynthetic light response (A–Q) curves of the two aspen clones were measured at saturating CO_2 concentration (2000 μl l^{-1}) in June, July and August, 2000, at ambient temperature (18–32 °C) and vapor pressure deficit (0.3–2.7 kPa) with an LI-6400 Portable Photosynthesis System (Li-Cor Inc., Lincoln, Nebraska, USA). Leaves were selected from 4 to 5 levels per tree, depending on its position in canopy, with a total of 97, 114 and 108 leaves sampled in June, July and August, respectively. Assimilation rates were measured in the following sequence of light levels – 1000, 500, 300, 100, 30, 0, 1000, 2000, 2000, 2000 μmol m^{-2} s^{-1}. Reading was taken when the change in the rate of assimilation was less than 5%, but no sooner than 2 min after changing the light intensity. The apparent quantum yield (ϕ) was determined with linear regression in the 0–100 μmol m^{-2} s^{-1} PAR range and P_{max} was determined as the average light-saturated assimilation of all measurements (PAR ≥ 1000 μmol m^{-2} s^{-1}). Photosynthesis CO_2 response (A-Ci) curves were measured in July and August using LI-6400. CO_2 concentration was changed in the order 360 (or 520 for leaves grown at elevated CO_2 treatments) 250, 100, 700, 1000, 1500 ppm. Maximum carboxylation capacity (V_{cmax}) and electron transport capacity (J_{max}) were calculated from A–C_i curves according to (Farquhar et al., 1980). Leaf chlorophyll content of all measured leaves was estimated from optical transmittance in the red and infrared wavebands using SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). Chlorophyll meter readings were calibrated against area-based nitrogen and chlorophyll concentrations from leaf punches (diameter 18 mm). These samples were dried at 65 °C for 24 h and weighed, or frozen in liquid nitrogen and stored at −30 °C until further analysis. Frozen leaf discs were weighed, and used for determination of chlorophyll. Chlorophyll content of the frozen leaf samples was determined by measuring the absorbtance of *N*,*N*-dimethylformamide-extracted samples, as described by Inskeep and Bloom (1985). The nitrogen content of leaves was measured with an elemental analyzer (Carlo Erba Instruments, Model NA 1500 NC).

2.4. Estimation of light environment

The local light environment was assessed using hemispheric photographs taken at the position of each leaf with Nikon Coolpix 950 digital camera and a FC-2 "fisheye converter". The images were analyzed using WinScanopy software (Regent Instruments Inc., Quebec, Canada) for openness, and direct, indirect and total site factors. The indirect site factor (ISF) exhibited the lowest variance when regressed against percent PAR transmittance (%PPFD). The ISF was calibrated against a permanently installed vertical array of LI-190SA (Li-Cor) PAR sensors. The

Table 5
Transmittance-adjusted treatment means (±SE) of chlorophyll content (Chl, mg m^{-2}), apparent quantum yield (ϕ, mol CO_2 mol^{-1} PAR), leaf nitrogen content (N, g m^{-2}) and light-saturated photosynthesis (P_{max}, μmol m^{-2} s^{-1}).

Treatment	Clone 216	Clone 259
	Chl	
Control	386.3 ± 12.7^A	400.9 ± 13.2^A
$+CO_2$	426.3 ± 12.8^A	395.0 ± 13.9^A
$+O_3$	370.2 ± 13.9^A	259.5 ± 15.4^B
$+CO_2+O_3$	312.7 ± 13.3^B	224.3 ± 13.2^B
	ϕ	
Control	0.118 ± 0.0039^A	0.104 ± 0.0041^A
$+CO_2$	0.115 ± 0.0040^A	0.107 ± 0.0049^A
$+O_3$	0.101 ± 0.0043^B	0.082 ± 0.0048^B
$+CO_2+O_3$	0.093 ± 0.0041^C	0.086 ± 0.0041^B
	N	
Control	4.29 ± 0.13^A	4.20 ± 0.13^A
$+CO_2$	4.63 ± 0.13^A	4.05 ± 0.14^A
$+O_3$	4.21 ± 0.14^A	3.39 ± 0.16^B
$+CO_2+O_3$	3.65 ± 0.14^B	3.20 ± 0.13^B
	P_{max}	
Control	22.1 ± 1.17^A	15.9 ± 1.22^{AB}
$+CO_2$	21.9 ± 1.29^{AB}	17.2 ± 1.35^A
$+O_3$	16.9 ± 1.29^{BC}	10.1 ± 1.43^C
$+CO_2+O_3$	14.4 ± 1.24^C	11.3 ± 1.22^{BC}

Data from July and August have been combined. The groupings by Tukey's post-hoc multiple comparison tests are indicated by the superscript letters separately for each clone.

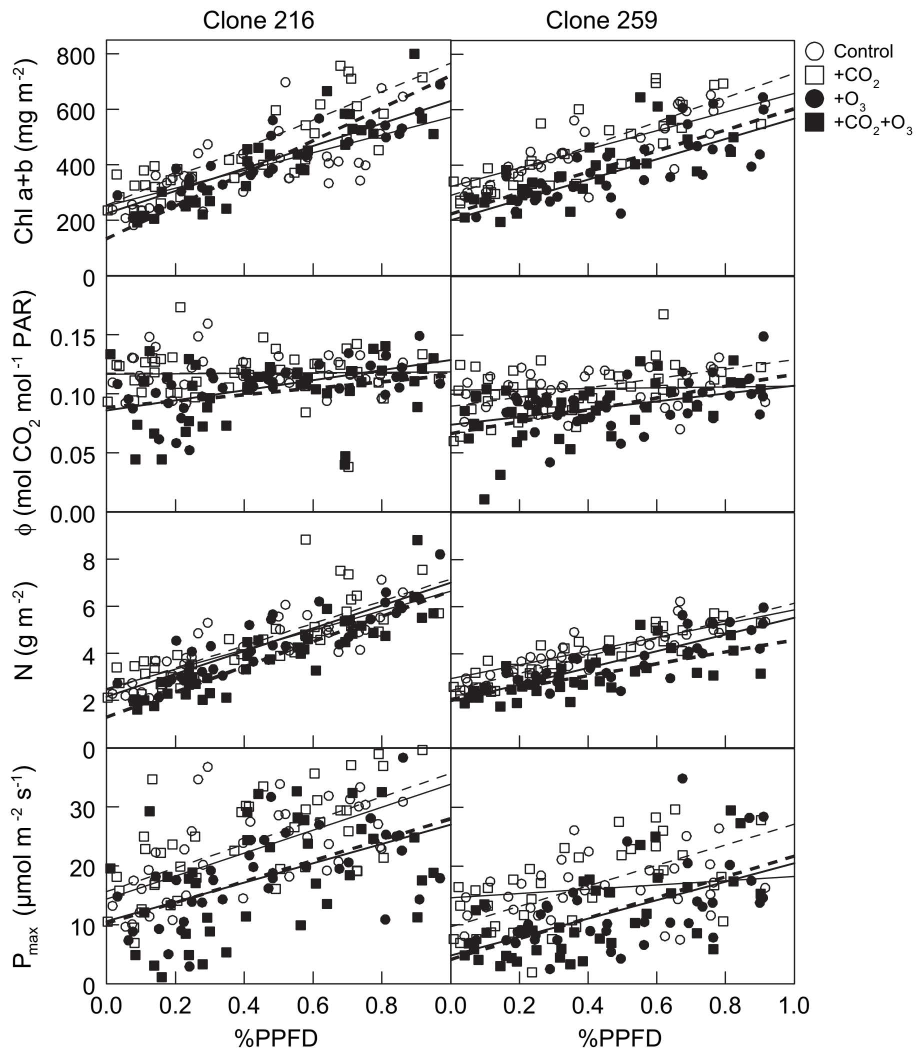

Fig. 2. Canopy profiles of leaf chlorophyll content (Chl), apparent quantum yield (ϕ), nitrogen content (N), and maximum photosynthetic capacity (P_{max}) during July and August 2000. Regression lines: solid, ambient CO_2; dashed, elevated CO_2; fine, ambient O_3; bold, elevated O_3.

following relationship, which remained constant throughout the growing season, was used to derive %PPFD from measured ISF:

$$\%\text{PPFD} = 1.2587 \times \text{ISF} - 0.2484\ (R^2 = 0.86) \quad (1)$$

The use of hemispherical photography for estimating %PPFD is not novel and has been reported to be reliable (Li-Cor, 1992; Rich et al., 1993). The coefficient of determination for the %PPFD–ISF relationship was $R^2 = 0.86$, which is higher than that observed by Machado and Reich (1999).

2.5. Statistical analysis

Leaf characteristics and the photosynthetic parameters were analyzed for the effects of CO_2, O_3, and clone as discrete factors, and the effect of %PPFD as a continuous factor and for all interactions using a general linear model (GLM) analysis. The relative effect size was calculated as a fraction relative to control (effect = (treatment–control)/control). All analyses were performed using Statistica software version 5.5 (StatSoft Inc., Tulsa, Oklahoma, USA). Effects were considered significant when the *P*-value of the *F*-test was ≤0.05. When the *F*-test showed significant interactions, *a posteriori* comparison of means was performed. *P*-values of these multiple comparisons were Tukey corrected, to reduce the chance of type I errors.

3. Results

Elevated CO_2 increased leaf *N* content and Chl, but did not alter any of the photosynthetic parameters (P_{max}, ϕ, J_{max} and V_{cmax}; Tables 3 and 4). At the same time, O_3 treatment suppressed all measured parameters except N content in the O_3-tolerant clone, and the effect on Chl, ϕ, and P_{max} got progressively greater with time (Fig. 1), especially in the O_3-sensitive clone. The combined treatment resulted in greater suppression of all photosynthetic parameters (Figs. 1 and 5), and like under elevated O_3 alone, the magnitude of the effect increased with time. The harmful effect of O_3 was either the same at both CO_2 levels, or it was greater at elevated than at ambient CO_2 (e.g. Chl and N content in both clones, J_{max} in the O_3-tolerant clone). Leaf nitrogen content was lower under $CO_2 + O_3$ than other treatments already in June, suggesting that leaf nitrogen status may depend on previous year's growing conditions (i.e. "memory effect"). In no situation did we observe elevated CO_2 ameliorating O_3 effects. The intrinsic difference in the O_3 tolerance between the two clones was detected as statistically significant only in Chl and V_{cmax} (Table 3, 4), although similar trend was observed in all parameters (Table 5). The difference in the magnitude of the CO_2 effect in the two clones decreased over time, whereas the difference in O_3 effect increased at both ambient and elevated CO_2 (Fig. 1). Maximum measured values of the apparent quantum yield (ϕ) reached 0.12 in the O_3-tolerant clone, which is close to the theoretical maximum of eight quanta per CO_2 molecule, whereas the O_3-sensitive clone had consistently lower ϕ.

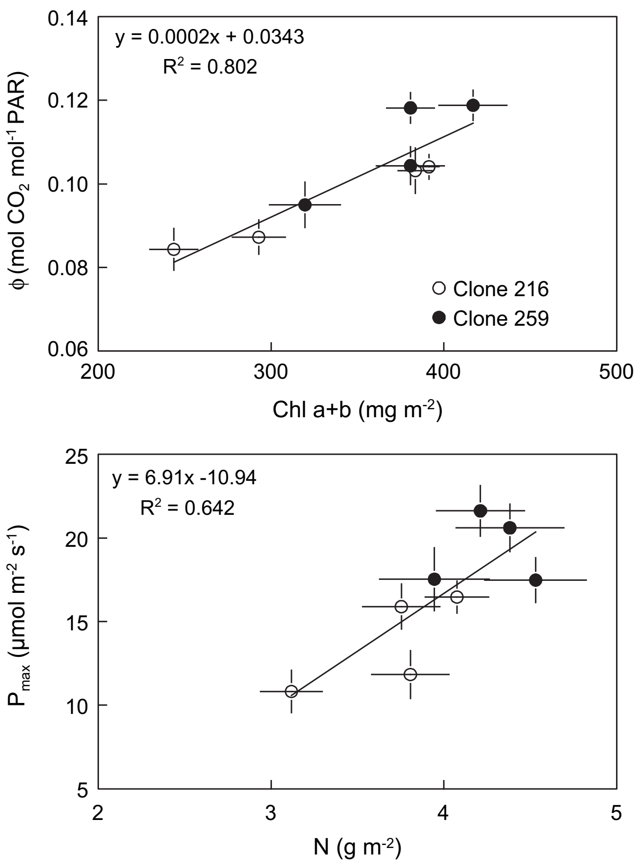

Fig. 3. Relationship between average chlorophyll content (Chl; averaged by clone and treatment) and apparent quantum yield (ϕ; top panel), and between leaf nitrogen content (N) and maximum light-saturated photosynthesis (P_{max}; bottom panel). Mean ± 1SE.

Most leaf parameters exhibited strong light-dependence. Some parameters, like leaf thickness and weight per area (LWA), exhibited similar trends with %PPFD in both clones, and under all treatments (data not shown). Leaf biochemical and photosynthetic properties, however, exhibited variable response to elevated CO_2 and O_3 depending on canopy position. Leaf nitrogen content per unit area decreased at all light levels (%PPFD × O_3 term was not significant, Table 3) at O_3 and $CO_2 + O_3$ treatments in the O_3-sensitive clone, and at the $CO_2 + O_3$ treatment in the O_3-tolerant clone (Fig. 2). The vertical gradient in Chl was weaker than that in N content (Fig. 2). In both clones the lowest Chl were observed under the $CO_2 + O_3$ treatment. The significant O_3 × %PPFD interaction in both clones for Chl, ϕ, J_{max} and V_{cmax} (Table 3, 4), but not for N content and P_{max}, suggested that the O_3-induced damage to electron transport capacity was greater in the lower canopy and bigger than the damage to the carboxylation capacity.

Canopy profile of P_{max} paralleled N content profile, whereas ϕ was strongly related to leaf Chl (Fig. 2). These relationships remained valid for treatment average values (Fig. 3), revealing that changes in Chl and N content were responsible for shifts in the photosynthetic parameters. Since Chl and N content responded differently to treatments, the relationship between Chl and N content also changed and Chl:N ratio changed differently in relation to %PPFD (Fig. 4). The Chl:N ratio was about 70 mg g^{-1} in the upper canopy in both clones and under all treatments. In the lower canopy, however, the increase in the Chl:N ratio was suppressed by O_3 at both ambient and elevated CO_2.

The CO_2 response curves indicated changes in the stoichiometry of photosynthetic apparatus primarily in response to elevated O_3, decreasing both V_{cmax} and J_{max} by 30–50% in both clones (Fig. 5). Elevated CO_2, on the other hand, stimulated J_{max} only in the O_3-sensitive clone, and only at ambient O_3. This led to an increase in the J_{max}:V_{cmax} ratio under elevated CO_2 in the O_3-sensitive clone, whereas no such change was observed in the O_3-tolerant clone (Fig. 5, Table 4). However, the O_3-induced decrease was greater in V_{cmax} than J_{max} in both clones, leading to increased J_{max}:V_{cmax} ratio (Fig. 5) at both ambient and elevated CO_2, and indicating that photosynthesis became more limited by carboxylation than electron transport capacity.

4. Discussion

Ozone caused a general decline in leaf nitrogen content and photosynthetic capacity (Table 3) and it particularly decreased the relative share of light harvesting apparatus in the lower canopy (Fig. 4). We did not measure *in situ* stomatal conductance, but generally the ratio of stomatal conductance to actual photosynthesis is higher in the lower than in the upper canopy as revealed by direct and indirect measurements of canopy profiles of C_i:C_a ratio (Garten and Taylor, 1992; Kull and Niinemets, 1998). Consequently, the ratio of carbohydrates produced per ozone uptake is lower, which may decrease the leaf's ability to combat oxidative damage in low light conditions compared to the well-lit conditions in the upper canopy. Furthermore, the greater decrease in Chl content in the lower than upper leaves, and the decrease in Chl:N ratio under elevated O_3 may have further contributed to O_3 damage in the

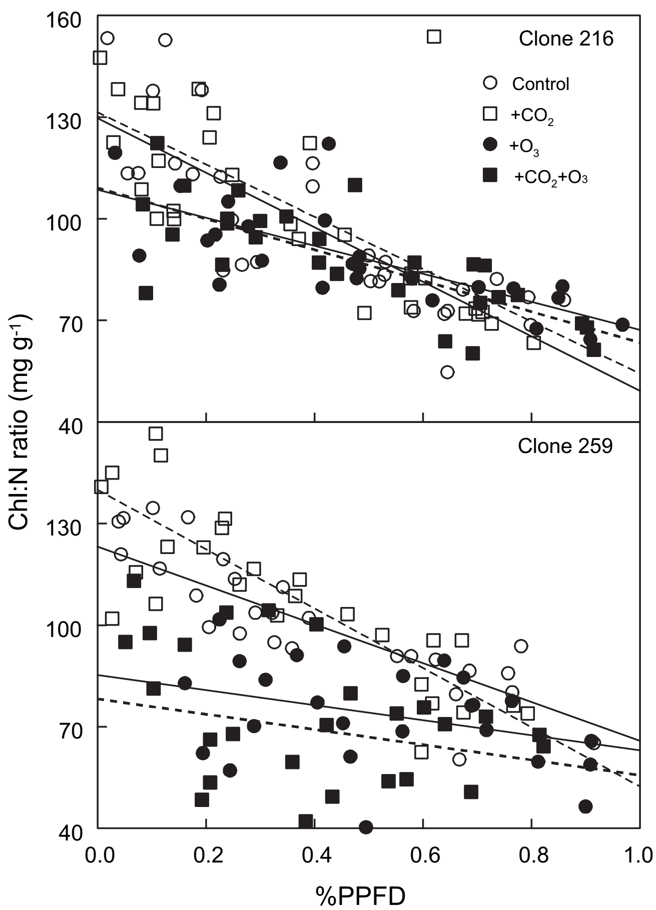

Fig. 4. Canopy profiles of foliar chlorophyll to nitrogen ratios. Data from July and August are combined. Regression lines: solid, ambient CO_2; dashed, elevated CO_2; fine, ambient O_3; bold, elevated O_3.

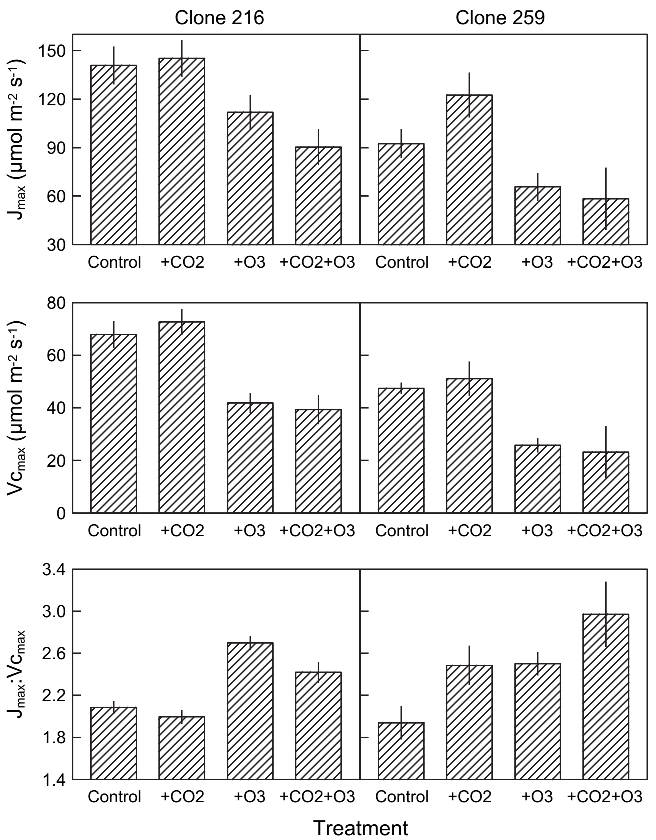

Fig. 5. Transmittance-adjusted mean electron transport capacity (J_{max}), carboxylation capacity (V_{cmax}) and their ratio in two aspen clones. Data from July and August, 2000 (mean ± 1 SE).

lower canopy. The steep decrease in Chl:N ratio and J_{max} imply that the light harvesting component may be the primary target of O_3 damage. Yet, this is at odds with earlier studies, which have attributed the primary response to the carboxylating component (Farage et al., 1991; Noormets et al., 2001). Furthermore, Eichelmann et al. (2005) reported that reallocation of N during shade-adaptation occurs first from carboxylation complexes and only later from light-reaction centres and Chl-associated proteins, and that the adjustment in photosystem I is coupled better to the changes the carboxylating component than it is in photosystem II. In the current study, we also observed an increase in the J_{max}:V_{cmax} ratio at elevated O_3, indicating that the biochemical component of the photosynthesis was damaged more than electron transport apparatus or that the down-regulation of the J_{max} overcompensated for the changes in V_{cmax}. Thus, the observed changes in Chl content and J_{max} may occur in response to declining Rubisco content and V_{cmax} (Rogers and Humphries, 2000).

The decrease in Chl was paralleled by a decrease in apparent quantum yield (Figs. 2 and 3). It has been found that when leaf chlorophyll content decreases below about 250 mg m^{-2}, the absorbance of PAR per leaf area decreases dramatically (Leverenz, 1987). In the current study, Chl did fall below this threshold at elevated O_3 levels, and more so in the O_3-sensitive clone 259 than in the O_3-tolerant clone 216, offering a potential explanation for the decreased ϕ. At the same time, the ratio of ϕ to Chl (Chl efficiency) increased in the O_3-sensitive clone in response to elevated O_3, particularly at elevated CO_2 (data not shown). While this may indicate a coordinated translocation of resources from the senescing leaf to match the contents of the carboxylating component (Fig. 5), the increased Chl efficiency may also just be a by-product of lower optical density of the leaves. Regardless, as assimilation in the lower canopy leaves operates in the linear portion of the light response function (Kull and Kruijt, 1998), the O_3-induced decrease in leaf absorption may have major implications for assimilatory capacity of leaves and their ability to sustain themselves.

It has been proposed that the stoichiometry of the photosynthetic apparatus adjusts to balance the capacities of light harvesting, electron transport and carboxylation in particular canopy conditions (Chen et al., 1993; Hikosaka and Terashima, 1995; Medlyn, 1996). Particularly, nitrogen as one of the most limiting constituents of the photosynthetic machinery is arguably distributed so that the total carbon gain is maximized (Hikosaka and Terashima, 1995). Furthermore, the turnover theory (Thornley, 1998; Kull, 2002) stipulates that the amount of nutrients needed to build photosynthetic tissue are in turn limited by the amount of carbohydrates available for root growth and nutrient acquisition. Given the strong feedback between leaf area and light in a canopy, maximum LAI is determined by light level that at given nitrogen availability still allows positive carbon balance for the lower leaves. Ozone had particularly strong effects on the photosynthetic properties of the lower leaves, thus apparently increasing the light level required where leaves could maintain positive carbon balance (and survive), leading to decreased LAI (Table 1). Lower LAI and leaf photosynthetic performance in turn decrease the energy and carbohydrates needed for nitrogen acquisition leading to decreased plant nitrogen pool and lower leaf nitrogen in all canopy positions.

The optimal distribution principle also suggests that at elevated CO_2 more resources should be allocated to light capture and electron transport, and less into carboxylation related components (Medlyn, 1996; Ainsworth and Long, 2005). At normal conditions photosynthesis is operating near the intersection of electron transport limited and carboxylation limited sections of the A–C_i curve (Farquhar, 1989). Increase in CO_2 concentration leads to the domination of limitation from electron transport and consequently, to optimize the capacities, the ratio of J_{max}:V_{cmax} should increase, which has been observed in many experiments (Farage et al., 1991; Rogers and Humphries, 2000; Ainsworth and Long, 2005). However, in the current study the ratio of J_{max}:V_{cmax} increased only in the O_3-sensitive clone whereas it decreased slightly in the O_3-tolerant clone at both O_3 concentrations. Even though the stimulation of P_{max} in the current study is smaller than observed earlier during more open canopy conditions (Noormets et al., 2001), we were unable to detect distinct acclimation response to elevated CO_2. This may indicate continuing sink strength as acclimation has been associated with source–sink balance. This was elegantly illustrated by Ainsworth et al. (2003), who showed that regular harvesting of foliage in a grassland ecosystem allowed sustained stimulation of photosynthesis by elevated CO_2 over the 10-year study period.

However, even though there was no universal acclimation of photosynthesis, elevated CO_2 suppressed most photosynthetic parameters in clone 216 at elevated but not at ambient O_3 (Table 5, Fig. 5). It is curious that this response was constrained to clone 216, which based on intrinsic photosynthetic capacities is considered relatively tolerant of O_3. In the O_3-sensitive clone 259 the response to elevated CO_2 was more consistent with that reported in literature, and the O_3 effects were comparable at both ambient and elevated CO_2 levels. Given that the ambient O_3 concentrations in Wisconsin, where this study was conducted, are among the lowest in the US and even the 1.5× ambient elevated O_3 treatment is lower than the ambient concentration in many places with stronger anthropogenic influence, the frequently observed CO_2-induced acclimation of photosynthesis could be related to the unrecorded, but likely significant, O_3 concentration in the air.

It still remains unclear what causes differential susceptibility to ozone in the two studied clones. In addition to differences in antioxidant capacities (Wustman et al., 2001) another factor potentially contributing to the differential O_3 sensitivity between the two clones comes from the intrinsically higher photosynthetic capacity in the O_3-tolerant clone. Furthermore, it is possible that photosynthetic capacity, foliar nitrogen content and secondary defence compounds, which correlate in the aspen clones used in current study (Noormets et al., 2001; Wustman et al., 2001) are functionally related (Polle et al., 2000). Therefore, it may be possible that O_3 tolerance is related to greater allocation to roots allowing better nutrient acquisition. While there is no clone-level data to allow testing this hypothesis, the treatment differences in root biomass (King et al., 2001) lend circumstantial support for this hypothesis.

5. Conclusions

A strong O_3 effect on leaf Chl and N content as well as on ϕ and P_{max} accumulated over time. This effect on Chl and ϕ was stronger in the lower canopy of shaded leaves. The effect of elevated CO_2 on these leaf parameters was small at ambient O_3, but at elevated O_3 CO_2 exaggerated the negative effects on Chl and ϕ. The evidence about the primary target of O_3-induced damaged was contradictory, as significant decreases in leaf chlorophyll content, Chl:N ratio and ϕ were accompanied by an increase in J_{max}:V_{cmax} ratio. The effect of elevated CO_2 on photosynthesis depended on clone as well as O_3 level. In the O_3-sensitive clone the responses to CO_2 conformed with theoretical expectations as based on optimality and turnover theories, and the response to O_3 was comparable at both CO_2 levels. In the O_3-tolerant clone, however, the slight stimulation of leaf photosynthetic properties by elevated CO_2 at ambient O_3 levels was replaced by a significant down-regulation at elevated O_3, implying potential connection between photosynthetic acclimation and background oxidant levels.

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