Interactions of chronic exposure to elevated CO$_2$ and O$_3$ levels in the photosynthetic light and dark reactions of European beech (Fagus sylvatica)

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SUMMARY

Young trees of European beech (Fagus sylvatica) acclimated for one growing season to ambient (c. 367 µl l$^{-1}$) or elevated CO$_2$ levels (c. 660 µl l$^{-1}$) were exposed during the subsequent year to combinations of the same CO$_2$ regimes and ambient or twice-ambient ozone (O$_3$) levels (generated from the database of a rural site). By the end of June, before the development of macroscopic leaf injury, the raised O$_3$ levels had not affected the light and dark reactions of photosynthesis. However, acclimation to elevated CO$_2$ had resulted in lowered chlorophyll and nitrogen concentrations, whereas photosynthetic performance, examined over a wide range of parameters from light and dark reactions, remained unchanged or showed only slight reductions (e.g. apparent electron transport rate, ETR; apparent quantum yield of CO$_2$ light and dark reactions, remained unchanged or showed only slight reductions (e.g. apparent electron transport rate, ETR; apparent quantum yield of CO$_2$ gas exchange, $\Phi_{\text{CO}_2}$; apparent carboxylation efficiency, CE; and photosynthetic capacity at light and CO$_2$ saturation, PC). In August, after the appearance of leaf necroses, plants grown under ambient CO$_2$ and twice-ambient O$_3$ conditions declined in both the photosynthetic light reactions (optimum electron quantum yield, $F_v/F_m$, non-photochemical energy quenching, NPQ, reduction state of Q$_A$, apparent electron quantum yield, $\Phi_{\text{qmol}}$, maximum electron transport rates) and the dark reactions as reflected by CE, $\Phi_{\text{qmol}}$ as well as the maximum CO$_2$ uptake rate (i.e. PC). CE, $\Phi_{\text{qmol}}$ and PC were reduced by 75, 40 and 75%, respectively, relative to plants exposed to ambient CO$_2$ and O$_3$ levels. By contrast, plants exposed to twice-ambient O$_3$ and elevated CO$_2$ levels maintained a photosynthetic performance similar to individuals grown either under ambient CO$_2$ and ambient O$_3$ or elevated CO$_2$ and ambient O$_3$ conditions. The long-term exposure to elevated CO$_2$ therefore tended to counteract adverse chronic effects of enhanced O$_3$ levels on photosynthesis. Possible reasons for this compensatory effect in F. sylvatica are discussed.

Key words: European beech (Fagus sylvatica), elevated CO$_2$, ozone (O$_3$), chlorophyll fluorescence, gas exchange, photosynthesis, stomatal conductance, chronic effects.

INTRODUCTION

The increase in the atmospheric carbon dioxide (CO$_2$) concentration from the pre-industrial 280 µl l$^{-1}$ to the present 360 µl l$^{-1}$ (Neftel et al., 1985), arising from extensive burning of fossil fuels and deforestation mainly in the tropics (IPCC, 1996), has progressed since 1980 by c. 1.5 µl l$^{-1}$ yr$^{-1}$ (Keeling et al., 1995). CO$_2$ levels are expected to exceed 700 µl l$^{-1}$ by the end of the next century (King et al., 1992; IPCC, 1996), while tree response to elevated CO$_2$ is still under debate (Saxe et al., 1998). Various studies have shown that short-term exposure of trees to elevated CO$_2$ for less than one growing season may increase photosynthesis and biomass production.
(Eamus & Jarvis, 1989; Long, 1991; Ceulemans & Mousseau, 1994), but attention has only recently been directed towards longer-term studies. In European beech (Fagus sylvatica), these longer term studies revealed minor down-regulation in photosynthesis (Epron et al., 1996) or even the absence of any effect (El Kohen et al., 1993; Mousseau et al., 1996; Heath & Kerstiens, 1997), while biomass production could be increased by changes in the whole-plant carbon allocation (El Kohen et al., 1993; Rey & Jarvis, 1997; Curtis & Wang, 1998). Remarkably, stomatal conductance was often unaffected or showed only minor reductions (Drake et al., 1996; Heath & Kerstiens, 1997; Curtis & Wang, 1998; Saxe et al., 1998). It is most likely that these findings represent a general response pattern of deciduous trees to extended high CO₂ supply (Curtis & Wang, 1998; Saxe et al., 1998).

Tropospheric ozone (O₃) has also increased, far above pre-industrial levels, during the past century (on average by a factor of two to four, and even higher with respect to peak concentrations; Stockwell et al., 1997). Chronic exposure to enhanced O₃ levels is regarded to be injurious, in particular, to broad-leaf trees including F. sylvatica, as a decline in photosynthesis, premature leaf loss and increased dark respiration may limit the biomass production (Matyssek et al., 1991, 1993; Pearson & Mansfield, 1993, 1994; Mikkelsen, 1995; Lippert et al., 1996b; Mikkelsen & Heide-Jørgensen, 1996; Langebartels et al., 1997; Zeuthen et al., 1997; Skárby et al., 1998). Plant sensitivity to ozone has often been found to relate to the stomatal conductance, as high O₃ influx through the stomata may increase the risk of injury (Reich, 1987; Barnes & Wellburn, 1998; Volin et al., 1998). Although both elevated CO₂ and chronic O₃ exposure have the potential of reducing stomatal aperture (Ceulemans & Mousseau, 1994; Matyssek et al., 1995), interactions on and, thus, impact of O₃ uptake, have rarely been investigated with respect to broad-leaf trees.

In herbaceous crop species, elevated CO₂ is known to counteract adverse effects of O₃ on photosynthesis and production (McKee et al., 1995, 1997a; Mulholland et al., 1997; Reid & Fiscus, 1998), which is ascribed, at least in part, to a CO₂-induced decline in stomatal conductance (Allen, 1990; McKee et al., 1995, 1997b; Fiscus et al., 1997; Volin et al., 1998). Neither in young Norway spruce (Barnes et al., 1995; Lippert et al., 1996a, 1997; Pfrimmer et al., 1996; Schner et al., 1998) nor in O₃-tolerant and O₃-sensitive aspen clones (Kull et al., 1996), however, could any counteracting effect of elevated CO₂ on O₃-induced damage be detected. In oak and poplar species, CO₂-induced compensation of O₃ impact appeared to be mediated through stomatal narrowing similar to that found in crop plants (Volin & Reich, 1996; Manes et al., 1998; Volin et al., 1998), although the type and extent of the tree response might depend on the species and experimental conditions (Saxe et al., 1998). The issue of CO₂-O₃ interaction has not been addressed, however, for F. sylvatica, one of the most important broad-leaf tree species in Central Europe.

The present study aims, therefore, to clarify whether long-term acclimation to elevated CO₂ may counteract the impact of chronic O₃ exposure in the photosynthetic performance of F. sylvatica, and if such a compensation might be mediated by stomatal limitation on O₃ uptake. The analysis focuses on the light and dark reactions of photosynthesis, that is chlorophyll fluorescence and leaf gas exchange.

**MATERIAL AND METHODS**

**Plants and treatments**

One-yr-old plants of European beech (F. sylvatica L.) grown from seeds were planted in 1995 into 10-l pots containing a mixed soil (1/3 quartz sand and 2/3 B-horizon of a dystric cambisol from the Bavarian Forest) and transferred into a shaded glasshouse. Plants were amply provided with deionized water and fertilized regularly during irrigation to ensure non-limiting water and nutrient supply throughout the entire experiment. During the second growing season in 1995, plants were acclimated in the glasshouse to ambient (c. 367 μl l⁻¹; Amb. CO₂) or elevated (ambient +300 μl l⁻¹; +300 CO₂) CO₂ concentrations. During the winter months of 1994–95 and 1995–96, plants were placed outside the glasshouse and exposed to field conditions. In April 1996 (before bud break), the plants were moved back into the glasshouse to their corresponding CO₂ regimes, and, on 2 May, transferred into the four phytotrons of the GSF National Research Center (Payer et al., 1993). Here, the soil moisture of each pot was continuously controlled by tensiometers, triggering irrigation whenever soil water tension had reached 300 hPa. The amounts of water applied to each pot throughout the year to compensate for evapotranspiration were integrated by 1-wk intervals. Whole-tree transpiration rate was related to the actual foliage area present each week and corrected for soil evaporation (estimated 40 ml per pot per d; Jungermann, 1998).

Each phytotron was divided into four sub-chambers (made of plexiglass and containing six plants each) with independent fumigation control to allow the two CO₂ treatments to be combined with the fluctuating regimes of ambient (1×O₃) or proportionally increased, twice-ambient O₃ concentrations (2×O₃). In total, each of the resulting four CO₂/O₃ regimes (i.e. Amb. CO₂/1×O₃; Amb. CO₂/2×O₃; +300 CO₂/1×O₃ and +300 CO₂/2×O₃) was replicated four times across the phytotrons. The 1×O₃ regime was based on the 2-h means.
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Fig. 1. Time course of (a) daily mean and (b) maximum ozone concentrations as measured inside the fumigation chambers during the growing season. Data are means from eight fumigation chambers. Black bars indicate the measurement periods, with AOT 40 (accumulated O₃ exposure above a threshold of 40 nl l⁻¹) values at the beginning and end of each period.

Assessment of leaf gas exchange

Measurements were conducted in 1996 on one individual, fully developed, attached and unshaded leaf per tree during early summer (18 June–7 July, before high O₃ levels in mid-July resulted in visible leaf injury) and midsummer (11–26 August, after O₃-caused leaf necroses had established; Fig. 2). Expressing the ‘critical level for ozone’ according to Kärenlampi & Skärby (1996) as an AOT 40 dose (i.e. accumulated O₃ exposure above a threshold of 40 nl l⁻¹), a value of 50 nl l⁻¹ h, as found by Lippert et al. (1996b) to lower the photosynthetic capacity of F. sylvatica by 10%, had not been reached during the first measurement period (Fig. 1). By the beginning of the second measurement period, however, AOT 40 of the 2 × O₃ treatment had amounted to 89.1 nl l⁻¹ h in the presence of visual leaf injury. The steady-state dependence of the leaf gas exchange (net CO₂ uptake rate and stomatal conductance) on the CO₂ concentration (A/ci-curves) and PPFR (light response curves) was assessed within the phytotrons, using a mini-cuvette system (H. Walz, Effeltrich, Germany) and a CO₂/H₂O diffusion porometer (HCM-1000, H. Walz). Measurements were performed during the morning hours under standardized conditions (O₂-free air of 20.0°C, dewpoint at 10.0°C), resulting in a relative humidity of c. 53% inside the gas exchange...
Fig. 2. Characteristic necrotic lesions on four different leaves of *Fagus sylvatica* exposed to the four fumigation regimes: (a) Amb. CO$_2$/1 × O$_2$; (b) Amb. CO$_2$/2 × O$_2$; (c) +300 CO$_2$/1 × O$_2$; (d) +300 CO$_2$/2 × O$_2$. Pictures were taken on August 13 (ambient CO$_2$ levels) and August 28 (+300 CO$_2$ levels).

cuvette (cf. Lange et al., 1986). Light responses of leaf gas exchange were measured at PPFR of c. 0, 5, 40, 80, 150 and 550 μmol m$^{-2}$ s$^{-1}$ and saturating CO$_2$ supply (1900 μl l$^{-1}$), whereas responses to CO$_2$ supply were assessed at c. 50, 150, 250, 350 and 2000 μl l$^{-1}$ and saturating PPFR (1800 μmol m$^{-2}$ s$^{-1}$). Photosynthetic capacity was derived from both saturating PPFR and CO$_2$ conditions; apparent carboxylation efficiency (CE) and quantum yield (Φ$_{\text{CO}_2}$) were calculated as already explained from the linear sections of the photosynthetic CO$_2$ and light response functions, respectively. A total of 64 response functions were recorded for each of light and CO$_2$ dependence of the leaf gas exchange. Each measured leaf was harvested to determine its area and concentrations of chlorophyll and nitrogen (see later section). Rates of gas exchange were calculated according to von Caemmerer & Farquhar (1981).

Assessment of chlorophyll a fluorescence

Chlorophyll fluorescence of leaves of the same plants that were subjected to the gas exchange analysis was assessed with a pulse-amplitude modulation fluorometer (PAM 2000, H. Walz), resulting in a total of 64 response functions to PPFR. The leaf was positioned with a clip at a constant distance (approx. 1 cm) and angle (60°) to the fibre optics of the fluorometer (Bilger et al., 1995). PPFR was determined closely above the leaf surface by means of a micro-quantum sensor that had been calibrated with a LI-COR 190 sensor (Li-Cor Inc., Lincoln, NE, USA) as a reference. In all treatments, light responses were measured during the early morning (to prevent photoinhibition by high midday irradiance) and at ambient CO$_2$, while leaf temperature was 23.2 ± 0.3°C (mean ± SE) and 24.3 ± 0.1°C in June/July and August, respectively. The sequence of readings was started after 30 min of dark adaptation with the determination of minimum and maximum fluorescence, $F_o$ and $F_m$, and continued with the assessment of responses to PPFR levels of c. 8, 12, 20, 30, 50, 105, 150, 230, 330, 480 and 1500 μmol m$^{-2}$ s$^{-1}$ using the internal LED and halogen lamp of the PAM 2000 as a light source. Each level of PPFR was applied for 6.5 min to ensure steady-state in the photosynthetic response. Parameters of chlorophyll fluorescence were calculated as described by van Kooten & Snel (1990; non-photochemical quenching, NPQ; reduction state of Q$_A$, 1 – q$_P$), Schreiber & Bilger (1993; optimum electron quantum yield, $F_o/F_m$; effective electron quantum yield, Δ$F/F_m$) and Bilger et al. (1995; relative excessive PPFR as a measure for the amount of light absorbed by the leaf that was not used for photosynthesis). The apparent electron transport rate through PSII
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Fig. 3. Light response curves of apparent electron transport rate of Fagus sylvatica in August grown under ambient ozone levels in combination with (a) ambient and (b) +300 CO$_2$ regimes. Data shown are means (± 1 SE) from eight light response curves of eight different plants per treatment. All measurements were conducted under ambient CO$_2$ concentrations.

(ETR) was estimated according to Krall & Edwards (1992) as $\text{ETR} = \Delta F/F^m \times \text{PPFR} \times a \times f$, assuming the absorptivity of photosynthetic active radiation, $a$, in the leaves of F. sylvatica as 0.84, and setting the light distribution factor between photosystem I and II, $f$, as 0.5 (cf. Krall & Edwards, 1992).

Analysis of leaf chlorophyll and nitrogen

After completion of the gas exchange analysis in the mini-cuvette, each measured leaf was excised and frozen immediately in liquid nitrogen to be stored at −80°C. Chlorophyll concentration was determined according to Porra et al. (1989) after chlorophyll extraction in 80% acetone. Nitrogen concentration was assessed by elementary analysis in samples of dried leaf material (Schramel, 1988).

Statistical analysis

Response curves of chlorophyll fluorescence and gas exchange to variable PPFR and CO$_2$ supply were fitted by means of the Sigma Plot 3.0 fit function (Jandel Scientific, Erkrath, Germany):

$$f(x) = a \times (1-e^{-bx}) + c$$

According to this equation, the photosynthetic capacity at saturating light and CO$_2$ supply was calculated as the saturation level ($a+c$) of the fitted CO$_2$ response curves. The quantum yield of net CO$_2$-uptake ($\Phi_{\text{CO}_2}$) was calculated from the PPFR response function of the net CO$_2$ uptake rate as the first derivative at 50 µmol photons m$^{-2}$ s$^{-1}$, and CE was derived accordingly from the CO$_2$ response function at 100 µl l$^{-1}$ CO$_2$. For fitting the light response of ETR, $c$ was set to zero, while saturation level and initial slope (at 50 µmol photons m$^{-2}$ s$^{-1}$) were derived according to the analysis of the gas exchange data (cf. Fig. 3). Means of each fumigation treatment ($n = 8$) were compared through two-factorial (CO$_2$ and O$_3$) analysis of variance (ANOVA), using the Statistical Analysis Software (SAS version 6.12, SAS Institute Inc., NC, USA). Subsequently, the Tukey Studentized Range Test was applied whenever the null hypothesis was rejected (Dufner et al., 1992).

RESULTS

The optimum electron quantum yield ($F^m/F^v$) did not show significant differences in June/July between the treatments (Table 1/1), whereas acclimation to elevated CO$_2$ had raised, to some extent, the relative excessive PPFR (Table 1/2). This latter effect may relate to the significantly raised reduction state of Q$_A$ at light saturation (Table 1/4). Reduced electron transport was also indicated by the lowered saturation level of ETR after acclimation to +300 CO$_2$ (Table 1/6). However, non-photochemical quenching (NPQ) was not increased at elevated relative to ambient CO$_2$ (Table 1/3). Likewise, the apparent quantum yield of ETR ($\Phi_{\text{pq}}$) and CO$_2$ uptake ($\Phi_{\text{CO}_2}$) remained unchanged (Table 1/6,7). Regarding the photosynthetic dark reactions, acclimation to +300 CO$_2$ induced minor reductions in CE and PC (Table 1/8,9). Overall, effects by elevated CO$_2$ were rather small and not significant in June/July, while O$_3$ impact was not detectable in leaf photosynthesis. Furthermore, leaves did not display macroscopic injury in June/July (not shown).

During both measurement periods (June/July and August), the fresh-weight : area ratio of leaves (f. wt : a) were slightly but significantly increased in +300 CO$_2$ relative to Amb. CO$_2$ plants (Tables 2, 3), in the absence of any apparent ozone effect. The fresh-weight related chlorophyll (a and b) concentration displayed an overall decline from June/July to August, with the lowest levels occurring in leaves of +300 CO$_2$ plants at each sampling date. However, chlorophyll a:b-ratios remained unchanged during the annual course with slightly
Table 1. Photosynthetic parameters of Fagus sylvatica leaves under four different fumigation regimes in June/July

<table>
<thead>
<tr>
<th>No.</th>
<th>Photosynthetic parameter</th>
<th>Evaluated part of the curve</th>
<th>Fumigation treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amb. CO₂</td>
</tr>
<tr>
<td>1</td>
<td>Optimum electron quantum yield ($F_{v}/F_{m}$)</td>
<td>–</td>
<td>1 x O₂</td>
</tr>
<tr>
<td>2</td>
<td>Relative excessive PPFR</td>
<td>Initial slope</td>
<td>0.0026 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saturation level</td>
<td>0.74 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>Non-photochemical-quenching (NPQ)</td>
<td>Initial slope</td>
<td>0.0048 ± 0.0009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>2.00 ± 0.26</td>
</tr>
<tr>
<td>4</td>
<td>Reduction state of $Q_{A}$ (1 – qₐ)</td>
<td>Initial slope</td>
<td>0.0019 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saturation level</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>Apparent electron quantum yield ($Φ_{PSII}$) (mole electrons mol⁻¹ photons⁻¹)</td>
<td>Initial slope</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>Apparent electron transport rate (ETR) (µmol m⁻² s⁻¹)</td>
<td>Saturation level</td>
<td>124 ± 15</td>
</tr>
<tr>
<td>7</td>
<td>Apparent quantum yield of CO₂ gas exchange ($Φ_{CO₂}$) (µmol CO₂ mol⁻¹ photons)</td>
<td>Initial slope</td>
<td>0.042 ± 0.004</td>
</tr>
<tr>
<td>8</td>
<td>Carboxylation efficiency (CE) (µmol CO₂ m⁻² s⁻¹ µl⁻¹ CO₂)</td>
<td>Initial slope</td>
<td>0.041 ± 0.005</td>
</tr>
<tr>
<td>9</td>
<td>Photosynthetic capacity of CO₂ gas exchange (PC) (µmol m⁻² s⁻¹)</td>
<td>Saturation level</td>
<td>12.9 ± 1.0</td>
</tr>
</tbody>
</table>

$F_{v}/F_{m}$ values were derived from measurements under growth conditions. Initial slopes and saturation levels of light response curves of chlorophyll fluorescence were measured under ambient CO₂-concentrations (relative excessive PPFR, NPQ, reduction state of $Q_{A}$, apparent electron quantum yield, ETR), light response curves of CO₂-exchange were recorded under CO₂-saturating conditions (apparent quantum yield of CO₂ gas exchange and CO₂-response curves were conducted under light saturating conditions (PC and CE). Data are expressed as means ± 1 SE, n = 8.
Table 2. Fresh weight to area ratio (f. wt:a), fresh weight related chlorophyll content (Chl a+b), chlorophyll a:b ratio and dry weight related nitrogen concentration of leaves of Fagus sylvatica used in gas exchange measurements during June/July and August

<table>
<thead>
<tr>
<th>Parameter</th>
<th>June/July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amb. CO₂ 1×O₃</td>
<td>Amb. CO₂ 2×O₃</td>
</tr>
<tr>
<td>f. wt:a (g m⁻²)</td>
<td>100.04±2.41</td>
<td>102.99±2.54</td>
</tr>
<tr>
<td>Chl a+b (µg g⁻¹ f. wt)</td>
<td>1939.22±138.56</td>
<td>1839.12±123.18</td>
</tr>
<tr>
<td>Chl a:b ratio</td>
<td>4.33±0.15</td>
<td>3.84±0.13</td>
</tr>
<tr>
<td>N (mg g⁻¹ d. wt)</td>
<td>17.3±0.8</td>
<td>18.1±0.8</td>
</tr>
<tr>
<td>Chl a (µg g⁻¹ f. wt)</td>
<td>1436.14±101.82</td>
<td>1218.97±47.08</td>
</tr>
<tr>
<td>Chl a:b ratio</td>
<td>3.84±0.16</td>
<td>3.35±0.32</td>
</tr>
<tr>
<td>N (mg g⁻¹ d. wt)</td>
<td>16.6±0.8</td>
<td>16.2±0.4</td>
</tr>
</tbody>
</table>

Data are expressed as means±1 SE, n = 16.

Table 3. Main effects of CO₂, O₃ and CO₂–O₃ interactions (CO₂×O₃) on leaf characteristics and photosynthetic parameters of Fagus sylvatica in June/July and August

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Evaluated part of the curve</th>
<th>June/Jul</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>O₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO₂</td>
<td>O₃</td>
</tr>
<tr>
<td>Fresh weight to area ratio (g m⁻²)</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chlorophyll a+b (µg g⁻¹ f. wt)</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chlorophyll a:b ratio</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N (mg g⁻¹ d. wt)</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Optimal electron quantum yield (Fᵥ/Fₚₚ)</td>
<td></td>
<td>Initial slope</td>
<td>–</td>
</tr>
<tr>
<td>Relative excessive PPFR</td>
<td></td>
<td>Saturation level</td>
<td>–</td>
</tr>
<tr>
<td>Non-photochemical-quenching (NPQ)</td>
<td></td>
<td>Initial slope</td>
<td>–</td>
</tr>
<tr>
<td>Reduction state of Qₐ (1–qₐ)</td>
<td></td>
<td>Initial slope</td>
<td>–</td>
</tr>
<tr>
<td>Apparent quantum yield (Φᵥ) (mol electrons mol⁻¹ photons)</td>
<td></td>
<td>Initial slope</td>
<td>–</td>
</tr>
<tr>
<td>Maximum apparent electron transport rate (ETR) (µmol m⁻² s⁻¹)</td>
<td></td>
<td>Saturation level</td>
<td>–</td>
</tr>
<tr>
<td>Apparent quantum yield of CO₂ gas exchange (Φᵥ(O₂)) (µmol CO₂ mol⁻¹ photons)</td>
<td></td>
<td>Initial slope</td>
<td>–</td>
</tr>
<tr>
<td>Carboxylation efficiency (CE) (µmol CO₂ m⁻² s⁻¹ µl⁻¹ CO₂)</td>
<td></td>
<td>Initial slope</td>
<td>–</td>
</tr>
<tr>
<td>Photosynthetic capacity of CO₂ gas exchange (PC) (µmol µmol⁻² s⁻¹)</td>
<td></td>
<td>Saturation level</td>
<td>–</td>
</tr>
</tbody>
</table>

Significant levels of main effects are classified as *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Reduced values at Amb. CO₂/2×O₃. At each date, the leaf nitrogen concentration (expressed on a dry-weight basis) was also significantly reduced by +300 CO₂, while no effect of ozone was detectable (Tables 2, 3).

By August, spot-like brownish necroses had mainly developed at the Amb. CO₂/2×O₃ treatment, whereas such symptoms were less pronounced at +300 CO₂/2×O₃ and +300 CO₂/1×O₃, and injury was hardly visible at Amb. CO₂/1×O₃. Characteristic ozone-induced necroses in each treatment are shown in Fig. 2. Plants grown at Amb. CO₂/2×O₃ had, in August, distinctly reduced values of ETR (i.e. lower initial slope and saturation level of the light dependence) compared with plants of the Amb. CO₂/1×O₃ regime (Fig. 3a). At +300 CO₂/1×O₃, initial slope and saturation level of the light dependence of ETR were slightly increased relative to those of the Amb. CO₂/1×O₃ plants (Figs 3a,b). In addition, the reduction in ETR as induced by 2×O₃ was less pronounced in the presence of elevated CO₂ than at ambient CO₂ supply. Light and CO₂ response curves of other photosynthetic parameters, analysed for their initial slopes and saturation levels, proved to be highly affected by ozone (Tables 3, 4). The optimum electron quantum yield (Fᵥ/Fₚₚ) was only c.
Table 4. Photosynthetic parameters of *Fagus sylvatica* leaves under four different fumigation regimes in August, otherwise as Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Photosynthetic parameter</th>
<th>Evaluated part of the curve</th>
<th>Fumigation treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amb. CO₂ 1 × O₂</td>
</tr>
<tr>
<td>1</td>
<td>Optimum electron quantum yield ($F_v/F_m$)</td>
<td>–</td>
<td>0.70 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Relative excessive PPFR</td>
<td>Initial slope</td>
<td>0.00020 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saturation level</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>Non-photochemical-quenching (NPQ)</td>
<td>Initial slope</td>
<td>0.0053 ± 0.0009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maximum</td>
<td>1.87 ± 0.14</td>
</tr>
<tr>
<td>4</td>
<td>Reduction state of Qₐ (1 – qₑ)</td>
<td>Initial slope</td>
<td>0.0022 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Saturation level</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>Apparent electron quantum yield (Φᵦᵦ) (mole electrons mol⁻¹ photons⁻¹)</td>
<td>Initial slope</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>Apparent electron transport rate (ETR) (μmol m⁻² s⁻¹)</td>
<td>Saturation level</td>
<td>113 ± 6</td>
</tr>
<tr>
<td>7</td>
<td>Apparent quantum yield of CO₂ gas exchange (Φᵦᵦ) (mol CO₂ mol⁻¹ photons)</td>
<td>Initial slope</td>
<td>0.37 ± 0.002</td>
</tr>
<tr>
<td>8</td>
<td>Carboxylation efficiency (CE) (μmol CO₂ m⁻² s⁻¹ μl⁻¹ CO₂)</td>
<td>Initial slope</td>
<td>0.024 ± 0.002</td>
</tr>
<tr>
<td>9</td>
<td>Photosynthetic capacity of CO₂ gas exchange (PC) (μmol m⁻² s⁻¹)</td>
<td>Saturation level</td>
<td>5.9 ± 0.5</td>
</tr>
</tbody>
</table>
CO₂ saturation level of ETR by a factor of two at ambient conditions by elevated CO₂ affected CE or PC, which was distinctly reduced, however, by ozone in August (Fig. 4a). Elevated CO₂ prevented reduction of both, CE and PC, by the 2 × O₃ regime, and low O₃ stress enabled the high CO₂ supply to slightly increase CE.

The transpiration rate of all plants was highest in July (Fig. 5). During the first half of the growing season, plants exposed to ambient CO₂ tended to show, regardless of the ozone regime, a significantly higher transpiration rate than plants at +300 CO₂, whereas in August and early September the influence of CO₂ supply appeared to diminish. Throughout the whole period depicted in Fig. 5, trees grown at +300 CO₂ transpired c. 25% less water (73.0 and 75.11 m⁻² at 1 × O₃ and 2 × O₃, respectively) than did plants at Amb. CO₂ (98.8 and 101.31 m⁻² at 1 × O₃ and 2 × O₃, respectively). In June/July the stomatal conductance (g_H₂O) ranged between 90 and 180 mmol m⁻² s⁻¹ in all treatments, when the apparent CO₂ concentration in the intercellular space of the mesophyll (c_i) was <300 μl l⁻¹ (Fig. 6a,b). At high c_i, however, g_H₂O was lowered to 50 mmol m⁻² s⁻¹ in plants grown at 2 × O₃ regardless of the CO₂ regime as well as in plants grown at +300 CO₂/1 × O₃. The stomatal conductance stayed as high as 100 mmol m⁻² s⁻¹ in plants exposed to the Amb. CO₂/1 × O₃ regime. In August, plants exposed to 2 × O₃ displayed, at high c_i, levels of g_H₂O being doubled relative to the findings in June/July. At 1 × O₃ and at c_i <300 μl l⁻¹ for plants exposed to 2 × O₃, seasonal changes in g_H₂O were less pronounced (Fig. 6d). However, plants grown at Amb. CO₂/1 × O₃ had reached the highest g_H₂O levels between 140–170 mmol m⁻² s⁻¹ in August (Fig. 6c).

0.6 at 2 × O₃ and c. 0.7 at 1 × O₃, regardless of the CO₂ supply (Table 4/1). Subsequently, plants exposed to 2 × O₃ displayed, at low irradiance, increases in the relative excessive PPFR (Table 4/2), whereas the saturation level remained unchanged in all treatments. Significant increases were also found in the light dependence of the reduction state of QA (Table 4/4), with the saturation level being highest at Amb. CO₂/2 × O₃. The +300 CO₂/2 × O₃ exposure did not result in substantial changes of NPQ (Table 4/3) relative to the Amb. CO₂/1 × O₃ treatment. However, NPQ was reduced at +300 CO₂/1 × O₃ and low irradiance. By contrast, the Amb. CO₂/2 × O₃ regime raised NPQ under low-light conditions by c. 135% , and the saturation level was increased by c. 40% compared with Amb. CO₂/1 × O₃. Doubling of the O₃ concentration significantly lowered both the quantum yield and the light saturation level of ETR by a factor of two at ambient CO₂ concentration (Table 4/5 and 6), and these O₃ effects were paralleled in the CO₂ uptake rate by a marked decline in Φ_{CO₂} under ambient rather than elevated CO₂ conditions (Table 4/7). CE and PC were affected most by the enhanced O₃ regime (significant reductions of c. 75% each, Table 4/8,9) but the O₃ effects were strongly counterbalanced by elevated CO₂ supply. When the O₃ impact was low, elevated CO₂ supply slightly increased ETR at both low and high-light conditions (Table 4/5,6), although Φ_{CO₂} dropped by c. 40% relative to the Amb. CO₂/1 × O₃ regime (Table 4/7). The statistical analysis indicated, in June/July, CO₂–O₃ interaction in the chlorophyll a : b ratio and, in August, in the leaf differentiation and reduction state of Qₐ, PC and Φ_{CO₂} (Table 3). In addition to the main effects on leaf differentiation, elevated CO₂ in August also caused NPQ, Φ_{PSII}, maximum ETR and CE to respond significantly. The enhanced O₃ regime displayed main effects on most of the investigated photosynthetic parameters only in August, but, as already seen in June/July, did not influence the leaf differentiation (Table 3).

To exclude bias by necroses, CE and PC, which appeared to be strongly affected by the CO₂/O₃ regimes when expressed on a leaf area basis, were also related to the chlorophyll concentration of the living leaf tissue (Fig. 4). This recalculation confirmed the conclusions drawn before: in June/July, neither elevated CO₂ nor O₃ levels significantly affected CE or PC, which was distinctly reduced, however, by ozone in August (Fig. 4a). Elevated CO₂ prevented reduction of both, CE and PC, by the 2 × O₃ regime, and low O₃ stress enabled the high CO₂ supply to slightly increase CE.

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Fig. 4. Carboxylation efficiency (a) and photosynthetic capacity (b) as leaf chlorophyll in June/July and August. Each bar represents a mean (±1 SE) of eight measurements of eight different plants. Treatments: Amb, CO₂/1 × O₃, open bars; Amb. CO₂/2 × O₃, diagonally-hatched bars; +300 CO₂/1 × O₃, bars with horizontal lines; +300 CO₂/2 × O₃ cross-hatched bars.
Fig. 5. Transpiration rates of *Fagus sylvatica* from 22 May to 4 September 1996 as derived from the amounts of irrigation water supplied each week. Data are expressed as means ± 1 SE, n = 16. Significance levels between treatments (same O$_3$ levels, Amb. CO$_2$ vs. +300 CO$_2$) according to Student's t-test: *, P < 0.05; **, P < 0.01; ***, P < 0.001. Ambient CO$_2$/1 × O$_3$ (open circles); +300 CO$_2$/2 × O$_3$ (closed squares); ambient CO$_2$/2 × O$_3$ (closed circles); +300 CO$_2$/1 × O$_3$ (open squares).

**Discussion**

While the nitrogen concentration of the beech leaves was stable throughout the year under the ambient CO$_2$ regime, chlorophyll levels showed some decrease in August compared with June/July. However, long-term acclimation to elevated CO$_2$ resulted in a significant decrease in both the chlorophyll and N levels (cf. Tables 2, 3), as previously shown in other studies on trees (Eamus *et al*., 1995; Epron *et al*., 1996; Curtis & Wang, 1998), and by August, the chlorophyll a : b ratio was increased at elevated CO$_2$ supply (cf. Table 2). Nevertheless, most parameters of electron transport (cf. Table 1) remained unchanged, with the exception, however, of the reduction state of Q$_A$ which was increased at photosynthetic light saturation. This increase probably resulted from analysing the light dependence of chlorophyll fluorescence under ambient CO$_2$ supply, which caused an approx. 20% decrease in ETR between the photosystems and may have led to an electron overflow at PSII. However, Ψ$_{CO_2}$, CE and PC were hardly reduced at elevated CO$_2$ supply, which has been confirmed by C. Lütz (pers. comm.) who also found, in the same experimental plants, a rather stable PC when assessed as photosynthetic oxygen evolution. Slight reductions in Ψ$_{CO_2}$, CE and PC under elevated CO$_2$ supply are in agreement with earlier reports on *F. sylvatica* (El Kohen *et al*., 1993; Epron *et al*., 1996; Mousseau *et al*., 1996; Grams & Matyssek, 1999) and with a recent meta-analysis by Curtis & Wang (1998) of >500 reports on CO$_2$ effects in trees.

In August, O$_3$ effects became apparent in the photosynthetic performance of the beech plants (cf. Fig. 2, Tables 3, 4) and were most probably the consequence of high episodic O$_3$ levels which had occurred since mid-July (cf. Fig. 1). Macroscopic O$_3$ injury of leaves (i.e. bronze discoloration and spot-like necroses) became visible towards the end of July, and premature leaf loss set in during early August (S. Anegg & C. Langebartels, pers. comm.). Lippert *et al*., (1996b) observed a similar progression of O$_3$ injury when beech was exposed to similar growth conditions at ambient CO$_2$ and four different O$_3$ regimes between 0.2 and 2.0 × ambient O$_3$. Also the O$_3$-induced decline in CE, PC, Ψ$_{CO_2}$ was confirmed by Lippert *et al*., (1996b) and was consistent with several other studies on *F. sylvatica* (Mikkelsen, 1995; Zeuthen *et al*., 1997; Dixon *et al*., 1998) and other tree species (Matyssek *et al*., 1991, 1993; Samuelson, 1994; Kull *et al*., 1996; Lippert *et al*.,
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Fig. 6. CO$_2$ response curves of stomatal conductance of Fagus sylvatica in June/July (a, b) and August (c, d) grown under ambient (open symbols) and twice-ambient (closed symbols) ozone exposure and in combination with ambient (circles) or +300 CO$_2$ supply (squares). The arrows mark stomatal conductance of plants at growth conditions. Data shown are means (±1 SE) from eight CO$_2$ response curves of eight different plants per treatment. All measurements were conducted under saturating light conditions.

The observed decline in the area-based CO$_2$ uptake rate was not only due to the spreading of necrotic lesions in the leaf area, but relating CE and PC to the leaf chlorophyll content (cf. Fig. 4) also indicated lowered photosynthesis in the remaining, still living leaf tissue. Thus, reductions in CE (cf. Table 4) as also found in other studies (Lehnherr et al., 1987; Matyssek et al., 1991; Lippert et al., 1996b; Reichnauer et al., 1997) may be explained, at least in part, by an inhibited Rubisco activity in the living cells. In fact, such an inhibition was found by C. Lütz (pers. comm.) in the same experimental plants by directly assessing the activity of Rubisco. The loss of Rubisco activity is generally associated with decreasing protein levels as observed in herbaceous plants under O$_3$ stress (Sane et al., 1996; Pell et al., 1997). However, the signal transduction from the apoplast as the primary site of the O$_3$ impact to the chloroplasts with their photosynthetic processes still needs to be clarified (Sandermann et al., 1998).

While elevated CO$_2$ did not cause a significant down-regulation in photosynthesis (cf. Tables 1, 4), it counteracted the adverse effects of the 2 × O$_3$ regime on the investigated photosynthetic parameters (cf. Fig. 3, Tables 3, 4). Thus, the photosynthetic dark and light reactions were hardly affected under co-occurring elevated CO$_2$ and O$_3$ levels relative to the Amb. CO$_2$/1 × O$_3$ regime. Several other studies on broad-leaf trees report on a similar buffering capacity of elevated CO$_2$ supply towards O$_3$ impact (Volin & Reich, 1996; Manes et al., 1998; Volin et al., 1998), although such a compensation did not occur in aspen clones (Kull et al., 1996) and Norway spruce (Barnes et al., 1995; Lippert et al., 1997). The compensation of O$_3$-induced reductions in the area-based CO$_2$ uptake rate cannot only be explained by a diminished proportion of the necrotic leaf area at elevated CO$_2$ supply, as indicated by expressing CE and PC on a leaf chlorophyll basis (cf. Fig. 4 and discussion above). Most studies on broad-leaf trees and herbaceous plants claim a lowered O$_3$ uptake rate, mediated through stomatal narrowing in response to elevated CO$_2$, as the cause of reduced O$_3$ injury (McKee et al., 1995, 1997b; Volin & Reich, 1996; Fiscus et al., 1997; Manes et al., 1998, Volin et al., 1998). However, in broad-leaf trees long-term exposure to elevated CO$_2$ often appears to reduce stomatal conductance only in the range of 10–20% (Heath & Kerstiens, 1997; Curtis & Wang, 1998; Saxe et al., 1998). In this present study, $g_{H_2O}$ of plants grown at +300 CO$_2$ tended to be reduced in June/July (Figs 5, 6). At the end of the growing
season, the influence of CO$_2$ supply appeared to diminish (Figs 5, 6; K.-H. Häberle, pers. comm.). Thus, the compensatory effect of high CO$_2$ supply on the photosynthetic decline caused by ozone, as found in this study, appears to be only in part mediated through stomatal behaviour. Moreover, one has to keep in mind that episodes of high O$_3$ levels most likely coincide with high leaf-to-air water vapour pressure deficit and soil drought. Because of the reduced responsiveness of stomata to elevated CO$_2$ under low water availability, the protective effect of elevated CO$_2$ against O$_3$ injury may be much less than is often assumed (Heath, 1998). Rather, metabolic compensation in the leaf mesophyll needs to be considered as concluded by S. Anegg & C. Langebartels (pers. comm.) for same experimental beech plants. Overall, long-term acclimation to elevated CO$_2$ supply does counteract the O$_3$-induced decline of photosynthetic light and dark reactions in *F. sylvatica*.

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