

MODERATION OF [CO₂]-INDUCED GAS EXCHANGE RESPONSES BY ELEVATED TROPOSPHERIC O₃ IN TREMBLING ASPEN AND SUGAR MAPLE

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Abstract

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The greenhouse gases CO₂ and O₃ are increasing in the earth's atmosphere. Little is known about long-term impacts of these two co-occurring gases on forest trees. We have been examining the impacts of these two gases on the physiology and growth of trembling aspen (*Populus tremuloides*) and sugar maple (*Acer saccharum*) in an open-air exposure system in northern Wisconsin, USA for the past 5 years.

Stimulation of photosynthesis by elevated [CO₂] has been consistently found for aspen but not for maple. In contrast, [O₃] causes decreased levels of photosynthesis in aspen but does not appear to significantly impact sugar maple. When the pollutants co-occur, CO₂-induced enhancements in photosynthesis are moderated so that trees in elevated [CO₂]+[O₃] treatments respond similarly to those in control rings. However, in some cases, the effects of co-occurring pollutants were more detrimental than either of the pollutants applied singly. For example, chlorophyll levels per unit leaf area were lowest in aspen exposed to [CO₂]+[O₃] and nitrogen levels per unit leaf area were the lowest in both aspen and maple leaves exposed to the combination treatment.

Key words: greenhouse gases, ozone, carbon dioxide, interacting pollutants

Introduction

The global atmospheric CO₂ concentration has risen by nearly 30% since the preindustrial time period (Barnola et al., 1995). This increase is likely largely due to industrial emissions (Keeling et al., 1995). Similarly, emissions of oxidized nitrogen (NO_x) and volatile organic compounds from fossil fuel emissions related to human activities, such as industrial production and transportation, have increased resulting in increased background concentrations of [O₃] (Finlayson-Pitts, Pitts, 1997; Fowler et al., 1998b; Stevenson et al., 1998; IPCC, 2001). Fowler et al. (1999a, b) suggest that nearly one-quarter of the earth's forests are currently at risk from tropospheric O₃ where the July peak concentrations exceed 60 ppb.

Elevated [CO₂] and [O₃] impact trees in diametrically opposite ways. Elevated [CO₂] stimulates photosynthesis (Tjoelker et al., 1998; Noormets et al., 2001a, b), delays foliar senescence in autumn (Karnosky et al., 2002), and stimulates aboveground (Volin, Reich, 1996; Volin et al., 1998; Norby et al., 1999) and belowground (King et al., 2001; Kubiske, Godbold, 2001) growth. Trees grown under elevated [CO₂] generally have lower nitrogen concentrations in their foliage, lower Rubisco concentrations (Moore et al., 1999), altered defense compounds (Lindroth et al., 1993, 1997) and decreased levels of antioxidants (Niewiadomska et al., 1999; Wustman et al., 2001).

In contrast to the largely beneficial effects of elevated [CO₂], [O₃] is generally detrimental to aspen growth and productivity. Ozone has been shown to induce foliar injury (Karnosky, 1976), decrease foliar chlorophyll content (Gagnon et al., 1992), accelerate leaf senescence (Karnosky et al., 1996), decrease photosynthesis (Coleman et al., 1995a), alter carbon allocation (Coleman et al., 1995b), alter epicuticular wax composition (Maňkiovská et al., 1998; Karnosky et al., 2002; Percy et al., 2002) and decrease growth (Wang et al., 1986; Karnosky et al., 1992, 1996, 1998). Extrapolation of open-top chamber data from O₃ impacts on aspen to the natural aspen suggests that 14–33% biomass loss may be occurring over 50% of their distribution in the eastern U.S. (Hogsett et al., 1997).

Current climate change scenarios predict further increases in [CO₂] (Stott et al., 2000) and [O₃] (Stevenson et al., 1998; Fowler et al., 1999a,b) over the next century. Because little research has been done on the impacts of these interacting pollutants and since conflicting results have been found even for the same species [e.g. Volin, Reich (1996) and Volin et al. (1998) suggest that [CO₂] ameliorates the effects of [O₃] on photosynthesis and growth of trembling aspen while Kull et al. (1996), McDonald et al. (2002), Oksanen et al. (2001), Sôber et al. (2002), Isebrands et al. (2001), and Wustman et al. (2001) suggest that [CO₂] does not ameliorate and in some cases it exacerbates the negative impacts of [O₃], it is difficult to predict how future forests will respond to these interacting pollutants.

Furthermore, no studies with [CO₂] or [O₃] have examined the long-term growth responses throughout the life history and most growth studies have been documented with young, open-grown trees in confined pots, greenhouses or chambers without realistic competitive interactions that forest trees face over much of their life (Körner, 2000).

In 1997, we established the Forest Atmospheric Carbon Transfer and Storage-II (FACTS II) Aspen FACE (Free-Air CO₂ and O₃ Exposure) experiment in Rhinelander, Wisconsin,

as a means to examine the long-term impacts of elevated atmospheric CO₂ and tropospheric O₃ on an aggrading northern forest ecosystem. In this paper, we present results from our Aspen FACE gas exchange studies with two species of differing shade tolerance and inherent growth rates: the rapid-growing, shade-intolerant, pioneer species trembling aspen (*Populus tremuloides* Michx.) and the slower-growing, shade-tolerant, later successional species sugar maple (*Acer saccharum* Marsh.).

Materials and methods

The Aspen FACE facility is located at the USDA Forest Service, Harshaw experimental farm near Rhinelander, Wisconsin, USA (Karnosky et al., 1999; Dickson et al., 2000). The experimental site encompasses 32 ha of land with twelve treatment rings (30-m diameter). The 12 rings are composed of 3 control rings, 3 rings with elevated [O₃], 3 rings with elevated [CO₂], and 3 rings with elevated [CO₂]+[O₃].

One half of each ring was planted in 1997 with 5 clones of trembling aspen, one quarter with trembling aspen (clone 216) and paper-birch and one quarter with trembling aspen (clone 216) and sugar maple (for details see Dickson et al., 2000). Soil in the rings was fertile (nitrogen content 0.12–0.15%).

The Aspen FACE gas dispensing apparatus was modified from that developed earlier (Lewin et al., 1992; Lipfert et al., 1992) as described by Dickson et al. (2000). Control plants were exposed to ambient air. In 1998, ambient [CO₂] averaged 350 μmol mol⁻¹ between 0700 h and 1900 h and at 390 μmol mol⁻¹ between 1900 h and 0700 h. Ambient daytime [O₃] averaged 36 μmol mol⁻¹. Similar ambient [CO₂]+[O₃] were found in 1999 and 2000. Elevated [CO₂] and [O₃] were applied from the middle of May (bud break) to late September (leaf abscission). Elevated [CO₂] treated plants were exposed to 550 μmol mol⁻¹ [CO₂] during daylight hours. Ozone was administered during the daylight hours at approximately 1.5 × ambient. There was no [O₃] fumigation during rain, fog, or dew conditions or during periods of cold weather (when daytime maximum temperatures were less than 15°C).

Photosynthetic parameters were measured on leaves of 4-year-old aspen (Clone 216 which is moderately tolerant to [O₃] was used [Karnosky et al., 1996; Noormets et al., 2001a, b]) and on leaves on maple trees in the maple quadrant in July and August 2000. AC_i curves were measured at different positions of tree crown in all rings. Data were grouped according to three vertical (top, middle, low) positions. A minimum of 12 curves per treatment were measured. The portable gas-exchange measurement system LiCor 6400, (Li-Cor Inc., Lincoln, NE) was used to measure photosynthetic parameters and stomatal conductance. Photosynthetic parameters were always measured at leaf temperatures 24 to 26°C, air humidity 40 to 60% and PPFD 1000 to 1500 μmol m⁻² s⁻¹. Initial slope, CE, and maximal (light and CO₂-saturated) value, P_m, and net photosynthesis were found from AC_i curves.

Chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan) readings were taken for all measured leaves. Chlorophyll meter readings were also taken for 100 randomly selected leaves from each ring. Area of these leaves was also measured (Li-Cor Model LI-3000A leaf area meter) to estimate percentage of leaf area in certain intervals of chlorophyll-content. Chlorophyll meter readings were calibrated against area-based chlorophyll concentrations from leaf punches (diameter 1.8 cm). Leaf punches from measured leaves were also collected. These samples were (i) dried at 60°C and weighed for N analyses, or (ii) frozen in liquid nitrogen and stored at -80°C for chlorophyll analyses.

The nitrogen concentration in leaves was measured with a nitrogen-analyzer (Carlo Erba Instruments, Model NA 1500 NC). Frozen leaf samples with known area were weighed, and used for determination of chlorophyll. Chlorophyll was extracted with N,N-dimethyl-formamide and light absorbance by chlorophyll solution was measured with a spectrophotometer (model 690, Turner Corp. Mountain View, Ca, USA) as in Inskeep and Bloom (1985).

Potential gas exchange of whole trees was calculated by a simple model using data of chlorophyll distribution between leaves, total leaf area of trees and correlation functions found to exist between chlorophyll content and light-saturated photosynthetic rate.

Analysis of variance (ANOVA) was used to test for significant differences between treatments. Least significant differences (LSD) were calculated for the determination of significant differences between treatments. F-test was significant at $P < 0.05$ level (Sokal, Rohlf, 1995). Average values \pm SE are presented and different letters in figures indicate significant differences.

Results and discussion

Gas exchange: CO_2

Both light-saturated photosynthesis and stomatal conductance of trembling aspen were on average 2 to 3 times higher than that recorded for sugar maple (Figs 1 and 2). Although variability in both components of gas exchange was very large, the stimulation of photosynthesis across conductance values was consistent for both species (Fig. 1). Instantaneous photosynthesis averaged over the growing season increased under elevated $[CO_2]$ by 36% in trembling aspen but was not changed in sugar maple (Fig. 2). Stomatal conductance was reduced under elevated $[CO_2]$ by nearly 50% in both species.

The values of $[CO_2]$ -induced stimulation of aspen photosynthesis and decrease of stomatal conductance are similar to those reported for upper canopy aspen leaves exposed to elevated $[CO_2]$ (Noormets et al., 2001a, b; Takeuchi et al., 2001). Average $[CO_2]$ -induced enhancement of photosynthesis for a number of tree species has been about 60% (Norby et al., 1999). Similarly, the average $[CO_2]$ -induced depression of stomatal conductance has been 21% (Medlyn et al., 2001). The lack of a $[CO_2]$ -induced photosynthesis for sugar maple is consistent with results presented by Karnosky et al. (2003).

While we have seen a slight stimulation of photosynthesis per unit of stomatal conductance, the reduced conductance levels associated with elevated $[CO_2]$ have resulted in no

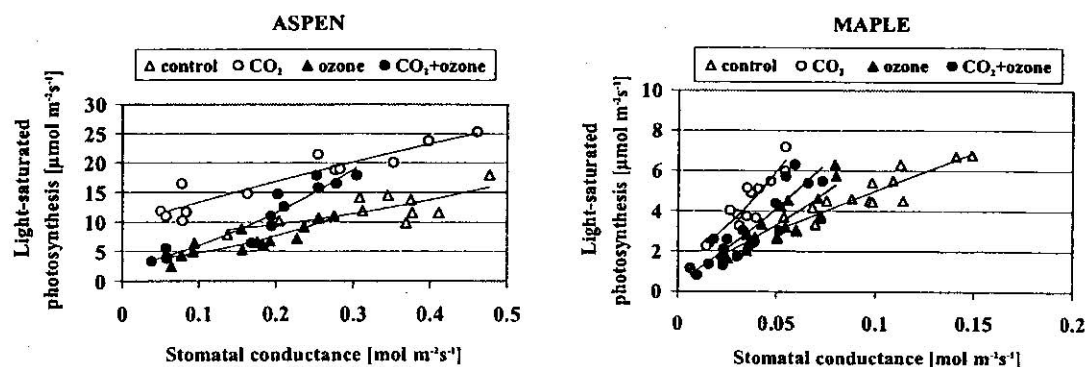


Fig. 1. Light saturated photosynthesis versus stomatal conductance for trembling aspen (A) and sugar maple (B) trees exposed to elevated $[CO_2]$ and $[O_3]$, alone and in combination, in the Aspen FACE experiment. All data were taken in July and August, 2000 and they represent 3 leaves per tree (one each for lower, middle, and upper canopy) from each of 2 to 3 trees of each species per ring and 3 replicate rings of each treatment. Different trees were randomly selected each month.

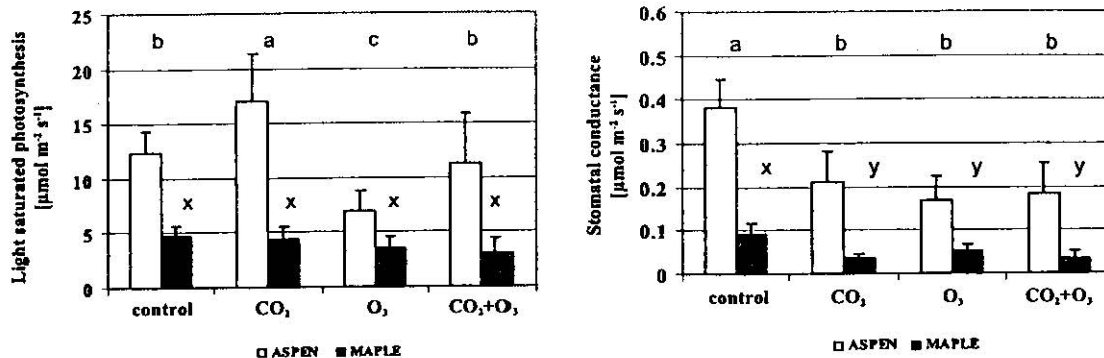


Fig. 2. Light-saturated photosynthesis (top) and stomatal conductance (bottom) for trembling aspen and sugar maple trees exposed to elevated [CO₂] and [O₃], alone and in combination, in the Aspen FACE experiment. All data were taken in June, July, and August, 2000 and they represent a minimum of 3 leaves per tree (one each from lower, middle, and upper canopy) from each of 2 to 3 trees of each species per ring, and 3 replicate rings of each treatment. Different trees were randomly selected each month. Data shown are means and standard errors. Values with different letters are significantly ($p < 0.05$) different as determined by least significant different methods (Fowler et al., 1998a).

net increase in mean values of instantaneous photosynthesis for sugar maple over the growing season. This is similar to results for other maple trees measured at our Aspen FACE site (Karnosky et al., 2003). Other researchers have found a significant enhancement in mean photosynthesis values (Tschaplinski et al., 1995; Volin et al., 2002), but these studies were with young (<1-year-old) seedlings exposed for single growing seasons. Our sugar maple were well-established 4-year-old seedlings that had been exposed continuously to elevated [CO₂] for four growing seasons.

Gas exchange: O₃

Elevated [O₃] depressed mean instantaneous values of trembling aspen photosynthesis by nearly 60%, while there was no significant depression of photosynthesis in sugar maple trees exposed to [O₃]. The aspen clone used, clone 216, is moderately tolerant to [O₃] (Coleman et al., 1995a, b; Isebrands et al., 2001), but as can be seen in Fig. 2, photosynthesis was severely decreased under [O₃]. These results are similar to those reported for aspen by Noormets et al., 2001a, b and for sugar maple (Karnosky et al., 2003). No impact of O₃ on early- or mid-season photosynthesis, but a significant late-season impact on sugar maple photosynthesis was found by Bäck et al. (1999) and Topa et al. (1999, 2001). The light environment has been shown to be important for the response of sugar maple to O₃ (Tjoelker et al., 1993, 1995).

Stomatal conductance was significantly decreased in both aspen and sugar maple trees exposed to elevated [O₃].

Gas exchange: CO_2+O_3

The enhancement of photosynthesis by elevated $[CO_2]$ for both trembling aspen and sugar maple was negated by the addition of $[O_3]$ (Figs 1 and 2). For aspen, this meant that trees exposed over the growing season to $[CO_2]+[O_3]$ had similar mean photosynthesis values to controls. While no significant differences were detected for mean photosynthesis in sugar maple, the seasonal mean values for the trees in the combination treatment were 32% less under $[CO_2]+[O_3]$.

Similar offsets of $[CO_2]$ enhancement of photosynthesis by elevated $[O_3]$ have been reported for aspen (Kull et al., 1996; Noormets et al., 2001a, b; Karnosky et al., 2003). While little work has been done on the effects of $[CO_2]+[O_3]$ on sugar maple seedlings, Noble et al. (1992) found no offset in $[CO_2]$ -enhanced photosynthesis and Karnosky et al. (2003) reported no significant effect of either $[CO_2]$, $[O_3]$, or $[CO_2]+[O_3]$ on photosynthesis of sugar maple.

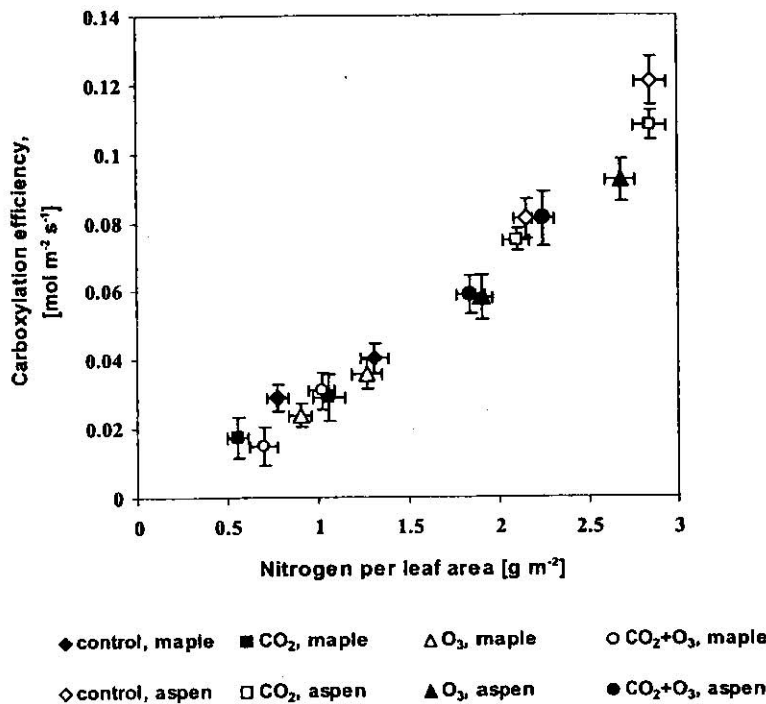


Fig. 3. Carboxylation efficiency, calculated from A_C curves plotted against foliar nitrogen values for trembling aspen and sugar maple trees exposed to elevated $[CO_2]$ and $[O_3]$, alone and in combination, in the Aspen FACE experiment. Data were taken from a minimum of 2 to 3 trees per species per ring and from 3 to 4 leaves, and at least 2 crown positions, per tree over the months of June to August, 2000. Values represented are means and standard errors.

Carboxylation efficiency

The carboxylation efficiency, calculated from the initial slope of AC_i curves, represents the increase in photosynthesis achieved per unit increase in CO_2 at the site of CO_2 fixation. In Fig. 3 carboxylation efficiency values from our experiment are plotted against nitrogen per unit leaf area. It is interesting to see how nitrogen drives the increase in carboxylation efficiency, regardless of species or treatment.

Chlorophyll and nitrogen

The impacts of $[CO_2]$ and/or $[O_3]$ on photosynthesis are strongly dependent on chlorophyll and nitrogen concentrations of the foliage. As can be seen in Fig. 4, chlorophyll values for leaves of trembling aspen and sugar maple were affected very differently by treatment. While no significant treatment effects on chlorophyll concentration were detected for sugar maple, decreases in chlorophyll were found in trembling aspen with $[O_3]$ additions, regardless of $[CO_2]$ treatment. The largest reductions in chlorophyll occurred in the combination treatment.

For foliar nitrogen, only the sugar maple had significantly decreased concentrations in the elevated $[CO_2]$ treatments, regardless of $[O_3]$ concentration. The lowest foliar nitrogen concentrations for both species were found in the combined $[CO_2]+[O_3]$ treatment.

It is well known that chlorophyll of aspen leaves exposed to elevated $[O_3]$ is subject to degradation (Farage, 1996; Gagnon et al., 1992). While we found no chlorophyll decrease in sugar maple leaves under elevated $[O_3]$, others have reported $[O_3]$ -induced decreases in chlorophyll in sugar maple (Tjoelker et al., 1995; Bäck et al., 1999). It is likely that there is genetic variation in $[O_3]$ sensitivity in sugar responses and that we are dealing with relatively $[O_3]$ -tolerant trees in our study. While elevated $[CO_2]$ has been shown to decrease

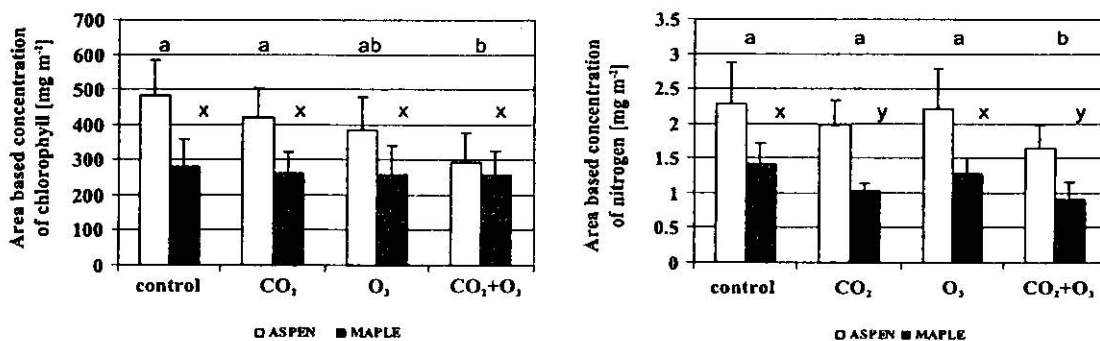


Fig. 4. Concentration of chlorophyll (top) and nitrogen (bottom) for trembling aspen and sugar maple trees exposed to elevated $[CO_2]$ and $[O_3]$, alone and in combination, in the Aspen FACE experiment. Values were determined from 2 to 3 trees of each species per ring over the months of June through August, 2000. Values represented are means and standard errors. Values with different letters are significantly ($p < 0.05$) different as determined by least significant difference methods (Fowler et al., 1998a).

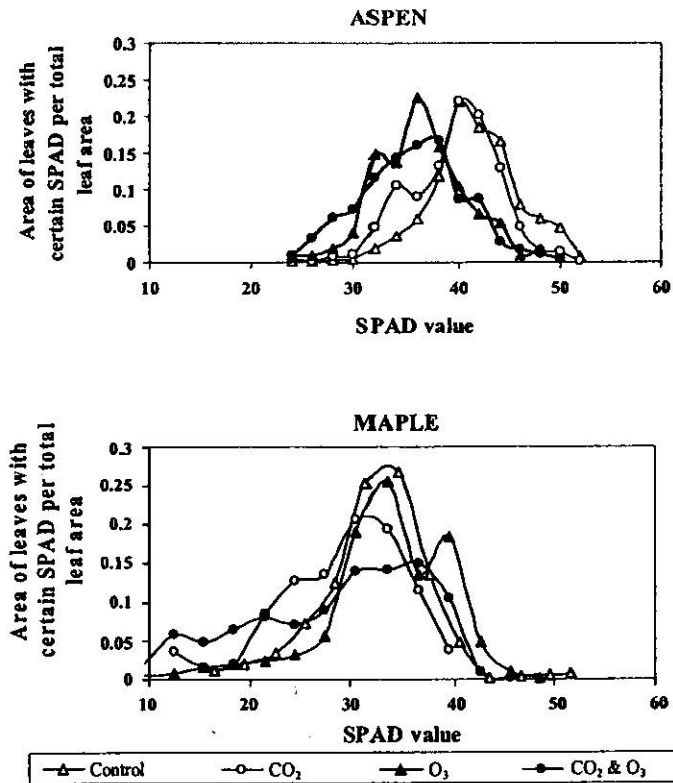


Fig. 5. The distribution of leaf area with various SPAD readings for trembling aspen (top) and sugar maple (bottom) trees exposed to elevated $[\text{CO}_2]$ and $[\text{O}_3]$, alone and in combination, in the Aspen FACE experiment. SPAD readings are the difference between light absorbance values in leaves at 650 and 750 nm. This difference in absorbance values has a high correlation with leaf chlorophyll and nitrogen, calculated per leaf area. These values were determined from August, 2000 whole-tree leaf harvests from 2 to 3 trees per ring per species and 3 replicates of each treatment.

sugar maple chlorophyll concentrations (Kubiske et al., 2002), we did not see any significant decreases in $[\text{CO}_2]$ alone in our study.

Our finding of decreased nitrogen levels in elevated $[\text{CO}_2]$ treatments for sugar maple is not unexpected. Elevated $[\text{CO}_2]$ has been shown to decrease nitrogen on an area basis in a number of tree species (Rey, Jarvis, 1998; Norby et al., 1999), including trembling aspen (Wang, Curtis, 2001) and sugar maple (Kubiske et al., 2002).

Trends in individual leaf chlorophyll and nitrogen effects were tested over the entire crown of a few candidate trees by measurements of SPAD values and calculation of leaf area with various SPAD values (Fig. 5). As can be seen in this figure, lower peak SPAD values for trembling aspen leaves were seen for the $[\text{O}_3]$ treatments, regardless of $[\text{CO}_2]$ concentration.

While $[\text{O}_3]$ did not impact SPAD values in sugar maple, the presence of elevated $[\text{CO}_2]$, with or without $[\text{O}_3]$, resulted in a shift in the curves with a larger number of leaves with

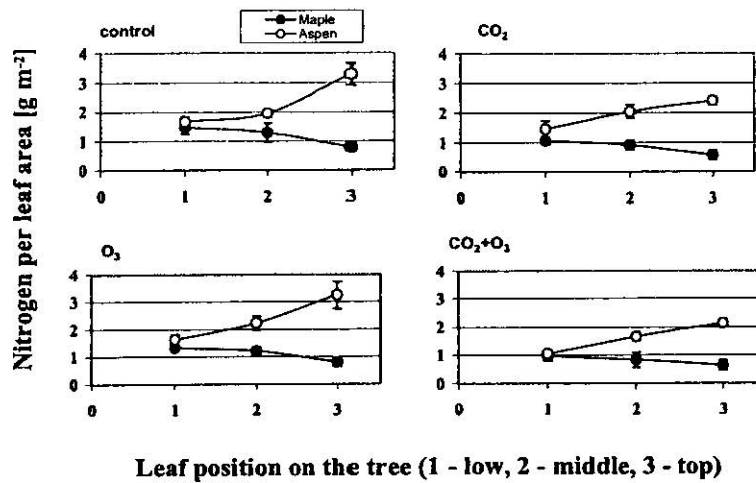


Fig. 6. Nitrogen concentration in leaves from various crown sections of trembling aspen and sugar maple trees exposed to elevated $[\text{CO}_2]$ and $[\text{O}_3]$, alone and in combination, in the Aspen FACE experiment. These are year 2000 seasonal average values from an average of 3 leaves per tree and 2 to 3 trees per ring per species. The values are means and standard errors.

lower SPAD values. Both of these results are consistent with the predicted responses from the individual leaf chlorophyll and nitrogen responses.

To determine how these two species allocate nitrogen in their crown, we examined nitrogen concentrations in foliage in three crown positions (lower, middle and upper crown). As can be seen in Fig. 6, the two species allocate nitrogen very differently. The highest nitrogen values in all aspen trees occurred in the upper canopy while just the reverse occurred for sugar maple.

In order to estimate the potential for carbon uptake from these two species, we modeled whole-tree carbon uptake by multiplying instantaneous photosynthesis values from three positions in the crown (lower, middle, and upper crown) with the leaf area for each crown position (Fig. 7). The importance of lower- and middle-crown for carbon uptake can be seen for both species. The positive responses of aspen to elevated $[\text{CO}_2]$ and negative responses to $[\text{O}_3]$ are clearly seen in this figure, as is the offset of $[\text{CO}_2]$ enhancement by elevated $[\text{O}_3]$ in the combination treatments. Also, clearly shown in this figure is the projection of reduced carbon gain under elevated $[\text{CO}_2]$ in sugar maple, with or without $[\text{O}_3]$ present.

Conclusions

In this study, we examined the impacts of elevated levels of two greenhouse gases $[\text{CO}_2]$ and $[\text{O}_3]$, alone and in combination, on gas exchange and foliar chlorophyll and nitrogen dynamics in two tree species with differing growth rates, shade tolerances, and succes-

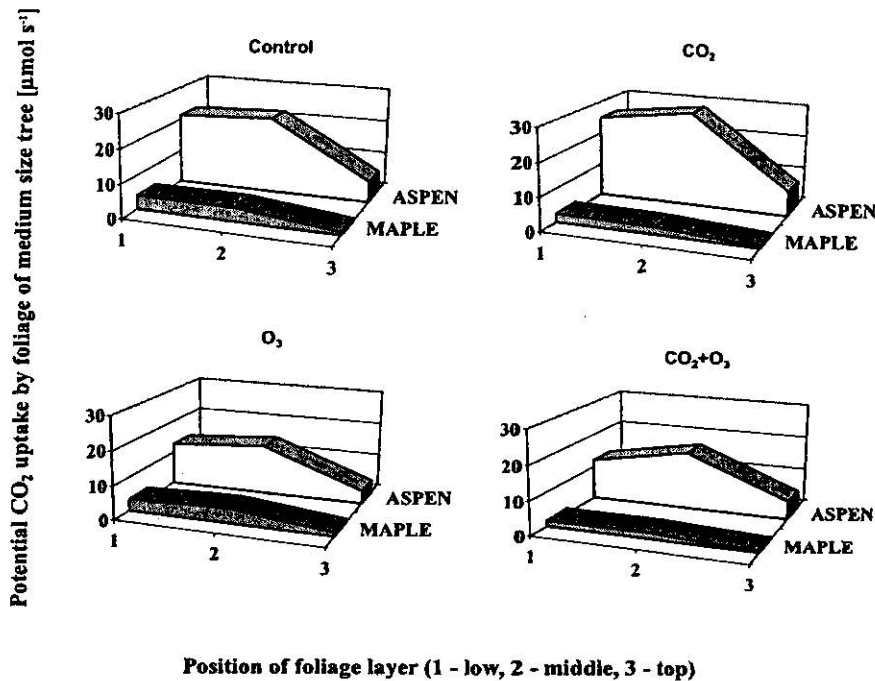


Fig. 7. Potential CO_2 uptake for various portions of the crown of a simple tree as estimated by multiplying the mean instantaneous photosynthesis value with the leaf area of each position in the crown, for trembling aspen and sugar maple trees exposed to elevated $[\text{CO}_2]$ and $[\text{O}_3]$, alone and in combination, in the Aspen FACE experiment.

sional roles in northern forest ecosystems. As expected, the responses varied greatly by treatment and by species as shown below:

For CO_2

- photosynthesis was dramatically enhanced by elevated $[\text{CO}_2]$ in trembling aspen but not in sugar maple
- stomatal conductance was decreased in both species under elevated $[\text{CO}_2]$
- sugar maple growing under elevated $[\text{CO}_2]$ had foliage with decreased levels of nitrogen and often showed visibly chlorotic foliage by mid-summer.

For O_3

- photosynthesis significantly decreased under elevated $[\text{O}_3]$ in trembling aspen but not sugar maple
- stomatal conductance was significantly decreased in both species under elevated $[\text{O}_3]$
- the photosynthesis effect in aspen was largely driven by decreases in chlorophyll.

For CO_2+O_3 ,

- $[O_3]$ offset the enhancement in trembling aspen photosynthesis seen under elevated $[CO_2]$ so that no difference from control levels were seen for trembling aspen trees grown under elevated $[CO_2]+[O_3]$
- the lowest whole-tree potential CO_2 uptake in sugar maple occurred under elevated $[CO_2]+[O_3]$.

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