Effects on photosynthesis and stomatal conductance in Trembling aspen (*Populus Tremuloides* Michx. *)* after more than 10 years of growth in free air CO_2 and/or O_3 enrichment

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ABSTRACT

Since the beginning of the industrialism, the levels of CO_2 and tropospheric O_3 in the environment have increased largely in the atmosphere. Elevated concentrations of both CO_2 and O_3 are known to have effects on stomatal conductance and photosynthesis in plants, but while the short-term responses are relatively well investigated, the knowledge about long-term effects is not that clear. This study was performed on trembling aspen (*Populus Tremuloides*) at the Aspen FACE (Free air carbon enrichment) research station located in Wisconsin, USA, to investigate effects of long-term growth in elevated CO_2 and/or O_3. During three weeks in august 2008, measurements of stomatal conductance, photosynthesis and leaf temperature was conducted. Leaf analyses were made for the investigation of N content and sap flow data achieved from study was used in addition. In earlier studies of aspen in the experiment, photosynthetic capacity was down regulated and N content was reduced in aspen from growth in elevated CO_2. Stomatal conductance was reduced in response to elevated CO_2 early in the experiment, while there were no effects on sap flow, which can be an estimation of canopy conductance, later in the experiment. The results in the present study showed that earlier effects were not present anymore, since no significant treatment effects were observed. This study also showed that stomatal sensitivity to CO_2 was preserved in aspen, both on a leaf and canopy level.

Sedan början av industrialismen har nivåerna av CO_2 och troposfäriskt O_3 i atmosfären ökat i stor utsträckning. Ökade koncentrationer av både CO_2 och O_3 har visat sig påverka fotosyntes och stomata konduktans hos växter, men medan effekter på kort sikt är relativt väl undersökta, är vetskapen om långtidseffekter inte lika klara och tydliga. Denna studie utfördes på asp (*Populus Tremuloides*) vid forskningsstationen Aspen FACE (Free air carbon enrichment) i Wisconsin, USA, för att undersöka långtidseffekterna av att växa förhöjd halt CO_2 och/eller O_3. Under tre veckor i augusti, 2008, utfördes mätningar av stomatakonduktans, fotosyntes och bladtemperatur. Bladanalyser gjordes för att undersöka N innehåll och sap flow data som erhållits från en annan studie användes i tillägg. Tidigare studier på asp i experimentet har visat att fotosynteskapaciteten var nedreglerad och N innehållet reducerat efter att ha växt i ökad CO_2-halt. Stomata konduktansen var reducerad som svar på ökad CO_2 halt, medan det inte fanns några effekter på sap flow, som kan användas för att uppskatta lövverkskonduktans, senare i experimentet. Resultaten från denna studie visade att tidigare effekter ej längre fanns kvar, då inga signifikanta behandlingseffekter observerades. Denna studie visade också att stomatas sensitivitet för CO_2 fanns bevarad hos asp, både på blad och lövverksnivå.

CONTENTS

1. INTRODUCTION

CO_2 and tropospheric O_3 are two of the most abundant green-house gases in the atmosphere and the concentrations of those gases are still increasing (IPCC, 2001). Plants are in direct contact with the atmosphere through stomata, and because of the necessity of CO_2 for photosynthesis and because of the phytotoxic effects of O_3 (Percy et al. 2002) it is important to investigate what effects the increasing levels of those gases might exert on plants in the future.

1.1 CO_2 and O_3 in the atmosphere

About 52-60 million years ago, the atmospheric carbon dioxide of the earth might have exceeded concentrations of 2000 ppm, but this was followed by a huge decline between 40 and 55 million years ago. The last 23 million years, carbon dioxide concentrations [CO_2], seems to have been relatively stable and to have stayed at levels below 370 ppm (Pearson and Palmer, 2000). Since the industrial revolution in the end of the 18:th century CO_2 emissions have steadily increased and has now reached a level of 385 ppm compared to the pre-industrial level of 280 ppm. While it took about 200 years before the first half of this increase was reached, the last half was attained in only little more than 30 years (IPCC, 2007). The most important source of CO_2 emissions is the burning of fossil fuels, but deforestation with subsequent CO_2 release and reduced CO_2 uptake from trees is also important. Except for anthropogenic sources, CO_2 is also released in natural processes, such as decomposition of organic matter (IPCC 2007). Estimations of future CO_2 emissions indicate levels of about 560 ppm in 2050 and as much as 760 ppm in 2100, if emissions continue at the same rate as today (IPCC 2001).

Fossil fuel burning does not only contribute to increasing atmospheric CO_2 emissions, it is also the largest single source of nitrogen oxides (NO_x) emissions, which are important compounds in the formation of tropospheric O_3 (Jenkins & Clemitshaw 2000). Many forests that before was relatively unexposed to NO_x and volatile organic compounds (VOCs) are now exposed to much higher levels of both compounds because of the fast expansion of urban areas (Percy et al., 2003). NO_x is to the largest part released as nitrogen oxide (NO), but is converted to nitrogen dioxide (NO_2) when reacting with O_3. By photolysis, NO_2 will then be reconverted to NO and O will be released. NO can also be converted to NO_2 by radicals formed from VOC and carbon monoxide but without the consumption of O_3, which results in a net production of O3 (Jenkins & Clemitshaw 2000). Compared to long-lived gases, its difficult to determine global average O_3 levels because of its unstable properties. While a

concentrations over distant tropical oceans may not exceed 10 ppm, the same compound can occur in levels as high as 100 ppm in highly polluted regions (IPCC 2001). Consequently, it is not easy to determine pre-industrial levels, but measurements from Central Europe shows evidence that [O_3] was around 10 ppb about 100 years ago (Volz and Kley, 1998). Surface O3 abundances during July over the industrialized continents of the industrialized continents of the northern hemisphere are about 40 ppb with 2000 emissions, and under SRES scenarios A2 and A1F1 they would reach 45 to 50 ppb with 2030 emissions, 60 ppb with 2060 emissions and > 70 ppb with 2100 emissions (IPCC 2001).

1.2 CO_2 and O_3- effects on plants

1.2.1 Plant responses to CO_2

The short-term stimulation of photosynthesis to elevated [CO_2] is well documented, although there is variability between growth conditions and species (e.g. Drake et al. 1997). There are two different reasons to why short-term increases in [CO_2] will have effects on ribulose 1.5-bisphosphate carboxylase/oxygenase (Rubisco) and thus photosynthesis. The first one is that since the atmospheric [CO_2] of today is substrate limiting to Rubisco, the carboxylation rate will increase in elevated [CO_2]. The second one is that the net CO_2 uptake efficiency will increase as a result of inhibition of the oxygenation reaction. The CO_2 lost in photorespiration will decrease and a larger part of the energy achieved from the light reactions as ATP and NADPH will be used for assimilation instead of photorespiration (Long et al., 2004). Responses of elevated [CO_2] in the long-term are not that clear and there are uncertainties regarding the effects on net photosynthesis (A_{net}), if photosynthetic capacity will be down regulated or not and if A_n will be sustained in the long term, even under resource limitations (e.g. Nowak et al., 2004). Photosynthetic acclimation can be defined as “a physiological change that occurs with growth at high [CO_2]” and the implication of this is that after medium or long term growth in elevated [CO_2], the initial stimulation of A_{net} from elevated CO_2 might be reduced (Drake et al., 1997).

There are uncertainties regarding both the carboxylation capacity and the electron transport capacity in future atmospheric [CO_2]. The carboxylation capacity will have effects on the CO_2 fixation in plants, i.g., the short-term stimulation of A_n id dependent on the long-term preservation of carboxylation capacity (Ellsworth et al. 2004).

Results summarized from free air carbon enrichment experiments (FACE) have demonstrated significantly reductions in the maximum carboxylation rate (V_{cmax}) (Ainsworth

et al. 2007, Long et al. 2004) and in the maximum rate of electron transport (J_{max}) (Ainsworth et al. 2007).

N is essential for photosynthesis, except for being essential in the formation of enzymes, like Rubisco, N is also a necessary component in chlorophyll. If other factors are not limiting, photosynthetic capacity will increase with increasing N content (Larcher 2003). Elevated [CO_2] have been shown to change the relationship between C and N, so that the carbohydrate content will be larger and the N content smaller. The [N] will then decrease, and if the [N] per unit leaf area decreases, photosynthesis might be downregulated as an effect from N dilution (e.g. Luo et al., 1994). Lower leaf N content as a consequence of elevated CO_2 have also been attributed to reallocations of N from the leaves to other parts of the plant (Nowak et al. 2003).

The most important factors that cause changes in stomatal aperture are water availability, light and CO_2 concentration. Stomata close in response to low water availability in the air or soil and in most species open in response to light and close in the dark (Raven et al., 2003). It is generally accepted that stomatal conductance (g_s,) decreases in response to short-term increase in [CO_2], although the amount of decrease to certain raises in CO_2 have differed between studies. The long term effects on g_s from growth in elevated [CO_2] are on the other hand more unclear and variable (Morison, 2001).

While short-term responses results from changes in stomatal aperture, long-term responses could be physiological (acclimations) or anatomical/morphological (adjustments) (Morison, 1998). Stomatal response to CO_2 and the way this response affects photosynthesis and transpiration will have effects on plant water regulation and growth, since virtually all of the CO_2 used by the plant passes through stomata. These responses might have influences on climate change through changes in the hydrological and carbon cycles (Morison, 2001). If stomatal acclimation to elevated [CO_2] occurs and if this acclimation is independent of photosynthesis, global models including stomatal conductance and photosynthesis will be more complex since each [CO_2] of interest would require re-parameterization (Ainsworth & Rogers, 2007). In a meta-analysis of different FACE experiments, Medleyn et al. (2001) concluded that on average, g_s was decreased by 21% in plants grown in elevated [CO_2], while there were no evidence for g_s acclimation.

1.2.2 Plant responses to O_3

Elevated [O_3] is generally detrimental to forests (Karnosky et al. 2003). O_3 have been shown to have phytotoxic properties (Percy et al. 2002) and Fowler et al (1999) proposed that nearly

a quarter of the worlds forests are currently at risk of damage, (at [O_3] > 60 ppb) and reduced productivity, and that by 2100 this will expand to half of the worlds´ forests.

O_3 may cause significant damage to photosynthesis because of its strong oxidizing effects (Reich et al. 1987) and decreases in photosynthesis have been observed as a consequence of loss of Rubisco activity (Farage et al. 1991). Wittig et al. (2007) made an analysis of the results from different experimental settings with O_3 fumigation to estimate effects on A_{net} at current but also at future O_3 concentrations. The conclusion was that [O_3] of today (approximately 40 ppb) suppresses A_{net} by on average 11% compared with pre-industrial O_3 levels (10 ppb). When growth in ambient background levels were compared with elevated O_3 treatment, A_{sat} was decreased by 18% in elevated [O_3].

Although stomatal closure is a well investigated response to O_3, there are uncertainties whether this is a direct response on stomata or an indirect response from the effects on photosynthesis (Robinson et al., 1997). It has been proposed that the decrease in stomatal aperture is an indirect affect as a result of decreasing carboxylation capacity and hence declining intercellular [CO_2] (C_i) (e.g. Farage et al., 1991). Wittig et al. (2007) found that the O_3 levels in the atmosphere today suppresses g_s by on average 13% compared to pre-industrial O_3 levels. When ambient background versus elevated [O_3] was compared, g_s decreased by 6% in the elevated O_3 treatment.

1.2.3 Interaction effects between CO_2 and O_3

There is limited knowledge about possible interaction effects of CO_2 and O_3, but since plants will be exposed to both gases at the same time, it is important to take this issue into consideration. Since CO_2 causes decreased stomatal aperture, it is expected that the short-term response to elevated [CO_2] reduces the amount of O_3 passing through stomata (Paoletti and Grulke, 2005). On the other hand, because O_3 also might induce stomatal closure, rising O_3 levels are likely to decrease the capacity of the terrestrial biosphere assimilate CO_2, and thereby also decreases the potential to offset rising global CO_2 levels (Wittig et al., 2007). Therefore, it is important to investigate the long term effects of these gases, both alone and in combination. In a summary of effects from CO_2 and O_3 during the first years of the Aspen FACE experiment, Karnosky et al. (2003) showed that growth enhancement by elevated CO_2 can be significantly reduced by relatively low [O_3]. They emphasized the importance of knowledge about combined O_3 and CO_2 effects when using global models for the prediction of primary productivity.

1.3 Aspen FACE

1.3.1 FACE versus other experimental settings

Much knowledge about physiological and biological functions of plants is derived from short-term, restricted environmental settings, but for the prediction of long-term responses on whole ecosystems, experiments of this kind are inadequate (Dickson et al., 2000). Artificially illuminated controlled environment chambers, pots, greenhouses, transparent enclosures and open-top chambers (OTC´s) all have size limitations and therefore much of the research using these experiments is based on young plants. Although OTC´s are open to the atmosphere, the environment will still differ between the chamber and the environment and rainfall, wind and the spreading of pests and pathogens will be decreased. Because of size limitation, OTCs only have room for a few trees and canopy closure is therefore not possible (Long et al., 2004). The progress of research on forest trees have evolved from controlled environment chambers to open-top chambers and later to free air carbon dioxide exposure (FACE) experiments (Karnosky et al., 2003).

A typical FACE plot is circular and enclosed by pipes releasing CO_2 or air enriched with CO_2. Fumigation flow rate is controlled by a computer system which measures wind velocity and direction in the middle of each plot (Long et al., 2004). A considerable advantage with FACE is the ability to study biological responses together with the interacting effects trees experience in the natural environment. The present study was conducted at the Aspen FACE site, outside Rhinelander, Wisconsin. Aspen FACE has some features which together make the project unique of its kind: 1) It is the only FACE experiment in the world with both CO_2 and O_3 fumigation, both separately and in combination. 2) Each ring (plot areas) is 30 m in diameter, which makes the total experimental area huge, 8400 m^2. 3) Exposure of the trees started in 1998, when they were only 1-year-old plants and have continued until now, when the plants have developed to mature trees. 4) Three tree species are used in the experiment (trembling aspen, paper birch and sugar maple) and five different clones of aspen with different sensitivity to O_3 (Dickson et al., 2000). The experiment is planned to close down in 2009.

1.3.2 Earlier studies on trembling aspen at the Aspen FACE site

The current study was performed on trembling aspen (*Populus tremuloides* Michx.*)*, which is the most widespread native tree species in North America. It survives in both wet and dry soils, and is present from the sea level to the tree line and in a range of different plant communities because of its great genetic diversity (Dickson et al., 2000). The species is

convenient for the study of O_3 effects since both ozone-tolerant and ozone-sensitive genotypes have been identified.

Noormets et al (2001) showed that A_n was higher in trees grown in elevated [CO_2] when measured at growth concentration early in the experiment. The same result was observed by Ellsworth et al. (2004) when *A*n was measured at growth concentration, but when measured at a common concentration, *A*n was significantly decreased by growth in elevated [CO_2]. Ellsworth et al. (2004) also observed that V_{cmax} and J_{max} were significantly decreased by elevated CO_2 in aspen. Early in the experiment, leaf N concentration (in % of dry mass) was lower in elevated [CO_2] compared to control (Lindroth et al. (2001), but in a later stage there was no longer any treatment effects on N concentrations (Zak et al., 2007).

Early measurements of g_s indicated lower values in elevated [CO_2] (Noormets et al., 2001). Uddling et al. (2008) measured sap flow, which is a way to estimate tree water use and therefore also canopy conductance in the pure aspen and the mixed aspen/birch community after more than 6 years. On a stand level, sap flow was increased by elevated [CO_2], but divided by leaf area, there were no effects from increased [CO_2].

Total biomass accumulation during the first 7 years of the experiment (six years with fumigation treatment), as well as coarse and fine root growth was increased by elevated [CO_2] in both aspen and aspen/birch communities. Fine root growth compared to foliage and wood growth was larger in elevated CO_2 compared to control and in elevated CO_2+O_3 compared to elevated O_3 (King et al. 2005).

There are also different results regarding the O_3 effects on aspen. Measured at growth [CO_2], elevated [O_3] decreased *A*n in mature and old leaves of an ozone-sensitive clone, at both ambient and elevated [CO_2], but not in an ozone-tolerant clone (Noormets et al., 2001).
N concentration was decreased by O_3 early in the experiment (Lindroth et al., 2001), but later, this effect on N concentration disappeared (Zak et al. 2007).

Stand level tree water use was not affected by elevated O_3, but was higher when measured on a leaf area basis (Uddling et al. 2008). O_3 decreased g_s in mature and old leaves of an ozone-sensitive clone (Noormets et al. (2001).

The present study aim to answer the following questions: Will photosynthetic capacity (carboxylation capacity and electron transport capacity) acclimate/ down regulate after more than ten years of growth in elevated CO_2 and or O_3? If there is an effect, will this response be related to lower N content? Do stomata of aspen exhibit significant responses to short-term changes in [CO_2]? Is this direct stomatal response affected by long term growth in elevated CO_2 and/or O_3, i.g. will there be an acclimation stomata?

2. MATERIALS AND METHODS

2.1 Research site

The research station is located outside Rhinelander in northern Wisconsin, USA (45.6° N, 89.5° W) on the Harshaw Experimental Farm of the USDA Forest Service. Before the US Forest Service purchased the farm in 1972 the sandy loam soil was used for agriculture (Dickson et al. 2000). Mean annual temperature in the area is 4.9°C and mean annual precipitation 810 mm. Twelve 30-diameter rings in which the concentrations of CO_2 and O_3 can be controlled are placed with 100 m space between each other on a 32 ha field (Figure 1). The Aspen FACE is designed as a full factorial experiment with three rings of each treatment (control, elevated CO_2, elevated O_3 and elevated CO_2 +O_3), constituting three blocks (replicates). The fumigation is controlled by a computer system which maintains stable CO_2 and O_3 concentrations by signal feedback technology. A number of vertical ventpipes surrounding each ring disperse the gases into the center of each ring (FACTS II: The Aspen FACE experiment 2009). The fumigation of CO_2 and O_3 began in 1998 and have continued from budbreak to budset during daylight hours since then. The target concentration for elevated CO_2 treatment is 560 µmol/mol and for O_3 1.5 x ambient concentration. For the first four growing seasons daytime concentrations for the CO_2 and O_3 treatments were 530, 548, 548 and 541 µmol mol^{-1} and 54.5, 51.1, 48.9 and 52.8 nL L^{-1}, respectively. The target concentrations were meant to simulate the levels in the Great Lakes Region in the year 2050 (Karnosky et al., 2003).

Figure 1. Aerial view of the Aspen FACE site showing the twelve rings.

3-6-month-old trees of trembling aspen, paper birch (*Betula papyrifera*) and sugar maple (*Acer saccharum*) was planted in 1997. Five different aspen clones were used in the experiment; 8L, 216 and 271 (relatively ozone-tolerant) and 42E and 259 (relatively ozone-sensitive) (Karnosky et al., 2003). In the eastern half of each ring, only aspen trees are planted (all clones represented), while the western half of each ring is split into further two halves. The south-west quadrant is planted with alternating birch and aspen (clone 216) and the northwest quadrant with mixed aspen and maple clones (Dickson et al., 2000) (Figure 2). By 2003, canopy closure occurred in the mixed aspen and aspen-birch sections (Zak et al., 2007).

Figure 2. Each ring is divided into three sections planted with pure Aspen, mixed Aspen/Birch and Aspen/Maple).

2.2 Gas exchange measurements

During three weeks in August 2008 gas exchange measurements were conducted in leaves of trembling aspen (clone 271) on the aspen FACE site. Photosynthesis versus intercellular [CO_2] (A/C_i) curves and stomatal response curves were measured in one leaf from each ring, e.g. totally in twelve leaves. In addition, A/C_i curves were conducted for five extra leafs from different rings. The three blocks were measured one at a time and one ring per day was selected randomly within each block. The leaves were collected in the morning, only from the aspen section, with the aid of 7 m high scaffolds placed in each ring (Figure 3). Healthy looking, sun-exposed shoots from the current year were selected from the upper part of the canopy in the core areas of the rings (the outer five tree rows were avoided). The reason for

measuring on excised shoots instead of directly on the trees was to permit measurements of individual leaf responses and prevent confounding effects from whole tree and soil water status. The twigs were cut under water to prevent embolism in the xylem and brought indoors for measurements to be carried out in a relatively stable climate. Measurements were conducted with an open gas exchange system, the Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) with the leaves enclosed in the connected leaf chamber. The system calculates g_s and A_{net} from differences in [CO_2] and H_2O vapour pressure in chamber and pre-chamber conditions.

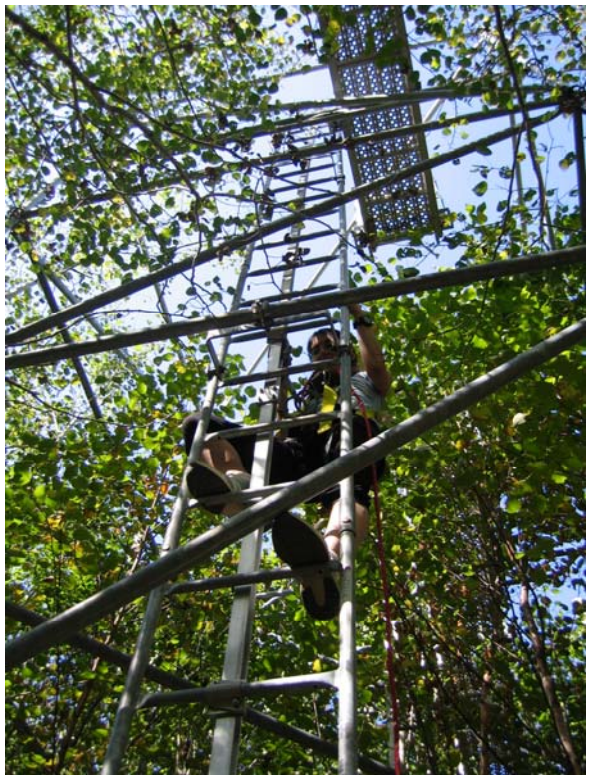


Figure 3. Leaves were collected from the upper canopy with the aid of scaffolds.

2.2.1 A/C_i curves

Each leaf was aloud to acclimate for about 20 minutes and the chamber conditions were set to a saturating light intensity of 1800 µmol m^{-2} s^{-1}, a temperature of 25 °C and the reference [CO_2] of incoming air [CO_2R] to 400µmol mol^{-1}. After that, and if g_s was > 0.05 mol m^{-1} s^{-1}, an A/C_i response curve was run with [CO_2R] set to 400, 60, 150, 225, 300, 400, 600, 800, 1000 and 1200 µmol mol^{-1}. An additional measurement at 1500 µmol mol^{-1} was made if g_s was between 0.05 and 0.1 µmol m^{-2} s^{-1}. The measurements were taken every minute.

Photosynthetic parameters were calculated from the A/C_i curve fitting model by Sharkey et al. (2007) that originally is based on a the model by Farquhar (1980). The theory behind this model is based on the prediction that the biochemical reactions of photosynthesis could be in three different steady-states (Sharkey et al., 2007). In the first state, the carboxylation rate of Rubisco is limiting to photosynthesis and this occur at low [CO_2], usually < 200 µmol mol^{-1}. The assumption for this state is that enough substrate (RuBP) is available for CO_2 binding. State number two occurs at higher [CO_2], typically >300 µmol mol^{-1} and assumes that the regeneration rate of RuBP is limiting to photosynthesis. There is a correlation between RuBP limiting photosyntheis and higher [CO_2] since the oxygenation rate will decrease and more RuBP will be carboxylated. The state occurs when photosynthesis is limited by light but also when other enzymes in the Calvin cycle than Rubisco limit the rate of photosynthesis. In the third state, the leaf is not able to keep pace with the triose phosphate production capacity of the chloroplasts and take care of these products, therefore, this state is called TPU (triose phosohate use) limiting. The model assumes that A is 100% of the lowest rate allowed by the three states, or biochemical conditions. Since the CO_2 fixation occurs in the chloroplast, it is the [CO_2] inside the chloroplast (C_c) that needs to be plotted against A. It is possible to estimate C_c if A, C_i and g_m are known (Sharkey et al., 2007).

In addition to stomata, there is one more resistance that the CO_2 from the atmosphere have to pass before reaching the site of carboxylation. From the substomatal cavities the CO_2 molecules have to pass the intercellular air spaces of the mesophyll and to diffuse across the cell wall, plasmalemma, cytosol and chloroplast envelope before reaching the carboxylation site in the chloroplast stroma (Warren C.R., 2008) This pathway is more often talked about as a conductance rather than a resistance and is usually called mesophyll conductance (g_m).
One advantage of using the A/C_i curve fitting model by Sharkey et al. (2007) is that mesophyll conductance is specificially calculated. It is possible to estimate g_m directly from A/C_i data and since especially V_{cmax} is sensitive to this estimation it is important (Sharkey et al., 2007).

Data on A_n and C_i achieved from the measured A/C_i curves, as well as temperature, atmospheric pressure and O_2 partial pressure was used as inputs to fit the curves in the model. Limiting factors was estimated both relative to C_c and relative to C_i concentrations, although the same results were achieved for both. Concentrations < 200 µmol mol^{-1} was regarded as Rubisco limited while concentrations >400 µmol mol^{-1} was considered as RubP limited, while TPU was never limiting for A_n (Figure 4). V_{cmax}, J_{max}, R_d and g_m was calculated for each A/C_i curve both at mean T and when T was adjusted to 25ºC. Predicted A_n at ambient (380 µmol mol^{-1}) and elevated (540 µmol mol^{-1}) [CO_2] was also calculated by the model, using C_i values

of 266 and 378 µmol mol^{-1}, respectively (C_i must be multiplied by 0.7 for conversion to C_a and adjustment for stomatal resistance). A_n was then tested for treatment effects both at 70% of growth concentration and at common [C_i] of 266 and 378 µmol mol^{-1} (which indicates effects on photosynthetic capacity).

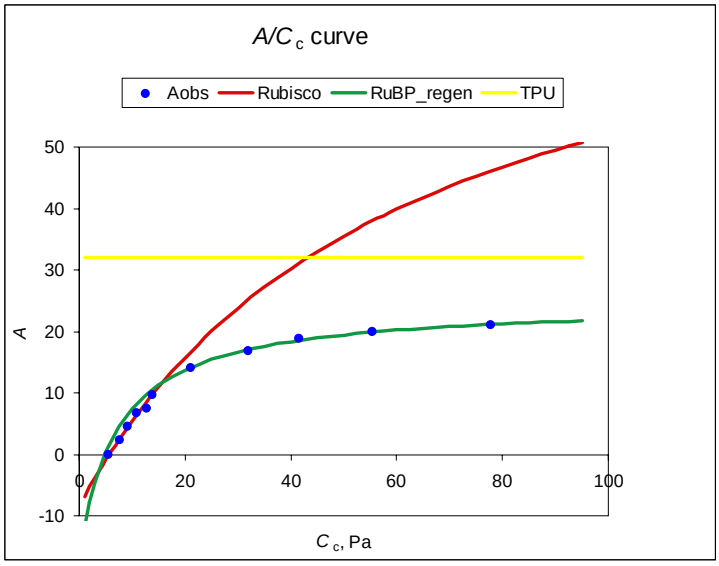


Figure 4. A/C_i curve fitted in the model by Sharkey et al. (2007). A_n is first Rubisco and then RubP limited, while TPU was never limiting. Most A/C_i curves showed a similar pattern.

2.2.2 Stomatal response curves

After finishing the mneasurement of an A/C_i curve, stomatal response curves were measured with the same temperature and light conditions. The sample CO_2 concentration [CO_2S] was changed as follows; 380, 540, 380, 280, 380, 760 and 380 µmol mol^{-1}. The water vapour deficit (VPD) in the chamber was held stable around 1.0-1.2 kPa. The CO_2 conditions represent ambient (380), pre-industrial (280), and levels expected in 2050 (540) and 2100 (760) µmol mol^{-1}. When g_s was stable (less than 1% change during 5 minutes) and after waiting for at least 30 minutes, [CO_2S] was changed. If one hour passed without g_s reaching stability, [CO_2] was changed anyway.

If g_s varied more than 30% between the readings at C_a = 380 µmol mol^{-1} before and after each measurement at C_a = 540, it was assumed that the variation was dependent on other factors than stomatal responses. If this requirement was not fulfilled, i.e. if the limit of 30% was exceeded, this data was excluded from the analysis. Stomatal responses to increasing/decreasing [C_a] was calculated as the difference in % between what g_s at C_i = 380 µmol mol^{-1} would have been expected to be at the time for measuring g_s at C_a = 540 µmol mol^{-}

[1] and g_s at C_i = 540 µmol mol^{-1}. The same 30% limitation level and calculations were made for g_s at C_a = 280 and 760 µmol mol^{-1} as for g_s at C_a = 540.

To get a picture of the drift in g_s over time, relative g_s at C_a = 380 µmol mol^{-1} was calculated by normalization.

2.3 Leaf analysis- leaf mass per area and N content

After gas exchange measurements leaves were separated from the twigs and three leaf discs (12 mm in diameter) were taken from each leaf. Leaf and bulk material was dried in the microwave for ten seconds and then stored in a freezer. Before analysis leaves were dried in the oven three times (24 h, 70 ºC). The leaf discs were weighted for calculations of leaf mass per area (LMA), while the bulk material was ground using a mixer mill (MM 301, Retsch GmbH, Germany) before analysis of N concentration by an elemental analyzer CHNS-O (EA 1108, Fisons Instruments, Italy). LMA was calculated from weight and area of the leaf discs and N content per area (g/m^2) could then be calculated by multiplying LMA and N concentration.

2.4. Leaf temperature measurements and energy balance

The energy from solar radiation reaching leaves is partly used for warming up the leaf and partly to evaporate water. If the stomatal opening is small, more energy will be used for warming up the leaf and less will be used for transpiration compared to a leaf with larger stomatal opening. By measuring leaf temperature (T_{leaf}), the relative amount of energy used for warming up the leaf and for transpiration can therefore be estimated (Campbell & Norman, 1998).

2.4.1 Leaf and air temperature measurements

Leaf temperature (T_{leaf}) measurements were performed on the 20th of August on healthy sun leaves in the upper canopy using radiometric infrared thermometers. Measurements were conducted placing the thermometers 2-3 cm from the leaf surface, holding the leaf horizontally and only under sunny conditions. In order to avoid confounding influences of climatic variation, the leaves in one block measured simultaneously. With four persons measuring at the same time, there was one person in each treatment/ring. Totally 30 leaves were measured in each ring, 15 from each section (aspen/birch and aspen). During the measurements, air temperature (T_{air}) in the canopy was logged every minute, since T_{leaf} will also be dependent on T_{air}.

The thermometers were calibrated before and after the measurements, so that data could be corrected for thermometer failure. The difference between T_{air} and T_{leaf} was calculated.

2.4.2 Energy balance

Transpiration (E) and g_s was calculated from energy balances. Since energy can not be destroyed, only transformed into different kinds of energy, knowledge of T_{leaf} makes it possible to derive values of E and g_s from energy balance calculations. The energy absorbed by the leaf can be divided into sensible heat loss, emitted long wave thermal radiation and latent heat loss (transpiration). E can be achieved if the other parameters are solved by a number of calculations (including knowledge of T_{leaf}, T_{air}, relative humidity, wind speed, leaf dimensions etc). The vapour conductance (g_v) can then be calculated if E and vapour pressure deficit (VPD) is known and g_s is achieved by subtracting boundary layer conductance (g_b) from g_v (Campbell & Norman, 1998).

2.5 Sap flow measurements

Xylem sap flow data from a one day in June 2004 without CO_2 fumigation was used to get an estimation of tree water use and conductance on a canopy level, canopy conductance (g_c). Data on sap flow per unit base area (g m^{-2} s^{-1}) was achieved from pure aspen and aspen/birch communities by Uddling et al. (2008), where the measurement procedure is also described. Since sap flow was measured on a number of trees the day for the fumigation gap, it was possible to compare these data with data from the same trees during 7 “reference days”, when fumigation worked. Data from the day without fumigation was divided by data from the same trees one day when fumigation worked to get a relative value. Data was normalized and a mean relative value was achieved for each ring. This value is an estimation of the difference in g_c in each ring with and without CO_2 fumigation.

2.6 Statistics

All parameters described above were tested for treatment effects. All parameters were fully replicated (3 replicates for each treatment) except for g_s and sap flow fumigation gap data. Two-factor analysis of variance (ANOVA) with replication was used to test for treatment effects and 90% confidence intervals were calculated. Regression was used to test for possible correlations between N per unit area and V_{cmax}, J_{max}, g_m and R_d.

3. RESULTS

3.1 Photosynthetic parameters and N content

V_{cmax}, $J_{max,}$ g_m and R_d were calculated from the A/C_i curve fitting model by Sharkey et al. (2007). Ten or eleven pairs of data of C_i and A_n from A/C_i curves measured with a [CO_2] between 60 and 1500 µmol mol^{-1} were used as input values for the model. The results shown are from values adjusted to 25 ºC by the model. There were no significant treatment effects on V_{cmax}, J_{max} (Figure 5, Table 1), R_d or g_m. The results were similar whether limiting factors were set based on C_i or C_c values.

A_n at C_i values of 266 and 379 µmol mol^{-1} was predicted from the model for all rings. When treatment effects on A_n were tested at common C_i values of both 266 and 378 µmol mol^{-1}, there were no significant effects (Figure 6 a). There was a significant effect of elevated CO_2 when effects on A_n were tested at 70% of growth concentration, 266 and 378 µmol mol^{-1} (P = 0.049; Figure 6 b, Table 1).

The leaf analyses of LMA (g m^{-2}) (Figure 7, Table 1), N concentration (in % of biomass) and N per area (g m^{-2}) (Figure 8, Table 1) did not indicate significant effects from growth in elevated CO_2 or O_3. From the linear regression analysis of N per area versus V_{cmax}, J_{max}, g_m and R_d, significance was only observed from the relationship between N and g_m (p = 0.042).

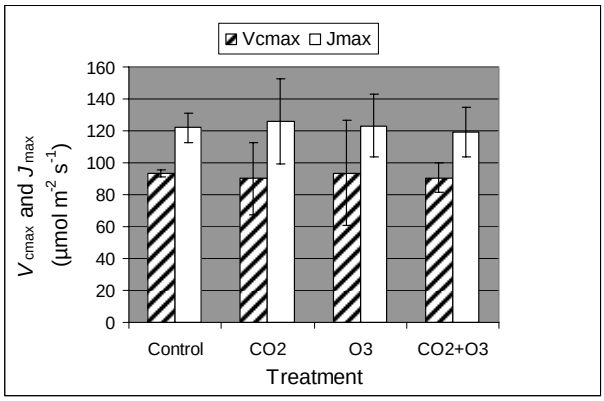
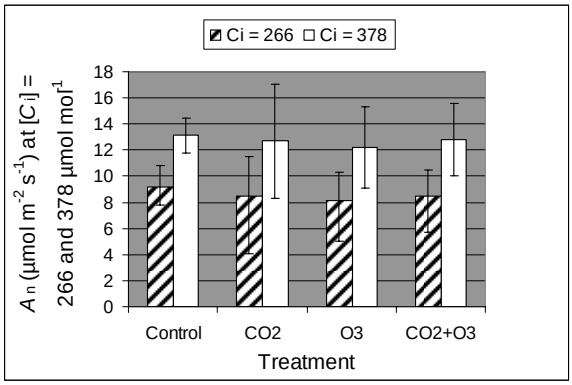


Figure 5. Treatment effects on maximum rate of carboxylation (V_{cmax}) and maximum rate of electron transport (J_{max}). Error bars represent 90% confidence intervals.

a)



b)

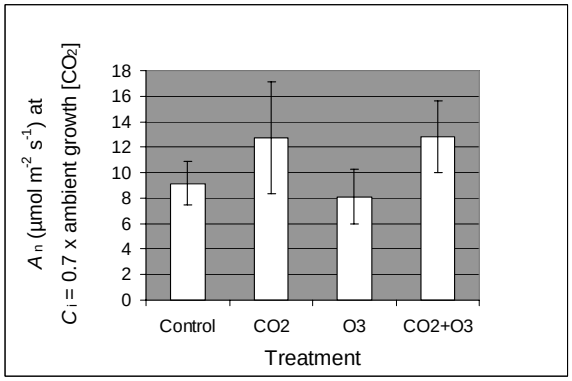


Figure 6. Treatment effects on net photosynthesis (A_n) at (a) common intercellular CO_2 concentrations [C_i] of 70% of ambient and elevated [CO_2]; 266 and 378 µmol mol^{-1} and (b) at [C_i] = 70% of growth concentrations; 266 µmol mol^{-1} for control and O_3 and 378 µmol mol^{-1} for CO_2 and CO_2+O_3. Error bars are representing 90% confidence intervals.

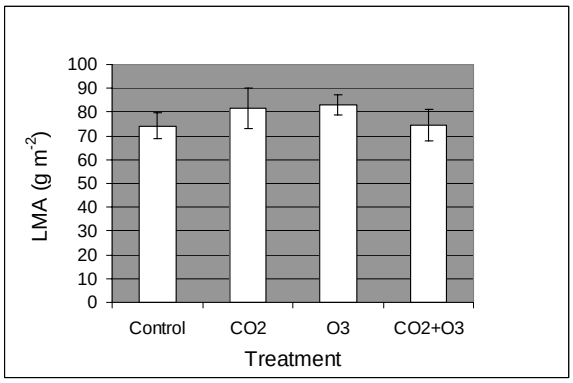
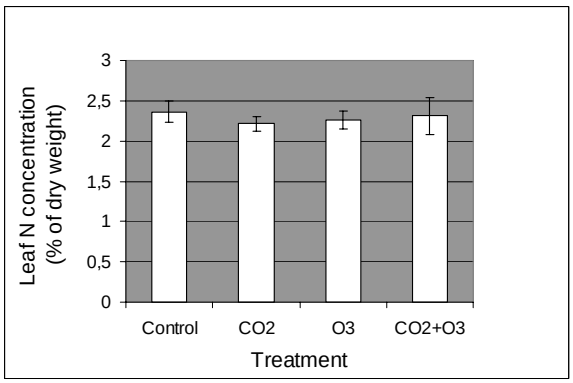


Figure 7. Leaf dry mass per area (LMA). Error bars are representing 90% confidence intervals.

a)



b)

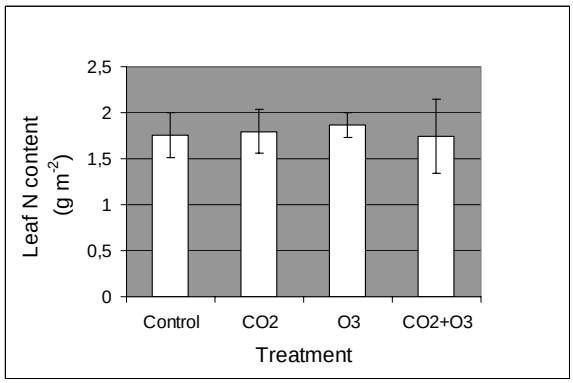


Figure 8. (a) Leaf N concentration (as % of dry weight) and (b) leaf N content per area (g m^{-2}) for the different treatments. Error bars are representing 90% confidence intervals.

Table 1. P-values of treatment effects (elevated CO_2, O_3 and CO_2 x O_3 interaction) on A_n at C_i = 70 % of ambient, elevated and growth [CO_2], V_{cmax}, J_{max}, LMA and N concentrations expressed on dry mass (% N) and area (g m^{-2}) basis. * indicates significiant effects.

	Elevated [CO_2]	Elevated [O_3]	Elevated [CO_2] and [O_3], interaction
A_n (µmol m^{-2} s^{-1}) at:			
Ci = 70 % of ambient [CO_2] (266 µmol mol^{-1})	0,92	0,728	0,699
Ci = 70 % of elevated [CO_2] (378 µmol mol^{-1})	0,966	0,836	0,79
Ci = 70% of growth [CO_2] (266 or 378 µmol mol^{-1})	**0,049***	0,801	0,752
V_{cmax} (µmol m^{-2} s^{-1})	0,805	0,969	--
J_{max} (µmol m^{-2} s^{-1})	0,983	0,827	0,752
LMA (g/m^2)	0,938	0,897	0,26
N (% of biomass)	0,63	0,984	0,305
N (g/m^2)	0,808	0,861	0,629

3.2 Stomatal conductance and sap flow

The drift in g_s, A_n and C_i over time are displayed as relative values at C_a = 380 µmol mol^{-1}, before and after measuring at C_a = 540 µmol mol^{-1}. Only rings from block 1 that could be used in the data analysis are included. There is a small decrease over time in relative g_s (Figure 9 a). There is a similar decrease in A_n (Figure 9 b) that seems to be of approximately the same magnitude, although a bit delayed compared to g_s. C_i (Figure 9 b) is very stable over time.

There were no significant treatment effects on stomatal sensitivity to changes in [C_a] from 380 to 540 µmol mol^{-1}. There were very few replicates, totally 7, that could be used in the analysis, after the exclusion of data that changed more than 30% when the values of g_s at C_a = 380 µmol mol^{-1} before and after the measurements at 540 µmol mol^{-1} were compared. There was no significant treatment effect on the change in g_s when [C_a] was changed from 380 to 280 µmol mol^{-1} either, but for this analysis even fewer replicates (5) could be used. Despite

the very few replicates it was possible to see a significant short-term closure response from 380 to 540 µmol mol^{-1} when pooling results for all 7 leaves (p-value = 0.007; Figure 10).

The analysis of sap flow data from a day without CO_2 fumigation compared to reference days with CO_2 fumigation demonstrates a significantly stronger increase in elevated [CO_2] treatment (p-value = 0.088; Figure 11, Table 2) in the pure aspen community. Relative sap flow was 3.2 % higher in elevated CO_2 than in control and 9.1 % higher in elevated CO_2+O_3 than in elevated O_3. In the mixed aspen/birch section relative sap flow was 3.1 % and 12.5 % higher in elevated CO_2 than in control and in elevated CO_2+O_3 than in O_3, respectively (Figure 6), but in this section there were fewer replicates and the effect was not significant.

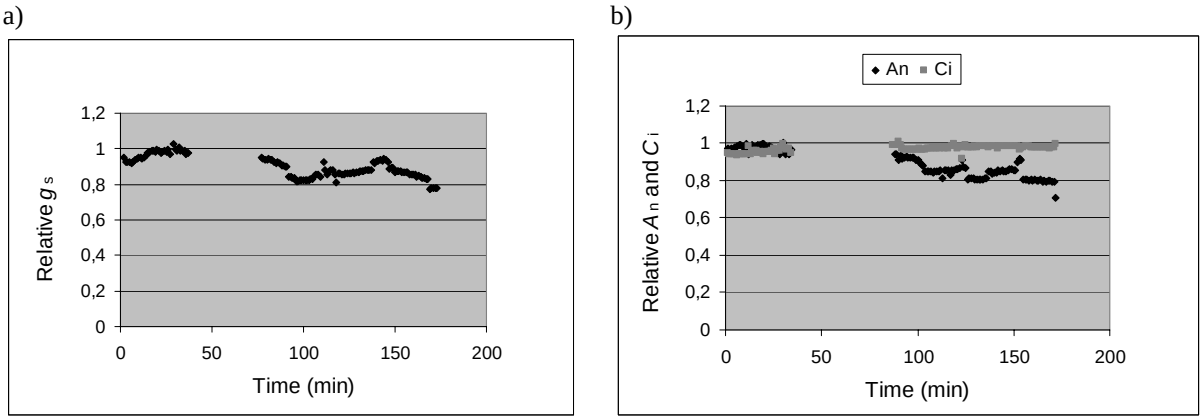


Fig 9. Drift in (a) stomatal conductance (*g*s), (b) net photosynthesis (A_n) and intercellular CO_2 concentration (C_i) over time measured at C_a = 380 µmol mol^{-1} shown as relative values. Relative values were obtained by dividing *g*s, *A*n and *C*i logged every minute by the maximum values of respective variable from each leaf. The drift in *g*s, *A*n and *C*i shows mean values of the leaves from ring 1.1, 1.2, 1.3 and 1.4 (all treatments were represented).

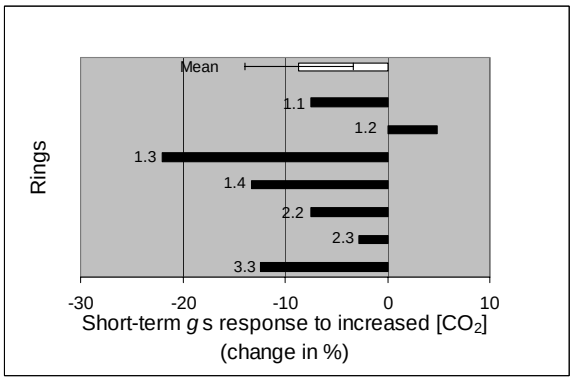


Figure 10. Short-term stomatal conductance (*g*s) response when C_a was increased from 380 to 540 µmol mol^{-1}. The response is showed as g_s change in % where negative values means stomatal closure and positive values stomatal opening. All rings that could be used for data-analysis (see text) are included in the figure. The error bars represent 90% confidence intervals. The over-all stomatal closure response was significant, p-value = 0.007.

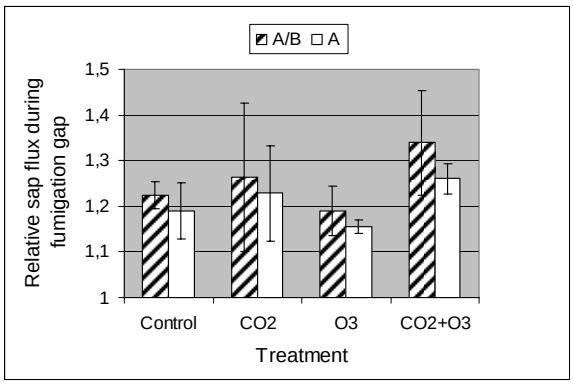


Fig 11. Sap flow on one day in the summer of 2004 when there was accidentally no CO_2 fumigation divided by data from the same trees during 7 reference days (when fumigation worked). The bars display ratios of both mixed aspen/birch (A/B) communities and pure aspen (A) communities. The error bars represent 90% confidence intervals.

Table 2. P-values of treatments effects on short-term *g*s response to increased [CO_2], as well as on sap flux during a fumigation gap (lack of fumigation compared to fumigation), for mixed aspen-birch (A/B) and pure aspen (A) sections. *indicates significant effects.

	Elevated [CO_2]	Elevated [O_3]	Elevated [CO_2] and [O_3], interaction
Short-term g_s response to elevated [CO_2] (mol m^{-2} s^{-1})	0,876	0,649	0,928
Relative sap flux (no unit)			
A/B	0,331	0,9718	0,543
A	**0,088***	0,8941	0,309

3.3 Leaf and air temperatures and energy balance derived transpiration and stomatal conductance

Leaf and air temperature data from the 20th of August 2008 as well as the energy balance derived values of transpiration (E) and g_s obtained from these data, displayed a large variability within treatments. There were no indications of significant treatment effects on the difference between T_{leaf} and T_{air} (Figure 12, Table 3), E or g_s (Figure 13, Table 3), but the errors were to large for this result to be reliable.

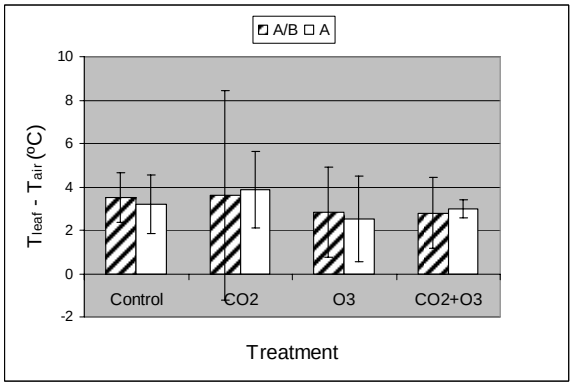


Figure 12. Difference between leaf and air temperature (T_{leaf}-T_{air}) on August 20 in 2008 in the Aspen FACE experiment. Measurements were conducted in both mixed aspen/birch communities (A/B) and pure aspen communities (A). The error bars represent 90% confidence intervals.

a b

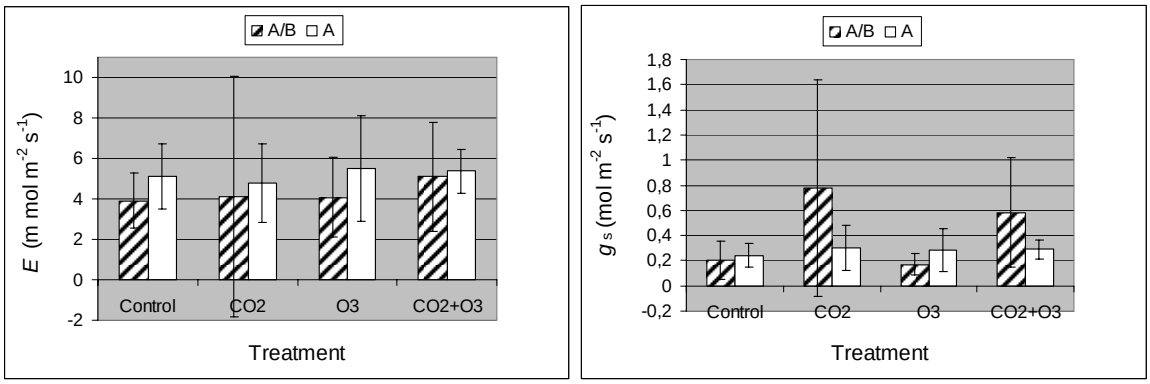


Figure 13. Leaf (a) transpiration (E) and (b) stomatal conductance (g_s) obtained from energy balance calculations. A/B shows mixed aspen/birch communities and A communities with aspen only. The error bars represent 90% confidence intervals.

Table 3. P-values of treatment efects on T_{leaf} -T_{air} and E and gs derived from energy balance calculations for A/B and A communities.

	Elevated [CO_2]	Elevated [O_3]	Elevated [CO_2] and [O_3], interaction
T_{leaf}-T_{air}			
A/B	0,988	0,677	0,974
A	0,545	0,424	0,908
E			
A/B	0,78	0,797	0,854
A	0,856	0,683	0,927
g_s			
A/B	0,137	0,71	0,799
A	0,712	0,872	0,766

4. DISCUSSION

4.1 Photosynthesis

The lack of significant treatment effects on V_{cmax} and J_{max} (Figure 5, Table 1) differ from earlier results in aspen, where both V_{cmax} and J_{max} was decreased by elevated CO_2 (Ellsworth et al., 2004). Even though photosynthetic capacity (carboxylation capacity and electron transport capacity) was down regulated in the young aspen trees, it seems like this effect has disappeared as trees have grown older and reached and canopy closure. Ellsworth et al. (2004) also found that A_n measured at common C_i values was lower in the CO_2 treatment, which was in agreement with the decreases in V_{cmax} and J_{max} because of the indication of down regulation. This effect had also disappeared in the previous study (Figure 6 a, Table 1), which further confirm that photosynthetic down regulation have disappeared.

A_n at 70% of growth concentration was significantly higher in elevated [CO_2] (Figure 6 b, Table 1), which would also be expected if photosynthesis in was not affected by growth in elevated O_3 or CO_2. The same result was reported by both Noormets et al. (2001) and Ellsworth et al. (2004), although the first observed decreases in A_n in old and mature leaves of an ozone sensitive clone.

Ellsworth et al (2004) also investigated N content, although the result was presented as an average across species in different FACE experiments and not specified for aspen. Mass based N concentration was significantly reduced and area based N concentration marginally significantly reduced by CO_2 treatment. Therefore, Ellsworth et al. suggested that since both A_n, V_{cmax}, and N content decreased by about the same magnitude, the responses could possibly be consequences from carbohydrate accumulation and subsequent N dilution. Lindroth et al. (2001) also saw that N concentration was decreased by elevated CO_2 early in the experiment and this effect was seen in aspen. Later, this effect was gone (Zak et al., 2007), which matches the results from the N analysis in this study (Figure 8, Table 1).

One explanation to why these early CO_2 effects were not observed any longer, could be that the relationship between fine root mass and canopy and stem mass differed from the same relationship in the beginning of the experiment in elevated CO_2. Compared to the other treatments fine root growth in elevated CO_2 was faster compared to canopy and stem growth (King et al. 2005). The relationship between decreased N content and photosynthetic capacity observed in the earlier therefore suggests that N uptake was lower in the beginning than now and therefore less N could be used for photosynthesis (in enzymes like Rubisco as well as in chlorophyll). As roots grew larger, N uptake was facilitated. Because elevated CO_2 result in

more carbohydrate accumulation than in ambient CO_2 levels, N concentration might also have decreased as a result of dilution effects (see e.g. Luo et al. 1994).

4.2 Stomatal conductance

Noormets et al. (2001) calculated g_s from A/C_i measurements in one ozone sensitive and one ozone tolerant clone of aspen in the beginning of Aspen FACE. Stomatal conductance was significantly reduced by elevated CO_2 in both clones, whereas elevated O_3 only had a decreasing effect on mature and old leaves of the ozone-sensitive clone. In the present study, no significant treatment effects were found on g_s (Table 2), but the limited number of replicates also have to be considered. Later in the experiment, Uddling et al. (2008) found no CO_2 effects on sap flow, indicating that g_s was no longer reduced under elevated CO_2. King et al. (2005) found substantially (+63%) larger fine root biomass in response to elevated CO_2 in 2003, and litter accumulation was also larger in elevated CO_2 which increases the water holding capacity of the upper soil and consequently also the tree water use (Uddling et al., 2008). These effects may have contributed in counteracting the leaf-level stomatal closure response.

Relative sap flow (sap flow during a day without CO_2 compared with sap flow during reference days with CO_2 fumigation) was significantly higher in elevated CO_2 in the pure aspen section (Figure 11, Table 2). Averaged over all rings and treatments, sap flow was higher the day without fumigation than the reference days due to differences in vapour pressure deficit of the air. But, the enhancement was particularly large in rings with CO_2 treatment. This was most likely because stomata responded to the decreases and that the sensitivity to changes in $[CO_2]$ is not lost after more than 10 years of exposure. This observation on canopy-level was supported by the highly significant short-term CO_2 response on leaf-level (Figure 10, Table 2). These two results together shows that the reason to why *g*s was reduced in the beginning of the experiment (Noormets et al., 2001), but not later (Uddling et al., 2008) is not because of reduced stomatal sensitivity, the effect could instead be the result of larger transpiring leaf area compared to root mass. Later, the relationship between roots and leaves was more balanced as canopy closure was reached.

The drift in relative g_s showed a comparatively small decrease over time (Figure 9, Table 2). Stomata does not only respond to environmental factors, there are also daily rhythms that seem to be controlled from within the plants (Raven et al., 2003). This decrease might be because of this daily rhythm. The small decrease in relative g_s shows that it was possible to

study direct responses of g_s for some leaves. However, almost half of the measured leaves had to be excluded from the analysis.

4.3 Temperature measurements and energy balance

Unfortunately, the data from the leaf and air temperature measurements (T_{air} - T_{leaf}) displayed a very large variation (Figure 12, Table 3), which makes it impossible to draw any conclusions from these results. The same problem applies to the energy balance derived E and g_s (Figure 13, Table 3). Since the values of E and g_s were reasonable, the results would probably have been useful if T_{leaf} measurements were repeated. Therefore, more measurements and a larger amount of data would have been desirable.

5. CONCLUSIONS

The present study on aspen has revealed important results about photosynthetic responses to long-term growth under elevated CO_2 and/or O3. The down regulation of A_n, V_{cmax} and J_{max} found earlier (Ellsworth et al., 2004) was not observed after more than 10 years of treatment and after canopy closure. Since neither N content was decreased any longer, the early down regulations of photosynthetic capacity might have been dependent on N dilution effects or limited N uptake due to less relative root growth. No O_3 effects or interactions effects between CO_2 and O_3 was observed on photosynthesis.

There was no evidence for stomatal acclimation from growth in CO_2 or O_3 in this study. There was still a stomatal CO_2 response left in aspen which was seen on both leaf and canopy level. The reason to why the earlier observed reduction in g_s (Noormets et al. 2001) was gone later in the experiment (Uddling et al., 2008) was therefore most likely not dependent on loss of stomatal sensitivity.

It is important to be able to predict the responses of CO_2 assimilation and g_s in the future, not only because of effects on plants themselves, but also because of feed-back from plants on the environment. Altered photosynthetic capacity and g_s are likely to affect both the ability of plants to sequester carbon, but also plant water use, which in turn can affect whole carbon and hydrological cycles. Studies like this could therefore be useful for the development of climate models. One interpretation of the result in this study is that that water use efficiency and carbon assimilation is not increased in aspen, although many other factors interact on these functions. This study clearly reveals the importance of long-term experiments, but also that it can be hard to predict future scenarios even from FACE experiments, but it is also important to keep in mind that if the experiment was aloud to continue even longer, the outcome of the

results maybe would have been different because of effects on carbon, water and nutrient balances.

REFERENCES

Ainsworth E.A. and Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant, Cell and Environment 30: 258-270.

Campbell G.S. & Norman J.M. 1998. An Introduction to Environmental Biophysics. 2nd edition. New York. Springer Verlag. 978-0-387-94937-6.

Dickson R.E., Lewin K.F., Isebrands J.G., Coleman M.D., Heilman W.E. et al. 2000. Forest atmospheric carbon transfer storage-II (FACTS II)- The aspen free air CO_2 and O_3 enrichment (FACE) project in an overview. USDA Forest Service North Central Experiment Station. General Tech. Rep. NC-214.

Drake B.G., Gonzàlez-Meler M.A. & S.P. Long. More efficient plants: A consequence of rising Atmospheric CO_2. Annu. Rev. Plant. Physiol. Plant Mol. Biol. 1997. 48: 609-39.

Ellsworth D.S., Reich P.B., Naumburg E.S., Koch G.W., Kubiske M.E. et al. 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forests, grassland and desert.

FACTS II: The Aspen FACE Experiment. Homepage [online](2009-03) Available from: www.aspenface.mtu.edu. 2009-06-15

Farage P.K., Long S.P., Lechner E.G. & Baker N.R. 1991. The sequence of change within the photosynthetic apparatus of wheat following short-term exposure to ozone. Plant Physiology 1991. 95: 529-535.

Farquhar G.D., von Caemmerer S. & Berry J.A. (1980). A biochemical model photosynthetic of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149: 78-90.

Fowler D., Cape N.J., Coyle M., Flechard C., Kuylenstierna J. et al., 1999. Water, Air and Soil Pollution 116: 5-32.

IPCC 2001. The Scientific basis. Contributions of working group 1 to the fourth assessment report of the IPCC. Available at:
www.grida.no/publications/other/ipcc_tar/?src=/CLIMATE/IPCC_TAR/WG1/index.htm

IPCC 2007. The physical science basis. Contribution of working group I to the fourth assessment report of the IPCC. Available at: www.ipcc.ch/

Jenkin M.E. & Clemitshaw K.C. 2000. Ozone and other secondary photochemically pollutants: chemical processes governing their formation in the planetary boundary layer. Atmospheric Environment 2000: 2499-2527.

Karnosky D.F., Zak D.R., Pregitzer K.S., Awmack C.S., Bockheim J.G. et al. 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2. Functional Ecology 17: 289-304.

King J.S., Kubiske M.E., Pregitzer K.S., Hendrey G.R., McDonald E.P. et al. 2005. New Phytologist 168: 623-636.

Lindroth R.L., Kopper B.J., Parsons W.F.J., Bockheim J.G., Karnosky D.F. et al., 2001. Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). Environmental Pollution 115: 395-404.

Larcher W. 2003. Physiological Plant Ecology- Ecophysiology & Stress Physiology of Functional Groups. 4th edition. Berlin. Springer Verlag. 978-3-540-43516-7.

Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. 2004. Rising atmospheric carbond cioaxide: Plants FACE the future. Annu. Rev. Plant Biol. 55: 591-628.

Lou Y., Field C.B. & Mooney H.A. 1994. Predicting responses of photosynthesis and root fraction to elevated $[CO_2]_a$: interactions among carbon, nitrogen and growth. Plant, Cell and Environment 17: 1195-1204.

Medlyn B.E., Barton C.V.M., Broadmeadow M.S.J., Ceulemans R., De Angelis P. et al. 2001. Stomatal conductance of forest species after long-term exposure to elevated CO_2 concentration: a synthesis.

Morison J.I.L. 1998. Stomatal response to increased CO_2 concentration. Journal of Experimental Botany. 49: 443-452.

Morison J.I.L. 2001. Increasing Atmospheric CO_2 and stomata. New Phytologist 149: 154-156.

Noormets A., Sôber A., Pell E.J., Dickson R.E. and Podila G.K. 2001. Stomatal and non-stomatal limitation to photosynthesis in two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated CO_2 and/or O_3. Plant, Cell and Environment 24: 327-336.

Nowak R.S., Ellsworth D.S., & Smith S.D. 2004. Functional responses of plants to elevated atmospheric CO_2- do photosynthetic and productivity data from FACE experiments support early predictions? New Phytologist 162: 253-280.

Percy K.E., Legge A.H., Krupa S.V. 2003. Tropospheric ozone: A continuing threat to global forests? Edt. Karnosky D.F., Percy K.E., Chappelka A.H., Simpson C. and Pikkarainen J. 2003. Air pollution, global change and forests in the new millennium. Developments in environmental science vol 3 First edition. Kidlington, Oxford, UK. Elsevier Ltd. ISBN: 0-08-044317-6

Pearson P.N. & Palmer M.R., 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. Nature 406: 695-699.
Raven P.H., Evert R.F. & Eichhorn S.E. 2003. Biology of plants. 6:th ed. W.H. Freeman and Company. New York. ISBN: 1-57259-041-6

Paoletti E. & Grulke N.E. 2005. Does living in elevated CO_2 ameliorate tree response to ozone? A review on stomatal responses. Environmental Pollution 137: 483-493.

Pearcy K.E., Awmach C.S., Lindroth R.L., Kubiske M.E. & Kopper B.J. Altered performance on forest pest under atmospheres enriched by CO_2 and O_3. Nature 420: 403-407.
Robinson M.F., Heath J. & Mansfield T.A. 1997. Disturbances in stomatal behaviour caused by air pollutants. Journal of Experimental Botany 49: 461-469.

Sharkey T.D., Bernacchi C.J., Farquhar G.D. & Singsaas E.L. 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. Plant, cell and Environment 30: 1035-1040.

Uddling J., Teclaw R.M., Kubiske M.E., Pregitzer K.S. & Ellsworth D.S. 2008. Sap flux in pure aspen and mixed aspen-birch forests exposed to elevated concentrations of carbon dioxide and ozone. Tree Physiology 28: 1231-1243.

Volz A. and Kley D. 1998. Evaluation of the montsouris series of ozone measurements made in the nineteenth century. Nature 332: 240-242.

Warren C.R. 2007. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO_2 transfer. Journal of experimental Botany. 59(7): 1475-1487.

Wittig V.E., Ainsworth E.A. & Long S.P. 2007. To what extent do current and projected increases in surface ozone affect photosynthesis and stomatal conductance of trees? A meta-analytic review of the last 3 decades of experiments. Plant, Cell and Environment 30: 1150-1162.

Zak D.R., Holmes W.E. & Pregitzer K.S. 2007. Atmospheric CO_2 and O_3 alter the flow of ^{15}N in developing ecosystems. Ecology 2007: 2630-2639.