EFFECT OF WARMING AND TROPOSPHERIC OZONE ON SILVER BIRCH *(Betula pendula* ROTH*)* GROWTH, LEAF AREA AND SOIL RESPIRATION

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ABSTRACT

In this work, the effect of warming and tropospheric ozone (O_3), singly and interactively on stem, leaf growth and soil respiration rates of two silver birch *(Betula pendula* Roth*)* genotypes (14 and 15) were studied. The measured parameters were stem height, stem base diameter, leaf count, leaf area, and soil CO_2 efflux (root+microbial respiration). The experimental trees were exposed to increased temperature (+0.9 ^oC) and O_3 level (1.4 times the ambient O_3) using open-air exposure system in the experimental field. In 2009, the exposure period lasted from mid-June to the end of September. In both clones, temperature increment enhanced the stem height regardless of O_3 levels, but O_3 did not have any statistically significant effect on stem elongation. Temperature treatment effects on stem height growth were more pronounced in the beginning of the exposure season. In both clones, the stem diameter growth was decreased (more in gt 15) under elevated O_3 alone at the end of season 2009, but temperature cancelled this O_3 effect. A significant temperature×genotype effect on leaf count and leaf area showed that genotypes did differ in their temperature responses partly. In gt 15, leaf count increased more than in gt 14 due to temperature treatments, but on the other hand, leaf area was reduced in temperature-treated trees in gt 15, whereas in gt 14 temperature treatments increased the total leaf area at the same time. On the other hand, temperature alone and in combination with O_3 increased the soil respiration, but in gt 15 only. Hence, on most measurement dates temperature treatments increased gt 15 soil respiration rates, whereas in gt 14 both temperature and O_3 treatments were highly variable and did not seem consistent over time. In general, temperature increment affected silver birch above-ground growth to some extent, but there were also some genotype-dependent responses, whereas ozone effects on tree growth and soil respiration were minor.

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ABBREVIATIONS

ANOVA	analysis of variance
AOC	average ozone concentration
AOT40	accumulated exposure over a threshold 40 ppb
CO_2	carbon dioxide
GHG	greenhouse gases
IPCC	Intergovernmental Panel on Climate Change
IRGA	infra-red gas analyzer
N_2O	nitrous oxide
NO_X	nitrogen oxides
O_3	ozone
OTC	Open top Chamber
ppm	parts per million
ppb	parts per billion
ROS	Reactive Oxygen Species
VOC	volatile organic compound

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1. INTRODUCTION

Due to human activities, the global atmospheric concentrations of greenhouse gases (GHG) and the surface temperature of the Earth are likely to increase (by 1.1-6.4°C by the end of the 21st century), altering the energy balance of the climate system (IPCC, 2007). Temperature has an important influence on plant phenotype and canopy duration (Vitasse et al., 2009), and in the Northern Hemisphere, changes in the duration of growing season may be observed (Steltzer and Post, 2009). In a recent meta-analysis, Way and Oren (2010) showed that several degrees temperature increase stimulates deciduous forest trees growth more pronouncedly than that of conifers, but there are also some studies which state that both deciduous and coniferous forest trees might be affected negatively especially if temperature increase is high enough (Wertin et al., 2011). Simultaneously with global warming, the global background concentrations of tropospheric ozone (O_3) have more than doubled since the pre-industrial level of about 10 ppb to the present concentrations of 25 - 40 ppb (Vingarzan, 2004; The Royal Society, 2008), and are predicted to continue to increase by another 40 to 70 % by the year 2100 (Grenfell et al., 2003; Zeng et al., 2008). Tropospheric O_3 is one of the most phytotoxic air pollutants causing reductions in the photosynthetic capacity, biomass allocation and carbon sequestration of forest trees (Felzer et al., 2007; Wittig et al., 2007), and according to a recent meta-analysis, the current O_3 concentration is decreasing the net photosynthesis of forest trees by 11% and the tree biomass by 7% (Wittig et al., 2009).

Silver birch *(Betula pendula*) is an autochthonous pioneer species, and also one of the economically and ecologically most important deciduous tree species in the boreal region. Though it has been predicted that the proportion of silver birch will increase relative to coniferous trees with the expected climatic warming (Talkkari, 1998), simultaneous ozone stress might also modify and probably reduce the warming effects on birch trees. In order to gain a better understanding of climate change effects on boreal deciduous trees, two silver birch genotypes were exposed to elevated temperature and elevated O_3 alone and in combination from mid-June to the end of September 2009 in open field conditions.

2. LITERATURE REVIEW

2.1 Effect of temperature increase on forest trees and soil respiration

Temperature is known to affect tree growth through photosynthesis, mitosis, respiration, and water transport (Thomas et al., 2007; Way, 2011). There is an optimum temperature for photosynthesis, generally between +15-+20^{o}C for C3 plants and between +25-+35^{o}C for C4 plants. If temperature increases over the range of +25 to +40^{o}C (Ranney and Peet, 1994), it can denature the enzymes, cause functional disorders in the photosynthetic apparatus, damage the photosystem II (Jin et al., 2009), and thus decrease plant growth.

Tree growth at higher latitudes is suggested to be temperature-limited and thus show an initial positive growth response to warming, whereas trees from tropical regions are likely to benefit less from increasing temperatures (Ghannoum et al., 2010). Way and Oren (2010) reviewed the temperature effects on tree physiology and growth, and noticed that a + 5 ^{o}C temperature increase stimulated temperate and boreal tree species growth, as trees grown under elevated temperature were taller, had more foliage, and smaller root systems than those growing under ambient temperature conditions. One explanation for the growth stimulation at higher temperatures might be that respiration is more acclimated to increasing temperature than photosynthesis and thus results into increasing carbon assimilation (Way and Oren, 2010). Some experiments indicate that stem elongation growth is usually more enhanced than that of stem diameter growth (Arend et al., 2011) or stem biomass accumulation (Ghannoum et al., 2010), and in a longer term, this might cause tapering of trees as well as result in difficulties in water transport to the canopy.

In contrast to Way and Oren (2010), Carter (1996) did not find clear differences between deciduous and coniferous species responses to a few degrees (+4 ^{o}C) temperature increase. In addition, some deciduous tree species can actually suffer from warming. For instance, Wertin et al., (2011) exposed red oak *(Quercus rubra)* seedlings to three different temperature regimes (ambient temperature, +3°C increase and +6 °C increase) using chambers, and found that both elevated growth temperatures reduced net photosynthesis, increased respiration and reduced stem

height, diameter and biomass production. If temperature increase is high enough, there is also a risk of drought stress occurring simultaneously. Centritto et al., (2011) and Levanic et al., (2011) reported that a temperature increase (+10°C) caused a drought stress and thus significant reduction in photosynthesis rates and mortality, due to water stress. In experiments where the temperature increase has been low or moderate, (i.e., only circa +1°C increase), Riikonen et al., (2009) and Mäenpää et al., (2011) noticed an increase in the total leaf area, leaf size as well as number of leaves in silver birch and European aspen. Altogether, low to moderate increase (circa +1°C - +3°C increase) in growth temperature can have a stimulatory impact on tree physiology and growth, but large temperature increases (circa +5°C - +10°C) can cause e.g. drought stress and therefore are detrimental to trees.

Soil respiration, i.e., CO_2 released from the soil surface to the atmosphere, represents the second-largest terrestrial carbon flux (Bond-Lamberty and Thomson, 2010). Soil respiration consists of CO_2 released from soil microbial activity and plant roots (Högberg et al., 2001; Epron et al., 2001; Bradford et al., 2008; Niinistö et al., 2011), but in addition to the above biotic factors, this efflux is also controlled by various abiotic factors, e.g. soil temperature, soil moisture and soil texture (Tang et al., 2004). Soil respiration rates are expected to alter under warming climate (Hartley et al., 2007).Several soil warming experiments (Bond-Lamberty and Thomson, 2010; Allison et al., 2010) show at least an initial increase in soil respiration rates, but there are also opposite results. Several researchers (Andrews et al., 2000; Zhang et al., 2005; Hartley et al., 2008; Bradford et al., 2008; Castro et al., 2009) observed that warming decreased soil carbon pools and microbial biomass. Another possible explanation for the decrease in soil respiration rate under warming treatment is that microbial enzymes are active in a certain temperature range (Aguiar-Oliveira and Maugeri, 2011) and under warming conditions, enzymes adapted to lower temperatures can be denatured and thereby alter the functioning of the soil microbes. If warming causes depletion in labile C pools (Kirschbaum, 2004; Eliasson et al., 2005; Knorr et al., 2005), soil respiration might actually decrease over a longer time period.

In forest ecosystems, soil CO_2 efflux rates have also been shown to depend on the amount and velocity of carbon transfer from stems to the roots (Högberg et al., 2001; Sampson et al., 2007; Kuzyakov and Gavrichkova, 2010; Epron et al., 2011). If temperature increase stimulates the tree

growth or C transfer rates in general, it could be also reflected in the soil respiration rates. So far the most of the field experiments studying the effects of warming on soil respiration rates have been performed with soil warming cables (either with electrical heating cables,infrared-heaters or glasshouses). Bronson et al., (2008) used both soil warming only and air+soil warming designs (in both systems temperature increase was $+5^{o}C$) and they found that soil CO_2 efflux was stimulated in the soil-only warming design, whereas in soil+air warming design it decreased. In fact in soil+air warming design affects plant processes such as stomata conductance, photosynthesis, allocation of photosynthates, belowground processes and thus alters the soil respiration (Bronson et al., 2008).

2.2 Effect of tropospheric ozone on forest trees and soil respiration

Tropospheric O_3 is generated from nitrogen oxides (NO_x) and volatile organic compounds (VOCs) in the presence of sunlight, and is currently an important stressor of over 30% of the world's forests (Fowler et al., 1999). However, there are tree species-specific differences in O_3 responses. Fast-growing tree species, such as poplar species (e.g: *Populus nigra)* are more sensitive and responsive to O_3 than slower-growing ones, such as beech *(Fagus sylvatica),* (Bortier et al., 2000). Silver birch is also classified as O_3 sensitive species though ozone-sensitivity varies between genotypes (Matyssek, 2001; Oksanen, 2003).

The level of O_3 injury depends on O_3 concentrations, i.e., acute versus chronic stress (Vahala et al., 2003). Since O_3 damages cell structures (Oksanen et al., 2004), it also reduces the ability of plants to photosynthesize and thus decreases their CO_2 uptake (Sitch et al., 2007). Acute O_3 stress leading to programmed cell death refers to short-term high O_3 concentrations in the range of 120-500 ppb (Kangasjärvi et al., 2001) and chronic O_3 stress means long-term O_3 exposure with peak daily concentrations in the range of 40-120 ppb (Rao et al., 2000). Crown exposure of European Beech *(Fagus sylvatica* L.*)* trees to 150 ppb of O_3 resulted in a change in lignin contents which indicates that acute O_3 stress may have the potential to disrupt the defence and signaling systems in the foliage (Jehnes et al., 2007). Matyssek et al., (2010) used 140 ppb O_3 level and noticed that O_3 did not affect photosynthesis in adult beech trees directly, but reduced CO_2 assimilation by stomatal closure and thereby decreased the stem growth. This result

indicates the high sensitivity of stem growth to acute O_3 stress and is consistent with previous chamber studies with juvenile beech trees (Kolb and Matyssek, 2001). Several other O_3 experiments with various tree species and using chronic O_3 levels have shown that trees can develop foliar injuries, visible cell death lesions, decrease in photosynthesis, growth reductions, and accelerated leaf senescence (Matyssek et al., 1995; Pääkkönen et al., 1997; Chappelka and Samuelson, 1998; and Matyssek Sandermann, 2003; Karnosky et al., 2005; Gielen et al., 2006; Nunn et al., 2005; Oksanen et al., 2009; Mäenpää et al., 2011).

There is also an ongoing debate whether the tree age affects the O_3 response. Mature trees were found to be more sensitive to O_3 than the seedlings and carbon retention to leaves and branches of mature trees was increased, whereas in seedlings there was no clear response (Edwards et al., 1994; Samuelson and Kelly, 1997; Zhang et al., 2010). In contrast, an earlier experiment (Grulke and Miller, 1994) with giant sequoia (*Sequoiadendron giganteum* Bucholz*)* showed that the seedlings were in fact more sensitive to O_3 when compared to mature trees. In several other tree species, O_3 has been found to decrease chlorophyll contents, the rate of photosynthesis and thus the growth (e.g., Kolb and Matyssek, 2001; Matyssek and Sandermann, 2003; Nunn et al., 2005; Matyssek et al., 2010; Mäenpää et al., 2011) and a reduction in biomass as well as carbon allocation to repair damages due to O_3 have been observed (Karnosky et al., 2005).

If O_3 affects tree carbon allocation, it can change tree biomass accumulation patterns, e.g., root: shoot-ratios (Grantz et al., 2006; Wittig et al., 2009). Elevated O_3 is also thought to decrease carbon allocation to roots, mycorrhizas and extramatrical mycelium (Matyssek et al., 2010), cause enhanced nutrient uptake by roots or increase root turnover due to O_3 stress (Andersen , 2003), and also decrease soil respiration rates (Pregitzer et al., 2006). In contrast, Kasurinen et al., (2004), after exposure of two silver birch clones to elevated O_3, found that soil CO_2 efflux response to ozone stress varied depending on the genotype.

2.3 Interactive effects of warming and ozone

There is not much previous information about the interactive effects of temperature and ozone on forest trees. Recently, Riikonen et al., (2009) and Mäenpää et al., (2011) reported that elevated

temperature diminished the ozone effect on photosynthesis by decreasing stomatal conductance, and result suggests that elevated O_3 reduces the ability of trees to benefit from the warming. Possible explanation is that elevated temperature increases VOC emissions (Hartikainen et al., 2009; Ibrahim et al., 2010) and protects leaves against O_3 stress.

2.4 AIMS OF THE STUDY

The main aim of this work was to assess the effects of climate warming and O_3 stress, alone and in combination, on above–ground growth and soil respiration of fast-growing silver birch trees. A more specific aim was to examine if the two genotypes (gt14 and gt15) differ in their responses to climate warming and O_3 stress. The measured parameters were stem growth (stem height and diameter), soil respiration (i.e., soil CO_2 efflux), leaf count and specific leaf area. The following hypotheses were tested: birch growth may benefit from warming, but on the other hand, birches may be susceptible to O_3 stress and in combination treatment O_3 might counteract at least partly temperature-induced changes in tree growth. Temperature treatment may also increase soil CO_2 efflux rates beneath birch trees, whereas O_3 effects can be more variable. However, there may be also genotype-dependant differences in all of these responses. The experimental trees were exposed to increased temperature and O_3 treatments by deploying open-air exposure system in the field (infrared heaters and free-air O_3 fumigation in Ruohoniemi) in growing season 2009.

3. MATERIALS AND METHODS

3.1 Experimental site and design

The experimental site (Fig 1 a) is located in Ruohoniemi field (62° 13' N, 27° 13' E: 80 m a.s.l), near Kuopio Campus Research Garden in central Finland. The open-air field exposure system consists of eight circular 10m-diameter plots, which means that there are four replicates for both ambient air and elevated O_3 (Fig 1 b). The O_3 fumigation system in O_3 plots consists of horizontal and vertical pipe systems, with total height of about 2 m.

Experimental subplots were prepared in the beginning of growing season 2009 (in early June) by mixing mull: sand (2:1 v/v) and laying it on the top of the original field soil. In each subplot, this mixed layer was approximately 30 cm thick and subplots had wooden frames to keep the soil in the subplot area. For this experiment, two different silver birch genotypes (gt14 and gt15) were micropropagated. Birch material was obtained from a naturally regenerated birch stand from FFRI (Finnish Forest Research Institute) Punkaharju Research Station (Laitinen et al., 2000), where it has been previously studied for variation in leaf chemistry, genetic resistance mechanisms against O_3, frost, insect and mammal herbivores (e.g. Oksanen et al., 2005). The parent tree genotypes were randomly selected. On the basis of previous pot experiment (Kasurinen et al., 2012, in press), these two genotypes also partly differed in their ozone and warming responses.

In the field, each ambient and ozone plot were thus divided into heated and non-heated subplots and there were six trees per clone in each subplot. Treatments were: (1) control (ambient ozone+ambient air temperature), (2) elevated temperature alone, (3) elevated ozone concentration alone and (4) elevated temperature and ozone concentration in combination (n = 4 per each treatment). Both genotypes were planted in each subplot into their own sections: soil was divided with root exclusion cloth between these two sections in order to prevent root mixing.

(a)

(b)

(c)

Fig. 1a-c. a) Ruohoniemi open-air exposure field near the Kuopio Campus Research Garden (http://maps.live.fi/), b) ozone exposure plots and temperature subplots in birch experiment (photo Anne Kasurinen) and c) soil respiration measurement with Licor soil respiration chamber attached to infrared gas analyzer (photo Anne Kasurinen).

3.2 Ozone and temperature exposures in the field

The free-air ozone enrichment system in the Kuopio University Garden has been technically described in Karnosky et al., (2007). Ozone was produced from pure oxygen using an ozone generator (G21, Pacific Ozone Technology Inc., Brentwood, CA, USA), and dispensed from the upwind vertical tubes daily from June to the end of September. O_3 concentrations were constantly monitored from the centre of each plot using UV photometric ozone analysers (Model 1008-RS, Dasibi Environmental Corp., Glendale, CA; ModelO342 Module, Environnement S.A., Poissy). The O_3 fumigation was run 14 h day^{-1} (from 08:00 to 22:00 h) during study period, 7 days a week, except during very low wind velocities, or if the ambient O_3 concentration was below 10 ppb. These low O_3 concentrations occurred mainly during high humidity conditions, e.g., in early morning hours and during rain. Warming treatment was realized using IR-heaters (Model Comfort intra CIR 105–220,230–400V, FricoAB, Sweden). One heater was installed above the canopy in the middle of each warming treatment plot. Heaters were lifted during the growing season to keep the distance between the heater and the canopy constant. In each plot, wind speed and direction were measured using a cup Anemometer (AnemometerA100; WindvaneW200, Vector Instruments). The relative humidity (RH) (HMP35A, Vaisala) and temperature (Humiter50Y, Vaisala) within the canopy (15 cm below the top of the canopy) and in the soil were measured at 7 cm depth. In addition, soil moisture was measured three times a week (and the trees were watered according to the weather conditions) using a Theta Probe soil moisture sensor and plants were watered when necessary with lake water. Each plot was also continuously monitored for wind speed and direction, and each subplot for relative humidity.

The average ozone concentrations for the whole exposure period were 24.2 ppb in ambient plots and 33.4 ppb in O_3 plots, resulting in approximately 1.4 times higher O_3 levels in ozone treatment (Table 1). Average AOT40 (accumulated exposure over a threshold 40 ppb) values for ozone treatment and control were 4.4 ppm.h and 0.1 ppm.h, respectively. Average air temperatures in ambient and warmed subplots over the whole period were 14.5 °C and 15.4 °C,

Table 1. Ozone and air temperature exposure data during the growing season 2009 (June-September). Values are mean O_3 concentrations in ambient and elevated O_3 plots, and mean temperature values in ambient and temperature subplots. (AOT40 = accumulated exposure over a threshold 40 ppb).

	Ambient plots	O_3 Plots
O_3 (ppb)	24.2	33.41
AOT40 (ppm.h)	0.1	4.4
	Ambient subplots	Temperature subplots
Air temperature (°C)	14.5	15.4

respectively. Warming thus resulted in approximately of +0.9 °C air temperature increase over the whole experiment period (Table 1).

3.3 Tree growth measurements

3.3.1 Stem height and base diameter

Stem height and diameter of all experimental trees (N=192) were measured every three weeks from June to September. The base of the stem was marked with a marker pen and the diameter was measured from the base at the mark by using a vernier caliper (from two directions at the marked point). Stem height was measured from the same marked base point to the tip of the stem.

3.3.2 Leaf area and leaf count

Two branches per tree from two trees per genotype per subplot were marked and selected for leaf growth measurements in the field. Every three weeks the leaf length and width was measured from each leaf in the selected branches with a ruler, and all of these measurements were conducted from June to mid-August. In addition, the total number of leaves (branch leaves and stem leaves) was counted similarly every three weeks from all experimental trees (N =192) from

June to mid-August. In August 2009, 96 leaf samples (3 leaves per genotype per subplot) were also collected, placed into small paper bags and transferred to the laboratory. For leaf area measurements, leaves were first scanned and then they were oven-dried (at +60 °C for 3 days) for dry weight measurements. Leaf area was determined from fresh leaves using Adobe Photoshop Elements 7.0 programme.

To correct the leaf area data measured with a ruler, scanned leaf area data was plotted against it in linear regression, and this was done separately for both genotypes. Following linear regression equations were then used to correct field measurement data: Y = 0.9337x + 3.2921 for gt 15 (R^2=0.73) and Y = 0, 9737x + 1.1256 for gt 14 (R^2=0.97).

3.4 Soil respiration measurements

Soil respiration was measured using Licor soil respiration chamber (LICOR 6400-09, LiCor, Lincoln, NE, USA) attached to infrared CO_2 analyzer (IRGA, LICOR 6400, Licor, Lincoln, NE, USA) from soil collars which were inserted into soil at the depth of 2 cm (Fig. 1c). Soil temperature and soil moisture content (vol %) were measured simultaneously with the soil CO_2 efflux measurements. There was one measurement site per genotype per subplot, and measurements were performed once a month from June to September. Mosses and grasses were removed from the collars in the beginning of the experiment and before the measurements when necessary.

3.5 Statistical analysis

Stem and leaf growth as well as soil respiration data were analyzed by linear mixed model ANOVA using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA). Ozone, temperature, genotype and measurement date were used as fixed factors and plot identity as the random factor in the model. Before testing, data were aggregated to obtain plot/subplot means for each treatment per genotype. Differences were considered statistically significant when $P < 0.05$, and marginally statistically significant when $P < 0.10$.

4. RESULTS

4.1 Stem growth responses

According to the results (Table 2), temperature main effect on stem height growth was significant ($p < 0.0001$), as stem height growth increased by 4 % under elevated temperature treatments in 2009 (Fig. 2a). In addition, temperature×time interaction effect on stem height was statistically significant ($p < 0.0001$, Table 2). Thus, the temperature effect on stem height was most pronounced in the mid-July (temperature-caused increase in stem height was circa 9 %), and decreased thereafter (in August temperature-caused increase in stem height was only 3 %).

The stem base diameter results showed that gt 15 had thicker stems in all treatments than gt 14 trees (Fig. 2b, genotype main effect, $p = 0.008$), and this genotype difference remained similar throughout the growing season 2009 (Table 2). However, a statistically significant Temp×O_3×Genotype×Time effect on stem diameter growth was also found ($p=0.016$, Table 2). In gt 14, ozone exposure decreased the stem diameter growth, but this occurred only at the end of the exposure season, and only under ambient temperature (Fig. 3a). In gt 15 these trends did not occur (Fig. 3b).

4.2 Leaf growth responses

Genotype main effect on the total leaf count was significant (Table 2). More precisely, gt15 had 9% more leaves than gt 14 (fig 4a); but this genotype difference on the total leaf count decreased over the growing season (genotype × time interaction, $p = 0.05$, Table 2). There was also a statistically significant temperature×genotype interaction effect on the total leaf count ($p = 0.018$). Temperature increased the total leaf count somewhat more in gt 15 than in gt 14, since gt 15 had 8% more leaves than gt 14 at the end of July, and 10% more in mid-August 2009.

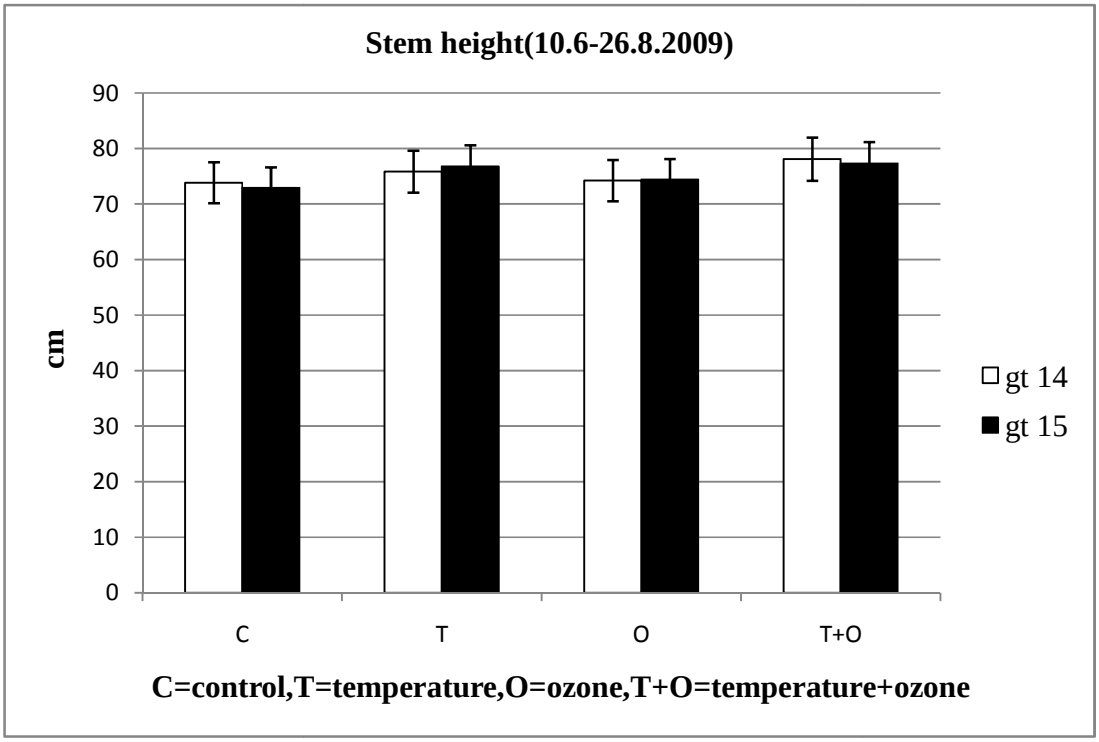
On the other hand, genotype main effect on the total leaf area was significant (Table 2).In fact, the total leaf area was reduced in gt 15, but increased in gt 14 at the same time (fig 4b). However, this genotype difference on the total leaf area decreased over the growing season (genotype×time interaction, $p=0.019$, Table 2).There was also a marginally statistically

significant temperature × genotype interaction effect on total leaf area (p = 0.077).In both genotypes, temperature effects on the leaf area were clearest from Mid-July to mid August 2009.

Table 2. Ozone, temperature and genotype main and interaction effects on stem and leaf growth parameters as well as on soil respiration rates in 2009. P-values are from mixed linear ANOVA, and differences were considered statistically significant when $P < 0.05$, and marginally statistically significant when $P < 0.10$. NS = non-significant.

	Stem height	**Stem base diameter**	**Total leaf area**	**Total leaf count**	**Soil respiration**
Genotype	NS	**0.008**	**<0.0001**	**<0.0001**	NS
Temp	**<0.0001**	NS	NS	NS	**<0.0001**
O_3	NS	NS	NS	NS	NS
Temp × Genotype	NS	NS	**0.077**	**0.018**	NS
O_3 × Genotype	NS	NS	NS	NS	NS
Temp ×O_3	NS	NS	NS	NS	NS
Temp ×O_3 ×Genotype	NS	NS	NS	NS	NS
Time	NS	**<0.001**	**<0.001**	NS	**<0.0001**
Genotype × Time	NS	NS	**0.019**	**0.05**	NS
Temp × time	**<0.001**	NS	NS	NS	**0.09**
O_3 × Time	NS	NS	NS	NS	NS
Temp×Genotype×Time	NS	NS	NS	NS	NS
Temp ×O_3 ×Time	NS	NS	NS	NS	NS
Temp×O_3×Genotype×Time	NS	**0.016**	NS	NS	**0.086**

(a)



(b)

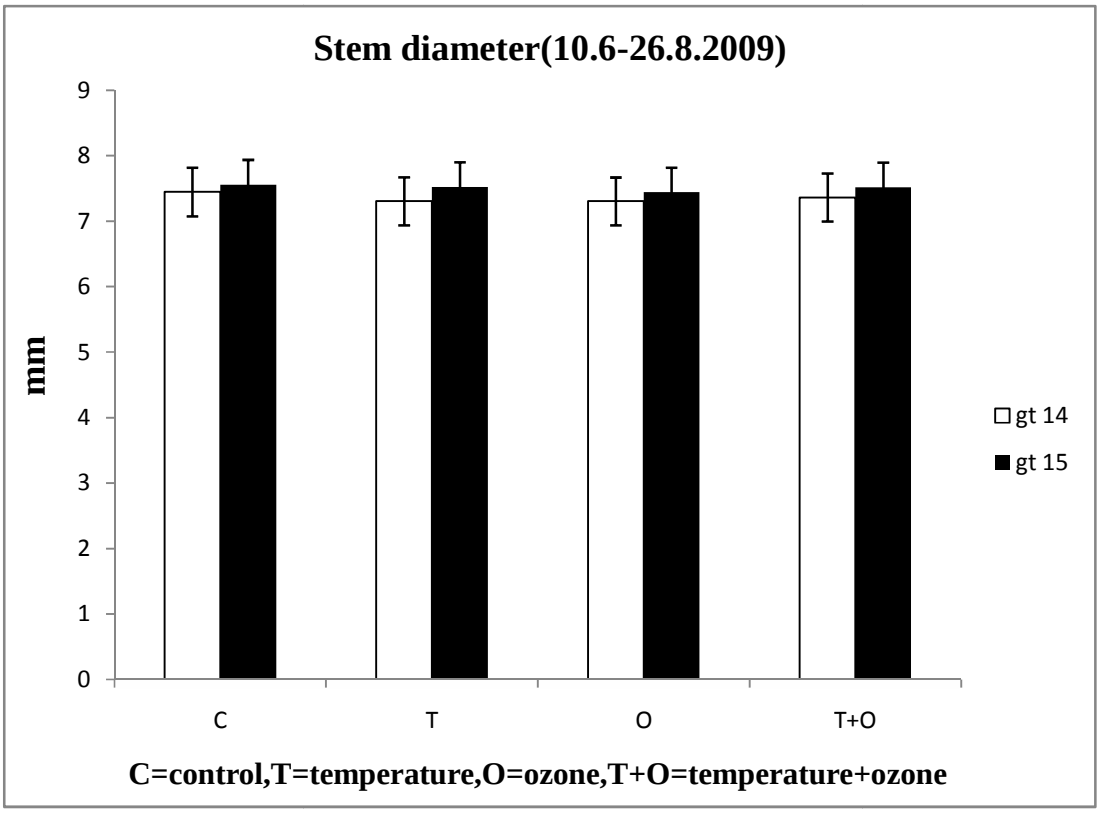
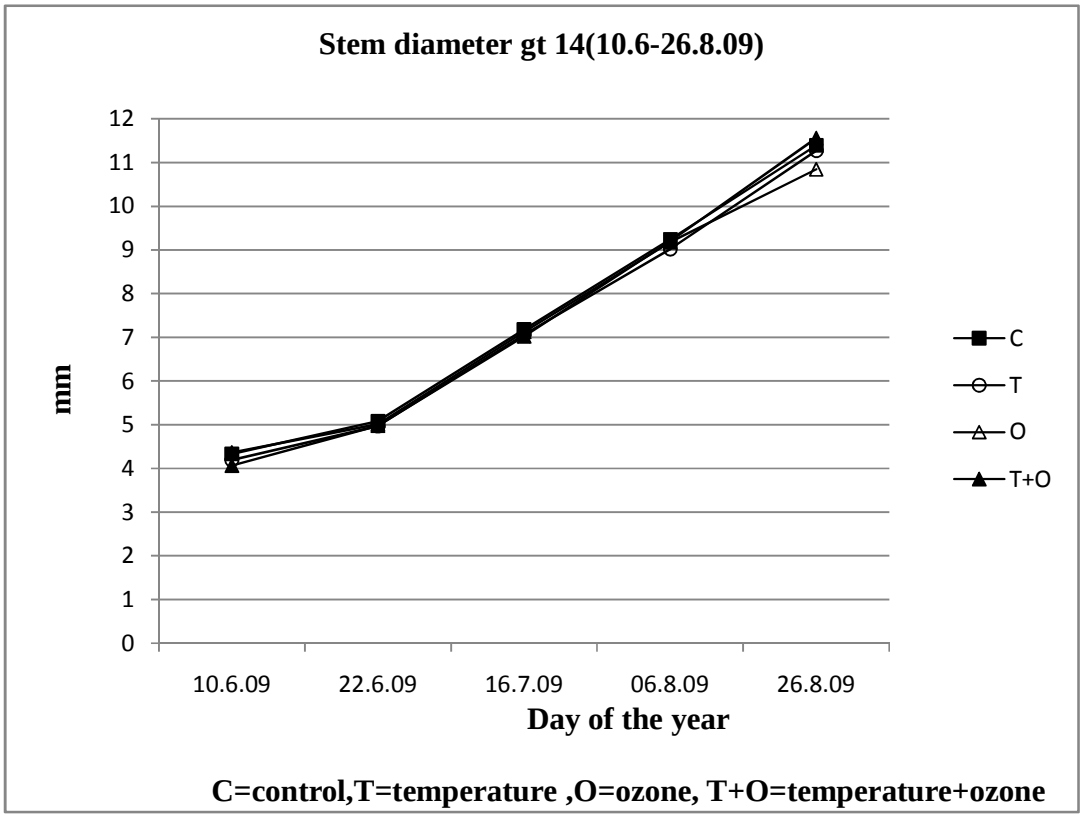


Fig 2. a) Overall stem height and b) stem base diameter (mean ± SE) in different temperature and O_3 treatments (n = 4 per treatment per genotype) during the growing season 2009.

(a)



(b)

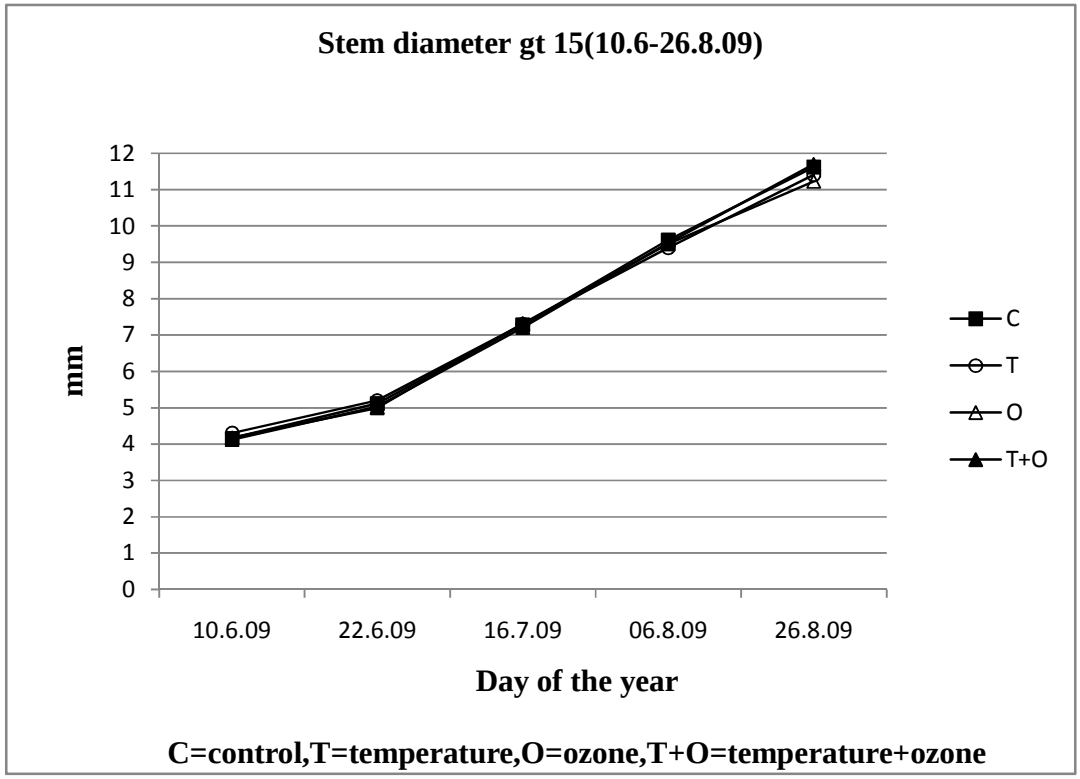
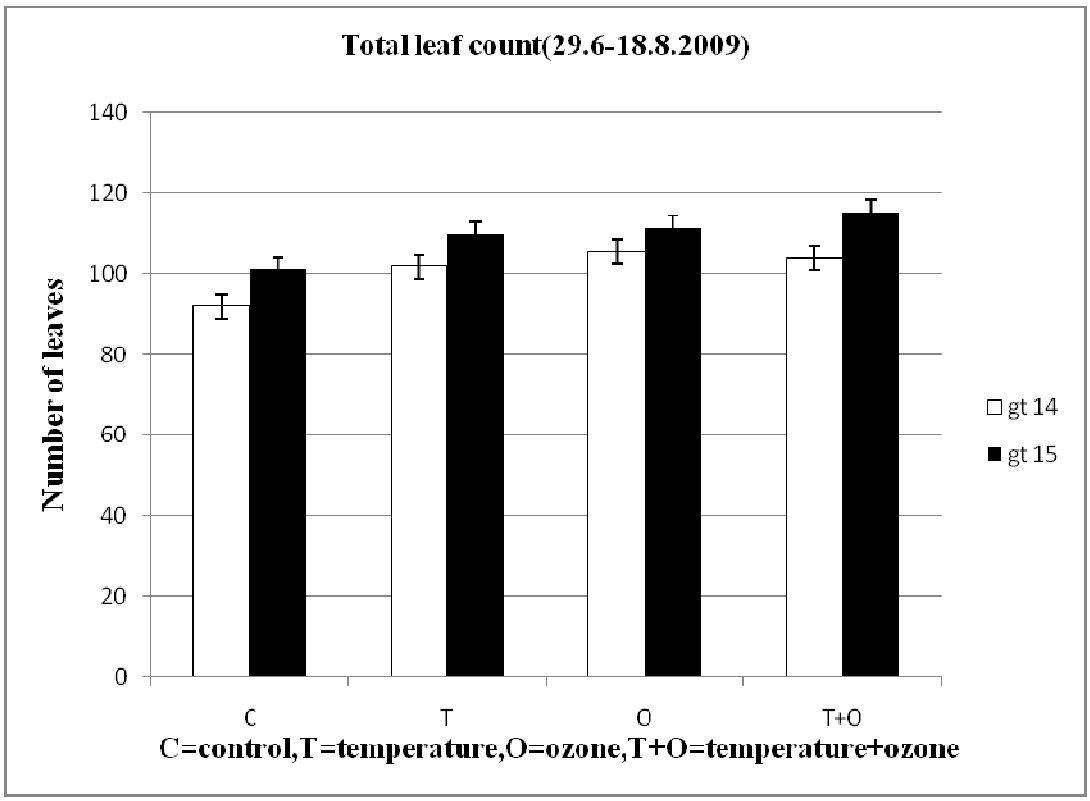


Fig 3. a) Mean stem diameter for gt 14 over time, and b) for gt 15 over time in different temperature and O_3 treatments (n = 4 per treatment per genotype).

(a)



(b)

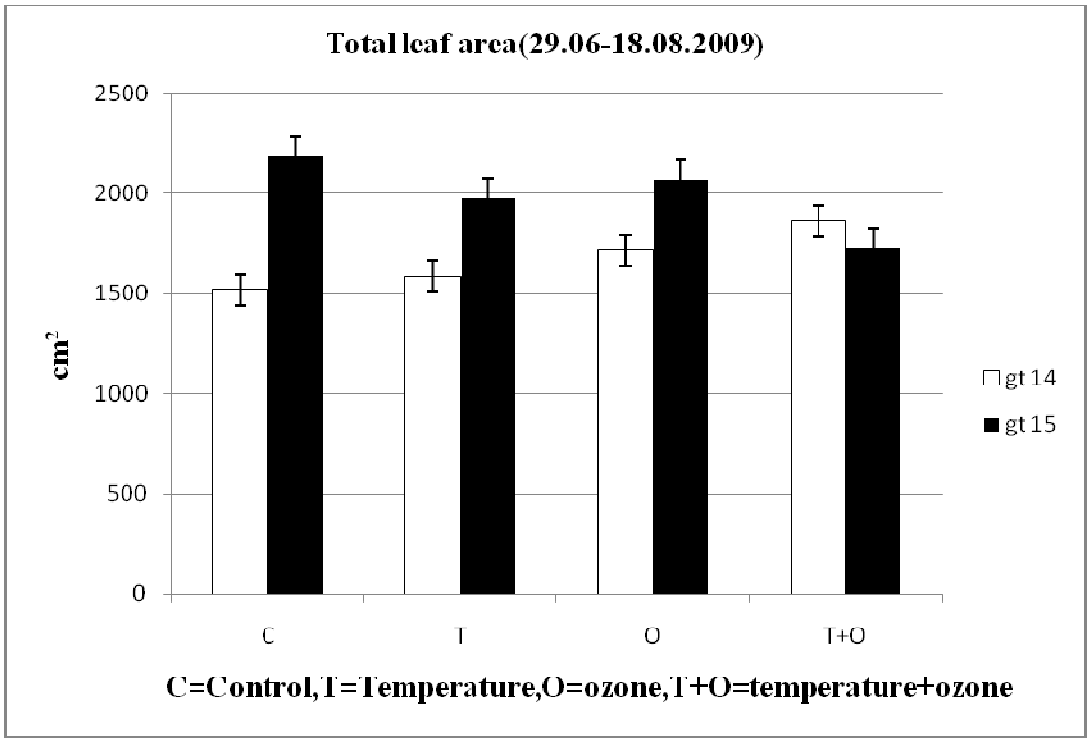


Fig 4. a) Overall total leaf count and b) total leaf area (mean ± SE) in different temperature and O_3 treatments (n=4 per treatment per genotype) during the growing season 2009.

4.3 Soil respiration

The results showed that temperature main effect ($p < 0.0001$) on soil respiration rates were statistically significant (Table 2, Fig.5). Hence, in both genotypes temperature increased the soil respiration rates by 36% (gt 15) and 24% (gt 14).On the other hand, Temperature×O_3×Genotype×Time interaction effect on soil respiration was marginally statistically significant ($p=0.086$,Table 2). Thus, temperature treatment alone and in combination with O_3 stimulated gt 15 soil respiration rates while O_3 seemed to decrease it and these trends could be observed on most measurement days, whereas in gt 14 both temperature and O_3 treatments on soil respiration varied and did not have consistent pattern over time (Fig. 6a-b).

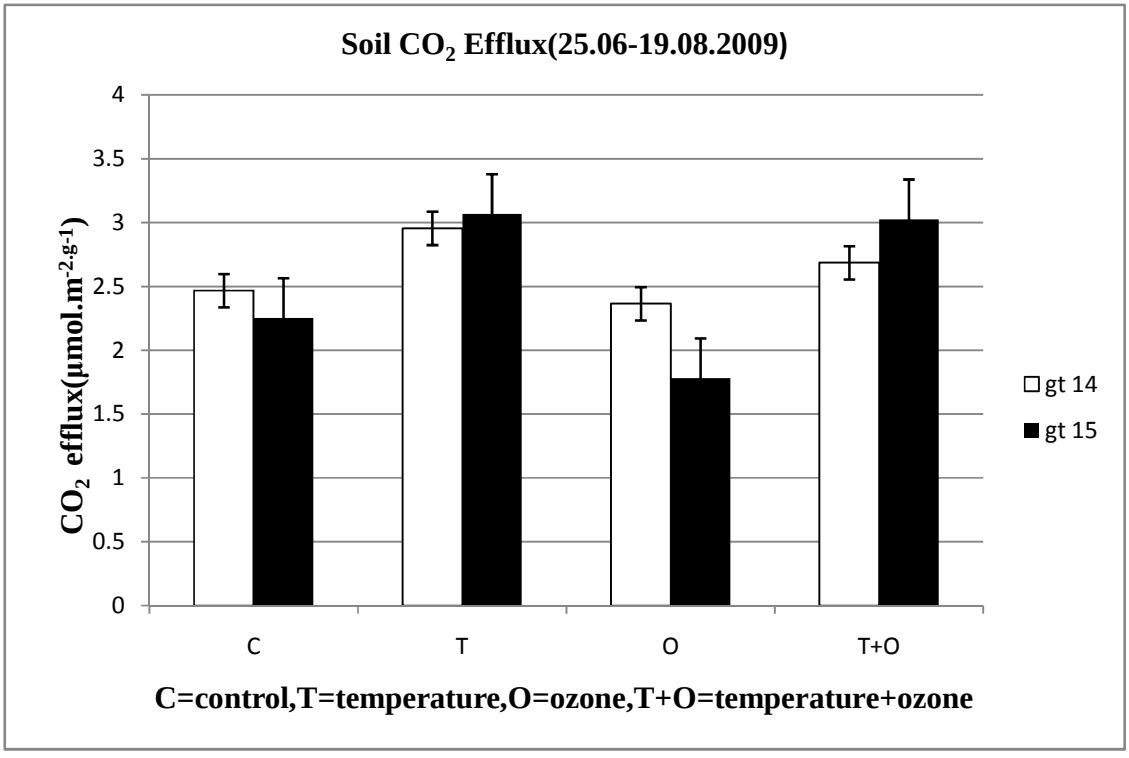
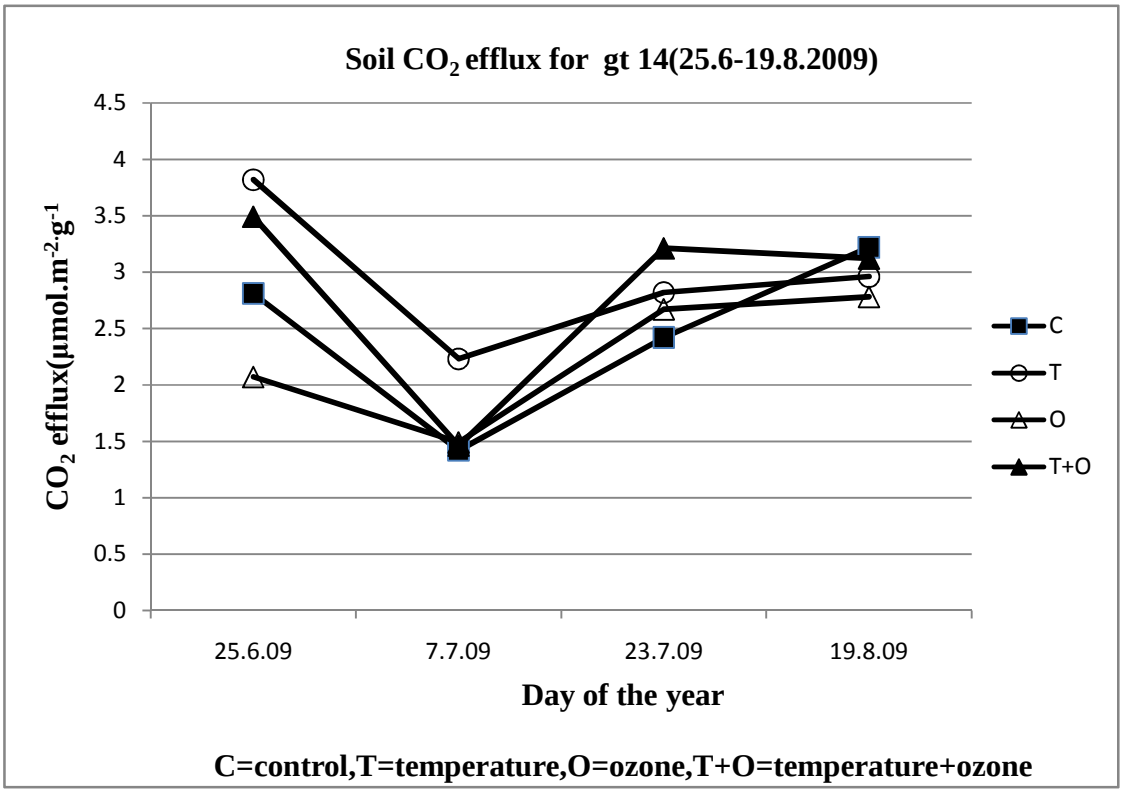


Fig 5. Overall soil CO_2 efflux (mean ± SE) in different temperature and O_3 treatments (n = 4 per treatment per genotype) in both genotypes during the growing season 2009.

(a)



(b)

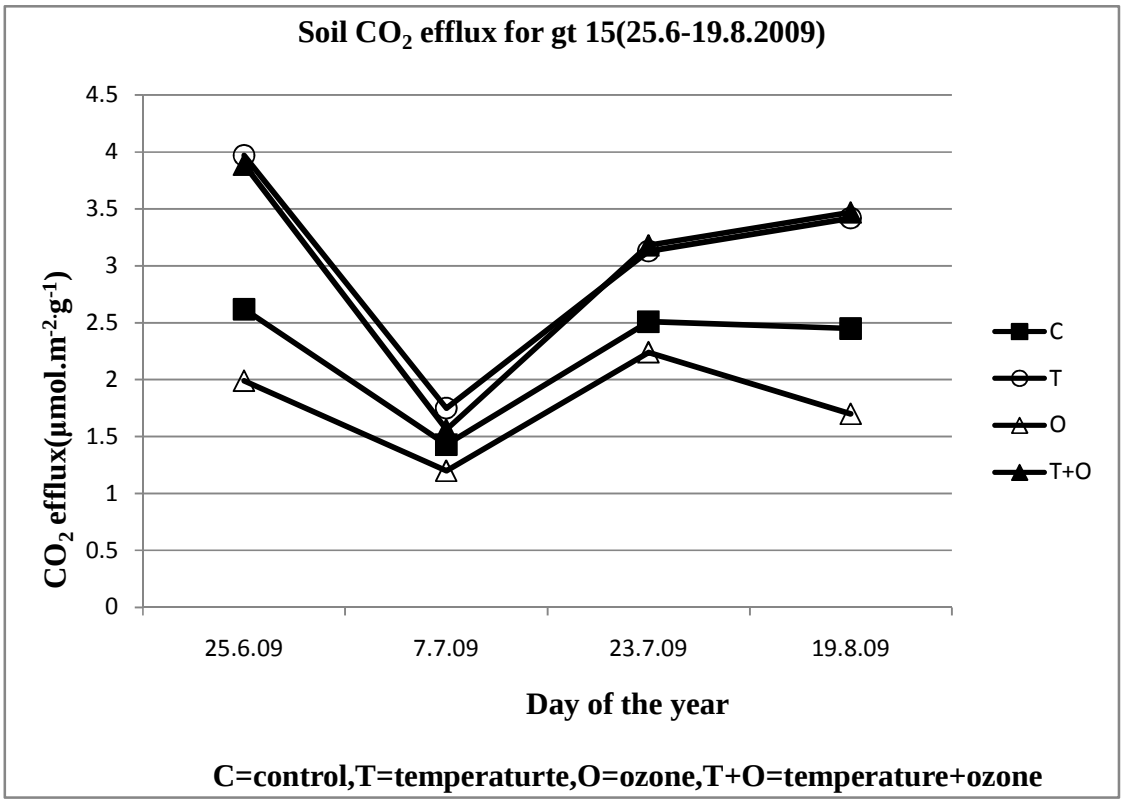


Fig 6. a) mean soil CO_2 efflux for gt 14 over time, and b) mean soil CO_2 efflux for gt 15 over time (n = 4 per treatment per genotype).

5. DISCUSSION

5.1 Temperature increase affects silver birch growth and soil respiration responses

In this study, elevated temperature increased the stem height in both genotypes, this result being in accordance with some previous experiments with birch trees (Xu et al., 2007; Hofgaard et al., 2010; Mäenpää et al., 2011). Higher carbon assimilation under warmer conditions can be expected and this likely explains the increase in stem height growth. In general, trees from high latitude may benefit from a certain degree of warming. Way and Oren (2010) found that the growth, including stem height and stem diameter was more promoted in deciduous species than in conifers, and possible explanation is that the morphogenetic responses of species to elevated temperature differ. Thus, tree species may have different shoot growth strategies in a warming climate (Way and Oren, 2010) and also higher growing temperatures could extend the growing season length, i.e. alter the phenology. The largest temperature effects on stem height growth were observed in mid-July. Previous studies have shown that mid-summer environmental conditions are important for the stem height growth (MacDonald and Little, 2006).

The increase in stem height growth could be the result of high carbon assimilation. In fact, warming is generally thought to enhance plant biochemical and physiological processes, facilitate carbon allocation to internal growth processes as long as optimum temperatures are not exceeded (Saxe et al., 2001; Arend et al., 2011). In fact, short-term moderate warming tends to increase the rate of photosynthesis, but may decline after long exposure to warming (Way and Sage, 2008). On the other hand, temperature treatment increased the total number of leaves in both genotypes (more in gt 15), but the total leaf area increased in gt 14 and decreased in gt 15.Thus gt 15 had smaller leaves. This decrease is may be due to the lack of acclimation (Athanasiou et al., 2010) and under elevated temperature, the oxygenating reaction of Rubisco increases more than the carboxylating reaction (Long et al., 2004; Raines, 2006), and photorespiration becomes more important, resulting in a decline in net photosynthesis in leaves and decrease in leaf area.

Temperature seems to play an important role during leaf growth since Haldimann and Feller (2004) found that low temperature did not decrease the leaf growth in oak trees, but temperatures above +30ºC reduced the stomatal conductance. This may be because high temperature causes physiological perturbations and reduces photosynthesis and thus the total leaf area (Hofgaard et al., 2010), whereas in the current work moderate temperature effects on leaf growth depended partly on the tree genotype. In this study, a significant temperature×genotype interaction effect on leaf area was found (gt 14 had larger leaf area than gt 15), which is in accordance with previous studies (Hartikainen et al., 2009) where a significant interaction temperature×genotype was found in one clone of European aspen *(Populus tremula)* and elevated temperature increased the leaf area.

Soil respiration rates increased under elevated temperature regardless of O_3 level, but mainly in gt15 on most measurement days whereas in gt14 temperature effects were inconsistent. Also, in a previous pot experiment, Kasurinen et al., (in press) observed that temperature-treated silver birch trees were simply larger and had larger root systems in general, and thereby soil respiration increased also beneath them. The effect of warming on soil respiration observed in this study is in contradiction with previous studies (Niu et al., 2008; Liu et al., 2009), in which reductions in soil respiration and microbial biomass were found under elevated temperature. The difference could be explained by the duration of exposure; in fact, long-term exposure to elevated temperature seems to elicit a different respiratory response from short-term exposure to elevated temperature, as a result of temperature acclimation (Bryla et al., 2001).

5.2 Ozone effects were negligible after the first exposure season

O_3 did not significally change the stem height or leaf growth. Sometimes there has been compensatory O_3 response in birch stem height (Oksanen and Rousi, 2001; Oksanen and Holopainen, 2001; Yamaji et al., 2003; Prozherina et al., 2005). It seems that trees with more foliage could be more resistant against O_3 (Gerosa et al., 2003; Ribas et al., 2005). However, O_3 exposure over several growing seasons is likely to result in growth losses (Oksanen, 2003; Kostiainen et al., 2006; Oksanen et al., 2007). For instance, McLaughlin et al., (2007) observed seasonal losses in stem growth of 30-50% under elevated O_3 concentration.

There was an interaction effect (Temperature×O_3×Genotype×Time) on stem diameter indicating that in gt 14, O_3 alone reduced stem diameter growth but this trend was observed only at the end of season 2009 and under ambient temperature only. The stem diameter growth variation among genotypes is related to the physiology and the tree architecture, in addition to the number of branches produced on the main stem (Calfapietra et al., 2001), and this finding partly explain the results. Some previous experiments with silver birch have also shown that stem diameter growth is decreased due to elevated O_3 levels (Matyssek et al., 1992; Oksanen and Rousi, 2001). Richet et al., (2011) found that the reduction the biosynthesis of cellulose and lignin together with cambial growth decreased under O_3. Ozone seems to decrease cellulose: lignin-ratio and thus wood formation in the vascular cambium (Matyssek et al., 2002; Richet et al., 2011).

Ozone did not significantly change the total number of leaves, in contradiction with some previous studies. In fact, Kull et al., (2003) and Schreuder et al., (2001) observed significant effects of ozone on the total number of leaves in silver birch *(Betula pendula).* Temperature×O_3×Genotype×Time interaction effect showed that only in gt15 soil respiration was decreased only when trees were exposed to O_3 alone, whereas in combination treatment O_3 effects were cancelled. Previously, Kasurinen et al., (2004) have also shown that soil respiration O_3 response may vary according to silver birch genotype. Pregitzer et al., (2006) found that O_3 induces some physiological changes in the tree, and thus can cause a decrease in root carbohydrates and thereby affect soil respiration rates. However, in some field and pot experiments have also shown that O_3 stress can also stimulate soil respiration beneath forest trees (Kasurinen et al., 2004; Andersen and Scagel, 1997; Andersen, 2003; Tingey et al., 2006; Grebenc and Kraigher, 2007).

6. CONCLUSIONS

In this field study, there were some genotype-dependent temperature and ozone responses, but sometimes these genotype-dependent response patterns developed over time. For instance, moderate warming increased silver birch stem height growth in both genotypes, but on most

measurement days, soil respiration rates were increased in gt 15 only. When there were some O_3 effects on tree growth or soil respiration responses, temperature treatment cancelled them, and sometimes O_3 effects became clearer towards the end of the exposure season.

Silver birch has thus a potential to enhance its biomass accumulation under warming during the growing period, but there is not knowledge how the response would change if trees would have been subjected to warming treatment throughout the whole year (i.e., during the winter-time too). On the other hand, this study also indicated that soil respiration rates in some birch genotypes can be also increased under warming and in fact may lead into increased C cycling between soil and atmosphere.

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