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Species-specific responses to ozone and drought in six deciduous trees

Running head: OZONE AND DROUGHT RESPONSES OF TREES

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Abstract

Saplings of alder (*Alnus glutinosa*), birch (*Betula pendula*), hazel (*Corylus avellana*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and oak (*Quercus robur*) were exposed to five episodic ozone regimes in solardomes, with treatment means between 16 and 72 ppb. All trees were kept fully watered for the first five weeks of exposure, after which half the trees continued to be well-watered, whereas the other half were subjected to a moderate drought by applying approximately 45% of the amount of water.

Species-specific reductions in growth in response to both ozone and drought were found, which could result in reduced potential carbon sequestration in future ozone climates. In well watered conditions the ozone treatments resulted in total biomass reductions for oak (18%), alder (16%), beech (15%), ash (14%), birch (14%) and hazel (7%) in the 72 ppb compared to the 32 ppb treatment. For beech there was a reduction in growth in response to ozone in the well watered

treatment, but an increase in growth in response to ozone in the drought treatment, in contrast to the decreased growth that would occur as a result of stomatal closure in response to either the ozone or drought treatment, and therefore assumed to result from changes in hormonal signalling which could result in stomatal opening in combined ozone and drought conditions.

For alder, in addition to a decrease in root biomass there was reduced biomass of root nodules with high compared to low ozone for both drought treated and well-watered trees. There was also a large reduction in the biomass of nodules from drought trees compared to well-watered. It is therefore possible that changes in the nitrogen dynamics of alder could occur due to reduced nodulation in both drought and elevated ozone conditions.

Introduction

Tropospheric ozone concentrations have been increasing since industrial times from a background of 10-15 ppb in the 1900s, due to increased emissions from anthropogenic sources (Solberg et al. 2005, Volz and Kley, 1988). A recent meta-analysis has suggested that the increase in ozone since the industrial revolution has been responsible for a reduction in photosynthesis of approximately 11% in trees (Wittig et al. 2007), which may have reduced tree productivity by approximately 7% (Wittig et al. 2009). Ozone concentrations have continued to increase over recent years, despite reductions in European precursor emissions (Wilson et al. 2012) and a further increase in background ozone concentration throughout the northern hemisphere has been predicted due to hemispherical transport of ozone precursor molecules (Royal Society 2008), with annual mean ozone concentrations reaching up to 68 ppb by 2050 (Meehl at al. 2007). These concentrations correspond with a predicted reduction in total tree biomass of approximately 11% (Wittig et al. 2009). World-wide, forest ecosystems store 80% of the world's above-ground carbon and 40% of the below-ground carbon (Brunner and Godbold 2007) and play a significant role in sequestering atmospheric CO_2 (Bonan 2008). Therefore, any impacts of ozone on carbon sequestration by trees could have a significant effect on the global carbon budget.

Studies of the effects of ozone on trees have shown responses such as visible leaf injury (Gerosa et al. 2009), elevated senescence (e.g. Mikkelsen and Jorgensen 1996, Pääkkönen et al. 1997) and reduced growth, e.g. on *Quercus rubra* (Samuelson et al. 1996). Some studies have indicated that a change in biomass partitioning can occur in response to ozone, for example, a decrease in the dry mass of roots and branches of *Betula pendula* attributed to ozone has been shown at the end of the exposure (Riikonen et al. 2004). It is thought that decreased partitioning to the roots may occur with increasing ozone exposure because the mature, lower leaves act as the main source of assimilate for root growth, and these are frequently the most damaged by ozone (Grantz et al. 2006, Cooley and Manning 1987, Okano et al. 1984). However, this has not been demonstrated for all species and some e.g. *Fagus sylvatica* and *Picea abies* showed no effect of ozone exposure on carbon allocation to roots (Andersen et al. 2010).

Concurrent with the predicted increases in ozone concentration, over the coming decades, summer rainfall is expected to be reduced across many temperate regions, with an increase in the frequency and severity of summer droughts predicted across much of Europe (Bates et al. 2008; Blenkinsop et al. 2007; Lehner et al. 2006). Although drought itself has been shown to reduce growth in some tree species (e.g. *Fagus sylvatica,* Thiel et al., 2014; *Picea abies,* Jyske et al. 2010; *Pinus spp,* Sanchez-Salguero et al. 2012), there can be interactive effects between ozone and drought stress. For *Betula pendula* drought stress alone has been shown to reduce stomatal density and stomatal conductance; the combined effects of drought and ozone were additive for some responses (Pääkkönen et al. 1998), for example, mild drought combined with 1.5 x ambient ozone concentrations caused an additive reduction in leaf number and total foliage area and also increased the N concentration of the leaves. In some species ozone exposure has been shown to decrease the ability of a plant to respond to subsequent drought, e.g. for the herbaceous species *Rancunculus acris* and *Dactylis glomerata* (Wagg et al., 2013), which could lead to further soil drying to increase the severity of a prolonged drought. In contrast, some other studies have demonstrated that drought has a protective effect against ozone as drought can induce stomatal closure (e.g. for *Populus spp.,* Silim et al. 2009). This can reduce ozone uptake and protect plants from injury caused by ozone exposure for some species (e.g. *Fagus sylvatica,* Löw et al. 2006). However, the meta-analysis of Wittig et al. (2009) on tree responses found no conclusive evidence for a protective role of drought against ozone induced effects on growth and biomass as there were insufficient published studies of ozone and drought interactions on trees available.

This study investigated the potential impacts of increasing background ozone concentration in combination with moderate drought after prior ozone exposure on six important tree species: alder (*Alnus glutinosa*), beech (*Fagus sylvatica*), oak (*Quercus robur*), ash (*Fraxinus excelsior*), hazel (*Corylus avellana*) and birch (*Betula pendula*). In this study, young trees were used, which allowed investigation of impacts of ozone on total root biomass avoiding the need for estimates of root turnover by methods such as root ingrowth cores, and plants were harvested before leaf-fall to obtain information on leaf number and leaf weight. Data on the biomass of leaves, stems and roots in response to ozone and drought for these six species is presented and used to indicate the relative sensitivity of these species to both stresses, including in combination.

Methods

Plant material

Trees of alder (*Alnus glutinosa*), birch (*Betula pendula*), hazel (*Corylus avellana*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and oak (*Quercus robur*) were all obtained from Cheviot Trees (Berwick-upon-Tweed, UK) as UK origin, cell-grown (10cm deep pots) seedlings. These were planted in 2-litre pots (14 cm diameter, 18 cm deep), which were lined with perforated plastic to discourage roots from growing outside the pot. All trees were planted in topsoil (Humax, UK), but retaining the soil around the existing root system to avoid disturbing the fine roots and established mycorrhizae. Trees were two years old and of initial height 35 cm (alder), 65 cm (birch), 40 cm (hazel), 45 cm (beech), 40 cm (ash) and 25 cm (oak). Alder, birch and beech were planted into their pots on 29th April 2009 whilst hazel, oak and ash were planted on 21st April 2010 and all trees were kept well-watered until the start of the experiment. Prior to the start of the experiment the height of each tree was measured. For each species, trees were separated into five size classes based on initial tree height and one tree of each size class was assigned to each solardome per watering regime. Altogether, ten trees of each species were exposed per solardome.

Ozone exposure

Plants were exposed to ozone in solardomes (hemispherical greenhouses 3m diameter, 2m tall). Ozone was generated from oxygen concentrated from air (Workhorse 8, Dryden Aqua, UK) using an ozone generator (G11, Dryden Aqua, UK) and distributed to each solardome via PTFE tubing. Ozone was delivered to each solardome using mass flow controllers (Celerion, Ireland) controlled by computer software (Labview version 7). Ozone concentrations were continuously monitored in one solardome using a dedicated ozone analyser (Thermoelectron, Model 49C), allowing feedback to compensate for small variations in ozone production. In all solardomes the ozone concentration was measured for 5 minutes in every 30 minutes using two additional ozone analysers (Envirotech API 400A) of matched calibration. Five ozone treatments were randomly allocated to the solardomes, with one solardome for each treatment. The weekly ozone profile used was based on an ozone episode from a UK upland site (Keenley Fell, Northumberland, (Grid Reference NY793561, 21st -28th May 2008) and target ozone concentrations were increases or decreases below this profile. This profile was repeated for each week of the experiment, giving target mean ozone concentrations of 16 ppb (O_316), 32 ppb (O_332), 48 ppb (O_348), 56 ppb (O_356) and 72 ppb (O_372). The mean weekly ozone regime applied in each treatment is shown in Figure 1.

In 2009, the ozone exposure over the 12 week experimental period ranged from a seasonal mean of 15.7 ppb to 74.1 ppb (Table 1), with the AOT40 (accumulated over 24 h) ranging from 0.2 ppm.h to 82.4 ppm.h. The AOT40 accumulated over 12 h (07:00 to 19:00) ranged from 1.7 ppm.h to 45.2 ppm.h. In 2010, the ozone exposure was similar, with seasonal means of 19.0 ppb to 73.4 ppb, and with the AOT40 accumulated over 12 h ranging from 0.8 ppm.h to 77.1 ppm.h. To reflect rising background ozone, the profile used involved significant ozone exposure during the night-time as well as during the day in both years; therefore, the AOT40 accumulated over 24h was much larger than that accumulated over 12h.

The mean temperature within the solardomes (over 24h) for the duration of the ozone exposure was 18.6°C in 2009 and 17.5°C in 2010.

For all trees, ozone exposure did not start until after bud-break and early leaf expansion. For alder, birch and beech, ozone exposure started on 20th May 2009 and finished on 11th August. Watering occurred by hand three times per week for all trees. All trees were kept fully watered for the first 5 weeks of ozone exposure to ensure that soil water availability was not limiting. To give a drought treatment, water was given at the same time as for the well-watered (WW) trees, but the volume was reduced and was approximately 45% of the volume given to the WW treatment. The soil moisture content of a sample of WW and drought trees was measured twice per week using a hand-held theta probe (Delta-T) to assess the irrigation requirements. The drought treatment started on 24th June and continued until the plants were harvested on 11th August. For hazel, oak and ash ozone exposure started on 21st April 2010. The drought treatment started on 25th May and continued until the plants were harvested on 19th July.

Harvest

At the end of the ozone exposure the height of all trees was determined before they were cut to soil level. For each tree, leaves > 1cm long were separated from stems and counted and weighed. Leaves < 1cm long were not counted or weighed. Roots were washed for all replicate trees from two ozone treatments ($O_3$32 and $O_3$72), and nodules were separated from the roots for alder. All plant material was oven-dried at 65°C for a minimum of seven days before weighing.

Data analysis and statistics

All data except that for root biomass were analysed using General Linear Model analysis (GLM) in Minitab (Version 16) using the mean value per solardome as the input data. Root weight data and for alder, root nodule biomass, were only available from the $O_3$32 and $O_3$72 treatments and therefore comparisons of root weights and total tree biomass were made using two-way ANOVA, using individual plants as replicates.

Results

Leaf weight

For beech there was a significant interaction (*P*=0.01) between ozone and watering regime for the leaf weight per tree, with the leaf weight decreasing with increasing ozone exposure for those trees that remained well-watered (Figure 2a, r^2=0.43, *P*=0.24), whilst for the drought-treated beech trees there was the opposite response of an increase in the total leaf weight per tree with increasing ozone exposure (r^2=0.94, *P*=0.01). This was partly due to an increase in the number of leaves per tree with increasing ozone in the drought treatment (Figure 2b, r^2=0.67, *P*=0.097). As a consequence of this interaction, although there was a large effect of watering regime at low ozone concentrations, with fewer leaves and lower leaf weight in the drought treatment, at high ozone concentrations these differences were lost.

There were no significant effects of ozone on the total leaf weight per tree for birch, hazel, oak, alder and ash, and no significant interactions between ozone and watering regime for these species. However, there were some effects of watering regime. There was a significant reduction in the leaf weight per tree in the drought treatment compared to WW (mean reduction across all ozone treatments) for alder (40%, *P*=0.017), hazel (45%, *P*=0.016), birch (27%, *P*=0.003) and oak (55%, *P*=0.008), but no significant effects of watering regime on the leaf weight of ash (data not presented).

Height and stem weight

For all tree species there was a significantly larger increase in height between the start and end of ozone exposure in the WW treatment compared to the drought treatment (Table 2). Mean values across all ozone treatments are presented and these show a range from a 7cm height increase in drought-treated hazel, to a 65 cm increase in height in WW alder. However, there was no statistically significant effect of ozone on height of any of the species, and no significant interaction between ozone and watering regime (data not presented).

There was a trend for a reduction in stem weight with increasing ozone exposure for hazel (*P*=0.058, Figure 3a). There was also a reduction in stem weight of hazel in the drought treatment compared to WW of approximately 30% (*P*=0.069), and this difference was consistent across all ozone treatments. There was no significant effect of ozone and no interaction between ozone and watering regime on the stem weight of oak, birch, alder or ash. However, there were large reductions in stem weight in the drought treatment compared to WW (mean reduction across all ozone treatments) for birch (30%; *P*=0.043), alder (40%; *P*=0.053) and oak (50%; *P*=0.005) and no significant reduction for ash (data not presented).

In contrast for beech, overall there was a significant increase in stem weight with increasing ozone exposure (*P*=0.047, Figure 3b). However, as for leaf weight for this species, there was a significant interaction between ozone and watering regime (*P*=0.010). For WW beech there was no effect of ozone on stem weight, but for drought-treated beech trees there was an increase in stem weight with increasing ozone exposure (r^2=0.99, *P*=0), so that the difference in stem weight between WW and drought trees was lost in the highest ozone treatments.

Root weight

Root weight was determined in the $O_3$72 and $O_3$32 treatments only. Root weight was significantly decreased in the $O_3$72 treatment compared to $O_3$32 for birch (*P*=0.025, Figure 4) and there was significant interaction between ozone and watering regime (*P*=0.05). Increased ozone corresponded with a large decrease in root biomass of approximately 23% in the WW birch only (*P*=0.021) and there were no effects of ozone on drought-treated birch. For beech there was also a significant interaction between ozone and watering regime (*P*=0.05). However, in contrast for this species there was a decrease in root biomass with increasing ozone concentration in WW trees compared to an increase in root biomass with increasing ozone concentration in drought-treated trees (Figure 4). There was no significant reduction in root weight in the $O_3$72 treatment compared to $O_3$32 for oak, ash or hazel. There was a significant reduction in root weight in drought compared to WW for birch (27%; *P*=0), alder (20%; *P*=0.007), oak (30%; *P*=0.004) and hazel (40%; *P*=0.005).

For alder, there was a small decrease in root biomass with increasing ozone for both the WW and drought-treated plants (10%, ns), and no significant interaction between ozone and watering regime. However, there was a large effect on the biomass of root nodules, with a large reduction in drought-treated compared to WW (mean reduction across both ozone treatments) of approximately 60% (*P*=0.001; Figure 5). There was also a reduced biomass of root nodules with high ozone exposure compared to low exposure for both drought-treated and WW trees of approximately 25% (*P*=0.046), but no significant interaction between ozone and drought on the weight of root nodules. The relative weight of nodules per gram of root was also reduced by approximately 25% with increasing ozone under both WW and drought conditions (not statistically significant) and by approximately 60% with drought (*P*=0.001; data not presented). The number of nodules and mean weight per nodule was not determined, however, it was noticed that the nodule size was smaller with elevated ozone conditions.

Total biomass

Total biomass data was only available for two ozone treatments because root biomass measurements were only carried out in the $O_3$32 and $O_3$73 treatments due to the length of time required for root washing. In WW conditions the ozone treatments resulted in a total (above and below-ground) biomass reductions for oak (18%), alder (16%), beech (15%), ash (14%), birch (14%) and hazel (7%; Figure 4). For alder there was a decrease in total biomass in the $O_3$72 treatment compared to $O_3$32 of approximately 16% (*P*=0.003), with a similar magnitude of reduction in both the WW and drought treatments. There was a reduction in total biomass in the drought compared to WW alder trees of 36% (*P*=0), but no significant interaction between ozone and watering regime (Figure 4). In contrast there was an interaction between ozone and watering regime for beech (*P*=0.056). In well-watered beech there was a decrease in biomass with increasing ozone of 15% (*P*=0.031), however, in drought treated trees there was an increase in biomass with increasing ozone of 25% (*P*=0.07; Figure 4). For oak, birch and hazel there was no significant effect of ozone on total biomass, however there was a large reduction in drought compared to WW plants of 45% (*P*=0) for oak, 32% (*P*=0) for birch and 43% (*P*=0.001) for hazel (Figure 4). There were no significant effects of either ozone or watering regime on the total biomass of ash.

Biomass of roots in the $O_3$72 treatment was maintained at the expense of allocation to the stems and leaves for oak. Although the root weight was reduced by approximately 30% in the $O_3$72 treatment, stem weight was reduced by approximately 50% and leaf weight was reduced by approximately 55% (Figure 4, Table 3). Differences in biomass allocation between treatments for the other species were small.

Discussion

The ozone treatments resulted in total (above and below-ground) biomass reductions of between 7% and 18% when the $O_3$72 treatment was compared with the $O_3$32 treatment. These changes are in broad agreement with those found by Wittig et al. (2009), who showed in a meta-analysis of responses of trees to ozone that ozone concentrations of 64 ppb compared to ambient concentrations were associated with biomass reductions of 11%. The biomass effects shown in the current study were found using two-year old trees and are therefore of particular relevance to afforestation using young trees. However, if such effects also occur in mature trees, these results suggest that elevated ozone could reduce carbon sequestration in future ozone climates if background ozone concentrations continue to rise, as suggested in modelling studies (e.g. Meehl et al. 2007, Sitch et al. 2007). The biomass reductions demonstrated in this study included stem and root biomass, both of which represent reductions in long-term carbon storage and support the hypothesis that increased ambient ozone could further exacerbate climate change.

Any decrease in root biomass as a result of ozone exposure could decrease the ability of the tree to take up water and nutrients. Reductions in root weight can be a consequence of either an overall reduction in availability of photosynthate for root growth or reduced allocation to the roots as resources are preferentially used to replace damaged leaves. In this short-term study there were larger effects on roots than above ground biomass for birch as has previously been reported for several species including trembling aspen (*Populus tremuloides*, Coleman et al. 1996) and birch (*Betula pendula*; Riikonen et al. 2004). This could be evidence of reduced partitioning to roots, however, it has been shown that for trees the main source of photosynthate for the roots is from the lower leaves, and it is these older leaves that tend to be most affected as a consequence of ozone exposure (Grantz et al. 2006). Therefore, it is possible that further reductions in partitioning to roots may have occurred if the exposure had occurred over a longer timescale, although subsequent root re-growth after relief from a period of ozone stress may occur for some species. Reduced root growth would also indicate that a drought following the occurrence of elevated ozone could have a more severe effect due to the decrease in ability to take up water and nutrients, although it is also possible that less water usage early in a drought period would help retain moisture during an extended drought and therefore benefit the long-term survival of the tree.

Although it could be considered that drought protected some species (birch, ash and oak) from the negative effects of ozone exposure, the decrease in biomass as a result of the drought outweighed any benefit as large biomass reductions of up to 45% in response to drought were shown for all species in this study. Drought had a large impact on stem weight in five out of the six species tested, confirming the strong impact that drought may have on carbon sequestration. Naturally occurring droughts in China in the twentieth century have been related to strong decreases in net primary production, which was inferred from tree-ring width chronologies (Xiao et al. 2009). Stomatal closure in response to drought has been shown to protect against ozone in some species e.g. *Populus spp* (Silim et al. 2009), however, there was no evidence of this in the current study.

In addition to effects on root biomass, over the longer term, indirect effects of ozone such as decreased nodulation of roots of alder may also have a large impact. This study showed large effects of ozone and drought on nodule biomass, but did not consider any impact on nodule activity. It has previously been demonstrated that the host plant can influence root nodule activity (Verghese and Misra 2000), but the influence of ozone on this signalling from the host plant has not been studied. Nitrogen transfer from clover to grass in grass-clover swards has been demonstrated in several studies (e.g. Sincik and Acikgoz 2007, Goodman 1988) and reduced sensitivity to ozone of *Lolium perenne* occurred when this was grown in mixture with *Trifolium repens*, which was attributed to an increased availability of nitrogen to *L. perenne* when it was grown with *T. repens* (Hayes et al. 2010). Therefore, in addition to effects of reduced nodulation on the host plant which may contribute significantly to changes in growth, other ecosystem services such as nitrogen cycling within the vegetation community could also be affected indirectly as a consequence of decreased nitrogen transfer from alder to the ecosystem.

Alder showed an additive effect of the combination of ozone and drought on both root biomass and total biomass. In contrast, whilst under well-watered conditions the effects of ozone on beech were small, the interaction between drought and ozone for beech resulted in growth stimulation with increasing ozone exposure for drought-treated trees, resulting in increased root and total biomass, stem weight and the number and total weight of leaves. The plant hormone abscisic acid (ABA) is released under drought conditions, resulting in reduced stomatal conductance and therefore water loss in the leaves. A mechanism to explain ozone-induced reductions in stomatal sensitivity to ABA has been proposed by Wilkinson and Davies (2010) whereby ethylene, released as a response to ozone stress, antagonises the ABA response. They hypothesize that although both ethylene and ABA individually close stomata and reduce growth, when these combine, such as in the presence of ozone and drying soil, stomata could be opened and that growth could be promoted via greater throughput of nutrients, as seen in beech in the current study. Ethylene emission from leaves of *Leontodon hispidus* have been shown to increase with elevated ozone (Wilkinson and Davies 2009) and a reduced sensitivity to ABA in ozone treated plants has also been demonstrated (e.g. Mills et al. 2009, Wilkinson and Davies, 2009), with increased stomatal conductance in combined elevated ozone and ABA-treated (to simulate drought) conditions for *Leontodon hispidus* (Wilkinson and Davies 2009). The results for beech from the current study therefore support the hypothesis of Wilkinson and Davies (2010), although this effect was not observed in the other species tested. Published data on the response of *F. sylvatica* in response to ozone have shown very mixed results with some studies showing large significant responses with increasing ozone exposure e.g. reduced photosynthesis (Paoletti et al. 2002); reduced biomass (Landolt et al. 2000, Matyssek et al. 2010), however, some other studies have shown no significant differences for growth or photosynthesis of *F. sylvatica* due to ozone (Bortier et al. 2000a, Wipfler et al. 2005). The differential response to ozone in varying soil moisture conditions as demonstrated in the current study may explain some of the discrepancies between the different studies.

Although the current study used young trees, there is some evidence that mature trees are as sensitive to ozone as younger trees. Epidemiological analysis of effects of ozone on *Fagus sylvatica* indicated that the reduction in shoot growth due to ozone was similar in both seedlings and mature trees (Braun et al. 2007). In addition, in the Aspen-FACE experiment facility in Wisconsin, USA, biomass loss after 6 years of growth and treatment was proportionally similar to the loss at 2 years (King et al. 2005). The occurrence of visible injury attributed to ozone for *Fagus sylvatica* in phytotrons under an ambient ozone regime was induced at AOT40 levels similar to those experienced by mature trees at a nearby field site (Baumgarten et al. 2000). However, other studies have shown young beech in phytotrons to be more sensitive to ozone than adult beech in the field, which was attributed to enhanced ozone uptake compared to field conditions (Nunn et al. 2005).

This study has shown that typical deciduous woodland species vary in their sensitivity to rising background ozone, although the ranking of the species in terms of sensitivity to either ozone or drought depended on the parameter used. It has been suggested that faster growing species e.g. poplar are more sensitive to ozone than slower growing species e.g. beech (Bortier et al. 2000b), although there was no evidence to suggest that this was the case in the current study. Reducing water availability by 45% had even more pronounced effects on both above and below-ground biomass, with positive and negative interactions with elevated ozone exposure occurring in some species. However, the variation in the response to both ozone and drought between species indicates that future ozone conditions may affect both above- and below-ground competition between tree species, and that these effects could be further modified by drought as the relative sensitivity to ozone of different tree species may depend on water availability.

Conclusions

Both elevated ozone and drought have been demonstrated to have a large influence on biomass of some species of young deciduous trees. If a similar magnitude of response were to occur with more mature trees this could result in a reduction in carbon sequestration, with long-term climatic consequences. Ideally, further experiments using mature species from a wide variety of species would need to be carried out to ascertain the response of mature trees to ozone and drought. However, this is difficult and expensive. The use of younger trees, as in this study, offers a valuable insight into the potential effects on a wider range of tree species. In this case, significant reductions in biomass in response to ozone were found for two species and significant reductions in biomass in response to drought were found for all six of the species tested during the study, implying that sensitivity of trees to ozone and drought may be widespread.

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Figure legends

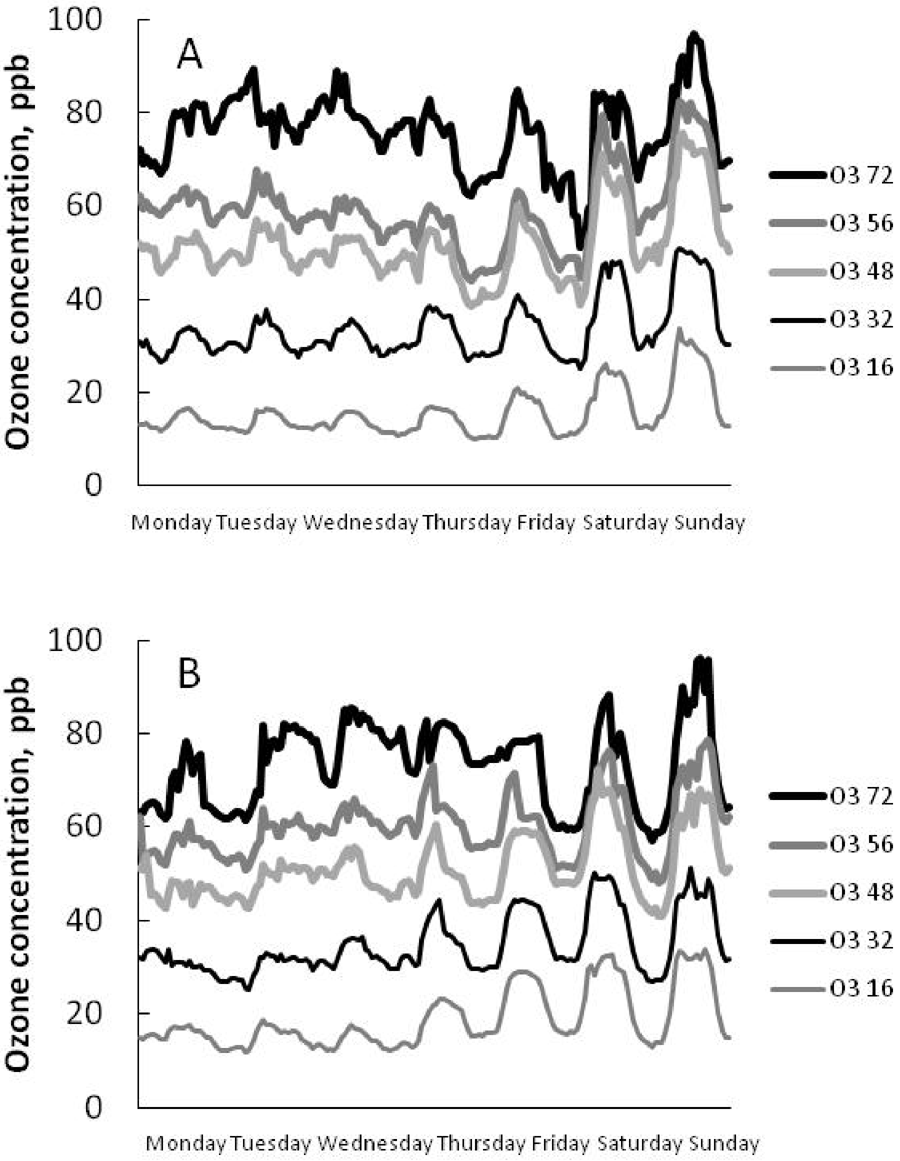
Figure 1: Mean weekly profile of ozone concentrations in the solardomes for the duration of the experiment in a) 2009 and b) 2010.

Figure 2: Leaf weight (a) and leaf number (b) of beech in response to ozone, in both well-watered (WW) and drought conditions, where each datapoint is the mean of five trees.

Figure 3: Stem weight of hazel (a) and beech (b) in response to ozone, in both well-watered (WW) and drought conditions.

Figure 4: Biomass partitioning to roots, stems and leaves for alder, birch, hazel, beech, ash and oak in well-watered (WW) and drought (D) conditions in the $O_3$32 and $O_3$72 treatments. Bars are standard errors based on individual pots. For significant differences, please refer to the main text.

Figure 5: Weight of nodules (per tree) on roots of alder from two ozone treatments, in well-watered (WW) and drought conditions. Bars are standard errors based on individual pots.



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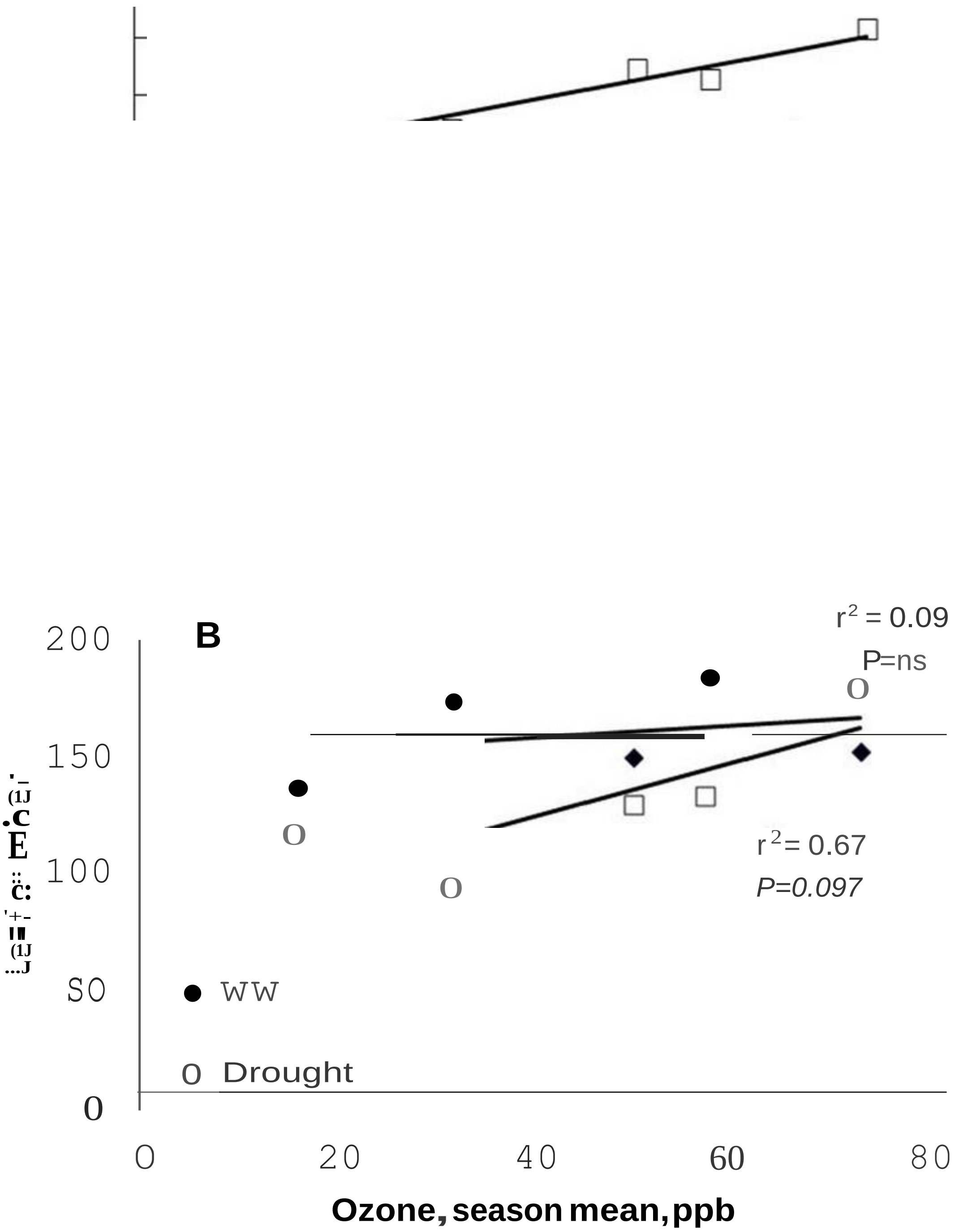
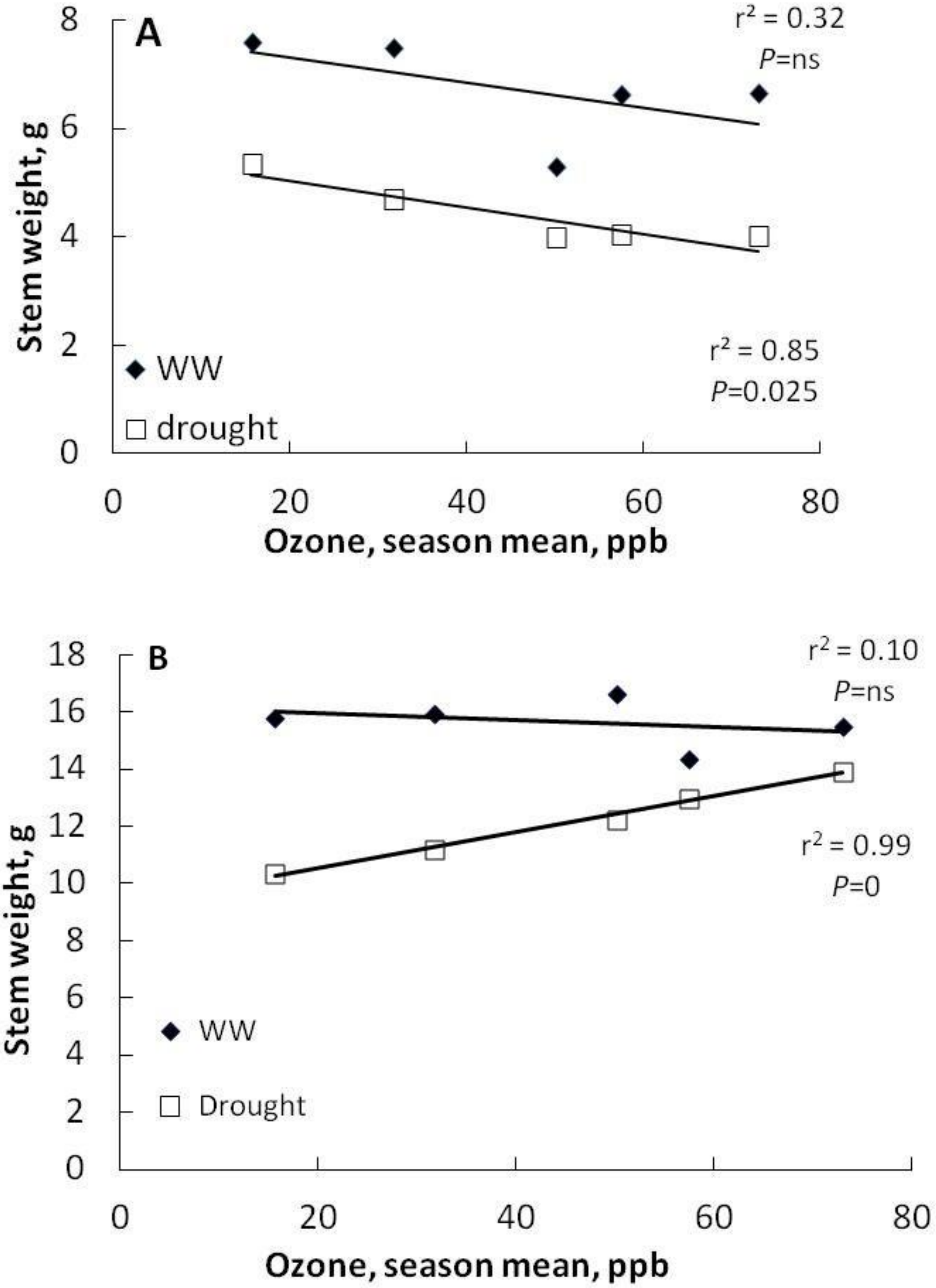


Figure 2: Leaf weight (A) and leaf number (B) of beech in response to ozone, in both well-watered (WW) and drought conditions, where each datapoint is the mean of five trees.



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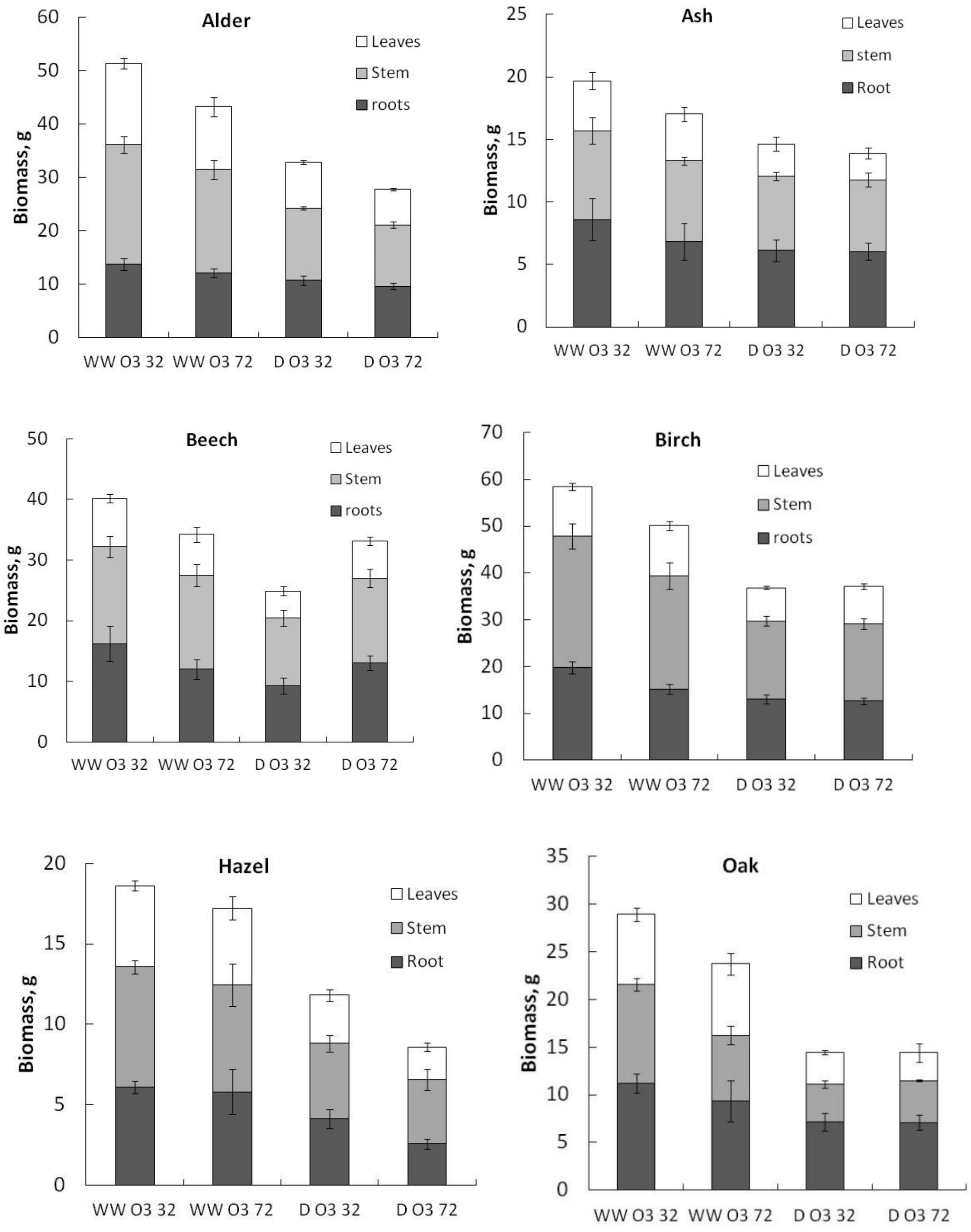

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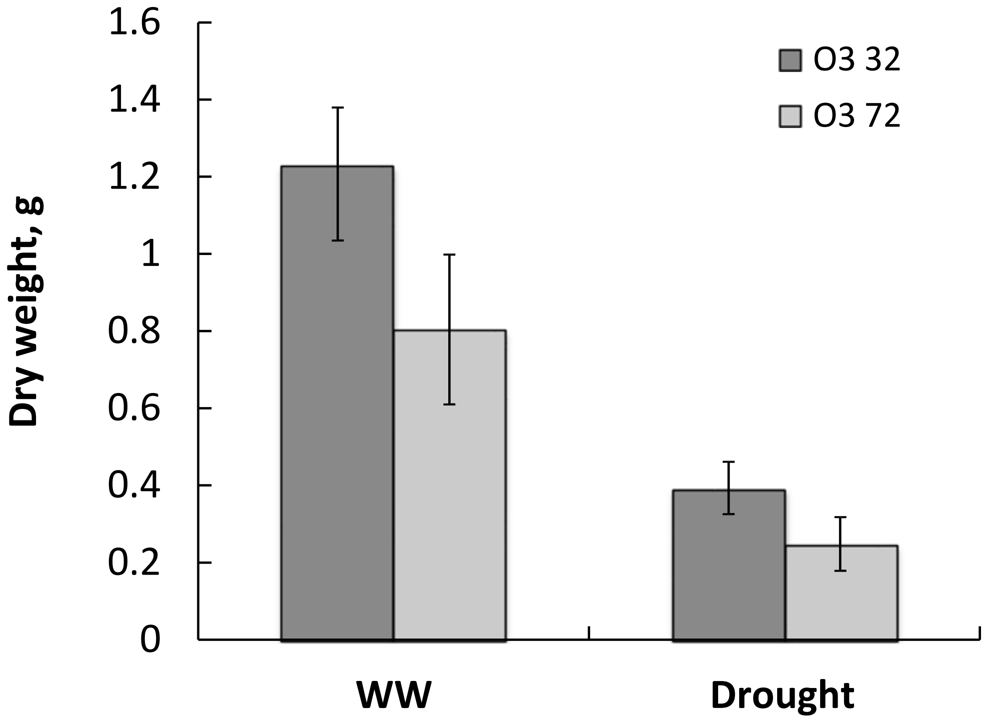

Figure 5: Weight of nodules (per tree) on roots of alder from two ozone treatments, in well-watered (WW) and drought conditions. Bars are standard errors based on individual pots.

Table 1: Mean ozone concentration, $AOT40_{24}$ and $AOT40_{12}$ (07:00-19:00) for the five treatments used in 2009 and 2010.

Treatment	2009 Mean ozone (ppb)	2009 $AOT40_{24}$ (ppm.h)	2009 $AOT40_{12}$ (ppm.h)	2010 Mean ozone (ppb)	2010 $AOT40_{24}$ (ppm.h)	2010 $AOT40_{12}$ (ppm.h)
$O_3$16	15.7	0.2	0.2	19.0	0.8	0.8
$O_3$32	33.3	4.2	3.5	34.8	5.3	4.3
$O_3$48	50.2	28.7	18.6	51.2	30.5	18.8
$O_3$56	57.7	44.1	26.2	60.3	47.0	27.2
$O_3$72	74.1	82.4	45.2	73.4	77.1	42.8

Table 2: Height increase from the start to the end of ozone exposure in the well-watered and drought treatment for the 6 tree species. Values shown are the mean across all ozone treatments. ***, ** and * indicate statistically significant differences between the WW and drought treatments at p=0.001, p=0.01 and p=0.05 respectively.

	WW (increase, cm)	D (increase, cm)
Alder	65.0	43.6***
Ash	25.4	18.0**
Beech	18.9	11.8**
Birch	64.2	53.1***
Hazel	12.3	7.1*
Oak	33.1	13.9***

Table 3: Size of biomass reductions due to ozone ($O_3$32 vs $O_3$72) and watering (WW vs drought), and significances of these differences and the interaction between ozone and drought, for each species tested, for stem weight, root weight and total biomass. (*), *, ** and *** indicate significant differences from two-way ANOVA at $p<0.1$, $p<0.05$, $p<0.01$ and $p<0.001$ respectively.

Species	**ozone**	**watering**	**Interaction**
Stem weight			
Alder	14% ns	40% *	ns
Birch	9% ns	30% *	ns
Hazel	13% (*)	30% (*)	ns
Beech	+	+	**
Ash	7% ns	14% ns	ns
Oak	21% ns	50% **	ns
Root weight			
Alder	11% ns	21% **	ns
Birch	15% *	27% ***	*
Hazel	18% ns	40% **	ns
Beech	+	+	*
Ash	13% ns	15% ns	ns
Oak	10% ns	30% **	ns
Total biomass			
Alder	16% **	36% ***	ns
Birch	8% ns	32% ***	ns
Hazel	15% ns	43% ***	ns
Beech	+	+	(*)

Ash	10% ns	22% ns	ns
Oak	12% ns	45% ***	ns

+ For beech there were interactions between ozone and watering regime, with opposite responses to ozone in WW and drought conditions.

Species-specific responses to ozone and drought in six deciduous trees

Running head: OZONE AND DROUGHT RESPONSES OF TREES

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Abstract

Saplings of alder (*Alnus glutinosa*), birch (*Betula pendula*), hazel (*Corylus avellana*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and oak (*Quercus robur*) were exposed to five episodic ozone regimes in solardomes, with treatment means between 16 and 72 ppb. All trees were kept fully watered for the first five weeks of exposure, after which half the trees continued to be well-watered, whereas the other half were subjected to a moderate drought by applying approximately 45% of the amount of water.

Species-specific reductions in growth in response to both ozone and drought were found, which could result in reduced potential carbon sequestration in future ozone climates. In well watered conditions the ozone treatments resulted in total biomass reductions for oak (18%), alder (16%), beech (15%), ash (14%), birch (14%) and hazel (7%) in the 72 ppb compared to the 32 ppb treatment. For beech there was a reduction in growth in response to ozone in the well watered

treatment, but an increase in growth in response to ozone in the drought treatment, in contrast to the decreased growth that would occur as a result of stomatal closure in response to either the ozone or drought treatment, and therefore assumed to result from changes in hormonal signalling which could result in stomatal opening in combined ozone and drought conditions.

For alder, in addition to a decrease in root biomass there was reduced biomass of root nodules with high compared to low ozone for both drought treated and well-watered trees. There was also a large reduction in the biomass of nodules from drought trees compared to well-watered. It is therefore possible that changes in the nitrogen dynamics of alder could occur due to reduced nodulation in both drought and elevated ozone conditions.

Introduction

Tropospheric ozone concentrations have been increasing since industrial times from a background of 10-15 ppb in the 1900s, due to increased emissions from anthropogenic sources (Solberg et al. 2005, Volz and Kley, 1988). A recent meta-analysis has suggested that the increase in ozone since the industrial revolution has been responsible for a reduction in photosynthesis of approximately 11% in trees (Wittig et al. 2007), which may have reduced tree productivity by approximately 7% (Wittig et al. 2009). Ozone concentrations have continued to increase over recent years, despite reductions in European precursor emissions (Wilson et al. 2012) and a further increase in background ozone concentration throughout the northern hemisphere has been predicted due to hemispherical transport of ozone precursor molecules (Royal Society 2008), with annual mean ozone concentrations reaching up to 68 ppb by 2050 (Meehl at al. 2007). These concentrations correspond with a predicted reduction in total tree biomass of approximately 11% (Wittig et al. 2009). World-wide, forest ecosystems store 80% of the world's above-ground carbon and 40% of the below-ground carbon (Brunner and Godbold 2007) and play a significant role in sequestering atmospheric CO_2 (Bonan 2008). Therefore, any impacts of ozone on carbon sequestration by trees could have a significant effect on the global carbon budget.

Studies of the effects of ozone on trees have shown responses such as visible leaf injury (Gerosa et al. 2009), elevated senescence (e.g. Mikkelsen and Jorgensen 1996, Pääkkönen et al. 1997) and reduced growth, e.g. on *Quercus rubra* (Samuelson et al. 1996). Some studies have indicated that a change in biomass partitioning can occur in response to ozone, for example, a decrease in the dry mass of roots and branches of *Betula pendula* attributed to ozone has been shown at the end of the exposure (Riikonen et al. 2004). It is thought that decreased partitioning to the roots may occur with increasing ozone exposure because the mature, lower leaves act as the main source of assimilate for root growth, and these are frequently the most damaged by ozone (Grantz et al. 2006, Cooley and Manning 1987, Okano et al. 1984). However, this has not been demonstrated for all species and some e.g. *Fagus sylvatica* and *Picea abies* showed no effect of ozone exposure on carbon allocation to roots (Andersen et al. 2010).

Concurrent with the predicted increases in ozone concentration, over the coming decades, summer rainfall is expected to be reduced across many temperate regions, with an increase in the frequency and severity of summer droughts predicted across much of Europe (Bates et al. 2008; Blenkinsop et al. 2007; Lehner et al. 2006). Although drought itself has been shown to reduce growth in some tree species (e.g. *Fagus sylvatica,* Thiel et al., 2014; *Picea abies,* Jyske et al. 2010; *Pinus spp,* Sanchez-Salguero et al. 2012), there can be interactive effects between ozone and drought stress. For *Betula pendula* drought stress alone has been shown to reduce stomatal density and stomatal conductance; the combined effects of drought and ozone were additive for some responses (Pääkkönen et al. 1998), for example, mild drought combined with 1.5 x ambient ozone concentrations caused an additive reduction in leaf number and total foliage area and also increased the N concentration of the leaves. In some species ozone exposure has been shown to decrease the ability of a plant to respond to subsequent drought, e.g. for the herbaceous species *Rancunculus acris* and *Dactylis glomerata* (Wagg et al., 2013), which could lead to further soil drying to increase the severity of a prolonged drought. In contrast, some other studies have demonstrated that drought has a protective effect against ozone as drought can induce stomatal closure (e.g. for *Populus spp.*, Silim et al. 2009). This can reduce ozone uptake and protect plants from injury caused by ozone exposure for some species (e.g. *Fagus sylvatica*, Löw et al. 2006). However, the meta-analysis of Wittig et al. (2009) on tree responses found no conclusive evidence for a protective role of drought against ozone induced effects on growth and biomass as there were insufficient published studies of ozone and drought interactions on trees available.

This study investigated the potential impacts of increasing background ozone concentration in combination with moderate drought after prior ozone exposure on six important tree species: alder (*Alnus glutinosa*), beech (*Fagus sylvatica*), oak (*Quercus robur*), ash (*Fraxinus excelsior*), hazel (*Corylus avellana*) and birch (*Betula pendula*). In this study, young trees were used, which allowed investigation of impacts of ozone on total root biomass avoiding the need for estimates of root turnover by methods such as root ingrowth cores, and plants were harvested before leaf-fall to obtain information on leaf number and leaf weight. Data on the biomass of leaves, stems and roots in response to ozone and drought for these six species is presented and used to indicate the relative sensitivity of these species to both stresses, including in combination.

Methods

Plant material

Trees of alder (*Alnus glutinosa*), birch (*Betula pendula*), hazel (*Corylus avellana*), beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*) and oak (*Quercus robur*) were all obtained from Cheviot Trees (Berwick-upon-Tweed, UK) as UK origin, cell-grown (10cm deep pots) seedlings. These were planted in 2-litre pots (14 cm diameter, 18 cm deep), which were lined with perforated plastic to discourage roots from growing outside the pot. All trees were planted in topsoil (Humax, UK), but retaining the soil around the existing root system to avoid disturbing the fine roots and established mycorrhizae. Trees were two years old and of initial height 35 cm (alder), 65 cm (birch), 40 cm (hazel), 45 cm (beech), 40 cm (ash) and 25 cm (oak). Alder, birch and beech were planted into their pots on 29th April 2009 whilst hazel, oak and ash were planted on 21st April 2010 and all trees were kept well-watered until the start of the experiment. Prior to the start of the experiment the height of each tree was measured. For each species, trees were separated into five size classes based on initial tree height and one tree of each size class was assigned to each solardome per watering regime. Altogether, ten trees of each species were exposed per solardome.

Ozone exposure

Plants were exposed to ozone in solardomes (hemispherical greenhouses 3m diameter, 2m tall). Ozone was generated from oxygen concentrated from air (Workhorse 8, Dryden Aqua, UK) using an ozone generator (G11, Dryden Aqua, UK) and distributed to each solardome via PTFE tubing. Ozone was delivered to each solardome using mass flow controllers (Celerion, Ireland) controlled by computer software (Labview version 7). Ozone concentrations were continuously monitored in one solardome using a dedicated ozone analyser (Thermoelectron, Model 49C), allowing feedback to compensate for small variations in ozone production. In all solardomes the ozone concentration was measured for 5 minutes in every 30 minutes using two additional ozone analysers (Envirotech API 400A) of matched calibration. Five ozone treatments were randomly allocated to the solardomes, with one solardome for each treatment. The weekly ozone profile used was based on an ozone episode from a UK upland site (Keenley Fell, Northumberland, (Grid Reference NY793561, 21st -28th May 2008) and target ozone concentrations were increases or decreases below this profile. This profile was repeated for each week of the experiment, giving target mean ozone concentrations of 16 ppb ($O_3$16), 32 ppb ($O_3$32), 48 ppb ($O_3$48), 56 ppb ($O_3$56) and 72 ppb ($O_3$72). The mean weekly ozone regime applied in each treatment is shown in Figure 1.

In 2009, the ozone exposure over the 12 week experimental period ranged from a seasonal mean of 15.7 ppb to 74.1 ppb (Table 1), with the AOT40 (accumulated over 24 h) ranging from 0.2 ppm.h to 82.4 ppm.h. The AOT40 accumulated over 12 h (07:00 to 19:00) ranged from 1.7 ppm.h to 45.2 ppm.h. In 2010, the ozone exposure was similar, with seasonal means of 19.0 ppb to 73.4 ppb, and with the AOT40 accumulated over 12 h ranging from 0.8 ppm.h to 77.1 ppm.h. To reflect rising background ozone, the profile used involved significant ozone exposure during the night-time as well as during the day in both years; therefore, the AOT40 accumulated over 24h was much larger than that accumulated over 12h.

The mean temperature within the solardomes (over 24h) for the duration of the ozone exposure was 18.6°C in 2009 and 17.5°C in 2010.

For all trees, ozone exposure did not start until after bud-break and early leaf expansion. For alder, birch and beech, ozone exposure started on 20th May 2009 and finished on 11th August. Watering occurred by hand three times per week for all trees. All trees were kept fully watered for the first 5 weeks of ozone exposure to ensure that soil water availability was not limiting. To give a drought treatment, water was given at the same time as for the well-watered (WW) trees, but the volume was reduced and was approximately 45% of the volume given to the WW treatment. The soil moisture content of a sample of WW and drought trees was measured twice per week using a hand-held theta probe (Delta-T) to assess the irrigation requirements. The drought treatment started on 24th June and continued until the plants were harvested on 11th August. For hazel, oak and ash ozone exposure started on 21st April 2010. The drought treatment started on 25th May and continued until the plants were harvested on 19th July.

Harvest

At the end of the ozone exposure the height of all trees was determined before they were cut to soil level. For each tree, leaves > 1cm long were separated from stems and counted and weighed. Leaves < 1cm long were not counted or weighed. Roots were washed for all replicate trees from two ozone treatments ($O_3$32 and $O_3$72), and nodules were separated from the roots for alder. All plant material was oven-dried at 65°C for a minimum of seven days before weighing.

Data analysis and statistics

All data except that for root biomass were analysed using General Linear Model analysis (GLM) in Minitab (Version 16) using the mean value per solardome as the input data. Root weight data and for alder, root nodule biomass, were only available from the $O_3$32 and $O_3$72 treatments and therefore comparisons of root weights and total tree biomass were made using two-way ANOVA, using individual plants as replicates.

Results

Leaf weight

For beech there was a significant interaction (*P*=0.01) between ozone and watering regime for the leaf weight per tree, with the leaf weight decreasing with increasing ozone exposure for those trees that remained well-watered (Figure 2a, r^2=0.43, *P*=0.24), whilst for the drought-treated beech trees there was the opposite response of an increase in the total leaf weight per tree with increasing ozone exposure (r^2=0.94, *P*=0.01). This was partly due to an increase in the number of leaves per tree with increasing ozone in the drought treatment (Figure 2b, r^2=0.67, *P*=0.097). As a consequence of this interaction, although there was a large effect of watering regime at low ozone concentrations, with fewer leaves and lower leaf weight in the drought treatment, at high ozone concentrations these differences were lost.

There were no significant effects of ozone on the total leaf weight per tree for birch, hazel, oak, alder and ash, and no significant interactions between ozone and watering regime for these species. However, there were some effects of watering regime. There was a significant reduction in the leaf weight per tree in the drought treatment compared to WW (mean reduction across all ozone treatments) for alder (40%, *P*=0.017), hazel (45%, *P*=0.016), birch (27%, *P*=0.003) and oak (55%, *P*=0.008), but no significant effects of watering regime on the leaf weight of ash (data not presented).

Height and stem weight

For all tree species there was a significantly larger increase in height between the start and end of ozone exposure in the WW treatment compared to the drought treatment (Table 2). Mean values across all ozone treatments are presented and these show a range from a 7cm height increase in drought-treated hazel, to a 65 cm increase in height in WW alder. However, there was no statistically significant effect of ozone on height of any of the species, and no significant interaction between ozone and watering regime (data not presented).

There was a trend for a reduction in stem weight with increasing ozone exposure for hazel (*P*=0.058, Figure 3a). There was also a reduction in stem weight of hazel in the drought treatment compared to WW of approximately 30% (*P*=0.069), and this difference was consistent across all ozone treatments. There was no significant effect of ozone and no interaction between ozone and watering regime on the stem weight of oak, birch, alder or ash. However, there were large reductions in stem weight in the drought treatment compared to WW (mean reduction across all ozone treatments) for birch (30%; *P*=0.043), alder (40%; *P*=0.053) and oak (50%; *P*=0.005) and no significant reduction for ash (data not presented).

In contrast for beech, overall there was a significant increase in stem weight with increasing ozone exposure (*P*=0.047, Figure 3b). However, as for leaf weight for this species, there was a significant interaction between ozone and watering regime (*P*=0.010). For WW beech there was no effect of ozone on stem weight, but for drought-treated beech trees there was an increase in stem weight with increasing ozone exposure (r^2=0.99, *P*=0), so that the difference in stem weight between WW and drought trees was lost in the highest ozone treatments.

Root weight

Root weight was determined in the $O_3$72 and $O_3$32 treatments only. Root weight was significantly decreased in the $O_3$72 treatment compared to $O_3$32 for birch (*P*=0.025, Figure 4) and there was significant interaction between ozone and watering regime (*P*=0.05). Increased ozone corresponded with a large decrease in root biomass of approximately 23% in the WW birch only (*P*=0.021) and there were no effects of ozone on drought-treated birch. For beech there was also a significant interaction between ozone and watering regime (*P*=0.05). However, in contrast for this species there was a decrease in root biomass with increasing ozone concentration in WW trees compared to an increase in root biomass with increasing ozone concentration in drought-treated trees (Figure 4). There was no significant reduction in root weight in the $O_3$72 treatment compared to $O_3$32 for oak, ash or hazel. There was a significant reduction in root weight in drought compared to WW for birch (27%; *P*=0), alder (20%; *P*=0.007), oak (30%; *P*=0.004) and hazel (40%; *P*=0.005).

For alder, there was a small decrease in root biomass with increasing ozone for both the WW and drought-treated plants (10%, ns), and no significant interaction between ozone and watering regime. However, there was a large effect on the biomass of root nodules, with a large reduction in drought-treated compared to WW (mean reduction across both ozone treatments) of approximately 60% (*P*=0.001; Figure 5). There was also a reduced biomass of root nodules with high ozone exposure compared to low exposure for both drought-treated and WW trees of approximately 25% (*P*=0.046), but no significant interaction between ozone and drought on the weight of root nodules. The relative weight of nodules per gram of root was also reduced by approximately 25% with increasing ozone under both WW and drought conditions (not statistically significant) and by approximately 60% with drought (*P*=0.001; data not presented). The number of nodules and mean weight per nodule was not determined, however, it was noticed that the nodule size was smaller with elevated ozone conditions.

Total biomass

Total biomass data was only available for two ozone treatments because root biomass measurements were only carried out in the $O_3$32 and $O_3$73 treatments due to the length of time required for root washing. In WW conditions the ozone treatments resulted in a total (above and below-ground) biomass reductions for oak (18%), alder (16%), beech (15%), ash (14%), birch (14%) and hazel (7%; Figure 4). For alder there was a decrease in total biomass in the $O_3$72 treatment compared to $O_3$32 of approximately 16% (*P*=0.003), with a similar magnitude of reduction in both the WW and drought treatments. There was a reduction in total biomass in the drought compared to WW alder trees of 36% (*P*=0), but no significant interaction between ozone and watering regime (Figure 4). In contrast there was an interaction between ozone and watering regime for beech (*P*=0.056). In well-watered beech there was a decrease in biomass with increasing ozone of 15% (*P*=0.031), however, in drought treated trees there was an increase in biomass with increasing ozone of 25% (*P*=0.07; Figure 4). For oak, birch and hazel there was no significant effect of ozone on total biomass, however there was a large reduction in drought compared to WW plants of 45% (*P*=0) for oak, 32% (*P*=0) for birch and 43% (*P*=0.001) for hazel (Figure 4). There were no significant effects of either ozone or watering regime on the total biomass of ash.

Biomass of roots in the $O_3$72 treatment was maintained at the expense of allocation to the stems and leaves for oak. Although the root weight was reduced by approximately 30% in the $O_3$72 treatment, stem weight was reduced by approximately 50% and leaf weight was reduced by approximately 55% (Figure 4, Table 3). Differences in biomass allocation between treatments for the other species were small.

Discussion

The ozone treatments resulted in total (above and below-ground) biomass reductions of between 7% and 18% when the $O_3$72 treatment was compared with the $O_3$32 treatment. These changes are in broad agreement with those found by Wittig et al. (2009), who showed in a meta-analysis of responses of trees to ozone that ozone concentrations of 64 ppb compared to ambient concentrations were associated with biomass reductions of 11%. The biomass effects shown in the current study were found using two-year old trees and are therefore of particular relevance to afforestation using young trees. However, if such effects also occur in mature trees, these results suggest that elevated ozone could reduce carbon sequestration in future ozone climates if background ozone concentrations continue to rise, as suggested in modelling studies (e.g. Meehl et al. 2007, Sitch et al. 2007). The biomass reductions demonstrated in this study included stem and root biomass, both of which represent reductions in long-term carbon storage and support the hypothesis that increased ambient ozone could further exacerbate climate change.

Any decrease in root biomass as a result of ozone exposure could decrease the ability of the tree to take up water and nutrients. Reductions in root weight can be a consequence of either an overall reduction in availability of photosynthate for root growth or reduced allocation to the roots as resources are preferentially used to replace damaged leaves. In this short-term study there were larger effects on roots than above ground biomass for birch as has previously been reported for several species including trembling aspen (*Populus tremuloides,* Coleman et al. 1996) and birch (*Betula pendula*; Riikonen et al. 2004). This could be evidence of reduced partitioning to roots, however, it has been shown that for trees the main source of photosynthate for the roots is from the lower leaves, and it is these older leaves that tend to be most affected as a consequence of ozone exposure (Grantz et al. 2006). Therefore, it is possible that further reductions in partitioning to roots may have occurred if the exposure had occurred over a longer timescale, although subsequent root re-growth after relief from a period of ozone stress may occur for some species. Reduced root growth would also indicate that a drought following the occurrence of elevated ozone could have a more severe effect due to the decrease in ability to take up water and nutrients, although it is also possible that less water usage early in a drought period would help retain moisture during an extended drought and therefore benefit the long-term survival of the tree.

Although it could be considered that drought protected some species (birch, ash and oak) from the negative effects of ozone exposure, the decrease in biomass as a result of the drought outweighed any benefit as large biomass reductions of up to 45% in response to drought were shown for all species in this study. Drought had a large impact on stem weight in five out of the six species tested, confirming the strong impact that drought may have on carbon sequestration. Naturally occurring droughts in China in the twentieth century have been related to strong decreases in net primary production, which was inferred from tree-ring width chronologies (Xiao et al. 2009). Stomatal closure in response to drought has been shown to protect against ozone in some species e.g. *Populus spp* (Silim et al. 2009), however, there was no evidence of this in the current study.

In addition to effects on root biomass, over the longer term, indirect effects of ozone such as decreased nodulation of roots of alder may also have a large impact. This study showed large effects of ozone and drought on nodule biomass, but did not consider any impact on nodule activity. It has previously been demonstrated that the host plant can influence root nodule activity (Verghese and Misra 2000), but the influence of ozone on this signalling from the host plant has not been studied. Nitrogen transfer from clover to grass in grass-clover swards has been demonstrated in several studies (e.g. Sincik and Acikgoz 2007, Goodman 1988) and reduced sensitivity to ozone of *Lolium perenne* occurred when this was grown in mixture with *Trifolium repens*, which was attributed to an increased availability of nitrogen to *L. perenne* when it was grown with *T. repens* (Hayes et al. 2010). Therefore, in addition to effects of reduced nodulation on the host plant which may contribute significantly to changes in growth, other ecosystem services such as nitrogen cycling within the vegetation community could also be affected indirectly as a consequence of decreased nitrogen transfer from alder to the ecosystem.

Alder showed an additive effect of the combination of ozone and drought on both root biomass and total biomass. In contrast, whilst under well-watered conditions the effects of ozone on beech were small, the interaction between drought and ozone for beech resulted in growth stimulation with increasing ozone exposure for drought-treated trees, resulting in increased root and total biomass, stem weight and the number and total weight of leaves. The plant hormone abscisic acid (ABA) is released under drought conditions, resulting in reduced stomatal conductance and therefore water loss in the leaves. A mechanism to explain ozone-induced reductions in stomatal sensitivity to ABA has been proposed by Wilkinson and Davies (2010) whereby ethylene, released as a response to ozone stress, antagonises the ABA response. They hypothesize that although both ethylene and ABA individually close stomata and reduce growth, when these combine, such as in the presence of ozone and drying soil, stomata could be opened and that growth could be promoted via greater throughput of nutrients, as seen in beech in the current study. Ethylene emission from leaves of *Leontodon hispidus* have been shown to increase with elevated ozone (Wilkinson and Davies 2009) and a reduced sensitivity to ABA in ozone treated plants has also been demonstrated (e.g. Mills et al. 2009, Wilkinson and Davies, 2009), with increased stomatal conductance in combined elevated ozone and ABA-treated (to simulate drought) conditions for *Leontodon hispidus* (Wilkinson and Davies 2009). The results for beech from the current study therefore support the hypothesis of Wilkinson and Davies (2010), although this effect was not observed in the other species tested. Published data on the response of *F. sylvatica* in response to ozone have shown very mixed results with some studies showing large significant responses with increasing ozone exposure e.g. reduced photosynthesis (Paoletti et al. 2002); reduced biomass (Landolt et al. 2000, Matyssek et al. 2010), however, some other studies have shown no significant differences for growth or photosynthesis of *F. sylvatica* due to ozone (Bortier et al. 2000a, Wipfler et al. 2005). The differential response to ozone in varying soil moisture conditions as demonstrated in the current study may explain some of the discrepancies between the different studies.

Although the current study used young trees, there is some evidence that mature trees are as sensitive to ozone as younger trees. Epidemiological analysis of effects of ozone on *Fagus sylvatica* indicated that the reduction in shoot growth due to ozone was similar in both seedlings and mature trees (Braun et al. 2007). In addition, in the Aspen-FACE experiment facility in Wisconsin, USA, biomass loss after 6 years of growth and treatment was proportionally similar to the loss at 2 years (King et al. 2005). The occurrence of visible injury attributed to ozone for *Fagus sylvatica* in phytotrons under an ambient ozone regime was induced at AOT40 levels similar to those experienced by mature trees at a nearby field site (Baumgarten et al. 2000). However, other studies have shown young beech in phytotrons to be more sensitive to ozone than adult beech in the field, which was attributed to enhanced ozone uptake compared to field conditions (Nunn et al. 2005).

This study has shown that typical deciduous woodland species vary in their sensitivity to rising background ozone, although the ranking of the species in terms of sensitivity to either ozone or drought depended on the parameter used. It has been suggested that faster growing species e.g. poplar are more sensitive to ozone than slower growing species e.g. beech (Bortier et al. 2000b), although there was no evidence to suggest that this was the case in the current study. Reducing water availability by 45% had even more pronounced effects on both above and below-ground biomass, with positive and negative interactions with elevated ozone exposure occurring in some species. However, the variation in the response to both ozone and drought between species indicates that future ozone conditions may affect both above- and below-ground competition between tree species, and that these effects could be further modified by drought as the relative sensitivity to ozone of different tree species may depend on water availability.

Conclusions

Both elevated ozone and drought have been demonstrated to have a large influence on biomass of some species of young deciduous trees. If a similar magnitude of response were to occur with more mature trees this could result in a reduction in carbon sequestration, with long-term climatic consequences. Ideally, further experiments using mature species from a wide variety of species would need to be carried out to ascertain the response of mature trees to ozone and drought. However, this is difficult and expensive. The use of younger trees, as in this study, offers a valuable insight into the potential effects on a wider range of tree species. In this case, significant reductions in biomass in response to ozone were found for two species and significant reductions in biomass in response to drought were found for all six of the species tested during the study, implying that sensitivity of trees to ozone and drought may be widespread.

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Figure legends

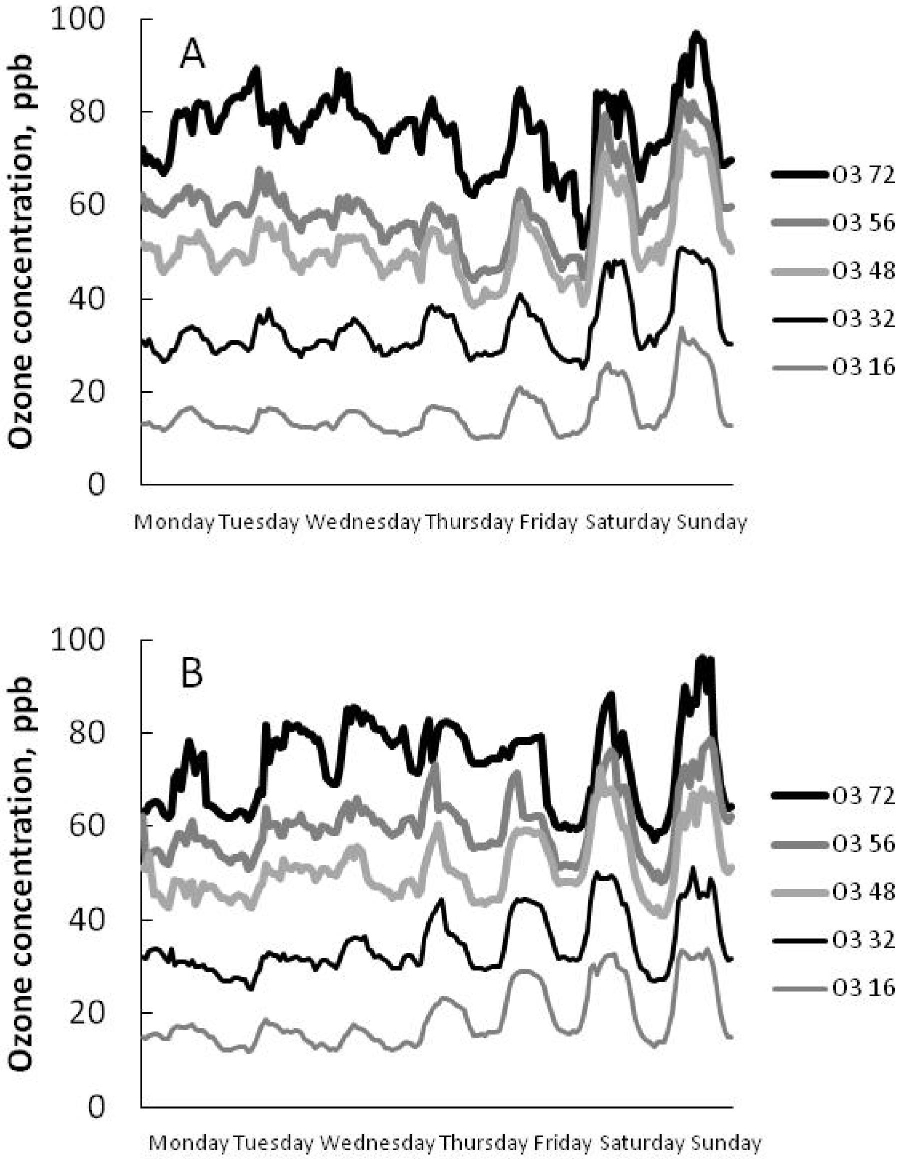
Figure 1: Mean weekly profile of ozone concentrations in the solardomes for the duration of the experiment in a) 2009 and b) 2010.

Figure 2: Leaf weight (a) and leaf number (b) of beech in response to ozone, in both well-watered (WW) and drought conditions, where each datapoint is the mean of five trees.

Figure 3: Stem weight of hazel (a) and beech (b) in response to ozone, in both well-watered (WW) and drought conditions.

Figure 4: Biomass partitioning to roots, stems and leaves for alder, birch, hazel, beech, ash and oak in well-watered (WW) and drought (D) conditions in the $O_3$32 and $O_3$72 treatments. Bars are standard errors based on individual pots. For significant differences, please refer to the main text.

Figure 5: Weight of nodules (per tree) on roots of alder from two ozone treatments, in well-watered (WW) and drought conditions. Bars are standard errors based on individual pots.



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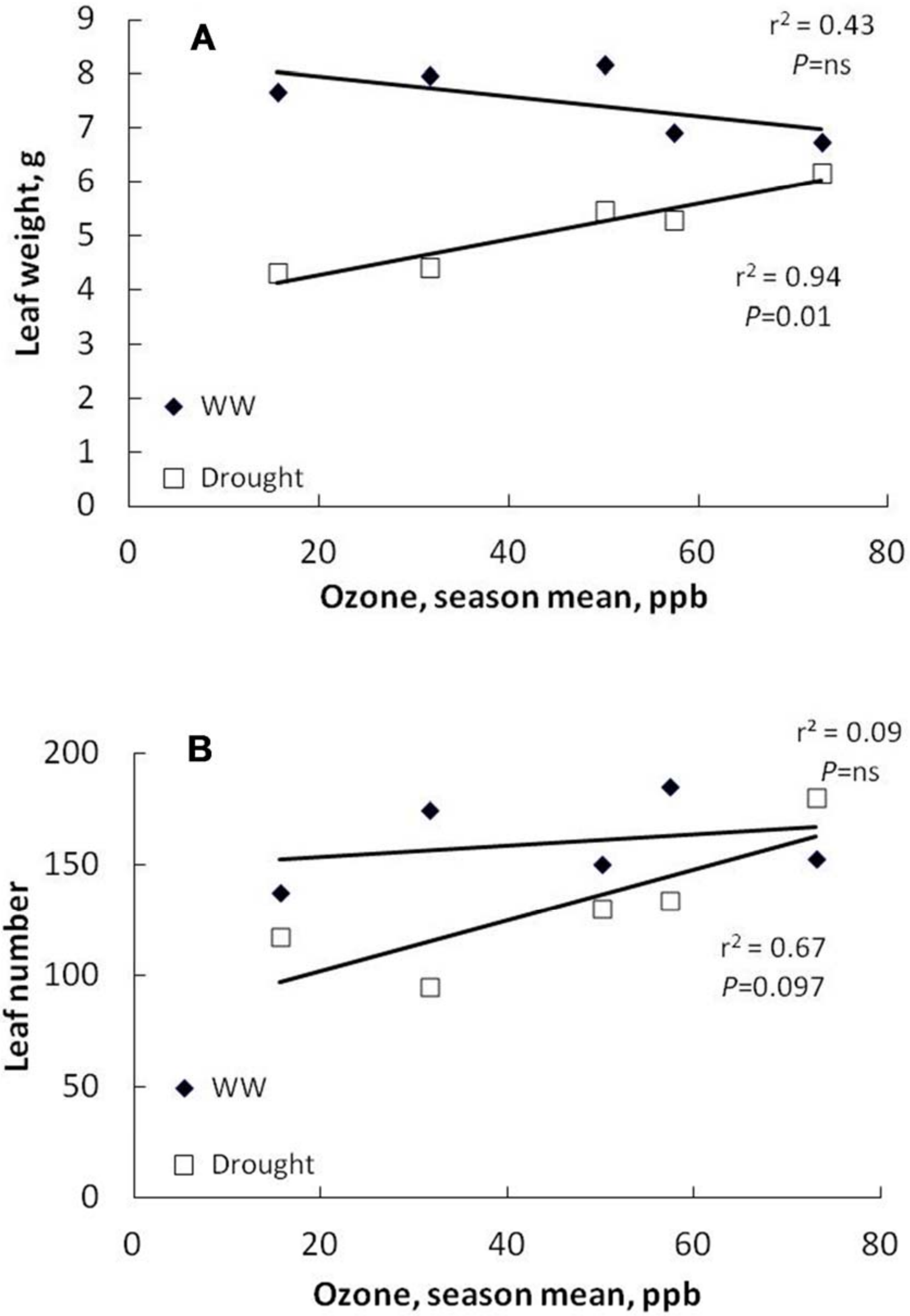

Figure 2: Leaf weight (A) and leaf number (B) of beech in response to ozone, in both well-watered (WW) and drought conditions, where each datapoint is the mean of five trees.

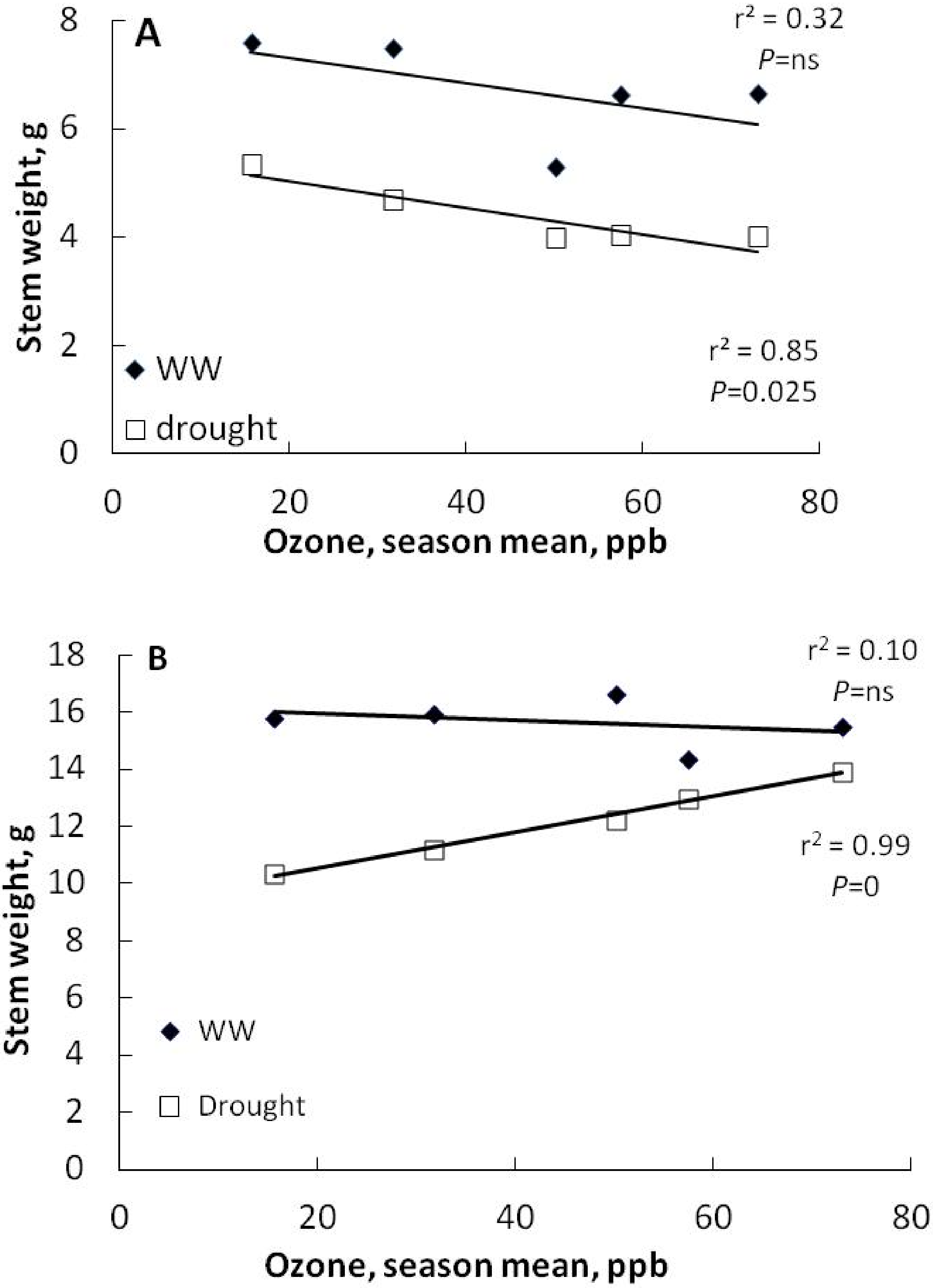

Figure 3: Stem weight of hazel (A) and beech (B) in response to ozone, in both well-watered (WW) and drought conditions.

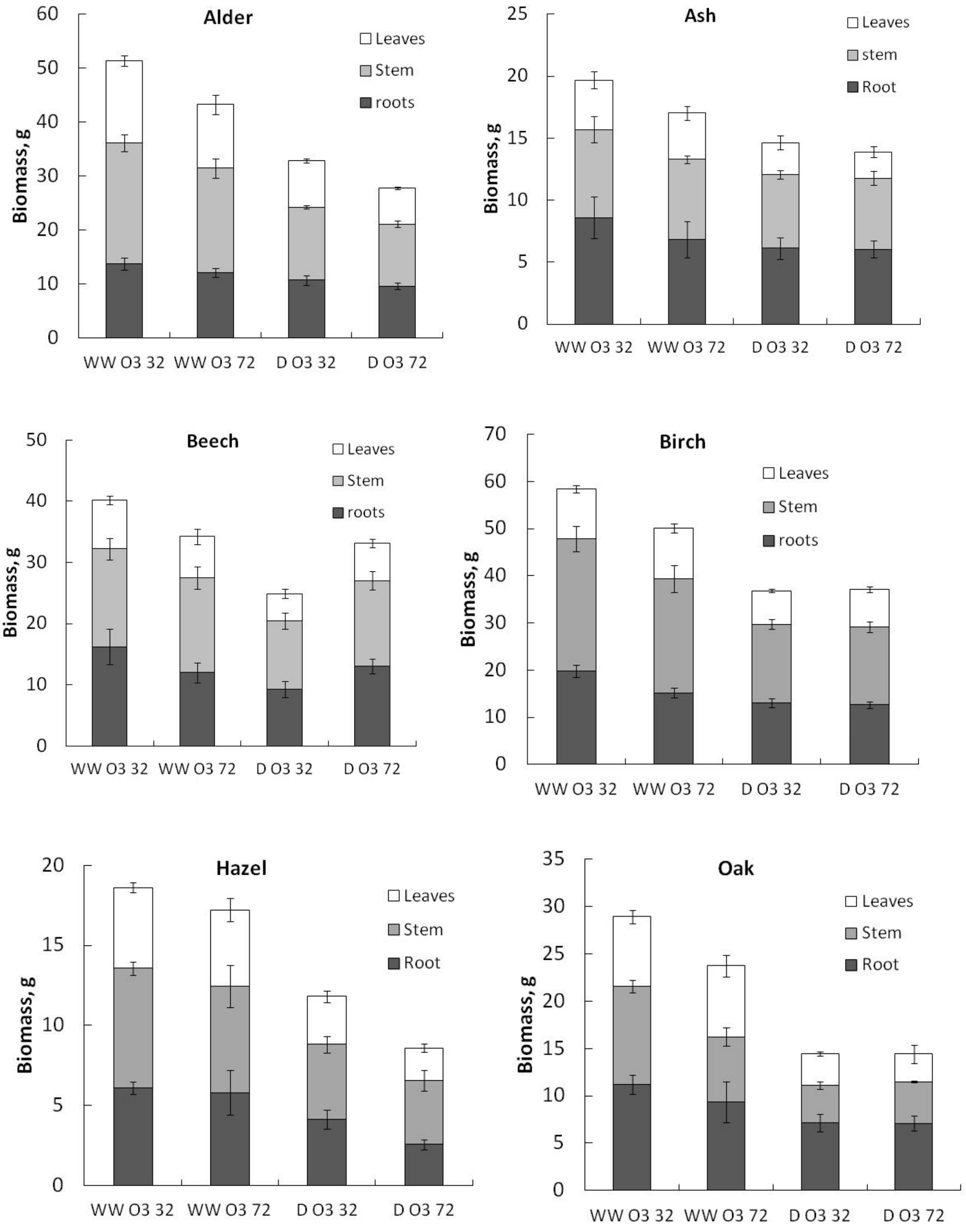

Figure 4: Biomass partitioning to roots, stems and leaves for alder, birch, hazel, beech, ash and oak in well-watered (WW) and drought (D) conditions in the $O_3$32 and $O_3$72 treatments. Bars are standard errors based on individual pots. For significant differences, please refer to the main text.

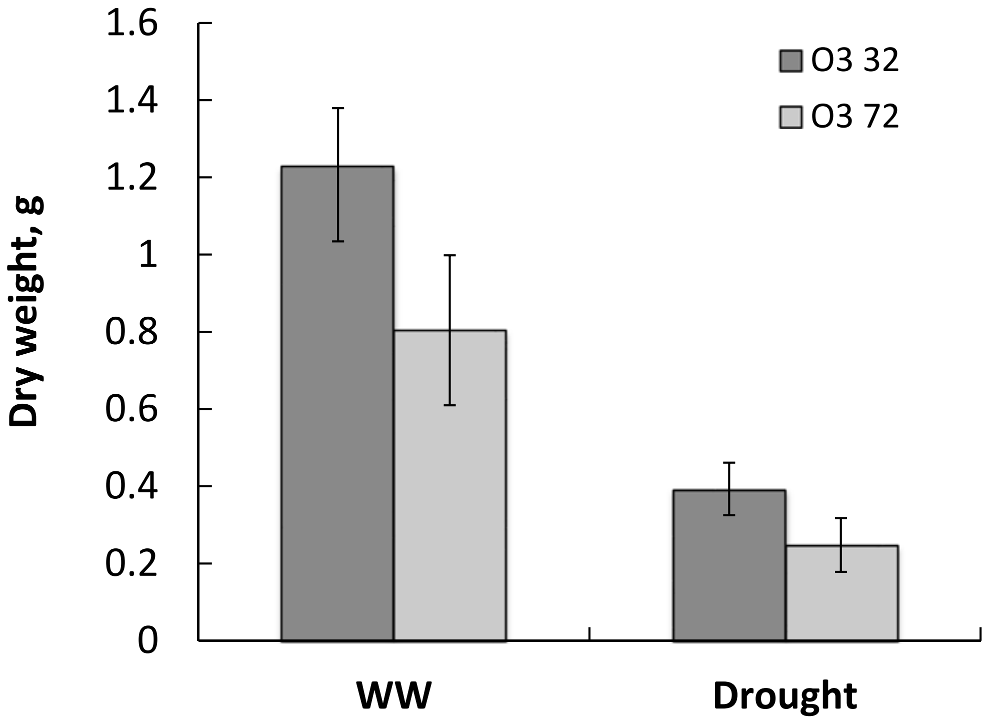

Figure 5: Weight of nodules (per tree) on roots of alder from two ozone treatments, in well-watered (WW) and drought conditions. Bars are standard errors based on individual pots.

Table 1: Mean ozone concentration, $AOT40_{24}$ and $AOT40_{12}$ (07:00-19:00) for the five treatments used in 2009 and 2010.

Treatment	2009 Mean ozone (ppb)	2009 $AOT40_{24}$ (ppm.h)	2009 $AOT40_{12}$ (ppm.h)	2010 Mean ozone (ppb)	2010 $AOT40_{24}$ (ppm.h)	2010 $AOT40_{12}$ (ppm.h)
$O_3$16	15.7	0.2	0.2	19.0	0.8	0.8
$O_3$32	33.3	4.2	3.5	34.8	5.3	4.3
$O_3$48	50.2	28.7	18.6	51.2	30.5	18.8
$O_3$56	57.7	44.1	26.2	60.3	47.0	27.2
$O_3$72	74.1	82.4	45.2	73.4	77.1	42.8

Table 2: Height increase from the start to the end of ozone exposure in the well-watered and drought treatment for the 6 tree species. Values shown are the mean across all ozone treatments. ***, ** and * indicate statistically significant differences between the WW and drought treatments at p=0.001, p=0.01 and p=0.05 respectively.

	WW (increase, cm)	D (increase, cm)
Alder	65.0	43.6***
Ash	25.4	18.0**
Beech	18.9	11.8**
Birch	64.2	53.1***
Hazel	12.3	7.1*
Oak	33.1	13.9***

Table 3: Size of biomass reductions due to ozone ($O_3$32 vs $O_3$72) and watering (WW vs drought), and significances of these differences and the interaction between ozone and drought, for each species tested, for stem weight, root weight and total biomass. (*), *, ** and *** indicate significant differences from two-way ANOVA at $p<0.1$, $p<0.05$, $p<0.01$ and $p<0.001$ respectively.

Species	**ozone**	**watering**	**Interaction**
Stem weight			
Alder	14% ns	40% *	ns
Birch	9% ns	30% *	ns
Hazel	13% (*)	30% (*)	ns
Beech	+	+	**
Ash	7% ns	14% ns	ns
Oak	21% ns	50% **	ns
Root weight			
Alder	11% ns	21% **	ns
Birch	15% *	27% ***	*
Hazel	18% ns	40% **	ns
Beech	+	+	*
Ash	13% ns	15% ns	ns
Oak	10% ns	30% **	ns
Total biomass			
Alder	16% **	36% ***	ns
Birch	8% ns	32% ***	ns
Hazel	15% ns	43% ***	ns
Beech	+	+	(*)

Ash	10% ns	22% ns	ns
Oak	12% ns	45% ***	ns

+ For beech there were interactions between ozone and watering regime, with opposite responses to ozone in WW and drought conditions.