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Does a simulated upland grassland community respond to increasing background, peak or accumulated exposure of ozone?

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Abstract

Tropospheric ozone concentrations are increasing, which may result in elevated background concentrations at rural high-altitude sites. In this study simulated upland grassland communities containing seven species were exposed to ozone treatments in solardomes for 12 weeks in each of two consecutive summers. Ozone profiles, based on future ozone predictions, were of elevated background concentrations, episodic peaks of ozone and a combination of the two. During the winter between the two exposures the communities were kept outdoors in ambient air. Whereas previous studies have demonstrated that peaks of ozone cause detrimental effects to vegetation, this study shows that for simulated grassland communities an increase in background ozone concentration in the absence of peaks of ozone also corresponded with increased senescence. In many cases senescence was further increased when peaks of ozone were also present. The species used showed no acclimation to ozone and the same relationship between senescence and ozone dose occurred in both years of the study. A decrease in cumulative biomass was demonstrated for *Anthoxanthum odoratum,* which contributed to a decrease in total community biomass. These results indicate that current and future ozone concentrations could cause detrimental effects on growth and vitality of natural grassland communities and that for some species the consequences of increased background ozone concentration are as severe as that of increased peaks.

Key words

Grassland; ozone profiles; senescence; biomass; competition

1. Introduction

Tropospheric ozone concentrations have been increasing during the last century due to increasing anthropogenic emissions of ozone precursors (Volz and Kley, 1988). In urban and lowland areas elevated ozone occurs as ozone episodes, where ozone concentrations are increased for several days in a pronounced diurnal profile, with the highest peak during the day and low concentrations at night (Garland and Derwent, 1979). In contrast, at remote, high-altitude sites ozone concentrations remain high at night-time, because the air remains turbulent and losses of ozone from the lowest air layers due to dry deposition are replaced from ozone-rich layers above (Coyle et al., 2002; Ashmore et al., 2002). In the last 15 years, there has been evidence of a change in the ozone profile over Europe. Peak concentrations have fallen (Vingarzan, 2004; Szopa et al., 2006) whilst background ozone concentrations have steadily increased (Solberg et al., 2005, Derwent et al., 2006). Current predictions indicate that the rising background may stabilise by ca. 2030, but peak ozone concentrations may again rise as global warming increases the incidence of climatic conditions conducive to ozone formation (Royal Society, 2008). In this study, we investigated the implications of the potential changes in ozone profile for an upland grassland since the effects are likely to be felt first in remote upland regions (Coyle et al., 2003). For these communities the diurnal cycles are less pronounced and the background ozone concentration is already in the 30 to 40+ ppb range in many such areas of Europe (e.g. Marchlyn Mawr, UK, altitude 610 m, www.welshairquality.co.uk, grid reference SH604627).

Ozone has been shown to cause adverse effects on individual plant species, with effects including visible injury and premature senescence (e.g. Bergmann et al., 1995) and effects on growth (e.g. Franzaring et al., 2000; Gimeno et al., 2004a). Different effects have been reported on different species and a wide range in sensitivity to ozone has been observed (Hayes et al., 2006). In addition to these experimental studies, effects of ozone on naturally occurring plants in ambient air have been demonstrated across Europe (Hayes et al. 2007a). However, few studies have investigated the effects of ozone at mean levels of 30 - 50 ppb without including some peak exposures and this has been identified as a large gap in current knowledge that makes it very difficult to predict the impact of changes in the background ozone concentration on vegetation (Coyle et al., 2003). One of the few studies to investigate the effects of elevated background ozone concentrations demonstrated that for the grass species *Anthoxanthum odoratum,* elevated background ozone concentrations induced premature senescence to a similar extent to episodic peaks superimposed on a low background (Dawnay and Mills, 2009).

It is possible that changes in community dynamics could occur with increased ozone exposure due to the differential responses to ozone of the component species, however, there have been comparatively few studies on the effects of ozone on plant communities. Studies to date have been almost exclusively on grassland/pasture vegetation. The majority of these studies carried out using two or three species mixtures (e.g. Bender et al., 2002, 2006; Gimeno et al., 2004b; Tonneijck et al., 2004), and a few additional studies used larger model communities or established vegetation (e.g. Ashmore and Ainsworth, 1995; Rämö et al., 2006; Volk et al., 2006). The studies have shown that some species respond differently to ozone depending on which species they are growing in competition with, for example *Poa pratensis* showed reduced growth with ozone exposure when growing with *Veronica chamaedrys*, but not when grown with other species such as *Achillea millefolium* (Bender et al., 2006). Elevated ozone has also been shown to have carry-over effects the following spring in species that did not respond to summer ozone exposure (Hayes et al., 2006). Longer-term studies involving field release of ozone in Switzerland have indicated that effects on biomass can take some time to manifest. For a species-rich hay pasture grassland, no effects were found in the first year of exposure, but after 5 years of exposure there was a significant decrease in total yield and the percentage of legumes (Volk et al., 2006) with effects being cumulative with time. In a similar ongoing study for sub-alpine pasture, no effects on biomass of the component species was evident after three years (Bassin et al., 2007b), but some species had a reduced chlorophyll content associated with yellowing (Bassin et al., 2009).

Despite the evidence for a changing ozone profile, with the exception of Dawnay and Mills (2009), conducted in our laboratory, very few studies have investigated the relative effects of increasing peak versus increasing background ozone concentration. Oksanen and Holopainen (2001) exposed saplings of birch to three ozone profiles with the same AOT40 (the sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb for each hour when the concentration exceeds 40 ppb) presented at 70 ppb for 24h d^{-1}, 100 ppb for 12h d^{-1} or 200 ppb for 4.5 h d^{-1}. It was found that high peak ozone concentration was important for visible injury and reductions in stomatal conductance whilst growth reductions were more related to total accumulated exposure. Exposure of mixtures of *Lolium perenne* and *Trifolium repens* also indicated that clover yield was similar with an episodic profile compared to a fairly constant ozone exposure with a similar AOT40 (Nussbaum et al., 1995), although in this case the total forage yield was affected more by the episodic treatment. More recently, Wang et al. (2008) reported that ozone with a diurnal profile but the same overall mean concentration and accumulated dose as constant ozone exposure had a greater negative effect on the growth and yield of oilseed rape. Heath et al., 2009, reviewed evidence of the temporal responses to ozone and provided some clues as to why different processes in plants respond differently to different ozone profiles. He found that the time of maximum anti-oxidant defence within plants was early-mid morning, well before the mid-afternoon peak ozone concentration associated with a rural profile and suggested that due to higher antioxidant activity morning ozone fluxes were less biologically effective than afternoon fluxes. Heath et al (2009) re-iterated the importance of nocturnal fluxes (see Musselman et al., 2000 for further details), and stressed the need for further research into long-term effects of ambient exposures.

Using species from a typical upland grassland (National Vegetation Classification (NVC) U4 community (*Festuca ovina-Agrostis capillaris-Galium saxatile* grassland, Rodwell, 1992) growing together as a simulated community, this study investigated the effects of increasing background ozone (by 20 – 27 ppb) and added peaks (by 50 ppb), singly and in combination over two consecutive summer exposures. The aim was to determine the relative importance of these simulated current and future ambient ozone concentrations on the development of senescence, above ground biomass and competitiveness of the component species and functional groups. The model communities were maintained in shallow containers to simulate a below-ground competitive environment typical of upland grasslands.

2. Methods

Plant communities

Plants of *Anthoxanthum odoratum, Carex echinata, Carex bigelowii, Potentilla erecta* and *Galium saxatile* were propagated from stock plants that originated from Snowdonia, UK (grid reference SH646606), an area dominated by *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland (NVC U4, Rodwell, 1992). Seeds of *Festuca ovina* and *Agrostis capillaris* were sown into cell trays from seed obtained from a commercial seed supplier with seed originating from the UK (Emorsgate, UK).

Straight-sided containers (27cm diameter, 11cm deep; LBS horticulture) were lined with perforated plastic to discourage root growth through the drainage holes and filled with a mixture of ericaceous compost and sharp sand (in a ratio of 40 litres to 25 kg). Each pot was planted with *A. capillaris* (2 plants), *A. odoratum* (2 plants), *F. ovina* (2 plants), *C. echinata* (1 plant), *C. bigelowii* (1 plant), *P. erecta* (1 plant) and *G. saxatile* (1 plant) to create a model plant community. Each pot had the same arrangement of species, with plants of the lower growing species *P. erecta* and *G. saxatile* in the centre of the pot and the other species around the pot edge. The communities were established in a cool greenhouse (approximately 18°C) for 8 weeks, by which time the plants had grown together to form a closed canopy. During this time the systemic pesticide imidachloprid (Provado) was applied as a soil drench. Immediately prior to the first exposure to ozone in the solardomes the vegetation was cut back to 11cm and all leaves growing outside the pot perimeter were also removed.

Communities were categorised according to the size of the component species at the end of the establishment phase. One community of the eight in each size category was randomly allocated to each solardome. Five communities were exposed to ozone in each solardome. Each community was moved within the solardome every two weeks in year 1 and every three weeks in year 2 to avoid any potential confounding effect of location.

Over-wintering

Following the first period of ozone exposure, the communities were left to over-winter in sheltered but outdoor conditions. A weak nutrient solution was applied every eight weeks over the winter period (half-strength "Phosphrogen", PBI Home and Garden). After cutting back the vegetation in June, the communities were transferred to the solardomes on 4th July to acclimatise before ozone treatments started on 8th July 2005.

Throughout both periods of ozone exposure the model communities were watered during the early morning (4 am) using an automated misting system, with additional hand-watering applied as necessary during periods of hot weather.

Ozone exposure

Eight solardomes (large, hemispherical glasshouses, 3m diameter, 2.1m high) were used for ozone exposure. Ozone was generated by a G11 ozone generator (Dryden Aqua, UK) using oxygen supplied by a Workhorse 8 oxygen generator (Dryden Aqua, UK) and added to charcoal filtered air to give the required ozone concentrations using a computer controlled (LabView version 6.0) mass-flow controller system. The ozone concentration in each solardome was recorded every 30 minutes using two photometric ozone analysers of matched calibration (Environmental Technology Services 400A). The first ozone exposure started on 14th July 2004 and the second exposure started on 8th July 2005. Each exposure period was for 12 weeks.

Ozone treatment was allocated to each solardome in a randomised block arrangement, with two blocks of four solardomes. An individual solardome received the same treatment in both years. The treatments applied and abbreviations for these treatments were:

LL: Low background (15 ppb), low peaks (20 ppb)

LH: Low background (15 ppb), high peaks (65 ppb)

HL: High background (35 ppb), low peaks (40 ppb)

HH: High background (35 ppb), high peaks (85 ppb)

'Peaks' of ozone were applied for eight hours per day, automated to start to increase at 09:00, reaching a peak at 11:00, then decreasing over 2 hours starting at 18:00. These peaks were applied for four consecutive days in each 7-day period. The concentrations indicated for these peaks are the maximum target ozone concentration during the peak. The ozone concentrations remained at the appropriate 'background' levels for the treatment at all other times.

The mean ozone concentration during the establishment of the communities was 24.8 ppb, with an AOT40 over the establishment period of approximately 80 ppb.h. Ozone concentrations of the ambient air were not measured on site throughout the overwintering period, but started on 8th July in 2005 (year 2), however, the AOT40 prior to 8th July was negligible, based on measurements from a nearby monitoring station.

Simulated meadow cuts and final harvest

The plant canopies were cut back to 7 cm to simulate meadow cuts at the end of the ozone exposure period in Year 1, after over-wintering and early season growth (June 2005), and to soil level after exposure to ozone in Year 2. The above-ground vegetation was separated into the component species prior to drying at 65°C. Below-ground biomass was not determined. Due to a system fault in one of the replicate solardomes for the 'HH' treatment on 25th August (week six), plants from this dome were discarded in Year 1. The five original communities and five spare communities (which had received the same ozone treatment) from the replicate dome were randomly allocated between the two HH treatments for the exposure in Year 2.

Visible injury and senescence assessments

Communities were checked weekly for ozone injury. The percentage of ozone-injured leaves and senesced leaves (a leaf was classified as senesced if >25% of the leaf was senesced) per species per pot was recorded fortnightly in Year 1 and three-weekly in Year 2.

Statistical analysis

All senescence data were arcsine transformed prior to analysis. Data from the discarded plants from the HH treatment in year 1 were included in this assessment for the weeks 0-6 (prior to system fault); after this in year 1, for weeks 7-12, data from the additional pots in the replicate solardome were used. Senescence at harvest and biomass were separately analysed using two-way analysis of variance in GenStat (Version 8) using the mean value per solardome. Data was analysed to investigate whether 'background', 'peak' or an interaction between 'background' and 'peak' ozone concentrations influenced plant response. Differences in the rates of progression of senescence between treatments were assessed for each species using a Repeated Measures test in SPSS (Version 12), based on the mean per replicate solardome at each assessment. Comparison of the development of senescence between Year 1 and Year 2 was made using the general linear model function within Minitab Version 14, using AOT0 and AOT40 as the ozone parameter.

3. Results

Ozone exposure

Figure 1 illustrates the mean weekly profile in the solardomes in years 1 and 2. In both years, the difference in mean concentration between the two replicate solardomes for each treatment was < 2 ppb. Ozone concentrations in all treatments were reduced to 10-20 ppb on day 7 allowing access for plant measurements. In year 1 mean background ozone concentrations were 20.3 ppb in both the LL and LH treatments and 48.7 ppb and 47.6 ppb in the HL and HH treatments respectively, with mean peak heights of an additional 2.9 ppb for the low peaks, and 49.8 ppb for the high peaks treatments. In year 2, the mean background ozone concentrations were 17.2 ppb and 18.1 ppb in the LL and LH treatments and 37.1 ppb and 39.8 ppb in the HL and HH treatments respectively, with mean peak heights of an additional 5.8 ppb for the low peaks and 52.3 ppb for high peaks treatments. The mean ozone concentrations for the background and peaks for each treatment, and the AOT0 (the sum of the differences between the hourly mean ozone concentration (in ppb) and 0 ppb for each hour when the concentration exceeds 0 ppb) and AOT40 in each year of the study are shown in Table 1. There was good replication between the solardomes for each treatment in each year (Figure 1), however, the ozone concentrations were generally lower in year 2 than in year 1, particularly the background ozone concentrations for the 'HL' and 'HH' ozone treatments.

Ozone-specific visible injury

No ozone-specific visible injury was observed on any of the component species in any treatment during either of the twelve-week exposures of the communities to ozone.

Senescence

At harvest in year 1, *P. erecta, C. echinata* and *F. ovina* showed increased senescence in response to increasing background ozone concentrations at $p<0.05$ (Figure 2a), with *A. odoratum* and *A. capillaris* showing strong trends towards the same effect ($p<0.1$). However, in year 2 the only species to show a significant increase in senescence in response to increasing background ozone concentrations was *F. ovina* ($p<0.01$; Figure 2b). Significant increases in senescence corresponding with increased peaks of ozone at harvest in year 1 were shown for *P. erecta* ($p<0.01$) and *F. ovina* ($p<0.05$; Figure 2c). Strong trends for increased senescence with increased peaks of ozone in year 1 were also observed for *C. echinata* and *A. odoratum* ($p<0.1$). At harvest in year 2, the only species that showed a significant effect of increased peaks of ozone was *F. ovina* ($p<0.01$; Figure 2d).

In year 1, the extent of senescence at harvest of both *P. erecta* and *A. odoratum* showed a significant interaction between increased background and peaks of ozone ($p<0.05$ for both species (Table 2), with a greater increase in senescence than the sum of effects of background and peaks individually. In year 2, the only species to show such an interaction was *F. ovina* ($p<0.05$), where the combination of increased background and peak ozone concentrations again corresponded with a synergistic increase in senescence.

The difference in the extent of senescence between the 'HL' and 'LL' treatments for *F. ovina* was larger in year 1 (19.1% and 8.5% respectively) than in year 2 (8.9% and 7.0% respectively). This corresponded with a larger difference in mean ozone concentration between these treatments in year 1 than in year 2 (Table 1).

The AOT40 (calculated over 24 h per day) for the LH treatment and the HL treatments were similar in Year 1 (16.0 and 13.3 ppm.h respectively). There were no significant differences in the extent of senescence between these two treatments in

Year 1 for any of the species (data not presented). Comparisons were not appropriate in Year 2 because the difference in AOT40 between the two treatments was larger.

Repeated measures analysis, comparing the rate of development of senescence to that of the ‘LL’ treatment for each species based on the time*treatment effect showed that for *F. ovina* there was accelerated senescence during the exposure period in year 1 for the HL ($p<0.05$), LH ($p<0.05$) and the HH ($p<0.05$) treatments (Figure 3). In year 2 for *F. ovina* there was a significant increase in the rate of development of senescence in the HH treatment compared to LL only ($p<0.05$). *P. erecta* showed accelerated senescence during the exposure period in year 1 for the HL ($p<0.01$), LH ($p<0.05$) and the HH ($p<0.05$) treatments compared to the LL ozone treatment (Figure 3). However, there were no significant differences in the rate of development of senescence in year 2 for this species. For both *F. ovina* and *P. erecta* differences in the extent of senescence were first apparent after exposure to the ozone regime for 8 weeks ($p<0.05$ and $p<0.01$ respectively). Although some effects of elevated ozone had been shown in cumulative biomass for *A. odoratum,* no differences in the rate of development of senescence were apparent in either year (Figure 3), although this may have been higher in the HH treatment in year 1. There were no significant differences in the rate of development of senescence in response to ozone in either year 1 or year 2 for the other species (data not presented).

Although there was reduced senescence in the HH treatment in year 2 compared to year 1, the ozone concentrations and AOT40 in year 2 were also lower. Regression analysis showed that there was no significant difference in the sensitivity to cumulative ozone (AOT0 or AOT40) between the two years of study for any of the species used. Using the data for both years, the relationship between cumulative ozone (AOT0) and senescence showed linear relationships for *F. ovina* (r^2=0.79, p<0.001), *A. odoratum* (r^2=0.12, p=0.106), and *P. erecta* (r^2=0.57, p=0.001), with similar relationships using AOT40 (Figure 4).

Species abundance based on above-ground biomass

The above-ground biomass of each species at each harvest, and the total harvested biomass are shown in Table 3. There were no significant differences in the total above-ground biomass of the communities between the different treatments at harvest in year 1, after overwintering or at harvest in year 2. However, the total cumulative above-ground biomass showed a significant effect of increasing peaks of ozone (p<0.05) and a significant interaction between increasing background and increasing peaks (p<0.5), with a synergistic decrease in biomass. These effects on total harvested biomass corresponded with effects on the biomass of *A. odoratum*, which had the largest contribution to the total biomass of the communities. *A. odoratum* had a total cumulative biomass which showed a significant effect of increasing peaks of ozone (p<0.05) and a significant interaction between increasing background and increasing peaks (p<0.05), corresponding with a further decrease in biomass amounting to 15% in the HH treatment compared to LL. In addition to the decrease in *A. odoratum* biomass, there was also a decrease in the grass:forb ratio with increasing background (p<0.1 for year 1; p<0.05 using total cumulative biomass), but there was no significant effect of increasing peak concentration on this ratio.

Some differences in above-ground biomass were apparent for individual species. *F. ovina* showed significant reductions in biomass at the harvest after exposure to ozone in year 1 due to both the influence of increased ‘background’ (p<0.05) and ‘peaks’

($p<0.05$) of ozone exposure. There was no significant interaction between increased 'background' and increased 'peaks' for this species. The differences in biomass between treatments for *F. ovina* did not persist for the duration of the experiment and there were no significant differences after over-wintering, at the final harvest or in cumulative biomass.

After overwintering, significant alterations in biomass due to the influence of increases in both 'peaks' ($p<0.05$) and 'background' ($p<0.05$) were shown for *P. erecta* (Table 3). Although these alterations in biomass were no longer evident after exposure to ozone in Year 2 there was an effect on the cumulative biomass for this species with a significant influence of increasing background ($p<0.05$) and a strong trend for an influence of peaks ($p<0.1$).

4. Discussion

The relationship between the extent of senescence with both AOT0 and AOT40 showed no difference in the sensitivity of the communities to ozone between the two years detected, indicating that the plants responded to the ozone dose received in the growing season with no carry-over effect or acclimation to ozone. This is in contrast to some other studies, such as Tonneijck et al. (2004), Barbo et al (1998) and Bungener et al (1999), which have all observed a decrease in response of perennial plants over years in multi-year experiments, attributed to physiological or morphological adaptations that had occurred within the three years. It has been suggested that the limitations for root growth during the second and subsequent years of study may decrease sensitivity to ozone by reducing plant relative growth rates, as higher relative growth rates may be associated with ozone sensitivity (Bassin et al., 2007a). However, it was not possible to determine whether the relative growth rates of the communities changed between exposure seasons in this study, because the harvested biomass at the end of the exposures was at different cutting heights in the different years.

The accelerated rates of senescence with increasing ozone treatment usually did not correspond to reductions in biomass during the exposure period within an individual growing season. The exception was *F. ovina*, which showed reductions in biomass in year 1 only. Senescence was also more frequently detected than changes in biomass in species from wetlands (Franzaring et al., 2000) and from upland grasslands (Hayes et al., 2006). Similarly, visible injury in the absence of biomass changes has frequently been demonstrated (e.g. Pleijel and Danielsson, 1997). However, although not always considered to be an ecological impact on plants, premature and enhanced senescence could be detrimental because this would mean reduced assimilation of resources in a growing season, resulting in a more gradual reduction in the overall ability of a plant species to survive and withstand other stresses.

Small increases in background ozone concentration in the absence of any peaks of ozone, from pre-industrial to current levels, were sufficient to induce a significant increase in senescence of *F. ovina.* The effect was larger at the higher ozone concentrations used in year 1 (backgrounds of 20.3 and 48.7 ppb for LL and HL respectively) compared to those of year 2 (background concentration of 17.2 ppb and 37.1 ppb for LL and HL respectively). Other species such as *C. echinata* and *P. erecta* were less sensitive to the small increase in background during year 2, but did show enhanced senescence in the larger concentration range used in year 1. There was also a linear response of increasing senescence with increasing ozone exposure (AOT0 and AOT40), indicating that there was no threshold for response in the species studied. Increasing background ozone in the absence of peaks has also been demonstrated to accelerate senescence in *A. odoratum* (Dawnay and Mills, 2009). The current study and that of Dawnay and Mills (2009) both show effects that are at relatively low ozone concentrations that are within the range already experienced within upland areas of Europe.

Effects of ozone on the competitive balance of this community were small despite the reduction in total biomass. There was a decrease in cumulative above-ground biomass with increased background ozone concentration for *A. odoratum,* with decreased biomass of similar magnitude in the highest ozone treatments observed throughout the study for this species which only reached significance by the end of the second exposure. This decrease in *A. odoratum* biomass contributed to a reduction in grass:forb ratio. Previous studies have demonstrated an increase in the grass:forb ratio with ozone exposure (e.g. Wilbourn et al., 1995) however, this is dependant on the relative sensitivity to ozone of the species involved. *A. odoratum* has been shown to be more sensitive to ozone than some other grasses such as *Lolium perenne* (Hayes et al., 2007b), which was frequently used in the earlier studies.

The responses of the species to ozone when grown as a community were not as large as expected based on their responses when they were grown and exposed to ozone as individual plants in a previous study, where widespread visible injury was observed on *C. echinata* and *P. erecta* when the plants were grown individually (Hayes et al., 2006). Reductions in ozone concentration of up to 30% have been demonstrated within a grassland canopy (e.g. Jäggi et al., 2006). Together with increased boundary layer thickness due to reduced windspeed within a canopy this could combine to give lower uptake of ozone into the plants when grown as part of a community rather than as individuals, particularly for species such as *P. erecta*, which were protected by other taller vegetation.

The contrasting ozone profiles with similar AOT40 during Year 1 of this study indicate that an increase in background ozone concentration, such as that predicted from increased hemispheric ozone concentrations, is as damaging to plants as an episodic profile from regional ozone pollution. Similar increases in senescence compared to the lowest ozone treatment were found for both *F. ovina* and *P. erecta*. This is in agreement with a previous study using mixtures of *L. perenne* and *Trifolium repens,* which indicated that clover yield was similar with an episodic profile compared to a fairly constant ozone exposure with a similar AOT40 (Nussbaum et al., 1995), although in this case the total forage yield was more affected by the episodic treatment.

The largest effects on both senescence and biomass were found with a combination of increased peaks and increased background ozone concentration simulating that predicted for future decades. In some cases a synergistic effect of increased background with increased peaks of ozone was apparent, including for total biomass. Previously critical levels for ozone have been based on data obtained using episodic ozone treatments (LRTAP Convention, 2004). AOT40 (the sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb for each hour when the concentration exceeds 40 ppb, accumulated during daylight hours) has been used to establish critical levels for ozone (LRTAP Convention, 2004, 2006). However, small changes in background ozone concentration around the 40 ppb threshold would have a large influence on the cumulative AOT40, therefore this index may not be appropriate for regions where the background is already at or close to 40 ppb and is likely to increase in the future. The predicted decrease in peak height during ozone episodes over recent years (NEGTAP, 2001) coupled with the predicted rise in background ambient ozone levels over the next 50 years suggests that data from experiments such as that described here would need to be taken into account in future revisions of the critical level.

5. Conclusions

The ozone treatments used in this study simulated changes in background ozone that may occur in future decades, with or without modest peaks of ozone that were well within the current normal ambient range. The effects of these treatments on the seven species within the simulated communities were mixed and were species-dependant, making generalisations difficult. Significant effects were observed on both the grasses and the forbs and occurred in response to increased background and/or peaks of ozone. Increased senescence of *F. ovina* and *P. erecta* was observed when the background ozone concentrations were increased in the absence of any additional peaks of ozone. These effects were also seen when the background ozone concentrations were raised in addition to peaks of ozone. Overall, the species used showed no acclimation to ozone and the same relationship between senescence and ozone dose occurred in the second year as the first year of this study.

In this community, *A. odoratum* was most affected in terms of above-ground biomass, but other species such as *F. ovina* showed transient significant differences between ozone treatments. It is likely that effects on biomass were occurring more slowly due to a gradual reduction in overall plant vitality. This study highlights the need for long-term experiments to study the effects of ozone on plant communities, because some of the significant effects were not observed until after exposure to ozone in the second year.

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Figure Legends

Figure 1: Season average weekly profile of ozone data in a) 2004 and b) 2005.

Figure 2: Senescence of the component species of the community at harvest in response to A) background ozone in year 1 B) background ozone in year 2 C) peaks of ozone in year 1 D) peaks of ozone in year 2. Bars are standard errors. **, * and (*) indicate differences at $p<0.01$, $p<0.05$ and $p<0.1$.

Figure 3: Development of senescence for species in the community in the different ozone treatments. A) *A. odoratum*, year 1. B) *A. odoratum*, year 2. C) *F. ovina*, year 1. D) *F. ovina*, year 2. E) *P. erecta*, year 1. F) *P. erecta*, year 2
Bars are standard errors.

Figure 4: Senescence at harvest in year 1 and year 2 in relation to AOT0 and AOT40 for A and B) *A.odoratum* C and D) *F.ovina* and E and F) *P. erecta.*