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Species-specific effects of elevated ozone on wetland plants and decomposition processes

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Abstract

Seven species from two contrasting wetlands, an upland bog and a lowland fen in North Wales, UK, were exposed to elevated ozone (150 ppb for 5 days and 20 ppb for 2 days per week) and low ozone (20 ppb) for four weeks in solardomes. The fen species were: Molinia caerulea, Juncus subnodulosus, Potentilla erecta and Hydrocotyle vulgaris and the bog species were: Carex echinata, Potentilla erecta and Festuca rubra. Senescence significantly increased under elevated ozone in all seven species but only Molinia caerulea showed a reduction in biomass under elevated ozone. Decomposition rates of plants exposed to elevated ozone, as measured by carbon dioxide efflux from dried plant material inoculated with peat slurry, increased for Potentilla erecta with higher hydrolytic enzyme activities. In contrast, a decrease in enzyme activities and a non-significant decrease in carbon dioxide efflux occurred in the grasses, sedge and rush species.
Key words
Tropospheric ozone, wetlands, senescence, chlorophyll content, enzyme activity,

Capsule:
Short-term, episodic ozone exposure increased senescence and changed short-term decomposition processes in wetland plant species.

Introduction
Tropospheric ozone concentrations have been increasing for the past century from pre-industrial levels of approximately 10ppb to current background concentrations of 35-50ppb (Laurila et al., 2004) and are predicted to continue to rise by between 0.5 and 2% per year in the northern hemisphere (Vingarzan, 2004). The effects of ambient ozone on plants were first observed in the 1950s as an increase in the incidence of plant injury in areas affected by photochemical smog (Laurence and Andersen, 2003) and it is now known that the principle phytotoxic component of such smog is ozone. Tropospheric ozone is a major secondary air pollutant formed during a series of reactions between oxides of nitrogen and volatile organic compounds in the presence of sunlight (NEGTAP, 2001). Elevated concentrations are mainly associated with periods of hot, sunny weather, which are predicted to increase as global warming continues (Ashmore, 2005). Although regulations to control the emissions of ozone precursor chemicals are in place in most European countries, the background concentration of tropospheric ozone is continuing to increase, in part due to transboundary transport of precursor compounds through the troposphere (Fiscus et al., 2005). The current tropospheric ozone concentration of 35-50ppb found in
Northern Europe is considered to be high enough to be a significant threat to semi-natural vegetation and hence biodiversity (Ramo et al., 2006a). Ozone toxicity in plants causes visible injury to leaves, often coupled with reductions in photosynthesis and biomass accumulation (Ramo et al., 2006a). Current background levels of ozone in Europe have already been found to cause visible injury in over 80 species of crops and natural vegetation with yield/biomass reductions reported in some areas (Fuhrer et al., 1997; Hayes et al., 2007). As an ecosystem, peat-forming wetlands are of particular concern because of their ability to store large quantities of carbon with vegetated upland peat showing carbon sequestration values between 67 and 183 gCm$^{-2}$ yr$^{-1}$ depending upon the dominant vegetation type present (Bortoluzzi et al., 2006). The majority of these peat-forming wetlands in northern Europe are in upland areas where ozone concentrations are comparatively high compared to adjacent low-lying areas (Royal Society, 2008), and thus any changes that affect plant growth and physiology have the potential to affect carbon storage within peatlands.

Recent research has focused on a variety of semi-natural ecosystems but there is relatively little published information on the effects of ozone exposure on wetland plants. These species are likely to be relatively sensitive to ozone exposure as ozone sensitivity is associated with high levels of stomatal conductance, relatively high growth rates and specific leaf area; all characteristics shared by many wetland species (Power and Ashmore, 2002). Stomatal uptake of ozone is likely to be relatively high in wetland plants as they are not normally water-limited and as such may not close their stomata during the day (Busch, 2000; Koch and Rawlik, 1993; Li et al., 2004; Mann and Wetzel, 1999; Smith and Houpis, 2004) thereby taking up more ozone (Power and Ashmore, 2002). Studies on wetland plants have shown that elevated
ozone can cause specific visible ozone injury (Power and Ashmore, 2002), an increase in senescence and premature senescence (Franzaring et al., 2000) and decreases in above ground biomass (Power and Ashmore, 2002), below ground biomass and root:shoot ratio (Franzaring et al., 2000).

Research has shown that the carbon flux to soil is affected when plants are exposed to elevated ozone; both by altered rhizodeposition and changes in leaf litter quality and quantity (Andersen, 2003). Plants exposed to elevated ozone have been shown to contain a greater proportion of foliar nitrogen (Andersen et al., 2001; Berg and Staaf, 1980) and are likely to decompose more rapidly, potentially releasing more carbon compounds to the microbial community. This increased level of foliar nitrogen has been found to be particularly present when exposure to elevated ozone has caused leaves to senesce prematurely. However, plants exposed to elevated ozone have also been shown to contain higher concentrations of protective compounds such as phenolics (Liu et al., 2005; Paakkonen et al., 1998; Saleem et al., 2001) which would reduce the rate of decomposition of leaf litter (Kim et al., 1998) as phenolic compounds inhibit the activity of hydrolase enzymes (Freeman et al., 2001). Ozone exposure has also been found to reduce the below ground biomass of some species (Grantz and Farrar, 2000; Grantz and Yang, 2000) which could lead to a reduction in the amount of labile carbon available to the bacterial community. This is further supported by results from Larson et al. (2002) who found that activity of extracellular hydrolase enzymes was reduced in soils that had been exposed to elevated ozone. However, McCrady and Andersen (2000) found that ozone exposure increased root exudation in spring wheat seedlings, which would lead to an increase in substrates available to soil microbes.
Below ground microbial biomass has been found to be significantly reduced by elevated ozone in crop systems (Islam et al., 2000) and microbial respiration is also reduced by elevated ozone (Phillips et al., 2002). This is unlikely to be a direct effect of ozone, even though it is toxic to bacteria, because ozone reacts with vegetation and the soil surface meaning very little will diffuse into the soil and reach the bacterial community (Turner et al., 1974). Furthermore, recent isotopic studies using $^{18}$O have found that, following an 11 hour exposure to 100ppb ozone, there was no ozone derived $^{18}$O in root tissue of white clover (*Trifolium repens*) (Toet et al., 2009).

However, the diversity of bacterial communities found when plants had been exposed to elevated ozone was not significantly reduced (Dohrmann and Tebbe, 2005) and, in peatlands, exposure to elevated ozone was found to increase total microbial biomass by 24% (Morsky et al., 2008).

This study sets out to investigate the effects of short term (four weeks) ozone exposure on seven wetland vascular plant species commonly found in fen and bog systems in Central and Northern Europe. Percentage senescence and chlorophyll content were assessed throughout the experimental period, above and below ground biomass were measured at the end of exposure and the short-term decomposability of the above ground plant material exposed to elevated ozone was determined post-ozone exposure. The tested hypotheses are that: exposure to elevated ozone will increase senescence and decrease chlorophyll content; exposure to elevated ozone will cause a decrease in both above and below ground biomass with a relatively larger decrease being seen in the below ground biomass and exposure to ozone will reduce the rates of decomposition of leaves within wetland soil.
Methods

Plant selection and propagation

Plants were collected from two wetland sites in North Wales, UK: Cors Erddreiniog, a low-lying fen site on Anglesey (SH 465 822) just above sea level and Marchlyn Mawr, an upland bog site in Snowdonia (SH 611 624) at 550m altitude. Cors Erddreiniog is an alkaline fen and is part of the Anglesey Fens special area of conservation (SAC). The national vegetation classification (NVC) communities found at this site are M22 (Juncus subnodulosus – Cirsium dissectum fen meadow) and M25 (Molinia caerulea - Potentilla erecta mire) (www.jncc.gov.uk). Marchlyn Mawr is on the border of the Snowdonia National Park and contains typical upland bog vascular plant species although Sphagnum mosses dominate the area. The site has not had its NVC classification published, the flora dominant at the site place it as being M6 (Carex echinata - Sphagnum recurvum/auriculatum mire) (www.eryri-npa.gov.uk). Four species from Cors Erddreiniog and three from Marchlyn Mawr that were representative of the dominant vegetation at each site were used in this experiment. The fen species were Molinia caerulea, Juncus subnodulosus, Hydrocotyle vulgaris and Potentilla erecta. The species from the bog site were Carex echinata, Festuca rubra and Potentilla erecta.

Individual plants of each species were collected from the field and potted up using peat compost (HUMAX 100% peat) in a greenhouse with controlled lighting and heating (day 18°C, night 16°C) until they were large enough for propagation. One month before plants were placed in the solardomes, 24 individual plants of each
species were planted into one-litre pots (10x10x10cm). The plants were matured for
three weeks in the greenhouse and were moved to a sheltered outdoor location a week
prior to being placed in the solardomes. Plants of each species were then allocated into
three size classes with eight individuals in each size class. Within each group of eight
individuals, one plant was randomly allocated to each solardome so there was one
“small”, one “medium” and one “large” plant per replicate solardome.

Experimental Design

Plants were exposed to elevated ozone at the CEH solardome facility at
Abergwyngregyn from 22nd August 2006 to 19th September 2006. This facility
consists of eight hemispherical, glass domes 2.2 metres high and 3 metres in diameter,
situated on an East-West line to minimise differences in shading (Rafarel et al., 1995)
and receiving two complete air changes per minute. The experiment was designed to
see how plants reacted to a relatively short term, high dose ozone exposure, with peak
values matching those found in some parts of the UK during the summer of 2006
(www.welshairquality.co.uk). Four solardomes were set to receive a constant ozone
concentration of 20ppb throughout the experiment (control) and the other four were
set to an episodic regime with ozone concentrations increasing from 20ppb to 150ppb
over one day, remaining at 150ppb for three days and returning to 20ppb on the fifth
day and remaining at 20ppb for two days (elevated ozone). This profile was repeated
over the four weeks of the experiment. The solardomes were arranged as a split block
design with two blocks of four domes. Within each block, two domes with high
ozone concentrations and two with low ozone concentrations were randomly assigned.
Ozone was generated by passing oxygen (from a Workhorse 8 oxygen generator,
Ozone Industries Ltd.) through a G11 ozone generator (Ozone Industries Ltd.) where
electricity was used to dissociate oxygen molecules that recombined to form ozone. A computer-controlled (LabVIEW version 7) mass-flow controller system delivered the correct amount of ozone to the solardomes. The ozone concentration within the domes was measured on a 30 minute cycle by two API400 ozone analysers (Envirotech) with matched calibrations. Ozone concentrations in one dome were continually sampled to provide a feedback system using a Model 49C ozone analyser (Thermo Electron) and the ozone supply to all domes was adjusted accordingly.

Plant measurements

Whole plant necrotic senescence was measured when the plants were first placed in the solardomes and weekly throughout the experiment. Senescence was recorded as the percentage of senesced leaves on a plant. A leaf was counted as senesced if more than 25% of the leaf showed necrotic senescence. Senescence was chosen as a measure of ozone stress as it is a general response to photo-oxidant stress and the symptoms are not species-specific. Relative senescence was calculated as the difference between the mean senescence under elevated ozone and the mean senescence from the control.

An estimate of leaf chlorophyll content of non-senesced leaves was taken weekly using a Minolta SPAD meter. Measurements were taken on the second youngest, fully expanded leaf and only leaves with no visible senescence or ozone damage were used. *Festuca rubra* and *Juncus subnodulosus* were not included in this analysis as their leaves were too narrow and did not fill the sample window.
Above-ground material was removed from the pot and weighed immediately after harvest to determine fresh weight before being dried to constant weight at 65°C. Root weight was determined by washing the root mass through a sieve, removing attached soil and substrate particles and drying to constant weight at 65°C. From this data, the above ground to below ground biomass ratio was calculated. After measurements of the dry biomass were made, above ground plant material was mixed to provide composite dome samples for each species and ground using a ball mill.

Decomposition assay and sampling

A microbial inoculum slurry was prepared using 2kg of fresh fen peat from Cors Erddreiniog (SH 465 822) and 6 litres of deionised water and filtered to remove large particulate matter. Approximately 1g of dried, ground plant material of each species (except H. vulgaris) was accurately weighed and put in individual 125ml glass bottles with 80ml of the pre-prepared slurry. Blank samples consisted of 80ml of slurry without the addition of any plant material. Immediately after sample inoculation, bottles were sealed and gases were allowed to accumulate for 1 hour. During the accumulation of gases, bottles were kept in the dark and constantly shaken at 50rpm to encourage mixing. Background samples of laboratory air were taken at the start of the gas accumulation and samples of the gases within the bottles were taken after 1 hour. Gas samples were taken using the same method after 3, 5, 7 and 10 days of incubation. Carbon dioxide was measured using a Perkin Elmer Gas Chromatograph (GC) fitted with a flame ionisation detector (FID) to detect methane and a methaniser to convert carbon dioxide to methane. The GC was calibrated using bottled gas with a known concentration of carbon dioxide (BOC gases) and this gas was used for quality control (QC) at set points throughout each sample run.
Twenty ml water samples were taken and filtered through a 0.45μm filter after the 10-day incubation period. These were analysed for total dissolved carbon (TC), phenolics and dissolved nitrogen. TC was measured using a Thermalox™ elemental analyser. Samples were injected over a platinum-coated, mesh catalyst. Oxygen was used as the carrier gas and thermal catalytic oxidation was used to oxidise carbon compounds in the sample to carbon dioxide. Carbon dioxide was detected and measured using a non-dispersive infrared detector. Standards consisted of Potassium Hydrogen Phthalate dissolved in distilled, de-ionised water and known concentrations were used to create the calibration curve and for QC standards. The concentration of total soluble phenolics was measured using Folin-Ciocalteau reagent following the methods of Box (1983). This measures polyphenolic compounds including phenolics, tannins and lignin. Dissolved organic nitrogen was measured using the Thermalox™ machine used for TC measurements and ammonium ions were measured using a SKALAR. After 10 days of decomposition, unfiltered water samples were taken and analysed for phenol oxidase, beta glucosidase and N-acetylglucosaminidase activities. Phenol oxidase assays followed the procedure of Pind et al. (1994) except the liquid from the assay was used rather than creating a slurry from peat samples. Beta glucosidase and N-acetylglucosaminidase were assayed fluorimetrically following the method of Freeman et al. (1995).

Statistical Analysis

Values from the three plants per species per dome were averaged to provide four replicates per ozone treatment at each time point prior to analysis. The effects of ozone were assessed using general analysis of variance (GENSTAT version 7).
measured as a percentage was arc-sine transformed in Minitab ver14 prior to analysis and back-transformed for presentation. Ozone dose-response for each species was analysed in Sigma-Plot by linear regression of relative senescence (as difference from the control) against AOT0_{24hr} using treatment means from each week of ozone exposure. The significance of the regression and the percentage variation in senescence explained by ozone were analysed using GENSTAT version 8. Carbon dioxide emissions were calculated to give cumulative results over the 10 days of decomposition. General analysis of variance was used to calculate the significance of any differences at each time point and repeated measures ANOVA was used to analyse the change in gas exchange over time. Analysis of variance was used to calculate any differences in enzyme activity and water chemistry after the 10 days of incubation.

Results

Ozone Exposure in the Solardomes

Average ozone concentrations measured in the solardomes over the four week experiment are shown in Table 1. Mean peak ozone concentrations were within 10% of the target value of 150ppb and background concentrations were 20ppb for the elevated ozone treatment and 13-14ppb for the control treatment. AOT0_{24hr} values after 28 days showed a mean value of 12 ppmh in the control treatment and a mean of 76ppmh in the elevated ozone treatment while AOT40 (daylight hours) ranged from 0ppmh in the control treatment to a mean of 27ppmh in the elevated ozone treatment (Table 1). Temperatures in the solardomes followed ambient temperatures but were
generally 1-2°C higher, with a mean daytime temperature of 20°C and a range of 15-28°C and a mean overnight temperature of 14.9°C and a range of 10-20°C.

Senescence

All of the species included in this experiment showed an increase in senescence during the four weeks of ozone exposure compared to those under control conditions (Figures 1 and 2). *M. caerulea* showed a significant increase in senescence under elevated ozone in weeks two, three, and four with time also being a significant factor, suggesting the difference in mean senescence values became more pronounced over time (P<0.001). *J. subnodulosus* showed the same pattern with a significant increase in senescence under elevated ozone from week two of the experiment (P<0.05) and time through the experiment also being a highly significant factor (P<0.001). *P. erecta* plants from the fen exposed to high ozone showed a significant increase in senescence over the four week experimental period (P<0.05) although the difference in senescence measured weekly was only significant in week two. In weeks three and four the data showed a trend towards significance (P<0.1) but variation within treatments was too high for a statistically significant difference to be measured. *H. vulgaris* showed a trend towards a significant increase in senescence under elevated ozone by the fourth week of the experiment (P<0.1) but it did not senesce in the first two weeks suggesting that it was slower to respond to ozone than other species.

Senescence on *Potentilla erecta* plants from the bog showed a trend towards a significant increase with elevated ozone over the four week experimental period (P<0.1) and a highly significant effect of time, meaning that senescence increased in plants from both the treatment and the control. However, percentage senescence
values only differed significantly in week two (P<0.05) suggesting a transient increase in senescence. *C. echinata* plants showed a significant increase in senescence under elevated ozone by week four of the experiment (P<0.05) and time was again a highly significant factor in the senescence measurements (P<0.001). *F. rubra* showed the same pattern as *M. caerulea* and *J. subnodulosus* with plants exposed to elevated ozone showing significantly more senescence by week two (P<0.05) and the difference becoming progressively more significant over time (P<0.001 for ozone*time interaction).

Six of the seven species exposed showed a significant (P<0.05) increasing linear relationship with AOT024h (Table 2). The only species not to show a significant relationship was *P. erecta* from the fen. When considering the percentage variance in the relative senescence that could be explained by ozone dose for the six species that did show a significant difference, at least 70% of the variation could be explained by the increasing ozone dose (Table 2).

Chlorophyll content of non-senescing leaves

Species tested for their chlorophyll content over the course of the experiment differed in their response to ozone exposure (Figure 2). *M. caerulea* showed no significant difference between individuals exposed to high or low ozone but both sets of plants showed a significant decrease in chlorophyll content over the four week exposure period (P<0.001). *P. erecta* plants from both the fen and the bog showed a significant reduction in chlorophyll content when they had been exposed to elevated ozone by week four of the experiment (P<0.05). *C. echinata* plants showed a transient increase in week three in chlorophyll content in plants exposed to elevated ozone but this did
not continue to week four. *H. vulgaris* plants showed significantly reduced chlorophyll contents in plants exposed to elevated ozone in weeks two, three and four.

Plant biomass

In contrast to the increase in senescence, only *M. caerulea* showed a significant decrease in above-ground fresh and dry weight at the end of the exposure period (P<0.05) (Table 3). Of the other species tested, the above ground biomass of *P. erecta* from the bog exposed to high ozone was slightly lower and *C. echinata* biomass exposed to high ozone was slightly higher when compared to their respective controls (P<0.1).

Plant decomposition

Cumulative carbon dioxide emissions from the decomposition of the five species used in the assay are shown in Figure 3. *Potentilla erecta* plants from the fen that had been exposed to elevated ozone caused a significant increase in carbon dioxide emissions from peat after five days of aerobic decomposition (P<0.05) and emissions continued to be higher for the remainder of the assay (P<0.1). Carbon dioxide emissions from *P. erecta* plants from the bog showed a similar trend although the differences were not large enough to be significant. Carbon dioxide emissions from the other four species did not differ according to past ozone exposure.

Total carbon and phenolic compounds after 10 days of decomposition were very similar from plants that had and hadn’t been exposed to elevated ozone (Table 4) with only total carbon from *Festuca rubra* showing a trend towards a reduction under
elevated ozone (P<0.1). This reduction in total carbon led to the proportion of carbon as phenolic compounds being increased under elevated ozone for *F. rubra* (P<0.1). The concentration of ammonium ions after 10 days of aerobic decomposition did not change for any of the five species and the only difference in the concentrations of total nitrogen compounds was a trend towards a reduction under elevated ozone seen in the *Carex echinata* decomposition assay (P<0.1).

Of the three enzymes whose activity was measured after ten days of decomposition, beta glucosidase and N-acetylglucosaminidase showed significant differences with ozone treatment (Table 5). Phenol oxidase activity did not show any variation under elevated ozone, but within treatment variation was high and enzyme activity was very low (data not presented). Beta glucosidase activity showed a significant reduction under elevated ozone in *Molinia caerulea* and *Juncus subnodulosus* (P<0.05 and P<0.01 respectively) and a non-significant reduction in *C. echinata* and *F. rubra*. However, beta glucosidase activities increased under elevated ozone for the decomposition assays using *P. erecta* from the fen and the bog (P<0.1 and P<0.05 respectively). A similar pattern was seen with N-acetylglucosaminidase; activities increased in the assays for plants exposed to elevated ozone for *P. erecta* from the fen and the bog (P<0.1 and P<0.01) but decreased significantly for plants exposed to elevated ozone for *J. subnodulosus* (P<0.001), *C. echinata* (P<0.05), and *F. rubra* (P<0.05). Enzyme activities in the slurry containing *M. caerulea* exposed to elevated ozone showed a non-significant decrease (Table 5).

Discussion
Plant senescence is defined as “the deteriorative processes that are the natural causes of death” (Leopold, 1980) and is characterised by a decrease in leaf chlorophyll content and photosynthetic activity (Wingler et al., 2006). Accelerated foliar senescence is a common response for many plant species treated with elevated ozone (e.g. (Bergmann et al., 1999; Gielen et al., 2007; Mikkelsen and Heide-Jorgensen, 1996; Paakkonen et al., 1996; Pell et al., 1997) and is often coupled with biochemical changes within the plant such as increases in ethylene emission, a cause of senescence (Schraudner et al., 1997). The link between ozone exposure and premature senescence has been found to be more marked in Northern latitudes because summer nights are shorter meaning there is less time for plants to recover from ozone injury through the repair processes that are driven by dark respiration (De Temmerman et al., 2002). Northern latitudes are also characterised by cooler and more humid conditions, both of which tend to lead to higher levels of stomatal conductance and hence higher ozone uptake (Yamaji et al., 2003), meaning that wetland plants in Northern latitudes are likely to be particularly affected by elevated ozone as they are characterised by high levels of stomatal conductance and leaf area (Power and Ashmore, 2002). This is shown in this experiment as all seven species showed an increase in senescence over the 28 days of exposure. For *H. vulgaris* and *P. erecta* plants from the bog, weekly differences in senescence were not significant over the experimental period but the correlation with AOT0_{24hr} was significant, showing that as ozone dose increased the amount of senescence also increased. This is in agreement with other experiments on the effects of ozone exposure on wetland plants with five out of ten wet meadow species tested showing increased senescence (Franzaring et al., 2000) and five wetland species also showing increased injury under elevated ozone (Power and Ashmore, 2002). The effects of elevated ozone on plant
senescence had been previously assessed for three of the species used in this experiment: *P. erecta*, *F. rubra* and *C. echinata* (Hayes et al., 2006). In that study, *F. rubra* and *C. echinata* had significant increases in senescence after ten weeks of ozone exposure but *P. erecta* did not show as high an increase in senescence as found in this experiment (Hayes et al., 2006). This is possibly because Hayes et al. (2006) used an episodic regime with a maximum concentration of 100ppb ozone over four days per week whereas in this experiment the ozone concentration was around 140ppb in the treatment domes for five days out of seven. As a further comparison, the elevated ozone treatment of Hayes et al (2006) had an AOT 40 (daylight hours) of 18.3 ppmh accumulated over ten weeks, whereas in this experiment the AOT 40 (daylight hours) was 24.8 ppmh accumulated over only four weeks.

The increase in senescence caused by elevated ozone has also been shown to be accompanied by an increase in the nitrogen content of abscised leaves (Findlay and Jones, 1990) which could have an effect on the subsequent decomposition of plant biomass as more fertilisation has been found to speed up the decomposition of plant litter (Allison and Vitousek, 2004). In the current study, carbon dioxide efflux was increased from plant material exposed to elevated ozone during the *P. erecta* decomposition with a corresponding increase in hydrolase activity suggesting that initial rates of decomposition had increased. However, there was no change in the nitrogen content of the slurry. Extra-cellular enzyme activity was found to decrease in the slurries containing *M. caerulea*, *J. subnodulosus*, *Carex echinata* and *F. rubra* that had been exposed to elevated ozone suggesting a reduction in the decomposition of the plant material. This is in agreement with previous work carried out on blackberry and broomsedge (Kim et al., 1998) that found exposure to elevated ozone
reduced litter decomposition. Exposure to elevated ozone also caused a reduction in decomposition of soybean residues (Booker et al., 2005). However, although hydrolytic enzyme activities were changed by exposure of the plants to elevated ozone, phenol oxidase activity was unaffected, there were no significant changes in phenolic concentrations after ten days of decomposition and only *F. rubra* showed a decrease in TC concentrations after elevated ozone. This suggests that, in contrast to previous results (Booker et al., 2005; Iglesias et al., 2006; Saleem et al., 2001), these plants did not upregulate their production of anti-oxidant compounds such as ascorbate and phenolics.

In this experiment, the chlorophyll content of healthy leaves of *P. erecta* from the fen and the bog and *H. vulgaris* was reduced under elevated ozone. In contrast, exposure to elevated ozone increased the chlorophyll content of healthy leaves in *C. echinata*. A reduction in chlorophyll content in leaves exposed to elevated ozone was also found for birch (*Betula pendula*) (Paakkonen et al., 1996) and strawberry (*Fragaria vesca*) (Ramo et al., 2007). However, there was no change in leaf chlorophyll content in *Centaurea jacea* after exposure to elevated ozone (Ramo et al., 2006b). This suggests that reduction in chlorophyll content of healthy leaves is not always a symptom of ozone damage. Chlorosis, or the bleaching of chlorophyll during cell damage, has been seen under elevated ozone as a precursor to elevated senescence (Heath, 2008); over a longer experimental period it is possible that the percentage of senesced leaves would have increased further.

Only *M. caerulea* plants exposed to elevated ozone showed a reduction in fresh and dry above-ground biomass compared to plants that received a constant 20ppb ozone.
This is in contrast to previous published results (Franzaring et al., 2000) that found the biomass of *M. caerulea* increased under elevated ozone. The difference in findings could be due to the type of ozone regime experienced by the plants; growth could be stimulated by moderate ozone exposure but reduced by higher ozone concentrations. *P. erecta* showed a trend towards a reduction in dry above-ground biomass under elevated ozone, which is in contrast to the results of Hayes et al (2006) where *P. erecta* showed a non-significant increase in biomass under elevated ozone. The results of this experiment show that, as in previous studies (Davison and Barnes, 1998), increases in senescence are not necessarily associated with reduction in plant growth, making it difficult when considering the wider ecological significance of elevated ozone. This result has also been found for some herbs and grasses; enhanced visible injury and senescence under elevated ozone did not necessarily lead to a reduction in biomass (Pleijel and Danielsson, 1997). The overall lack of change to above and below-ground biomass is unexpected as ozone exposure has been found to inhibit growth in a variety of species e.g. (Grantz, 2003; Grantz and Yang, 2000; Hayes et al., 2006; Peltonen et al., 2005). Inhibition of plant growth by ozone exposure may have been absent in this experiment because of the short-term nature of the experiment and it could be that it takes longer for changes in biomass to appear. In some experiments, it has been found that the biomass allocation to plant roots is reduced under elevated ozone (Andersen, 2003; Grantz and Yang, 2000). This may be because allocation to the roots is dependent on the source strength (Andersen, 2003) and plant repair after ozone exposure requires the diversion of fixed carbohydrate from other plant sinks (Dizengremel, 2001). This was not seen in this experiment; again possibly because of the short time scale of the exposure period.
Conclusions

From this experiment it can be seen that wetland plant species are affected by ozone, with senescence being increased under elevated ozone in all species studied. However, plant biomass was only negatively affected in one species (*M. caerulea*), suggesting that over short-term exposures, increases in senescence do not lead to decreases in plant growth. Chlorophyll content was affected in some species, with *P. erecta* plants from the fen and bog and *H. vulgaris* showing a decrease in chlorophyll content and *C. echinata* showing a transient increase. This could have a negative effect on carbon dioxide fixation during photosynthesis if the chlorophyll content of healthy leaves is reduced prior to visible senescence. The results of the plant decomposition suggest that the effects of elevated ozone on forb decomposition differ from the effects of elevated ozone on grass and sedge decomposition. *P. erecta* plants showed higher carbon dioxide efflux and higher rates of hydrolase activity whereas the other species tested showed a non-significant decrease in carbon dioxide efflux and a decrease in hydrolytic enzyme activity. If exposure to elevated ozone does change the decomposition rates and enzyme activities in wetland areas it could change the potential for wetlands to act as carbon sinks. If plant decomposition increases more carbon could be released at the end of the growing season; whereas if plant decomposition is reduced more carbon fixed during plant growth could be stored. However, for overall carbon storage to increase, it would be necessary for plant biomass to be unaffected by exposure to elevated ozone over a longer period. This seems unlikely and further studies are needed to fully comprehend the implications of rising ozone concentrations for wetland carbon cycling and storage.


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<th>week 2</th>
<th>week 3</th>
<th>week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>high ozone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ppb) peak</td>
<td>147±1.51</td>
<td>139±1.90</td>
<td>146±1.93</td>
<td>146±1.14</td>
</tr>
<tr>
<td>background</td>
<td>20±0.17</td>
<td>19±0.89</td>
<td>20±0.80</td>
<td>19±0.97</td>
</tr>
<tr>
<td>low ozone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ppb) peak</td>
<td>19±0.37</td>
<td>20±0.43</td>
<td>20±0.29</td>
<td>20±0.47</td>
</tr>
<tr>
<td>background</td>
<td>13±0.74</td>
<td>14±0.44</td>
<td>13±2.7</td>
<td>13±0.65</td>
</tr>
<tr>
<td>high ozone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ppm.h) AOT0</td>
<td>20.1±0.2</td>
<td>38.9±0.2</td>
<td>55.8±0.4</td>
<td>75.7±0.5</td>
</tr>
<tr>
<td>(ppm.h) AOT40</td>
<td>7.1±0.1</td>
<td>13.9±0.2</td>
<td>19.8±0.3</td>
<td>27.0±0.3</td>
</tr>
<tr>
<td>low ozone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ppm.h) AOT0</td>
<td>3.1±0.1</td>
<td>6.2±0.1</td>
<td>9.1±0.3</td>
<td>12.3±0.4</td>
</tr>
<tr>
<td>(ppm.h) AOT40</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 1**: Peak and background ozone concentrations, cumulative AOT0\textsubscript{24hr} and
AOT40 (daylight hours) for the two ozone treatments over the 28 day ozone exposure
period. Values are the means of the four domes in each treatment and are shown ± 1
standard error.
<table>
<thead>
<tr>
<th>Plant</th>
<th>$R^2$ value</th>
<th>P value</th>
<th>% variance accounted for</th>
<th>P values for repeated measures ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. caerulea</td>
<td>0.967</td>
<td><strong>0.003</strong></td>
<td>95.5</td>
<td><strong>0.004</strong></td>
</tr>
<tr>
<td>J. subnodulosus</td>
<td>0.959</td>
<td><strong>0.004</strong></td>
<td>94.6</td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td>P. erecta (fen)</td>
<td>0.442</td>
<td>0.221</td>
<td>25.5</td>
<td></td>
</tr>
<tr>
<td>H. vulgaris</td>
<td>0.835</td>
<td><strong>0.030</strong></td>
<td>78.0</td>
<td>0.162</td>
</tr>
<tr>
<td>C. echinata</td>
<td>0.895</td>
<td><strong>0.015</strong></td>
<td>86.0</td>
<td>0.589</td>
</tr>
<tr>
<td>F. rubra</td>
<td>0.964</td>
<td><strong>0.003</strong></td>
<td>95.0</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>P. erecta (bog)</td>
<td>0.796</td>
<td><strong>0.042</strong></td>
<td>72.7</td>
<td><strong>0.076</strong></td>
</tr>
</tbody>
</table>

**Table 2:** Regression analysis of relative senescence against AOT0$_{24hr}$ for each species together with repeated measures ANOVA for weekly senescence measurements. Significant P values at P<0.05 are in bold and values 0.05<P<0.1 are in italics.
<table>
<thead>
<tr>
<th>Species</th>
<th>Above ground fresh weight</th>
<th>Above ground dry weight</th>
<th>Root dry weight</th>
<th>Root:shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High ozone</td>
<td>Low ozone</td>
<td>Sig?</td>
<td>High ozone</td>
</tr>
<tr>
<td><strong>Fen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. caerulea</em></td>
<td>1.91±0.3</td>
<td>2.53±0.3</td>
<td>*</td>
<td>0.85±0.1</td>
</tr>
<tr>
<td><em>J. subnodulosus</em></td>
<td>1.91±0.2</td>
<td>1.97±0.2</td>
<td>NS</td>
<td>0.52±0.1</td>
</tr>
<tr>
<td><em>P. erecta</em></td>
<td>0.95±0.1</td>
<td>0.96±0.1</td>
<td>NS</td>
<td>0.36±0.1</td>
</tr>
<tr>
<td><em>H. vulgaris</em></td>
<td>0.88±0.1</td>
<td>1.18±0.2</td>
<td>NS</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Bog</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. echinata</em></td>
<td>0.97±0.1</td>
<td>0.75±0.1</td>
<td>NS</td>
<td>0.32</td>
</tr>
<tr>
<td><em>F. rubra</em></td>
<td>0.87±0.1</td>
<td>0.87±0.1</td>
<td>NS</td>
<td>0.26</td>
</tr>
<tr>
<td><em>P. erecta</em></td>
<td>0.72±0.1</td>
<td>0.95±0.1</td>
<td>NS</td>
<td>0.24</td>
</tr>
</tbody>
</table>

*Table 3:* The mean biomass for the 7 species exposed to elevated ozone. Figures are shown as the mean for each treatment ± 1 standard deviation. Where standard deviations are not shown they were less 0.05g. *P<0.05  (*) P<0.1  NS non-significant
Table 4: Total carbon and total nitrogen in the slurries after 10 days of aerobic decomposition. Values are concentrations in mg/l and are shown as the treatment mean ± 1 standard error.

<table>
<thead>
<tr>
<th></th>
<th>Total dissolved carbon</th>
<th>Total dissolved nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High ozone</td>
<td>Low ozone</td>
</tr>
<tr>
<td>M. caerulea</td>
<td>266.4±3.0</td>
<td>264.0±18.4</td>
</tr>
<tr>
<td>J. subnodulosus</td>
<td>364.8±16.4</td>
<td>414.3±45.0</td>
</tr>
<tr>
<td>P. erecta (fen)</td>
<td>861.3±38.6</td>
<td>831.0±39.2</td>
</tr>
<tr>
<td>P. erecta (bog)</td>
<td>892.0±59.0</td>
<td>802.8±38.4</td>
</tr>
<tr>
<td>C. echinata</td>
<td>283.6±10.3</td>
<td>286.3±27.6</td>
</tr>
<tr>
<td>F. rubra</td>
<td>339.7±14.6</td>
<td>522.7±80.9</td>
</tr>
</tbody>
</table>

Table 5: Beta glucosidase and N-acetylglucosaminidase activities after 10 days of aerobic decomposition. Values are the enzyme activity per gram of plant weight and are shown as the mean ± 1 standard error.

<table>
<thead>
<tr>
<th></th>
<th>Beta Glucosidase activity</th>
<th>N-acetylglucosaminidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High ozone</td>
<td>Low ozone</td>
</tr>
<tr>
<td>M. caerulea</td>
<td>1.14±0.62</td>
<td>2.41±0.22</td>
</tr>
<tr>
<td>J. subnodulosus</td>
<td>8.17±2.10</td>
<td>14.55±0.70</td>
</tr>
<tr>
<td>P. erecta (fen)</td>
<td>10.24±1.48</td>
<td>7.71±0.78</td>
</tr>
<tr>
<td>P. erecta (bog)</td>
<td>13.93±8.50</td>
<td>0.60±0.16</td>
</tr>
<tr>
<td>C. echinata</td>
<td>6.13±4.50</td>
<td>10.29±1.88</td>
</tr>
<tr>
<td>F. rubra</td>
<td>0</td>
<td>10.52±5.62</td>
</tr>
</tbody>
</table>

Figure 1: Weekly percentage senescence measured on the four fen species. Statistical tests were performed on arc-sine transformed data and data was back-transformed for presentation. * P<0.05, ** P<0.01, *** P<0.001 for differences between ozone treatments at each time point.

Figure 2: Weekly percentage senescence measured on the three bog species. Statistical tests were performed on arc-sine transformed data and data was back-
transformed for presentation. * P<0.05, ** P<0.01, *** P<0.001 for differences between ozone treatments at each time point.

**Figure 3:** Changes in chlorophyll content for five species over the 4 week exposure period. * P<0.05, ** P<0.01, *** P<0.001 for differences between ozone treatments at each time point.

**Figure 4:** Cumulative carbon dioxide efflux during the 10 day decomposition assay. (*) P<0.1, * P<0.05 for differences between ozone treatments at each time point.
C. echinata

F. rubra

P. erecta (bog)
Molinia caerulea

Juncus subnodulosus

Potentilla erecta (fen)

Potentilla erecta (bog)

Carex echinata

Festuca rubra