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How do increasing background concentrations of tropospheric ozone affect peatland plant growth and carbon gas exchange?

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Abstract

In this study we have demonstrated that plants originating from upland peat bogs are sensitive to increasing background concentrations of ozone. Peatland mesocosms from an upland peat bog in North Wales, UK were exposed to eight levels of elevated background ozone in solardomes for 4 months from May to August, with 24 hour mean ozone concentrations ranging from 16 to 94 ppb and cumulative $AOT0_{24hr}$ ranging from 45.98 ppmh to 259.63 ppmh. Our results show that plant senescence increased with increasing exposure to ozone, although there was no significant effect of increasing ozone on plant biomass. Assessments of carbon dioxide and methane fluxes from the mesocosms suggests that there was no change in carbon dioxide fluxes over the 4 month exposure period but that methane fluxes increased as cumulative ozone exposure increased to a maximum AOT 0_{24hr} of approximately 120 ppm h and then decreased as cumulative ozone exposure increased further.

Key words: tropospheric ozone; methane; peatlands; wetlands; senescence

Highlights

- Peatland plant senescence is increased by season-long exposure to elevated ozone but above and below ground plant biomass is not significantly affected.
- Methane emissions increase at low to moderate cumulative ozone exposure but decrease as cumulative ozone exposure increases further.
- Dissolved organic carbon in the peat pore water and carbon dioxide exchange are not significantly affected by increasing background ozone concentrations.

Introduction

Peat-forming wetlands are an important carbon storage ecosystem with global estimates of carbon sequestration in the region of 20-30 $gCm^{-2}yr^{-1}$ (Wieder, 2001). Carbon dioxide is taken up by vascular plants and mosses during photosynthesis and, although some is released back to the environment during plant respiration, the remainder is stored in plant tissue or transported through the plant and released as exudates of dissolved organic material (Schutz et al., 1991). Once the plants die, the carbon in their tissues is not broken down and released to the atmosphere as peatland decomposition rates are low so the plant material is laid down as peat. The low molecular weight exudates such as sugars and amino acids form an energy source for bacteria and archaea living in the peatland and their metabolism contributes to the carbon dioxide release and to the release of methane. The dissolved carbon in the pore water can be exported out of the peatland in streams and this can be an important loss point for carbon in the peatland carbon cycle. The majority of peat-forming wetlands in northern Europe are in upland areas where ozone concentrations are higher than adjacent low-lying areas (Royal Society, 2008), and thus any changes that affect plant growth and carbon gas exchange have the potential to affect carbon storage within peatlands.

Annual mean tropospheric ozone concentrations in Northern Europe are currently in the region of 30-35 ppb, having increased from less than 20 ppb in the mid-20th century. Although there has been little change in mean concentrations in the past decade (Hartmann *et al.,* 2014), climate change and hemispheric transport of pollutants may affect future ozone levels. One future scenario predicts that background ozone concentrations in Northern Europe will continue to increase during the 21st century due to hemispheric transport of ozone precursor molecules (Royal Society 2008).

Tropospheric ozone is a phytotoxic pollutant and wetland vascular plants have been found to be relatively sensitive to elevated ozone concentrations (Franzaring *et al.*, 2000, Power & Ashmore, 2002, Williamson *et al.,* 2010) with symptoms including premature senescence, reductions in photosynthesis and reduced biomass. However, *Sphagnum* mosses have been shown to be relatively tolerant to elevated ozone concentrations (Rinnan 2003) during both short term acute ozone fumigation (Potter et al., 1996a) and during longer term exposure with only *Sphagnum recurvum* showing a reduction in shoot growth under elevated ozone (Potter et al., 1996b).

Carbon dioxide uptake in wetland mesocosms takes place during photosynthesis and, if plant growth is reduced by increasing background ozone, it may be expected that carbon dioxide uptake would be reduced. Increasing tropospheric ozone concentrations during short-term exposure was found to transiently increase the rate of dark respiration (Niemi et al., 2002, Rinnan et al., 2003), possibly as a result of the plants repairing ozone damaged tissues. Under a doubling in ambient ozone concentrations Haapala et al., (2011) showed that photosynthesis was reduced during the first year of a four year exposure period but during the fourth year both photosynthesis and total respiration showed a tendency to be higher than under ambient conditions.

As wetland plants play a major role in methane emission from wetlands it is possible that any damage to wetland plant functioning by ozone could have a secondary effect on these. Two possible routes via which methane emissions are affected by plant growth are: through the provision of a conduit for gas exchange via the aerenchyma (Chanton et al., 1997; Ding et al., 2005; Greenup et al., 2000; Thomas et al., 1996) and the exudation of low molecular weight compounds to provide an energy source for microbes (Schutz et al., 1991). A range of wetland adapted species demonstrate active pressurised gas flow from the leaves to the roots to allowed continued oxygen supply in waterlogged soils, whereby air is forced downwards through the aerenchyma as a result of the pressure generated through the gradient in temperature and water vapour pressure. The return flow of gas is through the older leaves of the plants as they are unable to support the pressure gradients required to force air down into the roots (Mitsch and Gosselink 2000). Ozone-induced increased senescence could increase the available pathways for gas release and hence the flow of methane between the substrate and the atmosphere. Previous studies have shown that recently fixed photosynthate is preferentially retained in leaves rather than transported through the plant (Andersen, 2003; Grantz and Farrar, 1999, 2000), which could lead to a reduction in root exudates meaning that there would be less energy available for methanogens.

Previous published research on the effect of elevated ozone on methane emissions from peatlands ranged from showing an increase when mesocosms were exposed to 100ppb ozone (Niemi *et al.*, 2002), a non-significant increase after 50 days exposure to 200ppb ozone (Rinnan *et al.*, 2003), a transient decrease at ozone concentrations double the current ambient (Morsky *et al.*, 2008) to a significant decrease in methane emissions during the growing seasons of a two year exposure period (Toet *et al.*,

2011). A previous study investigating the impacts of elevated background ozone using open top chambers showed that seasonal exposure of meadow mesocosms to elevated ozone over a three year period did not change methane fluxes (Kanerva *et al.*, 2007).

Here, we have studied the effects of increasing background ozone concentrations on plant senescence, plant growth and carbon gas exchange in peatlands, an ecosystem recognised as having the potential to exert profound changes to the planet's climate through the storage of carbon and the emission of methane (Bridgham *et al.,* 2013, Freeman *et al.*, 2001). By using a wide range of ozone treatments, we hoped to increase our understanding of tropospheric ozone effects on plant growth and carbon gas exchange from peatlands and shed some light on the conflicting results found in other studies.

Materials and Methods

Ozone exposure:

Forty-eight mesocosms (diameter 16cm, depth 40cm) were collected from the Migneint, a large area of oligotrophic, blanket bog in North Wales, UK (3°48.8′ W, 52°59.6′ N) dominated by the NVC vegetation type M6 (*Carex echinata-Sphagnum recurvum/auriculatum* mire) (Buckton & Ormerod, 1997), following the method of Freeman *et al.,* (1993) and exposed to ozone in specially constructed greenhouses (solardomes), with 6 replicate mesocosms per ozone treatment. Mesocosms were selected to ensure as uniform as possible vegetation cover, with the vascular plants being *Juncus effusus* and *Carex echinata*. *Sphagum* mosses were not identified to species level but the majority of the *Sphagnum* present in the area sampled was *Sphagnum fallax*. The water table was maintained within 2cm of the surface of the

mesocosms throughout the exposure period.

The solardome facility consists of eight hemispherical glass domes, 3 m in diameter and 2 m tall, situated on an East-West line to minimise differences in shading as used in previous experiments including: Hayes et al (2015), Hayes et al (2011), and Mills et al (2009). Ozone was generated by passing oxygen (from a Workhorse 8 oxygen generator, Ozone Industries Ltd.) through a G11 ozone generator (Ozone Industries Ltd.). A computer-controlled (Lab-VIEW version 7) mass-flow controller system was used to deliver ozone to the solardomes, where it was mixed with charcoal filtered air and the fan system ensured two complete air changes per minute. The ozone concentration in the centre of each of the solardomes was measured on a 30 minute cycle by two API400 ozone analysers (Envirotech) with matched calibrations. Ozone concentrations in one solardome 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.

The ozone profile used in the solardomes was based on concentrations measured at the Snowdonia ozone monitoring site at Marchlyn Mawr, Wales, UK(4°03.4′ W, 53°08.2′ N) during a typical week with no marked ozone episodes but relatively high background ozone: 31st May – 6th June 2006 (AA treatment) and with incremental starting points. The target treatments consisted of a sub-ambient treatment (AA-20 ppb), a simulated ambient treatment (AA) and six treatments with increasing background ozone (AA+12 ppb, AA+24 ppb, AA+36 ppb, AA+48 ppb, AA+60 ppb and AA+72 ppb). These were applied as a continuous, repeated, weekly regime designed to simulate increased background ozone concentrations. The exposure period within the solardomes was from 9th May 2008 -2nd September 2008.

153 Gas and water sampling and analysis:

154 Gas exchange samples were taken fortnightly by placing a two litre transparent,
155 plastic chamber over the mesocosms and attaching with a rubber seal between the
156 headspace and the outer casing of the mesocosm to ensure that the soil structure was
157 not disturbed by the attachment of the chamber. A 30ml sample of the background
158 gas was taken at the moment of capping and a second sample of the gas within the
159 chamber was taken after one hour. The accumulation of methane and carbon dioxide
160 within the chamber was found to be linear over this time period when measured in a
161 preliminary experiment prior to the mesocosms being placed in the solardomes. Gas
162 samples were stored under positive pressure in airtight glass vials (Perkin Elmer) that
163 were evacuated prior to use and analysed within 24 hours of sample collection. Gas
164 samples were analysed for the concentration of methane and carbon dioxide using a
165 Perkin Elmer Gas Chromatograph (GC) fitted with a flame ionisation detector (FID)
166 to detect methane and a methaniser to convert carbon dioxide to methane. Gas
167 samples were pressurised with a known amount of nitrogen in the headspace
168 autosampler (Turbo-Matrix) and samples were injected into the GC at 23.2psi with
169 nitrogen carrier gas. Samples were passed through a Poropak QS ceramic column,
170 hydrogen flow was set at 45ml min^{-1} and airflow was set to 450 ml min^{-1}. The FID
171 (flame ionisation detector) temperature was 375^{o}C.

172 Pore water samples were taken at three weekly intervals from the wetland mesocosms.
173 Samples were filtered through a 0.45µm cellulose acetate filter immediately following
174 collection. Total dissolved carbon was measured using a Thermalox^{TM} elemental
175 analyser. Samples were injected over a platinum-coated, mesh catalyst. Oxygen was
176 used as the carrier gas and thermal catalytic oxidation was used to oxidise carbon
177 compounds in the sample to carbon dioxide, which was detected and measured using

a non-dispersive infra-red detector.

Plant Growth:

During the growing season visible senescence on vascular plants growing in the mesocosms was assessed at two week intervals. Vascular plant leaves were counted as senesced if more than 25% of an individual leaf had died back and the percentage of the entire plant that was senesced was calculated.

Above and below ground vascular plant and moss biomass present in the mesocosms was measured following 16 weeks of ozone exposure. Plant biomass was harvested and dried to constant mass at 65^{o}C.

Statistical analysis:

Relationships between ozone exposure, plant senescence, plant biomass and methane emissions were analysed using regression analysis in R v 2.14.2. Ozone exposure is reported as accumulated hourly mean ozone concentration over 24 hours without a threshold ozone concentration ($AOT0_{24hr}$). This measure incorporated the effects of elevated ozone throughout the night, rather than daylight hours as is more usually used. It also included the potential effects of ozone in treatments that were below 40 ppb, which would be omitted if the more commonly used parameter AOT40 had been calculated. Use of $AOT0_{24hr}$ also allowed the cumulative effect of ozone to be assessed, irrespective of the time scale of ozone exposure. Gas exchange and senescence measurements are plotted grouped by cumulative ozone exposure; dome mean values for senescence, carbon dioxide and methane fluxes measured through the 4 month ozone exposure were ordered by cumulative ozone dose and averaged by each 20 ppm h increase in ozone exposure.

Results

Seasonal mean ozone concentrations ranged from 16 ppb in the lowest ozone treatment to 94 ppb in the highest treatment, while $AOT0_{24hr}$ and daylight AOT40 ranged from 45 to 260 ppm h and 0 to 73 ppm h respectively (Table 1).

Vascular plant species emerging or germinating in the mesocosms consisted of *Juncus effusus, Carex echinata* and small quantities of *Poa triviata*. Although each mesocosm did not have the same number of plants per species, there was no significant difference in the species present across the eight ozone treatments. Using combined data from all ozone treatments and assessments, vascular plant senescence on the species growing in the mesocosms showed a positive relationship ($P<0.05$) with increasing $AOT0_{24hr}$ (Figure 1a), indicating senescence increased to a greater extent in the mesocosms exposed to higher doses of background ozone. Elevated background ozone over the 16 week period caused an increase in the percentage of senesced vascular plant material from 5% in the lowest exposure to 25% in the highest accumulated ozone exposure. There were no significant differences in senescence seen in the individual vascular plant species present in the mesocosms so senescence data was pooled across all vascular plant species for analysis and presentation. After 16 weeks of ozone exposure there was a significant relationship between ozone exposure and senescence, with the mesocosms exposed to higher ozone showing higher vascular plant senescence (Figure 1b). In contrast, there was no significant effect of ozone on vascular plant cover, above or below ground vascular plant biomass (for all biomass combined and for individual species present in each mesocosm) or *Sphagnum* spp. moss biomass (Table 2).

Methane fluxes showed an inverse polynomial relationship with accumulated ozone exposure. At low to moderate $AOT0_{24hr}$ values (ranging from 0 – 120 ppm h) methane fluxes increased as accumulated ozone exposure increased, whereas from $AOT0_{24\ hr}$ values of 120 ppm h to 220 ppm h methane fluxes decreased as $AOT0_{24hr}$ values increased (Figure 2). When methane fluxes after 16 weeks of ozone exposure were correlated with vascular plant senescence, vascular plant biomass and moss biomass there was no correlation between methane fluxes and above or below vascular plant biomass, vascular plant cover or moss biomass (Table 3). However, there was a trend towards a significant correlation ($P = 0.08$) between vascular plant senescence and methane fluxes (Table 3). Carbon dioxide fluxes did not show a statistically significant change with increasing exposure to ozone and showed high levels of variability within mesocosms exposed to similar levels of ozone, though mesocosms showed a net uptake of carbon dioxide throughout the exposure period (data not presented).

Dissolved organic carbon within the pore waters of the wetland mesocosms showed no relationship with increasing exposure to elevated background ozone, remaining unchanged over time and as ozone exposure increased (data not presented).

Discussion

This experiment has shown that elevating the background ozone throughout the growing season increases vascular plant senescence and changes methane fluxes from wetlands, although plant biomass, carbon dioxide fluxes and dissolved organic carbon concentrations were unchanged.

The increase in vascular plant senescence caused by exposure to elevated ozone agrees with published results showing that wetland plants were sensitive to mean daily peak concentrations of ozone of 77 ppb, 80 ppb and 150 ppb respectively (Franzaring *et al.*, 2000, Power & Ashmore, 2002, Williamson *et al.,* 2010). In this study the linear relationship between AOT $0_{24\ hr}$ indicates that vascular plants exposed to lower concentrations of ozone for a longer time period showed similar levels of senescence to those exposed to higher ozone concentrations for shorter time periods at any given value of AOT $0_{24\ hr}$. This suggests that a growing season with moderately high background tropospheric ozone could be as detrimental to plant health as one where there are a small number of high peaks in tropospheric ozone.

Above and below ground vascular plant biomass was unaffected by increasing exposure to ozone, a finding that agrees with published results from Toet et al (2011) who found that vascular plant biomass in wetland mesocosms was unaffected by two years exposure to elevated ozone. However, this is in contrast to studies on other semi-natural vegetation types where increasing ozone exposure reduced vascular plant biomass with examples from grasslands (Barbo et al., 1998; Hayes et al., 2006; Ramo et al., 2006; Ramo et al., 2007) and trees (Paakkonen et al., 1996; Saleem et al., 2001). Similarly to the vascular plants in this study *Sphagnum* moss biomass was unaffected by elevated ozone exposure, which agrees with previous studies showing that the majority of *Sphagnum* species are relatively tolerant to ozone (Morsky et al., 2011, Toet et al., 2011). In addition, as carbon dioxide fluxes were unchanged by increasing ozone exposure, this suggests that ozone exposure does not have a significant impact on wetland plant photosynthesis and respiration.

Methane emissions increased with increasing $AOT0_{24hr}$ to a maximum at approximately 120 ppm h and then decreased as $AOT0_{24hr}$ continued to increase. It is

277 possible that effects of ozone on methane fluxes seen in published papers, ranging
278 from large increases through to decreases in methane emissions, are due to differences
279 in cumulative ozone exposure between experiments taking into account the ambient
280 ozone concentrations at the experimental sites used. The results of Lloyd (2004)
281 showed large increases in methane emission but the cumulative ozone exposure of the
282 highest treatment was an $AOT0_{24hr}$ of 120 ppm h which coincides with the peak in
283 methane emissions shown in this experiment. The positive effects of ozone on
284 methane emissions shown by Niemi *et al.,* (2002) occurred at an estimated cumulative
285 ozone exposure of up to 48.8 ppm h in their highest ozone treatment, which, when
286 compared with an $AOT0_{24hr}$ of 9.7 ppm h in their control exposure would be in the
287 range of the results in this experiment that show an increase in methane. The results
288 of Morsky *et al.,* (2008) and Toet *et al.,* (2011), showing a decrease in methane
289 emissions after exposing peatland mesocosms to increases in ozone above current
290 ambient concentrations of tropospheric ozone, potentially coincided with the
291 decreasing methane phase of the relationship found in our experiment. Using ozone
292 concentrations measured at the Snowdonia ozone monitoring site at Marchlyn Mawr
293 over a four month summer growing season the average ozone exposure ($AOT0_{24hr}$)
294 between 2006 and 2010 was 101 ppm h, a value that is close to the ozone exposure
295 corresponding to the highest methane emissions seen in our study. This could be one
296 possible explanation for the decrease in methane emissions measured here and in
297 other studies under ozone concentrations above ambient levels, although it should be
298 remembered that the different studies took place in very different locations with
299 different ambient ozone characteristics.
300 The increase in methane fluxes from peatlands seen following exposure to low to
301 moderate accumulated levels of ozone in this study could either be due to an increase

in methanogenic activity, a decrease in methanotropic activity or an increase in the release of methane through the aerenchyma of the vascular plants. Toet *et al.,* (2009) showed that ozone does not diffuse more than a few millimetres into the substrate, particularly in waterlogged soils, suggesting that it is unlikely that the change in methane fluxes seen is a result of the direct impact of ozone on methanogenic or methanotrophic bacteria. This is corroborated by results from Morsky *et al.,* (2008) and Rinnan *et al.,* (2003) showing that exposure to elevated ozone had no impact on potential methane production or consumption in peat taken from mesocosms. Wetland vascular plants show two main types of gas exchange between the atmosphere and their roots: passive diffusion and active pressurised flow (Brix *et al.*, 1992, Whiting & Chanton, 1993) and the major flow mechanism differs between species (Roura-Carol & Freeman, 1999, Thomas *et al.*, 1996, Van der Nat *et al.*, 1998). Passive molecular diffusion occurs as a result of a concentration gradient between the methane within the substrate and the atmosphere. If this were the dominant gas exchange mechanism then an increase in methane production would have to occur for methane emissions to increase. DOC concentrations were unchanged by exposure to elevated ozone, which suggests that substrate availability to methanogens is not increasing. However, active pressurised flow occurs because of a pressure differential developing between green, photosynthesising leaves and older, senescing leaves (Chanton & Whiting, 1996, Chanton *et al.*, 1997, Chanton *et al.*, 1993, Shannon *et al.*, 1996, Yavitt & Knapp, 1998) forcing the flow of methane into the roots, through the aerenchyma and out through inter-cellular pore spaces in senesced leaves. As the senesced leaf area was increased by elevated ozone, then the lower pressure "leaky" leaf area is increased, thus there may be more gas flow through plants resulting in more methane being transported from the peat to the

atmosphere. Senesced leaves are also unlikely to show any stomatal control and it has been shown that methane emissions from *Carex* species are partially under stomatal control (Morrissey *et al.*, 1993), which suggests that this could also be a factor behind the increasing methane emissions seen in this experiment as many of the mesocosms were dominated by *Carex echinata*. A further indication that the influence of elevated ozone on methane fluxes may be under stomatal control comes from Mills *et al.,* (2009) and Wagg *et al.,* (2012) who showed that grassland plants exposed to elevated ozone lost their usual response to ABA and no longer had stomatal control when exposed to extreme drought. The natural variation in species cover and composition of the mesocosms means that further work would be required to further explore this relationship and test the hypothesis that the change in methane emissions seen following exposure to elevated background ozone is because of increased senescence present on aerenchymatous vascular plants.

As a recent review by Rinnan et al., (2013) concludes; methane and carbon dioxide fluxes from peatland systems are under the control of many different factors including temperature, water table height and fluctuation and light availability meaning that there are many ways the impact of elevated tropospheric ozone on these carbon gas fluxes may be masked. This study has provided a potential explanation for the seemingly contradictory methane emissions from previous studies but further investigation of the interactions between the factors that affect methane emission would increase our understanding of the effect of ozone exposure on methane emissions. We have shown that peatland ecosystems have the potential to be changed by relatively low accumulations of tropospheric ozone, when considered over the period of a growing season. Increases in plant senescence may affect the long-term viability of sensitive peatland plants, and, although biomass was unaffected by

elevated background ozone over one growth season, it is possible that the increased resources needed by the plants to replenish damaged tissue may have longer-term implications for plant health and carbon sequestration by wetlands.

Conclusions:

Our results have shown that peatland plants are sensitive to increasing background ozone concentrations, which adds new knowledge to previous published work showing that peaks in tropospheric ozone also damage wetland plants. The gas exchange measurements made during this study suggest that methane fluxes from wetlands can be very sensitive to relatively small changes in background ozone concentrations, and that the results from five separate studies on the impacts of elevated ozone on methane fluxes fit within the pattern found in our study. We hypothesize that there is a relationship between plant senescence resulting in changes in methane fluxes ($P = 0.08$ in our study). Further work carrying out more intensive gas exchange sampling from peatlands would indicate whether the effects we have seen occur on a wider scale. Further understanding of the mechanism for how changes in plant growth following ozone exposure are resulting in changes in methane fluxes and the interactions between the factors that affect methane emission and tropospheric ozone exposure are needed to fully assess the implications of tropospheric ozone increases for global greenhouse gas budgets.

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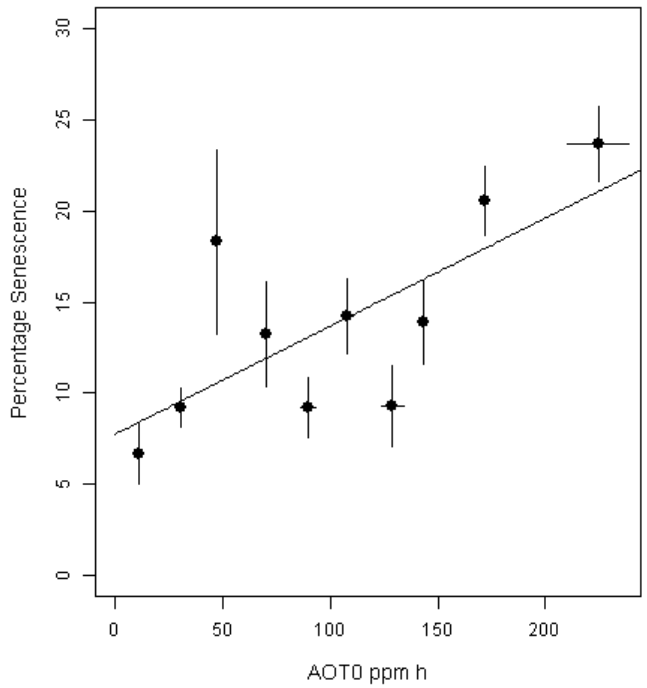

Figure 1a: Vascular plant senescence against AOT0 ppm h through the 16 week experimental period. $P < 0.05$, $R^2 = 0.509$, F stat = 8.30. Error bars show the standard error of the mean for each data point with AOT0 meaned per 20 ppm h intervals.

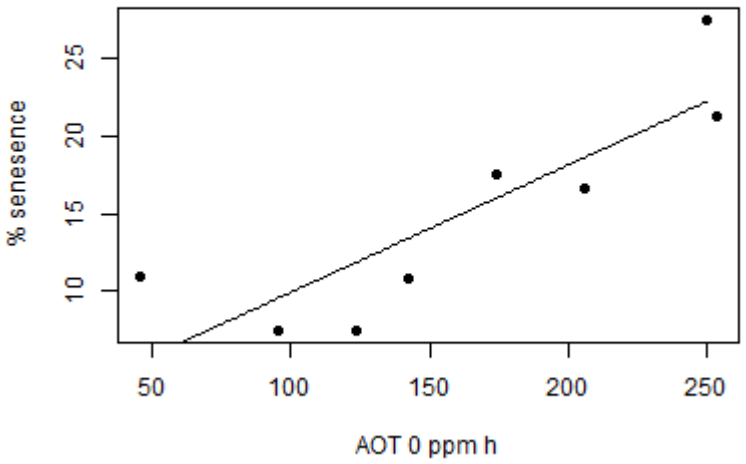

Figure 1b: Vascular plant senescence at the end of the 16 weeks of ozone exposure period. See Table 2 for statistical relationships.

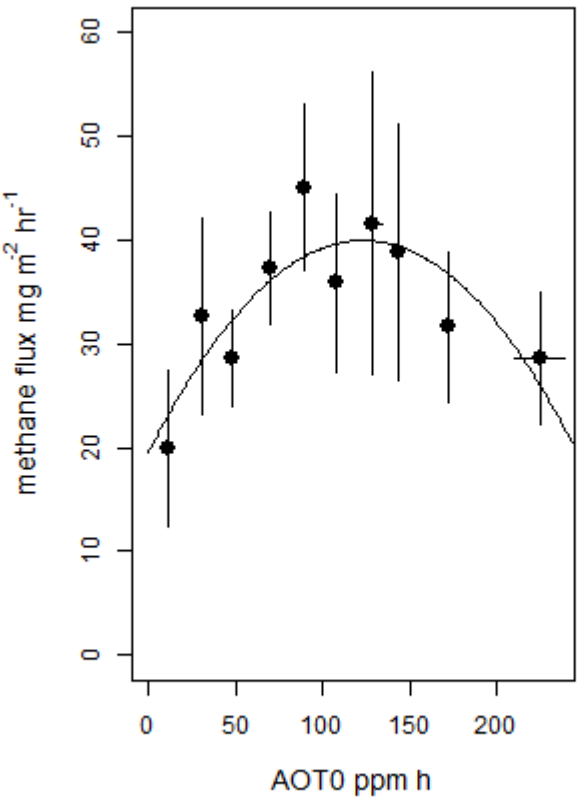

Figure 2: Methane flux plotted against AOT0 ppm h accumulated throughout the 16 week experimental period. $P < 0.01$, $R^2 = 0.719$, F stat = 8.93. Values are shown as the mean ± SE and where SE bars are not present variation was within the size of the points on the plot.