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The impacts of elavated ozone on plant vitality and carbon cycling in wetlands

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Abstract

This study investigated the effects of elevated background ozone on plant growth and carbon cycling in wetland ecosystems. Tropospheric ozone concentrations have been increasing since pre-industrial times and are currently approximately 30-40ppb in Europe. Background concentrations of ozone are predicted to increase in Northern Europe as hemispheric transport of ozone and its precursor molecules occurs from Asia and America. At the same time, because of emissions legislation in Europe, peak concentrations of ozone are predicted to decrease.

Previous research has found that wetland vascular plants are relatively sensitive to ozone and that *Sphagnum* mosses may be less sensitive, despite their lack of a cuticle and their leaves that are only one cell thick. Short-term exposure to high peak ozone concentrations has previously shown that ozone may cause an increase in methane emissions from wetland mesocosms, but longer-term, chronic ozone exposure has been shown to cause a decrease in methane emissions.

The effects of elevated ozone on the growth and physiology of wetland species was assessed on individual species and on naturally germinating communities within mesocosms collected from North Welsh fen and bog habitats. Gas exchange of methane and carbon dioxide from the wetland mesocosms was measured using headspace sampling and pore water carbon content was also measured. During the first year of study the effects of high peaks of ozone on wetland mesocosms and plants was assessed whilst during the second and third years of study the impacts of increasing background ozone was assessed over the growing season.

Vascular plant senescence was found to increase following both short and long-term exposure to ozone in individual species and in the communities. However, these increases in senescence were not always accompanied by significant decreases in biomass. Short-term ozone exposure consisting of five days of 150ppb peaks followed by two days background of 20ppb for four weeks did not significantly change methane emissions from wetland mesocosms. However, there was a trend towards an increase in methane emissions under elevated ozone during this

experiment which, when measured again in bog mesocosms over a longer-term ozone exposure simulating predicted increases in background ozone (eight treatments ranging from a mean of 16ppb to 102ppb) was significant. When vascular plants were removed from bog mesocosms as they germinated by excision below the water-table methane emissions no longer increased as background ozone concentrations increased, suggesting that vascular plants control a large part of the increase in methane emissions that had been measured. Methane emissions also dropped if the water-table was lowered, irrespective of ozone exposure or wetland type. Carbon dioxide exchange however, was not consistently affected by elevated ozone and in the majority of results, carbon dioxide exchange was unchanged as background ozone concentrations were increased.

Methane production and consumption from fen and bog mesocosms, both in-situ and when measured using laboratory assays, was not significantly affected by elevated ozone suggesting that the increase in methane emissions from bog mesocosms is linked to the increases in plant senescence induced by ozone. The majority of methane fluxes through wetland plants occur as a result of pressurised ventilation where gas flows from high-pressure regions such as photosynthesising green leaves, down into the roots to supply them with oxygen and then flows out through low-pressure, senesced, "leaky" leaves. As plant senescence is increased by elevated ozone it is supposed that this could increase the gas flow through the plants resulting in higher methane emissions.

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1 Introduction

Tropospheric ozone has been recognised as having the potential to damage vegetation for over 60 years (Royal Society 2008), but it is only in the last 30 years that these impacts have been intensively studied and attempts have been made to quantify the damage. One area that has so far received little attention is the impact of tropospheric ozone on peat-forming wetlands. Such effects would be of particular concern because any influence that causes a change in peatland carbon dynamics may affect the ability of peatlands to act as a sink for carbon dioxide, thereby also having an effect on global climate change. Although atmospheric ozone concentrations are unlikely to have any direct effect on carbon cycling processes within the peat because of the spatial separation of decomposition sites and the air (Niemi et al. 2002), it is possible that ozone damage to plants present in peatlands could have an effect on the carbon cycle thus changing the amount of carbon sequestered by peatlands.

1.1 What is Tropospheric ozone?

1.1.1 Formation of tropospheric ozone

Ozone (O_3) is an essential component of the stratosphere where approximately 90% of atmospheric ozone is found (NEGTAP 2001). This ozone layer filters out many of the harmful UV rays entering the atmosphere. Tropospheric, or ground level, ozone however, is a major secondary air pollutant (Ashmore 2005) and is considered to be one of the most phytotoxic pollutants found in the atmosphere (Potter et al. 1996a) with current atmospheric concentrations having adverse effects on many economically important crop species (Ashmore 2005) as well as a range of semi-natural vegetation types (Hayes et al. 2007a; Mills et al. 2007).

Tropospheric ozone concentrations are directly related to emissions of the main precursor compounds, nitrogen oxides (NO_x) and non-methane volatile organic compounds (NMVOCs) (NEGTAP 2001). These compounds react together in the presence of sunlight to form ozone (see Equation 1.1 and NEGTAP 2001 for more details). During the past century ozone concentrations have between doubled and

trebled from background concentrations of 10-15 ppb to 30-40 ppb annual 24 hour mean ozone concentration (Royal Society 2008) and peak concentrations worldwide are in excess of 50ppb (WHO 2006). The reasons for this will be discussed in more detail in section 1.1.3.

$NO + O_3 \rightarrow NO_2 + O_2$
$NO_2 + h\nu \rightarrow NO + O$ ($h\nu$ is sunlight in the wavelength 280-430 nm)
$O + O_2 \rightarrow O_3$

Equation 1.1: The reactions between NO_x gases in the presence of sunlight that lead to ozone formation (NEGTAP 2001).

Although the troposphere extends to the tropopause at 10 km above sea level, the majority of ozone forming pollutants are released from biological and industrial activity at the Earth's surface, within the planetary boundary layer (NEGTAP 2001). Within this layer ozone concentration is increased by photochemical production from ozone precursors, estimated to be in the region of 4500 Tg y^{-1} (Royal Society 2008), and decreased by chemical destruction through reaction with nitrogen monoxide, atmospheric transport vertically out of the boundary layer and surface deposition, either through reaction with surface material or by plant uptake (NEGTAP 2001). It is this last method of removal that is of interest in this study as increases in ozone uptake have been shown to cause damage to plants.

1.1.2 Distribution of tropospheric ozone

Tropospheric ozone concentrations across the UK are measured using automatic UV absorption analysers at 22 rural and 55 suburban and urban sites (Coyle et al. 2002). This data can be obtained from the National Air Quality Information Archive (Jenkin et al. 2002) and it is possible to determine trends in ozone concentrations from day to day changes to a multi-decade timescale.

In rural areas diurnal cycles of ozone concentration occur with a mid afternoon peak in ozone concentration and a night-time minimum, typically at about 4am (Coyle et

al. 2002; NEGTAP 2001). Diurnal cycling is caused by changes in the rates of ozone formation and destruction in the troposphere. Emissions of ozone precursors increase during the day, with peaks in emissions occurring during the morning and evening rush hours, whilst UV levels are at a maximum in the mid-afternoon. Although plant stomata are open during the day increasing uptake of ozone, the formation of ozone is greater than the rate of uptake leading to the mid-afternoon peak. During the night the boundary layer becomes thermally stratified with little mixing between the layers meaning that as the ozone concentration in the planetary boundary layer decreases through deposition onto the Earth's surface, it is not replaced by ozone from the upper layers leading to the night-time minimum concentration (Coyle et al. 2002). Diurnal cycling tends to be most pronounced in rural lowland areas and less pronounced in coastal and upland areas. In coastal areas, ozone concentration tends not to show such a pronounced decrease in concentration because deposition of ozone over water is lower than over land and during periods of onshore wind increases of 5-7ppb can occur relative to inland sites (Entwhistle et al. 1997). In upland areas, the air flow is more turbulent as it encounters areas of raised land mass leading to replacement of ozone depleted air at the boundary layer with comparatively ozone-enriched air from the upper layers (Coyle et al. 2002). As a result, ozone concentrations of over 50 ppb can persist in upland areas for several days (Potter et al. 1996a). In Figure 1.1 it can be seen that the lowland sites follow the typical diurnal profile with a mid-afternoon peak and an overnight low while the upland sites have continuously high ozone concentrations throughout the 24 hour period (Royal Society 2008; Sandroni et al. 1994).

As well as the daily cycling of ozone concentration in the troposphere, the UK also experiences weekly cycles in ozone concentration (Jenkin et al. 2002). A study examining the number of hours ozone concentration exceeded 90 ppb (Jenkin et al. 2002) found that there was a peak on Fridays when averaged over a number of rural UK sites. This is most likely to be because of the dependence of ozone precursor emissions on the day of the week; more cars are used on weekdays than at the weekend. Friday values were probably highest because ozone concentrations built up during the week.

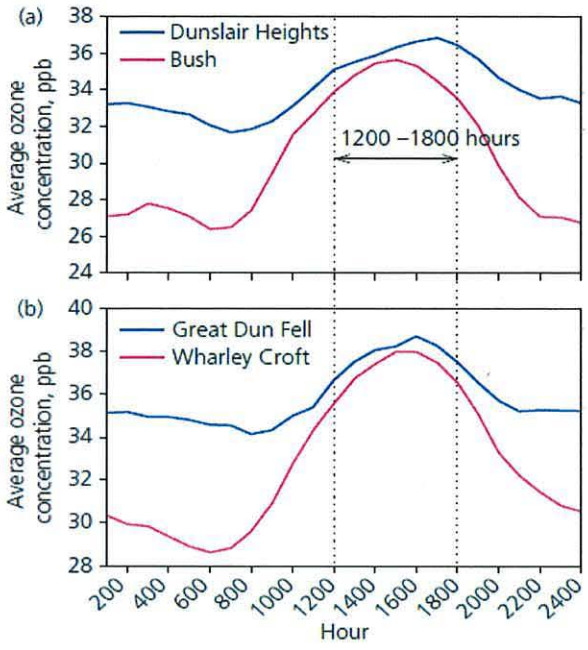


Figure 1.1: The diurnal cycling in ozone concentration at two hill top sites, Dunslair Heights and Great Dun Fell compared to their corresponding low-lying sites, Bush and Wharley Croft showing the much larger diurnal variation in the low-lying sites compared to the hill top sites (From Royal Society 2008).

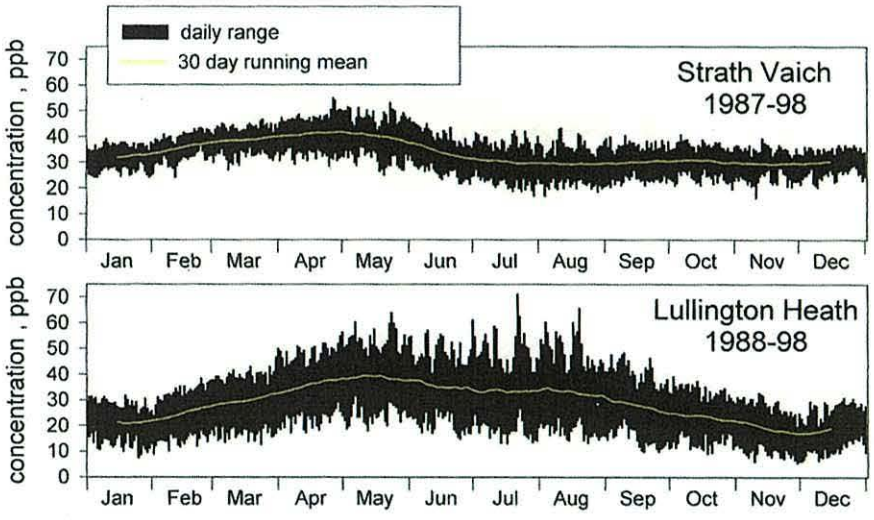


Figure 1.2: Yearly ozone profiles at Strath Vaich in Northern Scotland and Lullington Heath in Kent (NEGTAP 2001) showing the pronounced early season peak in ozone concentration at Strath Vaich between April and May and the broad peak in ozone concentration between April and September at the more polluted site in Kent.

Diurnal cycles of ozone concentration are more pronounced during the summer months when warm anti-cyclonic conditions occur (Coyle et al. 2002). This is because the slow moving warm air allows high concentrations of ozone to build up during the day and clear night skies allow pronounced temperature inversions to occur (NEGTAP 2001). During the winter, there is less ozone formation because solar intensity is lower and anti-cyclonic conditions are not common. The lower concentrations of ozone that are present react with nitrogen monoxide in the atmosphere thus reducing ozone concentration further (Carpenter et al. 1998). This leads to yearly cycles of ozone concentration with a broad peak spread from April to September in more polluted sites such as Lullington Heath in Kent and a pronounced peak in April at more northerly, unpolluted sites such as Strath Vaich in the north of Scotland (Figure 1.2) (NEGTAP 2001).

1.1.3 The future UK ozone climate

There has always been a low concentration of ozone (<10ppb) in the troposphere because transfer from the stratosphere occurs during events such as tropopause folding (Grewe 2007; NEGTAP 2001) and low levels of compounds from natural sources such as isoprene from vegetation contribute to the background ozone concentration (NEGTAP 2001; Smith et al. 2003). However, ozone concentrations in the troposphere have risen from 10 ppb prior to the industrial revolution to summer maximum concentrations of over 70 ppb today (Karberg et al. 2005). This increase in ozone concentration is predominantly caused by the increase in emissions of ozone precursors, primarily nitrogen oxides (NO_x) and volatile organic compounds (VOCs), since the industrial revolution (Coyle et al. 2002; Smith et al. 2003; West and Fiore 2005). Although there are now emissions controls in place in Europe to reduce the amount of ozone precursor compounds released into the atmosphere, background levels of ozone are predicted to continue to rise (Royal Society 2008). This is because hemispheric transport of gaseous pollutants brings precursor compounds over Europe from Asia (Derwent 2008; Vingarzan 2004). Emissions controls in place across the UK and Europe mean that peak concentrations of ozone are predicted to drop leading to a change in the ozone climate of the UK with a higher background but fewer and lower peaks rising above this concentration (NEGTAP 2001). Figure 1.3 shows

ozone concentrations measured at Strath Vaich and Leeds city centre between 1990 and 2006. At the city centre site, peak emissions show a significant downward trend whilst there was an increase in the 95th to the 25th percentiles of the ozone concentrations. In contrast, the maximum concentrations at Strath Vaich, which are less affected by air masses from Europe and local pollution events, did not show a reduction in peak ozone concentrations but the 95th to the 5th percentiles all show a significant increase in ozone concentration (Jenkin 2008).

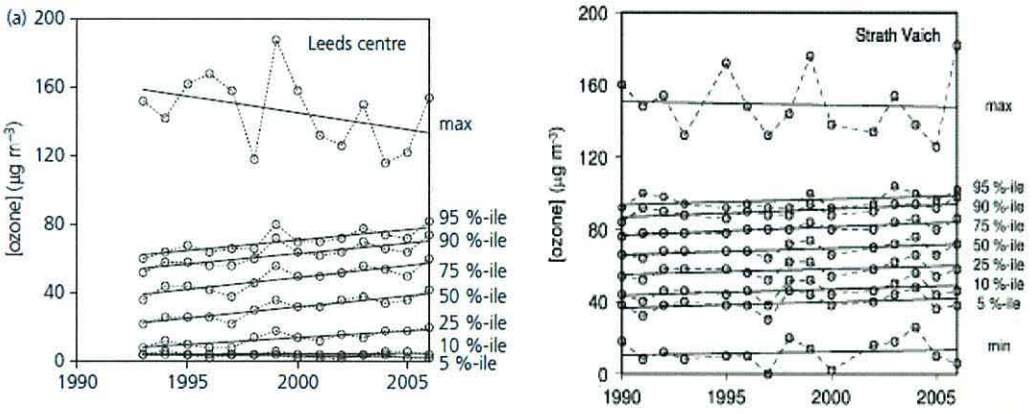


Figure 1.3: Peak and average ozone concentrations at a) Leeds city centre and b) Strath Vaich, showing the reduction in peak ozone concentrations at the more urban site although not at the remote site and the increase in average ozone concentrations at both sites. From Jenkin (2008).

Mean annual trends in background ozone concentration at sites across the northern hemisphere have been increasing by 0.5 and 2% per year over the past decade (Fuhrer 2009). Model predictions for 2030 vary from a 5% decrease in ozone concentration under a maximum feasible reduction scenario to a 15% increase under the IPCC's A2 "business as usual" scenario (Fuhrer 2009; Stevenson et al. 2006). Current and predicted future AOT40 concentrations are shown in Figure 1.4. Differing results for scenarios A1B and A2 refer to different emissions controls for the predicted scenarios. Due to the importance of ozone as a phytotoxic pollutant the United Nations Economic Commission for Europe (UNECE) has set critical levels, above which adverse effects on semi-natural vegetation can be expected, based on hours of ozone exposure above a threshold of 40ppb (Coyle et al. 2002). The indices are

calculated as the accumulated concentration of ozone over 40ppb during daylight hours and the critical level for semi-natural vegetation dominated by annuals has been determined as 3000ppb.h over a three month growing season from May until July (LRTAP 2004). 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 and reductions in yield of crop species (Fuhrer et al. 1997; Hayes et al. 2007b).

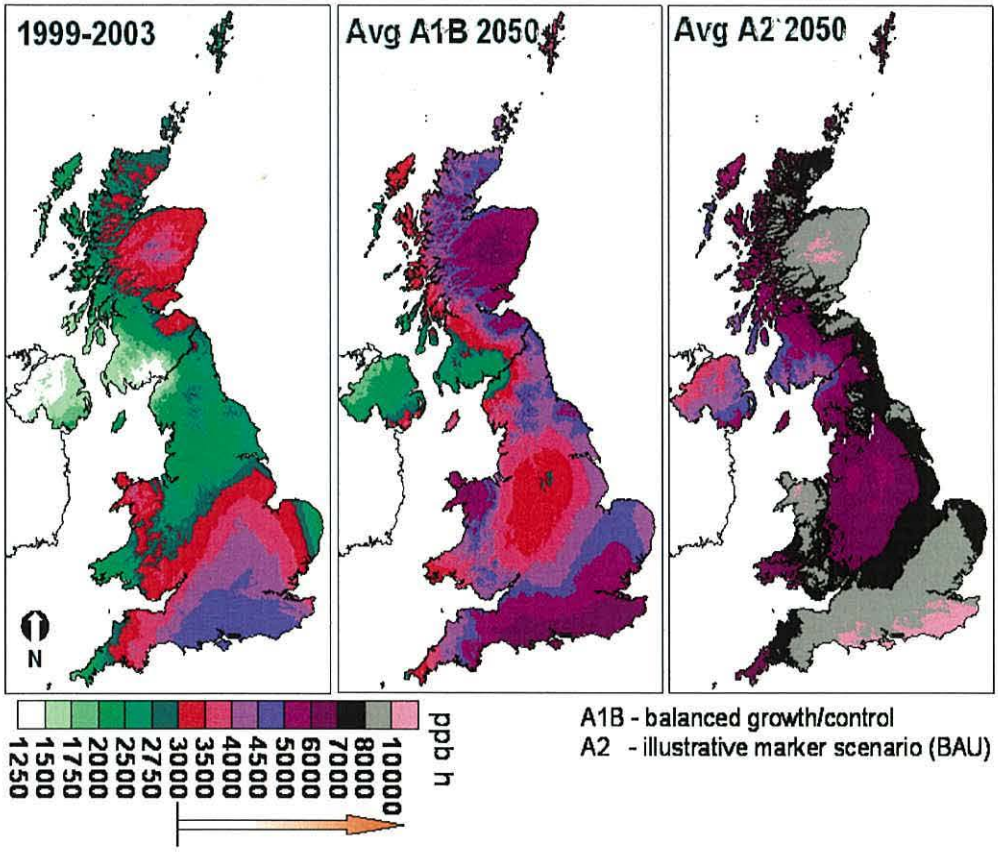


Figure 1.4: 1km x 1km maps of current AOT40 values across the UK (left) compared with future predicted AOT40 values for 2050 derived from data from UK monitoring sites Values to the right of the arrow exceed the 3000ppb h critical level for semi-natural vegetation (Mhari Coyle, pers comm.). The A1B scenario refers to an emissions scenario based on current legislation continuing to be implemented while the A2 scenario refers to incremental growth based on "business as usual".

1.2 Peat forming ecosystems

1.2.1 Habitat

The term "wetlands" covers a variety of habitats and is defined by the RAMSAR Convention as: "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt including areas of marine water, the depth of which at low tide does not exceed 6 metres" (Mitsch and Gosselink 2000). This wide-ranging definition was designed to cover all wetland habitats that are likely to be used by migratory water birds (Scott and Jones 1995).

"Peatlands" are a specific type of wetland habitat, predominantly found at northern latitudes that are characterised by the formation of peat because plant productivity exceeds decomposition. There are two main types of peatlands in the UK; bogs and fens. Bogs are generally upland, low nutrient (oligotrophic), acidic systems with very little water inflow from the surrounding area. In the UK, they tend to be dominated by *Sphagnum* mosses with few vascular plant species present. Fens are generally lower-lying and receive higher inputs of nutrients (eutrophic) via water inflow from the surrounding area. They are also less acidic, mainly due to the influx of base rich ions from groundwater and the surrounding areas. In the UK, fens tend to be dominated by a variety of vascular plant species such as *Juncus* sp, *Carex* sp and *Phragmites* sp (Mitsch and Gosselink 2000).

1.2.2 Formation of peatlands

There are two criteria necessary for the formation of peatlands; a positive water balance and conditions conducive to the accumulation of peat (Mitsch and Gosselink 2000). For a positive water balance to occur, water inputs must be greater than outputs via throughflow and evapotranspiration. This results in the waterlogging of the area thus reducing microbial activity (see Section 1.2.3.2). The formation of peat relies on the productivity and accumulation of dead plant material being greater than

the rate of decomposition. The acid, cold and waterlogged conditions found in most northern wetlands restrict decomposition (Rinnan et al. 2003) so once peatland areas have started accumulating they tend to be resistant to alteration providing that the positive water balance and the conditions suitable for the accumulation of peat remain (Mitsch and Gosselink 2000).

The formation of peatlands tends to follow one of two main trajectories, terrestrialisation or paludification (Mitsch and Gosselink 2000). Terrestrialisation is the classic process of peatland development, first described in the 1900s by Weber (Mitsch and Gosselink 2000) where peat gradually infills a lake basin leading first to a fen and then, as peat development continues, the surface is raised above the lateral water inflow and a raised bog develops (Kratz and DeWitt 1986). Paludification occurs when blanket bogs spread beyond their basin boundaries and begin to cover the surrounding dry land (Crawford et al. 2003; Mitsch and Gosselink 2000). This tends to happen as a result of some kind of change in climatic variables, land use or land morphology (Mitsch and Gosselink 2000). Once the peat covers the surrounding mineral soil it compresses and becomes impermeable to water resulting in a raised water-table, leading to conditions favourable for the growth of *Sphagnum* (Fenton et al. 2005) and eventually the formation of an acidic, low nutrient raised bog (Lavoie et al. 2005).

1.2.3 Carbon fixation, storage and export

1.2.3.1 Fixation

Photosynthetically produced carbon is the main carbon input into the peatland system and it enters the substrate through two main routes; the release of dissolved organic material via root exudates and the input of plant material at plant death (Freeman et al. 2004a). Although *Sphagnum* moss species may dominate peatland systems, Rinnan et al. (2003) showed that vascular plants (in their experiment, *Eriophorum vaginatum*) accounted for the majority of plant productivity especially when the water-table was near the level of the surface.

1.2.3.2 Storage

As previously mentioned, peatlands are accumulative systems with more carbon entering the system than leaving (Gorham 1991). Global estimates of this carbon sequestration are in the region of 20-30 gC m^{-2} yr^{-1} (Wieder 2001). The dominant factor that regulates wetland biogeochemistry is waterlogging of the peat (Blodau et al. 2004; Freeman et al. 1997) which is necessary for the continued preservation of the peat (Van Breemen 1995), sometimes for many centuries as seen by the discovery of "bog bodies" that are almost perfectly preserved. Other factors that limit the decomposition of organic matter include soil temperature, plant community structure (Strom et al. 2005) and the chemical composition of plant tissues (Blodau et al. 2004). This latter point is very important in peatland systems because of the predominance of *Sphagnum* mosses that help to create the low nutrient, acidic conditions found in peatlands (Van Breemen 1995). As well as being an efficient interceptor of nutrients entering the peatland system, *Sphagnum* contains high concentrations of galacturonic acid which give it its high cation exchange capacity (Van Breemen 1995). *Sphagnum* also contains sphagnan, a polyuronic acid that suppresses microbial activity (Scheffer and Aerts 2000) by inactivating exo-enzymes and binding nitrogen containing compounds so they are not available to microbes (Van Breemen 1995).

Enzyme activity in wetlands has been found to provide a vital "latch" mechanism for carbon storage in wetlands (Freeman et al. 2001b). Specifically, recalcitrant, phenolic compounds from plant polysaccharides such as cellulose and lignin build up in wetlands (McLatchey and Reddy 1998); these are broken down by phenol oxidase in aerobic conditions. However, phenol oxidase activity is severely restricted in the absence of oxygen (McLatchey and Reddy 1998; Pind et al. 1994), as regularly occurs in peat forming wetlands, allowing phenolic compounds to build up. These phenolic compounds restrict the activity of other decomposing enzymes such as hydrolases allowing the build up of peat (Freeman et al. 2004b; Freeman et al. 2001b). Extra-cellular enzymes are the main way that micro-organisms degrade insoluble macro-molecules such as cellulose, facilitating the assimilation of the smaller, soluble molecules that are produced as a result (Wallenstein and Weintraub 2008). Thus, a measure of enzyme activity is a good estimate of microbial activity within wetlands (Freeman et al. 1995).

1.2.3.3 Export

Carbon is lost from the peatland ecosystem through respiration of organic compounds by plants and microbes and via outflow of dissolved organic carbon (DOC) in water leaving the system. Net ecosystem exchange of carbon dioxide has been found to be consistently higher in peatland microcosms containing vascular plants than in those that only contain *Sphagnum* species (Rinnan et al. 2003). This implies that vascular plants contribute the majority of plant productivity in peatlands (Rinnan et al. 2003). The main output of carbon dioxide from wetlands is from dark plant respiration and microbial respiration of root exudates, decomposing plant material and peat (Moore and Dalva 1997; Rinnan et al. 2003). Such respiration has been found to be highest in microcosms containing vascular plants indicating that either vascular plant respiration dominates peatland respiration or that root exudates from vascular plants increase microbial respiration (Rinnan et al. 2003). Experiments have found that aerobic carbon dioxide production is highest at the surface of the peat and is quickly attenuated with depth. It has also been found that carbon dioxide production is linked to the predominant type of organic matter available with vegetation of herbaceous origin having a higher carbon dioxide production level than lignified vegetation (Moore and Dalva 1997).

As peat-forming wetlands are predominantly anaerobic, the complete decomposition of organic products to carbon dioxide may not occur (Freeman et al. 2004a). This incomplete decomposition results in the formation of DOC, including phenolic compounds that inhibit degradation (Wetzel 1992) and can only be decomposed by certain enzymes (Freeman et al. 2001b). Another important source of DOC is exudation of low molecular weight compounds from plant roots. If DOC compounds are not broken down within the wetland they are exported out of the wetland in the outflow of water (Kang et al. 1998). DOC has been shown to be increasing in water draining from peatlands in recent years (Freeman et al. 2001a; Freeman et al. 2004a; Monteith et al. 2007; Worrall et al. 2006b) possibly because of acid rain reductions, changing land use or increasing temperature.

Peatlands are an important worldwide source of methane with approximately 20-30% of global methane emissions originating from northern peat-forming wetlands (Cicerone and Oremland 1988). European wetlands have been estimated as being a yearly source of approximately 5.2Tg methane, with the majority (48%) of methane emissions coming from minerotrophic mires (Saarnio et al. 2009). Methane is a greenhouse gas with a warming potential approximately twenty times that of carbon dioxide (Niemi et al. 2002). The process by which methane is emitted from wetlands has been increasingly studied in recent years as the concentration of methane in the atmosphere has more than doubled since pre-industrial times (Smith et al. 2003). Methane emission has been found to be a result of whole ecosystem processes acting together beginning with inputs of carbon from plants to the soil in the form of detritus and exudates followed by bacterial fermentation of the organic matter into substrate that can be utilised by methanogenic archaea with methane as the end by-product (Cao et al. 1998). Although a large proportion of carbon entering the system is from decaying plant material, the flux of methane from wetland soils is affected by the amount of photosynthate entering the soil as low molecular weight exudates from plant roots (Rinnan et al. 2003; Strom et al. 2005; Van Veen et al. 1989). This means that the rate of photosynthesis may be linked to the rate of methanogenesis as increasing carbon fixed through photosynthesis may increase the amount of carbon available in the root, thereby increasing concentrations of labile carbon around the plant roots (Greenup et al. 2000). The presence of vascular plants can also decrease methanogenesis as many plants that are adapted to living in waterlogged conditions where methanogenesis occurs have vascular tissue called aerenchyma (Figure 1.5) that allow oxygen to reach the roots deep in the anoxic substrate (Greenup et al. 2000; Roura-Carol and Freeman 1999). Since methanogenic bacteria are obligately anaerobic such an input of oxygen to the roots may inhibit their action, reducing methane production (Roura-Carol and Freeman 1999; Thomas et al. 1996; Watson et al. 1997).

Vascular plants have been widely shown to increase methane release from wetlands, for example Greenup et al., (2000) showed that more methane was released from areas containing *Eriophorum vaginatum* than from areas without vascular plant cover. Of the two impacts vascular plants have on methane release (provision of a conduit

and as a source of carbon), over the short-term the provision of a conduit appears to be the more significant at affecting methane release. Greenup et al. (2000) found that short-term methane release was unaffected by removing photosynthesising plant tissue suggesting that methane emission is more affected by the build up of methane in the peat rather than instantaneous production (Conrad 1993). It has also been found that methane release is reduced at the end of the growing season when plants undergo senescence (Kim et al. 1998a). The type of plant material available to methanogens also affects the rate of methane emission, with sites dominated by grasses and sedges having a higher proportion of labile organic matter and showing higher rates of methane emission than sites dominated by shrubs that contain a higher proportion of lignin (Shannon and White 1994; Yavitt and Lang 1990).

There are three routes by which methane produced in the peat can reach the atmosphere: molecular diffusion, ebullition or bubbling and plant mediated transport (Greenup et al. 2000; Shannon et al. 1996; Smith et al. 2003). Diffusion of methane through the substrate to the atmosphere is unlikely to be a significant release route as methanotrophs in the aerobic areas of the substrate have been found to convert up to 90% of diffusing methane into carbon dioxide (Fechner and Hemond 1992). Ebullition of methane only occurs when concentrations in the substrate build up to high levels and bubbles of methane rise through the substrate to the atmosphere. The speed of this process prevents methane oxidation as it passes through the aerobic layers (Smith et al. 2003). The major route by which methane reaches the atmosphere is through plant mediated transport through plant aerenchyma (Figure 1.5) with up to 90% of methane released through this route (Shannon et al. 1996). There is a concentration gradient between the substrate around the roots and the plant aerenchyma and between the aerenchyma and the atmosphere (Smith et al. 2003) so plants can serve as a direct conduit between the substrate and the atmosphere (Chanton et al. 1992). There are two main types of methane flow through plant aerenchyma: passive molecular diffusion and active pressurised flow (Brix et al. 1992; Whiting and Chanton 1993). The major route of methane flow differs between species (Roura-Carol and Freeman 1999) and rates of methane production differ between wetland types (Whiting and Chanton 1993).

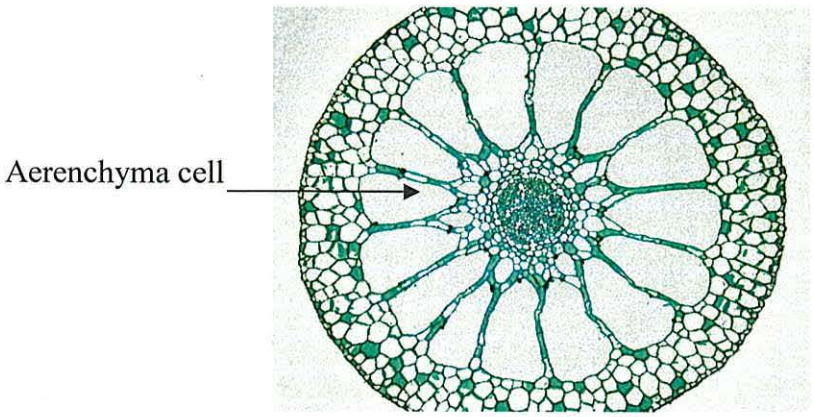


Figure 1.5: plant aerenchyma in a generalised wetland vascular plant (www.uri.edu)

Passive molecular diffusion occurs because of the concentration gradient between the methane pool in the peat and methane concentration in the atmosphere. Experimental results have shown that methane emissions from plots of peatland containing intact plants of *Eriophorum vaginatum* were not significantly different from plots where *E. vaginatum* stems were cut above the surface (Greenup et al. 2000). Active pressurised flow occurs when green, emergent leaves are heated and pressurised which causes methane to be driven into the roots and through the old, brittle leaves into the atmosphere (Shannon et al. 1996; Yavitt and Knapp 1998). The pressure gradients are caused by differences in temperature or water vapour pressure between internal air spaces and the atmosphere (Brix et al. 1992). Plants that release methane through pressure induced flow show considerably greater diurnal variation than those where gas is released by molecular diffusion (Sebacher et al. 1985). *Carex* species show methane emissions controlled by stomatal opening (Morrissey et al. 1993).

When considering the importance of carbon gas fluxes from wetlands it is important to take into account the global warming potential of the different greenhouse gases. When measuring the sequestration rates of various peatlands various authors have found that peatlands are a large sink for carbon, with values of 14-72 gC m^{-2} yr^{-1} being reported in the literature (Belyea and Malmer 2004; Hargreaves et al. 2003; Nilsson et al. 2008; Rivers et al. 1998). However, when the increased global warming potential of methane compared to carbon dioxide is taken into account then it has been found that most wetlands are a source of CO_2-C equivalents when measured over a 50 year time-scale, many are a small sink when measured over a 100 year time-

scale and if they are measured over a 500 year time horizon they are considered a large sink of CO_2-C equivalents (Whiting and Chanton 2001). More recently, the carbon budget of a restored wetland in the Netherlands has been calculated in terms of CO_2-C equivalents and over the three year measurement period gas fluxes showed the wetland to be a substantial sink for carbon (Hendriks et al. 2007). When dissolved carbon fluxes were included in the calculations the wetland was still a small sink for carbon (Hendriks et al. 2007). In percentage terms, vitualy all of the carbon equivalents entered the ecosystem via plant growth and, of the carbon equivalents exported, 75% was lost via ecosystem respiration, 23% left as methane emissions and the remaining 2% was lost via surface water flow and percolation to groundwater (Hendriks et al. 2007).

Further breakdown of the carbon gas fluxes in wetland systems shows that, at most wetland systems where long-term monitoring has taken place, they are an overall sink for carbon with the growing season being a large sink for carbon while the autumn and winter season shows a small rate of loss of carbon (Griffis et al. 2000; Hendriks et al. 2007; Nilsson et al. 2008). This is exacerbated when measuring carbon equivalents because methane emissions are highest from fens in the autumn (Rinne et al. 2006).

1.3 The effects of ozone on plant growth

Ozone has been recognised as causing damage to crop plants since the Second World War when crops were reported to be suffering from leaf injuries attributable to chemicals found in smog (Andersen 2003). Further research on the forests in the San Bernardino Mountains in California found that ozone was the component of smog that caused the damage. Ozone pollution has been found to have long-term impacts on ecosystems as ozone sensitive plants are outcompeted by those tolerant of ozone exposure (Andersen 2003). In Europe, long term monitoring of the impacts of air pollutants on vegetation has been carried out by the International Cooperative programme on Effects of Air Pollution on Natural Vegetation and Crops (ICP Vegetation) with particular focus on ozone in recent years (Harmens et al. 2005). A recent review of ozone effects on vegetation across Europe reported that ozone

damage had been recorded in sixteen European countries, with 30 crop species and 80 semi-natural vegetation species affected (Hayes et al. 2007b).

1.3.1 Plant uptake and detoxification of ozone

Plants exchange gases with the atmosphere during the processes of photosynthesis, transpiration and respiration, all of which are vital for plant survival. However, this means that pollutant gases such as ozone can also be taken up by plants thus exposing them to the toxic effects. There are three main flux processes controlling the transfer of gases into leaves (Fiscus et al. 2005). Firstly, within the turbulent boundary layer gases are mixed by the turbulence which can lead to them entering the laminar boundary layer where turbulence is much lower and molecular diffusion dominates. Once within the laminar boundary layer, gases are either deposited onto the leaf surfaces or taken up via the stomata into the sub-stomatal cavity (Fowler 2002).

The main sinks for tropospheric ozone are dry deposition onto plant surfaces and uptake via stomata into the leaves of vascular plants (Fowler et al. 2001). Since deposition of ozone onto the outer cuticle is not thought to be a major cause of injury to plants it will not be covered further here. Uptake of ozone by the stomata has been shown to be the dominant entry route into the plant (Davison and Barnes 1998; Long and Naidu 2002; Schraudner et al. 1997). This can be affected by factors that control stomatal opening such as leaf temperature, vapour pressure deficit, photosynthetically active radiation, soil water availability and carbon dioxide concentration (Jarvis 1976). Peaks in stomatal conductance may not necessarily coincide with peaks in ozone concentration thus reducing ozone uptake (Bergweiler et al. 2008), particularly as ozone concentrations tend to be highest around midday and early afternoon, whereas stomatal opening is often reduced during the hottest part of the day, particularly during the hot, dry conditions that favour elevated ozone (Bergweiler et al. 2008). Stomatal conductance has also been found to decline later in the growing season after an early summer maximum (Bergweiler et al. 2008). Further evidence that stomatal conductance may have a major effect on ozone damage is the observation that ozone damage to plants was reduced when they were grown under water stress (Tingey and Hogsett 1985) and a relationship has been observed between

leaf injury as a result of ozone and stomatal conductance in clover (*Trifolium repens*) (Becker et al. 1989) with those varieties with reduced stomatal conductance showing less ozone injury.

Once inside the leaves, ozone rapidly breaks down to form reactive oxygen species (ROS) such as superoxide anions, hydroperoxyl radicals, hydroxyl radicals and hydrogen peroxide (Kangasjarvi et al. 2005; Pell et al. 1997). The primary reaction sites for these ROS and any remaining ozone are in the extracellular matrix (Baier et al. 2005) where there are numerous sites for oxidation reactions such as unsaturated fatty acids and phenolic compounds (Fiscus et al. 2005; Pell et al. 1997; Schraudner et al. 1997). Plant defence responses to this increased level of oxidising compounds include the metabolisation of antioxidants such as superoxide dismutase, ascorbate peroxidase and glutathione reductase to reduce the oxidising environment (Kangasjarvi et al. 2005; Sharma and Davis 1997; Sharma et al. 1996). There is evidence that the increase in oxidative burst is sufficient to induce defence gene expression thereby increasing the production of anti-oxidant compounds (Rao and Davis 2001; Sharma and Davis 1997; Sharma et al. 1996). More recent research has suggested that sensitive cultivars of *Medicago truncatula* show a delayed response in the change in gene expression compared to resistant cultivars when exposed to acute ozone concentrations of 300ppb for one hour (Puckette et al. 2008). In addition to this, non-enzymatic, low molecular weight anti-oxidants such as ascorbate and glutathione and secondary metabolites such as phenolics, carotenoids and flavonoids are produced and these neutralise the oxidising molecules formed during the process of ozone damage (Noctor and Foyer 1998; Srivastava 1999). Some of these compounds are continually present in plant tissue and production of them is upregulated in response to ozone exposure, whereas others are not usually present and are induced as a result of ozone stress (Sharma and Davis 1997; Srivastava 1999). If plants cannot produce enough antioxidant compounds to react with the ROS, metabolic stress occurs (Baier et al. 2005), the effects of which will be described in the next section.

1.3.2 Biochemical and physiological effects of ozone on plants

Exposure to elevated concentrations of ozone results in a variety of plant responses including programmed cell death, increased senescence and reductions in growth and photosynthesis. Plants from northern, temperate latitudes are generally most affected by elevated ozone because of enhanced uptake due to long summer day length and the relatively high humidity levels (De Temmerman et al. 2002). However, there is a wide variation in the sensitivity of plant species to ozone, both within and between communities (Fuhrer et al. 1997). Figure 1.6 shows an overview of the sequence of ozone reactions with plant tissues and some of the common plant responses.

1.3.2.1 Programmed and uncontrolled cell death

Uncontrolled cell death is generally seen in response to acute ozone exposure for several hours (Long and Naidu 2002) and occurs when ozone and ROS damage membrane proteins to the extent that ion balance cannot be maintained and a loss of cell water and hence turgor pressure follows (Long and Naidu 2002). However, exposure to concentrations of ozone high enough to cause uncontrolled cell death is rare in natural systems and a more common response is programmed cell death. The initiation and execution of various cell death processes are under tight genetic control (Rao and Davis 2001) as this is a common plant response to pathogen attack known as the hypersensitive response (Pell et al. 1997). The entry of ozone into the plant leaf mimics the oxidative burst associated with the hypersensitive response and triggers the onset of programmed cell death (Rao and Davis 2001).

Programmed cell death is evident on plant leaves as visible ozone injury such as necrotic stippling, whitish spots and generalised reddening (Gielen et al. 2007). Visible injury has been seen on a number of species following ozone exposure including *Centauria jacea* (Ramo et al. 2006b), *Betula pendula* (Paakkonen et al. 1996), *Fagus sylvatica* (Gielen et al. 2007), *Populus nigra* (Bortier et al. 2000) and various grassland species (Bergmann et al. 1999; Pleijel and Danielsson 1997).

1.3.2.2 Senescence

Senescence has been described as "the deteriorative processes that are natural causes of death" (Leopold 1980); the first symptoms of which are reductions in total protein content of the leaves, a drop in chlorophyll content and the degradation of cellular contents such as plastids (Mikkelsen and HeideJorgensen 1996; Wingler et al. 2006). Chronic exposure to elevated ozone has been found to increase and accelerate senescence in many species of crops, trees and semi-natural vegetation (Biolley et al. 2002; Hayes et al. 2006; Long and Naidu 2002; Mikkelsen and HeideJorgensen 1996; Ramo et al. 2006b). Plant senescence is triggered by the presence of ethylene, the production of which is upregulated following exposure to ozone (Mehlhorn and Wellburn 1987; Tingey et al. 1976), as a generalised stress response to the rapid increase in ROS caused by the entry of ozone into the leaf tissue (Rao and Davis 2001). This accumulation of ethylene following exposure to ozone is under genetic control as it has been found that ozone sensitive clones of various species produce ethylene in response to ozone but ozone tolerant clones upregulate anti-oxidant production to increase free radical scavenging (Schraudner et al. 1997).

Other factors also affect the onset of senescence including the concentration of Rubisco (ribulose bisphosphate carboxylase/oxygenase) (Pell et al. 1997) and the accumulation of soluble sugars (Wingler et al. 2006). For example, ozone exposure has been found to increase the accumulation of sugars in the leaf at the expense of root growth (Grantz 2003; Grantz and Yang 2000; Liu et al. 2005) because of a detrimental impact of ozone exposure on phloem loading (Grantz and Farrar 2000) which is likely to further contribute to the increased senescence observed under elevated ozone. Rubisco concentration in plant leaves has been shown to be negatively affected by ozone exposure (Fiscus et al. 2005; McKee et al. 1995; Pell et al. 1997) as its degradation is enhanced (Fiscus et al. 2005); such a drop in Rubisco concentration could also increase senescence.

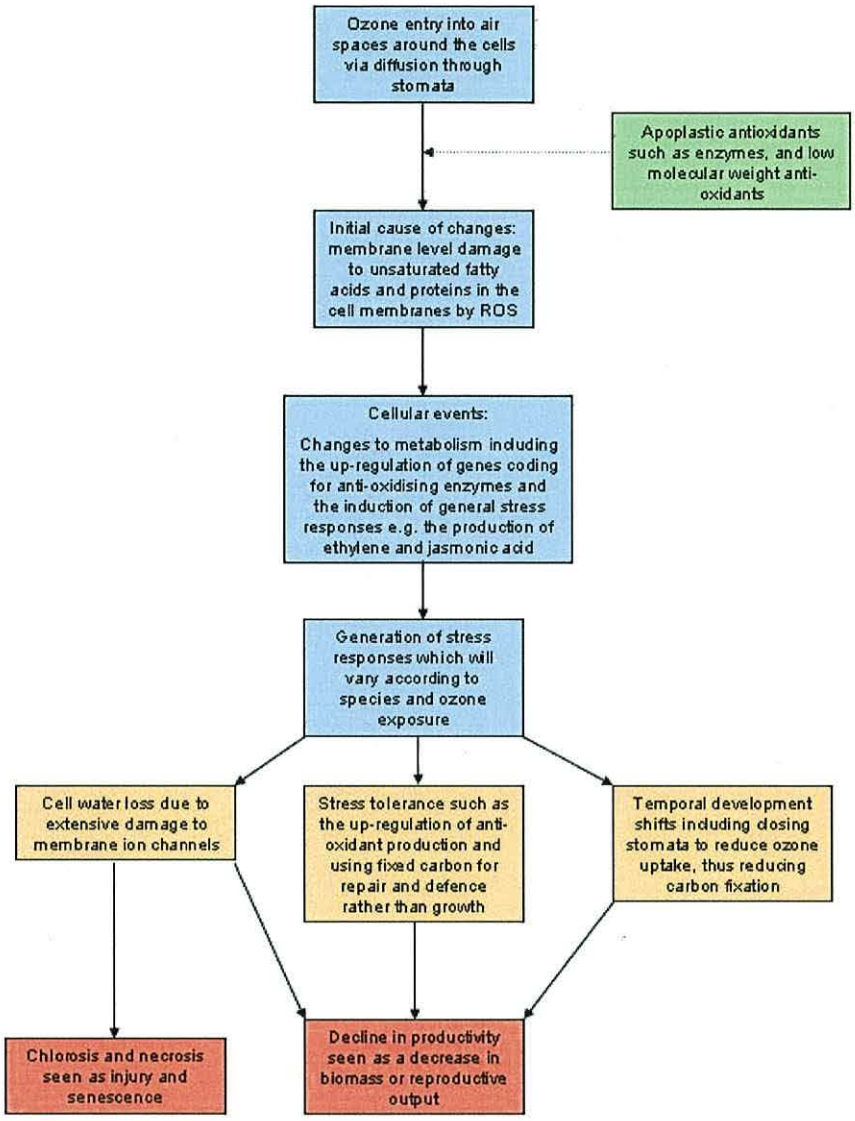


Figure 1.6: Sequence of ozone interaction with plant tissues and plant responses beginning with ozone entry into the leaf, covering the important biochemical changes that occur within plant leaves as a result of ozone stress and ending with the visible symptoms of ozone damage (Heath 2008).

1.3.2.3 Growth and photosynthesis

Plant growth occurs as the carbon captured during photosynthesis is used to make new tissue. Therefore, if photosynthesis is affected by elevated ozone, plant growth is also likely to be affected. As mentioned in the previous section, elevated ozone has been found to reduce Rubisco concentration in plant leaves. Rubisco plays an

essential role in the Kalvin cycle so if concentrations are lowered a reduction in plant growth is likely. Indeed, reductions in plant growth have been found for many species of crops, trees and semi-natural vegetation (Barbo et al. 1998; McCrady and Andersen 2000; Paakkonen et al. 1996; Ramo et al. 2006a; Ramo et al. 2007; Saleem et al. 2001).

The allocation of fixed carbon within plants can also be affected by elevated ozone (Andersen 2003). Ozone exposure reduced root biomass to a greater extent than shoot biomass for Pima cotton (Grantz 2003; Grantz and Yang 2000) and these effects could not be replicated by pruning plants to imitate ozone effects on above ground biomass (Grantz and Yang 2000), suggesting that ozone had more effect on carbon allocation than just the reduction in source strength. Carbon allocation has also been found to be switched towards the production of anti-oxidant compounds such as phenolics (Saleem et al. 2001; Yamaji et al. 2003) and tannins (Liu et al. 2005). This suggests that exposure to elevated ozone causes an increasing metabolic cost to the plant in terms of synthesis of new compounds and potentially the repair of damaged tissues (Andersen 2003). Research on *B. pendula* clones found that changes in the shoot:root ratio were a result of the plant defence strategy (Yamaji et al. 2003). Those where the shoot:root ratio increased showed an increase in the production of antioxidant compounds, whereas those where the shoot:root ratio decreased tended to close their stomata to reduce ozone uptake at the expense of further plant growth (Yamaji et al. 2003).

Elevated ozone has also been found to reduce stomatal conductance, especially following short-term ozone exposure (Andersen 2003). This reduction in stomatal conductance may not be linked to a reduction in biomass as some studies have found a reduction in stomatal conductance without any impacts on biomass (Bortier et al. 2000; McKee et al. 1995; Paakkonen et al. 1998b). However, recent results have shown that the stomatal control by absiscic acid is lost after plants are exposed to season-long, near ambient concentrations of ozone (Mills et al. 2009), potentially meaning that plants could not control water loss so the effects of drought would be greater.

1.3.3 Specific effects on wetland plants

Results from experiments by Franzaring et al. (2000) and Tonneijik et al. (2004) showed that senescence was significantly increased in species from fen meadows in response to exposure to elevated ozone. Power and Ashmore (2002) found that fen and fen meadow species showed significant responses to ozone exposure with nine out of the twelve species studied negatively affected by two months ozone exposure of 80ppb during daylight hours, either showing visible injury or detrimental effects on the growth patterns and physiology of the plants. Five of these species showed significant reductions in photosynthesis and stomatal conductance with a general trend towards reduced above and belowground biomass. They also found that species with small leaves shed their leaves under ozone stress whereas species with larger leaves retained them in spite of the damage (Power and Ashmore 2002). There have been few studies on the effects of ozone exposure on vascular plants from peatlands but exposure to ozone concentrations of 50-150 ppb has been shown to change the ultrastructure of the shrub species *Vaccinium oxycoccus* and *Andromeda polifera* and the graminoid *Eiriophorum vaginatum* (Rinnan and Holopainen 2004). Exposure of upland species to elevated ozone during daylight hours over a period of 10 weeks by Hayes et al. (2006) included some vascular plants commonly found in wetland areas such as *Juncus effusus*, *Potentilla erecta* and *Carex echinata* and these were found to show a variety of negative responses to ozone exposure including leaf injury, increased senescence, reduced biomass and a reduction in regrowth the following spring.

Although vascular plants are important components of wetland systems, many ombrotrophic bogs lack vascular vegetation and bryophytes form the majority of plant biomass (Potter et al. 1996a). Bryophytes are an ecologically significant component of peatland ecosystems as they regulate acidity and also peat growth (Niemi et al. 2002) and have been hypothesised to be sensitive to ozone because their leaves are only one cell thick and lack a protective cuticle (Niemi et al. 2002; Potter et al. 1996a). The genus primarily associated with peatlands is *Sphagnum* and within this genus, species have been shown to have differing sensitivities to ozone exposure (Potter et al. 1996b; Rinnan and Holopainen 2004). Chronic exposure of *Sphagnum* monocultures to ozone concentrations double the current ambient showed that

physiological responses such as photosynthesis and chlorophyll content were most reduced whereas growth and biomass were relatively unaffected (Gagnon and Karnosky 1992). Other effects of chronic ozone exposure on bryophytes include: increased ion leakage from membranes (Niemi et al. 2002), a decrease in chloroplast area and granum stack thickness of chlorophyllose cells, and a decrease in the area of stored cytoplasmic lipids (Rinnan and Holopainen 2004). It has been suggested that the energy usually stored in the lipids had to be used to maintain cell functioning during ozone stress (Rinnan and Holopainen 2004).

1.4 The effects of ozone on carbon cycling

Thus far there have only been a small number of studies assessing the impacts of elevated ozone on carbon cycling in wetlands. Those studies that have been published have not provided a clear picture of the effects of tropospheric ozone on carbon cycling in wetlands.

1.4.1 Effects on carbon gases

Exposure of peatland mesocosms to elevated ozone of 100-200ppb in growth chambers has shown that emissions of methane are increased, although in some cases only transiently, especially when the mesocosms contain *E. vaginatum* (Niemi et al. 2002; Rinnan et al. 2003). This is unlikely to be because of a direct effect of ozone on the methanogens as ozone is too reactive to diffuse through the substrate to the anoxic areas of the peat (Niemi et al. 2002). It has been suggested that oxidative damage to stomata may be a reason for the increased methane emission (Niemi et al. 2002) but this is unlikely to be the case for plants that do not show diurnal variation in methane emissions (Greenup et al. 2000). Unpublished results have found that exposure of fen mesocosms to elevated ozone with a background concentration of 45ppb and peak concentrations of 100ppb resulted in a 350% increase in methane emission from wetland mesocosms (Lloyd 2004). However, more recent results from an open field exposure system have found that one season's exposure of wetland microcosms to elevated ozone double that of ambient concentrations caused a decrease in methane emissions, with subsequent exposure seasons showing no effect of ozone on methane

emissions (Morsky et al. 2008). Measurements of carbon gas fluxes from wet meadow mesocosms also found that exposure to elevated ozone did not change methane emissions (Kanerva et al. 2007)

Peatlands are seen as long-term sinks of carbon dioxide as the plants take up more in photosynthesis than is released during decomposition and respiration (Gorham 1991). Peat microcosm experiments have found that net ecosystem exchange of carbon dioxide is transiently reduced under a high ozone concentration of 200ppb suggesting that as ozone levels in the troposphere increase, the strength of peatlands as a sink of carbon dioxide could be reduced (Rinnan et al. 2003). Dark ecosystem respiration has been found to increase slightly when peatland microcosms are exposed to elevated ozone (Niemi et al. 2002) suggesting that, as well as reducing the sink strength of peatlands, elevated ozone could also increase carbon dioxide release from wetlands. This increase in dark ecosystem respiration could be because of plant stress responses to ozone exposure (Niemi et al. 2002) or it could be because ozone breaks down carbon compounds in the surface waters allowing them to be more easily assimilated by microbes (Gul 2002). However, the latter is unlikely to be the case in wetlands showing complete vascular plant or moss cover because ozone is too reactive to diffuse through the plant cover to reach the pore water (Turner et al. 1974).

1.4.2 Effects on water chemistry

There has been little published research on the impacts of increasing tropospheric ozone on concentration and type of DOC present in peatland porewater although Morsky et al. (2008) found that concentrations of acetate, formalate and oxalate increased after 29 weeks of exposure to double ambient ozone concentrations. Recent data from Jones et al. (2009) found that DOC concentrations in the pore waters of fen microcosms dropped after exposure to elevated ozone but the relative molecular weight of the carbon compounds increased. Research on *Betula pendula* has found that leaves on trees exposed to elevated ozone had a higher phenolic content (Liu et al. 2005; Saleem et al. 2001). The increase has been found to be a response to exposure to elevated ozone concentrations as plant phenolic compounds can have a protective effect against ozone stress (Sgarbi et al. 2003). Thus, it is possible that the

partial decomposition of plant tissues exposed to ozone could increase the phenolic content in peatland pore waters.

1.4.3 Effects on enzymes

Enzyme activity and microbial biomass are interlinked because microbes release extra-cellular enzymes to break down complex organic molecules into simpler ones that can be easily assimilated resulting in greater microbial biomass. However, so far there has been no published research on the effects of tropospheric ozone on enzyme activities in peatlands although Morsky et al. (2008) found that exposure of peatland microcosms to elevated ozone resulted in an increase in microbial biomass.

Elevated tropospheric ozone has been hypothesised to have a negative effect on enzyme activity and microbial biomass through its negative effects on plant growth because both are affected by inputs of carbon from plants in the form of exudates, and root and stem death (Phillips et al. 2002). It is unlikely that there will be any direct effect of ozone on below ground microorganisms because the ozone is too reactive to diffuse through the substrate (Dohrmann and Tebbe 2005; Niemi et al. 2002).

Research on enzyme activity under elevated tropospheric ozone in forest ecosystems has so far found that increasing ozone concentrations has no effect on extra-cellular enzyme activity including that of phenol oxidase (Larson et al. 2002). Microbial community structure in the rhizosphere has also been found to be unaffected by elevated concentrations of ozone (Dohrmann and Tebbe 2005) although increases in microbial respiration under elevated carbon dioxide were reduced in the presence of elevated ozone concentrations suggesting the elevated ozone concentrations may have a negative effect on bacterial biomass and functioning (Phillips et al. 2002). Elevated tropospheric ozone has also been found to decrease microbial biomass in the soil (Islam et al. 2000b) and a long-term study looking at the impacts of elevated ozone on enzyme cycling in forest soils found a 25% reduction in the activity of beta glucosidase (Chung et al. 2006). A long-term study of meadow ecosystems found that exposure to elevated ozone resulted in a reduction in bacterial and fungal biomass (Kanerva et al. 2008).

1.5 Ozone, wetlands and climate change

As well as being a major phytotoxic pollutant, ozone is a greenhouse gas and is ranked as the third most important anthropogenically affected greenhouse gas after carbon dioxide and methane (Royal Society 2008). It has been estimated that the increase in ozone concentration in the troposphere since pre-industrial times has caused a contribution of between 0.25 and 0.65Wm^{-2} to global radiative forcing (Royal Society 2008). In the atmosphere, temperature, humidity and UV radiation (or sunshine levels) affect the formation and deposition of ozone. As temperature increases the rates of reaction between NO_x and VOCs increases. Increasing temperature also increases the rate of biogenic VOC emissions; for example during the heat wave in the summer of 2003 daytime isoprene concentrations in the south-east of England were measured at 1600ppt, a concentration typical of the levels found above tropical forests (Lee et al. 2006). If isoprene emissions increase in areas with high NO_x concentrations then ozone formation could increase further. Increasing sunshine levels will also increase the formation of ozone as the energy from sunlight is required in the reactions between NO_x and VOCs. A decrease in relative humidity could reduce stomatal uptake of ozone if plant stomata are not extensively damaged by ozone leading to an increase in the atmospheric concentration of ozone and increasing radiative forcing due to ozone. A further potential cause of increasing tropospheric ozone concentrations is the predicted increase in the Brewer-Dobson circulation which will result in more stratospheric ozone being transported down into the troposphere (Butchart et al. 2006).

Wetlands are considered to be an important sink for carbon dioxide through the formation and storage of peat, although the emission of methane from wetlands contributes to the overall radiative forcing in the atmosphere. The effects of elevated tropospheric ozone on wetland plants and carbon cycling may change their functioning and have a further impact on greenhouse gas concentrations and climate change. The main impact seen in studies of ozone exposure on gas exchange from peatland systems is a possible increase in methane release. Methane is a greenhouse gas with the second highest impact on global warming after carbon dioxide and a

warming potential approximately twenty times that of carbon dioxide (Niemi et al. 2002). Methane in the atmosphere also contributes to ozone formation in the same way as non-methyl VOCs (West and Fiore 2005). Thus, if ozone causes an increase in methane emission these increased concentrations of methane could cause an increase in ozone formation. VOC release from peatland microcosms has also been found to increase under elevated ozone concentrations which could again increase ozone formation especially if the peatland is near to an anthropogenic NO_x source (Rinnan et al. 2005).

Tropospheric ozone exposure has been found to potentially increase dark ecosystem respiration (Rinnan et al. 2003) and decrease wetland plant growth (Franzaring et al. 2000; Power and Ashmore 2002) reducing the sink strength of peatlands in respect of carbon dioxide. This has the potential to increase global warming if peatland areas "mop up" less carbon dioxide from the atmosphere in the future.

Changes in dissolved organic carbon composition can also have an indirect impact on global warming. If acetate concentrations do increase following exposure to elevated ozone (Morsky et al. 2008) then methanogenesis could also increase potentially increasing the rate of methane emissions to the atmosphere. If the concentration of phenolic compounds increases, decomposition could be inhibited, thereby increasing the DOC load to ecosystems downstream of the peatland. This may change the functioning of that ecosystem through the increased organic nutrient loading and contribute to the effects of a changing climate. Linked to this are many possible changes in microbial biomass and enzyme functioning. Even if changes have not been found in terrestrial systems (Larson et al. 2002) it is possible that changes may occur in the aquatic environment and these could affect carbon cycling in the peatland.

1.6 Summary of Current Knowledge

Although peak concentrations of tropospheric ozone are decreasing in Europe, background levels are still increasing meaning that tropospheric ozone will remain a significant phytotoxic pollutant for many years (NEGTAP 2001). The main effects of

ozone on wetland vascular plants are: reductions in stomatal conductance and photosynthesis and a general trend towards reduced above and below-ground biomass (Power and Ashmore 2002). Other effects include an increase in plant defence compounds such as leaf phenolics (Saleem et al. 2001) and visible signs of injury such as leaf senescence, necrotic spots on the leaves, and rolling and bronzing of the leaves (Vandermieren et al. 2005). These symptoms can result from programmed cell death which is a short-term response to ozone exposure and accelerated leaf senescence which occurs more slowly but over a larger leaf area (Vollenweider et al. 2003). Ozone has also been found to affect bryophyte species in peatlands with the main effects being on physiological responses such as photosynthesis, with growth and biomass being less affected (Gagnon and Karnosky 1992).

Peatland ecosystems have received much research attention because of their slow rates of decomposition meaning that they act as a sink for carbon dioxide (e.g. Gorham (1991)). Huge amounts of carbon dioxide are taken up by plants during photosynthesis which is then stored as peat as the anaerobic conditions reduce decomposition rates (Niemi et al. 2002). This store of carbon is not necessarily stable over time because changes in atmospheric conditions can alter conditions within the peat, potentially increasing decomposition (Davidson and Janssens 2006).

Although there has been little work published so far on the impacts elevated tropospheric ozone may have on carbon cycling in wetlands, the main effect that has been found from growth chamber and solardome studies is a possible increase in methane release from fen microcosms (Lloyd 2004; Niemi et al. 2002). However, studies in an open-field system have so far not replicated these results (Morsky et al. 2008). Studies have also shown that ozone exposure causes an increase in ecosystem respiration and a decrease in uptake of carbon dioxide (Niemi et al. 2002; Rinnan et al. 2003). There have not been any published studies on the effect of tropospheric ozone on enzyme activities in wetlands despite the fact that enzymes are of critical importance in peatland carbon cycling (Freeman et al. 2001b). Enzyme activities were found to be unaffected by elevated ozone in forest soils (Larson et al. 2002), whereas phenol oxidase activities have been found to increase in peat mesocosms under elevated ozone (Lloyd 2004).

1.7 Aims of study and hypotheses

The aims of this study were to identify the direct effects of elevated ozone on wetland plants and the indirect effects of ozone on wetland gas and dissolved carbon fluxes. Furthermore, it set out to determine the mechanisms behind any effects of ozone on wetland processes that lead to changes in carbon fluxes.

The hypotheses are as follows:

1. Elevated ozone exposure will reduce plant growth and cause the early onset of plant senescence.
2. Exposure to elevated ozone will result in increased efflux of methane from wetland mesocosms, with relatively more of an effect being seen in mesocosms containing vascular plants and from higher nutrient environments.
3. Carbon dioxide uptake by wetland mesocosms will decrease under elevated ozone as a result of a reduction in plant growth.
4. Plant decomposition rate will be increased following exposure to elevated ozone because of a potential reduction in the translocation of nutrients out of the leaves prior to ozone-induced senescence (Long and Naidu 2002).
5. Methane production and consumption potentials will be affected by the effects of elevated ozone on plant growth, both because of the presence of vascular plants as a conduit and as a source of bio-available carbon in the form of root exudates.

Initial experiments focussed on the effects of ozone on wetland plants as they play a major role in carbon gas flux from wetlands. Microcosm experiments took place to attempt to quantify the changes in carbon gas fluxes caused by elevated ozone and finally laboratory assays and stable isotope carbon tracing techniques were used to look at the mechanistic processes involved in the exchange of carbon dioxide and methane from wetlands when exposed to elevated ozone.

2 Effects of short-term ozone exposure on wetland plants

2.1 Introduction

Although most early work on ozone phytotoxicity was carried out on economically important crop species, more recent work has focused on the responses of semi-natural vegetation to ozone exposure. Ozone toxicity has been found to have two components; acute toxicity, symptoms of which are unregulated cell death and programmed cell death; and chronic toxicity, seen as accelerated senescence and decreases in growth rate (Fiscus et al. 2005). It is chronic exposure to ozone that is thought to have increased ecological significance (Franzaring et al. 2000) as semi-natural vegetation is rarely exposed to concentrations of ozone that are high enough to be acutely toxic. The main effects on above ground biomass have been found to include reductions in biomass accumulation (Paakkonen et al. 1996; Ramo et al. 2006a; Vandermieren et al. 2005), increases in senescence (Ashmore 2002) and reductions in chlorophyll content (Bortier et al. 2000; Paakkonen et al. 1996). As ozone is highly reactive it is unlikely to diffuse into the soil matrix, meaning that any effects on below ground biomass are likely to be a result of changes in above ground biomass. Results in the literature are conflicting; some experiments show no change in carbon allocation to roots during ozone exposure (McCrady and Andersen 2000; Ramo et al. 2006a) whereas others show that reductions in root growth and root carbohydrate concentration occur under elevated ozone (Andersen 2003; Andersen and Rygiewicz 1991). There are three main reasons why a decrease in root growth under elevated ozone may occur: a malfunction of phloem loading caused by damage to cell membranes, an increased allocation of fixed carbon to leaf injury repair and antioxidant synthesis and an altered balance between leaves and roots caused by a decrease in carbon assimilation (Andersen 2003; Fiscus et al. 2005).

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 conductance is likely to be of high importance in wetland plants as they are unlikely to be water limited and as such will not close their stomata (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).

In upland areas, ozone concentrations tend to remain high for several days during an ozone episode, with no overnight drop in concentration that is more usually seen in lowland, rural profiles (Section 1.1.2). With this in mind, this study was designed to investigate the effects of an ozone profile that remained at an elevated concentration for several days before dropping to background concentrations. This is in contrast to previous screening experiments carried out in the solardomes where upland plants were exposed to an ozone regime based on daily elevated concentrations of ozone that dropped back to background concentrations overnight (Hayes et al. 2006).

This study sets out to investigate the effects of short-term (4 weeks) ozone exposure on seven wetland vascular plant species; assessing percentage senescence and chlorophyll content through the experimental period and measuring above and below ground biomass at the end of exposure. The hypotheses are that: exposure to elevated ozone will increase senescence; exposure to elevated ozone will decrease chlorophyll content and 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.

2.2 Methods

2.2.1 Site selection and background ozone measurements

Two wetland sites in North Wales were chosen; Cors Erddreiniog, a low-lying fenland 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 (Figure 2.1). 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 situated on the border of the Snowdonia National Park and contains typical upland bog vascular plant species although the area is dominated by *Sphagnum* mosses. Although 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). Marchlyn Mawr was chosen as the upland bog site because the hourly ozone monitoring station was sited there and Cors Erddreiniog was chosen as the lowland fen site because of its status as a special area of conservation and because there was ongoing research taking place at the site.

Hourly measurements of ozone concentration are made at Marchlyn Mawr as part of the Welsh air quality monitoring network (www.welshairquality.co.uk). As a preliminary comparison between the two sites to assess whether there was a difference in ozone exposure, diffusion tubes (Gradko Int.) were used to monitor background ozone. A diffusion tube was placed at each site so that it was protected from rainfall but air could circulate freely around the tube and these were changed monthly. Analysis of the average monthly ozone concentration from the diffusion tubes was done by Gradko International. These diffusion tubes were a passive sampler with a chemical reagent at the closed end of the glass tube. Unfortunately, it was not possible to have replicate diffusion tubes at each site because the cost was prohibitive.

2.2.2 Plant selection and propagation

Seven wetland vascular plant species were used in this experiment. Four species were collected from Cors Erddreiniog and three species were collected from Marchlyn Mawr. The fen species chosen were; *Molinia caerulea, Juncus subnodulosus, Hydrocotyle vulgaris and Potentilla erecta.* The species chosen from the bog site were; *Carex echinata*, *Festuca rubra* and *Potentilla erecta.* These species were chosen to be representative of the natural vegetation found at each site.

Figure 2.1: Map of Wales showing the location of the two sites and pictures representative of each site. The left hand photograph is Cors Erddreiniog, the lowland fen and the right hand photograph is Marchlyn Mawr, the upland bog.

Individual plants of each species were collected from the field and potted up using peat compost (HUMAX 100% peat) in a greenhouse with supplemental lighting and controlled 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 grew for three weeks in the greenhouse and were moved to a sheltered outdoor location a week prior to being placed in the solardomes. This was to acclimatise them to the windier conditions found in the solardomes as previous experiments had found that leaf damage from the airflow over them was an extra cause of senescence if plants were moved directly from the greenhouse to the solardomes (L. Taylor *pers. comm*). Before plants were put in the solardomes each species was split 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 ozone treatment.

2.2.3 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 two metres high and three 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. Four solardomes were set to receive a constant ozone concentration of 20ppb throughout the experiment and the other four were set to an episodic regime with ozone concentrations of 150ppb for five days and 20ppb for two days. The solardomes were arranged as a split block design with two blocks of four domes. Within each block there were two domes with high ozone concentrations and two with low ozone concentrations. These were randomly assigned within the block.

Figure 2.2: Solardomes at Abergwyngregyn, situated on an East-West line to minimise differences in shading.

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 recombine to form ozone. A computer-controlled (Lab-VIEW version 7) mass-flow controller system was used to deliver 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.

2.2.4 Plant measurements

Whole plant 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 is not species-specific. Relative senescence was calculated as the difference between the mean senescence under elevated ozone and the mean senescence under low ozone on a weekly basis.

An estimate of leaf chlorophyll content was taken weekly using a Minolta SPAD meter. The SPAD meter measured light at two wavelengths passing through the leaf; infra-red light at 940nm and red at 50nm. Chlorophyll absorbs red light but not infra-red light so the difference between the two values provides a non-destructive measure of chlorophyll content. Measurements were taken on the second youngest, fully expanded leaf and care was taken to use only leaves with no visible senescence or ozone damage. *Festuca rubra* and *Juncus subnodulosus* were not included in this analysis as their leaves were too narrow and did not fill the sample window.

At the end of the experiment, plants were removed from the solardomes and photographed against a matt, black background prior to harvesting. 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.

2.2.5 Statistical Analysis

General analysis of variance was carried out on the senescence data in GENSTAT version 7. Initial analysis was carried out using the block effect to assess whether each block had a differing effect on senescence. This is because the block effect uses up one degree of freedom meaning results are less likely to be found to be significant if the block is used. If the block F ratio was less than one then the block was not having a significant effect meaning that it does not have to be used in the statistical analysis. Only 5 results out of the 35 tests for block effects indicated that the block could be having an effect so it was not used. The treatment structure was ozone*size class and the block structure was the dome as this is where the level of replication occurs for ozone in this experiment. As all senescence data was measured as a percentage it was arc-sine transformed in Minitab version 14 prior to analysis and back-transformed before presentation. Analysis of variance (ANOVA) was done for each species at each time point. This was repeated using the base-line senescence data as a co-variate to allow for any initial variation in senescence. Finally all of the senescence data was assessed using Repeated Measures ANOVA to look at the effect of ozone exposure, time and the interaction between the two in GENSTAT version 7. The treatment and the block structure remained the same for these analyses.

As the plant weights all showed similar values there was no need to transform the data. General analysis of variance was done on all species individually with the treatment structure being ozone* size class and the block structure being the dome.

Ozone dose response for each species was analysed in Sigma-Plot by linear regression of relative senescence (as difference from the control) against $AOT0_{24hr}$ (accumulated ozone over a 24 hour period without a threshold concentration) using treatment means from each week of ozone exposure. The significance of the regression and the

percentage variation in senescence explained by ozone was analysed in GENSTAT version 8.

For all statistical tests, results were taken as being significant at $P<0.05$ and showing a trend towards significance at $P<0.1$.

2.3 Results

2.3.1 Ozone exposure at Marchlyn Mawr and Cors Erddreiniog

Measurements of monthly ozone concentration at the two wetland sites during the year showed that concentrations were consistently higher at the upland site, Marchlyn Mawr. This was more pronounced during the winter and early spring (Figure 2.2). Ozone concentrations were not analysed from Cors Erddreiniog during July as the diffusion tube was missing from its holder, presumably because of vandalism.

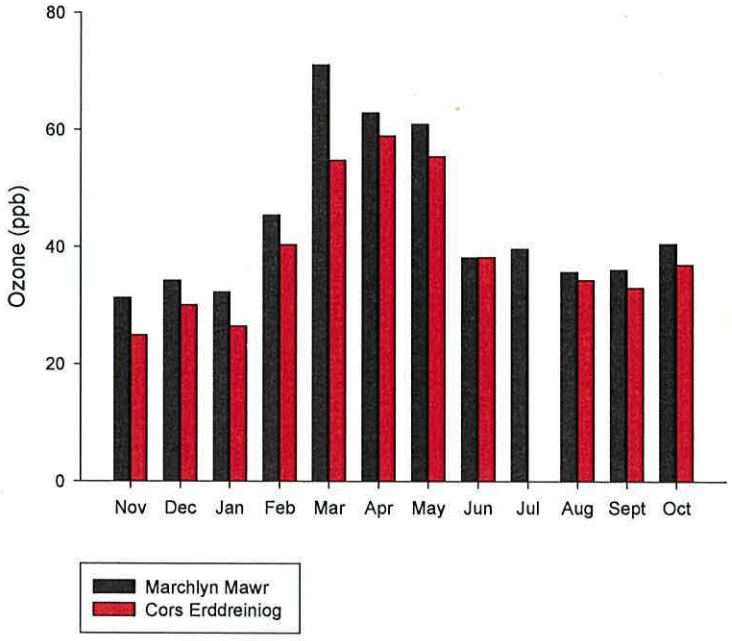


Figure 2.3: Monthly average ozone concentrations measured using diffusion tubes at Marchlyn Mawr (upland site) and Cors Erddreiniog (lowland site). Ozone concentrations were higher at Marchlyn Mawr than Cors Erddreiniog throughout the year.

Hourly ozone concentration measurements from the continuous analyser at Marchlyn Mawr between November 2005 and November 2006 showed that ozone concentrations were around 40ppb throughout the winter and were between 40 and 60ppb with occasional peaks for most of the growing season from March until July (Figure 2.4). Concentrations dropped in August, coinciding with a period of poor weather conditions, and then remained at around 40ppb for the rest of the year (Figure 2.4).

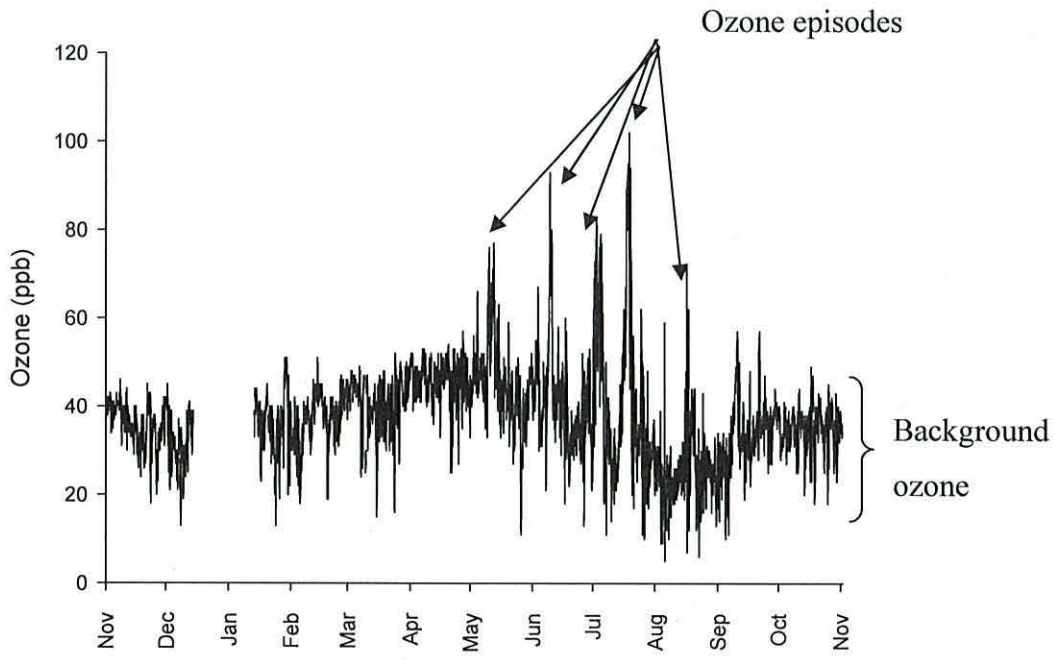


Figure 2.4: Measured ozone concentrations at Marchlyn Mawr 2005-2006. Data taken from the Welsh Air Quality Network. The background concentration is approximately 40ppb and peaks above this ozone concentration are shown as episodes. These lasted for several days.

When the measurements of ozone concentration made with the diffusion tubes were compared with the monthly average ozone concentration from the hourly Marchlyn Mawr data most of the months had comparable concentrations but in March, April and May the diffusion tubes indicated much higher ozone concentrations than the fixed monitor (Figure 2.5).

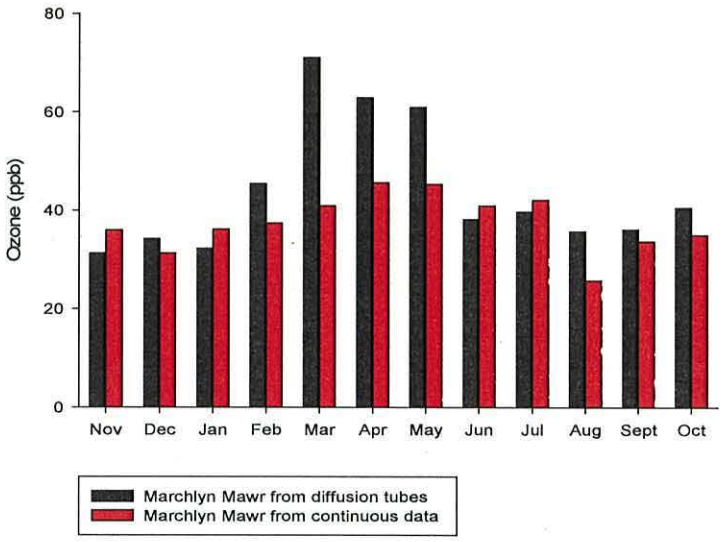


Figure 2.5: A comparison of the diffusion tube data and the monthly average ozone concentration taken from the measured hourly values at Marchlyn Mawr. For most of the year ozone concentrations were similar using both methods but in March, April and May the diffusion tubes over-estimated the monthly ozone concentration compared to the continuous monitoring data.

2.3.2 Ozone exposure in the Solardomes

The actual ozone concentrations measured within the solardomes over the four week experimental period are shown in Figure 2.6. In the first week of ozone exposure the ozone concentration in one of the solardomes reached 350ppb because of mechanical failure of the mass-flow controller controlling ozone supply to that dome. When average peak ozone concentrations were calculated for the treatment over the four weeks, the peak ozone concentration in week one remains comparable with weeks two, three and four (Table 2.1) and average peak ozone concentrations were within 10% of the target value of 150ppb.

In the solardomes receiving a constant supply of ozone at 20ppb, average weekly ozone concentration remained at 20ppb during peak episodes but dropped to 13-14ppb during background periods. This is because ozone supply to the solardomes

was turned off during measurement periods and the solardomes received charcoal filtered air with less than 10ppb ozone. "Background" ozone concentrations remained around 20-25ppb in the high ozone solardomes and were reduced to 13-14ppb at times when the ozone supply was turned off for entry into the solardomes.

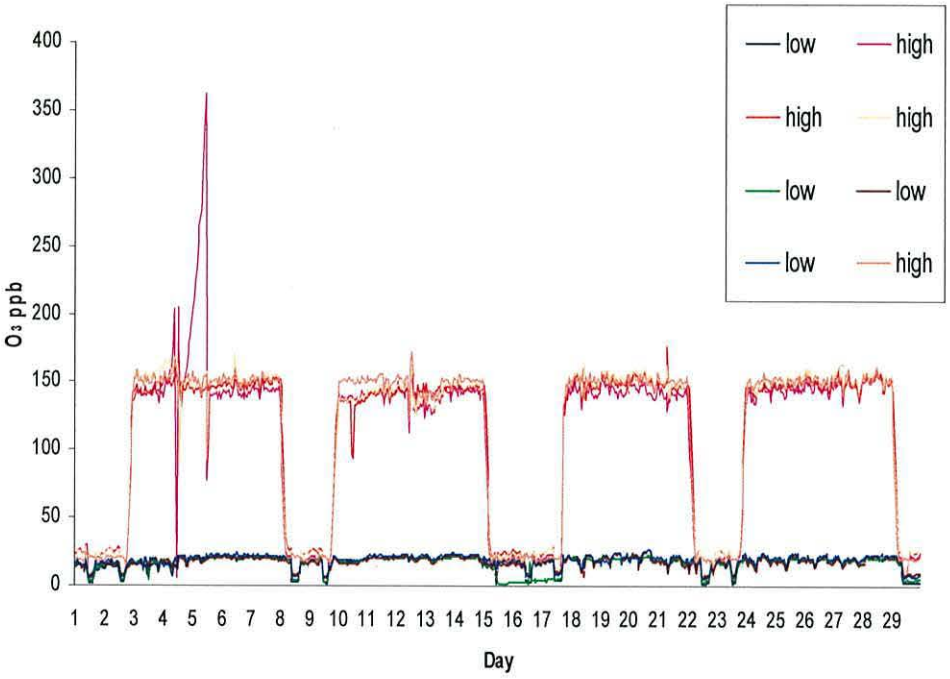


Figure 2.6: The measured ozone concentrations in the solardomes over the 28 days of ozone exposure showing the four peaks of elevated ozone lasting five days in weeks one, two and four and four days in week three.

		average ozone concentration (ppb) ±sd			
		week 1	week 2	week 3	week 4
high ozone	peak	147±3.02	139±3.79	146±3.85	146±2.27
	background	20±0.34	19±1.78	20±1.59	19±1.94
low ozone	peak	19±0.73	20±0.86	20±0.58	20±0.93
	background	13±1.47	14±0.87	13±5.40	13±1.30

Table 2.1: Peak and background ozone concentrations in the solardomes over the 28-day ozone exposure period. Values are the means of the four domes in each treatment and are shown ± 1 standard deviation.

After the four week treatment period the accumulated ozone over a threshold (AOTXppm.h), over 24 hours was calculated with threshold values of 0 and 40ppb. Accumulated ozone exposure increased linearly in the high ozone treatments suggesting that the shortened ozone peak in week three did not affect the overall ozone dose (Figure 2.7). Using the threshold value of 40ppb made no difference to the significance of the results in this experiment so the accumulated ozone dose without a threshold over 24 hours was used. This is because ozone concentrations remained high overnight and this is not taken into account if accumulated ozone in daylight is used.

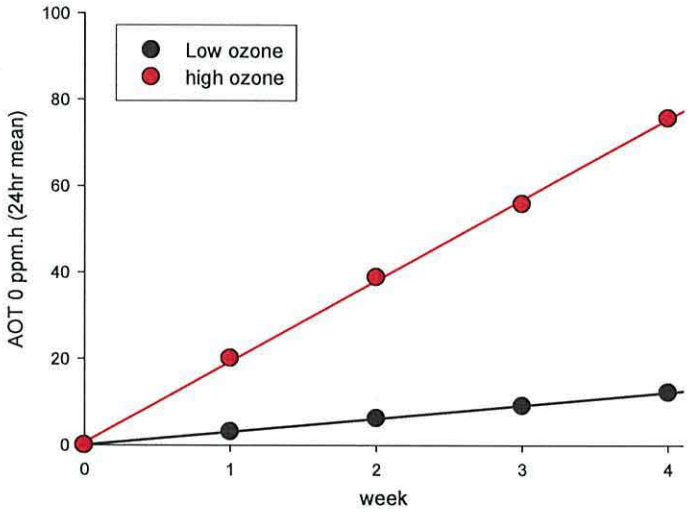


Figure 2.7: $AOT0_{24hr}$ ppmh using the 24 hour mean values for the 28 day exposure period. Points on the graph are the mean of four domes ± 1 SE. (note: SE bars are within the scale of the points.)

2.3.3 Senescence

In general, all of the species included in this experiment showed a tendency towards increased senescence after four weeks of ozone exposure compared to those under control ozone conditions (Figure 2.8).

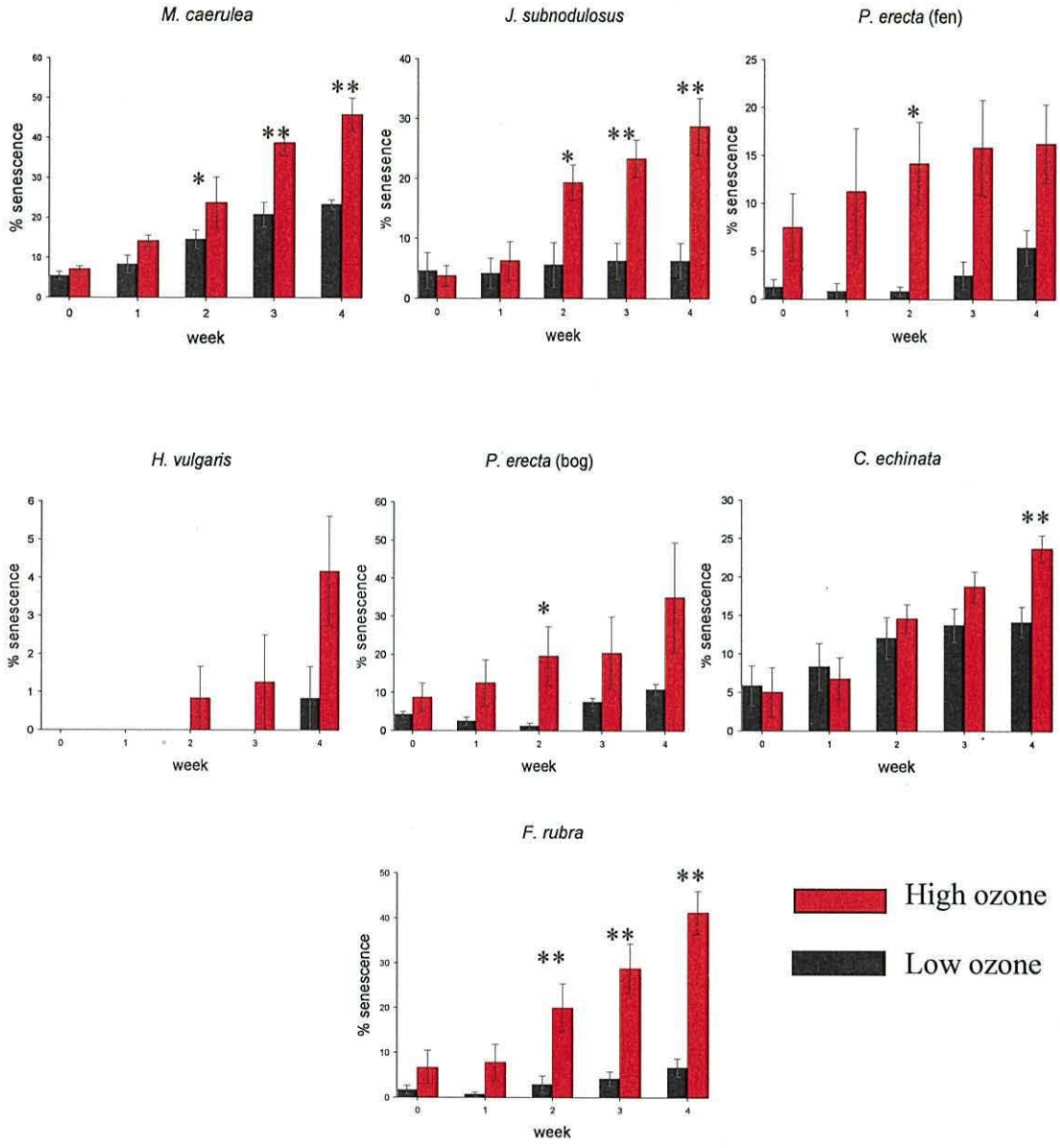


Figure 2.8: Weekly percentage senescence measured on the seven plant species used in the experiment. Statistical tests were performed on arc-sine transformed data and data was back-transformed for presentation. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

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 period through the experiment also being a highly significant factor ($P<0.001$). *P.erecta* plants from the fen 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 significance by the 4th week of the experiment ($P<0.1$) but it did not show any senescence under elevated ozone for 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 significance over the four week experimental period ($P<0.1$) and showed a highly significant effect of time, meaning that senescence increased in plants from both the treatment and the controls over the four week period. 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 linear relationship with AOT0 (Figure 2.9). The only species not to have 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 2.2).

The difference in senescence between plants exposed to low and high ozone can clearly be seen when comparing individuals from the two treatments. The photographs in Figure 2.10 compare plants after four weeks exposure to low and high

ozone.

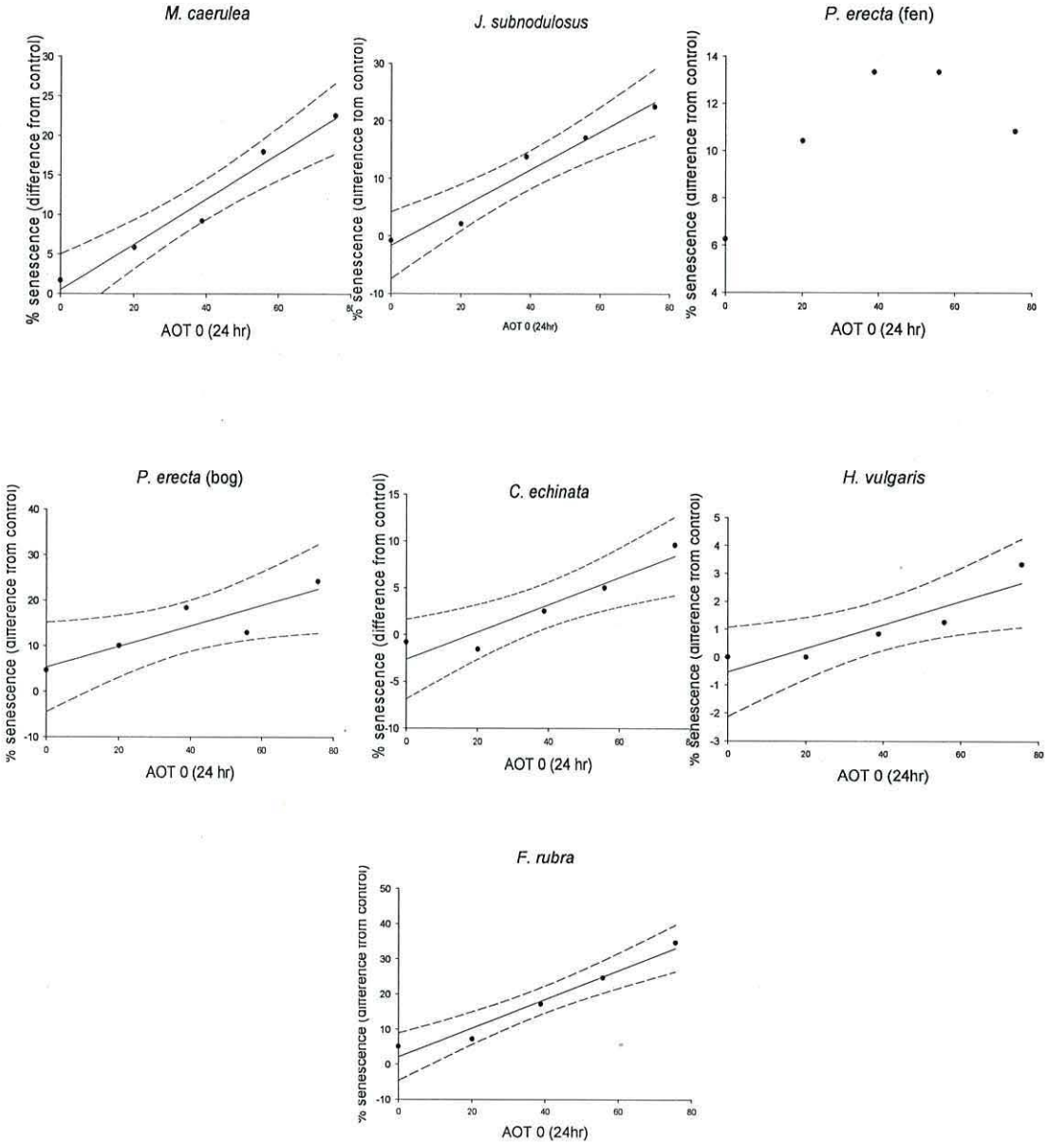


Figure 2.9: Relative senescence after four weeks exposure against the $AOT0_{24hr}$ for the four weeks. Graphs show the regression lines and 95% confidence limits for each species. See Table 2.2 for regression analysis.

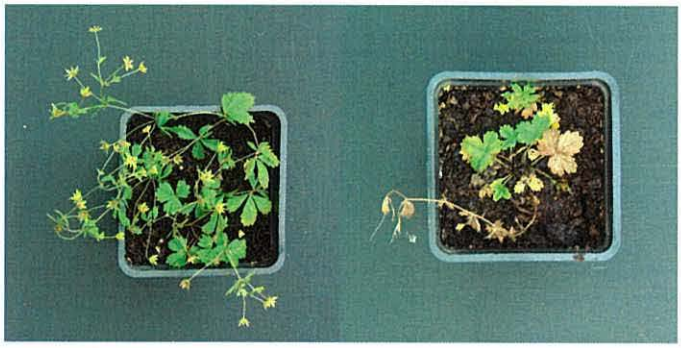
Plant	R^2 value	Regression slope	t-value	Degrees of freedom	P value	% variance accounted for
M. caerulea	0.967	0.286	88.03	1	**0.003**	95.5
J. subnodulosus	0.959	0.329	69.26	1	**0.004**	94.6
P. erecta (fen)	0.442	0.065	2.38	1	0.221	25.5
P. erecta (bog)	0.796	0.227	11.67	1	**0.042**	72.7
C. echinata	0.895	0.146	25.69	1	**0.015**	86.0
H. vulgaris	0.835	0.042	15.18	1	**0.030**	78.0
F. rubra	0.964	0.408	80.07	1	**0.003**	95.0

Table 2.2: Regression analysis for each species for the data shown in Figure 2.9. Significant P values at $P<0.05$ are in bold.

(a) *M. caerulea*

(b)

J. subnodulosus



(c)

P. erecta (fen)

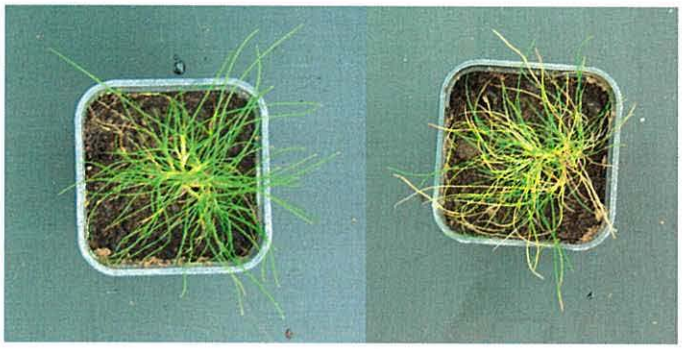
(d)

P. erecta (bog)

(e)

C. echinata

(f) *H. vulgaris*



(g) *F. rubra*

Figure 2.10: Pictures showing plants exposed to low ozone (left picture) and high ozone (right picture) after 28 days ozone exposure.

2.3.4 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 ($P<0.05$). Although none of the other species tested showed a significant difference in fresh weight, dry weight of shoots or roots and above-ground: below-ground biomass ratio (Table 2.3) *P. erecta* plants from the bog and *C. echinata* plants showed a trend towards a difference in the dry weight of their above ground biomass. *P. erecta* exposed to high ozone had dry weights slightly lower than those exposed to low ozone ($P<0.1$). *C. echinata* showed the opposite trend with the dried biomass of plants exposed to high ozone being slightly higher than those exposed to low ozone ($P<0.1$). When the total biomass of each species was tested, only *M. caerulea* showed a significant decrease in total biomass under elevated ozone (results not shown).

	Above ground fresh weight			Above ground dry weight			Root dry weight			Root:shoot ratio		
Species	High ozone	Low ozone	Sig?	High ozone	Low ozone	Sig?	High ozone	Low ozone	Sig?	High ozone	Low ozone	Sig?
M. caerulea	1.91±0.3	2.53±0.3	*	0.85±0.1	1.08±0.1	*	1.44±0.2	1.79±0.2	NS	0.61±0.1	0.63±0.1	NS
J. subnodulosus	1.91±0.2	1.97±0.2	NS	0.52±0.1	0.53±0.1	NS	0.75±0.1	0.95±0.1	NS	0.73	0.58	NS
P. erecta (fen)	0.95±0.1	0.96±0.1	NS	0.36±0.1	0.41	NS	0.52±0.1	0.61±0.1	NS	0.84±0.1	1.28±0.6	NS
P. erecta (bog)	0.72±0.1	0.95±0.1	NS	0.24	0.33±0.1	(*)	0.41±0.1	0.56±0.1	NS	2.09±1.4	0.68±0.1	NS
C. echinata	0.97±0.1	0.75±0.1	NS	0.32	0.25	(*)	0.41	0.32	NS	0.80	0.81±0.1	NS
H. vulgaris	0.88±0.1	1.18±0.2	NS	0.11	0.15	NS	0.19	0.31	(*)	0.65±0.1	0.52±0.1	NS
F. rubra	0.87±0.1	0.87±0.1	NS	0.26	0.3	NS	0.20	0.28	NS	0.48±0.1	1.22±0.2	NS

Table 2.3: The mean biomass for the seven species after four weeks exposure 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. For each analysis there is 1 degree of freedom for ozone and 23 degrees of freedom for the total analysis.

2.3.5 Chlorophyll content

Species tested for their chlorophyll content over the course of the experiment differed in their response to ozone exposure (Figure 2.11).

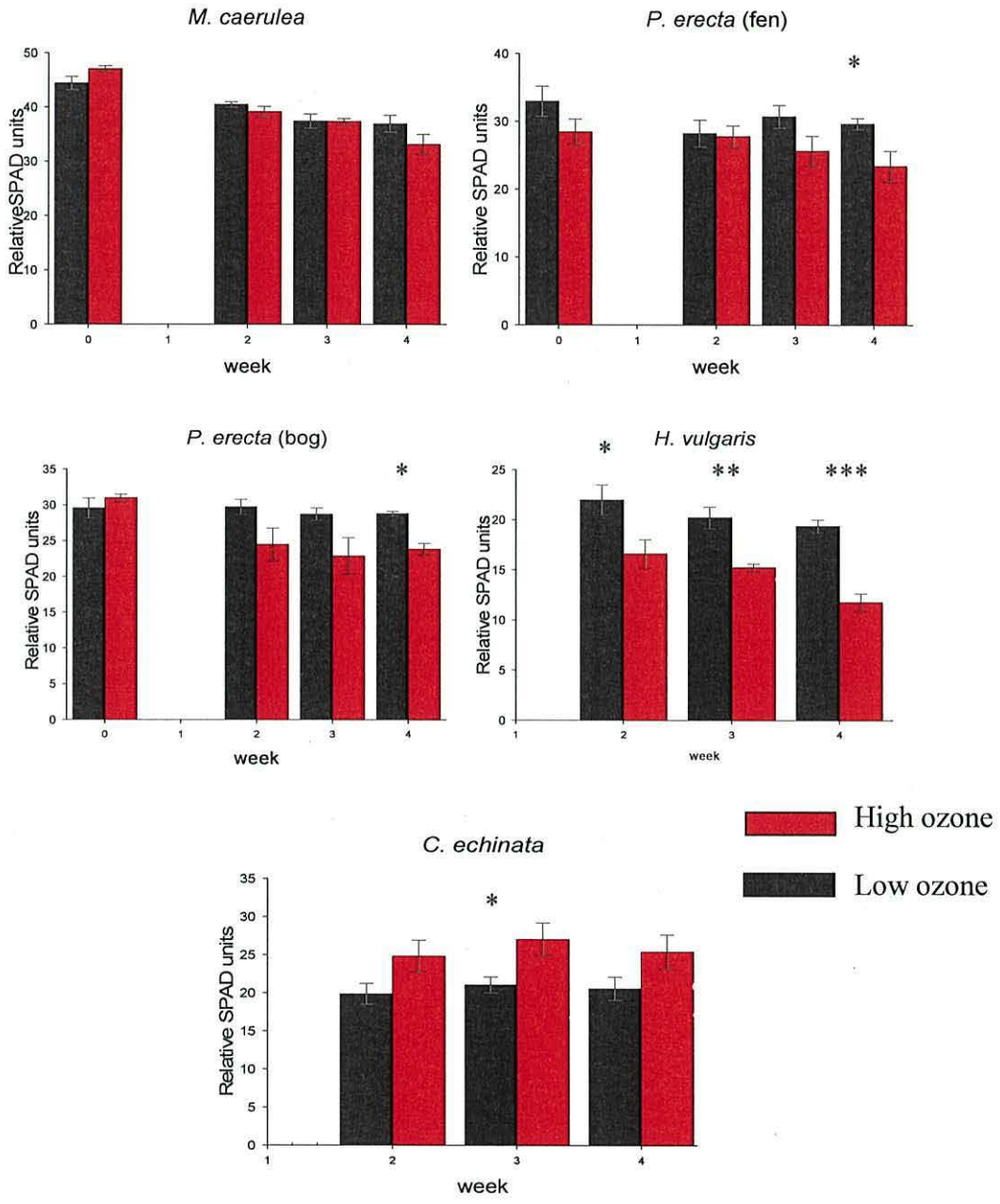


Figure 2.11: Changes in chlorophyll content for five species over the 4 week exposure period. * P<0.05, ** P<0.01, *** P<0.001 For each sampling point there is 1 degree of freedom for ozone and 23 degrees of freedom in total.

M. caerulea showed no significant difference between individuals exposed to high or

low ozone, whereas *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. The leaves of the individuals were too small in weeks zero and one to make measurements so this could be because of a pre-existing difference.

2.4 Discussion

2.4.1 Ozone concentration

Monitoring of the ozone concentrations at the two wetland sites showed that average monthly ozone concentration was consistently higher at Marchlyn Mawr, the upland site, compared to Cors Erddreiniog, the lowland site. This is consistent with previous monitoring that has shown ozone concentrations at various sites in the Alps can vary by up to 30ppb between lowland sites (less than 300m) and Alpine sites (~3000m) (Sandroni et al. 1994). As the difference in altitude between the two sites used in this study is in the region of 600m it is to be expected that concentration differences are considerably less than those found by Sandroni et al. (1994) but the differences are comparable to other sites in the UK where nearby upland and lowland areas are compared. For example the measured overnight difference between ozone concentrations at Great Dunn Fell (hilltop) and Wharley Croft (valley) is approximately 5ppb (Royal Society 2008) (Figure 1.1).

Hourly ozone monitoring at Marchlyn Mawr showed that during the year from November 2005 to November 2006, ozone concentrations were consistently around 40ppb, the threshold point above which damage to plants may be seen (Fuhrer et al. 1997). It is particularly significant that ozone concentrations were higher than 40ppb during the early part of the growing season between April and July meaning that plants growing in upland areas of Snowdonia are exposed to relatively high concentrations of ozone throughout their main growth period. When ozone episodes

occurred, concentrations tended to remain high for several days, a factor that was taken into account when designing the ozone profile used in the experiment.

2.4.2 Senescence and chlorophyll content

Plant senescence has been 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). Premature senescence was seen in six out of the seven species at some point during the 28-day ozone exposure with five of the seven species having a significant increase in senescence by the end of the exposure period. When looking at the relative senescence six of the seven species tested showed a significant linear relationship with $AOT0_{24hr}$ over time. This 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 HeideJorgensen 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 and susceptibility to ozone in general 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). The 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). This means that wetland plants in Northern latitudes are likely to be particularly affected by elevated ozone as wetland plants 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 the *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 in an experiment by

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 likely to be 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 AOT40 (daylight hours) of 18.3 ppm h accumulated over ten weeks, whereas in this experiment the AOT40 (daylight hours) was 24.8 ppm h accumulated over only four weeks.

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*. Healthy leaves of *M. caerulea* showed a reduction in chlorophyll content over the 28 day experiment irrespective of ozone exposure. A reduction in chlorophyll content in leaves exposed to elevated ozone was also found for birch (*Betula pendula*) (Paakkonen et al. 1996) and wild 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.

2.4.3 Plant Biomass

Only one species exposed to ozone showed a significant reduction in above-ground biomass under elevated ozone in this experiment (*M. caerulea*). This is in contrast to

previous published results (Franzaring et al. 2000) that found that the biomass of *M. caerulea* was 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. *C. echinata* plants showed a trend towards an increase in biomass under elevated ozone after the 28 days ozone exposure. 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). This inhibition may have been absent in this experiment because of the short-term nature of the experiment and it could be that it takes a longer period of time 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.

2.4.4 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.

3 Effects of short term ozone exposure on carbon cycling in wetlands

3.1 Introduction

Anthropogenic changes in the climate have been occurring since the industrial revolution and of particular concern is the increase in tropospheric ozone (NEGTAP 2001). Coupled with this is an increase in the deposition of nitrogen compounds (Galloway and Cowling 2002), which is of particular concern in areas of low nutrient input such as wetlands. The UK climate is predicted to move towards hotter, drier summers and this has the potential to increase the frequency of drought events. Wetlands contain many plants and micro-organisms adapted to water-logged conditions so the effect of a reduction in available water could have a particularly negative effect on these ecosystems.

Wetland plants have been found to be particularly sensitive to elevated concentrations of tropospheric ozone (Chapter 2) and it is possible that the impacts of ozone on plant vitality and growth could affect carbon storage and cycling within wetland ecosystems as they have all been found to be linked (Zak et al. 1993). Potential ozone effects on methane and carbon dioxide fluxes from wetlands are reviewed in Section 1.4.

There have been relatively few studies on the effects of elevated ozone on carbon cycling in wetlands and those that have taken place have found conflicting results. Studies have identified either a large increase in methane production under elevated ozone (Lloyd 2004), a slight increase (Morsky et al. 2008; Niemi et al. 2002), no effect (Morsky et al. 2008; Rinnan et al. 2003) or a decrease (Morsky et al. 2008). Carbon dioxide fluxes show a similar disparity in their results; Neimi et al. (2002) showed a transient increase in dark ecosystem respiration but no effect on net ecosystem exchange, while Rinnan et al. (2003) found that elevated ozone reduced net ecosystem exchange and increased dark respiration. When investigating the effects of elevated ozone on gas fluxes from a meadow ecosystem, Kanerva et al. (2007) found

no effect of elevated ozone on methane or carbon dioxide fluxes over one growth season of exposure. There have only been a few studies assessing the effect of elevated ozone on the activity of micro-organisms and their extra-cellular enzymes. Larson et al. (2002) found that elevated ozone had no effect on phenol oxidase activity in forest soils while studies on microbial biomass and community structure have found either a decrease under elevated ozone (Islam et al. 2000b) or no effect (Dohrmann and Tebbe 2005). A long-term study looking at the impacts of elevated ozone on enzyme cycling in forest soils found a 25% reduction in the activity of beta glucosidase (Chung et al. 2006).

Very few studies have assessed the effect of elevated ozone on dissolved carbon in the pore water of wetland areas. Jones et al. (2009) found that elevated ozone exposure reduced the concentration of dissolved organic carbon compared to a control ozone treatment. The nature of dissolved carbon may also be changed under elevated ozone as a result of the effect of ozone on vascular plants. In response to oxidative stress many plants produce protective phenolic compounds (Biolley et al. 2002; Liu et al. 2005; Peltonen et al. 2005; Saleem et al. 2001). Since these compounds can be exuded into the below ground system, it is possible that phenolic compounds may be increased in pore water as a result of ozone exposure.

Drought has been found to have either a protective or additive effect on ozone damage to forest trees depending on the timing and duration of ozone exposure and drought (Matyssek et al. 2006). If ozone exposure precedes drought, stomatal functioning can be damaged, thus decreasing the ability of the plants to conserve water during periods of drought (Mills et al. 2009; Wilkinson and Davies 2009). However, if a drought event occurs first, the closure of plant stomata could have a protective effect against ozone uptake. This could affect the amount of carbon dioxide taken up by wetland plants, thus potentially changing the ability of wetlands to act as a sink for carbon dioxide.

Lowering of the water-table has been found to have a direct effect on methane emission from wetlands. Methane production is an obligately anaerobic process and the influx of oxygen into pore spaces as the water-table falls suppresses microbial

activity and reduces methane production (Dowrick et al. 2006; Freeman et al. 1993; Whalen and Reeburgh 2000). Carbon dioxide efflux was found to increase under drought conditions (Freeman et al. 1993), possibly as a result of increasing aerobic decomposition which has carbon dioxide as its end point, rather than anaerobic decomposition which results in the production of various, more complex carbon molecules. Lowering the water-table could also have an impact on DOC concentrations in pore water as if aerobic decomposition increases, the amount of DOC could be reduced as it would be broken down to carbon dioxide.

The effects of lowered water-table or increased drought frequency have been found to increase beta glucosidase and phenol oxidase activity in wetlands (Fenner et al. 2005). Increased phenol oxidase activity is likely to be because of the influx of oxygen into the pore spaces left by the drop in the water-table allowing the enzyme to function (Freeman et al. 2001b). The increase in beta glucosidase activity is likely to be as a result of the drop in phenolic compounds as phenol oxidase activity increases (Freeman et al. 2004b). However, this is not always the case, as earlier results had found that drought increased the activity of beta glucosidase but not phenol oxidase (Freeman et al. 1996). If the drought regime is very severe beta glucosidase activity is negatively affected; for example Sardans and Penuelas (2005) found that drought reduced enzyme activity by up to 80% in a Mediterranean forest ecosystem.

Although nitrogen is a vital plant nutrient and is limiting in many low-nutrient systems such as wetlands, excess concentrations are toxic to plants (Lee and Caporn 1998). As oligotrophic systems, wetlands contain species adapted to low levels of nitrogen availability and an increase in its availability has been found to cause a change in species composition (Bobbink 1998). This could cause a change in wetland functioning, especially as nitrogen has been found to decrease the root: shoot ratio (Bobbink 1998), potentially meaning less carbon is transported below ground and less carbon is available for microbial respiration due to reduced exudation.

Long-term nitrogen inputs have not been found to have any effect on DOC fluxes from peatland catchments (Worrall et al. 2006a) but in forested ecosystems elevated nitrate deposition increased both total DOC and phenolic compounds being leached

out of the system (Smemo et al. 2007). Previous experimental results had found that nitrogen deposition can either increase (Pregitzer et al. 2004) or decrease (Park et al. 2002) DOC leaching from soil solution.

The addition of nitrogenous compounds to semi-natural ecosystems has been found to have a variety of effects on enzyme activity. Nitrogen fertilisation has been found to decrease phenol oxidase activity (DeForest et al. 2005) or have no effect (Allison and Vitousek 2004; Blackwood et al. 2007). The addition of nitrogen in the form of nitrate caused a non-significant decrease in beta-glucosidase activity (DeForest et al. 2005) but additions of more complex nutrients caused an increase in enzyme activity (Allison and Vitousek 2005). Over a longer-term experiment, additions of nitrate caused a decrease in soil respiration that was not due to changes in root growth or respiration (Burton et al. 2004) suggesting microbial activity was reduced. Recent results have shown that the effect of increased nitrate deposition on microbial activity may depend on the litter quality. Leaf litter high in polyphenolics exposed to increased nitrate showed a reduction in phenol oxidase activity whereas leaf litter that contained less than 25% polyphenolics showed an increase in phenol oxidase activity when exposed to increased nitrate (Waldrop and Zak 2006).

The aim of this experiment was to determine the effects of short-term elevated ozone on carbon cycling in wetland mesocosms. The hypotheses were that: elevated ozone will reduce carbon dioxide uptake and increase methane release, drought will reduce methane fluxes irrespective of ozone exposure, phenolic compounds in pore water will increase and drought will increase phenol oxidase activity.

3.2 Methods

3.2.1 Mesocosm collection

Two locations were used for the collection of peat mesocosms: Cors Erddreiniog; a low-lying fenland site on Anglesey (SH 465 822) and Marchlyn Mawr; an upland bog site in Snowdonia (SH 611 624). The two sites were chosen as they are typical of the two main peat forming wetland types found in the UK. See section 2.2.1 for the site

descriptions. Twenty four mesocosms (10.5cm diameter by 20cm depth) were taken from each wetland following the method of Freeman et al. (1993). Briefly, this method involves using a PVC pipe as the template, cutting round the outline with a long knife, pushing the pipe into the peat and continuing until the pipe surrounds the peat mesocosm. It is then dug out, ensuring the peat remains within the mesocosm liner. The method ensures that mesocosms are kept intact, with the peat horizons maintained at the same level as in the bog. It also ensures that the anoxic layers of peat are not exposed to air, so methanogenesis and other anaerobic processes are not interfered with. Once collected, the mesocosms were semi-sealed around the base with plastic sheeting to ensure that peat could not fall out of the bottom of the plastic liner but a certain amount of water could be exchanged between the mesocosm and the surrounding water to prevent them drying out. Prior to being placed in the solardomes, mesocosms were kept in individual buckets with the water-table at the surface of the mesocosms to ensure they remained waterlogged. These were kept in a sheltered outdoor location for one week until the start of the experiment. Mesocosms from Cors Erddreiniog were dominated by *Juncus subnodulosus* and *Molinia caerulea* while mesocosms from Marchlyn Mawr had a covering of *Sphagnum* with some *Carex echinata* germinating during the experiment.

3.2.2 Experimental design

Peat mesocosms were exposed to elevated ozone at the CEH solardome facility at Abergwyngregyn from 22nd August 2006 to the 19th September 2006. This experiment was designed to see how carbon cycling within peat mesocosms responded to a relatively short-term, high dose of ozone exposure. The experiment ran at the same time as the individual species experiment described in Chapter 2 and the mesocosms were exposed to the same ozone conditions with four solardomes receiving elevated ozone and four receiving low ozone (Section 2.2.3).

Three mesocosms from each wetland type were placed in each dome with the following arrangement for the bog and fen mesocosms in each dome. One mesocosm was designated a control with the water-table kept at the surface; one mesocosm had the water-table lowered by drilling holes in the side of the plastic liner and the side of

the buckets to keep the water-table 5cm below the peat surface; and the third mesocosm had a normal water-table level but had ammonium nitrate solution added to it to simulate the increase in nitrogen deposition across the UK (Bobbink 1998). Nitrogen was added at a concentration designed to simulate a deposition rate of 100kg N/ha/yr and this was added in the form of 100ml of ammonium nitrate solution each week. The mesocosms were randomly assigned a position within each dome and were rotated each week to reduce any effects of within dome variation.

3.2.3 Mesocosm measurements

Water and gas samples were taken from the peat mesocosms at the start of the experiment and weekly during the four-week exposure period. Gas samples were taken by placing a two litre transparent, plastic headspace over the mesocosms and inserting it between the peat and the liner to create an airtight seal. A sample of the background gas was taken at the moment of capping and a second sample of the gas within the headspace was taken after one hour. Gas samples were analysed for the concentration of carbon dioxide and methane 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. Briefly, gas samples were pressurised with a known amount of nitrogen in the headspace autosampler (Turbo-Matrix) and samples were injected into the GC at 23.2psi with nitrogen carrier gas. Once in the GC, samples were passed through a Poropak QS ceramic column to separate out different molecular weight compounds and, as the sample entered the detector, it was mixed with hydrogen and burned in air. This generated ions, which are collected and measured by the detector. Ion collection within the detector was enhanced by a polarised electric field applied across the tip of the detector (Clarus 500 GC users' guide). Hydrogen flow was set at 45ml min^{-1} and airflow was set to 450 ml min^{-1}. The FID (flame ionisation detector) temperature was 375°C. The GC was calibrated using bottled gas with a known concentration of carbon dioxide and methane (BOC gases) and this gas was used as a quality control (QC) at set points throughout each sample run.

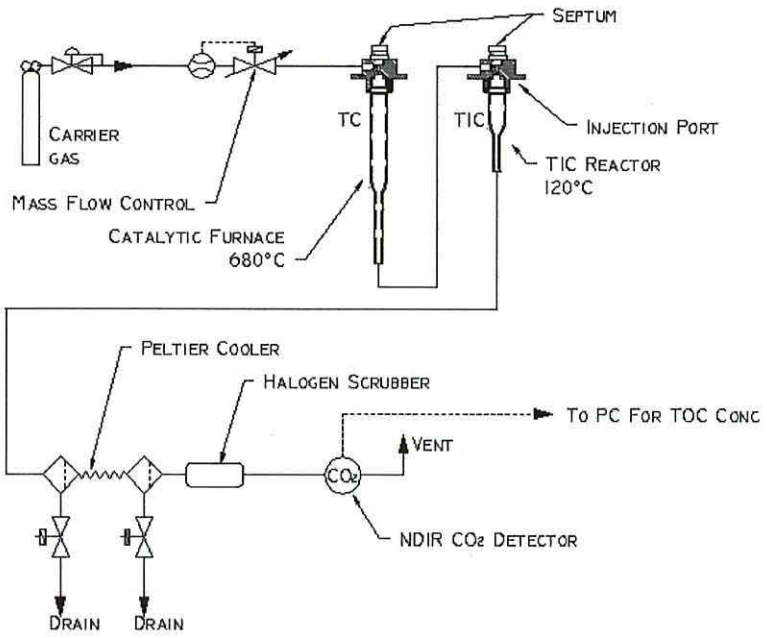


Figure 3.1: Schematic showing how the Thermalox TC works (from www.analyticalsciences.com). Water samples enter through the injection port, pass through the furnace, over a platinum catalyst and the output of carbon dioxide is measured with ans infra-red gas analyser.

Water samples were taken weekly from 10cm depth through capillary tubing 1mm in diameter with a glass wool filter to remove coarse material and filtered through a 5μm filter then a 0.45μm filter (Millipore) to remove particulate matter and micro-organisms. Samples were refrigerated prior to analysis. Water samples were analysed for dissolved total carbon (TC) and phenolic compounds. TC was measured using a Thermalox^{TM} 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. The carbon dioxide was detected and measured using a non-dispersive infra-red detector (Figure 3.1). Standards were made up using Potassium Hydrogen Phthalate dissolved in distilled, de-ionised water and known concentrations were used to create the calibration curve and for QC standards at set points throughout each sample run. TC was measured rather than DOC because of methodological limitations on the available machine. In acidic water samples there are generally very few inorganic carbonate compounds, so the concentrations of TC can approximate to DOC. This is

shown in Figure 3.2 where total and inorganic carbon concentrations from pore water samples taken at Marchlyn Mawr between February and July are compared. At each sampling time point, the concentration of IC was less than 5% of the TC.

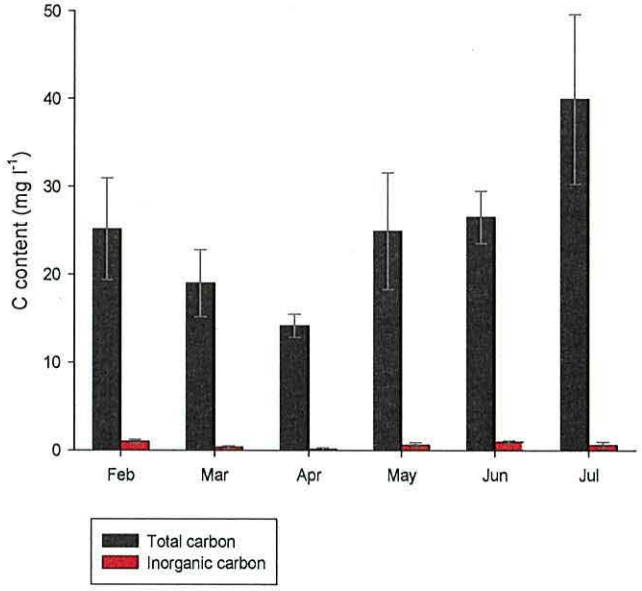


Figure 3.2: Total and inorganic carbon content of pore water samples taken from Marchlyn Mawr between February and July 2006 showing that inorganic carbon comprises less than 5% of the total carbon concentration present in the pore water at each sampling point.

The concentration of total soluble phenolics was measured using Folin-Ciocalteau reagent following the methods of Box (1983) which measures polyphenolic compounds including phenolics, tannins and lignin.

At the end of the exposure period, peat samples were taken from the surface and from 10cm depth and the activities of the enzymes phenol oxidase and beta glucosidase was measured. Phenol oxidase activity was measured following the method of Pind et al. (1994). A $1cm^3$ block of peat from each sample was homogenised in 9ml of milliQ water and 300µl of the homogenate was transferred to two centrifuge tubes to which 450µl of milliQ water was added. A 750µl aliquot of water was added to the control centrifuge tubes and 750µl of the model substrate L-dihydroxyphenylalanine (L-DOPA) was added to the active centrifuge tubes. These were stored at 25°C (the

average midday temperature in the solardomes) for nine minutes and the reaction was terminated by centrifuging the tubes at 10 000 rpm for 5 minutes. The absorbance at 460nm was measured using a Fluostar Galaxy Spectrophotometer. Final phenol oxidase activity was expressed as μmol 2,3-dihydroindole-5,6-quinone-2-carboxylate (dicq) per minute per gram of peat (Pind et al. 1994).

Beta glucosidase activity was measured following the methods of Freeman et al., (1995). This method involves the use of the fluorogenic substance methylumbelliferone to which a range of compounds can be chemically attached. Substrates are dissolved in methylcellusolve as this increases the solubility of the compound. To correct for the colouration of phenolic compounds present in peat samples Quench correction is used which involves making up replicate standards of the sample mixtures but without the fluorogenic substrates (Freeman et al. 1995). 1cm^3 peat from each sampling depth in each mesocosm was homogenised for 30 seconds with 7ml of methylumbelliferone and left for 45 minutes at field temperature. Fluorescence of the samples was measured using a Fluorstar Galaxy Spectrophotometer and Quench corrected using the replicate standards for each treatment.

3.2.4 Statistical Analysis

Results were analysed using GENSTAT version 7. Differences were taken as being significant at $P<0.05$ and showing a trend towards significance at $P<0.1$. The error bars in the Figures are the standard errors of the means.

The experimental set-up consisted of a 2*3 factorial design. General analysis of variance was performed with the treatment structure of ozone*wetland*treatment and the block structure being the dome level. General analysis of variance was carried out on all data on a week by week basis. This was then repeated using the values measured at the start of the experiment as a co-variate to account for any pre-existing variability in the samples. Finally, a repeated measures analysis was carried out to look for any variability in treatment effect over time. The percentage and actual change between the start and the end of the experiment for water and gas samples

respectively was calculated and differences between the treatment types was analysed in GENSTAT version 8 as above.

For the results of the enzyme activities a split, split plot design was used with three levels of strata; the dome, the mesocosm and the depth within the mesocosm. General analysis of variance was performed with a treatment structure of ozone*wetland*treatment*depth. The block structure used was dome/mesocosm. Further analysis of differences within each wetland and treatment was carried out in SPSS version 12. For each wetland and depth, analysis of variance and Tukey's HSD post-hoc test were carried out to analyse differences between each treatment (control/drought and control/nitrogen) and differences caused by high ozone compared to the low ozone control.

3.3 Results

3.3.1 Methane

Although there was a large increase in methane emissions from mesocosms exposed to elevated ozone over the four week exposure period there was no significant effect on the weekly gas exchange of methane irrespective of the statistical test used because of the variability within treatments (Figure 3.3). For methane the wetland type had a significant effect in weeks three and four with emissions from the fen mesocosms being significantly higher than those from the bog mesocosms in both weeks ($P<0.05$). This was unchanged by the addition of baseline methane fluxes as a co-variate. Although methane emissions from mesocosms subjected to drought were lower than those from the water-logged mesocosms by the end of the experiment, large standard errors meant this was not significant on a week-by-week basis (Table 3.1). A repeated measures test looking at effects over time found no significant effect of ozone, but found that wetland type, the treatment applied and the time through the experiment had a significant effect on methane emissions. In general, over all of the mesocosms used in the experiment, methane emissions increased over the four week period ($P<0.001$). The fens showed higher rates of methane emission than the bogs ($P<0.05$) and drought caused a significant reduction in methane emissions irrespective of wetland type and ozone exposure regime ($P<0.05$). This difference was only

noticeable when using all of the results in the repeated measures analysis as variation on a week-by-week basis was too high.

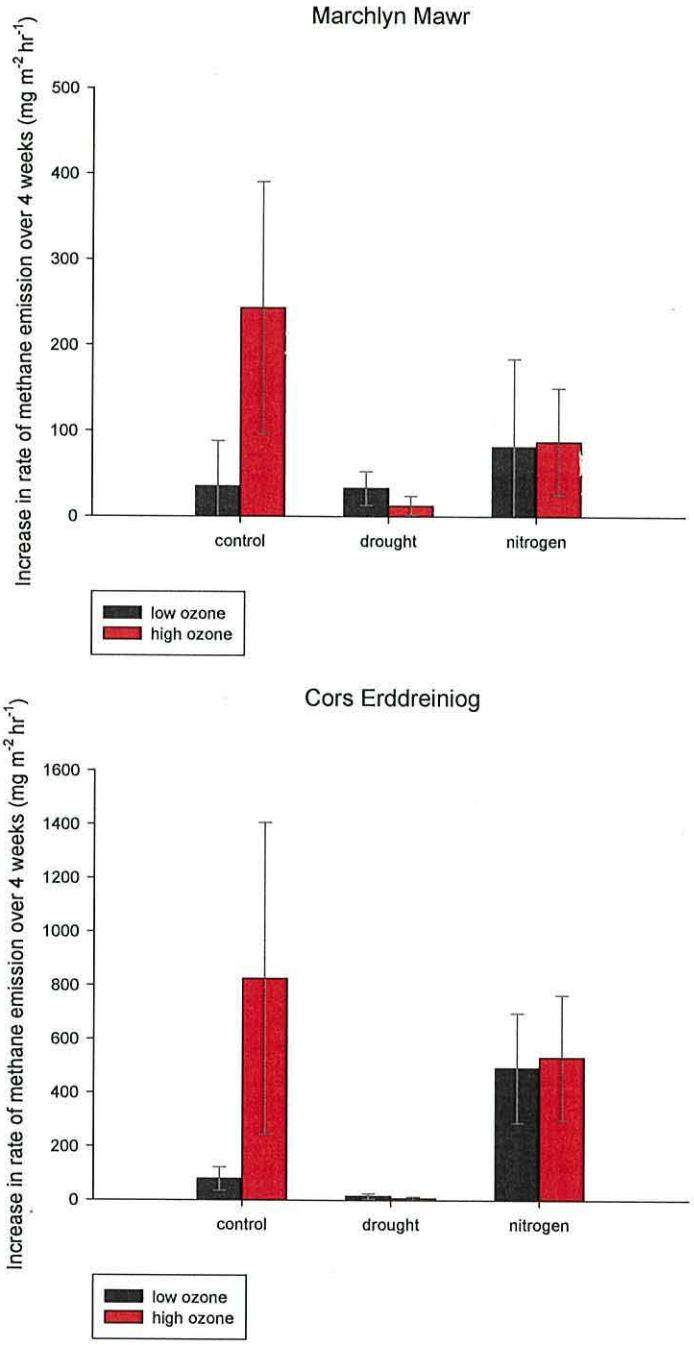


Figure 3.3: The change in the rate of methane emissions after four weeks of ozone exposure for mesocosms from Marchlyn Mawr (top) and Cors Erddreiniog (bottom). P>0.05 in all cases.

Fen	week 0		week 1		week 2		week 3		week 4	
treatment	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone
control	1.50±1.27	1.95±1.24	-15.4±24.5	134±121	2.42±1.29	20.2±15.0	534±386	506±290	80.6±42.8	828±580
drought	0.68±0.81	1.27±0.59	0.49±0.63	0.60±0.51	0.02±0.19	196.1±75.9	7.88±4.76	6.57±4.61	15.3±9.43	8.22±5.53
N	1.59±0.87	1.13±2.64	8.72±4.47	65.3±35.7	71.0±43.0	0.52±0.46	347±249	591±239	495±204	534±232
significance										
ozone	NS		NS		NS		NS		NS	
drought	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N	NS	NS	NS	NS	NS	*	NS	NS	NS	NS
Interactions										
Ozone* drought	NS		NS		NS		NS		NS	
Ozone* nitrogen	NS		NS		*		NS		NS	

Bog	week 0		week 1		week 2		week 3		week 4	
Treatment	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone
control	68.4±64.1	-0.74±2.58	7.16±5.55	28.1±14.7	240.9±233	167±159	77.8±37.0	90.7±52.7	104±44.7	243±149
drought	1.08±0.61	6.68±4.96	6.50±4.72	18.5±13.5	17.9±10.3	6.93±7.21	61.4±45.4	13.7±12.8	34.0±19.0	19.2±9.68
N	1.57±1.63	2.83±1.21	-0.74±15.49	30.1±12.0	6.38±11.0	30.2±17.8	192±171	34.6±12.3	82.9±104	90.2±62.7
significance										
ozone	NS		NS		NS		NS		NS	
drought	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions										
Ozone*drought	NS		NS		NS		NS		NS	
Ozone*nitrogen	NS		NS		NS		NS		NS	

Table 3.1: Methane emissions over the four weeks of ozone exposure. Values are given as mg m^{-2} hr^{-1} methane emission. * $P<0.05$, NS not significant.

Fen	week 0		week 1		week 2		week 3		week 4	
treatment	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone
control	65.0±115	18.0±234	98.0±329	2791±1415	102±117	544±422	1030±651	2490±908	-465±278	780±958
drought	78.0±188	19.0±86	137±172	-31.0±69.0	35.0±160	-640±208	946±624	-102±258	-403±185	-499±169
N	60.0±136	173±299	638±623	1550±634	-107±245	1410±985	1780±1070	1870±1150	-294±714	641±646
significance										
ozone	NS		NS		NS		NS		NS	
drought	NS				NS		NS		NS	
drought	NS	NS	NS	(*)	NS	NS	NS	NS	NS	NS
N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions										
ozone*drought	NS		NS		NS		NS		NS	
ozone*nitrogen	NS		NS		NS		NS		NS	

Bog	week 0		week 1		week 2		week 3		week 4	
treatment	control	elevated ozone	control	elevated ozone	control	elevated ozone	control	elevated ozone	Control	elevated ozone
control	306±211	-473±325	-523±219	-1020±299	-375±381	-707±206	-120±62	-369±174	-1240±96	-1080±312
drought	-128±233	120±178	180±478	-506±193	-480±281	-157±215	-480±80	-75±161	-765±252	-515.±75
N	-181±257	-60.0±161	-851±448	-769±175	0.56±191	-371±352	-325±233	-665±145	-1050±146	-628±306
significance										
ozone	NS		NS		NS		NS		NS	
drought	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interactions										
ozone*drought	NS		NS		NS		NS		NS	
ozone*nitrogen	NS		NS		NS		NS		NS	

Table 3.2: Carbon dioxide exchange over the four weeks of ozone exposure. Values are given as mg m^{-2} hr^{-1} carbon dioxide exchange.

NS = not significant, (*) $P<0.1$.

Although there was no significant difference between methane emissions from mesocosms exposed to high and low ozone because of the variability in the data there did tend to be more methane emitted from wetland mesocosms exposed to high ozone (Figure 3.3). Wetland type did have a significant effect on methane emissions with fens showing a significant increase in methane emission rates over the four weeks ($P<0.05$). Treatment type showed a trend towards having a significant effect on the difference in methane emissions between week zero and week four ($P<0.1$) with mesocosms that had received a lowered water-table having lower rates of methane emission.

3.3.2 Carbon dioxide

Rates of carbon dioxide exchange also did not show a significant effect of ozone exposure although the wetland type showed a significant effect from week one onwards. Bog mesocosms showed an uptake of carbon dioxide over the exposure period whereas for weeks one, two and three the fen mesocosms showed a net emission of carbon dioxide and in week four they showed a low rate of uptake in comparison to the bogs ($P<0.005$) (Table 3.2). By the end of the first week of ozone exposure the interactions between ozone and wetland type and wetland type and treatment applied were significant ($P<0.05$) but this effect was transient and had disappeared by week two. In week one, bog mesocosms showed a net uptake of carbon dioxide whereas fen mesocosms showed a net emission in both ozone exposures. Within fens, mesocosms exposed to elevated ozone emitted approximately 20 times the amount of carbon dioxide compared to mesocosms exposed to low ozone ($P<0.05$ for the ozone-wetland interaction). In contrast, bog mesocosms took up nearly double the rate of carbon dioxide when exposed to elevated ozone. The wetland-treatment interaction showed that fens emitted carbon dioxide, whereas bogs were net sinks ($P<0.05$). Over the three treatments an effect of drought was seen, with fen mesocosms with a lowered water-table emitting less carbon dioxide but bog mesocosms with a lowered water-table taking up less carbon dioxide ($P<0.05$). The addition of ammonium nitrate did not appear to affect carbon dioxide emissions from the mesocosms.

The use of repeated measures to look at effects over time found a significant effect of wetland type and time and the interaction between the two ($P<0.001$). In general carbon dioxide uptake increased over the four week experimental period and bogs showed an overall uptake of carbon dioxide whereas fens showed a net output of carbon dioxide.

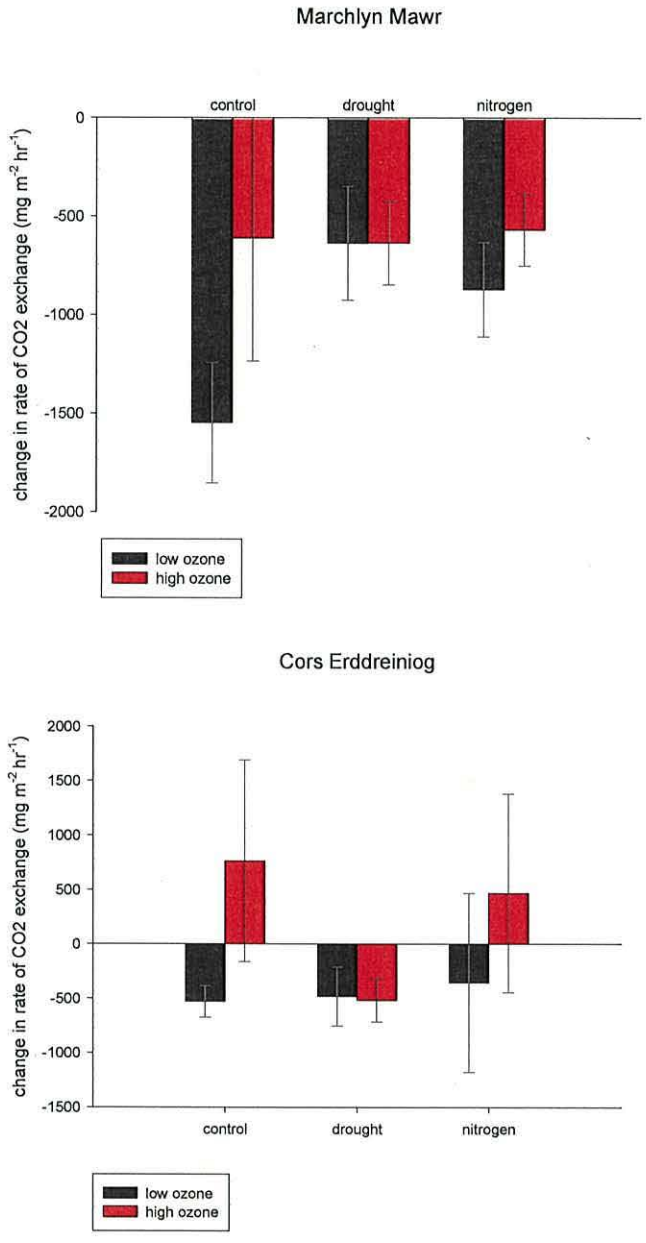


Figure 3.4: The change in the rate of carbon dioxide after four weeks of ozone exposure for mesocosms from Marchlyn Mawr (top) and Cors Erddreiniog (bottom).

When the difference in rate of gas exchange between the start of the experiment and the end was analysed, ozone exposure showed a trend towards having a significant

effect on carbon dioxide exchange ($P<0.1$) (see Figure 3.4) with more uptake in mesocosms exposed to low ozone concentrations compared to mesocosms exposed to high ozone concentrations over all of the data. Wetland type also showed a significant effect with more carbon dioxide being taken up by bog mesocosms over all the treatment types ($P<0.05$).

3.3.3 Dissolved carbon

Total carbon (TC) concentrations in pore water samples were not significantly affected by elevated ozone over the four week experimental period. Wetland type had a significant effect on TC concentrations in weeks three and four with fens having higher concentrations of TC in the pore water ($P<0.01$). Wetland type and treatment applied had a significant interactive effect in weeks three and four with pore water TC concentrations being lower in bog mesocosms subjected to a lowered water-table compared to control values but higher in fen mesocosms subjected to a lowered water-table when compared to the control treatment. This effect occurred irrespective of ozone exposure applied. The effects of ozone exposure, wetland type and treatment applied on TC concentrations were not altered by the addition of initial TC concentrations as a co-variate to the statistical analysis. When the percentage change of TC present in pore water was calculated over the four weeks ozone concentration and treatment type did not have a significant effect but wetland type did ($P<0.05$) with fens showing a higher percentage increase in TC than bogs over all treatment types and ozone levels (Figure 3.5). The ozone-wetland interaction showed a trend towards significance ($P<0.1$) with elevated ozone reducing the concentration of TC in the pore water in both wetland types but to a far greater extent in the fens. This was irrespective of treatment type received by the mesocosms.

The concentration of phenolic compounds in the pore water was not significantly affected by elevated ozone. However, wetland type did have a transient effect in week two ($P<0.05$) and treatment applied had an effect in week four ($P<0.05$) although this effect was removed by the addition of phenolic concentrations at the start of the experiment as a co-variate. In week two, phenolic concentrations were significantly higher in water samples from fen mesocosms when averaged over all

ozone exposures and treatments applied. In week four, the effect of drought and of ammonium nitrate addition was to reduce phenolic concentration compared to the control. Repeated measures analysis showed a significant effect of wetland type ($P<0.05$) and time ($P<0.001$).

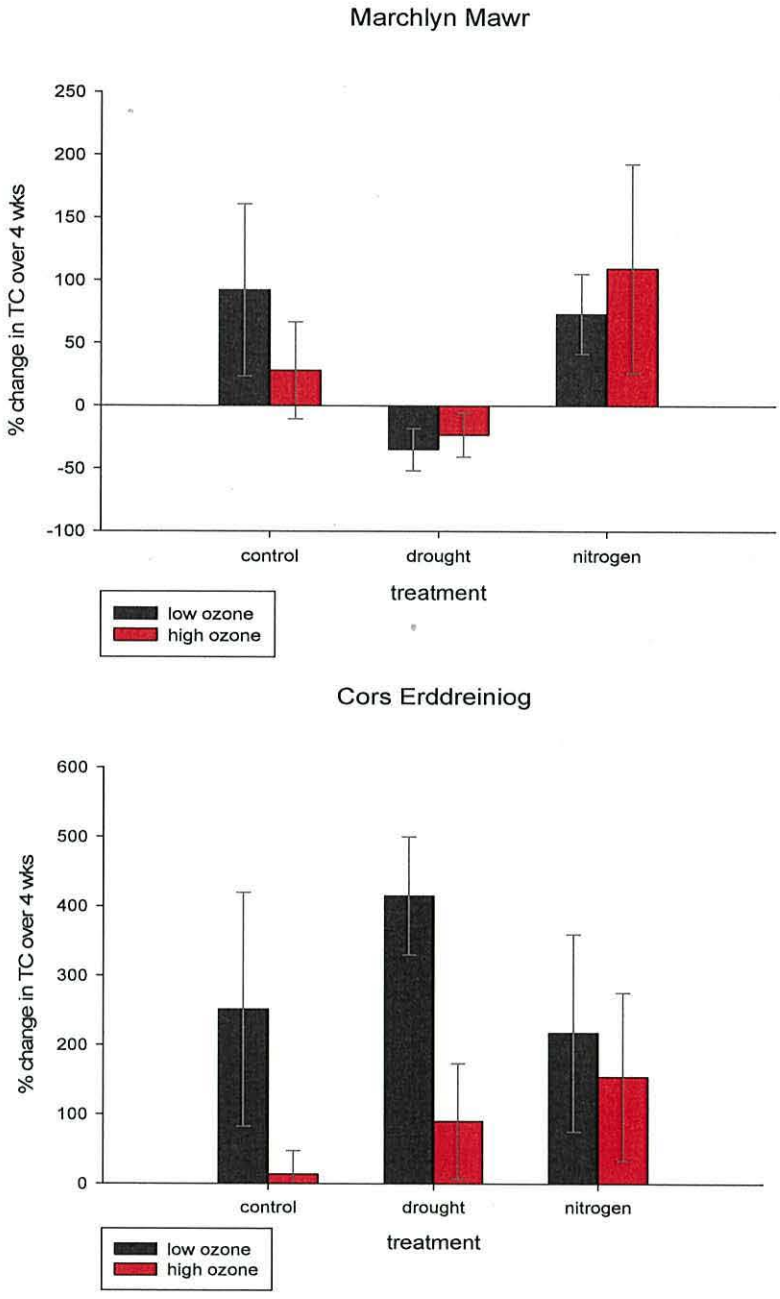


Figure 3.5: % change in total carbon content of pore water from mesocosms from Marchlyn Mawr (top) and Cors Erddreiniog (bottom) measured in mg l^{-1}.

In general, fens had a higher concentration of phenolic compounds present in the pore

water. Over the four week experimental period phenolic concentrations showed a trend towards increasing concentration. When the percentage change in the concentration of phenolic compounds over the four weeks was analysed ozone concentration and treatment type did not have any effect but wetland type showed a trend towards significance ($P<0.1$) with fens having a greater percentage increase in phenolic concentration than bogs (Figure 3.6).

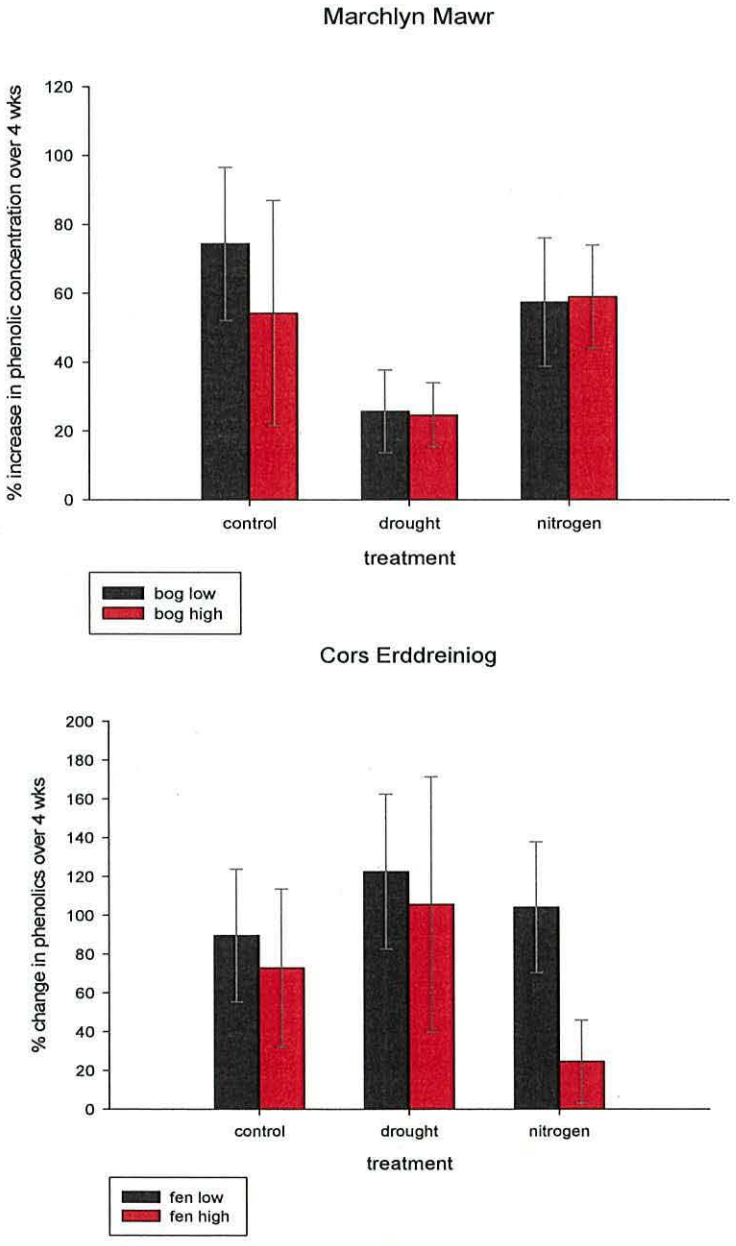


Figure 3.6: % change in phenolic content of pore water from mesocosms from Marchlyn Mawr (top) and Cors Erddreiniog (bottom) measured in mg l^{-1}.

3.3.4 Enzyme activity

Beta glucosidase activity was significantly affected by ozone over the four week experimental period (Table 3.3). Irrespective of wetland type or sub-treatment (watered, droughted or nitrogen addition) elevated ozone caused beta glucosidase activity to increase ($P<0.05$). The depth of the soil sample within the mesocosm also had a significant effect ($P<0.001$) with surface samples showing higher activities than those from 10cm depth. The interaction between ozone and depth was also significant ($P<0.05$) with peat samples from the surface of mesocosms exposed to elevated ozone showing the highest beta glucosidase activity. Wetland type and depth also showed a significant interaction with samples from the fens having a mean surface activity over three times that of bog samples ($P<0.001$). However, at 10cm depth, bog samples showed enzyme activities that were an order of magnitude higher than at the surface. The interaction between ozone exposure, wetland type and peat depth also showed a significant interaction: samples from the surface of fen mesocosms showed higher levels of activity at both ozone levels but samples from bogs showed higher levels of activity at 10cm depth for both ozone concentrations. The wetland type and interactions between ozone and wetland type, ozone and treatment, treatment and depth, and the interactions between all factors showed a trend towards significance ($P<0.1$).

When beta glucosidase activities were separated by wetland type and depth, significant differences between ozone concentration and treatment type applied could be seen. Peat samples at the surface and at 10cm depth from the bog showed an increase in beta glucosidase activity under elevated ozone when mesocosms had been subject to a lowered water-table (drought) compared to mesocosms that had received low ozone concentrations but had a lowered water-table. This pattern was repeated when the beta glucosidase activity in fen peat from both the surface and 10cm depth was analysed.

Bog surface	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$8.82\pm1.48_{a}$	$11.98\pm3.84_{ab}$	$0.14\pm0.10_{a}$	$0.04\pm0.03_{a}$
Drought	$12.11\pm1.28_{a}$	$3.10\pm2.01_{b}$	$0.11\pm0.06_{a}$	$0.04\pm0.03_{a}$

Bog surface	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$8.82\pm1.48_{a}$	$11.98\pm3.84_{a}$	$0.14\pm0.10_{a}$	$0.04\pm0.03_{a}$
Nitrogen	$14.43\pm3.86_{a}$	$8.14\pm4.14_{a}$	$0.04\pm0.04_{a}$	$0.08\pm0.06_{a}$

Bog 10cm depth	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$13.76\pm5.73_{a}$	$13.19\pm4.94_{ab}$	$0.23\pm0.15_{a}$	$0.07\pm0.07_{a}$
Drought	$12.27\pm2.26_{a}$	$4.27\pm2.25_{b}$	$0.19\pm0.08_{a}$	$0.07\pm0.03_{a}$

Bog 10cm depth	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$13.76\pm5.73_{a}$	$13.19\pm4.94_{a}$	$0.23\pm0.15_{a}$	$0.07\pm0.07_{a}$
Nitrogen	$11.5\pm4.57_{a}$	$21.18\pm13.82_{a}$	$0.07\pm0.05_{a}$	$0.16\pm0.06_{a}$

Fen surface	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$25.93\pm11.98_{a}$	$12.93\pm5.34_{ab}$	$0.17\pm0.06_{a}$	$0.21\pm0.10_{ab}$
Drought	$49.37\pm10.39_{a}$	$18.7\pm4.48_{b}$	$0.04\pm0.01_{a}$	$0.14\pm0.01_{b}$

Fen surface	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$25.93\pm11.98_{a}$	$12.93\pm5.34_{a}$	$0.17\pm0.06_{a}$	$0.21\pm0.10_{a}$
Nitrogen	$57.06\pm12.8_{a}$	$25.54\pm2.89_{a}$	$0.17\pm0.07_{a}$	$0.36\pm0.09_{a}$

Fen 10cm depth	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$2.31\pm2.31_{a}$	$8.78\pm2.25_{a}$	$0.10\pm0.03_{a}$	$0.13\pm0.09_{a}$
Drought	$5.28\pm3.03_{ab}$	0_{b}	$0.18\pm0.05_{a}$	$0.12\pm0.04_{a}$

Fen 10cm depth	Beta Glucosidase ($\mu mol\ g^{-1}\ min^{-1}$)		Phenol Oxidase ($\mu mol\ dicq\ min^{-1}\ g^{-1}$ dry soil)	
Treatment	High ozone	Low ozone	High ozone	Low ozone
Control	$2.31\pm2.31_{a}$	$8.78\pm2.25_{a}$	$0.10\pm0.03_{a}$	$0.13\pm0.09_{a}$
Nitrogen	$2.40\pm1.42_{a}$	$2.20\pm1.54_{a}$	$0.18\pm0.07_{a}$	$0.15\pm0.02_{a}$

Table 3.3: Enzyme activities after 4 weeks of ozone exposure. Values with a different letter are significant at $P<0.05$.

In contrast, the only significant effect on phenol oxidase activity was the wetland type with fens showing higher levels of activity when averaged over the whole data set ($P<0.05$). The interaction between ozone and wetland and ozone and sub-treatment (drought or addition of ammonium nitrate) showed a trend towards significance ($P<0.1$). When looking at the difference between phenol oxidase activity in mesocosms that had been exposed to different ozone concentrations and different treatments, the only significant difference occurred in surface samples from droughted fen mesocosms, where elevated ozone caused a significant decrease in phenol oxidase activity compared to mesocosms with a lowered water-table that received low ozone. (See Table 3.3 for a summary of enzyme results.)

3.4 Discussion

3.4.1 Carbon gases

Elevated ozone did not affect weekly carbon dioxide fluxes from the wetland mesocosms, which agrees with results from Niemi et al. (2002) who found that net ecosystem exchange was not affected by elevated ozone. However, when the change in carbon dioxide flux over the four week period was assessed, ozone exposure did show a trend towards significance with mesocosms exposed to high ozone taking up less carbon dioxide. This suggests that either carbon dioxide uptake via photosynthesis was reduced or that respiration was increased. There are two mechanisms by which carbon dioxide uptake could be reduced: plant stomata could close under elevated ozone as a preventative measure to reduce uptake or photosynthetic capacity of the plants could be reduced. The synthesis of Ribulose-1,5-bisphosphate (Rubisco) has been found to be reduced under elevated ozone (Heath 2008; Pell et al. 1997) which would reduce the photosynthetic capacity of plants and hence reduce carbon dioxide uptake. Reduction in stomatal conductance under elevated ozone has been found to occur in some plant species. Depending on plant growth strategy, plants may close their stomata as a preventative measure rather than relying on the synthesis of energetically expensive antioxidant compounds (Yamaji et al. 2003). Alternatively, respiration could be increased during the synthesis of antioxidant compounds and the detoxification of ozone once it has

entered the plant cells (Pell et al. 1997).

Net ecosystem exchange from wetlands has been found to decrease under drought conditions (Bortoluzzi et al. 2006; Roulet et al. 2007) and this agrees with the results of the carbon dioxide flux from bog mesocosms subjected to a lowered water-table in this study. Compared to well-watered mesocosms, droughted mesocosms showed less carbon dioxide uptake. This could be due to reduced net ecosystem exchange or it could be due to increased aerobic decomposition in the drier conditions emitting more carbon dioxide as the endpoint of decomposition. However, the opposite scenario occurred in fen mesocosms with a lowered water-table: these emitted less carbon dioxide than their well-watered counterparts. A possible reason for this is that beta glucosidase activity was lower in mesocosms with a lowered water-table, suggesting that microbial activity was lower under reduced water conditions. This has been found in previous studies looking at the effects of summer drought on phenol oxidase activity; phenol oxidase appears to have an optimum water level, above and below which enzyme activity declined (Toberman et al. 2008). This is likely to be because enzymes tend to be strongly adsorbed onto soil particles and as the water content of the soil is decreased the transport of suitable substrates for the enzyme is decreased (Poll et al. 2006).

Over the four week experimental period, bogs were a net sink of carbon dioxide but fens were a net source. This could be due to increased respiration in the fen mesocosms, both from higher microbial activity because of higher availability of labile carbon from vascular plants and from increased levels of dark respiration from the vascular plants themselves compared to the mosses that dominated the bog mesocosms.

The addition of ammonium nitrate had no effect on gas exchange of carbon dioxide or methane in this experiment irrespective of ozone treatment. This finding agrees with those of Gerdol et al. (2008) who found that fertilisation did not affect net ecosystem exchange in an Alpine bog. Research into the effects of ammonium nitrate on carbon cycling in a Swedish mire also found fertilisation had no effect on carbon dioxide flux (Saarnio et al. 2003) or methane flux (Saarnio et al. 2000).

Ozone did not have a significant effect on methane emissions over the four week exposure period although methane emissions were higher under elevated ozone in mesocosms that did not receive a sub-treatment (labelled control). These results are in agreement with findings from Rinnan et al. (2003), Morsky et al. (2008) and Kanerva et al. (2007) who all found that ozone exposure did not significantly change methane flux from both wetland and grassland mesocosms. In this experiment, within treatment variation was too high to be able to determine any difference between ozone treatments. The lack of a significant effect of ozone exposure on methane fluxes suggests that the amount of methane production and consumption was not consistently affected by elevated ozone and that the release mechanism to the atmosphere for methane was not changed in all mesocosms. Either methane flux through plant aerenchyma did not occur via plant stomata, as found in some wetland species (Greenup et al. 2000) or; if the species did show stomatal control of gas fluxes (Chanton et al. 1993; Morrissey et al. 1993), it was not affected by the elevated ozone. Unfortunately, because of time and equipment constraints it was not possible to measure stomatal conductance in this experiment.

Methane emissions were higher from fen mesocosms than bog mesocosms, potentially because of the "chimney" effect of vascular plant material increasing methane flux from wetlands (Chanton and Whiting 1996; Chanton et al. 1997; Chanton et al. 1993; Ding et al. 2005; Greenup et al. 2000; Kim et al. 1998a; Morrissey et al. 1993; Whiting and Chanton 1996). Vascular plants may have further increased methane emissions via increased root exudation of labile carbon which would supply a readily accessible energy source for methanogenic bacteria (Moore and Dalva 1997; Rinnan et al. 2003) (Chanton et al. 1997; Popp et al. 2000; Saarnio et al. 1997; Segers 1998).

Lowering of the water-table by 5cm significantly reduced methane emissions from wetland mesocosms. This is likely to be because of increased oxygen diffusion into the peat matrix that both directly limits methanogensis (Dowrick et al. 2006; Freeman et al. 1993; Whalen and Reeburgh 2000) and increases oxidation of methane produced deeper in the peat as the aerobic layer increases (King 1996; Whalen and Reeburgh

2000).

3.4.2 Dissolved carbon

In general, ozone did not have a statistically significant impact on the dissolved carbon concentrations present in the pore water irrespective of wetland type or treatment applied. However, when averaged over the three treatment types, wetland type and elevated ozone did have an interactive effect, with elevated ozone reducing the magnitude of the TC percentage change of the pore water, particularly in the fen mesocosms. This finding agrees with that of Jones et al. (2009), who also found that elevated ozone reduced DOC concentrations. Phenolic concentrations in pore water were hypothesised to increase under elevated ozone because of increased production of protective phenolic compounds in vascular plants (Biolley et al. 2002; Liu et al. 2005; Peltonen et al. 2005; Saleem et al. 2001). However, if this did occur, there was no change in the phenolic content of the pore water, possibly because protective compounds would be retained in the plant leaves rather than exuded through plant roots; and the four week experimental period was not long enough to take into account the input of dissolved carbon from leaf litter at the end of the growing season.

Pore water taken from the fen mesocosms had higher concentrations of both TC and phenolic compounds, probably because of the increased carbon loading from vascular plant exudation and the sloughing of root tissue into the peat matrix.

The sub-treatment applied had differing effects on the TC in pore water depending on wetland type. TC concentrations reduced in bog mesocosms that had a lowered water-table but increased in fen mesocosms with a lowered water-table. The reduction in TC concentrations in the bog mesocosms agrees with the hypothesis that lowered water-table would increase aerobic metabolism meaning carbon dioxide would be the end product rather than DOC end products. Conversely, the increase in TC concentration in fen mesocosms is in agreement with published results that found at a catchment scale, drought caused an increase in DOC loading to streams (Worrall et al. 2006b). The addition of ammonium nitrate did not have any effect on TC concentrations in either wetland type. This is in agreement with the results of Worrall

et al. (2006a) who found that addition of nitrogen had no effect on the export of DOC from peatlands. Results from experiments looking at the effects of nitrogen addition on DOC production in forested ecosystems have found conflicting results (Park et al. 2002; Pregitzer et al. 2004; Smemo et al. 2007) suggesting that any changes are due to more complex mechanisms, possibly linked to vascular plant functioning under elevated nitrogen.

3.4.3 Enzymes

The increase in beta glucosidase activity under elevated ozone in this experiment is in contrast to the previous results of Chung et al. (2006) who found a 25% reduction in beta glucosidase activity after exposure of forest soils to elevated ozone. In mesocosms that had received a lowered water-table, beta glucosidase activity was higher under elevated ozone at both sampling depths from both fens and bogs. Previous results have found that as the water-table is lowered beta glucosidase activity is increased (Fenner et al. 2005; Freeman et al. 1996) but this did not occur in this experiment; in three out of four comparisons under low ozone concentrations lowering the water-table caused a non-significant decrease in enzyme activity. The increase in beta glucosidase activity under elevated ozone could be due to ozonation of DOC in surface pore water breaking down complex aromatic compounds (Gul 2002) into aliphatic compounds that beta glucosidase can break down.

Exposure to elevated ozone had no overall effects on phenol oxidase activity, which is in agreement with results from Larson et al. (2002) for the forest ecosystems study. Within fen mesocosms that received a lowered water-table, elevated ozone caused a significant decrease in surface phenol oxidase activity. This could be because the lowered water-table allowed ozone to diffuse a certain distance into the peat and, as ozone is used as a disinfectant, it could have reduced microbial activity, in agreement with Islam et al. (2000b). However, as beta glucosidase activity showed the opposite trend under similar conditions this may not be the case.

Phenol oxidase activities did not respond to the lowered water-table in this experiment which is unexpected as previous results have found that lowering the water-table

caused a highly significant increase in phenol oxidase activity with potential to change the carbon storage of peatland ecosystems (Freeman et al. 2004b; Freeman et al. 2001b). However, this is not always the case as found by Freeman et al. (1996) and it could be that the four week drop in the water-table was not long enough to have an effect on the synthesis of phenol oxidase.

The addition of nitrogen had no effect on phenol oxidase or beta glucosidase activity, which is in agreement with the results of Allison and Vitousek (2004) and Blackwood et al. (2007). Microbial communities have been found to increase the production of extracellular enzymes when nutrients are relatively scarce (Wallenstein and Weintraub 2008) so it may have been expected that nitrogen inputs would decrease enzyme activity. However, the four week exposure period may have been too short for any changes to take place.

3.4.4 Conclusions

Short-term exposure to elevated ozone concentrations does not appear to have significantly increased methane fluxes from wetland mesocosms, although the magnitude of the non-significant increase seen suggests that, over a longer time period, elevated ozone may increase methane fluxes from peatlands. Increasing methane fluxes could potentially lead to a higher warming potential in the atmosphere, hence increasing global warming. A second effect could be an increase in ozone production resulting from the increased methane levels as methane reacts in a similar way to other VOCs (West and Fiore 2005). However, if the frequency and duration of drought events increase, the reduction in methane emissions following a lowering of the water-table could have a protective effect against the impacts of ozone exposure.

The trend towards mesocosms exposed to elevated ozone either taking up less or emitting more carbon dioxide suggests that the sink strength of peatlands for carbon could be changed if tropospheric ozone concentrations continue to increase.

During the short-term ozone exposure TC and phenolic concentrations were not

significantly affected, but elevated ozone did cause a reduction in the magnitude of the percentage change in TC concentration in fen mesocosms, which could result in a reduction in dissolved carbon leaving peatlands in pore water.

4 How does increasing background ozone affect plant growth and gas exchange from wetlands?

4.1 Introduction

Ozone is well-established as a phytotoxic secondary air pollutant (Bobbink 1998) and is formed during a series of complex photochemical reactions between oxides of nitrogen (NO_x) and volatile organic compounds (VOCs) (Ashmore 2005; Carpenter et al. 1998; Coyle et al. 2002; Sandroni et al. 1994). Background ozone concentrations across Europe are increasing (Sandroni et al. 1994) due to worldwide increases in emissions of precursor compounds (Ashmore 2005; Fiscus et al. 2005; Jenkin et al. 2002) and have increased by 35% since the 1750s (IPCC 2001). However, peak concentrations of ozone over Europe are decreasing because of regulations controlling the localised emission of precursors (Ashmore 2005).

As previously shown by Power and Ashmore (2002), Franzaring et al. (2000) and in Chapter 2, wetland plants are negatively affected by periods of elevated ozone. As peak ozone concentrations are predicted to decrease and background concentrations are predicted to increase this study focuses on the effects of small increases in elevated background ozone on wetland plants. Peaks of ozone cause increases in senescence and injury, whereas it is possible that lower peak ozone concentrations but continually higher background concentrations will have differing effects on wetland vascular plants, possibly including changes to stomatal conductance as has been seen in some upland vegetation (Mills et al. 2009). As wetland plants are the primary means by which methane is transported from the anaerobic zone to the atmosphere (Chanton and Whiting 1996; Rinnan et al. 2003; Shannon et al. 1996), any ozone-induced changes to plant physiology and morphology are likely to affect wetland methane emissions.

As described in Section 1.4.1 there has been little work done on the effects of elevated ozone on methane fluxes from wetlands, but previous results show that there may be a transient increase in methane emissions under elevated ozone (Lloyd 2004; Niemi et

al. 2002). However, more recent open field exposure systems have found that season long exposure to ozone concentrations double the current ambient resulted in a reduction in methane emissions from wetland mesocosms (Morsky et al. 2008).

The aim of this work was to evaluate the effect of raising the background ozone concentration on wetland plant growth, both on individual species and within intact peat microcosms and to assess any changes in carbon gas exchange under elevated ozone. The effect of plant growth on carbon gas fluxes from the bog mesocosms was also assessed to see if there were any secondary effects of ozone exposure. The hypotheses were that: plant senescence would increase and plant biomass would decrease, season-long exposure to elevated background ozone would cause an increase in methane emissions from both fen and bog mesocosms as a possible change was seen in Chapter 3 and that any changes in methane and carbon dioxide fluxes would be correlated with plant senescence and growth.

4.2 Methods

4.2.1 Mesocosm collection

The wetlands used in this experiment were the Migneint (SH:785,455) and Cors Goch (SH:500,814). The Migneint is an upland, oligotrophic, blanket bog and the two main NVC vegetation types found at the site are M6 (*Carex echinata-Sphagnum recurvum/auriculatum* mire) and M15 (*Scirpus cespitosus-Erica tetralix* wet heath) (Buckton and Ormerod 1997). Cors Goch is adjacent to Cors Erddreiniog and is also part of the Anglesey fens SAC (see Chapter 2). The wetland areas used for mesocosm collection were changed between 2006 and 2007 because there were access problems at Cors Erddreiniog at the time of mesocosm collection and, at Marchlyn Mawr, First Hydro were carrying out extension work on the dam above the wetland. Part of this work involved pumping highly alkaline grouting into the dam base, which could have changed the conditions in the overlying wetland below the dam. There were also potential problems with access to the site for health and safety reasons.

Thirty-two intact peat mesocosms (10.5cm diameter, 20cm depth) were taken from

each site following the method of Freeman et al., (1993). (See Chapter 3 for more details.) The bases of the mesocosms were sealed using clear, non-reactive plastic film and they were stored under ambient conditions for one month until the start of the ozone exposure. During this period, the water-table was kept at the surface of the mesocosms to ensure that conditions matched the source wetland area as much as possible.

4.2.2 Plant Propagation

Individual plants of *Molinia caerulea* and *Juncus subnodulosus* were propagated from stock plants, originally collected from Cors Erddreiniog (Chapter 2), and maintained under controlled greenhouse conditions at the Pen-y-Ffridd research centre. Individual plants were potted into three litre containers using 100% peat compost (HUMAX) and stored in plastic troughs with the water-table 5cm below the surface to simulate growing conditions within a natural wetland as closely as possible. Two weeks before the plants were transferred to the solardomes they were watered with water originating from Cors Erddreiniog to establish a representative microbial community. The plants were grown for four weeks in greenhouse conditions (supplemental daylight lighting, daytime temperature 18°C, night time temperature 16°C) before being transferred to the solardomes. Five replicates of each species were randomly assigned to each solardome and were kept in troughs with continuous drip watering using porous piping.

4.2.3 Ozone exposure

Ozone concentrations in the solardomes (see Chapter 2 for a detailed explanation of the solardome set-up) followed the trajectory of ozone concentrations measured at the Snowdonia monitoring site at Marchlyn Mawr (SH 621 624) during a typical week with no marked ozone episodes but relatively high background ozone: 31st May – 6th June 2006 but with incremental starting points (Figure 4.1).

Ozone exposure concentrations ranged from pre-industrial levels up to those predicted to occur by the end of the century. These were applied as a continuous weekly regime

designed to simulate the projected increase in background ozone concentrations. The exposure period within the solardomes was 5 months from 9th May-24th September 2007 for the peat mesocosms and 23rd May-4th September 2007 for the individual species. During this period, the water-table was maintained at the surface of the mesocosms through continual drip watering using standard porous tubing fed from the mains water supply. Supplemental watering by hand took place as required. Four replicate mesocosms from each wetland were randomly assigned to each dome and positioning was repeated between domes. Within each dome, the mesocosms from each wetland had a shared water supply.

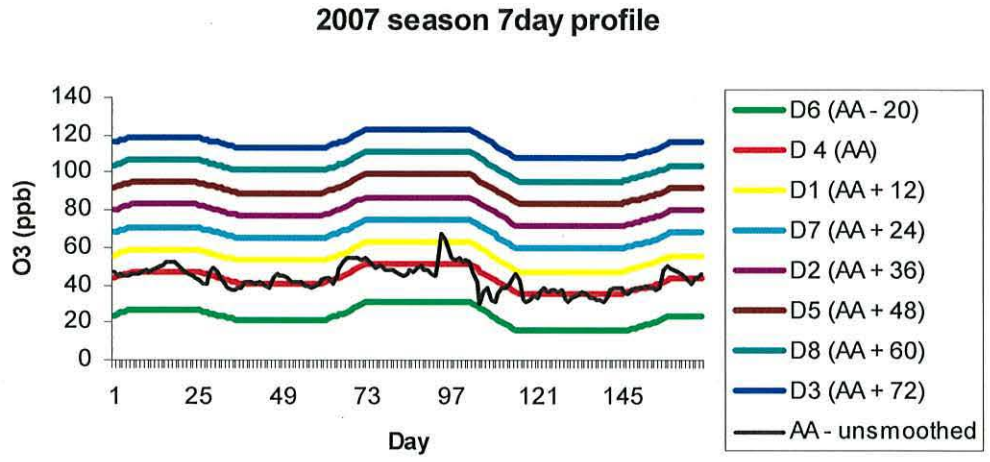


Figure 4.1: Planned weekly ozone regime. The black line is the unsmoothed weekly data from the Marchlyn Mawr monitoring station that the profile is based on.

4.2.4 Measurements of ozone effects

Samples of the headspace gases above the mesocosms were taken on a monthly cycle. A two-litre, transparent, sealed container was placed over each mesocosm and 20ml headspace gas samples were taken at the point of sealing and after one hour. These were analysed for carbon dioxide and methane within 24 hours of samples being taken and the results were used to calculate the net flux of gases from the mesocosm in mg m^{-2} hr^{-1} (See Chapter 3 for details of gas analysis). After 20 weeks of ozone exposure the percentage oxygen content of the pore water within the mesocosms was measured using an oxygen sensor calibrated in oxygen saturated water.

Plant physiological variables were measured on vascular plants that had germinated in the mesocosms originating from the Migneint immediately after the ozone supply ended. Percentage cover of vascular plants and the percentage of the base of the mesocosms covered with root material was assessed using a $1cm^2$ grid placed over the mesocosm and the presence of plant material in squares was counted. Whole plant senescence was visually assessed. Leaves were counted as senesced if more than 25% of a leaf had died back. These measurements were only taken on the plants germinating in the bog mesocosms because the fen mesocosms had become pot-bound after approximately two months of ozone exposure. Plant cover and root cover at the base of the mesocosm was close to 100% in all fen mesocosms and methane emissions had dropped to virtually nothing.

Senescence was assessed on the plants grown in individual containers at regular intervals through the ozone exposure and above ground biomass was measured at the end of the exposure period (see Chapter 2 for further details of plant senescence and biomass measurements).

4.2.5 Statistical Analysis

Statistical analyses of the effects of ozone concentration on plant growth and gas exchange were conducted on the mean dome data using Minitab version 13. The plant growth effects on carbon gas exchange were analysed by correlation at the individual mesocosm level irrespective of ozone concentration. Data was analysed using ANOVA general linear model. Although this analysis did not test for a direct effect of ozone it was possible to find out if there was a significant interaction between the different variables and ozone exposure. Data that was collected as percentage values was arc-sine transformed prior to analysis and back-transformed for data presentation. Results were accepted to be showing a significant interaction at $P<0.05$, and at $0.05>P<0.1$ results were taken as showing a trend towards significance.

4.3 Results

4.3.1 Ozone exposure

Weekly ozone exposure followed the smoothed profile of the concentrations measured in Snowdonia during a typical week in summer 2006. Mean values of the weekly ozone concentrations experienced by the mesocosms and the individual species over the five-month growing season are shown in Tables 4.1 and 4.2 respectively. The two highest treatments tracked each other very closely resulting in a very similar $AOT0_{24hr}$ value (see Figures 4.2 and 4.3). AOT40 (daylight hours) values at the end of the ozone exposure ranged from 0.07 ppm.h to 104 ppm.h and were strongly correlated with the weekly average ozone concentrations ($P<0.001$). Mesocosms and plants experienced different $AOT0_{24hr}$ values because the individual plants went into the solardomes later than the mesocosms.

	Dome 6 (AA-20)	Dome 4 (AA)	Dome 1 (AA+12)	Dome 7 (AA+24)	Dome 2 (AA+36)	Dome 5 (AA+48)	Dome 8 (AA+60)	Dome 3 (AA+72)
AOT0 24hr	71.29	132.65	166.86	197.28	248.78	276.61	336.16	340.23
24 hr mean	21.44	39.94	50.24	59.40	74.89	83.28	101.28	102.46
AOT 40 day	0.07	4.63	19.66	34.92	57.24	70.95	99.57	103.79

Table 4.1: $AOT0_{24hr}$ (ppm.h), 24 hour season mean (ppb) and AOT40 daylight hours (ppm.h) over the 5 months the mesocosms were in the solardomes

	Dome 6 (AA-20)	Dome 4 (AA)	Dome 1 (AA+12)	Dome 7 (AA+24)	Dome 2 (AA+36)	Dome 5 (AA+48)	Dome 8 (AA+60)	Dome 3 (AA+72)
AOT0 24hr	52.12	99.15	125.50	145.84	187.75	207.36	256.25	252.95
24hr mean	20.83	39.60	50.12	58.24	74.98	82.85	102.38	101.06
AOT 40 day	0.07	3.25	14.79	25.34	43.25	53.00	76.80	76.61

Table 4.2: $AOT0_{24hr}$ (ppm.h), 24 hour season mean (ppb) and AOT40 daylight hours (ppm.h) over the 4 months the plants were in the solardomes

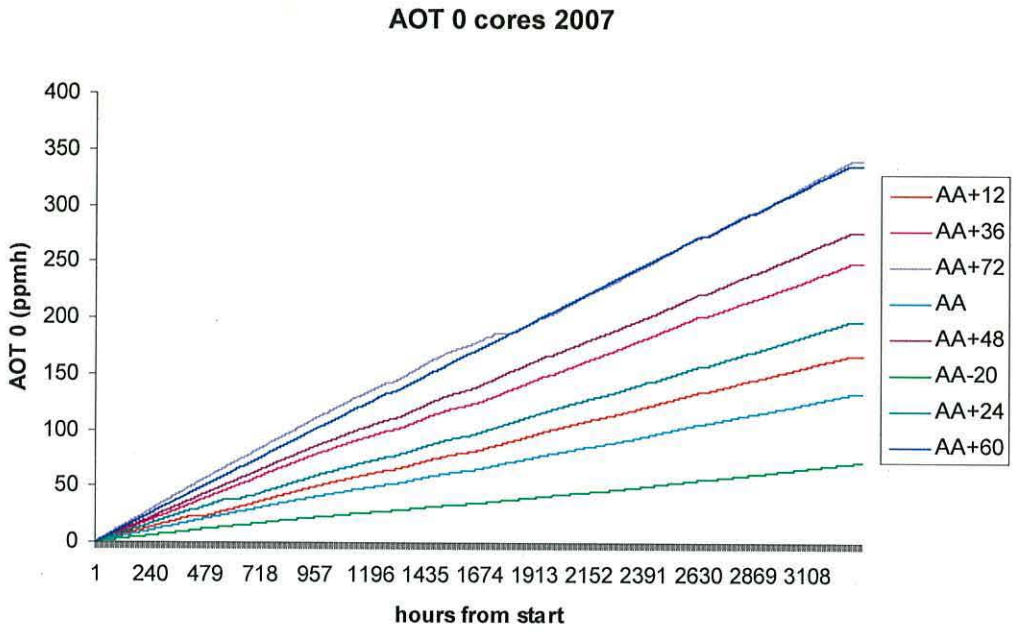


Figure 4.2: $AOT0_{24hr}$ values for the mesocosms in the solardomes

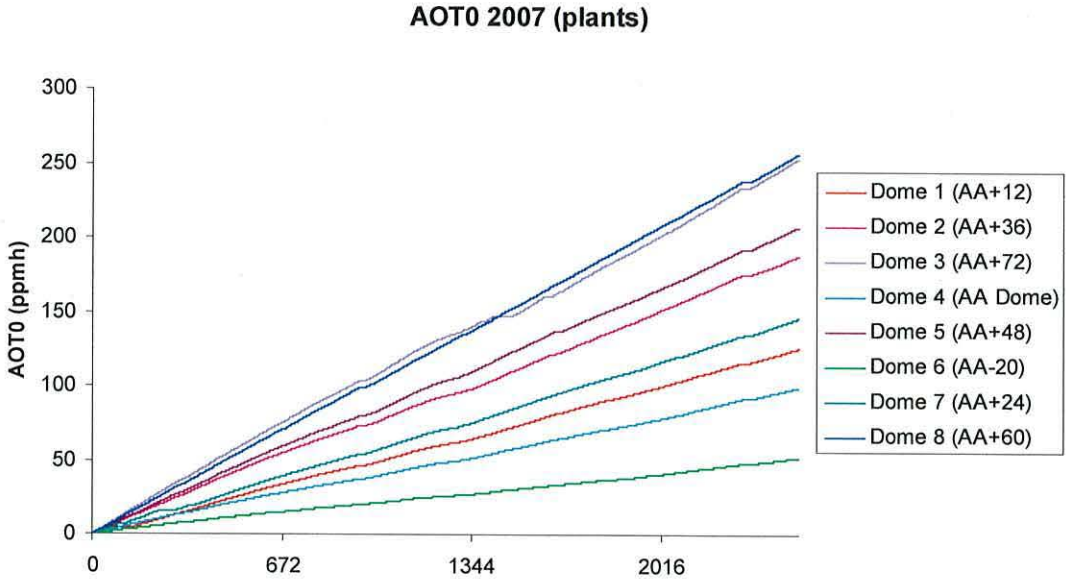


Figure 4.3: $AOT0_{24hr}$ values for the individual species in the solardomes.

4.3.2 Ozone effects on wetland plants

At the start of the ozone exposure, all bog mesocosms had 100% moss cover, with no

vascular plants. During the five months of the experiment, vascular plants, mainly *Carex echinata* with some *Juncus effusus* and *J. squarrosus*, emerged. Ozone concentration had no effect on the presence or absence of vascular plants but the effect on the percentage cover of vascular plants became more significant over time (Table 4.3). By the end of the experiment ozone exposure, both in terms of weekly mean ozone concentrations and $AOT0_{24hr}$, showed a significant correlation with the percentage cover of vascular plants (Figure 4.4).

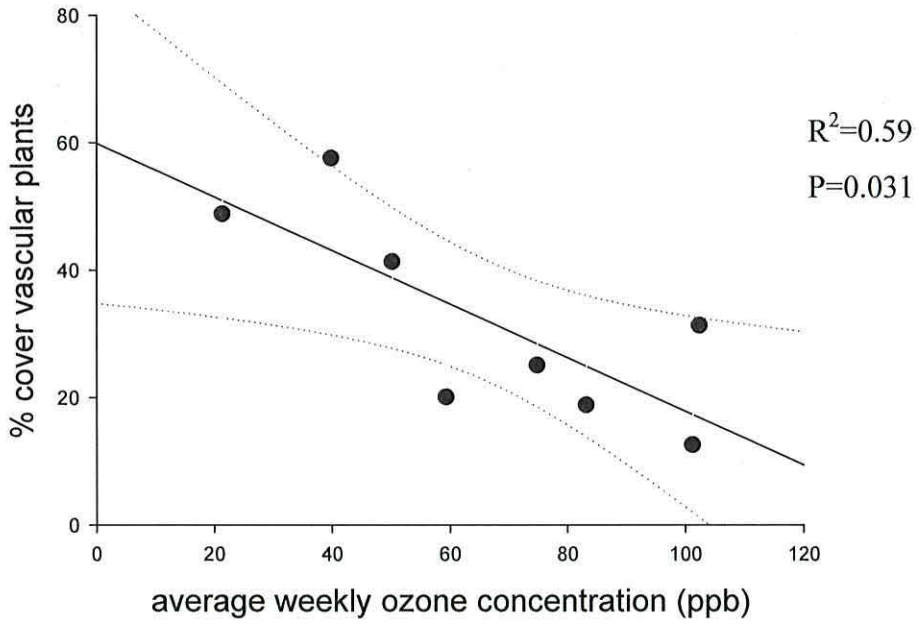


Figure 4.4: % cover of vascular plants in bog mesocosms after 20 weeks ozone exposure

As ozone concentration increased, the cover of vascular plants decreased ($P<0.05$). After five months of ozone exposure the number of leaves per mesocosm showed a strong trend towards a significant decrease with increasing ozone concentration ($P<0.1$) (Table 4.3). The correlation between ozone exposure and the height of the vegetation above the surface of the moss was not significant, although plant height did decrease as ozone exposure increased (Table 4.3). Above-ground vascular plant biomass also showed a slight trend towards significance with biomass decreasing as ozone concentration increased (Figure 4.5 and Table 4.3). Ozone exposure did not have any significant linear relationship with the percentage senescence seen on the

plants in the mesocosms (Table 4.3). However, not all of the mesocosms contained vascular plants so this value could be skewed by a relatively small number of samples.

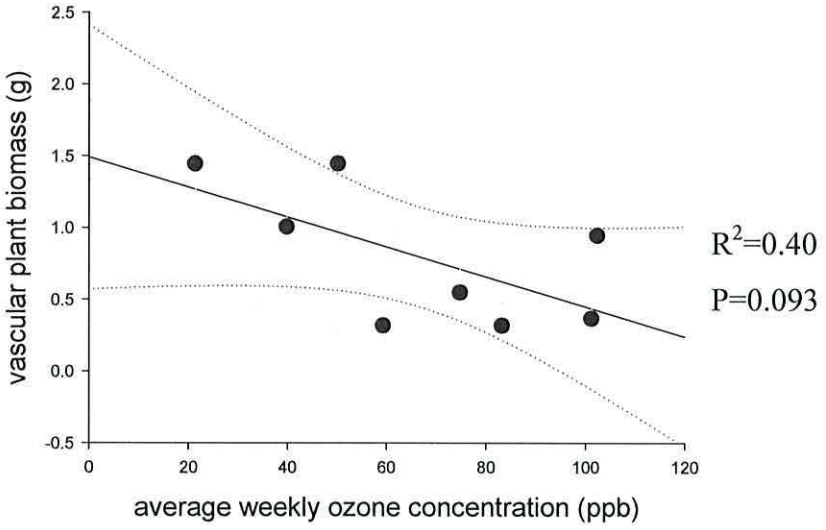
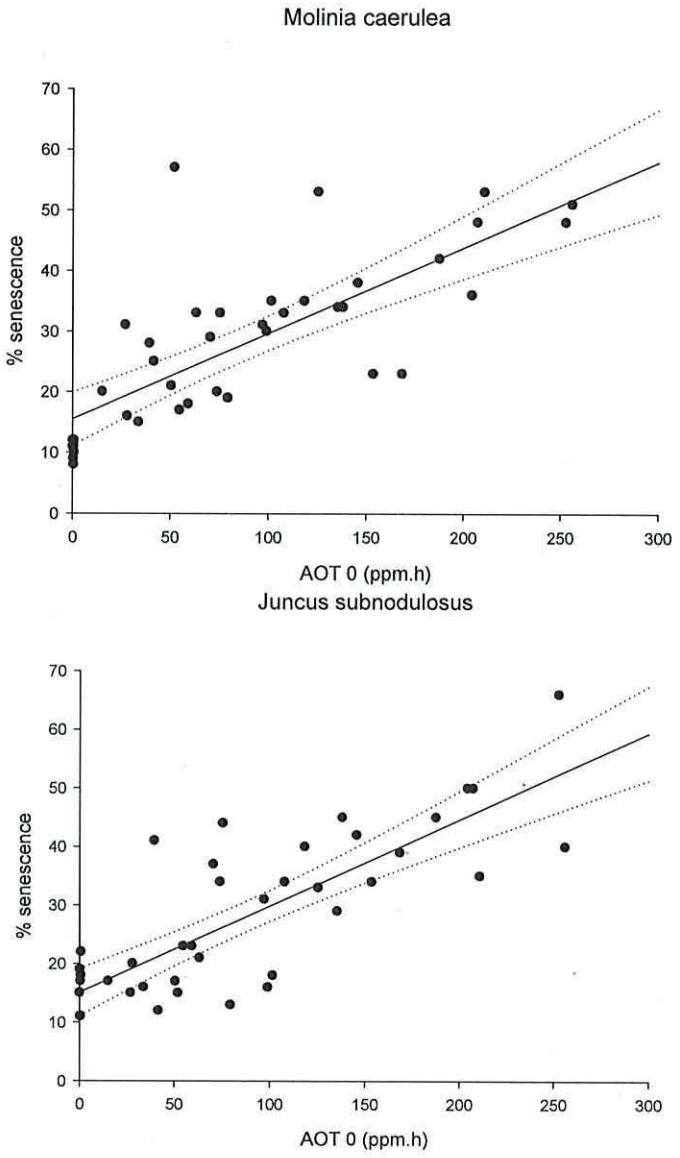


Figure 4.5: Vascular plant biomass in bog mesocosms after 20 weeks ozone exposure

Plant variable	Effect of ozone	P
% cover plants	--	**0.031**
% root cover	(--) NS	0.111
Plant biomass	--	*0.093*
Senescence	NS	0.468
Canopy height	(--) NS	0.144

Table 4.3: Effect of ozone on plant measurements in bog mesocosms after five months of ozone exposure

Individual plants however, did show an increase in senescence with increasing ozone exposure. Both *M. caerulea* and *J. subnodulosus* showed a significant relationship between $AOT0_{24hr}$ and percentage senescence ($P<0.001$) with 48% and 69% of the variation in senescence data respectively being explained by $AOT0_{24hr}$ (Figure 4.6). Ozone exposure did not have a significant effect on above ground biomass of either species after 16 weeks (Figure 4.7).



R^2=0.59

P<0.0001

R^2=0.65

P<0.0001

Figure 4.6: Senescence measurements on *M. caerulea* and *J. subnodulosus* over the four months ozone exposure. Each data point is a dome mean with senescence measured at the start of the ozone exposure then monthly for four months.

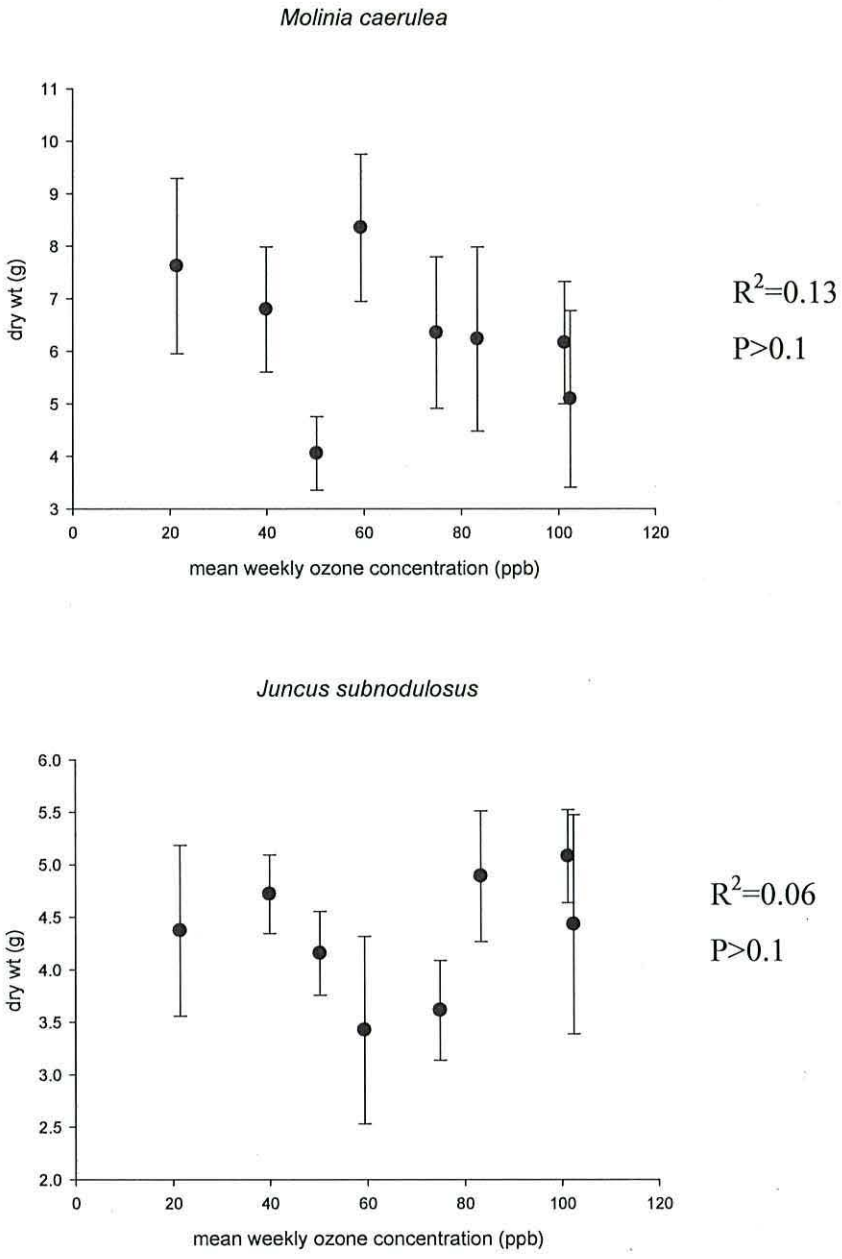


Figure 4.7: Dry weight of individual plants after 4 months ozone exposure. *Molinia caerulea* is shown in the top graph and *Juncus subnodulosus* is shown in the bottom graph.

4.3.3 Effects of ozone exposure on carbon gas fluxes

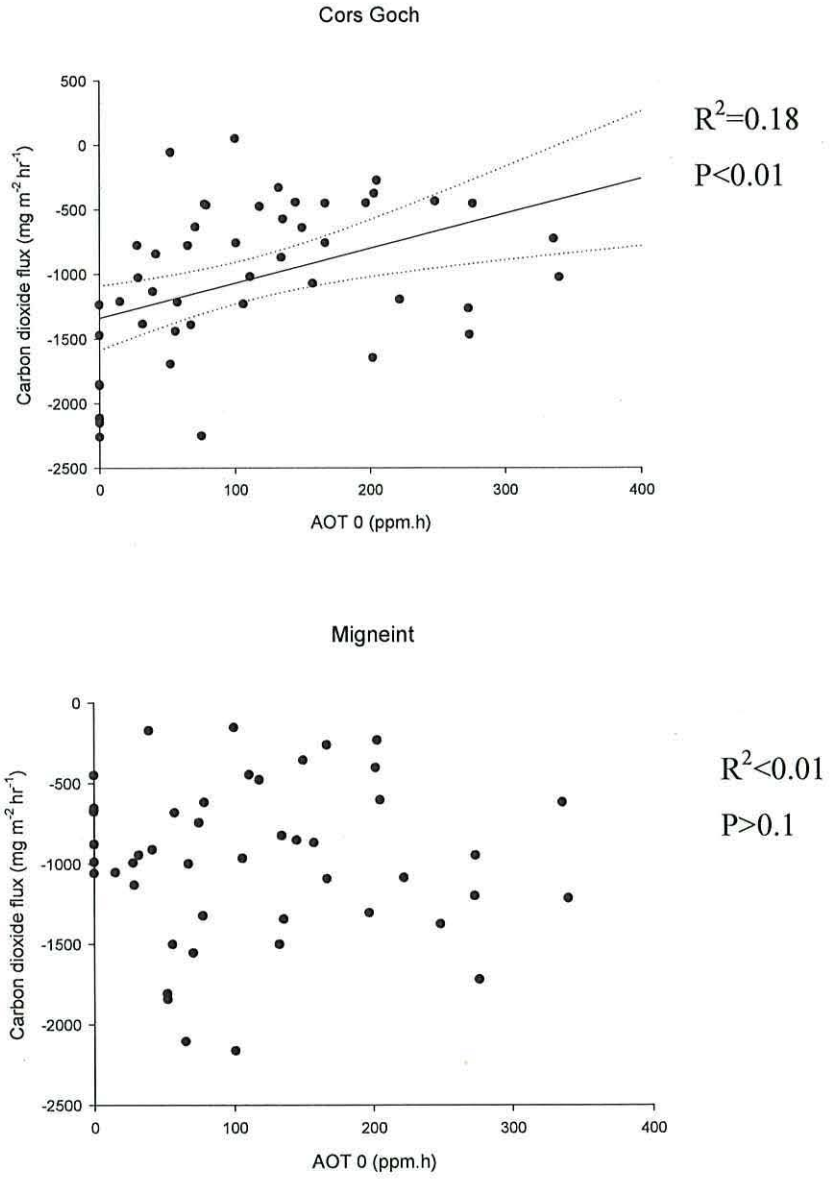


Figure 4.8: Carbon dioxide flux from the fen (top) and bog (bottom) mesocosms against $AOT0_{24hr}$ incorporating all data form the 5 month monitoring period. Each data point is an ozone treatment mean from the time of sampling.

When carbon dioxide fluxes were analysed against ozone exposure for each monthly sampling point separately there was no significant effect of ozone on fluxes from fen or bog mesocosms. However, when all of the carbon dioxide flux data was analysed

against $AOT0_{24hr}$ at the time of sampling, carbon dioxide exchange from fen mesocosms showed a significant relationship with $AOT0_{24hr}$ ($P<0.01$) with carbon dioxide uptake reducing under increasing $AOT0_{24hr}$ values (Figure 4.8). This relationship was not found when analysing carbon dioxide exchange from bog mesocosms (Figure 4.8).

Monthly data analysis of methane fluxes from fen and bog mesocosms against ozone concentration did not show any significant differences but when all methane flux data for each wetland type was analysed against $AOT0_{24hr}$, methane fluxes from the bog mesocosms increased as $AOT0_{24hr}$ increased ($P<0.01$) (Figure 4.9). The methane flux data from the fen mesocosms had a slightly negative relationship with $AOT0_{24hr}$ ($P<0.05$) (Figure 4.9) but from month two onwards methane fluxes from all fen mesocosms dropped to almost zero.

Thus, this negative relationship is strongly influenced by the initial high methane emissions. If the methane emissions are plotted in pairs of adjacent ozone treatments over the five months (Figures 4.10) emissions from the bog mesocosms are variable but the effect of any elevated ozone treatment was to increase methane emissions compared to the lowest pair of mesocosms. This effect was not significant at $P<0.1$ because of the variability within the groups. There was no such trend in methane emissions from the fen mesocosms and it can be seen that, irrespective of ozone concentration, there was very little methane emitted by month two of the exposure period.

At the end of the experiment, pore water oxygen concentrations were higher in the fen mesocosms than the bog mesocosms (Figure 4.11). However, although there was a reduction in pore water oxygen content with increasing $AOT0_{24hr}$ in the fen mesocosms (data not presented), the R^2 value was only 0.225 and the relationship was not significant at $P<0.1$.

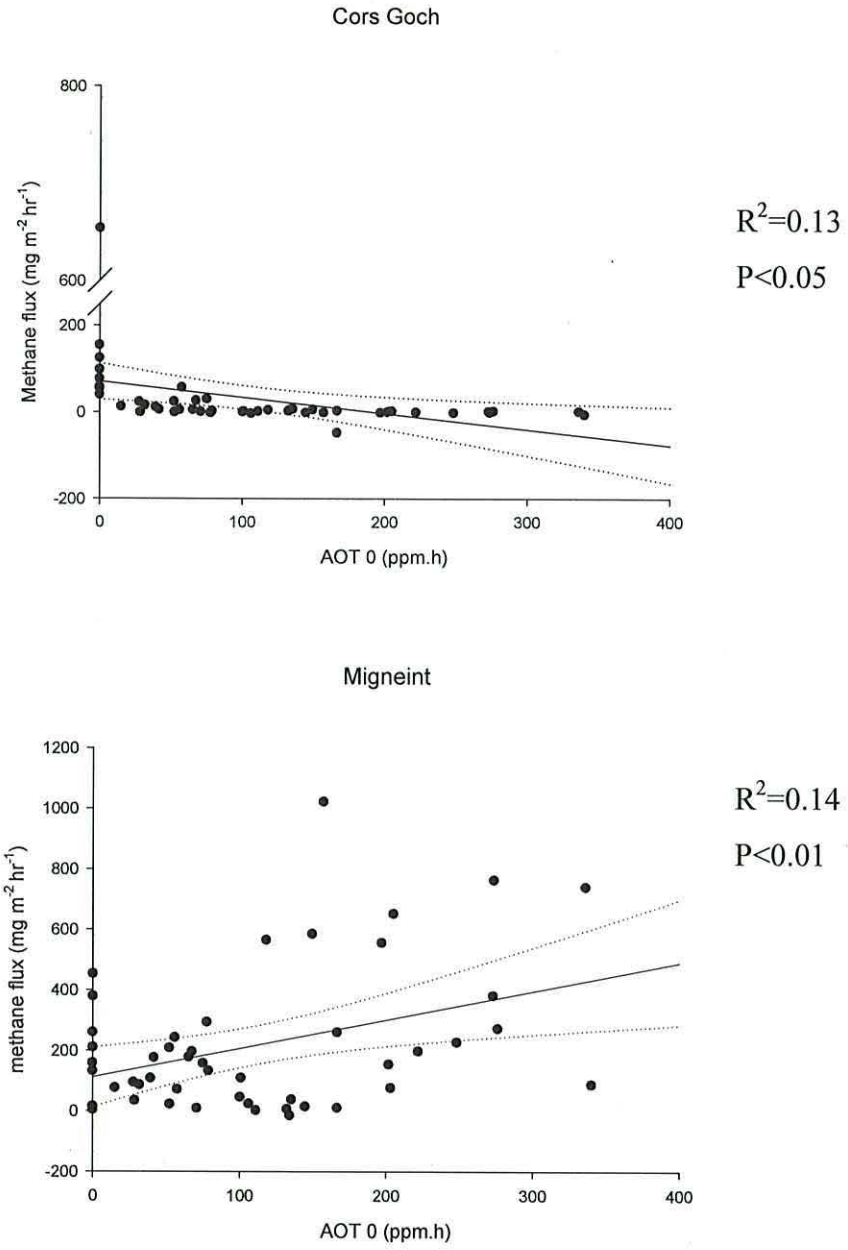


Figure 4.9: Methane fluxes from fen (top) and bog (bottom) mesocosms over the 5 months of ozone exposure. Each data point is an ozone treatment mean from the time of sampling.

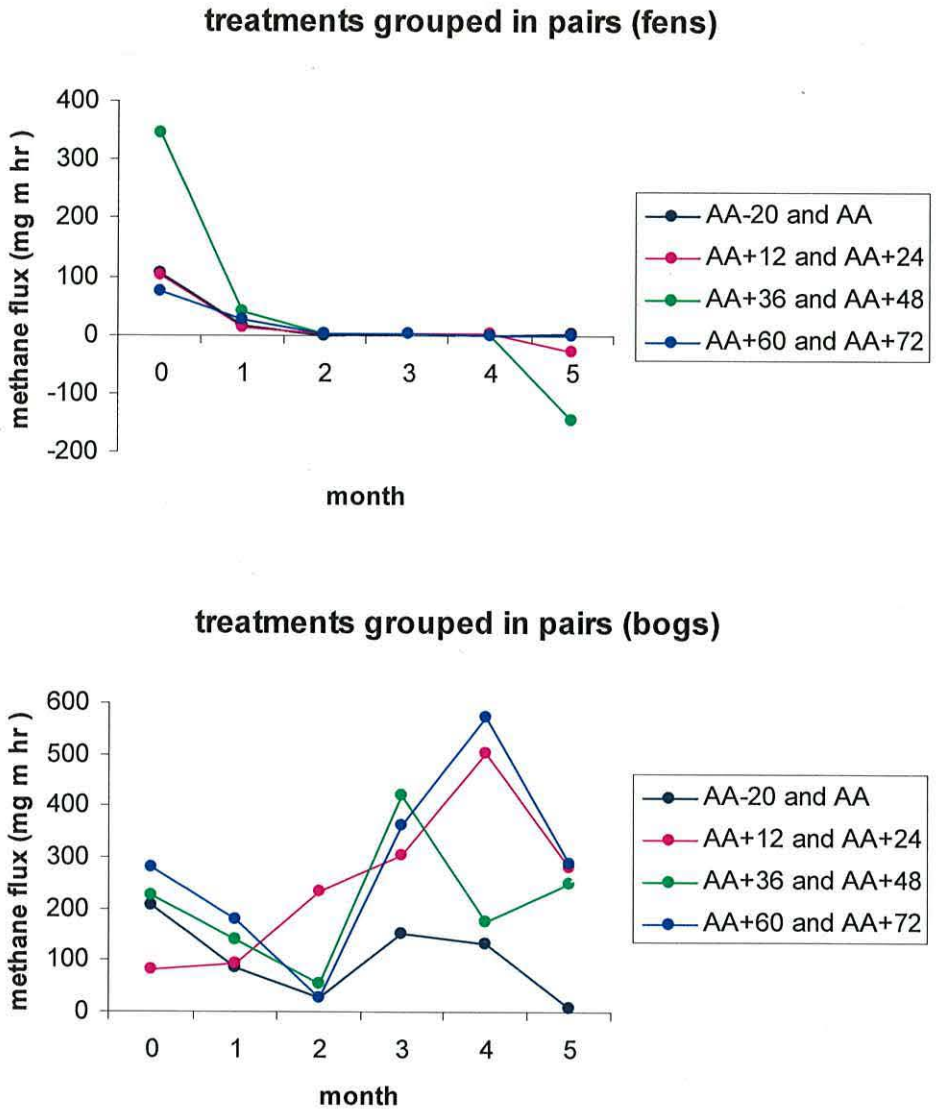


Figure 4.10: Monthly time course of methane flux with the solardomes grouped in pairs to show that ozone may be having a positive effect on bog methane fluxes so the overall regression is probably correct in saying increasing $AOT0_{24hr}$ increases methane efflux. However, the decreasing regression slope for the fens is probably due to the decrease over time rather than an ozone effect as samples from month 0 will have the lowest $AOT0_{24hr}$ values. Flux values are in mg m^{-2} hr^{-1}

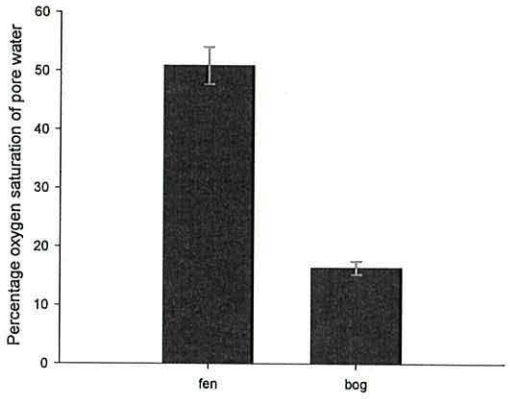
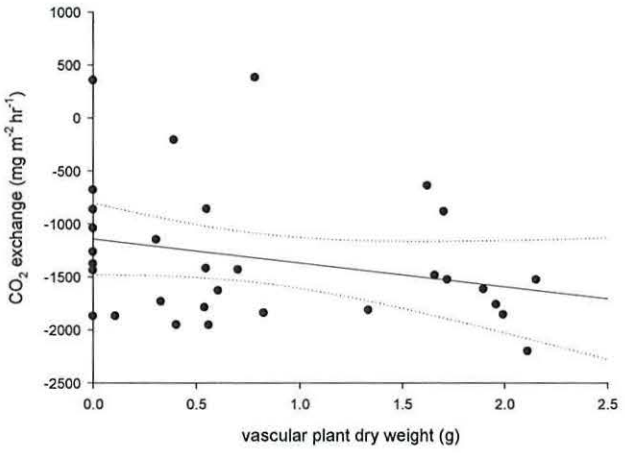


Figure 4.11: Oxygen content of pore water from fen and bog mesocosms after 20 weeks exposure. Water samples were compared to oxygen saturated water, meaning that the mean fen pore water oxygen content was 50% that of the oxygen saturated water, while the bog pore water oxygen content was 15% that of oxygen saturated water.

4.3.4 Effects of wetland plants on carbon gas fluxes from bogs

The third part of the experiment was to investigate the effects of vascular plant cover on the fluxes of carbon dioxide and methane from bog mesocosms only, irrespective of the ozone dose the mesocosms received. Fen mesocosms were not used because they had became pot-bound across all ozone treatments and they all had 100% plant cover. Methane fluxes were also negligible from fen mesocosms in all ozone treatments.

Carbon dioxide fluxes from the mesocosms were generally negative, suggesting that these wetlands are a sink for carbon dioxide. By the end of the experimental period carbon dioxide flux was found to significantly correlate with the dry weight of vascular plants within the mesocosm (Figure 4.12, Table 4.4), the percentage cover of roots around the base of the mesocosm (Table 4.4) and the canopy height of the vegetation (Table 4.4). This suggests that as the amount of vascular plants increases, the uptake of carbon dioxide through photosynthesis also increases.



R^2=0.07

P=0.05

Figure 4.12: The relationship between carbon dioxide flux and plant dry weight in bog mesocosms after 5 months ozone exposure. Each data point represents an individual mesocosm as this analysis was carried out irrespective of ozone exposure.

	CH4 Production		CO2 consumption	
	P value	F value	P value	F value
Ozone effect	0.365	1.19	0.247	1.48
% cover vascular plants	**0.039**	**5.11**	0.212	1.70
Interaction	0.232	1.53	**0.040**	**2.88**
Ozone effect	0.440	1.05	0.160	1.80
Canopy height	0.179	1.99	**0.005**	**10.75**
Interaction	0.259	1.45	**0.048**	**2.74**
Ozone effect	0.586	0.82	0.158	1.81
Number of leaves	0.120	2.71	0.735	0.12
Interaction	0.821	0.50	**0.009**	**4.29**
Ozone effect	0.329	1.27	0.217	1.58
Biomass (dry weight)	0.205	1.75	**0.050**	**4.53**
Interaction	*0.084*	*2.29*	**0.044**	**2.82**
Ozone effect	0.544	0.88	0.166	1.77
Senescence	*0.094*	*3.20*	0.735	0.12
Interaction	0.705	0.66	**0.010**	**4.16**
Ozone effect	0.376	1.17	0.111	2.08
% root cover	***0.051***	***4.49***	**0.023**	**6.41**
Interaction	0.231	1.53	**0.008**	**4.34**

Table 4.4: The significance of ozone, plant variables and the interactive effects of them on methane and carbon dioxide fluxes from bog mesocosms. Values in bold are significant at P<0.05.

Methane fluxes from the peat mesocosms were generally positive, suggesting that

these wetlands are a source of methane rather than a sink. At the end of the five month period methane fluxes were found to significantly correlate with the percentage cover of vascular plants; as the percentage cover of plants increased the flux of methane decreased (Figure 4.13, Table 4.4). The same correlation was seen between methane flux and the percentage cover of roots (Table 4.4). This suggests that as the amount of plant material in the mesocosms increases the flux of methane decreases.

Although methane fluxes from peat mesocosms in the solardomes were correlated with plant cover and plant growth was negatively affected by elevated ozone concentrations; the concentration of ozone that the mesocosms were exposed to did not have a direct effect on methane fluxes from the mesocosms. Over the variables measured at the end of the experiment, ozone exposure, alone or via an interaction with plant growth variables, did not show any significant effect on methane fluxes (Table 4.4). However, the interaction between ozone exposure and plant dry weight showed a slight trend towards a significant relationship with methane fluxes ($P<0.1$).

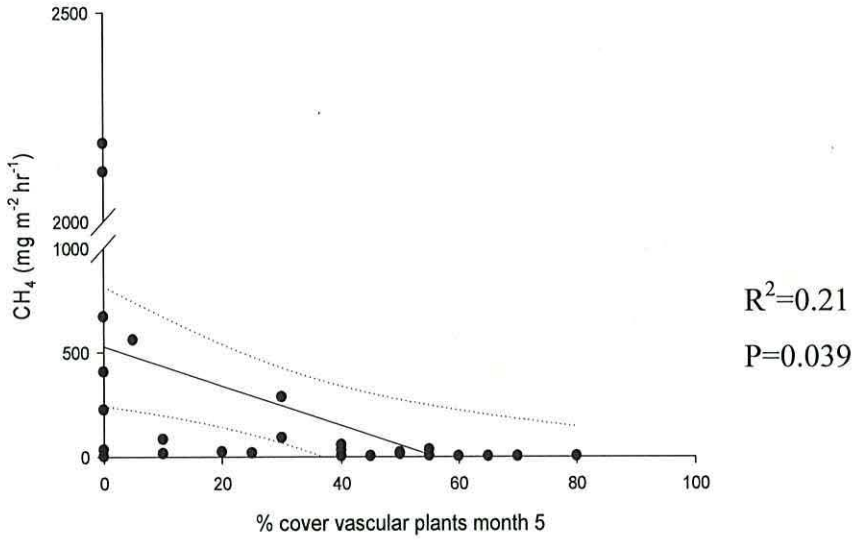


Figure 4.13: The relationship between methane flux and vascular plant cover after 5 months of ozone exposure. The data points refer to individual mesocosms rather than dome means.

In contrast to the methane fluxes, ozone had more of an interactive effect on carbon dioxide uptake. There was no direct effect of elevated ozone on carbon dioxide

exchange from the peat mesocosms. However, carbon dioxide exchange was significantly affected ($P<0.05$) by an interaction with the following plant variables and ozone exposure (Table 4.4): percentage cover of vascular plants, the canopy height of the vegetation, the number of leaves per mesocosm, the dry weight of the vascular plant biomass, the percentage senescence on the leaves in each mesocosm and the percentage of root material covering the base of the mesocosm.

4.4 Discussion

This research investigated the effect of elevated ozone concentrations on wetland plant growth and on carbon gas exchange from wetlands. Three hypotheses were tested: plant senescence would increase and plant biomass would decrease, season-long exposure to elevated background ozone would cause an increase in methane emissions from both fen and bog mesocosms as a possible change was seen in Chapter 3 and that any changes in methane and carbon dioxide fluxes would be correlated with plant senescence and growth.

4.4.1 Why does ozone have an effect on wetland plants?

The results show that ozone reduces plant cover and the number of leaves per mesocosm in peat mesocosms from bog ecosystems. However, there was not a significant effect on biomass or senescence after the five-month ozone exposure period. Plants take up ozone through the stomata and, once inside the leaves, ozone breaks down to form reactive oxygen species, which cause damage at a cellular level within the leaf (Long and Naidu 2002). Previous studies have found that ozone exposure negatively affects photosynthesis, stomatal conductance and biomass production (Power and Ashmore 2002; Yamaji et al. 2003) and relatively more carbon is used for anti-oxidant synthesis and in the repair of ozone damage than for new growth (Andersen 2003). There is no evidence that vascular plants grown under elevated ozone will not germinate, meaning that ozone concentrations have no effect on the absolute presence or absence of plant material. However, once vascular plants are growing they will have to expend relatively more resources on repair resulting in less ground cover and growth of fewer new leaves. This agrees with the results from

this experiment as elevated ozone had no effect on the presence of absence of vascular plants but did decrease the percentage cover of plants once they had germinated. A possible reason for the lack of difference in biomass between different ozone exposures could be that root growth within the mesocosm became limiting to extra biomass production in the final weeks of the experiment. In mesocosms with higher percentage cover of vascular plants there was more root growth across the base of the mesocosm and it has been found that once plants have become pot-bound they no longer show any further growth in response to outside stimuli (Baldwin 1988; Herold and McNeil 1979).

4.4.2 How does this change carbon gas fluxes from wetlands?

Previous work on methane fluxes from wetlands found that elevated ozone caused a transient increase in methane emissions from bog microcosms (Niemi et al. 2002) but this was not seen at any individual time point in this experiment. When all of the methane flux data from the bog mesocosms was correlated against $AOT0_{24hr}$ methane emissions increased as $AOT0_{24hr}$ increased. However, this did not occur in the fen mesocosms. The latter is in contrast to previous results from Lloyd (2004) who found that methane fluxes were increased under elevated ozone in fen mesocosms but were unaffected by elevated ozone in bog mesocosms. However, the exposure period only lasted six weeks so increasing plant growth could have had less of an impact. Increasing methane efflux under elevated ozone is also in contrast to the results of Morsky et al. (2008), who found that season-long exposure to elevated ozone reduced methane release. Methane fluxes from a meadow system exposed to elevated ozone were not changed over two years of experimental ozone exposure but in the final year fluxes showed a trend towards reduction with decreased biomass under elevated ozone (Kanerva et al. 2007).

Carbon dioxide fluxes from the bog mesocosms did not correlate with ozone exposure at any point in the experiment, suggesting that ozone exposure did not have enough of an effect on photosynthesis and respiration to change carbon dioxide flux. This is in agreement with results from Potter et al. (1996a) who found that elevated ozone did not have a significant effect on the carbon dioxide uptake of mosses. Many of the

mesocosms contained a high percentage cover of *Sphagnum* mosses so this could have had an effect on carbon dioxide uptake. However, overall $AOT0_{24hr}$ did correlate with carbon dioxide fluxes from the fen mesocosms, and as $AOT0_{24hr}$ increased, carbon dioxide uptake decreased. This could be because of higher rates of respiration from higher plants as they repair ozone damage rather than store the recently fixed carbon (Andersen 2003) or because of a reduction in photosynthesis by higher plants (Power and Ashmore 2002; Yamaji et al. 2003).

When the link between plant cover and carbon gas exchange was analysed irrespective of ozone exposure it was found that methane fluxes showed a negative correlation with both the percentage cover of vascular plants and the percentage root cover. This is in contrast to the hypothesis that increasing vascular plant cover would increase methane emissions as wetland plants provide a conduit for gas emissions and also provide increased root exudation as a source of simple carbohydrates (Chanton et al. 1997; Popp et al. 2000; Saarnio et al. 1997; Whiting and Chanton 1993). It has been found by many authors (e.g. (Chanton et al. 1997; Rinnan et al. 2003; Whiting and Chanton 1993) that methane emissions are positively correlated with vascular plant growth which is the opposite of what happened in this experiment. However, methanogenesis is an obligately anaerobic process (Peters and Conrad 1996; Segers 1998) and one function of the aerenchyma is to transport oxygen down to the rhizosphere (Roura-Carol and Freeman 1999). As the roots in this experiment reached the base of the mesocosms (20cm) by three months into the study period, it could be that they were contributing to the oxygenation of the peat and potentially decreasing methanogenesis while simultaneously increasing methanotrophy, a proposal supported by Figure 4.10. Other experiments have also found impacts of root growth, Thomas et al. (1996) found that root material extended to the base of intact peat mesocosms and that methane concentrations within peat mesocosms (at 15cm depth) were lower during daylight hours and it has been found that methane oxidation is generally higher in experiments using peat mesocosms than in intact wetlands because of the higher root density associated with potted plants (Lombardi et al. 1997; Schipper and Reddy 1996).

Both methane production and methane consumption are microbiological processes

and, as ozone does not penetrate into the soil (Turner et al. 1974), elevated ozone can only cause a change in carbon gas fluxes through altered plant processes and resource allocation (Kanerva et al. 2006; Kanerva et al. 2007). Many studies have found that elevated ozone reduces carbon partitioning to the roots of plants (Andersen 2003; Grantz and Farrar 2000; Grantz and Yang 2000) and in this system it could be that a reduction in vascular plant cover under elevated ozone may have led to a reduction in root biomass. If the root biomass is lower in mesocosms with less vegetation it could be that there is less oxygenation of the soil resulting in less methanotrophy and more methane production. A further possibility is that ozone caused a change in the below-ground availability of labile carbon but other results have found that exposure to elevated ozone had little effect on microbial respiration and structural diversity of the bacterial community in the rhizosphere (Chapman et al. 2005; Dohrmann and Tebbe 2005).

4.4.3 What are the implications for wetland functioning?

As elevated ozone reduces vascular plant cover in wetlands there is the potential for the amount of carbon stored over the growing season to decrease. Peat forming wetlands function as a carbon sink because the plant biomass laid down at the end of the growing season is not fully decomposed but instead undergoes anaerobic decay (Belyea and Clymo 2001). If there is less plant growth because of increasing background ozone there will be less carbon stored in the peat. Coupled with this is the reduction in carbon dioxide uptake as vascular plant cover is reduced. This will further decrease the amount of carbon dioxide taken out of the atmosphere by the vascular plants and reduce the sink strength of wetlands for carbon. Over a longer period of time this could reduce microbial activity in peatlands as decomposition is frequently carbon limited (Fontaine et al. 2007; Wetzel 1992).

Wetlands are already a major source of methane (Whiting and Chanton 1993, 2001) and a reduction in plant cover caused by elevated ozone appears to further increase the emission of methane from wetland mesocosms. This has the potential to increase global warming as methane has a warming potential twenty times that of carbon dioxide. A further potential problem with wetlands emitting more methane under

elevated ozone is that research has found that reducing methane emissions results in lower ozone concentrations over a widespread area (West and Fiore 2005). If this is the case increasing methane emissions from peatlands has the potential to increase ozone concentration further.

5 Does plant cover affect carbon cycling responses to elevated ozone?

5.1 Introduction

In the previous Chapter the focus of the study was on the effects of increasing background ozone on wetland plants and carbon cycling within wetlands. Results from this suggested that the effects of ozone on vascular plants may have important impacts on the carbon gas exchange from wetland mesocosms. In this experiment this effect was examined further by exposing mesocosms from an upland bog with and without vascular plants to increasing background ozone. This allowed the importance of the presence of vascular plants to carbon gas exchange to be assessed. Vascular plants can affect methane emission from peatlands through the provision of a conduit from the anaerobic peat to the atmosphere (Chanton et al. 1997; Ding et al. 2005; Greenup et al. 2000; Thomas et al. 1996). Many wetland plants have specialised aerenchyma tissue to allow oxygen down into the roots but this also provides a passage for the released methane to reach the atmosphere without passing through the oxic peat layer at the surface (Whiting and Chanton 1996) where methanotrophs consume a significant fraction of produced methane that diffuses through the peat matrix. An alternative method by which vascular plants can modify wetland systems is through the exudation of low molecular weight sugars that provide an easily assimilable energy source for microbial activity.

Peat-forming wetlands are an important carbon storage ecosystem with global estimates of carbon sequestration in the region of 20-30 g C m^{-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 because peatland decomposition rates are low (Rinnan et al. 2003) so the plant material is laid down as peat. 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 (Freeman et al. 2001a; Monteith et al. 2007).

The aim of this experiment was to determine what effects elevated background ozone has on carbon gas exchange and water chemistry in wetland mesocosms and whether the presence of vascular plants changes those effects. The hypotheses are that there will be more methane emissions and more carbon dioxide uptake from mesocosms containing vascular plants and that increasing ozone will have a negative impact on the vascular plant growth. This may lead to greater reductions in carbon dioxide uptake in the mesocosms exposed to increasing background ozone. A reduction in carbon dioxide uptake by vascular plants will reduce the source strength of plant partitioning meaning that less carbon will be transported below ground. This could lead to a reduction in methane emissions as, in an anaerobic environment, organic substrate availability is often a major limiting factor for methanogenesis (Segers 1998).

5.2 Methods

5.2.1 Mesocosm collection

Following the results of the ozone exposure in 2007, where mesocosms taken from the Migneint (SH:785,455) showed a greater response to elevated ozone than mesocosms from Cors Goch, it was decided that this experiment would only use mesocosms taken from the Migneint to maximise replication within the available space. Full details of the Migneint site are given in Chapter 4. Ninety-six mesocosms (diameter 16cm, length 40cm) were taken following the method of Freeman et al. (1993). For full details of the methodology, see Chapter 3, although it should be noted that the surface area and the depth of the mesocosms were much larger in this experiment compared to previous Chapters. Once collected, mesocosms were sealed using plastic trays around the base and encased in non-reactive plastic sheeting to ensure the water supply to each remained separate. Mesocosms were placed in pairs in boxes approximately 38 cm high, which were filled with water to reduce fluctuations in temperature around the mesocosms. The mesocosms were watered

daily throughout the experiment using mains water supply to keep the water-table at the peat surface.

Half of the mesocosms were designated "plant free" before the start of the ozone exposure and any vascular plants that germinated were removed by excision below the peat surface as soon as they appeared. Six mesocosms with plants allowed to grow and six with the plants removed were placed in each solardome.

5.2.2 Ozone exposure

Peat mesocosms were exposed to elevated ozone at the CEH solardome facility at Abergwyngregyn for 16 weeks during the summer of 2008. For full details of the ozone exposure set-up, see Chapter 2. The ozone exposure regime was the same as that used during the summer of 2007 (see Chapter 4) except mesocosms were exposed for 16 weeks between 9th May and 2nd September 2008 rather than for 20 weeks.

5.2.3 Gas and water sampling

As the mesocosms used during the 2008 exposure season were larger than those used in the previous experiments a slightly different method of sampling the headspace gases was required. Transparent "orchid pots" with a diameter of 16cm, a height of 25cm and a volume of two litres were used to make the headspace as these were the correct circumference and the plastic attenuated less than 5% of photosynthetically active radiation (PAR). An airtight seal was created using ozone-resistant neoprene and this fitted over the rim of the mesocosm (Figure 5.1). This was an improvement on the previous method, as the peat surface was not disturbed when the headspace was put over the mesocosm. 20ml gas samples were extracted through rubber septa and analysed for carbon dioxide and methane within 24 hours of sampling. Full details of the analysis method are in Chapter 3. Gas samples were taken from the mesocosms at approximately fortnightly intervals over the sixteen weeks.

Figure 5.1: Mesocosms with the headspaces on. The neoprene seal is between the transparent pot and the plastic mesocosm liner. Gas samples are taken through the rubber septa at the top of the headspace chamber.

Pore water samples were taken from 10cm depth using non-reactive, plastic tubing and were filtered through 0.45μm filters to remove particulate and microbial material. Water samples were refrigerated after filtering and were analysed for total carbon (TC) concentration within 3 days of the samples being taken. Full details of the method used for TC analysis are provided in Chapter 3. Water samples were taken at approximately three week intervals during the experimental period but had to finish after 13 weeks due to constraints on time and the carbon 13 labelling of the mesocosms (Chapter 6).

5.2.4 Plant physiology measurements

Assessments of visible senescence on plants growing in the mesocosms were taken approximately fortnightly throughout the growing season. 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 as the number of senesced leaves divided by the total number of leaves then multiplied by 100.

Senescence on moss was visually assessed based on area. Visual assessments of the percentage cover of plants were undertaken at fortnightly intervals over the 16-week exposure period. Measurements of stomatal conductance on *Carex echinata*, the most common vascular plant species to emerge, were taken on three separate days in weeks 13, 14 and 15 once the plants had grown big enough for the leaves to fill the porometer window. Although published work has used conductance measurements taken between the 1st and 3rd quartile for temperature (Mills et al. 2009), the temperature range for the measurements taken in this experiment was only 4°C so the whole data set was used. Measurements were taken using a Delta T Porometer calibrated under experimental conditions before each set of measurements. After sixteen weeks of ozone exposure, above ground vascular plant biomass was harvested, green moss and the brown moss under-layer were harvested and the vascular plant roots were washed and dried to constant mass at 65°C.

5.2.5 Statistical analysis

Statistical analysis of the results was conducted using GENSTAT version 8. Linear regression was used to analyse the relationships between variables. The slope of the regression line y=mx+c (i.e. m) between senescence and time and percentage cover and time was used to determine whether the change over time was linked to ozone exposure. Results expressed as a percentage, such as senescence and plant cover, were arc-sine transformed prior to analysis and back-transformed prior to presentation. For analysis of the gas, total dissolved carbon, plant cover and senescence data from mesocosms where vascular plants were allowed to grow only used mesocosms that contained vascular plants at each time point. (i.e. mesocosms that were designated as containing vascular plants but that did not contain any vascular plants were excluded from the analysis.) The standardised mean difference in methane emissions between mesocosms that contained vascular plants and those that did not was assessed in STATA using meta-analysis techniques to allow the differences between the two mesocosm types in all of the ozone treatments were analysed with one test (Stewart et al. 2008).

5.3 Results

5.3.1 Ozone exposure

Weekly ozone exposure in each solardome followed the smoothed profile of a typical weekly ozone concentration measured at Marchlyn Mawr between the 31st May 2006 and the 6th June 2006 but with the incremental starting concentrations to simulate increasing background ozone. Average daily ozone concentration, $AOT0_{24hr}$ and AOT40 (daylight hours) after 16 weeks of ozone exposure are shown in Table 5.1. The two highest treatments (AA+60 and AA+72) followed each other closely throughout the experiment (Figure 5.2) because the AA+60 treatment consistently ran slightly high and the AA+72 treatment ran low. Average ozone concentrations ranged from 16ppb in the lowest treatment to 94ppb in the highest treatment while the 24hour AOT0 ranged from 45 to 260ppmh over the four month period. AOT40 measured over the daylight hours ranged from 0.003ppm.h to 73ppm.h.

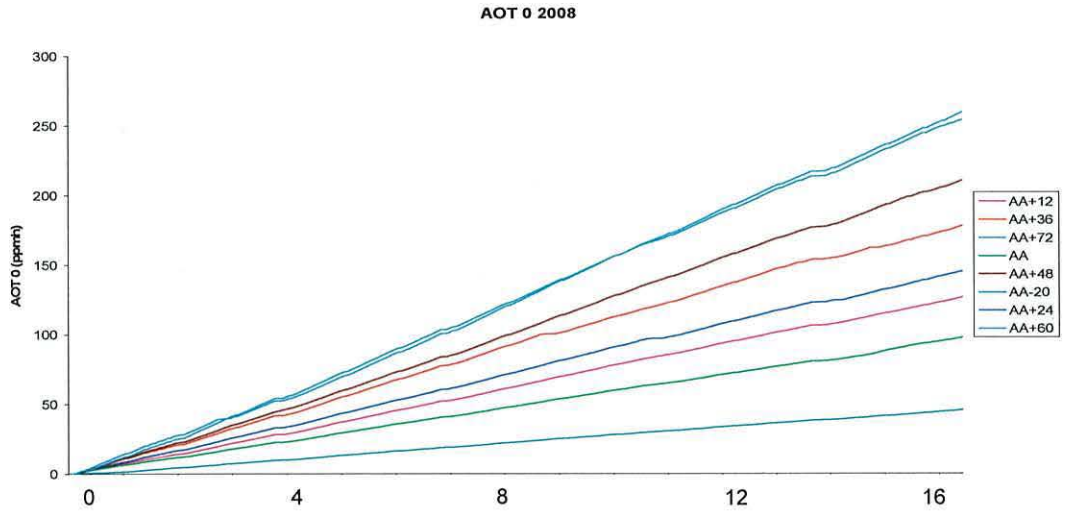


Figure 5.2: Continuous $AOT0_{24hr}$ values from the 8 ozone treatments over the 16 week exposure period.

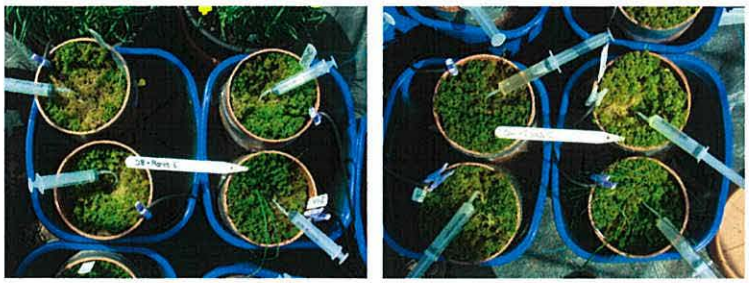
	AA-20	AA	AA+12	AA+24	AA+36	AA+48	AA+60	AA+72
AOT0 (ppm.h)	45.98	98.00	126.68	145.54	178.02	210.57	254.24	259.63
average ozone concentration	16.66	35.51	45.90	52.73	64.50	76.29	92.12	94.07
AOT 40 (ppm.h)	0.003	1.98	11.43	20.81	37.63	50.22	71.78	73.12

Table 5.1: $AOT0_{24hr}$, AOT 40 (daylight hours) and average ozone concentrations of the eight ozone treatments.

5.3.2 Plant physiology

When the mesocosms were first collected vascular plants were not present in any of the mesocosms. They were not seeded with any particular species but in the mesocosms that were designated as "containing vascular plants", individual plants were allowed to germinate throughout the ozone exposure period. The most common species to emerge in the mesocosms was *Carex echinata*, with some upland grass species, such as *Festuca ovina*, in some mesocosms, particularly those with lower percentage cover. The cover of vascular plants in the background treatment (AA) and the second highest treatment (AA+60) are shown at the start of the ozone exposure, after 9 weeks and after 16 weeks in Figure 5.3. The pictures show the mesocosms in the solardomes and the graphs show the percentage cover at the corresponding time points. In some mesocosms there was no emergence of any vascular plant species. The data from these mesocosms were removed from the analysis of all other measurements and are not included in any of the Figures and Tables presented here.

The percentage cover of vascular plants was measured regularly in all treatments. Over the 16-week exposure period, average vascular plant cover increased linearly with time over all ozone treatments (Figure 5.4) (R^2=0.298) and within each ozone treatment the response was linear for seven of the eight treatments (Figure 5.5). The gradient of the regression slope differed between treatments but when the gradient was correlated against ozone exposure (Figure 5.6) there was not a significant linear relationship (R^2=0.063, P>0.1). This is likely to be because seed germination is partially dependent on the seed bank available in each mesocosm. The AA+12 treatment did not show a strong linear relationship with ozone exposure, suggesting that another factor was affecting plant cover in these mesocosms. When it was removed from the regression analysis the R^2 increased to 0.288 with 14.5% of the variation in the data accounted for by ozone exposure. However, although this gave an indication of a negative effect, there was not a significant linear relationship (P>0.1).



AA+60 (left) and AA (right) mesocosms containing plants at time 0.

AA+60 (left) and AA (right) mesocosms containing plants on 15 July (9 wks)

AA+60 (left) and AA (right) mesocosms containing plants after 16 weeks.

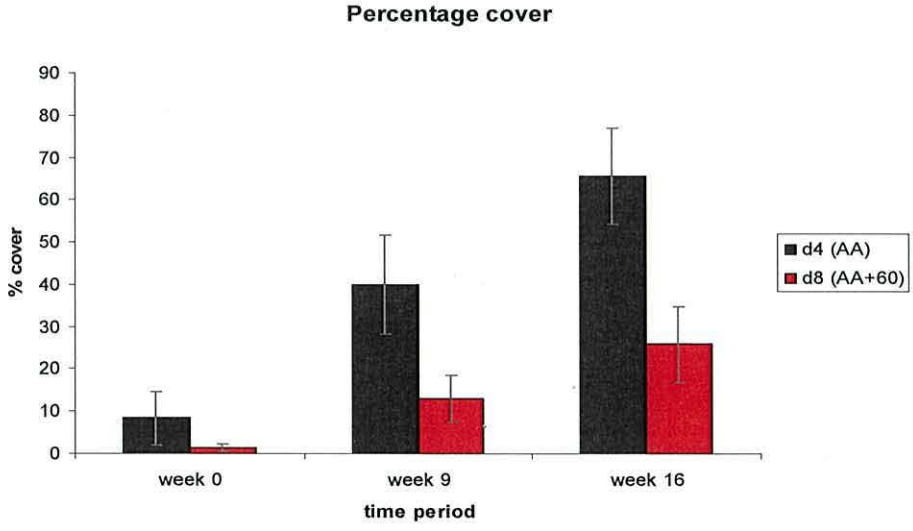


Figure 5.3: Early, mid and late ozone exposure period % cover of vascular plants for AA and AA+60. Graph shows mean value ± standard error for each treatment and time point.

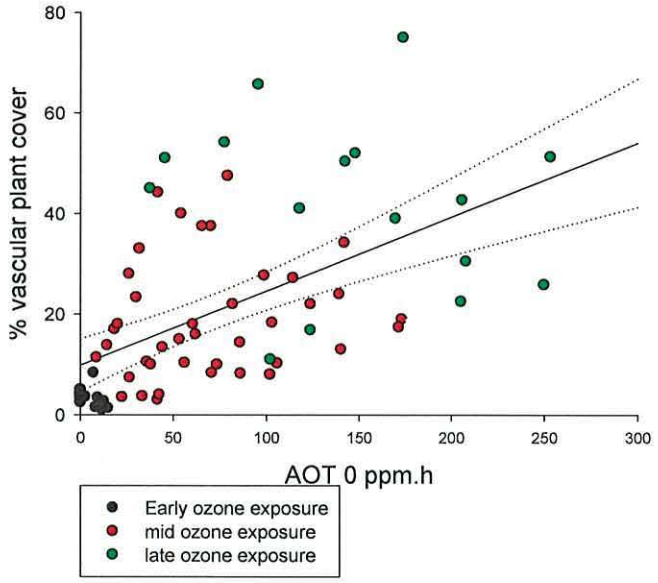


Figure 5.4: Percentage cover of vascular plants. R^2=0.298 Early ozone exposure refers to the first month, mid ozone exposure refers to the second and third month and late ozone exposure refers to the fourth month.

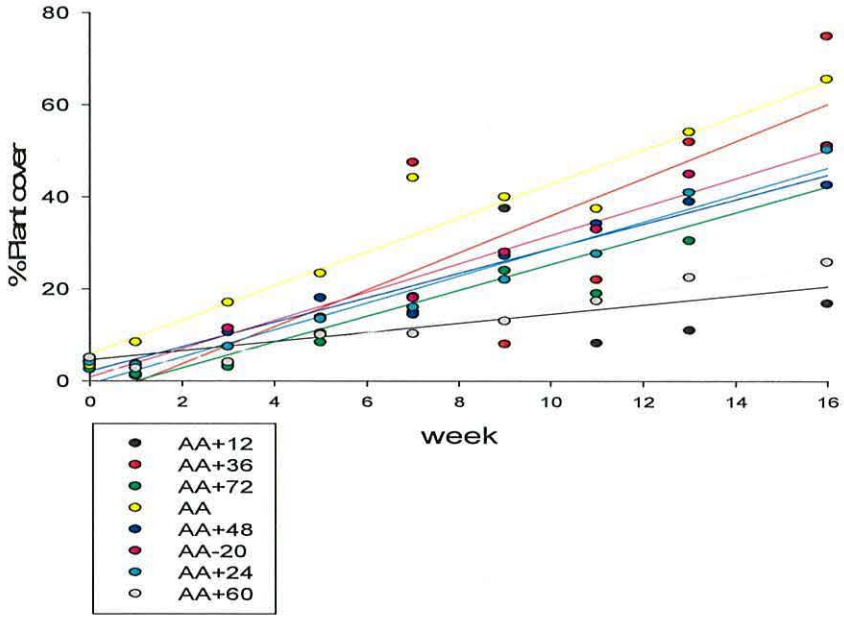


Figure 5.5: The increase in vascular plant cover over time in the 8 ozone treatments.

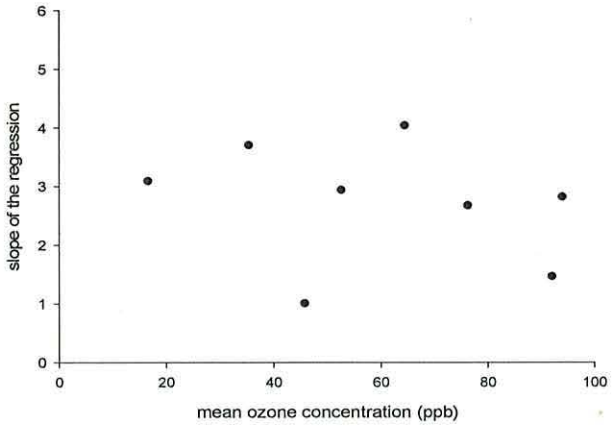


Figure 5.6: The relationship between average ozone concentration and the gradient of the regression slope for percentage plant cover. R^2=0.063, P>0.1

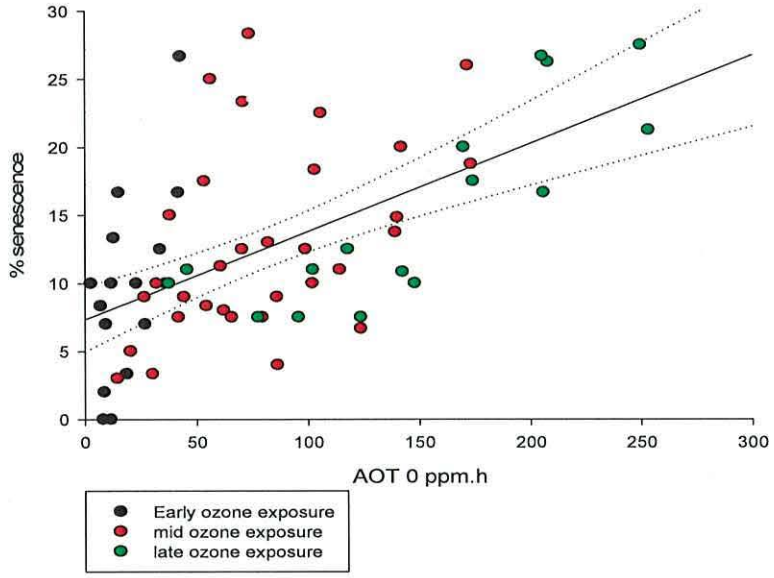


Figure 5.7: All vascular plant percentage senescence data plotted against $AOT0_{24hr}$ at the time of measurement. R^2=0.336, P<0.01. Early, mid and late ozone exposure times defined in Figure 5.4.

The total vascular plant senescence against $AOT0_{24hr}$ is shown in Figure 5.7. This relationship is significant at P<0.01 meaning that as $AOT0_{24hr}$ increases, visible

senescence on the vascular plants also increases. When the mean senescence values for each dome are regressed over time, senescence increases linearly with increasing ozone exposure in each dome (Figure 5.8). When the gradient of the regression slope is correlated with the average ozone concentration in each solardome (Figure 5.9) the gradient increases linearly with ozone concentration (R^2=0.819, P<0.01).

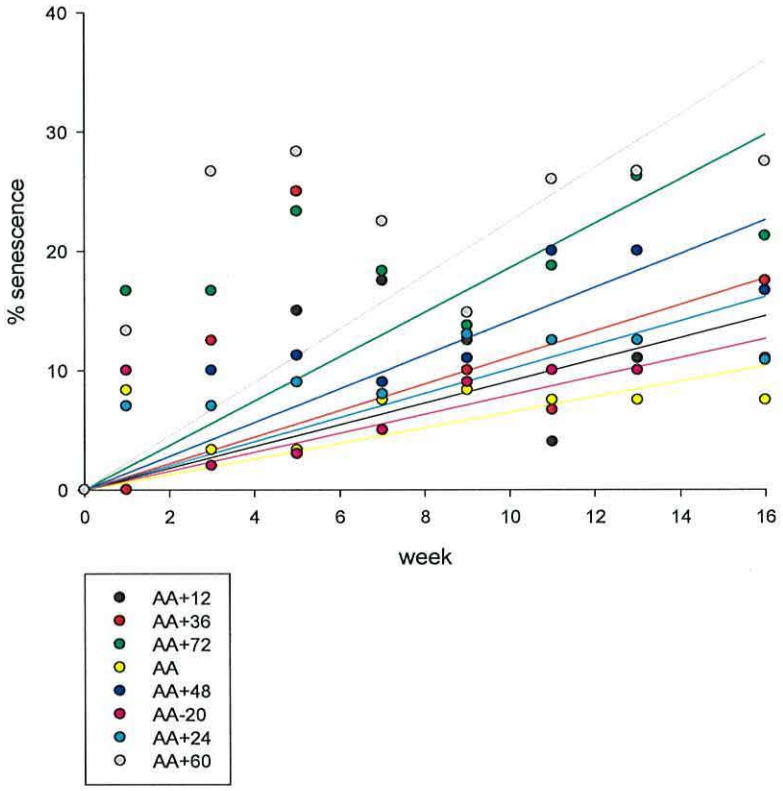


Figure 5.8: Vascular plant senescence over the ozone exposure period in the eight ozone treatments.

The percentage senescence observed on *Sphagnum* within the mesocosms without any vascular plants is shown against $AOT0_{24hr}$ in Figure 5.10. As $AOT0_{24hr}$ increases, the percentage senescence also increases (P<0.001) with 50% of the variance in senescence accounted for by the increase in $AOT0_{24hr}$. Over time, mean senescence values increased linearly in each treatment (Figure 5.11) but when the gradient of the resulting regression lines was correlated with average ozone concentration per dome the increase in gradient with increasing ozone was not significant (P>0.1) (Figure

5.12) suggesting that elevated ozone had less of an effect on moss senescence than vascular plant senescence.

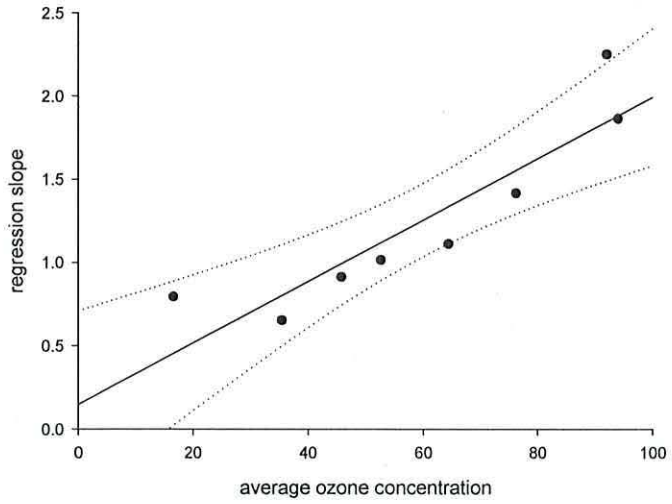


Figure 5.9: The gradient of the regression against average ozone concentration for vascular plant senescence. R^2=0.819, P<0.01

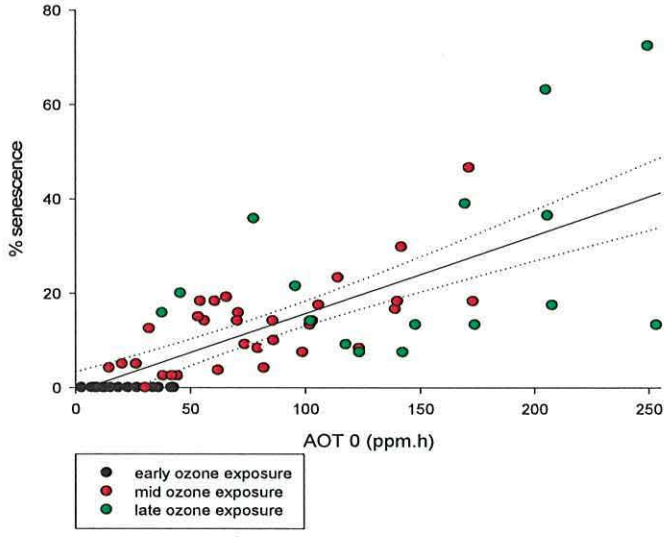


Figure 5.10: All of the moss senescence data plotted against AOT0 (24 hour). R^2=0.524, P<0.001

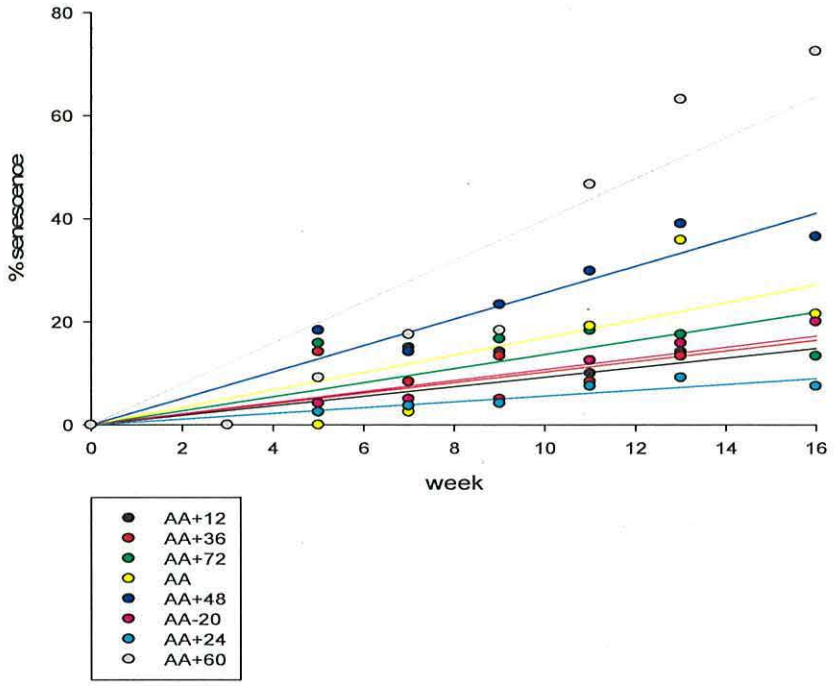


Figure 5.11: Mean moss senescence values for each treatment over the 16 week exposure period.

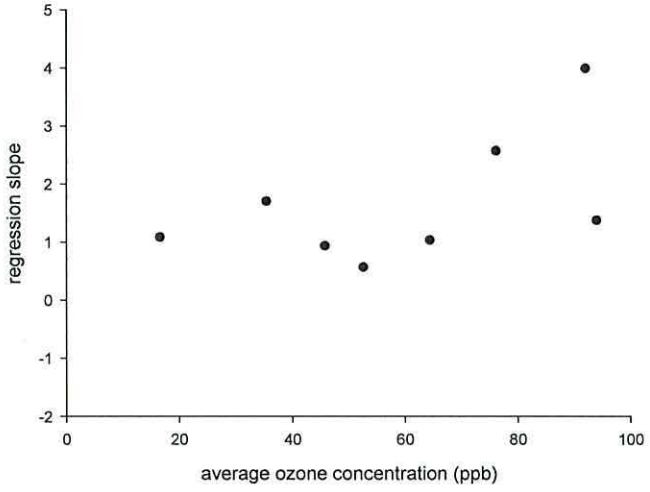


Figure 5.12: The gradient of the regression for moss senescence for each ozone treatment against the average ozone concentration. R^2=0.311, P>0.1

Stomatal conductance was measured on *Carex echinata*, the most common vascular plant in the mesocosms but was not found to have a significant relationship with

ozone concentration (Figure 5.13).

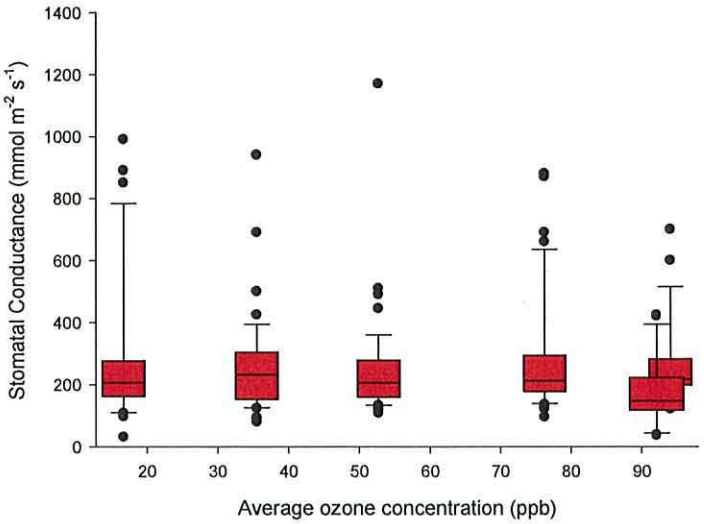


Figure 5.13: Stomatal conductance measurements on *Carex echinata* at three time points after 15 weeks of ozone exposure. Increasing background ozone did not affect stomatal conductance.

After 16 weeks of ozone exposure above and below ground vascular plant biomass was measured. Although root, shoot and total plant biomass all showed a reduction under elevated ozone (Figure 5.14), it was not significant ($P>0.1$ in all cases). Moss biomass was also measured in mesocosms without vascular plants, and, although biomass reduced under elevated ozone it was not significant ($P>0.1$). Following the initial regression analysis, data that were found to have a large standardised residual were removed from the analysis and the statistical tests were repeated. This did not make any difference to the trends seen so the original data is presented here.

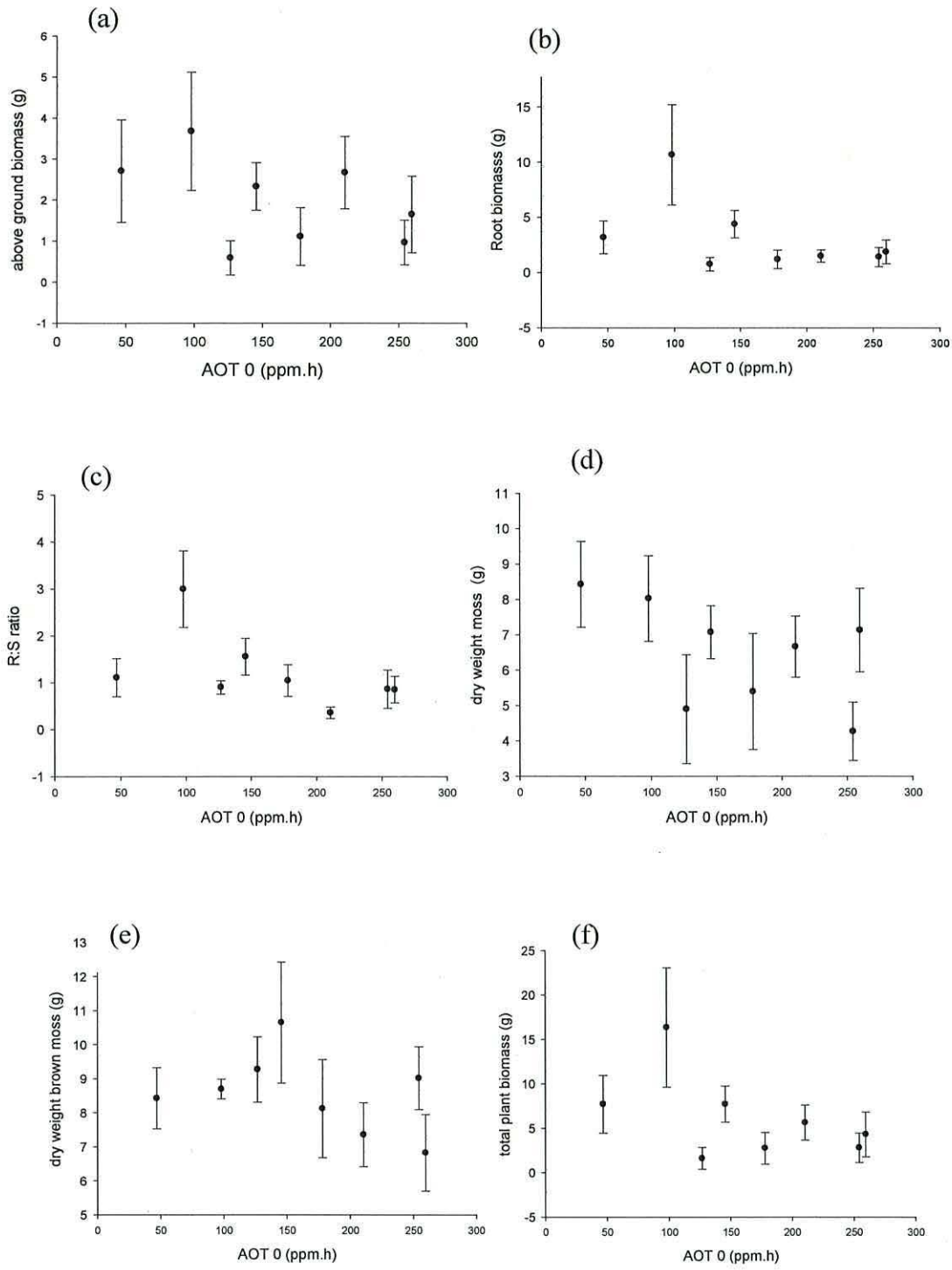


Figure 5.14 (a-f): Biomass of plants after 16 weeks of ozone exposure with regression slopes and 95% confidence intervals shown. a) above ground vascular plant biomass P=0.252; b) root biomass P=0.234; c) root:shoot ratio P=0.208; d) live moss dry biomass P= 0.164; e) dry weight of senesced moss P=0.334; f) total plant biomass P=0.197.

5.3.3 Water measurements

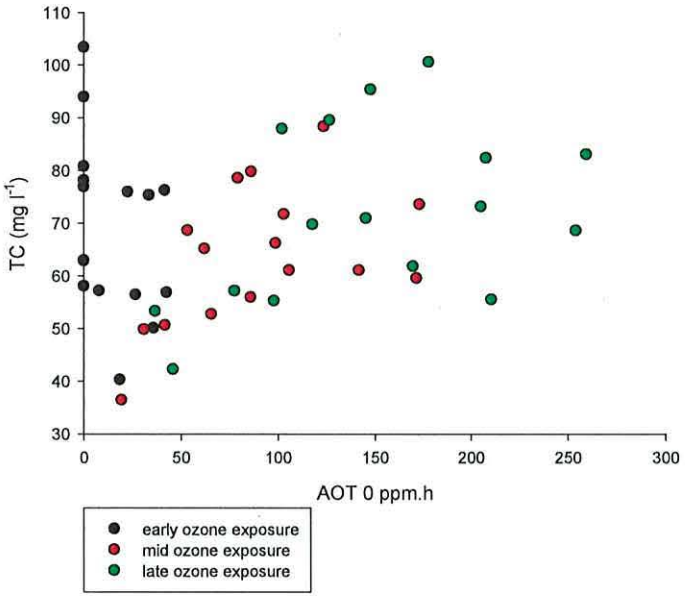


Figure 5.15: Total dissolved carbon concentration against $AOT0_{24hr}$ for mesocosms that did not contain any vascular plants. R^2=0.052, P>0.1

When considering the relationship between total dissolved carbon (TC) concentration and $AOT0_{24hr}$ in mesocosms without vascular plants there was not a significant change in total carbon over increasing $AOT0_{24hr}$ (P>0.1) (Figure 5.15). However, when the percentage change in total carbon concentration from the mesocosms without vascular plants between the start of the ozone exposure and 13 weeks was correlated, the lowest ozone treatments had a decrease in TC, whereas those exposed to higher concentrations of ozone did not change over time (Figure 5.16). This relationship was not significant at P<0.1 but 26% of the variation in TC percentage change could be explained by $AOT0_{24hr}$ so there could be some effect of long term elevated ozone on total carbon concentrations. However, no such relationship was seen in mesocosms containing vascular plants, either over all the data collected (Figure 5.17) or when considering the percentage change in total carbon concentrations (Figure 5.18).

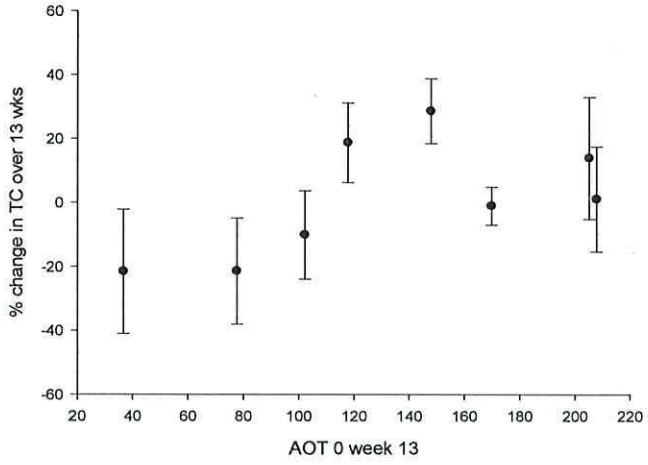


Figure 5.16: The percentage change in total dissolved carbon between the start of the ozone exposure and week 13 for mesocosms that did not contain any vascular plants. R^2=0.368, P>0.1

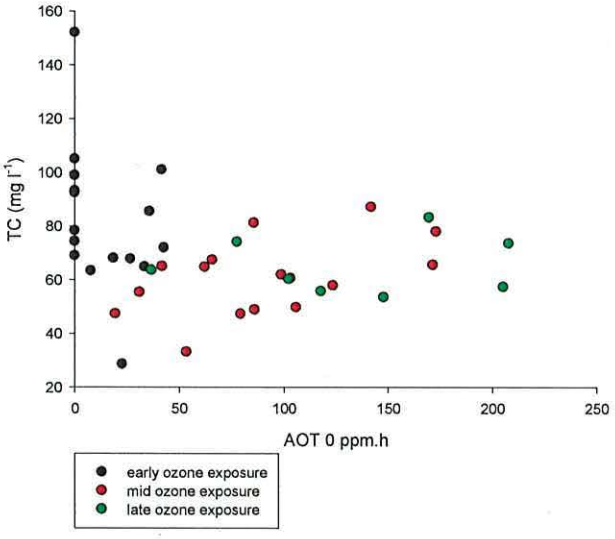


Figure 5.17: Total dissolved carbon concentrations from mesocosms that contained vascular plants against $AOT0_{24hr}$. R^2=0.062, P>0.1

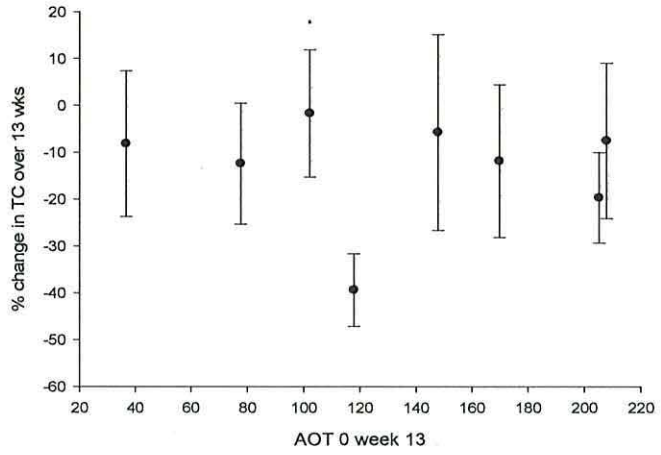


Figure 5.18: Percentage change in total dissolved carbon between the start of the ozone exposure and 13 weeks for mesocosms that contained vascular plants. R^2=0.005, P>0.1

5.3.4 Gas measurements

Mesocosms where vascular plants were present did not show any significant relationship between $AOT0_{24hr}$ and carbon dioxide exchange (P>0.1, R^2=0.014) (Figure 5.19). The majority of mesocosms containing vascular plants were net sinks for carbon dioxide with only five measurements showing net emission of carbon dioxide. These were not related to the length of exposure or to the ozone concentration received by the peat mesocosms. Mesocosms where vascular plants were not present also showed no relationship between $AOT0_{24hr}$ and carbon dioxide flux (P>0.1, R^2= 0.032) suggesting that for ombrotrophic peatlands ozone does not affect carbon dioxide exchange. The majority of mesocosms with *Sphagnum* cover were net sinks for carbon dioxide with only three measurement points showing a small rate of emission of carbon dioxide (Figure 5.20).

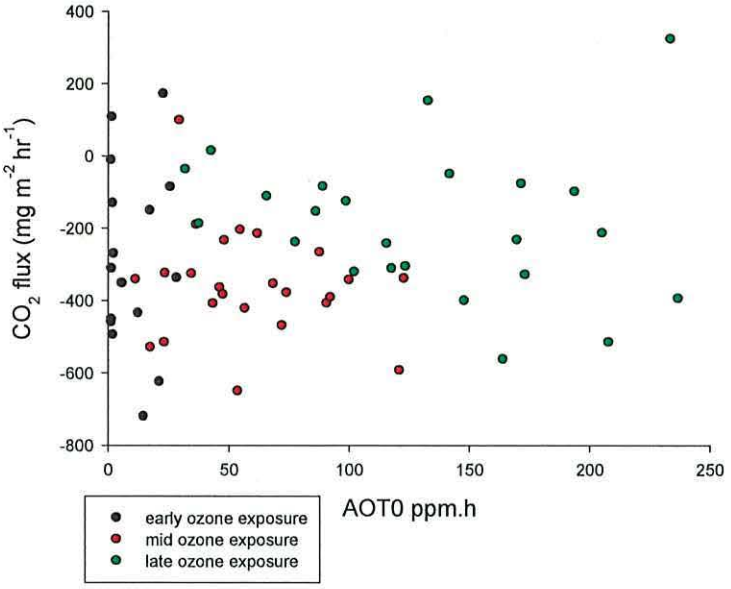


Figure 5.19: Carbon dioxide flux from mesocosms containing vascular plants. R^2=0.014, P>0.1

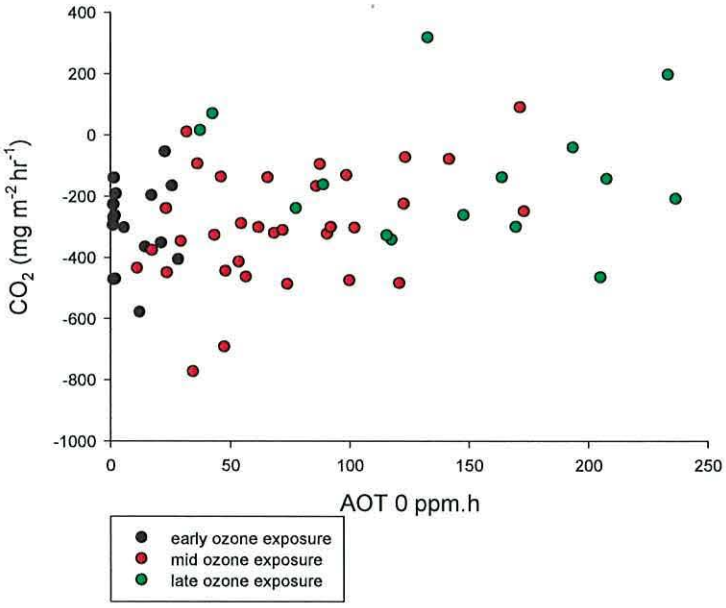


Figure 5.20: Carbon dioxide flux from mesocosms that did not contain any vascular plants. R^2=0.032, P>0.1

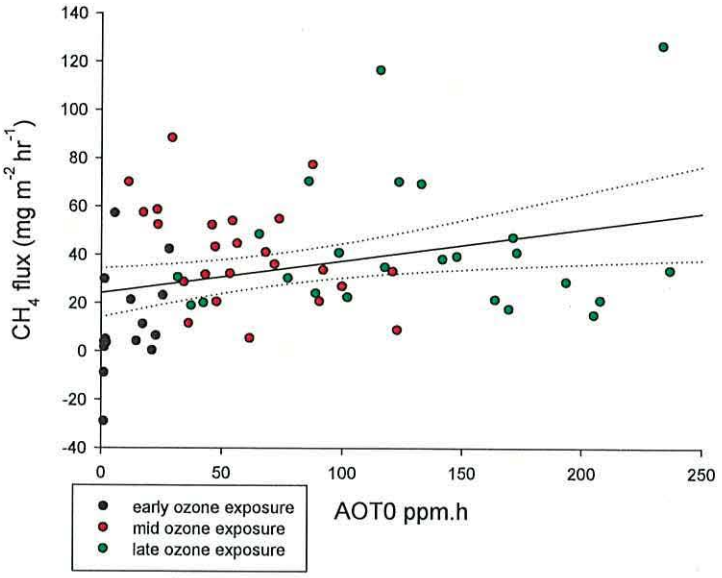


Figure 5.21: Methane fluxes from mesocosms containing vascular plants. R^2= 0.095, P<0.05

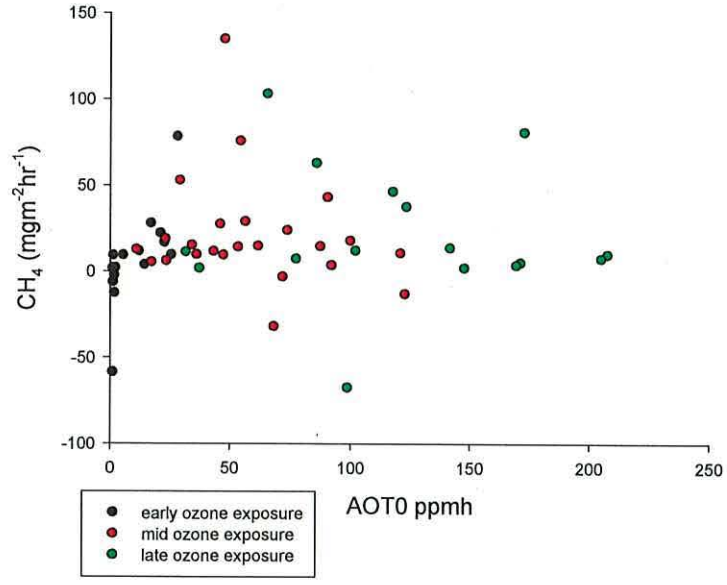


Figure 5.22: Methane fluxes from mesocosms that did not contain any vascular plants. R^2=0.007, P>0.1

When the exchange of methane was correlated against $AOT0_{24hr}$ there was a significant increase in methane efflux as $AOT0_{24hr}$ rose (P<0.05) in mesocosms

containing vascular plants (Figure 5.21). Over the measured values of $AOT0_{24hr}$ methane fluxes doubled. However, the R^2 value for the relationship is only 0.095 so this relationship may not be highly significant as there is a large amount of scatter about the plotted regression. When methane fluxes were analysed against $AOT0_{24hr}$ for mesocosms that did not contain vascular plants there was no relationship ($P>0.1$, $R^2=0.007$) (Figure 5.22). When methane fluxes from mesocosms containing vascular plants were compared to methane fluxes from those without vascular plants over all ozone concentrations methane emissions were significantly higher from mesocosms containing vascular plants ($P<0.05$) (Figure 5.23).

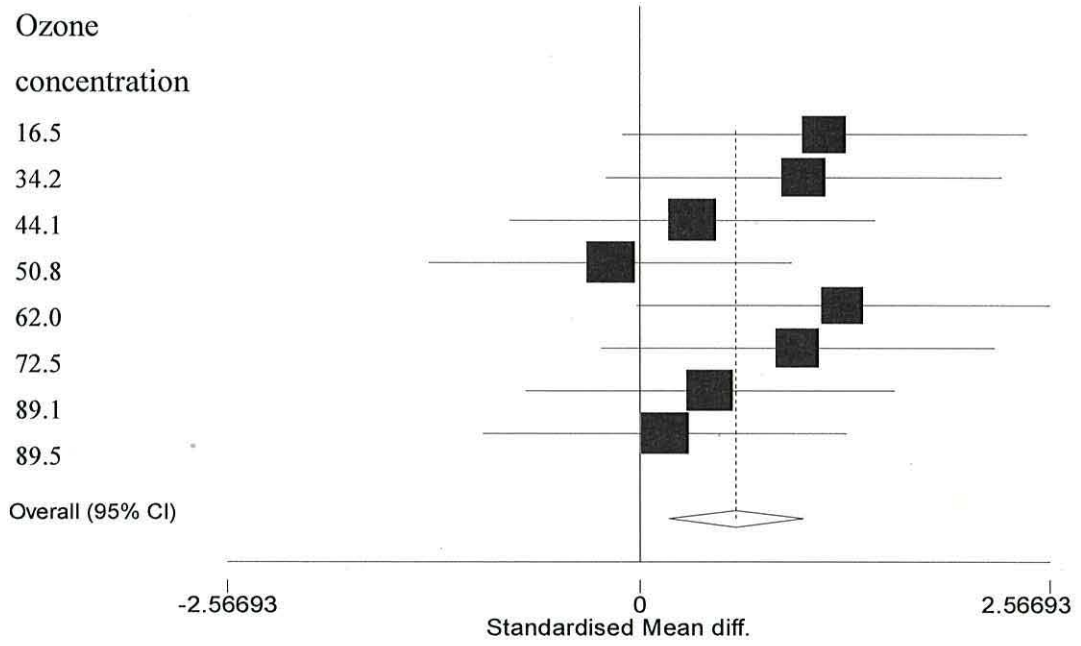


Figure 5.23: The difference in methane fluxes between mesocosms with and without vascular plants. The diamond shows the difference between vegetation cover is significant ($P<0.05$) as it does not pass over the centre line and the difference is positive meaning that over all the ozone treatments methane fluxes are higher from mesocosms containing vascular plants.

5.4 Discussion

The aims of this experiment were to quantify the impacts of increasing background

ozone on carbon fluxes through wetland mesocosms and to further understand the additional effects of the presence of vascular plants on these fluxes. The hypotheses tested were that ozone would reduce vascular plant growth and that this would lead to a reduction in carbon supply below ground, thus leading to a reduction in methanogenic activity. This would mean a reduction in carbon dioxide uptake and a decrease in methane emissions under elevated ozone. A second hypothesis was that at matching ozone exposure, methane emissions and carbon dioxide uptake would be higher from mesocosms containing vascular plants compared to those with 100% moss cover.

5.4.1 The effects of ozone on plant physiology

As peak concentrations of ozone have decreased by approximately 30% over the past decade (NEGTAP 2001) but background ozone concentrations have shown an annual increase of 0.1 ppb y^{-1} over the same period (Coyle et al. 2003) this experiment focussed on the effects of increasing background ozone on plant physiology. Over the 16 week exposure period both vascular plant and moss senescence were found to increase as $AOT0_{24hr}$ increased. However, the gradient of the regression slope for each ozone treatment only showed a significantly increasing relationship with average ozone concentration for vascular plant senescence. This indication that wetland plant species are sensitive to increasing ozone concentrations agrees with published results (Franzaring et al. 2000; Power and Ashmore 2002). Although the senescence data would indicate that wetland plants are sensitive to increasing background ozone concentrations, percentage cover data analysis and the analysis of all of the biomass data showed that increasing ozone concentrations resulted in a non-significant downward trend, indicating that the observed ozone effects were not sufficiently strong to override the natural variation within the mesocosms. The lack of significant effects on biomass agrees with findings from Tonneijik et al. (2004) who found that three seasons of ozone exposure had no significant effect on plant biomass from wet meadows. Similar results were also found by Hayes (2007); in an analysis of sensitivity of a variety of upland species, some that were common in wetland areas such as *Carex echinata*, *Potentilla erecta* and *Juncus squarrosus*, showed increases in senescence and injury without any loss of biomass after exposure to elevated ozone.

The increasing susceptibility of vascular plants to increased senescence compared to mosses agrees with the findings of Potter et al. (1996a; 1996b) and Bosley et al. (1998) who found that *Sphagnum* species and *Polytrichum commune* were relatively insensitive to rising ozone concentrations despite their tissues being only one cell thick and lacking a protective cuticle.

5.4.2 The effects of ozone on wetland carbon fluxes

The effects of elevated ozone on carbon fluxes through the mesocosms used in this experiment appeared to be affected by the presence or absence of vascular plant species. The total dissolved carbon present in the pore water of the mesocosms without vascular plants showed a slight trend towards an increasing percentage change in concentration with increasing background ozone after 13 weeks. Although this was not significant, the P value was 0.11 with 26% of the variation in total dissolved carbon data being explained by increasing $AOT0_{24hr}$. However, in mesocosms that did contain vascular plants there was no relationship between ozone concentration and total carbon concentration or the percentage change in total dissolved carbon in the pore water. This is in contrast to previous results (Jones et al. 2009) that measured dissolved organic carbon concentrations under elevated ozone from fen and bog microcosms. In that experiment, it was found that DOC concentrations decreased under increased peak ozone concentrations in the fen microcosms dominated by vascular plants but DOC concentrations in the bog microcosms, dominated by *Sphagnum*, were not affected by elevated ozone. However, recent results analysing the concentration of organic acids in pore water of bog mesocosms exposed to elevated ozone double the current ambient found an increase in acetate, formalate and oxalate concentrations compared to the controls after 29 weeks of ozone exposure (Morsky et al. 2008). This increase in organic compounds under long-term elevated ozone agrees with the trend found in the current experiment that the percentage change in dissolved carbon is higher in mesocosms exposed to elevated ozone although only in mesocosms that did not contain any vascular plants. It has been hypothesized that the relative increase in organic compounds was most likely to be due to an increase in microbial activity and biomass

(King et al. 2001; Morsky et al. 2008) breaking down root and litter material. Increased senescence could also have contributed to an increase in degradable substrate as dead leaves are lost from the plants. However, such an increase in microbial activity under elevated ozone is not seen in all experiments and in all soil types, for example in forest soils results have shown that elevated ozone had no effect on enzyme activity (Larson et al. 2002), and only a small effect on the structural diversity of rhizospheric bacteria (Dohrmann and Tebbe 2005).

Methane emissions from mesocosms containing vascular plants showed an increase during the exposure to elevated ozone although there was no effect seen in mesocosms without vascular plants. Acetate is a major substrate for methanogenic bacteria and if that does increase in concentration under elevated ozone (Morsky et al. 2008) it could be a reason why the increase in methane emission is seen as, in anaerobic peat, substrate availability is the major limiting factor for methanogenesis (Segers 1998). In this experiment however, the dissolved carbon concentration was not significantly affected by ozone in mesocosms containing vascular plants and methane emissions did not change in mesocosms without vascular plants. Published results show differing responses of methane emissions from peat mesocosms exposed to elevated ozone. Niemi et al. (2002) found that ozone concentrations of 100ppb caused an increase in methane emissions, while Rinnan et al. (2003) found that elevated ozone caused a non-significant increase in methane emissions. However, Morsky et al. (2008) found that one season of exposure to elevated ozone caused methane emissions from mesocosms to decrease. These conflicting results suggest that the response of methane emissions to elevated ozone is a complex process and may be dependent on plant species present.

Methane emissions were higher from mesocosms that contained vascular plants. This is most likely to be because wetland plants provide a conduit for gases to diffuse from the substrate to the atmosphere through the aerenchyma (Chanton et al. 1997; Ding et al. 2005; Greenup et al. 2000; Thomas et al. 1996). If methane does not pass through the aerenchyma it diffuses through the peat and the majority is oxidised by methanotrophs as it passes through the oxic/anoxic interface where oxygen is available (Segers 1998; Whalen and Reeburgh 2000). This could explain why

methane emissions did not increase from mesoscosms without vascular plants even though dissolved carbon concentrations did increase. It could also be that exudates from vascular plant roots provide an energy source (Schutz et al. 1991) that can be broken down to acetate and hydrogen by various bacteria and archaea to supply the substrates to methanogens.

In this experiment, exposure to elevated ozone had no effect on carbon dioxide exchange in the mesocosms irrespective of the presence of vascular plants. This is in agreement with previous results that found that ozone exposure did not cause a significant change in net carbon dioxide exchange (Niemi et al. 2002) and only transiently decreased net ecosystem exchange (Rinnan et al. 2003). This suggests that elevated ozone did not have any effect on plant uptake of carbon dioxide, which is backed up by stomatal conductance measurements on *Carex echinata* present in the mesocosms. There was no significant change in stomatal conductance as ozone concentrations got higher suggesting that carbon dioxide uptake is unlikely to be affected by elevated ozone. This is in contrast to previous research that showed stomatal conductance and photosynthesis were reduced after exposure to elevated ozone (Andersen 2003; McKee et al. 1995; Paakkonen et al. 1996; Paakkonen et al. 1998b; Pell et al. 1997). However, recent results from Mills et al. (2009) found that stomatal control was impaired in grassland species after chronic exposure to elevated ozone, leading to a potential increase in stomatal conductance.

5.4.3 Implications for wetland functioning

As background ozone concentrations are predicted to continue to rise it is useful to understand what the implications are likely to be for wetland functioning as they are important carbon storage systems (Gorham 1991). Ozone is a phytotoxic pollutant that has been shown in this experiment to increase plant senescence. This could lead to a reduction in productivity of already nutrient poor systems, leading to a reduction in carbon sequestered below ground. However, plant biomass did not seem to have been significantly reduced by elevated ozone over the experimental period. What has not been studied in this experiment is the regrowth after wintering, which has been found to be reduced in some species after ozone exposure, including those that

showed no change in biomass after one season of ozone exposure (Hayes et al. 2006). Methane emissions increased under elevated ozone but only when vascular plants were present, so it could be that increasing tropospheric ozone will increase methane emissions from wetlands. As wetlands already contribute approximately 45% of global methane emissions (Segers 1998) this could increase the concentration of methane in the atmosphere and increase the effect of methane on global warming. If increasing background ozone does cause a small increase in total dissolved carbon then that could increase the amount of dissolved carbon leaving the peatlands in streams; a process that already seems to be occurring in some places, possibly as a result of acid rain reductions, changing land use or increasing temperature (Freeman et al. 2001a; Freeman et al. 2004a; Monteith et al. 2007; Worrall et al. 2006b).

6 How does ozone exposure affect mechanistic processes in wetlands?

6.1 Introduction

Recent estimates have shown that approximately 5.2Tg yr^{-1} of methane is released from European wetlands (Saarnio et al. 2009) and these emissions are a result of the difference between methane production and oxidation (Saarnio et al. 1997; Whalen and Reeburgh 1992), meaning the net flux of methane can be affected by a change in either process and this can have further effects on the ability of peat-forming wetlands to act as carbon storage systems.

Methane production occurs when organic material is degraded anaerobically by a group of obligately anaerobic archaea known as methanogens (Segers 1998). Once the redox potential of the soil has been reduced to a level below which alternative electron acceptors cannot be used (Jakobsen et al. 1981; Lovley et al. 1996; Peters and Conrad 1996) methanogens use hydrogen and acetate as substrates to produce methane as an end product (Goodwin and Zeikus 1987; Lovley and Klug 1983; Peters and Conrad 1996). Any influx of oxygen into the anaerobic zone can result in a drop in methanogenic activity that persists after anaerobic conditions are resumed (Pavlostathis and Giraldogomez 1991; Whalen and Reeburgh 2000). However, so long as the peat remains anaerobic the methane produced is very stable (McNamara et al. 2006).

In contrast to methane production, methane oxidation is an aerobic process with the highest oxidation rates occurring at the aerobic/anaerobic interface where both oxygen and methane are present (Segers 1998; Whalen and Reeburgh 2000). Methanotrophic bacteria have been shown to oxidise up to 90% of the potential methane flux from wetlands (King 1996; Whalen and Reeburgh 2000) with approximately 25% of the potential flux being consumed by bacteria associated with the roots and rhizomes of

vascular plants (King 1996). If plant biomass is removed from the wetland system oxygen concentration in the water is reduced meaning that methane oxidation deeper in the peat is likely to be reduced (Ding et al. 2005). In contrast with methanogens, methanotrophic bacteria have been found to recover rapidly after a period of anoxia (Whalen and Reeburgh 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) meaning that they are likely to decompose more rapidly, potentially releasing more carbon compounds to the microbial community. 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. 1998a; Saleem et al. 2001) which would reduce the rate of decomposition of leaf litter (Kim et al. 1998b). Elevated ozone has been found to cause an overall reduction in decomposition rate in soy bean leaves that was correlated with increased lignin and reduced starch levels (Booker et al. 2005).

Ozone exposure has 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. 2000a) 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). 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).

The research described in this Chapter aimed to elucidate any potential mechanisms behind increases in methane emissions seen in the previous Chapters. The potential of microbes within peat that has been exposed to ozone to produce and consume methane was analysed, as was in-situ methane consumption using stable carbon isotopes. The decomposition of plant material that has been exposed to ozone was measured to see if there were any differences in nutrient input into the peat that may affect microbial activity. Finally, the flow of recently fixed carbon through the peatland mesocosms was measured using ^{13}C labelled carbon dioxide to follow carbon from the point of fixation through respiration, growth and exudation.

6.2 Methods

6.2.1 Decomposition Assay

This assay was conducted using the plants exposed to four weeks of high or low ozone as described in Chapter 2.

6.2.1.1 Site description and ozone treatment

Six species of vascular wetland plants were used in this experiment; three species from Cors Erddreiniog, a lowland fen, and three species from Marchlyn Mawr, an upland bog, (See Chapter 2 for more details). Species indicative of the NVC community dominant in each area were selected and propagated in controlled greenhouse conditions (supplemented daytime lighting, 18°C during the day and 16°C during the night) until the onset of ozone exposure.

Ozone exposure took place in hemispherical solardomes at Abergwyngregyn (see Chapter 2 for more details). Eight domes were used with four replicate domes per

treatment. The low ozone treatment consisted of a constant background concentration of 20ppb and the high ozone treatment consisted of five days at 150ppb ozone and two days at 20ppb. This ozone exposure continued over a four-week period. Three plants per species per dome were used in this experiment. See Chapter 2 for more details about the ozone exposure and plant harvest. At the end of the ozone exposure period, above ground plant biomass was harvested and dried to constant weight at 65°C. Once dried, composite samples at the dome level were made for each species and ground using a ball mill.

6.2.1.2 Decomposition assay and sampling

The decomposition assay followed the procedure set out by Bragazza et al. (2006). A microbial inoculum was prepared using 2kg of fresh fen peat from Cors Erddreiniog (SH 465 822) and 6 litres of deionised water. The slurry was stirred for 30 minutes then filtered through a coarse filter to remove large particulate matter. Approximately 1g of dried, ground plant material was accurately weighed and put in individual 125ml glass bottles and inoculated with 80ml of the pre-prepared slurry. Blank samples consisted of 80ml of slurry without the addition of any plant material. Bottles were stored in the dark at 10°C and aerobically incubated for 10 days. Immediately after sample inoculation, bottles were sealed and gases were allowed to accumulate for one 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 one hour. Gas samples were then taken using the same method after 3, 5, 7 and 10 days of incubation. Gas samples were analysed for CO_2 using a gas chromatograph fitted with an FID and a methaniser to convert carbon dioxide to methane (see Chapter 3 for more details). Twenty ml water samples were taken and filtered through a 0.45µm filter after the 10 days incubation period. These were analysed for total dissolved carbon (TC), phenolics and dissolved nitrogen as described in Chapter 3. Dissolved organic nitrogen was measured using the Thermalox machine used for TC measurements and ammonium ions were measured using a SKALAR SA 40. After 10 days, unfiltered water samples were taken and analysed for phenol oxidase, beta glucosidase and N-acetylglucosaminidase. Phenol

oxidase assays followed the procedure outlined in Chapter 3 (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 (see Chapter 3) following the method of Freeman et al. (1995).

6.2.1.3 Statistical analysis

Results were analysed using GENSTAT version 8. Carbon dioxide emissions were measured at each time point and 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. Each plant's measurements were analysed separately. Analysis of variance was used to calculate any differences in enzyme activity and water chemistry after the 10 days of incubation.

6.2.2 Methane Production and Consumption

These assays were conducted using peat samples collected from bog and fen mesocosms that had been exposed to increasing background ozone treatments for 20 weeks in 2007 (Chapter 4).

6.2.2.1 Site description and ozone treatment

Two contrasting wetland sites were used in this experiment, the Migneint (SH:785,455); an upland, oligotrophic bog dominated by *Sphagnum* mosses and Cors Goch (SH:495,813); a lowland fen site on Anglesey which is largely covered by *Phragmites australis*. Sixteen intact peat mesocosms (10.5cm diameter, 20cm depth) were taken from each site following the method of Freeman et al., (1993).

Mesocosms were exposed to elevated ozone at the CEH solardome facility at Abergwyngregyn, North Wales (see Chapter 2 for more details). In this experiment four ozone treatments were used; AA+72, AA+48, AA+12 and AA-20 (Chapter 4). These represented the highest ozone concentration, the third highest, sixth highest and the lowest ozone concentrations used in the experiment described in Chapter 4. These

followed the trajectory of ozone concentrations measured over a typical week at the Snowdonia ozone monitoring site at Marchlyn Mawr during the summer of 2006 from May 31st to June 6th but with incremental (+12ppb) starting points. The exposure period within the solardomes was five months from May-September 2007. During this period the water-table was maintained at the surface of the mesocosms through continual drip feeding using standard porous tubing fed from the mains water supply. Supplemental watering by hand took place as required. Eight mesocosms (four from each site) were exposed to elevated ozone in each solardome.

6.2.2.2 Methane production potentials

Following exposure to ozone, vegetation was removed to the level of the peat surface and mesocosms were stored in the dark at 4°C for two weeks. The water-table was maintained at the surface throughout this period. The peat was then removed from the PVC outer mesocosm and cut into two sections; upper and lower. Each section was then cut in half lengthways and the four sections were weighed. One section from each layer (upper and lower) was dried at 105°C for 36 hours to determine the dry weight. 15ml of peat from each remaining section was placed into 125ml Wheaton bottles with 15ml deionised water to make a slurry. Once thoroughly mixed these were flushed with nitrogen (N_2) at a rate of approximately two litres per minute for two minutes. Bottles were immediately capped with gas tight septa following flushing and were over-pressurised with 5ml N_2. 0.5ml headspace samples were taken and analysed following capping and after 24, 48 and 120 hours. During the incubation period, samples were kept at 20°C and were continuously shaken at a rate of 100rpm. Gas samples were measured on a Perkin Elmer GC using an FID to measure methane (see Chapter 3 for more details).

After the end of the incubation period, soils in the bottles were freeze-dried to determine the dry weight of peat used in the incubation so final rates of methane production could to be reported as $\mu g\, g^{-1}\, hr^{-1}$. The rate of methane emission increased slowly over the first 24 hours and the final rate was calculated from the increase in concentrations between 48 and 120 hours as the increase was linear over this time period.

6.2.2.3 Methane consumption potentials

Using the same peat sections as for the anaerobic potentials, 15ml of peat from each section was placed into 125ml Wheaton bottles and made into a slurry with 15ml deionised water. Methane consumption is an aerobic process so these bottles were sealed after the sample was added. 0.3ml methane in air was added to produce a headspace concentration of approximately 140ppm methane. 1ml headspace samples were taken after the addition of methane and after 24 hours. For the next two sampling time points (48 and 120 hours) 5ml of headspace gas was taken and stored in 3.6ml gas-tight Exetainer vials until analysis took place. After the 48 hour sampling point the 5ml taken out was replaced with 5ml 2.1ppm methane in air to prevent a negative pressure forming in the bottles. These soils were also freeze-dried after the end of the incubation period.

6.2.2.4 Stable isotope measurements – methane oxidation

After the peat mesocosms had been exposed to ozone for the five-month growing season the ozone supply to the solardomes was switched off. Following this a two hour time course of methane emissions in the dark took place using two litre PETE plastic covers that had been wrapped in silver foil to remove light and concurrently reduce any change in temperature between the atmosphere and the air above the mesocosms. These were pressed into the peat and sealed below the waterline to make them gas tight. This was to check that methane emissions from the mesocosms remained linear over the time period used.

Mesocosms were exposed to ^{13}C labelled methane in the headspace above the mesocosms. This had to take place in the dark to remove any effect of plant photosynthesis taking up labelled CO_2 that was produced during methane oxidation. ^{13}C labelled methane was passed over sodium hydroxide (NaOH) crystals to remove any $^{13}CO_2$ that could have been present in the cylinder. The $^{13}CH_4$ was mixed with compressed air in an airtight container providing a final concentration of approximately $400ml l^{-1}$. Mesocosms were capped using the same darkened headspaces and 10ml of the $^{13}CH_4$ was added to the headspace. Mesocosms were left for five minutes to allow the gas to mix before 5ml headspace samples were taken to

measure the starting concentration of methane. Headspace samples were taken from the mesocosms every half hour for two and a half hours and the samples were stored until analysis in gastight 3.6ml Exetainer vials. Once the two hour time period had elapsed mesocosms remained capped overnight and final headspace samples were taken the next morning, approximately 18 hours after the start of the incubation. Samples were analysed for $^{13}CO_2$ concentration using an infra-red mass spectrometer (Sercon Hydra 20-20) attached to a GC (Sercon Cryoprep) and the atom percent ^{13}C was calculated from the results using the following equation (Fenner et al. 2004):

$$^{13}C \text{ atom\%} = [R_{sample}/(R_{sample}+1)]*100$$

where R_{sample} is the $^{13}C/^{12}C$ ratio that has been determined from analysis of the gas using infra-red mass spectrometry. From the atom% values the F value (the fraction of the sample derived from 99.99 atom% $^{13}CH_4$) was calculated using the following equation:

$$F = (\text{sample atom\%-NAB atom\%})/(99.99-\text{NAB atom\%})$$

where NAB is the natural abundance control, i.e. the same sampling measure but without the addition of $^{13}CH_4$.

6.2.2.5 Statistical Analysis

The initial analysis was conducted using GENSTAT version 7. General analysis of variance was carried out to test for effects of wetland and depth over time and to see if ozone had an interactive effect on those factors. The effect of ozone cannot be tested for directly as there is only one replicate of each concentration at the dome level. The treatment structure was set as POL(ozone:1)*wetland*depth. The contrast was set as polynomial for ozone with one level. This should show if ozone has a linear effect on the methane concentrations. The block structure was set as dome/mesocosm. Testing the effects of ozone on the four wetland type and depth combinations was conducted using Minitab version 14. The data was first log-transformed to linearise the values then an ANOVA general linear model looked at the ozone concentration, the

sampling date and any interactions between them. Correlations between ozone concentrations and the rate of methane production and consumption were analysed in Minitab version 14. Repeated measures was used to test for differences in carbon dioxide stable isotope measurements in GENSTAT version 10. The correlation between initial F values for carbon dioxide and ozone concentration was measured using Minitab version 15.

6.2.3 Carbon Isotope Tracing

This study was carried out using mesocosms from the Migneint that had been exposed to increasing background ozone concentrations at the solardomes for 16 weeks during summer 2008.

6.2.3.1 Site description and ozone treatment

Mesocosms 16cm in diameter and 40 cm in depth were taken from the Migneint (see Chapter 4 for more details) and exposed to elevated background concentrations of ozone at the CEH solardome facility at Abergwyngregyn. Three of the eight ozone treatments were used for the carbon 13 labelling study, simulated ambient air (AA), ambient air plus 24ppb ozone (AA+24) and ambient air plus 60ppb ozone (AA+60). (See Chapter 5 for more details of the ozone exposure.) Mesocosms had been exposed to ozone for 16 weeks prior to labelling and exposure to ozone continued during the labelling process.

6.2.3.2 Carbon labelling and measurements

Four mesocosms containing vascular plants were labelled in each of the three ozone treatments and two mesocosms were the unlabelled natural abundance controls. Prior to labelling with $^{13}CO_2$ all mesocosms used in the experiment were capped with transparent headspaces (see Chapter 5) to ensure the natural abundance controls were not exposed to $^{13}CO_2$. Two millilitres of 99% atom $^{13}CO_2$ were injected into the headspaces of the mesocosms to be labelled and the headspaces were left on for two hours to allow the plants to take up the labelled carbon dioxide. Before the headspaces were removed gas samples were taken to provide starting measurements

of $^{13}CO_2$, both from the labelled mesocosms and the unlabelled controls. The headspace chambers were removed from the labelled mesocosms first and the solardomes were left to ventilate for five minutes (approximately 10 air changes) before the headspace chambers were removed from the controls to reduce the possibility of exposure to labelled carbon dioxide. Once headspace chambers had been removed, pore water samples were taken from all mesocosms and frozen at -20°C until analysis. Water samples were freeze-dried and the remaining dry material was analysed for ^{13}C content.

Initial labelling took place between 12 midday and 2pm and further headspace gas and pore water samples were taken after five hours and nine hours. At each sampling point gases were allowed to accumulate in the headspace for one hour before gas samples were taken. After 24 hours, final headspace gas and pore water samples were taken and mesocosms were destructively harvested to obtain above-ground vascular plant, moss and root samples. The plant samples were dried to constant mass at 65°C and were then ground using a ball mill for ^{13}C analysis.

Gas, plant and water samples were analysed for ^{13}C content using an infra-red mass spectrometer connected to a GC and an elemental analyser (Costech). Plant and water samples were analysed for total ^{13}C content while gas samples were analysed for $^{13}CO_2$ and $^{13}CH_4$.

6.3 Results

6.3.1 Decomposition assay

Cumulative carbon dioxide emissions from the decomposition of the six plants used in the assay are shown in Figure 6.1. *Potentilla erecta* plants from the fen that had been exposed to elevated ozone showed a significant increase in carbon dioxide emissions 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 significantly according to past ozone exposure, although carbon dioxide emissions were slightly lower from plants that had been exposed to elevated ozone.

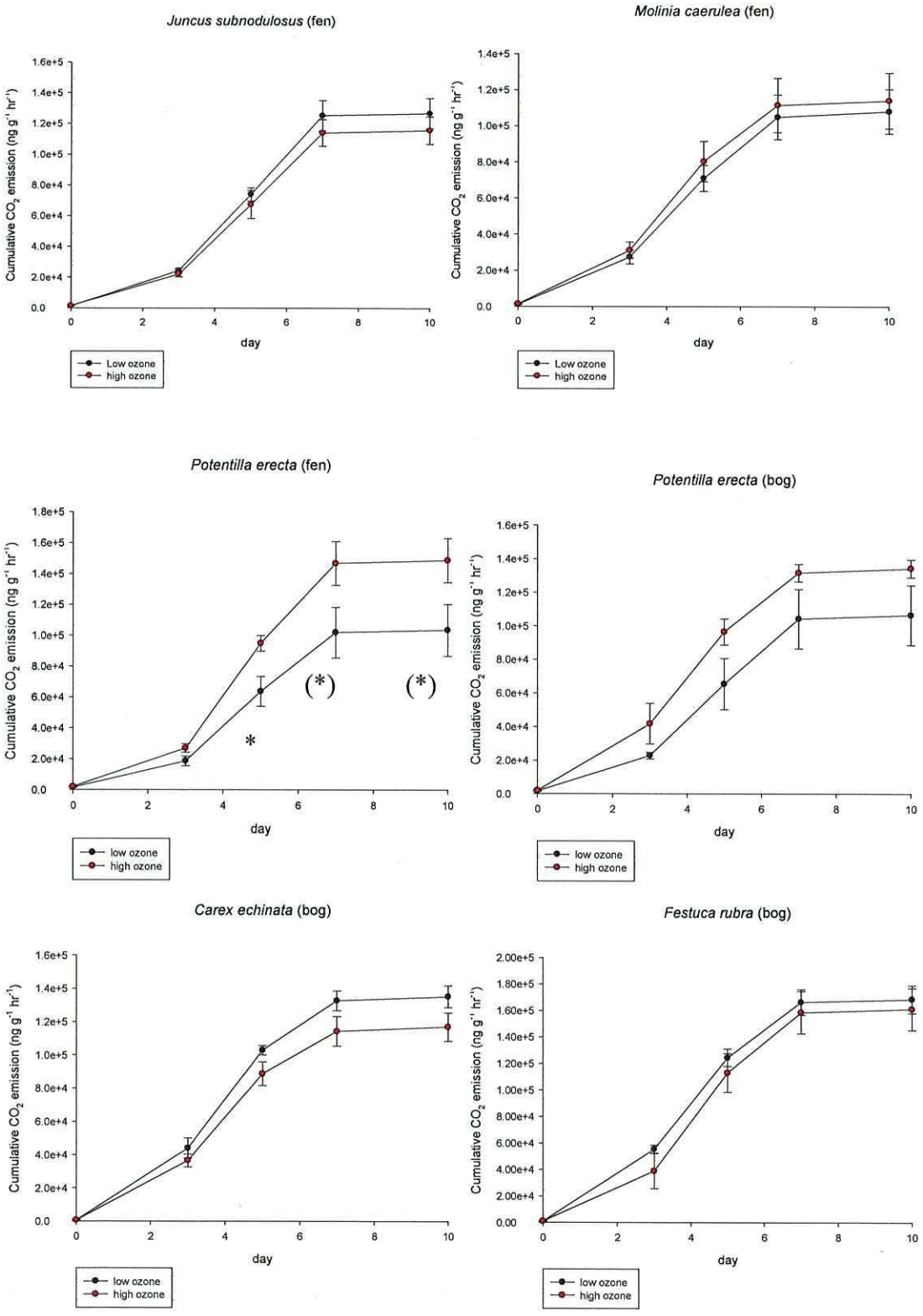


Figure 6.1: Cumulative carbon dioxide flux over the 10 day decomposition period for the five species used. * $P<0.05$, (*) $P<0.1$.

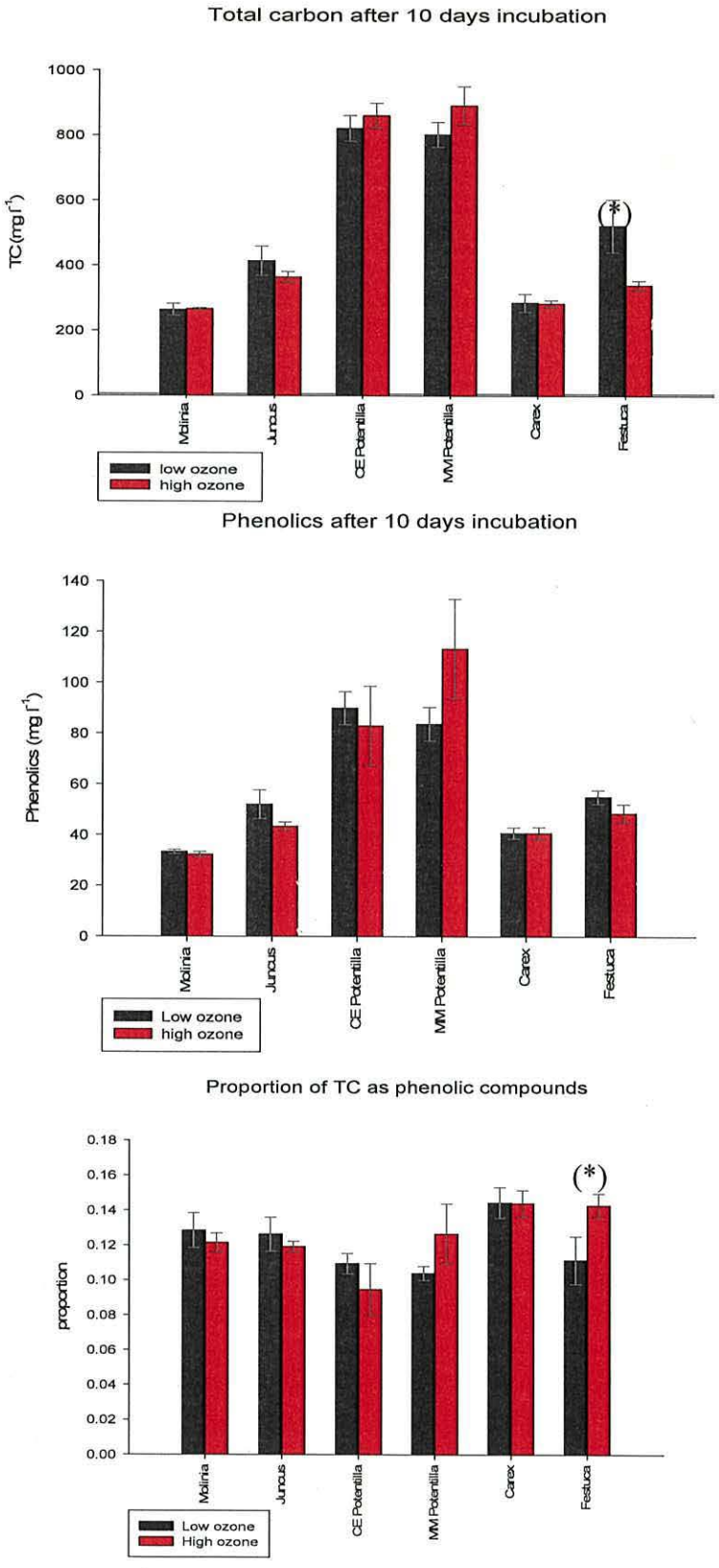


Figure 6.2: Total carbon, phenolic content and the proportion of carbon compounds that are phenolics in the slurry after 10 days of decomposition. (*) $P<0.1$

Total carbon and phenolic compounds after 10 days of decomposition were very similar from plants that had and had not been exposed to elevated ozone (Figure 6.2) with only total carbon from the *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 phenolics compounds being increased under elevated ozone for *F. rubra* (P<0.1).

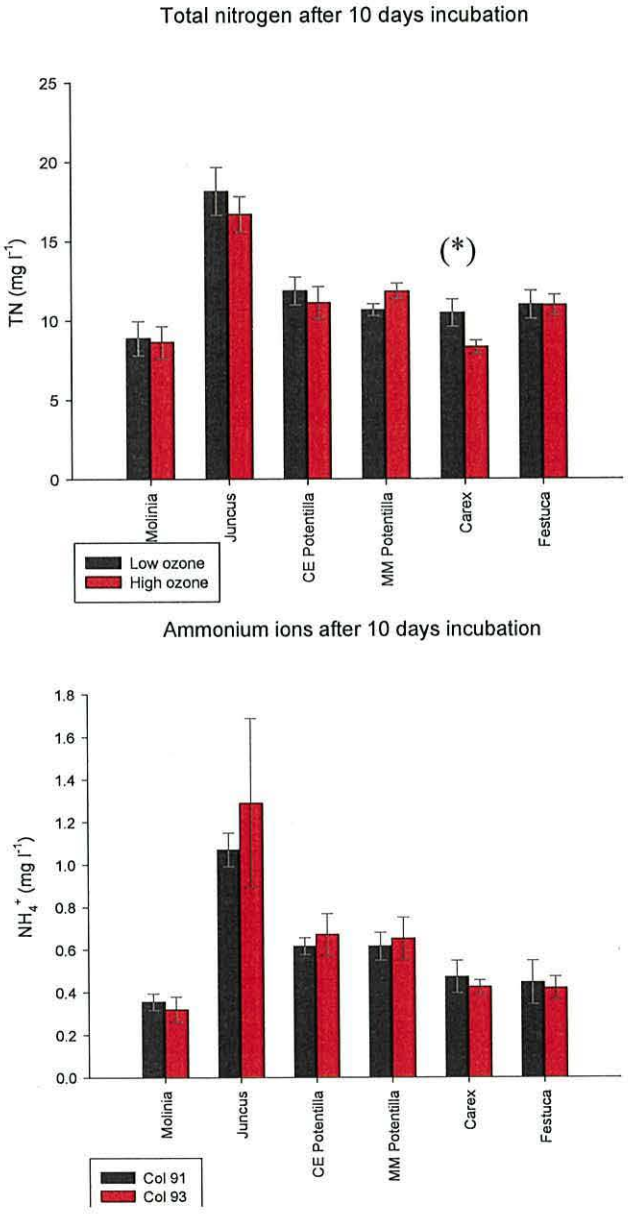


Figure 6.3: Total nitrogen and ammonium ion content of decomposing slurry after 10 days of decomposition. (*) P<0.1

The concentration of ammonium ions after 10 days of aerobic decomposition did not change for any of the six species (Figure 6.3) 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 (Figure 6.4) and N-acetylglucosaminidase (Figure 6.5) showed significant differences with ozone treatment. Phenol oxidase activity did not show any variation under elevated ozone, but within treatment variation was high and enzyme activity was very low (Figure 6.6). 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).

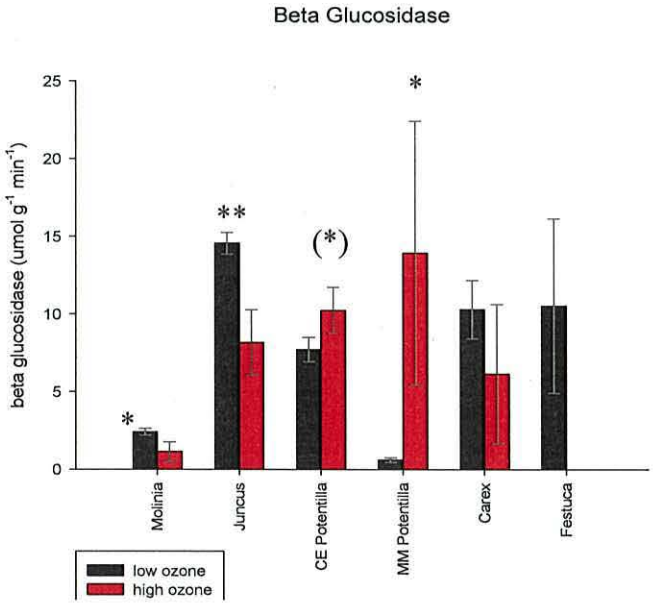


Figure 6.4: Beta glucosidase activity after 10 days of decomposition ** P<0.01, * P<0.05, (*)P<0.1

A similar pattern was seen with N-acetylglucosaminidase; activities increased in the assays performed using 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.

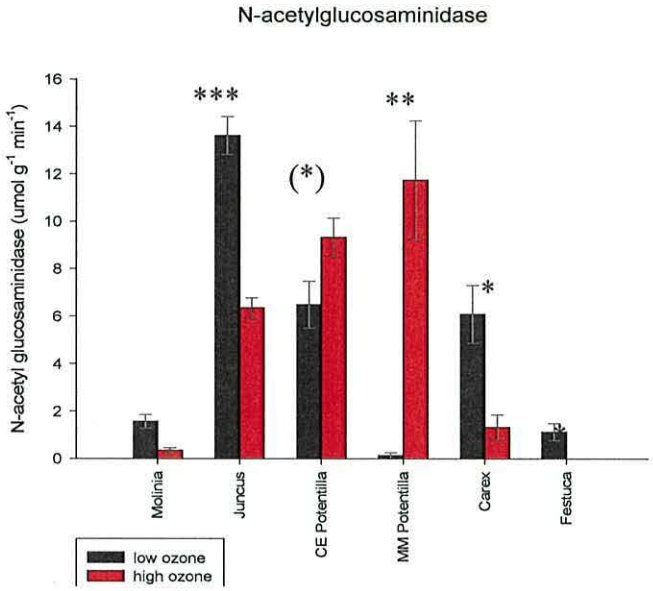


Figure 6.5: N-acetylglucosaminidase activity after 10 days of decomposition

** P<0.01, * P<0.05, (*)P<0.1

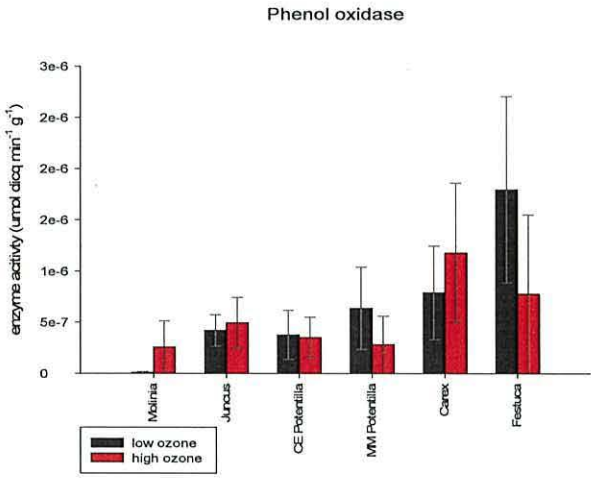


Figure 6.6: Phenol oxidase activity in slurries containing the six species after 10 days of decomposition.

6.3.2 Methane production

Depth of the mesocosm that peat samples were taken from had a significant effect on methane production from both fens and bogs across the whole time period that

samples were taken for ($P<0.001$). Methane production rates were higher in the upper 10cm of the peat from bog mesocosms, whereas they were higher in the lower 10cm of peat from fen mesocosms (Figure 6.7). The wetland type did not have any significant effect on methane production. When the concentrations of methane were split by wetland type and depth the date of sampling had a highly significant effect meaning that changes were occurring over time but ozone did not show any significant effect on methane production from either wetland or depth. When the effect of ozone on maximum methane production rate was tested the only significant effect of ozone was on peat samples from the upper half of the fen mesocosms ($P<0.05$) with methane production decreasing as $AOT0_{24hr}$ increased (Figure 6.7).

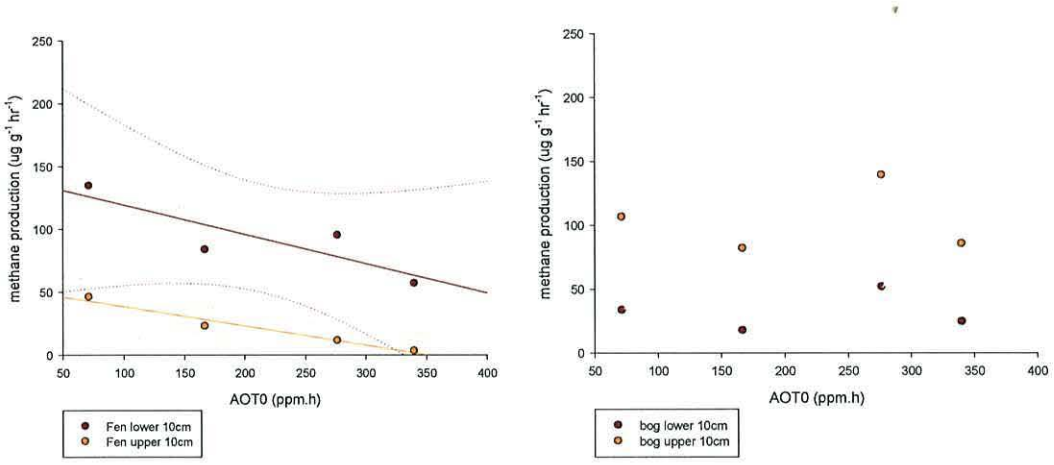


Figure 6.7: Rates of methane production from fen (left) and bog (right) mesocosms. The relationship between $AOT0_{24hr}$ and methane production is significant for samples from the upper 10cm of the fen mesocosms ($P<0.05$).

6.3.3 Methane consumption

In contrast, depth of peat sample did not have a significant effect on methane consumption but wetland type did over the first three days with bogs having higher methane consumption than fens ($P<0.05$). When samples were analysed by wetland type and depth separately ozone did not have a significant effect on methane concentration although samples taken from the lower 10cm of the fen mesocosms showed a significant time*ozone interaction ($P<0.05$). Rates of initial methane

consumption were not significantly correlated with $AOT0_{24hr}$ for either the fens or the bogs (Figure 6.8).

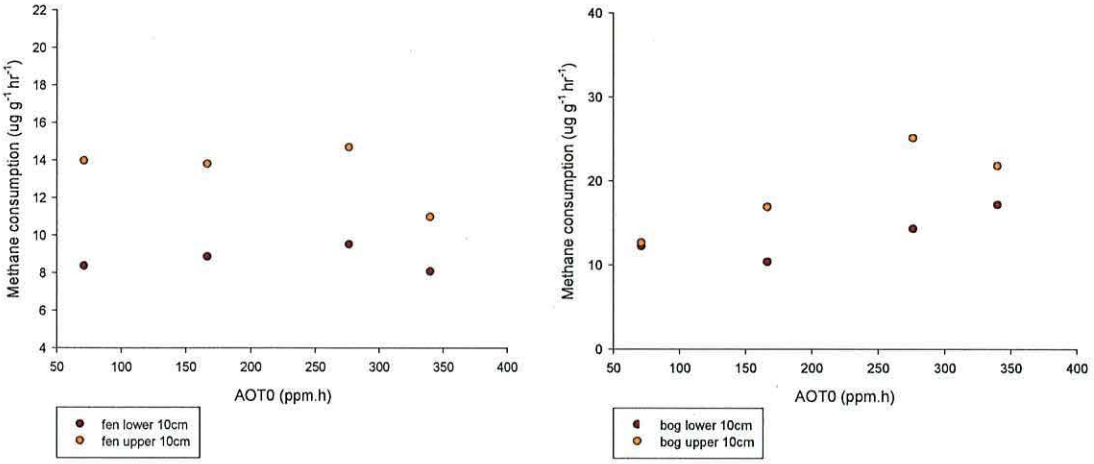


Figure 6.8: Rates of initial methane consumption for samples taken from fen (left) and bog (right) mesocosms (P>0.1 in all cases).

6.3.4 Stable isotope measurements of methane consumption

Measurements of ^{13}C labelled carbon dioxide that was emitted from the mesocosms following labelling with labelled methane are shown in Figure 6.9. ^{13}C levels dropped over time and were highest immediately post labelling. This suggests that methane oxidation is a fairly rapid process as the injected methane was quickly oxidised to carbon dioxide. There is no difference in rates of methane oxidation as measured by the production of labelled carbon dioxide between the treatments and although there is a linear relationship between initial carbon dioxide F values and ozone exposure (Figure 6.10) it is not significant (P=0.109).

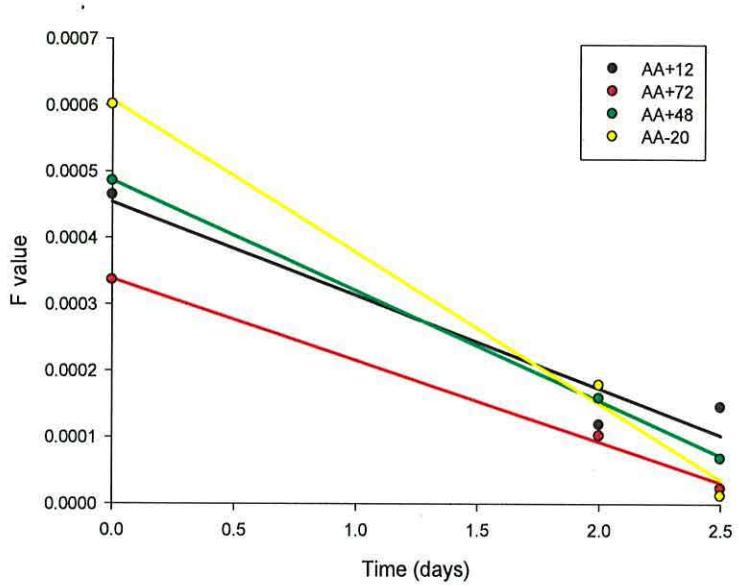


Figure 6.9: Carbon 13 present in emitted carbon dioxide that originally came from the labelled methane injected into the headspace. Differences between the treatments are not significant (P>0.1).

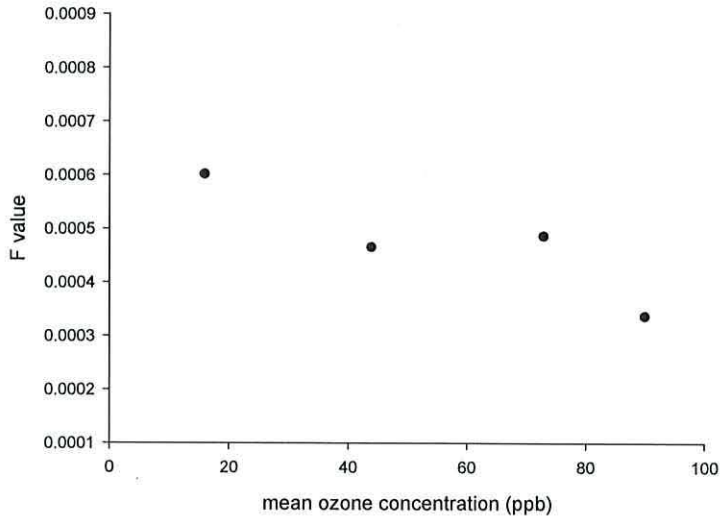


Figure 6.10: Initial F values correlated against the mean ozone concentration. This relationship is not significant (P=0.109).

6.3.5 Carbon tracing (2008)

Unfortunately, the results from the carbon tracing that took place during the summer

of 2008 are unable to be presented at this stage. There have been ongoing mechanical problems with the mass spectrometer mainly caused by disruptions to the building's power supply over the past eight months. Currently, work is ongoing to fix the machine and it is hoped that these results will be presented as an appendix as soon as possible and discussed further in the viva.

6.4 Discussion

In this experiment, the *P. erecta* plants from the fen that had been exposed to elevated ozone showed an increase in short-term microbial decay, a similar result to that found for *Betula papyrifera* (Parsons et al. 2008). A possible reason for this would be an increase in foliar nitrogen caused by incomplete remobilisation of nutrients during premature senescence (Andersen 2003; Findlay and Jones 1990). Although there was no significant difference in total nitrogen concentrations between ozone treatments in the decomposing slurries after ten days the activity of N-acetylglucosaminidase was significantly higher in slurries containing *P. erecta* exposed to elevated ozone suggesting that there may have been more labile nitrogen containing compounds available in the slurry but that they had been metabolised more rapidly. Beta glucosidase activity also increased in slurries containing *P. erecta* exposed to elevated ozone suggesting that either plant material exposed to elevated ozone is more easily degraded for this species or that the availability of easily assimable carbon was reduced so that microbes have had to produce more enzymes to break down more recalcitrant organic material (Allison and Vitousek 2005).

The other species (sedges, rushes and grasses) exposed to elevated ozone did not show a significant change in carbon dioxide emissions under decomposition but in many cases hydrolytic enzyme activities decreased following the plants' exposure to elevated ozone. In the slurry containing *F. rubra* the proportion of dissolved carbon present as phenolics increased under elevated ozone, which could have inhibited enzyme activity (Liu et al. 2005; Peltonen et al. 2005; Saleem et al. 2001; Yamaji et al. 2003). However, this was not seen for any of the other species so it is likely that there is another reason for lower enzyme activities seen. Results have found that the quality of carbon compounds in plant materials is changed by ozone exposure

(Paakkonen et al. 1998b; Schraudner et al. 1997) so it could be that the molecular weight of carbon compounds is changed even though the total concentration of dissolved carbon is unchanged by elevated ozone in this experiment. Extra-cellular enzymes are the primary way microbes degrade larger molecules present in the soil (Wallenstein and Weintraub 2008) but the production of enzymes is energetically expensive (Allison and Vitousek 2005) and thus it is likely that when nutrients are readily available microbes produce fewer enzymes. Exposure to elevated ozone could have increased the availability of easily assimable nutrients in these species, possibly due to reduced translocation of fixed photosynthate out of the leaves (Andersen 2003; Grantz 2003; Grantz and Farrar 1999, 2000) meaning that the production of enzymes was not necessary for the microbes to obtain carbon compounds.

From these results, it appears that the short-term decomposition of *P. erecta* is affected in the opposite way to the decomposition of *M. caerulea, J. subnodulosus, F. rubra* and *C. echinata*. A possible reason for this is that forbs tend to be more sensitive to elevated ozone than grasses (Bergmann et al. 1999). It is also possible that monocotyledon species store and translocate nutrients differently to dicotyledonous species, which could lead to a difference in nutrient availability for microbes and could affect the production of hydrolytic enzymes.

Potential methane production and consumption potentials provide a method of quantifying the maximum possible methanogen and methanotroph activity under "ideal" conditions. Following exposure of peatland microcosms to elevated ozone concentrations it was found that the methane consumption potential showed a slight decline under elevated ozone in peat from the Migneint but it was unchanged in peat from Cors Goch irrespective of the sampling depth. This is in agreement with results from Rinnan et al. (2003) who found that elevated ozone had no effect on potential methane consumption and those from Morsky et al. (2008) who found that elevated ozone caused a non-significant decrease in potential methane consumption. The slight decline in potential methane consumption in bog microcosms under elevated ozone could be linked to the decrease in plant cover as ozone exposure increased. It is likely that if above ground plant growth is decreased, below ground plant growth will also be decreased and it has been found that higher rates of methanotrophy in

peatlands are associated with the roots and rhizomes of vascular plants (Roura-Carol and Freeman 1999) with up to 27% of the potential methane flux being consumed by root associated methanotrophy in a Maine wetland (King 1996). This could mean that reduced plant growth is reducing aerobic microsites within the peat. The mesocosms from Cors Goch contained large volumes of root material irrespective of ozone exposure meaning that this pattern is less likely to be seen. Previous experiments have shown that methane oxidation rates are highest at the level of the water-table, at the oxic/anoxic interface (Moore and Dalva 1997; Segers 1998; Whalen and Reeburgh 2000), but in this experiment, peat depth was not a significant factor in methane oxidation rates, possibly because the water-table remained at the surface throughout the ozone exposure. The conversion of ^{13}C labelled methane to carbon dioxide was also unchanged in bog mesocosms by exposure to elevated ozone, suggesting that as well as the potential methane consumption being unchanged the in-situ methane consumption was also unchanged.

In contrast to rates of potential methane consumption, potential methane production showed a significant decrease with elevated ozone exposure in peat from the Cors Goch microcosms. This is in contrast to measurements made by Rinnan et al., (2003) and Morsky et al., (2008) who found that elevated ozone did not significantly affect methane production potentials. However, in those experiments, they used microcosms from a lower nutrient bog and methane production potentials were unchanged in peat from microcosms taken from the Migneint in this experiment. Providing the peat is anaerobic, methane production is usually limited by the concentrations of suitable substrates (hydrogen and acetate) and microbial biomass only limits production when the microbial uptake capacity is lower than the supply of substrate (Segers 1998). This means that potential methane production in peat exposed to elevated ozone is either reduced by reduced substrate supply or by reduced methanogen biomass. Although methanogen populations are slow to recover after oxygen influx into the peat (Whalen and Reeburgh 2000), the water-table remained at the surface in all ozone exposures throughout the experiment so those microcosms exposed to higher ozone concentrations are unlikely to have become aerobic meaning that methanogen population size is unlikely to be the main factor reducing potential methane production. Although wetlands contain high concentrations of phenolic

compounds these were unchanged by exposure to elevated ozone and are unlikely to have had a differentially inhibitory effect on the methanogen population in the microcosms. Experiments measuring the concentrations of organic acids in pore waters of mesocosms exposed to elevated ozone found that concentrations of acetate, formalate and oxalate increased following ozone exposure (Morsky et al. 2008). If this is the case then it would be expected that potential methane production would increase following exposure to elevated ozone but the opposite occurred in the experiments described in this Chapter.

From these results, it appears that elevated ozone does not cause a consistent change in the activity of methanogens or methanotrophs meaning that it is unlikely that the increased methane emissions seen under elevated ozone are the result of changes to the microbial population. Although ozone is unlikely to directly affect microbial activity below ground because of its reactivity it may have been expected that effects on plants such as reduced growth and changes to carbon allocation would have an effect on microbial populations via reductions in substrate availability. This suggests that the reason for the increased methane emissions is an effect on plant transport of methane to the atmosphere from the anaerobic peat layer.

The changes caused by elevated ozone to short-term plant decomposition appear to be different depending on the species. The majority of species tested showed a reduction in enzyme activity following plant exposure to elevated ozone, which would suggest a possible slowing down of decomposition activity. If this occurs in the field then elevated ozone could result in an increase in carbon storage in wetlands as the already slow rate of decomposition is reduced further. However, the slurries containing *P. erecta* showed an increase in enzyme activity, suggesting that for some species decomposition may be increased by exposure of the plants to elevated ozone. Therefore, overall effects on carbon storage in wetland systems may depend on the effects of ozone on the dominant species present in each system.

7 Overall Discussion

The original hypotheses and main findings were:

1. Elevated ozone exposure will reduce plant growth and cause the early onset of plant senescence.
   - Wetland vascular plants are sensitive to elevated ozone when grown individually with increased and premature senescence being seen in all species tested. However, these effects in senescence did not necessarily translate into reductions in biomass.
   - When naturally occurring communities are grown in wetland mesocosms elevated ozone still appears to increase senescence. Reductions in vascular plant cover and growth are also seen, although these are not always significant at $P<0.05$.
2. Exposure to elevated ozone will result in increased efflux of methane from wetland mesocosms, with relatively more of an effect being seen in mesocosms containing vascular plants and from higher nutrient environments.
   - Methane emissions from upland bog mesocosms are increased by elevated ozone over a long-term exposure. Methane emissions do increase from mesocosms containing vascular plants following exposure to elevated ozone but they are not affected by increased nutrients.
3. Carbon dioxide uptake by wetland mesocosms will decrease under elevated ozone as a result of a reduction in plant growth.
   - Elevated ozone does not have a consistent effect on carbon dioxide emissions from wetland mesocosms. During 2007, ozone exposure decreased carbon dioxide uptake from fen mesocosms but this did not occur at any other points in the experiments.
4. Plant decomposition rate will be increased following exposure to elevated ozone because of a potential reduction in the translocation of nutrients out of the leaves prior to ozone-induced senescence (Long and Naidu 2002).
   - The hydrolytic enzyme activities of beta glucosidase and N-acetylglucosaminidase measured in peat slurry containing vascular plants

exposed to ozone is changed by the concentrations of ozone the plants received. The direction of the response also depends on the species, with the forb *Potentilla erecta* showing an increase in hydrolytic enzyme activity in plants that had been exposed to elevated ozone whereas the monocots showed a decrease in hydrolytic enzyme activity following elevated ozone exposure.

5. Methane production and consumption potentials will be affected by the effects of elevated ozone on plant growth, both because of the presence of vascular plants as a conduit and as a source of bio-available carbon in the form of root exudates.
   - Methane production and consumption potentials were mainly unaffected by exposure of the mesocosms to elevated ozone with only peat from the upper layer of fen mesocosms showing a decrease in potential methane production as $AOT0_{24hr}$ increased.
   - The in-situ methane consumption, as measured by the oxidation of ^{13}C labelled methane was not significantly affected by elevated ozone. However, initial values of the fraction of the labelled carbon dioxide retrieved from the initial label showed a possible correlation with ozone concentration but this cannot be proven as an actual effect without more treatment replication.

These findings are displayed in Figure 7.1 and discussed in more detail in Sections 7.2 and 7.3. The primary site of action for the effects of elevated ozone appeared to be the vascular plants present in the mesocosms, and the increase in methane emissions from mesocosms containing vascular plants appeared to be as a result of the changes occurring in the vascular plants.

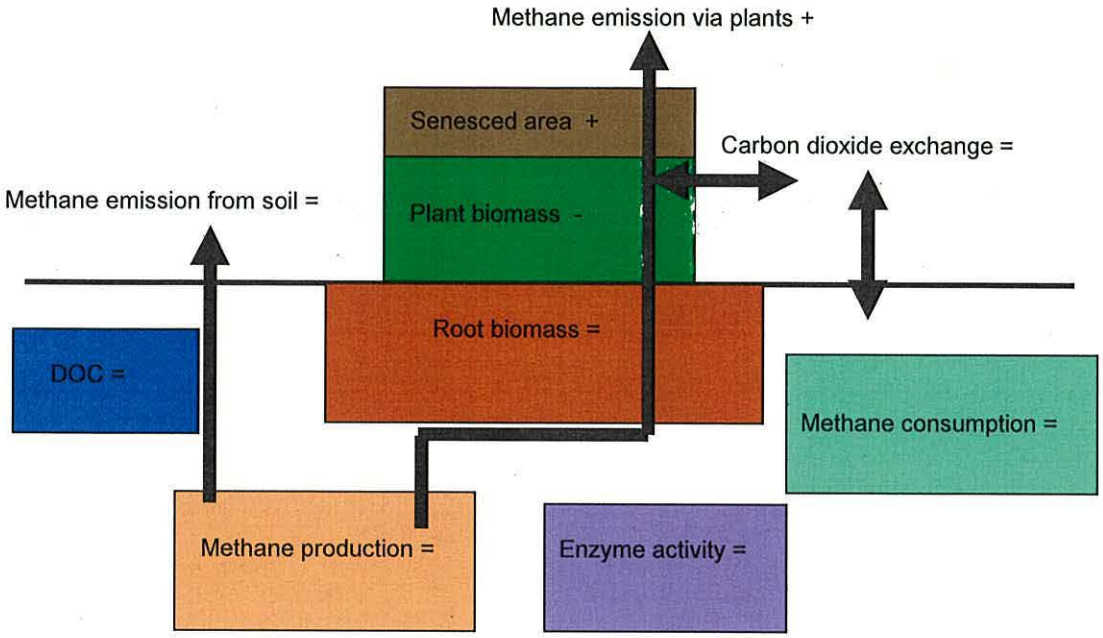


Figure 7.1: Schematic diagram showing the changes to wetland plants and carbon gas exchange caused by exposure to elevated background ozone. Above-ground plant biomass decreases but the senesced area of the plants increases. Potential methane production and consumption is unchanged, as is the emission of methane when plants are not present. However, methane emissions when plants are present are increased. Overall carbon dioxide exchange is unchanged and stomatal conductance is also unchanged. The activities of phenol oxidase and beta glucosidase in the peat are unchanged and the pool of dissolved carbon available in the peat does not appear to change either. + indicates an increase under elevated ozone, - indicates a decrease and = indicates no change.

7.1 Methodological Considerations

Throughout this study, accumulated ozone exposure has been measured as $AOT0_{24hr}$ rather than the more usual AOT40 measured over daylight hours. This is partially because of the measurement of ozone effects by regression; if AOT40 was used then the lowest treatment would be recorded as having an AOT40 of zero even though it did receive an accumulation of ozone over the experimental period. It is also possible that the species used in this study would show effects of ozone below the threshold

value of 40ppb and it was wished to include these effects. Finally, many previous ozone exposure studies have only exposed plants and mesocosms to elevated ozone during daylight hours (e.g. Hayes 2007, Power and Ashmore 2002, Rinnan et al. 2003) and in these studies plants were also exposed to elevated ozone throughout the night as occurs in upland areas. The use of AOT40 during daylight hours would not have taken into account the elevated concentrations of ozone overnight and it was thought necessary to include these as elevated background concentrations of ozone are likely to be higher overnight as well, whereas if a rural profile had been used then ozone concentrations drop overnight.

This study assessed the effects of elevated ozone on wetlands using mesocosms taken from natural wetlands and exposing them to ozone in solardomes. It is possible that there was a chamber effect caused by the solardomes although they have been designed to maintain near ambient light, temperature and humidity by having a rapid airflow through the solardomes (Hayes 2007). Artificial watering had to take place and because of time and feasibility the mesocosms were watered using tap water. This could have increased nutrient inputs into the mesocosms compared to watering with rain water but as all of the mesocosms received the same treatment it is unlikely that this would have differentially changed the effects of ozone on mesocosms receiving different ozone concentrations. The constant air supply within the solardomes could also have had an effect on species response to ozone although, unlike more sheltered ecosystems, upland bogs tend to occur in exposed, windswept places.

The main limitation of using the solardomes and measuring ozone response by regression was the number of treatments available to be used. This was unavoidable but in some results, such as plant biomass (Chapter 5), where there is a good relationship but it is not significant, more treatments may have reduced the effects of the scatter about the regression on the P values. Alternatively, more replicate mesocosms could have been used per solardome or more replicate solardomes per treatment.

The size of the mesocosms used was changed for the final ozone exposure as it was

found that the smaller mesocosms became pot-bound. The larger size reduced the edge effects experienced by the mesocosms compared to the internal volume. It also reduced the artificial effect of roots filling up the mesocosms and making the peat more aerobic than would be the case in a natural system.

Gas exchange was measured using transparent headspaces. During the 2006 and 2007 experiments headspaces that were inserted into the peat were used but these would have disrupted the peat surface and potentially affected gas fluxes. These were modified to have a neoprene seal that was attached around the outside of the mesocosm to reduce disturbance to the peat. Gas fluxes were taken over a period of one hour throughout the experiment. This was partially a result of methodological limitations of getting round all of the mesocosms and partially to coincide with laboratory protocols that were already in place (Ellis et al. 2009). Although the temperature within the mesocosms did increase over the one hour, initial measurements found that methane and carbon dioxide emissions remained linear over the first 90 minutes after capping with the headspaces (data not presented) and published results show that methane emissions accumulated linearly for 105 minutes despite temperature differences of up to 7°C (King 1996).

The biggest source of variability in the data was because of the natural variability of vegetation germinating within the wetland mesocosms. When the purpose of the experiment was to assess the effect of vascular plants and elevated ozone on gas fluxes, mesocosms that did not contain any vascular plants, but were in the group labelled as containing vascular plants, were removed from the analysis. The germination of species and the cover of vascular plants and mosses was recorded and where necessary used as a co-variate in the analysis.

7.2 Plant Responses to Ozone

This study has assessed the response of wetland plants to elevated tropospheric ozone. Species were grown both individually and as part of wetland plant communities germinating in natural mesocosms collected from peat-forming wetlands in North Wales. Responses measured have included increased and premature senescence,

above and below ground biomass and chlorophyll content.

The individual species grown in the experiment looking at the effects of a relatively short-term, high-dose ozone exposure with ozone concentrations at 150ppb for five days out of seven for four weeks (Chapter 2) all showed an increase in senescence following four weeks of ozone exposure. The following season two of the species, *Molinia caerulea* and *Juncus subnodulosus*, were exposed to elevated background ozone at lower concentrations than the previous year (highest average exposure of 90ppb) but for 16 weeks and these also showed a significant increase in premature senescence as $AOT0_{24hr}$ increased. However, over the same ozone exposure, mesocosms containing natural communities of vascular plants (mainly *Carex echinata* with some *Juncus effusus* and *J. squarrosus*) did not show a significant relationship between premature senescence and $AOT0_{24hr}$ suggesting that there was a differing effect of ozone on species grown in a mixed community compared to those grown as individuals. The following year larger mesocosms, both in terms of depth and cross-sectional area, were used and in this case there was a significant relationship between premature senescence and $AOT0_{24hr}$ over a range of predicted background ozone concentrations with senescence increasing as $AOT0_{24hr}$ increased. The vascular plant community that germinated was very similar in composition to the previous year with mesocosms again being dominated by *Carex echinata* with some *Juncus* species and a few grasses germinating later in the growing season.

The ozone regime used in 2007 and 2008 followed the same methodology, with the eight solardomes receiving incremental ozone doses designed to simulate predicted increases in background ozone. The ozone exposure period ran from May until September in both seasons, which would suggest that the differential response in senescence to elevated ozone is not the result of changes in the ozone exposure received by the plants. The mesocosms used in each season were collected from the same area of wetland flush on the Migneint to minimise differences between seasons and each year they were collected at the same time of year, approximately one month prior to the mesocosms being placed in the solardomes. This suggests that the only major difference between the experiments was the size of the mesocosms used. Between 2007 and 2008 the cross-sectional diameter of the mesocosms was increased

from 10.5cm to 16.5cm and the depth was increased from 20cm to 40cm. This was mainly because the mesocosms appeared to get pot-bound during the 2007 experiment, with large quantities of root material filling the mesocosms. It is possible that the restrictions to root growth caused by the use of the smaller mesocosms had an effect on the vascular plant responses to elevated ozone. Similar results have been found in experiments on upland plant communities (Hayes 2007) with some species showing larger effects of ozone when growing in a community and some showing smaller effects. In those experiments the communities planted in containers giving a smaller root volume (pots 27cm diameter, 11cm deep; 10 plants per pot) showed reduced effects compared to individuals (pots 1 litre or 2 litre depending on species size, 1 plant per pot), whereas a different set of communities planted in containers giving a relatively larger root volume per plant showed increased effects of ozone on *Trifolium repens* compared to when they were grown individually. This is possibly linked to water and nutrient availability as *Plantago major* plants grown with a reduced soil volume showed a reduction in nitrogen content, stomatal conductance and therefore ozone uptake, and reduced response to ozone (Whitfield et al. 1996). When plants from a wet meadow grassland were grown either in a two species competition or as a monoculture at the same planting density there were significant differences in the plant senescence response with *Molinia caerulea* and *Holcus lanatus* showing increased sensitivity to ozone when grown in competition with *Agrostis capillaris* (Tonneijik et al. 2004) compared to plants grown individually. This suggests that there is an effect of inter-species competition on the onset of premature senescence in some cases. However, this could be linked to the competitiveness of the plants grown in the species mixtures; if *Agrostis capillaris* is more competitive than the other species then it could restrict root growth and nutrient availability in a similar way to reduced root volume caused by smaller pot size. Whitfield et al. (1996), noted that plants grown in the most restrictive individual pots most resembled field grown plants, suggesting that root restriction is common in species mixes found in the natural environment. It has also been found that once plants become pot-bound they no longer show a response to outside stimuli (Baldwin 1988; Herold and McNeil 1979), which further suggests that the lack of significant senescence response to increasing ozone during the 2007 experiment was due to the plants becoming pot-bound.

When assessing the impacts of elevated ozone on natural plant species it is important to consider whether the observed changes will have any effect on the plants' competitive ability. Although premature senescence has been seen on many wetland species in this study it has not always been translated to reductions in biomass with only *Molinia caerulea* showing a significant reduction in biomass following short-term ozone exposure (Chapter 2). This reduction in biomass was not seen during longer-term ozone exposure (Chapter 4) and previous experiments have found that ozone exposure actually increased the biomass of *M. caerulea* (Franzaring et al. 2000). When the biomass of the natural communities that germinated in the wetland mesocosms was measured elevated ozone did not significantly reduce end of season biomass during the 2007 season but the percentage cover of vascular plants did decrease in bog mesocosms as $AOT0_{24hr}$ increased. This suggests that elevated background ozone does have a negative effect on plant growth but one growing season's exposure was not long enough to see significant differences in biomass. Biomass measurements taken from the end of the ozone exposure during 2008 found that above and below ground biomass and moss biomass were reduced under elevated ozone but the differences between treatments were not marked enough to be significant (P values between 0.164 and 0.252). A possible reason for this could be that the ozone exposure was not long enough for significant differences to be seen. A second reason could be the methodological limitations of using eight solardomes. This meant that regression analysis of biomass against ozone was limited to eight points on the graph so that relatively small deviations from the regression reduce the chance of obtaining a significant result.

Part of this research focussed on the effects of elevated ozone on the short-term decomposition of plant material to see if exposure of wetland systems to elevated ozone is likely to have an effect on the speed of decomposition and hence the storage of carbon within wetlands. As wetlands are an important carbon storage system, anything that affects their ability to store carbon could have a knock on effect at a wider scale. Results from this research appear to show that the effects on plant decomposition may be species-specific. Carbon dioxide emissions from the decomposition of *Potentilla erecta* were increased by exposure of the plants to

elevated ozone. However, carbon dioxide emissions from the decomposition of monocotyledon species used were unchanged by exposure of the plants to elevated ozone. Various factors can affect the decomposition of plant tissues, including the nutrient content of the tissues and the content of inhibitory compounds such as those based on phenolic rings. These inhibitory compounds are produced by plants in response to many stresses, including ozone (Sgarbi et al. 2003), and leaves of plants exposed to ozone have been found to have higher concentrations of phenolic compounds (Peltonen et al. 2005; Saleem et al. 2001; Yamaji et al. 2003). However, nitrogen mobilisation out of plant leaves following senescence has been found to be reduced if plants have been exposed to elevated ozone (Findlay et al. 1996; Findlay and Jones 1990), meaning that plant material exposed to ozone may be higher in nitrogen. Higher levels of fertilisation have been found to cause faster rates of decomposition in plant litter (Allison and Vitousek 2004) so the higher concentrations of nitrogen in plant leaves may increase the rate of decomposition. It has also been found that some plant species, such as cotton, that have been exposed to elevated ozone show inhibited transport of recently assimilated carbon out of the leaves meaning that it is preferentially retained in above-ground plant parts rather than being translocated into the roots (Andersen 2003; Grantz 2003; Grantz and Farrar 1999, 2000; Grantz and Yang 2000). Overall, in this experiment, it appears that nitrogen and phenolic contents are mainly unchanged, although this could be partly because the nitrogen and carbon content of the slurry as a whole was measured and this could have masked relatively small changes in leaf nutrient content. The main changes seen were an increase in beta glucosidase and N-acetylglucosaminidase activity in *P. erecta* slurries and a decrease in the same enzyme activities in the slurries containing the monocotyledon plants. This suggests that the effects of elevated ozone on plant decomposition at wetland sites are likely to be linked to the dominant plant material found there. Most upland wetlands are dominated by *Sphagnum* mosses and the main vascular plants are sedges and rushes. These species showed a reduction in enzyme activity following ozone exposure so it may be that in many wetland areas vascular plant decomposition will be reduced further potentially storing more carbon.

Many wetlands are in upland areas of the UK where ozone concentrations are generally higher, meaning that as background ozone concentrations rise they are

likely to be exposed to ozone concentrations high enough to damage wetland plants. This study has shown that wetland vascular plants commonly found in upland areas are particularly sensitive to ozone so it is probable that vascular plant growth will be increasingly negatively affected in natural systems as background ozone concentrations continue to rise. If plant senescence is elevated over a number of growing seasons it is likely to reduce plant cover, potentially decreasing the amount of carbon stored in wetlands and offsetting the reduction in plant decomposition caused by exposure of vascular plants to elevated ozone.

7.3 Effects of ozone on Greenhouse Gas Exchange from Wetlands

7.3.1 Carbon dioxide exchange

Wetlands are generally considered to be a sink for carbon dioxide (Whiting and Chanton 2001) and this was found to be the case in the majority of the experiments in this study. The short-term ozone exposure found that elevated ozone did not significantly affect carbon dioxide exchange and it was also unaffected by the addition of nitrogen-based fertiliser or the onset of drought. In many species the onset of drought causes stomatal closure to reduce water loss from the plants but the lack of significant response of carbon dioxide exchange to drought suggests that this was not the case for the plants present in the mesocosms. There are two probable reasons for this: one is that although the mesocosms were droughted relative to the controls enough water was still available to the plants for them to maintain sufficient water supply. The second possible reason is that exposure to elevated ozone has been found to cause a loss of stomatal control in some species (Mills et al. 2009) with stomata not responding to severe drought. Unfortunately, because of time limitations it was not possible to measure stomatal conductance of the species present in the mesocosms in this experiment. However, during the first long-term ozone exposure (Chapter 4) carbon dioxide uptake from the fen mesocosms decreased as $AOT0_{24hr}$ increased, suggesting that increasing background ozone may decrease the sink strength of wetlands. This decrease in uptake is likely to be because of a reduction in plant growth under elevated ozone rather than an increase in microbial respiration as

enzyme activities were unchanged by elevated ozone in the absence of drought (Chapter 3). A second possibility is that plant respiration increased in those growing under elevated ozone as relatively more resources were invested in repairing ozone damaged tissue. This effect was not seen so clearly in bog mesocosms during the same experiment although carbon dioxide exchange from the bogs did correlate with plant dry weight and there was a significant interaction between plant cover and ozone exposure and their effects on carbon dioxide exchange. This further suggests that the effect of ozone on vascular plants is the main route via which changes in carbon dioxide exchange occurs. However, in the 2008 exposure season there was no significant correlation between $AOT0_{24hr}$ and carbon dioxide exchange irrespective of the presence of vascular plants. This suggests that the growth limitations caused by the smaller mesocosm size also had an effect on carbon dioxide exchange. Measurements of stomatal conductance on *Carex echinata*, which was the dominant vascular plant present in the mesocosms suggested that elevated ozone had no effect on stomatal closure so gas exchange via plant stomata was not impaired.

7.3.2 Methane Emissions

Methane emissions from wetland mesocosms were generally shown to increase under elevated ozone in these experiments. In the preliminary study, methane emissions appeared to increase more from the higher nutrient fens than the lower nutrient bogs, although possibly due to the short-term ozone exposure these differences were not significant. The hypothesis put forward by Lloyd (2004) is that the higher nutrient status of the fen ecosystems is responsible for the higher rates of methane emission compared to the bog and is also responsible for the increase in methane emissions under elevated ozone. However, in the experiment described in Chapter 3 the addition of a nitrogen-based fertiliser to wetland mesocosms did not change methane emissions suggesting that there is another mechanism controlling the patterns of methane release. Drought did reduce methane emissions irrespective of ozone concentration suggesting that the background water-table overrides the ozone effect with the influx of air into the peat as the water-table is lowered disrupting methanogen populations. Periods of elevated ozone in the UK are associated with hot, dry, anticyclonic weather (Royal Society 2008) so it is possible that elevated ozone and

drought will combine in future. These results appear to suggest that drought will reduce methane emissions so the longer-term increases in methane emissions seen in later experiments may not occur to such a great extent. However, these results came from small mesocosms with a limited depth (20cm) and it is likely that in natural wetlands the zone of methane production extends far below that. In natural systems the drop in the water-table is more likely to have an effect on the oxidation of dissolved methane diffusing through the peat to the atmosphere and it has been found that approximately 90% of methane reaching the atmosphere from the anaerobic zone is transported through vascular plants (Shannon et al. 1996) so it is unlikely that a slight drop in the water-table would have such a great effect in natural systems.

In the second year of experiments (Chapter 4), fen mesocosms became pot bound and methane emissions dropped to almost zero. Oxygen concentrations were also higher in fen mesocosms compared to bog mesocosms suggesting that the presence of an extensive root system allowed oxygen into the root zone, reduced the potential of methanogens to produce methane and provided oxygen for methanotrophs to oxidise methane. Potential methane production in fen mesocosms decreased as ozone increased but did not change in bog mesocosms whereas potential methane consumption increased slightly in bog mesocosms under elevated ozone and decreased slightly in fen mesocosms under elevated ozone although these differences were not significant. This suggests that potential microbial activity is not a driving force behind the changes in methane emissions. This is in agreement with published studies that found that elevated ozone did not have a significant effect on methane production and consumption (Morsky et al. 2008; Rinnan et al. 2003). Measurements of in-situ methane consumption using ^{13}C labelled methane showed that there was not a significant effect of elevated ozone although increasing ozone did cause a slight decrease in initial methane consumption as measured by the percentage of carbon 13 present from the initial label added.

Methane emissions from the bog mesocosms during the 2007 experiment (Chapter 4) and methane emissions from bog mesocosms containing vascular plants from 2008 experiment (Chapter 5) showed a positive relationship with increasing $AOT0_{24hr}$. Previous results have shown varying effects of elevated ozone on methane emissions

from large increases (Lloyd 2004), to transient effects (Niemi et al. 2002), no significant effects (Rinnan et al. 2003) to a decrease (Morsky et al. 2008). The effect of elevated ozone on methane emissions appears to depend on the plant cover in the mesocosms as when plant cover was removed entirely (see Chapter 5) there was no relationship between methane emissions and ozone exposure. There were no other differences between the mesocosms in the experiment during summer 2008 except plant cover so this would appear to be the main factor. Methane emissions were also higher from mesocosms with vascular plants compared to those with no vascular plant cover during summer 2008, suggesting that plant cover does increase methane emissions. One of the initial hypotheses was that methane emissions would be increased from mesocosms with more vascular plants because of the potential increase in exudates providing an easily available, high-energy source for the microbes. However, during the summer 2007 experiment methane emissions showed a negative correlation with plant cover showing that as plant cover increased methane emissions decreased. The reasons for this could be that as ozone concentrations increased plant cover decreased so high ozone meant lower plant cover or it could be that reduced plant cover meant less possibility of mesocosms being pot bound with the substrate being too high in oxygen for methanogenesis to occur (Roura-Carol and Freeman 1999). This means that the increase in methane emissions under elevated ozone is unlikely to be because of greater root exudation.

The higher methane emissions from mesocosms containing vascular plants compared to those that did not (Chapter 5) is likely to be due to gas transport through the plants allowing methane to bypass the aerobic layer of the peat and avoid being oxidised at that point. There are different ways gas can be transported through wetland vascular plants, many of which are characterised by the presence of aerenchyma (large pore-filled spaces to allow oxygen to reach the roots). Gas transport can either be via passive diffusion where the gas passes along a concentration gradient between the atmosphere and the roots and vice-versa or be via pressurised throughflow. A major gas transport mechanism in wetland plants involves the pressurised flow of atmospheric gas from higher pressure green leaves down through the aerenchyma into the roots to allow oxygen to reach the roots and then the gas flows out of the plants at lower pressure through the older senesced leaves (Chanton and Whiting 1996;

Chanton et al. 1997; Chanton et al. 1993; Whiting and Chanton 1996). It has been found that concentrations of atmospheric methane in senesced leaves of *Typha latifolia* are up to 500 times higher than concentrations in green leaves (Whiting and Chanton 1996). This suggests that the pressurised flow through the plant is a major emission route for methane as it can bypass the aerobic zone at the soil surface and be forced out through the older senesced leaves. As the senesced area is increased by elevated ozone (Chapters 2, 4 and 5) then the lower pressure "leaky" leaf area is increased meaning that there may be more gas flow through the plants, therefore taking more methane out of the peat into the atmosphere. The connections holding together plant cells in senesced leaves are likely to be reduced by ozone and once leaves are senesced they are unlikely to have any stomatal control meaning that the pressure would be further reduced thus causing a greater pressure differential between healthy green leaves and prematurely senesced leaves.

A further possibility that has not yet been discussed relates to the recent findings by Keppler et al. (2006) that plants can emit methane that has been aerobically produced. Although these findings have not been replicated in all experiments since (Beerling et al. 2008; Dueck et al. 2007; Kirschbaum and Walcroft 2008) it is possible that the effects of ozone breakdown and the production of reactive oxygen species within the leaves of vascular plants could initiate reactions leading to the formation of methane. It has been found that under high UV levels plant tissues do break down and a minor amount of methane is produced (Bruggemann et al. 2009; McLeod et al. 2008; Nisbet et al. 2009) but not at concentrations high enough to cause a detectable difference in methane fluxes from wetlands.

7.4 Implications for the future

This study focussed on the effects of elevated ozone on wetland plants and on the effects of elevated ozone on carbon gas exchange to detect any change in wetland functioning and carbon storage. Wetland plants have previously been found to be relatively ozone sensitive (Franzaring et al. 2000; Power and Ashmore 2002) and this was also found in this study, although the measure used to detect sensitivity would affect whether the plants are considered sensitive. Senescence was found to increase

in all species tested and although biomass generally decreased under elevated ozone these changes were not significant. As increased senescence may affect later season plant growth and the ability of the plants to store enough reserves to survive over-wintering and regrowth the following spring, the predicted increases in background ozone over the next 50 years (Royal Society 2008) are likely to have a significant long-term effect on wetland plant growth and thus wetland functioning.

The majority of peat-forming wetlands are found in the Northern hemisphere where ozone is predicted to continue to increase in most climate change scenarios. If current legislation is implemented then ozone concentrations across the Northern hemisphere during the summer are predicted to rise by 3-5ppb by 2030 whereas if a "business as usual" model is followed then ozone concentrations are predicted to rise by up to 25ppb by 2030 (Royal Society 2008).

Using the current concentrations measured at Marchlyn Mawr in Snowdonia and increasing the background by 5ppb, 10ppb and 20ppb future effects of increasing ozone can be estimated (Figure 7.2). At current ozone concentrations vascular plant senescence is 100% higher than at an estimate of senescence caused by pre-industrial ozone concentrations. A pre-industrial estimate of 5ppb was used as, although extrapolations of ozone concentrations are variable, many papers suggest that pre-industrial concentrations of ozone were less than 10ppb (FinlaysonPitts and Pitts 1997; Karberg et al. 2005; Vingarzan 2004; West and Fiore 2005). If background ozone concentrations increase by an average of 5ppb then vascular plant senescence would increase by a further 20% higher than current levels and if background ozone is increased by 20ppb then vascular plant senescence would be 170% higher than pre-industrial times and 70% higher than current levels (Table 7.1). This increase in plant senescence is likely to impact on species decomposition in wetlands as monocotyledons exposed to ozone showed less enzyme activity and therefore potentially less decomposition. This could have knock-on effects on nutrient availability for further plant growth and over the long-term reduce plant growth, both by the direct effects of elevated ozone and the indirect effects of reduced decomposition.

Current levels of ozone in the uplands appear to have reduced plant biomass by approximately 20% compared to pre-industrial ozone and, should background ozone concentrations increase by a further 20ppb above current concentrations, the reduction in vascular plant biomass could be reduced by as much again, giving a total reduction of over 50%. The predicted reduction in plant growth could lead to a reduction in carbon dioxide uptake via photosynthesis, which would lead to an overall reduction in the sink strength of wetlands. This could have implications for the modelling of global climate change as wetlands are currently considered to be a sink for carbon dioxide and if this is reduced by elevated ozone then less carbon dioxide could be stored than is currently estimated to be the case.

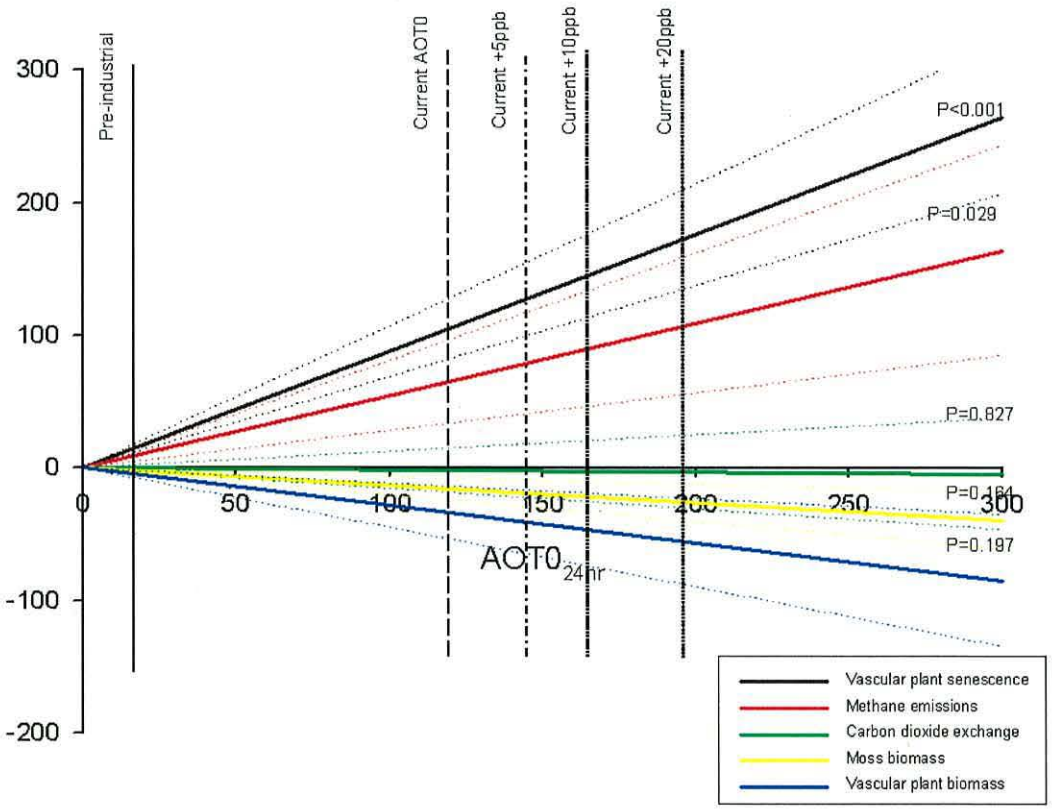


Figure 7.2: The percentage effects of increasing ozone exposure on the main findings from the 2008 experiment. P values were measured using linear regression and future $AOT0_{24hr}$ were calculated from measurements taken at Marchlyn Mawr between May and September 2006.

	current and future ozone concentrations			
	current	+5ppb	+10ppb	+20ppb
Vascular plant senescence	102%	122%	132%	172%
Methane emissions	73%	83%	93%	113%
Vascular plant biomass	-23%	-33%	-43%	-53%

Table 7.1: The percentage change in vascular plant senescence, methane emissions and vascular plant biomass estimated from future increases in background ozone of 5ppb, 10ppb and 20ppb against a starting point based on an estimated pre-industrial ozone concentration of 5ppb.

The results from this study seem to suggest that the observed increase in methane emissions following the exposure of mesocosms to elevated ozone is not caused by a change in methane consumption or methane production. The current hypothesis is that the increase in vascular plant senescence is causing the increase in methane emissions via changes to the internal pressure within plants resulting in higher concentrations of methane being forced out through senesced leaves. As shown in Figure 7.2, methane emissions do not increase as rapidly as percentage vascular plant senescence but they both increase as $AOT0_{24hr}$ increases. Current background concentrations of ozone appear to be causing an 73% increase in methane emissions compared to pre-industrial ozone concentrations and if ozone concentrations increase by 5ppb then a further 10% increase will occur. If background ozone concentrations are increased by 20ppb then an increase methane emissions in the order of 40% above current percentage increases is predicted to occur. This predicted increase in methane emissions is likely to have even more of an effect on the carbon export from wetlands than reductions in plant growth because of the higher warming potential of methane compared to carbon dioxide. The increase in methane emissions could result in wetlands becoming a net source of carbon to the atmosphere further reducing their ability to potentially mitigate the effects of climate change. Peat-forming wetlands are currently estimated to store 20-30 gC m^{-2} yr^{-1} (Wieder 2001) and if this store is

significantly reduced or removed then carbon dioxide concentrations in the atmosphere will accumulate at faster rates than are currently predicted, potentially increasing global warming.

The inferences and extrapolations shown in this section assume that all other variables will remain constant over the projected increases in background ozone concentration. This is not necessarily the case: for example, carbon dioxide concentrations are also predicted to increase, which in some experiments have been found to mitigate some of the effects of elevated ozone (McKee et al. 1995; Pregitzer et al. 2008). However, other experiments looking at lowland hay meadows found that elevated carbon dioxide failed to mitigate the effects of elevated ozone (Ramo et al. 2006) so it appears to be valid to use these results to predict what may happen under elevated ozone. The predictions that methane emissions will continue to increase are based on the assumption that plant cover will not be reduced by exposure to elevated ozone to levels so low that methane emissions through senescing plant culms will no longer be a significant part of the overall methane flux. Although these results show that increasing ozone concentrations will reduce vascular plant biomass it is unlikely that they will be reduced by so much that they will disappear.

7.5 Further Work

This study has shown that increasing background ozone increases methane emissions from wetland mesocosms. The hypothesis that is currently suggested is that this is due to increased gas transport through the vascular plants caused by increased pressure differentials between green photosynthesising leaves and senesced leaves. A priority for future work would be the testing of this hypothesis. This could potentially be done by measuring gases within the culms of vascular plants to see if the methane concentrations are higher in the efflux culms of plants exposed to elevated ozone. Further work on the stomatal control of wetland plants under elevated ozone may help to link gas exchange in the plants and methane emissions and may also help to determine whether carbon dioxide fluxes are affected by elevated ozone as the results from this study proved inconclusive. It would also be useful to have measurements of stomatal conductance in various wetland plant species as the measurements of the

effects of ozone are moving towards more flux-based approaches taking into account ozone uptake by the plants rather than just ozone concentration above the canopy. This is because ozone damage is seen in areas such as Scandinavia where ozone concentrations are relatively low but because of the cooler temperature and higher humidity the amount of ozone taken up by plants can be as high as plants growing in areas such as the Mediterranean where the ozone concentration is much higher, but frequent drought means that plants tend to close their stomata, which has the effect of reducing ozone uptake.

It is also possible that effects seen in mesocosms in solardomes may be exacerbated or under-estimated compared to effects that would occur in intact systems. Although free air ozone enrichment systems are expensive, such an approach would provide useful data to assess the effects of elevated ozone in intact wetland communities where there is no limitation of pot-binding or edge effects within mesocosms. The use of field-release technology would also allow carryover effects of ozone exposure to be measured without the problem of pot-binding. This would be a useful measure as it has previously been found that plants can show effects of ozone when re-growing the following spring even though they have not shown any effects the previous season (Hayes et al. 2006).

This study has focussed on the effects of elevated ozone on vascular plants and it would be useful to have more information about the effects of ozone on *Sphagnum* mosses. This has previously been studied (Gagnon and Karnosky 1992; Potter et al. 1996a; Potter et al. 1996b) to a certain extent but it would be interesting to find out if the effects of ozone on moss decomposition are similar to those on vascular plant decomposition, as *Sphagnum* mosses tend to dominate upland peatland systems. The long-term decomposition of mosses and vascular plants would be a useful measure as this study only assessed the effects of ozone exposure on initial decomposition.

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