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Evidence Project Final Report

- **Note**

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■ Project identification

1. Defra Project code

    AQ0815

2. Project title

    EFFECTS OF OZONE ON SEMI-NATURAL VEGETATION OF HIGH CONSERVATION VALUE

3. Contractor organisation(s)

    NERC Centre for Ecology & Hydrology

4. Total Defra project costs (agreed fixed price)

    £   435,282

5. Project: start date 1/4/2010

                end date 31/3/2013

6. It is Defra's intention to publish this form.

Please confirm your agreement to do so...**YES** ☒ **NO** ☐

    (a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

    Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

    In all cases, reasons for withholding information must be fully in line with exemptions under the Environmental Information Regulations or the Freedom of Information Act 2000.

    (b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Summary conclusions

Controlled experiments in the laboratory, open-top chambers and solardomes have provided quantifiable responses of important habitats and species to small increases in ozone exposure, typical of those expected to occur across much of the upland UK in the next few decades. The open-air field fumigation of a grassland at Keenley showed similar effects of small increases in ozone, both in the responses of individual species, and in the patterns of response between different plant functional groups. Man-made ozone, now and in the future, is expected to cause adverse changes in UK semi-natural vegetation not just from extreme 'episodes' of exposure associated with polluted air in summer, but also from chronic exposure to rising 'background' ozone concentrations across the northern hemisphere. Ozone acts by perturbing plant hormone ratios; this fundamental understanding will help in predicting responses at different scales (leaf, plant, community, landscape) but empirical parameterisations of simple models using measurements of individual species can be used already to set ozone concentration thresholds for effects.

1. Background

This project had the overall aim of investigating the effects of small increases in ambient ozone concentrations, likely to occur over the next few decades, on semi-natural ecosystems in the UK. It builds on previous research under contract CPEA33. Details have been reported annually – this report summarises the key findings and policy implications. **Measurements** have been made **in the field** at Keenley Fell, a novel field exposure system over a managed conservation grassland; in **open-top chambers** and **solardomes**, where different ozone exposure regimes have been applied to mesocosms representing important habitats; and in the **laboratory**, where the mechanisms of ozone effects have been examined. **Outcomes** are recorded in terms of biomass (both above and below ground), changes in species composition, flowering, mycorrhizal infection of roots, and soil characteristics. **Dose-response relationships** have been measured, and used to estimate parameters for models of ozone uptake by plants, which are then related to the observed effects to estimate **critical levels** for plant exposure to ozone. Finally, the implications are evaluated for the provision of several **ecosystem services**.

2. Field exposure at Keenley Fell (conservation grassland)

- A gradient in ozone exposure was created along 3 independent transects, from 2007 to 2012
- Ozone had little effect on total above-ground production but reduced below-ground root biomass
- Ozone changed the balance between functional groups, significantly reducing the forb component
- Ozone changed the species composition of the sward
- Ozone reduced the biomass of individual forb species within 1-2 years, and changed the biomass of individual grass species, but only after 3-4 years of treatment

- Ozone reduced the biomass of *Rhinanthus minor*, a keystone species which promotes species diversity
- Ozone reduced overall mycorrhizal infection of roots
- Ozone both significantly increased and decreased the flower density of individual species, but had no detectable effect on the timing of flowering
- Visible injury caused by ozone broadly confirms species sensitivity in terms of biomass and flowering
- Ozone reduced forage quality
- There was no significant effect of added nitrogen to the ozone responses

Policy implications

The effects of a slowly increasing background concentration of ozone, caused by emissions of precursors on a hemispheric scale, are likely to be seen most clearly in windy upland areas which are exposed to ozone day and night throughout the year. At risk are several habitats of conservation value, including hay meadows that are actively managed to conserve biodiversity, of which Keenley is an example. The effect of only a small increase in ozone concentrations has been to shift species composition in favour of grasses (against the direction of conservation management), and reduce root growth, even though above-ground growth was not affected overall. Reduced root growth implies increased susceptibility to drought, and reduced availability of nutrients. Changes in flowering are important for amenity, and for pollinators, but no clear response was seen overall. Reduced forage quality has implications for grazing. These effects were seen with very modest (few ppb) increases of average ozone concentration.

3. Controlled exposure of mesocosms representing key natural habitats

- In **MG3b grassland** communities, ozone caused a consistent significant decline of key species such as *Briza media* and *Lotus corniculatus* over 9 years, which was not simply related to the cumulative dose. A key species (the hemiparasite *Rhinanthus minor*), which promotes species of conservation value by inhibiting grass growth, was adversely affected by ozone. Although the long-term changes to both control and ozone-treated mesocosms showed better growth of species of conservation value, these positive changes were smaller in the ozone treatment.
- In **SD9b legume-rich sand dune** communities initial responses to ozone exposure, particularly of legumes, disappeared after 4 years as plants developed longer root systems over time
- In **calcareous grassland** communities, no effects of ozone were seen on total above-ground biomass, although growth of some individual species was reduced; root biomass was reduced. Significant effects on flowering were seen, either as accelerated flowering or decreased maximum flower number.
- In **acid heath** communities, an increase in above-ground biomass was seen for one species, but otherwise there were no significant effects of ozone.
- In **flood meadow** communities a decrease in net ecosystem exchange (of carbon) was observed with increasing ozone exposure, and a decrease in mass of one species. Soils of mesocosms exposed to ozone were less able to withhold flood water and had reduced soil-wetting characteristics.

Policy implications

These experiments, some over many years of exposure, show that ozone does change species composition (usually in favour of grasses at the expense of forbs and legumes, and contrary to measures to conserve plant diversity) without necessarily reducing overall above-ground productivity. However, there has been a consistent negative effect of ozone on root growth and function, implying increased susceptibility to drought, and possibly access to nutrients. Grasslands appear to be more sensitive to ozone than acid heaths. This type of experiment is necessary for developing dose-response relationships used in predictive models. The benefits of long-term experiments have been seen in the consistency of effects in the MG3b grassland community, and the ephemeral nature of effects of ozone in the sand-dune community; while some effects take time to develop, others may disappear as treatment continues, reflecting the changes in community dynamics, and the need for long-term field-based studies. While the direct influence of ozone on plant communities affects conservation and biodiversity issues, the indirect effects on pollinators (through flowering) and physical soil behaviour (seen in the flood meadows) point to wider aspects of ozone's involvement in the provision of ecosystem services.

4. Physiological Measurements

- Ozone affects hormonal control of stomatal opening (water regulation) in plants.
- The interactions of the plant hormones ethylene and abscisic acid determine plant behaviour; ozone and other stresses alter the ratio of these two hormones, and therefore plant responses.
- Plant responses are different among species and depend on other factors such as soil water status.
- Responses of whole plant communities may differ from those of individual species, partly because of varying effects of competition.
- Changes below ground (e.g. rhizobacteria) may influence hormonal responses in ways which affect responses to ozone exposure, and may be useful in mitigating adverse effects.
- Parameterisations of key physiological responses to ozone have been developed, based on measurements, permitting the development of dose-response models for 12 species of natural vegetation based on ozone flux.
- These models have identified critical levels of ozone exposure that produce measureable plant

responses, which differ for different species and different effects (e.g. flowering or growth).

Policy implications

Model predictions of the effects of ozone on semi-natural vegetation in the field can only be made where the underlying principles of ozone's mode of action are understood. Model parameterisations can (and have been) established based on measurements, but this approach can only be applied to a limited number of species. Generalisations to whole communities requires better understanding of the respective functions and responses of individual species, competition among species, and interactions of ozone stress with other environmental stresses such as drought and flooding. The improved understanding of the biochemical pathways affected by ozone provides a means of generalising response models, but too little information is as yet available for many quantitative models to be built. However, empirical parameterisations of physiological responses can be used to assess risks to individual key species.

5. Quantifying effects of ozone on ecosystem services in the UK

- Dose-response functions for ozone impacts on above-ground biomass in grassland predict that rising ozone concentrations have resulted in a **net loss in carbon sequestration** of 188,000 tonnes between 1987 and 2007; a **further loss** of 623,000 tonnes is projected between 2007 and 2020.
- Harvested plant material from Defra funded studies of ozone impacts on grasslands was analysed for **forage quality parameters**. 'Undesirable' quality parameters (ADF, crude fibre, lignin), which hinder digestibility of the pasture, showed an increase in concentration with increasing ozone exposure. **Live weight gain of lambs** was predicted to be **reduced by ozone** by approximately 4% for the period 2007 to 2020.
- **Impacts on biodiversity** were determined by estimating dose-response functions for ozone and plant species richness by spatial gradient analysis of national landscape surveys, and corresponding average ozone concentration data. Some habitats suggested positive relationships with ozone (Fen, Marsh and Swamp) while others suggested negative relationships with ozone (Heathlands and Bogs).

Policy implications

Quantifying impacts of pollutants, including ozone, in a form that allows direct comparison with effects of other drivers of change will provide an invaluable tool for policy makers. For this contract, it has been possible to quantify impacts on three ecosystem services, with two taken through to economic valuation (C sequestration and forage quality). Both suggest that losses will increase in the future as the ozone profile changes. Further experimental and modelling work is needed to expand the economic valuation to other services.

6. Proposals for future work – details and recommendations for possible future research to elucidate the effects of ozone on semi-natural ecosystems in the UK have been provided to the Defra Project Officer (March 2013).

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Exchange).

Objectives:

1. Two further seasons of ozone exposure of the upland conservation grassland at Keenley in Northumberland, to follow the significant effects of small ozone enhancement on forb and grass distribution, and to measure ozone, CO_2 and water vapour fluxes at the site as a means of interpreting and modelling the exposure regime. Extended for further year (SID3 2/12/11).
  Completed, other than final year when storm damage and flooding prevented full exposure.
2. A 7th year of exposure of grassland mesocosms in the open-top chamber (OTC) facility at Newcastle University, following the ongoing changes in species composition in response to moderate ozone enhancement. 9th year of exposure and soil sampling (SID3 2/12/11).
  Completed.
3. Determination of dose-response relationships for sensitive spring bulbs, and for legume-rich sand dune communities, based on OTC experiments (Newcastle). 4th year of exposure of sand dune community (SID3 2/12/11).
  Completed.
4. Measurement of the effects on growth and biodiversity at the Keenley field site, as moderated by the effects of ozone on Rhinanthus (hay rattle), which inhibits competitive grasses, including assessment of effects on flowering (York). Additional sampling quadrats established in 2012 (SID3 2/12/11).
  Completed, other than final year when storm damage and flooding prevented full exposure.
5. Measurement of below-ground effects of ozone at Keenley field site, including root biomass and mycorrhizal infection, and soil nutrient status (York).
  Completed.
6. Assess the extent to which any effects of ozone on community composition and biodiversity at Keenley Fell are reversed in the year following the ending of the ozone fumigation.
  Not pursued because fumigation extended; work continued under objective 4 instead (SID3 2/12/11)
7. Determination of ozone exposure thresholds for impacts on acid heath and calcareous grassland BAP Priority Habitats under current and predicted future reductions in precipitation, using controlled exposure to a range of ozone concentrations in the Bangor solardome facility.
  Completed as planned.
8. Determination of ozone exposure thresholds, and implications of prior ozone exposure for recovery from flooding or drought in Coastal and Floodplain Grazing Marsh, also in Bangor solardomes.
  Completed as planned.
9. Assessment of effects of ozone on dynamic phenology of stomatal aperture, leaf growth rates, plant fresh biomass and shoot water potential in dominant Keenley species using controlled laboratory experiments to provide input data for upscaling models (Lancaster).
  Completed as planned.
10. Assessment of effects of ozone on mesocosm / community soil water content and plant quality, for use in catchment scale modelling (Lancaster).
  Completed; complexity of ecosystem response precluded use in large-scale modelling pending further investigation of key processes.
11. Valuing ozone impacts on ecosystem services in UK habitats (SID3 2/12/11); construction of database from Defra-funded experiments, used to evaluate ozone effects on carbon sequestration, appreciation of biodiversity (flowering) and pasture quality.
 *Completed where possible – appropriate financial models for full evaluation are still under development.*

Methods, results and discussion (paragraph numbers refer to objectives above):

1. 1 Long-term field experiment at Keenley Fell (CEH Edinburgh and Newcastle)
Details of the methods were provided in the final report on contract CPEA33 (March 2010). In brief, 3 replicate ozone exposure transects were established over conservation-managed grassland at Keenley Fell, Northumberland (NY792558), in 2007. Ozone was released across the plots under computer control when the wind was between south and west, to give a gradient of decreasing ozone exposure downwind of the release points, with target concentrations 10m downwind from the ozone source of 10 ppb above ambient. Ozone concentrations and meteorological data were monitored continuously, and ozone, water vapour and CO_2 fluxes were measured during the growing season using eddy covariance for the upwind (untreated) area of the field. Exposure continued in 2010 and 2011, and was planned for 2012, but severe wind and lightning damage, and flooding in early 2012 precluded a full season of exposure. Moreover, a fault in spring 2012 led to the unplanned exposure of the plots to short-lived but large ozone concentrations which caused visible injury to some species (see annual report March 2013). Exposure was stopped prematurely in August, and (by agreement) the time released was spent on sampling and analysis of small sub-plots which had been subjected to a combined assessment of additional nitrogen and ozone. Various measures of response to ozone treatments were made at the site (reported below, and sections 4 & 5) including above-ground growth (by plant species) and below-ground growth, flowering timing and numbers, visible foliar injury, mycorrhizal infection and soil chemistry.

Ozone exposure at Keenley is summarised in Fig 1.1. Ambient ozone (during March to September) varied between years from 26 to 33 ppb; the range is similar to the average enhancement in ozone concentration at 10m from the source (the target distance), which was between 3 and 10 ppb (excluding 2012). Effects of ozone on vegetation are therefore likely to be variable between years, with the addition of only small concentrations on top of natural variability. There were some differences between transects, especially for distances closer to the source than 10m.
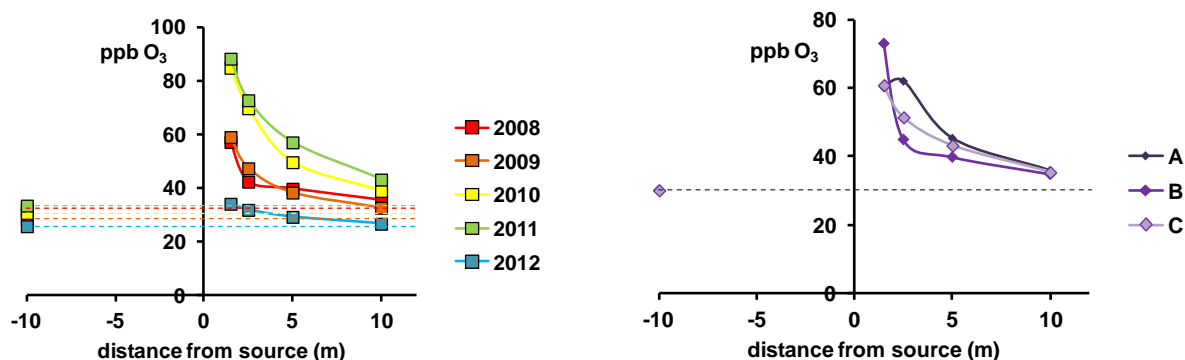
Figure 1.1: average concentrations of ozone at Keenley Fell (March-September) in each year averaged across transects (left) and for each transect, averaged across years (right). Ambient levels are shown by dashed lines.

Fluxes of ozone, CO_2 and water vapour at the site have been reported previously – only short periods of measurement were possible in 2012. The site was decommissioned as planned by the end of December 2012.

Policy implications

The very modest increases in ozone concentrations and exposure used in the Keenley Fell experiment nevertheless showed measurable effects on this upland conservation grassland, as detailed below, and demonstrate the potential for current and future impacts of ozone on plant species composition, soil carbon content, and ultimately agricultural production in similar upland areas of the UK.

4 & 5. Effects of ozone on plant growth and biodiversity at Keenley Fell (York)

The experiment at Keenley Fell that started in 2007 finished in August 2012, a year later than the original end-date of August 2011. However, interpretation of the additional year of experimental fumigation is complicated by (a) the very abnormal weather conditions, (b) the exposure to peak ozone levels causing visible injury (see annual report for 2012, March 2013), and (c) the higher grazing intensity at the site. We highlight the main findings for the whole experimental period below in turn, with relevant supporting data; more detailed analyses from 2012 are included in the annual report for 2012. In some cases, response variables were only measured on one occasion, whereas for others (such as biomass from the annual above-ground biomass harvest) a time-series of data is available. Because of the unusual conditions in 2012, when relevant, we present a time-series from 2007-2011 with the 2012 data separated, to indicate the importance differences between experimental conditions in 2012 and those in previous years.

In any field manipulation experiment, interpretation of the results needs to consider the initial vegetation composition, and possible variation in soil conditions across the site. In the case of Keenley Fell, initial data from 2007, before any significant ozone exposure, showed no significant differences between treatment plots in total biomass, or in the biomass of functional groups (see annual report, March 2013, Table 4.1) but the biomass of some individual species did differ. There were also significant differences between the treatment plots in soil water content and soil organic matter content. In the text below, we are confident, from additional statistical analyses not reported here, that the key bullet points do represent the effects of ozone, rather than confounding by initial vegetation composition or soil characteristics. However, for some of the specific references to individual species in the accompanying text, there can be less confidence in attributing the effects only to the effect of the imposed ozone treatment.

4.1 Ozone had little effect on total above-ground production

Although there were some differences between treatments in individual years, total above ground biomass showed no clear effect of ozone exposure and no clear trend over time (Fig 4.1a).

4.2 Ozone changed the balance between functional groups, significantly reducing the forb component

One aim of the high-level stewardship management of the site was to increase the proportion of forbs in the sward and reduce the dominance of grass species. This aim was being achieved in the initial part of the experiment, as seen in the trend of increased forb biomass over the period 2007-10 (Fig 4.1c), but this increase was significantly slower in the elevated ozone treatments. However, after 2010, forb biomass

declined again, possibly reflecting a more intensive management of the site. Over this period, forb biomass in the 5m plots has remained significantly lower than in the control plots, but that in the 10m plots has become more comparable to the control plots. Grass biomass fell over the period 2007-10 (Fig 4.1b), to a greater extent in the control treatments than in elevated ozone, possibly reflecting competition from the increased forb biomass. However, the differences in grass biomass between treatments were small at the end of the experiment. Legume biomass was consistently low and showed no clear treatment effects (Fig 4.1d).
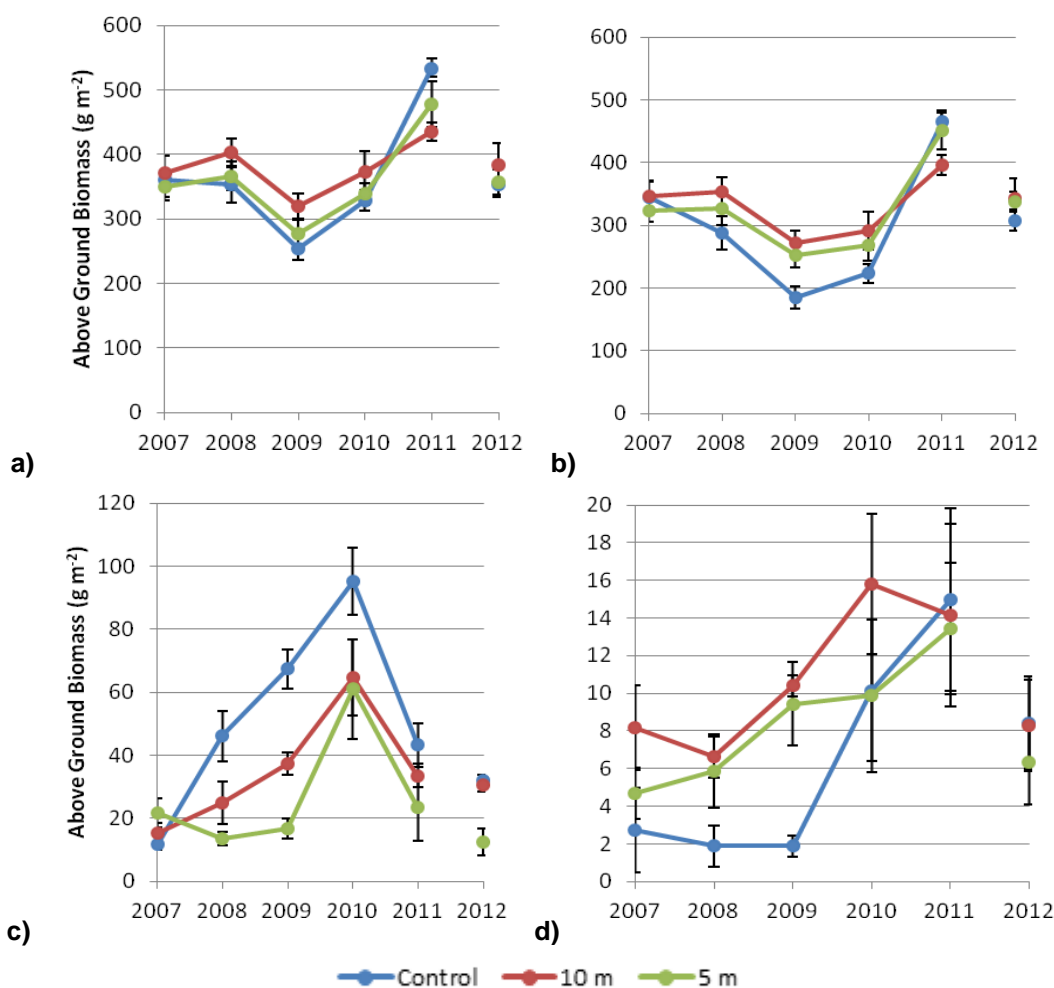
Figure 4.1: Effect of ozone treatment on above ground biomass for all years between 2007 and 2011. (a) Total biomass; (b) grass biomass; (c) forb biomass; (d) legume biomass. Data of 2012 are plotted separately. Error bars represent the standard error between replicate sub-plots.

4.3 Ozone changed the species composition of the sward

The effect of ozone in shifting species composition is illustrated in Fig 4.2, which summarises the different functional group composition in the three treatments in the 2012 biomass harvest. This shows the increased contribution of grasses to the sward biomass in elevated ozone, the diminished contribution of major forb species, such as the *Ranunculus* species, in elevated ozone, and differences between treatments in the relative proportions of individual grass species. Data by species are presented in the annual report for 2012.

4.4 Ozone treatment reduced the biomass of individual forb species within 1-2 years

There was a substantial number of individual species which showed no significant treatment effect in any year, although for some of these the low overall biomass and patchy distribution meant that the power of the experiment to detect a treatment effect was limited. Nevertheless, a large number of species did show significant ozone treatment effects, as summarised in the annual report for 2012, which lists all species for which above-ground biomass averaged at more than 1 g m^{-2} in 2012. Significant effects on individual forb species were apparent at an early stage of the experiment, from 2008, with many, but not all of these effects continuing throughout the experiment. Fig 4.3a shows data for all *Ranunculus* species, which closely mirror the effects on total forb biomass (Fig 4.1c). The rapid increase in biomass of these species from 2007 to 2010 in the control plots was suppressed in the elevated ozone treatments, and these large treatment differences remained in 2011 and 2012.
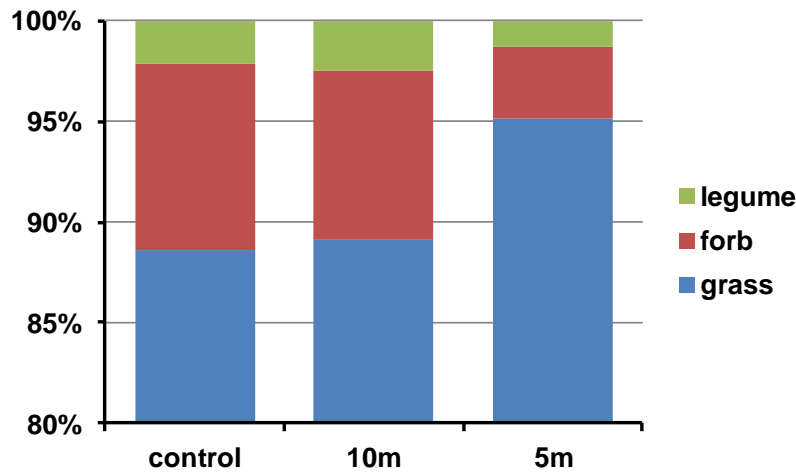
Figure 4.2: average proportion of above-ground biomass of grasses, forbs and legumes at 2012 harvest at different ozone exposures. Exposure at 5m was higher than at 10m.

4.5 Ozone reduced the biomass of *Rhinanthus minor*, a keystone species which promotes species diversity

Fig 4.3b shows a large reduction in biomass of *Rhinanthus minor* exposed to ozone in 2008 and 2009 relative to the control, although this was only significant in the 5m plots. This hemi-parasitic species is important in restricting the growth of competitive grass species and promoting greater species diversity in managed grassland. The adverse effects on this species are consistent with findings in the Newcastle OTCs (Section 3), and can be explained by the high stomatal conductance and limited stomatal control of this species. In the final years of the experiment, however, there was little of this species present in the Keenley sward at the annual harvest, and no significant effects of ozone were apparent.

a)   **b)**
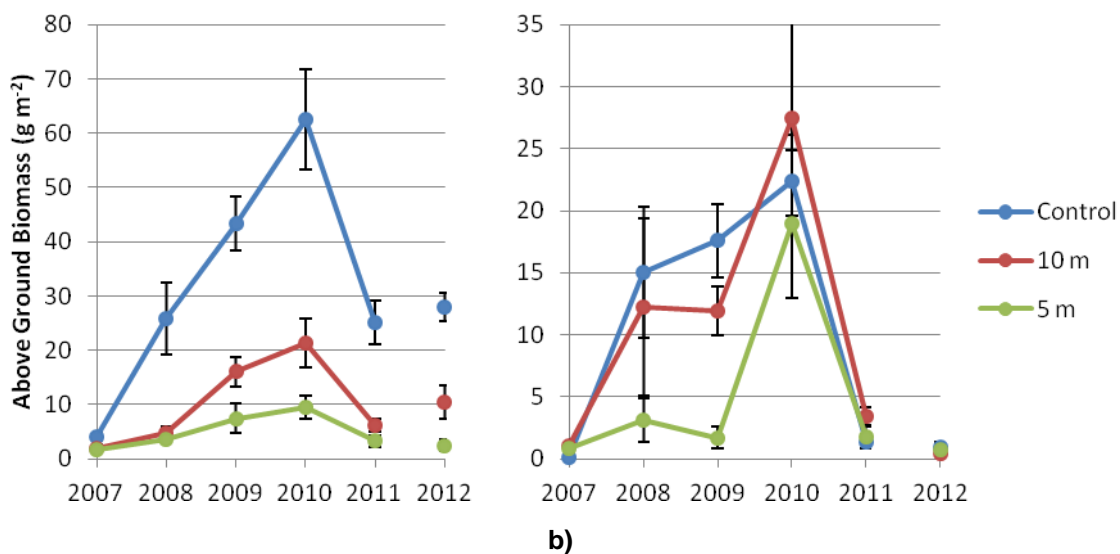
Figure 4.3: Effects of ozone on above ground biomass of (a) *Ranunculus sp.* (b) *Rhinanthus minor* between 2007 and 2011. Data of 2012 are plotted separately to the trend line. Error bars represent standard error between replicate sub-plots.

4.6 Ozone changed the biomass of individual grass species, but only after 3-4 years of treatment

Although no significant treatment effects on individual grass species were found in the first few years of the experiment (see annual report for 2012), by 2010/11 several species showed significant effects of ozone. Nevertheless, some of these effects were confounded by initial differences in biomass between treatment plots, while, for other species, there was no consistent trend in ozone treatment effects over time, e.g. *Festuca pratensis* (Fig 4.4b). Several species showed a trend of increased biomass in ozone, e.g. *Dactylis glomerata* (Fig 4.4a). Although initial biomass of this species was higher in elevated ozone at the start of the experiment (Fig 4.4a), it is also clear that the increase in biomass over the experiment was greater in elevated ozone than in the control treatment.

### 4.7 Ozone reduced below-ground root biomass

Although elevated ozone did not significantly reduce above ground biomass, it did significantly reduce root biomass extracted from the first 10 cm of soil. These measurements were only made in 2011. Lower fine root biomass was found at the highest ozone treatment both at the start of the growing season (Fig 4.5a) and shortly before harvest (Fig 4.5b), although the greater variability of the pre-harvest data meant that the

effect was not statistically significant.

**a)**   **b)**
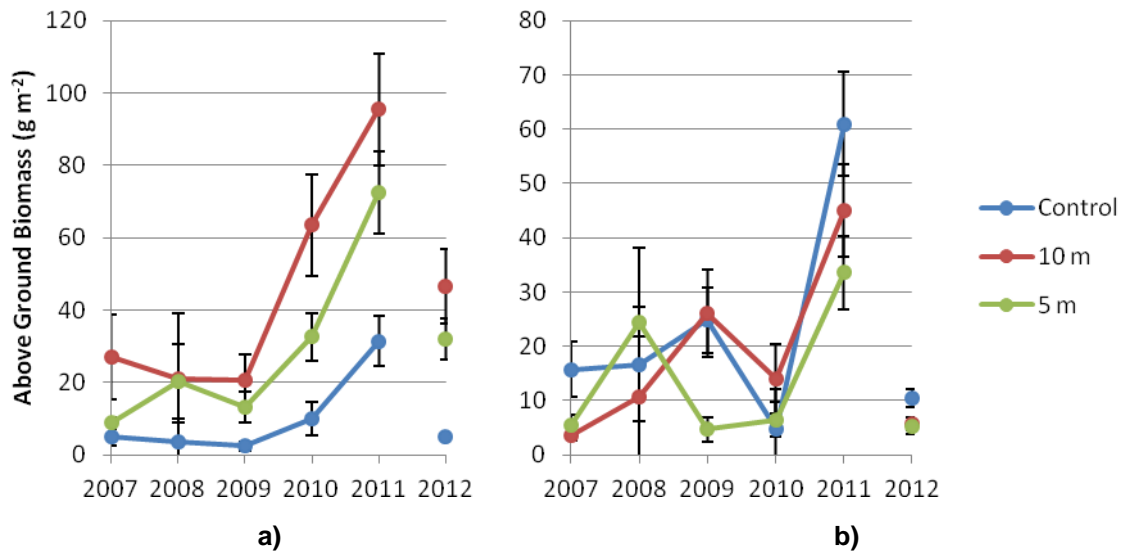
Figure 4.4: Effects of ozone on above ground biomass of (a) *Dactylis glomerata* and (b) *Festuca pratensis* between 2007 and 2011. 2012 data are plotted separately to the trend line. Error bars represent standard error between replicate sub-plots.

a)   **b)**
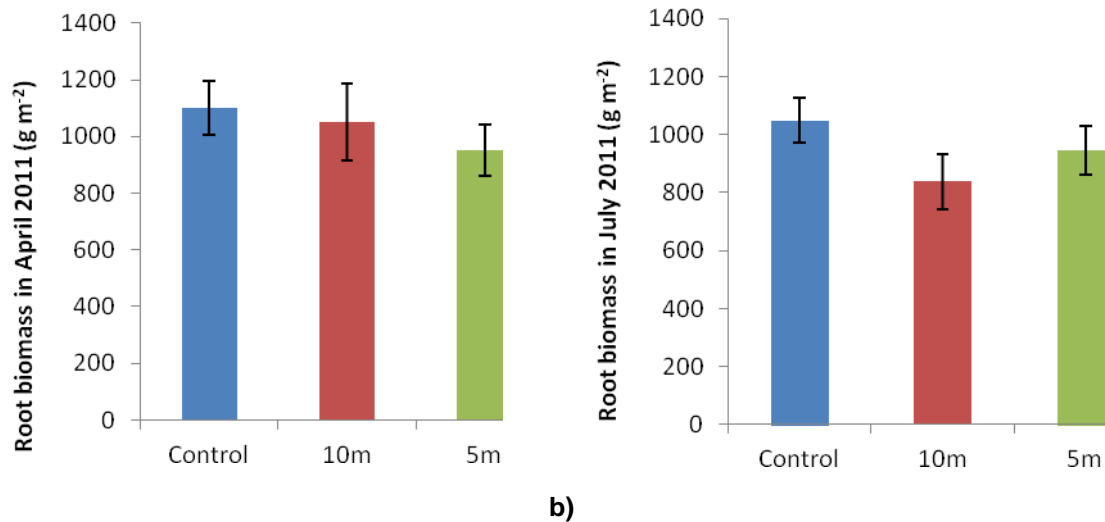
Figure 4.5: Effect of ozone on total root biomass in April 2011 (a) and July 2011 (b). Error bars represent standard error between replicate sub-plots.

4.8 Ozone reduced overall mycorrhizal infection

The frequency of mycorrhizal infection was also examined in July 2011 in roots from the top 5cm of soil. Both total mycorrhizal infection (Fig 4.6a) and arbuscule infection (Fig 4.6b) were significantly reduced in the 5m plots, and, for arbuscule infection there was also a significant reduction in the 10m plots.

a)   **b)**
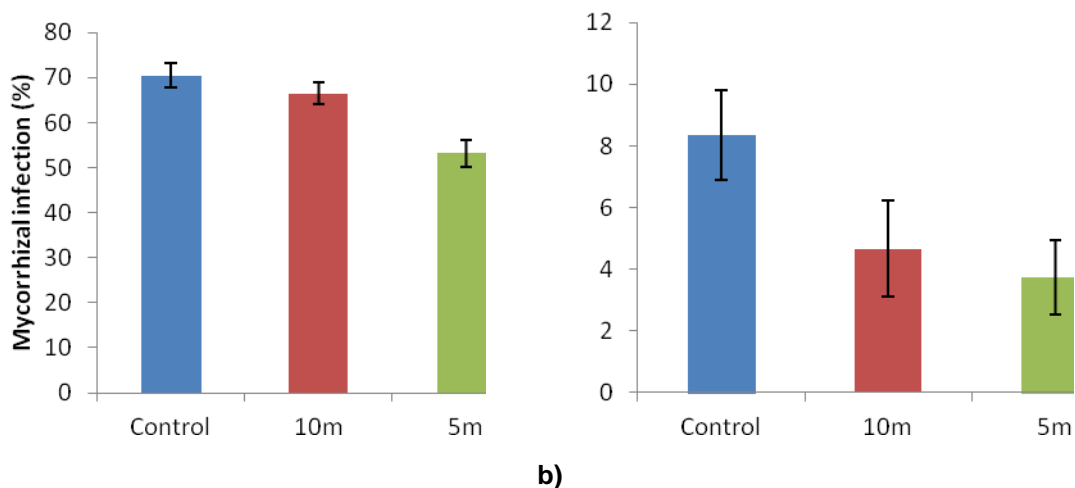
**Figure 4.6:** Effects of ozone on (a) total mycorrhizal infection of plant roots and (b) arbuscule infection.

Error bars represent standard error between replicate sub-plots.

4.9   Ozone both significantly increased and decreased the flower density of individual species, but had no detectable effect on the timing of flowering

Counts of flowers of the most important flowering species were carried out in 2010, 2011, and 2012. The results for 2012 are illustrated in Fig 4.7. Elevated ozone caused a large and significant decrease in flower density for *Ranunculus acris* and *Trisetum flavescens*, but a large and significant increase for *Dactylis glomerata* and *Conopodium majus,* with other species showing smaller or less consistent effects. For some species, these significant treatment effects were consistent throughout the three years 2010-2012, while for other species, the effect varied from year to year (annual report March 2013, Table 4.2). There was no evidence that elevated ozone delayed or accelerated flowering, although the frequency of recording meant that only changes of a week or more could have been detected.

4.10   Ozone effects on flowering and biomass are not always consistent

For most species, the effects of elevated ozone on flowering and biomass were comparable in size and statistical significance, for example the positive effects on *Dactylis glomerata* and the negative effects on *Ranunculus acris.* However, in some cases significant effects were found on flower density but not biomass, for example in *Lolium perenne* and *Anthoxanthum odoratum.* Details are tabulated in the annual report for 2012. These differences may reflect phenological characteristics (e.g. *Conopodium majus* produced a high density of flowers but contributed little biomass), or may indicate an effect of ozone in changing allocation of resources between vegetative and reproductive development.
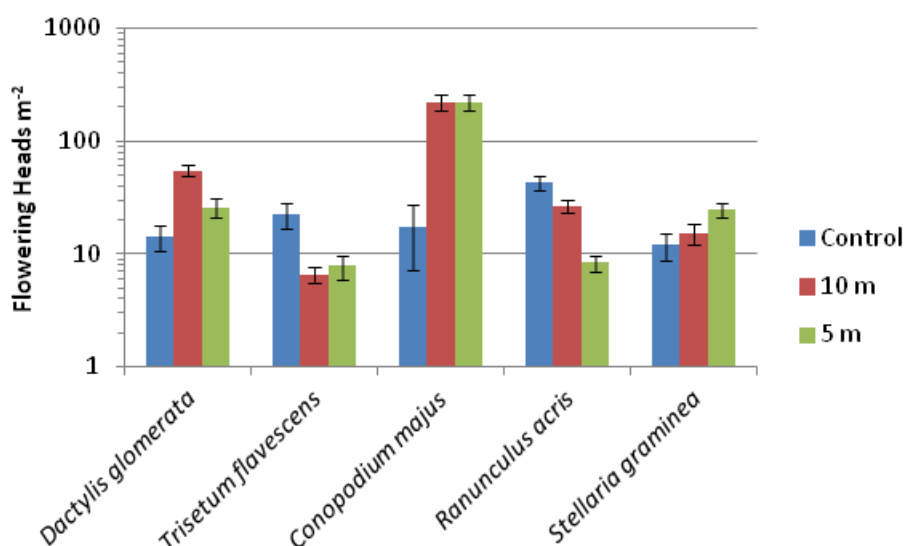

Figure 4.7: Effects of ozone on the maximum flowering density of individual species in 2012. This includes all species which had a significant treatment effect. Error bars represent standard error between replicate sub-plots. Note that data are plotted on a log scale to facilitate comparison of species with different flowering densities.

4.11   Visible injury caused by ozone broadly confirms species sensitivity in terms of biomass and flowering

Visible foliar injury was identified for the first time in the experimental plots at Keenley in June 2012 after accidental ozone release (see annual report for 2012). A total of 13 species showed characteristic ozone injury in the 5m plots, but only 9 species showed injury in the 10m plots (see 2012 report). The majority (5) of the species were forbs, including three (*Ranunculus acris, Ranunculus bulbosus* and *Rhinanthus minor*), which also showed significant negative effects of elevated ozone on biomass. Only two grasses showed visible injury, reflecting their lower response to ozone. These were *Holcus lanatus* and *Trisetum flavescens*, neither of which was among the grass species showing a strong positive response to ozone. *Trifolium repens* and *Trifolum pratense* both showed visible injury, reflecting their known sensitivity to ozone, and the low and variable cover of these legume species most likely accounts for the lack of significant ozone effects on other variables. The other two forb species showing significant visible injury, *Stellaria graminea* and *Rumex acetosa*, were also present at relatively low cover and showed inconsistent effects of ozone on other variables.

4.12   Ozone reduced forage quality

Samples of the above-ground biomass harvest in 2010 and 2011 were analysed by CEH Bangor, alongside samples from other experiments, for forage quality. From the different chemical components, an overall feed quality index was calculated. The results (Fig 4.8) indicate that feed quality was reduced by both ozone treatments in 2010, but only at 5 m in 2011. Feed quality was markedly lower in 2011 than in 2010.
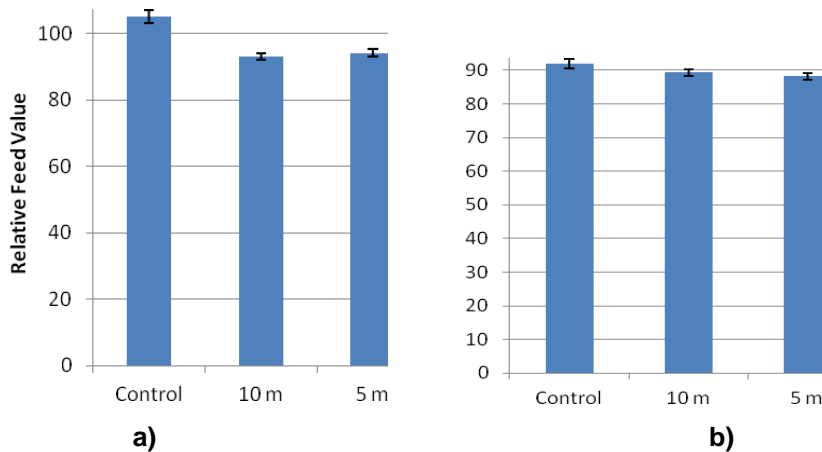
Figure 4.8: Effects of ozone on forage quality of above-ground biomass harvested from Keenley Fell in (a) 2010 and (b) 2011. This is shown as a relative feed value for each treatment. Error bars represent standard error between replicate sub-plots.

1.2 Ozone and nitrogen interaction plots at Keenley Fell (CEH Bangor and Newcastle)

Independent measurements were made of above-ground biomass from separate plots used in an experiment to test for interactions between ozone exposure and nitrogen supply. 36 plots (12 per transect) of area $1.2m^2$ were established in 2008 at 1m and 10m from the ozone source ('high' and 'low' ozone) and 10m upwind ('ambient'); two N addition treatments equivalent to 22 ('low') or 72 ('high') kg N ha^{-1}y^{-1} were assigned randomly. N was applied weekly as NH_4NO_3 solution. Plant cover, above-and below-ground biomass and soil cores were recorded – details are in the annual report for 2012 (March 2013). There was no significant effect of O_3 or N on total above-ground biomass in any of the years 2008-2012, but there was a significant effect of elevated ozone on below-ground biomass in both years where this was measured (2010 and 2012 – Fig 1.2).
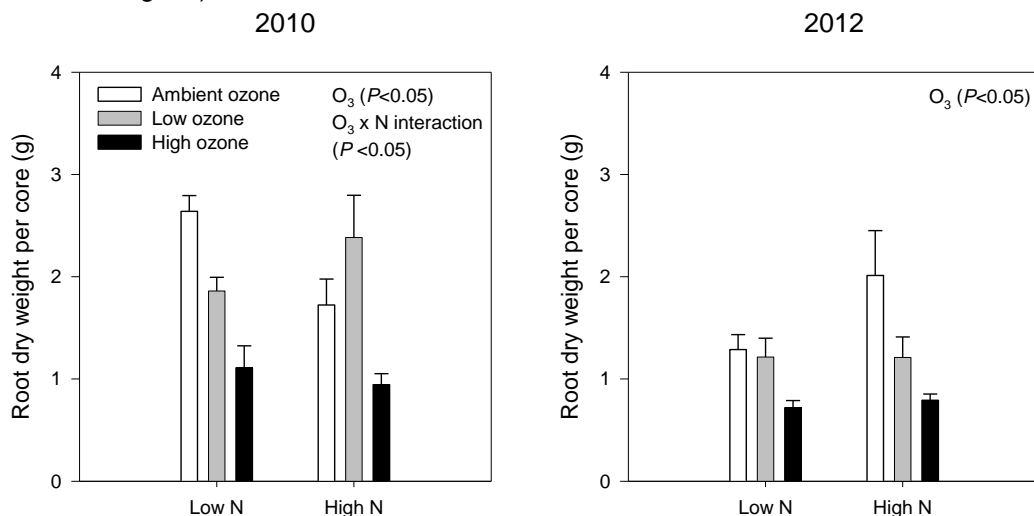

Figure 1.2: biomass in soil cores (5 cm width, 15 cm depth) taken from each plot in August 2010 and 2012. Values are means ± SE (*n*=6). Ozone x N interaction was seen only in 2010.

There were inter-specific differences in the effect of ozone on cover in the experimental plots, but no significant effect of elevated N. Ozone impact on the vegetation in these plots needs to be considered in the context of dynamic changes in sward composition and taking into account other factors which are known to influence competitive interactions between species. For example, soil moisture was found to be significantly lower 10 m downwind of the O_3 release pipes in 2010 (*P*≤ 0.01) than in other plots. Consistent with the data reported above in Section 4, some grasses had greater cover in high ozone plots, and the forb *Ranunculus acris* had significantly less ground cover in higher ozone.

2. Grassland mesocosms in open-top chambers (Newcastle)

Ozone fumigation in the open-top chambers at Newcastle continued, as planned, during the 2012 season, mainly accommodating collaborative Newcastle–York Umbrella experiments. MG3b grassland communities were exposed to simulated present-day versus '2050' ozone climate for a 9th season. Previous reports have outlined the significant decline of key species in this community such as *Briza media* and *Lotus corniculatus* in the elevated ozone climate, and results from the 2012 harvest are consistent with those of preceding

years (Fig 2.1).
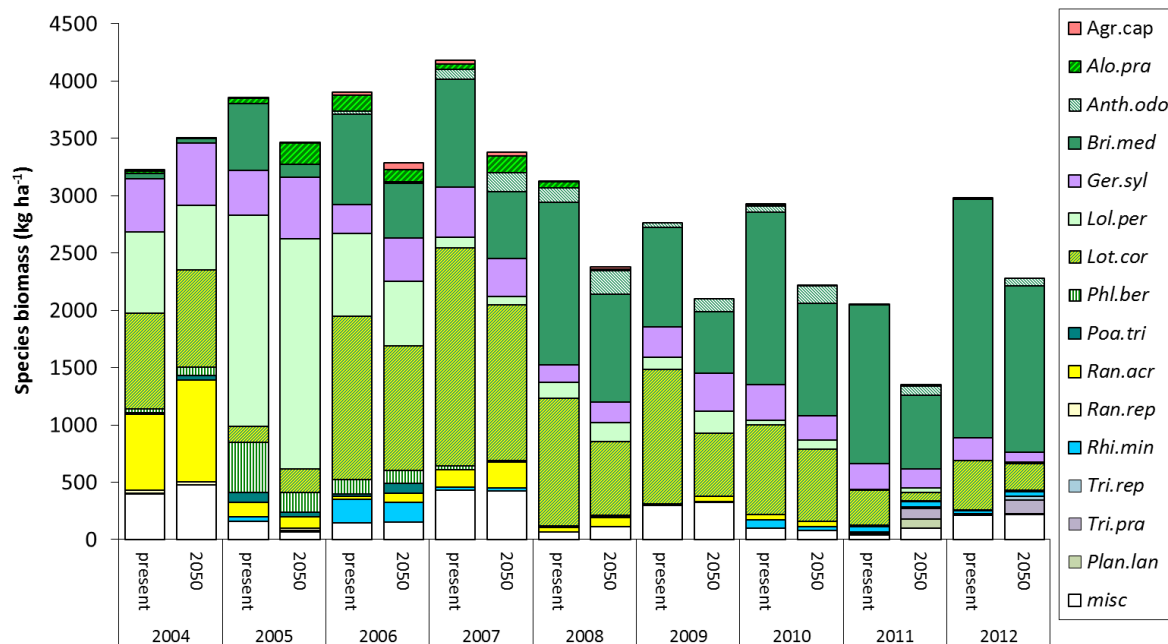
Figure 2.1: Impact of nine years of exposure to present-day and '2050' UK upland ozone climate on the productivity of MG3b grassland mesocosms.

However, regression analysis of cumulative sward biomass with cumulative ozone exposure revealed that the elevated ozone scenario did not match the more linear response observed in the 'present-day' scenario; an effect only apparent after several years of ozone exposure (see annual report March 2013). This result emphasises the need for such long-running studies on established complex species-mixtures to better understand how grassland communities are likely to respond to future ozone climates.

A key objective over the past two years has been to monitor the impacts of elevated ozone on key early season drivers of community dynamics such as the hemiparasite *Rhinanthus minor*. Although the dried biomass of *R. minor* measured during the July harvests was not significantly affected by the '2050' ozone climate, this annual species peaks in biomass and abundance in late May/early June. Therefore the effect of elevated ozone was assessed by quantifying the frequency of this species in June, in two separate seasons. In 2010 elevated ozone significantly reduced the frequency of *R. minor* plants from an average of 44.5 compared with 10.8 plants per mesocosm in the 'Present' ozone climate. The effect of ozone was similar in 2012 (but overall plant numbers were lower, possibly due to a mild 2011 winter) with an average of 14.6 and 5.8 plants per mesocosm in the 'present' and '2050' treatments respectively (reduction due to ozone $P \le 0.05$).

Overall the MG3b mesocosms under both the 'present' and the '2050' ozone climate showed signs of shifting towards a community dominated by species typical of meadows with higher conservation value (Fig 2.2). This could be attributed to the management practices applied including the sowing of *R. minor*, cessation of fertilizer input, and allowing time for seed set before annual harvesting. However, the significant impact of ozone on the total biomass, and the relative proportion of the species of high conservation value in these ecosystems (principally *Briza media* and *Lotus corniculatus* and *Rhinanthus minor*), indicate that an average increase of just 13 ppb over nine years will reduce the effectiveness of conservation and restoration schemes on upland MG3b grasslands.

Soil cores were taken following the 2012 above-ground harvest. Average pH was unaffected by O_3, but soil from mesocosms subject to elevated ozone exhibited significantly higher loss on ignition (LOI %) compared with controls (P≤ 0.05). These preliminary results suggest that 9 years of ozone exposure may have changed key soil properties such as organic carbon content/turnover. The implication is that rising levels of ozone pollution may enhance litter recalcitrance and slow decomposition and carbon release from upland soils – a hypothesis proposed to be explored as part of an extension of the ongoing umbrella – important because such impacts would profoundly influence carbon turnover in these habitats.

A manuscript from the MG3b mesocosm experiment will be submitted to *Oecologia* in March 2013.

Policy implications

Long-term exposure to elevated ozone has inhibited growth of the very species that the grassland management regime is designed to promote, and while no total loss of species has been observed, the progression towards a species-rich grassland has been slowed.
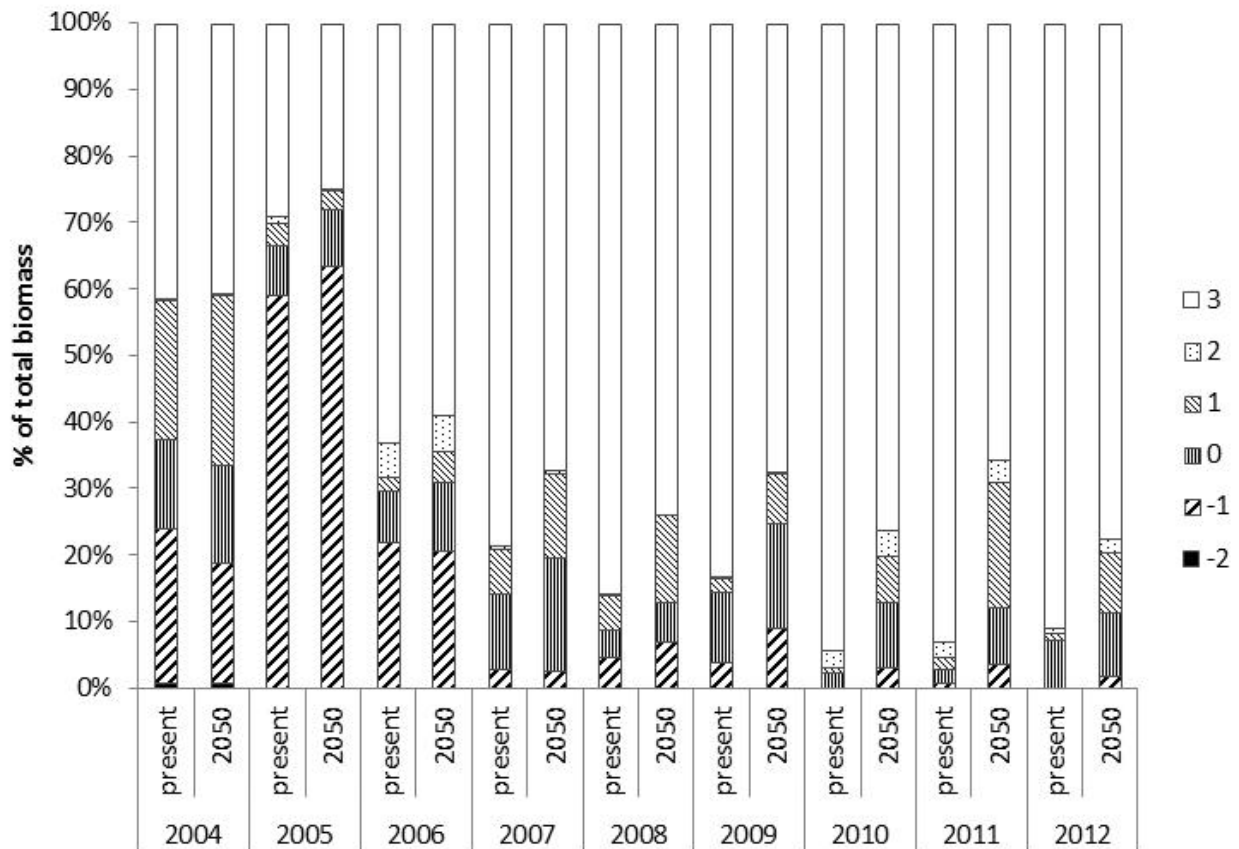
Figure 2.2: Percentage of the total annual sward biomass consisting of species with negative and positive indicator scores for upland hay-meadows in the North Pennines Area of Outstanding Natural Beauty (AONB). Negative scores indicate the presence of troublesome weeds and signs of agricultural improvement, whereas positive scores indicate species only present in rich restored meadows. Species present in the mesocosms had scores of -2: *Ranunculus repens;* -1: *Lolium perenne, Phleum bertolonii, Trifolium repens*; 0: *Alopecurus pratensis, Poa trivialis*; 1: *Agrostis capillaris, Anthoxanthum odoratum, Plantago lanceolata, Ranunculus acris, Trifolium pratense*; 2: *Rhinanthus minor;* 3: *Briza media, Geranium sylvaticum, Lotus corniculatus*.

3a. Spring bulbs: dose-response to ozone exposure (Newcastle)

More than 4000 measurements of stomatal conductance were made over a range of conditions on *Allium ursinum* and *Tulipa sylvestris* (previously identified as particularly sensitive to O_3) in collaboration with the York team, and a DO_3SE-style model was derived from boundary line analyses. The resultant algorithms are provided in the annual report (March 2013). Environmental data are being collated and these will be used to develop flux-response relationships, and facilitate risk assessment in connection with future predicted impacts of changes in spring O_3 concentrations. This work is due for submission for publication before the end of April 2013.

3b. Legume-rich sand dune communities in open-top chambers (Newcastle)

Assessment of effect on SD9b community productivity was undertaken for a 4th season of ozone exposure. The significant negative impact of ozone on the biomass of several leguminous species, such as *Ononis repens, Lathyrus pratensis* and *Lotus corniculatus* was no longer evident. This change was attributed to the growth forms of the most dominant species, *Ononis repens* and *Lathyrus pratensis*, which had developed long tap-roots. These results suggest that competitor interactions and access to available nutrients strongly influence ozone impacts in this community, a hypothesis which we believe is worthy of further detailed investigation. The use of field pollution exposure experiments, where larger areas of established vegetation could be monitored, is necessary to further our understanding of ozone dose-response relationships for the SD9b community.

Policy implications

The first few years of experiment showed an inhibitory effect of ozone on the growth of legumes, but this was not maintained in year 4. This result highlights the importance of using long-term experiments to advise policy, rather than simply single-season experiments, particularly for plant communities where interactions and competition may take several growing seasons to be established.

7 & 8. Determination of ozone exposure thresholds for impacts on acid BAP Priority Habitats under current and predicted future reductions in precipitation, using controlled exposure to a range of ozone concentrations in the Bangor solardome facility (CEH Bangor).

7.1 Introduction and policy relevance

Experimental research at CEH Bangor and by other members of this consortium has shown that many species of (semi-)natural vegetation are sensitive to ozone within the ambient range experienced in the UK (e.g. Hayes et al., 2007, Mills et al., 2009, Wilkinson and Davies, 2009, Contract reports for CPEA 33). In this work-package, we investigated effects of ozone on mixed species communities representative of three important BAP Priority habitats found in relatively high ozone areas within the UK (Morrissey et al., 2007): Lowland/Upland calcareous grassland, Lowland/Upland acid heath, and Coastal and floodplain grazing marsh. We considered these effects within the context of biodiversity and ecosystem service policy objectives. By using eight precision controlled ozone regimes, we investigated effects of rising background ozone (2009, 2010) and different approaches to reducing ozone concentrations (2011, CEH funded in 2012), with peaks decreasing more than background to simulate more rapid European than Global control of ozone precursors. These effects of ozone were set within the context of an increasingly unstable climate by including combined effects of ozone and reduced precipitation (Lowland/Upland calcareous grassland, Lowland/Upland acid heath) and ability to withhold water and subsequently recover from a flooding event (Coastal and floodplain grazing marsh). In summary, for the three BAP priority habitats, we investigated effects on the following ecosystem services:

          Provisioning services: genetic diversity, water supply, forage quality
          Regulating services: reducing flooding, C sequestration
   Intermediate services: pollination
          Cultural services: appreciation of biodiversity, aesthetic appreciation of the natural environment

Note: CEH contributed >50% of the costs for experiments conducted in 2000–2011 including the costs of running and maintaining the solardome-based ozone exposure system, and fully funded the experiment in 2012.

7.2 Experimental approach

In all the experiments (see annual report, March 2013) mesocosms were constructed in the year before ozone exposure from young plants purchased from specialist native plant species nurseries or grown from seed collected in the UK at CEH Bangor. Depending on experiment, there were 5 or 6 replicate mesocosms per watering treatment per solardome, with two watering treatments during ozone exposure for calcareous grassland and acid heath, and three post-exposure ozone treatments for the flood meadow communities (well-watered, drought and flooding). For each community, watering was applied by hand as needed to ensure soil moisture levels met the target values.

The mesocosms were exposed to ozone in 8 solardomes, where CEH-funded state-of-the-art ozone exposure enables naturally occurring episodes to be simulated or modified, with differences between treatments as small as 5 ppb being consistently reproducible. Details are in the annual reports. In 2009 and 2010, the calcareous grassland and acid heath communities were exposed to eight rural ozone upland profiles based on an episode at Keenley in May, 2008. One treatment simulated this episode, and the other seven increased or decreased the episode ozone concentrations by -30, -20, -10, +10, +20, +30 or +40 ppb (Fig 7.1a). In 2011, the floodplain communities were exposed to ozone profiles representative of lowland rural areas. The profile was based on an ozone episode at the Aston Hill monitoring station (16-22 July 2006). The highest ozone treatment was this 7-day long episode repeated every week (Fig 7.1b). The other 7 treatments had increasingly large reductions in the peak and background ozone concentrations (with larger decreases for the peak exposure than for the background concentrations) to represent the effects of predicted changes in precursor emissions.
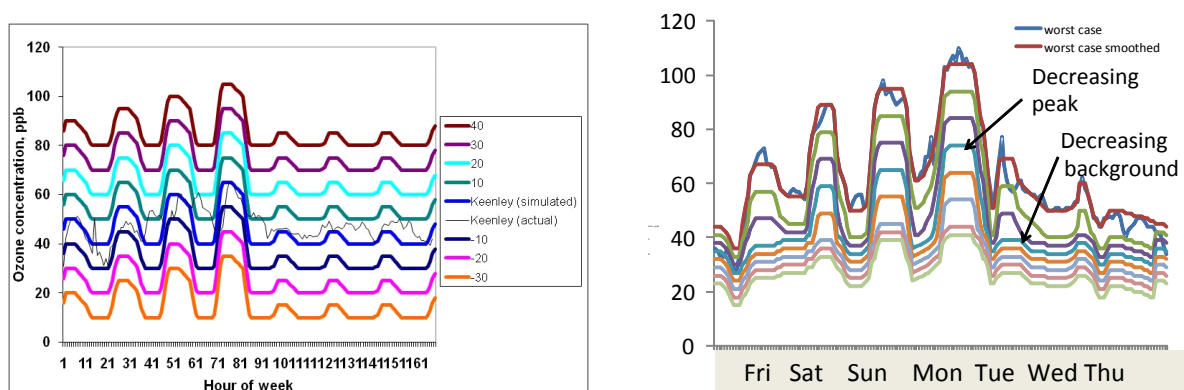
Figure 7.1: The target ozone regime used in the solardomes in (a) 2009 and 2010, and (b) 2011

The effects of ozone on canopy senescence, leaf area index, flower number and duration, above and below-ground biomass, net ecosystem exchange and stomatal conductance were all measured to a varying degree for each community. An important deliverable from these studies has been to increase the availability of flux models for native species in the UK. This is only possibly by making a large number of stomatal conductance measurements (> 300 per species) over a wide range of environmental conditions during the exposure period. We have made such measurements on 10 species and used these to compile DO_3SE models; measurements were also made on a further 7 species and will be retained for future use. An example of the parameterisation process is provided for *Campanula rotundifolia* (harebell) in Fig 7.2. Application of the DO_3SE models has been used to investigate the potential effects of emission controls on natural vegetation – see annual report for 2012 for details.
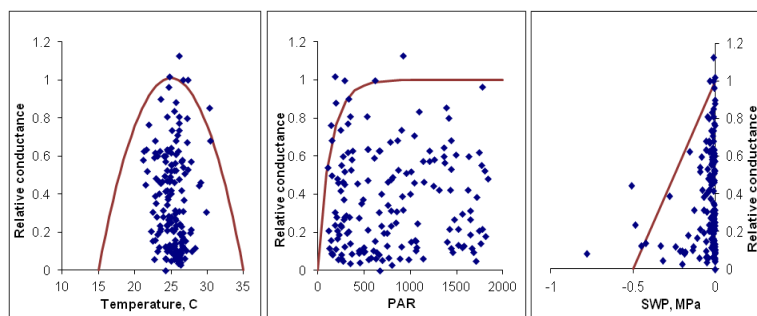
Figure 7.2: An example of the flux parameterisation for *Campanula rotundifolia* (harebell), showing stomatal conductance plotted against (a) temperature, (b) Photosynthetically active radiation (PAR) and (c) soil water potential (SWP).

7.3 Summary of key results and policy implications
#### 7.3.1 Effects of ozone on flowering
Flowering is a critical stage in the life-cycle of a plant and alterations to this process could influence species survival within a plant community and reduce the important ecosystem services related to pollination and nectar sources. A recent meta-analysis of effects of ozone on plant reproductive growth and development revealed that current ambient ozone concentrations significantly reduced seed number, fruit number and fruit weight compared to charcoal-filtered air conditions (Leisner and Ainsworth, 2012). Despite evidence of alterations in flower numbers/biomass following ozone exposure, comparatively few studies have investigated the effects of ozone on the timing of flowering. For the calcareous grassland communities, weekly counts of flower number for each species revealed species-specific effects of increasing background ozone on both the timing and number of flowers.

Early season formation of flowers on *Lotus corniculatus* (Birdsfoot Trefoil) was accelerated with increasing ozone concentration during the second year of ozone exposure; the date on which 20% of the maximum number of flowers (used as a surrogate or the start of flowering) was reached correlated linearly with 24h mean ozone concentration (p = 0.017; Fig 7.3a) . The difference in the time taken to reach 20% of the maximum number of flowers varied across the range of ozone exposures by 9 days in the well-watered (WW) treatment and by 7 days in the reduced water (RW) treatment. In the early weeks of flowering this resulted in increased numbers of flowers in the higher ozone treatments. For example, on 27th May 2010, after exposure to the ozone regime for 5 weeks, the earlier flowering in the highest ozone treatments resulted in a linear increase in flower number with increasing ozone exposure for the WW treatment (r^2 = 0.67, p = 0.013) and a non-significant increase for the RW treatment. Despite the differences in flower number between treatments in the early weeks of flowering, there were no differences in the maximum number of flowers between ozone treatments for this species.

A contrasting response was found for harebell (*Campanula rotundifolia*), an iconic species of calcareous grassland. For the WW treatment, an increase in mean ozone concentration from 30 ppb to 70 ppb corresponded to a 40% decline in flower number. Combining both watering treatments, the decline in maximum flower number for *C. rotundifolia* showed a significant linear relationship with POD_1 (r^2 = 0.33, p = 0.02; Fig 7.3b). Based on 95% confidence intervals for this relationship, the POD_1 needed to give a significant change in flower number was 12.2 mmol m^{-2}. For small scabious (*Scabiosa columbaria*), a later flowering species, there was a linear decline in the number of buds in the well-watered treatment with increasing ozone (r^2 = 0.65, p = 0.016). Other species in the mesocosms showed no effect on flowering.

Policy implications: These detailed assessments of flowering have revealed effects that might previously have been overlooked in more usual intermittent counts of flowering. The high proportion of species from the calcareous grassland community showing altered flowering in response to increasing background ozone is of concern for the viability of this habitat in future ozone conditions. Given that a recent study indicated that 72% of UK lowland calcareous grassland occurs in regions where the AOT40 is greater than 6.5 ppm h (averaged over 1999-2003; Morrissey et al., 2007), it is possible that effects on flowering may already be occurring at current ambient, compared to pre-industrial, ozone concentrations. The consequence of earlier flowering of a species in a community as a result of ozone exposure could be a lack of synchronicity with

pollinating species. For example, Kudo et al. (2004) found a mismatch between early flowering plants in Japan, which advanced their flowering time in a warm spring, and bumble bee emergence, which did not advance, resulting in a decreased seed-set in bumble bee pollinated plants. Such effects could already be occurring in the UK. Furthermore, it has also been shown that for some species the abundance of other flowers before or during its own flowering can influence reproductive success due to competition for pollination (Brown et al., 2002). With two of the seven species showing reduced flower or bud number with increasing ozone and one showing earlier flowering, it is likely that the reproductive potential of several species within calcareous grassland may be being impacted, with effects likely to worsen if ozone concentrations continue to rise. See Hayes et al. (2012a) for further details.
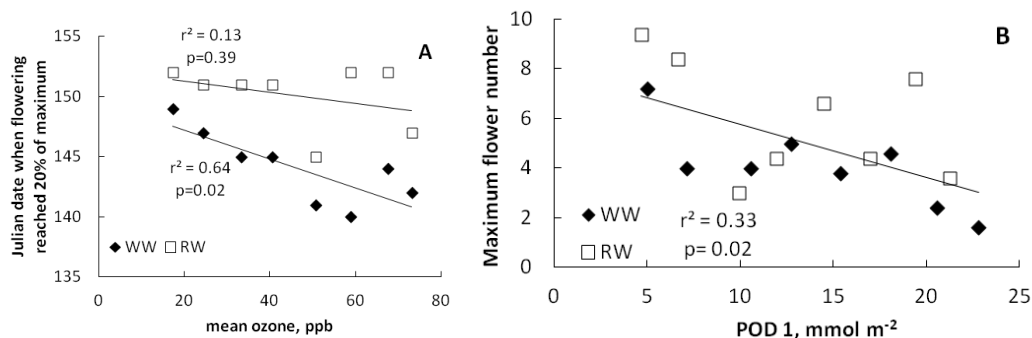
Figure 7.3: (a) Timing of the start of flowering in *Lotus corniculatus* (Birdsfoot Trefoil) in relation to 24h mean ozone concentration. Data are presented as the day of year on which 20% of maximum flower number was achieved. (b) Relationship between maximum flower number and POD_1 for *Campanula rotundifolia* (harebell). From Hayes et al.(2012a)

7.3.2 Effects of ozone on community composition, including carry-over effects

An effect on biodiversity would require loss of a species from a community, with effects increasing as ozone concentration increases. For the three communities studied, there was no loss of any species during or after ozone exposure, but there were shifts in species composition that with extended ozone exposure may have eventually led to a loss of species. In summary, we found:

Calcareous grassland: In June 2011, after overwintering and regrowth following the second season of ozone exposure, there was no significant effect on the total biomass of the community. However, the total biomass of lesser salad burnet (*Sanguisorba minor*) decreased with increasing ozone in well-watered (WW) communities (Fig 7.4a), but not the reduced watering (RW) communities. Although it was not possible to separate out the roots by species, root cores taken from the centre of each pot in June 2011 showed a significant decrease in root mass with increasing ozone for both the WW and RW communities (Fig 7.4b). There was also a significant effect of the RW regime, with reduced root growth in RW conditions (p=0.006) and an interaction between watering regime and ozone exposure (p=0.053).

Acid heath communities: Following two seasons exposure to ozone, overwintering and re-growth in 2011, an increase in biomass of *P. erecta* with increasing ozone was found (p=0.004), with no effect of watering regime and no interaction between ozone and watering regime. This increase may have been in response to the (non-significant) decrease in *F. ovina* biomass with increasing ozone seen at this and in the previous harvest. For the biomass of *Calluna vulgaris* there was a significant interaction between ozone and watering regime (p=0.039, data not presented), with no response to ozone in the WW treatment, but an increase in biomass with increasing ozone in the RW treatment. There was no significant effect of either ozone or watering regime on the root biomass of the heath communities.

Flood meadow communities: A decrease in Net Ecosystem Exchange was found with increasing ozone exposure (Fig 7.4c). For greater salad burnet (*Sanguisorba officinalis*) there was reduced stomatal conductance with increasing ozone exposure. This may indicate reduced demand for water at higher ozone concentrations. At the end of the two year exposure there was a decrease in biomass of *S. officianalis* (r^2 = 0.11, p = 0.008) with increasing ozone.

Policy implications: Although no individual species were lost from the communities, all three BAP Priority habitats showed biomass responses to increasing background ozone. Effects were most frequent and pronounced for the calcareous grassland communities, with both above-and below-ground biomass being reduced with a linear increase in ozone concentration. These effects were detected at relatively low ozone – well within the range we could expect in a high ozone year such as 2003 and 2006. Potentially, such effects could ultimately lead to loss of a species by competition from more ozone–resistant species, if there were several consecutive high ozone years.
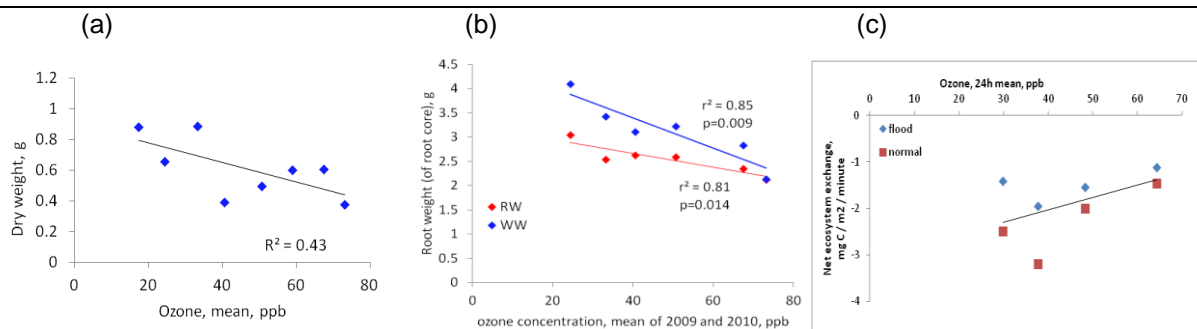
Figure 7.4: (a) Above ground biomass of *Sanguisorba minor* (lesser salad burnet, WW only) in June 2011, following ozone exposure in 2009 and 2010. (b) Root weight (per root core) of WW and RW calcareous communities in June 2011, following ozone exposure in 2009 and 2010.(c) Net ecosystem exchange of the flood meadow communities during ozone exposure in 2011

7.3.3 Effects of ozone on pasture quantity and quality

(Semi-)natural grasslands can also have an important role in an agricultural context as the sward constitutes an important nutritional resource, particularly for sheep. Although changes in forage quantity can influence animal performance, quality of the vegetation is particularly important. Along with grassland samples collected under previous contracts, vegetation samples from the calcareous grassland mesocosm experiments were analysed for forage quality. For several of the 'desirable' characteristics of forage – protein, sugar and fat content – there were no significant changes in concentration within the foliage. However, there were elevated concentrations of lignin, crude fibre (Fig 7.5a) and acid detergent fibre with increasing ozone concentrations. These changes could be a result of either changes in species composition, or effects on individual species that may cause changes such as an increased proportion of stems compared to leaves or an increased proportion of senescent leaves. In addition to the quality parameters directly measured, further quality parameters that give an indication of the digestibility of the forage were calculated for each sample. These showed that the relative feed value (Fig 7.5b) and metabolizable energy of the forage decreased with increasing ozone concentration, indicating reduced nutritive quality for the grazing animals. Further details are given in Section 11 below.

Policy implications: These studies have shown that the quality of pasture for these examples of calcareous grassland decreased with elevated ozone exposure. This has financial implications for the livestock owners, as increased supplementary feeding may be required to made up for the shortfall in nutrition from the forage.
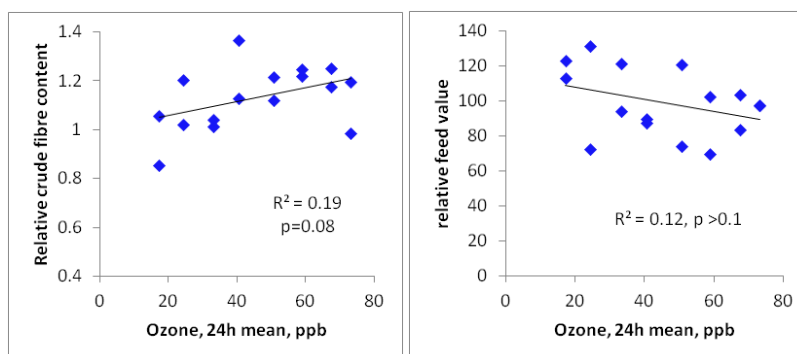
Figure 7.5: Effects of ozone on fodder quality for the calcareous grassland community (a, left) crude fibre content and (b, right) relative feed value.

7.3.4 Interactions between ozone and drought

In the experiments conducted as part of contract CPEA33, and by CEH funded PhD student Serena Wagg at Bangor, we have shown that chronic ozone reduces the sensitivity of stomata to several environmental stimuli, including temperature, light, humidity and water potential – all components of the DO$_3$SE flux model (Wagg et al., 2013). For one of these variables, soil moisture, inclusion of the modifying effect of ozone on stomatal response within the DO$_3$SE model indicated that ozone fluxes could be substantially higher under conditions where soil moisture is limiting compared to those calculated with the standard DO$_3$SE model (Hayes et al., 2012b). Thus, rather than protecting plants from ozone damage, the combination of chronic exposure to ozone and reduced water availability could potentially make some species more sensitive to ozone as there is potentially greater ozone uptake than would be expected under normal water availability. For the current contract, we also investigated the implications of this effect for intact grassland communities and their response to reduced water availability as may be anticipated in the coming decades. For example, the roots of the calcareous grassland community were (as expected) smaller in the reduced water treatment than the well-watered treatment at low ozone, but at high ozone there was no difference in root growth

between the two watering treatments (Fig 7.4b). In the flood meadow communities that had water completely withheld for 3 weeks in ambient air after 18 weeks of ozone exposure, the photosynthetic rates of the canopy were higher and there was a greater proportion of green leaf area in the highest ozone treatment. Possibly the smaller roots and canopy had lower water demand and plants were thus able to maintain functioning for longer as the soil dried.

Policy implications: These studies have provided clear evidence that continuous ozone exposure can interfere with the functioning of plants by reducing their capacity to respond to environmental stimuli. Whereas drought may provide some protection against ozone damage in some species, it is clear that when drought or reduced watering is accompanied by prolonged ozone exposure no such protection occurs in some grassland species. Generalising on which species might respond to ozone by stomatal closure and which by enhanced stomatal opening has proven to be difficult. A literature review conducted within the ICP Vegetation contract (AQ0816) investigated effects for 71 species and showed that overall 30% demonstrated enhanced conductance or reduced stomatal response to environmental stimuli, 46% enhanced stomatal closure, and 24% had no effect on conductance (Mills et al., in prep.).

7.3.5 Effects of ozone on soil water retention and re-wetting capacity in flood meadow communities
Flood meadow communities conduct a vitally important role in withholding flood water during periods of excess rainfall. In the UK, it is becoming increasingly common for a dry period in spring with relatively high ozone levels to be followed by a period of excessive rainfall in summer often resulting in flooding in many areas. The CEH Bangor flood meadow community experiment aimed to simulate this occurrence with flooding of one third of the communities following April, May and June ozone exposure. A further one third were maintained at field capacity and the remaining third were subjected to a severe drought as described above. To investigate how well these communities could withhold soil moisture, excess water was drained from the flooded communities and then no further watering was provided for a week. It was found that there was decreased soil moisture content with increasing ozone exposure (Fig 7.6a). This faster drainage with increased ozone exposure may also indicate reduced root biomass of the mesocosms from higher ozone treatments. In addition, the flood meadow communities experiencing drought did not retain as much soil moisture when re-wetted (Fig 7.6b).

Policy implications: This simulation has clearly shown that ozone effects during the early growing season can reduce the ability of flood meadow communities to withhold water in soil and re-wet after drought.
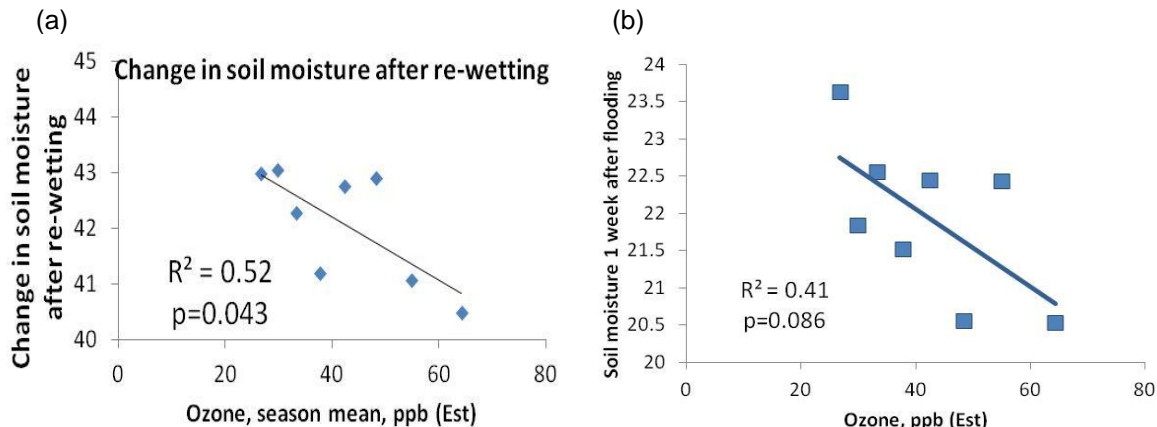
Figure 7.6: Effects of prior ozone exposure on (a) soil moisture retention after three weeks of flooding and (b) soil re-wetting after three weeks of drought for the flood meadow communities.

7.3.7 Ozone exposure thresholds for effects and insights into impacts of policy measures
Flux-response relationships have also been derived for several species – see examples in this section of the report, using POD_1 (Phytotoxic Ozone Dose over a flux threshold of 1 nmol m^{-2} PLA s^{-1}) – and used to derive critical levels (Table 7.1). Because of the different flux model parameterisations for each species, it cannot be assumed that the lower the flux-based critical level, the more sensitive the species is to ozone. The most sensitive response was found for accelerated flowering in *Lotus corniculatus* (critical level = a 24h mean of 34 ppb, accumulated during the exposure period only). Critical levels for biomass tended to be higher than those for flowering and flood water retention. The new flux-based critical levels listed in Table 7.1 will add to the scarcity of information available for natural vegetation. We have also analysed the data in the context of precursor emission controls using dose response relationships where $p<0.05$. A decrease in background ozone from 40 to 30 ppb, increases canopy photosynthesis and biomass of sensitive species by 8 – 22% (Table 7.2).

Policy implications: Some of the flux-effect relationships derived during the solardome study provided invaluable input into the discussions on deriving flux-based critical levels at the LRTAP Convention Critical

Levels Workshop (Ispra, 2009) and ICP Vegetation Task Force meeting (Tervuren, 2010). The more recently derived flux-effect relationships have been presented at subsequent ICP Vegetation meetings. Using the precision control of the solardome facility, it has been possible to show that there could be large benefits of decreasing the 24h mean by 10 ppb for ozone-sensitive species and communities, and a 10 ppb decrease in peak and 3 ppb decrease in background ozone could lead to 10% more photosynthesis in flood meadow communities, a 21% increase in biomass of some ozone-sensitive species and 2% more flood water retained.

Table 7.1: New critical levels for effects of ozone on ecosystem services provided by calcareous grassland, acid heath and flood meadow BAP priority habitats. These values were calculated as the lowest concentration or flux causing a significant effect.

	Response variable	Ecosystem service	24h mean	POD1
			Critical level (ppb)	Critical level (mmol m^{-2} PLA)
Scabiosa columbaria	Biomass	C seq., biodiv.	56	21.4
Sanguisorba minor	Biomass	C seq., biodiv.	53	16
Campanula rotundifolia	Cover	C seq., biodiv.	49	14.3
Sanguisorba minor	Cover	C seq., biodiv.	57	16.4
Festuca ovina	Cover	C seq., biodiv.	60	n.a
Lotus corniculatus	Early Flower.	Biodiv.	34	2.5
Potentilla erecta	Early Flower.	Biodiv.	50	n.a
Campanula rotundifolia	Fewer flowers	Biodiv.	47	12.6
Scabiosa columbaria	Fewer flowers	Biodiv.	52	26.9
Flood meadow community	Soil water ret.	Flood prev.	47	n.a.

Table 7.2: Beneficial effects of precursor emission control scenarios based on significant response functions from the solardome experiments.

Ecosystem service	Parameter	Benefit of:	
		Decrease in 24h mean from 40 to 30 ppb	decrease in peak by 10 ppb, decrease in background by 3 ppb
C sequestration	Flood meadow NEE	12% more photosynthesis	10% more photosynthesis
	Calcareous, root biomass	8% more root	n.a.
Biodiversity	Calcareous – sensitive species biomass (*S. minor*)	9% more biomass	n.a.
	Flood meadow – sensitive species biomass (*S. officianalis*)	22% more biomass	21% more biomass
	Timing of flowering (*L. corniculatus*)	Flowers 1 day earlier	n.a.
	Max. flower number (*C.rotundifolia*)	13% more flowers	n.a
Flood prevention	% soil water, flood meadow communities	2% more water retained	2% more water retained

**9. Laboratory experiments to elucidate the mechanism of ozone effects on upland plant species (Lancaster)**

9.1 Experiments to quantify physiological responses and developmental effects of ozone on growth and functioning of plants from upland ecosystems

During the course of this study, numerous experiments have highlighted a novel effect of ozone on plants, namely that it interferes with the stomatal functioning of a range of species, including those commonly found at our upland experimental site (Keenley Fell). Invariably stomata show only limited responses to soil drying (and to the drought stress hormone abscisic acid (ABA) (Mills et al. 2009; Wilkinson and Davies, 2009; Wilkinson and Davies, 2010; Wagg et al. 2012). We report first on experimental effects on growth that may have some mechanistic commonality with the stomatal effects. Fig 9.1B shows that in lab experiments with an upland forb *Anthoxanthum*, ozone opened stomata in well-watered plants in comparison to non-ozonated controls in weeks 1 and 2, but that it had no effect or closed stomata for the following 4 weeks. From week 6 it was necessary to measure stomatal conductance (gs) in a new cohort of leaves, and again ozone opened stomata over the following 3 weeks (7-9), suggesting an effect of leaf age on ozone sensitivity. In drying soil there did not seem to be a clear effect of time on differences between control and ozonated stomatal conductance. Rather, gs was the same or higher than that of non-ozonated plants in a seemingly random pattern over the course of the experiment. We have concluded that responses by this species may differ from those of other species because of interactions with plant water relations. Ozone increased the average leaf length of *A. odoratum* in both well-watered and drying soil over the first 5 weeks of growth (Fig 9.1A). Other experiments have shown limitations in root growth induced by ozone treatment.

In this study (see also Wilkinson and Davies, 2010), we predicted that species in which ozone opened

stomata, increased leaf growth and decreased root growth might generate unsustainable decreases in leaf water potential which would result in subsequent hydraulic limitations to leaf growth and/or stomatal conductance, particularly under drought (Fig 9.2). However, surprisingly, it was only when a new cohort of leaves began to develop that ozone treatment failed to increase leaf length, even in plants growing in drying soil. Thus it may not necessarily always be the case that ozone-induced increases in leaf growth result in secondary reductions in growth over a longer time frame. Stomatal conductance may have been more sensitive to water potential, however, because ozone only opened stomata in well-watered plants for the first 2 weeks of treatment, and then these began to close (first cohort). This did not, however, result in decreased growth. One reason for this divergence from the predicted response (for which there is some evidence in tree species), may be the growth habit of grasses. They continually produce new shoots that have water transport systems that are not necessarily connected to those of the older leaves, such that the water potential of an older blade, however much it is affected by ozone, will not necessarily affect the water potential of the newly developing leaves. This may not be the case in forbs or larger broad-leafed species. We discuss below the counteracting effects on plant water balance of higher stomatal conductance and reduced leaf area as a result of enhanced senescence under elevated ozone.
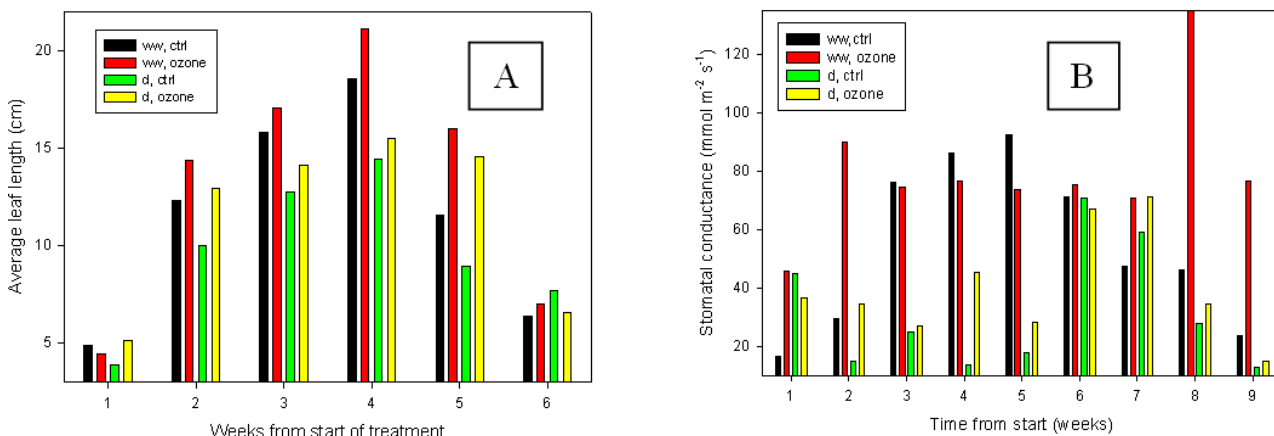
Figure 9.1: Effect of ozone +/- drying soil (ww=well-watered, d=drying) on (A) average leaf length and (B) stomatal conductance in *Anthoxanthum odoratum*.
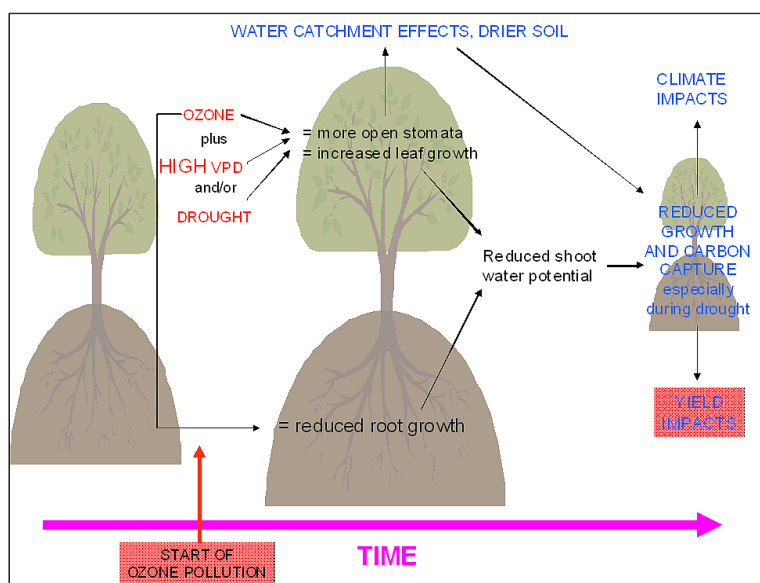
Figure 9.2: Schematic representation of hypothetical longer term effects of ozone pollution on plant growth, yield and carbon capture.

9.2 Experiments to provide further understanding of the mechanistic basis of the effects of ozone on plants (hormonal changes, whole plant and cellular signalling)

As a result of the work funded through this project, we have proposed (Wilkinson and Davies, 2010, Wilkinson et al. 2012) that stomatal response patterns can be interpreted to be a function of the combined influence of the hormones ABA and ethylene, accumulation of which is affected by changes in tropospheric ozone concentrations. This understanding is more generally useful given that ABA and ethylene often exhibit different patterns of accumulation in response to changes in several other environmental perturbations (e.g. Sharp and LeNoble, 2002; Sobeih et al. 2004)). We argue that a given plant has the capability both to reduce gas exchange in response to ABA or ethylene accumulation, and to exhibit ABA-ethylene antagonism that results in higher rates of gas exchange, dependent on prevailing conditions. To test this hypothesis we have generated a range of ABA-ethylene concentration combinations relevant to

field-grown plants, by exposing wheat plants to a variable environment and/or to combinations of externally applied hormones and hormone precursors. Effects on stomatal conductance (gs) were tested in intact plants (where higher rates of gs equate to more open stomata). Ozone and other stresses probably have a wider impact than this, however, through effects on roots and reproductive development of changes in the ABA/ethylene hormone ratio.

In identical sets of plants, or in some cases within the same experiments that tested effects of ACC/ethylene on gs, we applied solutions containing a range of ABA concentrations to foliage. Those sprays generating concentrations observed in leaves of intact plants experiencing soil moisture deficit, showed reduced gs. Endogenous ABA accumulation generated by imposing a soil drying (drought) treatment was also associated with reduced gs. Such effects of drought and ABA on stomata have frequently been documented (e.g. Hussain et al 2009; Wilkinson and Davies, 2010). However when ACC (an ethylene precursor) was co-applied in the foliar spray solution, gs was not reduced as much as expected: ACC antagonised the stomatal closing effect of ABA/drought. This is consistent with the stomatal responses described by Tanaka and co-workers (2005) in *Arabidopsis* epidermal tissue and intact plants exposed to ethylene gas. We also observed a corresponding effect whereby applied ABA and/or early-stage soil drying antagonised ACC-induced reductions in gs. Thus, in contrast to the traditionally accepted view that ABA accumulation in plants in drying soil induces stomatal closure, we have shown that an increase in ABA concentration and/or a reduction in soil moisture co-incident with exposure to ethylene can actually be associated with more open stomata, in plants accumulating ethylene.

Within the same, or identical set(s) of plants we have identified four stomatal 'response classes' to ABA and ethylene, that can occur independently of species, tissue type or, in some cases, experimental environment: a) reduced stomatal aperture via ABA, b) reduced aperture via ethylene, c) antagonism, by ethylene, of the closing effect of ABA effect, d) antagonism, by ABA, of the ethylene 'closing' effect. Crucially, by demonstrating that ethylene accumulation can both reduce stomatal aperture and antagonise ABA- and drought-induced stomatal 'closure', and that neither effect precludes the other, we have established that there is a broad-ranging role for ethylene in mediating stomatal responses. This may be one explanation for the apparent lack of responsiveness of stomata to ABA often observed in the field. Here we term this effect ABA-ethylene synergism in stomatal control.

To further develop and substantiate the paradigm of ABA-ethylene synergism in stomatal control, intact plants were manipulated to produce an extended range of tissue ABA-ethylene concentration combinations, or 'ratios'. A 5-6 day soil drying and re-watering regime was imposed with and without ozone, both with and without supplemental ACC. Differing accumulation responses, between the two hormones, to the fluctuating soil moisture availability, resulted in dynamic, wide-ranging changes in the ABA-ethylene concentration ratio over the course of the experiments. Critically, data show clearly that gs could be explained more effectively by the hormone interaction, than by the action of one or other hormone in isolation, over a broad range of hormone combinations/soil moisture conditions. Our data show that ethylene may affect many stomatal responses to the changing external environment, such that its involvement in the regulation of plant gas exchange is likely to be much more important, and prevalent, than has previously been suspected.

We have defined a relationship between an ABA-ethylene concentration 'ratio', and gs in a changeable environment, that is described by a parabolic curve (Fig 9.3). Peak gs (required for 'maximum carbon gain) occurred at low-to-intermediate ABA-ethylene concentration combinations, however gs could be reduced from this point either when the relative concentration of ABA decreased (a left shift to a lower ratio) or when the relative concentration of ABA increased (a right shift to a higher ratio). There are thus several means of "escape", in terms of reduced transpiration (with a C penalty) under a harsh environment, for genotypes that are able to respond by altering either ABA accumulation/sensitivity, ethylene accumulation/sensitivity, or both. Crucially, in variable environments, positive selection for stomatal sensitivity and plasticity has been demonstrated (Nicotra and Davidson, 2010; Piovani et al 2011; Duan et al. 2007). In terms of the modelled curve, sensitive shifts to lower ratios could allow protection against and recovery from certain stresses (e.g. UVB and, oxidative stress) likely to increase ethylene accumulation, or protection against and recovery from stresses that may reduce ABA concentrations (e.g. anaerobic soil environments: Grichko and Glick, 2001). Sensitive alteration in the ratio to higher values could confer resilience against, and sensitive recovery from soil drying (where ABA is known to accumulate), or resilience to heat stress through transpirational cooling (Lopes and Reynolds, 2010; ethylene accumulation is a consequence of heat: Hays et al. 2007). In this model, labile stomatal apertures shifting freely in and out of maximum carbon acquisition would confer an advantage in circumstances where stomata effectively regulate gas exchange.

In combination, our data elucidate a mechanistic basis for the plasticity often observed in this major plant response to its erratic and stressful environment. Plant environmental responses are more accurately explained by corresponding changes in an ABA-ethylene concentration 'ratio' than in the concentration of each hormone taken individually. Such integrated multi-hormone signalling systems seem generally to provide greater flexibility in plant responses (Sankar et al. 2011). Significant genetic variability exists in

ethylene and/or ABA accumulation in response to stress and in the sensitivity of plant responses to ABA, and developmental stage and past environment also alter production of, and/or sensitivity to, these hormones. Our data thus allow us to propose that ABA-ethylene synergy in the control of plant gas exchange can dynamically integrate broadly different combinations of genetic (G), developmental stage (D), and environmental (E) complexity (GxDxE). We suggest that ABA-ethylene concentration ratios can provide explanatory power to help explain effects of a range of ozone concentrations on plants and genotypic variation in this property.
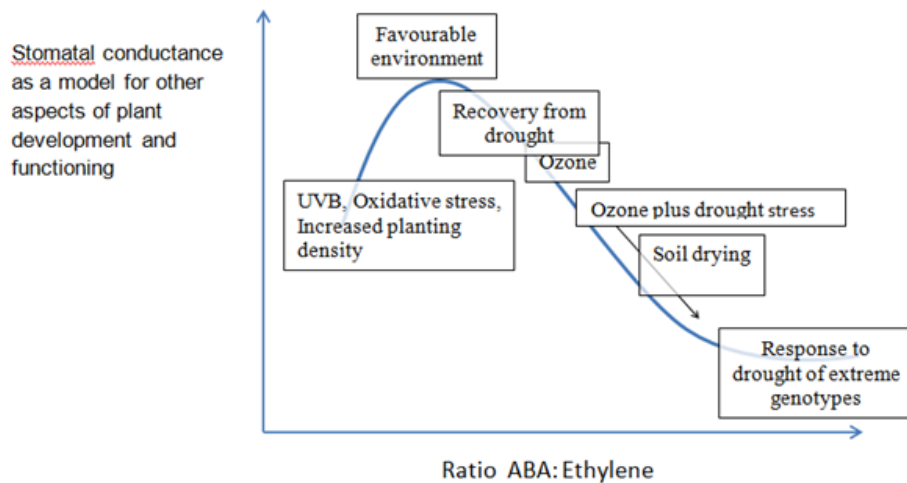
Figure 9.3: Proposed relationship between plant functioning and development and the hormone ratio ABA: Ethylene. See text for literature references to individual responses.

Interactions between ABA and ethylene may also effectively regulate grain filling rate (Yang et al. 2006), root and shoot extension growth (Sharp and LeNoble 2002; Pierik et al 2007), and seed germination (Gassemian et al 2000). We need to determine whether relationships between ABA-ethylene synergism and several physiological responses are conserved in plants, such that systemic changes in hormones, induced for example by ozone exposure can induce multiple effects on growth and functioning. Fig 9.4 shows our proposed model for whole plant signalling involving hormones and hormone ratios and illustrates the potential interactions between soil water and nitrogen availability, soil biology (see below) and atmospheric factors such as ozone (see e.g. Wilkinson et al 2012). McAinsh et al (2012) have recently published work on ozone and cellular signalling and these Lancaster workers with Sally Wilkinson (Lancaster) and Julia Davies (Cambridge) have recently obtained funding from BBSRC to follow up on the present project.
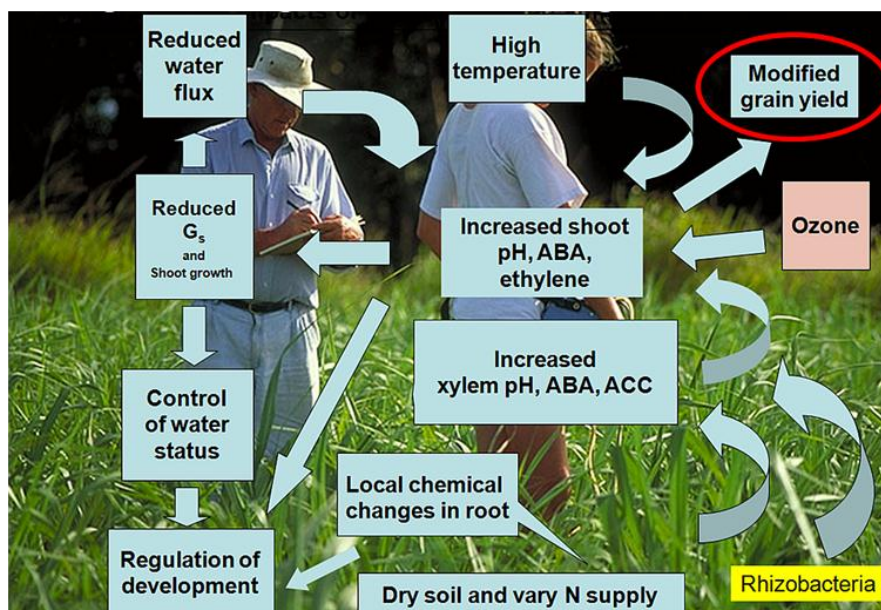

Figure 9.4: Linking the below-ground and above-ground environment

9.3 Experiments to help in the assessment of the efficacy of tools for mitigating ozone effects on plants (ethylene binding inhibitors and rhizobacteria)

In this project we have worked extensively on the impact of rhizobacteria on the ethylene signalling pathway. Fig 9.4 shows the model which we propose to explain the interactive effect of ozone and soil water availability on plant functioning and development. We have shown that there is substantial genetic variation in the sensitivity of plant ethylene production to ozone exposure. Importantly, as well as reducing plant sensitivity to ABA, ethylene also reduces the production of ABA and there is genetic variation also in this response. Fig 9.5 shows how the stomatal response to ABA and hence the plant's capacity to regulate water balance is reduced *in vivo* by the impact of ethylene (e.g. by ozone exposure of the plants). If an ethylene binding inhibitor (1-MCP) is used to negate the impact of this hormone, then stomatal sensitivity is restored. We are investigating the appropriateness of this binding inhibitor as a tool for ameliorating the ethylene effect. This experiment also substantiates our proposal that ethylene/ozone opens stomata at low ABA/stress levels and closes stomata at high stress/ABA. On a larger (e.g. pasture) scale manipulation of soil microbiology may be a more effective intervention.
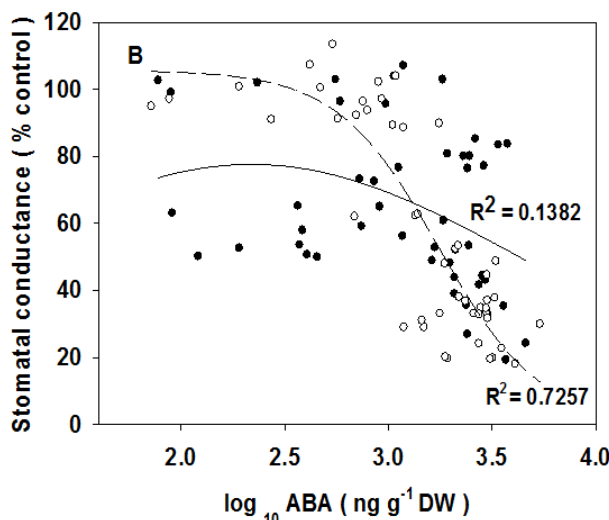
Figure 9.5: Stomatal ABA response in plants in the field (_____) and with 1-MCP applied to inhibit the action of ethylene (-----).
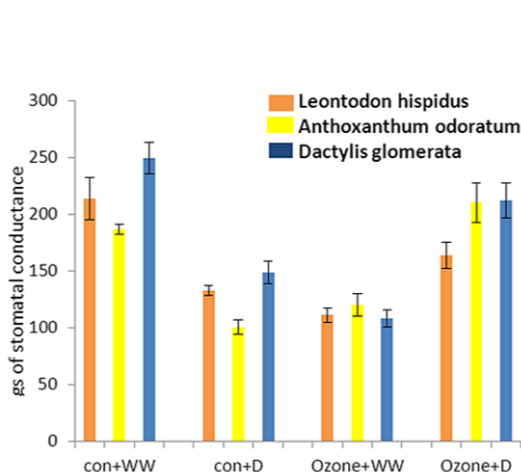
Figure 9.6a: Ozone and drought (D) close stomata when applied individually to upland plants in mesocosms in the glasshouse in Lancaster, but not when appled at the same time.
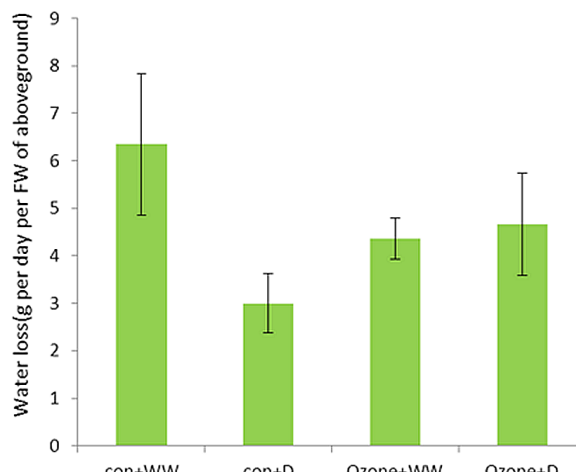
Figure 9.6b: Water loss from droughted mesocosms per unit plant fresh weight is eventually enhanced when air contains additional ozone, but less than on a leaf area basis.

9.4 Experiments to assess the impact of interactions of changing soil water availability and increasing tropospheric ozone on productivity and competitive potential of upland plants in the UK

Given clear effects of ozone and drought on growth and water loss from upland plants (above), we have grown mesocosms containing simple mixtures of species in a glasshouse in Lancaster. Impacts of drought and ozone on stomata of the individual species were as predicted from previous studies (Fig 9.6a), i.e. ozone prevented the complete closure of stomata as a result of soil drying; the extra water loss under combined ozone and drought conditions could be deleterious to vegetation. There was no clear inter-specific variation in sensitivity to ozone in this experiment. However, the effects of the combined stresses on

community water loss relative to plant fresh weight (Fig 9.6b) were not as clear in this experiment as had been expected. This may be because of an interacting impact of extra leaf senescence from the combined stress effects (smaller live leaf area), counteracting the effects of high stomatal conductance per unit leaf area observed for the individual species.

10. Extension of modelling plant responses to catchment scales
The additional complexity demonstrated by the above experiments (i.e. hormonal interactions, and differences between the behaviour of individual plants and communities) make extension of existing models to larger spatial scales impossible at this stage.

11. Valuing Ozone impacts on carbon sequestration, livestock yield and biodiversity in the UK
This research aims to estimate a financial value for ozone impacts on three ecosystem services and follows an ecosystem services approach based on the impact pathway for ozone developed in a previous study (Jones et al. 2012). The chain of ozone impacts is identified starting from changes in ozone concentrations to calculating altered impact on ecosystem processes. The next step is to conceptually link changes in processes to a quantifiable impact on potential ecosystem service supply. Lastly, an estimate of the financial value of ozone impact is calculated by applying environmental economic analysis techniques including market values or benefit transfer valuation as appropriate, for historical changes (1987 to 2007) and projected future changes (2007 to 2020). Uncertainty estimates are produced using Monte Carlo analysis.

11.1 Ozone impacts on carbon sequestration
Dose response functions for ozone impacts on above-ground biomass in grassland were derived based on a meta-analysis of seven UK studies on grassland species mixes from this consortium (current and previous contracts). On average over the UK, rising ozone concentrations have resulted in a net loss in carbon sequestration of 188,000 tonnes in the historic scenario and a further loss of 623,000 tonnes is projected for the future scenario. The equivalent annual value (EAV) of that loss is calculated as £1.5million in the historical scenario, and £9.2million in the future scenario.

11.2 Ozone impacts on livestock yield
Harvested plant material from a range of the Defra funded UK studies of ozone impacts on grasslands, including calcareous grassland, mesotrophic grassland, sand dune grassland and hay meadow grassland (Keenley), was sent for analysis to the same specialist laboratory (AUNIR, Towcester, UK). 'Desirable' quality parameters, which increase the nutritional value of forage (sugar, crude protein and fat), showed no statistically significant effects of increasing ozone concentration. In contrast, 'undesirable' quality parameters (ADF, crude fibre, lignin), which hinder digestibility of the pasture, showed an increase in concentration with increasing ozone exposure. Metabolisable energy (for sheep) was calculated for each sample and this decreased with increasing ozone concentration (p=0.002), with no differences in the slope of the regression line between the grassland types. Using mapped data of 1km resolution for ozone concentration and distribution of lambs, pasture quality and therefore predicted live weight gain of lambs was calculated. This showed that over the period 2007 to 2020, the total daily live weight gain of lambs across the UK was reduced by approximately 4%. The monetary value of this change, considering the length of time of lamb rearing between weaning and slaughter and the proportion of the flock that is slaughtered will be presented in the full Ozone and Ecosystem Services report.

11.3 Ozone impacts on biodiversity
Ozone impacts on biodiversity were determined by estimating dose-response functions for ozone and plant species richness using spatial data. Within the funding constraints, this was the limit to which the assessments for biodiversity could be taken at this stage. Dose-response relationships were based on spatial gradient analysis of national UK landscape surveys over 3 (approximately decadal) time intervals, and corresponding average ozone concentration data. The results were slightly counter-intuitive, in that some habitats suggested positive relationships with ozone (Fen, Marsh and Swamp) while others suggested negative relationships with ozone (Heathlands and Bogs). Further work is required to understand better the outcomes of this gradient approach.

Policy implications Quantifying impacts of pollutants, including ozone, in a form that allows direct comparison with effects of other drivers of change will provide an invaluable tool for policy makers. For this contract, it has been possible to quantify impacts on three ecosystem services, with two taken through to economic valuation (C sequestration and forage quality). Both suggest that losses will increase in the future as the ozone profile changes. Further experimental and modelling work is needed to expand the economic valuation to other services.

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9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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