DETECTING THE $\delta^{15}N$ SIGNAL FROM AGRICULTURAL POINT SOURCES OF AMMONIA

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It has been established that agriculture contributes almost 90% of the anthropogenic ammonia (NH_3) emissions over Europe. This project aims to examine the spatial distribution of NH_3 deposition derived from intensive animal farms, concentrating on pig and chicken units around North Yorkshire. Modelling work can assist in determining the affected area in a particular ecosystem however, to attribute NH_3 to a specific point source, background signals must be separated from those derived from individual animal units. Naturally occurring isotopic ratios may be used to achieve this, based on the hypothesis that deposition signals from an animal point source can be identified from background values due to their differing $\delta^{15}N$ signatures. These values arise from small differences in the distribution of the stable isotopes ^{15}N and ^{14}N. Several studies have been undertaken to determine a suitable sampler to provide enough material for isotope ratio mass spectrometry (IRMS), and a device has been constructed which allows increased capture potential than most other passive samplers.

Current field trials have utilised this experimental passive sampler in an NH_3 release site run by CEH Edinburgh. Sensors placed in a transect downwind of the source show good agreement between $\delta^{15}N$ values and NH_3 concentration data. A second campaign has been implemented at Bishop Burton agricultural college, where $\delta^{15}N$ signals have been monitored in atmospheric NH_3, pig feed, manure, hair and surrounding vegetation to trace the path of this signal. Initial results indicate uniformity in the atmospheric $\delta^{15}N$ signal throughout the various stages of animal production and in the pig manure, hair and feed. Changes in $\delta^{15}N$ signals along the various transects away from the unit also correlate with NH_3 concentration data.

Several studies have also looked at the use of *Calluna Vulgaris* as a bio-monitor for N deposition. Results from the open top chambers (OTC's) at CEH Edinburgh show good correlation between deposition and $\delta^{15}N$ values. These values can also be linked to source signal and percent N in shoot tissue. Testing has currently moved to the field site at Whim Bog to examine this effect on a totally natural environment.

MODELLING THE WET AND DRY INPUTS OF REDUCED, OXIDISED AND ORGANIC FORMS OF NITROGEN TO A TEMPERATE WOODLAND SYSTEM

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The atmospheric deposition of nitrogen has been studied at Rothamsted for a number of years, with much of the work focused on the calculation of inputs and the exceedance of critical loads.

Data from the monitoring of weather variables, along with reduced, oxidised and organic forms of N to an area of natural regeneration (Geescroft Wilderness) over the last 10 years were used to produce empirical relationships. These have been used to predict the wet and dry deposition of these forms of N to the tree canopy and woodland floor. The model relationships were tested on measured values from Knott Wood, a woodland area about 1km away, and on literature data sets from temperate woodlands.

Annual mean measured and modelled deposition of NO_2-N to the tree canopy were the same at 10.60 kg ha^{-1} yr^{-1}. Deposition to the woodland floor was 2.44 kg ha^{-1} yr^{-1} for the measured and for the modelled, 2.38 kg ha^{-1} yr^{-1}.

The mean annual wet deposition model to the tree canopy gave accumulated depositions for measured and modelled data of 4.24 and 3.65 kg ha^{-1} yr^{-1} for NH_4-N and 3.15 and 3.11 kg ha^{-1} yr^{-1} for NO_3-N. The DON-N data set was shorter, but gave annual depositions, for the measured and modelled, of 1.39 and 1.75 kg ha^{-1} yr^{-1}, respectively.

The mean annual measured accumulated deposition of NH_4-N in throughfall over the period of study (2.5 years), was 5.49 kg ha^{-1} yr^{-1} in good agreement with the modelled results of 5.07 kg ha^{-1} yr^{-1}. Measured mean annual NO_3-N deposition was 3.66 kg ha^{-1} yr^{-1} compared with 3.45 kg ha^{-1} yr^{-1} from the modelled data. The accumulated annual mean of DON-N from the measured results was 3.45 kg ha^{-1} yr^{-1} and from the modelled results 3.75 kg ha^{-1} yr^{-1}. Overall, the total mean annual measured N deposition in throughfall was 12.61 kg ha^{-1} yr^{-1}, compared to 12.25 kg ha^{-1} yr^{-1} from the modelled deposition.

Conclusions drawn from the predictions of the model of future deposition can be made from simple model parameters. It shows that amounts of N above the critical loads are deposited to the woodland site. These calculated accumulations will be used to predict changes in the groundcover species, as early indicators of the enhanced deposition of N.

WINTER-INJURY IN NITROGEN-POLLUTED HEATHER *(CALLUNA VULGARIS)*

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Elevated nitrogen supply is believed to reduce frost tolerance in higher plants, but the response is complex, with some cases of improved tolerance reported, particularly in early-winter. Field observations suggest that, in general, cold-related damage to heather (*Calluna vulgaris*) in upland regions occurs mainly in late-winter and is not simply a result of direct frost-damage but is due to a particular combination of climatic factors and phenology. Our observations in a nitrogen addition experiment on heather moorland in north Wales have shown that late winter-injury of this type was greatest in nitrogen treated heather, when compared with water-fed control plants. The injury became obvious during spring in several years but the relationships with environmental conditions and the underlying plant physiological events were never satisfactorily established. The work described here analyses in greater detail several possible components of winter injury in *Calluna*. These include the relationship between visible injury, bud burst and frost hardiness, wintertime shoot water relations, and the possible influence of high light in combination with cold, as a major cause of physiological stress and visible damage.

THE EFFECT OF NITROGEN ON HEATHLAND SYSTEMS

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Since the 1850s, scientists have investigated the biological and physico-chemical properties of nitrogen (N) and much is now known of its cycle through ecosystems and its oxidation/reduction status under varying environmental conditions. However, the prediction of the impact of N on ecosystems when it accumulates above natural levels is still the focus of much scientific attention.

In recent years, strong evidence has emerged showing that enhanced rates of nitrogen deposition throughout Europe have resulted in nutrient poor, semi-natural ecosystems, such as lowland heath, becoming less limited by nitrogen. Lowland heath has been described by English Nature as a priority habitat for conservation. Observations have shown that heathland is one of the fastest declining habitats in the UK and Europe. The UK currently has approximately 58,000 ha of lowland heath, which accounts for 20% of the international total of this habitat.

The current research aims to determine the effects and fate of deposited nitrogen on a heathland system with particular reference to the effects on nutrient cycling and the soil microbial community. This is being achieved by applying new approaches to a long term manipulation study at Thursley Common, Surrey. The initial experiment of four replicate blocks was set up in 1989, and four additional replicate blocks were added in 1998. At present there are two ongoing experiments; 1) nitrogen additions following four different management regimes and 2) ecosystem recovery, with no N addition.

We present results to date, indicating that the composition of the soil microbial community has been significantly altered by both nitrogen addition and habitat management. In particular, plots receiving ongoing nitrogen additions showed a significant effect of nitrogen on both the number of culturable fungal communities and the bacteria : fungi ratio. The persistence of effects of nitrogen deposition is clearly shown in the recovery plots, as effects of both N addition and management are still apparent within the microbial community some seven years after applications of N ceased.

THE EFFECTS OF URBAN AIR POLLUTION ON HERBACEOUS PLANT SPECIES

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Road traffic is a major source of air pollution in this country, particularly in urban areas. Motor vehicles emit a complex mixture of gases including particulates, nitric oxide (NO), nitrogen dioxide (NO_2) and volatile organic compounds (VOCs), leading to elevated levels of these gases in towns and cities.

Work presented at previous conferences has demonstrated measurable effects on herbaceous plants of exposure to a simulated urban pollution climate. Further work has been carried out to assess the relative roles of two of the most important components of traffic pollution, NO and NO_2. We present results from a fumigation study aimed at evaluating the relative toxicities of these pollutants, singly and in combination.

Seven herbaceous plant species were exposed to either charcoal-filter air, 150ppb NO, 150ppb NO_2 or 75ppb NO & 75ppb NO_2, for 7 weeks. Measurements of nitrate reductase activity, plant growth and plant physiology were carried out. The results are discussed with reference to other plant exposure experiments and current critical levels legislation.

EFFECTS OF SIMULATED POLLUTANT N DEPOSITION AND PHOSPHORUS LIMITATION ON ROOT-SURFACE PHOSPHATASE ACTIVITIES OF THREE PLANT FUNCTIONAL TYPES OF A CALCAREOUS GRASSLAND

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N enrichment of nutrient limited systems can result in N saturation and a shift from N to phosphorus (P) as the limiting nutrient in plant productivity. Such P limitation has been observed in a Peak District calcareous grassland subjected to long term simulated enhanced N deposition. Root-surface phosphatase enzymes (which catalyse mineralisation of organic P) may play an important role in plant P supply and therefore P limitation of N enriched systems. However, the effects of N enrichment on root-surface phosphatase activities and consequences for plant P uptake are poorly understood. This study was therefore undertaken to determine the effects of long-term enhanced N deposition on root-surface phosphatase activities of three calcareous grassland plants of differing functional type.

Bioassay seedlings of *Carex flacca* (sedge), *Koeleria macrantha* (grass) and *Leontodon hispidus* (forb), were grown in turfs removed from experimental plots of a Peak District calcareous grassland. The experimental plots had previously received eight years of simulated pollutant N deposition (ambient deposition +0, +3.5 and +14 g N m^{-2} yr^{-1}) with and without additional P at 3.5 g P m^{-2} yr^{-1} (to reduce P limitation). Seedlings were harvested after 14 and 28 days and root-surface phosphatase activities, shoot N and P, and soil extractable N and P were determined. Phosphatase activities were greatest in *Koeleria* being approximately two fold greater than in *Carex* and five fold greater than in *Leontodon*. N enrichment increased root-surface phosphatase activities of all three species at both harvest dates. In all species, both the +3.5 and + 14 g N m^{-2} yr^{-1} treatments induced similar responses in doubling phosphatase activities (compared to control levels). By the day 28 harvest, phosphatase activities followed a more N dose-dependant trend with phosphatase activities being greatest under the +14 g N treatment and intermediate under the +3.5 g N treatment. Phosphorus addition reduced phosphatase activities across all levels of N treatment in both Carex (-50%) and *Koeleria* (-65%). Phosphatase activities of *Leontodon*, however, appeared unresponsive to P addition. Changes in root-surface phosphatase activities in response to N and P enrichment will be discussed in the context of plant P uptake and P limitation.

DEGRADATION OF MONTANE MOSS HEATH DUE TO NITROGEN POLLUTION

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N deposition has been shown to cause degradation of montane moss heath in Britain. An experiment in NE Scotland, now entering its 6th year, is investigating the effect of long-term N additions on *Racomitrium lanuginosum* and the mechanisms driving a reduction in its performance and survival within the moss heath. Permanent plots have been sprayed with KNO_3 or NH_4Cl solutions at doses equivalent to 10 kg N ha^{-1} yr^{-1} and 40 kg N ha^{-1} yr^{-1} over 6 applications each summer. Control plots were sprayed with distilled water.

A significant loss of *Racomitrium* cover was found with increasing N dose, coupled with an increase in the proportion of dead shoots. At high dosage, effects of NH_4^+ were more detrimental than those of NO_3^-. Measurements were also made of nitrate reductase activity and membrane potassium leakage from the *Racomitrium*. Results indicated that the critical load of the moss was exceeded by additions of 10 kg N ha^{-1} yr^{-1}, and that physiological damage had occurred, demonstrating a direct toxic effect of the N additions.

The effect of grazers at the site were also investigated, using sheep and mountain hare exclosures factorial with N addition. A negative effect of grazing on moss growth in addition to the detrimental effect of N deposition was observed after 2 years.

The differential toxicity of the two forms of N (NH_4^+ and NO_3^-) suggests that the predominant ion type in deposition should be taken into consideration when critical loads are set. It is also important to consider any amplification of N deposition effects by grazers.

IMPACTS OF NH_4^+ AND NH_3 ON *CALLUNA VULGARIS* WATER LOSS AND PHOTOSYNTHESIS

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The impacts of two elevated forms of Atmospheric Nitrogen (NH_4^+ and NH_3) on *Calluna vulgaris* were investigated using open top chambers (OTC's) during March 2003. Each OTC had been treated over a five-year period with either increasing concentrations of NH_4Cl (equivalent to 8, 16, 32, 64, 128 kg $ha^{-1}y^{-1}$), increasing concentrations of gaseous NH_3 (equivalent to 6, 20, 50, 90 µg m^3), or deionised water (control chambers).

Effects of these increased atmospheric N forms on photosynthesis, transpiration, and stomatal conductance were measured using a Handy Pea meter (Chlorophyll Fluorescence) and an LC Pro. Changes in chlorophyll content were also measured. Alterations in the physiological mechanisms of *C. vulgaris* may play an important role in the susceptibility of this plant to environmental stresses such as winter desiccation, and its ability to recover from this, via effects on water conservation. Current work, being carried out at the Whim Moss field site will address whether the effects observed in this study are alleviated by Potassium and Phosphorous additions to the system.

DIAGNOSTIC INDICATORS OF NITROGEN CRITICAL LOAD EXCEEDENCE ON MOORLANDS

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The setting of critical loads for N deposition in moorlands is not based on such clear guidelines nor on such widely tested soil-based data as those used for forests[1], moreover the consequences of exceeding the critical load for moorlands are not clear[2]. This study aims to investigate the fate of long-term increased N deposition on moorland plots through the chemistry of leachate taken from under the organic (mor) layer and the mineral layer respectively. It aims to explore the relationships between N inputs and outputs, well established in the context of UK forests, as well as nitrogen and phosphorus transformation processes governing these relationships, and so broaden and inform current methods for setting critical loads for moorlands. Other potential biotic indicators are reviewed.

The work was carried out on a *Calluna* dominated upland moor using the long-running field manipulation experiment at Ruabon, North Wales[3,4]. These plots have experienced monthly application of ammonium nitrate sprayed on at rates of 0, 40, 80 and 120 kg N ha^{-1} yr^{-1} since 1989.

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ATMOSPHERIC POLLUTION: ALL AT SEA

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Alteration of atmospheric composition by man's activities can influence marine biota in a number of ways. Increased levels of atmospheric combined nitrogen can be deposited in surface ocean waters even at large distances from land, and act as a fertilizer for nitrogen-limited phytoplankton growth in the oligotrophic ocean. The large pH buffering capacity of the oceans means the acidifying effects of NH_y (after nitrification) and NO_x (and SO_2) are not significant in the ocean. Changes in land use, and especially desertification, can increase dust input to the ocean, and significantly increase the availability of iron to phytoplankton. As well as increasing photosynthesis in 'high nutrient (N, P) – low chlorophyll' parts of the ocean, the iron inputs can also increase the rate of biological nitrogen fixation in parts of the ocean where low availability of combined nitrogen means that added iron does not directly stimulate photosynthesis by non-diazotrophs.

Less direct changes in the composition of the atmosphere above oceans by man's activities come from riverine input of plant nutrients which stimulate the growth of algae. Gaseous products of such blooms include dimethylsulfide (from, for example, *Phaeocystis* in the North Sea) which, by atmospheric oxidation to (ultimately) SO_2 and H_2SO_4 can impact on poorly pH-buffered communities on nearby land. *Phaeocystis* also produces surfactants which could facilitate sea to atmosphere transfer of microbes in aerosols and be involved in dispersal.

These atmospheric effects on, and of oceanic biota, involve biota which contribute almost half of global net primary productivity, and deserve further study.

AIR QUALITY – THE POLICY PERSPECTIVE

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The sources of air pollution are many and varied, ranging from industrial, agricultural and road transport to domestic and natural sources. It is recognised that air pollution can adversely affect health, materials and vegetation, and its transboundary nature calls for international action to curb emissions. Good progress is being made. For example, UK emissions of sulphur dioxide have been cut by 80% since their peak in the 1970s, and emissions of nitrogen oxides have nearly halved since the early 1990s, bringing significant improvements in air quality.

Reducing the adverse effects of air pollution on ecosystems continues to be a major driver of international agreements on emission reductions. The EC National Emissions Ceilings Directive (NECD) and the UNECE Gothenburg Protocol recently set emission targets for sulphur dioxide, nitrogen oxides, ammonia and volatile organic compounds, to be achieved from 2010.

The National Expert Group on Transboundary Air Pollution's recent report demonstrated that levels of sulphur dioxide and acid rain are generally decreasing and damaged ecosystems are showing signs of recovery. However, nitrogen deposition and ground-level ozone will continue to threaten some areas, and combating these problems will require further concerted international efforts, such as in the forthcoming review of the NECD and Gothenburg Protocol.

CRITICAL LEVELS FOR OZONE AND CRITICAL LOADS FOR NITROGEN – AN OVERVIEW OF RECENT DEVELOPMENTS

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Two UN/ECE workshops held during 2002 have resulted in significant changes to critical levels for effects of ozone and critical loads for effects of nutrient nitrogen on vegetation. In the case of ozone, changes have been made to the methods used to calculate AOT40-based critical levels and to map their exceedance, and flux-based critical levels have been defined for the first time for wheat and potato. In the case of nutrient nitrogen, critical load ranges have been defined for the first time for some communities, and significant changes have been made to critical load ranges for grasslands and other communities. Mapping of critical loads for nutrient nitrogen is now based on the EUNIS classification. We will discuss the implications of these changes for risk assessment and for research priorities in the UK.

THE CEH O3GRASSLAND PROJECT: IMPACTS OF OZONE ON GRASS:CLOVER MIXTURES

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Ozone exposure experiments were conducted at CEH Bangor in 2002 as part of the CEH O3GRASLAND project. The aim was to provide information on the growth and physiological responses of the component species of grassland to ozone, for use in mechanistic models developed by Marcel van Oijen and colleagues at CEH Edinburgh (see separate abstracts).

Plants of perennial rye grass (*Lolium perenne*) and white clover (*Trifolium repens*) from the grassland site adjacent to CEH Edinburgh that was used for flux measurements, were sent to CEH Bangor several months before the start of the exposure experiment. Clonal material from these parent plants was grown on into sufficient individual plants for use in solardome exposure experiments. The plants were transplanted to large containers of size 35 x 45 x 25cm deep of commercial compost at a density of 12 plants per container. Three containers each of the following combinations were placed in each of four solardomes: all clover, all perennial rye grass, or 4 clover plants on the inside, surrounded by 8 outer rye grass plants. Two solardomes were ventilated with filtered air to which 30 ppb of ozone was added, and two were ventilated with the following 7 day episodic ozone regime for 12 weeks: 7h per day at 80 ppb for day 1, at 100 ppb for days 2 and 3, and 80 ppb for day 4; 30 ppb of ozone was added at all other times. The episodic ozone treatment amounted to an AOT40 of 21.9 ppm.h over the 12 weeks.

Six weeks after the start of exposure, all of the plants in the containers were cut to 7 cm above soil level. Sixty nine percent of the clover leaves per pot showed visible injury in the high ozone treatment compared to 0.5% in the background ozone treatment. The leaves of *Lolium* showed a significant increase in senescence in the high ozone treatment compared to the background treatment, but there was not a significant ozone effect on total dry weight or leaf dry weight for this species. At the final harvest, the canopy was divided into layers: significant effects of ozone on clover occurred in the uppermost layers of the canopy, but not in the lowest layer; no significant effects on the dry weight of *Lolium* were found even though the canopy was visibly and quantifiably more senescent. Results of physiological measurements such as stomatal conductance measurements, A-Ci response curves and C:N ratios for the leaves will also be presented.

THE CEH O3GRASSLAND PROJECT: MODELLING OZONE FLUXES & DAMAGE

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Ozone damages plants in various ways. We can distinguish four damage mechanisms: (1) disrupting photosynthesis, (2) increasing respiration, (3) accelerating senescence, and (4) altering root-shoot allocation. Damage depends on the ozone deposition rate and how it is distributed in time, but it also depends on the plant species, it's physiological state, and the environmental conditions. Because of these interactions, it is desirable to quantify damage under field conditions, particularly if the goal is to predict when real vegetation is at risk from ambient ozone. However, the four damage mechanisms can only be quantified well under controlled conditions. An instrument for scaling up leaf-scale data measured under controlled conditions to the field-scale would thus be useful for predicting ozone damage.

In this project (O3GRASSLAND), the solardomes at CEH-Bangor were used for detailed measurements of damage caused by ozone to grass (*Lolium perenne*) and clover (*Trifolium repens*). The plants had been cloned from material taken from a grassland field near CEH-Edinburgh. At this latter field site, measurements of fluxes between vegetation and atmosphere of O_3, CO_2 and H_2O were made by the eddy covariance method. Vegetation growth rate was also measured at this field site.

A simulation model that accounts for the four damage mechanisms triggered by ozone was constructed and applied to the field site. Parameters of the model were taken from the solardome experiments. We will report the accuracy with which the model was able to simulate the fluxes of O_3, CO_2 and H_2O in the field during two consecutive years. An analysis is presented of the conditions identified by the model as conducive to high ozone risk.

THE CEH O3GRASSLAND PROJECT: FLUX MEASUREMENTS AT THE FIELD SCALE

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Ozone damages plants in various ways, which depend on the ozone deposition rate and how it is distributed in time, as well as the plant species, it's physiological state, and the environmental conditions. Because of these interactions, it is desirable to quantify damage under field conditions, particularly if the goal is to predict when real vegetation is at risk from ambient ozone. However, damage mechanisms can only be accurately quantified under controlled conditions. A model that scales up leaf-scale data measured under controlled conditions to the field-scale would thus be useful for predicting ozone damage. The aim of the O3GRASSLAND project is to develop such a model. Measurements under controlled conditions (in the Solar Domes at CEH Bangor, see other abstracts) are used to parameterise the model and measurements at the field-scale to test it.

Field-scale measurements of fluxes between vegetation and atmosphere of O_3, CO_2 and H_2O were made at a site grassland site, near CEH Edinburgh, during the 2001 and 2002 growing seasons. The grass is cut for silage twice during the season and so 3 periods of canopy growth were observed each year. The H_2O flux results are used to estimate the bulk-canopy stomatal conductance and hence the proportion of the ozone flux that is taken up by the vegetation through its stomata or onto other plant surfaces and the soil. The results show the percentage of the total ozone flux that is stomatal increases from ~20% to ~60% over a growth period. On average the stomatal component is only about 30% of the total flux and the flux is dominated by non-stomatal uptake. An overview of results for each year will be given and the factors controlling non-stomatal uptake examined.

MONITORING OZONE IMPACT ON VEGETATION IN GERMAN MIDLANDS: AN ANN-BASED FLUX-RESPONSE MODEL FOR WHITE CLOVER

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In collaboration with the UNECE ICP Vegetation (International Cooperative programme on effects of air pollution on vegetation and crops) field experiments with two white clover (*Trifolium repens*) clones differing in ozone sensitivity were conducted at three sites in the Trier region (Rhineland-Palatinate, Germany) in 1998-2001. The aim was to develop an ozone flux model for the Trier region based on physiological and microclimatic parameters in order to determine and locate potential ozone-induced damage to vegetation.

20 plants of both NC-S and NC-R clones of white clover (*Tifolium repens* cv. Regal) were exposed to ambient air according to a standard protocol developed by the Coordination Centre of the ICP Vegetation at three sites (Deuselbach, Trier-University and Trier-City) differing in altitude and ozone profile. During each experimental period of about five months foliage damage was assessed every three to four days and plants were cut every 28 days to a height of 7 cm in order to determine biomass. Stomatal conductance measurements were conducted on fully developed leaves (4^{th} leaf from tip of stolon) on sunny and overcast days using a mobile porometer (WALZ, Germany).

Artificial Neural Networks (ANN) were chosen to model stomatal ozone uptake. They were trained using backpropagation algorithms with multiple hidden layers and varying number of hidden neurons with different activation functions (Neuroshell-2, Ward Systems Ltd.). The parameters air temperature (°C), vapour pressure deficit of air (VPD, kPa), photosynthetic active radiation (PAR, µmol m^{-2} s^{-1}) ozone concentration (ppb), day of year (DOY), daytime and genotype (NC-S- or NC-R-clone) were included as inputs, whereas the stomatal conductance was the output. Eventually, the model was applied to data from different weather stations located in the Trier region and maps of spatial distribution of ozone fluxes were created using ArcView 3.2 (ESRI).

In all years the NC-S clone showed higher visible foliar injury than the NC-R clone. Plants exposed in Deuselbach, the site with the highest AOT40, always showed most severe visible injury. However, the relationship between AOT40 and amount of visible injury was poor as was the relationship between AOT40 and NCS/NC-R biomass ratio. Replacing the AOT40 with ozone fluxes slightly improved these relationships leaving space to include further parameters (ozone concentration at plant canopy height, detoxification potential, soil moisture deficit) to optimize the model.

Nevertheless, a comparison of fluxes calculated with measured and modelled stomatal conductance clearly indicated the ability of the model to predict ozone fluxes. Furthermore, the results showed that the model is applicable to meteorological data available on half-hour or hour scale only, thus forming the basis for spatial distribution maps of ozone fluxes as an indication of enhanced risk to vegetation by ozone.

BIOSTRESS: EMPLOYING OZONE TO INVESTIGATE WHETHER THE IMPACTS OF EARLY SEASON STRESS ARE AMPLIFIED BY COMPETITION?

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Troposhperic ozone (O_3) is regarded as the most widespread pollutant to which plants are exposed. It is known that many regions of Europe already experiencing episodes that exceed UNECE critical level guidelines for the protection of sensitive vegetation. Modelled forecasts predict that O_3 levels are likely to continue rising by *c.* 1-2% per annum for the foreseeable future. Although, adverse effects of the air pollutant on agricultural/horticultural crops are well documented, little is known about the impacts imposed on more complex communities, with virtually nothing about the way in which the rising levels of this ubiquitous air pollutant are likely to alter species diversity within natural ecosystems.

Three years ago, an **EU-FP5** project, **BIOSTRESS**, was initiated to investigate (i) the role played by tropospheric O_3 in driving shifts in the diversity of natural/semi-natural plant communities and (ii) the importance of the timing of stress events

Studies undertaken at Newcastle during the third (and final) year of the BIOSTRESS project will be presented. Data reveal substantial differences in ozone exposure-response relationships dependent on the nature of competitive restraints.

EXAMINING THE ROLE OF CELL WALL-LOCALIZED ASCORBATE IN OZONE DETOXIFICATION

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Ozone (O_3) is a phytotoxic gaseous air pollutant that enters the plant through the stomates, dissolves in the extracellular matrix (leaf apoplast) and imposes a potentially damaging oxidative burden on the plasmalemma. There is growing evidence that vitamin C (ascorbate), localized in leaf cell walls, is capable of intercepting and detoxifying a significant proportion of the ozone flux entering vegetation under environmentally-relevant conditions.

We will present findings from studies performed on new tobacco lines in which it has proved possible to manipulate the size of the ascorbate pool in the leaf apoplast through not only the over-expression of ascorbate oxidase (AO) in the leaf cell walls (*sensu* Sanmartin *et al*. *Planta* 2003), but also the under-expression of this poorly understood enzyme. The reaction of these engineered plants to acute and chronic O_3 fumigation will be reported and the role of cell wall-localized ascorbate in ozone scavenging discussed.

EXPLORING THE ROLE OF OZONE-INDUCED OXIDATIVE STRESS IN THE INDUCTION OF CAM IN *MESEMBRYANTHEMUM CRYSTALLINUM*

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In recent years, the model halophyte *Mesembryanthemum crystallinum* has come under increased scrutiny and now represents the best studied CAM plant at both biochemical and molecular level. A sodium accumulator, *M.crystallinum* exhibits a rapid switch from C_3 photosynthesis into CAM in response to high salinity, preceded by an up regulation of key genes and proteins in the CAM pathway, including Imt leading to the production of cyclitols and PEPc, the enzyme of nocturnal phosphorylation.

Our investigations focus on the signals and constraints underpinning this C_3-CAM transition. Our starting hypothesis is that oxidative stress at the cellular level triggers this well-studied switch in terms of salt and drought stress. Exposure to the atmospheric pollutant ozone (O_3) is a novel way to impose oxidative stress and constitutes a useful tool to explore signalling and mechanistic aspects of the C_3-CAM switch. We have examined metabolites and proteins, as well as gene expression of key components of the CAM pathway. Evidence from this study shows up regulation of both Imt and PEPc in response to ozone, but no switch into CAM was observed. This suggests that there is some other constraint on the system preventing the full CAM pathway from being implemented.

To look at these constraints, a recently isolated putative null CAM mutant of *Mesembryanthemum crystallinum*, identified by its failure to conduct nocturnal acidification provided an ideal system for comparative study and to further understand plant structure, development and metabolism. Similar analysis of this mutant line suggests that leaf starch content is a key limiting factor, both for the mutant line and for wild-type plants subjected to ozone.

SETTING VISIBLE SYMPTOMS OF "OZONE INJURY" IN AN ECOLOGICAL CONTEXT

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Visible foliar symptoms of ozone injury are commonly employed to identify 'sensitive' genotypes and/or ozone 'damage' in both an ecological and molecular genetics context. Indeed, molecular biologists employ screening programmes based upon visible symptoms to identify prospective genes determining 'ozone sensitivity'. We report on a study that questions the validity of such screening programmes and has endeavoured to assess the meaningfulness of visible injury in an ecological and agricultural context.

A range of *Arabidopsis thaliana* mutants donated by colleagues in Japan and America were exposed to either long-term 'low' ozone concentrations (chronic exposure) or short- term 'high' ozone concentrations (acute exposure). Stomatal conductance, relative growth rates, fecundity and visible symptoms were recorded. A key questions to be addressed will be: Are visible symptoms a reliable indication of effects on growth and fecundity?

EFFECT OF O_3 ON MALAYSIAN CROPS

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Awaiting abstract

HORMONAL CHANGES IN FOREST TREES UNDER ATMOSPHERIC POLLUTION

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Cytokinin levels in trees growing on polluted sites as well as trees exposed experimentally to excess influx of nitrogenous compounds have been shown to contain increased levels of the growth regulator cytokinin. As an experimental system to study effects of elevated cytokinin levels in trees, transgenic poplar hybrids carrying a bacterial or Arabidopsis derived *ipt* gene – a gene which codes for an enzyme catalysing a key step in cytokinin biosynthesis - were generated. These plants showed elevated cytokinin levels and a distinctively changed phenotype.

As basis for the study of changes in the expression of genes linked to primary metabolism, homologues of genes coding for nitrate reductase, glutamine synthethase and sucrose synthase were cloned from poplar. To investigate whether cytokinin signalling in trees is based on a two-component signalling system of histidine kinase-cytokinin receptor and response regulators as described for Arabidopsis, maize and bacteria, the homologs of genes coding for these proteins were cloned from poplar. Expression levels of these genes in wildtype and transgenic plants and plants treated with nitrate are being studied by Northern blotting and semi-quantitative pcr. The data are expected to contribute to our understanding of the significance of changed hormone levels in trees exposed to air pollution.

CRITICAL LOADS OF NITROGEN IN SAND DUNES – ARE THEY BEING EXCEEDED

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Eleven sand dune sites in England and Wales were chosen with a range of atmospheric N inputs, in order to test for possible critical load exceedance. Each site was surveyed for a range of soil, vegetation and groundwater parameters. Survey locations at each site incorporated the dominant successional habitats of mobile, semi-fixed, fixed dunes, dune grasslands and dune slacks where possible.

Significant correlations of N inputs with a range of parameters were found. These parameters included biomass in mobile dunes; species diversity, soil C:N ratio and available inorganic N in the fixed dune grasslands. Other near-significant correlations with DON in the groundwater and sward height in the mobile dunes and the fixed dune grasslands were observed.

LEAF ZINC AS AN ENVIRONMENTAL INDICATOR OF TRAFFIC POLLUTION

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Analysis of *Sambucus nigra* and *Crataegus monogyna* leaves sampled along a transect adjacent to the busy A1M showed that zinc content was highest in those leaves collected close to the motorway. This difference was attributed to emissions from car engine and mechanical wear. Zinc concentration was shown to negatively correlate with rainfall suggesting that the majority of the zinc was loosely bound to external leaf cuticle surfaces. Leaves of *Sambucus nigra* and *Crataegus monogyna* did not contain equal amounts of zinc. Differences in cuticle structure and composition may have been responsible for this difference. A mixture of biochemical and electron microscope analyses were employed to investigate the retention of leaf surface zinc.

MOSSES AS INDICATORS OF TRAFFIC POLLUTION – THE RISE OF ZINC AND FALL OF LEAD

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Mosses have been collected and sampled from transects next to busy roads in urban and rural areas. The moss tissues were measured for Pb and Zn content. Zinc content was found to range from background amounts of 20-40 µg g^{-1} DWt in mosses from traffic-free rural areas, to over 1000 µg g^{-1} DWt in mosses collected from roadsides in urban areas. Lead was also higher in some urban compared to rural areas suggesting recalcitrant amounts of lead can still be detected, despite the use of lead-free fuel. We also present repeat transect surveys from the 1980's compared to 2002 and show that soil and moss Pb concentrations still remain high in some urban areas. However, the trend is for a decline in Pb and rise in Zn that is associated with increasing traffic density. As Zn is largely produced from mechanical wear rather than from exhaust emissions it is a useful marker for changes in traffic density or use.