

# **CAPER 2006**

## EDINBURGH, POLLOCK HALLS

31<sup>st</sup> Annual Meeting 10<sup>th</sup>-12<sup>th</sup> April 2006

## **Book of Abstracts**

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## **CAPER 2006**

### COMMITTEE ON AIR POLLUTION EFFECTS RESEARCH CEH Edinburgh and Pollock Halls, Edinburgh University 10<sup>th</sup> – 12<sup>th</sup> April 2006

### Programme

#### Afternoon Programme Monday 10<sup>th</sup> April Venue CEH Edinburgh

#### OPTIONS

- 14.00 Welcome by *Neil Cape* 
  - 1. Visit to Whim Bog N manipulation experiment on an ombrotrophic bog.

Or

2. Overview of the NITROEUROPE Project - Eiko Nemitz

Low cost pollution monitoring using the delta system - Sim Tang

Introduction to the EMEP Super Site at Auchencorth Moss - Neil Cape

There will be an opportunity to talk with staff making flux measurements of  $NH_3$ ,  $CH_4$ ,  $O_3$  and aerosols using micrometeorology and also to look at the equipment.

PP systems will be on site to demonstrate their equipment for measuring  $CO_2$  exchange.

- 16.00 **TEA**
- 16.30 David Fowler: Changes in N concentration and deposition in the UK 1986-2006 and a brief overview of heavy metal deposition in the UK
- 17.10 *Neil Cape*: Quantifying NH<sub>4</sub> N inputs to vegetation
- 18.30 HOT BUFFET
- 19.30 Poster Pub
- 21.00 Depart for Pollock Halls

### Tuesday 11<sup>th</sup> April St Trinians Room, Pollock Halls

Breakfast from 7.30am John McIntyre Centre Dining Room

	Session Chair: Colin Gillespie	- Policy Issues - Introduction
8.55	Scott Mathieson (SEPA)	Air pollution science; needs of the UK environmental regulators
09.20	Bill Bealey (CEH Edinburgh)	Application of the SCAIL model to support environmental regulation
09.40	Rob Kinnersley (Environment Agency)	Practical science for environmental protection – evolving towards outcome- based regulation
10.00	Simon Bareham (CCW)	Assessing the impacts of air pollution on nature conservation in the UK
10.20	Jill Edmondson (MMU)	Indicators of nitrogen pollution on heather moorland from long term experiments to regional surveys
10.40	COFFEE	
	Session Chair: Simon Caporn	- N effects in the Field
11.10	Jacky Carroll (MMU)	Recovery of ombrotrophic Sphagnum species in relation to air pollution in the Southern Pennines
11.30	Andrea Britton (Macaulay Institute)	The effect of nitrogen deposition and management on the structure and function of low-alpine heathland
11.50	Laurence Jones (CEH Bangor)	Does atmospheric nitrogen contribute significantly to the nutrient budget in a South Wales sand dune?
12.10	Elleke van Zetten and Irene de Lange (Radboud University, Nijmegen, Netherlands)	Effects of dry and wet N deposition on vegetation and biogeochemistry of an ombrotrophic bog

12.30	Gareth Phoenix (Sheffield University)	Nitrogen dynamics in grasslands: indirect effects of species change are greater than direct effects of N deposition
12.50	LUNCH	Will be held in the <b>Nelson Room</b>
	Session Chair: Sally Power	
14.00	Odhran O'Sullivan (Sheffield University)	Cation depletion by long-term pollutant N deposition assessed by organic acid extractants
14.20	Leon van den Berg (University of York)	Dissolved Organic Carbon (DOC) concentrations and variations in UK soils
14.40	Tony Miller (Rothamsted Research)	Moss tissue structure, pH regulation and sensitivity to atmospheric nitrogen pollution
15.00	John Pearson (UCL)	Can mosses and atmospheric nitrogen pollution provide answers to current controversies in cell pH homeostasis?
15.20	William Purvis (Natural History Museum)	Multi-element composition of historical lichen collections and bark samples, indicators of changing atmospheric conditions
15.40	Ana-Maria Rusu (The Natural History Museum)	Biomonitoring using lichen transplants following industrial closure at Zlatna smelter town, Romania
16.00	ΤΕΑ	
	Session Chair: David Fowler Students at the Macaulay In	- New work from 1 <sup>st</sup> Year PhD stitute and CEH Edinburgh
16.30	Heather Armitage (Macaulay Institute University of Aberdeen)	Assessing the potential for recovery of degraded montane heaths
16.40	Niki Papanikolaou (Macaulay Institute University of Aberdeen)	Impacts of increased nitrogen deposition on carbon and nutrient dynamics in a montane heathland

16.50	Pauline Leterme (University of Aberdeen)	Influence of nitrogen deposition on carbon dynamics in peatlands
17.00	Kerry Dinsmore (CEH Edinburgh)	Atmosphere-soil-stream greenhouse gas fluxes from peatlands
17.10	Comfort break	
17.20	Professor Terry Mansfield	
18.30	DINNER	To be held in the <b>John McIntyre</b> Centre restaurant
20.00	Committee Meeting	

### Wednesday 12<sup>th</sup> April St Trinians Room, Pollock Halls

### Breakfast from 7.30am John McIntyre Centre Dining Room

Session Chair: David Fowler			
09.00	Felicity Hayes (CEH Bangor)	Effects of ozone on a plant community exposed for two consecutive summers	
09.20	Simon Peacock (University of Newcastle)	Studies of the sensitivity to ozone of natural plant species at an individual and community level	
09.40	Gina Mills (CEH Bangor)	Critical levels of ozone for vegetation: an update on international developments	
9.50	Sally Gadsdon (Imperial College London)	Ecosystem health in Epping Forest	
10.10	Becky Keelan (University of York)	The effects of ozone on woodland ground flora	
10.30	Louise Taylor (University of Wales)	Intraspecific ozone sensitivity in Anthoxanthum odoratum (L)	
10.50	COFFEE		
	Session Chair: Anne Borland		
11.20	Jeremy Barnes (University of Newcastle)	Ascorbate-oxidase-dependent changes in the redox state of the apoplast modulate gene transcription leading to modified hormone signalling and defence in tobacco	
11.40	Chris Callaghan (University of Newcastle)	Quantitative trait loci (QTL) governing ozone tolerance and foliar antioxidative properties in Arabidopsis thaliana	
12.00	Chris Malkin (University of Newcastle)	Impacts of ground-level ozone concentrations on the contamination of salad crops by human pathogens	
12. 20	Agnieszka Kaminska (University of Newcastle)	QTLs governing ozone impacts on wheat yield	

12.40	Emma Glen (Manchester Metropolitan University)	A novel technique for reducing soil fertility in ecological restoration projects
13.00	LUNCH	Will be held in the <b>Nelson Room</b>
14.00	Close of Conference	

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## Talk Abstracts

## Air pollution science; needs of the UK environmental regulators

Scott Mathieson<sup>1</sup> and Colin Gillespie<sup>2</sup>

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The UK's environmental regulatory agencies (Environment Agency, SEPA and the Environment and Heritage Service Northern Ireland) are charged with the complex task of implementing a range of environment protection legislation. In making their regulatory decisions, they rely on sound science and must also provide protection to a wide range of internationally and nationally important habitats. In respect of the regulation of air emissions, this is now largely through the implementation of the Pollution Prevention and Control (PPC) regime in the UK. Few of the PPC permitting staff have specific ecological expertise and therefore rely on the input of agency ecology advisors and conservation agency specialists. Even those who do, typically have an aquatic ecology background, while the majority of concerns about potential air pollution impacts relate to terrestrial habitats. Regulators are also being faced with regulating industries new to the air pollution control regime, such as the intensive pig and poultry sector, which brings new challenges in understanding potential impacts.

Accordingly, there is a need to provide risk assessment and modelling tools, such as SCAIL for ammonia emissions, and straightforward, relatively simple-to-use decision support systems, such as the APIS system, that interprets air pollution/ ecological research for use by non-ecological regulatory staff. These systems need to be based on the outputs of a strong research sector. In addition, the strength of environmental regulatory agencies' monitoring science has been in the aquatic realm, reflecting the historic origins of the agencies as water protection authorities. The need to account for and understand ecological impacts associated with licensed emissions to air has exposed the shortcomings of the agencies' terrestrial ecological monitoring capacity in recent years. As such, the agencies are collaborating with research institutes, JNCC and others in developing their capacity to undertake, and understand the results of, terrestrial biomonitoring. The example of SEPA's research and development funding is used to illustrate how the agencies are seeking to pull together these various requirements into coherent programmes of research which feed, ultimately, through to regulatory decisions.

## SCAIL – Simple Calculation of Ammonia Impact Limits

M.R. Theobald, W.J. Bealey and M.A. Sutton

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Emission of ammonia (NH<sub>3</sub>) from agriculture and its subsequent deposition to sensitive ecosystems imposes a major environmental burden both nationally and internationally [Bobbink *et al.*, 1998; Pearce and van der Wal, 2002]. At a local scale the deposition of nitrogen in the form of ammonia can result in eutrophication of sensitive ecosystems and the acidification of the soil. The gradual recognition of these problems has led to  $NH_3$  being included in UNECE and EC emissions abatement agreements for the first time, under the Gothenburg Protocol, the National Emissions Ceilings Directive and the Directive on Integrated Pollution Prevention and Control (IPPC). Under the IPPC Directive, permits are required for pig and poultry systems with more than a certain number of livestock (40,000 places for poultry, 2000 fattening pigs or 750 places for breeding sows). Applications for IPPC permits need to be assessed for their potential impacts on the environment including the impact of  $NH_3$  deposition downwind of the installation.

The SCAIL (Simple Calculation of Ammonia Impact Limits) model was developed by the Centre for Ecology and Hydrology (CEH) to provide a screening model that could help assess applications for IPPC permits. The objective of the model was to screen the applications to determine if there is the possibility of impacts from deposited NH<sub>3</sub>, which would then indicate that more detailed assessment is required.

SCAIL produces an estimate of the  $NH_3$  deposition at a certain distance downwind of the source; the concentration estimate is multiplied by a 'deposition velocity' specific to the habitat of interest, e.g. raised bog. The model also estimates the nitrogen deposition critical load exceedance at the nearest edge of the habitat taking into account the background deposition at that location, and the critical load of the habitat.

Bobbink, R., M. Hornung, and J. G. M. Roelofs (1998), The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation, J. Ecol., 86, 717-738.

Pearce, I. S. K., and R. van der Wal (2002), Effects of nitrogen deposition on growth and survival of montane Racomitrium lanuginosum heath, Biol. Conserv., 104, 83-89.

## Practical science for environmental protection – evolving towards outcome-based regulation

Rob Kinnersley

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Principal Scientist – Air, Environment Agency

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The Environment Agency has statutory duties to protect the environment from adverse influences arising from the industries it regulates. This must be achieved using regulatory measures that are both proportionate to the threat and testable. While physical measurements of air concentrations of air pollutants provide a useful indicator of the likelihood of harm, the ultimate test for the success of regulation in protecting the environment must be whether or not harm takes place, or is effectively reduced by the imposition of regulations. Such an approach takes into account *all* of the pressures on a particular ecosystem or habitat, allowing cumulative and synergistic effects to be accounted for. To this end the concept of "Outcomes-based regulation" is rising to prominence.

For environmental change to be regarded as an 'outcome', it must be tangible (i.e. of significance to the vitality or survival of an ecosystem or one or more of its components), and evidence-based (i.e. with a demonstrated causal link between exposure to the pollutants of concern and the resulting effect). An 'outcome' must also be quantifiable, in that the degree of damage (or recovery) must be assessed in the context of the value of the biota affected, whether in terms of cash value, loss/gain of amenity, or loss/gain of biodiversity.

EA Air Science, in partnership with CEH, has been working to define outcomes-based regulation concepts for air quality. The use of Air Quality Outcomes for regulatory purposes requires techniques that demonstrate such changes over appropriate temporal and spati al scales. We have been exploring the practical use of biological measurements to assess the air-mediated impacts of industrial activity on the surrounding environment. In particular, we have looked at the use of such measurements of the existing flora and fauna in identifying temporal changes caused by changed patterns of emission, such as might occur following the implementation of emission controls.

## Assessing the impacts of air pollution on nature conservation in the UK

Simon Bareham<sup>1</sup> and Zoe Masters<sup>2</sup>

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Air Pollution Lead Co-ordination Network

<sup>1</sup>Senior Pollution and Climate Change Impacts Advisor, Countryside Council for Wales <sup>2</sup>Air Pollution Advisor and Network Officer, Joint Nature Conservation Committee

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Despite large reductions in emissions of the main air pollutants in the UK, substantial areas of semi-natural habitat remain at risk from atmospheric deposition (in particular nitrogen deposition) and elevated concentrations of pollutant gases (such as ozone).

The UK nature conservation agencies are responsible, on behalf of the government, for protecting and monitoring the condition of nationally and internationally important sites for biodiversity. The conservation agencies are statutory consultees under planning and pollution regulation, as well as conservation legislation (e.g. Habitats Regulations 1994 and Countryside Rights of Way Act 2000). At a local level, staff work closely with the environment agencies to assess permits for industrial processes, agricultural units etc that may impact on nature conservation sites, using an approach based on 'critical loads' and 'critical levels'.

The inter-agency Air Pollution Lead Co-ordination Network (LCN) has been set up by the Joint Nature Conservation Committee to carry out its work with respect to air pollution and involves specialist staff from the four country nature conservation agencies and JNCC Support Co. The network provides policy advice to government departments and others, at the UK and European level. For example, advising DEFRA on air quality standards for ecosystem protection in the revision of the UK Air Quality Strategy and leading work to produce a nitrogen deposition indicator, as part of a major pan-European programme (SEBI2010), under the auspices of the Convention on Biological Diversity. The network chair is also a member of the National Expert Group on Transboundary Air Pollution (NEGTAP). In addition to this, the network supplies technical/operational advice to the conservation agencies and manages a small research programme.

This talk will explain in more detail the work of the conservation agencies and Air Pollution LCN in relation to air pollution risk assessment and the need for sound science (a solid evidence base) to underpin this work.

### Indicators of nitrogen pollution on heather moorland from long term experiments to regional surveys

Jill Edmondson, Simon Caporn, Jacky Carroll and Elizabeth Price

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- 1. Semi-natural vegetation systems such as upland moors are adapted to very low levels of nutrient supply, and under natural conditions usually show strong nitrogen limitation. Enhanced nitrogen deposition can alter ecosystem dynamics, resulting in a loss of species diversity, particularly bryophytes and lichens, and in some cases lead to a change in dominant plant species from *Calluna vulgaris* to a grass dominated moor. Bio-indicators of N status of an ecosystem at a site specific level would prove a valuable tool for assessing the impact of enhanced N deposition in an area.
- 2. A long term nitrogen manipulation experiment, established in 1998, on a heather moorland at Ruabon, Wales was used to identify possible bio-indicators of nitrogen pollution. The bio-indicators identified ranged from *C. vulgaris* and *Hypnum jutlandicum* foliar nutrient content, higher and lower plant vegetation survey data and nitrogen storage and enzyme activity within heather litter and peat. These bio-indicators were then used in a regional survey covering moorland areas in Wales, the Peak District and Scotland in spring 2005.
- 3. C. vulgaris and H. jutlandicum foliar N, litter total N and extractable N responded positively to N addition at the Ruabon experiment. Bryophyte and lichen species diversity and cover showed a negative response to N addition, with the addition of phosphorus alleviating the detrimental effects of artificially elevated N levels. Litter phosphatase enzyme activity increased with N addition, indicating increased demand for P as N limitation was reduced.
- 4. The regional survey revealed interesting trends in litter extractable N, foliar nutrient content of *C. vulgaris* and *H. jutlandicum* and bryophyte species diversity. Variation between these possible bio-indicators at a regional scale may provide an insight into the legacy and levels of N pollution these regions have received.

## Recovery of ombrotrophic *Sphagnum* species in relation to air pollution in the Southern Pennines

Jacky Carroll<sup>1</sup>, Simon Caporn<sup>1</sup>, John Lee<sup>2</sup>, Colin Studholme<sup>3</sup>

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The well documented loss of *Sphagnum* species from the ombrotrophic moorlands of the Peak District during the first half of the 20<sup>th</sup> century has generally been attributed to their proximity to major centres of the Industrial Revolution and their very high emissions of atmospheric pollutants, notably sulphur dioxide and its solution products.

From the mid 1970's onwards (Ferguson *et al* 1983, 1984) two *Sphagnum* re-introduction experiments were established at Holme Moss in the southern Pennines, with very limited success. During the 1980's a series of studies was also undertaken of Southern Pennine bog pools and their vegetation (Studholme 1989). The pools were surveyed for the presence of the ombrotrophic species *S. cuspidatum* and all plant species within 100m radius of the pools were also recorded. *S. cuspidatum* was found at only three localities and very few bryophyte species were recorded at any of the survey sites.

The original survey was carried out between 1983-1986, near the end of a period of sharply declining sulphur dioxide concentrations in the surrounding industrial cities, dating back to the 1940's, and levels recorded over the Peak District continued to fall steadily. Atmospheric nitrogen pollution however, increased markedly over the same period and the levels of other pollutants, such as ozone and heavy metals, also remained high. The original transplantation and survey sites were resurveyed in the summer of 2005, with the aim of assessing the level of recovery of *Sphagnum* and other bryophyte species, following the changes in environmental conditions.

Significant, but not dramatic, recovery of both *Sphagnum* and other bryophytes was found over the twenty year period between the surveys, with many key species still poorly represented. The improvement was consistent with the fall in sulphur dioxide concentrations, but also suggested that other limiting factors were involved. Comparisons were therefore made between the levels of a number of compounds in *Sphagnum* tissues and the surrounding bog-waters at Holme Moss and cleaner sites in Wales and Cumbria, in order to assess the possible influence of other aspects of air pollution, on *Sphagnum* recovery. Marked differences between the clean and polluted sites in the levels of nitrogen species and certain metals were still present in 2005 and these could play a part in the continued absence of many of the more sensitive *Sphagnum* species from the moorlands of the southern Pennines.

Ferguson P., Lee JA. (1983). The growth of *Sphagnum* species in the southern Pennines. J.Bryol. **12** 579-586.

Ferguson P., Robinson RN., Press MC., Lee JA. (1984). Element concentrations in five *Sphagnum* species in relation to atmospheric pollution. J.Bryol. **13** 107 -114.

Studholme C. (1989). Isozyme variation, physiology and growth of *Sphagnum cuspidatum* in a polluted environment. PhD thesis. University of Manchester.

### The effect of nitrogen deposition and management on the structure and function of low-alpine heathland

Andrea Britton

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Low-alpine environments in the UK frequently experience high rainfall and prolonged cloud cover resulting in enhanced inputs of nitrogen compared with surrounding low lying areas. Climatic constraints on soil development and nutrient cycling in this environment mean that plant nutrient availability is generally low and the plant communities present have developed under these low nutrient conditions. Although there is relatively little experimental evidence, low alpine plant communities are considered to be potentially sensitive to enhanced nitrogen inputs and this is reflected in a low critical load of 5-15 kg N ha<sup>-1</sup> y<sup>-1</sup>. In this talk we will describe the results of a 5-year nitrogen addition experiment where treatments of 0, 10, 20 & 50 kg N ha<sup>-1</sup> y<sup>-1</sup> as ammonium nitrate were added to a prostrate Calluna dominated heathland rich in lichen species (NVC H13). Nitrogen addition treatments were applied in combination with management treatments of burning and simulated grazing (clipping) in order to investigate the potential interactions. Various aspects of community structure and function were monitored including plant species composition, tissue chemistry and growth and also the effects on soil and soil water chemistry. All aspects of the system were sensitive to the nitrogen treatments although the magnitude and speed of response varied. There were also significant interactions between management and nitrogen additions, especially in the case of burning.

### Does atmospheric nitrogen contribute significantly to the nutrient budget in a South Wales sand dune?

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Merthyr Mawr is a large dunes system in South Wales. This study was funded by Countryside Council for Wales to ascertain whether atmospheric N deposition, and polluted groundwater, contributed significantly to the nutrient budget at the site.

Gaseous NOx and  $NH_3$  were measured by diffusion tubes and ALPHA samplers respectively over a 12-month period. Rain chemistry was collected monthly and groundwater and surface water samples were collected monthly for nutrient chemistry analysis. Soil and vegetation N pools were assessed in winter and summer.

The results suggest that ammonia levels are moderate to low at the site, with the greatest contribution coming from grazing land to the east of the site, and from localised areas of sea buckthorn (*Hippophae rhamnoides*) clearance on-site. Nitrate pollution in some groundwater sources was considerable, affecting certain areas subject to regular winter flooding. However, neither effects of atmospheric nitrogen nor of groundwater contamination were detectable within the typical dune habitats across the site.

## Effects of dry and wet N deposition on vegetation and biogeochemistry of an ombrotrophic bog

Elleke van Zetten<sup>1</sup>, Irene de Lange<sup>1</sup>, Lucy J Sheppard<sup>2</sup>, Ian D Leith<sup>2</sup>, Alan Crossley<sup>2</sup>, Leon van den Berg<sup>1,3</sup>, Jan Roelofs<sup>1</sup>

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It has repeatedly been shown that increased nitrogen (N) deposition results in dramatic shifts in vegetation composition. The sources of N-deposition vary from agriculture (mainly  $NH_3$  and  $NH_4$ ) to industry and traffic (mainly  $NO_x$ ).

Effects of these different N forms on the vegetation and biogeochemistry of an ombrotrophic peat bog, Whim Moss (~15km southwest of Edinburgh), have been investigated since April 2002, by employing an automate N manipulation system. This field experiment, uniquely, offers the possibility to investigate the effects of the different N forms at the same site and at application rates and deposition scenarios simulating natural variation in rainfall. Within the manipulation system there are two N manipulations: **Dry**, where gaseous NH<sub>3</sub> is released over a 60 m transect at concentrations that simulate a 100,000 bird poultry unit (0.4-200  $\mu$ g m<sup>-3</sup>), and **Wet**, as soluble nitrate or ammonium, covering the full range of UK wet N-deposition (8 – 64 kg N ha<sup>-1</sup>yr<sup>-1</sup>).

The effects of dry N deposition on the vegetation and biogeochemistry at different distances from the  $NH_3$  source have been analysed. In the wet N deposition experiment, 5 treatments were followed, ranging from 8-64 kg N ha<sup>-1</sup>yr<sup>-1</sup> and differing in N form as either oxidised or reduced N.

Samples of soil water were obtained using mini-rhizon samplers and were tested for pH, NH<sub>4</sub>, NO<sub>3</sub>, P and base cations. Young (1 year old) shoots of *Calluna vulgaris L. (Hull), Erica tetralix L.* and *Sphagnum capillifolium Ehrh. (Hedw.)* were harvested and tested for chlorophyll concentrations, aminoacids and P and base cation concentrations in acid digests.

The preliminary results show differences between treatment N forms and N doses and along the  $NH_3$  gradient. In this presentation we will focus on the plant responses to the changes in the biochemistry.

### Nitrogen dynamics in grasslands: indirect effects of species change are greater than direct effects of N deposition

Gareth Phoenix, David Johnson<sup>+</sup>, Rosemary Booth, Philip Grime

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Calcareous grasslands are one of the most floristically rich ecosystems in the UK, yet that floristic composition is threatened by atmospheric N deposition. These ecosystems have recently been shown to provide an important ecosystem service through the accumulation and long-term storage of pollutant N deposition, significantly limiting the re-release of the pollutant N to groundwater or back to the atmosphere in gaseous form. It is of concern, therefore, that a number of biodiversity manipulation studies (e.g. BIODEPTH and Cedar Creek), have suggested that ecosystem function and provision of ecosystem services declines with loss of biodiversity.

Here we report on the first study to assess the impacts of plant community change and biodiversity loss on N pools and fluxes in calcareous grassland to understand how species change resulting from N deposition may impact on grassland N dynamics and the provision of ecosystem services.

In 1995, experimental assemblages of different composition and biodiversity were established using mature plant cuttings removed from Cressbrookdale (an ancient limestone grassland, Peak District, UK) and planted into microcosms of natural calcareous soil. Following 8 years of community establishment, N & P leaching, soil extractable N & P and vegetation biomass and N & P were quantified over a 12 month period.

Across most parameters of leachate and soil nutrients, leachate volumes, biomass and biomass nutrients, we found large differences between assemblages (large community change effects), but consistently no effect of biodiversity (loss of functional group richness or species richness). Greatest assemblage differences occurred in nitrate leaching, where sedges only communities, leached a considerable 40 to 110 fold more nitrate than grass only communities and communities of mixed plant functional groups. Soil extractable nutrients were closely correlated to (and appeared to explain) changes in leached nutrient losses. While there were assemblage differences in vegetation biomass and biomass N and P pools, the pattern of differences between communities were different to those seen for leaching and were therefore not (or only very weakly) related to soil and leachate nutrients.

Overall, our findings show that community change is of far greater consequence for ecosystem functioning than biodiversity loss *per se*, and also, that indirect effects of community change on N leaching are far greater than the direct effects of increased N deposition inputs.

## Cation depletion by long-term pollutant N deposition assessed by organic acid extractants

Odhran O'Sullivan and Jonathan Leake

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Semi-natural grasslands receiving high N deposition loads are typically highly P-limited as a consequence of N saturation. A major mechanism for increasing P mobilisation by plants is the release of low molecular weight organic acids, such as citric and oxalic acid. One of the potentially most serious consequences of N saturation of grassland soils is enhanced leaching of cations leading to base depletion. The organic acids secreted by roots and the acidification caused by N deposition are implicated in the mobilisation of base-cations.

Here we report the use of two organic acids as extractants to investigate the effects on extractable cations of simulated reactive N deposition over a 10 year time period. Soil samples were taken from calcareous and acid grasslands that have received simulated enhanced N deposition since 1995 (at rates of 0-140 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Use of citric and oxalic acid (two of the low molecular weight organic acids released by plant roots) as extractants revealed a decline in Ca and K (by 30% and 65% respectively in the 35 kg N ha<sup>-1</sup> yr<sup>-1</sup> treated plots) as well as an increase in Fe availability (by 24-27% in the 35 kg N ha<sup>-1</sup> yr<sup>-1</sup> treated plots) in response to the enhanced N deposition. The organic acid extractants were found to provide a more sensitive, and arguably more biologically meaningful, measure of change in soil base status than conventional extractants such as Mehlich III, which may include element pools that are not normally bioavailable.

## Dissolved Organic Carbon (DOC) concentrations and variations in UK soils

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From earlier studies it is known that dissolved organic carbon (DOC) strongly complexes metals, influencing metal partitioning between the soil and soil solution and metal leaching from the soil profile. DOC is, therefore, an essential parameter in the calculation of critical loads. Because DOC data are scarce, we analysed DOC concentrations in six different vegetation types from over 30 sites throughout Yorkshire, the Peak District, Scotland and Wales. Soil water for DOC analysis was collected using Rhizon samplers every three weeks for one year. Results show substantial variation within vegetation types and between vegetation types. Correlations for soil parameters are shown.

## Moss tissue structure, pH regulation and sensitivity to atmospheric nitrogen pollution

Tony Miller, John Pearson, Sue Smith and Catherine Stanley

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Within plant cells the vacuole functions as a nutrient storage reservoir. It also has an important role in maintaining homeostasis of the cytoplasm thereby sustaining the conditions for life biochemical processes. During atmospheric nitrogen deposition leaf tissues can be exposed to sudden changes in supply that could overload the tissues capacity to cope and maintain cellular homeostasis. The structure and organisation of tissues, including vacuoles, may be important factors in determining a species ability to cope with atmospheric pollution. These ideas will be discussed and data presented for two types of moss that each have differing tissue structure.

# Can mosses and atmospheric nitrogen pollution provide answers to current controversies in cell pH homeostasis?

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Recently the view that a pH-stat mechanism centred on PEP carboxylase activity and organic acid production operates to maintain cytosol pH has been brought in to question (Britto & Kronzucker, 2005). Cell pH maintenance was thought to be particularly important when cells or tissues undergo inorganic nitrogen uptake and assimilation. Here we show why mosses are particularly appropriate for a study and elucidation of the problems in this debate. We also present a preliminary study that models ammonia uptake and proton ( $H^+$ ) production in the woodland floor moss *Mnium hornum*.

Britto & Kronzucker (2005). Plant, cell & Environment, 28, 1396-1409.

### Multi-element composition of historical lichen collections and bark samples, indicators of changing atmospheric conditions

William O. Purvis<sup>a\*</sup>, P.J. Chimonides<sup>a</sup>, T.E. Jeffries<sup>a</sup>, G.C. Jones<sup>a</sup>, A-M. Rusu<sup>a</sup> and H. Read<sup>b</sup>

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Thirty six element signatures were compared in 11 historical samples from the Natural History Museum herbarium spanning the period 1797-1967 with those recorded in

different stages of the life cycle in the lichen Parmelia sulcata and tree bark sampled from Burnham Beeches, Buckinghamshire, England in 2000. Nineteen elements reached highest concentrations in herbarium samples, signatures consistent with a pollution legacy and dust contamination (especially for As) during storage. Highest V, Ni, Zn, Cd, Se Ge, elements characteristic of fuel combustion, were recorded from a healthy sample from Kent with a low  $\delta$ 34S and high S and N contents sampled east and down-wind of London at a farm during peak SO<sub>2</sub> emissions in 1967. Lowest V, Co, Ni, Cu, Zn, Sn, Ba, Pb, Mo, Sb, Li, B, Cs, U, Th, Ga and S were recorded in a sample with a high  $\delta$ 34S collected from a rock in 1887 from Ross-shire, Scotland and lowest N in Parmelia from Ben Lawers in 1867. Both areas were remote from local pollution sources. Zn concentrations broadly followed modelled temporal deposition trends. Zn emissions have declined by 73% since 1970, largely due to the decline in coal combustion and improvements in abatement measures in the iron and steel industry. Herbarium lichen samples help interpret changes in element deposition, for a broad suite of elements, including those near detection limits, where few direct deposition measurements are available.

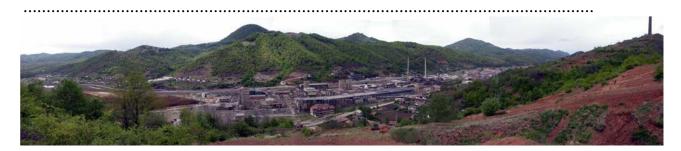
Purvis, O.W., Chimonides, P.J., Jeffries, T.E., Jones, G.C., Rusu, A.-M. & Read, H. Multielement composition of historical lichen collections and bark samples, indicators of changing atmospheric conditions. *Atmospheric Environment* [submitted].

## Biomonitoring using lichen transplants following industrial closure at Zlatna smelter town, Romania

Ana-Maria Rusu, P. Jim Chimonides, Gary C. Jones, Raquel Garcia-Sanchez and O. William Purvis

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The Natural History Museum, Cromwell Road, London SW7 5BD, UK



Lichens were transplanted in May 2004, over a 40 km long NW-SE transect centred on a major metallurgical waste dump close to Zlatna town surrounded by deciduous forests (Quercus / Carpinus / Fagus) two weeks after closure of a major industrial source - a 'natural laboratory' to evaluate metal accumulation in lichens. Lichens, bark, soil and waste dump materials were analysed for 56, including Rare Earth Elements (REE). The study considers chemical signatures in lichens and highlights the role of the tree canopy in leading to enhanced deposition to lichens. The negative europium anomaly in lichens and soil, similar to that in upper crust, confirm a strong crustal influence on lichen signatures across the transect area. REE analysis supports the view that epiphytic lichens, unlike trees, are not influenced by lower groundwater, and are therefore potentially, excellent indicators for REE and other rare elements entering the surface environment, difficult to detect by conventional means. Understanding metal fixation and release by lichens (and mosses) and the origin of elements is necessary to refine biomonitoring protocols and to understand the influence of metals, from both natural and anthropogenic sources, on biodiversity. There are further implications for conservation of rare fauna and flora and for habitat restoration.

Rusu, A-M, Jones, GC, Chimonides, PJ and Purvis, OW Biomonitoring using the lichen *Hypogymnia physodes* and bark samples near Zlatna, Romania immediately following closure of a copper ore processing plant. *Environmental Pollution* [in press]

Rusu, A-M, Chimonides, PJ, Jones, GC, Garcia-Sanchez, R and Purvis, OW Multielement including rare earth element composition of lichen and other environmental samples following industrial closure at Zlatna smelter town, Romania. *Environmental Science and Technology* [submitted].

## Assessing the potential for recovery of degraded montane heaths

Heather Armitage – PhD Research Student (1st Year)

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Racomitrium heath is an important semi-natural montane community. The condition and extent of this habitat, however, is in decline due to increased atmospheric nitrogen pollution and grazing pressure. Previous research, including nitrogen addition experiments and grazing exclosures, has shown the sensitivity of the dominant moss Racomitrium lanuginosum to these factors, resulting in disruption to N-metabolism, loss of cell integrity, reduced growth and ultimately shoot death. Conservation agencies have a duty to preserve British Racomitrium heaths but require more information in order to do this effectively. The habitat's potential for recovery is a fundamental concern. Would a reduction in grazing pressure be sufficient to initiate recovery or will the effects of atmospheric pollution and climate change limit its effectiveness? This project aims to assess the current condition of UK montane heath in relation to similar European sites, by comparing vegetation composition and Racomitrium health (in-situ growth, tissue chemistry and physiology). Field manipulation experiments (grazing exclosures and reciprocal transplants) will aid in the evaluation of recovery potential, as will controlled environment experiments aimed at assessing the relative impacts of climate and Ndeposition. It is hoped that this project will contribute to the production of guidance notes for the restoration and management of montane moss-heath and provide further evidence to support the continuing refinement of critical loads of nitrogen deposition.

# Impacts of increased nitrogen deposition on carbon and nutrient dynamics in a montane heathland

Niki Papanikolaou<sup>1, 2</sup>, Dave Johnson<sup>1</sup>, Andrea Britton<sup>2</sup> and Rachel Helliwell<sup>2</sup>

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Global environmental change, in particular increased atmospheric nitrogen deposition and climate change are currently posing a threat for semi natural ecosystems. Their impact on carbon and nutrient dynamics is studied on a montane heathland in the Cairngorm Mountains dominated by the ericaceous shrub Calluna vulgaris. Regular additions of  $NH_4NO_3$  (0, 10, 20 and 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were used to simulate atmospheric nitrogen deposition over a period of 6 years. In addition, warming chambers were used over the last two years to simulate global warming, and half of the plots were burnt at the beginning of the experiment so that the influence of current land management practice could be investigated. So far, in order to test the hypothesis that increased nitrogen availability leads to increased phosphorus demand by soil microorganisms and plants, we determined the activity of phosphomonoesterase (PME), a widespread extracellular soil enzyme that catalyses the hydrolysis of phosphate monoesters. PME activity increased with each increment of nitrogen addition, supporting our hypothesis, whereas warming and burning had also a combined treatment effect. The results suggest that the turnover of phosphorus in montane ecosystems may increase alongside nitrogen deposition. Further studies are underway to determine how these treatments may affect litter quality and decomposition, nitrogen mineralization, key enzymes involved with carbon turnover and the composition of the microbial community.

### Influence of nitrogen deposition on carbon dynamics in peatlands

Pauline Leterme<sup>1,2</sup>, David Johnson<sup>2</sup>, Rene van der Wal<sup>3</sup>, Lorna Dawson<sup>1</sup>, Rebekka Artz<sup>1</sup>

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The impact of high levels of nitrogen deposition from the atmosphere (primarily from the combustion of fossil fuels and transportation) on soil carbon fluxes and carbon sequestration pathways in peatlands are uncertain and limit our understanding of its consequence on peatlands' role as global carbon sinks. An alteration in peatlands' natural carbon accumulation process could result in the increased release of  $CO_2$  into the atmosphere, potentially increasing the greenhouse effect and contributing to climate change.

Recent studies in forest soils have shown that high concentrations of inorganic nitrogen accelerate the activity of key soil enzymes involved in the degradation of easily decomposable litter (low lignin content) but slow down the decomposition of lignin abundant litter. Peatland soils are adapted to slow rates of nitrogen mineralization; therefore increasing the nitrogen supply in these environments may have an even deeper impact on litter quality, potential litter decomposability, and overall carbon storage capacity.

The aim of this study is to use plant wax biomarkers as proxies of vegetational change in litter profiles. An alkane and alcohol profile database of peatland vegetation is currently being characterized for comparison with samples taken from the Whim Moss experimental site (Edinburgh) where different levels of nitrogen has been added to peatland soil since 2002. A temporal study combining the MicroResp technique (community level physiological profiles) and enzyme activity assays is considered to look at the effect of litter compositional changes on soil microbial diversity and biological activity. To better understand how nitrogen deposition in peatland soil affects the mechanisms controlling carbon storage, the incorporation of stable isotope labelling (<sup>13</sup>C) would allow direct determination of the fate of carbon into the different carbon pools and better pin-point the changes in litter composition.

## Atmosphere-soil-stream greenhouse gas fluxes from peatlands

Kerry Dinsmore, Mike Billett and Ute Skiba

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The project aims to produce a complete inventory of greenhouse gas fluxes and emissions from a Scottish peatland. Carbon dioxide, methane and nitrous oxide emissions from the land surface (soil and vegetation) to the atmosphere, losses to streamwater and degassing will all be considered. The study is carried out at Auchencorth Moss, Midlothian, with intensive monitoring and measurements being made over a 2-year period, starting March 2006. The site consists of a patchwork of different vegetation communities including areas dominated by Calluna or Juncus, grassy hummocks and hollows and a narrow riparian zone again dominated by Juncus. GHG flux measurements will be made using chambers covering each vegetation type allowing for both a comparison between vegetation types and the subsequent scaling up to catchment level emissions. A flux tower on site provides further data on CO<sub>2</sub> net exchange. In addition the concentrations of GHG in the soil are measured using gas permeable tubing. Other land based measurements will include water table depth, soil moisture, soil temperature and soil NO<sub>3</sub>, NH<sub>4</sub> and DOC content. A datalogger is in place adjacent to the stream allowing for almost continuous measurements of stream temperature, conductivity and height; this data along with regular measurements of stream solute and dissolved gas concentrations will be used to estimate both stream gaseous emissions and lateral outputs. Routine measurements of carbon (DOC, DIC, POC, CO<sub>2</sub> and CH<sub>4</sub>) and nitrogen (NO<sub>3</sub>, NH<sub>4</sub>, DON, N<sub>2</sub>O) will also be made along the stream length to measure spatial variability.

An introduction to the project will be presented.

### Effects of ozone on a plant community exposed for two consecutive summers

Felicity Hayes, Laurence Jones, Andrew Lloyd, Philip Williams, Gina Mills

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A model plant community, representing NVC U4 (*Agrostis capillaris-Festuca ovina-Galium saxatile* grassland) containing *Anthoxanthum odoratum, Agrostis capillaris, Festuca ovina, Carex echinata, Carex bigelowii, Potentilla erecta* and *Galium saxatile* was exposed to ozone in the solardomes at CEH Bangor for two consecutive summers. Eight solardomes, with 2 replicate domes of each of 4 treatments were used in 2004 and 2005. The treatments used represented current ozone climates and predicted scenarios for 2050. Two background ozone treatments were used, low background at 20-25 ppb ozone and high background (predicted for 2050) at 45-50 ppb ozone. In addition, an episodic ozone regime for four days every week was applied to each of the background treatments. The treatments were applied for twelve weeks in each summer.

Increased/premature senescence was observed on five of the seven species in the higher ozone treatments, and differences between the treatments were starting to show after 6-8 weeks of the first ozone exposure. There was most senescence in the high background-high peaks treatment in each case, and no differences in the sensitivity to ozone between the two years. Interestingly, *Festuca ovina*, had a significant increase in the rate of development of senescence in the high background-low peaks treatment compared to low background-low peaks, indicating that this species responded to the rise in background ozone concentrations tested.

There were no significant effects on biomass of component species or total mesocosm biomass in any one season, however cumulative biomass was reduced by 15% for Anthoxanthum odoratum in the high background-high peaks treatment (p=0.043). In addition, after 2 seasons, the percentage cover of *Anthoxanthum odoratum* decreased from 53% (Low background-Low peaks) to 42% (High background-High peaks). There had been no differences in percentage cover between treatments prior to this.

Effects of ozone on this community were gradual, with some differences not present until after exposure to ozone for a second summer season.

## Studies of the sensitivity to ozone of natural plant species at an individual and community level

Maria Samuelsson, Simon Peacock and Jeremy Barnes

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This presentation describes the impacts of simulated 2050 upland ozone climate on the species composition of long-established upland mesotrophic grassland mesocosms of contrasting residual soil fertility and presence and absence of *R. minor*. In addition, a future project to investigate the effect of a present and future predicted ozone climate on above and below-ground species richness in a leguminous fixed-dune grassland community will be introduced.

Furthermore, we present studies to determine i) whether night-time ozone fluxes are more damaging than equivalent daytime fluxes by investigating growth analysis, ascorbate activity and enzyme activity in leaf cell walls in *Caltha palustris* (Marsh Marigold). ii) the impact of ozone on the hemi-parasitic *Rhinanthus minor* (Hay Rattle) and potential interactions with two host plants of contrasting ozone sensitivity, and iii) the effect of ozone and low temperature on the growth of *Digitalis purpurea* (Foxglove).

Further information: simon.peacock@ncl.ac.uk

### Critical levels of ozone for vegetation: an update on international developments

Gina Mills

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A workshop on the critical levels of ozone and further applying and developing the fluxbased concept took place on 15-19 November 2005 in Obergurgl, Austria. It was attended by nearly 100 people from 18 countries together with representatives of the International Cooperative Programme (ICP) on Forests, ICP Vegetation, the EMEP Meteorological Synthesizing Centre – West (MSC-W) and the UNECE secretariat.

The overall aim of the workshop was to validate the flux concept and the primary objectives were to:

- Further develop methods for applying flux-effect relationships for impact assessments at different geographical levels including consideration of uncertainties;
- Review the provisional flux-based critical level for forest trees, and to consider progress in establishing flux-based critical levels for additional crops not currently included in the mapping manual;
- Assess progress with the development of canopy and stand level ozone flux-effect models and methods for crops and forest trees;
- Assess progress with the development of flux-effect models for (semi-) natural vegetation;
- Identify areas of further work for crops, (semi-) natural vegetation and forest trees.

The outcomes from this meeting will be described together with the ways in which the new and existing critical levels and methods for ozone will be used within the LRTAP Convention<sup>1</sup> to further develop European air pollution control policy.

<sup>1</sup>Long-range Transboundary Air Pollution Convention

#### **Ecosystem health in Epping Forest**

Sally Gadsdon<sup>1</sup>\*, Sally Power<sup>1</sup> and Jeremy Dagley<sup>1</sup>

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Epping Forest is on the edge of London. As a SSSI and SAC, the site is notified for its beech forest and wet and dry heathlands. There is concern over the health of beech trees within Epping Forest; recent surveys revealed that more than one third of the trees are in particularly poor health.

Paired sample sites were set up to investigate the relationship between biotic and abiotic variables and beech tree health. Soil and leaf samples were analysed for nutrient and heavy metal contents. Root samples were taken to measure the proportion of live and mycorrhizal roots. Results were analysed using multivariate analysis and generalised linear modelling. In addition, long term monitoring of canopy characteristics (crown density and architecture), as a measure of beech vitality, has allowed a comparison between the health of trees at Epping Forest and those at the New Forest (Hampshire) and Burnham Beeches (Buckinghamshire).

The proximity of Epping Forest to London and the high volumes of road traffic within the Forest have led to concern over the deposition of nitrogenous pollutants to sensitive habitats, particularly heathlands. Calluna shoots were sampled as biomonitors of atmospheric nitrogen deposition along a transect from the north of the Forest towards London, and along transects away from a busy local road. In addition, detailed condition assessments were carried out on remnant heathland areas to provide an accurate assessment of their status. These data will be discussed in relation to current heathland management practices.

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#### The effects of ozone on woodland ground flora

Becky Keelan and Mike Ashmore

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Fourteen species, collected as seed from 3 broad habitat types from within the Yorkshire Dales National Park, were grown and exposed to ozone in an indoor fumigation system. Included within these were 3 species represented from more than one habitat type to allow between population comparison. These species were exposed for 3 weeks at 80ppb ozone. The results indicated that species from the limestone pavement areas were insensitive, whereas the species from meadows and woodlands showed a greater sensitivity to ozone. Effects on root biomass were much greater than those on shoot biomass.

Three further experiments were conducted to assess the effect of ozone on the emergence and development of woodland ground flora using mesocosms; plants were allowed to emerge from soil collected from an upland oak-ash woodland. The mesocosms were exposed to 80ppb ozone over periods of 2-3 months. Cover, abundance and diversity were measured throughout the experiments and the experiments were concluded with a destructive harvest for above-ground biomass. Visible injury to a number of species was found in all three experiments, but significant effects on the above-ground biomass of typical woodland species were only found in one of the three experiments. This may reflect the variation in the time and location at which soils were taken from the field site. There was evidence that ozone had a greater effect on typical woodland species than those adapted to higher levels of light.

A further screening experiment of spring bulb species *Hyacinthoides non-scripta*, *Allium ursinium* and *Narcissus pseudonarcissus* suggest that these species are also particularly sensitive to ozone.

Levels of ozone were recorded in an upland woodland. These suggest that the levels of ozone reaching the field layer are similar to those outside the woodland in the open. Woodland ground flora is of high conservation value in many European countries, and more understanding is needed of how the modification by the woodland canopy of ozone exposure patterns and microclimate influences its responses to ozone.

# Intraspecific ozone sensitivity in anthoxanthum odoratum (L.)

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The aim of this study was to investigate the sensitivity of the perennial grass *Anthoxantum odoratum* L. to elevated tropospheric ozone at the species, population and individual level. A total of 76 individuals sourced from four populations in North Wales were exposed to four ozone treatments for 12 weeks in the solardomes at CEH Bangor from July to October 2005. Two background ozone concentrations of 20-25 ppb and 45-50 ppb were applied and combined with an episodic ozone regime in one treatment at each background level. Each episode consisted of ozone concentrations increased to 50ppb above the background level during daylight hours over four days. The effects of elevated ozone on dry weight biomass, senescence and tillering were assessed. Despite the highly significant inherent inter- and intra-population variation observed in this species, exposure to elevated ozone resulted in increased/premature senescence at the species and population levels, a varied response for tillering dependant on the level considered and no significant effect on dry weight biomass.

Further information: bspc2b@bangor.ac.uk

#### Ascorbate-oxidase-dependent changes in the redox state of the apoplast modulate gene transcription leading to modified hormone signaling and defence in tobacco

Cristina Pignocchi<sup>1,2\*</sup>, Guy Kiddle<sup>1</sup>, Iker Hernández<sup>2#</sup>, Simon J Foster<sup>1\*</sup>Amparo Asensi<sup>2</sup>, Tahar Taybi<sup>2</sup>, Christine H. Foyer<sup>1</sup>, **Jeremy Barnes<sup>2</sup>** 

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To date, the role of cell wall-localised ascorbate (AA) in determining ozone susceptibility has remained focused on the role of this forward defensive barrier as sink for the gas and it's potentially reactive intermediates. This talk will focus on recent studies revealing the potentially central role played apoplast redox status (modulated via the key component: AA) in regulating hormone-related growth responses, signaling cascades and gene expression. The studies have been performed using transgenic tobacco plants with modified cell wall-localized ascorbate oxidase (AO) as a tool. High AO activity specifically decreased the ascorbate (AA) content of the apoplast and altered plant growth responses triggered by hormones. Auxin stimulated shoot growth only when the apoplastic AA pool was reduced in the wild type (WT) or in AO antisense lines. Oxidation of the apoplast AA pool in AO sense lines was associated with the loss of auxin response, higher MAP kinase activities, increased sensitivity to ozone-induced oxidative stress as well as enhanced susceptibility to a virulent strain of the pathogen Pseudomonas syringae. The total leaf glutathione pool, the ratio of reduced glutathione to glutathione disulphide and glutathione reductase activities were similar in the leaves of all lines. However, AO sense leaves exhibited significantly lower dehydroascorbate reductase and ascorbate peroxidase activities than WT and antisense leaves. The abundance of mRNAs encoding antioxidant enzymes was similar in all lines. However, the day/night rhythms of abundance of transcripts encoding the three catalase isoforms were changed in response to the AA content of the apoplast. Other transcripts influenced by AO include photorespiratory genes and plasma membrane-associated Ca<sup>2+</sup> channel-associated genes. We conclude that the redox state of the apoplast modulates plant growth and defence responses by regulating signal transduction cascades and gene expression patterns. Hence AO activity, which modulates the redox state of the apoplastic AA pool, strongly influences the responses of plant cells to external stimuli, including ozone (and potentially other pollutants generating ROS externally/internally)

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# Quantitative Trait Loci (QTL) governing ozone tolerance and foliar antioxidative properties in *Arabidopsis thaliana*

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Fourteen partially-mapped parental lines of *Arabidopsis thaliana* were screened for ozone sensitivity based on rosette diameter and seed weight. Total antioxidative capacity (TAC) and ascorbate-related ROS scavenging capacity were also determined. The aim of this exercise was not only to investigate relationships between 'ozone sensitivity' and several morphological and biochemical traits, but also to identify parental lines exhibiting genetic variation in the traits identified. Plants were fumigated with charcoal/Purafil<sup>®</sup>-filtered air (CFA) or CFA + ozone (15 nmol mol<sup>-1</sup> overnight rising for 8 h d<sup>-1</sup> to 250 ppb). Based on the outcome of this study, controlled environment studies were performed on a population of 94 recombinant lines (plus parents) of *Arabidopsis thaliana* resulting from a cross between Nok-3 and Ga-0 mapped using 58 markers at the John Innes Centre (JIC)

Impacts of ozone on seed weight, rosette diameter, TAC and ASC were determined on > 500 plants and QTL's were mapped and ranked. Considerable genetic variation in responses to ozone were observed between lines for all traits measured. QTL's specific for the effects of ozone on seed production were identified on chromosomes 4 and 5 and TAC and ASC on chromosomes 3 and 4. Downstream investigations will involve searching the QTL's found with the goal of identifying candidate genes governing ozone tolerance.

#### Impacts of ground-level ozone concentrations on the contamination of salad crops by human pathogens

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Ready-to-eat salads occasionally cause food poisoning as a consequence of microbial contamination with pathogenic bacteria. Experimentation has revealed that the source of such contaminants is via foliage rather than soil-mediated root uptake. To determine the effect of ozone as a bactericide, *in vivo* experiments were conducted in which bacterial cultures on agar plates were exposed to various ozone levels. A two-log reduction was observed after exposure at 200 ppb for 30 minutes: a level of ozone sometimes experienced briefly at ground-level due to photochemical pollution. To harness this effect for industrial applications a shorter treatment-time is required. Visible injury occurs after 10 minutes of exposure at 5 ppm, at this level *in vivo* studies yield a 2-log reduction. The reason for the discrepancy in inactivation rates between bacteria on an inert surface and on the surface of leaves is under investigation.

#### QTLs governing ozone impacts on wheat yield

**Agnieszka Kaminska**<sup>1,2</sup>, Ali Dodmani<sup>1</sup>, Ignacio Gonzalez<sup>1</sup>, Colin Gillespie<sup>5</sup>, Paul Bilsborrow<sup>3</sup>, Steve Quarrie<sup>1,4</sup>, Jeremy Barnes<sup>1</sup>

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Despite more than 30 years' relatively-intensive research, few efforts have been directed toward the identification of genetic traits governing the impacts of ozone on vegetation. In this paper we will report progress with ongoing studies performed in open top chambers on a population of 95 double haploid lines of wheat (plus parents; Chinese Spring and SQ1) for which there is a comprehensive genetic map (Quarrie *et al.*, 2005 *J. Exp. Bot.* **110**: 865-880) in a bid to identify QTLs (quantitative trait loci) governing ozone "tolerance" and foliar oxidative defence mechanisms.

Studies were conducted in 2003 and 2005 involving work on >7000 plants over the two years, raised in open top chambers (OTCs) exposed to NFA or NFA+50 ppb. In both years, parental lines displayed considerable variation in measured traits, and there was considerable genetic variation between lines raised in 'clean air' as well as in the response of the lines to challenge by ozone. In sensitive lines, ozone exposure reduced grain yield and thousand grain weight by up to 50%, whilst in others ozone exerted no significant effects. Data were consistent between years and revealed much coincidence with yield and component QTLs identified in previous independent experiments conducted over the past decade on the same mapping population (see Quarrie *et al.*, 2006 *J. Exp. Bot. In Press*). Weak QTLs for ozone impacts were identified in 2003 (due to a general lack of  $O_3$  impacts on yield in the 2003 experiment) whilst strong (and coincident between years) QTLs were evident for ozone impacts on yield and yield components in 2005. Pronounced ozone-related QTL clusters were identified on chromosomes 1DL, 2AS, 4AL, 4DL, 5BL, 6AL, 6BL and 7D.

Measurements of foliar antioxidative capacity and ascorbate-related scavenging capacity on flag leaves of the 95 lines (plus parents) conducted during the 2005 experiments revealed marked variation in these traits between lines and a different pattern of reaction between genotypes in response to ozone (illustrative of genetic variation in the extent of perception and signalling of ozone-induced oxidative stress). Data revealed ASC to be the principal contributor to measured TAC (total antioxidative capacity). Data indicated little relationship between foliar TAC or ASC and ozone 'sensitivity' (in terms of impacts on yield). This observation suggests that antioxidant-based redox signalling and/or subcellular compartmentation maybe more important than foliar antioxidant content *per se* in determining responses to ozone. Strong QTLs governing foliar antioxidantive properties were identified on chromosomes 2D, 3A, 3B, 7A and 7D.

We are currently probing the bins identified as containing key QTLs for candidate genes governing ozone tolerance, foliar antioxidative properties and signaling networks.

<sup>5</sup>currently: SEPA, Glasgow

### A novel technique for reducing soil fertility in ecological restoration projects

E. Glen<sup>1</sup>, E.A.C. Price<sup>1</sup>, S.J.M. Caporn<sup>1</sup>, J. Carroll<sup>1</sup>, M.L.M. Jones<sup>2</sup> and R. Scott<sup>3</sup>

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Surface soil eutrophication hinders ecological restoration projects by favouring communities of low biodiversity. This study assesses the effectiveness of a novel technique known as topsoil inversion that may promote recovery from eutrophication. Topsoil inversion is undertaken by a deep plough, which buries 30 cm of topsoil under approximately 40 cm of subsoil. The main study site is within new community woodland on former agricultural land. It comprises deep ploughed and conventionally ploughed plots, to compare two planting types: wildflowers only, and wildflowers with trees. This presentation will discuss some preliminary findings of the effect of topsoil inversion on soil properties and plant tissue nutrient content. Surface soil fertility is lowered following inversion treatment, and this appears to affect plant nutrient sequestration. These results suggest that topsoil inversion has the potential to facilitate ecological restoration on eutrophic soil. This technique may have benefits for restoration projects taking place in a variety of habitats affected by air pollution, including former agricultural land and lowland heaths.

### **Poster Abstracts**

#### Can Ellenberg numbers predict ozone sensitivity?

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There have been relatively few studies of ozone effects on semi-natural vegetation in comparison to crops and trees. It is impossible to screen all species for ozone response. Therefore, a method was developed to try and predict ozone sensitivity on the basis of Ellenberg Indicator values.

The model was developed using the OZOVEG database held at CEH Bangor which contains empirically-derived ozone response functions for 83 semi-natural species. The database defines Relative Sensitivity (RS) to ozone for a species as the change in above-ground biomass at an AOT40 of 15 ppbh relative to that at 3 ppbh (Hayes et al., submitted), where RS > 1 responds positively and RS < 1 responds negatively. Stepwise multiple regression (backwards elimination) was used to select the most appropriate combination of Ellenberg indicators to predict RS. Five equations were tested using jacknifing techniques, and a performance test which assessed the risk of misclassification of 'sensitive' species (RS < 0.80 or RS > 1.20).

The following equation was selected (details in bold, Table 2) for Predicted Relative Sensitivity (RSp):

 $RS_{p} = 1.805 - 0.118Light - 0.135\sqrt{Salinity}$ 

### World biodiversity hotspots threatened by atmospheric nitrogen deposition

Gareth Phoenix<sup>1</sup>, Kevin Hicks<sup>2</sup>, Steve Cinderby<sup>2</sup>, Frank Dentener<sup>3</sup>, Mike Ashmore<sup>2</sup>, Phil Ineson<sup>2</sup>

<sup>1</sup>Department of Animal and Plant Sciences, University of Sheffield, Sheffield <sup>2</sup>Stockholm Environment Institute, University of York, Heslington York <sup>3</sup>Institute for Environment and Sustainability, Ispra, Italy

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Increased atmospheric N deposition is known to reduce plant diversity in natural and semi-natural ecosystems, yet our understanding of these impacts comes almost entirely from studies in northern Europe and North America. Currently, we lack an understanding of the threat of N deposition to biodiversity at the global scale. In particular, rates of N deposition within the newly defined 34 world biodiversity hotspots, to which 50% of the world's floristic diversity is restricted, has not been quantified previously.

Using output from global chemistry transport models, we provide the first estimates of recent (mid 1990s) and future (2050) rates and distributions of N deposition within biodiversity hotspots. Our analysis shows that the average deposition rate across these areas was 50% greater than the global terrestrial average in the mid-1990s and could more than double by 2050, with 33 of 34 hotspots receiving greater N deposition in 2050 compared with 1990. By this time, 17 hotspots could have between 10% and 100 % of their area receiving greater than 15 kg N ha<sup>-1</sup> yr<sup>-1</sup>, a rate exceeding critical loads set for many sensitive European ecosystems. Average deposition in four hotspots is predicted to be greater than 20 kg N ha<sup>-1</sup> yr<sup>-1</sup>.

This elevated N deposition within areas of high plant diversity and endemism may exacerbate significantly the global threat of N deposition to world floristic diversity.

From this equation, two tools were developed (ORI% and CORI) to predict ozone effects at the community level. These tools were tested against field data from Le Mouret, Switzerland, and with UK NVC communities.

### Has ammonia Fumigation affected enchytraeid worms at Whim Moss

M. Prendergast<sup>1</sup>, V. Standen<sup>2</sup>, L. Cole<sup>3</sup>, B. Rees<sup>4</sup>, J. Parker<sup>4</sup>, I. Leith<sup>5</sup> and L. Sheppard<sup>5</sup>

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The Whim Moss experimental site was established in 2002, for the UK Natural Environment Research Council's GANE programme (Global Atmospheric Nitrogen Enrichment). This site enables the study of *in situ* enhanced N effects (as NH<sub>3</sub>, NH<sub>4</sub><sup>+</sup> and  $NO_3 - N$ ) on a sensitive semi-natural habitat, where N applications are dependent upon suitable meteorological conditions. 3 years (2002-2005) of NH<sub>3</sub>-N fumigation along a 60 m transect has resulted in an exponential decline in NH<sub>3</sub>-N concentrations from the NH<sub>3</sub>-N source to 60 m. On this transect, *Calluna* and sensitive moss species are now in decline. Below-ground effects were investigated in a short-term<sup>\*</sup> study that focused on Enchytraeid worms (Oligochaeta): Enchytraeids are the dominant indicator species in wet acidic habitats, with key roles in biogeochemical cycling. Results showed that changes to the peat pH and mineral N correlated (p < 0.05) with the decline of NH<sub>3</sub>-N concentrations down the transect. It was expected that NH<sub>3</sub>-N fumigation would increase the N content of the litter layer, the main Enchytraeid food source; an improved litter quality would thus increase the Enchytraeid population on the transect. At Whim, 3 acidophilic Enchytraeid species were identified; however Enchytraeid species and total abundance were not affected by NH<sub>3</sub>-N concentrations, pH or mineral N. Both Enchytraeid abundance and litter N content were similar on the transect and ambient control. It is proposed that 3 years of ammonia fumigation at Whim is not yet long enough for plant matter with an increased N content to become incorporated into the litter layer. Future long-term monitoring, with more systematic sampling, will confirm any N effect on the Enchytraeids.

<sup>\*</sup> MSc dissertation, University of Edinburgh.

### The influence of nitrogen deposition on phosphatase activity in *Cladonia portentosa*

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We have demonstrated that the common heathland lichen Cladonia portentosa (Dufour) Coem. expresses both acid phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activity. A capacity to utilise the organic fraction of phosphorus available in atmospheric deposits may confer an ecological advantage to C. portentosa growing under nutrient-limiting conditions. Nitrogen enrichment of oligotrophic habitats can lead to increased plant demand for phosphorus. Previous studies on C. portentosa have demonstrated a strong covariance between thallus nitrogen and phosphorus concentrations. Therefore we investigated the relationship between N enrichment and phosphomonoesterase activity in the apices (top 10 mm) of this common heathland lichen. Under field conditions at Whim Bog, C. portentosa cushions were subject to either  $NO_3^-$  or  $NH_4^+$  treatments at 8 (control), 16, 32 and 64 kg N ha<sup>-1</sup> yr<sup>-1</sup>. The effect of enhanced deposition of both phosphorus and potassium was also investigated. There was a significant increase in PMEase activity with increasing N deposition (as either NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>) suggesting that as nitrogen supply increases, C. portentosa has the capacity to allocate an increasing quantity of nitrogen to phosphatase synthesis. Such high levels of activity may also help to explain the observed relationship between thallus nitrogen and phosphorus concentrations. Phosphomonoesterase activity was not stimulated in treatments receiving P and K in addition to N; this was interpreted as an effect of increased availability of inorganic P. It was concluded that N enrichment promotes phosphatase synthesis and phosphorus capture in C. portentosa thus maintaining N:P stoichiometry.

# Additional Talk Abstracts

### Direct measurement of ozone deposition to soil using isotopically labelled O<sub>3</sub>

Jens-Arne Subke<sup>1</sup>, Sylvia Toet<sup>2</sup>, David D'Haese<sup>3</sup>, Jeremy D. Barnes<sup>3</sup>, Lisa Emberson<sup>1</sup>, Mike Ashmore<sup>2</sup>, Phil Ineson<sup>1</sup>

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Ozone is widely acknowledged as a major air pollutant affecting both crops and wild plants. Modelling ozone flux to vegetation in order to assess potential damage and make predictions for future impacts now rely on a flux based approach, but there is still a lack of understanding regarding ozone flux to stomata, plant external surfaces, and soil that is needed to parameterise such models. We describe a new approach in which ozone is generated from the stable isotope <sup>18</sup>O, to allow tracing of ozone deposition in plant-soil systems. This first part of a joint presentation (see complementary abstract by Toet *et al*) describes the general research method and results for the O<sub>3</sub> deposition to mineral soil.

Mineral soil samples were exposed to different ozone concentrations and over increasing lengths of time to measure the accumulation of ozone derived <sup>18</sup>O. Soil parameters that were varied within these treatments were either soil water content, or microbial activity (through sucrose additions before fumigation). Based on analysis of dry soil samples, the results show a linear increase of <sup>18</sup>O content with time. Soils with no or low water content (0 or 30% of water-holding capacity, WHC) showed similar <sup>18</sup>O accumulation , while a higher soil water content (60% WHC) resulted in a reduction to about 30% of dry soil <sup>18</sup>O accumulation. Variation in soil microbial activity resulted in no measurable variation in soil <sup>18</sup>O accumulation. <sup>18</sup>O accumulation in soil under 50ppb was approximately 45% of the accumulation under 100ppb over the same length of time, indicating an approximately linear relationship between ozone concentration and ozone deposition.

# <sup>18</sup>O tracer studies to identify the fate of ozone in plants

Sylvia Toet<sup>1</sup>, Jens-Arne Subke<sup>2</sup>, David D'Haese<sup>3</sup>, Jeremy D. Barnes<sup>3</sup>, Phil Ineson<sup>2</sup>, Lisa Emberson<sup>2</sup> and Mike Ashmore<sup>1</sup>

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Ozone is a secondary air pollutant that causes damage to sensitive crops, trees and semi-natural vegetation in Europe and other parts of the world at current levels. Risk assessment of ozone impacts on vegetation is essential, since background ozone concentrations are expected to increase further during the next decades. A model (DO<sub>3</sub>SE) has been developed to quantify and predict ozone deposition and stomatal flux, but some of its components are severely limited by current knowledge. A novel approach with isotopically labelled ozone (<sup>18</sup>O-ozone) was applied to trace ozone and its derivatives directly in plants and soil (also see companion abstract by Subke *et al.*).

Ozone-sensitive white clover (*Trifolium repens*, NC-S) plants grown in pots were exposed to <sup>18</sup>O-ozone at two ozone concentrations during the photoperiod under summer conditions in a climate-controlled chamber. The aims of the study were to assess the temporal change in accumulation of ozone-derived <sup>18</sup>O in different plant parts and soil, and to determine the effects of ozone concentration and pre-exposure with ozone on rates of <sup>18</sup>O accumulation. Results on the fate of <sup>18</sup>O-ozone in the dry fraction of plant parts and the soil surface are presented.

Fumigation of plants with <sup>18</sup>O-ozone during the photoperiod resulted in a continuous increase in  $\delta^{18}$ O of dry leaf biomass, indicating accumulation of ozone-derived <sup>18</sup>O in the dry constituents of leaves. Dry biomass of petioles was a very small sink for <sup>18</sup>O originating from ozone, while no <sup>18</sup>O accumulation was detected in the dry fraction of root biomass and soil. <sup>18</sup>O-enrichment of dry leaf biomass was 3.5 times smaller at 50 ppb than at 100 ppb of <sup>18</sup>O-ozone, which was probably largely the result of differences in ozone concentration and stomatal conductance.  $\delta^{18}$ O of dry leaf biomass was significantly higher in plants which experienced a 1-month ozone pretreatment than in plants grown in filtered air prior to the 100 ppb <sup>18</sup>O-ozone fumigation. However, stomatal conductance was significantly lower in plants which experienced the ozone pretreatment. This may imply that defence mechanisms were stimulated in plants that have been pre-exposed to ozone. Initial results also suggest that a large part of ozone-derived <sup>18</sup>O associated with dry leaf biomass was located in the cell walls.