RESPONSES TO, AND BIOINDICATORS OF, ATMOSPHERIC NITROGEN DEPOSITION ON LOWLAND HEATHLAND ECOSYSTEMS AT BOTH EXPERIMENTAL AND FIELD-SCALE LEVELS

Georgina Elizabeth Southon

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Imperial College London
Division of Biology
To recline on a stump of thorn, where the eye could reach nothing of the world outside the summits and shoulders of heathland, which filled the whole circumference of its glance, and to know that everything around and underneath had been from prehistoric times as unaltered as the stars overhead, gave ballast to the mind adrift on change, and harassed by the irrepressible New. The great inviolate place had an ancient permanence which the sea cannot claim. The sea changed, the fields changed, the rivers, the villages, and the people changed, yet the heath remained.

The Return of the Native, Thomas Hardy, 1878

The contents of this thesis are the result of my own work and all else, has been appropriately referenced. The work outlined in Chapter 3, however, forms part of a collaborative project; as such, some survey data and data resulting from chemical analyses have been provided by collaborative colleagues. Their contributions have been duly referenced where applicable.
Abstract

Reactive nitrogen (N) as a result of anthropogenic activities (e.g. fossil fuel combustion, fertiliser manufacturing and intensive agricultural practices), now dominates the global nitrogen cycle. For sensitive ecosystems across the globe, perturbation of the nitrogen cycle as a result of increased inputs of reactive N, has been seen to have a profoundly detrimental impact on biodiversity and ecosystem structure and function. Concerns surrounding the conservation and protection of both natural and semi-natural habitats from the damaging effects of N deposition, have prompted widescale research, that aims to understand and quantify ecosystem responses to elevated N inputs. Much of this research is in the experimental domain, based upon the simulation of N deposition within controlled conditions. The research outlined in this thesis, is principally focused on the findings of a long-term experimental approach on a lowland heathland system in the south east of England. N additions of 30 kg N, ha\(^{-1}\), yr\(^{-1}\) were seen to cause large, persistent effects on Calluna growth, phenology and chemistry, retard the development of lower plant groups and alter soil biogeochemical processes. Interactions between N inputs and climatic stress were also observed, with N additions seen to significantly modify Calluna’s response to drought conditions.

Interactions between N deposition and climate were also evident at a larger scale, following a nationwide survey of UK heathlands across broad pollution, climatic and geographic gradients. Representing one of the first surveys to test both above and below ground responses to ambient N deposition across UK heathlands, the findings provide compelling evidence that many N driven changes observed within an experimental context, are also occurring at a nationwide scale. Such findings facilitate the identification of robust bioindicators of N deposition that could be successfully integrated into heathland conservation measures. Given that current emissions and deposition rates of N are predicted to double by 2050, and in regard to the future climate changes that are expected, research of the kind presented within this field of research is of the upmost importance if heathlands, and other sensitive ecosystems, are to be preserved.
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Chapter 1

INTRODUCTION

Abstract
Increased atmospheric nitrogen (N) deposition is considered to be one of the main drivers of pan-habitat biodiversity loss and ecosystem degradation at a global scale. This chapter outlines the factors and issues that have contributed to the creation of reactive N in the atmosphere, and synthesises wide-scale research across diverse systems, in relation to the effect of elevated N on ecosystem structure and function. In particular, the impacts of increased N deposition on heathland (predominantly lowland heathland) ecosystems are introduced, along with the central research questions that will be discussed in the subsequent chapters.
Nitrogen: A biological necessity

The cyclical transformations of nitrogen (N) represent, after the carbon cycle, one of the most important nutrient cycles in the world. Nitrogen is an essential component of amino and nucleic acids, the building blocks of life. It is therefore a critical element for the development and sustenance of every living organism (Galloway et al. 2003). Nitrogen gas (N\textsubscript{2}) constitutes approximately 79% of the Earth’s atmosphere, however, despite its abundance, this elemental form is inaccessible to most organisms due to the strength of its triple atomic bond (Erisman, 2011). In order to be biologically useful, N\textsubscript{2} must be converted to a reduced or oxidised state. Because of the stable nature of N\textsubscript{2}, this conversion can only occur with considerable quantities of energy, or by enzymatic processes that occur within specialised bacteria (Galloway et al. 2004). The processes which enable the conversion of non-reactive N\textsubscript{2} into available forms are both biotic and abiotic.

The biological fixation of N\textsubscript{2} is performed by specific microorganisms (prokaryotic bacteria) that contain the enzyme complex, nitrogenase (Sprent, 1987). Nitrogenase catalyses N\textsubscript{2} to produce biologically available reduced forms of nitrogen such as ammonia, amines and amino acids (Dixon & Kahn, 2004). These specialised organisms include symbiotic bacteria (those that have developed metabolic relationships with the roots of some leguminous plant species) and free-living soil bacteria. Oxidative fixation of N\textsubscript{2} can occur during high temperature natural processes such as volcanic activity, thunderstorms (lightning strikes) and wildfires, to produce nitrogen oxides (NO, NO\textsubscript{2}, HNO\textsubscript{3}, NO\textsubscript{3}, N\textsubscript{2}O) (Galloway & Cowling, 2002).

The industrial fixation of N occurs during the Haber-Bosch process which combines nitrogen and hydrogen under elevated temperatures to form ammonia (NH\textsubscript{3}) for nitrogen fertilisers (Smil, 2001). Unintentional nitrogen fixation also occurs during various high energy combustion processes; depending on the nature of the fuel (coal, wood, diesel oil etc.) the resulting product may be nitrogen oxides, ammonia or hydrogen cyanide (Sprent, 1987).

Once fixed, nitrogen is cyclically transformed by passage through the various stages of the global nitrogen cycle.

Nitrogen in soils

Soil nitrogen exists in either organic or inorganic forms, the latter comprised of ammonium (NH\textsubscript{4}\textsuperscript{+}), nitrate (NO\textsubscript{3}\textsuperscript{-}) and nitrite (NO\textsubscript{2}\textsuperscript{-}) ions (Rosswall, 1976). The
largest nitrogen pool at any given time is the organic pool, where nitrogen is locked in relatively stable organic matter from plant and animal residues, or in soil microbial communities (Rosswall, 1976). This nitrogen is not directly available to plants until it has been converted to an available form by microbial activity.

The majority of plant available nitrogen is in the inorganic forms of $\text{NH}_4^+$ and $\text{NO}_3^-$. Positively charged ammonium ions can bind to the soil’s negatively-charged cation exchange complex, and function in a similar way to other cations in the soil. Nitrate ions carry negative charges and cannot, therefore, bind to soil solids, yet exist in soluble form in soil water (Rosswall, 1976; Sprent, 1987). Nitrogen is cyclically transformed in the soil through pathways described as the nitrogen cycle (Figure 1.1).

![Figure 1.1: The nitrogen cycle (www.learner.org).](image_url)
The conversion of organic nitrogen to inorganic (and vice versa) forms, is regulated by soil microorganisms (Tietema & Wessel, 1992). As microorganisms develop, they remove NH$_4^+$ and NO$_3^-$ from the inorganic pool for their own requirements, converting it into organic forms through the process of immobilisation (Stockdale et al. 1994; Booth et al. 2005). Microbial death and subsequent decomposition releases NH$_4^+$ back into the inorganic pool through the process of mineralisation, rendering it available for plant use (Jarvis et al. 1996; Jackson et al. 2008). The degree of immobilised or mineralised nitrogen will depend on the carbon: nitrogen (C:N) ratio of the substrate in comparison to that of the decomposing material (Lewis, 1986). High C:N ratios denote available carbon for microbial respiration and protoplasmic development, whilst lower C:N ratios indicate an excess of N which is converted to inorganic forms for plant use (Schulten, 1997).

Ammonium ions not immobilised or assimilated by plants are usually converted to NO$_3^-$ by the process of nitrification. This is a two step process whereby *Nitrosomonas* bacteria convert NH$_4^+$ to nitrite (NO$_2^-$) which is then converted by *Nitrobacter* bacteria to NO$_3^-$ (Wrage et al. 2001; Costa et al. 2006). Nitrate carries a negative charge, and as a consequence is excluded from cation exchange sites (Johnson, 1993). This renders it more mobile than ammonium, and therefore readily leached, particularly in mineral soils (Broschat, 1995; Schulten, 1997). In saturated soils, nitrate can also be lost through denitrification, a series of reactions that reduce nitrate ultimately to molecular nitrogen (N$_2$) or gaseous oxides of N (e.g. N$_2$O, NO, NO$_2$) (Sprent, 1987). These processes are carried out by denitrifying bacteria and fungal species under predominantly anaerobic (yet, also aerobic) conditions (Bateman & Baggs, 2005; Hayatsu et al. 2008). Ammonia can be lost from the soil via the process of volatilisation (Jarvis et al. 2011). Ammonium ions are constituted of anhydrous ammonia (NH$_3$) with a hydrogen molecule attached (H$^+$) (Killham, 1994). The removal of the hydrogen ion by negatively charged ions such as hydroxyl (OH-) can result in the evaporation or volatilisation of the NH$_3$ molecule (Killham, 1994).

**Assimilation**

For plants, nitrogen is an important component of many intracellular structural, genetic and metabolic compounds. It is a major component of chlorophyll (the principal compound for photosynthesis) and amino acids, the building blocks of proteins (Taiz et al. 2006). Plants acquire nitrogen from the soil in organic form...
through the uptake of amino acids (Näsholm et al. 2009), and through inorganic forms of nitrate (NO$_3^-$) and ammonium (NH$_4^+$), incorporating them into organic nitrogen compounds via enzymatic processes (INDITE, 1994). The assimilation of ammonium is energetically less costly than nitrate which has to be reduced to ammonium before it can be assimilated (de Graaf et al. 1998; Glass et al. 2002), however, due its inherent cytotoxicity, ammonium must be assimilated into amino acids immediately (Neuhauser, 2007; Butterbach-Bahl & Gundersen, 2011) whereas nitrate can be stored in plant vacuoles prior to assimilation (de Graaf et al. 1998).

**Anthropogenic perturbation of the nitrogen cycle: N emissions and deposition**

*Emissions*

Intensive anthropogenic activity has had a substantial effect on the global nitrogen cycle by increasing inputs of reactive N by emissions of oxidised and reduced nitrogen (Vitousek et al. 1997). From 1860, to the early 1990’s, anthropogenic N inputs are considered to have increased from ~ 15 Tg N yr$^{-1}$ to 156 Tg N yr$^{-1}$ across regions spanning Asia, Europe and North America (Galloway et al. 2004). By 2050, these inputs are predicted to increase to ~ 270 Tg N yr$^{-1}$, and will have a broader global impact (Galloway et al. 2004).

Emissions of nitrogen oxides (NO$_x$) primarily result from road traffic and other combustion processes such as the power supply industry. Global emissions are estimated to be in the region of 23 Tg N yr$^{-1}$ (Galloway & Cowling, 2002). In the UK, NO$_x$ emissions for 2009 represented 1.1 Mt (1100,000 tonnes) N yr$^{-1}$ (NAEI, 2011) (Figure 1.2a). European targets for a 50 % reduction of NO$_x$ by 2010 were agreed at the UN Convention on Long-Range Transboundary Air Pollution (UN-CLRTAP) in 1998 and supported by the 1999 Gothenburg Protocol (Erisman et al. 2003). Whilst total emissions have decreased in the EU by 36 % (EEA, 2008), many member states, including the UK, have failed to reach the 2010 emissions target (ROTAP, in press), consequently the Gothenburg Protocol is currently under revision to include more stringent emissions ceilings for 2020 (UNECE, 2010).

Recent emission inventories from several countries attribute approximately 90 % of the total ammonia (reduced N) released to the atmosphere, to agricultural sources. Livestock waste management and inorganic N fertilisers are the critical emission factors, releasing in both cases volatile ammonia, amines and nitrogen oxides to the atmosphere. These substances then form ammonium nitrate or
ammonium sulphate aerosols, or, alternatively they may leach into groundwater (Misselbrook, et al. 2000). In 2007, total NH$_3$ emissions from the European Union were in the region of 4 Tg (4000 kt) N yr$^{-1}$. Figures from the UK estimate annual total ammonia emissions as at 2009 to be in the region of 0.29 Mt (290, 000 tonnes) N yr$^{-1}$ (NAEI, 2011) (Figure 1.2b). As at 2009, the UK was on track to reach UNECE ammonia emissions targets (ROTAP, in press).

**Figure 1.2**: 2009 UK emissions maps for (a) nitrogen oxides (NOx) and (b) ammonia (NH$_3$). (courtesy of www.defra.gov.uk/pollutant-maps).

**Nitrogen deposition**

Nitrogen (oxidised and reduced N) is deposited from the atmosphere in both wet and dry forms. Wet deposition (or scavenging) occurs when nitrogenous gases and aerosols are removed from the atmosphere by precipitation and fog and as such, is considered to dominate N loads in the uplands of Europe where precipitation rates are (a) high, and (b) the topography influences orographically enhanced cloud precipitation over hilly or mountainous areas (Hertel et al. 2011). Dry deposition is the direct input of nitrogenous gases or aerosols to terrestrial and marine surfaces, and
is a continuous process that is influenced by turbulent transport processes in the atmosphere’s boundary layer, the surface capacity to capture or absorb particles and gases, and the nature of the depositing N species (Hertel et al. 2011).

Modelled N deposition at the European scale show that north-westerly regions receive the highest loadings of N deposition, and, that at an ecosystem level, deposition to forests is significantly higher than to semi-natural areas (up to 60 kg N ha\(^{-1}\) yr\(^{-1}\)) is deposited to forests in Central and Western Europe, whilst the range for short vegetation is roughly half, i.e. 3 – 30 kg N ha\(^{-1}\) yr\(^{-1}\), Dise et al. 2009). In Nordic countries, the N load is dominated by both wet and dry fractions of oxidised N, however wet deposition of reduced N is believed to account for around 20% - 30% of the total N deposited to forest ecosystems, and a larger amount for non-forested systems (Simpson et al. 2011). The most significant contributor to many areas of Europe, however, (i.e. central Europe, parts of France, UK, Ireland and the Netherlands) is dry deposited reduced N, and this is especially the case in the drier areas of southern Europe (Simpson et al. 2011).

Total N deposition in the United Kingdom is approximately 400 kt yr\(^{-1}\), and is apportioned fairly evenly between oxidised and reduced forms (Figure 1.3) (ROTAP, in press). Wet deposition of NOx is higher in the north-westerly hill areas due to greater precipitation (inclusive of the orographic effect), whilst reduced N is highest at the source area due to the solubility of NH\(_3\). In respect of dry deposition, oxidised N is highest near major roads and urban conurbations, whilst the reduced form is strongly correlated to livestock emissions, chiefly in the west of the country (ROTAP, in press). Overall, the total N deposition to the UK is dominated by the wet component in precipitation, with NOx and NH\(_3\) making similar contributions to this total (ROTAP, in press).
Ecological impacts of elevated N deposition

The anthropogenic creation of reactive N can initiate a cascade of N impacts through the global environment, resulting in a number of intended, and unintended effects (Galloway et al. 2003). Each molecule of reactive N can contribute to soil fertility, increasing crop yields that subsequently nourish livestock, and ultimately humans. In an efficient system, this reactive N is re-circulated back through the agricultural system with no losses (Sutton et al. 2011). Reactive N, is however, highly mobile, and emissions from agriculture, industry and transport, have led to an unintentional cascade of reactive N in the Earth’s atmosphere, hydrosphere and biosphere, the consequences of which, are magnified with time as the reactive N proceeds through its biogeochemical pathway (Galloway et al. 2003). The main impacts are considered

Figure 1.3: 2009 UK deposition maps of (a) total oxidised N, and (b) total reduced N. (courtesy of www.defra.gov.uk/pollutant-maps).
to include: (i) the accumulation of reactive N, causing declines in biodiversity through the facilitation of nitrophilous species and competitive exclusion of characteristic species (Bobbink et al. 1998); (ii) toxic effects on species through the accumulation of NH$_4^+$ ions, in systems where NO$_3^-$ is the dominant N form (Roelofs et al. 1996; Stevens et al. 2011); (iii) reduced resistance of plants to secondary stresses such as frost (Caporn et al. 2000), herbivory, pathogen attacks (Brunsteig & Heil, 1985; Sheppard et al. 2008) and soil mediated effects of acidification and eutrophication (Bowman et al. 2008; Horswill et al. 2008); (iv) feedbacks between plant community change and N cycling, resulting in changes to foliar (and litter) N concentrations, increased litter decomposition and plant productivity all resulting in changes to N availability or residence time in N pools (Bobbink et al. 2010; Butterbach-Bach & Gundersen, 2011).

Concern about the ecological impacts of N deposition, has given rise to a large body of studies over the last two decades, with much focus on observing these impacts within N deposition manipulation experiments (Phoenix et al. 2011). Forest ecosystems were among the first ecosystems to be studied for impacts of reactive N, following the realisation that these N limited systems were becoming saturated with deposited N (Aber, 1989). Individual experiments within Europe were integrated into the NITREX project in 1991, and work carried out under this umbrella has been influential in informing policy regarding the abatement of N emissions, and the determining of critical loads (Wright & van Breemen, 1995). Broadly speaking, observed effects of enhanced N on forest ecosystems include increased tree growth rates (Crossley, 2001; Pregitzer et al. 2008), increased tree foliar N concentrations (Eriksson, 1992; Emmett et al. 1995) and decreased frequency of arbuscular mycorrhizal infection (Cawley, 2001; Read & Perez- Moreno, 2003; Liu & Crowley, 2009). Declines in the biodiversity of understory vascular and cryptogamic flora have also been recorded (Hallingback, 1992; Lamers et al. 2000; Gilliam, 2007), along with the increased retention of deposited N in the forest floor compartment (as a result of increased litterfall N fluxes and associated changes in C:N ratios (Gundersen et al. 1998; Nadelhoffer et al. 1999) and increased potential for carbon sequestration (Magnani et al. 2007; De Vries et al. 2009).

The impact of N on the diversity and species composition of grassland systems (acidic and calcareous) have been relatively well studied (Bobbink et al. 2003). Prolonged exposure to enhanced rates of N has been seen to initiate severe declines in
the species diversity of vascular plant species (Stevens et al. 2004; Clark & Tilman, 2008) and cryptogams (Morecroft et al. 1994; Arroniz-Crespo et al. 2008) with shifts towards grass dominance (Dodd, 1995; Lee & Caporn, 2001). Other impacts include increased foliar N concentrations (Morecroft, 1994; Carroll et al. 2003; Phoenix, et al. 2003a) increased inorganic soil N availability (Horswill et al. 2008; O Sullivan, 2008), soil mineralisation rates (Morecroft et al. 1994) and phosphomonoesterase (PME) enzyme activity (Phoenix et al. 2003b) and soil acidification (Pilkington et al. 2005).

Another example (and there are many) of N driven ecosystem disturbance can be drawn from bog ecosystems. Ombrotrophic bogs receive all of their nutrients from the atmosphere and are therefore highly sensitive to increased N loads. Responses include the decline (or disappearance) of sphagnum species (Press et al. 1988) and increases in vascular, or nitrophilous species (Greven, 1992; Hogg, 1995; Heijmans et al. 2001). Sheppard et al. (2008) report overall decreases in bog vegetation (Calluna vulgaris (the dominant shrub species)) bryophyte and lichen abundance. Additionally, increased concentrations of dissolved organic nitrogen (DON) in peat water have been related to N saturation of bog systems (Williams et al. 1999; Williams & Silcock, 2000).

Rapid changes to the vegetation structure of Dutch heathlands during the 1980s stimulated much research into the detrimental effects of N deposition. Dwarf shrub dominance was seen to decline dramatically in favour of nitrophilous grass species and this was attributed to eutrophication of the system (Heil & Diemont, 1983). Subsequent studies of heathland and moorland ecosystems in the UK have found strong evidence of N effects on the growth, phenology and chemistry of Calluna vulgaris (Power et al. 1998; Carroll et al. 1999), the dominant vegetative element of these habitats. Recent research has shown that reduced N has the most detrimental effect on Calluna physiological processes, inducing stomatal closure and subsequent leaf desiccation, increased foliar N uptake and reduced frost tolerance (van den Berg, 2008).

The biogeochemical consequences of increased N inputs to sensitive ecosystems also range beyond direct changes of N cycling and plant responses. Leaching of surplus N and cations in soils potentially threatens the integrity of aquatic systems; acidification and eutrophication via nitrate and cation runoff have been
reported to contribute to the degradation of freshwater, estuarine and coastal marine ecosystems (Grennfelt & Hultberg, 1986; Vitousek et al. 1997; Howarth et al. 2000). Additionally, it is estimated that an additional 10 % of anthropogenic carbon emissions may be sequestered by the land and ocean by 2030, as a result of increased forest growth due to elevated N inputs (Karjalainen et al. 2003; Reay et al. 2008). The impact on soil carbon sinks, however, depends on the balance between N induced increases in carbon inputs via stimulated plant growth and increased rates of litter production and the subsequent impact on carbon losses through decomposition, respiration and mineralisation processes (Townsend et al. 1996; Evans et al. 2006; Reay et al. 2008).

A balance of soil N and P (phosphorus) is believed to be a critical factor in the maintenance of ecosystem diversity (Elser et al. 2007), however, widespread evidence suggests that high inputs of atmospheric N has changed the nutrient dynamics in many systems from N limitation to P limitation (Kirkham, 2001; Bobbink & Lamers, 2002). In such cases, plant productivity, although no longer limited by N, becomes limited by P, indicating that floristic change in some N saturated systems may be driven by mechanisms other than competitive exclusion by nitrophilous species, such as the promotion of P loving species or soil acidity (Roem & Berendse, 2002; Phoenix et al. 2003).

Critical loads
Widening international concern in regard to the observed impacts of atmospheric N deposition, have led to the development of the critical load concept (Nilsson & Grenfelt, 1988; Hettelingh et al. 2001). The critical load, as defined by the Convention of long-range transboundary air pollution (CLRTAP) (UNECE, 1999), is a quantitative measure that represents the maximum level of pollutant deposition an ecosystem can be exposed to before detrimental, possibly irreversible, changes occur. Exceedance is the term used for excess over the critical load, and the greater the exceedance, the greater the risk of damage. Critical loads vary between habitats and are subject to review. For lowland heath ecosystems, The critical load range is currently set as 10 - 20 kg N ha \(^{-1}\) yr \(^{-1}\) for lowland heath, 15 - 25 kg N ha \(^{-1}\) yr \(^{-1}\) for calcareous grasslands and 5 - 10 kg N ha \(^{-1}\) yr \(^{-1}\) for bog ecosystems (Bobbink et al. 2011).
The heathland habitat

Heathlands occur across the world and are defined by similar soil and climatic characteristics (Gimingham, 1972). In Europe, heathlands are restricted to the North-Western areas of the continent extending from the north coast of Spain northwards through Brittany and Normandy in France, continuing into Belgium, the Netherlands, the north German plain up to Jutland in Denmark, the British Isles and the southern provinces of Norway and Sweden (Figure 1.4; Webb, 1986).

![Map of Europe showing the distribution of heathlands](image)

**Figure 1.4:** The Distribution of European heathlands (Webb, 1986).

Heathland areas generally occur on free draining, nutrient poor, acidic soils that have developed a characteristic podsolic profile (Gimingham, 1960). Mineral and organic matter are leached through the soil via rainwater and deposited at water table level, depleting the upper horizons of the nutrients needed by plants to survive (Symes, 2003). The geographical distribution of suitable soils is influenced by underlying geology and historical geophysical processes (glaciation, erosion and leaching). Climate is another important factor as moderate and regular rainfall (between 600 mm
and 1,100 mm per year) in mild conditions is key to the formation and maintenance of podsolic soils (Symes, 2003).

The European geographical distribution of heathlands (and its wide gradient of climatic and geological factors) results in a varied plant community composition despite the restrictions of a generally acidic, oligotrophic habitat (Gimingham, 1972). Gimingham (1972) classifies the main community compositions found in the heathlands of Western Europe as: (1) Montane heaths (Scandinavia, Britain and central and southern Europe); (2) Dry heaths: lowland and upland (subdivided into three broad categories (i) highly oceanic western seaboard (western Britain, Brittany, Scottish highlands, coastal south west Norway); (ii) predominantly northern (Norway, south-west Sweden, Denmark and Scotland); (iii) Southern heaths (north Germany, south-west France and northern Spain); (3) Humid / Wet heaths (widespread in oceanic and some northern areas). Floristically, heathland vegetation can be considered as a continuum of variation, with dwarf shrub species (*Calluna vulgaris* and members of the *Ericaceae*) dominant within most communities (Gimingham, 1972; Rodwell, 1998), however, distinct phytosociological communities can be determined within this. Quantitative floristic analyses carried out in the UK by Rodwell et al. (1998) characterised 22 discrete communities within the broader *Callunetum*; five types of lowland heath (including three transitions to damper sub-shrub vegetation), two types of maritime heath, four sub-montane heaths with two transitions to sub-alpine vegetation and six lichen or moss dominated (*Racomitrium languginosum*) montane heaths.

**Conservation of heathland**

The heathland habitat is largely a semi-natural one; the result of man’s influence upon the landscape (English Nature, 2002). It is accepted that the post-glacial primeval forest that covered much of what is now Europe was cleared by early humans for animal husbandry and settlements; the continual pressure of clearing and grazing suppressed natural forest regeneration and as a result vast expanses of heathland developed (Webb, 1986). Due to the infertile nature of heathland areas, agrarian activity was confined to animal grazing and fuel collection, however, the industrialisation of Britain in the eighteenth century meant that, with improved technology and the means to manufacture inorganic fertilisers, previously infertile
land could be utilised for agricultural and silvicultural purposes (English Nature, 2002). The most rapid losses of European heathland occurred after the second World War with an increase in urban development and a decline of pastoralism in favour of intensive farming practices (Symes, 2003). For areas not converted for modern purpose, the neglect brought about by the cessation of traditional management resulted in the degradation of many heathland areas by encroaching scrub and inevitable natural succession to woodland (English Nature, 2002). By way of example, in the UK, approximately 86 % of the Breckland heathland was lost between 1934 – 1980 to forestry, agriculture and military bases (JNCC, 2001). Similarly, loss of Dorset heathland is estimated as being 75 % during the 20th Century (JNCC, 2001). Overall, an estimated 375,000 hectares of the UK’s heathland has been lost to agricultural, forestry, mining and urban development (roads and housing) since the 1800s (English Nature, 2002).

The recognition of the need to protect this habitat from further degradation has led to the inclusion of heathlands as a conservation priority in the EC Habitats Directive (92/43/EEC). Lowland heathland in particular has high conservation importance due to its status as a rare and threatened habitat (JNCC, 2001), and many of these areas in the UK are designated Sites of Special Scientific Interest (SSSI) (Wildlife and Countryside Act, 1981) in recognition of the wide range of plant, inveterbrate and bird species that are solely restricted to this habitat (English Nature, 2002). Additionally, the Government’s ratification of the Convention on Biological Diversity (CBD) in 1992 led to the establishment of the UK Biodiversity Action Plan (1995) which cites lowland heath as a priority habitat for conservation.

Lowland heathland

The UK represents a large and important proportion of Europe’s lowland heathland (approximately 20%). Covering an area of approximately 58,000 hectares, 55% of this area is represented in England alone (JNCC, 2001). The habitat is generally found below 300 m altitude from East Anglia across the south of England to Cornwall. Scattered deposits occur in the northerly parts of England, Wales and Scotland, however, these are typically small remnant areas (English Nature, 2002).

The habitat is characterised by a few ericaceous dwarf shrub species that are adapted to survive in relatively impoverished conditions. The dominant species are
Calluna vulgaris (Heather), Erica cinerea (Bell Heather) and Erica tetralix (Cross-leaved Heath), depending on the level of the water table. Other important plants characteristic of lowland heathland are fabaceous shrub genera Ulex, Genista and Cytisus, and Poaceae species Molinia caerula and Deschampsia flexuosa in particular. Rodwell (1998) describes the following lowland heath communities and their typical geographical distribution in the British Isles.

- **H1** – Calluna – Festuca. East Anglia and the South Eastern counties.
- **H2** – Calluna – Ulex minor. From the Weald to the Hampshire Basin.
- **H6** – Erica vagans – Ulex europaeus. South Western Peninsula.
- **H8** – Calluna – Ulex gallii. Westerly regions.
- **H9** – Calluna – Deschampsia. Southern Pennines.

Lowland heathland supports breeding populations of rare birds such as the nightjar, woodlark and Dartford warbler. It is the exclusive habitat of the sand lizard and smooth snake (both endangered species) as well as the rare ladybird spider, southern damselfly and large marsh grasshopper, the presence of which highlights the conservation importance of this habitat (English Nature, 2002).

**Atmospheric nitrogen effects on lowland heathland**

As a typically N limited system, increases of atmospheric N inputs threaten to unbalance and possibly permanently alter the specialised mechanisms that have developed over thousands of years to facilitate growth in lowland heathland systems. The physiognomical features of lowland heathland vegetation reveal physical adaptations to the environment. For example, the dominant plant species Calluna vulgaris, is an evergreen, closed-canopy forming plant of low stature. These provide a competitive advantage over species that cannot colonise under the intact canopy or have shorter growing seasons (Gimingham, 1972). Retarded nutrient cycling as a result of low nutrient availability benefits Calluna’s slow growth rate at the expense of faster growing, nutrient demanding species (Gimingham, 1972; Heil & Diemont, 1983). Additionally, the productivity of Calluna is supported by mycorrhizal associations with ericoid fungi that enable increased tolerance to soil acidity and provide access to valuable soil nitrogen and phosphorus (Read, 1991; Caporn et al.
In respect to these adaptations, increased N has been shown to accelerate *Calluna* growth rates, increased shoot extension, canopy height (Caporn et al. 1995b; Carroll et al. 1999; Sheppard et al. 2008) and phenological processes such as flowering (Britton & Fisher, 2007) and springtime bud-burst (Power et al. 1998). A direct correlation has been seen to exist between N addition and a decline in mycorrhizal infection (Caporn et al. 1995a; Egerton-Warburton et al. 1997) and increased levels of foliar tissue N have also been observed (Bobbink, 1994; Pitcairn et al. 1995; Power et al. 1998). Alterations in the nutrient balance of foliar tissue has shown to increase *Calluna* susceptibility to frost and drought damage as well as increasing vulnerability to insect attack (specifically the heather beetle) due to higher nitrogen availability (Pitcairn et al. 1995; Carroll et al. 1999; Saebo et al. 2001).

Evidence also indicates that enhanced N deposition modifies the soil environment, by decreasing soil pH and increasing demand for other nutrients such as phosphorus (Pilkington et al. 2005; Green, 2005). Nitrogen manipulation experiments have shown that additions of N increased both litter production and litter N concentrations (Lee et al. 1998). As a result of higher N inputs from organic matter, a decrease in soil C:N ratios can be expected, leading to faster nutrient turnover through increased rates of N mineralisation by microbial activity (and the subsequent release of NH$_4^+$) (Waldrop et al. 2004; Rao et al. 2009). A surplus of mineralised N in the system can then lead to increased fluxes of trace gases by nitrification / denitrification, leaching or stimulated rates of immobilisation (Johnson et al. 1998). Increases in nutrient turnover threaten the integrity of the *Calluna* dominated system, potentially altering species composition. Heil & Diemont’s (1983) seminal work of elevated N impacts on Dutch heathland systems, observed that at relatively low inputs of N (1.75 – 28 kg N ha$^{-1}$, yr$^{-1}$) *Calluna* was replaced by the grass species *Festuca ovina*. The mechanism enabling this shift in species competition was the opening of the *Calluna* canopy by heather beetle attack, which created space for grass invasion. The temporary replacement of *Calluna* with the grass *Deschampsia flexuosa* following N addition was also observed at Budworth Common, Cheshire by Cawley (2001) with *Deschampsia* abundance increasing at N loads of 20 – 120 kg N ha$^{-1}$, yr$^{-1}$.
The impacts of nitrogen deposition on heathland ecosystems: research aims and thesis outline

The work presented herein, aims to provide further supporting evidence of atmospheric N impacts on heathland (predominantly lowland heath) ecosystem structure and function, and to present novel findings that will contribute to this globally important issue. The central research questions investigated in this study are as follows:

- Chapter 2 – What are the long term responses of heathland ecosystems to nitrogen deposition, climate stress and wildfire?

In this chapter, data collected from the Thursley Common N manipulation study from 2008 to 2010 is viewed in relation to past findings (since 1998) in order to provide a long-term overview of continued enhanced N inputs to lowland heathland structure and function. This long-term approach (as opposed to previous discrete three year studies) aims to facilitate the identification of trends and threshold responses to N addition over time, as well as providing an opportunity to investigate the impact of stochastic events (e.g. wildfire, extreme weather conditions) on heathland systems exposed to elevated N deposition.

- Chapter 3 - Can heathland responses to, and indicators of, N deposition be identified on a nationwide scale?

In this chapter, a range of biological and biogeochemical indicators of N deposition that have been identified in manipulation experiments, are investigated on a broader, nationwide scale, to include lowland and upland heathland habitats. This work specifically aims to quantify relationships between atmospheric N deposition and plant community composition as well as N driven effects on biogeochemical processes (e.g. nutrient turnover, enzyme activity). The findings from this study aim to provide a current view of the status of British heathlands in regard to differing N pollution loads, and discusses whether robust, repeatable indicators of ecosystem change can be identified.

- Chapter 4 – How do N deposition inputs influence the post-fire recolonisation and diversity of heathland cryptogamic flora?
The long-term data from the Thursley experimental site highlight the sensitivity of heathland lower plants to N additions, however, measurements have typically been made in relation to functional types *en masse* rather than identifying species-specific responses. This study aims to provide a detailed floristic study of lower plant species found within the experimental plots five years after a wildfire had eradicated all vegetation from the site. Based solely on floristic data, the influence of N additions on the species composition and diversity of recolonising lower plants are presented, and species either tolerant of, or highly adverse to, N inputs and N mediated effects are identified.

Finally, Chapter 5 synthesises the findings outlined in the previous chapters, and where possible, contextualises them in relation to each other, and in regard to the wider field of N deposition research. Relevance of this study to the overall field of N deposition impacts research is also discussed, along with recommendations for further work.
Chapter 2

LONG TERM RESPONSES OF HEATHLAND ECOSYSTEMS TO NITROGEN DEPOSITION, CLIMATE STRESS AND WILDFIRE

Abstract

Increases in the emissions and associated atmospheric deposition of nitrogen (N) have the potential to cause significant changes to the structure and function of N-limited ecosystems. Here we present the results of a long-term (13 year) experiment assessing the impacts of N addition (30 kg ha\(^{-1}\) yr\(^{-1}\)) on a UK lowland heathland under a wide range of environmental conditions, including the occurrence of prolonged natural drought episodes and a severe summer fire. Our findings indicate that elevated N deposition results in large, persistent effects on \textit{Calluna} growth, phenology and chemistry, severe suppression of understorey lichen flora and changes in soil biogeochemistry. Growing season rainfall was found to be a strong driver of inter-annual variation in \textit{Calluna} growth and, although interactions between N and rainfall for shoot growth were not significant until the later phase of the experiment, N addition exacerbated the extent of drought injury to \textit{Calluna} shoots following naturally occurring droughts in 2003 and 2009.

Following a severe wildfire at the experimental site in 2006, heathland regeneration dynamics were significantly affected by N, with a greater abundance of pioneering moss species and suppression of the lichen flora in plots receiving N additions. Significant interactions between climate and N were also apparent post-fire, with the characteristic stimulation in \textit{Calluna} growth in +N plots supressed during dry years. Carbon (C) and N budgets demonstrate large increases in both above- and below-ground stocks of these elements in N-treated plots prior to the fire, despite higher levels of microbial activity and organic matter turnover. Although much of the organic material was removed during the fire, pre-existing treatment differences were still evident following the burn. Post-fire accumulation of below-ground C and N stocks was increased rapidly in N-treated plots, highlighting the role of N deposition in ecosystem C sequestration.
Introduction

Emissions and deposition of reactive N have increased substantially since preindustrial times (Galloway et al., 2004). Perturbation of the global N cycle has far reaching implications for the structure and function of sensitive ecosystems that have low nutrient demands but a high capacity for nutrient retention and recycling. Studies across a broad range of ecosystems have demonstrated responses to elevated N inputs that range from changes in plant growth and community composition to effects on microorganisms and biogeochemical cycling (Emmett et al., 1998; DeVries et al., 2006; Madan et al., 2007; Bobbink et al., 2010; Pardo et al., 2010).

Heathlands form part of the biodiversity of temperate zones, with their high nature conservation value recognised through international frameworks such as NATURA 2000 and associated habitat- and species action plans for rare or endangered (typically habitat specialist) species. Their oligotrophic, low nutrient soils make heathlands highly susceptible to the threats of eutrophication from atmospheric N deposition, potentially altering species composition through the facilitation of and invasion by nitrophilous plant species (Bobbink et al. 2010). As a semi-natural system, active management underpins heathland integrity and habitat diversity. However, agricultural intensification, urban expansion and elevated inputs of atmospheric N have contributed to a decline in European heathland cover over recent decades (Rose et al., 2000; Smart et al., 2003; Barker et al., 2004).

A considerable amount of research has been undertaken to investigate the ecological implications of elevated N inputs on the structure and function of heathland ecosystems. Experimental additions of N have been shown to stimulate above-ground Calluna productivity (Heil & Diemont 1983; Power et al., 1995, Carroll et al., 1999; Marcos et al., 2003, Barker et al. 2004) and increase foliar N concentrations (Pitcairn et al., 1995; Bobbink, 1998; Power et al., 1998a). These responses have been linked with faster Calluna life cycles and increased susceptibility to damage from insect herbivores, frost or drought (Power et al., 1998b; Carroll et al., 1999; Saebo et al., 2001). Additionally, N deposition promotes the build up of soil (microbial) N stores and increases rates of nutrient cycling (Berendse, 1990; Power et al., 1998a; Nielsen et al., 2000; Green, 2005), facilitating invasion by nitrophilous grasses, particularly in
areas of high N deposition and where gaps exist in the dwarf shrub canopy (Van der Eerden et al., 1991; Hartley and Amos, 1999; Calvo et al., 2007). Nitrogen additions have also been seen to have dramatically detrimental effects on lichen and bryophyte groups, with declines in non-vascular plant cover observed in ecosystems ranging from arctic tundra (Soudzilvskaia et al., 2005) to alpine heath (Britton & Fisher, 2007).

Long-term experiments play a fundamental role in identifying signals of environmental change and the drivers that prompt it (Silvertown et al., 2010). The long-term N manipulation study at Thursley Common (Surrey, UK), established in 1998, has assessed ecosystem responses to global change events over a significant proportion of the 20-30 year Calluna life cycle (sensu Gimingham, 1972). Long-term experiments such as this provide unique opportunities to identify the effects of stochastic climate-driven events (e.g. drought and wildfire) on ecosystems under differing N loads. Importantly, they provide valuable information on ecosystem function under a range of (naturally varying) climatic conditions, offering insight into the way in which future climate change will modify the ecological impacts of continued elevated inputs of reactive N.

Fire, in the form of a controlled burn, is a vital element of heathland management, removing nutrients accumulated in above-ground biomass and surface litter, whilst ensuring a vigorous, mixed aged stand of Calluna (Webb, 1998). Wildfires, however (whether natural or anthropogenic in origin), are detrimental to regeneration dynamics, potentially generating temperatures high enough to destroy valuable surface organic horizons, plant roots, buds and the seedbank (Gimingham, 1972; Legg et al., 1992; Hawley et al., 2008). In Mediterranean dwarf-shrub and forest systems, increased wildfire frequency has been seen to drive changes in the biomass and composition of soil microbial communities (Barcenas-Moreno et al., 2011) and shifts in vegetation structure from woody- to herbaceous- dominated communities (Malkinson & Wittenberg, 2011). Similar, wildfire-driven changes to characteristic successional pathways have been observed in Chilean Nothofagus stands (Vidal & Reif, 2011) and in temperate forests in the Western United States (Peppin et al., 2011). High soil nutrient contents after a fire can also promote a
dominance of invasive plant species, potentially altering community diversity, structure and ecological function (Brooks et al., 2004; Coffman et al., 2010).

During the very dry UK summer of 2006, the long-term experimental plots at Thursley Common National Nature Reserve, and much of the surrounding heath, were substantially burnt by a severe fire. Given the importance of understanding (and predicting) the impacts of future global change on habitats of nature conservation importance, this paper presents a long-term perspective on interactions between N deposition, climate stress and fire in a heathland ecosystem. Specifically, we test the hypotheses that: (i) plant and biogeochemical responses to N are highly persistent over time and are influenced by climatic conditions; (ii) elevated N deposition accelerates the rate of vegetation recovery following fire; and (iii) prolonged N addition offsets the effects of fire on ecosystem C and N budgets.

Materials and Methods
Experimental site and design
The experimental site is situated within an area of Calluna-dominated lowland heath (NVC type H2, Eunis code F4.2) at Thursley Common National Nature Reserve, Surrey, UK (Latitude: 51°.9’22”.N; Longitude: 0°.41’, 58”.W). The site’s physical and chemical characteristics are summarised in Table 2.1. Of particular note is the very shallow (pre-fire) humus layer and low (7% pre-fire, 2004) soil organic matter content; in 2006, the fire removed the majority of the organic layer and this remained negligible for the following 4 years.

Background N deposition at the site is estimated to be approximately 8-12 kg ha⁻¹ yr⁻¹ (Power et al., 2006). The experimental area comprises four replicate blocks, separated by a distance of 20-100m, encompassing the topographic heterogeneity within the site. Each block contains four 4 x 4 m plots, all of which are separated by a 1m wide buffer strip. Two plots per block are assigned to control and two to N addition treatments; these are treated as duplicate plots for statistical analysis. Each plot is further sub-divided into four 2 x 2 m sub-plots, reflecting different initial (1998) management treatments. These are described in Barker et al. (2004) but, since management effects were not generally significant after the first three years of the experiment, management treatments are not considered here. Nitrogen, in the form of
(NH₄)₂SO₄, has been added every two weeks since April 1998, at a rate of 0 (control) or 30 (+N) kg ha⁻¹ yr⁻¹ (equivalent to 0 or 4.56mM NH₄⁺, respectively). Ammonium sulphate was used for comparability of results with an earlier experiment at the same site which started in 1989, when ammonium co-deposition with sulphate occurred widely across the UK (NEGTap, 2001). Treatments are added as 15 l per 16 m² plot on 26 occasions each year, with additions therefore representing 7% of the annual average rainfall amount recorded on site (Barker et al., 2004). Solutions are applied using a fine nozzle knapsack sprayer in order to simulate as closely as possible the natural mode of atmospheric deposition.

**Table 2.1:** Physical and chemical properties of the Thursley site.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>O horizon</th>
<th>A horizon</th>
<th>E horizon</th>
<th>B horizon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm)</td>
<td>0 - 1</td>
<td>1 - 5</td>
<td>5 - 22</td>
<td>22 - 50 +</td>
</tr>
<tr>
<td>% sand</td>
<td>99.3</td>
<td>99.3</td>
<td>99.2</td>
<td>99.1</td>
</tr>
<tr>
<td>% silt</td>
<td>0.66</td>
<td>0.68</td>
<td>0.73</td>
<td>0.75</td>
</tr>
<tr>
<td>% clay</td>
<td>0.01</td>
<td>0.03</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Bulk density (g / cm³)</td>
<td>1.37</td>
<td>1.45</td>
<td>1.36</td>
<td>1.41</td>
</tr>
<tr>
<td>pH</td>
<td>4.91</td>
<td>4.82</td>
<td>4.74</td>
<td>4.71</td>
</tr>
<tr>
<td>Loss on ignition (%)</td>
<td>1.8</td>
<td>2.13</td>
<td>2.56</td>
<td>1.16</td>
</tr>
<tr>
<td>Management</td>
<td>Plots burnt/mowed February 1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N additions commenced</td>
<td>April 1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil type</td>
<td>Podsol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humus layer depth (2004)</td>
<td>1.0 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Measurement of plant responses**

Point quadrat measurements (Hill et al., 1992) were carried out at the end of every growing season (mid-October to early-November). A pin was lowered through the canopy of each subplot at 25 evenly distributed points to collect data on each of the following: Calluna annual shoot length (measured as the distance from the tip of the shoot to the bud scales denoting the start of the current year’s growth), shoot flowering (whether the first Calluna plant touched by the pin had flowers on its terminal shoot), canopy height (at first pin touch) and cover repetition (i.e. the number of times the pin touched Calluna as it was lowered through the canopy). Percentage frequency of Calluna, other vascular plants (specifically Erica cinerea and Ulex
laborious) lichens and bryophytes were also recorded, based on the number of pin touches out of 25 points per sub-plot.

The widespread appearance of brown Calluna shoots across the site in 2004 led to the initiation of annual assessments of shoot discolouration from this time. This was achieved by noting the presence or absence of damaged (brown) shoots at every sampled point per sub-plot during autumn point quadrat assessments. Annual assessments of the timing of spring bud burst were made in April / May by recording the presence or absence of open buds on sixteen randomly selected Calluna shoots per sub-plot.

Calluna foliar N concentrations were measured using current year’s shoot material collected at the end of the growing season (mid October-mid November). Samples were oven dried at 80°C for 48 hours and then ground into a fine powder in a ball mill (Glen Creston Ltd., London, UK). Every year from 1998 to 2005 samples (200 mg) were digested (following the Kjeldahl method, Allen et al., 1989) in 3 mL of sulphuric acid with a selenium catalyst before being analysed using a SKALAR sanflow ++ segmented flow analyser (Skalar UK (Ltd), York, UK). In 2008 foliar N and carbon (C) concentrations were determined using a FlashEA 1112 CN analyser (Thermo Scientific, UK). Between 15 –20 µg of milled Calluna material was weighed into tin cups prior to analysis. Duplicates were run for each sample, with greater than 95% precision obtained between sample replicates; samples were calibrated against standards prepared with aspartic acid.

Soil pH
In 1999, 2002, 2004, 2007 and 2010, 15 g of fresh soil from each sub-plot was mixed with 30 ml of distilled water and shaken on a rotary shaker for 30 minutes. Samples were measured for pH using a Mettler Toledo (Switzerland) pH meter which had been calibrated using pre-prepared solutions at pH 4 and 7.

Soil phosphatase enzyme activity assay
A 1 g sub-sample of fresh soil collected in May 2009 was placed in a 50cl centrifuge tube, with 4 ml of distilled water, 0.25 ml of toluene and 1 ml of 0.115 M p-
Nitrophenyl phosphate (PNP) solution (following the method described by Tabatabai & Bremner, 1969). Samples were mixed on a rotary shaker for one hour, after which 1 ml of 0.5 M calcium chloride (CaCl$_2$) and 4 ml of 0.5 M sodium hydroxide (NaOH) were added. Samples were then centrifuged for 5 minutes at 3000 rpm and the resulting supernatant liquid was analysed colorimetrically (using a Perkin Elmer Lambda 3 UV spectrophotometer, Perkin Elmer, USA) at a wavelength of 460 nm. Paired, non reactive controls were prepared for each of the samples by adding the PNP and toluene solution immediately after the CaCl$_2$ and NaOH to prevent chemical reaction. The readings were compared with pigment densities from a calibration curve obtained using six standards prepared within the absorbance range of the samples (0 – 1000 ppm p-Nitrophenyl).

**Microbial biomass N**

Microbial biomass N was measured during the summer of 2002 using the chloroform fumigation extraction method developed by Brookes *et al.* (1989). In order to minimise degradation, samples were processed within 24 hours of collection. 40 g of fresh soil was extracted with 1 M KCl. Samples were digested and analysed colourimetrically for total N concentration using the SKALAR san flow ++ segmented analyser described above. A paired sample was fumigated with ethanol-free chloroform for 48 hours before being processed in the same way as the unfumigated samples. Microbial biomass N was calculated from the difference in the amounts of N extracted in the fumigated and unfumigated soils and expressed as mg per g soil.

**Soil C and N concentrations**

Soil was sampled (0-10cm depth) in June 2003, October 2006 and May 2009. Samples were dried at 80°C for 24 hours and then ground to a fine powder using a ball mill (Glen Creston Ltd., London, UK). 15-20 µg of ground material was weighed into tin capsules and C and N concentrations were determined using a Thermo Scientific FlashEA 1112 CN analyser (Thermo Scientific, UK).
Carbon and N stock calculation

Above-ground: The relationship between canopy density (as determined by point quadrat measurements) and Calluna biomass was derived by measuring the mean number of pin-hits in replicated 1m$^2$ quadrats and the dry weight of all harvested biomass within these, for a range of Calluna growth phases (pioneer to mature, *sensu* Gimingham, 1972) outside of the experimental area. The regression equation for this relationship ($y_{(biomass)}= 117.4 \times (pin \text{ hits})+38.9; \ r^2=0.743, \ RSD=347.3, \ d.f.=43$) was then applied to annual canopy density measurement data taken from the experimental blocks to derive treatment level biomass estimates. The potential for effects of treatment N addition on biomass-canopy density relationships was tested using destructively harvested data and concurrent point quadrat canopy density measurements from within treatment plots in an earlier experiment at the same site, following 7 years of experimental N additions (Power *et al.*, 1998). Density-biomass relationships did not differ significantly between control and +N treatments, validating use of the regression equation obtained from outside of experimental plots.

Standing stock estimates were derived by multiplying biomass with measured C and N concentration values from that same year. Above-ground stocks have been calculated for 2005 (the year before the fire) and 2008 (the first year of vegetation analysis post-fire).

Below-ground: Total soil C and N concentrations were multiplied by plot-specific measurements of bulk density to calculate soil C and N contents (g m$^{-2}$) in the top 10 cm of soil. Below-ground stocks were calculated for 2003, 2006 and 2009; C and N concentration data were not available for 2005, so 2003 data have been used to provide an assessment of treatment effects on pre-fire stocks.

The overall retention of experimental N inputs within the system (plant + top 10 cm soil) has been calculated for pre- and post-fire periods. Pre-fire calculations are based on treatment inputs of 30 kg N ha$^{-1}$ yr$^{-1}$ from April 1998 (when N additions began) to June 2003 (when the last set of pre-fire soil N concentration measurements were made) and, since no 1998 baseline soil N data are available, N accumulation was taken as the difference between control and +N above and below-ground stocks in June 2003. Post fire calculations are based on N accumulation in +N plots from
October 2006 measurements (when treatments re-started following a fire-related 3 month suspension of additions) to October 2009, when concentrations were again measured.

Data analysis
Replicate plot data were averaged prior to analysis using the ‘R’ statistical package (version 2.10.1) (R foundation for statistical computing, 2009). The effects of N and management were investigated using analysis of variance (ANOVA). Generalised linear models (GLM) were used for percentage and count data. Binomial error correction was applied in GLM to percentage and count data and poisson error correction was applied to proportional data. Mixed-effects models were used for repeated measures analysis of the effects of N treatment across the full dataset.

The effects of climatic variables, and interactions with N, were analysed using regression models for *Calluna* growth parameters (annual shoot length, flowering, canopy height and cover repetition) and foliar N concentration, with N treatment, rainfall and temperature means modeled as explanatory variables. Temperature and rainfall data (courtesy of the British Atmospheric Data Centre (BADC)) were averaged for a) the year, b) the growing season (April – September). Analyses were run separately for (i) the full 13 year data set, (ii) the 8 year pre-fire dataset (1998-2005) and (iii) the 4 year post fire dataset (2007-2010). Maximal models (inclusive of the main effects and their possible interactions) were simplified using likelihood ratio deletion tests, and compared using ANOVA, until the minimal adequate model was attained (Crawley, 2007).

Results

*Calluna productivity and chemistry*

Table 2.2 summarises *Calluna* shoot length, canopy height, cover repetition and percentage frequency data, highlighting highly significant effects of N in most years since the experiment commenced, and significant overall effects of N using repeated measures analyses. Treatment differences in annual shoot extension are illustrated in
Figure 2.1, showing negligible effects of N additions in the first growing season of the experiment (treatment additions began only in April 1998) but, consistent, significant effects of N addition on shoot length from 1999-2005; treatment differences ranged from 8% (2000) to 106% (2002) increases in +N plots compared to controls. Rapid shoot growth was observed in the two years of vegetation development following initial management removal of all standing biomass in 1998 (as were increases in *Calluna* frequency and cover repetition), and this can be attributed to the higher availability of nutrients (and light) following burning and mowing managements. A similar response was observed in 2007 where *Calluna* shoot growth was invigorated immediately following the 2006 wildfire. Despite the severity of the fire, the effects of N additions on regenerating *Calluna* were highly significant in the first year after the site burnt, with shoot growth 75% greater in +N plots compared to controls in 2007 ($F = 26.0, P < 0.001$). Effects of N addition were, however, not significant from 2008-2010.

Inter-annual variation in shoot length is closely related to rainfall patterns. Figure 2.1 demonstrates the influence of precipitation as a driver of inter-annual variability in plant growth, with *Calluna* shoot length significantly related to the amount of rainfall which fell during the growing season (April - September) (+N plots: $F = 11.9, P <0.01$, control plots: $F = 8.5, P < 0.05$). Temperature was not found to be an important driver of shoot length on its own. However, interactions between growing season rainfall and temperature were statistically significant, with greater growth in warmer, wetter years ($F = 14.86, P <0.01$). Interactions between climate (temperature or rainfall) and N treatment on shoot growth were not significant either across the whole (13 year) dataset or for the pre-fire period (1998-2005). However, there was a significant interaction between growing season rainfall and N following the fire ($F=8.5, P<0.05$), with the characteristic stimulation in growth in +N plots lost during drier years.
N additions consistently increased canopy height, cover repetition (density) and the percentage frequency of *Calluna* within plots from 1999-2005 (Table 2.2). However, although repeated measures analyses revealed a significant overall effect of N on these canopy characteristics across the full 13 year dataset, this appears to be driven by the consistent pattern of treatment stimulation prior to the fire in 2006 since no effects of N were apparent after this time (Table 2.2).

A statistically significant proportional increase in foliar N concentrations in +N plots relative to controls has been a consistent feature of the experiment in all years when these analyses were carried out (Table 2.2), with repeated measures analysis demonstrating a highly significant response overall (F=59.7, P<0.001). Inter-annual variations in foliar N concentration are apparent, however, with the highest concentrations evident in the years immediately following management/fire. Regression analyses show that, whilst trends in relation to climate are evident (e.g. lower N concentrations in years with higher temperatures and lower rainfall), these were not statistically significant.

**Phenology and flowering**

Repeated measures analyses show a consistent, highly significant effect of N addition on springtime phenological activity. Typically, twice as many buds had burst on plants in +N plots compared to controls on census dates in early spring each year,
with treatment effects highly significant both before and after the fire (Table 2.2). Similarly, highly significant positive effects of N addition on the proportion of flowering shoots have been observed in most years (Figure 2.2); repeated measures analysis also showed a highly significant increase in flowering in +N plots across the full 13 year dataset (Table 2.2). The extent of flowering was also related to annual rainfall levels, with a greater proportion of flowering shoots seen in wetter years (F=5.5, P<0.05). Interactions between N and rainfall were not statistically significant, although the direction of (significant) treatment effects was reversed in 2009, one of the driest years of the experiment.

**Drought injury**

Drought injury was first recorded in 2004, following a particularly warm and dry year in 2003 (Green, 2005; Power et al., 2006). At this time, the proportion of brown, damaged shoot tips was significantly greater in plots which had received N additions (control: 12.3 %, +N: 43.3 %, F = 12.6, P < 0.001). Drought effects were again apparent five years later, following very dry conditions experienced during the early part of the 2009 growing season (April - June); these conditions also had a marked detrimental effect on *Calluna* growth and flowering which was not evident in previous years. Drought injury was significantly exacerbated by N inputs, with *Calluna* plants in the +N plots displaying substantially greater damage than those in

![Figure 2.2: Effects of N addition on the percentage of flowering *Calluna* shoots.](image)

(n = 64; * p<0.05, ** p<0.01, *** p<0.001). (error bars = ± 1 SEM).
the control plots (control: 40.2 % shoots injured, + N: 70.4 %; $F = 43.49$, $P < 0.001$). Drought effects were still apparent in 2010, with a significantly greater proportion of dead shoots in the N addition plots (58.3 %) compared to controls (37.4 %, $F = 16.37$, $P < 0.001$).

Other vascular plant responses

*Erica cinerea* was present only at very low frequencies at the site (< 2 % in 2010), with no evidence of treatment-related effects on abundance. Despite the transient occurrence of a very small number of grass seedlings post-fire, these failed to establish and grasses are virtually absent from experimental plots. In contrast, *Ulex minor*, whilst representing only a small part of the overall vegetation cover (typically <5% in most years), was found to be highly sensitive to N additions following the fire, with significantly lower post-fire percent frequency in the +N plots in 2010 (control: 16 %, +N: 2 %, $F = 18.14$, $P < 0.05$).

Non-vascular plant abundance

In the early years of this study, lichens had a higher percent frequency in the +N plots (Table 2.2, Barker, 2001). However, this effect rapidly diminished from 2001 onwards, with lichen abundance consistently and significantly reduced in the +N plots in all subsequent years (Figure 2.3). The strong treatment-related suppression of lichens was again evident following the 2006 fire, with values in +N plots as low as 5% of those recorded in control plots (2009). Repeated measures analyses revealed highly significant overall negative effects of N addition on lichen percent frequency across the 13 year dataset ($F=83.5$, $P<0.001$, Table 2.2), although N x time interactions were not significant.

Repeated measures analysis showed a significant effect of N addition on the percent frequency of bryophytes across all years, although treatment effects were less consistent between years than those observed for lichens (Table 2.2). Post-fire bryophyte recovery was, however, greatly stimulated by N addition, with significantly greater abundance in +N plots from 2007-2010.
Soil pH

Despite small inter-annual fluctuations, soil pH has been significantly and consistently reduced by N addition (Table 2.2), reflecting the form of N ((NH$_4$)$_2$SO$_4$) in which treatments are applied. Reductions of 0.3 units were apparent within 16 months of N treatments commencing (June 1999), increasing to a difference of 0.5 units after 6 years (2004). The 2006 fire resulted in an overall increase in pH of control and +N plots, although absolute treatment differences remained similar (reductions of 0.5 to 0.8 pH units in +N plots, relative to controls) after the fire (Table 2.2).

Soil microbial biomass and enzyme activity

Much of the added N stored in the soil appears to accumulate in the microbial community. Indeed, measurement of microbial biomass N in 2002 demonstrated that +N plots (12.85 mg N g$^{-1}$ soil) had significantly more microbial biomass N compared to control plots (8.3 mg N g$^{-1}$ soil) ($F = 8.94$, $P < 0.01$).

Significant N treatment effects post-fire soil PME activity were also apparent, with almost three times the level of activity in +N plots (14.1 nmol g$^{-1}$ soil hr$^{-1}$) compared to controls (4.8 nmol g$^{-1}$ soil hr$^{-1}$) ($F = 21.03$, $P < 0.001$).
**Carbon and nitrogen stocks**

Pre-fire calculations indicate that N addition significantly increased C storage in above ground biomass (84% greater than control plots in 2005, $F = 123.29$, $P < 0.001$, Figure 2.4a) (Table 2.3). Above-ground N stocks were increased by 114%, with +N plots having 0.0363 g N m$^{-2}$ compared to control plot means of 0.0169g N m$^{-2}$ ($F=215.82$, $P<0.001$) (Figure 2.4b). Post-fire (2008) estimation of regenerating *Calluna* biomass suggests a 22% increase in C accumulated in N addition plots compared to controls, and a 42% increase in stored N, just 2 years after fire had removed all above-ground material. Treatment differences at this time were, however, not statistically significant ($F=4.1$, $P=0.08$).

Based on 2003 soil C and N concentration data, five years of N addition had increased the total N content of the (top 10cm of) soil by 92% (Table 2.3, Figure 2.4d). Soil C stocks had also increased in +N plots, though by a smaller (17%), not significant amount (Figure 2.4c). In 2006, immediately after the fire, soil C and N contents were reduced by similar amounts in control (45%) and +N (46%) plots, relative to pre-fire levels. However, significant treatment effects remained for N stocks ($F=5.1$, $P<0.05$), with post fire values in +N plots N plots more than double those in the controls. Three years later, stocks of C and N in the top 10cm of the soil were again significantly enhanced in N-treated plots; in 2009 soil N stocks were 106% greater in the +N plots compared to controls, with C stocks also 54% higher in N treated plots.
Figure 2.4: Effects of N addition on above-ground (a-b) and below ground (c-d) carbon and nitrogen stocks. (n = 16; * p<0.05, ** p<0.01, *** p<0.001). (error bars = ± 1 SEM).
### Table 2.2: Treatment means for above- and below-ground parameters.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Years of N addition</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
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<td>13</td>
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<tr>
<td>Years since fire</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Annual rainfall (mm)</td>
<td>885</td>
<td>854</td>
<td>1122</td>
<td>924</td>
<td>1020</td>
<td>690</td>
<td>749</td>
<td>799</td>
<td>850</td>
<td>925</td>
<td>954</td>
<td>830</td>
<td>745</td>
<td></td>
</tr>
<tr>
<td>Growing season rainfall (mm)</td>
<td>378</td>
<td>297</td>
<td>357</td>
<td>357</td>
<td>129</td>
<td>203</td>
<td>307</td>
<td>259</td>
<td>459</td>
<td>335</td>
<td>443</td>
<td>410</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>Annual mean temperature</td>
<td>10.0</td>
<td>10.5</td>
<td>10.2</td>
<td>8.6</td>
<td>10.6</td>
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<td>10.2</td>
<td>10.1</td>
<td>10.4</td>
<td>10.6</td>
<td>10.6</td>
<td>9.3</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Growing season mean temperature</td>
<td>13.3</td>
<td>14.5</td>
<td>13.6</td>
<td>11.4</td>
<td>13.9</td>
<td>14.4</td>
<td>14.0</td>
<td>13.8</td>
<td>14.7</td>
<td>14.1</td>
<td>13.7</td>
<td>12.9</td>
<td>11.6</td>
<td></td>
</tr>
</tbody>
</table>

**Calluna shoot length (mm)**
- Control: 75 ± 103, 135 ± 18, 17 ± 26, 20 ± 21, 11 ± 37, 52 ± 41, 40 ± 40
- N: 74 ± 128, 174 ± 21, 54 ± 36, 27 ± 24, ns = 0.10
- F = 5.46, *p = 0.01

**Calluna cover repetition**
- Control: 1.6 ± 1.6, 2.0 ± 2.0, 0.8 ± 0.8, 0.9 ± 1.5, 1.6 ± 1.6, - ± -
- N: 5.1 ± 8.3, 13.0 ± 18.9, 25.0 ± 27.3, 28.4 ± 25.4, - ± -
- F = 5.7, *p = 0.03

**Calluna canopy height (cm)**
- Control: 4.9 ± 6.3, 9.1 ± 14.2, 17.3 ± 17.7, 19.8 ± 15.9, - ± -
- N: 5.1 ± 8.3, 13.0 ± 18.9, 25.0 ± 27.3, 28.4 ± 25.4, - ± -
- F = 5.7, *p = 0.03

**Calluna % flowering shoots**
- Control: 48.5 ± 67.5, 65.2 ± 56.5, 72.5 ± 36.3, 28.8 ± 25.4, 9.3 ± 52.9, 41.2 ± 46.3, 62 ± 72
- N: 52.9 ± 84.6, 91.5 ± 66.1, 95.7 ± 94.6, 24.1 ± 35.7, 42.0 ± 74.1, 20.0 ± 64.0, 64 ± 88
- F = 5.7, *p = 0.03

**Calluna frequency (%)**
- Control: 42.4 ± 73.3, 71.9 ± 50.6, 53.5 ± 70.1, 76.4 ± 79.9, 18.5 ± 45.5, 64.0 ± 50.7, 69 ± 69
- N: 51.7 ± 79.8, 88.2 ± 74.6, 78.1 ± 88.1, 89.1 ± 92.5, 20.4 ± 50.9, 66.5 ± 46.4, 70 ± 70
- F = 5.7, *p = 0.03

**Bryophyte frequency (%)**
- Control: 6.6 ± 8.0, 30.6 ± 19.0, 16.8 ± 16.8, 22.6 ± 19.6, 7.7 ± 35.7, 41.4 ± 46.3, 69 ± 69
- N: 6.7 ± 19.4, 25.9 ± 20.0, 21.0 ± 21.0, 27.4 ± 22.3, 17.1 ± 65.5, 75.8 ± 81.8, 79 ± 79
- F = 5.7, *p = 0.03

**Lichen frequency (%)**
- Control: 12.0 ± 14.0, 65.0 ± 35.0, 44.0 ± 45.0, 57.0 ± 53.0, 5.0 ± 20.0, 21.0 ± 15.0, 69 ± 69
- N: 21.0 ± 22.0, 32.0 ± 10.0, 8.0 ± 3.0, 6.0 ± 6.0, 6.0 ± 1.0, 3.0 ± 7.0, 7.0 ± 7.0
- F = 5.7, *p = 0.03

**Calluna foliar N (%)**
- Control: 1.13 ± 1.04, 0.98 ± 0.76, 0.80 ± 0.89, 1.00 ± 1.00, - ± -
- N: 1.20 ± 1.40, 1.08 ± 0.82, 0.86 ± 1.03, 1.09 ± 1.09, - ± -
- F = 5.7, *p = 0.03

**Calluna bud burst (%)**
- Control: - ± -
- N: - ± -
- F = 5.7, *p = 0.03

**Calluna drought damage (%)**
- Control: - ± -
- N: - ± -
- F = 5.7, *p = 0.03

**Soil pH**
- Control: 4.0 ± 4.0, 3.9 ± 4.8, - ± -
- N: 3.8 ± 3.8, 4.5 ± 4.5, - ± -
- F = 5.7, *p = 0.03

**Soil carbon stocks (g m⁻²)**
- Control: 1547.1 ± 1547.1, 707.6 ± 707.6, 646.9 ± 646.9
- N: 1620.7 ± 1620.7, 735.4 ± 735.4, 1001.4 ± 1001.4
- F = 5.7, *p = 0.03

**Soil nitrogen stocks (g m⁻²)**
- Control: 13.9 ± 13.9, 5.8 ± 6.0, 9.7 ± 9.7
- N: 26.7 ± 26.7, 12.1 ± 12.1, 19.9 ± 19.9
- F = 5.7, *p = 0.03

---

1 = dead Calluna plants in 2005, 2010. * Asterisks indicate significance level of treatment effect = * p < 0.05, ** p < 0.01, *** p < 0.001

Rep. Mean = repeated measures analysis; statistics relate to treatment effects of N across the full dataset.

---

### Table 2.3: Above and below ground carbon and nitrogen stocks, representing pre- and post-fire pools (2006 - 2009).

<table>
<thead>
<tr>
<th>Year</th>
<th>2003</th>
<th>2005</th>
<th>2006</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calluna carbon stocks (g m⁻²)</td>
<td>-</td>
<td>0.75</td>
<td>-</td>
<td>0.40</td>
<td>-</td>
</tr>
<tr>
<td>+ N</td>
<td>-</td>
<td>1.39</td>
<td>-</td>
<td>0.49</td>
<td>-</td>
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<tr>
<td>F</td>
<td>-</td>
<td>123.3</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Calluna nitrogen stocks (g m⁻²)</td>
<td>-</td>
<td>0.017</td>
<td>-</td>
<td>0.009</td>
<td>-</td>
</tr>
<tr>
<td>+ N</td>
<td>-</td>
<td>0.036</td>
<td>-</td>
<td>0.013</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>215.8</td>
<td>-</td>
<td>4.3</td>
<td>-</td>
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<td>P</td>
<td>-</td>
<td>***</td>
<td>-</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Soil carbon stocks (g m⁻²)</td>
<td>-</td>
<td>1547.1</td>
<td>-</td>
<td>707.6</td>
<td>-</td>
</tr>
<tr>
<td>+ N</td>
<td>-</td>
<td>1620.7</td>
<td>-</td>
<td>735.4</td>
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<td>F</td>
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<td>Soil nitrogen stocks (g m⁻²)</td>
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<td>+ N</td>
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<td>P</td>
<td>-</td>
<td>*</td>
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</tr>
</tbody>
</table>

(* = p < 0.05, ** = p < 0.01, *** = p < 0.001)
Discussion

The findings of this study provide clear evidence of sustained, N deposition-driven changes to the structure and functioning of a lowland heathland, and of increased sensitivity to drought under elevated deposition loads.

**Vascular plant responses to N deposition**

N additions of 30 kg N ha\(^{-1}\) yr\(^{-1}\) (above a background level of 8–12 kg N ha\(^{-1}\) yr\(^{-1}\)), consistently stimulated *Calluna* performance prior to the fire, with significant increases apparent in annual shoot extension, canopy height, density, abundance and flowering. Long-term datasets such as the one presented here make it possible to evaluate ecosystem responses over ecologically realistic timescales, whilst also identifying the role of drivers of observed inter-annual variation (such as precipitation and temperature) and the effects of disturbance (e.g. fire). Annual growth of *Calluna* shoots was, for example, clearly driven by the amount of rainfall in a given year. Similar climate-driven effects on the performance of ericaceous plant species and associated ecosystem processes have also been reported from a multi-site study along a naturally occurring N-S climatic gradient across Europe (Beier et al., 2004; Emmett et al., 2004; Penuelas et al., 2004; Sowerby et al., 2005). Whilst N was not explicitly manipulated in these studies, the potential for complex interactions between climate and N was highlighted, and some evidence of this is provided in the current study.

The fire which took place in the 9\(^{th}\) year of the experiment also appears to have had some effect on ecosystem responses to N. For example, post-fire re-establishment of *U. minor* was significantly lower in +N plots, compared to controls. In a similar vein, significant interactions between growing season rainfall and N treatment were only seen following fire, with N-driven stimulation of *Calluna* shoot growth lower, or even reversed, in low rainfall years after 2006. This may be associated with combustion of the soil organic layer and the implied decrease in soil water holding capacity, as has been found following intense fires elsewhere (Wright & Bailey, 1982).

Nitrogen also appeared to affect *Calluna* sensitivity to climate stress, with greater levels of shoot browning (“drought injury”) in plots receiving N additions in 2003, 2009 and 2010. Whilst the appearance of brown shoots cannot be categorically
attributed to drought damage, the fact that this phenomenon directly followed notably dry periods, was widespread across the surrounding heath area and was not related to any other damage agents (such as insect herbivory) makes this assumption likely. In addition to being one of the hottest European summers on record, 2003 experienced below average rainfall - 203 mm of rain in April-September, against a 12 year average value of 360 mm (UK Meteorological Office). Summer temperatures exceeded 30°C on 9 days (and 36 days >25°C), compared to the long-term average of 2 days (and 16 days >25°C). Whilst 2009 was nowhere near as hot, growing season rainfall was again low (264 mm, compared to 410 mm in 2008 and 443 mm in 2007). Carry over effects of the 2009 drought were further compounded by a subsequent dry spring in 2010 (the driest since 1984 with less than 50% of the average April rainfall recorded over much of England - UK Met Office, 2011); drought damage seen in 2010 may, therefore, reflect the cumulative effects of two years of unusually low rainfall.

Reduced biomass allocation to plant roots has frequently been found in response to increased N availability, and the associated reduction in root:shoot ratio has been cited as one of the reasons behind a reduction in drought tolerance of vegetation receiving high deposition inputs of N (Bobbink et al. 2010). Earlier investigation at Thursley revealed no change to root biomass in N-treated plots (Power et al., 1998b); given the observed treatment-related increases in above-ground biomass, the implied reduction in root:shoot ratios may explain the greater levels of drought injury observed in plots receiving elevated N inputs, as has been reported elsewhere for Calluna (Aerts, 1990; van der Eerden et al., 1991; Gordon et al., 1999). Similar effects on drought tolerance and associated mortality have also been seen in forest ecosystems (Archaux and Wolters, 2006; van Mantgem et al., 2009).

**Non-vascular plant community response**

Cryptogams have shown considerable sensitivity to enhanced N additions in ecosystems ranging from tundra (Baddeley et al., 1994; Soudzilovskaia et al., 2005) and semi-arid deserts (Evans and Belnap, 1999) to alpine, lowland and upland heathland (Carroll et al., 1999; Power et al., 2006; Britton and Fisher, 2007; Haworth
et al., 2007; Pilkington et al., 2007). Profound effects on lichen abundance have been observed in response to modest N inputs in the present study, although the direction of response has changed over time; early treatment-related stimulation in the percent abundance of lichens (1998-2000, when the regenerating Calluna canopy was relatively open) was followed by significant (and increasing) decline in N-treated plots (2001-2005). N-driven stimulation of the Calluna canopy (and, as a consequence, reduced light transmission) has been suggested as the principal driver of declines in understorey communities (Lee & Caporn, 1998). After the fire in 2006, strong effects of N treatment on re-vegetation dynamics were apparent, with lichen regeneration severely suppressed in the +N plots despite the total removal of the Calluna canopy by fire. This highlights the likelihood of direct effects of deposited NH$_4^+$ on this sensitive ecosystem component, particularly in the early post-fire re-vegetation phase when over-storey light interception is unlikely to have limited lichen re-colonisation. Additional detrimental effects of the sulphate counter ion used in this experiment cannot, however, be ruled out as lichens have been shown to respond negatively to sulphate deposition in earlier studies (Showman & Long, 1992).

Nitrogen-driven reductions in bryophyte abundance have also been observed in many studies (Morecroft et al., 1994, Carroll et al., 2000), although the underlying mechanisms for such declines are not fully understood (Koranda et al., 2007; Arroniz-Crespo et al., 2008). At Thursley, there were large N-driven increases in bryophyte re-colonisation following the fire, despite a lack of significant treatment effects in the first 8 years of the experiment. Interestingly, typical heathland moss pioneers (e.g. Ceratadon purpureum, Polytrichum juniperinum) appear to have been out-competed in +N plots by the vigorous post-fire colonisation of Campylopus introflexus. This is an introduced and rapidly spreading species which has previously been shown to respond positively to both fire and N (Ketner-Oostra, 2002). C. introflexus poses a threat to local flora, due to its tendency to form monotonous, dense carpets (Biermann & Daniels, 1997; Ketner-Oostra & Sykora, 2004; Vanderpoorten & Goffinet, 2009) which impede germination and establishment of Calluna (Equihua & Usher, 1993). Considered in conjunction with observed negative effects of N deposition on Calluna seedling establishment (Helsper et al., 1983; Britton et al., 2000) and post-fire re-
establishment of *U. minor* (this study), increases in abundance of *C. introflexus* could have important consequences for heathland composition and integrity in areas of high N deposition, particularly as summer fires are predicted to become a more frequent phenomenon in the future (IPCC, 2007).

**Effects on plant and soil chemistry**
Plant and soil N concentrations have been consistently higher in N treated plots. Coupled with increased *Calluna* productivity and litter production, faster rates of decomposition (1999), mineralisation (2003) and enzyme activities (2009) measured in the current experiment (Barker, 2001; Green, 2005), this provides clear evidence of rapid changes in the rates of biogeochemical cycling in response to increased N deposition.

Plant and soil N stocks were almost doubled by just 5 years of N addition. Indeed, we calculate that 82% of experimental inputs up to June 2003 (when the most recent pre-fire soil chemical analyses were carried out) were immobilised in the vegetation and top 10cm of the soil. Post-fire figures suggest that increases in ecosystem N stocks in +N plots between 2006 and 2009 represent ~96% of experimental additions during this period, implying a very high degree of N retention within the system. Increases in soil N appear to be largely driven by the rapid immobilisation of N by the microbial community at Thursley, the activity and biomass of which were significantly increased by experimental N addition in the early part of the experiment, in line with reports from studies elsewhere in the literature (Nielsen *et al*., 2000; Berendse *et al*., 1990; De Boer *et al*., 1989). This N-driven stimulation in the microbial community has occurred despite the observed treatment-driven reduction in soil pH, a widely reported phenomenon (Stevens *et al*., 2006). Addition of N in the reduced form, especially in association with sulphate, is frequently associated with a fall in soil pH, as a result of plant H+ excretion in the rhizosphere, microbial oxidation and depletion of soil cations (Ulrich, 1991, Stevens *et al*., 2011). Soil pH is considered to be an important influence on the size and activity of microbial communities (Wardle, 1992), however, the observed increases in microbial biomass and rates of associated biogeochemical processes suggest that the
stimulatory effect of N in this N-limited ecosystem outweighs any negative effects of associated acidity.

Strong plant and microbial immobilisation at Thursley has resulted in generally low concentrations of extractable N and negligible leaching losses of inorganic N (Barker, 2001, Green 2005), despite increases in nutrient turnover rates (litter decomposition, mineralisation). This highlights a conserved N cycle and high internal N demand, even under prolonged N loading. Whilst fire can affect the activity, composition and biomass of soil microorganisms (Barcenas-Moreno et al., 2011; Bogorodskaya et al., 2011), its effects on the immobilisation capacity of the system appear to have been short-lived. However, whether the microbial pool can continue to retain pollutant N in this way depends on the long-term immobilisation capacity of the system, and studies elsewhere suggest that ecosystems which have received historically high N inputs, or very large experimental additions, have a finite capacity to immobilise N in the soil (Phoenix et al., 2003; Pilkington et al., 2005; Beier et al. 2008).

Despite relatively low organic matter contents in sandy heathland soils (pre-fire ~7% SOM at Thursley), budget calculations imply that soil accounts for >99% of the ecosystem C stocks at this site. Beier et al. (2009) recently estimated that carbon storage across European shrublands ranged from 1160-18500 g C m\(^{-2}\), highlighting the C storage potential of shrub-dominated ecosystems. N addition resulted in a pre-fire doubling of above-ground C stocks at Thursley and, whilst proportional increases in below-ground C stocks were more modest, this nevertheless represented an increase in total ecosystem C storage after only five years. Fire clearly reduced soil C stocks, although significant N treatment effects were apparent within three years (equivalent to an additional ~115 g soil C m\(^{-2}\) yr\(^{-1}\) in +N plots, compared to controls). So, whilst our study site is at the low end of the range of reported soil C contents for shrub-dominated systems (Beier et al., 2009), N deposition at a rate of 30 kg ha\(^{-1}\) yr\(^{-1}\) – representing relatively modest ambient inputs across Europe (Bobbink, 2004) – substantially increased ecosystem accumulation of C.

A link between N deposition and increased soil organic matter accumulation has also been highlighted for ecosystems elsewhere (e.g. Waldrop et al., 2004; Adams
et al., 2005; Pregitzer et al., 2008). However, the long-term stability of N-driven increases in soil C sequestration will depend upon the rate at which labile C is converted to more stable forms with slower turnover rates, and these are processes which are currently poorly understood (Hendrikson, 2003). The evidence at this stage, however, suggests that the C storage potential of heathland and other low productivity systems, whilst relatively small compared to forests and peatlands (Sabine et al., 2004; Parish et al., 2008), is likely to be increasing in areas experiencing elevated inputs of atmospheric N. This suggestion is supported by findings of a recent field survey of UK lowland heaths which demonstrated significant increases in the depths of both litter and humus layers at sites along a gradient of increasing (ambient) N deposition (Jones & Power, 2012). The positive impact of N deposition on C sequestration should not, however, overshadow its widely observed negative effects on plant communities and other aspects of ecosystem function, and the urgent need to reduce N emissions as a way of safeguarding sensitive ecosystems from future damage (de Vries et al., 2009).

**Conclusion**

More than thirteen years of experimental manipulation and monitoring at Thursley Common have greatly enhanced our understanding of the long-term impacts of atmospheric N deposition on heathland ecosystems, and provided evidence of significant interactions with climate (and climate-related phenomena such as wildfire). In regard to our initial hypotheses, we have shown that modest increases in N inputs have the potential to affect the productivity, diversity and biogeochemical cycling within lowland heathlands and that these effects persist over time. There is evidence that N increases plant sensitivity to naturally occurring drought episodes, with significant interactions between N and rainfall for shoot growth following fire and greater levels of drought injury in plots receiving additional N.

Nitrogen inputs affected the post-fire regeneration of non-vascular plant groups, severely impeding lichen recolonisation whilst facilitating the rapid expansion of invasive moss species. Both N and fire, therefore, have profound effects on the
structure and composition of heathland plant communities. Treatment additions of N increased above- and below-ground stocks of C and N, although wildfire reduced the size of these stocks, treatment differences persisted, and continued N addition promoted rapid post-fire accumulation of both C and N. Finally, Given that 21st Century climate models predict lower summer rainfall and more prolonged dry periods in the northern hemisphere there is a clear need to understand interactions between climate and N deposition, two of this century’s most important drivers of ecological change. Long-term experiments such as the one described here provide a unique frame of reference from which ecosystem change can be identified, as well as important insight into the nature of interactions between multiple global change drivers, both experimental and naturally occurring.
Chapter 3

HEATHLAND RESPONSES TO ATMOSPHERIC NITROGEN DEPOSITION: RESULTS FROM A NATIONWIDE UK SURVEY

Abstract

Findings from nitrogen manipulation studies have provided strong evidence of the detrimental impacts of elevated N deposition on both the structure and function of heathland ecosystems. Few studies, however, have sought to establish whether these experimentally observed responses are also apparent under natural, field conditions. This work addresses a lack of research in this area, by way of presenting the findings of a nationwide field scale evaluation of British heathlands, across broad geographical, climatic and pollutant gradients. Across 52 heathland sites - (evenly distributed between upland and lowland areas), along a gradient of N deposition spanning from low (5.9 kg N ha\(^{-1}\), yr\(^{-1}\)) to high (32.4 kg N ha\(^{-1}\), yr\(^{-1}\)) - plant community and biogeochemical responses were quantified in conjunction with climatic variables (rainfall and temperature). Increasing ambient N deposition was significantly correlated with widespread declines in heathland species richness and diversity, as well as an increase in the abundance of nitrophilous plant species. Relationships were also evident between increasing N deposition and altered biogeochemical processes, and in many cases, these responses were seen to be significantly modified by climatic factors. Compelling evidence of N driven changes to heathland systems across a nationwide scale, are herein presented, lending weight to the experimental findings and highlighting the continued threat that elevated N deposition poses to these sensitive systems, and how this threat is further amplified by climatic factors.
**Introduction**

Human activities associated with the production of energy, fertilisers and crops (particularly nitrogen-fixing species) have had a substantial effect on the global nitrogen (N) cycle (Vitousek *et al.* 1997). Recent estimates suggest that anthropogenic contributions to the global reactive N pool have increased 10-fold since pre-industrial times, with estimated annual emissions of 16 Tg in 1860, having risen to ~160 Tg in 2000 (Galloway *et al.* 2004). Although fossil fuel combustion has resulted in a steady increase in oxidised N emissions over the last century, both emissions and deposition are currently dominated by reduced N, with more than half of current day N emissions associated with fertiliser production (Galloway *et al.* 2004). There is also large spatial variability in the amount of N deposited. For example, across Europe, N deposition rates currently range from ~1 kg ha$^{-1}$ yr$^{-1}$ in the relatively pristine areas of northern Scandinavia to >30 kg ha$^{-1}$ yr$^{-1}$ in areas dominated by industry (e.g. Northern Italy) or intensive agriculture (e.g. Netherlands) (EMEP). Global emissions and deposition of reactive N are predicted to increase further in the 21st century, with a doubling of current day levels by 2050 (Galloway *et al.* 2004).

The link between N deposition and changes in the structure and functioning of terrestrial ecosystems is now acknowledged, with N cited as one of the leading drivers of biodiversity loss at a global scale (Sala *et al.* 2000, Phoenix *et al.* 2006). Manipulation experiments have demonstrated a wide range of plant responses to N, including changes in plant phenology, physiology, biochemistry, productivity and assimilate allocation (Pearson & Stewart, 1993; Pilkington *et al.* 2007; Kleijn *et al.* 2008).

Resulting changes in competition between neighbouring species, as well as N-driven changes in soil pH/NH$_4^+$ concentrations and/or increased sensitivity to environmental stresses, are believed to be responsible for observed shifts in plant community composition (Bobbink *et al.* 2010). Similarly, N-driven changes in the activity and composition of the soil microbial community (Egerton-Warburton & Allen, 2000; Schimel *et al.* 2005) have been linked with changes in key soil processes, such as nutrient turnover rates and nitrous oxide production (Vitousek *et al.* 1997; Aber *et al.* 2003; Phoenix *et al.* 2004), with implications for the functioning of affected ecosystems. Nitrogen manipulation experiments have been carried out in a wide range of habitats, from bogs (Bragazza *et al.* 2004) and dune grasslands (Plassman *et al.* 2009) to deserts (Allen *et al.* 2009) and arctic tundra (Schmidt *et al.*
2000), establishing a general pattern in responses across terrestrial ecosystems. However, many of these have been of short duration and/or involving very high rates of N deposition (Phoenix et al. 2011).

Fewer studies have explicitly sought to establish whether the responses observed in manipulation experiments are apparent under natural, field conditions. Notable exceptions to this include surveys along gradients of ambient N deposition on acid grassland (Stevens et al. 2004, 2006), dune grassland (Remke et al. 2009), lowland heath (Power & Collins, 2010; Jones & Power, 2011), North American Forests (Gilliam et al. 2005) and upland moorland (Edmondson et al. 2010). Some of these have principally focused on evaluating patterns in plant tissue chemistry or soil biogeochemical indicators, including nutrient availability and enzyme activities, in relation to gradients of ambient N deposition. Where field surveys have been used to assess patterns in plant diversity or community composition in relation to N deposition, evidence of biodiversity loss and/or increased abundance of nitrophytic species has been reported (Smart et al. 2003; Stevens et al. 2004, 2006; Dupre et al. 2010, Maskell et al. 2010). To date, however, there has been no field-scale evaluation of the relationships between community-level (compositional) bioindicators, biogeochemical (functional) indicators and N deposition in heathland ecosystems, despite the fact that these have been shown to respond strongly to experimental N manipulation at a wide range of sites (e.g. Aerts et al. 1990; Marcos et al. 2003; Barker et al. 2004; Phoenix et al. 2011).

Although many of the responses reported in N-manipulation experiments are not specific to N and will be affected by factors such as climate and habitat management, several have been proposed as potential, robust bioindicators of the effects and/or amounts of N deposition. These include foliar N accumulation, activities of enzymes involved in nutrient metabolism, soil C:N ratios and concentrations of N in soil solution and leachate (Sutton et al. 2004; Pitcairn et al. 2006). This study presents the results of a nationwide survey of biological and biogeochemical indicators of N deposition at lowland and upland heathlands. It explicitly aims to evaluate relationships between N deposition and: (1) detrimental changes to plant community composition and species richness; (2) changes to ecosystem functioning, as measured by increased soil nutrient availabilities, turnover rates and enzyme activities. In addition, the strength of relationships with oxidised and reduced N are examined to determine whether the form, as well as amount, of N
is contributing to observed patterns in vegetation and soils at heathland sites across the UK.

Methods

Site selection and field surveying

52 *Calluna vulgaris* dominated heathland sites (25 upland and 27 lowland) representing building to mature phase (*sensu* Gimingham, 1972) were selected across a gradient of modelled N deposition (data provided by Ron Smith and Jane Hall, Centre of Ecology & Hydrology, Edinburgh) spanning 5.9 kg N ha\(^{-1}\) yr\(^{-1}\) to 32.4 kg N ha\(^{-1}\) yr\(^{-1}\). In addition, sites were chosen to be representative of the geographical, climatic and altitudinal (25 upland and 27 lowland heaths) variation encountered in heathland ecosystems across the United Kingdom.

Sites were visited during the summer of 2009 and grid reference, altitude, aspect and slope details were recorded at each site. Vascular and non-vascular plant species were recorded and relative abundance was estimated by percentage cover in five randomly placed quadrats (2 m x 2 m) at each site. Canopy height and *Calluna* shoot length (current year’s growth) were measured to the nearest mm and 5-10 g of *Calluna* shoots were collected into paper bags and air dried for later chemical analyses. Three soil cores (15 cm depth x 4 cm diameter) were extracted from the surveyed area for chemical analyses; these were stored in an ice filled cool-box before transfer to controlled temperature rooms upon return to the laboratory, typically within 1-3 days. Fresh *Calluna* litter (5-10g) was also collected from each quadrat for enzyme assays. The litter was air dried and stored in paper bags.

Chemical analyses

To determine extractable soil ammonium and nitrate concentrations, 40 g of fresh soil was mixed with 150 ml of M KCl and shaken for one hour on a rotary shaker before filtering. The filtrate was measured for inorganic N (NH\(_4^+\) and NO\(_3^-\)) using a SKALAR san flow\(^++\) segmented flow analyser (Skalar UK (Ltd), York, UK). Litter phosphomonoesterase (PME) activity was assayed using the artificial substrate p-nitrophenyl phosphate (p-NPP), in accordance with the method outlined by Johnson *et al.* (1998). Samples were centrifuged for 5 minutes at 3000 rpm and the resulting
liquid was analysed colourimetrically (using a Perkin Elmer Lambda 3 UV spectrophotometer, Perkins Elmer, USA) at a wavelength of 460 nm. Paired non-reactive controls were prepared for each of the samples by adding the PNP and toluene solution immediately after the stopping chemicals to prevent chemical reaction. The readings were compared with pigment densities from a calibration curve obtained using six standards of p-nitrophenol prepared within the absorbance range of the samples.

Total N and carbon (C) concentrations were determined using a FlashEA 1112 CN analyser (Thermo Scientific, UK). *Calluna* shoot and litter samples were dried at 80°C for 24 hours before being ground into a fine powder with a ball mill for two minutes. 15-20 µg of milled material was weighed into tin capsules and combusted. The evolved oxides, once chromatographically separated, were quantified by a thermal conductivity detector. Standard samples were prepared using certified reference materials for soil in order to monitor analytical precision. Concentrations of standards were within 5-10% of the original certified value.

Soil pH$_{(H_2O)}$ was measured using 10g field moist soil to which 25ml deionised water was added. This was then mixed thoroughly and allowed to stand for 30 minutes; pH was then recorded using a CORNING 220pH meter (Corning Inc, USA).

Litter phenol oxidase activity was measured by vortex-mixing 1 gram of ground *Calluna* litter with 9 ml deionised water. 3 ml of this solution was then added to 4.5 ml water and 7.5 ml of 1 M dihydroxyphenylalanine (DOPA) and then shaken for 9 minutes. After shaking the mixture was centrifuged at 6000 rpm for 5 minutes, a sub-sample was then taken and filtered to 0.2 µm and absorbance measured at 460 nm.

**Data analyses**

Relationships between response and explanatory variables (N deposition, rainfall and temperature) were analysed using the R statistical package, version 2.12.2 (R development core team, 2011). The climate data used were based upon UK 5 km$^2$ gridded data sets from the UK Meteorological Office (Met Office, 2009). Variables representing total annual precipitation and temperature were used, the latter represented by growing degree data (GDD, sum of degree days above 5°C). Both precipitation and growing degree data were averaged over the period 1997-2006.
Since total deposition is the product of oxidised and reduced N deposition these three explanatory variables could not be included in the same model. In order to separate the combined effects of total N deposition from the individual influences of oxidised and reduced N forms, two separate models were, therefore, run for each response variable. Model 1 = total N deposition, rainfall and temperature; Model 2 = reduced N, oxidised N, rainfall and temperature. Percentage cover data for vegetation were arc-sine transformed prior to analysis. Log or square root transformations were used to transform other non-normally distributed data prior to modelling. Relationships were tested using multiple regression analysis or, for count data (e.g. species richness), generalised linear models (GLM) with poisson errors. The most complicated models were fitted (to include interactions between explanatory variables, plus quadratic terms to test for non-linearity of responses) and then simplified using likelihood ratio deletion tests until the minimum adequate model was attained (Crawley, 2007). Three analyses were performed for each parameter to encompass (a) all sites, (b) upland sites only and (c) lowland sites only.

**Results**

*Higher plant responses*

A significant decline in overall species richness was observed across all sites in relation to increasing levels of total N deposition (F=27.5, P<0.001, Figure 3.1a), with oxidised N driving relationships in both upland (F=14.5, P<0.001, Table 3.1, model 2) and lowland systems (F=8.7, P<0.01, model 2). The most dramatic losses were to be seen at the lower end of the N deposition gradient, with an average of 13 species lost per site between 5 – 10 kg N ha\(^{-1}\) yr\(^{-1}\), and an average of 3 species lost between increases of 10 – 20 kg N ha\(^{-1}\) yr\(^{-1}\). Beyond this, declines in species richness plateaued, indicating a reduction in sensitivity as N loading increased. Temperature was also a significant factor, both solely and in interaction with N deposition (F=4.4, P<0.05, model 1, Figure 3.1b). Species richness was seen to decline by 50 % around a temperature threshold of 1000 growing degree days (gdd) and total N inputs of 15 kg N ha\(^{-1}\) yr\(^{-1}\), and declined by a further 50 % around the region of 2000 gdd.

Graminoid species richness was significantly lower across all sites with higher total N inputs (F=9.3, P<0.01, Figure 3.1c), as well as with increasing temperature (F=10.9, P<0.01). Sensitive species were seen to be most at threat by increases at the
lower end of the N deposition gradient (an average of 4 species lost per site between 5 – 10 kg ha\(^{-1}\) yr\(^{-1}\)) with little change evident as the pollution scale increased. Additionally, each incremental temperature increase of 500 growing degrees corresponded to an average loss of 1 species per site. Both forms of N (oxidised and reduced) were associated with graminoid species diversity decline in the lowland and upland sites independently, however, stronger relationships with NOx were found in the lowland sites, and with NH\(_4\) in the upland sites. Graminoid abundance, by contrast, was seen to increase in relation to increasing N (F=15.8, P<0.001), indicating a shift towards more grass dominated systems with greater N deposition (Figure 3.1d). Reduced N was the most significant driver of this response (F=19.5, P<0.001, Table 3.2, model 2). Increases in grass abundance were, however, found to be chiefly characterised by a few species, specifically *Molinia caerulea* and *Deschampsia flexuosa*, the latter being significantly more abundant at sites receiving higher rates of reduced N inputs (F=13.1, P<0.01, Table 3.2, model 1).

Overall forb richness reduced in relation to increasing total N deposition (F=21.2, P<0.001, Table 3.1, model 1), with the rate of decline steepest with modest increases of N at the lower end of the deposition gradient (4 species lost on average per site between 5 – 10 kg N ha\(^{-1}\) yr\(^{-1}\) with negligible losses thereon). Detailed analysis of the role of N forms indicate that stronger relationships were found with oxidised N (F=16.0, P<0.001, model 2). Significant interactions between total N deposition and rainfall, in upland and lowland datasets, indicate that forb diversity decline is exacerbated in wetter areas receiving higher levels of N deposition (uplands, F=6.9, P<0.05, model 1; lowlands, F=4.6, P<0.05, model 2).
Significant positive relationships between *Calluna* canopy height and both N deposition (F=15.3, P<0.001, Table 3.2, model 1) and temperature (F=12.6, P<0.001, model 1) were apparent across all sites. Whilst height increased in a linear fashion in relation to both N forms, the strongest statistical relationship across all sites was with reduced N (F=24.8, P<0.001, model 2).

**Lower plant responses**

Significant negative relationships between lichen species richness and total N deposition were found across all sites (F=7.8, P<0.01, Table 3.1, model 1, Figure 3.2a). An average of 5 lichen species per site were recorded in areas receiving less than 10 kg ha$^{-1}$ yr$^{-1}$ and this had declined to an average of 1.4 species at input rates of 20 kg ha$^{-1}$ yr$^{-1}$ indicating that lichen species richness is most at threat from even relatively modest rates of N loading. Analysis in relation to N forms, indicates that reduced N was the principal driver of lichen diversity decline (F=14.2, P<0.001, model 2), however, strong correlations with oxidised N were also found in the upland

**Figure 3.1**: Relationships between N deposition and plant species richness and abundance.
Significant interactions indicate that the negative effects of N deposition on lichen richness were greater at sites with high temperature (F=4.8, P<0.005, model 1).

Considered independently, patterns in lichen abundance at the upland and lowland sites were best explained by the different forms of N. The relationship with oxidised N was statistically stronger in the upland sites (F=4.5, P<0.05, Table 3.2, model 2), whilst the relationship was stronger with reduced N in the lowlands (F=8.9, P<0.01, model 2). Climatic conditions were also important, with abundance generally greater in areas receiving more rainfall (F=4.4, P<0.05, model 1). Lower plant cover was also generally lower where Calluna canopies were taller in wetter areas (F=9.1, P<0.01, model 1). Temperature was the main driver influencing bryophyte species richness and abundance across all sites (F=21.3, P<0.001, Table 3.1, model 1, Figure 3.2b), with less species recorded at the warmer end of the temperature gradient (<1000 gdd = 10 species (on average) per site; > 2000 gdd = 5 species (on average) per site).

Figure 3.2: The influence of N deposition and climate on lower plant species richness and abundance.
Whilst a general downward trend in bryophyte diversity was apparent in relation to N deposition across all sites, the only significant N signal was found in the lowland sites in the form of oxidised N ($F=11.2$, $P<0.01$, model 2).

Stronger relationships with N were, however, found at the species level: the characteristic heathland mosses *Hylocomium splendens* (Figure 3.2c) and *Pleurozium schreberii* were seen to severely decline in relation to increasing N whilst, conversely the nitrophilous species (Siebel et al. 2005), *Brachythecium rutabulum* (Figure 3.2d), was more abundant at sites receiving higher N deposition ($F=6.1$, $<0.05$). A general upward trend in bryophyte diversity and cover was found in relation to increasing precipitation, although these relationships were not statistically significant.

*Plant tissue chemistry*

The main factor affecting *Calluna* tissue nitrogen concentrations across all sites, was temperature (Figure 3.3a). Concentrations were higher as temperatures increased, however a significant decline was observed around a temperature equal to 1500 growing degree days ($F=4.3$, $P<0.05$). Significant relationships between nitrogen deposition and climate were only found in the lowland sites, where interactions between increasing total N and higher rainfall indicate a positive effect on *Calluna* tissue nitrogen concentrations ($F=4.5$, $P<0.05$, Table 3.3, model 1). In addition, model 2 analyses revealed that combined effects of increased reduced N and higher temperature describe a reduction in lowland heath tissue N concentrations ($F=6.1$, $P<0.05$).

*Soil pH*

Soil pH ranged from 3.5 to 5.4, decreasing significantly in relation to total N deposition ($F=12.4$, $P<0.01$, Table 3.3, model 1) and increasing at sites with higher temperatures ($F=7.5$, $P<0.01$, model 1) across all sites. Soil pH was influenced more by oxidised N ($F=13.7$, $P<0.001$, model 2) (predominantly in the uplands) and interactions between precipitations and total N deposition ($F=6.3$, $P<0.05$, model 1).

*Extractable soil nutrients*

A positive relationship between reduced nitrogen deposition and extractable soil nitrate (NO$_3^-$) concentrations was found only in the upland sites ($F=9.6$, $P<0.05$, Table 3.3, model 2). Concentrations were also greater at sites with higher moisture levels
Across all sites, however, interactions between temperature and soil moisture suggest that extractable nitrate concentrations are greater in areas with clement climatic conditions (warm and wet).

Extractable soil ammonium (NH$_4^+$) concentrations also increased with increasing total N in the upland sites, driven principally by oxidised N. As with nitrate, significant relationships with climate indicate that extractable ammonium concentrations are higher in warmer, wetter areas particularly in the uplands.

**Total soil nitrogen and carbon concentrations**

Significant interactions between total N deposition and rainfall were related to increases in total soil nitrogen and carbon concentrations in the upland sites. Model 2 analyses show that oxidised N is the form most significantly associated with soil nutrient increases across sites receiving greater levels of precipitation. A significant relationship between oxidised N and total soil nitrogen concentrations was also seen in the lowland sites, however conversely, this relationship displayed a negative trend. Climate is clearly also an important driver of soil C and N, with significantly higher concentrations at sites receiving more rainfall. Indeed, at upland sites, an interaction between temperature and precipitation indicates that soil N and C concentrations are highest where rainfall and temperature are both high. A significant upward trend in soil C:N ratios with increasing oxidised N deposition was evident in the lowland sites. C:N ratios were also related to climate, with lower ratios at wetter sites.
Nitrogen concentrations in *Calluna* litter were significantly higher (F=5.3, P<0.05, Table 3.3, model 1, Figure 3.3d) and C:N ratios lower (F=8.6, P<0.01, model 1) at sites receiving higher total inputs of N. In the upland sites, litter N was positively related to increased reduced N inputs (F=34, P<0.05, model 2), whilst also negatively related to temperature (F=82.3, P<0.05, model 2). Across all sites, temperature was a significant factor on its own (F=15.0, P<0.05, model 1) and in combination with precipitation (F=6.7, P<0.05, model 1). The relationship with temperature, although negative, is nonlinear, and indicates that whilst enzyme activity initially declines along an increasing temperature gradient, it begins to increase around a threshold of 2000 growing degrees.
A positive relationship between total N deposition and litter PME activity was found only for upland sites (F=4.3, P<0.05, model 1), however, analysis of N forms revealed that, across all sites, activity was positively related with reduced N inputs (F=4.3, P<0.05, model 2, Figure 3.4b). Again, across all sites, the influence of climate was significant, with a negative trend in activity occurring as precipitation levels increase. Conversely, a combination of higher N deposition (predominantly reduced N (F=9.3, P<0.01, model 2) and rainfall are related with an increase in PME activity.

Figure 3.4: Relationships between reduced N deposition and Calluna litter enzyme activity.
Table 3.1: Summary table of optimal models for plant species richness in relation to N deposition and climatic variables.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model 1 (Total nitrogen deposition + environmental variables)</th>
<th>Model 2 (oxidised/reduced nitrogen + environmental variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All sites d.f. P</td>
<td>Uplands d.f. P</td>
</tr>
<tr>
<td>Total species</td>
<td>(-) N 51 ***</td>
<td>(-) N 23 ***</td>
</tr>
<tr>
<td></td>
<td>(-) gdd ***</td>
<td>(-) gdd ***</td>
</tr>
<tr>
<td></td>
<td>N x gdd *</td>
<td>N x gdd *</td>
</tr>
<tr>
<td>Total lichen species</td>
<td>(-) N 51 **</td>
<td>(-) N 24 **</td>
</tr>
<tr>
<td></td>
<td>(-) gdd *</td>
<td>(-) gdd *</td>
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<tr>
<td></td>
<td>N x gdd **</td>
<td>N x gdd **</td>
</tr>
<tr>
<td>Total moss species</td>
<td>(-) gdd 51 ***</td>
<td>(-) gdd 26 **</td>
</tr>
<tr>
<td></td>
<td>(-) N x gdd *</td>
<td>(-) N x gdd *</td>
</tr>
<tr>
<td>Total graminoids</td>
<td>(-) N 51 **</td>
<td>(-) N 24 *</td>
</tr>
<tr>
<td></td>
<td>(-) gdd *</td>
<td>(-) gdd *</td>
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<tr>
<td></td>
<td>N x gdd *</td>
<td>N x gdd *</td>
</tr>
<tr>
<td>Total forb species</td>
<td>(-) N 51 ***</td>
<td>(-) N 24 **</td>
</tr>
<tr>
<td></td>
<td>(-) N x rain *</td>
<td>(-) N x rain *</td>
</tr>
</tbody>
</table>
| (+, -) = direction of response. (x) = interaction. P values = * <0.05; ** <0.001; *** <0.001.

Table 3.2: Summary table of optimal models for vegetation responses in relation to N deposition and climatic variables.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Model 1 (Total nitrogen deposition + environmental variables)</th>
<th>Model 2 (oxidised/reduced nitrogen + environmental variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All sites d.f. P</td>
<td>Uplands d.f. P</td>
</tr>
<tr>
<td>Graminoid cover (%)</td>
<td>(+) N 50 ***</td>
<td>(+) N 23 **</td>
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<tr>
<td></td>
<td>(+) gdd ***</td>
<td>(+) gdd ***</td>
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<tr>
<td></td>
<td>N x gdd x rain x c.ht *</td>
<td>N x gdd x rain x c.ht *</td>
</tr>
<tr>
<td>Moss cover (%)</td>
<td>(-) gdd 50 ***</td>
<td>(-) gdd 23 *</td>
</tr>
<tr>
<td></td>
<td>(-) gdd *</td>
<td>(-) gdd *</td>
</tr>
<tr>
<td></td>
<td>N x gdd *</td>
<td>N x gdd *</td>
</tr>
<tr>
<td>Lichen cover (%)</td>
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<td>(-) N 20 *</td>
</tr>
<tr>
<td></td>
<td>(+) rain *</td>
<td>(+) rain *</td>
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<tr>
<td></td>
<td>rain x c.ht *</td>
<td>rain x c.ht *</td>
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<tr>
<td>Canopy height (cm)</td>
<td>(+) N 49 ***</td>
<td>(+) N 22 ***</td>
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<td></td>
<td>(+) gdd ***</td>
<td>(+) gdd ***</td>
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<tr>
<td></td>
<td>N x rain x c.ht *</td>
<td>N x rain x c.ht *</td>
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<tr>
<td>Pleurozium (%)</td>
<td>(-) N 48 ***</td>
<td>(-) N 22 ***</td>
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<td></td>
<td>(+) rain *</td>
<td>(+) rain *</td>
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<tr>
<td></td>
<td>N x rain x c.ht *</td>
<td>N x rain x c.ht *</td>
</tr>
<tr>
<td>Brachythecium (%)</td>
<td>(+) N 50 *</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>Hylocomnium (%)</td>
<td>(-) N 49 **</td>
<td>(-) gdd 23 *</td>
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<td></td>
<td>(-) gdd *</td>
<td>(-) gdd *</td>
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<tr>
<td>Deschampsia (%)</td>
<td>(+) N 50 ***</td>
<td>(+) N 23 **</td>
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<tr>
<td></td>
<td>(-) gdd *</td>
<td>(-) gdd *</td>
</tr>
</tbody>
</table>

(+, -) = direction of response. (x) = interaction. P values = * <0.05; ** <0.001; *** <0.001. 63
Table 3.3: Summary table of optimal models for soil, plant and litter chemistry in relation to N deposition and climatic variables.

<table>
<thead>
<tr>
<th>Model 1 (Total nitrogen deposition + environmental variables)</th>
<th>Model 2 (oxidised/reduced nitrogen + environmental variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response variable</td>
<td>Response variable</td>
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<td>All sites</td>
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<td>d.f.            P</td>
<td>Uplands       d.f.            P</td>
</tr>
<tr>
<td>d.f.            P</td>
<td>Uplands       d.f.            P</td>
</tr>
<tr>
<td>Soil pH</td>
<td>(-) N 49 **</td>
</tr>
<tr>
<td>(-) gdd</td>
<td>gdd x rain</td>
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<tr>
<td>Extractable NO3 (mg/kg)</td>
<td>(+) gdd 38 **</td>
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<tr>
<td>(-) NOx</td>
<td>gdd x moist</td>
</tr>
<tr>
<td>Extractable NH4 (mg/kg)</td>
<td>(+) gdd 44 **</td>
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<tr>
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<td>X rain</td>
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<td>Total Soil Carbon (%)</td>
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<td>Soil C:N ratio (%)</td>
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<td>gdd x rain</td>
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<td>Calluna litter N (%)</td>
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<td>(-) gdd</td>
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<td>Calluna litter C:N (%)</td>
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</tr>
<tr>
<td>Calluna foliar N (%)</td>
<td>(-) gdd 48 *</td>
</tr>
</tbody>
</table>

(+, -) = direction of response. (x) = interaction. P values = * <0.05; ** <0.001; *** <0.001.
Discussion

Above-ground responses

A decline in plant species diversity in relation to increasing atmospheric N deposition has been observed in a variety of ecosystems ranging from acid and calcareous grasslands (Stevens et al. 2004; Clark and Tilman, 2008) to boreal and temperate forests (Strengbom et al. 2001; Gilliam and Roberts, 2003) and even arctic tundra (Gordon et al. 2001). The findings from this survey provide clear evidence that elevated N deposition significantly reduces the floristic diversity of heathland systems too, either solely or in combination with climatic drivers – particularly increasing temperature – a major driver of global biodiversity loss in both terrestrial and non-terrestrial ecosystems (Sala et al. 2000; van Vuuren et al. 2006). The nature of the relationship between total N deposition and species richness suggests similar patterns in both upland and lowland systems; chiefly that, the greatest reduction of species richness, on average, is induced by relatively low incremental increases of N input. It appears that both oxidised and reduced forms of N are contributing to heathland biodiversity loss, but that slightly stronger relationships are apparent with oxidised N. Given the broad cover of spatial, climatic and pollution indices, the magnitude of diversity loss that has been observed amongst forb, graminoid, bryophyte and lichen functional groups in this study not only validates experimental findings from many N manipulation studies (Carroll et al. 2003; Soudzilovskaia et al. 2005; Clark & Tilman, 2008) but, more profoundly, provides compelling evidence that N deposition is driving biodiversity loss amongst UK heathlands on a national scale.

In N-limited environments, increased N availability frequently leads to increased plant growth and/or expansion of nitrophilous species, potentially leading to competitive exclusion of slower growing habitat-specialist species adapted to low levels of N availability (Stevens et al. 2004). Effects of N on soil pH, particularly in its reduced form (Sheppard & Wallander, 2004), can also result in the decline of sensitive plant species, particularly those with narrow pH niches or low tolerance of high NH$_4^+$ ion concentrations (Nordin et al. 2009; Stevens et al. 2009). In the current study, Calluna plants were taller and soil pH lower in areas receiving high N inputs, suggesting that reduced understorey light availability and/or unfavourable soil pH may be mechanisms contributing to biodiversity loss in British heathlands. Whilst bryophyte cover was not related per se to N deposition, shifts within bryophyte communities, notable at the species level, were significantly related to N inputs. For
example, the characteristic heathland moss species *Hylocomnium splendens* and *Pleurozium schreberii* were seen to decline as N deposition increased, whilst *Brachythecium rutabulum*, a nitrophilous species more commonly associated with base rich, eutrophicated habitats (Hill *et al.* 2007) was more abundant at sites with higher N deposition. Similar species shifts have been seen in boreal forest systems (Hallingback, 1992; Strengbom *et al.* 2001) with *H. splendens* declining, and being replaced by *B. rutabulum*, at relatively modest input rates of 10 kg N ha$^{-1}$ yr$^{-1}$.

Although graminoid abundance increased significantly with increasing N deposition, a concurrent decrease in graminoid diversity, coupled with sharp declines in the abundance of typical heathland grass species (e.g. *Festuca ovina, Danthonia decumbens*), indicates that grass invasion at higher N deposition loads is dominated by only a small number of species (specifically *Deschampsia flexuosa* or *Molinia caerulea*). These findings support earlier observations of *D. flexuosa* and *M. caerulea* increases in high N deposition areas in the Netherlands (Heil & Aerts, 1993) and an associated conversion of 35 % of Dutch heathlands to grasslands in the 1980s (Bobbink *et al.* 1993). These species have also been found to increase in Danish bog systems exposed to N levels of 10-15 kg ha$^{-1}$ yr$^{-1}$, to the detriment of characteristic ombrotrophic vegetation (Aaby, 1994).

N deposition is associated with negative impacts on lichen communities within many ecosystems (Baddeley *et al.* 1994; Britton and Fisher, 2007; Fenn *et al.* 2008), a phenomenon often linked to increased competition for light as a result of N-driven stimulation in higher plant growth (Barker, 2001; Bobbink *et al.* 2010). However, direct toxicity of NO$_2^-$, NO$_3^-$ and NH$_4^+$ is also likely to be a contributing factor in the decline of non-vascular plants (Hyvärinen & Crittenden, 1998; Remke *et al.* 2009; Hogan *et al.* 2010). Whilst lichen abundance was negatively related to both reduced and oxidised forms of N in this study, declines in lichen species richness were most strongly associated with reduced N. As with most higher plants, lichens can effectively assimilate ammonium at modest levels (Miller & Brown, 1999; Hauck, 2010), rapidly converting it into amino acids to mitigate its cytotoxicity (Neuhauser *et al.* 2007). However, for acidophytic species (such as those found in heathlands) this assimilation capacity is limited and toxic levels of unassimilated NH$_4^+$ can accumulate in the lichen thallus (Dahlman *et al.* 2003). It seems likely, therefore, that both competitive exclusion and potentially also direct toxicity effects are contributing
to the decline in abundance and diversity of lichens observed with increasing N deposition in the current study.

*Calluna* responses to experimental N additions are generally represented by increased biomass production (Heil and Diemont, 1983; Carroll *et al.* 1995, Power *et al.* 1998). Increased tissue N concentrations have also been observed both experimentally and from field-scale surveys (Pitcairn *et al.* 2001; Sheppard *et al.* 2004; Rowe *et al.* 2008; Power & Collins, 2010). In this study, *Calluna* canopy height and (at lowland sites) foliar N concentrations were greater at sites receiving higher N inputs (and rainfall), providing further, field scale evidence of N-driven changes in plant growth and biochemistry.

*Below-ground responses*

In this study, litter N concentrations increased, and C:N ratios decreased, with increasing N inputs, suggesting greater availability of organic N for soil microorganisms and thus faster rates of nutrient cycling. The strongest relationships were seen in upland sites, for reduced N. Microbial enzyme activity provides a useful proxy for decomposition rates and has been frequently used to monitor the functional responses of microbial populations to global change perturbations (Carreiro *et al.* 2000, Schimel *et al.* 2005). Our results show that overall the activity of phenol-oxidase – an enzyme responsible for breakdown of the structural components of plant material (Sinsabaugh *et al.* 2002) – in litter was greater at sites receiving higher reduced N inputs. This relationship was seen in both the upland and lowland systems, the latter however, stronger in areas of greater rainfall. Significant interactions between oxidised N and rainfall in upland systems were also apparent, possibly reflecting the wider span of oxidised N inputs (3 – 15 kg ha\(^{-1}\) yr\(^{-1}\)) compared to lowland sites (3 – 9 kg ha\(^{-1}\) yr\(^{-1}\)). The pattern of increased litter/soil enzyme activity with increasing N deposition is in line with findings of a recent survey of biochemical indicators in lowland heathlands (Jones & Power, 2012). However, contrasting results have been reported previously for moorlands (Edmondson *et al.* 2010) and temperate forests (Carriero *et al.* 2000; Sinsabaugh *et al.* 2002).

Whilst conflicting results make it difficult to generalise, research carried out by Berg and Matzner (1997) and Berg (2000) implies that the effects of N inputs on decomposition rates are highly dependent on the stage of decomposition at the time of sampling. They found that whilst high litter N concentrations and low litter C:N ratios
accelerate the initial stages of litter decomposition in forest ecosystems, they appeared to have an inhibitory influence on the latter stages of decomposition due to a reduction in the efficiency of lignolytic enzymes in older litter (Berg, 1986; Knoor et al. 2005). *Calluna* litter has a high lignin content (Gimingham, 1972) and factors such as stand age and development phase are, therefore, likely to influence decomposition rates and their responses to N deposition (Berg & McClaugherty, 2008), as are disturbance events such as fire and management (Delcourt & Harris, 1980; Johansson, 1987; Emmett et al. 1998).

The positive relationship between N deposition and litter PME activity provides the first field scale evidence of an increase in P demand at heathland sites receiving high rates of N deposition. These findings are in line with those from manipulation experiments across a range of ecosystems (Saiya-Cork et al. 2002; Phoenix et al. 2003; Pilkington et al. 2005), and with survey evidence of increased plant uptake of P across a transect of increasing N deposition (Rowe et al., 2008; Power & Collins, 2010). Across all sites combined, the relationship was driven by reduced N while uplands appeared to be more influenced by total N. Whilst the N deposition gradient was wider across upland sites (upland: 32.4 – 7.4; lowland: 23.7 – 5.9), climatic drivers are also strong influences on the activity of plants, microorganisms and associated enzymes.

The main factors regulating decomposition are moisture and temperature (Criquet et al. 2004; Gillier et al. 2005); this is also apparent in our survey results with enzyme activities highest in warmer, wetter areas. Climate change predictions for warmer, drier summers and cooler, wetter winters will, therefore, have implications for the activities of enzymes involved in the decomposition process, with likely consequences for nutrient availabilities and uptake. Concentrations of soil extractable NO$_3$ and NH$_4$ were significantly higher with increasing N deposition in the upland sites, although no such relationship was evident in the lowlands. The strong influence of climate on rates of nutrient turnover and availability (Morecroft et al. 1992; Jamieson et al. 1998), and generally lower annual rainfall in lowland (600-1300 mm) compared to upland (800-2500 mm), sites may explain the lack of relationship with N in lowlands. This may be particularly the case in this survey since sampling was carried out in the summer when soil moisture levels are at their lowest and plant and microbial immobilisation of N are at their highest (O’Sullivan et al. 2011). Caution must be exercised, however, when making assumptions based on
‘snapshot’ measurements of mineral pools (particularly across broad temporal and spatial gradients) that are inherently subject to large inter-seasonal and inter-annual variations (Pilkington et al. 2005; O’Sullivan et al. 2011). Furthermore, recent N focused surveys carried out by Jones & Power (2012) and Stevens et al. (2011) found that soil types, underlying geology, climatic and altitudinal variation were important drivers of soil and plant responses, influencing the shape of responses to N deposition.

Higher soil C:N ratios at sites with higher N inputs, specifically in the lowlands, indicate a shift towards immobilisation with increasing N deposition. Lower litter C:N ratios at high N sites contrast with this pattern, although may be explained by the previously mentioned N-driven stimulation at the early stage of decomposition where labile, energy rich substrates are abundant, followed by a slowdown in later stages as the relative proportions of recalcitrant compounds (such as lignin) progressively increase (Kirk & Farrell, 1987). Whilst many studies of N deposition impacts have found that total soil N content increased with elevated N inputs (Aber, 1992; Power et al. 1998; Pilkington et al. 2005), no evidence of N or C accumulation in relation to elevated N as a sole driver was found in this survey. Across all sites, the most significant driver influencing soil total N and C content was precipitation, however significant upward increases in soil nutrient concentrations were evident where oxidised N and rainfall levels were higher. Since climatic factors are known to strongly influence the production of plant material (and, subsequently, plant residues) (Wells et al. 1997), the dominant influence of rainfall is not surprising. Indeed, our results suggest that climatic variables and seasonality are important drivers of many ecosystem processes and, therefore, are likely to influence responses to N deposition. Given that climate models predict altered precipitation and temperature patterns for the Northern hemisphere (IPCC, 2007), and that widespread exceedance of critical N loads is predicted across Europe for the foreseeable future (Galloway et al. 2004; Matejko et al. 2009), the implications of global change for the biodiversity, structure and function of these internationally important heathland ecosystems are very considerable.
Conclusion

The findings from this survey provide novel support to a growing body of evidence demonstrating a strong link between N deposition and an increase in nitrophilous plant species across a wide range of habitats (Maskell et al. 2010; Stevens et al. 2010). An associated decline in the diversity of both higher and lower plants across broad spatial and climatic gradients – in both upland and lowland environments - highlights the ubiquity of relationships with N, providing clear field evidence of widespread N-driven changes in the composition, diversity and productivity of British heathlands. There is also strong evidence to suggest that current rates of N deposition are altering a range of biogeochemical processes, particularly in relation to nutrient cycling and availability, and that these relationships are influenced by climatic conditions. Litter N concentrations, litter enzyme activity (phenol-oxidase and PME) and soil extractable N concentrations all appear to have strong relationships with N deposition, and represent a suite of biochemical indicators of, and responses to, N deposition. Given future predictions for climate change and continued wide scale exceedance of critical N loads across Europe, further effects of these global change phenomena on the diversity and service provision of heathland ecosystems can be expected.
Chapter 4

HOW DOES NITROGEN DEPOSITION AFFECT THE POST-FIRE RECOLONISATION AND DIVERSITY OF HEATHLAND CRYPTOGRAMIC FLORA?

Abstract

Cryptogamic (or lower plant) flora is widely recognised as being highly sensitive to elevated N inputs across many ecosystems. In typically species poor heathland systems, cryptogams can represent the bulk of floristic diversity, therefore, increased N deposition poses a substantial threat to the biodiversity of heathland ecosystems. Previous quantification of mosses and lichens growing in the experimental plots at Thursley Common, quantified annual changes of abundance in relation to functional groupings, rather than at a species level. In order to provide greater detail as to how N additions influence lower plant community composition, species dynamics and reproduction, a survey was carried out five years after a wildfire event. Findings demonstrate that early-stage recolonisation of lower plant species, is detrimentally influenced by N additions, characterised by overall declines in species diversity, reduced reproductive viability of typical heathland species and shifts towards more nitrophilous assemblages.
Introduction

The increase of atmospheric nitrogen deposition as a result of anthropogenic activities, is considered to be, in addition to global warming, one of the major threats facing global biodiversity (Krupa, 2003). A combination of fossil fuel combustion, chemical fertiliser production and intensive farming has been attributed to an increase in N fixation at levels over and beyond those occurring through natural processes (Galloway et al. 2004).

For N limited ecosystems, exposure to increased loads of biologically active N can result in profound and multiple impacts on their biodiversity and function (Vitousek et al. 1997; Duce et al. 2008). From a floristic perspective, plant communities within a wide range of habitats across Europe and North America have been seen to shift towards higher N status assemblages where N deposition is high (Bobbink et al. 1998). These shifts, in association with losses of species diversity, have been observed occurring within both vascular and non-vascular plant groups, with the latter proving to be the most sensitive element to increased N across many ecosystems (Bobbink et al. 1998).

Because lichen and bryophytes do not possess roots, stomata or a hydrophobic cuticle, nutrients, water and gases are directly absorbed from the atmosphere via the entire leaf or thallus surface (Nash, 2008; Vanderpoorten & Goffinet, 2009). This renders them highly sensitive to changes in atmospheric purity, and thus susceptible to the potentially toxic accumulation of N in excess of their nutritional requirements (Glavich & Geiser, 2008). Indeed, a wealth of evidence from both experimental and observational studies across a vast range of global ecosystems suggests that increased N deposition is having a detrimental impact on both lichen and bryophyte species, from arctic tundra (Soudzilovskaia et al. 2005), ombrotrophic bogs (Woodin & Lee, 1987) and Boreal forests (Hallingback, 1992) to European calcareous grasslands (During & Willems, 1986) and semi-arid deserts (Evans & Belnap, 1999). In the UK, experimental studies have also demonstrated the sensitivity of lower plant groups to enhanced N deposition. An overall decrease in lichen cover was observed in sub-alpine heathlands receiving inputs of N between 10-50 kg N ha\(^{-1}\) yr\(^{-1}\) (Britton & Fisher, 2007), whilst the addition of approximately 80 kg N ha\(^{-1}\) yr\(^{-1}\) at an upland heathland site, almost eradicated the associated lower plant community (Lee & Caporn, 1998; Carroll et al. 1999). Furthermore, bryophytes were seen to be
detrimentally affected in acid grassland sites receiving 140 kg N ha$^{-1}$ yr$^{-1}$ (Morecroft et al. 1994).

The mechanisms underlying N related suppression of lower plants have been defined as relating to either direct toxicity, or indirect competition for light (Barker, 2001). Ammonium, although readily taken up by lower plants, is cytotoxic, therefore it must be rapidly assimilated into amino acids if toxicity is to be avoided (Neuhauser et al. 2007). Nitrate assimilation follows similar constraints to ammonium, however, due to the increased metabolic costs involved in rapidly reducing nitrate, via nitrite (also toxic) to ammonium, it is considered to be the least preferred form (Hauck, 2010). The ability of a plant to assimilate nitrogen depends on the production of carbon skeletons via photosynthesis, which bind with N, reducing the amount of free intracellular ammonium (Hauck, 2010). In the case of lichens, photosynthetic capability is determined by the allocation of N to the photobiont (the algal partner in symbiosis), therefore, under ambient conditions, a state of positive feedback will exist between nitrogen availability and photosynthetic capacity (Gaio-Oliveria et al. 2005).

Where N availability increases, species that are tolerant of low to medium concentrations of ammonium (e.g. acidophytic species), can be severely affected due to their limited ability to compensate for elevated N through higher carbon assimilation (Dahlman et al. 2003). These species are thus susceptible to a reduction of photosynthetic efficiency and the subsequent toxic accumulation of unassimilated ammonium (Munzi et al. 2010). Direct negative effects of enhanced experimental N addition have been observed in mosses (Paulissen et al. 2005; Sheppard, 2009; Verhoeven et al., 2011) and lichens (Munzi et al. 2009; Pirintsos et al. 2009), with effects ranging from necrosis of plant material, damage of cytoplasmic membranes and marked chlorophyll degradation.

Alternatively, increased vascular plant growth as a consequence of higher N availability is considered to have an indirect negative impact on lower plant communities as a result of increased shading (Biermann & Daniels, 1997; Cornelissen et al. 2001; Hauck, 2010). In heathland systems, a vigorous Calluna canopy may reduce understory photosynthetically active radiation (PAR) to less than 1 %, however, heathland Cladonia species have been found to become metabolically inactive at PAR levels below 2 % (Gimmingham, 1972).
Negative impacts of nitrogen deposition can be further enhanced within the context of global climate change, particularly in regard to predicted increases in wildfire occurrences as a consequence of hotter, drier summers (IPCC, 2007). Fire has long been an integral part of the ecology of many ecosystems (Bradshaw et al. 2010; Gimingham, 1972) contributing to their diversity as well as influencing carbon and nutrient cycling (Bachelet et al. 2005). The sheer intensity of wildfires however, can severely damage lower plant communities to the point of eradication, and can be detrimental to typical regeneration dynamics (Clément & Touffet, 1981; Romme et al. 1998; Davies & Legg, 2008). For lower plant populations that are already negatively affected by increased atmospheric nitrogen inputs, interactions with wildfire events are therefore potentially damaging to typical vegetation community structure.

Evidence of this has been found at the long term lowland heathland N manipulation study at Thursley Common NNR, UK (now in its fourteenth year), following a severe wildfire in 2006. N additions of 30 kg N ha\(^{-1}\) yr\(^{-1}\) have induced profound declines in lichen abundance, and changes to bryophyte cover (Barker, 2004; Green, 2005; Jones, 2009) throughout the duration of the study and, despite the severity of the fire, this effect was seen to persist in the recolonising vegetation. Given that in species-poor lowland heath habitats the bulk of floristic diversity can often be found within the lower plant community (Rodwell, 1998), the interactive effects of both nitrogen deposition and wildfire not only pose a serious threat to the biodiversity of this habitat, but also to its structural and functional integrity.

Lower plant responses to N additions at the Thursley field site, have historically been measured as a functional type response, i.e. lichen and moss abundance *en masse*. This approach does not, however, provide information on species-specific responses to N additions, or allow for a better understanding of the effects of elevated N on species dynamics and composition. The primary aim of this study therefore, is to test the hypotheses that (a) added N alters typical regeneration dynamics of lichen and bryophyte species five years after a major fire, and (b) that N additions have a detrimental impact on the species diversity, composition and the reproductive fitness of lichen and bryophyte species. Furthermore, possible mechanisms pertaining to nitrogen tolerance of species will be discussed.
Materials and Methods

Experimental site and design

The experimental site is situated within an area of Calluna dominated lowland heath (Calluna -Ulex minor NVC type H2) at Thursley Common National Nature Reserve, Surrey, UK (Latitude: 51° 9’ 22” N; Longitude: 0° 41’ 58” W). The soil is a sandy podsol, with a shallow humus layer and low organic matter content. Background nitrogen deposition at the site is estimated to be approximately 12 kg ha\(^{-1}\) yr\(^{-1}\) (Power et al. 2006). The experimental area comprises four replicate blocks, each containing four 4 x 4 m plots. Two plots per block are assigned to a control treatment and a further two plots to nitrogen addition treatments. N additions in the form of (NH\(_4\))\(_2\)SO\(_4\) were applied to the site at two weekly intervals throughout the year, from April 1998 to date, at a rate of 0 (control) or 30 (+N) kg ha\(^{-1}\) yr\(^{-1}\). Treatments were applied using a fine nozzle knapsack sprayer (nozzle ref: 15-061) in order to simulate as closely as possible the natural mode of atmospheric deposition. Control plots received an artificial rain solution consisting of K\(^{+}\), Mg\(^{2+}\), Na\(^{+}\), SO\(_4\)^{2-}, Cl\(^{-}\) and Ca\(^{2+}\) at concentrations measured in rainfall at a nearby site (Uren, 1997). Additions represented 7% of the annual average rainfall recorded on site (Barker et al, 2004).

Field Sampling

Field sampling was carried out in February and March of 2011. Within each 2 x 2 m sub-plot, a 1 x 1 m quadrat (with 25, 20 x 20 cm sub-divisions) was placed centrally. Lichen and bryophyte species were identified and recorded as being present or absent within each 20 x 20 cm square. Algae, lichen crusts and squamules were also recorded. Additional observations were made on the reproductive status of each species present (e.g. gametophyte, sporophyte, sporophyte development, squamule, podetia and apothecia). Individuals were identified in the field using botanical field guides (Hodgetts, 1992; Dobson, 2005; Atherton et al. 2010). Specimens that could not be identified confidently in the field were sampled for subsequent laboratory analysis.

Laboratory examination

Collected samples were examined using a dissection microscope (max x40 magnification) and when necessary, a compound microscope (max x1000). Morphological and chemical characteristics were assessed using botanical keys.
Lichen chemical reagent tests were carried out using potassium hydroxide (K) and bleach (C). Para-phenylenediamine (Pd) was used sparingly in cases where samples could not be distinguished between their K and C reactions in accordance with the methodology outlined in Smith et al. (2009). In addition, UV-fluorescence was measured using a Mineralight ® lamp (model UVSL-58) at 350 nm.

**Data analysis**

The abundance of each species (hereon expressed as the response variable $Y$) was derived from its percentage frequency (e.g. the total number of times species X occurred in 25 quadrats). The percentage frequencies were then analysed to show changes in abundance, following the methodology outlined in Hill et al. (2005). Whilst the frequency of a species indicates how common it is, it does not provide information on how much of it is present (i.e. its spatial distribution). A species may have a high frequency, indicating that it is widespread over a particular habitat, yet it may also have a low mean % cover, which implies that whilst it is a commonly occurring species, it is not very dominant in the habitat in terms of occupied spatial area. In order to assess the relative dominance of a recorded species at Thursley, frequency measurements for each species recorded were weighted using percent cover values for lichen and bryophytes (as functional groups, not individual species) derived from Thursley 2010 vegetation sampling data (methods outlined in Barker, 2001; Green, 2005; Jones, 2009).

$Y$ variables were analysed in relation to the following explanatory variables derived from data collected from the field site in 2009 and 2010 (detailed methodologies pertaining to these parameters are outlined in Chapter 2, however, mean values have been included in Table 4.1): N treatment (a two factor variable comprising control and +N), soil pH, soil moisture, bryophyte cover and canopy height. Binomial generalized linear models (GLM) were fitted on proportional data and checked for statistical significance using chi squared-tests. GLM with poisson errors were fitted to count data (i.e. total number of species), and assessed for statistical significance using Fishers F test. Evidence of overdispersal (i.e. the residual deviance being larger than the residual degrees of freedom) was corrected by re-fitting the model with quasi-empirical error structures, followed by F tests, as outlined by Crawley (2007). All models were simplified using likelihood ratio deletion tests until
the minimum adequate model was attained (Crawley, 2007). Data were analysed using the statistical package R (version 2.10.1) (R foundation for statistical computing, 2009).

Results
Species diversity
The number of lower plant species (inclusive of bryophyte and lichen species, but excluding algal species) recorded was significantly less in the +N plots (mean values: +N plots: 10, Control plots: 13, P<0.01). The number of bryophytes recorded were also significantly less in the +N plots (mean values: +N: 3, Control: 4, P<0.05). Treatment means for lichen species diversity were lower in the +N plots (mean values: +N: 13, Control: 8), however these differences were not statistically significant.

Bryophytes
*Campylopus introflexus* was the most abundant moss species in both the control and +N treated plots with only small, non-significant treatment differences recorded in relation to the frequency of actively growing individuals (+N: 95 %, Control: 89 %, Table 4.1). The proportion of dead individuals, however, was significantly greater in the +N plots (+N: 20 %, Control: 5 %, P<0.01, Table 4.3). At the time of sampling, the proportion of current season sporophytes were higher in the +N plots, but not significantly so (Figure 4.1a). The previous years sporophytes, were however, significantly greater in the +N plots (+N: 90 %, control: 25 %, P<0.001, Table 4.3). Cover weighted values for this species (based on moss percent cover data from the 2010 Thursley vegetation survey, however, indicate a profound influence of N inputs on its spatial distribution, with 86 % of the total moss cover in the +N plots represented by *C. introflexus*, compared with 60 % in the control plots (Figure 4.2).

By contrast, the abundance of *Polytrichum juniperinum* was more than doubled in the control plots compared with the +N plots (+N: 11 %, control: 28 %, Table 4.2) and was additionally seen to be positively influenced by soil moisture availability however these results were not significant. Current season sporophytes of this species were significantly higher in the control plots (+N: 1 %, control: 12 %, P<0.01, Figure 4.1b), as were male inflorescences (+N: 4 %, control: 12 %, P<0.05,
Table 4.3), however treatment effects on previous years sporophytes and number of dead individuals were not significant, despite having greater abundance in the control plots. Again, by cover weighting the frequency of *P. juniperinum* as before, it is evident that the spatial abundance of this species is much reduced by N inputs (+N: 7 %, control: 23 %, Figure 4.2).

Other recorded moss species (see Table 4.2) appeared in frequencies so low that a viable treatment effect was not discernable; however, an exception to this was *Hypnum jutlandicum*, which only occurred in the +N plots (P<0.001).

*Cephaloziella divaricata* (the only liverwort recorded) was seen to be highly sensitive to N inputs and associated N mediated effects (e.g. N driven declines in soil pH and increases in moss cover). Severe declines in abundance were observed in relation to N treatment (+N: 10 %, Control: 82 %, P<0.001) (Figure 4.3a) and decreasing soil pH (P<0.001) and greater moss cover (P<0.05).

![Figure 4.1](image_url)

**Figure 4.1:** The proportion of fruiting organs in two moss species (a – b) and two lichen species (c – d) in relation to control or +N treatments (error bars = ± 1 SEM; n = 48).
Algae

N additions significantly increased the abundance of the filamentous algae *Desmococcus sp.* (+N: 1.33 %, Control: 0.16 %, P<0.05). In addition, a slimey algae (tentatively identified as either *Cylindrocystis sp.* or *Oocystis sp.*) was also seen to proliferate in the +N plots (+N: 33 %, Control: 15 %, P<0.05) (see Table 4.2).

Lichens

All lichens recorded were of the genus *Cladonia* with the exception of a few diminutive individuals of *Hypogymnia physodes* found on the stems of *Calluna vulgaris*. *Cladonia crispata*, *C. furcata*, *C. gracilis*, *C. fimbriata*, *C. strepsilis* and *C. cervicornis* were all negatively associated with N additions, decreasing soil pH and
increased bryophyte cover. In all cases the responses were statistically significant (Table 4.2). *Cladonia coccifera* and *C. macilenta agg.* were less abundant in the +N plots, however, the most significant factor negatively associated with these species was increased bryophyte cover (P<0.01, P<0.001 respectively). Undifferentiated lichens at the crustose stage were significantly retarded by N additions (P<0.001) and N treatment related declines in soil pH (P<0.01). Whilst increased bryophyte cover was seen to be influential, it was just short of being statistically significant (Table 4.2).

Not all species responded negatively to N inputs, *C. floerkeana, C. squamosa* and *C. pyxidata* were significantly increased in +N plots compared to control plots (Table 4.2). In addition, they also responded positively to an N driven decrease of soil pH (P<0.05). *C. polydactyla* was more abundant in the +N plots (11 % compared to 7 % in control plots) as was *C. ramulosa* (+N plots: 6%, Control: 4 %), however these increases were not statistically significant. Additionally, a decline of abundance in the latter species was related to decreasing soil pH, however, this was just short of being a significant effect. *Cladonia portentosa* was present in very low frequencies, with a slight (statistically significant) increase observed in the +N plots (Table 4.2).

In regard to lichen reproductive biology, N additions significantly reduced the proportion of fruiting individuals of *C. coccifera* (P<0.01) and *C. crispata* (P<0.001) (Figure 4.1c). By contrast, N additions significantly increased the proportion of fruiting in *C. floerkeana* (P<0.01) (Figure 4.1d) and *C. squamosa var. squamosa* (P<0.001). Fruiting individuals of *C. ramulosa* and *C. polydactyla*, whilst greater in the +N plots, were not significant (Table 4.3).

### Table 4.1: Treatment means of co-variates, derived from Thursley 2010 pin quadrat data and soil analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>+N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bryophyte cover (%)</td>
<td>46 +/- 15.6</td>
<td>82 +/- 5.2</td>
</tr>
<tr>
<td>Canopy height (cm)</td>
<td>15 +/- 2.3</td>
<td>16 +/- 1.4</td>
</tr>
<tr>
<td>Soil pH</td>
<td>4.8 +/- 0.2</td>
<td>4.1 +/- 0.2</td>
</tr>
<tr>
<td>Soil moisture (%)</td>
<td>8 +/- 1.2</td>
<td>11 +/- 2.4</td>
</tr>
</tbody>
</table>

Error given according to +/- s.e. of the mean. n = 16.
Table 4.2: Table of mean abundance of lower plant species recorded at Thursley, and species response to explanatory variables

<table>
<thead>
<tr>
<th>Species</th>
<th>Control (%)</th>
<th>N+ (%)</th>
<th>Treatment</th>
<th>P-value</th>
<th>F-value</th>
<th>Df.</th>
<th>pH</th>
<th>Bryophyte</th>
<th>Calluna canopy</th>
<th>Soil moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bryophytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylopus introflexus</td>
<td>89</td>
<td>95</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephaloziella divaricata</td>
<td>83</td>
<td>10</td>
<td>***-</td>
<td>94.56</td>
<td>47</td>
<td>***-</td>
<td>-</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceratodon purpureus</td>
<td>0.1</td>
<td>0.1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicranum scoparium</td>
<td>14</td>
<td>8</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypnum jutlandicum</td>
<td>0</td>
<td>1</td>
<td>***+</td>
<td>47.82</td>
<td>47</td>
<td>ns</td>
<td>ns</td>
<td>**+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pohlia nutans</strong></td>
<td>8</td>
<td>4</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Polytrichum juniperinum</strong></td>
<td>28</td>
<td>11</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(+,-) = direction of response. P value significance = *<0.05; **<0.01; ***<0.001. n=48

Table 4.3: treatment means (%) of fruiting and dead individuals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Fruit (this season)</th>
<th>P value</th>
<th>F</th>
<th>Df.</th>
<th>Fruit (previous season)</th>
<th>P value</th>
<th>F</th>
<th>Df.</th>
<th>Inflorescence</th>
<th>P value</th>
<th>Dead plants</th>
<th>P value</th>
<th>F</th>
<th>Df.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylopus introflexus</td>
<td>72</td>
<td>85</td>
<td>ns</td>
<td>-</td>
<td>25</td>
<td>90</td>
<td>***+</td>
<td>9.054e46</td>
<td>-</td>
<td>ns</td>
<td>5</td>
<td>20</td>
<td>**+</td>
<td>9.1</td>
</tr>
<tr>
<td>Polytrichum juniperinum</td>
<td>12</td>
<td>1</td>
<td>**+</td>
<td>8.9</td>
<td>46</td>
<td>11</td>
<td>2</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>4</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cladonia coccifera</td>
<td>20</td>
<td>4</td>
<td>**+</td>
<td>11</td>
<td>46</td>
<td>32</td>
<td>2</td>
<td>***+</td>
<td>40</td>
<td>46</td>
<td>5</td>
<td>17</td>
<td>9</td>
<td>5.5</td>
</tr>
<tr>
<td>Cladonia crispata</td>
<td>1</td>
<td>9</td>
<td>ns</td>
<td>-</td>
<td>2</td>
<td>46</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>31</td>
<td>4</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cladonia floerkeana</td>
<td>13</td>
<td>10</td>
<td>ns</td>
<td>-</td>
<td>2</td>
<td>46</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>ns</td>
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</tr>
<tr>
<td>Cladonia gracilis</td>
<td>6</td>
<td>2</td>
<td>ns</td>
<td>-</td>
<td>2</td>
<td>46</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cladonia macilenta</td>
<td>9</td>
<td>11</td>
<td>ns</td>
<td>-</td>
<td>2</td>
<td>46</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cladonia polydactyla</td>
<td>1</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>2</td>
<td>46</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cladonia portentosa</td>
<td>2</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>2</td>
<td>46</td>
<td>1</td>
<td>ns</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>4</td>
<td>ns</td>
<td>-</td>
</tr>
<tr>
<td>Cladonia pyxidata</td>
<td>0.1</td>
<td>1</td>
<td>*+</td>
<td>5.52</td>
<td>43</td>
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<td>9.054e46</td>
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<td>**+</td>
<td>9.1</td>
<td>46</td>
</tr>
<tr>
<td>Lichen crust</td>
<td>0.2</td>
<td>3</td>
<td>*+</td>
<td>4.61</td>
<td>43</td>
<td>**+</td>
<td>9.054e46</td>
<td>-</td>
<td>ns</td>
<td>5</td>
<td>20</td>
<td>**+</td>
<td>9.1</td>
<td>46</td>
</tr>
</tbody>
</table>

(+,-) = direction of response. P value significance = *<0.05; **<0.01; ***<0.001. n=48
Discussion

*N deposition impacts on lower plant diversity*

The gamma and alpha diversity (i.e. the total number of species recorded, and the mean of this total, respectively *(sensu* Whittaker, 1960; 1972)), of bryophyte and lichen species recorded in the experimental plots, do not necessarily indicate large diversity losses in relation to N inputs, however, analyses demonstrate that at a beta diversity level (i.e. the differentiation implicit within the treatment levels), N inputs are having a significant, detrimental effect on lower plant species diversity. This is best demonstrated at the community level, whereby N inputs can be seen to severely reduce the abundance of sensitive species, and promote the dominance of those either tolerant of, or stimulated by, N inputs, resulting in less diverse species assemblages (Figure 4.4).

![Control plots - species composition (%)](image1)

![+ N plots - species composition (%)](image2)

**Figure 4.4:** The composition of lower plant species within the control and +N treatments. Species with less than 2% abundance are not represented.

Much of the work on lower plant, compositional change and diversity loss as a function of increasing N pollution, has focused on epiphytic species (Nimis *et al.* 2002; van Herk, 2003; Wolseley *et al.* 2006; Perlmutter, 2010), however, the findings from Thursley are more closely aligned to studies of terricolous cryptogamic communities. For example, Britton & Fisher (2007) observed changes in alpine heath lichen species composition and an overall reduction in diversity, within four years of (relatively low, 10 – 20 kg ha\(^{-1}\) yr\(^{-1}\)) N inputs. In addition, Haworth *et al.* (2007), recorded calcicole – calcifuge shifts in bryophyte species composition, and a reduction in sensitive calcareous grassland species, following inputs of 35 kg N ha\(^{-1}\) yr\(^{-1}\).
Given that within heathland systems, the bulk of floristic diversity is represented by cryptogamic groups, the reduction, or absence of low frequency species as a result of N driven shifts in community structure, not only signals that the biodiversity of these systems is under threat, but also the ecological functions associated with this biodiversity (Phoenix et al. 2011). Such functions include reducing N leaching to groundwaters by filtering deposited N (Curtis et al. 2005); sequestering carbon and nitrogen (Lamers et al. 2000) and the provision of habitats for insect species and thus a food source for insectivores, and larger ungulates (Klein, 1990).

**N deposition alters early-stage succession**

Severe wildfires, such as that experienced at Thursley in 2006, have been seen to modify the re-establishment of characteristic heathland vegetation, and in some cases, facilitate a secondary successional pattern that is much different in structure and function to that occurring pre-fire (Clément & Touffet, 1990). In addition, elevated atmospheric N deposition has also been seen to have a modifying effect on the nature and rate of early stage secondary succession (Ketner-Oostra, 2002; Sparrius & Kooijman, 2011).

Initial post-fire recolonisation in heathland systems is characterized by pioneer bryophyte species *Ceratodon purpureus* and *Funaria hygrometrica* (Gimingham, 1972; Rodwell, 1998). These early occupants modify the environment, and by doing so, facilitate the establishment of early successional (vascular and non-vascular) species (Connell & Slatyer, 1977). The duration of this initial stage has been seen to last up to three years in moorland and heathland systems, and is dependent on availability of diaspores, water and nutrient resources (Maltby, 1980; Clément & Touffet, 1990). Four and a half years after the fire at Thursley, vegetation has differentiated beyond the initial stage, and clear treatment effects are apparent in the emerging successional patterns. Typically, early stage succession within *Calluna – Ulex minor* heaths, is characterized by the high abundance of *Polytrichum* species (Rodwell, 1998), however, within the Thursley +N plots, *Polytrichum juniperinum* has largely been replaced by *Campylopus introflexus* as the dominant moss species. The suppression of recolonising *Polytrichum* species by *C. introflexus* has also been reported by Hasse (2007) and Sparrius & Kooijman (2011), the latter observing that shifts towards *C. introflexus* dominance occurred primarily in areas receiving high N deposition. Whilst *Polytrichum* can also form vast mats, Sparrius & Kooijman (2011)
postulate that the mode of N uptake via below-ground rhizoids, renders this species at a competitive disadvantage to *C. introflexus*, the latter forming extensive ectorganic layers of dead moss which can effectively intercept and recycle N before it reaches the soil.

*Cladonia* species are also characteristic of early stage succession, however their recolonisation after intense fire is often a slow process due to the eradication of living fragments and their lack of underground, regenerative parts (Johansson, 2005; Ketner-Oostra, 2006). Establishment of terricolous lichen species is, therefore, contingent on the formation of suitable substrates by initial or early stage occupants (Hobbs *et al.* 1984) and the dispersal of viable diaspores from nearby populations or via animal, bird or inveterbrate vectors (Daniels *et al.* 2008; Nash, 2008). The cover of regenerating lichens at the Thursley site has been severely inhibited by N treatments every year since the fire (e.g. Pre-fire (2005) Control: 53 %, +N: 6 %. Post fire (2010) Control: 15 %, +N: 7 %). In addition, this survey found that the *Cladonia* species that dominate either the +N or control plots, are characterized by different species assemblages. *Cladonia crispata*, *C. furcata* and *C. fimbriata* (representing the dominant lichen species in the control plots) are absent at high frequencies in the +N plots, which in contrast, are characterised by *C. macilenta*, *C. floerkeana* and *C. coccifera*, all of which are considered to be generalist species (Sparrius & Kooijman, 2011). High survival rates of *C. floerkeana* and *C. macilenta* in N polluted Danish dune systems have been attributed to their ability to grow upon litter mats of *C. introflexus* (Ketner-Oostra & Sykora, 2008), however, more sensitive species are often restricted to mineral soil substrates, with very little organic matter content (Nijssen *et al.* 2010). Increasing *C. introflexus* cover therefore, not only usurps the substrate required for many *Cladonia* species, but it has also been hypothesized that it encloses and strangles less vigorous individuals, using them as a temporary nutrient source (Sparrius & Kooijman, 2011).

The species composition within the Thursley experimental plots, implies therefore, that secondary successional patterns are being altered by N inputs (Figure 4.4). Whilst *C. introflexus* is abundant within the control plots, it is associated with other equally abundant species that are characteristic of heathland early stage secondary succession. As such, this pattern resembles the facilitation succession model defined by Connell & Slatyer (1977) in which fast growing pioneer species modify the environment for subsequent colonizing species, which themselves pave
the way for later incoming species. The species assemblage in the +N plots however, is dominated by *C. introflexus*, with other species playing a subordinate role. Connell & Slatyer (1977) define this successional pattern as the inhibition model, whereby invading colonists suppress the growth of other species, potentially engendering a successional stasis.

Not only are early stage successional patterns being modified by N inputs, there is some evidence to suggest that N is promoting the establishment of species typically found in later successional phases; albeit at low frequencies, *Cladonia portentosa* and the pleurocarpous moss *Hypnum jutlandicum* were only recorded in the +N plots. Given that these species are considered to be stress-tolerators, characteristic of later successional stages (Magnusson, 1982; Rose, 1994), it could be inferred that N inputs are accelerating secondary succession – a phenomenon noted in other studies where N deposition was high (e.g. Dutch sand dune communities (Riksen et al. 2006) and temperate grasslands (Tilman et al. 1984, 1987)). Alternatively the presence of stress-tolerant strategists at this stage could denote a gradual transition to characteristic successional patterns. Connell & Slatyer (1977) suggest that the presence of later stage ‘stress-tolerant species’ within an inhibition model assemblage, such as this, could eventually result in the re-establishment of a traditional successional sequence if there are enough reproductive propagules available. Such a case was observed by Minarski & Daniels (2006) who reported a recovery of lichen vegetation and a resumption of typical succession in dune grasslands that had been dominated by *C. introflexus* for 10 years. Similar findings were also reported by Hasse (2007), who observed that initial losses in species abundance in relation to increased *C. introflexus* mats were compensated within two years following the initial disturbance. Where diaspores are not available however, the long-term threat to native vegetation by *C. introflexus* invasion could be profound.

**Reproductive Biology and life cycles**

The detrimental effect of air pollution on the reproductive biology of lower plants has been quantified in many systems, with observations of diminished sporophyte production in bryophytes (Rao, 1982; Giordano *et al.* 2004) and reduced lichen apothecia in areas exposed to high levels of heavy metals, and nitrogenous pollutants (Le Blanc & De Sloover, 1970; Estrabou *et al.* 2004; Mikhailova, 2007). A reduction in reproduction capacity will necessarily result in reduced or inhibited spor
germination, and thus detrimentally impact on species fitness, population dynamics and community structure (Zedda & Rambold, 2009; Holt 2011). At Thursley, all sexual reproductive functions in *Polytrichum juniperinum* were suppressed by N additions, as were the number of apothecia recorded for the majority of *Cladonia* species (significantly so for *C. crispat* and *C. coccifera*). The implications of such a decline in species fitness bode ill in regard to the observed N driven stimulation of reproductive function in *Campylopus introflexus*.

Even without the enriching effect of N deposition, *C. introflexus* is described as having a ‘colonist life strategy’, characterized by high output of sexual and asexual diaspores and a long gametophytic life span (Siebel & During, 2006), excess N therefore, only serves to contribute to its prolificacy (Ketner-Oostra & Sykora, 2008; Sparrious & Kooijman, 2011). In this study, the fecundity of *C. introflexus* was stimulated by N additions, with the proportion of fruiting individuals from the current and past year higher in the +N plots (significantly so for the latter). Given that the proportion of dead individuals was also greater in the +N plots, it is clear that N additions are accelerating the life cycle of this species, and, thus, its facility for the competitive exclusion of other species. In regard to *Cladonia* species, the presence of spore bearing organs (apothecia or pycnidia) was markedly (in some cases significantly) reduced in the +N plots for the majority of species recorded. Conversely, for *C. floerkeana* and *C. polydactyla* the proportion of apothecia observed was significantly greater in the +N plots, indicating an element of tolerance to N inputs in regard to their overall reproductive fitness. Given that both these species are considered to be somewhat generalist in their ecological distribution (Smith et al. 2009), the decline of fruiting capacity in typically characteristic heathland species (e.g. *C. crispat*, *C. gracilis*) is concerning, particularly in regard to the potential decline in heathland lichen biodiversity that this inevitably signals.

**Possible mechanisms and processes influencing responses to N**

Competition for light, as a result of N facilitated increases in vascular plant biomass, has been often cited as factor negatively affecting lower plant communities (Bobbink *et al.* 2010; Barker, 2001; Hauck, 2010). In this study, however, no significant effect of increased *Calluna* cover on lichen and bryophyte species was found. At the time of surveying, the *Calluna* plants at Thursley were in their fifth (post-fire) year, with negligible treatment differences observed in relation to their height (control plots: 15
cm, +N plots: 16 cm), therefore, it is evident that other processes are responsible for the observed declines in recolonising lower plant species.

Lichen species that were positively promoted by N additions (C. floerkeana, C. polydactyla, C. ramulosa, C. squamosa var. squamosa) were not necessarily the same as those with the highest overall abundance in the +N plots (C. floerkeana, C. macilenta, C. coccifera). As mentioned above, however, the capacity of the latter to grow on older mats of C. introflexus has been attributed to their broad ecological amplitude in terms of substrate (Ketner-Oostra & Sykora, 2008) rather than any inherent nitrophilous tendencies. Besides direct competition from vigorous C. introflexus mats, soil pH is also considered to be a key factor in the exclusion of species (Spire, 2010). It is interesting to note therefore, that the most abundant Cladonia species in the control plots (C. crispata, C. furcata and C. fimbriata) demonstrated a significant negative correlation with increasing soil acidity (an associated effect of (NH4)2SO4 additions at Thursley, Chapter 2). In contrast, species more prevalent in the +N plots (C. floerkeana, C. squamosa var. squamosa and C. ramulosa) all increased with increasing soil acidity. Literature relating to the response of terricolous lichen species to soil pH changes is rather scarce, however, Vagts & Kinder (1999), in their heathland manipulation study, found that increasing soil pH from 4 to 7 resulted in severe growth retardation and eventually death for C. floerkeana, whilst C. furcata flourished as conditions became less acidic.

For bryophytes, the only significant response associated with increased soil acidity was represented by Cephaloziella divaricata, a liverwort commonly associated with the early colonization of bare ground (Atherton et al. 2010). A decline in the abundance of this species in regard to N mediated soil pH changes could have ecological implications for heathland functioning. Duckett & Read (1995) observed a shared fungal association between Calluna (and other major ericoid) and liverworts in the Cephaloziaceae. Their study demonstrated that seeds from ericaceous plants germinated in close proximity to liverworts possessing the endophyte Hymenoscyphus ericae. In cases where this endophyte was absent, the delicate hair roots of ericaceous seedlings were unable to extend into acidic substrates, and therefore, failed to establish (Read, 1991; Duckett & Read, 1995). The loss of Cephaloziella through N deposition impacts could therefore potentially affect the ability of Calluna to compete effectively in N polluted environments.
Increased soil acidification as a result of anthropogenic N deposition has been reported in many ecosystems and has been associated with observed reductions in plant biodiversity (Bowman et al. 2008; Nordin et al. 2009; Stevens et al. 2009). Declines in soil pH are coupled with declines in base cation buffering capacities and as a consequence, aluminum (Al\(^{3+}\)) and iron (Fe\(^{3+}\)) become freely available. Soluble Al\(^{3+}\) and Fe\(^{3+}\) are both toxic to plants in high concentrations and have been linked, in much the same way as excess ammonium (Pearce et al. 2003), to chlorophyll degradation in lichens (Hauck et al. 2003). The production of secondary metabolites by some lichen species, however, can facilitate the uptake of and tolerance to, cations, metals and various protons (Hauck & Jurgens, 2008). It is possible, therefore, that the observed tolerance of *C. squamosa* to N mediated soil acidification is a result of its secondary metabolite, squamatic acid, a depside that chelates Fe ions to a more stable form (Williams, 1974; Lawrey, 1975).

Secondary metabolites can also protect certain lichen species against the toxic effects of elevated nitrogen. Species with a higher tolerance to nitrogen have been found to produce anthraquinones, such as parietin, the substance found in the nitrophytic epiphyte *Xanthoria parietina* (Bell & Treshow, 2003; Hauck, 2011). Anthraquinones are secondary defence metabolites, which, in the case of the pigment parietin, are instrumental in protecting photosynthetic apparatus in areas of high pollution (Silberstein & Galun, 1988). Given that almost all of the N tolerant species recorded in the +N plots at Thursley (*C. floerkeana*, *C. macilenta*, *C. polydactyla*, *C. coccifera*) share the anthraquinone derivative, rhodacladonic acid (the pigment found in all red fruited *Cladonia*, Baker & Bullock, 1969), we postulate that this compound has some underlying protective function in respect of nitrogen tolerance within these species, however, as literature relating to the function of this compound is scarce, further research would be required to test this theory.

**Conclusion**

This study has demonstrated that enhanced N deposition affects the typical post-fire regeneration dynamics of heathland cryptogamic flora, by facilitating the growth and development of the invasive moss *Campylopus introflexus*. Sensitive species, characteristic of early-stage succession appear to be competitively excluded by dense mats of *C. introflexus* in which only generalist species thrive, resulting in a decline in species diversity and a loss of characteristic species. In addition, an observed
relationship between N inputs and reduced sexual reproductive capability in sensitive species implies a likely continued decline in subsequent generations.

Lichen species seemingly tolerant to N addition or N associated soil acidification, were identified as potentially possessing secondary compounds that enable them to mitigate the damaging effects of acidification and or/ eutrophication on photosynthetic processes, however, only further monitoring will reveal if these species are effective tolerators of N deposition on heathland systems in the longer term.

Many ecosystems have experienced elevated N inputs over long time periods and can provide evidence of N associated impacts on soil pH and diversity loss within functional groups, few studies have focused on these changes at a species level. It is likely therefore, that the N impacts on species community composition, diversity and successional patterns, could be potentially widespread.
Chapter 5

GENERAL DISCUSSION

Abstract

This final chapter synthesises the findings presented in those previous, to provide an overview of how this research has added to studies of nitrogen deposition effects on heathland ecosystems, and how both above and belowground responses to N inputs are modified by climatic factors. Limitations arising within the research approach are discussed, along with suggestions for further areas of study that could potentially add new dimensions to our current knowledge of N deposition effects research.
The principal aim of this study was to investigate the impacts of atmospheric N deposition on the structure and function of lowland heathland ecosystems in the UK. The main focus for this research was the long-term, field scale N manipulation experiment at Thursley Common, Surrey; an area that has been dedicated to N deposition research for the past 14 years. By way of continuing with the N additions and subsequent annual vegetation and biogeochemical sampling, this work is intended to add to previous findings, and to further in some way, our current understanding of how N additions have and are, altering heathland processes and variability in interaction with climatic influences.

Long-term experiments offer the possibility of providing new insights into the nature of ecosystem change as a result of ecological disturbances, however, the breadth and scope of the findings from one site can be not be wholly representative of heathlands nationwide if their findings are not realistic with ‘real-world’ scenarios (Phoenix et al. 2011). In order therefore, to validate the results from the manipulation approach, the experimental methodology was broadened to encompass a number of heathland sites across the British Isles, in order to determine whether signals of N deposition change were detectable at a nationwide scale.

The findings arising from these approaches have been discussed in detail within each paper presented within this thesis, however, a synthesis of key findings arising from each are now discussed and, where possible, contextualised in relation to each other and for their contribution to the wider area of N deposition research.

Lowland heathland responses to N addition, over the long-term, indicate that ecosystem processes are being accelerated under elevated N loads as a result of increased primary production via plant and microbial uptake / immobilisation of added N. In addition to increases in Calluna biomass, N additions have also increased foliar N concentrations throughout the duration of the study, which, through foliar senescence, is translocated into litter, enriching litterfall N fluxes (Barker, 2001), and accelerating internal N cycling through increases in net mineralisation, inorganic N concentrations and plant uptake. A consequence of higher N availability across many systems, has been an increase in nitrophilous plant species (Bobbink et al. 2010), particularly in continental heathlands, where significant shifts from heathland to
grassland vegetation were attributed to elevated N deposition (Heil & Diemont, 1983; Aerts, 1990).

At the Thursley site, however, this has not been the case, despite the fact that the *Calluna* canopy has been removed twice (firstly as part of Barker’s management study (2001), and secondly as a result of the wildfire in 2006) throughout the duration of the study. Findings elsewhere suggest that *Calluna* can maintain a competitive advantage over faster growing species, if its canopy remains intact (Aerts, 1990, 1993). Whilst Barker (2001) did report a temporary increase of *Deschampsia flexuosa* in the +N plots, following management, this was a transient response, and no further grass invasion has been recorded at the site since.

Negligible grass cover, in addition to low frequencies of other higher plant species (notably *Erica cinerea* and *Ulex minor*) make the site currently unsuitable for the study of N impacts on vascular plant diversity and species dynamics, however, this limitation is addressed within the context of the nationwide heathland survey (chapter 3), which, by virtue of encompassing a greater spatial area, includes much of the floristic variation that exists in heath communities. Across a broad gradient of N deposition (from 5.9 kg N ha⁻¹, yr⁻¹ to 32.4 kg N ha⁻¹, yr⁻¹), significant relationships were found between increased grass cover and increasing N deposition (predominantly comprised of the nitrophilous species *Molinia caerulea* and *Deschampsia flexuosa*), in tandem with a general decline of overall species richness (inclusive of both higher and lower plant species). The survey approach also took into consideration the naturally occurring climatic variation across the UK, and as such, temperature and precipitation gradients were included in the multivariate statistical analyses, along with N deposition. In regard to this, N driven declines in species richness were also seen to be impacted by increasing temperature, signifying the importance of climate as an influential modifier of N driven responses.

The influence of climatic drivers (including climate change impacts) and N deposition (and other atmospheric pollutants) have been traditionally investigated separately, however, there is awareness that the combined effects of climatic and pollution factors may significantly differ from those observed independently, due to the effects of various synergistic or antagonistic interactions (Bytnerowicz et al. 2007; De Wit, 2008). The survey data provide evidence of how ecosystem responses to N inputs can be modified by climatic conditions, and this is further endorsed by the experimental
study, where ecosystem responses to drought conditions were seen to be significantly influenced by N additions. Long-term data relating to *Calluna* productivity was seen to be significantly influenced by climatic patterns (rainfall in particular), with seasonal fluctuations in precipitation driving observed inter-annual variability across the years. Furthermore, evidence of *Calluna* drought damage (e.g. reduced plant growth, flowering, visible injury) following dry (and / or hot) periods, was significantly exacerbated with N additions.

The negative impacts of severe drought damage potentially compromises the reproductive output of vegetation, under climate stress (Gordon *et al.* 1999; Jentsch *et al.* 2009) and has detrimental implications on subsequent *Calluna* generations as a result of diminished seed set, ripening and seed rain abundance, but also for ecosystem service areas such as plant-pollinator interactions. Given current predictions of climate and N deposition future scenarios, such observed negative effects could pose a significant threat to the integrity of heathland systems. Lowland England is one of the driest regions within the UK – and this is further compounded, by progressive trends towards hotter, yet drier summers, with average summer temperatures expected to rise between 2° C – 6.4° C in the South East (Met Office, 2011). In addition, N emissions and deposition rates in the UK, are currently in excess of the National Emissions Ceilings Directive, European Union air quality targets and empirical critical loads, and globally, these are expected to double from current values within the next few decades (Galloway *et al.* 2004).

A potential consequence of hotter, drier summers is an increase in the frequency and severity of naturally occurring heathland wildfires (Berry *et al.* 2004). Whilst wildfires have long coexisted with human activities and management of the landscape (Butterbach-Bahl & Gundersen, 2011), severe fires can be highly detrimental to ecosystem regeneration dynamics (Gimingham, 1972) initiating shifts in vegetation structure and diversity (Acacio *et al.* 2009; Coffman *et al.* 2010) and in some cases, facilitating a secondary successional pattern that is different from that of before (Clément & Toufflat, 1990), especially in areas subject to high levels of N deposition (Sparrius & Kooijman, 2011).

Whilst N additions at Thursley were seen to have a significant positive influence on the initial post-fire regeneration of *Calluna* (in terms of shoot length) and
moss cover (the latter at a magnitude not observed before the fire), the significant negative influence of N on pre-fire lichen abundance was seen to persist in the recolonising vegetation, highlighting the profound sensitivity of this element to ecosystem perturbation. As for the modifying effect of wildfire and N deposition on heathland regeneration dynamics, the in-depth cryptogamic survey carried out at the beginning of 2011 (five and a half years after the fire), provided evidence that N deposition can alter early stage secondary successional patterns in regenerating heathland vegetation. The increase of moss cover observed post-fire was seen to be dominated by the invasive species *Campylopus introflexus* (at the expense of more sensitive, yet characteristic heathland bryophyte species), the growth and reproductive development of which, were seen to be stimulated by N additions. As dense carpets of *C. introflexus* have been seen to contribute to a significant reduction in the germination and establishment of *Calluna* (Equihua & Usher, 1993), the increase of this species in N enriched areas poses a potential threat to the post-fire regeneration of *Calluna*, and, consequently, typical heathland structure.

Despite negligible changes to lichen species alpha diversity, a reduction of species at the beta diversity level was seen to be significantly related to N additions. The most profound effects, however, were at the community level, where significant reductions in the abundance of characteristic, or sensitive heathland species, were observed in relation to N inputs. Given that lower plants represent the bulk of floristic diversity in lowland heathland habitats (Rodwell, 1998), such declines are of great concern in regard to the conservation of heathland biodiversity.

Whilst the in-depth lower plant survey revealed that early stage post-fire successional patterns were being changed by N additions, findings from other studies report that, in the longer term, mono-specific dominance of *Campylyopus introflexus* is often temporary, and does not influence later successional pathways or compromise heathland structure in the long term. Despite this, the prognosis for heathland lower plant diversity could be less favourable, particularly in regard to the significant (N associated) reduction of reproductive viability in characteristic lichen and moss species.

The findings arising from the lower plant survey at Thursley, provide a detailed, and thus valuable insight into the community dynamics of cryptogamic species - a group of which indepth research has tended to be overlooked in favour of higher plant
dynamics (Phoenix et al. 2011). Indeed, despite being rich habitats for lower plants, there is a paucity of research in relation to lowland heathland lichen assemblages and responses to disturbances events (Sanderson, 2012), that goes beyond the widely reported, yet often generic, observations of declining abundance (particularly in regard to N manipulation studies (Carroll et al. 1999; Power et al., 2006; Pilkington et al. 2007)). These findings, however, when positioned in relation to the broader context of declining lower plant abundance and species diversity observed at the nationwide scale, suggest that N impacts on community composition and successional relationships could be potentially widespread.

Wildfires have the capacity to export large quantities of nitrogen from above and below ground ecosystem compartments (Butterbach-Bahl & Gundersen, 2011) through the removal of vegetation and soil organic matter. At Thursley, pre-fire N stock calculations suggest that approximately 80 % of the experimental N added to the systems was retained within the vegetation and top 10 cm of the soil. Whilst the fire of 2006 eradicated above ground stores and substantially decreased soil N stores, treatment effects on soil N concentrations were immediately apparent and rapidly increased in magnitude in the subsequent years, representing approximately 99 % retention of experimentally added N over the period spanning 2006 – 2009. N-fate studies carried out by Green (2005) found that the majority of the N added to the site was rapidly immobilised by the microbial community, the biomass and activity of which were significantly increased by experimental N additions. Elsewhere, increases in soil N % have also been observed at the Ruabon upland heathland manipulation experiment, (Field, 2010). In regard to lowland systems, however, experimentally added N was seen to readily leach from poor, mineral soils, resulting in inconsequential N and C pools (Evans et al. 2006). Similarly, soil analyses from the nationwide heath survey found no robust evidence of N or C accumulation in relation to elevated N deposition, despite findings from an earlier gradient study by Jones & Power (2011), that reported strong correlations between increasing soil organic matter and increasing (ambient N deposition).

Sandy lowland heath soils, such as those at Thursley, have relatively low organic matter content in comparison to upland heaths (the latter tending to be wetter and colder, and therefore losing N and C less quickly through slower decomposition rates (Evans et al. 2006)) and as such, literature pertaining to the C sequestration
potential of lowland heathland soils is limited. Indeed, when compared to forest and peatland systems, the C storage potential of lowland heathland is relatively small (Parish et al. 2008), however, this study provides evidence that, despite occupying the lower end of the range in respect to C contents for shrub-dominated systems (Beier et al. 2009), N deposition at a rate of 30 kg ha\(^{-1}\) yr\(^{-1}\), can facilitate substantial C accumulation in low nutrient, mineral soils.

Identifying limitations within the research

The modifying effect of climatic variables on N driven ecosystem responses have been identified at Thursley through the occurrence of stochastic events such as drought and wildfire and this inter-relationship has been further elucidated in the wider, multi-driver survey. Measured concentrations of extractable nitrate and ammonium have been seen to be significantly increased by N inputs at Thursley, however, at a nationwide level, no pattern was detected in the lowland heath soils, which in general, displayed a negative relationship between extractable nitrogen and N deposition. Given that microbial cycling of N and N mineralisation is regulated by climatic and seasonal factors such as soil moisture and temperature (Morecroft et al. 1992; Jamieson et al. 1998) and that 2009 experienced lower than average annual precipitation rates, it is likely that this result is closely aligned to low soil moisture status at the time of sampling. Indeed, within the upland heathland soils, increases in extractable ammonium concentrations were significantly greater in warmer, wetter areas. Similarly, an observed lack of relationship between N deposition and soil N and C accumulation at a national scale, is in contrast to the experimental findings.

The biogeochemical element of the gradient survey is thus potentially confounded by both climatic and temporal factors. Firstly, a lack of moisture (as a consequence of lower annual precipitation rates) found in the inherently drier lowland soils at the time of sampling, may confound the identification of N impacts. It has been suggested that summer sampling periods (such as that carried out in this survey) can fail to adequately detect the effect of elevated N on soil N pools due to a masking effect from plants and microbes that are actively immobilising N during the summer months (O’Sullivan et al. 2011). These authors go on to suggest that the optimum period for studying pollutant impacts on mineral N pools would be between February and April. Secondly, the collection of lowland heathland samples was restricted to the month of July in order to minimise the effects of climate along the geographical
gradient (i.e. samples from the warmer drier South-westerly region were collected before those in the cooler north of the country). Upland heathland samples however were collected within a broader timescale, increasing the likelihood of larger inter-seasonal variation in moisture and moisture-regulated microbial activity.

In regard to the lower plant survey at Thursley, it is possible that a complete picture relating to the nature of early stage recolonisation by lower plant species, has not been presented, due to the inability of the author to identify Cladonia squamules (small, scale-like primary thalli from which the Cladonia podetia grows) to species level. Whilst experienced lichenologists have determined morphological characteristics to aid field identification, it is considered that the only accurate way to confidently name squamules is through the use of thin-layer chromatographical techniques (pers. comm. Dr. Brian Coppins, RBG Edinburgh). Due to time constraints, the author chose to consider squamules as an aggregate response variable, rather than ascribe a specific epithet.

In this study, N deposition impacts on heathland systems have been investigated using two approaches – an experimental and a gradient study. The experimental approach facilitates control of the experimental conditions, thus enabling the investigation of cause and effect relationships on response parameters under a specific N load. This approach is not without its limitations, however, with questions arising regarding the wider applicability of results to non-experimental heathland areas, and indeed in comparison to findings from other experimental studies that may differ in duration, and N loads. More specifically, making point measurements in relation to manipulated exceedance of current critical loads, does not consider the historic legacy of ambient N from previous exposure, and the inherent variability in emission and deposition inputs over time. In contrast, the gradient study provides an opportunity to test the robustness of observed indicators and responses of N depositions, upon a wider spatial scale, however, the identification of cause and effect can be confounded by the influence of other environmental and climatic factors. Additionally, measuring real-world responses to N in respect to modelled N deposition values contains an inherent degree of uncertainty (Skeffington et al. 2007), and again does not account for the influence of historic N loading and threshold effects from N accumulating in the system.
The critical load concept aims to establish deposition levels that ecosystems can be exposed to without incurring harmful effects to its biodiversity or processes (Nillson & Grenfelt, 1988). Based, however, on findings from empirical N manipulation studies, critical load values may become lower with increased duration of treatment and, therefore, may not represent realistic biological thresholds from cumulative N effects over a substantial time-course (Bobbink et al. 2010). This view is further supported in the literature by Nordin et al. (2005), who observed that changes in important ecosystem functions were discernable at rates of N input, markedly lower than the recommended critical load values. In addition, De Schrijver et al. (2011) found that declines in species richness occurred faster where N deposition was low, yet had accumulated in the system.

The element of uncertainty pertaining to the current modelling of deposition data is largely based on discrepancies between modelled parameters (often complex in a paradoxical effort to emulate real world conditions as closely as possible) and actual observed parameters from monitoring networks (ROTAP, in press). Comparisons between the national scale modelled data sets that underpin the critical load concept, and data collected derived from site-specific measurement have been investigated by Heywood et al. (2007) and Skeffington et al. (2007). They postulate that national scale data may not accurately represent individual sites due to scaling issues and the simplifying assumptions that underly these national models, and that the use of modelled critical loads and modelled deposition data to calculate exceedances may lead to either under-estimation, or over-estimation of a site’s vulnerability. Heywood et al. (2007) conclude that in some cases, site-specific data, and national input parameters, differed significantly, indicating that the application of nationwide modelled data, at a site specific scale should be approached with care.

Analysing ecosystem responses in relation to perceived exceedance of a critical load value may, therefore, not be enough to protect ecosystems from N deposition impacts, especially those systems currently lying under a critical load threshold. Whilst inputs at the Thursley experiment are currently in excess of critical loads for heathlands (10 – 20 kg ha\(^{-1}\) yr\(^{-1}\) (UK National Focal Centre, 2003) and we have found robust indicators of N impacts over the duration of the study, what the data cannot tell us, however, is how long these impacts took to become apparent, and indeed if damage had already occurred under ambient conditions, before experimental N additions were
applied. In respect to lichen diversity at Thursley (the most sensitive parameter), the indepth-survey encapsulates the current assemblages and demonstrates that whilst species richness is not affected by N deposition inputs, species assemblages and frequency are severely affected. Unfortunately, the survey cannot inform us to the fate of sensitive species which may have already been lost, and whether the lichen diversity currently represented in Thursley is representative of lowland heathlands in general, as there is a marked dearth in lichen recording in lowland heath areas (Sanderson, 2011). Implications from the cumulative dose approach, however, intimate that lichen diversity (and other sensitive parameters also) can be severely impacted by low-level, but continued, N deposition inputs.

Phoenix et al. (2011) applied an accumulated dose methodology to data arising from the UKCREATE network of long-term N manipulation sites (Thursley being one of these sites), and found that sites inclusive of historically low levels of ambient N deposition, displayed steeper responses in relation to lichen cover than those with higher ambient N deposition. This strongly implies that the early stage of enhanced N loading is the most detrimental to sensitive species, even at low rates of input, and that continued deposition at the same rate can have serious consequences in the longer term as N accumulates in the system.

As critical loads are partly derived from long-term averages of climatic variables (e.g. precipitation and temperature, Posch, 2003), future changes in climate can potentially influence the critical load via these parameters (Wilby, 1995) and thus, their robustness as a tool for gauging ecosystem changes and emission reduction policies (Posch, 2003). This study, in both the experimental and nationwide contexts, has shown the importance of climate on ecosystem processes both solely, and in interaction with N deposition. It is intuitive to expect, that as ecosystem responses to N will be further modified under changing climatic conditions, critical load methodologies will have to be revised. The accumulated dose approach, however, due to its basis on historical data, will be able to provide more accurate information on how an ecosystem will respond not only to current, and historic N deposition inputs, but also highlight where these responses have been, and will be, further impacted by climatic changes.
Further work

Pollutant-mixing analyses

The focus of the nationwide heathland survey was principally directed upon the impacts of increasing nitrogen deposition, in totality, and as a sum of its oxidised and reduced forms. Analyses, however, did not encapsulate deposition partitioning into wet and dry forms, which, if combined with environmental data, could add another valuable dimension in identifying those heathland areas at risk from increasing atmospheric N inputs.

In addition, nitrogen deposition may not be the only pollutant driving changes in semi-natural ecosystem processes, especially in areas proximal to multiple-pollutant emission sources. Using a multivariate approach, the survey analyses could be expanded to include other pollutant forms (e.g. heavy metals, SO$_2$, O$_3$) in conjunction with climatic, geographic and edaphic data. This could help to uncouple confounding effects of ambient pollutants on key processes and bring more insight into the potential complications of multiple pollutant interactions with climatic change factors at a nationwide scale.

Furthermore, as the work outlined here demonstrates, findings from the experimental manipulation study have provided a baseline premise in regard to potentially robust bioindicators of N driven changes in heathland systems, therefore a multiple pollutant approach could also be factored into the Thursley experiment as a means of testing the repeatability of above and below ground responses to N inputs in relation to other pollutants, under controlled conditions.

Further Lower plant studies

Chemical analyses of lichen and moss species from the Thursley experimental plots, would greatly elucidate the findings from the floristic survey. Total N concentrations of moss and lichen tissue would provide valuable information as to the N status of species, and total P concentrations and PME (phosphomonoesterase) assays, would be beneficial in quantifying the role of P as either a mitigating or contributing factor to N impacts. Additionally, more information is needed on the role that secondary metabolites play in a lichen species’ tolerance or sensitivity to N pollution impacts, particularly in the case of the red-pigment rhodocladonic acid, the ecological properties of which are under researched. Furthermore, the use of thin-layer chromatography could be applied to identify lichens at the initial squamular stage, and
thereby gain a better understanding of those species that are among the first colonisers of burnt heathland areas.

**Climate at the macro and micro scale**

This research has shown, at both an experimental and broader spatial scale, that climate has a two fold impact on vegetation. Firstly, climate directly governs the distribution of vegetation types, and secondly, the soil conditions (including key chemical and biological processes) which support the vegetation, are equally dependent on climate conditions. Vegetation, in turn, influences soil conditions and also regulates changes at the microclimatic scale (typically 0.5 – 1 metre from the soil surface) (Stoutjesdijk & Barkman, 1987). Where atmospheric N is increased (either at experimental or ambient levels), the responses of soil and vegetation to changes in climate, have been seen to be (often detrimentally) modified, and it is likely that this will inevitably influence changes at the microclimatic level also.

The microclimate is, therefore, directly relevant to the distribution and population dynamics of plants and, despite being a little studied area, has been shown to be a vital factor in the colonisation and subsequent development of heathland vegetation (Stoutjesdijk & Barkman, 1987). Data on lower plant species composition arising from the lower plant survey (chapter 4), could be used, in relation with radiation indices and soil data (e.g. temperature, moisture etc.), to study the effect of N mediated microclimate and micromorphology on the lower plant species distribution and *Calluna* establishment at Thursley.

**Ecosystem recovery**

Findings from the Thursley ecosystem recovery experiment have been well documented in previous studies (Barker, 2001; Green, 2005; Power *et al.* 2006; Jones, 2009) and the long-term nature of this experiment (since 1996), represents a valuable resource for understanding the resilience of sensitive ecosystems to N deposition disturbance, and the rate of recovery following an abatement of elevated N loads. Throughout the duration of the recovery study, the afore-mentioned authors consistently found that the lichen group was the most sensitive ecosystem element to former N additions, with abundance values severely inhibited in the historic N addition plots. In regard to this, a survey of lower plant abundance and reproductive viability in the recovery plots was carried out in 2011, and the data arising from this
study will be presented in a forthcoming publication, thereby contributing to this important body of research.
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